Preparation and evaluation of two types of films made by electrospray technique

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**PREPARATION AND EVALUATION OF TWO
TYPES OF FILMS MADE BY ELECTROSPRAY
TECHNIQUE**

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SCHOOL OF MATERIALS SCIENCE AND ENGINEERING

2016

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**A thesis submitted to the Nanyang Technological University
in partial fulfilment of the requirement for the degree of
Master of Engineering**

2016

Statement of Originality

I hereby certify that the work embodied in this thesis is the result of original research and has not been submitted for a higher degree to any other University or Institution.

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Abstract

Electrospray technique has been massively studied in the recent years owing to its simple experimental setup, broad applications and cost-effective attribute. In this project, electrospray technique has been adopted to generate micro- or nanostructure with controllable size and morphology. The function of each parameter in altering structures' size and shape has been explored. Three optimized formulations used to produce nearly uniform spheres with average diameters of 300nm, 5 μ m and 10 μ m has been achieved accordingly. In addition, electrospray technique has been used to fabricate oral film with metoprolol tartrate salt loaded for the treatment of heart disease. The physicochemical properties of the oral film and the in-vitro drug release profile have been studied. Obtained results have been analyzed and compared with the oral film made by solvent casting. It was found that oral film made by electrospray technique has higher drug encapsulation efficiency up to 99.29%. The cumulative drug release profile for the electrospray oral film was found to be fully released within an hour; where the disintegration time of the electrospray oral film is 2.5 times faster than the oral film made by solvent casting. Apart from this, it is efficient to produce oral films using electrospray technique since the processing time is significantly reduced by several hours and it is less labor intensive. In summary, electrospray technique is highly recommended to fabricate oral films for drug delivery applications.

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Abbreviations

PD	Parkinson's disease
BBB	Blood-Brain Barrier
COMT	Catechol-O-Methyltransferase
3-OMD	3-O-Methylation
PLGA	Poly (Lactic-Co-Glycolic Acid)
PLA	Poly Lactic Acid
PLLA	Poly-L-Lactide
PDLA	Poly-D-Lactide
PGA	Poly Glycolic Acid
MT	Metoprolol Tartrate Salt
HPMC	Hydroxypropyl Methycellulose
SEM	Scanning Electron Microscope
BEI	Backscatter Electron Imaging
SEI	Secondary Electron Imaging
HPLC	High-Performance Liquid Chromatography
UV-Vis	Ultraviolet-Visible
DSC	Differential Scanning Calorimetry
EE	Encapsulation Efficiency
LC	Loading Capacity
MW	Molecular Weight
DCM	Dichloromethane
DI water	Distilled water
SGF	Simulated Gastric Fluid
SIF	Simulated Intestinal Fluid
PBS	Phosphate Buffered Saline
FDA	Food and Drug Administration
USP	United States Pharmacopoeia
PEG	Polyethylene glycol
T _g	Glass transition temperature

Chapter 1

Introduction

This chapter is aimed to give a very first impression about this project. Short background knowledge about the oral film will be introduced. This brief introduction shows the promising potential of oral film drug delivery system. The challenges of using conventional methods to fabricate oral films are listed in the problem statement which includes the low drug loading capacity and the limitations of the conventional methods. Thereafter, electrospray technique as an inventive method is proposed to be used to produce the oral film. A hypothesis is made that electrospray technique can fabricate oral films with even better properties compared with the conventional method. The objectives and outstanding findings are included in the chapter.

1.1 Background

Oral film drug delivery is described as using a dissolving film to release drugs through the oral route. The oral film has emerged as an advanced alternative to the traditional dosage forms like tablets, capsules and liquids. It is reflected from the statistics that four out of five patients prefer orally disintegrating dosage forms over conventional forms [1]. The oral film delivery mitigates any possibility of choking while at the same time allows for a comfortable oral consumption. These features of oral films remove the need for paediatrics and geriatrics. On top of this, oral film delivery is easy to administer, has a rapid onset of action, accurate of dosage, improve patients' compliance, and removes the need of water for consumption [1, 2]. There is a wide range of applications for oral films on the market. One report revealed that the oral film manufacturers had had rapid revenue growth from 2009 to 2014. A similar tendency is expected at least until 2019 with an annual growth of 17.1% [3].

1.2 Problem Statement

The biggest challenge for the fabrication of oral film using conventional method is the low drug loading capacity. The conventional methods used to produce oral films are solvent casting, hot melt extrusion and rolling [2]. The limitations of using these methods are summarized below. Solvent casting and rolling are time-consuming processes. For hot melt extrusion, heating process degrades bioactivity of therapeutic elements. Henceforth, there is an impending need to develop a new fabrication process where drugs can be released immediately and with high encapsulation efficiency.

1.3 Hypothesis

Electrospray technique is found to be a promising approach since there is no heating involved which can guarantee the bioactivity of drugs. The solvent almost dries out upon reaching the collector which prominently reduces the

fabrication time. High encapsulation efficiency and high drug loading capacity are always reported by using electrospray technique [4, 5]. Based on the features of electrospray technique, it is hypothesized that electrospray technique can produce oral films with even better properties compared with the conventional method.

1.4 Objectives and Scope

The objectives of this project are listed below:

1. Understand the effect of electrospray parameters on the size and morphology of micro- or nanospheres.
2. Materials selection and electrospraying optimization for oral film production.
3. Use various characterization methods to test and compare the properties of oral films made by electrospray technique and solvent casting.

The scope of this project is to apply the knowledge and skills of electrospray technique to fabricate two types of films with different drugs loaded inside. Drug encapsulation efficiency and in vitro drug release profile are studied. In vivo drug release study is beyond the scope of this project.

1.5 Findings and Originality

To our knowledge, no resource has reported using electrospray technique to make oral films. This project demonstrated the possibility of this method. Surprisingly, the oral film made in this way has better drug encapsulation and release properties.

By using electrospray technique, film fabrication process can be simplified,

where a mold was used as a collector, hence obviating the cutting of solvent casted film. The customized collector dimension is moulded according to the prescribed drug intake amount. With this method, it promotes the lab scale fabrication to large scale manufacture. The packing material is also considered and selected where aluminum is more suitable compared to plastics.

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Chapter 2

Literature Review

This chapter reviews the historical and contemporary literature concerning to electrospray technique, oral film technology, drugs and major materials used in this project. The literature review about electrospray technique illustrates the principle and the underlying mechanism of this method. The development of oral film technology, the applications of oral films and the composition of oral films are reviewed. Conventional methods for oral film fabrication and the innovative method for oral film fabrication are introduced and compared. The reason for choosing major materials like poly (lactic-co-glycolic acid) and hydroxypropyl methylcellulose are reviewed. The properties of these materials are also stated.

2.1 Electrospray Technique

The discovery of electrospray phenomenon can date back to the late 16th century when William Gilbert [1] observed the surface transformation of a water droplet in the presence of electrically charged amber. In 1882, Lord Rayleigh [2] theoretically studied the maximum surface charge density a liquid droplet can hold prior to disruption. This value is known as the “Rayleigh limit”. He also predicted that a fine jet would emanate from a droplet upon reaching the Rayleigh limit. This prediction was confirmed experimentally more than 100 years later [3]. A breakthrough in this field happened when Sir Geoffrey Ingram Taylor [4] mathematically modelled the Taylor cone which formed initially from a liquid droplet under the effect of an electrical field.

Indeed, electrospray is a liquid atomization process where liquid jet breaks up into small droplets under the effect of an external electrical field. In most electrospray apparatus, it consists of a syringe pump (to dispense polymer solution), a syringe (to load polymer solution), a metal needle (served as a nozzle), high voltage via a power supply (to create an external electrical field) and a grounded collector. The whole setup is enclosed in a chamber for the safety issue.

Figure 1 Electrospray process [Taken from Hartman et al,1999].

2.1.1 Theoretical Principles of Electrospray

The principle of electrospray technique is based on the theory of charged droplets. [5] When a droplet exposes to an electrical field, surface charges are induced. Electrostatic force and surface tension become two competing forces in the charged droplet. At a critical point, when Coulombic repulsion is equivalent to the surface tension, the droplet's surface starts to deform into a conical shape with a thin jet at its apex. This conical shape is named as Taylor Cone.

Sir Geoffrey Ingram Taylor [4] is the first one to derive a mathematical model for the formation of the Taylor cone and analytically calculates the conical shape. This model is based on two assumptions:

1. The cone surface is equipotential;
2. The cone exists only in an equilibrium steady state.

The equation used to illustrate this model is:

$$V=V_0 + AR^{1/2}P_{1/2}(\cos\theta_0)$$

To overcome the effect of surface tension acting on the cone, the electrical potential must have an azimuthal symmetry and $R^{1/2}$ dependence. As the cone surface is equipotential meaning $V=V_0$, the equation is only valid when $P_{1/2}(\cos\theta_0)$ equals to zero. Between 0 and π , there is only one solution: $\theta = 130.7099^\circ$. This angle is the complement angle of Taylor angle which is 49.3° [6]. It can be concluded that a conducting liquid with a conical surface at its equilibrium state can only exist when the semi-vertical angle is close to 49.3° .

However, the flaw of this model is that the thin jet is not concerned. Besides, it only balances surface tension and electrical force. To fix it, Hartman [7] established a new model taking into account a series of forces depicted in figure 2. By applying this model, the shape of the liquid cone and thin jet can be calculated accurately.

Figure 2 Liquid cone with a thin jet at its apex depicting forces acting on a cone-jet [Taken from Hartman et al, 1999].

After formation of the Taylor cone, the induced electrical field further pushes charges to the tip of the cone. The jet with high electrical charge density experiences an accelerated motion until it breaks up into primary, secondary and satellite droplets under different conditions [8, 9, 10].

The theory of jet fission has been widely studied. The pioneer is Lord Rayleigh [2] who derives an equation to indicate the instability of a conducting droplet in term of the maximum surface charges it can hold before breaking up. This critical value is called Rayleigh limit reckoned in the following equation.

$$Q^2 = 64\pi^2 \epsilon \gamma r^3$$

Where Q is the surface charge of the droplet, ϵ is the permittivity of the surrounding medium, γ is the liquid surface tension and R is the drop radius.

A droplet is stable only if the fissility $X = Q^2 / (64\pi^2 \epsilon \gamma r^3)$ is less than the unity. The stable situation implies that the intrinsic cohesive force referring to the surface tension is larger than the Coulombic repulsion force. Thus, surface tension is still capable of binding the droplet.

Hartman [11] investigated the mechanism of jet breakup using an analytical model. According to this model, the situation is simplified: the jet is set to move at a constant velocity in a cylindrical coordinate system and only minor disruptions are presented on the jet surface. The surface can be depicted using the equation below.

$$r_s = r_{jet} + \alpha_0 e^{(wt + jm\theta - jkz)}, \quad k = 2\pi/\lambda$$

The value of m determines the jet disintegration modes. When $m = 0$, the breakup of the jet is independent of the angular component θ . These axisymmetric instabilities are named varicose instabilities. If $m=1$, they are lateral instabilities also called kink instabilities. There is 50% of chance that whipping motion will occur when r_s is larger than r_{jet} . This situation occurs when the jet is charged or the jet is slowed down by an additional force. When $m = 2$, the jet breakup mode is called ramified breakup. In this circumstance, strong perturbations take place as the jet is highly charged.

Figure 3 Three jet breakup modes, the axisymmetric varicose breakup, the lateral kink breakup and the ramified breakup [Taken from Hartman, 2000].

Denis Duft [12] did an investigation to gain a better understanding of the mechanism for Coulomb fission. He captured the real-time pictures of the jet fission. The results shown below confirm that the Coulomb fission happens almost exactly at the Rayleigh limit.

From process a to f, the spherical droplet is first stretched to an ellipsoid shape predicted by Rayleigh (fig.4a). Then it forms two sharp tips at its apex (fig.4b). Nearly at the same moment, a fine jet of liquid is emitted from both tips in the opposite direction (fig.4 c). The jet later disintegrates into small droplets repelled from the original droplet by Coulomb repulsion (fig.4e). The

Figure 4 High-speed imaging of the disintegration of a charged droplet to the Rayleigh limit [Taken from Denis Duft, 2003].

tips disappear after jet ejection and contract back to the spherical shape.

Size distribution of the produced particles counts on the diameter of the jet and the way that jet breaks up into droplets [11]. There is a minimum flow rate for each electrospray solution below which the system cannot form a stable emission mode. At the minimum flow rate, varicose instability is the reason for jet fission. Increasing the flow rate, current through the liquid is increased together with the surface charges. In this situation, kink instability contributes to a wider particle size distribution leading to the presence of secondary and satellite particles. Clouseau [13] discovered that flow rate is a dominant factor responsible for the transition from varicose jet breakup mode to kink jet breakup mode. Hartman's experimental results [11] confirm that flow rate affects the number of secondary and satellite particles present in the system.

Figure 10a to 10c are real-time images obtained from the experiment with the flow rate increasing each time. From the result, it shows that the possibility of getting a broad size distribution is greater with a higher flow rate. When the flow rate is relatively low just above the minimum flow rate (fig10.a), the filament between primary droplet a and b is intact. The filament between primary droplet b and c is united first but is further broken by charge near c. However, the pressure at the tip is high because of the surface tension. The filament flows back to primary droplet b and forms monodispersed particles. When a higher flow rate (fig10.b) is applied, the filament cannot flow back to the primary droplet. Instead, it breaks and forms a secondary droplet. Under

the highest flow rate (fig10.c), the filament breaks between primary droplet a

Figure 5 Three regimes of the varicose jet breakup mode [Taken from Hartman, 2000].

and c and forms one secondary droplet b. If the filament between a and b also breaks, a satellite droplet will form between a and b with a particle size smaller than the secondary droplet.

2.1.2 Electrospray Modes

Figure 6 Various electrospray modes [Taken from Jaworek and Krupa, 1999b].

