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PREPARATION AND EVALUATION OF SUSTAINED RELEASE BIODEGRADABLE MICROSPHERES OF FOLIC ACID

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งานวิจัยนี้ศึกษาหาสภาวะการเตรียมสูตรตำรับยาในรูปแบบไมโครแคปซูลเพื่อควบคุมการปลดปล่อยกรดโฟลิก โดยกระบวนการ อิมัลซิฟิเคชัน/การระเหยตัวทำละลายโดยวัฏภาคกระจายตัวซึ่งประกอบด้วยเอทิลเซลลูโลสและยาอยู่ในระบบตัวทำละลายผสมระหว่าง อะซีโตนและเมทานอลในอัตราส่วน 9:1 ขณะที่น้ำมันพาราฟินทำหน้าที่เป็นวัฏภาคต่อเนื่องและมี Span 80 เป็นสารลดแรงตึงผิวเพื่อทำให้ระบบอิมัลชันเสถียร จากการศึกษาด้วย SEM พบว่าไมโครแคปซูลที่ได้มีลักษณะเป็นทรงกลม คุณลักษณะทางกายภาพและรูปแบบการปลดปล่อยยาของไมโครแคปซูลขึ้นอยู่กับปัจจัยในการเตรียมไมโครแคปซูล อาทิเช่น ความเข้มข้นของพอลิเมอร์ ความเข้มข้นของสารลดแรงตึงผิว สารปรุงแต่งที่ทำให้เกิดรูพรุนและปริมาณยาที่เติมลงไป โดยพบว่าเมื่อความเข้มข้นของพอลิเมอร์เพิ่มขึ้นจาก 2.5% เป็น 7.5% ทำให้ขนาดของไมโครแคปซูลเพิ่มขึ้นจาก 300 ไมโครเมตร เป็น 448 ไมโครเมตร ขณะที่อัตราการปลดปล่อยยาลดลงจาก 52% เหลือเพียง 40% เมื่อเพิ่มความเข้มข้นของสารลดแรงตึงผิวจาก 1% เป็น 4% ทำให้ไมโครแคปซูลมีขนาดเล็กลงจาก 300 ไมโครเมตร เหลือเพียง 141 ไมโครเมตรและอัตราการปลดปล่อยยาเพิ่มขึ้นจาก 52% เป็น 79% ในขณะที่การเติมสารปรุงแต่งที่ทำให้เกิดรูพรุน เช่นน้ำตาลซูโครสสามารถช่วยปรับปรุงการปลดปล่อยยาจากไมโครแคปซูลได้เป็นอย่างดี และความสามารถในการเก็บกักยาของไมโครแคปซูลเพิ่มขึ้นจาก 64% เป็น 88% เมื่อเพิ่มปริมาณยาจาก 20 มิลมิกรัมเป็น 60 มิลลิกรัม

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SATIT PRASERTMANAKIT: PREPARATION AND EVALUATION OF SUSTAINED RELEASE BIODEGRADABLE MICROSPHERES OF FOLIC ACID. THESIS PRINCIPAL ADVISOR: ASSOC. PROF. NONGNUJ MUANGSIN, Ph.D., THESIS COADVISOR: NALENA PRAPHAIRAKSIT, D.V.M. Ph.D., 125 pp.

The preparation of controlled release folic acid-loaded ethyl cellulose microcapsules by oil in oil emulsion solvent evaporation was investigated. A mixed solvent system (MSS) consisting of acetone and methanol in a 9:1 ratio was selected as a dispersed phase and light liquid paraffin as a continuous phase. Span 80 was used as an emulsifier for stabilizing the emulsion. The SEM showed that the microcapsules had a spherical shape. The particulate properties and the *in vitro* release profile depended on the processing and formulation parameters such as concentration of ethyl cellulose, concentration of emulsifier, pore inducer and initial drug feed. The average diameter of microcapsules increased from 300 μm to 448 μm whereas release rate decreased from 52% to 40% as the concentration of ethyl cellulose increased from 2.5% to 7.5%. When the concentration of emulsifier increased from 1% to 4%, the average diameter of microcapsules decreased from 300 μm to 141 μm and release rate increased from 52% to 79%. An addition of small amounts of sucrose, a water-soluble agent, improved the release of drug from the microcapsules matrix without influencing the morphology and particulate properties of microcapsules. The encapsulation efficiency increased from 64% to 88% as the initial drug feed increased from 20 mg to 60 mg.

Field of study: Petrochemistry and Polymer Science..Student's signature: Satit. Prasertmanakit
Academic year:.....2008..... Principal Advisor's signature: N.Muangsin
Co-Advisor's signature: .....

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CONTENTS

Page

Page

LIST OF TABLES

LIST OF FIGURES

Figure **Page**

Figure **Page**

LIST OF ABBREVIATIONS AND SYMBOLS

°C	Degree Celsius
cm^{-1}	Unit of wave number
conc.	concentration
D_{50}	mean particle size
EC	Ethyl cellulose
EE	The encapsulation efficiency
FA	Folic acid
FTIR	Fourier transform infrared spectroscopy
HPLC	High Performance Liquid Chromatography
O/O	Oil in Oil Emulsion
O/W	Oil in Water Emulsion
PCA	Pterine carboxylic acid
PGA	*p*-aminobenzoyl-L-glutamic acid
pH	The negative logarithm of the hydrogen ion concentration
ppm	Part per million
r^2	The correlation coefficient
RSD	Relative standard deviation
S.D.	Standard deviation
SEM	Scanning electron microscope
SGF	Simulated Gastric Fluid
SIF	Simulated Intestinal Fluid
SPAN	Polydispersity Index
W/O	Water in Oil Emulsion
W/O/W	Water in Oil in Water Emulsion
w/v	Weight by volume
v/v	Volume by volume

CHAPTER I

INTRODUCTION

1.1 Introduction

Nutrition has always been recognized as having a significant impact on health. Folic acid, also known as B9 or folate, is an important class of water-soluble B-vitamins that were discovered by researcher Lucy Wills in 1941. The vitamin folic acid was initially investigated as a dietary antianemia factor distinct from the pernicious anemia factor. A recent historical review details early studies on the isolation of the vitamin and on the establishment of its role as a cofactor in one-carbon metabolism. Nowadays, an increased interest in the folate status of population groups has occurred largely as the result of three aspects of the vitamin: (1) the association of low plasma folate levels with elevated plasma homocysteine concentrations and an increased risk of arteriosclerosis, (2) the benefits of folate in reducing the incidence of neural tube defects, and (3) the effects of folate on the occurrence of cancer [1].

Folate plays a crucial role in the biosynthesis of nucleotides that are essential for nucleic acid metabolism, cell division, and genetic expression. Folate-activated enzymes are required for the synthesis of certain amino acid and for various methylation reactions. Because of the role of folate coenzymes in the synthesis of DNA precursors, folate antagonists have found widespread clinical use as antiproliferative and antimicrobial agents. It has been known for many years that the pernicious anemia that results from defects in vitamin B12 availability, is due to the induction of a secondary functional folate deficiency. More recently, the demonstration that periconceptional supplementation with low doses of folic acid reduces the incidence of neural tube defects has generated considerable clinical and public health interest, with proposals that the food supply be supplemented with folic acid, although the metabolic basis for this effect is not understood [1].

Folate deficiency is an important problem in areas of the world where there is poverty and malnutrition. There appears to be a link between lack of folate and neural tube defects such as spina bifida, where the spinal cord does not develop correctly in the early fetus. Several studies have shown that giving folate supplements to women who have previously given birth to a child with a neural tube defect can reduce the risk of the same problem arising in a subsequent pregnancy by almost 75%. Alcohol affects the uptake of folate from the digestive system into the blood; so alcoholics are at risk of folate deficiency for this reason as well as because their diet may be lacking in folate. Other population groups who do not have a balanced diet, due to poverty, poor food choices, or illness, may also be at risk [2]. In addition, multiple studies of women taking oral contraceptives show decreased folic acid levels relative to negative controls. Postulated mechanisms reported for this phenomenon include decreased absorption of polyglutamates, increased excretion of folic acids, increased production of folic acid-binding proteins, and induction of folic acid-dependent hepatic microsomal enzymes [3]. Therefore, women on birth control pills should regularly eat good sources of folic acid [4].

Food sources of folic acid include dark-green leafy vegetables such as spinach, fruits particularly citrus such as oranges, bean and nuts. These natural found folic acids are highly sensitive to sunlight, ultraviolet light, visible light, heat oxygen and pH. Therefore processing food both by industrial and household preparation can reduce the amount of folic acid and cause negative effects on folate stability in these natural food sources [5-8].

There is a need for extensive studies, especially to develop new techniques for enhancing folate content, stability and bioavailability in food products. Encapsulation is an inclusion technique for confining a substance into a polymeric matrix coated by one or more semi-permeable polymers, by virtue of which the encapsulated compound becomes more stable than its isolated or free form [9-10]. In the recent literature, the microencapsulation of folic acid has been reported by several authors.

Shrestha *et al.* studied the technical feasibility of adding folic acid on to rice and coating with edible polymers. Rice premixes coated with locust bean gum, agar, and xanthan gum, pectin, and some of their composite mixtures retain more folic acid

during washing test than other edible polymers used in this study. The loss of folic acid in washing was lowest in rice premixes coated with ethyl cellulose followed by pectin, composite mixtures of locust bean and other coating materials with highest loss in gum arabic coated rice. No edible polymer could satisfactorily retain folic acid during boiling in excess water. Edible polymers failed to mask the yellow color of folic acid and additional masking agent was needed [11].

Madziva *et al.* prepared folic acid incorporated microcapsules using alginate and combinations of alginate and pectin polymer to improve stability. The results showed that the microencapsulation with a mixture of alginate and pectin, aided by freeze-drying, has the potential to protect folic acid from adverse environmental factors that lead to its degradation. Overall, the use of alginate in combination with pectin conferred greater folic acid stability compared to free folic acid and alginate alone [12].

In 2006, Madziva *et al.* studied the evaluation of alginate-pectin capsules in Cheddar cheese as a food carrier for the delivery of folic acid. They found that the combination of alginate and pectin polymers resulted in capsules with high encapsulation efficiency, notable stability in a milk system, significantly improved stress tolerance properties as seen by high folic acid retention during cheese pressing and even distribution in a cheese matrix. These results suggest that Cheddar cheese may be an effective medium for folic acid delivery particularly if alginate–pectin capsules are used [13].

Microencapsulation has been used in the pharmaceutical industry for the conversion of liquid to solid, taste-masking of bitter drugs, prolonged or sustained release, reduced gastric irritation, and environmental protection of labile moieties [14]. There are several techniques used to produce sustained release dosage form, which include physicochemical processes (such as solvent evaporation method or phase separation method), mechanical processes (such as spray drying), and a non-solvent addition process [15-21]. Polymeric microcapsules have received much attention as drug delivery systems in recent years and used to modify and retard drug release [22]. Ethyl cellulose is a non-biodegradable and biocompatible polymer, one of the extensively studied encapsulating materials for the controlled release of

pharmaceuticals [23]. In the recent literatures, the ethyl cellulose microcapsules have been reported by several authors for encapsulation of a variety of drugs such as Song *et al.* prepared the sustained release amoxicillin ethyl cellulose microcapsules by adjusting the various process parameters, Bagory *et al.* prepared sustained release microspheres of theophylline, using ethylcellulose as a release retarding material and Zinutti *et al.* prepared ethyl cellulose microspheres containing 5-fluorouracil by solvent evaporation technique [24-26].

The present study was designed to develop sustained release microcapsules of folic acid by oil in oil (O/O) emulsion solvent evaporation technique. For drugs or vitamins with water solubility, water in oil in water technique can be used but it is complicated to produce the stability of single emulsion. Thus, the O/O emulsion solvent evaporation technique was applied in this study as a way of protecting folic acid from deteriorative reactions or adverse environmental conditions.

1.2 The objectives of this research

1) To prepare the sustained release of folic acid from ethyl cellulose microcapsules by O/O emulsion solvent evaporation method.
2) To evaluate the effect of formulation variables such as concentration of polymer, concentration of emulsifier and the proportions of polymer matrix/drug.
3) To determine the efficiency of release behaviors of each formulation as well as folic acid commercial tablets.

1.3 The scope of research

The scope of this research was carried out by stepwise investigation as follows:

1) Review literatures survey for related research work.
2) Determine the emulsion solvent evaporation technique.
3) Prepare the microcapsules by varying the proportions of polymer, emulsifier and drug.
4) Determine the encapsulation efficiency of microcapsules.
5) Characterize and study the morphology of the microcapsules using SEM, FT-IR, and laser particle size distribution analyzer.
6) Study the release behavior of the microcapsules in simulated gastric-intestine fluids using HPLC method.
7) Summarize the results.

CHAPTER II

BACKGROUND AND LITERATURE REVIEWS

2.1 Microencapsulation

Microencapsulation is a process in which tiny particles or droplets are surrounded by a coating to give small capsules with many useful properties. In a relatively simplistic form, microcapsules are small particles that contain an active agent or core material surrounded by a coating or shell [27]. The core may be solid, liquid, or even gas. Microcapsules can have a variety of structure. Some have a spherical geometry with a continuous core region surrounded by a continuous shell or at least one discrete domain of active agent. Some variations on microcapsules structure are given in Figure 2.1 [28]. Microcapsules usually have a particle size range between 1 to 5000 μm [29-30].

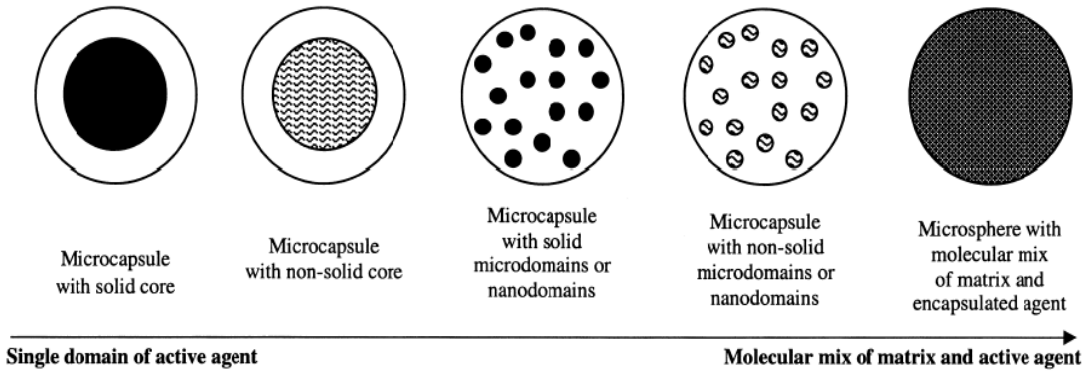


Figure 2.1 Variations of microparticle formulation [28].

Microencapsulation may be used for a number of reasons [31]. These include:

1. Protection of unstable, sensitive materials from their environments prior to use.
2. Controlled, sustained, or timed release.
3. Retarding evaporation of volatile core.

4. Masking of odor and/or taste of encapsulating materials.
5. Better processability (improving solubility, dispersibility, flowability).
6. Reducing gastric irritation.
7. Self-life enhancement by preventing degradation reactions (oxidation, dehydration).
8. Safe and convenient handling of toxic materials.
9. Enzyme and microorganism immobilization.
10. Handling liquids as solids.

2.2 History of microencapsulation technique

The first research leading to the development of microencapsulation procedures for pharmaceuticals was published by Bungenburg de Jong and Kaas in 1931 and dealt with the preparation of gelatin spheres and the use of a gelatin coacervation process for coating. In the late 1930s and 1940s, Green and co-workers of the Nation Cash Register Co., developed the gelatin coacervation process to prepare carbonless carbon paper. The microcapsules, containing a colorless dye precursor (3,3-bis-(p-dimethylaminophenyl)-6-dimethyamin-phthalide), were affixed to the under surface of the top of page and released the dye precursor upon rupture by pressure from the tip of a writing tool. The liberated dye precursor then reacted with an acidic clay (attapulgite) coating on the top surface of the underlying page to form a copy image [30].

2.3 Microencapsulation procedures

Microcapsules have been prepared by various techniques, which feature partly competing, partly complementary characteristics. Many microencapsulation processes are modifications of the three basic techniques: solvent extraction/evaporation, phase separation (coacervation) and spray-drying [6]. These major microencapsulation procedures are summarized briefly in Table 2.1 [29, 30, 32-35].

Table 2.1 Summary of major microencapsulation procedures

Process	Principle	Type of core	Type of coating
1. Physico-chemical Methods Coacervation/Phase separation	The salvation of polymeric solute(s) in a medium is reduced to form coacervate droplets to deposit and coat the dispersed phase.	Vehicle insoluble drug(s).	Vehicle soluble drug(s).
Solvent Evaporation	The emulsification of a polymer solution containing drug into an immiscible liquid phase containing an emulsifier and the solvent is removed from the dispersed droplets to leave a suspension of drug containing polymer microcapsules.	Solvent soluble and solvent insoluble drug(s), but insoluble in manufacturing vehicle.	Solvent soluble polymer(s), but insoluble in manufac-turing vehicle.
2. Chemical Methods Interfacial Polymerization	Various monomers are reacted at the interface of two immiscible liquid phases to form a film of polymer that encapsulated the dispersed phase.	High-molecular weight materials such as enzymes and hemolysates.	Water soluble and water-insoluble monomers.

Process	Principle	Type of core	Type of coating
3. Mechanical Methods			
Air Suspension	Polymer solution is spray applied to the suspending and moving particles in the coating zone portion of the coating chamber of air suspension apparatus.	Non-volatile and solid drug(s).	Water soluble or organic solvent soluble polymer (s).
Pan Coating	Polymer solution is spray applied to the desired solid core material, which is deposited onto spherical substrate.	Non-volatile and solid drug(s).	Water-soluble or organic solvent-soluble polymer(s).
Spray Drying	A core material is dispersed into a coating solution and then the mixture is atomized into a hot air stream to remove the solvent from the coating material.	Solvent-insoluble drug(s)	Solvent-soluble polymer(s)

Various microencapsulation processes give rise to the formation of microcapsules with various characteristic size ranges as shown in Table 2.2 [29, 33].

Table 2.2 Microcapsule size ranges produced by various microencapsulation processes

Microencapsulation process	Size range (μm)
Coacervation / Phase separation	1-5000
Solvent evaporation	5-5000
Interfacial polymerization	2-2000
Air suspension	35-5000
Pan coating	200-5000
Spray drying	5-800

2.4 Emulsification solvent evaporation procedures

Microencapsulation by emulsification/solvent evaporation basically consists of four major steps (Figure 2.2):

1. Dissolution or dispersion of the bioactive compound often in an organic solvent containing the matrix forming material.
2. Emulsification of this organic phase in a second continuous (frequently aqueous) phase immiscible with the first one.
3. In order for the microspheres to form, the organic solvent must first diffuse into the aqueous phase and then evaporate at the water/air interface.
4. Harvesting and drying of the microspheres.

This technique can be tailored to produce microspheres over a wide size range, from less than 200 nm to several hundred microns, and by choice of suitable solvent systems drugs have high or low aqueous solubilities can be encapsulated into a wide range of polymers [36].

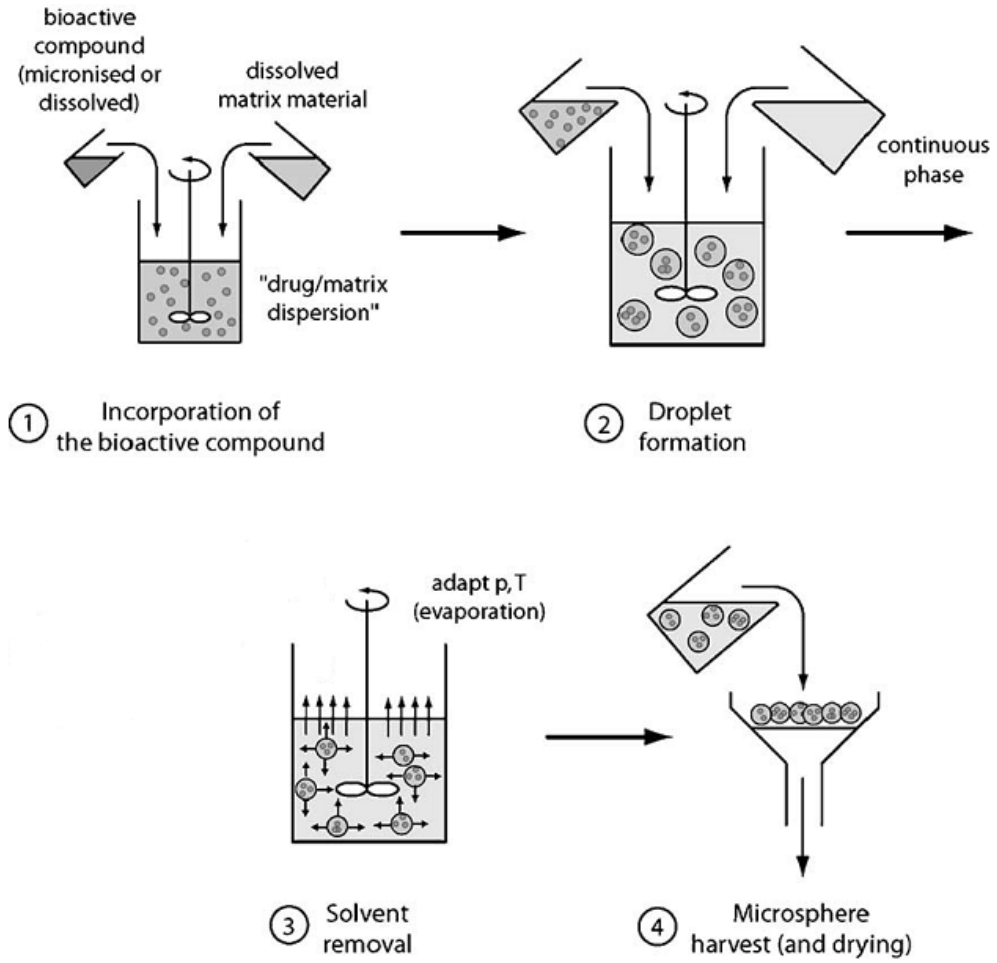


Figure 2.2 Schematic overview over the four principal process steps in microcapsules preparation by solvent evaporation [36].

There are two types of emulsification solvent evaporation, each having the concept of emulsion and are as follows [34, 37]:

1. Single-emulsion solvent evaporation
2. Multiple-emulsion technique

Single-emulsion solvent evaporation

For single-emulsion solvent evaporation, there are two systems to choose: oil in water (O/W) or water in oil (W/O)

2.4.1.1 Oil in water emulsion solvent evaporation technique

The O/W emulsion is produced by the agitation of two immiscible liquids. The drug substance is either dispersed or in solution in the polymer/solvent system or is captured in the dispersed phase of the emulsion. Agitation of the system is continued until the solvent partitions into the aqueous phase and is removed by evaporation. This process results in hardened microspheres which contain the active moiety [38].

This technique, particularly suitable for the encapsulation of lipophilic active principles, has since been widely used for the encapsulation of different classes of therapeutic agents such as clonazepam, testosterone, cisplatin and diclofenac sodium [37].

