Electrohydrodynamic Processing for Preparation of Advanced Drug Delivery Systems

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

Doctor of Philosophy

By

Talayeh Shams

Department of Mechanical Engineering

University College London

Torrington Place, London WC1E 7JE

U.K

May, 2018

Declaration

I, Talayeh Shams, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Signature:

Date

Abstract

This research explores the feasibility of the electrohydrodynamic processing using single and co-axial set-up as a single step processing tool for preparation of advanced drug delivery systems. A number of synthetic biodegradable and non-biodegradable polymers were used in order to prepare formulations incorporating drugs of different physicochemical characteristics. Based on the focus and the desired applications, the polymeric carrier and solvent system as well as the model drug of interest were selected to develop the drug delivery systems. Firstly, core-shell microparticles were prepared and optimized using co-axial electrohdrodynamic processing with precise control over the averaged particle size and size distribution. This was followed by integration of model drugs with different water-solubility. In this study, the release characteristics of the developed particles were investigated with single and simultaneous encapsulation of the drugs. Successful preparation of fixed dose combination formulation with high processing yield and encapsulation efficiency was reported. Secondly, single and co-axial electrohydrodynamic processing was utilized for preparation of smart drug delivery system for targeted release of prednisolone. Colon targeted drug delivery systems were developed using a pH-responsive polymer. Varying polymer drug ratio was applied to further enhance the release profiles and obtain an efficient delivery system whereby local delivery of prednisolone is made possible. Finally, microspheres were developed for co-encapsulation of anti-diabetic drugs with different water-solubility. The successfully developed sustained release formulations have the potential to overcome the existing limitations of conventional formulations by enhancing patient compliance and efficacy of the treatment of any chronic conditions.

Peer-reviewed Journal Articles

- Shams T., Parhizkar M, Illangakoon U. E., Orlu-Gul M., Edirisinghe M. (2017), Core/shell microencapsulation of indomethacin/paracetamol by co-axial electrohydrodynamic atomization, *J. R. Materials and Design*, 136, 204-213

- Shams T., Illangakoon U. E., Parhizkar M., Harker A. H., Edirisinghe S., Orlu M., Edirisinghe M. (2018), Electrosprayed microparticles for intestinal delivery of prednisolone, *Journal of Royal Society Interface,*15(145), 20180491.

- Shams T., Brako F., Harker A. H., Edirisinghe M. (2018), Understanding drug release from microparticles: experimental data analysis and mathematical modelling (In preparation).

- Orlu-Gul M., Topcu A. A., Shams T., Mahalingham S., Edirisinghe M. (2014), Novel encapsulation systems and processes for overcoming the challenges of polypharmacy, *Current Opinion in Pharmacology*, 18, 28-34.

- Ahmed J., Matharu R. K., Shams T., Illangakoon U. E., Edirisinghe M. (2018), A comparison of electric-field-driven and pressure-driven fiber generation methods for drug delivery, *Mater. Eng.* 303 (5), 1700577.

- Zhang Y., Shams T., Harker A. H., Parhizkar M., Edirisinghe M. (2018), Effect of copolymer composition on particle morphology and release behaviour *in vitro* using progesterone, *Material & Design,* 159, 57-67.

Conference Presentations

- Shams T., Orlu-Gul M., Edirisinghe M., 'Meeting the Polypharmacy Challenge: Co-encapsulation of Drugs with Different Solubility Using Electrohydrodynamic Atomization', PhD Student Conference, Department of Mechanical Engineering, UCL, London, June 2015 (oral and poster presentation).

- Shams T., Parhizkar M, U. E. Illangakoon U. E., Orlu-Gul M., Edirisinghe M., 'Meeting the Polypharmacy Challenge: Development of Polymeric Carriers Co-encapsulationg Multiple Drugs Using Electrohydrodynamic Atomization', PhD Student Conference, Department of Mechanical Engineering, UCL, London, June 2016 (oral and poster presentation).

- Shams T., Parhizkar M, U. E. Illangakoon U. E., Orlu-Gul M., Edirisinghe M., 'Meeting the Polypharmacy Challenge: Development of Polymeric Carriers Co-encapsulationg Multiple Drugs Using Electrohydrodynamic Atomization', UK Society for Biomaterials, London, UK, 30th June 2016 (oral presentation).

- Shams T., Parhizkar M, U. E. Illangakoon U. E., Harker A. H., Orlu M., Edirisinghe M, 'Meeting the Polypharmacy Challenge: Development of Polymeric Carriers Co-encapsulationg Multiple Drugs Using Electrohydrodynamic Atomization', 28th Annual Conference of the European Society for Biomaterials, Athens, Greece 4-8 September 2017 (poster presentation).

Acknowledgements

I wish to thank my supervisor Prof. Mohan Edirisinghe for his continuous guidance, support and supervision in my project. His patience and enthusiasm has been a driving force for pursuing this research work.

My thanks are equally due to my secondary supervisor Dr. Mine Orlu for her advice, enthusiasm and support throughout this research study.

I would like to thank Prof. Anthony Harker for providing knowledge, his invaluable input is greatly appreciated.

I am grateful to the supportive staff at UCL Mechanical Engineering and School of Pharmacy for all their help in using the equipment. My special thanks go to Dr. Maryam Parhizkar, Dr. Tom Gregory, Dr. Steven Firth, Dr. Suguo Huo, Timea Grego and Isabel Goncalves. I would like to thank all my colleagues at UCL for support and good company during my PhD.

Last but not least, I would like to give my eternal thanks to my loving family for their love, support and sacrifice in the past years.

Dedication

To

My lovely family

Your love and sacrifices for me

are exceptional.

Table of Contents

List of Figures

List of Tables

1 Introduction and background

1.1 Background

Electrohydrodynamic (EHD) atomization has proven its potential for preparation of encapsulated particles in the micro/nanometric ranges. The main advantage of this technique lies in its technical simplicity and flexibility. It allows formation of uniform and monodispersed particles. Moreover, with tuning of the apparatus set up, including the needle diameter and the working distance between the nozzle and collection platform, one can obtain products of desired morphology and structure at ambient temperature and relative humidity (Enayati *et al*., 2011a). For development of drug delivery systems, if a single needle is used, the active ingredients or other biological agents may be mixed with the polymeric solution prior to electrospraying to form a suspension that can be infused into the conductive needle and produce polymeric structures with entrapped agents (Zeng and Sun, 2009).

In co-axial electrohydrodynamic processing, two or more different solutions or suspensions are fed through concentric needles, that are subjected to an applied electrical potential and the co-flowing liquids are prone to experiencing different modes of atomization. Subsequent jet break-up can lead to near monodisperse compound aerosols, while the outer material can engulf or encapsulate the inner core. This phenomenon is of great importance for encapsulation of food additives, targeted drug delivery, and material processing (Loscertales *et al.*, 2002). Another important advantage of this method is the ability to process biocompatible products from non-toxic solvents in a single step that is cost effective and reproducible. Also, with careful selection of the material and

processing properties, multiple active ingredients can be processed using this technique in order to prepare a single dosing unit consisting of multiple drugs, which is highly attractive in the arena of geriatric therapy for overcoming the challenges of polypharmacy and increasing the patient compliance in order to improve the treatment efficacy. It has also been utilized for preparation of solid dispersion formulations, whereby the dissolution rate of the incorporated drug of poor aqueous solubility is optimized, this in turn results in higher bioavailability (Bohr *et al.*, 2011).

Recently, there has been great interest in the use of advanced drug delivery systems and tissue engineering including encapsulated particles in the medical field and pharmaceutical industries. These structures may be used to release drugs and other bioactive agents in a sustained and controlled manner. The incorporated drugs can be entrapped on to the polymer surface or into the polymeric matrix be encapsulated in the polymer core in order to shield it from the immediate environment and release it at different time points either by diffusion or biodegradation, depending on the nature of the polymer used.

In an ideal drug delivery device, the incorporated drug is released in a controlled manner and hence a constant drug concentration level is maintained over a given amount of time. An ideal drug carrier should be biocompatible in order to inhibit any harmful side effects and preferably be biodegradable in order to be removed physically from the human body (Leong and Langer, 1988). Thus, the preparation of effective drug delivery systems is yet an ever-challenging endeavour in medicine. Many techniques have been used for the preparation of drug delivery systems. Nonetheless, EHD and co-axial EHD processes are promising techniques that enable preparation of drug delivery systems in one-step, time-

efficient process and in an economical manner (Wu and Clark, 2008). Currently, development of smart drug delivery systems for targeted drug release are reliant on responsive polymeric materials (Alvarez-Lorenzo and Concheiro, 2014). With the application of different stimuli including oxidation, pH, temperature, ionic strength, light, electric fields, magnetic fields and ultrasound the release of the incorporated drug can be triggered based on desired applications. With the development of smart drug delivery systems, it is possible to achieve site-specific release whereby the local delivery of drug results in reduced frequency and lowered required administered dosing unit and therefore limited undesired side effects. This thesis will explore the prospect of EHD and co-axial EHD processing for preparation of advanced drug delivery systems and examine its potential as a platform for preparation of these formulations.

1.2 Aims and objectives

This research study is aimed at the exploration of the prospect of utilization of electrohydrodynamic processing for development of novel drug delivery systems. The initial objective was to understand the fundamentals of EHD processing and get familiarised with the technique and carry out experiments to study the effect of key parameters that influence the process. For this purpose, a number of experimental factors including solution flow rate, the applied electrical voltage, the working distance and a number of solution parameters including electrical conductivity, surface tension and viscosity were investigated to further understand the effects of each factor for optimization of the process in order to produce particles of desired size with narrow size distribution.

The second objective of this study was to prepare double-layered drug delivery systems for incorporation of model drug with different aqueous solubility and

examine the feasibility of the process for preparation of multi-drug delivery systems that are aimed at the development of fixed dose combination formulations. This was followed by confirmation of formation of different layers. The drug release profiles were studied to observe any possible interference in their release mechanism.

The third objective of this study was to explore the prospect of EHD processing for preparation of smart drug delivery systems that enables the site-specific delivery of the active compound. The prepared targeted drug delivery systems were examined in order to investigate its potential application for colon targeted delivery using a pH-responsive synthetic polymer. This was done by studying the release profiles of the active compound.

The fourth objective of this work was to prepare microcapsules for co-delivery of anti-diabetic drug for treatment of type 2 diabetes mellitus. The extended release of active pharmaceutical ingredients is of great importance for treatment of chronic conditions that require frequent administration of drugs at high dosages, whereby a controlled delivery device may aid reduce the prescribed drugs and increase patient compliance while enhancing the treatment efficacy.

1.3 Structure of the thesis

Chapter 1 gives a brief description on the background of the thesis. It highlights the requirements for successful drug delivery systems and their application in a number of areas that are of current interest in the field of biopharmaceutical developments.

Chapter 2 provides a detailed literature review encompassing the underlying principles and aspects of the EHD processing associated with this research. In

this section, particulate drug delivery systems and conventional encapsulation techniques has been reviewed. Moreover, the EHD technique was compared to few other current techniques for preparation of advanced drug delivery systems. A brief account is given on the materials used for the preparation of polymeric drug delivery systems. Moreover, areas in which the EHD processing has the potential to overcome current limitations were highlighted.

Chapter 3 describes the experimental set-up, materials used, the techniques carried out for characterization, experimental procedure and a detailed description of the experimental tools employed.

Chapter 4 covers the experimental results obtained for preparation of core/shell structures for development of drug delivery systems whereby model drugs of different aqueous solubility were encapsulated, this was done using co-axial EHD in conjunction with use of poly(lactic-co-glycolic acid) (PLGA) and polyethylene glycol) (PEG) as polymeric carriers. The experimental parameters were adjusted in order to optimize the developed systems. The release profile and state of the model drugs in the polymeric drug carriers were further investigated in order to assess the feasibility of EHD for preparation of fixed dose combination formulations.

Chapter 5 includes the experimental results obtained for development of targeted drug delivery system using Eudragit L100-55 as the pH-sensitive polymer carrier for site-specific release of prednisolone for colon delivery. A number of different formulations with varying polymer and drug content were implemented and assessed in terms of their applicability *in vitro* test. The state of the drug and the compatibility of the proposed system were investigated.

Chapter 6 focuses on preparation of multiple drug delivery systems using microcapsules and incorporating drugs that have potential synergetic effect for development of sustained drug delivery system. In this work, polymethylsilsesquioxane (PMSQ) was used as the polymeric carrier encapsulating metformin HCL and glibenclamide, both of which are anti-diabetic substances.

Chapter 7 summarises what has been achieved in this work and discusses some recommendations for the future experimental plans

2 Literature review

2.1 Drug delivery concept and goals of drug delivery systems

In order to achieve a successful therapy, adequate amount of active pharmaceutical ingredient needs to reach the desired site of action over an appropriate time period. Consequently, effective drug delivery systems have to exhibit appropriate bioavailability. Effectively, they should facilitate the release of drug at the desired place in appropriate dosage and within the correct therapeutic window. In addition to this, they should minimize adverse reaction and side effects while at the same time, not introducing any new ones (Gregoriadis, 1981). By definition, controlled release is “the tailorable delivery of compounds (e.g., drugs, proteins, fertilizers, nutrients, and other biologically active agents) at an effective level in response to time and stimuli (e.g., pH, temperature, enzymes, UV light, magnetic fields, osmosis)” (Huynh and Lee, 2014).

Various factors including the release environment, diffusion, molecular weight and solubility of the active pharmaceutical ingredients (APIs), pore size and polymer carrier degradation affects the release of the incorporated APIs (Siepmann *et al.*, 2012). In conventional release systems (e.g., tablets, capsules, etc.) the therapeutic agent is released almost immediately once administered in the body. This results in an abrupt increase in concentration level of the API in the bloodstream, which thereafter is followed by a fast reduction within a short period of time.

In order to maintain the drug concentration at the therapeutic level, multiple administration is necessary to sustain the drug concentration at an effective

range (Cohen *et al.*, 1991). These oscillations of plasma drug level may be toxic and reduce drug effectiveness (Figure 2.1), which in turn also reduces the patient compliance. Moreover, use of high drug concentration may induce adverse toxic effects since delivery of the therapeutic agent to the site of action is dependent on diffusion and partition from blood stream.

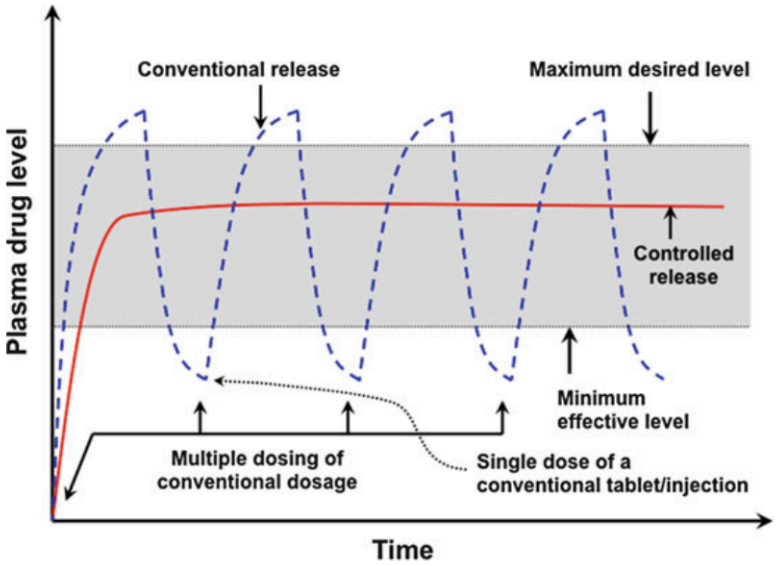


Figure 2.1 Drug level in the plasma released from multiple conventional capsules or injection dosing, and controlled release system (Huynh and Lee, 2014).

A standard drug release profile and concentration level, characteristic of a conventional drug delivery system, is shown in Figure 2.2a. For the therapeutic agent to be effective the drug concentration has to be lower than the toxic level (the upper limit) and higher than that of the effective level (the lower limit), which is indicated as the therapeutic window (Park *et al.*, 1998). Conventional drug delivery systems are highly favourable due to their cost effectiveness in terms of processing and administration. Figure 2.2b shows the sustained release of drug over an extended period of time whereby the drug concentration level falls in the therapeutic window with a constant release rate (Leong and Langer, 1988). In Figure 2.2c, a “pulsatile release” profile is shown, where the drug is released only

when it is required, an example of the use of delivery system is the treatment of an imbalance in the biological homeostasis (Kikuchi and Okano, 2002). Various targeting strategies may be used in order to achieve highly localized drug release, as shown in Figure 2.2d, the release is restricted to the targeted site at high local concentration for a prolonged period of time. This type of drug delivery systems offers significantly high therapeutic efficiency where side effects are kept at minimum.

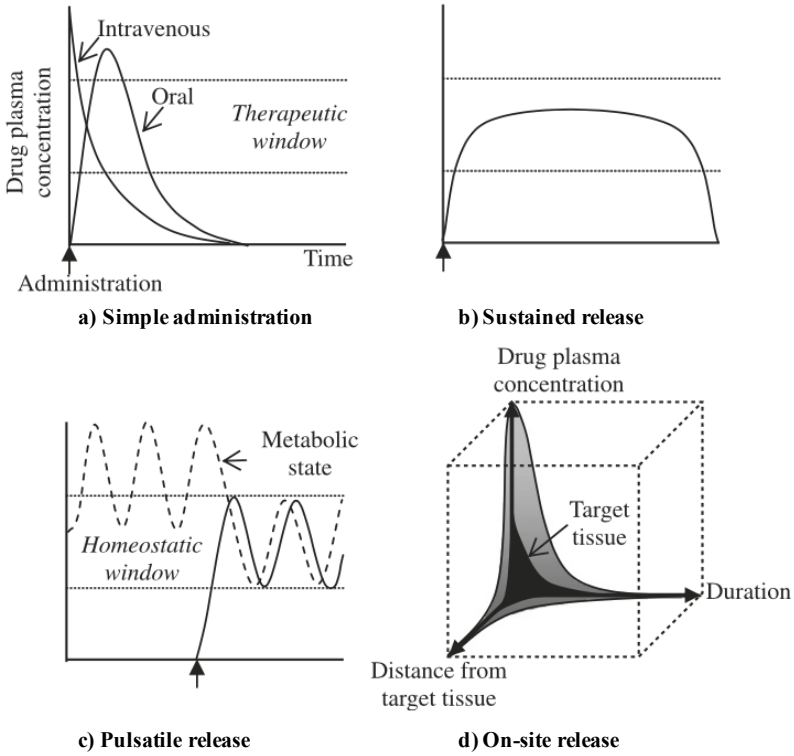


Figure 2.2 Drug delivery systems with a) drug delivery based on diffusion and partition, b) Sustained release over an extended period of time, c) Pulsatile release to closely maintain homeostasis, d) Site-specific release to enhance therapeutic efficiency and minimize side effects (Kim *et al.*, 2009).

In brief, drug delivery and targeting systems are developed to surpass the limitations that the conventional drug delivery systems encounter, in order to enhance the drug performance.

The key goals of these systems include targeting the drug to the desired cells or tissue and retaining on-site for the required amount of time, reducing the drug leakage while in transit to the desired site of action and keeping the drug out of non-target tissues, shielding the incorporated drug from undergoing metabolism and clearance, facilitating the transportation of the drug into the target tissue. Drug delivery systems need to be biocompatible, biodegradable and non-immunogenic.

2.2 Routes of drug delivery

There are different routes for administration of drug loaded particles such as oral, nasal, subcutaneous, intramuscular, transdermal and pulmonary. In this section, some of the most popular ones are briefly described.

Oral delivery ranks the highest and is the most frequently used route of administration (Chen *et al.*, 2010). This is due to high patient compliance and ease of administration. Nonetheless, the main drawback is the potential bio-inequivalence between orally administered drugs (Zhang *et al.*, 2002).

Parenteral drug delivery is chosen when the oral route is not possible. This may be due to poor drug absorption properties as the drug undergoes degradation in the gastrointestinal tract (Sinha and Trehan, 2005). The injection can be administered in the form of an intramuscular, intravenous or subcutaneous dosage. Parenteral administration can also be used for site-specific drug delivery as a direct route.

Pulmonary drug delivery is used in direct administration of therapeutics to the pulmonary system by inhalation and is a significant method of achieving highly localized treatment (Clark, 1995). The inherent limitation of using this route lies in the physical characteristic of the drug carriers. As an example, particle size 1 μm

to 5 μm is required to ensure successful drug delivery. Moreover, different particle size are also seen to deposit preferentially in different sites of the lungs (Schreier *et al.*, 1993; Fernández Tena and Casan Clarà, 2012). With the use of biodegradable polymers as carriers the drug can be released in a sustained manner and hence improve the treatment efficiency (Kawashima *et al.*, 1998). Furthermore, with the inhalation of drug loaded particles, the presence of the drug in the airways is prolonged. This in turn reduces the plasma drug concentration, and enhances patient compliance as the treatment is less frequent (Schreier *et al.*, 1993).

Transdermal drug delivery administers the therapeutic agents through the skin with the use of drug loaded adhesive patches. The incorporated drug passively diffuses across the skin as the patch is kept at constant contact. Examples of this include delivery of contraceptives and nicotine patches (Singh and Roberts, 1989).

2.3 Particulate drug carriers

A number of different structures including micro/nano spheres, micro/nano particles, liposomes, vesicles, micelles and dendrimers are collectively known as "particulate drug carriers" as shown in Figure 2.3 (Kaparissides *et al.*, 2006). Microspheres refer to spherical units of few micrometer diameter in size that are made of either natural or synthetic materials whereby therapeutic agents are dispersed or dissolved in their matrix (Kreuter, 2001). Nanospheres are spherical structures in nano-scale range in which the incorporated therapeutic agent is either absorbed at their surface or entrapped/dissolved in their matrix. Nanocapsules integrate the dissolved therapeutic agent in an inner core that is engulfed by a polymeric shell (Enayati *et al.*, 2011a).

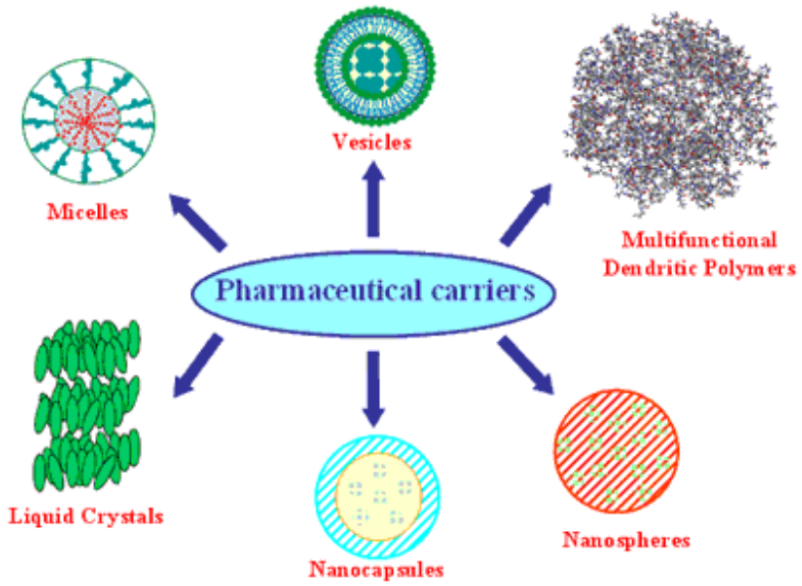


Figure 2.3 Pharmaceutical carriers (Kaparissides *et al.,* 2006).

Liposomes are comprised of an aqueous core enclosed by a lipid bilayer and are in a sub-micron size range. They incorporate the potential drug in either their lipid bilayer or aqueous core (Bangham *et al.*, 1965). Micelles constitute of a cluster of amphiphilic surfactant molecules that aggregate almost immediately in water and form into a spherical vesicle. Hydrophobic drugs may be incorporated into the core of micelles due to its hydrophobic nature and then release thereafter by some drug delivery mechanism. Micelles are shaped from small molecules with a polar, charged or hydrophilic “head” group and a hydrophobic “tail”, that often constitutes of the hydrocarbon portion of long fatty acids. The geometry and molecular size of the surfactant sets the size of the micelles (Nishiyama and Kataoka, 2003; Savic *et al.*, 2003).

A dendrimer is a macromolecule with characteristic highly branched 3D structure (Lee *et al.*, 2005). Owing to their unique surface structure, high degree of branching, multivalency, globular architecture and well defined molecular weight, dendrimers are excessively utilized as nano-carriers for medical applications,

ranging from drug delivery, gene transfection, tumour therapy to diagnostics (Boas and Heegaard, 2004).

2.4 Mechanisms of drug release

The classification of the controlled drug release systems can be based on the mechanism that governs the drug release, this necessitates the dissolution of the drugs to be followed by diffusion through the polymeric carrier in order to reach the release medium. Diffusion controlled systems are divided into two subcategories: the reservoir device and the matrix device (Huang and Brazel, 2001). The drug is entrapped inside a polymeric carrier in the reservoir device; which can be administered via injection or be inserted in the body as an implant. It constitutes of a polymeric membrane that shield the drug from the immediate surroundings, this way the drug gradually diffuses through the polymeric barrier as shown in Figure 2.4**.** In the first stages of development, non-biodegradable polymers (e.g. silicone) were used that enables prolonged release of lipophilic drugs with low molecular weight (Folkman and Long, 1964).

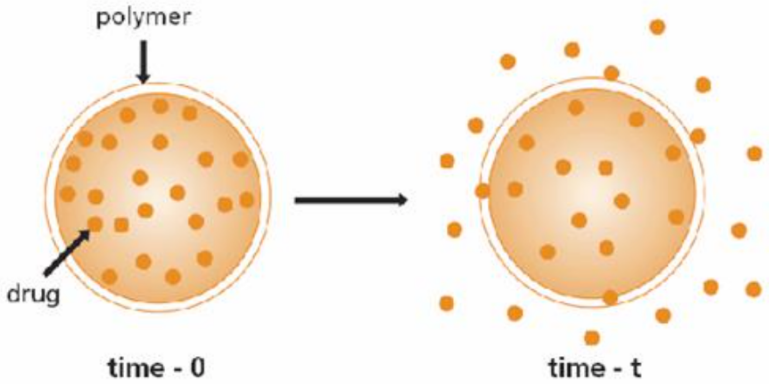


Figure 2.4 Schematic representation of a reservoir diffusion controlled drug delivery device.

Development of the Norplant capsules (Sivin *et al.*, 1998) is based on this technique, whereby contraceptives are enclosed onto small silicone capsules that are then gradually released by diffusion through the polymer over 5 years. The process of diffusion is generally described using a set of equations based on Fick's first law of diffusion:

$$\boldsymbol{J} = -\boldsymbol{D}\frac{\boldsymbol{\partial C}}{\boldsymbol{\partial X}} \quad \text{(Equation 1)}$$

Where J [mol/m^2 sec] is the flux density, that is the amount of drugs crossing the membrane per unit area per unit time; and $\frac{\partial C}{\partial X}$ is the rate of change of concentration "C" [mol/m^3], which is relative to the distance "X' [m] in the membrane, D is the diffusion coefficient [m^2/sec] of the drug in the membrane. D is a reflection of ability of the drug molecule to diffuse through the polymeric matrix, this is dependent on factors including the molecular size and charge. Matrix systems are deemed the most common devices utilized in controlled drug release; this is due to the ease of preparation when compared to reservoir devices. Also, there is a lower risk of high dosage accidents involved, which is likely to happen when the membrane is compromised in the case of reservoir devices. As shown in Figure 2.5, in these type of systems, drugs are distributed throughout the polymeric carrier that are then diffused out (Langer and Folkman, 1976; Langer, 1990). In matrix devices, the solubility of the drug in the polymer matrix governs the release profile. In porous matrix devices, this is dependent on the solubility of the drug in the sink solution within the particle's pore network as well as the tortuosity of the network (Huang and Brazel, 2001).

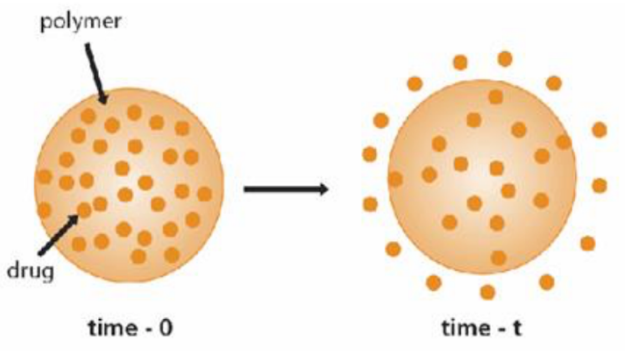


Figure 2.5 Schematic representation of a monolithic (matrix) diffusion controlled drug delivery device.

A combination of diffusion and degradation processes govern the drug release in the other drug delivery systems (Ogawa *et al.*, 1988). In these systems, drug carriers constitute of either biodegradable or bioerodible polymers, for this reason, these drug delivery devices are ultimately absorbed into the body which in turn negates the need for their surgical removal. In bioerodible matrix systems, the alteration of the matrix composition as it shrinks and ultimately completely dissolves, leads to release of the incorporated drug (Heller *et al.*, 1987).

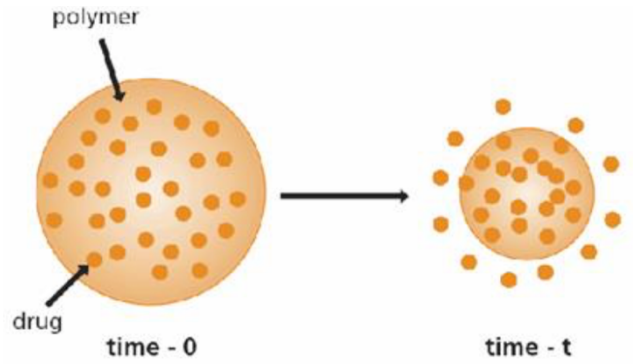


Figure 2.6 Schematic representation of biodegradable (bioerodible) drug delivery device.

In these systems, the drug is distributed uniformly throughout the polymeric carrier as is in the monolithic systems, whereby polymer erosion results in drug release as shown in Figure 2.6.

2.5 Control and regulation of drug release profiles

Polymeric controlled release systems are developed in order to reduce the amount of administered drug and at the same time achieve the desirable therapeutic effect. The main objective of these delivery systems is to enable delivery of the active pharmaceutical ingredient at the desired site and facilitate release at a specified rate over a defined period of time. In order to attain this, one strategy is to control the burst release phase and in turn increase the efficiency of the developed system. Another approach is to develop a smart delivery system that responds to external stimuli and release the drug at the preferred site. In this thesis, pH responsive polymer was used as part of a project in order to prepare targeted drug delivery system.

2.5.1 Significance of the burst release in drug delivery systems

Polymeric drug delivery systems have been studied as drug loaded vehicles in order release the active pharmaceutical ingredient in a sustained way. Nonetheless, these polymeric particles still suffer from the undesired burst release. The burst release is signified, as it occurs within a short period of time as the release is initiated when compared to that of the complete release profile (Huang and Brazel, 2001).

In general, burst release (see Figure 2.7) is associated with high toxicity and reduced life time of the incorporated active ingredient. Hence, this introduces limitations in terms of robustness of these types of drug delivery systems, consequently it is an undesirable phenomenon. Nonetheless, there exist situations in which rapid initial release may be anticipated. As an example, in pulsatile drug delivery systems, repeated burst release is one of the criteria, so that the active ingredient is delivered fast at a precise time.

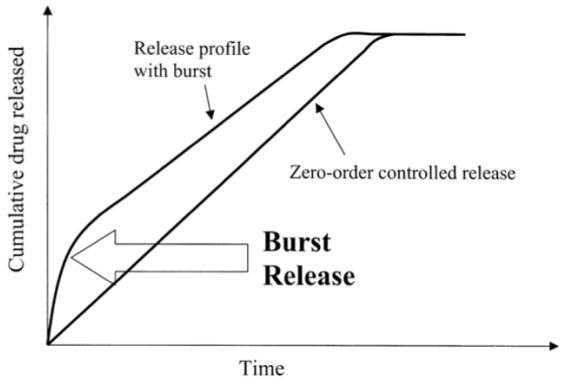


Figure 2.7 The burst release effect in drug delivery systems with a zero-order release pattern (Huang and Brazel, 2001).

Also, in targeted drug delivery systems, when the polymeric vehicle reaches the desired site, an immediate release may be required depending on the specific application. The occurrence of burst release may be attributed to few causes, the heterogeneous distribution of the incorporated drug in the polymeric system, caused due to porosity of the prepared system as it is processed or due to the drug properties (Huang and Brazel, 2001). In most cases, the burst release phase is primarily resultant of the surface associated drugs (Pitt, 1990; Cohen *et al.*, 1991). This phenomenon chiefly happens due to swelling of the polymer chains near the surface that significantly enhances the diffusion process of the active ingredient molecules dispersed in this region. Another contributor may be the entrapment of the drug on the surface of the polymer matrix (Batycky *et al.*, 1997), in particular if the drug loading is too high. Moreover, the diffusion and migration of the active ingredient will consequently lead to a heterogeneous distribution.

2.5.2 Controlling the burst release phase

The extent of burst release may be controlled by using a number of different approaches. One strategy is to use a polymeric carrier that is hydrophobic and

hence reduce the rate of water intake. Another strategy is to increase the diffusional resistance to the drug, this can be done by increasing the length of the diffusion pathways to the drug molecules. Prevention of drug accumulation on the surface is another strategy which can result in lowered burst release. These methods can be applied by increasing the polymer concentration, tuning the particle size and by surface modification (Huang and Brazel, 2001). In a study by Busatto *et al*. (Busatto *et al.*, 2018), the effect of particle size on progesterone release from PLGA particles were studied. A biphasic release profile for smaller microspheres was observed, comprising an initial burst effect and drug release controlled by the diffusion of the drug through the polymeric matrix. As for larger particles, a tri-phasic release profile was shown, that included an additional phase controlled by polymer degradation. This behaviour was explained on the basis that the complete drug release was achieved within a few days for smaller microparticles, during which polymer degradation effects are still insignificant. Nonetheless, all of these approaches may require extra costs that may in turn result in reduced drug loading and processing efficiency. Nevertheless, another approach is introducing an additional coating which in itself is a type of surface modification, that is utilizing an outer drug-free layer. This method is not only cost effective but also efficient. By using an erodible coating layer in the particulate drug delivery system, the extent of drug release is governed by the dissolution or erosion of the outer coat that engulfs the active ingredient. With optimization of the thickness of the outer layer, a time dependent release of the incorporated drug is obtainable. For this purpose, the processing parameters including type and concentration of the coating agent need to be optimized with care depending on the specific application.

2.6 Stimuli drug delivery systems

Recent developments in polymer science and engineering has brought newly formed polymers for advanced controlled delivery of drugs. Smart polymers that are sensitive to stimuli and are able to respond to environmental changes are in demand. In general, these stimuli are categorized into three groups that may be firstly physical (temperature, ultrasound, light, electricity and magnetism), or secondly chemical so changes in pH or ionic strength, and thirdly biological signals, such as enzymes and biomolecules. These signals may be applied externally or via an internal environment of a certain pathophysiological condition. A number of these stimuli will be discussed briefly.

2.6.1 Temperature triggering

The temperature-sensitive drug delivery is considered to be one of the simplest techniques of externally induced delivery system. The temperature-responsive drug delivery systems are based on components that tune their affinity for water as a function of environmental temperature, that in turn results in swelling or shrinkage of the network (Alvarez-Lorenzo and Concheiro, 2014). It originates from the balance in between hydrophobic and hydrophilic segments that result in polymer chain aggregation by physical cross-linking.

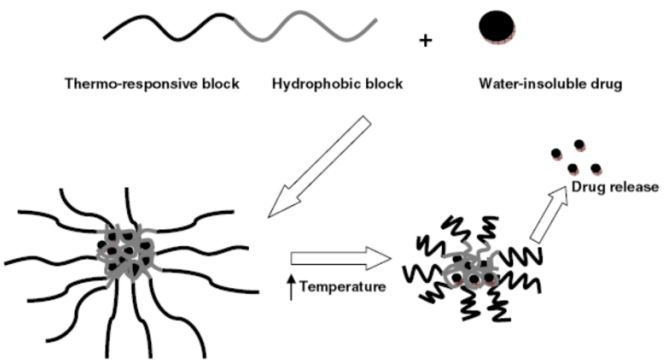


Figure 2.8 Structure and drug release from a thermo-responsive polymeric micelle (Klouda and Mikos, 2008).

In a study by Kaneko et al., it was reported that poly(N-isopropylacrylamide) (PNIPAAm) is soluble in water at the ambient temperature range of 25-32^{o}C (Kaneko *et al.*, 1999). The solution becomes opaque and turns into gel above the lower critical temperature. This phenomenon has been used for the development of thermos-responsive drug carriers, and the gelation process takes place as shown in Figure 2.8.

2.6.2 Light sensitivity

Light-responsive drug delivery systems have gained much attention as means of taking advantage of either daylight and seasonal exposure to natural solar irradiation or artificial sources of electromagnetic radiation of specified wavelengths (2500 to 380 nm) (Alvarez-Lorenzo and Concheiro, 2014). Ultraviolet and visible light can activate drug release from formulations that are topical or that circulate in blood vessels that are placed close to the surface. It was shown by Suzuki *et al*. (Suzuki and Tanaka, 1990), that the synthesis of light-responsive polymers is possible by incorporation of a light-sensitive element such as a chromophore. Light exposure onto the hydrogel results in local dissipation of the chromophore as heat by the absorbing light, inducing raised temperature of the hydrogel. The elevation of temperature modifies the swelling behaviour of the hydrogel and creates a path for controlled release application. Visible light-responsive polymers and hydrogels are more anticipated as they are safe, cost-efficient, widely available and easily tuneable (Qiu and Park, 2001).

2.6.3 Ultrasound triggering

Common physiotherapy equipment can be used to apply ultrasound to the body, to enable the penetration of nano-structures into desired regions and to activate

drug release (Alvarez-Lorenzo and Concheiro, 2014). Ultrasound belongs to a subsection of acoustics concerning vibratory or stress waves at frequencies beyond the human hearing range i.e., >20 kHz (Wu and Nyborg, 2008). As therapeutic ultrasound is relatively a non-invasive technique and highly applicable to a variety of cells, it has attracted much attention (Gao *et al.*, 2005). Owing to its versatility and cost efficient modality in various clinical applications, it has been extensively studied for the development of simulated pulsatile drug delivery systems (Lin and Thomas, 2004). Early research has demonstrated that acoustic cavitation is the main factor to accelerate the degradation rate of the biodegradable polymers and up to 20-fold increase in the drug release rate from polymer matrices (Kost *et al.*, 1989). In a study by Miyazaki *et al*. (Miyazaki *et al.*, 1988), the release rate of bovine insulin from ethylenevinyl alcohol copolymer drug delivery system was investigated. It was shown that by application of ultrasound at frequency of 1MHz, the insulin release was improved, which consequently lead to a significant reduction in blood glucose level.

2.6.4 pH sensitive systems

The changes in the pH gradient that may be found in the body has markedly gained the attention of researchers as one of the first and most assessed stimuli (Alvarez-Lorenzo and Concheiro, 2014). In general, the pH-responsive polymers that are sensitive to changes in environmental pH, contain pendant acids e.g. carboxylic and sulfuric acids, or basic e.g. ammonium salts group, that can either accept or release protons, therefore resulting in conformational changes of the polymers (Gupta *et al.*, 2002). Under healthy conditions, pH of the body tissue is kept at about 7.4, pH in the gastrointestinal tract changes from pH 1 to 3 in the stomach, and pH 7 in the intestine (Phang *et al.*, 2004). Development of pH-

responsive drug delivery systems has been based on these incremental changes, which utilizes the biochemical properties at the desired region for target specific delivery of active ingredients. In a study by Foss et al. (Foss *et al.*, 2004), insulin loaded poly acrylic acid-g-PEG nanoparticles were formulated for oral delivery. It was reported that at pH 6, the size of the nanoparticles became over 600 nm, therefore escaping uptake by the intestinal Peyer's patch and enabled treatment that played a crucial role in intestinal immunization and in the elimination of foreign elements. In a study by Kendall *et al*. (Kendall *et al.*, 2009), oil-in-oil emulsion technique was utilized for fabrication of pH-responsive acrylic microparticles for targeted gastrointestinal delivery of prednisolone; where they used Eudragit L55, S and L. It was discovered that Eudragit L was the most fitting polymer for this purpose, by displaying minimal drug release in acidic conditions and achieving optimum drug release when reaching the pH threshold of the corresponding polymer carrier. Moreover, pH-sensitive polymers with narrow pH range for stimuli release can be utilized for preparation of anti-cancer drug delivery; this is a requirement as the changes in the pH within bodily tissues are significantly refined. The extracellular pH of blood and healthy tissues is about 7.4, which is higher than that of the recorded value in tumour tissues which is typically in the range of pH 6.5-7. This is primarily due to over production of lactic acid as the result of hypoxic environment and fast metabolic rates. However, if overlooked, it may result in sever toxicity or poor therapeutic efficacy (Alvarez-Lorenzo and Concheiro, 2014).

2.7 Drug carrier materials

As the materials used for the development of drug delivery systems come in contact with the physiological environment of the human body, it is essential that

they are non-toxic and non-viable. Also, as they reside temporarily or permanently or undergo elimination by excretion, their proper functionality is limited by their long-term biocompatibility and their behaviour in the host. Moreover, they should offer adequate combination of the desired biological agent against the inflammatory response and bioactivities (Tang and Eaton, 1995). Polymers are the most versatile class of materials that have been used for development of drug delivery systems (Li and Vert, 1999). They are categorized as degradable and non-degradable. Biodegradable polymers can be further categorized as synthetic and natural.

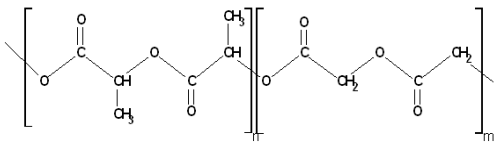
A number of biocompatible polymers have been used in this work: Poly(lactic-co-glycolic acid), Poly(ethylene glycol), Eudragit L100-55 and Polymethylsilsesquioxane. All these polymers are approved by The U.S. Food and Drug Administration (FDA), and have been widely used in many pharmaceutical formulations over a number of years.