There are various electrospray modes named according to the geometrical

figure of the liquid at the apex of the nozzle and the spray behavior.

The formation of different electrospray modes depends on processing parameters (e.g. distance from the nozzle to the collector, applied voltage and flow rate) and physical properties of the polymeric solution (e.g. viscosity, electrical conductivity and surface tension) [14]. The stability, reproducibility of the jet, and the morphology of the products are closely related to the electrospray mode.

The electrospray modes can be divided into two major groups. The first group is characterized by small fragments ejecting from the nozzle including dripping, micro-dripping, spindles and ramified meniscus modes. The second group consists of cone-jet, multi-jet, oscillating-jet, ramified-jet and precession modes as they all form a jet first followed by jet disintegration [15, 16].

Most often, it is highly possible to have the first group of modes when a small voltage is applied. Under high voltage, the second group of modes is predominantly formed [17]. The application of high voltage creates a stable and continuous jet. The jet later breaks up into numbers of droplets a few millimeters away from the nozzle tip flying towards the collector at a threshold applied voltage. Need to mention that the cone-jet mode is the preferred mode as it is the most stable and reproducible mode to fabricate fine particles.

2.1.3 Electrospray Parameters

Electrospray is a novel and easy approach to fabricate uniform particles. Although it might look like a straightforward process, there are many parameters to play with which can have tremendous impacts on the final morphology and performance of the formed particles. Some of the most important parameters are polymer molecular weight, polymer solution concentration, electrical conductivity of the solvent, volatility of the solvent,

surface tension and viscosity of the fluid, applied voltage, flow rate, nozzle gauge, and distance from the nozzle tip to the collector. In this section, some of the parameters will be elucidated in detail.

2.1.3.1 Polymer Solution Concentration

The concentration of polymer solution plays a significant role in polymer chain entanglement which has a direct impact on the final morphology of the particles. An element called critical chain overlap concentration (C_o) is introduced to explain this phenomenon.

The critical chain overlap concentration C_{ov} is when the concentration inside the radius of gyration of every single macromolecular chain is equivalent to the solution concentration. C_{ov} is inversely proportional to the intrinsic viscosity η , as shown in the equation below.

$$C_{ov} \propto 1/\eta$$

Figure 7 The relevance of solution concentration, chain entanglements and final morphology of the products [Taken from N.Bock, 2012].

When the polymer concentration C is less than C_{ov} , there is no chain overlap and the regime is known as the dilute regime. When C is larger than C_{ov} , the chain can overlap but insufficient for the occurrence of chain entanglements. The regime is called semi-diluted regime. It is not preferred as particles are not reproducible and can deform during the process. When $C_{ent} < C < 3C_{ov}$, it is

known as the semi-diluted moderately entangled regime. Under this system, sufficient chain entanglements result in the production of reproducible particles. The semi-dilute highly entangled regime describes the situation where polymer concentration is three times larger than C_{ov} . In this case, the intermolecular force is too high to form particles instead bead on fibers or only fibers are produced [18].

The molecular weight distribution and molecular weight (MW) affect intrinsic viscosity which further influences C/C_{ov} . Pankaj's experimental result [12] shows that increasing MW reduces the working window to get semi-dilute moderately regime. On the other hand, a broad MWD enlarges the range of semi-dilute moderately regime for the production of optimal particles.

2.1.3.2 Electrical Conductivity

Electrospray is a charge involved process. The electrical conductivity of the solution is a crucial factor in optimizing the process and has become a powerful tool to alter the size of particles [5].

Electrical conductivity reflects the ability of material to conduct an electric current. High electrical conductivity indicates more charges can be carried by an electrospray jet. In 1997, Ganán-Calvo [19] conducted series of experiments to measure the current and the primary droplet size by electrospray different types of liquid various with electrical conductivity, surface tension, permittivity, density and viscosity. Scaling laws were derived to show the relationship among the current, particle sizes, and particle charges. The scaling laws are applied to the solution with high electrical conductivity and high viscosity or the solution with low electrical conductivity and low

$$\delta\mu \cdot \delta^{1/3} \sim \left[\frac{\gamma^3 \epsilon_0^2}{\mu^3 K^2 Q} \right]^{1/3}$$

viscosity. The parameter used to govern the process is written in the equation below:

Where Q is the flow rate of liquid, ϵ_0 is the permittivity of vacuum, K is the electrical conductivity, γ is the surface tension and μ is the viscosity of the solution.

If $\delta\mu^*\delta^{1/3} \leq 1$, indicating a high electrical conductive and high viscous solution, the current I and size of droplets d in a cone-jet electrospray mode are:

$$\begin{aligned} I/I_0 &= 6.2(Q/(\beta - 1)^{1/2} \cdot Q_0)^{1/2}; \\ d/(\beta - 1)^{1/3}d_0 &= 1.6(Q/(\beta - 1)^{1/2}Q_0)^{1/3} - 1.0. \end{aligned}$$

$$I \sim Q^{1/2} \quad d \sim Q^{1/3}$$

If $\delta\mu^*\delta^{1/3} \geq 1$, indicating a low electrical conductive and low viscous solution, the current I and size of droplets d in a cone-jet electrospray mode are:

$$I/I_0 = 11.0(Q/Q_0)^{1/4} - 5.0; \quad d/d_0 = 1.2(Q/Q_0)^{1/2} - 0.3,$$

$$I \sim Q^{1/4} \quad d \sim Q^{1/2}$$

Where $I_0 = (\epsilon_0\gamma^2/\rho)^{1/2}$, $Q_0 = \epsilon_0\gamma/(\rho K)$, $d_0 = (\gamma\epsilon_0^2(\rho K^2))^{1/3}$, β and ρ are the liquid to vacuum permittivity ratio and density of the liquid, respectively.

It is reflected from the above scaling laws that higher electrical conductivity gives rise to smaller particle size.

In another point of view, high electrical conductivity results in greater Coulombic repulsion force. Under this circumstance, it is easier to break chain entanglements which lead to the formation of small particles. Further increasing the electrical conductivity, the Coulombic repulsion force is large enough to overcome viscoelastic force before the formation of any strong chain entanglements. It results in the formation of small offspring droplets and bimodal size distributions which are not favored [5].

Nanoparticles can be fabricated by using high electrical conductivity solution. In this situation, sufficient viscosity needs to be granted to avoid producing offspring due to the early occurrence of Coulomb fission [20].

Aqueous solvents are more conductive than organic solvents. When the electrical conductivity of the solution is lower than $0.01 \mu\text{S}/\text{m}$, [5] the liquid cannot be electrosprayed due to insufficient current. Change the solvent or add electrolytes such as didodecyldimethylammonium bromide [20] or ammonium hydroxide [21] can solve this problem. When using water as the solvent, a nozzle-ring device, a grounded ring placed opposite to the charged nozzle, is recommended to discharge the highly charged droplets [22].

Figure 8 A nozzle-ring electrospray apparatus setup.

2.1.3.3 Flow Rate

Flow rate is an important parameter as it affects not only the size of particles but also particles' morphology. It works by controlling polymer chain entanglements and Coulomb fission together with other parameters [23].

Chain entanglement is a physical interlock in the polymer solution. It happens due to chain overlap and is functioned to retard the polymer chain movement [89]. Coulomb fission describes the process of a charged particle emanating some small but charged offspring. It happens when the surface charge of the

primary droplet reaches the Rayleigh limit.

Chain entanglements and Coulomb fission are two competing forces. Chain entanglements preserve the integrity and monodispersity of particles electrospray from the primary droplets. On the contrary, Coulomb fission enlarges particle size distribution.

High flow rate produces bimodal size distribution by chance of triggering secondary and satellite particles. It can be explained based on the concept of polymer volume fraction of a droplet at Rayleigh limit: ϕ_{ray} [5]. According to the equation, flow rate is closely related to the ϕ_{ray} .

$$\phi_{\text{Ray}} = \phi \frac{288\epsilon_{\text{air}}\epsilon\gamma}{(I/Q)^2 d^3}$$

Where Q is the liquid flow rate, I is the current, ϵ_{air} is the permittivity of air, γ is the surface tension of solution in ambient air, and d is the initial droplet diameter.

During the electrospray process, the electrospray droplets experience solvent evaporation and polymer chain diffusion simultaneously. It is necessary to choose a flow rate that allows the solvent to evaporate completely upon reaching the collector. Otherwise, it is likely that particles are still solvated with an inconsistent shape for collection [24, 25]. Thus, high flow rates are not favoured.

2.1.3.4 Applied Voltage

The electrical field strength is a critical parameter to determine a reproducible spray pattern [26]. Since applied voltage is closely related to the electrical field strength, so that, it becomes a dominant role in altering the electrospray mode. The electrospray mode changes from dripping mode to cone-jet mode by increasing the applied voltage [27]. Further increasing the applied voltage leads to the formation of multiple cone-jet mode. It should be avoided as this

mode is unstable and unpredictable. Only the stable cone-jet mode is capable of producing monosized particles along with careful control of other parameters. Therefore, moderate voltage is favoured.

The influence of applied voltage on the size of particles in cone-jet mode is not evident. The morphology of particles does not change with the applied voltage unless the polymer concentration is significantly high. In this case, the shape of particles changes from spheres to elongated spheres or even fibres [28, 29]. The enhanced electrostatic repulsive force resulting from the increasing voltage is the reason for the formation of stretched droplets.

2.1.3.5 Distance from the Nozzle Tip to the Collector

Distance from the nozzle tip to the collector determines the flight time of a solution. A short distance may cause partially solvent evaporation leading to coalescence and collapse of particles giving a broad particle size distribution [30]. Increasing the distance which in turn increases the flight time, provides enough time for polymer chains to diffuse and entangle. Hence, monosized spherical particles are more likely to be achieved.

$$E = \frac{U}{d}$$

Under a constant voltage, the strength of an electrical field is enhanced via shortening the distance. The increased strength of an electrical field triggers a reduction in particle's size [31].

The lower limit of the distance should be guaranteed with the purpose to avoid equipment breakdown which is determined by electric discharge [5].

2.2 Oral Film Technology

2.2.1 Oral Drug Delivery System

The definition of drug delivery system is a formulation or a device that enables the administration of a pharmaceutical agent in the body and improves its safety and efficacy by controlling the rate, time, and place of drug release in the body [32]. To be more specific, this process includes three steps: First, the administration of the pharmaceutical product; Secondly, the release of the active ingredients within the product; and lastly, the transportation of the active agent to the site of action.

There are various routes for drug administration such as oral route, parenteral route, transdermal route and so on. The selection of route is based on the availability of the product, types of disease and the designed effect. Hereinafter, the oral route will be introduced.

Issue	Oral	Intravenous	Intramuscular/ subcutaneous	Transnasal	Transdermal	Pulmonary
Delivery to blood circulation	Indirect through GI tract	Direct	Indirect absorption from tissues	Indirect	Indirect	Indirect
Onset of action	Slow	Rapid	Moderate to rapid	Rapid	Moderate to rapid	Rapid
Bioavailability	Low to high	High	High	Moderate	Low	Moderate to high
Dose control	Moderate	Good	Moderate	Moderate to good	Poor	Moderate to good
Administration	Self	Health professional	Self or health professional	Self	Self	Self
Patient convenience	High	Low	Low	High	Moderate	High
Adverse effects	GI upset	Acute reactions	Acute reactions	Insignificant	Skin irritation	Insignificant
Use for proteins and peptides	No	Yes	Yes	Yes	No	Yes

GI gastrointestinal

Figure 9 Comparison of major routes of drug delivery for systemic absorption [Taken from Jain et al, 2008].

Oral drug delivery system is one of the most utilized drug administration methods in both conventional and novel drug delivery systems. It is due to its outstanding attributes like high patient compliance, ease of administration and non-invasive behaviors.

When a drug is introduced via the oral route, it can be absorbed through oral absorption or gastrointestinal track. There are some obvious limitations for gastrointestinal absorption. In a low pH environment, drugs may degrade, and some of them become insoluble. Hence, the bioavailability of drugs is declined. And the administration of drugs is constrained accordingly. Another concern is the first-pass metabolism. When passing through the hepatic area, drugs will experience sharp losses. An extra addition of drug dosage is required to achieve the same therapeutic effect. Because of this, there is a hidden safety issue like overdosing.

For oral absorption, drugs are absorbed through buccal mucosa or sublingual. The relative thin buccal layer accelerates drug absorption. Moreover, the drug loss issue is improved as the drug is not subjected to bile, stomach acid and first pass metabolism.

Figure 10 Simplified scheme with different terminologies [Taken from Jain, 2008].

2.2.2 Development of Oral Film Technology

The oral delivery market holds 52% of the market share being the biggest sector in the overall drug market [33]. Around 28% of the population encounters problems of swallowing drugs [34]. The swallowing issue involves synchronized actions of several muscles and nerves. The ability of swallowing drugs is assumed to be safe amongst children as young as 12 years old [35]. Dysphagia is an illness for elderly who has swallowing difficulties [36]. Hence, oral films are developed to cater the need for the pediatric and geriatric

population.

In fact, eight in ten patients have the preference of taking oral dispersible dosage form instead of the conventional solid oral dosages [33]. The oral film is proved to be a prominent commercial platform with a growing rate of 500 million dollars since 2006 and reached 2 billion dollars in 2010 [37]. From the recent market analysis, it revealed that the oral film manufacturers had had rapid revenue growth from 2009 to 2014. A similar tendency is expected at least until 2019 with an annual growth of 17.1% [38].

There is no reliable reference to the date for the appearance of the first oral film. It was not until 2001 when Pfizer introduced Listerine® Pocket Packs® to the market, and oral film concept had finally become a reality. After that, the oral film industry has experienced a significant development.

