EFFECT OF DUAL SURFACTANTS AND PROCESSING FACTORS ON THE CONTROLLED RELEASE OF A POORLY WATER-SOLUBLE DRUG FROM ETHYL CELLULOSE MICROSPHERES.

by

MOHAN THAKARE

(Under the Direction of Anthony Capomacchia and James Price)

ABSTRACT

The objective of this work was to study effects of different formulation (dual surfactants, surfactant structure), and processing factors on different formulation parameters of ethyl cellulose microspheres fabricated by a non-aqueous emulsion solvent evaporation method. Ethyl cellulose microspheres containing theophylline were prepared at different combined hydrophilic lipophilic balance (CHLB) by using different dual surfactants combinations of high HLB (Tween 40, Brij 58) and low HLB (Span 65) surfactants in an emulsion-solvent evaporation process. The external phase used was light mineral oil and the internal phase was acetone. Individual and combination effects of combined hydrophilic lipophilic balance (CHLBs) of dual surfactants; surfactant structures, different preparation temperature (22^0C and 35^0C) on microsphere properties like yield, particle size distribution, geometric mean diameters, initial drug release, and drug release characteristic of the microspheres were evaluated. With an increase in CHLB of

the dual surfactant combination of span 65+Tween40, we noted decrease in the geometric mean diameter, and an increase in the dissolution rate and initial drug release. When surfactants with similar or closely matching HLB values but with different chemical-structure including polyoxyethelene sorbitan monopalmitate with HLB 15.6, and polyoxyethylene cetyl ether with HLB 15.7 were studied, we noted decrease in the geometric mean diameter, increase in the dissolution rate and initial drug release of microspheres. With increasing microsphere preparation temperature from 22^0C to 35^0C, geometric mean diameter of microspheres decreased, the particle size distribution widened, the sphericity of microspheres was improved, and the % drug loading, the initial drug release rate, the t50 release time increased. By changing the surfactant structure from polyoxyethelene sorbitan monopalmitate to polyoxyethylene cetyl ether in combination with microsphere preparation temperature, percent drug loading and drug release was altered, and drug release mechanism was affected with some microsphere batches showing near zero-order release.

INDEX WORDS: Microspheres, surfactants, non-aqueous solvent evaporation, ethyl cellulose, theophylline, *in vitro* dissolution, particle size, drug loading.

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DEDICATION

This dissertation is dedicated to my advisors Dr. Anthony Capomacchia and Professor Emeritus Dr. James Price.

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CHAPTER 1

INTRODUCTION

Controlled release dosage forms have many advantages over conventional dosage forms because they allow a more convenient dose regimen resulting in better patient compliance [1,2]. Their controlled release properties provide more consistent blood levels and reduced toxic effects [1]. Controlled release dosage forms improve long term chronic disease management by therapeutic and non-therapeutic objectives. Therapeutic objectives include: improvement of patient compliance by reducing dosing frequency, minimizing local and systemic side effects, and reducing fluctuation of drug blood levels [3]. Non-therapeutic objectives include reduced hospitalization, fewer physician visits, taste masking, odor masking, separation of incompatible components, financial saving to the patient in terms of lost workdays and overall economy of patient's time. The benefits of controlled-release technology are currently being expanded with the advent of genetic engineering techniques. As a result, more remarkable progress should be seen within the next decade. The advanced understanding of drug delivery mechanisms will decrease adverse effects and increase drug efficacy, patient safety and convenience.

Microencapsulation has been recognized as an effective method to achieve controlled release of drug formulations [4,5]. After oral administration, microcapsules are often distributed throughout the GI tract. The wide distribution potentially improves drug absorption and may reduce local irritation to the mucosa of the GI tract [6]. Different methods of microencapsulation are grouped under two basic categories – 1) Mechanical and 2) Physiochemical. Mechanical microencapsulation methods are pan coating, air suspension, multi-orifice centrifugal technique, and modified spray drying techniques. Physiochemical methods include coacervation, phase

separation, emulsion-solvent evaporation, both aqueous and non-aqueous. The method chosen depends on the physical characteristics of the drug to be encapsulated such as solubility, stability, therapeutic index and chemical structure.

Emulsion-solvent evaporation is a widely used method to prepare matrix microspheres [4]. There are several advantages of non-aqueous emulsion-solvent evaporation over other methods. In the non-aqueous emulsion solvent evaporation method, pH adjustment is not required, the process can be carried out at low or moderate temperature, and reactive agents are not required [4,6]. Microspheres are matrix systems in which the drug molecules are dispersed throughout the particles [7]. The formulation and properties of microspheres are affected by processing factors such as type and molecular weight of the polymer, drug particle size, drug: polymer ratio and solubility of the drug in polymer [1,8]. The important processing factors for microspheres prepared by the non-aqueous emulsion solvent evaporation method are phase ratio, mixing intensity, temperature during processing and viscosity of polymer and external phases [9,10]. Surfactants, which are emulsifying agents, play a major role in promoting the stability of the emulsion for preparation of controlled release of microspheres [10-12]. The characteristics of the surfactant and its uses are determined by its unique hydrophilic and lipophilic nature, quantitatively described by the hydrophilic-lipophilic balance (HLB) value[13].

The aims of the current research are to investigate the influence of a formulation factor 'dual surfactants' on the controlled release of ethyl cellulose microspheres prepared by the non-aqueous emulsion solvent evaporation process. This work investigates the influence of type, concentration, and structure of the differing non-ionic surfactants (Tween 40, Brij 58 and Span 65) on the physical and drug release characteristics of ethyl cellulose microspheres containing model drug theophylline. This work also investigates the effects of a processing factor such as

temperature on different formulation parameters of ethyl cellulose microspheres prepared using dual surfactants. Tweens and Spans are high HLB and low HLB non-ionic surfactants respectively. Brij 58 is a high HLB surfactant like Tween 40 but with a different chemical structure. Microspheres will be prepared with differing ratios of Span 65 and Tween 40 in one differing ratios of Span 65 and Brij 58 to examine the effect of HLB value and chemical structure. Theophylline will be used as a model drug as it has a short elimination half-life (3.5hrs for children and 5-8hrs for adults) in humans and hence sustained release formulation is desirable [4]. It is widely used in treatment and prophylaxis of bronchial asthma [14]. It has low water solubility (8 mg/mL at 25°C), and has a narrow therapeutic index [4,14]. Physical properties such as particle size distribution, geometric mean diameter, yield, drug loading and *in vitro* drug-release behavior of the resultant microspheres etc. will also be examined.

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CHAPTER 2

LITERATURE REVIEW

Part I: Non-immediate Drug Release

The objective of any drug delivery system is to provide a therapeutic amount of the drug to the proper site in the body to achieve and maintain the desired therapeutic drug concentration [1-3]. Development of such drug delivery system can be as complex as the development of the drug itself [4,5]. An appropriately designed non-immediate release drug delivery system can be a major advance toward solving problems concerning the targeting of a drug to a specific organ or tissue and controlling the rate of drug delivery to the target tissue. Non-immediate release delivery systems can be classified into following categories [1]:

a) **Delayed release:** It utilizes repetitive, intermittent dosing of a drug from one or more immediate release units incorporated into single dosage form. A delayed release dosage form does not produce or maintain uniform drug blood levels within the therapeutic range; however it is more effective than the conventional dosage forms [1,6].

b) **Sustained release**: This type of delivery system achieves slow release of drug over an extended period of time[1,7]. These dosage forms are further classified into following sub categories:

   i) Controlled release: Where drug is released slowly over an extended period of time by maintaining constant drug levels in blood or target tissue[1,8].

   ii) Prolonged release**:** Where drug is releases slowly over an extended period of time but it is unsuccessful at maintaining a constant drug levels in blood or target tissue [1].

c) **Site specific release**: The drug is targeted directly to certain biological location, where the target is certain tissue or organ [1,9]. This system satisfies the spatial aspect of drug delivery [10].

d) **Receptor release**: The drug is targeted directly to certain biological location, where the target is a particular receptor for a specific drug within an organ or tissue [1,10]. This system satisfies the spatial aspect of drug delivery [10].

The objective in designing a sustained-release system is to deliver the drug at a rate necessary to achieve and maintain a constant therapeutic blood level of the drug [1].

Oral Controlled Release Solid Dosage Forms:

Controlled release solid oral dosage forms have been widely used for decades [11,12]. Among all the routes of drug administration, the oral route has been the most popular route of drug delivery due to its ease of administration, patience compliance, low sterility constraints and flexible design of dosage forms [11]. Oral routes of the drug administration have been most widely and successfully used compared to any other route of drug administration [13]. It enables drugs to be administered more comfortably while at the same time provides a sustained and reproducible method of drug release [11]. Controlled-release technology has evolved with matrix technology. Many researchers in the 1950s and 1960s reported simple matrix tablets or monolithic granules [11]. In 1952, Smith Kline & French introduced a timed-release formulation known as Spansule that called for a widespread search for other applications in the design of the dosage forms [11]. At that time, the objective behind the development of oral controlled-release formulations was the achievement of a constant release rate of the entrapped drug [13]. Using the same concept, the zero-order osmotic delivery system used in Procardia XL became one of the top 10 bestselling

medicines in the past century [12]. Procardia XL is used for treating hypertension and angina and total brand sales for both strengths was $299.7 million USD [12]. In recent years, the industry has seen a number of innovative oral controlled-release dosage forms patented at a rapid pace using different technologies.

Research in Controlled Release Dosage form:

Controlled release may be defined as a technique or an approach by which the chemical entity is made available to the specific target at a rate and duration design to accomplish an intended therapeutic effect [14]. The future of controlled-release products is promising. There are reports that more than 76 of the 100 top selling drugs are available in oral formulation [13,15]. Different forces are driving research in controlled release dosage forms [13]. To develop a drug for patient compliance, with reduced cost is one of the reasons driving research in controlled release dosage forms [1,13]. Controlled oral drug delivery could be the answer to the problems for administration of biotechnological molecules which otherwise are not possible to delivery orally [16]. FDA's review process and demand for a greater number of complex clinical trials are increasing the time for NCE (New Chemical entity) to enter as a drug into the market [12]. In addition, FDA's compliance with 21 CFR Part 11 and the new Health Insurance Portability and Accountability Act (HIPAA) is affecting the clinical trial process required for the drug approval process. In 1996, FDA approved 53 NMEs (New Molecular Entities); this figure dropped to 27 in 2000; 20 in 2005; 22 in 2006; 24 in year 2008 and is expected to be even lower in coming years [17]. Expenses accrued from drug development are hitting the roof, true innovation is at an all-time low, and the regular introduction of new molecular entities (NME), is currently weak. In coming years, at least 20 major products with combined sales of billions will lose patent

protection. Patent expiration always hit pharmaceutical companies drastically because of very high cost of drug development. Hence the developing effective controlled-release formulations of existing immediate-release products, as an attractive financial option for pharmaceutical companies, in addition to seeking new therapeutic indications for these "new" products. Introduction of different products, such as Augmentin XR by GlaxoSmithKline and Cipro XR by Bayer, are harbingers of this option becoming a trend [12].

Different technologies to develop oral controlled-release system:

Different technologies can be used to develop oral controlled release dosage forms [18]. Many researchers have focused on developing oral controlled drug delivery as follow:

1) Coating technology using different polymers.
2) Matrix systems using swellable or non-swellable polymers
3) Slowly eroding devices
4) Osmotically controlled devices

Conventional tablet formulations are still popular in the design of controlled- release dosage forms [19]. Because of simplicity of the design, matrix devices made with cellulose or acrylic acid derivatives, which release the homogeneously dispersed drug based on the penetration of water through the matrix, have gained steady popularity. However the drawback of matrix type delivery systems is their first-order drug delivery mechanism caused by changing surface area and drug diffusional path length with time. This drawback could be addressed by osmotic delivery systems. Osmotic delivery systems maintain a zero-order drug release irrespective of the pH and hydro-dynamics of the GI tract [20]. Multiparticulate drug delivery systems are gaining favor over single-unit dosage forms because of their desirable distribution characteristics,

reproducible transit time, and low gastric irritation [21]. Although several technologies for the production of microparticulate systems have been designed, thus far the mainstream technologies are still based on spray-drying and film-coating technology [22]. Our research focuses on microparticulate systems to develop oral controlled release of the drug dosage using the solvent evaporation technique.

Types of oral controlled release dosage forms:

Based on the mechanism of the drug release the oral controlled release system can be classified into following types [1]:

1) Ion Exchange Resin
2) pH Dependent and Independent Formulations
3) Osmotically Controlled Formulations
4) Diffusion and Dissolution Controlled System
5) Dissolution Controlled Release
    a) Encapsulated Dissolution Controlled System
    b) Matrix Dissolution
6) Diffusion Controlled System
    a) Reservoir System
    b) Matrix System

1) Ion Exchange Resins

Ion-exchange resins used in pharmaceutical preparations serve several different functions, including taste-masking, tablet disintegration, extended drug release and improve chemical stability of the active ingredients [23,24]. Ion exchange resins are water-insoluble cross-

linked polymers containing salt forming groups in repeating positions on the polymer chain [24]. Resins may be either a cationic exchanger or anionic exchanger. In the design of the oral controlled release dosage forms, the ion exchange resins forms a complex with the drug substance [25]. Drug molecule in this complex is released by exchanging appropriately charged ion in the GI tract [1]. Since the ion must diffuse into and out of the resin complex for exchange to occur, different factors significantly affect the rate of the release of basic drugs from cation-exchange resins [25]. These factors includes diameter of resin beads, degree of cross-linking within the resin, the pKa of the ionizable resin group, and electrolyte concentration of the drug release microenvironment [26]. A drug resin complex can be encapsulated or suspended in solution to further improve oral controlled drug delivery.

2) pH Dependent and Independent Formulations

The rate of release from oral controlled release dosage forms is independent of GI tract pH [27,28]. Appropriate buffering agents are needed used in this type of pH independent formulation, which adjusts the pH of the fluid entering the formulation to a constant, desirable pH thereby allowing a zero order release of the drug at a controlled rate [28,29]. The normal pH range of gastric juices is between pH 1 and 3, while the pH in the intestinal tract averages about pH 7 [29,30]. This fact has been used to good advantage for years in different oral controlled release formulations. These formulations are usually in the form of tablets coated with a drug substance which is insoluble or sparingly soluble in acidic solutions, but which dissolves rapidly at higher pH [30]. Drugs which would present a problem if released in the stomach, such as gastric irritation would be delivered using this pH dependent formulation. Moreover, such pH dependent formulation also permits extending the release of a drug over time [31]. For example, a controlled

release tablet can be formulated by compressing granules containing the drug, where some of which granules are enteric coated and some are not enteric coated. As the solid dosage form such as tablet disintegrates, the non-enteric coated granules dissolve in the stomach, dependent on pH, immediately releasing the drug, while the enteric coated granules pass to the intestine before dissolving to release the drug [31]. In this way, release of the drug can be controlled over an extended period of time. In this case, the drug is resident in both the stomach and intestine because of the different pH of stomach and intestine. Such an extended controlled release system is crude; essentially releasing the drug in a bi-modal manner dependent on the pH [31].

3) Osmotically controlled Formulations [32]

Osmotically controlled oral drug delivery systems utilize osmotic pressure as the energy source for controlled delivery of drug. The delivery of the drug is controlled by the solvent influx across a semi-permeable membrane [33]. Controlled release using osmotic pressure, to a large extent, is independent of the physiological factors of the gastrointestinal tract [33]. These systems can be used for developing systemic as well as targeted delivery of drugs. Various formulation factors affect the release of drug(s) from osmotic systems such as solubility and osmotic pressure of the core component(s), size of the delivery orifice, and nature of the rate-controlling membrane [34]. The controlled release dosage form, when it comes in contact with the aqueous fluids, imbibes water at a rate determined by the fluid permeability of the membrane and osmotic pressure of the core formulation [34]. This osmotic imbibition of water results in formation of a saturated solution of drug within the core. This solution is then dispensed at a controlled rate from the delivery orifice in the membrane. Osmatically controlled systems are suitable for controlled delivery of drugs having moderate water solubility [32]. Incorporation of excipients in

the formulation that modulate the drug solubility within the core can be one approach to control the release of drugs from the osmotic systems. The first oral osmotic pump was developed around 31 years ago. Today there are number of modifications available to meet a variety of drug delivery demands in oral controlled release delivery. Osmatically controlled systems hold a major market share in the drug delivery products as exemplified by the number of products in the market and patents granted in the last few years. By modulating various formulation factors, it is possible to use these systems to deliver drugs of diversified nature at a pre-programmed rate for oral controlled release.

4) Diffusion and Dissolution Controlled System

In addition to polymer swelling and drug diffusion, in diffusion and dissolution controlled system there is slow entanglement of the polymer chain leading to complete dissolution of the polymer. It is characterized by a phase erosion of the polymer carrier that is associated with fast or slow dissolution of the macromolecular polymeric chains. This type of controlled system is only possible in uncross-linked polymers.

a. Dissolution Controlled System

Dissolution controlled release systems are obtained by slowing the dissolution rate in the GI medium by incorporating the drug into insoluble polymeric material and or coating a drug into polymers of varying thickness. The rate limiting step for the dissolution controlled release system is the diffusion of the drug across an aqueous boundary layer. The solubility of the drug provides the source of energy for release of the drug. Controlled release using the dissolution mechanism can be achieved by encapsulating the drug into insoluble polymer. The coated beads can be encapsulated or compressed into tablets as was done to produce Spansule products. The rate of the dissolution (dm/dt) can be explained by following equation-

$$dm/dt = ADS/h \text{ ----------Equation \# 1.}$$

Where dm/dt is dissolution rate; s is aqueous solubility of drug; A is surface area of dissolving particle or tablet; D is the diffusivity of the drug; h is the thickness of the boundary layer.

This approach however does not allow for a constant release rate because the surface area changes with the time. The solubility of weak acids or weak bases drug is affected by the variable pH in the GI tract. One of the approaches to attain sustained release is to use hydrophobic polymers or waxes as the matrix to incorporate the drug.

b. Diffusion Controlled System

The basic principle of this type of controlled release is diffusion of drug particle through a polymeric membrane [1]. Similar to the dissolution control system, the diffusion controlled devices are manufactured by encapsulating a drug into a polymeric membrane or by dispersing the drug into polymeric matrix [1]. Unlike the dissolution control system, the diffusion controlled devices makes the drug available by partitioning through the polymer [1,35].There are two types of diffusion control release systems.

1) Reservoir type diffusion controlled release system: In reservoir type of diffusion system, a core of a drug is surrounded by a polymeric membrane. Generally the drug diffusion through the coating barrier controls the release rate. Two types of reservoir systems can be distinguished [36].
   a) Device with non-constant activity source[36]. In this reservoir type of system only the dissolved drug exists within the systems core upon penetration of water. More specifically the drug molecules that diffuse out of the device are not replaced and thus the inner drug concentration at the inner membrane surfaces decreases with the time. In this case, irrespective of the geometry of the device, first order of drug release observed if the

system does not swell; the perfect sink conditions are maintained (negligible drug concentration in the release medium; no hindrance of the further drug release by already released drug, and the membranes properties are not changed with time [36].

b) Device with constant activity source [36]. In this system the initial drug loading is much higher than the amount of drug is soluble within the wetted core. Thus during the major part of the drug release the dissolved and non-dissolved drug co-exists More importantly, the drug molecules that diffuse out of the device are replaced by the partial dissolution of the excess amount of the drug. Irrespective of the geometry of the drug, the resulting drug release is constant as long as the drug excess is provided in the device core; the system does not swell, perfect sink conditions are met, and membranes property does not change[36].

2) Matrix type diffusion controlled release system [37]: In matrix type of diffusion system, drug is distributed uniformly throughout an inert polymeric matrix [1,36]. Diffusion control involves dispersion of drug in either water soluble or insoluble polymer. In this type of oral controlled release system the release rate is dependent on the rate of the drug diffusion from the polymer matrix but not on the rate of solid dissolution.

Three major types of materials used in the preparation of matrix devices preparation are insoluble polymers; hydrophilic polymers, and fatty compounds. Plastic matrices include methyl methyl acrylate, polyvinyl chloride and polyethylene etc. Hydrophilic polymers include methylcellulose, hydroxyl-propyl-methylcellulose, and sodium hydroxyl-methylcellulose [1]. Fatty compounds include various waxes such as glyceryl tristerate, and carnauba wax [1].

Advantages and Disadvantage of oral controlled release dosage forms:

The major goal of controlled-release dosage forms is the improvement of drug therapy to the patients.

1) Advantages of oral controlled release [12,14,38]:

It is well known that for some drugs, controlled release of the drug over time may offer a number of advantages relative to immediate release dosage form of the same drug [38].

The advantages are listed below [39].

		Advantages
1	Therapeutic advantage	1. Reduced drug plasma level fluctuations. 2. Steady plasma level of the drug over an extended time period.
2	Reduction in adverse side effects and improvement in tolerability	3. Drug plasma levels are maintained within a narrow window with no sharp peaks. 4. Greatly reduces the possibility of side effects, because the scale of side effects increases as we approach the maximum safe concentration.
3	Patient comfort and compliance	1. Oral drug delivery is the most common and convenient for patients. 2. Reduction in dosing frequency enhances compliance.
4	Reduction in healthcare cost	1. The total cost of therapy of the controlled release product could be comparable or lower than the immediate-release product as the

		number of dosage are reduced for a specific treatment compared with the immediate release dosage forms 2. Overall expense in disease management also would be reduced with reduction in the side effects.

2) Disadvantages of oral controlled release [1]

a) Difficulty of quick stoppage of pharmacological action of the given drug.

b) The rate of gastric emptying affects the drug release especially in cases of serious poisoning or intolerance.

c) If the drug is not absorbed by intestinal mucosa there will be a little or no efficacy of pharmaceutical dosage form.

d) There may be difficulty of adjusting the posology to individual pharmacokinetics.

e) Drug release rate dependent on pharmaceutical dosage form integrity.

f) The pharmaceutical dosage form size may be large.

g) The manufacturing cost may be greater than conventional dosage forms, as more processing is required.

Factors affecting the development of oral controlled release dosage form:

a) Dose: Developing oral controlled-release dosage forms would require approximately two or three times greater amount of drug than the oral conventional dosage forms. The

prohibitively high dose of poorly soluble drugs can limit their suitability for developing oral controlled release form [14].

b) **Aqueous solubility [14]:** The aqueous solubility of drug is a very important factor that affects its incorporation into oral pharmaceutical dosage forms [14]. The drugs with high or low solubility are not a suitable candidate for developing oral controlled release dosage forms. The poorly soluble drug has a slow dissolution rate and thus absorption will occur for extended period of time. However, the dissolution kinetics are however linear and vary with surface properties, particle size and particle distribution [14]. The *in vivo* absorption of such drug may remain incomplete and variable if the drug is to be absorbed past the ileocecal junction (more than 7-8 hrs).

c) **Stability:** Drugs need to be stable to pH, enzyme, GI flora throughout its time in GI tract. For example – Drugs that are that is not stable to GI tract flora or pH are not suitable candidates for once or twice a daily dosing in order to maintain a desirable concentration for over 12 hrs in GI tract.

d) **Lipophilicity/Permeability:** Absorption of drugs having poor permeability is limited with by membrane permeation [14]. Change in release rate will have little effect on plasma profile and may attain poor absorption.

e) **Partition coefficient:** Partition coefficient explains the ability of a drug to cross the biological membranes and interact with the receptor [14]. As a first approximation, the more effectively a drug crosses membranes, the greater is its activity. Drugs with a partition coefficient that is either extremely high or low are poor candidates for formulation into controlled-release dosage forms [14].

f) **Elimination Half Life**: One of the basic reasons to develop controlled release is short (2-6 hrs) elimination half-life. Other variables such as minimum therapeutic effective concentration and dose are also important parameters to consider. Two drugs with similar half-life, may not be equally suitable to develop oral controlled release dosage forms.

g) **Therapeutic Window:** The drug needs to maintain the minimum therapeutic concentration in plasma with reduced fluctuation. Fluctuations in plasma levels is undesirable for drugs with a narrow therapeutic index [14].

h) **First-pass metabolism:** Drug bioavailability will be decreased for drugs with saturable first pass metabolism as it slows the systemic input from the controlled release system.

The future of oral controlled release:

Researchers are making great efforts to discover new methods for delivery and controlled release of drugs that will be efficient and economically acceptable for drug manufacturers. The future of controlled-release products is promising, especially in areas that present greater acceptability from patients:

1) **Chronopharmacokinetic systems:** The drug can be effectively delivered with an oral controlled drug delivery with a pulsatile release regimen. A need exists to counter naturally occurring processes such as bacterial/parasitical growth patterns. For example, cnce-daily oral Amoxicillin Pulsys by Advancis Pharmaceutical Corp is a pulsatile-release formulation of amoxicillin for oral administration that could potentially inhibit the emergence of resistant strains of microorganisms.

2) **Targeted oral controlled drug delivery**: Oral controlled drug delivery that can target regions in the GI tract and release drugs only upon reaching the site could offer effective treatment for certain disease states, specifically in cases of cancers. For example, colon targeted delivery of anti-neoplastics in the treatment of colon cancers.
3) **Mucoadhesive delivery** [40]: This is a promising technique for buccal and sublingual oral drug delivery. This type of drug delivery can offer rapid onset of action and superior bioavailability compared with simple oral delivery because it bypasses first-pass metabolism in the liver.

Particulate systems

The micro and nanoparticle approach involves packaging drug into a particulate carrier system. Different polymers are employed and aimed at the uptake of intact drug-loaded particles via the Peyer's patches located in the small intestine. This could be useful for delivery of different drugs including those of protein origin that cannot, in general, be given orally. Incorporation of the drug into a microparticulate carrier system can protect it against degradation *in vitro* as well as *in vivo*.

Microencapsulation techniques can be used to incorporate polymeric or other protective material with active ingredients. One approach is to package drugs into a micro-particulate carrier system. Microcapsules, microspheres, and nanospheres are some of the options [41]. Our research focuses on developing microspheres to deliver theophylline, a weakly basic, relatively low water soluble, narrow therapeutic index drug and to study the effects of dual surfactants on the formulation process.

Micro-particulate Drug Delivery System

Micro-particulate drug delivery systems have found wide application in the pharmaceutical industry, first for external use in creams and ointments, later for subcutaneous drug delivery and then in oral and intravenous administration. The terminology used to describe micro-particulate formulations can sometimes be inconsistent and confusing to those not familiar with the field of pharmaceutics [42]. The term "microparticle" refers to a particle with a diameter of 1–1000 µm, irrespective of the precise interior or exterior structure [43]. Further classification of microparticles, "microspheres" specifically refers to spherical microparticles where drug is dispersed homogenously throughout the polymer. The subcategory of "microcapsules" applies to microparticles that have a core surrounded by a material that is distinctly different from that of the core. In this case, the core may be solid, liquid, or even gas. The term nanoparticles refers to particles of equal or less than a size of 1 µm. Particles greater than 1000 µm are called macro-particles or beads [43]. Commercial microspheres have a diameter in the range of 3 to 800 µm [43]. Despite the specific and logical subcategories as mentioned in different literature reports, many researchers use these terms interchangeably, that often leads confusion to the reader. It is usually assumed that a microparticle formulation is comprised of a fairly homogeneous mixture of polymer and active agent, whereas microcapsule formulations have at least one discrete domain of active agent and sometimes even more [44]. Microparticles are of special importance for developing a controlled release drug delivery system [44,45]. They play an important role to improve bioavailability of conventional dosage forms and reducing side effects [44]. Pharmaceutical application of microspheres involves different routes of administration including oral, pulmonary and parental [44,45]. For parental drug delivery, the diameter of the microparticles should be less than 250 µm, ideally 125 µm [44,45].

Microspheres

Microspheres are a type of microparticles in which the drug particles are homogenously dissolved and dispersed in a polymeric matrix [44]. Microspheres show different release properties than true microcapsules with an additional feature that there is no possibility of catastrophic drug burst due to rupture of a shell [44]. Our current research will focus on how to control release by different formulation and processing variables by use of dual surfactants. Many investigators have focused on studying microspheres for delivering controlled release oral solid dosage forms [46-53]. Studying different microencapsulation techniques is important in order to understand the basis of microsphere formation and drug release [51].

Microencapsulation

Microencapsulation techniques are widely used for developing a oral controlled release drug delivery system [53,54]. Microencapsulation technology emerged in the mid-1950s and has been used for various applications, including graphic products, optics, agricultural chemicals, adhesives, perfumes, food and flavorings [44]. The first significant application of microencapsulation in 1950 was in the development of carbonless paper and is based on coacervation [43]. Interest in microencapsulation technology for the formulation of controlled delivery of drug has increased in the last decade owing to numerous advantages [43,44]. The term microencapsulation is used to designate a category of technology to entrap solid, liquid or gases inside a polymeric matrix or shell. The advantages of microencapsulation includes [43,44,55]:

a) Protecting microencapsulated compound from the surrounding environment.
b) Keeping a non-compatible substance separate from other substances of the product.

c) Mixing immiscible or incompatible ingredients.

d) Produces physical stability against heat, moisture, oxidation/reduction, light etc.

e) Physicochemical properties of the micro-particulate system remain unaltered.

f) Prolongs the shelf life of the active components.

g) Micro-particulate systems can be administered via various routes (I/V. I/M. S/C, Oral).

h) Micro-particulate systems are practically scalable to the industrial level.

i) Controlling the release of the drug or core material through the surrounding shell. Such release from microparticles is ensured by various mechanisms:

    i) Mechanical rupture of capsule wall

    ii) Dissolution of the wall

    iii) Melting the wall

    iv) Diffusion through the wall

    v) Albation (Slow erosion of the wall)

    vi) Biodegradation

In matrix type microparticles (known as microsphere) the active ingredients are entrapped within the microparticles [55]. Microspheres provide encapsulation of an active ingredient homogenously throughout the whole particle including the external layer [43]. Microspheres have significant importance in biomedical applications [55,56]. Administration of drug in the form of microspheres usually improves the treatment by localizing active ingredients at the site of action and by prolonging the release of the drug. Additionally, sensitive drugs such as proteins and peptides may be protected against the chemical and enzymatic degradation.

Micro-encapsulation Methods

Various microencapsulation methods are described in literature for the preparation of pharmaceutical microspheres [43,57-61]. Research to find new and improved methods of microencapsulation to encapsulate new invented active molecule is always in progress [43,60]. Microencapsulation technique depends on the physical and chemical properties of the drug to be encapsulated [60]. Existing methods of microencapsulation are classified as follows [43,55,60]:

1) **Physicochemical methods [43,59]**
    a) Simple and complex Coacervation / Phase separation
    b) Complex precipitation
    c) Solvent Evaporation
    d) Solvent Extraction
    e) Layer by layer adsorption
    f) Ionic geletion
    g) Salting out
2) **Chemical methods [43,59]**
    a) Interfacial polymerization
    b) In-situ polymerization
    c) Matrix polymerization
3) **Mechanical Methods [43,59]**
    a) Coating
    b) Extrusion
        i) Simple or double capillary
        ii) Ionic Gelation

c) Spray

i) Spray drying

ii) Spray Cooling

iii) Spray Chilling

Amongst these different methods the following are commonly used to develop microspheres: phase separation by polymer-polymer incompatibility and coacervation, solvent evaporation and solvent removal, hot-melt microencapsulation, spray drying, interfacial polymerization, and supercritical fluid processing techniques.

1) Phase separation/Coacervation:

The Dutch scientists Bungenburg de John and Kruyt introduced the term coacervation in 1929 [44]. The term coacervation came from the Latin word 'coacervus' which means heap or pile [43]. This method involves the dissolution of the polymer in a liquid in which the insoluble core material to be encapsulated is suspended. The process involves formation of a polymer rich separate phase known as a coacervate [18,43]. Phase separation is a non aqueous method that is suitable for microencapsulation of both water soluble and water insoluble drugs and is generally used to microencapsulate protein or peptides [18,44]. While the o/w solvent evaporation/extraction method is only suitable for water insoluble drugs, the phase separation, being a non-aqueous method is suitable for both water soluble and insoluble drugs [18,62]. In short, developing microspheres by phase separation method involves dissolving the polymer in organic solvents; and then dissolving or dispersing the drug in this polymer organic solvent [63]. In the second step, organic non-solvent (called the first non-solvent) is slowly added to the polymer-drug-solvent system with stirring. The polymer forms a phase separate, a coacervate droplet that contains the

drug. Since the coacervate droplets are too soft at this stage, the emulsion system is transferred to a third body of the second organic non-solvent (referring to the 2nd non-solvent) with stirring. The microspheres, thus prepared ,are washed with the second organic non-solvent and then collected by sieving or centrifugation and dried at ambient conditions under vacuum [18,64]. This method is used to produce double walled microspheres [65].

2) Interfacial Polymerization:

Interfacial polymerization involves dissolving or dispersing the drug in an organic solution of a monomer that can be polymerized to solid [66,67]. The organic solution that serves as a dispersed phase is emulsified into a continuous aqueous phase. Emulsification can be induced by adding catalyst [68]. This method can be used to prepare the nanospheres [66,68].

3) Spray Drying:

Spray drying techniques can be used to encapsulate sensitive substances including drugs, fragrances, essential oils or vitamins [69,70]. This technique involves spraying the suspension of a drug in an organic solution of the polymer. The evaporation of the solvent is achieved by a special temperature controlled cyclone [70]. Microspheres are obtained by selection of appropriate conditions and solvents [69]. Spray drying is defined as the transformation of the feed from a fluid state such as solution, dispersion or paste into a dried particulate form by spraying the feed into a dried hot gaseous drying medium [44]. Spray drying is a continuous one step process that includes atomization of the feed, mixing of spray and air, evaporation of solvent and separation of the final product [44,70]. Owing to readily available equipment and high output, spray drying is a viable commercial method. The disadvantages include limitation on spraying the high viscous solution that limits core loading and exposure of active ingredients to high temperature [71].

Hot-Melt Method:

The hot-melt method can be used to encapsulate drugs or dyes [72]. This method involves melting a polymer slightly above its melting point temperature. The drug is then added and mixed well [73]. This mixture of molten polymer-drug is poured into a heated bath of non-solvent with stirring. Typical non-solvents used for the hot-melt method are silicon or corn oil, and the stirring action causes emulsion formation. Once the microspheres are formed, the desired mass is quench-cooled to harden the microsphere[74]. The microspheres are harvested by washing and drying to remove the non-solvent [73]. This method is used for thermally stable drugs that need encapsulation to achieve desired drug delivery characteristics.

4) Supercritical fluid processing techniques:

Microencapsulation is achieved in supercritical fluid rather than in organic solvents. In this method, the coating material and core particles are placed in an autoclave heated and pressurized with CO_2 until supercritical conditions are achieved [43,75,76]. The coating material is solubilized in supercritical fluid. After phase change from supercritical fluid to liquid stage the core particles are precipitated into microparticles [43].

5) Precipitation:

The precipitation process of microencapsulation involves precipitation of a polymer around the drug selected for encapsulation [77]. This method involves numerous variations such as precipitation of water soluble polymers such as gelatin, with water miscible solvents such as isopropanol [78]. Other examples include precipitation of water insoluble polymers such as ethyl cellulose from cyclohexane by cooling; gelation of sodium alginate with aqueous calcium salt solution, and the thermally induced process of precipitation of protein to form microspheres [78]. The limitation of this process is oxidation of the active ingredients.

6) Salting Out:

This technique involves addition of salts to the aqueous polymer solution ultimately causing the polymer to phase separate from the solution [79]. One potential problem is incorporating a high amount of salt that will show up in the final product of microsphere wall. This is undesirable in preparing the formulation for diffusion based delivery.

7) Solvent Extraction Technique:

In the emulsification-evaporation methods, elimination of the organic solvent is accomplished two ways:

a) Diffusion of solvent in the dispersing phase, defined as solvent extraction [44].

b) Elimination of solvent at the dispersing phase-air interface, defined as solvent evaporation [44].

In the solvent extraction process, theoretically, if one uses a continuous phase that extracts dispersed phase solvent(s), the solvent evaporation stage is not needed. However in practice, this is not the case. Solvent extraction is normally achieved by using large volumes of dispersing phase with respect to the continuous phase or by choosing a dispersed phase of co-solvents. The co-solvent selection should be done with an objective that one of the co-solvents will have greater affinity for the dispersing phase [44]. However, this method may develop crystal formation of active ingredients at the microsphere's surface [44]. The solvent extraction method is commonly employed to encapsulate protein drugs in polymeric microparticles [80].

8) Solvent Evaporation Technique:

The solvent evaporation technique is one of the oldest and commonly applied methods of microsphere preparation [81-83]. This method can be used to produce microspheres (size more than 1 µm) as well as nanospheres (size less than 1000 nm). This technique is mainly based on the

evaporation of the internal phase by agitation. This method involves emulsification of an organic solvent, containing dissolved polymer and dissolved/dispersed drug in an excess amount of continuous phase (that could be aqueous or non-aqueous) with the aid of an agitator. Subsequent evaporation of dispersed phase solvents yields solid polymeric microparticles entrapping an active ingredient such as drug. The solid microspheres are recovered by filtration, centrifugation or by lyophilization [84]. This technique is simple and economical.[84,85]. Depending on the solubility of the active agent in polymer solution, the product can be homogenous or heterogeneous microcapsules. This method is usually used for low to completely water insoluble drugs [84]. The polymer used in this method must be water insoluble such as ethyl cellulose [86]. The basic prerequisite for this method is the use of a solvent that is able to efficiently dissolve the drug to be encapsulated as well as the wall-forming polymeric material. Although the solvent evaporation process is relatively simple, the physiochemical phenomenon governing this process is very complex [87]. In general, the diameter of the particle depends upon the size of the micro droplets formed in the emulsion before evaporation of the solvents [81]. The polymeric encapsulating material (i-e d,l-lactide-co-glycolide, chitosan, ethyl cellulose), often dictates the choice of the solvent(s) employed in the process of emulsification. Solvent(s) such as halogenated alkanes such as methylene chloride and chloroform that often employed as the dispersed phases are not desirable because of human safety concerns. The toxicity of the solvents does prevent microspheres made by this process from meeting FDA regulations since residual amounts of chlorinated solvents may be retained in the sample. Use of safer solvents such as ethyl acetate and acetone has since been preferred and recommended. Our research employs acetone as the choice of solvent for developing microspheres. In order to avoid the use of chlorinated and other solvents in the microsphere preparation, the hot-melt microencapsulation

technique was developed [88]. The physiochemical properties of both these processes of forming the microsphere wall are the same [88]. However the solvent removal process differs significantly from the hot-melt method in that the solvent removal process requires milder conditions for extraction of solvent that prevents destruction of unstable drug substances [88]. Several methods have been developed and depend on the variables of processing factors such as the elimination procedure for the solvents (extraction or evaporation) and formulation factors such as the type of the external phase (aqueous or non-aqueous) [89].

Emulsions- General consideration

Emulsions are dispersed systems consisting of two or more mutually insoluble, immiscible or sparingly soluble liquids. The liquid usually present in excess is termed the continuous or external phase, while the dispersed liquid is called the dispersed, discontinuous or internal phase. If the continuous or external phase consists of water, and the dispersed or internal phase consists of an organic liquid, such as mineral oil, the term oil-in-water (O/W) emulsion is used. If the continuous or external phase consists of organic or non-aqueous liquid, the dispersed or internal phase consists of water, the term water-in-oil (W/O) emulsion is used. O/W and W/O emulsions may contain a non-polar oil such as silicone, however in O/O emulsions it is relatively rare to have both phases non-polar.

(Water-in-oil)-In-Water Multiple Emulsion Technique (W/O/W)

Multiple emulsion or double emulsion techniques are used to encapsulate water soluble drugs such as proteins or peptides, efficiently. In brief, polymer is dissolved in an organic solvent and emulsified into an aqueous drug solution to form a *water-in-oil* emulsion [90]. This primary emulsion is re-emulsified into an aqueous solution containing an emulsifier to produce a multiple

w/o/w emulsion [84,89,90]. This method is based on the premise that an organic phase would act as a barrier between the two aqueous phases, preventing the diffusion of active ingredient towards external aqueous phase [91]. This method yields high encapsulation efficiency of highly water soluble drugs. Microspheres manufactured by this method exhibit various morphologies such as porous or non-porous external shell layers enclosing hollow, macro-porous, micro-porous internal structure [89,92].

Oil-in-Water Emulsion Technique (O/W)

The solvent evaporation method is essentially an *oil-in-water* dispersion or emulsion process. An organic (solvents) phase containing polymer and drug is mixed in an aqueous phase containing an emulsifier. The emulsified droplets are then hardened into a microsphere by removing the solvent. This method is usually used to encapsulate lipid soluble drugs and is limited to the lipid soluble drugs because even drugs that are slightly hydrophilic may be partitioned into an aqueous phase [89,93].

Oil-in-Oil Emulsion Technique (O/O) or Non-aqueous emulsion solvent evaporation technique:

Oil-in-oil emulsion technique is also known as the non-aqueous emulsion solvent evaporation method. The major disadvantage of this method is insufficient encapsulation efficiency to encapsulate low water soluble and water insoluble compounds [18,89,94]. This method is modified from the *oil-in-water* emulsion method. In this method, an organic liquid such as castor oil, mineral oil, or cottonseed oil is used as a continuous phase. Oil soluble surfactants such as lecithin, span or brij are used as an emulsifier [18,89]. Using oil as an external phase is a disadvantage of this system, as it makes cleaning the final product difficult. The advantages include prevention of hydrolysis of active drugs and higher microsphere yield. There are several formulation and processing variables that affect the characteristics and properties of the

microsphere. These factors include the type of polymer used, drug, solvent, emulsifier and different ratio among them, stirring speed, and temperature.

Role of different factors on properties of microspheres

The solvent evaporation method makes possible the encapsulation of a wide range of drugs having different physical properties and solubility characteristics[44]. Although the solvent evaporation method is a simple process, a number of variables affect the microsphere characteristics. These variables include the polymer composition, molecular weight, the nature and solubility of the drug to be encapsulated, the organic solvent used, the temperature of emulsion, the external to internal phase ratio, and the amount of emulsifier used. Different studies are conducted to determine the effect of stir rate, type and amount of the dispersing agents, the viscosity of emulsion phases, the configuration of vessel and stirrer, and the quantity of emulsion phases on the size of microsphere [55,81]. Formation of a stable emulsion is an important step in solvent evaporation. It makes the polymer globule stable so that it does not coalesce during solvent evaporation. This allows for hardened microspheres as a final product. There are reports of using single surfactants for the preparation of microspheres of water-soluble pharmaceuticals [95,96], [97]. In formulating protein drugs, the use of a single surfactant was reported to attenuate the burst release of PGLA microsphere [98]. After an extensive search of the scientific literature, we could not find any report/s on use of two (dual) surfactants on controlled release of sparingly water soluble drug produced by non-aqueous solvent evaporation method, although there are some non-published studies conducted at the University of Georgia, Athens, USA [99].

The use of the hydrophilic-lipophilic balance system (HLB) is widely known for choosing surfactants for microsphere preparation [100]. When using two or dual surfactants to

stabilize an emulsion, usually the HLB is stated as the required hydrophilic-lipophilic balance (RHLB) to account for the use of two or more surfactants in the emulsion. The term combined hydrophilic-lipophilic balance (CHLB) has been coined by Professor Emeritus Price, at the University of Georgia to define the number that is combined to achieve a required HLB number to stabilize the emulsion. Since a dual surfactant emulsion system is obtained by combining a Low HLB (LHLB) surfactant and a High HLB (HHLB) surfactant the term CHLB makes more sense than the use of RHLB.

In our current studies the objective is to study the effects of dual surfactants (HHLB and LHLB surfactants) per cent ratio as a function of different variables such as temperature and mixing speed on the different physical properties of microspheres prepared by non-aqueous emulsion solvent evaporation method.

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Part II: Surfactants

Our current work studies the effects of the combined use of dual surfactants, a low HLB surfactant and a high HLB surfactant as a function of temperature and mixing speed on the different physical properties of microspheres prepared by non-aqueous emulsion solvent evaporation method.

Surfactants are amphipathic substances with hydrophilic and hydrophobic groups making them capable of adsorbing at the interfaces between liquids, solids and gases [1]. The term surfactant was coined by Antara products in the year 1950 [2]. Surfactants are used in many chemical industries, paints, detergents, dyestuffs, paper coatings, inks, plastics and fibers, personal care and cosmetics, agrochemicals, pharmaceuticals, and food processing [3,4]. They are low to moderate molecular weight compounds that contain a hydrophobic part that is readily soluble in oil but sparingly soluble or insoluble in water and another part that is hydrophilic part which is readily soluble in water but sparingly soluble or insoluble in oil [5]. If the hydrophobic segment is very large, then the surfactant will not be water soluble and vice versa. Surfactants form self-associated clusters, which normally lead to organized molecular assemblies, monolayers, micelles, vesicles and membranes [6]. The formation of such structure depends on different parameters such as the size of hydrophobic domain, the nature and size of the polar head group, temperature, and salt concentration [7]. Surfactants are extensively used as excipients in drug delivery because of their capability of reducing surface tension that allows drugs to mix or disperse readily as emulsions in water or other liquids [7].

Understanding of the physiochemical properties and behavior of surfactants in solution and at interfaces has undergone dramatic development in the past few decades. Scientists both in academia and industry are giving serious attention to the study of surfactants and their

application to develop controlled release drug delivery. Hydrophobic domains of surfactant molecules can associate to form various structures including micelles, micro-emulsions, and microspheres that offer opportunities for drug delivery applications (Sydney Ross 1988).

Classification of surfactants

Traditionally, surfactants are chemically classified according to the nature of hydrophilic group present [8,9]. If the surfactant molecule is not ionized, then it is called a non-ionic surfactant [9]. If the surfactant molecule carries a negative charge it is called anionic, and if it carries a positive charge it is called a cationic. If it carries both charges (positive and negative), it is frequently called zwitterionic but this terminology applies to surfactants only at pH values at which both positive and negative charges occur concomitantly on the same molecule. In all other circumstances, the use of term amphoteric surfactant is more acceptable. The most commonly used zwitterionic surfactant is lecithin (phosphotidylcholine), usually employed in oil-in-water formulations. Anionic surfactants are the first surfactants used for emulsions. Early era soaps were produced using such surfactants derived from animal fats. Early in the 20th century, synthetic detergents were produced by using the processes of sulfation and sulfonation which still dominate the manufacture of anionic surfactants today [10].

Non-ionic surfactants generally possess a polar, but uncharged, head group and one or more non-polar hydrocarbons (C10-C18 chains) which are also uncharged. The hydrophobic and hydrophilic moieties are generally linked by ether, ester or amide bonds. The most commonly used non-ionic surfactants are ethoxylate, ethylene, and propylene oxide copolymers, and sorbitan esters. The chemical structure and electrical neutrality of non-ionic molecules imparts a lower sensitivity to the presence of electrolytes in the GI tract. These surfactants are less toxic than anionic surfactants. Chemicals in this class are active if they carry at least one free –OH or

ether group normally derived from ethylene or propylene oxide. Non-ionic surfactants are most useful in pharmaceutical preparation because of their compatibility with other pharmaceutical excipients, stability and low toxicity [11,12]. Non-ionic surfactants can be further divided into water soluble and water insoluble compounds. Long chain fatty acid analogs such as fatty alcohols, glyceryl and fatty acid esters are examples of water insoluble non-ionic surfactants [8] while polyoxyethelene (POE) sorbitan fatty acid esters are an example of water soluble non-ionic surfactants. The latter is mostly used in oral, topical and parenteral formulations [8]. There are non-traditional surfactants used in many industries such as bile salts. Certain drugs also exhibit surfactant like activity for example ibuprofen, benzocaine etc [8]. Non-ionic surfactants have the advantage over ionic surfactants in that they are 1) compatible with most other types of surfactants; 2) minimally affected by moderate pH changes and by moderate electrolyte concentrations [13].

