An Investigation into UV-Curable Gel Formulations for Topical Nail Medicines

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Declaration

'I, Laxmi Valji Kerai confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.'

December 2016

In loving memory of my grandmother, Prembai Shivji Kerai (1928 – 2013),

who encouraged me to start this Ph.D.

Acknowledgements

Abstract

UV gels are nail cosmetics which are applied on the nail plate surface and polymerised by placing the nail under a UVA nail lamp. The polymeric film formed can reside on the nail plate for up to 3 weeks without developing any visible defects. Using such a formulation as a drug carrier for the treatment of nail diseases, e.g. fungal infections, could address current issues with topical formulations, such as the failure to maintain a drug depot at the desired site and the need for frequent applications.

The aim of this thesis was thus to formulate pharmaceutical UV-curable gels, using antifungals as test drugs, and characterise the resulting film's properties, such as morphology, thickness, macro- and micro-structure, residual monomer content, stability, *in vivo* nail residence, drug release, ungual drug permeation and efficacy.

The gels formulated contained diurethane dimethacrylate, different reactive diluent (meth)acrylate-based monomers, a photoinitiator, antifungal drugs (amorolfine HCl or terbinafine HCl), solvents and penetration enhancers. The diluent monomer, solvent and penetration enhancer choice and amount varied in the gel in order to develop an optimised formulation.

Upon application to a nail model and exposure to UVA for 2 minutes, the pharmaceutical gels polymerised and formed smooth, transparent, thin and highly cross-linked films, containing negligible levels of residual monomers. The formulations were stable and were able to reside on the nails of volunteers for up to two weeks. Despite drug release studies showing incomplete release from the formulations developed, the amount of drug that permeated across the nail was sufficient to arrest the growth of the fungus *Trichophyton rubrum* (the most common fungus causing nail infections) in an *in vitro* model.

In conclusion, it has been demonstrated that the UV-curable gel formulations show potential as drug carriers for the topical treatment of nail diseases, in this instance for fungal infections.

Table of Contents

List of Tables

Chapter 3

Chapter 4

Chapter 5

Chapter 6

List of Figures

Chapter 1

Chapter 2

Chapter 3

Chapter 4

Chapter 5

Chapter 6

Abbreviations

AH   Amorolfine hydrochloride

ANOVA   Analysis of variance

ASTM   American Society for Testing and Materials

BA   Butyl acrylate

1,4-BDA   1,4-Butanediol acrylate

1,4-BDDMA   1,4-Butanediol dimethacrylate

BMA   Butyl methacrylate

BNF   British national formulary

CIR   Cosmetic ingredient review

CND   Creative Nail Design, Inc.

DC   Degree of conversion

DEGDA   Diethylene glycol diacrylate

DMA   Dynamic mechanical analysis

DSC   Differential scanning calorimetry

DUDMA   Diurethane dimethacrylate

EA   Ethyl acrylate

ECA   Ethyl cyanoacrylate

EGDMA   Ethylene glycol dimethacrylate

EMA   Ethyl methacrylate

ETOH   Ethanol

FID   Flame ionisation detector

Fig.   Figure

FT-IR   Fourier transform infrared

GC   Gas chromatography

HEA   Hydroxyethyl acrylate

HEMA   2-Hydroxyethyl methacrylate

HDPE   High density polyethylene

HPLC   High-performance liquid chromatography

HPMA   Hydroxypropyl methacrylate

IBOMA	Isobornyl methacrylate
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
ISO	International Organisation for Standardisation
IUPAC	International Union of Pure and Applied Chemistry
LoD	Limit of detection
LoQ	Limit of quantification
M	Monomer
MIC	Minimum inhibitory concentration
MMA	Methyl methacrylate
MPE	2-Mercaptoethanol
NF	Not formulated
NMP	1-Methyl-2-pyrrolidinone
NSI	Nail Systems International
PAF	Peak adhesive force
PBS	Phosphate buffer solution
PE	Penetration enhancer
PEG 200	Poly(ethylene glycol) 200
PI	Photoinitiator
PLM	Polarised light microscopy
SDA	Sabouraud dextrose agar
SEM	Scanning electron microscopy
Tg	Glass transition temperature
TGA	Thermal gravimetric analysis
TH	Terbinafine hydrochloride
TOWL	Transonychial water loss
TREGDA	Triethylene glycol diacrylate
TREGDMA	Triethylene glycol dimethacrylate
TRPGDA	Tripropylene glycol diacrylate
UDA	Urethane diacrylate
XRD	X-ray diffraction

Chapter 1: Introduction to the human nail, its diseases, current treatments, and UV-curable gels

In order to fully appreciate the need for a new topical nail medicine, knowledge regarding nail diseases, why these diseases require treatment, current treatment options, and the barriers faced for a successful response is required. This in turn requires an understanding of the nail's structure and function. This chapter therefore covers these bases, and eventually provides a detailed overview of UV-curable gels and why they have been researched extensively, in this thesis, as a candidate for topical nail therapy.

1.1 The structure of the human nail

The human nail is the thin, translucent, horny sheath on the dorsal surface of the distal end of each terminal phalanx of fingers and toes. It consists of the nail plate, nail matrix, proximal and lateral nail folds, nail bed, and hyponychium, which are collectively known as the nail unit (Gonzalez-Serva, 1997) (Fig. 1.1). The nail plate is produced by the nail matrix, emerges via the proximal nail fold and is secured in place by the lateral nail folds. It lies on the nail bed and detaches from it at the hyponychium, which is the region underneath the free edge of the nail plate. The nail plate is the most obvious part of the nail unit and its complex nature is discussed below. The nail unit is supported by the distal phalanx, which incidentally governs the shape of the proximal aspect of the nail plate (Parrinello et al., 1995). It has a rich blood supply maintained by two lateral digital arteries, which course down either side of a digit and eventually form arches that deliver blood to the nail matrix and nail bed. It additionally has a rich sensory nerve supply which also courses down either side of a digit in close association with the arteries (Rich and Scher, 2003).
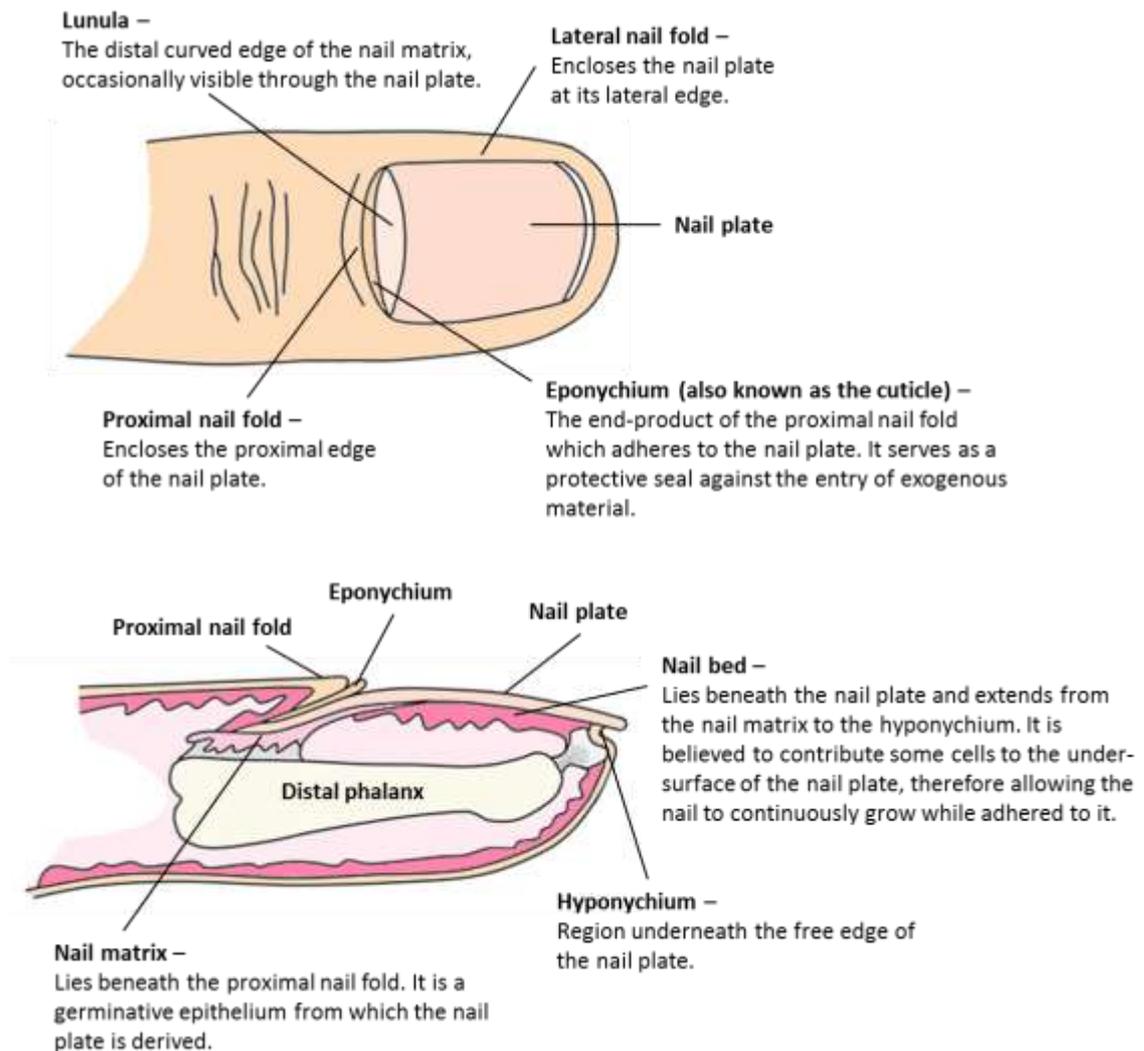
Fig. 1.1 Schematic diagram of the nail unit – external appearance and cross-section. (Adapted from Jiaravuthisan et al., *Journal of the American Academy of Dermatology,* 57, 1, 2007.)

1.1.1 The nail plate

The nail plate is the end-product of the keratinocyte differentiation in the nail matrix. The nail plate grows throughout life, but with varying rates among individuals (Fleckman, 2005). On average, it grows at a rate of approximately 3 mm per month for fingernails, and a significantly slower 1 mm per month for toenails (Rich and Scher, 2003). Therefore fingernails take 6 months to grow out completely, while toenails take between 12 – 18 months. Furthermore, the thickness of the fingernail plates (approximately 0.5 mm at the distal edge) is around half the thickness of the big toe (up to 1 mm at the distal edge) (Hamilton et al., 1955).

1.1.1.1 Nail plate layers

The nail plate is composed of approximately 80 – 90 layers of dead keratinised cells (Achten et al., 1991). It is considered to consist of three histological layers of keratinous tissue known as the dorsal, intermediate, and ventral layers, and the thickness ratio of each layer, i.e. dorsal: intermediate: ventral, is believed to be 3:5:2 (Kobayashi et al., 1999). The cells of the dorsal nail plate undergo extensive keratinisation and flattening during their complete maturation process. These cells overlap as seen in Fig. 1.2, resulting in the characteristic smooth surface of the nail which provides a barrier between the external environment and the body at the distal tips. In comparison, the cells of the ventral nail plate do not undergo keratinisation to the same extent, hence its softer and more pliable nature (Zaias and Alvarez, 1968); this can be seen as the smooth region in the cross-section image of the human nail clipping (Fig. 1.2B).
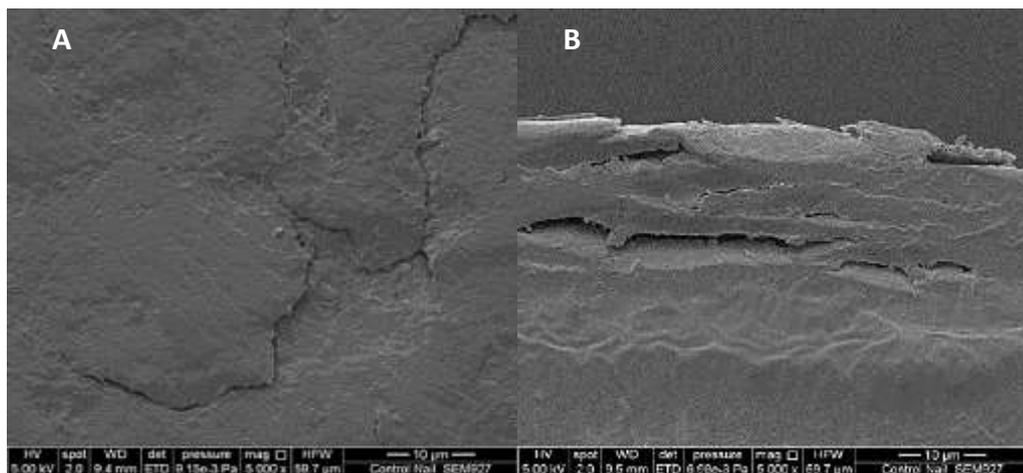
Fig. 1.2 Scanning electron micrograph of (A) the dorsal surface of a human nail plate and (B) the cross-section of a human nail clipping.

1.1.1.2 Nail plate composition

The nail plate contains both 'hard' hair-type keratin and 'soft' epithelial-type keratin, though the majority (80 – 90%) is the more stable hair-type (Lynch et al., 1986). The hair-type keratin are only present in the intermediate layer and are oriented perpendicular to the growth axis, in the nail plane, while the epithelial-type keratin are found in the dorsal and ventral layers and are oriented in two privileged directions; parallel and perpendicular to the growth axis (Garson et al., 2000). The keratin is

thought to be held together by globular, cysteine rich proteins, whose disulphide links behave like an adhesive (Fleckman, 1997). This 'sandwich' structure and the strong intercellular junctions give the nail great mechanical rigidity and hardness, both in the transverse direction and in the growth direction.

The nail plate also contains 0.1% calcium, but this barely contributes to its hardness (Rich and Scher, 2003). It contains water at 10 – 30%, which is directly related to the environmental relative humidity and is important for nail flexibility and elasticity (Forslind, 1970, Baden et al., 1973), while lipids account for less than 5% of the nail's content (Helmdach et al., 2000).

1.2 The function of the human nail

It is evident that the nail unit is a complex unique structure; it is to no surprise then that it serves numerous functions. These include protecting the distal digits, improving fine touch sensation, assisting in picking up and manipulating small objects, scratching and grooming, and enhancing the aesthetic appearance of the hands and feet. It is commonly used as a cosmetic organ, and by some, to communicate social class (Gonzalez-Serva, 1997, Rich and Scher, 2003). However it is often also abused by biting, which is thought to contribute to providing a sense of relief and alleviating boredom (Williams et al., 2007).

1.3 Nail diseases and current treatments

Unfortunately, the nail can suffer from a very wide range of disorders, from benign, for example, nail discoloration following the administration of drugs such as chloroquine, to extremely painful and serious, for example, malignant tumours of the nail apparatus. The majority of nail disorders arise from two nail diseases - onychomycosis and nail psoriasis. These diseases are therefore explored in the following sections.

1.3.1 Onychomycosis

Onychomycosis is a fungal nail infection, with a mean prevalence of 4.3% in Europe and North America (Sigurgeirsson and Baran, 2014). Over 50 million people worldwide suffered from the disease in 2012, and it is forecasted that cases of onychomycosis across the US, France, Germany, Italy, Spain, UK and Japan will increase by 15% over the next decade (De Angelis, 2013). Onychomycosis is more frequent amongst the elderly, diabetics, individuals with peripheral artery disease, and sports-active individuals. Its increase in occurrence is in part a reflection of the rise in individuals in these categories, together with the increasing numbers of individuals who are immunodeficient (resulting from an increased use of immunosuppressant drugs), lifestyle factors such as wearing ill-fitting footwear, and improved detection and greater public awareness (Piérard, 2001, Gupta et al., 1998, Gupta et al., 2000b, Caputo et al., 2001, Scher, 1996).

1.3.1.1 Etiology

The dermatophytes, particularly *Trichophyton rubrum* and *Trichophyton mentagrophytes*, yeasts such as *Candida albicans*, and non-dermatophytes such as *Scytalidium dimidiatum*, *Scytalidium hyalinum*, and *Fusarium* species have all been identified as causative agents for the disease (Midgley et al., 1994). In a recent study, the main causative agent was found to be a dermatophyte, accounting for 65% of cases, with *Trichophyton rubrum* identified as the single most common fungus (45%). Yeasts were found on average in 21% of cases, and non-dermatophyte moulds in 13% of cases (Sigurgeirsson and Baran, 2014). Onychomycosis predominately affects toenails compared to fingernails, with the ratio of toenail to fingernail onychomycosis ranging from 4:1 to 19:1 (Gupta et al., 2014). Onychomycosis can adopt several clinical patterns depending on the causative organism and site of infection, and is classified accordingly as explained in the following section.

1.3.1.2 Disease features and classification

The five main categories of onychomycosis are (A) distal and lateral subungual onychomycosis, (B) proximal subungual onychomycosis, (C) superficial white onychomycosis, (D) endonyx onychomycosis, and (E) total dystrophic onychomycosis, depending on where the infection begins (Ameen et al., 2014) (Fig. 1.3).

(A) Distal and lateral subungual onychomycosis

Distal and lateral subungual onychomycosis is the most common form, in which the nail bed is invaded by fungal (mainly *T. rubrum*) penetration through the distal or lateral margins. The affected nail can become thick, discoloured, and onycholysis may present to an extent. The infection can be confined to one side of the nail or spread to involve the whole nail bed. Over time the nail plate can therefore become friable and break.

(B) Proximal subungual onychomycosis

Proximal subungual onychomycosis is usually caused by *T. rubrum*, and can originate in the proximal nail fold, with subsequent penetration into the newly forming nail plate or beneath the proximal nail plate. It produces a white discolouration in the area of the lunula, and as the nail plate grows, the white discolouration moves distally.

(C) Superficial white onychomycosis

White superficial onychomycosis arises as the surface of the nail plate is invaded by the fungal organism, usually *T. mentagrophytes*. Crumbling white lesions appear on the nail surface, and the infection can spread to the deeper layers to involve the entire nail plate.

(D) Endonyx onychomycosis

Endonyx onychomycosis is most commonly caused by *T. soudanense* and *T. violaceum*, which invade the nail by immediately penetrating the nail plate keratin. It causes the nail plate to be discoloured white with the absence of onycholysis and subungual hyperkeratosis.

(E) Total dystrophic onychomycosis

Total dystrophic onychomycosis results from the unchecked progression of any of the other forms of onychomycosis, especially of distal and lateral subungual onychomycosis. It causes the nail plate to become hyperkeratotic and crumble, therefore the nail plate is essentially completely destroyed.

Mixed pattern onychomycosis

Occasionally, the same individual may present with different patterns of nail plate infection, with the most common combinations including proximal subungual onychomycosis with superficial white onychomycosis, and distal and lateral subungual onychomycosis with superficial white onychomycosis (Hay and Baran, 2011).
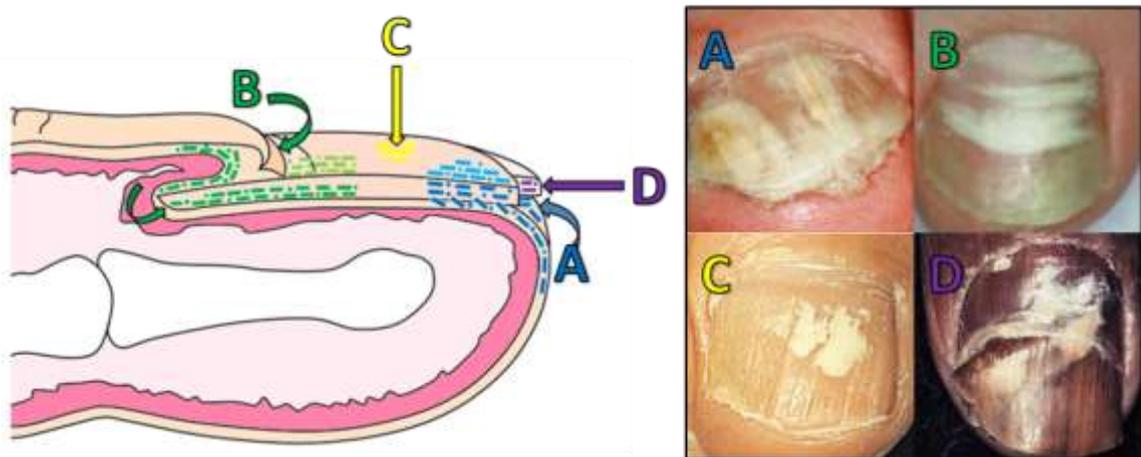
Fig. 1.3 Schematic diagram of the nail unit cross-section highlighting the sites of infection and hence types of onychomycosis (left), and corresponding images of its clinical appearance (right). (A) Distal and lateral subungual onychomycosis; (B) proximal subungual onychomycosis; (C) superficial white onychomycosis; (D) endonyx onychomycosis. (Adapted from Hay, R. J. and Baran, R., Baran & Dawber's Diseases of the Nails and their Management, Forth Edition, 2012.)

1.3.1.3 Why treat onychomycosis?

There are numerous reasons as to why the successful treatment of onychomycosis is an absolute necessity. For one, it can prevent the progression of the disease to its total dystrophic form and help restore the nails' natural beauty. While this may appear as tackling what looks to be merely a cosmetic nuisance, the reality is in fact far from this, as in addition to causing obvious changes to the appearance of the nail unit, onychomycosis presents with physical, psychosocial, and occupational consequences (Scher, 1996, Lubeck et al., 1993, Drake et al., 1999, Elewski, 1997, Whittam and Hay, 1997, Turner and Testa, 2000). One study which surveyed a total of 258 patients with confirmed onychomycosis, found that the disease caused nail-trimming problems (76%), embarrassment (74%), pain (48%), nail pressure (40%), discomfort wearing shoes (38%),

and an impaired ability to pick up small objects by those with fingernail involvement (41%). Furthermore, during a 6-month period, more than 58 onychomycosis-related sick days and 468 medical visits (1.8 per subject) were reported (Drake et al., 1998). A more recent article on the burden of onychomycosis revealed that up to 93% of patients believe that other people are repulsed by the site of their infected nails, and that nearly one-fifth of patients avoid various social activities due to the disease (Daniel, 2013).

If left untreated, an onychomycotic nail not only progresses to its total dystrophic form, but acts like a fungal reservoir with the potential to spread to other nails, body sites such as the groin, skin and scalp, and to other people (Ameen et al., 2014). It has been suggested that the presence of sensitising dermatophyte antigens in an infected nail unit may predispose to clinical conditions such as erythema nodosum, urticaria, atopic dermatitis and asthma or sensitisation of the bronchia and upper airways (Hicks, 1977, Weary and Guerrant, 1967, Wilson et al., 1993, Ward et al., 1989, Schwartz and Ward, 1995, Gumowski et al., 1987, Ameen et al., 2014). An onychomycotic nail can also disrupt the integrity of the skin providing an entry port for bacteria, and this may explain why diabetic patients with onychomycosis have a high predisposition to foot ulceration and gangrene (Joseph, 2002, Ameen et al., 2014).

The rise in the incidence of onychomycosis is of significant importance as it is further increasing the pool of infection. This urgently needs to be controlled and reversed, through education for prevention and treatment for elimination. The next section walks through the treatment options currently available for onychomycosis.

1.3.1.4 The current armamentarium for onychomycosis treatment

A range of treatment options are available for onychomycosis, with the main aim to permanently eliminate the infecting organism as demonstrated by culture and microscopy. In the UK, the options include eliminating the infectious agent by topical or systemic therapy; details of the antifungal prescription-only–medicines (POMs) available can be found in Table 1.1. There are further topical therapies for onychomycosis treatment which are available outside the UK, and all the available systemic and topical therapies are discussed in detail below. Laser and light therapies are also available outside the UK; however at the time of writing they had FDA clearance for only a

'temporary increase in clear nail in patients with onychomycosis' rather than cure, and will therefore not be discussed further.

Table 1.1 Onychomycosis treatment options in the UK as detailed in the British National Formulary (BNF) 71 (March 2016).

Therapy	Formulation	Dose	Duration
Systemic	Terbinafine (as hydrochloride) 250 mg tablet	250 mg daily	6 weeks – 3 months (occasionally longer in toenail infections).
	Itraconazole 100 mg capsule	200 mg daily	3 months
		200 mg twice daily	7 days, repeated after 21 day interval; fingernails 2 courses, toenails 3 courses.
	Griseofulvin 500 mg tablet	500 – 1000 mg daily	6 – 9 months for fingernails and 12 – 18 months for toenails.
Topical	Amorolfine (as hydrochloride) 5% w/v nail lacquer	Applied to infected nails 1 – 2 times weekly after filing and cleansing.	6 months for fingernails and 9 – 12 months for toenails (review at intervals of 3 months).
	Tioconazole 28% w/v solution (Trosyl®)	Applied to nails and surrounding skin twice daily.	Up to 6 months (may be extended to 12 months).

Systemic therapies

The BNF states that for onychomycosis treatment, a systemic antifungal is more effective than topical therapy. These agents enter the systemic circulation following oral administration and absorption, and diffuse from the blood vessels into the nail plate via the nail bed. The two most widely used drugs are terbinafine and itraconazole, with terbinafine considered the drug of choice, and itraconazole reserved for Candida and non-dermatophyte infections (Sá et al., 2014). Terbinafine and itraconazole both concentrate and persist in the nail plate following therapy cessation. Itraconazole can persist for up to 6 – 9 months, compared to the 3 – 6 months for terbinafine (Baran et al., 2008a, Baran et al., 2008b), and this explains the success of the 'pulsed' administration of itraconazole. Griseofulvin is rarely considered for onychomycosis treatment given its lower efficacy, higher relapse rates, longer treatment duration and hence lower compliance rates, and greater risk of drug interactions when compared to the others. Fluconazole is not licensed for the treatment of onychomycosis, but may be a useful alternative for patients who cannot tolerate terbinafine or itraconazole (Ameen

et al., 2014). These antifungals' mechanism of actions, spectrum of activity and mycological cure rates are detailed in Table 1.2.

Table 1.2 Mechanism of action, spectrum of activity and mycological cure rates of systemic antifungals used for onychomycosis treatment.

Antifungal	Mode of action	Spectrum of activity	Mycological cure rate (toenail infections)
Terbinafine	Terbinafine is an allylamine which inhibits the enzyme squalene epoxidase of the ergosterol synthetic pathway. Intracellular accumulation of squalene initiates a cascade of events that leads to the fungicidal activity, whereas the depletion of ergosterol (needed for integrity of the fungal cell membranes) leads to fungistatic activity (Ryder and Favre, 1997).	Terbinafine has a broad and potent fungicidal effect against dermatophytes, particularly *T. rubrum* and *T. mentagrophytes*, but a lower fungistatic activity against Candida species (Bueno et al., 2010).	74 – 82% (Goodfield, 1992, Drake et al., 1997).
Itraconazole	Itraconazole is a triazole which inhibits the fungal cytochrome P450-mediated 14-alpha-lanosterol demethylation, an essential step in fungal ergosterol biosynthesis. This results in the depletion of ergosterol and fungistatic activity (Vanden Bossche et al., 1989).	Itraconazole is active against a range of fungi including yeasts, dermatophytes and some non-dermatophyte moulds. When compared to terbinafine, it is not as active *in vitro* against dermatophytes – it has a 10 times greater minimum inhibitory concentration (MIC) (Bueno et al., 2010).	Pulse therapy: 88.2 – 90.6% (Gupta et al., 2001, Gupta et al., 2006).
Griseofulvin	Griseofulvin acts by inhibiting fungal nucleic acid synthesis, arresting cell division and inhibiting fungal cell wall synthesis (Roobol et al., 1977).	Griseofulvin is weakly fungistatic against species of *Trichophyton, Microsporum*, and *Epidermophyton*. Studies comparing griseofulvin therapy with terbinafine and itraconazole have demonstrated lower cure rates for griseofulvin (Faergemann et al., 1995, Haneke et al., 1995, Korting et al., 1993).	30 – 40% (Davies et al., 1967).
Fluconazole	Fluconazole is also a triazole and therefore its use results in the depletion of ergosterol in a similar manner to itraconazole.	Fluconazole has some activity against dermatophytes and some Candida species. However, it is less effective than itraconazole or terbinafine (Arca et al., 2002).	47 – 62% (Scher et al., 1998).

Unfortunately, systemic therapy can cause adverse reactions such as headache, gastrointestinal symptoms, taste disturbances, and more rarely, liver and kidney

disturbances. Furthermore, while terbinafine displays minimal drug–drug interactions, itraconazole and fluconazole can inhibit the cytochrome P450 3A4 isoenzyme system, with fluconazole additionally inhibiting CYP2C9. They therefore have the potential to increase the plasma concentration of other concomitantly administered drugs that are metabolised by these enzyme systems, which can cause major problems. Oral therapy with standard antifungal drugs is also subject to a consistent failure rate of 20%, as well as a relapse rate of 25% (Roberts, 1999, Gupta, 2012). Reasons identified for treatment failure include poor patient compliance, misdiagnosis, low bioavailability, lack of drug penetration into the nail due to extensive onycholysis for example, and the presence of dormant, drug-resistant fungal spores (Pierard et al., 2000, Baran et al., 2008a, Baran et al., 2008b) .

Topical therapies

Therapy with topical antifungals is limited to mild cases of distal and lateral subungual onychomycosis, for superficial white onychomycosis, or where there are contraindications to systemic therapy. As shown in Table 1.1, amorolfine (as hydrochloride) 5% w/v nail lacquer and tioconazole 28% w/v solution (Trosyl®) are treatment options in the UK, with amorolfine 5% w/v nail lacquer considered as the more superior agent due to its greater efficacy against onychomycosis-causing pathogens. While both are available as POMs, amorolfine nail lacquer is also available to the public over-the-counter (OTC) for the treatment of mild cases of distal and lateral subungual onychomycosis caused by dermatophytes, yeasts and mould; subject to treatment of a maximum of two nails (BNF 71, March 2016).

Outside the UK, two ciclopirox 8% w/v nail lacquers are available, Penlac® (also marketed as Batrafen®, Loprox® and Mycoster®) and Ciclopoli® (also marketed as RejuveNail®). Both should be applied once daily, for up to 24 weeks on the fingernails and up to 48 weeks on the toenails. Two recently FDA approved formulations for toenail onychomycosis – Jublia® (efinaconazole) 10% topical solution and Kerydin® (Tavaborole) 5% topical solution – are also available. These are applied to the affected toenails once daily for 48 weeks. These antifungals' mechanism of actions, spectrum of activity and mycological cure rates are shown in Table 1.3.

Table 1.3 Mechanism of action, spectrum of activity and mycological cure rates of topical antifungals used for onychomycosis treatment.

Formulation	Antifungal's mode of action	Spectrum of activity	Mycological cure rate
Amorolfine 5% w/v nail lacquer	Amorolfine is a morpholine antifungal agent that inhibits the delta 14 reductase and delta 8 and delta 7 isomerase enzymes in the ergosterol biosynthetic pathway which leads to fungistatic and fungicidal activities (Ameen et al., 2014).	It exhibits a broad spectrum of activity against dermatophytes, non-dermatophyte moulds and yeasts, and is fungicidal against *C. albicans* and *T. mentagrophytes* (Ameen et al., 2014).	52% for toenail onychomycosis (Zaug, 1995).
Tioconazole 28% w/v solution (Trosyl®)	Tioconazole is an imidazole derivative which inhibits the CYP450 dependent enzyme lanosterol 14 alpha-demethylase, involved in the ergosterol biosynthetic pathway. This results in ergosterol depletion and fungistatic activity.	It exhibits a broad spectrum of activity against common dermatophytes and *C. albicans* (Jevons et al., 1979).	22% (Hay et al., 1985).
Ciclopirox 8% w/v nail lacquer (Penlac®, Batrafen®, Loprox®, Mycoster® Ciclopoli® or RejuveNail®)	Ciclopirox is a hydroxypyridone and by binding to trivalent cations such as Fe^{3+}, it inhibits several metal-dependent enzymes such as cytochromes. It has been shown to affect the energy production of mitochondrial electron transport processes, to reduce the activity of catalase and peroxidase which are responsible for the degradation of toxic peroxides, and to affect the nutrient uptake and associated synthesis of protein and nucleic acid. Ciclopirox is therefore fungicidal and also has anti-inflammatory and anti-allergic properties (Bohn and Kraemer, 2000).	Ciclopirox is active against dermatophytes, as well as yeasts and non-dermatophyte moulds (Bohn and Kraemer, 2000).	29 – 36% for toenail onychomycosis (Gupta et al., 2000a).
Efinaconazole 10% topical solution (Jublia®)	Efinaconazole is a triazole antifungal which inhibits ergosterol biosynthesis by inhibiting the enzyme 14 alpha-demethylase. The resulting ergosterol depletion and accumulation of precursor sterols may secondarily affect cell membrane integrity and function (Tatsumi et al., 2013).	Efinaconazole has shown activity against Trichophyton, Microsporum, Epidermophyton, Acremonium, Fusarium, Paecilomyces, Pseudallescheria, Scopulariopsis, Aspergillus, Cryptococcus, Trichosporon and Candida (Jo Siu et al., 2013).	53 – 55% for toenail onychomycosis (Elewski et al., 2012).
Tavaborole 5% topical solution (Kerydin®)	Tavaborole is a novel oxaborole antifungal drug that exerts its antifungal activity by blocking cellular protein synthesis via inhibition of fungal cytoplasmic leucyl-aminoacyl transfer RNA (tRNA) synthetase. Formation of a stable tRNA[Leu] – tavaborole adduct inhibits leucyl-tRNA[Leu] synthesis, thus blocking protein synthesis (Markham, 2014).	Tavaborole is fungicidal against *T. rubrum* and *T. mentagrophytes*. It is also active against *Trichophyton tonsurans, Epidermophyton floccosum, Microsporum audouinii, Microsporum canis and Microsporum gypseum, Aspergillus fumigatus, Candida* spp., *Cryptococcus neoformans, Fusarium solani, Malassezia* spp. and *Saccharomyces cerevisiae* (Markham, 2014).	31 – 36% for toenail onychomycosis (Elewski et al., 2015).

As can be seen when comparing Tables 1.2 and 1.3, the mycological cure rates are lower with topical antifungals than systemic antifungals, and this justifies why systemic therapy is ideal when infections become more complex.

Another product available for fungal nail infections in the UK and mentioned in the BNF 71 (March 2016) is a Phytex® paint. It contains salicylic acid 1.46%, tannic acid 4.89% and boric acid 3.12% (as borotannic complex), and should be applied twice daily after washing. However it is considered by the Joint Formulary Committee to be less suitable for prescribing, as there is limited data to support its use as a therapy for onychomycosis (Ameen et al., 2014).

There is a vast range of other products (in addition to the amorolfine 5% w/v nail lacquer) that are available OTC for the treatment of mild fungal nail infections, examples of which are shown in Table 1.4. While some contain known actives such as urea, miconazole, and benzalkonium chloride, published studies regarding the efficacy of these products are lacking and their use cannot therefore be recommended.

Table 1.4 Products available OTC from UK pharmacies (Boots, Lloyds and Tesco) for the topical treatment of fungal nail infections (these products were available on 11th August 2015).

Boots Advanced Footcare Fungal Nail Treatment Solution	Canespro® Fungal Nail Treatment Set	Daktarin® 2% Cream	Excilor® Treatment for Fungal Nail Infections
Contains pentylene glycol, dimethyl isosorbide, olive-leaf active-TM, hydrolyzed silk fibroin, 2-phenoxyethanol and water.	Contains one 10g urea ointment tube (40% urea), 22 specially designed waterproof plasters, and one plastic scraper.	Contains miconazole nitrate, PEG-6, PEG-32, glycol stearate, oleoyl macroglycerides, liquid paraffin, benzoic acid (E210), butylated hydroxyanisole (E320) and purified water.	Contains ethyllactate, acetic acid, penetration enhancer, film-forming agent, water, glycerol, polysorbate 80, cetyl acetate, acetylated lanolin alcohols, biotin and preservatives (contains no parabens).

Profoot Mycosan Fungal Nail Treatment	Nailner Repair Brush	Scholl Fungal Nail Treatment	ClearZal® BAC Antimicrobial Solution
Contains aqua, rye ferment filtrate, pentylene glycol, dimethyl isosorbide and hydroxyethylcellulose.	Contains ethyl lactate, glycerin, aqua, lactic acid and citric acid.	Contains an 'advanced liquid'. Neither the packaging, nor the bottle nor the enclosed instruction leaflet gives any indication as to what the product's active ingredients are.	Contains benzalkonium chloride (0.1%), purified water (aqua), cocamidopropylbetaine, cocamidopropylamine oxide, hypromellose, centrimonium chloride, organic aloe vera, didecyldimonium chloride, diazolidinyl urea, quaternium-15, methylisothiazolinone, phenoxyethanol, triethanolamine and citric acid.

Systemic and topical therapy combination

Some studies suggest a topical and systemic antifungal therapy combination for severe onychomycosis, as this can achieve a higher cure rate, reduces the oral drug intake, and demonstrates a better cure per cost ratio (Baran et al., 2000, Lecha, 2001, Avner et al., 2005, Baran et al., 2007). However, there are also a few studies which have failed to find

statistical differences between topical and systemic antifungal combination therapies and oral alone therapies (Rigopoulos et al., 2003, Jaiswal et al., 2007). There appears to be a need for further rigorously conducted, randomised, controlled trails in order to draw firm conclusions regarding the efficacy of combination therapy.

1.3.2 Nail psoriasis

Psoriasis is a chronic, immune-mediated inflammatory disease of the skin which typically causes red, flaky, crusty patches covered with silvery scales. At the cellular level, it is characterised by epidermal hyperproliferation, incomplete differentiation and accumulation of glycogen, and it affects between 1.5 – 1.9% of the UK population (Rosa et al., 2012). As skin appendages, nails can be affected by psoriasis, and the life time incidence of nail involvement in psoriatic patients is as high as 80 – 90% (Tripuraneni and Kerdel, 2014). Nail psoriasis without any cutaneous involvement is rare but does occur, the reasons for which are unknown, and it accounts for 1 – 3% of cases (Wozel, 2008).

1.3.2.1 Disease features

The main features of nail psoriasis are pitting, discoloration, onycholysis, subungual hyperkeratosis, nail plate dystrophy, and splinter hemorrhages, which can be correlated with the site of nail involvement (Holzberg and Baran, 2012) (Fig. 1.4). Proximal nail fold involvement results in its psoriatic scaling accompanied with soft tissue swelling. Involvement of the matrix causes changes which are reflected in the outgrowing nail plate, and these changes depend on disease duration as well as location. For instance, lesions may be limited to nail pitting and transverse furrows reflecting transient matrix dysfunction, while superficial pits indicate matrix disease in the proximal region; trapped parakeratotic cells in the plate (which cause the clinical appearance of leukonychia – white discoloration of the nail plate) reflect matrix disease in the distal region, and sustained nail abnormalities such as nail plate thickening or nail loss reflect persistence disease. Nail bed involvement results in splinter hemorrhages (which are longitudinal collections of extravasated blood under the nail plate), an oil-droplet like discoloration (which is caused by serum glycoprotein accumulation in and under the nail), subungual hyperkeratosis and/or onycholysis.
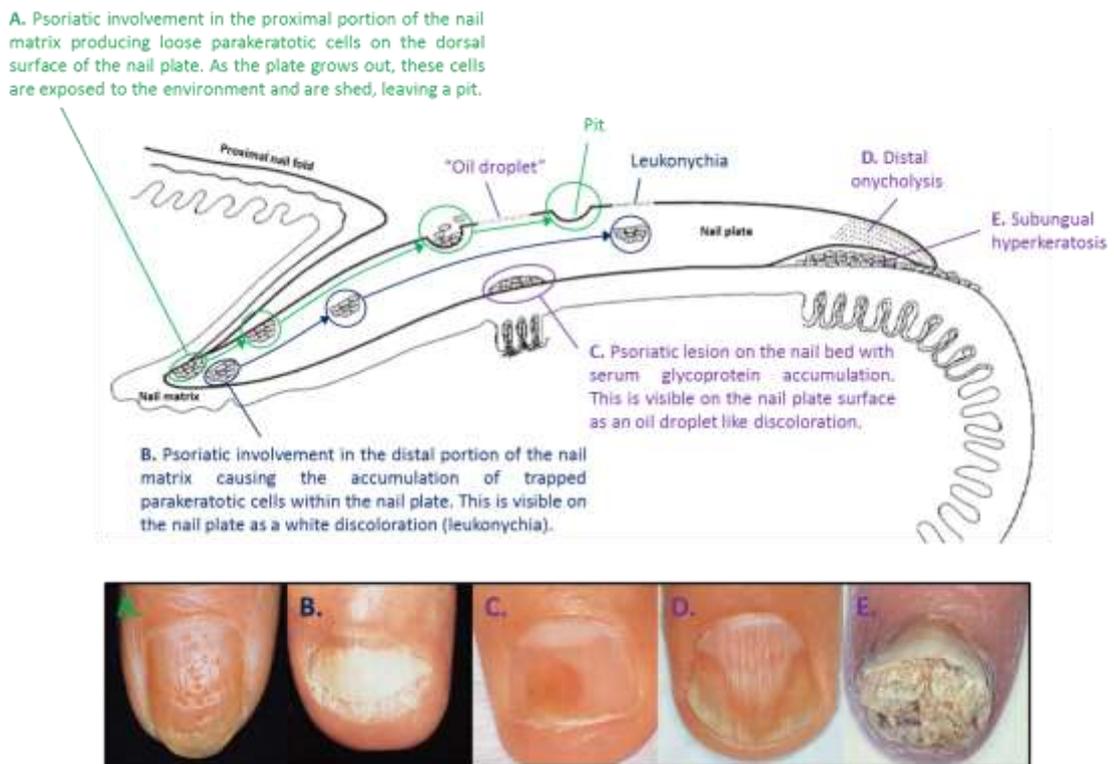
Fig. 1.4 Top – schematic diagram of the nail unit cross-section highlighting:
(i) the main clinical features of nail psoriasis – A. pitting; B – C. discoloration (leukonychia (B) and "oil-drop" (C)); D. onycholysis; E. subungual hyperkeratosis, and
(ii) the site of nail involvement – proximal portion of the matrix, distal portion of the matrix and nail bed.
Bottom – corresponding images of clinical appearances. (Adapted from Camisa, C., Handbook of Psoriasis, Second Edition, 2007 and Holzberg, M. and Baran R., The Nail in Dermatological Disease, 2012).

Psoriatic nail disease can lead to significant functional impairment, pain, an altered sense of touch, and psychological stress. It can also have a negative impact on social and work activities, and hence quality of life (van der Velden et al., 2014, Baran, 2010, de Jong et al., 1996). The management of nail psoriasis is therefore necessary, and in the next section the options available are discussed.

1.3.2.2 Options available for the management of nail psoriasis

Unfortunately, a standardised therapeutic regimen for nail psoriasis does not currently exist (Crowley et al., 2015). However, based on the studies available for its management, the options could include topical, intralesional, radiation, and systemic therapies, which are discussed below.

Topical therapies

Topical therapies can be applied to the nail plate, nail fold and/or nail bed depending on the location of the psoriatic lesion. In order to facilitate a successful therapeutic response, nails with onycholysis present are trimmed to the point where it separates from the nail bed, bulky nails are chemically avulsed, and patients may be advised to cover the topical formulation with an occlusive dressing, such as non-porous tape or plastic gloves, to enhance drug movement into the nail unit (Holzberg and Baran, 2012). Therapies with drugs such as glucocorticoids, vitamin D3 analogues, 5-flourouracil, dithranol, tazarotene and ciclosporin in vehicles such as creams, ointments, gels, solutions and lacquers have all been investigated, and Table 1.5 provides a summary of these. In general, topical therapies appear to be an effective form for treating subungual hyperkeratosis, but lack convincing evidence for other dystrophies. Glucocorticoids and vitamin D3 analogues are the more popular choices of topical therapy, however as clinical outcomes are not standardized, comparison among treatment options is virtually impossible.

Table 1.5 Topical therapies for nail psoriasis management, their efficacies and side-effects.

Topical active	Formulation and efficacy	Side-effects
Glucocorti-coids	**Betamethasone dipropionate (64 mg/g) with salicylic acid (3%) in an ointment base (Diprosalic® ointment in UK)**: Reduced <u>subungual hyperkeratosis</u> by 52% when used twice a day for 5 months (Tosti et al., 1998). **Clobetasol-17-propionate (8%) nail lacquer (not available in the UK):** Daily use for an average of 2.5 months lead to a 69% improvement, while using the formulation 2 – 3 times per week for an average of 7 months lead to an 80% improvement, particularly in <u>nail pitting and onycholysis</u> (Holzberg and Baran, 2012).	Erythema, skin atrophy and tapering of the distal phalange on the treated digit with prolonged use.
Vitamin D3 analogues	**Calcipotriol (50 µg/g) ointment (also available as Dovonex®)**: Reduced <u>subungual hyperkeratosis</u> by 49% for fingernails and 41% for toenails when used twice a day for 5 months (Tosti et al., 1998). **Calcipotriol (50 µg/g) cream (no longer marketed in the UK):** 3 months use showed improvement of <u>subungual hyperkeratosis and onycholysis</u> in five of seven patients (Holzberg and Baran, 2012). **Tacalcitol (4 µg/g) ointment (Bonalfa® ointment, ISDIN S.A, marketed as Curatoderm® in the UK):** Found to be effective in all nail parameters, especially <u>onycholysis and subungual hyperkeratosis</u>, followed by oil-drop discoloration and pitting. The trial included 15 patients and the ointment was used daily at night in occlusion (with cotton gloves) over a 6-month period (Márquez Balbás et al., 2009).	Erythema, periungual irritation, burning at the site of application, and diffuse urticaria.

Glucocorti- coids and Vitamin D3 analogues combination	**Betamethasone dipropionate and calcipotriol ointment (Dovobet® ointment in UK):** Application once daily for 12 weeks in a study involving 25 patients resulted in a significant improvement for <u>hyperkeratosis and onycholysis</u>, moderate improvement for <u>oil drops</u> and slight improvement for pitting (Rigopoulos et al., 2009).	Refer to side- effects for glucocorticoids and vitamin D3 analogues.
	Combination of calcipotriol cream (no longer marketed in the UK) and clobetasol propionate cream (Dermovate® in UK): Use of calcipotriol cream daily during the weekday and clobetasol propionate cream daily over the weekend for 6 months, followed by the use of only clobetasol propionate cream daily over the weekend for a further 6 months, was found to improve <u>subungual hyperkeratosis</u> by 77% at 12 months in a study involving 60 patients (Rigopoulos et al., 2002).	
5-Fluorouracil	**Fluorouracil (1%) solution (not available at this strength in the UK):** Twice daily application for 6 months around the margin of the nail improved <u>pitting and hyperkeratosis</u> by 75% in 17 of 20 patients, however onycholysis worsened (Frederiksson, 1974).	Transient rhabdomyoly- sis, yellow nail discoloration, pain, swelling, discoloration, inflammation, onycholysis and nail perforations.
Dithranol	**Anthralin (0.4 – 2.0%) ointment (Dithranol ointment, BP in the UK):** In a study of 20 patients, 5 months of 30-min short contact dithranol 0.4 – 2.0% ointment showed a fair response in 60% of patients for <u>onycholysis and subungual hyperkeratosis</u> and, to a lesser extent, pitting (Yamamoto et al., 1998).	Irritation and considerable temporary staining.
Tazarotene	**Tazarotene (0.1%) gel (Zorac® in the UK):** One study found a statistically significant improvement in <u>onycholysis</u> (occluded and non-occluded nails) and <u>nail pitting</u> (if applied under occlusion) following daily evening application for 24 weeks (Scher et al., 2001). In another study 19 of 25 patients, showed a statistically significant improvement in <u>onycholysis, hyperkeratosis, pitting and oil spots</u>, following daily evening application for 12 weeks. However the effects started to lapse after 24 weeks (Bianchi et al., 2003).	Proximal nail fold peeling and erythema, periungual irritation, paronychia and skin irritation.
Ciclosporin	**70% of ciclosporin (Neoral® oral solution) and 30% of maize oil:** This solution was applied to affected fingernails with a little brush twice daily for a period of 12 weeks. A mean improvement of 77% was observed, with the best results obtained for <u>onycholysis and hyperkeratosis</u> (Cannavò et al., 2003). However, to date, finding an effective vehicle for topical cyclosporine delivery has been challenging (Prins et al., 2007, Holzberg and Baran, 2012).	No side-effects were noted.

Intralesional therapy

An intralesional corticosteroid injection (most commonly of triamcinolone acetonide) is considered effective owing to the anti-inflammatory and anti-proliferative effect of the corticosteroid which is commonly administered through a 28 gauge needle directly to the target site (via the nail folds, usually the proximal nail folds). Clinicians typically determine frequency of injection by response and often taper the regimen over a 12-

month period. The therapy is more effective in treating ridging, nail thickening and subungual hyperkeratosis. The main disadvantage is pain; however a local anaesthetic may be used to overcome this. Other adverse effects include subungual haemorrhage, reversible atrophy at the injection sites, periungual hypopigmentation and potential atrophy of the underlying bone (Holzberg and Baran, 2012).

Radiation therapies

Radiation therapy is not routinely used in the treatment of psoriatic nails, however a number of therapies have been investigated for their potential use. One form of radiation therapy is photochemotherapy with UVA in addition to the photosensitizer psoralen (PUVA). PUVA has been found to improve subungual hyperkeratosis, onycholysis, discoloration, nail crumbling and proximal nail fold symptoms. However, complete clearing is uncommon and pitting may not respond. Moreover there is a potential for adverse side-effects such as subungual haemorrhage, photo-onycholysis and local pigmentation (Holzberg and Baran, 2012). Other types of radiation therapies include Grenz rays, superficial radiotherapy and electron beam therapy. However, there are a limited number of studies available assessing their efficacy, from which the response rates have been found to be variable (Jiaravuthisan et al., 2007).

Systemic therapies

The systemic therapies available include acitretin, methotrexate, cyclosporine, and biological immunomodulators such as adalimumab, etanercept, golimumab, infliximab and ustekinumab. For disease limited to the nails, the toxicity of the medication used against the benefits of treatment need to be weighed, while also considering the degree of debilitation of the patient's nail psoriasis. Therefore due to the associated toxicities and cost of the systemic therapies available, systemic treatment is only recommended when there is widespread skin or joint involvement in addition to nail psoriasis (Holzberg and Baran, 2012).

Best practice recommendations from the medical board of the National Psoriasis Foundation/ USA

In order to help guide treatment decisions for clinicians who are treating patients with nail psoriasis, treatment recommendations for four clinical nail psoriasis scenarios have recently been developed based on a review of evidence and expert opinion of the Medical Board of the National Psoriasis Foundation (Crowley et al., 2015). The recommendations are summarised in Table 1.6 and are consistent with the discussions above.

Table 1.6 Treatment recommendations for four clinical nail psoriasis scenarios (Crowley et al., 2015).

Clinical nail psoriasis scenario	Treatment Recommendation
Disease limited to the nails	High-potency topical corticosteroids with or without calcipotriol. Intralesional corticosteroids can also be considered.
Significant nail disease and where topical therapy has failed	Adalimumab, etanercept, intralesional corticosteroids, ustekinumab, methotrexate, and acitretin.
Significant skin and nail disease	Adalimumab, etanercept, and ustekinumab are strongly recommended, and methotrexate, acitretin, infliximab, and apremilast (not licensed in the UK) are recommended.
Significant nail, skin, and joint disease	Adalimumab, etanercept, ustekinumab, infliximab, methotrexate, apremilast, and golimumab.

1.4 Topical drug delivery

As discussed in the previous section, nail diseases are mostly treated with therapeutic agents administered systemically or topically. Systemic therapy can be seen to be more efficacious; however it is associated with a number of serious side effects, drug interactions, contraindications, high recurrence rates, and can be considerably costly. Topical therapies bypass the side effects, drug interactions and contraindications associated with systemic therapy; it is therefore the ideal mode of therapy. However topical therapies for nail diseases such as onychomycosis and nail psoriasis remain unsatisfactory. The following sections explore the barriers to a successful therapeutic response following topical therapy, and past and current attempts to improve their efficacy.

1.4.1 The barriers to a successful therapeutic response

The efficacy of therapy with topical therapeutic agents can be limited by several factors, one of which is poor patient compliance (Zhou et al., 2011) due to the requirement of regular topical drug application until all the affected nail tissue has grown out, which can take up to several months. The other more important factor, (which comes into play even when adherence is not an issue), is the limited penetration of the therapeutic agent through the nail plates' firmly bound layers of dead keratinous cells and compact dorsal structure. One study actually found the total drug uptake into the nail to be less than 0.2% of the applied dose after 6 weeks of application twice daily (vanHoogdalem et al., 1997). Other factors dig deeper and include those that affect the ungual permeability of the permeant, such as the permeants' molecular weight, partition coefficient, keratin affinity, and ionisation, in combination with the diseased nail plate properties, all of which are discussed briefly below.

1.4.1.1 Permeant properties

The most important factor affecting ungual permeation is molecular size, as the extent of permeation is significantly decreased when the molecular weight of the permeant increases (Kobayashi et al., 2004, Mertin and Lippold, 1997c). This is why it has been suggested that antifungal drugs considered for the topical management of onychomycosis should have a molecular weight not larger than 350 g mol^{-1} (Elsayed, 2015). The permeant's partition coefficient was previously thought to also be an

important factor for ungual permeation, given that the nail plate is believed to behave like a hydrophilic gel membrane rather than a lipophilic partition membrane. As expected, a decrease in nail permeability with increasing lipophilicity of the permeant had been found (Walters et al., 1983, Mertin and Lippold, 1997b, Kobayashi et al., 2004), but this association was disregarded and instead attributed to the increasing molecular weight of the permeant. It should not be neglected altogether however, as the effect of the permeant lipophilicity is expected to be prominent for permeants with similar molecular weights and wide lipophilicity differences.

The permeant's affinity to keratin also limits its nail permeability. This is particularly important to consider for onychomycosis treatment as *in vitro* antifungal assays have demonstrated than keratin binds to and reduces the antifungal activity, i.e. increases the MICs (minimum inhibitory concentrations) or the MFCs (minimum fungicidal concentrations), of amorolfine and terbinafine. However this binding is often reversible (Tatsumi et al., 2002), and keratin can thus act as a reservoir that prolongs antifungal drug effect.

Finally, vehicle pH and permeant ionisation is expected to affect the nail permeability of a permeant. The isoelectric point of nail keratins is in the range of 4.0 – 5.0 (Elsayed, 2015), and they therefore carry a net positive charge at a pH below their isoelectric point and a net negative charge at a pH above their isoelectric point. The apparent pH at the nail plate surface is 5.0 and at the nail plate interior is 4.1 (Murdan et al., 2011). The nail plate is therefore almost neutral under normal conditions, while at pH 7.4 the nail plate surface charge is negative. As expected the nail permeability of water and non-ionisable compounds are unaffected by varying the formulation pH in the range of 2 – 11. However, charges on ionised permeant molecules are expected to electrostatically interact with the charges on nail keratins, thus enhancing or reducing nail permeability. Furthermore, permeant ionisation can increase its aqueous solubility which in turn is expected to increase ungual flux, while ion hydration and the consequent increase of the permeant's apparent molecular weight can decrease the nail diffusivity and permeability (Elsayed, 2015). Disappointingly, contradictory results have been reported in studies investigating the effect of vehicle pH and permeant ionisation on nail permeability (Mertin and Lippold, 1997b, Walters et al., 1985a, Kobayashi et al., 2004).

1.4.1.2 Diseased nail plate properties

The nail plate's diseased state can also affect nail permeability with the direction and extent of change dependent on the clinical features of the affected nail. For instance, an onychomycotic or psoriatic nail plate can be much thicker than a healthy nail plate, and its permeability is therefore expected to be considerably lower (Kobayashi et al., 2004). In an onychomycotic nail, the presence of dermatophytoma, which is a dense focus of fungi with thick shortened hyphae, is thought to reduce the success of oral therapy (Roberts and Evans, 1998), and topical therapy could potentially follow a similar fate as drug diffusion within the dermatophytoma is expected to be reduced. An onychomycotic or psoriatic nail plate can also present with onycholysis, thus presenting a barrier for drug movement from the formulation to the nail bed. However, in such cases, if suitable, the formulation can be applied to the exposed portion of the nail bed. On the other hand, a 'crumbly' onychomycotic nail could have greater porosity and thereby increase drug permeability. Likewise, pitting, a symptom of psoriatic nail disease, may enhance drug permeability in a similar manner that microporation of the nail plate does (which is discussed in the next section); however, this requires investigation.

1.4.2 Enhancing the nail permeability of topically applied drugs

As previously mentioned, the nail plate behaves as a hydrophilic gel membrane, and when the nail plate is hydrated, the nail keratin networks contain pores filled with aqueous solution. Using a cylindrical pore model, the effective size of the pores radius in a fully hydrated nail has been estimated to be around 0.7 nm (Hao and Li, 2008), which is the same order of magnitude as the Stokes-Einstein radii of most drugs. When the pore dimensions are of the same order as those of permeants, the permeants' transport (via diffusion and convection) through the aqueous pore pathways can be significantly restricted (Deen, 1987, Hao and Li, 2008), and this explains why the molecular size and shape of the permeant is an important factor in transungual transport. At the same time the pore size and nail porosity are equally important, with nail permeability boosted by enlarging the nails' effective pore size and/or increasing its porosity by enlarging pre-existing pores or creating larger pores, both of which can be achieved by hydrating the nail and/or treating the nail with ungual penetration enhancers. When considering the nail plate layers, the dorsal layer is the main barrier to permeant transport through the

nail plate into the nail bed (Nair et al., 2009a, Kobayashi et al., 1999). Ungual permeation of topically applied drugs can therefore also be enhanced by disrupting the dorsal layer by physical means, and the following section explores the more popular physical and chemical approaches to enhance ungual penetration. It should be noted at this point that the drug permeation into an intact nail plate may be encouraged by formulating the drug within a vehicle which enables drug partition out of the vehicle and into the nail plate; however topical vehicles will be covered extensively in Section 1.5.

