Using pressurised gyration to generate mucoadhesive progesterone-loaded fibres for drug delivery applications

A thesis submitted in partial fulfilment of the requirements for the degree of

Doctor of Philosophy

By

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Declaration

I, Francis Asamoah Brako, confirm that work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated.

Signature:

Date

Abstract

This work explores the prospects of polymeric micro and nanofibres as drug delivery systems intended to facilitate transport of progesterone across vaginal mucosa by mucoadhesion. These fibres, due to their physical attributes, ability to improve drug solubility and high adsorption efficiency may be adapted for improved trans-mucosal drug delivery. Mucoadhesion on the other hand is being explored for improved dosage form residence times, targeting and therapeutic efficacy. Notwithstanding the potential utility of mucoadhesion and nanofibres, generating substantial amounts of mucoadhesive fibres is fraught with many challenges.

In this work, pressurised gyration, a novel approach combining centrifugal force and pressure was used to produce fibres from combinations of polyethylene oxide (PEO), carboxymethyl cellulose sodium (CMC), sodium alginate and polyacrylic acid; polymers with inherent mucoadhesive properties. Nanofibres generated were characterised using scanning electron microscopy, infra-red and x-ray diffraction analyses to determine their morphology, size distribution and molecular composition. Furthermore, they were assessed by texture analyser and atomic force microscope for mucoadhesive performance after which PEO/CMC blends were selected for drug (progesterone) loading. The progesterone-loaded fibres were assessed, mainly for drug release and mucoadhesion. A new methodology based on classical mucoadhesion theories, where atomic force microscopy was used to map interfacial roughness and voids in adhering surfaces was developed for quantifying mucoadhesive properties of systems produced.

In conclusion, this work has demonstrated the possibility of generating drug-loaded fibres as potential constructs for developing vaginal dosage forms for improved performance facilitated by mucoadhesion. Furthermore, a new approach to quantifying mucoadhesion between fibres and mucosa by AFM was developed, with outcome correlating favourably with forces required to detach interacting surfaces measured by texture analyser.

Publications

Refereed Journal Papers

Brako, F., Mahalingam, S., Rami-Abraham, B., Craig, D. Q., & Edirisinghe, M. (2016). Application of nanotechnology for the development of microbicides. *Nanotechnology, 28*(5), 052001.

Brako, F., Raimi-Abraham, B., Mahalingam, S., Craig, D. Q., & Edirisinghe, M. (2015). Making nanofibres of mucoadhesive polymer blends for vaginal therapies. *European Polymer Journal, 70*, 186-196.

Brako, F., Raimi-Abraham, B., Mahalingam, S., Craig, D. Q., & Edirisinghe, M. Mucoadhesion of progesterone-loaded vaginal drug delivery nanofibre constructs. (Submitted to ACS Applied Materials and Interfaces).

Brako, F., Raimi-Abraham, B., Mahalingam, S., Craig, D. Q., & Edirisinghe, M. The Use of Pressurised Gyration to generate Progesterone-Poly (ethylene oxide) (PEO) Nanofibres for Vaginal Drug Delivery Applications, in preparation.

Conference Presentations

Brako, F., Craig, D. Q., & Edirisinghe, M. *Novel Vaginal Drug Delivery Systems from Nanofibres.* PhD Student Conference, Department of Mechanical Engineering, UCL, London, UK. 18th July, 2014.

Brako, F., Craig, D. Q., & Edirisinghe, M. *Making Mucoadhesive Nanofibres by Pressurised Gyration for Drug Delivery*. PhD Student Conference, Department of Mechanical Engineering, UCL, London, UK. 25th June, 2015.

Brako, F., Craig, D. Q., & Edirisinghe, M. *Nanofibre Based System Delivering Progesterone for Preventing Preterm Birth.* 10th World Biomaterials Congress, Montreal, Canada. 22nd May, 2016.

Brako, F., Craig, D. Q., & Edirisinghe, M. *Using Pressurised Gyration in Generating Nanofibre Systems for Improved Delivery of Progesterone.* 15th UK Society for Biomaterials Annual Conference, University of Westminster, London, UK. 1st July, 2016.

Brako, F., Craig, D. Q., & Edirisinghe, M. *Making progesterone-loaded Nanofibres by the Pressurized Gyration Method.* Academy of Pharmaceutical Sciences Conference, University of Strathclyde, Glasgow, UK. 7th September, 2016.

Brako, F., Craig, D. Q., & Edirisinghe, M. *Progesterone-loaded nanofibres for enhanced release and bioavailability*. EPSRC EHDA Network: Intl. Pharm. Tech. Conference 2016, Leicester, UK. 4th November, 2016.

Brako, F., Craig, D. Q., & Edirisinghe, M. *Exploring Pressurised Gyration and Electrohydrodynamic Atomisation (EHDA) Methods for Producing Drug-loaded Mucoadhesive fibres.* Society for Biomaterials Annual Meeting and Exposition, Minneapolis, USA. 6th April, 2017.

Brako, F., Craig, D. Q., & Edirisinghe, M. *Making progesterone-loaded Nanofibres* by the Pressurized Gyration Method. Academy of Pharmaceutical Sciences Conference, De Havilland Campus, University of Hertfordshire, Hatfield, UK. 5th September, 2016.

Brako, F., Martha Gyansa-Lutterodt, M., Craig, D. Q., & Edirisinghe, M. *Pharmacy-focused interventions for improving utility and sustainability of the national health insurance scheme (Ghana).* 77th International Pharmaceutical Federation World

Congress of Pharmacy and Pharmaceutical Sciences, Seoul, South Korea. 10-14 September, 2017.

Workshop/Symposium

Particle Engineering Workshop by Electrohydrodynamic Atomisation (EHDA) Network /Engineering and Physical Sciences Research Council (EPSRC) in University of Portsmouth, UK. 25th April, 2017.

Symposium on Pharmaceutical Formulation by Electrohydrodynamic Atomisation (EHDA) Network /Engineering and Physical Sciences Research Council (EPSRC) in University College London, UK. 15th July, 2017.

Dedication

To

My lovely wife, Georgia Brako.

Your love and sacrifices for our family and many others are exceptional and worthy of praise.

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'Except the Lord build the house, they labour in vain that build it: except the Lord keep the city, the watchman waketh but in vain.' Psalm 127:1(King James Version).

I am first and foremost grateful to God Almighty for the gift of life, health and sound mind. His mercies and kindness has seen me through this endeavour.

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Contents

List of Figures

List of Tables

Chapter 1

Introduction

1.1 Introduction and background

The increasing utility of nanofibres in diverse fields including bone and tissue engineering, drug delivery, filtration, conducting composites, photonics and fuel cells has been reported extensively (He et al., 2008). It has been shown for instance how extremely light nanofibre offer excellent filtrations properties, support the growth of human, animal and bacteria cells and thus proving valuable in some decontamination technologies and tissue engineering (Leung and Ko, 2011, Pham et al., 2006)

In addition, the unique physical properties of nanofibre can have remarkable utility in the area of drug delivery. A large surface area, specifically characterised by a high surface-to-volume ratio and porosity defined by relatively small pore size is usually the most cited benefit of nanofibre (Frenot et al., 2007, Bhardwaj and Kundu, 2010). The prospects for enhancing delivery and improving performance and safety of drugs explains the increasing demand for highly structured materials such as nanofibres for drug delivery systems. The benefit of the large surface area of nanofibre is even clearer when illustrated quantitatively, considering the entire cylindrical surface. For instance, a fabric made with 10nm fibres will have a surface area in the region of $350m^2/g$ compared to only $0.35m^2/g$ for that made of 10µm fibres. Although higher surface areas are achievable with some nano-porous granules and powders, nanofibre units are easier handled and manipulated than powders (Schreuder-Gibson and Gibson, 2006). The easier manipulation of nanofibres implies a flexibility that can allow design for highly targeted and efficient drug delivery.

One of the areas within drug delivery, where the properties of nanofibres can be most valuable is delivery through mucosa aided by adhesion. For instance, with vaginal drug delivery, the high surface area of nanofibre may aid extensive contact with the

vaginal mucosa and thereby facilitating drug transport. There is also flexibility with choice of material where particular polymers and their combinations, e.g. mucoadhesive polymers may be used for delivery systems with exceptional adhesive capabilities. Encapsulation of drugs such as tetracycline hydrochloride, captopril and hydrochlorothiazide in biodegradable nanofibres have been attempted in the past, though mainly by electrospinning (Qi et al., 2010, Beck-Broichsitter et al., 2010, Wei et al., 2011). Considering the limitations of electrospinning, particularly with energy (electricity) costs associated and the relatively low yield, even over long periods of spinning, a newly invented pressurised gyration system for producing nanofibres (Mahalingam and Edirisinghe, 2013) offers fresh prospects for a nanofibre-based drug delivery system. Pressurised gyration offers flexible processing environment which can be manipulated to produce structures fit for function and purpose.

In order to produce nanofibres to meet the objective of a superior drug delivery system for topical mucosa application, an understanding of the starting material properties – polymers, in our case is essential. Till date, much of the attempt to understand and describe the behaviour of polymers has been with reference to their chain characteristics, usually including their dimensions, stiffness or flexibility and interaction with neighbouring chains (Shaw, 2012). Of these chain characteristics, as Shaw (2012) further explains, rheological behaviour properties, justifiably so is progressively utilised in research and industry as it closely links the physical characteristics of polymers to their processing and quality of finished products. As with many operations dependent on the physical transformation of polymer from one matter state to the other, the process of spinning fibre into the solid state from liquid polymer solution could be optimised by rheology. An understanding of polymer - solvent molecular interaction, and to some extents, polymer – polymer interaction within blends would be helpful for the objective of this study. Mucoadhesive properties of nanofibres, especially in this case, will be critical for the performance of system

intended as topical application through the mucosa is often reliant on the extent of adhesion to the site of drug application.

Furthermore, some principles in rheology could be useful in putting into context some often complex relationships among flow, working pressure, molecular nature of polymer and particularly in this case, the angular forces from the gyrating apparatus being utilised. Because some industrial polymer materials are expressed in ranges, the viscosities of the solutions which offer a more specific characteristic in reference to the amount of polymer and solvent system are utilised. Several studies including those based on the Mark-Houwink equation, $[\eta] = KM^a$ (where $[\eta]$ is intrinsic viscosity, M is molecular weight and K and a are values dependent on the polymer-solvent system in question) have been used to explain the relationship between polymer viscosity and molecular weight (Wang et al., 1991, Beer et al., 1999, Flory, 1954, Chuah et al., 2001, Kasaai, 2007). An analysis of the surface properties, particularly through the measurement of surface tension of various polymer solutions are also helpful in harnessing properties of starting materials for optimal production of nanofibres.

1.2 Aim

The aim of this study was to identify and analyse the various conditions required for optimal production of drug-loaded mucoadhesive nanofibres utilising blends of polymers with different adhesive profiles. Fibres obtained from this project are intended as materials for design of a drug delivery system to be applied vaginally. It begun with some rheological analyses i.e. measuring the viscosities and surface properties of polymer solutions and finding their correlation with fibre produced. Relationships established may be helpful in predicting outcomes such as fibre quality, yield and dimensions. Once the fibres were obtained, characterisation studies were completed using such analytical methods as scanning electron microscopy, FTIR, and X-ray analysis to offer insights into the exact morphology, surface structure and

composition of the nanofibres, which are essential for effective design for a drug delivery system. An approach based on the fracture theory of mucoadhesion as well as a novel method purposely developed for this study were utilised in studying the mucoadhesive properties of fibres generated.

1.3 Objectives of research

The objectives enumerated below we set out to guide various tasks needed to achieve the aim of this project,

1.3.1 Identification of suitable materials for nanofibre production

Various polymers confirmed to have intrinsic mucoadhesive properties in different blends will be selected to produce fibres by PG. Preliminary literature search will be conducted, particularly on polymers widely known and confirmed to be safe for drug formulation, as the intended purpose of fibres to be generated is for drug delivery. Solvents or systems containing suitable proportions of different kinds of solvent will also be preselected, based on their utility, safety and capability of making suitable forms of liquid of materials to be converted to nanofibres

1.3.2 Generating nanofibres from selected materials

Optimising the production of nanofibres using a newly invented PG system. By way of optimisation, the major parameters that affect the structural integrity and appearance of fibres are investigated to determine which set of conditions produced nanofibres most suited for the design. Specifically, a relationship between the amount of polymer and quality of fibre is determined by varying material concentrations and blends. The influence of solution properties such as viscosity and surface tension on outcome of nanofibres, especially size distribution will be investigated.

1.3.3 Characterisation to identify physical attributes of fibres

Fibres produced will be examined by SEM analyses for their morphology and cross-sectional diameters (sizes). Relationships established between solution properties such as viscosity and surface tension and average fibre diameters will be noted and analysed to determine if they will be useful for predicting fibre outcome in production of subsequent batches.

1.3.4 Characterisation to identify molecular composition and changes

Further characterisation procedures including thermal analysis, FTIR and variable temperature FTIR, X-ray diffraction were carried out to identify any changes in terms of molecular composition and rearrangement during nanofibre formation. These analyses also provided a basis for classification and determining which batches of fibre will be more suited for the drug delivery systems anticipated

1.3.5 Investigating mucoadhesive properties of fibres

Forces required to detach a probe from a mixture of fibres and mucin in a simulated environment to mimic adhesion of fibres onto mucosa surfaces will be measured to help predict the relative mucoadhesive potential of various batches of fibres produced. The extent of fibre adhesion to mucosa surfaces is anticipated to be useful for predicting performance of our delivery systems. This concludes the first part of the study where the encapsulating system to be used for the delivery of the chosen drug is produced and characterised.

1.3.6 Encapsulating the model drug progesterone in fibres

The second part of the work will begin with encapsulating the active drug, progesterone in systems identified from previous work to have the best prospects in terms of mucoadhesion capabilities. The drug-loaded systems produced will be characterised to determine if inclusion of the model drug affected the physical and

molecular attributes of the nanofibre, as well as tested for mucoadhesive and drug release performance.

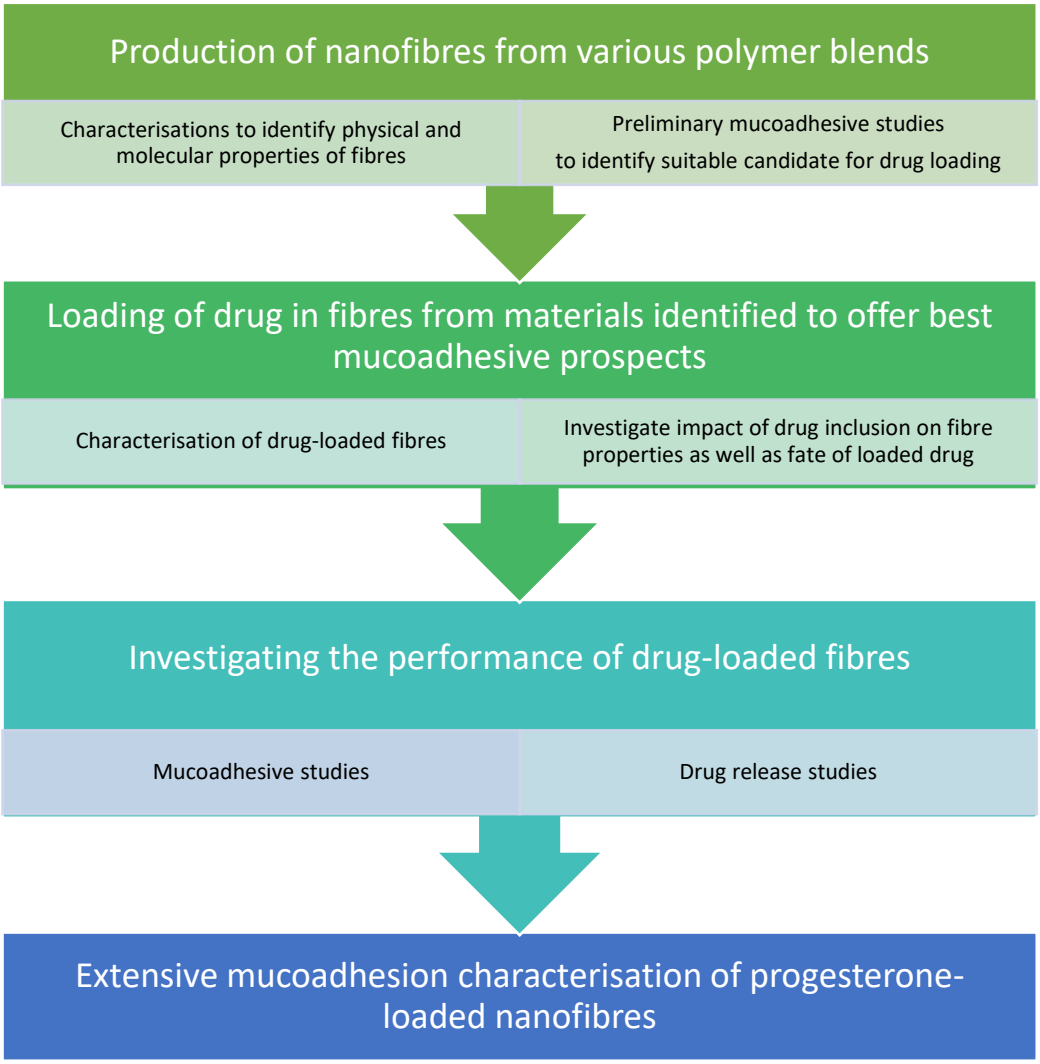


Figure 1-1: Summary of study objectives

1.3.7 Investigating the performance of drug-loaded systems

The utility of progesterone-loaded nanofibres as drug delivery constructs will be examined. Two key areas crucial to the usefulness of systems for drug delivery through mucosa are permeation/release properties and their mucoadhesive capabilities. Permeation/release studies will be conducted using artificial membranes and Franz cells. This will end the second part of the work.

1.3.8 Investigating the mucoadhesion of fibres

Mucoadhesion will be crucial to superior performance of delivery systems developed from nanofibres generated. Therefore, extensive investigation into this phenomenon will be major part of this work. Existing methodologies such as use of texture analyser to quantify fracturing forces between fibres interacting with mucosal surfaces to study extent of mucoadhesion occurring will be utilised. A suitable methodology based on atomic force microscopy analysis of points of interaction between fibres and mucosal surfaces will be explored and compared with outcomes from other methodologies

1.3.9 Novelty in this research work

The possibility of making mucoadhesive drug-loaded fibres from combination of inherently mucoadhesive polymers by simultaneous use of pressure and centrifugal force (pressurised gyration) is demonstrated for the first time. Furthermore, a new approach based on analysing interfacial roughness by atomic force microscopy for the quantification of mucoadhesion between polymeric nanofibers and mucosa membrane has been proposed. Results obtained correlates favourably with those from quantifying mucoadhesion by measuring force required to detach two interacting surfaces. Both findings are contributions towards exploring applications and assessing the performance of drug-loaded fibres as drug delivery systems.

1.4 Structure of thesis

The figure below summarises the organisation of this thesis. Briefly, the main parts are introduction and literature review, three consecutive studies, conclusions and future recommendations.

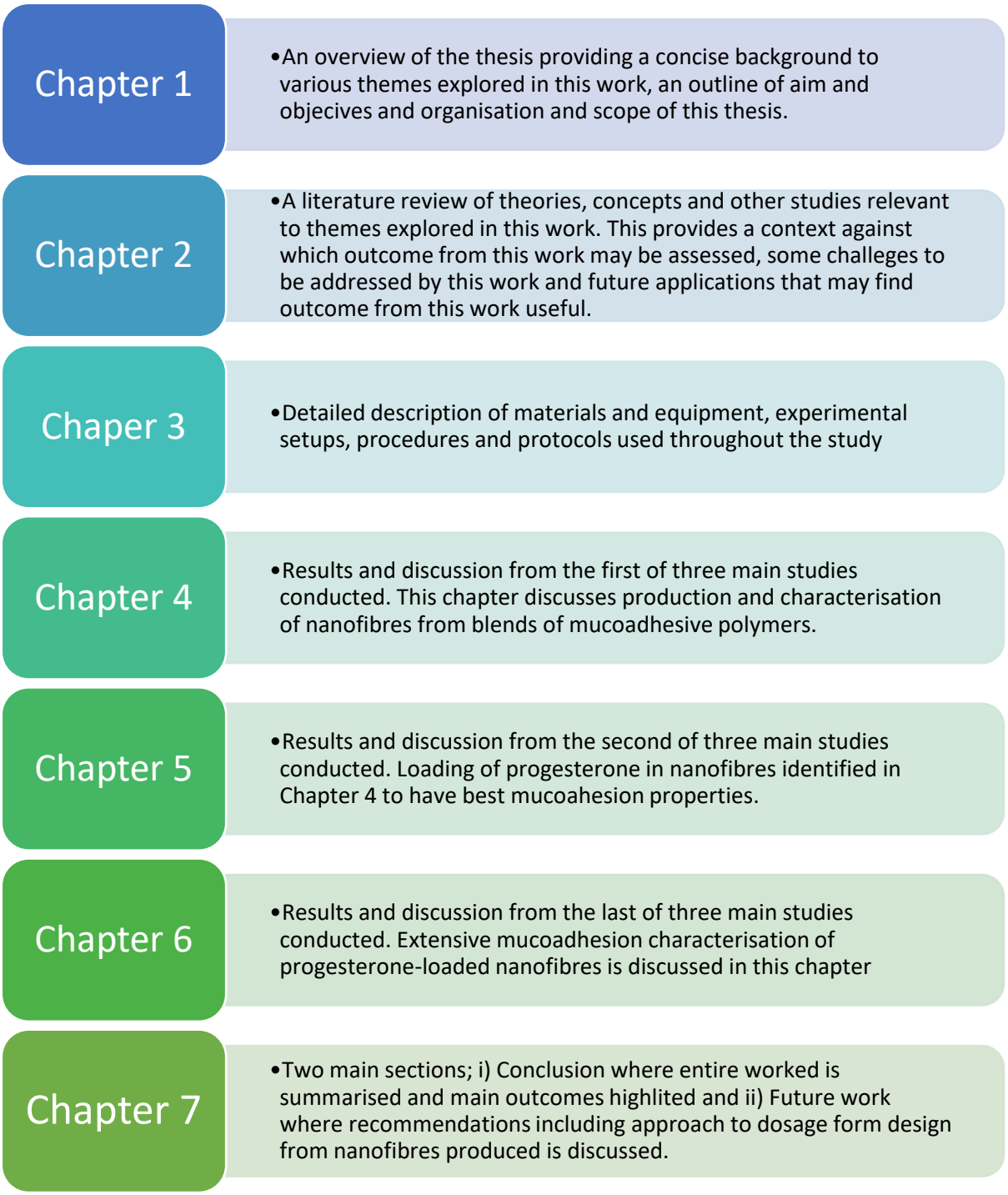


Figure 1-2: Chapter organisation of thesis

Table 0-1: An overview of main themes discussed in Literature review.

Nanotechnology	Drug Delivery	Mucoadhesion
Nanotechnology	Overview on drug deliver	Mucoadhesion
Nanotechnology for drug delivery	Health outcomes today	Overview
Nanostructures	Prospects for improvement	Two-step principle of mucoadhesion
Nanoparticles	Overview on vaginal delivery	Theories of mucoadhesion
Dendrimers	Model drug: Progesterone	Factors affecting mucoadhesion
Liposomes	History of discovery	Hydrophilicity
Nanofibres	Progesterone as of today	Molecular weight
Nanofibres	Chemistry and pharmacology	Spatial conformation
Applications	Delivery of Progesterone	Drug /excipients
Methods of production	Oral	Quantifying mucoadhesion
Self-assembly	Parenteral	*Proposal of novel methods*
Phase separation	Vaginal	Nanotechnology and mucoadhesion for drug delivery
Spinning (e.g. gyration)	Proposal of new approach	
Pressurised Gyration	Rational for this work	
Factors affecting outcome		
Solution properties		
Working pressure		
Rotation speed		

Chapter 2

Literature review

2.1 Introduction

Utilising nanofibres for various applications including drug delivery is still an emerging area in nanotechnology. This literature review aims to put the research outcomes reported in this thesis in proper context by highlighting and exploring relevant theories and concepts within nanotechnology, specifically as applied to drug delivery. This evaluation looks broadly into types and current applications of nanofibre and methods employed in developing these nanostructures. Secondly, the unique properties of nanofibres and how they can influence the structure and performance of various delivery systems when utilised for such purposes are discussed. Drug delivery aided by mucoadhesion of delivery systems onto mucosa membrane of vagina is a key strategy guiding the course of this project. Therefore, principles and theories underlying mucoadhesion, polymeric materials with inherent mucoadhesive properties and various methods of assessing mucoadhesion have been discussed. This research work is seeking to develop materials that can be utilised for optimal delivery of progesterone, specifically for the prevention of preterm labour among women considered high risk. Therefore, the chemistry and pharmacology of this drug has been explained briefly. Various methods and routes for administering the drug presently as well as lapses or shortfalls associated with their delivery have also been deliberated. And finally, suggestions for improving delivery of progesterone, especially through recent technologies such as nanofibre based dosage forms as demonstrated in this project have also been discussed. The review has been organised under three main themes as summarised in Table 2-1. The first is nanotechnology, a broad multidisciplinary endeavour offering principles and tools, some of which enabled the production of materials central to this study. The second

theme reviewed is drug delivery where current challenges and prospects in this area are discussed, with special emphasis on vaginal delivery of progesterone. The last part involves mucoadhesion, where various theories and factors affecting the process are discussed.

2.2 What is nanotechnology?

Nanotechnology as a word was first used in 1974 by Norio Taniguchi in a conference paper titled “On the Basic Concept of Nano-Technology’’ where he described it as a concept of processing i.e. separation, consolidation and deformation of materials by one atom or one molecule (Rogers et al., 2014). ‘Nano’, from which several other fields derive their names, and more commonly known to be the prefix representing 10^{-9} comes from the Greek word *nano* meaning dwarf, rightly reflects the size of substances denoted as such.

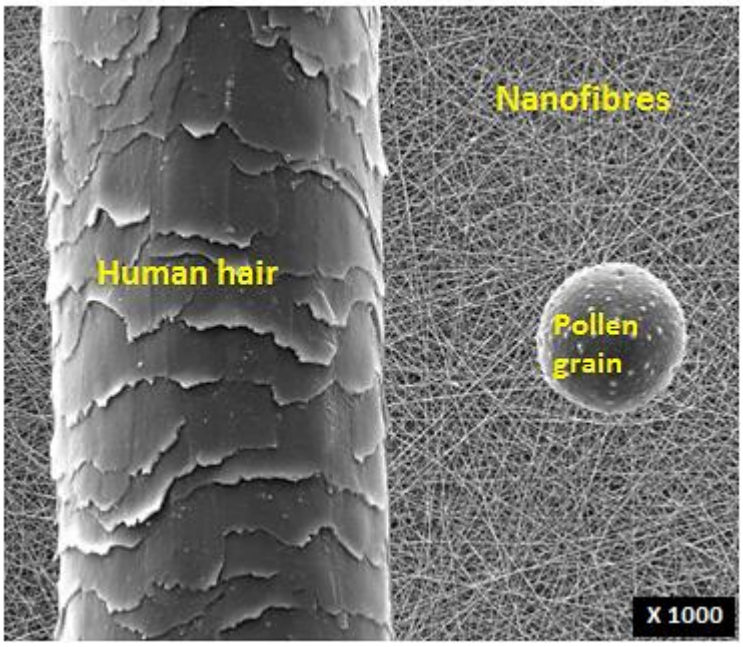


Figure 2-1: Dimensions of nanofibre and how they compare with human hair and pollen grain (Image magnification: X1000)

Nanotechnology is largely considered a product of nanoscience and a definition of nanoscience has been suggested as a good starting point for explaining what

nanotechnology actually involves (Schmid, 2008). A report from Royal Society and the Royal Academy of Engineering defines nanoscience aptly as 'the study of phenomena and manipulation of materials at atomic, molecular and macromolecular scales, where properties differ significantly from those at larger scale' (Dowling et al., 2004). Findings and outcomes from nanoscience typically inform nanotechnology where the design, characterisation and production of structures and systems are achieved by controlling the physical properties of materials involved at the nanometre scale. Nanotechnology based applications have and continue to support the generation of nanostructures which are crucial elements in a recent phenomenon, nano-formulation – a field within pharmaceutical research and development gaining much attention due to its attractive prospects. Nano-formulation is a broad concept involving various formulation strategies that aim to improve performance of therapeutic agents principally by particle size reduction of materials employed (Wais et al., 2016). The nanostructures employed, widely varying in size, physical characteristics and functionality are already demonstrating various benefits which may be summarised under three main themes; improved bioavailability, efficient drug loading and better targeted delivery (Kumar, 2006). Nanofibre is an example of material that can be generated using suitable nanotechnology. The central theme of this project is to utilise nanotechnology-based approach in generating drug loaded nanofibres for developing delivery systems for better therapeutic outcomes.

2.3 Nanotechnology for drug delivery: lessons from microbicide development

For over three decades, many challenges yet to be surmounted have kept humans from developing an ideal microbicide capable of preventing HIV transmission in the safest possible manner. A detailed review of issues responsible for this slow progress points to formulation approach as a major concern. The majority of microbicide which has been in various phases of clinical trials (some examples shown in Table 2-1) are conventional semi-solid formulations, particularly in the gels designed to effectively

deliver a single dose of an antiviral agent at a time (Stone, 2002, Di Fabio et al., 2003, Ndesendo et al., 2008, Van Herrewege et al., 2004). Several of these formulations have failed to demonstrate adequate efficacy, safety and tolerability, thus prompting a re-evaluation of the current development paradigm (Hendrix et al., 2009, Abdool Karim and Baxter, 2014). Indeed the focus on formulating an effective microbicide for the prevention of HIV transmission is gradually expanding from earlier concepts that centred on gel and cream formulations to include such forms as films and fast dissolving solids (Rohan et al., 2013). However, there is still more room for improving upon the existing range of vaginal dosage forms intended for the delivery of microbicides.

For a microbicide to effectively deliver the protection expected, highly complex multi-level biochemical interaction among the host, virus and the drug would have to occur. Some details in these interaction remain unknown but recent efforts in establishing a framework for advancing knowledge required for developing microbicides has improved our understanding of the subject and has actually been translated into practical methodological approaches that are bringing us closer to achieving a suitable microbicide (Hendrix et al., 2009). Some of the key challenges identified during trial of these formulations, including disruption or inflammation of mucosal epithelium, effect of pH conditions and proteolytic enzyme action in the genital tract, inadequate delivery of active drug to target sites due to poor retention and inconvenience associated with use frequency of application are likely to be surmounted through strategies utilising nanotechnology applications (D'Cruz and Uckun, 2014).

Thus, a strong case has been made for taking on new formulation approaches such us exploring the many options available in the area of nanotechnology. The possibility of utilising the unique physical and structural properties of nanostructures such as nanofibres, liposomes, dendrimers and nanoparticles (NPs) for addressing some of the specific issues seen in the current stock of microbicides are discussed.

Table 2-1: Some microbicidal candidates that has been in various phases of clinical trials recently

Trial Name	Phase	Start Date	Countries	Candidate(s)	Completion Date
FACTS 002 An adolescent safety study designed to test the safety and acceptability of tenofovir gel in 16- and 17-year-old South African young women	II	July 1, 2015	South Africa	1% Tenofovir gel	December 2016
A13-128 Safety of the TFV/LNG*, and TFV-only intravaginal rings pharmacokinetics of TFV and LNG acceptability of intravaginal rings	I	November 30, 2014	United States of America, Dominican Republic	TFV ring, TFV/LNG* ring	November 2015
MTN 017 Assess the safety, acceptability, systemic and local absorption, and adherence of reduced glycerine tenofovir gel applied rectally	II	September 30, 2013	Thailand, South Africa, United States of America, Puerto Rico, Peru	Reduced Glycerine 1% Tenofovir Gel, TDF/FTC** (Truvada)	June 2016
IPM 027 (The Ring Study) To assess the safety and efficacy of a silicone elastomer vaginal matrix ring	III	April 30, 2012	South Africa, Uganda	Dapivirine Ring	December 2016

** TFV/LNG = Tenofovir / levonorgestrel; ** TDF/FTC =Tenofovir disoproxil / emtricitabine*

Nanotech inspired approaches to formulating drugs and medical devices have proved to be successful in some therapeutic areas like cancer management. When extensively researched and adequately applied, these same technologies could be resourceful in the development of vaginally applied interventions such as microbicides for HIV prevention or progesterone inserts for preventing premature

labour. Some significant transformations happening with drug delivery, brought about by applications of nanotechnology utilising nanostructures are discussed further. Structural description, processing methods for their fabrication, various interventions aimed at enhancing drug delivery and their future prospects are discussed specifically for each of the nanostructures cited.

2.3.1 Polymeric NPs for microbicides

NPs for drug delivery has been broadly defined to include particulate systems with mean diameter between 50 and 1000 nm (zur Mühlen et al., 1998). Polymeric NPs are typically the result of spontaneous self-assembling of amphiphilic polymers when dispersed in an aqueous medium (Uchegbu et al., 2013). The self-assembly is usually driven by methods such as probe sonication, micro-fluidisation and high pressure homogenisation. The therapeutic agents in NPs could be dissolved, encapsulated within or adsorbed onto the constituent polymer matrix.

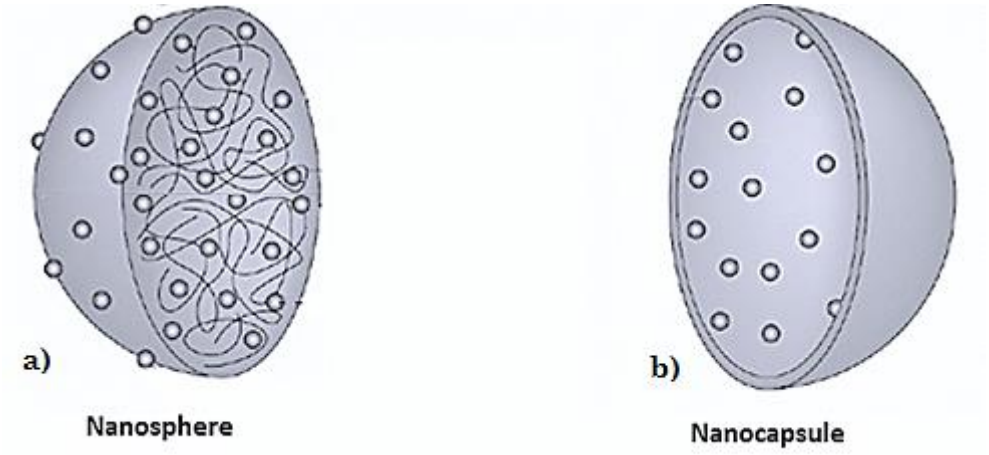


Figure 2-2: A schematic illustration of a) nanosphere showing how drug (small spheres) is dispersed throughout the polymer matrix and b) nanocapsule in which drug is confined in a reservoir bound by single membrane.

Depending on the processing method utilised, the resulting structure could either be a nanosphere (Figure 2-2a) in which the active agent is dispersed throughout a polymeric matrix system in the particle or as a nanocapsule (Figure 2-2b) in which the

drug exist in a vesicular reservoir enclosed by single polymeric membrane (Letchford and Burt, 2007).

NPs, due to their ability to overcome physiological barriers while delivering drug molecules to specific cells or tissue compartments either by passive or ligand mediated movements make them a highly versatile class of drug delivery systems (Mallipeddi and Rohan, 2010). NPs have demonstrated promising prospects for superior drug delivery, especially in the area of cancer pharmacotherapy (Prabhu et al., 2015) and this novel approach could be extended into the area of vaginal drug delivery for better outcomes. For instance targeted delivery of microbicide drugs into vaginal tissues through the use of NPs has recently been developed to overcome issues such as drug movement across mucosa barriers, physicochemical stability, solubility, and immunogenic response typically associated with conventional hydrogel formulations (Rohan and Sassi, 2009). If microbicides are to offer the necessary protection against HIV infection, it will be crucial for the active components of the formulation to navigate the many barriers in order to reach tissues hosting these viruses for optimal antiviral activity. Nanoparticles, when employed as carrier systems could aid the delivery of active drug to these tissues. Surface engineered dapirivine-loaded polycaprolactone NPs have been demonstrated to have the potential to facilitate movement of the antiviral drug across mucosa barriers in the cervico-vaginal region (das Neves et al., 2012). Furthermore, microbicide formulations delivering active drug (dapirivine) through NPs have been confirmed to exhibit better antiviral activity due to increased intracellular drug delivery facilitated by better cellular uptake of the drug loaded NPs (das Neves et al., 2012).

In addition to enhancing antiviral activity through more efficient drug transport across barriers and better cellular uptake, NPs can be instrumental in improving the acceptability and use of microbicides when their potential to protect active agents from untimely metabolism are applied to prolonging microbicidal activities.

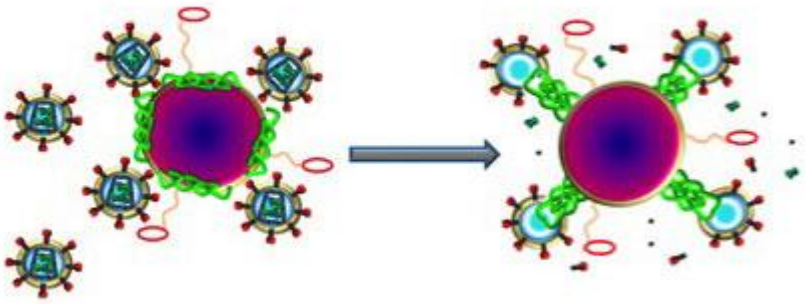


Figure 2-3: Nanoparticle (purple) as carrier system for melittin (green) and how they inhibit infection by fusing with HIVs (spiked small circles), subsequently destroying their protective envelopes. Molecular bumpers (small red ovals) shield the system from attacking much larger mammalian cells thereby enhancing selectivity for the viruses. (Photo credit: Washington University in St. Louis)

A poly (D,L-lactide-co-glycolide) NP delivering the highly potent anti-HIV protein PSC-RANTES was shown to offer superior antiviral activity over an extended period, thus confirming the prospects of achieving longer acting microbicide action when active drugs are presented in NP carrier systems (Ham et al., 2009). Some pioneering and innovative work in utilising NPs to interfere with HIV infectivity is currently underway. Melittin, a cytolytic peptide component of bee venom, incorporated into shells of perfluorocarbon NPs (Figure 2-3) has been proven to inhibit HIV infectivity by disrupting lipid viral envelopes while remaining safe and actually therapeutic for host cells (Hood et al., 2013, Jallouk et al., 2014). Chitosan NPs containing tenofovir with exceptional mucoadhesive properties are also being developed to target mucosa reservoirs of HIV in areas such as the vaginal epithelial tissues (Meng et al., 2011). Some of these positive developments strengthen arguments that nanotechnology offers immense opportunities for developing ideal microbicides in the near future.

2.3.2 Dendrimers for microbicides

Dendrimers (illustrated in Figure 2-4) are highly branched and usually symmetrical three-dimensional structures with a well-defined architecture where peripheral groups are joined to the core by branching units (Murugavel, 2014). They are classified as supramolecular (Astruc et al., 2010) in that they are typically a chemical system made

up of a discrete number of assembled subunits rather than a single unit (Lehn, 1995, Lehn, 1988). Dendritic molecules can be visualised as repetitive layers of multifunctional blocks of a protected and unprotected scheme of complimentary monomers typically resulting in a fractal-like tree in which each incorporated layer serves as a platform for the successive layer (Tomalia and Cheng, 2012). The first completely characterised repetitively branched and polyfunctional molecule was generated by Vogtle and co-workers from a protocol based on cycles of nucleophile amine addition to electron-poor cyanoalkene, followed by reduction of the cyano groups which in turn yields new amine moieties for further reactions (Buhleier et al., 1978).

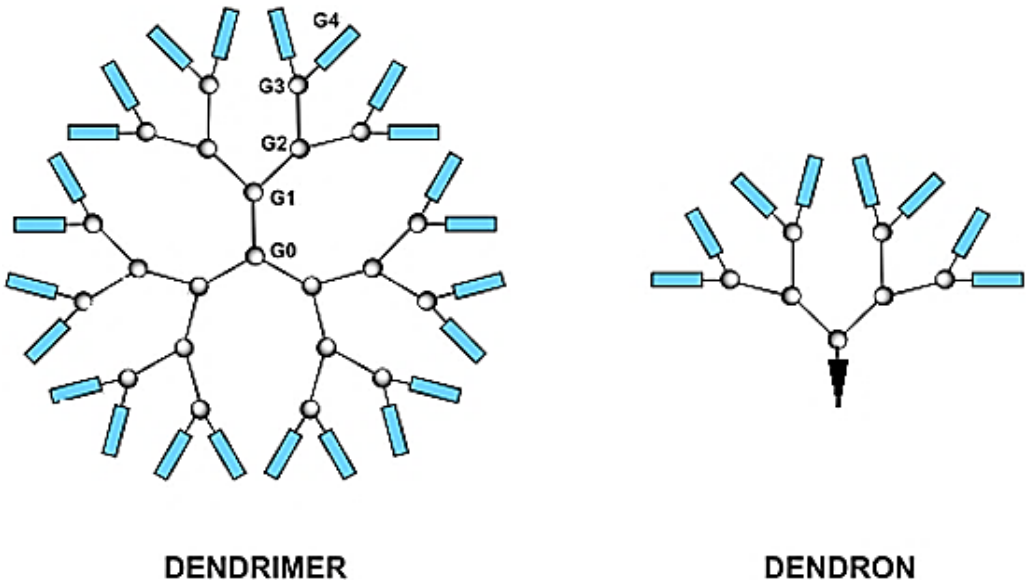
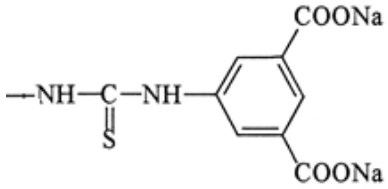
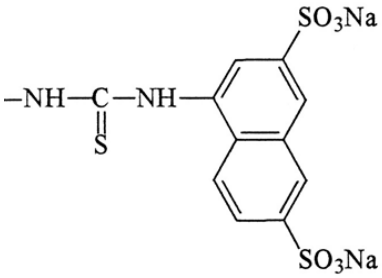
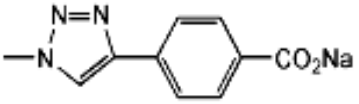
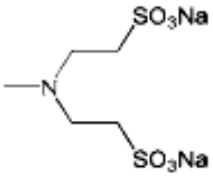


Figure 2-4: Schematic illustration of dendrimer showing the core (G0) and branching points (G1 – G4) which serve as platforms for either expanding the network of molecules with more basic units (Dendron) or with different functional groups for a specific activity (Photo credit: Oleg Lukin)

Several other contributions like Vogtle's, particularly those from the research groups of Newkome, Denkewalter and Tomalia (Newkome et al., 1985, Denkewalter et al., 1982, Tomalia et al., 1985) build upon experiments setting out some fundamental principles of molecular organisation pioneered by Flory in the middle of the 20th century (Flory, 1942)

Table 2-2: Chemical groups used in modification of some classical dendrimers for enhanced antiviral activity against HIV

Dendrimer type [Code name of specific compound and reference]	Functional group delivering anti-HIV activity	Site and mode of anti-HIV activity
Polyamidoamine (PAMAM) [BRI2932, (Witvrouw et al., 2000)]		Attaches to gp120 to inhibit binding to MT-4 cells
Polyamidoamine (PAMAM) [BRI6195, (Witvrouw et al., 2000)]		Attaches to gp120 to inhibit binding to MT-4 cells and capable of permeating host cell to inhibit reverse transcriptase and integrase activities during replication
Gallic acid-triethylene glycol (GATG) [[G1]-CO2Na (Doménech et al., 2010)]		Complexes with C-terminal domain of viral capsid protein which results in disruption of capsid assembly for maturation of HIV type 1
Carbosilane [2G-S16, (Chonco et al., 2012)		Combines with both gp120 on viral surface and CD4 on host cells to disrupt fusion of virus onto host cell

Extensive research into the possible applications of dendrimers in diverse biological and medical fields is currently ongoing (Caron et al., 2010, Mintzer and Grinstaff, 2011, Nasibullah et al., 2013, Noriega-Luna et al., 2014). Some properties of dendrimers including their nanoscopic size and uniformity, flexible molecular structure

and presence of multiple peripheral functional groups to facilitate conjugate formation with a wide range of drug molecules make dendrimers highly suited for targeted delivery of drugs (Kesharwani et al., 2014), and hence the rigorous investigations into their potential as drug delivery systems, especially in the area of cancer pharmacotherapy and delivery of macromolecules (Cheng et al., 2011, Brannon-Peppas and Blanchette, 2012, Lim and Simanek, 2012, Parhi et al., 2012, Parveen et al., 2012).

Furthermore, the unique architecture of dendritic molecules, especially that of its interior with enough void volume makes it ideal for doping with a wide range of molecules for desirable functioning (Tomalia and Cheng, 2012). Presently, drug-dendrimer conjugates of anticancer drugs including fluorouracil, methotrexate, doxorubicin, paclitaxel, camptotecin and a few more are being investigated for improved physical attributes and performance. In addition to anticancer drugs, several other classes of medicines including anti-inflammatory and antimicrobial agents are being investigated for presentation as drug-dendrimer units (Tomalia and Cheng, 2012)

The attractive characteristics of dendrimers are being utilised for the development of safer and more efficient microbicides. First of all, dendrimers by themselves can be inherently antiretroviral when some functional groups capable of interfering with viral adhesion to cells are incorporated onto their surface during synthesis (Jiménez et al., 2012). Some functional groups designed to confer anti-HIV activity on certain classes of dendrimers are shown in Table 2-2. Proteins present on surface of viruses bind multiple carbohydrates on target host cells during invasion (Gajbhiye et al., 2009). Groups similar to these carbohydrates on host cell surfaces can be incorporated onto dendrimers peripheries to act as preferred receptors for invading viruses thus sparing host cells and therefore preventing infection. These have been successfully tested with some influenza viruses and the principle can be explored for the synthesis of dendrimers to be used as microbicides (Tsvetkov et al., 2002, Roy, 1996). In addition

to preventing viral binding to host cells, polyanionic dendrimers have been shown to affect the life cycle of viruses, including HIV (Tephly, 1991). Carboxylated fullerene-based dendrimers for instance are known to inhibit viral protease and reverse transcriptase in acutely HIV infected primary human lymphocytes (Schinazi et al., 2001). The molecular structure of dendrimers, apart from allowing the incorporation of specific functional groups to confer antiviral capabilities also facilitates the conjugation of other antiretroviral compound for possible multi-targeted activities against the transmission of viruses. Two of the most widely studied antiretroviral for microbicide development, tenofovir and maraviroc are reported to have been successfully conjugated onto polyanionic carbosilane dendrimers in separate formulations (Sepúlveda-Crespo et al., 2015). Formulations combining two different dendrimers to be used as microbicides have also been reported (Sepúlveda-Crespo et al., 2014). In both dendrimer-dendrimer and dendrimer-drug combinations, where lower overall concentrations offered greater antiviral activity than usually seen in monotherapies, a case for using a combination formulation to offer optimal activity from low doses for minimal incidence of toxicity and emergence of resistant viral strain has firmly been established (das Neves et al., 2012)

Irritations, possible epithelial injury and inflammation from local application of microbicides have emerged as serious concerns (Beer et al., 2006, W Buckheit, 2012). It is therefore interesting to learn that many dendrimer-based microbicides currently in development appear to be less irritant to the mucosa environment where their application is intended. Following the formulation and evaluation of polyanionic carbosilane dendrimers G3-S16 and G2-NF1, it was observed that in addition to blocking the entry of HIV into target host cell, these dendrimers protected the epithelial layer from cell disruption. Furthermore, these dendrimers did not induce any inflammatory cytokines or caused an irritation or vaginal lesion upon application (Córdoba et al., 2013).

A similar observation of biocompatibility and encouraging antiviral activity was recorded when water-soluble anionic carbosilane dendrimer (2G-S16) was studied (Chonco et al., 2012) confirming the consistent safety profile among these kinds of dendrimers when employed as microbicides. The activity and safety profiles of dendrimer based formulations has so far been promising. The nano-range microbicide which is furthest in the development phases, dendrimer based Vivagel®(Starpharma, Melbourne, Australia) performed well on safety and efficacy at preliminary animal testing and is progressing into human trials (Roy et al., 2015). Phase I clinical trials have been completed with some favourable general outcomes though further progression seems to have been halted because of excessive inflammation and damage to epithelial tissue (McGowan et al., 2011, Moscicki et al., 2012). It has been observed though that current nanotechnology being explored for microbicide formulation is shifting from utilising inherent antiviral activity of the nanostructures to using them as systems to deliver highly active antiretroviral drugs (das Neves et al., 2010). Therefore, the prospects of utilising the flexible structure of dendrimers to deliver active drug for prevention of HIV transmission remains positive and worth considering.