Physical properties of surfactants

Physical properties of surface active agents differ from those of electrolyte or non-electrolyte molecules [9]. At low concentrations surfactants have similar physical properties to electrolytes except regarding surface tension, and surface tension decreases rapidly with increase in surfactant concentration. At a certain point called the critical micelle concentration (CMC) unit surface active ions form an association to form larger, generally spherical molecules known as micelles which occurs at the minimum concentration of surfactant [9]. The CMC decreases with increasing chain length of the hydrophobic portion (alkyl group) in any class of surfactants. As a rule of thumb the CMC decreases by a factor of two for ionic surfactants (with no salt added) and by a factor of three for non-ionic surfactants upon adding one methylene group to the alkyl

chain. With nonionic surfactants, the increase in CMC is determined by increasing the length of the poly (ethylene oxide) hydrophilic group. Nonionic surfactants usually have lower CMC than corresponding ionic surfactants with the same alkyl chain length. Non-electrolytes, such as alcohols, can also cause a decrease in the CMC, since alcohols are less polar than water are distributed between the bulk solution and the micelles.

Surfactants in drug delivery

Surfactants are commonly used as excipients in all major drug delivery systems [14,15]. They are used in all disperse systems employed in pharmaceutical drug delivery. Relatively few surfactants exhibit pharmacological effects alone and hence the major use of surfactants in drug delivery comes from their application as an adjuvant. They can be used as an emulsifier, stabilizer, suspending agent and as an agent to increase drug absorption. Drug absorption mostly depends on two basic processes: (1) the dissolution of the drug in physiological fluids and (2) the process of absorption itself, i.e., the process by which a drug in physiological solution crosses the membrane at the site of absorption and, enters the general circulation. A pharmaceutical formulation may utilize one or more mechanism to increase the extent to which the administered drug is absorbed, especially in cases of poor absorption. Inclusion of the surfactant in oral solid and liquid dosage forms is expected to increase the rate of drug dissolution as a result of lowering the surface tension [16].

The level of surfactant in a particular dosage form depends on its role in formulation. In solid dosage forms, the level may be used at less than 0.1% to increase the dissolution rate and to help wetting the drug particles. A wetting effect is found at concentrations below the CMC. Surfactant effect on dissolution of solid is a complex phenomenon. Surfactants can affect the

surface area, and increase drug solubility, and hence the effective concentration gradient. They may also affect the effective rate of drug diffusion as a consequence of drug solubilization within the micelles. For solid dosage forms where dissolution is surface area dependent, surfactants can greatly affect the dissolution rate. The bioavailability of hydrophobic drugs can be increased by increasing their dissolution rate by the application of dissolution enhancers such as surfactants. Surfactants have been added to conventional drug-polymer solid dispersions to improve the release characteristics of the drug. In self-emulsifying drug delivery of liquid or semi-solid dosage forms, the level of surfactant may vary from 10% to 40% [7].

Surfactant molecules incorporated in formulations can affect drug availability and interaction by different ways. It may influence the disintegration and dissolution of solid dosage forms by controlling the rate of drug precipitation, and by increasing membrane permeability and integrity. Release rates of poorly soluble drugs from tablet or capsule can be increased by using surfactants. Surfactants decrease the aggregation of drug particles and create a greater surface area on the drug particle for dissolution to occur. Drug release is increased because the surface tension is lowered. Concentration of surfactant in solid dosage forms affects the release rate of drug. Currently used pharmaceutical surfactants with low-molecular-weights have low toxicity and high solubilization power towards poorly soluble drugs.

Effect of surfactants on GI absorption of drug

Surfactants have been one of the most investigated chemicals to enhance GI drug absorption [17]. They have been used to examine enhancement of GI absorption of drug under different test conditions using model membranes, everted intestinal sacs, and intestinal epithelia in diffusion chambers [18]. Increasing drug absorption from the gut wall is not only dependent upon a surfactant's activity with drug but also its ability to alter the barrier property of the

membrane through which the drug must diffuse. Bile salts, gut pH and bacteria in GI tract affect the role of a surfactant as a GI absorption enhancer, hence in vitro experiments cannot fully predict their behavior in vivo [19,20]. In the late 1960s, Engle and coworkers proposed surfactants as GI absorption promoters for insulin and heparin [21]. In their studies, sodium laurel sulfate was shown to enhance the absorption of heparin but not of insulin [21]. In 1978, researchers from Japan and Israel reported successful rectal absorption of insulin by use of polyoxyethylene ether, a non-ionic surfactant [22].

Application of surfactants in emulsions

One of the most important applications of surfactants in pharmaceutical drug delivery is emulsification [23]. This phenomenon has been extensively studied. Based on droplet size the emulsions can be classified as micro-emulsions (10-100nm) and macro-emulsions (0.1 to 50 µm). Micro-emulsions are transparent dispersions while macro-emulsions are opaque emulsions[23]. Surfactants are commonly applied to stabilize the emulsion. During emulsion formation one of the two immiscible liquids is broken up into droplets and dispersed into the other immiscible liquid. An increase in the interfacial free energy is formed because of increase in the area of interface. The surfactant adsorbs at the liquid-liquid interfaces by forming an oriented interfacial film. This film reduces the interfacial tension and reduces the coalescence of the dispersed droplet by forming a mechanical, steric and/or electrical barrier around them. The interfacial film formed by surfactants, produces repulsive forces in approaching droplets. This repulsion is due to the charges on the surfactant molecules. This surface charge plays an important role in o/w emulsions. In case of non-ionic surfactants, the charge may arise from adsorption of ions from the aqueous phase, from friction between droplets and the aqueous

phase, or mechanical repulsion. Micro-emulsions are more thermodynamically stable than macro-emulsions most likely because the former are produced by gentle mixing while the latter are produced using intense agitation.

Surfactant selection for pharmaceutical formulations [24]

The selection of surfactants for use in pharmaceutical applications depends to some extent on the route of administration. There are limitations on choice of surfactants since many surfactants are not tolerated physiologically well enough for pharmaceutical use. For example ionic surfactants may not be suitable since they may cause hemolysis of red blood cells and destruction of T lymphocyte cells at low concentrations. The most accepted surfactants for oral use, non-ionic surfactants are usually preferred but ionic surfactants used in low concentrations have been shown to be acceptable in many cases [23]. Non-ionic surfactants used in pharmaceutical applications today include mixtures such as ethoxylated castor oil (Cremophor EL), ethoxylated sorbitan fatty acid esters, e.g. polyoxyethylene sorbitan monooleate (Tween 80), sorbitan fatty acid esters, e.g. sorbitan monooleate (Span 80), ethoxylated hydroxystearic acid, e.g. polyethylene glycol 660 (12-)hydroxystearate (Solutol HS15), etylene and propylene oxide block copolymers (Pluronic F68) and fatty acid esters of glycerol (Imwitor 742) [24]. The above described non-ionic surfactants do, however, exhibit a number of disadvantages. For instance, the commercial non-ionic surfactants available for pharmaceutical formulators are complex mixtures of different molecules which make the pharmaceutical characterization of these products very difficult [24]. It makes the analytical processes expensive and tedious to ensure adequate quality of pharmaceutical dosage forms. Surfactant selection should be based on the toxicology profile of the surfactant, drug solubility in surfactant, and drug-surfactant

compatibility. Drug solubility can be measured by adding a drug into the surfactant while stirring at room temperature. Semisolid surfactants can be heated to a temperature that is slightly higher than its melting point before addition of drug. The solubility of water insoluble drugs can be enhanced by adding a co-surfactant. Physical and chemical compatibility of water insoluble drugs with surfactant should be tested before using surfactant [24].

Surfactant selection on the basis of HLB

The diverse and multifunctional role of surfactants in emulsion systems makes their application in developing pharmaceutical dosage forms difficult and empirical at best [24]. Although Bancroft in 1913 recognized the role of the emulsifier on the type of emulsion formed, it was not until 1949 when Griffin introduced the concept of HLB (hydrophilic lipophilic balance); and then subsequently by 1950s formulators, who used the HLB system to characterize surfactants in a manner that was relevant to formulation[25,26]. The HLB scale provides a scale of hydrophilicity (0-20) and is based on the relative percentage of hydrophilic to lipophilic (hydrophobic) groups in surfactant molecule; this simplified emulsifier selection and blending [27]. Surfactants with a low HLB (≤ 6) tend to provide more stable water-in-oil (w/o) stable emulsion and those with high HLB (≥ 8) provide more stable oil-in-water (o/w) emulsions. For an O/W emulsion droplet the hydrophobic chain resides in the oil phase whereas the hydrophilic head group resides in the aqueous phase. For a W/O emulsion droplet, the hydrophilic group(s) reside in the water droplet, whereas the lipophilic groups reside in the hydrocarbon phase.

HLB values and activity of surfactants

HLB Range	Application
1-3	Antifoaming
3–6	W/O emulsifier
7–9	Wetting agent
8-18	O/W emulsifier
13-16	Detergent
15-20	Solubilizers

Approximation of HLB for the surfactants not described by the HLB scale developed of Griffin can be made by their water dispersible characterization [6].

HLB values and water dispersibility

HLB Range	Water Dispersibility
1-4	Not dispersible
3-6	Poor dispersion
6-8	Milky dispersion only after vigorous agitation
8-10	Stable Milky Dispersion
10-13	Clear dispersion
$\geq$ 13	Clear solution

While using Griffins HLB system it is important to understand that the HLB system has a few limitations. The nature of immiscible phases, other adjutants, emulsifier concentrations,

phase volume, temperature and processing methods can influence the HLB system [6]. The calculated HLB doesn't consider the effect of temperature or additives. Salting in or out the surfactant by using NaCl, will make the molecule less hydrophilic thus one can expect a higher HLB compared to the one calculated using Griffins' HLB system. Emulsion stability affected by rheological behavior is not accounted for by the HLB relationships. In fact emulsion stability may be unrelated to HLB value, since, for instance stable O/W emulsions can be prepared throughout the wide range of HLB values (≤ 2 to ≥ 17). Direct calculation of HLB values is only applicable to non-ionic surfactants.

Surfactant toxicity

Surfactants act as a solubilizers, stabilizers, emulsifiers and wetting agents. They can also cause toxicity if not used in the proper concentration. One of the very first studies during the 1960s, explored the effects of different surfactant types on mouse GI mucosa [28]. Significant pathological changes were observed following exposure to ionic surfactants but not with non-ionic surfactants. Non-ionic and anionic surfactants were less toxic than cationic surfactants. After GI absorption, surfactants are metabolized rapidly and metabolites are excreted in the urine and feces. Most metabolic activity takes place in the liver. Food additive surfactants such as polysorbate 60, 80 greatly affected rat intestinal mucosa. Topical application of surfactants may cause irritation. Regardless of routes of administration, there is no surfactant that is 100% safe, however administration of low levels of surfactants is GRAS (Generally Recognized As Safe) [29]. The use of high levels of surfactants in solid dosage forms rarely practical and as a result they may not exert any toxic reactions.

Future investigation and prospects

There are reports exploring new ways to apply surfactant science to pharmaceutical drug delivery [5]. Although the field of dual surfactant systems is less studied, mixed surfactant systems (dual surfactants) are of great theoretical importance and interest [30]. It can be speculated that the tendency of the aggregated structure to form in solution containing mixed surfactants would be substantially different from that of the single surfactant and this could be used in drug delivery applications. Using non-ionic surfactants, it may also be possible to use niosomes for pharmaceutical drug delivery [31]. Niosomes are non-ionic surfactant vesicles obtained on hydration of synthetic nonionic surfactants [32]. Niosomes can be formed with or without incorporation of cholesterol or other lipids [33]. Niosomes are vesicular systems similar to liposomes that can be used as carriers of amphiphilic and lipophilic drugs. Niosomes are promising vehicles for drug delivery and being non-ionic; are less toxic and may improve the therapeutic index of drugs by restricting action to target cells as in the cases of liposomal drug delivery. In the future it may be possible to develop artificial bio-surfactants from bacteria in the laboratory. Bio-surfactants are similar to chemical surfactants and could be applied to many scientific and industrial uses [5]. There are also studies being conducted to use biological surfactants such as obtained from mammalian organs such as lung surfactants for use in drug delivery applications [34].

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CHAPTER 3

INFLUENCE OF DIFFERENT COMBINED HYDROPHILIC-LIPOPHILIC BALANCE (CHLBS) OF DUAL SURFACTANT SYSTEM ON FORMULATION PROPERTIES OF THEOPHYLLINE LOADED ETHYL CELLULOSE MICROSPHERES FOR ORAL SOLID DOSAGE FORMS.

Research Paper to be submitted to the Journal of Microencapsulation

Thakare Mohan, Capomacchia Anthony, Price James

Abstract: The purpose of this study was to evaluate the effects of different combined hydrophilic-lipophilic balance (CHLBs) of dual surfactant system on different physical formulation parameters and the *in vitro* release of ethyl cellulose microspheres. Microspheres containing theophylline were prepared at different CHLBs using different combinations of dual surfactants in an emulsion-solvent evaporation process that used light mineral oil as the external phase with acetone as the internal phase. The effects of CHLBs of dual surfactants yield, particle size distribution, geometric mean diameters, initial drug release, and drug release characteristics of the microspheres were evaluated. The *in vitro* drug release from the microspheres was studied using simulated intestinal fluid (pH 6.8, 37° C) containing 0.1% Tween 20 but no enzymes. The geometric mean diameter of the microsphere batches decreased with an increase in CHLB. The dissolution rate and initial drug release from the microsphere batches increased with an increase in CHLB. According to scanning electron microscopy observations, microspheres prepared with dual surfactants showed well-formed spherical particles with solid interiors. This particulate system, in which the microsphere matrix is a hydrophobic polymer, and the release rate can be modulated by the ratio of dual surfactants is a promising formulation system for drug delivery.

Keywords: Dual surfactants, ethyl cellulose, microspheres, emulsion solvent evaporation, drug release

(Total words: 201)

Introduction

In controlled release drug delivery systems, the drug is released at controlled rates for long periods of time, ranging from hours to months [1]. Controlled drug delivery can be achieved by polymeric microspheres due to their ability to encapsulate a variety of drugs, biocompatibility, high bioavailability and sustained drug release characteristics [2-4] . Microsphere characteristics including particle size is crucial in designing a controlled drug delivery system. Various formulation and processing parameters affect the microsphere characteristics and thus drug release [5].

Theophylline cannot be loaded efficiently in microspheres prepared with o/w emulsion systems because a significant amount of drug is lost in the external phase [6]. Many factors affect the characteristics of microspheres such as type and molecular weight of the polymer, the core drug particle size, the drug to polymer ratio, drug solubility in the polymer, mixing intensity, and polymer phase viscosity [3,7-10]. There are reports that the emulsifying agent (surfactant) also affects the characteristics of polymeric microspheres [11,12].

The task of selecting a suitable surfactant as an emulsifier for a particular system often involves a great deal of experimentation. The hydrophile-lipophile balance (HLB) system introduced by Griffin is based on the hydrophilic-lipophilic balance of a surfactant [13-15]. This HLB system can be used to select the surfactant for optimum efficiency [16]. A low and a high HLB surfactant can be blended together in order to stabilize emulsion at a particular HLB known as required or combined HLB (CHLB).

Many investigators reported the application of a single surfactant like Span 80, Span 85, aluminum stearate, or magnesium stearate for stabilizing the emulsion to prepare microspheres [16-25]

Based on the literature, the tendency of the aggregated structure to form in solution containing mixed surfactant would be substantially different than that from a single

surfactant. Based on this premise the aim of this research was to study the effects of using dual surfactants (one with a high HLB and another with a low HLB) at different CHLBs on physical and drug release characteristics of ethyl cellulose microspheres prepared by the with emulsion-solvent evaporation method.

Experimental

Materials

The following chemicals were used: Ethyl cellulose (Scientific Polymer Products, New York), micronized theophylline (Gift sample from BASF), light mineral oil (Central scientific store, UGA, Athens, GA), Span 65, Tween 40, Brij 58 (Ruger Chemical Company Inc., Irvington, NJ), methylene chloride (Fisher Scientific, NJ), acetone, monobasic potassium phosphate and sodium hydroxide (J.T. Baker, Phillipsburg, NJ).

Instruments

The following instruments were used: Stirrer (Lab Stirrer LR 400D, Yamato Scientific Company Ltd., Tokyo, Japan), Dissolution Apparatus II USP (Dissolution Test system 5100, Distek Inc., North Brunswick, NJ and Prolabo dissolution), Aquamate (UV Spectrophotometer, Thermo Electron Corporation, Mercer's Row, Cambridge, UK), Accumet 5 pH meter (Fisher Scientific, NJ) and, USP Standard sieve series for PSD studies.

Preparation of microspheres

The preparation of ethyl cellulose microspheres containing theophylline was accomplished by the emulsification-solvent evaporation method in a 1 L tall glass beaker. For the preparation of all the different batches of microspheres, experimental conditions were kept identical. Light mineral oil (300 ml) containing the low HLB surfactant was used as an external or continuous phase (phase A). In a separate glass vessel, a 5 % solution of ethyl cellulose in acetone was prepared (phase B). Micronized anhydrous theophylline was

dispersed in phase B to give a 33% theoretical drug loading (1 part theophylline to 2 parts ethyl cellulose). The entire contents of this vessel (phase B) were added into the glass beaker containing a solution of light mineral oil and a low HLB surfactant (phase A) under vigorous agitation. Agitation was continued until the acetone evaporated and the microspheres were firm. The major part of the mineral oil was decanted from the microspheres which were then placed on a filter and washed with mineral spirits to remove the residual light mineral oil. The clean microspheres were dried in an oven at 50^0C overnight.

Particle size distribution

The size distribution of microspheres was determined by sieving the microspheres through a set of standard sieves of range 90-710 μm. A pan was placed underneath the sieves to collect the particles that passed through the last sieve. To perform sieving, the aggregate sample was placed on the sieve of largest size, covered, and then tapped by hand till no change in weight was observed in the sieves. Manual checking and brief hand sieving was done to assure that all particles retained on a sieve were larger than the sieve apertures. After sieving, the quantity of each fraction of particles was weighed. Particle size distribution and geometrical mean diameters were calculated.

Determination of Drug loading

The 355 μm fraction of each batch of ethyl cellulose microspheres was analyzed for drug loading. Drug loading was determined spectrophotometrically at 276.5 nm by placing accurately weighed samples (in triplicate) in 25 ml volumetric flasks and dissolving in methylene chloride. Spectrophotometric interference from ethyl cellulose was not observed at this wavelength.

SEM analysis of microspheres

The surface morphology of microspheres was observed by scanning electron microscope (SEM) using a Zeiss model 1450EP scanning electron microscope. Microspheres

were mounted onto metal multi-stubs using double-sided adhesive tape and SEM images were taken at specific magnifications.

In vitro dissolution studies

In vitro release studies of ethyl cellulose microspheres were performed using a USP dissolution apparatus II consisted of Distek dissolution tester (Distek Inc.,New Jersey). The 355 μm fraction of each batch of microspheres was selected for evaluation. Microsphere samples in triplicate for each batch were suspended in 900 ml of simulated intestinal fluid with 0.1% Tween 20, and no enzymes. The dissolution study was carried out at 37±0.5°C at 100 r.p.m for 12-24 hours. Three ml of sample were withdrawn at specific time intervals and replaced with fresh simulated intestinal fluid medium. The drug released was determined spectrophotometrically at 274 nm. The dissolution data was evaluated for initial release, dissolution rate and the mechanism of drug release.

Release kinetics

Data obtained from *in vitro* release studies were fitted to Higuchi kinetic equations to find out the mechanism of drug release from ethyl cellulose microspheres [26,27].

Result and discussion

Theophylline was used as the model drug in this experiment. Theophylline is a sparingly water soluble drug with a narrow therapeutic index [28-32]. The effects of using dual surfactants at different CHLBs on the physical and drug release parameters of the model drug theophylline encapsulated in ethyl cellulose microspheres using emulsion solvent evaporation method were evaluated.

Microsphere preparation

Ethyl cellulose microspheres were prepared by the emulsion solvent evaporation method. Microspheres were formed after agitation of the emulsion and evaporation of the

solvent. It is very important to carefully select the solvent combination that is non-toxic and pharmaceutically acceptable. We selected acetone as a choice of solvent because it is a unique organic solvent that is polar and there are reports stating that microsphere formation is easier in acetone using different approach [33]. This attribute is an advantage in scaling up at the industrial level. Initial experiments were performed to optimize the formulation process and each step of the preparation of microsphere was keenly observed. Dual surfactants used in this process are presented in Table 3.1 with their respective HLBs and CHLBs. Dual surfactant combination is expected to have a high disparity for the present emulsion system by reducing the surface tension at the interface.

Particle size distribution (PSD) and geometric mean diameter (GMD)

The microspheres prepared with a single surfactant such as Span 65, Tween 40 and Brij 58 showed variations in the GMD, as shown in Figure 3.2. Microsphere prepared with the single surfactant Span 65 showed lower GMD compared to the microspheres prepared with the single surfactant Tween 40. Application of the single surfactant Brij 58 showed the lowest GMD. When the Span 65 and Tween 40 served as dual surfactants, we noticed change in the GMD of microspheres. The GMD of microspheres prepared with dual surfactants (Span65 and Tween 40) was lower compared with the GMD of microspheres prepared with single surfactant Tween 40 alone (Figure 3.1 and 3.2). Microspheres prepared from Tween 40 alone showed some fused particles when observed under microscope. This might have caused an increase in the GMD of microspheres. During emulsification process dual surfactants (Span 65 and Tween 40) would have produced a strong interfacial film ultimately producing more separated microspheres. We attempted to separate the aggregated microspheres prepared with Tween 40 alone, by applying a rubbing pressure using a wooden spatula. We observed that the likelihood of aggregation increased as the mean diameter of the microspheres decreased. Adding surfactant aids in to maintain a monodispersed emulsion and

thus helps separation of the microspheres. Surfactant typically provides a short-range repulsion or disjoining pressure that prevents droplet coalescence and more separated microspheres are formed. We expect this effect to occur with microspheres prepared using dual surfactant versus single surfactant. At different CHLBs ranging from 4.5 to 7.5 the dual surfactants Span 65 and Tween 40, appear to influence the geometric mean diameter (GMD) of the microspheres, as shown in Figure 3.1. An increase in the CHLBs of dual surfactants from 4.5 to 7.5 reduced the GMD of microspheres for respective batches. Incorporation of dual surfactants decreased the particle size of microspheres at different CHLB levels. This reduction of the mean particle size may be attributed to the presence of surfactants at the interface of phases in emulsion, which facilitates the formation of smaller emulsion droplets that is dependent on the HLB value and thereby reducing the size of the final microspheres.

As shown in figures 3.3 and 3.4, the particle size distribution (PSD) was varied significantly for single and dual surfactant microspheres. Microspheres prepared using the single surfactant Tween 40 showed one sided skewed PSD with the majority of the particles in 710µm fraction. Brij 58 showed most particles in the size 125 µm fraction. Span 65 showed a bimodal distribution with particles in both the 710µm and 355µm fractions. This irregular PSD of microspheres was not observed when dual surfactants were used. Microspheres developed using dual surfactant; showed the PSD range mostly in the 355 µm fraction for all the CHLBs. We also observed that the percentage of particles in the 710µm and 500µm fractions was decreased with an increase in the CHLB level of dual surfactants. With an increase in CHLB from 4.5 to 6.5 we noted an increase in the particles in 355 µm fraction. The particles with the 7.5 CHLB were found with highest percentage in the 125 µm fraction compared to other CHLBs. These changes may be attributed to the stabilization of the emulsion that was expected to occur with the increase in CHLB. Thus coalescence is

retarded, leading to smaller microsphere particle size, especially in cases of using dual surfactants.

Scanning electron microscopy (SEM) analysis

The SEM micrographs of microspheres prepared from single as well as dual surfactants are shown in Figure 3.13 to 3.19. Microspheres with the single surfactant in the preparation procedure were spherical and denser structure (Figure 3.13 to 3.15). Using dual surfactants at different CHLBs strongly affected microsphere morphology and resulted in less dense microsphere with wrinkled surfaces (Figure 3.16. and 3.19). This may be attributed to stabilization of the emulsion and thus formation of more uniform droplet dispersion in oil phase, which after solidification could lead to a more porous structure. Studying the effect of dual surfactants on surface morphology revealed that by using dual surfactants at certain CHLBs, a less dense matrix structure could be formed.

Drug loading

As seen in Table 3.2, the drug loading studies of the 355 μm fractions of the microsphere batches revealed that there was no significant difference between the drug loadings with the change in most CHLBs. At 6.5 CHLB the drug loading was higher than at all other CHLBs. The single surfactant Span 65 had increased drug loading compared to dual surfactants (Table 3.2). These batches were prepared with 33% theoretical drug loading. We observed that none of the microsphere batches has drug loading more than the theoretical drug loading. This indicates that there is less air trapped in the microsphere while preparation and this is desired in pharmaceutical preparation.

In vitro drug release behavior

The *in vitro* release profiles of ethyl cellulose microspheres are depicted in figure 3.5 and 3.6. The microspheres produced with dual surfactants (Span 65+Tween 40) showed differences in release profiles at different CHLBs (Figure 3.5). Theophylline release was

effectively increased with the dual surfactants formulations, while the single surfactant retarded the drug release in most of the formulations. Microspheres made using the dual surfactants exhibited adequate controlled-release profiles in all CHLB formulations. The effects of the dual surfactants on the t_{50} release are shown in Figure 3.7. The t_{50} drug release was effectively increased with an increase in CHLB. The t_{50} drug release is a time taken by 50% of the drug to release from the dosage form. Microsphere prepared with different single surfactants also showed different effects on the t_{50} drug release. The single surfactant, Tween 40, formulation showed a lower t_{50} than the single surfactant Span 65. The drug release mechanism is naturally influenced by many factors such as the presence and the location of drug and surfactant molecules in the microsphere particle. The possible sites of location of the surfactant molecules could be either on the surface of the microsphere, within the matrix isolated from the interior environment or within the microsphere but connected with its outer surface possibly by means of channels. As discussed earlier, the microspheres prepared with the single hydrophobic surfactant, Span 65, had the slowest release. When the hydrophobic surfactant Span 65 that has low HLB is combined with the hydrophilic surfactant Tween 40 that has high HLB, the increased dissolution rate was noted. This is because the hydrophilic surfactant may dissolve in the polymer solution in the polar solvent phase and trapped in the microspheres. In the dissolution process the hydrophilic surfactant dissolves in the aqueous dissolution medium and thus facilitates medium entry into the system. The amount of hydrophilic surfactant is greater with an increase in CHLB formulations. Therefore the amount of hydrophilic surfactant in the microsphere matrix also increases and that causes the increased dissolution rate as shown in Figure 3.5. Other factors such as the effect of surfactant as a solubilizer might also increase the drug dissolution and this effect is increased with dual surfactants system. We have observed slower dissolution rates with the microspheres prepared with the single surfactants. This could be due to some of the single

surfactant collecting at the interface of the polar phase droplets and the external mineral oil phase causing a slow release of the drug. Single surfactant may not be as effective as dual combination in solubilizing the drug. The hydrophilic surfactants if remained on the microsphere surface may not allow the drug to come easily in dissolution medium and slow down the release.

Initial drug release

Figure 3.7 shows the initial drug release of dual surfactants at different CHLBs. The initial drug release percent within the first 30 minutes is analyzed for the microspheres fraction of 355 μm size. In the case of 4.5 CHLB, the initial drug release was lowest amongst all CHLBs. The highest initial drug release was for 7.5 CHLB amongst all CHLBs. The increased hydrophilic surfactant concentration that occurs when CHLB was increased affected the initial drug release. There seems to be an increased solubilizing effect because of increased in CHLB.

Drug release kinetics

Figures 3.8 and 3.9 depict the drug release kinetics. The solvent evaporation method usually develops non-porous or relatively less porous microsphere in which drug is distributed homogenously throughout the polymeric matrix. The release from such a matrix is either by erosion or by diffusion [34]. The release can be described by explained by three stages i-e initial diffusional release from the superficial region of microsphere, followed by slower release by a polymer hydrolysis and then finally a rapid release resulting from a polymer erosion. Our current study is not dependent on the chemical or physical erosion of the polymer. The release occurs by the diffusion through the pores. Higuchi equation analysis for spherical matrices indicated that the drug release from the microspheres was primarily by diffusion (Table 3.3).

Conclusion

The CHLBs of dual surfactants play an important role on the physical characteristics and drug release behavior of microspheres prepared by the emulsion solvent evaporation method. The different CHLB values of dual surfactants have shown that the different effects on physical as well as drug release properties of microspheres etc. We noted a decrease in the geometric mean diameter of the microsphere batches with an increase in CHLB. There was a high initial drug release in the microspheres prepared with the dual surfactants. We noted an increase in the initial drug release with an increase in CHLB. The *in vitro* drug release was altered by the dual surfactants. The release was faster in the dual surfactant prepared at 7.5 CHLB compared to other CHLBs. The drug release was slowest in microspheres prepared with dual surfactant at 4.5 CHLB compared to other CHLBs. We also noted that with the single surfactant, the drug release was slow. The release kinetics showed a diffusion type of release from dual surfactant microsphere.

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Table 3.1. Different dual surfactant combinations with HLB and CHLBs.

Surfactants used	**CHLB or HLB**	**Effective ratio**
Span 65 (HLB=2.1) + Tween 40 (HLB=15.6)	4.5	82:18
Span 65 (HLB=2.1) + Tween 40 (HLB=15.6)	5.5	77:23
Span 65 (HLB=2.1) + Tween 40 (HLB=15.6)	6.5	67:33
Span 65 (HLB=2.1) + Tween 40 (HLB=15.6)	7.5	60:40
Span 65 (HLB=2.1)	2.1	100
Tween 40 (HLB=15.6)	15.6	100
Brij 58 (HLB=15.7)	15.7	100

Table 3.2. Effect of 355 µm fractions of microspheres prepared at different CHLBs using dual surfactants on actual drug loading.

Surfactants used	CHLB	Actual drug loading (%)
Span 65 (HLB=2.1) + Tween 40 (HLB=15.6)	4.5	21.62 ± 1.64
Span 65 (HLB=2.1) + Tween 40 (HLB=15.6)	5.5	21.90 ± 0.42
Span 65 (HLB=2.1) + Tween 40 (HLB=15.6)	6.5	25.47 ± 1.83
Span 65 (HLB=2.1) + Tween 40 (HLB=15.6)	7.5	22.52 ± 0.46
Span 65 (HLB=2.1)	2.1	22.58 ± 0.46
Tween 40 (HLB=15.6)	15.6	31.70 ± 1.73
Brij 58 (HLB=15.7)	15.7	27.86 ± 0.86

Table 3.3. Higuchi equation regression analysis of different microsphere batches of single and dual surfactants.

Surfactants used	**CHLB or HLB**	**Higuchi equation regression R^2**
Span 65 (HLB=2.1) + Tween 40 (HLB=15.6)	4.5	0.9636
Span 65 (HLB=2.1) + Tween 40 (HLB=15.6)	5.5	0.9860
Span 65 (HLB=2.1) + Tween 40 (HLB=15.6)	6.5	0.9881
Span 65 (HLB=2.1) + Tween 40 (HLB=15.6)	7.5	0.7669
Span 65 (HLB=2.1)	2.1	0.8369
Tween 40 (HLB=15.6)	15.6	0.9695
Brij 58 (HLB=15.7)	15.7	0.9285

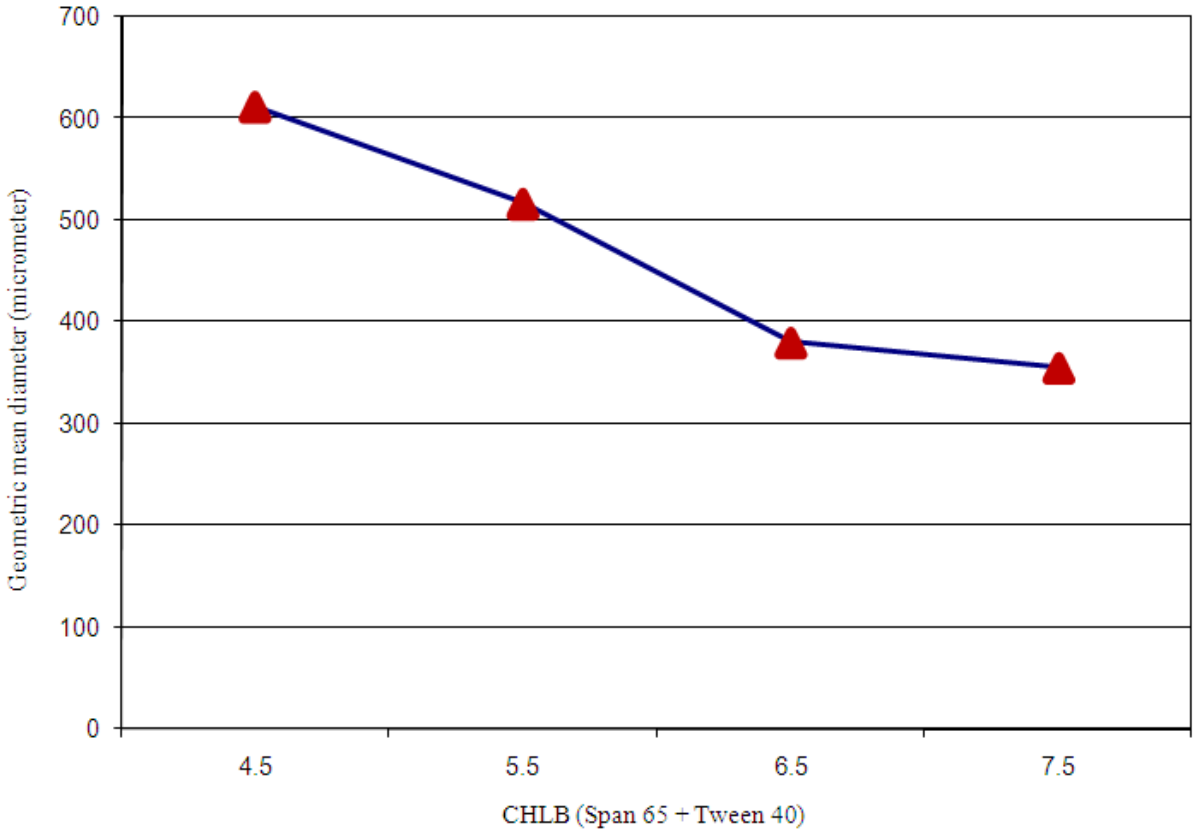

Figure 3.1. Effect of different CHLBs on geometric mean diameter of microsphere batches prepared with dual surfactants (Span65+Tween40).

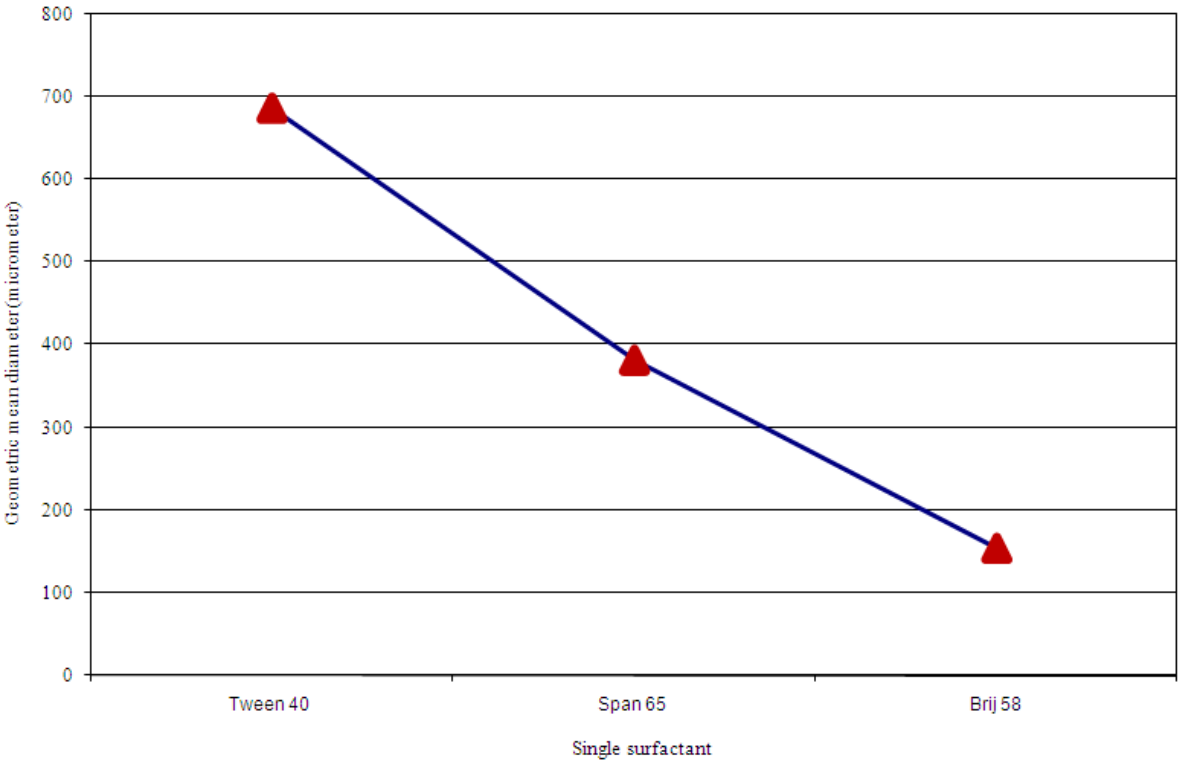

Figure 3.2. Effect of different HLBs on geometric mean diameter of microsphere batches prepared with different single surfactants.

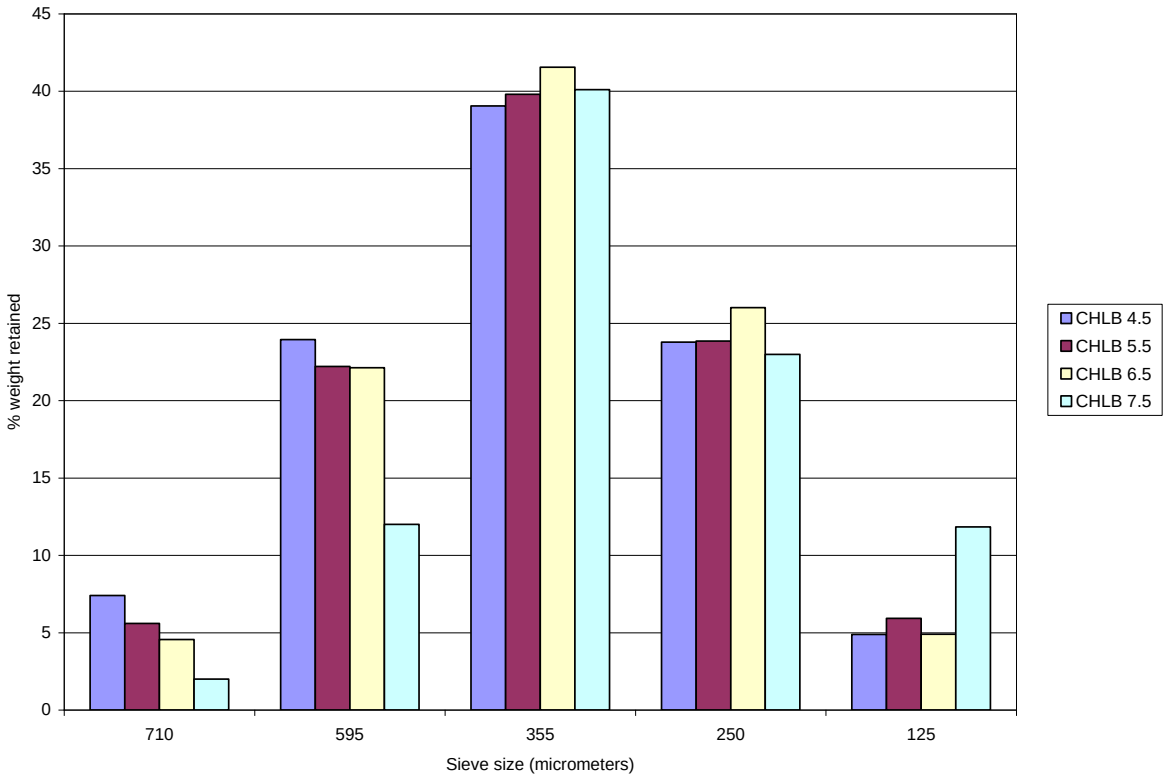

Figure 3.3. Effect of different CHLBs on particle size distribution of microsphere batches prepared with dual surfactants (Span 65+Tween 40).

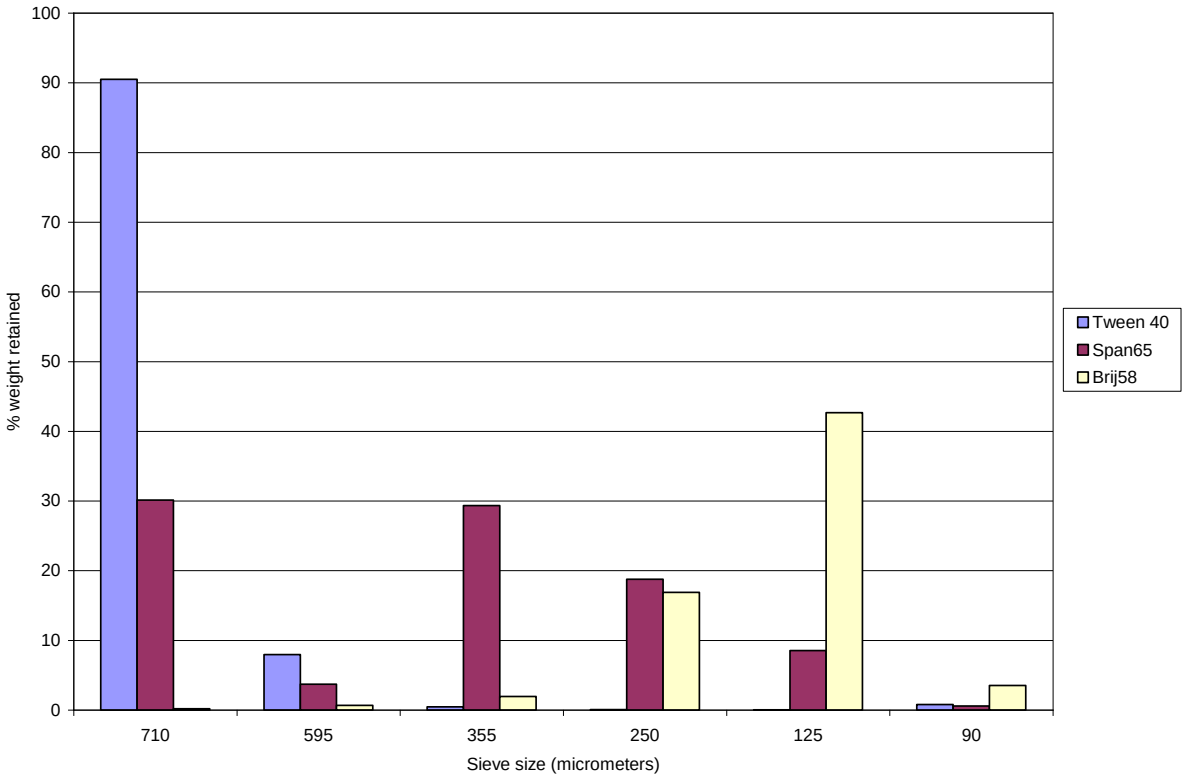

Figure 3.4. Effect of HLB on particle size distribution of microsphere batches prepared with different single surfactants.

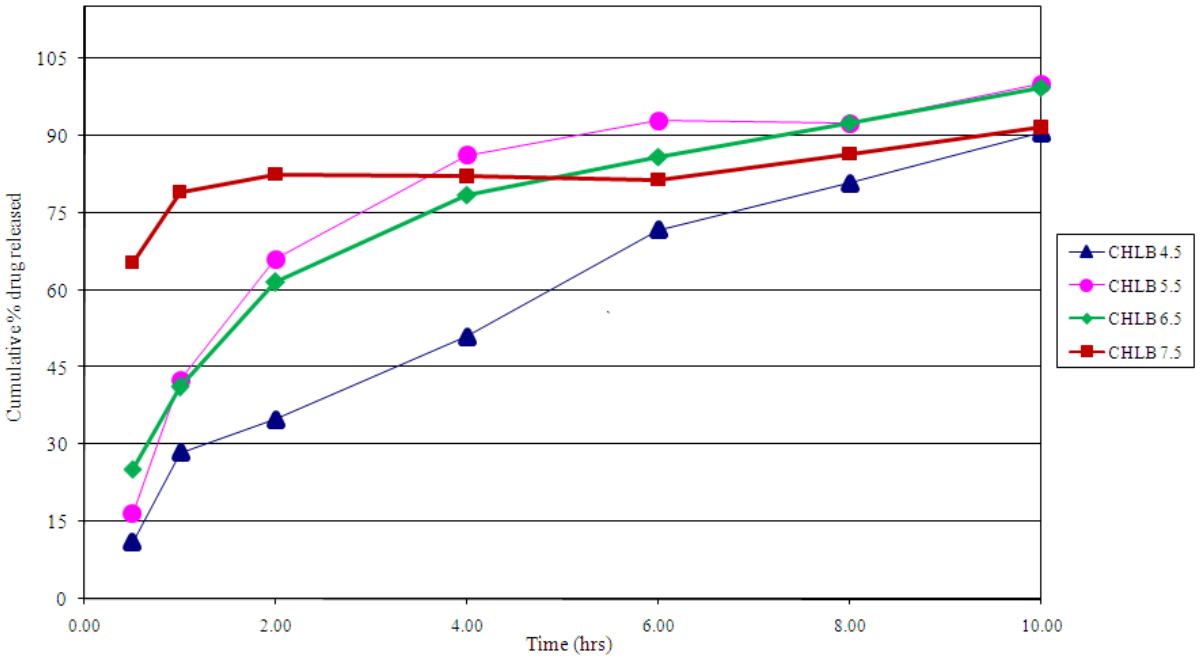

Figure 3.5. Effect of different CHLBs on *in vitro* dissolution studies of microsphere batches prepared with dual surfactants (Span 65+Tween 40).