Nevertheless, the microencapsulation of hydrophilic active principles by this process can pose problems. In actual fact, a partitioning phenomenon operates between the dispersed and the continuous phases which contribute to a substantial lowering of microencapsulation yields [39].

2.4.1.2 Oil in oil emulsion solvent evaporation technique

Oil in oil, sometimes referred as water in oil emulsification process, was developed for the encapsulation of highly water soluble drugs. The encapsulation of water soluble drugs by the conventional O/W solvent evaporation method will generally result in rapid partitioning of the drug from the organic phase and into the aqueous phase, resulting in microspheres with little or no drug loading. Innovative modifications to the conventional O/W solvent evaporation method have been reported to circumvent this problem.

Anhydrous systems, which are comprised of an organic polymer phase emulsified in the immiscible oil, have been used to produce microspheres of the O/O type. The elimination of water significantly reduces the tendency of the drug to partition into the continuous phase, provided that the drug is insoluble in the external

oil [38]. However, an important drawback of using an oil external phase is cleaning up the final product. The oil has to be removed using organic solvent such as n-hexane, which may present problems in terms of completeness of removal [34].

2.4.2 Multiple-emulsion technique

Multiple-emulsion or double emulsion technique is use for the efficient incorporation of water-soluble peptide, protein and other macromolecules. In this technique, the polymers are dissolved in an organic solvent and emulsified into an aqueous drug solution to form the primary emulsion then reemulsified into an aqueous solution containing an emulsifier to produce the multiple W/O/W dispersion. Herrmann and Bodmeier have reported that the biodegradable somatostatin acetate-containing microspheres could be achieved by W/O/W solvent evaporation technique with high drug encapsulation efficiency [40].

2.5 Factors affecting on microencapsulation using solvent evaporation

2.5.1 Solvent type

Central to the process of microencapsulation by emulsification/solvent evaporation is the selection of two liquid phases, one to contain drug and polymer (dispersed phase) and one to contain the emulsifier (continuous phase) [34].

Important criteria for dispersed phase solvent:

a) Ability to dissolve chosen polymer
b) Ideally, the solvent should be able to dissolve the drug
c) Immiscibility with the continuous phase solvent
d) Lower boiling point than continuous phase solvent
e) Low toxicity

Important criteria for continuous phase solvent:

a) Immiscibility with dispersed phase solvent

b) Inability to dissolve polymer
c) Low solubility toward drug
d) Higher boiling point than dispersed phase solvent
e) Low toxicity
f) Allows easy recovery and clean-up of microspheres

The final structure and composition of a microsphere will result from a complex interplay between polymer, drug, solvent, continuous phase, and emulsifier. Since the drug and polymer to be used are usually mixed, solvent choice can be of crucial importance. For example, in a detailed study of the effects of solvent properties on the preparation of poly (lactic acid) (PLA) microspheres a high efficiency of quinidine sulfate entrapment was favored by dissolving the drug and polymer in water-immiscible solvents with significant water solubility. It was considered that such solvents cause rapid precipitation of polymer at the droplet interface, thereby creating a barrier to drug diffusion out of the forming microsphere. Methylene chloride which has the highest water solubility (2% w/w) can be achieved the highest drug loading (23% w/w). Whereas insignificant loading (0.4% w/w) was achieved using chloroform, which has a solubility of only 0.8% w/w [41].

In the case of the O/O emulsion process, the volatile solvent depends upon the nature of the continuous phase. When the continuous phase is a nonpolar liquid like paraffin oil, the volatile solvent is typically acetone or acetonitrile. Acetone is volatilized from the system at room temperature while acetonitrile often is removed at 50 to 85 ^{o}C [42].

2.5.2 Emulsifier

The emulsifier (stabilizer) is an important parameter that provides a thin protective layer surrounding the oil droplets and reduces the coalescence and coagulation of microparticles during the solvent evaporation process.

The surfactant used to stabilize O/O emulsions normally is dissolved in the continuous phase. Since the continuous phase for O/O emulsion process may be a high-boiling nonpolar liquid like paraffin oil or mineral oil thus the surfactant should

be oil soluble [42]. A large number of such surfactants are available. Polysorbate is an oily liquid. It is a class of emulsifiers used in some pharmaceuticals and food preparation. It is often used in cosmetics to solubilise essential oils into water based products. Polysorbates are derived from PEG-ylated sorbitan (a derivative of sorbitol) esterified with fatty acids. Surfactants that are esters of plain (non-PEG-ylated) sorbitan with fatty acids are usually referred to by the name Span [43].

2.5.3 Stirring rate

Stirring rate is a parameter of primary importance in emulsification steps. In the forming droplets, the energy and the surface active agent decrease the interfacial tension between the organic droplets and the aqueous phase. The stirring rate providing the energy which is appropriate for the division of the organic phase, so if high energy, small particle and narrow particle size distribution are obtained [44].

2.5.4 Polymer type

Polymers are generally used as the structural backbone for controlled drug release system. The characteristics of polymers selected in the preparation of the dosage form must comply with the following requirement [45].

a) Biocompatibility: Harmful and toxic impurities must be eliminated from polymer before their inclusion in dosage forms. There must be minimal tissue response after injection or implantation into the body.
b) Physical and mechanical properties: Properties of polymer should be the same as natural tissues or required for the dosage form design such as: elasticity, resistance to tensile, swelling, etc.
c) Biodegradability: the polymer should be degraded by the body or in nature by enzymes at a well defined rate to non-toxic and rapidly excreted degradation product.
d) Pharmacokinetic properties: Chemical degradation of the polymer matrix must be non-toxic, non-immunogenic and non-carcinogenic.

e) Cost-effectiveness: the polymer should be low-cost or abundantly available, which could easily be adapted to the standard manufacture procedures.

2.5.4.1 Ethyl cellulose

Cellulose is the most abundant and widely used organic material in the world, with a worldwide consumption that is higher than steel, coal, cereals or sugar. Cellulose can be converted to useful derivatives by etherification of the various ring hydroxyl groups. A variety of cellulose ether derivatives are manufactured annually at an industrial scale [46].

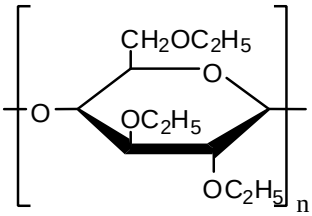


Figure 2.3 Ethyl cellulose structure

Ethyl cellulose is an ethyl ether cellulose derivative (Figure 2.3) and has been used either as modified drug release dosage form for oral administration or a release retardant polymer in controlled-release matrix dosage forms [47]. The drug release for extended duration, particularly for highly water-soluble drugs, using a hydrophilic matrix system is restricted due to rapid diffusion of the dissolved drug through the hydrophilic gel network. For such drugs with high water solubility, hydrophobic polymer like ethyl cellulose is suitable as matrixing agents for developing sustained-release dosage forms [48]. Hydrophobic polymers provide several advantages, ranging from good stability at varying pH values and moisture levels to well-established safe applications [49].

In the recent literature, the ethyl cellulose microcapsules have been reported by several authors for encapsulation of a variety of drugs such as zidovudine, aspirin, potassium chloride, isosorbide dinitrate, metformin hydrochloride, isoniazid, etc. for a variety of reasons [50-55].

2.6 Folic acid

Folic acid (FA) is known chemically as N-[4(2-amino-4-hydroxy-pteridin-6-ylmethylamino)-benzoyl]-L(+)-glutamic acid or pteroyl-L-glutamic acid. The other name of folic acid was known as vitamin B9/vitamin M or folacin [56].

2.6.1 Clinical importance of folic acid

FA, also known as B9 or folate, is a class of water-soluble B-vitamins discovered by researcher Lucy Wills in 1941. In the general form of folates are made up of a pteridine ring, p-aminobenzoic acid and one to six glutamic acids moieties (Figure 2.4). Dietary folates are in the form of pteroylpolyglutamate that the small intestine can not absorb thus it must be cleaved or hydrolyzed to the pteroylmonoglutamate form for absorption [57].

FA is the folate vitamin that is traditionally utilized to fortify cereals/grains and dietary supplement. FA is a synthetic form of this vitamin, and it is the fortificant of choice because of its relative stability and increased bioavailability compared to natural folate forms [58-59].

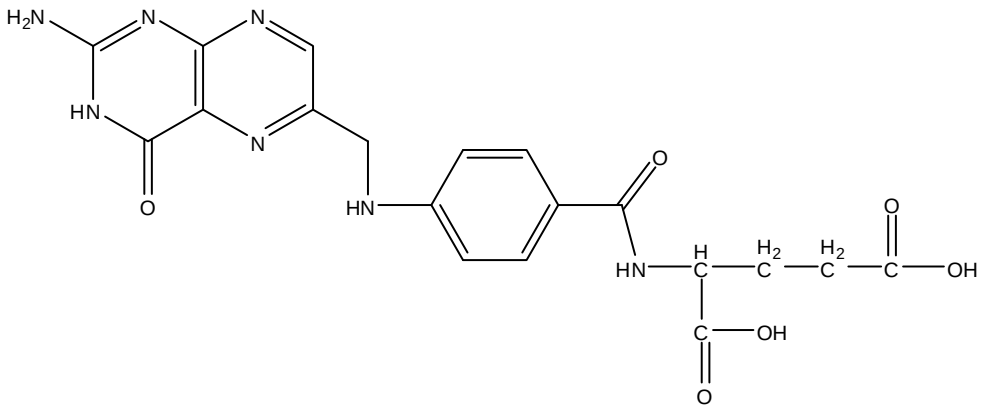


Figure 2.4 Structure of folic acid.

FA has been used for the treatment of megaloblastic and macrocytic anemias result from folate deficiency. A deficiency of folate can occur when the need for

folate is increased, when dietary intake of folate is inadequate, and when the body excretes (or loses) more folate than usual [60].

Some situations that increase the need for folate include [61]:

- Pregnancy and lactation (breastfeeding)
- Alcoholism
- Tobacco smoking
- Malabsorption, including celiac disease
- Kidney dialysis
- Liver disease
- Certain anemias

Medications can interfere with folate utilization, including:

- Rheumatoid arthritis patients receiving long-term methotrexate therapy.
- AIDS patients receiving sulfa drugs or pentamidine pnemocystis prophylaxis.

Folate is necessary for the production and maintenance of new cells. This is especially important during periods of rapid cell division and growth such as infancy and pregnancy. Folate is needed to replicate DNA. Thus, folate deficiency hinders DNA synthesis and cell division, affecting most clinically the bone marrow, a site of rapid cell turnover. Because RNA and protein synthesis are not hindered, large red blood cells called megaloblasts are produced, resulting in megaloblastic anemia. Both adults and children need folate to make normal red blood cells and prevent anemia [62].

2.6.2 Biochemical of folic acid

FA is not the active form of the vitamin. It needs to be reduced to tetrahydrofolate (THF also H_4folate). FA is reduced within cells (principally the liver where it is stored) to THF through the action of dihydrofolate reductase (DHFR), an NADPH-requiring enzyme [63].

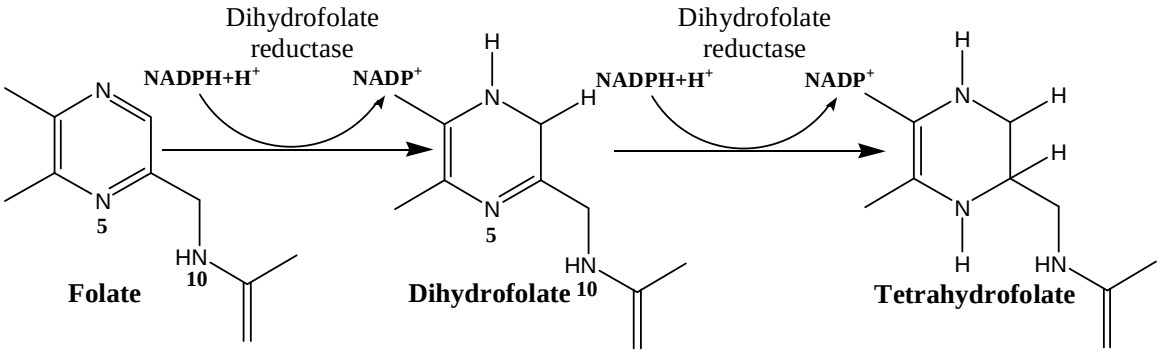


Figure 2.5 Activation of folic acid [63].

The function of THF derivatives is to carry and transfer various forms of one carbon units during biosynthetic reactions. The one carbon units are either methyl, methylene, methenyl, formyl or formimino group [62].

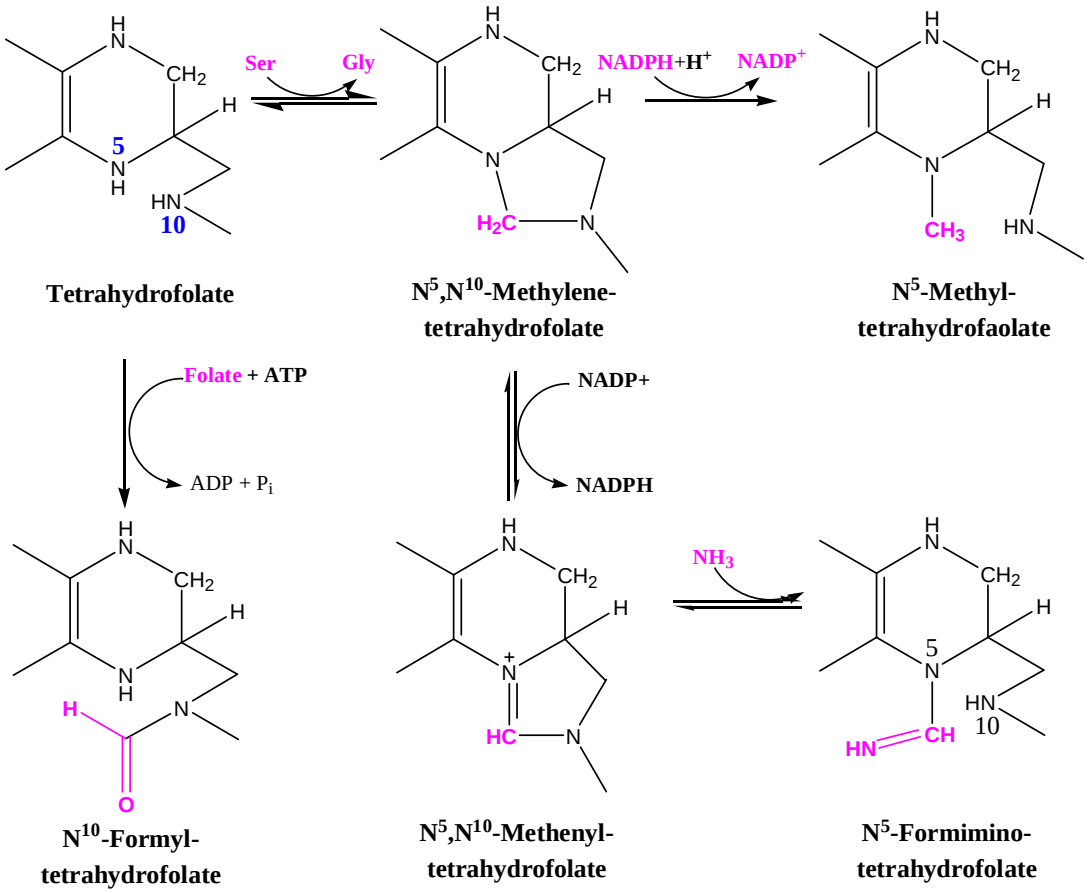


Figure 2.6 Carbon transfer reaction of THF [63].

These one carbon transfer reactions are required in the biosynthesis of serine, methionine, glycine, choline and the purine nucleotides and dTMP (2-deoxythymidine-5-phosphate). The ability to acquire choline and amino acids from the diet and to salvage the purine nucleotides makes the role of N^5, N^{10}-methylene-THF in dTMP synthesis the most metabolically significant function for this vitamin. N^5, N^{10}-methylene-THF is absolutely essential for DNA synthesis. This is important in cells that are dividing rapidly such as red blood cell producing bone marrow cells, hair follicles, intestinal mucosa cells and cancer cells (rapidly dividing cells need to replicate their DNA often). The role of vitamin B12 and N^5-methyl-THF in the conversion of homocysteine to methionine also can have a significant impact on the ability of cells to regenerate needed THF. Methionine is the precursor to S-adenosylmethionine (SAM), which is considered to be the universal methyl-group donor that is involved in many metabolic reactions [63].

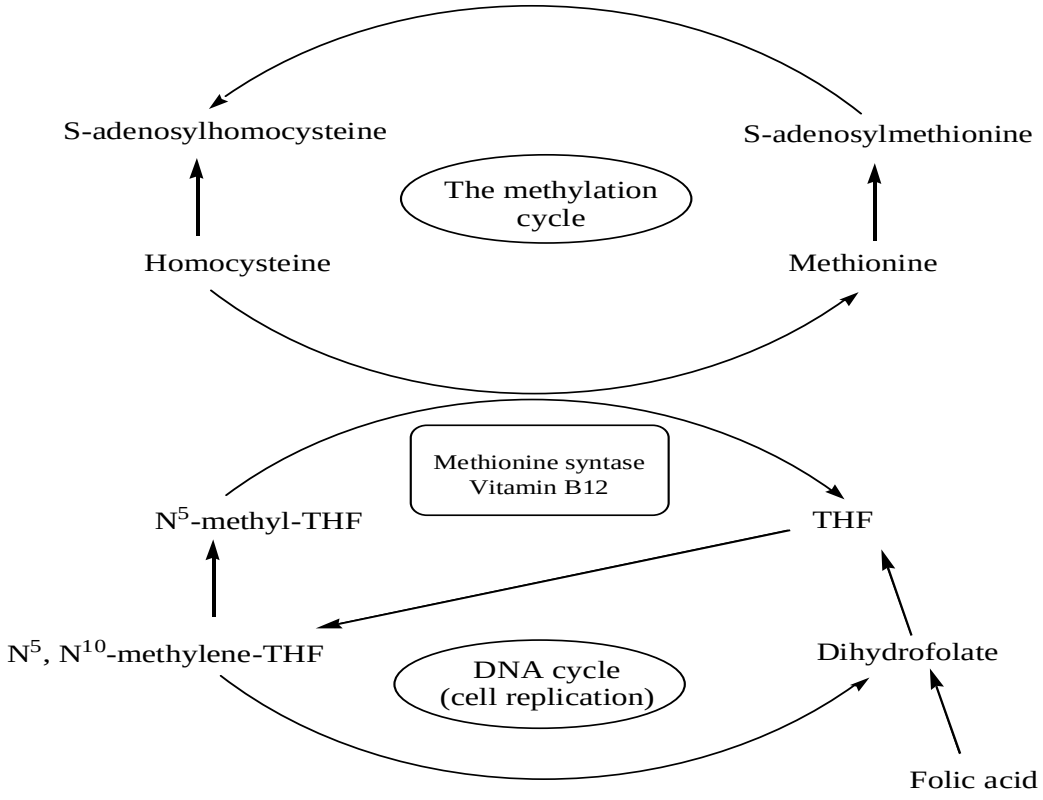
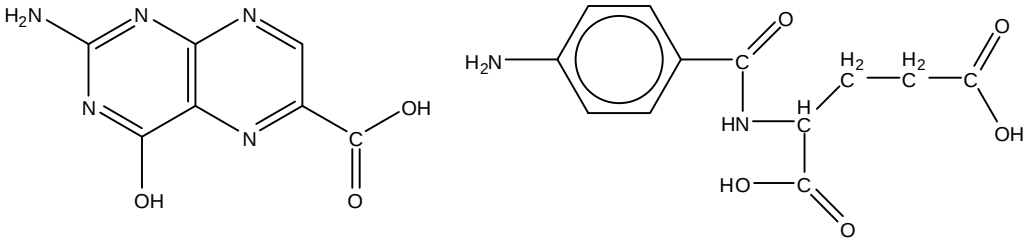


Figure 2.7 Biochemical path way of folic acid [64].

2.6.3 Stability of folic acid

FA like all other folates is in danger of oxidative degradation, which is enhanced by oxygen, light, heat and a shift in pH either way from 7.6 [65]. FA is a photosensitive compound and is degraded in aqueous solution by sunlight. Para-aminobenzoyl-L-glutamic acid and pterine-6-carboxylic acid are the major degradation products of folic acid, along with traces of *p*-aminobenzoic acid. Jamil Akhtar et al proposed the degradation pathway of folic acid [66].



Pterine-carboxylic acid (PCA) **p-Aminobenzoyl-L-glutamic acid (PGA)**

Figure 2.8 Pterine-6-carboxylic acid and *p*-Aminobenzoyl-L-glutamic acid structures [67].

2.6.4 Folate deficiency

A deficiency of folate can occur when an increased need for folate is not matched by an increased intake, when dietary folate intake does not meet recommended needs, and when folate excretion increases. Medications that interfere with the metabolism of folate may also increase the need for this vitamin and risk of deficiency [68].

2.6.4.1 Risk of megaloblastic anemia

Folate deficiency can cause megaloblastic (or microcytic) anemia. In this type of anemia, red blood cells are larger than normal, and the ratio of nucleus size to cell cytoplasm is increased. There are other potential causes of megaloblastic anemia, including vitamin B12 deficiency or various inborn metabolic disorders. If the cause is folate deficiency, then treatment with folate is the standard approach.

Patients with anemia should be evaluated by a physician in order to diagnose and address the underlying cause [69].

2.6.4.2 Prevention of neural tube defect

FA is very important for all women who may become pregnant. Adequate folate intake during the periconceptional period, the time just before and just after a woman becomes pregnant, helps protect against a number of congenital malformations including neural tube defects. Neural tube defects result in malformations of the spine (spina bifida), skull and brain (anencephaly). Babies with anencephaly do not develop a brain and are stillborn or die shortly after birth. Those with spina bifida have a defect of the spinal column that can result in varying degrees of handicap, from mild and hardly noticeable cases of scoliosis (a curvature of the spine) to paralysis and bladder or bowel incontinence [60]. With proper medical treatment, most babies born with spina bifida can survive to adulthood. However, they may require leg braces, crutches, and other devices to help them walk, and they may have learning disabilities. About 30 percent have slight to severe mental retardation.

In the developing fetus, the neural tube closes early in pregnancy at 23 to 27 days after conception, a time when many women do not realize that they are pregnant. The risk of neural tube defects is significantly reduced when supplemental folic acid is consumed in addition to a healthy diet prior to and during the first month following conception. Women who could become pregnant are advised to eat foods fortified with folic acid or take supplements in addition to eating folate-rich foods to reduce the risk of some serious birth defects. Taking 400 micrograms of synthetic folic acid daily from fortified foods or supplements has been suggested for adult men and women [70]. The Recommended Dietary Allowance (RDA) for folate equivalents for pregnant women is 600 micrograms [71].

2.6.4.3 Prevention of heart disease

Homocysteine is an amino acid that normally found in blood. There is evidence that an elevated homocysteine level is an independent risk factor heart disease and stroke. The evidence suggests that high levels of homocysteine may

damage coronary arteries or make it easier for blood clotting cells called platelets to clump together and form a clot. The risk of high homocysteine is similar to the risk of high cholesterol but it is much easier to lower homocysteine levels through increased intake of folic acid. The earlier studies found that folic acid reduces levels of homocysteine, which lowers a person's chances of developing heart disease [70, 72-73].