As outcomes from clinical trials on conventional formulations of microbicides has largely been negative (Grant et al., 2008), shifting our focus onto nanostructures such as dendrimers in pursuit of an ideal microbicide seem to be a step in the right direction.

2.3.4 Liposomal microbicide

Liposomes (Figure 2-5), from a Greek root word meaning 'fat body' is a multi-layered phospholipid structure with a hollow core, the inner portion usually made of a polar phosphate group and the outer consisting of one or more bilayers of natural or synthetic lipids (Watwe and Bellare, 1995). Liposomes made of natural phospholipids are physiologically inert, weakly immunogenic and of low toxicity (Immordino et al.,

2006). Furthermore, due to their combined hydrophilic and lipophilic nature, a wide range of drugs with different lipophilicities can be effectively encapsulated within liposomes, the highly hydrophilic ones staying in the polar compartment, the lipophilic ones in the lipid layers and those with intermediate partition coefficients easily apportioning between the polar and lipid portions of the liposome (Gulati et al., 1998). The applicability of liposomes have been further enhanced recently due to a steady progression from conventional liposomes to a new generation of liposomes developed through modulation of lipid constituents, size and charge adjustments and surface modification (Torchilin, 2005).

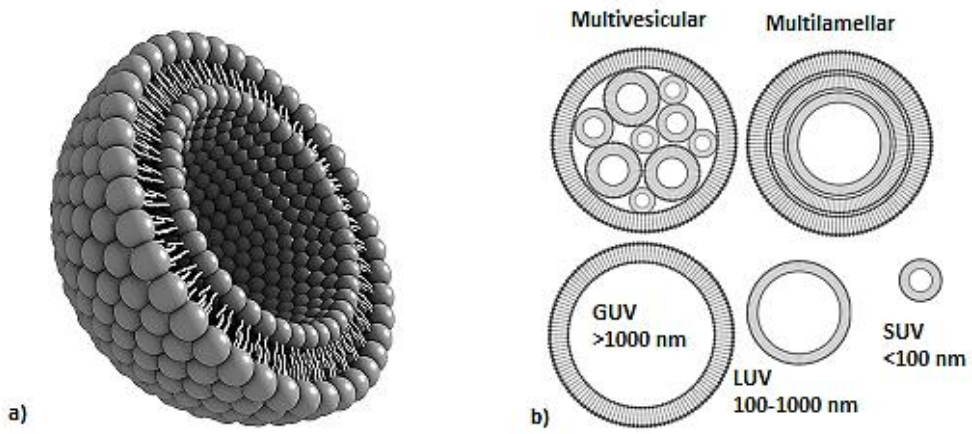


Figure 2-5: Schematic representation of a) Liposome showing assembly of phospholipids in a bilayer that yields both aqueous and lipid compartments within the structure and b) various lamellarity and sizes; small unilamellar vesicles (SUV), large unilamellar vesicles (LUV), giant unilamellar vesicle (GUV), multilamellar and multivesicular

Structurally, the lipid microenvironment of some newer generations of liposomes, the so-called lipid raft achievable from peculiar lipid composition utilising glycosphingolipids (GSLs), sphingomyelins and cholesterols make them capable of serving as platforms of membrane with associated activities such as signal transduction, cell adhesion and lipid/protein organisation, thus increasing their appeal

for biomedical applications (Anderson and Jacobson, 2002, Helms and Zurzolo, 2004, de Gassart et al., 2003).

These physical characteristics of liposomes and their widening applicability due to their continuous improvement make them very attractive for consideration as delivery systems, especially in cases where drug solubility is an issue. With regards to microbicide activity against HIV transmission in the vagina, some potential challenges are anticipated. In order to inhibit activities of viruses that have broken through the physical barrier provided by microbes, active drug, ideally in nano delivery systems, capable of matching the ease with which viruses travel through epithelial layers to infect cells would be required to inhibit viral activity either at point of entry or within tissues (Pope and Haase, 2003, Vanić and Škalko-Basnet, 2013). Furthermore, the carrier system delivering the active drug ought to have minimal interference with vaginal flora and pH, minimal irritation to the mucosa as well as being effective in protecting the drug from sudden changes in the vaginal environment e.g. vaginal fluids due to arousal and release of semen (Vanić and Škalko-Basnet, 2013).

Studies looking into the potential of liposomes as delivery systems for microbicides and capable of meeting these requirements have reported some optimistic outcomes indicating that these nanostructures could be the future of effective microbicides. The membrane-like characteristics of liposomes are also thought to have a potential of fusion with viron material, thus giving these structures some possibility of interfering with HIV transmission by competing with host membrane for uptake of the virus. Several liposomal membranes based on their lipid composition have been assessed for the potential of fusion with HIV-1 virus and found to be in the order cardiolipin (CL) $\gg$ phosphatidylinositol > CL/dioleoylphosphatidylcholine (DOPC) (3:7), phosphatidic acid > phosphatidylserine (PS), PS/cholesterol (2:1) > PS/PC (1:1), PS/phosphatidylethanolamine (1:1) > DOPC, erythrocyte ghosts (Larsen et al., 1993, Malavia et al., 2011)

In a study evaluating a liposome formulation of MC-1220, a highly potent and selective NNRTI on the prevention of HIV transmission through the vagina of non-human primate models (Caron et al., 2010), it was found that formulating MC-1220 in a liposomal gel allowed high amount of drug loading in a small volume of formulation, thus solving some of the bioavailability issues typically seen in conventional gel formulations (Loftsson and Masson, 2001). In addition, the liposomal formulations of the NNRTI were seen to be less irritating to mucosa tissues. Finally, and most importantly, it was observed that the liposomal formulation offered some protection against viral transmission and in fact reduced the viral load in infected models.

Another work that investigated the feasibility of liposomes for use as microbicides (Wang et al., 2012) utilised octylglycerol (OG), a synthetic lipid derived from human breastmilk and has been shown to destabilise viral envelopes and therefore a potential microbicide (Isaacs, 2001, Isaacs and Thormar, 1991, Skinner et al., 2010). In this study, liposomes were produced from combinations of OG and phosphatidyl choline in ratios that ensured *in vitro* antiviral activity and at the same time sparing the natural vaginal flora. Activities of the liposomes were compared to two conventional gel formulations.

2.2.5 RNA interference (RNAi) as strategy for microbicidal action

Nanotechnology offers platforms for drug delivery strategies utilising biologics, especially where highly specific molecular or biological interference is required for therapeutic outcomes (Farokhzad and Langer, 2009). One of such interventions principally disrupt RNA activity to yield desired outcome. RNAi is described as a post-translational and post-transcriptional inhibition of gene expression typically brought about by destruction of specific RNA molecules and this biological process has been demonstrated as capable of preventing HIV transcription, thus having some promising prospects in the prevention of viruses infecting host cells (Lee et al., 2002, Zhang et al., 2006). With regards to preventing HIV transmission through microbicidal

action in the female reproductive mucosa, some pioneering work utilising small-interfering RNA (siRNA) densely packed into biodegradable polymer NPs have been used to bring about silencing of endogenous genes in the genital track, ultimately resulting in protection against challenge from the infectious pathogens (Woodrow et al., 2009). In another study detailing the impact of siRNA on viral transmission, vaginal instillation of siRNA targeting Herpes Simplex virus 2 (HSV2), an important cofactor in HIV transmission was confirmed to reduce overall lethal viral challenge in mice thus suggesting siRNAs as an important and a suitable component of microbicide formulation (Palliser et al., 2006). Outcomes from these studies and several others have established RNAi firmly as a strategy for preventing viral infections and hence siRNAs as valuable components for the development of future microbicides. However, a massive challenge remains with delivery of siRNAs as these structures are highly unstable in serums and delivery across cell membranes. Delivery strategies showing promise so far, such as liposomal, viral or NPs delivery are heavily reliant on nanotechnology and therefore adds to the compelling case being made for utilising nanotechnology for developing the next generation of microbicides (Nguyen et al., 2008). The future of RNAi as a strategy for disease cure and prevention is promising and this is attested by the vibrant pharmaceutical companies' involvement in research currently ongoing in this field, especially with regards to delivery of these nucleic acids.

2.3.6 Nanofibre

A nanofibre typically has two similar external dimensions (making up the cross-sectional area) within the nanoscale and the third dimension, usually the length, significantly larger (Glavas-Dodov et al., 2002). Nanofibres, described as slender, elongated thread-like structures within the nanoscale are characterised by exceptionally high specific area that allows a higher proportion of atoms of interest to be on the fibre surface (He et al., 2008).

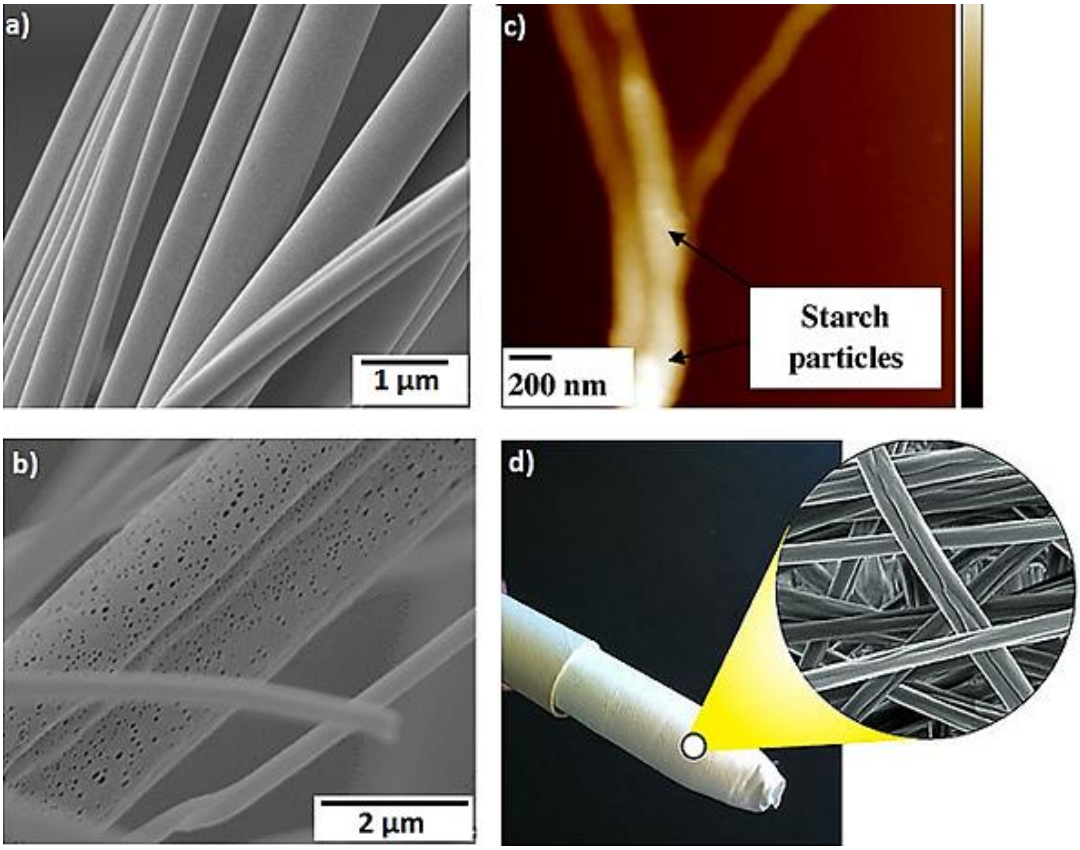


Figure 2-6: a) Nanofibres produced by pressurised gyration (Mahalingam and Edirisinghe, 2013) b) revealing porosity that can be utilised for optimal drug delivery (Illangakoon et al., 2016) c) atomic force micrograph showing encapsulating abilities of nanofibers (Mahalingam et al., 2014) and d) nanofibre based tampon (photo credit: University of Washington) intended for protection against HIV transmission

This physical attribute has been claimed to enable 'quantum efficiency, nanoscale effect of unusually high surface energy, surface reactivity, high thermal and electrical conductivity and high strength'(He et al., 2008). Nanofibres are currently produced by widely varying methods including molecular self-assembly, thermally induced phase separation and fibre spinning (Luo et al., 2012). Of all these methods, fibre spinning, a term used to describe the various methods of fibre formation by extrusion through a spinneret appears to be the most widely used method. Electrospinning and centrifugal spinning are two of fibre spinning methods commonly used.

Nanofibres, depending on constituent materials and conditions of fabrication could exhibit widely varying morphology and structural properties. However, features such

as high surface area to volume ratio and well defined porosity are typical of nanofibres and have often been reasons why they are desirable for drug delivery applications (Pillay et al., 2013). High surface area per unit mass of nanofibres could be harnessed to overcome solubility issues seen in many active pharmaceutical ingredients (API) e.g. ibuprofen, usually by combining them with hydrophilic materials of nanofibres (Williams et al., 2012). In addition, controlling the matrix properties such as fibre diameter and porosity by manipulating fabrication parameters allows for the incorporation of delicate molecules such as proteins and DNA into nanofibre constructs such as meshes for site-specific delivery in the body (Pillay et al., 2013). Significantly higher encapsulation efficiency is attainable when active ingredients are incorporated into nanofibres (Xie and Wang, 2006, Liao et al., 2006). Achieving drug loading more than 90% offers the possibility of designing highly efficient delivery systems with fewer additives and excipients. This ultimately enhances the safety profile of the formulation since unwanted materials, which have to be metabolized and eliminated are already in minimal quantities. Drug delivery systems utilising polymeric nanofibres as basic units can be designed, e.g. using electrospinning with multi-axial needles to obtain multi-compartment assembly delivering different active drugs from a single unit. Basically, multiple APIs may be encapsulated in different nanofibres and combined to be presented as one unit. The multi-targeted approach is a viable option since the individual encapsulations prevents the active ingredients from interacting among themselves regardless of their proximities, until onset of drug action. Finally, the potential of modulating drug release from nanofibre systems by varying their material constituents makes these structures attractive for drug delivery (Kenawy et al., 2009). Considering the wide array of suitable materials available for making nanofibres, several possible combinations may be selected for a specific release kinetic desired. The starting point for most nanofibre production requires the materials to be in solution or melt. Therefore, most materials, especially polymers, once converted to the suitable liquid state and such properties as viscosity and

surface tension optimized may be transformed into nanofibres by the appropriate method. This feature of making nanofibres enable the accommodation of a wide range of materials such as drugs e.g. paclitaxel, biologicals e.g. human nerve growth factor (hNGF) and functional polymers e.g. surface-glycosylated polycaprolactone thereby (Hu et al, 2014) enhancing the prospects of manipulating release kinetics through choice of materials.

2.4 Applications of nanofibre

Many unique physical properties of nanofibres, arising mainly from their ultrahigh surface area and well-defined porosity, enable them to be suitable for several different applications. Most of the applications of nanofibres presently being explored can be classified under one of these main areas – filtration, drug delivery, tissue engineering, microelectronics and sensing, protective clothing and food processing (Wei, 2012).

Of all the areas of nanofibre application, it appears their utility in drug delivery and tissue engineering has seen unprecedented levels of research and publicity, possibly because of the crucial developments and solutions being offered (Sill and von Recum, 2008, Cui et al., 2016, Sill and von Recum, 2015).

Widely varying materials, both biodegradable e.g. poly (lactic-co-glycolic acid) and non-biodegradable e.g. graphene can be selected as materials for fabrication of nanofibres. For this reason, suitable combinations of materials with physical and chemical properties complimenting each other can be used in developing nanofibre drug delivery systems with such desirable properties as an ideal solubility for an optimal release profile. The possibility of forming nanofibres from a wide range of materials also gives better prospects for compatibility, in that materials most compatible with the active ingredient can be selected for developing the delivery systems. Furthermore, the wider surface area of nanofibre which makes it suitable platform to take on a higher proportion of atoms of interest (He et al., 2008), in this

case active pharmaceutical ingredients allows for efficient loading of drug and therefore cutting out the use of unnecessary amounts of excipients during formulation. Due to their exceptionally desirable physical properties which supports such benefits as efficient drug loading, the flexibility with choice of materials for fabrication and their structural versatility that allows them to be delivery systems through adsorption or encapsulation, nanofibres have been used extensively for the delivery of antibiotics, anticancer drugs and various macromolecules such as DNA, siRNA and other proteins such as bovine serum albumin (Hu et al., 2014).

In the area of biomedicine and specifically tissue engineering, the same enhanced surface properties of nanofibres described above have been identified to enable these nanostructures to perform as platforms that support the interaction of cells and growth factors for the repair or replacement of damaged tissues (Rim et al., 2013). This performance as a functional platform supporting cell adhesion and growth, particularly derived from the continuous structure has conferred attractive prospects in areas such as orthopaedics (Christenson et al., 2007)

2.5 Methods for nanofibre generation

As mentioned earlier, most nanofibres are currently produced by one of three methods – self-assembly, phase separation or spinning. Nanofibre generation by spinning, which includes electrospinning and centrifugal spinning, is by far the predominant method in use today. All structures used in this project were obtained by spinning and specifically, by the simultaneous use of high speed centrifugal spinning and high pressure.

2.5.1 Molecular self-assembly

Molecular self-assembly is responsible for formation of a wide variety of complex biological structures. Molecular self-assembly is the spontaneous reorganisation of

molecules in equilibrium conditions into stable and structurally well-defined aggregates joined by non-covalent bonds (Whitesides et al., 1991). Observations from this phenomenon have informed strategies being utilised in chemical synthesis of supramolecular structures typically within the nano-range e.g. peptide amphiphiles (PA) which is presented as nanofibre (Chen and Liu, 2015). Thus self-assembly is one of the main methods of producing nanofibres and PA nanofibres for instance can have fibre diameters as small as 10nm, a size significantly smaller than fibres generated from other methods such as electrospinning (Zhang, 2003).

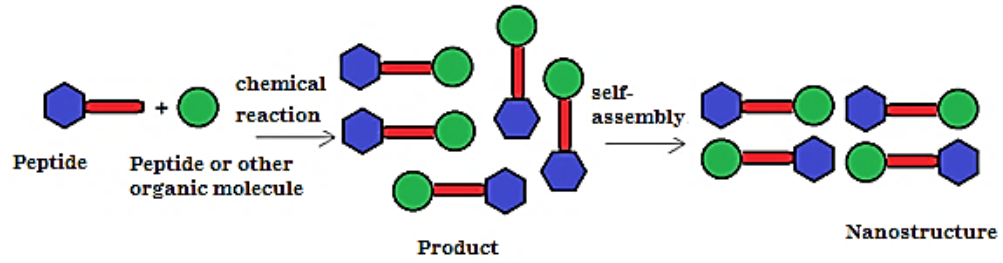


Figure 2-7: A scheme illustrating a self-assembly process

Notwithstanding, poor mechanical strength due to fragility of molecules e.g. peptides involved and high complexity associated with generation of nanofibre by self-assembly has limited the use and applicability of this method, and subsequently impeded its progress into being useful and capable of large-scale fabrication (Chen and Liu, 2015).

2.5.2 Phase separation

Another method of fabricating nanofibres is by phase separation. In phase separation, a homogeneous polymer solution is thermodynamically separated typically by thermal induction or in few instances, by addition of a nonsolvent to cause two phases of polymer rich gel and a solvent rich component. Any remaining solvent in polymer rich

component is extracted, first by using water, then cooling below the glass transition temperature and finally freeze-dried under vacuum to obtain a nanofibrous scaffold (Barnes et al., 2007). Unlike self-assembly, this method is very simple as it does not require any specialised equipment and can easily offer batch-to-batch consistency. The utility of this method is however seriously limited by choice of materials as only a narrow range of polymers can yield nanostructures by this method and so upscaling prospects has not been fully explored thus confining phase-separation to a laboratory scale method for nanofibre production (Jayaraman et al., 2004)

2.5.3 Spinning

Lastly, methods based on spinning such as electrospinning and centrifugal spinning are being used to produce nanofibres. Nanofibres are basically generated by electrospinning through the uniaxial stretching of a viscoelastic solution (Teo and Ramakrishna, 2006).

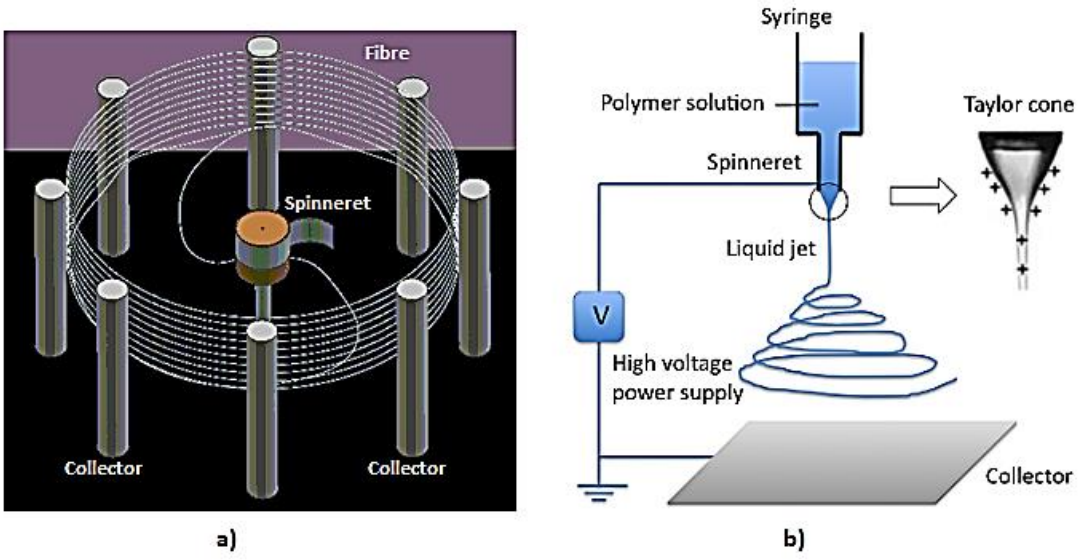


Figure 2-8: Schemes illustrating a) Centrifugal spinning and b) Electrospinning, two main methods for generating nanofibres by spinning (Ren et al., 2013, Li et al., 2010)

In this method of generating nanofibres, where the setup is essentially made up of high voltage supply, a capillary tube typically in the form of a needle and a metal

collecting screen, high voltage is used to create electrically charged jet of polymer solutions through a capillary and before reaching the collection plate, solvent evaporates from the jet thereby solidifying to become fibres (Huang et al., 2003).

Other methods for fibre generation, based on spinning include use of centrifugal spinning with or without pressure. In this method, a polymer solution contained in a closed vessel with orifices is forced out upon high speed rotation and fibre forming during flight of solution jets with simultaneous solvent evaporation and drying up (Padron et al., 2013, Mahalingam and Edirisinghe, 2013). In pressurised gyration for instance, several variables such as pressure, rotation speed, solution properties and room conditions such as temperature and humidity can all be adjusted to yield fibres with specific physical attributes that are just right for applications being sought.

2.5.3.1 Nanofibre generation using Pressurised gyration

The potential utility of nanofibres in diverse arrears including engineering, biomedicine, pharmaceutical and textile industries has driven demand for these structures in recent times. The unique characteristics of nanofibres makes them versatile and hence easily adaptable to meet varied requirements wherever needed. In this light, extensive research into the efficient and up-scaled production of nanofibre materials is on the rise in recent times.

Nanofibres till date have largely been produced by fibre spinning, thermal controlled phase separation and bio-fabrication usually by molecular self-assembly (Luo et al., 2012). Fibre spinning, specifically electrospinning appears to be the method mostly used in generating nanofibres (Zhou et al., 2009). Notwithstanding its popularity, this method of production is yet to see any meaningful upward transition into mass scale level to meet the ever-increasing demand of nanofibres because of a number of challenges, notably the need for high voltage and very low rate of production. These challenges clearly present the need for simpler and versatile production methods that can easily be up-scaled into production levels capable of meeting existing demands.

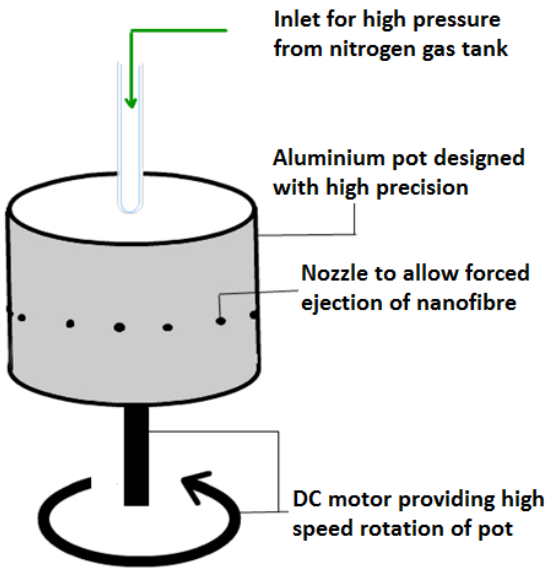


Figure 2-9: Schematic diagram of a pressurised gyration apparatus

Fibre production method driven by centrifugal forces and with prospects of easy adaptation for mass production of nanofibres because of its simplicity, higher production rate and versatility is gaining attention in recent times(Padron et al., 2013). A variant of using centrifugal forces in drawing out fibres from solutions and melts, where the spinning is carried out under pressure has been reported (Mahalingam and Edirisinghe, 2013). The process has been termed *'Pressurised gyration'*. Spinning fibres under pressurised conditions has the benefit of being able to manipulate an additional parameter, pressure and thus a wider flexibility in producing fibres with different cross-sectional diameters.

As shown in the schematic diagram in Figure 2-9, the setup is made of a cylindrical aluminium vessel of approximately 60mm in cross-sectional diameter and 35mm in height. There are nozzles approximately 0.5mm wide surrounding the pot, about 10mm from each other. The vessel is attached to a DC motor capable of rotations of up to 36,000 rpm. Finally, there is a lid connected to a source of high pressure to enable fibre spinning in a pressurised environment.

In order to generate fibres from this set up, the vessel with all its connections are held in place securely by a retort stand and clamps. The gyration process is carried out in a Perspex box to ensure safety. Aliquots of the solution to be spun into fibres are placed in the vessel and covered. Rotation begins at the same time as pressure is being applied. Each cycle takes between 2-5 minutes depending on the experimental conditions. Fibres ejected through the nozzles are collected and stored until required for further analyses.

2.6 Factors affecting outcome of fibre formation

2.6.1 Solution properties

Two of solution properties widely reported to have effect on fibre formation by spinning are viscosity and surface tension(Padron et al., 2013, Lu et al., 2013). Basically solution concentration, type of solvent system and molecular weights of materials affect fibre production outcomes by influencing their rheological properties and hence studying the viscosity and surface tension of solutions offer an effective means of quantifying the extent of correlation between solution properties and fibre characteristics (Lu et al., 2013). There ought to be some minimum forces to overcome the surface tension of liquids for polymer jets and subsequently fibre formation to occur. Again, solutions or melts ought to have optimal viscosities, which usually imply suitable chain entanglement for each material in order for fibres to be drawn out of the liquids(Padron et al., 2013). Therefore, for any material to be transformed into nanofibres, an optimal combination of viscosity and surface tension which reflects such properties as suitable amount of chain entanglement will be required.

2.6.2 Working pressure

It has been demonstrated that increasing the working pressure can drastically reduce the fibre diameter. A three-fold increase in working pressure, for instance has been reported as capable of reducing fibre diameter six times (Mahalingam and

Edirisinghe, 2013). The effect of pressure on the outcome of fibre formation has been explained by its role in rate of solvent evaporation and their interaction with centrifugal force at the orifice against surface tension of the solution.

2.6.3 Rotational velocity

It is firmly established that the angular velocity with which fibres are spun out affect the physical properties of the fibre, especially the fibre thickness. The effect of velocity on the fibre size has been explained by further expansion of fibre trajectory outward. The effect of increasing velocity, apart from reducing fibre size also results in more uniformly sized fibres (Padron et al., 2013). In this regard, for a fairly uniform sized fibre to produced, especially when those in the nano range is desired, spinning ought to occur in appreciably high speed.

2.7 Drug delivery

2.7.1 Overview

Drug delivery systems (DDS) have seen remarkable improvements recently and as discussed earlier, strategies utilising polymer-based nanostructures e.g. liposomes and nanoparticles have been and continue to improve pharmacological and therapeutic performance of many drugs (Allen and Cullis, 2004). Notwithstanding these positive developments in drug delivery, there remain more room for improving drug delivery via vaginal route, especially for systemic effects as this area has not seen as much interest and developments as others like the oral and parenteral routes for drug delivery. There are now advances in nanofabrication that makes possible the generation of materials that could be utilised in developing more efficient systems for systemic delivery of drugs via the vaginal route. A group of the population that stand to benefit from improved drug delivery via the vaginal route are women considered at risk of going into early labour (Dodd et al., 2008, Fuchs et al., 2014), as presently, a

more likely clinical intervention involves daily administration of intramuscular injection of progesterone, an approach that can be very inconvenient. Vaginal delivery systems whose performance may be aided by mucoadhesion, for instance in terms of improved resident times for better bioavailability and lower dosage frequency could replace the current regimen dominated by parenteral administration.

2.7.2 Vaginal drug delivery

The human vagina has been a route for administering drugs since ancient times (Hussain and Ahsan, 2005). However, it was mainly used to deliver drugs for local effects until 1918 when it was found to be capable of systemic delivery (Macht, 1918). Since then, this route of administration has gained relevance as a viable option for drug delivery in modern medicine. Several classes of medicine are currently approved for vaginal application (some listed in Figure 2-10). In this era of increasing discovery of poorly soluble new chemical entities (NCE), protein based therapeutics and other biologics, vaginal delivery of drugs for systemic use is increasingly being considered as alternative to oral administration (Baloglu et al., 2009, Bassi and Kaur, 2012, Hussain and Ahsan, 2005). Furthermore, where local effect or less invasive route of administration is desirable, vaginal delivery of a drug presents a more viable therapeutic strategy than many other routes (Fallowfield et al., 2006)

The human vagina is a fibromuscular S-shaped canal, between 6 and 12 cm long and connecting the cervix to the vulva vestibule (Neves et al., 2014). The upper portion is wider and almost horizontal when in upright posture and the lower part is convex in shape (Funt et al., 1978). This anatomical positioning and shape contributes to the retention of objects inserted deep into the vagina, thus making it a suitable destination for application of such materials as dosage forms and medical devices (Barnhart et al., 2004). In addition, the increased surface area of the vagina arising from several rugae and extensive vascularisation facilitating access to major blood vessels and

organs like the inferior vena cava and uterus offer immense potential for systemic drug delivery (Katz et al., 2007, De Ziegler et al., 1997).

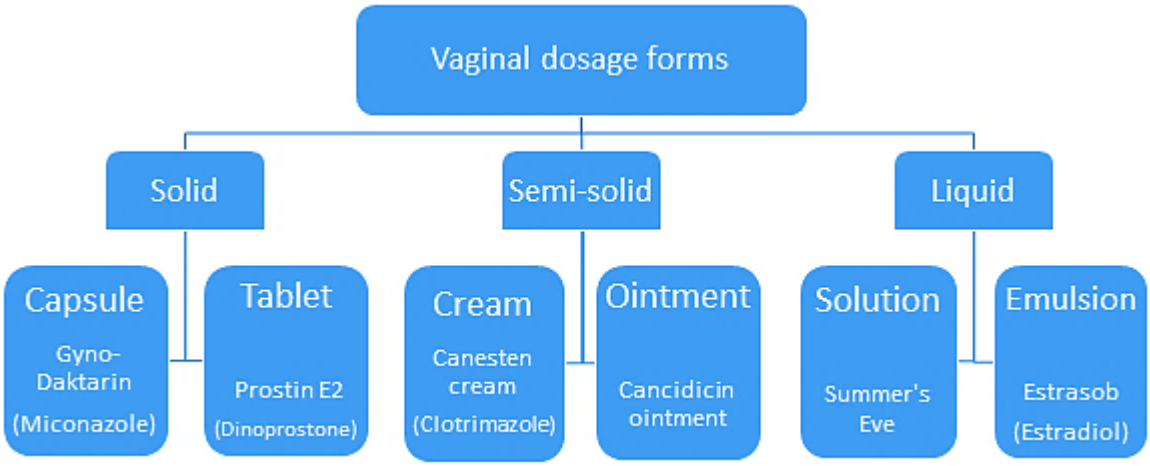


Figure 2-10: Types of vaginal dosage form presently in use.

Some advanced methods of drug delivery to the vagina has recently been reported. Coconut-oil core cationic nanocapsule of clotrimazole prepared from Eudragit® RS100 polymer has been reported to offer prolonged delivery of the antifungal drug to the vagina, offering better antifungal activity against *Candida sp.* compared to ordinary clotrimazole creams (Santos et al., 2014). In another example, Paclitaxel delivered as mucus-penetrating nanoparticles made form poly(lactic- co -glycolic acid) was reported as being more effective in suppressing tumour growth and prolonging the median survival in animal models (Yang et al., 2014).

Apart from the possibility of being useful for the systemic delivery of some drugs, the vaginal route offer prospects for other drug delivery strategies. For instance, the vaginal structure and environment can be adapted for long term delivery of some medications, e.g. contraceptives, where strict adherence is required for desired effect.

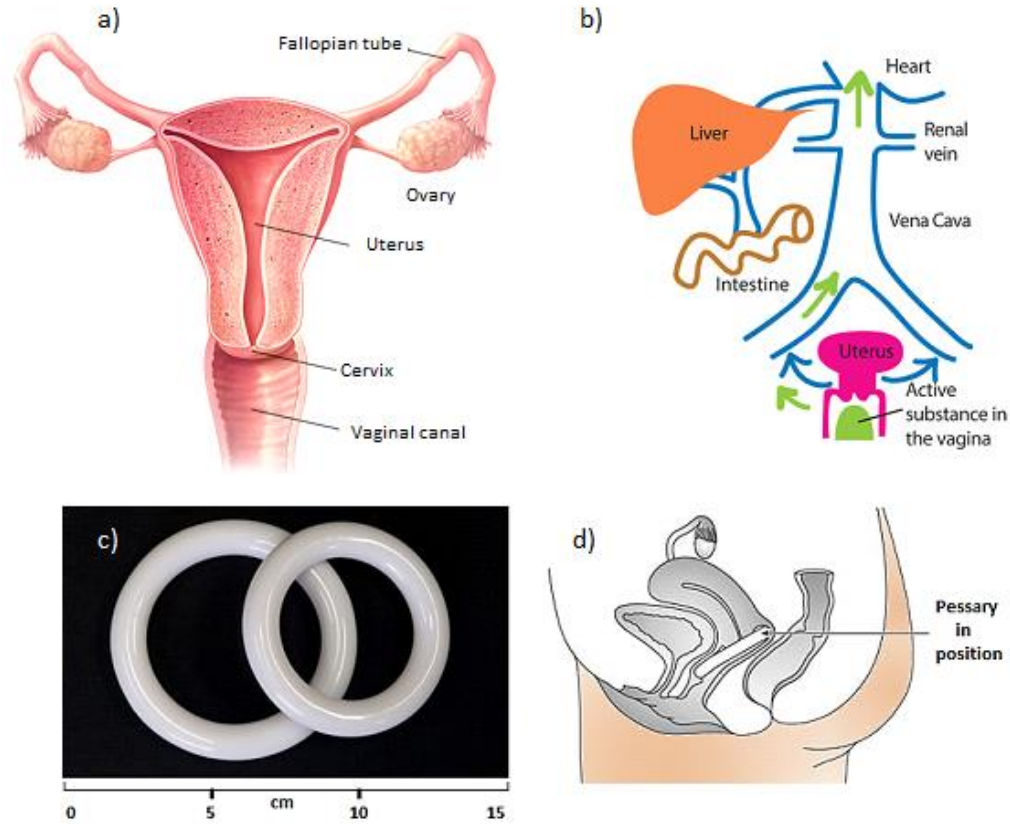


Figure 2-11: a) Cross-section of female reproductive tract showing vaginal canal, b) schematic illustrating active substance pathways for systemic circulation following application of a vaginal formulation, c) silicone elastomer vaginal ring and d) an inserted ring in a female reproductive tract

Vaginal rings for delivering contraceptives over several months have been shown to maintain constant serum levels of the drug, thus offering an effective solution to problems often arising from nonadherence to oral contraceptives such as through missed dose (Potter et al., 1996). This also takes away the daily burden of taking pills orally and therefore a more convenient approach. Adverse drug effects such as gastrointestinal disturbances, typically occurring after oral administration of a drug can be mitigated by delivering via vaginal route. For instance, gastrointestinal disturbances resulting from the oral administration of bromocriptine were drastically reduced when given vaginally, in addition to improved bioavailability (VERMESH et al., 1988). Vaginal route is also particularly helpful for systemic delivery of drug

susceptible to extensive hepatic metabolism or when intestinal absorption capacity is impaired and also helpful for avoiding possible drug-drug or drug-food interactions in the gastrointestinal system (Tozer, 1996). In a more recent study, vaginal delivery of subunit vaccines (plasmid DNA) was confirmed to induce better immunity when compared to rectal or intranasal delivery (Lowry, 2015)

Notwithstanding the promising prospects of optimized pharmacotherapy offered by this route, there remains challenges. Perhaps the first and most obvious disadvantage of vagina as a route of drug administration is its gender specificity (Neves et al., 2014). Drug delivery through the vagina is only possible for women. The other challenges associated with vaginal delivery of drug mainly involves cultural perceptions about insertions into the vagina, perceived interference with personal hygiene and possible interference with coitus in sexually active women. Likely local irritations, urination and widely varying pharmacokinetics following vaginal administration of drugs also pose some challenges in this route of administration (Srikrishna and Cardozo, 2013).

2.8 Progesterone

2.8.1 History of the discovery of progesterone

The discovery of progesterone hormone, its role in reproduction and much of what we know about it presently, though largely attributed to the works of Corner and his colleagues in the early to mid-twentieth century actually started several centuries before, when some fundamental discoveries in anatomy were made (Corner Sr, 1974). The history of progesterone discovery could be traced to a young Dutchman, Regner de Graaf who wrote extensively on the female reproductive system in the seventeenth century (De Graaf, 1965).

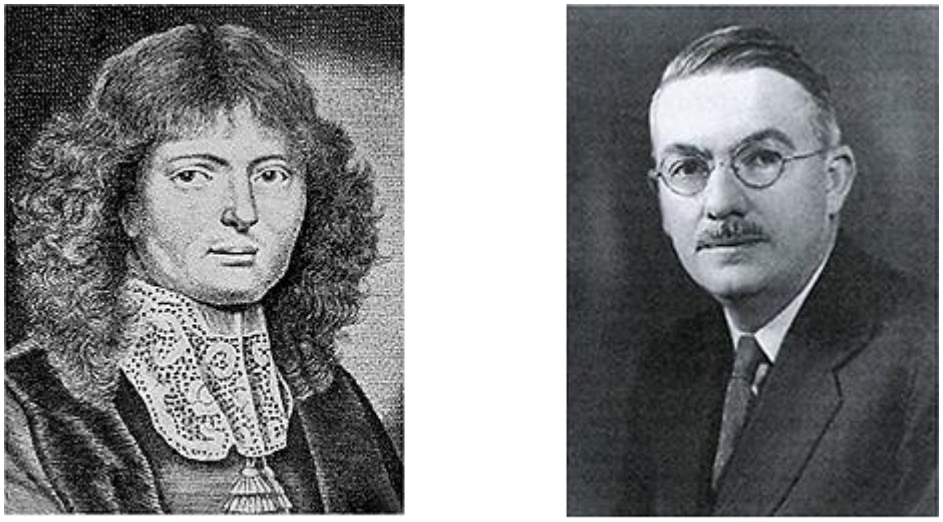


Figure 2-12: Regner de Graaf whose study of female reproductive anatomy laid the foundations upon which George Washington Corner built on to discover and isolate progesterone (photo credit: Museum Boijmans Van Beuningen and Wellcome Images from Wikimedia Commons)

He was the first to describe the corpus luteum in 1672, after which Louis-Auguste Prenant suggested the corpus luteum was an internal organ of secretion (Prenant, 1898). Thereafter, several experiments to ascertain the function of this organ, including its action on the endometrium were undertaken and reported by renowned anatomists including Ludwig Fraenkel, Paul Ancel and Paul Bouin between 1903 and 1910 (Corner Sr, 1974).

The tipping point in the discovery of progesterone and its function was when George Washington Corner demonstrated that the corpus luteum was necessary for survival of the pre-implantation embryo, and subsequently working with G W Allen to isolate the hormone which was used to maintain pregnancy in a rabbit with ablated ovaries (Corner, 2015).

Following Corners work which brought much clarity to the subject of corpus luteum and the hormone it secrets, there appear to have been, as one publication puts it ‘a dramatic neck-and-neck scientific race’ towards the isolation of pure progesterone (Frobenius, 1997). By 1934, at least four scientific groups working independently from

each other had reported on the isolation and purification of progesterone (Butenandt and Westphal, 1934, Hartmann and Wettstein, 1934, Slotta et al., 1934, Allen, 1935). So building on their predecessors work in area of human reproduction, various scientists contributed their due in the discovery of the hormone progesterone and its functions and several decades on, this wonderful hormone and our understanding of its role have remarkably improved healthcare through many of its interventions including those in the area of Assisted Reproductive Technology (ART), infertility and preterm delivery and catamenial epilepsy (Chakravarty et al., 2005, Devinsky et al., 2005). There had to be an efficient and cost-effective way of obtaining substantial quantities of progesterone if they were to be useful in healthcare. Between isolating and purifying progesterone and producing commercial quantities of the compound as we see today, there had been serious challenges with synthesis of practically useful amounts. At the early stages of progesterone production, according to one account, considerable amounts of cholesterol, about a ton from the brain and spinal cord of farm animals was required for just 20 pounds of the starting material from which progesterone would be synthesised (Hudson). Not long afterwards, by 1940, the pioneering works of Russel Marker which led to the partial chemical synthesis of progesterone from steroids derived from some varieties of yams (Marker and Krueger, 1940) ushered in an era of cost efficient production of progesterone in commercial quantities thus facilitating the use of this hormone in diverse fields of medicine, especially in reproductive healthcare.

2.8.2 Progesterone as of today

2.8.2.1 Assisted reproduction

The discovery, isolation and purification of progesterone, a hormone so crucial for conception, pregnancy and other related activities in humans remains one of the most remarkable developments in health science. In fact, progesterone supplementation

has become a routine in most assisted reproductive activities including IVF as it is thought to help create a conducive environment for successful embryo implantation (Williams et al., 2001, Yanushpolsky et al., 2008). Progesterone has been confirmed to bring about uterine relaxation i.e. decreasing contraction frequency which is typically associated with embryo displacement and implantation failure in IVF procedures (Fanchin et al., 1998, Fanchin et al., 2001). A thorough review by the Food and Drug Authority (FDA) on relevant scientific data established that IVF cycles utilising long acting Gonadotropin-releasing hormone analogues (GnRH) and progesterone supplements resulted in significantly higher pregnancy rates compared to placebo or no hormonal supplementation (Medicine, 2008). Furthermore, a newer understanding of the role of this hormone regarding women's wellbeing in general and particularly in their reproductive activities have ensured better outcomes in this area of healthcare.

The birth of Louise Jay Brown on 25th July, 1978 signified landmark achievement in reproductive healthcare and medical science in general. The first ever in vitro fertilisation (IVF) had resulted in the live birth of a healthy and normal baby girl. Gynaecologist Patrick Steptoe and physiologist Robert Edward's many years journey along an uncharted course characterised by obtaining eggs from ovaries, developing ways of fertilising them in a laboratory and hundreds of embryo transfers had finally paid off with a revolutionary outcome never seen in human history (Eley, 2015).

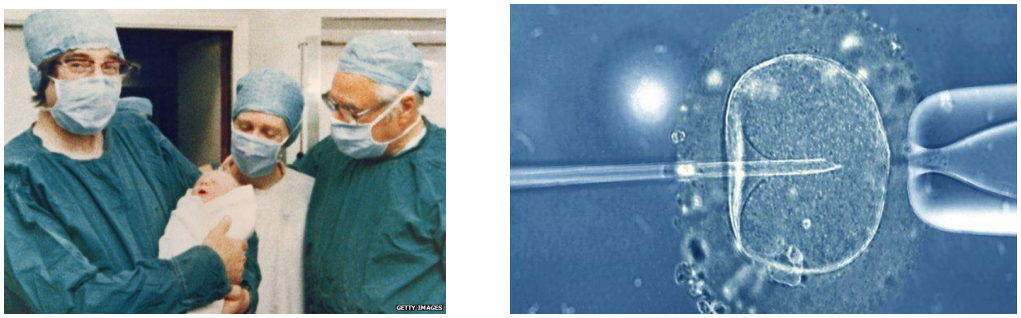


Figure 2-13: First IVF baby Louise, a midwife and the pioneers of the procedure Robert Edwards (L) and Patrick Steptoe (R) and b) an egg being injected with a single sperm using a micro-needle during an IVF procedure (photo credit: Getty Images and Science Photo Library)

Of course, such an endeavour calls celebrations with all the fanfare and media attention that could be mustered. Some breakthroughs in medicine prior to this great achievement, and contributed to the success of the first IVF, though kept silent over the years ought to be applauded as ground-breaking in their era. Indeed, the fact that progesterone supplementation has become part of most assisted reproductive activities today confirms the contribution of discovery and isolation/synthesis of progesterone to the current advancement we see in the area of reproductive healthcare.

In addition to its control of the female reproductive function, progesterone has been identified to play key roles in development of lobular-alveoli structures in the breast during puberty, sexual receptive behaviour in the brain and in bone remodelling, though information currently available on all of these additional functions are very limited (Graham and Clarke, 1997).

2.8.2.2 Supporting pregnancy

Progesterone's intervention in preparing the female reproductive system for conception and supporting pregnancy has been known for nearly a century and the exogenous form was widely used in preventing preterm birth for much of the 1980s and 90s until safety concerns halted its use (Fuchs et al., 2014). Thereafter, outcome from several clinical trials reassessing the significance of exogenous progesterone in maintaining pregnancy to full term, especially in women considered high risk has revived interest in this treatment regimen, thus triggering efforts in developing dosage forms capable of better control of serum levels of the administered hormone to ensure minimal side effects (Dodd et al., 2008, Valenta et al., 2001).

This renewed interest in using progesterone for medical interventions, especially in supporting pregnancies in women at risk of going into early labour would translate into actual benefit for users only when a number of provisions are made. These include the availability of a range of dosage forms capable of effective delivery of

progesterone to extents where suitable serum levels required for optimal outcomes are obtained. Typically classified as Class II under the biopharmaceutical classification system, progesterone's bioavailability is significantly limited by its low solvation rate and often require some formulation interventions if intended for oral administration (Dahan and Hoffman, 2006, Reddy and Karunakar, 2011). In addition, rapid metabolism of the hormone mainly in the liver following ingestion presents significant limitations to oral administration of progesterone (Levy et al., 1999). Other routes of administering exogenous progesterone would be preferred considering the many drawbacks associated with oral administration. However, these alternate methods of administering the hormone are not without challenges. Injecting oil-based progesterone intramuscularly ensures reliable absorption but the pain associated and possibility of local irritation and cold abscess make patients less compliant with this route (Devroey et al., 1989). Limitations in oral and parenteral routes of administration have made vaginal method the most established way of delivering progesterone for optimal outcomes. Notwithstanding, inconsistent serum concentrations have been identified among pessaries or suppositories utilising different fat or glycol bases (Price et al., 1983) thus creating the need for a formulation strategy capable of delivering more predictable outcomes in progesterone pharmacotherapy.

Nearly all of the dosage forms of progesterone intended for local application are fat-based (Convention, 2011). There are strong indications suggesting significant differences in bioavailability of progesterone following administration of dosage forms with different fat bases (Price et al., 1983). So clearly, a dosage form formulated with entirely different material, in this case from a combination of polymers in nanostructure basic units offers new opportunities for adaptation to suit specific release patterns. This would be a positive addition to the overall strategy of managing preterm labour with progesterone.

2.8.3 Chemistry of progesterone

Progesterone is a steroidal hormone. Steroids are a group of natural or synthetic fat soluble organic compounds among lipids, characterised by a molecular core of four fused rings (three 6-Carbon rings and one 5-Carbon ring) typically made of 17 carbon atoms (Lednicer, 2011).