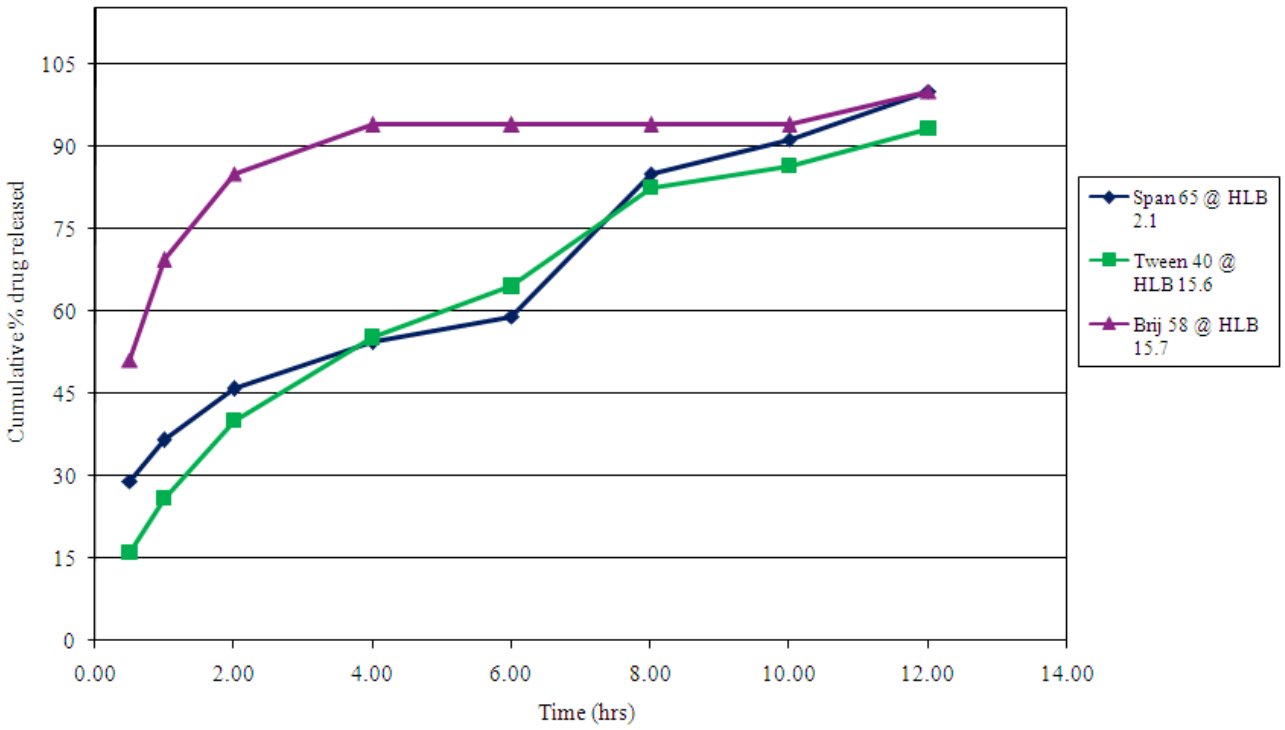

Figure 3.6. Effect of different HLBs on *in vitro* dissolution studies of microsphere batches prepared with different single surfactants (Span 65; Tween 40, and Brij 58).

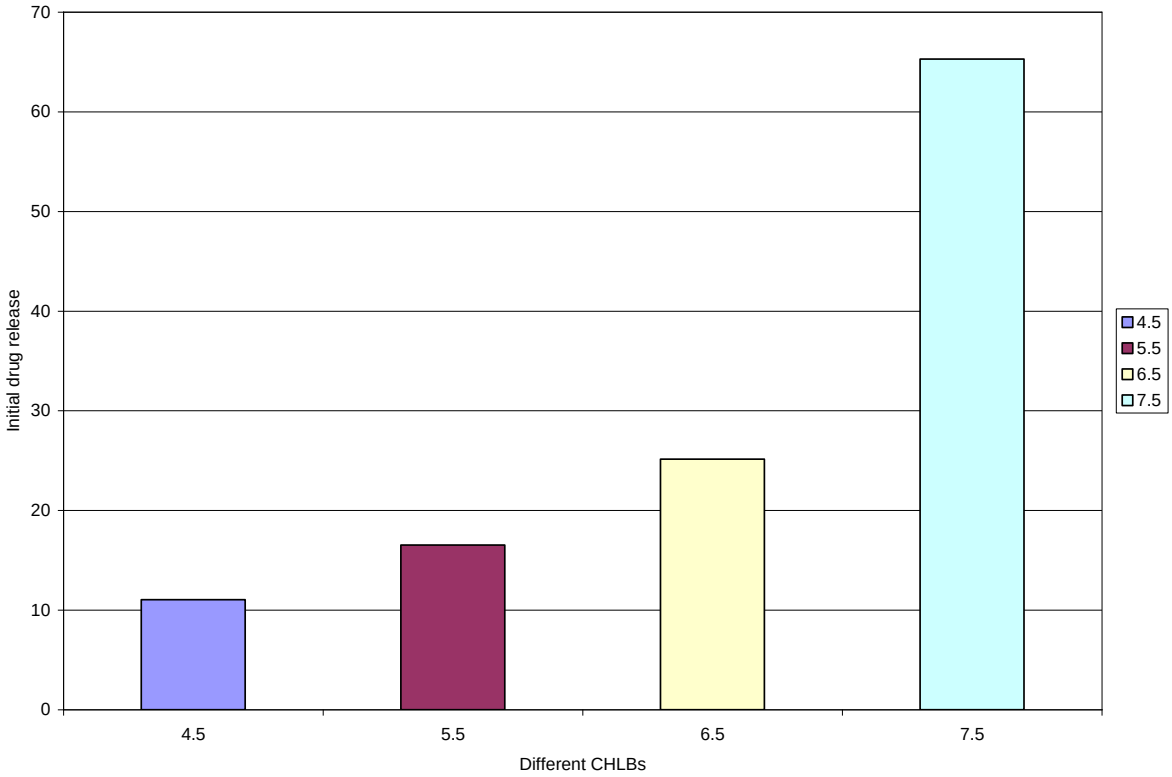

Figure 3.7. Effect of different CHLBs on the initial drug release rate of microsphere batches prepared with dual surfactants (Span 65+Tween 40).

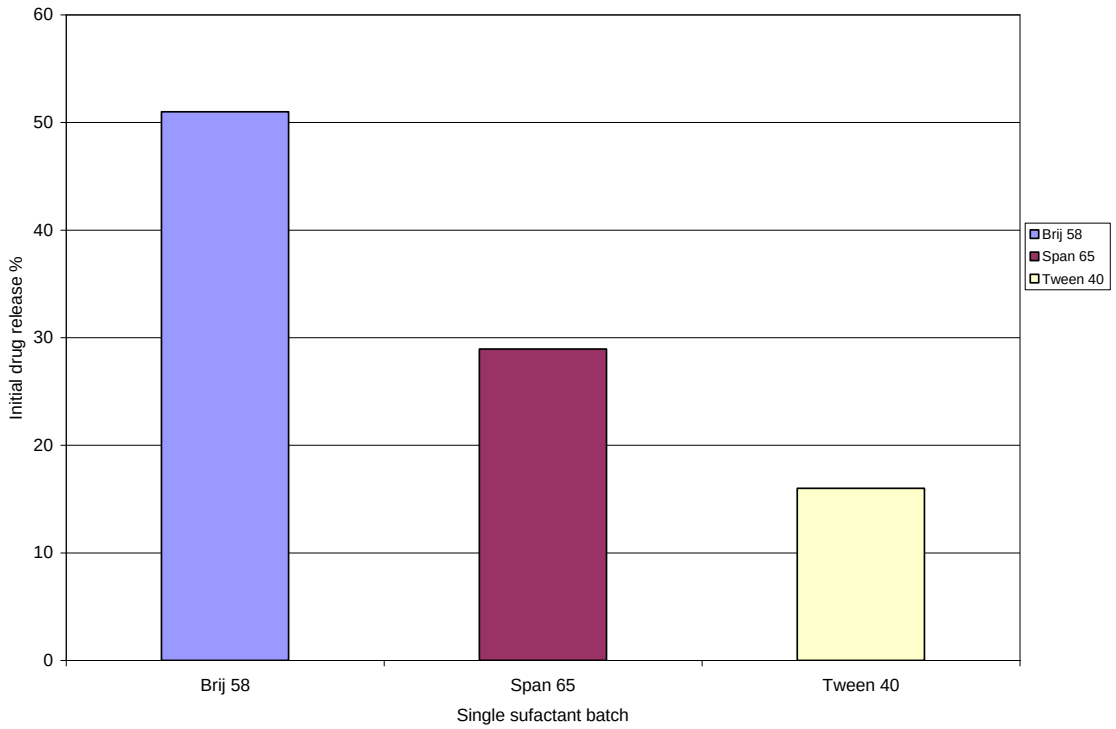

Figure 3.8. Effect of different HLBs on the initial drug release rate of microsphere batches prepared with different single surfactants (Span 65; Tween 40, and Brij 58).

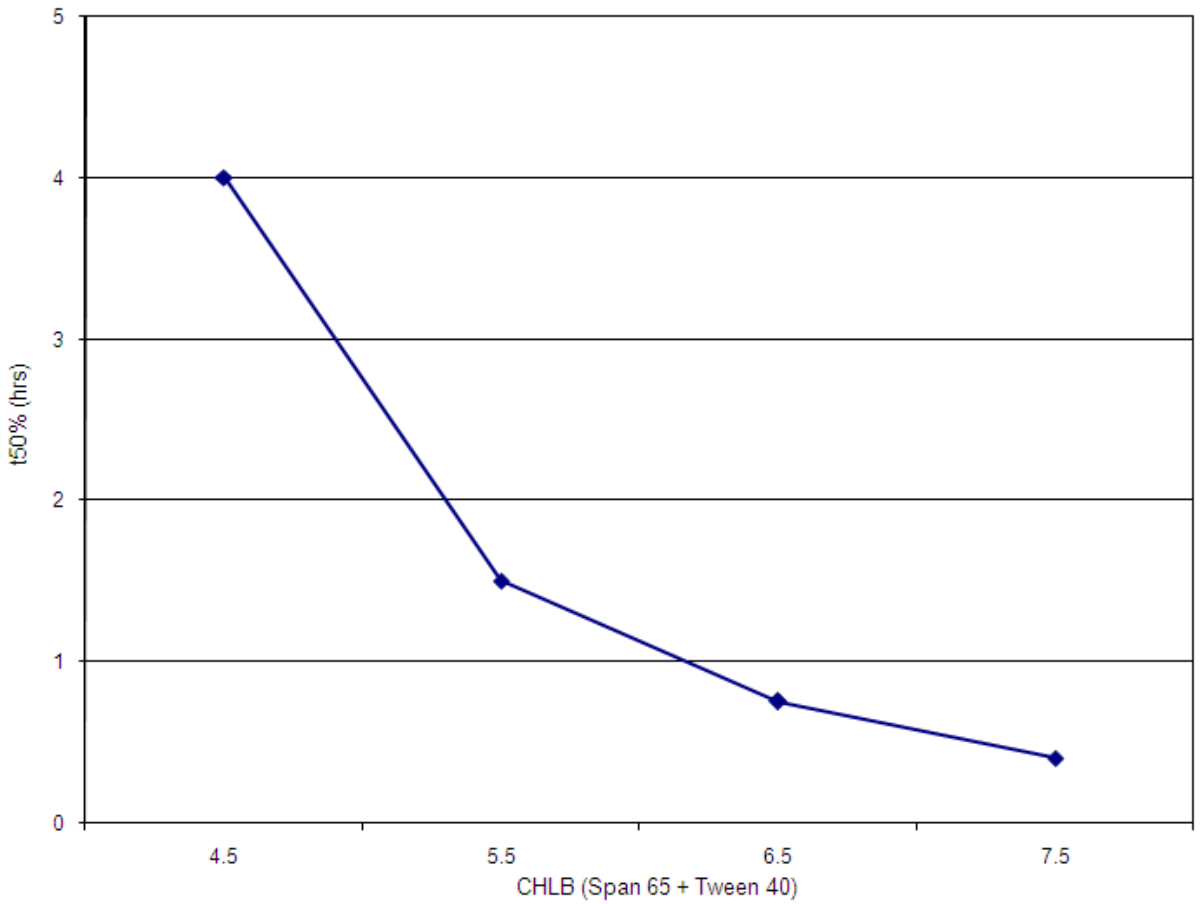

Figure 3.9. Effect of different CHLBs on the dissolution rate of microsphere batches prepared with dual surfactants (Span 65+Tween 40) at t50.

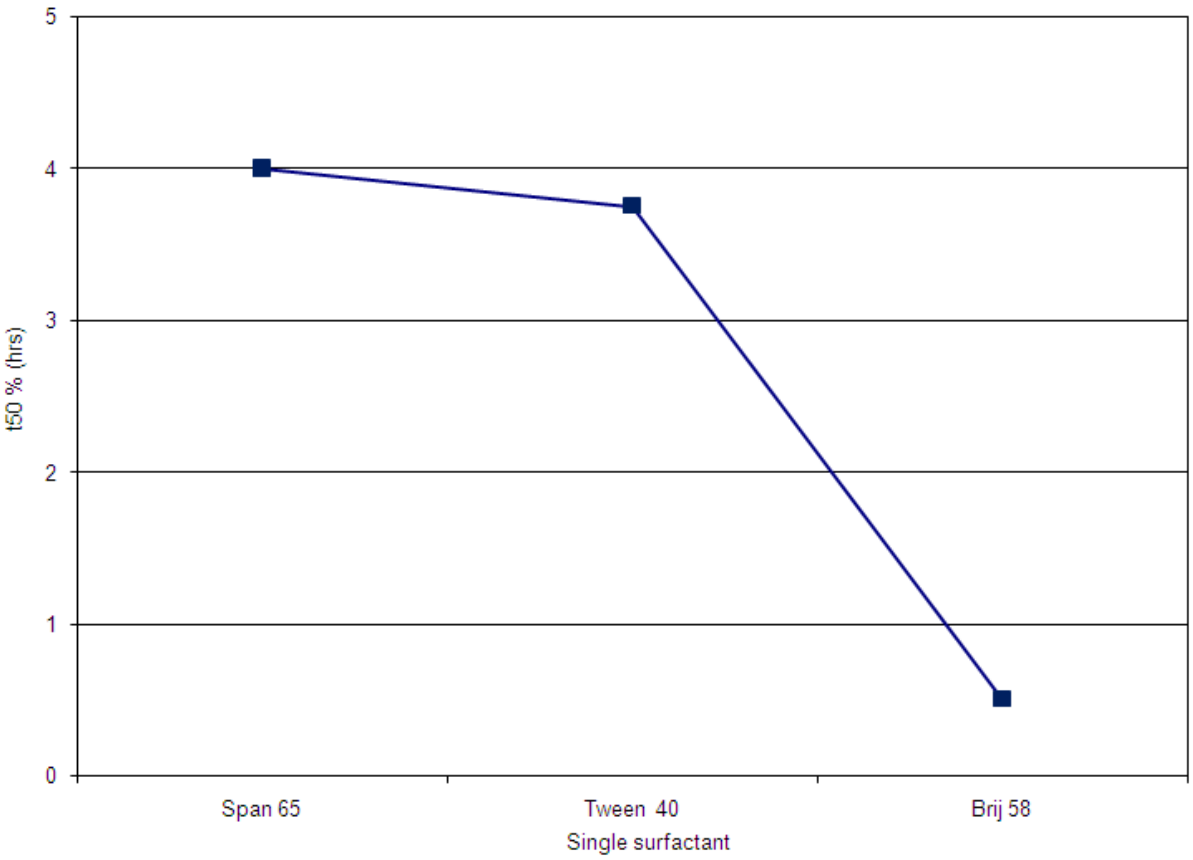

Figure 3.10. Effect of different HLBs on the dissolution rate of microsphere batches prepared with different single surfactants at t50.

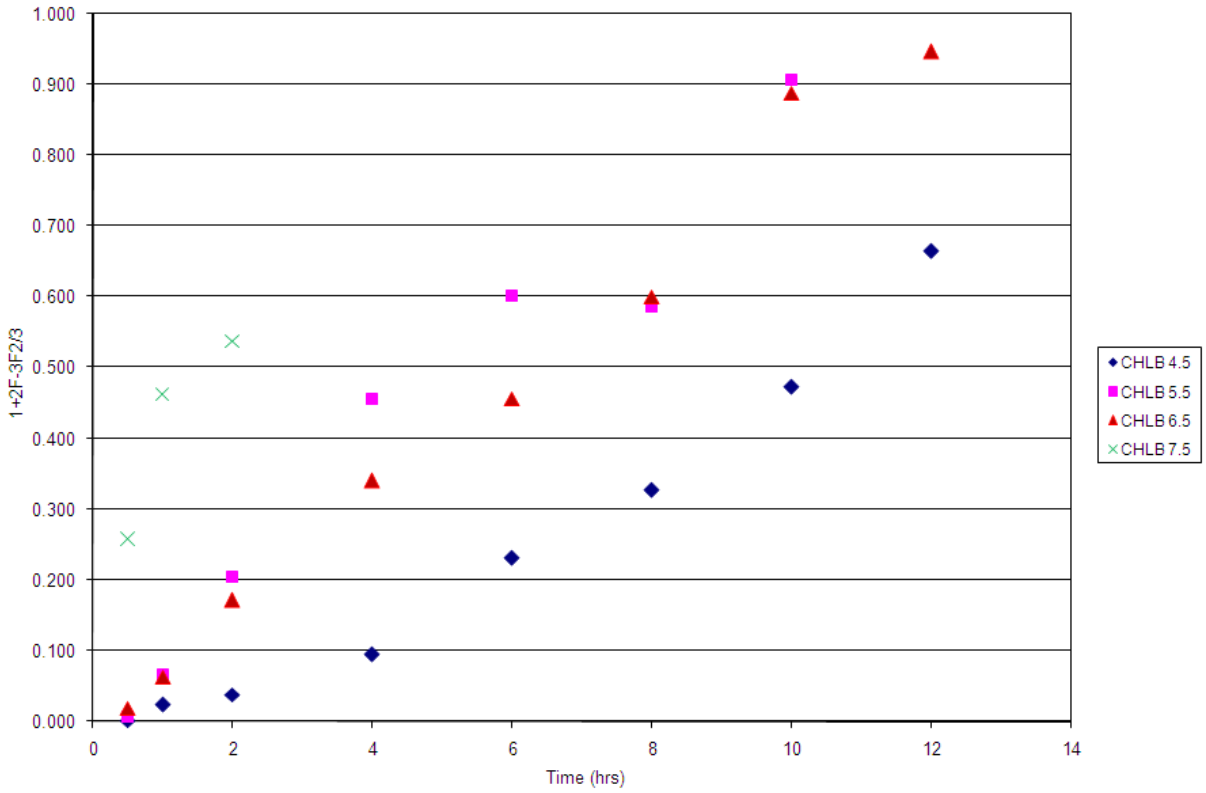

Figure 3.11. Effect of different CHLBs on the dissolution mechanism (Higuchi equation) of microsphere batches prepared with dual surfactants (Span 65+Tween 40).

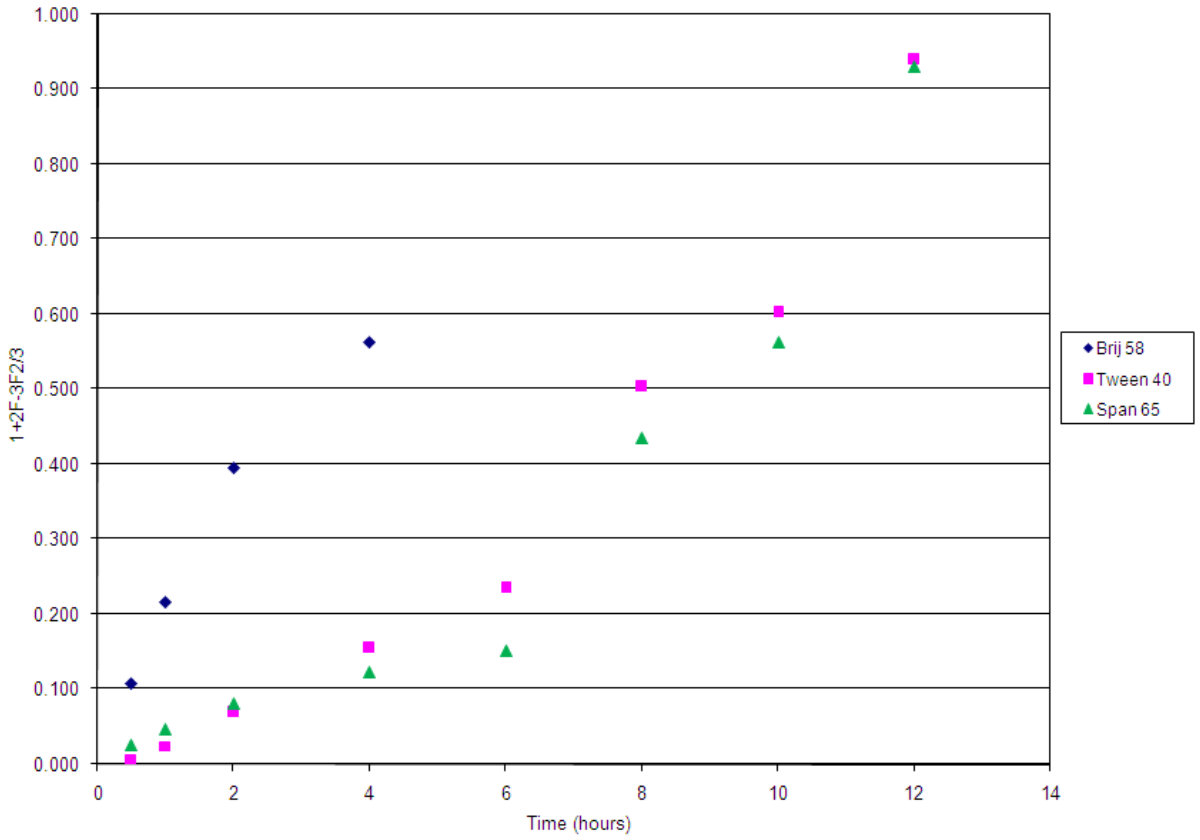

Figure 3.12. Effect of different CHLBs on the dissolution mechanism (Higuchi equation) of microsphere batches prepared with different single surfactants.

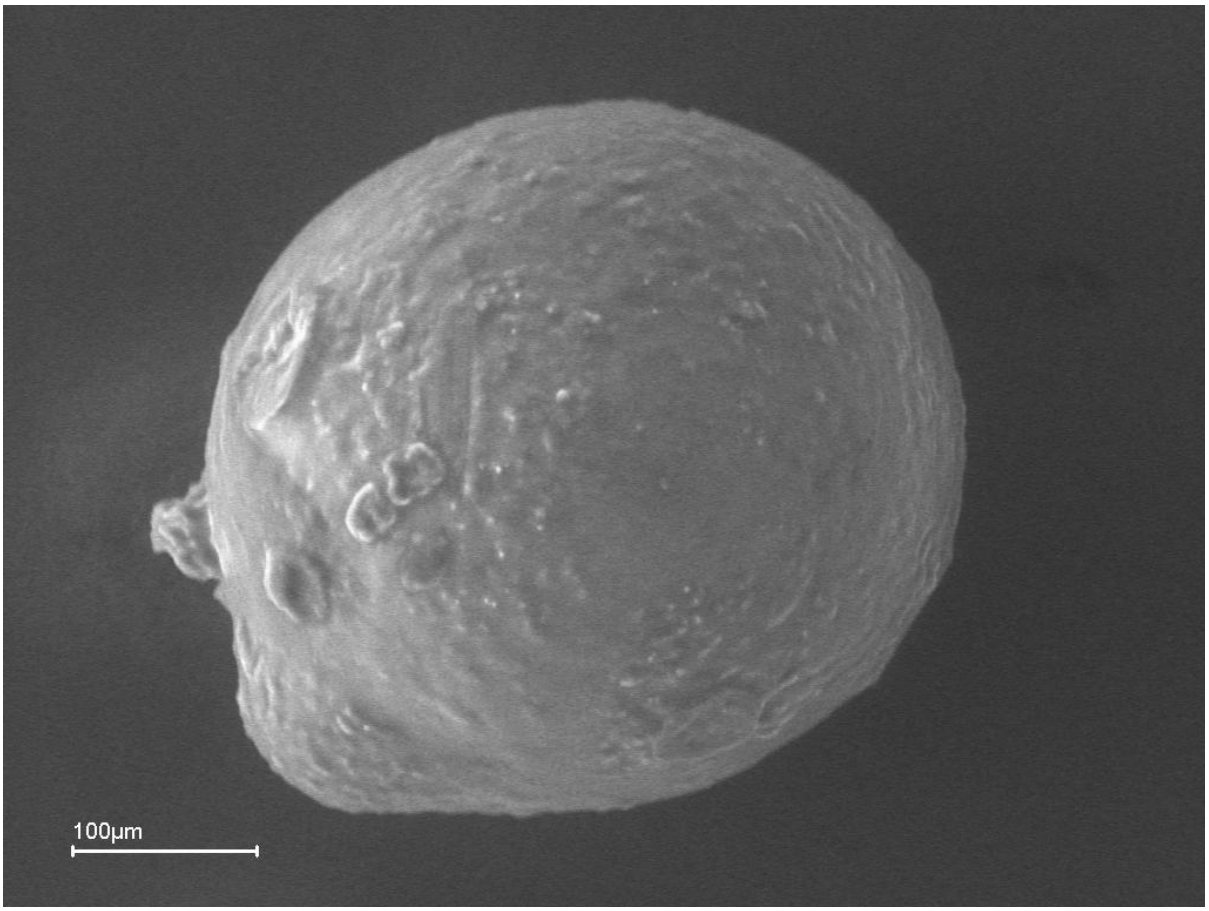

Figure 3.13. Scanning electron micrograph of microspheres prepared with single surfactant Span 65 (HLB 2.1).

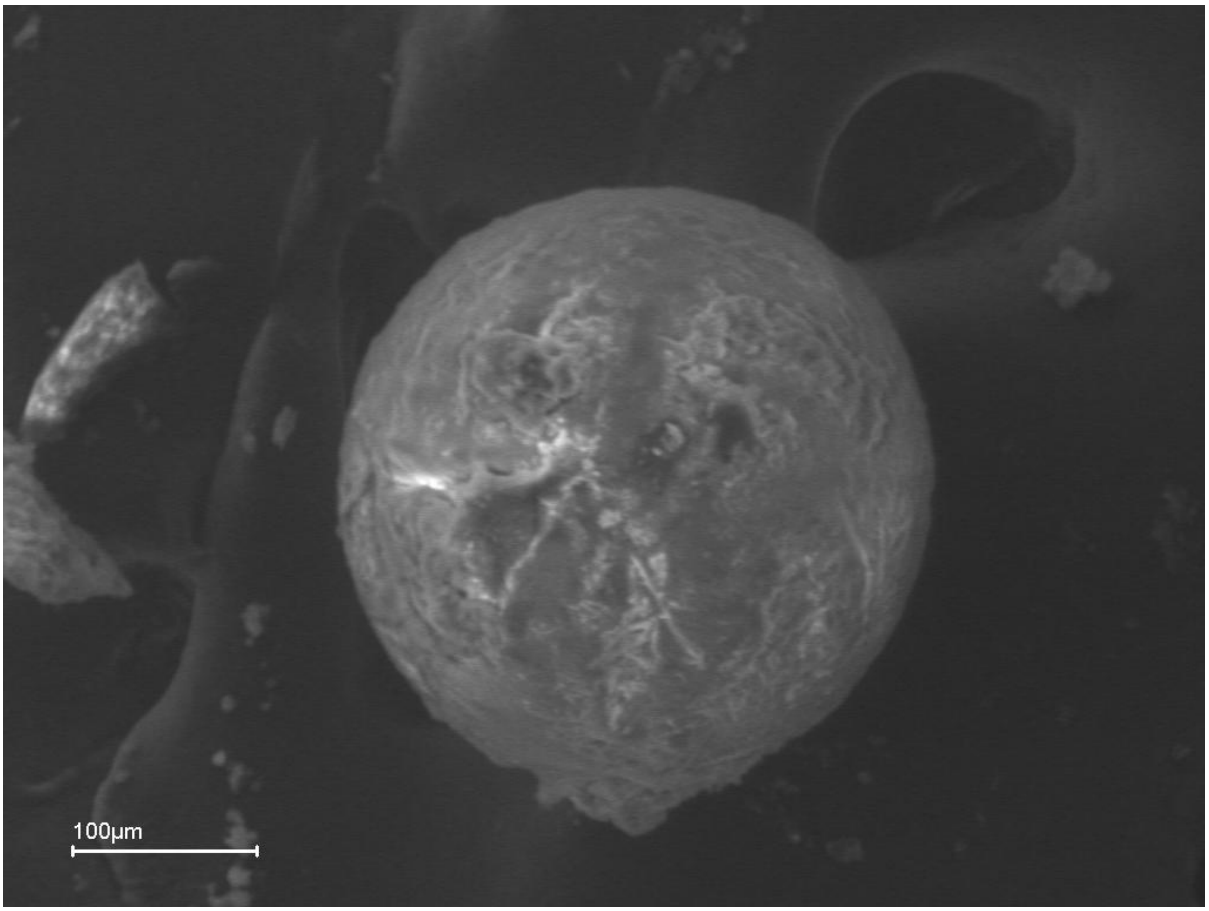

Figure 3.14. Scanning electron micrograph of microspheres prepared with single surfactant Tween 40. (HLB 15.6)

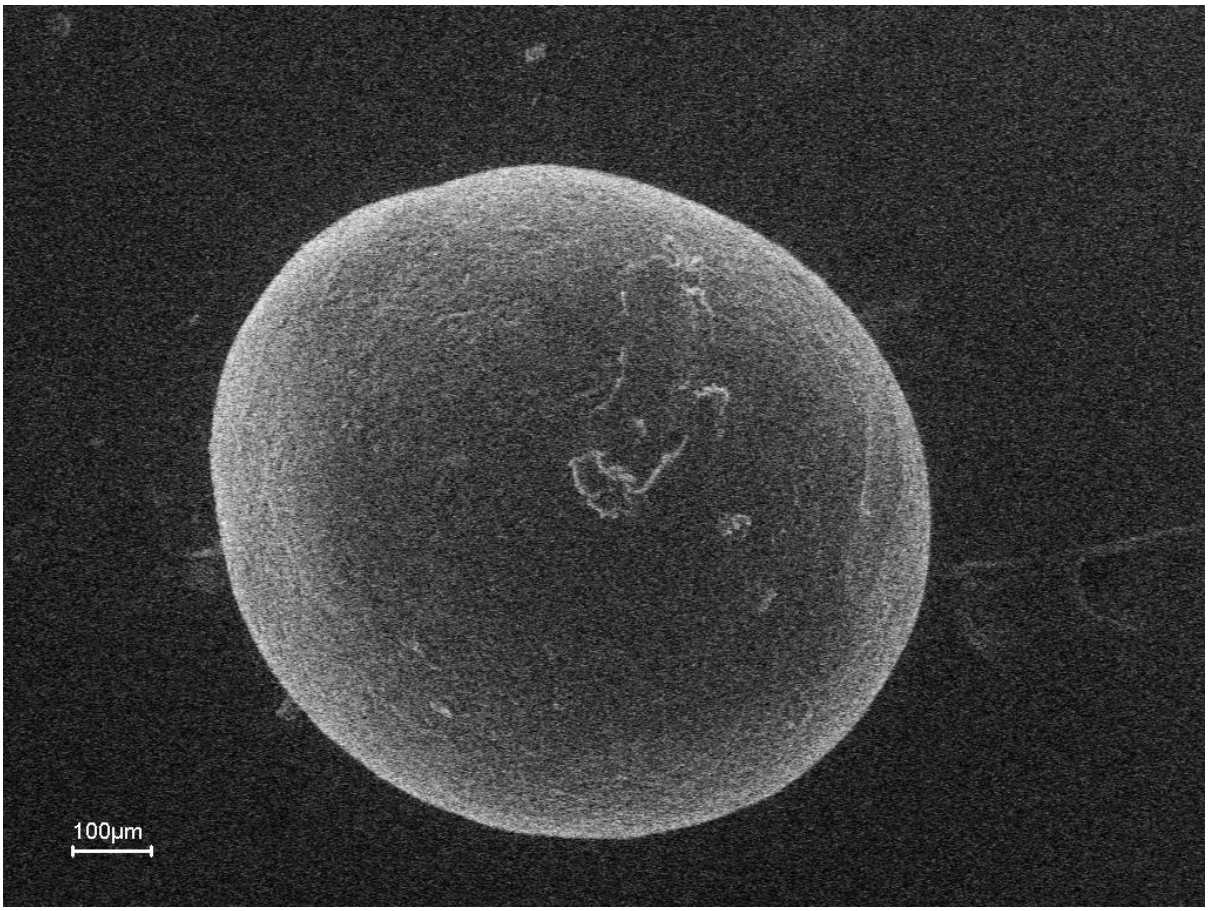

Figure 3.15. Scanning electron micrograph of microspheres prepared with single surfactant Brij 58. (HLB 15.7)

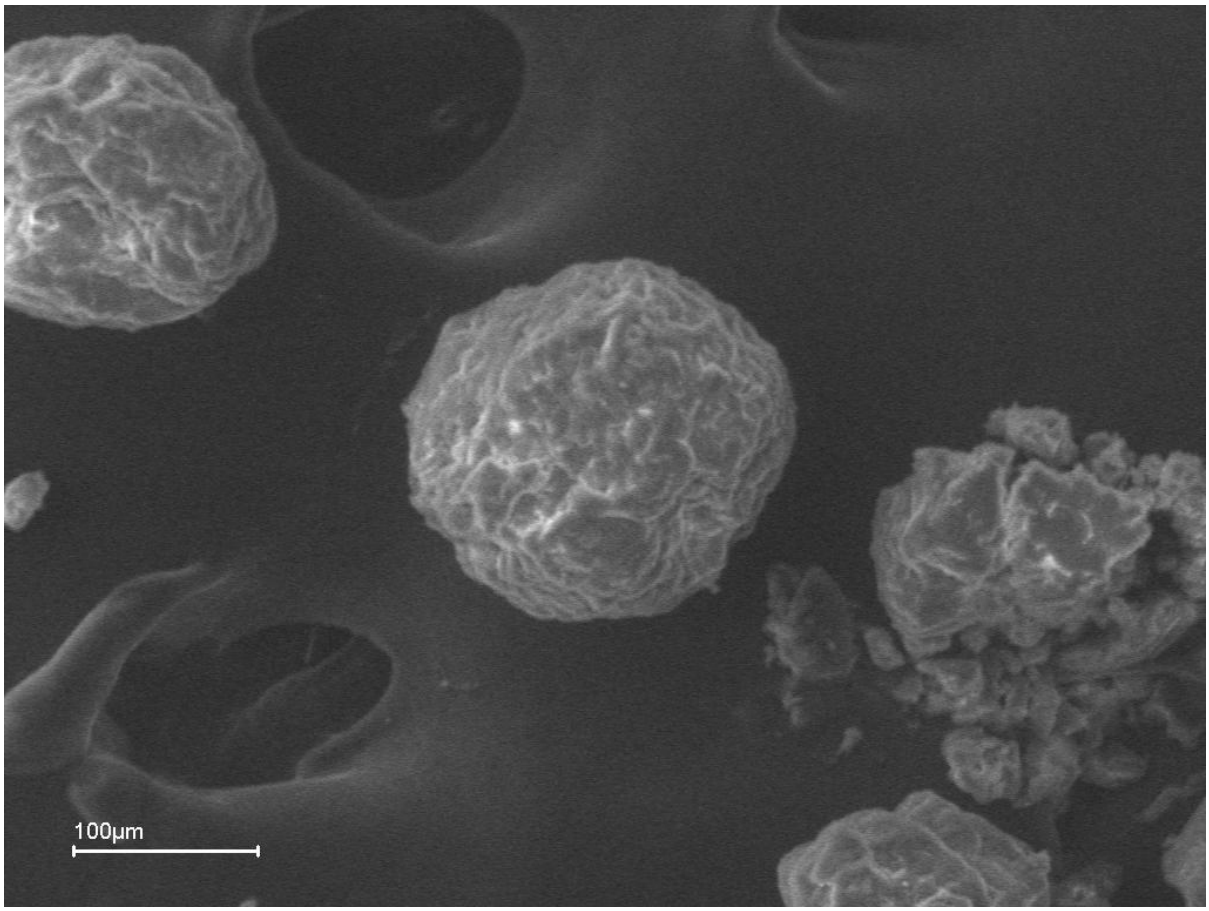

Figure 3.16. Scanning electron micrograph of microspheres prepared with dual surfactants (Span 65+Tween 40) at 4.5 CHLB.

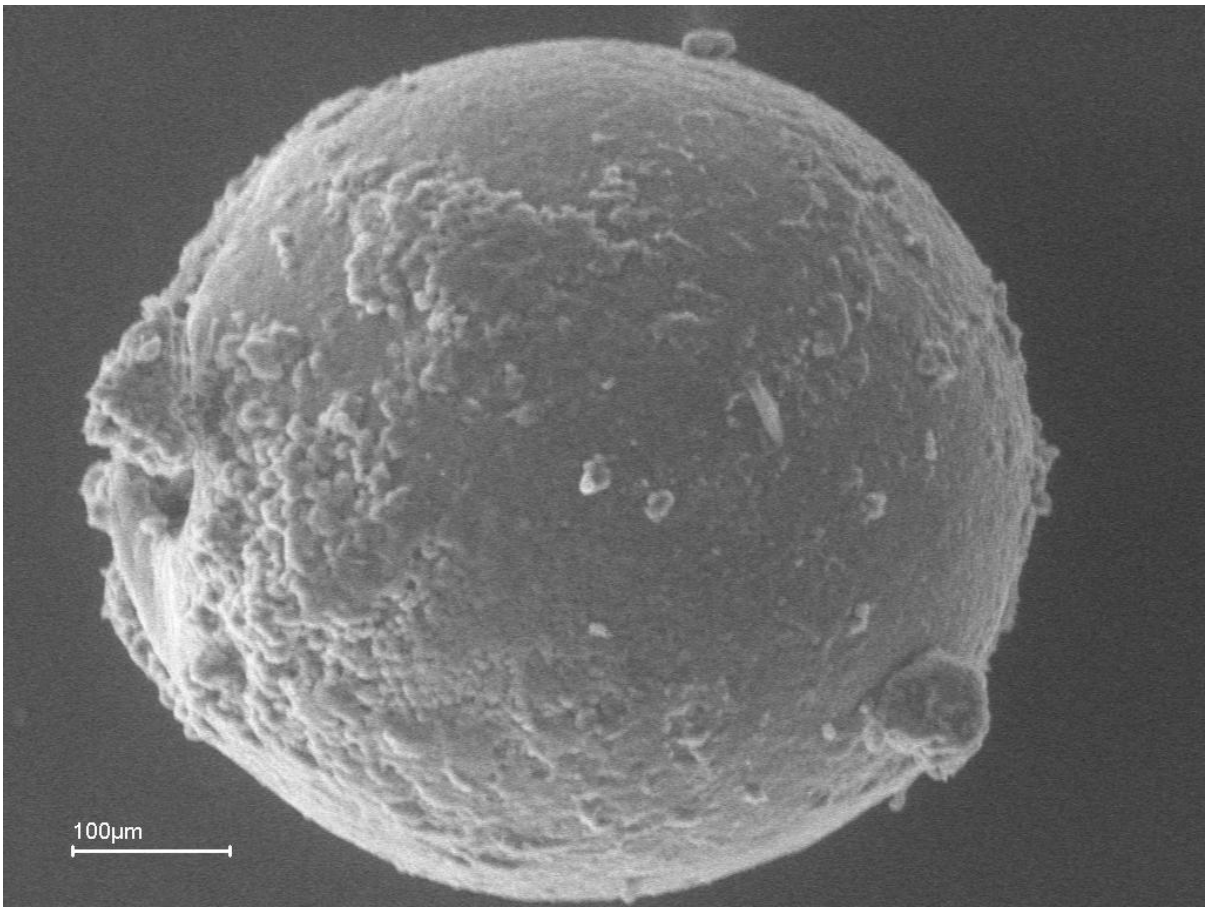

Figure 3.17. Scanning electron micrograph of microspheres prepared with dual surfactants (Span 65+Tween 40) at 5.5 CHLB.

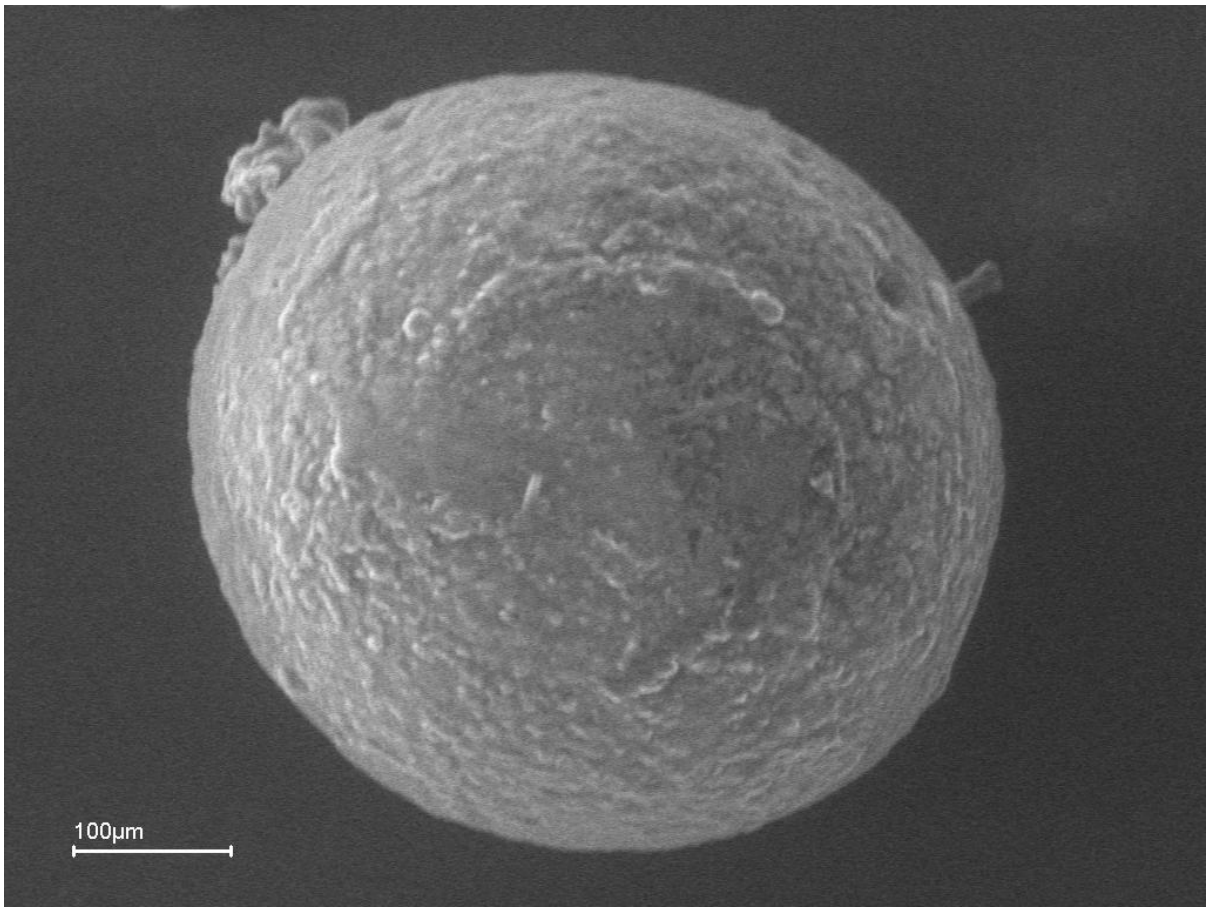

Figure 3.18. Scanning electron micrograph of microspheres prepared with dual surfactants (Span 65+Tween 40) at 6.5 CHLB.

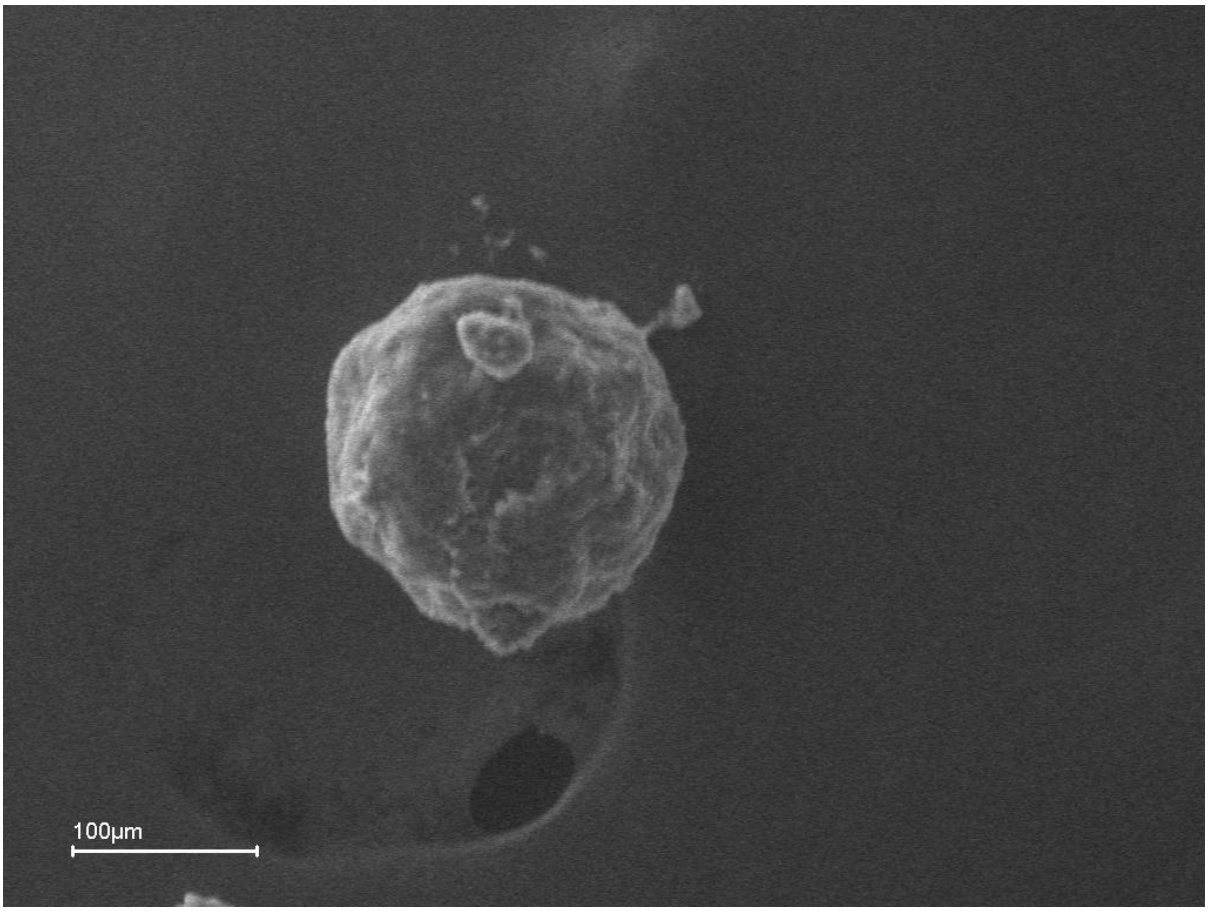

Figure 3.19. Scanning electron micrograph of microspheres prepared with dual surfactants (Span 65+Tween 40) at 7.5 CHLB.

CHAPTER 4

CHEMICAL STRUCTURE-ACTIVITY RELATIONSHIP OF NON-IONIC DUAL SURFACTANTS USED IN PREPARATION OF ETHYL CELLULOSE MICROSPHERES WITH DIFFERENT PHYSICAL AND DRUG RELEASE FORMULATION PARAMETERS.