2.6.4.4 Risk of cancer

Since folate is involved in the synthesis, repair, and functioning of DNA, our genetic map, a deficiency of folate may result in damage to DNA that may lead to cancer. The association between folate and cancer appears to be complex. It has been suggested that folate may help prevent cancer, as it is involved in the synthesis, repair, and functioning of DNA, our genetic map, and a deficiency of folate may result in damage to DNA that may lead to cancer. Conversely, it has been suggested that excess folate may promote tumor initiation. Although diets high in folate are associated with decreased risk of colorectal cancer, the association is stronger for folate from foods alone than for folate from foods and supplements, and a 2007 randomized clinical trial found that folate supplements did not reduce the risk of colorectal adenomas [74-75].

A 2006 prospective study of 81,922 Swedish adults found that diets high in folate from foods, but not from supplements, was associated with a reduced risk of pancreatic cancer. Most epidemiologic studies suggest that diets high in folate are associated with decreased risk of breast cancer, but results are not uniformly consistent: one large cancer screening trial reported a potential harmful effect of high folate intake on breast cancer risk, suggesting that routine folate supplementation should not be recommended as a breast cancer preventive, but a 2007 Swedish prospective study found that a high folate intake was associated with a lower incidence of postmenopausal breast cancer [75].

2.6.4.5 Risk of depression

Folate deficiency has been found among people with depression and has been linked to poor response to antidepressant treatment. Folate supplements have been used for enhancing treatment response to antidepressants. Limited clinical research suggests that folic acid is not effective as a replacement for conventional antidepressant therapy. Depression should be treated by a qualified healthcare provider [76].

2.6.4.6 Risk of stroke

FA appears to reduce the risk of stroke. The reviews indicate only that in some individuals the risk of stroke appears to be reduced, but a definite recommendation regarding supplementation beyond the current recommended daily allowance has not been established for stroke prevention [75].

FA is gaining more clinical importance as a preventative agent for many diseases. Extensive clinical research is underway to elucidate its mechanism of action in preventing a disease, or to establish other potential clinical uses. Folic acid is likely going to play a critical and major role in the prevention therapy of many diseases in the future.

2.6.5 Folic acid and oral contraceptives

Oral contraceptives are prescription birth control pills taken by women to prevent pregnancy. Although very effective at preventing pregnancy, about 8% of women taking the pill become pregnant during the first year, according to Planned Parenthood. With perfect use, less than 1% of women will get pregnant. Failure rates with oral contraception occur when they are not taken at almost exactly the same time every day or when they are not taken daily [77]. Thus, women with low folate levels in the failure cases have an increased risk, compared with those having sufficient high folate levels, of giving birth to children suffering from congenital malformations such as neural tube, ventricular valve and urogenital defects [78].

In several cases, women taking oral contraceptives developed folic acid deficiency. However, it appears that many of these women had low intake of folic acid or problems with intestinal absorption prior to taking birth control pills. Again, women on birth control pills should regularly eat good sources of folic acid. Good folate nutrition is especially important for women who become pregnant shortly after they stop taking oral contraceptives [4]. The addition of folic acid to oral contraceptives may prevent birth defects in infants born to women who either accidentally become pregnant while taking the pill or become pregnant shortly after stopping the pill.

CHAPTER III

EXPERIMENTAL

3.1 Materials

The following materials were obtained from commercial suppliers.

3.1.1 Model drug

- Folic acid, Lot No. 455042/1(Fluka, UK) using without further purification.

3.1.2 Polymers and chemicals

- Ammonium hydroxide solution 25 %, AR grade (Merck, Germany)

- Ethyl cellulose, Lot No. 417820/1 (Fluka, UK)

- Acetone, AR grade (Merck, Germany)

- Acetonitrile, HPLC grade (Merck, Germany)

- Methanol, HPLC grade (Merck, Germany)

- Hydrochloric acid fuming 37 %, AR grade (Merck, Germany)

- Sodium hydroxide, AR grade (Merck, Germany)

- Sodium hydrogen phosphate, AR grade (Merck, Germany)

- Span 80 Lot No. 1097347 (Fluka, UK)

- Paraffin oil, Food grade (UCS, Thailand)

- Phosphoric acid 85%, AR grade (Merck, Germany)

- Potassium chloride, AR grade (Merck, Germany)

- Potassium dihydrogen phosphate, AR grade (Merck, Germany)

- Potassium bromide, AR grade (Merck, Germany)

- Tetrabutyl ammonium bisulfate, Lot No. 1303236 (Fluka, UK)

3.2 Instruments

The instruments used in this study are listed in Table 3.1

Table 3.1 Instruments

Instrument	Manufacture	Model
Analytical balance	Mettle	AT 200
HPLC	Thermo Finnigan	P4000
Fourier transform infrared spectrometer	Nicolet	Impact 410
Microscope	Olympus	CH-30
Scanning electron microscope	Jeol	JSM-5800 LV
Digital camera	Olympus	C-4040
	Canon	A 80
pH-meter	Metrohm	744
Horizontal shaking water-bath	Lab-line instrument	3575-1
Centrifuge	Sanyo	Centaur 2
Ultrasonic bath	Ney Ultrasonik	28 H

3.3 Methods

3.3.1 Preparation of folic acid microcapsules by emulsion solvent evaporation technique

The microcapsules of folic acid were prepared by o/o solvent evaporation technique modified from a method described by Song et al [24]. 1.0 g of ethyl cellulose was dissolved in 20 ml of organic solvent. Next, 20 mg of folic acid was dispersed in the polymer solution to give a final concentration of 1 mg/ml. The drug-polymer mixture was thoroughly mixed and slowly emulsified into 100 ml of paraffin oil that contained 1 ml of Span 80 as an emulsifier. The whole system was continuously stirred at 2000 rpm (electric overhead stirrer IKA RW 20) for 5 h at room temperature. Organic solvent was completely removed by evaporation technique and the microcapsules were separated from the solution by vacuum filtration. The filtered microcapsules were then washed three times with 50 ml of hexane. The microcapsules were collected, dried at room temperature overnight and stored in a desiccator. The schematic of the method of preparation is illustrated in Figure 3.1.

3.3.1.1 Effect of solvent

The microcapsules of folic acid were prepared by the above method with varying organic solvent such as dichloromethane, acetone and a mixed solvent of methanol and acetone. The ratio of mixed solvent of methanol and acetone was 1/2, 1/4 and 1/9, respectively.

The appropriate solvent system was selected from the preparation with the well-shaped of microcapsules.

3.3.1.2 Effect of concentration of wall former

The microcapsules of folic acid were prepared by the above method with varying concentration of ethyl cellulose, namely, 2.5, 5.0, and 7.5% (w/v). The other parameters were fixed, i.e., 2.0% (v/v) Span 80, 1 mg/ml of folic acid, and stirring rate of 2000 rpm.

The appropriate concentration of ethyl cellulose was selected from the preparation with the highest encapsulation efficiency and well-shaped release profile of microcapsules.

3.3.1.3 Effect of emulsifier concentration

The microcapsules of folic acid were prepared using various emulsifier concentration; i.e. 1.0%, 2.0% and 4.0% (v/v) while other parameters were fixed; i.e. 2.5% (w/v) ethyl cellulose, 1 mg/ml of folic acid and stirring rate of 2000 rpm.

The particle size distribution, encapsulation efficiency and release profile of microcapsules were determined.

3.3.1.4 Effect of concentration of pore inducer

Water-soluble powder or liquids can be added to the formulation of microcapsules by Emulsion Solvent Evaporation process to obtain desirable release of active ingredients and these powders or solvents are called pore inducers.

The microcapsules of folic acid were prepared by the same method described in the section 3.3.1 except varying amounts of a pore inducer (sucrose), i.e., 2.5%, 5.0% and 7.5% (w/v). The other parameters were kept the same.

The morphology, encapsulation efficiency and release profile of microcapsules were determined.

3.3.1.5 Effect of drug content

The microcapsules of folic acid were prepared using various concentration of drug in ethyl cellulose solution; i.e. 1, 2 and 4 mg/ml. The other parameters were fixed; i.e. 2.5% (w/v) ethyl cellulose, 1.0% (v/v) Span 80, 5.0% (w/v) sucrose and stirring rate of 2000 rpm.

The morphology of microcapsules, encapsulation efficiency and release profile of microcapsules were determined.

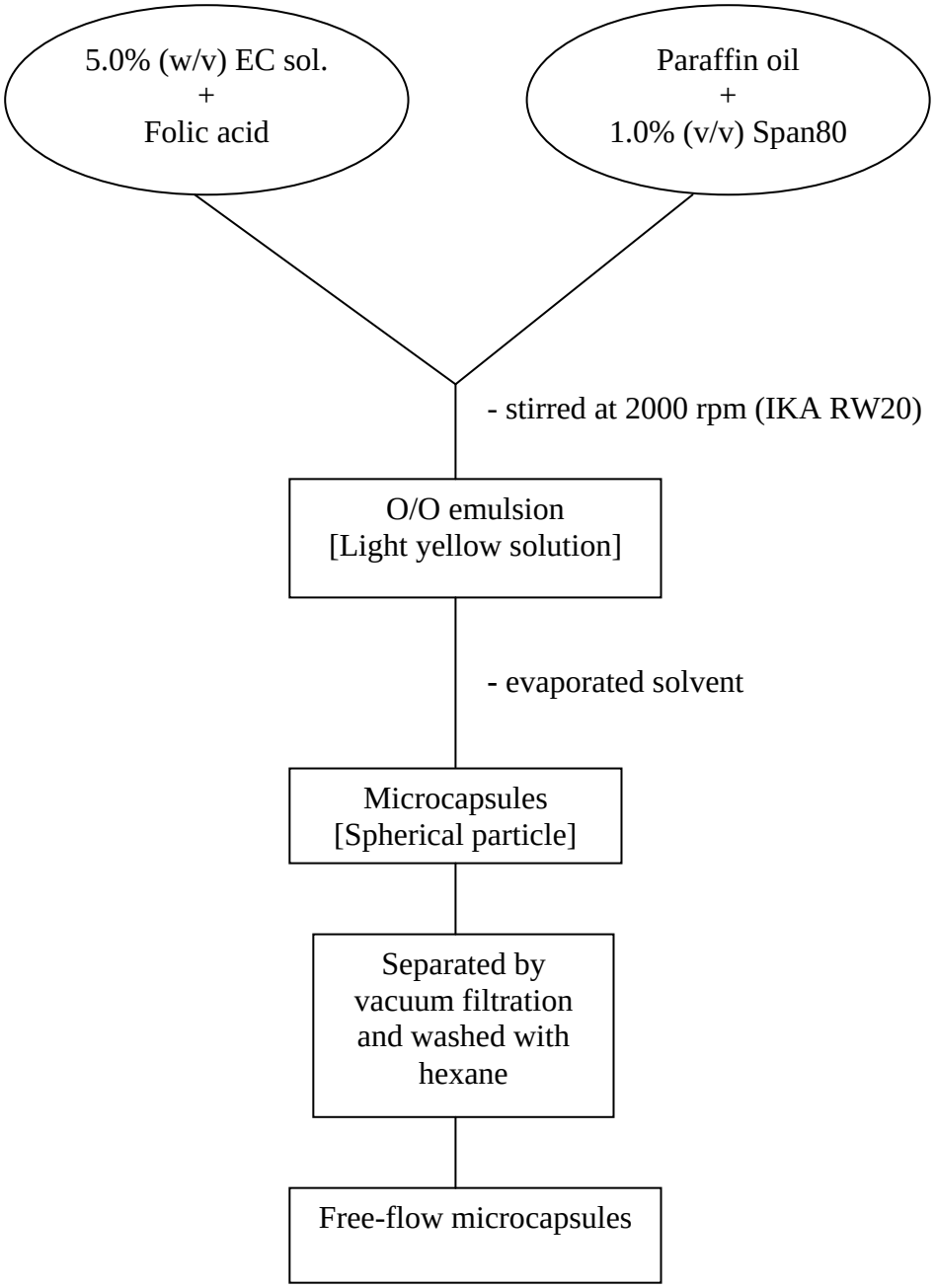


Figure 3.1 Schematic of O/O emulsion solvent evaporation technique for the microcapsule of folic acid

3.3.2 Determination of folic acid by HPLC technique

Quantitative analysis of folic acid was usually associated with analytical problems. HPLC analysis with UV detection was a technique commonly used for the determination of folic acid, since folic acid fluoresced after activation by ultraviolet radiation. Determination of folic acid content in the microcapsules was modified from a method described by Andrisano *et al.* [79].

3.3.2.1 HPLC assay of folic acid

Mobile phase: 0.01 M phosphate buffer (pH 5.0) containing tetrabutyl ammonium bisulfate 4 mM: acetonitrile (76: 24)

HPLC system
Pump: Spectra SYSTEM P 4000
Detector: Spectra SYSTEM UV 6000 LP detector
Column: Pinnacle II C 18 5µm 250 x 46 mm.
Flow rate: 1.0 ml/min
Detection wavelength: 280 nm

3.3.3 Encapsulation efficiency

Microcapsules (50 mg) were dissolved in 5 ml of dichloromethane, 20 ml of water was added and mixed well. The mixture was then centrifuged at 4000 rpm for 10 minutes. 10% (v/v) ammonium hydroxide was added to the supernatant and diluted with distilled water. The diluting solution was filtrated through 0.45 µm nylon membrane filter, 20 µl of filtrate was withdrawn and determined the encapsulation efficiency by HPLC assay with the same conditions described in Section 3.3.2.

The encapsulation efficiency was calculated according to the following equation. All experiments were performed in triplicates.

$$EE(\%) = \frac{Actual\ drug\ content}{Theoretical\ drug\ content}\ X\ 100\%$$

3.3.4 Morphological characterization of the microcapsules

3.3.4.1 Scanning electron microscope (SEM)

The samples for the SEM analysis were prepared by sprinkling the microcapsule on one side of a double adhesive stub. The stub was then coated by gold under vacuum. The microcapsules were then observed with the scanning electron microscope (JSM-5800 LV, JEOL, Japan).

3.3.4.2 Fourier transform infrared spectroscopy (FT-IR)

The infrared spectra of all formulations were recorded with FT-IR (Impact 410, Nicolet). The dried sample was mixed with potassium bromide in agate mortar and pestle. The mixture was then transferred to a hydraulic pressing machine and pressed into a thin disc. The KBr disc was then measured within the wave numbers of 4000-400 cm^{-1}.

3.3.4.3 Particle size and size distribution

The particle size and distribution of microcapsules were tested by a laser particle size analysis instrument (Mastersizer S long bed Ver. 2.19). Microcapsules were joined into ounce distilled water and 0.1% Nondiet P40 to prepare suspension. After the parameters of laser particle size analysis instrument, ultrasonic power and liquid velocity, were regulated, specimen uniformity was joined to beaker and the particle size and distribution of microcapsules are tested.

3.3.5 *In vitro* drug release

3.3.5.1 Folic acid release behavior in SGF (pH 1.2)

Accurately weighed quantities of 100 mg microcapsules were loaded into a bag and then immersed in 250 ml of 0.1 N HCl pH 1.2 in a conical flask. The flask was then placed in a shaken water bath at a speed of 100 rpm with the temperature maintained at 37±1^{o}C. At appropriate intervals, 3 ml of samples were

collected and neutralized with 1 ml of 0.3 M NaOH. The samples were then filtered through a 0.45 µm nylon membrane filter and determined by a HPLC assay as the same conditions described in Section 3.3.2. Samples (3 ml) were withdrawn at 0.25, 0.5, 1, 2, 3, 4, 5 and 6 h, and replaced by the same volume of fresh dissolution medium. Drug release tests were performed in triplicate for each formulation.

3.3.5.2 Folic acid release behavior in SIF (pH 7.4)

Accurately weighed quantities of 100 mg microcapsules were loaded into a bag and then immersed into conical flask with 250 ml of 0.1 M phosphate buffer saline (pH 7.4) and incubated at 37±1^{o}C under shaking speed of 100 rpm. At appropriate intervals, 3 ml of samples were collected. The samples were then filtered through a 0.45 µm nylon membrane filter and determined by a HPLC assay as the same conditions described in Section 3.3.2. Samples (3 ml) were withdrawn at 0.25, 0.5, 1, 2, 3, 4, 5, 6 and 24 h, and replaced by the same volume of fresh dissolution medium. For each formulation, the samples were analyzed in triplicates.

3.3.5.3 Folic acid tablet release behavior in SIF (pH 7.4)

Accurately weighed quantities of 100 mg folic acid tablet were placed into conical flask with 250 ml of 0.1 M phosphate buffer saline (pH 7.4) and incubated at 37±1^{o}C under shaking speed of 100 rpm. At appropriate intervals, 3 ml of samples were collected. The samples were then filtered through a 0.45 µm nylon membrane filter and determined by a HPLC assay as the same conditions described in Section 3.3.2. Samples (3 ml) were withdrawn at 0.25, 0.5, 1, 2, 3, 4, 5, 6 and 24 h, and replaced by the same volume of fresh dissolution medium. For each formulation, the samples were analyzed in triplicates.

Chapter IV

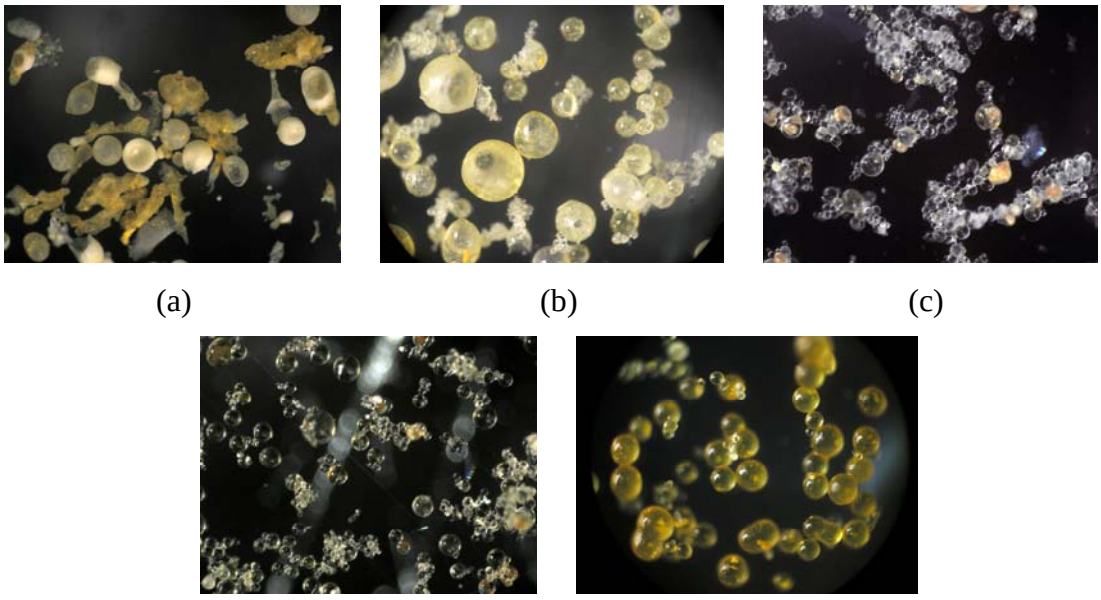
RESULTS AND DISCUSSION

4.1 Preliminary study

4.1.1 Effect of solvent on emulsion solvent evaporation technique

Microencapsulation by the solvent evaporation method was a complex process, which was influenced by several parameters such as solvent evaporation rate, solubility of polymer and drug in both emulsion phases. The organic solvent was crucial factor that could be affected in the forming of microcapsules in this method. The solvent of the dispersed phase must be slightly soluble in the continuous phase so that partitioning into the continuous phase could lead to precipitation of the matrix material. Then the solvent must evaporate from the surface of the dispersion to yield sufficiently hardened microcapsules. In this study three kinds of organic solvents, dichloromethane, acetone, and binary mixture of methanol with acetone, were used in the preparation of microcapsules.

Oil in oil emulsion solvent evaporation technique was used in the preparation of folic acid-loaded microcapsules. First, ethyl cellulose was dissolved in three kinds of organic solvents: dichloromethane, acetone, and binary mixture of methanol with acetone. Folic acid, as a model drug, was suspended in the ethyl cellulose solution and this polymer/drug solution was emulsified in the paraffin oil. Span 80 was used as stabilizer in order to make the emulsion stable during dispersing polymer/drug solution in paraffin oil. Stirring was continued for 5 hours in order to ensure complete evaporation of the organic solvent, resulting in a thin polymer film coated around the emulsion droplets.



(a) (b) (c)

(d) (e)

Figure 4.1 Microphotographs of microcapsules prepared with various solvents (a) dichloromethane (b) acetone (c) methanol/acetone (1/2) (d) methanol/acetone (1/4) (e) methanol/acetone (1/9).

Figure 4.1 showed the photomicrograph of the resulting microcapsules prepared with various solvents. The microcapsules had irregular shapes when dichloromethane was used as solvent. This might be due to the miscibility of dichloromethane and paraffin oil which diffused out as soon as the dispersed phase was pour into the oil phase. The fast solvent removal rate probably encouraged the formation of coagulates during the early stages of droplet formation. To form stable emulsion droplets, a certain amount of times was required before the organic solvents diffused into paraffin oil phase and the polymer solidified. The result showed that when using oil as a processing medium and only dichloromethane as a dispersed medium it did not ensure formation of a stable emulsion. Therefore the primary requirement of this method to obtain stable well-shaped microcapsules was the selected solvent system for polymer.

From the previous study, acetone was chosen as a solvent because it was soluble in liquid paraffin to a limited extent. The resulting microcapsules were spherical and rough surface with some agglomerates (Figure 4.1b). In this case, some of the acetone was partitioned into the liquid paraffin external phase when acetone

was added. The extent of acetone partitioning depended on the affinity between liquid paraffin oil and acetone. Note that even in solvent evaporation, the solvent must first diffuse into the suspension medium before it was removed by evaporation. The limited solubility in oil of acetone affected to low partitioned from the polymer droplets. As a result, it was more difficult to break the polymer droplets.

Another strategy, that affected the solvent composition studied here, was the use of a binary mixture of methanol with acetone, a water-miscible organic solvent. Methanol was chosen since it diffused out into oil phase slowly due to its low solubility in oil phase. In this case, microcapsules were formed by the following steps. When the polymeric solution was poured into the continuous phase, emulsion droplets were formed. Acetone quickly diffused out from each emulsion droplet and the remaining methanol was then removed from the system, causing the droplets to solidify and finally formed polymeric microcapsules.

The effect of solvent ratio on the morphology of microcapsules was investigated (Figure 4.1). The varying volume ratios of methanol/acetone were tested at 1/2, 1/4 and 1/9, respectively. The volume of methanol played a key role in the formation of microcapsules. The proportion of methanol was larger such the volume ratio as 1/2 and 1/4, the resulting microcapsules were smaller with irregular shapes whereas the volume ratio as 1/9 gave the well-shaped of microcapsules. As the volume of acetone was increased, the microcapsules were formed in a spherical shape.

