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The Effect of Formulation Parameters on the Physical Properties and In-Vitro Dissolution Profile of Ethyl Cellulose-Theophylline Microspheres Using the Emulsion-Solvent Evaporation Process: Nonionic Surfactant Structure and Concentration, Temperature and Solvent

By
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I dedicate this thesis to my loving family who always supported me
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The Effect of Formulation Parameters on the Physical Properties and In-Vitro Dissolution Profile of Ethyl Cellulose-Theophylline Microspheres Using the Emulsion-Solvent Evaporation Process: Nonionic Surfactant Structure and Concentration, Temperature and Solvent
Hassan A. Rammal

ABSTRACT

Controlled-Release drug delivery using microspheres depends on drug-encapsulation formulation, processing conditions, and excipient physical properties. Several parameters affect the microspheres’ formation and properties, including Physical, Chemical, and dissolution profile. This thesis aims to gain a better understanding of the number and type of parameters which affect the properties of Ethyl-Cellulose-Theophylline microspheres, and also attempts to explain the reason why said parameter affects said property in a certain specific way. In order to fully explore by which means drug-encapsulated microspheres are affected, this thesis looked at eight different parameters which were suspected to trigger changes in the properties of microspheres. The final aim of this thesis was to develop the most effective, efficient, and environmentally friendly method to form microspheres possessing the most beneficial properties for controlled release drug delivery.

Keywords: Microspheres, Drug-Encapsulation, Method Optimization, Ethyl-Cellulose, Theophylline.
TABLE OF CONTENTS

CHAPTER ONE .................................................................................................................. 1

1.1 Background.............................................................................................................. 1

1.2 Introduction............................................................................................................. 2

1.3 Microencapsulation Objectives ............................................................................... 3

1.4 Microencapsulation Methods.................................................................................... 4
  1.4.1 Physico-Chemical Methods.................................................................................. 4
    1.4.1.1 Coacervation............................................................................................... 5
    1.4.1.2 Ionotropic Gelation................................................................................... 5
    1.4.1.3 Supercritical Fluid Technology................................................................... 6
  1.4.2 Physical Methods................................................................................................. 8
    1.4.2.1 Spinning Disk............................................................................................ 8
    1.4.2.2 Spray Drying............................................................................................. 8
    1.4.2.3 Fluid-Bed Coating..................................................................................... 9
  1.4.3 Chemical Methods............................................................................................... 9
    1.4.3.1 Interfacial Polycondensation..................................................................... 9
    1.4.3.2 Interfacial Cross-Linking.......................................................................... 10
    1.4.3.3 Emulsification-Solvent Evaporation......................................................... 10

1.5 Materials in Emulsification-Solvent Evaporation Method ........................................ 12
  1.5.1 Solvents........................................................................................................... 12
  1.5.2 Coating Materials............................................................................................ 14
  1.5.3 Surfactants....................................................................................................... 14

1.6 Factors Affecting the Properties of Microspheres.................................................... 15
  1.6.1 Temperature.................................................................................................... 15
  1.6.2 Stirring Speed.................................................................................................. 16
  1.6.3 Polymer Content of the Solvent....................................................................... 16
  1.6.4 Type of Solvent.............................................................................................. 16
  1.6.5 Type of Surfactant......................................................................................... 17

CHAPTER TWO ................................................................................................................. 18

2.1 Introduction.............................................................................................................. 18

2.2 Theory ..................................................................................................................... 18
  2.2.1 Absorption Spectrum....................................................................................... 19
  2.2.2 Electronic Transitions...................................................................................... 20
    2.2.2.1 σ to σ* Transition.................................................................................... 21
    2.2.2.2 π to π* Transition.................................................................................... 21
    2.2.2.3 n to σ* Transition.................................................................................... 21
    2.2.2.4 n to π* Transition.................................................................................... 21
    2.2.2.5 σ to π* Transition and π to σ* Transition................................................. 22
    2.2.2.6 Important Electronic Transitions............................................................... 22
  2.3 Beer-Lambert Law.................................................................................................. 22

2.4 Components of the Spectrophotometer.................................................................... 23
  2.4.1 Light Source.................................................................................................... 23
  2.4.2 Monochromator............................................................................................... 23
  2.4.3 Sample Holder............................................................................................... 23
  2.4.4 Photosensitive Detector.................................................................................. 24
CHAPTER THREE .............................................................................................................. 25
3.1 Introduction ........................................................................................................... 25
3.2 Theory .................................................................................................................. 25
3.3 Instrumentation .................................................................................................. 28
  3.3.1 Infrared Source ......................................................................................... 28
  3.3.2 Interferometer ......................................................................................... 28
  3.3.3 Detector .................................................................................................. 29
  3.3.4 Computer ............................................................................................... 29
3.4 Applications ....................................................................................................... 29
3.5 Advantages ......................................................................................................... 30

CHAPTER FOUR .......................................................................................................... 31
4.1 Introduction ....................................................................................................... 31
4.2 Theory ................................................................................................................ 31
4.3 Solubility ............................................................................................................ 32
  4.3.1 Factors Affecting Solubility ..................................................................... 33
  4.3.2 Solvent Selection ..................................................................................... 33
  4.3.3 Particle Size ............................................................................................ 33
  4.3.4 Temperature ............................................................................................. 34
4.4 Mathematical Modeling .................................................................................... 34
  4.4.1 Zero-Order Kinetics ............................................................................... 34
  4.4.2 First-Order Kinetics ................................................................................ 35
  4.4.3 Higuchi Model ........................................................................................ 36
  4.4.4 Hixson-Crowell Model .......................................................................... 37
4.5 Instrumentation .................................................................................................. 37

CHAPTER FIVE ............................................................................................................. 39
5.1 Materials ............................................................................................................. 39
5.2 Drug - Theophylline ......................................................................................... 39
5.3 Coating Material - Ethylcellulose .................................................................... 42
5.4 Solvents .............................................................................................................. 42
  5.4.1 Acetone ................................................................................................... 42
  5.4.2 Dichloromethane .................................................................................... 43
  5.4.3 Tetrahydrofuran ..................................................................................... 44
  5.4.4 Ethyl Acetate .......................................................................................... 44
  5.4.5 Tetrachlorocarbon ................................................................................ 45
5.5 Surfactants ......................................................................................................... 45
  5.5.1 Hydrophilic-Lipophilic Balance (HLB) ..................................................... 46
  5.5.2 Critical Micelle Concentration (CMC) .................................................... 47
  5.5.3 Surfactant Packing Parameter ................................................................. 47
5.6 Oils ....................................................................................................................... 47
  5.6.1 Paraffin Oil .............................................................................................. 47
  5.6.2 Isopropyl Myristate ................................................................................ 48
5.7 Methods .............................................................................................................. 48
  5.7.1 Microspheres Preparation ...................................................................... 48
  5.7.2 Process Variables ..................................................................................... 49
5.7.3 Microspheres Yield

5.7.4 Drug Content Determination

5.7.5 Drug Entrapment Efficiency

5.7.6 Surface Drug Accumulation

5.7.7 Particle Size and Shape Analysis

5.7.8 Fourier Transform Infrared Spectroscopy

5.7.9 Stability Studies

5.7.10 In-Vitro Drug Release

5.7.11 Microspheres Drug Release Kinetics

CHAPTER SIX

6.1 Yield and Physical Properties

6.1.1 Solvent Variations

6.1.2 Propeller Variations

6.1.3 Type of Oil Variations

6.1.4 Temperature and Stirring Speed Variations

6.1.5 Drug to Polymer Ratio Variations

6.1.6 Solvent Layer to Oil Layer Variations

6.1.7 Surfactant Variations

6.2 Physicochemical Properties

6.2.1 Drug Entrapment Efficiency

6.2.2 Fourier-Transform Infrared Spectroscopy

6.2.3 Stability Studies

6.3 Drug Release Kinetics

6.3.1 Batch 9

6.3.2 Batch 10

6.3.3 Batch 15

6.3.4 Batch 17

CHAPTER SEVEN

References
LIST OF TABLES

Table 1: Results of Varying Solvents ................................................................. 55
Table 2: Results of Varying Propeller Type ....................................................... 58
Table 3: Results of Varying the Type of Oil ....................................................... 59
Table 4: Results of Varying Temperature and Stirring Speed .......................... 61
Table 5: Results of Varying Drug to Polymer Ratio ........................................ 62
Table 6: Yield and Size of Microspheres .......................................................... 62
Table 7: Results of Varying Solvent Layer to Oil Layer Ratio ......................... 63
Table 8: Results of Varying the Surfactant Type ............................................. 64
Table 9: Yield and Physical Characteristics of Microspheres ......................... 65
Table 10: Effects of Temperature on Entrapment Efficiency ........................... 67
Table 11: Effect of Drug to Polymer Ratio on Entrapment Efficiency ............. 68
Table 12: Effect of Solvent Layer to Oil Layer on Entrapment Efficiency ........ 68
Table 13: Effect of Surfactant Type on Entrapment Efficiency ....................... 69
Table 14: Entrapment Efficiency Over Time .................................................... 72
# LIST OF FIGURES

Figure 1: Microencapsulation Objectives............................................................4
Figure 2: Preparation of microparticles by Ionotropic Gelation..........................6
Figure 3: Rapid Expansion of Supercritical Solution Microencapsulation Technique 7
Figure 4: Scheme Representing Energy Transition ...........................................19
Figure 5: Absorption Spectrum of Acetone..........................................................20
Figure 6: Electronic Transitions (Black: Important. Grey: Not Very Useful)..........22
Figure 7: Symmetrical Stretching .......................................................................26
Figure 8: Asymmetrical Stretching .....................................................................26
Figure 9: In-Plane Bending: Scissoring ...............................................................27
Figure 10: In-Plane Bending: Rocking .................................................................27
Figure 11: Out-of-Plane Bending: Wagging .........................................................27
Figure 12: Out-of-Plane Bending: Twisting ..........................................................27
Figure 13: Schematic Representation of the Interferometer ..................................29
Figure 14: Dissolution Process ............................................................................32
Figure 15: Concentration at the Surface of Drug and in Bulk Solution ................35
Figure 16: 1-Methylxanthine ..............................................................................41
Figure 17: 3-Methylxanthine ..............................................................................41
Figure 18: Theophylline .......................................................................................41
Figure 19: 1,3-Dimethyluric Acid .......................................................................41
Figure 20: Caffeine ............................................................................................41
Figure 21: Ethylcellulose Structure .....................................................................42
Figure 22: Acetone Structure .............................................................................43
Figure 23: Dichloromethane Structure ...............................................................43
Figure 24: Tetrahydrofuran Structure .................................................................44
Figure 25: Ethyl Acetate Structure .....................................................................45
Figure 26: Tetrachlorocarbon Structure .............................................................45
Figure 27: Batch 3 ............................................................................................59
Figure 28: Batch 2 ............................................................................................59
Figure 29: Theophylline Standard Curve (Absorbance taken at 272nm)............66
Figure 30: Theophylline IR Spectrum .................................................................70
Figure 31: Ethylcellulose IR Spectrum .................................................................70
Figure 32: IR Spectrum of a Mixture of Theophylline and Ethylcellulose ..........71
Figure 33: IR Spectrum of Microspheres ............................................................71
Figure 34: Zero-Order Model for Batch 9 ............................................................74
Figure 35: First-Order Model for Batch 9 .............................................................74
Figure 36: Hixson-Crowell Model for Batch 9 .....................................................75
Figure 37: Higuchi Model for Batch 9 .................................................................75
Figure 38: Zero-Order Model for Batch 10 ..........................................................76
Figure 39: First-Order Model for Batch 10 ..........................................................76
Figure 40: Higuchi Model for Batch 10 ...............................................................77
Figure 41: Hixson-Crowell Model for Batch 10 ...................................................77
Figure 42: Zero-Order Model for Batch 15 .......................................................... 79
Figure 43: First-Order Model for Batch 15 .......................................................... 79
Figure 44: Higuchi Model for Batch 15 ............................................................ 80
Figure 45: Hixson-Crowell Model for Batch 15 .............................................. 80
Figure 46: Zero-Order Model for Batch 17 ......................................................... 82
Figure 47: First-Order Model for Batch 17 ......................................................... 82
Figure 48: Higuchi Model for Batch 17 ............................................................ 83
Figure 49: Hixson-Crowell Model for Batch 17 .............................................. 83
CHAPTER ONE

Microencapsulation

1.1 Background

Previous research pertaining to the study of drug encapsulated microspheres examined the effect of combining two surfactants on the physical properties and dissolution profile of the formulated microparticles. One study tested a pair of surfactants having different Hydrophilic-Lipophilic-Balance (HLB) values, namely Span65 (HLB = 2.1) and Tween40 (HLB = 15.6) (Mohan, et al., 2011). Another team investigated the effects of a pair of surfactants having similar HLB values, namely Tween40 and Brij58 (Mohan, et al., 2016). Despite the multitude of surfactants, research related to Theophylline encapsulated microspheres focused only on the three aforementioned surfactants, along with Span80, which gives this thesis the chance to further examine the effect of other types of surfactants, individually or in combination, such as Poloxamer188, Poloxamer407, Tween80, Tween20, Polyethylene Glycol (400, 800, and 1200), among others.

In an attempt to assess the effect of Theophylline to Ethyl-Cellulose ratio on the microspheres, Mitra, et al. (Mitra, Siavoush, & Nazila, 2010), varied the drug to polymer ratio between 0.25:1 and 1:1. In the same study, three stirring speeds were examined, 500, 1000, and 1500 rpm. However, the two extremities are far from the speed used in the majority of studies (1000 rpm), hence a narrower investigation would be more appropriate.
Most of the studies available in the literature used dichloromethane as the evaporating solvent and paraffin oil as the non-aqueous medium. This points to a gap in research, which can be fulfilled by testing different types of oils such as isopropyl myristate, or olive oil. Moreover, other types of solvents should be investigated.

