Appendices

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A1. HPLC method validation

HPLC was used for the analyses of antifungal, i.e. amorolfine HCl or terbinafine HCl, containing samples. HPLC assays for the two antifungals in ethanol were used to quantify the saturation solubilities of the drugs in different monomers and to quantify drug present inside the nail (following extraction), and assays in a pH 5 phosphate buffer solution (PBS) were used to quantify drug concentrations in drug release and permeation experiments. Details of the HPLC method conditions used for each drug are shown in Chapter 2 (Table 2.2). The HPLC method was validated for specificity, calibration and linearity, accuracy and precision, and limit of detection (LoD) and limit of quantification (LoQ), and was carried out in accordance to the School of Pharmacy's standard operating procedure SOP-014 (Gill, 2014).

A1.1 Validity parameters

A1.1.1 Specificity

Specificity is the ability to accurately and specifically measure the analyte of interest, (i.e. the antifungal drugs), in the presence of other components that may be expected to be present in the sample, (such as residual monomers and solvents). Specificity was therefore established by (i) injecting blank solutions of ethanol and pH 5 phosphate buffer to assess interference from the solutions used in the preparation of standards and/or mobile phase, and (ii) injecting ethanolic solutions of excipients present in the formulations with and without known quantities of drug to ensure that there was no interference from the formulation's excipients.

A1.1.2 Calibration and linearity

Stock solutions of 200 µg/ml and 50 µg/ml were prepared by accurately weighing the required quantity of drug, (20mg for the 200 µg/ml solution and 5mg for the 50 µg/ml solution), and dissolving it in ethanol or pH 5 PBS in a 100 ml volumetric flask. Subsequently, standard solutions (with concentrations ranging between 0.5 to 200 µg/ml) of the two antifungals were obtained by diluting the stock solutions with ethanol or pH 5 PBS where needed. These standard solutions were prepared in triplicates. Calibration curves were constructed by assaying the standard solutions. The linearity of

the HPLC detection assays was assessed by plotting the peak area against the drug concentration.

A1.1.3 Accuracy and precision

Accuracy is the level of agreement between the true value and measured value (Lindsay and Barnes, 2003). The accuracy of the analysis obtained for each drug (n=3) expressed as a percentage of the expected concentration (also known as percentage recovery), was determined according to Equation 1.

$$\text{Accuracy} = \frac{C_c}{C_s} \times 100 \qquad \textbf{Equation 1}$$

where C_c is the calculated concentration and C_s is the expected standard concentration.

Precision is the measure of the variability of a set of measurements and is usually expressed as a percent relative standard deviation (% RSD) (Jones, 2002), as shown in Equation 2.

$$\%\ \text{RSD} = \frac{\text{SD}}{[\text{Drug}]} \times 100 \qquad \textbf{Equation 2}$$

The precision of the methods was determined by evaluating repeatability (intra-day) and intermediate precision (inter-day). Repeatability was determined by performing three repeated analysis of the same standard solution on the same day, under the same experimental conditions. The intermediate precision was assessed by carrying out the analysis on three different days. For each drug, the percentage recovery of the standard solutions and % RSD were determined.

A1.1.4 Limit of detection (LoD) and limit of quantification (LoQ)

The LoD is the lowest concentration of an analyte in a sample that can be detected, not quantified. LoDs were determined based on the standard deviation of the response and the slope, as shown in Equation 3.

$$\mathrm{LoD} = \frac{3.3\sigma}{\mathrm{S}} \qquad \textbf{Equation 3}$$

where σ= the standard deviation of the response which is standard error of the predicted 'y' value (AUC response value) for each 'x' (concentration of standard) in the regression (which can be determined from the y-intercepts of regression lines), and S is the slope of the calibration curve.

The LoQ is the lowest concentration of any analyte in a sample that can be determined with acceptable precision and accuracy. Similarly to LoD, LoQs were determined based on the standard deviation of the response and the slope, as shown in Equation 4.

$$\mathrm{LoQ} = \frac{10\sigma}{\mathrm{S}} \qquad \textbf{Equation 4}$$

A1.2 Amorolfine HCl HPLC method validation

Amorolfine HCl was easily discriminated from the solutions used in the preparation of the standards and the mobile phase, as well as the excipients present in UV-curable gel formulations during a HPLC run. The method is therefore of an acceptable specificity.

The calibration curve of standards, prepared both in ethanol and the pH 5 PBS, were linear over the analysed concentration ranges, with $R^2 > 0.999$, as shown in Fig. A1.1. The % RSD values calculated to determine precision were ≤ 1 in all cases as shown in Table A1.1, and the % recovery values calculated to determine accuracy were between 98 – 102% in all cases as shown in Table A1.2. The method is therefore of an acceptable accuracy, precision and linearity over the tested concentration ranges.

Furthermore, the LoD and LoQ values (Table A1.2) are low and therefore also acceptable, thus enabling quantification of the lower concentration ranges required for drug permeation studies.

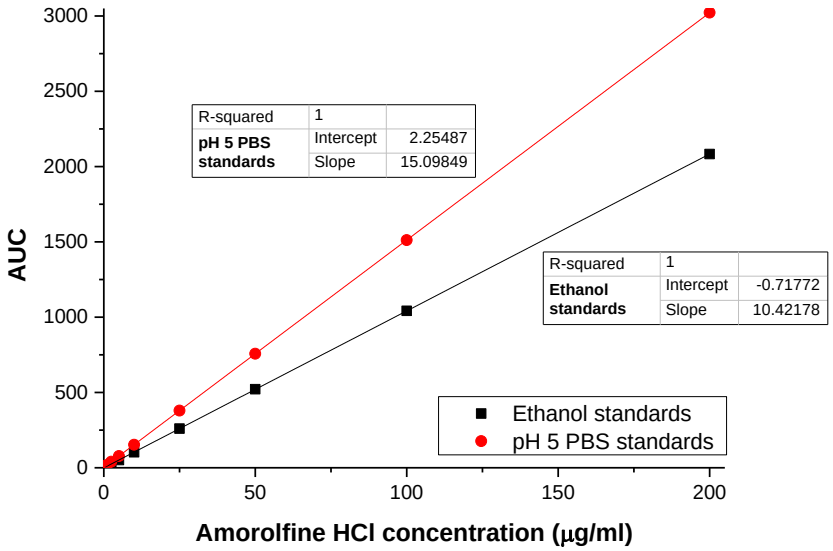


Fig. A1.1 Calibration curves of amorolfine HCl HPLC assays.

Table A1.1 Precision of amorolfine HCl HPLC assays.

Ethanol standards			pH 5 PBS standards		
Standard concentration (µg/ml)	Intra-day % RSD	Inter-day % RDS	Standard concentration (µg/ml)	Intra-day % RSD	Inter-day % RDS
0.5	0.5	0.8	**0.5**	0.8	1.0
1	0.5	0.6	**1**	0.7	1.0
2.5	0.7	0.6	**2.5**	0.9	0.9
5	0.7	0.8	**5**	0.8	0.9
10	0.9	0.8	**10**	0.2	0.9
25	0.6	1.0	**25**	0.8	0.9
50	0.4	0.6	**50**	0.6	1.0
100	0.2	0.3	**100**	0.4	1.0
200	0.4	0.3	**200**	0.4	0.5

Table A1.2 The accuracy, LoD and LoQ of amorolfine HCl HPLC assays.

Ethanol standards				pH 5 PBS standards			
Test solution concentration (µg/ml)	% Recovery	LoD (µg/ml)	LoQ (µg/ml)	Test solution concentration (µg/ml)	% Recovery	LoD (µg/ml)	LoQ (µg/ml)
25	99.8 ± 0.6	0.15	0.45	**25**	99.4 ± 0.9	0.03	0.08
50	100.3 ± 0.4			**50**	99.9 ± 0.1		
100	99.7 ± 0.7			**100**	100.5 ± 1.0		
200	100.2 ± 0.1			**200**	100.9 ± 1.0		

A1.3 Terbinafine HCl HPLC method validation

Like amorolfine HCl, terbinafine HCl was easily discriminated from the solutions used in the preparation of the standards and the mobile phase, as well as the excipients present in UV-curable gel formulations during a HPLC run. The method is therefore of an acceptable specificity.

The calibration curve of standards, prepared both in ethanol and the pH 5 PBS, were linear over the analysed concentration ranges, with $R^2 > 0.999$, as shown in Fig. A1.2. The % RSD values calculated to determine precision were ≤ 1 in all cases as shown in Table A1.3, and the % recovery values calculated to determine accuracy were between 98 – 102% in all cases as shown in Table A1.4. The method is therefore also of an acceptable accuracy, precision and linearity over the tested concentration ranges.

Furthermore, the LoD and LoQ values (Table A1.4) are low as required for quantifying the amount of drug permeated through a human nail plate.

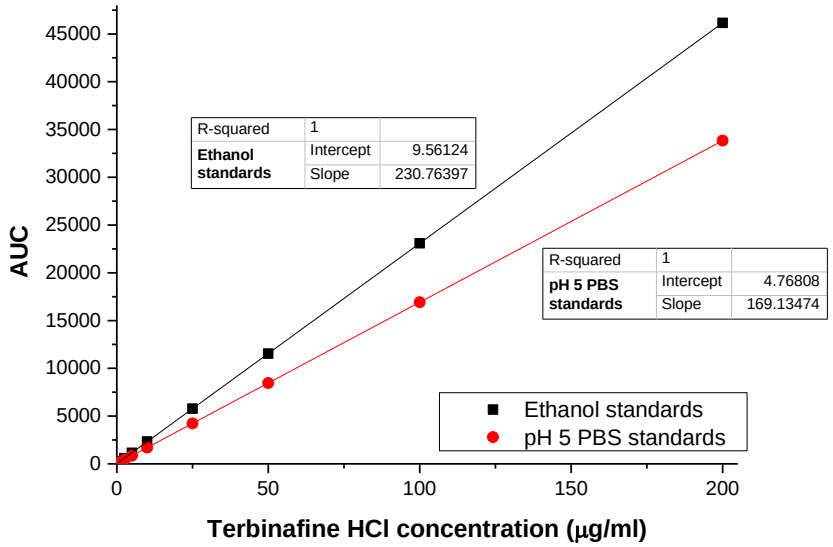


Fig. A1.2 Calibration curves of terbinafine HCl HPLC standards.