These results showed that solvent composition could also be a key factor in the characteristic of the microcapsules. In this study, the mixed solvent system comprising 1/9 proportion of methanol and acetone was used.

4.2 Fourier transform infrared spectroscopy (FTIR)

FTIR spectroscopy was used to determine the chemical interaction of the samples as displayed in Figures 4.2 to 4.6.

The IR spectrum for the pure drug and ethyl cellulose was presented in Figure 4.2. The IR spectrum for ethyl cellulose (Figure 4.2(a)) showed a distinct peak at 3482 cm^{-1} which was due to the O-H groups present on the closed ring structure of the polymer's repeating units. The same also represented the intra- and intermolecular hydrogen bonding due to the O-H groups. The asymmetric peak around 2976-2877 cm^{-1} might be due to –CH stretching vibration. The peak at 1379 cm^{-1} was due to $–CH_3$ bending and the small peak near 1447 cm^{-1} was due to $–CH_2$ bending. The broad distinct peak near 1112 cm^{-1} may be due to the C-O-C stretch in the cyclic ether.

In the IR spectrum of folic acid (Figure 4.2(b)), band related to N-H stretching vibration of the amine terminal groups could be observed at 3413cm^{-1} and 3323 cm^{-1}. The presence of absorption at 1700 cm^{-1} for C=O stretching of carboxylic group which was presented in folic acid but not in ethyl cellulose. The peak at 1605 cm^{-1} and 1339 cm^{-1} were attributed to N-H bending vibration and C-N stretching vibration of amide groups.

The IR spectra of folic acid-loaded microcapsules prepared with different concentration of polymer were shown in Figure 4.3. All of the IR spectra showed the characteristic absorption band of ethyl cellulose. The band centered at around 3480 cm^{-1} was assigned to O-H stretching vibration and the band at around 2900 cm^{-1} was assigned to -CH stretching vibration. The peak at around 1380 cm^{-1} was due to $-CH_3$ bending and the broad distinct peak near 1100 cm^{-1} was attributed to the C-O-C stretch in the cyclic ether. Whereas the characteristic peak of folic acid around 1700 cm^{-1}, concerned to the stretching vibration of C=O bond of carboxylic acid group, shifted to the wave number around 1740 cm^{-1}. It could imply that there was the non covalent intramolecular interaction between O-H group of ethyl cellulose and C=O of folic acid. At the same time, the peak at 1600 cm^{-1} which was corresponding to N-H bending vibration was not shifted and the new absorption bands of drug-loaded microcapsules were not presented.

Figure 4.4 illustrated the IR spectra of microcapsules prepared with different concentration of emulsifier. The IR spectrum of all formulations exhibited characteristic band of ethyl cellulose which were around 3400 cm^{-1} for the O-H stretching vibration, 2970 cm^{-1} for the -CH stretching vibration, 1370 cm^{-1} for the -CH_3 bending and 1100 cm^{-1} corresponding to C-O-C stretching vibration. While the characteristic peak of folic acid around 1700 cm^{-1} shifted to the wave number around 1740 cm^{-1}. The result showed that there was the non covalent intramolecular interaction between polymer and drug. In addition there were no new absorption bands of drug-loaded microcapsules.

Figure 4.5 presented the IR spectra of folic acid-loaded microcapsules with different amount of sucrose as the pore inducer. The IR spectrum of sucrose presented the peak at 910 cm^{-1} and 990 cm^{-1} assigned to -CH_2 out of plan wag and -CH_2 out of plan deformation and the peak at 1068 cm^{-1} was attributed to C-O stretching. The shoulder peak at 1120 cm^{-1} assigned to C-O-C stretching of aliphatic ethers that found in both structure of sucrose and ethyl cellulose. Whereas the characteristic of folic acid exhibited the peak at 1740 cm^{-1} that corresponding to C=O of carbonyl group. The peak shifted due to the non covalent intramolecular interaction between polymer and drug. Again, there were no new adsorption peaks of drug-loaded microcapsules.

Figure 4.6 illustrated the IR spectra of folic acid-loaded microcapsules with different amount of drug. The characteristic peak of folic acid showed at 1740 cm^{-1} corresponding to C=O of carbonyl group. The IR spectrum of all formulations exhibited characteristic band of ethyl cellulose which were around 3400 cm^{-1} for the O-H stretching vibration, 2930 cm^{-1} for the -CH stretching vibration. While The IR spectrum of sucrose presented the peak at 910 cm^{-1} and 990 cm^{-1} assigned to -CH_2 out of plan wag and -CH_2 out of plan deformation and the peak at 1068 cm^{-1} was attributed to C-O stretching.

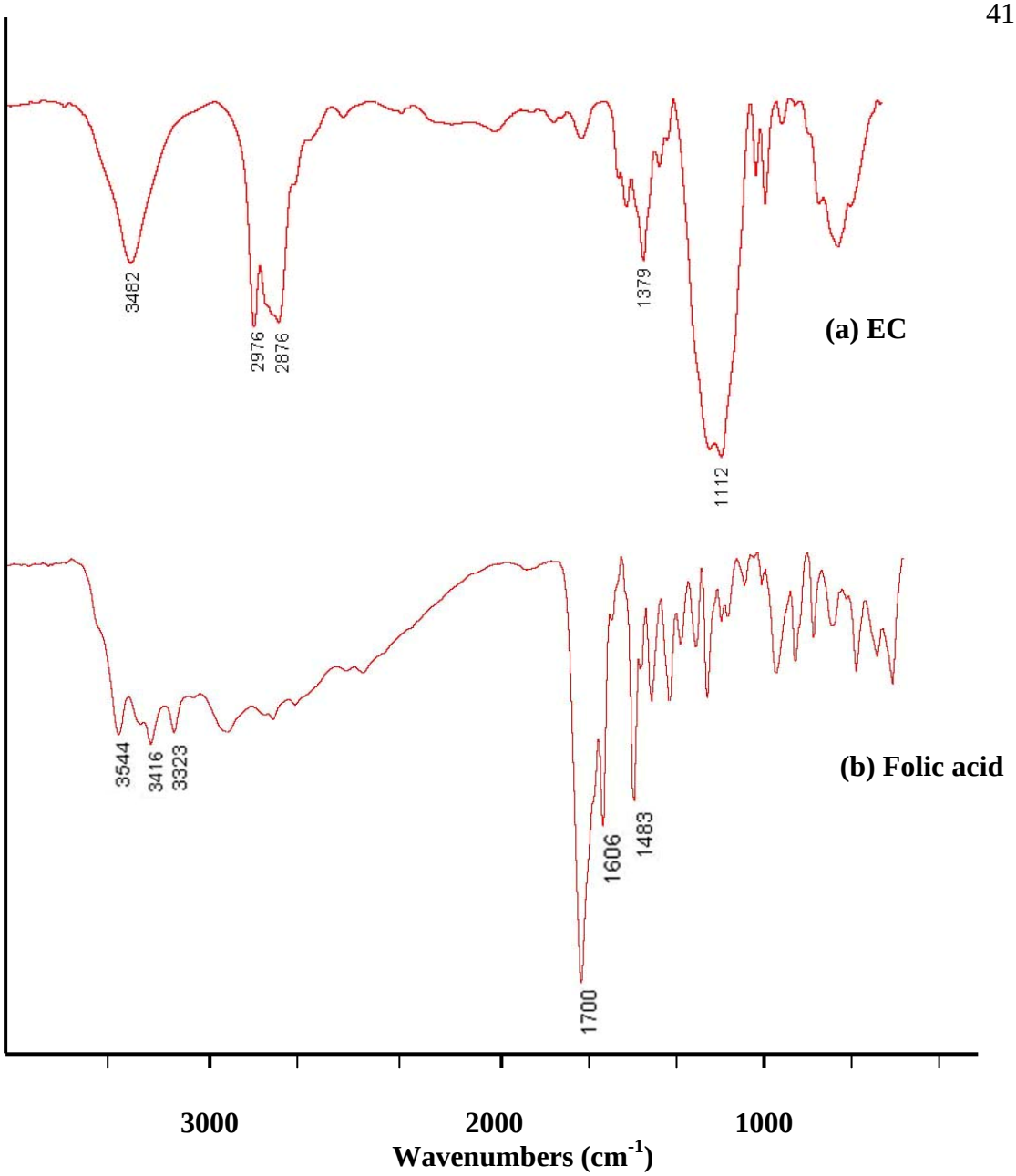


Figure 4.2 IR spectra of (a) pure ethyl cellulose, (b) pure folic acid.

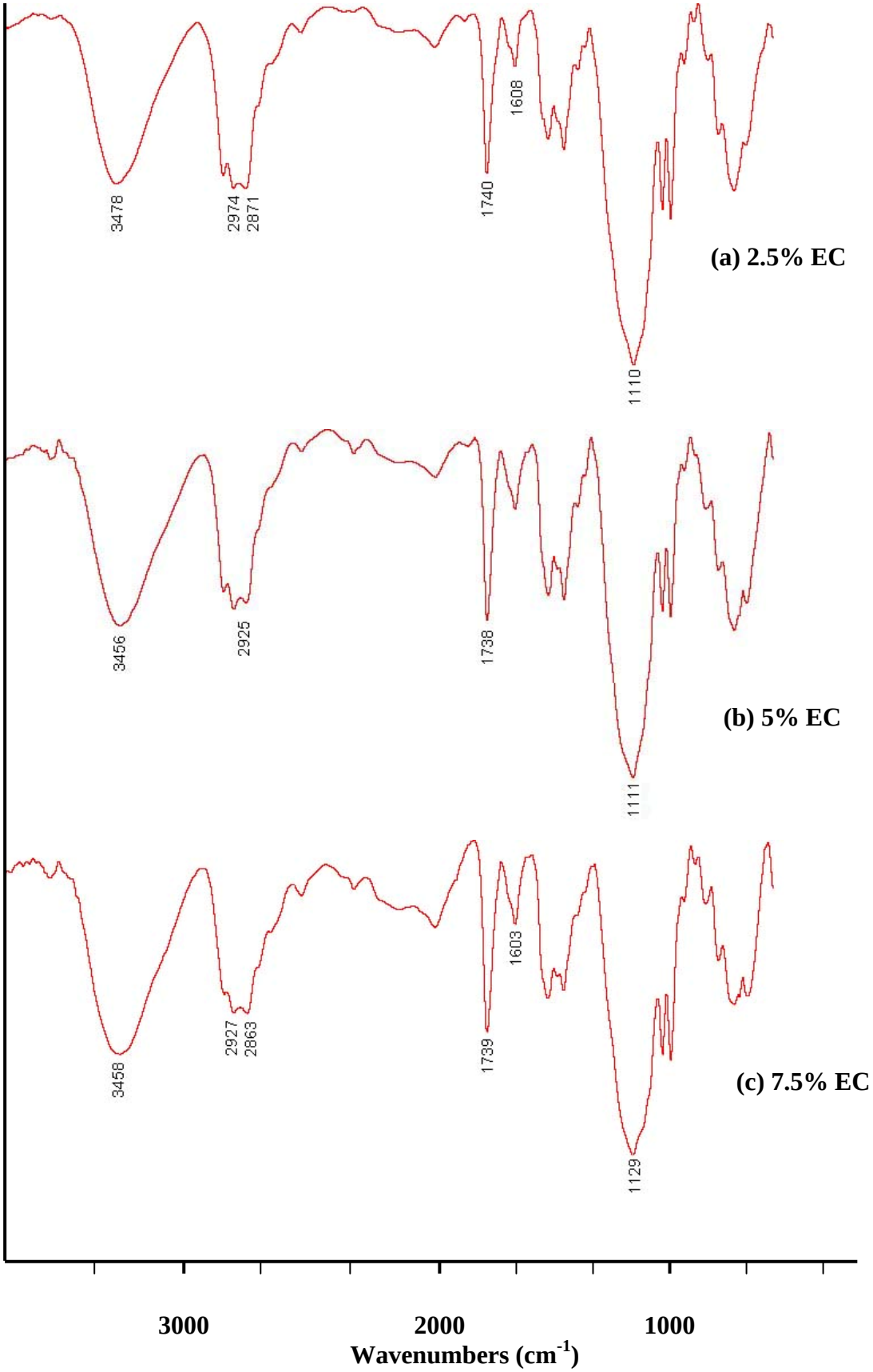


Figure 4.3 IR spectra of folic acid-loaded microcapsules prepared with different polymer concentrations (a) 2.5% (w/v) EC, (b) 5% (w/v) EC, (c) 7.5% (w/v) EC.

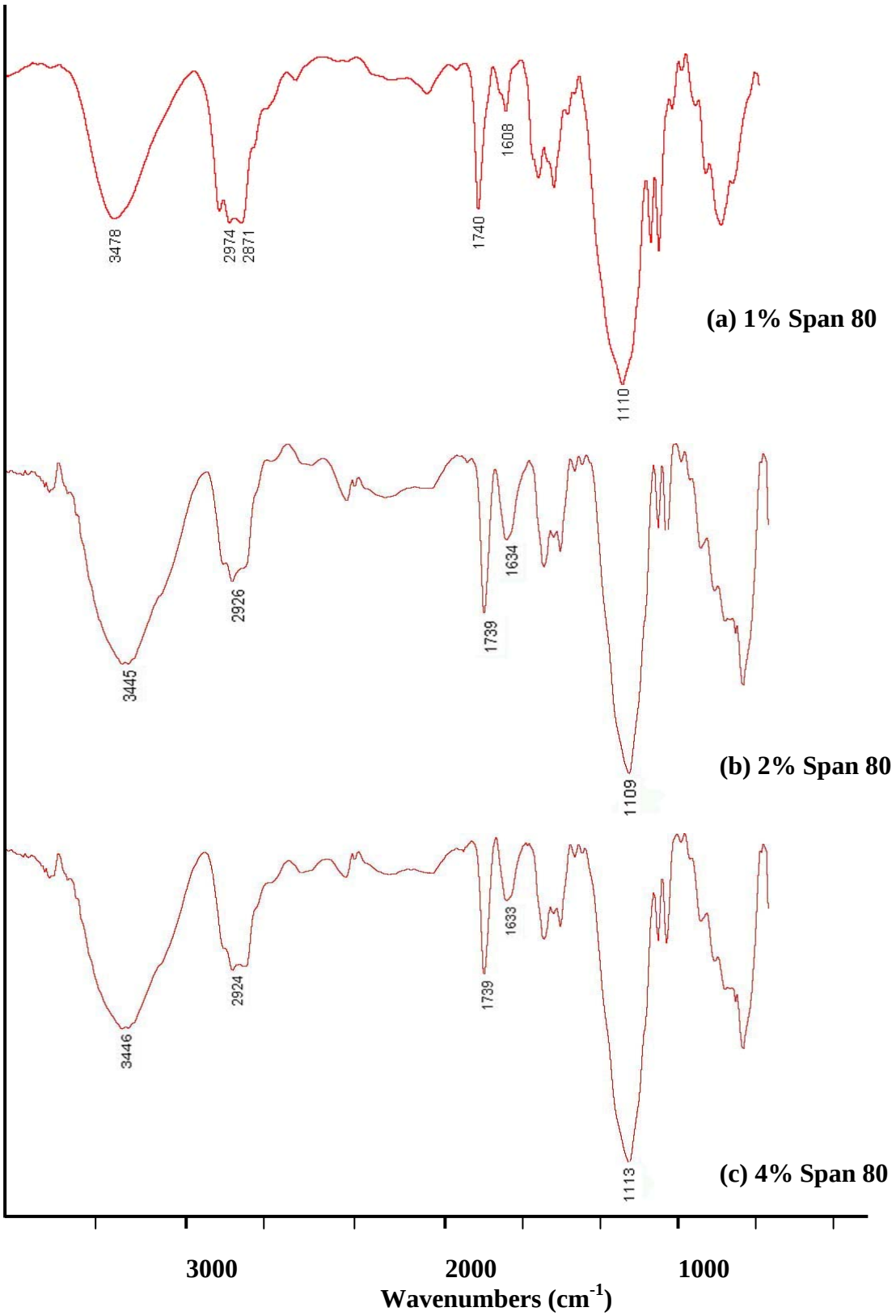


Figure 4.4 IR spectra of folic acid-loaded microcapsules prepared with different emulsifier concentrations (a) 1% (v/v) Span80, (b) 2% (v/v) Span80, (c) 4% (v/v) Span80.

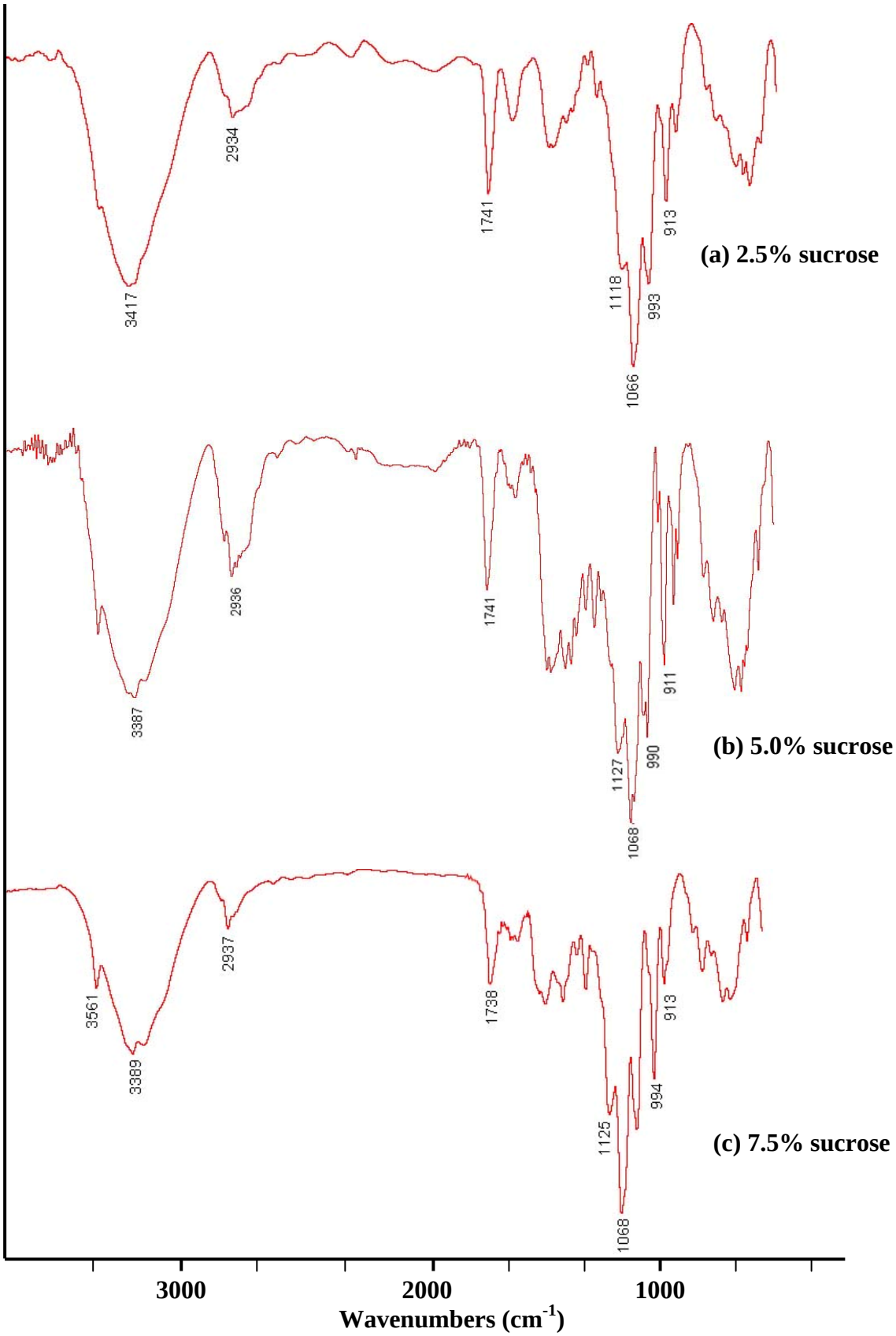


Figure 4.5 IR spectra of folic acid-loaded microcapsules prepared with different amount of pore inducer (a) 2.5% (w/v) sucrose, (b) 5.0% (w/v) sucrose, (c) 7.5% (w/v) sucrose.

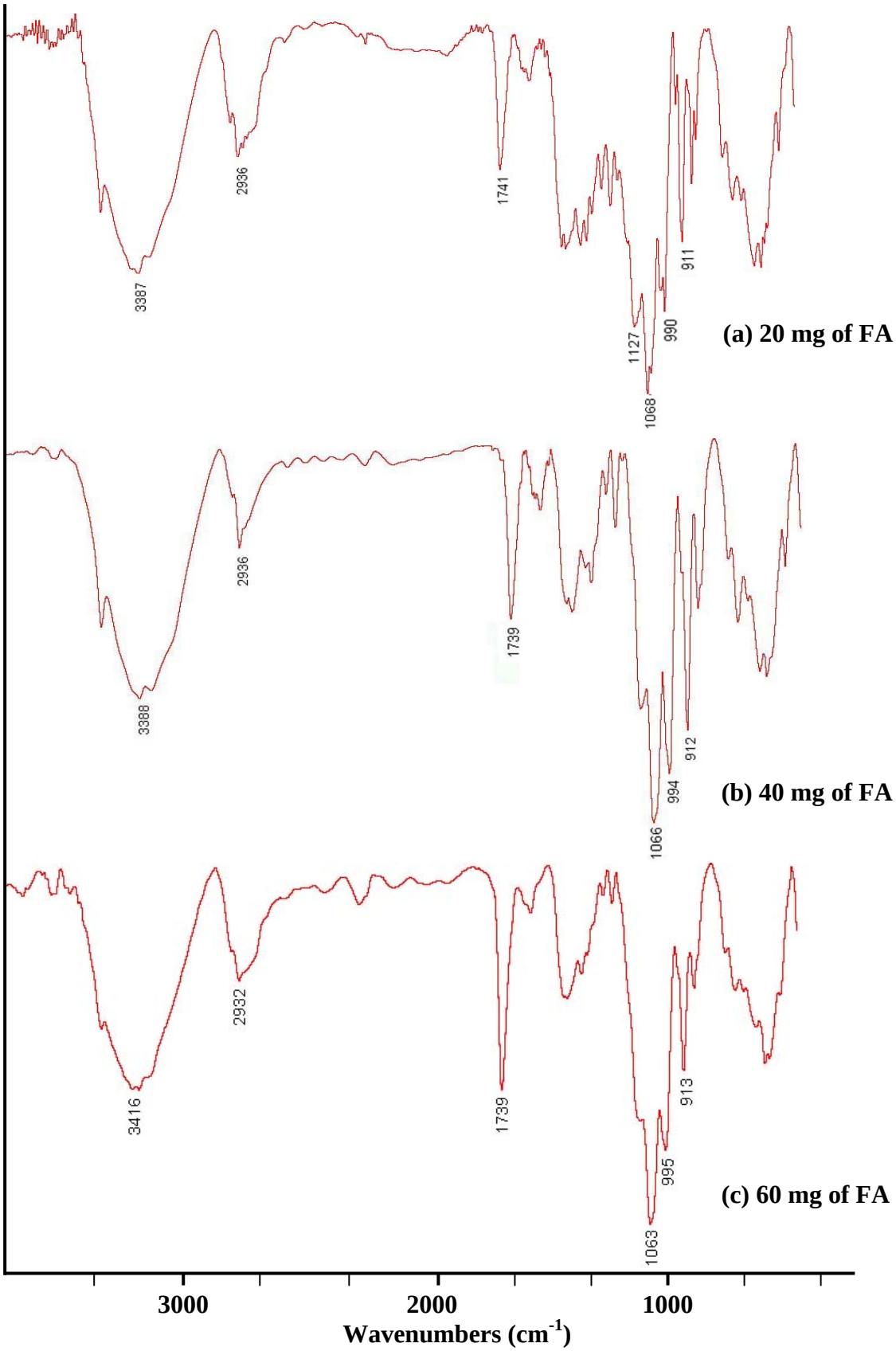


Figure 4.6 IR spectra of folic acid-loaded microcapsules prepared with different amount of folic acid (a) 20 mg of FA, (b) 40 mg of FA, (c) 60 mg of FA.