In 2003, Chloraseptic® Relief Strips, the first drug-loaded oral strip appeared on the market with the function to relief throat pain [39]. In 2004, Triaminic® Thin Strips and Theraflu® Thin Strips were produced to treat cold for young children by Novartis® which was rewarded as the “Best Product of 2004” [40]. In 2010, the launch of the Rx oral film, the ondansetron Rapidfilm® (Setofilm®) and the ondansetron Pharmfilm® (Zuplenz®), aimed to prevent nausea and vomiting, gained 1.9 billion dollars in the same year [41]. One year later, the revenue for Suboxone® thin film was 513 million and occupied 96% of the oral transmucosal film market [42]. In 2012, the income for Suboxone® sublingual film exceeded 1.5 billion and continued to grow [43]. From the information above, it tells the patients’ acceptance of oral films is increasing every year. Also, it shows the great potential for this market.

2.2.3 Methods for Oral Film Fabrication

There are three major conventional methods used to prepare oral films which are: Solvent casting [44, 45], hot melt extrusion [46, 47], and rolling method. The following paragraphs introduce the general procedures for each technique.

For solvent casting, a clear and viscous solution composing of excipients and drugs is firstly prepared. The mixture is then cast on a surface, allowed to dry and then cut into desired shapes.

Hot melt extrusion [48] is a process of applying heat and pressure to melt and shape a pre-dissolved liquid consisting of polymers, drugs and other agents. The liquid is forced through an orifice in a continuous process which is finally moulded into a uniform film. Solvent casting is frequently used. Probably due to the high cost and particular machine needed for hot extrusion method.

A master batch is prepared in advance including a forming polymer, solvent and other additives. The procedures of the rolling method are described as follow. The master batch is controllably fed into the first mixer via the first metering pump. A certain amount of drug is added to the mixer through an opening. Sufficient time is required to achieve a uniform blend of master batch and drugs. This uniform matrix is then deposited on a pan via the second metering pump. The thickness of the film is determined by the metering roller. Eventually, the film is formed on a substrate and carried away through the support roller waiting to be dried [49].

An inventive way of making films is printing technology. Although this technique is widely used for label and identification, it steps into the pharmaceutical industry during the 80s when Anhauser, Klein and Nick et al. used screen printing to load drugs in a transdermal patch. Nevertheless, the low yielding rate limits its large scale production. Inkjet printing was introduced to improve the efficacy of output later by GlaxoSmithKlein (GSK) who holds all intellectual properties of this technology until 2028. Apart from this, flexographic printing [50] is another highly flexible and cost-effective method used for large scale manufacturing.

Compared to conventional methods, printing technology can achieve homogeneous oral films with certain drug loaded inside [35].

So far, no resource has reported using electrospray to make oral films. However, using electrospray some shortages of conventional methods for

preparing film can be avoided. For examples, the drying process can be shortened sharply. Electrospray is a liquid atomization process. Products are almost dried upon reaching the collector. It is also not necessary to cut the film. Instead, electrospray is able to generate films with the required size in a single process. Prepare individual collectors with dimensions according to the stipulations. There is no heating process involved in electrospray, so the bioactivity of active pharmaceutical ingredients can be maintained. Most importantly, electrospray gives high drug encapsulation efficiency. With these excellent points, electrospray is a promising approach to prepare oral films. The production efficiency should be improved as well.

2.2.4 Composition of Oral Film

Oral film normally consists of film forming polymers, plasticisers, active pharmaceutical ingredients, sweetening agents, saliva stimulating agents, flavoring agents, coloring agents, and stabilizing and thickening agents [51].

The general formulation for the oral strip composition is listed below [52].

Drug	5%-30%w/w
Water soluble polymer	45%w/w
Plasticizers	0-20%w/w
Surfactants	q.s.
Sweetening agent	3%-6%w/w
Saliva stimulating agent	2%-6%w/w
Fillers, colors, flavors etc.	q.s

2.2.4.1 Film Forming Polymer

The oral film is a polymeric matrix which consists of one or more than one polymers with distinct physical and chemical properties. Hydrophilic polymers are used for the purpose of achieving the fast disintegration performance. The forming polymer have similar properties to an oral film,

such as disintegration time, drug loading capacity, mechanical strength, handling properties, mucoadhesiveness and so on [35].

Some of the celluloses, starch, semi-synthetic or synthetic polymers are used as film formers. Celluloses, including hydroxypropyl methylcellulose (HPMC), hydroxypropyl cellulose (HPC) and carboxymethylcellulose (CMC) are frequently used.

Native starch has a lack in strength, water resistibility, and thermal stability. Processing with it is difficult [53]. Hence, pure starch films are very brittle and tacky. Starch pre-modification is the solution to the problem. Pullulan, modified starch, is a good choice for polymeric matrix.

Some semi-synthetic or natural polymers are also candidates for film former, such as rosin and rosin derivatives, gelatin, sodium alginate, pectin, gum and so on [54]. In this batch, gelatin is the popular alternative.

For synthetic polymers, polyvinyl alcohol (PVA), polyvinylpyrrolidone (PVP) and polyethylene oxide (PEO) are intensively explored and managed to produce oral films [35].

There are plenty qualified polymers can be chosen for film former either alone or in combination. The criteria should be based on the designed effects and the required properties.

Figure 11 Overall scenario of polymers usage [Taken from Borges et al, 2015].

2.2.4.2 Plasticizers

Plasticizer is used in order to improve the mechanical properties of the oral film by reducing the glass transition temperature (T_g) of the polymer [48]. With the addition of plasticizer, the oral film tends to be less brittle and more flexible. Film mechanical properties are dependent on plasticizer concentration. Typically, plasticizer with 0-20% w/w of dry polymer weight is used to avoid cracking and peeling of the film [55]. The selection of plasticizer is based on its compatibility with the polymer and its solubility in the solvent. Glycerol, polyethylene glycol (PEG) and di-butylphthalate are frequently used plasticizers.

2.2.4.3 Sweetening Agents

The addition of sweetening agents is to enhance the taste of the overall oral film. There are two types of sweetening agents: natural sweeteners and artificial sweeteners. For natural sweeteners, the major sources are from glucose, sucrose, dextrose and so on. The natural sweeteners are restricted to diabetic patients. Hence, artificial sweeteners are more prevalent in food and pharmaceutical preparations. The artificial sweeteners are classified into two categories: I generation (saccharin, aspartame) and II generation (Acesulfame-K, sucralose) [52].

2.2.4.4 Saliva Stimulating Agents

The usage of saliva stimulating agent is to accelerate the disintegration time for oral films. Generally, edible acids are used to stimulate the secretion of saliva, like citric acid, lactic acid and ascorbic acid. At times, sweeteners can also act as salivary stimulants [56].

2.2.4.5 Others

The component of an oral film also includes surfactants, flavoring agents, coloring agents and other optional elements. Surfactants play the role of wetting in the formulation. By adding surfactants, it can achieve a fast dissolution of the oral film thus a rapid release of active therapeutic ingredients. Flavoring agent is added for the taste-masking purpose. The selection of flavoring agents is determined by the preference of targeted population. For example, the elderly prefers mint flavor while young people like fruit flavor [56]. There is a broad range of selection for pigments, like titanium dioxide, EU Colors, Natural Colors and so on [52]. Each ingredient in an oral film should be approved by FDA.

2.3 Drugs Used for Parkinson's Disease

In 1817, Jams Parkinson became the first person to depict the “paralysis agitans” situation which was later renamed Parkinson's disease (PD) [57]. PD is one of the most common neurodegenerative disorder which results from the malfunction and death of dopaminergic neurons (nerve cells that secrete dopamine) in the substantia nigra, an area of the midbrain. Loss of dopaminergic neurons reduces the amount of generated dopamine. In the brain, dopamine as a neurotransmitter has several distinctive pathways one of which is the nigrostriatal pathway. It particularly involves in the production of movement. Because of this, lack of dopamine is the reason of movement-related symptoms such as tremor, slow movement and rigidity.

The number of PD patients in the world is larger than 4 million [58]. In the USA, 500,000 people are believed to have PD with 50,000 new cases reported each year [59]. The situation in Singapore is not bright on account of the significant aging problem. Three out of every thousand individuals, aged 50 years and above, suffer from this disease [60].

2.3.1 Levodopa

The chemical formula of Levodopa is $C_9H_{11}NO_4$ and its molecular weight is 197.19g/mol. Levodopa is hydrophilic as $\log P = -1.66$ [61]. It is sensitive to air and light and degrades or oxidizes quickly. Hence, Levodopa is stored in a freezer using an opaque bottle. The UV detectable peak wavelength for Levodopa is at 280nm [62, 63].

Figure 12 Left is the chemical structure of dopamine and right is the chemical structure of levodopa.

Levodopa can be orally or intravenously administered. Oral intake of levodopa is absorbed along the gastrointestinal tract via the saturable large neutral amino acid transport system. 1-2 hours after administration, the maximum plasma concentration is reached under the fasting condition. The half-life ($T_{1/2}$) of levodopa is 1.5 hours. Levodopa is distributed all over the body with a few amount managed to cross the blood-brain barrier (BBB) through a stereospecific process by the large neutral amino acid transport carrier system. The rest is metabolized via four major pathways reviewed by Nutt and Fellman [64]:

- Decarboxylation by aromatic amino acid decarboxylase;
- Metabolism via catechol-O-methyltransferase (COMT) to form 3-O-methylation (3-OMD) metabolite;
- Transamination by tyrosine aminotransferase
- Oxidation by oxidants like tyrosinase.

The decarboxylation pathway is the most prominent one which accounts for 95% of levodopa's metabolism in the peripheral nervous system [65]. The initial metabolite of decarboxylation is dopamine which can be further broken down into different kinds of acids. Other research results revealed that, via intravenous injection, 10% levodopa underwent COMT process translating

itself to 3-OMD [66].

Levodopa is used for PD based on the concept of dopamine replacement therapy proposed by Hornykiewicz and coworkers from Vienna [67]. In 1960, they identified dopamine deficiency as a characteristic biochemical abnormality in PD. This finding helped to determine that dopamine is a neurotransmitter involved in the normal regulation of movement. More importantly, it gave the rationale and supporting information to the idea of dopamine replacement therapy. However, dopamine cannot be directly used for the treatment of PD because it is unable to cross the BBB. In this case, levodopa was introduced as it is the pro-drug of dopamine and able to enter BBB. A pro-drug is a pharmacologically inert form of an active drug that must undergo transformation to the parent compound in vivo either by a chemical or an enzymatic reaction to exert its therapeutic effect. Once entering the brain, levodopa is metabolized to dopamine and will be released into the synaptic cleft when needed. In this way, the concentration of dopamine in the brain increases.

The levodopa therapy was put into practice one year later when the same group published the paper reporting that small doses of levodopa provided some improvements in 20 PD cases [68]. This observation, together with work done by Carlsson and colleagues [69] showed that levodopa reverses akinesia. Cotzias and coworkers [70] also contributed to the research progress of PD. In 1967, they reported significant improvements in PD cases by using a large amount of levodopa. These studies contributed to the widespread introduction of levodopa as a treatment for PD.

Although levodopa is the most efficient agent in the treatment of PD, it is highly dissipated along the way. Merely 1% [71] orally intake levodopa can enter the brain as it rapidly decarboxylates into dopamine in the peripheral nervous system. Excessive peripheral dopamine results in many adverse side effects such as hypotension, nausea and so on. One standard clinical practice is applied to overcome these side effects by giving a peripheral decarboxylase inhibitor at the same time.

2.3.2 Carbidopa

The chemical formula of Carbidopa is $C_{10}H_{14}N_2O_4 \cdot H_2O$ and its molecular weight is 226.229g/mol. Carbidopa, a white compound, is soluble in water and diluted HCl. It is hydrophilic as $\log P = -1.3$ [61]. Carbidopa is sensitive to air, light and temperature. It needs to be sealed in an opaque bottle and stored in the fridge. The half-life ($T_{1/2}$) of Carbidopa is 1-2 hours. The UV detectable wavelength for Carbidopa is around 280nm [62, 63].

Figure 13 The chemical structure of Carbidopa.

Carbidopa is a decarboxylase inhibitor which inhibits decarboxylation process of levodopa. Thus, prevent the formation of dopamine from levodopa metabolism. With the usage of Carbidopa, the plasma concentration and plasma half-life of levodopa are increased. The plasma concentration of levodopa keeps for 4-5 hours before declining, and the plasma half-life of levodopa prolongs from 50 minutes to 1½ hours [72]. Carbidopa cannot cross the BBB; thus, it inhibits only peripheral decarboxylase. As a result of this, it reduces the side effects caused by excessive dopamine in the peripheral nervous system, as well as increases the concentration of levodopa and dopamine in the brain.

Apart from this, adding Carbidopa reduces the amount of levodopa required to achieve the same therapeutic effect of 80% [72]. The reduction in dosage is extremely useful due to the toxicity that may occur from an overdose of levodopa.

2.3.3 Entacapone

Entacapone is a yellow chemical compound with chemical structure showed below. The chemical formula of Entacapone is $C_{14}H_{15}N_3O_5$ and its molecular weight is 305.286 g/mol. Entacapone is a hydrophobic drug as $\log P = +2.12$ [73]. It is sensitive to air. The half-life ($T_{1/2}$) of Entacapone is 0.4-0.7 hours. The UV detectable wavelength for Entacapone is from 300nm to 390nm based on the pH of the release medium [74].

Figure 14 The chemical structure of Entacapone.

Entacapone is a selective COMT inhibitor. COMT is the primary enzyme responsible for the decomposition of levodopa. The metabolite is 3-OMD which cannot cross the BBB. Neither can Entacapone pass through the BBB.

By adding Entacapone, one of the levodopa metabolic pathways is blocked. As a result of this, the bioavailability of Levodopa is raised. Thus, permit a greater amount of Levodopa stored in the brain, at the same time, increase the amount of available dopamine in the brain.