Table A1.3 The precision of terbinafine HCl HPLC assays.

Ethanol standards			pH 5 PBS standards		
Standard concentration (µg/ml)	Intra-day % RSD	Inter-day % RDS	Standard concentration (µg/ml)	Intra-day % RSD	Inter-day % RDS
0.5	0.8	0.7	**0.5**	0.4	0.8
1	1.0	0.8	**1**	0.6	0.9
2.5	0.9	0.7	**2.5**	0.6	0.8
5	0.7	0.8	**5**	0.4	0.9
10	0.4	0.9	**10**	0.4	0.9
25	0.1	0.7	**25**	0.8	0.8
50	0.2	0.4	**50**	0.3	0.9
100	0.3	1.0	**100**	0.4	0.9
200	0.5	0.3	**200**	0.8	0.9

Table A1.4 The accuracy, LoD and LoQ of terbinafine HCl HPLC assays.

Ethanol standards				pH 5 PBS standards			
Test solution concentration (µg/ml)	% Recovery	LoD (µg/ml)	LoQ (µg/ml)	Test solution concentration (µg/ml)	% Recovery	LoD (µg/ml)	LoQ (µg/ml)
25	99.6 ± 0.9	0.16	0.48	**25**	99.4 ± 1.0	0.01	0.04
50	100.6 ± 0.2			**50**	99.3 ± 0.9		
100	100.0 ± 0.9			**100**	100.7 ± 0.9		
200	100.7 ± 0.9			**200**	100.5 ± 0.8		

A2. GC method validation

GC assays for monomers, i.e. DUDMA, EMA, IBOMA and HEMA, in methanol were used to quantify unreacted monomers remaining in cured polymer films (following extraction). Details of the GC method conditions used for the monomers are shown in Chapter 2 (Section 2.4.6.2). A GC equipped with a FID system was selected as it has great sensitivity, is very robust and has been shown to accurately quantify (meth)acrylate monomers (Khosrou et al., 2005, Sun et al., 2010). The GC method was validated for specificity, calibration and linearity, accuracy and precision, and LoD and LoQ in a similar manner to the HPLC method validation, but for quantifying residual monomers as oppose to drug content (Section A1.1).

Specificity was established by (i) injecting blank solutions of methanol to assess its interference, and (ii) injecting methanolic solutions of excipients present in the formulations, with and without known quantities of specific monomers, to ensure that there were no interferences between the monomers and other excipients. Each monomer was easily discriminated from methanol which was used in the preparation of the standards, as well as the other excipients present in UV-curable gel formulations during the GC run. The method is therefore of an acceptable specificity for the monomers.

Stock solutions were prepared by accurately adding the required quantity of monomer (i.e. 5ml of DUDMA or 1ml of EMA, IBOMA or HEMA) and dissolving it in methanol in a 100 ml volumetric flask. Subsequently, standard solutions (between 0.6 – 55.5 mg/ml, 0.004 – 9.2 mg/ml, 0.005 – 9.8 mg/ml and 0.005 – 10.7 mg/ml for DUDMA, EMA, IBOMA and HEMA respectively) were obtained by diluting the stock solutions with methanol. These standard solutions were prepared in triplicates. Calibration curves were constructed by assaying the standard solutions. The calibration curves, accuracy and precision data, and LoD and LoQ values for DUDMA, EMA, IBOMA and HEMA are shown in Sections A2.1, A2.2, A2.3 and A2.4 respectively.

The calibration curves for all monomers were linear over the analysed concentration ranges with $R^2 > 0.999$. The % RSD values calculated to determine precision were ≤ 1 in all cases, and the % recovery values calculated to determine accuracy were 98 – 102% in

all cases. The method is therefore of an acceptable accuracy, precision and linearity over the tested concentration ranges for the four monomers. Furthermore, the LoD and LoQ values are low and therefore also acceptable, thus enabling quantification of lower concentration ranges which are expected.

A2.1 DUDMA GC method validation

Calibration curve and linearity

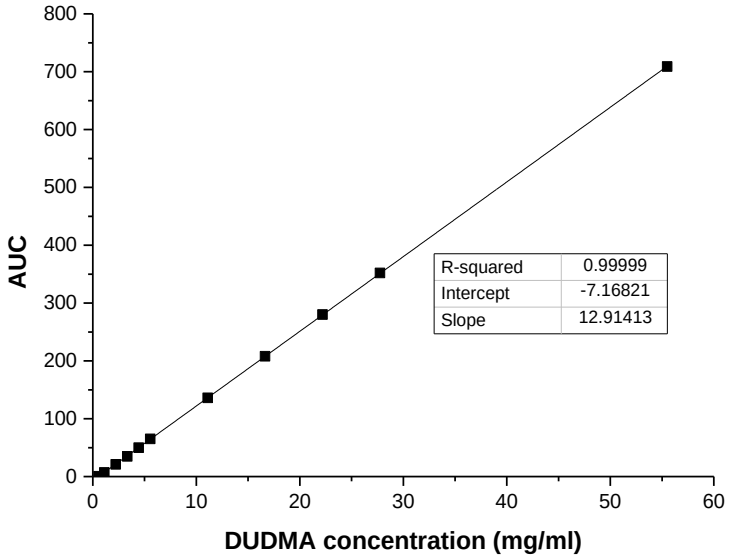


Fig. A2.1 Calibration curve of DUDMA (n=3).

Accuracy and precision

Table A2.1 Precision of DUDMA GC assays.

Standard concentration (mg/ml)	Intra-day % RSD	Inter-day % RDS
0.6	0.6	0.6
1.1	0.6	0.3
2.2	0.8	0.7
3.3	0.9	0.9
4.4	0.9	0.7
5.6	0.8	0.8
11.1	0.7	0.9
16.7	1.0	0.8
22.2	0.7	0.5
27.8	0.8	0.4
55.5	0.8	0.1

Table A2.2 Accuracy, LoD and LoQ of DUDMA GC assays.

Test solution concentration (mg/ml)	% Recovery	LoD (mg/ml)	LoQ (mg/ml)
0.5	100.0 ± 0.9	0.14	0.43
5	98.6 ± 0.6		
25	101.0 ± 0.5		
50	101.3 ± 0.3		

A2.2 EMA GC method validation

Calibration curve and linearity

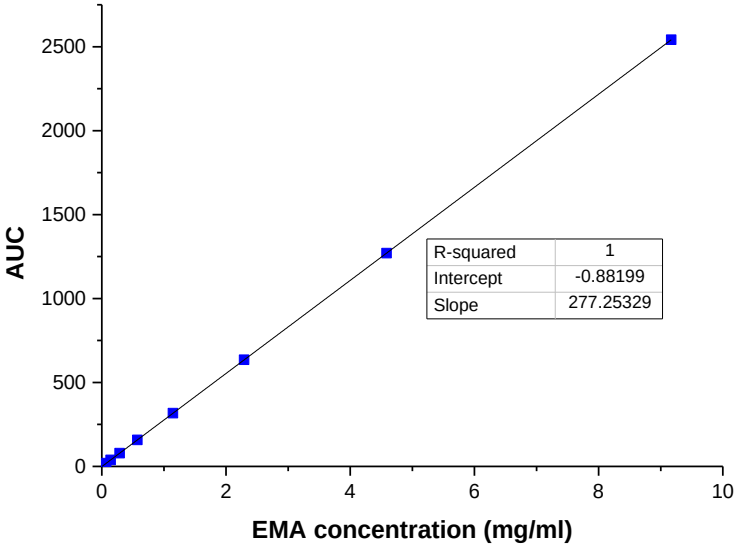


Fig. A2.2 Calibration curve of EMA (n=3).

Accuracy and precision

Table A2.3 Precision of EMA GC assays.

Standard concentration (mg/ml)	Intra-day % RSD	Inter-day % RDS
0.004	1.0	1.0
0.01	0.6	0.4
0.02	0.2	0.9
0.04	0.7	0.7
0.07	0.6	0.9
0.1	0.5	0.6
0.3	0.6	0.9
0.6	0.5	0.8
1.1	0.4	0.9
2.3	1.0	0.9
4.6	0.2	0.9
9.2	0.9	0.9

Table A2.4 Accuracy, LoD and LoQ of EMA GC assays.

Test solution concentration (mg/ml)	% Recovery	LoD (µg/ml)	LoQ (µg/ml)
0.005	100.4 ± 0.7	1.2	3.5
0.05	101.6 ± 0.7		
0.25	99.5 ± 1.0		
0.5	101.4 ± 0.9		

A2.3 IBOMA GC method validation

Calibration curve and linearity

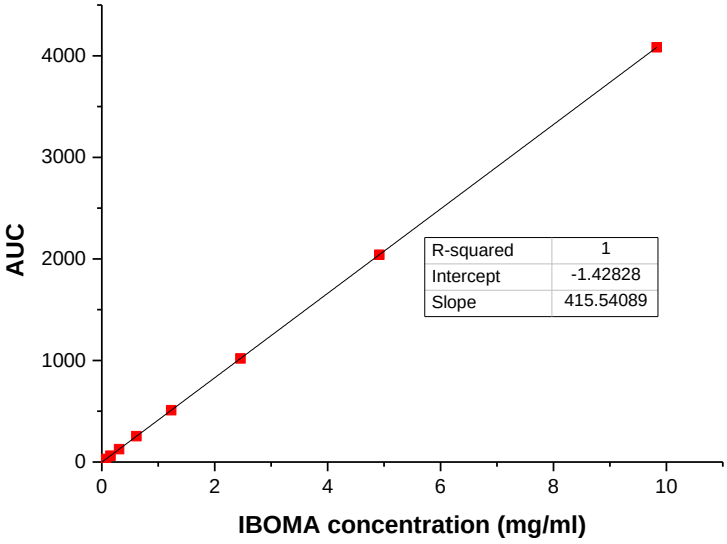


Fig. A2.3 Calibration curve of IBOMA (n=3).