1.2 Introduction

The field of drug microencapsulation has recently attracted the attention of many scientists due to its controlled drug-delivery implications. With the ability to regulate the release of therapeutic molecules and their localization, humanity would have solved the problem of drugs possessing a short half-life, which by nature, are administered to patients more frequently in view of maintaining the desired plasma concentration, hence causing more undesirable side effects than molecules with a longer half-life (Young, Hyungsoo, & Kyekyoon, 2008). On top of that, patient compliance will certainly improve upon administering such sustained release dosage forms.

In addition, it is worthy to note that microencapsulation is not only limited to drug molecules, as many attempts to encapsulate a variety of products have been successful, such as stem cells, proteins, liquids, and bacterial cells, thus enabling the delivery of microorganisms, as well as artificial cell and tissue delivery (Catherine, Shyamali, Meenakshi, Imen, & Satya, 2013), which paves the way to several scientific and medical breakthroughs.

Microencapsulation methods can be categorized under three general methods, namely Chemical, Physical, and Physicochemical. Several techniques exist under each method, such as ionotropic gelation and supercritical fluid technology under the physicochemical method, spray drying and spinning disk under the physical method,
and finally interfacial cross-linking and solvent evaporation under the chemical method (Catherine, Shyamali, Meenakshi, Imen, & Satya, 2013). The three general methods will be further examined later in the chapter. The focus of this thesis will be on the latter method, using the solvent evaporation technique.

1.3 Microencapsulation Objectives

The general goal of microencapsulation is to shield a compound of interest by an agent called the wall material, which acts as an isolation medium, whose mission is the protection of the active compound. The latter will be released from the protecting matrix upon sensing a certain stimulus, a change in temperature or in pH for instance.

The first microencapsulated materials surfaced as early as 1954, in the food industry. Applications of this technology in such an industry had several uses, mainly to prevent lipid oxidation and the loss of volatile compounds (Pedro, Patrícia, & Leilane, 2018). In the pharmaceutical industry, microencapsulation takes place in order to serve two major purposes: The protection of an active compound, and to release an active agent in a controlled-fasion. The former goal is necessary when dealing with pH-sensitive substances, which might be degraded when exposed to the high acidity of the upper gastrointestinal tract, while the latter goal is sought when a drug molecule needs to be delivered at a certain rate or a specific site of action (Catherine, Shyamali, Meenakshi, Imen, & Satya, 2013).
1.4 Microencapsulation Methods

As previously mentioned, three methods of encapsulation exist, and multiple techniques can be found under each method. This multitude is explained by the fact that the selection of a certain method and technique is dependent upon the desired final product. This section will be divided into three sub-sections, each one dealing with one of the aforementioned methods.

1.4.1 Physico-Chemical Methods

Physico-Chemical methods of encapsulation include coacervation, ionotropic gelation and supercritical fluid technology. Each technique will be discussed below.
1.4.1.1 Coacervation

Coacervation was first discovered in the 1940s, making it one of the oldest microencapsulating techniques. There are two types of coacervation, simple and complex, the first of which involves using only one colloidal substance, while the second involves the use of two or more colloids. Both types of coacervation processes start by the dispersion of the active substance (to be coated) into the colloidal solution (e.g. gelatin). Secondly, the separation of the colloidal solution into two phases – one having a high concentration of the polymer and the other a low concentration – is induced by a change in temperature, pH, addition of a nonsolvent, or electrolytes in the case of simple coacervation, or the addition of an oppositely charged colloid in the case of complex coacervation. Following this separation, colloidal droplets will precipitate on the surface of the active substance. Finally, a cross-linking agent, such as formaldehyde is added in order to solidify the coating (Salaün, 2016).

1.4.1.2 Ionotropic Gelation

In this method, natural polyelectrolytes are used in order to encapsulate the desired substance, such as chitosan, or carboxymethyl cellulose. The basic idea of this method is that polyelectrolytes will cross link in the presence of counterions, thus forming micro- or nanoparticles. The therapeutic agent is dissolved in the acidic chitosan solution, which is then added dropwise to a polyanionic solution, such as tripolyphosphate, under continuous stirring. Chitosan will thus precipitate under the form of small spherical particles due to the cross-linking between oppositely charged species (Giri, 2106).
1.4.1.3 Supercritical Fluid Technology

By definition, a supercritical fluid is a substance at a pressure and temperature above its critical point. At this stage, the substance can exhibit liquid properties, such as dissolving solids, as well as gas properties, such as effusing through solids (Samadzadeh, 2019). In the pharmaceutical industry, Carbon Dioxide (CO₂) is the most widely used substance for encapsulating drug molecules using the supercritical fluid technology, given that the conditions which render CO₂ supercritical are easily accessible: A pressure of 73.8 bar and a temperature of 31.1°C. On top of its critical temperature being relatively low and very close to the suitable temperature at which processes involving biological entities are conducted (i.e. 37°C), supercritical CO₂ is
non-toxic, non-flammable, possess microbial inactivation properties, and has a low cost. The process of microencapsulation starts by dissolving the active ingredient and the coating agent in supercritical CO$_2$, which is maintained at a high pressure before being expanded through an orifice nozzle. This rapid and sudden expansion causes CO$_2$ to revert to its normal gaseous phase, thus leading to the supersaturation of the coating material which deposits on the surface of the active ingredient, hence producing microcapsules (Soon Hong & Lai Yeng, 2019).

Figure 3: Rapid Expansion of Supercritical Solution Microencapsulation Technique
1.4.2 Physical Methods

Physical methods of encapsulation involve spinning disk, spray drying and fluid-bed coating. Each technique will be discussed below.

1.4.2.1 Spinning Disk
The spinning disk system makes use of rotational forces to create droplets. The active substance is dispersed in the wall material, and the mixture is sprayed onto a rotating disk, hence throwing the droplets outward from the rim of the disk, which are solidified by chilling. The velocity at which the disk revolves can be precisely controlled, which allows the formation of microparticles within a wide range of diameters, spanning from 5 to 3,000 micrometers (Microencapsulation Innovations, 2011).

1.4.2.2 Spray Drying
Spray drying is a process through which a fluid feed, typically a dispersion, is transformed into a dry small particulate (Ré, 2007). Usually, an oil or a water-soluble active substance is dissolved/dispersed in the wall material, and this emulsion is then atomized into droplets which are exposed to a brief high temperature in a heating medium. The simplicity as well as the high throughput of this method makes it particularly attractive for the food industry, however its main disadvantage when it comes to pharmaceutical preparations is due to the difficulty in controlling the particle size, and its incompatibility with temperature-sensitive ingredients (Benita, 2006).
1.4.2.3 Fluid-Bed Coating

The fluid-bed coating technique is a variation of the pan coating technique, an old method which was developed in the late second half of the 1800s (Sanjoy, et al., 2011). In this technique, a jet of air with a controllable rate suspends the solid particles, onto which a liquid coating material is sprayed. These newly formed coated particles are then dried by either solvent evaporation, or cooling (Guignon, Duquenoy, & Dumoulin, 2002).

1.4.3 Chemical Methods

Chemical methods of encapsulation comprise interfacial polycondensation, interfacial cross-linking, and emulsification-solvent evaporation. Each technique will be discussed below.

1.4.3.1 Interfacial Polycondensation

Polycondensation reactions are widely used to produce a range of products, such as nylon, polyurethanes, and capsule walls for microspheres. The Schotten-Baumann reaction forms the basis of this method, first pioneered in 1883 by two German chemists, describing the reaction between a species containing an active hydrogen atom (amines, alcohols, polyesters, etc.) and an acid chloride. (Organic Chemistry Portal, 2002). This technique involves the utilization of two immiscible phases (oil and water phases), wherein one monomer (the active-hydrogen-bearing species or the acid chloride) is dissolved in each phase, followed by the dispersion of the water phase in the oil phase. A polymerization reaction occurs between the two reactants at the surface of the dispersed particles, and at the interface of the two phases, thus forming the capsule walls (Nelson, 2103).
1.4.3.2 Interfacial Cross-Linking
This technique involves the same reaction employed in the previously described method (Schotten-Baumann reaction). The difference resides in the addition of a polysaccharide, polyanine, or protein, although the latter is the most widely used. This biopolymer will react with the active-hydrogen-bearing species and replace its protons, followed by a reaction between the functional groups of the biopolymer and the acid chloride at the interface of the emulsion, resulting in the formation of the capsule membrane (Poncelet, Perignon, & Ongmayeb, 2013).

1.4.3.3 Emulsification-Solvent Evaporation
Four different processing conditions exist under this technique, the use of which is dependent on whether the drug to be encapsulated is hydrophilic or hydrophobic. The conditions are denoted oil-in-water (o/w) co-solvent, o/w dispersion, w/o/w double emulsion, and o/o non aqueous. The first of which involves a drug which is insoluble in the solvent consisting the oil phase, so a co-solvent is used to dissolve the drug, followed by the dispersion of the whole system in the aqueous phase. The o/w dispersion describes a method in which the drug of interest is dispersed in the organic phase containing the polymer (oil phase), which is then dispersed in a second aqueous phase. The w/o/w double emulsion system makes use of a hydrophilic drug which is dissolved in water, followed by the emulsification of this aqueous phase in an organic liquid, and finally the dispersion of this w/o system in a second aqueous phase. Lastly, the o/o emulsion consists of the same principles of the o/w dispersion, however replaces the aqueous phase with an oil (BO'Donnell & WMcGinity, 1997).

Upon the diffusion of the polymeric organic phase containing the drug, in the aqueous phase or in the second oil phase in case of an o/o emulsion, small droplets will start forming due to high speed mixing, and the microspheres will start to appear.
as the organic solvent starts to evaporate at the continuous phase/air interface (Mendoza-Muñoz, Alcalá-Alcalá, & Quintanar-Guerrero, 2016). Multiple parameters affecting the properties of microspheres have been characterized, including processing temperature, type of surfactant, stirring speed, type of solvent, drug loading, drug solubility, among others (Neeta, Saurabh, Parijat, & Mandeep, 2016). Each factor and its influence on the microspheres properties will be discussed in the next section.

Two mechanisms by which micro- or nanoparticles form using the emulsification-solvent evaporation technique exist. The first mechanism depicts that each particle is produced from only one droplet. Indeed, by monitoring and varying the evaporation rate of the solvent, no change in size of the particle was evident, suggesting that there was no coalescence, which points to the fact that one micro- or nanoparticle is formed from only one emulsion droplet (Sergio, et al., 2005). On the other hand, a second mechanism was proposed, stating that one nanoparticle is formed through the fusion of multiple emulsion droplets when the solvent is evaporating. This suggestion was based on the observation that the diameter of the spheres increased after 30 minutes, which is explained by the fusion of droplets together (Stéphanie, et al., 2003). It is worth noting that the contradiction between these two mechanisms might be due to the fact that Sergio et al. used poly(L-lactide acid) as a polymer, while Stéphanie et al. used ethylcellulose. Nonetheless, most studies confirm that the main mechanism through which the formation of nanoparticles occur is the one suggesting that each nanosphere is formed by one droplet, given that the size of the primary emulsion and the diameter of the nanospheres are highly correlated. Coalescence, which results in the formation of one particle by multiple droplets is a
rare phenomenon, which occurs in less than 10% of cases (Mendoza-Muñoz, Alcalá-Alcalá, & Quintanar-Guerrero, 2016).

1.5 Materials in Emulsification-Solvent Evaporation Method

Three main materials are used in order to successfully encapsulate a drug molecule inside a wall matrix. Each material should be chosen based on what physical, chemical, and biological properties are expected.

1.5.1 Solvents

The choice of the solvent to be used is dependent on multiple factors and considerations. In the intent to use the final product in human beings, the appropriate solvent would have to be unharmful, or at least possess a minimal amount of undesirable effects. This is due to the fact that conventional methods of removing the solvent from the final product, such as high-temperature drying under vacuum, are often unachievable due to the possibility of damaging the product (Eun-Jung, Ae-Hee, & In-Joon, 2012). In an attempt to clarify this concept, the International Conference on Harmonization grouped solvents in three classes based on their toxicity, and quantified the allowed amount of each solvent. Class 1 comprises “solvents to be avoided”, such as strongly suspected human carcinogens. Class 2 includes “solvents to be limited” which are of two types: agents causing irreversible toxicity such as neurotoxicity, and agents causing significant but reversible toxicities. Lastly, class 3 solvents are the ones which present a “low toxic potential”, which do not require an exposure limit, and can have a permitted daily exposure as high as 50mg or more per day (International Conference on Harmonization, 2019). These guidelines are behind the reason why most studies and manufacturing plants are switching to the use of ethyl acetate (class 3) instead of chloroform and
dichloromethane, both of which classified under class 2, for the production of microspheres.

Aside from safety concerns, the chemical properties of the solvent must be suitable in sustaining the microencapsulation procedure. Solvency plays a major role in deciding which solvent is to be used. In fact, the solvent must be able to dissolve the proper amount of polymer – hence its second dependability is on the type of wall matrix to be used – as well as display a good ability in dissolving the drug molecule of interest, the third dependence factor. Furthermore, the right solvent must be poorly miscible or ideally immiscible with the continuous phase, which is water in case of an o/w or w/o/w emulsions, or the type of liquid used in an o/o emulsion. Regarding, the boiling point, it must be higher, however not too far, from the processing temperature, in order to avoid boiling or rapid evaporation rate, and must be low enough in order for it to be able to evaporate. Evaporation is also enhanced if the solvent has a high vapor pressure. The ability of dichloromethane to dissolve fairly high amounts of polymers, its practical insolubility in water – which does not exceed 2% w/v – as well as its relatively low boiling point settled at 39.6°C, allowed it to be the most widely used solvent in microencapsulation by the solvent evaporation method (Carmen, Katharina, Daniel, & Alexander, 2013). In fact, dichloromethane replaced chloroform as the most widely used solvent, due to high toxic and cancerous potential of the latter. Gradually, two other solvents emerged, namely ethyl acetate and ethyl formate, with the advantages of being less toxic than the two aforementioned solvents (Ming, Olivier, & Denis, 2008).
1.5.2 Coating Materials

The chosen coating material must be able to fulfill a number of requirements, mainly being capable of enveloping and be cohesive with the drug molecule, being inert vis-à-vis the latter, and have a consistent strength, impermeability and stability (Murtaza, 2012). In addition, the wall matrix must be able to exhibit controlled release properties when presented with specific stimuli, be tasteless and ideally mask the bitter taste of some drugs. On another note, the polymer should be economical in order for the final product to be affordable by the general public (Bansode, Banarjee, Gaikwad, Jadhav, & Thorat, 2010).