The synthetic polymers that are used as drug delivery systems often include esters such as poly(lactic acid) (PLA), poly(glycolic acid) (PGA), and the copolymer poly(lactic-co-glycolic acid) (PLGA). These are considered aliphatic polyesters and have gained much interest due to their biodegradability and biocompatibility. This class of polymers undergo degradation by the hydrolytic cleavage of the ester bond in their backbone (Sinha and Trehan, 2005).

2.7.1 Poly (lactic-co-glycolic acid)

Lactic acid is more hydrophobic when compared to glycolic acid and therefore lactide-rich PLGA copolymer has less hydrophilicity and consequently absorb less water, therefore undergoes degradation over longer period of time (Witschi and Doelker, 1998). The mechanical strength and the ability of the polymer to

form as a drug carrier is influenced by its physical properties including molecular weight and the polydispersity index. Moreover, these characteristics also affect the rate of polymer biodegradation and hydrolysis. Different grades of PLGA polymers are generally characterized according to their intrinsic viscosity, that is a direct indication of their molecular weight. Moreover, the degree of crystallinity of PLGA polymer affects the mechanical strength, capacity to undergo hydrolysis and in turn the biodegradation rate. The type and the molar ratio of each monomer, lactide and glycolide, in the copolymer chain influences the resultant crystallinity of the PLGA (Jalil and Nixon, 1990). The PLGA (50:50) that equally contains lactic and glycolic acid undertakes hydrolysis fastest when compared to any other monomer ratio of this copolymer (see Figure 2.9) (Sinha and Trehan, 2005).



PLGA: poly(lactide-*co*-glycolide)

Figure 2.9 Poly(lactic acid-co-glycolic acid) chemical structure.

2.7.2 Polyethylene glycol

Polyethylene glycol is a synthetic water-soluble polymer that is synthetized by the interaction of ethylene oxide with water, ethylene glycol, or ethylene glycol oligomers (Figure 2.10) (Kadajji and Betageri, 2011). It has been widely used in biological applications due to its hydrophilicity and low intrinsic toxicity. As a result of its highly hydrophilic nature, it has often been used for preparation of solid dispersion formulations in order to improve the solubility of hydrophobic drugs or carriers when conjugated with them. Furthermore, it also enhances the

physical and chemical stability of drugs and inhibits aggregation of the drug *in vivo* and upon storage (Knop *et al.*, 2010). In addition, it has been used for preparation of temperature-responsive hydrogels that are attracting much attention as injectable drug delivery systems (Ruel-Gariépy and Leroux, 2004).

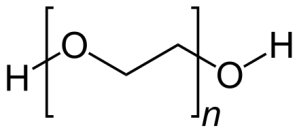


Figure 2.10 Polyethylene glycol chemical structure.

2.7.3 Poly (meth) acrylate

Eudragit L 100-55 is an ester fabricated by Evonik Industries, based on methylacrylic acid and methyl methacrylate (as shown in Figure 2.11) (Bando and McGinity, 2006). Eudragit L100-55 is a pH dependent polymer that is soluble in solutions of pH 5 or above, this characteristic has been utilized to develop drug delivery systems that are capable of protecting the active pharmaceutical ingredient in the stomach environment and facilitate the release only in the lower parts of the intestinal tract (Wang *et al.*, 2015).

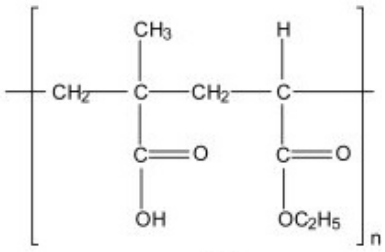


Figure 2.11 Eudragit L100-55 chemical structure.

Eudragit polymers have been extensively used for preparation of oral drug delivery systems including tablet coatings, tablet matrices as well as microspheres and nanoparticles for development of controlled drug delivery in the gastrointestinal (GI) tract (Kendall *et al.*, 2009; Alhnan *et al.*, 2010, 2011). These

applications arise mainly due to the pH-responsiveness solubility of the polymeric carriers.

2.7.4 Polymethylsilsesquioxane (PMSQ)

Polymethylsilsesquioxane is another polymer that is used frequently in pharmaceutical industry and biomedical applications for preparation of particles, fibres and also film fabrication (Figure 2.12)(Colombo, 2008). It has gained much attention for development of drug delivery matrices due to its inherent properties including non-toxicity, hydrophobicity, chemical and thermal stability (Ye *et al.*, 2010). It has also been used *in-vivo* owing to its physical stability, bio-durability and biocompatibility for several decades (Zhuo *et al.*, 2005).

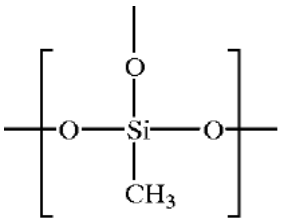


Figure 2.12 Polymethylsilsesquioxane chemical structure.

2.7.5 Polymer's biocompatibility, degradation and erosion

There are various description of the terms biocompatibility, degradation and erosion in the literature (Hong *et al.*, 2012; Ammala, 2013). To ensure a cohesive thesis, these terms are defined as follows:

Polymer biocompatibility: Biocompatibility is a prerequisite for polymers to be candidate for pharmaceutical application, meaning that they are non-toxic and that are able to co-exist in physiological conditions without imposing any harm to the surrounding living tissue (Sofokleous *et al.*, 2013).

Polymer degradation: Degradation is one of the key processes that lead to drug

release. The degradation takes place as a chemical process, it results in bond cleavage and chain scission, meaning the cleavage of the polymer chains to form oligomers and monomers. Polymeric biomaterials are further divided, based on their mode of degradation, to those that undergo enzymatic degradation and those that degrade hydrolytically. Natural biopolymers including proteins such as gelatin and collagen, polysaccharides and poly(β-hydroxy acids) degrade enzymatically. Synthetic polymers mainly undergo hydrolytic degradation, which is affected by the copolymer composition, water uptake, pH and the type of chemical bond. Degradation is characterized by the loss in molecular weight as well as the loss in mechanical integrity and effectively the complete degradation into monomers or monomer release (Huang and Brazel, 2001; Ammala, 2013).

Polymer erosion: Erosion refers to complete depletion of materials via physical processes of dissolution and diffusion (Tamada and Langer, 1993). It is subdivided to surface erosion and bulk erosion (Göpferich, 1996). As the name reflects, surface erosion indicates material loss from the surface, which occurs when the rate of erosion exceeds the rate of water permeation into the bulk of the polymer. As a result, polymer size is reduced while maintaining the initial structure. For this reason, undergoing surface erosion can lead to a steady drug release over time that is extremely reproducible and tuneable by modifying the surface area of the drug delivery device. Another feature that this process entails is the ability to protect the more water-labile drugs until the time of drug release, this is due to the slow water permeation in the bulk of the surface eroding drug delivery systems (Göpferich, 1996). Poly(anhydrides) and poly(ortho esters) are examples of surface eroding polymers that are among biodegradable classes of polymers that possess highly labile groups; this confirms a faster hydrolysis of

polymer chains that come in contact with water molecules. Lower water permeation can be achieved by designing the polymers with hydrophobic monomer units. Based on the type of monomers, their respective ratio and the molecular weight of each component, the duration of the degradation as well as its behaviour and mechanism can be altered from days to months (Modi *et al.*, 2005; Hussein *et al.*, 2013).

As opposed to surface erosion, in bulk eroding polymers, the mass loss occurs in the whole structure of the device. Bulk erosion occurs when water permeation rate into the polymer bulk surpasses the rate of erosion. Consequently, there may be no change in the size of the device for a prolonged period of time. The bulk polymer molecules hydrolyse, this in turn complicates the kinetics of polymer degradation/erosion when compared to surface eroding polymers (Makadia and Siegel, 2011). Almost all biodegradable polymers undergo combined surface and bulk erosion. Nonetheless, the extent of each mechanism is dependent on the chemical structure of the polymer.

2.8 Existing techniques for particle preparation

There are various techniques to prepare advanced particles, among them are emulsion solvent evaporation/extraction, spray drying, coaccervation phase separation, suspension cross linking and electrohydrodynamic processing.

2.8.1 Single/double emulsion

In single emulsion technique, a water-insoluble polymer is dissolved in an appropriate solvent that is immiscible with water. Subsequently, the drug of interest is dissolved in the polymer solution. The resulting solution is then mechanically emulsified in water with the aid on an emulsifier or surfactant to

help keep the emulsion stable (Filho *et al.*, 2012). Single emulsion is also known as oil-in-water emulsion (o/w) and is suitable for encapsulation of hydrophobic drugs since the encapsulation efficiency for hydrophilic drugs are significantly low as they may diffuse out into the aqueous phase and be lost during processing (Hombreiro Pérez *et al.*, 2000). Double emulsion is used to encapsulate water-soluble drugs by the water-in-oil-in-water (w/o/w) method (Jain, 2000). For this purpose, the polymer is first dissolved in an organic solvent. The aqueous solution of the hydrophilic drug is then emulsified in to achieve a water-in-oil (w/o) emulsion. Successively, the w/o emulsion is added to a large volume of water that contains an emulsifier under vigorous stirring, in order to obtain a w/o/w emulsion. In both cases, the residual solvent is removed either through evaporation or extraction, this in turn hardens the oil droplets (Jain, 2000). There are also alternative routes to obtain emulsions, including sonication (Zambaux *et al.*, 1998) instead of magnetic stirring or utilization of microfluidics in order to have better control over encapsulation (Chu *et al.*, 2007). Microfluidics technique has been widely used to encapsulate both hydrophilic and hydrophobic drugs and is proven to be an accessible and simple process, nonetheless, it is worth mentioning that it requires multiple steps for preparation of drug delivery systems with low production rate.

2.8.2 Coacervation phase separation

The coacervation phase separation process is carried out as a three-step process that occurs under constant agitation. It includes formation of three chemical phases that are immiscible, followed by deposition of the coating, and lastly the rigidization of the coating (Mallardé *et al.*, 2003). The three immiscible phases include the liquid vehicle phase, the core material phase and the coating

material phase. These are formed via dispersion of the core material in a solution that contains the coating polymer. The vehicle phase is used as the polymer solvent. To prepare the coating material phase, the immiscible polymer that is in liquid phase is made by varying the temperature of the polymer solution, with the addition of either salt, a non-solvent or an incompatible polymer to the polymer solution (Madan, 1978). This is followed by depositing the liquid polymer coating onto the core material. This step requires the polymer to be adsorbed at the formed interface in-between the core material and the liquid vehicle phase, which in turn ensures effective coating. lastly, continuous deposition of the coating material is promoted by a reduction in the total free interfacial energy of the system via rigidization of the coating. The main advantage of this process is the simplicity of the preparation that includes jacketed tanks with tuneable speed agitators. However, formation of aggregates is the main drawback due to lack of emulsifiers, which require large amount of solvent leading to complications with regards to solvent removal.

2.8.3 Suspension cross-linking

Preparation of polymeric particles by suspension cross-linking is carried out in three steps. This includes formation of a stable droplet suspension of the polymeric solution (or melt) in an immiscible liquid. The formed droplets are then gradually hardened by covalent cross-linking. Lastly, the resulting cross-linked polymer particles have to be recovered. The core material, either dispersed or dissolved, need to have much higher affinity for the droplet phase as opposed to the suspension medium, in order to ensure successful encapsulation. This requires the average size of the core particles to be at least one order of magnitude smaller than the droplets (Arshady, 1989).

2.8.4 Spray drying

Spray drying is a well-established applied atomization technique. As the name reflects, the polymer solution is pumped through a spray nozzle to disperse the liquid into a fine spray in the drying chamber. As the atomized droplets come into contact with the heated gas (air or nitrogen dependent on the application) (Zbicinski, 2002), they form dry particles due to evaporation of the excess liquid.

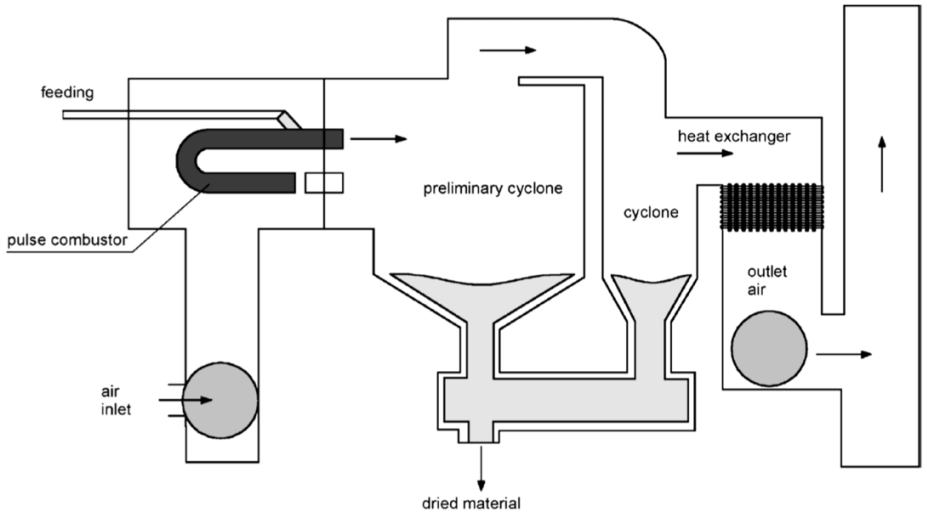


Figure 2.13 Schematic of the spray drying process (Zbicinski, 2002).

The solid particulate is then directed towards a cyclone and the gas directed away, the formed particles are collected in a container (Figure 2.13) The particle size range is affected by the size and design of the nozzle as well as the characteristic properties of the liquid used (Schuck *et al.*, 2009). The main advantages that this technique offers include improved reproducibility and high production rate (Burke *et al.*, 2004). Nonetheless, the common drawbacks associated with this technique comprise particle agglomeration and low collection yield caused by particles adhering to the walls of the spray drier before completion of solvent removal.

2.9 Electrohydrodynamic processing

The existing processes for development of polymeric drug delivery systems are well accepted and some used commercially as well. Nonetheless, there are some inherent drawbacks and disadvantages that cause limitations and necessitates further enhancement and improvement. Some of these restrictions include high processing costs, poor encapsulation efficiency, wide particle size distribution, multiple processing steps and the need for additional solvents and additives that may not be fully biocompatible, which are all associated with the techniques previously mentioned. These limitations compromise fabrication robustness and result in low controllability and biological stability of the bioactive agents (Sofokleous *et al.*, 2011). In distinction, electrohydrpdynamic processing is comparably simple and cost efficient. It enables production of particles and fibres with narrow size distribution at micro/nano scale under consistent ambient conditions and in a single step. It also negates the need for toxic and non-biocompatible additives that counterpart technique may require. Moreover, it offers high production yield and efficiency (Orlu-Gul *et al.*, 2014).

2.9.1 Principles and theoretical aspects of the EHD process

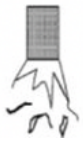
The term electrohydrodynamic processing, encompasses both electrospraying and electrospinning techniques, that is used to prepare particles and fibres, respectively. The underlying phenomenon that is the foundation of the modern EHD processing is based on what was initially defined by William Gilbert in the late 16th century, when he explained a droplet of water underwent deformation into a cone shape in presence of a charged piece of amber. Thereafter, there was not much progress to follow the initial observations. Only until the late 19th century when Lord Rayleigh helped advance the field of fluid dynamics that

greatly influenced EHD processing (Rayleigh, 1882). The Rayleigh instability, which is a term coined by Lord Rayleigh, is defined as the phenomenon observed as a streaming flow of liquid breaks up to smaller droplets that rises due to the perturbations in the fluid flow. He also defined the Rayleigh limit, which is the theoretical maximum charge a liquid can withhold before Coulombic repulsion forces surpass the surface tension, which leads to fission of the liquid. In the late 1960's, Taylor contributed to a deeper understanding of EHD processing by studying the effects of droplets when in presence of an electric field. He concluded that in order to attain a stable conical meniscus, it is essential to achieve a balance between surface tension stresses and the electrostatic force; this is referred to as the Taylor cone (Taylor, 1964). The most commonly used laboratory setup for electrohydrodynamic processing consists of a pump that is used to feed a steady flow of solution, a metal needle that the solution flows through, a high voltage power supply unit to provide a high voltage between the needle and a grounded base. When the liquid is fed through the needle orifice, the electric field generated by the applied voltage induces a charge within the flowing solution. The meniscus formed at the exit of the needle orifice adopts a conical form (the Taylor Cone) as a critical voltage is reached. This indicates that the applied electric stresses have overcome the surface tension of the liquid, which in turn results in jet initiation. The term "cone-jet mode" refers to the formation of a stable cone that is stationary but not static as the liquid jet is emitted from the apex. This mode is proven to be the most desirable one in electrohydrodynamic processing, since it results in formation of end-products with narrow size distribution. The main factors that affect the EHD processing can be categorized into three groups. These include the processing parameters, the

solution properties and the environmental conditions. The processing parameters consist of applied voltage, solution flow rate, needle diameter and the working distance, which is defined as the distance between the needle tip and the collector. The solution properties include electrical conductivity, surface tension, viscosity and dielectric constant. The environmental conditions that are known to have an effect on the EHD processing are temperature, relative humidity and the medium surrounding the needle (Ramakrishna *et al.*, 2005).

2.9.2 Electrospraying

An early contributor to further the advancement of electrospraying was John Zeleny. His work focuses on describing the charged droplet at the end of the glass capillaries that exhibited several modes of electrospraying (Zeleny, 1914). Since then, the functional modes in electrospraying have been further classified as shown in Figure 2.14 (Cloupeau and Prunet-Foch, 1994; Jaworek and Krupa, 1999). Based on the geometry that the liquid assumes at the capillary outlet and the type of jet behaviour as it disintegrates into droplets, different modes of EHD spraying have been classified.

Mode of spraying	Forms of liquid	Dynamics of meniscus/jet	Spray pattern
		Fragments of liquid	
1. Dripping 	Meniscus: Semi-spherical. Drop: simple spherical (with trailing thread or sibling).	Axially vibrating	Single drop (with siblings)
2. Microdripping 	Meniscus: cone/hemispherical. Drop: small, simple spherical (in some cases with trailing/leading thread).	Axially stable	Linear series of droplets (accompanied with fine mist)
3. Spindle 	Meniscus: cone/semi-spherical. Drop: elongated fragment of liquid (spindle) (with trailing thread).	Axially vibrating	Spindles disrupting into small droplets
4. Multispindle 	Meniscus: flat/multi-cone. Drops: multiple spindles (with leading/trailing thread).	Stable/Lateral vibrating	Spindles around the axis disrupting into small droplets.
5. Ramified-meniscus 	Meniscus: irregular short jets oriented in random directions. Drops: irregular fragments of liquids.	Irregularly deformed	Fragment of liquids sputtered in different directions

6. Oscillating-jet	Meniscus: skewed, oscillating cone. Jet: linear, oscillating in a plane.	Oscillating in a plane, kink instabilities at its end	Droplets sprayed in ellipsoidal-base cone
7. Precession	Meniscus: skewed, rotating cone. Jet: spiral, rotating around the capillary axis.	Rotating around the capillary axis, spiral instabil.	Fine aerosol sprayed in a regular cone
8. Cone-jet	Meniscus: cone (linear, concave, convex, skewed) Jet: simple straight linear.	Axially stable, varicose or kink instabilities.	Fine aerosol sprayed in regular cone
9. Multijet	Meniscus: flat, with small cones on the rim. Jet: linear, multiple.	Stable (usually with kink instabilities)	Fine aerosol sprayed in distinct directions
10. Ramified-jet	Meniscus: cone. Jet: linear, ramified in random directions.	Mother jet with randomly changed sub-jets	Droplets sprayed around the capillary axis

Figure 2.14 Characteristic features of the modes of EHD spraying (Jaworek and Krupa, 1999).

These modes are further divided into two groups: modes in which only liquid fragments are emitted and modes where a continuous jet is formed (Jaworek and Krupa, 1999). In the absence of the applied voltage, the dripping mode is observed, which occurs due to gravity. With a gradual increase in the applied voltage the rate of dripping is increased, which is also called micro-dripping. With further increase in the applied voltage, the micro-dripping mode is followed by the spindle mode, where discharge of a strand of continuous liquid is observed. As voltage is further increased, a multi-spindle mode is noted. This is where few columns of liquid are emitted concurrently from the needle meniscus.

Additional increase in the applied voltage gives rise to formation of the ramified meniscus mode where fragments of liquid are discharged and deposited in a random manner onto the collection platform. These are the modes in which only fragments of liquid are emitted. It is worth mentioning that liquids that experience spindle, multi-spindle and ramified meniscus modes of spraying cannot be electrosprayed. This is caused by having incompatible properties that do not meet the criteria to be processed via electrohydrodynamic technique.

As for the second group where a continuous jet is achieved, oscillating, precession and cone-jet mode can be formed at high applied voltages where the liquid surface tension is overcome by electric stresses. Sprays of liquid droplets are emitted from the tip of the jet in these modes. With the application of higher applied voltage multi-jet or ramified jet is formed.

The cone-jet mode is the most studied of the modes in electrospray processing since it leads to the formation of droplets that are relatively monodisperse. In the cone-jet mode, the applied electric field accelerates and directs the surface charge towards the apex of the cone. As a result of mutual charge repulsion and

the applied electric stresses, a jet is formed from the end of the apex. The formed jet travels a distance from the needle before it breaks up due to a combination of Rayleigh instability and Coulomb repulsion. As the liquid droplet travels, solvent evaporation also takes place which results in higher charge density within the droplet. When the charges reach the Rayleigh limit, droplets undergo Coulomb fission as the electrical repulsive forces overcome the liquid surface tension (Rayleigh, 1882; Gomez and Tang, 1994). When Coulomb fission takes place, there is subsequently a reduction in mass and charge of the droplet that are carried away by the daughter droplets. This leads to formation of a cycle whereupon further solvent evaporation results in a reduction in droplet volume whilst the charge is retained constant which result in approaching the Rayleigh limit and undergoing Coulomb fission.

This is an ongoing cycle until either the droplet impacts the target, solidifies before reaching the target or does not have sufficient charge density for Coulomb fission to occur (Jaworek and Sobczyk, 2008). Owing to the complex multifactorial nature of EHD processing, it is still not yet fully understood. Nonetheless, there are numerous groups and studies investigating this process. Theoretical scaling laws for the prediction of particle size exist, however their use is remained controversial which is due to discrepancies between theoretically predicted and experimental values (Barrero and Loscertales, 2007). Unfortunately, this lack of in depth understating results in undertaking the experimental approaches by trial and error when using a material or solvent combination that has not been used previously.

2.9.2.1 Post production of droplet evolution

The droplets behaviour during spraying has been investigated and it has been suggested that the radial velocities of the droplets are lower than that of their axial velocities. It has also been indicated that both primary and secondary droplets have the equal initial radial and axial velocities (Hartman *et al.*, 2000). Nevertheless, due to size difference, the primary droplets have higher inertia and therefore their acceleration due to the electric field is lower. Therefore, smaller droplets attain a higher radial velocity and consequently the main and secondary droplets will be divided. As a result, smaller droplets are formed at the edge of the spray and the main droplets are located in the centre (Hartman *et al.*, 1999). The circular spraying pattern that forms during EHD or co-axial EHD, as well as the higher coating growth rate at the centre can be explained by this phenomenon.

2.9.2.2 Droplet evaporation and drying of products

As mentioned briefly, the droplet size reduces considerably when the solvent evaporation takes place without changing any of its transport properties. The outcome is greatly influenced by the properties of the solvent and environmental factors including temperature, humidity and wind. As the droplet is evaporating, the charges density increases and therefore its size will be reduced and consequently the Rayleigh limit for the droplet's charge may be reached. If this occurs, the droplet size will be reduced to a greater extent due to droplet fission (Wilhelm *et al.*, 2003).

2.9.2.3 Jet break-up

Jet break-up is the process of particle formation and it takes pace by two distinctive mechanisms (Hartman *et al.*, 1999). Based on the first one, the applied electric field at the apex of the Taylor cone stimulates instabilities that results in emission of ions, neutral atoms and droplets are discharged from the surface of the liquid. According to the second and the common mechanism, the surface instabilities causes formation of a liquid jet that breaks up into droplets (Rulison and Flagan, 1994). In order to establish a stable cone-jet, the set flow rate needs to be in a specified range for every given applied voltage. At lower flow rates, the jet-mode breaks up to a multi-jet mode due to axisymmetric instabilities. These instabilities are also called varicose instabilities. At flow rates that are higher than that of the critical rate, the current through the liquid cone increases. As a result, surface charge on the jet will be much higher and hence, lateral instabilities will affect the jet break-up resulting in wider droplets; this is desirable for processing even and uniform coatings (Cloupeau and Prunet-Foch, 1989). At flow rates, well above a maximum value, the jet-mode adopts micro-dripping mode, as shown in Figure 2.14 (Gañan-Calvo *et al.*, 1996).

2.9.3 Parameters influencing EHD process

As briefly mentioned before, there is a number of factors that affect the electrohydrodynamic process. These include the intrinsic properties of the solution used that include viscosity, electrical conductivity, surface tension. The processing parameters also have an effect, these include the set flow rate, applied voltage, needle dimension and the working distance (Sofokleous *et al.*, 2011). Each of these factors is described herein. It should be noted that it is

almost impossible to denote boundary values for one single parameter that influence the EHD process. The reason being is that the precise value of an EHD parameter is linked with other parameters in turn. In other words, when one parameter is altered it will affect the value of the other one in order to establish and maintain a stable jet.

2.9.3.1 Processing parameters

2.9.3.1.1 Applied Voltage

The applied voltage determines the electric field that is formed between the capillary and the ground electrode, which regulates the electrohydrodynamic atomization process. The meniscus of the liquid forms a stationary conical shape, thus a cone-jet, when subjected to a well-defined voltage range (Tang and Gomez, 1994). When this range is defined, the mean droplet size reduces as the applied voltage is increased. Therefore, in order to achieve the minimum droplet size, it is crucial to select the highest possible value of the voltage in conjunction with the lowest possible value of the set flow rate, within the stable jet range (Jayasinghe and Edirisinghe, 2002). A pulsating mode is formed when the applied voltage is lower than the defined range (Gañán-Calvo, 1997; Gañán-Calvo *et al.*, 1997). When processing solutions that are highly conductive and viscous, the size of the produced droplets in the cone-jet mode was found to be impartial to the applied voltage (Ku and Kim, 2002).

2.9.3.1.2 Flow rate

The size distribution of the formed particles produced by utilizing electrohydrodynamic atomization on the cone-jet mode, is dependent on the jet diameter as well as the break-up of this jet into droplets. There exists a minimum

flow rate for every solution, below which a stable cone-jet mode cannot be achieved for a given applied voltage. It is due to the axisymmetric instabilities that the jet breaks up at this minimum flow rate. At higher flow rates, the current through the solution cone increases, which in turn results in higher accumulation of surface charge on the jet. If the surface charge is high enough, the jet undergoes lateral or azimuthal instabilities (also called kink instabilities). As the influence of the so-called kink instabilities becomes more prominent, the droplets exhibit wider size distribution (Hartman *et al.*, 2000).

2.9.3.1.3 Needle size and electrode configuration

In general, the nozzles used for electrohydrodynamic processing are made of metal. Cloupeau and Pruner-Foch reported that for a given solution, the applicable range of flow rates is dependent on the needle size (Cloupeau and Prunet-Foch, 1989). Tang and Gomez (Tang and Gomez, 1994) showed in 1994, that the stable cone-jet domain of electrospraying is very sensitive to the needle size; however, the size of the droplet is entirely independent. In another study carried out by Tang and Gomez (Tang and Gomez, 1996), it was indicated that with an increase in the needles size, the required maximum flow rate for formation of a stable cone-jet decreases.

Jayasinghe and Edirisinghe (Jayasinghe and Edirisinghe, 2002) studied the effects of the ground electrode configuration on trajectories of the formed droplets and it was shown that for a given point shaped ground electrode, the concentration of the formed droplets in inversely proportional to the radius of the point.

2.9.3.1.4 Working distance

The working distance is defined as the set distance between the nozzle and the ground collector, this is also called the collection distance. It is directly proportional to the strength of the applied electrostatic field and the evaporation time of the solution before the end product reaches the collection platform (Collins *et al.*, 2012). This distance in turn affects the morphology and the geometrical features of the end products. In order to achieve the desired particle shape, the working distance should be optimized so as to determine an optimum range. When the working distance is lower than the optimum range, the collected products may be wet due to inadequate solvent evaporation (Jalili *et al.*, 2005).

2.9.3.2 Liquid properties

2.9.3.2.1 Surface tension

In order to obtain a stable cone-jet, the electric field has to overcome the surface tension of the solution. There exists a correlation between the two, as the surface tension increases there is the need for higher electric field strength. The required threshold voltage for formation of a stable cone-jet increases with the solution surface tension.

However, if the liquid surface tension is too high, a stable jet may not be achievable as the required field surpasses that for the electric break down in the gas surrounding the cone (i.e. air, under environmental conditions). In a study by Tang and Gomez (Tang and Gomez, 1995), they used gases that held higher electrical break down as the surrounding medium, and managed to achieve a stable cone-jet mode with water as the solution.

2.9.3.2.2 Viscosity

The effect of the viscosity on the droplet size was studied by Hartman et al. (Hartman *et al.*, 2000), where they reported that for solutions with viscosity lower than 100 mPa s, the droplet size is directly proportional to the viscosity, in the stable cone-jet mode. Nonetheless, a stable cone-jet mode is not attainable if the solution viscosity is too high. This is due to insufficient electrical stresses acting on the solution that are incapable of overcoming its surface tension. In co-axial electrohydrodynamic processing, solutions with sufficiently high viscosity may be used as the outer liquid in order to enable diffusion of the electrical stresses that act on the liquid-air interface into the liquid bulk (López-Herrera *et al.*, 2003).

2.9.3.2.3 Electrical conductivity

The electrical conductivity of the solution is of crucial importance in electrohydrodynamic processing. Sufficient electrical conductivity is prerequisite for the liquid droplet at the capillary exit to assume a conical form. Liquids with low conductivity (insulators, e.g. oil), cannot be processed by electrospraying, unless additives are used in order to increase their conductivity (López-Herrera *et al.*, 2003). The extent of the liquid conductivity also affects the morphology of the stable cone-jet (Gañán-Calvo, 1997). An increase in the conductivity is associated with a decrease in the capillary width, length and flow rate for formation of a stable cone-jet mode, and subsequently lower droplet size. It has been reported that increasing the electrical conductivity results in a reduction in the size of the formed particles (Gañán-Calvo *et al.*, 1997).

2.9.3.2.4 Dielectric constant

Dielectric constant is a measure of the extent of polarizability of a material in an electric field. Polarization effectively lowers the magnitude of the external electric

field. The electrical relaxation time is determined by the dielectric constant and the vacuum permittivity. It is defined as the time required for a perturbation in the electric charge to be smoothed out and is given by:

$$t_e = \varepsilon_r \varepsilon_o / K \quad \text{(Equation 2)}$$

Where ε_r is the dielectric constant of the liquid and $\varepsilon_o$is the dielectric constant of the vacuum [C/V m], and K is the electrical conductivity of the vacuum [S/m] (Gañán-Calvo, 1997).

2.9.3.2.5 Density

For liquids of sufficiently high viscosity and electrical conductivity, the electrical charge is conveyed across the jet via viscous drag. The latter is dependent on the liquid density. Therefore, the formation of a stable cone-jet mode is significantly affected by density (Gañán-Calvo, 1997). A summary of the factors influencing the size of the electrosprayed products is shown here:

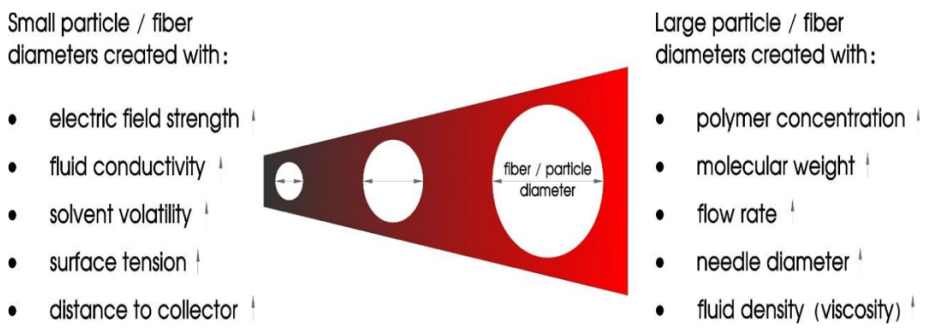


Figure 2.15 The influence of processing and solution parameter on particle/fibre formation

2.9.4 Co-axial electrohydrodynamic atomization (CEHDA)

Current techniques for effective encapsulation in micro/nano particles has been achieved by utilizing various methods including sol-gel encapsulation (Gouin,

2004), emulsification of core liquid in a continuous phase (Jafari *et al.*, 2006) and coacervation (Fery *et al.*, 2004). Nonetheless, most of these technique for preparation of encapsulating drug delivery systems entail complicated, time consuming procedures that are not cost efficient. In addition, they require use of solvents and additives that are not fully biocompatible. In contrast, co-axial electrohydrodynamic atomization processing offers efficient preparation of particles for application in a number of sensitive fields including medicine, cosmetic and food processing (Enayati *et al*., 2011b; Ammala, 2013). It can be used for preparation of particles at micro/nano scale for encapsulation of a sensitive core (e.g. cells, enzymes or drugs) engulfed in a protecting shell (Enayati *et al*., 2011a). Moreover, it can be utilized for preparation of microbubbles that have promising applications including targeted drug delivery, diagnostic imaging, thrombolysis and focused ultrasound surgery (Mizushige *et al.*, 1999; Liu *et al.*, 2006; Schmidt and Roessling, 2006; Poliachik *et al.*, 2018). Co-axial EHD has a rather brief history with just over a decade's worth of exploration into it (Zhang *et al.*, 2012). With the addition of a coaxial needle, comes a great deal of complexity into the process. The governing parameters in the single needle EHD also applies to the co-axial needle configuration. However, due to the interaction of the two or more co-flowing solutions, additional factors need to be taken into account in order to obtain a stable cone-jet. It is prerequisite for the EHD forces to act upon at least one of the solutions in order to successfully attain a stable cone-jet. This solution is referred to as the driving liquid, a term first coined by Loscertales *et al*. (Loscertales *et al.*, 2002). The driving characteristic of the liquid can be determined by making a comparison between their electrical relaxation times (t_e), as previously defined. The solution

with the smaller relaxation time can be placed onto inside or outside needle to form a cone-jet. If placed as the outer solution, the electric stresses directing toward the apex of the cone have to be transmitted throughout the solution by viscous diffusion. This requires the solution to have sufficiently high viscosity to obtain a cone-jet.

2.9.5 Recent advances in electrohydrodynamic atomization

Various configurations of needles facilitate great potential for encapsulation. The flexibility of co-axial EHD to prepare various novel morphologies of the end products was further extrapolated in a study by Ahmad *et al*. (Ahmad *et al.*, 2008). In this study, co-axial EHD was used to prepare double-layered bubbles, porous encapsulated threads and nano-capsules that constituted of three distinct layers. It was reported that successful preparation of multilayered structures has the potential to process particles exhibiting multi-stage controlled release, and *in-situ* encapsulation of nanoparticles, liquids and/or gases (Figure 2.16a).

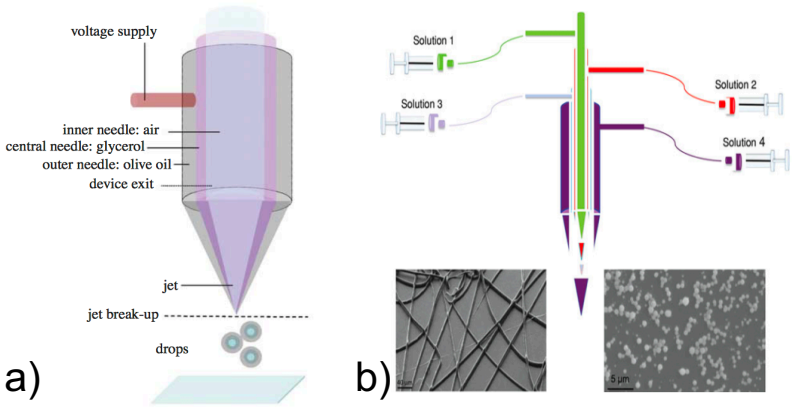


Figure 2.16 Schematic representation of the co-axial EHD set-up, a) three-needle set-up for preparation of particles with novel morphologies (Ahmad *et al.*, 2008), b) four-needle set up to prepared four-layered particles and fibres (Labbaf *et al.*, 2014).

The potential and adaptability of the co-axil EHD processing for preparation of micro/nano capsules have led to further advancements and developments of

systems with up to four concentric needles. Labbaf *et al*. (Labbaf *et al.*, 2014) pioneered utilization of four concentric needles for EHD processing, and confirmed the feasibility of the technique for successful preparation of both particles and fibres in nano scale with four different layers (Figure 2.16b).

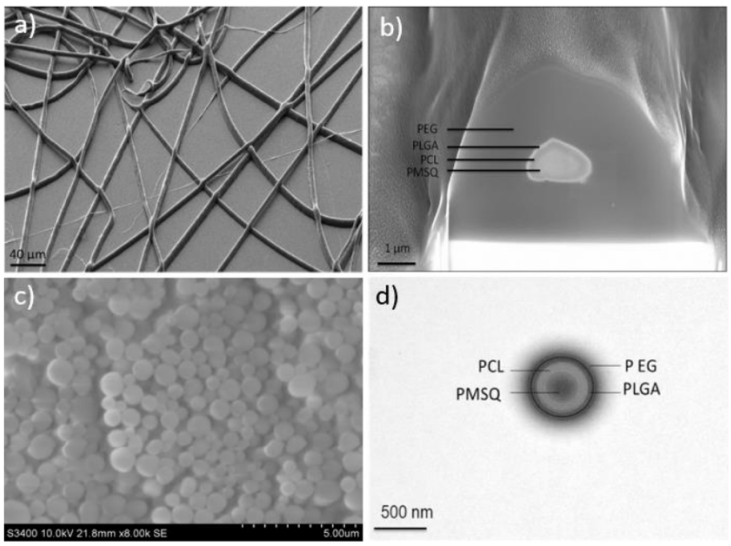


Figure 2.17 Prepared products via four-needle co-axial EHD processing, a) fibres, b) fibre cross section, c) nanoparticle, d) nanoparticle cross section (Labbaf *et al.*, 2014).

The morphology, distribution and layer formation of the prepared nanoparticles and fibres were investigated using transmission and scanning electron microscopy (TEM and SEM), as well as focused ion beam (FIB). The results showed uniform fibres possessing four layers (Figure 2.17a and b), and spherical nanoparticles with average size distribution of 620 nm ± 120 constituting of four layers (Figure 2.17c and d).

In a recent study by Parhizkar *et al*. (Parhizkar *et al.*, 2017), multiplex electrospray systems were developed in order to further enhance the processing throughput. In order to investigate the applicability of the EHD processing at larger industrial scale, four different needles in a circular and a rectangular

metallic plate (Figure 2.18a and b), as to study possible interactions of the formed jets emerging the nozzles with the neighbouring ones, were connected to a high voltage power supply. Stable cone-jet mode was achieved for all needles in both configurations. However, the multi-needle circular plate proved to enable more control over particle uniformity while it required lower applied voltages. The production rates of both systems were found to be considerably higher than that of single electrospraying, whilst achieving particles with narrow size distributions.

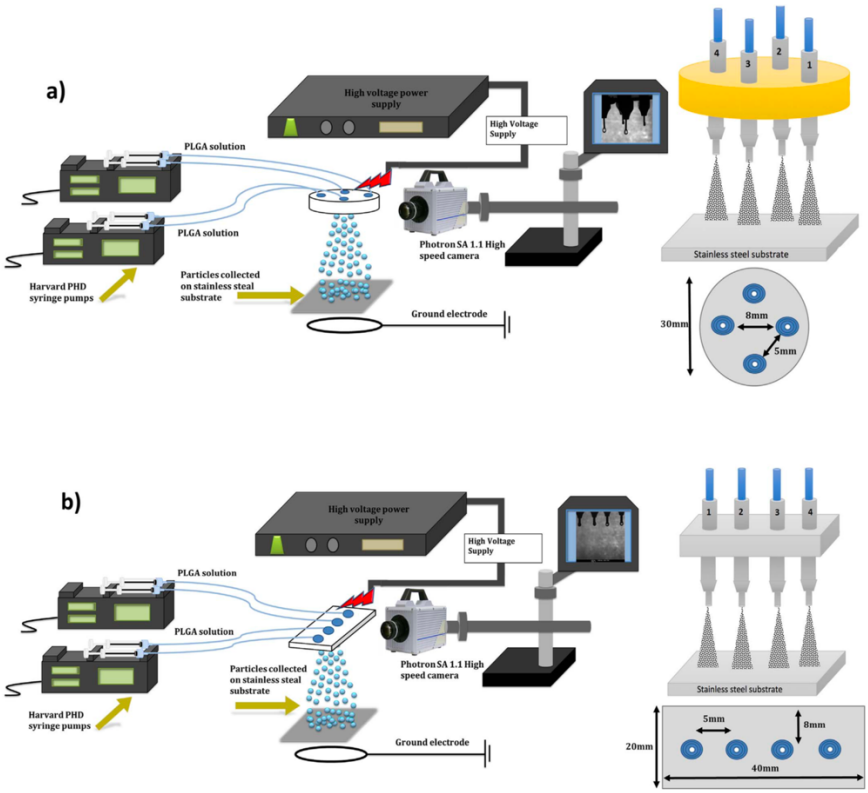


Figure 2.18 Schematic diagram of the multiples four needle electrospraying set-up with a) circular plate and b) rectangular plate configurations (Parhizkar *et al.,* 2017).