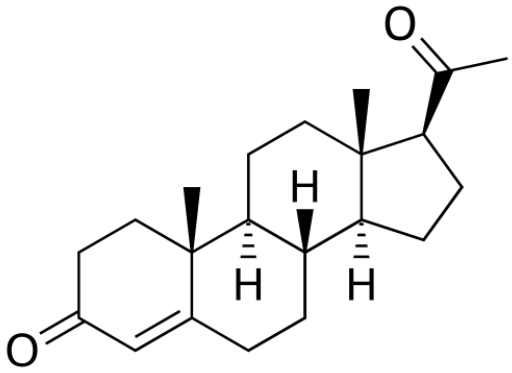


Figure 2-14: Progesterone molecule with four fused rings, double bond between Carbon position 4 and 5 and two ketone groups at 3 and 20, giving the name Pregn-4-ene-3,20-dione.

A 5-Carbon naturally occurring diene hydrocarbon, isoprene pyrophosphate (IPP) serves as starting synthon for many natural compounds in plants and animals, including steroids. IPP itself is formed by addition of acetyl group to activated acetoacetate to form glutarate which is then reduced by enzyme HMG-CoA-reductase (3-hydroxy-3-methylglutaryl-CoA) to mevalonic acid. This intermediate undergoes further reactions including esterification, decarboxylation and isomerisation to become IPP. Transformations through a cascade of several reactions make up the biosynthesis of steroidal hormones.

Progesterone, as used today, is usually obtained either by semi-synthesis from phytochemicals such as diosgenin and soy-bean derived stigmasterol or by total synthesis through cascade of ring closures such as a type utilising aldehydes as starting material (Lednicer, 2010)

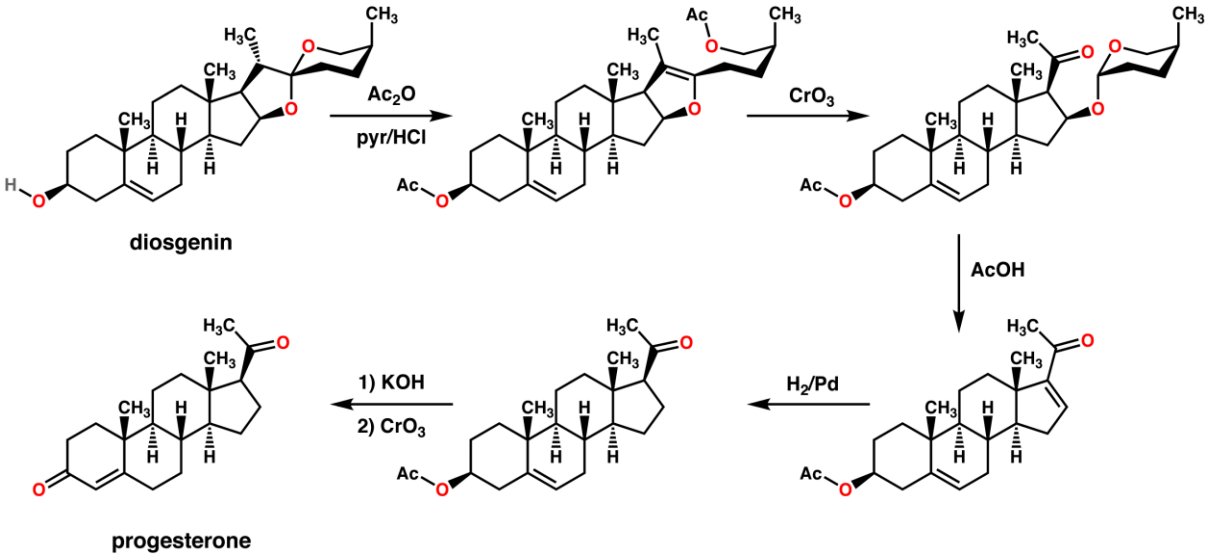


Figure 2-15: Semi-synthesis of progesterone from diosgenin using Marker's degradation process

The scheme above illustrates steps required for semi-synthesis of progesterone from diosgenin. This method, pioneered by Russel Marker (Marker and Krueger, 1940), sometime known as Marker's degradation basically through kinetic control degrades the sapogenin side-chain of diosgenin while leaving functional groups in the steroid nucleus intact. In this approach, the pyran ring on the side-chain is opened by hydrolysis while the resulting 26-hydroxyl and the 3-hydroxyl on the steroid nucleus acetylated and esterified respectively to render the remaining furan on the side-chain more susceptible to opening by oxidation. Further selective oxidation of 20, 22 double bond by chromic acid and conversion of the ester at position 3 to ketone yields progesterone (Dewick, 2009). Alternatively, semi-synthesis of progesterone can be done through formation of a conjugated system by Oppenauer oxidation of 3-hydroxyl to ketone, side-chain degradation of stigmasterol through ozonolysis, iminium-enamine tautomerisation and selective oxidation of double bonds. Total synthesis of progesterone in three main schemes, the first being synthesis of aldehyde from a Grignard reagent. This is then converted into a phosphonium ylide in the second

stage before finally being transformed through a series of reactions, including a biomimetic cationic cyclization reaction to yield progesterone (Johnson et al., 1971).

2.8.4 Pharmacology of progesterone

The actions of progesterone, like all its agonists and antagonists are facilitated in target tissues by progesterone receptor (PR) a type of nuclear receptor (Giangrande and McDonnell, 1998). Nuclear receptors are a class of structurally related gene products that act as receptors for some compounds including several steroids, thyroid hormones, retinoids and some fat soluble vitamins (Tsai and O'Malley, 1994, Mangelsdorf et al., 1995). An insight into the structure and role of PR will be helpful for better understanding of physiological activities of progesterone. PRs exist mainly as proteins, PR-A and PR-B though another isoform PR-C which lacks DNA binding domain (DBD) and confined primarily to cytosolic compartment in cells has been identified (Spitz, 2008). PR-B differs from PR-A only by an additional sequence of amino acid in the N-terminus, and this difference has been identified as reason why PR-B in general is a stronger activator than PR-A (Conneely et al., 2003, Spitz, 2008). Progesterone's interaction with PR triggers a significant conformational changes in these proteins which in turn initiate a cascade of activities leading to transcription initiation complex in specific target genes and thereby eliciting response in a variety of female reproductive activities as well as non-reproductive interventions like neuroendocrine actions (Conneely et al., 2003). Or understanding of progesterone induced reproductive functions has improved considerably in recent times thanks to extensive research into tissue specific roles PR-A and PR-B in mediating activities in the female reproductive system. For instance PR is now definitely known to be essential mediators of ovulation after experiments analysing ovarian phenotypes of progesterone receptor knockout (PRKO) mouse established that PR is specifically required for luteinising hormone (LH) dependent rapture that brings about ovulation (Conneely et al., 2003, Lydon et al., 1995). Similar studies have also confirmed the

mediating role of PRs in other activities such as uterine development, preparation for and support of pregnancy and mammary gland development (Conneely et al., 2002)

2.8.5 Delivery of progesterone

2.8.5.1 Oral administration

Until micronized progesterone formulation begun, oral administration of progesterone was seriously limited by low bioavailability, thus making synthetic progestin with much higher oral bioavailability widely used for conditions requiring the hormone (Remington and Allen, 2013). Oral formulations utilising micronized progesterone, though with still low bioavailability have been found to be useful for some conditions when given 100-400mg daily. Extensive intestinal and hepatic metabolism of progesterone have account for the poorly sustained serum levels and low bioavailability following oral administration of this hormone drug (Levy et al., 1999)

Since the oral route remains the easiest, most convenient and preferred method of drug administration, more efforts are being channelled into developing dosage forms that can deliver this hormone orally without compromising its bioavailability.

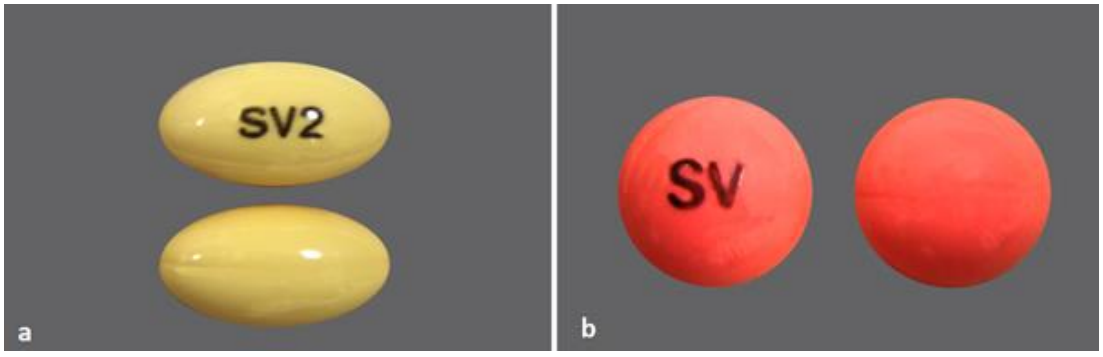


Figure 2-16: Progesterone presented as a) Oval and b) round shaped gelatine capsules containing an oil suspension of the drug for oral administration (photo credit: Pill Identifier, drug.com)

Progesterone is now commonly administered orally as a soft gel capsule containing an oil suspension of the micronized active drug (Figure 2-16). Several patents

claiming procedures for formulating micronized suspension in oils for superior performance and bioavailability have been filed (Maxson et al., 1992, Liu et al., 2015). A particularly interesting claim involved wet-milling the micronized progesterone with a mixture of oils and subsequently using the wet-milled composition for producing the final dosage form (Abidi et al., 2012). These and many other claims reflect efforts put into improving the oral delivery of progesterone. Two of the most widely circulated oral progesterone-in-oil formulation which are very popular for hormone replacement therapy (HRT) and menstrual disorders are Prometrium® and Utrogestan® (British Medical and Royal Pharmaceutical Society of Great, 2015). These soft gel capsules are remarkable improvement over conventional oral formulations such as compressed tablets. But their serum concentrations and bioavailability profiles following their administration are still far less desirable compared to other routes, thus limiting their usefulness in managing other conditions requiring sustained levels of the hormone (Nahoul et al., 1993)

Still within the scope of oral administration of drugs, newer strategies such as formulations that allow complete disintegration and absorption of active drug within the buccal cavity could be explored for systemic delivery of progesterone. The earliest record of a solid dosage form intended for complete dissolution and absorption in the oral cavity is seen in patent documents filed by Tanaku et al in 1975 (Tanaka et al., 1977). This formulation was however designed to release its active ingredient over prolonged periods and therefore depending on the palatability or otherwise of ingredients therein, or even the feeling of an unusual object at the site of administration over a long period, it probably was not very convenient mode of drug administration albeit the intent of improving drug delivery. Several attempts at improving upon this pioneering work in orally dissolving solid formulations resulted in a number of technologies for producing fast-dissolving oral tablets by the early 1990s (Kearney et al., 2002). Most popular among these technologies, Zydis® which utilise a freeze-drying process to create an extremely porous structure that allows ingress

of saliva to facilitate rapid disintegration of the tablet, usually in a few seconds (Katou et al., 1993).

Significant limitations associated with conventional orally administered dosage forms such as swallowing difficulties among a segment of the population including those with dysphagia, elderly or children, onset of drug action and bioavailability has inspired technologies and innovations that still allow for oral administration of these dosage forms while mitigating the problems limiting their use (Hirani et al., 2009). These innovations can be extended to making oral administration of progesterone more effective and worthwhile as current orally disintegrated tablets (ODTs) technologies are capable of formulating steroids that can escape the dreaded first pass effect for desirable bioavailability profiles (Slavkova and Breitkreutz, 2015).

Table 2-3: An overview of existing dosage forms of Progesterone and their routes of administration

Route of administration	Dosage form	Indication	Unit presentation
Oral	Capsule	Preventing endometrial hyperplasia in hormone therapy for postmenopausal women Amenorrhoea	200 mg 400 mg
Parenteral	Solution for injection	Dysfunctional uterine bleeding Amenorrhoea	50 mg/ml
Vaginal	Gel Suppositories	Amenorrhoea Infertility procedures Maintaining pregnancy Preventing preterm labour	4% 8% 25 - 200 mg

Progesterone absorbed through oral mucosa directly into systemic circulation may be effective for obtaining appreciable bioavailability and thereby making it useful for managing a variety of medical conditions requiring progesterone. Indeed, some

formulations of progesterone, capable of rapid disintegration sublingually have been carried out and tested, albeit on a very limited scale, some as far back as 2001 (Ruiz and Daniels, 2013, Vaugelade et al., 2001). Progress with developing progesterone dosage forms in this direction has been palpably slow, and as indicated in the current edition of Remington: The Science and Practice of Pharmacy, there is still not 'good clinical data' on this dosage form (Remington and Allen, 2013). There are immense opportunities presented by ODT technology for effective oral delivery of progesterone and moving more rapidly in this direction will ensure overall improvement in pharmacotherapy dependent on exogenous progesterone.

2.8.5.1 Parenteral delivery

Progesterone, when administered by the parenteral route is usually given intramuscularly. High plasma concentration of the drug is seen within 2 hours of administration through this route with peak serum levels attained after 8 hours (Nillius and Johansson, 1971).

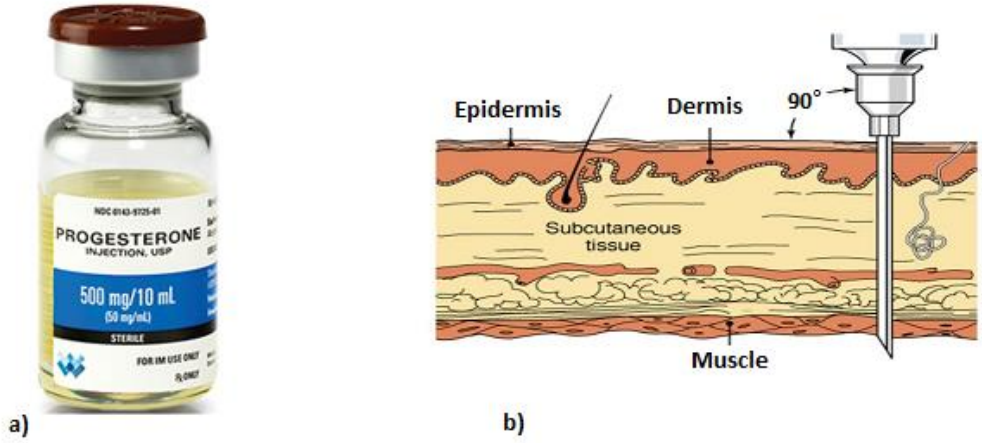


Figure 2-17: Progesterone-in-oil injection (a). The formulation must be administered intramuscularly (b) which makes it more invasive and problematic over long period of use (photo credit: Westward pharmaceuticals, NJ, USA and Prentice Hall Health Drug Guides).

Serum levels equivalent to those seen during luteal phase are reported to be obtained following intramuscular administration of 25mg progesterone (Johansson, 1972). In

many respects, the parenteral route of administering progesterone, compared to the oral route seem to offer better prospects for attaining desirable serum levels in a timely manner. In a study comparing the performance of various formulations of progesterone, it was found that 200mg of micronized progesterone given orally attained only about 10% of the bioavailability of 50mg progesterone given parenterally (Simon et al., 1993). Furthermore, the drug administered as injection reached serum peak levels in about 2.5 hours compared to the oral formulation which required nearly 9 hours to reach peak concentrations.

Notwithstanding the benefits of using parenteral progesterone, including rapid onset of action and desirable serum levels attained in shorter time, this method of administration has not been widely accepted as the first line approach due to a number of issues. Patients requiring long term use of the drug, e.g. for pregnancy support will need daily injections to maintain appropriate serum levels over long periods. This can be uncomfortable, painful and most likely affect compliance with this regimen. These injections have actually been confirmed as causing inflammation a characterised by redness and sometimes abscess at the site of administration (Tavaniotou et al., 2000).

Long-acting (LA) progesterone formulations could be helpful in mitigating the associated discomfort as less frequent dosing and hence fewer injections would be required to maintain desirable serum levels. Solid lipid structures within nanoscale range such as solid lipid nanoparticles (SLN), nanostructured lipid carriers (NLC) and lipid drug conjugate (LDC) are increasingly being used to improve parenteral application of drugs(Mehnert and Mäder, 2001, Wissing et al., 2004, Yoon et al., 2013, Dolatabadi et al., 2015). Although these formulations are usually utilised as carrier systems to confer stealth and targeting functions for optimised drug action especially in cancer pharmacotherapy, varying their material for composition and modification of processing conditions could make them useful as long acting progesterone systems for parenteral use.

LA parenteral formulations are well accepted and used extensively in managing conditions such as psychotic disorders, for contraception/hormone therapy and currently being considered for the application of antiretroviral in the prevention and management of HIV infections (Baert et al., 2009). Current technologies for formulating nanosuspensions and solid nanoparticles and the availability of a wide range of biocompatible materials can be utilised for the development of long-acting progesterone for less frequent parenteral application. In addition, technologies available today which facilitate the formulation of long-acting parenterals in different forms such as injectable monoliths and in situ-forming depots (Owen and Rannard, 2016) further broaden the prospects of developing suitable progesterone injectables that offer desirable treatment outcomes. These new technologies for developing LA parenterals, when fully explored could offer options that can make the full clinical benefits of progesterone be realised when administered as injections.

2.8.5.2 Vaginal progesterone

Progesterone administered vaginally appear to be the most effective and practical method as many of the issues relating to the oral or parenteral route are usually non-existent. Nonetheless, formulations containing higher amounts of progesterone are typically required for optimal serum levels. For instance in supporting pregnancies in women with history of recurrent miscarriage and confirmed progesterone deficiency, while 100mg daily dose of progesterone injection given intramuscularly may be adequate, 200-400 mg progesterone in vaginal formulations would be required for the same clinical effect (Brayfield, 2014).This relatively inefficient delivery via vaginal route has performance and toxicity implications, highlighting the need for improved formulations via this route.

Figure 2-18: Cyclogest, a brand of progesterone administered vaginally. Release from this formulation was compared to progesterone-loaded fibres produced in this study (photo credit: LD Collins & Co Ltd, UK).

Formulations commonly employed for vaginal application of progesterone are either in the form of semisolids such as gels and creams or as solids, commonly pessaries. Gel preparations are typically presented in strengths around 8% and often employed in managing infertility due to inadequate luteal phase (Committee, 2015). Progesterone pessaries on the other hand come in strengths of 200 or 400 mg and usually better suited for daily administration in support of pregnancy as well as managing premenstrual syndrome and post-natal depression.

As with most conventional formulations delivering drugs via the vaginal route, progesterone gels and pessaries presently in use present many challenges (Hussain and Ahsan, 2005). First, low retention time for these formulations within the vagina imply less opportunity for the absorption of adequate amounts of the active drug for optimal therapeutic outcomes. Then there is the issue of leakage which often results in various extents of messiness around site of application and ultimately an unpleasant patient experience. One formulation strategy currently being considered as capable of mitigating some of the issues currently seen in administration of conventional vaginal formulations is the design of mucoadhesive dosage forms (Hussain and Ahsan, 2005). In this approach, interactions between components of the vaginal epithelium, mainly glycoproteins (mucin) and those of the dosage forms

are expected to bring about suitable level of adhesion capable of holding the delivery system in place long enough for optimal release and uptake of active drug.

Mucoadhesive systems are usually designed from polymers such as polyacrylic acids and cellulose derivatives which typically have appreciably high adhesive capabilities. Some mucoadhesive preparations for vaginal application currently in trials such as ACIDFORM® (lactic acid, citric acid and potassium bitartrate formulated with preservatives, gelling agents and humectants (glycerine)) have demonstrated better intra-vaginal retention and potential for drug release up to 12 hours , two characteristics that can be utilised for improved delivery of drug via the vaginal route (Andrews et al., 2009). Other attempts at utilising nanofibre properties for improved drug delivery via the vagina include use of electrospun fluconazole-loaded nanofibres which showed prolonged and superior anti-microbial activity (Sharma et al., 2016). Furthermore, possibility of designing multiple delivery systems from nanofibres and upscaling their manufacture to meet realistic demand as alternative materials for making vaginal dosage forms have been reported (Hou et al., 2013, Krogstad and Woodrow, 2014). All of these affirm the promising prospects of drug loaded nanofibres as candidates for superior delivery of drugs via the vaginal route.

An investigation into making progesterone-loaded nanofibres which combines enhanced mucoadhesive capabilities with other known nanofibre material properties is one of the main objectives of this study. Materials generated are expected to be formulated into appropriate dosage forms administered vaginally for supporting pregnancies in women considered at risk of going into preterm labour. This could potentially replace the invasive regimen of intramuscular injection applied daily over several weeks (Fonseca et al., 2007, Simon et al., 1993), a significant contribution towards antenatal healthcare among women in need of such therapy

2.9 Mucoadhesion

2.9.1 Overview

Mucoadhesion is defined as an interactive state in which two material surfaces, at least one being biological in nature and typically a mucosa membrane are held together by interfacial forces for a prolonged period of time (Smart, 2005).

Table 2-4: Some mucoadhesive polymers used to improve performance of various pharmaceutical preparations (Wang et al., 2000, Dehghan and Kha, 2009, Akiyama *et al., 1998)*

polymer	Drug incorporated	Feature
Polyacrylic acid with PEG	Botulinum toxin	Mucoadhesion from polymers prolong gastric retention of this formulation when administered orally
Sodium alginate	Captopril	This polymer confers substantial mucoadhesion capabilities to captopril microcapsules for prolonged release in stomach
Carboxymethyl cellulose	Famotidine	Increasing amounts of carboxymethyl cellulose in this formulation improved mucoadhesion of famotidine microspheres
Chitosan	Amoxicillin	Chitosan improves *in situ* gelation nanoparticle formulation of amoxicillin
Positively charged gelatine	Amoxicillin	Improved mucoadhesive properties of modified gelatine microspheres of amoxicillin
polyglycerol esters of fatty acids	Furosemide	Improved mucoadhesive properties of microspheres for better bioavailability
Polyethylene oxide	Famotidine	Confers mucoadhesive properties to nanosuspensions of famotidine

Utilising mucoadhesion for more effective drug delivery has and continues to attract more attention within the pharmaceutical sciences as substantial evidence exists to support claims of improved dosage form residence time, therapeutic efficacy,

improved drug targeting in cancer therapy and delivery of biologicals such us peptides and antibodies through a variety of routes of administration such as ocular, nasal, buccal and vaginal (Andrews et al., 2009, Mansuri et al., 2016).

2.9.2 Two-step principle of mucoadhesion

The exact mechanism underlying mucoadhesion remains under discussion (Carvalho et al., 2010). Notwithstanding this uncertainty, classical observational theories deduced from several investigations into polymer-mucin interactions explain mucoadhesion in two main steps, regardless of underlying theory. These are the contact stage and the consolidation stage (Huang et al., 2000, Hägerström and Edsman, 2003). The first step involves the spreading and swelling of the mucoadhesive material following moisture absorption to facilitate extensive contact with the mucosal membrane. At the consolidation stage, the mucoadhesive materials interact with the membrane; one suggestion being that moisture plasticizes the systems allowing molecules from these materials to break free and form linkages with mucins in the mucosal layer by weak Van der Walls and hydrogen bonds (Smart, 2005). Both the contact and the consolidation stage working together to bring about mucoadhesion.

2.9.3 Theories of mucoadhesion

In addition to the two-step principle of how two surfaces are held together during mucoadhesion, several theories have been used to explain this complex phenomenon. These include the electrostatic explanation where opposing electrical charges from interacting surfaces sustains mucoadhesion. Others are the adsorption theory which suggests that a mucoadhesive device is held to the mucosa surface by secondary chemical interactions such as hydrogen bonding and electrostatic attraction while the wetting theory describes affinity between surfaces facilitated by

surface energetics, predominantly in liquid bioadhesive systems (Kaelble and Moacanin, 1977, Peppas and Buri, 1985). On the other hand, the diffusion theory which is used quite extensively explains how mucoadhesion is brought about by interpenetration of polymer and mucin chains into each other. The rate and extent of penetration, dependent on such factors as diffusion coefficient and nature of mucoadhesive chains, their mobility and contact time, determines the strength of mucoadhesion (Leung and Robinson, 1990).The fracture theory, presently used widely in assessment of mucoadhesion, explains mucoadhesion in terms of the amount of force required to completely detach interacting surfaces (Chickering and Mathiowitz, 1995, Mathiowitz et al., 1999). Last of all, there is the mechanical theory which describes mucoadhesion in terms of surface roughness and the filling up of irregular surface spaces, interfacial surface behaviour and surface energy dissipation (Peppas and Sahlin, 1996).

2.9.4 Factors affecting mucoadhesion

Mucoadhesion is a highly dynamic process typically influenced by several factors (Ahuja et al., 1997, Peppas et al., 2000). Some of the most important factors affecting mucoadhesion such as molecular structure (chain arrangement) and weight, spatial conformation and concentration are derived from the non-biological system involved in adhesion, typically materials easily wettable and swellable e.g. most hydrophilic polymers (Ahuja et al., 1997). Secondly, there are factors contributed by the biological system, often a mucosal surface, such as mucin turnover which influence the process usually by limiting contact or residence times or by amount of functional groups available for interlocking (Swarbrick and Boylan, 2000). Furthermore, when occurring in vivo, conditions such as disease states where microbial activities or inflammatory interventions affect the physiology of mucosal tissues can greatly affect mucoadhesion (Swarbrick and Boylan, 2000). Lastly, environmental factors such as pH and temperature and concentration of active drug in system where mucoadhesion

is occurring may affect the process. Some of these factors relevant to this study are discussed further (Blanco-Fuente et al., 1996).

2.9.4.1 Hydrophilicity

The ease with which mucoadhesive polymers may be wetted and swell is known to influence mucoadhesion significantly. In aqueous condition, many of the functional groups such as hydroxyl and carboxyl are freed up to facilitate adhesion by interactions such as hydrogen bonding (Carvalho et al., 2010). In addition, swollen polymers have maximum distance between their chains making them more flexible and allowing for more and efficient interpenetration which eventually influence mucoadhesion. (Rahamatullah Shaikh et al., 2011).

2.9.4.2 Molecular weight of polymer

Interpenetration of polymer bringing about mucoadhesion is known to be favoured at lower molecular weights (less steric hindrance to interpenetration) while higher molecular weights (enough to reach critical length required for entanglement) results in stronger entanglement, both conditions greatly influencing mucoadhesion. Therefore, a balance between interpenetration and entanglement will be required for optimal adhesion. Furthermore, it has been established that bioadhesion increases with molecular weight up to 100,000, beyond which no further gain dependent of weight alone is realised (Gurny et al., 1984).

2.9.4.3 Spatial conformation

In addition to molecular weight and chain length of polymers, the spatial arrangements of molecules are known to influence bioadhesion. For instance PEO with molecular weight of 200,000 is known to have bioadhesion similar to dextrans with weights up to 19,500,00 under similar conditions (Jiménez-castellanos et al., 1993). The helical structure of dextrans shielding many of the functional groups like

hydroxyl primarily responsible for adhesion is attributed to this unusual observation, thus confirming the influence of spatial conformation in mucoadhesion.

2.9.4.4 Drug/Excipients concentration

Drug or excipient concentrations can affect mucoadhesion in a number of ways. It has been established that drug complexation with polymers may either limit amount of water, increase elasticity between interacting systems and resulting in stronger adhesion or in the presence of large amounts of water, precipitation of drug-polymer complexes leading to decreased adhesion (Blanco-Fuente et al., 1996). In addition, different charge distribution over drugs and polymers e.g. a cationic drug and anionic polymer may bring about electrostatic interactions leading to internal cohesion, thus influencing bioadhesion (Donnelly et al., 2007).

2.9.5 Quantifying mucoadhesion

In the last three decades, mucoadhesion has continued to gain attention as a viable approach for improving drug delivery. However, a comprehensive assessment of this phenomenon still appears difficult. Factors relevant to an approach for quantifying mucoadhesion has been the subject of several investigations (Mortazavi and Smart, 1995, Leung and Robinson, 1990). Several methods, including measuring forces required to detach two surfaces (Carvalho et al., 2010), tracking the extent of polymer reaction with mucin, for instance by measuring fluorescence intensities, zeta potential or levels of turbidity (Cook and Khutoryanskiy, 2015, Rençber et al., 2016) and more recently, by using atomic force microscopy (AFM) to study footprints such us dried out surfaces prior to mucoadhesion (Joergensen et al., 2011, Davidovich-Pinhas and Bianco-Peled, 2010, Brako et al., 2015)have been attempted for quantifying mucoadhesion. Tremendous efforts and resources have been dedicated to accurately quantifying mucoadhesion but findings from these investigations often point to

conflicting outcomes (Joergensen et al., 2011). A key reason assigned to the inconsistent outcomes from studying mucoadhesion is the lack of standardised protocol for assessment.

2.9.5.1 Combining mucoadhesive theories for comprehensive quantification

A novel method of assessing mucoadhesion was developed as part of the study reported in this thesis. An AFM assessment of the roughness at the interface between nanofibre and mucosa was conducted and compared to see how well they correlated with forces required to detach them. The extent of filling up of irregular spaces was also analysed. According to the mechanical theory, adequate filling up of cavities on mucosa surface results in stronger mucoadhesion. Deducing from the diffusion and mechanical theories of mucoadhesion described, it was hypothesised that interfacial roughness may correlates to degree of interpenetration between polymer functional groups and mucin, hence a smoother interface may imply closer interaction, sufficient filling up of surface cavities and therefore stronger mucoadhesion. Testing this hypothesis is the basis for studying mucoadhesion by quantifying the roughness at intersection of the two layers. The physical attributes of nanofibres that make them attractive material for mucoadhesive drug delivery are the surface properties i.e. area and topology. The experimental design in this work combines the diffusion and mechanical theories which typically utilise surface topology as a prime determinant of mucoadhesion and is hence more likely to give a more effective assessment of the interactions taking place. Fracture and interfacial properties of two surfaces in mucoadhesive interaction were studied using texture analyser and AFM respectively. This approach of mucoadhesion quantification was used to investigate the effects of varying amounts of carboxyl methylcellulose included in polymer blends on adhesive the adhesion prospects of nanofibres generated by pressurised gyration.

2.9.6 Nanotechnology and mucoadhesion for drug delivery

Nanotechnology enables encapsulation of active drug into nanofibres. Selecting appropriate material and applying further modification, suitable mucoadhesive properties may be conferred onto these drug-loaded nanostructures. Nanofibres are emerging strongly as material of choice when mucoadhesion is desirable. Due to their large surface area, unique surface topology, porosity and minimal moisture content, nanofibres are known to significantly improve adhesiveness of systems utilising them (Malik et al., 2015, Singh et al., 2015). Furthermore, their ability to enhance drug solubility and high adsorption efficiency potentially make them suitable carriers from trans-mucosal drug delivery (Malik et al., 2015). These sum up the rationale for producing drug-loaded nanofibres by PG which has good prospects of upscaling for yields to meet realistic demands. This approach to producing drug-loaded mucoadhesive nanofibres offer many parameters that may be adjusted to optimise outcome of production. These include flexibility with choice of material, solution properties e.g. viscosity, production conditions such as rotation speed and pressure and environmental factors e.g. room temperature and humidity.

Producing substantial quantities of nanofibres with good mucoadhesive prospects will be a positive addition to efforts in improving drug delivery across mucosa membranes. It is expected that outcomes from the work reported subsequently in this thesis will be a significant contribution to improving drug delivery across mucosal membranes.

Chapter 3

Experimental details

3.1 Introduction

In this chapter, materials and equipment used throughout the project are described in detail. With respect to materials used, a thorough description, rational for selection as well as details of suppliers are given. For equipment, details of the manufacturers or suppliers in addition to an overview of their operation and instrumental settings are set out in relevant sections discussing various procedures. The parameters analysed by these equipment and their relevance to the objectives of this project are highlighted in this section.

3.2 Materials

In generating various kinds of nanofibre materials, which was the predominant procedure throughout the project, polymers – specifically polyethylene oxide (facilitating fibre generation), and carboxymethyl cellulose, sodium alginate and polyacrylic acid; polymers with enhanced mucoadhesive properties in acidic conditions (Khutoryankiy, 2011). Further details on these polymers are given later on in this section. Various mixtures of alcohols and water in different proportions were also utilised in efforts to determine the best possible solvent system capable of facilitating the production of high quality drug loaded fibres. The active drug used in all experiments is Progesterone. Several other compounds were also used in buffer and physiological fluid preparations which were required for conducting some of the experiments.

3.2.1 Polyethylene oxide

White to off-white in colour and with slightly ammoniacal odour, polyethylene oxide is a free-flowing powder with crystalline melting point between 62-67 ^{0}C (Dhawan et al., 2005). Chemically, it is describes as a non-ionic homopolymer of ethylene oxide units with formula $(CH_2CH_2O)_n$, where *n* is the average number of oxythylene groups present (Row et al., 2009). Its desirable characteristics including extremely low toxicity, as confirmed in animal studies, rapid and complete elimination after ingestion and appreciable swelling capacity have contributed to its extensive use in the food and pharmaceutical industry.

Polyethyleneoxide, due to its molecular structure and response when subjected to deformation (Peterlin, 1971) is an extremely useful material for nanofibre production. In addition, it has been identified as a valuable spinning agent capable of improving the prospects of other materials such as polysaccharides to be spun into nanofibres (Kriegel et al., 2009, Desai et al., 2008, Duan et al., 2004).

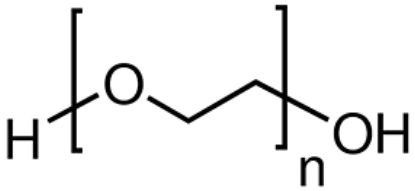


Figure 3-1: Molecular structure of Polyethylene oxide

Lack of adequate chain entanglement has been identified among the main reasons for the difficulties associated with producing nanofibres from alginates and cellulose based materials by themselves (Lee et al., 2009). Hence PEO, known for its excellent properties as a carrier for spinning materials into nanofibres was used to aid the successful spinning of our mucoadhesive polymers into fibres. It is worth noting that

PEO itself also has desirable mucoadhesive properties and hence the additive benefits from individual polymers is utilised when blends are used for production of the nanofibres.

3.2.2 Carboxymethyl cellulose sodium

Carboxymethyl cellulose sodium, a sodium salt of polycarboxymethyl ether of cellulose is white to off-white granular powder without taste and odour. It is a soluble derivative of cellulose in which carboxymethyl groups are bound to some hydroxyl groups on the cellulose backbone structure made up of glucopyranose monomer units. Largely considered as non-irritant and nontoxic material, its viscosity increasing property in addition to its desirable safety profile make it useful for many pharmaceutical applications, including suspension and stabilising agents as well as tablet binder and disintegrant (Row et al., 2009).

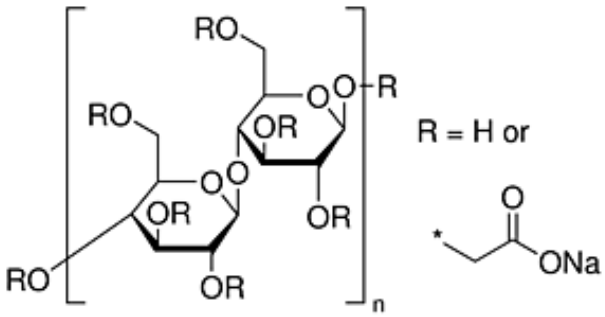


Figure 3-2: Molecular structure of Carboxymethyl cellulose sodium

Since becoming commercially available in the early half of the previous century, this polymer has been found so useful in several industries including those of food, drugs and cosmetics, paper, ceramics, paints and adhesives (Hollabaugh et al., 1945). With regards to nanofibre production and their applications, cellulose-based materials for

membranes for antimicrobial activity, enzyme immobilisation and drug delivery have been used and reported (Taepaiboon et al., 2007, Lee et al., 2009). Electrospinning has been the method employed in all of the nanofibre production cited. Processing nanofibres from carboxymethyl cellulose by pressurised gyration would be among the pioneering works utilising cellulose derivative for drug delivery at nanoscale level.

3.2.3 Sodium alginate

Sodium alginate is presented as a white to pale yellowish-brown powder produced by neutralising alginic acid extracted from brown seaweed with a base, typically sodium bicarbonate. Alginate has been used extensively in diverse biomedical and pharmaceutical systems because of its favourably physical and chemical properties, with gel formation, low toxicity and biocompatibility being the more obvious reasons. The relatively mild crosslinking structure makes alginate a preferred biopolymer for encapsulation of delicate macromolecules including proteins, cells and nucleotides as the structure and function of these biologics remain intact to a significant extent within the alginic systems (Gombotz and Wee, 2012, Lee and Mooney, 2012).

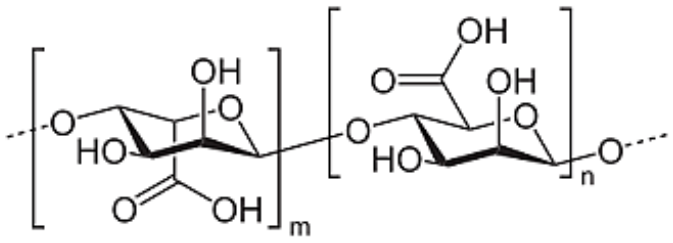


Figure 3-3: Molecular structure of Sodium alginate

Another interesting feature of alginates that inspired its selection for this study is the strength of its interaction with mucosa surfaces. This feature partly explained by the charge distribution around the molecule makes alginates good choice of polymer for

drug delivery through mucosa where mucoadhesive properties. There are studies to confirm how alginates compares favourably to other known mucoadhesive polymers such as carboxymethyl cellulose, polystyrenes and chitosan (Chickering and Mathiowitz, 1995). It is expected that nanofibres incorporating alginates would contribute to a superior performance by enhanced attachment to the mucosa area for drug application.

3.2.4 Polyacrylic acid

Polyacrylic acid is white lightweight powder with a slight characteristic odour principally used in pharmaceutical semisolids as rheology modifiers. A smooth feel obtained from its aqueous mixtures makes them ideal for inclusion in several pharmaceutical and cosmetic products for topical application.

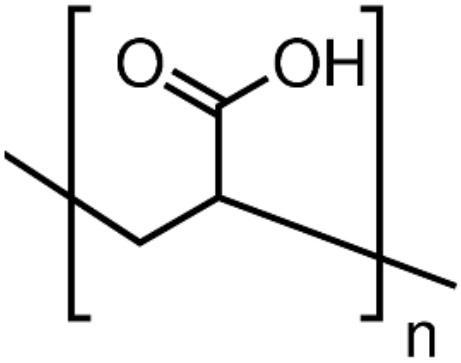


Figure 3-4: Molecular formula of Polyacrylic acid

Mucoadhesive properties of polyacrylic acid though highly dependent on pH make them valuable polymers for drug delivery, especially through the mucosa. It has been demonstrated that the carboxyl groups need to be in an acidic environment for significant mucoadhesive ability and sharply diminish in environments when pH begins to rises above 4 (Park and Robinson, 1987). As mentioned earlier, the typical

acidic environment of the vagina for instance could improve the strength of attachment and thereby enhancing the performance of a delivery system whose mucoadhesive properties are potentiated in acidic environment. This consideration explains the reason for selecting polyacrylic acid as one of the materials for making nanofibres intended to be used for the design of a drug delivery system for vaginal application.

3.2.5 The active drug – Progesterone

Progesterone has been discussed extensively in section 2.5. Briefly, it is an endogenous steroidal hormone with crucial roles in human reproduction and activities such as metabolic intermediate in the synthesis of other hormones such as corticosteroids (King and Brucker, 2010). Chemically, as typical of steroids, it is synthetic fat soluble organic compounds characterised by a molecular core of four fused rings (three 6-Carbon rings and one 5-Carbon ring) and made of 17 carbon atoms (Lednicer, 2011). Progesterone is known to exist in two crystal forms (α and β types which melt at 129°C and 121°C) which are easily interconverted and have similar physiological activities (Payne et al., 1999). Laboratory grade progesterone are available in different physical forms and two of these with average particle size 100 and 10 μm were employed in this project. The rationale was to investigate the effect of drug particle size on the performance of delivery systems developed from them. This drug is considered to be effective for supporting pregnancy to term in women considered at risk of early labour has been firmly established through various studies (Dodd et al., 2008) and hence the choice as active drug for this project.

3.2.6 Solvents

Water and alcohols were used in developing solvent systems capable of forming consistent polymer-drug mixtures for best possible fibre outcomes. Ethanol proved to be the preferred alcohol to be used in solvent systems for producing high quality

progesterone-loaded nanofibres. Ethanol's miscibility with water has made it an attractive and a general-purpose solvent in several operations, including formulations in the pharmaceutical industry. Other alcohols investigated for their suitability as component of solvent system for generating nanofibres were propanol and butanol.

3.2.7 Surfactants

Polysorbate 80 (Tween 80) was used in attempts to formulate polymer-drug solution to determine its suitability in making constituent mixture of materials with different water solubilities in place of alcohol. Polysorbate 80 is slightly yellowish oily liquid with faint and characteristic odour. It is an emulsifying agent particularly useful for solubilising oil-soluble APIs such as progesterone.

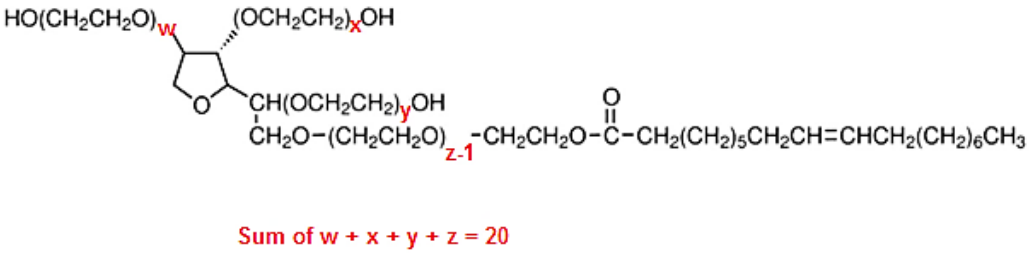


Figure 3-5: Polysorbate 80 with hydrophilic and lipophilic groups aiding its function as surfactant and emulsifier.

3.2.8 Physiological fluids and buffers

Drug permeation and mucoadhesive assessments were conducted with the help of simulated vaginal fluid (SVF) and phosphate buffer. The rationale for choosing these was to observe the performance of materials produced under conditions closely resembling vaginal environments. The SVF was prepared according to an existing formulation (Marques et al., 2011) and contained sodium chloride, potassium hydroxide, calcium hydroxide and bovine serum albumin (BSA). The remaining ingredients were acetic acid, lactic acid, glucose, urea, glycerol and mucin.

3.2.9 Membranes for mucoadhesive study

Cellulose acetate of pore size 0.2µm, used as artificial membrane in mucoadhesive studies was obtained from Sartorius, Gottingen, Germany. Fresh mucosa for mucoadhesive study was from lamb oesophageal tissue arranged and delivered from a local abattoir by Giggly Pigs, Romford, UK.

3.3 Procedures

3.3.1 Characterisation of solutions

Solution characterisation mainly involved measuring viscosity and surface tension of liquid preparations form which fibres were spun. These are the two most important properties affecting nanofibre processing and outcome, as established by Mahalingam and Edirisinghe (2003) in their pioneering experiments on fibre generation by PG.

3.3.1.1 Viscosity

The viscosity for each polymer solution or blend was measured using Brookfield DV-111 viscometer (Harlow, Essex UK) at a specific shear stress indicated where relevant. The Brookfield DV-111 is a rotational viscometer that measures the absolute viscosity of fluids using the torque of a rotating spindle submerged in the fluid being analysed to calculate the resistance to flow. In simple terms, the viscosity measured by this equipment is a function of the resistance encountered by the spindle as it rotates through the liquid being analysed at a particular shear stress. In measuring viscosity, 5ml of solution was placed in a tube and spindle (Number 18) lowered into the tube such that it was completely submerged in the solution being analysed.

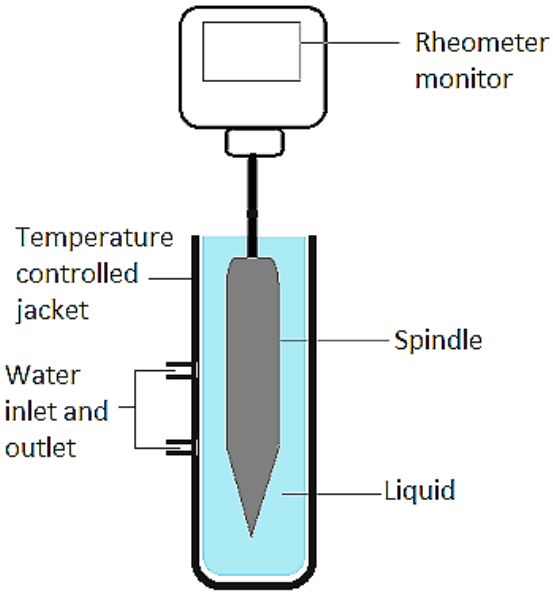


Figure 3-6: Schematic illustration of rotational viscometer

At a shear stress of 3.5 Pa, suitable point for consistent readings for the viscosity range of all liquids analysed, the dynamic viscosity of each solution was taken and noted. Four additional measurements were taken for each batch of solution. The mean and standard deviation from the five measurements were used in subsequent analyses.

3.3.1.2 Surface tension

Solution surface tension measurements were done using Kruss K9 tensiometer (Hamburg, Germany). The Wilhelmy plate method, in which a thin platinum plate (Kruss PL21, Hamburg, Germany) is positioned perpendicular through the liquid being analysed and the force required to break contact between the plate tip and liquid surface measured was used in assessing the surface tension of solutions. The surface tension is given as a function of the force (*F*), the wetted perimeter of the platinum tip (l) and contact angle θ (Butt et al., 2006). The equation is given below:

$$\gamma = \frac{F}{l\cos(\theta)}$$

When complete plate wetting by the liquid being analysed has occurred, which is usually the case in this method, the contact angle, θ is 0. Cos 0 is 1 therefore effectively, surface tension measured by this method is given as a ratio of F and l, calculated as $2w + 2d$ (Figure 3-7b) where w and d are width and thickness of the platinum plate respectively.

In assessing these, the platinum plate connected to a balance was dipped into 20ml of solution in a 25ml beaker on a fixed stage. The platinum plate was gradually removed from the liquid with the automated balance recording the tension at the air-liquid interface. In all, five measurements were taken for each solution sample and the mean and standard deviations used in subsequent analyses

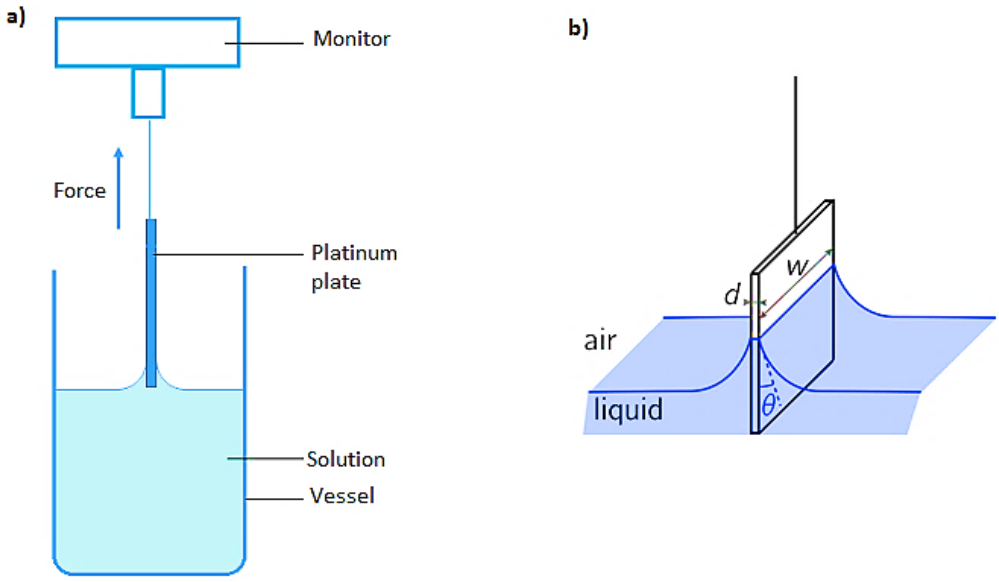


Figure 3-7: Schematic illustration of tensiometer and 3D of the plate's interaction with liquid sample (Butt et al., 2006)

3.3.1.3 Particle size analysis

In search of optimal conditions for producing good quality nanofibres with suitable level of drug loading, two batches of the active drug presented in different particle size classes were analysed to determine how their sizes affected outcome of fibre produced.