The chemical properties of the phase containing the polymer affects the properties of microspheres. For instance, the particle size and the concentration of polymer in the organic phase are positively correlated, due to an increase in the viscosity of the solution (Mendoza-Muñoz, Alcalá-Alcalá, & Quintanar-Guerrero, 2016).

Furthermore, coating materials can be classified as water soluble, such as Gelatin, Polyvinylpyrrolidone, Hydroxyethylcellulose, water insoluble, including Ethylcellulose, Cellulose Nitrate, Polymethacrylate, waxes such as Paraffin, Stearic Acid, Stearyl Alcohol, and finally enteric resins, such as Cellulose Acetate Phthalate (Bansode, Banarjee, Gaikwad, Jadhav, & Thorat, 2010).

1.5.3 Surfactants

Surfactants play a major role in the manufacturing of micro and nanoparticles, due to their ability to stabilize the organic droplets during the emulsification process. Indeed, the surfactant helps reduce the aggregation of particles once they form after the solvent starts evaporating. The ability of stabilizers to achieve this goal is attributed to its reduction of interfacial tension between the continuous and discontinuous phase, as well as the creation of electrostatic/steric repulsive forces.
between the formed particles (Mendoza-Muñoz, Alcalá-Alcalá, & Quintanar-Guerrero, 2016). The amount of surfactant added to the emulsion affects the size of the formed particles, and is explained by a logarithmic relation between the particle size reduction and the concentration of the surfactant. This phenomenon can be explained by the adsorption of the surfactant on the particle’s surface, thus preventing aggregation (Galindo-Rodriguez, Allémann, & Doelker, 2004). Most commonly, polyethylene glycol derivatives such as Tween and Poloxamer, as well as polyvinyl alcohol are used as surfactants in the preparation of microspheres by the solvent evaporation method (Mendoza-Muñoz, Alcalá-Alcalá, & Quintanar-Guerrero, 2016).

1.6 Factors Affecting the Properties of Microspheres
Several parameters affect the properties of microspheres, including processing temperature, type of surfactant, stirring speed, type of solvent, polymer concentration in organic layer, among others (Neeta, Saurabh, Parijat, & Mandeep, 2016).

1.6.1 Temperature
Processing temperature affects several characteristics of the microspheres, mainly due to its impact on the evaporation rate of the organic solvent. Higher temperatures have been linked with greater microspheres diameters and a wider size distribution, which is something to be avoided. However, low temperatures induce irregular microspheres shapes, whereas higher temperatures have been linked with a more spherical shape (Mateović-Rojnik, Frlan, Bogataj, Bukovec, & Mrhar, 2005). These findings suggest that a sweet-spot must be sought in order to come up with the best outcome.
1.6.2 Stirring Speed

High stirring speeds provide more energy and thus maximize the division of the oil phase, resulting in smaller droplets. As previously mentioned, these droplets are the basis of microspheres formation, thus smaller droplets will lead to smaller particles, which means that as the stirring rate increases, the particle size decreases, and so does the size distribution (Sansdrap & Moës, 1993).

1.6.3 Polymer Content of the Solvent

An increase in polymer percent concentration of the organic phase leads to an increase in the solution’s viscosity. Higher viscosities improve the encapsulation efficiency, and significantly decrease the initial release of the drug. However, as the volume of organic solvent is increased while the mass of dissolved polymer remains constant (lower concentration, hence lower viscosity), the mean diameter of the particles was reduced (Obeidat & Price, 2003).

1.6.4 Type of Solvent

Different solvents exhibit different chemical properties, which in turn affect the properties of microspheres. Such chemical properties include boiling point, which must be higher, however not too far, from the processing temperature, in order to avoid boiling or rapid evaporation rate, and must be low enough in order for it to be able to evaporate. Another property is solvency, which must be appropriate to dissolve a sufficient quantity of the polymer, and maintain a proper viscosity. Moreover, the solvent must be immiscible with the aqueous continuous phase (or oil continuous phase in case of an o/o emulsion) and unharmful in case the final product is to be used in humans. The most widely used solvent, Dichloromethane, does not fulfill the last requirement, given its carcinogenic properties. It also suffers a
drawback in its low boiling point (39.6°C), which is very close to the common processing temperature of 37°C (Nagiat, Prakash, Babu, & Shanta, 2103).

1.6.5 Type of Surfactant

Previous studies reported that surfactant chemical structure affected microsphere physical properties and drug release. It is suggested that surfactant concentration is also a factor. The main factor in structural differences appears to be surfactant bulkiness whereas bulky surfactants will pack drug, polymer, and surfactant in the forming microsphere differently than linear surfactants, especially if surfactant melting point is near formulation processing temperature. For example, the bulky sorbitan moiety in Tween 40 (polyoxyethelene-20-sorbitan palmitate) versus the linear cetyl ether in Brij 58 (polyoxyethelene - cetyl ether) (Mohan, et al., 2016).
CHAPTER TWO

Ultraviolet-Visible Spectroscopy

2.1 Introduction
Electromagnetic radiation is comprised of energy packages formed by multiple discrete species called photons, consisting of perpendicular oscillating electric and magnetic fields. One of the main components of the electromagnetic radiation is its wavelength, the distance between two nearest crests or troughs. The most basic division of the electromagnetic spectrum is on the basis of wavelengths, which ranges from $10^{-4}$ nanometer (Gamma Rays) to $10^{9}$ nanometers (Radio waves). The ultraviolet region lies between 10nm and 400nm, while the visible region extends from 400nm to 800nm, (Watson, 2012) however for quantification of chemical species, the lowest wavelength used is 180nm (Worsfold & Zagatto, 2019). UV-Vis spectroscopy is a method used in qualitative and quantitative studies, however is more prevalent in the latter type. This method is used in multiple settings, including quality assurance and quality control, as well as analytical and regulatory laboratories (Bain, 2017).

2.2 Theory
UV-Vis spectroscopy measures the attenuation of electromagnetic radiation in the region of the ultraviolet and visible light, after it passes through a sample. Simply put, the absorption of radiation – be it a single wavelength or an extended range – by a sample is measured. The phenomenon of absorption is accomplished by the
transition of an electron from a lower energy to a higher energy state upon being hit by a photon.

![Diagram of energy transition](image)

**Figure 4: Scheme Representing Energy Transition**

### 2.2.1 Absorption Spectrum

The atoms which form a molecule merge their atomic orbitals together thus producing molecular orbitals, which will be occupied by electrons. Upon being hit by energy under the form of electromagnetic radiation, these electrons undergo a transition from the highest occupied molecular orbital, to the lowest unoccupied molecular orbital, thus transforming the molecule from a ground state species to an excited species (Watson, 2012).

Electrons in different molecules do not absorb radiations possessing the same wavelength. In fact, the energy of the radiation (dictated by its wavelength) must match the energy gap between the ground state and the excited state within a molecule. Once this match is achieved, a portion of the energy of the light is
absorbed, and a spectrometer then detects the extent of absorption at different wavelengths. Once the range of wavelength is complete, the Absorbance (A) is plotted versus the wavelength ($\lambda$), and $\lambda_{\text{max}}$ (the wavelength at which maximum absorption is attained) is then chosen as the reference wavelength at which quantitative measurements will be performed (Satinder & Stephen, 2012).

![Absorption Spectrum of Acetone](image)

Figure 5: Absorption Spectrum of Acetone

### 2.2.2 Electronic Transitions

Molecular orbitals can be classified into five types, going from the lowest energy state to the highest energy state:

1. Bonding ($\sigma$)
2. Bonding ($\pi$)
3. Non-bonding ($\text{n}$)
4. Anti-bonding ($\pi^*$)
5. Anti-bonding ($\sigma^*$)
2.2.2.1 σ to σ* Transition

Upon being hit by a photon, an electron jumps from the bonding σ orbital to the anti-bonding σ* orbital. Such a transition requires a high amount of energy, thus a low wavelength (energy and wavelength are inversely proportional), which explains why for instance methane (CH₄) has an absorption maximum at 125nm, given that it only possess σ and σ* orbitals, hence the only possible transition is σ to σ* (Satinder & Stephen, 2012).

2.2.2.2 π to π* Transition

Bonding π orbitals and anti-bonding π* orbitals only exist in molecules containing multiple (double or triple) bonds. Hence, this type of transition is achievable in compounds like alkenes or alkynes, and those possessing carbonyl, nitrile and phenyl groups, among others (Satinder & Stephen, 2012).

2.2.2.3 n to σ* Transition

The n to σ* type of transition requires an amount of energy lesser than that required by a σ to σ* transition, and are less frequent, which is explained by the low number of n to σ* peaks in the UV region. Such a transition is accomplished through atoms possessing lone pairs of electrons, such as Oxygen, Nitrogen, Sulfur, and halogens (Satinder & Stephen, 2012).

2.2.2.4 n to π* Transition

This type of transition is observed in compounds having multiple bonds between unlike atoms (C=O, N=O, etc.), and requires a minimal amount of energy due to the proximity of these two orbitals, which explains the fact that absorption peaks of this type of transition are observed at longer wavelengths (Satinder & Stephen, 2012).
2.2.2.5  \( \sigma \) to \( \pi^* \) Transition and \( \pi \) to \( \sigma^* \) Transition

Those two types of electronic transitions are forbidden (Satinder & Stephen, 2012).

2.2.2.6  Important Electronic Transitions

Not all types of electronic transitions are useful. As previously mentioned, the absorption peak of methane is at a wavelength of 125nm, which is below the threshold of 180nm, hence making the transition from \( \sigma \) to \( \sigma^* \) not very useful. Transitions between energetically closely related orbitals are the most useful, namely \( n \) to \( \pi^* \) and \( \pi \) to \( \pi^* \) transitions, which are possible in extended systems of double bonds, called chromophores. The most common chromophore in drugs is the benzene ring (Satinder & Stephen, 2012).

![Diagram of Electronic Transitions](image)

Figure 6: Electronic Transitions (Black: Important. Grey: Not Very Useful)

2.3  Beer-Lambert Law

The Beer-Lambert law establishes the relationship between the absorbance and the concentration of an analyte, and acknowledges that the type and concentration of the absorbing species affect the process of photon absorption (Wypych, 2018). The intensity of the incident light \( I_0 \) diminishes upon passing through a medium of thickness \( b \), and containing an absorbing species at a concentration \( c \). The reduced light intensity is termed \( I_s \), and the Beer-Lambert law is shown in the equation below
where \( \varepsilon \) is the molar extinction coefficient, also known as constant of absorptivity (Watson, 2012).

\[
\frac{\log I_0}{I_e} = A = \varepsilon bc
\]

Equation 1: Beer-Lambert Law

2.4 Components of the Spectrophotometer

The UV-Vis spectrometer is usually composed of five components, namely a light source, a monochromator, a sample holder, a photosensitive detector and an interpreter.

2.4.1 Light Source

The light source should provide a continuous and stable radiation. Three main types of light sources are used: A Deuterium or Hydrogen lamp provides light with wavelengths covering the ultraviolet region (190nm to 420nm), while the Tungsten filament lamp is used to cover the visible range. The third type, a Xenon lamp covers both the ultraviolet and visible regions, providing light with wavelengths ranging from 190nm to 800nm (Jayakumar, 2016).

2.4.2 Monochromator

This device breaks the continuous range of wavelengths of the incident radiation into component wavelengths. It consists of an entrance slit, which lets in a narrow beam of light incoming from the source, a prism which disperses the light into singular component wavelengths, and an exit slit which allows the passage of the selected wavelength only (Jayakumar, 2016).

2.4.3 Sample Holder

The sample solution to be measured is placed in a sample holder called a cuvette. The latter can be made from several materials, including glass, quartz, and plastic. If
the wavelength irradiating the sample belongs in the UV region, the sample holder must be made of quartz, since glass absorbs UV radiations (Jayakumar, 2016)

2.4.4 Photosensitive Detector
The detector is a device which permits the conversion of photonic energy into an electrical signal. The latter’s intensity would be directly proportional to the intensity of the transmitted light, and a spectrum is generated by comparing the received intensity to the reference intensity, which is generated by measuring the blank. Detectors should be sensitive and their response must be fast (Jayakumar, 2016)

2.5 Applications
UV-Vis spectroscopy is widely used in many industries, including the food and beverages industry, the chemical industry, the health institutes, and others. The reason lies in the fact that UV-Vis spectroscopy requires little time in performing different types of analyses and is highly reliable. It is used to indicate whether olive oil is “virgin” or “extra virgin”, to determine the concentration of an analyte, to measure benzene impurities in organic solutions, to quantify blood hemoglobin, the analysis of phosphorus content in beverages, detergents, and fertilizers, the analysis of various samples in the cosmetic industry, among many other functions (Cosimo, 2015).