Figure 15 Levodopa metabolism pathways in peripheral and central nervous systems. Dopamine and 3-OMD are two metabolites of levodopa via DDC and COMT processes, respectively. [Taken from A Abbott, 2010].

Figure 15 briefly summarizes metabolism pathways of Levodopa. Carbidopa and Entacapone cannot cure PD. These two drugs are administered with the purpose to elevate the bioavailability of Levodopa which in turn increases the concentration of Levodopa in the brain. These two drugs also help to cut levodopa intake and weaken side effects caused by excessive peripheral dopamine.

2.4 Metoprolol Tartrate Salt

Metoprolol tartrate salt (MT) is in a white powder form. The chemical formula of MT is $(C_{15}H_{25}NO_3)_2 \cdot C_4H_6O_6$ with the molecular weight of 684.81g/mol. MT has a low melting point, around 120°C. Hence, it is always manufactured in a salt based solution. MT is highly soluble in water and soluble in methanol, chloroform and acetic acid.

Figure 16 The chemical structure of metoprolol tartrate salt.

Metoprolol tartrate belongs to β blockers a type of medication that treats cardiac arrhythmias and hypertension. β blockers are competitive antagonists which block the receptor sites for adrenaline and noradrenaline on β receptors. The interference of the binding activity weakens the effects of stress hormones. MT is known as a selective β_1 blocker which is used to block β_1 receptor located mainly in the heart and kidney. By taking MT, heart rate, blood pressure and strain on the heart can be suppressed [75, 76].

MT can be easily absorbed (95%) [77,78] through the oral administration. The peak plasma concentration reaches after 2-3 hours [79]. The first-pass metabolism of MT is obvious as it experiences 50% loss of [77, 78] bioavailability in the liver. The plasma half-life is near 3-4 hours [80].

2.5 Poly (Lactic-Co-Glycolic Acid)

Poly (lactic-co-glycolic acid) is the most commonly used biodegradable and

Figure 17 The chemical structure of PLGA.

biocompatible copolymer approved by FDA. PLGA is utilized to make suitable drug delivery carriers because of its long clinical experience, appropriate degradation behaviors and tunable chemical and physical properties. The synthesis of PLGA is through a ring-opening copolymerization of two different monomers, glycolic acid and lactic acid. During the polymerization process, successive monomers are linked together via an ester linkage.

Poly lactic acid (PLA) is biodegradable polyester containing an asymmetric α -carbon which is known as the D or L form. Poly-L-lactide (PLLA) is the highly crystalline form of PLA while Poly-D-Lactide (PDLA) is the completely amorphous form of PLA. Poly glycolic acid (PGA) is a linear polyester possessed with high crystallinity because of the absence of methyl side group [81].

Figure 18 The left is the chemical structure of PLA and the right is the chemical structure of PGA.

By tuning the ratio of lactide to glycolide used for polymerization, various forms of PLGA can be obtained. The composition affects crystallinity, hydrophilicity and biodegradation rate of PLGA. PGA is hydrophilic while

PLA is hydrophobic. PLGA tends to be hydrophilic with a higher percentage of PGA. The presence of PGA accelerates water intake and facilitates hydrolysis process. As a consequence, fast degradation rate and rapid drug release rate can be achieved. Experimental results also prove that increase the glycolic acid percentage in the oligomers accelerates the weight loss of polymer. Among all the combination, PLGA 50:50 (PLA/PGA) exhibits the fastest degradation [82].

PLGA has a good solubility in many solvents such as chlorinated solvents, tetrahydrofuran, acetone or ethyl acetate [83]. The degradation process of PLGA contains bulk diffusion, bulk erosion, surface diffusion, and surface erosion. The degradation process depends on various parameters like the molar ratio of lactic to glycol acid, the degree of crystallinity, the molecular weight of the polymer and the Tg of the polymer. In vivo, PLGA degrades by hydrolyzing its ester bonds producing biocompatible, toxicity-free side products that can be further eliminated via normal metabolic pathways.

2.6. Hydroxypropyl Methylcellulose

Hydroxypropyl methylcellulose (HPMC) is the frequently used cellulose for oral film fabrication. The chemical structure of HPMC is illustrated in figure 19. It has the polymeric backbone of cellulose containing a basic repeating structure of anhydroglucose unit [84]. The substituent R represents either a CH_3 , or a H , or a $\text{CH}_2\text{CH}(\text{CH}_3)\text{OH}$ group.

Figure 19 The chemical structure of HPMC.

HPMC is white and odorless powder. It is soluble in water and in some mixed solvents like water and ethanol mixture, water and propanol mixture. But it is insoluble in chloroform.

The USP distinguishes and categorizes HPMC into four groups by using four digits with the first two digits indicating the percentage of $-\text{CH}_3$ groups and the last two digits telling the percentage of $-\text{CH}_2\text{CH}(\text{CH}_3)\text{OH}$ groups. The average level of $-\text{CH}_3$ substitution on the polymeric chain is designated as the degree of substitution. And molar substitution represents the average level of $-\text{CH}_2\text{CH}(\text{CH}_3)\text{OH}$ substitution on the cellulose chain. The degree of substitution determines some properties of HPMC such as solubility and thermal gelation temperature of the solution [84].

According to the Dow Chemical Grade classification, there are two types of HPMC usually used for oral film preparation: type K and type E. Type K, consisting of 22% $-\text{CH}_3$ and 8.1% $-\text{CH}_2\text{CH}(\text{CH}_3)\text{OH}$, [85] is often used to fabricate drug carriers with controlled or delayed drug release profile. On the other hand, type E composed of 29% $-\text{CH}_3$ and 8.9% $-\text{CH}_2\text{CH}(\text{CH}_3)\text{OH}$ is utilized as an excipient for oral film fabrication [86].

The mechanical properties of HPMC are significantly influenced by the degree of substitution and its intrinsic viscosity. HPMC with high viscosity and degree of substitution is more likely to produce stiff and extensible films. High viscosity implies high molecular weight or long chain length. Large strength input is needed to disrupt the chain entanglements. Besides, compared to the original hydroxyl group, the dimension of the methoxyl group is larger

providing an anchoring effect on HPMC chains giving a high tensile strength. The degree of substitution does not show an exact impact on Young's modulus [35].

The overall mechanism for drug release from the HPMC-based system is hydration followed by HPMC swelling then drug dissolution and diffusion. Upon contact with the release media, it diffuses into the matrix, leading to the chain relaxation and volume expansion [87]. $-\text{CH}_2\text{CH}(\text{CH}_3)\text{OH}$ is a hydrophilic group facilitating the rate of hydration. $-\text{CH}_3$ is a hydrophobic group. Hence, HPMC with less $-\text{CH}_3$ substituent will establish a gel barrier rapidly which will, in turn, delay the drug release from an oral film [88].

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Chapter 3

Experimental Methodology

This chapter introduces materials used to synthesize two types of films. The main materials used to synthesize the Parkinson's disease (PD) drugs loaded thin film are poly (lactic-co-glycolic acid), chloroform and acetone. The main materials used for fabrication of Metoprolol tartrate salt (MT) loaded oral film are hydroxypropyl methylcellulose, glycerol and PEG400. The method used to produce PD drugs loaded thin film is electrospray technique. Solvent casting and electrospray technique are used to synthesize MT loaded oral film. Characterization technique such as scanning electron microscope (SEM) is applied for identification of size and morphology of final products. Differential scanning calorimetry (DSC) is used to evaluate the thermal properties of oral films. Folding test, mechanical test and disintegration test are applied to know the physical properties of oral films. High-performance liquid chromatography (HPLC) is used to examine the drug release profile and drug encapsulation efficiency.

3.1 Introduction

The choice of technique used to fabricate films is not limited. Electrospray is selected because of its unique advantages. Nowadays, people are longing for novel and simple process. Comparing to other techniques, electrospray this one-step method is much appreciated. It needs to be emphasized that electrospray can load hydrophobic and hydrophilic drugs at the same time. Cost-effective is another particular merit of electrospray technique.

Reasons for using PLGA and HPMC in this project are adequate. First, both of them are FDA approved materials. PLGA is a biodegradable and biocompatible polymer while HPMC is frequently used cellulose. The long clinical experiences for both materials further prove that PLGA and HPMC are safe and mature candidates for the fabrication of drug carriers. Secondly, the composition of PLGA and HPMC can be modified easily. For PLGA, the molecular weight and the ratio of lactide to glycolide can be altered easily. The physicochemical properties of PLGA are changed accordingly. Various batches of PLGA increase the chance of producing ideal drug carriers. The same situation applies to HPMC. There are numerous grades of HPMC classified by the degree of substitution. The physiochemical properties of HPMC vary according to the amount of methoxyl and hydropropoxyl groups represented. Apart from this, it is easy to purchase different compositions of PLGA and HPMC on the market.

This project is designed first to understand the role that some involved factors played in altering the final morphology of the electrospray products. The achieved regulations are used as a guidance to optimise final recipes.

Next, films loaded with PD drugs and MT are undergone several characterization tests to evaluate film properties and drug release performances. Thin films encapsulated with Parkinson's disease drugs, SEM and HPLC tests are utilized. Oral films loaded with Metoprolol Tartrate Salt, folding, dissolution, mechanical, SEM, differential scanning calorimetry (DSC) and HPLC tests are applied.

3.2 Materials

In this research work, PDLG 5010, a copolymer of Lactide and Glycolide in a 50/50 molar ratio with an inherent viscosity of 1.0dl/g, was purchased from Purac[®]. The METHOCEL[™] E3, E5, E6 and E15 Premium LV Hydroxypropyl Methylcellulose were free gifts from the Colorcon[®] company. Gum Arabic was a free gift from a bakery store. Hydroxypropyl cellulose and gelatin were purchased from Sigma-Aldrich. Drugs like Levodopa (Sigma-Aldrich), Carbidopa (Sigma-Aldrich), Entacapone (European Pharmacopoeia Reference) and Metoprolol Tartrate Salt (Sigma-Aldrich) were purchased directly from the supplier. Solvent such as chloroform, acetone, acetonitrile, DCM and methanol were merchandised from Tedia Co.Inc. HCL solution (37% v/v Fuming), Tween 20, Tween 80, Phosphate buffered saline (PBS), Acetic Acid, Monopotassium phosphate (KH_2PO_4) and Sodium hydroxide(NaOH) were purchased from Sigma Aldrich. Plasticizers like glycerol and PEG 400 were also bought from Sigma-Aldrich. Sodium Dihydrogen Phosphate (NaH_2PO_4) was bought from Emsure. Distilled water (DI water) was used. All the mentioned polymers, drugs and solvents were used in the experiment as received unless noted otherwise.

3.3 Synthesis of PD Drugs Loaded Thin Film

3.3.1 Solution Preparation

To learn the role of some important factors that played in determining the size and morphology of final products made by electrospray technique, pre-mixed electrospray solutions were prepared beforehand.

Chloroform was the solvent of choice. 1%-6% (w/v) PDLG 5010 was dissolved in it. A 10ml solution was prepared for each concentration of PDLG 5010. A magnetic stir was placed alongside the polymer and solvent. The mixture was made to stir overnight to become homogenous.

The solvent changed to acetone, when preparing the drug loaded electrospray solution. This is because the PD drugs have high solubility in acetone. Also, comparing to chloroform, acetone is less toxic.

1%-3% (w/v) PDLG 5010 was dissolved in acetone. A 10ml solution was prepared for each concentration of PDLG 5010. A magnetic stir was placed alongside the polymer and solvent and the mixture was made to stir overnight for the solution to become homogenous. This experiment is aimed to find the optimized condition for getting monosized microparticles.

Drug-loaded polymeric solutions were prepared by adding 0.1% (w/v) of each Levodopa, Carbidopa and Entacapone in 10ml acetone together with 3% (w/v) PDLG5010. The bottle was covered with aluminum foil to avoid drug degradation. A magnetic stir was placed alongside the polymer and solvent and the mixture was made to stir overnight for the solution to become homogenous.

The following solutions were prepared for the usage in conducting drug encapsulation efficiency experiment and drug release study when doing HPLC. The drug release media were PBS, simulated gastric fluid (SGF) and simulated intestinal fluid (SIF). The HPLC mobile phase was not the same for all PD drugs.

PBS was prepared by using 900ml pH=7.4 PBS solution to dissolve 0.5% (v/v) Tween 80. The mixture was made to stir overnight for later usage.

SGF was made by dissolving 2.61ml fuming HCL and 0.1g Tween 20 in 500ml DI water. The mixture was made to stir overnight. The pH value of SGF, was tested using a pH probe.

SIF was prepared by dissolving 0.02% (w/v) Tween 80 in phosphate buffer whose pH is 6.8. The mixture was made to stir overnight. The phosphate buffer was made by adding 6.8g KH_2PO_4 and 0.94g NaOH in 1000ml DI water. The pH value of phosphate buffer was tested using a pH probe.

Based on the work done by Gandhi [1] and Vemić [2], HPLC can separate Levodopa and Carbidopa under the same system. The mobile phase used to distinguish Levodopa and Carbidopa was 100% acetic acid (2% v/v), which was made by dissolving 36ml acetic acid in 1800ml DI water. The mixture was sonicated for 15-30 mins before usage.

The mobile phase used to elute Entacapone was 60% Sodium Di-hydrogen Phosphate/40% Methanol Mixture, which was prepared by adding 20g NaH_2PO_4 in 2L DI water then topped up with methanol according to the ratio. The mixture was filtered and sonicated before usage.

3.3.2 Electrospray

The electrospray machine used in this project is NANON-01A from MECC CO., LTD. The specification of the machine is stated. The range of applied voltage is from 10kV to 30kV; the range of the distance from the nozzle tip to the collector is from 10cm to 14cm and the range of the flow rate is from 0.1ml/h up to hundreds ml/h.