In another study, Yan *et al*. (Yan *et al.*, 2017) designed multiplex double-needle system for co-axial EHD processing (Figure 2.19), and investigated the electrospraying characteristics, deposition pattern, particle morphology and structure. The obtained results were then compared to that of a simple co-axial EHD set-up. It was determined that in order to ensure the integrity of the

prepared particles, there is an optimum distance in-between the needles at which the particle size distribution and the assumed core-shell structure may be maintained.

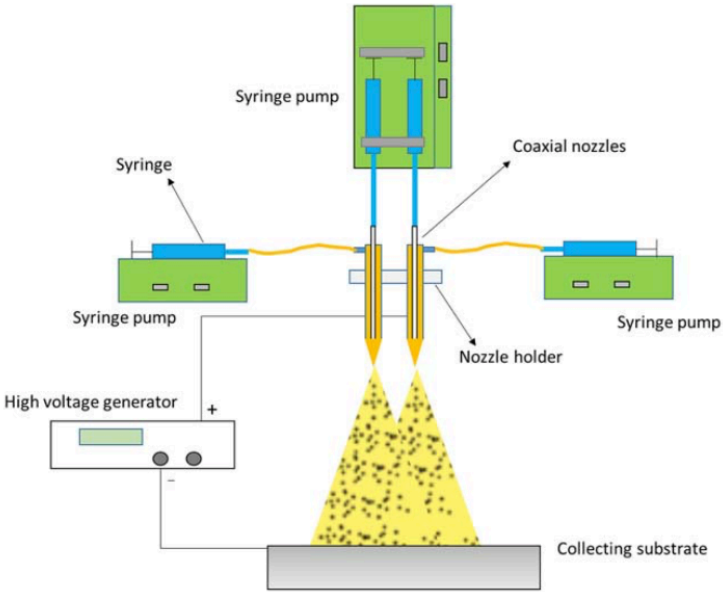


Figure 2.19 Experimental set-up for double-needle co-axial electrohydrodynamic systems (Yan *et al.,* 2017).

2.10 Pharmaceutical application

2.10.1 Enhancing the solubility of poorly water-soluble drugs

Sufficient aqueous solubility of the drug is a critical factor for drugs that are specifically administrated orally. Poorly water-soluble drugs, due to their low aqueous solubility and their poor capacity to dissolve in the gastrointestinal fluid within a relevant absorption window, have low bioavailability. Therefore, the drug dissolution is considered the main limitation and a challenging task to improve upon in order to develop oral formulations (Fahr and Liu, 2007). To tackle the solubility issue, and ultimately enhance drug absorption and in turn the bioavailability, various strategies have been developed over the last decade (Nguyen et al., 2016b). some of these strategies include modification of the drug at molecular level, such as formation of prodrugs and salts (Singh *et al.*, 2011), or

via using lipid based formulation (e.g. emulsions) (Gursoy and Benita, 2004); or by modifications at particulate level, for example through particle size reduction or use of solid dispersion formulations (Rabinow, 2004; Van Den Mooter, 2012). Nonetheless, all the existing techniques have disadvantages although having shown success. The prospect of using electrohydrodynamic processing for improving the dissolution rate of poorly water-soluble drugs is promising.

2.10.2 Preparation of amorphous solid dispersions by electrospraying

The EHD processing affects the poorly water-soluble drugs at particulate level, more precisely by nanoionization and amorphization (Nguyen *et al*., 2016b). As with the application of electrical forces, a drug loaded solution is atomized to small droplets with diameters ranging from micron to nanometer which facilitates fast solvent evaporation and in turn, rather instantaneous solidification. Owing to exceedingly high solvent evaporation rates, amorphous state of the incorporated drug is made; that is, amorphous solid dispersions as drug molecules are nearly “frozen” in a random manner and immediately in the solid polymer matrix and conserved as solid solution. As mentioned previously, spray drying is a rather comparable technique to electrospraying in terms of preparation of solid dispersion formulations. However, spray drying suffers from wide particle size distribution, particle agglomeration and stability issues for thermosensitive molecules (Singh and Van Den Mooter, 2016). On the basis of the higher free energy of the drugs when in an amorphous state, and also the higher surface area to volume ratio that are resultant from submicron particle size, enhancement of the dissolution is expected once prepared by means of EHD processing.

In the studies by Bohr *et al*. (Bohr *et al.*, 2011, 2012), celexocib microspheres (a BSC Class II compound) were prepared using PLGA as the polymeric carrier. It

was shown that by tuning the experimental parameters and the drug content, they had control over both size and morphology of the prepared formulation. Further physical characterization analyses including X-ray powder diffraction (XRD) and differential scanning calorimetry (DSC) were taken out in order to investigate the state of the celecoxib, which was found to be in an amorphous form and stable over the course of 8 months. Moreover, faster release of celexocib from the prepared microparticles was reported as opposed to the pure form, which was due to the amorphous state of the drug. Amorphous solid dispersion formulation of ketoprophen, a BSC Class II drug, was prepared using EHD and polyvinylpyrrolidone (PVP) as the carriers, where rapid release of the drug was reported in one minute (Yu *et al.*, 2011). Yu *et al*. (Yu *et al.*, 2014) utilized EHD to prepare oral fast-dissolving formulation of acetaminophen using PVP K25, that due to the amorphous state of the drug post processing, exhibited rapid release within one minute when compared with casted films containing the same component and pure powders. Nevertheless, it has been reported that some macromolecules may be degraded during the electrospraying process, which could be induced by the stress involved in the operation parameters such as thermal stress in drying and the shear stress in the nozzle (Sridhar and Ramakrishna, 2013).

2.10.3 Preparation of targeted drug delivery systems

There are numerous cases of successful development of targeted drug delivery system utilizing the electrohydrodynamic processing in the literature. In a study by Jing *et al*. (Jing *et al.*, 2011), paclitaxel loaded core-shell structures were prepared using TiO_2 as the shell using co-axial EHD. Where the inner core contained a dispersion of paclitaxel and Fe_3O_4 in olive oil, and the outer shell

consisted of a mixture of tetrabutyl titanium and PVP K30 in a blend of ethanol, dimethylformamide and acetic acid as the solvent. Magnetic targeting feature of the prepared system was resultant of Fe_3O_4 inclusion in the core structure. Using ultrasonic stimulation, release of the paclitaxel was initiated via ultrasonic simulation, whilst the duration of the ultrasonication period regulated the release profile.

Gambogic loaded nanoparticles using poly-DL-lacride as the carrier was developed by Yin *et al*. (Yin *et al.*, 2014), for targeted delivery using EHD processing. It was demonstrated that with modification of the material and processing parameters, the particle size distribution and the release profile can be modulated. The developed formulation demonstrated higher efficacy when compared to that of the gambogic solution administration when tested *in vivo*.

Jain *et al*. (Jain *et al.*, 2014) developed indomethacin loaded particles for colon targeted delivery by using inulin acetate as the polymeric carrier system, and confirmed the release of indomethacin as particles were placed in inulinase containing simulated colonic fluid with pH 6.8.

In a study by Nguyen *et al*. (Nguyen *et al*., 2016a), solid dispersion formulation of darunavir in hydroxypropyl methylcellulose was encapsulated in Eudragit L100 using co-axial EHD. It was shown that by fine tuning the experimental parameters, it is possible to prepare drug delivery systems of narrow size distribution with high encapsulation efficiency utilizing a single step technique. The release of darunavir in acidic medium HCL 0.1M was well minimized to less that 20% due to use of the enteric shell.

2.10.4 Preparation of controllable drug release systems

The preparation of sustained drug release delivery systems that necessitates the

incorporation of the drug into a biodegradable and biocompatible polymeric matrix, has been of great interest in the recent years. Various types of synthetic polymers with different degradability profile, including PLGA, PLA and polycaprolactone (PCL), have been developed for clinical applications as they are FDA approved, owing to their biocompatibility and low toxicity. Alongside different configuration of single nozzle and co-axial dual capillary nozzle, EHD enables preparation of drug-loaded nano/micro particles with various microstructures ranging from polymeric matrix to core-shell structures and multi-compartment particles (Labbaf *et al.*, 2014).

In a study by Eltayeb *et al*. (Eltayeb *et al.*, 2013), co-axial EHD was utilized in order to investigate its application for encapsulation of hydrophilic components (vanillin, ethylmaltol and matol) into polymer-lipid core-shell nanoparticles. It was reported that by using the lipid component, the stability of the nanoparticles against aggregation and collapse was enhanced while achieving increased encapsulation efficiency. Electrospraying has been proven to be single-step technique that is capable of preparing solid based lipid-based particles (Nguyen *at al*., 2016b).

2.10.5 Preparation of multiple drug delivery systems

The potential of incorporation of multiple drugs in one drug delivery system is a promising prospect of application of electrohydrodynamic processing. Co-delivery of multiple drugs offers various advantages in terms of preparation including manufacturing costs, storage and distribution. From patient perspective, the synergistic action of multiple active ingredients, suppressed drug resistance and convenience are among the benefits of these formulations (Desai *et al.*, 2012). There is a limited number of studies for development of multiple drug delivery

systems that are prepared using EHD processing in the literature. A combination of two or more APIs can be incorporated into polymer matrix or core-shell structured particles, therefore they can facilitate drug release profiles that are programmable and distinct if required (Nguyen *et al*., 2016b).

In a study by Sakuma *et al*. (Sakuma *et al.*, 2015), co-axial electrospraying was used in order to prepare drug delivery systems containing dissolved lopinavir (LPV) and ritonavir (RTV) in ethanol as the inner solution and Eudragit L100 as the outer one. The aim of this study was to enhance the oral absorption of LPV through coencapsulation with RTV via electrospraying. They reported successful preparation of particles with improved dissolution properties for both drugs when compared to pure substances, as a result of amorphization, improved dispersity and reduced particle size. In addition, *in vivo* testing demonstrated higher oral absorption of LPV from the co-formulated particles when compared to that of the single encapsulation. This was explained by the synergistic effect of combining the drugs which lead to the boosting effect.

Cao *et al.* (Cao *et al.*, 2014) used co-axial EHD to obtain distinct release profiles of rhodamine B (RhB) and Nap, whereby they proposed using a blend of hydrophilic and hydrophobic polymer (PVP/PLGA or PCL/PLGA) for regional encapsulation of hydrophilic RhB and hydrophobic Nap into core-shell nanoparticles. Different release patterns were based on the drug distribution.

In a similar study by Wang *et al*. (Wang *et al.*, 2013), emulsion electrospraying was used in a single-needle set-up whereby RhB and Nap were used as model drugs to develop dual drug-loaded micro/nano particles with core-shell structures. Two different type of particles were prepared by incorporating RhB in the core or the shell of the developed systems, and Nap in the opposing phase as to RhB.

Different *in vitro* drug releases were obtained by careful selection of the carrier polymers, chitosan as core and PVP as shell. The different drug release profiles were resultant of the regional drug loading.

2.10.6 Preparation of inhalable drug systems

Electrohydrodynamic processing has also been used for development of drug delivery systems for administration through inhalation that enables local delivery of active ingredients. This can be used to treat lung disease including asthma, bronchitis and chronic obstructive pulmonary disease (Nguyen *et al*., 2016b), as well as for delivery of nano-therapeutics such as proteins, peptides for treatment of several diseases including cancer, pain management and diabetes (Shoyele and Cawthorne, 2006; Chow *et al.*, 2007; Goldberg and Wong, 2015).

Administration of therapeutics via inhalation offers a non-invasive path that is beneficial when compared to conventional delivery routes. Attributed to the physiological function of the respiratory system, some of the benefits that this route offers include high surface area of the mucous membranes of the respiratory tracts and pulmonary epithelium, the fine membranes in-between the pulmonary alveoli and circulation, and also the high blood flow to and from these specific organs that in turn results in high and fast absorption into the circulation (Patton and Byron, 2007; Beck-Broichsitter *et al.*, 2012). Furthermore, the absorbed drugs do not suffer from undergoing first pass metabolism. These factors lead to a fast onset of action that enables smaller dosages to be administered. Nonetheless, there are a number of challenges in terms of development of the drug delivery systems for inhalation. The particle size and the size distribution affect the deposition and diffusion as they are being transported (Garbuzenko *et al.*, 2014). The optimum particle size range that offers the highest

potential to reach the lower airways and alveoli and be deposited, is 0.5-20 μm. Another factor that influences the efficacy of the deposition is size distribution. Consequently, controlled monodisperse particles with narrow size distribution and of specified size range are required to prepare successful drug delivery system for inhalation. Electrohydrodynamic processing meets the requirements for production of drug delivery systems for inhalation as it facilitates preparation of polymeric particles of controllable size and morphology that exhibit narrow size distribution. Ijsebaert *et al*. (Ijsebaert *et al.*, 2001) used EHD atomization processing to prepare aerosol generator of beclomethasone dipropionate particles. They reported successful preparation of the aerosols of required size with narrow size distribution, where they utilized a configuration of nozzle-discharge needle.

2.10.7 EHD jet printing and patterning

Electrohydrodynamic processing has been widely utilized to perform jet printing, which enables printing patterns and different topographies on different surfaces at micro/nano scale (Youn *et al.*, 2009). One application of this techniques lies in preparation of pre-programmed Hydroxyapatite nanoparticles deposition on patterned tracks, that has the potential to guide the attachment of osteoblast cells and direct their alignment and is thereof highly attractive for development of implants (Thian *et al.*, 2008). In a recent study by Wang *et al*. (Wang *et al.*, 2016), EHD printing was used to fabricate tetracycline hydrochloride loaded patterned microstructures with composite fibres. The prepared structures exhibited typical drug release characteristics conforming with literature, this proves their performance and feasibility in the innovative field of personalized medicine. However, the use of EHD printing is not relevant to this thesis.

2.11 Polypharmacy

By definition, polypharmacy is the “use of multiple drugs by a patient” (Mizokami *et al.*, 2012). As a consequence of the prevalence of multiple chronic conditions, geriatric population is inclined to be exposed to polypharmacy and hence the associated high risks of disability and hospitalization (Gijsen *et al.*, 2001). In order to enhance geriatric drug therapy, the targeted elderly patients must be considered with regards to their individual capabilities and limitations throughout the development of pharmaceutical products. The major age-related variations observed in the elderly population include physiological functioning of the organs, the cognitive, visual, motoric and swallowing capabilities; all of which must be reflected in selecting the proper medication regimen (Stegemann *et al.*, 2010).

The administration of multiple active pharmaceutical ingredients in one unit form offers a potential solution for overcoming the polypharmacy challenge where patients can benefit from treatment for several conditions by the release of drugs incorporated in a single dosage. Nevertheless, this necessitates the rapid development of engineering technologies to encapsulate and control the release of multiple drugs contained in single dosage form (Orlu-Gul *et al.*, 2014).

2.11.1 Fixed Dose Combinations (FDCs)

Fixed dose combination drugs also known as FDCs are essentially two or more active pharmaceutical ingredients combined in a single dosage form (Gautam and Saha, 2008). FDCs are relatively common for nearly all therapeutic areas and are available in different formulations including oral, parenteral and inhalation; among which the oral delivery route ranks the highest (Desai *et al.*, 2012).

2.11.1.1 Advantages and disadvantages of FDC products

Fixed dose combination products are of key importance in treating numerous types of diseases such as cardiovascular, HID/AIDS, malaria and tuberculosis where polypharmacy potentially exists. However, they present both pros and cons regarding their therapeutic and non-therapeutic effects.

Therapeutic advantages

Different pharmacological mechanisms can be introduced into one single dosing unit offered by FDC products. This presents further substantial clinical values in various therapeutic areas such as treatments of anti-hypertensive, anti-viral, anti-glycaemic and anti-cholesterol. Some of these advantages comprise:

- Synergistic therapeutic effects where the efficacy is enhanced compared to co-administration of the individual drugs (Serebruany *et al.*, 2004).
- Potential drug abuse can be minimised by use of combined drugs where one would diminish the unintended side effects of the other.
- Incorporation of both short acting and long acting active pharmaceutical ingredients where coverage of an extended period is made possible.
- Reduction in drug-resistant micro-organisms, more specifically for anti-viral and anti-tubercular drugs.
- Enabling combination of drugs where one improves the safety and/or tolerability of the other.
- Minimising dosing unit burden and improving patient compliance which is of great importance in order to avoid development of drug resistance.

Non-therapeutic advantages of the FDC products

They are more cost effective for the consumer as they are potentially more economical than when sold individually, hence enhancing the patient compliance. FDC products also reduce the cost from the manufacturing point of view as well as simplification of logistics of distribution (Desai *et al.*, 2012).

Disadvantages of the FDC Products

In spite of the aforementioned, the FDC products also exhibit some disadvantages including:

- The reduced dosage flexibility is a down side of FDCs as there is a constraint within which only predetermined dosing combinations are available. Although multiple strength combinations are developed, some patients may need frequent dose adjustments if the FDCs come short.
- It is also challenging to distinguish the possible cause of adverse drug reactions of FDCs since more than one drug is being administered in one particular dosing unit.
- The possible occurrence of the limits of each constituent being overlooked by the pharmacist or the physicians.
- Tablet size also imposes a limitation where there is high dosage to be covered, hence rises the issue of swallowing in geriatric drug therapy.

Nevertheless, design and process development of FDC formulations are challenging compared with single entity products (Orlu-Gul *et al.*, 2014). Various factors complicate the development of these formulations. These are the size of the prepared tablet of the FDC product, disproportionate drug dose combination and different aqueous solubility (Gautam and Saha, 2008).

3 Experimental details

3.1 Overview

In this chapter, a brief description of the materials used, the suppliers and product details are given. The methods and procedures taken to prepare and characterise the solutions and the techniques used to carry out further analysis are explained. This is followed by a description of the EHD set-up configuration with the multi-needle assembly and the protocols in the work presented.

3.2 Materials

The biocompatible polymers used in this work were poly (lactic-co-glycolic acid) (PLGA), poly (ethylene glycol), Eudragit L100-55 and polymethylsilsesquioxane. The drugs used include indomethacin, paracetamol, prednisolone, metformin and glibenclamide. Other subsidiary materials used were distilled water, ethanol, methanol, dimethylcarbonate (DMC), isopropanol and phosphate buffer saline (PBS).

3.2.1 Poly (lactic-co-glycolic acid)

Poly (lactic-co-glycolic acid) (PLGA) was chosen as a suitable copolymer of glycolic and lactic acid that is approved by the Food and Drug Administration for usage in therapeutic devices due to its biocompatible and biodegradable properties. Based on the ratio of lactide to glycolide used for the polymerization, various forms of PLGA can be obtained, that in turn leads to different degradation rate that is controlled merely by tuning the proportion of the starting monomer units (Mohamed and Van Der Walle, 2018). In this work, PLGA Resomer® 503H (Boehringer Ingelheim, Germany) was used. PLGA Resomer® 503H has a M_w of

24,000-38,000 g mol^{-1}, a 50:50 ratio of lactic acid to glycolic acid. This M_w was chosen as it is sufficient enough to form particles at low concentrations.

3.2.2 Polyethylene glycol

Polyethylene glycol (PEG) with M_w of 8,000 g mol^{-1} was obtained from Sigma-Aldrich (Poole, UK). PEG is an attractive polymer in pharmaceutical industry due to its hydrophilicity and high solubility in organic solvents. Higher molecular weights of PEG have been used for fibre preparation via electrohydrodynamic processing. However, the chosen molecular weight was deemed suitable as per formation of particles.

3.2.3 Eudragit L100-55

Eudragit L100-55 with M_w of 320,000 g mol^{-1} was a gift from Evonik GmbH (Germany). It is soluble in solutions of pH 5 or above. The pH dependent solubility of Eudragit polymers has been utilized for development of pH-sensitive drug delivery systems that are able to prevent and minimize the drug release in the stomach and facilitate the target delivery of the incorporated APIs in the lower parts of the intestinal tract (Wang *et al.*, 2015).

3.2.4 Polymethylsilsesquioxane

Polymethylsilsesquioxane (PMSQ) with an average M_w of 7465 g mol^{-1} was provided by Wacker Chemie AG, GmbH (Burghausen, Germany). It has been greatly used in pharmaceutical and cosmetic industry due to its biocompatibility, non-toxicity and good chemical stability (Gunduz *et al.*, 2012). It has also been used to produce solid and encapsulated particles and therefore obtain prolonged release time of the incorporated components (Chang *et al.*, 2010).

3.2.5 Methanol

Methanol or methyl alcohol is the simplest alcohol with the following specifications: CH_3OH 99.8%, M_w 32.04 g mol^{-1}, density 0.791 g ml^{-1}, boiling point 64.7^oC. Methanol used in this work was purchased from Sigma-Aldrich (Poole, UK).

3.2.6 Dimethyl carbonate (DMC)

Dimethyl carbonate ($C_3H_6O_3$) with 99% purity (molecular weight 90.08 g mol^{-1}, density 1.069 g cm^{-3}, boiling point 90^oC) was obtained from Sigma-Aldrich (Poole, UK). It is considered as a “green” solvent that is environmentally friendly.

3.2.7 Isopropanol (Isopropyl alcohol)

Isopropanol also known as isopropyl alcohol was used in this work as a solvent and was obtained from Sigma-Aldrich (Poole, UK). It is a colourless compound with strong odour and formula C_3H_8O, molecular weight 60.10 g mol^{-1}, viscosity 1.96 mPa s and boiling point of 82.6^oC.

3.2.8 Ethanol

Ethanol or ethyl alcohol (C_2H_5OH) is a common organic solvent that has extremely low toxicity. In our research, general purpose research grade ethanol (99% purity) was purchased from Sigma-Aldrich (Poole, UK) with the following physical properties, density 789 kg m^{-3}, M_w 46 g mol^{-1}, viscosity 1.3 mPa s, boiling point 78^oC. Moreover, ethanol was used for cleaning purposes if the single and co-axial needle assembly device following experiments, as well as for calibration of the characterization apparatus.

3.2.9 Hydrochloric Acid

Hydrochloric acid (HCL, 1 N) was obtained from Sigma-Aldrich (Poole, UK). It constitutes the majority of the gastric acid, the human digestive fluid. Appropriate amount of HCL was diluted in distilled water to prepare 0.1 N HCL as the release medium for dissolution studies that will be described hereafter.

3.2.10 Phosphate buffer saline

Phosphate buffer saline (PBS) is a buffer solution that is commonly used in biological research as it resembles the osmolarity and ion concentration of the human body. The PBS in this work was obtained from Sigma-Aldrich (Poole, UK).

3.2.11 Dimethylacetamide

Dimethylacetamide (DMAc) is a colourless solvent that is miscible with water with high boiling point, molecular weight of 87.12 g mol^{-1}, density of 0.937 g cm^{-3}. DMAc used in this project was purchased from Sigma-Aldrich (Poole, UK).

3.2.12 Sodium phosphate

Sodium phosphate 96% with 163.94 g mol^{-1} molecular weight was obtained from Sigma-Aldrich (Poole, UK). Appropriate amount of this compound was dissolved in distilled water in order to prepare buffer solution of 0.20 M for release studies.

3.2.13 Paracetamol

Paracetamol ($C_8H_9NO_2$) (M_w 151.16 g mol^{-1}) was used as a model drug in part of this work and was purchased from Sigma-Aldrich (Poole, UK). Paracetamol is an analgesic used for reducing mild to moderate body pain. It also acts as an antipyretic that helps for lowering body temperature (Illangakoon *et al.*, 2014). It is completely adsorbed from the intestinal tract and metabolized by the liver. At

high concentrations, it can result in hepatic injury (Granberg and Rasmuson, 1999). Attempts have been made to prepare oral controlled release formulations of paracetamol to allow slow release in a controlled manner into the gastrointestinal tract. With this aim, in a recent study, mucoadhesive microspheres of xanthan gum and guar were prepared containing paracetamol (Nathaji *et al.*, 2017). Endo *et al*. (Endo *et al.*, 2000) has reported successful preparation of gel formulation for the oral delivery of paracetamol with high bioavailability of 90% in rabbit. In-situ gelling formulations containing gellan gum and sodium alginate have also been prepared for oral delivery of aqueous paracetamol solution (Kubo *et al.*, 2003). For this purpose, functionalised depots were developed and were able to introduce controlled release paracetamol for 6 hours, with similar bioavailability to those of commercial suspensions.

3.2.14 Indomethacin

Indomethacin ($C_{19}H_{16}ClNO_4$) (M_w 357.79 g mol^{-1}) was used as a model drug that is practically insoluble in water and was obtained from Sigma-Aldrich (Poole, UK). It is a light-sensitive nonsteroidal anti-inflammatory drug (NSAID), that is used as a prescription medication to relieve pain and inflammatory conditions. Indomethacin has poor water-solubility and is also classified as a highly permeable drug (Shokri *et al.*, 2006). In an amorphous solid state, it is found to be the most soluble of all solid forms (Terada *et al.*, 2000). Indomethacin was electrosprayed onto reduced and atmospheric pressure in a study done by Nyström *et al*. (Nyström *et al.*, 2011) and it was reported that the reduced particle size gave rise to higher surface area to volume ratio. In addition to that, the resultant amorphous state of the indomethacin particles enhanced its solubility.

3.2.15 Prednisolone

Prednisolone crystalline powder was acquired from Sigma-Aldrich (Poole, UK) (M_w 360.44 g mol^{-1}). Prednisolone ($C_{21}H_{28}O_5$) is among the most frequently prescribed drugs for treatment of inflammatory bowel disease. It is a corticosteroid drug that is widely used as immunosuppressant and anti-inflammatory drug, used to treat number of inflammatory and autoimmune conditions. However, due to use of high dosages at frequent predetermined time intervals, it also induces adverse side effects (Campieri *et al.*, 1997). Furthermore, it has poor water-solubility, characteristic of class II substances according to the Biopharmaceutics Classification System (Vogt *et al.*, 2007). This is one of the major challenges in development of oral formulations and is proven to be a limiting factor in the drug bioavailability, which is directly related to the dissolution rate of the drug. Accordingly, various techniques including solid dispersion and preparation of liquisolid compacts (Spireas and Sadu, 1998; Zakeri-Milani *et al.*, 2011) have been used for enhancement of its water solubility. Nonetheless, recent reports on prednisolone loaded particles suffer from low encapsulation efficiency (Lau *et al.*, 2017).

3.2.16 Metformin hydrochloride

Metformin hydrochloride (HCL) ($C_4H_{12}ClN_5$) with M_w of 165.62 g mol^{-1} was obtained from Sigma-Aldrich (Poole, UK). It is antidiabetic agent that is used to reduce glucose levels and improve insulin sensitivity (Wang *et al.*, 2017), and is one of the most widely prescribed drugs in the treatment of type 2 diabetes mellitus. After oral administration, it is mostly absorbed from the small intestine and has a rather low bioavailability (Cetin and Sahin, 2016). Bioadhesive and

gastroretentive drug delivery systems of metformin have been developed in order to enhance its incomplete absorption (Adikwu *et al.*, 2003; Murphy *et al.*, 2012). Repeated daily administration of metformin is required to achieve an effective treatment, which in turn results in poor patient compliance and greater possibility of side effect occurrence such as diarrhoea, nausea, anorexia, vomiting, weight loss and taste disturbance (Corti *et al.*, 2008). Therefore, development of novel drug delivery systems for preparation of metformin formulations is necessary in order to enhance its bioavailability and decrease the dosing frequency and to reduce the unwanted gastrointestinal side effects and toxicity (Marathe *et al.*, 2000; Cetin and Sahin, 2016).

3.2.17 Glibenclamide

Glibenclamide ($C_{23}H_{28}ClN_3O_5S$) is an antidiabetic drug with molecular weight of 494.61 g mol^{-1} and it belongs to Class II of the Biopharmaceutical Classification Systems, it has the characteristic of high permeability and poor aqueous solubility of 4 mg/L at 27^{o}C (Yalkowsky and Dannenfelser, 1992). Glibenclamide used in this work was purchased from MP Biomedicals, UK. The low aqueous solubility of glibenclamide leads to poor and variable bioavailability and therefore potential side effects including variable clinical outcomes such as hypoglycaemia or hyperglycemia (Guan *et al.*, 2014). It also suffers from short elimination half-life that necessities the need for high dose frequency (Essa *et al.*, 2015). Development of extended release oral formulation of glibenclamide has the potential to overcome the associated challenges. For this purpose, a number of techniques including solid dispersion, amorphization and floating tablets have been implemented (Betageri and Makarla, 1995; Wojnarowska *et al.*, 2010; Essa *et al.*, 2015).

3.3 Preparation of solutions

3.3.1 Core/shell encapsulation of paracetamol/indomethacin

3.3.1.1 Particle preparation

PLGA solutions of different concentrations including 4% w/w, 6% w/w and 8% w/w were made by dissolving appropriate amount of PLGA polymer in DMC followed by mechanical stirring for 900s to obtain clear solutions that is indicative of complete dissolution of the PLGA content.

Appropriate amount of PEG was dissolved in methanol in order to obtains solution with 2% w/w, 4% w/w and 6% w/w concentration of PEG in the used solvent system. This was followed by magnetic stirring to ensure complete dissolution of PEG. The prepared solutions were used in order to prepare the microparticles.

3.3.1.2 Drug loaded particles preparation

Upon optimization of the prepared particles, the model drugs were incorporated into the polymeric solution in order to develop drug loaded microparticles. For this purpose, 0.4% w/w indomethacin was added to PLGA 4% w/w in DMC under ambient conditions and were stirred to facilitate total dissolution of the prepared solution. Moreover, 0.2% w/w paracetamol was added to the prepared PEG 2% w/w in methanol.

3.3.2 Targeted delivery of prednisolone

Eudragit L100-55 solutions were prepared by dissolving sufficient amount of polymer in isopropanol in order to obtain 1.0% w/w and 2.0% w/w concentration, that were then stirred thoroughly. Prednisolone solutions with 0.1% and 0.2% w/w

were made by dissolving required amount of drug in methanol. Solution of prednisolone 0.2% w/w and Eudragit L100-55 1.0% w/w were made by dissolving suitable amount of drug and polymer in methanol. Prednisolone 0.1% w/w and Eudragit L100-55 1.0% w/w were dissolved in isopropanol.

3.3.3 Co-encapsulation of metformin HCL and glibenclamide

10% w/v and 15% w/v PMSQ were dissolved in ethanol and fully mixed to obtain complete dissolution of the polymer. In order to prepare the drug solutions, 10 mg metformin HCL was added to 80 mL of ethanol, 20 mg of glibenclamide was added to 80 mL ethanol, and lastly, 10 mg metformin HCL combined with 20 mg glibenclamide were dissolved in 80 mL of ethanol.

3.4 Characterization of the solution

3.4.1 Density

A standard density bottle with the volume of 25 mL (VWR International, Lutterworth, UK) was used in order to obtain the density (volumetric mass density) of the prepared solutions. The mass of the density bottle being empty and also filled with the solution was measured using an electronic balance (AND HF-1200G A&D Instruments Ltd., Japan). The mean value of the measured density of the liquid/polymer solutions was obtained from five consecutive calculations as reported in this work. Ethanol was used in order to calibrate the density bottle and procedure. The density ρ [kg m^{-3}] was calculated using the following equation:

$$\rho = \frac{W_2 - W_1}{25} \qquad \text{(Equation 3)}$$

Where W_1 [g] and W_2 [g] correspond to the mass of the empty density bottle and the bottle filled with solution and V [cm^3] is the bottle volume.

3.4.2 Viscosity

A U-Tube glass viscometer (BS/U type, VWR International Ltd, Lutterworth, UK) was used to determine the viscosity of the prepared solutions. In order to calculate the viscosity with a U-Tube viscometer, ethanol or acetone was filled onto it in order to clean in between readings. Then the time taken for a standard volume of distilled water to pass through the U-Tube capillary, from the upper region to the lower marked region, was recorded. The procedure was then repeated five times, in order to calculate the mean time value. Same method was performed with the polymeric solutions. The following equation was used in order to calculate the viscosity mean value:

$$n_{solution} = n_{water} \frac{\rho_{solution} \times t_{solution}}{\rho_{water} \times t_{water}} \quad \text{(Equation 4)}$$

Where $n_{solution}$ is the solution/liquid viscosity [mPa s], n_{water} is the viscosity of water [1 mPa s], ρ_{water} is the water density [998 kg m^{-3}], $\rho_{solution}$ is the solution/liquid density [kg m^{-3}], t_{water} is the time taken for water to pass through the U-Tube [3.5 s] and $t_{solution}$ is the time taken for the liquid/solution to pass through the U-Tube.

3.4.3 Surface tension

The surface tension of the solutions was measured using a Kruss Tensiometer K9 (standard Wilhelmy's plate method). Beaker containing the solution was

placed on the tensiometer platform and the probe (rectangular shape plate) was hung from the tensiometer hook. The tensiometer was set to zero and calibrated before carrying out measurements. The edge of the probe was moved at the surface of the liquid in such a way that a meniscus could be formed between the liquid and the probe owing to the surface tension of the liquid. The probe was thereafter moved steadily and the surface tension value was recorded as the probe was just about to detach from the liquid surface. Total of five measurements were taken for each sample in order to minimise errors involved and the mean value and standard deviation was calculated. The probe was cleaned in-between each measurement.

3.4.4 Electrical conductivity

With the use of Jenway 3540 pH/conductivity meter (Bibby Scientific Ltd, Staffordshire, UK) the electrical conductivity of the solutions was determined. The instrument was calibrated before taking readings. The conductivity probe was cleaned with acetone or ethanol to remove any liquid or polymeric solution residue on the probe. The probe was also rinsed off with distilled water, after cleaning with the selected solvents and air-dried before taking further measurements. The electrode was immersed and kept in the solutions for 300s prior to reading the electrical conductivity values. The mean value of five consecutive readings was calculated for each sample.

3.5 Sample characterization

3.5.1 Optical microscopy

The prepared particles were collected on glass slides with or without

ethanol and they were initially studied using optical microscopy (Micropublisher 3.3 RTV, 3.3 megapixel CCD Color-Bayer Mosaic, Real Time Viewing camera, MediaCybernetics, Marlow, UK). MediaCybernetics Image-Pro Insight software was then used for the analysis of the samples.

3.5.2 Scanning electron microscopy

Hitachi S-3400N and JEOL JSM-6301F field emission scanning electron microscope (SEM) was utilized in order to study the surface morphology and size of the prepared particles. JEOL JSM-6301F is fitted with an emitter that can obtain a resolution of ~1.5 nm. The samples were collected on glass slides and then left to air dry under ambient conditions for 24 hours beforehand. Then, they were mounted using carbon tape onto metal stubs. The materials used were non-conductive, hence, a conductive layer was applied to the surface of the samples and they were vacuum-coated with gold for 120s at 20 mA using an ion sputter coater machine (Quorum Q150R ES). The SEM images were acquired at accelerating voltage of 5 kV. The working distance between the emitter and the sample was set at 20mm. Analysis of the formed particles was carried out using the Image-Pro Insight software (MediaCybernetics Ltd., Marlow, UK).

3.5.3 Transmission electron microscopy

Transmission electron microscopy (TEM) was used in order to discern the potential presence of layers. The images of the samples were recorded on a JEM 2100F field emission instrument (JEOL). TEM samples were directly taken on a fixed lacey carbon coated copper grid that was placed on the grounded collection platform that the particles were sprayed onto.

3.5.4 Fourier transform infrared (FTIR) spectroscopy

Fourier transform infrared spectroscopy was conducted to confirm the presence of different functional groups corresponding to the model drugs used within the prepared formulations. The studies were carried out using a Perkin-Elmer 100 FTIR spectrometer (Massachusetts, USA) fitted with an ATR attachment. The scanning range was 600-4000 cm^{-1} and the resolution set at 1 cm^{-1}.

3.5.5 X-ray diffraction calorimetry

X-ray diffraction (XRD) patterns were obtained utilizing a MiniFlex 600 diffractometer (RigaKu, Tokyo, Japan) with Cu Kα radiation. Data were collected over the 2θ range 5^o– 45^o at 40 mV and 15 mA.

3.5.6 Differential scanning calorimetry

Differential scanning calorimetry (DSC) analyses were carried out using of the two different DSC machines:

(a) A Netzch STA 449C Jupiter, Netzch, USA. Data were recorded over the range of 30^oC to 200^oC at a rate of 5^oC/min.

(b) A TS Instruments DSC Q1000 calorimeter, New Castle, DE, USA. Data were collected over the temperature range of 40^oC to 270^oC at a heating rate of 10^oC/min.

3.5.7 Focused ion beam microscopy

The internal structure of the prepared particles was investigated using SEM in conjunction with a focused-ion-beam (FIB) microscope for sectioning the particles utilizing Carl Zeiss XBI 1540 “Cross-Beam” that was equipped with the Gemini SEM column.

This system is comprised of a field emission SEM system of top-down Gemini SEM column from Carl Zeiss and an add-on focused ion beam (FIB) system of Orsay Physics Canion 31 FIB column, 54 degrees related to the SEM column. Both columns function at up to 30 kV. For the FIB studies, particles were collected and left to air-dry at ambient conditions (21^{o}C). Prior to FIB studies, dried samples were gold sputtered for 60 seconds. The accelerating voltage ranged from 5 kV to 10 kV during scanning.

3.5.8 UV spectroscopy

UV spectrophotometer was used in order to quantify the drug release. A brief account of UV spectroscopy and how UV absorption spectra can be used to detect compounds and to obtain the concentrations of it in a solution with initially unknown quantity of that compound. In order to obtain the drug concentration in any formulation, ultraviolet wavelength light in the region of 200 nm to 700 nm is transmitted by the UV spectrometer through the sample. The light absorbance detected at a specified wavelength corresponding to the drugs' characteristic wavelength in a given medium is directly proportional to the concentration of the drug in question in the sample.

At sufficiently low concentrations of the compound in the solution, there exists a linear correlation in between the absorbance and concentration of the drug in the solution. This relationship is given by the Beer-Lambert law (Parnis and Oldham, 2013), as it follows:

$$A = \varepsilon \times \ell \times c \quad \text{(Equation 5)}$$

Where: A is the absorbance (arbitrary units, $A = \log_{10} \frac{P_o}{P}$, P: the intensity of transmitted light, P_o: the incident intensity),

ε is the attenuation coefficient [L mol^{-1} m^{-1}],

ℓ is the distance which the light travels across the material [m],

and c represents the concentration of the drug [mol L^{-1}].

In order to obtain the drug release profile and the experimental entrapment efficiency of the used drugs in the prepared formulations, calibration curves corresponding to each component were obtained and utilized for determination of the correlation in between the absorbance and the drug concentrations.

In this work, the UV/VIS spectrometry measurements were obtained using a UV/VIS Spectrometer (Cary 300 UV-Vis, Agilent Technologies) in the wavelength range of 200 nm to 400 nm, depending the characteristic wavelength of the drug in use at the time.

3.5.8.1 Paracetamol and Indomethacin calibration curves

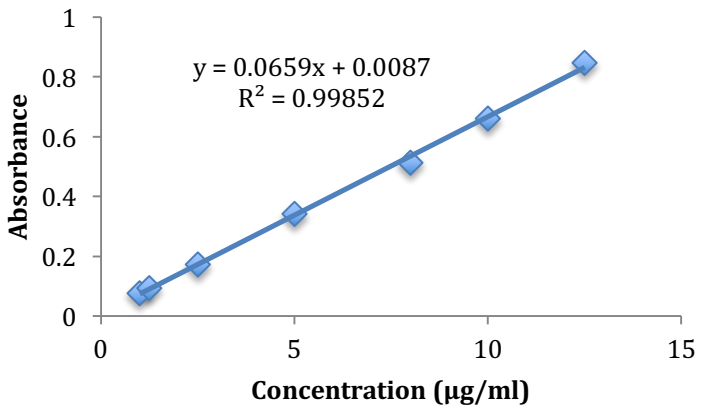


Figure 3.1 Paracetamol calibration curve in PBS at 243 nm.

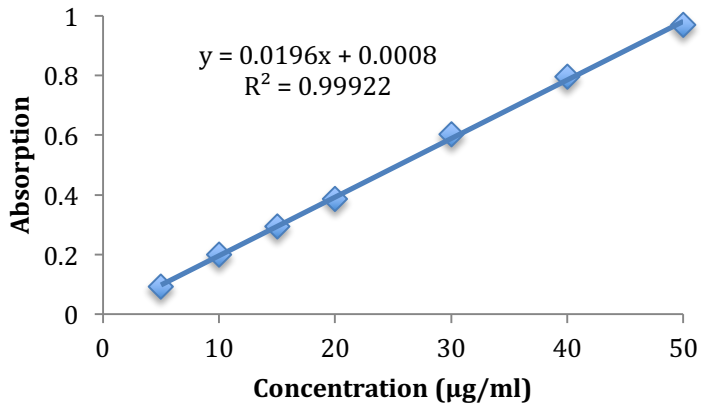


Figure 3.2 Indomethacin calibration curve in PBS: EtOH (9:1) at 320 nm.

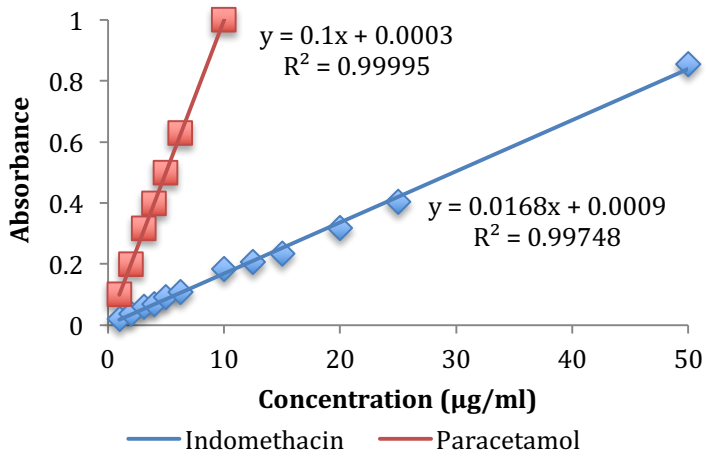


Figure 3.3 Paracetamol and indomethacin calibration curve in PBS: EtOH (9:1)

3.5.8.2 Prednisolone calibration curves

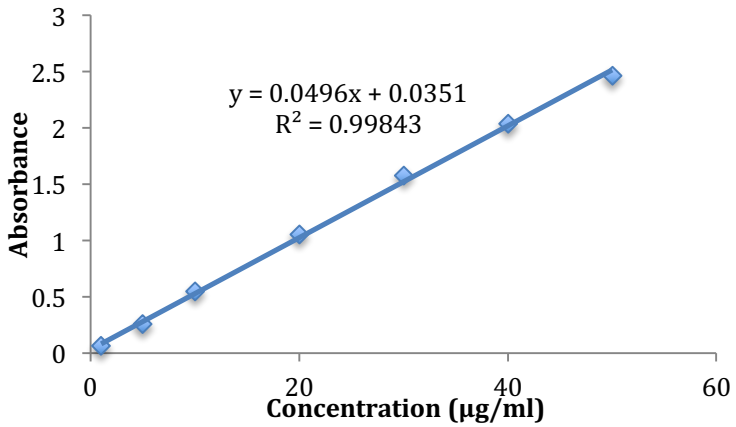


Figure 3.4 Prednisolone calibration curve in EtOH at 247 nm.