Research Paper to be submitted to Journal of Microencapsulation

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Abstract

The objective of this study was to evaluate the chemical structure-activity relationship of polysorbate based and alkyl-ether based non-ionic dual surfactants on different formulation properties of ethyl cellulose microspheres. Microspheres containing theophylline were prepared at different combined HLBs (CHLBs) by using different combinations of dual surfactants. Surfactants with similar or closely matching HLB values but with different chemical-structure including sorbitan tristearate (STE) with HLB 2.1, polyoxyethelene sorbitan monopalmitate (POE-SM) with HLB 15.6, and polyoxyethylene cetyl ether (POE-CE) with HLB 15.7 were selected. The emulsion-solvent evaporation process with an acetone/light mineral oil system was used to produce microspheres. The microspheres produced were spherical, discrete and free-flowing. The geometric mean diameter of the microspheres decreased with the change in chemical-structure of dual surfactant combinations from POE-SM to POE-CE. The dissolution rate and initial drug release in the microsphere batches increased with an increase in CHLB. The initial drug release was higher with the dual surfactant combination STE+POE-CE compared with STE+POE-SM. Microspheres prepared with dual surfactants STE+POE-SM, were well-formed spherical particles with solid interiors. Microspheres prepared with STE+POE-CE surfactants were well-formed spherical particles with more porous structures. All batches demonstrated diffusion type of drug release. We conclude that the chemical structure of non-ionic dual surfactants could play an important role to alter physical, and drug release characteristics of ethyl cellulose microspheres to modify drug delivery properties of microspheres.

Keywords: Dual surfactants, polysorbate, alkyl ether, ethyl cellulose microspheres, drug release.

(Total words: 225)

Introduction

Polymeric microspheres are ideal vehicles for many controlled delivery applications due to their ability to encapsulate a variety of drugs, biocompatibility, high bioavailability, and rugged nature [1]. The therapeutic agents may be released from such microspheres for long periods of time, ranging from hours to months [2,3]. Drug release can be tailored and compliance can be increased by using such a system. Various formulation factors can affect microsphere characteristics such as type and molecular weight of the polymer, core drug particle size, drug to polymer ratio and drug solubility [1,4-6]. In the emulsion-solvent evaporation process, emulsifying agents stabilize the emulsion by accumulating at the polar-non-polar interface to reduce the interfacial tension and thus can affect the properties of the resultant drug dosage forms [2].

The classical useful HLB (Hydrophile-Lipophile Balance) values of non-ionic surfactants devised in 1949 by Griffin have been widely used to indicate the balance between these hydrophilic and hydrophobic parts. Selection of such surfactants for a specific application mostly depends on their hydrophilic-lipophilic balance (HLB) values. Non-ionic surfactants may have similar or close HLB values, however may have different chemical structures. For example, a non-ionic surfactant type such as Brij 58, chemically known as 'polyoxyethylene-(20)-cetyl ether' (POE-CE), has an HLB value of 15.7. Another non-ionic surfactant Tween 40, a palmitic acid ester chemically known as 'polyoxyethelene sorbitan monopalmitate' (POE-SM) has an HLB value of 15.6 It would be expected from Griffin's HLB scale that these two surfactants would have similar surfactant effects on emulsion systems because they have similar HLBs.

It could be observed from research literature that the hydrophilic–lipophilic balance (HLB) alone is not always a reliable predictor for a particular application of surfactants, since the size and shape of both the alkyl chain and the polar group influence different properties

and thus the results of their applications [7]. Even if the HLB is the same, a surfactant with a different chemical structure may result in a different interfacial tension, altered micelle formation, altered wetting ability, and dispersion of solids. When used in the emulsion-solvent evaporation process, the length and nature of the hydrophobic group, branching or unsaturation in the hydrophobic group, the nature of the hydrophilic group and its position in the molecule may affect the ethyl cellulose microsphere formation and its characteristics. Non-ionic surfactants may be classified into four major classes, polysorbate based, alkyl ether and ester based, alkyl phenols based and polyxamer based.

The widespread interest in microencapsulation of drugs brought forth the need to prepare such micro-particles in larger quantities and in sufficient desired quality suitable for further use. Our approach was to study the chemical structure-activity relationship of the dual surfactants on the properties of microspheres prepared using the emulsion solvent evaporation method. Theophylline, a model drug in this study, is reported as a sparingly water soluble drug with a narrow therapeutic index [8,9]

The present study was undertaken to study the chemical structure-activity relationship of polysorbate based and alkyl-ether based non-ionic dual surfactants to the various formulation parameters of ethyl cellulose microspheres prepared with the emulsion solvent evaporation method. The non-ionic surfactants used in this study were polyoxyethelene sorbitan monopalmitate (POE-SM) and polyoxyethylene-(20)-cetyl ether (POE-CE). POE-SM is polysorbate based and POI-CE is alkyl-ether based. POE-SM has a HLB OF 15.6 and POE-CE has a HLB of 15.7.

Experimental

Materials

The following chemicals were used: Ethyl cellulose (Scientific Polymer Products, New York), micronized theophylline (gift sample from BASF), light mineral oil (Ruger Chemical Company Inc., Irvington, NJ), Sorbitan tristearate (STE), Polyoxyethelene sorbitan monopalmitate (POE-SM), Polyoxyethylene-(20)-cetyl ether (POE-CE) (Ruger Chemical Company Inc., Irvington, NJ), methylene chloride (Fisher Scientific, NJ), acetone, monobasic potassium phosphate and sodium hydroxide (J.T. Baker, Phillipsburg, NJ).

Instruments

The following instruments were used: Stirrer (Lab Stirrer LR 400D, Yamato Scientific Company Ltd., Tokyo, Japan), Dissolution Apparatus II USP (Dissolution Test system 5100, Distek Inc., North Brunswick, NJ and Prolabo dissolution), Aquamate (UV Spectrophotometer, Thermo Electron Corporation, Mercer's Row, Cambridge, UK), Accumet 5 pH meter (Fisher Scientific, NJ) and, USP Standard sieve series for PSD studies.

Preparation of microspheres

The preparation of ethyl cellulose microspheres containing theophylline was accomplished by the emulsification-solvent evaporation method in a 1 L, tall glass beaker. For preparation of all batches, experimental conditions were identical. Light mineral oil (300 ml) containing the low HLB surfactant such as sorbitan tristearate (STE) was used as the external or continuous phase (phase A). In a separate glass vessel, a 5 % solution of ethyl cellulose in acetone and the high HLB surfactant, polyoxyethelene sorbitan monopalmitate (POE-SM), was prepared (phase B). Micronized anhydrous theophylline was dispersed in this solution to give a 33% theoretical drug loading (1 part theophylline to 2 parts ethyl cellulose by weight). Subsequently, the entire contents of this vessel (phase B) were added into the glass beaker containing the solution of light mineral oil and low HLB surfactant (phase A)

under vigorous agitation. Agitation was continued until the acetone evaporated and the microspheres were firm. The microspheres were allowed to settle to the bottom of the container and most of the mineral oil was decanted. Microspheres were separated from the remaining oil by filtering and washing with mineral spirits to remove the residual light mineral oil. The clean microspheres were then dried in an oven at 50^0C overnight. Dual surfactants used in this process are listed in Table 4.1 with their respective HLBs. Figures 4.1 and 4.2 show the chemical structures of these surfactants.

Particle size distribution

The size distribution of microspheres was determined by sieving through a set of standard sieves ranges from 90-710μm. A pan was placed underneath the sieves to collect the particles that were small enough to pass through the last sieve. To perform sieving, the aggregate sample was placed on the sieve of largest size, covered, and then tapped by hand until no change in weight was observed in the sieves. After sieving, the quantities of each fraction of particles were measured by weighing. Particle size distribution and geometric mean diameter were calculated using methods in literature.

Determination of Drug loading

The 355 μm fraction of each batch of ethyl cellulose microspheres was used for all studies including drug loading. Accurately weighed samples of microspheres (in triplicate) were placed in 25 ml volumetric flasks and dissolved in methylene chloride. Drug concentration in the methylene chloride solution was then determined spectrophotometrically 276.5 nm. Spectrophotometric interference was not observed at this wavelength with ethyl cellulose microspheres that did not contain drug.

SEM analysis of microspheres

The surface morphology of microspheres was examind by scanning electron microscope (SEM) using the Zeiss model 1450EP SEM. Microspheres were mounted onto

metal multi-stubs using double-sided adhesive tape and SEM images were taken at the magnification shown on the photos.

In vitro dissolution studies

In vitro release studies of ethyl cellulose microspheres were performed using USP dissolution apparatus II (Distek Inc., New Jersey) at 100 r.p.m. using the paddle method as described in USP 31. The 355 μm fraction of each batch of microspheres was selected for evaluation. Microsphere samples in triplicate for each batch were suspended in 900 ml of simulated intestinal fluid with 0.1% Tween 20, and no enzymes. The dissolution study was carried out at 37±0.5°C at 100 r.p.m for 12-24 hours. Three ml of sample was withdrawn at specific time intervals and replaced with fresh simulated intestinal fluid medium. The drug released was determined spectrophotometrically at 274 nm. The dissolution data was evaluated for initial release, dissolution rate and the mechanism of drug release.

Release kinetics

Data obtained from *in vitro* release studies were fitted to Higuchi kinetic equations to help determine the mechanism of drug release from ethyl cellulose microspheres.

Results and discussion

Particle size distribution (PSD) and geometric mean diameter (GMD)

The microspheres prepared with dual surfactant combination of sorbitan tristearate (STE) with polyoxyethelene-(20)-sorbitan monopalmitate (POE-SM) showed variations in the GMD at different CHLBs, as shown in Figure 4.3. Combination of STE+POE-SM dual surfactants, showed decreased in the GMD with an increase in CHLB. The GMD was highest at 4.5 CHLB and lowest at 7.5 CHLB (Figure 4.3). The microspheres prepared with dual surfactant combination of sorbitan tristearate (STE) and polyoxyethylene-(20)-cetyl ether (POE-CE) showed variations in GMD as well (Figure 4.3).

STE+POE-CE dual surfactants, showed decreased GMD with an increase in CHLB l from 4.5 to 6.5 CHLB. No difference was noted with the microspheres prepared with 6.5 and 7.5 CHLB. The GMD was highest at 4.5 CHLB and lowest at 6.5 CHLB (Figure 4.3). Microspheres prepared with dual surfactant combinations of STE+POE-CE showed lower GMD compared to the microspheres prepared with dual surfactants combination of STE+ POE-SM. Dual surfactants STE+POE-CE would have produced a strong interfacial film while emulsification process that resulted in more separated microspheres and a lower GMD. The STE+POE-CE would have caused more monodisperse emulsion that resulted in more separated microspheres. Strong short-range repulsion or disjoining pressure caused by POE-CE when combined with STE might have prevented droplet coalescence and more separated microspheres are resulted. We found that the chemical structure of surfactant thus affects the GMD regardless of their similar HLB value. It appears that incorporation of dual surfactants with different chemical structures (POE-CE and POE-SM) decreased the particle size of microspheres at different CHLB levels.

As shown in Figures 4.4 and 4.5, the chemical structure of surfactants affects the particle size distribution of microspheres when used in dual surfactant combinations. Microspheres developed using dual surfactants (STE+POE-SM) showed the particle size distribution range mostly in 355 µm fraction for all the CHLBs (Figure 4.4). We also observed that the percentage of particles in the 710 µm and 500 µm fractions is decreased with increase in the CHLB level of dual surfactants (STE+POE-SM). The particles in 355 µm fractions were increased with an increase in the CHLB from 4.5 to 5.5 to 6.5. The particles with 7.5 CHLB were found with the highest percentage in the 125µm fraction compared to other CHLBs. These changes may be attributed to the stabilization of emulsion that occurs with an increase in CHLB, and thus coalescence is retarded, leading to a smaller microsphere particle size especially in cases of using two surfactants. Microspheres prepared using dual

surfactants (STE+ POE-CE) showed a variable range in particle size distribution for all the CHLBs (Figure 4.5). The percentage of particles in 250 µm and 125 µm fractions is decreased with increase in the CHLB level of dual surfactants STE+POE-CE (Figure 4.5).

Overall, lower microsphere particle size (125 to 355 µm) was seen in batches prepared with STE+POE-CE, compared to the microspheres prepared with STE+POE-SM (250 to 710 µm). These changes were attributed to the solubilization and stabilization of emulsion by the change in the chemical structure from polyoxyethelene sorbitan monopalmitate to polyoxyethylene (20) cetyl ether, leading to smaller microsphere particle size.

Scanning electron microscopy (SEM) analysis

The SEM micrographs of microspheres prepared from dual surfactants STE+POE-SM are shown in Figures 4.14 to 4.17. The microspheres prepared from dual surfactants STE+POE-CE are shown in Figures 4.18 to 4.21. Microspheres with dual surfactants STE+POE-SM were spherical with a less porous structure (Figures 4.14 to 4.17). Dual surfactants STE+POE-SM at CHLB 4.5, 5.5 and 6.5 strongly affected microsphere morphology and resulted in dense surface (Figures 4.14 and 4.16). This was attributed to the stabilization of the emulsion and thus formation of more uniform and dense droplets dispersion in oil phase. After solidification such dense droplets lead to a more dense surface structure. Microspheres with dual surfactants STE+POE-CE were spherical with a more porous structure (Figure 4.18 to 4.21). This porous nature was increased with an increase in CHLB from 4.5 to 7.5.

We found that by using dual surfactants with certain chemical structure such as polyoxyethylene (20) cetyl ether, at certain CHLB, a denser and porous matrix structure could be formed compared with using chemical structure polyoxyethelene sorbitan monopalmitate regardless of their HLB values.

Drug loading

As seen in Table 4.2, the drug loading studies of the 355 μm fractions of the microsphere batches prepared with STE+POE-SM revealed that there was no significant difference between the drug loadings with the change in most CHLBs. At 6.5 CHLB the drug loading was higher than all other CHLBs. Microspheres prepared with STE+POE-CE dual surfactant combinations showed a reduction in drug loading with an increase in CHLB from 4.5 to 7.5. These batches were prepared with 33% theoretical drug loading. None of the microsphere batches has drug loading more than the theoretical drug loading. This indicates that there is less air trapped in the microsphere during the preparation. The drug loading percent was higher in microspheres prepared with STE+POE-CE dual surfactants compared with STE+POE-SM dual surfactants. This indicates that the chemical structure of surfactants play an important role in drug loading irrespective of their HLB value.

In vitro drug release behavior

The *in vitro* release profiles of ethyl cellulose microspheres prepared by using dual surfactants are shown in Figures 4.6 and 4.7. The microspheres produced with dual surfactants STE+POE-SM showed differences in drug release profiles at different CHLBs (Figure 4.6). Theophylline release was effectively increased with an increase in CHLB of the dual surfactant combination STE+POE-SM. These microspheres exhibited adequate controlled-release profiles in all CHLB formulations.

The microspheres produced with dual surfactants STE+POE-CE showed differences in release profiles at different CHLBs as well (Figure 4.7). Theophylline release was effectively increased with an increase in CHLB of the dual surfactant combination STE+POE-CE. These microspheres exhibited adequate controlled-release profiles in all CHLB formulations. Studies on the effects of dual surfactants STE+POE-SM and STE+POE-CE effect on the t_{50} release (Figure 4.10 and 4.11) showed that the t_{50} drug release was

effectively decreased with an increase in CHLB. The t_{50} drug release is the time required by 50% of the drug to be released from the dosage form.

The drug release mechanism is influenced by many factors such as the presence and the location of drug and surfactant molecules in the microsphere particle. The possible sites of location of the surfactant molecules could be either on the surface of the microsphere, within the matrix isolated from the interior environment or within the microsphere but connected with its outer surface possibly by means of channels. In the dissolution process, the hydrophilic surfactant dissolves in the aqueous dissolution medium and thus facilitates medium entry into the system. In microspheres the amount of hydrophilic surfactant is greater with an increase in CHLB formulations that caused increased dissolution rate as seen in Figures 4.6 and 4.7. Surfactant also worked as a solubilizer that resulted in increased drug dissolution. This effect has increased with dual surfactants system. We observed comparatively slower dissolution rate with the microspheres prepared with the dual surfactants STE+POE-SM. This could be because the chemical structures of surfactant played a role in slowing the theophylline release. Dual surfactants combination STE+POE-SM may not be as effective as dual combination of STE+POE-CE in solubilizing the drug.

Initial drug release

Figure 4.8 shows the initial drug release of dual surfactants STE+POE-SM at different CHLBs. The initial drug release percentage within the first 30 minutes was analyzed for the microspheres fraction of 355 μm. At 4.5 CHLB, the initial drug release was lowest amongst all CHLBs. The highest initial drug release was for 7.5 CHLB amongst all CHLBs. In microsphere formulation, the hydrophilic surfactant concentration increases with an increase in CHLB. There seems to be increased solubilizing effect because of increased in CHLB that affect the initial drug release.

Figure 4.9 explains the initial drug release of dual surfactants STE+POE-CE at different CHLBs. The initial drug release percentage within the first 30 minutes was analyzed for the microspheres fraction of 355 μm. An increase in the initial drug release percent with an increase in CHLB value was observed. At 4.5 CHLB, initial drug release was lowest amongst all CHLBs. The highest initial drug release was at 7.5 CHLB amongst all CHLBs. In general an increased hydrophilic surfactant concentration in microspheres formulation, when CHLB was increased has affected the initial drug release from microspheres.

It appears that microspheres prepared with dual surfactant combination STE+POE-CE has greater initial drug release percentage compared to microsphere prepared with STE+POE-SM. This is due to the change in the chemical structure of the dual surfactants. There is an increased solubilizing effect because of the change in the chemical structure when the POE-CE surfactant is used. As discussed earlier, the SEM analysis showed more porous microsphere prepared with STE+POE-CE dual surfactant combination (Figures 4.18 to 4.21). Additionally, this porous structure may have contributed to an increased initial drug release percentage from the microspheres prepared with the STE+POE-CE dual surfactant combination.

Drug release kinetics

Figures 4.12 and 4.13 depict the drug release kinetics. Usually, the release from the matrix of the microsphere is either by erosion or by diffusion [3,10,11]. Drug release occurs in three stages i-e an initial drug release by diffusion from the superficial region of microsphere, followed by slower release by a polymer hydrolysis and then finally a rapid release resulting from polymer erosion. Current study is not dependent on the chemical or physical erosion of a polymer for the release. The release is by diffusion through the pores. Higuchi equation analysis for spherical matrices for the microspheres prepared with dual surfactants STE+POE-SM indicated that the drug release from the microspheres was primarily by

diffusion (Table 4.3). The microspheres prepared with dual surfactants STE+POE-CE also showed a diffusion process of drug release.

Conclusion

Chemical structure of surfactant play an important role on physical characteristics and drug release behavior of microspheres prepared by emulsion solvent evaporation method. We noted a decrease in the geometric mean diameter of the microsphere batches with an increase in CHLB for both of the dual surfactant combinations STE+POE-SM and STE+POE-CE. The range of microsphere size was smaller (125 to 355 μm) when prepared with STE+POE-CE surfactant combinations compared with STE+POE-SM combination (250 to 710 μm). There was a high initial drug release in the microspheres prepared with the dual surfactants STE+POE-CE compared to STE+POE-SM. We noted an increase in the initial drug release with an increase in CHLB for both the surfactant combinations STE+POE-SM and STE+POE-CE. The *in vitro* drug release was affected by dual surfactants. The release was faster in the dual surfactant prepared by STE+POE-CE dual surfactant combinations. The release kinetics showed a diffusion type of release from dual surfactant microspheres. Thus, we conclude that the chemical structure of surfactants affects different formulation parameters.

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Table 4.1. Combinations of low HLB sorbitan tristearate (STE) with two high HLB surfactants of different chemical structures, polyoxyethelene sorbitan monopalmitate (POE-SM) and polyoxyethylene cetyl ether (POE-CE) to obtain various combined HLBs.

Surfactants used with STE (HLB 2.1)	**CHLB**	**Effective ratio**
POE-SM (HLB=15.6)	4.5	82:18
POE-SM (HLB=15.6)	5.5	77:23
POE-SM (HLB=15.6)	6.5	67:33
POE-SM (HLB=15.6)	7.5	60:40
POE-CE (HLB=15.7)	4.5	82:18
POE-CE (HLB=15.7)	5.5	77:23
POE-CE (HLB=15.7)	6.5	67:33
POE-CE (HLB=15.7)	7.5	60:40

Table 4.2. Effect of different CHLBs and high HLB surfactant chemical type on drug loading of 355 µm diameter microspheres.

.

Surfactants used	**CHLB**	**Drug loading %**
POE-SM (HLB=15.6)	5.5	21.90 ± 0.42
POE-SM (HLB=15.6)	6.5	25.47 ± 1.83
POE-SM (HLB=15.6)	4.5	21.62 ± 1.64
POE-SM (HLB=15.6)	7.5	22.52 ± 0.46
POE-CE (HLB=15.7)	4.5	28.94 ± 1.97
POE-CE (HLB=15.7)	5.5	24.49 ± 1.39
POE-CE (HLB=15.7)	6.5	24.15 ± 0.73
POE-CE (HLB=15.7)	7.5	22.18 ± 0.10

Table 4.3. Higuchi spherical matrix equation regression analysis of drug release from microsphere batches prepared using sorbitan tristearate (HLB 2.1) with polyoxyethelene sorbitan monopalmitate (POE-SM) or polyoxyethylene cetyl ether (POE-CE), surfactants with different chemical structures.

Surfactants used with sorbitan tristearate (HLB=2.1)	**CHLB**	**Higuchi spherical matrix equation regression R2**
POE-SM (HLB=15.6)	4.5	0.9636
POE-SM (HLB=15.6)	5.5	0.9860
POE-SM (HLB=15.6)	6.5	0.9881
POE-SM (HLB=15.6)	7.5	0.7669
POE-CE (HLB=15.7)	4.5	0.9880
POE-CE (HLB=15.7)	5.5	0.9533
POE-CE (HLB=15.7)	6.5	0.9650
POE-CE (HLB=15.7)	7.5	0.8257

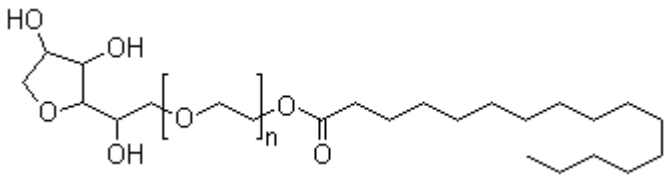

Figure 4.1. Chemical structure of non-ionic surfactant polyoxyethylene sorbitan monopalmitate (POE-SM) [12].

Figure 4.2. Chemical structure of non-ionic surfactant polyoxyethylene-(20)-cetyl ether (POE-CE) [12].

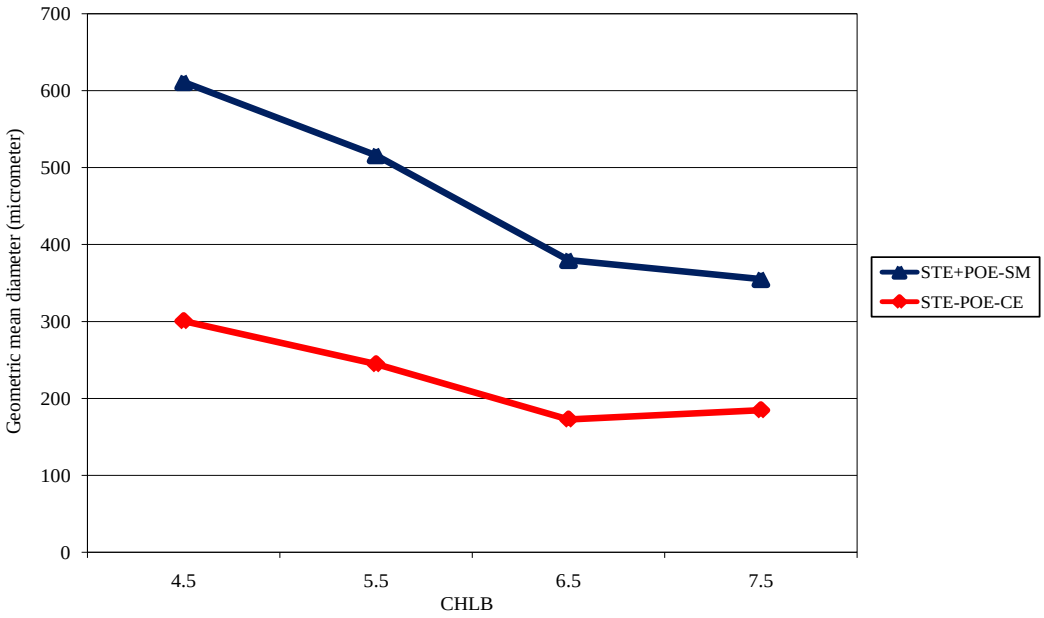

Figure 4.3. Comparison of the effect of chemical structure and CHLB on geometric mean diameter of microspheres prepared with dual surfactants (Sorbitan tristearate and polyoxyethelene sorbitan monopalmitate (STE+POE-SM), and sorbitan tristearate and polyoxyethylene-(20)-cetyl ether (STE+POE-SM).

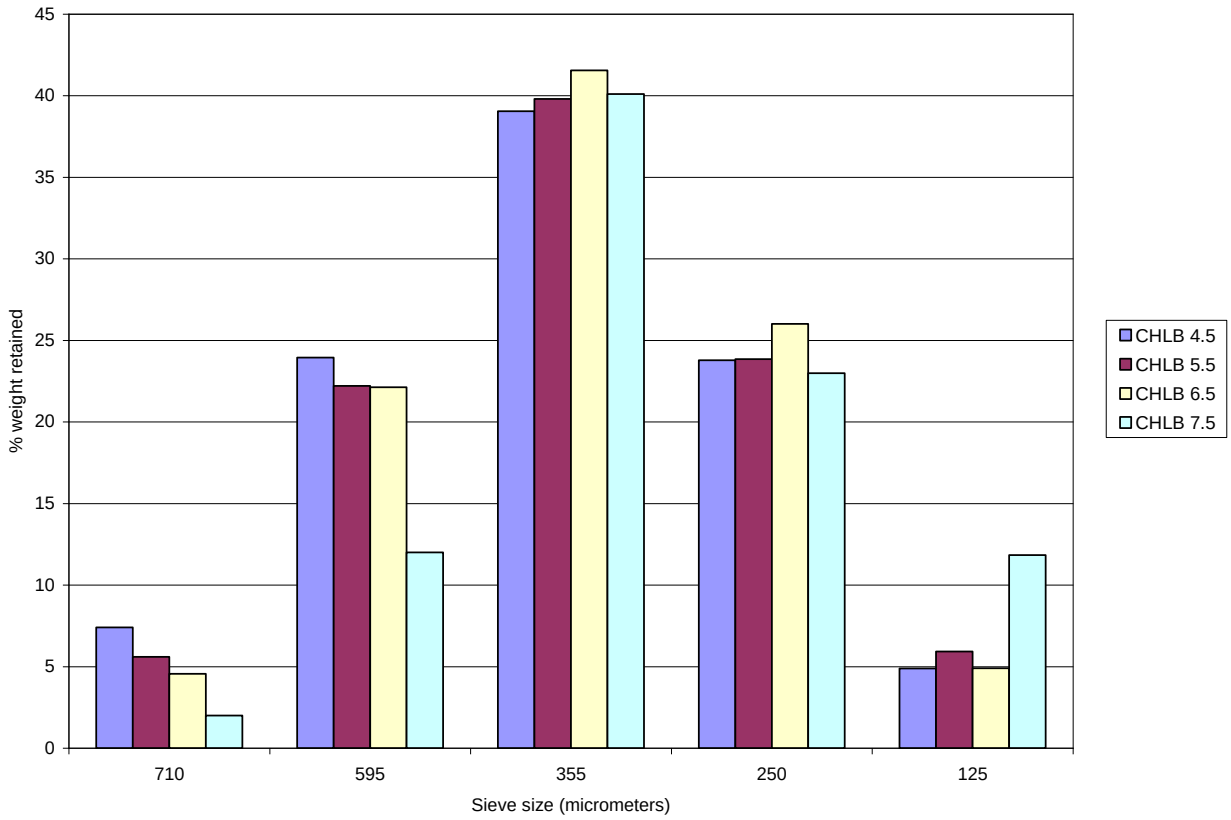

Figure 4.4. Effect of CHLB on particle size distribution of microsphere batches prepared with dual surfactants sorbitan tristearate and polyoxyethelene sorbitan monopalmitate (STE+POE-SM).

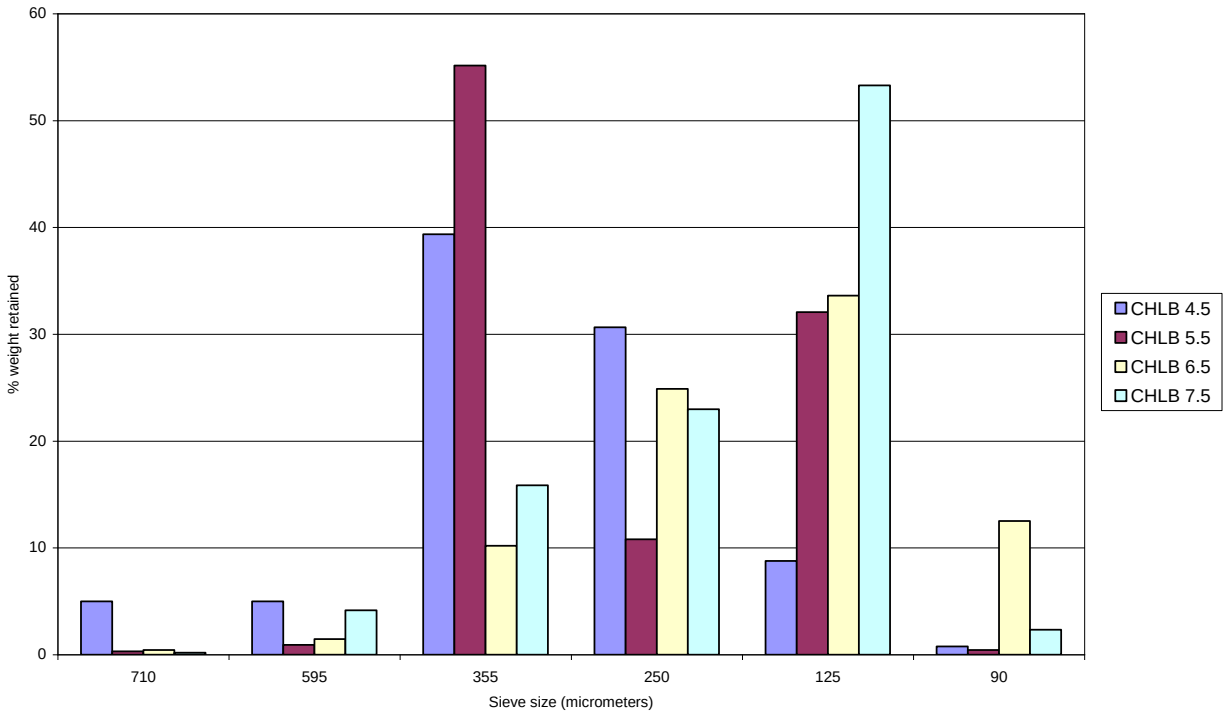

Figure 4.5. Effect of CHLB on particle size distribution of microsphere batches prepared with dual surfactants sorbitan tristearate and polyoxyethylene cetyl ether (STE+POE-CE).

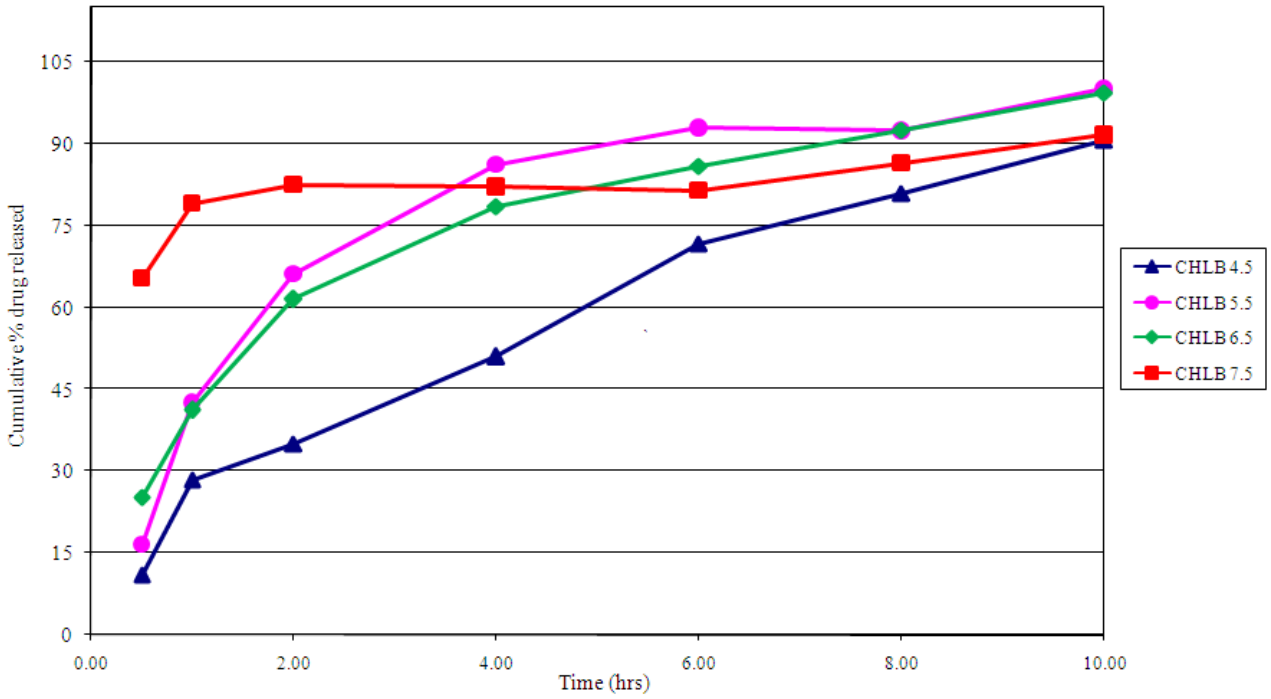

Figure 4.6. Effect of CHLB on in vitro dissolution studies of microsphere batches prepared with dual surfactants sorbitan tristearate and polyoxyethelene sorbitan monopalmitate (STE+POE-SM).

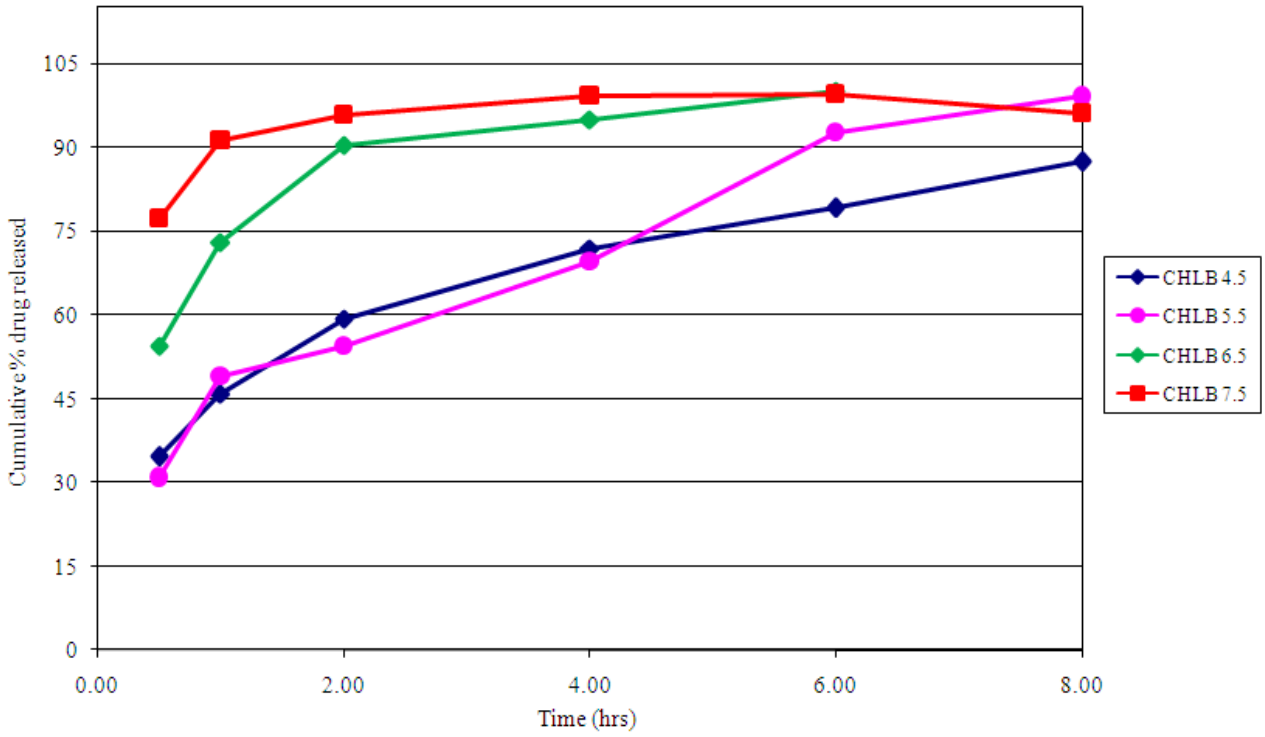

Figure 4.7. Effect of CHLB on in vitro dissolution studies of microsphere batches prepared with dual surfactants sorbitan tristearate and polyoxyethylene cetyl ether (STE+POE-CE).

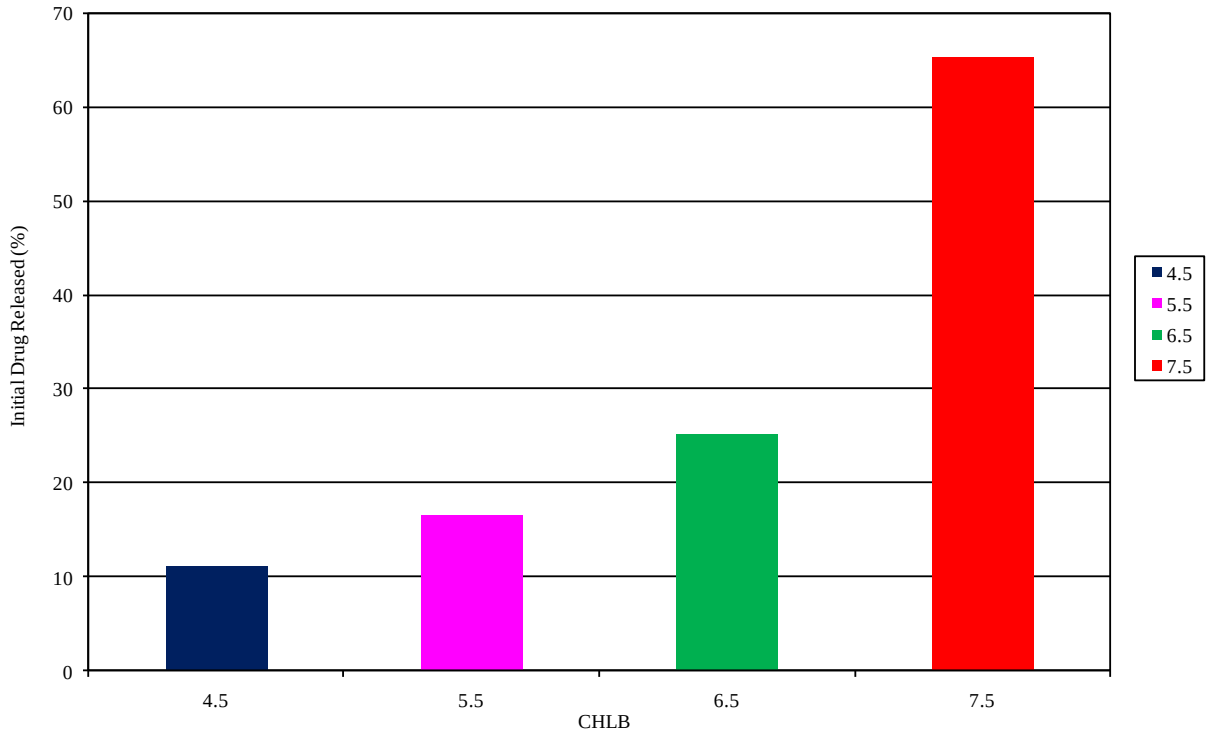

Figure 4.8. Effect of CHLB on change initial drug release of microsphere batches prepared with dual surfactants sorbitan tristearate and polyoxyethelene sorbitan monopalmitate (STE+POE-SM).

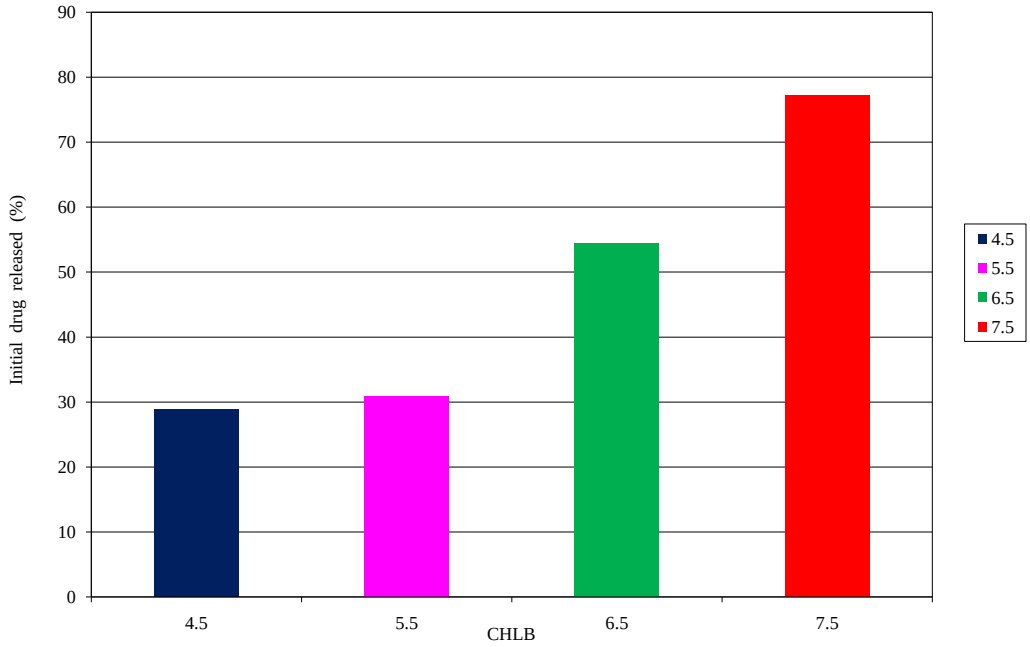

Figure 4.9. Effect of CHLB on initial drug release of microsphere batches prepared with dual surfactants sorbitan tristearate and polyoxyethylene cetyl ether (STE+POE-CE).

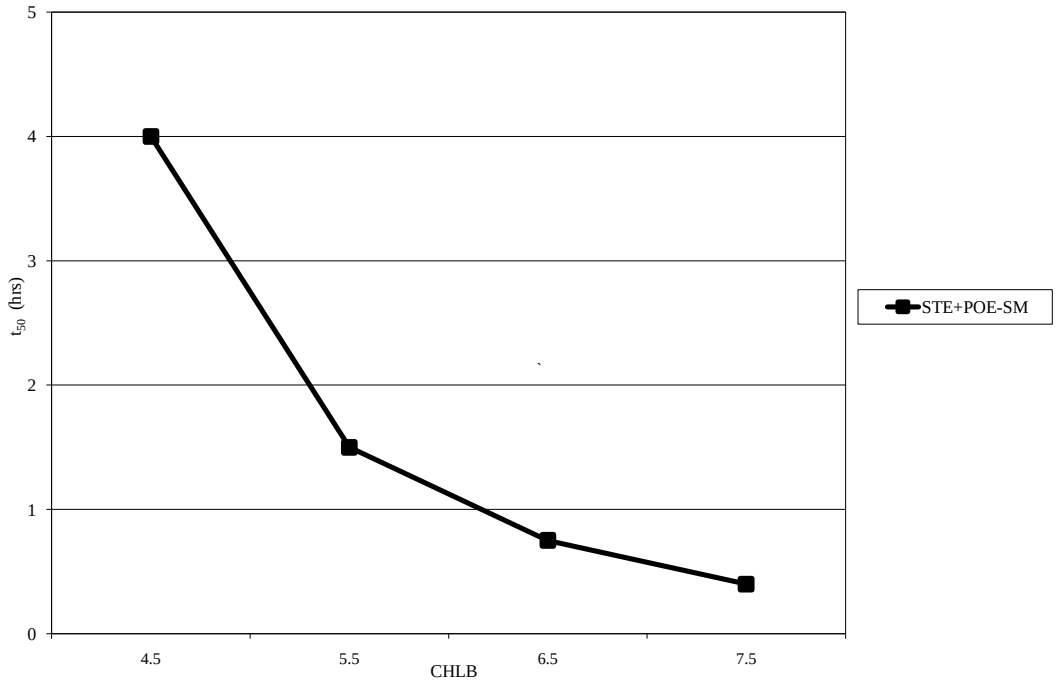

Figure 4.10. Effect of CHLB on dissolution t_{50} of microsphere batches prepared with dual surfactants sorbitan tristearate and polyoxyethelene sorbitan monopalmitate (STE+POE-SM) at t_{50}.

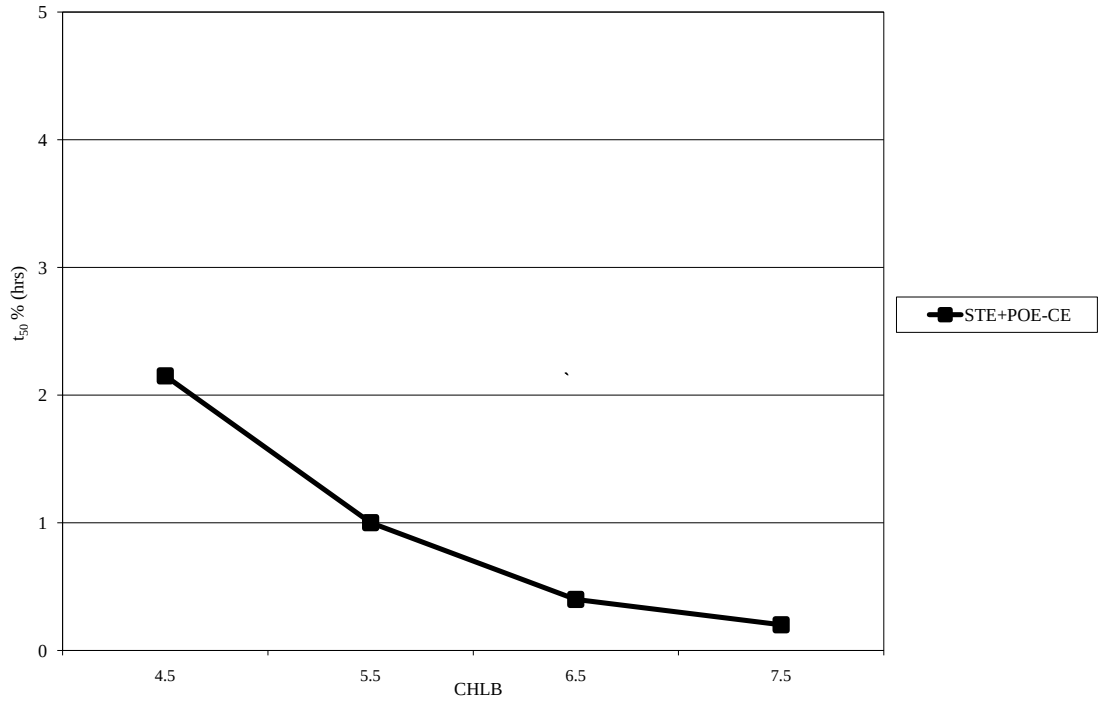

Figure 4.11. Effect of CHLB on dissolution t_{50} of microsphere batches prepared with dual surfactants sorbitan tristearate and polyoxyethylene cetyl ether (STE+POE-CE).