1.4.2.1. Physical approaches to enhance ungual drug delivery

Physical techniques can enhance ungual permeation by disrupting the dorsal nail plate layer prior to the application/re-application of drug-loaded formulations, and simple techniques such as nail filing using an abrasive have shown success (Pittrof et al., 1992, Lauharanta, 1992). This is why amorolfine 5% w/v nail lacquer is provided with an abrasive stick and why filing the nail is recommended prior to formulation application/re-application. However, despite this, as shown in Table 1.3, the mycological cure rate following the use of this nail lacquer remains inadequate.

Etching the dorsal nail plate surface with brief applications of 20% tartaric acid solution or a 10% phosphoric acid gel prior to the application of drug-loaded topical formulations has shown to enhance the formulations' adhesion to the nail plate as well as drug permeation. Etching with acid increases the surface roughness and hence surface area of the nail, providing a greater opportunity for drug-loaded films to bond with the nail plate, and permeation is enhanced by the decreased membrane thickness of the etched nail plate (Repka et al., 2002, Repka et al., 2004).

Other physical approaches to disrupt the dorsal nail plate surface include abrasion of the nail plate surface in a more aggressive manner (i.e. by using electrical equipment and dental drills), ablation of the nail plate using pulsed lasers, microporation of the nail plate, and application of low-frequency ultrasound (Murdan, 2008). While these approaches have been shown to increase ungual drug penetration, little has been done to examine how these techniques can be used in practice.

Finally, iontophoresis, which is a method to deliver a compound across a membrane with the assistance of an electric field, has also been shown to enhance ungual drug delivery by influencing the movement of the permeant into and through the nail (Delgado-Charro, 2012, Hao et al., 2009, Nair et al., 2009c, Narasimha Murthy et al., 2007). It is unique to the other methods as it avoids the need to reduce the integrity of the nail plate. Donor pH is an important factor in influencing iontophoretic flux due to its effects on the ionisation of the permeant and the nail plate. For greatest iontophoretic drug flux, the pH of the donor drug solution should allow the full ionisation of the permeant, and the nail plate should be oppositely charged. However, further work is required to establish the safety of the technique on repeated application, and the clinical potential of the technique to treat onychomycosis and nail psoriasis has not been fully established.

1.4.2.2 Chemical approaches to enhance ungual drug delivery

The drug penetration into the nail plate can be enhanced using chemical agents that break the physical and chemical bonds which maintain the integrity of the nail keratin, such as the disulphide, peptide, hydrogen and polar bonds. These penetration enhancers can be concomitantly applied with the penetrant or used prior to application of the penetrant, depending on their compatibility status.

Thiols – which are compounds containing a sulfhydryl group – are the most effective ungual penetration enhancers. Examples include N-acetylcysteine, 2-mercaptoethanol, thioglycolic acid and N-(2-mercaptopropionyl) glycine. These compounds promote nail plate softening and swelling, and increase nail plate porosity by irreversibly reducing and therefore disrupting the disulphide bonds in keratin (Nogueiras-Nieto et al., 2011, Chouhan and Saini, 2012, Khengar et al., 2007, Hao et al., 2008, Kobayashi et al., 1998, Malhotra and Zatz, 2002b, Brown et al., 2009). Another compound capable of disrupting the disulphide bonds in keratin by reduction is sodium sulfite. While this inorganic salt has shown success in enhancing ungual penetration, it has been hypothesized that it may also act by increasing the hydration of the nail plate and the thermodynamic activity of the drug (Nair et al., 2009b).

Keratolytic agents, such as urea and salicylic acid, also promote nail softening and swelling (Chouhan and Saini, 2012, Hao et al., 2008, Kobayashi et al., 1998), but via keratin denaturation. Unfortunately, when used alone these agents are unable to enhance ungual drug flux through the nail (Kobayashi et al., 1998, Malhotra and Zatz, 2002b, Quintanar-Guerrero et al., 1998). However a combination of two keratolytic agents or a keratolytic agent and a thiol can enhance ungual drug flux by supposedly working synergistically, with urea, for example, capable of augmenting the transungual permeation enhancing effects of N-(2-mercaptopropionyl) glycine and N-acetyl-cysteine (Malhotra and Zatz, 2002b, Quintanar-Guerrero et al., 1998). Hydrogen peroxide, which is an oxidising agent, is thought to oxidise and therefore cleave disulphide bonds. When complexed with urea, it promotes nail plate softening and swelling (Khengar et al., 2007), and similar to keratolytic agents, it augments the transungual permeation enhancing effect of thiols and is ineffective when used alone (Brown et al., 2009).

Finally, keratolytic enzymes which have the ability to hydrolyze nail keratins are expected to weaken the nail plate's barrier properties and enhance transungual permeation. A keratinase enzyme from the fungus *Paecilomyces marquandii* did precisely that and enhanced the permeability of a model permeant through a model nail plate (Mohorcic et al., 2007). However, further investigations are required to demonstrate the efficacy of keratinase on human nail plates.

Other ungual penetrations enhancers have also been identified and include 2-*n*-nonyl-1,3-dioxolane (Hui et al., 2003), hydroxypropyl-β-cyclodextrin (Chouhan and Saini, 2014), hydrophobins (Vejnovic et al., 2010b, Vejnovic et al., 2010a), sodium phosphate (Nair et al., 2009b), and solvents such as water (Walters and Flynn, 1983, Walters et al., 1985b), dimethyl sulfoxide (DMSO) (Stüttgen and Bauer, 1982, Franz, 1992, Hui et al., 2002, Vejnovic et al., 2010b), methanol (Vejnovic et al., 2010b), low molecular weight polyethylene glycol (PEG) (Nair et al., 2010, Ahn et al., 2013) and *N*-methyl-2-pyrrolidone (NMP) (Ahn et al., 2013, Myoung and Choi, 2003). These enhancers were tested as excipient in formulations and do not necessarily work by breaking bonds which maintain the integrity of the nail keratin, but rather promote nail hydration, increase the aqueous solubility of the drug, possibly induce nail lipid depletion, promote a higher affinity of

the drug towards the nail surface or promote an improved adhesion of the formulation to the nail plate.

1.5 Topical drug vehicles

It was previously mentioned that drug permeation into an intact nail plate may be encouraged by formulating the drug within a vehicle which enables drug partition out of the vehicle and into the nail plate. Even when factors that can enhance permeation are considered, such as disrupting the dorsal nail plate layer prior to formulation application or using chemical enhancers, if the vehicle itself is inadequate, the success rate following therapy will reflect this. There were various topical nail formulations for onychomycosis and nail psoriasis which had been discussed earlier which included solutions, lacquers, gels, ointments and creams. Other formulations investigated or under investigation include films, patches, and colloidal carriers. These are all discussed in detail below in order to highlight why there remains a need for a new topical nail medicine.

1.5.1 Nail solutions

Nail solutions are highly concentrated solutions of a drug in a suitable solvent. When the nail solution is a concentrated aqueous or alcoholic antimicrobial solution in a volatile vehicle, it is called a paint (Shivakumar et al., 2013). Following application of the nail solution on the nail surface, the solvent evaporates to leave a highly concentrated deposit of drug on the nail (not necessarily in the form of a film), which is expected to penetrate and diffuse through the nail plate. The depth of penetration of topically applied therapeutic agents depends on the solvents used to make up the nail solution (Hui et al., 2002), and Table 1.7 shows the excipients used in some of the topical solutions mentioned in previous sections. Unfortunately, such a topical dosage form needs to be applied frequently, and fails to maintain therapeutic drug levels at the desired site due to its ease of removal.

Table 1.7 Excipients of topical solutions currently approved for treating nail diseases (onychomycosis).

Product	Active Pharmaceutical Ingredient	Solvent base
Trosyl® nail solution	Tioconazole 28% w/v	Undecylenic acid and ethyl acetate
Jublia® topical solution	Efinaconazole 10% w/w	Alcohol, anhydrous citric acid, butylated hydroxytoluene, C12-15 alkyl lactate, cyclomethicone, diisopropyl adipate, disodium edetate and purified water.
Kerydin® topical solution	Tavaborole 5% w/w	Alcohol, propylene glycol and edetate calcium disodium
Phytex® paint	Salicylic acid 1.46% (total combined), tannic acid 4.89% and boric acid 3.12% (as borotannic complex)	Alcohol and ethyl acetate

1.5.2 Nail lacquers

Nail lacquers contain a therapeutic agent in a suitable lacquer base. Following a nail lacquer's application on the nail plate surface, its volatile solvent(s) evaporate leaving behind a highly concentrated deposit of drug in a polymeric film on the nail, from which the drug is released and permeates through the nail plate. The polymeric film formed can reduce transonychial water loss (TOWL), and is therefore likely to hyper-hydrate the upper nail layers, which can aid the drug permeation into and through the nail plate (Spruit, 1971, Marty, 1995). This hydration property is useful in onychomycosis treatment, as the hydration can also facilitate germination of drug-susceptible fungal hyphae and limit the formation and persistence of drug-resistant fungal spores, thus limiting the risk of reinfection. The polymeric film formed can additionally inhibit the adhesion of fungal propagules on and underneath the nail plate, which can prevent fungal infection/reinfection in its initial step (Flagothier et al., 2005), a useful property even when non-infectious nail diseases are being treated. Table 1.8 provides a list of excipients for the nail lacquers mentioned in previous sections, and identifies film-formers and solvents as key ingredients alongside the pharmaceutical active.

Table 1.8 Excipients of the nail lacquers currently approved for treating nail diseases (onychomycosis).

Product	Active Pharmaceutical Ingredient	Lacquer base
Amorolfine 5% w/v nail lacquer (Loceryl®)	Amorolfine 5% w/v, as the hydrochloride salt	Water-insoluble film-former: Ammonio methacrylate copolymer A (Eudragit® RL,) Plasticizer: triacetin Solvents: butyl acetate, ethyl acetate and ethanol
Penlac® (also marketed as Batrafen®, Loprox® or Mycoster®) nail lacquer	Ciclopirox 8% w/v	Water-insoluble film-former: Butyl monoester of poly(methylvinyl ether/maleic acid) Solvents: ethyl acetate and isopropyl alcohol
Ciclopoli® (also marketed as RejuveNail®) nail lacquer	Ciclopirox 8% w/v	Water-soluble film-former: Hydroxypropyl chitosan Solvents: cetostearyl alcohol, ethyl alcohol, ethyl acetate and purified water

The amount of drug permeated through the nail plate and the drug uptake by the nail has been shown to be influenced by the solvent constituting the lacquer (Franz, 1992). Similarly, the drug concentration in the deposited lacquer film following application influences drug permeation and uptake into the nail plate (Mertin and Lippold, 1997a, Pittrof et al., 1992), with increasing concentrations showing to be favourable as long as the drug is maintained in its soluble/amorphous form after evaporation of the solvent(s). The addition of penetration enhancers such as 2-*n*-nonyl-1,3-dioxolane or hydroxypropyl-β-cyclodextrin in a lacquer formulation can further increase ungual flux (Hui et al., 2003, Chouhan and Saini, 2014).

Finally, the choice of the film-former used in the nail lacquer can affect several factors in addition to transungual permeation. The water-insoluble films, such as methacrylic polymer and vinyl resin based films, provide sustained drug release and are resistant to washing, however, they require weekly removal either by mechanical means or by using a suitable solvent, which may affect the surrounding skin. Water-soluble films, such as hydroxypropyl chitosan based films, adhere to the nail plate to a greater extent and facilitate a greater drug partitioning/ release (by directly promoting nail hydration) when compared to the water-insoluble films (Monti et al., 2010, Monti et al., 2005), however, they can be easily washed off from the nail surface. In order to combine the advantageous occlusiveness and wash-resistance of water-insoluble films with the adhesion and drug release properties of water-soluble films, a bilayer nail lacquer

composed of an underlying drug-loaded hydrophilic layer and overlying hydrophobic vinyl layer was suggested (Shivakumar et al., 2010). This formulation although promising complicates the topical drug administration protocol, which requires the application of a drug-loaded hydrophilic nail lacquer, followed by the application of a hydrophobic nail lacquer once the hydrophilic layer has dried.

Another novel drug-loaded nail lacquer formulation has recently been suggested to facilitate an effective topical response. The vehicle was an anhydrous/alcohol composition with a dual acrylate–silicone hybrid copolymer system that offers film forming and occlusion properties due to synergistic plasticizing properties. The vehicle base contained ethanol, polysilicone-8, panthenol, acrylates copolymer, tocopheryl acetate, phytantriol, butylene glycol, benzophenone-3, calcium chloride, and fragrance (Hafeez et al., 2013). However *in vivo – in vitro* correlations are required to define the clinical relevance of the results obtained.

1.5.3 Semi-solids

Semi-solids such as gels, ointments and creams are also available or have been investigated for the treatment of nail disorders, as these formulations can provide the necessary chemical gradient to deliver the drug to the infected site.

1.5.3.1 Gels

Gels can hold a considerable amount of water and therefore have the potential to hydrate the nail plate upon application, even more so than nail lacquers (Shivakumar et al., 2013). This in turn can enhance drug permeation due to the formation of a less dense structural keratin matrix with large pores. One study actually demonstrated the better efficacy of gel formulations compared to a lacquer in enhancing the permeation of antifungals across the nail (Hui et al., 2004). The gel used was a Loprox® gel which is marketed outside the UK for dermatological infections, and contains ciclopirox (0.77%) in a base consisting of octadecanol, dimethicone copolyol 190, carbomer 980, isopropyl alcohol, sodium hydroxide, docusate sodium and purified water. The lacquer used was Penlac® (which contains ciclopirox 8% w/v), and this study highlights how the type of formulation plays a more crucial role in determining drug penetration than drug concentration in the formulation.

Zorac® 0.1% gel is another gel used for nail diseases. It contains tazarotene (0.1%) in a gel base consisting of benzyl alcohol, macrogol 400, hexylene glycol, carbomer 974P, trometamol, poloxamer 407, polysorbate 40, ascorbic acid, butylhydroxyanisole (E320), butylhydroxytoluene (E321), disodium edetate and purified water. While it has shown to be of some benefit for nail psoriasis management (Table 1.5), like the Loprox® gel, it was initially formulated for use on the skin, in this case for mild to moderate plaque psoriasis affecting up to 10% of the skin area.

There are a few studies available that have investigated gels formulated specifically for application to the nail plate, but with the overall aim to evaluate the effect of nail etching or various penetration enhancers on the permeability of the human nail plate (Repka et al., 2004, Malhotra and Zatz, 2002b, Nair et al., 2009b). These studies suggest that gels could be the most ideal formulations for etched nails as they could easily flow into the microporosities at the nail surface and therefore establish good contact at the microscopic level, and also for incorporating water soluble penetration enhancers such as sodium phosphate. Gels are also the most suitable formulation for iontophoresis, but unfortunately, they can be easily wiped off the surface of the nail plate during regular activities and so their use is not popular.

1.5.3.2 Ointments

Ointments have been tested for use in the topical treatment of various nail disorders. Table 1.9 shows the collection of excipients that make up the ointment bases for the formulations which have been tested for nail psoriasis management. Unfortunately the efficacies of these formulations are below par. Formulations tested for onychomycosis include a bifonazole (1%) and urea (40%) ointment which has been shown to adequately soften a diseased nail for atraumatic removal (Hardjoko et al., 1990), and a tolnaftate (2%) in urea 20% ointment which has shown some success (Ishii et al., 1983). However these formulations required the use of an occlusive dressing, and the overall success following therapy was still inadequate. Furthermore, like gels and solutions, ointments can be easily wiped off the surface of the nail plate during regular activities, and this factor limits their use.

Table 1.9 Excipients of the ointments tested for management of nail psoriasis.

Product	Active Pharmaceutical Ingredient	Ointment base
Diprosalic® ointment	Betamethasone dipropionate 0.064 % w/w (equivalent to 0.05 % w/w betamethasone) and salicylic acid 3.0 % w/w	Liquid paraffin and white soft paraffin
Dovobet® ointment	Betamethasone dipropionate 0.05% and calcipotriol monohydrate 50 μg/g	Liquid paraffin, all-rac-α-tocopherol, polyoxypropylene-11stearyl ether, white soft paraffin and butylhydroxytoluene (E321)
Dovonex® ointment	Calcipotriol 50 μg/g	Disodium edetate, disodium phosphate dihydrate, DL-α tocopherol, liquid paraffin, macrogol (2) stearyl ether, propylene glycol, purified water and white soft paraffin.
Curatoderm® ointment	Tacalcitol monohydrate 4.17 μg/g (tacalcitol 4 μg/g)	White petrolatum, liquid paraffin and diisopropyl adipate
Dithranol ointment, BP	Dithranol 0.1 – 2.0%	Yellow soft paraffin

1.5.3.3 Creams

Creams are emulsion-based topical formulations and are typically water-in-oil (w/o) or oil-in-water (o/w) emulsions, depending on the solubilities of the actives that need to be incorporated. Table 1.10 shows the list of excipients that make up a cream base for some of the formulations tested for nail diseases. Clinical experts do not find a good rationale for treating onychomycosis with antifungal creams applied to intact nail, apart from cases of superficial white onychomycosis (de Berker, 2009), hence creams studied for onychomycosis have been applied following mechanical reduction of the nail plate or (non)surgical nail avulsion (Davies, 2006, Baden, 1994). Creams have been studied for nail psoriasis without compromising the nail plate structure, and the use of calcipotriol cream daily during the weekday and clobetasol propionate cream daily over the weekend for 6 months, followed by the use of only clobetasol propionate cream daily over the weekend for a further 6 months, was found to improve subungual hyperkeratosis (Rigopoulos et al., 2002). However this treatment regimen can be

considered as tedious and response rates are inadequate. Furthermore, creams can also be easily wiped off the surface of the nail plate during regular activities.

Table 1.10 Excipients of the creams tested or available for the management of onychomycosis.

Product	Active Pharmaceutical Ingredient	Cream base
Loprox cream (not available in the UK)	Ciclopirox 0.77%	Benzyl alcohol, cetyl alcohol, cocoamide diethanolamine, lactic acid, mineral oil, myristyl alcohol, octyldodecanol, polysorbate 60, purified water, sorbitan monostearate and stearyl alcohol.
Clotrimazole cream	Clotrimazole 1%	Benzyl alcohol, cetostearyl alcohol, cetyl palmitate, octyldodecanol, polysorbate 60, sorbitan stearate and purified water.
Nizoral® cream	Ketoconazole 2% w/w	Propylene glycol, stearyl alcohol, cetyl alcohol, sorbitan stearate, polysorbate 60, isopropyl myristate, sodium sulphite anhydrous (E221), polysorbate 80 and purified water.

1.5.4 Hot-melt extruded films

Hot melt extrusion is the process of applying heat and pressure to melt an active compound embedded in a carrier formulation (consisting of polymeric materials or low melting point waxes) and forcing it though an orifice in a continuous process. One study investigated the use of a Killion extruder (Model KLB 100) to produce hot-melt extruded films for the topical treatment of onychomycosis. The excipients included ketoconazole (20%), hydroxypropylcellulose, polyethylene oxide and Noveon AA1, and these were blended thoroughly, dried at 50 ^{o}C for 24 hours, and then extruded at 115 – 120°C to form films with good content uniformity, drug content, bioadhesion, and drug permeation through the nail (Repka et al., 2004). While further work was conducted on hot-melt extruded films, these mainly revolved around the study of the films' physio-chemical and mechanical properties, and drug stability (Mididoddi and Repka, 2007, Mididoddi et al., 2006), and the efficacy of such a topical therapy was never established.

1.5.5 Patches

A bioadhesive patch was also investigated for the ungual delivery of 5-aminolevulinic acid (ALA), a naturally occurring precursor of the photosensitiser protoporphyrin IX, which could be useful in the treatment of onychomycosis. The patch was prepared by a film-casting technique and was produced from an aqueous solution of ALA, methyl vinyl

ether and maleic anhydride copolymer, tripropylene glycol methyl ether and ethanol. This mixture formed a gel which was casted into a mould consisting of a release liner placed on a levelled surface and dried under a warm air flow to prepare a 2.5 x 3 cm patch. A backing layer consisting of medical grade polyvinyl chloride plasticised with dibutyl phthalate (Plastisol®) was attached to one side of the formed patches. Patch application for 24 hours allowed drug permeation into the nail, and it was suggested that drug penetration across nail may be improved using penetration enhancers or by filing of the impenetrable dorsal surface of the nail (Donnelly et al., 2005).

Sertaconazole nail patches were also investigated for the treatment of onychodystrophy and onychomycosis. The sertaconazole-containing nail patches were 2.2 cm² in area and contained sertaconazole, lactate, aerosol and aluminium acetylacetonate in the adhesion layer and Durotak® was used as the backing layer. Sixteen healthy adults were treated with this patch in a double-blind study, for a treatment period of 6 weeks with the patches replaced weekly. The drug was detected in all sertaconazole-treated nail samples (Susilo et al., 2006), however further work is required to establish the clinical relevance of the results.

The onychomycotic drug, ciclopirox, was also formulated into a patch to investigate the effect of various pressure sensitive adhesives on its penetration. It was found that the patch could enable ciclopirox release and hence permeation, with the highest permeability from an acrylic adhesive with a hydroxyl functional group, followed by styrene-isoprene-styrene, styrene-butadiene-styrene, silicone and polyisobutylene adhesives in order (Myoung and Choi, 2003). More recently, terbinafine was formulated into a patch to investigate the same parameter. Similar to ciclopirox, terbinafine release was possible but this time the permeation was highest from the silicone adhesive matrix, followed by polyisobutylene and most of the acrylic adhesives, polystyrene–polyisoprene–polystyrene and finally polystyrene–polybutadiene–polystyrene (Ahn et al., 2013).

While patches address problems associated with the conventional topical dosage forms, such as the need for frequent applications and failure to maintain therapeutic drug

levels at the desired site, they do not have an aesthetically pleasing appearance. Furthermore, studies have not demonstrated the clinical efficacies of such formulations.

1.5.6 Colloidal drug carrier systems

Colloidal drug carrier systems are well known for their penetration enhancing effects on the skin, and consist of an oil and water phase stabilized by emulsifiers in a certain ratio which has to be ascertained. The penetration behavior of a novel highly lipophilic antifungal agent (EV-086K) for the treatment of onychomycosis was investigated in one study in four different formulations. The first formulation was a hydrogel consisting of the active, Tylose H 30.000 P2 PHA hydroxyethylcellulose, glycerol, and distilled water. The second was a colloidal carrier system which contain the active, propylene glycol and distilled water (hydrophilic phase), PEG-20 glyceryl oleate and Poloxamer 331 (emulsifiers), and butylphthalimide isopropylphthalimide (oil component). The third was a nail lacquer consisting of the active, ethanol, Eudragit®E100 (a cationic copolymer based on dimethylaminoethyl methacrylate, butyl methacrylate and methyl methacrylate), and the solubiliser diethylene glycol monoethyl ether. The final formulation was a solution which contained the active, ethanol, distilled water, the solubiliser diethylene glycol monoethyl ether, the pH adjusters citric acid and sodium phosphate monobasic, and the antioxidants butylated hydroxytoluene and ethylenediaminetetraacetic acid. The investigations found that the developed colloidal drug carrier system significantly increased the penetration rate of an extremely lipophilic antifungal drug through the human nail plate compared to other formulations (nail lacquer, solution and hydrogel). The high drug penetration rate following the application of the colloidal carrier system was down to the presence of propylene glycol in the system, which improved the solubility of the drug in the hydrophilic phase and therefore enabled the system to obtain a high drug concentration, and hence high thermodynamic activity and drug flux. Propylene glycol may have also acted as a penetration enhancer (Naumann et al., 2014). It should be noted though that while the developed active penetrated into the human nail plate in high concentrations, it did not completely penetrate through the nail plate.

NB-002 is an antifungal, oil-in-water nanoemulsion containing highly purified oil, ethanol, polysorbate 20, water, and cetylpyridinium chloride, with activity against

dermatophytes, including spores. It consists of nanodroplets that have an average diameter of 180 nm which can be taken up through skin pores and hair follicles when applied to the skin surrounding the nail, where they laterally diffuse to the site of infection (nail bed). In patients with distal subungual onychomycosis, NB-002 was applied to all 10 toenails and 5 mm of adjacent skin for 42 weeks. While NB-002 showed clear antifungal activity with clinically significant nail clearing, at 4 or 8 weeks post-treatment, the mycological cure rates were low and ranged from 25.0% to 31.7% (Ijzerman et al., 2010).

Finally, TDT 067, which is terbinafine in soybean phosphatidylcholine – Tween 80 deformable lipid vesicles (Transfersomes®), is a new terbinafine preparation that has been formulated for topical delivery to the nail, nail bed, and surrounding tissue for onychomycosis treatment. *In vitro* studies have shown that TDT 067 has inhibitory and fungicidal activity against dermatophytes and the non-dermatophytic pathogens of onychomycosis. It has a higher activity than conventional terbinafine preparations, and the Transfersome® is believed to accelerate entry of terbinafine into the fungal cells and enhance its fungicidal effects. A clinical study which involved treating infected nails as well as its surrounding skin with the TDT 067 liquid spray twice daily for 12 weeks demonstrated reasonable mycological cure rates and few local reactions, however, an ongoing Phase-III study is investigating the efficacy and safety of TDT 067 further (Dominicus et al., 2012, Ghannoum et al., 2011, Ghannoum et al., 2012, Sigurgeirsson and Ghannoum, 2012).

1.5.7 The need for a new nail medicine

As demonstrated, there is a vast range of topical drug vehicles available for nail diseases with the aim to deliver a therapeutic level of drug at the desired site. Unfortunately, as also seen, these vehicles require frequent applications, which reduce patient compliance, and/or are easily removed from the surface of the nail plate, thus failing to maintain therapeutic drug levels. There is therefore an urgent need for a nail medicine which can overcome these limitations, while being aesthetically acceptable in appearance at the same time.

1.6. UV-curable gels as potential topical nail medicines

The concept of using a nail product as a drug delivery vehicle was borrowed from the cosmetic industry. The cosmetic industry also uses artificial nail enhancements for decorative purposes or to hide unattractive nail deformities such as discoloration, splitting, and breaking, which can be a result of median canal dystrophy or splinter hemorrhages for example. The first artificial nail appeared in the United States around 1935. Throughout the years their quality has improved and as a result they have become more and more popular. At one point they were almost exclusively applied by professional trained manicurist in nail salons, which can be quite expensive, however artificial nail kits designed for home use are now available over-the-counter (Andre et al., 2014). There are three main types of artificial nail enhancements which include nail wraps, liquid and powders, and UV gels (Schoon, 2010). These are all more durable than the conventional nail lacquer (Schoon and Baran, 2012), and the following section explores these enhancements and their safety, and outlines why UV-curable gels were investigated as potential topical nail medicines in this thesis.

1.6.1 Artificial nail enhancements – an introduction

1.6.1.1 Nail wraps

Nail wraps are also known as fiberglass wraps, resin wraps, no-light gels, silk or paper wraps, and are a less popular nail enhancement technique, accounting for about 1 - 2% of the worldwide market (Jefferson and Rich, 2012, Schoon and Baran, 2012). They are created from methyl and ethyl cyanoacrylate monomers, with the cyano functional group believed to provide tremendous adhesion to the natural nail plate, and are applied onto a clean, dehydrated nail surface, with dehydration achieved by wiping the nail surface with a nail wipe coated with isopropyl alcohol. Typically, thin layers of a fibrous substance (silk, linen or fiberglass) are applied within multiple layers of cyanoacrylate monomers. Alternatively, the cyanoacrylate monomers can be thickened with polymers such as polymethyl methacrylate and applied without a reinforcing fibrous substance; such systems are termed no-light gels. Cyanoacrylate monomers are applied to the nail plate directly from its container's nozzle and cure upon exposure to nail plate moisture. The curing process is accelerated to 5 – 10 seconds by solvents containing a tertiary aromatic amine such as dimethyl tolylamine (0.5 – 1 %), which can

be sprayed on, applied with a dropper or impregnated into the woven fabric. Following the cure, the nail wrap coating can be shaped as desired. This technique is often used to mend cracks or tears in the nail plate, by using the monomer to adhere a small piece of fabric over the broken or damaged area of the nail plate (Schoon and Baran, 2012, Schoon, 2010). Nail wraps are often referred to as three-component systems, as they commonly require a fabric mesh, resin (cyanoacrylate monomer) and activator (tertiary aromatic amine) (Newman, 2005b).

Nail wraps are removed using acetone, and the removal process involves placing the nails in a small bowl and immersing them in the solvent, a process that requires less than 10 minutes (Schoon and Baran, 2012).

1.6.1.2 Liquid and powders

The liquid and powder system is a two-component system, which is also known as acrylic, porcelain or solar nails (Schoon and Baran, 2012). It came directly from the dental industry, was the first enhancement to be used commercially, and is popular in the UK and the US, holding approximately 50% of the nail market (Newman, 2005b). The 'liquid' component is a mixture of ethyl methacrylate (60 – 95%), other di- or tri-functional methacrylate monomers (3 – 5%), stabilisers such as hydroquinone or methyl ether hydroquinone (100 – 1000 ppm), catalysts such as dimethyl tolylamine (0.75 – 1.25%), and other additives such as soluble colorants and plasticizers. The 'powder' component consists of poly methyl and/or ethyl methacrylate powder beads (approximately 50 – 80 μm in size) coated with 1 – 2% benzoyl peroxide (which is a polymerization initiator), and other additives such as colorants and opacifiers (e.g. titanium dioxide) (Schoon, 2010, Schoon and Baran, 2012, Andre et al., 2014).

Mixing the liquid and the powder components initiates polymerisation; the two components are therefore mixed before use and immediately applied and smoothed into shape on the nail surface. The actual procedure for liquid and powder application involves dipping a brush into the monomer liquid, wiping off the excess and drawing the tip of the brush through the polymer powder to form a small bead at the end of the brush. Typically three to six beads are normally smoothed into shape with the brush per nail plate. The mixed components polymerise by free radical polymerisation and harden

on the nail surface within 2 – 3 minutes. Over 95% of the polymerisation occurs in the first 5 – 10 minutes, however complete polymerisation can take anywhere between 24 – 48 hours. After hardening, the nail is shaped either by hand filing or with an electric file, and the product formed is highly durable (Schoon, 2010, Schoon and Baran, 2012).

Prior to the application of the liquid and powder system, the natural nail is cleaned with a disinfected soft-bristled brush to remove surface oil and debris, lightly filed with a low grit abrasive file (180 – 240 grit) to increase the surface area, wiped with a nail wipe coated with nail surface dehydrators containing isopropyl alcohol to remove surface moisture and residual oils, and primers are also applied to aid adhesion by increasing the surface compatibility between the natural nail and product. The primers usually contain, for example, mixtures of hydroxylated monomers or oligomers which adhere well to both the nail plate and product. Removal of the liquid and powder product also requires acetone, and the removal process is the same as that for the nail wraps, but longer in duration (30 – 40 minutes) as the polymer is highly cross-linked. However, this duration can be greatly accelerated by pre-filing to remove the bulk of the system (Schoon, 2010, Schoon and Baran, 2012).

1.6.1.3 UV gels

UV gels are also known as gel nails (Schoon and Baran, 2012), and have for the most part, also been derived from the dental industry, where light-cured materials are used for dental bonding. UV gels are becoming increasing popular, and in some countries such as Germany, they are favoured over liquid and powder systems (Newman, 2005b). UV gels are one-component systems which typically contain a urethane methacrylate based monomer (75 – 85%), mono- and multi-functional (meth)acrylate based monomers (15 – 25%), and a photoinitiator (1 – 4%) such as 1-hydroxycyclohexyl phenyl ketone or 2-hydroxy-2-methylpropiophenone. The gel cures under low intensity UVA light typically in the range of 435 – 325 nm, and this is provided by a UVA nail lamp, which can hold one to five 6 – 9 W bulbs depending on the design. An alternative UVA light source is a light-emitting diode lamp, suitable for gels which contain photoinitiators that respond to a narrower spectrum of UVA light (Newman, 2005b, Jefferson and Rich, 2012, Schoon and Baran, 2012).

Prior to gel application, in order to ensure good adhesion, the natural nail is cleaned, lightly filed, and dehydrated in the same manner as liquid and powder systems. A layer of gel is then applied on the nail plate surface and the nail is placed under a UVA lamp for 1 – 3 minutes. The UVA light activates the photoinitiator in the gel to form free radicals which facilitate a polymerisation process to produce a polymeric film on the nail plate. Further gel layers are sequentially applied and cured as the UVA light does not efficiently penetrate more than a few millimetres into the UV gel. Following the curing process, an occlusive polymer film is produced on the nail surface. This film is wiped with isopropyl alcohol in order to remove an unreacted monomer layer on its surface, caused by an oxygen-induced inhibition of polymerisation. The resulting film is highly durable and can be worn for up to 3 weeks without developing any visible defects (Schoon, 2010, Jefferson and Rich, 2012, Schoon and Baran, 2012).

Removal of the UV gel requires acetone, and the removal process is the same as that for the nail wrap and liquid and powder system, but longer in duration (45 – 60 minutes) as the highly cross-linked urethane methacrylate based polymers have an inherently greater solvent resistance (Schoon, 2010). Alternatively, it can be removed with a wooden stick 10 minutes after applying a cotton pad soaked with acetone over the coating (Schoon and Baran, 2012).

1.6.2. Artificial nail enhancement - safety concerns

1.6.2.1 Allergic contact dermatitis

Unfortunately, the monomers used in artificial nail enhancement products are known allergens (Lazarov, 2007), and their use poses a risk of developing allergic contact dermatitis which results from a T-cell mediated, delayed-type hypersensitivity response (Type IV hypersensitivity) (Gober and Gaspari, 2008). Typical clinical manifestations in sensitised patients include nail fold dermatitis, while less frequent reactions include onycholysis, paronychia and nail bed hyperkeratosis. Table 1.11 is a collection of case reports and identifies monomers which the patients were sensitised to.

Table 1.11 Case reports of allergic contact dermatitis following the use of artificial nail enhancements. The monomers in bold were present in the formulation believed to trigger allergic contact dermatitis.

Case	Manicurist or client	Clinical presentation	Positive reaction to the following (Meth)acrylates	Reference
1	Manicurist	Fissure and dryness, followed by exudative, itchy lesions on or around the nails of fingers that had been in contact with a (meth)acrylate resins. The manicurist had previously used liquid-powder systems without any problems.	EMA MMA HEMA	(Conde-salazar et al., 1986)
2	Client	Dermatitis affecting the fingers, dorsa of hands, arms, upper trunk and face, and mild onycholysis of the fingernails. This began 6 months after beginning regular applications of the liquid-powder system.	EMA BMA Polymethacrylate powder	(Fitzgerald and English, 1994)
3	Client	Itching within one hour of applying liquid-powder system. Blisters, erythema and oedema on the fingertips and around the nail folds and paraesthesia in 2 fingers on the left hand the next day. The client had previously used liquid-powder systems without any problems.	MMA EGDMA	(Freeman et al., 1995)
4	Client	Nail fold dermatitis following use of liquid-powder system for up to 18 months. Examination showed erythema, scaling and fissuring of the nail folds and tips of the fingers. The right index and left 5th finger nail were dystrophic.	MMA	(Freeman et al., 1995)
5	Manicurist	Erythema on ulnar border of the left hand and mild dermatitis on the nail folds. The manicurist had previously worn artificial acrylic nails for 8 years without problems.	EGDMA	(Freeman et al., 1995)
6	Manicurist	Dermatitis affecting eyelids and neck. Wearing artificial acrylic nails did not affect the fingers.	EGDMA	(Freeman et al., 1995)
7	Client	Fingertip rash following application of acrylic nails. Dermatitis subsided after removal of acrylic nail; however persistent paresthesia in all fingertips had been experienced since (3 years).	HEMA HPMA EGDMA	(Slodownik et al., 2007)
8	Client	Severe paronychia, pulps fissure, nail dystrophy, onycholysis and nail bed hyperkeratosis affecting all fingers, 1 week following the application of a liquid-powder system.	EA MMA	(Cruz et al., 2011)
9	Manicurist	Dermatitis localised to the face and eyelid 4 months after first contact with liquid-powder system. No hand/periungual or other cutaneous lesions at distant sites present.	HEMA HPMA	(Paula et al., 2012)
10	Manicurist	Periungual eczema 4 months after first application of UV gel.	HEMA HPMA	(Paula et al., 2012)
11	Client	Periungual eczema 2 months after first application of UV gel.	HEMA HPMA	(Paula et al., 2012)

12	Client	Severe paresthesia of distal phalanges of all fingers following application of UV gel.	MMA	(Fisher, 1990)
13-17	4 Clients and 1 manicurist	Pruritic and painful perionychial and subonychial dermatitis for several months, 6 months to 3 years after first application of UV gels. Two patients also had dermatitis of the lower lids and cheeks. Monthly applications caused strong exacerbation of the dermatitis the next day.	**(Meth)acrylated urethanes, TREGDMA HEMA HPMA**	(Hemmer et al., 1996)
18	Manicurist	Dermatitis on right thumb within weeks after starting work with UV gels. This spread to both hands, and after stronger exposure led to severe hand and face dermatitis.	BA, EA, MMA, EMA, BMA, HEMA, HPMA, EGDMA, TREGDMA, 1,4-BDDMA, 1,4-BDA, DEGDA, TRPGDA, UDA, TREGDA	(Kanerva et al., 1996)
19	Client	Dermatitis of nail folds and fingertips following application of UV gels. The client had been applying it for 3 years previously with no problems.	**HEMA HPMA** MMA EGDMA	(Erdmann et al., 2001)
20	Manicurist	Dermatitis affected fingertips, nail folds, and eyelids.	**HEMA HPMA EGDMA** MMA	(Erdmann et al., 2001)
21-22	2 Clients	Periungual eczema 3-6 months after first application of UV gels.	HEMA HPMA	(Cravo et al., 2008)
23	Manicurist	Periungual and hand dermatitis after using UV gels professionally, in spite of having had UV gel nails for 2 years.	HEMA HPMA	(Cravo et al., 2008)
24	Manicurist	Eyelid dermatitis 5 months after starting work with no hand/periungual lesions.	TREGDA	(Cravo et al., 2008)
25	Manicurist	Fingertip dermatitis, nail dystrophy and periungual hyperkeratosis. Symptoms improved after avoiding contact with UV gels.	HEA HEMA EGDMA	(Vázquez-Osorio et al., 2013)
26	Manicurist	Airborne dermatitis (cheeks, eyelids, forehead and temple area) and fingertip dermatitis. Symptoms improved after avoiding contact with UV gels.	HEMA EGDMA	(Vázquez-Osorio et al., 2013)
27	Client	Fingertip dermatitis following use of UV gels. Dermatitis of the fingertips resolved following removal of the artificial nail, although nail dystrophy persisted for several months.	HEMA EGDMA	(Vázquez-Osorio et al., 2013)
28	Manicurist	Dermatitis of the fingers, dorsa of the hands, face including eyelids, and large areas of the trunk. Symptoms improved after avoiding contact with cyanoacrylate glues and wearing polypropylene gloves while working.	**ECA**	(Fitzgerald et al., 1995)
29	Client	Eczematous change over fingertips, subungual hyperkeratosis, and dystrophy of fingernails. Scattered areas of eczema (in a hand-print pattern) over the arms, shoulders, and trunk were also present.	EMA EA ECA	(Guin et al., 1998)

30	Client	Subungual hyperkeratosis and eczematous eruptions on the hands, behind the left ear and on the left neck. The client used artificial nails containing fiberglass, which were attached at 2-week intervals.	ECA	(Guin et al., 1998)
31	Client	Eczema of the tips of digits, especially periungually, elevated and dystrophic nails, and hyperkeratotic eczema in the mid-palmar skin.	ECA	(Guin et al., 1998)

Abbreviations: EMA, ethyl methacrylate; MMA, methyl methacrylate; HEMA, 2-hydroxyethyl methacrylate; BMA, butyl methacrylate; EGDMA, ethylene glycol dimethacrylate; HPMA, hydroxypropyl methacrylate; TREGDMA, triethylene glycol dimethacrylate; BA, butyl acrylate; EA, ethyl acrylate; 1,4-BDDMA, 1,4-Butanediol dimethacrylate; 1-4-BDA, 1,4-Butanediol acrylate; DEGDA, diethylene glycol diacrylate; TRPGDA, tripropylene glycol diacrylate; UDA, urethane diacrylate; TREGDA, triethylene glycol diacrylate; HEA, hydroxyethyl acrylate; ECA, ethyl cyanoacrylate.

As can be seen from Table 1.11, both the manicurist and those wearing artificial nails can develop allergic contact dermatitis, and it appears that the most frequent (meth)acrylates they are sensitised to include ethylene glycol dimethacrylate, hydroxyethyl methacrylate and hydroxypropyl methacrylate. Unfortunately, the majority of these case reports cannot identify which (meth)acrylates were present in the formulation that triggered the allergic contact dermatitis, and the positive (meth)acrylate sensitivities could therefore be attributed to cross-sensitisation (Kanerva, 2001) .

These allergic responses most frequently arise due to repeated (meth)acrylate monomer overexposure to the cuticles, lateral nail folds, or hyponychium. Following the polymerisation reaction, the artificial nail enhancement is not considered to be a sensitiser, and therefore the risk of developing allergic contact dermatitis can be overcome by simply avoiding skin contact with the uncured product. This can be achieved by applying the product to the nail plate ensuring a tiny product-free margin at the nail folds is kept. As evident from Table 1.11, allergic contact dermatitis can also affect the neck, face and eyelids, as a result of touching these areas with contaminated hands. Exposure to filings may also be responsible in such cases, as they can contain small amounts of unreacted monomers following the shaping of liquid and powder nail enhancements for example, which as mentioned can take up to 48 hours to cure fully. To avoid the non-local reactions, hands should be washed thoroughly after application

of a nail enhancement and contact with enhancement filings should be avoided (Schoon, 2010).

The safety of methacrylate ester monomers used in nail enhancements has been assessed by the cosmetic ingredient review (CIR) panel, and they concluded that butyl methacrylate, t-butyl methacrylate, cyclohexyl methacrylate, ethoxyethyl methacrylate, 2-ethoxy ethoxy ethyl methacrylate, ethyl methacrylate, ethylene glycol dimethacrylate, hexyl methacrylate, hydroxyethyl methacrylate, di-HEMA trimethylhexyl dicarbamate, hydroxyethyl methacrylate acetoacetate, hydroxypropyl methacrylate, isobornyl methacrylate, isobutyl methacrylate, isopropylidenediphenyl bisglycidyl methacrylate, lauryl methacrylate, methoxydiglycol methacrylate, PEG-4 dimethacrylate, pyromellitic glycidyl dimethacrylate, tetrahydrofurfuryl methacrylate, triethylene glycol dimethacrylate, trimethylolpropane trimethacrylate and urethane methacrylate are all safe to use in nail enhancement products when skin contact is avoided (Escobar and Yamarik, 2005).

1.6.2.2 Worn down nails

Worn down nails following the removal of a nail enhancement (Wu et al., 2015, Andre et al., 2014) is the most common non-allergic problem reported. This can be a result of nail filing with heavy grit abrasives (<180 grit) prior to the application of the nail enhancement. Avoiding the use of nail files or switching to smoother abrasive files (>180 grit) can avoid such nail damage (Schoon, 2010).

1.6.2.3 UVA radiation exposure

At one point there was a concern that the UVA radiation required for UV gel curing may be a risk factor for the development of skin cancer, as a case review on two women with a history of UVA nail light exposure developed squamous cell carcinoma on their dorsal hands (MacFarlane and Alonso, 2009). However, this concern has been put to rest with recent research suggesting that two hands (of a person) placed in a UV nail lamp for 10 minutes twice a month is equivalent to that person spending an extra 2.7 minutes in sunlight each day for a month (Schoon et al., 2010), a person could use a UV nail lamp for 2.8 hours every day without any requirement for warning or protective measures (Dowdy and Sayre, 2013), and that the UVA radiation produced by UVA nail lamps

specifically designed for UV gels pose a low risk of skin cancer even when used weekly for over 250 years (Markova and Weinstock, 2013).

1.6.3 Why investigate UV-curable gels as topical nail medicines?

Of the three artificial nail enhancements, UV gels appear to be more favourable as they are a much simpler and therefore convenient and consistent system to work with, their polymerisation process is controlled by an external source providing an unlimited application time, their complete cure time is relatively short (1 – 3 min), and they display an inherently greater solvent resistance. To add to this, a recent article highlighted that UV gels could be a valuable tool in improving cosmesis and satisfaction among patients presenting with nail plate surface abnormalities which included nail pitting (Nanda and Grover, 2014). These factors in combination with the UV gels' ability to produce a natural-looking, long-lasting, occlusive polymeric film on the nail plate surface makes it worthwhile to consider as a potential topical nail medicine, especially as it addresses current issues such as the need for frequent applications and failure to maintain therapeutic drug levels at the desired site.

In this thesis, the pharmaceutical potential of UV-curable gels using antifungals drugs as the test drugs, (as onychomycosis is the most common of nail diseases), is investigated by setting out the following aims:

(i) To formulate and characterise UV-curable gels in terms of formulation components and polymerisation.

(ii) To characterise the resulting film's properties, such as morphology, thickness microstructure, thermal and viscoelastic properties, occlusivity, adhesivity, water sensitivity, drug-loading and stability.

(iii) To follow the release and ungual permeation of the loaded drug, and determine the efficacy of the formulations against the most common onychomycosis-causing pathogen.

(iv) To optimise the formulations with penetration enhancers.

Chapter 2: Optimisation of the UV-curable gel formulation and of the polymerisation process

2.1 Introduction

To rationally formulate a UV-curable gel formulation for use as a topical nail medicine, a more detailed understanding of commercially available UV gels is required. As mentioned in Chapter 1, a UV gel is typically a blend of urethane methacrylate based monomers/oligomers, mono- and multi-functional (meth)acrylate monomers and a photoinitiator. However, a literature search on currently available cosmetic gels is required to identify the exact components, their proportions and reasoning behind selection. These excipients could then be screened as potential components for the pharmaceutical formulation, as their safety as a nail formulation component is expected to be established. To test the pharmaceutical potential of the formulation, a pharmaceutical active needs to be incorporated into the gel, which in this case is an antifungal agent for onychomycosis treatment. While there is a vast range of antifungal agents available to tackle onychomycosis, the most favourable agents are those that possess high potencies against onychomycosis-causing dermatophytes and Candida. With this in mind, at least one agent needed to be selected. As the concentration of this agent in the UV gel and therefore in the deposited film following application is expected to influence drug permeation and uptake into the nail plate, a high drug load in the gel is desired such that drug movement out of the preparation and into the nail along the thermodynamic activity is favoured. The solubility of the antifungal in the UV gel component mix is unknown, and hence if poor, a solvent may be necessary. Solvents miscible with both the drug and the other UV gel components can not only help incorporate drug into the gel, but also help achieve a high drug load. In this chapter, the exact components that constitute a UV gel, alongside the potential antifungals and solvents that could be incorporated into a gel are identified, and the information available on these excipients are used to aid their selection for the pharmaceutical UV-curable gel formulation.

Alongside excipient choice, the excipient proportion is vital to consider, as both can have a knock-on effect on the gel properties, curing conditions and film characteristics detailed in Fig. 2.1.

UV-cured film characteristics
- Mass yield
- Degree of conversion
- Residual monomer content

UV-curing conditions
- UVA device
- Cure time

UV-curable gel properties
- Gel viscosity
- Drug stability

Excipient selection
- Choice and quantity
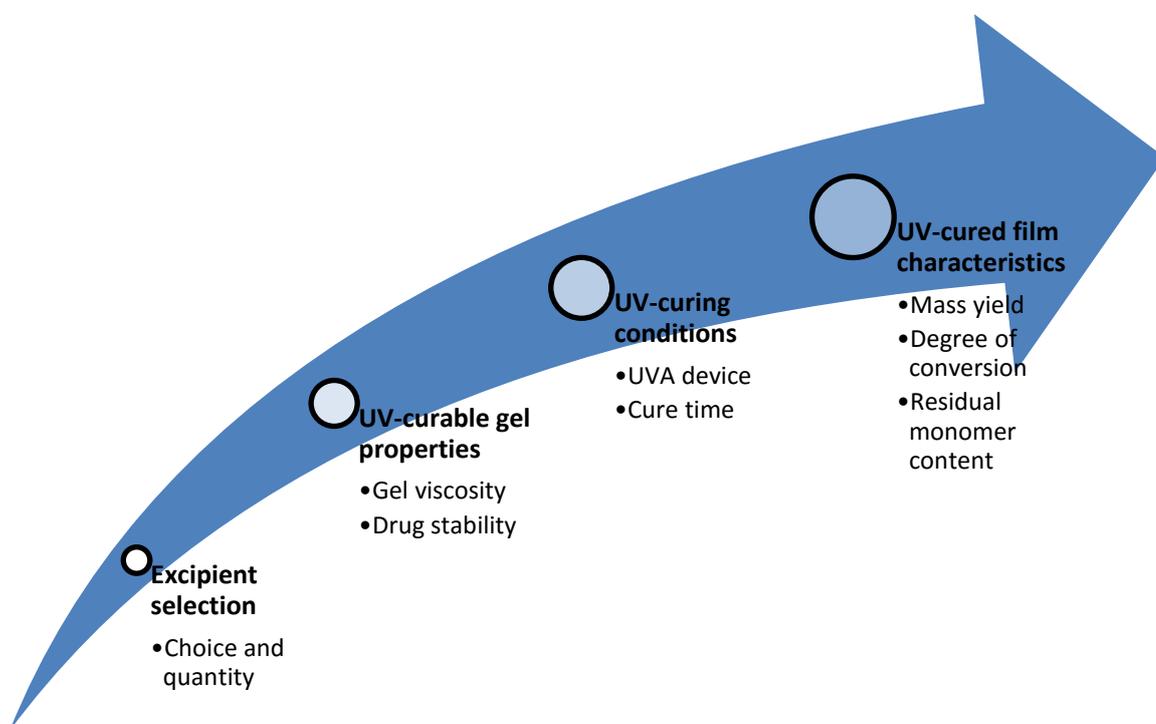
Fig. 2.1 Schematic diagram highlighting the importance of excipient choice and quantity.

UV gels are marketed in their gel form, and hence determining their properties is absolutely essential. One vital UV-curable gel property is its viscosity, which is important to consider as it can influence how well the formulation can be applied on the nail plate, and whether it easily flows towards the proximal and lateral nail folds – an undesirable property as repeated skin contact can trigger allergic contact dermatitis. Although, it should be noted that commercially-available cosmetic UV gels do not have a defined viscosity as such, with 'thin to medium', 'medium', 'medium to thick', and 'super thick' viscosity gels among the options available, and with the selection by a manicurist based on the desired finished look (Newman, 2005a). Nevertheless it is vital to define from a pharmaceutical prospective. Another vital UV-curable gel property, particularly for a pharmaceutical gel, is drug stability, and it is essential to ensure that the drug

incorporated in the UV gel does not react with the gel components, readily decompose upon storage, or precipitate out of the mixture. A stability study which examines these changes over time is therefore required. The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) have developed guidelines for accelerated testing conditions, and recommend that the formulation is stored at $40^{o}C \pm 2^{o}C$/ 75% RH $\pm$ 5% RH for a minimum time period of 6 months. With this in consideration, in this chapter, the UV-curable gel formulated is characterised for its viscosity and drug stability.

In order for the UV gel to cure, the correct curing conditions, i.e. UVA light source and cure time, is required, which is interrelated with the photoinitiator choice and photoinitiator concentration respectively. This is as the photoinitiator has to be capable of absorbing the wavelength of light emitted by the UVA light source in order to generate free radicals. With regards to photoinitiator concentration, increasing the concentration can increase the rate of cure, thus potentially reducing total cure time. However, there is an optimum concentration, beyond which a large fraction of the radiation is absorbed in the upper few micrometres of the film that minimal radiation reaches the lower layers. As the half-life of free radicals is short, they must be generated within a few nanometres of the depth in the film where they are to initiate polymerisation (Wicks et al., 2007), and if this is not achieved, an improper cure results. UV-curing is an exothermic reaction, and increasing the photoinitiator concentration beyond a certain point can also increase the maximum temperature of the UV gel curing process, which can "burn" the nail (Schoon, 2005). Another requirement for a UV gel to cure is an inert atmosphere. This is because as the radicals form by the photolysis of the initiator, they are rapidly scavenged by O_2 molecules and are unreactive towards the (meth)acrylate C=C bond; they therefore do not initiate or participate in the polymerisation process (Decker, 1989). However, an inert atmosphere is not practically feasible; UV gels are therefore cured in the presence of oxygen. As a consequence, the UV-curable monomers do not achieve 100% conversion due to this oxygen-induced inhibition of polymerisation, and an unreacted layer forms on the surface of the cured film which must be removed. The mass yield from monomer to cured polymer is a useful calculation to help determine loss due to the removal of the oxygen inhibition layer, and

can be a valuable tool for determining the effect of excipients, their concentrations and curing conditions on its formation. The polymerisation reaction itself can also be assessed by Fourier transform infrared (FT-IR) spectroscopy as the alkene bonds of the (meth)acrylate monomers are converted to alkane ones, thus enabling a degree of conversion (DC) percentage from monomer mixture to polymer film to be obtained. Determining DC can therefore aid in defining the effect of excipients, their concentrations and curing conditions on the polymerisation process. In this chapter, the mass yield and DC percentage from monomer mixture to polymer film are determined to aid in the identification of the optimal excipient concentrations and curing conditions for a pharmaceutical UV-curable gel.

Unfortunately, residual monomers may exist in a cured film, and this is an undesired property as they may penetrate into and through the nail plate, triggering either allergic contact dermatitis or irritation. To determine the risk that the residual monomers may cause, they can be extracted from the film into a solvent and quantified using analytical methods such as gas chromatography. Quantifying the residual monomer content in the film can also aid in the identification of optimal excipient concentrations and curing conditions, thus keeping the amount of residual monomers in the cured film to an absolute minimum. In this chapter, the amount of residual monomers in the cured film is therefore also determined and examined alongside the mass yield and DC percentage from monomer mixture to polymer film. In addition, these examinations are used to help determine the effects of excipients, in particular the drug and solvent, on the UV-curing process, to assess whether a pharmaceutical UV-curable gel formulation for use as a topical nail medicine is feasible.

2.2 Aims

(i) Select components for the pharmaceutical UV-curable gel formulation.

(ii) Develop a method to prepare the gel formulation and determine its key properties, i.e. viscosity and stability.

(iii) Develop a method to cure the gel formulation and form a UV-cured film, and assess the polymerisation process.

2.3 Materials

As detailed in Section 2.5.2, the excipients selected for the pharmaceutical UV-curable gel formulation were diurethane dimethacrylate in combination with either ethyl methacrylate, isobornyl methacrylate or 2-hydroxyethyl methacrylate, and the photoinitiator 2-hydroxy-2-methylpropiophenone. The antifungal drugs selected were amorolfine HCl or terbinafine HCl and the solvents – ethanol or NMP – were also found to be necessary. The chemical structures for these components can be found in Section 2.5, alongside their properties and reasonings behind selection.

Diurethane dimethacrylate, ethyl methacrylate, isobornyl methacrylate, 2-hydroxyethyl methacrylate, 2-hydroxy-2-methylpropiophenone, absolute ethanol, methanol, 1-methyl-2-pyrrolidinone (NMP), propan-2-ol, sodium chloride, triethylamine, phosphoric acid 85% wt. solution in water and trifluoroacetic acid were purchased from Sigma–Aldrich (Dorset, UK). Acetonitrile HPLC gradient grade was purchased from Fisher Scientific (Hertfordshire, UK). Amorolfine HCl was purchased from Ranbaxy Research Laboratories (Haryana, India) and terbinafine HCl from AK Scientific (CA, USA). The 36 Watt Cuccio Professional UVA nail lamp was purchased from Amazon UK. Nail & Beauty Emporium lint-free wipes (4 ply) were purchased from Just Beauty UK, an online retailer specialising in professional beauty, hair and skin products.

2.4 Methods

2.4.1 Identification of UV-curable gel components and proportions to be used

The web pages of four professional nail care product manufacturers, namely Creative Nail Design, Inc. (CND) (*CND*, [No date]), Nail Systems International (NSI) (*NSI*, [No date]), Kinetics – Professional Nail Systems (*Kinetics - Professional Nail Systems*, [No date]) and Jessica Cosmetics International (*JESSICA*, [No date]), were visited in order to obtain details of the UV gels they manufacture for salon and/or home cosmetic use. Details of the formulations' components and their contents were obtained from their Material Safety Data Sheets and product profiles where available. This information was used to identify potential excipients for a pharmaceutical UV-curable gel formulation.

2.4.1.1 Determination of drug solubility in monomers and in solvents

Saturation solubility studies were carried out to determine the solubilities of the antifungal drugs – amorolfine HCl or terbinafine HCl – in the monomers selected, i.e., ethyl methacrylate, isobornyl methacrylate and 2-hydroxyethyl methacrylate, and solvents selected, i.e. ethanol or NMP. The antifungals were added in excess to vials containing 2 ml of the monomers or solvents. The mixtures were left to stir on a magnetic stirrer placed in a water bath at 25°C for 72 hours. Subsequently, 1 ml of each sample was withdrawn from the vial using a syringe and passed through a 0.22μm MILLEX® GP filter unit into an Eppendorf tube. The samples were then centrifuged using a bench centrifuge for 20 minutes at a speed of 13.2 x 1000 rpm. The supernatant for each sample was then collected and diluted by 50, 100 or 1000 times with ethanol (or NMP – for the solubility determination in NMP) and analysed by HPLC (as per Section 2.4.6). Each test was carried out in triplicate.

2.4.2 UV-curable gel preparation

A total of four types of formulations were prepared (i) drug-free and solvent-free; (ii) drug-free containing solvent; (iii) drug and solvent containing; and (iv) solvent-free containing drug, the details for which are shown in Table 2.1. The types of formulations were further subdivided depending on whether they contained the monomer ethyl methacrylate, isobornyl methacrylate or 2-hydroxyethyl methacrylate, the solvent ethanol or NMP, and the drug amorolfine HCl or terbinafine HCl. Furthermore, the ratio of diurethane dimethacrylate to ethyl methacrylate, isobornyl methacrylate or 2-hydroxyethyl methacrylate was set at 85:15% v/v (unless otherwise stated) based on the findings from Section 2.5.1.