4.3 Stability of folic acid

Folic acid like all other folates was in danger of oxidative degradation, which was enhanced by oxygen, light, heat and a shift in pH either way from 7.6. In aqueous solution, *p*-aminobenzoyl-L-glutamic acid and pterine-6-carboxylic (Figure 4.7) acid were the major degradation products of folic acid, along with traces of *p*-aminobenzoic acid (Figure 2.8). Degradation of the active ingredient during release experiment might be the reason for failure released of active ingredient from the dosage unit. To address that, the HPLC methods were assessed for their ability to detect the degradable products, so as to determine qualitatively and quantitatively if major loss of the active ingredient had occurred.

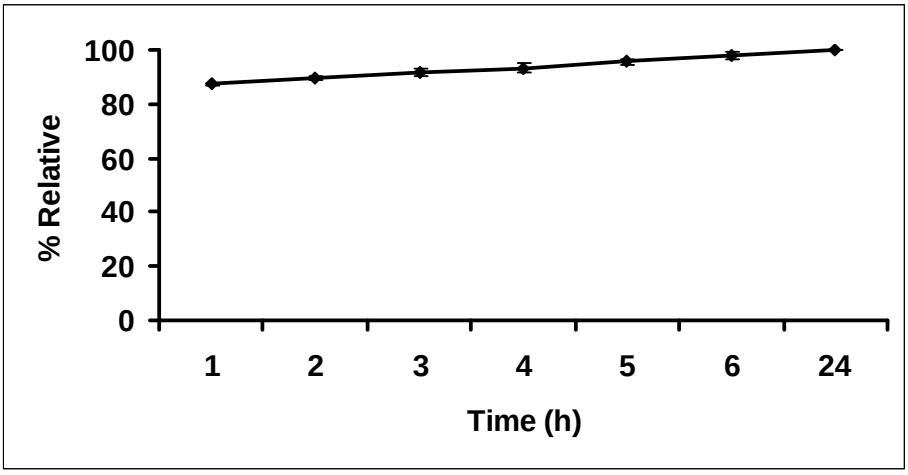


Figure 4.7 Percent relative of folic acid in acidic medium.

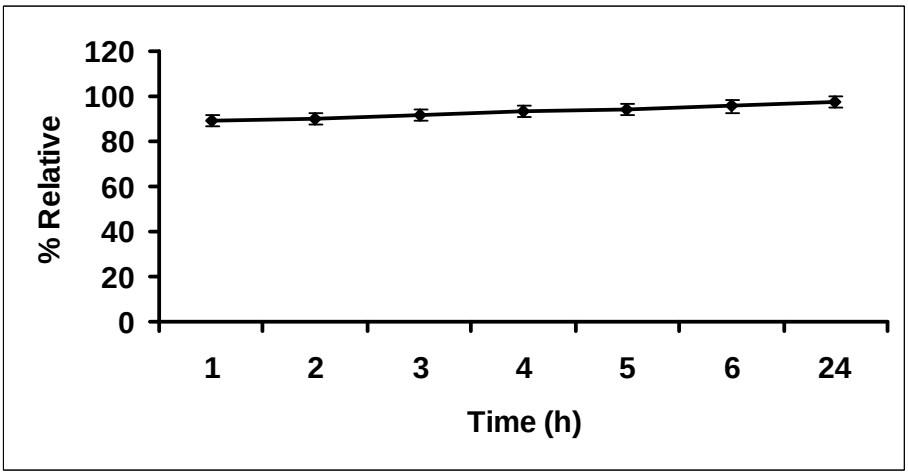


Figure 4.8 Percent relative of folic acid in pH 7.4 phosphate buffer.

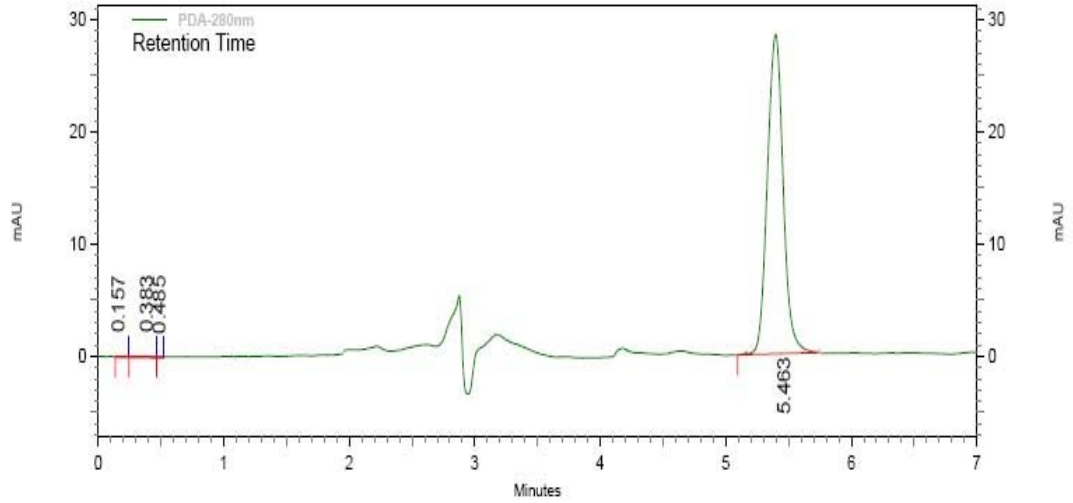


Figure 4.9 Chromatogram of folic acid in acidic simulating the gastric pH.

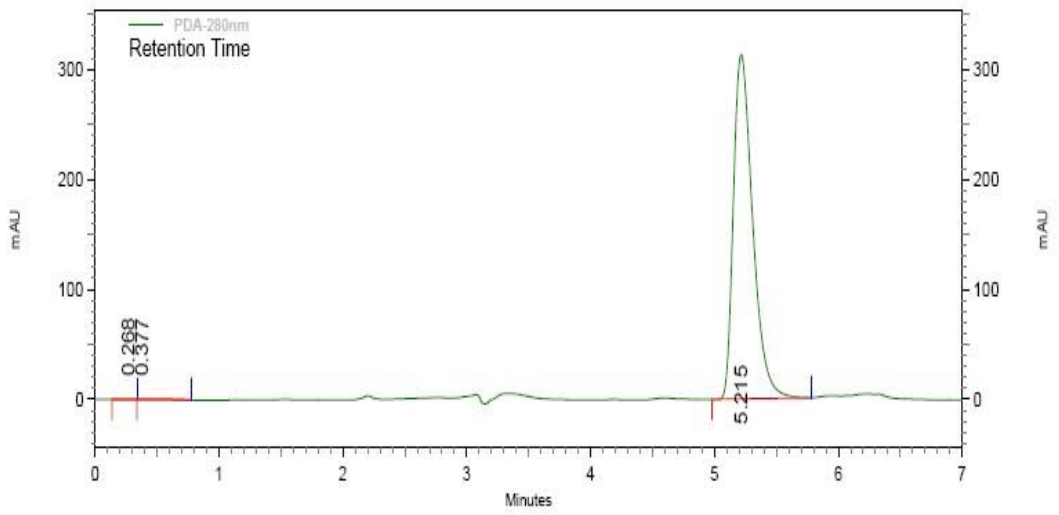


Figure 4.10 Chromatogram of folic acid in pH 7.4 simulating the intestinal pH.

The relative amount of folic acid with time was shown in Figures 4.7-4.8. It was observed that the amount of folic acid was in range of 88-98% in acidic simulating the gastric pH and about 89-95% of drug was observed in pH 7.4 simulating the intestinal pH. The chromatogram (Figures 4.9-4.10) showed the single peak of folic acid at 5.4 minutes in acidic simulating the gastric pH and at 5.2 minutes in pH 7.4 simulating the intestinal pH. There were no signs of major degradation of folic acid in both mediums. These results demonstrated that the folic acid had the stability in acidic simulating the gastric pH and pH 7.4 simulating the intestinal pH.

4.4 *In vitro* drug release

To evaluate the pH-dependent release profiles of folic acid from microcapsules, *in vitro* release tests were studied at two pH values, acidic (pH 1.2) simulating the gastric pH and simulating the intestinal pH (pH 7.4).

4.4.1 Folic acid release behavior

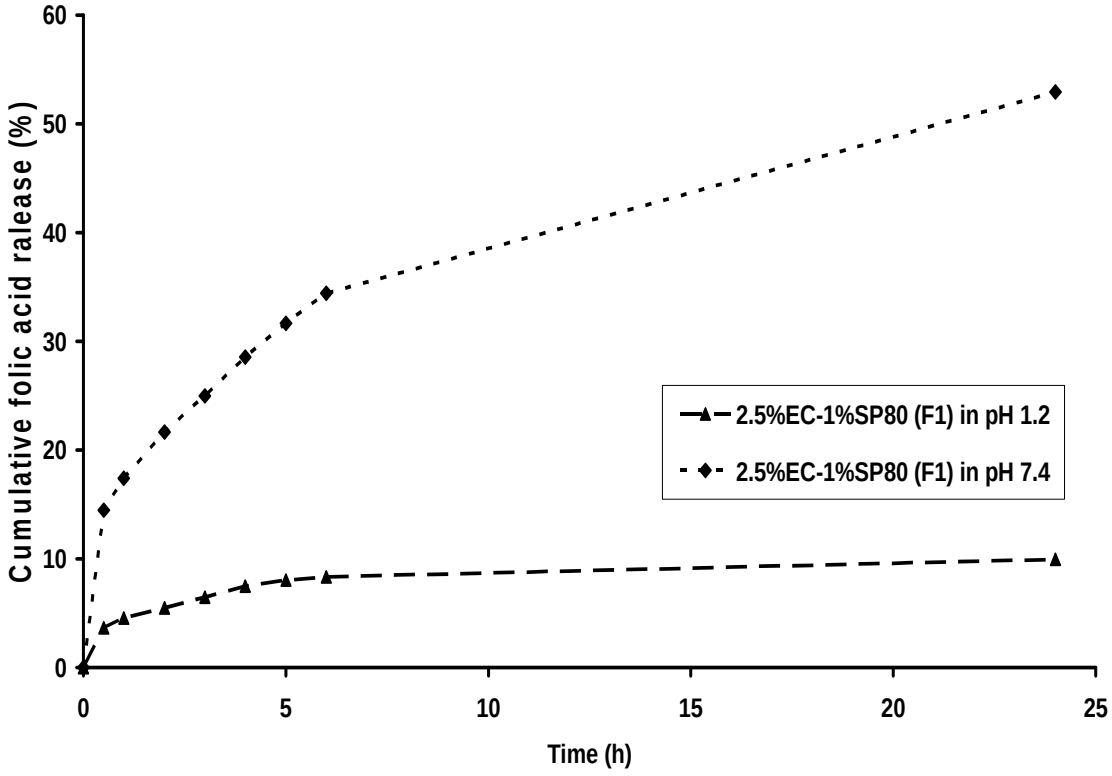


Figure 4.11 The release profiles of folic acid from the folic acid-loaded microcapsules (Formulation F1) in 0.1 N HCl pH 1.2 and pH 7.4 phosphate buffer.

Figure 4.11 illustrated the release behavior of folic acid-loaded microcapsules (Formulation F1) in the acidic medium (0.1 N HCl) and pH 7.4 phosphate buffer. In pH 7.4 phosphate buffer, the cumulative drug release of microcapsules (Formulation F1) reached to 35% during 6 hours and about 50% of the drug was released in 24 hours. At the same time, the cumulative drug release of microcapsules (Formulation F1) was about 10 % during 24 hours. The result was shown that ethyl cellulose could retard drug released from microcapsules in acidic medium. This was possibly due to

the hydrophobicity of polymer that provided the good stability at varying pH values. Thus folic acid-loaded microcapsules (Formulation F1) were relatively stable and incapable of preventing the drug release in acidic medium. This advantage of hydrophobic polymer provided the higher absorption of folic acid in the upper small intestine.

Table 4.1 Folic acid solubility in buffers at different pH [80]

pH	Folic acid solubility (mg/ml)	RSD (%)
1	0.029	4.02
3	0.840	1.66
4	1.050	5.33
7	5.330	0.77
10	19.470	2.84

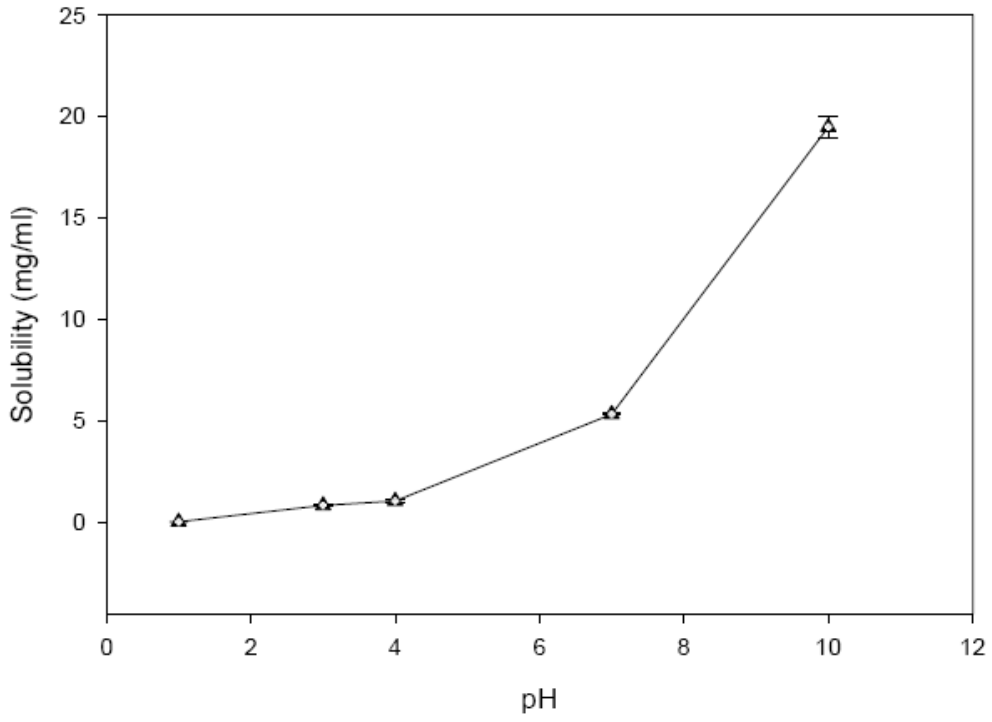


Figure 4.12 Folic acid solubility pH-profile [80].

In addition, the minimal release of folic acid in acidic medium depended on the pH-dependent solubility of folic acid. It was also seen in both of the commercial folic acid tablet and folic acid-loaded microcapsules (Formulation F1). Younis Islam *et al.* [80] reported that the solubility of folic acid at acidic pH might play a major role in the failure of the tested products to release. A folic acid solubility-pH profile was generated (Table 4.1 and Figure 4.12). The result showed that folic acid exhibited an increased solubility with increasing pH at 37 ^{o}C. The solubility of folic acid was 0.029 mg/ml at pH 1 while about 5.330 mg/ml of folic acid was soluble in pH 7. Folic acid was more highly ionized in basic pH. The ionic form of folic acid was more soluble in aqueous solutions than the non-ionic free acid form. This might be a contributing factor to the distinct changes in dissolution as a function of pH for commercial folic acid tablet and folic acid-loaded microcapsules.

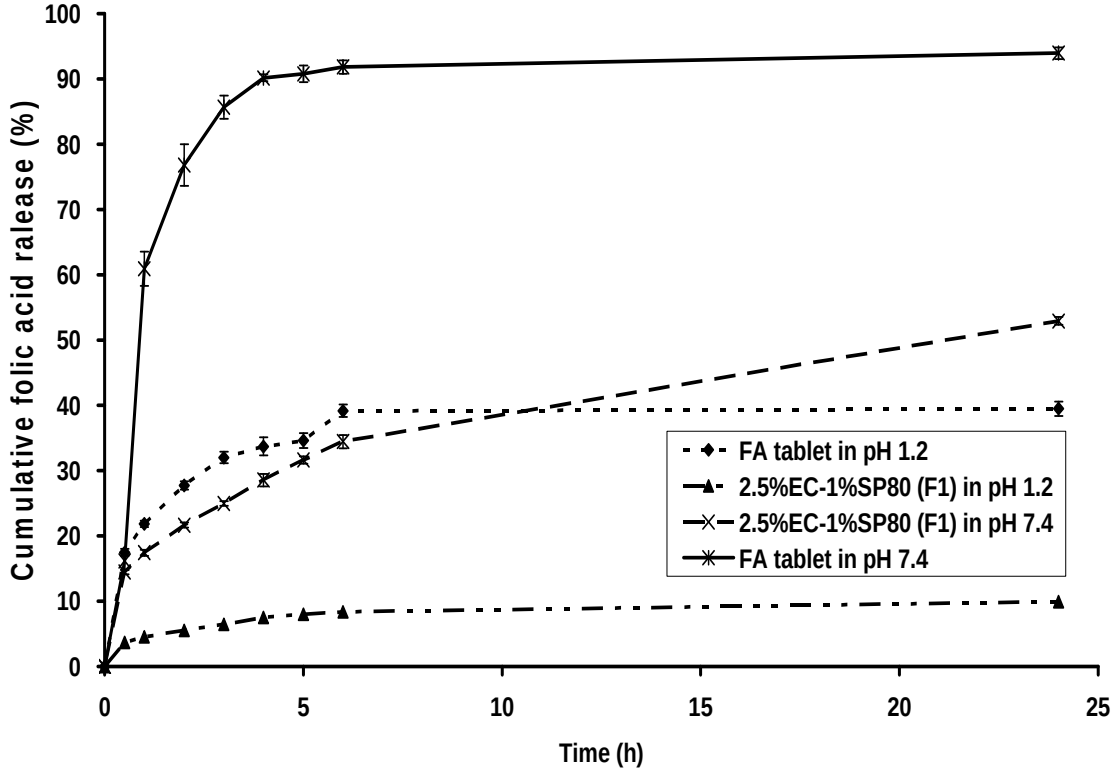


Figure 4.13 The release profiles of folic acid from the folic acid-loaded microcapsules (Formulation F1) and the commercial folic acid tablet in 0.1 N HCl pH 1.2 and pH 7.4 phosphate buffer.

Compared with the commercial folic acid tablet, the folic acid-loaded microcapsules exhibited more sustained release of the drug during 24 hours than ones in both mediums. In SIF, the commercial folic acid tablet extremely released about 90% of the drug entrapment while the cumulative drug release of microcapsules was about 52% during 24 hours. It was observed that the folic acid-loaded microcapsules could not completely release the drug in time.

From the above problem, the influence of various parameters on the microcapsule preparation was studied to improve the release behavior. Therefore, the further *in vitro* study of folic acid-loaded microcapsules was especially presented in pH 7.4 phosphate buffer medium.

4.4.2 Folic acid release behavior in SIF (pH 7.4 phosphate buffer)

4.4.2.1 Effect of an increase in concentration of wall former

The concentration of the ethyl cellulose in dispersed phase exerted a significant impact upon the microencapsulation process. A series of ethyl cellulose solutions with different concentrations, namely, 2.5%, 5.0%, and 7.5% (w/v) were used to prepare folic acid microcapsules while keeping all the other factors the same (1 mg/ml of folic acid, 1% (v/v) of Span80 and stirring rate of 2000 rpm). The particulate properties of microcapsules prepared with different concentrations of ethyl cellulose were listed in Table 4.2.

Table 4.2 Particulate properties of folic acid microcapsules prepared with different concentrations of ethyl cellulose (n = 3)

Formulation	Conc. of EC (%)	D_{50} (μm)*	SPAN**	E.E. (%)***
F1	2.5	300±9	2.12±0.06	80.29±1.46
F2	5.0	392±2	1.86±0.04	61.80±1.22
F3	7.5	448±9	1.42±0.04	41.85±1.33

*D_{50} = mean particle size (mean ± SD)

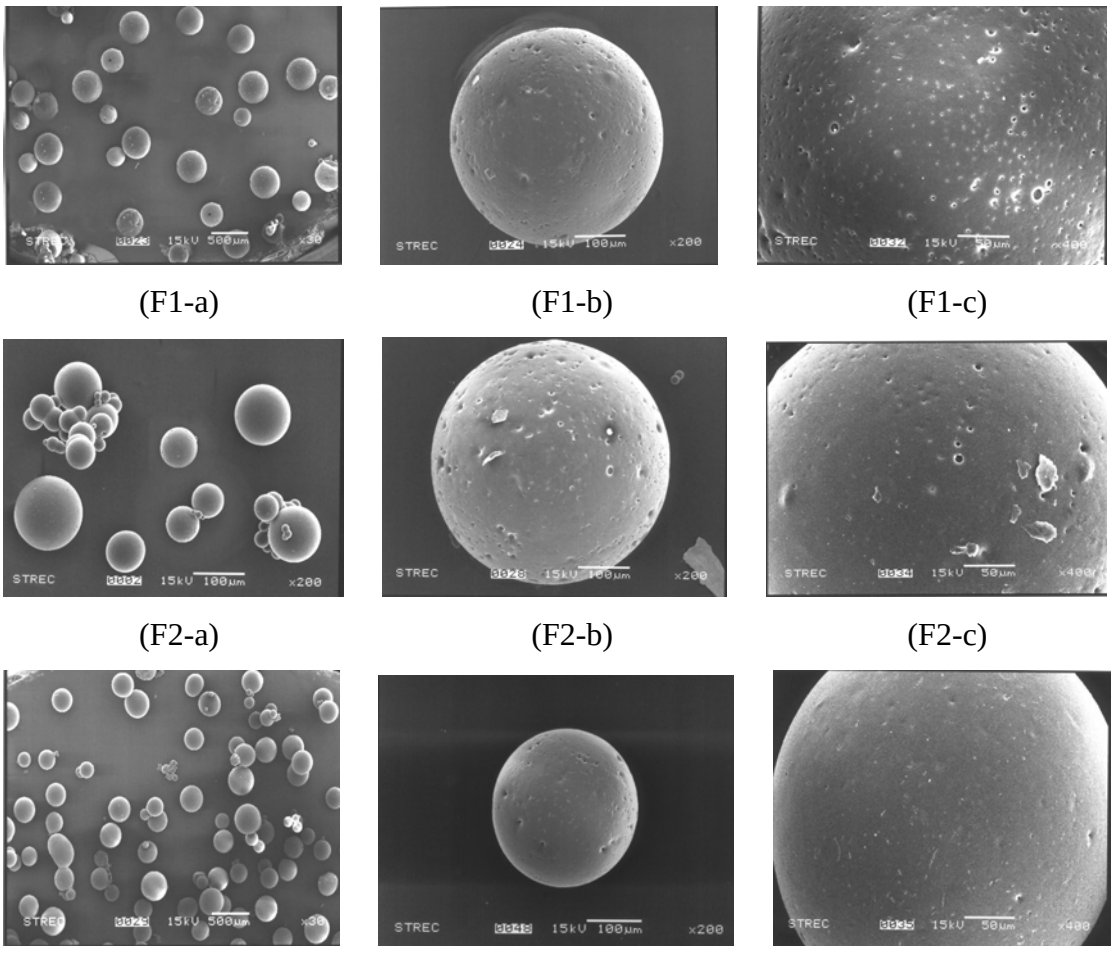
**SPAN = polydispersity Index (mean ± SD)

*** E.E. = encapsulation efficiency (mean ± SD)

The data showed that as the concentration of ethyl cellulose increased from 2.5% to 7.5% the average diameter of microcapsules increased within the range of 299 ± 9 μm to 447 ± 9 μm. This was due to the viscosity of the medium increased at a higher polymer concentration resulting in the enhancement of interfacial tension. At fixed stirring shear force, it was difficult for small emulsion droplets to form because higher shear forces were necessary for droplet disruption. This resulted in the formation of larger particles. While SPAN values, as an indicator of particle size distribution decreased from 2.12 to 1.42 with an increase in the concentration of ethyl

cellulose. These results showed that the size distribution of microcapsules was getting narrower with increasing the concentration of ethyl cellulose, because of the particle was separated as a stable form without the aggregation between the microcapsules when completed evaporation of the solvent.