Accuracy and precision

Table A2.5 Precision of IBOMA GC assays.

Standard concentration (mg/ml)	Intra-day % RSD	Inter-day % RDS
0.005	0.3	0.5
0.01	0.7	0.7
0.02	0.7	0.7
0.04	0.4	0.6
0.08	0.5	0.6
0.2	1.0	0.4
0.3	0.8	0.5
0.6	0.9	0.4
1.2	0.9	0.7
2.5	0.4	0.7
4.9	0.4	1.0
9.8	0.3	0.2

Table A2.6 Accuracy, LoD and LoQ of IBOMA GC assays.

Test solution concentration (mg/ml)	% Recovery	LoD (µg/ml)	LoQ (µg/ml)
0.005	100.5 ± 0.8	1.7	5.0
0.05	99.3 ± 0.5		
0.25	98.8 ± 0.9		
0.5	101.1 ± 0.5		

A2.4 HEMA GC method validation

Calibration curve and linearity

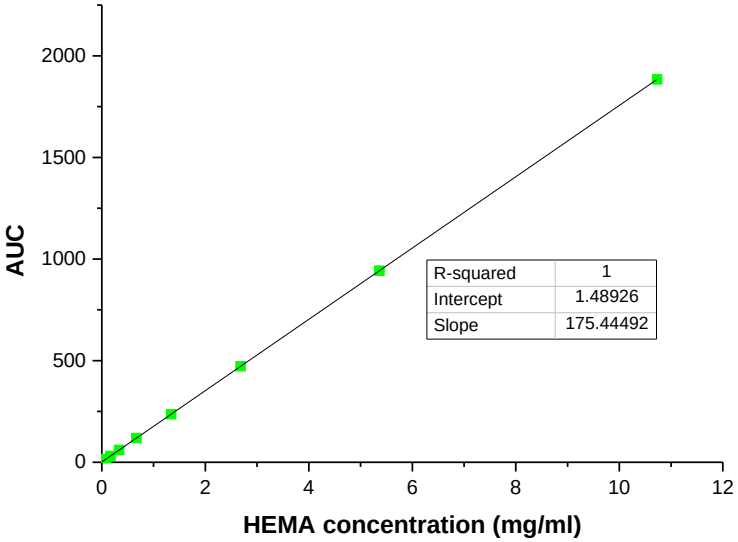


Fig. A2.4 Calibration curve of HEMA (n=3).

Accuracy and precision

Table A2.7 Precision of HEMA GC assays.

Standard concentration (mg/ml)	Intra-day % RSD	Inter-day % RDS
0.005	0.6	0.6
0.01	0.6	1.0
0.02	0.8	0.8
0.04	0.8	0.8
0.08	0.4	0.7
0.2	0.3	0.4
0.3	0.2	0.4
0.7	0.7	0.6
1.3	0.6	0.3
2.7	0.8	0.9
5.4	0.5	0.3
10.7	0.4	0.5

Table A2.8 Accuracy, LoD and LoQ of HEMA GC assays.

Test solution concentration (mg/ml)	% Recovery	LoD (µg/ml)	LoQ (µg/ml)
0.005	101.9 ± 0.6	1.2	3.6
0.05	99.7 ± 0.7		
0.25	101.2 ± 0.9		
0.5	98.7 ± 0.9		

A3. The levels of residual monomers in the polymer films - extraction method optimisation

Ultrasonication-assisted solvent extraction was used for determining the levels of residual monomers in the polymer films. To obtain a high recovery and make the extraction procedure efficient, the effects of different factors such as (i) the amount of solvent and (ii) the time of extraction on recovery were investigated using the drug-free and solvent-free formulation containing DUDMA, EMA and a photoinitiator. This formulation was prepared as described in Section 2.4.2 (Chapter 2), and immediately after curing and removal of the oxygen inhibition layer (as per Section 2.4.4 [Chapter 2]), one gram of the film was placed in a glass vial, to which the extraction solvent was added. Methanol was chosen as the extraction solvent as it (i) does not dissolve the polymer; (ii) is miscible with the monomers; and (iii) displays excellent chromatographic behaviour, whereby it is easily discriminated from the analyte of interest.

A3.1 Volume of extraction solvent

In order to study the effect of methanol volume on extraction, the volume was varied in the range of 1 – 6 ml at 1ml intervals, and the film and solvent mix was sonicated for up to 3 hours, after which the solvent was analysed by gas chromatography (as per Section 2.4.6 [Chapter 2]) to calculate the amount of residual monomers in the polymer film. Fig. A3.1 shows that increasing the volume of methanol to 3 ml increased the residual DUDMA and EMA content in the extraction solvent; however there was no further increase above 3ml for both monomers. Below 3ml, the residual monomers already extracted hinder the extraction of further monomers, possibly due to a reduction in their concentration gradient. Therefore 3ml was chosen as the optimal extraction volume – allowing maximal extraction without diluting the amount of extracted residual monomers unnecessarily.

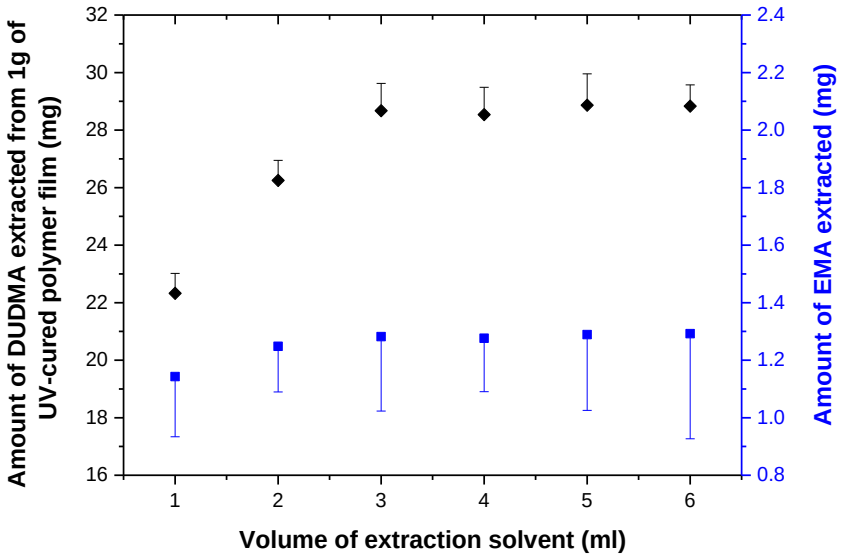


Fig A3.1 Mean residual DUDMA and EMA quantity ± standard deviation (n=3) in extraction solvent (methanol) at different volumes, following a 3 hour sonication.

A3.2 Selection of extraction time

To study the effect of extraction time, 3 ml of methanol was added to the vial containing 1g of film. Subsequently, the mixture was sonicated for up to 3 hours, with 50µl of solvent withdrawn from the mixture every 20 minutes for analysis to assess the time of extraction on recovery. Fig. A3.2 shows that the amount of residual monomers (especially DUDMA) extracted increased steadily between 20 – 100 minutes, after which there was no further increase. Therefore, 120 minutes was selected as the optimal extraction time. A methanol extraction volume of 3 ml and extraction time of 2 hours was thus used for determining the levels of residual monomers in the polymer films produced by the UV-curable gels formulated.

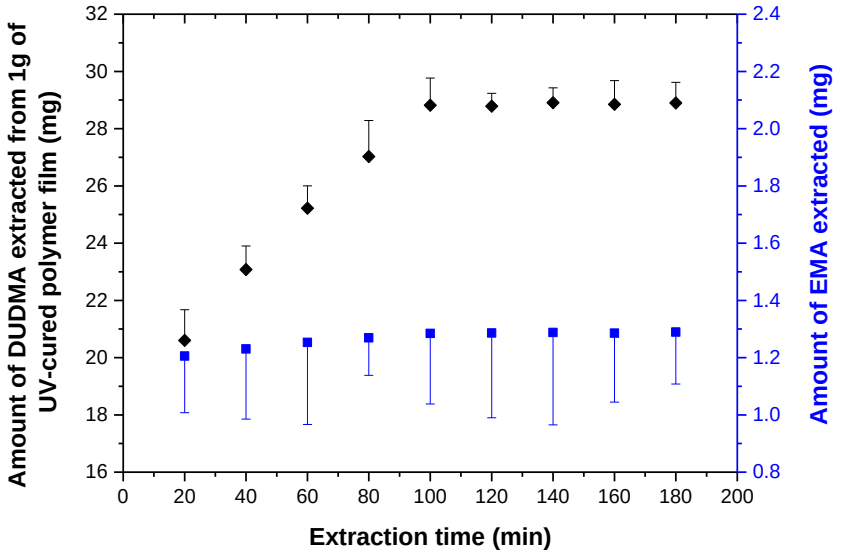


Fig A3.2 Mean residual DUDMA and EMA quantity ± standard deviation (n=3) in extraction solvent (3ml of methanol) at different ultrasonication time intervals.

A4. Stability of antifungals in UV-curable gel formulations over time

The drug-loaded UV-curable gel formulations were stored under accelerated stability testing conditions (as described in Section 2.4.3.2 [Chapter 2]) and assessed for any changes in their appearance over time. Photographic images were taken to visualise colour change due to drug degradation with time and polarised light microscopy images (as described in Section 3.4.5.1 [Chapter 3]) were taken to observe whether the drug had crystallised out of the formulation with time. Tables A4.1 and A4.2 show photographic images and polarised light microscopy images of the amorolfine HCl– and terbinafine HCl– loaded UV-curable gel formulations respectively. No colour changes were observed nor were drug crystals present for all formulations tested. The drug-loaded UV curable gels were therefore stable over six months.