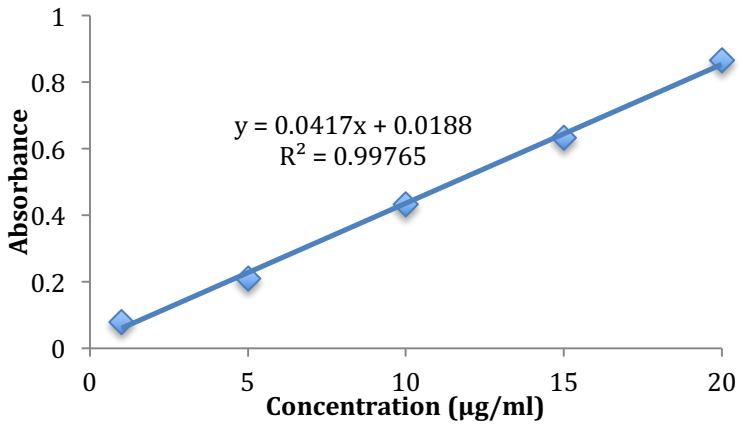


Figure 3.5 Prednisolone calibration curve in HCL at 247 nm.

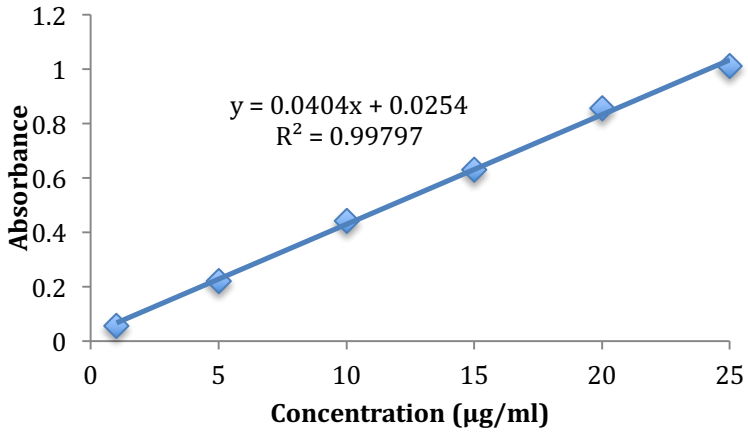


Figure 3.6 Prednisolone calibration curve in phosphate buffer at 247 nm.

3.5.8.3 Glibenclamide and metformin HCL calibration curves

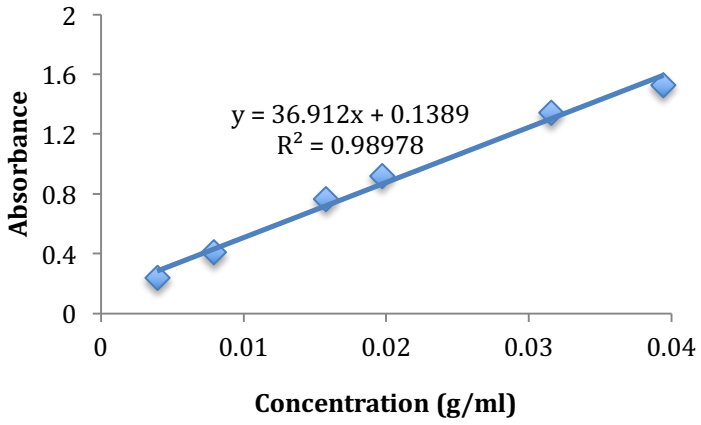


Figure 3.7 Glibenclamide calibration curve in PBS at 300 nm.

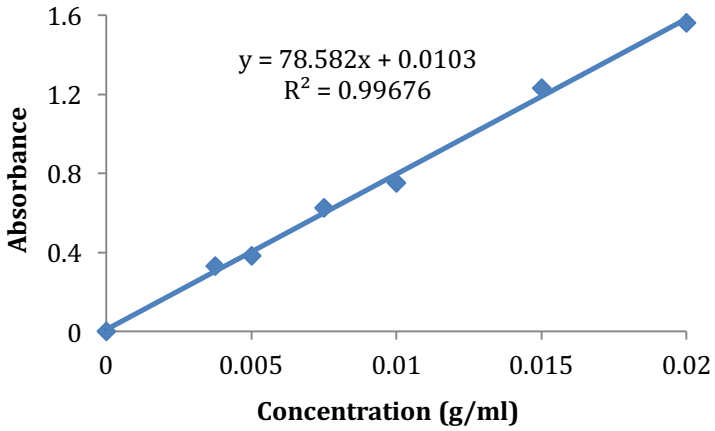


Figure 3.8 Metformin HCL calibration curve in PBS at 231 nm.

3.6 *In-vitro* release experiments

3.6.1 Core/shell microencapsulation of indomethacin and paracetamol by co-axial electrohydrodynamic atomization

In order to obtain the release of the encapsulated drugs, 20 mg of the developed formulations were dissolved in vials containing 50 mL of PBS pH 7.4 and placed in a water bath that was set at 37 ± 1^{o}C temperature to mimic the environmental body temperature. 3 mL of the samples were withdrawn at a predetermined time intervals over the course of 8 hours, which were then passed through 0.22 μm filters to confirm that no particles were collected at the time point of taking out aliquots. Each time 3 mL of fresh PBS that was kept at the same temperature were added to the vials in order to keep the volume of the release medium constant throughout the release study. Using UV/VIS spectrometer the samples were analysed to understand the release mechanism.

3.6.2 Electrosprayed microparticles for targeted delivery of prednisolone

In this study, the following protocol was used to study the release of prednisolone from the developed formulation. 50 mg of particles were placed in empty

capsules. The capsules were then loaded onto enclosed metallic sinkers. The sinkers were attached to the rotating rods in the dissolution apparatus. The beakers were filled in with buffer solution under 50 rpm continuous stirring at the set temperature of 37 ± 0.5°C. In vitro drug dissolution tests were conducted in 750 mL pH 1.2 hydrochloric acid solution for the first 2 h. Thereafter, the pH of the dissolution medium was adjusted to pH 6.8 for the following 6 h. At predetermined periodic intervals, 3 mL aliquots were withdrawn from the dissolution medium and replaced with 3 mL of preheated fresh buffer solution to ensure a constant volume and maintain sink conditions. The drug concentrations in the withdrawn aliquots were obtained using UV spectrometry. Experiments were conducted in triplicate and results recorded as mean ± S.D.

3.6.3 Co-encapsulation of metformin HCL and glibenclamide as oral drug delivery depots

In order to obtains the amount of drug released from drug-loaded microparticles, a set-up in which 40 mg of sample was enclosed in a fine metal mesh to ensure total immersion of particles and placed in a 40 mL volumetric vessel. Body temperature conditions and continuous agitation at 80 rpm were set throughout the release study using a benchtop incubated shaker (Sciquip, Newtown, UK). Aliquots of volume 2 mL were taken at different time points from the vessel. 2mL of blank media was then added after each sampling to conserve a constant volume. UV measurements were used to quantify amount of drug sampled during this work.

3.7 Measuring drug entrapment efficiency

In order to obtain the encapsulation efficiency of the prepared formulations, given amount of particles were dissolved in appropriate solvent system in order to extract the drug content. For this purpose, due to hydrophobicity of some of the used model drugs and polymers, a combination of relevant solvent and PBS was used, typically in ratio of 1:9. The solutions were then stirred thoroughly and filtered using cellulose acetate membranes before UV measurements were acquired. Unloaded polymeric particles were also used in the same manner in order to take UV measurements as control and therefore facilitate the subtraction of the UV background signal from the drugs' UV signal caused by the used polymers, solvent and PBS. The drug encapsulation efficiencies were calculated using:

$$Drug\ encapsulation\ efficiency\ (\%) = \left[\frac{actual\ amount\ of\ drug\ in\ the\ particles}{theoretical\ amount\ of\ drug\ in\ the\ particles}\right] \times 100$$

(Equation 6)

The studies were carried out in triplicates and the average values and standard deviations were calculated.

3.7.1 Core/shell microencapsulation of indomethacin and paracetamol by co-axial electrohydrodynamic atomization

30 mg of drug-loaded microparticles were dissolved in dimethylacetamide (DMAc) then diluted with PBS pH 7.4 and agitated for 1 hour under sealed conditions. The obtained solution was then filtered through 0.22 μm filters and the drug content was analysed using UV/VIS Spectrometer at corresponding detection wavelengths, 243 nm for paracetamol and 320 nm for indomethacin.

3.7.2 Electrosprayed microparticles for targeted delivery of prednisolone

20 mg of each formulation was dissolved in 20 mL ethanol in order to determine the actual amount of drug encapsulated in the particles with respect to its theoretical value. Ethanol was used as it can dissolve both the drug and polymer used in this part of the work. Moreover, it did not interfere with the characteristic wavelength of prednisolone at 247 nm.

3.8 Experimental set-up and equipment

The essential experimental set-up used for the assembly of electrohydrodynamic atomization processing constitutes of a single/co-axial needle configuration, infusion pumps, a high voltage power supply and grounded collection platform as depicted in Figure 3.9. Here, an account of the experimental design and specification of each equipment used is given in further details. The needle/s are connected to the syringes consisting of the solution of interest and also connected to the power supply. Syringes with 10 mL capacity were mounted onto the infusion pumps to enable a steady flow rate, they were also connected to the corresponding needle using silicone tubing. The prepared solutions were fed into the system through the needles and in a configuration relating to each study (specifics are given later). The distance in-between the needles' exit and the grounded electrode was set at a range of heights to obtain a narrow size distribution while achieving a stable cone-jet. A camera was used to visualize the jet at all times. The formation of a stable jet required fine tuning of the applied voltage on the needle and the set flow rate during electrohydrodynamic spraying.

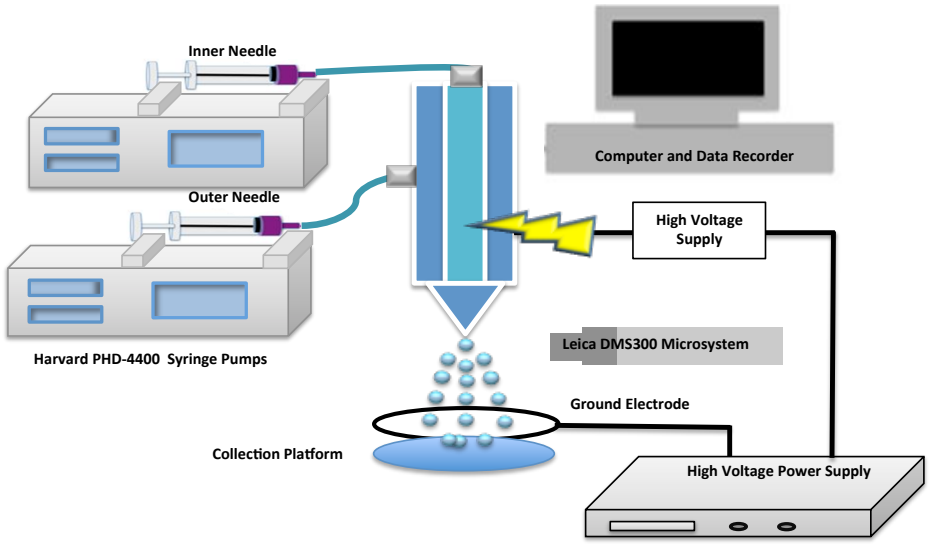


Figure 3.9 Schematic representation of the experimental set-up used to co-axial electrohydrodynamic atomization.

3.8.1 High voltage power supply

A high voltage DC power supply (FC30 P4 12w, Glassman Europe Limited, Bramley, UK) was utilized to deliver a high voltage to the needle by means of a high voltage cable, attached to a crocodile clip fixed onto a height adjustable stand. The grounded collection platform was connected to the ground terminal at the back of the power supply unit. The output voltage range was 0 kV to 30 kV, and the range of output current was 0 mA to 4 mA, with the minimum resolution of 0.1 kV.

3.8.2 Infusion pumps

All throughout the studies carried out in this work, the needles were connected to plastic syringes (BD Plastic^{TM}, VWR, Lutterworth, UK), filled with the prepared solutions. Harvard syringe pumps (Infuse/Withdraw PHD 4400 or PHD ULTRA high pressure programmable syringe pump, Harvard Apparatus Ltd., Edenbridge, UK) were utilized in order to deliver the solutions at a constant set flow rate. The

operation pad can be used in order to set the flow rate value, infuse/defuse mode and the syringe mode selection. In the co-axial EHD experiments, solutions were introduced into the system simultaneously and at appropriate inner/outer needle flow arte that was determined empirically to establish a stable cone jet based on the physicochemical characteristic of each solution and obtaining a narrow particle size distribution desired for the end application of each system.

3.8.3 Recording equipment

The formation of the jet and consequently the droplets were visualized using a camera (LEICA S6D JVC-colour video camera, Milton Keynes, UK) that was connected to a zoom lens and data DVD video recorder MP-600 using CDV Recorder/Editor DN-100 with a video screen for live feed monitoring. The camera was focused on the tip of the needle device, images stills form the recording were used to demonstrate the morphology and stability of the formed jet.

3.8.4 Needle configuration

The needles used in this work, were all made up of an inner stainless steel that were designed with screw and thread type in order to eliminate potential leakage of solutions throughout the application of high pressure. The needles' configuration in each part of the work is given in further details here.

3.8.4.1 Core/shell microencapsulation of indomethacin and paracetamol by co-axial electrohydrodynamic atomization

The co-axial needle configuration for preparation of core/shell particles and formulations that co-incorporated paracetamol and indomethacin consisted of an inner needle with inner diameter and outer diameter of 0.69 mm and 1.1 mm respectively; and those of the outer needle were 1.55 mm and 2.05 mm

accordingly. For optimization of the particle preparation, unloaded particles were developed initially using the same needle configuration. Upon inclusion of the model drugs, the paracetamol solution was delivered through the inner needle, while the outer one constituted of the indomethacin solution. The distance between the needles exit and the collection platform was kept at 150 mm for all experiments. This was done in order to ensure that the electrical stress that is obtained by the electric field in-between the needles and the ground electrode was the same for all studies.

3.8.4.2 Electrosprayed microparticles for targeted delivery of prednisolone

The needle system used for preparation of prednisolone loaded particles had the following dimensions: inner needle with inner diameter of 0.69 mm and outer diameter of 1.1 mm, outer needle with inner diameter of 1.55 mm and the outer diameter of 2.05 mm. The working distance was kept at 250 mm. In this part of the work, dissolved prednisolone solution was fed into the system using the inner needle and the polymeric solution throughout the outer one. Moreover, the needle with higher dimensions was used in order to electrospray the drug and polymer combined as one solution in order to determine the effects on the release studies.

3.8.4.3 Co-encapsulation of metformin HCL and glibenclamide as oral drug delivery depots

Using the same concentric needle configuration as previously mentioned (inner diameters of 0.90 mm and 1.55 mm and outer diameter of 1.10 mm and 2.05 mm for the inner and outer needle respectively), the working distance was fixed at 150 mm. The model drugs, metformin HCL and glibenclamide, were supplied to

the system using the inner needle separately and also combined, while the outer needle consisted of the polymeric shell.

4 Core/shell microencapsulation of paracetamol and indomethacin utilizing co-axial EHDA processing

4.1 Overview

In this chapter, co-axial EHDA processing was used in order to develop drug delivery systems for preparation of fixed dose combination formulations that incorporate model drug of different aqueous solubility. For this purpose, the capability of EHDA processing for preparation of polymeric drug carriers were explored, using polymer constituent of different concentration in order to optimise the system before loading the model drugs. In the first set of experiments, the objective was to further enhance the combination of key processing parameters in order to achieve a stable cone-jet and hence, produce polymeric drug carriers with narrow size distribution. This was followed by a final set of experiments incorporating the model drugs separately and in combination into the polymeric particles. The prepared formulations were further studied in terms of their morphology and drug release profiles and possible interactions.

4.2 Introduction

EHDA processing have been used for preparation of drug delivery systems incorporating both water soluble and water insoluble active ingredients with reported high encapsulation efficiency and production yield (Chakraborty *et al.*, 2009). It also offers great control over particle size distribution and morphology of the prepared formulations, which is of great importance for pharmaceutical applications. In this chapter, EHDA processing was utilized to encapsulate model drugs of different water solubility, which is a challenging endeavour in preparation of fixed dose combination formulations. We aim to present EHDA processing as a

potential platform for development of fixed dose combination formulations that are currently drawing growing attention in the area of geriatric and cancer therapy.

4.3 Selection of model drugs

Indomethacin was selected as a model drug due to its very low water solubility (0.937 mg/L) (Yalkowsky and Dannenfelser, 1992) that makes it difficult to formulate. It has been reported that indomethacin is found to be most soluble when in an amorphous solid state (Terada *et al.*, 2000). Figure 4.1 shows an SEM image of unprocessed indomethacin.

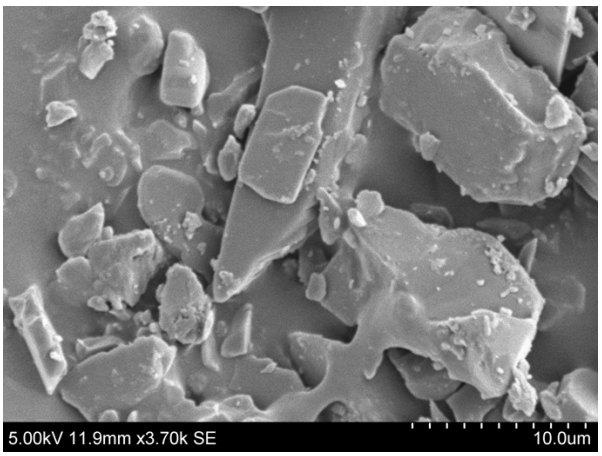


Figure 4.1 Scanning electron microscopy of crystalline indomethacin.

Paracetamol was chosen as the second model drug as it has relatively higher water solubility when compared to that of indomethacin (14 mg/mL at 25^{o}C) (Granberg and Rasmuson, 1999). Development of slow controlled release of paracetamol as oral formulation is desirable since at high concentration it may lead to hepatic injury.

4.4 Polymer selection

There are various polymers that are used extensively in the field of drug delivery, whereby each has advantages and disadvantages that may be appealing to one specific application or another. In this study, PLGA (50:50) that offers highest hydrophilicity among other monomer ratio of this copolymer was used as the shell incorporating indomethacin. PLGA is a well-known polymer that is FDA approved and has been studied greatly in conjunction with electrospraying (Enayati *et al.*, 2010). It has also been successfully used for preparation of solid dispersion formulation with the aim of increasing the dissolution rate of less hydrophilic drugs (Bohr *et al.*, 2011). PEG (8000 g mol^{-1}) was also used as the core polymer in combination with paracetamol. PEG has also been used for preparation of drug delivery systems and is approved by FDA. The proposed system was constructed on the hypothesis that the positioning of the water insoluble drug in the outer layer would lead to placement of the drug at the outermost surface, hence, having greater surface area to volume ratio, in addition to reducing diffusion distance when compared to the water-soluble drug in the inner layer. Inclusion of the hydrophilic paracetamol was accredited to the fact that the outer PLGA layer would prevent it from suffering burst release and therefore prolonging the release period. The aforementioned reasoning was the logic behind using PEG in the inner layer and PLGA in the outer one, also demonstrating that the system is adoptable for different polymer/drug composition, demonstrating it to be a viable platform for further improvement of development of the customized polymeric drug delivery system as necessitated by FDC developments.

4.5 Solvent selection

The selection of solvent was based on some conditions that allow consistent formation of nearly monodispersed particles while obtaining a stable cone-jet.

For this study, solvents were selected based on their physicochemical properties (Table 4.1) as well as their ability to fully dissolve both the polymer and drug constituent of the solutions. Moreover, the electrical conductivity and the surface tension of the chosen systems had to facilitate cone-jet formation without adding any salts or surfactants. DMC has a slightly higher viscosity than that of MeOH, and MeOH is more conductive than DMC. For this reason, MeOH was delivered through the inner needle while DMC was the sheath solution solvent. Higher conductivity in the inner polymeric solution helps formation of a stable cone-jet while the outer solution of higher viscosity aids in directing the electrical stress toward the apex of the cone-jet.

Table 4.1 Physical properties of the solvents used to prepare microparticles (Smallwood, 1996).

Property	MeOH	DMC
Viscosity (mPa s)	0.57	0.6
Boiling point (^{o}C)	64.0	90
Evaporation rate (nBuAc=1)	3.5	3.2
Dielectric constant	32.6	20.0
Electrical conductivity (μS/cm)	0.5	0.02
Surface tension (mN/m)	22.1	31.0

4.6 Characterization of polymeric solutions with different concentration

Preparation of particles utilizing co-axial EHD processing is highly governed by the physiochemical properties of the solutions including surface tension, viscosity and electrical conductivity (as shown in Table 4.2), as well as the processing parameters including the set flow rates and applied voltage. Various combination of these factors allow the formation of different jetting modes (Cloupeau and Prunet-Foch, 1990). Hence, the initial step was taken in order to characterize the prepared solutions.

Table 4.2 Properties of the solutions used to prepare PEG-PLGA microparticles.

Solution	Surface tension (mN/m)	Viscosity (mPa s)	Electrical conductivity (μS/cm)
2% w/w PEG in MeOH	22.8 ± 0.3	0.9 ± 0.1	22.3 ± 0.4
4% w/w PEG in MeOH	23.1 ± 0.6	1.0 ± 0.1	24.9 ± 0.9
6% w/w PEG in MeOH	22.9 ± 0.2	1.2 ± 0.1	29.5 ± 0.2
4% w/w PLGA in DMC	25.7 ± 0.2	1.9 ± 0.2	0.01 ± 0
6% w/w PLGA in DMC	27.9 ± 0.7	2.6 ± 0.1	0.03 ± 0
8% w/w PLGA in DMC	27.7 ± 0.2	4.6 ± 0.1	0.03 ± 0

The electrical conductivity of the PEG solutions increases with an increase in the polymer concentration from 2% w/w to 4% w/w and 6% w/w, the viscosity of the PEG solution increased slightly with an increase in polymer content, however, the

surface tension did not seem to differ significantly. When compared to viscosity of the parent solution, methanol, the addition of PEG resulted in an increase, nonetheless, the surface tension value stayed somewhat similar.

Measurement of electrical conductivity showed that addition of PLGA into DMC, did not alter the conductivity of the solution, nor did higher concentration of PLGA. The viscosity of the solution, however, increased with an increase in polymer content. The change in viscosity values were more noticeable with an increase in polymer content in case of PLGA when compared to that of PEG solutions, this may be due to higher molecular weight of PLGA as opposed to PEG. There was no noticeable difference in the surface tension measurement with increase in PLGA concentration. The surface tension of organic solvents is generally low and does not seem to be affected by the addition of solutes used in this work. A relatively low surface tension is required in order to achieve a stable cone-jet in electrospraying. The values attained here are far lower than the surface tension of water at 72 mN/m at 25^{o}C, and therefore they are presumed to be within an acceptable range.

4.7 Characterization of polymeric solutions incorporating the model drugs

With the inclusion of indomethacin onto PLGA 4% w/w, there was a slight increase in the electrical conductivity measurements, however, the viscosity and surface tension values remained almost unchanged (Table 4.3). The addition of PLGA has a bigger effect on the viscosity of the solution while the addition of indomethacin does not seem to have much impact on the viscosity of the parent solution. The greater influence of PLGA on the viscosity can be explained by much higher molecular weight and hydrodynamic volume of PLGA compared to that of indomethacin.

The addition of PEG 2% w/w in MeOH solution had a more pronounced effect on the electrical conductivity of the solution, more than four-fold, when compared to that of PEG combined with paracetamol. Measurements of the surface tension showed that there is no noticeable influence on the surface tension of the solution with the addition of PEG or paracetamol. The viscosity of the solutions containing PEG and paracetamol resulted in a higher measurement of viscosity when compared to that of the parent solvent.

Table 4.3 Properties of the solutions used to prepare drug loaded PEG-PLGA microparticles.

Solution	Surface Tension (mN/m)	Viscosity (mPa s)	Electrical Conductivity (μS/cm)
4% w/w PLGA in DMC	25.7 ± 0.2	1.9 ± 0.2	0.01 ± 0
4% w/w PLGA, 0.4% w/w Indomethacin in DMC	24.9 ± 0.2	1.9 ± 0.1	0.03 ± 0
2% w/w PEG in MeOH	22.8 ± 0.3	0.9 ± 0	22.3 ± 0.4
2% w/w PEG, 0.2% w/w Paracetamol in MeOH	23 ± 0.4	1.9 ± 0.1	23.8 ± 0.4

4.8 Electrospraying of polymeric particles

The prepared solutions were all electrosprayed using a co-axial needle set-up. It was demonstrated that a stable cone-jet can be obtained (Figure 4.2). The preparation of unloaded polymeric drug delivery systems was done prior to incorporation of the model drugs in order to allow optimization of the system,

including the experimental specifics, to best select the system that facilitates preparation of particles with narrow size distribution of desired size while offering consistent reproducible results.

Other experimental parameters including the collection distance was varied in order to determine the best fitted distance as to collect dry particles. Too low working distance resulted in collection of wet particles as the solvent evaporation process is still incomplete. Too high of working distance would also decrease the collection yield. Therefore, a distance of 150 mm was chosen and was fixed throughout the experiments to ensure that the applied electric field strength was altered in proportion to the applied voltage values. In this study, it was assumed that the majority of solvent had evaporated before the particles reach the collection platform.

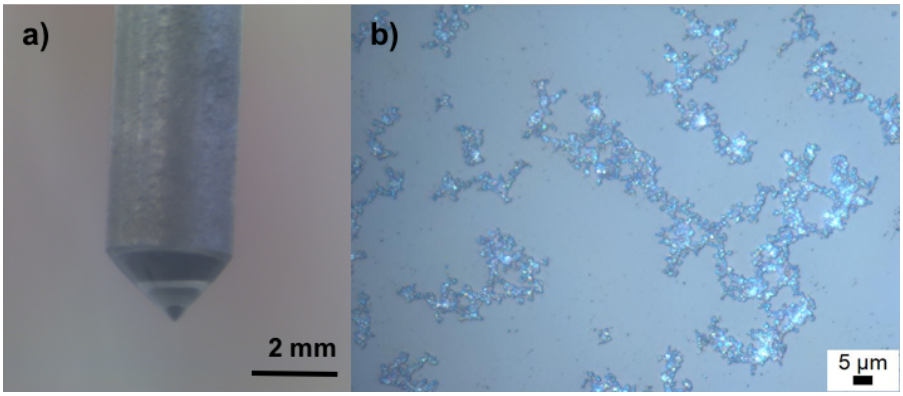


Figure 4.2 Video camera image of the stable cone-jet (a), optical microscopy image of polymeric particles collected on glass slide (b).

The composition of the developed polymeric system is shown in Table 4.4. The inner and outer layers had nearly the same surface tension values, this leads to formation of flowing mediums that are treated as one (Xie *et al.*, 2008). The flow rates for the inner and solutions in S1, were set at 4 µl/min and 10 µl/min, the higher set flow rate for the outer solution was as a result of greater electrical

conductivity of the inner layer, therefore directing the electrical stresses towards the apex of the Taylor cone, whilst surpassing the surface tension and consequently establishing a stable cone-jet. The small difference in the measured viscosities of the polymer solution is complimentary to formation of compact particles in the co-axial EHD processing (Labbaf *et al.*, 2014).

Table 4.4 The composition of the unloaded PEG-PLGA microparticles.

Polymer Systems	Inner Solution	Outer Solution	Mean Particle Size (μm)
S1	2% w/w PEG in MeOH	4% w/w PLGA in DMC	8.06 ± 0.84
S2	4% w/w PEG in MeOH	6% w/w PLGA in DMC	6.74 ± 0.70
S3	6% w/w PEG in MeOH	8% w/w PLGA in DMC	6.40 ± 0.85

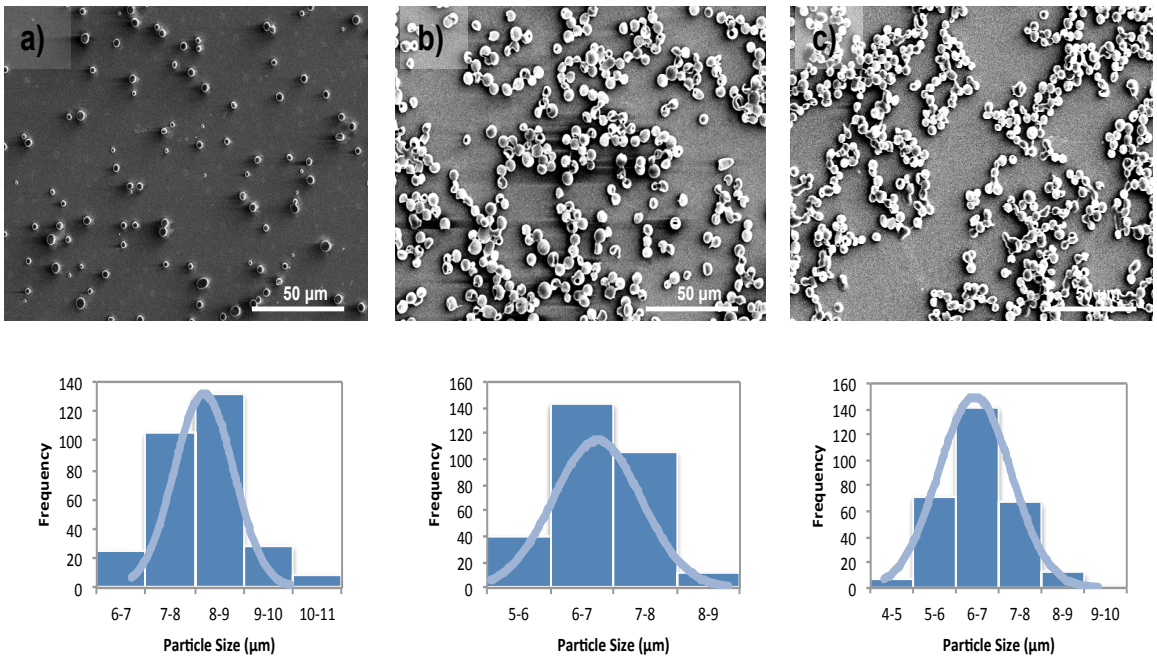


Figure 4.3 SEM images and size distribution graphs of particles with a) S1 (2% w/w PEG, 4% w/w PLGA), b) S2 (4% w/w PEG, 6% w/w PLGA) and c) S3 (6% w/w PEG, 8% w/w PLGA). The first mentioned is the inner layer (Shams *et al.*, 2017).

The mean particle size of S1 is slightly higher (8.06 ± 0.85 μm) (Figure 4.3a) than that of S2 and S3. This is explained by the higher flow rate in the outer needle and lower applied voltage (16.9 kV), when compared to other two systems.

For development of the other two polymeric systems, the flow rates for S2 (Figure 4.3b) were set to 4 μl/min and 8 μl/min and for S3 (see Figure 4.3c) 4 μl/min and 8 μl/min; with an applied voltage of 17.9 kV and 17.6 kV accordingly. The experimental parameters were selected as to obtain a stable cone jet that would lead to formation of nearly monodispersed particles. The acquired mean particle size for S2 and S3 were 6.74 ± 0.69 μm and 6.44 ± 0.86 μm. Upon evaporation of the solvents, microparticles are formed. The evaporation rate of the selected solvents affects the particle size distribution. The higher the evaporation rate and the lower the boiling point, the smaller the particles as there is successively a greater loss of solvents as the cone-jet discharges and lands on the collection platform. The yield of the process was obtained and a mean of 60–70% particle collection was achieved followed by optimization of the working distance as the compromise between dry particle formation and production yield.

4.9 Incorporation paracetamol and indomethacin

With the addition of paracetamol in the inner layer, the experimental parameters including flow rate and applied voltage were adjusted in order to obtain a stable cone-jet and achieve the desired particle size while maintaining the working distance unchanged. The composition of the developed formulations is shown in Table 4.5. The set flow rates of the inner and outer layer were 4 μl/min, 14 μl/min accordingly and at an applied voltage of 15.7 kV for the PCMP formulation. The mean particle size achieved was 5.3 ± 0.6 μm. For preparation of INDOP formulation, indomethacin was added to the PLGA solution and processed where

the flow rates of the inner and outer layer were set at 4 µl/min, 10 µl/min at an applied voltage of 16.4 kV. The mean particle size achieved was 7.4 ± 0.8 µm.

Table 4.5 Composition of the developed formulations. PCMP: paracetamol particles, INDOP: indomethacin particles, PCMINDOP: paracetamol-indomethacin particles.

Formulation	Inner Solution	Outer Solution
PCMP	2% w/w PEG, 0.2% w/w Paracetamol in MeOH	4% w/w PLGA in DMC
INDOP	2% w/w PEG in MeOH	4% w/w PLGA, 0.4% w/w Indomethacin in DMC
PDMINDOP	2%/w PEG, 0.2% w/w Paracetamol in MeOH	4% w/w PLGA, 0.4% w/w Indomethacin in DMC

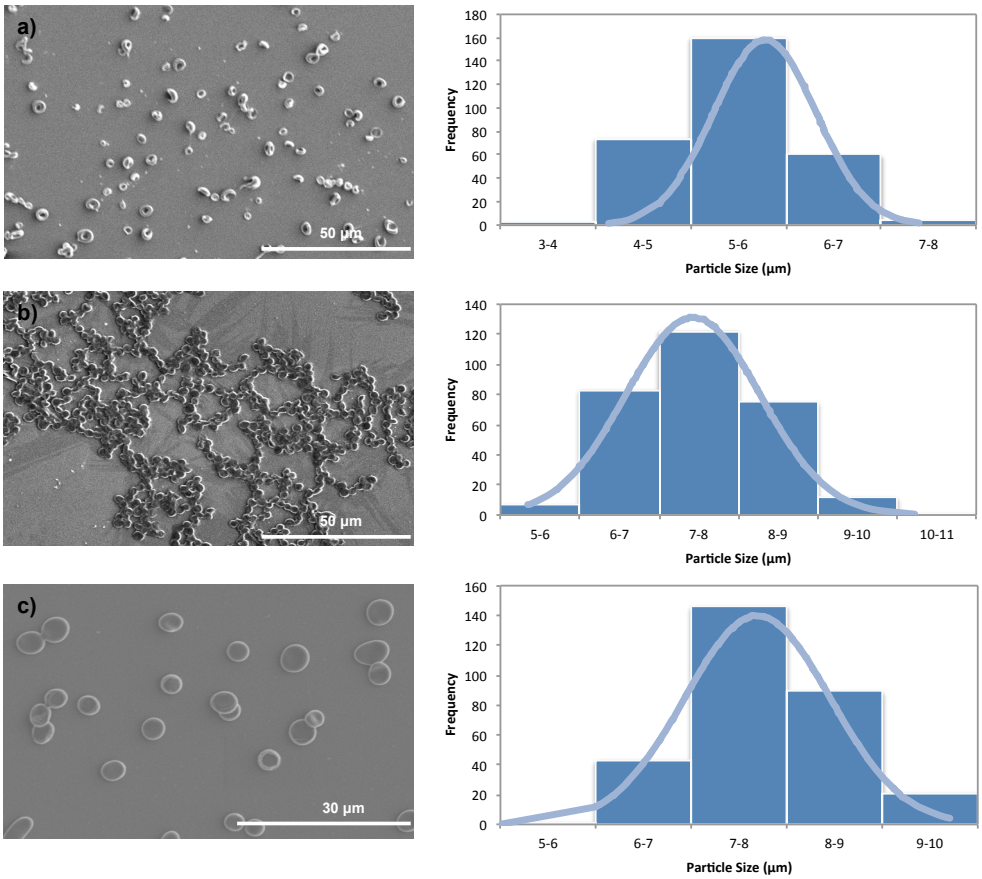


Figure 4.4 SEM images and particle size distribution histograms of a) PCMP, b) INDOP and c) PCMINDOP (Shams *et al.*, 2017).

Upon development of the drug delivery systems incorporating a single drug at a time, multiple drug delivery systems were prepared by simultaneous incorporation of paracetamol and indomethacin.

In order to prepare PCMINDOP formulation (Table 4.5), the processing parameters were fine tuned in order to establish a stable cone-jet, whereby the flow rates were set at 4 µl/min, 14 µl/min for the inner needle and outer needle accordingly, with an applied voltage of 15 kV. The mean particle size achieved for PCMINDOP was 7.7 ± 0.8 µm. The mean particle size was lowest for PCMP, which is accredited to the higher surface tension of the outer needle solution, even though the applied voltage is lower of that of INDOP. In the INDOP formulation, the flow rate of the outer solution is set at a lower value when compared with the other two formulations, this was due to marginally higher conductivity of the outer solution that in turn led to adaptation of a lower flow rate (Barrero and Loscertales, 2007).

TEM studies were conducted in this work in order to examine formation of different layers. The TEM images obtained demonstrated that the prepared formulations exhibit a double-layer structure as shown by the difference in contrast between the inner PEG and outer PLGA layer.

Arrows and dotted lines are indicative of different layers as it can be seen from Figure 4.5. It is evident that there is an overall size variance from one layer to another that is attributable to the varied flow rates set for each solution. It must be noted that changing the solution and processing parameters further regulates layer thickness. Moreover, the formation of a double-layered structure is conserved in all formulations.

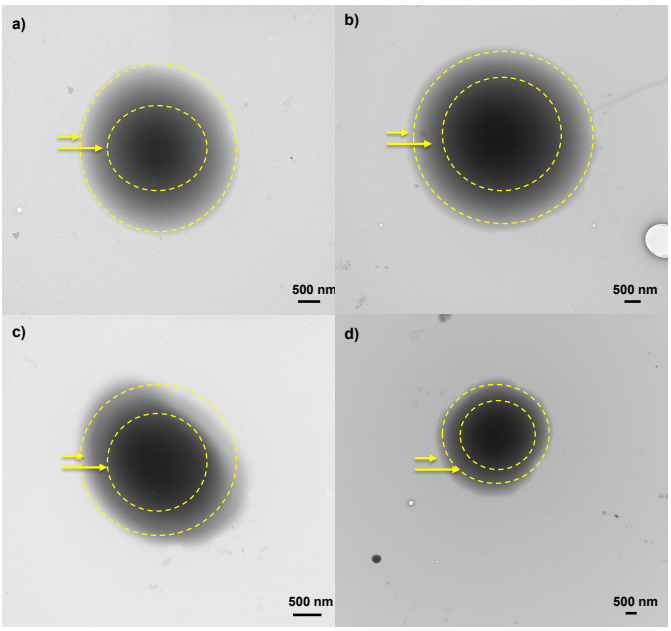


Figure 4.5 TEM micrographs of a) unloaded particles S1, b) PCMP, c) INDOP and d) PCMINDOP (with inner and outer flow rates of 4 µl/min and 10 µl/min for S1 and INDOP, and inner and outer flow rates of 4 µl/min and 14 µl/min for PCMP and PCMINDOP, respectively)(Shams *et al.*, 2017).

The morphology of the unloaded particles for S1 and the porosity of the prepared formulation were studied using FIB/SEM (Figure 4.6). The particles exhibit a smooth surface indicating high solubility of the polymers in the associated solvents used. The arrows are indicative of the cross-sectional cuts made across the microparticles.

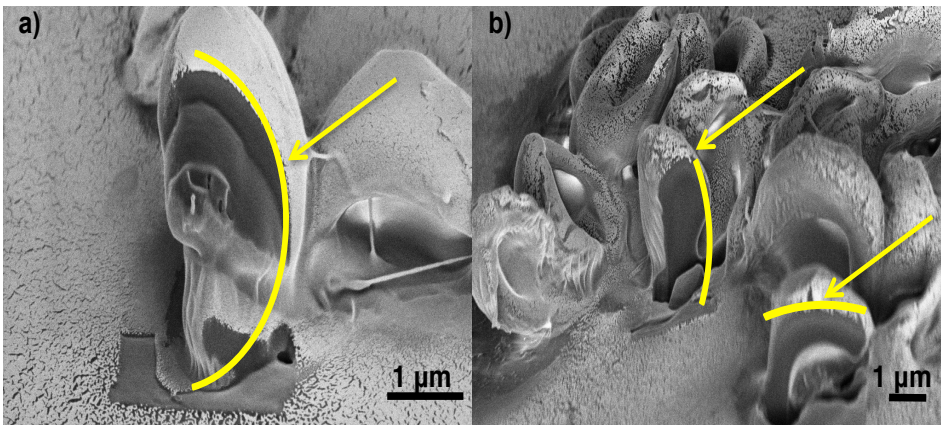


Figure 4.6 SEM/FIB cross-section images of unloaded microparticles S1 (yellow arrows are indicative of the cross-sectional cuts)(Shams *et al.*, 2017).