Figure 3-8: Mastersizer 3000 used in progesterone particle size analyses

Their particle size distributions were analysed using Mastersizer 3000 laser diffraction analyser (Malvern Instruments, Worcester, UK). 1g of progesterone from either batch was uniformly dispersed in 20ml of deionised water and transferred into the sample dispersion unit of the analyser. This amount of progesterone was enough for the needed obscuration for accurate measurement of its particle sizes. The automated process of applying the laser and measuring the intensity of light scattered was started and subsequently, the particle size classes in the sample, presented as a histogram were recorded. Six measurements were taken and used to determine the differences in particle size between the two samples.

3.3.2 Solutions for generating nanofibre

Solutions used throughout the project for generating nanofibres were classified into 4 groups, according to their components. These are solutions containing single polymer without active drug, single polymer with active drug, polymer blends without active drug and polymer blends with active drug. All solution constituents were measured by weight and specified in various sections discussing outcomes following their conversion into fibres. Solutions were typically made by weighing the dry constituents together before adding appropriate amount of solvent, except for those containing

more than one polymer where each polymer was made into a solution before mixing desirable amounts together to obtain various the needed blend.

For progesterone-loaded fibres, desirable proportions of polymers and drug (progesterone) occurring as mixtures were obtained by weighing out the powders and solvents separately and mixing together. Continuous magnetic stirring followed by sonication for 10 minutes using Branson Sonifier 250 (Danbury, Connecticut USA) ensured that liquid preparations were appreciably uniform. Mixtures prepared were stored in airtight bottles until required for fibre generation.

3.3.3 Fibre generation

Fibres were generated by pressurised gyration (PG) from each of the mixtures described in section 3.3.2. PG is a manufacturing approach that combines centrifugal force and applied pressure via a gaseous medium to generate fibres, usually in the nanoscale diameter range, from solutions (Mahalingam and Edirisinghe, 2013) and from molten liquids (Xu et al., 2016).

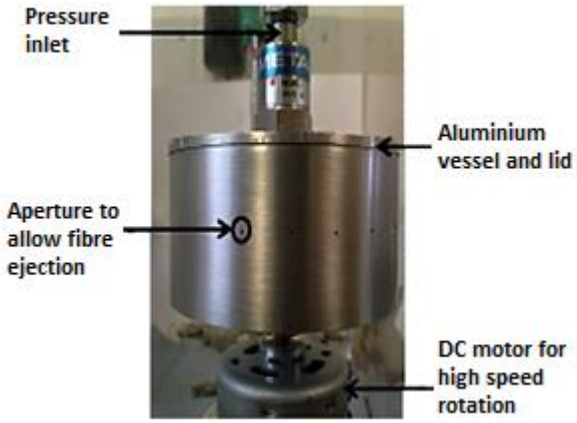


Figure 3-9: Pressurized gyration apparatus

The setup used, shown in Figure 3-9, comprises of a cylindrical aluminium vessel of approximately 60mm in cross-sectional diameter and 35mm in height. The pot incorporates orifices of approximately 0.5mm diameter dispersed horizontally around

the circumference, spaced 10mm apart. The vessel is attached to a DC motor capable of rotations of up to 36,000 rpm. Finally, there is a lid connected to a source of gas source to enable fibre spinning in a pressurised environment. To produce nanofibres, aliquots of the liquid preparation are placed in the vessel and covered. Rotation begins at the same time as pressure, typically from a nitrogen source, is being applied; as the fluid is forced through the orifices, the solvent is evaporated off the jet during flight leaving a formed fibrous material on the surrounding collecting shields. Each manufacturing cycle takes between 2-5 minutes depending on the experimental conditions. Fibres ejected through the nozzles are collected and stored until required for further analyses.

For each cycle of fibre production in this project, 3 ml of solution was placed in the aluminium vessel and spun at a rotation speed of 24,000 rpm and working pressure of 0.15 MPa room temperature conditions (approximately 25ºC/45% relative humidity). These conditions were selected after trial runs at 10000, 24000 and 36000 rpm rotation speeds and pressures 0.1, 0.15 and 0.2 MPa.

3.3.4 Characterisation of fibres

3.3.4.1 SEM imaging and analysis

Samples from each batch of fibres were analysed by scanning electron microscope (SEM), JEOL JSM 630 IF (Tokyo, Japan) for the morphology, and in particular the size distribution of fibres. Fibres were mounted on SEM stubs with the aid of two-sided adhesive carbon discs obtained from Agar Scientific, Stansted, UK. Each mounted sample of fibres was gold coated for 90 seconds using Quorum Q150R pumped sputter coaters (Quorum Technologies, Lewes, UK). Coated samples were analysed at operating voltage of 5kV. Record of images was produced with the aid of SemAfore software, also from the SEM manufacturer.

3.3.4.2 FTIR analyses of fibres

Fibre samples were analysed for different chemical properties to help verify the presence and extent of miscibility during blend formation. In addition, the content of nanofibres produced from solutions incorporating progesterone were analysed to confirm that indeed fibres generated contained active drug. All of these investigations were carried out using the Attenuated Total Reflection Fourier Transform Infrared spectroscopy (ATR–FTIR) Vertex 90 spectrometer (Bruker, Coventry, UK), and spectrographs were interpreted using OPUS Viewer version 6.5, a software also from Bruker, Coventry, UK. A baseline (background) scan at a resolution of 4 cm^{-1} before each sample was mounted on the platform, adjusted and secured to be in contact with the scanning probe and finally scanned 32 times using same parameters used for background scans. Secondly, variable temperature FTIR scans were taken using Specac Golden Gate (Orpington, UK) heating controller attached to the FTIR spectrometer to track chemical changes in nanofibres under elevated temperature conditions. Scans were taken at 2°C intervals between 50 and 80°C to study the effect of heat on the samples beyond the melting point of polyethylene oxide typically between 60 and 65°C for the grade used.

3.3.4.3 X-ray analyses of fibres

Structure and crystalline forms of the content of fibres were studied using D/Max-BR diffractometer (RigaKu, Tokyo, Japan) with Cu Kα radiation (λ = 1.5148 Å). Prior to measuring X-ray diffractions, materials in the form of either powders and nanofibres were pressed gently into a 20mm aluminium sample tray and the surface made even by levelling with a glass slide. This was then mounted onto the diffractometer and analyses conducted at 40mV and 30mA over 2θ range of 5−60° at a rate of 2°/min. Data obtained were converted to diffractograms and evaluated using OriginPro 7.0 software (OriginLab Corporation, MA, USA).

3.3.4.4 UV analysis of drug content in fibres

The progesterone content within the fibres generated was determined following the standard assay procedure of dissolution in ethanol for detection by UV at 241 nm (NCBI, 2004). UV analysis was chosen as PEO has no active UV chromophores and hence minimal interference with assay of progesterone was anticipated. 1 mg of fibres were dissolved in 10 ml of absolute ethanol in a volumetric flask. The flask was swirled gently over a period of 24 hours to ensure complete removal of progesterone from fibres into the ethanol. 3 ml of resultant solution was drawn and analysed spectrophotometrically at 241 nm using a Jenway 6305 UV/Visible spectrophotometer (Bibby Scientific, Staffordshire, UK).

3.3.4.5 Calibration and calculations for UV analyses

Standard solutions in concentration range 1.875 X 10^{-3} to 2.5 X 10^{-2} mg/ml of pure progesterone in ethanol were prepared by serial dilution.

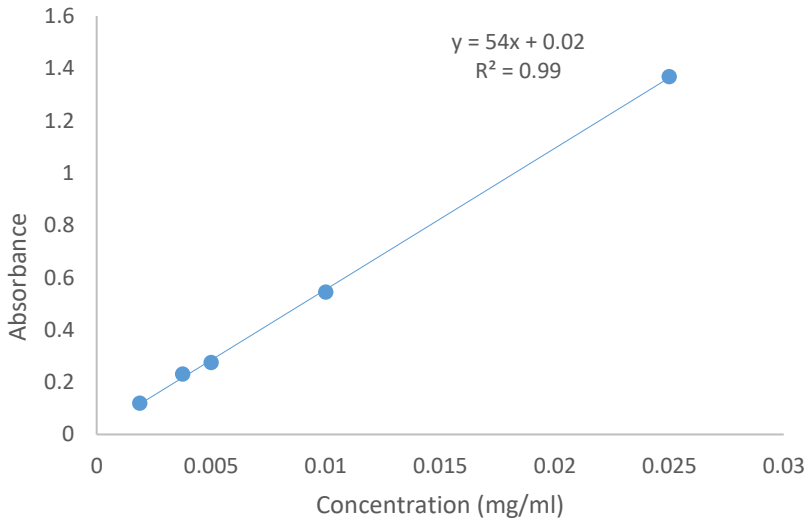


Figure 3-10: Calibration curve and equation used to calculate amount of progesterone in nanofibre samples

The absorbances of these solutions were taken at 241 nm (λmax of Progesterone) to obtain a calibration curve (Figure 3-10) from which progesterone content in nanofibres produced were quantified.

Using the equation of the calibration curve, $y = 54x + 0.02$, concentration (x) from absorbance readings (y) were obtained by the equation:

$$(Absorbance\ - 0.02) \div 54$$

Calculation example

1mg sample of nanofibre in 10ml of ethanol; this is equivalent to 0.1mg/ml

If this sample records an absorbance of 0.8 when measured at 241 nm, then using the calibration curve equation which is $(Absorbance\ - 0.02) \div 54$, progesterone concentration from this nanofibre sample is found to be 0.014 mg/ml

Total nanofibre weight is 0.1 mg

Progesterone content is 0.014 mg

Therefore % wt. of progesterone is $\frac{0.014}{0.1} \times 100$ = 14% wt.

3.3.4.6 Hot Stage Microscopy

Hot-Stage Microscopy (HSM) studies were conducted on a Leica DM 2700 M microscope (Leica Microsystems, Wetzlar, Germany) fitted with Infinity 2 digital camera (Lumenera Corporation, Ottawa, Canada). The unit was used to visually observe the melting of fibres on a FP5/FP52, heating stage unit controlled by FP90 central processor unit, both from Mettler-Toledo Ltd, Leicester, UK. Fibres containing progesterone were heated from 50 – 130 °C at a rate of 2 °C/min while plain PEO fibres were heated from 50 – 70 °C at the same rate.

3.3.5 Mucoadhesive studies

3.3.5.1 Measuring detaching force by Texture Analyser

In assessing mucoadhesion with texture analyser, three different methods were used. The first involved measuring tensile strength of gel prepared from nanofibre samples and SVF plus mucin mimicking the vaginal environment. A relationship between tensile strength of gels and mucoadhesion is well established (Hägerström and Edsman, 2001) and this was the basis for developing this methodology to compare mucoadhesive properties of samples produced. For many decades, different methods have been adapted for measuring tensile strength of gels (Ben-Arie, 1955). In this study, texture analyser was adapted for this purpose. The second and third methods involved separation of adhering surfaces and measuring the forces involved. The forces measured, which correlates extent of mucoadhesion were interpreted as such. Each of these methods are described further.

3.3.5.2 Assessing tensile strength of gels

The extent of interactions between fibres and mucin under simulated conditions similar to a vaginal environment were studied using Texture analyser, TA-XT2 (Food technology Corporation, Virginia, USA) shown in Figure 3-11. The approach used was to measure the breaking properties of mixes of the polymer and simulated mucus (Tamburic and Craig, 1997). A predetermined force of 20g was applied by an acrylic probe of cross sectional area of 50mm^2 for a contact period of 0.1 second. Pre-test speed of probe was 0.5mm/s while return speed was 0.5mm/s over 4mm distance. The force required to break up polymer/mucin gel by separating the probe from the sample was measured; it was ensured that the breakage occurred within the gel rather than between the gel and probe.

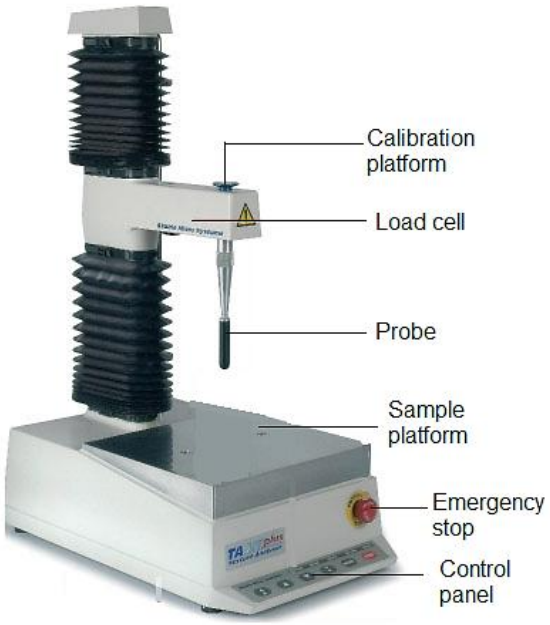


Figure 3-11: Texture Analyser TA-XT2 used for measuring fracture force in mucoadhesion assessment

3.3.5.3 Assessing adhesion between fibres and mucosal membrane

Interactions between nanofibres and mucosa membrane surface under simulated conditions mimicking a vaginal environment were studied using a Texture analyser, TA-XT2 (Food Technology Corporation, Virginia, USA). Mucosa membrane from lamb oesophageal tissue in dimensions of 40 X 30 mm was firmly attached onto the stage of the texture analyser with the help of adhesive tapes. Lamb oesophageal mucosa is non-keratinised and closely resemble those of humans and hence selected for this study (Prasanna, 2011). 100mg samples of nanofibres whose mucoadhesive potential are being assessed were securely attached to a cylindrical probe (Chen-Hoseney dough stickiness rig) with cross-sectional area of 0.785 cm^2 using a double sided adhesive disc and mounted on the movable part of the analyser. The analyser was set to measure force required to completely detach the nanofibre from the mucosa membrane after bringing the two materials together for a contact time of 5 minutes with a pre-test force of 20gF (0.2N). This routine was conducted in triplicates

for all samples and the means and standard deviations calculated and used in further analyses.

Table 3-1: Experimental condition selected for measuring forces required to detach fibres from mucosa.

Velocity	
• Pre-test	50 mm/s
• Test	50 mm/s
• Post-test	10 mm/s
• Tracking	5 mm/s
Force	
• Test force	40 gF
• Trigger force	10 gF
Distance	
• Return distance	4 mm
Time	
• Contact time	5 s

3.3.5.4 Assessing adhesion between fibres and artificial membrane

The same procedure was repeated using 0.2µm pore sized cellulose acetate membrane treated with SVF (with mucin) to confer some mucosa properties. Cellulose acetate with this pore dimension was selected for comparison with actual mucosa membrane as they were confirmed to correlate well with each other when utilised in some permeation studies (Khdair et al., 2013).

3.3.5.5 Atomic force microscopy (AFM)

Mucoadhesive properties of nanofibres were studied by AFM using two different methods developed based on theories (Carvalho et al., 2010) confirmed relevant to interactions resulting in mucoadhesion.

In the first approach, relative solubilities of nanofibre samples in the simulated mucus environment were determined by measuring the surface roughness of residual films from nanofibre and mucin mixtures using Bruker Multimode 3 atomic force microscope (Coventry, UK). A film area of 225µm^2 was scanned at a rate of 1 Hz

using a TESPA-V2 probe (Bruker, Coventry, UK) with cantilever having a spring constant of 42N/m. Height images were obtained using the tapping mode. Measurements from images were obtained using ImageJ software (National Institute of Health, USA).

In the second method, 100mg of nanofibre was mounted on the membrane of mucosa tissue sufficiently irrigated with SVF. This allowed sufficient time of interaction for mucoadhesion to occur, and then samples were kept in a desiccator to remove excess moisture. Transverse sections of the nanofibre on the mucosa were prepared by cutting extremely thin slices with razor blades such that they were thin and smooth enough for the AFM probes to be brought close to their interface to measure the level of roughness. An illustration of this procedure is shown in Figure 3-12. Dimension icon AFM (Bruker, Coventry - UK) using PointProbe® Plus Nanosensor probes in tapping mode was employed in measuring the roughness of the interface. Samples prepared in transverse sections as described above were carefully mounted such that the probe was nearly in contact with the interface area to obtain best the possible signal for quality imaging. Height images of interacting surfaces were taken and used in calculating level of roughness as a function of mucoadhesion.

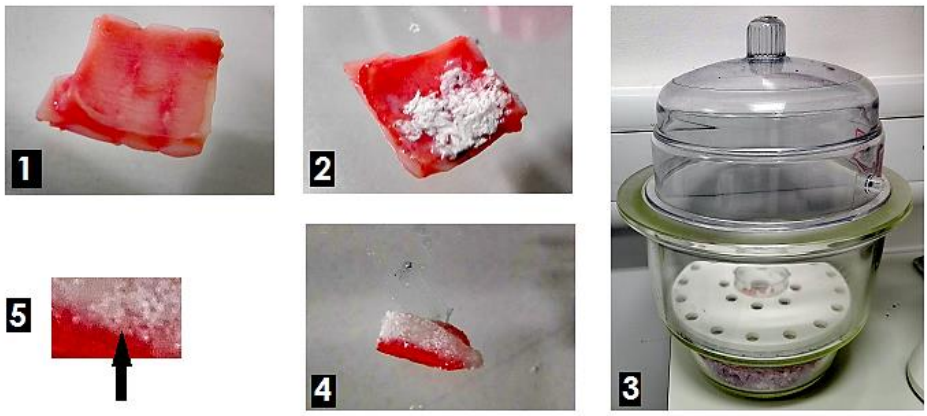


Figure 3-12: Steps taken in preparing mucosa-fibre samples for AFM: 1) Lamb oesophagus mucosa irrigated with simulated vaginal fluid 2) 100mg of nanofibre mounted onto irrigated mucosa surface and allowed to interact 3, 4) Sample kept in desiccator to remove excess moisture and 5) Thin transverse sections across both layers removed and portion indicated by arrow was examined by AFM

Chapter 4

Generating nanofibres from blends of mucoadhesive polymers

4.1 Introduction

The value of nanofibers in diverse applications, due to their unique physical attributes have been discussed extensively in previous chapters. Nanofibres are emerging strongly as material of choice for drug delivery through mucosa, as their exceptional surface properties could be adapted for superior adherence onto the mucosa and hence improving resident times significantly (Sharma et al., 2016, Zong et al., 2015). Notwithstanding these prospects, the availability of adequate quantities of nanofibre structures have been a drawback, in that there remains a considerable gap between demand and supply. Furthermore, in order to produce sufficiently mucoadhesive nanofibres for design of delivery systems with superior adhering features, polymers inherently mucoadhesive may be utilised. However, a significant proportion of mucoadhesive polymers such as polysaccharides, due to their chain structure, cannot be easily spun into fibres on their own (Lee et al., 2009). In this chapter, the possibility of using polymer blends for the preparation of nanofibres that offer substantial mucoadhesive capabilities is reported.

Fibres from four different polymers in various combinations were obtained by pressurised gyration at different working pressures and a rotation speed of 24,000 rpm. Electron microscopy indicates that structurally well-defined fibres with diameters from less than 100 nm upwards were successfully produced. A preliminary investigation looking into the effect of solution properties and production conditions such as working pressure on outcome of fibre was conducted to determine quantitative relationships that exist among these properties. In addition, the chemical composition of fibre from each batch was elucidated to confirm if all of the starting polymers were present as expected. Finally, a combination of texture analysis and

atomic force microscopy was used to verify the benefit of transforming polymer powders into nanofibre structures, as far as mucoadhesive potential is concerned.

4.2 Fundamental principles of nanofibre generation

Nanofibres in varying sizes were produced using procedures discussed in section 3.3.3. The differences observed in fibre size and morphology are very much dependent on material properties such as constituents and concentration of solution yielding the fibres as well as the experimental conditions such as rotation speed and working pressure. The relationships occurring between fibre properties and material or production conditions are discussed in sections further on.

The processes of converting polymer solutions or melts to nanofibres have been explained with theories from the Raleigh-Taylor instability and the Marangoni stress concepts (Mahalingam and Edirisinghe, 2013).

The Raleigh-Taylor (RT) instability in the simplest of terms is the instability at the interface of two fluids of different densities which occurs due to the light fluid pushing into the heavy one (Davies and Taylor, 1950). The behaviour of water suspended on top of oil in Earth's gravity is a classical everyday example often used to illustrate this instability. Lord Raleigh studied this instability using two immiscible fluids subjected to Earth's gravity with the denser fluid on top of the lighter one (Stone and Williams, 2015). Any disturbance at the interface of the two fluids makes their equilibrium unstable. The instability is characterised by continuous dissipation of potential energy as the heavier fluid moves downwards while the lighter fluid is displaced upwards under gravity, such that the potential energy configuration of the setup diminishes with time, compared to the initial state. Taylor discovered that this instability was dependent on the lighter fluid accelerating into the denser one (Strutt and Rayleigh, 1883) and hence this phenomenon referred to as the Raleigh-Taylor instability.

In pressurised gyration, the high rotational forces on system of fluids with different densities (basically polymer solution and air) creates the necessary fluid acceleration and instability at the interface that eventually cause a breakaway in the form of jets. These jets are further stretched due to sustained stress from the forces, primarily centrifugal and gravitational acting on the system. Evaporation of solvent during flight of jet from vessel through the nozzle ensures thinning and completes the fibre formation process.

It is also known that fibre formation is initiated when sufficiently high centrifugal force overcome surface tension in certain areas within the polymer liquid (Padron et al., 2013). This brings about surface tension gradient, which according to the Marangoni effect, results in mass transfer along the interface between the polymer solution and air and ultimately forming polymer jets. The Marangoni effect describes how a pocket of fluid can break free from the bulk due to difference in surface tension. This effect brought about due to variation in conditions such as solute or surfactant concentration and temperature at the interface is also exhibited as sheer stress, similar to wind effect at the fluid surface. The turbulence at the interface (Sharp, 1984), most likely due to the forces applied to the system also facilitate the fibre formation process. The underlying principles described give rise to jets, ultimately resulting in fibres or droplets preceding bubble or bead formation depending on conditions such as solution properties, particularly its viscoelasticity and surface tension as well as amount of centrifugal force being applied (Mahalingam and Edirisinghe, 2013, Padron et al., 2013)

4.3 Fibre generation

The strategy adopted for producing sufficiently mucoadhesive nanofibres is to utilise polymers with inherent mucoadhesive properties. As mentioned earlier, these polymers which are usually polysaccharides lack the necessary chain arrangement

to allow them to be drawn into fibres on their own. So PEO, a very good spinning agent with some inherent mucoadhesive properties was blended in to allow fibre formation.

Mucoadhesive polymers used in this study are polyacrylic acid, sodium alginate and carboxymethyl cellulose. These are anionic polymers proven to demonstrate their strongest mucoadhesion properties under acidic condition (Khutoryanskiy, 2011).

Table 4-1: Polymer quantities in blends used for generating nanofibers

Polymer	PEO	CMC
Concentration (%)	15	4
Proportions in blend of 100ml	75	25
Absolute ratios	$\frac{75}{100} \times 15 = 11.25$	$\frac{25}{100} \times 4 = 1$
*Absolute quantities in solution with 15% polymer and 85% solvent	13.8	1.2

Nanofibres generated in this project are intended as materials for developing delivery systems for vaginal application. Since the vaginal environment is usually acidic, it is anticipated that systems developed from these anionic polymers will exhibit substantial mucoadhesive properties when applied as such. Before these polymers were used for generating fibres, a preliminary investigation into optimal conditions required for production of fibres from polymer blends was carried out using mixtures with PEO and CMC in various ratios. Blends containing 15%wt PEO and 4%wt CMC in various proportions were used in this preliminary study. Specifically, polymer blends in PEO: CMC ratios of 90:10, 86:14 and 75:25 were utilised. In absolute terms, these translate to 0.49, 0.69 and 1.225% wt. of CMC (Calculations shown below

4.3.1 Relationships between solution properties and fibre outcome

4.3.1.1 Effect of CMC content on polymer blends properties

Generally, increasing amounts of CMC in the polymer blends increased both viscosity and surface tension of the mixtures. This is worth noting as the effect of these solution properties on outcome of nanofibres are well established (Mahalingam and Edirisinghe, 2013, Padron et al., 2013).

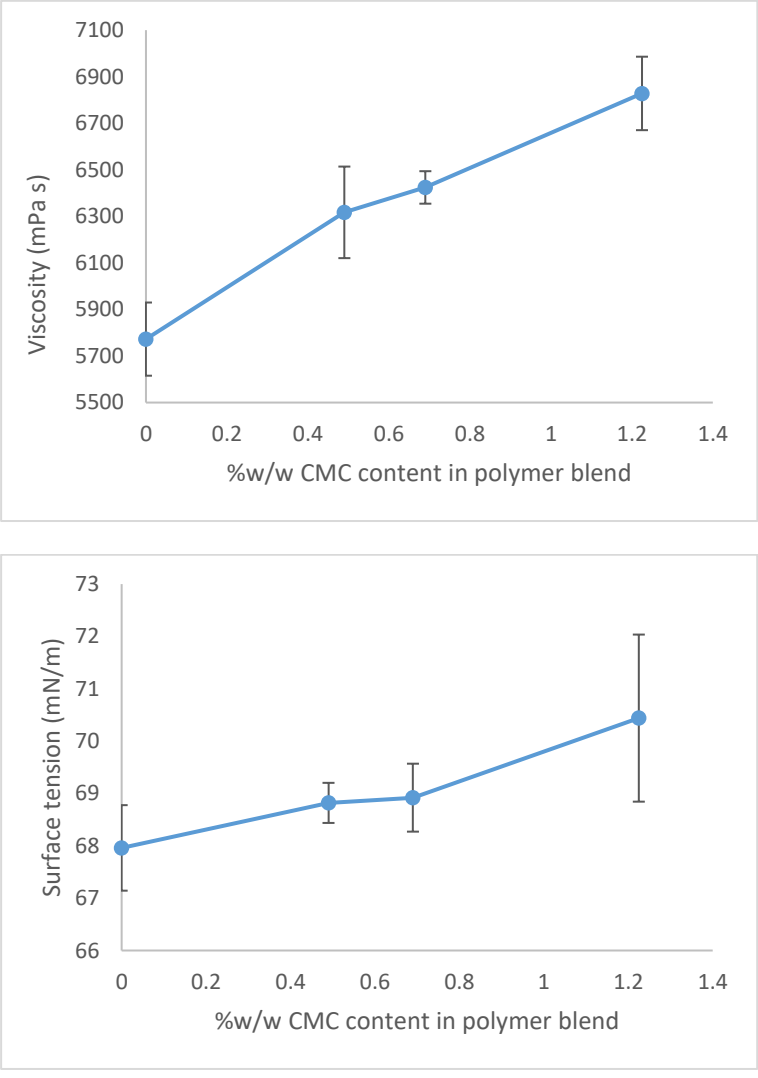


Figure 4-1: Effect of CMC content on blends' viscosities and surface tension

The elevated viscosity and surface tension arising from inclusion of CMC is expected as this polymer is characterised by yielding high viscosity solutions even in low concentrations (Yang and Zhu, 2007), a property which is largely dependent on

degree of substitution (DS) occurring on the cellulosic backbone and the intrinsic viscosity of plant pulp from which the polymer is derived (Barba et al., 2002).

The impact of varying viscosity and surface tension which depends on the overall concentration of polymer solution, and in this case, the amount of CMC was examined with respect to fibre outcome.

4.3.1.2 Effect of concentration of fibre outcome

Fibre morphology, particularly their sizes was observed to be dependent on solution properties. CMC content was the variable in solution samples whose influence on fibres outcomes were investigated. So, fibre characteristics observed have been defined in terms of amount of CMC in solutions from which fibres were generated. The relationship between fibre size and solution properties was also found to be significantly influenced by the working pressure. Therefore, looking at the influence of solution properties and working pressure simultaneously appears to give a better view of the effects of processing conditions on fibre formation. Table 4-2 summarises the average fibre sizes from their respective solution for each working pressure employed in their processing

Table 4-2: Solution and pressure working conditions for fibres generated from blends of PEO and CMC

% Content of CMC (In blend of 15% PEO and 4% CMC)	Average fibre diameter (nm) ±SD at pressures shown below		
	0.1MPa	**0.15MPa**	**0.2MPa**
10	266 ± 49	280 ± 48	234 ± 47
14	189 ± 23	270 ± 54	192 ± 29
25	202 ± 54	194 ± 34	161 ± 37

Generally, solutions containing higher proportions of CMC and characterised by higher viscosity and surface tension yielded smaller sized fibres. A closer examination indicates that the influence of solution properties on fibre outcome is better defined at higher working pressures. At 0.1MPa, there seemed to be no particular trend in fibre sizes with respect to solution properties. However, at higher pressure i.e. 0.15 and 0.2MPa, there was a clear trend among fibre size in relation to solution properties. Specifically, increasing amounts of CMC typified by higher viscosity and surface tension, resulted in smaller nanofibres. This observation highlights the benefit of spinning fibre from a pressurised system; the amount of pressure applied is an additional parameter that can be regulated in order to predict and actually yield a more specific outcome. Generally, spinning at higher working pressure results in fibres with smaller diameter. A more detailed analysis on the effect of pressure on fibre generation from various blends of polymers are in section 4.4.1.

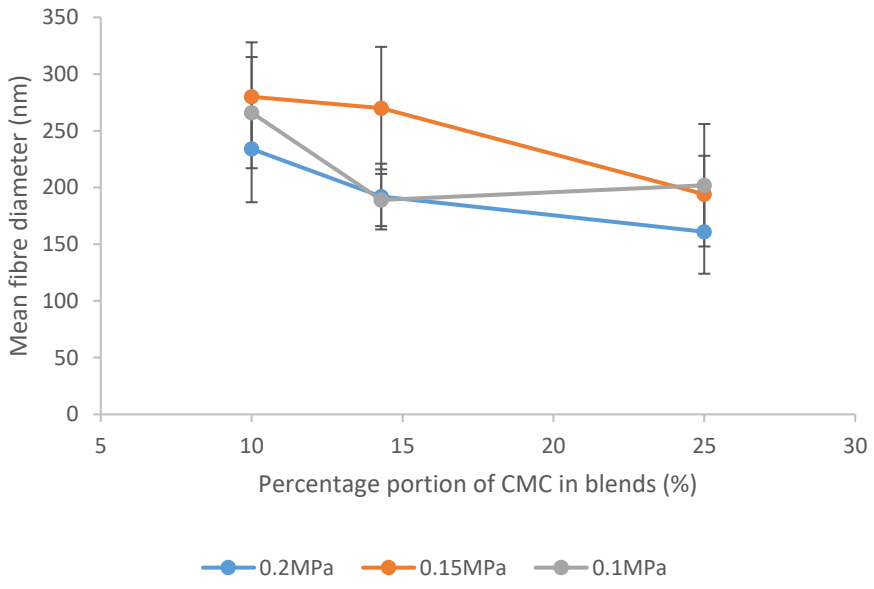


Figure 4-2: Effect of blend composition on mean fibre diameter.

Solution with highest concentration of mucoadhesive CMC (25%) showed uniform reduction in fibre size with increasing working pressure. Generally, higher CMC

content yielded fibres with smaller mean diameter demonstrating the effect of mucoadhesive CMC in fibre formation

4.3.1.3 Summary of preliminary investigations

Three parameters most influential on the outcome of fibre generated by pressurised gyration are solution properties (essentially expressed in terms of viscosity and surface tension), working pressure and rotation speed. Studies relating these parameters to fibre outcomes (Xu et al., 2015, Hong et al., 2016, Mahalingam et al., 2014, Raimi-Abraham et al., 2014, Mahalingam and Edirisinghe, 2013) have established the possibility of producing fibres with desired characteristics when a set of parameters are selected.

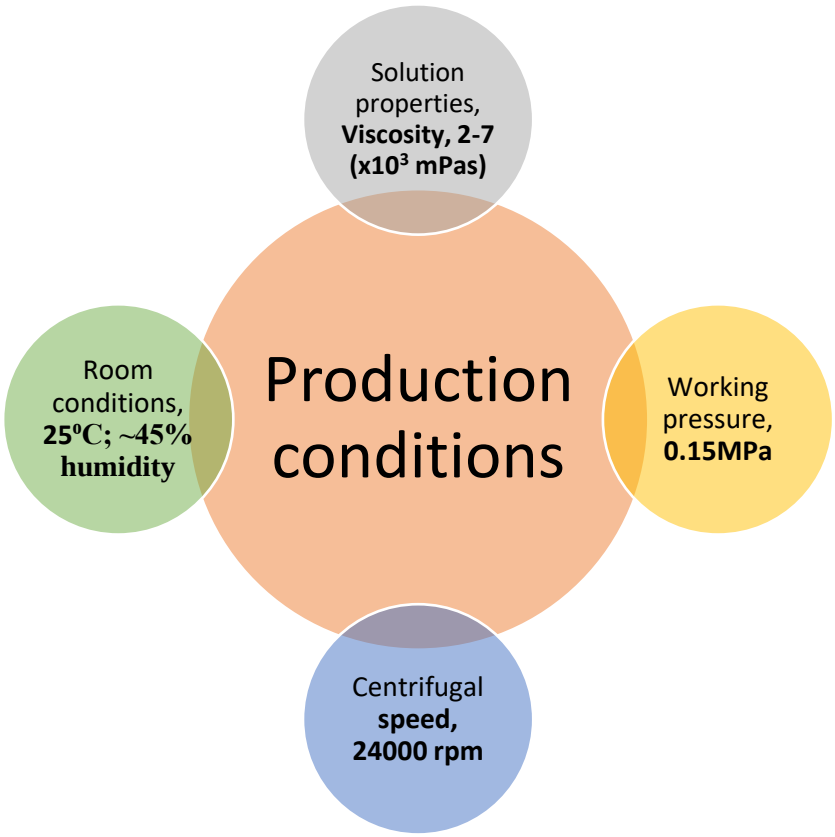


Figure 4-3: Summary of production conditions

In the study by Mahalingam and Edirisinghe (2013) which first reported use of pressurised gyration for nanofibre production, ideal rotation speeds (10-36,000 rpm) most likely to yield fibres from various polymer concentrations were shown. The volatility of solvents used in polymer liquid preparation prior to spinning is also important in determining an ideal speed for spinning. Based on data from this study, an average rotation speed of 24,000 rpm was selected for fibre spinning throughout this study unless indicated otherwise. At this speed, polymers capable of forming nanofibres will have a better yield, relative to those spun at lower rotational speeds. Furthermore, this rotation speed is a good balance between the upper limit of 36,000 rpm where solvent systems selected for this work may not cope due to rapid evaporation and a lower speed typically producing larger, often low-quality fibres

In terms of pressure, it was observed earlier that working pressure of 0.15MPa or above yielded smaller nanofibres and more importantly, fibres traceable to their solution properties i.e. a clear trend relating fibre size to solution composition. On this account, a working pressure of 0.15MPa was used throughout the study unless otherwise indicated. Results from this preliminary investigation indicate better outcome of fibres from solution with higher CMC content, both in terms of characteristics such as size and reaction to elevated working pressure. Therefore, polymer blends in proportions similar in viscosity to the 75:25 mixture of 15% PEO and 4% CMC respectively was used for subsequent production of plain and drug-loaded fibres from PEO/CMC as well as from blends incorporating sodium alginate and polyacrylic acid.

4.4 Nanofibres from PEO and other mucoadhesive polymers

The outcome from preliminary investigations informed the choice of parameters for producing nanofibres from PEO blended with alginate or polyacrylic acid in addition to those containing CMC. Each 100ml of polymer blend contained 75 ml of 15% PEO

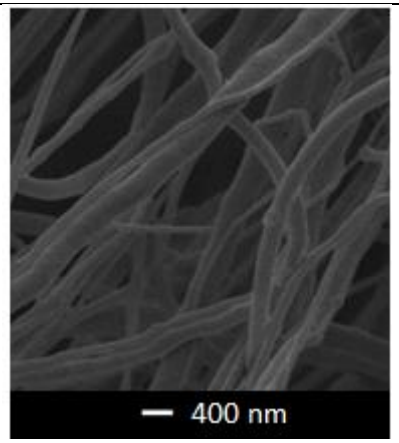
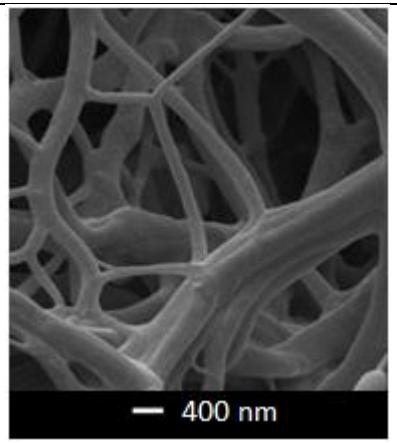
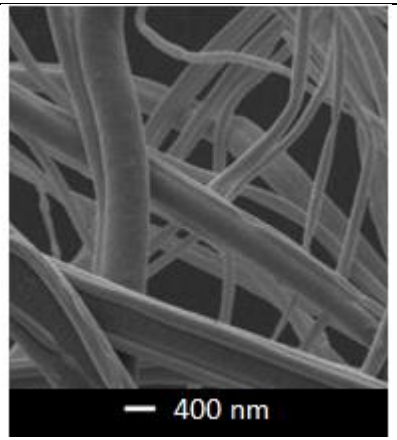
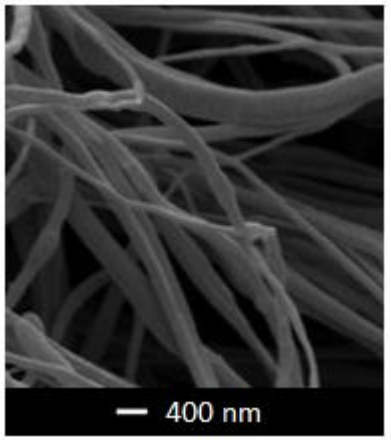
and 25 ml of 4-5% mucoadhesive polymer, depending on the viscosity. This was based on the blend composition for PEO and CMC which was responsive to increasing working pressure and yielding high quality nanofibers in the preliminary study. Also, a pressure of 0.15MPa was chosen as the effect of the lower pressure of 0.1MPa offered no desirable effect on fibre outcome on the blends. Centrifugal spinning at 24000rpm, a suitable balance between high speed and an environment to maintain material integrity, as explained earlier, was selected. Table 4-3 presents an overview of fibres and relationships between their physical characteristics and solutions from which they were produced.

4.4.1 Morphology of fibres

Generally, fibres from the three blends came out well-structured and of high quality. The production conditions adapted from the preliminary investigations with PEO and CMC were indeed helpful for ensuring good quality fibres were obtained. Various extents of entanglement among collected fibres were observed and these appeared to correlate to their corresponding solution properties, especially viscosity. For instance, among the fibres obtained from the three polymer blends, those from PEO/PAA (least viscosity and surface tension) were the least entangled. These were completely separated and well-aligned, unlike the other batches. Fibres from PEO/Alginate on the other hand exhibited the most entanglement and branching (Table 4-3). PEO/Alginate solution had the highest viscosity and surface tension among all blends. Relationships between fibre size and solution properties are discussed in detail in subsequent section but it is worth noting that different trends in terms of size i.e. absolute measurements and distribution (or dispersity) were seen in fibres from blend of polymers and fibres from PEO only. The role of polymer-polymer interaction at solution stage prior to spinning on the outcome of nanofibres are discussed subsequently.

A strong correlation with R^2 above 0.9 was seen between solution properties and fibre size. As seen in Figures 4-4 and 4-5, viscosity and surface tension increased in the order PEO/PAA > PEO/CMC > PEO/Alginate. The exact reverse is seen in the size distribution of fibres produced, thus establishing the order PEO/PAA < PEO/CMC < PEO/Alginate. The inverse relationship observed in the preliminary blends with PEO and CMC also holds true for these blends. The conventional observation in force spinning has been lower viscosities and surface tension usually resulting in fibres with smaller diameters and vice versa (Mahalingam and Edirisinghe, 2013, Padron et al., 2013).

Table 4-3: Overview of nanofibers and relationships between production conditions and outcome.

SEM image				
Material composition	PEO only	PEO/Alginate	PEO/PAA	PEO/CMC
Solution properties Viscosity x10^3 (mPa s) Surface tension (mN/m)	2.2 55	6.8 81	4.5 56	5.2 69
Size distribution (nm) (Mean ± SD)	172	176	217	194
Polydispersity (%)	12	5	2	12
Appearance	Fairly aligned but few attached to each other. Fibres appear thinnest among all batches	Slightly entangled and somewhat attached to each other, most likely due to the high PEO/alginate viscosity	Well aligned and separated from each other. Most fibres sampled closely related in size, hence the low polydispersity	Aligned and appearing mostly as individual fibres. Some minimal entanglement but generally well-structured fibres.

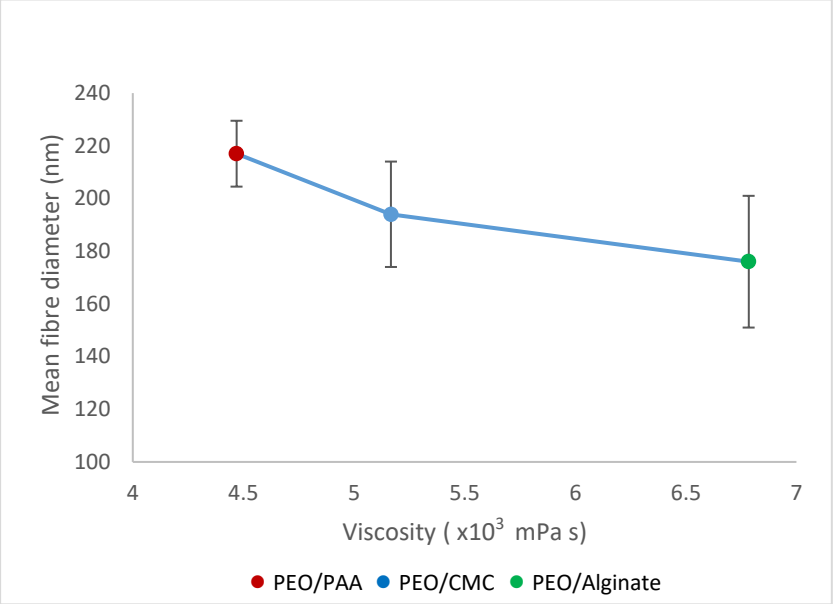


Figure 4-4: Correlation (R^2 = 0.91) between blends viscosities and size distribution of fibres by their mean diameters. Fibres are produced from blends incorporating 75ml of 15 wt.% w/w of PEO and 25ml of b) 5%w/w Alginate c) 5%w/w polyacrylic acid and d) 4%w/w carboxymethyl cellulose for any given 100ml solution

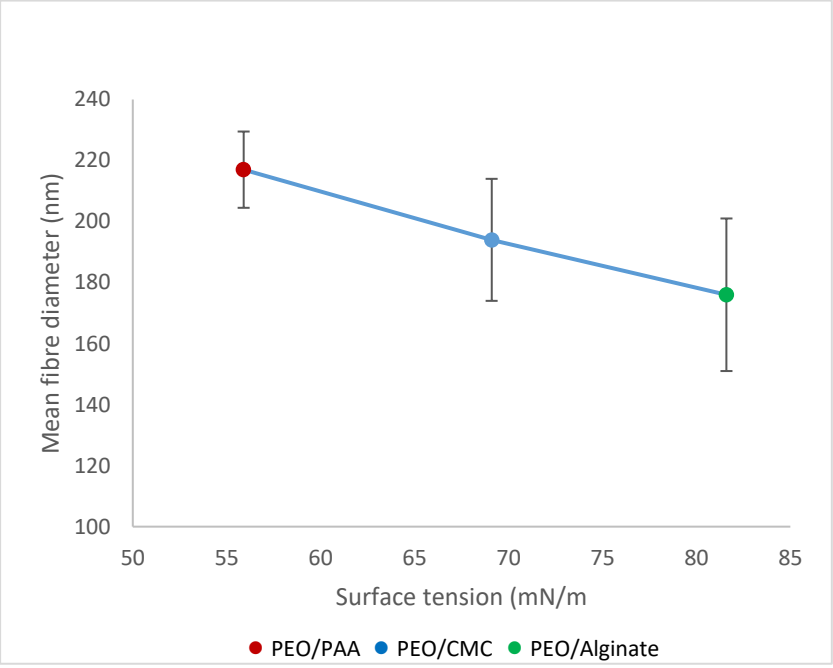


Figure 4-5: Correlation (R^2 = 0.99) between blends surface tension and size distribution of fibres by their mean diameters. Fibres are produced from blends incorporating 75ml of 15 wt. % w/w of PEO and 25ml of b) 5%w/w Alginate c) 5%w/w polyacrylic acid and d) 4%w/w carboxymethyl cellulose for any given 100 ml of solution

This was confirmed in the batch containing only PEO which had the lowest viscosity and surface tension and the least average fibre diameter among the batches. However, batches from solutions containing different polymers have consistently shown an inverse relationship between viscosity and surface tension on one side and fibre size on the other. The role of molecular interactions between various polymers making up the blends and their cascading effects explain these inverse relationships. Rheology of blends have establish, based on molecular interaction that systems containing different polymer constituents might show a positive or negative deviation from expected ideal behaviour of those containing a single polymer (Sionkowska et al., 2004)

4.4.2 Composition of fibres

A combination of DSC, FTIR and variable temperature FTIR were employed to verify the content of batches of nanofibres produced from the blends. Though steps were taken to ensure adequate mixing of various constituents for blend homogeneity, it was important to confirm constituents of a nanofibre produced. Firstly, the extremely high forces from the rotation and pressure applied during fibre formation could result in a phase separation, especially in the absence of appreciable molecular interaction between the separate polymers in solution. Experimental and mathematical models have been used to describe the fate of polymers blends, including possible separation under certain stress conditions (Zhang et al., 2001)

4.4.2.1 Differential scanning calorimetry

DSC investigations were conducted to identify differences in energy changes and melting points. Theoretically, different compositions making up the various blends would be expected to give different profiles in response to heat. However, the complexity of polysaccharides structures with associated molecular dynamics

presented a limitation to the extent of analysis by DSC. Besides the complexity, the issue of wide disparities in degradation temperatures of some constituents in blend meant that the degraded residual from a part of the blend possibly contaminated other constituents yet to undergo state transitions.

Notwithstanding these challenges, DSC was used, in addition to other analytical procedures to confirm the crystallinity state of progesterone, following their loading into fibers. This is reported in section 5.5.3.2.

4.4.2.2 FTIR

Variable temperature FTIR and conventional FTIR were also used to confirm the presence or otherwise of different polymers in fibres produced. The rationale for FTIR was to compare spectra for any peak shift usually associated with molecular interactions among polymers. Hydrogen bonding and complexation, for instance may occur when polymer blends are formed, resulting in changes in bond energies and frequencies of valence and deformation vibrations (Caykara, Demirci et al. 2005). These changes detectable on the FTIR spectra are also helpful in confirming the presence of polymers expected in the nanofibres.

FTIR spectra of PEO only and blends containing PEO, as expected gave profiles similar to those of pure PEO. This is because significant proportions of all blends are PEO. A peak at 843 cm-1 indicating a C-O-O bending is typical for PEO. Furthermore, spectra of the various blends showed peaks typical of constituent materials other than the PEO. For instance the blend containing alginate showed additional peak around 1613 cm-1 indicative of asymmetrical –COO. This is found in pure alginate spectra but clearly absent from that of PEO and this gives a hint of the presence of alginate in the fibre.

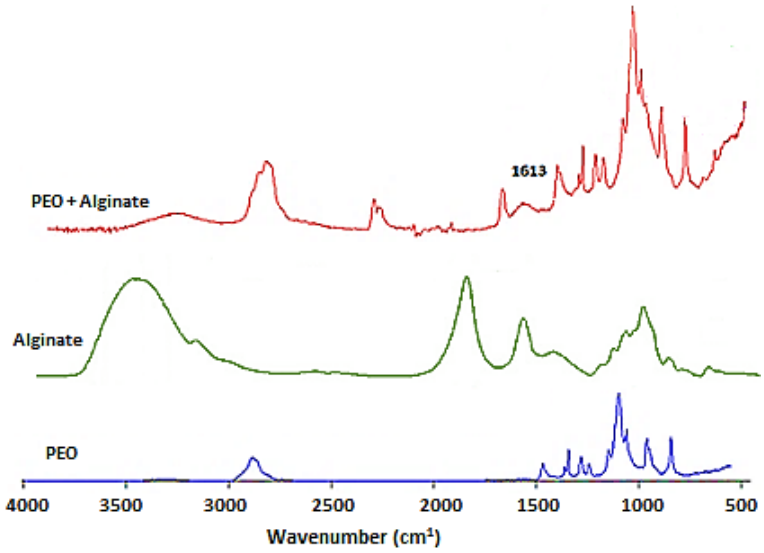


Figure 4-6: FTIR spectra of PEO and Alginate polymers and how they compare with PEO+Alginate nanofibres

Fibres from PEO-PAA blends also gave spectra indicating the presence of both polymers and the interactions that occurred during the formation of the blends. A carbonyl peak occurring approximately around 1702 cm-1 is typical for pure PAA compounds. This is confirmed in the spectra shown in Figure 4-7. The spectrum for the PEO-PAA blend also shows this carbonyl peak, though with diminished intensity.