Table A4.1 Photographic and polarised light microscopy (PLM) images of amorolfine HCl–loaded gel formulations to assess colour change due to drug degradation and presence of drug crystals over time. It should be noted that the vials containing the UV-curable gel formulation also contain magnetic stirrers within.

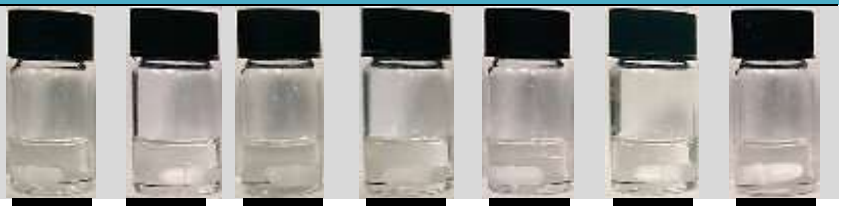
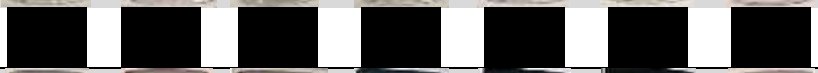
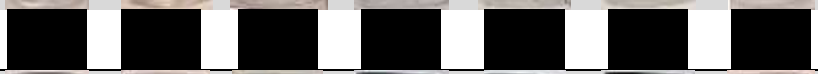
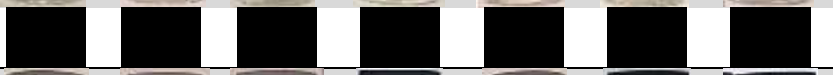
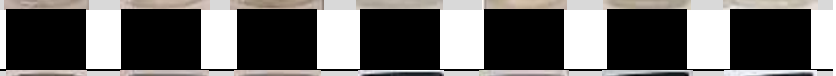
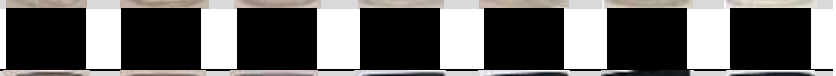
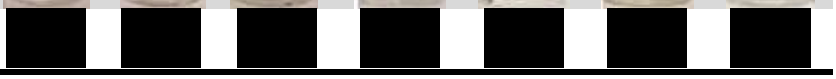
Formulation	Image	Time						
		Day 0	Week 4	Week 8	Week 12	Week 16	Week 20	Week 24
DUDMA & EMA gel containing ethanol and 3% w/v amorolfine HCl	Photographic							
	PLM							
DUDMA & IBOMA gel containing ethanol and 3% w/v amorolfine HCl	Photographic							
	PLM							
DUDMA & HEMA gel containing ethanol and 4% w/v amorolfine HCl	Photographic							
	PLM							
DUDMA & HEMA gel containing 2% w/v amorolfine HCl	Photographic							
	PLM							

Table A4.2 Photographic and polarised light microscopy (PLM) images of terbinafine HCl–loaded gel formulations to assess colour change due to drug degradation and presence of drug crystals over time. It should be noted that the vials containing the UV-curable gel formulation also contain magnetic stirrers within.

Formulation	Image	Time						
		Day 0	Week 4	Week 8	Week 12	Week 16	Week 20	Week 24
DUDMA & EMA gel containing ethanol and 4% w/v terbinafine HCl	Photographic							
	PLM							
DUDMA & IBOMA gel containing ethanol and 4% w/v terbinafine HCl	Photographic							
	PLM							
DUDMA & HEMA gel containing ethanol and 6% w/v terbinafine HCl	Photographic							
	PLM							
DUDMA & HEMA gel containing 2% w/v terbinafine HCl	Photographic							
	PLM							

A5. Temperature change inside UVA nail lamp during use

The 36 Watt Cuccio Professional UVA nail lamp (Amazon UK) was monitored for temperature change during use with the aid of a RS-1327 Infrared thermometer (-35^{o}C - +500^{o}C) (RS Components Ltd., UK), to determine whether it could be a potential contributor to affecting the polymerisation process of a gel formulation and therefore the characteristics of the resulting polymer film.

To monitor the temperature change, the nail lamp was switched on and a timer started (set for two minutes). At time intervals, i.e. 0, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110 and 120 seconds, the temperature of a marked point inside the lamp (where a substrate coated with a layer of photo-curable gel is usually placed) was measured and recorded. This was repeated a total of three times.

Fig. A5.1 shows the results obtained. The temperature inside the UVA nail lamp increased proportionally with time, with an average temperature rise of 3.0^{o}C ± 0.8^{o}C following a 2 minute cure. This increase in temperature of the UVA nail lamp during curing could potentially contribute to the evaporation of volatile components in the UV-curable gel formulations, such as ethanol or HEMA, and therefore affect the properties of the resulting polymer film, e.g. its mass conversion from monomer gel to polymer film.

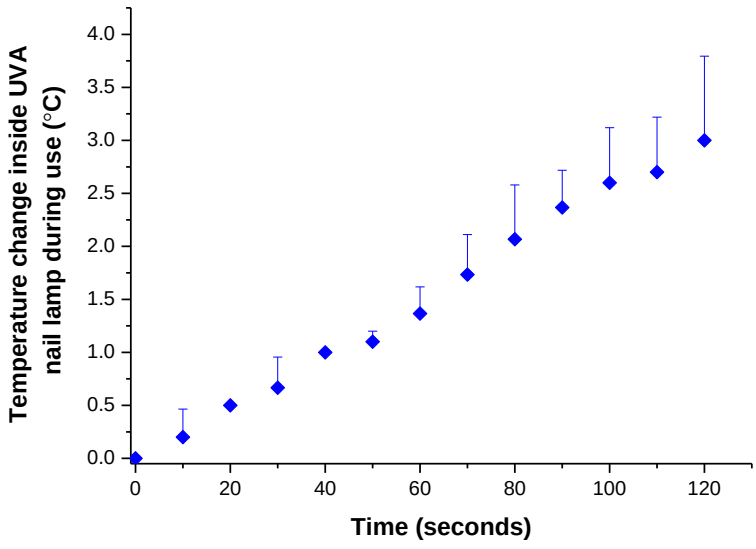
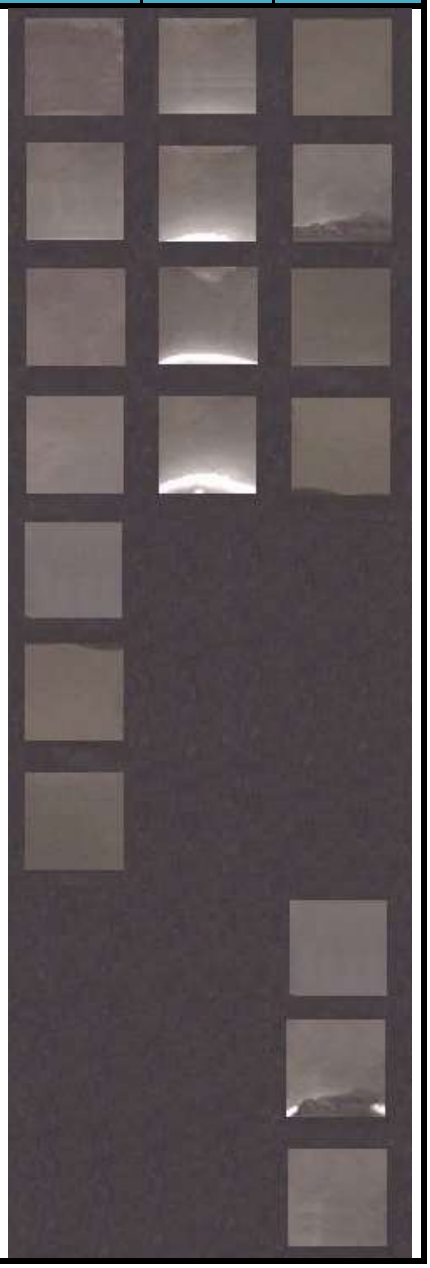


Fig. A5.1 Temperature change inside UVA nail lamp during use. Mean and standard deviation are shown, n=3.

A6. Photographic images of UV-cured films

The UV-cured films produced by formulations containing EMA, IBOMA or HEMA, with/without solvent and with/without drug were photographed and the images obtained are shown in Table A6.1. All the films were smooth and transparent, and therefore considered aesthetically acceptable and thus visually suitable as a means for delivering drug, in this case an antifungal, through the nail plate.

Table A6.1 Photographic images of UV-cured films.

Excipients	DUDMA	Solvent	Drug	Photographic image of UV-cured film produced from formulations containing: EMA / IBOMA / HEMA
Formulations	DUDMA 85 % v/v : diluent monomer 15 % v/v	None	None	
		Ethanol	None	
			AH	
			TH	
		NMP	None	
			AH	
			TH	
	DUDMA 75 % v/v : diluent monomer 25 v/v	None	None	
			AH	
			TH	

Abbreviations: AH, amorolfine HCl; TH, terbinafine HCl

A7. Adsorption isotherms of UV-cured polymer films

N_2 adsorption isotherms for the UV-cured polymer films were obtained by an automated surface area analyser (Chapter 3, Section 3.4.3). Figs A7.1, A7.2, A7.3 and A7.4 show the adsorption isotherms of the UV-cured films produced from DUDMA & EMA containing gel (±drug and ± solvent), DUDMA & IBOMA containing gels (± drug and ± solvent), DUDMA & HEMA containing gels with a DUDMA: HEMA ratio of 85:15 % v/v (± drug & ± solvent), and DUDMA & HEMA containing gels with a DUDMA: HEMA ratio of 75:25 % v/v (± drug) respectively. These isotherms are characteristic of adsorbents which are macroporous (i.e. contains pores with a diameter greater than 50 nm) and with weak interactions with the adsorbate (Sing et al., 1985), and the total pore volume (Chapter 3, Section 3.5.1.2) was calculated using these isotherms from the amount of nitrogen adsorbed at the relative pressure of 0.98.