Table 2.1 Excipients and their quantities for the different types of UV-curable gel formulations prepared. The drug-free & solvent-free formulation and solvent-free formulation containing drug which are shaded in grey were only prepared with 2-hydroxyethyl methacrylate (with the diurethane dimethacrylate to 2-hydroxyethyl methacrylate ratio set at 75:25% v/v). NMP was used as a solvent for formulations containing ethyl methacrylate only. For formulation containing drug, the drug and solvent mixture constituted 25% v/v of the formulation.

Formulation	Excipient				
	Diurethane dimethacrylate (% v/v)	Ethyl methacrylate, isobornyl methacrylate or 2-hydroxyethyl methacrylate (% v/v)	2-hydroxy-2-methylpropio phenone (% v/v)	Ethanol or NMP (% v/v)	Amorolfine HCl or Terbinafine HCl (% w/v)
Drug-free & solvent-free	82.45	14.55	3		
	72.75	24.25	3		
Drug-free containing solvent	61.2	10.8	3	25	
Drug and solvent containing	61.2	10.8	3	25	1-7
Solvent-free containing drug	72.75	24.25	3		1-7

Drug-free formulations were prepared by mixing the excipients and leaving the mixture to stir overnight. Drug-loaded formulations were prepared by first dissolving the drug in the chosen solvent, and then adding the required excipients to the drug solution and leaving the mixture to stir overnight. Each formulation was prepared in triplicate.

To obtain the optimal photoinitiator concentration, which was found to be 3% v/v, formulations containing diurethane dimethacrylate, ethyl methacrylate and the photoinitiator at concentrations of 1, 2, 3 and 4% v/v were prepared. Films were formed as described below and tested as described in Section 2.4.5.

To determine the optimal concentration of solvent, which was found to be 25% v/v, formulations containing diurethane dimethacrylate, ethyl methacrylate, a photoinitiator (3% v/v) and ethanol at concentrations of 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50% v/v were prepared. Films were formed as described below and tested as described in Section 2.4.5. The film's sensitivity to water was also tested as described in Chapter 3 (Section 3.4.11).

2.4.3 Assessment of the UV-curable gel properties

2.4.3.1 Viscosity of gel

The viscosity of the UV-curable gel formulations was determined using a Bohlin Gemini HR nano rheometer (Malvern Instruments, UK). This is a rotational rheometer which requires 2 ml of sample per test. The experimental temperature was maintained at 25°C and the experiment was conducted in a dark room to prevent the polymerisation of the test sample during viscosity measurements. A 4°/40mm cone and plate was selected for all measurements and a controlled stress from 0.02 – 15.76 Pa was applied. The test time was 220 seconds, and this produced 22 test points following a logarithmic scale. From the data generated, a shear rate (1/s) vs shear stress (Pa) plot was produced as shown in Fig. 2.2. As the samples behaved as a Newtonian fluid where shear stress was directly proportional to the shear rate, the gradient of the plot was recorded as the viscosity of the sample.
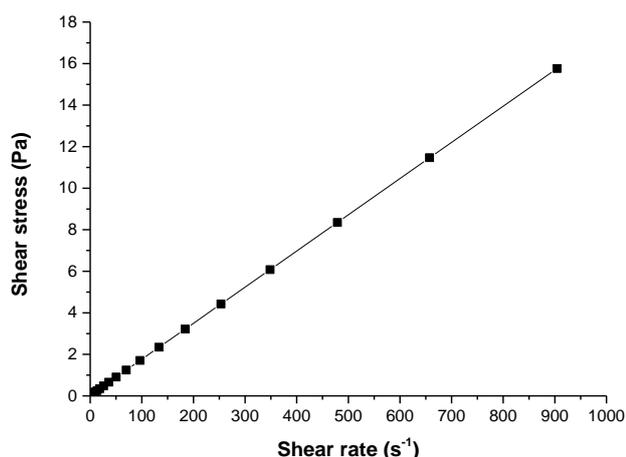
Fig. 2.2 Typical rheogram of UV-curable gel formulation

2.4.3.2 Stability of gel

The stability of drug-loaded gel formulations was tested over 6 months under accelerated testing conditions, i.e., 40°C ± 2°C and 75% RH ± 5% RH. This was conducted by placing the formulations in a desiccator containing a saturated solution of sodium chloride and placing this in a 40°C oven. At timed intervals (i.e. on Day 0 and every 28 days for 6 months) the formulations were assessed for (i) colour change (visually), (ii)

precipitation of loaded drug (by polarised light microscopy as described in Chapter 3 [Section 3.4.5.1]) and (iii) drug concentration over time (by HPLC as per Section 2.4.6).

2.4.4 UV-curing of formulations

The formulation (30 µl) was applied using a pipette tip on a microscope glass slide (to an area of 15 mm x 15 mm) as a single layer. The glass slide was placed under a 36 Watt Cuccio Professional UVA nail lamp for 2 minutes. This caused curing of the formulation and formation of a film. The surface of the film was wiped with propan-2-ol using a 4 ply lint-free nail wipe to remove the oxygen inhibition layer. This revealed a glossy polymer film, which was then collected from the glass slide using a scalpel and used in the assessments described below. The decision to use a 2 minute cure time came from the assessment of films (as described below) produced by curing formulations containing diurethane dimethacrylate, ethyl methacrylate and a photoinitiator for 0.5, 1, 2, 3, 4 or 5 minutes.

2.4.5 Assessment of the polymerisation process

2.4.5.1 Mass yield from monomer to cured polymer film

The mass yield from monomer mixture to polymer film was calculated using the following equation:

$$\text{Mass yield (\%)} = (W_t/W_0) \times 100$$

where W_0 is the weight of the monomer mixture before curing and W_t is the weight of the UV-cured film after removal of the oxygen inhibition layer.

2.4.5.2 FT-IR spectroscopy

Infrared spectroscopy was used to determine the DC from monomer mixture to polymer film by following the conversion of alkene bonds to alkane bonds. Spectra of the uncured and cured formulations were obtained using the OPUS 7.0 software and recorded by a Bruker Alpha IR Spectrophotometer (Bremen, Germany); taking 24 scans over the 400–4000 cm^{-1} range with background subtraction. The DC % was calculated from the ratio of the height of the absorbance peak of the aliphatic C=C bond (1636 cm^{-1}) relative to that of the carbonyl group (>C=O, 1702 cm^{-1}), used as an internal standard, using the following equation:

$$DC\ (\%) = \frac{(A_{1636}/A_{1702})_0 - (A_{1636}/A_{1702})_t}{(A_{1636}/A_{1702})_0} \times 100$$

where $(A_{1636}/A_{1702})_0$ and $(A_{1636}/A_{1702})_t$ are relative absorbance of C=C bonds to C=O before curing and after curing respectively.

2.4.5.3 The levels of residual monomers in the polymer film

The levels of residual monomers in the polymer film were quantified by ultrasonic extraction using an Elma Transsonic T460/H sonicator (Singen, Germany). Immediately after curing and removal of the oxygen inhibition layer, one gram of each film was placed in a glass vial and three millilitres of methanol was added. The mixture was sonicated for up to 2 hours, after which the solvent was analysed by gas chromatography (as per Section 2.4.6) to calculate the amount of residual monomers in the polymer film. This extraction procedure was optimised for volume of extraction solvent and extraction time (Appendix A3). Furthermore, to ensure that all the residual monomers had been extracted, the polymer film used in the first extraction was rinsed with methanol and blotted dry with Kimwipes, before placing in another vial containing 3 ml of methanol. This was also sonicated for 2 hours and the solvent was analysed by gas chromatography to confirm the absence of residual monomers. Each test was carried out in triplicate.

2.4.6 Analytical methods

2.4.6.1 High-performance liquid chromatography (HPLC)

The amount of amorolfine HCl and terbinafine HCl in samples was quantified by using a 1260 Infinity Agilent HPLC system equipped with an autosampler and a variable wavelength absorbance detector (Agilent Technologies, Germany). Elution was performed using a Luna C18 column (150 x 4.6 mm, 5 µm) at a temperature of 40°C. Table 2.2 shows the HPLC method developed, which was validated for specificity, calibration and linearity, accuracy and precision, and detection and quantification limit (Appendix A1).

Table 2.2 HPLC method for the quantification of amorolfine HCl and terbinafine HCl in samples.

Drug	Mobile Phase	Flow rate (ml/min)	Sample injection vol. (µl)	Wavelength (nm)	Retention time (min)
Amorolfine HCl	0.1% trifluoroacetic acid: acetonitrile (55:45 v/v)	1.0	20.0	220	5.8
Terbinafine HCl	0.012M triethylamine + 0.020M phosphoric acid: acetonitrile (65:35 v/v)	1.0	20.0	224	8.8

2.4.6.2 Gas chromatography (GC)

GC was conducted to determine the amount of diurethane dimethacrylate and ethyl methacrylate, isobornyl methacrylate or 2-hydroxyethyl methacrylate in samples using a 7890A GC System (Agilent, USA) equipped with a flame ionisation detector (FID) system. Chromatographic separation was achieved on a HP-5 column (30 m long x 320 µm inside diameter with 0.25 µm film thickness). The sample injection volume was 2 µl. The injector was in the split mode (100:1) and its temperature was maintained at 250°C throughout the experiments. The column temperature was raised from 45°C (hold 2 min) to 110°C (hold 2 min) at a 10°C/min heating rate, and then from 110⁰C to 280°C (hold 2 min) at a 20°C/min heating rate. The flow rate of the carrier gas (N_2) was 1.5 ml/min. The detection was carried out by the FID with the temperature of 280°C and the ratio of H_2/air at 25/250. Diurethane dimethacrylate, ethyl methacrylate, isobornyl methacrylate and 2-hydroxyethyl methacrylate were detected at 16.5, 6.5, 16.2 and 5.7 minutes respectively. The method developed was validated for specificity, calibration and linearity, accuracy and precision, and detection and quantification limit (Appendix A2).

2.4.7 Statistical analyses

Statistical calculations were conducted using IBM SPSS 22. The data was tested to determine whether they were normally distributed or not using the Shapiro-Wilk test and then analysed using either a t-test or Mann-Whiney U test for parametric and non-parametric data respectively. For multiple comparisons, one-way analysis of variance (ANOVA) followed by post hoc Tukey or the Kruskal Wallis test with post hoc analysis performed with Nemenyi's test was used for parametric and non-parametric data respectively.

2.5 Results and discussion

2.5.1 Components of cosmetic UV gels

A number of urethane-based (meth)acrylates, other (meth)acrylates, and a couple of photoinitiators were identified upon reviewing the commercial and scant academic literature on cosmetic UV gels. One of the more commonly used UV gel is the CND® Shellac® brand system. Unfortunately, Shellac® is a proprietary formula, with butyl acetate (≤50.0%) listed as the only chemical under composition and ingredient information. The compositions of other UV gels available, excluding pigments, are listed in Table 2.3.

Table 2.3 Composition of some of the UV gel products manufactured by NSI, Kinetics – Professional Nail Systems and Jessica Cosmetics UK.

Manufacturer	Product	Viscosity	Components			
			Urethane based monomers/ oligomers	Other functional ((meth)acrylate) monomers	Photoinitiator	Other
NSI	Balance Body Builder Gels	Medium – thick	Di-HEMA trimethylhexyl dicarbomate* (85 -95%)	Triethylene glycol dimethacrylate (5 -10 %) and glycerol propoxylate triacrylate (< 5%)	2-Hydroxy-2-methylpropiophenone (1-4%)	Triethylene glycol divinyl ether (< 3%)
	Balance Builder Gels	Medium	Di-HEMA trimethylhexyl dicarbomate* (80-90%)	Triethylene glycol dimethacrylate (5-10 %) and isopropylidenediphenyl bisoxyhydroxypropyl methacrylate (5-10 %)	2-Hydroxy-2-methylpropiophenone (1-3%)	Triethylene glycol divinyl ether (1-3%)
	Balance Colour Gels	Thin – medium	Di-HEMA trimethylhexyl dicarbomate* (70-80%)	2-Hydroxyethyl methacrylate (15-25%)	1-Hydroxycyclohexyl phenyl ketone (1-5%)	Benzophenone (1-5%)
	Balance One Step Clear Gel	Thin – medium	Urethane acrylate (55-65%) and Di-HEMA trimethylhexyl dicarbomate* (20-30%)	Isopropylidenediphenyl bisoxyhydroxypropyl methacrylate (5-10%), glycerol propoxylate (1PO/OH) triacrylate (3-5%) and triethylene glycol dimethacrylate (1-3%)	2-Hydroxy-2-methylpropiophenone (<1%)	

*Di-HEMA trimethylhexyl dicarbomate is also commonly referred to as urethane dimethacrylate or diurethane dimethacrylate.

Manufacturer	Product	Components			
		Urethane based monomers/ oligomers	Other functional ((meth)acrylate) monomers	Photoinitiator	Other
NSI	Glaze'n Go	Polyurethane acrylate oligomer (70-80%)	PEG-4 dimethacrylate (15-20%) and ethyl methacrylate (5-10%)	1-Hydroxycyclohexyl phenyl ketone (1-3%)	
	Illusion Colour Gels	Di-HEMA trimethylhexyl dicarbomate* (75-90%)	2-Hydroxyethyl methacrylate (5-10%) and triethylene glycol dimethacrylate (1-3%)	2-Hydroxy-2-methylpropiophenone (1-5%)	Triethylene glycol divinyl ether (1-3%)
	Polish Pro Now Colours	Di-HEMA trimethylhexyl dicarbomate* (50-60%)	2-Hydroxyethyl methacrylate (15-20%) and hydroxypropyl methacrylate (15-20%)	1-Hydroxycyclohexyl phenyl ketone (<1%)	Trimethylbenzoyl diphenylphoshine oxide (1-3%), butyl acetate (1-5%), cellulose acetate propionate (1-3%), methyl ethyl ketone (<2%), polysilicone-13 (<1%) and isopropyl alcohol (<2%)
Kinetics – Professional Nail Systems	Epsilon BioDefence Gel	Urethane acrylate oligomer (50-80%)	Isobornyl methacrylate (10-30%) and carboxyethyl acrylate (1-10%)	Photoinitiator identity not published (1-10%)	Calcium pantothenate (ppm)
	Kinetics SHIELD Coloured Gel Polish	Urethane acrylate (30-40 %) and polyester urethane acrylate (5-10 %)	Hydroxypropyl methacrylate (30-40%), tripropylene glycol diacrylate (<1%) and acrylates copolymer (<1%)	1-Hydroxycyclohexyl phenyl ketone (<1 %)	Nitrocellulose (5-10 %), ethyl acetate (1-5 %), butyl acetate (1-5 %), silica dimethyl silylate (1-5 %), ethyl trimethylbenzoyl phenylphosphinate (1-5 %), phthalic anhydride (<1 %), dilinoleic acid/ glycol copolymer (<1 %), acetyl tributyl citrate (<1 %), isopropyl alcohol (<1%), hydroxyethyl acrylate/ sodium acryloyldimethyl taurate copolymer (<1%) and p-hydroxyanisole (<0.005%).
Jessica Cosmetics UK	GELERATION Soak-Off Gel Polish	Di-HEMA trimethylhexyl dicarbomate* (50-60%)	2-Hydroxyethyl methacrylate (3-7%), hydroxypropyl methacrylate (3-7%) and isobornyl methacrylate (2-6%)	1-Hydroxycyclohexyl phenyl ketone (2-6%)	Butyl acetate (10-13%) and ethyl acetate (9-12%)

It can be clearly seen from Table 2.3 that urethane-based (meth)acrylates are the main components in cosmetic UV gels, (constituting between 50 – 95% of the formulation). They contain carbamate groups, -NHCOO-, which are also referred to as urethane groups, in their backbone structure, and are popular due to their favourable properties, namely high abrasion resistance and durability (Botero et al., 2014). Of the UV gel products listed, the Balance Body Builder gel is an example of a medium to thick viscosity gel, the Balance Builder gel is an example of a medium viscosity gel and the Balance Colour and Balance One Step Clear gels are examples of thin to medium viscosity gels. Urethane-based (meth)acrylates are viscous monomers, and it can be seen that it is the amount of this excipient which governs whether a UV gel is a thin or thick viscosity gel. It can also be seen that the most commonly used urethane-based (meth)acrylate is diurethane dimethacrylate, the structure and molecular weight for which is shown in Table 2.4.

Other (meth)acrylate monomers are also included in the gel mix, and their structures and molecular weights are also shown in Table 2.4. Carboxyethyl acrylate, ethyl methacrylate, 2-hydroxyethyl methacrylate, hydroxypropyl methacrylate and isobornyl methacrylate are all monomers with a single polymerisable C=C bond, and these shape the type and nature of the polymer backbone. They are used primarily as reactive diluents because of their low viscosities, but are also carefully selected to obtain a desired glass transition temperature, flexibility, mechanical strength and hydrophilic/hydrophobic character of the resulting polymer film. Isopropylidenediphenyl bisoxyhydroxypropyl methacrylate, PEG-4 dimethacrylate, triethylene glycol dimethacrylate and tripropylene glycol diacrylate are all monomers with two polymerisable C=C bond sites. These add crosslinks to the polymer architecture, with the group in between the reactive ends helping to determine the physical and mechanical attributes of the resulting cross-linked polymer structures. Glycerol propoxylate triacrylate is a monomer with three polymerisable C=C bond sites, and is typically used to generate a highly cross-linked polymer structure, with an improved polymer toughness and solvent resistance (Polysciences, 2011).

Table 2.4 Chemical structures and molecular weights of (meth)acrylate monomers identified in commercially available UV gels. The monomers shaded in white contain a single polymerisable C=C bond, those in light blue contain two polymerisable C=C bond sites, and the monomer shaded in dark blue contains three polymerisable C=C bond sites.

(Meth)acrylate ester monomer	Chemical structure & molecular weight (Daltons)
Carboxyethyl acrylate	144.13
Ethyl methacrylate	114.14
Hydroxyethyl methacrylate	130.14
Hydroxypropyl methacrylate	144.17
Isobornyl methacrylate	222.32
Diurethane dimethacrylate	470.56
Isopropylidenediphenyl bisoxyhydroxypropyl methacrylate	512.59
PEG-4 dimethacrylate	330.37
Triethylene glycol dimethacrylate	286.32
Tripropylene glycol diacrylate	300.35
Glycerol propoxylate triacrylate	428.48
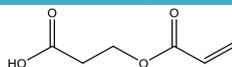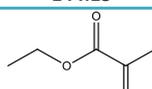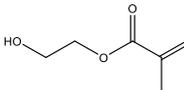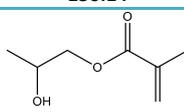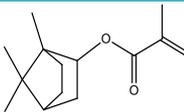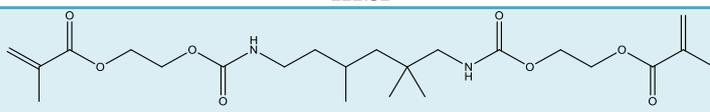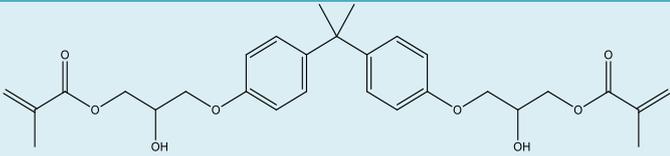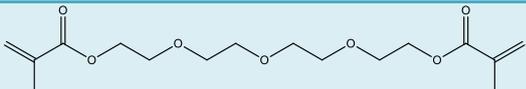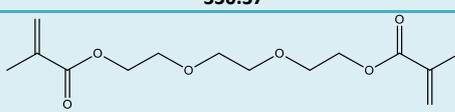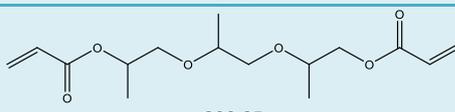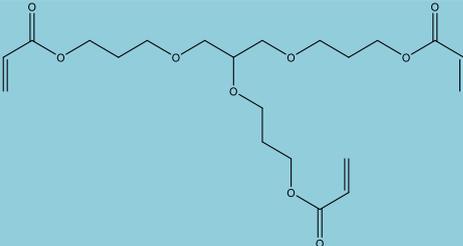
It can also be seen from Table 2.3 that the α-hydroxy ketones – 1-hydroxycyclohexyl phenyl ketone and 2-hydroxy-2-methylpropiophenone – are popular photoinitiator choices in cosmetic UV gels. This is as both are capable of absorbing photons upon irradiation with the UVA light provided by nail lamps. They both therefore undergo cleavage to produce free radicals to initiate polymerisation, with exceptionally low concentrations (<1%) being adequate. Table 2.5 shows both the photoinitiators' physical form, chemical structure, UV/VIS absorption peaks (nm) in methanol, quantum yields of dissociation (i.e. the number of dissociated molecules divided by the number of photons absorbed by the system), and the structure of the free radicals formed by the photoinitiators upon UVA light exposure (Schwalm, 2007). Both photoinitiators have similar UV/VIS absorption peaks, the same dissociation quantum yield value, and both are also useful for non-yellowing applications. Hence, from the cosmetic UV gels examined, both were equally popular.

Table 2.5 The physical form, chemical structure, UV/VIS absorption peaks (nm) in methanol and quantum yields of dissociation of 1-hydroxycyclohexyl phenyl ketone and 2-hydroxy-2-methylpropiophenone, along with the structure of the free radicals they form upon irradiation with UVA light (Schwalm, 2007).

Photoinitiator	Physical form	Chemical structure	UV/VIS Absorption peaks (nm) in methanol	Dissociation quantum yield	Free radicals formed
1-hydroxycyclohexyl phenyl ketone	Powder		246, 280, 333	0.8	
2-Hydroxy-2-methylpropiophenone	Liquid		245, 280, 331	0.8	
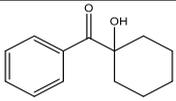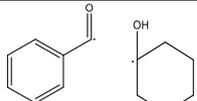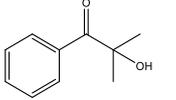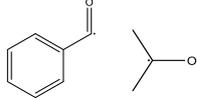
Finally, while other components may be present in the cosmetic UV gels, they are not as essential for the polymerisation reaction. One exception is the non-(meth)acrylate based monomer - triethylene glycol divinyl ether - which contains two polymerisable C=C bond sites, and can thus partake in the polymerisation process. However, it makes up no more than 3% of the formulation and was not popular in the majority of the cosmetic UV gels examined. Therefore, only the urethane-based (meth)acrylates, other functional (meth)acrylates and photoinitiators identified from the cosmetic UV gels were screened

as potential components for the pharmaceutical formulation, and the next section walks through the components selected.

2.5.2 Components of the pharmaceutical UV-curable gel formulation

2.5.2.1 The monomers

The cosmetic UV gels examined had a mixture of acrylate- and methacrylate- based monomers. (Meth)acrylates are α,β-unsaturated ester monomers, with the methacrylate esters differing in composition from their corresponding acrylate esters by the presence of a methyl group substituted at the alpha carbon, as highlighted in Fig. 2.3. This extra methyl group donates electrons to the double bond, thereby stabilising it. Furthermore it imposes sterile hindrance. Both factors render methacrylates less reactive than their corresponding acrylates, and this is reflected in their lower toxicity and lower sensitising capacity (Clemmensen, 1984, Yoshii, 1997, Rietschel et al., 2008). Due to this lower sensitising potential, methacrylates are generally more favourable than their corresponding acrylates for nail enhancements (Schoon, 2010).

Acrylate-based monomer   **Methacrylate-based monomer**
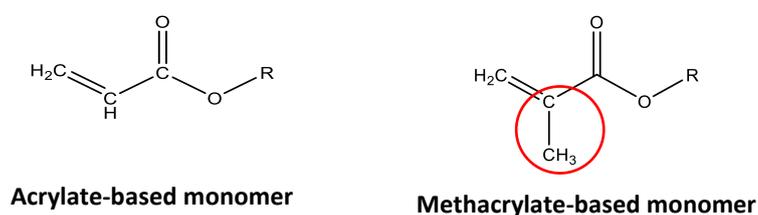
Fig. 2.3 Chemical structure of acrylate and methacrylate-based monomers.

As briefly mentioned in the previous chapter, methacrylate monomers used in nail enhancements have been assessed for safety by the cosmetic ingredient review panel (Escobar and Yamarik, 2005). To no surprise, the methacrylate-based monomers identified in the cosmetic UV gels examined were all regarded as safe to use when skin contact is avoided, and these methacrylate-based monomers were considered for inclusion in the pharmaceutical UV-curable gel formulation.

Diurethane dimethacrylate (DUDMA) was chosen as the backbone of the formulation, similar to cosmetic UV gels, because it is capable of producing a strong cross-linked polymer which imparts exceptional abrasion resistance and durability as mentioned in Section 2.5.1. While the addition of further cross-linking monomers such as

isopropylidenediphenyl bisoxyhydroxypropyl methacrylate, PEG-4 dimethacrylate and triethylene glycol dimethacrylate may improve polymer toughness and solvent resistance, they were not considered as this would compromise polymer flexibility and increase its brittleness (Daniels, 1989). The addition of a reactive diluent monomer with one methacrylate functional group was therefore considered instead, with ethyl methacrylate (EMA), hydroxyethyl methacrylate (HEMA), hydroxypropyl methacrylate (HPMA) and isobornyl methacrylate (IBOMA) among the choices available. Of these, three monomers were selected to investigate three different UV-curable gel blends due to their unique structures which could potentially impact the resulting polymers' properties: (i) EMA as it can enhance film flexibility, (ii) IBOMA as its cyclic group may affect the resulting film's mechanical strength and glass transition temperature, and finally (iii) HEMA as it contains a hydroxyl group which can affect the hydrophilic/hydrophobic character of the resulting polymer.

2.5.2.2 The photoinitiator

2-hydroxy-2-methylpropiophenone was selected as the photoinitiator for the formulation because it shows a high acrylate double bond conversion, is capable of forming hard films, and is available in a liquid form with good solvency properties (Segurola et al., 1999).

2.5.2.3 The antifungals and solvents

Two drugs – amorolfine hydrochloride and terbinafine hydrochloride – were selected to determine the pharmaceutical potential of UV-curable gel formulations. Their chemical structures and molecular weights are shown in Table. 2.6.

Table 2.6 Chemical structure and molecular weight of amorolfine hydrochloride and terbinafine hydrochloride.

Antifungal agent	Chemical Structure	Molecular Weight (Daltons)
Amorolfine hydrochloride	HCl	353.98
Terbinafine hydrochloride	HCl	327.90
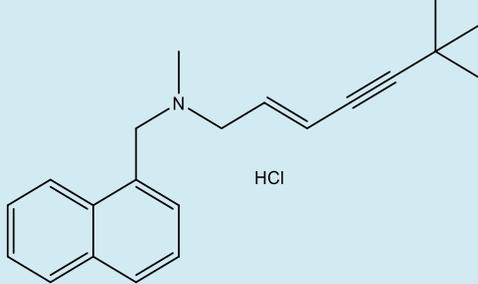
Amorolfine hydrochloride was selected since an amorolfine 5% w/v nail lacquer is licensed in Europe to treat onychomycosis. An amorolfine UV-curable gel formulation could therefore be compared with this lacquer to assess the vehicle's equivalence (or not). Terbinafine hydrochloride's choice was based upon its high potency against onychomycosis-causing dermatophytes (Badali et al., 2015), and also as it is sublimable at room temperature (23-25°C) and at physiological temperatures (37°C) (Polak et al., 2004, Jäckel et al., 2006). This sublimable ability should in theory enable terbinafine to overcome air cavities in mycotic lesions and reach tissue layers on the other side of cavities, thus contributing to its therapeutic efficacy against onychomycosis (Elsayed, 2015).

Unfortunately both drugs are insoluble in DUDMA, while their solubilities in the other methacrylate monomers are as shown in Table 2.7. The solubility of the drugs in EMA and IBOMA were extremely poor in comparison to their solubilities in HEMA. This is as HEMA contains a polar functional group (-OH-) which is capable of facilitating the dissolution of the antifungals. However, as DUDMA was the main component of the UV gel, a solvent was included in the UV-curable gel formulation to achieve a reasonable drug load. Two solvents – ethanol and NMP – were selected for their miscibility with the monomers and their solvency for the drugs. The solvents' chemical structures, molecular

weights, and the solubilities of amorolfine HCl and terbinafine HCl in the solvents are as shown in Table 2.8.

Table 2.7 Solubilities of amorolfine HCl and terbinafine HCl in methacrylate monomers used in the gel formulations.

	Solubility (mg/ml)		
	EMA	**IBOMA**	**HEMA**
Amorolfine HCl	0.10 ± 0.01	0.19 ± 0.02	209.3 ± 9.4
Terbinafine HCl	0.66 ± 0.19	0.12 ± 0.02	229.0 ± 9.8

Table 2.8 Chemical structures and molecular weights of the solvents used in the gel formulations and the solubilities of amorolfine HCl and terbinafine HCl in these solvents.

Solvent	Chemical Structure	Molecular Weight (Daltons)	Amorolfine HCl solubility (mg/ml)	Terbinafine HCl solubility (mg/ml)
Ethanol		46.07	119.5 ± 5.4	141.30 ± 1.7
NMP		99.13	56.2 ± 2.1	106.6 ± 1.3
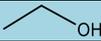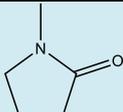
2.5.3 Proportions of the different gel components

2.5.3.1 Monomer proportions

The proportions of DUDMA and the diluent methacrylate monomers (EMA, IBOMA or HEMA) chosen were similar to those in cosmetic UV gels (of medium viscosity), with the ratio of DUDMA to EMA, IBOMA or HEMA kept at 85:15% v/v. While a higher diluent methacrylate monomer content would enhance its overall effect on the property of the resulting polymer, it was anticipated that the addition of further excipients to the gel, particularly the solvent, would reduce the overall proportion of DUDMA, which in turn could (i) reduce the viscosity of the gel and hence affect its application ability and (ii) compromise the formulations ability to produce a durable polymer.

2.5.3.2 Determination of the proportion of the photoinitiator

To determine the optimal amount of the photoinitiator, a DUDMA and EMA mix, with varying concentrations of the photoinitiator (1 – 4% v/v) was cured for 2 minutes, and the mass yield and DC from gel blend to polymer film, and levels of residual monomers in the polymer film were determined. It was assumed that the findings would be

applicable to formulations containing IBOMA and HEMA, due to their lower overall content in the formulations in comparison to DUDMA. It was found that the mass yield and DC from gel blend to polymer film increased as the photoinitiator concentration increased, while the levels of residual monomers in the polymer film decreased (Fig. 2.4 – 2.6). The decrease in the concentration of unreacted monomers extracted is consistent with previous findings, whereby the amount of unreacted monomer molecules decreased with an increase in the photoinitiator concentration and therefore the availability of radicals (Taki and Nakamura, 2011). Furthermore, it has been found that increasing the photoinitiator concentration can reduce the thickness of the oxygen inhibition layer, as the greater amount of radicals formed shifts the balance between the free radicals quenched by oxygen and the free radicals available for polymerisation (Taki and Nakamura, 2011); the higher mass yield and DC obtained can be considered to reflect this. However, the increase in mass yield and DC, and decrease in the concentration of unreacted monomers extracted was insignificant between a 3% v/v and 4% v/v photoinitiator concentration ($p>0.05$) (Fig. 2.4 – 2.6). A photoinitiator concentration of 3% v/v was therefore selected for the pharmaceutical formulations, (which is also within the concentration range found in cosmetic UV gels). A higher photoinitiator concentration was not considered, because while it can increase the rate of polymerisation, there is also a possibility of radical recombination of the photoinitiator fragments before they are able to initiate polymerisation, as well as early termination of growing chains due to their reaction with the primary photoinitiator radicals. Moreover as mentioned, a high concentration can increase the heat released during the UV gel curing process, which in turn would impact on the application of the UV gel in practice.
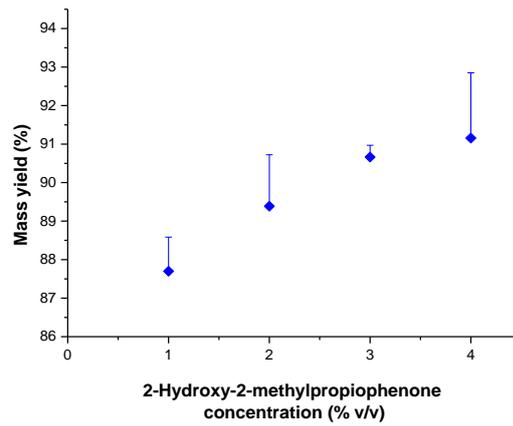
Fig. 2.4 Effect of photoinitiator concentration on mass yield from monomer to cured polymer film. Means and standard deviations are shown, n=3. The Kruskal Wallis test with post hoc analysis performed with Nemenyi's test showed a significant increase in mass yield ($p < 0.05$) between a 1% and 4% photoinitiator concentration. All other differences were insignificant ($p > 0.05$).
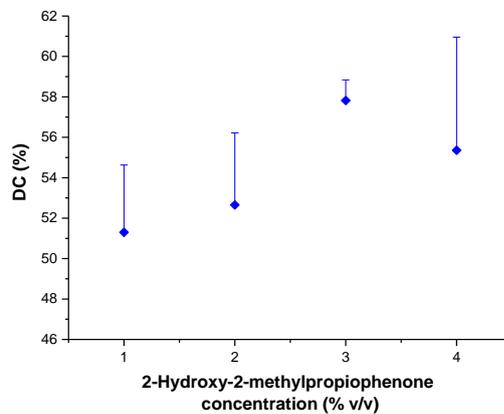
Fig. 2.5 Effect of photoinitiator concentration on percentage DC from monomer to polymer. Means and standard deviations are shown, n=3. The Kruskal Wallis test with post hoc analysis performed with Nemenyi's test showed no significant increase in DC % ($p > 0.05$) as the concentration of the photoinitiator increased.
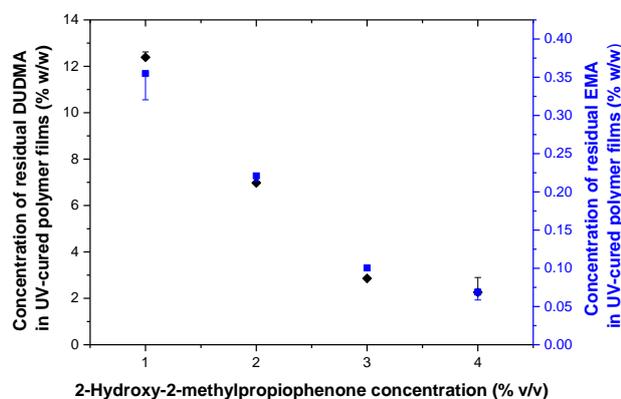
Fig. 2.6 Effect of photoinitiator concentration on concentration of residual DUDMA and EMA monomers extracted from UV-cured polymer film. Mean levels of monomers in film and standard deviations are shown, n=3. There was no significant decrease in the amount of residual monomers extracted ($p > 0.05$) between a 3% and 4% photoinitiator concentration. All other differences were significant ($p < 0.05$).

2.5.3.3 Determination of the proportion of the solvent

The amount of drug that could be loaded in the gel depended on the amount of solvent (ethanol/ NMP) that could be incorporated. Formulations were therefore developed which contained DUDMA, EMA, a photoinitiator and ethanol at concentrations ranging from 0 – 50% v/v, and the mass yield and DC from gel blend to polymer film, the levels of residual monomers in the polymer film and water sensitivity of the polymer film were determined. Once again, it was assumed that the findings would be applicable to formulations containing IBOMA and HEMA, or the solvent NMP. The % DC from monomer mixture to polymer film was found to increase as the ethanol concentration increased up until 25% v/v, after which DC % remained the same (Fig. 2.7). Similarly, the concentration of unreacted monomers extracted from the UV-cured polymer film decreased with increasing ethanol concentrations up till 25% v/v, after which no change was observed (Fig. 2.8). This apparent increase in polymerisation with increasing ethanol concentration, (which is in fact favourable), could be due to the lowering viscosity of the polymerising mixture, and a subsequent increase in the mobility of the DUDMA side chains, a phenomenon which is further discussed in Section 2.5.7.2. However at the same time, ethanol inclusion in the gel caused the mass yield percentage to decrease (Fig. 2.9) possibly due to the volatile nature of ethanol. Furthermore, the water-sensitivity of the UV-cured film increased in a concentration dependent manner (Fig. 2.10), and this could be attributed to the fact that ethanol is miscible in water, and hence if ethanol is present in the film, it would be expected to negatively affect the film's water-resistance. As the water-resistance of a nail preparation is critical to its residence on the nail (Murdan et al., 2015), a solvent concentration of 25% v/v was chosen as a compromise between the ability of the formulation to contain sufficient drug and to be water-resistant.
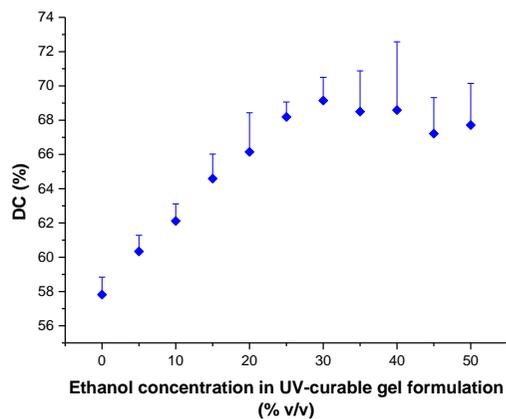
Fig. 2.7 Percentage DC from monomer to polymer for formulations containing between 0 and 50% v/v ethanol. Means and standard deviations are shown, n=3.
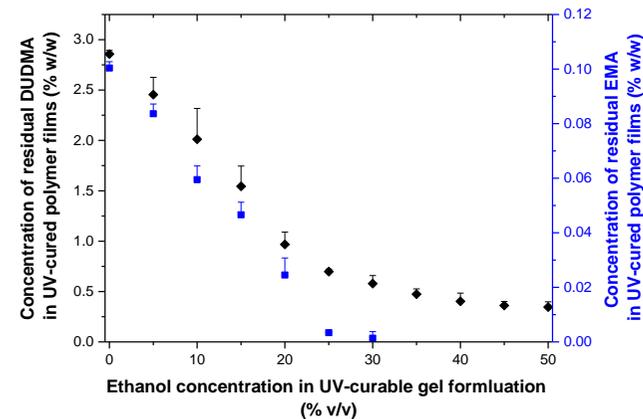
Fig. 2.8 Concentration of residual monomers in the UV-cured polymer films produced from formulations containing between 0 and 50% v/v ethanol. Means and standard deviations are shown, n=3.
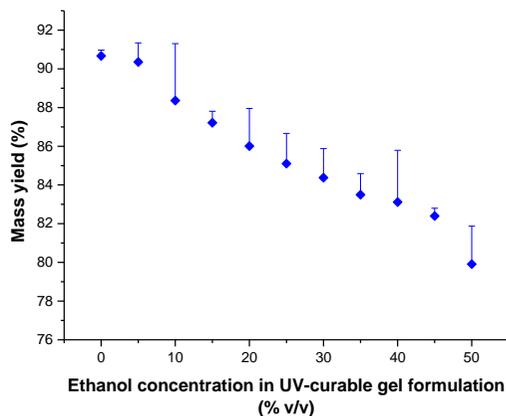
Fig. 2.9 Percentage mass yield of films produced by UV-curable gel formulations containing between 0 and 50% v/v ethanol. Means and standard deviations are shown, n=3.
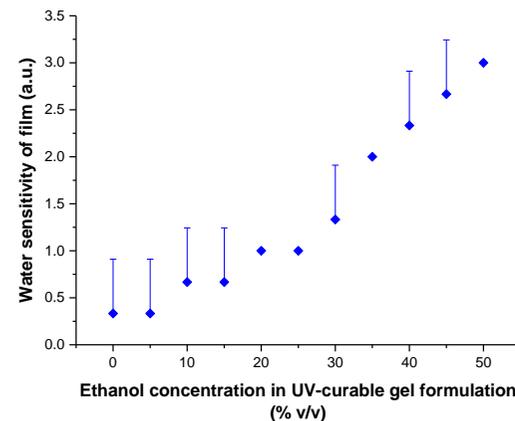
Fig. 2.10 Water sensitivity (at 48 hour incubation) of films produced by UV-curable gel formulations containing between 0 and 50% v/v ethanol. Means and standard deviations are shown, n=3.

2.5.4 Preparation of gel formulation

Drug-free gel formulations were prepared by mixing DUDMA with EMA, IBOMA or HEMA, the photoinitiator, with or without the solvent, and leaving the mixture to stir overnight, which produced a clear homogenous solution. Drug-loaded formulations were prepared by first dissolving the drug in the chosen solvent, and then adding the two monomers and photoinitiator to this drug solution and leaving the mixture to stir overnight. This also produced a clear homogenous solution.

2.5.5 Properties of the gel formulation

2.5.5.1 Drug-loading

Based on the solubility of the two antifungal drugs in the gel components (i.e. the monomers and solvents), theoretical drug-loadings were calculated (Table 2.9), and the actual drug load was also determined by visualising the absence/presence of drug crystals in the gel formulation at various drug concentrations. It was found that inclusion of ethanol (at 25% v/v) in an EMA-containing gel allowed the loading of 3% w/v amorolfine HCl or 4% w/v terbinafine HCl. Meanwhile, inclusion of NMP, also at 25% v/v, allowed the inclusion of 1% w/v of amorolfine HCl or 1% w/v of terbinafine HCl. The lower drug load enabled by NMP reflects its lower solvency for the drugs as shown in Table 2.8. Inclusion of ethanol (at 25% v/v) in an IBOMA-containing gel formulation also allowed the loading of 3% w/v amorolfine HCl or 4% w/v terbinafine HCl, which is as expected as both EMA and IBOMA have similar solvencies for the drugs. Inclusion of ethanol (at 25% v/v) in a HEMA-containing gel formulation allowed higher drug loadings (4% w/v amorolfine HCl or 6% w/v terbinafine HCl), reflecting HEMA's higher solvency for the drugs in comparison to the other diluent monomers (as shown in Table 2.7). Furthermore, as HEMA has a higher solvency for the antifungal drugs in comparison to ethanol, drug formulations with a DUDMA to HEMA ratio set at 75:25% v/v (without solvent) were also produced. However, as seen in Table 2.9, the actual drug-load in these formulations were much lower than anticipated due to the precipitation of drug upon mixing with DUDMA and the photoinitiator. An exception was seen with the EMA and IBOMA formulations which contained ethanol and terbinafine HCl, whereby the actual drug-load was slightly higher than the expected drug-load. This suggests that

terbinafine may be more soluble in an ethanol, DUDMA and EMA or IBOMA mix than in the individual components alone.

The drug-load in the gel formulations was considered acceptable, as both drugs were present in the formulations at concentrations much greater than their MIC's against the most common onychomycosis-causing pathogens (Elsayed, 2015).

Table 2.9 Theoretical and actual drug-load in UV-curable gel formulations.

Backbone and photoinitiator	Diluent monomer	Solvent (25% v/v)	Antifungal		
			AH or TH	Expected Drug load (% w/v)	Actual drug load (% w/v)
DUDMA and 2-hydroxy-2-methylpropiophenone	EMA	Ethanol	AH	3.0	3
			TH	3.5	4
		NMP	AH	1.4	1
			TH	2.7	1
	IBOMA	Ethanol	AH	3.0	3
			TH	3.5	4
	HEMA	None	AH	5.1	2
			TH	5.6	2
		Ethanol	AH	5.2	4
			TH	6.0	6

Abbreviations: AH, amorolfine HCl; TH, terbinafine HCl

2.5.5.2 Gel viscosity

The viscosities of the UV-curable gels (± solvent and ± drug) are as shown in Table 2.10. It can be seen that the viscosities of the drug-free, solvent-free gels are between that of DUDMA and the other monomer, (whose viscosities are also shown in Table 2.10). Of the drug-free and solvent-free gel blends with a DUDMA to diluent monomer ratio of 85:15% v/v, the formulation containing EMA has the lowest viscosity due to the lower viscosity of EMA when compared to IBOMA and HEMA. While the formulations containing IBOMA and HEMA have similar viscosities due to the comparable viscosities of the components. As expected, the solvent-free HEMA formulation with a greater concentration of diluent monomer, has a significantly ($p<0.05$) lower viscosity than the formulation containing a lower HEMA concentration.

All the formulations containing a solvent – ethanol or NMP – also have significantly ($p<0.05$) lower viscosity values when compared to the solvent-free formulations. The solvent makes up a quarter of the UV-curable gel formulation, and hence in its presence,

the viscosity reducing effects of the diluent monomers, while still evident, are statistically insignificant ($p > 0.05$). Furthermore, in this case, the choice of solvent does not affect the final viscosity of the gel, possibly as ethanol and NMP have similar viscosity values (1.08 and 1.67 for ethanol and NMP respectively). Finally, the presence of drug in the UV-curable gel does not significantly influence the viscosity of the gel ($p > 0.05$), possibly due to the very low drug levels in the formulation.

In order to deduce whether the viscosity of the drug-loaded UV gels is acceptable for the purpose of application on the nail plate, one would expect direct comparisons with cosmetic UV gels. However, this proved to be difficult, because as previously mentioned, they do not have a defined viscosity as such, and viscosity values for the different viscosity grade gels are not readily available. When considering other nail technologies such as nail enamels, a viscosity with a range of 300 – 400 mPas at 25°C is desired for ease of application (Schlossman, 1980). This is because a lower viscosity material is too thin to remain on the application brush and unable to deposit enough to cover the average nail, while a higher viscosity material is thick and streaky when applied. The viscosities obtained for the pharmaceutical UV-curable gels are significantly lower than this. However, the mode of gel application will be different to cosmetic nail products, and it is anticipated that the product will be applied on the nail plate in a manner that mimics application with the aid of a pipette. When taking this application procedure into consideration, the viscosity values obtained for the drug-loaded gels were considered acceptable for the purpose of application on the nail plate, particularly as it was found that the gel could be applied as desired and did not readily flow towards the proximal and lateral nail folds upon application (as found from *in vivo* studies described in Chapter 3 [Section 3.4.12]).

Table 2.10 Viscosities of gel components and UV-curable gel formulations. Means ± standard deviations are shown, n=3.

Excipients	DUDMA	Solvent		Drug	Viscosity (mPas) of gel formulations containing		
		Ethanol	NMP		EMA	IBOMA	HEMA
Formulations	DUDMA 85 % v/v : diluent monomer 15 % v/v	None	None	None	366.5 ± 27.4	672.4 ± 54.5	598.1 ± 34.9
		25% v/v	None	None	18.0 ± 4.6	25.3 ± 2.9	24.5 ± 8.7
				AH	15.5 ± 4.4	23.2 ± 8.3	23.0 ± 5.5
				TH	15.2 ± 4.5	24.3 ± 7.7	22.6 ± 6.1
		None	25% v/v	None	18.1 ± 6.7	NF	NF
				AH	18.7 ± 0.1	NF	NF
				TH	19.3 ± 1.2	NF	NF
	DUDMA 75 % v/v : diluent monomer 25 v/v	None	None	None	NF	NF	162.1 ± 33.7
				AH	NF	NF	188.9 ± 28.5
				TH	NF	NF	222.8 ± 69.5
Viscosity of excipients (mPas)	8500	1.08	1.67	-	0.62	7.40	6.79

Abbreviations: AH, amorolfine HCl; TH, terbinafine HCl; NF, not formulated.

Notes: DUDMA has a high viscosity of 8500 mPas, imparted by its high hydrodynamic volume, (due to its high molecular weight), and the presence of polar functional groups (-NH-) which form intermolecular hydrogen bonds. EMA, IBOMA and HEMA have viscosities of 0.62 mPas, 7.40 mPas and 6.79 mPas respectively. When compared to EMA, IBOMA and HEMA are significantly more viscous due to their higher molecular weights, and the presence of a polar functional group (-OH-) which can form intermolecular hydrogen bonds in the case of HEMA (Table 2.4).

2.5.5.3 Stability of antifungals in the gel formulation

The drug-loaded UV-curable gel formulations were stored under accelerated stability conditions and assessed for any changes in their appearance and drug concentration over time. Over a period of six months, the drug-loaded gel formulations showed no changes in colour, no visible signs of drug precipitation (Appendix A4), and no significant changes in drug concentration ($p > 0.05$) (Fig. 2.11 - 2.12). The UV-curable gel formulations were therefore considered to be stable and suitable for further consideration.
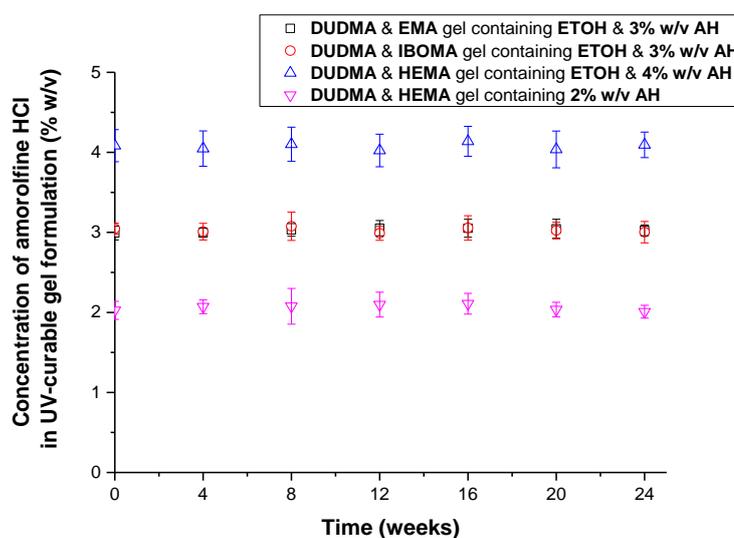
Fig. 2.11 Stability of amorolfine HCl in UV-curable gels over time. Means ± standard deviations are shown, n=3. Abbreviations: AH, amorolfine HCl; ETOH, ethanol.
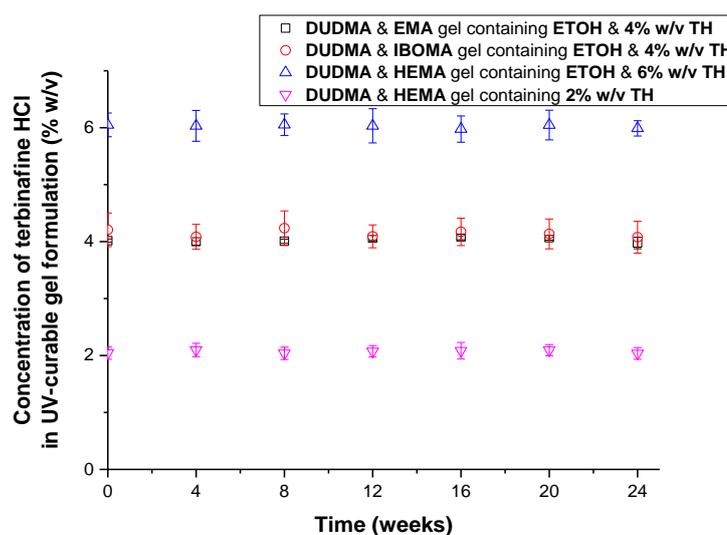
Fig. 2.12 Stability of terbinafine HCl in UV-curable gels over time. Means ± standard deviations are shown, n=3. Abbreviations: TH, terbinafine HCl; ETOH, ethanol.

2.5.6 Preparation of UV-cured film

2.5.6.1 Selection of UVA lamp and duration of UV exposure for polymerisation

The lamp used for curing was a 36 Watt Cuccio Professional UVA nail lamp (Fig. 2.13) which uses 4 x 9W UV bulbs, and is capable of emitting low intensity UVA light in the range of 320 – 400 nm, thus allowing the chosen photoinitiator to absorb the wavelength of light (331 nm) required for free radical production.

Fig. 2.13 Photographic image of the UVA nail lamp used for curing the gel formulations.

In order to determine the optimal duration of UV exposure, gel formulations containing DUDMA, EMA and the photoinitiator were exposed to this lamp for either 0.5, 1, 2, 3, 4 or 5 minutes, and the mass yield and DC from gel blend to polymer film, and the levels of residual monomers in the polymer film were determined. Once again, it was assumed that the findings would be applicable to formulations containing IBOMA and HEMA. As the cure time was increased from 0.5 to 1 minute, the mass yield from monomer mixture to polymer film significantly increased ($p < 0.05$), after which there was no further increase ($p > 0.05$) (Fig. 2.14). Similarly, the % DC from monomer mixture to polymer film significantly increased as the cure time was increased from 0.5 to 1 minute ($p < 0.05$), and beyond a 1 minute cure there was no further increase ($p > 0.05$) (Fig. 2.15). In terms of the concentration of unreacted monomers extracted from the UV-cured polymer film, significantly more DUDMA and EMA monomers were extracted from the films following a 0.5 minute cure compared to higher cure times ($p < 0.05$), and beyond a 2 minute cure time, there was no significant difference in the amount of unreacted monomers extracted ($p > 0.05$) (Fig. 2.16). It appears that a cure time of less than 2 minutes is insufficient for complete polymerisation, and with this in mind, a 2 minute cure time was selected for curing the pharmaceutical UV-curable gel formulations. A

higher cure time was not considered necessary, given the data in Figs 2.14, 2.15 and 2.16, and in order to keep UVA light exposure to a minimum.
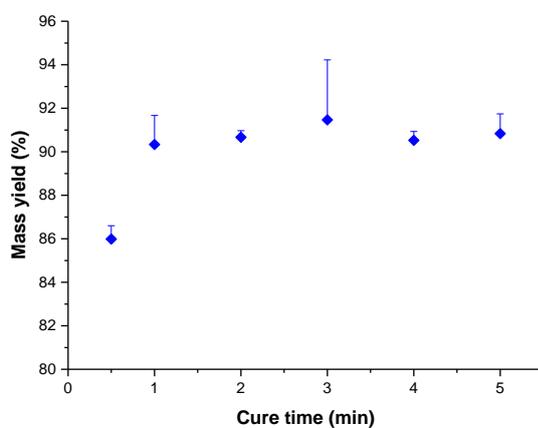
Fig. 2.14 Effect of UVA cure-time on mass yield from monomer to cured polymer film. Means and standard deviations are shown, n=3.
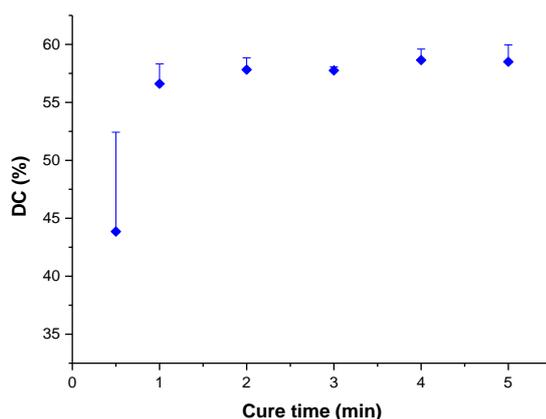
Fig. 2.15 Effect of UVA cure-time on percentage DC from monomer to polymer. Means and standard deviations are shown, n=3.
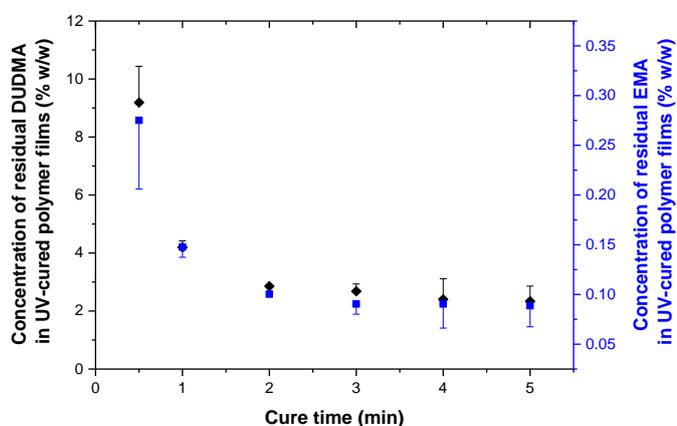
Fig. 2.16 Effect of UVA cure-time on concentration of residual monomers in the cured polymer films. Means and standard deviations are shown, n=3.

2.5.6.2 Polymerisation process

To prepare a UV-cured film, the gel formulation was applied on a glass slide and exposed to the UVA light provided by the UVA nail lamp. This exposure initiated polymerisation between DUDMA and the other monomer (EMA, IBOMA or HEMA), where the original C=C alkene bonds in the acrylate moieties of the monomers were converted to alkane ones. A suggested polymerisation pathway for UV-curable gel formulations containing DUDMA and EMA, and the basic structure of the polymer produced is shown in Fig. 2.17. The polymerisation pathways are expected to be similar when the other monomers – IBOMA or HEMA – are used instead of EMA, and the basic chemical structures of the polymers they produce are shown in Fig. 2.18.