4.10 Compositional features of the pure component and prepared formulations

FTIR spectra were obtained to study possible interaction between PLGA and PEG with the corresponding drugs, indomethacin and paracetamol, during EHD processing. FTIR spectra of PLGA, PEG, indomethacin and paracetamol before and after undergoing processing are presented in Figure 4.7.

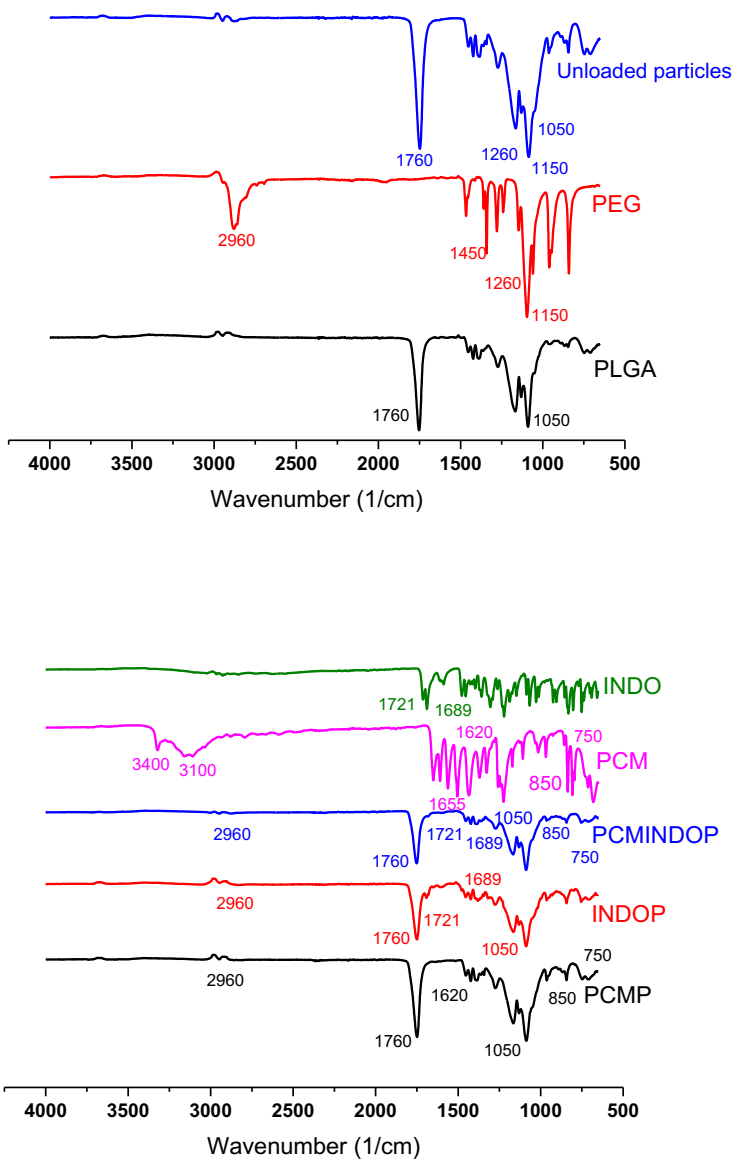


Figure 4.7 FTIR spectra of PLGA, PEG, unloaded particles, INDOP, INDO, PCMP, PCM and PCMINDOP(Shams *et al.*, 2017).

The pure indomethacin sample demonstrated characteristic peaks at 1689 cm^{-1} (amide group) and 1721 cm^{-1} (carboxyl stretching), the pure paracetamol exhibited O-H stretching at 3100 cm^{-1} to 3500 cm^{-1}, N-H stretching at 3200 cm^{-1} to 3400 cm^{-1}, and C=O stretching from 1620 cm^{-1} to 1655 cm^{-1}, also para-distributed aromatic ring from 750 cm^{-1} to 850 cm^{-1}.

Moreover, pure PEG showed stretching C-O from 1000 cm^{-1} to 1260 cm^{-1}, stretching C-O-C from 1050 cm^{-1} to 1150 cm^{-1}, stretching C-H from 1300 cm^{-1} to 1450 cm^{-1} and 2850 cm^{-1} to 2960 cm^{-1}. From pure PLGA, the bands at 1050 cm^{-1} to 1250 cm^{-1} are characteristic of C-O stretching of aliphatic polyesters and also at 1760 cm^{-1} C=O bond stretching was detected.

The drug loaded particles showed combination of peaks corresponding to polymers and model drugs confirming that there were no degrading interactions between the components used.

Differential scanning calorimetry studies (Figure 4.8 and Figure 4.9) were taken to investigate the physical state of paracetamol and indomethacin within the developed formulations (PCMP, INDOP and PCMINDOP). The thermographs acquired for pure PLGA and PEG indicated glass transition temperatures at 50°C and 62°C, respectively. However, these endothermic peaks were absent in the prepared formulations.

Paracetamol and indomethacin melting peaks in pure form were detected at 170°C and 160°C, respectively. These peaks were not observed in the prepared formulations (S1-S3). This suggests that the encapsulated model drugs were well integrated in an amorphous form, this leads to successful preparation of the formulations that included more water-soluble form of the model drugs.

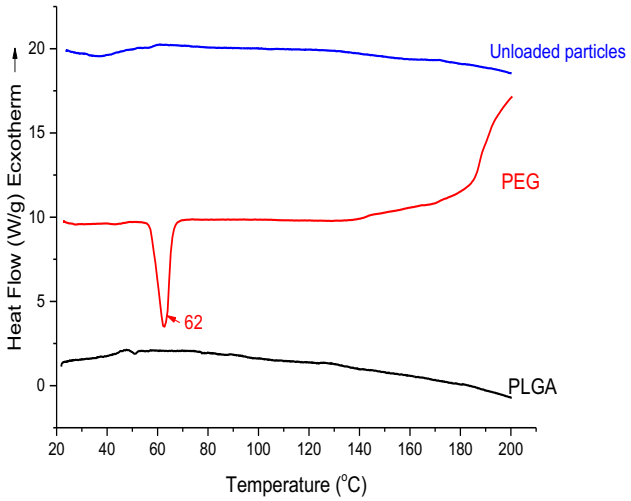


Figure 4.8 DSC thermograms of PLGA (50:50), PEG, unloaded particles S1 (endotherms are shown as peaks)(Shams *et al.*, 2017).

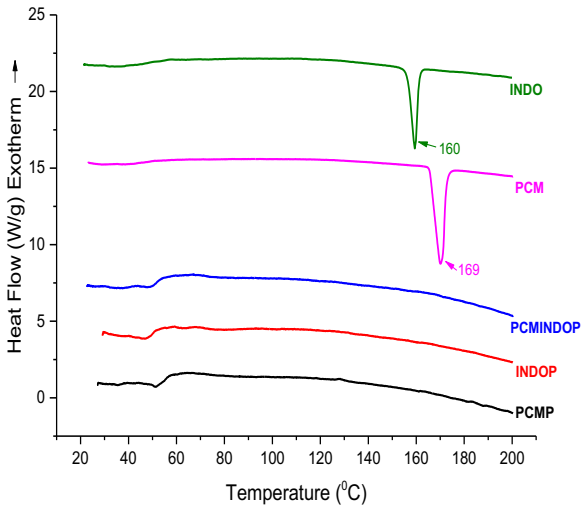


Figure 4.9 DSC thermograms of pure paracetamol, pure indomethacin, PCMP, INDOP and PCMINDOP (endotherms are shown as peaks)(Shams *et al.*, 2017).

This is accredited to the nature of EHD processing, whereby the polymeric system adopts a more amorphous state as a result of fast solvent evaporation. As a consequence, the degree of crystallinity of the drug is compromised. This is favourable in case of encapsulation of drugs that have low aqueous solubility,

leading to a higher degree of dissolution when introduced as a solid oral dosage unit (Nyström *et al.*, 2011).

4.11 Release Studies

The encapsulation efficiency is a crucial parameter of the developed microparticles and also an indicator of the efficiency of the co-axial EHDA forming process (Zamani *et al.*, 2013). The encapsulation efficiency of paracetamol from PCMP was found to be 69% compared to that of PCMINDOP, which was at 54%. The encapsulation efficiency of indomethacin in INDOP formulation was measured at 78%, which was higher compared to that of PCMINDOP, at 69%.

Drug release from biodegradable polymers occurs by several means that couple and control the drug release rate (Soppimath *et al.*, 2001). In matrix structures, drug release occurs primarily through desorption of surface bound drug, the diffusion of the drug through a polymeric matrix and the polymer matrix erosion. For particles of sub-micrometre size, owing to reduced size and increased surface area to volume ratio, faster release of drug and higher degradation rate of the polymeric system is enabled (Bohr *et al.*, 2011).

The release profiles of paracetamol were studied from PCMP and also PCMINDOP. As is shown in Figure 4.10, the release rate of paracetamol from PCMINDOP is slightly slower than that of PCMP. Nonetheless, the release pattern remained alike implying that drug dissolution occurred irrespective of the aqueous solubility of the incorporated drug. This is confirmed by Dubois and Ford (Dubois and Ford, 1985), who established that formulations containing low drug concentration lead to a polymer controlled dissolution profile that is predominantly reliant on the rate of dissolution of polymer as opposed to the properties of the drug. In the paracetamol release profile from PCMP, 59% of the drug is released

within the initial 2 hours. The corresponding value for PCMINDOP is 48%. The slightly higher release rate of paracetamol from the PCMP is credited to the smaller particle size of 5 μm compared to that of 7.4 μm, which further decreased the diffusion distance that the drug must travel in order to reach the dissolution medium.

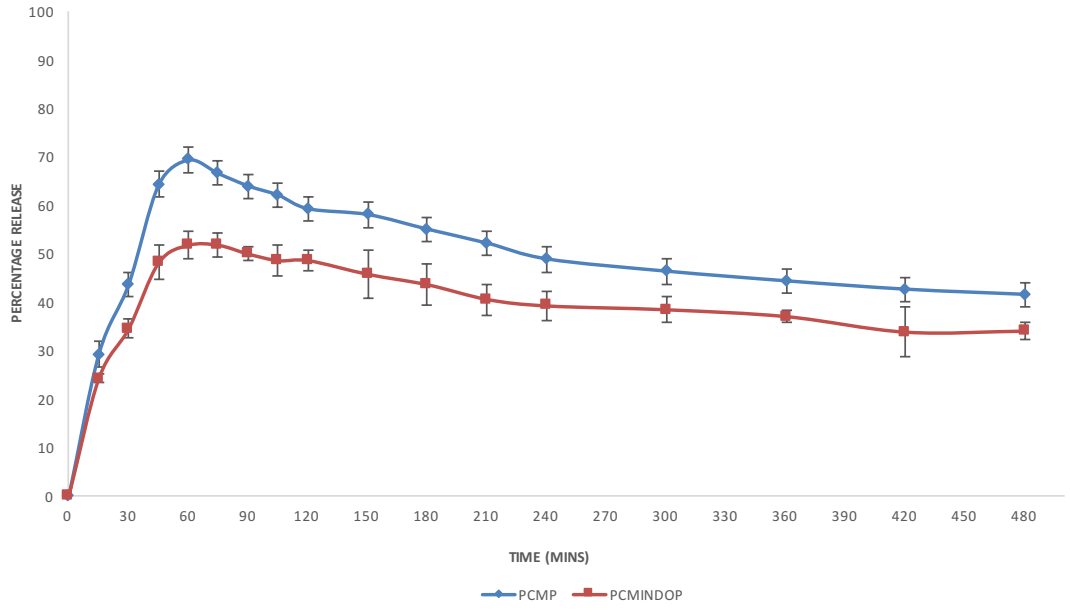


Figure 4.10 Release profiles of PCMP and PCMINDOP(Shams *et al.,* 2017).

In-vitro release profiles of indomethacin demonstrated minor difference in release rate and profile between INDOP and PCMINDOP formulation. This shows that there is no significant effect from incorporation of paracetamol into the polymeric drug delivery systems. The developed drug delivery systems incorporating indomethacin has considerably improved the dissolution of the active ingredient compared to that of the free drug. This is accredited to reduced particle size of the drug delivery carrier as previously reviewed. Increased surface area to volume ratio of the particles is highly favourable for development of these formulations.

Furthermore, as confirmed by DSC results, the developed formulation including indomethacin is in amorphous form, this further enhances the aqueous solubility of crystalline indomethacin and is accountable to the favourable nature of EHDA processing. The rate of oral absorption of the drug is often governed by the dissolution rate, which is a key factor of its oral bioavailability (Nokhodchi *et al.*, 2005).

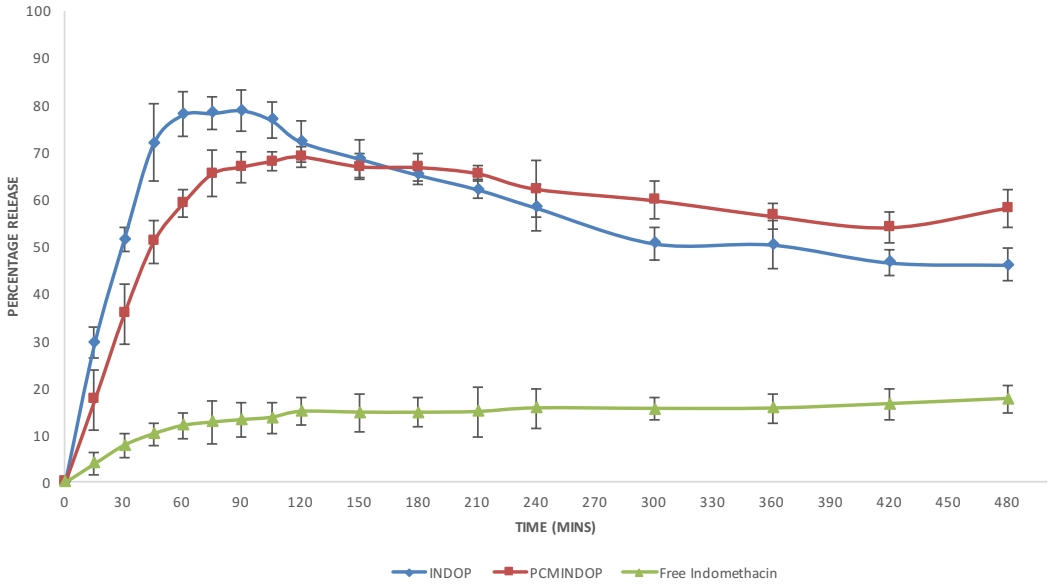


Figure 4.11 Release profiles of indomethacin from free drug, INDOP and PCMINDOP (Shams *et al.,* 2017).

As it can be seen from the release profile obtained for INDOP and PCMINDOP in Figure 4.11, micro-particles showed an initial burst release in the first 2 hours when introduced to the release medium and 72% and 68% of indomethacin is discharged, respectively. In contrast, there was only 14.8% release of free indomethacin after 2 hours. After 3 hours, the release rate of indomethacin from INDOP and PCMINDOP is decreased and follows a plateau. The initial release can also be due to the accumulation of the drug at the surface of the microparticles, which can be explained by difference in the degree solubility of

polymer and the incorporated drug in the solvent used for EHD processing. This subsequently lead to dislocation of the drug to a superficial level, as solvent evaporation occurs.

The monomer ratio of PLGA that was used in this study contains 50% lactic acid 50% glycolic acid. Among other existing monomer ratios of this polymer, PLGA (50:50) offers the highest hydrophilicity and undergoes degradation at a faster rate (Makadia and Siegel, 2011).

Besides, the water-soluble PEG core further enhances the hydration of the polymer matrix (Kim *et al.*, 2009). As the water permeates inside the drug carriers, it hydrolyses the polymer and subsequently creates a pathway for the drug to be released by diffusion and erosion until complete polymer solubilisation is achieved (Makadia and Siegel, 2011).

4.12 Summary

This sturdy demonstrates the successful preparation of micro scale polymeric drug carrier with double layer conformation using co-axial electrohydrodynamic atomization technique. This technique overcomes the limitations associated with conventional technique used for preparation of drug delivery systems whereby it negates the need for extreme temperatures, multi-step processing, or the use of additional substances such as surfactants and other additives.

The key processing parameters including flow rates, applied voltage and the working distance were optimized in order to achieve a stable cone-jet. Based on the solution key properties including electrical conductivity and viscosity, the experimental parameters had to be fine-tuned in order to obtain polymeric particles of sub-micron size with narrow size distribution. It was shown that higher electrical conductivity of the inner solution is favourable to formation of a cone-jet,

as is higher viscosity of the outer solution. It was also shown that application of high voltage combined with low flow rates results in formation of particles of smaller size. Moreover, it was confirmed that for solutions of higher viscosity, larger particles are formed.

Upon inclusion of the model drug onto the polymeric solution, few changes were noted in the physiochemical properties of the solutions, which in turn led to changes in the processing parameter in order to sustain a stable cone-jet without much compromise on the average size of the particles. Model drug with different aqueous solubility were successfully processed with high encapsulation efficiency and production yield. This is highly desirable for preparation of fixed dose combination formulation as a potential solution to tackle the challenges associated with polypharmacy in geriatric therapy.

5 Electrosprayed microparticles for targeted delivery of prednisolone

5.1 Overview

The main objective of a drug delivery system is to deliver the therapeutic agent at the desired site of action for a required amount of time at a therapeutic window concentration. In order to achieve this, targeted drug deliveries have been implemented, whereby site-specific delivery of the incorporated active ingredient results in minimisation of the unwanted side effects and reduced toxicity. In this study, single and co-axial EHD processing was utilized in order to develop pH-sensitive drug delivery systems for targeted release of prednisolone using Eudragit L100-55 as the polymer carrier. A number of formulations were implemented with varying drug: polymer ratios to minimize the possible burst release affect in drug release for the first two hours of release studies, in order to study the feasibility of the proposed system for site-specific release of prednisolone. The developed formulations were further studied using XRD, DSC and FTIR to better understand the composition and physical state of the prepared drug delivery systems. Preparation of prednisolone loaded pH-responsive drug delivery systems are of great interest for the treatment of conditions including inflammatory bowel disease and colon cancer.

5.2 Introduction

Variation in the environmental pH is one of the most commonly applied stimuli for the development of triggers for drug release. The development of pH sensitive drug delivery system is mainly established by adopting polymers that bear weak acid (e.g. carboxylic acid) or base (e.g. primary and tertiary amines) groups with

a pKa that allows sharp changes in the ionization state at the desired pH. An increase in the degree of ionization enables dramatic change in the conformation and also the affinity of the chains both for the solvent and the intrachain interactions, causing in either disassembly of the components or the swelling/shrinkage of the covalent network (Rodríguez *et al.*, 2003). The objective of this study was reducing prednisolone release in the acidic environment and achieving fast release when the pH is adjusted to the pH threshold of the polymeric carrier.

Colon specific drug delivery is especially beneficial for treatment of inflammatory bowel disease (IBD) that encompasses ulcerative colitis and Crohn's disease. Moreover, the oral delivery is proven to be the most convenient route of administration of drug to the patients (Chen *et al.*, 2010). Nonetheless, the change in pH that the drug is exposed to prior to reaching the desired release location, remains a hindrance where localized delivery of the drug is a requirement for optimal treatment of these chronic conditions that necessitates sustainable treatment. Thus, development of targeted delivery of active pharmaceutical ingredient (API) to a specific region of the colon, where the API is protected from premature absorption in the upper gastrointestinal tract (GIT), is of great significance. The variation in the pH along GIT has been exploited for this purpose. In a study oil-in-oil emulsion technique was utilized for fabrication of pH-responsive acrylic microparticles for targeted gastrointestinal delivery of prednisolone (Kendall *et al.*, 2009). Eudragit L55, S and L were deployed for this purpose, it was discovered that Eudragit L was the most appropriate polymer for this use, by showing minimal drug release in acidic conditions and achieving optimum drug release when reaching the pH threshold of the corresponding

polymer carrier.

5.3 Polymer selection

The sensitivity to change in pH can be tuned by changing the nature of the co-monomers that are used to synthetize the polymer. Eudragit L100-55 was used in this study, which dissolves when the pH is 5.5 (Joshi, 2013). Thus, it is resistant to gastric fluid and consequently it can bypass the stomach and release the incorporated active ingredient into the small intestine. Eudragit polymers have been used for preparation of microspheres and nanoparticles for controlled drug delivery system in the gastrointestinal tract (Shen *et al.*, 2009). In the past, Eudragit L100-55 has been used as an enteric coating agent for encapsulation of aspirin where the drug stability in acidic environment of the upper gastrointestinal tract was preserved (Hao *et al.*, 2014).

5.4 Model drug selection

Prednisolone is one of the most commonly prescribed drug for treatment of IBD. It is a corticosteroid drug that is generally used as immunosuppressant and anti-inflammatory drug to treat number of inflammatory and autoimmune conditions. However, due to use of high dosages at frequent predetermined time intervals, it also induces adverse side effects (Campieri *et al.*, 1997). Furthermore, it has poor water-solubility, characteristic of class II substances according to the Biopharmaceutics Classification System (Vogt *et al.*, 2007). This is one of the main challenges in preparation of oral formulations and is proven to be a limiting factor in the drug bioavailability, that is directly related to the dissolution rate of the drug. Therefore, numerous techniques such as solid dispersion and preparation of liquisolid compacts, have been used for enhancement of its water

solubility. However, recent reports on prednisolone loaded particles suffer from low encapsulation efficiency (Lau *et al.*, 2017).

5.5 Solvent selection

Solvent and solution for particle preparation were selected based on some conditions that allow consistent preparation of near-monodispersed particles as a result on formation of a stable cone-jet, as well as the ability to both dissolve Eudragit L100-55 and prednisolone. Based on the requirements, methanol and isopropanol were chosen as the solvent system with boiling points of 64.0^{o}C and 83.0^{o}C respectively, therefore volatile enough to help formation of dry particles upon collection. Methanol and isopropanol have similar density and surface tension, while isopropanol has a slightly higher viscosity and lower electrical conductivity than that of methanol (Table 5.1). In general, a minimum electrical conductivity of 0.01 μS m^{-1} is favourable for EHD processing of solutions (Ding *et al.*, 2005).

Table 5.1 Physical properties of solvents used to prepare the microparticles (Smallwood, 1996).

Property	MeOH	Isopropanol
Viscosity (mPa s) (at 25^{o}C)	0.5	2.0
Boiling point (^{o}C)	64.0	83.0
Evaporation rate (nBuAc=1)	3.5	1.7
Dielectric constant	32.6	17.2
Electrical conductivity (μS/cm)	0.50	0.06
Surface tension (mN/m)	22.1	20.9

5.6 Characterization of the prepared solutions

With the inclusion of prednisolone onto methanol solution to prepare 0.1% w/w and 0.2 % w/w concentrations, there was an increase in the electrical conductivity measurements at lower concentration of prednisolone, nonetheless the viscosity and surface tension values remained nearly the same (Table 5.2). With the addition of Eudragit L100-55 to 0.2% w/w in methanol, the electrical conductivity of the solution increased notably, there was also a slight increase in the viscosity and surface tension measurements of the prepared solution.

Table 5.2 Physiochemical properties of the prepared solutions.

Solution	Surface Tension (mN/m)	Viscosity (mPa s)	Electrical Conductivity (µS/cm)
Prednisolone 0.1% w/w in MeOH	23.4 ± 0.2	0.8 ± 0.3	10.0 ± 0.1
Prednisolone 0.2% w/w in MeOH	24.4 ± 0.3	0.8 ± 0.3	2.5 ± 0.1
Prednisolone 0.1% w/w + L100-55 1% w/w in Isopropanol	25.2 ± 0.5	2.5 ± 0.5	3.2 ± 0.1
Prednisolone 0.2% w/w + L100-55 1% w/w in MeOH	25.5 ± 0.4	0.9 ± 0.3	30.6 ± 0.4
L100-55 2% w/w in Isopropanol	26.2 ± 0.3	2.8 ± 0.1	5.3 ± 0.1
L100-55 1% w/w in Isopropanol	25.8 ± 0.2	2.0 ± 0.1	3.2 ± 0.1

Upon preparation of Eudragit L100-55 1% and 2% w/w in isopropanol, the electrical conductivity increased with the addition of higher concentration of the

polymer, as was the same for viscosity measurements. The surface tension of the solution remained almost constant for the prepared solution and the parent isopropanol solution. With the addition of prednisolone 0.1% w/w onto Eudragit L100-55 1% w/w in isopropanol, the electrical conductivity of the solution decreased slightly, as did the surface tension, however, it was insignificant.

5.7 Preparation of the prednisolone loaded drug delivery systems

The electrical potential was applied throughout a fixed distance of 250 mm between the concentric needles tip and the collection platform. The composition details of the prepared solutions are shown in Table 5.3, where they are denoted as F1-F5. The set flow rates were kept constant for formulations F1, F2, F3, F4 at 6 µl/min and 12 µl/min for the inner and outer solution, respectively. The flow rate for F5 was set at 12 µl/min. The applied voltage was varied from 14 kV to 19 kV, in order to establish a stable cone jet. For all formulations, any applied voltage lower or higher than that of the given range, resulted in formation of an unstable or multiple, which would in turn lead to formation of particles with large size distribution.

Table 5.3 Formulations compositions (F1-F5).

Formulation	Core Solution	Shell Solution
F1	Prednisolone 0.1% w/w in MeOH	L 100- 55 2% w/w in Isopropanol
F2	Prednisolone 0.2% w/w in MeOH	L 100- 55 1% w/w in Isopropanol
F3	Prednisolone 0.2% w/w in MeOH	L 100- 55 2% w/w in Isopropanol

F4	Prednisolone 0.2% w/w + L 100-55 1% w/w in MeOH	L 100- 55 2% w/w in Isopropanol
F5	Prednisolone 0.1% w/w + L100-55 1% w/w in Isopropanol	None

The morphology of the prepared formulations is shown in Figure 5.1. It can be deduced from the obtained SEM images, the acquired particles assumed the form of donut-shaped encapsulating matrices. It is believed that, the periphery of the droplet is pinned as the solvent is evaporating further. Solvent removal from the surface results in an increase in the concentration of the polymer near the surface; this culminates in the formation of toroid structures. Moreover, the high molecular weight of Eudragit L100-55 may result in a restricted mobility of the polymer as the shell formation occurs, this has led to formation of a ring-like structure as a result of solvent evaporation.

In terms of particle size, F1 and F3 formulations consisted of the same outer solution, with F3 having higher drug concentration in the core solution. The averaged applied voltage was slightly higher for F1, at 19 kV as compared to 16 kV for F3. Hence, as shown in Table 5.4**,** F1 averaged particle size was found to be 3.2 ± 1.0 μm as opposed to 4.2 ± 1.0 μm for F3 (Gañán-Calvo *et al.*, 1997). The outer solution in F4 is also has the same polymer concentration as in F1 and F3, only in this formulation, the inner solution constituted of both the drug and polymer, in order to further limit the drug release in the first two hours before it reaches the intestine. Inclusion of polymer, as well as having relatively lower applied voltage of 15-18 kV, resulted in higher average particle size when compared to that of F1, at 3.8 ± 1.4 μm.

Furthermore, when compared to F3, F4 has lower averaged particle size, where

the applied voltage is slightly lower for that of F3 at 16 kV.

Table 5.4 Encapsulation efficiency and mean particle size data for the prepared formulations.

Formulation	Mean Particle Outer Diameter (μm)	Mean Particle Inner Diameter (μm)	Encapsulation Efficiency
F1	3.2 ± 1.0	0.7 ± 0.2	93 %
F2	3.0 ± 0.6	0.5 ± 0.1	81 %
F3	4.2 ± 1.0	0.5 ± 0.2	83 %
F4	3.8 ± 1.4	0.4 ± 0.1	84 %
F5	1.7 ± 0.4	0.6 ± 0.2	76 %

The constituent of the inner needles solution for F2 and F3 was kept unchanged, nevertheless, the outer solution in F3 had higher polymer concentration compared to that of F2, and the averaged particle size was 3.0 ± 0.6 μm at an applied voltage range of 18-19 kV for F2, and 4.2 ± 1.0 μm at an applied voltage of 16 kV for F3. The marginally higher particle size of F3 can be justified by the higher polymer content, as well as the lower applied voltage.

Among all the developed formulations, F5 that used a single needle electrospraying, had the lowest particle size at 1.7 ± 0.4 μm and applied voltage of 14-19 kV. Although the drug: polymer ratio for F5 was the same of that in F2, having no outer shell solutions, resulted in lower particle size.

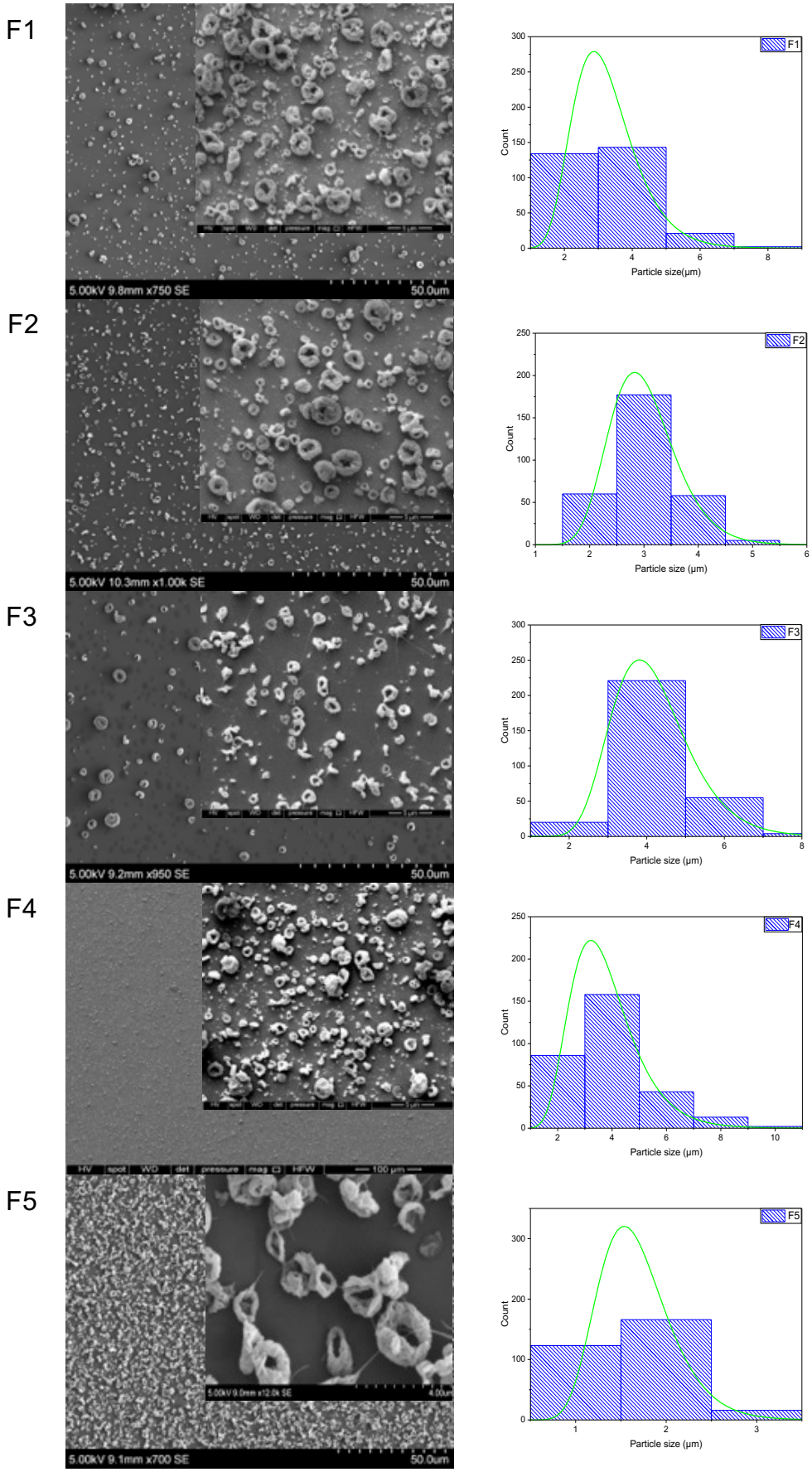


Figure 5.1 Size distribution graphs and scanning electron microscopy images of the prepared formulations F1-F5 (Shams *et al.*, 2018).

5.8 XRD results

The XRD patterns of the polymer, Eudragit L100-55 and the drug, prednisolone, and also those of the prepared formulations were obtained. Prednisolone exhibited a number of reflections and sharp peaks at 15.75°, 17.64°, 21.33°, 22.77°, 25.27° and 26.31° that is indicative of its crystallinity.

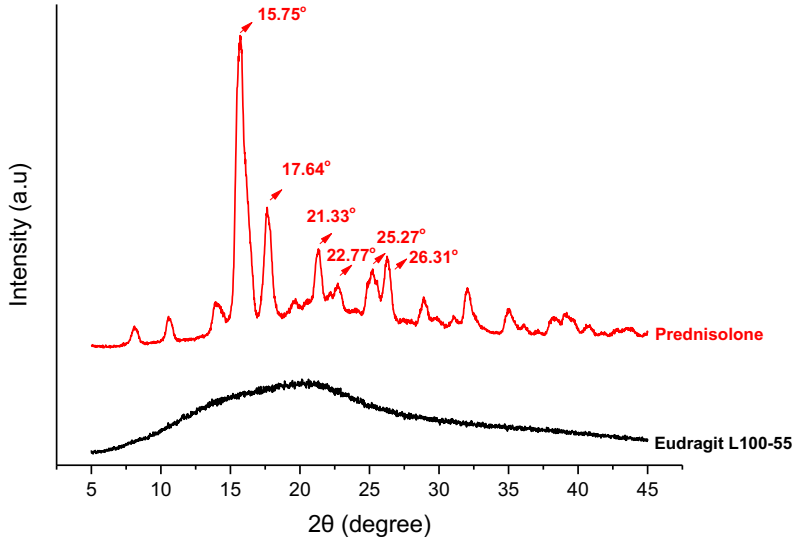


Figure 5.2 X-ray Diffractogram of prednisolone and Eudragit L100-55 (Shams *et al.,* 2018).

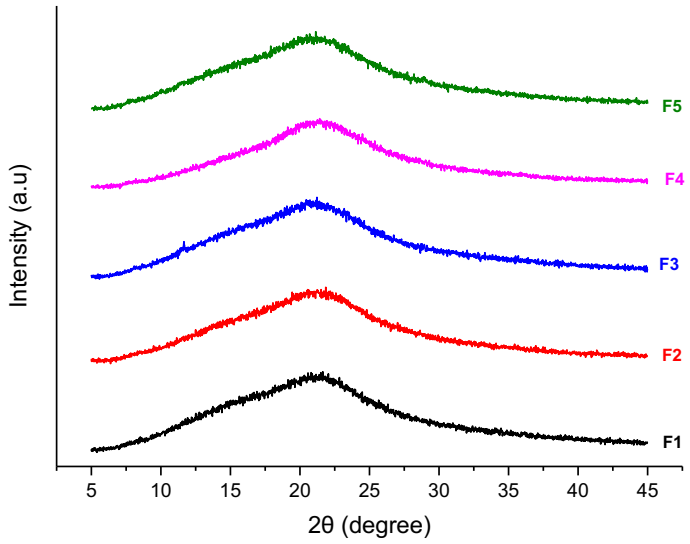


Figure 5.3 X-ray Diffractogram of the developed formulations (F1-F5) (Shams *et al.,* 2018).

Eudragit L100-55 showed the characteristic broad hump of amorphous materials. As revealed in the diffractograms obtained for the developed formulations (Figure 5.2 and Figure 5.3), amorphous nature was most noticeable. In all formulations, the characteristic peak of prednisolone at 2θ of 15.7^{o} was found unseen and overlaid with that of Eudragit L100-55 at 2θ of 20.76^{o}. As elucidated by the overlaid X-ray diffractograms of the formulations, the amorphous state of prednisolone within the formulations can be proved. This in turn confirms that the drug is molecularly dispersed in Eudragit L100-55.

5.9 Thermal properties

DSC studies were performed in order to assess possible solid-state interactions between the components and, consequently, to evaluate the actual drug-excipient compatibility in all the studied formulations. The thermal curves of pure components, prednisolone and Eudragit L100-55, and those of the developed formulations (F1-F5) are shown in Figure 5.4 and Figure 5.5.

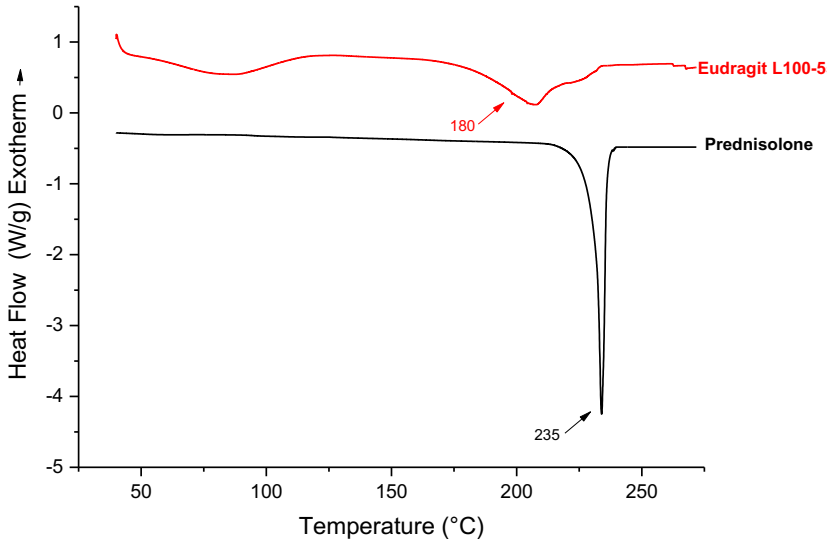


Figure 5.4 DSC thermograms of prednisolone and Eudragit L100-55 (Shams *et al.,* 2018).

The DSC curve of the pure drug was indicative of its crystalline state, showing a sharp endothermic peak at 235°C. The thermal profile of Eudragit L100-55 exhibited a broad endothermic band that ranged between 50°C and 100°C, due to dehydration as evaporation of unbound water took place, the second endothermic effect was seen at a higher temperature, accredited to the melting of its crystalline phase. Eudragit L100-55 is a heat labile polymer that undergoes thermal degradation at 150°C by decomposition of carboxylic side groups, followed by chain decomposition at 180°C (Lin and Yu, 1999). The thermal curves of the developed formulations almost corresponded to the superimposition of those of the pure components, demonstrating the absence of solid-state interactions and allowing assessment of drug-polymers compatibility in all the examined formulations.

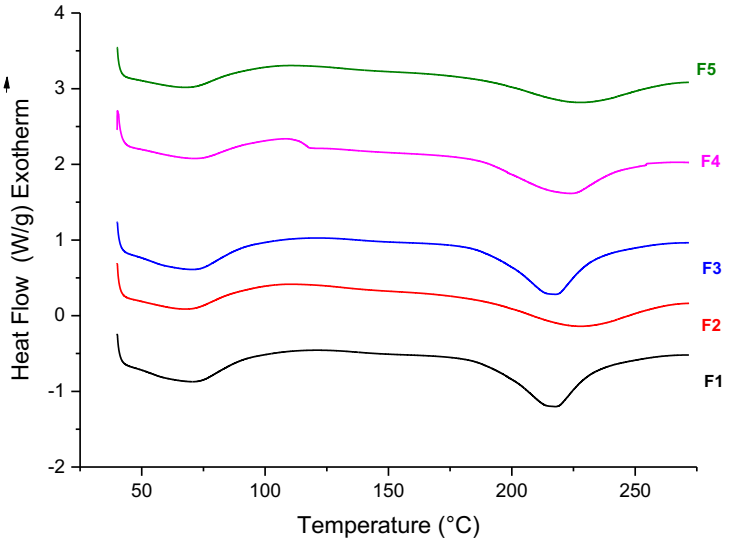


Figure 5.5 DSC thermograms of the prepared prednisolone formulations (F1-F5) (Shams *et al.,* 2018).

As shown from the thermograms obtained for F1-F5, the melting temperature of prednisolone is shifted to a lower value as the L100-55 has acquired a higher degradation temperature, and the new T_m is at an intermediate point of the two.

This shift is steepest for F1, where polymer: drug ratio is at its highest, this may be due to random dispersion of prednisolone molecules within the polymeric carrier and a semi-crystalline structure.

5.10 FTIR results

Fourier transform infrared spectroscopy was used in order to characterise possible interactions between prednisolone and Eudragit L100-55 in the solid state. FTIR spectra of unprocessed material and the prepared formulations were obtained as illustrated in Figure 5.6 and Figure 5.7**.** The spectrum of prednisolone showed characteristic bands of OH group at 3200-3500 cm^{-1} (OH involved in intermolecular association). The characteristic stretching vibration of C=O at 1700 cm^{-1} and C=C at 1660 cm^{-1} appear as very strong bands. The spectrum of Eudragit L100-55 shows characteristic bands of methyl and methylene CH stretch vibrations at 3000 cm^{-1} and 2936 cm^{-1}, a strong band owing to carbonyl groups at 1723 cm^{-1} (C=O stretch) and two bands at 1267 cm^{-1} and 1165 cm^{-1} that are due to ester linkages (COC stretches).

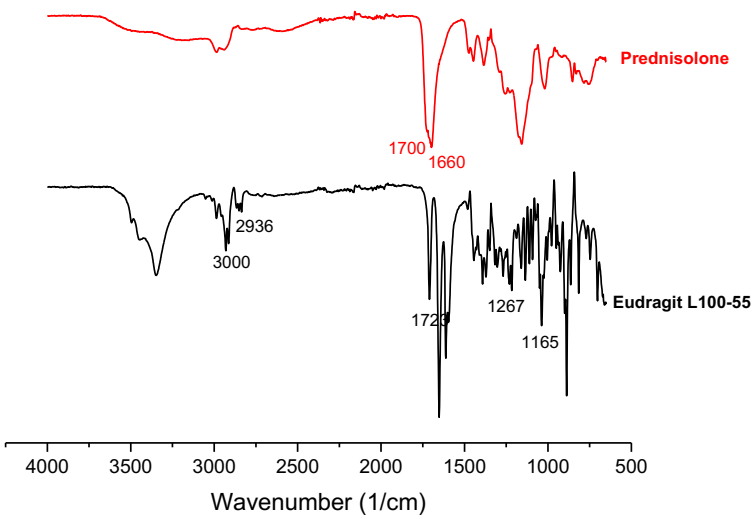


Figure 5.6 FTIR spectra of prednisolone and Eudragit L100-55 (Shams *et al.,* 2018).