The prepared electrospray solution (solvent: chloroform) was fed into a plastic syringe attached to a metal nozzle. An electric field was established between the syringe and collection plate covered with an aluminum foil. Parameters such as polymer solution concentration, applied voltage, flow rate and distance from the needle tip to the collector were played and studied. The rational for setting each parameter is based on equipment limitations and research work done by P. Fattahi [3].

The conditions to be examined are listed in the figure below. In total, there are: 540 ($6*6*3*5=540$) combinations.

Table 1 Electrospray conditions for particles fabrication using chloroform as the solvent.

Parameters	Polymer concentration (%)	Flow rate (ml/h)	Distance (cm)	Applied voltage (kV)
Conditions	1	0.1	10	10
	2	0.5	12	15
	3	1	15	20
	4	1.5		25
	5	2		30
	6	2.5		

The setup for this experiment is similar to the previous one, except for the used electrospray solution (solvent: acetone). The overall experimental conditions are listed in the figure below. In total, there are 180 ($3 \times 5 \times 3 \times 4 = 180$) combinations.

Table 2 Electrospray conditions for particles fabrication using acetone as the solvent.

Parameters	Polymer concentration (%)	Flow rate (ml/h)	Distance (cm)	Applied voltage (kV)
Conditions	1	0.1	10	10
	2	0.5	12	15
	3	1	15	20
		1.5		25
		2		

3.3.3 Drying Process

This step is aimed to dry the residue solvent if any. It is necessary as the wet particles give inconsistent shapes and cannot be used for SEM test as SEM samples must be in a dry form. Two frequently used drying methods: freeze

dry and vacuum dry were used and compared.

Freeze dry was conducted by collecting electrospray samples and putting them in a tube filled with DI water. The tube was placed in a freezer for freezing process. After several hours, the frozen tube was transferred to a manifold freeze-drier. The frozen water in the sample sublimated directly from the solid phase to the gas phase.

Another option was vacuum dry where the collecting samples were placed in a fume hood for couple of hours aiming to dissipate the residue solvent.

3.4 Synthesis of MT Loaded Oral Film

The first step of MT loaded film synthesis was formulation screening. Upon confirming the recipe used for producing a blank film, the mixed solution was then fed into the syringe for electrospray. The collected samples were then vacuum dried for further usage.

3.4.1 Selection of Polymer

According to the literature review, cellulose, modified starch and semi or fully synthetic polymers are usually used as film formers. In this experiment, HPMC (E5), Gelatin and Gum Arabic were studied and selected.

2g of each polymer was dissolved in 30ml DI water. Polymer dissolution is a slow process including polymer swelling followed by dissolution. Polymers mentioned above need to wait around 6 hours to achieve clear solutions. The stirring is not necessary for this process. On the contrary, to avoid bubbling, it is preferred not to stir the solution.

3.4.2 Selection of HPMC Type

As there are plenty grades of HPMC. This experiment is designed to choose the most suitable type of HPMC. The candidates of HPMC were selected referring to the work done by Chen Fang et al. [4, 5, 6] which are: E3, E5, E6 and E15. Either uses it alone or in combination. The ingredients for each condition are summarized in the table below.

In each condition, 2g of the polymer was dissolved in 30ml DI water. Wait at least 6 hours to achieve a clear solution. During the process do not stir the solution to avoid bubbling.

Table 3 Experimental conditions for selection of HPMC type.

Sample	E3 (g)	E5 (g)	E6 (g)	E15 (g)	Water (ml)
1	2				30
2				2	30
3	1			1	30
4		2			30
5			2		30
6		1	1		30

3.4.3 Selection of Plasticizer

The plasticizer was added to enhance the mechanical properties of the film. The type and amount of plasticizer were examined and selected. In this experiment, glycerol and PEG 400 were used for comparison. The experimental conditions are concluded in the table below. The amount of each ingredient added is within the range of the corresponding composition amount for oral film preparation showed in the previous literature review.

Table 4 Experimental conditions for selection of plasticizer.

Samples	E3 (g)	E5 (g)	E6 (g)	E15 (g)	Glycerol (g)	PEG 400 (g)	DI water (ml)
1		1	1		0.1		30
2		1	1		0.2		30
3		1	1		0.4		30
4		1	1		0.6		30
5		1	1			0.1	30
6		1	1			0.2	30
7		1	1			0.4	30

3.4.4 Selection of Amount of Drug Loaded

The amount of drug loaded in the film was evaluated to figure out the maximum drug loading capacity and the optimum drug amount which can give a good drug release profile. The experimental conditions are summarized in the table below.

Table 5 Experimental conditions for selection of amount of drug loaded.

Samples	E5 (g)	E6 (g)	Glycerol (g)	PEG 400 (g)	MT (g)	DI water (ml)
1	1	1		0.2	0.1 (4.3%)	30
2	1	1		0.2	0.2 (8.3%)	30
3	1	1		0.2	0.4 (15.4%)	30
4	1	1	0.4		0.1 (4.3%)	30
5	1	1	0.4		0.2 (8.3%)	30
6	1	1	0.4		0.4 (15.4%)	30

3.4.5 Electrospray

The electrospray condition was fixed for all the solution used to make blank and MT loaded oral films: applied voltage = 30kV, distance from the needle tip to the collector = 14cm, flow rate = 20ml/h. The reason for choosing these processing parameters is determined by the knowledge and principles from the literature review and previous experimental results.

3.4.6 HPLC Mobile Phase for MT

The mobile phase used to analyze MT was 30% 25mM KH_2PO_4 /70% Acetonitrile mixture [7]. It was prepared by adding 2.04g KH_2PO_4 in 600ml DI water topped up with 1400ml Acetonitrile according to the ratio. The mixture was filtered and sonicated before usage.

3.5 Characterization

3.5.1 Scanning Electron Microscope

3.5.1.1 Principles

SEM, an electron microscope, produces images by scanning the sample surface using a focused beam of electrons. Signals generated from electron-sample interactions form images on the screen. The backscatter electron imaging (BEI) and the secondary electron imaging (SEI) modes are utilized in SEM. The BEI mode is to detect backscattered electrons which give information to distinguish different components in a multicomponent material. For SEI mode, it detects and collects secondary electrons providing information regarding specimen morphology.

3.5.1.2 Operations

The size and morphology of the sample were viewed under Scanning Electron Microscopy (SEM, JEOL JSM_6360A and JSM-5410) at 5kV with 10cm working distance. Prior to analysis, samples were fixed on the sample holder using a carbon tape. To avoid charging effect, Copper tape was pasted on the sample holder. Subsequently, the whole thing was coated with gold using a sputter coater (SPI-Module) for 60 seconds. The images of samples were recorded and analyzed offline.

3.5.2 Folding Test

Folding endurance is another attribute to be evaluated. The purpose of this test is to select the proper formulations which give nice integrity of the film. This property makes sure the oral films can be safely handled and packed.

The folding test was done by cutting 2cm (length)*1cm (width) of each selected film. The film was folded in a repeated way using same strength until it breaks. The folding time was counted and compared.

3.5.3 Disintegration Test

Disintegration test is aimed to know the time needed for a certain amount of film getting dissolved in the release medium. It is an important criterion for oral film formulation selection.

In this experiment, the apparatus was a 37°C water bath. 15ml DI water was filled in every glass bottle with a magnetic stirrer bar inside. The stir rate was 250rpm. 50mg of each selected oral film was weighed carefully and put in the glass bottle. The glass bottles were placed in the water bath. Under the same experimental condition, the duration of each film to get fully dissolved was

recorded and compared.

3.5.4 Mechanical Test

3.5.4.1 Principles

Appropriate physical strength is one of the most critical attributes of oral films. Although, there is no strict guidance of which parameters should be tested for this quality evaluation, a consensus from literature reflects that elongation at break and tensile strength are two important determiners in this aspect.

It is desired to have an oral film with a good balance between these properties. A film should have sufficient tension to survive in all processes. However, too much tension is not preferred as it may be difficult to cut the film. Nevertheless, the possessed mechanical property of oral films is not only important during the product life-time but also necessary for the up-scale manufacturing process. Since, all the processes from coiling to packaging demand film robustness [8].

From this test, the value of tensile strength (σ) and tensile strain (ϵ) can be achieved. Tensile strength measures the maximum stress that a strip can bear while being pulled apart before breaking. The unit of tensile strength is Pa or MPa. Tensile strain measures the ratio of film extension to the original film length at the breaking point. There is no unit for tensile strain. The formulations used to calculate the above two values are shown below.

$$\text{Tensile Strength} = \frac{\text{force at break}}{\text{initial cross section area of the film}}$$

$$\text{Tensile Strain} = \frac{\text{Increase in length}}{\text{original length}} * 100\%$$

3.5.4.2 Operations

Mechanical properties were tested using Mech Tester Instron 5567 equipped with a 5kg load cell. Films were held in between two clamps. The distance between the two clamps was kept the same which was 1.8cm. The sample was cut into a dimension of 4cm (length)*1cm (width). The film thickness was determined by the average thickness value from three random points measured by a caliper. During measurements, the strip was pulled at the rate of 15mm/sec.

3.5.5 Differential Scanning Calorimetry

3.5.5.1 Principles

Differential Scanning Calorimetry (DSC) belongs to a thermal analysis method. It measures the heat flow difference between a sample and a reference to maintain at the same temperature.

Figure 20 An idealized DSC curve showing the shapes associated with particular phase transition.

An exothermic process is where to raise the temperature at the same rate; less

heat is needed for the sample than for the reference. On the contrary, if more heat flows to the sample, it is an endothermic process. The endothermic process includes melting (solid phase to liquid phase). The exothermic process comprises crystallization (amorphous solid to crystalline solid). DSC can detect subtle physical change too such as glass transitions. There is no formal phase change during this process. Instead, heat capacity changes [9].

3.5.5.2 Operations

The DSC was recorded for selected samples. The weight of the sample was measured accurately and the value was input in the system. The sample was prepared by sealing it in a standard aluminum pan. At the same time, an empty aluminum pan was also made as the reference for calibrating the DSC temperature and enthalpy scale. All the DSC experiments were done under the same condition. In the beginning, the system temperature was maintained at 10°C for 2mins. After that, the system was heated at a constant rate 10°C/min from 10°C up to 300°C. N₂ was used as the purging gas. The experimental results were analyzed offline using the software from DSC TA instrument Q10.

3.5.6 High-Performance Liquid Chromatography

3.5.6.1 Principles

HPLC is a powerful technique used in analytical chemistry. It can separate, identify and quantify each component in a mixture [10].

HPLC consists of a sampler, pumps, and a detector. The mobile phase is pumped into the system by a high-pressure pump. The sample is extracted by the sampler and added to the mobile phase. The mobile phase together with the sample is flushed into the stationary phase which is the column. Each sample component has different partition coefficient between mobile phase

and stationary phase. The sample experiences multiple absorptions and desorptions during the relative phase movement. The velocity of each element in the sample passing through the column is different. The elution time also named retention time for each component can be differentiated. Hence, each component can be recognized by the characterized elution time.

The underlying working principle for a UV/Vis detector is based on the fact of characteristic light absorption and Beer-Lambert Law. Each analyte selectively absorbs a particular wavelength. It can be detected by the UV/Vis detector. The information is translated into an electrical signal recorded and transferred to a recorder. Then, a unique UV peak appears on the chromatography at its corresponding elution time point. The peak area is proportional to the analyte concentration. The UV-Vis detector is typically used as it is responsive to most organic compounds, with broad detection range and high sensitivity.

3.5.6.2 Operations

HPLC characterization is used to differentiate each element presented in the sample and get to know its corresponding amount. Before HPLC analysis, pre-sample and mobile phase preparations are necessary to avoid column clogging or contamination.

Pre-sample preparation instruction is illustrated below. Use a syringe to extract 1ml sample solution. Attach the syringe with a 0.22 μ m filter. Push the solution through the filter to a brown HPLC vial. Make sure the amount of solution in the vial is more than 0.5 μ l. Cap and cramp the vial. Place the vial in the sample tray according to the set sequence. Next, load the mobile phase (HPLC analysis standard). Set up the processing parameters. After that, start to wash and stabilize the system. Once everything is done, run the system. In the end, collect and analyze the data offline.

3.5.7 Drug Encapsulation Efficiency

3.5.7.1 Principles

Extraction is always used to measure the drug encapsulation efficiency and drug loading capacity. First, weigh the sample and calculate the theoretical value of drug loading assuming that drugs are evenly distributed in the film. Then, dissolve the sample in the release media like PBS, SGF, SIF or just water. There are plenty ways to separate the drug from the polymeric matrix. In this project, both PD's drugs loaded films and MT loaded films use polarity difference and solubility difference to extract drugs. Add certain organic solvent to the solution and vortex the mixture for seconds. Take the drug from the most precipitated phase. Remove the phase through evaporation or other methods (this step is optional). Finally, use mobile phase or release media to dissolve the precipitates.

Encapsulation efficiency calculates the mass of therapeutic agent actually loaded in particles (m_{Load}) as a fraction of the initial mass of the particles(m_{Total}).

$$EE = \frac{m_{Loaded}}{m_{Total}}$$

EE is affected by the type of polymer, the weight ratio of polymer to therapeutic molecules and flow rates. Meanwhile, high loading causes a high burst release [11] but a low EE [12] which results from the partition coefficient referring to the drug equilibrium solubility in the polymer against with the drug equilibrium solubility in the solvent. To improve EE, Plutonic F-127 a surfactant can be added.

3.5.7.2 Operations

3.5.7.2.1 Levodopa

Weigh assured amount of film loaded with Levodopa and dissolve in 10ml SGF via sonication. The mixed solution was well shaken and placed for a while. After the film was fully dissolved, 1ml supernatant was extracted from SGF phase as Levodopa is hydrophilic. The new solution was pushed through a 0.22 μ m filter into an HPLC vial. It was later analyzed by HPLC using mobile phase (2% (v/v) acetic acid) at 1.2ml/min, wavelength 284nm.