In the attempt of determining the concentration of drug content, the solvent to be used should have its peaks at low wavelengths in order not to interfere with the absorption of the molecule of interest. Such solvents include water, methanol, acetone, ethanol, cyclohexane, and many more (Heinz-Helmut, 1992).
CHAPTER THREE

Fourier Transform Infrared Spectroscopy

3.1 Introduction

Fourier Transform Infrared Spectroscopy (FTIR) analyzes the vibrational transitions in a molecule upon being illuminated by infrared light (LibreTexts, 2019), hence it is also called vibrational spectroscopy, along with Raman Spectroscopy (Hainschwang, 2017). This technique works on the scientific principle that different atoms which are bonded together have a dipole as a result of their non-symmetrical bond, hence the infrared light interacts with this bond and causes a transition from the bottom vibrational state to the excited state (Watson, 2012). The main use of this technique is to elucidate the structure of the compound of interest, and determine its functional groups, a fact which led to the use of this technique in a wide array of settings, including dairy analysis (Subramanian & Rodriguez-Saona, 2016), gemstone analysis (Hainschwang, 2017), forensic science (Ferrer, 2107), chemistry labs (Avendaño & Menéndez, 2008), quality analysis and control in the food industry (Lin & Al-Holy, 2009), and pharmaceutical analysis (J.Palermo, 2001).

3.2 Theory

Unlike atoms have different electronegativity values, which results in a polar bond once they come together. A polar bond possesses a dipole moment, and each bond vibrates at a frequency that is different from that of other bonds, which gives rise to a distinctive infrared absorption pattern for each bond, thus correlating to the structure of the molecule (Watson, 2012). Drug molecules, which are very often organic
molecules, consist of multiple unsymmetrical bonds between different atoms, thus generating complex vibrational modes, hence the importance of FTIR in the pharmaceutical industry. Two types of modes of vibration exist, namely stretching (symmetrical and asymmetrical) and bending (in-plane and out-of-plane) vibrations. In a symmetrical stretching, two bonds either increase or decrease in length at the same time, while in an asymmetrical stretching one bond increases in length and the other decreases in length. Two types of bending exist under bending vibrations, in-plane bending and out-of-plane bending, under each also exists two ways of bending. In case of an in-plane bending, two atoms can approach each other leading to a decrease in the bond angle – a way of bending called scissoring – or two atoms can move in the same direction, keeping the bond angle unaltered – a way of bending called rocking. Regarding out-of-plane bending, two atoms can move either up or down the plane at the same time, which is called wagging, or one atom moves above the plane while the other moves below the plane, which is called twisting (Satinder & Stephen, 2012).

Figure 7: Symmetrical Stretching

Figure 8: Asymmetrical Stretching
The infrared part of the electromagnetic spectrum can be divided into three regions according to their wave number: the far infrared extending from 10 to 200 cm\(^{-1}\), the middle infrared ranging between 200 and 4000 cm\(^{-1}\), and lastly the near infrared stretching from 4000 to 12500 cm\(^{-1}\), and possessing the highest energy among those three regions. Regarding the analytical applications of FTIR in the pharmaceutical industry, the most commonly employed region is the middle infrared given that organic molecules highly absorb radiation in this particular region. When radiation is passed through the sample, the bonds of the molecules will absorb energy in the form
of light, thus causing them to undergo stretching and bending. The absorption of infrared light is not random, on the contrary, each absorbed radiation has a wavelength that is characteristic of a specific bond, and that radiation is also subject to a variety of absorption intensities, depending on the type of bond and the type of connected atoms. As a result, peaks at different wave numbers and with distinct intensities appearing on the IR spectrum are indicative of the molecular structure (Satinder & Stephen, 2012).

3.3 Instrumentation

An infrared spectrometer is composed of the following components: the infrared source, the interferometer, the detector, and the computer.

3.3.1 Infrared Source

Different types of filaments are used in order to produce infrared light, mainly metal oxides, which include zirconium, thorium, and yttrium oxides. The filament needs to be heated and its temperature must be controlled given that infrared radiation is dependent on temperature (Satinder & Stephen, 2012).

3.3.2 Interferometer

An interferometer employs two components, a beam-splitter which divides the incoming infrared beam into two optical beams, and mirrors. One of the two beams is reflected by the fixed mirror, while the other beam is reflected by a moving mirror. After each beam reflects off either one of the two mirrors, they are recombined, and the signal leaves the interferometer towards the sample. Since one of the beams travels a variable length due to the movement of one of the mirrors, each time the beams are recombined, they produce a signal having a different frequency, also called an interferogram. The need to know the intensity of each different frequency –
in order to identify the sample – requires decoding the interferogram, which is accomplished through the Fourier transformation (Satinder & Stephen, 2012).

![Schematic Representation of the Interferometer](image)

Figure 13: Schematic Representation of the Interferometer

### 3.3.3 Detector

After passing through the sample, the beam reaches the detector in order for its intensity to be measured, and converted into an electrical signal. Deuterated triglycine sulfate and mercury cadmium telluride are the most important elements in a detector (Watson, 2012).

### 3.3.4 Computer

The measurement obtained from the detector is transmitted to the computer. Several measurements can be performed and averaged in order to reduce noise. Finally, the infrared spectrum is presented on the screen of the computer (Watson, 2012).

### 3.4 Applications

As previously mentioned, FTIR is a versatile method that is used in multiple industries and settings, due to its convenience and non-destructive fashion (Goel, et
In the pharmaceutical industry, this method plays an important role in running a qualitative check for the identity of powders and different raw materials involved in the manufacturing process. It is also used for the sake of identifying any contaminants in manufactured drugs, and can be used on different dosage forms, including tablets, gels, creams, etc. (Bunaciu, Aboul-Enein, & Fleschin, 2010).

3.5 Advantages

The main reason why FTIR is such a versatile and widely used method is due to its numerous strengths. Firstly, it is a fast and reliable method, which provides a unique complex fingerprint for each specific compound within matter of seconds. Secondly, given that it is a computer controlled method, multiple scans can be performed and averaged which enhances the signal to noise ratio, leading to a clearer and neater spectrum. Moreover, matching a standard spectrum with that of an unknown is easily performed by the computer. Thirdly, FTIR is a very sensitive technique and is able to detect trace amounts of chemicals (Fels, Zamama, & Hafidi, 2015).
CHAPTER FOUR

Dissolution Studies

4.1 Introduction
Dissolution is the process in which a chemical compound in a solid state enters into a solution (Ansel, Popovich, & Allen, 2014). Dissolution studies are carried out in order to monitor the release profile of a drug from its dosage form, to prove that this release profile is the same across batches, and to compare it to that of drugs proven to be clinically effective (Aulton, 2013). The dissolution parameters of a drug are related to the specific characteristics of the dosage form as well as the chemical and physical properties of the drug itself, such as crystallinity, amorphicity, the wettability of the dosage form, the particle size, the binder concentration, the tablet hardness, among others (Sherif, Ajit, Keirnan, Ganeshkumar, & Sailes, 2019).

4.2 Theory
In the field of pharmaceutical sciences, a solution is defined as a liquid preparation consisting of one or multiple substances dissolved in a solvent or a mixture of solvents which are miscible (Reference.MD, 2012). The formation of a solution is governed by the laws of thermodynamics, and can be divided into three steps: First, the intermolecular forces in the solute must be overcome, meaning the molecules composing the solute must be separated, which is an endothermic process, i.e. requiring energy. Secondly, the same must happen in the molecules composing the solvent, which is also an endothermic process. Finally, the discrete molecules of the solvent and solute can interact and form attractive bonds between each other, a
process called solvation, which is exothermic, i.e. energy releasing (Chemistry LibreTexts, 2020).

The dissolution process starts by the disintegration of the solid dosage form into granules, which in turn undergo disaggregation into fine particles. Dissolution takes place simultaneously at each phase, and is considered the rate-limiting step in the absorption of drugs (Ansel, Popovich, & Allen, 2014).

Figure 14: Dissolution Process

4.3 Solubility

One of the most important factors affecting the dissolution of drugs is the solubility of the latter, which is correlated to the chemical nature of the drug itself and the solvent in which it is being dissolved. The solubility of a drug can be defined as the amount of drug molecules which can go into solution in a certain solvent, at a specific pressure and temperature (Bhavishya, 2017).
4.3.1 Factors Affecting Solubility

The chemical properties of the solute and the solvent play a key role in determining whether a given substance can go into solution in a given solvent. The dissolution process is governed by the principal of “like dissolves like”, implying that the solvent is best able to dissolve a certain solute if both chemical entities possess similar properties, such as polarity (Wagne, 2012). Other factors include particle size of the solute, and temperature.

4.3.2 Solvent Selection

In chemistry practices, the choice of a suitable solvent able to dissolve a certain chemical entity is relatively easy and rarely comes with restrictions. However, this is not the case in pharmaceutical sciences, given that dissolution studies must mimic the environment of the stomach and the intestine, thus leaving only water as the suitable solvent (Ansel, Popovich, & Allen, 2014).

4.3.3 Particle Size

The solubility of a drug increases as the particle size decreases, due to an increase in the surface area of the solute coming in contact with the solvent. Referring to Figure 14, this is why dissolution is minimal in the phase of “tablet or capsule”, becomes higher at the stage of “granules or aggregates”, and becomes maximal upon reaching the phase of “fine particles” (Aulton, 2013).
4.3.4 Temperature

Temperature and solvation are positively correlated, since heat provides energy to break-down the bonds in a solid. Nevertheless, dissolution studies are limited to being conducted at a temperature of 37°C in order to mimic the body temperature.

4.4 Mathematical Modeling

Values provided by the dissolution test are employed in quantitative analyses and expressed in mathematical models as a means to allow the classification of the mechanism by which the drug release process takes place. Swelling, diffusion, and chemically-based are the three identified mechanisms through which dissolution of a drug happens (Aleksander, et al., 2015). Furthermore, mathematical modeling can be used as a tool to enhance and optimize the development of pharmaceutical formulations with specific release profiles (Peppas & Narasimhan, 2014).

4.4.1 Zero-Order Kinetics

Zero-order release kinetics describe the release of a drug in a fashion that is only dependent on time, meaning that the drug concentration does not affect the speed at which the drug is released. Dosage forms exhibiting such kinetics include oral osmotic tablets, transdermal systems, matrix tablets with low-soluble drugs, and others (Bruschi, 2015). Such release kinetics can be described by the following mathematical relation:

\[ C_t = C_0 + K_0 t \]

Equation 2: Zero-Order Kinetics

Where \( C_t \) is the amount of drug released at time \( t \), \( C_0 \) the initial concentration of drug at time \( t = 0 \) (usually \( C_0 = 0 \)), and \( K_0 \) the zero-order release constant.
4.4.2 First-Order Kinetics

First-order kinetics describe the absorption and elimination of multiple drugs. In this model, the change in concentration during time is thought to be dependent on the concentration of the solution, represented in the following relation, where $C$ is the concentration of the drug, and $K$ is the first-order constant:

$$\frac{dc}{dt} = -KC \quad \text{Equation 3}$$

In 1897, Noyes and Whitney postulated that the concentration at the interface between the dosage form’s surface and the medium in which it is dissolving is different than the concentration of the bulk solution.

This interpretation signifies that the rate of dissolution of the drug is governed by Fick’s first law of diffusion, where drug molecules will be diffusing through a
diffusion layer of thickness \( h \), hence explaining why two concentration are considered in this model, \( C \) and \( C_s \), where the latter describes the concentration at the surface of the dosage form (which is the saturation concentration of the drug) (Ansel, Popovich, & Allen, 2014). This phenomenon can be described by the following equation:

\[
\frac{dc}{dt} = K(C_s - C) \quad \text{Equation 4: Noyes Whitney}
\]

After introducing Fick’s first law into the equation, integrating the equation, and transforming it to logarithmic terms, the relation can be written as the following:

\[
\log Q_1 = \log Q_0 + \frac{K_1 t}{2.303} \quad \text{Equation 5: First-Order Release Kinetics}
\]

Where \( Q_1 \) is the amount of drug at time \( t \), \( Q_0 \) is the initial amount of drug, and \( K_1 \) is the first-order release constant.

### 4.4.3 Higuchi Model

The dissolution of drugs from matrix systems was proposed in 1961 by Higuchi. Between this year and 1963, Higuchi evaluated the release of drugs from ointment bases, and proposed the “mechanism of sustained-action medication”. The works of Higuchi led to development of mathematical models which aim at analyzing the release of soluble and low-soluble active ingredients incorporated in solid and semi-solid matrices (Bruschi, 2015). This model is described by the following equation:

\[
Q = K_H t^{1/2} \quad \text{Equation 6: Higuchi Model}
\]

Where \( Q \) is the amount of drug released at time \( t \), and \( K_H \) the Higuchi release constant.
4.4.4 Hixson-Crowell Model

The Hixson-Crowell model relates to the geometrical form of the entity being studied rather than the diffusion of drug molecules through the medium. Dissolution processes in which the surface of the dosage form diminishes proportionally through time – thus leading to the conservation of the geometrical form – generally follow this model, which is represented by the following equation:

\[ W_0^{1/3} - W_1^{1/3} = K_{HC}t \]  

Equation 7: Hixson-Crowell

Where \( W_0 \) is the initial amount of drug, \( W_1 \) is the amount of drug remaining at time \( t \), and \( K_{HC} \) is the surface-volume relation constant.

4.5 Instrumentation

There are four basic types of dissolution apparatus, including the rotating basket (Apparatus 1), the paddle (Apparatus 2), the reciprocating cylinder (Apparatus 3), and the flow-through cell (Apparatus 4). Concerning the first two apparatus, the assembly include a container made of glass or other inert and transparent material, having a hemispherical bottom, and a 1L nominal capacity, a variable speed stirrer motor, and an apparatus which constitutes the water bath, preferably allowing the observation of the specimen and stirring element (Way, 2015).

Apparatus 1 is most commonly used for non-disintegrating products, given that the basket might be occluded by materials such as gelatin or wax, thus inhibiting the proper flow of media through the mesh of the basket. In case disintegrating products are tested using apparatus 1, 75% dissolution should be achieved within a specified period of time, before particles become small enough to pass through the mesh and sink to the bottom of the container (Way, 2015).
Apparatus 2 is used in case the product to be tested is known to release small particles which might clog the mesh of the basket used in apparatus 1, or pass through it and fall to the bottom. Such products include tablets manufactured by direct compression or capsules filled with powder. The usual stirring speed employed in apparatus 2 is between 50 and 75rpm (Way, 2015).
CHAPTER FIVE

Materials and Methods

5.1 Materials

In the following sub-sections, each material used in the emulsion-solvent evaporation manufacturing process of the microspheres will be described, including the drug, the coating material, the solvents, the surfactants, and the medium in which the microspheres were formed.