This polymerisation process and the conversion of C=C to saturated bonds was confirmed by FT-IR which showed that the C=C stretching related absorption band at 1636 cm^{-1} was much weaker upon UV-curing (Fig. 2.19).
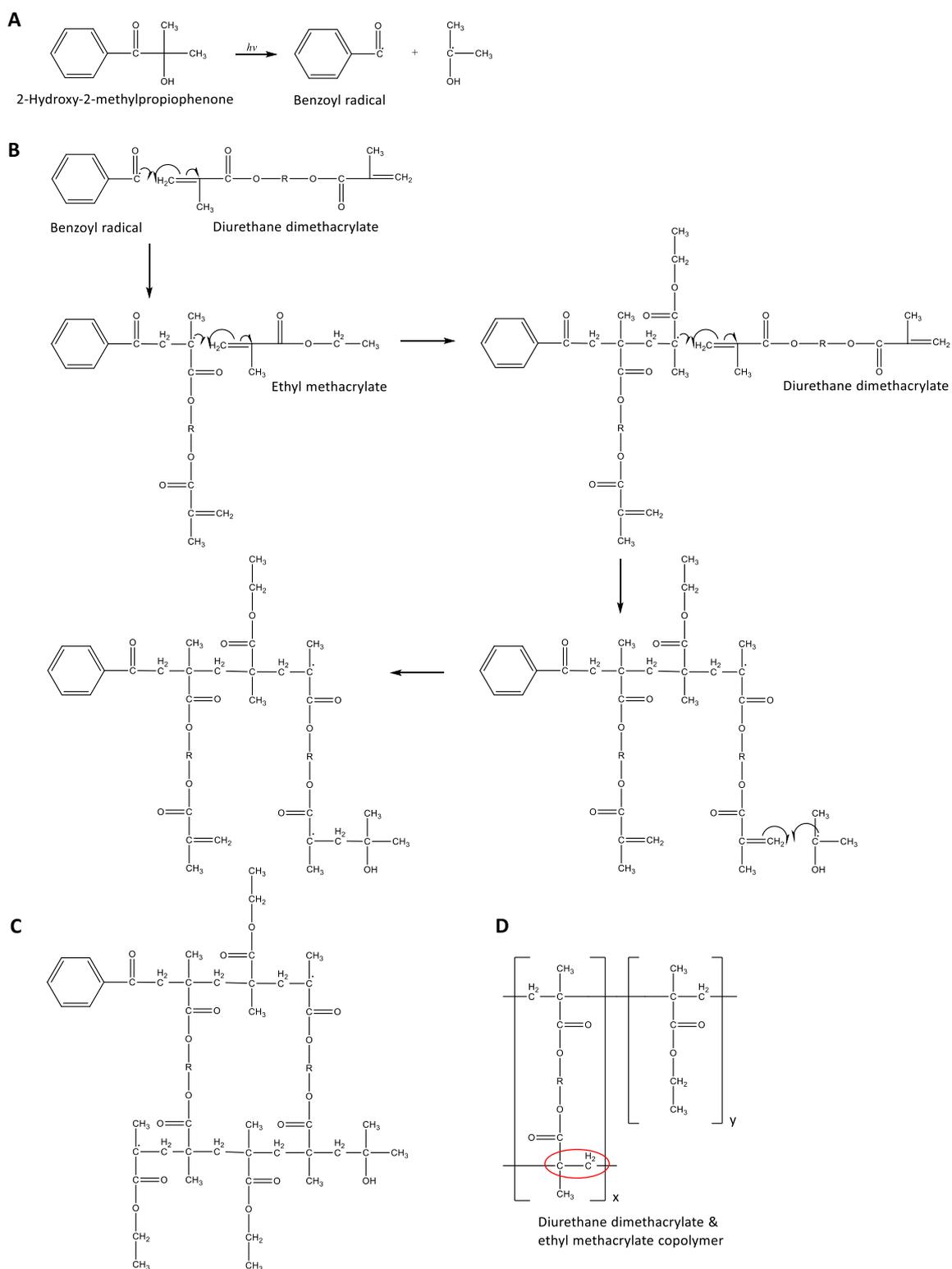
Fig. 2.17 Suggested synthetic pathway for diurethane dimethacrylate & ethyl methacrylate copolymer: A) Initiation step - formation of free radicals from 2-hydroxy-2-methylpropiophenone upon UVA exposure; B) Propagation steps; C) Example of cross-linked structure (polymer section); D) Basic chemical structure of diurethane dimethacrylate & ethyl methacrylate copolymer. It is possible that not all C=C alkene bonds in the acrylate moieties of the diurethane dimethacrylate convert to form the alkane ones (circled). R = CH_2-CH_2-O-CO-NH-CH_2-C $(CH_3)_2$-CH_2-CH (CH_3)-CH_2-CH_2-NH-CO-O-CH_2-CH_2.
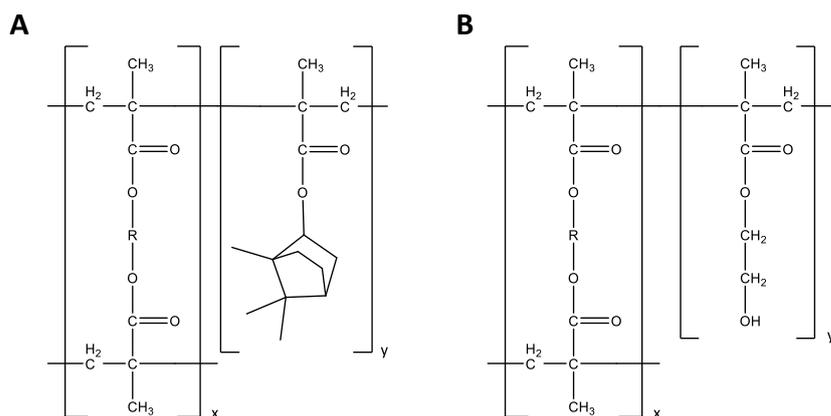
Fig. 2.18 Basic chemical structure of A) DUDMA & IBOMA copolymer and B) DUDMA & HEMA copolymer.
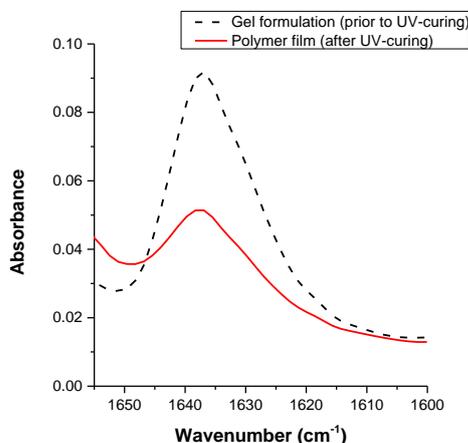
Fig. 2.19 FT-IR spectra of the gel (containing DUDMA, EMA and photoinitiator) and of the resulting polymer film after UV-curing (but before removal of the oxygen inhibition layer).

2.5.7 Assessment of the polymerisation process

2.5.7.1 Removal of the oxygen inhibition layer and mass yield from monomer mixture to polymer film

The existence of the small C=C peak in the UV-cured film shown in Fig. 2.19 indicates that conversion of the alkene to alkane bonds was not total and is likely due to the oxygen inhibition layer that is formed on the surface of the polymer film. As practised in the cosmetic industry, this unpolymerised layer was removed by wiping the film surface with a super absorbent 4 ply lint-free nail wipe soaked in propan-2-ol. Wiping the surface reduced the mass yield percentage from monomer mixture to polymer film to less than 100% as shown in Table 2.11.

Table 2.11 Mass yield of formulations after UV-curing and removal of oxygen inhibition layer. Means ± standard deviations are shown, n=3.

Ratio of DUDMA to reactive diluent monomer	Solvent	Drug	Mass yield (%) for gel formulations containing		
			DUDMA & EMA	DUDMA & IBOMA	DUDMA & HEMA
85:15% v/v	None	None	90.7 ± 0.3	84.5 ± 0.8	84.8 ± 0.6
	Ethanol	None	85.1 ± 1.6	80.1 ± 1.2	80.9 ± 1.7
		AH	85.9 ± 1.9	80.9 ± 1.9	80.8 ± 0.5
		TH	86.5 ± 1.7	80.7 ± 1.4	80.7 ± 1.1
	NMP	None	85.8 ± 1.3	NF	NF
		AH	85.9 ± 0.5	NF	NF
		TH	85.5 ± 1.5	NF	NF
75:25% v/v	None	None	NF	NF	81.4 ± 1.7
		AH	NF	NF	81.2 ± 2.0
		TH	NF	NF	81.1 ± 1.3

Abbreviations: AH, amorolfine HCl; TH, terbinafine HCl; NF, not formulated.

From Table 2.11, it can be seen from the drug-free and solvent-free gel blends with a DUDMA to diluent monomer ratio of 85:15% v/v, that the choice of reactive diluent monomer appears to affect the percentage mass yield from monomer to cured polymer film, as the EMA formulation shows a higher mass yield percentage than the other two monomer formulations, which have similar values. This could be attributed to the viscosities of the formulations. The EMA formulation is less viscous, as shown in Table 2.10, and this property could translate to a less restricted mobility of the reactants within the gel matrix during UV exposure and curing, thereby perhaps enabling a greater extent of polymerisation to take place. However, a lower viscosity can also increase oxygen diffusion during the cure, and this explains why the difference, while evident, is not significant ($p > 0.05$).

The solvent-free HEMA formulations with a greater concentration of diluent monomer have a small but significant reduction in mass yield ($p < 0.05$). This could be attributed to the volatile nature of HEMA, which has a boiling point of 67°C. As previously mentioned, free radical polymerisation is an exothermic reaction; therefore it is possible that the heat generated by the reaction coupled with the heat provided by the UVA lamp during the curing process (Appendix A5), causes some of the HEMA to evaporate off, thus reducing the mass yield from monomer to cured polymer film. In addition, it is possible that the lower viscosity of the formulation (as shown in Table 2.10) allows greater diffusion of oxygen into the formulation, such that a thicker inhibition layer is formed, and lost when the inhibition layer is removed by wiping with alcohol.

All the UV-curable gels containing the solvent ethanol have significantly lower mass yields ($p < 0.05$) when compared to the solvent-free formulations. This could be due to the volatile nature of ethanol, which has a boiling point of 78°C, in combination with the lower viscosity of the formulation, as explained above.

The EMA formulation containing NMP also has a significantly lower mass yield percentage when compared to the solvent-free formulation ($p < 0.05$). NMP has a high boiling point of 202°C, and is therefore unlikely to evaporate off during curing. Hence it appears that NMP might interact with the free radicals and prevent them from initiating

or participating in the polymerisation process, a phenomena which has previously been suggested following an investigation into the behaviour of free radicals in coal in various organic solvents (Li et al., 2002). Furthermore, the lower viscosity of the formulation may also be responsible, as explained above.

Finally, the incorporation of either amorolfine HCl or terbinafine HCl in the UV-curable gel formulations did not affect the mass yield from monomer to cured polymer film (p>0.05); possibly due to the very low drug levels in the formulation. Therefore, the mass yield from monomer mixture to cured polymer film for the pharmaceutical gel was considerable, and hence acceptable.

2.5.7.2 Degree of conversion and amount of residual monomers in cured polymer film

Following the removal of the oxygen inhibition layer, the polymer film was re-examined by FT-IR to measure the extent of polymerisation within the bulk of the film. The % DC – using the change in the peak of the alkene group – was found to be between 56% and 69% (Table 2.12). Such a DC reflects values (43-73%) reported for photo-activated methacrylate-based dental composites (Halvorson et al., 2003). To determine whether the less than 100% DC shown in Table 2.12 was due to the presence of unreacted monomers or the presence of unreacted groups within reacted monomers, the level of monomers in polymer films was quantified following their extraction. The amount of DUDMA and reactive diluent monomers in the polymer films were found to be extremely low (Table 2.13), which indicates that the 56-69% DC shown in Table 2.12 is due to the presence of unreacted groups within the polymer, rather than unreacted monomers. It seems that a significant proportion of the methacrylate groups of the DUDMA monomer are not involved in the polymerisation reaction, which could be due to the restricted mobility of some of the side chains during the polymerising reaction.

When considering the drug-free and solvent-free gel blends with a DUDMA to diluent monomer ratio of 85:15% v/v, it can be seen that the choice of reactive diluent monomer does not cause any significant changes in the % DC or the amount of unreacted monomers extracted, possibly indicating that they may have similar levels of

reactivity. A previous study has actually measured the reactivity of these methacrylates (5% w/w) in a Radical[TM] artificial nail monomer/polymer system. The reactivity was determined by using Differential Scanning Calorimetry (DSC) maximum peak exotherms while the nail enhancement product reacted in the test chamber. The maximum peak exotherm occurs at the gel point of a curing nail enhancement system, which is reached when at least 50% of the monomer has reacted and the material has a hardened surface. The changes in gel point time are directly proportional to the methacrylates' reactivity, and the readings obtained were 310, 327 and 285 mins for EMA, IBOMA and HEMA respectively. The reactivity of IBOMA and HEMA was thus considered similar to EMA (Escobar and Yamarik, 2005). However, a methacrylate concentration of 5% w/w to test reactivity could be considered low and hence tests conducted using higher methacrylate concentrations would enable the possibility of more realistic conclusions. Furthermore, one would expect the formulation containing EMA to have a higher DC % and lower amount of unreacted monomers in the film when compared to the other monomers, due to its lower gel viscosity which could increase the mobility of reactants and hence reactivity.

The HEMA formulation with a greater concentration of diluent monomer has a slightly (but significantly, $p<0.05$) higher % DC and much lower amounts of unreacted DUDMA and HEMA monomers ($p<0.05$). This increase in polymerisation could possibly be due to the lower viscosity of the polymerising mixture and a subsequent increase in the mobility of the chains. The UV-curable gel formulations containing the solvent – ethanol or NMP – also have significantly higher DC percentages ($p<0.05$) and lower amounts of unreacted monomers in their films, also possibly due to their lower viscosities when compared to the solvent-free formulations. The incorporation of an antifungal in the UV-curable gel formulations did not affect % DC or amount of unreacted monomers in the films ($p>0.05$); probably due to its very low level in the formulation.

The negligible amounts of monomers in drug-containing UV-cured films (Table 2.13) show that such UV-curable formulations can be used as topical nail medicines, especially if a formulation-free margin is left around the nail to avoid contact with the skin.

Table 2.12 Percentage DC from monomers to polymer. Means ± standard deviations are shown, n=3.

Ratio of DUDMA to reactive diluent monomer	Solvent	Drug	Degree of conversion (%) for gel formulations containing		
			DUDMA & EMA	DUDMA & IBOMA	DUDMA & HEMA
85:15% v/v	None	None	57.8 ± 3.0	56.2 ± 4.1	57.4 ± 0.9
	Ethanol	None	68.2 ± 0.9	65.7 ± 1.9	64.0 ± 2.2
		AH	68.4 ± 0.2	66.9 ± 1.2	64.7 ± 1.6
		TH	67.3 ± 2.0	65.8 ± 2.1	63.0 ± 3.2
	NMP	None	66.6 ± 12.4	NF	NF
		AH	68.1 ± 8.5	NF	NF
		TH	67.0 ± 2.7	NF	NF
75:25% v/v	None	None	NF	NF	61.2 ± 0.5
		AH	NF	NF	61.1 ± 0.9
		TH	NF	NF	60.2 ± 0.8

Abbreviations: AH, amorolfine HCl; TH, terbinafine HCl; NF, not formulated.

Table 2.13 Concentration of residual DUDMA and EMA, IBOMA or HEMA in the UV-cured polymer films. Mean levels of monomers in films ± standard deviations are shown, n=3. Residual DUDMA concentrations are those in black, while residual EMA, IBOMA and HEMA concentrations are in blue, red and green respectively.

Excipients	DUDMA	Solvent	Drug	Residual DUDMA and diluent monomer concentrations (% w/w) in films produced from gel formulations containing		
				EMA	**IBOMA**	**HEMA**
Formulations	DUDMA 85 % v/v : diluent monomer 15 % v/v	None	None	2.9 ± 0.04 0.1 ± 0.002	3.1 ± 0.3 0.2 ± 0.03	3.2 ± 0.3 0.2 ± 0.03
		Ethanol	None	0.7 ± 0.03 0.003 ± 0.0001	0.8 ± 0.1 0.004 ± 0.001	0.8 ± 0.1 0.004 ± 0.001
			AH	0.7 ± 0.03 0.003 ± 0.00008	0.8 ± 0.1 0.004 ± 0.001	0.8 ± 0.1 0.004 ± 0.002
			TH	0.7 ± 0.03 0.005 ± 0.002	0.7 ± 0.1 0.003 ± 0.001	0.8 ± 0.07 0.004 ± 0.001
		NMP	None	0.4 ± 0.009 0.003 ± 0.0001	NF	NF
			AH	0.4 ± 0.01 0.003 ± 0.00008	NF	NF
			TH	0.3 ± 0.008 0.002 ± 0.00001	NF	NF
	DUDMA 75 % v/v : diluent monomer 25 v/v	None	None	NF	NF	1.0 ± 0.02 0.005 ± 0.0004
			AH	NF	NF	1.0 ± 0.04 0.005 ± 0.0004
			TH	NF	NF	1.0 ± 0.07 0.005 ± 0.0009

Abbreviations: AH, amorolfine HCl; TH, terbinafine HCl; NF, not formulated.

2.6 Conclusions

It is possible to formulate a stable pharmaceutical UV-curable gel formulation using blends of an antifungal – amorolfine HCl or terbinafine HCl – and a solvent – ethanol or NMP, in combination with excipients commonly used in cosmetic UV gels, i.e. DUDMA and either EMA, IBOMA or HEMA, and the photoinitiator 2-hydroxy-2-methylpropiophenone. The solvent, at a concentration of 25% v/v, was found to be necessary for drug incorporation into the gel, and for such formulations a DUDMA to diluent monomer ratio of 85:15 % v/v, and a photoinitiator concentration of 3% v/v was found to be optimal. The ethanol and DUDMA & EMA- or IBOMA- containing gels allowed the loading of 3% w/v amorolfine HCl or 4% w/v terbinafine HCl, the ethanol and DUDMA & HEMA-containing gels allowed the inclusion of 4% w/v amorolfine HCl or 6% w/v terbinafine HCl, while the NMP and DUDMA & EMA-containing gels allowed the inclusion of 1% w/v amorolfine HCl or terbinafine HCl. Formulations containing HEMA, with a DUDMA to diluent monomer ratio of 75:15% v/v and no solvent, also enabled drug incorporation. However, the overall drug-load was considerably lower (2% w/v amorolfine HCl or terbinafine HCl).

The viscosities of the formulations were affected by monomer choice and the incorporation of a solvent, with formulations containing EMA and either ethanol or NMP displaying lower viscosities. However, the viscosity reducing effects of EMA was overpowered by the solvent present in the formulation. Furthermore the presence of drug in the formulation did not influence its viscosity, and the viscosity of the drug-loaded formulations was found to be acceptable for application on the human nail plate.

The formulations were able to cure sufficiently under a UVA nail lamp for 2 minutes. However, the mass yield from monomer mixture to cured polymer film was found to be affected by the presence of a solvent, with formulations containing either ethanol or NMP displaying lower mass yields. The presence of drug in the formulation did not influence this factor and the mass yields for the drug-loaded formulations were considerable, and therefore acceptable. The DC% and amount of unreacted monomers extracted from the films were also affected by the presence of solvent in the formulation, whereby the DC% had increased and the levels of unreacted monomers in

the films decreased. Once again, the drug in the formulation was not found to influence the polymerisation process, and the drug-loaded UV-cured films contained negligible levels of residual monomers.

A UV-curable gel formulation for use as a topical nail medicine appears feasible, and the formulations developed appear suitable for further consideration.

Chapter 3: UV-cured film characterisation

3.1 Introduction

UV gels can shrink between 12 – 18% upon curing (Schoon, 2005). When applied on the human nail plate, excessive shrinking can cause discomfort and trauma to the nail bed, rendering the nail more prone to infection. This shrinkage phenomenon is therefore minimised in practice by applying multiple thin coatings of UV gels instead of a single thick coating, which incidentally also avoids the likelihood of increasing the maximum temperature of the UV gel curing process (Schoon, 2005). For the pharmaceutical UV-curable gels formulated, the minimum amount of gel required to cover a given area while still capable of producing an adequate film was 13.3 µl/cm^2. Therefore, in order to minimise shrinkage upon curing, this volume per area ratio was used for assessing the polymerisation process in Chapter 2. In the cosmetic industry, UV gel layers are sequentially applied and cured. However, it would be more convenient to apply a pharmaceutical formulation as a single layer as this is not only simpler, but also reduces application time and minimises the time a patient has to expose their hand under a UVA nail lamp. With this in consideration, the pharmaceutical UV gel was designed for application on the nail plate as a single layer. This single cured layer produced a film which was visually smooth and transparent, as shown in Fig. 3.1.

Fig. 3.1 Photographic image of UV-cured film produced from a DUDMA & EMA containing gel formulation (drug-free and solvent-free).

In order to assess the formulations' appropriateness for topical nail drug delivery and understand the potential drug release and ungual drug permeation patterns, the film formed requires full characterisation, starting with film thickness and structure. While

simply quantifying the thickness of a polymeric film is in itself not crucial, assessing its thickness uniformity is an essential characteristic to ensure that consistent films are continually being produced for further determinations. Deducing the film's structure on the other hand, in particular the film's cross-link extent, porosity and microstructure, can not only help decipher the effect of excipients on the polymer's architecture, but can help investigate whether this in turn can affect drug release. In this Chapter, the UV-cured film's thickness uniformity and structure is therefore assessed. The extent of cross-linking in the UV-cured polymer film is determined by measuring the film's swelling in a solvent, given that the solvent uptake into the polymer matrix is expected to decrease with increased cross-linking (Flory and Rehner Jr., 1943).

As mentioned in Chapter 2, a high drug-load in a polymeric film is ideal for optimal drug release and hence optimal ungual drug penetration (Mertin and Lippold, 1997a, Pittrof et al., 1992). However, the drug should be maintained in its soluble/amorphous form following a UV-cure. Furthermore, the absence/presence of drug-polymer interactions should be confirmed, as it can be an important factor governing drug release patterns (Puttipipatkhachorn et al., 2001). In this Chapter, the film's maximum drug-load is therefore confirmed and the existence of any drug-polymer interactions is identified.

The film's thermal properties are also worth investigation to determine its thermal stability and whether there are excipients (such as solvents) remaining within the polymer film – a factor which may impact other properties including glass transition temperatures (Belfiore, 2010). The glass transition temperature of a UV-cured polymeric film could in turn impact the film's drug release properties. Glass transition temperatures can be determined by using techniques such as differential scanning calorimetry (DSC) and dynamic mechanical analysis (DMA). However, DMA is 1000 times more sensitive for detecting the glass transition temperature compared to DSC (Chartoff, 1997). DMA involves subjecting the polymer film to an oscillating stress, usually following a sinusoidal waveform:

$$\sigma(t) = \alpha_{max}\sin\omega t$$

where $\sigma(t)$ is the stress at time t, α_{max} is the maximum stress and ω is the angular frequency of oscillation.

The applied stress produces a corresponding deformation or strain. For an elastic material, the strain is proportional to the applied stress. A viscous material possesses a resistance to deformation proportional to the rate of application of strain. Therefore for a viscoelastic material, the measured strain will lag behind the applied stress by an angle of δ, the maximum lag being 90°. The ratio of peak stress to peak strain gives the complex modulus which consists of an in-phase component (storage modulus, E') corresponding to the sample's elastic response, and an out-of-phase component (loss modulus, E'') corresponding to the sample's viscous component. The ratio between the loss and storage moduli (E''/E') gives tan δ, which is a measure of the amount of deformational energy that is dissipated as heat (Price, 2002). At the glass transition temperature, the storage modulus declines rapidly and the loss modulus and tan δ curves rise to maximum values, the glass transition temperature can therefore be calculated as the inflection point in the storage modulus or as the tan δ peak. In this Chapter, the thermal properties and glass transition temperatures of the films are determined, and the effects of excipients on these properties are also examined.

An ideal pharmaceutical UV-cured film should be occlusive, a characteristic which is determined *in vivo* by measuring the reduction of transonychial water loss (TOWL) following the formation of the film on the nail plate surface. The occlusive effect of the formulation is expected to hyper-hydrate the upper nail layers, which can aid drug permeation into and through the nail plate (Spruit, 1971, Marty, 1995). Another ideal property is a long residence on the nail plate similar to its cosmetic counterpart, to minimize application frequency and consequently improve patient compliance, treatment efficacy and cost. A long residence on the nail plate requires good adhesion between the nail plate surface and UV-cured film, a factor which is considered to positively affect ungual drug permeation (Hui et al., 2003, Monti et al., 2005). Tests such as the cross-cut test to assess the resistance of the polymer films to separation from a substrate and adhesion studies using a texture analyser are therefore commonly used for assessing the adhesion properties of topical nail formulations (Murdan et al., 2015, Shivakumar et al., 2010). Ideally the substrate used in adhesion tests should be human nail plates. However, due to the small size and scarcity of human nail plates, alternatives are often used, examples include glass and the smooth side of high density polyethylene

(HDPE) sheets (Murdan et al., 2015). In addition to possessing excellent adhesive properties, an ideal pharmaceutical formulation should be water-resistant, as this is a major factor which governs the *in vivo* residence time of formulations on the human nail plate, and simple *in vitro* water sensitivity tests can give valuable insight into the *in vivo* residence of formulations on the nail plate (Murdan et al., 2015). Therefore, in this Chapter, the UV-cured films' occlusivity, adhesivity and water sensitivity are explored, while considering the influence of excipients on these properties. The *in vivo* residence of the UV-cured polymer films on fingernails is also investigated over 28 days in order to determine how long the films actually reside on the nail plate. Furthermore, the UV gels' occlusivity and *in vivo* residence is compared to the commercially available pharmaceutical nail lacquer – Curanail® – to assess whether the UV gel vehicle offers any advantages over a nail lacquer regarding these two essential properties.

Finally, the drug incorporated in the UV-cured films should be stable while the film resides on the nail plate surface in order for it to have a therapeutic effect following ungual penetration; hence drug stability studies are vital. Determining whether there is loss of any residual solvent from the films with time is just as important, as this may cause the drug in the film to crystallise with time and potentially affect its release. In this Chapter, a stability study is therefore carried out at 30°C ± 2°C/ 50% RH ± 5% RH over the time period that the film is likely to reside on the nail plate, and the suitability of the pharmaceutical UV-curable gel for further consideration is deduced.

3.2 Aims

To produce UV-cured films using the gel formulations developed in Chapter 2 and determine their:

(i) morphology, thickness uniformity and structure

(ii) maximum drug-load and identify any drug-polymer interactions

(iii) viscoelastic and thermal properties

(iv) occlusivity, adhesivity, water sensitivity and *in vivo* residence time on the human nail plate

(v) stability

and the influence of drug, solvent and monomer compositions on the above properties.

3.3 Materials

The monomers, photoinitiator, solvents and antifungal agents used to make the UV-curable gel formulations and the UVA nail lamp and nail wipes were obtained as detailed in Chapter 2 (Section 2.3). Toluene and magnesium nitrate were purchased from Sigma–Aldrich (Dorset, UK). High density polyethylene (HDPE) sheets were purchased from RS Components (Corby, UK). Double-sided Sellotape® Sticky Fixer Strips (25mm x 3m) were purchased from Robert Dyas, UK. Curanail® (5% w/v amorolfine hydrochloride) nail lacquer was purchased from Boots pharmacy, UK.

3.4 Methods

The UV-cured films produced from formulations containing DUDMA and a diluent reactive monomer (EMA, IBOMA or HEMA) at a ratio of 85:15% v/v (with and without ethanol or NMP and with and without amorolfine HCl or terbinafine HCl), as well as the formulations containing DUDMA and HEMA at a ratio of 75:25% v/v (with and without amorolfine HCl or terbinafine HCl) were prepared as described in Chapter 2 (Section 2.4.2 and 2.4.4). The concentration of amorolfine HCl and terbinafine HCl in the formulations was the maximum that could be incorporated in the gel as determined in Chapter 2 (Section 2.5.5.1), and Table 3.1 highlights this concentration of drug in the drug-loaded formulations.

Table 3.1 Excipients and their quantities required to produce the different drug-loaded UV-curable gel formulations. Two types of each formulation were prepared: (i) amorolfine HCl-loaded or (ii) terbinafine HCl-loaded.

Formulation		Excipient				Drug (% w/v)	
		DUDMA (% v/v)	EMA, IBOMA or HEMA (% v/v)	2-hydroxy-2-methylpropiophenone (% v/v)	Solvent (% v/v)	AH	TH
Drug and solvent containing	DUDMA 85%: EMA 15% v/v	61.2	10.8	3	25% ETOH	3	4
					25% NMP	1	1
	DUDMA 85%: IBOMA 15% v/v				25% ETOH	3	4
	DUDMA 85%: HEMA 15% v/v				25% ETOH	4	6
Solvent-free containing drug	DUDMA 75%: HEMA 25% v/v	72.75	24.25	3		2	2

Abbreviations: AH, amorolfine HCl; TH, terbinafine HCl; ETOH, ethanol

The resulting film's thickness, cross-link extent, porosity, microstructure, maximum drug-load, thermal properties, occlusivity, adhesivity, water sensitivity, *in vivo* residence and stability were characterised as detailed below. For the adhesion and water immersion studies, the smooth side of a HDPE sheet was used as a nail model, as its surface energy (35 mJ/m^2) (Yaoguang et al., 1993) is similar to that of the nail plate surface (34.1 ± 5.5 mJ/m^2) (Murdan et al., 2012), and surface energy is known to influence adhesion (Venkatraman and Gale, 1998). Unless otherwise stated, each test was conducted in triplicate.

3.4.1 Determining film thickness and uniformity of thickness

The thickness of the films produced was measured using a Sealey AK9635D 0–25 mm Digital External Micrometer (PVR Direct, Bristol, UK). Each film was measured at three separate points and an average was taken. Precautions were taken to measure the thickness without compressing the polymer film. The uniformity of thickness within each film and among films was calculated using the following formula:

$$\% \text{ Uniformity} = (1 - \frac{\text{standard deviation}}{\text{mean}}) \times 100$$

3.4.2 UV-cured film's swelling in toluene as an indication of its cross-link extent

In order to deduce the UV-cured polymer film's swelling behaviour when immersed in toluene, a 15 x 15 mm film was produced and weighed on a precision balance. This film was then placed in a glass vial containing three millilitres of toluene and left to stand at room temperature (22 ± 2°C) for 48 hours. Subsequently, the solvent was decanted and the liquid solvent adhering to the film's surface was blotted dry with Kimwipes. The film was then reweighed and its swelling (%) was determined using the following formula:

$$\text{Swelling (\%)} = \frac{\text{polymer mass after toluene immersion} - \text{polymer mass before toluene immersion}}{\text{polymer mass before toluene immersion}} \times 100$$

The mass at 48 hours was confirmed as the equilibrium swelling mass as soaking the film for a total duration of 7 days did not alter the mass obtained at 48 hours.

3.4.3 Determination of UV-cured polymer film's porosity using gas adsorption/desorption

The porosity of the UV-cured films was determined using N_2 adsorption/desorption isotherms obtained by an automated surface area analyser (Beckman Coulter SA-3100 apparatus, Beckman Coulter, Inc., California, USA). Samples (approx. 150 mg of 2 mm x 2 mm sized films) were added to a Beckman Coulter RapiTube sample tube and were firstly outgassed at 120°C for 15 minutes. Subsequently, the sample tube was immersed in liquid nitrogen and the samples N_2 adsorption/desorption isotherms were measured over a relative pressure (Ps/Po) range between 0 – 1 and back. The total pore volume was calculated (using the Coulter SA-3100 Surface Area and Pore Size Analyser software version 2.13) from the amount of nitrogen adsorbed at the relative pressure of 0.98.

3.4.4 Imaging using Scanning Electron Microscopy (SEM)

SEM was used for imaging the surfaces and cross-sections of the polymer films produced. The samples were gold sputter coated (10 nm) and imaged using a FEI Quanta 200F electron microscope (Eindhoven, The Netherlands).

3.4.5 Maximum drug-load determination

3.4.5.1 Polarised Light Microscopy (PLM)

To determine whether the gel formulations which were saturated with drug produced films with the drug remaining in their soluble form, the films were examined by PLM for the absence/presence of drug crystals using a Nikon Microphot-FXA microscope (Tokyo, Japan) and polarising filters. Images were taken using a Lumenera Infinity 2 digital camera (Ottawa, Canada) attached to the microscope.

3.4.5.2 X-Ray Diffraction (XRD)

XRD was used: (i) to determine the crystallinity/amorphousness of the UV-cured films and (ii) to confirm the maximum (soluble) drug-load of the films determined by PLM. X-ray diffraction spectra of the antifungals and of the polymer films were obtained using a Rigaku MiniFlex 600 X-ray diffractometer (Tokyo, Japan) equipped with MiniFlex Guidance software. The samples were scanned over an angular range of 2–60°, with a step size of 0.02° and step duration of 0.5°/min.

3.4.6 Quantification of drug in UV-cured polymer film

The exact drug-load in UV-cured polymer films was determined by ultrasonic extraction using an Elma Transsonic T460/H sonicator (Singen, Germany). Ten milligrams of each film was placed in a glass vial. Five millilitres of ethanol was added to the film and the mixture was sonicated for up to 2 hours. Subsequently the solvent was analysed by HPLC (as per Section 2.4.6 [Chapter 2]). The polymer film was rinsed with ethanol and blotted dry with Kimwipes, before placing in a vial containing 1 ml of ethanol. This was ultrasonicated for 2 hours and the solvent was analysed by HPLC, while the film retrieved was placed in another vial containing 1 ml of ethanol for a further two hour sonication. This extraction procedure was repeated until no further drug was extracted. The total amount of drug in the polymer film was then calculated.

3.4.7 Drug-polymer interactions

FT-IR spectroscopy was used to assess the UV-cured polymer films' structure and to identify any drug-polymer interactions. The equipment used is as detailed in Chapter 2 (Section 2.4.5.2).

3.4.8 Thermal properties of UV-cured polymer films

3.4.8.1 Thermal Gravimetric Analysis (TGA)

TGA was used to determine the thermal stability of the polymer films. It was conducted using the Discovery TGA Model (TA Instruments–Waters LLC, Delaware, USA). Oxygen-free nitrogen gas at a flow rate of 25 ml/min was used. The sample mass was approximately 4 mg, and each sample was heated from 25 to $500^{o}C$, with a heating rate of $10^{o}C$/min. Data analysis was carried out with TA Instruments TRIOS V3.1.0.3538.

3.4.8.2 Dynamic Mechanical Analysis (DMA)

DMA was used to study the viscoelastic behaviour of the polymer films as a function of temperature. Films with an area of 15 mm x 6.5 mm (produced after curing 13 µl of the formulation) were prepared and analysed using the Q800 Dynamic Mechanical Analyser (TA Instruments – Waters LLC, Delaware, USA). The mode was set at DMA multi-frequency strain using a film tension clamp. The purge gas used was nitrogen, and the heating range was from room temperature to $200^{o}C$ with a heating rate of $3^{o}C$/min. The oscillating frequency was set at 1 Hz. A preload force (0.01 N) was applied to the sample

prior to the dynamic oscillating force to prevent the film buckling. The data was collected using Advantage for Q Series Version 2.8.0.394, and the data analysis was carried out with TA Instruments Universal Analysis 2000.

3.4.8.3 Differential Scanning Calorimetry (DSC)

DSC was used mainly to confirm the results from DMA, and the thermal properties of the polymer films were analysed by using the Q2000 TA Instrument (Waters LLC, Delaware, USA), equipped with TA Universal Analysis 2000 software. Oxygen-free nitrogen gas with a purge rate of 50 ml/min was used. Approximately 8 mg of sample was contained within a T-zero pan following seal with a T-zero hermetic lid. Each sample was heated from -30 $^\circ$C to 250°C with a heating rate of 10 $^\circ$C/min.

3.4.9 Occlusivity of UV-cured polymer films

The occlusivity of UV-cured films was extrapolated from TOWL measurements taken from healthy fingernail plates of volunteers before and after the application of the gel formulations, (which were applied as described in Section 3.4.12). The occlusivity of the Curanail® lacquer film was also extrapolated in a similar manner with the nail lacquer applied as instructed on the packaging, (i.e. after filing and cleansing). Prior to TOWL measurements, the volunteers rested for at least 60 minutes in the laboratory, and avoided contact with water. TOWL measurements were obtained using a Biox Aquaflux Model AF200 equipped with an *in vivo* nail cap (Biox Systems Ltd, London, UK). In order to obtain readings, the finger was placed on a flat surface for support and the Aquaflux measurement head was placed on the centre of the nail plate. No pressure was applied as the weight of the measurement head itself ensured a good seal, and measurements were collected using AquaFlux version 4.8 (Fig. 3.2). Water vapour flux density vs time graphs were recorded until a steady-state TOWL reading was obtained (between 90 - 120 seconds, with a mean of 10 points), and five repeats were obtained per formulation tested (using a different fingernail plate each time). All measurements were conducted in the same laboratory, where the ambient temperature and air humidity fluctuated between 22–26°C and 38–48% respectively. The % reduction in TOWL was calculated using the following formula:

$$\text{Reduction in TOWL \%} = \frac{\text{TOWL (without formulation applied)} - \text{TOWL (with formulation applied)}}{\text{TOWL (without formulation applied)}} \times 100$$

A greater reduction in TOWL indicated a higher occlusivity.

Fig. 3.2 Photographic image of the TOWL instrument (Aquaflux) used to conduct film occlusivity tests.

3.4.10 UV-cured polymer film's adhesivity

3.4.10.1 Cross-cut test

The cross-cut test (adapted from ISO (International Organisation for Standardisation) 2409:2013 – Paints and Varnishes – Cross-cut Test (ISO-2409, 2013)) was used to assess the resistance of the polymer films to separation from a substrate (i.e. the films' adhesion to the substrate) when a right-angle lattice pattern is cut into the film, penetrating through the substrate. A single layer of the UV-curable gel formulation (1.68 ml) was cured onto a 70 x 180 mm area on the smooth surface of a HDPE sheet and was left to age for 24 hours. Prior to testing, the thickness of the film was measured using a micrometre. A cross-hatch pattern was then cut manually into the polymer film using a scalpel (blade thickness of 0.38 mm, Swann–Morton, Sheffield, UK) by applying uniform pressure at a uniform cutting rate. A total of six parallel cuts were made in the direction of gel application, followed by six perpendicular cuts to form a lattice. The cuts made were 3 mm apart as the film thickness was between 121 and 250 μm. Any loose film was then removed by brushing the substrate lightly with a soft brush several times backwards and forwards and several times forwards along each of the diagonals of the lattice pattern. This cross-hatch pattern was examined visually to assess the extent to which the polymer film had been removed off the polyethylene sheet. The film's

removal was scored as detailed in Table 3.2. A high score therefore reflected poorer resistance of the film to removal from the substrate (i.e. poor adhesion). Five repeats were conducted per formulation.

Table 3.2 Scoring of cross-cut test results (ISO 2409:2013).

Score	Description	Appearance of surface of cross-cut area from which flaking has occurred
0	The edges of the cuts are completely smooth; none of the squares of the lattice is detached.	
1	Detachment of small flakes of the coating at the intersections of the cuts. A cross-cut area not greater than 5 % is affected.	
2	The coating has flaked along the edges and/or at the intersections of the cuts. A cross-cut area greater than 5 %, but not greater than 15 %, is affected.	
3	The coating has flaked along the edges of the cuts partly or wholly in large ribbons, and/or it has flaked partly or wholly on different parts of the squares. A cross-cut area greater than 15 %, but not greater than 35 %, is affected.	
4	The coating has flaked along the edges of the cuts in large ribbons and/or some squares have detached partly or wholly. A cross-cut area greater than 35 %, but not greater than 65 %, is affected.	
5	Any degree of flaking that is greater than for a score of 4.	

3.4.10.2 Pull off test using a texture analyser

The adhesion of a UV-cured film to a HDPE sheet was determined using Instron 5567 equipped with a 5 kg load cell (Instron®, UK). A single layer of the UV-curable gel formulation (65.4 µl) was cured on the smooth surface of a circular HDPE sheet (which had a diameter of 25mm and a height of 3mm). The HDPE sheet with the film attached was secured to the base of the Instron equipment. Double-sided Sellotape® was attached to the entire surface of a circular attachment (diameter of 50mm) secured to the Instron cross-head. The crosshead was lowered from a height of 35 centimetres at a speed of 1mm/s till the tape touched the surface of the film on the substrate. When the tape detected the surface of the film, a trigger force of 20 N was applied for 30 seconds. After the contact time had lapsed, the cross-head was raised away from the surface at a pre-set speed of 0.5 mm/s, as shown in Fig. 3.3, and the force required to achieve this was recorded as peak adhesive force (PAF). The energy at break (i.e. work of adhesion)

was also determined from the force deflection profiles. The two parameters were recorded and interpreted using Instron Bluehill software, Version 2.6.
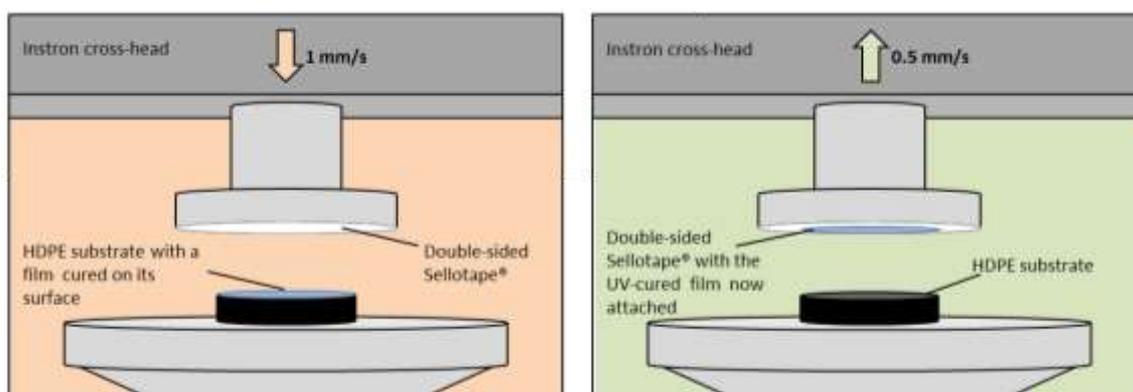
Fig. 3.3 Schematic of Instron set up for pull-off test. The cross-head was lowered at a speed of 1mm/s till the double-sided tape on the surface of an attachment secured to the cross-head touched the surface of the film adhered to the HDPE substrate. When the tape detected the surface of the film, a trigger force of 20N was applied for 30 seconds. After the contact time had lapsed, the cross-head was raised at a pre-set speed of 0.5mm/s. At this point, the UV-cured film was detached from the HDPE substrate and attached to the double-sided Sellotape®.

3.4.11 UV-cured polymer film's sensitivity to water

The water immersion test (adapted from ASTM (American Society for Testing and Materials) D870-15 – Standard Practice for Testing Water Resistance of Coatings Using Water Immersion (ASTM-D870, 2015)) was used to determine the resistance of the cured films to water. The formulation (140 µl) was cured to a 15 mm by 70 mm area onto the smooth side of a HDPE sheet. The latter was then placed in a distilled water bath (Grants Instruments Ltd., Cambridge, England) at room temperature (25°C) such that half the length of the film strip was immersed in water, while the other half was outside. At timed intervals, (initially every 10 minutes for 1 hour, then hourly for 8 hours and finally every 24 hours for two days), the HDPE sheets were taken out of the water bath to observe the effect of water immersion on the polymer film. The film's sensitivity/resistance to water was scored as 0 for no change in film, 1 for ≤25% blister formation, 2 for >25≤50% blister formation, 3 for >50≤75% blister formation and 4 for >75% blister formation. Therefore a high score indicated high sensitivity to water. The extent of blister formation was reported since other factors such as colour, were not affected.

3.4.12 *In vivo* fingernail residence of UV-cured polymer films

Following approval by the UCL School of Pharmacy's ethics committee for Project 5337/002: 'Determination of the *in vivo* residence of UV-cured polymer films to nails', 12 female volunteers (aged between 18 – 65 years) with healthy fingernails were recruited. For each volunteer, a single layer of the UV-curable gel formulation was applied on the fingernail plate using a pipette tip. This was initially done on each of the fingernails on one hand while leaving a formulation-free margin (approximately 1-2 mm) at the nail plate perimeter, ensuring that skin contact was avoided. This hand was then placed under a UVA nail lamp for two minutes, as shown in Fig. 3.4A. Subsequently, the surface of the cured gel on each nail plate was wiped with propan-2-ol using a super absorbent 4 ply lint-free nail wipe to remove the oxygen inhibition layer, thus leaving behind a thin, smooth, transparent film, as shown in Fig 3.4B. This procedure was then repeated for the other hand. The nails were then visually observed daily to estimate the percentage of UV-cured film remaining on the fingernails. Estimation was facilitated by visually dividing the nail plate into quadrants which were then further divided into sub-quadrants. This was continued until the UV-cured films had completely dislodged from the nail plate or for up to 28 days, whichever occurred sooner. After 28 days, the remaining film was removed with a wooden stick 10 minutes after applying a cotton pad soaked with acetone over the film. The experiment was then repeated with a different formulation. A total of four UV-curable gel formulations (which contained the monomer EMA, ± solvent and drug) were tested, and each formulation was tested on the 10 fingernails of a minimum of six volunteers.
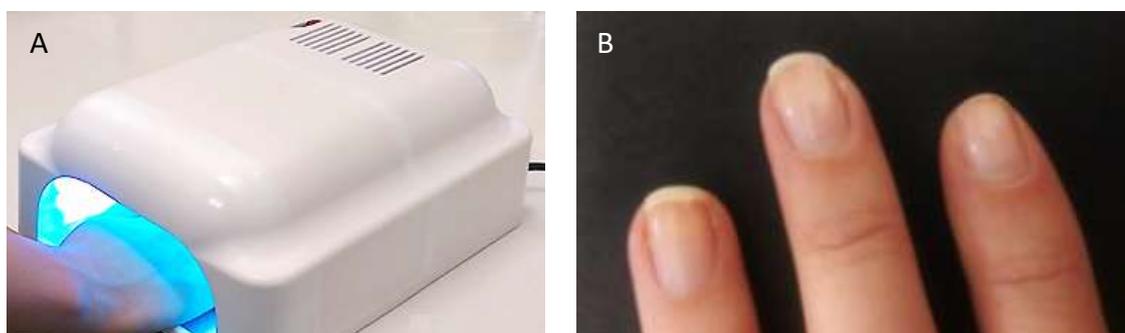
Fig. 3.4 Steps to apply UV-curable gel formulations. (A) Apply the UV gel on the nail surface and cure it under a UVA lamp for two minutes and (B) wipe the nail surface with a nail wipe soaked with isopropyl alcohol to reveal the polymer film adhered to it.

3.4.13 Mass change of UV-cured polymer films with time and drug stability in film

3.4.13.1 Mass change

The UV-cured films' mass change was followed over 28 days under the following testing conditions: 30°C ± 2°C and 50% RH ± 5% RH. These conditions were chosen as they were considered to resemble conditions the films would encounter when attached on the nail plate. The experiment was conducted by placing the films in a desiccator containing a saturated solution of magnesium nitrate and placing this in a 30°C oven. The UV-cured film's mass was recorded following its formation (day 0). At timed intervals (i.e. day 1, 2, 3, 7, 14, 21 and 28) the mass was recorded and any changes from day 0 calculated.

3.4.13.2 Drug stability in film

The drug stability in the UV-cured films was also tested over 28 days under the same testing conditions as above. At timed intervals (i.e. every 7 days), the incubated polymer films were observed for signs of drug crystallisation, after which the drug concentration in the film was determined. PLM was used to visualise the presence of crystals in the film, if any, as per Section 3.4.5.1. To determine whether the drug in the film was stable with time, the drug in the UV-cured polymer film was extracted and quantified as per Section 3.4.6.

3.4.14 Statistical analyses

Statistical calculations were conducted using IBM SPSS 22. The data was tested to determine whether they were normally distributed or not using the Shapiro-Wilk test and then analysed using either a t-test or Mann-Whiney U test for parametric and non-parametric data respectively. For multiple comparisons, ANOVA followed by post hoc Tukey or the Kruskal Wallis test with post hoc analysis performed with Nemenyi's test was used for parametric and non-parametric data respectively. Lastly, repeated measures ANOVA was used to determine whether there were differences in the *in vitro* water resistance, *in vivo* nail residence, and mass change of the UV-cured polymers over the experimental time.

3.5 Results and discussion

3.5.1 Morphology, thickness and structure of UV-cured films

3.5.1.1 Morphology and thickness

As mentioned previously (in Section 3.1), the UV-cured films produced were visually smooth and transparent, and this held true for the films produced by all the gels formulated containing EMA, IBOMA or HEMA, with/without solvent and with/without drug (Appendix A6). All formulations were therefore considered to produce films that were aesthetically acceptable and thus visually suitable as a means for delivering drug, in this case an antifungal, through the nail plate.

The thicknesses of the different films produced are shown in Table 3.3. As can be seen, the films produced from the drug-free and solvent-free gel blends with a DUDMA to diluent monomer ratio of 85:15% v/v are about 210 μm thick, with the choice of reactive diluent monomer in the gel displaying insignificant alterations in its film thickness ($p>0.05$). The drug-free and solvent-free HEMA formulation with a greater concentration of HEMA produced a slightly but statistically significantly thinner film ($p<0.05$), suggesting that the proportion of monomers, rather than diluent monomer choice, in the gel mixture predominately governs the thickness of the resulting film. Films produced from formulations containing the solvents ethanol or NMP are significantly thinner ($p<0.05$), at about 165 μm, than their solvent-free control counterparts due to the formulations' lower monomer content, with solvent choice displaying no impact on film thickness. Inclusion of the drug amorolfine HCl or terbinafine HCl in the gel also had no influence on its film thickness ($p>0.05$), due to their fairly low levels. While these films could be considered thin, in practice this would translate to a discreet drug-delivery vehicle, which is great when considering factors which could impact patient compliance.

Table 3.4 shows the uniformity of thickness within and among UV-cured films. In both instances, the thickness uniformity was equal to or greater than 95%, making the films suitable for further investigations.

Table 3.3 Thickness of UV-cured polymer films. Means ± standard deviations are shown, n=9. Abbreviations: AH, amorolfine HCl; TH, terbinafine HCl; NF, not formulated.

Ratio of DUDMA to reactive diluent monomer	Solvent	Drug	Film thickness (µm) for formulations containing		
			DUDMA & EMA	DUDMA & IBOMA	DUDMA & HEMA
85:15% v/v	None	None	211.1 ± 6.0	210.0 ± 10.0	212.2 ± 8.3
	Ethanol	None	161.1 ± 7.8	167.8 ± 6.7	168.9 ± 6.0
		AH	163.3 ± 8.7	165.6 ± 5.3	163.3 ± 7.1
		TH	164.4 ± 7.3	162.2 ± 6.7	164.4 ± 5.3
	NMP	None	165.6 ± 7.3	NF	NF
		AH	167.8 ± 8.3	NF	NF
		TH	166.7 ± 7.1	NF	NF
75:25% v/v	None	None	NF	NF	196.7 ± 7.1
		AH	NF	NF	193.3 ± 7.1
		TH	NF	NF	192.2 ± 6.7

Table 3.4 Uniformity of thickness (%) within and among three UV-cured polymer films.

Ratio of DUDMA to reactive diluent monomer	Solvent	Drug	Uniformity of thickness (%) for formulations containing					
			DUDMA & EMA		DUDMA & IBOMA		DUDMA & HEMA	
			Within film	Among films	Within film	Among films	Within film	Among films
85:15% v/v	None	None	97.3	97.2	97.3	95.2	95.2	96.1
	Ethanol	None	96.3	95.1	96.7	96.0	96.5	96.4
		AH	96.5	94.7	96.5	96.8	96.5	95.7
		TH	96.5	95.6	96.3	95.9	96.5	96.8
	NMP	None	96.5	95.6	NF		NF	
		AH	96.7	95.0	NF		NF	
		TH	96.5	95.8	NF		NF	
75:25% v/v	None	None	NF		NF		97.1	96.4
		AH	NF		NF		97.0	96.3
		TH	NF		NF		96.9	96.5

3.5.1.2 Structure of UV-cured films
Extent of cross-linking

The mean weight gain % of the UV-cured polymer films as a result of solvent-induced swelling is shown in Fig. 3.5. As mentioned in Section 3.1, the swelling of a polymer film can yield information on its cross-linking (Hirschl et al., 2013), with a higher weight gain % indicating that more solvent molecules have imbibed into the film, due to fewer cross-links in the polymer structure.

From Fig. 3.5, it can be seen that the UV-cured polymer films developed are highly cross-linked, with the highest weight gain being no greater than 10%. When considering the drug-free and solvent-free gel blends with a DUDMA to diluent monomer ratio of 85:15% v/v, it can be seen that the choice of reactive diluent monomer does not cause any significant changes in the swelling of the polymer films ($p>0.05$), which thus appear to have similar levels of cross-linking. This suggests that the diluent methacrylate monomers have a similar level of reactivity, as the proportion of the cross-linking monomer, i.e. DUDMA, and other monomers was kept constant. The HEMA formulation with a greater concentration of diluent monomer has a significantly lower weight gain % ($p<0.05$), indicating that fewer solvent molecules have imbibed into the film due to more cross-links. This increase in cross-link extent could possibly be due to the lower viscosity of the polymerising mixture (Chapter 2, Table 2.10), which would allow greater mobility of the reactants and hence greater reactivity and cross-linking. The UV-curable gel formulations containing the solvent ethanol also have a significantly lower weight gain % ($p<0.05$), also probably due to their lower viscosities when compared to the ethanol-free formulations. The incorporation of either amorolfine HCl or terbinafine HCl in the formulation does not appear to affect the film's property ($p>0.05$); possibly due to the small drug content in the films.

The results obtained follow the same trend as for degree of conversion (Chapter 2, Table 2.12), and in fact show that an increased degree of conversion from monomer gel to polymer film produces a film with a reduced swelling extent, i.e. increased polymer cross-linking, and that the swelling reduces exponentially with increased conversion

(R^2=0.871, p<0.01) (Fig. 3.6). This finding is consistent with previous reports which suggest that the density of cross-linkages increases markedly at higher monomer to polymer conversions (Flory, 1947).

However, the UV-curable gel formulations containing the solvent NMP did not swell, and instead the drug-free, amorolfine HCl–loaded and terbinafine HCl–loaded formulations obtained mass loss values of -9.6 % ± 2.9 %, -11.4 % ± 3.3 % and -9.4 % ± 2.9 % respectively. As mentioned in the previous Chapter, NMP has a higher boiling point compared to ethanol (202°C vs 78°C); therefore while the ethanol originally present in the gel formulation may evaporate off during UV-curing, NMP is likely to remain in the film. Consequently, it appears that when immersed in toluene, the loss of NMP residing in the film outweighs the amount of solvent molecules that have imbibed into the film; hence information regarding the film's cross-link extent cannot be ascertained. This highlights a limitation of using film swelling in a solvent as an indication of its cross-link extent. Inverse gas chromatography is an alternative method to measure the cross-link density of a UV-cured film (Burnett et al., 2007), and should be considered in future studies for films produced from gel formulations containing a solvent.
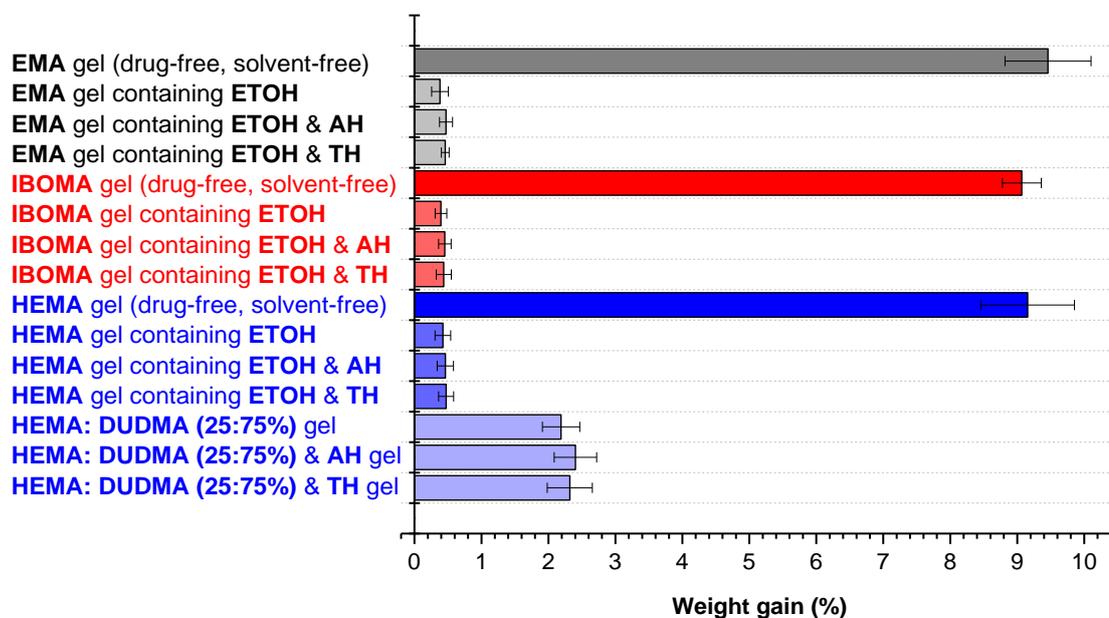
Fig. 3.5 Weight gain (%) for UV-cured films produced from DUDMA & EMA, IBOMA or HEMA containing gels (± solvent and ± drug) at 48 hours. Means and standard deviations are shown, n=3. Higher % weight gain indicates a lower extent of cross-linking. Abbreviations: AH, amorolfine HCl; TH, terbinafine HCl; ETOH, ethanol.
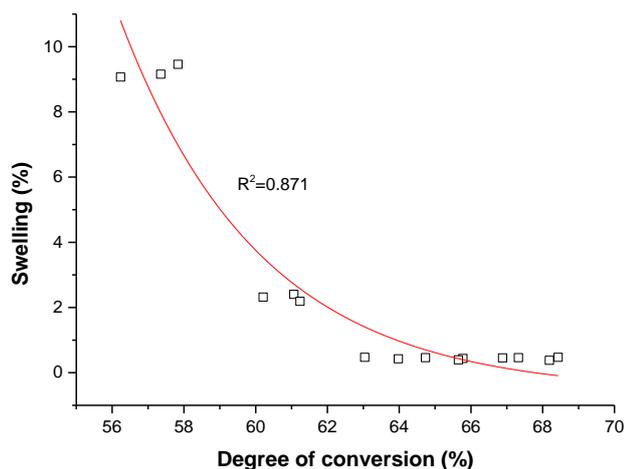
Fig. 3.6 Influence of degree of conversion (%) from monomer gel to polymer film on the resulting film's swelling ability, and hence cross-linking extent.