3.5.7.2.2 Carbidopa

The drug loading analysis method for Carbidopa is almost the same as Levodopa. Indeed, the analysis of Carbidopa and Levodopa can be done simultaneously. Weigh assured amount of film loaded with Carbidopa and dissolve in 10ml SGF via sonication. The mixed solution was well shaken and placed for a while. After the film was fully dissolved, 1ml supernatant was extracted from SGF as Carbidopa is hydrophilic. The new solution was pushed through a 0.22 μ m filter into an HPLC vial. It was later analyzed by HPLC with mobile phase (2% (v/v) acetic acid) at 1.2ml/min, wavelength 284nm.

3.5.7.2.3 Entacapone

Weigh a certain amount of film loaded with Entacapone and dissolve in 10ml SGF together with 1ml DCM via sonication. After the film was fully dissolved, the DCM phase was fully extracted and placed in a vacuum oven for evaporation as Entacapone is hydrophobic. The dried Entacapone was dissolved in 1ml mobile phase. The new solution was pushed through a 0.22 μ m filter into an HPLC vial. It was later analyzed using HPLC with mobile phase (60% Sodium Dihydrogen Phosphate/40% Methanol mixture) at 1.2ml/min, wavelength 284nm.

3.5.7.2.4 Metoprolol Tartrate Salt

Weigh assured amount of film loaded with MT and dissolve in 2ml DI water via sonication. After the film was fully dissolved, solvent extraction method was applied by adding 5ml chloroform into the solution. The mixed solution was well shaken and placed for a while. The chloroform phase was fully extracted and placed in a vacuum oven to evaporate chloroform. The dried MT was dissolved in 2ml mobile phase. The new solution was pushed through a 0.22 μ m filter into an HPLC vial. It was later analyzed using HPLC with mobile phase (30% 25mM KH_2PO_4 /70% Acetonitrile mixture) at 1ml/min, wavelength 260nm [7].

3.5.8 In Vitro Drug Release Study

3.5.8.1 Principles

There are three typical drug release profiles named: conventional release, sustained (first-order) release and controlled (zero-order) release shown in figure 21.

The conventional release is characterized by an apparent burst release and short drug release time. To extend the duration of drug release and reduce the frequency of dosing, sustained release is introduced which gives a prolonged or delayed release [13]. It is also called the first-order release as the rate of process is linearly proportional to the drug concentration. Controlled release is the most sophisticated way for drug delivery. It is known as the zero-order release as it has a constant drug release rate independent of the drug concentration and local environment.

The drug release profile elucidates the accumulative drug release amount with respect to time. The timescale depends on the drug's pharmacokinetics properties. There are usually seven or more data points that contribute to the release figure.

Figure 21 Drug release profile for zero-order controlled release, sustained release and conventional release.

3.5.8.2 Operations

3.5.8.2.1 PD Drugs Loaded Film

The media used for drug release study for PD drugs loaded films were PBS, SGF together with SIF. 10mg electrospray film was weighed and dissolved in 10ml PBS and 10ml SGF. All samples were incubated in a water bath at 37°C under magnetic rotation at 250rpm. At different time points, extract 5ml solution without destroying the films as kinetics samples. Immediately fill in 5ml of the same medium after the extraction.

The extraction time points for samples in PBS were: 1hr, 2hr, 3hr, 4hr, 5hr, 6hr, 10hr, 24hr, day5, day7 and day 9. The extraction time points for samples in SGF were: 15mins, 30mins, 1hr, 2hr, 3hr and 4hr. Right after extraction from 4hr time point, use DI water to clean electrospray film in SGF and replace the medium to SIF. Extract the sample after one hour which was denoted as 5hr, followed by 6hr, 8hr, 10hr, day1, day5, day7 and day9. Every obtained solution was analyzed using HPLC.

The above mentioned media were used for Levodopa and Carbidopa as they are hydrophilic drugs. However, Entacapone is a hydrophobic drug. The extraction time for Entacapone is the same as the other two drugs. The only difference is the release medium which was changed to 60% Sodium Dihydrogen Phosphate/40% Methanol Mixture.

3.5.8.2.2 MT Drug Loaded Film

MT loaded films were released in 100ml DI water. One and a half pieces of film were weighed accurately. The film was incubated in a 37°C water bath under magnetic rotation at 100rpm [14]. At different time points, 2ml supernatant was extracted and transferred to a plastic tube as kinetics samples. Then, immediately add another 2ml DI water to the water bath after each extraction. The time points chosen for extraction were: 1min, 5mins, 19mins, 15mins, 20mins, 30mins and 60mins.

Add 5ml chloroform to the plastic tube. Shake the extracted solution together with chloroform for 60s to get a well-mixed solution. The tube was placed for a while until seeing a clear partition. A syringe attached to a needle was used to extract the chloroform phase fully. The obtained solution was transferred into a glass bottle and was placed in a vacuum oven. The chloroform dissipated after 10-15mins. The precipitate was dissolved in 2ml mobile phase solution. The new solution was filtered using a 0.22µm filter to an HPLC vial ready to be analyzed later.

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Chapter 4

Results and Discussion

An optimal system using electrospray technique to fabricate oral films is achieved. The obtained oral film exhibits a fast-dissolving pattern and high drug encapsulation efficiency. The disintegration time of the electrospray oral film is 2.5 times faster than the oral film made by solvent casting. The drug encapsulation efficiency for the electrospray oral film is 99.29%. These two outstanding attributes show the possibility of using electrospray technique to fabricate oral films. Polymer concentration, flow rate, applied voltage and the distance from the nozzle tip to the collector in the way of controlling the results of electrospray products are discussed. The optimal conditions of fabricating uniform microspheres are hereby determined.

4.1 Particles Fabrication

The purpose of the series of experiments in the particle fabrication is to evaluate how each factor controls the result of the electrospray products. The optimised conditions of fabricating uniform microspheres are hereby determined.

4.1.1 Polymer Concentration

In this segment, the polymer concentration (C %) is the single variable while the other experimental conditions are fixed. The electrospray solution was prepared by dissolving different amounts of PDLG5010 polymer in 10ml chloroform. The electrospray processing conditions are summarized: Flow rate=1.5ml/h, distance from nozzle tip to the collector=10cm, applied voltage=15kV and the needle size=0.6mm. The obtained results were analyzed using SEM.

The SEM images in Table 6 show that the polymer solution concentration strongly affects the morphology of the electrospray results. Small cracks were formed when a 1% PDLG5010 solution was used while flatten microspheres were produced from 2% PDLG5010 solution. As the polymer concentration increased to a value of 3%-4%, microspheres were formed. When polymer concentration was 5%, conjugated microspheres with some concomitant nanofibers were produced. Further increasing the polymer concentration to 6%, a new structure bead-on-fiber was achieved. This phenomenon indicates that low polymer concentration tends to form microspheres while higher polymer concentration tends to form fibers. In between the transitional phase from microspheres to nanofiber structures, the bead-on-fiber structure is formed.

Table 6 Study of the polymer concentration in altering the morphology and size of particles.

SEM picture
C%	1%	2%
SEM picture
C%	3%	4%
SEM picture
C%	5%	6%

This transition phenomenon can be explained using the concept of entanglement number in solution $n_{e(sol)}$ proposed by Shenoy et al [1] where a

semi-empirical model was developed to predict the transition process from electrospray to electrospinning.

$n_{e(sol)}$ equals to the polymer average molecular weight M_w divides solution entanglement molecular weight $(M_e)_{soln}$. $(M_e)_{soln}$ is defined as the average molecular weight between two adjacent entanglements.

$$(n_e)_{soln} = \frac{M_w}{(M_e)_{soln}} = \frac{(\phi_p M_w)}{M_e}$$

In the polymer solution, polymer concentration (ϕ) and molecular weight affect the number of chain entanglements. The experimental results showed, $n_{e(sol)} = 2$, is the upper limit for pure particles formation. The lower limit for pure fibers formation is $n_{e(sol)} = 3.5$. Between these two boundaries, a mixture of fibers and particles are formed. This theory is in agreement with the experimental results.

Begoña Almería [2] used the polymer solution concentration at Rayleigh limit (ϕ_{ray}); the overlap volume fraction ($\phi_{ch.ov}$) and the entanglement volume fraction (ϕ_{ent}) to describe electrospray process. ϕ_{ent} is larger than $\phi_{ch.ov}$ because chain overlap is the onset of chain entanglement. These parameters can be utilized to explain scenarios in this experiment as well.

When the polymer concentration is 1%-2%, meaning $\phi_{ray} < \phi_{ch.ov}$, there is no chain overlap in this diluted solution, not to mention chain entanglement. Hence, Coulomb fission takes place giving charged offspring with irregular size and shape.

When the polymer concentration is 3%-4%, implying $\phi_{ray} > \phi_{ent}$, there are sufficient polymer chain entanglement before reaching the Rayleigh limit. The enhanced surface tension holds droplets from rupture and suppresses droplet deformation during Coulomb fission. Thus, uniform particles are produced.

When the polymer concentration is 5%-6%, indicating $\phi_{ray} \gg \phi_{ent}$, the solution is overly saturated. The Coulomb fission cannot overcome surface tension. As

a result, the liquid cannot be split, only stretched. Hence, fibers are formed.

Judging from the SEM images, 3wt% PDLG5010 polymer solution is the optimal condition in obtaining regular microspheres.

4.1.2 Flow Rate

This experiment is designed to study the function of flow rate in altering the electrospray results. In this regard, the flow rate is the single variable while the other experimental conditions are fixed. The electrospray solution was prepared by dissolving 3wt% PDLG5010 in chloroform. The electrospray processing conditions are summarized: Distance from the nozzle tip to the collector=10cm, applied voltage=15kV and the needle size=0.6mm. The obtained results were analyzed using SEM.

From Table 7, it can be seen that when the flow rate of the polymer solution is set at 1ml/h, small amount of porous microspheres of size 4.37 μm were formed. As the flow rate increases from 1ml/h to 1.5ml/h, the average diameter of microspheres also increases from 4.37 μm to 4.6 μm . Although there is a slight variation in the size of the microspheres, the amount obtained was significantly different; there are more microspheres formed in a 1.5ml/h flow rate than in a 1ml/h.

Further increasing the flow rate to 2ml/h, show no deviation in the pattern of the microsphere size. The average size of the microspheres obtained at 2ml/h flow rate is 5 μm . At this point, clusters of microspheres start to appear. When the flow rate is 2.5ml/h, bimodal size distribution of microspheres together with lumps was formed.

Table 7 Study of the flow rate in altering the morphology and size of particles using chloroform as the solvent.

SEM picture
Flow rate	1ml/h	1.5ml/h
SEM picture
Flow rate	2ml/h	2.5ml/h

By increasing the flow rate from 1ml/h to 2.5ml/h, the diameter of formed microspheres varied from $4.37\mu\text{m}$ to $7.4\mu\text{m}$, respectively. It can be concluded that microspheres grew in size as the flow rate increased. When the flow rate sharply reduced to 0.1ml/h, nanospheres can be formed showed in table 8.

By increasing the flow rate, microspheres tend to aggregate together and form a broad size distribution. This can be explained by the kink instability. Increasing the flow rate, not only increase the current through the liquid but also the surface charges. Increased surface charges result in a large electrostatic repulsive force. When droplets emit from the primary droplet,

Coulomb fission happens in an early stage. The droplet cannot flow back to the primary droplet leading to the formation of secondary or even satellite droplets.

In this project, as uniform microspheres are desired, the flow rate of 1.5ml/h is the chosen.

Table 8 Conditions for the formation of nanospheres.

SEM picture
Flow rate	0.1ml/h	
Description	Nanoparticles are formed with the condition of 100mg PLGA in 10ml acetone; applied voltage=10kV and the distance=10cm.	

4.1.3 Applied Voltage

The purpose of this experiment is to find the optimal voltage to produce monosized microspheres. In this regard, the applied voltage is the single variable while the other experimental conditions are fixed. The electrospray solution was prepared by dissolving 3wt% PDLG5010 in chloroform. The electrospray processing conditions are summarized: Flow rate=1.5ml/h, distance from the nozzle tip to the collector=10cm and the needle size=0.6mm. The obtained results were analyzed using SEM.

Table 9 Selection of the applied voltage.

SEM picture
Applied voltage	15kV	20kV

Judging from the SEM pictures, when the applied voltage is at 20kV, the microspheres produced were more clustered as compared to the 15kV one. Some bead-on-fiber structures were also seen. However in terms of the homogeneity, it is inferior to the 15kV produced microspheres. Hence, 15kV is the chosen applied voltage.

The following SEM pictures are used to study the function of applied voltage in altering the electrospray results. In this regard, the applied voltage is the single variable while the other experimental conditions are fixed. The electrospray solution was prepared by dissolving 4wt% PDLG5010 in chloroform. The electrospray processing conditions are summarized: Flow rate=2ml/h, distance from the nozzle tip to the collector=10cm and the needle size=0.6mm. The obtained results were analyzed using SEM.

Table 10 Study of the applied voltage in altering the morphology and size of particles.

SEM picture
Applied voltage	10kV	15kV
SEM picture
Applied voltage	20kV	25kV

Based on the SEM pictures, applied voltage influences the final electrospray products in various ways. Increasing the applied voltage from 10kV to 25kV, increases the fiber density. The average diameters of formed microspheres are 10 μ m and 5 μ m when the applied voltage is 10kV and 20kV, respectively. The bimodal size distribution of microspheres is evident when the applied voltage was 20kV.