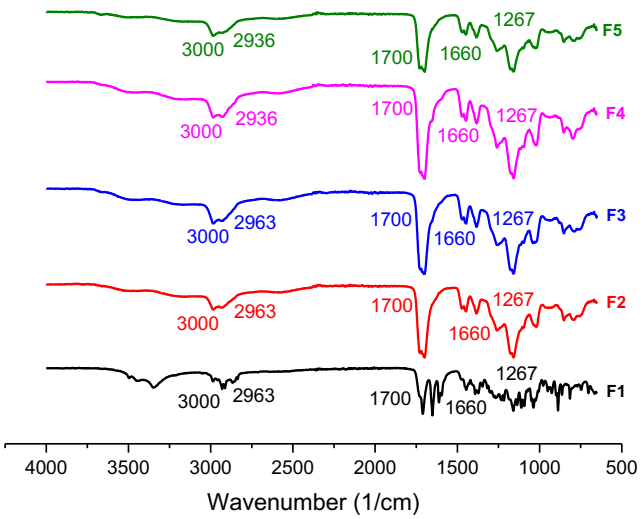


Figure 5.7 FTIR spectra prednisolone formulations (F1-F5)(Shams *et al.,* 2018).

5.11 Prednisolone release

To investigate prednisolone release profiles from Eudragit L100-55 developed formulations, in-vitro dissolution tests were conducted on F1-F5 under acidic conditions pH 1.2 for the first 2 hours that replicates the stomach passage transit time, followed by 6 hours at pH 6.8, which mimics intestinal conditions. The drug release profiles are shown below in Figure 5.8 and Figure 5.9. The encapsulation efficiencies obtained for all formulations are presented in Table 5.4**.** Prednisolone release from all formulations is hindered to different extents for the initial 2 hours of release in acidic environment, dependent on the composition of the formulation. This is due to insolubility of Eudragit L100-55 at pH < 5.5. It can be appreciated from the release graphs, that the prednisolone release during the first two hours is at its highest for F3 > F5 > F1 > F2 > F4. Although the drug: polymer ratio is kept the same for F2 and F5, the percentage release for the first two hours is higher for F5, where the polymer and drug were fed into single needle electrospraying, as opposed to F2, where drug solution was fed only into

the inner needle. Moreover, the mean particle size corresponding to F5 is lower at 1.7 ± 0.4 μm as opposed to F2 with mean particle size of 3.0 ± 0.6 μm.

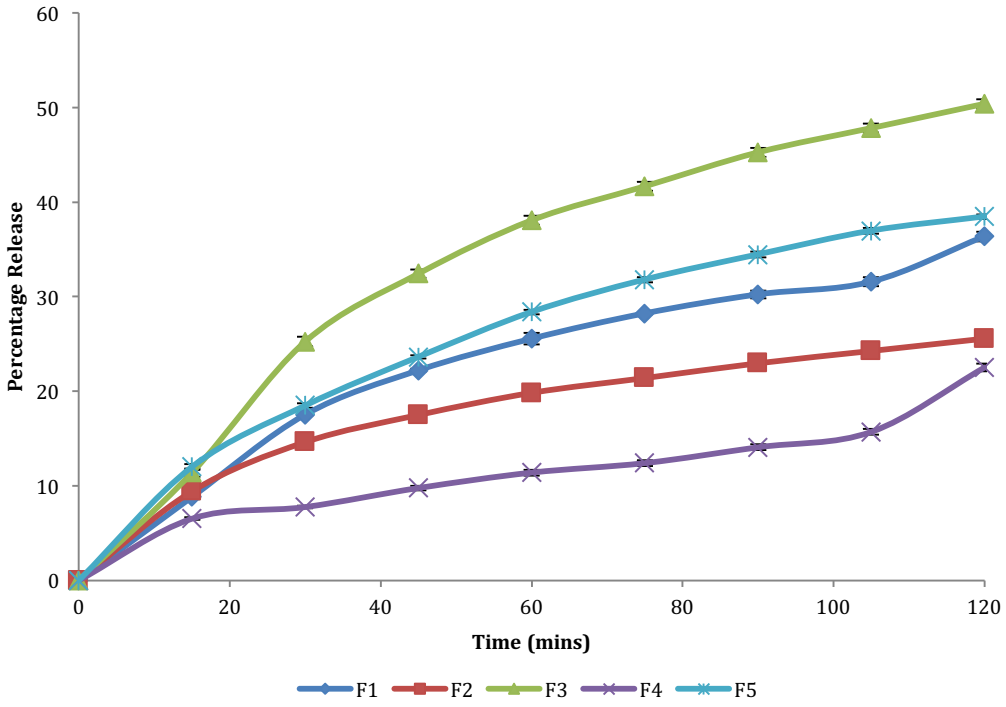


Figure 5.8 Prednisolone percentage drug release for the first two hours at pH 1.2 (F1-F5) (Shams *et al.,* 2018).

It was also found out that the drug encapsulation efficiency was slightly higher for F2 at 81% when compared to that of F5 at 76%. The drug release was at its least for F4, whereby Eudragit L100-55 was incorporated to both the inner and outer solutions fed into co-axial electrospraying, where prednisolone was only in the core solution. The encapsulation efficiency was at peak value for F1, at 93% where the polymer concentration with respect to drug was at its highest. After the first 2 hours, when the pH is at 6.8, F1 demonstrates the fastest initial dissolution rate. Prednisolone release shows a more sustained pattern for F4 and F5 where the drug release reaches a plateau after 3 hours.

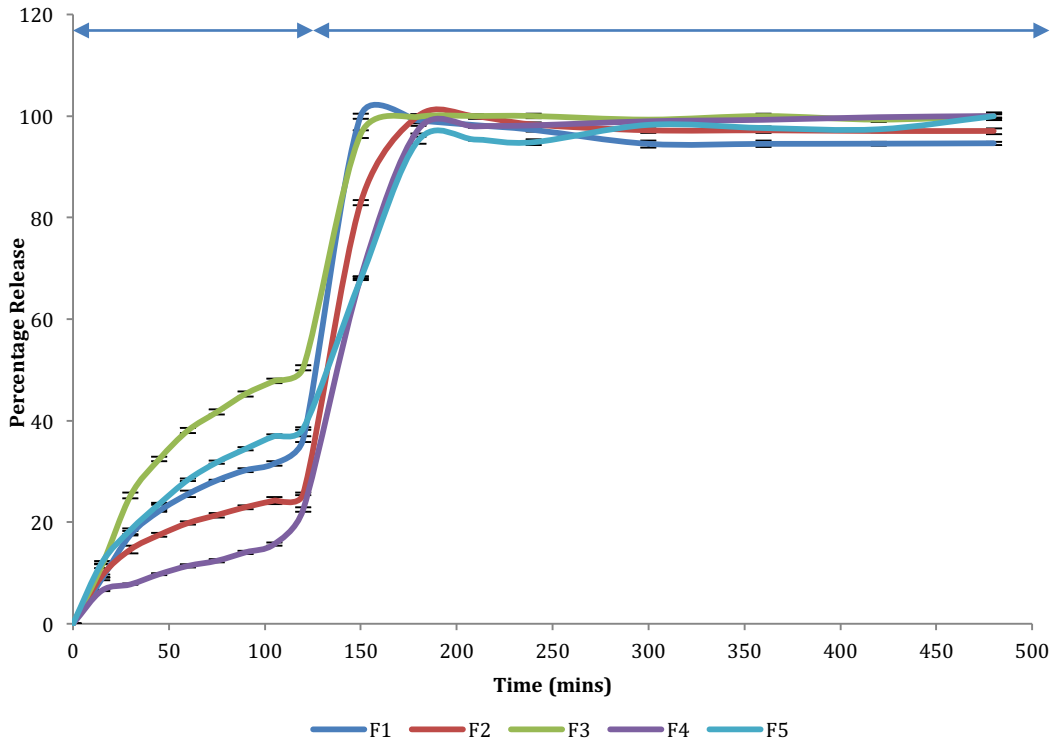


Figure 5.9 Prednisolone percentage drug release at pH 1.2 for 120 mins, followed by pH 6.8 for 360 mins (F1-F5) (Shams *et al.*, 2018).

The drug release rate in µg/ml follows the order F2 > F5 > F3 > F1 > F4. Total drug release over a longer time span is shown in Figure 5.9. Although Eudragit L100-55 is soluble at pH > 5.5, the drug dissolution process did not occur instantly, which shows that further physical processes take place before the incorporated drug reaches its maximum release amount in the release medium. Particles need to absorb water, swell and disentangle prior to complete dissolution of the incorporated drug. The obtained results confirm that both drug: polymer concentration ratio and solution compositions using single and co-axial electrospraying set-ups can affect both encapsulation efficiency and dissolution rate. It can be concluded that using polymer both in the core solution and the shell solution can further prolong the release and yield higher encapsulation efficiency.

5.12 Clinical Perspective

Inflammatory bowel disease comprises predominantly of ulcerative colitis and Crohn's disease, two complex chronic conditions. Crohn's disease is characterised by transmural inflammation affecting anywhere along the gastrointestinal tract, most commonly the terminal ileum. Ulcerative colitis is described as mucosal inflammation restricted to the colon often affecting the distal colon in a continuous manner. Symptoms can comprise severe diarrhoea and abdominal pain regularly showing with weight loss and malaise. Both conditions have the capacity to greatly affect an individual's day to day life and in some cases, introduce life-threatening emergencies. The variable nature of inflammatory bowel disease in each individual means that providing effective treatment is challenging. Targeted drug delivery to the bowel is greatly anticipated due the changing environment along the gastrointestinal tract. The steroids taken via the oral route are susceptible to changes in pH, enzymatic action and prolonged transit times, compromising absorption. Nevertheless, understanding of the gut physiology can be used to an advantage. In order to adapt the drug distribution to the affected section of bowel, it is crucial to understand the changes in pH along the gastrointestinal tract. The stomach tends to be extremely acidic, pH then becomes more alkaline moving along the small intestine reaching approximately 7.4 at the distal small intestine. Moving into the colon, the pH drops at the caecum to approximately 5.7, rising once again to 6.8 at the rectum. The pH can further be altered by dietary intake, resident gut flora and disease-induced inflammation. Having a prednisolone coating that is initially resilient to acidic pH but then highly responsive to a higher pH facilitates a much more efficient and successful means of drug delivery.

Prolonged use of steroids unfortunately comes with systemic side effects including hyperglycaemia, gastric irritation and osteoporosis. Formulations which are pH responsive are therefore highly attractive to both the prescriber and the patient. By optimising where the drug acts it is possible to reduce the required dose and frequency, limiting the undesired side effects. Through optimising the safety and efficacy of prednisolone, there exists the potential to greatly improve patient compliance and reduce costs of administering excessive quantities of steroids. The carefully composed drug formulations described are able to work in combination with the physiology of gut rather than against, which make the future applications vast.

5.13 Conclusions

In this study, colon-targeted prednisolone loaded micropartciles were sought. Initially, single and co-axial electrospraying with the pH-sensitive polymer Eudragit L100-55 was explored. A series of five different formulations were developed in order to assess the extent of drug release in the acidic environment and efforts were made in order to further minimize the drug percentage release for the initial 2 hours. It was found that significant amounts of drug release occurred at pH 1.2 despite the polymers being insoluble under these conditions for F1, F3 and F5 formulations. However, this effect was minimized in F2 and F4 formulations, which was followed by immediate release after the adjustment of the pH to 6.8 imitating the small intestine. It is proposed that the low molecular weight of prednisolone permitted it to diffuse through the Eudragit L100-55 microparticles. The slower release in the early stages could be described by a diffusion process. The developed formulations could offer localised delivery of prednisolone to colon, where site specific release of the active pharmaceutical

ingredient is anticipated for treatment of inflammatory bowel syndrome or colon cancer.

It was shown that EHD processing offers a versatile platform for preparation of targeted release formulations by careful selection of the polymeric system composition that is highly adoptable to obtain different modes of release based on the desirable requirements. The scanning electron microscopy images of the microparticles showed that all have particles were toroids in shape. X-ray diffraction and differential scanning calorimetry studies proved that prednisolone was converted into the amorphous form in the particles.

6 Co-encapsulation of metformin HCL and glibenclamide as oral drug delivery depots

6.1 Overview

The main objective of this study was to prepare spherical microparticles for development of drug delivery systems incorporating metformin HCL and glibenclamide. The incorporated drugs had different aqueous solubility, and suffered from low bioavailability and requirement of high dosing frequency in order to achieve the desired therapeutic effects. Electrohydrodynamic processing has been reported for successful preparation of the amorphous state of model drugs in microparticles and therefore enhancement of the dissolution rate. Moreover, it has been utilized for preparation of fixed dose combination formulations which are highly attractive for treatment of chronic conditions. For this purpose, oral sustained formulation of metformin HCL and glibenclamide were implemented using co-axial EHD processing. PMSQ was chosen as the polymeric carrier due to its extremely hydrophobic nature that favours the preparation of depot formulations. Three different drug delivery systems were developed incorporating single drug and combined drugs in order to assess possible interference of one drug on the other. Processing parameters were adjusted based on the solution properties in order to achieve a stable cone-jet and in turn microparticles of small size distribution.

6.2 Introduction

Oral administration of hypoglycaemic agents is in general the first line option when medication is required for managing type 2 diabetes mellitus. In most patients with type 2 diabetes two defects coexist. This includes defective insulin

sensitivity and defective insulin secretion. Both of these abnormalities contribute to hyperglycemia (DeFronzo, 1992). Thus, oral therapy with either biguanide metformin that increases sensitivity of peripheral tissues to insulin, or sulfonylureas glibenclamide, which stimulate insulin secretion, are sensible approaches to type 2 diabetes mellitus. Each of them can be used solely or in combined form when either of them on their own is not sufficient on bringing or maintaining blood sugar at safe levels. However, there remains the issues on patient adherence which are generally driven by multiple daily dosing requirements (Cramer, 2004). Reflecting on how effective these active pharmaceutical ingredients have been in developed oral formulations for the control of blood sugar levels, the potential of formulating these as depot preparations for long-term release is an appealing proposal. Due to a number of advantages, micro and nanoparticles as drug delivery systems have been transforming over the decades from scientific curiosity and research to valuable clinical applications (Kohane, 2007).

6.3 Polymer selection

Polymethylsilsesquioxane was used as carrier polymer (Figure 6.1). PMSQ can be directly converted to ceramic products via pyrolysis (Ma *et al.*, 2002). It is perceived as an interesting polymer for materials engineering of both polymeric and ceramic products. PMSQ is biocompatible, non-polar with good heat resistance and slip properties. It also disperses well into organic solvents, making it a suitable selection for a variety of applications (Luo *et al.*, 2015). It exhibits high thermal and chemical durability, low density and low dielectric constant. These properties mark PMSQ a desirable material for applications in medicine and biomedical engineering (Zhuo *et al.*, 2005; Nangrejo *et al.*, 2008).

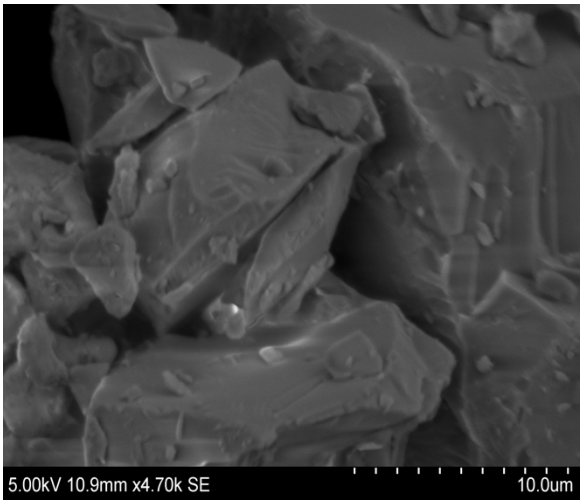


Figure 6.1 Scanning electron micrograph of PMSQ.

6.4 Model drug selection

Metformin HCL (Figure 6.2) was used as one of the model drugs in this work, it is widely used for management of diabetes mellitus type 2. It is classified as a class III compound based on Biopharmaceutics Classification Systems, this is due to its low permeability across the cell membranes and high water solubility (Cetin and Sahin, 2016). In pharmacological doses, it does not decrease basal blood glucose concentrations below the physiological range and therefore it is considered as an antihyperglycemic agent rather than a hypoglycemic agent. Metformin decreases hepatic glucose production, reduces the intestinal absorption of glucose and improves the insulin sensitivity by increasing peripheral glucose uptake and utilization (Meka *et al.*, 2013). Owing to its high aqueous solubility, novel formulations that enable controlled release of metformin HCL for prolonged duration in order to obtain maximum absorption and better bioavailability are highly desirable. In conventional oral controlled release formulations, majority of the incorporated drug content is released at the colon, this necessitates that the drug will be absorbed from the colon.

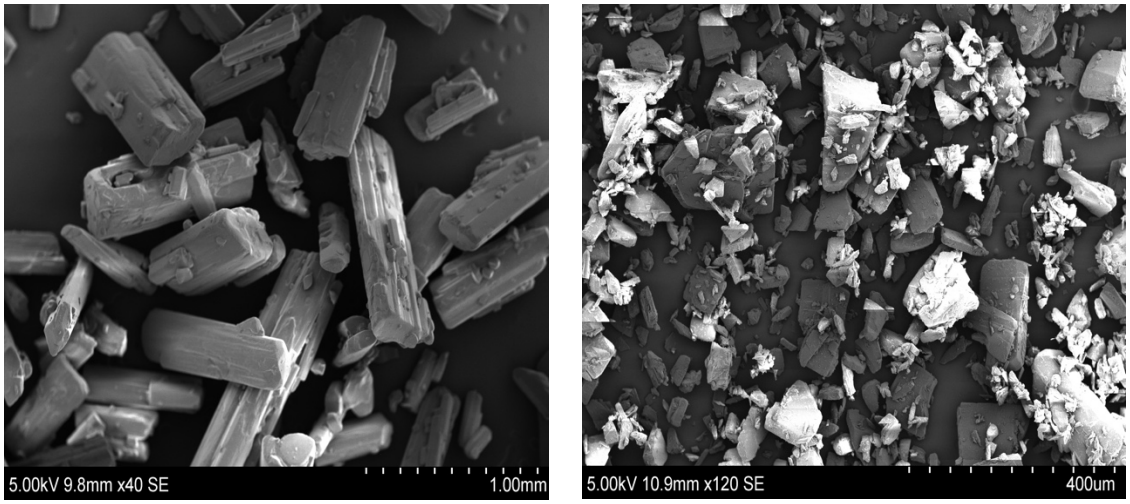


Figure 6.2 Scanning electron micrographs of metformin HCL (left) and crystalline glibenclamide (right).

Glibenclamide was selected as a model drug due to its poor solubility in water, which in turn results in its limited dissolution and absorption (Figure 6.2). It also has high molecular weight that makes it difficult to formulate. The low oral bioavailability of the conventional glibenclamide tablets necessitates the development of oral controlled release formulation and enhancement of its poor solubility. These specifications have led to large variation in glibenclamide bioavailability between different commercial brands with each brand presenting inter-subject variability. Being vital in the management of chronic disease (e.g. diabetes mellitus), patient compliance is a pivotal part of its therapeutic benefits. Floating drug delivery systems incorporating glibenclamide have been developed to enable slow release of the drug in the stomach with reported successful optimization of the dissolution rate (Essa *et al.*, 2015).

Herein, EHD processing was utilized in order to enhance the dissolution rate of poorly water-soluble drug by means of preparation of microparticle drug delivery systems, whereby the high surface area to volume ratio contribute to enhance the solubility of the incorporated active ingredient (Bohr *et al.*, 2011).

6.5 Solvent selection

The selection of appropriate solvent for the development of drug delivery systems was on the basis of the solubility of PMSQ, metformin HCL and glibenclamide in the selected solvent (Table 6.1). For this work, ethanol was used since both the polymer and model drugs were readily soluble in it. Moreover, ethanol had the required electrical conductivity and surface tension in a window that enables the cone-jet formation without the need to add extra salt or surfactants to the solutions. Ethanol boiling point is at 78^{o}C and it has high enough evaporation rate that resulted in formation of dry particles upon collection, it should be noted that the working distance was also optimized in order to achieve complete solvent evaporation without compromising on the production yield or premature solidification of the particles.

Table 6.1 Physiochemical properties of solvent used to prepare microparticles (Smallwood, 1996).

Property	EtOH
Viscosity (mPa s) (20 ^{o}C)	1.08
Boiling point (^{o}C)	78
Evaporation rate (BuAc=1)	1.4
Dielectric constant	22.4
Electrical conductivity (μS/cm)	0.014
Surface tension (mN/m)	22.3

6.6 Characterization of the prepared solutions

Measurements of the electrical conductivity showed that addition of 15% w/v PMSQ to the parent ethanol solution does not change much as PMSQ is an electrical insulator. However, with the addition of metformin HCL and glibenclamide onto the parent solution, the electrical conductivity increases significantly to the extent that the incorporation of metformin HCL was limited as the highly conductive solution could not be electrosprayed due to deficiency of achieving a stable cone-jet. For this reason, the content of the drug in the core solution was limited by an adequate drug concentration that would enable formation of a stable jet.

Table 6.2 Physicochemical properties of the solution used to prepare microcapsules containing model drugs.

Solution	Surface tension (mN/m)	Viscosity (mPa s)	Electrical conductivity (μS/cm)
Metformin HCL 10mg in 80ml EtOH	21.2 ± 0.3	0.92 ± 0.0	25.4 ± 0.3
Glibenclamide 20mg in 80 ml EtOH	21.4 ± 0.2	0.97 ± 0.0	32.3 ± 0.3
10mg Metformin HCL + 20mg Glibenclamide in 80ml EtOH	21.5 ± 0.2	0.93 ± 0.0	23.2 ± 0.4
PMSQ 15% w/v in EtOH	21.8 ± 0.3	1.36 ± 0.0	0.66 ± 0.0

Measurements of viscosity showed that the addition of metformin HCL or glibenclamide did not have a noticeable influence on the viscosity of the solutions when compared to that of inclusion of PMSQ. The greater effect of PMSQ on the viscosity is expected since it possesses much higher molecular weight. The surface tension values obtained for the solutions and the parent solvent seemed to be marginally equal. This is due to the inherent low surface tension of organic solvents and does not seem to be influenced by the addition of solutes used in this work.

6.7 Preparation of the unloaded drug delivery systems

Prior to incorporation of the model drugs, single needle EHD was used in order to prepare spherical microparticles with different concentration of PMSQ at 10% w/v and 15% w/v in EtOH to optimize the system (Figure 6.3 and Figure 6.4). The Experimental parameters were kept constant for this purpose, with set flow rate of 10 µl/min at an averaged applied voltage of 10.8 kV and an optimized working distance of 150 mm, in order to isolate the said factor.

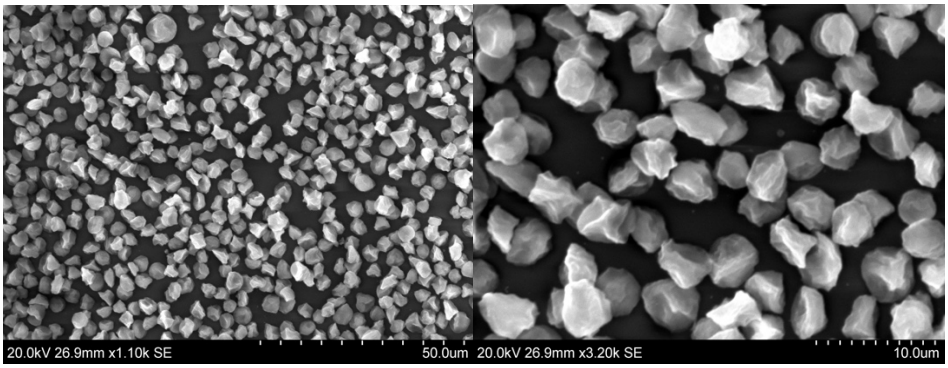


Figure 6.3 SEM images of electrosprayed microparticles with PMSQ 10% w/v in EtOH.

As it can be seen from the SEM images, an adequate concentration of 15% w/v resulted in optimum distribution of the polymeric shell as a smooth spherical

surface whereas the less content of the polymer led to formation of still mono dispersed particles but in a form of randomly shaped microparticles.

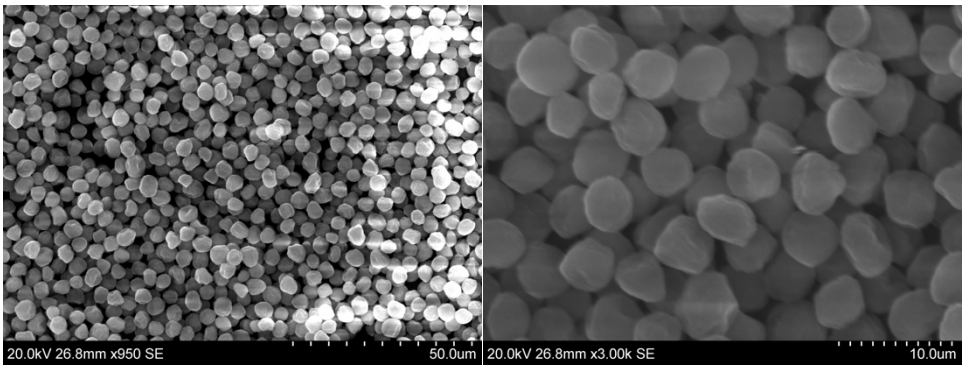


Figure 6.4 SEM images of electrosprayed microparticles with PMSQ 15% w/v in EtOH.

Upon optimization of the experimental parameters and solution properties, the co-axial EHD was utilized in order to prepare the microparticles with PMSQ 15% w/v in EtOH in the outer needle and EtOH in the inner needle.

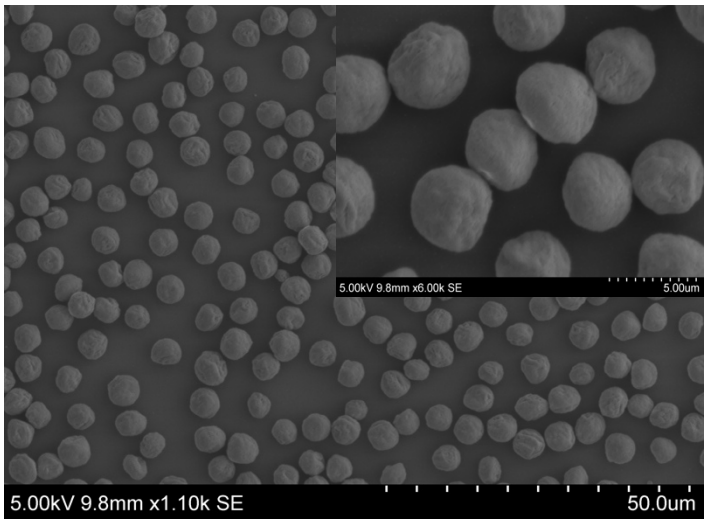


Figure 6.5 SEM images of co-axial electrosprayed microparticles with EtOH and PMSQ 15% w/v in EtOH in inner and outer needle respectively.

The flow rates were set at 5 μl/min and 10 μl/min for the inner and outer solutions, respectively. Smooth microcapsules with narrow size distribution were successfully made as it can be seen in Figure 6.5.

6.8 Preparation of the metformin HCL and glibenclamide loaded drug delivery systems

Electrical potential was applied throughout at fixed working distance of 150 mm. The prepared drug loaded polymeric systems are denoted as per description and are listed in Table 6.3.

Table 6.3 Formulation composition of metformin HCL and glibenclamide loaded particles.

Polymer System	Inner Solution	Outer Solution
S1	Metformin HCL 10mg/80ml EtOH	PMSQ 15% w/v in EtOH
S2	Glibenclamide 20mg/80ml EtOH	PMSQ 15% w/v in EtOH
S3	10mg Metformin HCL + 20mg Glibenclamide in 80ml EtOH	PMSQ 15% w/v in EtOH

The experimental parameters including the set flow rates and applied voltages (Table 6.4) was best selected as to achieve a stable cone-jet form given the physicochemical properties of the composition of the prepared systems. The mean particle size was lowest for S2 at 1.66 ± 0.74 μm, this may attributable to the highest electrical conductivity of the inner solution containing glibenclamide as well the highest averaged electrical potential. When compared to S3, with the same set flow rates, owing to a lower applied voltage and electrical conductivity of the inner solution, the average particle size is slightly higher at 4.79 ± 0.48 μm. Figure 6.6 shows the SEM images and the corresponding normal size distribution graphs of S1, S2 and S3. The same applies to S1, when compared to S2, it has lower electrical conductivity of the inner solution and slightly lower average applied electrical potential leading to slight increase in the average particle size at 3.44 ± 0.44 μm.

Table 6.4 Experimental parameter for drug delivery systems containing metformin HCL, glibenclamide, and combined metformin HCL and glibenclamide formulations.

Polymer System	Inner Flow Rate (µl/min)	Outer Flow Rate (µl/min)	Applied Voltage (kV)	Mean Particle Size (µm)
S1	2	8	11	3.44 ± 0.44
S2	6	18	12	1.66 ± 0.74
S3	6	18	11	4.79 ± 0.48

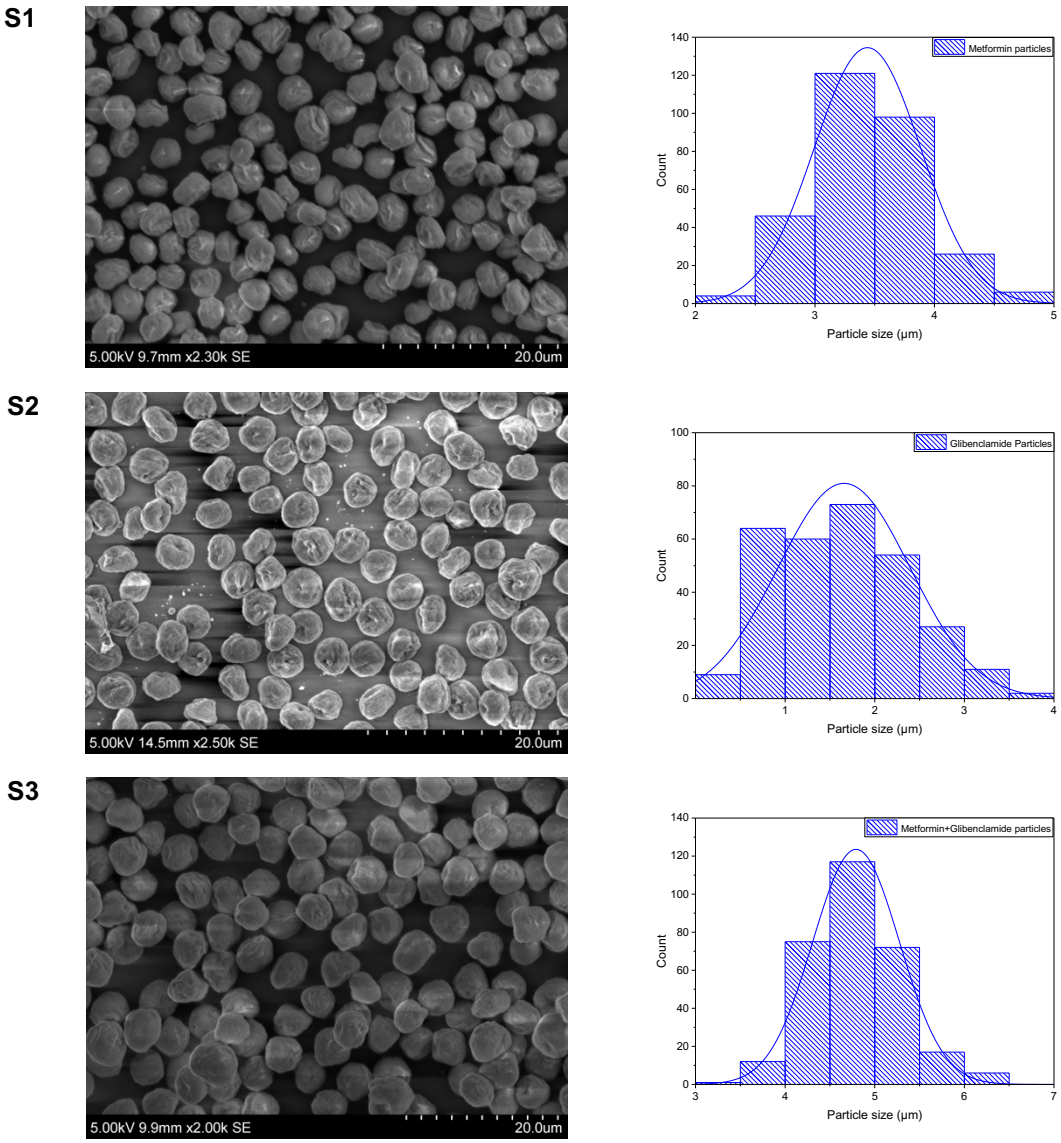


Figure 6.6 SEM images and the corresponding normal size distribution graphs of S1, S2 and S3.

The discrepancies in the set flow rates of S1 compared to that of S2 and S3 was due to formation of an unstable cone-jet that resulted in wide distribution size and inconsistency, therefore the selected values were chosen as to ensure narrow particle size distribution which is of high importance in development of drug delivery system.

6.9 XRD results

The XRD patterns of the unprocessed PMSQ, metformin HCL and glibenclamide as well as those of the prepared formulations were obtained (Figure 6.7). The sharp diffraction peaks that are representative of pure metformin HCL were notable at 2θ angles including 17^{o}, 22^{o}, 23^{o}, 25.27^{o} and 45^{o}. This series of sharp and intense diffraction peaks are indicative of the crystalline state of unprocessed metformin HCL. The diffraction spectrum of unprocessed glibenclamide also showed high peaks intensity in the region of 12^{o} to 34^{o} of 2θ, indicating its crystalline state (Elbahwy *et al.*, 2017). PMSQ showed the characteristic broad humps of amorphous materials.

As observed in the diffractograms acquired for the developed formulations, amorphous nature was most prominent. In all formulations, no characteristic peaks of metformin HCL and glibenclamide were detected. As revealed by the overlaid X-ray diffractograms of the formulations, the amorphous state of metformin and glibenclamide within the formulations can be proved. The polymorphic structure of drugs are important parameters that affect the dissolution rate and bioavailability of the drug.

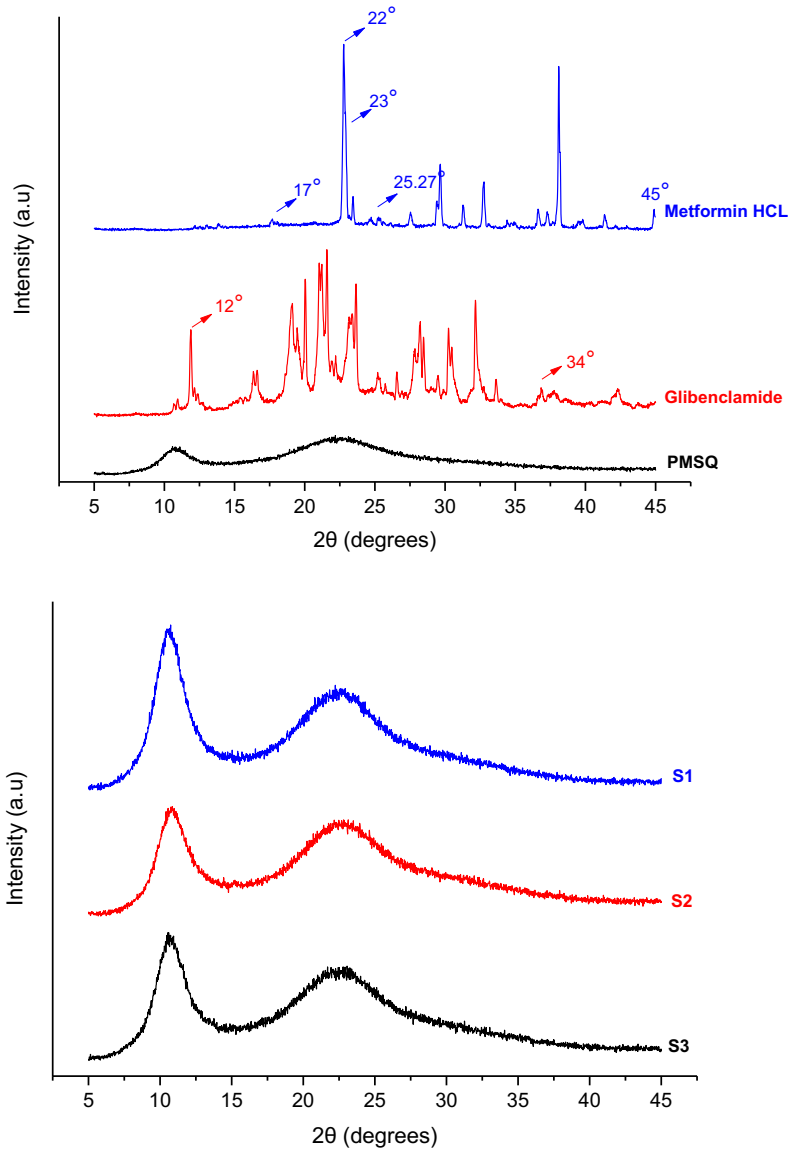


Figure 6.7 XRD spectrum of pure metformin HCL, glibenclamide, PMSQ and the developed formulations S1, S2 and S3.

6.10 FTIR results

Fourier transform infrared spectroscopy was utilized in order to characterise possible interactions between metformin HCL, glibenclamide and PMSQ in the solid state. FTIR spectra of the pure materials and the prepared formulations were obtained as shown in Figure 6.8. The characteristic band of PMSQ was observed at 1130 cm^{-1} (Chang *et al.*, 2010).

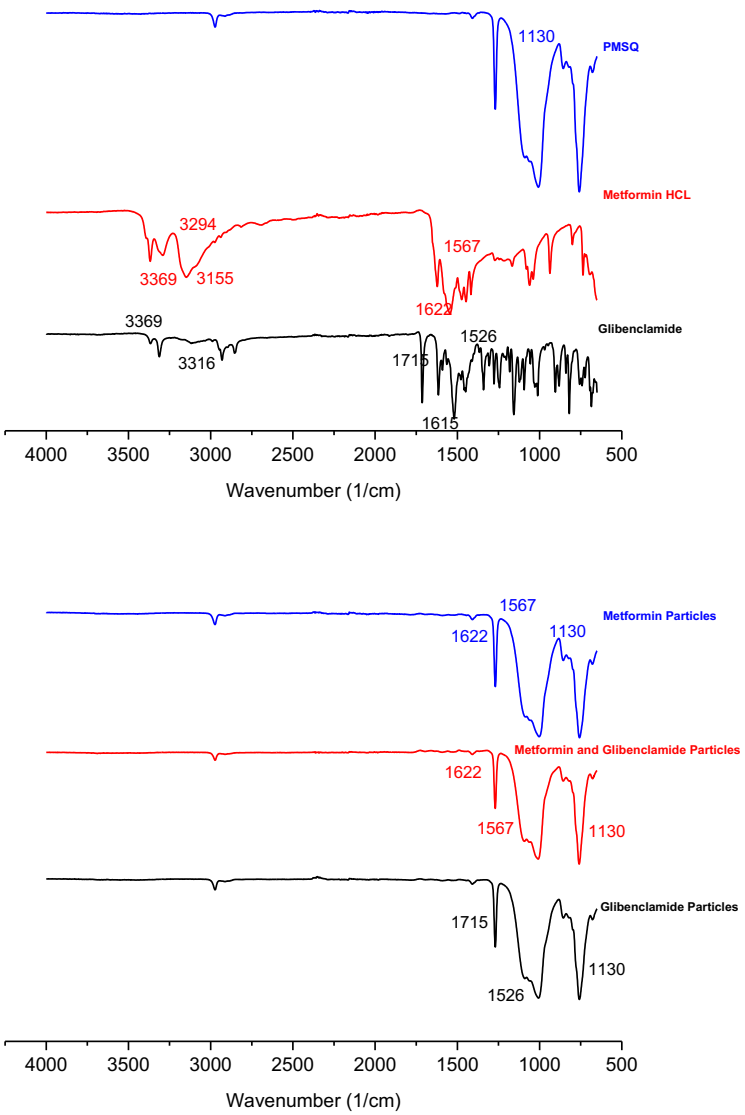


Figure 6.8 FTIR spectra of pure PMSQ, metformin HCL, glibenclamide and the developed formulations S1, S2 and S3.

The Spectrum of metformin HCL showed characteristic bands at 3369 cm^{-1} and 3294 cm^{-1}, relative to the N-H primary amine stretching vibration and a band at 3155 cm^{-1} owing to the N-H secondary amine stretching, also a characteristic band at 1622 cm^{-1} and 1567 cm^{-1} which are assigned to C-N stretching. The spectrum obtained for glibenclamide shoes the characteristic amide peaks at 3369 cm^{-1}, 3316 cm^{-1} and 1715 cm^{-1}. The urea carbonyl and N-H stretching vibrations were observed at 1615 cm^{-1} and 1526 cm^{-1}, accordingly. The

absorption bands between 2800 cm^{-1} and 3200 cm^{-1} were accredited to the aliphatic and aromatic C-H bond. The results obtained were in good agreement with the published spectrum for the pure drugs (Essa *et al.*, 2015). The FTIR spectra obtained for the developed formulations were an overlay of the acquired ones for the pure model drugs and polymer whereby the peaks have the highest intensity and bands corresponding to PMSQ.