Porosity - total pore volume analysis from gas adsorption profiles of UV-cured polymer films

The adsorption of N_2 onto the UV-cured polymer film's surface is governed by the interaction between N_2 and the polymer and also the polymer's pore size and shape. The adsorption isotherms obtained by the DUDMA & EMA, IBOMA or HEMA UV-cured copolymer films (produced from drug-free and solvent-free gels) are shown in Fig. 3.7. According to the International Union of Pure and Applied Chemistry (IUPAC) classification of adsorption isotherms, the isotherms obtained are characteristic of adsorbents which are macroporous (i.e. contains pores with a diameter greater than 50 nm [Table 3.5]) and with weak interactions with the adsorbate (Sing et al., 1985). The adsorption isotherms obtained by UV-cured films produced from gels containing DUDMA & EMA, IBOMA or HEMA with drug and/or solvent (Appendix A7) are also characteristic of adsorbents which are macroporous, and the total pore volume of the UV-cured films produced from DUDMA & EMA, IBOMA or HEMA containing gels (± solvent and ± drug) is shown in Fig. 3.8.
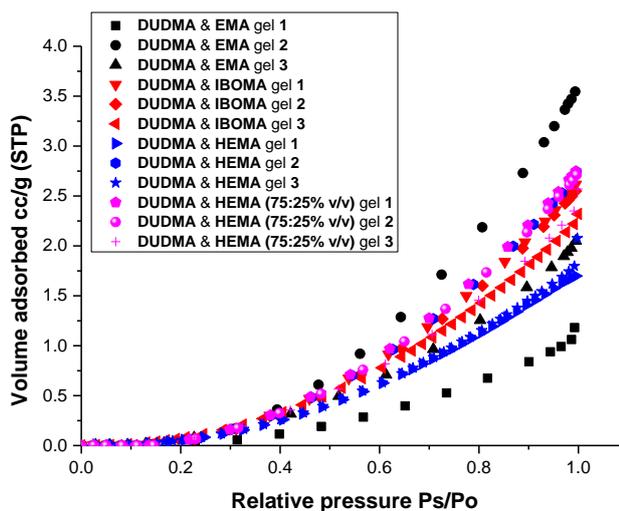
Fig. 3.7 Relative pressure vs volume adsorbed for UV-cured films produced from DUDMA & EMA, IBOMA or HEMA containing gels (drug-free, solvent-free, with a DUDMA: diluent monomer ratio of 85:15 % v/v unless otherwise stated).

Table 3.5 Classification of pores by the IUPAC (Sing et al., 1985), where width refers to diameter of a cylindrical pore or distance between opposite walls in case of slit pores.

Pore type	Width (nm)
Micropore	< 2
Mesopore	2 – 50
Macropore	> 50
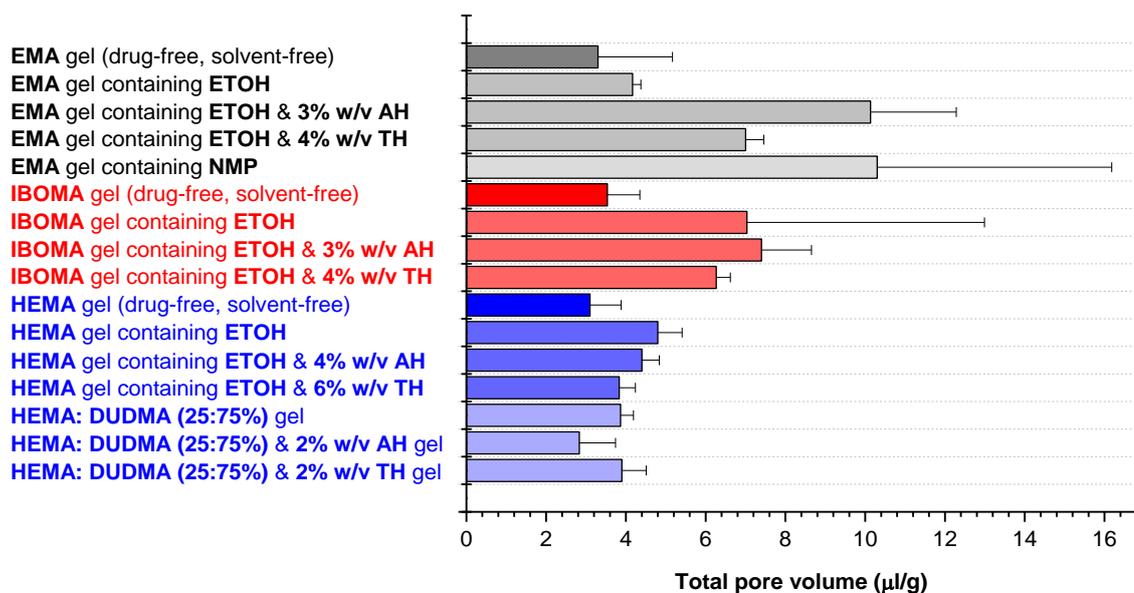
Fig. 3.8 Total pore volume of UV-cured films produced from DUDMA & EMA, IBOMA or HEMA containing gels (± solvent and ± drug). Means and standard deviations are shown, n=3. Abbreviations: AH, amorolfine HCl; TH, terbinafine HCl; ETOH, ethanol.

When considering the drug-free and solvent-free gel blends with a DUDMA to EMA, IBOMA or HEMA ratio of 85:15% v/v, it can be seen that their films have similar total pore volumes (3.1 – 3.6 µl/g) ($p>0.05$). These pores may have formed in response to shrinkage of the resin, i.e. UV gel, during polymerisation (Gilbert et al., 2000), a phenomenon introduced in Section 3.1, and it appears that the UV-curable gel formulations backbone, i.e. DUDMA, is the primary cause of the suspected 'shrinkage-induced porosity'.

The films produced from the DUDMA and HEMA gel blends with a DUDMA to HEMA ratio of 85:15% v/v and 75:25% v/v also have similar total pore volumes ($p>0.05$), indicating that increasing the HEMA concentration by 10% is not significant enough to affect the resulting film's total pore volume.

The presence of ethanol and NMP in the gel formulation appears to produced films which have a greater pore volume, however this difference is only significant for the HEMA and ethanol containing formulation ($p<0.05$), and may be down to the loss of volatile components, i.e. ethanol coupled with residual HEMA, during sample outgassing (where the temperature reached 120°C).

Interestingly, when observing whether the incorporation of either amorolfine HCl or terbinafine HCl in the formulation affects the total pore volume of the film, an increase in total pore volume can be seen by the amorolfine HCl–loaded DUDMA & EMA containing formulation compared to its drug-free counterpart ($p<0.05$). While an increase in total pore volume can also be seen by the terbinafine HCl–loaded DUDMA & EMA containing formulation compared to its drug-free counterpart, the difference is not significant ($p>0.05$), neither is the difference between the amorolfine HCl– and terbinafine HCl– loaded DUDMA & EMA copolymer. These findings suggest that despite a higher concentration of terbinafine HCl in a DUDMA & EMA gel formulation compared to amorolfine HCl (4% w/v vs 3% w/v), the incorporation of amorolfine HCl in the film produces a 'drug-modified' polymer, (i.e. a film with a greater total pore volume due to the presence of drug), while the incorporation of terbinafine HCl does not. A possible explanation for the observation could be the fact that terbinafine has the potential to

take part in the polymerisation process as it has an alkene group. However, the latter is not suspected as the incorporation of amorolfine HCl or terbinafine HCl in formulations containing DUDMA & IBOMA or DUDMA & HEMA (with a DUDMA to HEMA ratio of 85:15% v/v or 75:25% v/v) did not appear to affect the films' total pore volume ($p>0.05$). A plausible reason for the observations therefore remains to be seen.

Microstructure

Scanning electron micrographs of the UV-cured films produced from all the gels formulated containing EMA, IBOMA or HEMA, with/without solvent and with/without drug (Table 3.6), revealed a generally rough surface of the side that was exposed to UV light and a much smoother surface where the film had been formed in contact with the support. The latter provides a certain containment for the flow of the fluid during polymerisation and the smooth film surface reflects the smooth surface of the support. The cross-sectional views show a fairly dense film interior, which could be due to the polymers' highly cross-linked nature.

Films produced from the drug-free and solvent-free gel blends with a DUDMA to diluent monomer ratio of 85:15% v/v have comparable microstructures, whereas the films produced from the HEMA formulations with a DUDMA to HEMA ratio of 75:25% v/v have a slightly smoother top and cross-sectional surface compared to the films produced from the formulations with a DUDMA to HEMA ratio of 85:15% v/v. This suggests that the proportion of monomers, rather than monomer choice, in the gel mixture predominately influences the film's architecture.

Inclusion of the solvent ethanol in the gel formulation seems to have no influence on the film microstructure, while inclusion of NMP results in films with smoother top and cross-sectional surfaces. As mentioned above, NMP has a high boiling point, therefore while the ethanol originally present in the gel formulation may evaporate off during UV-curing, NMP is likely to remain in the film and influence the polymer formed.

Inclusion of the drug amorolfine HCl or terbinafine HCl in the gel formulation showed no visible changes in the resulting film's microstructure, possibly as the amount of drug included in the formulation was below the saturation limit, i.e. the drugs were molecularly dispersed in the formulation, and as the drug-load was fairly low. These findings will be used as a tool to discuss the film's drug release behaviour examined in the following Chapter.

Table 3.6 Scanning electron micrographs of top surfaces (i.e. exposed to UV light), under surfaces (i.e. in contact with the support), and cross-sections of UV-cured films.

Ratio of DUDMA to reactive diluent monomer	Solvent	Drug	SEM images of film produced from formulations containing DUDMA & EMA		
			Top	Under	Cross-section
85: 15% v/v	None	None			
	Ethanol	None			
		AH			
		TH			
	NMP	None			
		AH			
		TH			

Table 3.6 *Continued*

Ratio of DUDMA to reactive diluent monomer	Solvent	Drug	SEM images of film produced from formulations containing		
			DUDMA & IBOMA		
			Top	Under	Cross-section
85: 15% v/v	None	None			
	Ethanol	None			
		AH			
		TH			
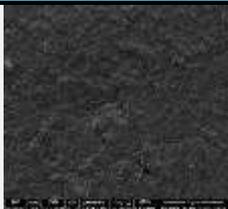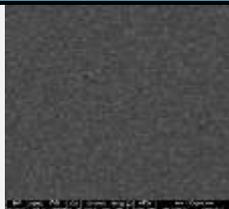
Table 3.6 *Continued*

Ratio of DUDMA to reactive diluent monomer	Solvent	Drug	SEM images of film produced from formulations containing		
			DUDMA & HEMA		
			Top	Under	Cross-section
85: 15 % v/v	None	None			
	Ethanol	None			
		AH			
		TH			
75: 25% v/v	None	None			
		AH			
		TH			
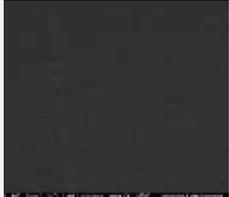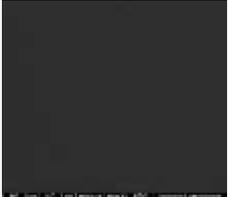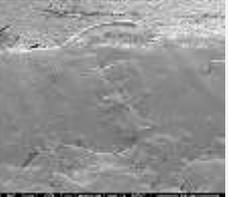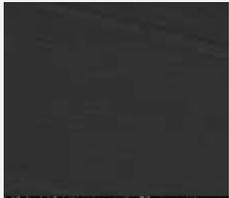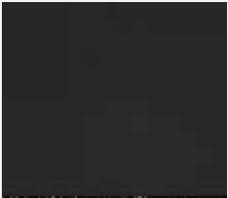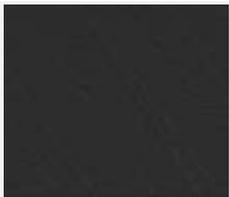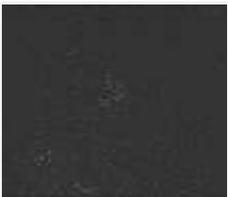
Abbreviations: AH, amorolfine HCl; TH, terbinafine HCl; NF, not formulated.

3.5.2 Drug-load in UV-cured film and drug-polymer interactions

3.5.2.1 Drug-load

The antifungals amorolfine HCl and terbinafine HCl are both crystalline powders as can be seen from their polarised light microscopy images and XRD patterns which reveal characteristic diffraction peaks (Fig. 3.9). DUDMA and EMA, IBOMA or HEMA copolymer films, on the other hand, are amorphous as can be seen in Fig. 3.10. As mentioned previously (in Section 3.4), the amount of drug incorporated in the gels formulated was the maximum that could be dissolved in the gel mixture (Table 2.9, Chapter 2). Upon UV-curing, the drug remained in the dissolved state with no crystals observed by polarised light microscopy, and this was confirmed by XRD which showed no change in the amorphicity of the polymer films (Table 3.7).
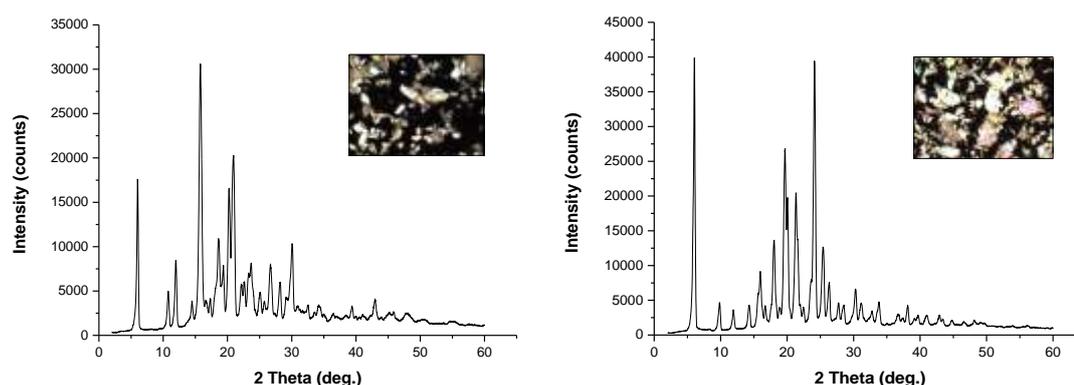
Fig. 3.9 Polarised light micrograph & corresponding XRD pattern of amorolfine HCl (left) & terbinafine HCl (right).
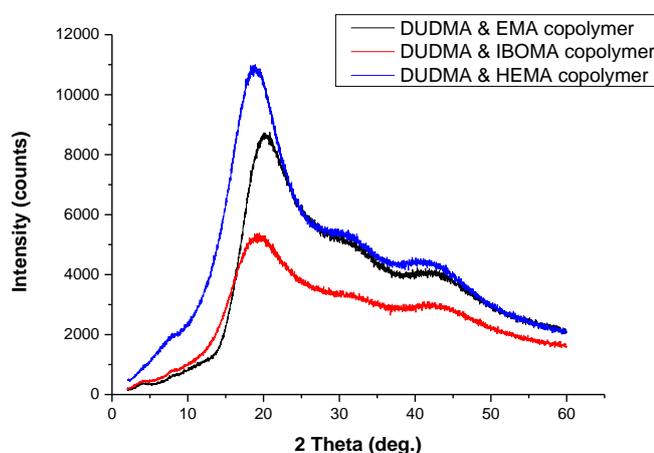
Fig. 3.10 XRD patterns of DUDMA & EMA, IBOMA or HEMA copolymer films (drug-free and solvent-free with a DUDMA: diluent monomer ratio of 85:15% v/v).

Table 3.7 Polarised light micrographs and corresponding XRD patterns of drug-loaded UV-cured films.

	Polarised light micrograph and corresponding XRD pattern of film produced from formulations containing	
	Amorolfine HCl	**Terbinafine HCl**
DUDMA 85%: EMA 15% v/v and 25% v/v ethanol	**3% w/v**	**4% w/v**
DUDMA 85%: EMA 15% v/v and 25% v/v NMP	**1% w/v**	**1% w/v**
DUDMA 85%: IBOMA 15% v/v and 25% v/v ethanol	**3% w/v**	**4% w/v**
DUDMA 85%: HEMA 15% v/v and 25% v/v ethanol	**4% w/v**	**6% w/v**
DUDMA 75%: HEMA 25% v/v	**2% w/v**	**2% w/v**

Table 3.8 shows the drug-load in the UV-cured films (following the removal of the oxygen-inhibition layer). The drug concentrations in all the films examined are similar to the drug concentration in their uncured gel formulation ($p>0.05$). The fact that the drug concentration did not increase or decrease in the film following the removal of the oxygen inhibition layer indicates that there was no drug migration to the film surface or interior during UV curing. This finding reveals an interesting difference between UV-curable gels and nail lacquers. In the latter, the drug concentration in the film formed is higher than that in the initial formulation as the solvent evaporates off upon application of a nail lacquer. For example, the pharmaceutical nail lacquer Curanail® contains amorolfine HCl at 5% w/v; however, upon application, a polymer film containing drug at a concentration of 31.5% ± 0.8% w/w is formed (measured by dissolving the Curanail® film in ethanol and quantifying by HPLC as per Section 2.4.6 [Chapter 2]). The lower solvent content of UV gel formulations and possible retention of some solvent in the UV-cured film may explain the similar drug concentrations in the gel and UV-cured film.

Table 3.8 Antifungal drug concentration in the gel formulations prior to curing and in UV-cured film following the removal of the oxygen inhibition layer.

Backbone and photoinitiator	Diluent monomer	Solvent (25% v/v)	Antifungal		
			AH or TH	Concentration in gel (% w/v)	Concentration in film (% w/w)
DUDMA and 2-hydroxy-2-methylpropiophenone	EMA	Ethanol	AH	3.0 ± 0.27	3.3 ± 0.16
			TH	4.0 ± 0.39	3.9 ± 0.11
		NMP	AH	1.0 ± 0.14	1.0 ± 0.04
			TH	1.0 ± 0.12	1.0 ± 0.01
	IBOMA	Ethanol	AH	3.0 ± 0.18	3.3 ± 0.20
			TH	4.0 ± 0.26	3.9 ± 0.06
	HEMA	Ethanol	AH	4.0 ± 0.49	4.4 ± 0.04
			TH	6.0 ± 0.22	5.9 ± 0.07
		None	AH	2.0 ± 0.37	2.2 ± 0.07
			TH	2.0 ± 0.12	2.0 ± 0.01

Abbreviations: AH, amorolfine HCl; TH, terbinafine HCl

3.5.2.2. Any drug-polymer interactions?

The FT-IR spectra of the DUDMA & EMA, IBOMA or HEMA UV-cured polymer films (drug-free and solvent-free) (Fig. 3.11) all show characteristic absorption bands at around 3360 cm^{-1} (N-H), 2957 cm^{-1} (C-H) and 1702 cm^{-1} (COO-R, R-O-CONH-R). The bands between 1300 – 1050 cm^{-1} relate to C-O stretches. These absorbances relate primarily to the functional groups of the DUDMA backbone and appear to overlap the absorption bands relating to IBOMA's cyclic group and HEMA's hydroxyl functional groups.
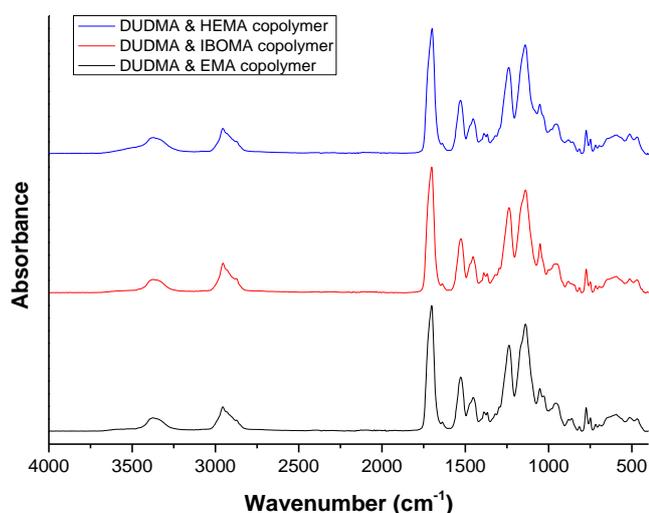
Fig. 3.11 FT-IR spectra of DUDMA & EMA, IBOMA or HEMA copolymer films (drug-free and solvent-free with a DUDMA: diluent monomer ratio of 85:15% v/v).

The FT-IR spectra of the drug-free polymer films produced from ethanol and EMA, IBOMA or HEMA containing gels (Table 3.9) are comparable to their solvent-free control counterparts. Interestingly, the FT-IR spectra of the film produced from the NMP containing formulation (Table 3.9) shows an absorption band between 1670 and 1710 cm^{-1} relating to the C=O stretch of NMP's five ring lactam, suggesting that NMP does remain in the polymer film upon curing of the gel formulation as hypothesised in earlier sections (Section 2.5.7 [Chapter 2] and 3.5.1.2).

The FT-IR spectra of the drug-free and drug-loaded polymer films show no obvious shifts in characteristic bands (Table 3.9), indicating the absence of chemical drug-polymer interactions in the film. This bodes well for drug release from film and ungual drug permeation.

Table 3.9 FT-IR spectra of UV-cured films. The spectra in black, red and blue are for drug-free, amorolfine HCl- and terbinafine HCl- loaded films respectively.

	FT-IR spectra of film produced from formulations containing
DUDMA 85%: EMA 15% v/v and 25% v/v ethanol	
DUDMA 85%: EMA 15% v/v and 25% v/v NMP	
DUDMA 85%: IBOMA 15% v/v and 25% v/v ethanol	
DUDMA 85%: HEMA 15% v/v and 25% v/v ethanol	
DUDMA 75%: HEMA 25% v/v	
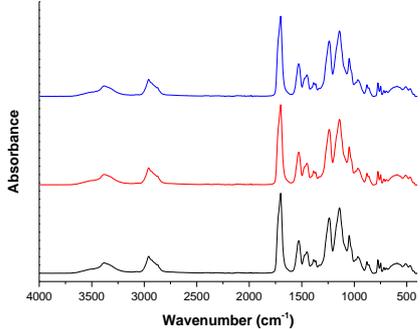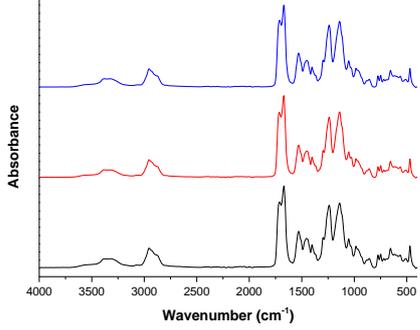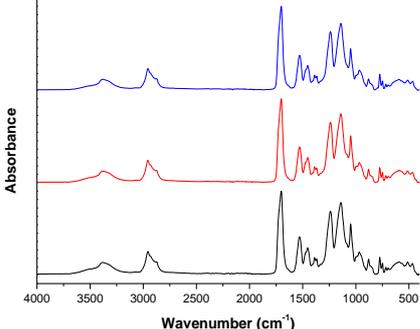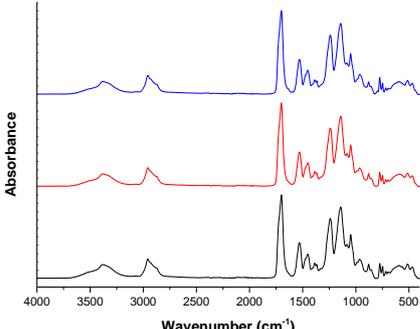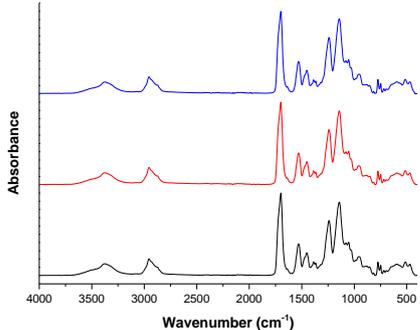
3.5.3 Thermal properties of UV-cured films

3.5.3.1 Polymer degradation

The thermal properties of the UV-cured films were determined using TGA, and the influence (if any) of monomer choice, monomer ratio, solvent and drug incorporation on the film's degradation were evaluated. The TGA curves for the drug-free polymer films (with and without solvent) are shown in Fig. 3.12. The profiles seem to consist of four phases, a–d, and is consistent with the profiles reported for UV-cured coatings based on urethane acrylate oligomers elsewhere (Kunwong et al., 2011, Alshuiref et al., 2013). The first phase relates to the loss of volatile materials from the film as the latter is heated. Films produced from solvent-containing formulations showed a greater loss in mass than the control solvent-free film during phase a ($p<0.05$). This indicates that some solvent has remained in the film upon UV-curing and was being lost as the film was heated. NMP-containing films showed a greater mass loss than ethanol-containing ones and a transition at around 200°C. This is linked to the greater amount of residual solvent in these films and to NMP's boiling point of 202°C. Phases b–d in the profiles are related to polymer decomposition via the degradation of polymer side-chains, followed by scission and depolymerisation, and finally complete de-crosslinking and thermal degradation, as suggested for similar films by other researchers (Chattopadhyay and Webster, 2009).

It can be seen from Fig. 3.12 that the films produced from the solvent-free DUDMA & EMA, IBOMA or HEMA gel blends with a DUDMA to diluent monomer ratio of 85:15% v/v have comparable thermal degradation profiles. It can also be seen that the ratio of DUDMA: diluent methacrylate monomer (i.e. HEMA) used in the gel does not affect the resulting polymers' thermal degradation. This could be as the higher HEMA concentration in the gel is not sufficiently different enough to significantly affect the resulting copolymers degradation profile. The presence of amorolfine HCl or terbinafine HCl in the formulation also did not alter the film's thermal stability (Appendix A8), possibly due to their low concentrations. The thermal stability of the drug-loaded UV-cured polymers at room temperatures therefore makes them acceptable for consideration as a nail drug delivery vehicle.
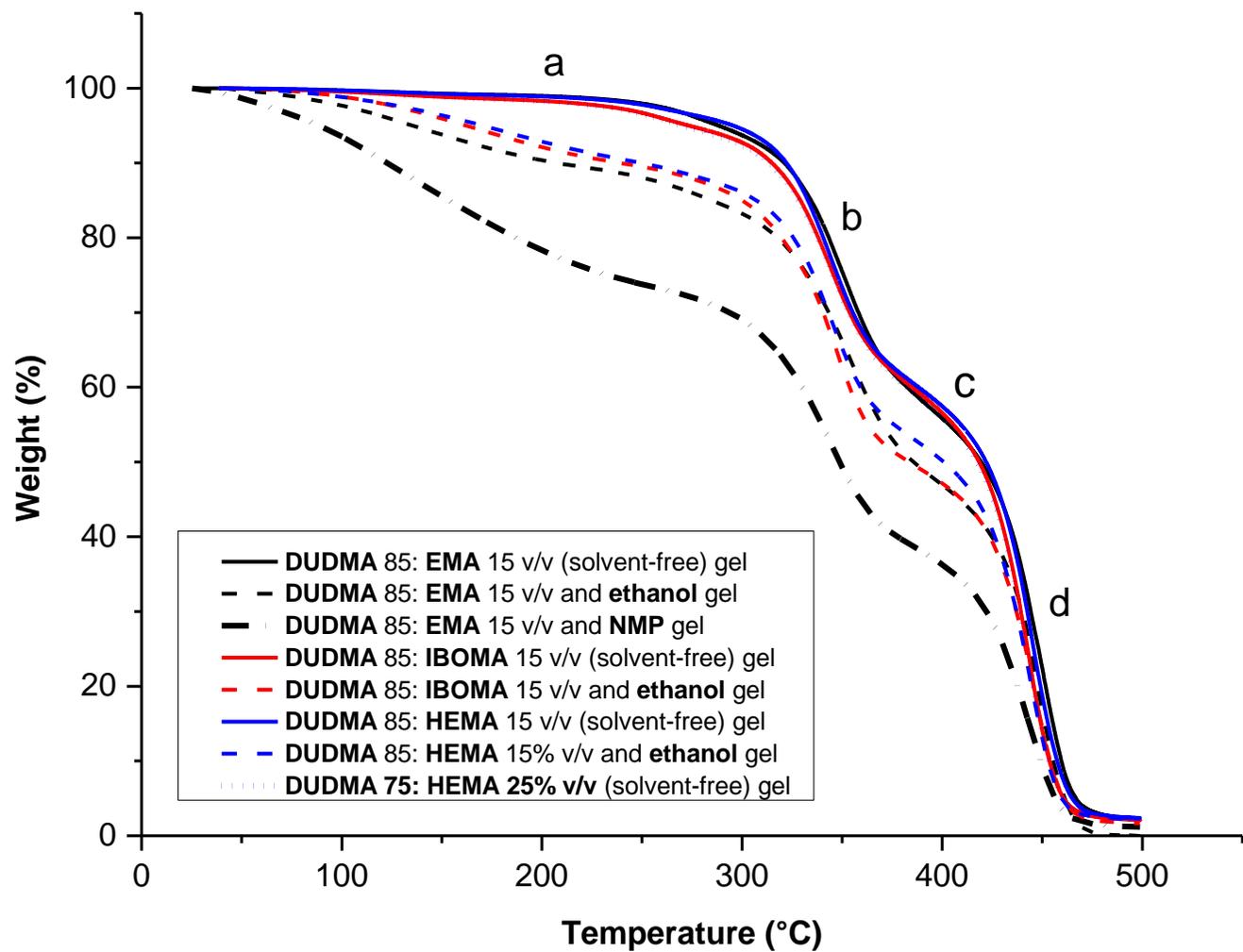
Fig. 3.12 TGA profiles of films produced from drug-free UV-curable gel formulations containing EMA, IBOMA and HEMA represented by black, red and blue respectively.

3.5.3.2 Glass transition temperatures of UV-cured films and the influence of film components

The UV-cured polymers' glass transition temperatures (Tg) were calculated as the tan δ peak from the DMA curves. Interestingly, the DMA curves for the film produced from the DUDMA & EMA containing formulation absent of solvent and drug showed two transitions as shown in Fig. 3.13. The first glass transition relates to the polymer film produced following UVA irradiation of the gel formulation, and the second glass transition could be in response to an additional thermal cure of the UV-cured polymer film owing to the high temperature range used in the DMA experiment (Jong Keun et al., 2003). A more detailed examination of the log [storage modulus] vs. temperature plot (Fig. 3.13) shows that the polymer film starts off glassy, and as the temperature is increased the first glass transition is evident. As the temperature further increases in this region there is a likely increase in the mobility of macromolecular chains of the film, and a possible additional cure with further crosslinking reaction induced by thermal energy. Once the second cure is completed, a second glass transition of the polymer film is evident, and a new material can be considered to have been created. This phenomenon is feasible given that the degree of conversion from monomer to polymer was not 100% for this formulation (Chapter 2, Table 2.12), the lifetime of residual free radicals at room temperature could reach several days or months (Zhu et al., 1990, Tian et al., 1992), and the reaction of an irradiated unsaturated monomer has previously been observed for several days in the dark (Marino et al., 1995). Furthermore, upon observation of the DSC thermogram for this polymer film (Fig. 3.13), an exothermic peak is evident (at a temperature beyond the first glass transition temperature) in response to the additional thermally induced polymerisation reaction. This trend is also evident with all the solvent-free polymer films containing IBOMA and HEMA, which also display two glass transition temperatures, the values for which are in Table 3.10. As the lower glass transition temperature corresponds to the polymer film produced following UV-irradiation of the gel formulation without a subsequent thermal reaction, as would be the case in practice, it was used when comparing the different formulations.

It can be seen from Table 3.10, that the choice of diluent monomer in a gel formulation absent of solvent and drug (and with a DUDMA to diluent reactive monomer ratio of 85:

15% v/v) significantly alters the resulting polymers' Tg ($p<0.05$), where DUDMA & EMA copolymer's Tg is greater than that of the DUDMA & HEMA copolymer, which is in turn greater than that of the DUDMA & IBOMA copolymer. This trend can be explained by the viscosity of the formulations, which have values of 366.5, 598.1 and 672.4 mPas respectively (Chapter 2, Table 2.10), and it can be seen that the lower the viscosity of the formulation, the higher the glass transition of its UV-cured polymer film. A formulation with a lower viscosity can increase the mobility of the reactants and therefore reactivity during polymerisation, this in turn can produce a highly cross-linked film with decreased chain mobility, hence the increased Tg observed. The amount of diluent monomer used in a gel formulation also affects the resulting polymers' Tg. The film produced from the HEMA formulation with a DUDMA to HEMA ratio of 75:25% v/v has a higher Tg compared to the film produced from the formulations with a DUDMA to HEMA ratio of 85:15% v/v, also due to its lower viscosity (Table 2.10).

Inclusion of ethanol or NMP in the gels resulted in films with only one Tg – which was significantly higher than the first glass transition temperature seen for the corresponding solvent-free films (Fig. 3.14, Table 3.10). It was mentioned earlier (in Chapter 2, Section 2.5.7.2) that inclusion of a solvent in a UV-curable gel considerably decreases its viscosity and hence significantly increases its polymerisation extent, which as mentioned above can increase Tg. The increased polymerisation can also decrease the likelihood of an additional thermal cure of the UV-cured polymer film, and this explains why the DSC thermogram for the films produced from the DUDMA & EMA gel formulations with solvent (ethanol or NMP), but without drug, displays no appreciable exotherm (Fig. 3.14), which in turn explains the absence of a second glass transition.

It can also be seen from Table 3.10, that the choice of diluent monomer in an ethanol-containing gel formulation significantly alters the resulting polymers' Tg ($p<0.05$), where DUDMA & IBOMA copolymer's Tg is greater than that of the DUDMA & EMA copolymer, which is in turn greater than that of the DUDMA & HEMA copolymer. As the viscosities of the solvent-containing gel formulations are similar (Chapter 2, Table 2.10), gel viscosity does not explain the observations. Instead this trend can be explained by the Tg of IBOMA, EMA and HEMA's homopolymers, which have values of 110°C, 65° and 57°C

respectively. IBOMA's bulky cyclic group is responsible for the much higher Tg displayed by IBOMA containing polymers due to its potential to hinder chain mobility.

Interestingly, films formed from EMA and NMP-containing gels have lower Tg values than EMA and ethanol-containing ones ($p<0.05$). The greater amount of residual solvent in the NMP-based film (as evidenced in Fig. 3.12) seems to result in a plasticising effect on the UV-cured polymer film, by spacing polymer chains apart and enabling chain mobility, such that Tg is lowered.

The presence of the drugs in the solvent-containing films' does not cause any significant changes in the films' Tg ($p>0.05$), while the presence of drugs in the solvent-free DUDMA & HEMA gel formulations with a DUDMA to HEMA ratio of 75:25% v/v, slightly increases the film's Tg ($p<0.05$). Both amorolfine HCl and terbinafine HCl have a molecular weight greater than 300 Daltons (Table 2.6), and when incorporated in a UV-cured film, both may restrict the mobility of polymer chain and increase Tg. In the presence of ethanol, this restriction is less evident; indicating that the residual ethanol in the film masks the effect of drug incorporation when considering the resulting film's Tg.
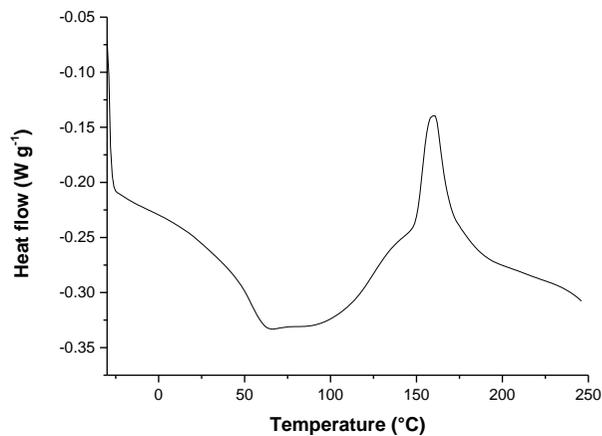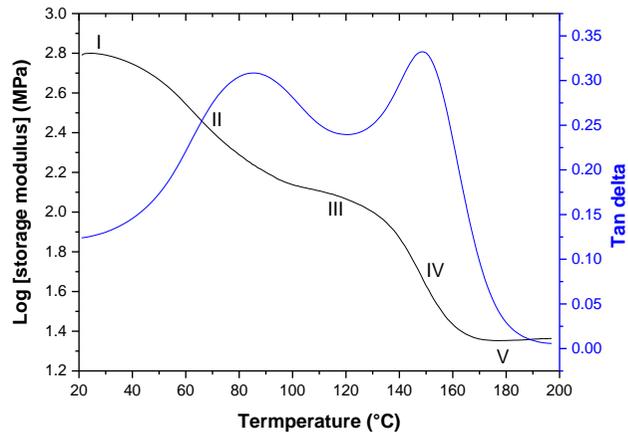
Fig. 3.13 Log [storage modulus] and tan δ from DMA and heat flow from DSC vs. temperature for UV-cured films produced from DUDMA & EMA gel formulations without solvent and without drug. The log [storage modulus] vs. temperature plot can be considered to show five different regions: I = glassy, II = glass transition, III = thermal reaction, IV = new glass transition, and V = rubbery (Jong Keun et al., 2003).
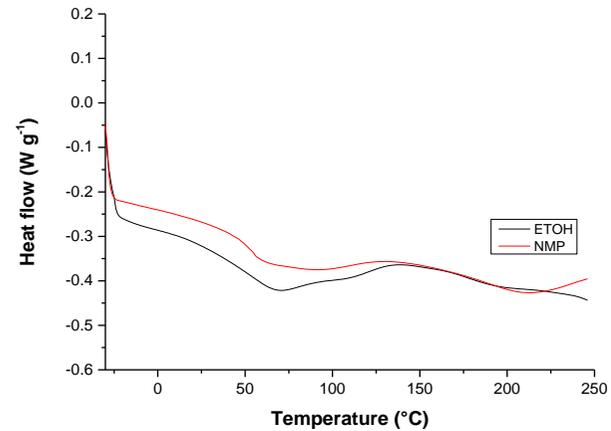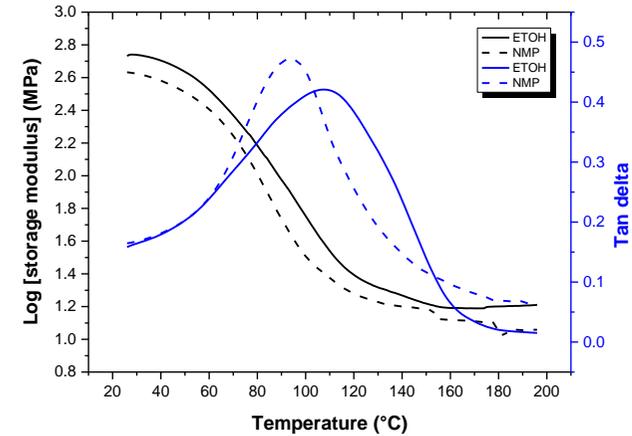
Fig. 3.14 Log [storage modulus] and tan δ from DMA and heat flow from DSC vs. temperature for UV-cured films produced from the DUDMA & EMA gel formulations with solvent (ethanol or NMP), but without drug. Abbreviations: ETOH, ethanol.

Table 3.10 Tg values of UV-cured films. Means ± standard deviations are shown, n=3.

| Excipients | DUDMA | Solvent | | Drug | Tg values of UV-cured films produced from formulations containing | | |
		Ethanol	NMP		EMA	IBOMA	HEMA
Formulations	DUDMA 85 % v/v : diluent monomer 15 % v/v	None	None	None	85.7 ± 1.2 and 146.0 ± 2.5	67.2 ± 2.4 and 164.0 ± 0.2	74.4 ± 2.5 and 147.2 ± 0.3
		25% v/v	None	None	112.5 ± 1.8	139.2 ± 9.0	98.1 ± 2.3
				AH	109.2 ± 1.1	137.2 ± 0.02	99.2 ± 1.8
				TH	109.6 ± 0.8	133.4 ± 3.6	94.7 ± 4.8
		None	25% v/v	None	95.1 ± 1.5	NF	NF
				AH	94.8 ± 2.0	NF	NF
				TH	95.0 ± 1.8	NF	NF
	DUDMA 75 % v/v : diluent monomer 25 v/v	None	None	None	NF	NF	81.8 ± 0.5 and 142.6 ± 0.4
				AH	NF	NF	92.0 ± 0.9 and 138.9 ± 0.5
				TH	NF	NF	92.4 ± 0.4 and 133.2 ± 0.2
Tg of homopolymers (ºC)	-	-	-	-	**65**	**110**	**57**

Abbreviations: AH, amorolfine HCl; TH, terbinafine HCl; NF, not formulated.

3.5.4 Occlusivity of UV-cured polymer films

UV gels produce an occlusive film on the nail plate surface following UV-curing and removal of the oxygen inhibition layer. Fig. 3.15 shows just how occlusive the polymeric film formed is by quantifying the suppression of TOWL following its formation on the nail plate surface. It can be seen that a film formed from a drug-free and solvent-free EMA-containing gel formulation reduces TOWL by as much as 48%. The choice of diluent monomer (i.e. EMA, IBOMA or HEMA) in the gel formulation does not significantly alter this % reduction ($p > 0.05$). The presence of solvent with and without drug in the EMA containing gel formulations also does not significantly alter this % reduction ($p > 0.05$). As seen from Table 3.3, formulations containing solvents produced films which are significantly thinner (by approximately 20%) than their solvent-free counterparts. However, the thickness of the UV-cured film does not appear to correlate with occlusivity as can be seen from Fig. 3.16. Therefore it appears that the choice of the polymer backbone, i.e. DUDMA, is primarily responsible for the reduction in TOWL.

When compared to the commercially available pharmaceutical nail lacquer – Curanail® – the UV-curable gel formulations reduce TOWL % to a greater extent ($p < 0.05$). In addition to containing the active amorolfine hydrochloride, Curanail® contains ammonio methacrylate copolymer A, triacetin, butyl acetate, ethyl acetate and ethanol. The Curanail® film produced has a thickness of 13.3 μm ± 2.9 μm, which is 10 times thinner than the drug-loaded DUDMA & EMA UV-cured films; therefore in this instance film thickness may indeed be responsible for the lower TOWL % reduction. The DUDMA containing copolymers may also have a lower permeability to water vapour compared to ammonio methacrylate copolymer A, a factor which can also contribute to TOWL reduction.
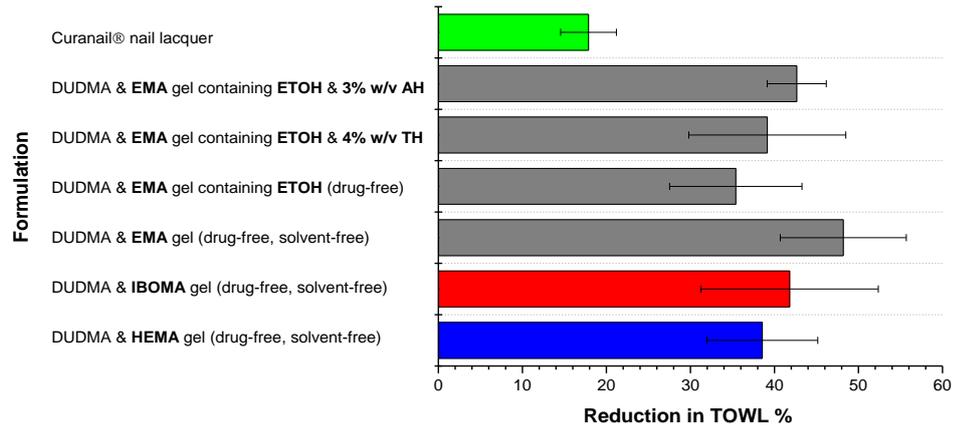
Fig. 3.15 Reduction in TOWL following application of a formulation on the nail plate surface. All gel formulations tested have a DUDMA to diluent monomer ratio of 85:15% v/v. Means and standard deviations are shown, n=5. Abbreviations: AH, amorolfine HCl; TH, terbinafine HCl; ETOH, ethanol.
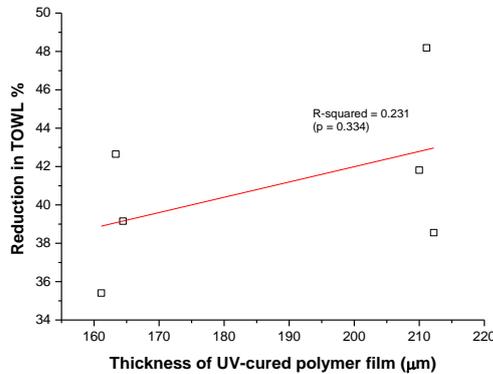
Fig. 3.16 Influence of film thickness on film occlusivity. Greater reduction in TOWL % = greater occlusivity.

As previously mentioned, a reduction in TOWL is likely to hyper-hydrate the upper nail layers. This can aid drug permeation into and through the nail plate (Spruit, 1971, Marty, 1995), given that *in vitro* nail hydration has been found to increase diffusivity of water and of the antifungal ketoconazole in the nail (Gunt et al., 2007, Gunt and Kasting, 2007). Nail hydration can also benefit in the treatment of onychomycosis by inducing the germination of drug-resistant fungal spore into drug-susceptible hyphae, which would enable fungi eradication (Flagothier et al., 2005). Therefore, it could be possible that since the drug-loaded UV-curable gel formulations reduce TOWL to a greater extent than Curanail®, they could cause a greater increase in nail plate hydration and thereby a greater enhancement of drug permeation into the nail to tackle the onychomycosis-causing pathogen.

It should be noted however, that these TOWL reduction readings were obtained using healthy fingernails, and TOWL reduction in practice, i.e. on a diseased nail, following the application of the UV-curable gel formulation could be significantly different due to possible differences in initial TOWL readings. For example, it has been found that onychomycotic nails show a significantly lower TOWL than healthy nails, as the diseased nail develops a granular layer (Krönauer et al., 2001). This is based on the speculation that the structure of a dystrophic nail is more similar to that of skin. To add to this, onychomycosis predominately affects toenails which are thicker than fingernails, and TOWL has also been found to be influenced by nail plate thickness (where it is lower for thicker nails) (Murdan et al., 2008). On the other hand, a more recent study found that TOWL measurements are similar for healthy and thicker diseased nails (McAuley et al., 2016). Whether the application of a UV-curable gel formulation reduces TOWL of diseased nails to the same extent as healthy nails could therefore be considered for further work.

3.5.5 UV-cured polymer film's adhesivity

Adhesion of a topical formulation to the nail plate surface is essential in order to allow the drug to leave the formulation and penetrate the nail. The adhesivity of UV-cured polymer films was measured both manually (cross-cut test) and by using a texture analyser (pull-off test).

3.5.5.1 Cross-cut test

The mean cross-cut score for the UV-cured polymer films is shown in Fig. 3.17. A high score means lower adhesion (or lower resistance to separation from a substrate). The greatest adhesion was shown by the films produced from the solvent-free formulations, with the choice of diluent monomer not altering adhesion ($p>0.05$). The DUDMA backbone is primarily responsible for the resistance to separation displayed as it inherently provides excellent adhesion and abrasion resistance (Botero et al., 2014). However as the DUDMA to diluent monomer ratio used (i.e. 85: 15% v/v or 75:25% v/v for HEMA) does not significantly alter the films' adhesion ($p>0.05$), it appears that the diluent monomer, in this case HEMA, also contributes to the copolymers' adhesive property. Presence of ethanol (with and without antifungal drugs) in the formulations

caused no statistically significant change in film adhesion ($p>0.05$). In contrast, presence of NMP (with and without antifungal drugs) in the formulation significantly reduced film adhesivity ($p<0.05$). As discussed earlier, more NMP (than ethanol) remains in the film upon gel curing. The presence of a greater amount of solvent seems to cause a larger change in the properties of the NMP-containing films, resulting in a significant decrease in film adhesion to the substrate. The incorporation of either amorolfine HCl or terbinafine HCl in the formulation did not affect the films' cross-cut score ($p>0.05$); possibly due to the small drug content in the film.
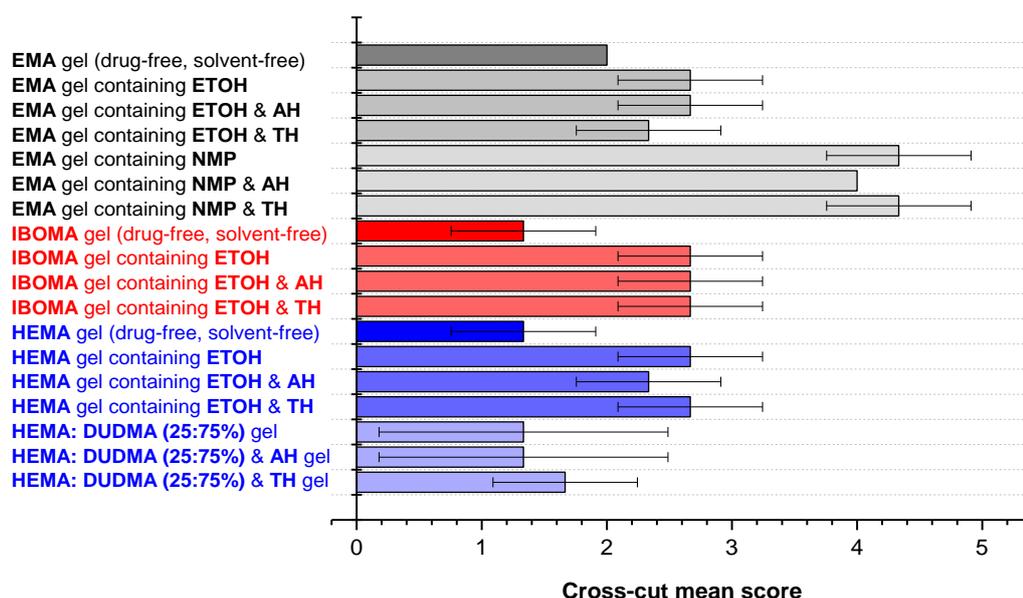
Fig. 3.17 Cross-cut scores for films produced from gels containing DUDMA & EMA, IBOMA or HEMA (± solvent and ± drug). Means and standard deviations are shown, n=5. High cross-cut scores mean lower adhesivity. Abbreviations: AH, amorolfine HCl; TH, terbinafine HCl; ETOH, ethanol.

3.5.5.2 Pull-off test

The pull-off test was used to determine the maximum force and work needed to separate two surfaces in intimate contact, i.e. the substrate and UV-cured film. The peak adhesive force (N) and work of adhesion (mJ) readings obtained for the UV-cured films examined are shown in Fig. 3.18. It can be seen that the choice and ratio of diluent monomer, and the incorporation of ethanol with and without amorolfine HCl or terbinafine HCl in DUDMA containing gel formulations does not significantly affect the resulting films' peak adhesive force or work of adhesion ($p>0.05$). In contrast, the presence of NMP (with and without antifungal drugs) reduces both parameters ($p<0.05$)

and thereby the film's adhesive properties. These results are comparable to the cross-cut test results shown in Fig. 3.17, and confirm that NMP negatively affects the films' adhesive properties. It also suggests that the drug-loaded UV-curable gel formulations (without NMP) have good adhesive properties when compared to the pharmaceutical nail lacquer Curanail®, (which has peak adhesive force and work of adhesion values of 48.4 ± 8.9 N and 2.3 ± 1.1 mJ respectively). This "better" adhesivity should aid ungual drug permeation.
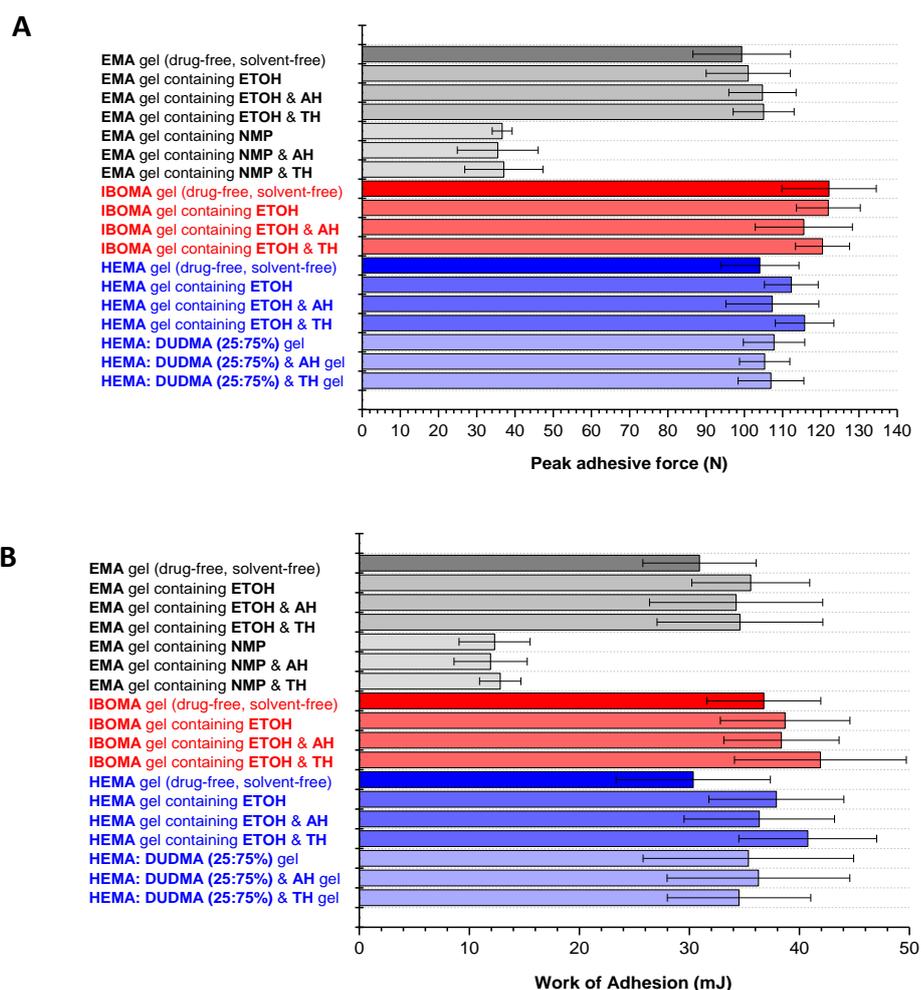
Fig. 3.18 Peak adhesive force (A) and work of adhesion (B) readings obtained for the UV-cured films produced from gels containing DUDMA & EMA, IBOMA or HEMA (± solvent and ± drug). Means and standard deviations are shown, n=3.

3.5.6 UV-cured polymer film's sensitivity to water

The water sensitivity scores of the UV-cured films are shown in Figs 3.19 - 3.22. Fig. 3.19 shows the water sensitivity score of UV-cured films produced from DUDMA & EMA gels

(± solvent and ± drug), and shows that films produced by the NMP-containing formulations are extremely water sensitive. By five hours, the films had detached from the substrate. NMP is miscible in water (Jouyban et al., 2010), and as it is present at a considerable level in the film, contact with water causes the film to blister and subsequently detach from the substrate. In contrast, solvent-free and drug-free films had the lowest water sensitivity. This was expected as urethane methacrylate based films are known for water resistance. Inclusion of ethanol caused no significant change in water sensitivity ($p<0.05$). Like NMP, ethanol is miscible in water and therefore one could have expected the water sensitivity of ethanol- and NMP- containing films to be similar. A possible explanation for the greater water sensitivity displayed by NMP-containing films is that the large planar non-polar region of NMP intercalates among polymer chains forming hydrophobic interactions with the polymer, whereas more of the ethanol evaporates off during and after curing due to its lower boiling point. The incorporation of either amorolfine HCl or terbinafine HCl in the formulation did not affect the films' water sensitivity, possibly due to the very low drug-load.

Films produced from the DUDMA & IBOMA or HEMA containing gels (± solvent and ± drug) show a similar trend to the EMA containing gels (Fig. 3.19 – Fig. 3.21), where the presence of ethanol (with and without drug) in the gel does not cause a significant change in the resulting film's water sensitivity ($p>0.05$). This could be as the ethanol concentration incorporated in all the gel formulations was the same and also as the drug-load in these formulations was low. The incorporation of drug in DUDMA & HEMA gels with a DUDMA: HEMA ratio of 75: 25% v/v also did not affect the film's water sensitivity ($p>0.05$) (Fig. 3.22), also probably due to its very low level in the formulation.

From Fig. 3.23, it can be seen that the choice of diluent monomer in the drug-free and solvent-free gel formulation does not affect the resulting films' water sensitivity score ($p>0.05$). This comes as a surprise given that a HEMA polymer is expected to swell in water due to the molecules' hydrophilic pendant group (Chapter 2, Table 2.4). A likely explanation for the similarities is therefore that the concentration of the diluent monomers present in the gel formulation is not high enough to affect the resulting films' water sensitivity.
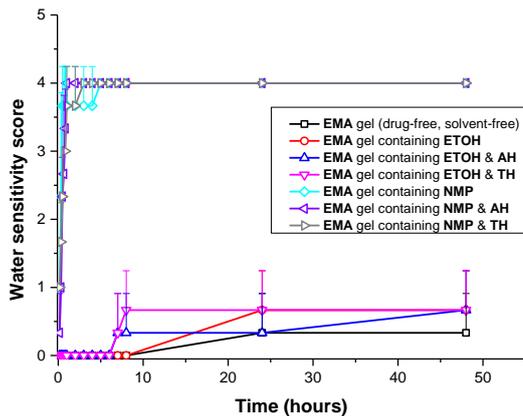
Fig. 3.19 Water sensitivity score of UV-cured films produced from DUDMA & EMA gels (± solvent and ± drug). Means and standard deviations are shown, n=3.
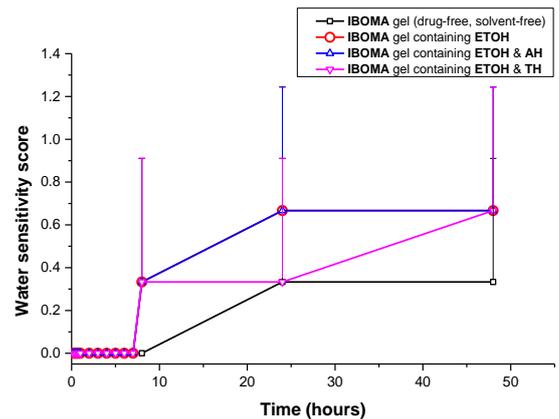
Fig. 3.20 Water sensitivity score of UV-cured films produced from DUDMA & IBOMA gels (± solvent and ± drug). Means and standard deviations are shown, n=3.
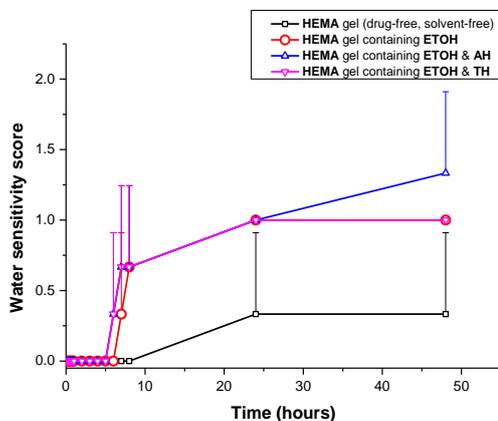
Fig. 3.21 Water sensitivity score of UV-cured films produced from DUDMA & HEMA gels (± solvent and ± drug). Means and standard deviations are shown, n=3.
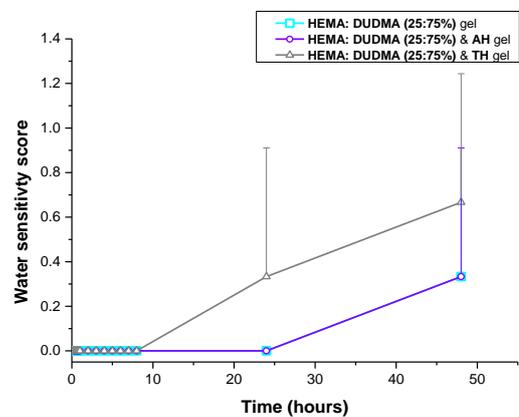
Fig. 3.22 Water sensitivity score of UV-cured films produced from DUDMA & HEMA gels (± drug) with a DUDMA: HEMA ratio of 75:25 % v/v. Means and standard deviations are shown, n=3.
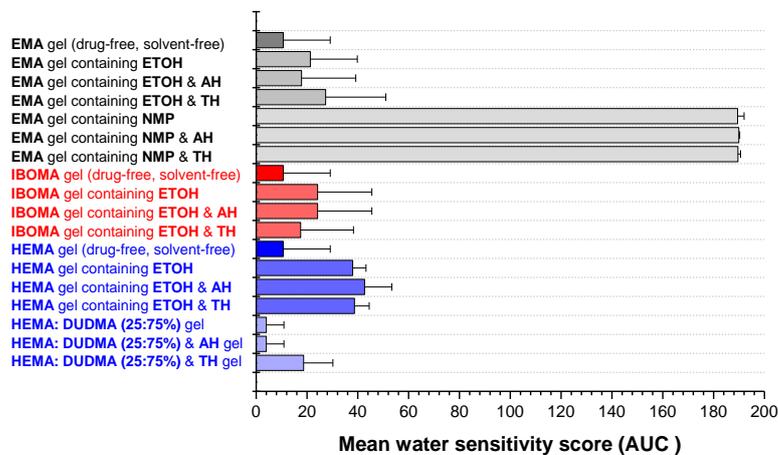
Fig. 3.23 Area under the curve values calculated from the curves in Fig. 3.19 – Fig. 3.22 for the UV-cured films produced from DUDMA & EMA, IBOMA or HEMA containing gels (± solvent and ± drug). Means and standard deviations are shown, n=3. AUC was determined using OriginPro 9.0.