According to the literature mentioned above, applied voltage determines the electrospray mode. The electrospray mode changes from dripping mode to

4.1.4 Distance from the Nozzle Tip to the Collector

According to the SEM pictures, the distance from the nozzle tip to the collector influence the morphology of produced microspheres. From 10cm to 13cm, the density of short fibres is increased. When the distance is at 10cm, the produced microspheres have an average diameter of $4.6\mu\text{m}$. The average diameter of microspheres increases from $5.4\mu\text{m}$ to $6.3\mu\text{m}$ when the distance between the nozzle tip to the collector increases from 11cm to 13cm. Based on

these results, it can be concluded that a shorter distance between the nozzle tip and the collector results in the formation of smaller sized microspheres. The reduced distance enhances the electrical field strength which in turn boosts the electrical repulsive force. In this case, the Coulomb fission happens in an early stage leading to the production of small size microspheres.

4.1.5 Drying Process

Freeze dry and vacuum dry are used for comparison. The results are showed below. When samples are vacuum dried, individual microspheres can be collected. On the contrary, agglomerated microspheres are collected when it is freeze dried. Since the electrospray experiments are conducted at room temperature, it is highly possible that the residual solvent, i.e. chloroform (boiling point: 61.2°C) is not completely evaporated upon reaching the collector. This forms solvated polymeric microspheres.

In freeze drying, because there is a need for the addition of water to the polymeric microspheres for the freezing process, the addition of water will enable movement of the microspheres which in turn cause them to agglomerate with one another. Because of the absence of the need to add water in vacuum drying, individual microspheres can be collected. Thus, vacuum drying is the choice of drying method.

Figure 23 The left side is the freeze dry SEM image and the right side is the vacuum dry SEM image.

In conclusion, if the solvent used is chloroform, the optimal conditions to produce the microspheres are:

Electrospray solution: 3% PDLG5010 in chloroform.

Electrospray processing condition: flow rate: 1.5ml/h, applied voltage: 15kV, distance from the nozzle tip to the collector: 10cm.

Drying process: Vacuum dry.

4.1.6 Different Solvents

This section is to discuss the solvent property in altering the electrospray products by using different solvents.

Evaporation rate and electrical conductivity are two criteria for electrospray solvent selection. During the flight time of the atomized droplets, in order to prevent the aggregation and collision of the microspheres, the solvent must be fully evaporated before it reaches the collector. This ensures that discrete and uniform particles can be obtained. So in order for this to happen, a solvent with a relatively low boiling point must be selected. The candidates are chloroform and acetone with boiling point of 61.2°C and 56°C, respectively.

As electrospray is a charge involved process, certain electrical conductivity is needed to guarantee the jet emission and Coulomb fission processes. The conductivity of chloroform is the same as acetone which is 0.0508 μ S/in. Based on these two criteria, besides chloroform, acetone is served as another candidate for electrospray solvent selection.

The solvent type is the only variable. The other experimental conditions are fixed. The electrospray solutions are 3wt% PDLG5010 in chloroform and acetone. The electrospray processing conditions are summarized: Flow rate=1.5ml/h, distance from nozzle tip to the collector=10cm, applied voltage=15kV and the needle size=0.6mm. The obtained results were analyzed using SEM.

Figure 24 Optimized conditions for monosized microparticles produced by using chloroform (left) and acetone (right).

Judging from the SEM pictures, using chloroform as the solvent, the average diameter of microspheres is around $5\mu\text{m}$. On the other hand, the average diameter of microspheres using acetone as the solvent is around $10\mu\text{m}$. When using acetone as the solvent, there is a larger quantity of microspheres being produced. At the same time, the microspheres are more uniform.

Solvent evaporation and polymer chain diffusion are two competing factors in forming fine particles [3]. Acetone is more volatile than chloroform because of its low boiling point. It can evaporate at a faster rate. In this case, insufficient time is provided for polymer chains to contract and rearrange. Thus, large and textured particles are formed. Chloroform has a higher boiling point. The evaporation process is slightly prolonged which gives time for polymer chain to rearrange leading to the formation of small and smooth particles.

To conclude, the morphology and size of the electrospray microspheres depend on various factors: Polymer concentration is the primary factor in determining the morphology of electrospray products. The dimensions of a product are decided by the flow rate, distance from the nozzle tip to the collector, the type of solvent and so on. High flow rate and long distance create large size particles. The applied voltage influences not only the morphology but also the size of particles.

Figure 25 Parameters in altering the diameter of particles.

4.1.7 HPLC Calibration Curves

In this project, HPLC is used to examine EE, LC and the drug release profile. A HPLC system has been established. Standard solutions are made for calibration and quantitative analysis. The drug loading and drug release experiments will be conducted, and results will be displayed in the future. The performance of the drug-loaded film is compared to Stalevo[®].

The high-performance liquid chromatography calibration is reported. The calibration curves of Levodopa, Carbidopa and Entacapone in their corresponding medium are displayed in the table below.

Table 11 Calibration curves regression analysis.

		PBS	SIF	60%NaH ₂ PO ₄ /40%CH ₄ O
Levodopa	Regression equation	Y=5952x-12.753	Y=4184.3x+2.4801	NA
	R ²	0.9993	0.9969	
Carbidopa	Regression equation	Y=390.15x-4.7215	Y=461.51x-1.9141	NA
	R ²	0.9626	0.998	
Entacapone	Regression equation	NA	NA	Y=20384x-39.864
	R ²			0.9998

4.2 Oral Film Fabrication

4.2.1 Selection of Polymer Type

In this experiment, according to the literature, HPMC (E5), HPC, Gelatin and Gum Arabic are chosen as candidates for film formers. Oral films made of these materials undergo the folding test, the disintegration test and the mechanical test. The appearance of these oral films is presented below. Based on the experimental results, the film formability of each polymer is judged and ranked. A good balance between these properties is needed for the selection of the polymer type.

Figure 26 The appearance of film made of HPMC, HPC, gelatin and Gum Arabic.

Based on the above images of the oral films, except for the film made of gelatin which is porous and irregular, the rest are smooth and flat. Hence, gelatin is eliminated.

The experimental data of each oral film from the folding test, the disintegration test and the mechanical test are summarized in the table below.

Table 12 Physical properties characterization for each oral film.

Materials	Folding Test	Disintegration Test (mins)	Mechanical Test	
			Tensile Strength (MPa)	Tensile Strain (%)
HPMC (E5)	9	7'35"	46.8987	24.7389
HPC	> 300	> 50'	3.8892	380.5954
Gelatin	13			
Gum Arabic	1			

The folding test is conducted first. The result shows that Gum Arabic film breaks within one fold. On the contrary, HPC film has the highest folding endurance, breaking only after 300 folding. Gelatin has a slightly higher folding endurance compare to HPMC (E5). Based on the folding test, Gum Arabic should be ruled out due to its low folding endurance.

The disintegration test is done subsequently. The result shows that HPC film takes a substantial amount of time (more than 50mins) to be fully dissolved. Long disintegration time may add inconvenience to patients. On the other hand, the disintegration time for HPMC (E5) is more acceptable. Because of the shorter disintegration time, HPMC (E5) is a much-preferred material for producing oral films. The mechanical test results also agree with this point. From the data, it shows that HPC film is a relatively ductile material; its tensile strain is up to 380.6%. On the contrary, HPMC (E5) has the proper

tensile strength (46.8987 MPa) and tensile strain (24.7389%). Enough tensile strength helps the film to withstand all the external forces during processing and packing. Suitable tensile strain makes the cutting easy to perform. The priority of polymer type selection is the disintegration time followed by the fabrication process. Hence, HPMC (E5) film has the most required mechanical properties.

In a word, based on the comparisons and selections, HPMC (E5) is the most rational film former material.

4.2.2 Selection of HPMC Type

From the previous results, HPMC is the selected polymer. There are however many grades of HPMC. The type of HPMC and the combination with different grades of HPMC need to be addressed in this experiment. E3, E5, E6, E15 are commonly used HPMCs due to their optimal T_g and low viscosity [4].

Pictures are taken for films made of HPMC E3, HPMC E5, HPMC E6, and HPMC E15 and displayed in Table 13. The folding tests results are recorded in Table 14.

From the images in Table 13, it can be seen that all films are smooth and transparent. According to these images, all of them are aesthetically qualified to make oral films. The folding test results become the critical criteria for selection.

From Table 14, it can be seen that there is a correlation between the folding time and polymer designation. The folding time increases along with the number of polymer designation. E15 has the highest folding endurance (29 folding times) while E3 has the lowest folding endurance (2 folding times). Among these polymers, the major difference is the chain length which associates with the polymers molecular weight. The fact is, M_w HPMC E3 < M_w HPMC E5 < M_w HPMC E6 < M_w HPMC E15. HPMC E15 has the

highest molecular weight together with the longest chain length which hinders the chain movements resulting in a great folding endurance.

Table 13 The appearance of films made of HPMC E3, HPMC E5, HPMC E6, and HPMC E15.

Picture
Film former	E3	E5
Picture
Film former	E6	E15

In this experiment, polymer with folding times larger than ten is preferred, since it will have better handling properties. According to this criterion, HPMC E15 can be used without any additives; while the rest should be used in combination to enhance its mechanical properties. In order to get a good balance of the folding endurance, the combinations are determined as HPMC E3+HPMC E15 and HPMC E5+ HPMC E6.

Table 14 Folding test results for oral films made of HPMC E3, HPMC E5, HPMC E6, and HPMC E15.

Materials	E3	E5	E6	E15
Folding times	2	9	3	29

The following tests are conducted by using films made of HPMC E15, HPMC E3+ HPMC E15 and HPMC E5+ HPMC E6. The façade of these films are showed in Table 15.

Table 15 The appearance of films made of HPMC E15, HPMC E3+ HPMC E15 and HPMC E5+ HPMC E6.

Picture
Film former	E15	E3+E15
Picture
Film former	E5+E6	

Based on the look of these films, they are all smooth and not tacky. Hence, they are all acceptable to make oral films.

The folding test results, the disintegration test results and the mechanical test results for the above films are reported in Table 16.

Table 16 The properties of films made of HPMC E15, HPMC E3+ HPMC E15 and HPMC E5+ HPMC E6.

Materials	Folding Test (times)	Disintegration Test (mins)	Mechanical Test	
			Tensile strength (MPa)	Tensile strain (%)
E15	29	20'	70.0441	38.9364
E3+E15	15	7'29"	36.2914	17.6666
E5+E6	35	13'30"	16.3857	9.8420

Based on the results showed in the Table 16, comparing to using one type of HPMC, the folding endurance is significantly improved by the usage of HPMC blends. It is obvious that HPMC E5+ HPMC E6 has the highest folding endurance (35 folding times) even larger than HPMC E15 (29 folding times). HPMC E3+ HPMC E15 has the shortest disintegration time (7'29"). HPMC E5+ HPMC E6 has the moderate disintegration time (13'30") which is also acceptable.

From the mechanical test, it can be observed that film made of HPMC E15 is the stiffest film with ductile deformation upon breaking. The mechanical properties decline with the reduction of HPMC E15. It is reflected from the tensile strength and tensile strain for the oral film made of HPMC E3+ HPMC E15. Both values are reduced compared to the oral film made of HPMC E15 only. On the other hand, the oral film made of HPMC E5+ HPMC E6 creates enough tensile strength (16.3857MPa) and relatively low tensile strain (9.8420%) which make the oral film withstand all external forces during processing.

In general, due to the outstanding merits of HPMC E5+ HPMC E6, it is the chosen polymeric matrix used to fabricate oral film in this project.

4.2.3 Selection of Plasticizer

Two types of plasticizers are used in this experiment for comparison they are: Glycerol and PEG400. The amount of glycerol used for analysis is 0.1g, 0.2g, 0.4g, and 0.6g. The PEG400 is used with the amount of 0.1g, 0.2g, and 0.4g. The look of oral films fabricated by different formulations is showed in the table below.

Table 17 Oral films made of HPMC E5+ HPMC E6 films with the addition of various amount of glycerol.

Picture
Glycerol amount	Glycerol 0.1g	Glycerol 0.2g
Picture
Glycerol amount	Glycerol 0.4g	Glycerol 0.6g

Judging from the look of these oral films, they are all clear, smooth and transparent. In addition, oral film made of 0.4g glycerol is the most flexible film.

Table 18 Oral films made of HPMC E5 + HPMC E6 with the addition of various amount of PEG400.

Picture
PEG400 amount	PEG400 0.1g	PEG400 0.2g
Picture
PEG400 amount	PEG400 0.4g	

These films are all suitable to make oral films as they are clear, smooth and transparent. However, film made of 0.2g PEG400 is the most flexible film among all.

The folding test, the disintegration test and the mechanical test are done for all the formulations mentioned above. The results are summarized in Table 19 below.

Table 19 The properties of oral films made of HPMC E5+ HPMC E6 with addition of various amount of glycerol.

Glycerol amount (g)	Folding test (times)	Disintegration test (mins)	Mechanical test	
			Tensile strength (MPa)	Tensile strain (%)
0.1	7	13'15"	30.1774	16.4333
0.2	2	22'53"	27.5385	12.7613
0.4	3	21'28"	20.9788	9.2590
0.6	26	23'34"	25.0563	11.8444

4 sets of films were fabricated with different amount of glycerol, and it has been observed that the folding times decline first followed by rising sharply from 2 times to 26 times with the addition of 0.4g glycerol. The disintegration time seems to have an ascendant pattern with the addition of glycerol. The disintegration time rises from 13'15" to 23'34" with the addition of six times amount of glycerol. The tensile strength and tensile strain share a similar trend that is drop to its lowest value, with 0.4g glycerol added to the system, at 20.9788Mpa and 9.25905%, respectively and then both value increase gradually.

The formulation of 0.4g glycerol was chosen because; it presented a lower mechanical strength hence a softer feel for the application of oral film. Secondly, it has a lower disintegration time of (21'28") along with lower mechanical properties. Henceforth, formulation of 0.4g glycerol is poised to be a better oral film candidate for its lower mechanical properties (softer feel) and faster disintegration time.

In addition, oral film with 0.4g glycerol added achieved the lowest tensile strength (20.9788MPa) and tensile strain (9.2590%) among others. With this tensile strength, it can bear external forces during processing. And the low tensile strength makes the cutting process easy to perform.