6.11 Metformin HCL and glibenclamide release studies

The release profile of metformin HCL was studied from S1 formulation and S3 formulation for a duration of 12 hours and 168 hours respectively, which are shown in Figure 6.9. The burst release can be observed for the first 5 hours. This can be explained by diffusion of metformin HCL towards the surface of the polymeric shell as the solvent evaporation takes place. The slightly higher dissolution rate of metformin HCL from S3 at 39% can be due to slightly larger particle size of 4.79 ± 0.48 μm that incorporates more surface bound drug per surface area. The burst release of metformin HCL from S1 accounts for 26% of the total drug content. Moreover, the flow rate ratio of metformin HCL solution to PMSQ solution is higher for S3 at 1:3 when compared to that of S1 at 1:4, this may also attribute to the slight discrepancies of metformin HCL burst release from the developed formulations. The same applied for glibenclamide, as it can be seen from Figure 6.10, the initial burst release was slightly higher form S3 when compared to S2. The flow rates ratio was kept the same for both formulations. Therefore, the slightly higher dissolution rate of glibenclamide from S3 may be explained by more surface bound drug content in the developed microparticles. The initial burst release studies were also compared for that of glibenclamide and metformin HCL from S3 formulations to better understand the

effect of drug aqueous solubility. As it can be concluded from Figure 6.11, metformin HCL suffered from notably higher dissolution rate which may be attributable to higher water solubility and hence relatively faster diffusion from the polymeric particles and onto the release medium. This effect is expected for highly water-soluble drugs.

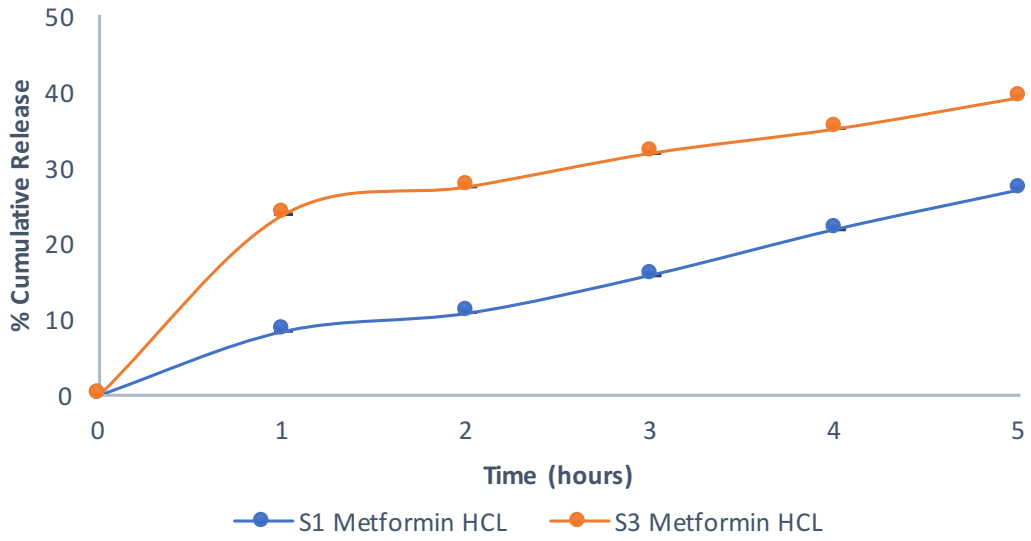


Figure 6.9 Metformin HCL percentage cumulative release from S1 and S3 formulations for the first 5 hours of dissolution studies.

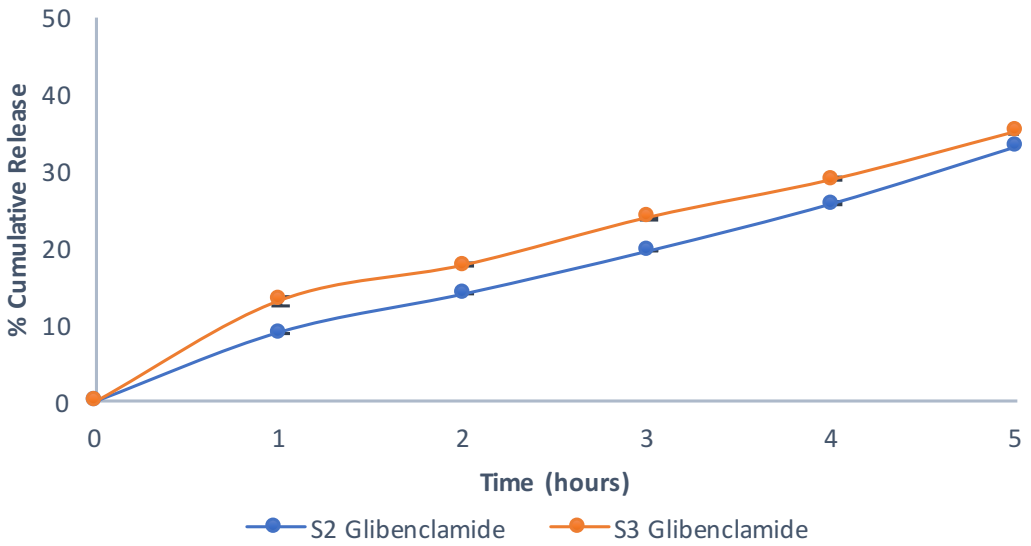


Figure 6.10 Glibenclamide percentage cumulative release from S2 and S3 formulations for the first 5 hours of dissolution studies.

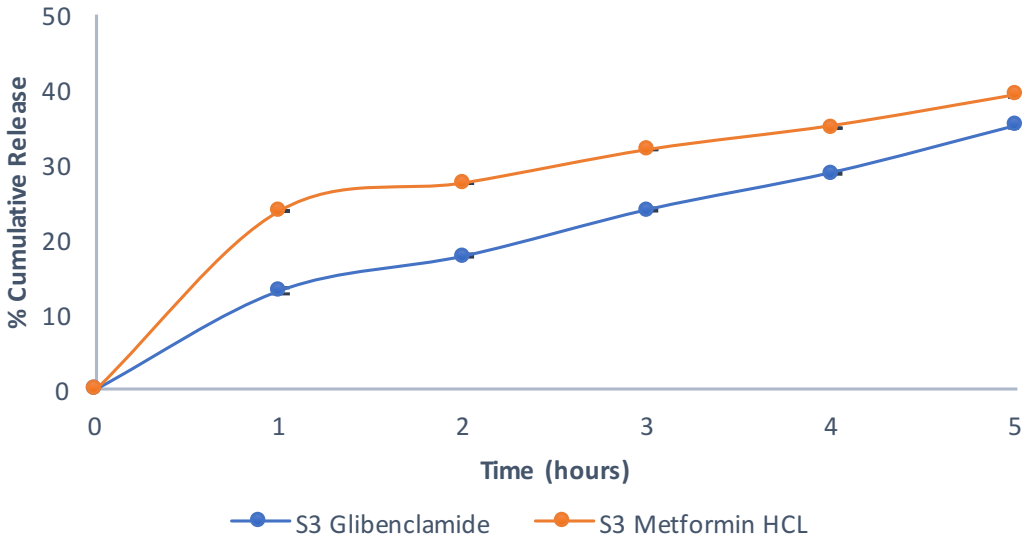


Figure 6.11 Glibenclamide and metformin HCL percentage cumulative release from S3 formulation for the first 5 hours of dissolution studies.

Passed the first 5 hours of burst release, metformin HCL and glibenclamide release from the combined S3 formulation nearly followed the same path (see Figure 6.12), resemblance of the first order release. Due to the hydrophobic nature of the PMSQ polymeric carrier, the drug release is mainly driven by diffusion against the concentration gradient. Whereby, higher drug content within the microparticles, diffuse through the polymer chains in order to reach an equilibrium. It is evident from the release profiles, that the frequency of sampling can also affect the dissolution rate. As, higher frequency causes more disturbance to the system and results in further agitation of the drug delivery systems, which in turn also increases the dissolution rate as the release medium is continuously refreshed after aliquots are taken. The dissolution rate decreases as there is a greater time interval in between each sampling. As it is shown in Figure 6.13, the glibenclamide release from S2 follows first order release after the initial bust release for the first 5 hours, at which point the frequency of sampling is decreased to be withdrawn on hourly basis.

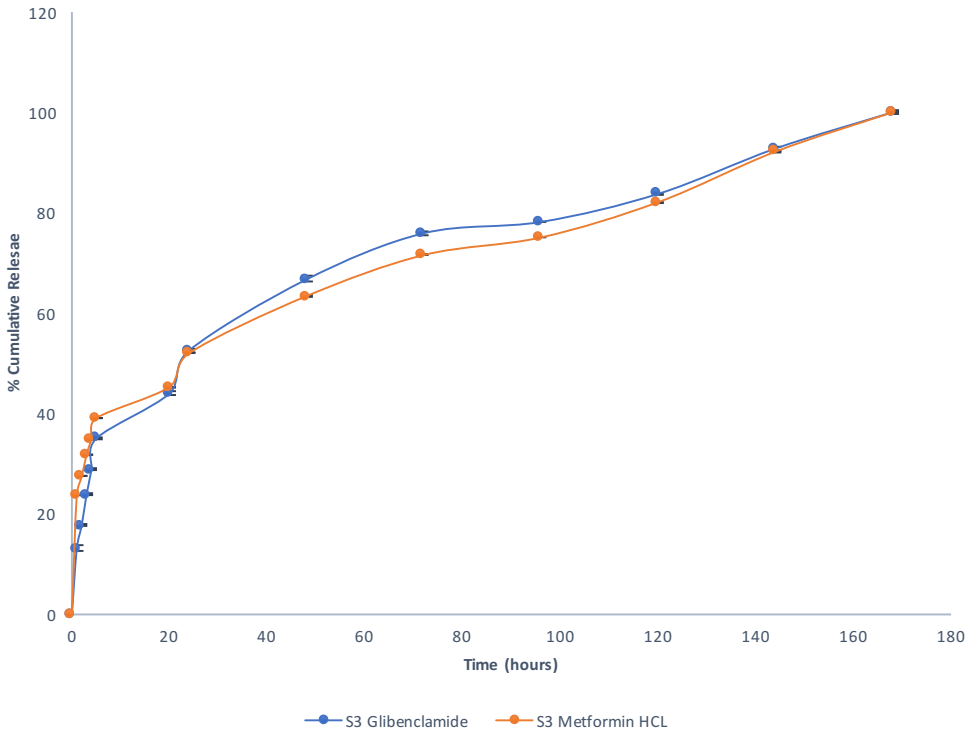


Figure 6.12 Glibenclamide and metformin HCL percentage cumulative release from S3 formulation.

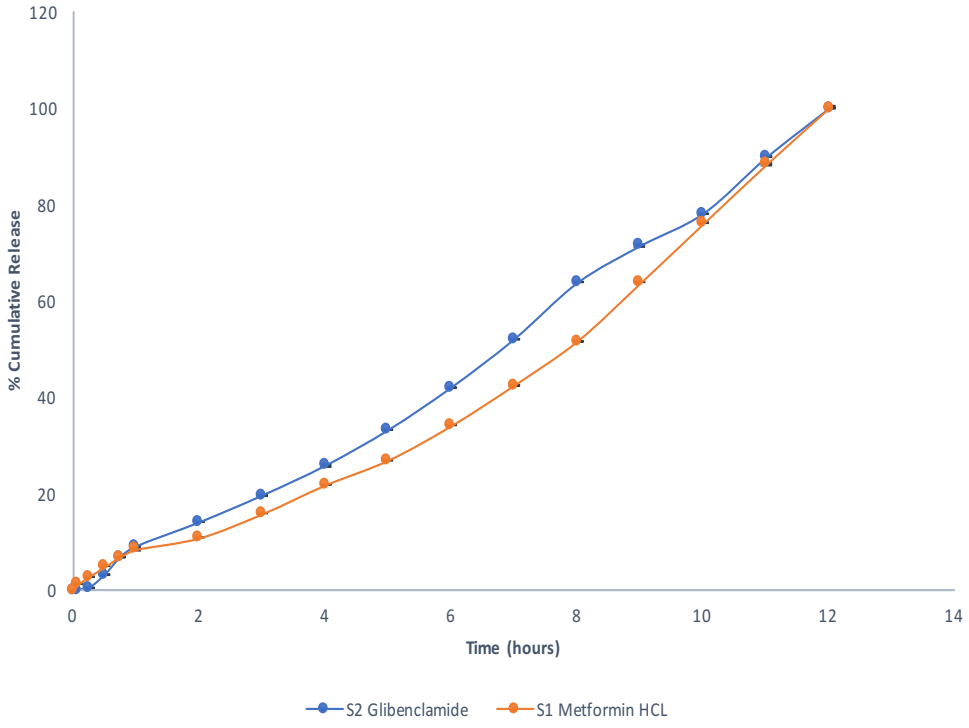


Figure 6.13 Glibenclamide and metformin HCL percentage cumulative release from S2 and S1. formulations.

The same was done for metformin HCL release studies from S1 formulation. Although glibenclamide is a poorly water-soluble drug, the dissolution rate is slightly higher when compared to metformin HCL, which may be accredited to smaller averaged particle size at 1.66 ± 0.74 μm. This occurs as there is higher surface area to volume ratio which results in higher dissolution rate of the incorporated drug and is a characteristic of microparticles that is highly favourable for encapsulation of drugs with low aqueous solubility that suffer from limited bioavailability, as there is a correlation between the dissolution rate and bioavailability of active ingredients. However, there is little difference between metformin HCL release and glibenclamide that suggests the hydrophilic nature of the incorporated drug has little effect on the dissolution rate. This is in agreement with previous studies that have reported that in general, electrospraying results in formation of microparticles that incorporate the active ingredient in their amorphous state hence the accelerated drug release profile is observed (Shams *et al.*, 2017).

6.12 Conclusions

In this work, successful preparation of sustained release drug delivery systems for simultaneous release of metformin HCL and glibenclamide was reported. This was achieved by processing them into electrosprayed microparticles using PMSQ as the polymeric carrier. Scanning electron microscopy showed that the prepared microparticles had spherical smooth surface with narrow size distribution and the averaged particle size of 1 μm to 5 μm. The X-ray diffraction study indicated that both incorporated drugs were fully converted to amorphous form in the microparticles. The FTIR studies indicated no destructive intermolecular interaction in between the metformin HCL, glibenclamide and PMSQ. The

dissolution studies showed fast dissolution rate of glibenclamide with poor water solubility due to reduced particle size and amorphous state of the drug in the developed formulations. Metformin HCL exhibited a sustained release which is of high interest in case of processing drugs that have short half-life and suffer from side-effects when formulated in high dosage forms. The developed system offers a high potential for preparation of depot formulations for long-term release of the active ingredients, that is an attractive proposition for treatment of chronic conditions including diabetes mellitus to increase patient compliance.

7 Conclusions and Future work

7.1 Conclusions

This research investigates the study of elecrohydrodynamic atomization as a single step processing platform for preparation of advanced drug delivery systems, whereby the flexibility and the simplicity of the process enables production of various drug delivery systems based on the application requirements. The adaptability of the processing parameters given the solution properties makes formulating drugs of different physiochemical properties possible, with fine tuning of the experimental parameters and careful selection of polymeric carrier and solvent system.

From Core-shell microencapsulation of indomethacin and paracetamol by co-axial electrohydrodynamic atomization

The co-axial electrohydrodynamic processing has proven to be an efficient one-step method of producing core-shell microparticles at ambient conditions with high processing yield. This technique overcomes the restrictions associated with conventional technique used for preparation of drug delivery systems whereby it eliminates the need for extreme temperatures, multi-step processing, or the use of additives. Unloaded polymeric drug delivery vehicles were developed and optimized in terms of average particle size, narrow size distribution and reproducibility. The key processing parameters such as flow rates, applied voltage and the working distance were optimized in order to achieve a stable cone-jet. It was reported that higher electrical conductivity of the inner solution is favourable to formation of a stable cone-jet, this also applies to higher viscosity of the outer solution. It was shown that with the combination of high electrical

potential and low flow rates particles of smaller size is achievable. It was also confirmed that for more viscous solutions larger particles are produced. Model drug with different water-solubility were successfully processed with high encapsulation efficiency. This provides a potential platform for preparation of fixed dose combination formulation to tackle the challenges related to polypharmacy in geriatric therapy.

Electrosprayed microparticles for targeted delivery of prednisolone

A pH-sensitive polymer, Eudragit L100-55, was used to prepare the smart drug delivery system for site specific release of prednisolone. Five different formulations incorporating different configuration of polymer and drug content were developed in order to examine the extent of drug release in the acidic environment. Efforts were made in order to lower the drug percentage release for the first 2 hours. Notable amounts of drug release occurred at pH 1.2 despite the polymers being insoluble under these conditions for F1, F3 and F5 formulations. Nonetheless, this effect was less pronounced for F2 and F4 formulations. Following the adjustment of release medium pH to 6.8 resembling the small intestine environmental pH, immediate release of prednisolone was observed. It was suggested that the low molecular weight of prednisolone compared to that of the polymer, allowed the drug to diffuse through the microparticles. The slower release in the early stages of release could be described by a diffusion process. Another aspect of application of EHD processing for development of advanced drug delivery systems was demonstrated. The scanning electron microscopy images of the microparticles showed that all the particles had toroid form. X-ray diffraction and differential scanning calorimetry studies showed that prednisolone was in an amorphous state in the prepared microparticles.

Co-encapsulation of metformin HCL and glibenclamide as oral drug delivery depots

Co-axial electrohydrodynamic processing was used for successful preparation of sustained release drug delivery systems for simultaneous release of metformin HCL and glibenclamide. The electrosprayed microparticles were prepared by using PMSQ as the polymeric carrier. Unloaded PMSQ microparticles were prepared and optimized in order to produce perfectly spherical microcapsules with narrow size distribution and the averaged particle size of 1 μm to 5 μm. The X-ray diffraction studies showed that both incorporated drugs were encapsulated in an amorphous state in the microparticles. The dissolution studies demonstrated fast dissolution rate of glibenclamide that suffers poor water solubility, this was due to reduced particle size and amorphous state of the drug in the developed formulations. Metformin HCL showed a sustained release which is highly desirable when processing drugs with short half-life and suffer from adverse side-effects when administered in high dosage forms. The developed drug system has the potential for preparation of depot formulations for long-term release of the active ingredients. This is especially valuable for treatment of chronic conditions including diabetes mellitus to increase patient compliance.

7.2 Future Work

Based on the studies conducted and the outcome of this work, several aspects of future work are suggested as follows:

Preparation of nanoparticles

Particles of submicron size range were prepared in this work as they suffice for preparation of oral dosage formulations. However, by fine tuning the experimental

parameter including the working distance and needle gauge, as well as solution parameter with careful selection of polymer and solvent system and the appropriate concentration of each, the production of nanoparticles is deemed possible. This is in particular attractive for development of drug delivery system for pulmonary delivery of active ingredient as well as targeting cancerous tissues.

Study the effect of needle geometry

Needle geometry is a crucial factor for electric field that is the driving force in electrohydrodynamic processing, in order to form and sustain the cone-jet mode. The needles used in this work possessed regular geometry. It has been reported in the literature that the needle geometry can affect the cone-jet domain, lacking further explanation on the impact phenomena and mechanism (Chen *et al.*, 1999). However, to the best of our knowledge, there has been no systematic investigation of how different factors including nozzle angle, tip length or shape may affect the formation of a stable cone jet.

Investigate the effect of the particle shape on drug delivery systems

In addition to particle size, there is a growing interest to further study the particles attributes including shape on the effectiveness of the developed drug delivery systems. As previously reported in the literature, particles of different shape can be prepared using electrohydrodynamic processing (Enayati *et al*., 2011a). However, there remains a gap for in depth understanding of how the shape of the prepared drug delivery system can influence the release profile, biocompatibility, biodistribution, clearance and desired applicability that would suit the goal of the intended drug delivery system.

Encapsulation of protein drugs

Protein drugs can be incorporated into the co-axial electrohydrodynamic processing to further study the feasibility of this technique for pharmaceutical applications while preserving the stability and bioactivity of the incorporated agents. Encapsulation of protein in the inner core of microparticles would lead to minimized distribution of the drug at the surface that is one of the leading factors to their inactivation and instability. By utilizing a suitable polymeric shell, drug stability and bioactivity can be prolonged, as the process enables control over the constituent distribution via use of different molecular weight polymers and concentration to further localise positioning of the active ingredient. Nonetheless, other factors including the molecular weight and net charge of the incorporated protein may also affect the overall displacement in prepared microparticles.

Preparation of solid dispersion formulations using water soluble polymers

The development of solid dispersion formulation has gained significant interest as a means of enhancing the dissolution rate and in turn the drug bioavailability. The term has been used to define a group of dosage forms whereby the drug is dispersed in an inert matrix (Craig, 2002). Solid dispersion formulation may be further developed using co-axial electrohydrodynamic processing whereby active ingredients of low aqueous solubility are incorporated into polymeric drug carriers with high water solubility. This was employed in this thesis at some extent but it was not the focus of the project. However, the anticipated future directions fall in parallel with preparation of solid dispersion formulations using water soluble polymers. This may be achieved by using natural polymer including alginate or collagen or hydrogels such as polyvinyl alcohol or cellulose.

Preparation of composite microspheres

By combining a pH-responsive polymer as the coating layer and inclusion of another polymer that results in sustained release of the incorporated agent, targeted delivery of drug is achievable while also acquiring a sustained release profile when the drug vehicle is at the desired site. This was partially implemented in this work; however, it would be interesting to take control over the release profile of the drug once the drug reaches the site of interest.

Particle preparation for diabetes treatment

Current therapy for diabetes mellitus is based on intravenous delivery of insulin. However, this therapy requires frequent administration and is associated with high possibility of infection, low patient compliance, low flexibility and needle phobia. The preparation of sustained release drug delivery system for extended release of insulin is highly desirable as diabetes mellitus is one of the most pressing global disease.

Preparation of fast dissolving formulations

Fast dissolving drug delivery systems have gained attention in the pharmaceutical industry (Illangakoon *et al.*, 2014). These formulations negate the need for water in order to be swallowed. These drug delivery systems dissolve or disintegrate very fast in the mouth and are of high interest for patients who find swallowing challenging and also in paediatric therapy. These formulations are advantageous as they enable fast onset of action and avoid undergoing the first pass metabolism. Moreover, they encompass convenient and non-invasive administration.

Preparation of fixed dose combination formulations for treatment of Parkinson's disease

Levodopa is the gold standard treatment for Parkinson disease. However, it suffers from short half-life and this leads to poor efficacy and fluctuating motor response in long term treatments. Levodopa combined with carbidopa formulations has shown promising results. When compared to the conventional immediate release formulations, the sustained delivery of these drugs is far more superior. Attempts have also been made to prepare dry powder inhalation formulations of levodopa (Luinstra *et al.*, 2015). Electrohydrodynamic atomization offers great potential for the development of combined formulation of levodopa for preparation of not only oral dosage units but also as dry powder for inhalation. As it has the ability to fine tune the end products size based on the desired application requirements.

Commercial viability and translation

The recent advancements in electrohydrodynamic processing have been briefly discussed in the literature. However, it is worth emphasizing the importance of translating this well-established technique with proven feasibility in numerous applications into commercial utilization. Attempts have been made to potentially scale up and increase the production rate, some of which include the use of multiplexed systems in single and co-axial configurations. Nonetheless, more investigation should be undertaken on the performance of the developed drug delivery systems using multiplexed EHD apparatus while comparing and contrasting with that of the regular set-up in order to ensure its clinical viability.

8 References

Adikwu, M. U., Yoshikawa, Y. and Takada, K. (2003) 'Bioadhesive delivery of metformin using prosopis gum with antidiabetic potential', *Biological and Pharmaceutical Bulletin*, 26(5), pp. 662–666. doi: 10.1248/bpb.26.662.

Ahmad, Z., Zhang, H. B., Farook, U., Edirisinghe, M., Stride, E. and Colombo, P. (2008) 'Generation of multilayered structures for biomedical applications using a novel tri-needle coaxial device and electrohydrodynamic flow', *Journal of The Royal Society Interface*. The Royal Society, 5(27), pp. 1255–1261. doi: 10.1098/rsif.2008.0247.

Alhnan, M. A., Cosi, D., Murdan, S. and Basit, A. W. (2010) 'Inhibiting the gastric burst release of drugs from enteric microparticles: The influence of drug molecular mass and solubility', *Journal of Pharmaceutical Sciences*, 99(11), pp. 4576–4583. doi: 10.1002/jps.22174.

Alhnan, M. A., Kidia, E. and Basit, A. W. (2011) 'Spray-drying enteric polymers from aqueous solutions: A novel, economic, and environmentally friendly approach to produce pH-responsive microparticles', *European Journal of Pharmaceutics and Biopharmaceutics*, 79(2), pp. 432–439. doi: 10.1016/j.ejpb.2011.03.015.

Alvarez-Lorenzo, C. and Concheiro, A. (2014) 'Smart drug delivery systems: from fundamentals to the clinic', *Chemical Communications*. The Royal Society of Chemistry, 50(58), pp. 7743–7765. doi: 10.1039/C4CC01429D.

Ammala, A. (2013) 'Biodegradable polymers as encapsulation materials for cosmetics and personal care markets', *International Journal of Cosmetic Science*, 35(2), pp. 113–124. doi: 10.1111/ics.12017.

Arshady, R. (1989) 'Microspheres and microcapsules: A survey of manufacturing

techniques. Part 1: Suspension cross-linking', *Polymer Engineering & Science*. Society of Plastics Engineers, 29(24), pp. 1746–1758. doi: 10.1002/pen.760292404.

Bando, H. and McGinity, J. W. (2006) 'Relationship between drug dissolution and leaching of plasticizer for pellets coated with an aqueous Eudragit® S100:L100 dispersion', *International Journal of Pharmaceutics*, 323(1), pp. 11–17. doi: https://doi.org/10.1016/j.ijpharm.2006.05.043.

Bangham, A. D., Standish, M. M. and Watkins, J. C. (1965) 'Diffusion of univalent ions across the lamellae of swollen phospholipids.', *Journal of molecular biology*. Academic Press Inc. (London) Ltd., 13(1), pp. 238–252. doi: 10.1016/S0022-2836(65)80093-6.

Barrero, A. and Loscertales, I. G. (2007) 'Micro- and nanoparticles via capillary flows', *Annual Review of Fluid Mechanics*, 39(1), pp. 89–106. doi: 10.1146/annurev.fluid.39.050905.110245.

Batycky, R. P., Hanes, J., Langer, R. and Edwards, D. A. (1997) 'A theoretical model of erosion and macromolecular drug release from biodegrading microspheres', *Journal of Pharmaceutical Sciences*, 86(12), pp. 1464–1477. doi: https://doi.org/10.1021/js9604117.

Beck-Broichsitter, M., Merkel, O. M. and Kissel, T. (2012) 'Controlled pulmonary drug and gene delivery using polymeric nano-carriers', *Journal of Controlled Release*, 161(2), pp. 214–224. doi: https://doi.org/10.1016/j.jconrel.2011.12.004.

Betageri, G. V. and Makarla, K. R. (1995) 'Enhancement of dissolution of glyburide by solid dispersion and lyophilization techniques', *International Journal of Pharmaceutics*, 126(1–2), pp. 155–160. doi: 10.1016/0378-5173(95)04114-1.

Boas, U. and Heegaard, P. M. H. (2004) 'Dendrimers in drug research', *Chemical Society Reviews*, 33, pp. 43–63.

Bohr, A., Kristensen, J., Stride, E., Dyas, M. and Edirisinghe, M. (2011) 'Preparation of microspheres containing low solubility drug compound by electrohydrodynamic spraying.', *International Journal of Pharmaceutics*, 412(1–2), pp. 59–67. doi: 10.1016/j.ijpharm.2011.04.005.

Bohr, A., Kristensen, J., Dyas, M., Edirisinghe, M. and Stride, E. (2012) 'Release profile and characteristics of electrosprayed particles for oral delivery of a practically insoluble drug', *Journal of The Royal Society Interface*, 9(75), pp. 2437–2449. doi: 10.1098/rsif.2012.0166.

Burke, P. A., Klumb, L. A., Herberger, J. D., Nguyen, X. C., Harrell, R. A. and Zordich, M. (2004) 'Poly(lactide-co-glycolide) microsphere formulations of darbepoetin alfa: Spray drying is an alternative to encapsulation by spray-freeze drying', *Pharmaceutical Research*, 21(3), pp. 500–506. doi: 10.1023/B:PHAM.0000019305.79599.a5.

Busatto, C., Pesoa, J., Helbling, I., Luna, J. and Estenoz, D. (2018) 'Effect of particle size, polydispersity and polymer degradation on progesterone release from PLGA microparticles: Experimental and mathematical modeling', *International Journal of Pharmaceutics*, 536(1), pp. 360–369. doi: 10.1016/j.ijpharm.2017.12.006.

Campieri, M., Ferguson, A., Doe, W., Persson, T. and Nilsson, L. G. (1997) 'Oral budesonide is as effective as oral prednisolone in active Crohn's disease. The global budesonide study group.', *Gut*, 41, pp. 209–214. doi: 10.1136/gut.41.2.209.

Cao, Y., Wang, B., Wang, Y. and Lou, D. (2014) 'Dual drug release from core-shell nanoparticles with distinct release profiles', *Journal of Pharmaceutical Sciences*, 103(10), pp. 3205–3216. doi: 10.1002/jps.24116.

Cetin, M. and Sahin, S. (2016) 'Microparticulate and nanoparticulate drug delivery systems for metformin hydrochloride', *Drug Delivery*, 23(8), pp. 2796–2805. doi: 10.3109/10717544.2015.1089957.

Chakraborty, S., Liao, I. C., Adler, A. and Leong, K. W. (2009) 'Electrohydrodynamics: A facile technique to fabricate drug delivery systems.', *Advanced Drug Delivery Reviews*, 61(12), pp. 1043–54. doi: 10.1016/j.addr.2009.07.013.

Chang, M. W., Stride, E. and Edirisinghe, M. (2010) 'Controlling the thickness of hollow polymeric microspheres prepared by electrohydrodynamic atomization.', *Journal of The Royal Society Interface*, 7, pp. S451–S460. doi: 10.1098/rsif.2010.0092.focus.

Chen, C. H., Emond, M. H. J., Kelder, E. M., Meester, B. and Schoonman, J. (1999) 'Electrostatic sol-spray deposition of nanostructured ceramic thin films', *Journal of Aerosol Science*, 30(7), pp. 959–967. doi: https://doi.org/10.1016/S0021-8502(98)00075-5.

Chen, X., Wen, H. and Park, K. (2010) 'Challenges and new technologies of oral controlled release', in Wen, H. and Park, K. (eds) *Oral Controlled Release Formulation Design and Drug Delivery*. John Wiley & Sons, pp. 257–277. doi: 10.1002/9780470640487.ch16.

Chow, A. H. L., Tong, H. H. Y., Chattopadhyay, P. and Shekunov, B. Y. (2007) 'Particle engineering for pulmonary drug delivery', *Pharmaceutical Research*, 24(3), pp. 411–437. doi: 10.1007/s11095-006-9174-3.

Chu, L. Y., Utada, A. S., Shah, R. K., Kim, J. W. and Weitz, D. A. (2007) 'Controllable monodisperse multiple emulsions', *Angewandte Chemie*, 119(47), pp. 9128–9132. doi: 10.1002/ange.200701358.

Clark, A. R. (1995) 'Medical aerosol inhalers: Past, present, and future', *Aerosol Science and Technology*, 22(4), pp. 374–391. doi: 10.1080/02786829408959755.

Cloupeau, M. and Prunet-Foch, B. (1989) 'Electrostatic spraying of liquids in cone-jet mode', *Journal of Electrostatics*, 22(2), pp. 135–159. doi: 10.1016/0304-3886(89)90081-8.

Cloupeau, M. and Prunet-Foch, B. (1990) ‘Electrostatic spraying of liquids: Main functioning modes’, *Journal of Electrostatics*, 25(2), pp. 165–184. doi: https://doi.org/10.1016/0304-3886(90)90025-Q.

Cloupeau, M. and Prunet-Foch, B. (1994) ‘Electrohydrodynamic spraying functioning modes: A critical review’, *Journal of Aerosol Science*, 25(6), pp. 1021–1036. doi: https://doi.org/10.1016/0021-8502(94)90199-6.

Cohen, S., Yoshioka, T., Lucarelli, M., Hwang, L. H. and Langer, R. (1991) ‘Controlled delivery systems for proteins based on poly(lactic/glycolic acid) microspheres’, *Pharmaceutical Research*, pp. 713–720. doi: 10.1023/A:1015841715384.

Collins, G., Federici, J., Imura, Y. and Catalani, L. H. (2012) ‘Charge generation, charge transport, and residual charge in the electrospinning of polymers: A review of issues and complications’, *Journal of Applied Physics*, 111(4), p. 044701. doi: 10.1063/1.3682464.

Colombo, P. (2008) ‘Engineering porosity in polymer-derived ceramics’, *Journal of the European Ceramic Society*, 28(7), pp. 1389–1395. doi: https://doi.org/10.1016/j.jeurceramsoc.2007.12.002.

Corti, G., Cirri, M., Maestrelli, F., Mennini, N. and Mura, P. (2008) ‘Sustained-release matrix tablets of metformin hydrochloride in combination with triacetyl-β-cyclodextrin’, *European Journal of Pharmaceutics and Biopharmaceutics*, 68(2), pp. 303–309. doi: https://doi.org/10.1016/j.ejpb.2007.06.004.

Craig, D. Q. M. (2002) ‘The mechanisms of drug release from solid dispersions in water-soluble polymers’, *International Journal of Pharmaceutics*, 231(2), pp. 131–144. doi: 10.1016/S0378-5173(01)00891-2.

Cramer, J. A. (2004) ‘A systematic review of adherence with medications for diabetes’, *Diabetes Care*, 27(5), pp. 1218–1224. doi: 10.2337/diacare.27.5.1218.

DeFronzo, R. A. (1992) ‘Pathogenesis of type 2 (non-insulin dependent) diabetes mellitus: A balanced overview’, *Diabetologia*, 35(4), pp. 389–397. doi: 10.1007/BF00401208.

Desai, D., Wang, J., Wen, H., Li, X. and Timmins, P. (2012) ‘Formulation design, challenges, and development considerations for fixed dose combination (FDC) of oral solid dosage forms’, *Pharmaceutical Development and Technology*, 18(6), pp. 1265–1276. doi: 10.3109/10837450.2012.660699.

Ding, L., Lee, T. and Wang, C. H. (2005) ‘Fabrication of monodispersed taxol-loaded particles using electrohydrodynamic atomization’, *Journal of Controlled Release*, 102(2), pp. 395–413. doi: https://doi.org/10.1016/j.jconrel.2004.10.011.

Dubois, J. L. and Ford, J. L. (1985) ‘Similarities in the release rates of different drugs from polyethylene glycol 6000 solid dispersions’, *The Journal of Pharmacy and Pharmacology*, 37(7), pp. 494–495. doi: 10.1111/j.2042-7158.1985.tb03048.x.

Elbahwy, I. A., Ibrahim, H. M., Ismael, H. R. and Kasem, A. A. (2017) ‘Enhancing bioavailability and controlling the release of glibenclamide from optimized solid lipid nanoparticles’, *Journal of Drug Delivery Science and Technology*, 38, pp. 78–89. doi: https://doi.org/10.1016/j.jddst.2017.02.001.

Eltayeb, M., Stride, E. and Edirisinghe, M. (2013) ‘Electrosprayed core-shell polymer-lipid nanoparticles for active component delivery’, *Nanotechnology*, 24(46), p. 465604. doi: 10.1088/0957-4484/24/46/465604.

Enayati, M., Ahmad, Z., Stride, E. and Edirisinghe, M. (2010) ‘One-step electrohydrodynamic production of drug-loaded micro- and nanoparticles’, *Journal of The Royal Society Interface*, 7(45), pp. 667–675. doi: 10.1098/rsif.2009.0348.

Enayati, M., Chang, M. W., Bragman, F., Edirisinghe, M. and Stride, E. (2011a) ‘Electrohydrodynamic preparation of particles, capsules and bubbles for

biomedical engineering applications', *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 382(1), pp. 154–164. doi: https://doi.org/10.1016/j.colsurfa.2010.11.038.

Enayati, M., Farook, U., Edirisinghe, M. and Stride, E. (2011b) 'Electrohydrodynamic preparation of polymeric drug-carrier particles: Mapping of the process', *International Journal of Pharmaceutics*, 404(1–2), pp. 110–115. doi: 10.1016/j.ijpharm.2010.11.014.

Endo, H., Watanabe, Y., Matsumoto, M. and Shirotake, S. (2000) 'Preparation and evaluation of heat-sensitive melting gel acetaminophen gel', *Japanese Journal of Hospital Pharmacy*, 26, pp. 250–258.

Essa, E. A., Elkotb, F. E., Zin Eldin, E. E. and El Maghraby, G. M. (2015) 'Development and evaluation of glibenclamide floating tablet with optimum release', *Journal of Drug Delivery Science and Technology*, 27, pp. 28–36. doi: 10.1016/j.jddst.2015.04.002.

Fahr, A. and Liu, X. (2007) 'Drug delivery strategies for poorly water-soluble drugs', *Expert Opinion on Drug Delivery*, 4(4), pp. 403–416. doi: 10.1517/17425247.4.4.403.

Fernández Tena, A. and Casan Clarà, P. (2012) 'Deposition of inhaled particles in the lungs', *Archivos de Bronconeumología*, 48(7), pp. 240–246.

Fery, A., Dubreuil, F. and Möhwald, H. (2004) 'Mechanics of artificial microcapsules', *New Journal of Physics*, 6, pp. 1–13.

Filho, D. C. M., Ramalho, J. B. V. S., Spinelli, L. S. and Lucas, E. F. (2012) 'Aging of water-in-crude oil emulsions: Effect on water content, droplet size distribution, dynamic viscosity and stability', *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 396, pp. 208–212. doi: 10.1016/j.colsurfa.2011.12.076.

Folkman, J. and Long, D. M. (1964) ‘The use of silicone rubber as a carrier for prolonged drug therapy’, *The Journal of Surgical Research*, 4(3), pp. 139–142. doi: 10.1016/S0022-4804(64)80040-8.

Foss, A. C., Goto, T., Morishita, M. and Peppas, N. A. (2004) ‘Development of acrylic-based copolymers for oral insulin delivery’, *European Journal of Pharmaceutics and Biopharmaceutics*, 57(2), pp. 163–169. doi: https://doi.org/10.1016/S0939-6411(03)00145-0.

Gañán-Calvo, A. M. (1997) ‘On the theory of electrohydrodynamically driven capillary jets’, *Journal of Fluid Mechanics*, 335, pp. 165–188. doi: 10.1017/S0022112096004466.

Gañán-Calvo, A. M., Dávila, J. and Barrero, A. (1997) ‘Current and droplet size in the electrospraying of liquids. Scaling laws’, *Journal of Aerosol Science*, 28(2), pp. 249–275. doi: 10.1016/S0021-8502(96)00433-8.

Gañan-Calvo, A. M., Pantano, C. and Barrero, A. (1996) ‘The equilibrium shapes of liquid menisci emitting liquid and charges in steady cone-jet mode’, *Journal of Aerosol Science*, 27, pp. 187–188.

Gao, Z. G., Fain, H. D. and Rapoport, N. (2005) ‘Controlled and targeted tumor chemotherapy by micellar-encapsulated drug and ultrasound’, *Journal of Controlled Release*, 102(1), pp. 203–222. doi: https://doi.org/10.1016/j.jconrel.2004.09.021.

Garbuzenko, O. B., Mainelis, G., Taratula, O. and Minko, T. (2014) ‘Inhalation treatment of lung cancer: the influence of composition, size and shape of nanocarriers on their lung accumulation and retention’, *Cancer Biology & Medicine*, 11(1), pp. 44–55. doi: 10.7497/j.issn.2095-3941.2014.01.004.

Gautam, C. S. and Saha, L. (2008) ‘Fixed dose drug combinations (FDCs): rational or irrational: a view point’, *British Journal of Clinical Pharmacology*, 65(5), pp. 795–796. doi: 10.1111/j.1365-2125.2007.03089.x.

Gijsen, R., Hoeymans, N., Schellevis, F. G., Ruwaard, D., Satariano, W. A. and Van Den Bos, G. A. M. (2001) 'Causes and consequences of comorbidity : A review', *Journal of Clinical Epidemiology*, 54, pp. 661–674.

Goldberg, T. and Wong, E. (2015) 'Afrezza (insulin human) inhalation powder: A new inhaled insulin for the management of type-1 or type-2 diabetes mellitus', *Pharmacy and Therapeutics*, 40(11), pp. 735–741.

Gomez, A. and Tang, K. (1994) 'Charge and fission of droplets in electrostatic sprays', *Physics of Fluids*, 6(1), pp. 404–414. doi: 10.1063/1.868037.

Göpferich, A. (1996) 'Mechanisms of polymer degradation and erosion', *Biomaterials*, 17(2), pp. 103–114. doi: 10.1016/0142-9612(96)85755-3.

Gouin, S. (2004) 'Microencapsulation: industrial appraisal of existing technologies and trends', *Trends in Food Science & Technology*, 15(7), pp. 330–347. doi: https://doi.org/10.1016/j.tifs.2003.10.005.

Granberg, R. A. and Rasmuson, Å. C. (1999) 'Solubility of paracetamol in pure solvents', *Journal of Chemical and Engineering Data*, 44(6), pp. 1391–1395. doi: 10.1021/je990124v.

Gregoriadis, G. (1981) 'Targeting of drugs: Implications in medicine', *The Lancet*, 318(8240), pp. 241–247.

Guan, J., Han, J., Zhang, D., Chu, C., Liu, H., Sun, J., He, Z. and Zhang, T. (2014) 'Increased dissolution rate and oral bioavailability of hydrophobic drug glyburide tablets produced using supercritical CO2 silica dispersion technology', *European Journal of Pharmaceutics and Biopharmaceutics*, 86(3), pp. 376–382. doi: https://doi.org/10.1016/j.ejpb.2013.10.008.