The encapsulation efficiency, expressed as the percentage of the actual loading to the theoretical loading, decreased from 78% to 41% with an increase in concentration of ethyl cellulose. The increasing viscosity of polymer solution at higher concentration yielded the formation of larger polymer/solvent droplets. It caused the drug diffusion out of the particle which tended to decrease encapsulation efficiency due to slower hardening of the larger particle [81].



(F1-a) (F1-b) (F1-c)

(F2-a) (F2-b) (F2-c)

(F3-a) (F3-b) (F3-c)

Figure 4.14 Scanning electron micrographs of each formulation (F1-F3): (a) overview of folic acid-loaded microcapsules, (b) and (c) the surface of microcapsules.

The SEM micrographs of microcapsules were shown in Figure 4.14. All of the microcapsules were spherical and smooth. The different concentrations of ethyl cellulose, namely; 2.5%, 5.0% and 7.5% (w/v) were resulted in difference in the morphology of the microcapsules. The morphology of the formulation F1 and F2 were spherical in shape and exhibited pinholes on the surface due to the rapid escape of the volatile solvent during formation. As the formulation F3 showed smooth surface. This was understandable since the more concentration of ethyl cellulose led to increase the viscosity of polymer solutions influencing the slow rate of solvent removal. The slow solidification favored denser over more porous microcapsules. Thus, the rate of removal of the solvent from embryonic microcapsules influenced the morphology of the product.

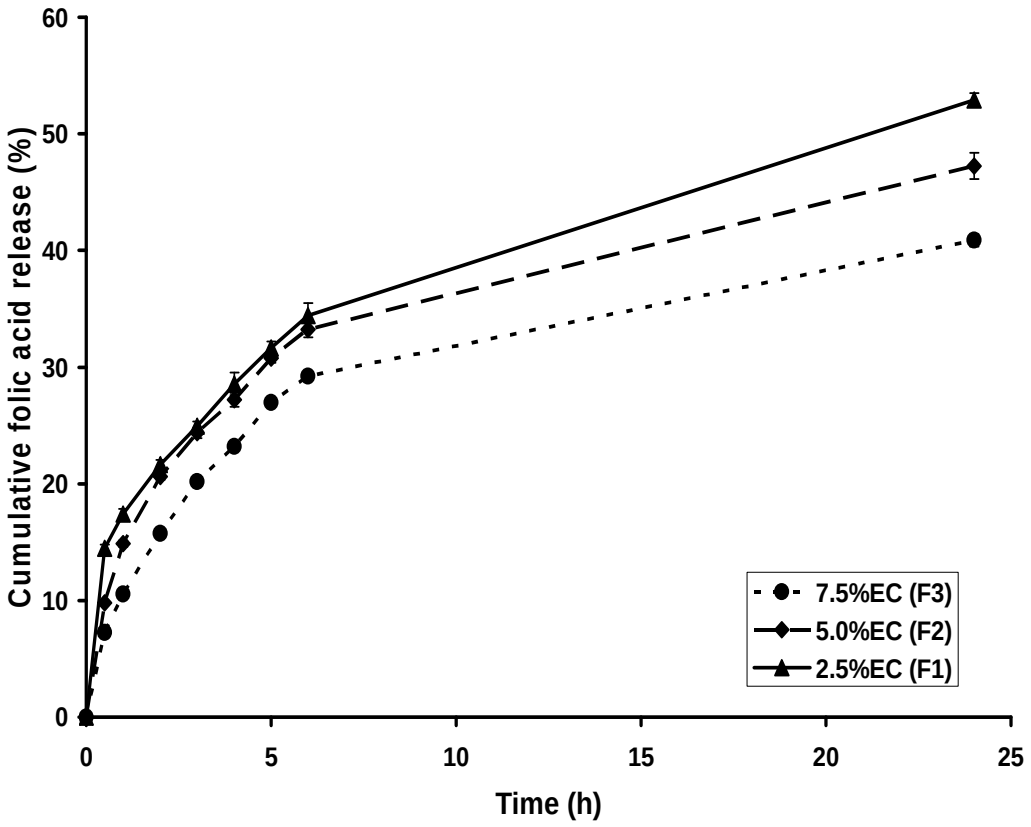


Figure 4.15 The release profiles of folic acid from microcapsules prepared with increasing concentrations of ethyl cellulose in SIF (pH 7.4 phosphate buffer).

Figure 4.15 showed the effect of the concentration of ethyl cellulose on the drug release behaviors. The release profile showed that ethyl cellulose microcapsules could retard the drug release. The drug was continuously release with constant rate during 24 hours. The release rate of folic acid from the microcapsules decreased with an increase in the concentration of ethyl cellulose. Retardation of drug release could be a result of increase of particle size as well as the increase in matrix density when the concentration of ethyl cellulose was increased. Furthermore larger microcapsules were formed at a higher polymer concentration and had a smaller surface area exposed to dissolution medium, giving reduce to drug release. At 24 hours, the formulation F1, F2, and F3 released about 52%, 47%, and 40% of the drug, respectively. In fact, ethyl cellulose was a non-water-soluble polymer. The release of water-soluble drugs was mainly driven by a permeation of the drug through the hydrophobic polymer membrane within water filled pores [82]. Therefore, the amount of ethyl cellulose increased, drug diffusion reduced correspondingly.

These results showed that suitable concentration of ethyl cellulose for further study should obtain desired entrapment efficiency and release profile. Therefore, the formulation F1 which produced microcapsules with desirable encapsulation efficiency (80%) and sustained release profile was selected.

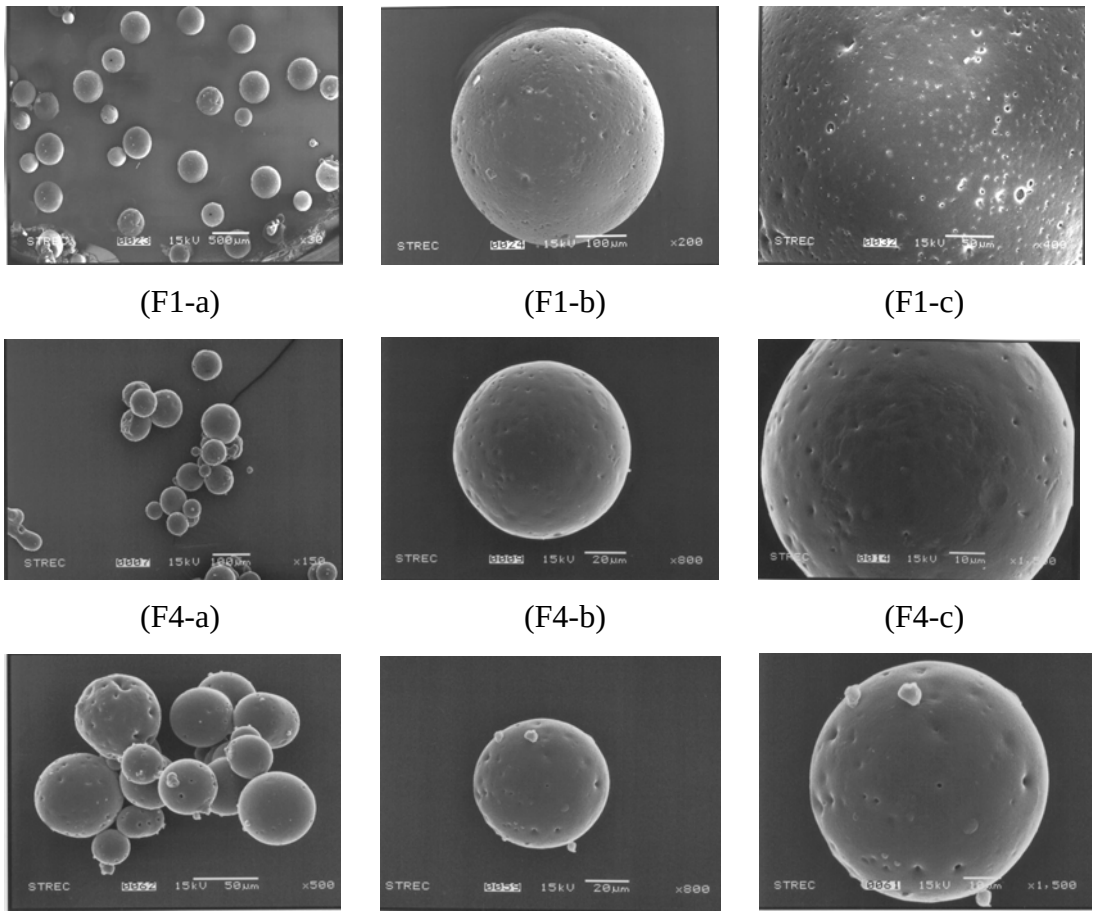
4.4.2.2 Effect of an increase in emulsifier concentration

The effect of emulsifier concentration on the properties of folic acid microcapsules was studied by comparing the size distribution and released of folic acid from microcapsules prepared with different concentrations of sorbitan monooleate (Span 80) when all other factors were kept the same (1 mg/ml of folic acid, 2.5% (w/v) of ethyl cellulose solution, and stirring rate of 2000 rpm). The mean diameter of the microcapsules prepared using various concentrations of emulsifier, namely 1%, 2% and 4% (v/v) were 300, 193, and 141 µm, respectively. Evidently, a significant decreased in mean particle size was achieved by the increasing the concentration of Span 80 in the continuous phase. It was clear that Span 80 played an important role in emulsion stability. Span 80 was a nonionic surface active emulsifying agent that also frequently useed in the oil in oil solvent evaporation technique. Among the properties of surface active agent, Span 80 had the capability to stabilize the interface between the two phases to form emulsion droplet by reduction the surface tension. An increase in the level of Span 80 would allow it to stabilize a greater interfacial surface area, thus leading to smaller particle size. In addition, SPAN values as an indicator of particle size distribution decreased from 2.12 to 1.65 with an increase in the concentration of emulsifier. The result showed that the size distribution became narrower with the increased in the concentration of emulsifier.

Table 4.3 Influence of concentration of emulsifier on microcapsules particle size and % encapsulation efficiency (n = 3)

Formulation	Conc. of emulsifier (%)	D_{50} (µm)	SPAN	E.E. (%)
F1	1	300±9	2.12±0.06	80.29±2.94
F4	2	193±3	1.78±0.04	65.77±1.39
F5	4	141±2	1.65±0.04	57.19±1.43

The effect of various emulsifier concentrations on the drug encapsulation efficiency of microcapsules were shown in Table 4.3. The encapsulation efficiency of microcapsules with 1%, 2%, and 4% (v/v) emulsifier was 80%, 65%, and 57%, respectively. There was a significant decreased in encapsulation efficiency of folic acid with an increase in the concentration of emulsifier for emulsification. This might be due to the fact that the drug diffused out of the microcapsules before they harden. It was found that the miscibility of solvent with liquid paraffin oil was increased with increasing emulsifier concentration. This might increase the diffusion of folic acid in to the continuous phase. The assumption of drug diffusion to the continuous phase was supported by SEM analysis, which showed the presence of pores and drug particles on the surface of microcapsules as shown in Figure 4.16(F5-c).



(F1-a) (F1-b) (F1-c)

(F4-a) (F4-b) (F4-c)

(F5-a) (F5-b) (F5-c)

Figure 4.16 Scanning electron micrographs of each formulation (F1, F4 and F5): (a) overview of folic acid-loaded microcapsules, (b) and (c) the surface of microcapsules.

Figure 4.16 showed SEM photographs of folic acid-loaded microcapsules prepared with different concentrations of emulsifier. The microcapsules had a spherical appearance, showing small pores on the surface (Figures 4.16(F1-c, F4-c, and F5-c)). It was observed that the image photographs (Figures 4.16(F4-a and F5-a)) were shown some aggregated microcapsules at 2% and 4% (v/v) emulsifier content. This was due to adhesion of the particles by increasing viscosity according to the increased of emulsifier concentration in solution. At 4% (v/v) emulsifier content, microcapsules exhibited porous surfaces (Figure 4.16(F5-c)). This might be due to the fact that the increase in emulsifier concentration proportionally increased miscibility of solvent (dispersed phase) with light liquid paraffin (continuous phase), which might increase the rate of solvent evaporation. The high rate of evaporation adopted for microcapsules hardening provoked the formation of pore and cavities within the polymeric matrix.

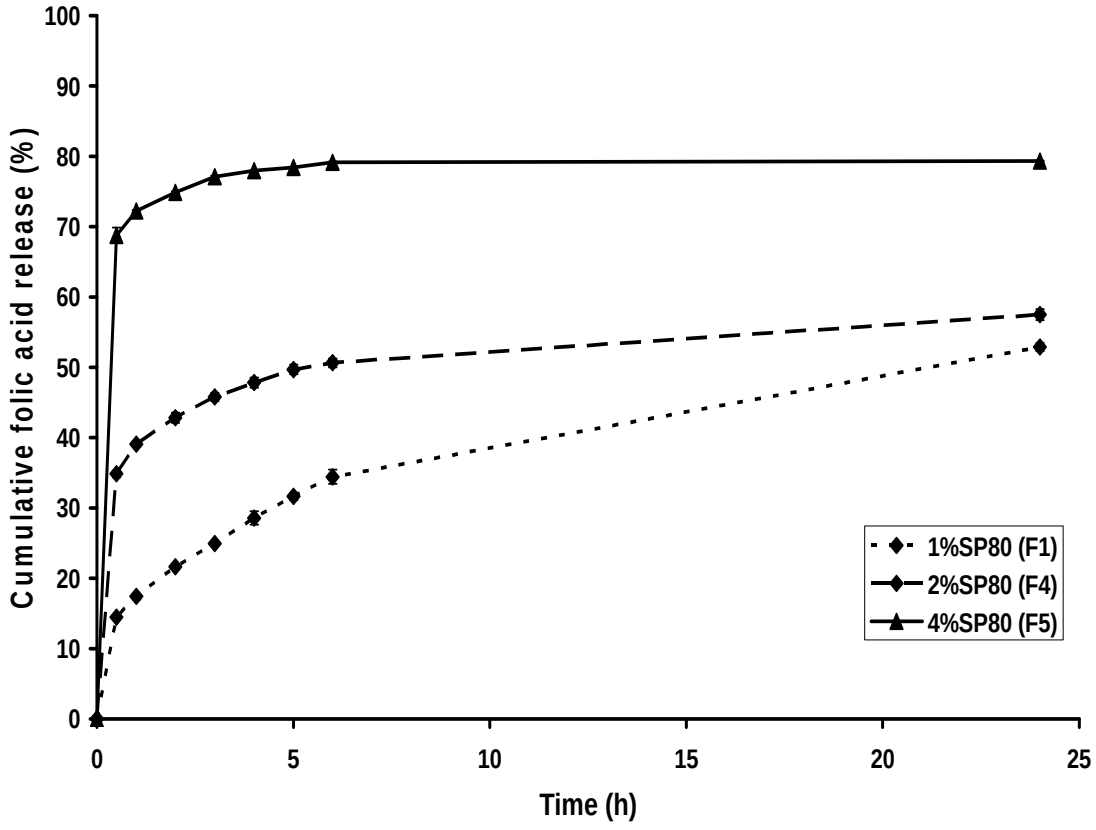


Figure 4.17 The release profiles of folic acid from microcapsules prepared with increasing concentrations of sorbitan monooleate in SIF (pH 7.4 phosphate buffer).

Figure 4.17 showed the drug release profiles of the microcapsules prepared with different emulsifier concentrations. At 30 minutes, the amount of drug released from the microcapsules prepared with 1%, 2%, and 4% (v/v) emulsifier, was approximately 14%, 34% and 68%, respectively. And about 52%, 57%, and 79% of the drug were released from the microcapsules prepared by 1%, 2%, and 4% (v/v) emulsifier in 24 h, respectively. Namely, the drug released rate increased as the emulsifier concentration increased.

The release profiles of the formulation F4 and F5 (as shown in Figure 4.18) showed the initial burst release in early stage. The burst release (within 30 minute) was considered to be due to the presence of more free drugs on the surface and larger diffusion area of the microcapsules [81]. It was attributed to the increasing the emulsifier concentration and thus decreasing the size of the microcapsules, resulting in increasing the interface area between the microcapsules and the release medium. Therefore smaller microcapsules with a greater surface area gave the faster drug release rate compared to the larger ones. On the contrary, the slow drug release in the formulation F1, the drug release was controlled by only diffusion through the polymer matrix.

Since the formation of the microcapsules was dramatically influenced by the stability of the oil in oil emulsion, it was important to select the optimal emulsifier concentration to prepare well-defined microcapsules with high drug entrapment and desirable drug release. These results showed that the formulation F1 obtain desired drug release and entrapment efficiency. Therefore, this formulation was considered for further study.

4.4.2.3 Effect of concentration of pore inducer

From the previous study, all of the formulation of microcapsules failed to complete release of the drug during 24 hours. This critical finding suggested that the microcapsules were very dense and the drug could not diffuse from the polymer matrix. Typically the drug release mechanism depended on diffusion though the porous matrix. Thus the additive like a pore inducer was added to the microcapsules for developed drug release profile.

Water-soluble powder or liquids was added to the formulation of microcapsules by the emulsion solvent evaporation process to obtain desirable release of active ingredients. These powders or solvents were called pore inducers. The dissolution of pore inducers into release media induced pores in the surface and channels in the matrix of the microcapsules, which eased the release of active ingredients [24]. Different amount of sucrose as a pore inducer was added to the formulation of folic acid microcapsules (1 mg/ml of folic acid, 2.5% (w/v) ethyl cellulose solution, 1% (v/v) emulsifier, and stirring rate of 2000 rpm) to study the effect on the particulate properties and drug release profiles.

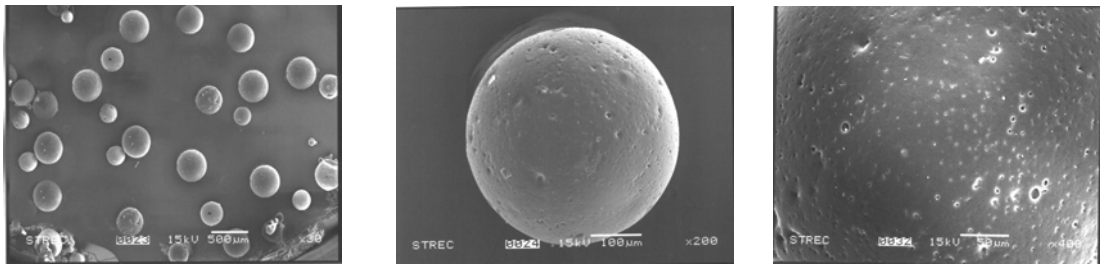
The various formulations that prepared with the different amounts of pore inducer gave the average size in the range of 489-740 µm. The addition of sucrose as pore inducer influenced the particle size of microcapsules. The result from the Table 4.4 showed that the mean size of microcapsules increased from 489 µm to 740 µm with the increasing amount of pore inducer from 2.5% (w/v) to 7.5% (w/v). It might be inferred that pore inducer could be increased matrix of the droplet of emulsion. Therefore the size of microcapsules depended on the size of the emulsion droplets formed during homogenization. The size distribution (polydispersity) was recorded as SPAN factor in the prepared formulations. The SPAN values decreased from 2.12 to 1.33 when the amount of pore inducer increased. This meant that the size distribution of microcapsules got narrower with increased amount of pore inducer, due to the uniformity of microcapsules.

Table 4.4 Effect of the amount of pore inducer on the properties of microcapsules (*n* = 3)

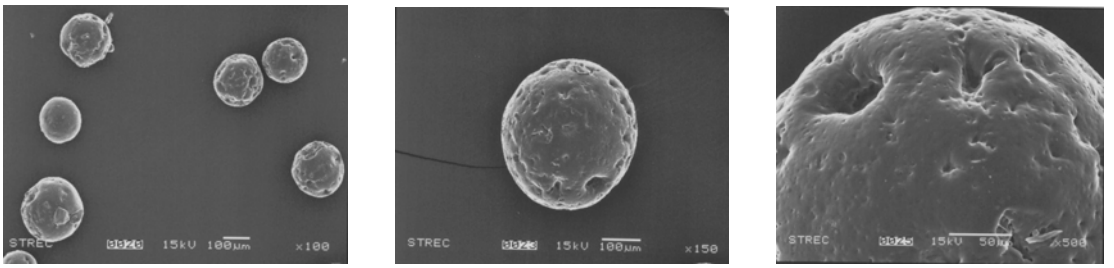
Formulation	Amount of Sucrose (%)	D_{50} (μm)	SPAN	E.E. (%)
F1	0.0	300±9	2.12±0.06	80.29±2.94
F6	2.5	489±9	1.73±0.03	56.64±3.43
F7	5.0	549±7	1.66±0.01	64.78±3.70
F8	7.5	740±9	1.33±0.05	52.12±3.59

The effect of amount of pore inducer on the drug encapsulation efficiency of microcapsules showed in Table 4.4. The encapsulation efficiency of microcapsules with pore inducer (Formulation F6-F8) ranged from 52.12% to 64.78% compared to 80.29% of encapsulation efficiency was given by microcapsules without pore inducer (Formulation F1). It was observed that encapsulation efficiency decreased with increasing amount of pore inducer. This might be contributed to the fact that more encapsulating materials were in the assembly and more materials stuck to the inside of microcapsules. Another possible explanation was that there was a migration through the pore of drug into the outer oil phase during droplet hardening which in turn resulted in drug loss.