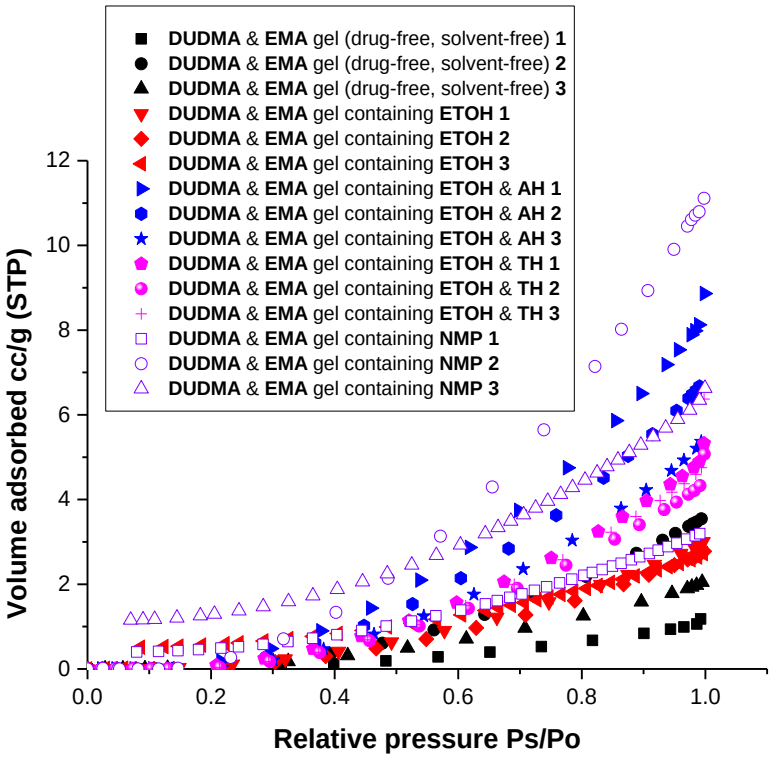


Fig. A7.1 Relative pressure vs volume adsorbed for UV-cured films produced from DUDMA & EMA, containing gels (± drug and ± solvent). Abbreviations: AH, amorolfine HCl; TH, terbinafine HCl; ETOH, ethanol.

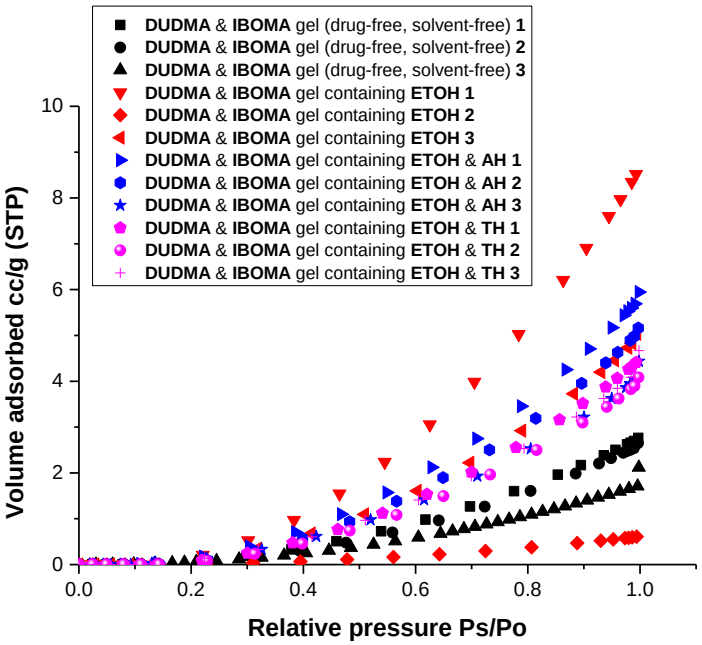


Fig. A7.2 Relative pressure vs volume adsorbed for UV-cured films produced from DUDMA & IBOMA, containing gels (± drug and ± solvent). Abbreviations: AH, amorolfine HCl; TH, terbinafine HCl; ETOH, ethanol.

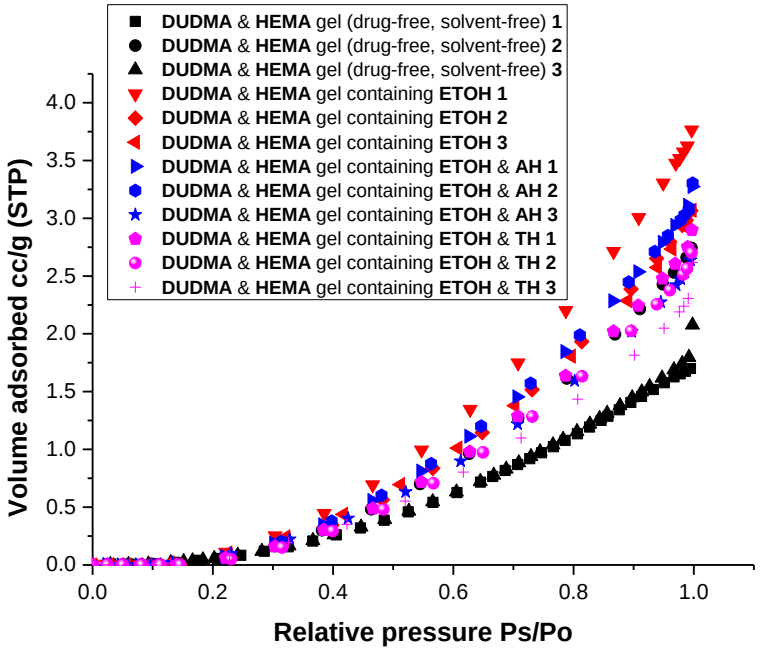


Fig. A7.3 Relative pressure vs volume adsorbed for UV-cured films produced from DUDMA & HEMA containing gels with a DUDMA: HEMA ratio of 85:15 % v/v (± drug & ± solvent). Abbreviations: AH, amorolfine HCl; TH, terbinafine HCl; ETOH, ethanol.

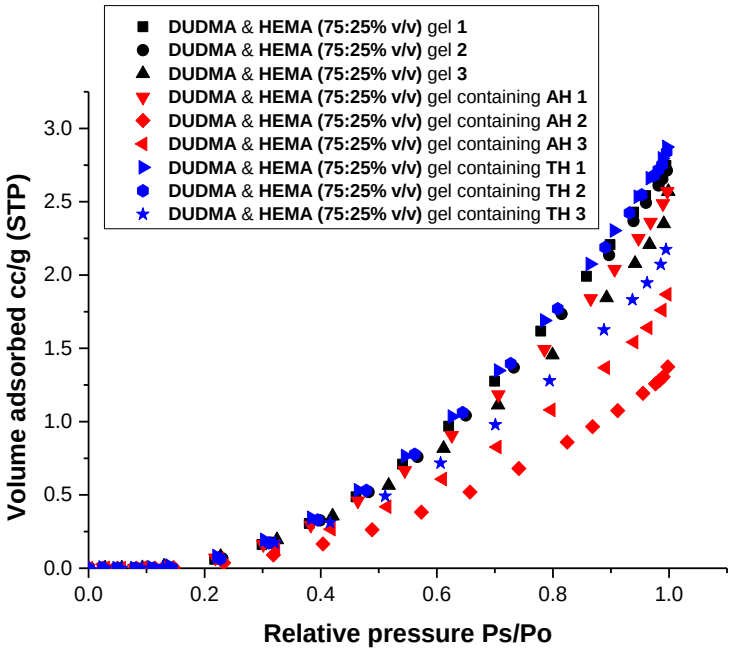


Fig. A7.4 Relative pressure vs volume adsorbed for UV-cured films produced from DUDMA & HEMA containing gels with a DUDMA: HEMA ratio of 75:25 % v/v (± drug). Abbreviations: AH, amorolfine HCl; TH, terbinafine HCl; ETOH, ethanol.

A8. The thermal stability of drug-loaded UV-cured films

TGA (as per Section 3.4.8.1 [Chapter 3]) was used to determine whether the presence of drug in the formulations containing DUDMA & EMA, IBOMA or HEMA, with or without ethanol or NMP alters the resulting films thermal stability. The TGA profiles of the drug-loaded films and their corresponding drug-free films are shown in Figs A8.1 – A8.5. It can be seen that the presence of drug in the formulation does not alter the film's thermal stability, as the TGA profiles obtained for films with and without drug are comparable.

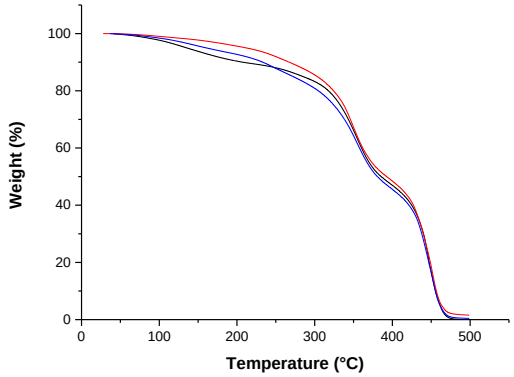


Fig. A8.1 TGA profiles of UV-cured films produced from formulation containing DUDMA: EMA (85:15 v/v) and ethanol ± drug. The spectra in black, red and blue are for drug-free, amorolfine HCl- and terbinafine HCl- loaded films respectively.