However, increasing the concentration of HEMA in the gel, (so that the DUDMA to HEMA ratio is 75: 25% v/v as oppose to 85: 15% v/v), does not significantly alter the resulting films' water sensitivity either ($p>0.05$). This therefore indicates that a HEMA concentration of 25% v/v or less can be incorporated in a gel formulation without having a significant impact on DUDMA's ability to produce a water-resistant film.

The low water sensitivity of films produced from ethanol- and drug- containing DUDMA & EMA, IBOMA or HEMA gels indicates that these films could be used as long-term topical nail medicines, and the next section explores just how long the film does reside on the nail *in vivo*. The high water sensitivity of NMP-containing films means that these films would not remain on the nail *in vivo* for prolonged periods (Murdan et al., 2015). These films were therefore not included in the work described in the following Sections and Chapters.

3.5.7 *In vivo* fingernail residence profiles of UV-cured polymer films

The mean *in vivo* residence of DUDMA & EMA containing gels (± drug and ± ethanol) and the Curanail® nail lacquer on fingernails over a 28 day period is shown in Fig. 3.24. A large variability in the residence of the same formulation in different individuals was seen as shown by the error bars. This is as expected, given that a formulations' residence on the nail is governed by the individual's activities such as hand washing, dish washing, swimming etc. Nonetheless, it was found that a significantly greater % of the film produced from the solvent-free gel formulation remained on the fingernail over the test period when compared to the gel containing ethanol ($p<0.05$). The TGA profile of films produced from gels containing ethanol confirmed that some ethanol does reside in the polymer (Fig. 3.12). As previously mentioned, ethanol is miscible with water, and while this factor did not affect the *in vitro* water sensitivity of the films produced from ethanol-containing gels (Fig. 3.19), it did affect the film's *in vivo* residence on the nail plate, possibly as the formulations' *in vivo* fingernail residence profile was tested over a longer period of time compared to the water sensitivity test (28 days vs 2 days). Furthermore, nail plates rapidly absorb water when exposed to it, and the HDPE substrate used for the water immersion test is known for its low water absorption. The use of HDPE sheet as a nail substitute may therefore limit how well the water sensitivity test predicts *in vivo* nail residence of ungual formulations. The incorporation of

amorolfine HCl or terbinafine HCl in the gel formulation did not significantly affect the resulting films' fingernail residence ($p>0.05$), possibly due to the very low drug-load.

Fig. 3.24 also shows that UV-cured polymers resides on fingernails for significantly longer than Curanail® ($p<0.05$). This could be explained by the more durable nature of the DUDMA backbone in the gel formulations in comparison to Curanail's polymer backbone, i.e. ammonio methacrylate copolymer A, and explains Curanail's 1 – 2 times weekly application regimen as detailed in the British National Formulary (BNF) 71 (March 2016). This result highlights durability as an advantage for considering a UV-curable gel over a lacquer as a nail drug delivery vehicle. In fact by day 14, greater than 50% of the films produced from the drug-loaded UV-curable gel still remains on the fingernail plate, indicating a possible fortnightly application regimen for fingernails. The residence profiles of films on the feet are generally longer than on hands (Murdan et al., 2015), due to the feet's lower exposure to abrasion during daily activities and lower washing frequency when compared to hands, therefore the % of film remaining on the toenail is expected to be much higher than that for fingernails on day 14.

As previously mentioned, commercially available cosmetic UV gels can be worn for up to 3 weeks without developing any visible defects (Schoon, 2010, Jefferson and Rich, 2012, Schoon and Baran, 2012). Fig. 3.24 reveals that this is not the case for the UV-curable gel formulations developed. The reason behind this is that prior to gel application, the nail is lightly filed with a low git abrasive file (180 – 240 grit) to increase the surface area, wiped with a nail wipe coated with isopropyl alcohol to remove surface moisture and residual oils, and coated with a layer of primer (containing mixtures of hydroxylated monomers or oligomers) to aid adhesion by increasing the surface compatibility between the natural nail and product (Schoon, 2010, Schoon and Baran, 2012). Nevertheless as the durability of the pharmaceutical UV-curable gel formulations was still long, it was considered acceptable. It was assumed that the findings would be applicable to formulations containing IBOMA and HEMA due to their identical occlusivity, adhesivity and water resistance. The next section looks at how the UV-cured films' mass changes over 28 days and whether the drug in the film is stable during this time period.
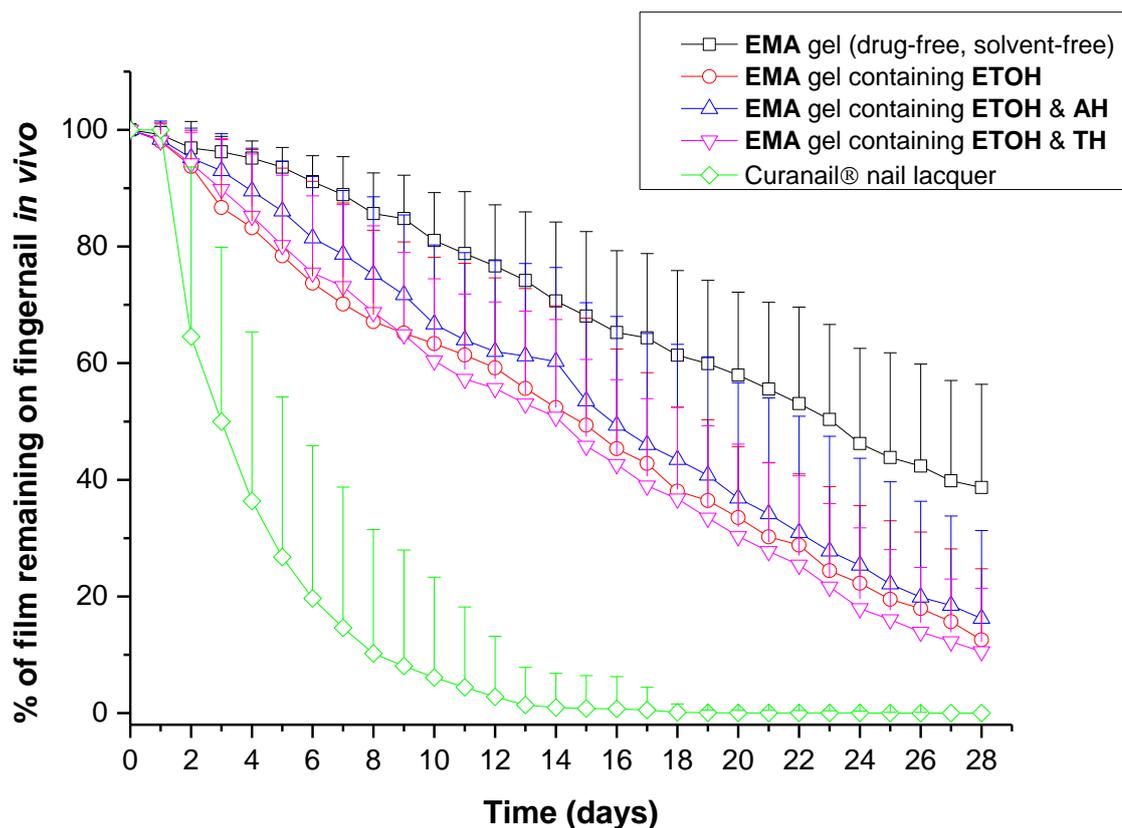
Fig. 3.24 *In vivo* residence profile of UV-cured films (± solvent and ± drug) and a commercially available nail lacquer on the ten fingernails in six volunteers. Means and standard deviations are shown, n=60. Abbreviations: AH, amorolfine HCl; TH, terbinafine HCl; ETOH, ethanol.

3.5.8 Stability of UV-cured polymer films - mass change of film with time and drug stability in film

3.5.8.1 Mass change

The UV-cured films were stored at 30°C ± 2°C and 50% RH ± 5% RH over the 28 days, and Fig. 3.25 – Fig. 3.27 follows the mass change of the films produced from gels containing DUDMA & EMA, IBOMA or HEMA (± solvent and ± drug) with time. It can be seen that while the films produced from solvent-free gel formulations display almost no mass change, the films produced from solvent-containing gel formulations lose approximately 3% of their mass, with no differences seen amongst formulations containing EMA, IBOMA or HEMA ($p > 0.05$). This mass loss is thought to be the loss of the ethanol that remains in the film following a UV-cure. The mass loss is rapid over the first three days, most probably due to the evaporation of ethanol trapped closest to the film's surface. After three days, there is a gradual mass loss probably as the remaining ethanol is

trapped deep within the film. The TGA curves of the films produced from the ethanol-containing gel formulations shown in Fig 3.12 suggest that approximately 40% of the ethanol incorporated in the gels remains in the film. This indicates that after 28 days, there is likely to be ethanol still trapped in the films. The presence of either amorolfine HCl or terbinafine HCl in the gel formulation does not significantly influence this mass loss either ($p > 0.05$), possibly as the drug-load in the film is too low to influence its properties significantly.
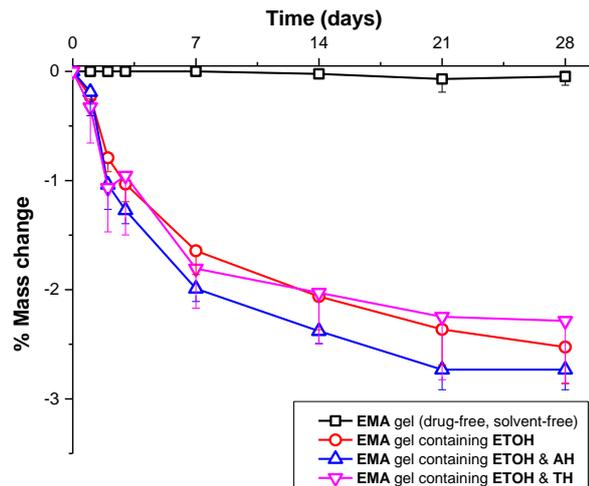
Fig. 3.25 % Mass change of UV-cured films produced from DUDMA & EMA gels (± solvent and ± drug). Means and standard deviations are shown, n=3.
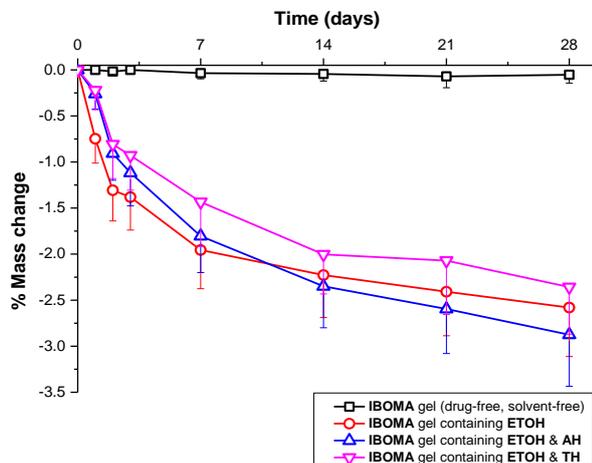
Fig. 3.26 % Mass change of UV-cured films produced from DUDMA & IBOMA gels (± solvent and ± drug). Means and standard deviations are shown, n=3.
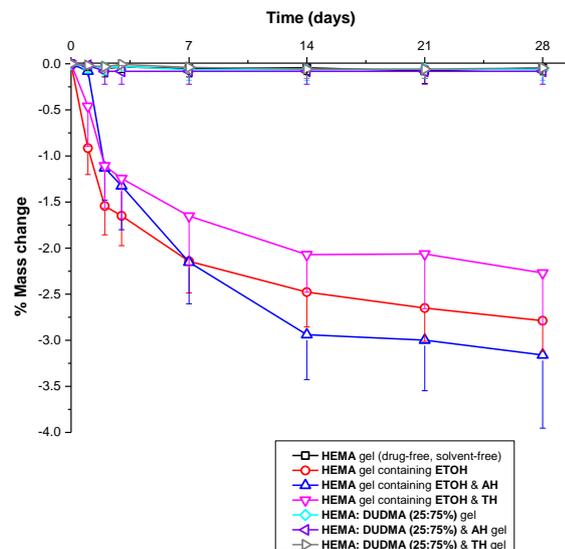
Fig. 3.27 % Mass change of UV-cured films produced from DUDMA & HEMA gels (± solvent and ± drug). Means and standard deviations are shown, n=3.

3.5.8.2 Drug stability in film

The stored UV-cured films were also assessed for any changes in their appearance and drug concentration over time. Over the 28 day period, the state of the drug in the film changed, with drug crystals being visible from week 3 (Table 3.11). This drug precipitation in the films could be a direct response to the loss of residual ethanol from the polymer film with time, as shown in Figs 3.25 – 3.27. However, the drug did not show signs of degradation with time as the films showed no significant changes in drug concentration ($p>0.05$) (Figs 3.28 – 3.29). The UV-curable gel formulations were therefore considered to be stable over the time period that they would likely reside on the nail (2 weeks) before reapplication, and are therefore suitable for further consideration.

Table 3.11 Polarised light microscopy images of drug-loaded (amorolfine HCl or terbinafine HCl) UV-cured polymer films with time.

Formulation	PLM of polymer films with time				
	Day 0	Day 7	Day 14	Day 21	Day 28
DUDMA & EMA gel containing ethanol and 3% w/w AH					
DUDMA & IBOMA gel containing ethanol and 3% w/w AH					
DUDMA & HEMA gel containing ethanol and 4% w/w AH					
DUDMA & HEMA gel containing 2% w/w AH					
DUDMA & EMA gel containing Ethanol and 4% w/w TH					
DUDMA & IBOMA gel containing ethanol and 4% w/w TH					
DUDMA & HEMA gel containing ethanol and 6% w/w TH					
DUDMA & HEMA gel containing 2% w/w TH					
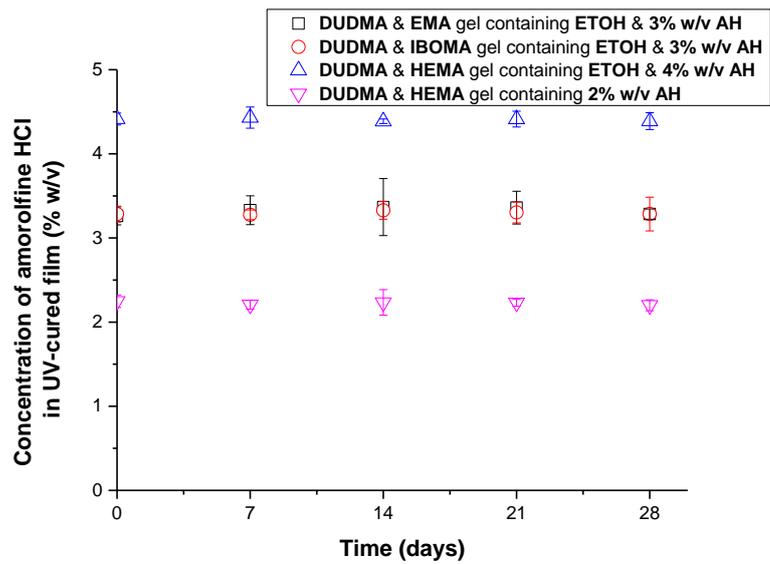
Fig. 3.28 Stability of amorolfine HCl in UV-curable films over time. Means ± standard deviations are shown, n=3. Abbreviations: AH, amorolfine HCl; ETOH, ethanol.
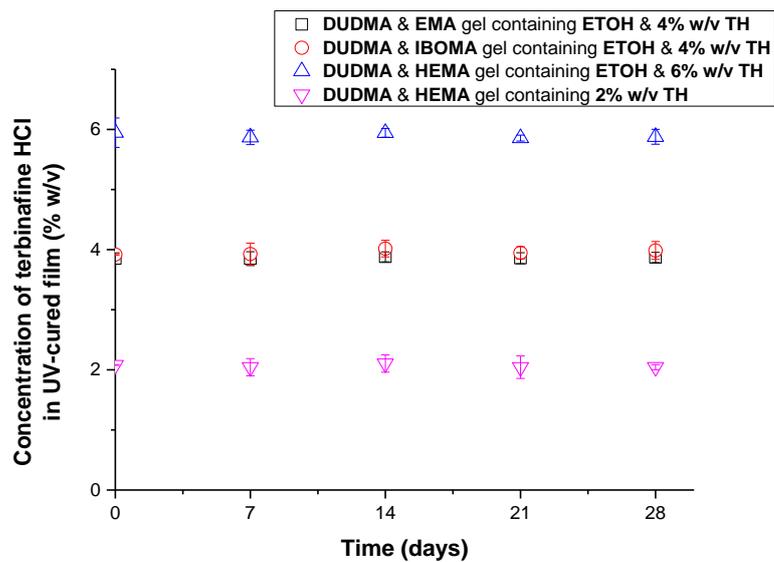
Fig. 3.29 Stability of terbinafine HCl in UV-curable films over time. Means ± standard deviations are shown, n=3. Abbreviations: TH, terbinafine HCl; ETOH, ethanol.

3.6 Conclusions

The DUMDA and EMA, IBOMA or HEMA UV-curable gels formulated (± drug and ± solvent) produced films which were visually smooth, transparent and thin (with an excellent uniformity of thickness).

These films were highly cross-linked as indicated by their low swelling in toluene, and the extent of cross-linking was related to the films' degree of conversion from monomer gel to polymer film. The film's interior was dense and did not vary with the choice of diluent monomer or presence of ethanol and drug in the gel; however, the addition of the solvent NMP or the use of a higher diluent monomer concentration in the gel reduced this compact microstructure.

The concentration of drug that could be incorporated into the gel, as determined in Chapter 2, was the maximum drug-load, and upon UV-curing, the drug remained in the dissolved state and did not appear to interact with the polymer. The solvents incorporated in the UV-curable gels resided in the cured film, although the amount of ethanol residing was much less than NMP due to ethanol's volatile nature.

The films produced from solvent-free UV-cured films displayed two glass transitions, with the higher glass transition a possible consequence of a thermal reaction to complete a previous "incomplete" cure. Films produced from formulations containing a solvent showed one glass transition which was in the range of $95 - 140^{\circ}C$, and which was influenced by the choice of diluent methacrylate monomer in the formulation and solvent, but not the presence of drug.

All UV-cured films, with the exception of those containing NMP, showed good adhesivity and water resistance *in vitro*. *In vivo*, the films also reduced TOWL and showed fairly long residence times (during which period the drug remained stable in the film). The adhesivity, TOWL reduction and nail residence displayed by UV-curable gels were greater than that displayed by a pharmaceutical nail lacquer, and thus, a UV-curable gel appears to be a promising drug delivery vehicle.

Chapter 4: UV-cured film's drug release, ungual drug permeation and efficacy against *Trichophyton rubrum*

4.1 Introduction

Drug release from an acrylic-based resin is feasible, via mechanisms such as (i) diffusion through the acrylic resin, and/or (ii) surface release combined with drug diffusion via cracks and channels in the resin formed by the incorporation of drug (Brook and van Noort, 1985). Given that drug release from a topical nail medicine is an essential step prior to drug permeation into and through the nail plate, the different UV-cured polymer films' drug release profiles are determined in this Chapter. Furthermore, the influence of the film's properties such as its structure, drug-load, glass transition and water sensitivity (as determined in previous Chapters) on drug release is also determined to understand the drug release mechanism from UV-cured films.

In addition to demonstrating drug release from the UV-cured film, the evaluation of ungual and trans-ungual drug penetration is fundamental, as the success of the UV-curable gel formulation as a topical nail medicine ultimately depends on its ability to deliver the drug into and across the nail plate. Such an evaluation typically involves the use of diffusion cells, (Franz or modified Franz diffusion cells), which consist of a donor compartment, receptor compartment, and a nail plate in between the two chambers. The donor compartment holds the drug-loaded formulation while the receptor compartment is generally filled with a suitable solvent system to provide sink conditions, i.e. where the concentration of the dissolved drug remains less than 10% of its saturation solubility (Kenneth et al., 1981, Kobayashi et al., 1998, Kierstan et al., 2001, Malhotra and Zatz, 2002a, Donnelly et al., 2005, Gunt and Kasting, 2007, Hui et al., 2002). Due to the difficulty in obtaining intact human nail for such studies, several *in vitro* models such as hoof membranes (cow, pig, sheep and horse) (Mertin and Lippold, 1997a, Mertin and Lippold, 1997b, Mertin and Lippold, 1997c, Monti et al., 2005, Monti et al., 2010, Pittrof et al., 1992, Kim et al., 2001, Yang et al., 1997, Khengar et al., 2007), keratin films (Lusiana et al., 2011), excised cadaver nail plates (Malhotra and Zatz, 2002a, Malhotra and Zatz, 2002b, Gunt et al., 2007, Gunt and Kasting, 2007, Nair et al., 2011) and nail clippings (Chouhan and Saini, 2014, Hossin, 2015) have been used. While

human cadaver nail presents the closest simulation to human nail, nail clippings obtained from volunteers can be considered an easily accessible and inexpensive alternative. In this Chapter, the ungual drug permeation profiles of the different UV-cured polymer films are determined using human nail clippings and modified Franz diffusion cells designed in-house, and the relationship between drug release from the film and drug permeation into and through the nail is explored.

While the *in vitro* ungual drug permeation study can provide insight into the feasibility of *in vivo* drug delivery and can predict whether the amount of drug delivered will be at therapeutic concentration against the fungal pathogen in question, a test to confirm the antifungal efficacy of the formulation adds value to the findings. Experimental models for evaluating the efficacy of formulations are available (Nair et al., 2013). An example is the TurChub® model, which uses a modified Franz cell and where sections of human nail are placed over an agar-filled receptor chamber (previously inoculated with dermatophytes). In this model, the test formulation is placed over the nail plate and incubated for a set duration at a set temperature. Subsequently, the extent of drug permeation is approximated by measuring the zone of fungal growth inhibition in the agar medium in the receptor compartment. Despite the availability of such experimental models, there is currently no standard *in vitro* method to assess the anti-fungal efficacy of anti-onychomycotic formulations. Therefore in this Chapter, the disc diffusion (Kirby-Bauer) method of antibiotic susceptibility testing is adapted and investigated as a method to test and compare the efficacies of the different antifungal-loaded UV-cured polymer films (when applied on human nail clippings) against *T. rubrum*, which as mentioned in Chapter 1, is the single most common fungus to cause onychomycosis (Sigurgeirsson and Baran, 2014). The UV-cured polymer film's *in vitro* efficacy against *T. rubrum*, alongside its drug release and ungual drug permeation profiles, is also compared against a commercially available pharmaceutical nail lacquer – Curanail® – to further assess the UV gel's potential.

4.2 Aims

Determine the UV-cured films':

(i) drug release

(ii) ungual drug permeation

(iii) *in vitro* antifungal efficacy against *T. rubrum*

and the influence of drug and monomer compositions on the above properties.

4.3 Materials

The monomers, photoinitiator, antifungal agents, solvents, acids, UVA nail lamp and nail wipes were obtained as detailed in Chapter 2 (Section 2.3). Curanail® (5% w/v amorolfine hydrochloride) nail lacquer was purchased as detailed in Chapter 3 (Section 3.3). Sodium phosphate monobasic dihydrate, sodium phosphate dibasic heptahydrate and a dialysis tubing cellulose membrane (MW 10281) were purchased from Sigma–Aldrich (Dorset, UK). Human nail clippings (fingernails) were obtained from healthy volunteers aged between 18 and 65 years. Sabouraud dextrose agar was purchased from Oxoid Ltd (Basingstoke, UK).

4.4 Isolate

One dermatophyte strain, *Trichophyton rubrum* (CBS 118892), which was obtained from Birkbeck, University of London was used.

4.5 Methods

The UV-cured films produced from formulations containing DUDMA and a diluent reactive monomer (EMA, IBOMA or HEMA) at a ratio of 85:15% v/v (with ethanol and with and without amorolfine HCl or terbinafine HCl), as well as the formulations containing DUDMA and HEMA at a ratio of 75:25% v/v (with and without amorolfine HCl or terbinafine HCl) were prepared as described in earlier Chapters (Chapter 3, Section 3.4 and Chapter 2, Section 2.4.4). These films' drug release profiles, ungual drug permeation profiles and efficacies against *T. rubrum* were determined as detailed below. Unless otherwise stated, each test was conducted in triplicate.

4.5.1 Drug release from UV-cured polymer films

The drug release experiments were carried out using Franz diffusion cells. The receptor fluid was a 0.1 M pH 5 phosphate buffer solution, as both amorolfine HCl and terbinafine HCl are stable at this pH (Hossin, 2015). Furthermore, the saturation solubilities of amorolfine HCl and terbinafine HCl in the pH 5 phosphate buffer solution are 9.27 ± 0.67 mg/ml and 7.91 ± 0.67 mg/ml respectively, and its use ensures that sink conditions were maintained throughout the duration of the study. Finally, it should be noted that both amorolfine HCl (pKa 6.6) and terbinafine HCl (pKa 7.1) are weak acids, and both are expected to be unionised in an aqueous environment at pH 5. For these experiments, the film produced from the Curanail® nail lacquer was used as a control.

To set up the Franz cell, a UV-cured film (circular with an area of 1.8 cm^2) was placed between the donor and receptor compartments of a Franz diffusion cell, with the bottom surface facing the receptor side, and the compartments were clamped together. For the Curanail® nail lacquer, a semi-permeable membrane was needed to support the film. A cellulose membrane was therefore cut into a circle (with an area of 4.909 cm^2) and the lacquer was applied onto the surface covering a circular area of 0.9503 cm^2. This was allowed to dry and the cellulose support with the air-dried Curanail film was then placed between the donor and receptor compartments of a Franz diffusion cell, with the test films facing the donor side, and the compartments were clamped together. Upon the assembly of the Franz diffusion cell, 4 ml of receptor fluid was added to the receptor compartment while ensuring that no air bubbles were introduced. A schematic of this set up can be seen in Fig. 4.1. The diffusion cells were immediately left to stir on a magnetic stirrer placed in a water bath maintained at 32°C. Samples of the receptor fluid were collected at pre-determined time intervals (i.e. every 20 minutes for the first 1 hour, then every 30 minutes for the second hour, then every hour for the following four hours, then daily till day 12 and finally every three days till day 30). At each sampling time, 0.5 ml of the receptor phase was collected via the receptor arm and replaced with 0.5 ml of fresh buffer. The samples were analysed by HPLC (as per Section 2.4.6 [Chapter 2]) to determine the amount of drug released, and the cumulative % drug release over time was plotted.
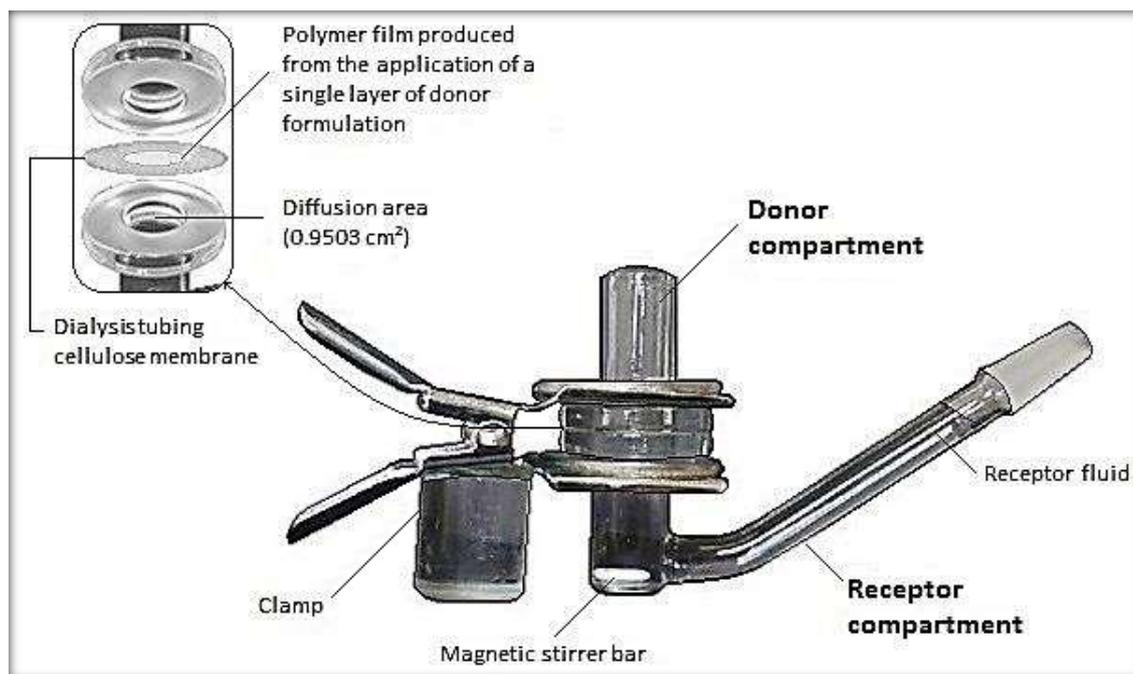
Fig. 4.1 Franz diffusion cell set up for release studies. The cellulose membrane was only used to support the Curanail® film. UV-cured films did not need any support.

4.5.1.1 Mass & polarised light microscopy examination of polymer films

All the films were weighed and observed by polarised light microscopy (as per Section 3.4.5.1 [Chapter 3]) prior to the release study and at day 30 (end of release study) to determine whether there was any film swelling or drug crystallisation during the release study.

4.5.1.2 Mathematical modelling of drug release

In order to describe drug release kinetics, three commonly used mathematical models for the characterisation of release from thin films were considered:

(i) **Zero order:**

Where drug release takes place at a constant rate, independent of its concentration. Modelling of the drugs' release using zero-order kinetics was carried out by plotting cumulative amount of drug released over time, and then calculating the linear regression coefficient value (R^2). The data was considered to fit a zero order profile if the R^2 value was equal to 1.

(ii) **First order:**

Where the drug release rate is dependent on drug concentration. Modelling of the drugs' release using first order kinetics was carried out by plotting log (cumulative percentage of drug remaining) over time. The data was considered to fit first order kinetics if the R^2 value was equal to 1.

(iii) **Higuchi model:**

Where the release rate through the film matrix is diffusion-controlled. The release profile of the drug from the polymer film was considered to fit this model if plotting cumulative % drug release against $\sqrt{time}$ yielded a R^2 value equal to 1.

4.5.2 Determination of ungual drug permeation

The ungual drug permeation experiments were carried out using modified Franz diffusion cells, as depicted in Fig. 4.2, and with the Curanail® nail lacquer used as a control. Human fingernail clippings were used as the nail model as opposed to toenails, as volunteers were more willing to grow and provide fingernails longer than 3 mm than their toenails. These fingernail clippings were washed with water and soaked in distilled water for one hour prior to use. They were then cut to size (circular, with a diameter of 0.3 cm) and measured for thickness using a micrometre. A layer of the formulation (using 2 µl) was applied on the nail surface with a pipette tip (while ensuring that a 0.2 mm formulation-free perimeter was left near the edge of the nail surface). In the case of Curanail®, the nail lacquer was applied and allowed to dry. For the UV-curable gel formulations, the formulation was applied on the nail surface and cured under an UVA lamp for 2 minutes. The surface of the UV-cured film was then wiped with propan-2-ol using a nail wipe. The nail was placed in the donor compartment and fixed into place, such that when assembled with the receptor compartment, the under-surface of the nail alone was exposed to the receptor fluid. The area exposed was 0.025 cm^2. Subsequently, 900 µl of receptor fluid was added to the receptor compartment. The receptor fluid was a 0.1 M pH 5 phosphate buffer solution. The diffusion cell was assembled, the receptor arm opening was covered with parafilm, and left to stir on a magnetic stirrer placed in a water bath at 32°C. Samples of the receptor fluid were collected every three days, up to

day 30, by taking 50 µl of receptor fluid via the receptor arm and replacing it with 50 µl of fresh buffer. The receptor fluid samples were analysed by HPLC (as per Section 2.4.6 [Chapter 2]) to determine the amount of drug permeated across the nail over time. Each experiment was repeated six times.
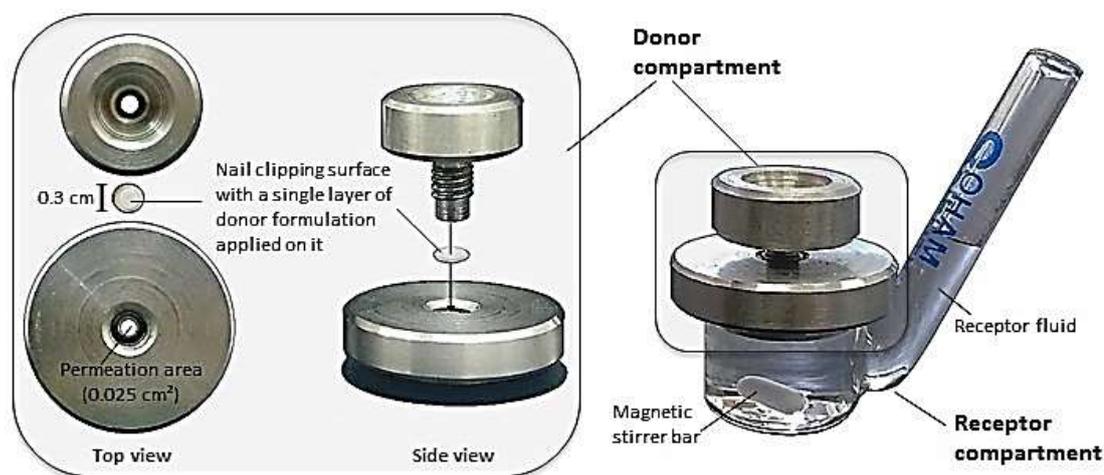
Fig. 4.2 Modified Franz diffusion cell used for permeation studies. The donor compartment was made from steel and the receptor compartment from glass.

4.5.2.1 Extraction of drug from nail following the permeation study

At the end of the permeation study, the nail clipping was removed from the donor compartment using forceps. The film on the nail surface was carefully lifted off the nail using a scalpel and placed in a vial containing 1 ml of ethanol. The mixture was ultrasonicated for 2 hours using an Elma Transsonic T460/H sonicator (Singen, Germany) and the liquid was analysed by HPLC to quantify the amount of drug remaining in the donor film. The nail plate was rinsed with distilled water and blotted dry with Kimwipes, before placing in a vial containing 1 ml of ethanol. This was also ultrasonicated for 2 hours and the liquid was analysed with HPLC while the nail clipping retrieved was placed in another vial containing 1 ml of ethanol for a further two hour sonication. This extraction procedure was repeated until no further drug could be extracted from the nail. The total amount of drug extracted from the nail was then calculated. Mass balance was carried out to ensure that most of the drug has been retrieved from the modified Franz-diffusion cell and that most of the drug remaining within the nail tissue had been extracted (Appendix A9).

4.5.2.2 Calculating the steady-state flux, permeability coefficient, lag time & diffusion coefficient

The steady-state flux (J) was calculated from the slope of the linear portion of the plot of the cumulative amount of antifungal drug permeated across the nail ($\mu g/cm^2$) (Q) against time (t):

$$J = \Delta Q/\Delta t$$

The apparent permeability coefficient (P) which is defined as the flux divided by the concentration of the permeant in the donor compartment (C_d) was calculated using:

$$P = J/C_d$$

The transport lag time (t_L) was estimated by extrapolating the linear portion of the Q versus t plot to the x-axis, (i.e. the x-intercept), and the effective diffusion coefficient (D_{eff}) was obtained from the transport lag time by the relationship:

$$t_L = h^2/6D_{eff}$$

where h represents the thickness of the nail plate.

4.5.3 Determination of the antifungal efficacy of UV-curable films against *T. rubrum*

The disc diffusion method of antibiotic susceptibility testing was adapted by replacing the 'disc' with a circular piece of nail clipping onto which the formulation was applied, such that the drug would have to be permeate into and through the nail plate to reach the agar gel.

4.5.3.1 Preparation of media

Sixty five grams of Sabouraud Dextrose Agar (SDA) was dissolved in 1000 ml of distilled water and sterilised by autoclaving at 121^0C for 15 minutes. Subsequently approximately 25 ml of agar was poured into 90 mm petri dishes and allowed to solidify overnight.

4.5.3.2 Preparation of test plates

A culture plate of *T. rubrum* was used to inoculate the test plates. Firstly, a blank SDA plate was labelled with the name of the organism and the date. A cork borer with an internal diameter of 8 mm was then dipped in ethanol, flamed to sterility, and punched

into the centre of the SDA plate to punch out a plug of agar. This agar plug was discarded. The cork borer was dipped once again in ethanol, flamed to sterility, and used to punch a *T. rubrum* plug from the culture plate. This *T. rubrum* plug was inserted into the slot created from the removal of the agar plug in the blank SDA plate. Twenty five SDA plates were set up in this way and incubated at 32°C for three days to ensure that the fungal culture was able to grow sufficiently and to identify defective plates. During this period, the *T. rubrum* culture grew to 10 mm in diameter.

4.5.3.3 Preparation of nails

Human nail clippings (fingernails) were washed with water prior to use. They were then cut to size (circular, with a diameter of 3 mm) and autoclaved at 120^{0}C for 20 minutes in order to sterilise them. This was possible as it was found that the process of autoclaving did not affect the solvent (water & ethyl acetate) uptake properties of the nail clippings (Appendix A10). The formulations tested were:

(i) Curanail® nail lacquer (containing 5% w/v amorolfine HCl)

(ii) DUDMA and EMA gel containing ethanol and 3% w/v amorolfine HCl

(iii) DUDMA and IBOMA gel containing ethanol and 3% w/v amorolfine HCl

(iv) DUDMA and HEMA gel containing ethanol and 4% w/v amorolfine HCl

(v) DUDMA and HEMA gel containing 2% w/v amorolfine HCl

(vi) DUDMA and HEMA gel containing ethanol and 6% w/v terbinafine HCl.

The drug-free counterparts of the above formulations (except for the Curanail® nail lacquer) were also tested to examine the influence, in any, of the UV-cured polymer vehicle against *T. rubrum.*

To prepare the nail clippings for placement on the SDA plates containing the fungal plug, a layer of the formulation (using 2 µl) was applied on the nail surface in the same manner as that for the ungual drug permeation studies described above in Section 4.5.2 (while ensuring that a 0.2 mm formulation-free perimeter was left near the edge of the nail surface).

4.5.3.4 Testing of formulations

In order to test the UV-curable gel formulations' efficacy against *T. rubrum*, the SDA plates containing the fungal plugs were set up in one of three ways on day 3 as depicted in Fig 4.3.

The first set up **(A)** was a **formulation-free negative control** to monitor the growth of *T. rubrum* under the same experimental conditions and compare it with the plates containing test formulations (a total of two plates were set up in this way).

The second set up **(B)** was a control containing nail clippings with **drug-free polymer films** cured on the surface. The plates were set up by firstly labelling it with the formulation to be tested, marking it into three segments, and marking a distance of 20 – 25 mm from the centre of the plate in the three segments for the nail clippings to be placed. This distance was chosen to allow sufficient time for the permeant to penetrate into and through the nail, a judgement based on the results obtained from the ungual drug permeation studies (Section 4.6.2) and *T. rubrum* growth rates from preliminary studies (Appendix A11). A sterile tweezer was used to pick up the prepared nail clippings, which were carefully placed onto the agar surface (with the under surface of the nail in contact with the agar). Each plate contained a triplicate set of the same formulation.

The third set up **(C)** contained the samples (i.e. three nail clippings with the **drug-loaded polymer films** cured on the surface) and one nail clipping with the corresponding drug-free polymer film cured on the surface. To set these plates up, the plates were labelled with the formulation to be tested and marked into four segments, one for the drug-free formulation (control) and the remaining three for a triplicate set of the same drug-loaded formulation. The nail clippings were placed onto the agar surface as described above.

These plates were incubated at 32°C for as long as it took the *T. rubrum* culture to grow over the entire SDA plate's surface (or when no further growth was detected). During this period, the plates were observed daily to monitor the growth of *T. rubrum* and to monitor the formation of a zone of inhibition, if any, around the samples.

Day 0

Trichophyton rubrum
plug (8 mm diameter)

Day 3
(T. rubrum culture approximately 10 mm in diameter)

Nail clipping (3 mm diameter) with a single layer of formulation (2 µl) applied on and therefore polymerised on the surface. A small formulation–free perimeter was left near the edge of the surface. The nail was placed on the agar at a distance of 20 – 25 mm from the centre.

(A) Control 1 – formulation-free

(B) Control 2 – Nail clippings with a drug-free formulation applied on the surface (n=3)

(C) Samples – Nail clippings with a drug-loaded formulation applied on the surface (n=3) and a nail clipping with the control drug-free formulation applied on the surface

Fig. 4.3 Photographic images of *T. rubrum* inoculated test plates set up on day 0 and day 3. The formulation in this case is the DUDMA & EMA gel formulations containing ethanol (with and without 3% w/v amorolfine HCl). Abbreviations: AH, amorolfine HCl; C, control; ETOH, ethanol.

4.5.4 Statistical analyses

Statistical calculations were conducted using IBM SPSS 22. The Student's t-test was carried out for testing differences, if any, between two data sets and the one-way ANOVA followed by post hoc Tukey were carried out for all statistical analyses involving more than two data sets. Repeated measures ANOVA was conducted to determine whether there were differences in drug release profiles and ungual drug permeation profiles of the formulations tested over the experimental duration.

4.6 Results and discussion

4.6.1 Drug release from UV-cured polymer films and from the control Curanail®

The drug release profiles of the amorolfine HCl–loaded and terbinafine HCl–loaded films are shown in Fig. 4.4 and Fig. 4.5 respectively. The polarised light microscopy images of the films pre- and post-release and the amount of drug released by day 30 are also shown alongside the drug release profiles.

4.6.1.1 Drug release profile of Curanail®

From Curanail's drug release profile (Fig. 4.4), a burst of drug release from the film over the first 24 hours is evident due to the release of drug found at the film surface. After this, the film gradually releases almost all of the incorporated drug. The polarised light micrograph of the Curanail® film pre-release shows that it contained precipitated amorolfine HCl crystals, (most probably as a result of its high drug-load of 31.5 ± 0.8% w/w following solvent evaporation and film formation), and it appears that these may have gradually dissolved and released, as no crystals were seen in the "tacky" film remains at the end of the study. The fact that an intact film was not retrieved at the end of the release study suggests that Curanail's polymer backbone, (i.e. ammonio methacrylate copolymer A), may have gradually swelled and partially disintegrated during the 30 day study, thereby enabling the gradual and almost complete drug release observed.

4.6.1.2 Drug release profiles of amorolfine HCl–loaded UV-cured polymer films

The amorolfine HCl–loaded UV-cured films' release profiles (Fig. 4.4) also displayed a burst of drug release over the first 24 hours due to the release of drug found at the surface of the film. However, after this point, the drug release slowed and eventually plateaued. Only part of the incorporated drug (42 – 48 %) had been released by the end of the study, with no significant differences amongst the different UV-curable gel formulations ($p > 0.05$). Visually, the films remained intact after the release study, and the negligible weight change of the films during the release experiment (Fig. 4.6) confirmed the absence of film swelling and dissolution. In addition, polarised light micrographs of the films (pre- and post-release) showed no obvious changes, i.e. any signs of drug precipitation following the release study (Fig. 4.4). This enabled the evaluation of

amorolfine HCl's release kinetics from the UV-cured films using zero order, first order and Higuchi models. From Table 4.1, it can be seen that the Higuchi model best describes amorolfine HCl release from the four amorolfine HCl-loaded UV-cured films, as it yielded the highest R^2 value. Therefore it appears that the drug released through the film matrix following the initial burst phase is diffusion-controlled. However, this release was minimal and eventually halted most probably due to the films' highly cross-linked nature and dense interior (Chapter 3, Section 3.5.1.2), both of which can limit the diffusion of drug molecules through the film.

4.6.1.3 Drug release profiles of terbinafine HCl–loaded UV-cured polymer films

In a similar manner to the amorolfine HCl–loaded UV-cured polymer films, the terbinafine HCl–loaded films release profiles (Fig. 4.5) showed a burst phase over the first 24 hours, which then slowed and eventually plateaued. Once again, no significant differences were seen amongst the different gel formulations ($p>0.05$). However, the percentage of drug released by the end of the study from all four formulations was significantly lower ($p<0.05$) than the same vehicle containing amorolfine HCl. Like the amorolfine HCl–loaded films, the terbinafine HCl–loaded films remained intact, showed negligible weight change (Fig. 4.6) and showed no signs of drug precipitation post-release (Fig. 4.5), which did not offer any positive clues to explain the lower terbinafine HCl release. Table 4.1 reveals that the Higuchi model best describes terbinafine HCl release from all four UV-cured films, and therefore it appears that the drug released through the film matrix following the initial burst release is also diffusion-controlled and eventually limited by the films cross-linked nature and dense film interior. Both amorolfine HCl and terbinafine HCl have similar molecular weights (353.98 and 327.90 Da respectively), similar logP values (5.8 and 5.3 respectively), and the DUDMA & EMA, IBOMA or HEMA copolymer film's structure (Chapter 3, Section 3.5.1.2), glass transition temperature (Chapter 3, Table 3.10) and water sensitivity (Chapter 3, Fig. 3.23) was unaffected by the incorporation of amorolfine HCl or terbinafine HCl; hence these drug and film characteristics do not explain why terbinafine release is almost half that of amorolfine. A low drug release could occur if terbinafine was incorporated in the polymer structure upon UV-curing. Terbinafine does have the potential to take part in the polymerisation process due to its alkene group as mentioned in the previous

Chapter (Section 3.5.1.2). However as most of the drug included in the gel mixture could be extracted from the UV-cured film (just as amorolfine HCl could be) (Chapter 3, Table 3.8), this is an unlikely occurrence. It is possible that terbinafine may have therefore bound to the polymer films, which would retard its release.

While FT-IR revealed the absence of drug-polymer interactions (Chapter 3, Table 3.9), an alternative experiment to investigate terbinafine binding or not would shed light on whether terbinafine does have a higher affinity for the UV-cured film compared to amorolfine. Therefore for future work, the affinity of the two antifungal drugs to the UV-cured films could be tested by adapting the experimental procedure used for determining the affinities of these drugs to keratin (Tatsumi et al., 2002, Sugiura et al., 2014). In this instance a drug-free UV-cured polymer film could be immersed in a drug solution and left shaking. Subsequently, at intervals, the drug concentration in this mixture could be determined to calculate the rate of drug–polymer film binding. When no further drug-polymer film binding is evident, the UV-cured polymer film with bound drug could be washed with a solvent and the drug concentration in the solvent could be measured. This washing procedure could be repeated several times using fresh solvent each time to determine the rate of release of drug from the UV-cured film, and confirm if the drug does have a high affinity for the polymer or not.

4.6.1.4 Influence of film components on drug release

The similarities in the drug release profiles of the UV-cured films produced from DUDMA & EMA, IBOMA or HEMA containing gels (with the DUDMA to reactive diluent monomer ratio set at 85:15% v/v) could be due to their similar degree of conversion from monomer gel to polymer film (Chapter 2, Table 2.12), similar extents of cross-linking (Chapter 3, Section 3.5.1.2), comparable microstructures (Chapter 3, Table 3.6) and comparable water sensitivities (Chapter 3, Fig. 3.23). In addition, the film produced from the DUDMA & HEMA gel (with a DUDMA to HEMA ratio of 75:25% v/v) appears to show a slightly higher percentage of drug release compared to the other formulations, possibly because of its lower degree of conversion and hence lower extent of cross-linking. In fact, linear regression analysis revealed a strong correlation between degree of conversion from monomer gel to polymer film and drug release (R^2 = 0.920, $p<0.05$)

for amorolfine HCl and (R^2= 0.938, p<0.05) for terbinafine HCl. On the other hand, the differences in the glass transition temperatures of the different UV-cured polymer films (Chapter 3, Table 3.10) was not found to influence drug release from the films (p>0.05). While a higher glass transition temperature of a film can indicate a more rigid structure and can decrease the rate of drug diffusion (Jenquin et al., 1992), in this instance it appears that the differences in glass transition temperatures did not play a role in drug release.

4.6.1.5 Amount of drug released by day 30

When considering the amount of drug released by the end of the study, it can be seen that the Curanail® film releases the highest amount of drug due to its greatest percentage of drug release and highest initial drug-load. Meanwhile, for the UV-cured polymer films (which contained the maximum amount of drug that could be loaded in the dissolved state in each film), the DUDMA & HEMA gel containing ethanol and 4% w/v amorolfine HCl has the greatest amount of drug released (p<0.05) (due to its higher drug-load) compared to the other gel formulations, which show similar amounts of drug release (p>0.05). Similarly, the DUDMA & HEMA gel containing ethanol and 6% w/v terbinafine HCl has the greatest amount of drug released (p<0.05) (also due to its higher drug-load) compared to the other terbinafine HCl–loaded gel formulations. The drug concentration in the film (which is influenced by the nature of the reactive diluent monomer used and the inclusion of solvent) is therefore an important parameter governing the extent of drug release. Whether the percentage of drug and/or amount of drug released influences the permeation of the drug across the nail plate is explored in the next section.
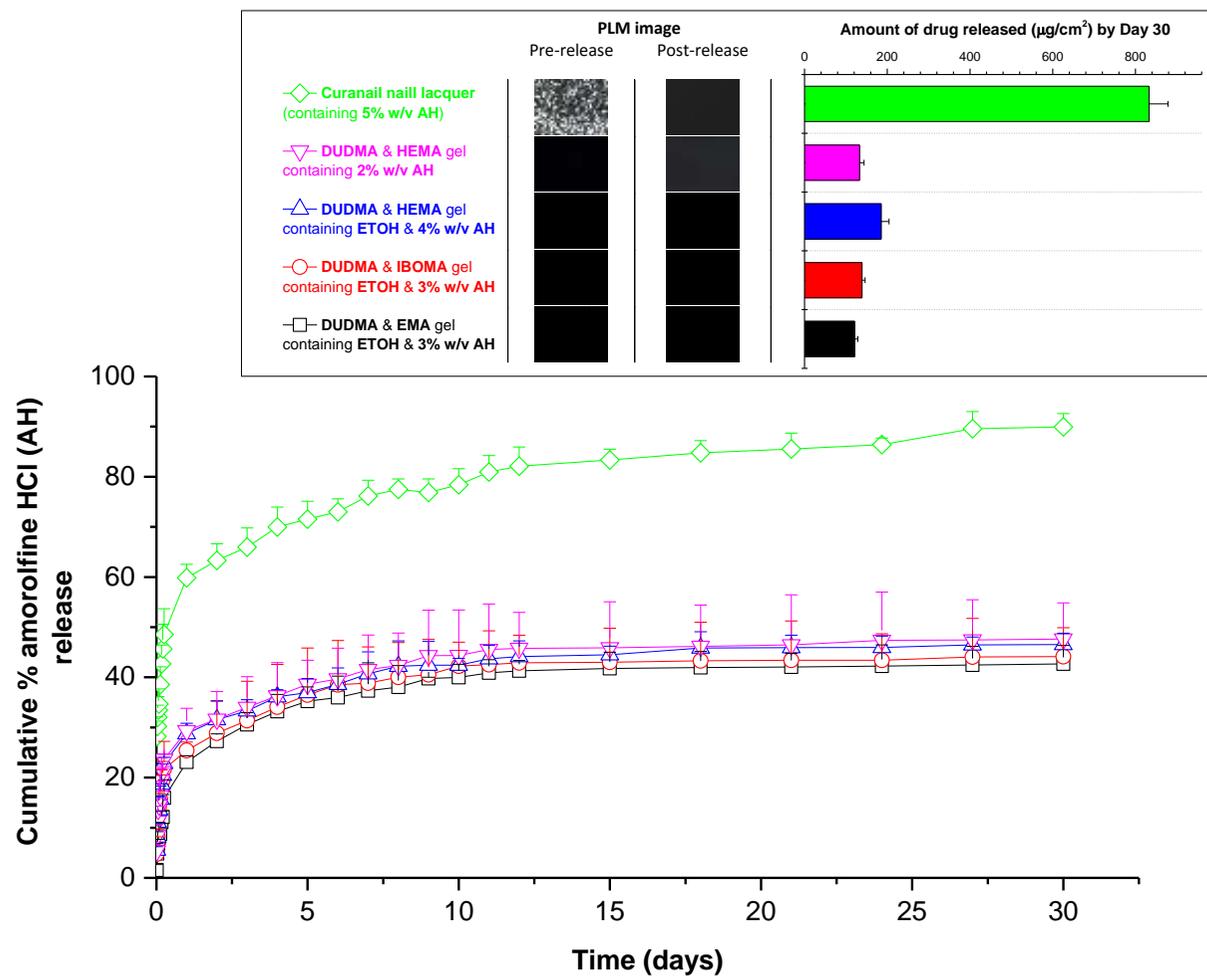
Fig. 4.4 Cumulative % drug release from the amorolfine HCl loaded UV-cured films and Curanail®, their corresponding polarised light microscopy images (pre- and post-release) and the amount of drug released by day 30. Means and standard deviations are shown, n=3. Abbreviations: AH, amorolfine HCl; ETOH, ethanol.
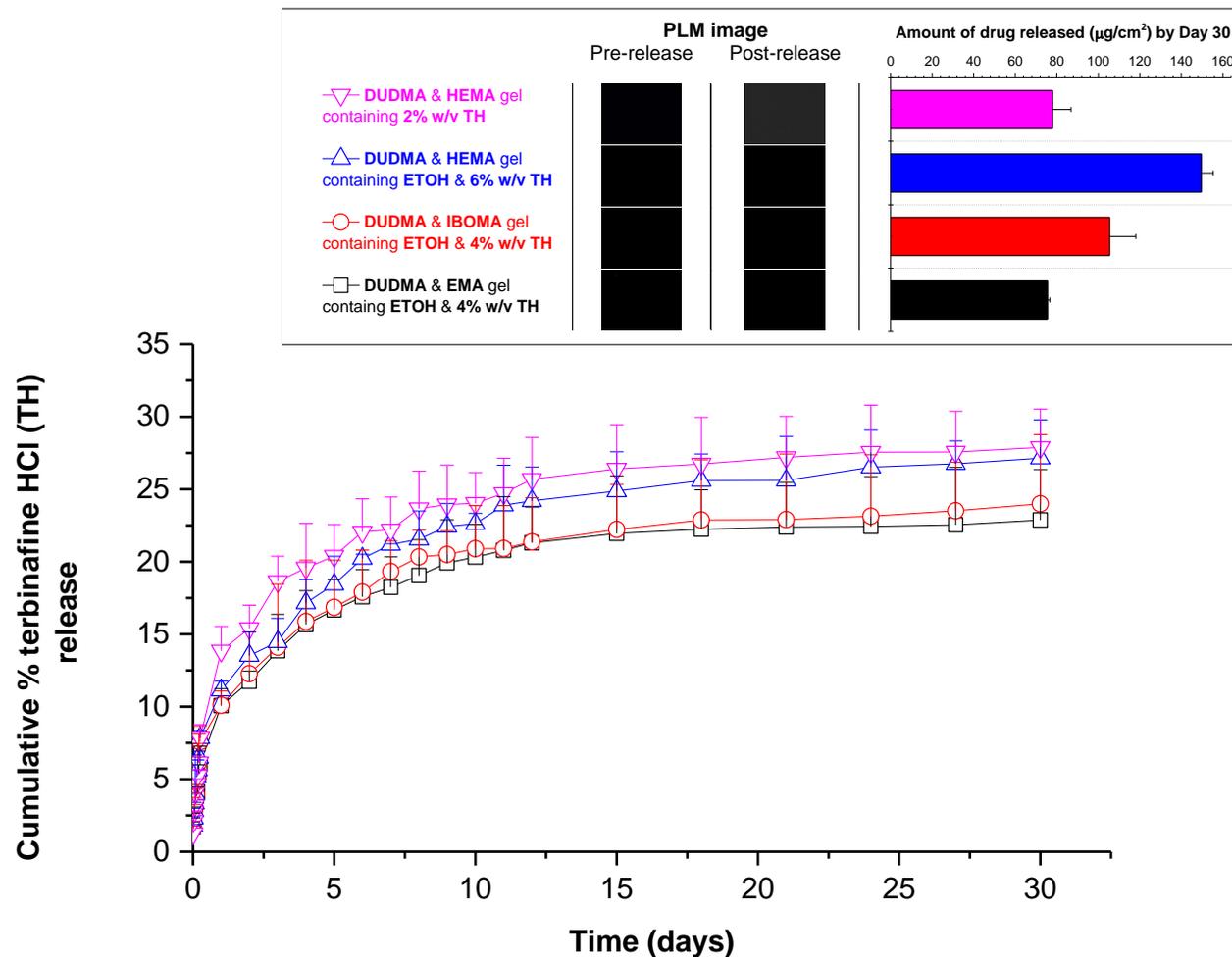
Fig. 4.5 Cumulative % drug release from the terbinafine HCl loaded UV-cured films, their corresponding polarised light microscopy images (pre- and post-release) and the amount of drug released by day 30. Means and standard deviations are shown, n=3. Abbreviations: TH, terbinafine HCl; ETOH, ethanol.
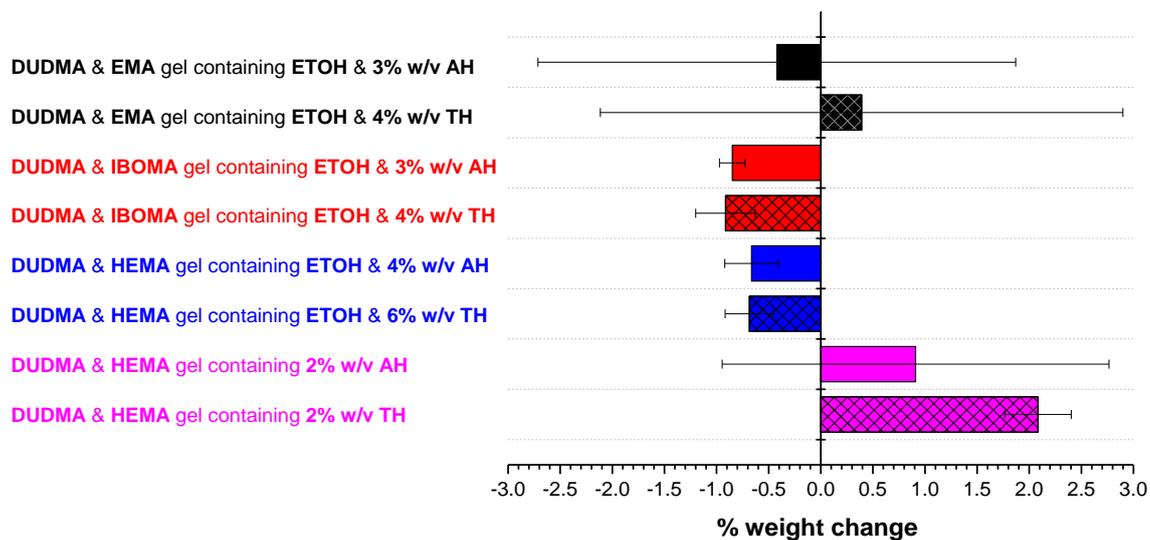
Fig. 4.6 Weight change (%) for the drug-loaded UV-cured films produced from DUDMA & EMA, IBOMA or HEMA containing gels following drug-release studies. Means and standard deviations are shown, n=3. Abbreviations: AH, amorolfine HCl; TH, terbinafine HCl; ETOH, ethanol.