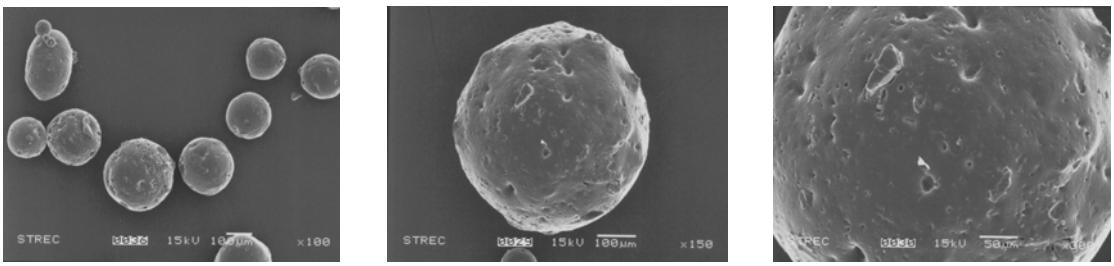
The scanning electron microphotograph (SEM) of folic acid-loaded microcapsules prepared with different amounts of pore inducer showed in Figure 4.18. The folic acid-loaded microcapsules without pore inducer (Formulation F1) showed a smooth surface while the microcapsules with pore inducer (Formulation F6, F7, and F8) exhibited a highly porous and coarser surface. It was assumed that the formation of pore was the result of sucrose leaching from the matrix during microcapsule formation.



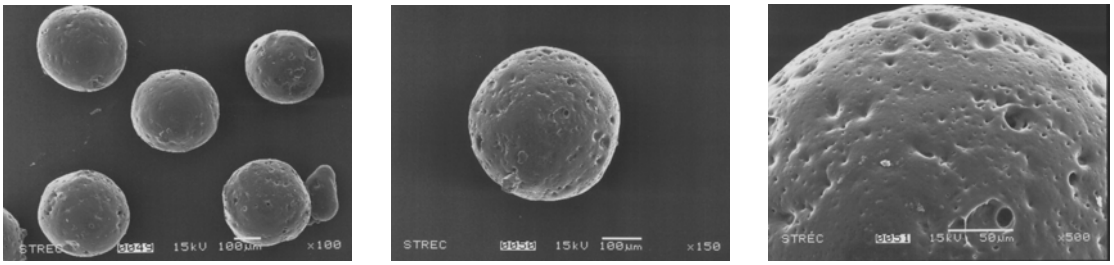
(F1-a) (F1-b) (F1-c)



(F6-a) (F6-b) (F6-c)



(F7-a) (F7-b) (F7-c)



(F8-a) (F8-b) (F8-c)

Figure 4.18 Scanning electron micrographs of each formulation (F1 and F6-F8): (a) overview of folic acid-loaded microcapsules, (b) and (c) the surface of microcapsules.

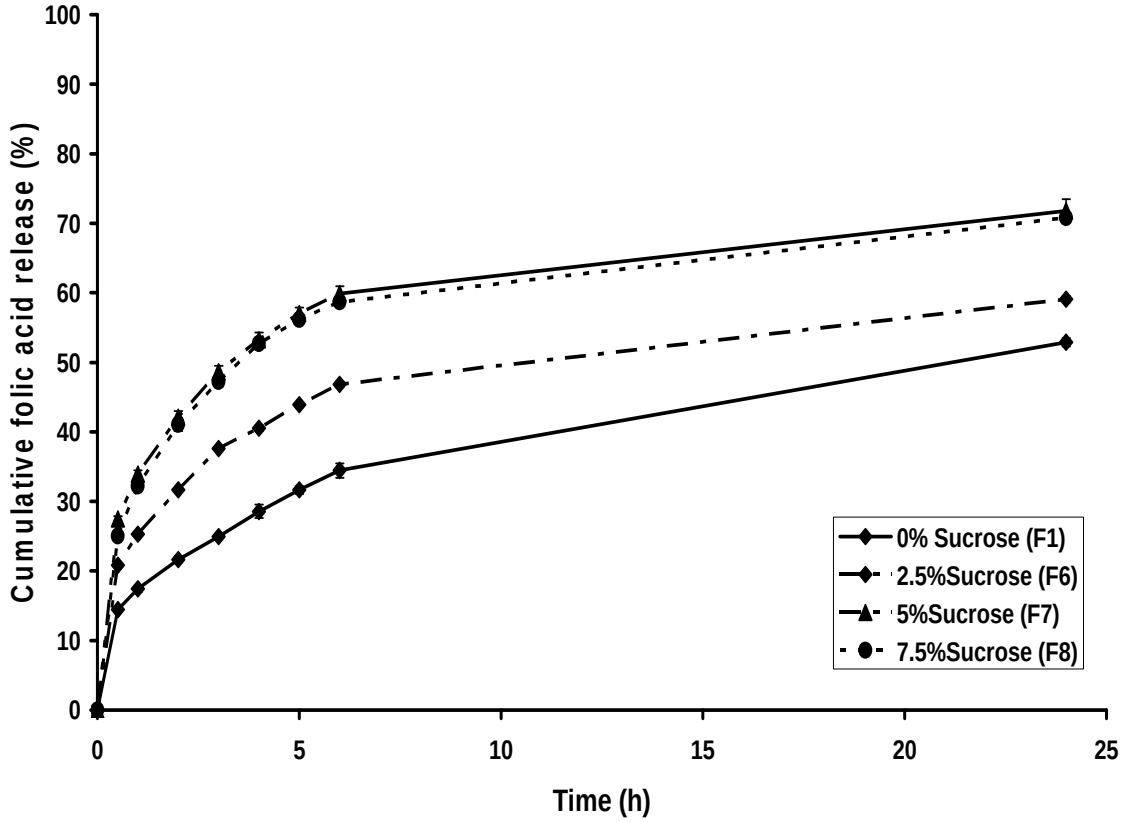


Figure 4.19 The release profiles of folic acid from microcapsules prepared with increasing concentrations of sucrose in SIF (pH 7.4 phosphate buffer).

It was well known that additives were able to modify the release rate of drug from microcapsules. The effect of these substances could be different. The addition of a pore inducer like sucrose could promote higher release of drug from ethyl cellulose microcapsules.

Figure 4.19 showed the drug release profiles of the microcapsules prepared with different amounts of sucrose. At the first hour, cumulative drug release of microcapsules prepared without sucrose (Formulation F1) was about 17% while about 25%, 33% and 32% of the drug were released from microcapsules prepared with 2.5%, 5.0% and 7.5% of sucrose, respectively. At 6 hours, the microcapsules prepared with 2.5%, 5.0% and 7.5% of sucrose released about 46%, 59% and 58%, respectively while only 33% of the drug was released from the formulation F1. It was observed that cumulative drug release increased when sucrose was added to the formulation. After 24 hours, about 59%, 71% and 70% of the total drug were released from

microcapsules prepared with 2.5%, 5.0% and 7.5% (w/v) of sucrose, respectively, while the cumulative percentage release of the formulation F1 was about 52%. Compared with the formulation F1, the microcapsules with sucrose (Formulation F6, F7, and F8) had a fast drug release. The higher release of drug was related to the porosity of the particle. A probable mechanism of the release was considered to be due to the diffusion through channels which were formed by sucrose. As the porosity of the formulation increased, the amounts of drug released also increased. While the microcapsules without sucrose (Formulation F1) resulted in denser morphology and slower release rate, probably because of the slower drug diffusion rate.

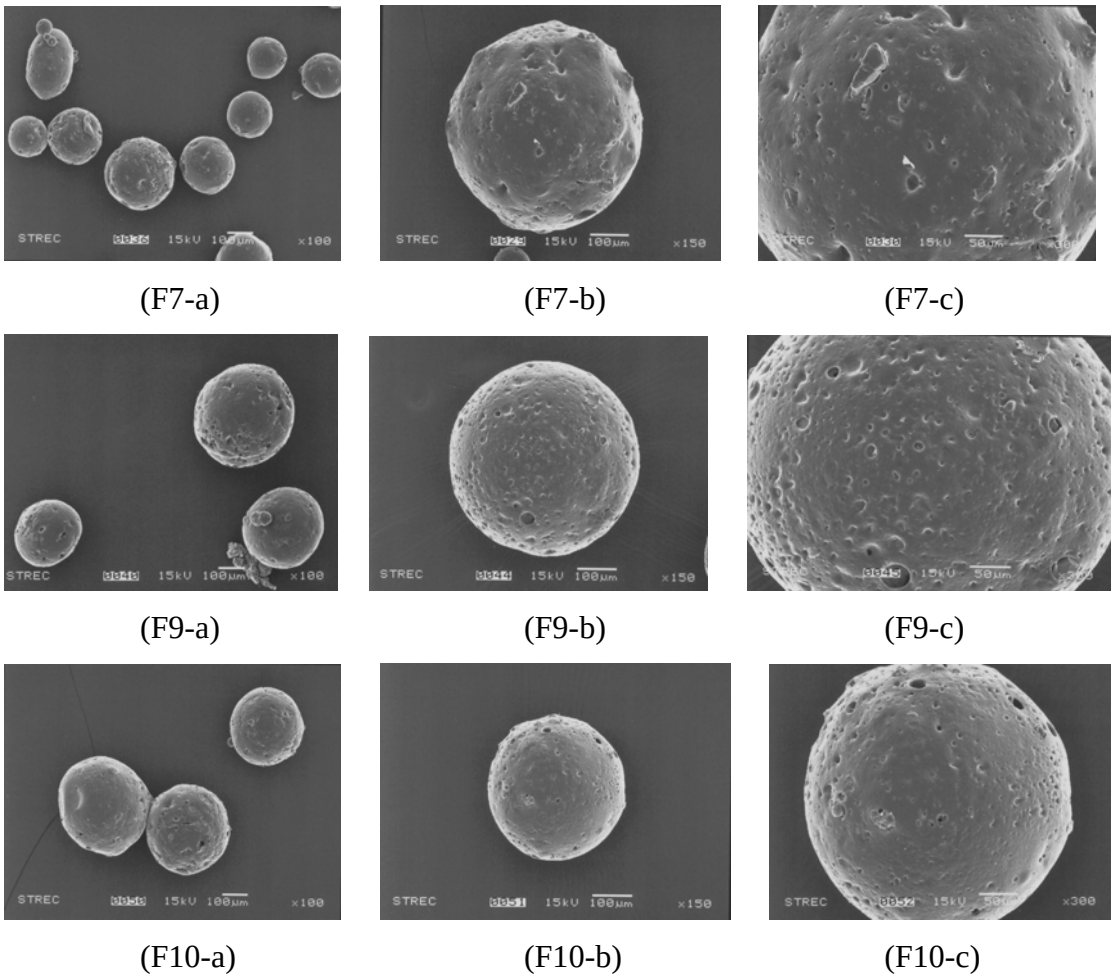
4.4.2.4 Effect of drug content

Folic acid-loaded microcapsules were prepared by using different amounts of folic acid namely, 20, 40, and 60 mg while keeping all other factors the same (2.5% (w/v) ethyl cellulose solution, 1% (v/v) emulsifier, 5% (w/v) of sucrose and stirring rate of 2000 rpm). The particulate properties of microcapsules and encapsulation efficiency were studied and summarized in Table 4.5. The amount of drug influenced both the particulate properties and encapsulation efficiency. The result was shown that there was an increased in mean particle size from 549 μm to 789 μm when the amount of drug was increased. This could be explained by the fact that a greater amount of drug resulted in a more viscous dispersed phase, leading to larger droplet formation. The size distribution (polydispersity) of microcapsules was recorded as SPAN factor. The SPAN value was varied in range of 1.30 to 1.82.

Table 4.5 Effect of the amount of folic acid on the particulate properties of microcapsules and encapsulation efficiency (n = 3)

Formulation	Amount of folic acid (mg)	D_{50} (μm)	SPAN	E.E. (%)
F7	20	549±7	1.66±0.01	64.78±3.70
F9	40	649±18	1.82±0.03	65.16±2.92
F10	60	790±13	1.30±0.02	88.36±1.58

The effect of drug content on the drug encapsulation efficiency of microcapsules was shown in Table 4.5. The encapsulation efficiency of microcapsules with 20, 40, and 60 mg of folic acid was 64%, 65%, and 88%, respectively. The variation in drug content had significant effect on the encapsulation efficiency of microcapsules. An increase in amount of drug resulted in increasing drug entrapment in microcapsules. The highest encapsulation efficiency of the formulation F10 could be explained through the fact that the amount of drug in per unit polymer was greater than that in other formulations.



(F7-a) (F7-b) (F7-c)

(F9-a) (F9-b) (F9-c)

(F10-a) (F10-b) (F10-c)

Figure 4.20 Scanning electron micrographs of each formulation (F7, F9 and F10): (a) overview of folic acid-loaded microcapsules, (b) and (c) the surface of microcapsules.

Figure 4.20 showed SEM photographs of folic acid-loaded microcapsules prepared with a different amount of drug. The microcapsules had spherical shape in all case. The increase in amount of drug resulted in an increase in microcapsule size and density combined with a decrease in porosity. This increase in size and density might be attributed to the higher viscosity of the dispersed phase, due to higher concentration of gradients, rather than to the formation of larger droplets during emulsification. As the result, the surfaces of the higher drug loading were relatively smoother and more presence of free drugs.

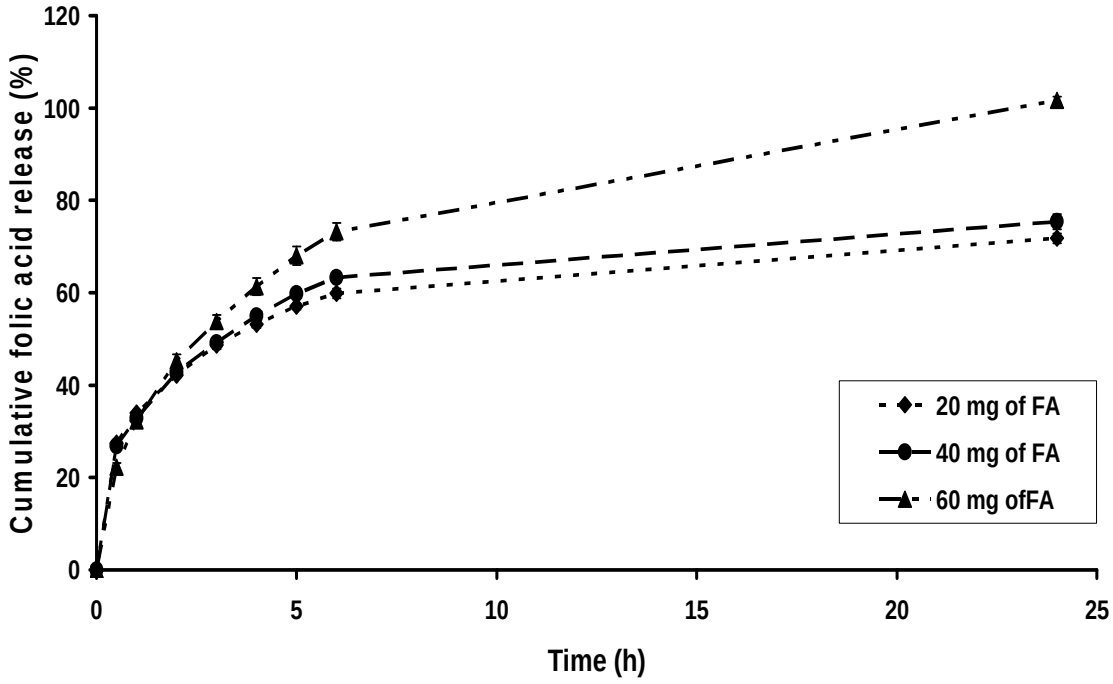


Figure 4.21 The release profiles of folic acid from microcapsules prepared with increasing amount of folic acid in SIF (pH 7.4 phosphate buffer).

Figure 4.21 showed the drug release profiles of the microcapsules prepared with different amount of folic acid. Folic acid entrapment efficiency was also a significant factor influencing drug release. The initial cumulative release of microcapsules prepared with 20 mg, 40 mg and 60 mg of folic acid was about 33%, 32% and 32% of the entrapped drug, respectively. At 6 hours, the microcapsules prepared with 20 mg, 40 mg and 60 mg of folic acid released about 58%, 63% and 69% of the drug, respectively. After 24 hours, about 71% and 75% of the total drug were released from microcapsules prepared with 20 mg and 40 mg of folic acid, while the cumulative percentage release of microcapsules prepared with 60 mg of folic acid reached to 100%. It was obvious that the cumulative release of folic acid increased with increasing amount of folic acid in the formulation. Higher level of folic acid corresponding to lower level of the polymer in the formulation resulted in an increase in the cumulative percentage release. As more drugs were released from the microcapsules, more channels were produced, contributing to faster drug released. In

addition, higher drug levels in the microcapsules formulation produced a higher drug concentration gradient between the microcapsules and dissolution medium, thus the cumulative release of drug was increased [83].

4.4.3 Comparative evaluation of *in vitro* release performance of commercial folic acid tablet and fabricated sustained release folic acid microcapsules

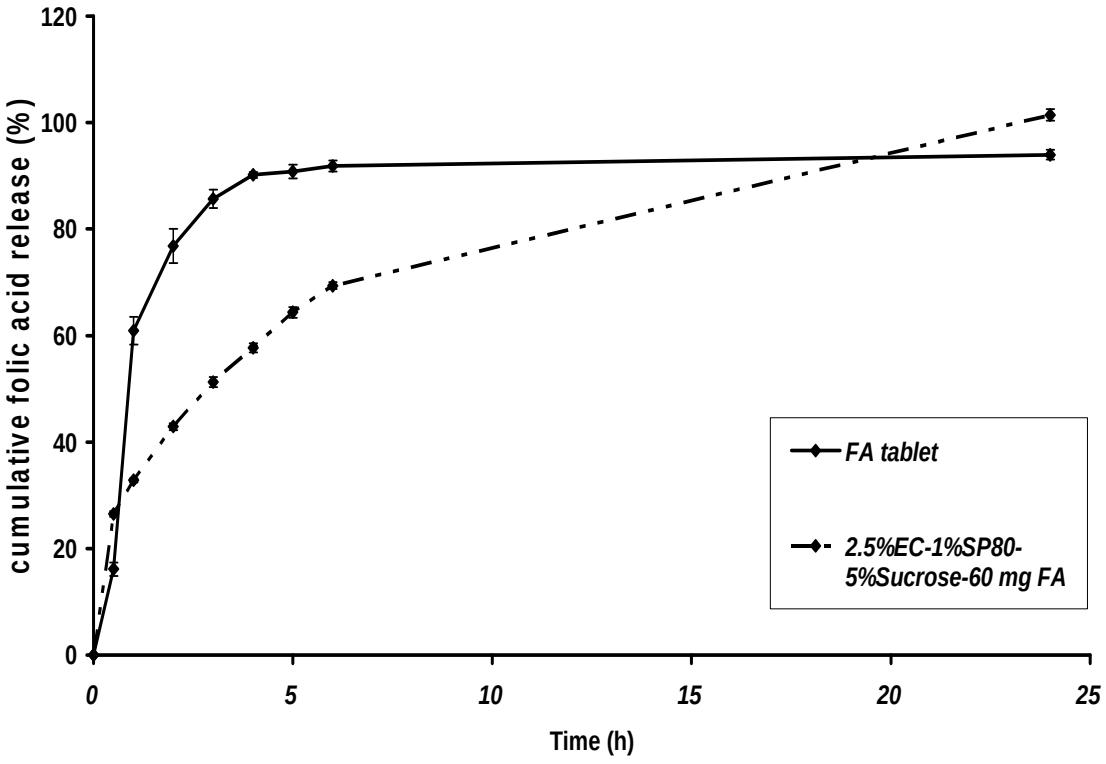


Figure 4.22 The release profiles of the commercial folic acid tablet compared with the folic acid-loaded microcapsules (Formulation F10) in SIF.

According to the previous results, the formulation F10 showed the most appropriate sustained release profile of the drug. In this section, the dissolution test of folic acid between the formulation F10 and the commercial folic acid tablet was studied (Figure 4.22).

The release profile of the folic acid tablet showed an extremely high burst effect about 60% of drug entrapment after 1 hour and reached to 90% of drug entrapment during 5 hours. While the formulation F10 showed an ability to delay the drug release. The cumulative drug release was 32% of entrapped drug after 1 hour and the drug was continuously released to 73% of entrapped drug during 6 hours. The complete release of drug from the formulation F10 was observed at 24 hours.

CHAPTER V

CONCLUSION AND SUGGESTION

5.1 Conclusion

In the present study, we prepared folic acid-loaded ethyl cellulose microcapsules using the oil in oil emulsion solvent evaporation technique. By using a mixture of methanol and acetone in the ratio of 1/9 as the dispersed phase, the more stable and well-defined microcapsules could be prepared. The surface structure of the microcapsules was spherical and smooth.

From the result of the *in vitro* release studies, the commercial folic acid tablet and the folic acid-loaded microcapsules (Formulation F1) failed to release in simulating gastric fluid (SGF, pH 1.2 HCl). This finding suggested that the folic acid dissolution process was solubility controlled. Folic acid had a pH dependent solubility profile with inherent low solubility in acidic pH. However the result from folic acid dissolution in simulating intestinal fluid (phosphate pH 7.4) was confirmed that folic acid-loaded ethyl cellulose microcapsules were achieved the sustained release of folic acid.

Process parameters, such as the concentration of polymer, the amount of drug, and concentration of emulsifier, were optimized to produce microcapsules with higher drug entrapment efficiency and the appropriate release profile of folic acid. Formulation F1 showed the high drug entrapment efficiency (80%) but the total release of folic acid from microcapsules was only 52% of the drug entrapment. To solve this problem, some additives were added to the formulation.

The addition of small amount of a water-soluble pore inducer, sucrose, was used to modify the release of active ingredient from the ethyl cellulose microcapsule matrix without influencing the morphology and particulate properties of the sustained release folic acid microcapsules. The formulation F10 showed the highest encapsulation efficiency (88%) and suitable sustained release profiles. The present

result showed that the formulation F10 had a long release period of about 24 hours. The release of folic acid from the sustained release microcapsules might be a process where drug diffusion occurred through the ethyl cellulose structure network that formed the shell around the folic acid particles.

5.2 Suggestion for the future work

Commonly, the microencapsulation of a hydrophilic drug like folic acid in a polymeric matrix via an ordinary oil in oil emulsification/solvent evaporation allowed entrapping limited drug amounts. To solve this problem, there are several alternatives that we intend to suggest as follows.

- The paraffin oil used in the experiment will be repeatedly recycled for use in the succeeding experiments. The old folic acid concentration in the recycled paraffin oil may increase in the encapsulation efficiency of the microcapsules

It is worthwhile to note that the recycling of the spent continuous phase for microencapsulation markedly reduces the oil waste and, thus, significantly minimizes the pollution (if any) to the environment.