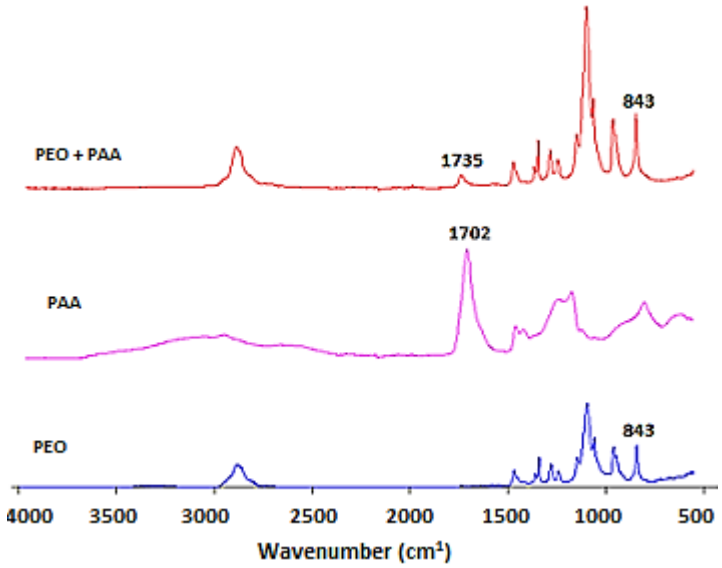


Figure 4-7: FTIR spectra of PEO and PAA polymers and how they compare with PEO+PAA nanofibres

The intensity of the peak reflects the relatively small proportion of PAA in the blend. There is a slight shift in peak, specifically at 1735 cm-1 confirming a complex formation. This shift has been explained by disruption of intramolecular hydrogen bonding in PAA that must occur to make way for interaction with the PEO, thus a confirmation of some self-associated PAA-PAA hydrogen bonds being replaced by PAA-PEO hydrogen bonds (Alkan et al., 2012).

Finally, determining the presence or otherwise of CMC in the PEO-CMC blends begun by assessing the spectrum of pure CMC compound. A strong peak around 1600 cm^{-1} indicative of a carboxylate ion, -COO is usually characteristic of carboxymethyl cellulose. In the PEO-CMC blend, all characteristic peaks of PEO are seen and in addition, a significant, though diminished peak representative of the carboxylate in CMC.

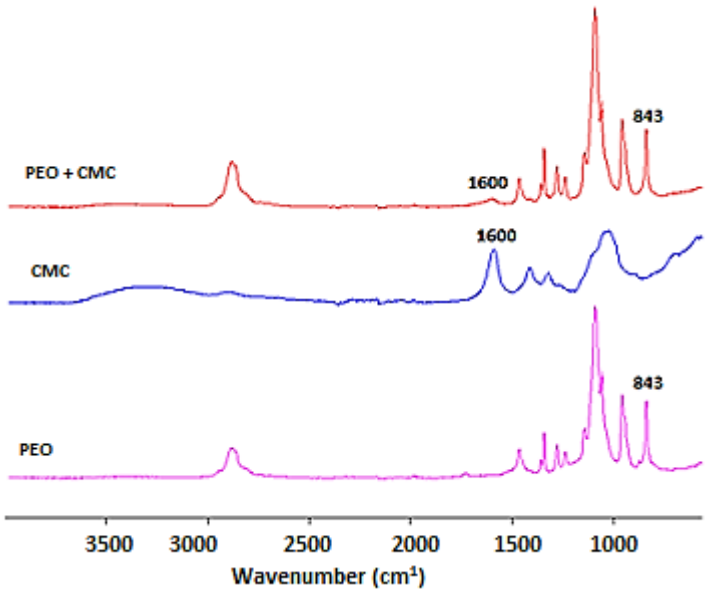


Figure 4-8: FTIR spectra from PEO, CMC and PEO+CMC nanofibres

This confirms the presence of CMC in the blend, with the reduction in peak intensity likely to be due to some interaction with the carboxylate and ether groups from the PEO to ensure miscibility during the blend formation.

4.4.2.3 FTIR at elevated temperature

A variable temperature FTIR was carried out to examine if the batches of fibres reacted differently to exposure heat. The spectra are shown in Figure 4-9. The blends largely containing PEO were scanned at 80°C, well above the melting point of PEO. It was observed that the characteristic peak at 843 cm-1 indicating a C-O-O bending for PEO was gradually diminishing with increasing temperature. A more interesting observation was the emergence of new peaks around 663 cm^{-1} for all fibres made up of the polymer blends. There was no significant peak in this region for the fibres made up of PEO alone. This observation also confirms the presence of additional material in the fibres from polymer blends.

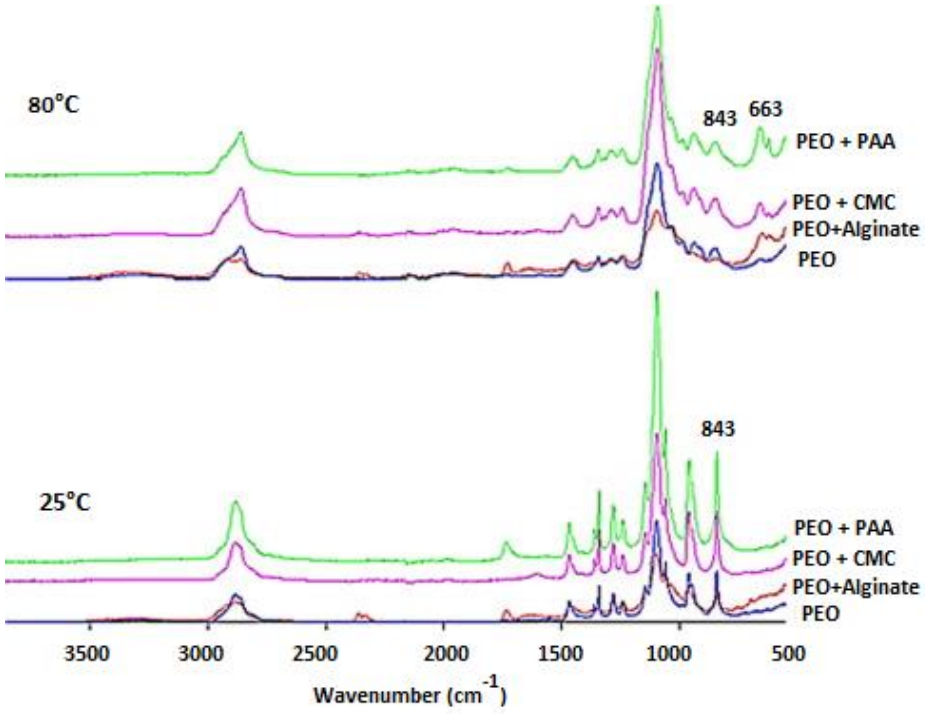


Figure 4-9: Elevated temperature (80°C) FTIR spectra of fibres from PEO and blends containing Alginate, CMC and PEO.

A peak in this region could signify the presence of several functional groups, notably alkyl-halides, specifically C-Br groups, thioether C-S stretch and alkyne C-H groups

(Coates 2000). Further analysis may be required to identify these new peaks seen upon exposure to heat beyond the melting point of PEO but the objective of a variable temperature of FTIR was basically to find out if the fibres from the blends reacted differently compared to those from only PEO in the presence of heat.

4.4.3 Mucoadhesive study

Measuring the force required to detach preparations of polymer from mucosa surfaces or environments mimicking mucosa has often been considered useful in predicting the extent of mucoadhesion capabilities of the materials (Lehr et al., 1992, Eouani et al., 2001). A relationship between viscosities or gel strengths of polymer-mucin systems have also been found to correlate strongly with mucoadhesion properties of polymers involved (Marschütz and Bernkop-Schnürch, 2002, Caramella et al., 1994, Tamburic and Craig, 1997). All these are useful for measuring such quantitative values for predicting the mucoadhesive potential of polymeric systems. Mucoadhesive studies till date, however remains an area with widely differing reports on the same materials by different research groups. The need for assessing each case within a context of methodology, experimental conditions and individual interpretation of results is worth noting (Grabovac et al., 2005, Hägerström and Edsman, 2003).

An approach based on measuring the force corresponding to the gel strength of polymer/mucin system in a simulated vaginal fluid condition was used to investigate adhesion capabilities of nanofiber samples. This force, according to theories cited, is a quantitative function of the extent and strength of interaction between the polymeric structures and mucosa surface. Baseline studies analysing the interactions between mucin and polymer powders in similar proportions as those used in blends for producing fibres were carried out.

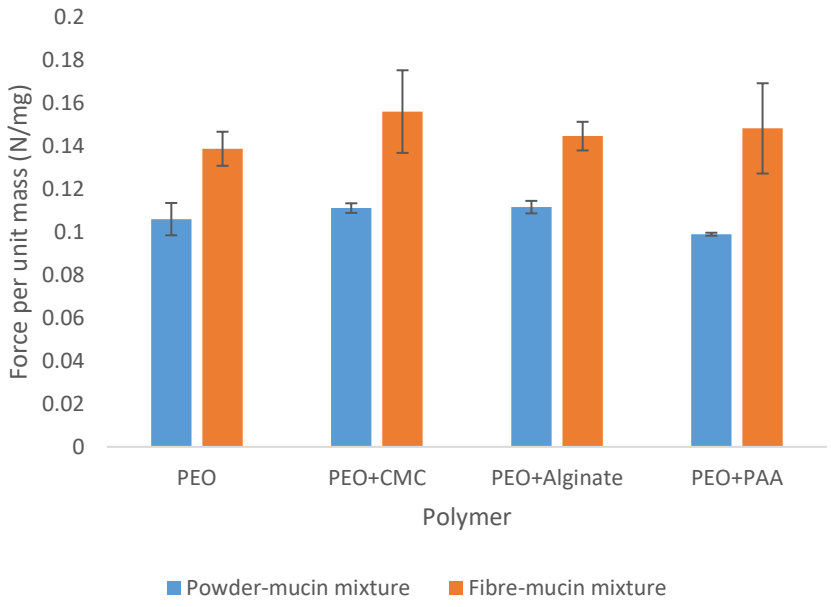


Figure 4-10: The effect of transformation from polymer powders to fibres on potential mucoadhesive properties. Higher adhesion capabilities were measured in fibre systems from all batches. Fibres were produced from blends incorporating 75 ml of 15 wt. % w/w of PEO and 25 ml of 5% w/w alginate, 5% wt. polyacrylic acid and 4% wt. carboxymethyl cellulose for any given 100 ml of solution.

The forces measured were compared to another set where the powdered mixtures were replaced with fibres. The aim was to see if transforming materials into fibre structures improved their mucoadhesive prospects.

As seen in Figure 4-10, an increase in mucoadhesive potential was observed in all fibre/mucin systems confirming better adhesive properties after transforming the powders into fibres. Furthermore, a paired sample t-test returned a p-value of 0.001 confirming a statistically significant difference in mucoadhesion averages of the powders are their respective fibres at 0.05 (95% confidence) level. A significant increase in surface area of the fibres, providing more sites for interaction with the mucin must have contributed to the increase in forces behind the gel strength.

Specifically, the mucoadhesive potential for the polymer powders occurred in the order PEO/Alginate > PEO/CMC > PEO> PEO/PAA implying that Alginate and CMC in blends with PEO in the powder forms are more likely to offer strong contact by adhesion on mucosa surfaces. Unlike the trend seen with the powder mixtures, fibres with PEO alone showed the weakest interaction with mucin while

the remaining batches made of blends demonstrated higher mucoadhesive potential thus confirming the value of producing fibres from blends of different polymers.

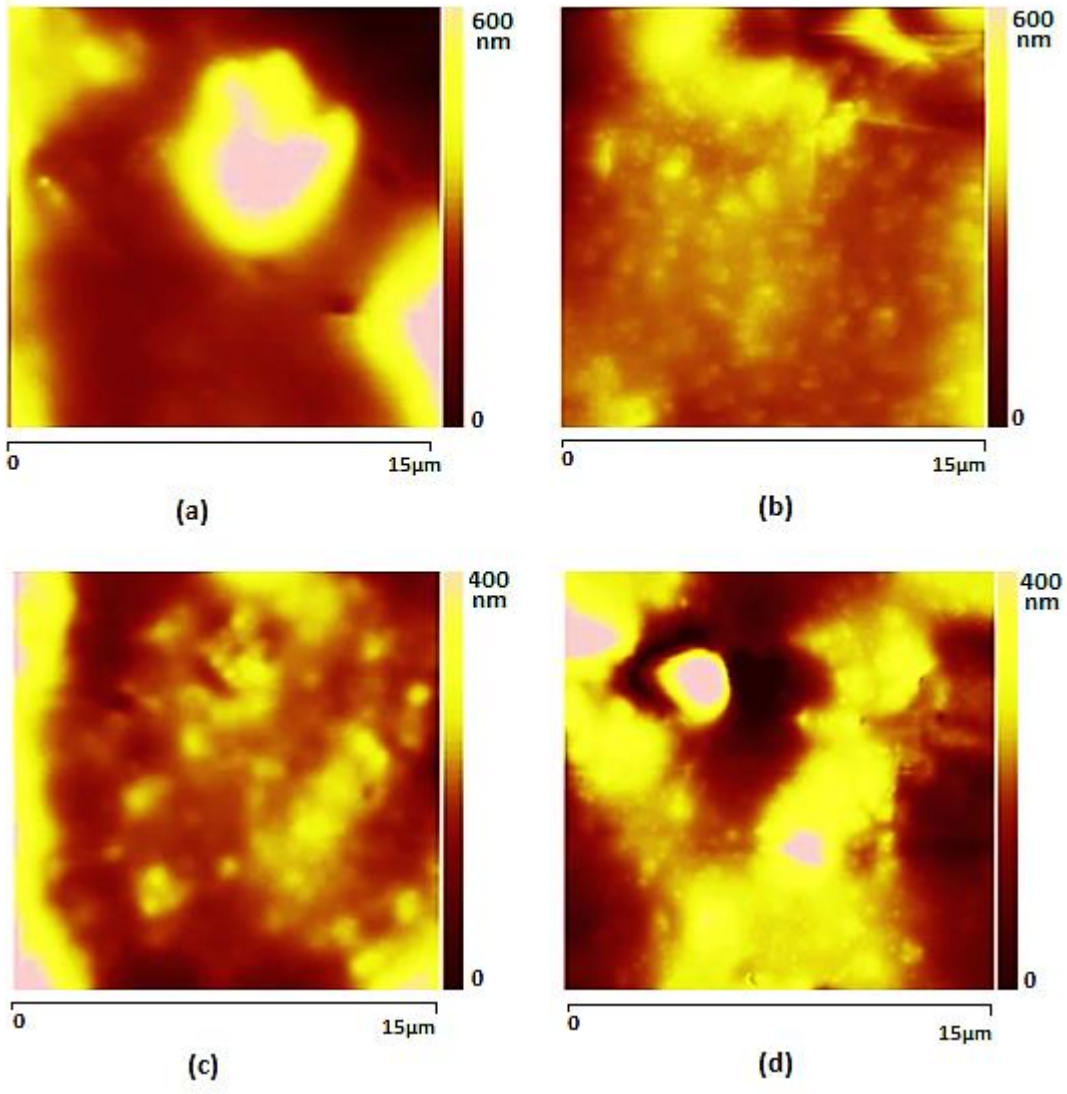


Figure 4-11: (a–d) Atomic force micrographs (height images) from residual films resulting from fibres/mucin mixtures in simulated vaginal fluid. Fibres produced are from blends incorporating 75 ml of 15 wt. % w/w of PEO and 25 ml of (b) 5% w/w alginate (c) 5% w/w polyacrylic acid and d (d) 4% w/w carboxymethyl cellulose for any given 100 ml of solution.

A number of characteristics, including polymer chain entanglement often relating to molecular weight (Thirawong et al., 2007) and net charge distribution, whether cationic, neutral or anionic (Khutoryanskiy, 2011) have often been used to explain

the mucoadhesive behaviour of polymers. However, none of these characteristics appear in certain terms to explain the trend seen considering the fact that all three polymers mixed with PEO to form blends are anionic with average molecular weight in the order PAA > CMC > Alginate, quite different from the trend CMC > PAA > Alginate seen in Figure 4-10. A possible intervention of a carboxylic group in mucoadhesion through hydrogen bonding may be helpful for explaining the trend seen from the mucoadhesive studies (Park and Robinson, 1987) since all three polymers in question yield different amounts of carboxylic acid groups. The blend containing carboxymethyl cellulose appear to offer the best mucoadhesive prospects throughout the study.

Several theories have been used to explain how mucoadhesion occurs. Two of these theories, the wetting theory including hydration also largely considered a prerequisite for facilitating hydrogen bonding for molecular interaction and the diffusion theory where interpenetration of polymer chains across an adhesive interface (Smart, 2005, Leung and Robinson, 1990) must have occurred prior to gel formation between the fibres and mucin. Dried residues resulting from fibre/mucin mixtures were analysed for surface roughness to determine the extent of dissolution of the fibres within the simulated vaginal environment to determine if any correlation exist between this and mucoadhesion. Extent of dissolution of fibres should offer some helpful insights of the level of hydration occurring prior to mucoadhesion.

Typically, if complete dissolution of fibres occurred, then the residual film upon drying should be smooth, hence an analysis of the surface roughness. The sum of all areas on the height images in Figure 4-11 above 300nm were calculated with the help of ImageJ software (National Institute of Health, USA) and taken to be a function of roughness of the film surfaces. Out of a total image area 225μm^2, PEO/Alginate, PEO, PEO/CMC, PEO/PAA had 133 μm^2, 114 μm^2, 104 μm^2 and 83 μm^2 respectively being above 300nm. This observation correlates with the mucoadhesion measurements carried out earlier where PEO and PEO/Alginate

shown to have left a rougher residual film recorded lower forces of adhesion while blends containing CMC and PAA which yielded a smoother residual film demonstrated higher adhesive forces. It's likely the extent of dissolution of the fibres in the mucin - simulated vaginal fluid mixture may actually have played a role in hydration prior to mucoadhesion and hence the trend seen.

Chapter 5

Producing progesterone-loaded mucoadhesive nanofibres

5.1 Introduction

The strategy adopted for the design of a vaginal delivery system from nanofibres is to first incorporate the model drug, progesterone in the polymeric fibres before using these drug-loaded nanofibres as the starting material for developing appropriate dosage forms. The first challenge in the drug loading process was getting a uniform solution from the active drug and polymer. Progesterone is insoluble in water but soluble in ethanol while the polymers used are soluble in water but only sparingly soluble in ethanol. Therefore, a suitable solvent system and methods capable of keeping all these materials in a uniform solution to allow generation of good quality drug-loaded nanofibres were investigated. Solutions of progesterone and the polymers containing different proportions of water and alcohols were prepared and spun into fibres. The solvent system whose solution yielded fibres with highest drug loading was selected and subsequently used throughout the study. Based on results from study reported in the previous chapter, PEO and CMC were chosen to be loaded with progesterone.

In optimising the drug loading process, various parameters such as solvent systems composition, progesterone particle size and solution preparation methods were varied and investigated to determine their influence on outcome of fibres generated. The physical attributes of fibres produced, mainly their morphology and size were investigated. Relationships between fibre outcomes and their respective processing conditions were also established.

Progesterone is known to exist in two forms, α and β crystal types (Payne et al., 1999). It has been established that the α crystal type, with a higher melting point at 129°C is thermodynamically more stable than the β type with melting point at 121°C though

very little conformational and no pharmacological differences exist between the two molecules (Muramatsu et al., 1979) .The ease with which inter-conversion occur between the crystal types of progesterone (Payne et al., 1999) means that an understanding of the effect of processing on the crystal behaviour of progesterone in the encapsulating material could be helpful in predicting such properties as stability and release profile of the drug in its delivery system. On this account, a combination of spectroscopic techniques such as X-ray diffraction and thermal analyses including DSC were employed to investigate the fate of the loaded model drug, in addition to the routine characterisation of the physical properties of the fibres produced.

5.2 Optimising drug loading

5.2.1 Solvent system

To generate nanofibres by pressurised gyration, materials will usually have to be in the liquid state. Progesterone is soluble in ethanol (up to 100 mg/mL) and insoluble in water (< 1mg/mL at 19 ºC) while PEO and CMC are insoluble in ethanol but soluble in water. To obtain a suitable liquid mixture of materials to allow for fibre generation, a series of ethanol and water mixtures were trialled to determine the ratio that allowed the highest possible drug loading. Specifically, solvent systems of ethanol and water containing 10, 20, 30, 40, and 50% of ethanol were used to prepare drug-polymer mixtures (15% wt. PEO and 1% wt. Progesterone) from which nanofibres were generated and assessed for drug content. As seen in Table 5-1, amount of drug loaded in fibre was directly proportional to that of ethanol. The solvent system containing highest amount of ethanol possibly allowed more of the progesterone to be reduced to size small enough to be incorporated into the fibres while being formed. The solvent system containing 50% ethanol resulted in fibres with considerably higher drug loading than the rest. While a correlation between drug loading and ethanol content in solvent system was established, mixtures containing more than 50% of ethanol could not adequately dissolve the polymer. A mixture of alcohol and water in

equal parts was therefore considered to make an ideal solvent system for efficient drug loading when generating nanofibres from PEO and progesterone.

Table 5-1: Percentage drug loading in fibres produced from different solvent systems

Sample	Solvent system		Drug content in fibre
	Ethanol (%)	Water (%)	(%)
1	10	90	0.1
2	20	80	0.4
3	30	70	0.6
4	40	60	1.0
5	50	50	1.5

Following the observation made from a 1:1 ethanol-water mixture, a series of simple primary alcohols, up to butanol were used in a similar manner to determine if any offered a better drug loading in the fibres.

Methanol was excluded due to its toxicity (Tephly, 1991). Solution made from the various water-alcohol solvent systems yielded fibres containing appreciable amounts of progesterone, though not as much as those previously recorded in the water-ethanol batches. Therefore a 1:1 mixture of ethanol and water was chosen subsequently as solvent system for producing drug-loaded nanofibres used throughout the study.

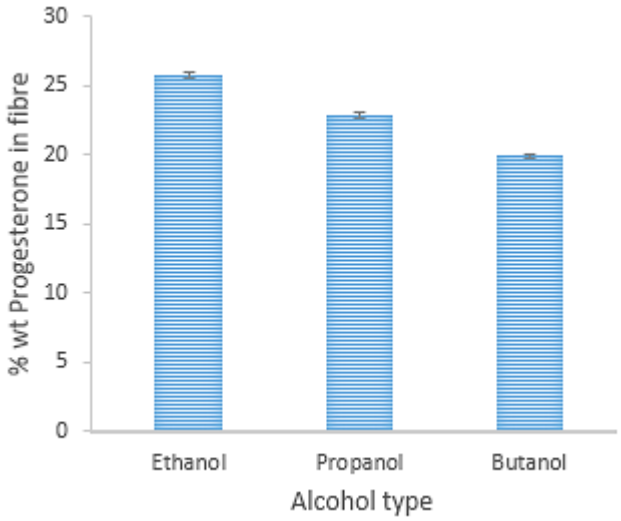


Figure 5-1: Progesterone content in fibres produced using different alcohols in liquid preparation

In investigating an ideal solvent system for solutions from which progesterone loaded nanofibres could be made, it was found that ethanol and water in equal parts offered the best prospects for loading the active drug into these structures.

5.2.2 Drug particle size

Progesterone of average particle size 100µm (A) was initially considered for producing drug-loaded fibres used in this work. During solution preparation, it was observed that any amount of progesterone beyond 1% wt. did not increase amount of drug loaded into fibres, possibly because the particle sizes averaging 100µm could not be reduced appreciably to a level where they could be incorporated into nanofibres forming. An inspection of the inner wall of the gyration vessel after each round of spinning revealed separated progesterone that could not be incorporated into the fibres during formation. Following this observation (a schematic illustration in Figure 5-4), it was hypothesised that a higher drug loading could be achieved with a batch of progesterone with smaller particle size as this could be reduced easier to a level which allows for their inclusion into fibres as they are being formed.

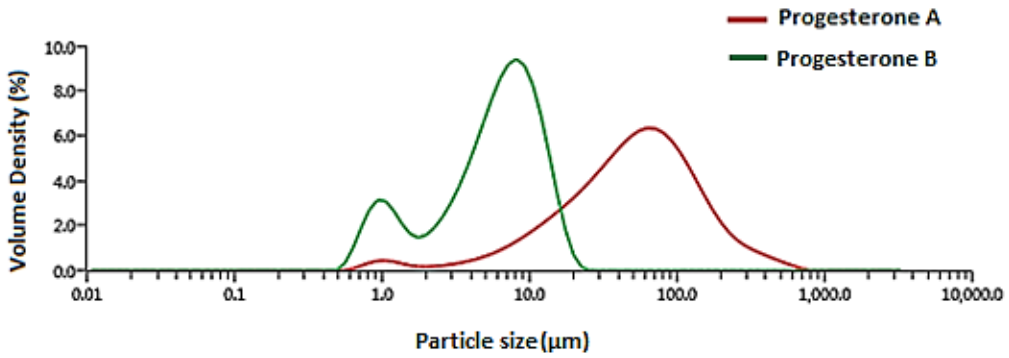


Figure 5-2: Size distribution curves from laser diffraction spectroscopy showing difference in particle sizes of Progesterone A and B

Therefore, another batch of progesterone with average particle size of 10µm (B) was used and compared with the former to determine which batch yielded fibre with higher drug content.

Drug-polymer mixtures from this batch of progesterone (B, with average particle size of 10), shown in Table 5-2 exhibited better prospects of drug loading, according to results from UV spec analysis.

Table 5-2: Viscosity and surface tension of drug-polymer mixtures used in generating fibres

Fibre composition (wt. %)			Viscosity	Surface tension
Polymer		Drug		
PEO	CMC	Progesterone	(mPa s) ± SD	(mNm − 1) ± SD
13.5	1.25	1	5284 ± 9	53.5 ± 0.5
15	0	1	4065 ± 3	47.8 ± 0.2
13.5	1.25	5	8220 ± 30	55.7 ± 0.4
15	0	5	7593 ± 13	54.0 ± 0.2

An inspection of the inner wall of the gyration vessel after spinning fibre with this batch of progesterone also indicated some amount of progesterone particles remaining, though much less than those of the former batch. Conclusion drawn from examining

the vessels after fibre formation is that the progesterone in the drug-polymer mixture remained suspended rather than completely dissolved.

As stated earlier, solution with more than 1% wt. Progesterone batch A could not yield fibres with higher drug content. Therefore, no data was available for 5% wt. Progesterone A as fibre formation was impossible at this concentration. Solutions with up to 5% wt. Progesterone B however yielded fibres with commensurate amount of drug within. Amounts of drug loaded in fibres generated from solutions with different amounts of Progesterone batches A and B are summarised in Table 5-2.

Table 5-3: Extent of drug loading in nanofibres produced using either PEO or PEO/CMC blend as polymer carrier and Progesterone A or B as the active drug.

	Progesterone content in nanofibre (%) ± SD		
Polymer constituent	1% Progesterone A solution	1% Progesterone B solution	5% Progesterone B solution
PEO Only	1.67 ± 0.02	7.67 ± 0.23	25.67 ± 0.21
PEO/CMC	1.42 ± 0.07	7.14 ± 0.01	25.20 ± 0.12

The effects of both initial drug loading and polymer composition on loading efficiency were examined. The solvent system chosen for this study allowed initial drug loading up to 5 wt. % of Progesterone B (average particle size, 10µm), beyond which fibres could not be formed. Figure 5-3 indicates that a 5% and 1% initial drug loading resulted in fibres with 25.7% and 7.7% by weight progesterone in PEO batches. A similar trend was observed in batches from PEO/CMC blends.

Entrapment efficiencies occurring in drug-loaded systems shown in Table 5-4 were calculated based on dry weight ratios of drug contents in fibres (final) to drug content in mixtures (initial) from which fibres were generated. For example, initial drug content

in a mixture containing 15% wt. PEO and 1% wt. Progesterone, based on dry weights, was calculated to be 6.3% ($1 \div 16 \times 100$) The results are shown in Table 5-3.

Table 5-4: Progesterone loading in fibres, calculated based on dry weights of final and initial drug loadings

Polymer	Progesterone content (%)		Loaded progesterone in fibre relative to dry powder (final/initial) %
	Initial drug loading	Final (in fibre)	
PEO only	6.3	7.7	122
PEO/CMC	6.3	7.1	113
PEO only	25.0	25.7	102
PEO/CMC	25.0	25.2	100

As seen in Table 5-3, more efficient drug loading occurred in batches with 1% initial drug loading (6.3% based on dry weights). The effect of drug concentration on encapsulation efficiency (Wang et al., 2004; Yang et al., 2013) is often associated with solubility of drug prior to encapsulation; here we see a greater efficiency with those polymer systems in which initial solubility is higher i.e. batches containing 1% initial drug loading.

Another observation was that polymer composition did not appear to affect drug loading even when the amount of drug in the starting mixture was varied. As seen in Figure 5-3, PEO only or PEO/CMC blends recorded similar drug loading efficiencies irrespective of the initial drug loading. This is a positive outcome as one can expect to vary polymer composition within reasonable limits in order to achieve some desirable properties such as mucoadhesion without compromising drug loading significantly.

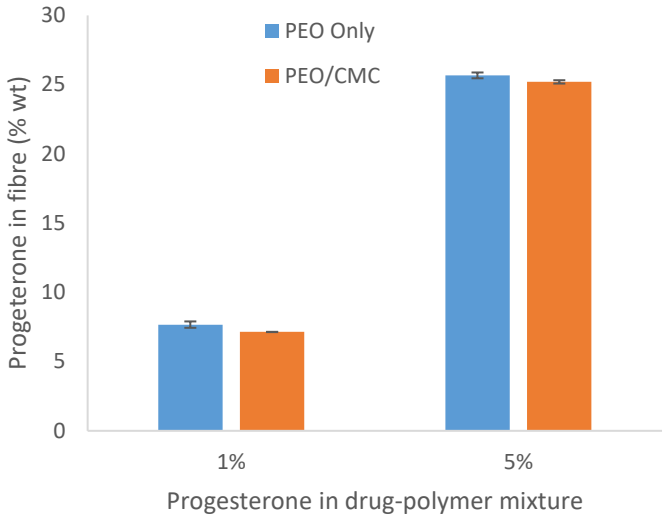


Figure 5-3: Drug-loading in fibres produced from progesterone and PEO or PEO/CMC mixtures

5.2.2.1 Theoretical basis for influence of drug particle size

As seen in Table 5-3, solutions containing 1% of Progesterone batch A yielded fibres with drug content about five times less than those generated with similar concentration of Progesterone batch B. Furthermore, drug-polymer mixtures containing as much as 5% of the progesterone with smaller particle size was stable and uniform enough to allow fibre generation yielding nanofibres with up to 25% drug content. It is therefore clear that drug particle size affects outcome of fibre generation and hence a suitable drug particle size will be required for generating fibres with appreciable amount of drug loading.

Progesterone embedded in nanofibres were found to be of much smaller particle size than those used to constitute the drug-polymer mixture (This is discussed further in section 5.5.1). Some level of solvation of drug particle that caused further size reduction possibly must have occurred prior to fibre formation. The difference in drug loading seen between the two batches with different particle size could be explained with the help of the Gibbs-Kelvin equation relating solubility, interfacial energies and particle size (Freundlich and Hatfield, 1926, Buckton and Beezer, 1992) which is:

$$log\frac{S_r}{S_\infty} = \frac{2\gamma V}{2.303RTr}$$

Where S_r is solubility of particle with radius *r* and S_∞ is the solubility of a particle with infinite radius compared to *r*. γ is the interfacial energy and *V, R, T* are the molar volume of the solid, gas constant and absolute temperature respectively.

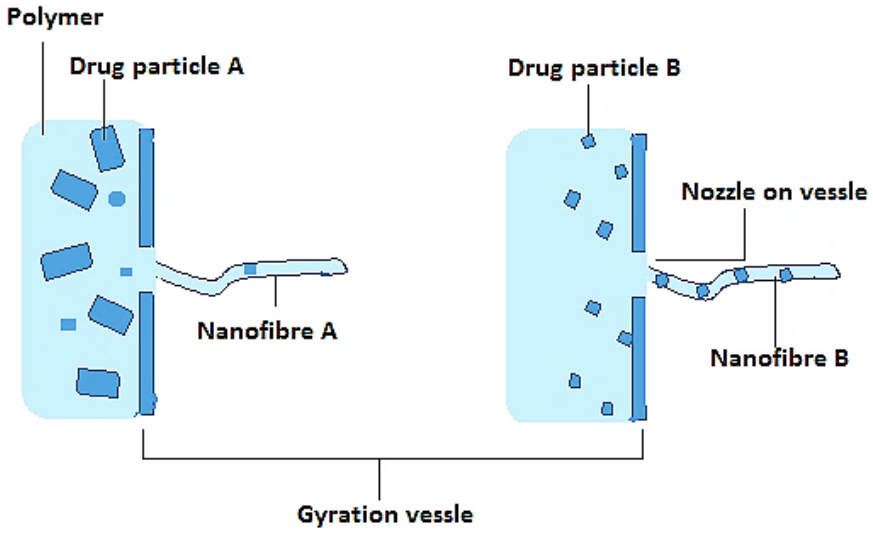


Figure 5-4: A scheme illustrating drug loading during fibre formation. System utilising progesterone A (Drug particle A) has more of its larger particles not incorporated in fibre resulting in less efficient loading while system forming fibres from progesterone B (Drug particle B) allows more drug to be loaded into fibres.

As can be inferred from equation above, the level of solvation occurring is always proportional to a ratio of the interfacial energy and particle size of the solute in question, implying that larger solid particles required higher energy for a particular level of dissolution to occur. As mentioned earlier, complete dissolution of progesterone was not possible in the polymer/drug systems. However, drug particles size reduction must have occurred prior to progesterone being included in fibres before ejection under forces of the pressurised gyration.

A much lower interfacial energy was required to bring appreciable level of solvation to already smaller progesterone batch A particles to a size capable of being embedded in the fibres readily occurred and hence a higher drug loading seen in that batch. On the other hand, much work had to be done to reduce the size of the larger drug particles in batch B to levels capable of being embedded in fibres and therefore a much lower drug loading occurred in fibres generated from mixtures containing large particle size progesterone.

5.2.3 Varying method of solution preparation

Having determined a suitable solvent system and drug particle size to be used in generating the progesterone loaded nanofibres, a different approach to preparing polymer-drug mixture from which fibres would be spun was attempted to investigate the possibility of further increasing the drug content in fibres.

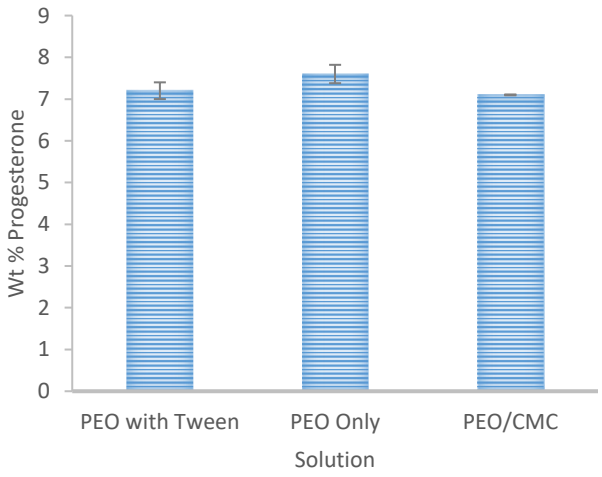


Figure 5-5: Drug loading seen in fibres from 1% Progesterone solution with or without a surfactant

The use of surfactants in improving the aqueous solubility of some drugs is a well-known approach(Liu, 2008). Furosemide (loop diuretic) for instance, is reported to have had its water solubility improved by utilising surfactants including Polysobate-80 (Tween 80) during formulation (Shihab et al., 1979). With both Furosemide and

Progesterone being practically insoluble in water, Tween 80 was selected as surfactant to improve the stability of progesterone in a mixture with PEO or PEO/CMC blend. The solvent this time was water only. Fibres obtained from polymer/drug system utilising a surfactant were compared to those obtained previously using the water-ethanol solvent system.

As seen in Figure 5-5, the solution utilising Tween 80 as a surfactant meant to keep more of the active drug in the aqueous polymer solution and subsequently offer a higher drug loading was not significantly different from other fibres generated from solutions without a surfactant. The drug-loaded fibres being produced are meant to be used in delivery systems deigned to perform by mucoadhesion. Surfactants are known to adversely affect mucoadhesion capabilities of systems in which they occur. (Mortazavi and Moghimi, 2010, Tobyn et al., 1997). On this account, and considering their minimal intervention in drug loading, surfactants were not utilised in drug-loaded fibres produced subsequently.

5.2.4 Summary of drug loading optimisation

Based on outcome from production variables investigated, the set of conditions likely to yield high quality progesterone-loaded nanofibres efficiently are a solvent system made up of equal parts of water and ethanol and use of progesterone presented in smaller particle sizes. The use of surfactants in improving the solubility of progesterone, considering its potential to reduce the systems mucoadhesive capabilities, was not selected. Using these sets of conditions, in addition to keeping all other parameters from previous experiments constant, two sets of drug loaded nanofibres were produced and analysed mainly for their morphology, molecular characteristics and performance as drug delivery constructs i.e. release profiles and mucoadhesion capabilities.

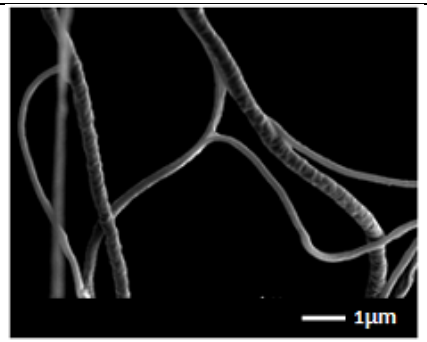
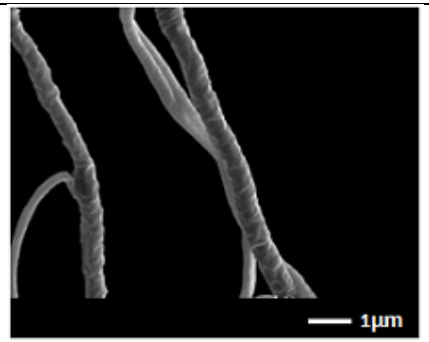
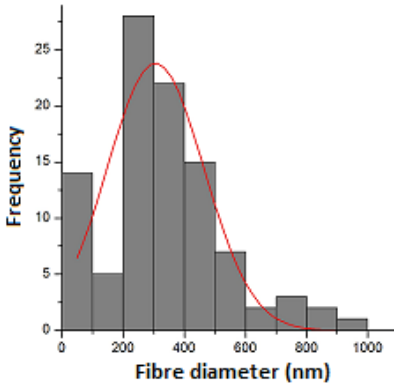
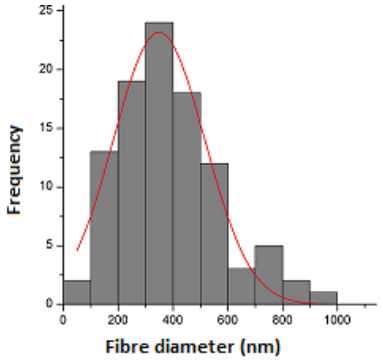
5.3 Fibre morphology and relationship to solution properties

The properties of polymer-drug mixtures used to generate nanofibres appeared to be influenced by such factors as drug particle size, amount of suspended drug and polymer constituent of the mixture. As seen in Table 5-1, different drug loadings in these mixtures resulted in different solution properties i.e. viscosity and surface tension. Mixtures containing 5% progesterone (from which fibres used throughout the study were generated) also had slightly higher viscosities and surface tension than solutions with similar polymer constituent but lower progesterone. In addition, those with CMC were more viscous than those made of only PEO.

Some physical characteristics of progesterone loaded fibres and their respective solution properties are summarised in Table 5-5. Fibres generated from 15% w/w PEO only (lower viscosity and surface tension) had mean diameter of 349nm with polydispersity of 22%. The higher viscosity and surface tension in the PEO/CMC polymer-drug mixture influenced outcome of fibres as expected, resulting in slightly larger nanofibres with mean diameter of 404nm and polydispersity of 15% (Table 5-5).

Polymer liquid preparations with higher viscosity and surface tension yielding nanofibres with larger diameters has been the conventional outcome of fibre formation from centrifugal spinning (Mahalingam and Edirisinghe, 2013, Padron et al., 2013). The improved uniformity seen in the size distribution of fibres generated from PEO/CMC blends may be due to molecular interactions (Sionkowska et al., 2004) between polymers in the blend, prior to fibre formation. The influence of inter-polymer activities on fibres generated from a blend, which has been observed throughout the study points to a possibility of utilising molecular interactions among polymers for specific outcome

Table 5-5: Overview of progesterone-loaded nanofibers and relationships between production conditions and fibre outcome

Polymer composition	15% wt. PEO	13.75% wt. PEO/ 1.25% wt. CMC
Solution properties Viscosity (mPa s) Surface tension (mNm $^{-1}$)	 7593 ± 13 54.0 ± 0.2	 8220 ± 30 55.7 ± 0.4
SEM Image		
Size Distribution		
Mean fibre diameter (nm)	349	404
Polydispersity index (%)	22	15
Comment	Average fibre size slightly smaller than that of PEO/CMC blends. The sizes observed are proportional to viscosity, indicating a relationship between fibre outcome and solution viscoelastic properties.	Fibres slightly larger than those produced from PEO only. The inclusion of CMC, as seen in the size distribution and dispersity offered some uniformity, an observation similar to the trend seen in section 4.4.1.

5.5 The loaded drug

5.5.1 Size

Drug particle size has long been established as crucial for their transport across physiological membranes (Lai et al., 2007, Win and Feng, 2005). The loaded drug particles were isolated by completely dissolving the fibre in water while the active drug remained undissolved, followed by air drying, leaving the undissolved progesterone crystals on the surface of the resulting dry film. Figure 5-6A is an image of progesterone crystals isolated from PEO/CMC fibre.

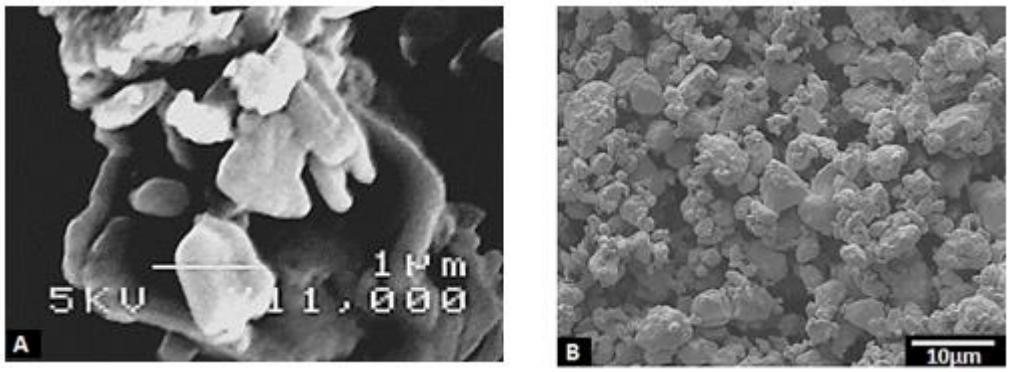


Figure 5-6: Scanning electron micrograph images of A) agglomerated progesterone crystals isolated from PEO/CMC fibre formulated from a mixture containing 5% wt. progesterone and B) Progesterone powder used in liquid mixtures for generating fibres

These were compared to the progesterone powder used in the polymer-drug mixture from which fibres were generated. Whereas the progesterone powder had particles with size up to 10μm, the loaded drug had drug particles typically less than a micron. Presenting the progesterone encapsulated in fibres therefore enables particle size reduction of the active drug, an opportunity that may be utilized for better drug delivery across membranes.

5.5.2 Physical properties

Getting progesterone encapsulated in polymeric fibres mean the drug had to be solvated to an extent within the continuous polymer phase before spinning into fibres.

The solvation process followed by solvent evaporating off after generating the fibres comes with the possibility of the drug crystallinity being altered or remaining in a similar form as they occur in the powder form (Benoit et al., 1986). This study looked into the possible changes in the physical characteristics of progesterone after its incorporation into nanofibres. Before the loaded drug was analysed, it was important to first confirm whether fibre formation using pressurised gyration allowed the drug's inclusion in the nanofibres. Therefore some spectroscopic and thermal analyses were carried out, first to confirm the presence of progesterone in the fibres and then to establish the physical state of the drug as encapsulated in the fibres.

5.5.2.1 Visual confirmation

Hot stage microscopy was used to visually track melting of the nanofibres in order to confirm the presence of active drug within the fibres, and the state in which they occur.

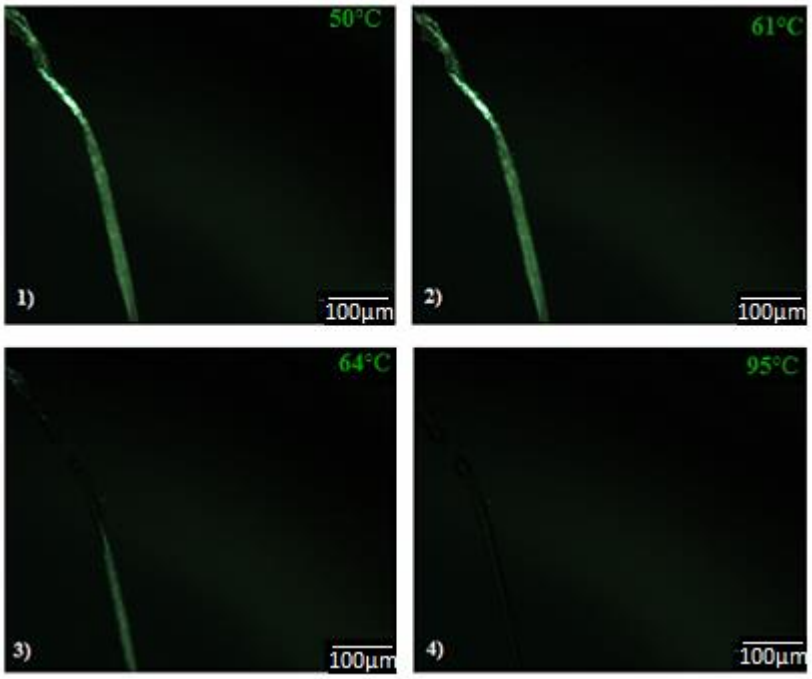


Figure 5-7: Melting of PEO fibre without progesterone (stages 1-4) visualised by hot-stage microscopy. By 67°C (stage 4) the fibre is completely melted but because no progesterone is contained within fibres, no particles are left behind.