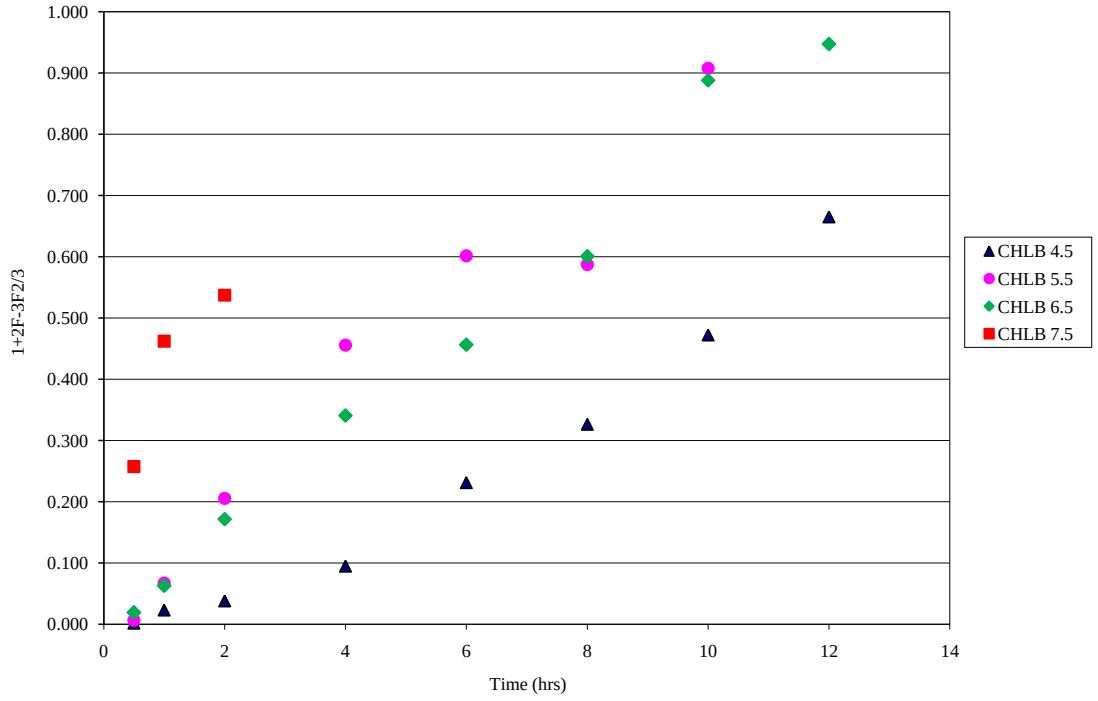

Figure 4.12. Effect of CHLB on dissolution mechanism (Higuchi spherical matrix equation) of microsphere batches prepared with dual surfactants sorbitantristearate and polyoxyethelene sorbitan monopalmitate (STE+POE-SM).

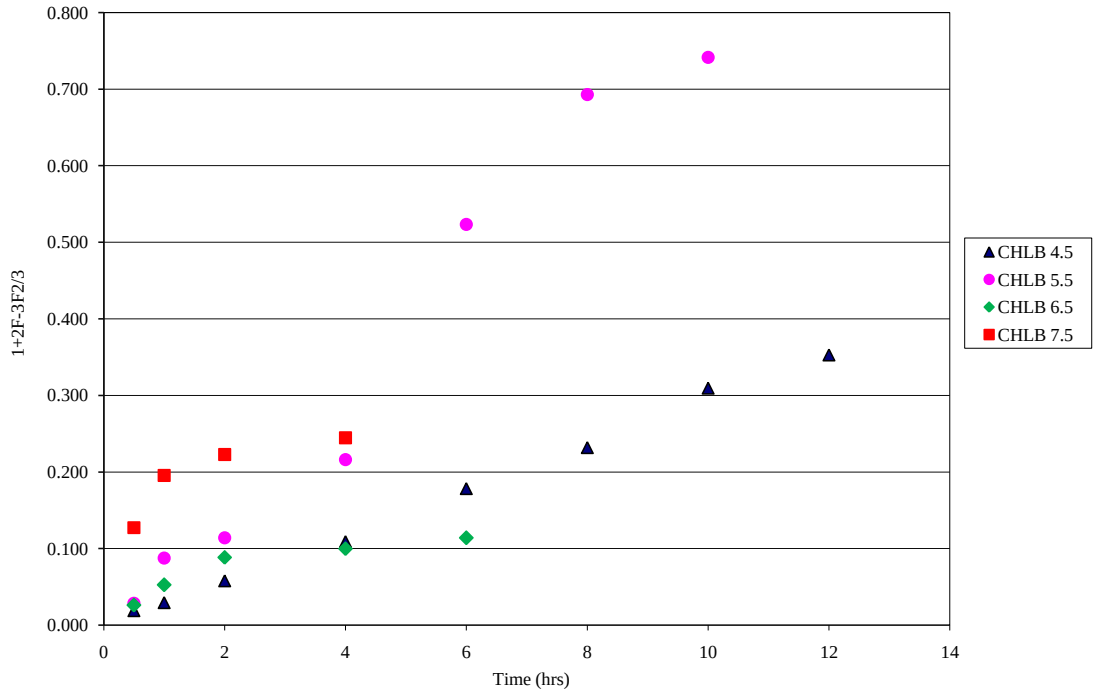

Figure 4.13. Effect of CHLB on dissolution mechanism (Higuchi spherical matrix equation) of microsphere batches prepared with dual surfactants sorbitan tristearate and polyoxyethylene cetyl ether (STE+POE-CE).

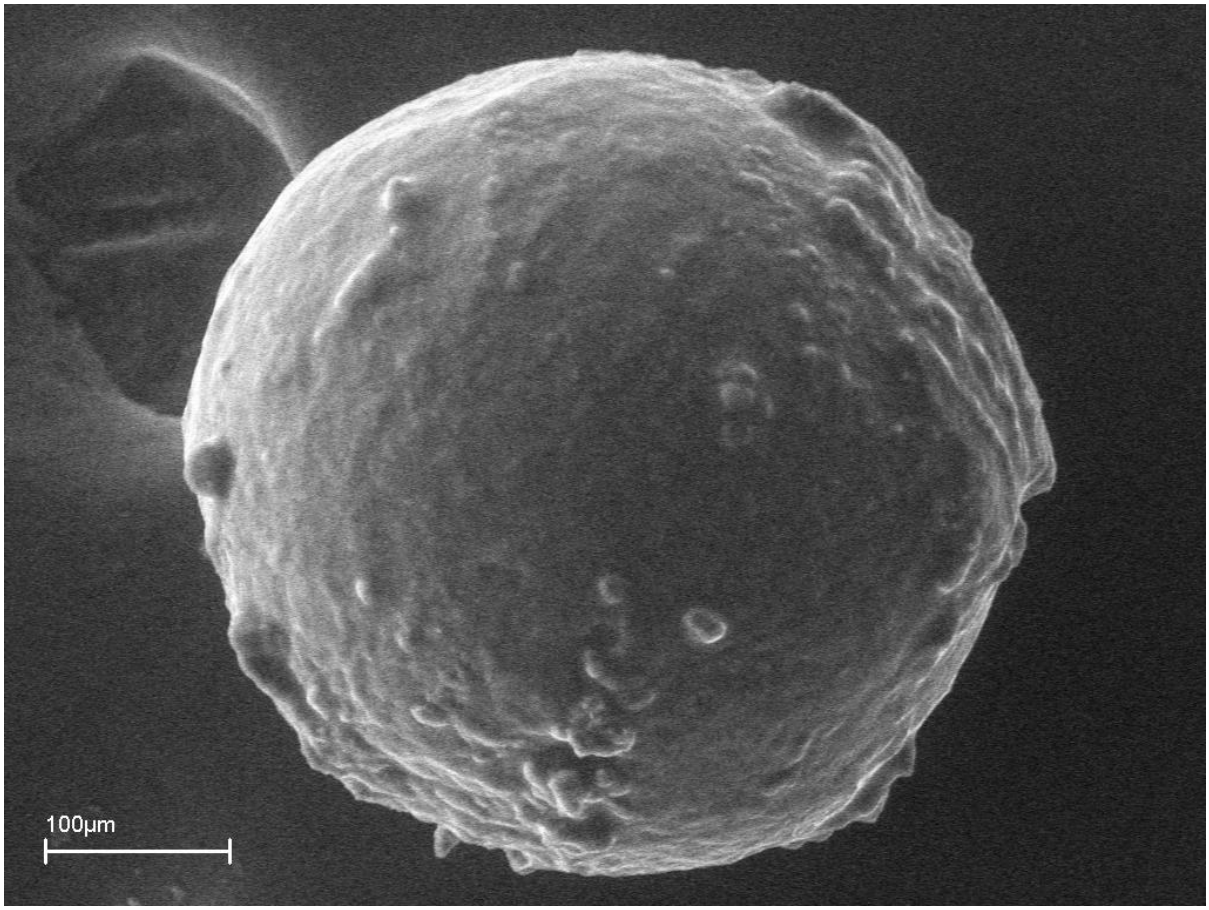

Figure 4.14. Scanning electron micrograph of a microsphere prepared with dual surfactants sorbitan tristearate and polyoxyethelene sorbitan monopalmitate (STE+POE-SM) at 4.5 CHLB.

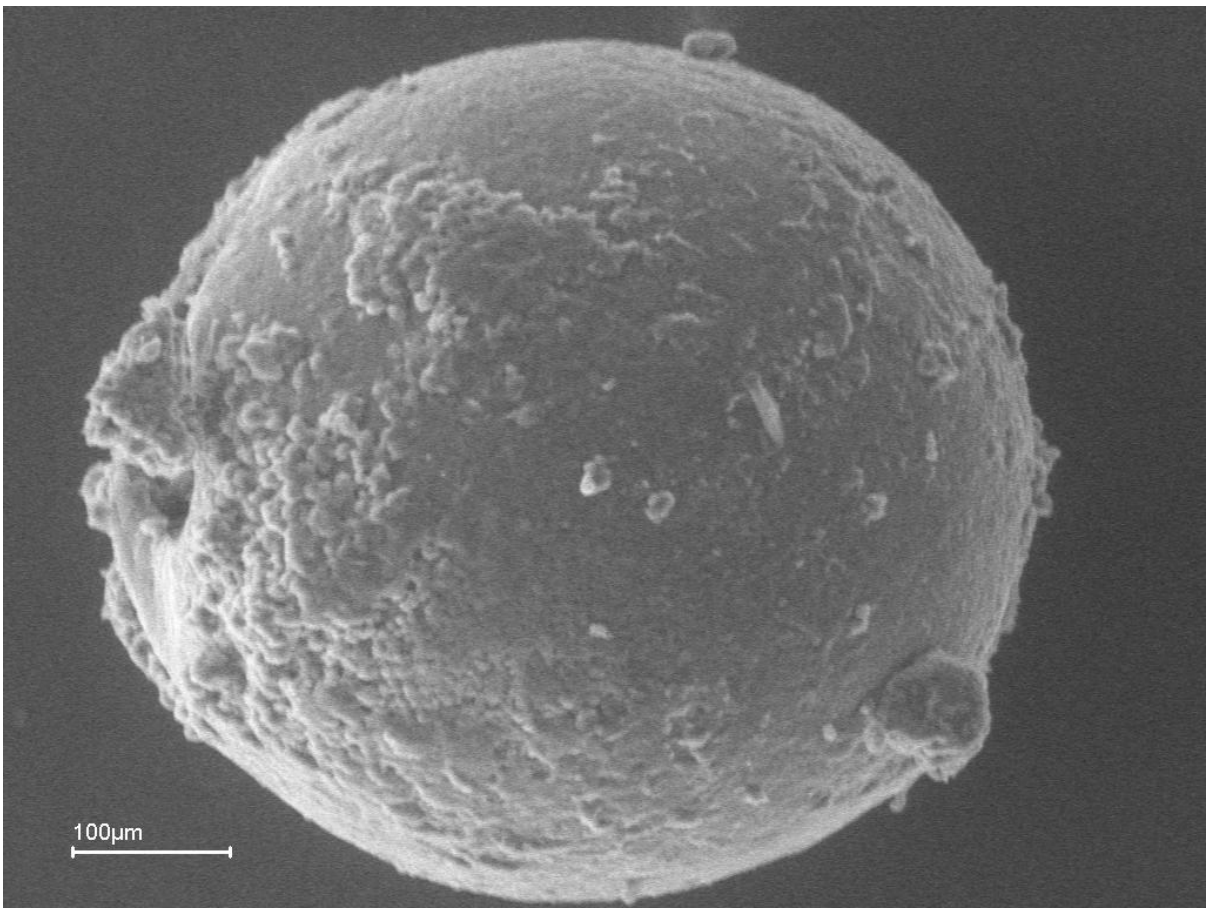

Figure 4.15. Scanning electron micrograph of a microsphere prepared with dual surfactants sorbitan tristearate and polyoxyethelene sorbitan monopalmitate (STE+POE-SM) at 5.5 CHLB.

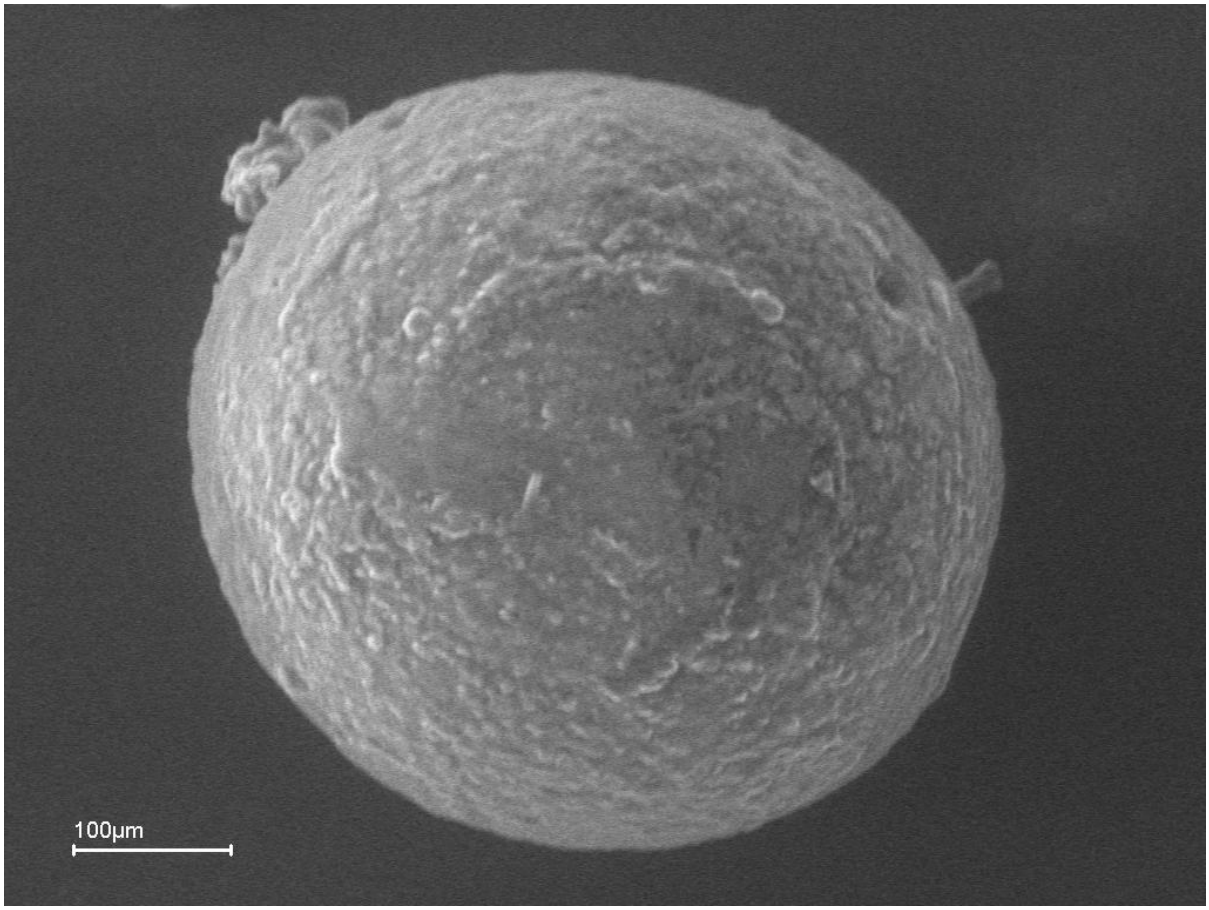

Figure 4.16. Scanning electron micrograph of a microsphere prepared with dual surfactants sorbitan tristearate and polyoxyethelene sorbitan monopalmitate (STE+POE-SM) at 6.5 CHLB.

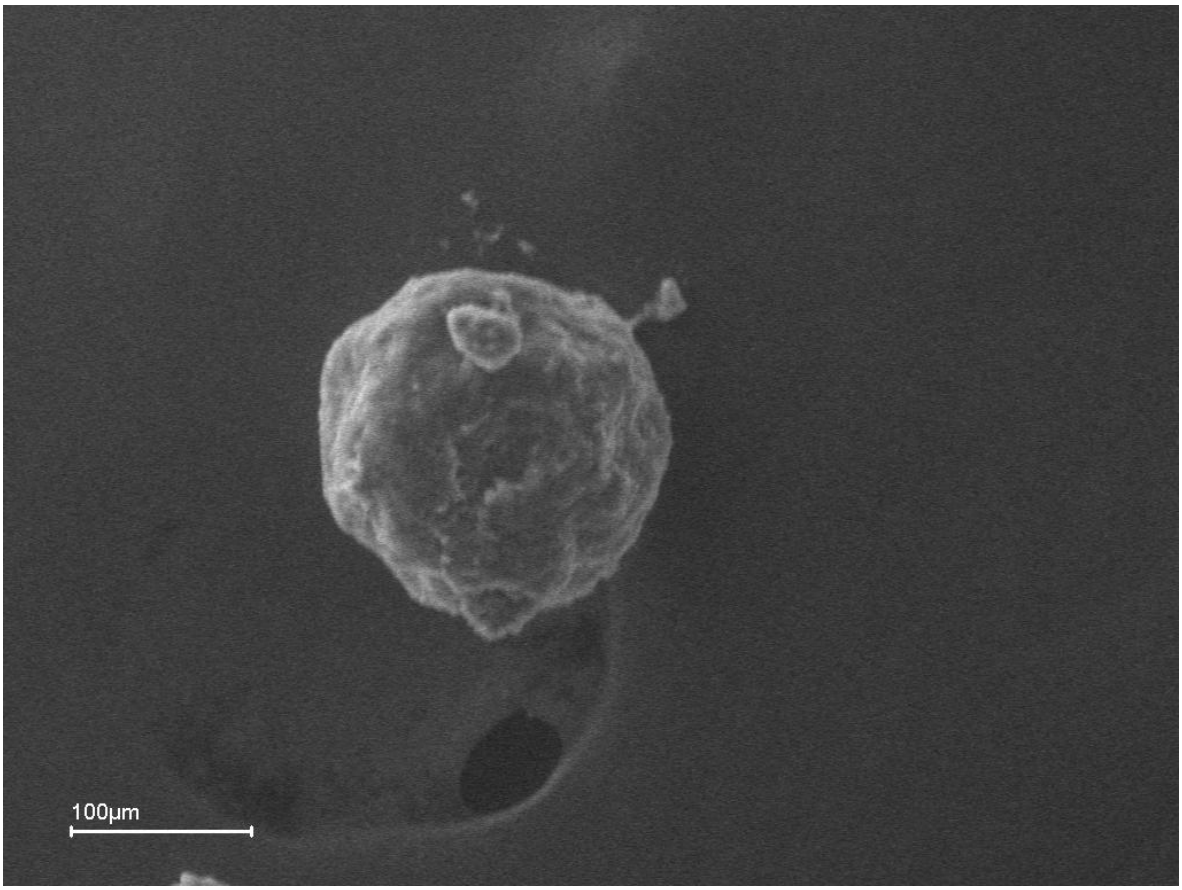

Figure 4.17. Scanning electron micrograph of a microsphere prepared with dual surfactants sorbitan tristearate and polyoxyethelene sorbitan monopalmitate (STE+POE-SM) at 7.5 CHLB.

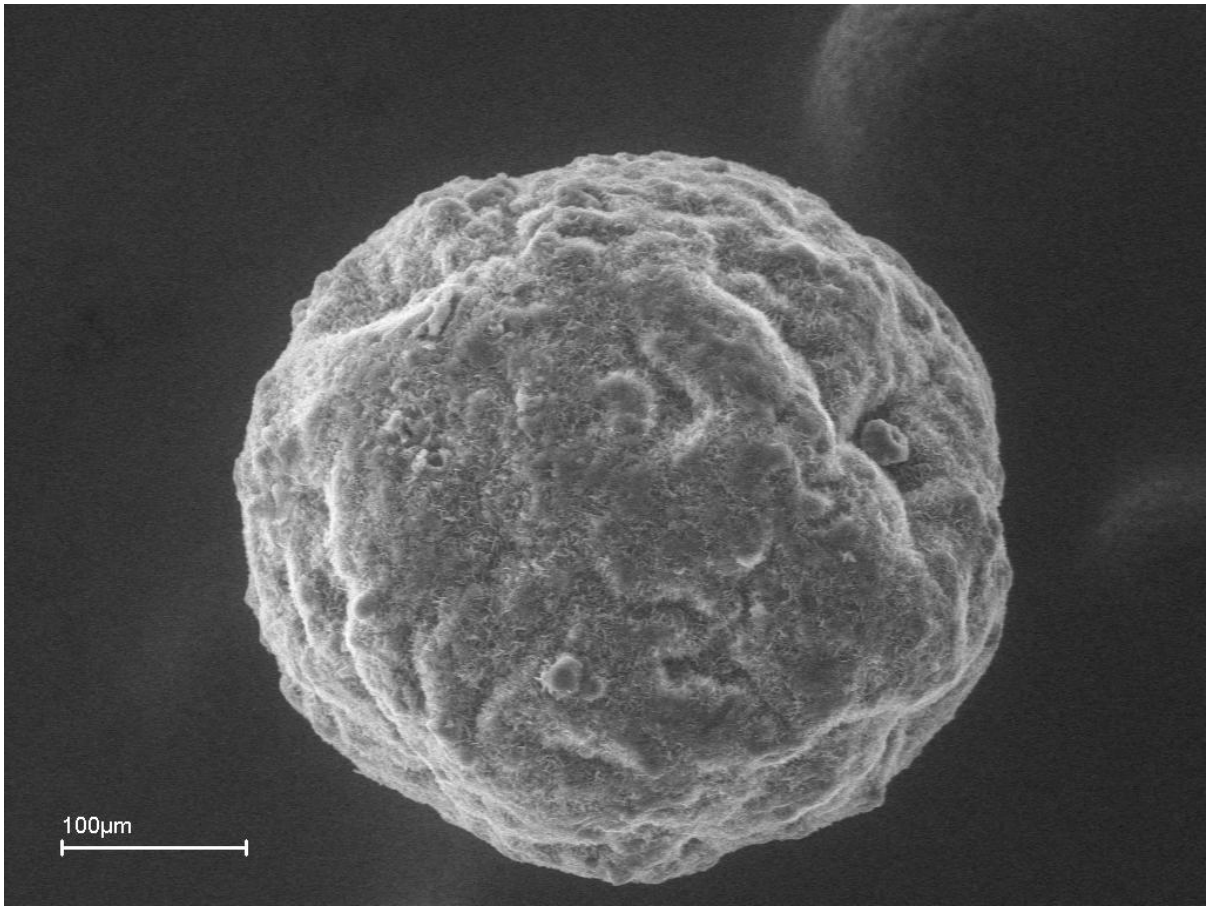

Figure 4.18. Scanning electron micrograph of a microsphere prepared with dual surfactants sorbitan tristearate and polyoxyethylene cetyl ether (STE+POE-CE) at 4.5 CHLB.

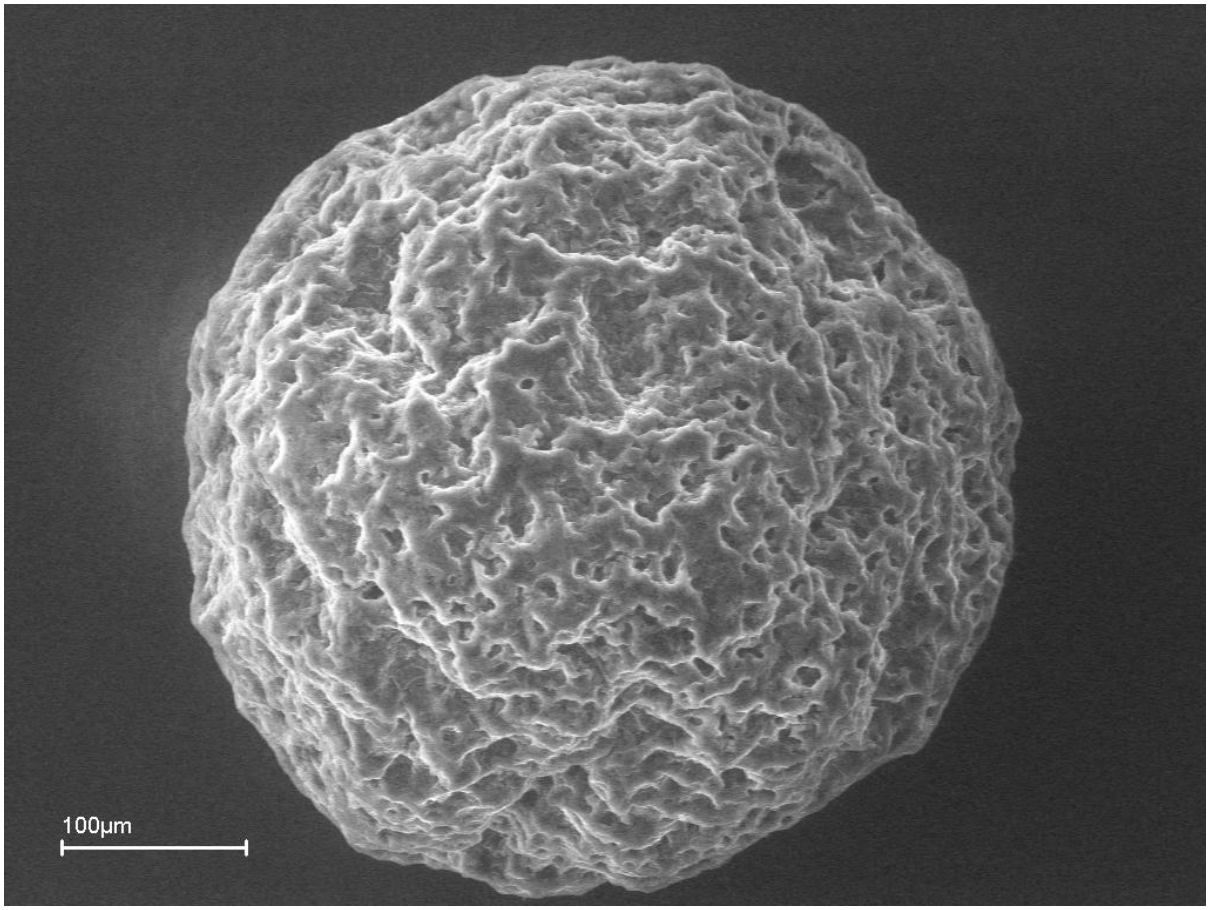

Figure 4.19. Scanning electron micrograph of a microsphere prepared with dual surfactants sorbitan tristearate and polyoxyethylene cetyl ether (STE+POE-CE) at 5.5 CHLB.

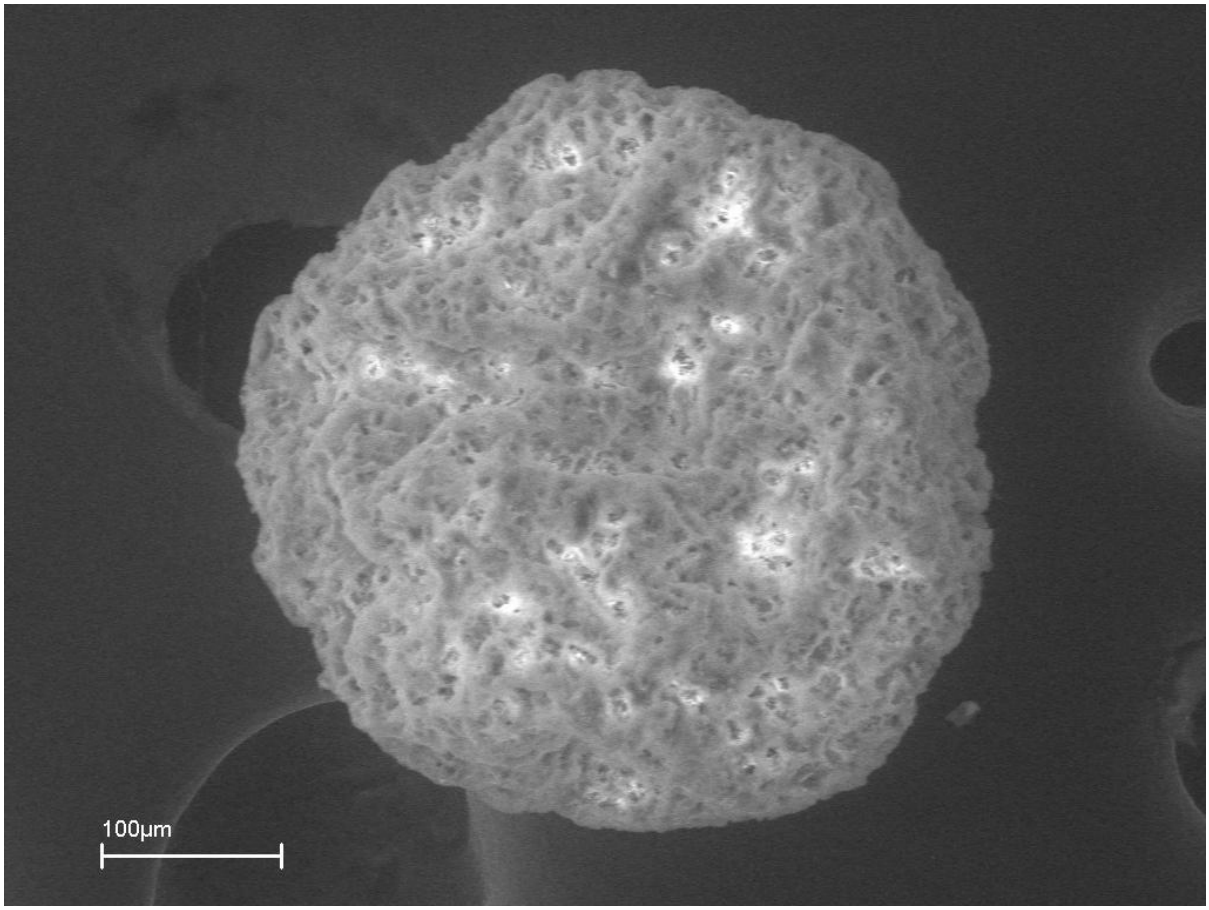

Figure 4.20. Scanning electron micrograph of a microsphere prepared with dual surfactants sorbitan tristearate and polyoxyethylene cetyl ether (STE+POE-CE) at 6.5 CHLB.

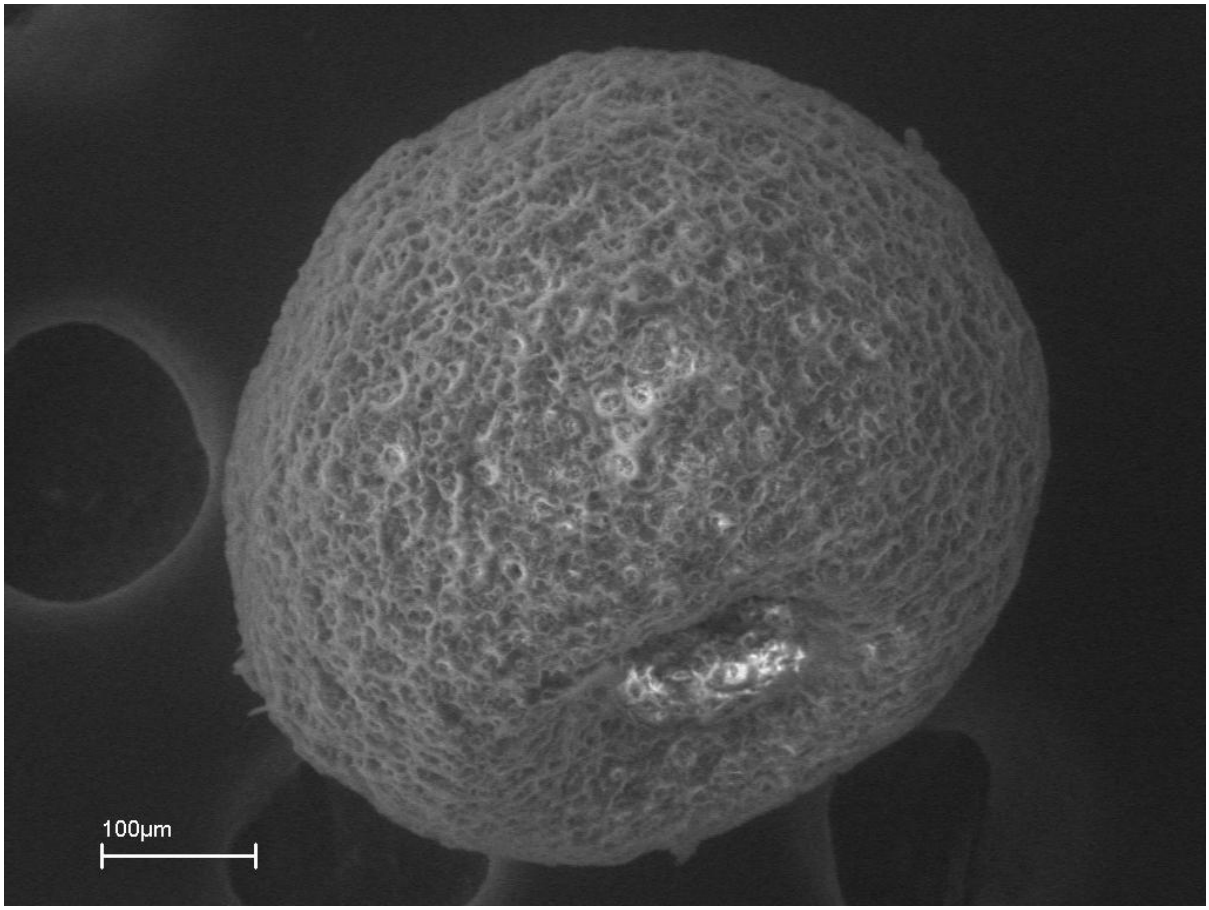

Figure 4.21. Scanning electron micrograph of a microsphere prepared with dual surfactants sorbitan tristearate and polyoxyethylene cetyl ether (STE+POE-CE) at 7.5 CHLB.

CHAPTER 5

EFFECT OF ELEVATED PREPARATION TEMPERATURE ON THE CHARACTERISTICS AND RELEASE PROFILES OF ETHYL CELLULOSE MICROSPHERES CONTAINING THEOPHYLLINE FABRICATED BY NON-AQUEOUS EMULSION-SOLVENT EVAPORATION

Research Paper to be submitted to Journal of Microencapsulation

Thakare Mohan, Capomacchia Anthony, Price James

Abstract

Ethyl cellulose microspheres containing theophylline as a model drug were prepared by the emulsion-solvent evaporation method using acetone as the polymer solvent internal phase, and mineral oil as the external phase. The influence of preparation temperature on particle size and morphology, drug content, drug release and kinetics was evaluated. With increasing preparation temperature from 22^0C to 35^0C, geometric mean diameter decreased, and the particle size distribution widened significantly. When the preparation temperature was increased from 22^0C to 35^0C, the sphericity of microspheres was improved. Scanning electron microscope photographs showed that the microsphere particle surface roughness decreased as preparation temperature increased from 22^0C to 35^0C. Increasing preparation temperature from 22^0C to 35^0C increased the percent drug loading, the initial drug release rate, and the t50 release time. The release kinetics followed the Higuchi model for release from the microspheres.

Keywords: Ethyl cellulose, microspheres, preparation temperature, particle size, drug loading, drug release.

(Total words: 138)

Introduction

One of the major objectives of drug delivery is sustained release of drugs [1]. The success of the sustained release of a drug for a specific duration of time with the optimum release mode depends on different factors. Such as the pharmaceutical properties of the drug and the drug-carrier matrix [2]. In recent years, microencapsulation by the emulsion-solvent evaporation method has been widely used for polymeric microspheres preparation for sustained release drug delivery [3,4]. The solvent evaporation method is a complex method [4]. From the number of studies being conducted all over the world, it is clear that many factors have been reported to affect the solvent evaporation process [3]. These factors include polymer solubility, viscosity, drug solubility, volume and the volume ratio between the inner and outer phases, the polymer: drug ratio, and the rate of evaporation of solvent.

One of the major issues with the solvent evaporation method is that solvent removal can become an extensive and time-consuming process involving several hours to days for complete evaporation of the solvent [5]. There are reports of studying the effects of preparation temperature on microsphere formation and characteristics [6,7]. There are no reports of the effect of temperature on ethyl cellulose microspheres prepared by using dual surfactants. This study investigated the effect of processing temperature on ethyl cellulose microsphere properties using non-ionic dual surfactants, at different combined HLBs (CHLBs).

Theophylline was used as the model drug because it has narrow therapeutics index, and short elimination half-life in humans [8,9]. The solvent used for the dispersed phase solution was acetone, while the continuous phase medium was mineral oil. The microspheres were characterized by their size, drug loading efficiency and drug release kinetics.

Experimental

Materials

The following chemicals were used: Ethyl cellulose (Scientific Polymer Products, New York), micronized theophylline (Gift sample from BASF), light mineral oil (Ruger Chemical Company Inc., Irvington, NJ), Span 65, Tween 40 (Ruger Chemical Company Inc., Irvington, NJ), methylene chloride (Fisher Scientific, NJ), acetone, monobasic potassium phosphate and sodium hydroxide (J.T. Baker, Phillipsburg, NJ).

Instruments

The following instruments were used: Stirrer (Lab Stirrer LR 400D, Yamato Scientific Company Ltd., Tokyo, Japan), Dissolution Apparatus II USP (Dissolution Test system 5100, Distek Inc., North Brunswick, NJ and Prolabo dissolution), Aquamate (UV Spectrophotometer, Thermo Electron Corporation, Mercer's Row, Cambridge, UK), Accumet 5 pH meter (Fisher Scientific, NJ), USP Standard sieve series for PSD studies, Jacketed beaker (with input and output for water flow), Haake, thermostatted circulator bath Type FJ (W. Germany), Temperature monitoring system using platinum resistance temperature detectors (RTD's) fabricated at the University of Georgia under the direction of Dr. Price.

Preparation of microspheres

For preparation of all batches of microsphere, experimental conditions including temperature were identical. The preparation of ethyl cellulose microspheres containing theophylline was accomplished by the emulsification solvent-evaporation method in a 1L tall jacketed glass beaker. The jacketed beaker had continuous water flow through the jacket from a controlled temperature water bath. Microsphere batches were prepared at two temperatures (22^0C ±1 and 35^0C ±1). The temperature monitoring system with the computer software was done with the platinum resistance temperature detectors (RTD) probe immersed in the emulsion mixture. In phase A, light mineral oil (300 ml) containing the low HLB surfactant

was used as an external or continuous phase and was placed in a 1 L jacketed glass beaker. In a separate glass vessel, A 5 % solution of ethyl cellulose in acetone with a high HLB surfactant was prepared. It is called as phase B. Micronized anhydrous theophylline was dispersed in this solution to give a desired (33%) theoretical drug loading (1 part theophylline to 2 parts ethyl cellulose). The entire contents of this vessel (phase B) were added into the glass beaker containing the solution of light mineral oil and low HLB surfactant (phase A) under vigorous agitation. Agitation was continued until the acetone was evaporated and the microspheres became firm. These microspheres were decanted, filtered and washed with mineral spirits to remove the residual light mineral oil. The clean microspheres were then dried in an oven at 50^0C overnight.

Particle size distribution

The size distribution of microspheres was determined by sieving with a set of standard sieves ranges from 90-710μm. A pan was placed underneath the sieves to collect any particles that passed through the last sieve. The aggregate sample was placed on the sieve of largest size, covered, and then tapped by hand until no change in weight was observed in the sieves. We applied manual checking and brief hand sieving to make sure that all particles retained on a sieve were larger than the sieve apertures. After sieving, the quantity of each fraction of particles was measured by weighing. Particle size distribution and geometric mean diameter were calculated. The 355 μm fraction was used for all drug loading and dissolution studies.

Determination of Drug loading

Drug loading was determined by placing accurately weighed samples (in triplicate) in 25 ml volumetric flasks and dissolving in methylene chloride. Drug concentration was then determined by taking absorbance at 276.5 nm. Spectrophotometric interference from the microsphere polymer was not observed at this wavelength.

SEM analysis of microspheres

The surface morphology of microspheres was observed by scanning electron microscope (SEM) using the model Zeiss 1450EP SEM. Microspheres were mounted onto metal multi-stubs using double-sided adhesive tape and SEM images were taken at specific magnification.

In vitro dissolution studies

In vitro release studies of ethyl cellulose microspheres were performed using a USP dissolution apparatus II (spindle method) (Distek Inc., New Jersey) at 100 rpm using the paddle method as described in USP 31. The 355 μm fraction of each batch of microspheres was selected for evaluation. Microsphere samples in triplicate for each batch were suspended in 900 ml of simulated intestinal fluid with 0.1% Tween 20, and no enzymes. The dissolution study was carried out at 37 ± 0.5^{0}C at 100 r.p.m for 12-24 hours. Three ml of sample were withdrawn at specific time intervals and replaced with fresh simulated intestinal medium. The drug released was determined spectrophotometrically at 274 nm. The dissolution data was evaluated for initial release, dissolution rate and the mechanism of drug release.

Release kinetics

Data obtained from *in vitro* release studies were fitted to Higuchi kinetic equations to find out the mechanism of drug release from ethyl cellulose microspheres [10].

Results and discussion

Microsphere preparation

Ethyl cellulose microspheres were prepared by the emulsion-solvent evaporation method. Microspheres were formed after a series of steps including evaporation of the solvent. Initial experiments were carried out to optimize the formulation process and each step of preparation of microsphere was carefully observed. The experimental temperature

conditions for preparation of microspheres are given in Table 5.1. Dual surfactants used in this process are listed in Table 5.1 with their respective HLBs and CHLBs. Almost a 50% reduction in microsphere preparation time resulted when the preparation temperature was elevated from 22^0C to 35^0C (Table 5.2). This indicated that the elevated temperature increased the speed of the evaporation of solvent.

Particle size distribution (PSD) and geometric mean diameter (GMD)

When the microspheres were prepared at 22^0C using the Span 65 and Tween 40 as dual surfactants, an increase in CHLB resulted in a decrease in the GMD (Figure 5.1). Microspheres prepared at 35^0C using the Span 65 and Tween 40 as dual surfactants, also showed change in the GMD at different CHLBs. With an increase in CHLB from 4.5 to 5.5 the GMD decreased (Figure 5.1). However no significant effect was observed in the GMD, when the CHLB was further increased to 6.5. A further increase in CHLB to 7.5 caused an increase in the GMD of the microspheres with the batches prepared at 35^0C (Figure 5.1).

An increase in preparation temperature from 22^0C to 35^0C led to a decrease in GMD (Figure 5.1). Microspheres prepared at 35^0C showed more free flowing particles when observed under the microscope. This might have caused increase in the GMD of microspheres 35^0C. Increase in the preparation temperature 22^0C to 35^0C increases acetone evaporation. As the acetone leaves, the particles shrink; the polymer congeals and ultimately becomes solid.

As shown in Figures 5.2 and 5.3, the particle size distribution (PSD) varied significantly for 22^0C and 35^0C at different CHLBs. Microspheres developed at 22^0C using dual surfactants showed the particle size distribution range mostly in the 125 µm fractions for all the CHLBs, while microspheres prepared at 35^0C extended to the range of 90 µm. At 22^0C we noted an increase in the percentage of the particles in 355 µm fraction for the CHLB from

4.5 to 5.5 to 6.5. The particles with 7.5 CHLB were found with highest percentage in 125µm fraction compared to other CHLBs at 22^0C.

The percentage of particles in the 710 µm and 595 µm fractions decreased with increase in temperature from the 22^0C to 35^0C. Smaller average particle size resulted with an increase in temperature from 22^0C to 35^0C. The changes in particle size may be attributed to an increase in the temperature and their effects in the presence of dual surfactants. Temperature affects the way and the rate at which microspheres form by speeding up the process of the evaporation of solvent. Hence, it is conceivable that the particle size characteristics of the microspheres would be affected by preparation temperature.

Scanning electron microscopy (SEM) analysis

The SEM micrographs of microspheres prepared at 22^0C using dual surfactants are shown in Figures 5.11 to 5.14. Microspheres prepared at 22^0C were irregularly spherical with a less porous structure (Figure 5.11 to 5.14). Increasing preparation temperature from 22^0C to 35^0C affected the surface morphology of microspheres and resulted in improved sphericity, and a more porous surface (Figures 5.15 and 5.17). An increase in CHLB at 35^0C preparation temperature changed the morphology as well. We observed that the microspheres were not spherical at the 7.5 CHLB at 35^0C. The particles were not completely spherical because of agglomeration of small microspheres into larger non-spherical units as seen in the SEM photograph. The change in the SEM appearance from 22^0C to 35^0C may be attributed to more rapid evaporation of solvent ultimately allowing less time for agglomeration of soft particles. Studying the effect of preparation temperature on surface morphology revealed that increasing preparation temperature from 22^0C to 35^0C at certain CHLBs, a porous matrix structure could be formed. The formation of the microsphere skin is often likened to the two basic processes such as phase separation and precipitation of asymmetric membranes. The

SEM analysis showed that the high-temperature (35^0C) solvent removal results in less defective membrane skin layer.

Drug loading

As seen in Table 5.3, the drug loading studies of the 355 μm fractions of the microsphere batches prepared at 22^0C revealed that there was no significant difference between the drug loadings with the change in most CHLBs. At 6.5 CHLB, the drug loading was higher than all other CHLBs at 22^0C. Increase in preparation temperature from at 22^0C to at 35^0C increased the drug loading percent with respective CHLBs. These batches were prepared with 33% theoretical drug loading. We observed that with an increased temperature to 35^0C at CHLB 5.5 and 7.5 the actual drug loading was more than the theoretical drug loading (Table 5.3). None of the microsphere batches prepared at 22^0C had drug loading greater than the theoretical drug loading at any CHLB levels. With an elevated temperature, the increase in the drug loading attributed to the rapid solidification of polymer leading to denser layer resulting in increased drug loading efficiency [11,12]. Many factors affect the fast solidification of the polymer such as a higher solvent removal rate, higher polymer concentration or a lower ratio of the dispersed phase to continuous phase DP/CP) [11,12]. In our case, the rate of solvent removal had been increased (Table 5.2) however, it didn't affect the encapsulation efficiency among the different microsphere batches.