Gunduz, O., Ahmad, Z., Stride, E. and Edirisinghe, M. (2012) 'A device for the fabrication of multifunctional particles from microbubble suspensions', *Materials*

Science & Engineering: C, 32(4), pp. 1005–1010. doi: 10.1016/j.msec.2012.01.018.

Gupta, P., Vermani, K. and Garg, S. (2002) 'Hydrogels: from controlled release to pH-responsive drug delivery', *Drug Discovery Today*, 7(10), pp. 569–579. doi: https://doi.org/10.1016/S1359-6446(02)02255-9.

Gursoy, R. N. and Benita, S. (2004) 'Self-emulsifying drug delivery systems (SEDDS) for improved oral delivery of lipophilic drugs', *Biomedicine & Pharmacotherapy*, 58(3), pp. 173–182. doi: https://doi.org/10.1016/j.biopha.2004.02.001.

Hao, S., Wang, B., Wang, Y. and Xu, Y. (2014) 'Enteric-coated sustained-release nanoparticles by coaxial electrospray: Preparation, characterization, and in vitro evaluation', *Journal of Nanoparticle Research*, 16(2204). doi: 10.1007/s11051-013-2204-2.

Hartman, R. P. A., Borra, J. P., Brunner, D. J., Marijnissen, J. C. M. and Scarlett, B. (1999) 'The evolution of electrohydrodynamic sprays produced in the cone-jet mode, a physical model', *Journal of Electrostatics*, 47(3), pp. 143–170. doi: 10.1016/S0304-3886(99)00034-0.

Hartman, R. P. A., Brunner, D. J., Camelot, D. M. A., Marijnissen, J. C. M. and Scarlett, B. (2000) 'Jet break-up in electrohydrodynamic atomization in the cone-jet mode', *Journal of Aerosol Science*, 31(1), pp. 65–95. doi: 10.1016/S0021-8502(99)00034-8.

Heller, J., Pangburn, S. H. and Penhale, D. W. H. (1987) 'Use of bioerodible polymers in self-regulated drug delivery systems', in *Controlled-Release Technology*, pp. 172–187. doi: 10.1021/bk-1987-0348.ch013.

Hombreiro Pérez, M., Zinutti, C., Lamprecht, A., Ubrich, N., Astier, A., Hoffman, M., Bodmeier, R. and Maincent, P. (2000) 'The preparation and evaluation of poly(ϵ-caprolactone) microparticles containing both a lipophilic and a hydrophilic

drug', *Journal of Controlled Release*, 65(3), pp. 429–438. doi: 10.1016/S0168-3659(99)00253-9.

Hong, K. H., Woo, S. H. and Kang, T. J. (2012) 'In vitro degradation and drug-release behavior of electrospun, fibrous webs of poly(lactic-co-glycolic acid)', *Journal of Applied Polymer Science*, 124(1), pp. 209–214.

Huang, X. and Brazel, C. S. (2001) 'On the importance and mechanisms of burst release in matrix-controlled drug delivery systems', *Journal of Controlled Release*, 73(2–3), pp. 121–136. doi: 10.1016/S0168-3659(01)00248-6.

Hussein, A. S., Abdullah, N. and Ahmadun, F. R. (2013) 'In vitro degradation of poly (D, L-lactide-co-glycolide) nanoparticles loaded with linamarin', *IET Nanobiotechnology*, 7(2), pp. 33–41.

Huynh, C. T. and Lee, D. (2014) 'Controlled Release', in *Encyclopedia of Polymeric Nanomaterials*. Springer-Verlag Berlin Hiedelberg, pp. 1–12. doi: 10.1007/978-3-642-36199-9.

Ijsebaert, J. C., Geerse, K. B., Marijnissen, J. C. M., Lammers, J. W. J. and Zanen, P. (2001) 'Electro-hydrodynamic atomization of drug solutions for inhalation purposes', *Journal of Applied Physiology*, 91(6), pp. 2735–2741. doi: 10.1152/jappl.2001.91.6.2735.

Illangakoon, U. E., Gill, H., Shearman, G. C., Parhizkar, M., Mahalingam, S., Chatterton, N. P. and Williams, G. R. (2014) 'Fast dissolving paracetamol/caffeine nano fibers prepared by electrospinning', *International Journal of Pharmaceutics*, 477(1–2), pp. 369–379. doi: 10.1016/j.ijpharm.2014.10.036.

Jafari, S. M., He, Y. and Bhandari, B. (2006) 'Nano-emulsion production by sonication and microfluidization - A comparison', *International Journal of Food Properties*, 9(3), pp. 475–485. doi: 10.1080/10942910600596464.

Jain, A. K., Sood, V., Bora, M., Vasita, R. and Katti, D. S. (2014) 'Electrosprayed inulin microparticles for microbiota triggered targeting of colon', *Carbohydrate Polymers*, 112, pp. 225–234. doi: https://doi.org/10.1016/j.carbpol.2014.05.087.

Jain, R. A. (2000) 'The manufacturing techniques of various drug loaded biodegradable poly(lactide-co-glycolide) (PLGA) devices', *Biomaterials*, 21(23), pp. 2475–2490. doi: 10.1016/S0142-9612(00)00115-0.

Jalil, R. and Nixon, J. R. (1990) 'Biodegradable poly(lactic acid) and poly(lactide-co-glycolide) microcapsules: problems associated with preparative techniques and release properties', *Journal of Microencapsulation*, 7(3), pp. 297–325. doi: 10.3109/02652049009021842.

Jalili, R., Hosseini, S. A. and Morshed, M. (2005) 'The effects of operating parameters on the morphology of electrospun polyacrilonitrile nanofibres', *Iranian Polymer Journal*, 14(12), pp. 1074–1081.

Jaworek, A. and Krupa, A. (1999) 'Classification of the modes of EHD spraying', *Journal of Aerosol Science*, 30(7), pp. 873–893. doi: 10.1016/S0021-8502(98)00787-3.

Jaworek, A. and Sobczyk, A. T. (2008) 'Electrospraying route to nanotechnology: An overview', *Journal of Electrostatics*, 66(3), pp. 197–219. doi: https://doi.org/10.1016/j.elstat.2007.10.001.

Jayasinghe, S. N. and Edirisinghe, M. J. (2002) 'Effect of viscosity on the size of relics produced by electrostatic atomization', *Journal of Aerosol Science*, 33(10), pp. 1379–1388. doi: 10.1016/S0021-8502(02)00088-5.

Jing, Y., Zhu, Y., Yang, X., Shen, J. and Li, C. (2011) 'Ultrasound-triggered smart drug release from multifunctional core−shell capsules one-step fabricated by coaxial electrospray method', *Langmuir*, 27(3), pp. 1175–1180. doi: 10.1021/la1042734.

Joshi, M. (2013) 'Role of eudragit in targeted drug delivery', *International Journal of Current Pharmaceutical Research*, 5(2), pp. 3–7.

Kadajji, V. G. and Betageri, G. V (2011) 'Water soluble polymers for pharmaceutical applications', *Polymers*, 3, pp. 1972–2009. doi: 10.3390/polym3041972.

Kaneko, Y., Nakamura, S., Sakai, K., Kikuchi, A., Aoyagi, T., Sakurai, Y. and Okano, T. (1999) 'Synthesis and swelling-deswelling kinetics of poly(N-isopropylacrylamide) hydrogels grafted with LCST modulated polymers', *Journal of Biomaterials Science, Polymer Edition*, 10(11), pp. 1079–1091. doi: 10.1163/156856299X00757.

Kaparissides, C., Alexandridou, C., Kotti, K. and Chaitidou, S. (2006) 'Recent advances in novel drug delivery systems', *Azonano Nanotechnology Online*, 2, pp. 1–11.

Kawashima, Y., Serigano, T., Hino, T., Yamamoto, H. and Takeuchi, H. (1998) 'A new powder design method to improve inhalation efficiency of pranlukast hydrate dry powder aerosols by surface modification with hydroxypropylmethylcellulose phthalate nanospheres', *Pharmaceutical Research*, 15(11), pp. 1748–1752. doi: 10.1023/A:1011916930655.

Kendall, R. A., Alhnan, M. A., Nilkumhang, S., Murdan, S. and Basit, A. W. (2009) 'Fabrication and in vivo evaluation of highly pH-responsive acrylic microparticles for targeted gastrointestinal delivery', *European Journal of Pharmaceutical Sciences*, 37(3–4), pp. 284–290. doi: 10.1016/j.ejps.2009.02.015.

Kikuchi, A. and Okano, T. (2002) 'Pulsatile drug release control using hydrogels', *Advanced Drug Delivery Reviews*, 54(1), pp. 53–77. doi: https://doi.org/10.1016/S0169-409X(01)00243-5.

Kim, S., Kim, J. H., Jeon, O., Kwon, I. C. and Park, K. (2009) 'Engineered polymers for advanced drug delivery', *European Journal of Pharmaceutics and*

Biopharmaceutics, 71(3), pp. 420–430. doi: 10.1016/j.ejpb.2008.09.021.

Klouda, L. and Mikos, A. G. (2008) 'Thermoresponsive hydrogels in biomedical applications', *European Journal of Pharmaceutics and Biopharmaceutics*, 68(1), pp. 34–45. doi: https://doi.org/10.1016/j.ejpb.2007.02.025.

Knop, K., Hoogenboom, R., Fischer, D. and Schubert, U. S. (2010) 'Poly(ethylene glycol) in drug delivery: Pros and cons as well as potential alternatives', *Angewandte Chemie - International Edition*, 49(36), pp. 6288–6308. doi: 10.1002/anie.200902672.

Kohane, D. S. (2007) 'Microparticles and nanoparticles for drug delivery', *Biotechnology and Bioengineering*, 96(2), pp. 203–209. doi: 10.1002/bit.21301.

Kost, J., Leong, K. and Langer, R. (1989) 'Ultrasound-enhanced polymer degradation and release of incorporated substances', *Proceedings of the National Academy of Sciences of the United States of America*, 86(20), pp. 7663–7666.

Kreuter, J. (2001) 'Nanoparticulate systems for brain delivery of drugs', *Advanced Drug Delivery Reviews*, 47(1), pp. 65–81. doi: 10.1016/S0169-409X(00)00122-8.

Ku, B. K. and Kim, S. S. (2002) 'Electrospray characteristics of highly viscous liquids', *Journal of Aerosol Science*, 33(10), pp. 1361–1378. doi: https://doi.org/10.1016/S0021-8502(02)00075-7.

Kubo, W., Miyazaki, S. and Attwood, D. (2003) 'Oral sustained delivery of paracetamol from in situ-gelling gellan and sodium alginate formulations', *International Journal of Pharmaceutics*, 258(1–2), pp. 55–64. doi: 10.1016/S0378-5173(03)00163-7.

Labbaf, S., Ghanbar, H., Stride, E. and Edirisinghe, M. (2014) 'Preparation of multilayered polymeric structures using a novel four-needle coaxial electrohydrodynamic device', *Macromolecular Rapid Communications*, 35, pp.

618–623. doi: 10.1002/marc.201300777.

Langer, R. (1990) ‘New methods of drug delivery’, *Science*, 249(4976), pp. 1527–1533. doi: 10.1126/science.2218494.

Langer, R. and Folkman, J. (1976) ‘Polymers for the sustained release of proteins and other macromolecules.’, *Nature*, 263(5580), pp. 797–800. doi: 10.1038/263797a0.

Lau, E. T. L., Johnson, S. K., Williams, B. A., Mikkelsen, D., McCourt, E., Stanley, R. A., Mereddy, R., Halley, P. J. and Steadman, K. J. (2017) ‘Optimizing prednisolone loading into distiller’s dried grain kafirin microparticles, and in vitro release for oral delivery’, *Pharmaceutics*, 9(4), p. 17. doi: 10.3390/pharmaceutics9020017.

Lee, C. C., MacKay, J. A., Frechet, M. J. and Szoka, F. C. (2005) ‘Designing dendrimers for biological applications’, *Nature Biotechnology*, 23(12), pp. 1517–1526.

Leong, K. W. and Langer, R. (1988) ‘Polymeric controlled drug delivery’, *Advanced Drug Delivery Reviews*, 1(3), pp. 199–233. doi: 10.1016/0169-409X(88)90019-1.

Li, S. and Vert, M. (1999) ‘Biodegradable polymers : polyesters’, in Mathiowitz, E. (ed.) *Encyclopedia of Controlled Drug Delivery*. John Wiley & Sons, pp. 71–93.

Lin, H. Y. and Thomas, J. L. (2004) ‘Factors affecting responsivity of unilamellar liposomes to 20 kHz ultrasound’, *Langmuir*, 20(15), pp. 6100–6106. doi: 10.1021/la049866z.

Lin, S. and Yu, H. (1999) ‘Thermal stability of methacrylic acid copolymers of Eudragits L, S, and L30D and the acrylic acid polymer of carbopol’, *Journal of Polymer Science Part A: Polymer Chemistry*, 37(13), pp. 2061–2067.

Liu, Y., Miyoshi, H. and Nakamura, M. (2006) 'Encapsulated ultrasound microbubbles: therapeutic application in drug/gene delivery', *Journal of Controlled Release*, 114(1), pp. 89–99. doi: 10.1016/j.jconrel.2006.05.018.

López-Herrera, J. M., Barrero, A., López, A., Loscertales, I. G. and Márquez, M. (2003) 'Coaxial jets generated from electrified Taylor cones. Scaling laws', *Journal of Aerosol Science*, 34(5), pp. 535–552. doi: 10.1016/S0021-8502(03)00021-1.

Loscertales, I. G., Barrero, A., Guerrero, I., Cortijo, R., Marquez, M. and Gañán-Calvo, A. M. (2002) 'Micro/nano encapsulation via electrified coaxial liquid jets', *Science*, 295(5560), pp. 1695–1698. doi: 10.1126/science.1067595.

Luinstra, M., Grasmeijer, F., Hagedoorn, P., Moes, J. R., Frijlink, H. W. and De Boer, A. H. (2015) 'A levodopa dry powder inhaler for the treatment of Parkinson's disease patients in off periods', *European Journal of Pharmaceutics and Biopharmaceutics*, 97, pp. 22–29. doi: https://doi.org/10.1016/j.ejpb.2015.10.003.

Luo, C. J., Okubo, T., Nangrejo, M. and Edirisinghe, M. (2015) 'Preparation of polymeric nanoparticles by novel electrospray nanoprecipitation', *Polymer International*, 64(2), pp. 183–187. doi: 10.1002/pi.4822.

Ma, J., Shi, L., Shi, Y., Luo, S. and Xu, J. (2002) 'Pyrolysis of polymethylsilsesquioxane', *Journal of Applied Polymer Science*, 85(5), pp. 1077–1086. doi: 10.1002/app.10576.

Madan, P. L. (1978) 'Microencapsulation I. Phase separation or coacervation', *Drug Development and Industrial Pharmacy*, 4(1), pp. 95–116. doi: 10.3109/03639047809055641.

Makadia, H. K. and Siegel, S. J. (2011) 'Poly lactic-co-glycolic acid (PLGA) as biodegradable controlled drug delivery carrier', *Polymers*, 3(3), pp. 1377–1397. doi: 10.3390/polym3031377.Poly.

Mallardé, D., Boutignon, F., Moine, F., Barré, E., David, S., Touchet, H., Ferruti, P. and Deghenghi, R. (2003) 'PLGA–PEG microspheres of teverelix: influence of polymer type on microsphere characteristics and on teverelix in vitro release', *International Journal of Pharmaceutics*, 261(1), pp. 69–80. doi: https://doi.org/10.1016/S0378-5173(03)00272-2.

Marathe, P. H., Wen, Y., Norton, J., Greene, D. S., Barbhaiya, R. H. and Wilding, I. R. (2000) 'Effect of altered gastric emptying and gastrointestinal motility on metformin absorption', *British Journal of Clinical Pharmacology*, 50(4), pp. 325–332. doi: 10.1046/j.1365-2125.2000.00264.x.

Meka, V. S., Gorajana, A., Dharmanlingam, S. R. and Kolapalli, V. R. M. (2013) 'Design and evaluation of a gastroretentive drug delivery system for metformin HCl using synthetic and semi-synthetic polymers', *Investigacion Clinica (Venezuela)*, 54(4), pp. 347–359.

Miyazaki, S., Yokouchi, C. and Takada, M. (1988) 'External control of drug release: controlled release of insulin from a hydrophilic polymer implant by ultrasound irradiation in diabetic rats', *Journal of Pharmacy and Pharmacology*, 40(10), pp. 716–717. doi: 10.1111/j.2042-7158.1988.tb07001.x.

Mizokami, F., Koide, Y., Noro, T. and Furuta, K. (2012) 'Polypharmacy with common diseases in hospitalized elderly patients', *The American Journal of Geriatric Pharmacotherapy*, 10(2), pp. 123–128. doi: 10.1016/j.amjopharm.2012.02.003.

Mizushige, K., Kondo, I., Ohmori, K., Hirao, K. and Matsuo, H. (1999) 'Enhancement of ultrasound-accelerated thrombolysis by echo contrast agents: Dependence on microbubble structure', *Ultrasound in Medicine & Biology*, 25(9), pp. 1431–1437. doi: 10.1016/S0301-5629(99)00095-2.

Modi, S., Jain, J. P. and Kumar, N. (2005) 'Synthesis, characterization, and degradation of poly(ester-anhydride) for particulate delivery', *Israel Journal of Chemistry*, 45(4), pp. 401–409. doi: 10.1560/0M15-CNAQ-BDR7-XFCM.

Mohamed, F. and Van Der Walle, C. F. (2018) 'Engineering biodegradable polyester particles with specific drug targeting and drug release properties', *Journal of Pharmaceutical Sciences*, 97(1), pp. 71–87. doi: 10.1002/jps.21082.

Van Den Mooter, G. (2012) 'The use of amorphous solid dispersions: A formulation strategy to overcome poor solubility and dissolution rate', *Drug Discovery Today: Technologies*, 9(2), pp. e79–e85. doi: https://doi.org/10.1016/j.ddtec.2011.10.002.

Murphy, C., Pillay, V., Choonara, Y. E., Du Toit, L. C., Ndesendo, V. M. K., Chirwa, N. and Kumar, P. (2012) 'Optimization of a dual mechanism gastrofloatable and gastroadhesive delivery system for narrow absorption window drugs', *AAPS PharmSciTech*, 13(1), pp. 1–15. doi: 10.1208/s12249-011-9711-1.

Nangrejo, M., Ahmad, Z., Stride, E., Edirisinghe, M. and Colombo, P. (2008) 'Preparation of polymeric and ceramic porous capsules by a novel electrohydrodynamic process', *Pharmaceutical Development and Technology*, 13(5), pp. 425–432. doi: 10.1080/10837450802247929.

Nathaji, R., Shahana, P., Binulal, C., Manikandan, P., N. Subramaniam, S. and Babu, G. (2017) 'Formulation and evaluation of paracetamol loaded mucoadhesive microspheres', *International Journal of Pharmaceutics & Pharmacology*, 1(2), pp. 1–106.

Nguyen, D. N., Palangetic, L., Clasen, C. and Van Den Mooter, G. (2016a) 'One-step production of darunavir solid dispersion nanoparticles coated with enteric polymers using electrospraying', *Journal of Pharmacy and Pharmacology*, 68(5), pp. 625–633. doi: 10.1111/jphp.12459.

Nguyen, D. N., Clasen, C. and Van Den Mooter, G. (2016b) 'Pharmaceutical applications of electrospraying', *Journal of Pharmaceutical Sciences*, 105(9), pp. 2601–2620. doi: 10.1016/j.xphs.2016.04.024.

Nishiyama, N. and Kataoka, K. (2003) 'Polymeric micelle drug carrier systems:

PEG-PAsp(Dox) and second generation of micellar drugs', in *Polymer Drugs in the Clinical Stage*. Boston: Kluwer Academic Publishers, pp. 155–177.

Nokhodchi, A., Javadzadeh, Y., Siahi-Shadbad, M. R. and Barzegar-Jalali, M. (2005) 'The effect of type and concentration of vehicles on the dissolution rate of a poorly soluble drug (indomethacin) from liquisolid compacts', *Journal of Pharmacy and Pharmaceutical Sciences*, 8(1), pp. 18–25.

Nyström, M., Murtomaa, M. and Salonen, J. (2011) 'Fabrication of amorphous pharmaceutical materials by electrospraying into reduced pressure', *Journal of Electrostatics*, 69(4), pp. 351–356. doi: 10.1016/j.elstat.2011.04.013.

Ogawa, Y., Yamamoto, M., Okada, H., Yashiki, T. and Shimamoto, T. (1988) 'A new technique to efficiently entrap leuprolide acetate into microcapsules of polylactic acid or copoly(lactic/glycolic) acid', *Chemical & Pharmaceutical Bulletin*, 36(3), pp. 1095–1103.

Orlu-Gul, M., Topcu, A. A., Shams, T., Mahalingam, S. and Edirisinghe, M. (2014) 'Novel encapsulation systems and processes for overcoming the challenges of polypharmacy.', *Current Opinion in Pharmacology*, 18, pp. 28–34. doi: 10.1016/j.coph.2014.08.001.

Parhizkar, M., Reardon, P. J. T., Knowles, J. C., Browning, R. J., Stride, E., Pedley, R. B., Grego, T. and Edirisinghe, M. (2017) 'Performance of novel high throughput multi electrospray systems for forming of polymeric micro/nanoparticles', *Materials & Design*, 126, pp. 73–84. doi: https://doi.org/10.1016/j.matdes.2017.04.029.

Park, T. G., Yong Lee, H. and Sung Nam, Y. (1998) 'A new preparation method for protein loaded poly(D, L-lactic-co-glycolic acid) microspheres and protein release mechanism study', *Journal of Controlled Release*, 55(2–3), pp. 181–191. doi: S0168-3659(98)00050-9 [pii].

Parnis, J. M. and Oldham, K. B. (2013) 'Beyond the Beer–Lambert law: The

dependence of absorbance on time in photochemistry', *Journal of Photochemistry and Photobiology A: Chemistry*, 267, pp. 6–10. doi: https://doi.org/10.1016/j.jphotochem.2013.06.006.

Patton, J. S. and Byron, P. R. (2007) 'Inhaling medicines: Delivering drugs to the body through the lungs', *Nature Reviews Drug Discovery*, 6(1), pp. 67–74.

Phang, J. S., Marsh, W. A., Barlows, T. G. and Schwartz, H. I. (2004) 'Determining feeding tube location by gastric and intestinal pH values', *Nutrition in Clinical Practice*, 19(6), pp. 640–644. doi: 10.1177/0115426504019006640.

Pitt, C. G. (1990) 'The controlled parenteral delivery of polypeptides and proteins', *International Journal of Pharmaceutics*, 59(3), pp. 173–196. doi: https://doi.org/10.1016/0378-5173(90)90108-G.

Poliachik, S. L., Chandler, W. L., Mourad, P. D., Bailey, M. R., Bloch, S., Cleveland, R. O., Kaczkowski, P., Keilman, G., Porter, T. and Crum, L. A. (2018) 'Effect of high-intensity focused ultrasound on whole blood with and without microbubble contrast agent', *Ultrasound in Medicine and Biology*, 25(6), pp. 991–998. doi: 10.1016/S0301-5629(99)00043-5.

Qiu, Y. and Park, K. (2001) 'Environment-sensitive hydrogels for drug delivery', *Advanced Drug Delivery Reviews*, 53(3), pp. 321–339. doi: https://doi.org/10.1016/S0169-409X(01)00203-4.

Rabinow, B. E. (2004) 'Nanosuspensions in drug delivery', *Nature Reviews Drug Discovery*, 3(9), pp. 785–796.

Ramakrishna, S., Fujihara, K., Teo, W. E., Lim, T. C. and Ma, Z. (2005) *An Introduction to Electrospinning and Nanofibers*, *World Scientific*. doi: 10.1142/9789812567611_0006.

Rayleigh, B. (1882) 'On the equilibrium of liquid conducting masses charged with electricity', *Philosophical Magazine Series 5*, 14(87), pp. 184–186.

Rodríguez, R., Alvarez-Lorenzo, C. and Concheiro, A. (2003) 'Cationic cellulose hydrogels: Kinetics of the cross-linking process and characterization as pH-/ion-sensitive drug delivery systems', *Journal of Controlled Release*, 86(2–3), pp. 253–265. doi: 10.1016/S0168-3659(02)00410-8.

Ruel-Gariépy, E. and Leroux, J. (2004) 'In situ-forming hydrogels - review of temperature-sensitive systems', *European Journal of Pharmaceutics and Biopharmaceutics*, 58(2), pp. 409–426. doi: https://doi.org/10.1016/j.ejpb.2004.03.019.

Rulison, A. J. and Flagan, R. C. (1994) 'Electrospray atomization of electrolytic solutions', *Journal of Colloid and Interface Science*, 167(1), pp. 135–145. doi: 10.1006/jcis.1994.1341.

Sakuma, S., Matsumoto, S., Ishizuka, N., Mohri, K., Fukushima, M., Ohba, C. and Kawakami, K. (2015) 'Enhanced boosting of oral absorption of lopinavir through electrospray coencapsulation with ritonavir', *Journal of Pharmaceutical Sciences*, 104(9), pp. 2977–2985. doi: 10.1002/jps.24492.

Savic, R., Luo, L., Eisenberg, A. and Maysinger, D. (2003) 'Micellar nanocontainers distribute to defined cytoplasmic organelles', *Science*, 300(5619), pp. 615–618.

Schmidt, W. and Roessling, G. (2006) 'Novel manufacturing process of hollow polymer microspheres', *Chemical Engineering Science*, 61(15), pp. 4973–4981. doi: 10.1016/j.ces.2006.03.021.

Schreier, H., Gonzalez-Rothi, R. J. and Stecenko, A. A. (1993) 'Pulmonary delivery of liposomes', *Journal of Controlled Release*, 24(1–3), pp. 209–223. doi: 10.1016/0168-3659(93)90180-D.

Schuck, P., Dolivet, A., Méjean, S., Zhu, P., Blanchard, E. and Jeantet, R. (2009) 'Drying by desorption: A tool to determine spray drying parameters', *Journal of Food Engineering*, 94(2), pp. 199–204. doi:

https://doi.org/10.1016/j.jfoodeng.2008.08.014.

Serebruany, V. L., Malinin, A. I., Sane, D. C., Jilma, B., Takserman, A., Atar, D. and Hennekens, C. H. (2004) 'Magnitude and time course of platelet inhibition with Aggrenox® and aspirin in patients after ischemic stroke: The aggrenox versus aspirin therapy evaluation (AGATE) trial', *European Journal of Pharmacology*, 499(3), pp. 315–324. doi: 10.1016/j.ejphar.2004.07.114.

Shams, T., Parhizkar, M., Illangakoon, U. E., Orlu, M. and Edirisinghe, M. (2017) 'Core/shell microencapsulation of indomethacin/paracetamol by co-axial electrohydrodynamic atomization', *Materials & Design*, 136, pp. 204–213. doi: https://doi.org/10.1016/j.matdes.2017.09.052.

Shams T., Illangakoon U. E., Parhizkar M., Harker A. H., Edirisinghe S., Orlu M., Edirisinghe M. (2018), Electrosprayed microparticles for intestinal delivery of prednisolone, *Journal of Royal Society Interface,*15(145), 20180491.

Shen, X. X., Yu, D. G., Zhu, L. M. and Branford-White, C. (2009) 'Preparation and characterization of ultrafine Eudragit L100 fibers via electrospinning', in *3rd International Conference on Bioinformatics and Biomedical Engineering (iCBBE 2009)*, pp. 9–12. doi: 10.1109/ICBBE.2009.5163230.

Shokri, J., Hanaee, J., Barzegar-Jalali, M., Changizi, R., Rahbar, M. and Nokhodchi, A. (2006) 'Improvement of the dissolution rate of indomethacin by a cogrinding technique using polyethylene glycols of various molecular weights', *Journal of Drug Delivery Science and Technology*, 16(3), pp. 203–209. doi: 10.1016/S1773-2247(06)50036-X.

Shoyele, S. A. and Cawthorne, S. (2006) 'Particle engineering techniques for inhaled biopharmaceuticals', *Advanced Drug Delivery Reviews*, 58(9), pp. 1009–1029. doi: https://doi.org/10.1016/j.addr.2006.07.010.

Siepmann, J., Siegel, R. A. and Rathbone, M. J. (2012) 'Overview of Controlled

Release Mechanisms', in *Fundamentals and Applications of Controlled Release Drug Delivery*. Springer, pp. 19–43. doi: 10.1007/978-1-4614-0881-9_2.

Singh, A. and Van Den Mooter, G. (2016) 'Spray drying formulation of amorphous solid dispersions', *Advanced Drug Delivery Reviews*, 100, pp. 27–50. doi: https://doi.org/10.1016/j.addr.2015.12.010.

Singh, A., Worku, Z. A. and Van Den Mooter, G. (2011) 'Oral formulation strategies to improve solubility of poorly water-soluble drugs', *Expert Opinion on Drug Delivery*, 8(10), pp. 1361–1378. doi: 10.1517/17425247.2011.606808.

Singh, J. and Roberts, M. S. (1989) 'Transdermal delivery of drugs by iontophoresis: A review', *Drug Design and Delivery*, 4(1), pp. 1–12. Available at: http://europepmc.org/abstract/MED/2673280.

Sinha, V. R. and Trehan, A. (2005) 'Biodegradable Microspheres for Parenteral Delivery', *Critical Reviews™ in Therapeutic Drug Carrier Systems*, 22(6), pp. 535–602.

Sivin, I., Campodonico, I., Kiriwat, O., Holma, P., Diaz, S., Wan, L., Biswas, A., Viegas, O., Abdalla, K. E. D., Anant, M. P., Pavez, M. and Stern, J. (1998) 'The performance of levonorgestrel rod and Norplant® contraceptive implants: A 5 year randomized study', *Human Reproduction*, 13(12), pp. 3371–3378. doi: 10.1093/humrep/13.12.3371.

Smallwood, I. M. (1996) *Handbook of Organic Solvent Properties*. Wiley. doi: https://doi.org/10.1016/B978-0-08-052378-1.50020-7.

Sofokleous, P., Chang, M. W., Ge, B. Y., Stride, E. and Edirisinghe, M. (2011) 'Controlled preparation of drug-exchange phase loaded polymeric fibres', *Bioinspired, Biomimetic and Nanobiomaterials*, 1(1), pp. 48–56. doi: 10.1680/bbn.11.00009.

Sofokleous, P., Stride, E. and Edirisinghe, M. (2013) 'Preparation,

characterization, and release of amoxicillin from electrospun fibrous wound dressing patches', *Pharmaceutical Research*, 30(7), pp. 1926–1938. doi: 10.1007/s11095-013-1035-2.

Soppimath, K. S., Aminabhavi, T. M., Kulkarni, A. R. and Rudzinski, W. E. (2001) 'Biodegradable polymeric nanoparticles as drug delivery devices', *Journal of Controlled Release*, 70(1–2), pp. 1–20.

Spireas, S. and Sadu, S. (1998) 'Enhancement of prednisolone dissolution properties using liquisolid compacts', *International Journal of Pharmaceutics*, 166(2), pp. 177–188. doi: 10.1016/S0378-5173(98)00046-5.

Sridhar, R. and Ramakrishna, S. (2013) 'Electrosprayed nanoparticles for drug delivery and pharmaceutical applications', *Biomatter*, 3(3), p. e24281. doi: 10.4161/biom.24281.

Stegemann, S., Ecker, F., Maio, M., Kraahs, P., Wohlfart, R., Breitkreutz, J., Zimmer, A., Bar-Shalom, D., Hettrich, P. and Broegmann, B. (2010) 'Geriatric drug therapy: Neglecting the inevitable majority', *Ageing Research Reviews*, 9(4), pp. 384–398. doi: 10.1016/j.arr.2010.04.005.

Suzuki, A. and Tanaka, T. (1990) 'Phase transition in polymer gels induced by visible light', *Nature*, 346, pp. 345–347.

Tamada, J. A. and Langer, R. (1993) 'Erosion kinetics of hydrolytically degradable polymers', in *National Academy of Sciences of the United States of America*, pp. 552–556. doi: 10.1073/pnas.90.2.552.

Tang, K. and Gomez, A. (1994) 'On the structure of an electrostatic spray of monodisperse droplets', *Physics of Fluids*, 6(7), pp. 2317–2332. doi: 10.1063/1.868182.

Tang, K. and Gomez, A. (1995) 'Generation of monodisperse water droplets from electrosprays in a corona-assisted cone-jet mode', *Journal of Colloid and*

Interface Science, 175(2), pp. 326–332. doi: 10.1006/jcis.1995.1464.

Tang, K. and Gomez, A. (1996) 'Monodisperse electrosprays of low electric conductivity liquids in the cone-jet mode', *Journal of Colloid and Interface Science*, 184(2), pp. 500–511. doi: https://doi.org/10.1006/jcis.1996.0645.

Tang, L. and Eaton, J. W. (1995) 'Inflammatory responses to biomaterials', *American Journal of Clinical Pathology*, 103(4), pp. 466–471. doi: 10.1093/ajcp/103.4.466.

Taylor, G. (1964) 'Disintegration of water drops in an electric field', in *Royal Society*, pp. 383–397.

Terada, K., Kitano, H., Yoshihashi, Y. and Yonemochi, E. (2000) 'Quantitative correletaion between initial dissolution rate and heat of solution of drug', *Pharmaceutical Research*, 17(8), pp. 920–924.

Thian, E. S., Ahmad, Z., Huang, J., Edirisinghe, M. J., Jayasinghe, S. N., Ireland, D. C., Brooks, R. A., Rushton, N., Bonfield, W. and Best, S. M. (2008) 'The role of electrosprayed apatite nanocrystals in guiding osteoblast behaviour', *Biomaterials*, 29(12), pp. 1833–1843. doi: https://doi.org/10.1016/j.biomaterials.2008.01.007.

Vogt, M., Derendorf, H., Krämer, J., Junginger, H. E., Midha, K. K., Shah, V. P., Stavchansky, S., Dressman, J. B. and Barends, D. M. (2007) 'Biowaiver monographs for immediate release solid oral dosage forms: Prednisone', *Journal of Pharmaceutical Sciences*, 96(6), pp. 1480–1489. doi: 10.1002/jps.20817.

Wang, J. C., Chang, M. W., Ahmad, Z. and Li, J. S. (2016) 'Fabrication of patterned polymer-antibiotic composite fibers via electrohydrodynamic (EHD) printing', *Journal of Drug Delivery Science and Technology*, 35, pp. 114–123. doi: 10.1016/j.jddst.2016.06.009.

Wang, X., Yu, D. G., Li, X. Y., Bligh, S. W. A. and Williams, G. R. (2015)

‘Electrospun medicated shellac nanofibers for colon-targeted drug delivery’, *International Journal of Pharmaceutics*, 490(1), pp. 384–390. doi: https://doi.org/10.1016/j.ijpharm.2015.05.077.

Wang, Y., Zhang, Y., Wang, B., Cao, Y., Yu, Q. and Yin, T. (2013) ‘Fabrication of core-shell micro/nanoparticles for programmable dual drug release by emulsion electrospraying’, *Journal of Nanoparticle Research*, 15(6), p. 1726. doi: 10.1007/s11051-013-1726-y.

Wang, Y. W., He, S. J., Feng, X., Cheng, J., Luo, Y. T., Tian, L. and Huang, Q. (2017) ‘Metformin: A review of its potential indications’, *Drug Design, Development and Therapy*, 11, pp. 2421–2429. doi: 10.2147/DDDT.S141675.

Wilhelm, O., Mädler, L. and Pratsinis, S. E. (2003) ‘Electrospray evaporation and deposition’, *Journal of Aerosol Science*, 34(7), pp. 815–836. doi: 10.1016/S0021-8502(03)00034-X.

Witschi, C. and Doelker, E. (1998) ‘Influence of the microencapsulation method and peptide loading on poly(lactic acid) and poly(lactic-co-glycolic acid) degradation during in vitro testing’, *Journal of Controlled Release*, 51(2), pp. 327–341. doi: https://doi.org/10.1016/S0168-3659(97)00188-0.

Wojnarowska, Z., Grzybowska, K., Adrjanowicz, K., Kaminski, K., Paluch, M., Hawelek, L., Wrzalik, R., Dulski, M., Sawicki, W., Mazgalski, J., Tukalska, A. and Bieg, T. (2010) ‘Study of the amorphous glibenclamide drug: analysis of the molecular dynamics of quenched and cryomilled material’, *Molecular Pharmaceutics*, 7(5), pp. 1692–1707. doi: 10.1021/mp100077c.

Wu, J. and Nyborg, W. L. (2008) ‘Ultrasound, cavitation bubbles and their interaction with cells’, *Advanced Drug Delivery Reviews*, 60(10), pp. 1103–1116. doi: https://doi.org/10.1016/j.addr.2008.03.009.

Wu, Y. and Clark, R. L. (2008) ‘Electrohydrodynamic atomization: A versatile process for preparing materials for biomedical applications’, *Journal of*

Biomaterials Science, Polymer Edition, 19(5), pp. 573–601. doi: 10.1163/156856208784089616.

Xie, J., Ng, W. J., Lee, L. Y. and Wang, C. H. (2008) 'Encapsulation of protein drugs in biodegradable microparticles by co-axial electrospray', *Journal of Colloid and Interface Science*, 317(2), pp. 469–476. doi: 10.1016/j.jcis.2007.09.082.

Yalkowsky, S. H. and Dannenfelser, R. M. (1992) *Handbook of Aqueous Solubility*. 5th edn. CRC Press.

Yan, W. C., Tong, Y. W. and Wang, C. H. (2017) 'Coaxial electrohydrodynamic atomization toward large scale production of core-shell structured microparticles', *AIChE Journal*, 63(12), pp. 5303–5319. doi: 10.1002/aic.15821.

Ye, C., Chen, A., Colombo, P. and Martinez, C. (2010) 'Ceramic microparticles and capsules via microfluidic processing of a preceramic polymer', *Journal of the Royal Society Interface*. The Royal Society, 7(Suppl 4), pp. S461–S473. doi: 10.1098/rsif.2010.0133.focus.

Yin, D., Yang, Y., Cai, H., Wang, F., Peng, D. and He, L. (2014) 'Gambogic acid-loaded electrosprayed particles for site-specific treatment of hepatocellular carcinoma', *Molecular Pharmaceutics*, 11(11), pp. 4107–4117. doi: 10.1021/mp500214a.

Youn, D. H., Kim, S. H., Yang, Y. S., Lim, S. C., Kim, S. J., Ahn, S. H., Sim, H. S., Ryu, S. M., Shin, D. W. and Yoo, J. B. (2009) 'Electrohydrodynamic micropatterning of silver ink using near-field electrohydrodynamic jet printing with tilted-outlet nozzle', *Applied Physics A*, 96(4), pp. 933–938. doi: 10.1007/s00339-009-5262-7.

Yu, D. G., Williams, G. R., Wang, X., Yang, J. H., Li, X. Y., Qian, W. and Li, Y. (2011) 'Polymer-based nanoparticulate solid dispersions prepared by a modified electrospraying process', *Journal of Biomedical Science and Engineering*, 4(12), pp. 741–749. doi: 10.4236/jbise.2011.412091.

Yu, D. G., Xu, Y., Luo, J. Q., Zhao, J. S., Teng, L., Kuang, Q. K. and Wang, X. (2014) 'Oral rapid-dissolving polymeric nanoparticles of acetaminophen fabricated using electrospraying', *Applied Materials Research*, 898, pp. 283–287. doi: 10.4028/www.scientific.net/AMR.898.283.

Zakeri-Milani, P., Nezhadi, S. H., Barzegar-Jalali, M. and Mohammadi, L. (2011) 'Studies on dissolution enhancement of prednisolone, a poorly water-soluble drug by solid dispersion technique', *Advanced Pharmaceutical Bulletin*, 1(1), pp. 48–53. doi: 10.5681/apb.2011.007.

Zamani, M., Prabhakaran, M. P. and Ramakrishna, S. (2013) 'Advances in drug delivery via electrospun and electrosprayed nanomaterials', *International Journal of Nanomedicine*, 8, pp. 2997–3017. doi: 10.2147/IJN.S43575.

Zambaux, M. F., Bonneaux, F., Gref, R., Maincent, P., Dellacherie, E., Alonso, M. J., Labrude, P. and Vigneron, C. (1998) 'Influence of experimental parameters on the characteristics of poly(lactic acid) nanoparticles prepared by a double emulsion method', *Journal of Controlled Release*, 50(1), pp. 31–40. doi: https://doi.org/10.1016/S0168-3659(97)00106-5.

Zbicinski, I. (2002) 'Equipment, technology, perspectives and modeling of pulse combustion drying', *Chemical Engineering Journal*, 86(1), pp. 33–46. doi: https://doi.org/10.1016/S1385-8947(01)00269-8.

Zeleny, J. (1914) 'The electrical discharge from liquid points, and a hydrostatic method of measuring the electric intensity at their surfaces', *Physical Review*, 3(2), pp. 69–91. doi: 10.1103/PhysRev.3.69.

Zeng, Q. and Sun, M. (2009) 'Poly(lactide-co-glycolide) nanoparticles as carriers for norcantharidin', *Materials Science and Engineering: C*, 29(3), pp. 708–713. doi: https://doi.org/10.1016/j.msec.2009.01.004.

Zhang, H., Zhang, J. and Streisand, J. B. (2002) 'Oral mucosal drug delivery: clinical pharmacokinetics and therapeutic applications', *Clinical*

Pharmacokinetics, 41(9), pp. 661–680. doi: 10.2165/00003088-200241090-00003.

Zhang, L., Huang, J., Si, T. and Xu, R. X. (2012) 'Coaxial electrospray of microparticles and nanoparticles for biomedical applications', *Expert Review of Medical Devices*, 9(6), pp. 595–612. doi: 10.1016/j.biotechadv.2011.08.021.Secreted.

Zhuo, R., Colombo, P., Pantano, C. and Vogler, E. A. (2005) 'Silicon oxycarbide glasses for blood-contact applications', *Acta Biomaterialia*, 1(5), pp. 583–589. doi: https://doi.org/10.1016/j.actbio.2005.05.005.