Table 4.1 R^2 values obtained for drug release modelling using zero order, first order and Higuchi models.

Formulation	R^2 value obtained for		
	Zero order	First order	Higuchi model
DUDMA & **EMA** gel containing **ETOH** & **3% w/v AH**	0.85	0.88	**0.96**
DUDMA & **EMA** gel containing **ETOH** & **4% w/v TH**	0.89	0.90	**0.98**
DUDMA & **IBOMA** gel containing **ETOH** & **3% w/v AH**	0.76	0.81	**0.89**
DUDMA & **IBOMA** gel containing **ETOH** & **4% w/v TH**	0.90	0.91	**0.98**
DUDMA & **HEMA** gel containing **ETOH** & **4% w/v AH**	0.75	0.79	**0.89**
DUDMA & **HEMA** gel containing **ETOH** & **6% w/v TH**	0.90	0.92	**0.98**
DUDMA & **HEMA** gel containing **2% w/v AH**	0.75	0.79	**0.88**
DUDMA & **HEMA** gel containing **2% w/v TH**	0.86	0.87	**0.97**

4.6.2 Ungual drug permeation profiles of UV-cured polymer films and Curanail®

The ungual drug permeation profiles are shown in Fig. 4.7 and Fig. 4.8 for amorolfine HCl– and terbinafine HCl– loaded films respectively. The % of drug permeated across the nail and remaining in the nail at day 30 is also shown alongside the permeation profiles, while the calculated lag times, study-state flux, apparent permeability coefficients, effective diffusion coefficients and amount of drug in nail clippings at the end of the experiment are shown in Table 4.2.

4.6.2.1 Amorolfine HCl–loaded UV-cured polymer films

From Fig. 4.7, when considering the UV-curable gel formulations, it can be seen that the film produced from the DUDMA & HEMA gel containing ethanol and 4% w/v amorolfine HCl displays greater ungual drug permeation ($p<0.05$) compared to the films produced from the DUDMA & EMA or IBOMA gels containing ethanol and 3% w/v amorolfine HCl, which were similar ($p>0.05$), and that these in turn display greater ungual drug permeation profiles ($p<0.05$) compared to the film produced from the DUDMA & HEMA gel containing 2% w/v amorolfine HCl. This indicates that the drug-load in the gel (which as mentioned earlier is influenced by the nature of the reactive diluent monomer used and the inclusion of solvent) is directly related to the extent of ungual drug permeation. A more detailed examination of the profiles reveals fairly lengthy lag times, which is typical of nail permeation studies (McAuley et al., 2016). A lag time of approximately 10 days was obtained for all the films apart from the DUDMA & HEMA film (containing 4% w/w amorolfine HCl), which had a shorter time of approximately 7 days, possibly due to its higher drug-load and hence greater concentration gradient between the donor and the receptor phases. The lag period was followed by a linear increase in the cumulative amount of drug permeated. The ungual drug flux in this region, i.e. steady-state flux, was similar for all films (2.2 – 2.4 µg/cm^2/day), apart from the DUDMA & HEMA film containing 2% w/w amorolfine HCl, for which it was significantly lower ($p<0.05$). This could be due to its lower drug-load compared to the other formulations and hence a lower drug concentration gradient between the donor and the receptor phases. Towards the end of the permeation study, the ungual drug permeation appears to slow down. In all cases less than 10% of the applied dose had penetrated into and through

the nail suggesting infinite dosing kinetics, and therefore a slowing down in permeation was not expected as this would suggest violation of sink conditions and/or the presence of an air bubble under the membrane (Williams, 2003), in this case the nail plate. However, sink conditions were maintained and no air bubbles were evident throughout the study, therefore these factors do not explain the observation. This suggests that perhaps amorolfine HCl's high affinity to nail keratin (Tatsumi et al., 2002) eventually leads to an increased amount of drug bound to keratin, thus retarding further permeation.

4.6.2.2 Terbinafine HCl–loaded UV-cured polymer films

In a similar manner to the amorolfine HCl–loaded formulations, the drug-load in the UV gel governs the ungual drug permeation patterns of the terbinafine HCl–loaded formulations (Fig. 4.8). Thus, the film produced from the DUDMA & HEMA gel containing ethanol and 6% w/v terbinafine HCl displayed greater ungual drug permeation ($p<0.05$) compared to the films produced from the DUDMA & EMA or IBOMA gels containing ethanol and 4% w/v terbinafine HCl, which were similar ($p>0.05$), and these in turn displayed greater ungual drug permeation profiles ($p<0.05$) compared to the film produced from the DUDMA & HEMA gel containing 2% w/v terbinafine HCl. Once again the DUDMA & HEMA film (containing the higher drug-load, i.e. 6% w/w terbinafine HCl) has a shorter lag time, and the steady-state flux was similar for all films (1.4 – 1.6 $\mu g/cm^2/day$) apart from the DUDMA & HEMA film (containing 2% w/w terbinafine HCl), for which it was significantly lower ($p<0.05$), again due to its lower drug-load and hence a lower drug concentration gradient between the donor and the receptor phases. Towards the end of the permeation study, the ungual drug permeation also appears to slow down, this time in response to possible terbinafine–nail keratin binding, a well-known phenomenon (Tatsumi et al., 2002). For the UV-cured films, it appears that most of the drug remains in the film and a very small proportion enters the nail plate and eventually the receptor medium. This very low ungual permeation reflects previous reports *in vitro* and in man. For example, van Hoogdalem et al. (1997) showed that less than 0.2% of the applied dose permeated fingernails after twice daily application for 6 weeks in a human volunteer study.

4.6.2.3 Amorolfine HCl–loaded vs. terbinafine HCl–loaded UV-cured polymer films

Compared to the amorolfine HCl–loaded gel formulations, the terbinafine formulations show comparable lag times and diffusion coefficients ($p>0.05$). This could be attributed to the small difference in the molecular weights of the two drugs being insignificant for altering their passage through the nail plate. However, the amorolfine HCl–loaded gel formulations resulted in a significantly higher ($p<0.05$) steady-state flux, permeability coefficient and percentage of drug permeated across the nail at day 30. This may be linked to the greater amount of drug released from the amorolfine HCl–loaded films and thereby available for ungual permeation compared to the terbinafine HCl–loaded films. Nonetheless, the amount of drug extracted from the nail plate and the percentage of drug found in the nail plate were similar for both drugs ($p>0.05$). Terbinafine has previously been shown to bind more strongly to keratin compared to amorolfine (Tatsumi et al., 2002), and this may explain terbinafine HCl–loaded films' lower drug–in–receptor phase compared to drug–in–nail. It also provides an alternative explanation for the terbinafine HCl–loaded films' lower steady-state flux, permeability coefficient, and drug permeation percentage compared to amorolfine. Greater nail keratin binding by a drug does not mean that it is less superior however. While drug-nail keratin binding has been correlated to a reduction in a drug's antifungal potency (Tatsumi et al., 2002), it could also result in the creation of a drug reservoir in the nail, which could be beneficial in the long run as the nail keratin can act as a drug depot releasing drug over time to (i) kill the fungus and any newly-germinating fungal spore residing in the nail plate, and (ii) permeate into, and therefore exert antifungal activity in, the nail bed.

4.6.2.4 UV-curable gels vs. Curanail® nail lacquer

In order to examine how the UV-curable gel formulations compared to a commercially available nail medicine as a drug delivery vehicle, the Curanail® nail lacquer (which contains 5% w/v amorolfine HCl) was compared with the DUDMA & HEMA gel containing 4% w/v amorolfine HCl (the best of the amorolfine HCl-loaded gel formulations). While the two vehicles displayed similar ungual drug permeation profiles ($p>0.05$) and displayed similar permeability coefficients, diffusion coefficients and amounts of drug in the nail clipping at day 30 ($p>0.05$), the Curanail® nail lacquer film

showed a slightly greater steady-state flux ($p<0.05$) probably due to its higher drug-load (32% w/w vs. 4% w/w). However, the UV-cured film displayed a shorter lag time possibly due to it greater occlusivity compared to the lacquer film (Chapter 3, Fig. 3.15), which is expected to increase nail hydration and reduce the time taken for the drug to reach a uniform concentration in the membrane, given that nail hydration is known to increase drug diffusivity in the nail (Gunt and Kasting, 2007). Furthermore, despite the slightly slower drug flux by the UV-cured film, a greater percentage of the drug permeated across the nail and remained in the nail at day 30. This finding along with the lengthy lag times and low ungual drug permeation obtained shows the critical role played by the nail plate barrier to ungual drug permeation and the comparatively lower significance of the film's drug release. In fact, as the amount of drug released from the film into an aqueous sink is greater than the amount permeated across the nail, correlations between the two studies could not be made (Guy and Hadgraft, 1992). The Curanail® nail lacquer's lag time of approximately 10 days suggests that the lacquer must reside on the nail plate surface during this period to allow sufficient drug penetration; however, its *in vivo* nail residence profile (Chapter 3, Fig. 3.24) shows that by day 2 more than a quarter of the lacquer film dislodges from the nail plate surface. Therefore in order to show effect, its twice weekly application process will need to be followed rigorously. The UV-curable gel formulations override the need for frequent applications, given their longer *in vivo* residence (Chapter 3, Fig. 3.24); and as such appears to be a more favourable form of topical nail medicine. However, whether the amount of drug permeated is therapeutically effective against an onychomycosis-causing dermatophyte or not still requires investigation, and this is explored in the next section.
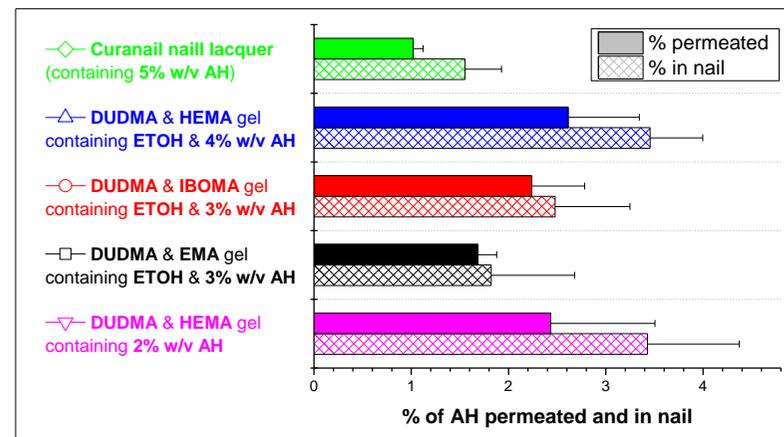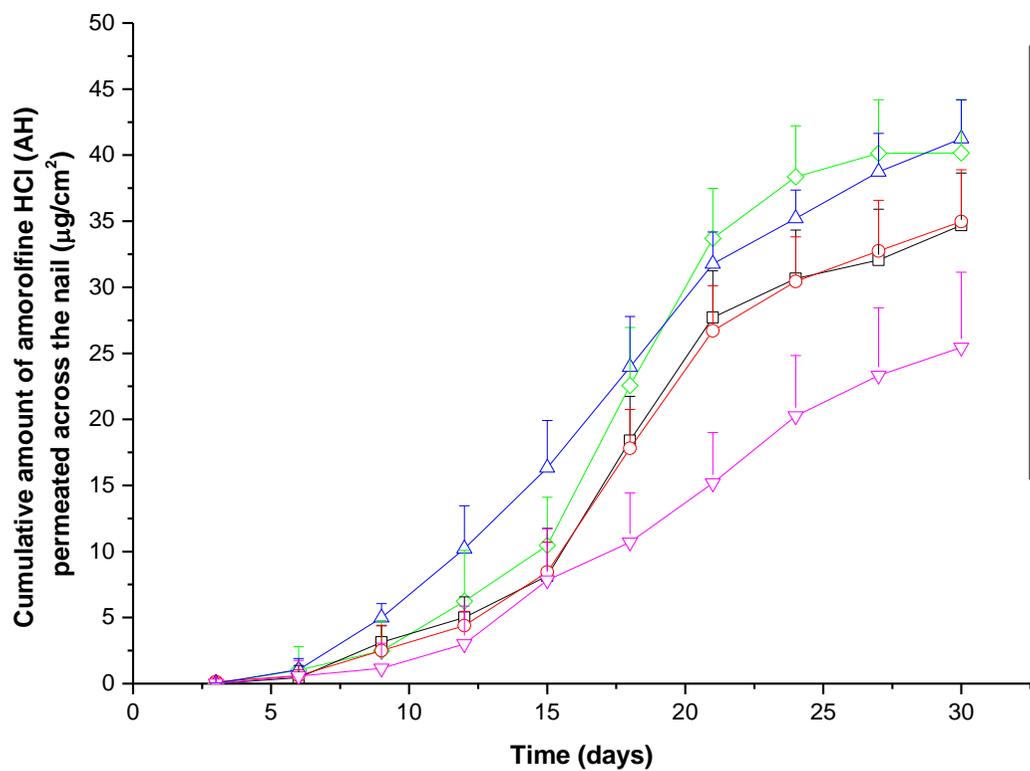
Fig. 4.7 Cumulative amount of amorolfine HCl permeated across the nail with time from the UV-cured and Curanail® films, and the % of amorolfine HCl permeated across the nail and remaining in the nail at day 30. Means and standard deviations are shown, n=6. Abbreviations: AH, amorolfine HCl; ETOH, ethanol.
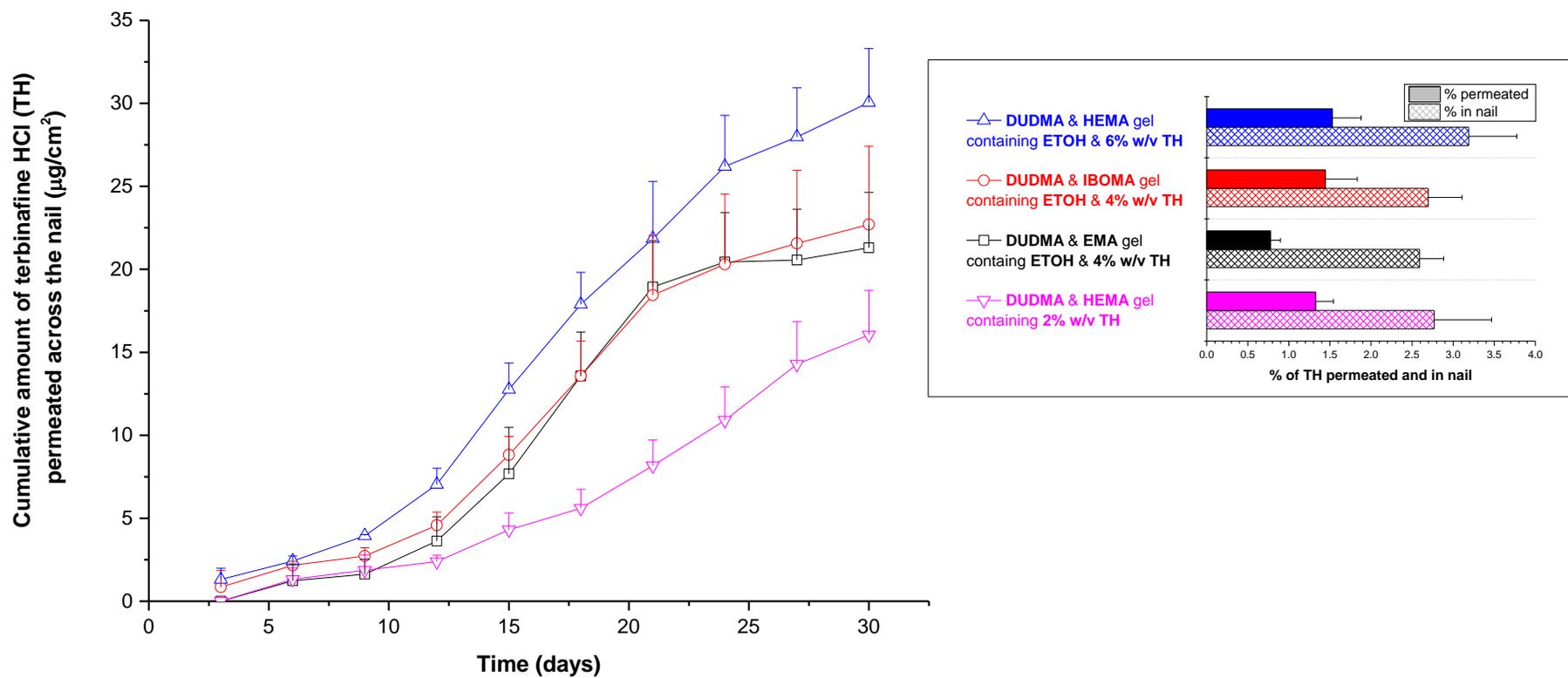
Fig. 4.8 Cumulative amount of terbinafine HCl permeated across the nail with time from the UV-cured films, and the % of terbinafine HCl permeated across the nail and remaining in the nail at day 30. Means and standard deviations are shown, n=6. Abbreviations: TH, terbinafine HCl; ETOH, ethanol.

Table 4.2 Lag time, steady-state flux, apparent permeability coefficient, effective diffusion coefficient and amount of drug in nail clippings. Means and standard deviations are shown, n=6. Abbreviations: AH, amorolfine HCl; TH, terbinafine HCl; ETOH, ethanol.

	Amorolfine HCl (AH)						Terbinafine HCl (TH)					
	% in formula-tion (% w/v)	Lag time (day)	Steady-state flux (µg/cm^2/day)	Permeability coefficient x 10^{-5} (cm/day)	Diffusion coefficient x 10^{-5} (cm^2/day)	Drug in nail clipping (mg/cm^3)	% in formula-tion (% w/v)	Lag time (day)	Steady-state flux (µg/cm^2/day)	Permeability coefficient x 10^{-5} (cm/day)	Diffusion coefficient x 10^{-5} (cm^2/day)	Drug in nail clipping (mg/cm^3)
Curanail® nail lacquer	5	10.4 ± 1.3	2.9 ± 0.2	5.8 ± 0.3	2.5 ± 0.5	0.6 ± 0.2	NA	NA	NA	NA	NA	NA
DUDMA & EMA gel containing ETOH & AH or TH	3	10.4 ± 0.9	2.4 ± 0.2	7.9 ± 0.7	2.7 ± 0.8	0.3 ± 0.2	4	9.5 ± 1.2	1.5 ± 0.1	3.7 ± 0.3	1.6 ± 0.4	0.9 ± 0.2
DUDMA & IBOMA gel containing ETOH & AH or TH	3	10.5 ± 0.6	2.3 ± 0.3	7.8 ± 1.0	2.2 ± 0.4	0.4 ± 0.1	4	8.3 ± 0.8	1.4 ± 0.3	3.4 ± 0.8	3.1 ± 0.9	0.4 ± 0.1
DUDMA & HEMA gel containing ETOH & AH or TH	4	7.1 ± 1.7	2.2 ± 0.3	5.5 ± 0.7	3.5 ± 1.4	0.6 ± 0.1	6	7.1 ± 1.0	1.6 ± 0.2	2.6 ± 0.4	3.0 ± 0.8	0.7 ± 0.2
DUDMA & HEMA gel containing AH or TH	2	10.0 ± 1.9	1.4 ± 0.1	7.0 ± 0.7	3.2 ± 0.4	0.3 ± 0.2	2	8.9 ± 1.0	0.7 ± 0.1	3.5 ± 0.7	2.6 ± 0.9	0.3 ± 0.1

4.6.3 Antifungal efficacy of formulations against *T. rubrum*

The results of the adapted disc diffusion method developed to test the efficacies of the different topical nail formulations (when applied on human fingernail clippings) against *T. rubrum* are shown in Table 4.3.

As expected, the *T. rubrum* inoculated SDA plates used as a negative control (i.e. formulation-free), allowed the *T. rubrum* culture to grow over the entire plate's surface. Similarly, *T. rubrum* inoculated SDA plates containing nail clippings with only drug-free formulations cured on the surface (n=3) also showed fungal growth over the entire plate's surface. This shows that the UV-cured film vehicle is not antifungal itself. In contrast, the *T. rubrum* inoculated SDA plates containing nail clippings with drug-loaded formulations cured on the surface (n=3) showed no fungal growth for the duration of the experiment following the introduction of the nail clippings. This suggests that the low percentage of applied drug which permeated into and through the nail plate (Fig. 4.7 – 4.8), is therapeutically effective against the single most common fungus to cause onychomycosis. It also suggests that the drug permeated through the nail plate may have diffused into the agar, thus preventing the growth of the culture towards the nail clippings containing the drug-free formulation on the same plate.

The MIC of amorolfine and terbinafine against *T. rubrum* has been reported to be 0.12-0.5 µg/ml and 0.004–0.06 µg/ml respectively (Tamura et al., 2014), and the drug levels achieved in the nail plate by the amorolfine HCl– and terbinafine HCl– loaded formulations were considerably greater (Table 4.2); the demonstrated inhibition of fungal growth by the formulations is therefore consistent with what would be expected theoretically. While it can be argued that the MIC values determined *in vitro* in broth are not expected to be directly applicable to MICs in the nail plate, and that the extent of drug permeation and drug-load is expected to be significantly lower for toenails (which are predominately affected) compared to fingernails (Nair et al., 2011), the drug levels achieved in the nail are more than sufficient to cause fungal kill. The UV-cured gels are therefore promising topical carriers for anti-onychomycotic drugs.

While the method developed was capable of yielding insightful qualitative data, due to the absence of a zone of inhibition, it could not be used to deduce which of the

amorolfine HCl–loaded UV gel formulations tested was the 'best', i.e. facilitated better ungual drug permeation, and whether this in turn correlated with the nail permeation studies conducted. Similarly the amorolfine HCl–loaded DUDMA & HEMA gel (containing ethanol) could not be compared with the terbinafine HCl–loaded DUDMA & HEMA gel (containing ethanol) or with the Curanail® nail lacquer. A future study containing one sample per *T. rubrum* inoculated SDA plate, (with the *T. rubrum* plug inserted at one end of the petri dish and the sample placed at the other end after allowing the plug to grow greater than 10 mm), may enable discrimination among the formulations or confirm anti-fungal equivalence despite different ungual permeations.

Table 4.3 Photographic images (at day 30) of *T. rubrum* inoculated SDA plates containing nail clippings with drug-free and drug-loaded formulations cured on the surface. The corresponding drug-free formulation for the Curanail® nail lacquer is a formulation-free nail clipping (as a drug-free formulation could not be produced in the lab due to lack of knowledge regarding the quantity of excipients).

Formulation applied on nail clipping surface	*T. rubrum* inoculated SDA plates containing nail clippings with drug-loaded formulations cured on the surface (n=3) and the corresponding drug-free formulation cured on the surface (C)		*T. rubrum* inoculated SDA plates containing nail clippings with drug-free formulations cured on the surface (n=3)	
	Top surface	Under surface	Top surface	Under surface
None (nail clipping-free & **formulation-free control**)				
Curanail® nail lacquer (containing **5% w/v AH**)				
DUDMA & EMA gel containing **ETOH ± 3% w/v AH**				
DUDMA & IBOMA gel containing **ETOH ± 3% w/v AH**				
DUDMA & HEMA gel containing **ETOH ± 4% w/v AH**				
DUDMA & HEMA gel **± 2% w/v AH**				
DUDMA & HEMA gel containing **ETOH ± 6% w/v TH**				

4.7 Conclusions

The drug release profiles were similar for all the UV gels formulated. However, the release of drug was limited (to less than 50%) possibly by the films' cross-linked and highly dense nature, and the release of terbinafine from the films was almost half that of amorolfine due to possible terbinafine-polymer binding.

Permeation studies revealed that all formulations were capable of enabling drug permeation into and through the nail *in vitro*. The drug-loaded DUDMA & HEMA gels containing ethanol displayed the greater ungual drug permeation profile due to their greater drug-load, and the amorolfine HCl–loaded DUDMA & HEMA gel containing ethanol was in fact found to be comparable to the commercially available pharmaceutical nail lacquer Curanail®. For the terbinafine HCl-loaded films, a lower percentage of drug permeated across the nail compared to amorolfine HCl-loaded films, most probably as terbinafine binds more strongly to keratin compared to amorolfine.

The amount of drug that permeated across the nail was sufficient to arrest the growth of the fungus *T. rubrum in vitro*. However, differences among the formulations regarding anti-fungal efficacy could not be discerned despite differences in ungual drug permeations.

The UV-cured films are thus promising candidates for the topical treatment of onychomycosis, and in the next Chapter, the incorporation of penetration enhancers are considered for optimising the more superior of the UV-curable gels, i.e. DUDMA & HEMA gels containing ethanol and either 4% w/v amorolfine HCl or 6% w/v terbinafine HCl.

Chapter 5: Optimising UV-curable gel formulations with the use of penetration enhancers

5.1 Introduction

The UV-curable gel formulations developed thus far contained the maximum amount of drug that could be loaded in the dissolved state in each film. The gel's maximum drug-load depended on the drug's solubility in ethanol and in the reactive diluent methacrylate-based monomer. Thus the gels containing HEMA and ethanol dissolved the most drug due to the greater drug solvency of HEMA (Chapter 2, Table 2.7). In the previous Chapter, the films' drug release and ungual drug permeation was the greatest from the film with the highest drug-load, and hence the DUDMA & HEMA gels (with a DUDMA to HEMA ratio of 85:15% v/v) containing ethanol and either 4% w/v amorolfine HCl or 6% w/v terbinafine HCl were the superior of the formulations developed. However the percentage of drug in the formulation that permeated across the nail and remained in the nail was less than 2.7% and 3.6% respectively, following the 30 day ungual drug permeation study using fingernail clippings as the nail model (Chapter 4, Figs 4.7 – 4.8). Despite this, the amount of amorolfine and terbinafine that had permeated across the fingernail clippings was sufficient enough to inhibit the growth of *T. rubrum*, the most common onychomycosis-causing pathogen (Chapter 4, Table 4.3). Nonetheless, as also mentioned in the previous Chapter (Section 4.6.3), the extent of drug permeation is expected to be significantly lower for toenails (which are predominated infected) compared to fingernails. These formulations are thus worth considering for optimisation to overcome the poor permeability percentage and guarantee treatment success.

In this Chapter, the incorporation of an ungual penetration enhancer in the pharmaceutical UV gel is therefore considered for boosting nail permeability. A chemical approach to enhance ungual drug permeation was considered as it does not add to the formulation's application process when the chemical is incorporated into the UV gel, and in turn will not compromise patient compliance. The penetration enhancers considered were 2-mepcaptoethanol (MPE), water, NMP and poly(ethylene glycol) 200 (PEG 200), the chemical structures and molecular weights for which are shown in Table 5.1.

Table 5.1 Chemical structures and molecular weights of the penetration enhancers considered for use in the UV-curable gel formulations.

Penetration enhancer	Chemical structure	Molecular wt. (Daltons)
MPE	HS—CH₂—CH₂—OH	78.13
Water	H_2O	18.02
NMP	(N-methyl-2-pyrrolidone structure)	99.13
PEG 200	H—[O—CH₂—CH₂—]ₙ—OH	~ 200
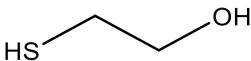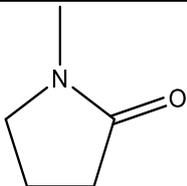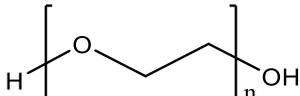
As mentioned in Chapter 1, MPE is a thiol capable of reducing the disulfide linkage in the keratin matrix of the nail, as shown below:

Nail-S-S-Nail + 2[HO-CH₂-CH₂-SH] ↔ 2Nail-SH + HO-CH₂-CH₂-S-S-CH₂-CH₂-OH

It is therefore capable of enhancing drug permeation across the nail by disrupting the disulphide bonds of keratin. This mechanism renders thiols as the most effective of the ungual penetration enhancers and worth considering for optimising the pharmaceutical UV gel. Other potential thiol compounds such as N-acetylcysteine and thioglycolic acid were not considered, as a recent study which investigated nail lacquers containing MPE, N-acetylcysteine or thioglycolic acid found MPE to enhance drug permeation to the greatest extent (Patel and Vora, 2016). MPE enabled 8.5, 1.5 and 3 times more terbinafine HCl to permeate through a nail model compared to the formulations with no penetration enhancer, N-acetylcysteine and thioglycolic acid respectively.

Water, NMP and PEG 200 can enhance ungual drug permeation without the need to disrupt the bonds maintaining the integrity of nail keratin. These enhancers instead promote nail swelling resulting in the formation of larger pores through which the drug can easily diffuse (Nair et al., 2010, Hossin et al., 2016). Of these enhancers, NMP and PEG 200 have been found to enhance drug flux through a nail model when incorporated in a terbinafine-loaded acrylic-based transungual patch, with enhancement ratios of 1.17 and 1.24 for NMP and PEG 200 respectively, where enhancement ratio = flux with enhancer/flux without enhancer (Ahn et al., 2013). Interestingly, these enhancement

ratios are higher than that achieved by MPE (which had an enhancement ratio of 1.03) when incorporated in the transungual patch, making NMP and PEG 200 also worth considering for optimising the pharmaceutical UV gel. Water was considered as its role as an ungual penetration enhancer when incorporated into a topical nail medicine has not been evaluated to date, despite its ability to facilitate the transport of both water-soluble and poorly water-soluble permeants across the nail plate (Walters and Flynn, 1983, Gunt and Kasting, 2007).

In this Chapter, either water, MPE, NMP or PEG 200 is incorporated in the DUDMA & HEMA gel formulations containing 4% w/v amorolfine HCl or 6% w/v terbinafine HCl, and their effects on the formulations' polymerisation process and the resulting films' chemical and physical structure, thickness, drug-load, thermal properties, water sensitivity, drug release profiles and ungual drug permeation profiles are investigated. This is in turn to deduce whether the selected enhancers are indeed capable of optimising nail drug delivery when incorporated in a UV-curable vehicle, and whether the enhancer and gel combination itself positively or negatively influences the other formulation properties investigated.

5.2 Aims

To incorporate a penetration enhancer in a drug-loaded UV-curable gel and determine its influence, if any, on the resulting formulation's polymerisation process, i.e. mass yield and degree of conversion from monomer gel to polymer film, and also its influence, if any, on the resulting film's:

(i) residual monomers content

(ii) physical and chemical structure, microstructure and thickness

(iii) drug-load

(iv) thermal properties, i.e. degradation and viscoelasticity

(v) water sensitivity

(vi) drug release and ungual drug permeation profiles.

5.3 Materials

The monomers, photoinitiator, antifungal agents, solvents, acids, UVA nail lamp and nail wipes were obtained as detailed in Chapter 2 (Section 2.3). Toluene and high density polyethylene (HDPE) sheets were obtained as detailed in Chapter 3 (Section 3.3). Sodium phosphate monobasic dihydrate, sodium phosphate dibasic heptahydrate, and human nail clippings (fingernails) were obtained as detailed in Chapter 4 (Section 4.3). 2-Mercaptoethanol (MPE) was purchased from Sigma–Aldrich (Dorset, UK) and poly(ethylene glycol) 200 was purchased from Fisher Scientific (Loughborough, UK).

5.4 Methods

5.4.1 UV-curable gel preparation

A total of five types of DUDMA & HEMA formulations (with a DUDMA: HEMA ratio of 85:15% v/v) were prepared containing ethanol and either (i) no penetration enhancer, i.e. control, (ii) water, (iii) MPE, (iv) NMP or (v) PEG 200. The ethanol and penetration enhancer together made up no greater than 25% v/v of the formulation, so that the penetration enhancer incorporation in the gel would not hugely (i) affect the viscosity of the gel, and hence affect its application ability, and (ii) compromise the formulations ability to produce a durable polymer.

The types of formulations were further subdivided depending on whether they contained the antifungal amorolfine HCl or terbinafine HCl. Details of the formulations' compositions are shown in Table 5.2.

Table 5.2 Excipients and their quantities. Two types of each formulation were prepared: (i) amorolfine HCl-loaded or (ii) terbinafine HCl-loaded.

Penetration enhancer in formulation	Excipient quantity						
	Penetration enhancer (% v/v)	Ethanol (% v/v)	DUDMA (% v/v)	HEMA (% v/v)	2-hydroxy-2-methyl-propiophenone (% v/v)	Drug (% w/v)	
						AH	TH
No enhancer	-	25	61.2	10.8	3	4	6
Water	10	15					
MPE	5	20					
NMP	5	20					
PEG 200	5	20					

The formulations (excluding the MPE–containing formulations) were prepared by first producing an ethanol and penetration enhancer mixture, and dissolving the drug in this mixture, and then adding the required excipients to the drug solution and leaving the mixture to stir overnight to produce a clear homogenous solution. Each formulation was prepared in triplicate.

The MPE–containing formulations were prepared in the same manner as the enhancer-free formulation; however the MPE was incorporated into the gel immediately prior to UV-curing to overcome the observed premature polymerisation of the monomer mixture during storage. It appears that the heat generated when mixing the gel components may have led to the formation of a thiyl radical, which in turn may have facilitated the premature polymerisation observed.

The penetration enhancer concentration in the gel was the maximum that could be incorporated to produce drug-loaded films with the drug remaining in the dissolved state. A maximal concentration was sought to achieve maximal enhancement, given that it has been found that as the concentration of penetration enhancer increases, the permeation of drug through the nail plate increases (Patel and Vora, 2016). To determine this maximum concentration, formulations containing DUDMA, HEMA, a photoinitiator, the antifungal drug and penetration enhancers at concentrations of 2.5, 5, 7.5, 10 and 12.5 % v/v with corresponding ethanol concentrations of 22.5, 20, 17.5, 15 and 12.5 % v/v were prepared. These formulations were visually observed for drug crystals and subsequently exposed to UV light for polymerisation. The resulting films were observed under a polarised light microscope (as described in Chapter 3, Section 3.4.5.1) and examined using XRD (as described in Chapter 3, Section 3.4.5.2) to confirm the absence or presence of drug crystals. The polarised light micrographs of the films and their corresponding XRD patterns can be found in Appendix A12.

5.4.2 UV-curing of formulations and UV-cured film characteristics

The formulations were cured to produce polymer films as per Section 2.4.4 (Chapter 2). The formulations' mass yield and degree of conversion from monomer to cured polymer film were determined as detailed in Sections 2.4.5.1 and 2.4.5.2 respectively, and the polymer films' thickness, residual monomer content, chemical structure, microstructure,

drug-load, thermal properties, water sensitivity, drug release profiles and ungual drug permeation profiles were determined as detailed in Sections 3.4.1, 2.4.5.3, 2.4.5.2, 3.4.4, 3.4.6, 3.4.8, 3.4.11, 4.5.1 and 4.5.2 respectively.

The polymer films were also imaged using a Nikon Microphot-FXA microscope (Tokyo, Japan) to assess the UV-cured polymer film's structure. Images were taken using a Lumenera Infinity 2 digital camera (Ottawa, Canada) attached to the microscope.

5.4.3 Statistical analyses

Statistical calculations were conducted using IBM SPSS 22. The data was tested to determine whether they were normally distributed or not using the Shapiro-Wilk test and then analysed using either a t-test or Mann-Whiney U test for parametric and non-parametric data respectively. For multiple comparisons, ANOVA followed by post hoc Tukey or the Kruskal Wallis test with post hoc analysis performed with Nemenyi's test was used for parametric and non-parametric data respectively. Repeated measures ANOVA was conducted to determine whether there were differences in drug release profiles and ungual drug permeation profiles of the formulations tested over the experimental duration.

5.5 Results and discussion

5.5.1 Assessment of the polymerisation process

5.5.1.1 Mass yield from monomer gel to polymer film and thickness of the resulting polymer film

The incorporation of water, MPE, NMP or PEG 200 in a drug-loaded UV-curable gel formulation did not affect the gels' ability to produce a polymeric film. Similarly to formulations without any penetration enhancer, an oxygen inhibition layer was formed on the films' surface, and removal of this layer reduced the % mass yield from monomer mixture to polymer film as shown in Table 5.3.

From Table 5.3, it can be seen that both the amorolfine HCl– and terbinafine HCl– loaded formulations containing MPE have a significantly higher mass yield percentage ($p<0.05$) compared to the other formulations, indicating the formation (and subsequent removal) of a thinner oxygen inhibition layer. As mentioned in Chapter 2, the oxygen inhibition layer is formed when O_2 reacts with the primary initiating or propagating radicals to form peroxyl radicals (as shown in Scheme 1) which are unreactive towards the (meth)acrylate C=C bond. These peroxyl radicals therefore terminate polymerisation through radical-radical recombination (Odian, 1991, Rabek, 1993). MPE is a thiol, and thiols are known to reduce the inhibitory effects of O_2 on resin formulations (Hoyle and Bowman, 2010). In the presence of initiating radicals, hydrogen transfer by the thiol provides a thiyl radical to propagate the polymerisation reaction. While a carbon-based radical can still react with O_2, the peroxyl radical formed can also abstract hydrogen from thiol, and as a result reinitiation occurs by a thiyl radical (Scheme 2) (Ligon et al., 2014). This phenomenon explains the observed reduction in the thickness of the oxygen inhibition layer.

The amorolfine HCl– and terbinafine HCl– loaded formulations containing water have a significantly higher % mass yield ($p<0.05$) compared to the formulations containing no penetration enhancer, NMP or PEG 200. This is most likely due to the formulations' lower concentration of the volatile component ethanol (15% vs. 20% or 25 %). As previously mentioned, it is possible that the heat generated by a free radical polymerisation reaction, coupled with the heat provided by the UVA lamp during the

curing process (Appendix A5), may cause some of the volatile components to evaporate off, thus reducing the mass yield from monomer mixture to polymer film.

The amorolfine HCl– and terbinafine HCl– loaded formulations containing no penetration enhancer, NMP or PEG 200 showed similar % mass yields from monomer mixture to polymer film (p>0.05). NMP and PEG 200 are both non-volatile compounds and thus do not evaporate off during curing, therefore it appears that at a concentration of 5% v/v, they do not significantly interfere with the polymerisation process.

The choice of drug in the formulation did not alter the % mass yield either (p>0.05), and this is consistent with the findings in Chapter 2 (Section 2.5.7.1). Furthermore as expected, linear regression analysis revealed that an increased % mass yield from monomer mixture to polymer film is correlated with a thicker film (R^2 = 0.982, p<0.01) (Table 5.3).

Therefore, it is seen that the incorporation of a penetration enhancer in a drug-loaded DUDMA & HEMA UV-curable gel formulation does not negatively affect its mass yield from monomer mixture to cured polymer film, which remains considerable and hence acceptable.

Table 5.3 Mass yield of AH- or TH- loaded DUDMA & HEMA gel formulations containing ethanol and different penetration enhancers (after UV-curing and removal of oxygen inhibition layer), and the thickness of the resulting film. Means ± standard deviations are shown, n=3 for mass yield % and n=9 for film thickness.

	Mass yield (%) of DUDMA & HEMA formulations containing		Film thickness (µm) of films produced from DUDMA & HEMA formulations containing	
	4% w/v AH	6% w/v TH	4% w/v AH	6% w/v TH
ETOH (25% v/v) & No PE	80.8 ± 0.5	80.7 ± 1.1	163.3 ± 7.1	164.4 ± 5.3
ETOH (15% v/v) & Water (10% v/v)	85.4 ± 1.5	86.6 ± 2.0	172.2 ± 6.7	174.4 ± 6.3
ETOH (20% v/v) & MPE (5% v/v)	94.0 ± 1.4	93.2 ± 0.9	181.7 ± 6.1	180.6 ± 6.8
ETOH (20% v/v) & NMP (5% v/v)	79.7 ± 1.4	79.2 ± 1.5	161.7 ± 7.1	162.2 ± 7.9
ETOH (20% v/v) & PEG 200 (5% v/v)	77.5 ± 1.9	77.4 ± 0.5	158.3 ± 5.0	158.9 ± 6.0

Abbreviations: AH, amorolfine HCl; TH, terbinafine HCl; ETOH, ethanol; PE, penetration enhancer.
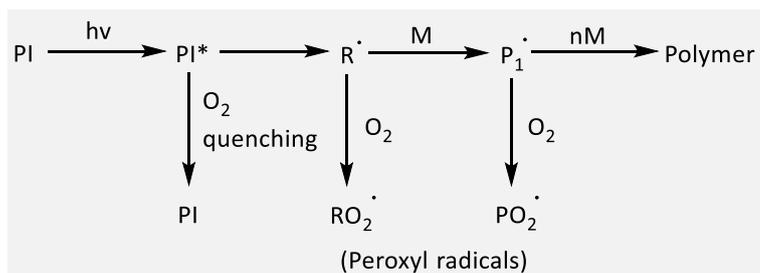
Scheme 1 Oxygen inhibition in a radical-type photoinitiated polymerisation reaction. Abbreviations: PI, photoinitiator; M, monomer. (Adapted from Studer et al., *Progress in Organic Coatings* 48, 2003).
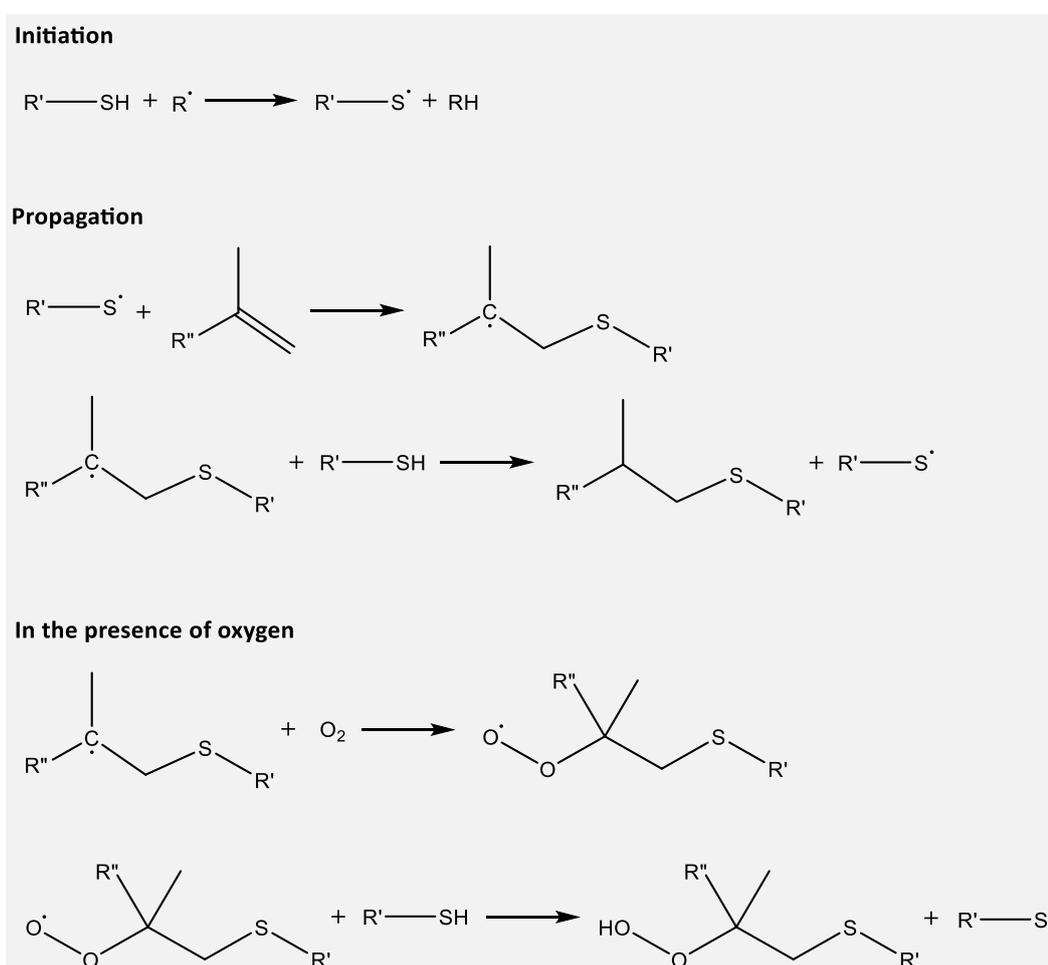
Scheme 2 Thiols in a radical-type photoinitiated polymerisation reaction. (Adapted from Ligon et al., *Chemical Reviews,* 114, 1, 2014).

5.5.1.2 Degree of conversion (DC) and amount of residual monomers in cured polymer film

In order to determine the influence of a penetration enhancer on the extent of polymerisation within the bulk of the film, the formulations' DC from monomer gel to polymer film and the films' residual monomer contents were measured, the results for which are in Table 5.4 and Fig. 5.1 respectively. The formulations containing the penetration enhancers obtained a DC % between 58 – 67% and extremely low levels of residual monomers (≤1.04% DUDMA and ≤0.007% HEMA) in the polymer films. This was similar to the formulations with no penetration enhancer present and indicates the presence of unreacted groups within the polymer, as opposed to unreacted monomers, as previously discussed in Chapter 2 (Section 2.5.7.2).

When considering the amorolfine HCl-loaded formulations with and without the different penetration enhancers, it can be seen that the formulation containing water has the highest DC % and the lowest amount of unreacted DUDMA and HEMA monomers in the film produced. It appears that water's low viscosity, (0.89 mPas at 25°C compared to 1.08, 1.67, 3.42 and 50 mPas for ethanol, NMP, MPE and PEG 200 respectively) coupled with its higher concentration in the gel, may have produced a formulation with a lower viscosity than the other formulations, given that the overall viscosity of the gel formulation is influenced by the viscosity of its components (Chapter 2, Section 2.5.5.2). The formulation's lower viscosity may have in turn facilitated greater mobility of the methacrylate side chains during curing and hence it's greater polymerisation. However, the other formulations' higher ethanol content (20% or 25% vs. 15%) may also be responsible for the observations. As mentioned, ethanol can evaporate during the curing process. This evaporation can somewhat limit the mobility of the methacrylate side chains, which already start to restrict due to the increasing viscosity of the formulation as the polymerisation reaction progresses, thus limiting the extent of polymerisation.

The formulations containing no penetration enhancer or NMP have similar DC percentages ($p>0.05$) and similar amounts of residual DUDMA and HEMA monomers in their films ($p>0.05$). This could be down to the similar viscosities of ethanol and NMP,

and therefore the similar viscosities assumed for the two formulations, with viscosity influencing extent of polymerisation as discussed above.

The formulations containing PEG 200 or MPE have the lowest DC percentages ($p<0.05$) and highest amounts of unreacted DUDMA and HEMA monomers in the films produced, due to the higher viscosities of PEG 200 and MPE. Interestingly, the formulation containing MPE has a slightly (but not significantly, $p>0.05$) lower DC % and slightly higher (but not significant, $p>0.05$) amounts of DUDMA and HEMA monomers remaining in its film, despite MPE's considerably lower viscosity than PEG 200. A possible explanation for this observation could be that during the polymerisation process, a large fraction of the UVA radiation is absorbed in the upper few micrometres of the MPE-loaded UV gel, that minimal radiation reaches the lower layers, resulting in an improper cure.

The terbinafine HCl-loaded formulations have similar DC percentages ($p>0.05$) and similar amounts of DUDMA and HEMA monomers remaining in their films ($p>0.05$) when compared to their corresponding amorolfine HCl-loaded formulations. This is as expected given that drug-loading was not found to affect the films DC % (Chapter 2, Table 2.12) or residual monomer content (Chapter 2, Table 2.13).

Overall, as the amounts of DUDMA & HEMA monomers remaining in the films produced from the drug-loaded UV-curable gel formulations containing water, MPE, NMP or PEG 200 were negligible, the extent of polymerisation was considered acceptable, and all the formulations were therefore characterised further.

Table 5.4 Percentage DC from monomer gel to polymer film for AH- or TH- loaded DUDMA & HEMA gel formulations containing ethanol and different penetration enhancers. Means ± standard deviations are shown, n=3.

	Degree of conversion (%) of DUDMA & HEMA formulations containing	
	4% w/v AH	**6% w/v TH**
ETOH (25% v/v) & No PE	64.7 ± 1.6	63.0 ± 3.2
ETOH (15% v/v) & Water (10% v/v)	66.6 ± 0.8	66.2 ± 4.5
ETOH (20% v/v) & MPE (5% v/v)	58.4 ± 1.4	58.2 ± 1.2
ETOH (20% v/v) & NMP (5% v/v)	63.9 ± 0.6	62.8 ± 2.7
ETOH (20% v/v) & PEG 200 (5% v/v)	59.5 ± 2.0	59.3 ± 1.3

Abbreviations: AH, amorolfine HCl; TH, terbinafine HCl; ETOH, ethanol; PE, penetration enhancer.
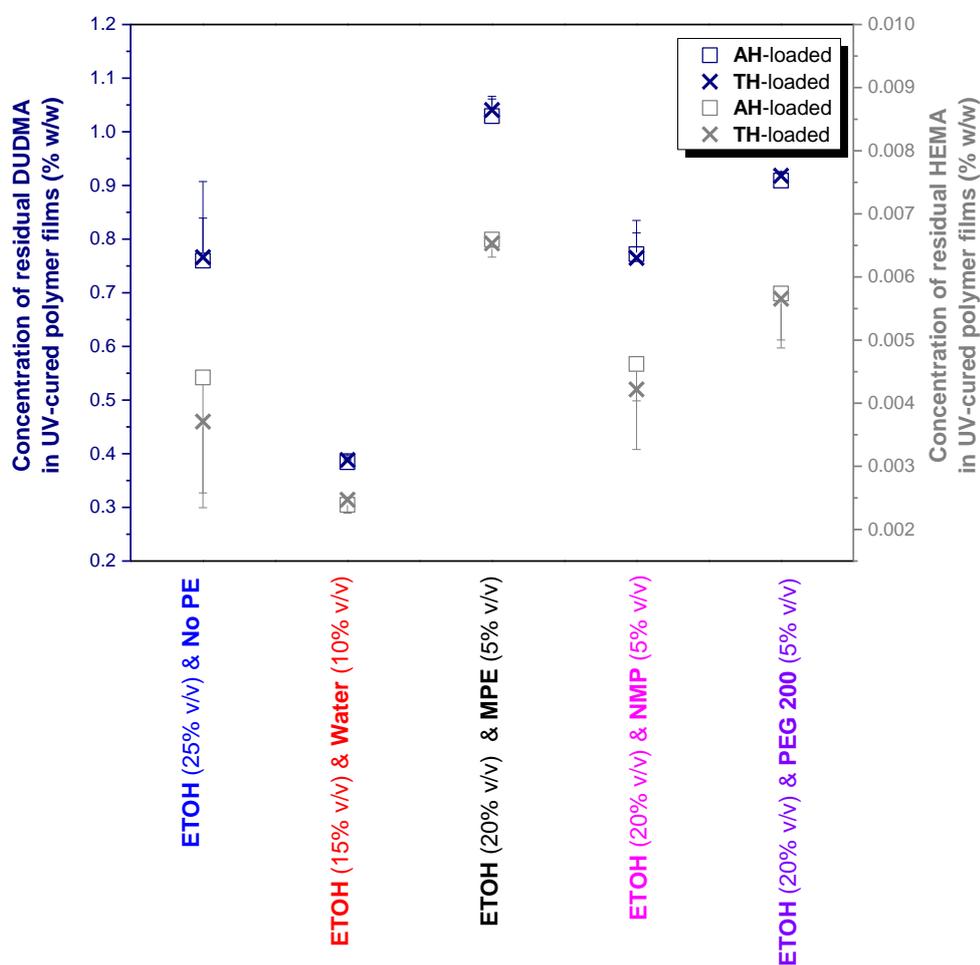
Fig. 5.1 Influence of penetration enhancer incorporation on concentration of residual monomers in AH- or TH- loaded UV-cured DUDMA & HEMA copolymer films. Means and standard deviations are shown, n=3.

5.5.2 Structure & microstructure of UV-cured polymer film

Water, MPE, NMP and PEG 200 have boiling points of 100°C, 157°C, 202°C and >250°C respectively. As such, they are not expected to evaporate off during curing, and instead are expected to remain in the film, perhaps influencing the structure of the polymer film formed. However, the incorporation of water, MPE, NMP or PEG 200 in a drug-loaded UV-curable DUDMA & HEMA gel formulation did not alter the visual appearance of the film produced, which remained smooth and transparent (Appendix A13), and therefore aesthetically acceptable. In addition, the incorporation of the penetration enhancers in the formulations did not produce any changes in the resulting films' FT-IR spectra when compared to the FT-IR spectra of the formulations containing no penetration enhancer (Appendix A14). Thus any chemical structural change in the film imposed by the presence of a penetration enhancer in the gel was not obvious by FT-IR. The films were therefore examined for visual changes in their microstructure using an optical microscope (Table 5.5) and a scanning electron microscope (Fig. 5.2).

From Table 5.5, it can be seen that the incorporation of MPE, NMP or PEG 200 in amorolfine HCl– or terbinafine HCl– loaded DUDMA and HEMA gels does not cause any noticeable changes in the microstructure of the films produced. However, the incorporation of water appears to produce films which contain trapped water droplets or air bubbles. The scanning electron micrograph of the cross-section of the film produced from the amorolfine HCl–loaded gel formulation containing water did not appear to show the presence of water droplets or air bubbles however (Fig. 5.2). In fact, the scanning electron micrographs of the cross-sections of the films produced from the amorolfine HCl–loaded formulations containing water, MPE, NMP or PEG 200 revealed dense film interiors, like the film produced from the formulation containing no penetration enhancer.

Scanning electron micrographs of the films produced from terbinafine HCl–loaded formulations containing penetration enhancers were not taken, as the choice of drug was not expected to affect the film's microstructure, given that in Table 3.6 (Chapter 3), the drug in the formulation showed no visible changes in the resulting film's microstructure.

Table 5.5 Optical microscopy images of films produced from amorolfine HCl- or terbinafine HCl- loaded DUDMA & HEMA gel formulations containing ethanol (ETOH) and different penetration enhancers (PE).