In vitro drug release behavior

The *in vitro* release profiles of ethyl cellulose microspheres prepared at different temperatures are shown in Figures 5.4 and 5.5. The microspheres produced at 22^0C with dual surfactants (Span 65+Tween 40) showed differences in release profiles at different CHLBs (Figure 5.4). With an increase in temperature from 22^0C to 35^0C, we noted changes in the theophylline release. Microspheres made at 35^0C using the dual surfactants exhibited adequate sustained-release profiles in all CHLB formulations (Figure 5.5).

Initial drug release

The initial drug release percentage within the first 30 minutes was analyzed for the fraction of 355 μm size microspheres batches prepared at 22^0C and 35^0C. In batches prepared at the 22^0C, the initial drug release was lowest at 4.5 CHLB and the highest drug release was at 7.5 CHLB (Figure 5.6). In batches prepared at 35^0C, the initial drug release was lowest at 4.5 CHLB (Figure 5.7). When the temperature was increased from 22^0C to 35^0C, the initial drug release was increased at 4.5, 5.5 and 6.5 CHLB and decreased at 7.5 CHLB.

t_{50} drug release

Studies on the 22^0C preparation temperature on the t_{50} release as shown in Figure 5.8 showed that the t_{50} drug release was effectively increased with an increase in CHLB. Studies on 35^0C preparation temperature on the t_{50} release as explained in Figure 5.8 showed that the t_{50} drug release did not show any effect with an increase in CHLB. The t_{50} drug release is the time taken for 50% of the drug to be released from the microsphere. With an increase in temperature from 22^0C to 35^0C, the t_{50} drug release was decreased. The drug release mechanism is naturally influenced by many factors such as the presence and the location of drug and surfactant molecules in the microsphere particle. The possible sites of location of the surfactant molecules could be either on the surface of the microsphere, within the matrix isolated from the interior environment or within the microsphere but connected with its outer surface possibly through channels. The differences in release profiles at different temperature are the consequence of differences in the surface area because of the particle surface roughness and porosity that changed with an increase in temperature from 22^0C to 35^0C.

Drug release kinetics

Figures 5.9 and 5.10 depict the drug release kinetics. Our study shows that the release occurs by diffusion through the pores for different batches of microspheres prepared at 22^0C and 35^0C. Although the Higuchi equation analysis for spherical matrices indicated that the

drug release from the microspheres was primarily by diffusion, the model seems to be an exact fit when there was an increase in temperature from 22^0C to 35^0C (Table 5.4).

Conclusion

The preparation temperature of ethyl cellulose microspheres fabricated by the non-aqueous emulsion solvent evaporation method affected different physicochemical and release properties of microspheres. With increasing preparation temperature from 22^0C to 35^0C, the geometric mean diameter decreased, the particle size distribution was significantly wider. With an increase in temperature from 22^0C to 35^0C, the sphericity of microspheres improved, and the drug loading percentage, and initial drug release rate from the microspheres increased. The t_{50} release time for theophylline release was increased with an increase in the preparation temperature from 22^0C to 35^0C. The release kinetics showed a diffusion type of release. The SEM photographs show that the microsphere particle surface roughness decreased as preparation temperature increased from 22^0C to 35^0C.

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Table 5.1. Different experimental conditions and dual surfactant combination for microsphere preparation.

Surfactants used	CHLB	Temperature
Span 65 (HLB=2.1) + Tween 40 (HLB=15.6)	4.5	22^0C
Span 65 (HLB=2.1) + Tween 40 (HLB=15.6)	5.5	22^0C
Span 65 (HLB=2.1) + Tween 40 (HLB=15.6)	6.5	22^0C
Span 65 (HLB=2.1) + Tween 40 (HLB=15.6)	7.5	22^0C
Span 65 (HLB=2.1) + Tween 40 (HLB=15.6)	4.5	35^0C
Span 65 (HLB=2.1) + Tween 40 (HLB=15.6)	5.5	35^0C
Span 65 (HLB=2.1) + Tween 40 (HLB=15.6)	6.5	35^0C
Span 65 (HLB=2.1) + Tween 40 (HLB=15.6)	7.5	35^0C

Table 5.2. Effect of microsphere preparation temperature on microsphere fabrication time.

Surfactants used	**Temperature**	**Microsphere preparation time**
Span 65 (HLB=2.1) + Tween 40 (HLB=15.6)	22^0C	16 to 18 hrs
Span 65 (HLB=2.1) + Tween 40 (HLB=15.6)	35^0C	8 to 9 hrs

Table 5.3. Effect of different preparation temperatures on the 355 μm fractions of microspheres prepared at different CHLBs using dual surfactants on actual drug loading.

Surfactants used	CHLB	Temperature	Actual drug loading (%)
Span 65 (HLB=2.1) + Tween 40 (HLB=15.6)	4.5	22^0C	21.62 ± 1.64
Span 65 (HLB=2.1) + Tween 40 (HLB=15.6)	5.5	22^0C	21.90 ± 0.42
Span 65 (HLB=2.1) + Tween 40 (HLB=15.6)	6.5	22^0C	25.47 ± 1.83
Span 65 (HLB=2.1) + Tween 40 (HLB=15.6)	7.5	22^0C	22.52 ± 0.46
Span 65 (HLB=2.1) + Tween 40 (HLB=15.6)	4.5	35^0C	22.52 ± 1.14
Span 65 (HLB=2.1) + Tween 40 (HLB=15.6)	5.5	35^0C	48.83 ± 1.15
Span 65 (HLB=2.1) + Tween 40 (HLB=15.6)	6.5	35^0C	26.43 ± 2.27
Span 65 (HLB=2.1) + Tween 40 (HLB=15.6)	7.5	35^0C	41.03 ± 0.84

Table 5.4. Higuchi equation regression analysis of dissolution from different microsphere batches prepared at different preparation temperature.

Surfactants used	**CHLB**	**Temperature**	**Higuchi equation regression R^2**
Span 65 (HLB=2.1) + Tween 40 (HLB=15.6)	4.5	22^0C	0.9636
Span 65 (HLB=2.1) + Tween 40 (HLB=15.6)	5.5	22^0C	0.9860
Span 65 (HLB=2.1) + Tween 40 (HLB=15.6)	6.5	22^0C	0.9881
Span 65 (HLB=2.1) + Tween 40 (HLB=15.6)	7.5	22^0C	0.7669
Span 65 (HLB=2.1) + Tween 40 (HLB=15.6)	4.5	35^0C	0.9942
Span 65 (HLB=2.1) + Tween 40 (HLB=15.6)	5.5	35^0C	0.9540
Span 65 (HLB=2.1) + Tween 40 (HLB=15.6)	6.5	35^0C	0.9454
Span 65 (HLB=2.1) + Tween 40 (HLB=15.6)	7.5	35^0C	0.9911

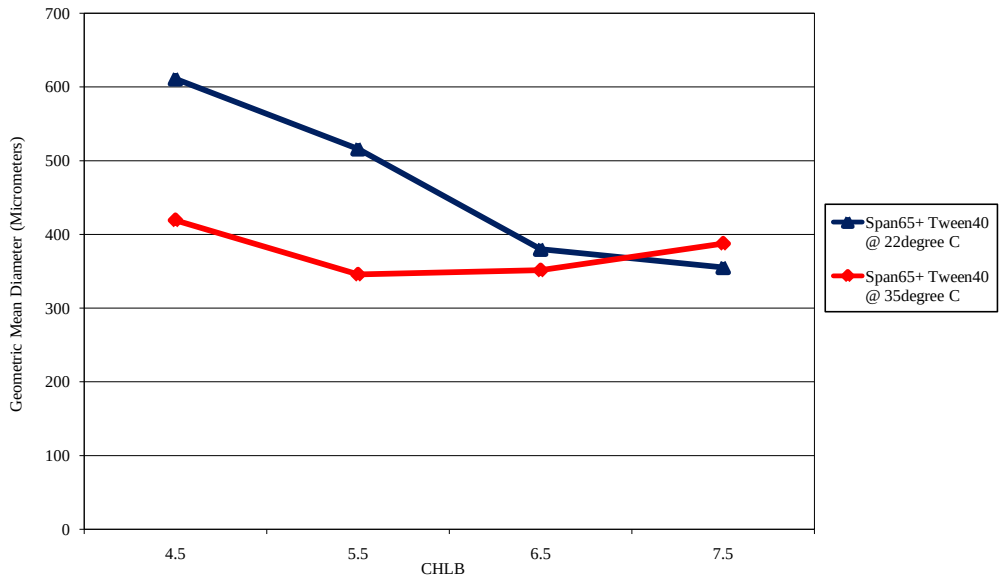

Figure 5.1. Comparative effect of CHLB on geometric mean diameter of microsphere batches prepared with dual surfactants (Span 65+Tween 40) at 22^0C and 35^0C.

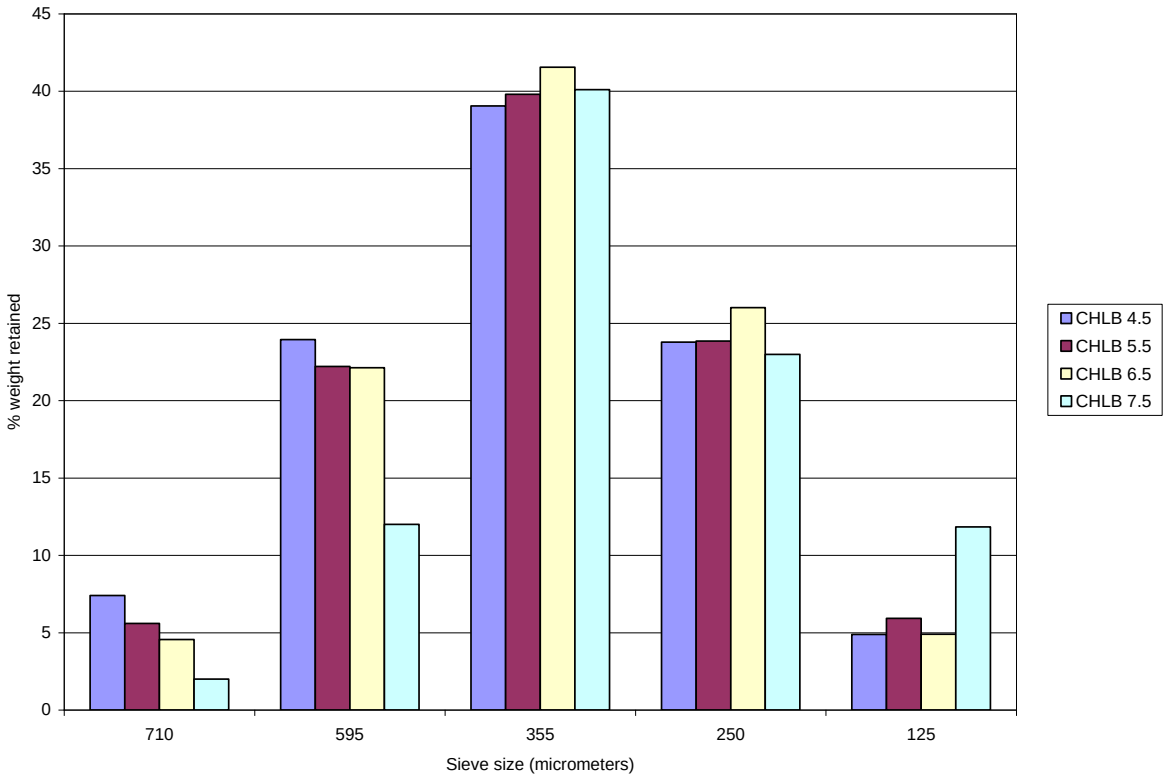

Figure 5.2. Effect of CHLB on particle size distribution of microsphere batches prepared with dual surfactants (Span 65+Tween 40) at 22^0C.

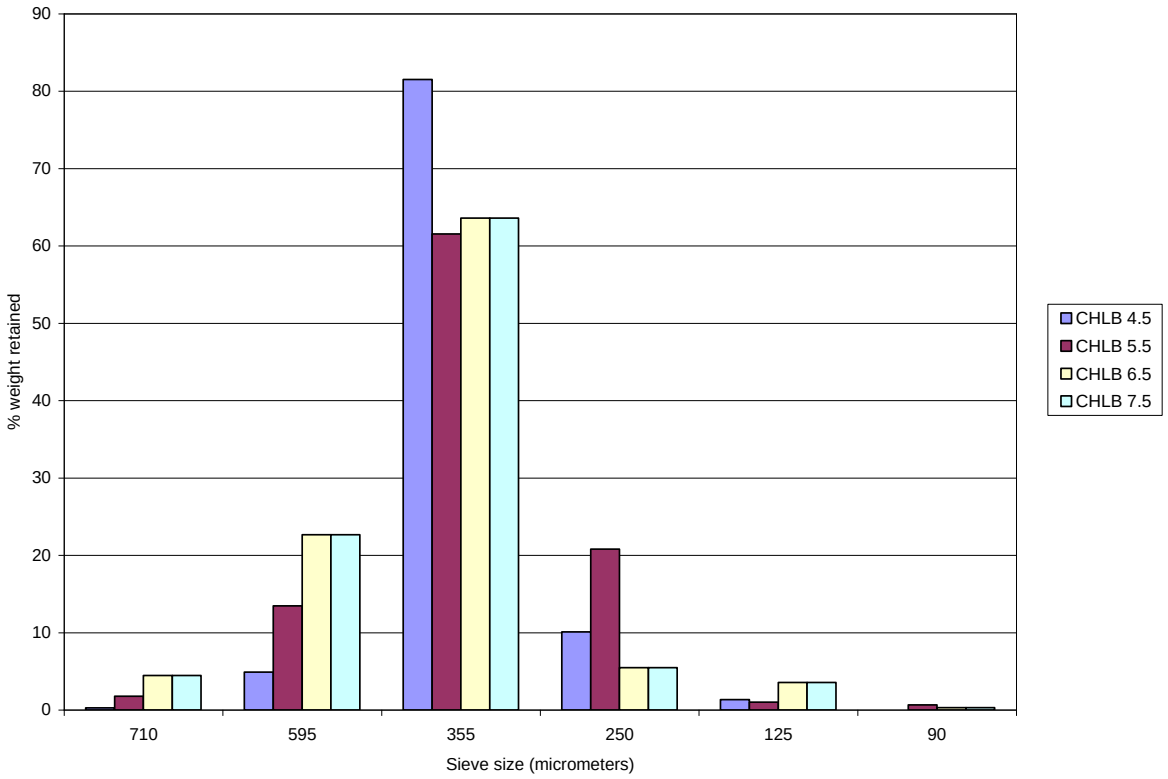

Figure 5.3. Effect of CHLB on particle size distribution of microsphere batches prepared with dual surfactants (Span 65+Tween 40) at 35^0C.

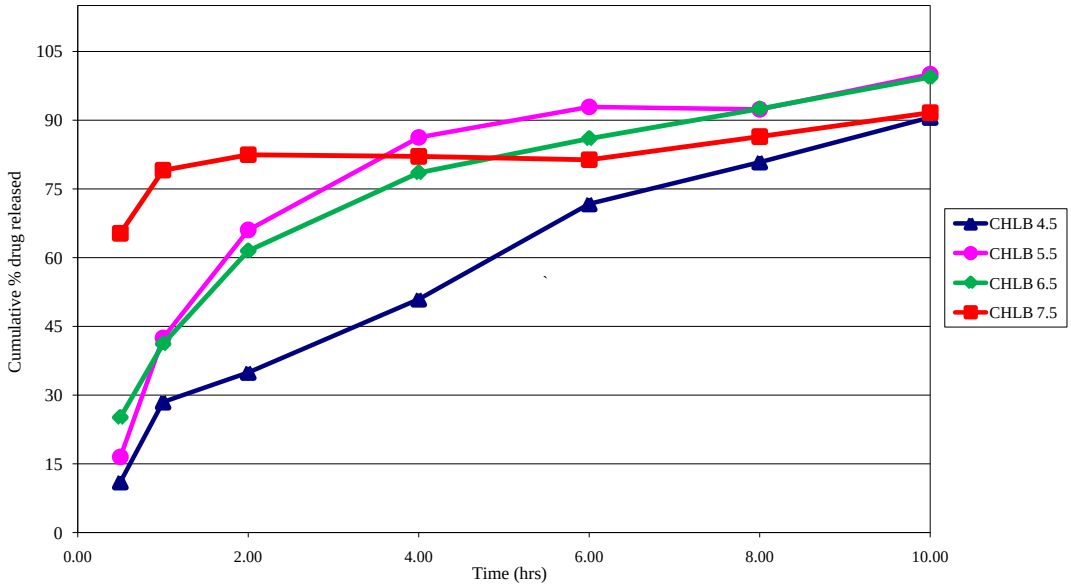

Figure 5.4. Effect of CHLB on *in vitro* dissolution studies of microsphere batches prepared with dual surfactants (Span 65+Tween 40) at 22^0C.

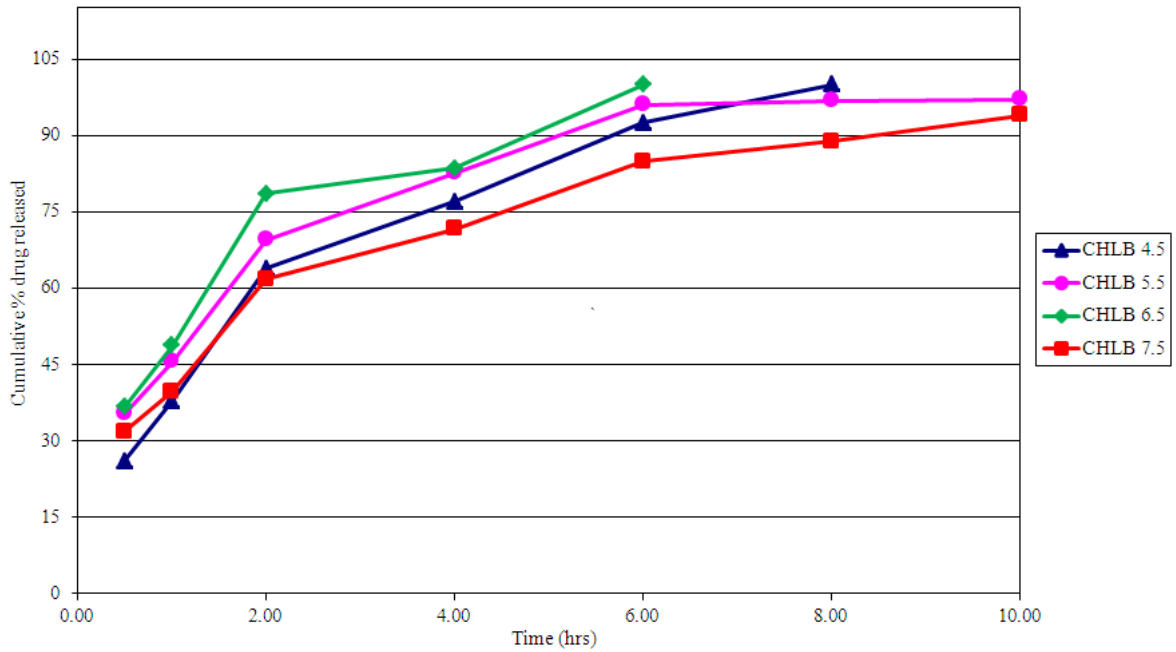

Figure 5.5. Effect of CHLB on *in vitro* dissolution studies of microsphere batches prepared with dual surfactants (Span 65+Tween 40) at 35^0C.

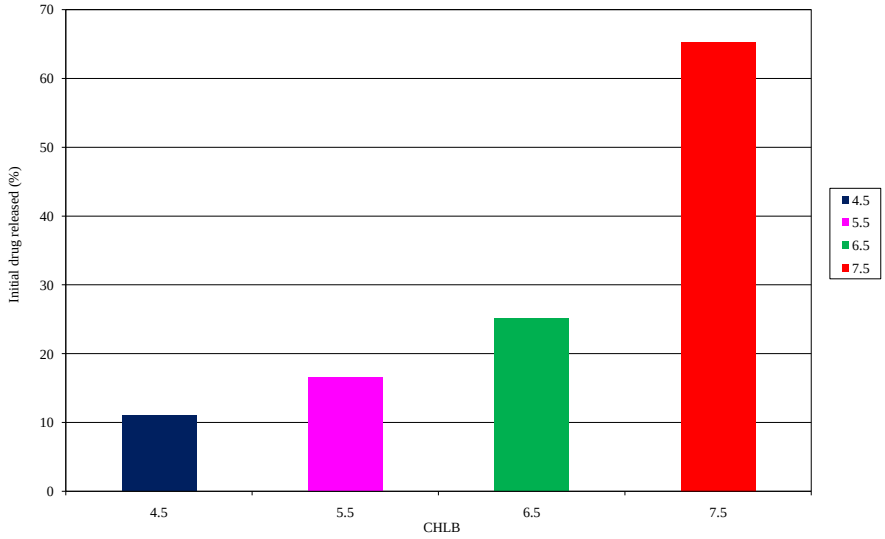

Figure 5.6. Effect of different CHLBs on initial drug release (drug released within first 30 min) of microsphere batches prepared with dual surfactants (Span 65+Tween 40) at 22^0C.

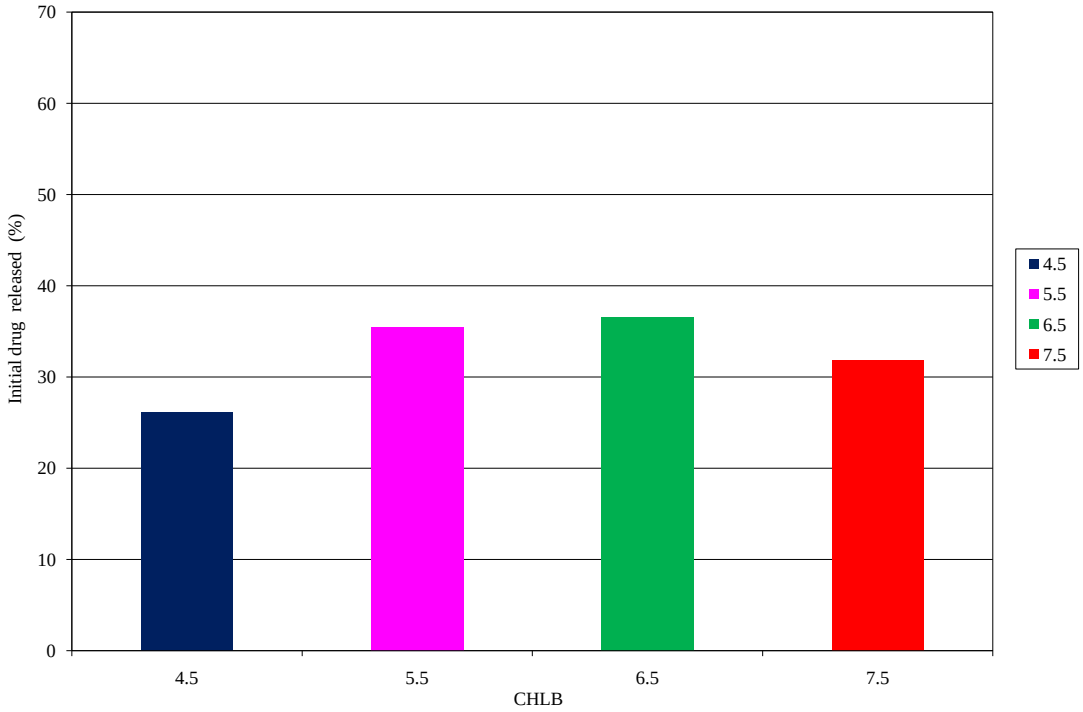

Figure 5.7. Effect of different CHLBs on initial drug release (drug released within first 30 min) of microsphere batches prepared with dual surfactants (Span 65+Tween 40) at 35^0C.

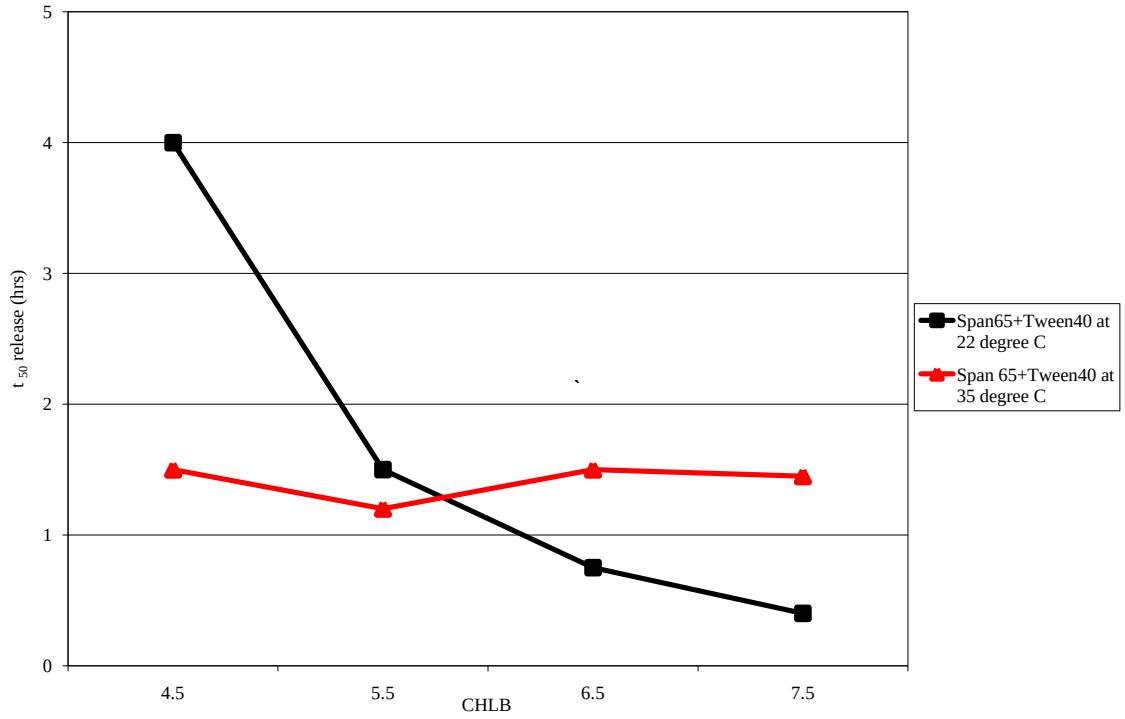

Figure 5.8. Effect of CHLB on t_{50} release (time required for 50% of drug released) from microsphere batches prepared with dual surfactants (Span 65+Tween 40) at 22^0C and 35^0C.

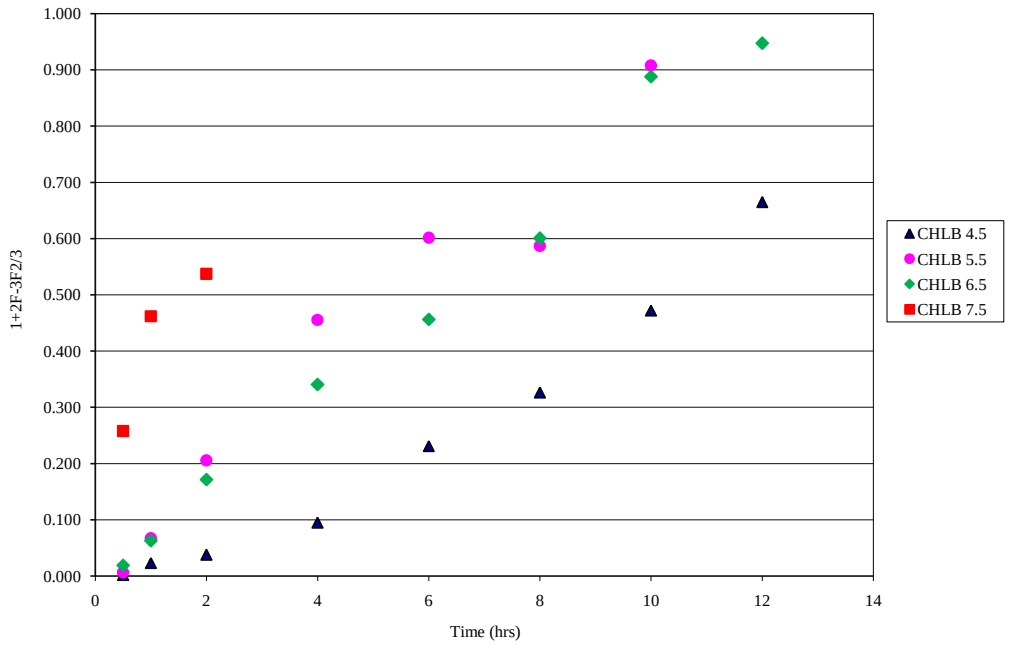

Figure 5.9. Effect of CHLB on dissolution mechanism (Higuchi equation) of microsphere batches prepared with dual surfactants (Span 65 + Tween 40) at 22^0C.

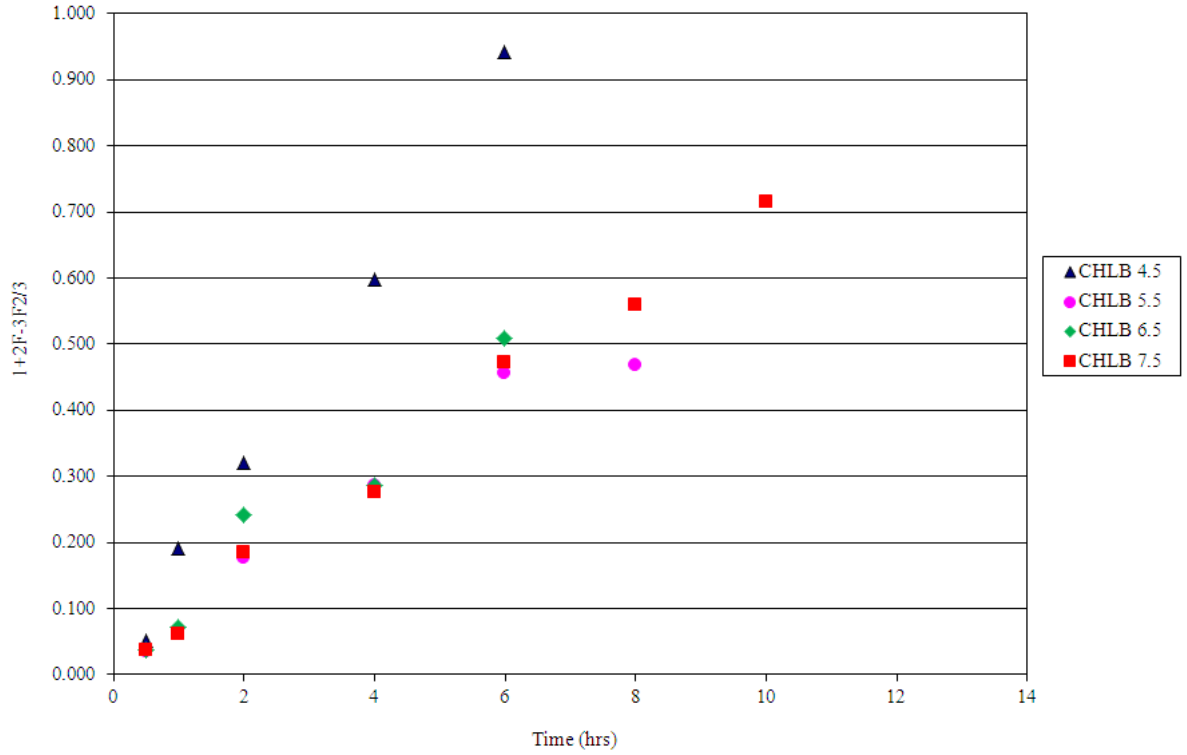

Figure 5.10. Effect CHLB on dissolution mechanism (Higuchi equation) of microsphere batches prepared with dual surfactants (Span 65+Tween 40) at 35^0C.

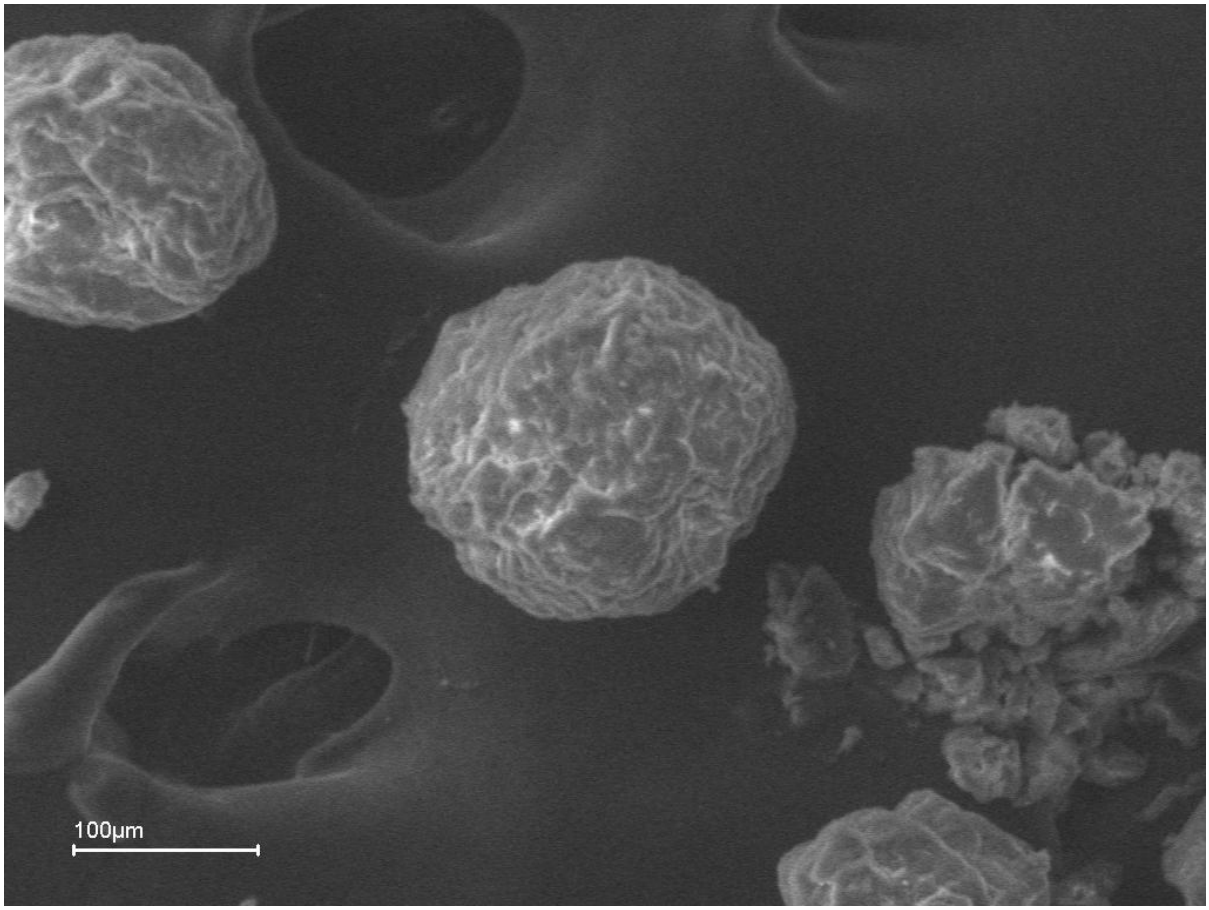

Figure 5.11. Scanning electron micrograph of microspheres prepared @ 22^0 C using dual surfactants (Span 65+Tween40) at 4.5 CHLB.

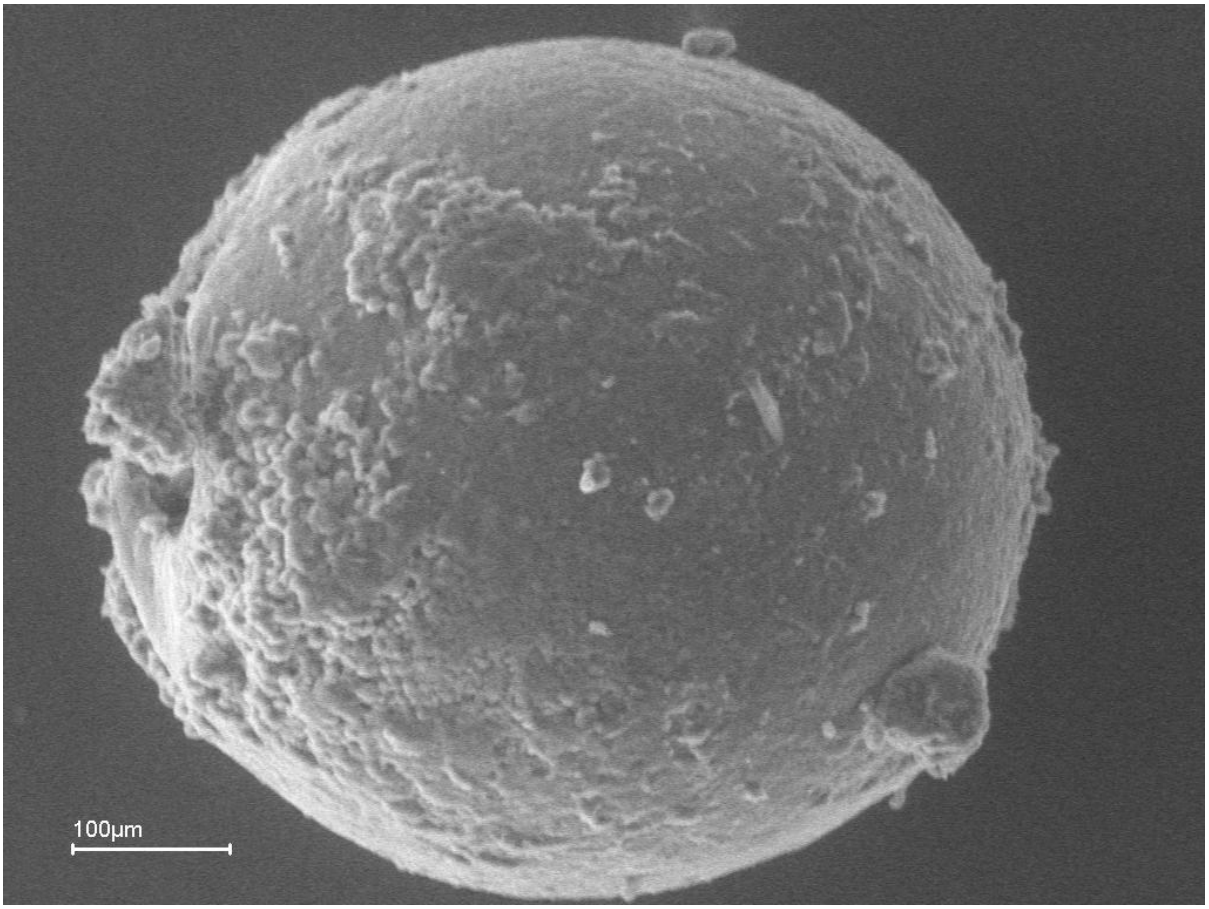

Figure 5.12. Scanning electron micrograph of microspheres prepared @ 22^0 C using dual surfactants (Span 65+Tween40) at 5.5 CHLB.

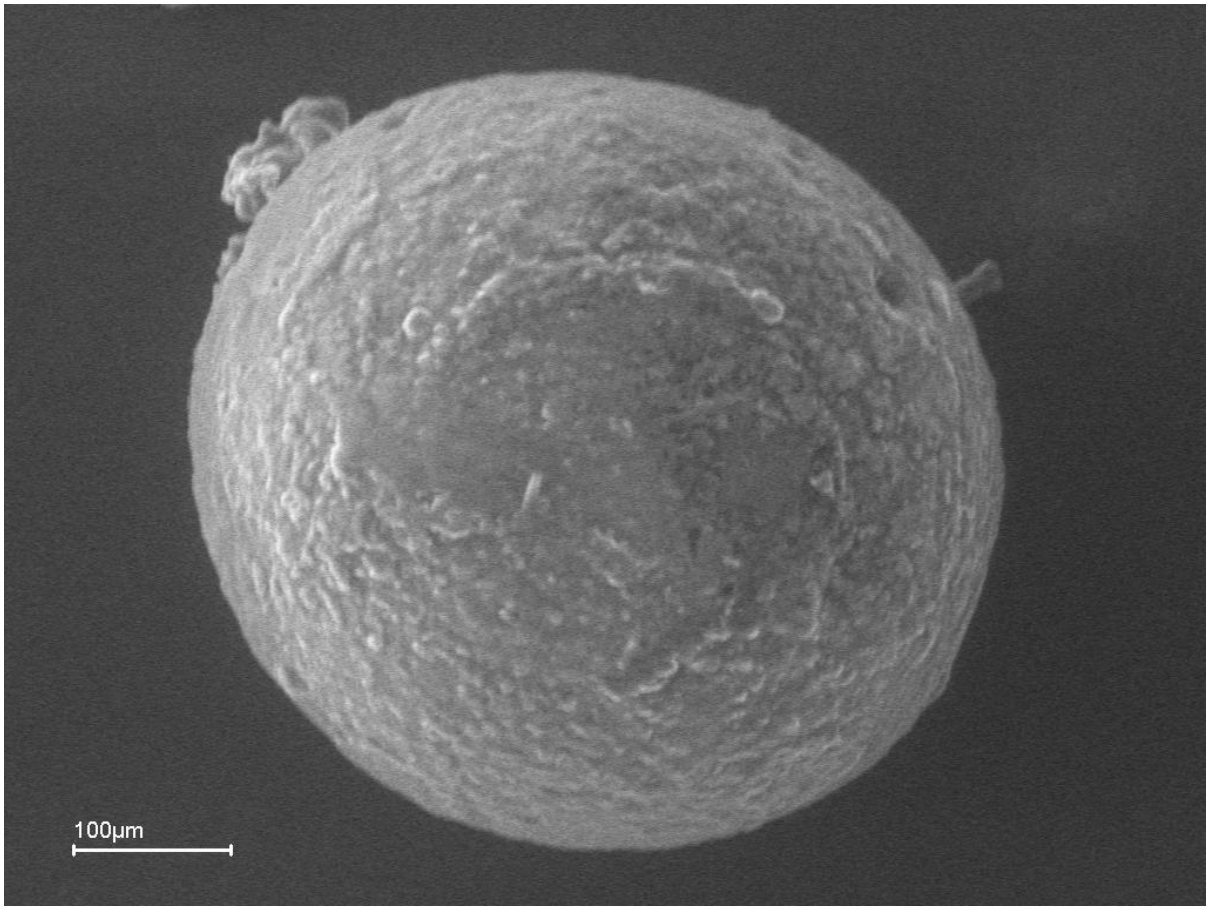

Figure 5.13. Scanning electron micrograph of microspheres prepared @ 22^0C using dual surfactants (Span 65+Tween40) at 6.5 CHLB.

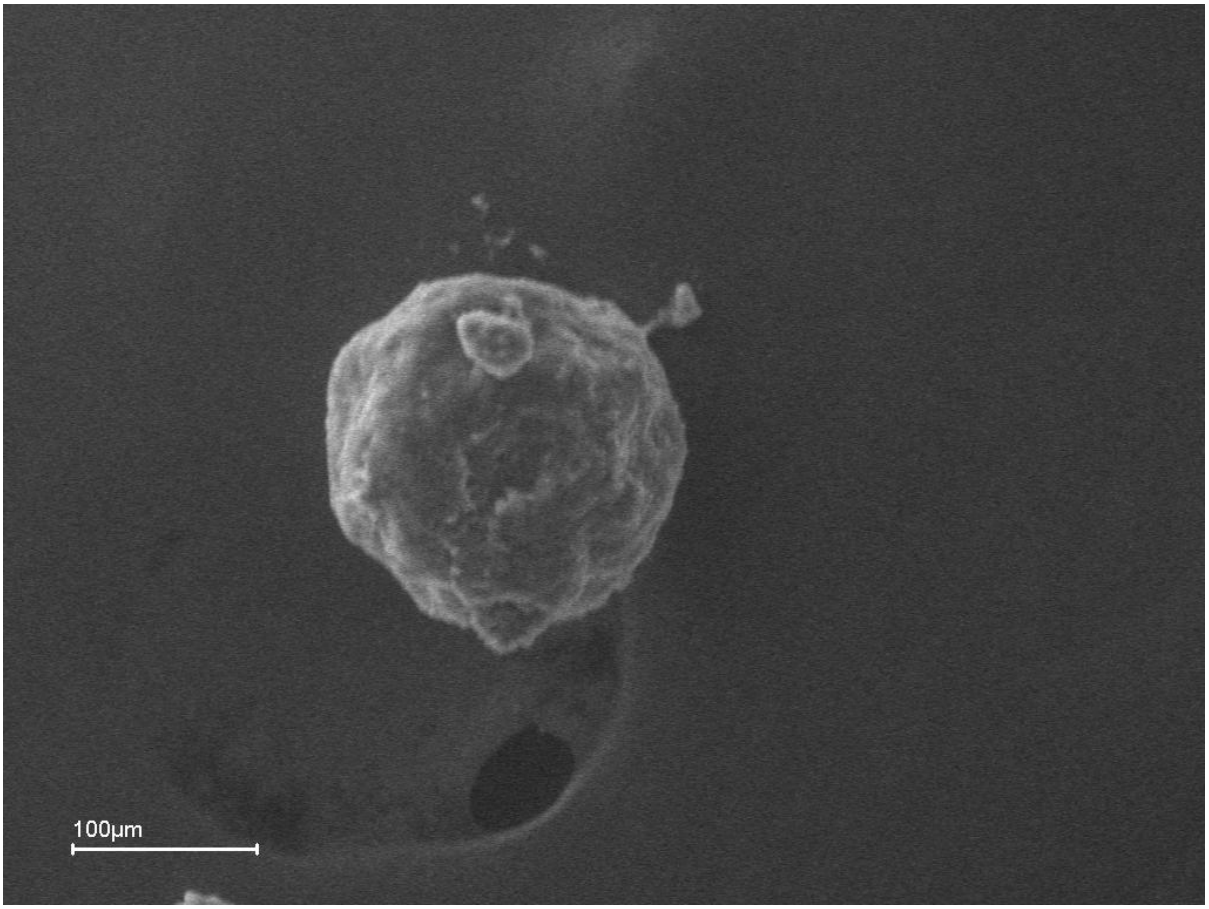

Figure 5.14. Scanning electron micrograph of microspheres prepared @ 22^0C using dual surfactants (Span 65+Tween40) at 7.5 CHLB.