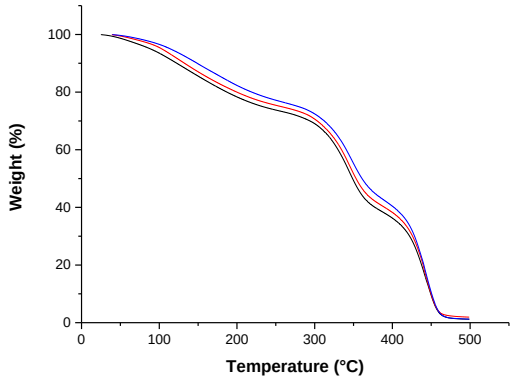


Fig. A8.2 TGA profiles of UV-cured films produced from formulation containing DUDMA: EMA (85:15 v/v) and NMP ± drug. The spectra in black, red and blue are for drug-free, amorolfine HCl- and terbinafine HCl-loaded films respectively.

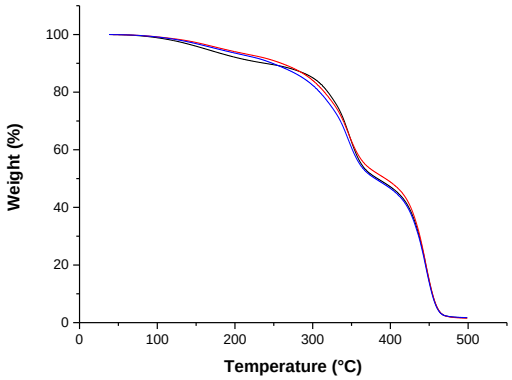


Fig. A8.3 TGA profiles of UV-cured films produced from formulation containing DUDMA: IBOMA (85:15 v/v) and ethanol ± drug. The spectra in black, red and blue are for drug-free, amorolfine HCl- and terbinafine HCl- loaded films respectively.

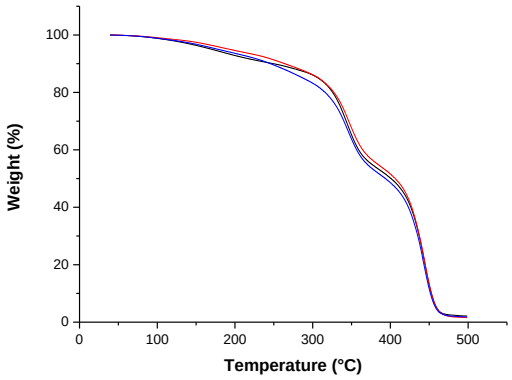


Fig. A8.4 TGA profiles of UV-cured films produced from formulation containing DUDMA: HEMA (85:15 v/v) and ethanol ± drug. The spectra in black, red and blue are for drug-free, amorolfine HCl- and terbinafine HCl- loaded films respectively.

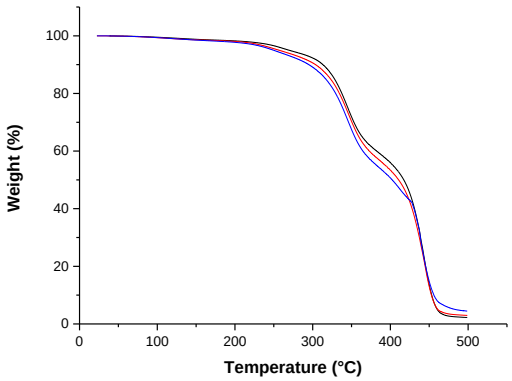


Fig. A8.5 TGA profiles of UV-cured films produced from formulation containing DUDMA: HEMA (75:25 v/v) ± drug. The spectra in black, red and blue are for drug-free, amorolfine HCl- and terbinafine HCl- loaded films respectively.

A9. Mass Balance for ungual drug permeation study

Mass balances were carried out for the permeation experiments conducted in Chapter 4 (Section 4.5.2) to ensure that most of the drug had been retrieved from the modified Franz diffusion cell, and therefore most of the drug remaining within the nail tissue had been extracted. As can be seen from Table A9.1, almost all of the drug which was introduced to the Franz cell had been successfully retrieved. Therefore the % of drug permeated across the nail and remaining in the nail at day 30 data was used for comparing the ungual drug permeation ability of the different formulations tested (Chapter 4, Section 4.6.2).

Table A9.1 Mass balance in permeation experiment at day 30. Means ± standard deviations are shown, n=6.

Formulation	Percentage drug recovered			
	Receptor (i.e. permeated)	Nail clipping	Donor (i.e. film remaining on the nail plate surface)	Total
Curanail	1.0 ± 0.1	1.6 ± 0.4	96.9 ± 1.4	99.5 ± 1.3
DUDMA & EMA gel containing ETOH & 3% w/v AH	1.7 ± 0.2	1.8 ± 0.9	96.4 ± 1.4	99.9 ± 1.4
DUDMA & EMA gel containing ETOH & 4% w/v TH	0.8 ± 0.1	2.6 ± 0.3	96.4 ± 1.1	99.8 ± 1.1
DUDMA & IBOMA gel containing ETOH & 3% w/v AH	2.2 ± 0.5	2.5 ± 0.8	92.4 ± 2.4	96.9 ± 2.0
DUDMA & IBOMA gel containing ETOH & 4% w/v TH	1.4 ± 0.4	2.7 ± 0.4	92.8 ± 1.3	96.9 ± 1.6
DUDMA & HEMA gel containing ETOH & 4% w/v AH	2.6 ± 0.7	3.5 ± 0.5	90.9 ± 1.8	97.0 ± 2.0
DUDMA & HEMA gel containing ETOH & 6% w/v TH	1.5 ± 0.3	3.2 ± 0.6	92.7 ± 2.0	97.4 ± 2.3
DUDMA & HEMA gel containing 2% w/v AH	2.4 ± 1.1	3.4 ± 0.9	91.2 ± 2.2	97.0 ± 1.2
DUDMA & HEMA gel containing 2% w/v TH	1.3 ± 0.2	2.8 ± 0.7	93.3 ± 1.7	97.4 ± 1.2

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

A10. Autoclaving effect on the nail plates ability to take up solvent

To test the efficacy of UV-curable gel formulations against *T. rubrum*, human nail clippings (fingernails) were used (Chapter 4, Section 4.5.3), and to avoid the likelihood of introducing contaminants into the SDA plates used for the study, the nails needed to be sterilised before use. The method of sterilisation chosen was autoclaving at 120^{o}C for 20 minutes. In order to determine whether this method would affect the permeation of the permeant into and through the nail clippings, the nail clippings ability to take up solvent with time after autoclaving was investigated and compared to non-autoclaved nails. Two solvents were chosen: (i) water, which is known to swell the nail plate, and (ii) ethyl acetate (Fisher Scientific, UK), which has been found to dehydrate the nail plate (Hossin, 2015).

Prior to using the fingernail clippings, they were washed with distilled water and any excess debris was removed. The nail clippings were then left to dry for one hour at room temperature. Subsequently, half the nails were kept as they were, while the other half were autoclaved at 120^{o}C for 20 minutes. The solvent uptake property of the nail clippings was then measured by placing the individual autoclaved and non-autoclaved nails in small glass vials containing 5ml of the test solvent and ensuring that the nail was completely submerged. The samples were then left in a water bath maintained at 25^{o}C, and at timed intervals, i.e. after 1, 2, 4, 8, 24, 48 and 72 hours, the nail clippings were removed from the vials using forceps, blotted dry with Kimwipes and re-weighed. The experiment was repeated in triplicates and the mass change of the nail clippings following immersion in a solvent was calculated using the following formula:

$$\text{Mass change (\%)} = \frac{\text{nail mass after solvent immersion} - \text{nail mass before solvent immersion}}{\text{nail mass before solvent immersion}} \times 100$$

Fig. A10.1 shows the mean mass change (%) of autoclaved and non-autoclaved nail clippings with time following immersion in water or ethyl acetate. As expected, immersion in water caused the nail plate to swell (as indicated by the increase in mass), while immersion in ethyl acetate caused the nail plate to dehydrate (as indicated by the decreases in mass). Autoclaving the nail plate, did not affect the solvent uptake ability of

the nail plate with time (p>0.05), and hence it can be considered a suitable method for nail clipping sterilisation.

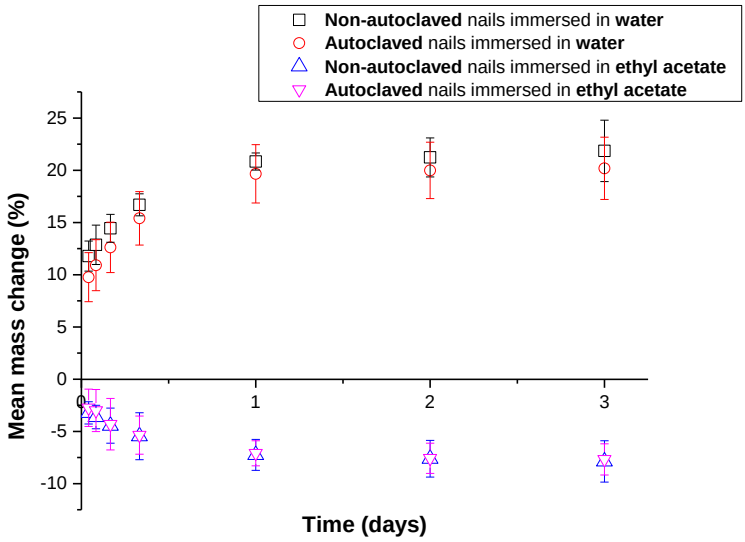


Fig. A10.1 Mass change (%) of autoclaved & non-autoclaved nail clippings with time following immersion in water or ethyl acetate. Mean and standard deviation are shown, n=3.