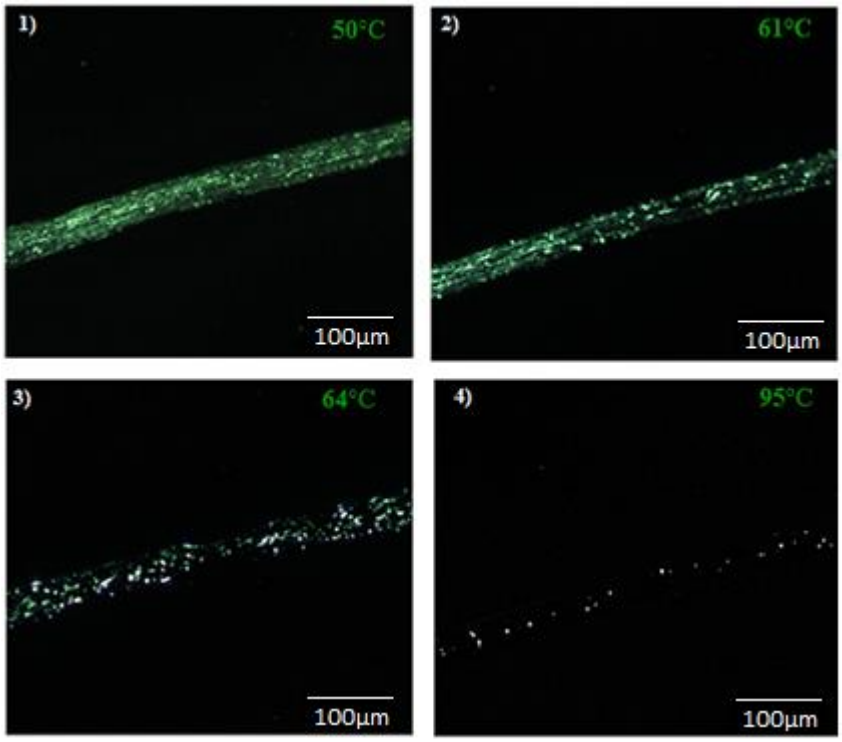


Figure 5-8: Melting of PEO fibres encapsulating progesterone (stages 1-4) visualised by hot-stage microscopy. Towards the melting point of PEO (stage 2), fibre begins to melt and by 95°C, (stage 4) fibre is completely melted leaving behind progesterone which has a higher melting point

The significant difference between the melting points of the polymer and drug (61 and 121°C) offered an opportunity for confirming the presence of progesterone in nanofibres by subjecting samples to temperature beyond the melting point of the PEO but below that of progesterone.

Plain PEO fibres were heated from 50 to 95°C as seen in Figure 5-7 (stages 1-4). From 61°C, the fibre begins to melt and by 95°C, the fibre had melted completely leaving only a trace along the position the fibre laid. This procedure was repeated using nanofibres generated from a liquid mixture containing progesterone. In contrast, Figure 5-8 stage 4 (i.e. 95 °C) shows the presence of birefringent particles most likely to be progesterone crystals as they are visible above the melting point of PEO. Further molecular characterisations were carried out to confirm the residual particles that remained after subjecting the drug-loaded fibre to temperature beyond the melting point of PEO.

5.5.3 Molecular properties

5.5.3.1 FTIR spectra

As PG nanofabrication involves a degree of material hydration, it was necessary to investigate the possibility of progesterone-PEO or progesterone-PEO-CMC complexation. The ATR-FTIR spectra of PEO and progesterone is shown in Figure 5-9. For the progesterone, peaks indicating carbonyl stretching bands at C3 and C20 within the molecule can be seen at 1661 and 1693 cm1 respectively (Benoit et al., 1986).

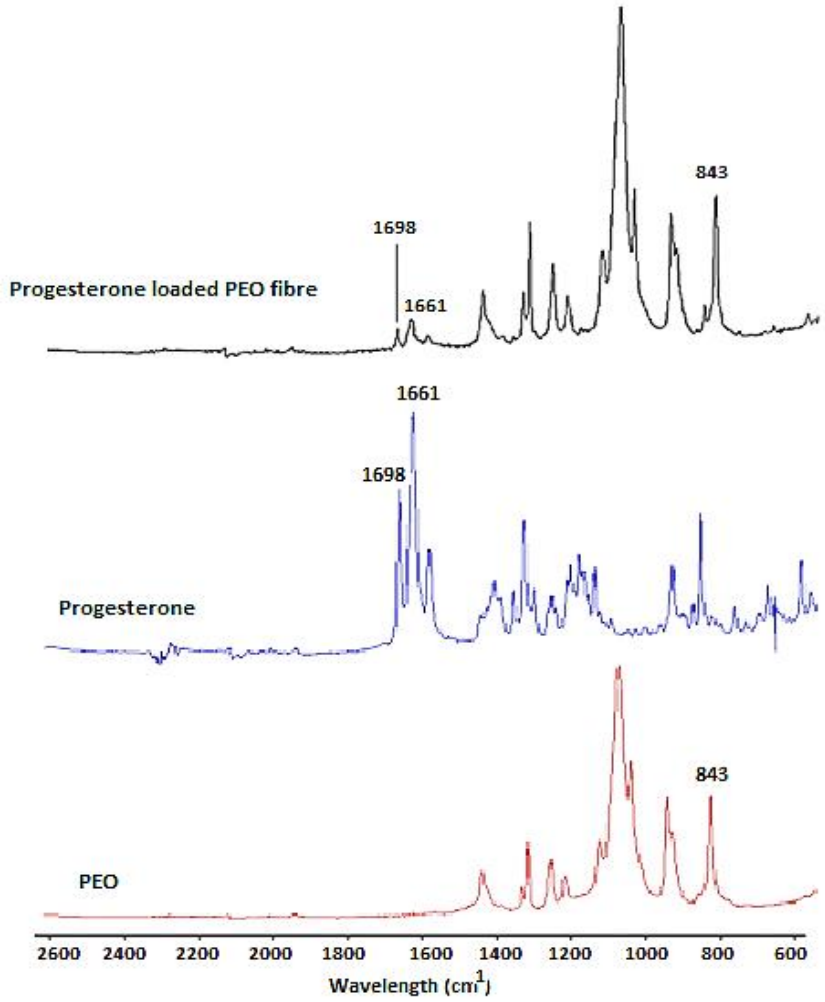


Figure 5-9: ATR-FTIR spectra of PEO and progesterone in the 600-2600 cm-1 region showing characteristic peaks and that of progesterone loaded PEO fibre confirming the presence of progesterone contained in the PEO fibre.

The characteristic peak at 843 cm1 resulting from –CH2–CO rocking/stretching in PEO is also shown (Frech and Huang, 1995, Li and Hsu, 1984). All characteristic peaks for both compounds can be seen in ATR-FTIR spectra of progesterone loaded PEO fibres (Figure 5-9). ATR-FTIR studies did not provide evidence of significant progesterone structural changes occurring during PG nanofabrication.

5.5.3.2 Differential scanning calorimetry trace

After identifying functional groups confirming presence of progesterone in the nanofibre samples by FTIR, DSC was carried out in an attempt to elucidate the structural characteristics i.e. crystallinity or otherwise of the loaded drug.

The melting points of progesterone and PEO used in generating nanofibres were confirmed by DSC. Results obtained, seen in Figure 5-10, were in agreement with observations from previous studies (Beech and Booth, 1970, Payne et al., 1999)

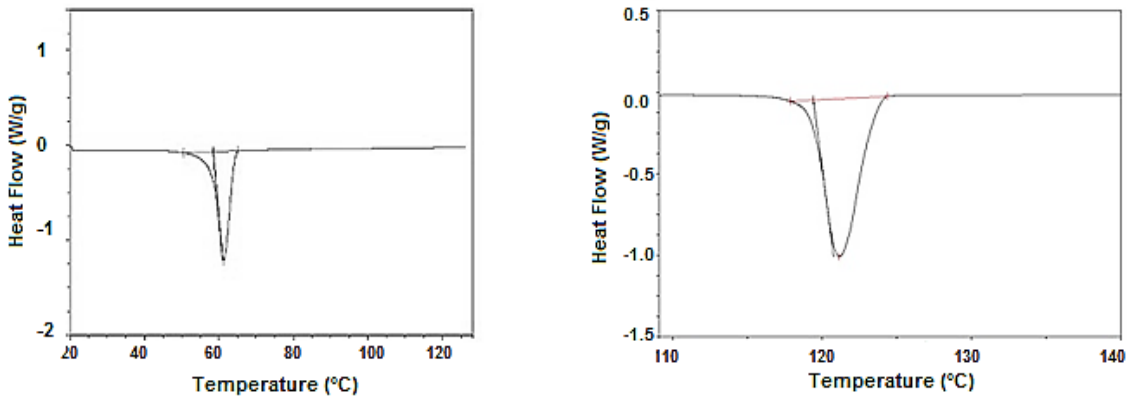


Figure 5-10: Melting point of a) PEO and b) progesterone

Fibres encapsulating progesterone were also analysed under the same experimental conditions. As shown in Figure 5-11, a slight elevation in melting point for PEO and a small melting point depression (approximately 5ºC) for progesterone were observed. The lower temperature is likely to be as a result of the smaller particle size progesterone in the fibres as reduction in particle size, especially towards or within

the nanoscale has been established to lower the melting point of materials, organic compounds included (Jackson and McKenna, 1990). In addition, the presence of polymer compounds acting as impurities must have contributed to the lower than expected melting point for progesterone in the fibres, what is usually considered as melting point depression by impurities (Hock et al., 2008). This peak identified at 114.5°C points to the likelihood of the loaded drug existing in the crystalline form.

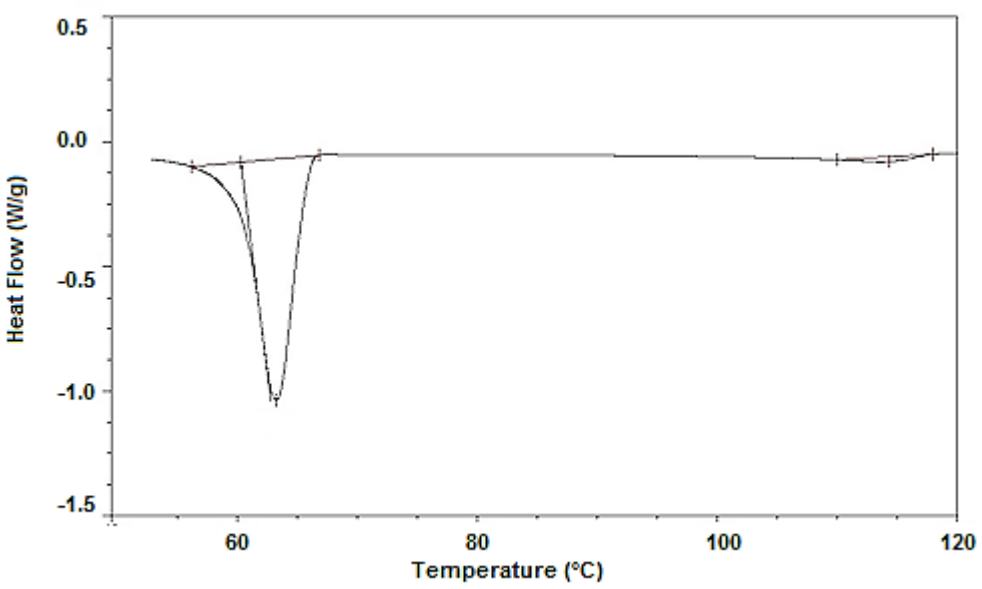


Figure 5-11: A representative DSC trace showing the Total Heat Flow signal of PEO fibre containing 25%w/w progesterone

Further analysis by hot-stage microscopy and XRD were carried out to rule out other possibilities as the melting point temperature discrepancies between the pure progesterone drug in powder form and the loaded drug were significant. Progesterone remained largely crystalline but a possibility of the processing condition converting some to the amorphous state was seen in the X-ray analysis.

5.5.3.3 X-rays powder diffraction patterns

DSC, possibly due to the reduced proportion of progesterone in the fibres yielded minimal information on the physical state of the loaded drug.

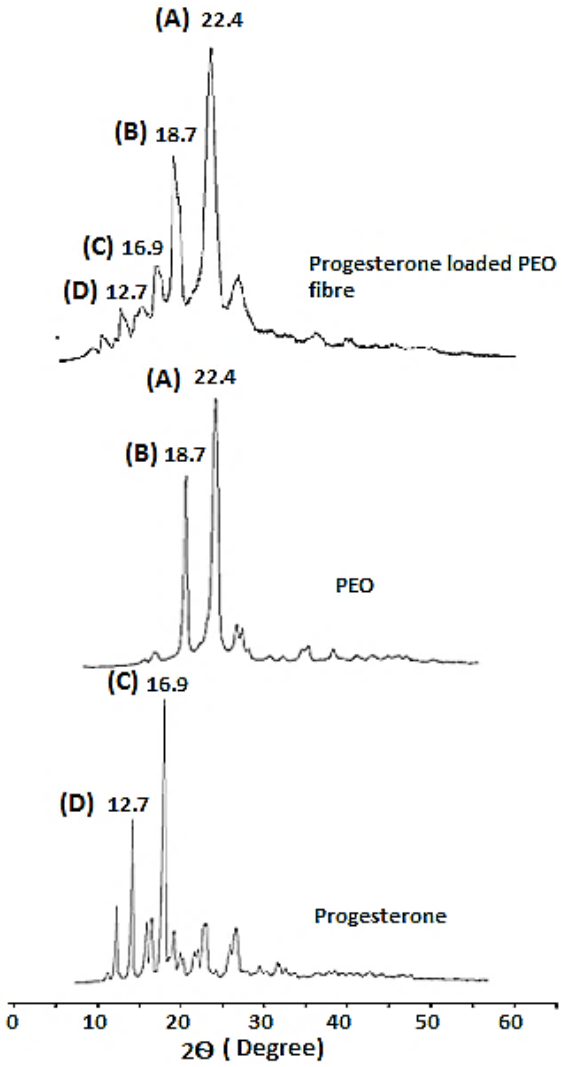


Figure 5-12: X-ray diffractograms of PEO and progesterone showing prominent and characteristic reflection peaks confirming their crystalline nature and progesterone loaded PEO fibre with characteristic peak indicating some of the loaded drug occurring in crystalline form.

Further investigations were conducted utilising the more sensitive X-ray diffraction approach for further and clearer insight into the physicochemical nature of the progesterone following its inclusion in a polymer carrier.

X-ray Powder Diffraction studies (XRPD) were conducted on PEO and progesterone raw materials and progesterone-PEO fibres to confirm the solid state of encapsulated progesterone in the PEO fibres. Several diffraction peaks are seen in both the PEO and progesterone XRPD patterns (Figure 5-12).

However, the most prominent among these confirming their crystallinity are in the region of 22° and 19° (Peaks A and B) for PEO and around 17° and 13° (Peaks C and D) for progesterone, the latter mainly in the α crystal form (Oliveira et al., 2013; Sangawar and Bhagat, 2013).

The progesterone loaded PEO fibre (Figure 5-12) shows all the characteristic peaks (A, B, C, and D) seen in each of the pure samples indicating that the progesterone was indeed encapsulated in the PEO in the predominantly α crystalline form. The halo diffraction pattern seen between 10 and 40 2Ɵ degrees in the drug loaded fibres is characteristic of amorphous material (Jain et al., 2008; Young, 2012). Therefore, the progesterone containing fibre, unlike the pure drug or polymer which appeared to be predominantly crystalline materials, occurs as a mix of amorphous and crystalline systems. The processing conditions, including conversion of the powders into liquid state by solvation and reconverting back into solid through solvent evaporation are likely to have disturbed the lattice arrangements of both materials, thereby resulting in some level of amorphicity in the fibres.

5.6 Performance assessment of progesterone-loaded nanofibres

5.6.1 In-vitro drug release studies

In vitro drug release studies were conducted on progesterone loaded PEO and PEO/CMC fibres and compared (under the same experimental conditions) to that of Cyclogest, a pessary containing progesterone. The experimental approach was to observe progesterone release from nanofibres across an artificial membrane mimicking the mucosa environment of the vagina. This analysis is helpful for

predicting the expected release profile from a drug loaded nanofiber based dosage form. The correlation coefficients from release profiles of nanofibre systems and Cyclogest® when fitted in a zero-order model were 0.95, 0.97 and 0.89 for PEO, PEO/CMC and Cyclogest formulations respectively.

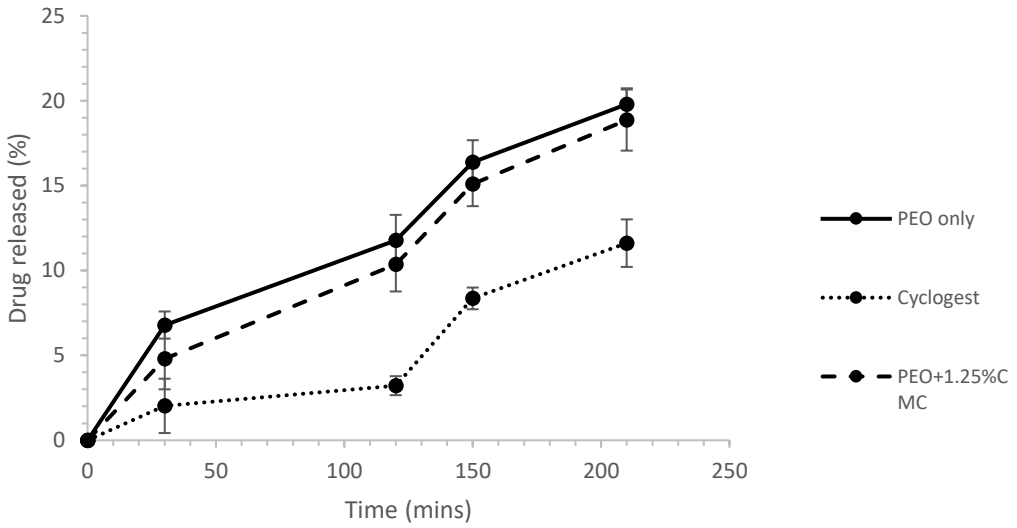


Figure 5-13: Release profiles of progesterone loaded PEO and PEO/CMC fibres and Cyclogest

The possibility of membrane deterioration and pore plugging after an extended period of use (Gekas and Hallström, 1990) was anticipated and hence measurements were performed over a period less than 4 hours. As seen in the release profiles (Figure 5-13), the rapidly dissolving drug-loaded nanofibres in simulated vaginal fluid (SVF) released higher amounts of drug than the Cyclogest during the period of study. Furthermore, it was observed that the inclusion of CMC affected drug release, and more importantly increased its tendency for a zero-order release (R^2 of 0.98, compared to that of Cyclogest which was 0.89 in a zero-order model plot) which is often desirable for delivery systems, as in principle, it provides the best control of plasma concentration levels and hence desirable therapeutic outcomes (Gokhale, 2014). CMC possibly interacting with PEO to modulate drug release has long been established as a useful strategy for achieving desirable release, particularly from

matrices (Palmer et al., 2011). Therefore in addition to improving the mucoadhesive prospects of the nanofibres, as demonstrated in our previous study (Brako et al., 2015), CMC demonstrated its usefulness for modulating release from these nanostructures. The possibility of fine-tuning the characteristics of these progesterone-loaded nanofibres during processing present opportunities for enhancing the functionality of the delivery system to be developed from them.

5.6.2 Mathematical fitting of release data

Data obtained from in vitro release studies may be fitted into mathematical models to help generate further information on systems being analysed. These models, when applied to release data enable clearer insights into underlying release mechanisms, help predict effects of design parameters such as geometry and size of dosage form and performance levels that may be expected from delivery systems (Siepmann and Peppas, 2012).

Table 5-6: Correlations (R^2), release constants (K) and release mechanism (n) returned from fitting release data into various mathematical models.

	Korsemeyer-Peppas			Higuchi		Hixson-Crowell		Zero-order	
	R^2	K	n	R^2	K_H	R^2	K	R^2	K
PEO + CMC	0.98	0.92	0.53	0.95	1.27	0.98	0.010	0.97	0.09
PEO Only	0.99	0.88	0.53	0.98	1.26	0.96	0.010	0.95	0.08
Cyclogest	0.78	0.14	0.40	0.78	0.72	0.89	0.009	0.89	0.05

In this study, progesterone release from nanofibre systems produced and a formulation presently available on market, Cyclogest were fitted into selected models

to gain further insights. In addition to zero order kinetics the models and rationale for selecting them are described briefly below.

5.6.2.1 Korsmeyer-Peppas

This is a simple but effective semi-empirical model relating amount of drug released exponentially to elapsed time (Costa and Sousa Lobo, 2001). A logarithmic plot of release data gives an equation with parts that may be analysed further for additional information. One of such, *n* indicates the drug release mechanism depending on the geometry of system. For instance in a cylindrical matrix, n closer to 0.45 indicates release predominantly driven by Fickian diffusion whereas 0.89 points to release controlled by swelling of the system (Costa and Sousa Lobo, 2001). Values ranging between 0.45 and 0.89 point to release mechanism controlled by diffusion and swelling concurrently. This model works well with polymeric matrices with well-defined solid geometry and such systems will usually return a high correlation following the logarithmic plot. A high correlation (>0.9) between release and time for the progesterone-loaded fibres following this modelling confirms their well-defined geometry (cylindrical) and polymeric character. In addition, an *n* value of 0.53 in both formulations indicate progesterone release driven by swelling and diffusion; a release mechanism expected in mucoadhesive polymeric systems. On the contrary, an *n* value of 0.4 seen in Cyclogest, a fat-based formulation implies a release mechanism not typically defined by the Korsemeyer-Peppas model. A relatively low correlation of 0.78 confirms this deduction.

5.6.2.2 Higuchi

The Higuchi model was developed from several mathematical formulae to describe release of both high and low water soluble drugs from units uniformly dispersed in a matrix (Higuchi, 1963, Higuchi, 1961, Siepmann and Peppas, 2012). In its simplest

form, the Higuchi model relates amount of drug release to the square root of time elapsed with a constant of proportionality known as the Higuchi dissolution constant, K_H. A system showing a high correlation in this model may imply a more uniform dispersion of active drug as this feature is a main assumption upon which the model was developed. A higher correlation seen in the progesterone-loaded fibres implies a more uniform dispersion of progesterone in these systems relative to the Cyclogest. Furthermore, the K_H values in the progesterone-loaded fibres (PEO fibre, 1.26 and PEO/CMC, 1.27) and Cyclogest (0.72) indicates remarkable differences in release between the fibre systems and fat-based Cyclogest suppository. The possibility of a different release kinetic and potential for a more uniform dispersion of progesterone in polymeric systems indicate a potential for developing an alternative vaginal dosage forms from these drug-loaded fibres.

5.6.2.3 Hixson – Crowell

This model, originally developed by observing the relationships among heterogeneous reactions, surface erosion and agitation in a solid-liquid dissolution system (Hixson and Crowell, 1931) is now useful for describing how surfaces erode gradually to bring about drug release. It relates drug release from matrix surface to the cube root of time (Costa and Sousa Lobo, 2001). The Hixson – Crowell model, a mathematical model specific for describing the influence of surface behaviour on drug release is particularly useful as a better understanding of surface activity may as well inform strategies for developing better adhering surfaces optimal mucoadhesion. As seen in Table 5-3, the progesterone-loaded nanofibres returned higher correlation when fitted in this model meaning the amount of drug released over a period is better defined in terms of their surface activity.

5.6.2.4 Summary of release modelling

For all models fitted, a higher correlation between respective functions of drug release and time was observed among the progesterone-lobed fibre systems. These mathematical models describe how material properties, geometry and release mechanism and phases influence release patterns from delivery systems (Peppas and Narasimhan, 2014). The polymeric composition of these fibre systems, better defined geometry and the enhanced surface area which may facilitate drug release Specifically, Korsemeyer-Peppas confirmed a release mechanism driven by simultaneous swelling and diffusion in the fibre systems while the Higuchi model indicated a more uniform dispersion of progesterone therein.

Chapter 6

Assessing mucoadhesion of progesterone-loaded nanofibres

6.1 Introduction

Progesterone-loaded nanofibre systems have so far proved to be suitable material candidates for design of systems delivering progesterone vaginally to support pregnancies to term, particularly in women at risk of going into early labour (Dodd et al., 2008). These nanofibres, essentially mucoadhesive systems can be expected to adhere firmly onto mucosa of vagina to facilitate transport of the active drug for improved bioavailability at a more convenient dose frequency.

Mucoadhesive delivery systems have good prospects for drug delivery, especially for their extraordinary potential in prolonging dosage form resident times at sites of application such as in vagina or nasal cavity thereby improving convenience and compliance as a result of less frequent dosage (Andrews et al., 2009, Mansuri et al., 2016). For a comprehensive assessment of the performance of these delivery systems, mucoadhesive capabilities ought to be quantified by accurate and reliable method. This is however presently difficult and often impedes the development of these systems. Moreover, obtaining and preparing mucosa membrane to test these system is logistically challenging and often fraught with inconsistent results (Khdair et al., 2013). Utilising artificial membranes as suitable alternative for quicker and easier analyses of mucoadhesion of these systems is currently being explored. In this work, the mucoadhesive interactions of various batches of nanofibres with either artificial or mucosa membranes are investigated and the results compared to determine how well they correlate. Furthermore, a novel approach dependent on diffusion and mechanical theories of mucoadhesion, which uses roughness and void spaces at the interface of interacting surfaces in quantifying extent of mucoadhesion is reported. A nanoscale examination of the point of interaction between the fibre and mucosa

membrane using AFM to identify evidence of depth of interpenetration and unfilled voids, which are crucial to mucoadhesion, was carried out. Trends obtained from analysing the interfacial roughness and voids were compared to those obtained by measuring forces required to detach fibres from mucosa membranes to determine the viability of this method as a reliable means of mucoadhesion quantification.

6.2 Fibre morphology – Surface properties

It is been established earlier on that CMC improved the mucoadhesive properties of nanofibres. Therefore in quantifying mucoadhesion additional batch of fibres containing 2.5% wt. of CMC was produced with the aim of identifying significant differences driven by CMC. Composition of nanofibres compared in the mucoadhesive quantification work are shown in Table 6-1

Table 6-1: Polymer and drug quantities used for liquid preparations before spinning into fibres

Sample	Progesterone (wt. %)	PEO (wt. %)	CMC (wt. %)
A	5	15.00	0
B	5	13.75	1.25
C	5	12.5	2.5

Crystalline materials including some drugs such as Vitamin B6, carbon nanotubes and metals, when embedded in nanofibres can cause extensive protrusion from within resulting in an uneven nanofibre surface. Several studies investigating nanofibres containing crystallites have reported this observation (Salalha et al., 2004, Nam et al., 2010, Llorens et al., 2013). The effect of crystalline material content on nanofibre

surface is particularly relevant in this work as several of the mucoadhesion theories emphasise the relationships between surface properties and their ability to adhere.

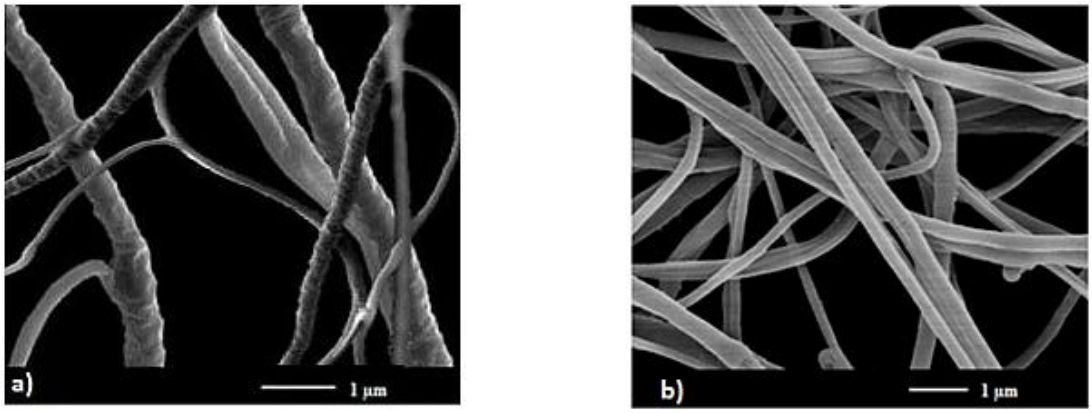


Figure 6-1: a) Progesterone-loaded nanofibre and b) nanofibre without any drug showing differences in surface morphology brought about by the crystalline nature of embedded drug

The effects of protrusion by crystalline material in fibres on surface morphology is clearly seen in Figures 6-1a, drug-loaded nanofibre (approx. 25 wt. % of progesterone), especially when compared to nanofibres without any drug (Figure 6-1b). Following this observation, necessary adjustments to manage fibre surface disruptions due to crystalline content (both qualitative and quantitative) during formation will be required in order to produce structures with best possible surface properties that support mucoadhesion. Furthermore, the chemistry of materials to be loaded into nanofibres can alter fibre dimensions while being formed. In a study exploring bioactive platforms for tissue engineering, Llorens et al. (2013) found that embedding crystalline pyridoxine (Vitamin B6) eventually increased fibre size while antioxidants such as p-hydroxycinnamic acid influenced generation of thinner fibres. The thinning of fibres by materials such as p-hydroxycinnamic acid was attributed to their chemistry, where dissociated ions possibly affected charge difference and thereby influencing elongation of fibres during formation by electrospinning. Indeed, a different method of producing nanofibres not dependent on charge difference, PG was employed and the latter observation may not be relevant as far as altering fibre

outcomes. However, the inclusion of crystalline progesterone was identified to affect fibre outcome in terms of surface appearance. In addition, when progesterone loaded fibres are compared with plain ones, e.g. from polymer blend with approximately 1.2% wt. CMC, the former was nearly twice the size of the latter. An increase in fibre size following embedding of crystalline progesterone is similar to electrospun fibres containing crystalline pyridoxine (Llorens et al., 2013). It can be therefore inferred that crystalline material, when incorporated into nanofibres, irrespective of fabrication method can affect their size and surface properties.

In terms of dimensions among drug loaded fibres, it is clear from the plot in Figure 6-3 that average diameter of nanofibres were significantly affected by variations in polymer constituents. Specifically, mixtures containing higher proportions of CMC yielded fibres with a larger diameter and increasing CMC quantities from 1.25 to 2.50 wt. % resulted in over a 40% increase in fibre diameter. In assessing solution properties before fibre production, it was observed that solution viscosity increased with increasing CMC content. Mixtures with high viscosity generally produced fibres with larger diameters. The trend seen here confirms viscosity as an effective variable for the control of fibre size and morphology, and in line with fundamental observation in generating nanofibres from polymer solution (Mahalingam and Edirisinghe, 2013, Deitzel et al., 2001, Zong et al., 2002). CMC, functioning as a viscosity modulator here is well known for its high viscosity in low concentrations (Yang and Zhu, 2007), a property also largely dependent on degree of substitution (DS) occurring on the cellulosic backbone and the intrinsic viscosity of plant pulp from which the polymer is derived (Barba et al., 2002).

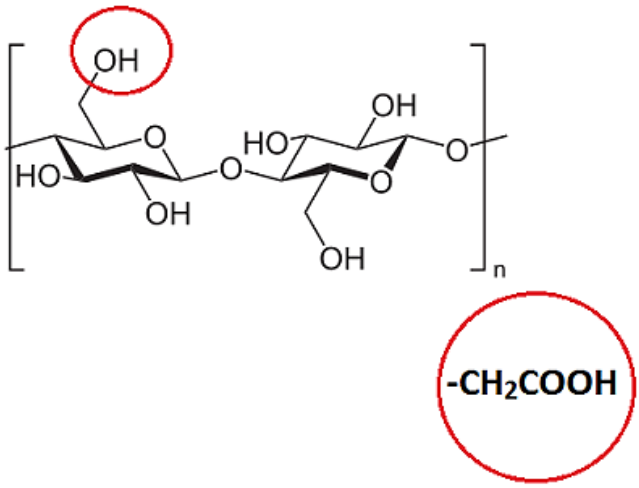


Figure 6-2: Cellulose backbone and groups substituted for -OH to yield carboxymethyl cellulose. Degree of substitution (DS) influence behaviour e.g. viscosity of resulting product

Mucoadhesive effect in fibres also derived from inclusion of CMC is discussed later in this paper. Controlling viscosity and mucoadhesive properties for better outcome and performance of nanofibres is possible because of our strategy to use blends of polymers in generating these fibres as this adds to existing variables that can be fine-tuned for desirable outcome.

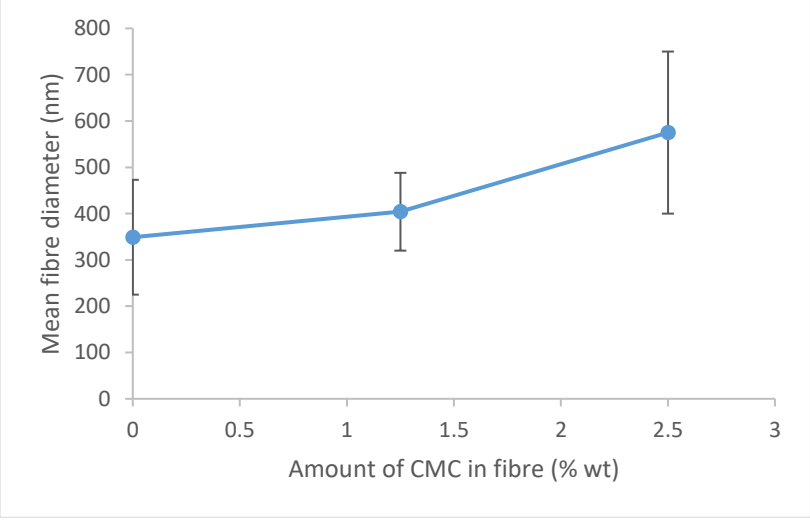


Figure 6-3: Graph illustrating the effect of CMC content on mean diameter (n=100) of nanofibres generated in this work. Error bars are standard deviations in size distributions.

6.3 Mucoadhesivity

6.3.1 Mucin-Polymer interactions

Polymer-mucin interactions is complex and often driven by multiple activities including physical chain interlocking, conformational adjustments, electrostatic interactions and interfacial chemical reactions through secondary bond formation between functional groups in adhering materials (Menchicchi et al., 2014, Madsen et al., 1998, Rossi et al., 1995). The interfacial bond formations are known to occur mainly through hydrogen bonding and van der Waal forces.

Various functional groups in materials interacting determine extent of interfacial bonding and hence strength of adhesion. Functional groups such as hydroxyl and carboxyl groups, typically on the oligosaccharide chains and amino groups in cysteine-rich domains in mucin could form bonds with similar functional groups in polymers or other materials such as artificial membranes) interacting with mucosa to keep both surfaces adhered to each other (Roy et al., 2009, Bansil and Turner, 2006). Figure 6-4 illustrates various points on the mucin molecule where interfacial bonding may occur for mucoadhesion.

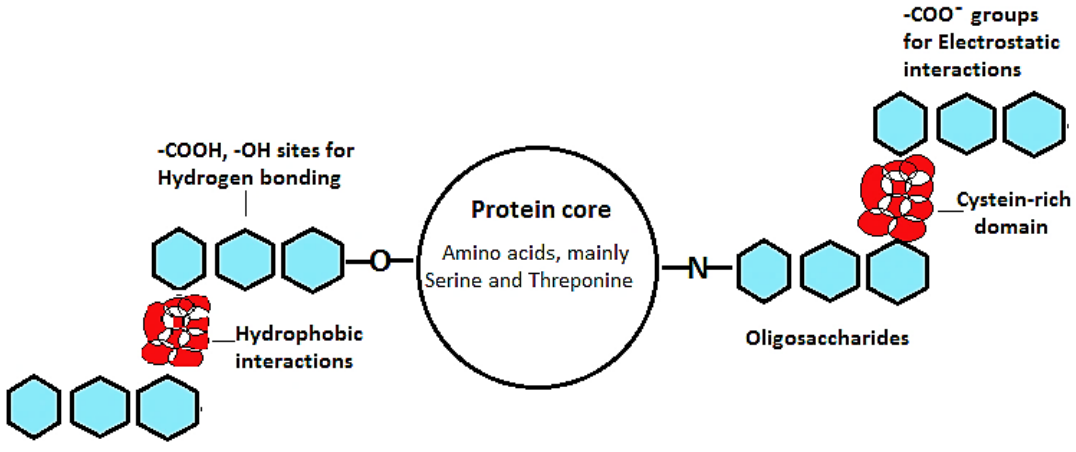


Figure 6-4: Schematic illustration of various active points on mucin molecule that may interact with other groups to facilitate mucoadhesion (Yang et al., 2012)

6.3.2. Artificial versus natural membrane

When the performance of mucoadhesive delivery systems are assessed, two features routinely quantified are the adhesion to and permeation through the mucosa (Boegh et al., 2013, Ivarsson and Wahlgren, 2012). Evaluating both features typically require the use of mucosa membranes from sacrificed animals. The use of actual mucosa tissues, in addition to being costly and logistically challenging, can give rise to inconsistent results due to the widely varying approaches to tissue preparation. In this regard, artificial membranes such as cellulose acetate have been utilised for both adhesive and permeation studies and confirmed to correlate well with those obtained from actual mucosa tissues (Khdair et al., 2013, Jain et al., 2002).

Mucoadhesive properties of various batches of drug-loaded nanofibres were measured using a texture analyser under test conditions shown in Table 3-2. A typical trace from stages A-C (Figure 6-5) encountered during testing is shown graphically in Figure 6-6. Basically, the nanofibre sample attached to the tip of a probe (cylindrical probe (Chen-Hoseney dough stickiness rig) with cross-sectional area of 0.785 cm^2) is brought in contact with membrane surface for 5 seconds with a test force of 40g. And then the force required to detach the fibre from membrane surface, which is a function of the extent of adhesion occurring at between the two surfaces, is measured and recorded.

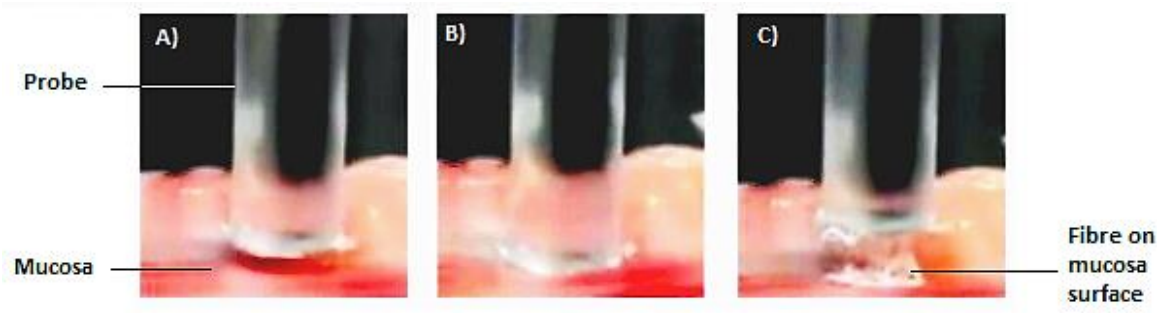


Figure 6-5: Stages in measuring strength of fibre adhesion to mucosa: a) analyser probe with fibre attached to the tip is brought in contact with mucosa membrane, b) fibre and mucosa are in contact for specified period and c) fibre is separated from mucosa while the force required is measured

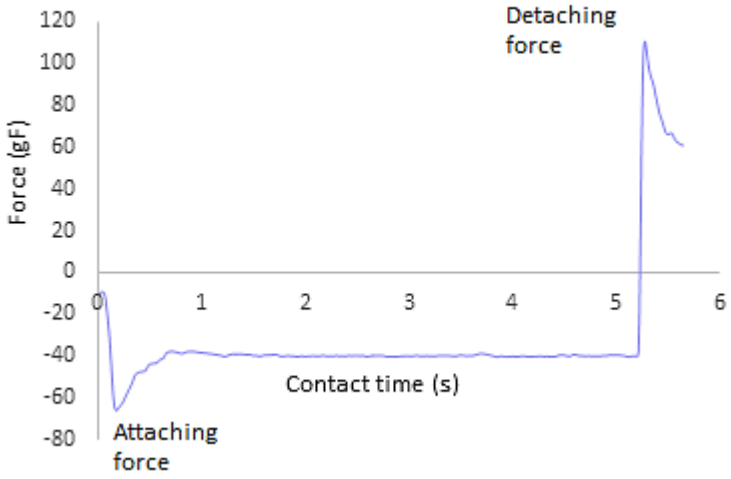
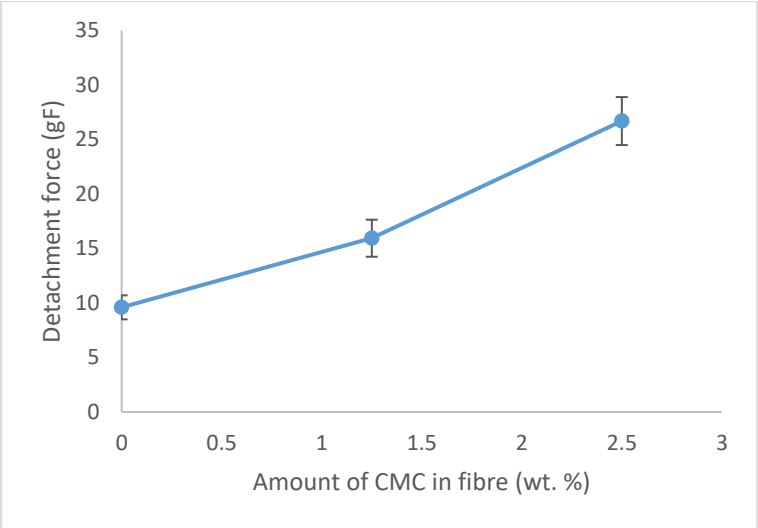


Figure 6-6: Typical trace recorded during attaching and detaching nanofibre samples from mucosa surfaces

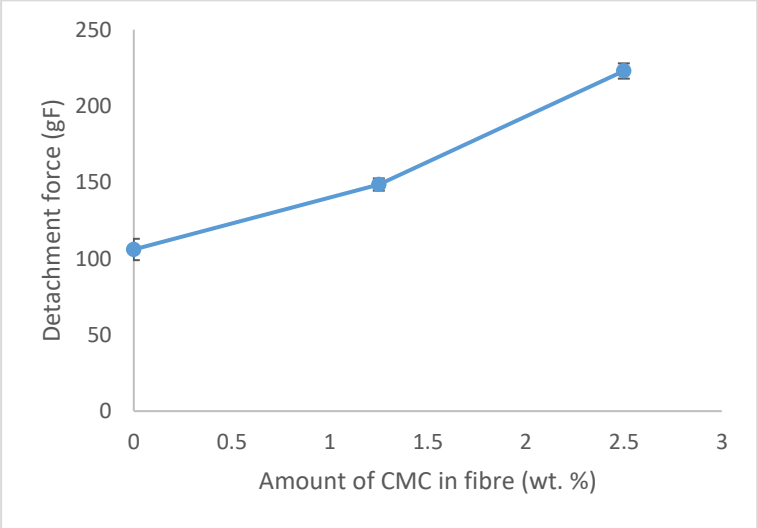
Firstly, the effect of increasing amounts of CMC in fibres on their mucoadhesive properties were studied using both artificial and mucosa membranes. In both instances, a clear trend of increasing mucoadhesive interactions with higher amounts of CMC was established (Figures 6-7a and b). The stronger mucoadhesion seen with increasing amounts of CMC may be explained using some well-established principles governing interactions between weakly anionic carboxyl containing polymers with oligosaccharides chains in mucins (Khutoryanskiy, 2011).

The carboxylic groups in these polymers are able to form strong hydrogen bonds with functional groups in mucin, thereby strengthening interactions between these two materials leading to appreciable levels of mucoadhesion. The formation of these bonds has been confirmed with displacement of infra-red absorption bands and nuclear magnetic resonance (Patel et al., 2003). Furthermore, these weakly anionic polymers demonstrated strongest mucoadhesive interactions in acidic conditions, with adhesive properties diminishing drastically at pH > 4 (Park and Robinson, 1987). These materials are for systems applied vaginally and hence experiments were conducted under simulated vaginal conditions which are acidic. Therefore CMC,

which offers stronger mucoadhesion in acidic conditions contributed significantly to the overall adhesive properties observed.



a)



b)

Figure 6-7: Effect of CMC content on nanofibre mucoadhesive properties as assessed by measuring forces required to detach them from a) sheep oesophagus mucosa (R^2=0.97) and b) cellulose acetate membrane (R^2=0.98).

Secondly, results from fibre mucoadhesive interactions with artificial membrane were compared to those obtained from mucosa. Generally, stronger interactions were

observed between the fibres and the artificial membrane than those seen with the sheep mucosa. This observation is expected due to the adhesive behaviour of cellulose-based materials mainly driven by formation of hydrogen bonds by hydroxyl groups present and partially by free energy interactions driven by apolar and electron donor components of cellulose (Gardner et al., 2008). Therefore the higher mucoadhesive interactions observed with the cellulose acetate membrane is the result of interactions between the fibre and cellulosic components of the membrane, as described above as well as with mucins. In contrast, the mucoadhesion occurring between the fibres and the sheep mucosa was largely due only to interactions with mucin in the mucosa and hence not as strong as those seen in the cellulosic membrane. In terms of trends however, a similar correlation between adhesion and CMC content in fibres was observed in both the artificial and the natural membrane (R^2 = 0.97 and 0.98 as seen in Figures 6-7a and b).

On the basis of these observations, artificial membranes may not be ideal substitutes for actual mucosa membrane for mucoadhesive studies, especially when scalar quantification of adhesion is relevant to the study as additional adhesive interactions not from samples being analysed can mask the actual observations of interest. However, they may be suitable for analysing trends such as the effect of varying constituents of a system on mucoadhesion, in which case superfluous adhesive interactions that may arise from the artificial membrane will be prevalent in all samples being studied, thus maintaining a trend that will correlate to the mucoadhesive feature being investigated.

6.3.3 AFM analyses of fibre – mucosa interface

As mentioned earlier, a standardised method for quantifying mucoadhesion remains to be adopted for use despite tremendous interest in mucoadhesive drug delivery (Woertz et al., 2013). Notwithstanding, some approaches based on one or a combination of mucoadhesion theories have been used to assess mucoadhesion,

albeit with different levels of reliability. Most of these approaches are based on the fracture method, where the forces required to separate two interacting surfaces are quantified and used to express extent of mucoadhesion. A few more depends on the investigation of surface properties, typically by AFM (Patel et al., 2000).

In this section, a novel approach where the interface between fibres and mucosa membrane, after mucoadhesive interaction, is visualised at the nanoscale for indications of extent of interpenetration between polymer and mucin chains and filling up of cavities, two of the most crucial activities leading to mucoadhesion according to the diffusion and mechanical theories. A schematic in Figure 6-8 illustrates how interfacial images were interpreted to imply extent of interpenetration between surfaces and hence extent of mucoadhesion. Extensive penetration ensures stronger binding of surfaces and thus a cross-section reveals a smoother (lower height) surface. In the same way, limited interpenetration leaves a rougher surface and that can be taken as minimal mucoadhesion.

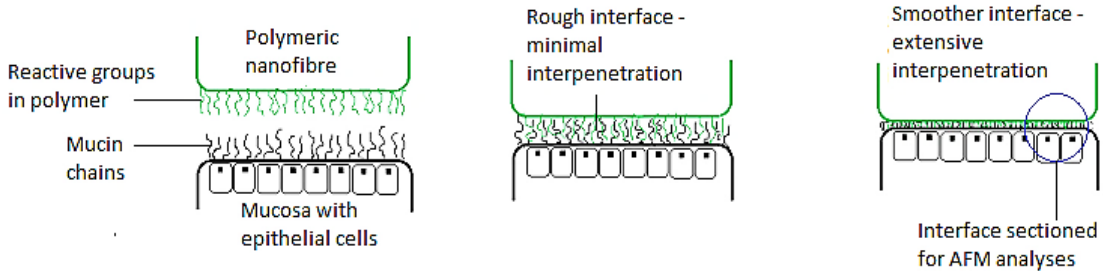


Figure 6-8: A schematic illustration of interaction between polymer and mucin reactive groups. An extensive interpenetration of groups from two surfaces, according to diffusion theory results in closer (smoother) and stronger adhesion.

In comparing roughness at the interface of various nanofibre samples interactions with mucosa, the parameter routinely used to measure surface roughness was Ra (arithmetic average of absolute height values). The Rmax values (the height from the lowest depth to the highest point) also gave a trend similar to the Ra, implying that the roughest of all the interfaces also had most voids that were left unfilled. This observation reinforces our hypothesis that using interfacial roughness to track extent

of mucoadhesion may reflect the diffusion and mechanical theories which are based on interpenetration and surface activity facilitating the filling up of cavities in order to bring about mucoadhesion. Furthermore, previous studies looking into these interactions have traced the depth of interpenetration between polymer and mucin groups to the characteristic of the interfacial layer (Ponchel et al., 1987).

Therefore a thorough examination of the point of interaction between the polymer and mucosa can help us compare extents of mucoadhesion that have occurred among batches of delivery systems and that is the basis of using AFM to study these interfaces. As shown in Figure 6-9, nanofibres containing CMC, after interaction with mucosa membrane come out significantly smoother than those from fibres without any CMC.