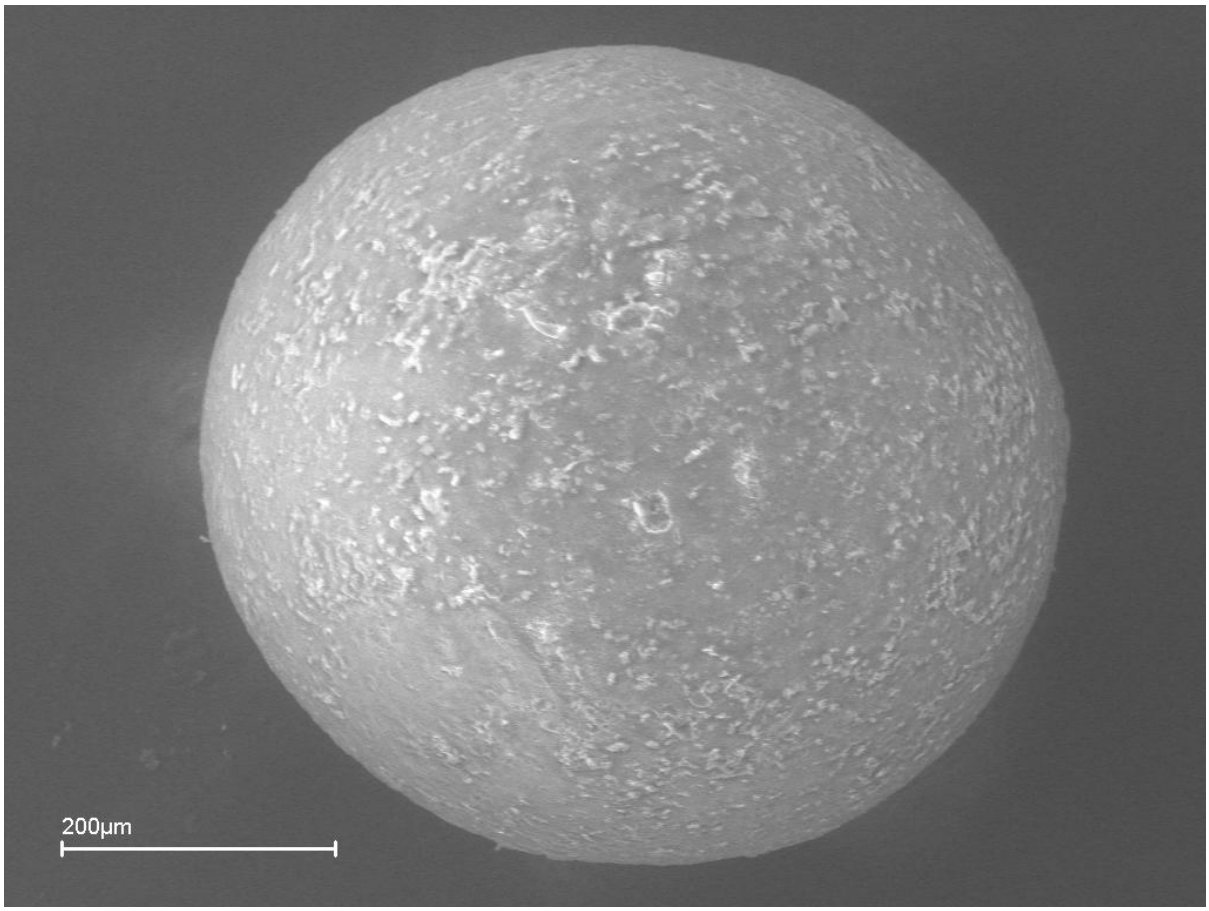

Figure 5.15. Scanning electron micrograph of microspheres prepared @ 35^0C using dual surfactants (Span 65+Tween 40) at 4.5 CHLB.

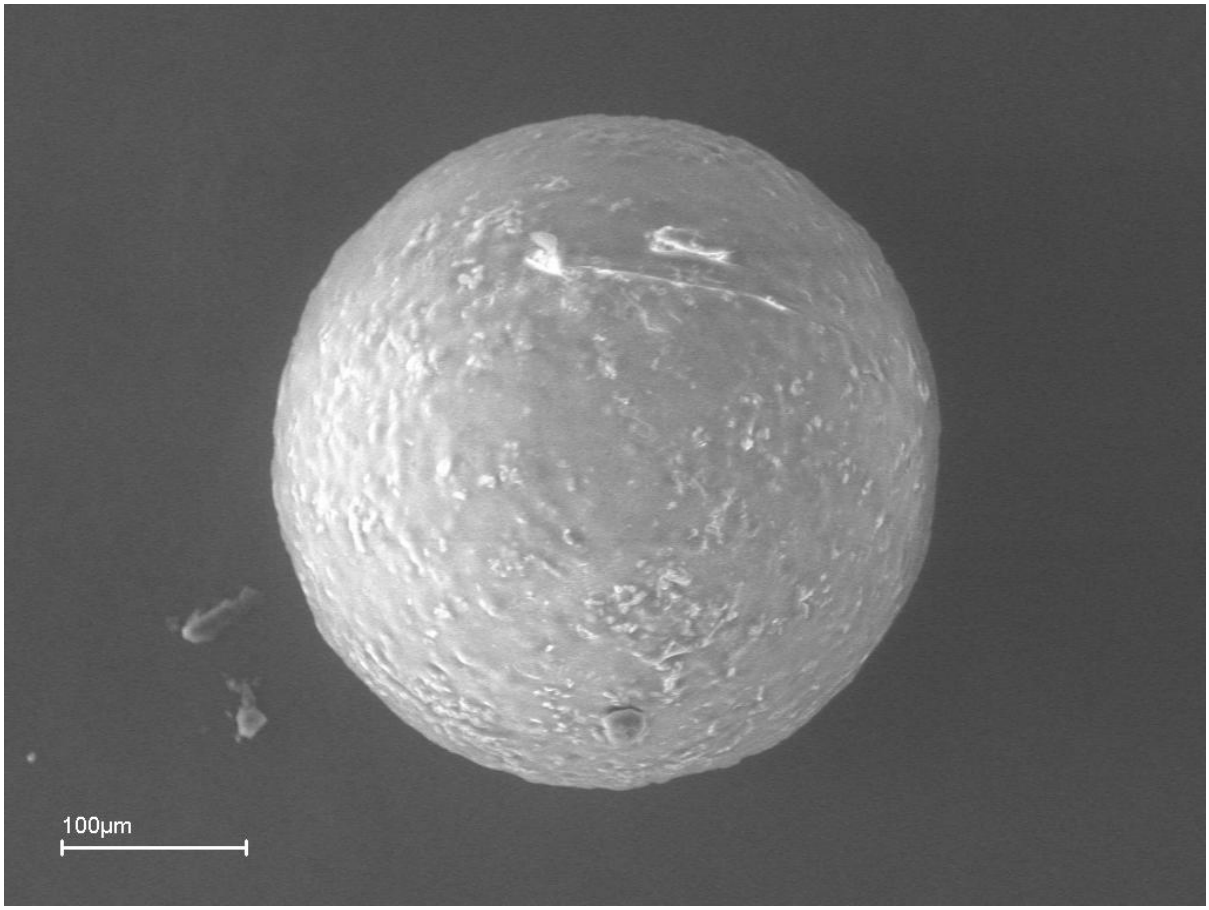

Figure 5.16. Scanning electron micrograph of microspheres prepared @ 35^0C using dual surfactants (Span 65+Tween 40) at 5.5 CHLB.

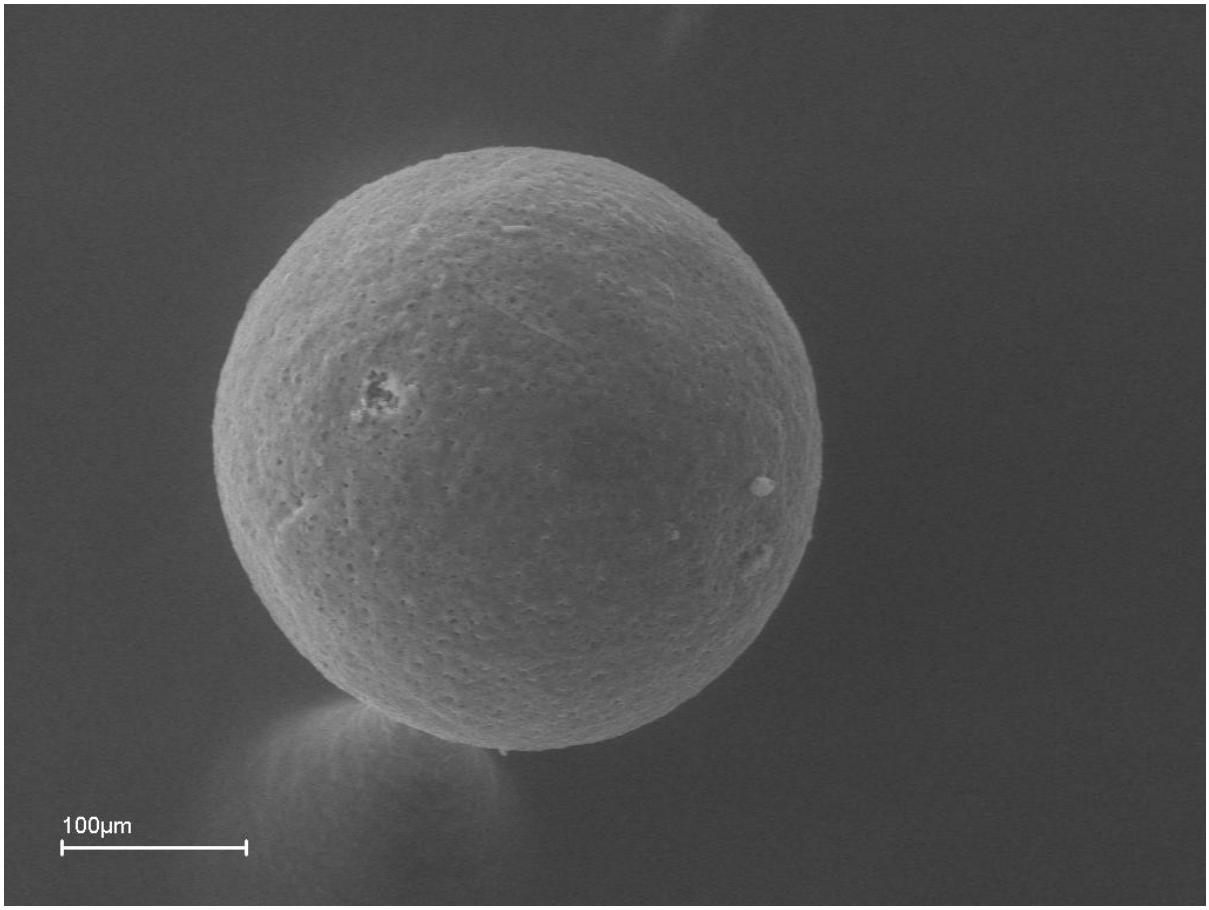

Figure 5.17. Scanning electron micrograph of microspheres prepared @ 35^0C using dual surfactants (Span 65+Tween 40) at 6.5 CHLB.

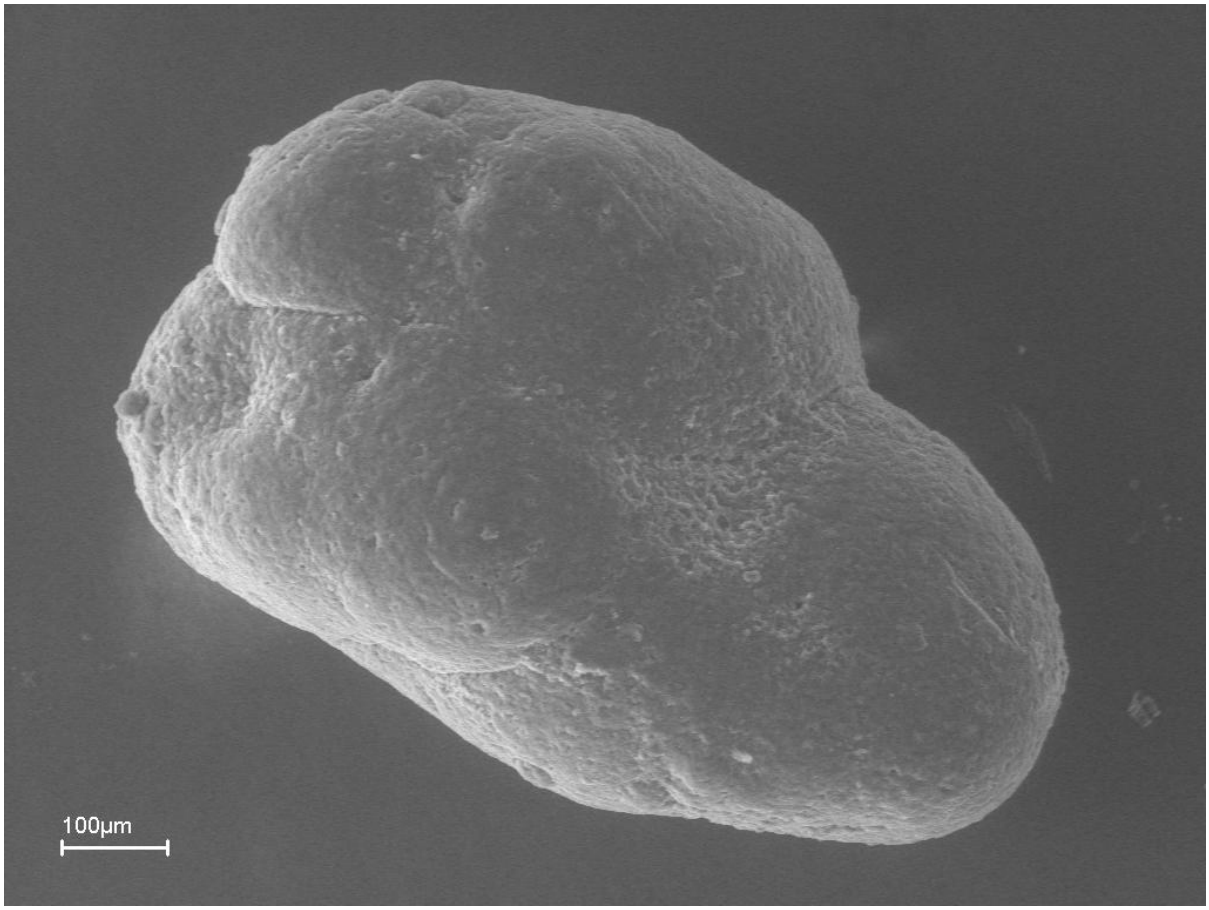

Figure 5.18. Scanning electron micrograph of microspheres prepared at 35^0C using dual surfactants (Span 65+Tween 40) at 7.5 CHLB.

CHAPTER 6

FORMULATION OF ETHYL CELLULOSE CONTROLLED RELEASE MICROSPHERES FOR SOLID DOSAGE: COMBINED EFFECTS OF SURFACTANT STRUCTURE AND PREPARATION TEMPERATURE

Research Paper to be submitted to Journal of Microencapsulation

Thakare Mohan, Capomacchia Anthony, Price James

Abstract

The aim of this work was to study the combined influence of dual surfactants structure and preparation temperature in the preparation of sustained release ethyl cellulose microspheres by the non-aqueous emulsion solvent evaporation technique. A factorial design of experiment (DOE) was designed to study the combined effects of the independent variables (X1) surfactant structures (polyoxyethelene sorbitan monopalmitate and polyoxyethylene cetyl ether) and (X2) temperature (22^0C and 35^0C) on the microsphere size, drug content, initial drug release etc. Temperature and surfactant structure, individually affected microsphere diameter and particle size distribution significantly. By changing the surfactant structure from polyoxyethelene sorbitan monopalmitate (POE-SM) to polyoxyethylene cetyl ether (POE-CE) in combination with temperature, percent drug loading and initial drug release increased significantly. Theophylline release kinetics showed diffusion type of release when surfactant changed from POE-SM to POE-CE only at 35^0C, all other microspheres formulations showed near zero-order release. The t_{50} release time was also increased. Scanning electron microscope photomicrographs of microsphere show that the particle surface roughness decreased as preparation temperature increased from 22^0C to 35^0C only if combined with POE-SM surfactant combination.

Keywords: Ethyl cellulose microspheres, surfactant structure, preparation temperature, particle size, drug loading, drug release.

(Total words: 179)

Introduction

Polymeric microspheres have received much attention for the delivery of therapeutically useful moieties in controlled release formulations. Various techniques can be employed to develop microparticulate systems including the solvent evaporation method for the preparation of microspheres [1]. One of the major issues with the solvent evaporation method is that solvent removal can become an extensive and time-consuming process involving several hours to days for complete solvent evaporation [2]. Process optimization, including temperature control for the solvent evaporation process, may be advantageous to develop microspheres for specific formulation release characteristics [3]. Therefore, investigating the effects of preparation temperature during the solvent evaporation process may be useful to achieve the desired drug release characteristics of microspheres.

Microsphere characteristics are also influenced by surfactant HLB. The HLB value is a measure of hydrophilic and lipophilic balance of the surfactant [4]. Surfactants may have similar or closely matching HLB but may have different chemical structures. For example the surfactant polyoxyethelene sorbitan monopalmitate (PSM) has 15.6 HLB, and polyoxyethylene-(20)-cetyl ether (PCE) has 15.7 HLB. Differences in the chemical structure may affect the activity of each surfactant regardless of their HLB.

The literature is replete with examples of surfactant use in different industries [5-7]. There are also reports on the effects of preparation temperature on microsphere formation and physical characteristics [8,9]. However, after literature search, we could not find any report on the combined effects of surfactant chemical structure and temperature on ethyl cellulose microspheres prepared by using dual surfactants.

Previous results from our lab indicate that surfactant as well as temperature independently may improve formulation and drug release characteristics of ethyl cellulose microspheres. These results indicate a need to study effect of dual surfactant structure in

combination with temperature as combined effect of two factors. We studied the combined influence of surfactant chemical structure and temperature as variables on different pharmaceutical properties of ethyl cellulose microspheres. Theophylline was used as the model drug as it has a narrow therapeutic index and it is sparingly soluble drug, and [10,11]. The solvent used for the dispersed phase solution was acetone, while the continuous phase medium was mineral oil.

The art of drug formulation can be strengthened by using statistical models such as factorial designs. Factorial design is an established method to study the effect of selected parameters. The surfactant chemical structure in dual surfactants in the formulation (X1) and temperature (X2) were selected as independent variables while the drug content, microsphere diameter, particle size and initial drug release were chosen as the dependent variables in the present investigation. The levels for the two parameters, X1 and X2 were determined from the preliminary trials; and selected formulations were evaluated for mechanism of *in vitro* drug release.

Experimental

Materials

The following chemicals were used: Ethyl cellulose (Scientific Polymer Products, New York), micronized theophylline (Gift sample from BASF), light mineral oil (Ruger Chemical Company Inc., Irvington, NJ), sorbitan tristearate (STE), polyoxyethelene sorbitan monopalmitate (PSM), and polyoxyethylene (20) cetyl ether (PCE) (Ruger Chemical Company Inc., Irvington, NJ), methylene chloride (Fisher Scientific, NJ), acetone, monobasic potassium phosphate and sodium hydroxide (J.T. Baker, Phillipsburg, NJ).

Instruments

The following instruments were used: Stirrer (Lab Stirrer LR 400D, Yamato Scientific Company Ltd., Tokyo, Japan), Dissolution Apparatus II USP (Dissolution Test system 5100, Distek Inc., North Brunswick, NJ and Prolabo dissolution), Aquamate (UV Spectrophotometer, Thermo Electron Corporation, Mercer's Row, Cambridge, UK), Accumet 5 pH meter (Fisher Scientific, NJ), USP Standard sieve series for PSD studies, Jacketed beaker (with input and output for water flow), Haake, thermostatted circulator bath Type FJ (W. Germany), temperature monitoring system fabricated at the Pharmaceutical Development Lab, University of Georgia.

Preparation of microspheres

For preparation of the different batches of microsphere, experimental conditions including temperature were constant. The preparation of ethyl cellulose microspheres containing theophylline was accomplished by emulsification solvent-evaporation method in a 1 L tall jacketed glass beaker. The jacketed beaker was temperature controlled using a water bath at either 22^0C ±1 or 35^0C ±1. The temperature monitoring system with a temperature monitoring computer software was attached to the jacketed beaker. Light mineral oil (300 ml), was used as the external or continuous phase, with the low HLB surfactant and placed in the1 L jacketed glass beaker (phase A). In a separate glass vessel, a 5 % solution of ethyl cellulose in acetone with the high HLB surfactant was prepared as phase B. Micronized anhydrous theophylline was dispersed in this solution to give a desired (33%) theoretical drug loading (1 part theophylline to 2 parts ethyl cellulose). The entire contents of this vessel (phase B) were added into the glass beaker containing the solution of light mineral oil and a low HLB surfactant (phase A) under rigorous agitation. Agitation was continued until the solution became clear after the acetone evaporated and all microspheres precipitated out of the solution. These microspheres were decanted, filtered and washed with a mineral spirit

solution to remove the residual light mineral oil. The clean microspheres were then dried in an oven at 50^0C overnight.

Particle size distribution

The size distribution of microspheres was determined by sieving the microspheres using a set of standard sieves ranging from 105-710μm. A pan was placed underneath the sieves to collect the particles that pass through the last sieve. The aggregate sample was placed on the sieve of largest size, covered, and then tapped by hand until no change in weight was observed in the sieves. Manual checking and hand sieving was applied to assure that all particles retained on a sieve were bigger than the sieve apertures. After sieving, the quantity of each fraction of particles was measured by weighing. Particle size distribution and geometrical mean diameter were calculated.

Determination of Drug loading

The 355 μm fraction of each batch of ethyl cellulose microspheres was calculated for drug loading. Actual drug loading was determined by dissolving accurately weighed samples (in triplicate) in methylene chloride using 25 ml volumetric flasks. Drug concentration was determined by measuring ultraviolet absorbance at 276.5 nm. Spetrophotometric interference from ethyl cellulose blanks was not observed at this wavelength.

In vitro dissolution studies

In vitro release studies of ethyl cellulose microspheres were performed using a USP dissolution apparatus II (Distek Inc., New Jersey) at 100 rpm as described in USP 31. The 355 μm fraction of each batch of microspheres was selected for evaluation. Microsphere samples in triplicate for each batch were suspended in 900 ml of simulated intestinal fluid with 0.1% Tween 20, and no enzymes. The dissolution study was carried out at 37 ± 0.5^0C at 100 r.p.m. for 12-24 hours. Three ml of sample were withdrawn at specific time intervals and

replaced with fresh simulated intestinal fluid medium. The drug released was determined spectrophotometrically at 274 nm. The dissolution data was evaluated for initial release, dissolution rate and the mechanism of drug release.

Release kinetics

Data obtained from *in vitro* release studies were fitted to Higuchi kinetic equations to determine the mechanism of drug release from ethyl cellulose microspheres [4].

Scanning electron microscope (SEM) analysis of microspheres

The surface morphology of microspheres was observed by scanning electron microscope (SEM) using a Zeiss model 1450EP SEM. Microspheres were mounted on metal multi-stubs using double-sided adhesive tape and SEM images were taken at specific magnifications.

Result and discussion

Microsphere preparation

Ethyl cellulose microspheres were prepared by the solvent evaporation method and initial experiments were carried out to optimize the formulation process. The formulations containing dual surfactants with different chemical structure at two different temperatures for microsphere preparation are shown in Table 6.1. Formulation preparation times are shown in Table 6.2. Formulations containing the dual surfactants sorbitan tristearate (STE) and polyoxyethelene sorbitan monopalmitate (POE-SM) are labeled DS1, whereas formulations containing dual surfactants sorbitan tristearate (STE) and polyoxyethylene cetyl ether (POE-CE) are labeled DS2. An almost 50% reduction in microsphere preparation time was observed when the preparation temperature was elevated from 22^0C to 35^0C in both the formulations DS1 and DS2, indicating that elevated temperature increased rate of solvent evaporation, thus reducing preparation time (Table 6.2).

Particle size distribution (PSD) and geometric mean diameter (GMD)

The data of particle size distribution was analyzed using the general factorial design, Stat-Ease Design-expert software®. The temperature and surfactant chemical structure both individually and significantly affected particle size distribution at 710 µm, 595 µm, 355 µm, 259 µm, 125 µm sieve sizes and particle size diameter of the microspheres as shown in Figures 6.1 to 6.6. The changes in particle size and their distribution are clearly the result of both the temperature increase from 22^0C to at 35^0C and the effects of changing the chemical structure of the dual surfactant (Figure 6.1). Formulation DS2 shows a greater proportion of smaller particles as particle size reduced from 770 µm to 125 µm, whereas formulation DS2 shows the opposite trend (Figures 6.2 - 6.6). The combination of manipulating temperature and surfactant chemical structure appears to control microsphere particle size. Particle size had a direct effect on drug loading and release.

Drug loading

As seen in Figure 6.7, the drug loading studies of the 355 µm fractions of the microsphere batches revealed that there was a significant increase in percent theophylline loading as a function of surfactant chemical structure and temperature. Increase in preparation temperature from at 22^0C to at 35^0C significantly increased the drug loading percent (Figure 6.7). The microsphere batches for both formulations DS1 and DS2 were prepared with 33% theoretical drug loading. With elevated temperature, the increase in percent drug loading was attributed to the rapid solidification of polymer that gave rise to a denser outer layer on the microsphere[12]. Many factors affect the fast solidification of the polymer such as a higher solvent removal rate, higher polymer concentration or a lower ratio of dispersed phase to continuous phase DP/CP)[13]. In our case, the rate of solvent removal was increased (Table 6.2). Although the rate of solvent removal and thickness of the microsphere outer layer were different, they were not reflected in the encapsulation efficiency between the

two different microsphere formulations DS1 and DS2. The increased percent drug loading seen in the latter was attributed, as referenced, to comparatively reduced surface tension activity due to the different chemical structures irrespective of their HLB value. Thus, this may have helped to form a more stable emulsion during the microsphere preparation process, increasing homogenous dispersion and stabilizing theophylline in the formulation

In vitro drug release behavior

The *in vitro* release profiles of ethyl cellulose microspheres prepared with formulations DS1and DS2 are shown in Figure 6.8. Changing the surfactant structure from polyoxyethelene sorbitan monopalmitate (POE-SM; DS1) to polyoxyethylene cetyl ether (POE-CE; DS2), and keeping the temperature constant at 22^0C showed little difference in the dissolution profile for DS1 or DS2. However DS1 showed an increase in the cumulative theophylline released as the temperature was increased to 35˚C. Figure 6.8 shows that the microspheres formulated as either DS1 or DS2 exhibited adequate sustained-release profiles. The combined effect of surfactant structure and temperature variables on initial percent theophylline release was examined. Release within the first 30 minutes was analyzed for the fraction of 355 μm size microspheres batches (Figure 6.9). The combined effect of surfactant structure and the temperature significantly affected initial drug release *(p value < 0.0001).* By changing the surfactant structure from DS1 to DS2 in combination with increasing temperature, the initial drug release was increased significantly *(p value < 0.0001*). Figure 6.10 shows that DS1 compared to DS2 demonstrate very different behavior. At a constant temperature of 22^0C DS1 has a lower $t_{50\%}$ than DS2. However as temperature is changed from 22^0C to 35^0C an increase in initial drug release for DS2 is observed but that for DS1 decreased. The mechanism of drug release is naturally influenced by many factors such as the presence and the location of drug and surfactant molecules in the microsphere particle. The possible sites of location of the surfactant molecules could be either on the surface of the

microsphere, within the matrix isolated from the interior environment or within the microsphere but connected with its outer surface possibly by channels. The differences in release profiles at different temperature are the consequence of differences in the surface area because of the particle surface roughness and porosity that has changed with different variables as observed in SEM analysis.

Drug release kinetics

Data obtained from *in vitro* release studies were fitted to Higuchi kinetic equations to determine the mechanism of drug release from ethyl cellulose microspheres [4]. Microsphere batches DS1+22^{0}C, DS2+22^{0}C, and DS2+35^{0}C showed near zero-order release. This is not a typical spherical matrix release. Developing matrix microsphere formulation with a a zero-order release has always been challenge to the pharmaceutical scientist [14]. The release from the monolithic matrix is inherently non-linear due to an increase in the diffusional length resistance and/or the decrease in the inwardly releasing surface area with time [14]. We propose different reasons for the near zero-order release behavior of our matrix formulations batches. There is a possibility of drug accumulation at the center and the matrix is behaving as a microcapsule. With the microsphere batches showed near order zero-release the exterior of the shell is dense and less porous, and interior of the shell is more porous. These structural changes are resulting in the zero order release. The dense exterior is giving an impact of the microcapsule.

Scanning electron microscopy (SEM) analysis

SEM micrographs of microspheres prepared with formulations DS1 and DS2 are contained in Figures 6.12 – 6.19. The SEM micrograph of microspheres prepared at 22^{0}C using dual surfactant formulation DS1 is shown in Figure 6.12, and a cross-section in Figure 6.16. The microspheres were spherical with rough surfaces and no agglomeration was observed. Formulation DS2 at 22^{0}C is shown in Figure 6.13, and was nearly spherical with

slightly rough surfaces. The cross section is shown in Figure 6.17 and was found to agglomerate. Microspheres from formulation DS1 at 35^0C preparation temperature presented as spheres with a smooth surface (Figure 6.14). The cross section is shown in Figure 6.18. These microspheres were free flowing with no agglomeration. Increasing preparation temperature from 22^0C to 35^0C affected the surface morphology of the microspheres and resulted in a dense and more porous surface (Figure 6.14). Microspheres from formulation DS2 at 35^0C preparation temperature were mostly spherical with a rough surface (Figure 6.15). The cross section is shown in Figure 6.19 and they showed agglomeration. Changing the surfactant structure from the polyoxyethelene sorbitan monopalmitate to polyoxyethylene cetyl ether (DS1 to DS2) affected the surface morphology of the microspheres with the DS2 formulations presenting with a rougher surface. Agglomeration seen in some of the micrographs might be due to the fact that small microsphere size tends to agglomerate and the resultant SEM photograph shows non spherical particles. The change in temperature from 22^0C to 35^0C caused evaporation of solvent and stabilization of the emulsion and thus formation of more uniform and dense droplets dispersed in the oil phase. After solidification these droplets lead to a more dense surface structure. The formation of the microsphere outer layer is often likened to the two basic processes such as phase separation and precipitation of asymmetric membranes. SEM analysis shows that the high-temperature (35^0C) solvent removal results in less defective membrane skin layer as discussed above specifically only for DS1 formulation. Microspheres at from DS1 formulations at high (35^0C) temperature, the dispersed phase will most likely take the coagulation path during formation. Because of high temperature (35^0C) the solvent removal is rapid. With this scenario out-flow of solvent would be higher than in-flow of mineral oil. This leads to an increase in polymer concentration within the dispersed phase that then solidifies into a dense layer. For microspheres made at low (22^0C) temperatures, polymer precipitation probably follows another path. Here, the

solvent (acetone) out-flow is slower allowing increased in-flow of mineral oil affecting the morphology of microsphere.

Conclusion

The surfactant chemical structure and preparation temperature of ethyl cellulose microspheres fabricated by non-aqueous emulsion solvent evaporation method has combined effect on different physicochemical and release properties of microsphere. The temperature and surfactant structure both individually and significantly affect the size diameters of the microspheres. There was a highly significant effect of combined temperature and surfactant structure variables on the particle size distribution. By changing the surfactant structure from polyoxyethelene sorbitan monopalmitate (POE-SM) to polyoxyethylene cetyl ether (POE-CE) in combination with temperature, percent drug loading, and initial drug release was increased significantly. The t_{50} release time for theophylline release was also found to increase. Theophylline release kinetics showed diffusion type of release for the batch DS1+35^{0}C. All other microsphere formulations showed near zero-order release.

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Table 6.1. Different formulation and experimental conditions for microsphere preparation.

Surfactant structure formulations [@]	**Temperature**
Sorbitan tristearate (HLB=2.1) + POE-SM* (HLB=15.6)	22^0C
Sorbitan tristearate (HLB=2.1) + POE-SM* (HLB=15.6)	35^0C
Sorbitan tristearate (HLB=2.1) + POE-CE** (HLB=15.7)	22^0C
Sorbitan tristearate (HLB=2.1) + POE-CE** (HLB=15.7)	35^0C

* polyoxyethelene sorbitan monopalmitate
** polyoxyethylene cetyl ether
[@] The formulation containing dual surfactants sorbitan tristearate (STE) and polyoxyethelene sorbitan monopalmitate (POE-SM) is labeled DS1 and the formulation containing dual surfactant sorbitan tristearate (STE) and polyoxyethylene cetyl ether (POE-CE) is labeled as DS 2.

Table 6.2. Effect of surfactant chemical structures and temperature on the microsphere preparations time at different CHLBs.

Dual surfactants used	**Temperature**	**Microsphere preparation time**
Sorbitan tristearate (HLB=2.1) + POE-SM (HLB=15.6)	22^0C	16 to 18 hrs
Sorbitan tristearate (HLB=2.1) + POE-SM (HLB=15.6)	35^0C	8 to 9 hrs
Sorbitan tristearate (HLB=2.1) + POE-CE (HLB=15.7)	22^0C	16 to 18 hrs
Sorbitan tristearate (HLB=2.1) + POE-CE (HLB=15.7)	35^0C	8 to 9 hrs

Table 6.3. Higuchi equation regression analysis of different microsphere batches prepared at different preparation temperature.

Surfactant structure	**Temperature**	**Higuchi equation R^2**
Sorbitan tristearate (HLB=2.1) + POE-SM* (HLB=15.6)	22^0C	0.9636
Sorbitan tristearate (HLB=2.1) + POE-SM (HLB=15.6)	35^0C	0.9942
Sorbitan tristearate (HLB=2.1) + POE-CE** (HLB=15.7)	22^0C	0.9880
Sorbitan tristearate (HLB=2.1) + POE-CE (HLB=15.7)	35^0C	0.9543

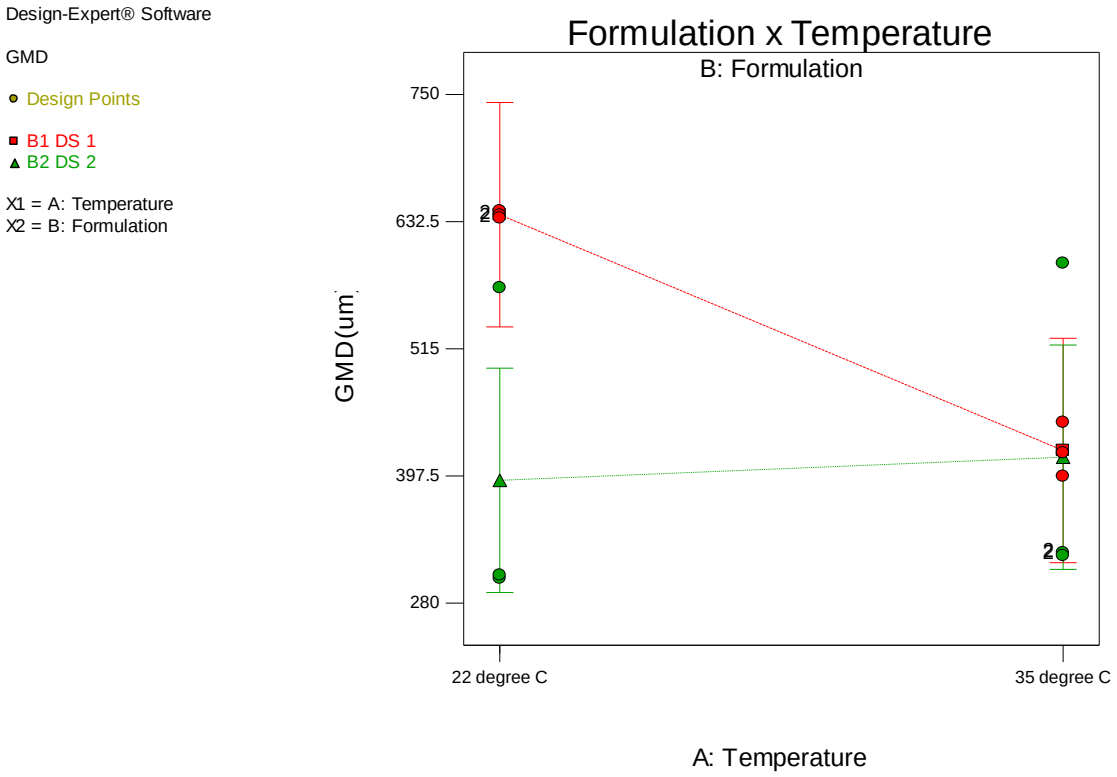

Figure 6.1. Combined effects of surfactant structures and temperature on geometric mean diameter of microsphere batches prepared with dual surfactants.

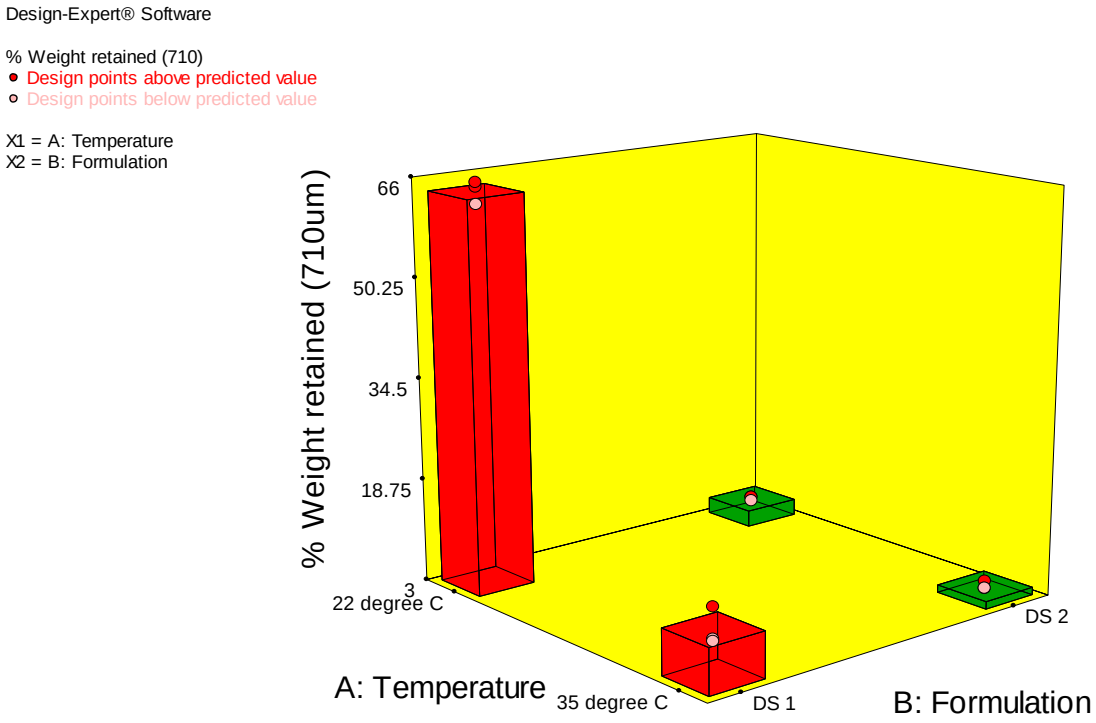

Figure 6.2. Combined effects of surfactant structures and temperature on particle size distribution of microsphere batches prepared with dual surfactants at 710 µm sieve size.

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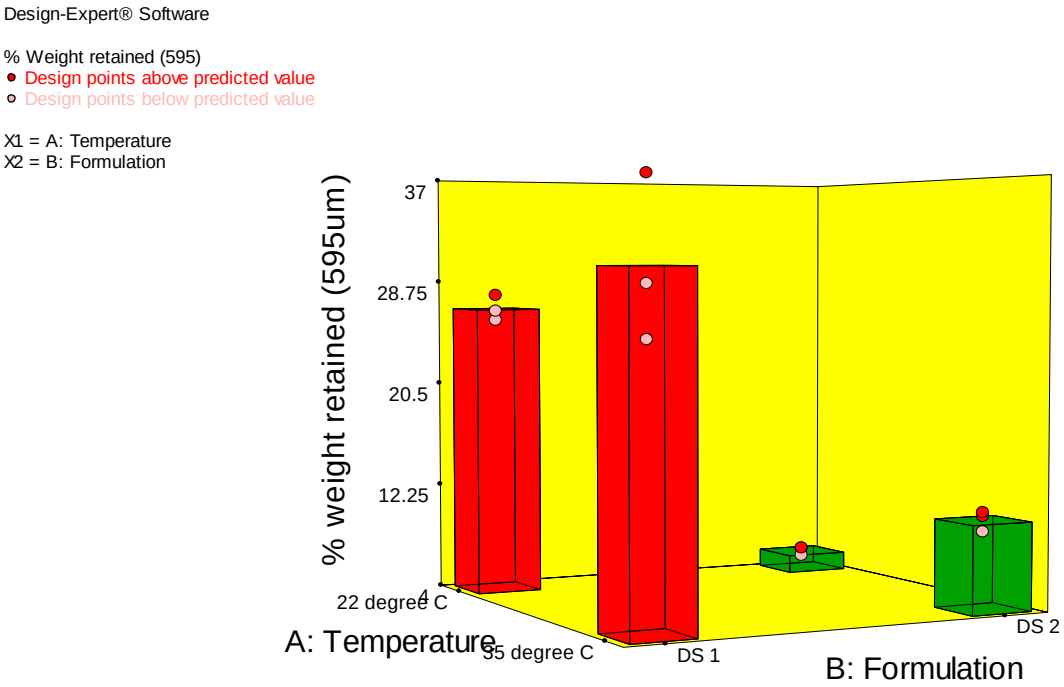

Figure 6.3. Combined effects of surfactant structures and temperature on particle size distribution of microsphere batches prepared with dual surfactants at 595 µm sieve size.

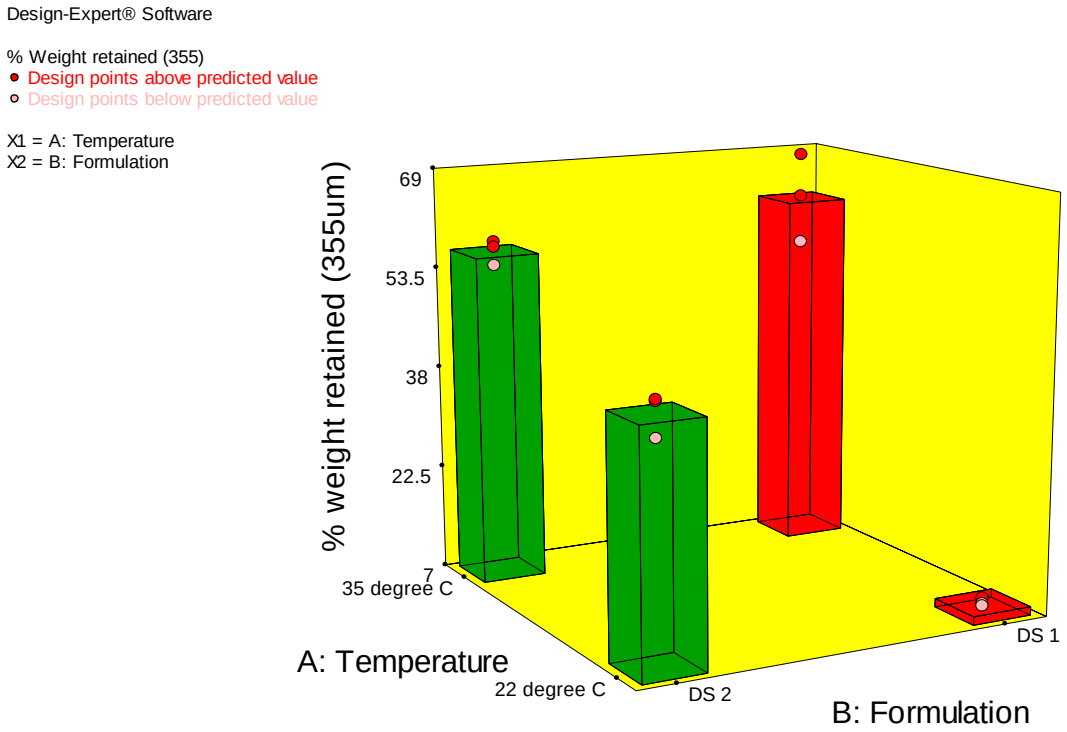

Figure 6.4. Combined effects of surfactant structures and temperature on particle size distribution of microsphere batches prepared with dual surfactants at 355 µm sieve size.

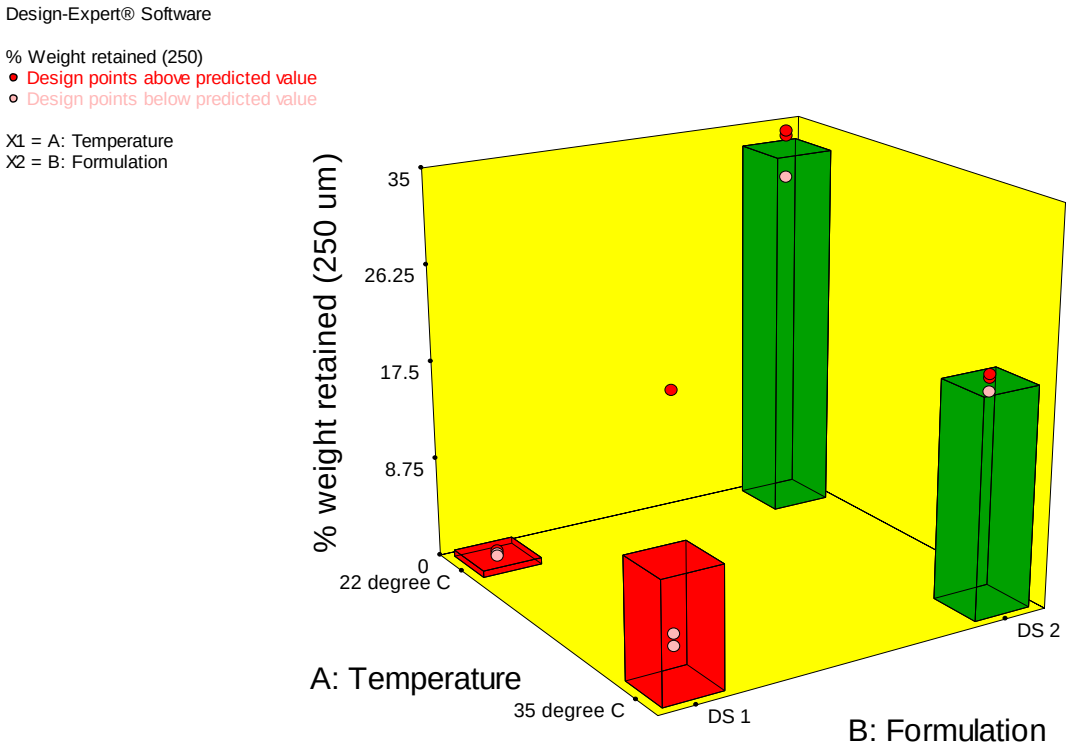

Figure 6.5. Combined effects of surfactant structures and temperature on particle size distribution of microsphere batches prepared with dual surfactants at 250 µm sieve size.