5.2 Drug - Theophylline

Theophylline, also known under the IUPAC name as 1,3-dimethylxanthine (C$_7$H$_8$N$_4$O$_2$), is a drug used to treat respiratory diseases, including asthma, Chronic Obstructive Pulmonary Disease (COPD), emphysema, and chronic bronchitis. This chemical can be found in tea (which might explain some of its relaxing effects), and has been shown to be a smooth muscle relaxant, possibly causing bronchodilation, along with having central nervous and cardiac stimulating activities. The pharmacodynamics behind such activities are thought to work through phosphodiesterase inhibition, adenosine receptor blocking, and histone deacetylase activation, thus relaxing the bronchial airways and pulmonary blood vessels, as well as reducing airway responsiveness to histamine, methacholine, adenosine and allergen. Theophylline has also been shown to bind the adenosine A2B receptor, hence blocking bronchoconstriction caused by the latter. Many dosage forms containing Theophylline as the Active Pharmaceutical Ingredient (API) are marketed under different names, and are mainly given orally due to Theophylline’s high
bioavailability, from which some are elixirs, others are tablets and capsules, which could be further divided into either immediate release or extended release. Yet, although less common, parenteral routes of administration have been marketed by several pharmaceutical companies (DrugBank, 2005).

Special care should be given to the process of formulating Theophylline tablets due to its narrow Therapeutic Index, which is partly due to the fact that some of its metabolites are pharmacologically active. Indeed, Theophylline undergoes several bio-transformations, including demethylation which yields 1-Methylxanthine and 3-Methylxanthine, along with hydroxylation which gives rise to 1,3-Dimethyluric Acid. Furthermore, Theophylline is N-methylated, thus producing Caffeine, and 1-Methylxanthine is hydroxylated by xanthine oxidase to yield 1-Methyluric Acid. Two of these metabolites, namely 3-methylxanthine and Caffeine are pharmacologically active (DrugBank, 2005). In addition to its narrow therapeutic index, Theophylline displays a short half-life, ranging from 5 hours for adult non-smokers, to 8 hours for adult smokers (Drugs.com, 2019), which might thus result in low patient compliance.

Bearing this in mind, the optimization of Theophylline encapsulated microparticles offers several advantages, including the avoidance of frequent administration of the drug, the reduction of peak plasma fluctuations, and a good dispersion in the gastro-intestinal tract which aids in maximizing absorption (G.J., et al., 2001).
5.3 Coating Material - Ethylcellulose

Ethylcellulose, a partly O-ethylated cellulose derivative, is synthesized by reacting alkali cellulose with ethyl chloride (Thomas, 2019). This chemical is water-insoluble, however shows good solubility profiles with a wide variety of solvents including tetrahydrofuran, alcohols, chloroform, methyl acetate, and a variety of organic compounds (National library of Medicine, 2020).

[Figure 21: Ethylcellulose Structure]

Ethylcellulose is entailed in multiple and varying uses: It is used in food packaging, printing inks, nail polish, and pharmaceutical applications. In the latter setting, it is employed in the manufacturing of cosmetics, the coating of vitamins, as well as a coating agent for modified release tablets and matrix tablets (McKeen, 2017).

5.4 Solvents

5.4.1 Acetone

Acetone belongs to the ketone family and has multiple uses in the pharmaceutical sector. It is a pharmacologically inactive substance, is colorless, volatile, and highly flammable. Under standard temperature and pressure, acetone takes the form of a liquid (Drugs.com, 2019). In cosmetics, acetone is used in the manufacturing of makeup and creams, while in the pharmaceutical industry, it is the most commonly
used organic solvent, from correcting the density of pharmaceutical preparations, to enhancing the efficacy of some medications (Industrial Degreasers, 2017).

5.4.2 Dichloromethane

Also known as Methylene Chloride, Dichloromethane is a chlorinated hydrocarbon in a liquid state, possessing a somewhat sweet aroma. This colorless chemical is miscible with many organic solvents such as chlorinated hydrocarbons, alcohols, and ether, but not with water, and is used in multiple industries besides the food sector due to its high toxicity and carcinogenic properties (National Library of Medicine, 2020). It is widely used in the pharmaceutical industry, mainly as a solvent for extraction purposes (Drug.com, 2018), and is suitable for the analysis of samples using the UV-Vis technique given that methylene chloride has a cutoff wavelength of 235nm, thus its absorption does not interfere with that of drug molecules (Landolt-Börnstein, 1991).
5.4.3 Tetrahydrofuran

Tetrahydrofuran is part of the class of five-membered oxygenated heterocycles which is water-miscible and used in a wide variety of chemical industries, including electrochemistry (Creager, 2007), organometallic chemistry (Sargent & Dean, 1984), molecular weight determination (Farah, Kunduru, & Domb, 2015), as well as cosmetic formulations (Pérez-Mayoral, Calvino-Casilda, & Martín-Aranda, 2015). In the pharmaceutical industry, tetrahydrofuran is primarily used as a reaction medium (Solventis, 2018).

![Figure 24: Tetrahydrofuran Structure](image)

5.4.4 Ethyl Acetate

Ethyl acetate is a naturally occurring ester, but can also be manufactured by reacting acetic acid with ethanol, a synthetic procedure known as Fischer esterification (Modla, 2019). This chemical is considered environmentally safe and can be used in the food and packaging industry (Auras, 2007). In the pharmaceutical industry, ethyl acetate is involved in extraction processes, and is considered an important component in some cosmetic preparations (Sekab, 2020).
5.4.5 Tetrachlorocarbon

Also known as tetrachloromethane, tetrachlorocarbon is a highly hepatotoxic chemical, which uses have diminished since the late 1990s due to its damaging effects on the environment and its health hazards (McCulloch & Midgley, 2015). Its main uses lie outside the pharmaceutical industry given that it can easily be substituted by less harmful chemicals. It is mainly used in the production of chlorofluorocarbons, and is employed as a degreasing and fire extinguishing agent (Fung, 2012).

5.5 Surfactants

Surfactants are a class of chemicals which once incorporated in a solution, form molecular clusters termed micelles. These structures are capable of adsorbing on the interface between two compounds having different polarities, namely water and oil,
and reducing the surface tension between these two phases. Accomplishing such a task is dependent on one major aspect of the surfactant molecule: having two functional groups with differing affinities to water and oil. The tail, which is a long alkyl chain, usually comprised of a minimum of eight carbon atoms, is the hydrophobic or lipophilic part, which is attracted to the oil layer. The head, which is the hydrophilic part of the molecule, is attracted to the water phase. The presence of such two groups within the same molecule renders the latter amphiphilic, a term used to describe molecules possessing affinity for both polar and non-polar compounds (Nakama, 2017).

Due to their capability in reducing the interfacial tension between oil and water, surfactants are known to be used across a wide variety of settings, including the petroleum and pharmaceutical industries, as well as in the synthesis of polymers and the field of microbiology (Sonawane, Pal, SindhuTayade, & Bisht, 2015). Surfactants are classified as non-ionic or ionic, with the latter group sub-divided into anionic, cationic, or zwitterionic. The latter term points to surfactants which can dissociate into both cations and anions once added to a solution, depending on the pH. Three major parameters control how surfactants interact with their medium and influence the characteristics of microemulsion products, namely the hydrophilic-lipophilic balance, the critical micelle concentration, and the surfactant packing parameter (Vaidya & Ganguli, 2019).

5.5.1 Hydrophilic-Lipophilic Balance (HLB)

Surfactants rely on their ability to be attracted to both polar and non-polar media in order to stabilize emulsions, hence a good balance between their hydrophilic and lipophilic character is required in view of achieving their goal. A systematic ranking ranging between 1 and 20 was put in place by Griffin, wherein the lower end of the
range depicts surfactants with a dominating hydrophobic character, while the higher end points to the most hydrophilic material. The HLB value dictates the type of emulsion which will form as a result of adding the surfactant, either a water-in-oil or an oil-in-water emulsion system (Vaidya & Ganguli, 2019).

5.5.2 Critical Micelle Concentration (CMC)
The critical micelle concentration is the minimum concentration of surfactant required in order for micellization to take place. As a result, the surfactant amount inside a solution should be monitored in a way which guarantees the achievement of the CMC in case surfactant aggregates formation is needed. These aggregates exist in multiple forms and types, depending on the nature of the head and tail of the surfactant. The classification goes as follows: Regular micelles, planar-lamellar, reverse micelles, cylindrical micelles, onion-like lamellar, and interconnected cylinders (Vaidya & Ganguli, 2019).

5.5.3 Surfactant Packing Parameter
The formation of the previously mentioned types of surfactant aggregates are dependent on the molecular structure of the surfactant. Some surfactants might have a more linear character than others, yet other surfactants might present themselves as bulky chains of hydrocarbons. The nature of the surfactant thus affects its packing constraints, which are governed by the laws of thermodynamics and chemical dynamic stability (Vaidya & Ganguli, 2019).

5.6 Oils
5.6.1 Paraffin Oil
Also known as light mineral oil, paraffin oil is obtained from crude petroleum refining. It is a colorless, tasteless, and oily hydrocarbon blend, which is considered a
laxative, and an indispensable compound in the pharmaceutical industry. It is insoluble in water and alcohol, but soluble in numerous organic material, including benzene, ether, chloroform, among others. Its uses in the pharmaceutical industry are multiple, ranging from the production of laxatives, to ointments and gelatin capsules (Lubricant World, 2016).

5.6.2 Isopropyl Myristate

Isopropyl myristate, having a solidification point at 3°C, takes the form of a liquid at room temperature, is a colorless oil with low viscosity, and is odorless. It is soluble in volatile oils, and a variety of organic compounds such as acetone, chloroform, ethyl acetate and many others (National Library of Medicine, 2020). This oil is considered a moisturizer, hence it is used in topical preparations in view of enhancing the skin absorption, as it has been shown to be a good skin penetration enhancer (DrugBank, 2020).

5.7 Methods

5.7.1 Microspheres Preparation

The emulsion-solvent evaporation technique was employed to produce ethylcellulose-theophylline microspheres. The preparation process started by placing a beaker filled with liquid paraffin oil or with isopropyl myristate (with a varying volume between 200 and 480mL) in an ice-water bath, or in a temperature-controlled water bath given that the temperature was varied between 2 and 25°C. Next, a small beaker was filled with a volume ranging between 30 and 60mL of 5% w/w ethylcellulose solution formed either from acetone, ethyl acetate, dichloromethane, tetrachlorocarbon, or tetrahydrofuran. The surfactant was then added to the oil in
case it has a low HLB value, or to the ethylcellulose solution in case it has a high HLB value. Theophylline (with a mass varying between 0.375g to 3.00g) was added to the ethylcellulose solution, while stirring at 750rpm. The theophylline suspension was then added dropwise to the paraffin oil, which was being stirred with a four-bladed propeller or a spherical propeller at a speed varying between 950 and 1250rpm. After 6 hours, the microspheres were collected by suction filtration and washed three times with 50mL portions of hexane. Subsequently the microspheres were air-dried for 24 hours before being weighed and analyzed.

5.7.2 Process Variables

A total of eight variables were monitored during the optimization of microspheres preparation. First of all, the solvent used to produce the ethylcellulose solution was closely studied while maintaining the other variables fixed, given that it plays a major role in formation process and has a strong effect on the characteristics of the microspheres. After testing all five solvents, the one which yielded microspheres presenting the best physical properties was chosen as the reference solvent.

Secondly, the type of propeller was investigated: Two identical preparations were conducted, with the type of propeller being the sole differing factor, wherein one preparation was done using a four-bladed propeller, while the second was done using a spherical propeller.

After choosing the propeller which gave rise to the best outcome, the type of oil used was put under investigation. One preparation was performed in liquid paraffin oil, while a second preparation under identical conditions was performed in isopropyl myristate.
Once the choice of oil has been made, the processing temperature was varied between 2 and 25°C. After that, the stirring speed was varied between 900 and 1250rpm.

After preparing multiple batches of microspheres while varying the aforementioned parameters, the chemical composition of the reaction medium was put to test. The drug to polymer ratio was varied between 1:1 and 1:4, followed by a variation of the organic layer to oil layer ratio, wherein 1:4, 1:8, and 1:16 organic:oil ratio were investigated. At the final stage, the effect of surfactant type and their combination was evaluated.

5.7.3 Microspheres Yield

After drying the microspheres for 24 hours, their mass was recorded, and the percent yield was calculated by dividing the experimental mass by the theoretical mass and multiplying by 100, according to the equation below:

\[ \text{Yield}(\%) = \frac{EM}{TM} \times 100 \quad \text{Equation 8: Percent Yield of Microspheres} \]

Where EM is the experimental mass, and TM is the theoretical mass.

5.7.4 Drug Content Determination

The total drug content of the microspheres was determined by dissolving microspheres weighing between 20 and 50mg (depending on the drug:polymer ratio) in 25mL of dichloromethane, followed by dilution by a factor of 40, and finally measuring the absorbance of the resulting solution at 272nm using a UV-Vis spectrometer. The dilution process was carried out in order to measure the absorbance at very low concentrations given that measurements at low molarities yield more accurate results.
5.7.5 **Drug Entrapment Efficiency**

The loading efficiency was calculated by dividing the experimental total drug content by the theoretical content of microspheres and multiplying by 100, according to the equation below:

\[
\text{Loading Efficiency} \%(\%) = \frac{\text{EDC}}{\text{TDC}} \times 100
\]

Equation 9: Drug Entrapment Efficiency

Where EDC is the experimental drug content, and TDC is the theoretical drug content.