	Light microscopy images of DUDMA & HEMA UV-cured films produced from formulations containing	
	4% w/v amorolfine HCl	**6% w/v terbinafine HCl**
ETOH (25% v/v) & No PE		
ETOH (15% v/v) & Water (10% v/v)		
ETOH (20% v/v) & MPE (5% v/v)		
ETOH (20% v/v) & NMP (5% v/v)		
ETOH (20% v/v) & PEG 200 (5% v/v)		
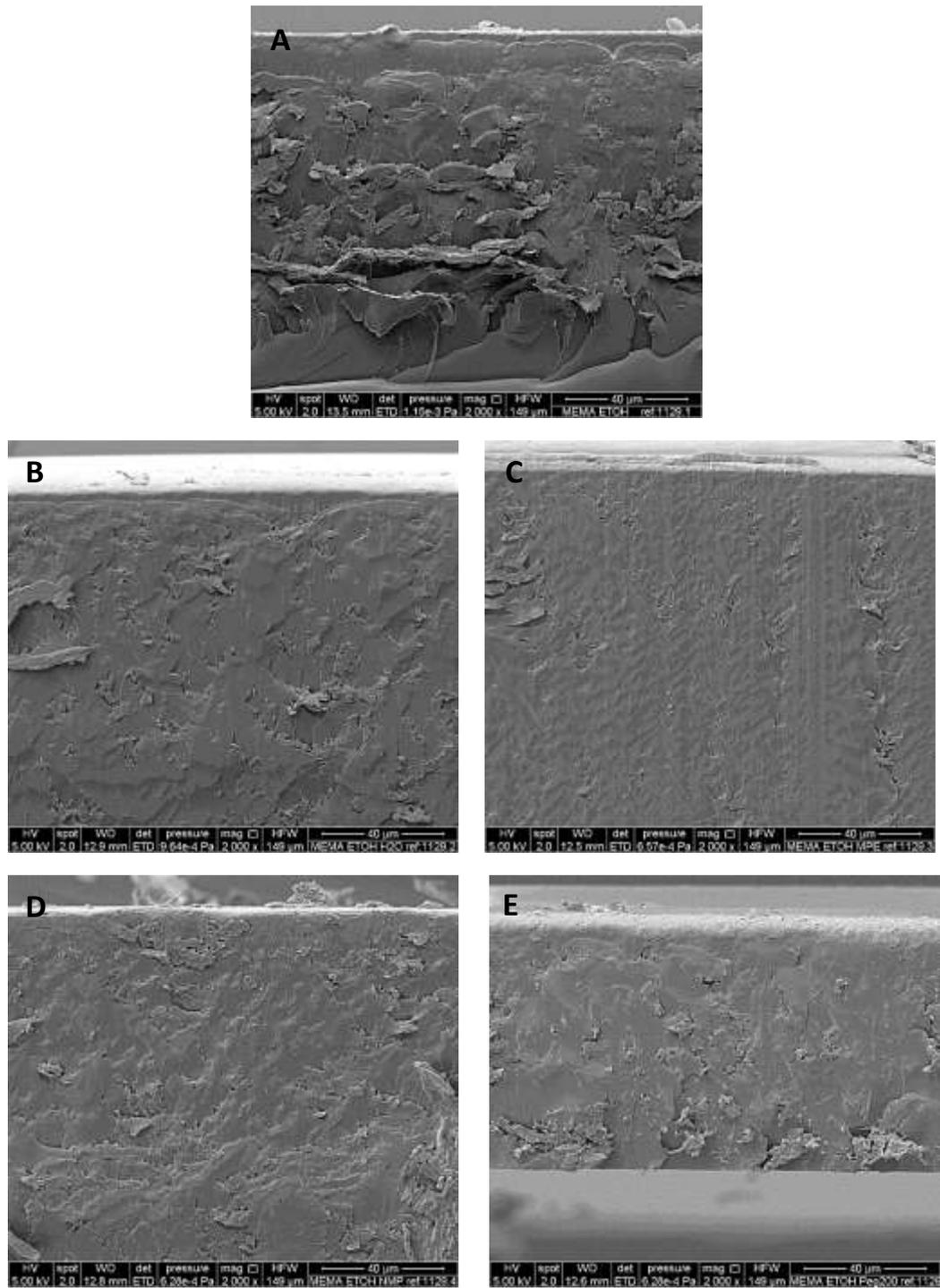
Fig. 5.2 Scanning electron micrographs of the cross-sectional surfaces of UV-cured films produced from amorolfine HCl-loaded DUDMA & HEMA gels containing ethanol and (A) no enhancer, (B) water, (C) MPE, (D) NMP and (E) PEG 200.

5.5.3 Drug-load in UV-cured polymer film

The drug-load in the UV-cured films (following the removal of the oxygen-inhibition layer) is shown in Table 5.6. In a similar manner to the drug-loaded DUDMA and HEMA films containing no penetration enhancer, the drug concentrations in the films containing water, MPE, NMP or PEG 200 are similar to that of the uncured gel formulation ($p > 0.05$). The fact that the drug concentration did not increase or decrease in the film following the removal of the oxygen inhibition layer, indicates that even in the presence of the penetration enhancer, there was no drug migration to the film surface or interior during UV-curing.

Table 5.6 Antifungal drug concentration in the gel formulations prior to curing and in UV-cured films following the removal of the oxygen inhibition layer.

DUDMA & HEMA formulation containing	Antifungal		
	AH or TH	Concentration in gel (% w/v)	Concentration in film (% w/w)
ETOH (25% v/v) & No PE	AH	4.0 ± 0.49	4.4 ± 0.04
	TH	6.0 ± 0.22	5.9 ± 0.07
ETOH (15% v/v) & Water (10% v/v)	AH	4.0 ± 0.02	4.2 ± 0.45
	TH	6.1 ± 0.08	5.8 ± 0.23
ETOH (20% v/v) & MPE (5% v/v)	AH	4.1 ± 0.28	4.3 ± 0.31
	TH	6.0 ± 0.22	5.8 ± 0.32
ETOH (20% v/v) & NMP (5% v/v)	AH	4.0 ± 0.21	4.4 ± 0.56
	TH	6.0 ± 0.13	5.9 ± 0.17
ETOH (20% v/v) & PEG 200 (5% v/v)	AH	4.0 ± 0.36	4.4 ± 0.34
	TH	6.0 ± 0.24	5.9 ± 0.19

Abbreviations: AH, amorolfine HCl; TH, terbinafine HCl; ETOH, ethanol; PE, penetration enhancer.

5.5.4 Thermal properties of UV-cured polymer films

5.5.4.1 Polymer degradation

The TGA profiles obtained for films produced from formulations containing the penetration enhancers were comparable to the films produced from the formulations containing no penetration enhancer (Appendix A15). The incorporation of water, MPE, NMP or PEG 200 in a drug-loaded UV-curable gel formulation therefore did not affect the gel's ability to produce a polymeric film that was thermally stable at room temperature.

5.5.4.2 Glass transition

The incorporation of water, MPE, NMP or PEG 200 in a drug-loaded UV-curable gel formulation did however cause noticeable changes in the glass transition temperature of the resulting polymeric film as shown in Table 5.7. The Tg's of the films produced from the formulations containing MPE were significantly lower ($p<0.05$) compared to the Tg's of the films produced from the formulations containing no penetration enhancer, water, NMP or PEG 200, which were similar ($p>0.05$). The lower Tg's for the films produced from the formulations containing MPE could be due to the thiyl radicals formed during the UV-curing of the formulation. It is possible that the thiyl radicals formed interfere with DUDMA's cross-linking ability, and thus increase the flexibility of the polymer chains, which in turn can decrease the Tg of the film.

A lower degree of conversion can also lead to a decrease in polymer cross-linking extent (Chapter 3, Section 3.5.1.2), and hence a decrease in the Tg of a film by increasing the flexibility of the polymer chains. In fact, a positive correlation between degree of cure and Tg has been reported previously (Keenan, 1987, Urbaniak, 2011). Thus, the lower Tg's for the films produced from formulations containing MPE could be also related to their lower degree of conversions from monomer gel to polymer film when compared to the films produced from formulations containing no penetration enhancer, water and NMP (Table 5.4). However, degree of cure does not explain why the Tg's of the films produced from the formulations containing PEG 200 is significantly higher than the Tg's of the films produced from the formulations containing MPE ($p<0.05$), as both formulations have similar degree of conversions from monomer gel to polymer film (Table 5.4). In this instance, it appears that the incorporation of PEG 200 in the formulation, decreases the free volume available for polymer chain mobility due its higher molecular weight (Table 5.1), thus resulting in a higher Tg.

The choice of drug in the formulation did not alter the Tg values obtained ($p>0.05$), and this is consistent with the findings in Chapter 3 which suggest that the drug-load is insufficient to significantly alter Tg in the presence of ethanol (Section 3.5.3.2).

Table 5.7 Tg values of amorolfine HCl- or terbinafine HCl- loaded DUDMA & HEMA gel formulations containing ethanol (ETOH) and different penetration enhancers (PE). Means ± standard deviations are shown, n=3.

	Tg values of DUDMA & HEMA UV-cured films produced from formulations containing	
	4% w/v amorolfine HCl	**6% w/v terbinafine HCl**
ETOH (25% v/v) & No PE	99.2 ± 1.8	94.7 ± 4.8
ETOH (15% v/v) & Water (10% v/v)	103.8 ± 7.9	98.2 ± 7.5
ETOH (20% v/v) & MPE (5% v/v)	65.8 ± 2.1	64.6 ± 0.2
ETOH (20% v/v) & NMP (5% v/v)	98.8 ± 0.3	97.0 ± 2.3
ETOH (20% v/v) & PEG 200 (5% v/v)	106.1 ± 0.3	103.9 ± 3.1

5.5.5 UV-cured polymer films' sensitivity to water

The water sensitivity scores of the UV-cured films produced from amorolfine HCl– or terbinafine HCl– loaded DUDMA & HEMA gel formulations containing no penetration enhancer, water, MPE, NMP or PEG 200 are shown in Figs 5.3 and 5.4, while Fig. 5.5 shows the AUC values calculated from the curves in Figs 5.3 and 5.4.

It can be seen that the films produced from water-containing formulations were the most sensitive to water ($p > 0.05$); they had detached from the substrate within a couple of hours. A possible reasoning is that the trapped water droplets or air bubbles within the film itself (as observed in Table 5.5) limited the film's adhesion to the substrate in the first place, thus facilitating detachment following blister formation. The films produced from formulations containing NMP or PEG 200 had similar water sensitivities ($p > 0.05$), and both were also more water sensitive compared the films containing no penetration enhancer ($p < 0.05$). NMP and PEG 200 are both miscible in water, and as both are incorporated into the UV-cured films, contact with water causes the films to blister. In contrast, the films produced from MPE-containing formulations were as water-resistant as those containing no penetration enhancer ($p > 0.05$), despite the fact that MPE is also miscible in water. The incorporation of MPE in the drug-loaded gel formulations may therefore produce films which adhere to the HDPE substrate to a greater extent than the other penetration enhancer-loaded films, thus minimising the extent to which it blisters when immersed in water. Once again, in a similar manner to previous findings (Chapter 3, Section 3.5.6), the choice of drug in the formulation did not alter the resulting films' water sensitivity ($p > 0.05$).

While the incorporation of water, NMP and PEG 200 in a gel formulation affected the films' ability to remain on the HDPE substrate *in vitro*, the extent to which the films' residence on the nail *in vivo* is affected can only be confirmed by determining the *in vivo* residence of the UV-cured films on fingernails and toenails. Therefore all the formulations were considered for the drug release and ungual drug permeation studies discussed in the following sections.
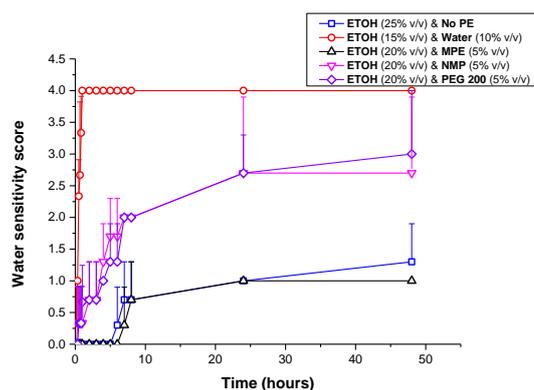
Fig. 5.3 Water sensitivity scores for amorolfine HCl-loaded UV-cured films produced from DUDMA & HEMA gels containing ethanol (ETOH) and different penetration enhancers (PE). Means and standard deviations are shown, n=3.
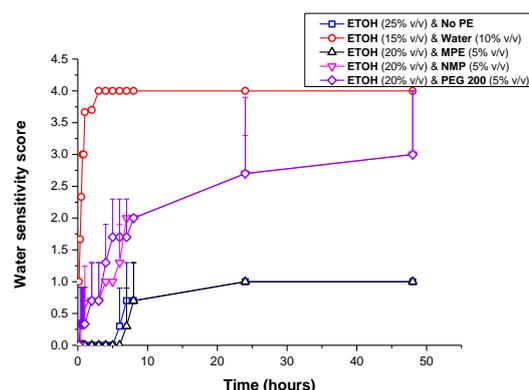
Fig. 5.4 Water sensitivity scores for terbinafine HCl-loaded UV-cured films produced from DUDMA & HEMA gels containing ethanol (ETOH) and different penetration enhancers (PE). Means and standard deviations are shown, n=3.
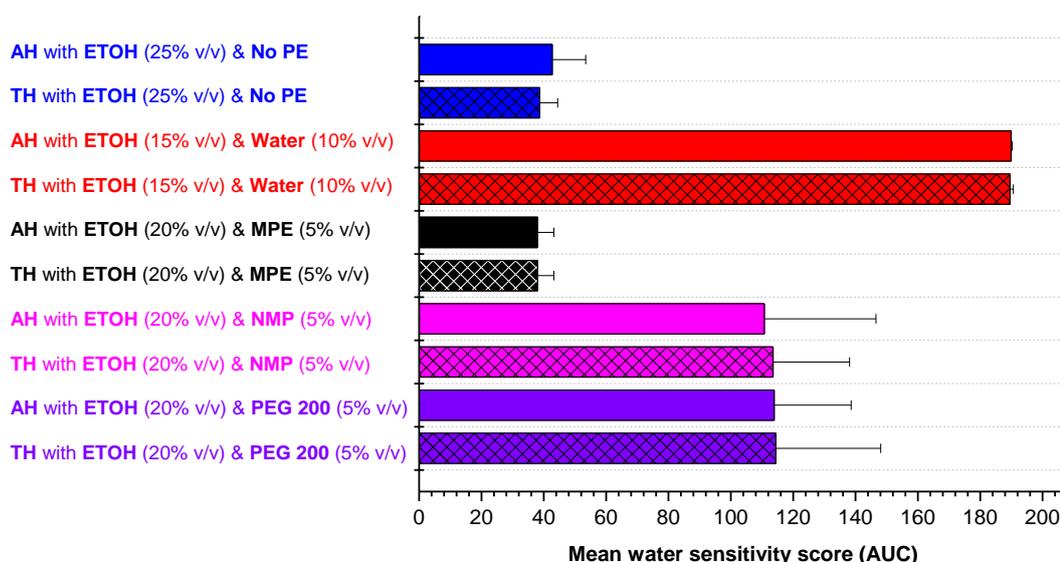
Fig. 5.5 Area under the curve values calculated from the curves in Fig. 5.3 & Fig. 5.4 for the UV-cured films produced from the drug-loaded DUDMA & HEMA gels containing ethanol (ETOH) and different penetration enhancers (PE). Means and standard deviations are shown, n=3. AUC was determined using OriginPro 9.0.

5.5.6 Drug release profiles of UV-cured polymer films

The drug release profiles of the amorolfine HCl-loaded DUDMA & HEMA films containing no penetration enhancer, water, MPE, NMP or PEG 200 are shown in Fig. 5.6, and the corresponding drug release profiles of the terbinafine HCl-loaded DUDMA & HEMA films are shown in Fig. 5.7. The polarised light micrographs of the films pre- and post-release experiment and the total amount of drug released by day 30 are also shown alongside the drug release profiles.

5.5.6.1 Drug release profiles of amorolfine HCl–loaded UV-cured polymer films

In a similar manner to the drug release profile of the amorolfine HCl–loaded formulation containing no penetration enhancer, the drug release profiles of the amorolfine HCl–loaded formulations containing water, MPE, NMP or PEG 200 display a burst of drug release over the first 24 hours due to the release of drug found at the surface of the film. After this point drug release slows, and by day 30 a greater percentage, i.e. between 50 – 75%, of the incorporated drug had been released (Fig. 5.6). Following the release study, all the films remained intact, showed negligible weight change (Fig. 5.8), and showed no signs of drug precipitation (polarised light micrographs in Fig. 5.6). This enabled the evaluation of the drug release kinetics from these films using zero order, first order and Higuchi models. Like the film containing no penetration enhancer, the Higuchi model was found to best describe amorolfine HCl release from the penetration enhancer-loaded films (Table 5.8), and therefore the drug released through the film matrix following the initial burst release was also diffusion-controlled.

When the influence of the different penetration enhancers on drug release was examined, it was found that drug release was the greatest from the formulation containing MPE, followed by the formulations containing PEG 200, NMP, water and no penetration enhancer. However, statistically only the formulations containing MPE, PEG 200 or NMP had greater drug release profiles than the formulation containing no penetration enhancer ($p<0.05$). These penetration enhancers increased drug release by reducing the formulations' degree of conversions from monomer gel to polymer film (Table 5.4). In fact, linear regression analysis confirmed that lower degree of conversions from monomer gel to polymer film was correlated with the higher release of amorolfine HCl ($R^2 = 0.824$, $p<0.05$) (Fig. 5.9). As mentioned previously (Chapter 3, Section 3.5.1.2),

a decrease in the degree of conversion from monomer gel to polymer film produces a film with decreased polymer cross-linking. Thus the penetration enhancers increased release by producing polymeric films with lower cross-link densities and hence a less rigid structure. Interestingly, the differences in the glass transition temperatures of the films did not significantly correlate with drug release ($p<0.05$), most probably as they were outweighed by other formulation properties, such as degree of conversion. Finally, in a similar manner to the film containing no penetration enhancer, the diffusion-controlled release through the penetration enhancer-loaded films' matrix was limited (in that % drug release did not reach 100%), most possibly due to the films' dense interior (Fig. 5.2).

5.5.6.2 Drug release profiles of terbinafine HCl–loaded UV-cured polymer films

In a similar manner to the amorolfine HCl–loaded formulations, the terbinafine HCl–loaded formulations' release profiles showed a burst of drug release over the first 24 hours, which then gradually slowed down (Fig. 5.7). Furthermore, like the amorolfine HCl–loaded films, the terbinafine HCl–loaded films remained intact, showed negligible weight change (Fig.5.8) and showed no signs of drug precipitation post-release (Fig. 5.7). Table 5.8 revealed that the Higuchi model best described terbinafine HCl release from the penetration enhancer-loaded films, and therefore the drug released through the film matrix following the initial burst release (due to the release of drug found at the surface of the film) was also diffusion-controlled.

The pattern of drug release obtained by the terbinafine HCl-loaded formulations containing no penetration enhancer, water, MPE, NMP or PEG 200 was similar to the corresponding amorolfine HCl–loaded formulations. The drug release was the greatest from the formulation containing MPE, followed by the formulations containing PEG 200, NMP, water and no penetration enhancer, and statistically only the formulations containing MPE, PEG 200 or NMP had greater drug release profiles than the formulation containing no penetration enhancer ($p<0.05$). As the choice of drug in the UV-curable gel formulation was not found to or expected to influenced the formulation's degree of conversion (Table 5.4), or the film's microstructure (Section 5.5.2), glass transition temperature (Table 5.7) and water sensitivity (Fig. 5.5), the mechanism by which

enhancement of drug release occurred is the same as that for the amorolfine HCl–loaded formulations, i.e. the penetration enhancers affected drug released by producing polymeric films with differing cross-link densities. However, once again the percentage of drug released by day 30 was significantly lower from the terbinafine HCl-loaded films compared to the amorolfine HCl-loaded films ($p<0.05$) due to possible terbinafine-polymer binding, as suggested in Section 4.6.1.3 (Chapter 4).

The incorporation of water, MPE, NMP or PEG 200 in an amorolfine HCl– or terbinafine HCl– loaded UV-curable gel formulation therefore positively influenced the formulation's ability to release drug, and in the next section, whether the incorporation of water, MPE, NMP or PEG 200 in the drug-loaded UV-curable gel formulation enhances its ungual drug permeation is explored.
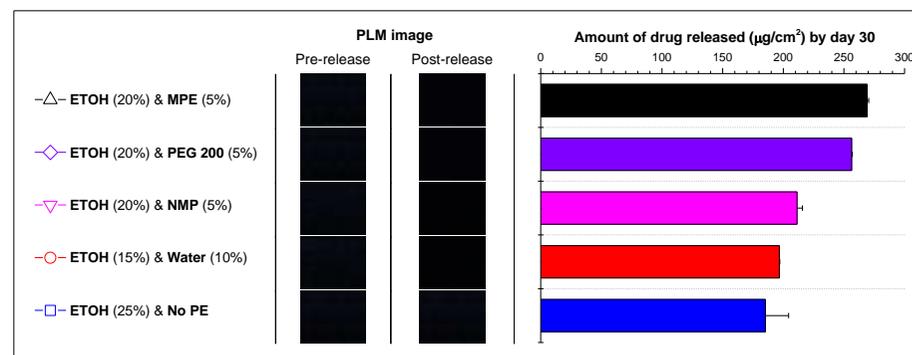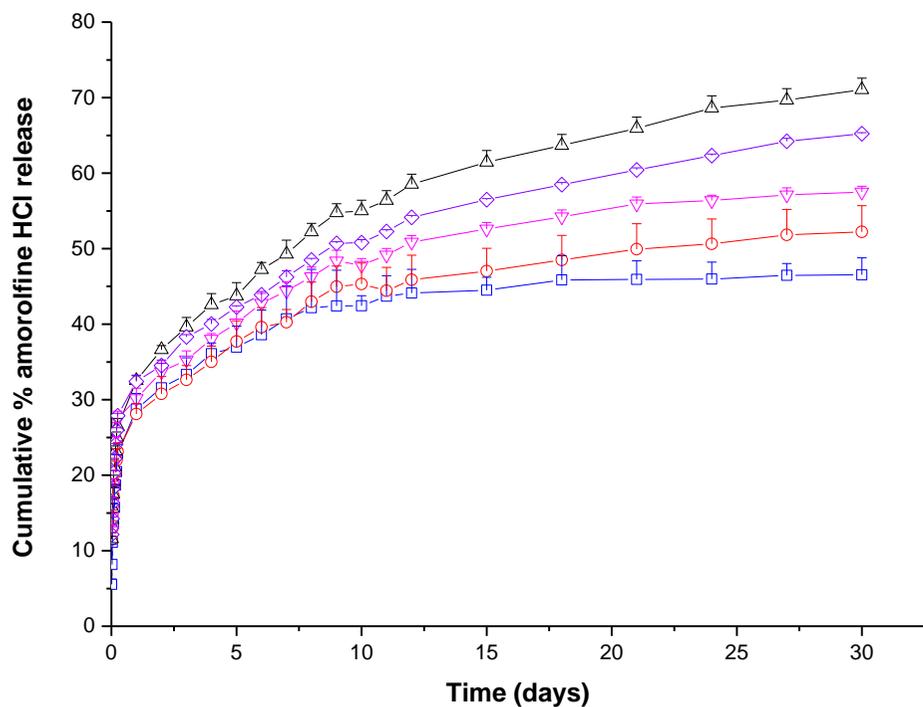
Fig. 5.6 Cumulative % drug release from the 4% w/v amorolfine HCl-loaded DUDMA & HEMA UV-cured films containing ethanol (ETOH) and different penetration enhancers (PE), their corresponding polarised light microscopy images (pre- and post-release) and the amount of drug released by day 30. Means and standard deviations are shown, n=3. Repeated measures ANOVA showed that the formulation containing MPE displays a similar drug release profile to the formulation containing PEG 200 (p>0.05) and greater drug release profile than the formulations containing NMP, water and no penetration enhancer (p<0.05). The formulation containing PEG 200 also has a similar drug release profile to the formulation containing NMP (p>0.05), and both have greater drug release profiles than the formulations containing water and no penetration enhancer (p<0.05), which in turn have similar drug release profiles (p>0.05).

Fig. 5.7 Cumulative % drug release from the 6% w/v terbinafine HCl-loaded DUDMA & HEMA UV-cured films containing ethanol (ETOH) and different penetration enhancers (PE), their corresponding polarised light microscopy images (pre- and post-release) and the amount of drug released by day 30. Means and standard deviations are shown, n=3. Repeated measures ANOVA showed that the formulation containing MPE displays a similar drug release profile to the formulation containing PEG 200 ($p > 0.05$) and greater drug release profile than the formulations containing NMP, water and no penetration enhancer ($p < 0.05$). The formulation containing PEG 200 also has a similar drug release profile to the formulation containing NMP ($p > 0.05$), and both have greater drug release profiles than the formulations containing water and no penetration enhancer ($p < 0.05$), which in turn have similar drug release profiles ($p > 0.05$).

Fig. 5.8 Weight change (%) for UV-cured films produced from drug-loaded DUDMA & HEMA gels containing ethanol (ETOH) and different penetration enhancers (PE) following drug-release studies. Means and standard deviations are shown, n=3. Abbreviations: AH, amorolfine HCl; TH, terbinafine HCl.

Table 5.8 R^2 values obtained for drug release modelling using zero order, first order and Higuchi models.

Formulation	R^2 value obtained for		
	Zero order	First order	Higuchi model
4% w/v AH with ETOH (25% v/v) & No PE	0.75	0.79	**0.89**
6% w/v TH with ETOH (25% v/v) & No PE	0.90	0.92	**0.98**
4% w/v AH with ETOH (15% v/v) & water (10% v/v)	0.82	0.85	**0.93**
6% w/v TH with ETOH (15% v/v) & water (10% v/v)	0.91	0.92	**0.98**
4% w/v AH with ETOH (20% v/v) & MPE (5% v/v)	0.81	0.86	**0.93**
6% w/v TH with ETOH (20% v/v) & MPE (5% v/v)	0.85	0.87	**0.95**
4% w/v AH with ETOH (20% v/v) & NMP (5% v/v)	0.77	0.81	**0.89**
6% w/v TH with ETOH (20% v/v) & NMP (5% v/v)	0.85	0.86	**0.95**
4% w/v AH with ETOH (20% v/v) & PEG 200 (5% v/v)	0.77	0.82	**0.90**
6% w/v TH with ETOH (20% v/v) & PEG 200 (5% v/v)	0.85	0.87	**0.95**
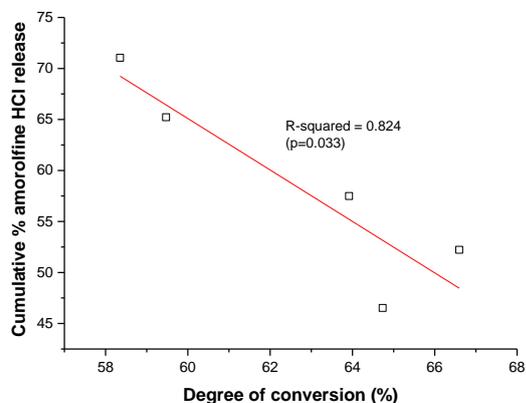
Fig. 5.9 Influence of degree of conversion from monomer gel to polymer film on amorolfine HCl release from the polymer film.

5.5.7 Ungual drug permeation profiles of UV-cured polymer films

The ungual drug permeation profiles of the films containing no penetration enhancer, water, MPE, NMP or PEG 200 are shown in Figs 5.10 & 5.11 for amorolfine HCl and terbinafine HCl respectively. The % of drug that permeated across the nail and that remained in the nail at day 30 is also shown alongside the permeation profiles, while the calculated lag times, study-state flux, apparent permeability coefficients, effective diffusion coefficients and amount of drug in nail clippings are shown in Table 5.9.

5.5.7.1 Amorolfine HCl–loaded UV-cured polymer films

In a similar manner to the ungual drug permeation profile of the amorolfine HCl-loaded DUDMA & HEMA formulation containing no penetration enhancer, the ungual drug permeation profiles of the formulations containing water, MPE, NMP or PEG 200 show a lengthy lag period prior to a linear increase in the cumulative amount of drug permeated across the nail (Fig. 5.10). However, the film containing MPE allows greater ungual drug permeation ($p<0.05$) compared to the films containing NMP, PEG 200 and water, which have similar ungual drug permeation profiles ($p>0.05$), and all the films apart from the film containing water display greater ungual drug permeation profiles compared to the film containing no penetration enhancer ($p<0.05$).

Table 5.9 shows that the MPE-loaded film obtained a shorter lag time ($p<0.05$) and an increased steady-state flux ($p<0.05$), permeability coefficient ($p<0.05$) and diffusion coefficient ($p<0.05$) compared to the film containing no penetration enhancer. It additionally obtained a higher percentage of drug permeated across the nail ($p<0.05$) and percentage of drug remaining in the nail at the end of the permeation experiment ($p<0.05$) (Fig 5.10). It therefore appears that despite MPE's possible role in facilitating the polymerisation of the gel formulation upon UVA-irradiation (as discussed in Section 5.5.1.1), some MPE molecules are still available intact to permeate into the nail, disrupt the disulphide bonds of nail keratin, and thus reduce the time taken for the drug to reach a uniform concentration in the membrane and increase ungual drug permeation.

In comparison, the incorporation of NMP, PEG 200 or water in a UV-cured film did not significantly influence the films' lag time, steady-state flux, permeability coefficient, diffusion coefficient and percentage of drug permeated across the nail at the end of the

permeation experiment ($p>0.05$) when compared to the film containing no penetration enhancer. These enhancers did however increase the percentage of drug remaining in the nail at the end of the permeation experiment ($p<0.05$) to similar extents. While water, NMP and PEG 200 enhance ungual drug permeation by promoting nail swelling (Nair et al., 2010, Hossin et al., 2016), and thus form larger pores through which drug can easily diffuse; in the permeation experiment set up used, the under-surface of the nail was in direct contact with the receptor phase which would also have promoted nail swelling to an extent. Therefore, NMP– and PEG 200– loaded films may have increased the drug-in-nail percentage by promoting greater water uptake (from the receptor phase) and hence further swelling of the nail, or the NMP–, PEG 200– and water– loaded films may have increased drug-in-nail percentage by swelling the surface of the nail in contact with the film.

5.5.7.2 Terbinafine HCl–loaded UV-cured polymer films

The terbinafine HCl–loaded formulations' permeation profiles also show fairly lengthy lag times followed by linear increases in the cumulative amount of drug permeated (Fig. 5.11). However, in a similar manner to the amorolfine HCl–loaded films, the film containing MPE allows greater ungual drug permeation ($p<0.05$) compared to the films containing NMP, PEG 200, and water, and all the films apart from the film containing water display greater ungual drug permeation profiles compared to the film containing no penetration enhancer ($p<0.05$).

Table 5.9 shows that once again the MPE-loaded film obtained a shorter lag time ($p<0.05$) and an increased diffusion coefficient ($p<0.05$) compared to the film containing no penetration enhancer. It additionally obtained a higher percentage of drug permeated across the nail ($p<0.05$) and percentage of drug remaining in the nail at the end of the permeation experiment ($p<0.05$) (Fig. 5.11). The reason behind this is as explained in the previous section. Furthermore, the water–, NMP– and PEG 200– loaded films, all increased the percentage of drug remaining in the nail at the end of the permeation experiment ($p<0.05$) to similar extents, compared to the film containing no penetration enhancer. The reason for which is also as explained in the previous section.

Interestingly, Fig. 5.11 also shows that while the films containing NMP and PEG 200 have similar ungual drug permeation profiles ($p>0.05$), the film containing NMP has a greater ungual drug permeation profile than the film containing water ($p<0.05$), whereas the film containing PEG 200 does not ($p>0.05$). Therefore of the terbinafine HCl-loaded formulations containing NMP and water, the formulation containing NMP appears to be superior in terms of enhancing ungual drug permeation. It appears that despite water being able to promote nail swelling to a greater extent when compared to NMP (Hossin et al., 2016), the possible entrapment of water in the film (Table 5.5) limits its ability to facilitate the transport of drug across the nail plate. This also explains why amorolfine HCl– or terbinafine HCl– loaded films containing water have comparable ungual drug permeation profiles to the films containing no penetration enhancer ($p<0.05$). In addition, the similarity in the ungual drug permeation profiles of the NMP– and PEG 200– loaded films suggests that both NMP and PEG 200 promote similar degrees of nail swelling.

Overall, it therefore appears that the incorporation of MPE, NMP or PEG 200 in an amorolfine HCl– or terbinafine HCl– loaded DUDMA & HEMA gel formulation enhances ungual drug permeation, with MPE as the strongest enhancer. Thus it seems that disrupting the disulphide linkages in the keratin matrix of the nail is more effective than promoting nail swelling, (with NMP or PEG 200 incorporation), for enhancing the ungual drug permeation of pharmaceutical UV-curable gel formulations.

5.5.7.3 Amorolfine HCl–loaded vs. terbinafine HCl–loaded UV-cured polymer films

When compared to the amorolfine HCl-loaded gel formulations, the terbinafine formulations obtain comparable lag times and diffusion coefficients ($p>0.05$), due to the small difference in the molecular weights of the two drugs.

However, the amorolfine HCl-loaded gel formulations obtained a significantly higher steady-state flux, permeability coefficient and percentage of drug permeated across the nail at day 30 ($p<0.05$) when compared to the terbinafine HCl-loaded formulations, due to amorolfine's weaker nail keratin-binding ability when compared to terbinafine. These

findings are consistent with the findings in Chapter 4 (Section 4.6.2.3), and confirm the significant impact of drug–nail keratin binding on ungual drug permeation. Nonetheless as also mentioned in Section 4.6.2.3, drug–nail keratin binding can be beneficial in the long run.
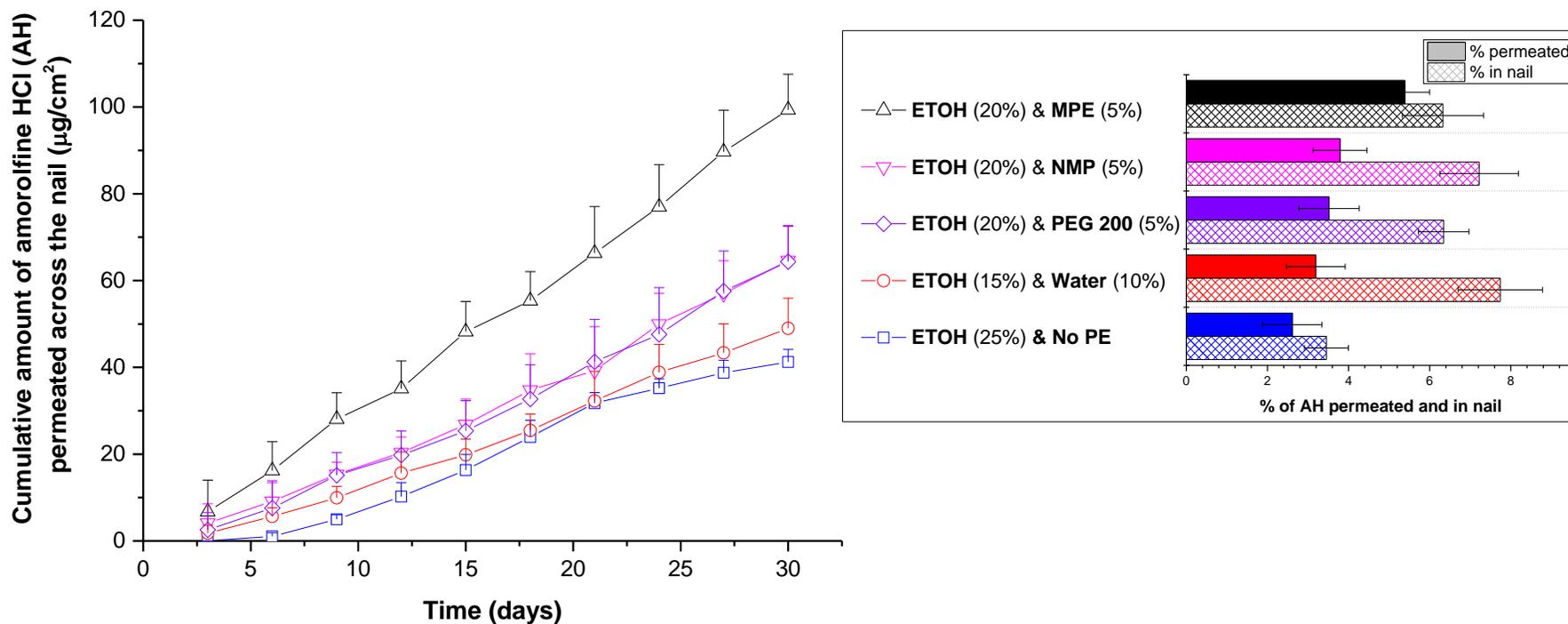
Fig. 5.10 Cumulative amount of amorolfine HCl permeated across the nail with time from the 4% w/v amorolfine HCl-loaded DUDMA & HEMA UV-cured films containing ethanol (ETOH) and different penetration enhancers (PE), and the % of amorolfine HCl permeated across the nail and remaining in the nail at day 30. Means and standard deviations are shown, n=6. A mass balance had been carried out for this experiment to ensure that most of the drug had been retrieved from the modified Franz diffusion cell, and that most of the drug remaining within the nail tissue had been extracted (Appendix A16).
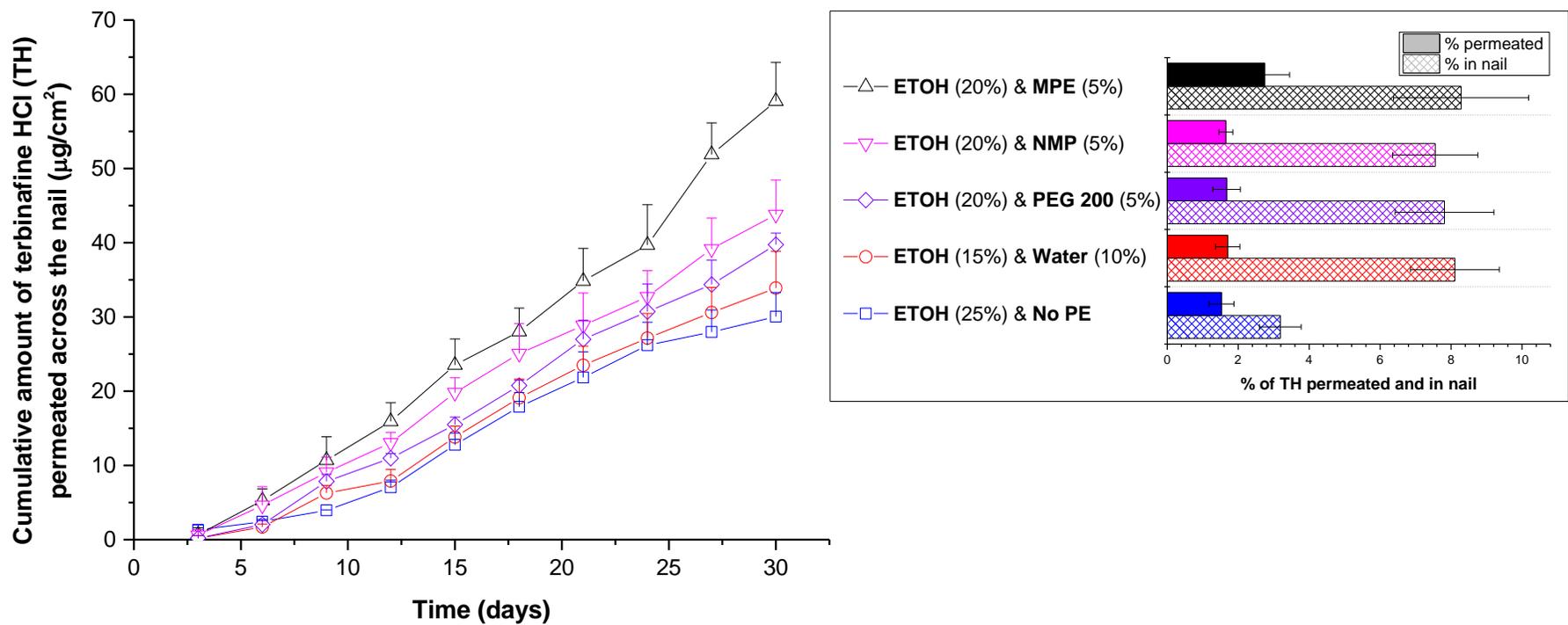
Fig. 5.11 Cumulative amount of terbinafine HCl permeated across the nail with time from the 6% w/v terbinafine HCl-loaded DUDMA & HEMA UV-cured films containing ethanol (ETOH) and different penetration enhancers (PE), and the % of terbinafine HCl permeated across the nail and remaining in the nail at day 30. Means and standard deviations are shown, n=6. A mass balance had been carried out for this experiment to ensure that most of the drug had been retrieved from the modified Franz diffusion cell, and that most of the drug remaining within the nail tissue had been extracted (Appendix A16).

Table 5.9 Lag time, steady-state flux, apparent permeability coefficient, effective diffusion coefficient and amount of drug in nail clippings. Means and standard deviations are shown, n=6.

DUDMA & HEMA formulation containing	4% w/v Amorolfine HCl					6% w/v Terbinafine HCl				
	Lag time (day)	Steady-state flux (µg/cm^2/day)	Permeability coefficient x 10^{-5} (cm/day)	Diffusion coefficient x 10^{-5} (cm^2/day)	Drug in nail clipping (mg/cm^3)	Lag time (day)	Steady-state flux (µg/cm^2/day)	Permeability coefficient x 10^{-5} (cm/day)	Diffusion coefficient x 10^{-5} (cm^2/day)	Drug in nail clipping (mg/cm^3)
Ethanol (20% v/v) & MPE (5% v/v)	1.4 ± 1.1	3.4 ± 0.5	8.5 ± 1.1	24.0 ± 16.4	1.2 ± 0.3	3.2 ± 2.3	2.0 ± 0.4	3.3 ± 0.7	12.4 ± 8.1	1.6 ± 0.2
Ethanol (20% v/v) & NMP (5% v/v)	3.2 ± 2.8	2.4 ± 0.6	6.0 ± 1.6	10.3 ± 8.8	1.4 ± 0.3	3.2 ± 0.9	1.6 ± 0.2	2.7 ± 0.3	6.6 ± 2.3	2.1 ± 0.5
Ethanol (20% v/v) & PEG 200 (5% v/v)	3.4 ± 3.0	2.4 ± 0.7	6.0 ± 1.8	12.8 ± 12.7	1.3 ± 0.3	5.4 ± 1.8	1.7 ± 0.3	2.8 ± 0.5	2.8 ± 1.0	2.4 ± 0.4
Ethanol (15% v/v) & Water (10% v/v)	4.4 ± 2.2	2.0 ± 0.4	4.9 ± 0.9	7.8 ± 5.2	1.1 ± 0.2	6.6 ± 0.8	1.6 ± 0.2	2.7 ± 0.3	4.5 ± 1.3	1.5 ± 0.4
Ethanol (25% v/v) & No PE	7.1 ± 1.7	2.2 ± 0.3	5.5 ± 0.7	3.5 ± 1.4	0.6 ± 0.1	7.1 ± 1.0	1.6 ± 0.2	2.6 ± 0.4	3.0 ± 0.8	0.7 ± 0.2

Abbreviations: ETOH, ethanol; PE, penetration enhancer.

5.6 Conclusions

It is possible to incorporate either 10% v/v of water, 5% v/v of MPE, 5% v/v of NMP or 5% v/v of PEG 200 in a 4% w/v amorolfine HCl– or 6% w/v terbinafine HCl– loaded DUDMA & HEMA UV-curable gel formulation without negatively affecting the formulation's mass yield from monomer gel to polymer film, and the film's visual appearance, drug-load, thermal degradation, glass transition, and drug release and ungual drug permeation.

When considering the influence of these agents on the polymerisation process, the incorporation of MPE and PEG 200 decreased the degree of conversion from monomer gel to polymer film and increased the levels of residual monomers in the polymer film. However despite this, the amounts of residual monomers remaining in the films were negligible and therefore considered acceptable. In terms of the films' microstructure, the formulations containing water, MPE, NMP or PEG 200 produced films with a dense interior, and the incorporation of water in the gel formulation appeared to produce films which contained trapped water droplets or air bubbles. Finally, when considering the films' glass transition and water sensitivity, MPE was the only agent which reduced the glass transition of the film produced following its incorporation in the gel, and the only one not to negatively affect the film's water sensitivity.

The drug release patterns obtained for both the amorolfine HCl– and terbinafine HCl– loaded formulations containing water, MPE, NMP or PEG 200 were found to be related to the formulations' degree of conversion from monomer gel to polymer film (and hence cross-linking). Once again, the release of terbinafine from the films was less than that of amorolfine, due to possible terbinafine-polymer binding.

Ungual permeation studies revealed that MPE, NMP and PEG 200 were capable of improving ungual amorolfine and terbinafine permeation when present in a UV-curable gel vehicle. Overall, MPE was found to be the superior of the penetration enhancers, not only because of the significant enhancement of ungual drug permeation, but for being the least likely to affect the UV-cured film's residence on the nail.

Chapter 6: Conclusions and future work

6.1 Introduction

The human nail acts as a fairly impervious barrier, presenting a challenge to the treatment of common nail diseases such as onychomycosis and nail psoriasis. In the past, treatment relied mainly on systemic therapy, which is associated with a number of serious side effects, drug interactions, contraindications and high recurrence rates. However, the recognition that drug penetration into the nail, although difficult, is achievable, sparked a race towards the development of topical therapies. Various topical nail drug delivery vehicles have been investigated to date, including solutions, lacquers, gels, ointments, creams, films, patches and colloidal carriers, and considerable effort has been made to enhance ungual drug delivery using physical, chemical, or a combination of physical and chemical means. Unfortunately, despite these efforts, currently available topical therapies for nail diseases such as onychomycosis and nail psoriasis remain unsatisfactory. Treatment failure could be a result of the limited penetration of the therapeutic agent through the nail plates' firmly bound layers of dead keratinous cells and compact dorsal structure. However, the formulations' inability to maintain therapeutic drug levels at the desired site and poor patient compliance can also contribute to the poor treatment response, especially when considering that topical therapies require regular application until all the affected nail tissue has grown out, a process which can take up to several months. There appears to be a special need for a topical nail medicine capable of overcoming these limitations. In this thesis, pharmaceutical UV gels were proposed as such a nail medicine, as UV gels are known to have a long residence on the nail plate and can therefore address the issues highlighted, i.e. the need for frequent applications and failure to maintain therapeutic drug levels at the desired site. The pharmaceutical potential of the UV gels was determined by formulating and characterising the gel and film as depicted in Fig. 6.1, while using antifungal drugs as model drugs, and the summary of the key findings and recommendations for future research are discussed in this Chapter.

UV-curable gel optimisation using penetration enhancers

UV-cured film characteristics
- Mass yield
- DC and cross-link extent
- Residual monomer content
- Morphology, thickness, microsturcture and porosity
- Drug-load
- Drug-polymer interactions
- Thermal stability and glass transition temperature
- Occlusivity, adhesivity and water sensitivity
- *In vivo* nail residence
- Film stability and drug in film stability
- Drug release and ungual drug permeation
- *In vitro* efficacy against *T. rubrum*

UV-curing conditions
- UVA device
- Cure time

UV-curable gel properties
- Gel viscosity
- Drug stability

Actives and excipients
- Choice and quantity

Fig. 6.1 Schematic diagram highlighting the investigations to assess the pharmaceutical potential of UV-curable gel formulations.

6.2 Summary of key findings

It is possible to formulate a pharmaceutical UV-curable gel formulation containing an antifungal (amorolfine HCl or terbinafine HCl) when incorporated within DUDMA-based UV gels containing the photoinitiator 2-hydroxy-2-methylpropiophenone, a solvent such as ethanol and one of three different reactive diluent methacrylate-based monomers (EMA, IBOMA or HEMA). The solvent, at a concentration of 25% v/v, was found to be necessary for drug incorporation into the gel, and for such formulations, a DUDMA to reactive diluent monomer ratio of 85:15 % v/v and a photoinitiator concentration of 3% v/v was found to be optimal. Formulations containing HEMA, with a DUDMA to reactive diluent monomer ratio of 75:25% v/v and no solvent, also enabled drug incorporation due to the greater drug solvency of HEMA. Penetration enhancers such as water (10% v/v), MPE (5% v/v), NMP (5% v/v) or PEG 200 (5% v/v) could also be incorporated into drug-loaded DUDMA & HEMA UV-curable gel formulations containing ethanol.

These formulations, when applied on the human nail and cured for 2 minutes under a UVA nail lamp, were capable of producing visually smooth and transparent films, all of which were capable of enabling sufficient ungual drug permeation *in vitro* to inhibit the growth of the most common onychomycosis-causing pathogen – *T. rubrum*. However there were differences amongst the UV-cured films regarding other properties, based on monomer choice, monomer ratio, presence or absence of ethanol, and nature of drug and of penetration enhancer, as summarised below. How UV gels compare to a commercially available pharmaceutical nail lacquer as a form of topical nail medicine is also summarised below.

6.2.1 Influence of reactive diluent monomer choice on UV-cured film properties

The nature of the reactive diluent methacrylate-based monomer used in the gel was found to influence the final viscosity of the gel formulation, which in turn affected the glass transition temperature of the resulting polymer film. However, the differences in the glass transition temperatures of the different films did not influence drug release from the films. Of the reactive diluent methacrylate-based monomers used, HEMA had a higher solvency for the antifungal drugs as mentioned above. As a result, in the presence of ethanol, the maximum drug-load in the DUDMA & HEMA gel was much higher (4% w/v amorolfine HCl or 6% w/v terbinafine HCl) compared to the drug-load in the DUDMA & EMA or DUDMA & IBOMA gels (3% w/v amorolfine HCl or 4% w/v terbinafine HCl). The films' drug release and ungual drug permeation were found to increase as the drug concentration in the film was increased. The nature of the diluent monomer therefore influenced drug release and ungual drug permeation via its influence on drug concentration in the film.

6.2.2 Influence of monomer ratio on UV-cured film properties

As mentioned earlier, increasing the proportion of the HEMA monomer in a UV-curable gel formulation to produce a formulation with the DUDMA: HEMA ratio of 75:25% v/v, (as opposed to 85:15% v/v) made it possible to incorporate antifungals in the gel without the need for ethanol. However, the total drug content in the film was fairly low (2% w/v for both amorolfine HCl and terbinafine HCl compared to the 4% w/v amorolfine HCl and 6% w/v terbinafine HCl with ethanol). Nonetheless, the higher HEMA

concentration in the gel did reduce the formulation's viscosity, which in turn increased its polymerisation, and subsequently its glass transition temperature.

6.2.3 Influence of ethanol incorporation on UV-cured film properties

Ethanol was incorporated in the gel for its ability to promote and increase drug loading. Its inclusion reduced the formulations' viscosity, which in turn increased its polymerisation, and subsequently the resulting film's glass transition temperature. However, its inclusion also negatively affected the film's *in vivo* residence on the nail, which would mean more frequent medicine application. Nonetheless, the greater drug loading enabled by ethanol resulted in higher drug release and ungual flux.

6.2.4 Influence of drug nature and presence on UV-cured film properties

The presence of the drugs – amorolfine HCl and terbinafine HCl – had negligible influence on the films' properties such as its morphology, structure, glass transition temperature, adhesion, water sensitivity and *in vivo* nail residence. In contrast, the drug nature did have a considerable influence on drug release and ungual flux. Amorolfine release from the film was much higher compared to that of terbinafine, possibly due to terbinafine-polymer binding. Similarly, amorolfine's ungual flux was higher compared to terbinafine's due to the latter's higher affinity for nail keratin. Nonetheless, as terbinafine HCl has a smaller MIC against the most common onychomycosis-causing pathogen, and presence of drug in the nail as well as drug movement out of the nail and into the nail bed is important, both drugs show potential when loaded in a UV-curable gel formulation for the treatment of onychomycosis.

6.2.5 Influence of penetration enhancer on UV-cured film properties

The incorporation of water, MPE, NMP or PEG 200 in 4% w/v amorolfine HCl– or 6% w/v terbinafine HCl– loaded DUDMA & HEMA UV-curable gel formulations containing ethanol did not negatively affect the formulations' mass yield from monomer gel to polymer film, and the films' visual appearance, drug-load, thermal degradation, drug release and ungual drug permeation. However, following its incorporation in the gel, MPE was the only agent not to negatively affect the film's water-resistance. Furthermore, MPE incorporation in the UV gel enhanced ungual drug permeation to the greatest extent compared to the other penetration enhancer-loaded formulations and

the penetration enhancer-free control. Disrupting the disulphide linkages in the keratin matrix of the nail is therefore more effective than promoting nail swelling, (with water, NMP or PEG 200 incorporation), for enhancing the ungual drug permeation of pharmaceutical UV-curable gel formulations.

6.2.6 UV-curable gels vs. nail lacquers

Comparisons between the Curanail® nail lacquer (which contains 5% w/v amorolfine HCl) with the best UV gel formulation (penetration enhancer-free) showed that drug release from the UV-cured polymer film was significantly lower than that from the nail lacquer film, possibly due to the UV-cured film's cross-linked and highly dense nature. Nevertheless, both lacquer and UV gel films displayed similar ungual drug permeation profiles, and this in turn confirmed the critical role played by the nail plate barrier to ungual drug permeation and the lesser role of drug release from the film. In comparison to the Curanail nail lacquer®, the UV-curable gel formulations reduced TOWL to a greater extent and resided on fingernails for considerably longer (even when containing ethanol); they therefore appear to be a more favourable form of topical nail medicine.

6.3 Considerations for future work

In the previous Chapters, the following was suggested for future work:

(i) measuring the cross-link densities of the UV-cured films using inverse gas chromatography (Chapter 3, Section 3.5.1.2),

(ii) determining whether the application of a UV-curable gel formulation reduces TOWL of diseased nails to the same extent as healthy nails (Chapter 3, Section 3.5.4),

(iii) determining the affinity of amorolfine and terbinafine to the UV-cured films (Chapter 4, Section 4.6.1.3), and

(iv) optimising the adapted disc diffusion method developed for testing the efficacies of topical nail formulations against *T. rubrum* (Chapter 4, Section 4.6.3).

Details as to why the above were suggested for future work and specifics about the experiments considered can be found in the relevant sections.

Other considerations for future work include looking at ways to further improve the pharmaceutical UV-curable gel formulation and thus guarantee treatment success, these are discussed below.

6.3.1 Physical approach to enhance the ungual drug delivery of pharmaceutical UV-curable gels

While the incorporation of a penetration enhancer (in particular MPE) in the pharmaceutical UV-curable gel enhanced ungual drug permeation, it could be further improved by also using a physical means to enhance ungual permeation. In Chapter 1, the dorsal layer of the nail plate was identified as the main barrier to permeant transport through the nail plate and into the nail bed, and methods of disrupting this layer by physical means to enhance ungual permeation were reviewed. One such technique which has shown success was nail filing using an abrasive (Pittrof et al., 1992, Lauharanta, 1992), which enhances permeation by decreasing the thickness of the dorsal layer. Furthermore, it increases the surface roughness and hence surface area of the nail as shown in Table 6.1, which in turn provides a greater opportunity for drug-loaded films to adhere to the nail plate and thereby facilitate drug permeation.

Table 6.1 Scanning electron micrographs of the dorsal surface and the cross-section of a human nail plate clipping, with and without filing the dorsal surface with the abrasive stick provided with the Curanail® nail lacquer.

	'Control' nail clipping	'Filed' nail clipping
Nail plate clipping dorsal surface		
Nail plate clipping cross-section		
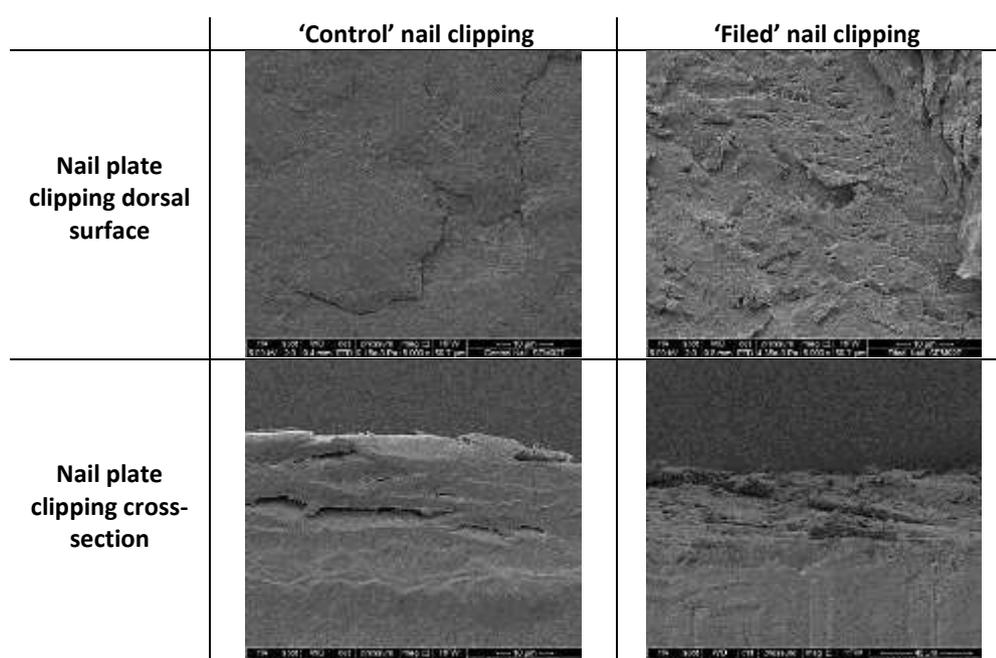
Coincidently, prior to the application of commercially available cosmetic UV gels, the nail is lightly filed with a low grit abrasive file to increase its surface area and ensure good adhesion. Therefore, disrupting the dorsal layer of the nail plate with an abrasive file prior to the application of a drug-loaded topical formulation to improve film adhesion and enhance ungual drug permeation further, seems fitting for a pharmaceutical UV gel. Hence as part of future work, the influence of nail filing on a UV-cured film's adhesion and ungual drug permeation can be investigated.

6.3.2 3D printed pens as a delivery device for UV-curable gel formulations to improve formulation application procedure

When considering the application process of a UV-curable gel formulation, the procedure is not so straight forward. Not only is there a need for a UVA nail lamp to facilitate the polymerisation of the gel formulation, the film that subsequently forms contains an oxygen inhibition layer which requires removal with a nail wipe soaked in alcohol. One way of addressing the issues highlighted is by investigating hand-held 3D printing pens that emit UV (such as CreoPop) as a formulation delivery device, where the ink inside the pen is replaced with the pharmaceutical UV-curable gel formulation. The application of the UV-curable gel formulation on the nail plate via a CreoPop-like device, would eliminate the need for a UVA nail lamp, prevent the formation of an oxygen inhibition layer and cut down total cure time, all of which could further tackle the issue of poor patient compliance.

6.4 Conclusions

It has been demonstrated that UV-curable gel formulations can be considered as an alternative vehicle for the delivery of topical nail medicines. Their use addresses current topical formulation issues such as

- (i) the need for frequent applications and
- (ii) failure to maintain therapeutic drug levels at the desired site

by residing on the nail longer than other nail formulations, like nail lacquers for example.

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