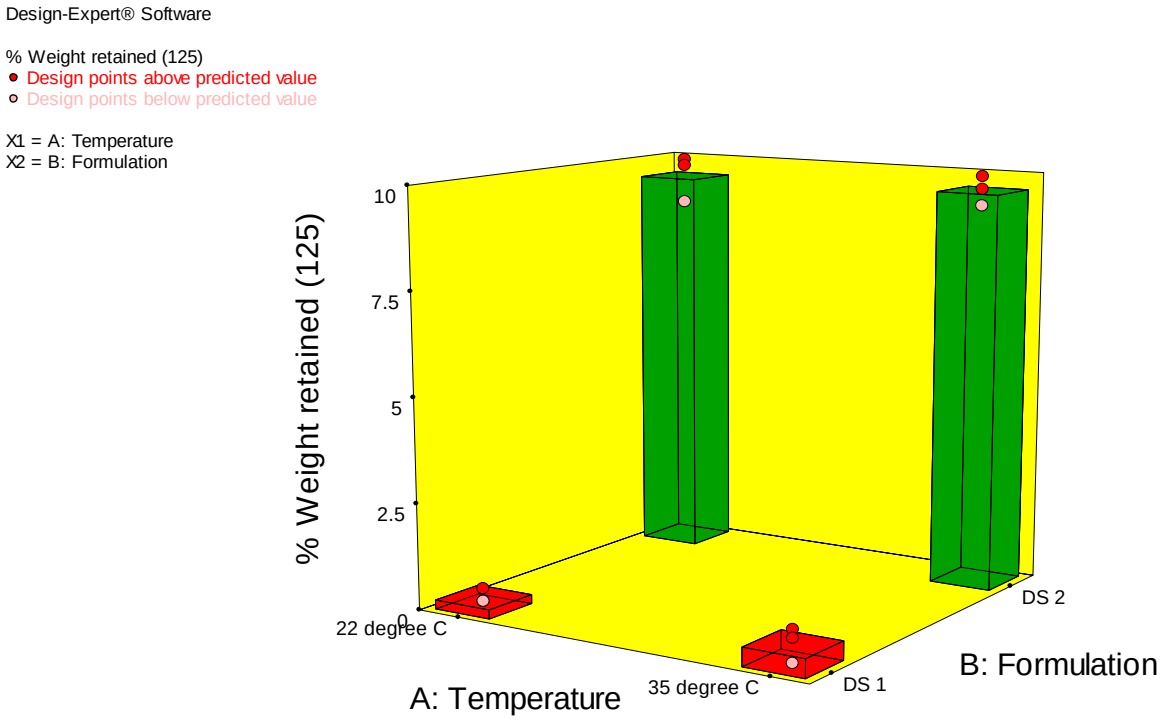

Figure 6.6. Combined effects of surfactant structures and temperature on particle size distribution of microsphere batches prepared with dual surfactants at 125 µm sieve size.

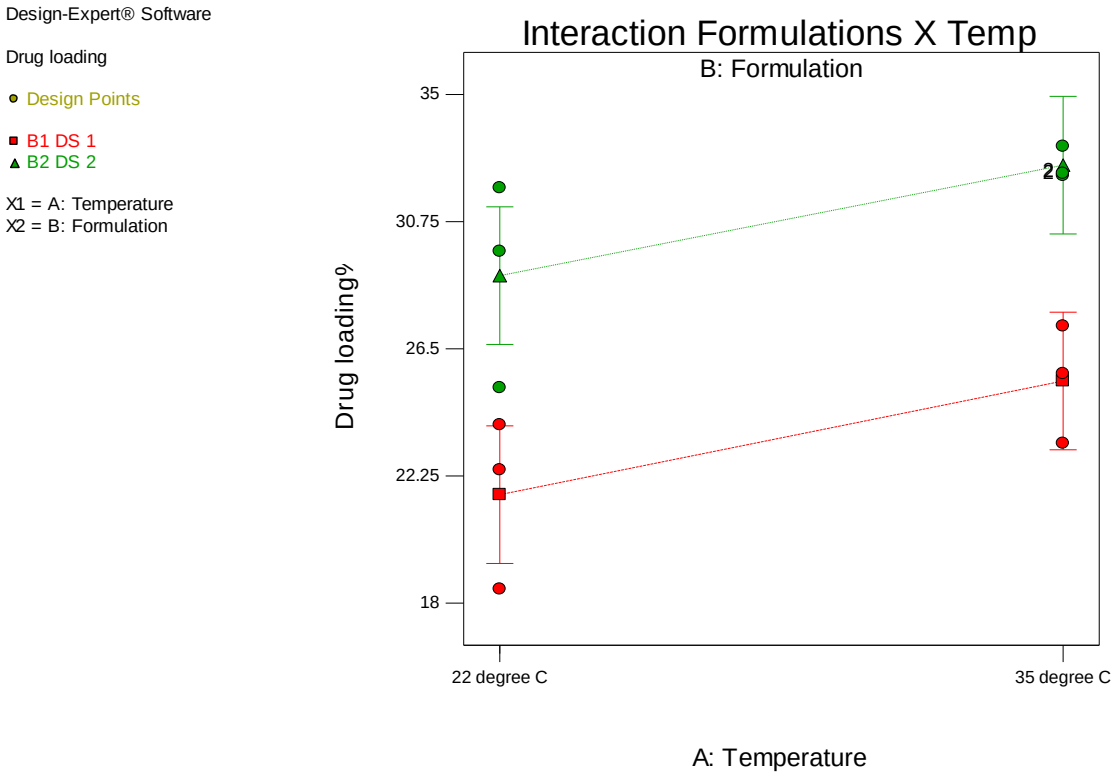

Figure 6.7. Combined effects of surfactant structure and temperature levels on drug loading percent of microspheres at 355 µm fraction size.

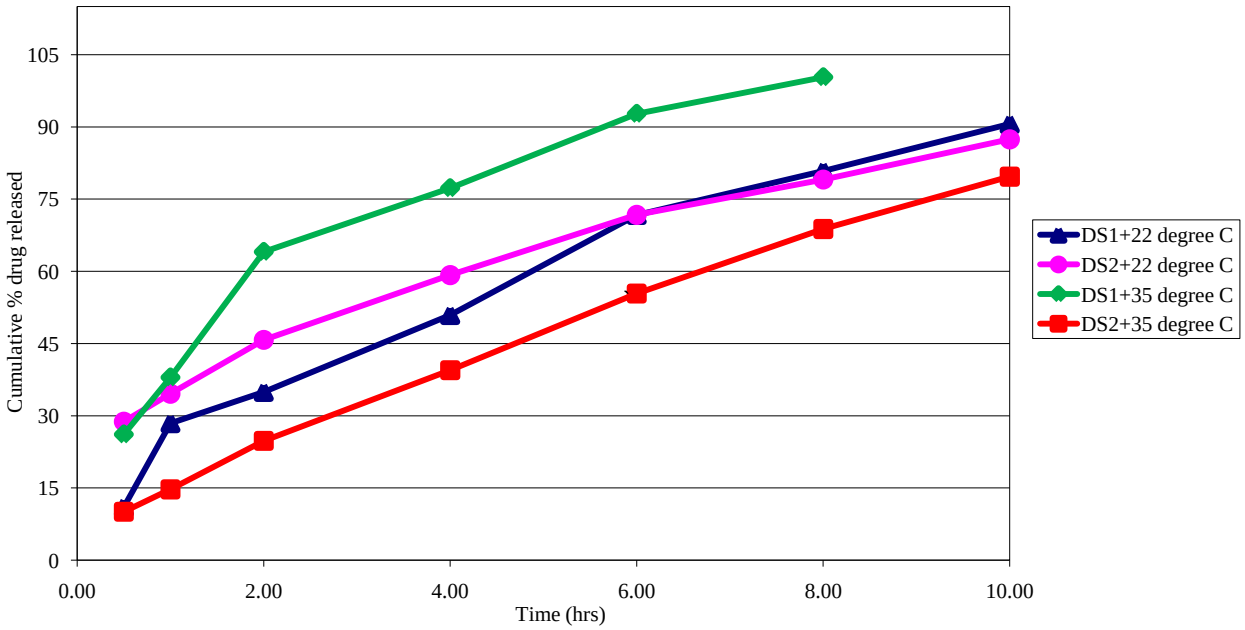

Figure 6.8. Combined effects of surfactant structure and temperature levels on *in vitro* dissolution studies of 355 µm fraction size of microsphere batches prepared with dual surfactants.

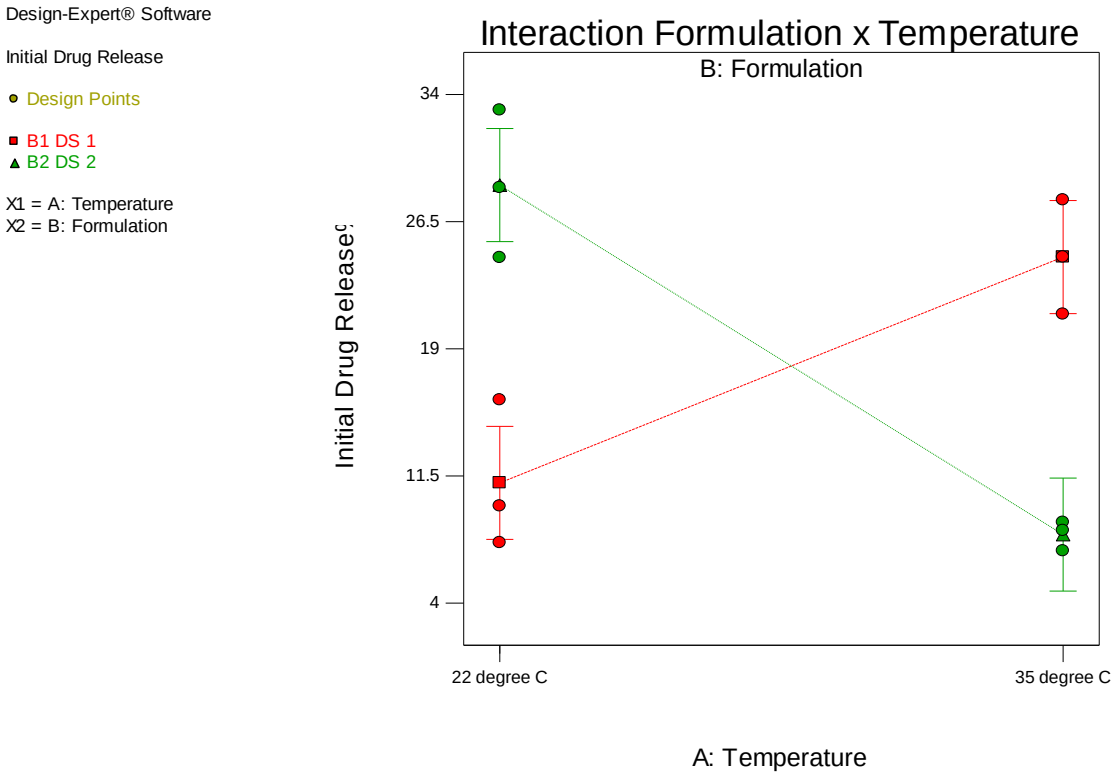

Figure 6.9. Combined effects of surfactant structure and temperature levels on initial drug release percent of 355 µm fraction size of microsphere batches prepared using dual surfactants.

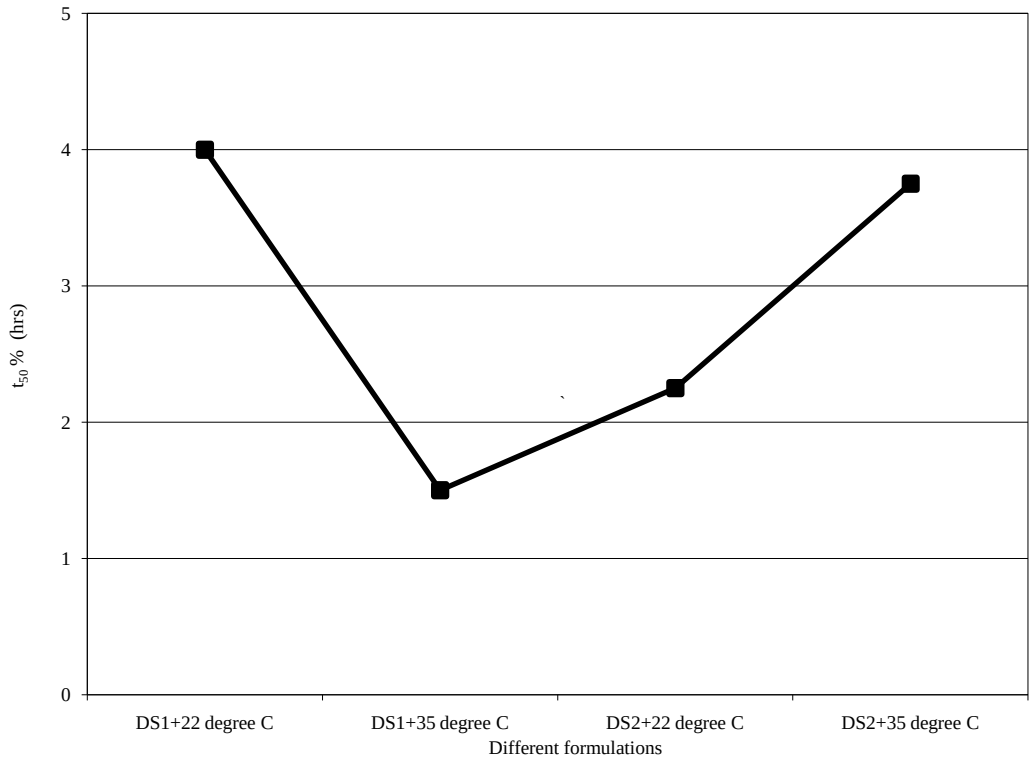

Figure 6.10. Combined effects of surfactant structure and temperature levels on drug release at t_{50} of 355 µm fraction size of microsphere batches prepared using dual surfactants.

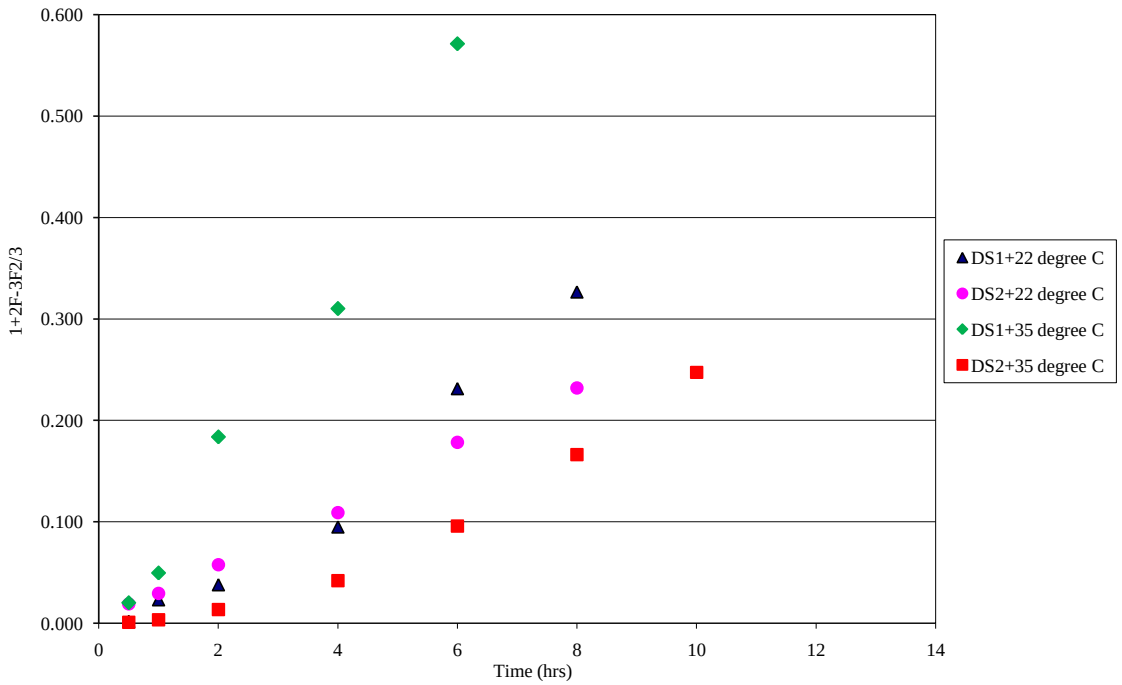

Figure 6.11. Combined effects of surfactant structure and temperature levels on dissolution kinetics of 355 µm fraction size of microsphere batch prepared with dual surfactants

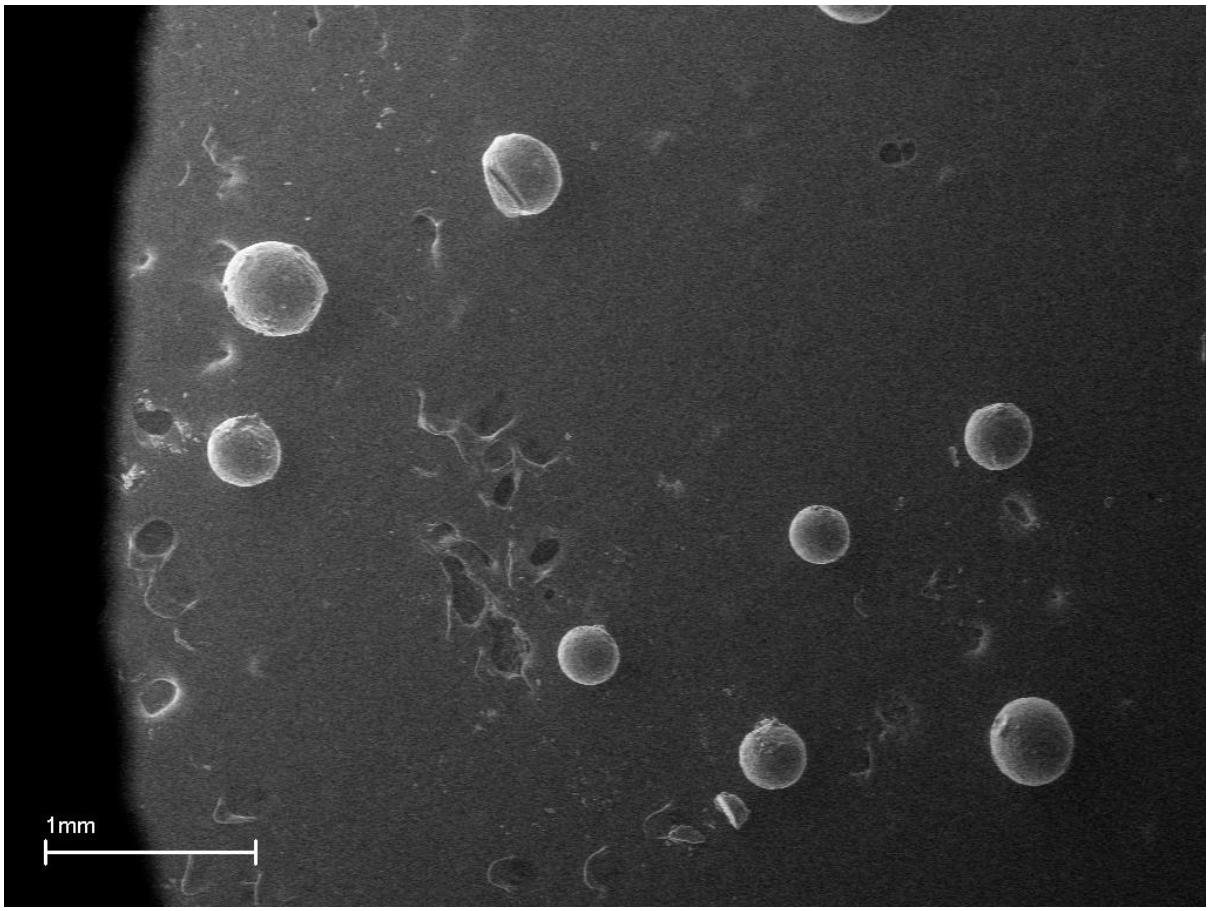

Figure 6.12. Scanning electron micrograph of microspheres prepared at 22^0C using formulation DS1.

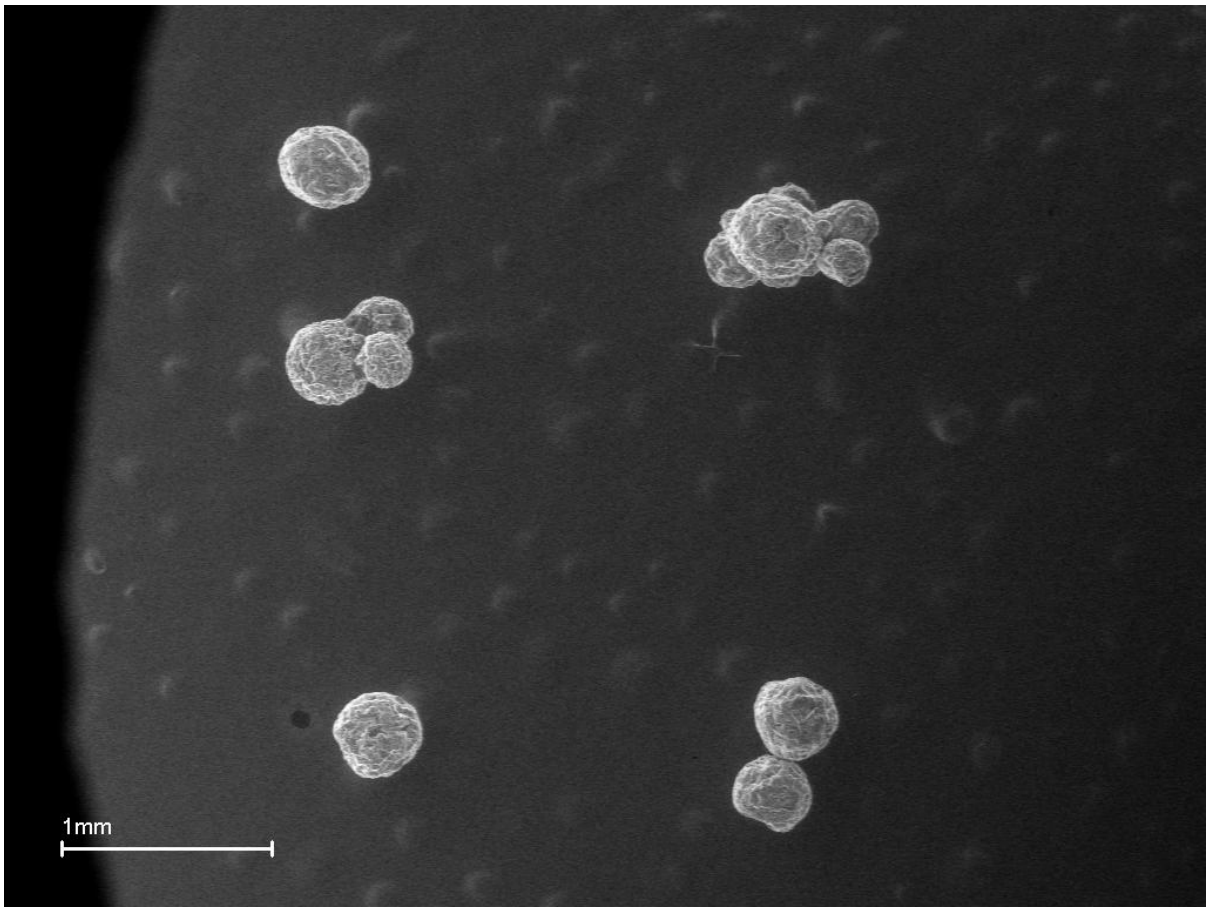

Figure 6.13. Scanning electron micrograph of microspheres prepared at 22^0C using formulation DS2.

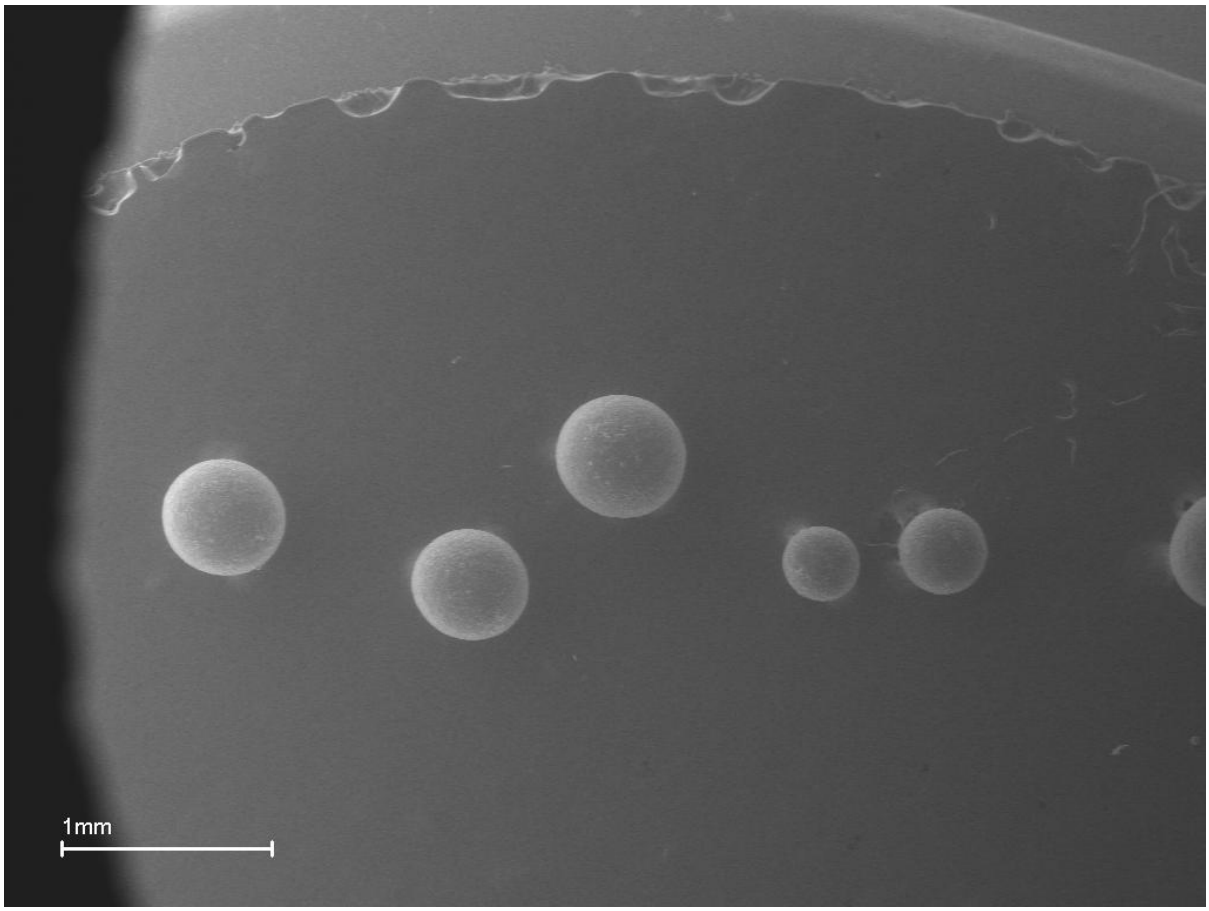

Figure 6.14. Scanning electron micrograph of microspheres prepared at 35^0C using formulation DS1.

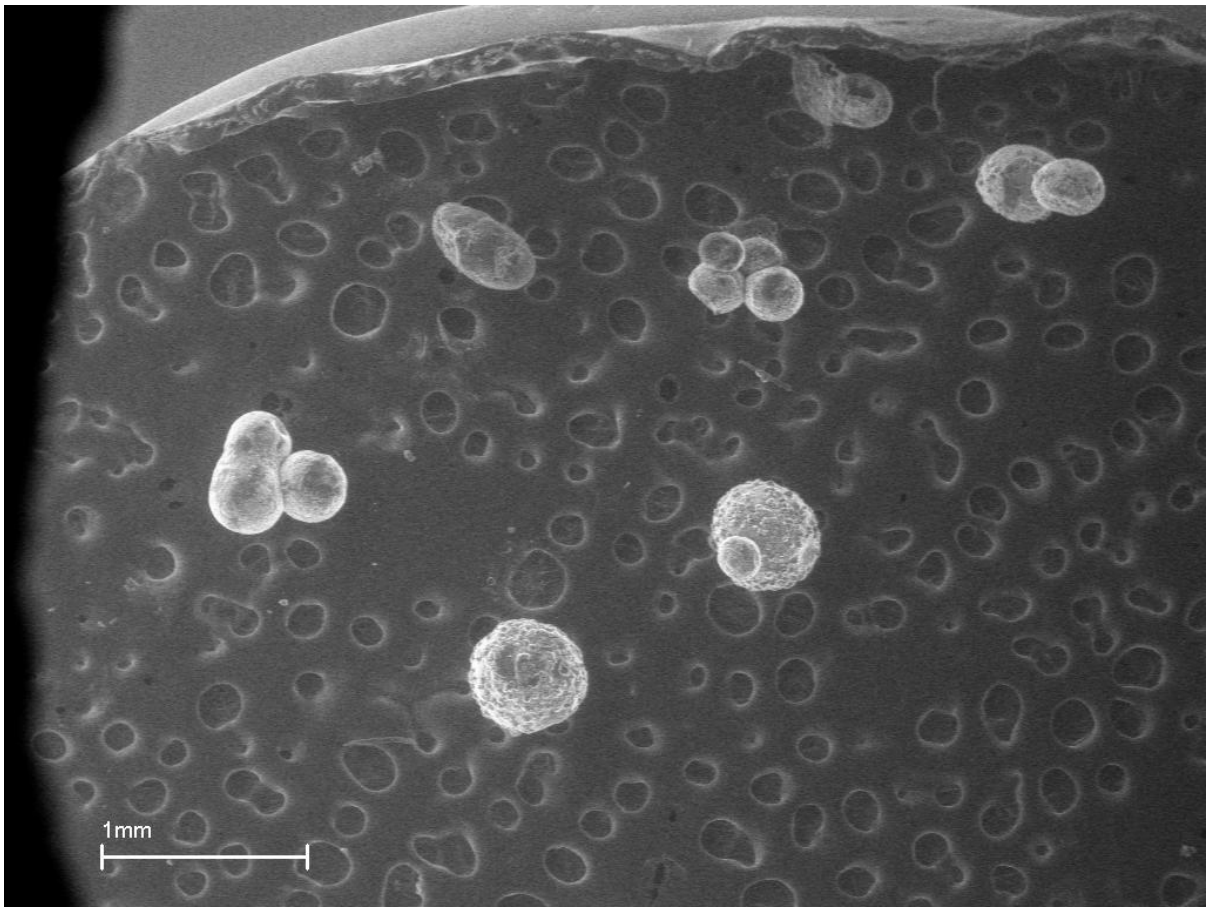

Figure 6.15. Scanning electron micrograph of microspheres prepared at 35^0C using formulation DS2.

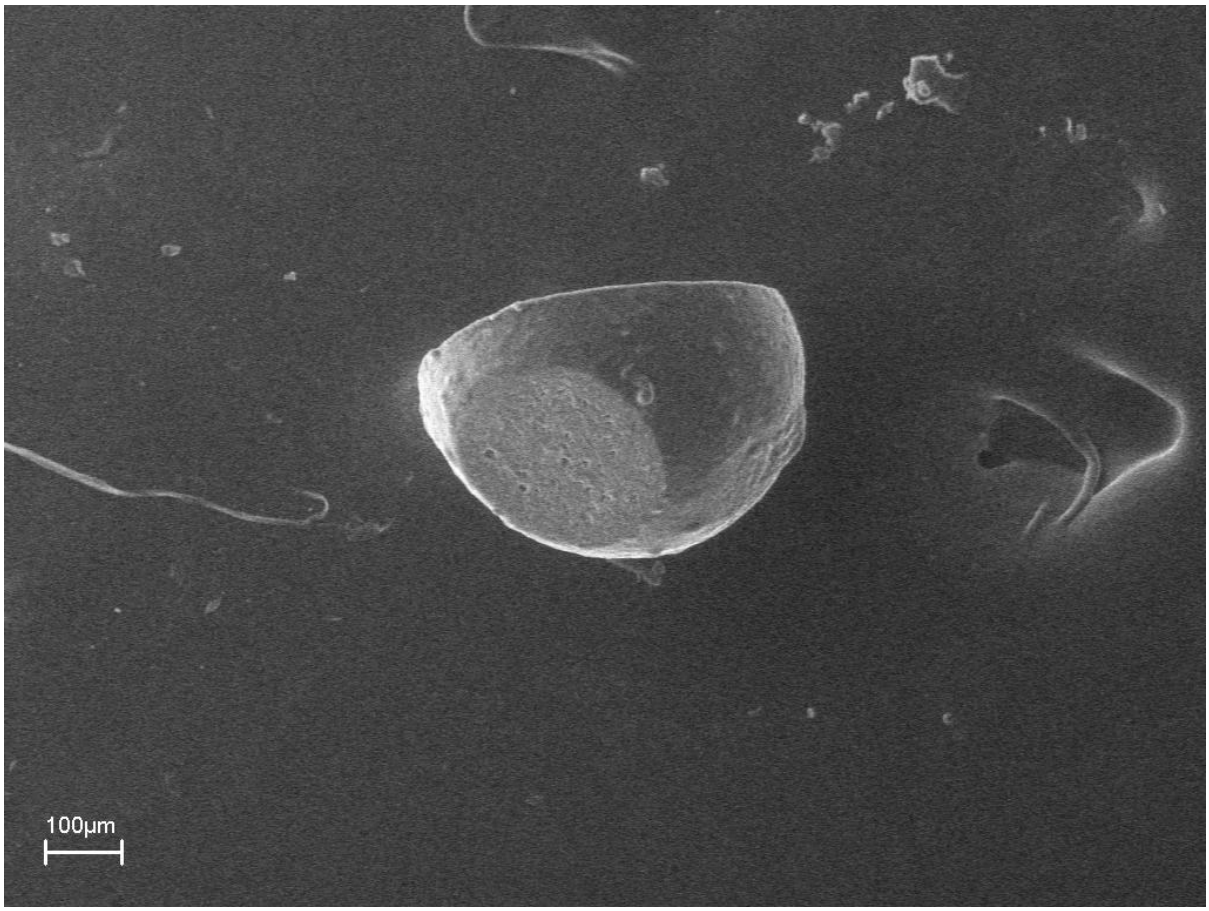

Figure 6.16. Scanning electron micrograph of cut section of microspheres prepared at 22^0C using formulation DS1.

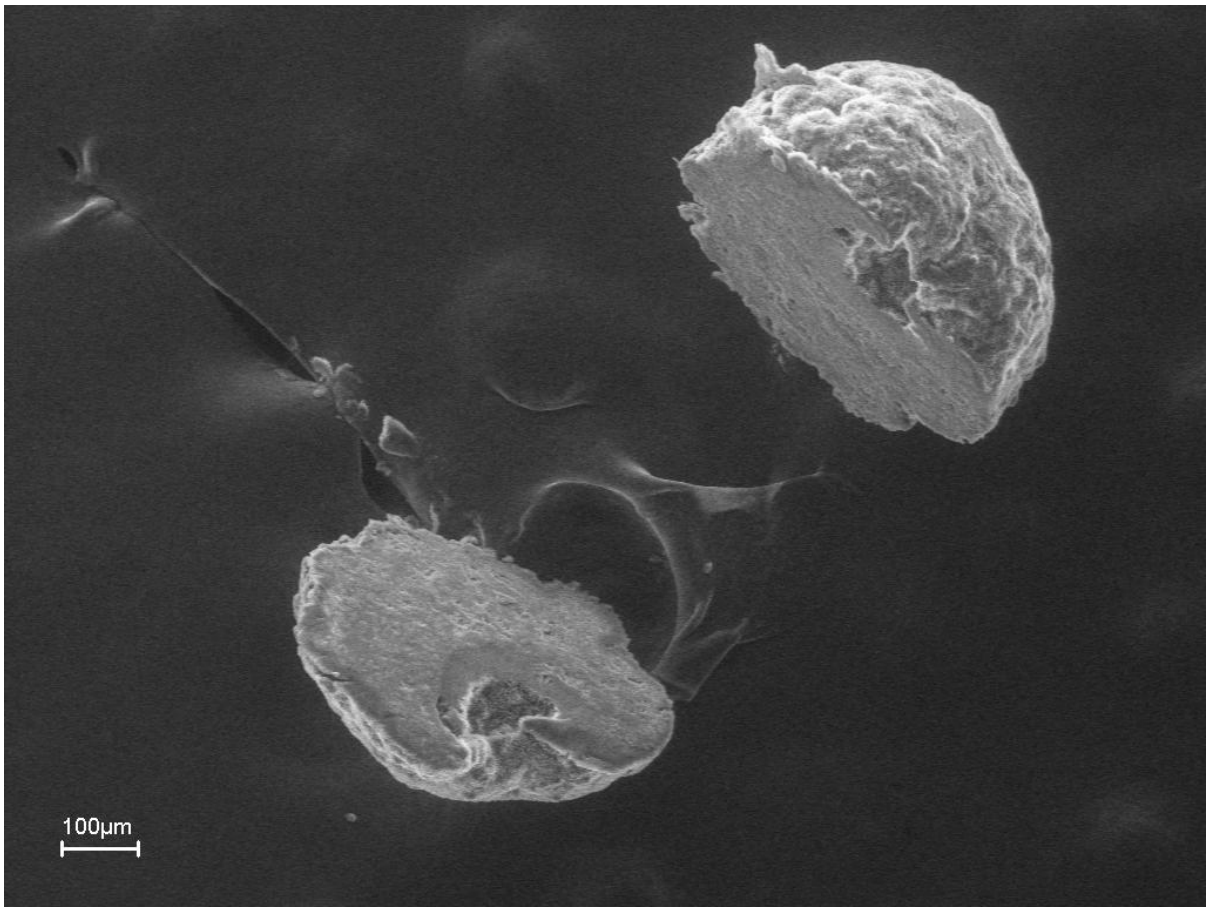

Figure 6.17. Scanning electron micrograph of cut section of microspheres prepared at 22^0C using formulation DS2.

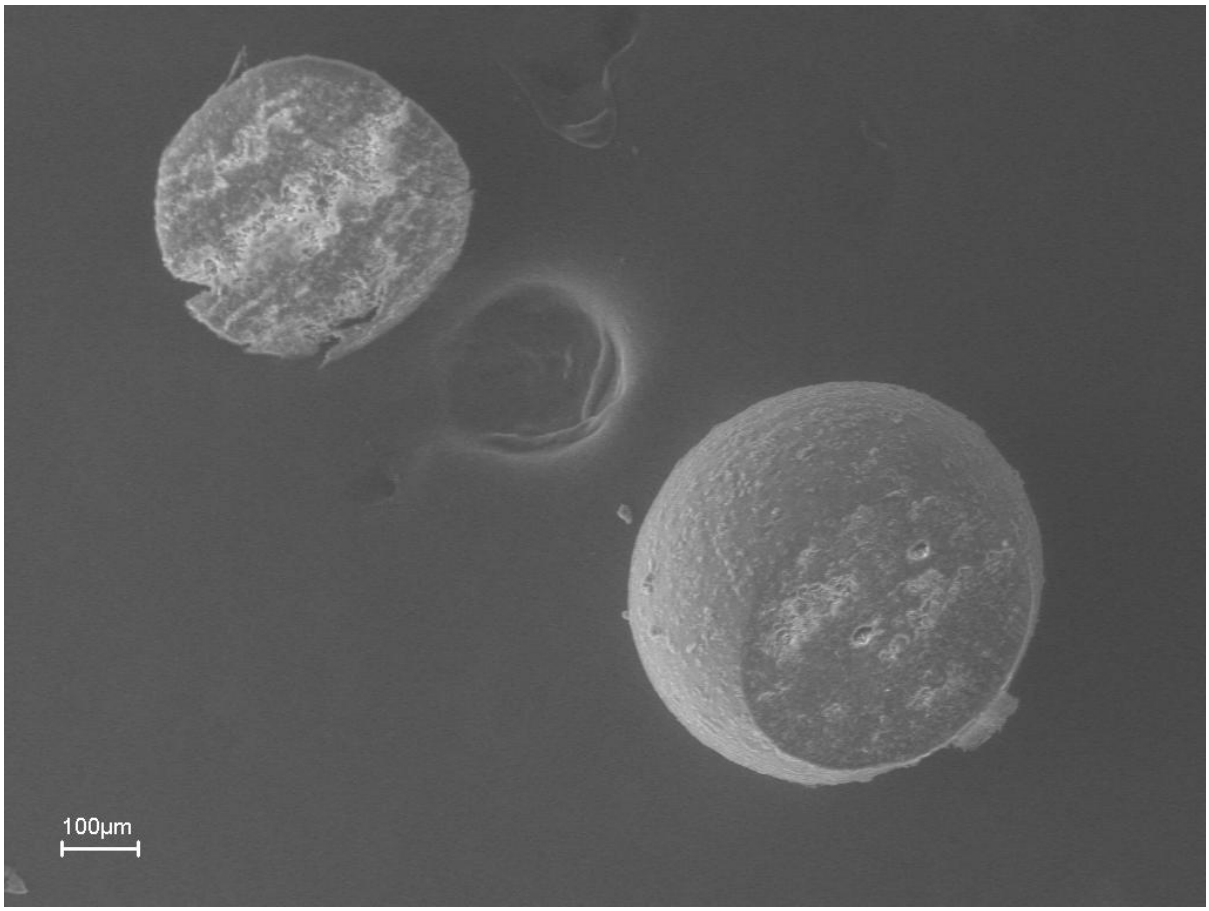

Figure 6.18. Scanning electron micrograph of cut section of microspheres prepared at 35^0C using formulation DS1.

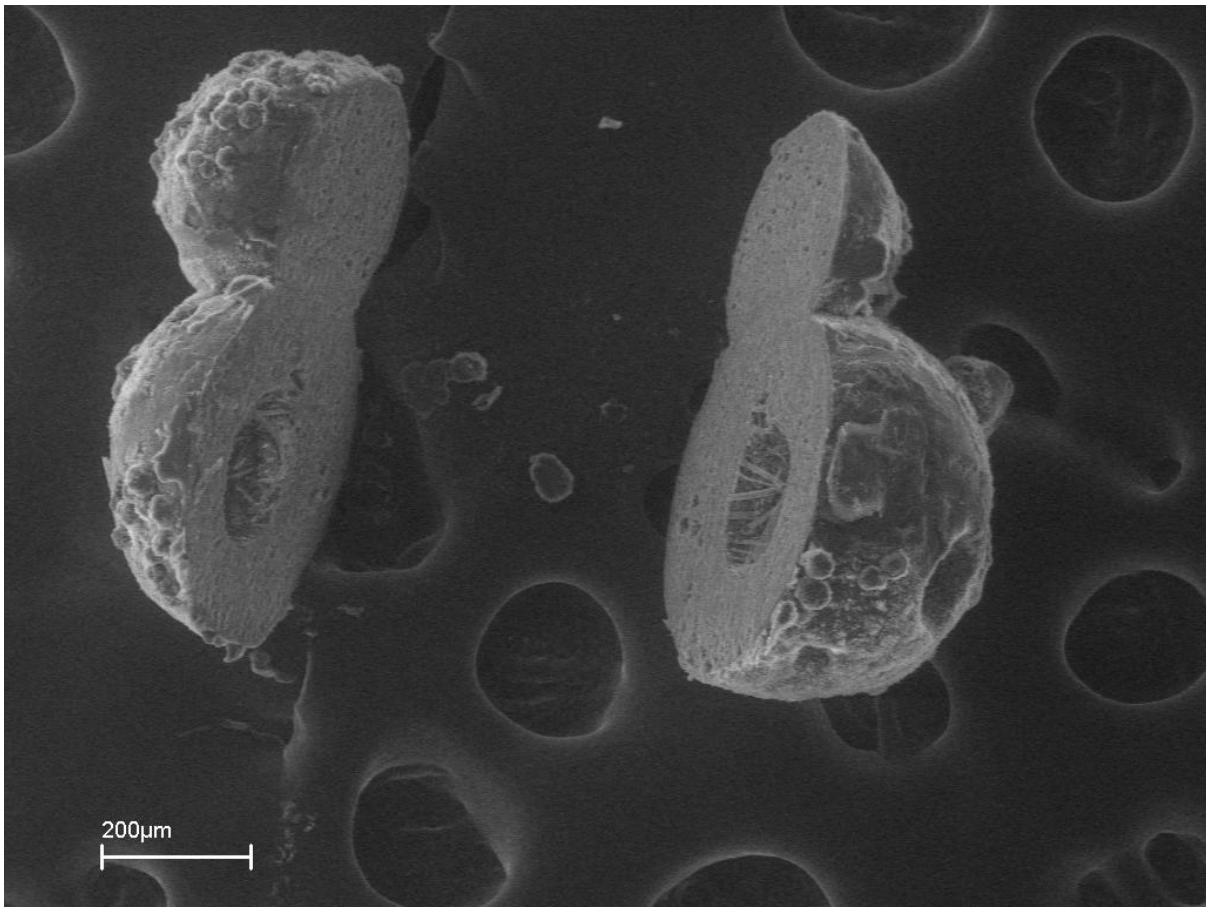

Figure 6.19. Scanning electron micrograph of cut section of microspheres prepared at 35^0C using formulation DS2.

CHAPTER 7

CONCLUSIONS

The combined hydrophilic and lipophilic balance (CHLBs) of dual surfactants played an important role on the physical characteristics and drug release behavior of microspheres prepared by the emulsion solvent evaporation method. The different CHLB values of dual surfactants have shown different effects on physical as well as drug release properties of microspheres. We noted a decrease in the geometric mean diameter of the microsphere batches with an increase in CHLB. There was a high initial drug release in the microspheres prepared with the dual surfactants. We noted an increase in the initial drug release with an increase in CHLB. The *in vitro* drug release was altered by the dual surfactants. The release was faster in the dual surfactant prepared at 7.5 CHLB compared to other CHLBs. The drug release was slowest in microspheres prepared with dual surfactant at 4.5 CHLB compared to other CHLBs. We also noted that with the single surfactant, the drug release was slow. The release kinetics showed a diffusion type of release from dual surfactant microsphere.

Chemical structure of surfactant played an important role on physical characteristics and drug release behavior of microspheres prepared by emulsion solvent evaporation method. We noted a decrease in the geometric mean diameter of the microsphere batches with an increase in CHLB for both of the dual surfactant combinations sorbitan tristearate and polyoxyethelene sorbitan monopalmitate (STE+POE-SM) and sorbitan tristearate and polyoxyethylene-(20)-cetyl ether (STE+POE-CE). The range of microsphere size was smaller (125 to 355 μm) when prepared with STE+POE-CE surfactant combinations compared with STE+POE-SM combination (250 to 710 μm). There was a high initial drug release in the microspheres prepared

with the dual surfactants STE+POE-CE compared to STE+POE-SM. We noted an increase in the initial drug release with an increase in CHLB for both the surfactant combinations STE+POE-SM and STE+POE-CE. The *in vitro* drug release was affected by dual surfactants. The release was faster in the dual surfactant prepared by STE+POE-CE dual surfactant combinations. The release kinetics showed a diffusion type of release from dual surfactant microspheres. Thus, we conclude that the chemical structure of surfactants affects different formulation parameters.

The preparation temperature of ethyl cellulose microspheres fabricated by the non-aqueous emulsion solvent evaporation method affected different physicochemical and release properties of microspheres. With increasing preparation temperature from 22^0C to 35^0C, the geometric mean diameter decreased, the particle size distribution was significantly wider. With an increase in temperature from 22^0C to 35^0C, the sphericity of microspheres improved, and the drug loading percentage, and initial drug release rate from the microspheres increased. The t_{50} release time for theophylline release was increased with an increase in the preparation temperature from 22^0C to 35^0C. The release kinetics showed a diffusion type of release. The SEM photographs show that the microsphere particle surface roughness decreased as preparation temperature increased from 22^0C to 35^0C.

The surfactant chemical structure and preparation temperature of ethyl cellulose microspheres fabricated by non-aqueous emulsion solvent evaporation method has combined effect on different physicochemical and release properties of microsphere. The temperature and surfactant structure both individually and significantly affect the size diameters of the microspheres. There was a highly significant effect of combined temperature and surfactant structure variables on the particle size distribution. By changing the surfactant structure from polyoxyethelene sorbitan monopalmitate (POE-SM) to polyoxyethylene cetyl ether (POE-CE) in

combination with temperature, percent drug loading, and initial drug release was increased significantly. The t_{50} release time for theophylline release was also found to increase. Theophylline release kinetics showed diffusion type of release for the batch DS1+35^{0}C. All other microsphere formulations showed near zero-order release.