5.7.6 **Surface Drug Accumulation**

In order to determine the amount of drug present on the surface of the microspheres rather than encapsulated inside the coating material, a mass varying between 20 and 50mg of microspheres (depending on the drug:polymer ratio) was agitated in 25mL of simulated gastric fluid for 5 minutes. The microspheres were then removed from the solution by suction filtration, and the solution’s absorbance was measured at 272nm using a UV-Vis spectrometer.

5.7.7 **Particle Size and Shape Analysis**

The particle size and the shape of microspheres were assessed using a light microscope with a field of view of 1350µm when then 10X lens is used. Placing the microspheres adjacent to each other in a straight line, counting the number of microspheres visible in the field of view, and dividing the diameter of the latter (1350µm) by the number of visible microspheres will yield their diameter. Measurements were done in triplicate on multiple microspheres in view of determining the size distribution.
5.7.8 Fourier Transform Infrared Spectroscopy
The infrared spectra of pure theophylline, pure ethylcellulose, a physical mixture of both ingredients, and microspheres were recorded over a range of 400 to 4000cm\(^{-1}\). Comparing these spectra will allow us to gain a better understanding of the interaction between ethylcellulose and theophylline in microspheres.

5.7.9 Stability Studies
Stability studies were conducted in order to determine whether the drug content of the microspheres would remain the same over several months, or the particles would otherwise leach some of the drug. Small samples from randomly selected batches of microspheres were put into glass vials at 45°C for 90 days. Each 30 days, an accurately weighed mass of microspheres was withdrawn from the vials and tested for its drug content.

5.7.10 In-Vitro Drug Release
In-vitro drug release studies were performed on selected batches of microspheres using Apparatus 1. 400mg of microspheres were put in a basket attached to a shaft connected to variable speed motor, and immersed into a hemispherical-bottom container filled with Simulated Gastric Fluid (SGF) at 37°C. The SGF without enzyme was prepared following USP recommendations, wherein sodium chloride (NaCl), hydrochloric acid (HCl) and distilled water were combined so as to have a final solution with a concentration of 0.2% (w/v) NaCl, and 0.7% (v/v) HCl, at a pH of 1.2. The container was filled with 900mL of SGF and the dissolution studies were run for 8 hours as per USP recommendations. Every hour, 1mL aliquot was collected for UV-Vis analysis at 272nm, and replaced by an equivalent volume of fresh medium.
5.7.11 Microspheres Drug Release Kinetics

In an attempt to evaluate theophylline release kinetics from the microspheres, the aforementioned collected data was fitted to four kinetic models, namely zero-order, first-order, Higuchi, and Hixson-Crowell. The model which best describes the release kinetics is the one having the highest correlation coefficient $R^2$. 
CHAPTER SIX

Results and Discussion

6.1 Yield and Physical Properties

In the following sub-sections, different batches formed under differing conditions will be compared with respect to their yield and physical properties. Chemical characteristics and release studies will follow.

6.1.1 Solvent Variations

Five solutions of 5% (w/v) of ethylcellulose in acetone, ethyl acetate, dichloromethane, tetrachlorocarbon, and tetrahydrofuran were prepared. 1.00g of theophylline and 3.75mL of Tween80 were added to 60mL of each of the above mentioned solutions, and mixed at 750rpm for five minutes using a magnetic bar and a magnetic stirrer. The resulting suspension was added dropwise to a beaker filled with 250mL of paraffin oil at 2°C, while being mixed with a spherical propeller at 900rpm. The processing conditions, namely theophylline:ethylcellulose ratio (TH:EC), solvent:oil ratio, type and volume of oil, type and volume of surfactant, temperature, type of propeller, and stirring speed were maintained the same for all five preparations, with the only variable being the solvent used to prepare the microspheres.
Table 1: Results of Varying Solvents

<table>
<thead>
<tr>
<th>Batch</th>
<th>Solvent (w/v)</th>
<th>TH:EC Ratio</th>
<th>Solvent:Oil Ratio</th>
<th>Temp °C</th>
<th>Speed (rpm)</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>N/A</td>
<td>5% EC in Ethyl Acetate</td>
<td>1:3</td>
<td>1:4</td>
<td>2.0</td>
<td>900</td>
<td>Failed</td>
</tr>
<tr>
<td>1</td>
<td>5% EC in Acetone</td>
<td>1:3</td>
<td>1:4</td>
<td>2.0</td>
<td>900</td>
<td>Successful</td>
</tr>
<tr>
<td>N/A</td>
<td>5% EC in Dichloromethane</td>
<td>1:3</td>
<td>1:4</td>
<td>2.0</td>
<td>900</td>
<td>Failed</td>
</tr>
<tr>
<td>N/A</td>
<td>5% EC in Tetrachlorocarbon</td>
<td>1:3</td>
<td>1:4</td>
<td>2.0</td>
<td>900</td>
<td>Failed</td>
</tr>
<tr>
<td>N/A</td>
<td>5% EC in THF</td>
<td>1:3</td>
<td>1:4</td>
<td>2.0</td>
<td>900</td>
<td>Failed</td>
</tr>
</tbody>
</table>

After 6 hours of processing, the microspheres were collected by suction-filtration, washed with three portions of 50mL n-hexane, and air-dried for 24 hours. Microsphere formation was only successful with a solution of 5% ethylcellulose in acetone, with a percent yield of 84.62%, and a diameter ranging between 650 and 750µm, while the use of other solvents did not prove to be successful in producing microparticles. The reason behind such a phenomenon might be due to the fact that all the aforementioned solvents except for acetone, are miscible with paraffin oil. This chemical aspect can be explained by the fact that acetone has the highest dielectric constant among the used solvents (Engineering ToolBox, 2008), making it the most polar. As a result, unlike the other solvents, acetone does not go into solution with paraffin oil, however acetone molecules do get close enough to the oil molecules thanks to the presence of the surfactant, which allows the formation of a stable emulsion, suitable for microsphere formation. The emulsion is composed of 19% solvent and 81% paraffin oil. A detailed study of the solubility profile of Paraffin-Acetone-Toluene system shows that dissolution of 19.68% acetone in
60.61% paraffin oil is not possible below 40°C (Grishin & Kosolapova, 1965), while the processing temperature was 2°C.

Apart from the miscibility component, the boiling point of the solvent coupled with the processing temperature play a major role in the formation of microspheres. While ethyl acetate, tetrachlorocarbon, and tetrahydrofuran have boiling points ranging between 65 and 77°C, which are considered high, and dichloromethane having a low boiling point (below 40°C), acetone has an intermediate boiling point of 54°C. Higher boiling points make it harder for the solvent to evaporate and induce microsphere formation, while lower boiling points lead to the fast evaporation of the solvent, hence an intermediate boiling point is most suitable for microsphere formation.

Moreover, the vapor pressure of the solvent may play an important role in microsphere formation. The vapor pressure of a substance can be explained as the pressure exerted by the vapor of a substance, which is in equilibrium with the liquid (or solid) phase, at a given temperature. The vapor pressure is indicative of the evaporation rate of a substance, as both dimensions are positively correlated, and substances are classified as volatile when they possess a high vapor pressure at a normal temperature (Speight, 2019). Vapor pressure of substances can be calculated by substituting the Antoine coefficients into the following equation:

\[ \log_{10} P = A - \frac{B}{T+C} \]  

Equation 10: Vapor Pressure Equation

Where \( P \) is the vapor pressure in mmHg, \( A, B, \) and \( C \) are the Antoine coefficients, and \( T \) is the temperature in degrees Celsius (Yaws & Satyro, 2015).
By substituting the respective Antoine coefficients of each solvent and a temperature of 2°C into the above equation we get the following vapor pressure values for each solvent, listed in increasing order:

- Tetrahydrofuran: 21.28mmHg
- Ethyl acetate: 28.55mmHg
- Tetrachlorocarbon: 37.00mmHg
- Acetone: 77.89mmHg
- Dichloromethane: 157.79mmHg

The high vapor pressure of dichloromethane as well as it having the lowest boiling point will lead to the fastest evaporation compared to the other solvents. Its rate of evaporation might be high to the point that it negatively affects the formation of microspheres. On the other hand, the very low vapor pressures of tetrahydrofuran, ethyl acetate, and tetrachlorocarbon will lead to minimal evaporation, on top of the fact that theophylline is soluble in the three aforementioned solvents, which hardens the process of microsphere formation. Finally, the moderate vapor pressure of acetone results in good evaporation rate which permits the formation of microspheres.

The final factor which may have played a role in the successful formation of microspheres is the solubility of theophylline in the solvent. While the combination of theophylline and acetone resulted in a suspension of the drug in the solvent (due to poor solubility), the drug was readily soluble with the other solvents and practically formed a solution. This fact may have hindered the ability of microspheres to form, given that a solution is thermodynamically more stable than a suspension, hence the reaction drive would be less shifted toward the formation of microspheres.
6.1.2 Propeller Variations

The second variable that was tested is the type of propeller used to mix the emulsion system comprising the oil, the organic phase, the drug, the surfactant, and the polymer. Acetone was the chosen solvent based on the results discussed in the section above. The formulation process parameters were all kept constant, except for the propeller type, wherein batch 3 was produced using a 4-bladed propeller, while batch 2 was produced using a spherical propeller.

The spherical propeller yielded free-flowing microparticles which are spherically shaped and have a diameter of 650-750µm, while the 4-bladed propeller produced microparticles which are fairly spherical in shape, however larger in size compared to those of batch 2, and are not free-flowing, on the contrary, the microparticles aggregated into large chunks.

Batch 2 has the exact same parameters of batch 1, which explains why both yields are very similar, with the first being 86.09% and the second being 84.62%, and their diameters are the same. Batch 3 gave a yield of 79.06%, a slightly diminished one compared to the first two batches.

Based on these findings, the spherical propeller was deemed more appropriate for the production of microspheres.
The reason behind such a difference in properties might be related to how the propeller shapes the liquid vortex. Whereas the spherical propeller induced a well-equilibrated vortex that is symmetrical overall, the 4-bladed propeller engendered an unsymmetrical and unstable liquid vortex. This difference in physical dynamics is the major factor which led to a difference in the properties of the microspheres.

### 6.1.3 Type of Oil Variations

After selecting the proper solvent and the most suitable propeller, the effect of the type of oil on microspheres properties was examined. For this purpose, two types of oil have been used, paraffin oil and isopropyl myristate. All the processing parameters were kept constant as for the first 3 batches, with the only differing factor being the type of oil. Moreover, a small change was included, which is the elevation of the temperature from 2°C to 4°C, given that isopropyl myristate freezes at 3°C.

<table>
<thead>
<tr>
<th>Batch</th>
<th>Solvent (w/v)</th>
<th>TH:EC Ratio</th>
<th>Oil</th>
<th>Temp °C</th>
<th>Propeller</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>5% EC in Acetone</td>
<td>1:3</td>
<td>Paraffin</td>
<td>4.0</td>
<td>Spherical</td>
<td>Successful</td>
</tr>
<tr>
<td>N/A</td>
<td>5% EC in Acetone</td>
<td>1:3</td>
<td>Isopropyl Myristate</td>
<td>4.0</td>
<td>Spherical</td>
<td>Failed</td>
</tr>
</tbody>
</table>

Table 3: Results of Varying the Type of Oil
Batch 4 recorded a yield of 85.33% of spherical and free-flowing microspheres, with a diameter range of 650 to 750 µm, similar to batches 1 and 2, indicating that the elevation of temperature by 2°C (from 2°C to 4°C) does not affect the properties of microspheres. When isopropyl myristate was used, no microspheres yield was recorded. No possible reason for such a phenomenon is present in the literature given that isopropyl myristate has not been employed in the production of microspheres. Nevertheless, the viscosity of the oil phase plays an important role in dictating the properties of the microspheres, as has been suggested by Khare and Jain. Their findings point to the fact that when the viscosity of the oil phase is increased, irregularities in shape and size distribution of the microspheres become more pronounced. They explain this occurrence by referring to Newton’s law which relates the viscosity of a liquid to the force required to produce a given rate of shear. That is, with increasing viscosity, a constant force produced by the rotation of the propeller will induce a lower shear stress, resulting in nonuniform microspheres (Khare & Jain, 2009). Hence, the only postulate to explain the failure of isopropyl myristate to yield microspheres is the fact that its viscosity at 4°C becomes higher than that of paraffin oil.

6.1.4 Temperature and Stirring Speed Variations

As previously mentioned in Chapter 1, the size of the microspheres is positively correlated with temperature and negatively correlated with the stirring speed. Thus, there was no need to replicate such studies. Instead, stirring speed and temperature variables were combined and they were increased simultaneously in an attempt to come-up with a sweet-spot for these two variables. An increase in temperature will lead to an increase in microsphere size, while an increase in stirring speed will induce the opposite effect, leading to a decrease in
size. Hence, the rationale here is to increase both temperature and stirring speed at the same time, which will thus determine which parameter has the overwhelming effect, and if a sweet-spot does exist.