A11. Growth of T. rubrum

The growth of a *T. rubrum* plug (with a diameter of 8mm) inserted in the centre of a SDA plate was followed over two weeks, in order to help develop an adapted disc diffusion method to test and compare the efficacies of different antifungal-loaded UV-cured polymer films (when applied on human nail clippings) against the fungus.

A total of five SDA plates were prepared with a mycelial plug inserted in the centre. This was done as described in Section 4.5.3.1 and 4.5.3.2 (Chapter 4). These plates were incubated at 32^{o}C, and at time intervals, i.e. on day 2, 3, 7, 8, 9, 11, 14 and 15, the longest diameter of the colony was measured using a caliper and recorded.

Fig. A11.1 shows the growth of *T. rubrum* with time. Under the test conditions it took on average a total of 1.9 ± 0.3 days for growth to be detected, and the culture grew an average of 5.8 ± 0.1 mm/day from day 2 (as determined from the linear growth region). From this it was decided that the culture should be allowed to grow for three days prior to being used for studying the efficacies of different antifungal-loaded UV-cured polymer films, as at this point, if growth is not detected, the plate is likely to be defective. Furthermore, based on the rapidity of fungal growth, it was decided that samples should be placed at least 20 mm from the centre of the plate to allow time for the drug in the UV-cured polymer film to permeate through the nail and into the agar.

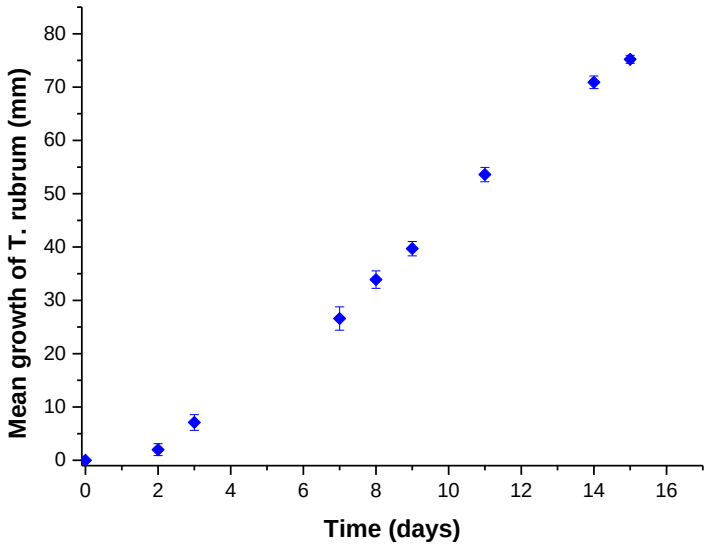


Fig. A11.1 Growth of T. rubrum with time. Mean and standard deviation are shown, n=5.

A12. Determining the drug-loaded UV-curable gels maximal penetration enhancer concentration

To determine the maximum concentration of penetration enhancer that could be incorporated into drug-loaded UV-curable DUDMA & HEMA gels containing ethanol to produced films with the drug remaining in the dissolved state, formulations containing DUDMA & HEMA (with a DUDMA to HEMA ratio of 85: 15% v/v), 3% v/v 2-hydroxy-2-methylpropiophenone, an antifungal drug (either 4% w/v amorolfine HCl or 6% w/v terbinafine HCl), and a penetration enhancer (i.e. water, MPE, NMP or PEG 200) 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 cured as per Section 2.4.4 (Chapter 2). The resulting films were observed under a polarised light microscope (as described in Section 3.4.5.1 [Chapter 3]) and examined using XRD (as described in Section 3.4.5.2 [Chapter 3]) to confirm the absence or presence of drug crystals.

The polarised light micrographs of the amorolfine HCl-loaded and terbinafine HCl-loaded films with their corresponding XRD patterns can be found in Tables A12.1 and A12.2 respectively. From both tables it can be seen that the maximum water concentration that can be used in the drug-loaded formulations to produce films with the drug remaining in the dissolved state is 10% v/v, while the maximum MPE, NMP or PEG 200 concentration that can be used is 5% v/v, as higher concentrations produces films containing drug crystals.

Table A12.1 Polarised light micrographs and corresponding XRD patterns of amorolfine HCl-loaded UV-cured films produced from DUDMA & HEMA gel formulations containing ethanol and water (10% or 12.5% v/v) or MPE, NMP or PEG 200 (5% or 7.5% v/v).

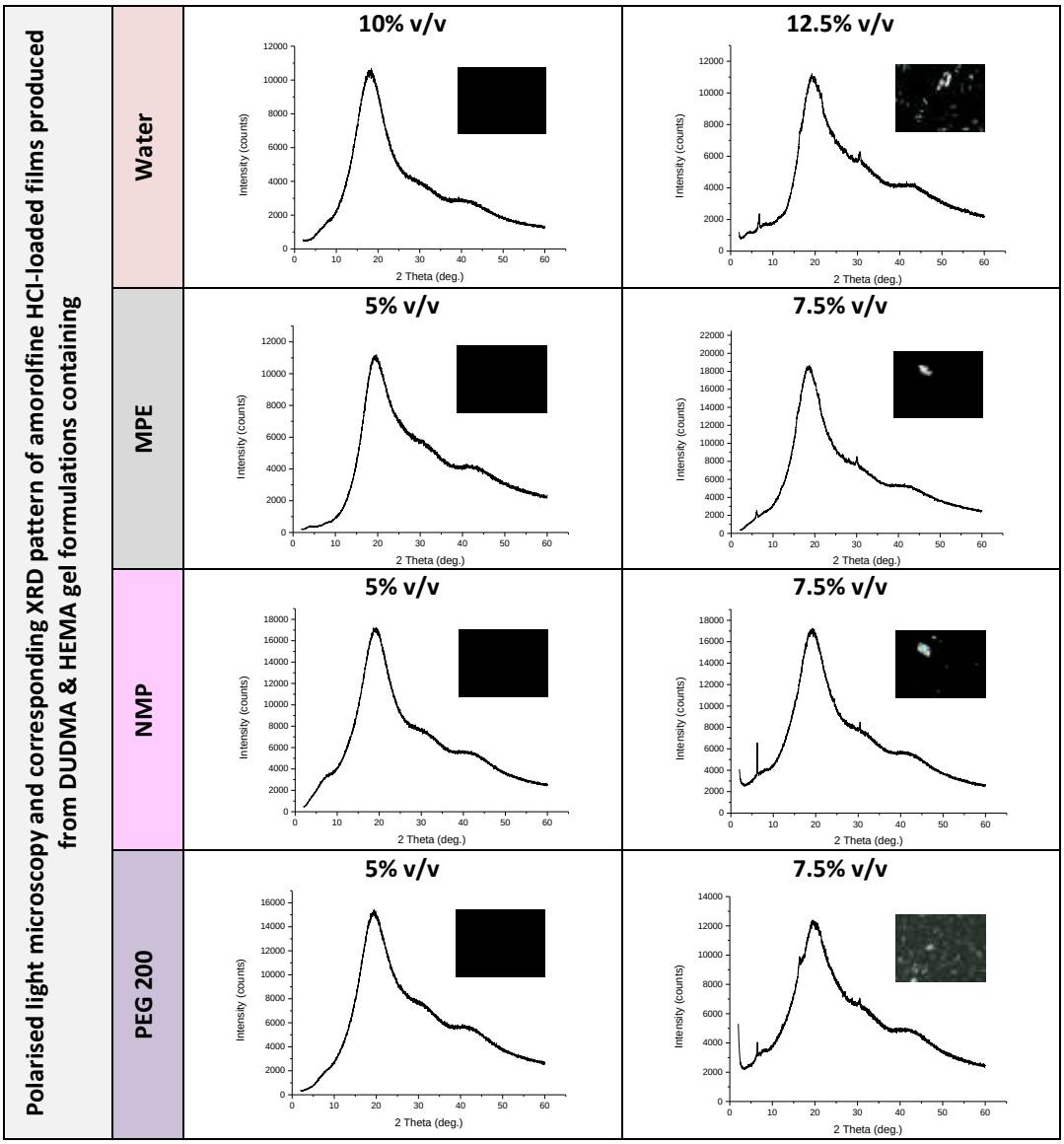
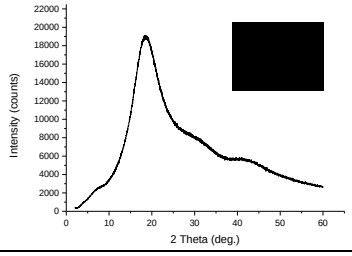
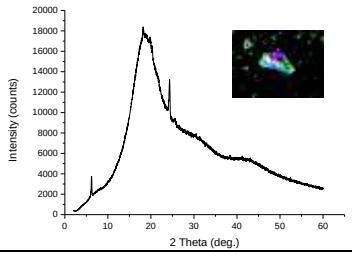
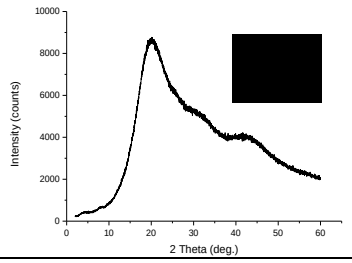
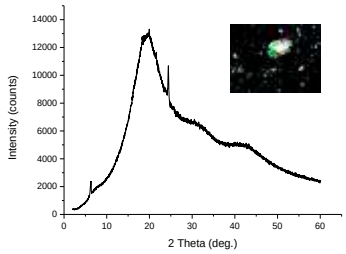
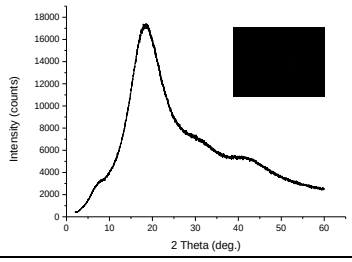
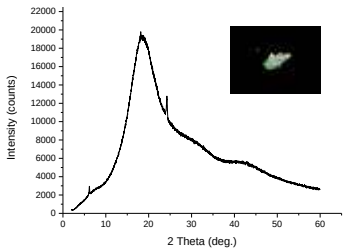
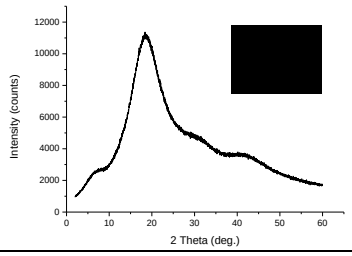
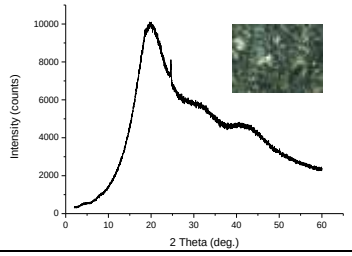


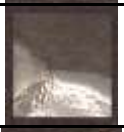
Table A12.2 Polarised light micrographs and corresponding XRD patterns of terbinafine HCl-loaded UV-cured films produced from DUDMA & HEMA gel formulations containing ethanol and water (10% or 12.5% v/v) or MPE, NMP or PEG 200 (5% or 7.5% v/v).