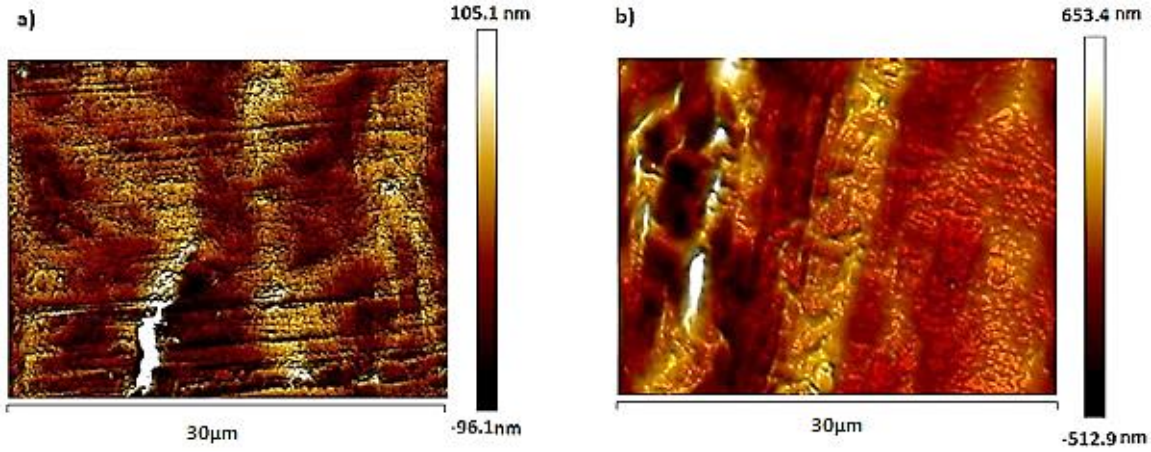


Figure 6-9: AFM images showing depths (dark regions) and heights (light regions) of sections of interface derived from mucosa membrane interactions with nanofibres a) containing 2.5% CMC and b) without CMC. PEO/CMC appear smoother.

Elevations on the image interpreted from the scale bars, which determines the surface roughness, are much lower on the image from samples containing CMC. Smoother surfaces imply closer and stronger interaction of surfaces and therefore higher level of mucoadhesion having occurred. Furthermore, according to the mechanical theory, surface energies and behaviour facilitating the filling up of voids on mucosa membrane results in stronger adhesion. From examining depths on these images, it is clear that interface sections from fibres without CMC had greater voids after fibre-

mucin interactions indicating limited surface activities leading to filling up of cavities in the membrane and hence giving inadequate mucoadhesion.

6.3.3.1 Phase images from AFM analyses of interfaces

Phase imaging provides additional information on material surface properties. In tap mode operation, these images are derived from the phase lag between the excitational signal and cantilever response due to interactions between the probe tip system and the material being analysed. Images seen in Figure 6-10 also confirmed samples with highest CMC content had most uniform phase image implying higher consistency in polymer-mucin residue left after interactions.

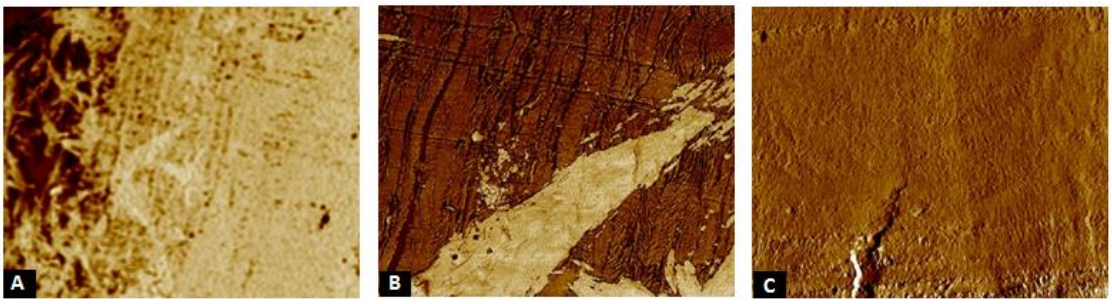


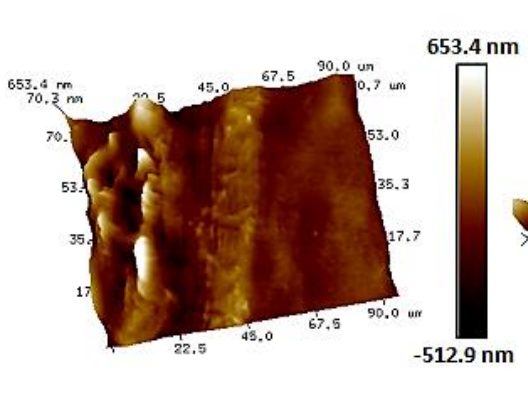
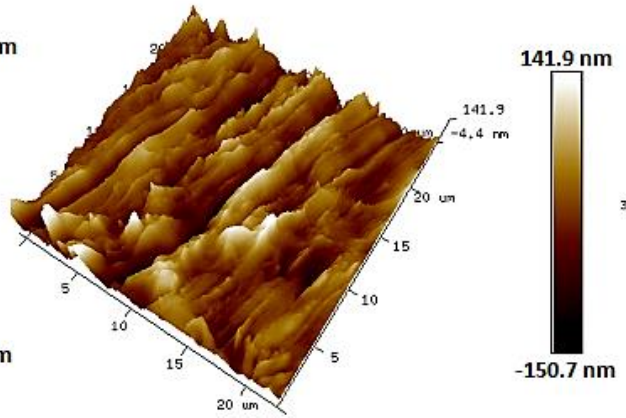
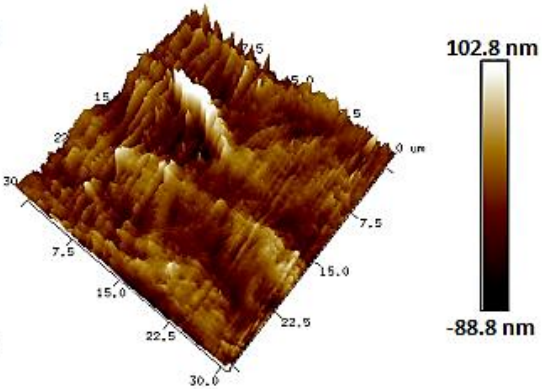
Figure 6-10: Interfacial phase images from: A) PEO only B) PEO/1.25 wt. % CMC and C) PEO/2.5 wt. % CMC after interactions with mucosa membrane. Images resized to represent an area of 900μm² with angular units between -30 and 15°.

As different material surfaces will interact uniquely with the tip system, phase imaging is found to go beyond simple topographical mapping to detect variations in composition, adhesion and viscoelasticity, among other properties (Tamayo and Garcia, 1996, Stark et al., 2001). Indeed different AFM procedures have been used to directly quantify adhesion in various materials (Yu et al., 2013)

AFM analysis was used to study trails left behind after mucoadhesion to examine degree of interactions. Though not directly detecting adhesion, these phase images (Figure 6-10) confirm highest interfacial uniformity between fibres containing 2.5 wt.

% CMC and mucosa surface. According to results from both texture analysis and AFM, fibres from this composition exhibited the highest mucoadhesive capabilities. 3D images of various nanofibre interactions with mucosa, parameters assessed and respective observations made are summarised in Table 6-2. In summary, the PEO only fibres, which according to the AFM images appeared roughest after being in contact with mucosa is the least mucoadhesive among the batches examined. This is because in addition to having highest Ra value indicative of it being roughest, it had the highest Rmax value which implies the deepest voids in its surface. Both features strongly imply limited interactions between polymer and mucosa. Conversely, the batch containing highest the amount of CMC (2.5 wt.%) had the least Ra and Rmax values implying the smoothest interface with least number of voids, which according to the diffusion and mechanical theories of mucoadhesion, indicate deeper interpenetration among interacting surfaces and therefore high mucoadhesion.

Table 6-2: Relationship between nanofibre content and the surface properties of interface arising from their interaction with mucosa

CMC content (wt. %)	0	1.25	2.50
AFM image at dried interface between nanofibre and mucosa			
Roughness data, Rq (nm)	101	29	19
Rmax (nm)	1716	480	439
Comment	Interactions between this batch of fibres (PEO only) and mucosa left the roughest interface. A rough residue, according to the diffusion and mechanical theories points to less interpenetration between polymer functional groups and mucin. A conclusion of least mucoadhesion from the roughest residue is confirmed by results from texture analysis which is based on the fracture theory.	Fibres containing 1.25% CMC showed much less rough interface following interactions with mucosa. According to the diffusion and mechanical theories, this showed more interpenetration activity between the two interacting surfaces than was observed in the batch containing only PEO. A much more even height distribution may also be due to more widespread interactions between fibres and mucins.	This batch of fibres, containing the highest amount of CMC yielded the smoothest interface following interactions with mucosa and therefore is interpreted as having the best interpenetrating activity between the two interacting surfaces. A widespread surface roughness may also be indicative of better interaction of surfaces to bring about mucoadhesion. This batch of fibres being most mucoadhesive is also confirmed by results from texture analyses.

Chapter 7

Conclusions and future recommendations

7.1 Conclusions

Progesterone, when used in supporting pregnancy is applied at least once daily for several weeks. Due to current limitations in formulation, the most practical route of administration for this indication is by intramuscular injection. For such long periods, parenteral administration is painful, inconvenient and in some cases, will require healthcare personnel to administer doses. Clearly, there is a need for alternate dosage forms that can suitably address these challenges. The vaginal route can be a suitable alternative for delivering progesterone both for local and systemic action. Therefore a formulation, based on entirely different design approach but capable of optimal trans-mucosal delivery of this drug would be a significant contribution towards improving the delivery of progesterone for such indications. The design approach reported in this thesis utilises mucoadhesive drug-loaded nanofibres which is expected to combine the qualities of a nanofibre and benefits from mucoadhesion for better drug delivery. The following conclusions are drawn from this study:

7.1.1 Generating well-structured and mucoadhesive fibres

Mucoadhesive fibres with size distribution from less than 100nm upwards have been produced from mucoadhesive polymer blends by a simple but efficient method of pressurised gyration. Biodegradable, water soluble and anionic polymers, known to exhibit mucoadhesive character in acidic conditions were selected as these were safe as well as suitable for use in the vagina, considering its slightly acidic environment. Blending these anionic polymers i.e. polyacrylic acid, sodium alginate

and carboxymethyl cellulose with PEO allowed successful formation of well-structured fibres. Fibres produced were subsequently assessed for their physical properties, mainly size and morphology as well as molecular compositions.

7.1.2 Outcome of mucoadhesive fibres

SEM analyses have confirmed that fibres from these blends were well defined, uniformly cylindrical throughout their lengths and of appreciably high structural integrity. Size distribution analyses confirmed average size for PEO, PEO/Alginate, PEO/CMC and PEO/PAA being 172, 176, 194 and 217 nm respectively. FTIR confirmed identical functional groups in polymer/blends and their corresponding fibres, thus validating PG as a method for the production of fibres from blends of polymers. Texture analysis established the trend PEO/CMC > PEO/PAA > PEO/Alginate > PEO in terms of adhesion strength.

7.1.3 Generating progesterone-loaded fibres

CMC/PEO blends, confirmed as having the best mucoadhesive prospects were used to produce well-structured progesterone-loaded fibres with diameters from 40 – 1400 nm. The inclusion of progesterone was found to increase average fibre size as well disparities in their size distribution. Thermal and spectroscopic analyses confirmed the loaded drug existed as crystals. Optimising the fibre formation process resulted in fibres that contained up to 25% wt. of the active drug, progesterone.

7.1.4 The loaded progesterone

Loaded progesterone isolated from fibres by selective dissolution was found to be of smaller particle size (< 1µm), compared to the original powder form of average

size 10μm. This is a positive outcome as active drug presented in smaller particle size is usually desirable considering their likelihood for easier dissolution and subsequent transport across membranes. In summary, encapsulating progesterone in polymeric fibre systems results in several benefits including efficient drug loading, increased surface area for effective mucosa contact and significant drug particle size reduction.

7.1.5 Drug release from progesterone-loaded fibre systems.

When compared to Cyclogest (a formulation of progesterone presently available on the market), progesterone release from all batches of drug-loaded fibres exhibited higher potential for zero order release. Specifically, a trend in the order PEO/CMC > PEO only > Cyclogest for release mimicking a zero order kinetic was established. In addition, both drug-loaded fibre systems released higher amounts of progesterone, compared to Cyclogest, a feature that may be further explored for solutions in addressing the various bioavailability issues presently limiting the use of progesterone.

7.1.6 Assessing mucoadhesion by texture analyser

Fibre adhesion to artificial and natural mucosa membranes was investigated. It was established that while artificial membranes may be a useful substitute for mucosa for determining general trends in mucoadhesive potential among a set of samples relative to each other, they exhibit different magnitudes of adhesion and hence may not be exact representation of mucoadhesion. Secondly, results compared within models confirmed that fibres containing higher amounts of CMC required higher detaching force for separation in both the natural and artificial membranes. Clearly, the inclusion of mucoadhesive polymer influenced mucoadhesion prospects of the fibres.

7.1.7 Assessing mucoadhesion by atomic force microscopy

A novel approach which relates interfacial properties such as roughness and void spaces to the degree of mucoadhesion was proposed. According to results from texture analysis, CMC content positively influenced mucoadhesion. AFM analysis of fibre-mucosa interfacial roughness gave a trend similar to that seen in the texture analysis. A non-parametric hypothesis test (Kendall's tau coefficient) confirms this correlation to be statistically significant.

Therefore fibre-mucin interfacial roughness may be a useful parameter for quantifying mucoadhesion

7.1.8 Summary of conclusions

In summary, the work reported in this thesis has demonstrated the possibility of using PG to produce well-structured drug-loaded mucoadhesive fibres. Analytical investigations used in characterising these systems confirmed their dimensions, morphology and molecular properties as suitable materials for further development into alternate dosage forms for improved delivery of progesterone. Furthermore, performance assessment such as permeation and mucoadhesive study confirmed desirable prospects of zero order release kinetics and sufficient mucoadhesion properties, all of which make them attractive materials for diverse pharmaceutical applications.

7.2 Future recommendations

Recommendations for addressing some challenges encountered in this study and suggestions to improve the utilisation of pressurised gyration for manufacture of progesterone-loaded fibres for various pharmaceutical applications are listed below:

- *Formulation strategy for developing dosage forms*

  First and foremost, a formulation strategy to convert the drug-loaded fibres into stable, practical and safe vaginal dosage forms will be needed to take this project further. A few formulation schemes seeking to convert fibres into suitable dosage forms have been attempted and reported (Poller et al., 2017, Blakney et al., 2013). The options include compression of fibres into tablets for vaginal insertion or winding bundles of the fibre into a miniature tampon. The unique but delicate nature of these micro and nano structures will certainly require modification of existing pharmaceutical formulation procedures for handling and further processing into useful A cross-disciplinary collaboration among pharmaceutical formulation and relevant materials research groups to investigate and develop options for designing an appropriate dosage form from these progesterone-loaded fibres is highly recommended.

- *Further improvement of the Pressurised Gyration setup*

  The pressurised gyration approach to producing fibres of various sizes and shapes from a wide range of materials, following reports of several studies, is proving to be a suitable alternative to electrospinning. The invention is relatively new and as expected, its setup still quite basic. A project dedicated to redesigning the setup in a suitable housing complete with tubes, buttons and dials to control production parameters would ensure better utility and productivity, make the process more efficient by reducing waste while tremendously improving its output. In addition, its operation would be further simplified, offer better control of production parameters and encourage usage among diverse research groups. Improving the usability of pressurised gyration will increase the body of work generated, thus stimulating improvements from variety of sources and establishing this

method as a mainstream approach to micro and nanofiber production. A modification of the present setup is therefore highly recommended.

- *Managing information arising from pressurised gyration experiments*

  Pressurised gyration has various production parameters such as rotational speed, pressure, amount and type of materials, flow-rate of liquid across different variations of the process such as pressured, flow-controlled or combination of pressure and flow control gyration. This has tendency to generate a lot of data. Some computational and statistical analyses of these data have begun and it is highly recommended that it continues and outcome of analyses applied to further improve the production process. In addition, a compilation of production protocols, parameters and expected outcomes, suitable material combinations such as polymers and ideal solvent systems will be helpful for utilisation of this relatively new method of fibre production.

- *Characterising mucoadhesion*

  Assessing mucoadhesion is still quite challenging, especially as the various methods used remain unstandardised. A new approach to studying mucoadhesion was developed in this work. Further quantification and interpretation of image data obtained would be very helpful in consolidating the use of interfacial scans by AFM and possibly SEM as a means of gaining further insight into mucoadhesion mechanism. Furthermore, a consortium of stakeholders in fields where mucoadhesion is relevant to their objectives could review assessments of mucoadhesion and adopt a set of standard operating procedures to help harmonise the process across various research themes. Such guidelines, when adopted would improve assessment and reproducibility of mucoadhesion measurements. Mucoadhesion characterisation is becoming more crucial as trans-mucosal

delivery of drugs is increasingly being considered for newer therapeutic entities such as structurally delicate macromolecules and biologicals, hormonal drugs and a variety of drugs that are practically impossible to be presented via the oral route.

- *Studying drug release from fibres*

  Progesterone-loaded fibres produced in this study are to be used in formulating dosage forms for vaginal application. Drug release assessment was therefore designed to track release of progesterone from the fibres as well as movement across membrane mimicking vaginal mucosa. Due to logistics and time constrains, cellulose acetate membranes treated in simulated vaginal fluid was used as models for release study reported. This approach has limitations. First of all, prolonged exposure of the cellulose acetate membrane to micro and nanostructures such as fibres analysed causes widespread blockade and render the membranes ineffective subsequently. This limited the time for release study to under four hours. Secondly, the absence of physiological factors present in actual mucosal membranes made this particular model an oversimplified approach to studying drug transport across the vaginal epithelia.

  Ideally, actual vaginal mucosa from mammals with non-keratinised membranes such as pig or lamb should have been used. These were attempted but the immense logistic challenge of obtaining viable vaginal mucosa membrane into the laboratory for this study prevented this study from being undertaken. Typically, excised mucosal freshly delivered within a short period from an abattoir to ensure tissues viability as at time of experimenting would have been required. This was difficult due to location of laboratories. It is therefore highly recommended that release studies are carried out on these progesterone-loaded membranes to investigate how

well drugs encapsulated within are release for onward transport across viable mucosal membranes.

References

ABDOOL KARIM, S. & BAXTER, C. 2014. Microbicides for Prevention of HIV Infection: Clinical Efficacy Trials. *In:* NUTTALL, J. (ed.) *Microbicides for Prevention of HIV Infection.* Berlin Heidelberg, Springer.

ABIDI, S. E., COUTINHO, E. R., GUMUDAVELLI, S., AGRAWAL, S. & GULLAPALLI, R. P. 2012. Methods of preparing progesterone pharmaceutical compositions. Google Patents.

AHUJA, A., KHAR, R. K. & ALI, J. 1997. Mucoadhesive drug delivery systems. *Drug Development and Industrial Pharmacy,* 23**,** 489-515.

AKIYAMA, Y., NAGAHARA, N., NARA, E., KITANO, M., IWASA, S., YAMAMOTO, I., AZUMA, J. & OGAWA, Y. 1998. Evaluation of oral mucoadhesive microspheres in man on the basis of the pharmacokinetics of furosemide and riboflavin, compounds with limited gastrointestinal absorption sites. *Journal of pharmacy and pharmacology,* 50**,** 159-166.

ALKAN, C., GÜNTHER, E., HIEBLER, S. & HIMPEL, M. 2012. Complexing blends of polyacrylic acid-polyethylene glycol and poly (ethylene-co-acrylic acid)-polyethylene glycol as shape stabilized phase change materials. *Energy Conversion and Management,* 64**,** 364-370.

ALLEN, T. M. & CULLIS, P. R. 2004. Drug delivery systems: entering the mainstream. *Science,* 303**,** 1818-1822.

ALLEN, W. 1935. The isolation of crystalline progestin. *Science,* 82**,** 89-93.

ANDERSON, R. G. W. & JACOBSON, K. 2002. Cell biology - A role for lipid shells in targeting proteins to caveolae, rafts, and other lipid domains. *Science,* 296**,** 1821-1825.

ANDREWS, G. P., LAVERTY, T. P. & JONES, D. S. 2009. Mucoadhesive polymeric platforms for controlled drug delivery. *European Journal of Pharmaceutics and Biopharmaceutics,* 71**,** 505-518.

ASTRUC, D., BOISSELIER, E. & ORNELAS, C. 2010. Dendrimers designed for functions: from physical, photophysical, and supramolecular properties to applications in sensing, catalysis, molecular electronics, photonics, and nanomedicine. *Chemical Reviews,* 110**,** 1857-1959.

BAERT, L., VAN 'T KLOOSTER, G., DRIES, W., FRANÇOIS, M., WOUTERS, A., BASSTANIE, E., ITERBEKE, K., STAPPERS, F., STEVENS, P., SCHUELLER, L., VAN REMOORTERE, P., KRAUS, G., WIGERINCK, P. & ROSIER, J. 2009. Development of a long-acting injectable formulation with nanoparticles of rilpivirine (TMC278) for HIV treatment. *European Journal of Pharmaceutics and Biopharmaceutics,* 72**,** 502-508.

BALOGLU, E., BERNKOP-SCHNÜRCH, A., KARAVANA, S. Y. & SENYIGIT, Z. A. 2009. Strategies to prolong the intravaginal residence time of drug delivery systems. *Journal of Pharmacy & Pharmaceutical Sciences,* 12**,** 312-336.

BANSIL, R. & TURNER, B. S. 2006. Mucin structure, aggregation, physiological functions and biomedical applications. *Current Opinion in Colloid & Interface Science,* 11**,** 164-170.

BARBA, C., MONTANÉ, D., FARRIOL, X., DESBRIÈRES, J. & RINAUDO, M. 2002. Synthesis and characterization of carboxymethylcelluloses from non-wood pulps II. Rheological behavior of CMC in aqueous solution. *Cellulose,* 9**,** 327-335.

BARNES, C. P., SELL, S. A., BOLAND, E. D., SIMPSON, D. G. & BOWLIN, G. L. 2007. Nanofiber technology: Designing the next generation of tissue engineering scaffolds. *Advanced Drug Delivery Reviews,* 59**,** 1413-1433.

BARNHART, K. T., PRETORIUS, E. S. & MALAMUD, D. 2004. Lesson learned and dispelled myths: three-dimensional imaging of the human vagina. *Fertility and Sterility,* 81**,** 1383-1384.

BASSI, P. & KAUR, G. 2012. Innovations in bioadhesive vaginal drug delivery system. *Expert Opinion on Therapeutic Patents,* 22**,** 1019-1032.

BECK-BROICHSITTER, M., THIEME, M., NGUYEN, J., SCHMEHL, T., GESSLER, T., SEEGER, W., AGARWAL, S., GREINER, A. & KISSEL, T. 2010. Novel 'Nano in Nano'Composites for Sustained Drug Delivery: Biodegradable Nanoparticles Encapsulated into Nanofiber Non-Wovens. *Macromolecular Bioscience,* 10**,** 1527-1535.

BEECH, D. & BOOTH, C. 1970. Thermodynamic melting point of poly (ethylene oxide). *Journal of Polymer Science Part B: Polymer Letters,* 8**,** 731-734.

BEER, B. E., DONCEL, G. F., KREBS, F. C., SHATTOCK, R. J., FLETCHER, P. S., BUCKHEIT, R. W., WATSON, K., DEZZUTTI, C. S., CUMMINS, J. E. & BROMLEY, E. 2006. In vitro preclinical testing of nonoxynol-9 as potential anti-human immunodeficiency virus microbicide: a retrospective analysis of results from five laboratories. *Antimicrobial Agents and Chemotherapy,* 50**,** 713-723.

BEER, M., WOOD, P. & WEISZ, J. 1999. A simple and rapid method for evaluation of Mark–Houwink–Sakurada constants of linear random coil polysaccharides using molecular weight and intrinsic viscosity determined by high performance size exclusion chromatography: application to guar galactomannan. *Carbohydrate Polymers,* 39**,** 377-380.

BEN-ARIE, M. M. 1955. Determination of the tensile strength of gels. *Journal of Polymer Science,* 17**,** 179-190.

BENOIT, J., COURTEILLE, F. & THIES, C. 1986. A physicochemical study of the morphology of progesterone-loaded poly (d, l-lactide) microspheres. *International Journal of Pharmaceutics,* 29**,** 95-102.

BHARDWAJ, N. & KUNDU, S. C. 2010. Electrospinning: a fascinating fiber fabrication technique. *Biotechnology Advances,* 28**,** 325-347.

BLAKNEY, A. K., BALL, C., KROGSTAD, E. A. & WOODROW, K. A. 2013. Electrospun fibers for vaginal anti-HIV drug delivery. *Antiviral Research,* 100**,** S9-S16.

BLANCO-FUENTE, H., ANGUIANO-IGEA, S., OTERO-ESPINAR, F. & BLANCO-MÉNDEZ, J. 1996. In-vitro bioadhesion of carbopol hydrogels. *International Journal of Pharmaceutics,* 142**,** 169-174.

BOEGH, M., FOGED, C., MÜLLERTZ, A. & NIELSEN, H. M. 2013. Mucosal drug delivery: barriers, in vitro models and formulation strategies. *Journal of Drug Delivery Science and Technology,* 23**,** 383-391.

BRAKO, F., RAIMI-ABRAHAM, B., MAHALINGAM, S., CRAIG, D. Q. & EDIRISINGHE, M. 2015. Making nanofibres of mucoadhesive polymer blends for vaginal therapies. *European Polymer Journal,* 70**,** 186-196.

BRANNON-PEPPAS, L. & BLANCHETTE, J. O. 2012. Nanoparticle and targeted systems for cancer therapy. *Advanced Drug Delivery Reviews,* 64, Supplement**,** 206-212.

BRAYFIELD, A. 2014. *Martindale : The complete drug reference,* London, Pharmaceutical Press.

BRITISH MEDICAL ASSOCIATION. & ROYAL PHARMACEUTICAL SOCIETY OF GREAT, B. 2015. *BNF 70 : September 2015 - March 2016*.

BUCKTON, G. & BEEZER, A. E. 1992. The relationship between particle size and solubility. *International Journal of Pharmaceutics,* 82**,** R7-R10.

BUHLEIER, E., WEHNER, W. & VÖGTLE, F. 1978. ' CASCADE'-AND' NONSKID-CHAIN-LIKE' SYNTHESES OF MOLECULAR CAVITY TOPOLOGIES. *Chemischer Informationsdienst,* 9.

BUTENANDT, A. & WESTPHAL, U. 1934. Zur Isolierung und Charakterisierung des Corpus-luteum-Hormons. *Berichte der Deutschen Chemischen Gesellschaft (A and B Series),* 67**,** 1440-1442.

BUTT, H.-J., GRAF, K. & KAPPL, M. 2006. *Physics and chemistry of interfaces,* Weinheim, John Wiley & Sons.

CARAMELLA, C., BONFERONI, M. C., ROSSI, S. & FERRARI, F. 1994. Rheological and tensile tests for the assessment of polymer-mucin interactions. *European Journal of Pharmaceutics and Biopharmaceutics,* 40**,** 213-217.

CARON, M., BESSON, G., ETENNA, S. L.-D., MINTSA-NDONG, A., MOURTAS, S., RADAELLI, A., MORGHEN, C. D. G., LODDO, R., LA COLLA, P. & ANTIMISIARIS, S. G. 2010. Protective properties of non-nucleoside reverse transcriptase inhibitor (MC1220) incorporated into liposome against intravaginal challenge of Rhesus macaques with RT-SHIV. *Virology,* 405**,** 225-233.

CARVALHO, F. C., BRUSCHI, M. L., EVANGELISTA, R. C. & GREMIÃO, M. P. D. 2010. Mucoadhesive drug delivery systems. *Brazilian Journal of Pharmaceutical Sciences,* 46**,** 1-17.

CHAKRAVARTY, B. N., SHIRAZEE, H. H., DAM, P., GOSWAMI, S. K., CHATTERJEE, R. & GHOSH, S. 2005. Oral dydrogesterone versus intravaginal micronised progesterone as luteal

phase support in assisted reproductive technology (ART) cycles: results of a randomised study. *The Journal of Steroid Biochemistry and Molecular Biology,* 97**,** 416-420.

CHEN, D. W.-C. & LIU, S.-J. 2015. Nanofibers used for delivery of antimicrobial agents. *Nanomedicine,* 10**,** 1959-1971.

CHENG, Y., ZHAO, L., LI, Y. & XU, T. 2011. Design of biocompatible dendrimers for cancer diagnosis and therapy: current status and future perspectives. *Chemical Society Reviews,* 40**,** 2673-2703.

CHICKERING, D. & MATHIOWITZ, E. 1995. Bioadhesive microspheres: I. A novel electrobalance-based method to study adhesive interactions between individual microspheres and intestinal mucosa. *Journal of Controlled Release,* 34**,** 251-262.

CHONCO, L., PION, M., VACAS, E., RASINES, B., MALY, M., SERRAMÍA, M., LÓPEZ-FERNÁNDEZ, L., DE LA MATA, J., ALVAREZ, S. & GÓMEZ, R. 2012. Carbosilane dendrimer nanotechnology outlines of the broad HIV blocker profile. *Journal of Controlled Release,* 161**,** 949-958.

CHRISTENSON, E. M., ANSETH, K. S., VAN DEN BEUCKEN, J. J., CHAN, C. K., ERCAN, B., JANSEN, J. A., LAURENCIN, C. T., LI, W. J., MURUGAN, R. & NAIR, L. S. 2007. Nanobiomaterial applications in orthopedics. *Journal of Orthopaedic Research,* 25**,** 11-22.

CHUAH, H., LIN-VIEN, D. & SONI, U. 2001. Poly (trimethylene terephthalate) molecular weight and Mark–Houwink equation. *Polymer,* 42**,** 7137-7139.

COLEMAN, M. M., SKROVANEK, D. J., HU, J. & PAINTER, P. C. 1988. Hydrogen bonding in polymer blends. 1. FTIR studies of urethane-ether blends. *Macromolecules,* 21**,** 59-65.

CONNEELY, O. M., MULAC-JERICEVIC, B., DEMAYO, F., LYDON, J. P. & O MALLEY, B. W. 2002. Reproductive functions of progesterone receptors. *Recent Progress in Hormone Research,* 57**,** 339-356.

CONNEELY, O. M., MULAC-JERICEVIC, B. & LYDON, J. P. 2003. Progesterone-dependent regulation of female reproductive activity by two distinct progesterone receptor isoforms. *Steroids,* 68**,** 771-778.

U. S. P. CONVENTION, 2011. *U.S. Pharmacopeia National Formulary 2011: USP 34 NF 29*, United States Pharmacopeial.

COOK, M. T. & KHUTORYANSKIY, V. V. 2015. Mucoadhesion and mucosa-mimetic materials—A mini-review. *International Journal of Pharmaceutics,* 495**,** 991-998.

CÓRDOBA, E. V., ARNAIZ, E., RELLOSO, M., SÁNCHEZ-TORRES, C., GARCÍA, F., PÉREZ-ÁLVAREZ, L., GÓMEZ, R., FRANCISCO, J., PION, M. & MUÑOZ-FERNÁNDEZ, M. Á. 2013. Development of sulphated and naphthylsulphonated carbosilane dendrimers as topical microbicides to prevent HIV-1 sexual transmission. *AIDS,* 27**,** 1219-1229.

CORNER, G. W. 2015. *Hormones in human reproduction*, New Jersey, Princeton University Press.

CORNER SR, G. 1974. The early history of progesterone. *Gynecologic and Obstetric Investigation,* 5**,** 106-112.

COSTA, P. & SOUSA LOBO, J. M. 2001. Modeling and comparison of dissolution profiles. *European Journal of Pharmaceutical Sciences,* 13**,** 123-133.

CUI, W., ZHOU, Y. & CHANG, J. 2016. Electrospun nanofibrous materials for tissue engineering and drug delivery. *Science and Technology of Advanced Materials*.

D'CRUZ, O. J. & UCKUN, F. M. 2014. Vaginal microbicides and their delivery platforms. *Expert Opinion on Drug Delivery,* 11**,** 723-740.

DAHAN, A. & HOFFMAN, A. 2006. Use of a dynamic in vitro lipolysis model to rationalize oral formulation development for poor water soluble drugs: correlation with in vivo data and the relationship to intra-enterocyte processes in rats. *Pharmaceutical Research,* 23**,** 2165-2174.

DAS NEVES, J., AMIJI, M. M., BAHIA, M. F. & SARMENTO, B. 2010. Nanotechnology-based systems for the treatment and prevention of HIV/AIDS. *Advanced Drug Delivery Reviews,* 62**,** 458-477.

DAS NEVES, J., MICHIELS, J., ARIËN, K. K., VANHAM, G., AMIJI, M., BAHIA, M. F. & SARMENTO, B. 2012. Polymeric nanoparticles affect the intracellular delivery, antiretroviral activity and cytotoxicity of the microbicide drug candidate dapivirine. *Pharmaceutical Research,* 29**,** 1468-1484.

DAVIDOVICH-PINHAS, M. & BIANCO-PELED, H. 2010. Mucoadhesion: a review of characterization techniques. *Expert Opinion on Drug Delivery,* 7**,** 259-271.

DAVIES, R. & TAYLOR, G. The mechanics of large bubbles rising through extended liquids and through liquids in tubes. Proceedings of the Royal Society of London A: Mathematical, Physical and Engineering Sciences, 1950. *The Royal Society*, 375-390.

DE GASSART, A., GEMINARD, C., FEVRIER, B., RAPOSO, G. & VIDAL, M. 2003. Lipid raft-associated protein sorting in exosomes. *Blood,* 102**,** 4336-4344.

DE GRAAF, R. 1965. *De mulierum organis generationi inservientibus, 1672*, B. de Graaf.

DEHGHAN, M. & KHA, F. 2009. Gastroretentive drug delivery systems: A patent perspective. *International Journal of Health Research,* 2.

DE ZIEGLER, D., BULLETTI, C., DE MONSTIER, B. & JÄÄSKELÄINEN, A. 1997. The first uterine pass effect. *Annals of the New York Academy of Sciences,* 828**,** 291-299.

DEITZEL, J. M., KLEINMEYER, J., HARRIS, D. & TAN, N. B. 2001. The effect of processing variables on the morphology of electrospun nanofibers and textiles. *Polymer,* 42**,** 261-272.

DENKEWALTER, R. G., KOLC, J. & LUKASAVAGE, W. J. 1982. Preparation of lysine based macromolecular highly branched homogeneous compound. Google Patents.

DESAI, K., KIT, K., LI, J. & ZIVANOVIC, S. 2008. Morphological and surface properties of electrospun chitosan nanofibers. *Biomacromolecules,* 9**,** 1000-1006.

DEVINSKY, O., PACIA, S. V. & SHACHTER, S. C. 2005. *Complementary and alternative therapies for epilepsy*, New York, Demos Medical Publishing.

DEVROEY, P., PALERMO, G., BOURGAIN, C., VANWAESBERGHE, L., SMITZ, J. & VANSTEIRTEGHEM, A. C. 1989. PROGESTERONE ADMINISTRATION IN PATIENTS WITH ABSENT OVARIES. *International Journal of Fertility,* 34**,** 188-193.

DEWICK, P. M. 2009. *Medicinal Natural Products : A Biosynthetic Approach,* Chichester, John Wliey & Sons Ltd.

DHAWAN, S., VARMA, M. & SINHA, V. 2005. High Molecular Weight Poly (ethylene oxide)–Based Drug Delivery Systems. *Pharmaceutical Technology,* 29**,** 72-80.

DI FABIO, S., VAN ROEY, J., GIANNINI, G., VAN DEN MOOTER, G., SPADA, M., BINELLI, A., PIRILLO, M. F., GERMINARIO, E., BELARDELLI, F. & DE BETHUNE, M.-P. 2003. Inhibition of vaginal transmission of HIV-1 in hu-SCID mice by the non-nucleoside reverse transcriptase inhibitor TMC120 in a gel formulation. *Aids,* 17**,** 1597-1604.

DODD, J. M., FLENADY, V. J., CINCOTTA, R. & CROWTHER, C. A. 2008. Progesterone for the prevention of preterm birth: a systematic review. *Obstetrics & Gynecology,* 112**,** 127-134.

DOLATABADI, J. E. N., VALIZADEH, H. & HAMISHEHKAR, H. 2015. Solid lipid nanoparticles as efficient drug and gene delivery systems: Recent breakthroughs. *Advanced Pharmaceutical Bulletin,* 5**,** 151.

DOMÉNECH, R., ABIAN, O., BOCANEGRA, R., CORREA, J., SOUSA-HERVES, A., RIGUERA, R., MATEU, M. G., FERNANDEZ-MEGIA, E., VELÁZQUEZ-CAMPOY, A. & NEIRA, J. L. 2010. Dendrimers as potential inhibitors of the dimerization of the capsid protein of HIV-1. *Biomacromolecules,* 11**,** 2069-2078.

DONNELLY, R. F., MCCARRON, P. A., TUNNEY, M. M. & WOOLFSON, A. D. 2007. Potential of photodynamic therapy in treatment of fungal infections of the mouth. Design and characterisation of a mucoadhesive patch containing toluidine blue O. *Journal of Photochemistry and Photobiology B: Biology,* 86**,** 59-69.

DOWLING, A., CLIFT, R., GROBERT, N., HUTTON, D., OLIVER, R., O'NEILL, O., PETHICA, J., PIDGEON, N., PORRITT, J. & RYAN, J. 2004. Nanoscience and nanotechnologies: opportunities and uncertainties. *London: The Royal Society & The Royal Academy of Engineering Report,* 61**,** e64.

DUAN, B., DONG, C., YUAN, X. & YAO, K. 2004. Electrospinning of chitosan solutions in acetic acid with poly (ethylene oxide). *Journal of Biomaterials Science, Polymer Edition,* 15**,** 797-811.

ELEY, A. 2015. *How has IVF developed since the first 'test-tube baby'?* [Online]. British Broadcasting Coporation. Available: http://www.bbc.co.uk/news/health-33599353 [Accessed 22/02/2016 2015].

EOUANI, C., PICCERELLE, P., PRINDERRE, P., BOURRET, E. & JOACHIM, J. 2001. In-vitro comparative study of buccal mucoadhesive performance of different polymeric films. *European Journal of Pharmaceutics and Biopharmaceutics,* 52**,** 45-55.

FALLOWFIELD, L., ATKINS, L., CATT, S., COX, A., COXON, C., LANGRIDGE, C., MORRIS, R. & PRICE, M. 2006. Patients' preference for administration of endocrine treatments by injection or tablets: results from a study of women with breast cancer. *Annals of Oncology,* 17**,** 205-210.

FANCHIN, R., RIGHINI, C., DE ZIEGLER, D., OLIVENNES, F., LEDÉE, N. & FRYDMAN, R. 2001. Effects of vaginal progesterone administration on uterine contractility at the time of embryo transfer. *Fertility and Sterility,* 75**,** 1136-1140.

FANCHIN, R., RIGHINI, C., OLIVENNES, F., TAYLOR, S., DE ZIEGLER, D. & FRYDMAN, R. 1998. Uterine contractions at the time of embryo transfer alter pregnancy rates after in-vitro fertilization. *Human Reproduction,* 13**,** 1968-1974.

FAROKHZAD, O. C. & LANGER, R. 2009. Impact of nanotechnology on drug delivery. *ACS Nano,* 3**,** 16-20.

FLORY, P. 1954. *Principles of polymer chemistry / Paul John Flory,* New York, Cornell University Press.

FLORY, P. J. 1942. Random Reorganization of Molecular Weight Distribution in Linear Condensation Polymers1. *Journal of the American Chemical Society,* 64**,** 2205-2212.

FONSECA, E. B., CELIK, E., PARRA, M., SINGH, M. & NICOLAIDES, K. H. 2007. Progesterone and the risk of preterm birth among women with a short cervix. *New England Journal of Medicine,* 357**,** 462-469.

FRECH, R. & HUANG, W. 1995. Conformational changes in diethylene glycol dimethyl ether and poly (ethylene oxide) induced by lithium ion complexation. *Macromolecules,* 28**,** 1246-1251.

FRENOT, A., HENRIKSSON, M. W. & WALKENSTRÖM, P. 2007. Electrospinning of cellulose-based nanofibers. *Journal of Applied Polymer Science,* 103**,** 1473-1482.

FREUNDLICH, H. & HATFIELD, H. S. 1926. *Colloid and capillary chemistry,* London, Methuen And Co. Ltd.

FROBENIUS, W. 1997. [Ludwig Fraenkel, corpus luteum and discovery of progesterone]. *Zentralblatt fur Gynakologie,* 120**,** 317-323.

FUCHS, F., AUDIBERT, F. & SENAT, M.-V. 2014. Progesterone and preterm delivery: back to the future? *Gynecologie, Obstetrique & Fertilite,* 42**,** 112-122.

FUNT, M., THOMPSON, J. & BIRCH, H. 1978. Normal vaginal axis. *Southern Medical Journal,* 71**,** 1534-5, 1552.

GAJBHIYE, V., PALANIRAJAN, V. K., TEKADE, R. K. & JAIN, N. K. 2009. Dendrimers as therapeutic agents: a systematic review. *Journal of Pharmacy and Pharmacology,* 61**,** 989-1003.

GARDNER, D. J., OPORTO, G. S., MILLS, R. & SAMIR, M. A. S. A. 2008. Adhesion and surface issues in cellulose and nanocellulose. *Journal of Adhesion Science and Technology,* 22**,** 545-567.

GIANGRANDE, P. H. & MCDONNELL, D. P. 1998. The A and B isoforms of the human progesterone receptor: two functionally different transcription factors encoded by a single gene. *Recent Progress in Hormone Research,* 54**,** 291-313; discussion 313-4.

GLAVAS-DODOV, M., GORACINOVA, K., MLADENOVSKA, K. & FREDRO-KUMBARADZI, E. 2002. Release profile of lidocaine HCl from topical liposomal gel formulation. *International Journal of Pharmaceutics,* 242**,** 381-384.

GOMBOTZ, W. R. & WEE, S. F. 2012. Protein release from alginate matrices. *Advanced Drug Delivery Reviews,* 64**,** 194-205.

GRABOVAC, V., GUGGI, D. & BERNKOP-SCHNÜRCH, A. 2005. Comparison of the mucoadhesive properties of various polymers. *Advanced Drug Delivery Reviews,* 57**,** 1713-1723.

GRAHAM, J. D. & CLARKE, C. L. 1997. Physiological action of progesterone in target tissues 1. *Endocrine reviews,* 18**,** 502-519.

GRANT, R. M., HAMER, D., HOPE, T., JOHNSTON, R., LANGE, J., LEDERMAN, M. M., LIEBERMAN, J., MILLER, C. J., MOORE, J. P. & MOSIER, D. E. 2008. Whither or wither microbicides? *Science,* 321**,** 532-534.

GULATI, M., GROVER, M., SINGH, S. & SINGH, M. 1998. Lipophilic drug derivatives in liposomes. *International Journal of Pharmaceutics,* 165**,** 129-168.

GURNY, R., MEYER, J.-M. & PEPPAS, N. A. 1984. Bioadhesive intraoral release systems: design, testing and analysis. *Biomaterials,* 5**,** 336-340.

HÄGERSTRÖM, H. & EDSMAN, K. 2001. Interpretation of mucoadhesive properties of polymer gel preparations using a tensile strength method. *Journal of Pharmacy and Pharmacology,* 53**,** 1589-1599.

HÄGERSTRÖM, H. & EDSMAN, K. 2003. Limitations of the rheological mucoadhesion method: the effect of the choice of conditions and the rheological synergism parameter. *European journal of pharmaceutical sciences,* 18**,** 349-357.

HAM, A. S., COST, M. R., SASSI, A. B., DEZZUTTI, C. S. & ROHAN, L. C. 2009. Targeted delivery of PSC-RANTES for HIV-1 prevention using biodegradable nanoparticles. *Pharmaceutical Research,* 26**,** 502-511.

HARTMANN, M. & WETTSTEIN, A. 1934. Ein krystallisiertes Hormon aus Corpus luteum.(Vorläufige Mitteilung). *Helvetica Chimica Acta,* 17**,** 878-882.

HE, J.-H., LIU, Y., MO, L.-F., WAN, Y.-Q. & XU, L. 2008. *Electrospun nanofibres and their applications*, Akron, Smithers Rapra Technology.

HELMS, J. B. & ZURZOLO, C. 2004. Lipids as targeting signals: Lipid rafts and intracellular trafficking. *Traffic,* 5**,** 247-254.

HENDRIX, C. W., CAO, Y. J. & FUCHS, E. J. 2009. Topical microbicides to prevent HIV: clinical drug development challenges. *Annual Review of Pharmacology and Toxicology,* 49**,** 349-375.

HIGUCHI, T. 1961. Rate of release of medicaments from ointment bases containing drugs in suspension. *Journal of Pharmaceutical Sciences,* 50**,** 874-875.

HIGUCHI, T. 1963. Mechanism of sustained-action medication. Theoretical analysis of rate of release of solid drugs dispersed in solid matrices. *Journal of Pharmaceutical Sciences,* 52**,** 1145-1149.

HIRANI, J. J., RATHOD, D. A. & VADALIA, K. R. 2009. Orally disintegrating tablets: a review. *Tropical Journal of Pharmaceutical Research,* 8.

HIXSON, A. & CROWELL, J. 1931. Dependence of reaction velocity upon surface and agitation. *Industrial & Engineering Chemistry,* 23**,** 1160-1168.

HOCK, C., STRASSBURG, S., HABERLAND, H., ISSENDORFF, B. V., AGUADO, A. & SCHMIDT, M. 2008. Melting-Point Depression by Insoluble Impurities: A Finite Size Effect. *Physical Review Letters,* 101**,** 23401.

HOLLABAUGH, C., BURT, L. H. & WALSH, A. P. 1945. Carboxymethylcellulose. Uses and applications. *Industrial & Engineering Chemistry,* 37**,** 943-947.

HONG, X., EDIRISINGHE, M. & MAHALINGAM, S. 2016. Beads, beaded-fibres and fibres: Tailoring the morphology of poly (caprolactone) using pressurised gyration. *Materials Science and Engineering: C,* 69**,** 1373-1382.

HOOD, J. L., JALLOUK, A. P., CAMPBELL, N., RATNER, L. & WICKLINE, S. A. 2013. Cytolytic nanoparticles attenuate HIV-1 infectivity. *Antiviral Therapy,* 18**,** 95-103.

HOU, Z., LI, X., LI, C., DAI, Y., MA, P. A., ZHANG, X., KANG, X., CHENG, Z. & LIN, J. 2013. Electrospun upconversion composite fibers as dual drugs delivery system with individual release properties. *Langmuir,* 29**,** 9473-9482.

HU, X., LIU, S., ZHOU, G., HUANG, Y., XIE, Z. & JING, X. 2014. Electrospinning of polymeric nanofibers for drug delivery applications. *Journal of Controlled Release,* 185**,** 12-21.

HUANG, Y., LEOBANDUNG, W., FOSS, A. & PEPPAS, N. A. 2000. Molecular aspects of muco- and bioadhesion:: Tethered structures and site-specific surfaces. *Journal of Controlled Release,* 65**,** 63-71.

HUANG, Z.-M., ZHANG, Y.-Z., KOTAKI, M. & RAMAKRISHNA, S. 2003. A review on polymer nanofibers by electrospinning and their applications in nanocomposites. *Composites Science and Technology,* 63**,** 2223-2253.

HUDSON, T. *Women's Health Update: Wild Yam, Natural Progesterone, Unraveling the Confusion* [Online]. Available: http://www.encognitive.com/node/12926 [Accessed 09/02/2016 2016].

HUSSAIN, A. & AHSAN, F. 2005. The vagina as a route for systemic drug delivery. *Journal of Controlled Release,* 103**,** 301-313.

ILLANGAKOON, U. E., MAHALINGAM, S., COLOMBO, P. & EDIRISINGHE, M. 2016. Tailoring the surface of polymeric nanofibres generated by pressurised gyration. *Surface Innovations,* 4**,** 167-178.

IMMORDINO, M. L., DOSIO, F. & CATTEL, L. 2006. Stealth liposomes: review of the basic science, rationale, and clinical applications, existing and potential. *International Journal of Nanomedicine,* 1**,** 297.

ISAACS, C. E. 2001. The antimicrobial function of milk lipids. *Advances in Nutritional Research.* Springer.

ISAACS, C. E. & THORMAR, H. 1991. The role of milk-derived antimicrobial lipids as antiviral and antibacterial agents. *Immunology of Milk and the Neonate.* Springer.

IVARSSON, D. & WAHLGREN, M. 2012. Comparison of in vitro methods of measuring mucoadhesion: Ellipsometry, tensile strength and rheological measurements. *Colloids and Surfaces B: Biointerfaces,* 92**,** 353-359.