Table 4: Results of Varying Temperature and Stirring Speed

<table>
<thead>
<tr>
<th>Batch</th>
<th>Solvent (w/v)</th>
<th>TH:EC Ratio</th>
<th>Solvent:Oil Ratio</th>
<th>Temp ºC</th>
<th>Speed (rpm)</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>5% EC in Acetone 1:3</td>
<td>1:4</td>
<td>2.0</td>
<td>900</td>
<td>Successful</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>5% EC in Acetone 1:3</td>
<td>1:4</td>
<td>10.0</td>
<td>1,250</td>
<td>Failed</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>5% EC in Acetone 1:3</td>
<td>1:4</td>
<td>25.0</td>
<td>1,000</td>
<td>Successful</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>5% EC in Acetone 1:3</td>
<td>1:4</td>
<td>35.0</td>
<td>1,100</td>
<td>Failed</td>
<td></td>
</tr>
</tbody>
</table>

Regarding the stirring speed, problems with microspheres formation were encountered once the stirring speed approaches and exceeds 1,100rpm. In fact, the microparticles become less spherical, and more needle-like, and the yield is much-reduced, a phenomenon which is observed in batch 5 and 6. The first gave a yield of 20.39% and the second a yield of 47.32%, compared to a yield equaling 86.09% and 81.39% for both batches 2 and 14 respectively, hence the yield is diminished with increasing stirring speed. Batches 2 and 14 had the same average diameter ranging between 650 and 750µm which indicates that the increase in diameter of microspheres induced by increasing temperature can be countered by an increase in stirring speed, to the limit of 1,000rpm. As a result, the rest of the batches are produced at a temperature of 2°C and a stirring rate of 950rpm.
6.1.5 Drug to Polymer Ratio Variations

Table 5: Results of Varying Drug to Polymer Ratio

<table>
<thead>
<tr>
<th>Batch</th>
<th>Solvent (w/v)</th>
<th>TH:EC Ratio</th>
<th>Solvent:Oil Ratio</th>
<th>Temp °C</th>
<th>Speed (rpm)</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>5% EC in Acetone</td>
<td>1:1</td>
<td>1:4</td>
<td>2.0</td>
<td>950</td>
<td>Successful</td>
</tr>
<tr>
<td>9</td>
<td>5% EC in Acetone</td>
<td>1:2</td>
<td>1:4</td>
<td>2.0</td>
<td>950</td>
<td>Successful</td>
</tr>
<tr>
<td>2</td>
<td>5% EC in Acetone</td>
<td>1:3</td>
<td>1:4</td>
<td>2.0</td>
<td>900</td>
<td>Successful</td>
</tr>
<tr>
<td>10</td>
<td>5% EC in Acetone</td>
<td>1:4</td>
<td>1:4</td>
<td>2.0</td>
<td>950</td>
<td>Successful</td>
</tr>
</tbody>
</table>

Once the solvent (acetone), the propeller type (spherical), the type of oil (paraffin), the temperature (2°C), and the stirring speed (950rpm) were chosen, several drug to polymer ratios (1:1, 1:2, 1:4) were examined, while keeping all the other parameters fixed. Batch 2 had a drug to polymer ratio of 1:3, and the exact same parameters as batches 8, 9, and 10, except for the stirring speed which is lower by 50rpm. Nevertheless, its comparison with the other batches would be appropriate. Results reveal that microspheres yield increases as the ratio gets closer to 1:1, as shown in the table below, however at the expense of the size.

Table 6: Yield and Size of Microspheres

<table>
<thead>
<tr>
<th>Batch</th>
<th>Solvent (w/v)</th>
<th>TH:EC Ratio</th>
<th>Yield (%)</th>
<th>Size (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>5% EC in Acetone</td>
<td>1:1</td>
<td>89.91</td>
<td>750-850</td>
</tr>
<tr>
<td>9</td>
<td>5% EC in Acetone</td>
<td>1:2</td>
<td>88.58</td>
<td>750-850</td>
</tr>
<tr>
<td>2</td>
<td>5% EC in Acetone</td>
<td>1:3</td>
<td>84.62</td>
<td>650-750</td>
</tr>
<tr>
<td>10</td>
<td>5% EC in Acetone</td>
<td>1:4</td>
<td>83.14</td>
<td>450-550</td>
</tr>
</tbody>
</table>
All batches gave microparticles which are spherical in shape. The yield increased by 8.14% when the TH:EC ratio is changed from 1:4 to 1:1, however the size of the microspheres is decreased by an average of 37.65% when the drug to polymer ratio is 1:4 compared to 1:1. Hence, the 1:4 TH:EC ratio was deemed as more successful than the 1:1 ratio, and subsequent batches were produced using the former ratio.

### 6.1.6 Solvent Layer to Oil Layer Variations

Batches 10, 11, and 12 had the exact processing conditions except for the organic layer to oil layer ratio, which were 1:4, 1:8, and 1:16 respectively.

<table>
<thead>
<tr>
<th>Batch</th>
<th>Solvent (w/v)</th>
<th>TH:EC Ratio</th>
<th>Solvent:Oil Ratio</th>
<th>Temp (°C)</th>
<th>Speed (rpm)</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>5% EC in Acetone</td>
<td>1:4</td>
<td>1:4</td>
<td>2.0</td>
<td>950</td>
<td>Success</td>
</tr>
<tr>
<td>11</td>
<td>5% EC in Acetone</td>
<td>1:4</td>
<td>1:8</td>
<td>2.0</td>
<td>950</td>
<td>Success</td>
</tr>
<tr>
<td>12</td>
<td>5% EC in Acetone</td>
<td>1:4</td>
<td>1:16</td>
<td>2.0</td>
<td>950</td>
<td>Not Bad</td>
</tr>
</tbody>
</table>

Varying the organic layer to oil ratio did not have an impact on the yield of microspheres, which were 83.14%, 82.25%, and 83.68% for batches 10, 11, and 12 respectively. However, this variable had an impact on the shape and the size distribution of the microparticles. Indeed, batch 12 yielded irregularly-shaped microparticles, batch 11 gave microparticles closer to being spherically-shaped with a wide size distribution ranging from 800 to 950µm. This effect can be attributed to changes in the viscosity of the processing medium, thus the best results were obtained when the organic layer to oil layer ratio is 1:4.
6.1.7 Surfactant Variations

Table 8: Results of Varying the Surfactant Type

<table>
<thead>
<tr>
<th>Batch</th>
<th>Solvent (w/v)</th>
<th>TH:EC Ratio</th>
<th>Solvent:Oil Ratio</th>
<th>Surfactant [HLB]</th>
<th>Temp °C</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>5% EC in Acetone</td>
<td>1:4</td>
<td>1:4</td>
<td>Tween80 [15.0]</td>
<td>2.0</td>
<td>Successful</td>
</tr>
<tr>
<td>13</td>
<td>5% EC in Acetone</td>
<td>1:4</td>
<td>1:4</td>
<td>Tween20 [16.7]</td>
<td>2.0</td>
<td>Failed</td>
</tr>
<tr>
<td>15</td>
<td>5% EC in Acetone</td>
<td>1:4</td>
<td>1:4</td>
<td>Brij58 [15.7]</td>
<td>2.0</td>
<td>Successful</td>
</tr>
<tr>
<td>16</td>
<td>5% EC in Acetone</td>
<td>1:4</td>
<td>1:4</td>
<td>Tween40 [15.6]</td>
<td>2.0</td>
<td>Successful</td>
</tr>
<tr>
<td>17</td>
<td>5% EC in Acetone</td>
<td>1:4</td>
<td>1:4</td>
<td>Span85 Tween80</td>
<td>2.0</td>
<td>Successful</td>
</tr>
<tr>
<td>18</td>
<td>5% EC in Acetone</td>
<td>1:4</td>
<td>1:4</td>
<td>Span80 Tween80</td>
<td>2.0</td>
<td>Successful</td>
</tr>
<tr>
<td>N/A</td>
<td>5% EC in Acetone</td>
<td>1:4</td>
<td>1:4</td>
<td>Span65 [2.1]</td>
<td>2.0</td>
<td>Failed</td>
</tr>
<tr>
<td>N/A</td>
<td>5% EC in Acetone</td>
<td>1:4</td>
<td>1:4</td>
<td>Span80 [4.3]</td>
<td>2.0</td>
<td>Failed</td>
</tr>
<tr>
<td>N/A</td>
<td>5% EC in Acetone</td>
<td>1:4</td>
<td>1:4</td>
<td>Span85 [1.8]</td>
<td>2.0</td>
<td>Failed</td>
</tr>
<tr>
<td>N/A</td>
<td>5% EC in Acetone</td>
<td>1:4</td>
<td>1:4</td>
<td>Poloxamer188 [29]</td>
<td>2.0</td>
<td>Failed</td>
</tr>
<tr>
<td>N/A</td>
<td>5% EC in Acetone</td>
<td>1:4</td>
<td>1:4</td>
<td>Poloxamer407 [22]</td>
<td>2.0</td>
<td>Failed</td>
</tr>
</tbody>
</table>

The effect of several surfactants on the properties of microspheres was explored.

After optimizing all the processing conditions, including solvent type, propeller, type of oil, temperature, stirring speed, drug to polymer ratio, and solvent layer to oil layer ratio, different surfactants – alone or in combination – were examined.

Regarding the Tween surfactants, Tween20 gave a yield of 72.52%, the lowest compared to Tween 40, Tween80, both of which had similar yields. Moreover, Tween20 did not give spherical microparticles, as opposed to the other two. The
highest yield, 97.9% was obtained by combining a 1:1 mixture of Span80 and Tween80 for a total of 3.75mL. The second highest yield, 93.77% was obtained from a combination of Span85 and Tween80 in a 1:1 ratio. The interesting part is that Span surfactants alone did not yield any microspheres, however when in combination with Tween, were able to significantly increase the yield and decrease the diameter of microspheres as compared to when Tween alone is used.

Regarding the microspheres size, the lowest diameter range, 250µm to 300µm was exhibited by batch 17. Batch 18 also displayed microspheres with a reduced diameter, ranging from 300µm to 350µm. Table 9 shows selected microsphere shapes, sizes and yields of successful batches and is further discussed below.

<table>
<thead>
<tr>
<th>Batch</th>
<th>Surfactant [HLB]</th>
<th>Yield (%)</th>
<th>Shape</th>
<th>Size (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>Tween80 [15.0]</td>
<td>83.14</td>
<td>Spherical</td>
<td>450-550</td>
</tr>
<tr>
<td>13</td>
<td>Tween20 [16.7]</td>
<td>72.52</td>
<td>Irregular</td>
<td>N/A</td>
</tr>
<tr>
<td>15</td>
<td>Brij58 [15.7]</td>
<td>79.55</td>
<td>Spherical</td>
<td>450-550</td>
</tr>
<tr>
<td>16</td>
<td>Tween40 [15.6]</td>
<td>82.03</td>
<td>Spherical</td>
<td>550-600</td>
</tr>
<tr>
<td>17</td>
<td>Span85, Tween80</td>
<td>93.77</td>
<td>Spherical</td>
<td>250-300</td>
</tr>
<tr>
<td>18</td>
<td>Span80, Tween80</td>
<td>97.90</td>
<td>Spherical</td>
<td>300-350</td>
</tr>
</tbody>
</table>

The combination of high HLB value and low HLB value surfactants led to the highest yields and the lowest microspheres diameter. This fact can be explained by the proper distribution of surfactants between the more polar solvent layer and the non-polar oil layer, which results in a more stable emulsion system and hence a
better interaction between the polymer and the drug, which in turn leads to higher packing capability. In conclusion, the best physical characteristics and the highest yield was obtained by choosing acetone as a solvent, a spherical propeller, paraffin oil, 950rpm stirring speed, a temperature of 2°C, a drug to polymer ratio of 1:4, an organic layer to oil layer ratio of 1:4, and combining a low HLB surfactant with a high HLB surfactant.

6.2 Physicochemical Properties

In the following sub-section, the drug entrapment efficiency, the interaction between the drug and the polymer, and the stability studies will be explored.

6.2.1 Drug Entrapment Efficiency

As mentioned in section 5.6.5, the drug loading efficiency will be calculated according to equation 9. A standard curve of theophylline dissolved in methylene chloride was built. The concentration ranged from 2 to 12mg/L as shown in the graph below.

![TH Standard Curve](image)

Figure 29: Theophylline Standard Curve (Absorbance taken at 272nm)
In order to measure the drug content of microspheres, a suitable amount of the drug (depending on the drug to polymer ratio) was accurately measured and dissolved in a 25mL volumetric flask filled with methylene chloride. A dilution by a factor of 40 was then carried out and the absorbance of the resulting solution was measured at 272nm. The measurement was fitted in the equation of the graph and hence the concentration of theophylline is obtained. This concentration is then divided by the theoretical one and multiplied by 100, according to equation 9. The table below shows the entrapment efficiency of selected batches.

Table 10: Effects of Temperature on Entrapment Efficiency

<table>
<thead>
<tr>
<th>Batch</th>
<th>Temp °C</th>
<th>MCP Mass (mg)</th>
<th>TDC (mg)</th>
<th>Abs</th>
<th>EDC (mg)</th>
<th>Loading Efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>40.800</td>
<td>10.123</td>
<td>0.197</td>
<td>4.325</td>
<td>42.73%</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>40.600</td>
<td>10.150</td>
<td>0.206</td>
<td>4.526</td>
<td>44.59%</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>41.800</td>
<td>10.450</td>
<td>0.211</td>
<td>4.637</td>
<td>44.38%</td>
</tr>
<tr>
<td>14</td>
<td>25</td>
<td>51.700</td>
<td>10.340</td>
<td>0.245</td>
<td>5.394</td>
<td>52.17%</td>
</tr>
<tr>
<td>6</td>
<td>35</td>
<td>47.200</td>
<td>11.800</td>
<td>0.306</td>
<td>6.753</td>
<td>57.23%</td>
</tr>
</tbody>
</table>

*MCP: Microparticles
*TDC: Theoretical Drug Content
*EDC: Experimental Drug Content

Batches 1, 2, and 4 display very similar loading efficiencies given that the conditions under which they were produced are the same, except for batch 4 which had a processing temperature greater than that of batches 1 and 2 by only 2°C. Batch 14 displays a higher loading efficiency than the three aforementioned batches, due to the higher temperature of production. The highest loading efficiency of 57.23% was achieved at the highest processing temperature (batch 6). Thus, as the stirring rate...
has the overwhelming effect regarding the shape and the yield of microspheres, temperature has the overwhelming effect when it comes to drug loading, regardless of stirring speed.