Polarised light microscopy and corresponding XRD pattern of terbinafine HCl-loaded films produced from DUDMA & HEMA gel formulations containing			
		10% v/v	12.5% v/v
	Water		
		5% v/v	7.5% v/v
	MPE		
		5% v/v	7.5% v/v
	NMP		
		5% v/v	7.5% v/v
	PEG 200		

A13. Photographic images of UV-cured films loaded with penetration enhancers

The UV-cured films produced by amorolfine HCl- or terbinafine HCl- loaded DUDMA & HEMA gel formulations containing ethanol and water, MPE, NMP or PEG 200 were photographed and the images obtained are shown in Table A13.1. All the films were smooth and transparent, and therefore considered aesthetically acceptable and thus visually suitable as a means for delivering drug, in this case an antifungal, through the nail plate.

Table A13.1 Photographic images of UV-cured films produced for drug-loaded DUDMA & HEMA gel formulations containing ethanol (ETOH) and water, MPE, NMP or PEG 200.

	Photographic images of DUDMA & HEMA UV-cured films produced from formulations containing	
	4% w/v amorolfine HCl	**6% w/v terbinafine HCl**
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)		

A14. FT-IR spectra of UV-cured films loaded with penetration enhancers

In order to determine whether the incorporation of water, MPE, NMP or PEG 200 in drug-loaded DUDMA & HEMA gel formulations produces any noticeable changes in the resulting films' chemical makeup, the films were examined using FT-IR (as per Section 2.4.5.2 [Chapter 2]). The FT-IR spectra of the films produced from amorolfine HCl-loaded and terbinafine HCl-loaded gel formulations are shown in Figs A14.1 and A14.2 respectively. It can be seen that the incorporation of the penetration enhancers in the drug-loaded 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. It therefore appears that the concentration of the penetration enhancers in the formulations were too low to produce any noticeable changes or changes that could be detected by FT-IR.

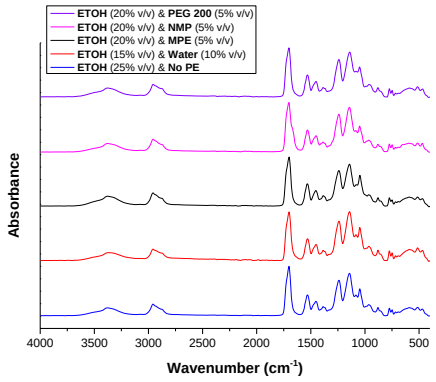


Fig. A14.1 FT-IR spectra of UV-cured films produced from 4% w/v amorolfine HCl-loaded DUDMA & HEMA gel formulations containing ethanol (ETOH) and no penetration enhancer (PE), water, MPE, NMP and PEG 200.

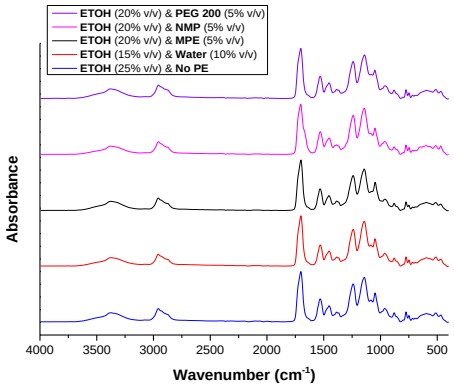


Fig. A14.2 FT-IR spectra of UV-cured films produced from 6% w/v terbinafine HCl-loaded DUDMA & HEMA gel formulations containing ethanol (ETOH) and no penetration enhancer (PE), water, MPE, NMP and PEG 200.

A15. Thermal stability of UV-cured films loaded with penetration enhancers

TGA (as per Section 3.4.8.1 [Chapter 3]) was used to determine whether the incorporation of water, MPE, NMP or PEG 200 in drug-loaded DUDMA & HEMA gel formulations alters the resulting films' thermal stability. The TGA profiles of the films produced from amorolfine HCl-loaded and terbinafine HCl-loaded gel formulations are shown in Figs A15.1 and A15.2 respectively. It can be seen that the presence of the penetration enhancers in the drug-loaded formulations does not alter the films thermal stability, as the TGA profiles obtained for films produced from formulations containing the penetration enhancers are comparable to the films produced from the formulations containing no penetration enhancer.

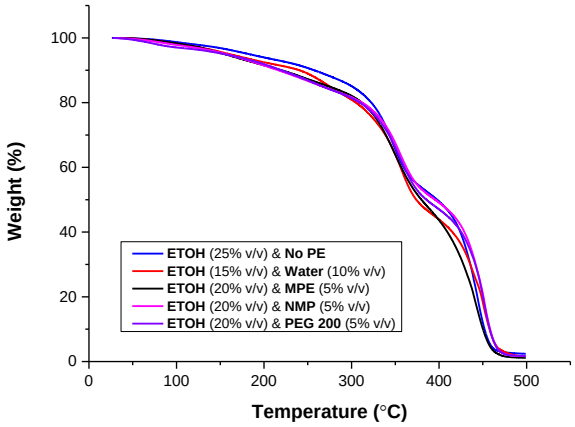


Fig. A15.1 TGA profiles of films produced from 4% w/v amorolfine HCl-loaded UV-curable DUDMA & HEMA gel formulations containing ethanol (ETOH) and no penetration enhancer (PE), water, MPE, NMP and PEG 200.

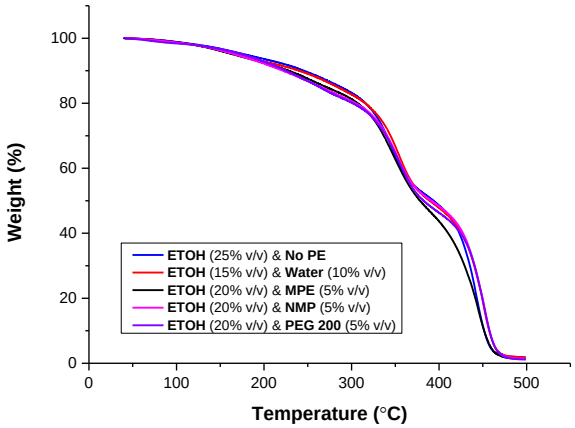


Fig. A15.2 TGA profiles of films produced from 6% w/v terbinafine HCl-loaded UV-curable DUDMA & HEMA gel formulations containing ethanol (ETOH) and no penetration enhancer (PE), water, MPE, NMP and PEG 200.

A16. Mass Balance for ungual drug permeation study using UV gels containing different penetration enhancers

Mass balances were carried out for the permeation experiments conducted in Chapter 5 (Section 5.4.2) to ensure that most of the drug had been retrieved from the modified Franz diffusion cell, and therefore most of the drug remaining within the nail tissue had been extracted. As can be seen from Table A16.1, almost all of the drug which was introduced to the Franz cell had been successfully retrieved. Therefore the % of drug permeated across the nail and remaining in the nail at day 30 data was used for comparing the ungual drug permeation ability of the different formulations tested (Chapter 5, Section 5.5.7).

Table A16.1 Mass balance in permeation experiment at day 30. Means ± standard deviations are shown, n=6.

Formulation	Percentage drug recovered			
	Receptor (i.e. permeated)	Nail clipping	Donor (i.e. film remaining on the nail plate surface)	Total
4% w/v AH with ETOH (15% v/v) & Water (10% v/v)	3.2 ± 0.7	7.7 ± 1.0	84.0 ± 2.5	94.9 ± 3.8
6% w/v TH with ETOH (15% v/v) & Water (10% v/v)	1.7 ± 0.3	8.1 ± 1.3	86.3 ± 3.0	96.2 ± 2.2
4% w/v AH with ETOH (20% v/v) & MPE (5% v/v)	5.4 ± 0.6	6.3 ± 1.0	83.6 ± 2.5	95.3 ± 2.6
6% w/v TH with ETOH (20% v/v) & MPE (5% v/v)	2.7 ± 0.7	8.3 ± 1.9	83.9 ± 3.7	94.9 ± 2.5
4% w/v AH with ETOH (20% v/v) & NMP (5% v/v)	3.8 ± 0.7	7.2 ± 1.0	83.9 ± 2.8	94.9 ± 2.8
6% w/v TH with ETOH (20% v/v) & NMP (5% v/v)	1.7 ± 0.2	7.6 ± 1.2	87.0 ± 1.7	96.2 ± 1.4
4% w/v AH with ETOH (20% v/v) & PEG 200 (5% v/v)	3.5 ± 0.7	6.3 ± 0.6	84.4 ± 3.3	94.3 ± 3.0
6% w/v TH with ETOH (20% v/v) & PEG 200 (5% v/v)	1.7 ± 0.4	7.8 ± 1.4	85.4 ± 2.0	94.9 ± 1.8

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

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