JACKSON, C. L. & MCKENNA, G. B. 1990. The melting behavior of organic materials confined in porous solids. *The Journal of Chemical Physics,* 93**,** 9002-9011.

JALLOUK, A. P., MOLEY, K. H., OMURTAG, K., HU, G., LANZA, G. M., WICKLINE, S. A. & HOOD, J. L. 2014. Nanoparticle incorporation of melittin reduces sperm and vaginal epithelium cytotoxicity. *PloS one,* 9**,** e95411.

JAYARAMAN, K., KOTAKI, M., ZHANG, Y., MO, X. & RAMAKRISHNA, S. 2004. Recent advances in polymer nanofibers. *Journal of Nanoscience and Nanotechnology,* 4**,** 52-65.

JIMÉNEZ-CASTELLANOS, M. R., ZIA, H. & RHODES, C. 1993. Mucoadhesive drug delivery systems. *Drug Development and Industrial Pharmacy,* 19**,** 143-194.

JIMÉNEZ, J. L., PION, M., DE LA MATA, F. J., GOMEZ, R., MUÑOZ, E., LEAL, M. & MUÑOZ-FERNANDEZ, M. A. 2012. Dendrimers as topical microbicides with activity against HIV. *New Journal of Chemistry,* 36**,** 299-309.

JOERGENSEN, L., KLÖSGEN, B., SIMONSEN, A. C., BORCH, J. & HAGESAETHER, E. 2011. New insights into the mucoadhesion of pectins by AFM roughness parameters in combination with SPR. *International Journal of Pharmaceutics,* 411**,** 162-168.

JOHANSSON, E. D. 1972. Plasma levels of progesterone achieved by different routes of administration. *Acta Obstetricia et Gynecologica Scandinavica,* 51**,** 17-20.

JOHNSON, W. S., GRAVESTOCK, M. B. & MCCARRY, B. E. 1971. Acetylenic bond participation in biogenetic-like olefinic cyclizations. II. Synthesis of dl-progesterone. *Journal of the American Chemical Society,* 93**,** 4332-4334.

KAELBLE, D. H. & MOACANIN, J. 1977. A surface energy analysis of bioadhesion. *Polymer,* 18**,** 475-482.

KASAAI, M. R. 2007. Calculation of Mark–Houwink–Sakurada (MHS) equation viscometric constants for chitosan in any solvent–temperature system using experimental reported viscometric constants data. *Carbohydrate polymers,* 68**,** 477-488.

KATOU, S., KEARNEY, P. & YARWOOD, R. 1993. The zydis fast dissolving oral dosage form. *Pharm Tech Japan,* 9**,** 713-719.

KATZ, V. L., LENTZ, G. M., LOBO, R. A. & GERSHENSON, D. M. 2007. *Comprehensive Gynecology*, Mosby Elsevier Philadelphia.

KEARNEY, P., RATHBONE, M. & HADGRAFT, R. 2002. The Zydis oral fast-dissolving dosage form. *Modified-release Drug Delivery Technology,* 2**,** 191-201.

KENAWY, E.-R., ABDEL-HAY, F. I., EL-NEWEHY, M. H. & WNEK, G. E. 2009. Processing of polymer nanofibers through electrospinning as drug delivery systems. *Materials Chemistry and Physics,* 113**,** 296-302.

KESHARWANI, P., JAIN, K. & JAIN, N. K. 2014. Dendrimer as nanocarrier for drug delivery. *Progress in Polymer Science,* 39**,** 268-307.

KHDAIR, A., HAMAD, I., AL-HUSSAINI, M., ALBAYATI, D., ALKHATIB, H. & ALKHALIDI, B. 2013. In vitro artificial membrane-natural mucosa correlation of carvedilol buccal delivery. *Journal of Drug Delivery Science and Technology,* 23**,** 603-609.

KHUTORYANSKIY, V. V. 2011. Advances in mucoadhesion and mucoadhesive polymers. *Macromolecular Bioscience,* 11**,** 748-764.

KING, T. L. & BRUCKER, M. C. 2010. *Pharmacology for Women's Health*, Burlington, Jones & Bartlett Publishers.

KRIEGEL, C., KIT, K., MCCLEMENTS, D. J. & WEISS, J. 2009. Electrospinning of chitosan–poly (ethylene oxide) blend nanofibers in the presence of micellar surfactant solutions. *Polymer,* 50**,** 189-200.

KROGSTAD, E. A. & WOODROW, K. A. 2014. Manufacturing scale-up of electrospun poly (vinyl alcohol) fibers containing tenofovir for vaginal drug delivery. *International Journal of Pharmaceutics,* 475**,** 282-291.

KUMAR, C. S. 2006. Biological and pharmaceutical nanomaterials. *Biological and Pharmaceutical Nanomaterials, by Challa SSR Kumar (Editor), pp. 425. Weinheim, Wiley-VCH*

LAI, S. K., O'HANLON, D. E., HARROLD, S., MAN, S. T., WANG, Y.-Y., CONE, R. & HANES, J. 2007. Rapid transport of large polymeric nanoparticles in fresh undiluted human mucus. *Proceedings of the National Academy of Sciences,* 104**,** 1482-1487.

LARSEN, C. E., NIR, S., ALFORD, D. R., JENNINGS, M., LEE, K.-D. & DÜZGÜNEŞ, N. 1993. Human immunodeficiency virus type 1 (HIV-1) fusion with model membranes: kinetic analysis and the role of lipid composition, pH and divalent cations. *Biochimica et Biophysica Acta (BBA) - Biomembranes,* 1147**,** 223-236.

LEDNICER, D. 2010. *Steroid chemistry at a glance : Chemistry at a Glance,* Chichester, John Wiley & Sons.

LEDNICER, D. 2011. *Steroid chemistry at a glance,* Chichester, John Wiley & Sons.

LEE, K. Y., JEONG, L., KANG, Y. O., LEE, S. J. & PARK, W. H. 2009. Electrospinning of polysaccharides for regenerative medicine. *Advanced Drug Delivery Reviews,* 61**,** 1020-1032.

LEE, K. Y. & MOONEY, D. J. 2012. Alginate: properties and biomedical applications. *Progress in Polymer Science,* 37**,** 106-126.

LEE, N. S., DOHJIMA, T., BAUER, G., LI, H., LI, M.-J., EHSANI, A., SALVATERRA, P. & ROSSI, J. 2002. Expression of small interfering RNAs targeted against HIV-1 rev transcripts in human cells. *Nature Biotechnology,* 20**,** 500-505.

LEHN, J.-M. 1988. Supramolecular chemistry—Scope and perspectives: Molecules—Supermolecules—Molecular devices. *Journal of Inclusion Phenomena,* 6**,** 351-396.

LEHN, J.-M. 1995. *Supramolecular chemistry,* Basel, Vch, Weinheim.

LEHR, C.-M., BOUWSTRA, J. A., SCHACHT, E. H. & JUNGINGER, H. E. 1992. In vitro evaluation of mucoadhesive properties of chitosan and some other natural polymers. *International Journal of Pharmaceutics,* 78**,** 43-48.

LETCHFORD, K. & BURT, H. 2007. A review of the formation and classification of amphiphilic block copolymer nanoparticulate structures: micelles, nanospheres, nanocapsules and polymersomes. *European Journal of Pharmaceutics and Biopharmaceutics,* 65**,** 259-269.

LEUNG, S.-H. S. & ROBINSON, J. R. 1990. Polymer structure features contributing to mucoadhesion. II. *Journal of Controlled Release,* 12**,** 187-194.

LEUNG, V. & KO, F. 2011. Biomedical applications of nanofibers. *Polymers for Advanced Technologies,* 22**,** 350-365.

LEVY, T., GUREVITCH, S., BAR-HAVA, I., ASHKENAZI, J., MAGAZANIK, A., HOMBURG, R., ORVIETO, R. & BEN-RAFAEL, Z. 1999. Pharmacokinetics of natural progesterone administered in the form of a vaginal tablet. *Human Reproduction,* 14**,** 606-610.

LI, F., ZHAO, Y. & SONG, Y. 2010. Core-shell nanofibers: nano channel and capsule by coaxial electrospinning. *Nanofibers.* InTech.

LI, X. & HSU, S. 1984. An analysis of the crystallization behavior of poly (ethylene oxide)/poly (methyl methacrylate) blends by spectroscopic and calorimetric techniques. *Journal of Polymer Science: Polymer Physics Edition,* 22**,** 1331-1342.

LIAO, I., CHEW, S. & LEONG, K. 2006. Aligned core-shell nanofibers delivering bioactive proteins. *Nanomedicine,* 1**,** 465-471.

LIM, J. & SIMANEK, E. E. 2012. Triazine dendrimers as drug delivery systems: From synthesis to therapy. *Advanced Drug Delivery Reviews,* 64**,** 826-835.

LIU, R. 2008. *Water-insoluble drug formulation,* Boca Raton, CRC Press.

LIU, Z., GOSANGARI, S. L., TOOPS, D. S. & FATMI, A. 2015. Progesterone solutions for increased bioavailability. Google Patents.

LLORENS, E., DEL VALLE, L. J., DÍAZ, A., CASAS, M. T. & PUIGGALÍ, J. 2013. Polylactide nanofibers loaded with vitamin B6 and polyphenols as bioactive platform for tissue engineering. *Macromolecular Research,* 21**,** 775-787.

LOFTSSON, T. & MASSON, M. 2001. Cyclodextrins in topical drug formulations: theory and practice. *International Journal of Pharmaceutics,* 225**,** 15-30.

LOWRY, D. 2015. Vaginal Delivery of Subunit Vaccines. *Subunit Vaccine Delivery.* Springer.

LU, Y., LI, Y., ZHANG, S., XU, G., FU, K., LEE, H. & ZHANG, X. 2013. Parameter study and characterization for polyacrylonitrile nanofibers fabricated via centrifugal spinning process. *European Polymer Journal,* 49**,** 3834-3845.

LUO, C., STOYANOV, S. D., STRIDE, E., PELAN, E. & EDIRISINGHE, M. 2012. Electrospinning versus fibre production methods: from specifics to technological convergence. *Chemical Society Reviews,* 41**,** 4708-4735.

LYDON, J. P., DEMAYO, F. J., FUNK, C. R., MANI, S. K., HUGHES, A. R., MONTGOMERY, C., SHYAMALA, G., CONNEELY, O. M. & O'MALLEY, B. W. 1995. Mice lacking progesterone receptor exhibit pleiotropic reproductive abnormalities. *Genes & Development,* 9**,** 2266-2278.

MACHT, D. I. 1918. On the absorption of drugs and poisons through the vagina. *Journal of Pharmacology and Experimental Therapeutics,* 10**,** 509-522.

MADSEN, F., EBERTH, K. & SMART, J. D. 1998. A rheological assessment of the nature of interactions between mucoadhesive polymers and a homogenised mucus gel. *Biomaterials,* 19**,** 1083-1092.

MAHALINGAM, S. & EDIRISINGHE, M. 2013. Forming of polymer nanofibers by a pressurised gyration process. *Macromolecular Rapid Communications,* 34**,** 1134-1139.

MAHALINGAM, S., REN, G. & EDIRISINGHE, M. 2014. Rheology and pressurised gyration of starch and starch-loaded poly (ethylene oxide). *Carbohydrate polymers,* 114**,** 279-287.

MALAVIA, N. K., ZURAKOWSKI, D., SCHROEDER, A., PRINCIOTTO, A. M., LAURY, A. R., BARASH, H. E., SODROSKI, J., LANGER, R., MADANI, N. & KOHANE, D. S. 2011. Liposomes for HIV prophylaxis. *Biomaterials,* 32**,** 8663-8668.

MALIK, R., GARG, T., GOYAL, A. K. & RATH, G. 2015. Polymeric nanofibers: targeted gastro-retentive drug delivery systems. *Journal of Drug Targeting,* 23**,** 109-124.

MALLIPEDDI, R. & ROHAN, L. C. 2010. Nanoparticle-based vaginal drug delivery systems for HIV prevention. *Expert Opinion on Drug Delivery,* 7**,** 37-48.

MANGELSDORF, D. J., THUMMEL, C., BEATO, M., HERRLICH, P., SCHÜTZ, G., UMESONO, K., BLUMBERG, B., KASTNER, P., MARK, M. & CHAMBON, P. 1995. The nuclear receptor superfamily: the second decade. *Cell,* 83**,** 835-839.

MANO, J., KONIAROVA, D. & REIS, R. 2003. Thermal properties of thermoplastic starch/synthetic polymer blends with potential biomedical applicability. *Journal of Materials Science: Materials in Medicine,* 14**,** 127-135.

MANSURI, S., KESHARWANI, P., JAIN, K., TEKADE, R. K. & JAIN, N. K. 2016. Mucoadhesion: A promising approach in drug delivery system. *Reactive and Functional Polymers,* 100**,** 151-172.

MARKER, R. E. & KRUEGER, J. 1940. Sterols. CXII. Sapogenins. XLI. The Preparation of Trillin and its Conversion to Progesterone. *Journal of the American Chemical Society,* 62**,** 3349-3350.

MARQUES, M. R., LOEBENBERG, R. & ALMUKAINZI, M. 2011. Simulated biological fluids with possible application in dissolution testing. *Dissolution Technology,* 18**,** 15-28.

MARSCHÜTZ, M. K. & BERNKOP-SCHNÜRCH, A. 2002. Thiolated polymers: self-crosslinking properties of thiolated 450 kDa poly (acrylic acid) and their influence on mucoadhesion. *European Journal of Pharmaceutical Sciences,* 15**,** 387-394.

MATHIOWITZ, E., CHICKERING III, D. E. & LEHR, C.-M. 1999. *Bioadhesive drug delivery systems: fundamentals, novel approaches, and development*, Boca Raton, CRC Press.

MAXSON, W. S., HARGROVE, J. T. & MEYERS, P. G. 1992. Oral administration of micronized progesterone in an oil vehicle high in glycerides of polyunsaturated fatty acids. Google Patents.

MCGOWAN, I., GOMEZ, K., BRUDER, K., FEBO, I., CHEN, B. A., RICHARDSON, B. A., HUSNIK, M., LIVANT, E., PRICE, C. & JACOBSON, C. 2011. Phase 1 randomized trial of the vaginal safety and acceptability of SPL7013 gel (VivaGel®) in sexually active young women (MTN-004). *AIDS (London, England),* 25**,** 1057.

MEDICINE, P. C. O. T. A. S. F. R. 2008. Progesterone supplementation during the luteal phase and in early pregnancy in the treatment of infertility: an educational bulletin. *Fertility and Sterility,* 89**,** 789-792.

MEHNERT, W. & MÄDER, K. 2001. Solid lipid nanoparticles: Production, characterization and applications. *Advanced Drug Delivery Reviews,* 47**,** 165-196.

MENCHICCHI, B., FUENZALIDA, J., BOBBILI, K. B., HENSEL, A., SWAMY, M. J. & GOYCOOLEA, F. 2014. Structure of chitosan determines its interactions with mucin. *Biomacromolecules,* 15**,** 3550-3558.

MENG, J., STURGIS, T. F. & YOUAN, B.-B. C. 2011. Engineering tenofovir loaded chitosan nanoparticles to maximize microbicide mucoadhesion. *European Journal of Pharmaceutical Sciences,* 44**,** 57-67.

MINTZER, M. A. & GRINSTAFF, M. W. 2011. Biomedical applications of dendrimers: a tutorial. *Chemical Society Reviews,* 40**,** 173-190.

MORTAZAVI, S. A. & MOGHIMI, H. R. 2010. Effect of surfactant type and concentration on the duration of mucoadhesion of carbopol 934 and HPMC solid compacts. *Iranian Journal of Pharmaceutical Research***,** 191-199.

MORTAZAVI, S. A. & SMART, J. D. 1995. An investigation of some factors influencing the in vitro assessment of mucoadhesion. *International Journal of Pharmaceutics,* 116**,** 223-230.

MOSCICKI, A.-B., RUPERT, K., YIFEI, M., SCOTT, M. E., DAUD, I. I., BUKUSI, E. A., SHIBOSKI, S., REBBAPRAGADA, A., HUIBNER, S. & COHEN, C. R. 2012. Measurement of mucosal biomarkers in a phase 1 trial of intravaginal 3% SPL 7013 gel (VivaGel®) to assess expanded safety. *Journal of Acquired Immune Deficiency Syndromes (1999),* 59**,** 134.

MURAMATSU, M., IWAHASHI, M. & TAKEUCHI, U. 1979. Thermodynamic relationship between α-and β-forms of crystalline progesterone. *Journal of Pharmaceutical Sciences,* 68**,** 175-177.

MURUGAVEL, K. 2014. Benzylic viologen dendrimers: a review of their synthesis, properties and applications. *Polymer Chemistry,* 5**,** 5873-5884.

NAHOUL, K., DEHENNIN, L., JONDET, M. & ROGER, M. 1993. Profiles of plasma estrogens, progesterone and their metabolites after oral or vaginal administration of estradiol or progesterone. *Maturitas,* 16**,** 185-202.

NAM, S. H., SHIM, H.-S., KIM, Y.-S., DAR, M. A., KIM, J. G. & KIM, W. B. 2010. Ag or Au nanoparticle-embedded one-dimensional composite TiO2 nanofibers prepared via electrospinning for use in lithium-ion batteries. *Acs Applied Materials & Interfaces,* 2**,** 2046-2052.

NASIBULLAH, M., HASSAN, F., AHMAD, N., KHAN, A. R. & RAHMAN, M. 2013. Dendrimers as Novel Polymeric Material: A Review on Its Synthesis, Characterization and Their Applications. *Advanced Science Focus,* 1**,** 197-204.

NCBI. 2004. *Compound Summary for Progesterone* [Online]. Bethseda, Maryland: National Center for Biotechnology Information. Available: https://pubchem.ncbi.nlm.nih.gov/compound/progesterone#section=Top [Accessed 20/06/2017 2017].

NDESENDO, V. M., PILLAY, V., CHOONARA, Y. E., BUCHMANN, E., BAYEVER, D. N. & MEYER, L. C. 2008. A review of current intravaginal drug delivery approaches employed for the prophylaxis of HIV/AIDS and prevention of sexually transmitted infections. *AAPS Pharmscitech,* 9**,** 505-520.

NEVES, J. D., PALMEIRA-DE-OLIVEIRA, R., PALMEIRA-DE-OLIVEIRA, A., RODRIGUES, F. & SARMENTO, B. 2014. *Vaginal mucosa and drug delivery,* New Jersey, John Wiley & Sons Ltd.

NEWKOME, G. R., YAO, Z., BAKER, G. R. & GUPTA, V. K. 1985. Micelles. Part 1. Cascade molecules: a new approach to micelles. A [27]-arborol. *The Journal of Organic Chemistry,* 50**,** 2003-2004.

NGUYEN, T., MENOCAL, E. M., HARBORTH, J. & FRUEHAUF, J. H. 2008. RNAi therapeutics: an update on delivery. *Current opinion in molecular therapeutics,* 10**,** 158-167.

NILLIUS, S. J. & JOHANSSON, E. D. 1971. Plasma levels of progesterone after vaginal, rectal, or intramuscular administration of progesterone. *American Journal of Obstetrics and Gynecology,* 110**,** 470-477.

NORIEGA-LUNA, B., GODÍNEZ, L. A., RODRÍGUEZ, F. J., RODRÍGUEZ, A., ZALDÍVAR-LELO DE LARREA, G., SOSA-FERREYRA, C., MERCADO-CURIEL, R., MANRÍQUEZ, J. & BUSTOS, E. 2014. Applications of Dendrimers in Drug Delivery Agents, Diagnosis, Therapy, and Detection. *Journal of Nanomaterials,* 2014.

OWEN, A. & RANNARD, S. 2016. Strengths, weaknesses, opportunities and challenges for long acting injectable therapies: Insights for applications in HIV therapy. *Advanced Drug Delivery Reviews*.

PADRON, S., FUENTES, A., CARUNTU, D. & LOZANO, K. 2013. Experimental study of nanofiber production through forcespinning. *Journal of Applied Physics,* 113**,** 024318.

PALLISER, D., CHOWDHURY, D., WANG, Q.-Y., LEE, S. J., BRONSON, R. T., KNIPE, D. M. & LIEBERMAN, J. 2006. An siRNA-based microbicide protects mice from lethal herpes simplex virus 2 infection. *Nature,* 439**,** 89-94.

PARHI, P., MOHANTY, C. & SAHOO, S. K. 2012. Nanotechnology-based combinational drug delivery: an emerging approach for cancer therapy. *Drug Discovery Today,* 17**,** 1044-1052.

PARK, H. & ROBINSON, J. R. 1987. Mechanisms of mucoadhesion of poly (acrylic acid) hydrogels. *Pharmaceutical Research,* 4**,** 457-464.

PARVEEN, S., MISRA, R. & SAHOO, S. K. 2012. Nanoparticles: a boon to drug delivery, therapeutics, diagnostics and imaging. *Nanomedicine: Nanotechnology, Biology and Medicine,* 8**,** 147-166.

PATEL, D., SMITH, J. R., SMITH, A. W., GRIST, N., BARNETT, P. & SMART, J. D. 2000. An atomic force microscopy investigation of bioadhesive polymer adsorption onto human buccal cells. *International Journal of Pharmaceutics,* 200**,** 271-277.

PATEL, M. M., SMART, J. D., NEVELL, T. G., EWEN, R. J., EATON, P. J. & TSIBOUKLIS, J. 2003. Mucin/poly (acrylic acid) interactions: a spectroscopic investigation of mucoadhesion. *Biomacromolecules,* 4**,** 1184-1190.

PAYNE, R., ROBERTS, R., ROWE, R. & DOCHERTY, R. 1999. Examples of successful crystal structure prediction: polymorphs of primidone and progesterone. *International Journal of Pharmaceutics,* 177**,** 231-245.

PEPPAS, N. A. & BURI, P. A. 1985. Surface, interfacial and molecular aspects of polymer bioadhesion on soft tissues. *Journal of Controlled Release,* 2**,** 257-275.

PEPPAS, N. A., LITTLE, M. D. & HUANG, Y. 2000. Bioadhesive controlled release systems. *Handbook of pharmaceutical controlled release technology. New York: Marcel Dekker***,** 255-69.

PEPPAS, N. A. & NARASIMHAN, B. 2014. Mathematical models in drug delivery: How modeling has shaped the way we design new drug delivery systems. *Journal of Controlled Release,* 190**,** 75-81.

PEPPAS, N. A. & SAHLIN, J. J. 1996. Hydrogels as mucoadhesive and bioadhesive materials: a review. *Biomaterials,* 17**,** 1553-1561.

PETERLIN, A. 1971. Molecular model of drawing polyethylene and polypropylene. *Journal of Materials Science,* 6**,** 490-508.

PHAM, Q. P., SHARMA, U. & MIKOS, A. G. 2006. Electrospinning of polymeric nanofibers for tissue engineering applications: a review. *Tissue Engineering,* 12**,** 1197-1211.

PILLAY, V., DOTT, C., CHOONARA, Y. E., TYAGI, C., TOMAR, L., KUMAR, P., DU TOIT, L. C. & NDESENDO, V. M. 2013. A review of the effect of processing variables on the fabrication of electrospun nanofibers for drug delivery applications. *Journal of Nanomaterials,* 2013.

POLLER, B., STRACHAN, C., BROADBENT, R. & WALKER, G. F. 2017. A minitablet formulation made from electrospun nanofibers. *European Journal of Pharmaceutics and Biopharmaceutics,* 114**,** 213-220.

PONCHEL, G., TOUCHARD, F., DUCHÊNE, D. & PEPPAS, N. A. 1987. Bioadhesive analysis of controlled-release systems. I. Fracture and interpenetration analysis in poly (acrylic acid)-containing systems. *Journal of Controlled Release,* 5**,** 129-141.

POPE, M. & HAASE, A. T. 2003. Transmission, acute HIV-1 infection and the quest for strategies to prevent infection. *Nature Medicine,* 9**,** 847-852.

POTTER, L., OAKLEY, D., DE LEON-WONG, E. & CAÑAMAR, R. 1996. Measuring compliance among oral contraceptive users. *Family Planning Perspectives***,** 154-158.

PRABHU, R. H., PATRAVALE, V. B. & JOSHI, M. D. 2015. Polymeric nanoparticles for targeted treatment in oncology: current insights. *International Journal of Nanomedicine,* 10**,** 1001.

PRASANNA, R. I., ANITHA, P. & CHETTY, C. M. 2011. Formulation and evaluation of bucco-adhesive tablets of sumatriptan succinate. *International Journal of Pharmaceutical Investigation,* 1**,** 182.

PRENANT, A. 1898. La valeur morphologique du corps jaune. Son action physiologique et therapeutique possible. *Rev. Gen. Sci. Pure Appl,* 9**,** 646-650.

PRICE, J., ISMAIL, H., GORWILL, R. & SARDA, I. 1983. Effect of the suppository base on progesterone delivery from the vagina. *Fertility and Sterility,* 39**,** 490-493.

QI, R., GUO, R., SHEN, M., CAO, X., ZHANG, L., XU, J., YU, J. & SHI, X. 2010. Electrospun poly (lactic-co-glycolic acid)/halloysite nanotube composite nanofibers for drug encapsulation and sustained release. *Journal of Materials Chemistry,* 20**,** 10622-10629.

RAHAMATULLAH SHAIKH, T. R. R. S., GARLAND, M. J., WOOLFSON, A. D. & DONNELLY, R. F. 2011. Mucoadhesive drug delivery systems. *Journal of Pharmacy and Bioallied Sciences,* 3**,** 89.

RAIMI-ABRAHAM, B. T., MAHALINGAM, S., EDIRISINGHE, M. & CRAIG, D. Q. M. 2014. Generation of poly(N-vinylpyrrolidone) nanofibres using pressurised gyration. *Materials Science and Engineering: C,* 39**,** 168-176.

REDDY, B. B. K. & KARUNAKAR, A. 2011. Biopharmaceutics classification system: a regulatory approach. *Dissolution Technology,* 18**,** 31-37.

REMINGTON, J. P. & ALLEN, L. V. 2013. *Remington : The science and practice of pharmacy,* London, Pharmaceutical Press.

REN, L., PANDIT, V., ELKIN, J., DENMAN, T., COOPER, J. A. & KOTHA, S. P. 2013. Large-scale and highly efficient synthesis of micro-and nano-fibers with controlled fiber morphology by centrifugal jet spinning for tissue regeneration. *Nanoscale,* 5**,** 2337-2345.

RENÇBER, S., KARAVANA, S. Y., YıLMAZ, F. F., ERAÇ, B., NENNI, M., ÖZBAL, S., PEKÇETIN, Ç., GURER-ORHAN, H., HOŞGÖR-LIMONCU, M., GÜNERI, P. & ERTAN, G. 2016. Development, characterization, and in vivo assessment of mucoadhesive nanoparticles containing fluconazole for the local treatment of oral candidiasis. *International Journal of Nanomedicine,* 11**,** 2641-2653.

RIM, N. G., SHIN, C. S. & SHIN, H. 2013. Current approaches to electrospun nanofibers for tissue engineering. *Biomedical materials,* 8**,** 014102.

ROGERS, B., ADAMS, J. & PENNATHUR, S. 2014. *Nanotechnology: understanding small systems*, Boca Raton, CRC Press.

ROHAN, L., DEVLIN, B. & YANG, H. 2013. Microbicide dosage forms. *Microbicides for Prevention of HIV Infection.* Berlin Heidelberg: Springer.

ROHAN, L. C. & SASSI, A. B. 2009. Vaginal drug delivery systems for HIV prevention. *The AAPS journal,* 11**,** 78-87.

ROSSI, S., BONFERONI, M. C., LIPPOLI, G., BERTONI, M., FERRARI, F., CARAMELLA, C. & CONTE, U. 1995. Influence of mucin type on polymer-mucin rheological interactions. *Biomaterials,* 16**,** 1073-1079.

ROW, R. C., SHESKEY, P. J. & QUINN, M. E. (eds.) 2009. *Handbook of pharmaceutical excipients.* London, Pharmaceutical Press.

ROY, R. 1996. Syntheses and some applications of chemically defined multivalent glycoconjugates. *Current Opinion in Structural Biology,* 6**,** 692-702.

ROY, S., PAL, K., ANIS, A., PRAMANIK, K. & PRABHAKAR, B. 2009. Polymers in mucoadhesive drug-delivery systems: a brief note. *Designed Monomers and Polymers,* 12**,** 483-495.

ROY, U., RODRÍGUEZ, J., BARBER, P., DAS NEVES, J., SARMENTO, B. & NAIR, M. 2015. The potential of HIV-1 nanotherapeutics: from in vitro studies to clinical trials. *Nanomedicine,* 10**,** 3597-3609.

RUIZ, A. D. & DANIELS, K. R. 2013. The effectiveness of sublingual and topical compounded bioidentical hormone replacement therapy in postmenopausal women: an observational cohort study. *International Journal of Pharmaceutical Compounding,* 18**,** 70-77.

SALALHA, W., DROR, Y., KHALFIN, R. L., COHEN, Y., YARIN, A. L. & ZUSSMAN, E. 2004. Single-walled carbon nanotubes embedded in oriented polymeric nanofibers by electrospinning. *Langmuir,* 20**,** 9852-9855.

SANTOS, S. S., LORENZONI, A., PEGORARO, N. S., DENARDI, L. B., ALVES, S. H., SCHAFFAZICK, S. R. & CRUZ, L. 2014. Formulation and in vitro evaluation of coconut oil-core cationic nanocapsules intended for vaginal delivery of clotrimazole. *Colloids and Surfaces B: Biointerfaces,* 116**,** 270-276.

SCHINAZI, R., BRETTREICH, M. & HIRSCH, A. 2001. *Water-soluble dendrimeric fullerene as anti-HIV therapeutic*.

SCHMID, G. 2008. *Nanotechnology: Principles and fundamentals*, Weinheim, Wiley-VCH.

SCHREUDER-GIBSON, H. L. & GIBSON, P. Applications of electrospun nanofibers in current and future materials. ACS Symposium series, 2006. ACS Publications, 121-136.

SEPÚLVEDA-CRESPO, D., GÓMEZ, R., DE LA MATA, F. J., JIMÉNEZ, J. L. & MUÑOZ-FERNÁNDEZ, M. Á. 2015. Polyanionic carbosilane dendrimer-conjugated antiviral drugs as efficient microbicides: Recent trends and developments in HIV treatment/therapy. *Nanomedicine: Nanotechnology, Biology and Medicine*.

SEPÚLVEDA-CRESPO, D., LORENTE, R., LEAL, M., GÓMEZ, R., DE LA MATA, F. J., JIMÉNEZ, J. L. & MUÑOZ-FERNÁNDEZ, M. Á. 2014. Synergistic activity profile of carbosilane dendrimer G2-STE16 in combination with other dendrimers and antiretrovirals as topical anti-HIV-1 microbicide. *Nanomedicine: Nanotechnology, Biology and Medicine,* 10**,** 609-618.

SHARMA, R., GARG, T., GOYAL, A. K. & RATH, G. 2016. Development, optimization and evaluation of polymeric electrospun nanofiber: A tool for local delivery of fluconazole for management of vaginal candidiasis. *Artificial Cells, Nanomedicine, and Biotechnology,* 44**,** 524-531.

SHARP, D. H. 1984. An overview of Rayleigh-Taylor instability. *Physica D: Nonlinear Phenomena,* 12**,** 3IN111-10IN1018.

SHAW, M. T. 2012. *Introduction to polymer rheology,* New Jersey, Wiley.

SHIHAB, F., EBIAN, A. & MUSTAFA, R. 1979. Effect of polyethylene glycol, sodium lauryl sulfate and polysorbate-80 on the solubility of furosemide. *International Journal of Pharmaceutics,* 4**,** 13-20.

SIEPMANN, J. & PEPPAS, N. A. 2012. Modeling of drug release from delivery systems based on hydroxypropyl methylcellulose (HPMC). *Advanced Drug Delivery Reviews,* 64, Supplement**,** 163-174.

SILL, T. & VON RECUM, H. Electrospun materials for affinity-based engineering and drug delivery. *Journal of Physics: Conference Series, 2015*. IOP Publishing, 012060.

SILL, T. J. & VON RECUM, H. A. 2008. Electrospinning: applications in drug delivery and tissue engineering. *Biomaterials,* 29**,** 1989-2006.

SIMON, J. A., ROBINSON, D., ANDREWS, M., HILDEBRAND 3RD, J., ROCCI JR, M., BLAKE, R. & HODGEN, G. 1993. The absorption of oral micronized progesterone: the effect of food, dose proportionality, and comparison with intramuscular progesterone. *Fertility and Sterility,* 60**,** 26-33.

SINGH, H., SHARMA, R., JOSHI, M., GARG, T., GOYAL, A. K. & RATH, G. 2015. Transmucosal delivery of Docetaxel by mucoadhesive polymeric nanofibers. *Artificial Cells, Nanomedicine, and Biotechnology,* 43**,** 263-269.

SIONKOWSKA, A., WISNIEWSKI, M., SKOPINSKA, J., KENNEDY, C. & WESS, T. 2004. Molecular interactions in collagen and chitosan blends. *Biomaterials,* 25**,** 795-801.

SKINNER, M., KISELEV, A., ISAACS, C., MIETZNER, T., MONTELARO, R. & LAMPE, M. 2010. Evaluation of WLBU2 peptide and 3-O-octyl-sn-glycerol lipid as active ingredients for a topical microbicide formulation targeting Chlamydia trachomatis. *Antimicrobial Agents and Chemotherapy,* 54**,** 627-636.

SLAVKOVA, M. & BREITKREUTZ, J. 2015. Orodispersible drug formulations for children and elderly. *European Journal of Pharmaceutical Sciences,* 75**,** 2-9.

SLOTTA, K. H., RUSCHIG, H. & FELS, E. 1934. Reindarstellung der Hormone aus dem Corpus luteum.(Vorläuf. Mitteil.). *Berichte der deutschen chemischen Gesellschaft (A and B Series),* 67**,** 1270-1273.

SMART, J. D. 2005. The basics and underlying mechanisms of mucoadhesion. *Advanced Drug Delivery Reviews,* 57**,** 1556-1568.

SPITZ, I. M. 2008. Progesterone receptor antagonists. *Nuclear Receptors as Drug Targets***,** 223-248.

SRIKRISHNA, S. & CARDOZO, L. 2013. The vagina as a route for drug delivery: a review. *International Urogynecology Journal,* 24**,** 537-543.

STARK, M., MÖLLER, C., MÜLLER, D. J. & GUCKENBERGER, R. 2001. From Images to Interactions: High-Resolution Phase Imaging in Tapping-Mode Atomic Force Microscopy. *Biophysical Journal,* 80**,** 3009-3018.

STONE, A. 2002. Microbicides: a new approach to preventing HIV and other sexually transmitted infections. *Nature Reviews Drug Discovery,* 1**,** 977-985.

STONE, M. C. & WILLIAMS, H. C. 2015. Clinical pharmacists in general practice: value for patients and the practice of a new role. *British Journal of General Practice,* 65**,** 262-263.

STRUTT, J. & RAYLEIGH, L. 1883. Investigation of the character of the equilibrium of an incompressible heavy fluid of variable density. *Proceedings of London Mathematical Society,* 14**,** 8.

SWARBRICK, J. & BOYLAN, J. C. 2000. *Encyclopedia of Pharmaceutical Technology: Volume 20-Supplement 3*, Boca Raton, CRC Press.

TAEPAIBOON, P., RUNGSARDTHONG, U. & SUPAPHOL, P. 2007. Vitamin-loaded electrospun cellulose acetate nanofiber mats as transdermal and dermal therapeutic agents of vitamin A acid and vitamin E. *European Journal of Pharmaceutics and Biopharmaceutics,* 67**,** 387-397.

TAMAYO, J. & GARCIA, R. 1996. Deformation, contact time, and phase contrast in tapping mode scanning force microscopy. *Langmuir,* 12**,** 4430-4435.

TAMBURIC, S. & CRAIG, D. Q. 1997. A comparison of different in vitro methods for measuring mucoadhesive performance. *European Journal of Pharmaceutics and Biopharmaceutics,* 44**,** 159-167.

TANAKA, W., AKITO, E., YOSHIDA, K., TERADA, T. & NINOMIYA, H. 1977. Pharmaceutical preparation for oral cavity administration. Google Patents.

TAVANIOTOU, A., SMITZ, J., BOURGAIN, C. & DEVROEY, P. 2000. Comparison between different routes of progesterone administration as luteal phase support in infertility treatments. *Human Reproduction Update,* 6**,** 139-148.

TEO, W. E. & RAMAKRISHNA, S. 2006. A review on electrospinning design and nanofibre assemblies. *Nanotechnology,* 17**,** R89.

TEPHLY, T. R. 1991. The toxicity of methanol. *Life Sciences,* 48**,** 1031-1041.

THIRAWONG, N., NUNTHANID, J., PUTTIPIPATKHACHORN, S. & SRIAMORNSAK, P. 2007. Mucoadhesive properties of various pectins on gastrointestinal mucosa: An in vitro evaluation using texture analyzer. *European Journal of Pharmaceutics and Biopharmaceutics,* 67**,** 132-140.

TOBYN, M. J., JOHNSON, J. R. & DETTMAR, P. W. 1997. Factors affecting in vitro gastric mucoadhesion IV. Influence of tablet excipients, surfactants and salts on the observed mucoadhesion of polymers. *European Journal of Pharmaceutics and Biopharmaceutics,* 43**,** 65-71.

TOMALIA, D. A., BAKER, H., DEWALD, J., HALL, M., KALLOS, G., MARTIN, S., ROECK, J., RYDER, J. & SMITH, P. 1985. A new class of polymers: starburst-dendritic macromolecules. *Polymer Journal,* 17**,** 117-132.

TOMALIA, D. A. & CHENG, Y. 2012. *Dendrimer-based drug delivery systems: From theory to practice,* New Jersey, John Wiley & Sons.

TORCHILIN, V. P. 2005. Recent advances with liposomes as pharmaceutical carriers. *Nature Reviews Drug Discovery,* 4**,** 145-160.

TOZER, T. N. 1996. *Clinical Pharmacokinetics Concepts and Applications,* New Dehli, BI Waverly.

TSAI, M. & O'MALLEY, B. W. 1994. Molecular mechanisms of action of steroid/thyroid receptor superfamily members. *Annual Review of Biochemistry,* 63**,** 451-486.

TSVETKOV, D., CHESHEV, P., TUZIKOV, A., CHINAREV, A., PAZYNINA, G., SABLINA, M., GAMBARYAN, A., BOVIN, N., RIEBEN, R. & SHASHKOV, A. 2002. Neoglycoconjugates based on dendrimer poly (aminoamides). *Russian Journal of Bioorganic Chemistry,* 28**,** 470-486.

UCHEGBU, I. F., SCHÄTZLEIN, A. G., CHENG, W. P. & LALATSA, A. 2013. *Fundamentals of Pharmaceutical Nanoscience,* New York, Springer.

VALENTA, C., KAST, C. E., HARICH, I. & BERNKOP-SCHNÜRCH, A. 2001. Development and in vitro evaluation of a mucoadhesive vaginal delivery system for progesterone. *Journal of Controlled Release,* 77**,** 323-332.

VAN HERREWEGE, Y., MICHIELS, J., VAN ROEY, J., FRANSEN, K., KESTENS, L., BALZARINI, J., LEWI, P., VANHAM, G. & JANSSEN, P. 2004. In vitro evaluation of nonnucleoside reverse transcriptase inhibitors UC-781 and TMC120-R147681 as human immunodeficiency virus microbicides. *Antimicrobial Agents and Chemotherapy,* 48**,** 337-339.

VANIĆ, Ž. & ŠKALKO-BASNET, N. 2013. Nanopharmaceuticals for improved topical vaginal therapy: Can they deliver? *European Journal of Pharmaceutical Sciences,* 50**,** 29-41.

VAUGELADE, C., ROHMER, A. C., BUREL, F., BELLENEY, J., DUCLOS, R. & BUNEL, C. 2001. Progesterone freeze-dried systems in sublingual dosage form. *International Journal of Pharmaceutics,* 229**,** 67-73.

VERMESH, M., FOSSUM, G. T. & KLETZKY, O. A. 1988. Vaginal bromocriptine: pharmacology and effect on serum prolactin in normal women. *Obstetrics & Gynecology,* 72**,** 693-698.

W BUCKHEIT, K. 2012. An algorithm for the preclinical development of anti-HIV topical microbicides. *Current HIV Research,* 10**,** 97-104.

WAIS, U., JACKSON, A. W., HE, T. & ZHANG, H. 2016. Nanoformulation and encapsulation approaches for poorly water-soluble drug nanoparticles. *Nanoscale,* 8**,** 1746-1769.

WANG, J., TAUCHI, Y., DEGUCHI, Y., MORIMOTO, K., TABATA, Y. & IKADA, Y. 2000. Positively charged gelatin microspheres as gastric mucoadhesive drug delivery system for eradication of H. pylori. *Drug delivery,* 7**,** 237-243.

WANG, L., SASSI, A. B., PATTON, D., ISAACS, C., MONCLA, B., GUPTA, P. & ROHAN, L. C. 2012. Development of a liposome microbicide formulation for vaginal delivery of octylglycerol for HIV prevention. *Drug Development and Industrial Pharmacy,* 38**,** 995-1007.

WANG, W., BO, S., LI, S. & QIN, W. 1991. Determination of the Mark-Houwink equation for chitosans with different degrees of deacetylation. *International Journal of Biological Macromolecules,* 13**,** 281-285.

WATWE, R. M. & BELLARE, J. R. 1995. Manufacture of liposomes-a review. *Current Science,* 68**,** 715-725.

WEI, A. F., WANG, J., WANG, X. Q., WEI, Q. F. & HOU, D. Y. 2011. Biodegradable Electrospun Fibers Containing the Compound Antihypertensive Drugs. *Advanced Materials Research,* 332**,** 1218-1222.

WEI, Q. 2012. *Functional nanofibers and their applications,* Cambridge, Woodhead Publishing.

WHITESIDES, G. M., MATHIAS, J. P. & SETO, C. T. 1991. Molecular self-assembly and nanochemistry: A chemical strategy for the synthesis of nanostructures. Document from Defene Technical Information Centre, Virginia.

WILLIAMS, G. R., CHATTERTON, N. P., NAZIR, T., YU, D.-G., ZHU, L.-M. & BRANFORD-WHITE, C. J. 2012. Electrospun nanofibers in drug delivery: recent developments and perspectives. *Therapeutic Delivery,* 3**,** 515-533.

WILLIAMS, S. C., OEHNINGER, S., GIBBONS, W. E., VAN CLEAVE, W. C. & MUASHER, S. J. 2001. Delaying the initiation of progesterone supplementation results in decreased pregnancy rates after in vitro fertilization: a randomized, prospective study. *Fertility and Sterility,* 76**,** 1140-1143.

WIN, K. Y. & FENG, S.-S. 2005. Effects of particle size and surface coating on cellular uptake of polymeric nanoparticles for oral delivery of anticancer drugs. *Biomaterials,* 26**,** 2713-2722.

WISSING, S., KAYSER, O. & MÜLLER, R. 2004. Solid lipid nanoparticles for parenteral drug delivery. *Advanced Drug Delivery Reviews,* 56**,** 1257-1272.

WITVROUW, M., FIKKERT, V., PLUYMERS, W., MATTHEWS, B., MARDEL, K., SCHOLS, D., RAFF, J., DEBYSER, Z., DE CLERCQ, E. & HOLAN, G. 2000. Polyanionic (ie, polysulfonate) dendrimers can inhibit the replication of human immunodeficiency virus by interfering with both virus adsorption and later steps (reverse transcriptase/integrase) in the virus replicative cycle. *Molecular Pharmacology,* 58**,** 1100-1108.

WOERTZ, C., PREIS, M., BREITKREUTZ, J. & KLEINEBUDDE, P. 2013. Assessment of test methods evaluating mucoadhesive polymers and dosage forms: an overview. *European Journal of Pharmaceutics and Biopharmaceutics,* 85**,** 843-853.

WOODROW, K. A., CU, Y., BOOTH, C. J., SAUCIER-SAWYER, J. K., WOOD, M. J. & SALTZMAN, W. M. 2009. Intravaginal gene silencing using biodegradable polymer nanoparticles densely loaded with small-interfering RNA. *Nature Materials,* 8**,** 526-533.

XIE, J. & WANG, C.-H. 2006. Electrospun micro-and nanofibers for sustained delivery of paclitaxel to treat C6 glioma in vitro. *Pharmaceutical Research,* 23**,** 1817-1826.

XU, Z., MAHALINGAM, S., BASNETT, P., RAIMI-ABRAHAM, B., ROY, I., CRAIG, D. & EDIRISINGHE, M. 2016. Making Nonwoven Fibrous Poly (ε-caprolactone) Constructs for Antimicrobial and Tissue Engineering Applications by Pressurized Melt Gyration. *Macromolecular Materials and Engineering*.

XU, Z., MAHALINGAM, S., ROHN, J., REN, G. & EDIRISINGHE, M. 2015. Physio-chemical and antibacterial characteristics of pressure spun nylon nanofibres embedded with functional silver nanoparticles. *Materials Science and Engineering: C,* 56**,** 195-204.

YANG, M., YU, T., WANG, Y. Y., LAI, S. K., ZENG, Q., MIAO, B., TANG, B. C., SIMONS, B. W., ENSIGN, L. M. & LIU, G. 2014. Vaginal Delivery of Paclitaxel via Nanoparticles with Non-Mucoadhesive Surfaces Suppresses Cervical Tumor Growth. *Advanced Healthcare Materials,* 3**,** 1044-1052.

YANG, X., FORIER, K., STEUKERS, L., VAN VLIERBERGHE, S., DUBRUEL, P., BRAECKMANS, K., GLORIEUX, S. & NAUWYNCK, H. J. 2012. Immobilization of pseudorabies virus in

porcine tracheal respiratory mucus revealed by single particle tracking. *PloS One,* 7**,** e51054.

YANG, X. H. & ZHU, W. L. 2007. Viscosity properties of sodium carboxymethylcellulose solutions. *Cellulose,* 14**,** 409-417.

YANUSHPOLSKY, E., HURWITZ, S., GREENBERG, L., RACOWSKY, C. & HORNSTEIN, M. D. 2008. Comparison of Crinone 8% intravaginal gel and intramuscular progesterone supplementation for in vitro fertilization/embryo transfer in women under age 40: interim analysis of a prospective randomized trial. *Fertility and Sterility,* 89**,** 485-487.

YOON, G., PARK, J. W. & YOON, I.-S. 2013. Solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLCs): recent advances in drug delivery. *Journal of Pharmaceutical Investigation,* 43**,** 353-362.

YU, X., BURNHAM, N. A., MALLICK, R. B. & TAO, M. 2013. A systematic AFM-based method to measure adhesion differences between micron-sized domains in asphalt binders. *Fuel,* 113**,** 443-447.

ZHANG, S. 2003. Fabrication of novel biomaterials through molecular self-assembly. *Nature Biotechnology,* 21**,** 1171-1178.

ZHANG, Y., CRISTOFARO, P., SILBERMANN, R., PUSCH, O., BODEN, D., KONKIN, T., HOVANESIAN, V., MONFILS, P. R., RESNICK, M. & MOSS, S. F. 2006. Engineering mucosal RNA interference in vivo. *Molecular Therapy,* 14**,** 336-342.

ZHANG, Z., ZHANG, H., YANG, Y., VINCKIER, I. & LAUN, H. 2001. Rheology and morphology of phase-separating polymer blends. *Macromolecules,* 34**,** 1416-1429.

ZHOU, F. L., GONG, R. H. & PORAT, I. 2009. Mass production of nanofibre assemblies by electrostatic spinning. *Polymer International,* 58**,** 331-342.

ZONG, S., WANG, X., YANG, Y., WU, W., LI, H., MA, Y., LIN, W., SUN, T., HUANG, Y. & XIE, Z. 2015. The use of cisplatin-loaded mucoadhesive nanofibers for local chemotherapy of cervical cancers in mice. *European Journal of Pharmaceutics and Biopharmaceutics,* 93**,** 127-135.

ZONG, X., KIM, K., FANG, D., RAN, S., HSIAO, B. S. & CHU, B. 2002. Structure and process relationship of electrospun bioabsorbable nanofiber membranes. *Polymer,* 43**,** 4403-4412.

ZUR MÜHLEN, A., SCHWARZ, C. & MEHNERT, W. 1998. Solid lipid nanoparticles (SLN) for controlled drug delivery – Drug release and release mechanism. *European Journal of Pharmaceutics and Biopharmaceutics,* 45**,** 149-155.