Table 11: Effect of Drug to Polymer Ratio on Entrapment Efficiency

<table>
<thead>
<tr>
<th>Batch</th>
<th>TH:EC</th>
<th>MCP Mass (mg)</th>
<th>TDC (mg)</th>
<th>Abs</th>
<th>EDC (mg)</th>
<th>Loading Efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>1:1</td>
<td>21.200</td>
<td>10.600</td>
<td>0.146</td>
<td>3.190</td>
<td>30.09%</td>
</tr>
<tr>
<td>9</td>
<td>1:2</td>
<td>31.100</td>
<td>10.367</td>
<td>0.187</td>
<td>4.103</td>
<td>39.57%</td>
</tr>
<tr>
<td>2</td>
<td>1:3</td>
<td>40.600</td>
<td>10.150</td>
<td>0.206</td>
<td>4.526</td>
<td>44.59%</td>
</tr>
<tr>
<td>10</td>
<td>1:4</td>
<td>50.200</td>
<td>10.040</td>
<td>0.362</td>
<td>8.000</td>
<td>79.68%</td>
</tr>
</tbody>
</table>

The drug entrapment efficiency was maximized with a decrease in the drug to polymer ratio. In fact, the entrapment efficiency of batch 10 increased by 164.8% when compared to batch 8. This observation might be attributed to changes in the processing medium at the molecular level, where a high drug content may be leading to an overcrowding in the processing vessel, thus hindering the ability of the polymer to properly pack the drug.

Table 12: Effect of Solvent Layer to Oil Layer on Entrapment Efficiency

<table>
<thead>
<tr>
<th>Batch</th>
<th>Solvent:Oil</th>
<th>MCP Mass (mg)</th>
<th>TDC (mg)</th>
<th>Abs</th>
<th>EDC (mg)</th>
<th>Loading Efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>1:4</td>
<td>50.200</td>
<td>10.040</td>
<td>0.362</td>
<td>8.000</td>
<td>79.68%</td>
</tr>
<tr>
<td>11</td>
<td>1:8</td>
<td>52.000</td>
<td>10.400</td>
<td>0.314</td>
<td>6.931</td>
<td>66.65%</td>
</tr>
<tr>
<td>12</td>
<td>1:16</td>
<td>52.800</td>
<td>10.560</td>
<td>0.239</td>
<td>5.261</td>
<td>49.82%</td>
</tr>
</tbody>
</table>
When the oil volume is increased while keeping the solvent volume fixed, i.e. when the organic layer to oil layer is decreased, the loading efficiency also decreases. The organic layer has a greater viscosity than the oil layer, hence when the volume of oil increases, the viscosity of the whole medium decreases. This is the reason behind a decrease in the loading efficiency.

Table 13: Effect of Surfactant Type on Entrapment Efficiency

<table>
<thead>
<tr>
<th>Batch</th>
<th>Surfactant [HLB]</th>
<th>MCP Mass (mg)</th>
<th>TDC (mg)</th>
<th>Abs</th>
<th>EDC (mg)</th>
<th>Loading Efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>Tween80 [15.0]</td>
<td>50.200</td>
<td>10.040</td>
<td>0.362</td>
<td>8.000</td>
<td>79.68%</td>
</tr>
<tr>
<td>15</td>
<td>Brij58 [15.7]</td>
<td>50.300</td>
<td>10.060</td>
<td>0.299</td>
<td>6.597</td>
<td>65.58%</td>
</tr>
<tr>
<td>16</td>
<td>Tween40 [15.6]</td>
<td>50.900</td>
<td>10.180</td>
<td>0.242</td>
<td>5.328</td>
<td>52.33%</td>
</tr>
<tr>
<td>17</td>
<td>Span85[1.8]</td>
<td>51.600</td>
<td>10.320</td>
<td>0.429</td>
<td>9.492</td>
<td>91.98%</td>
</tr>
<tr>
<td></td>
<td>Tween80</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>Span80[4.3]</td>
<td>49.700</td>
<td>9.940</td>
<td>0.421</td>
<td>9.314</td>
<td>93.70%</td>
</tr>
</tbody>
</table>

The highest entrapment efficiency was achieved by combining low HLB and high HLB surfactants. The higher packing capability induced by combining surfactants will lead to a lesser amount of drug left un-encapsulated, hence the drug loading efficiency is maximized.

6.2.2 Fourier-Transform Infrared Spectroscopy

Samples of pure theophylline, pure ethylcellulose, a physical mixture of both chemicals, and microspheres were analyzed spectroscopically. The spectra are shown below.
Figure 30: Theophylline IR Spectrum

Figure 31: Ethylcellulose IR Spectrum
Figure 32: IR Spectrum of a Mixture of Theophylline and Ethylcellulose

Figure 33: IR Spectrum of Microspheres
The IR spectrum of theophylline shows two peaks at roughly 1,700 cm\(^{-1}\), exhibited by the two carbonyl groups present on the six-membered ring of the drug. These two peaks are also present on the spectrum of the mixture of theophylline and ethylcellulose, as shown in figure 32. However, these peaks are absent from the spectrum of the microspheres, while the double-dented peak at 2900 cm\(^{-1}\) – characteristic of ethylcellulose – is present on both the spectrum of the mixture and the microspheres. These findings suggest that the drug is properly packed inside the core material and no drug is present on the surface, and that no interaction occurs between the polymer and the drug. Furthermore, the IR analysis was repeated 3 months after microspheres synthesis, and the same spectra were shown, suggesting that the microspheres are stable and do not leach any drug material through time.

### 6.2.3 Stability Studies

In order to test the stability of microspheres, small samples retrieved from randomly selected batches were exposed to a temperature of 45°C for 90 days. Drug entrapment efficiency was tabulated at 30-day intervals in an attempt to assess whether the microspheres might be leaching some of the drug over time.

<table>
<thead>
<tr>
<th>Batch</th>
<th>Abs(_{30})</th>
<th>Entrapment Efficiency</th>
<th>Abs(_{60})</th>
<th>Entrapment Efficiency</th>
<th>Abs(_{90})</th>
<th>Entrapment Efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0.360</td>
<td>79.24%</td>
<td>0.357</td>
<td>78.57%</td>
<td>0.365</td>
<td>80.35%</td>
</tr>
<tr>
<td>15</td>
<td>0.304</td>
<td>66.68%</td>
<td>0.302</td>
<td>66.24%</td>
<td>0.295</td>
<td>64.69%</td>
</tr>
<tr>
<td>17</td>
<td>0.430</td>
<td>92.20%</td>
<td>0.427</td>
<td>91.55%</td>
<td>0.433</td>
<td>92.84%</td>
</tr>
<tr>
<td>18</td>
<td>0.419</td>
<td>93.26%</td>
<td>0.417</td>
<td>92.81%</td>
<td>0.424</td>
<td>94.38%</td>
</tr>
</tbody>
</table>
As per the table above, the entrapment efficiency was not affected by exposing the microspheres to a high temperature over a period of time extending for 90 days. This has been confirmed by taking the exact same mass of microspheres which had been taken when the first measurement was performed, dissolving them in the same volume of methylene chloride, carrying out the exact same dilution process, and measuring the absorbance of the solution at 272nm. The stability of the microspheres had also been proven by the IR spectra.

### 6.3 Drug Release Kinetics

Batches 9, 10, 15, and 17 were selected to conduct in-vitro dissolution studies. The comparison of the release kinetics exhibited by batches 9 and 10 will offer insight into the effect of varying the drug to polymer ratio. Moreover, microspheres of batch 10 are roughly 38% smaller than those of batch 9, hence the effect of the size of microspheres on the release kinetics will be examined. Batches 15 and 17 will permit the analysis of surfactant variations on the release kinetics of microspheres, as well as to gain further insight into the effect of decreasing size given that batch 17 comprises microspheres having the smallest diameter among all the batches.

400mg of microspheres were taken from each batch and placed inside the basket of dissolution apparatus 1, which was then attached to a shaft connected to a varying speed motor. The basket was then immersed in 900mL of simulated gastric fluid (SGF) without enzyme, maintained in a water bath at 37±0.5°C, and rotated at a revolution rate of 100rpm for 8 hours. Sampling was performed at 1-hour intervals by collecting 1mL aliquot from the dissolution medium and replacing it by the same amount of fresh medium.
6.3.1 Batch 9

Figure 34: Zero-Order Model for Batch 9

Figure 35: First-Order Model for Batch 9
Microspheres of batch 9 follow the Hixson-Crowell kinetic model given that it has the highest correlation coefficient ($R^2 = 0.9901$). In fact, batch 9 and 10 comprise microspheres which are perfectly spherical possessing the least irregularities among all the batches, which might explain why the dominant factor relating to the dissolution process is the geometrical form, explained by the Hixson-Crowell model.
6.3.2 Batch 10

![Zero-Order Model](image)

**Figure 38: Zero-Order Model for Batch 10**

\[ y = 8.7265x + 5.3621 \]
\[ R^2 = 0.9877 \]

![First-Order Model](image)

**Figure 39: First-Order Model for Batch 10**

\[ y = -0.0672x + 2.0116 \]
\[ R^2 = 0.9919 \]
Just like batch 9, batch 10 also follows Hixson-Crowell kinetics, as expected, with a correlation coefficient of \( R^2 = 0.998 \). The perfectly spherical microparticles dissolve in a manner which always keep their geometrical form stable. Such findings reveal that the microspheres of batches 9 and 10 do not contain the drug only in their core, surrounded by ethylcellulose. It seems like multiple layers of the polymer are on top
of each other, and a somewhat uniform amount of theophylline is present between
the layers. However, the difference between batch 9 and 10 is that the latter leached
72% of its drug content during 8 hours, whereas the former released 94% of its
theophylline content. The variation in drug to polymer ratio is the factor which
induced such a change. Batch 10 which was formulated with a 1:4 drug to polymer
ratio – as compared to 1:2 ratio for batch 9 – contains a higher amount of protecting
material, which further retards the drug release.
6.3.3 Batch 15

**Zero-Order Model**

- Equation: \( y = 9.9778x + 9.4476 \)
- \( R^2 = 0.9612 \)

**Log Cumulative Percent of Drug Release**

- Equation: \( y = -0.0953x + 2.0197 \)
- \( R^2 = 0.962 \)

Figure 42: Zero-Order Model for Batch 15

Figure 43: First-Order Model for Batch 15
Batch 15, which was produced under the same conditions of batch 10, except for the surfactant used, being Brij58 instead of Tween80, follows the Hixson-Crowell kinetic model, given that it has the highest correlation coefficient $R^2 = 0.9824$. Using Brij58 did not change the way microspheres dissolve compared to Tween80, however the rate of release increased, since microparticles of batch 15 released 86%
of their theophylline content over a period of 8 hours compared to 72% for batch 10, meaning a roughly 20% faster release. Such a difference in release rate can only be attributed to the structure of the surfactant, since all the other processing conditions for batch 10 and 15 are identical. Furthermore, Brij58 and Tween80 have very similar HLB values, 15.7 and 15.0 respectively. Brij58 has a linear structure with 36 carbons aligned in a straight chain, whereas Tween80 has a bulky sorbitan structure. This variation in structure will lead to different steric hindrance and strains when the microcapsules are forming, which will probably result in different microsphere matrices, thus affecting the release rate.
6.3.4 Batch 17

**Zero-Order Model**

\[ y = 6.9507x + 8.4504 \]

\[ R^2 = 0.9328 \]

**First-Order Model**

\[ y = -0.0465x + 1.9729 \]

\[ R^2 = 0.9745 \]

Figure 46: Zero-Order Model for Batch 17

Figure 47: First-Order Model for Batch 17
By employing a dual-surfactant system, comprising Span85 and Tween80, a low-HLB and high-HLB surfactant respectively, the release kinetics shifted from the Hixson-Crowell model to the Higuchi model, which has the highest correlation coefficient $R^2 = 0.9836$. The latter describes the dissolution of water soluble and low-soluble active ingredients (like theophylline) incorporated in solid and semi-
solid matrices. The incorporation of Span85 alongside Tween80 results in a system where Tween80 will interact with the polar organic phase, and Span85 with the non-polar oil layer, thus forming a more stable emulsion leading to the production of a matrix system that is different from that produced in batch 10 (where only Tween80 is used). In fact, such a matrix closely resembles and mimics that of sustained-action medication.

Furthermore, microspheres of batch 17 were the ones to release the least amount of drug over 8 hours, releasing only 57% of their drug content compared to 94%, 72%, and 85% for batches 9, 10, and 15 respectively. Batch 17, with a 57% release after 8 hours makes it a good choice for a BID formulation with 85% of the drug undergoing controlled release over 12 hours.
CHAPTER SEVEN

Conclusion

The processing conditions of theophylline-loaded ethylcellulose microspheres production by the emulsion-solvent evaporation technique were closely analyzed and optimized to give the highest yield, the best physical and chemical properties, and the most suitable drug release kinetics. Eight parameters in total were examined, including the solvent, the propeller, the type of oil, the temperature, the stirring rate, the drug to polymer ratio, the organic layer to oil layer ratio, and the type of surfactant. All parameters were kept constant while only one was varied, thus ensuring step-by-step optimization of parameters. The size and shape of microspheres were determined using a light microscope, the yield was calculated based on the expected mass and experimental mass, the interaction of the drug and the polymer and the packing method were investigated through Fourier-Transform Infrared spectroscopy, the drug content was analyzed using a UV-Vis spectrometer, the stability of the microspheres was examined over a period of 90 days using FTIR and UV-Vis spectroscopy, and the drug release profile was investigated using USP Apparatus 1, UV-Vis spectroscopy, and fitted into four different models. The best outcome occurred when microspheres were produced from acetone as a solvent, paraffin oil as a continuous phase, while mixing with a spherical propeller at 950rpm, at a temperature of 2°C, with both a drug to polymer ratio and organic to oil ratio of 1:4, and a combination of low HLB and high HLB surfactants, namely Span85 and Tween80. These parameters produced a high yield of the smallest and
perfectly spherical microspheres, having the most suitable dissolution profile and the highest drug content.


Guerrero, *Polymer Nanoparticles for Nanomedicine* (pp. 87-121). Springer, Cham.