The table below lists the experimental results of oral films with addition of

different amounts of PEG400.

Table 20 The properties of oral films made of HPMC E5+ HPMC E6 with addition of various amount of PEG400.

Plasticizer amount	Folding test (times)	Disintegration test (mins)	Mechanical test	
			Tensile strength (MPa)	Tensile strain (%)
PEG400 0.1g	24	8'18"	27.7277	28.5442
PEG400 0.2g	52	14'35"	17.0228	10.3470
PEG400 0.4g	2	9'32"	10.1540	20.9798

According to Table 20, the folding times and disintegration time of films ascend by adding PEG400 from 0.1g to 0.2g. Then both of these two factors descend when there are 0.4g PEG400 in the system. The tensile strain has an opposite trend. It experiences a descendant first followed by an ascendant. The turning point is where 0.2g PEG400 is added to the system. The tensile strength decreases along with the addition of PEG400.

The formulation of 0.2g PEG400 has been chosen, since it has the highest folding endurance (52 folding times). The longest disintegration time (14'35") may become a shortage but it is still acceptable. The more important thing is that this oral film has a suitable tensile strength (17.0228MPa) which can endure the stress during processing and also has the lowest tensile strain (10.3470%) that makes the cutting process easy to conduct. Hence, by comparing the above factors, 0.2g PEG400 is equipped with the most desired qualities.

The plasticizers are treated as a softener in the system. Hence, by adding the right amount of plasticizer, the two systems (adding glycerol or PEG400) achieve their particular maximum folding endurance conditions.

The plasticizer should be compatible with the polymer. It is worked by embedding themselves between the chains of polymers, spacing them apart.

By doing that, the intermolecular distance is increased the same as the free volume [5]. The enlarged intermolecular distance gives rise to a weakening effect of secondary bonding including Van der Waal's force and hydrogen bonding. The impaired function of hydrogen bonding results in the increasing of disintegration time.

The present of glycerol or PEG400 in this system always increases the disintegration time. However, the disintegration time starts to decrease with the addition of 0.2g PEG400. Glycerol is a small molecule with molecular weight of 92.09382g/mol. While, PEG400 is a polymer with molar mass around 380-420g/mol. Increasing the amount of PEG400, the saturation point to separate polymer chains reaches quickly due to the large size and long chain length of PEG400. Instead, a significant amount of hydrogen bonds contributed by PEG400 accelerates the dissolve time.

By adding different plasticizers into the system, tensile strength and tensile strain have a similar tendency that is decreasing with some plasticizers added to the lowest point then increasing again. It can be explained by the proposed underlying mechanism. Due to the addition of plasticizers, the increased free volume enhances polymer chain mobility. Polymer chains are more easily to slip. The T_g is significantly reduced which permits the polymer to remain more ductile and tough at low temperature. Conversely, the Young's modulus and strength of material decline. Upon an applied load, the stiffness of the material is reduced because the polymer chains can move easily and pull out of the polymer mass. To an extent, when plasticizers reach its saturation point, the film shows its stiffness again.

To conclude, the selections of plasticizer are glycerol (0.4g) and PEG400 (0.2g).

4.2.4 Selection of Amount of Drug Loaded

At this moment, the selected recipes used to make blank oral films are:

1. HPMC E5 (1g) + HPMC E6 (1g) + Glycerol (0.4g)
2. HPMC E5 (1g) + HPMC E6 (1g) + PEG400 (0.2g)

The amount of drug loaded in the oral film is discussed and determined according to the experimental data showed in the tables below.

Table 21 HPMC E5+ HPMC E6+Glycerol(0.4g) films with various amount of drug.

Picture
Drug amount	MT: 0.1g	MT: 0.2g
Picture
Drug amount	MT: 0.4g	

Table 22 Oral film made of HPMC E5+ HPMC E6+PEG400 (0.2g) films with addition of various amount of drug.

Picture
Drug amount	MT: 0.1g	MT: 0.2g
Picture
Drug amount	MT: 0.4g	

Judging from the façade of the oral films, they are all smooth, flat and translucent. It can be detected that there are white mists on the surface of the films and can be removed. It might be the drug that attached on the film's surface. Although, it is hard to tell the difference based on the appearance of the oral films. There are some precipitates when adding 0.4g of drugs into the polymeric solution. On the contrary, 0.1g and 0.2g drugs can fully dissolve in the solution. Hence, the amount of drug loaded in the oral film is selected at 0.2g.

To this point, the final recipes for MT loaded oral films are confirmed which are:

Recipe one: E5(1g) +E6 (1g) +glycerol (0.4g) + MT (0.2g)

and

Recipe two: E5(1g) +E6 (1g) +PEG400 (0.2g) + MT (0.2g)

The following section of this project is to compare oral films made by electrospray and solvent casting using the recipes mentioned here. Due to the limitation of time, only **Recipe one: E5(1g) +E6 (1g) +glycerol (0.4g) + MT (0.2g)** is fabricated and tested.

4.3 Electrospray VS Solvent Casting

In this section, oral films made by electrospray and solvent casting using recipe one are compared. It is aimed to choose an efficient fabrication method which can achieve better physiochemical properties of the oral film.

4.3.1 Physical Properties

Table 23 The look of oral films made by electrospray and solvent casting using recipe one.

Picture
Fabrication method	Electrospray	Solvent casting

There is not much difference based on the look of the films. Both of them are clear and smooth. The oral film made by electrospray is slightly transparent than the oral film made by solvent casting. In this case, electrospray technique has a slight advantage.

Folding test, disintegration test and mechanical test are done for film properties comparison. The experimental data for both films are presented in the table below.

Table 24 The properties of films made by electrospray and solvent casting.

Fabrication Method	Folding test (times)	Disintegration test (mins)	Mechanical test	
			Tensile strength (MPa)	Tensile strain (%)
Electrospray	23	4'42"	9.3312	21.4445
Solvent casting	23	11'02"	6.6722	14.2271

From Table 24, it can be seen that the folding endurance doesn't change with the fabrication method. The disintegration time showed a huge difference. The disintegration time for the oral film made by electrospray technique and solvent casting is 4'42" and 11'02", respectively. The electrospray oral film disintegrates around 2.5 times faster. It is an outstanding point as it improves patients' compliance, and speed up the drug intake process. Oral Films produced by electrospray has a higher tensile strength (9.3312MPa) and tensile strain (21.4445%). During the electrospray process, the solvent evaporation occurs in a fast speed. On the other hand, using solvent casting method, the solvent evaporation is slowly. More voids are produced by solvent casting comparing to electrospray method. Hence, the tensile strength for oral film fabricated by electrospray technique is higher than the one applying solvent casting method.

4.3.2 SEM Images

SEM is used to check the microstructure of oral films. It is aimed to figure out the relevance of film properties and microstructure. In this part, SEM pictures of the plain drug; drug loaded oral films produced by electrospray and solvent casting are displayed.

Figure 27 Plain metoprolol tartrate salt.

Figure 28 Plain oral film made by electrospray.

Figure 29 Drug loaded oral film made by electrospray.

Figure 30 Plain oral film made by solvent casting.

Figure 31 Drug loaded oral film made by solvent casting.

From the SEM images, the morphology of drug loaded film doesn't show significant difference whether it is produced by electrospray or solvent casting. However, there are still some minor differences. Under the same magnification(X3500), the density of pillars showed in electrospray SEM image is slightly higher than the density of pillars showed in solvent casting SEM image. This result is in agreement with the previously reported data that the oral film made by electrospray technique has higher tensile strength due to the absence of fewer voids compared to the one produced by solvent casting. Using electrospray technique, pillars seem to be thinner and have more branches. This phenomenon can be explained by electrosprayed materials have more distortion since it is being sprayed from needle. Using solvent casting, the pillars are thicker and larger and the size is more uniform. The morphology differences are the reason for the fast dissolution of oral films made by electrospray technique. Thinner and smaller shape of pillars can be dissolved easily.

4.3.3 DSC Results

The DSC test is a thermoanalytical technique. It is used to know what happens in the polymeric matrix during heating. The following figures are DSC images of MT and various oral films.

Figure 32 Metoprolol tartrate salt powder.

Figure 33 E5+E6+glycerol (0.4g) oral film.

Figure 34 Electro spray recipe 1 oral film.

Figure 35 Solvent casting recipe 1 oral film.

The characteristic sharp endothermic melting peak for metoprolol tartrate salt is at 123.39°C. The plain oral film has a corresponding melting peak at 79.36°C. The oral film produced by electro spray technique has a melting peak at 100.57°C. Lastly, oral film fabricated by solvent casting has a melting peak at 88.07°C. The drug peak (123.39°C) doesn't show in figure 34 and 35 meaning the drug is not in a crystalline form. It might be in an amorphous form dispersing in the oral film. The polymeric matrix peak (79.36°C) also doesn't show in figure 34 and 35. Instead, the new melting peaks are detected: 100.57°C and 88.07°C for electro spray technique and solvent casting, respectively. It implies that there are some reactions between drug and the polymeric matrix.

4.3.4 In Vitro Drug Release Study

The drug release study figures for oral film made by electrospray technique and solvent casting are showed below.

Figure 36 Drug release profile for the oral film made by electrospray technique.

Figure 37 Drug release profile for oral film made by solvent casting.

Figure 38 Comparison for drug release from electrospray oral film and solvent casting oral film.

Oral films fabricated by both conventional and innovative methods exhibit a fast dissolving pattern. By using electrospray method, the burst release is even faster. The cumulative release reaches 85.63% at five minutes. Meanwhile, the cumulative release for solvent casting oral film at five minutes is 73.99%. At 30 minutes, the cumulative drug releases for both oral films are around the same. This is because, at this moment, both oral films are fully dissolved in the water. Within 1 hour, the cumulative drug release for film made by electrospray is 100%. The cumulative drug release for film made by solvent casting is 98.55%. The drug release profiles for both oral films are around the same. If it is for the application of an acute medication, oral film made by electrospray technique is preferred because of the rapid burst release.

4.3.5 Drug Encapsulation Efficiency

Encapsulation efficiency calculates the mass of therapeutic agent actually loaded in particles (m_{Load}) as a fraction of the initial mass of the particles (m_{Total}).

$$EE = \frac{m_{\text{Loaded}}}{m_{\text{Total}}}$$

The drug encapsulation efficiency is calculated for the oral film made by electrospray technique. The weighed drug loaded oral film is 995mg. Assumed the drug is homogeneously distributed in the oral film, the theoretical drug loaded amount should be 76.54mg. From the previous drug release result, the actual amount of drug that loaded in the film is 76mg. Hence, the drug encapsulation efficiency is 99.29%.

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Chapter 5

Conclusion and Recommendations

The tested optimal recipe for Metoprolol tartrate salt (MT) loaded oral film is: HPMC E5(1g) +HPMC E6 (1g) +Glycerol (0.4g) + MT (0.2g). This electrospray oral film can dissolve fast and has high drug encapsulation efficiency. The processing time for oral films made by electrospray technique is significantly reduced. These advantages make up for the limitation of conventional methods. Hence, it is recommended to use electrospray technique to produce oral films. The electrospray thin film loaded with PD's drugs has been studied, where uniform size of microparticles (5 μ m and 10 μ m) or nanoparticles (300nm) has been embedded in the film.

5.1 Conclusion and Recommendations

Electrospray technique is considered to be simple, cost-effective and robust. However, the processing parameters will largely affect the particles' size and morphology. Hence, proposed underlying mechanisms for electrospray have been reviewed and summarized.

In this project, Poly (lactic-co-glycolic acid) is used to explore the general role of each parameter that contributes to altering particles' size and morphology. It was found that polymer concentration affects the morphology of particles. The change of polymer concentration leads to morphology transition from flattened cracks to particles to bead-on-fiber and to fibers. Flow rate plays a significant role in altering the particles size. Sharply reducing flow rate can produce nanoparticles. The distance from the nozzle tip to the collector influences not only the particles' morphology but also the size of particles. Applied voltage does not change the size of particles in a tremendous way. However, it is likely to get fibers when applied voltage is increasing. The selection of solvent is important as it should possess a good solubility for polymer and drugs. In addition, the solvent type impacts the size and shape of particles.

In this experiment, monodispersed microparticles and nanoparticles are achieved under optimal conditions, and summarized as shown below:

- Nanoparticles with size around 300nm are obtained under the condition of: 1% PLGA in 10ml acetone; 10kV; 0.1ml/h; d=10cm.
- Microparticles with size around 5 μ m are achieved under the condition of: 3% PLGA in 10ml chloroform; 15kV; 1.5ml/h; d=10cm.
- Microparticles with size around 10 μ m are achieved under the condition of: 3% PLGA in 10ml acetone; 15kV; 1.5ml/h; d=10cm.

This project has also shown a promising prospect of using electrospray technique to fabricate oral films. Oral film loaded with MT, compared to the oral films made by solvent casting, the experimental results have proved that oral films produced by electrospray technique gives better drug release profile (100% drug release in one hour) and high drug encapsulation efficiency 99.29%. Apart from this, using electrospray technique, the processing time is remarkably shortened and the cutting process can be eliminated. With these two merits, the production efficiency is significantly increased.

The optimal recipes for drug loaded electrospray oral films are summarized below:

- HPMC E5(1g) +HPMC E6 (1g) +Glycerol (0.4g) + MT (0.2g)
- HPMC E5(1g) + HPMC E6 (1g) +PEG400 (0.2g) + MT (0.2g)

Electrosprayed film loaded with PD's drugs has been studied, where uniform size of micro-particles (5 μ m and 10 μ m) or nanoparticles (300nm) has been embedded in the film. The drug release study for this new structure is of interest and can be studied in the future work. Another interesting aspect of this work could focus on the improvement of the mouth feel and taste of oral film by adding some additives.