- Develop the multiple emulsion system like water in oil in oil in oil (W/O/O/O) for the microcapsule preparation. Utilization of this type of multiple emulsion system may obtain the high entrapment efficiency of water soluble compounds. Yet the various parameters on the preparation can affect on the properties of the resulted microcapsules.

- It is possible that other polymers may deliver higher encapsulation efficiencies or even confer greater stability and so more research into the polymers to increase the application base and variety is recommended.

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APPENDICES

APPENDIX A

Calibration curve of folic acid

Calibration curve of folic acid

The concentration versus peak area of folic acid by HPLC assay as the same conditions described in Chapter III is presented in Tables A1 and A2. The plot of calibration curve of folic acid is illustrated in Figures A1 and A2.

Table A1 Absorbance of various concentrations of folic acid determined by HPLC

Concentration (ppm)	Peak area
0.1	72988
1	577999
2	113447
4	2223718
6	3246990

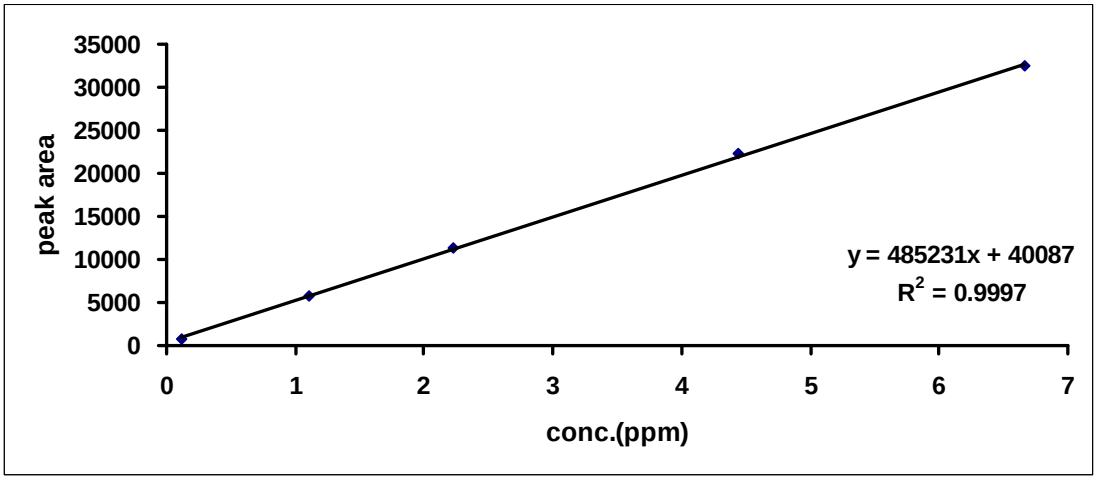

Figure A1 Calibration curve of folic acid for determined the folic acid microcapsules.

Table A2 Absorbance of various concentrations of folic acid determined by HPLC

Concentration (ppm)	Peak area
0.1	72818
2	707145
5	1210632
10	2687962
50	21911866

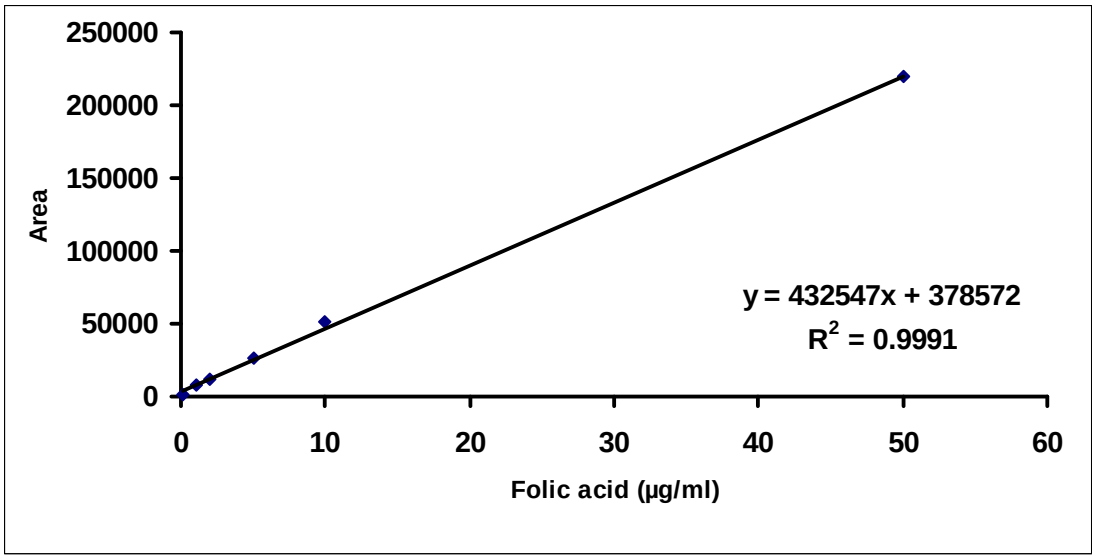

Figure A2 Calibration curve of folic acid for determined commercial folic acid tablet.

APPENDIX B

Preparation stock solution

Reagents

1 N Hydrochloric Acid

Exactly measured 8.3 ml of 12.1 N HCl was added to 50 ml distilled water in 100 ml volumetric flask and the volume was adjusted with distilled water.

0.2 N Sodium Hydroxide

0.8 grams of NaOH were placed in a 100 millilite volumetric flask, distilled water was added to volume and the solution was mixed.

1 N Sodium Hydroxide

Four grams of NaOH were placed in a 100 ml volumetric flask and diluted with distilled water to volume and the solution was mixed.

3 N Phosphoric acid

Exactly measured 16.82 ml of 85% HPLC grade o-phosphoric acid was transferred to a 250 ml volumetric flask, distilled water was added to volume and the solution was mixed.

6 N Ammonium Hydroxide

Exactly measured 111.1 ml of 25% ammonium hydroxide solution was transferred to a 250 ml volumetric flask, distilled water was added to volume and the solution was mixed.

10% Ammonium Hydroxide

Forty ml of 25% ammonium hydroxide solution was transferred to a 100 ml volumetric flask, distilled water was added to volume and the solution was mixed.

Simulated Gastric Fluid

Six grams of sodium chloride were placed in a 3-liter round bottom flask, and 2500 ml of distilled water and 21 ml of 1 N HCl were added. The volume was adjusted and the solution pH was adjusted to 1.5 with either 1 N HCl or 0.2 N NaOH. No enzymes were added to the fluid.

Simulated Intestinal Fluid

20.4 g of potassium monobasic phosphate were placed in a 3-liter round bottom volumetric flask, and 570 ml of 0.2 NaOH were added. The volume was adjusted with distilled water. The solution pH was adjusted to 7.4 with either 1 N HCl or 0.2 N NaOH. No enzymes were added to the fluid.

APPENDIX C

Percentage of drug release

Table C1 Percentage of folic acid release in SIF (pH 7.4 phosphate buffer)

Time (h)	Percentage of folic acid release (%) ± S.D.					
	Commercial	Formulation F1	Formulation F2	Formulation F3	Formulation F4	Formulation F5
0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
0.5	16.15 ± 1.26	14.47 ± 0.32	9.80 ± 0.20	7.26 ± 0.42	34.85 ± 0.47	68.78 ± 1.06
1	60.91 ± 2.63	17.42 ± 0.44	14.88 ± 0.28	10.54 ± 0.31	39.11 ± 0.39	72.25 ± 0.13
2	76.83 ± 3.19	21.66 ± 0.40	20.63 ± 0.28	15.74 ± 0.37	42.85 ± 0.72	74.89 ± 0.15
3	85.68 ± 1.74	24.96 ± 0.38	24.37 ± 0.47	20.18 ± 0.40	45.78 ± 0.59	77.12 ± 0.14
4	90.16 ± 0.55	28.57 ± 0.96	27.21 ± 0.60	23.19 ± 0.21	47.83 ± 0.73	77.93 ± 0.11
5	90.79 ± 1.28	31.64 ± 0.55	30.75 ± 0.38	26.96 ± 0.24	49.69 ± 0.70	78.42 ± 0.02
6	91.81 ± 1.04	34.43 ± 1.04	33.23 ± 0.67	29.21 ± 0.13	50.64 ± 0.62	79.15 ± 0.14
24	93.97 ± 0.88	52.92 ± 0.60	47.24 ± 1.13	40.87 ± 0.54	57.51 ± 0.77	79.31 ± 0.08

Table C2 Percentage of folic acid release in SIF (pH 7.4 phosphate buffer)

Time (h)	Percentage of folic acid release (%) ± S.D.				
	Formulation F6	Formulation F7	Formulation F8	Formulation F9	Formulation F10
0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
0.5	20.84 ± 0.24	27.49 ± 0.36	25.01 ± 0.54	26.85 ± 0.29	26.53 ± 0.52
1	25.29 ± 0.27	33.95 ± 0.51	32.21 ± 0.34	32.86 ± 0.38	32.81 ± 0.39
2	31.68 ± 0.29	42.13 ± 0.83	41.07 ± 0.95	42.81 ± 0.60	42.89 ± 0.65
3	37.61 ± 0.32	48.66 ± 0.84	47.27 ± 0.84	49.22 ± 0.91	51.27 ± 0.98
4	40.54 ± 0.46	53.21 ± 1.06	52.72 ± 0.57	55.03 ± 0.81	57.68 ± 0.85
5	43.93 ± 0.39	57.05 ± 0.81	56.13 ± 0.56	59.79 ± 0.66	64.36 ± 1.01
6	46.83 ± 0.39	59.88 ± 1.07	58.66 ± 0.62	63.30 ± 1.04	69.37 ± 0.67
24	59.11 ± 0.47	71.78 ± 1.69	70.77 ± 0.19	75.38 ± 1.05	101.43 ± 1.10

Table C3 Percentage of folic acid release in SGF (pH 1.2 HCl)

Time (h)	Percentage of folic acid release ± S.D.	
	Commercial	Formulation F1
0	0.00 ± 0.00	0.00 ± 0.00
0.5	17.47 ± 0.51	3.67 ± 0.02
1	21.82 ± 0.49	4.53 ± 0.05
2	27.71 ± 0.63	5.50 ± 0.03
3	32.01 ± 0.87	6.47 ± 0.15
4	33.71 ± 1.39	7.50 ± 0.01
5	34.59 ± 1.14	8.04 ± 0.09
6	39.17 ± 0.95	8.33 ± 0.13
24	39.47 ± 1.07	9.95 ± 0.09

APPENDIX D

Representative Folic acid HPLC Chromatogram

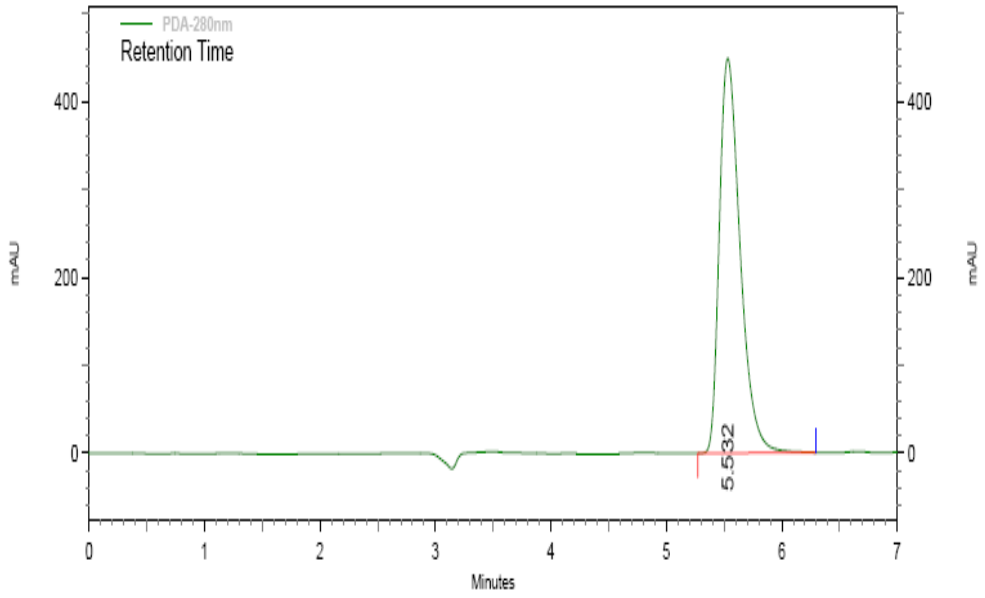

Figure D1 HPLC Chromatogram of folic acid (10 µg/ml) using HPLC assay for folic acid calibration curve.

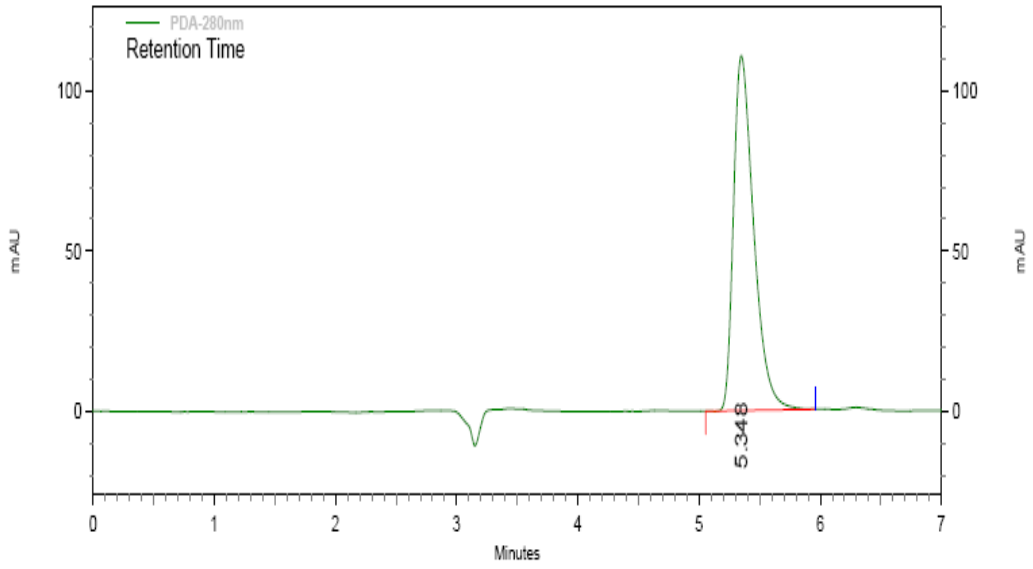

Figure D2 HPLC Chromatogram of folic acid using HPLC assay for encapsulation efficiency of folic acid-loaded microcapsules.

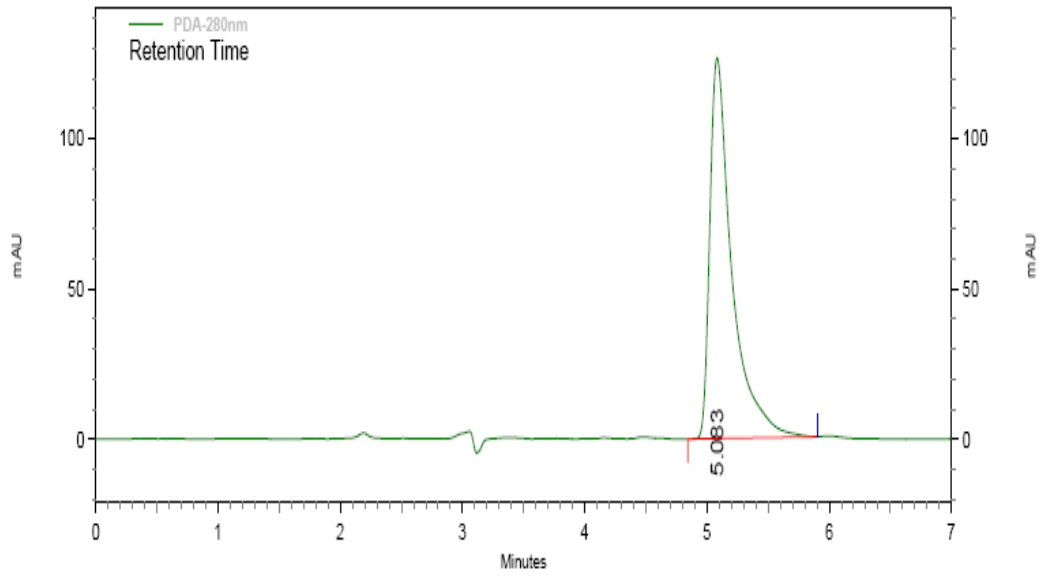

Figure D3 HPLC Chromatogram of folic acid using HPLC assay for folic acid dissolution from folic acid-loaded microcapsulse.

APPENDIX E

Particle size distribution

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อาคารสถาบัน 2 จุฬาลงกรณ์ ซอย 62 ถ.พญาไท ปทุมวัน กรุงเทพฯ 10330 โทร 2188029-32, 2188101 โทรสาร 2540211
Scientific and Technological Research Equipment Centre Chulalongkorn University
Building 2-3 Chula Soi 62 Phaya-Thai Rd. Phatumwan Bangkok 10330 Tel. 2188029-32, 2188101 Fax. 2540211

Result: Analysis Report

Sample Details

Sample ID: 2.5EC-1%SP80
Sample File: CHULA6
Sample Path: C:\SIZERS\DATA\
Sample Notes: Wet Analysis System
Dispersing Medium : Water
Ultrasonic : No
Additive : 0.1% Nonidet P40

Run Number: 3
Record Number: 1352

Measured: 21 Feb 2008 13:06PM
Analysed: 21 Feb 2008 13:06PM
Result Source: Analysed

System Details

Range Lens: 1000 mm
Presentation: 3_ECL
Analysis Model: Polydisperse
Modifications: None

Beam Length: 2.40 mm
[Particle R.I. = (1.4790, 0.1000); Dispersant R.I. = 1.3300]

Sampler: MS1

Obscuration: 5.9 %
Residual: 0.549 %

Result Statistics

Distribution Type: Volume
Mean Diameters:
D [4, 3] = 291.19 um

Concentration = 0.1363 %Vol
D (v, 0.1) = 76.97 um
D [3, 2] = 161.28 um

Density = 1.000 g / cub. cm
D (v, 0.5) = 239.60 um
Span = 2.099E+00

Specific S.A. = 0.0372 sq. m / g
D (v, 0.9) = 579.78 um
Uniformity = 6.493E-01

Size Low (um)	In %	Size High (um)	Under%	Size Low (um)	In %	Size High (um)	Under%
4.19	0.00	4.88	0.00	120.67	5.19	140.58	26.46
4.88	0.00	5.69	0.00	140.58	5.94	163.77	32.40
5.69	0.00	6.63	0.00	163.77	6.65	190.80	39.05
6.63	0.00	7.72	0.00	190.80	7.23	222.28	46.28
7.72	0.00	9.00	0.00	222.28	7.64	258.95	53.92
9.00	0.00	10.48	0.00	258.95	7.87	301.68	61.79
10.48	0.00	12.21	0.00	301.68	7.97	351.46	69.76
12.21	0.00	14.22	0.00	351.46	7.35	409.45	77.11
14.22	0.04	16.57	0.04	409.45	6.40	477.01	83.51
16.57	0.07	19.31	0.11	477.01	5.25	555.71	88.75
19.31	0.13	22.49	0.25	555.71	4.10	647.41	92.85
22.49	0.23	26.20	0.48	647.41	3.04	754.23	95.89
26.20	0.38	30.53	0.86	754.23	2.14	878.67	98.03
30.53	0.59	35.56	1.45	878.67	1.37	1023.66	99.40
35.56	0.87	41.43	2.31	1023.66	0.60	1192.56	100.00
41.43	1.21	48.27	3.53	1192.56	0.00	1389.33	100.00
48.27	1.62	56.23	5.15	1389.33	0.00	1618.57	100.00
56.23	2.09	65.51	7.23	1618.57	0.00	1885.64	100.00
65.51	2.60	76.32	9.84	1885.64	0.00	2196.77	100.00
76.32	3.17	88.91	13.01	2196.77	0.00	2559.23	100.00
88.91	3.79	103.58	16.80	2559.23	0.00	2981.51	100.00
103.58	4.47	120.67	21.27	2981.51	0.00	3473.45	100.00

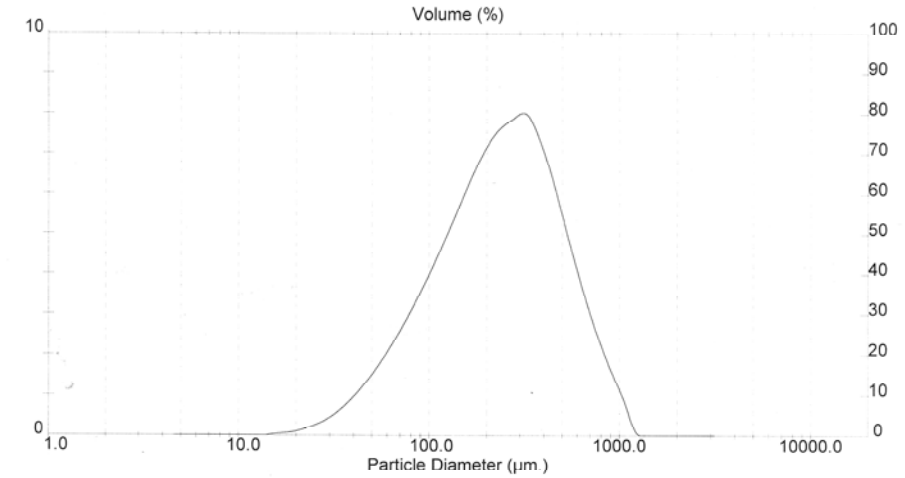

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Figure E1 Particle size distribution data of Formulation F1.

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Scientific and Technological Research Equipment Centre Chulalongkorn University
Building 2-3 Chula Soi 62 Phaya-Thai Rd. Phatumwan Bangkok 10330 Tel. 2188029-32, 2188101 Fax. 2540211

Result: Analysis Report

Sample Details

Sample ID: 2.5EC-1%SP80
Sample File: CHULA6
Sample Path: C:\SIZERS\DATA\
Sample Notes: Wet Analysis System
Dispersing Medium : Water
Ultrasonic : No
Additive : 0.1% Nonidet P40

Run Number: 4
Record Number: 1353

Measured: 21 Feb 2008 13:06PM
Analysed: 21 Feb 2008 13:06PM
Result Source: Analysed

System Details

Range Lens: 1000 mm
Presentation: 3_ECL
Analysis Model: Polydisperse
Modifications: None

Beam Length: 2.40 mm
[Particle R.I. = (1.4790, 0.1000); Dispersant R.I. = 1.3300]

Sampler: MS1

Obscuration: 5.9 %
Residual: 0.300 %

Result Statistics

Distribution Type: Volume
Mean Diameters:
D [4, 3] = 309.39 um

Concentration = 0.1313 %Vol
D (v, 0.1) = 77.60 um
D [3, 2] = 156.98 um

Density = 1.000 g / cub. cm
D (v, 0.5) = 249.67 um
Span = 2.188E+00

Specific S.A. = 0.0382 sq. m / g
D (v, 0.9) = 623.94 um
Uniformity = 6.830E-01

Size Low (um)	In %	Size High (um)	Under%	Size Low (um)	In %	Size High (um)	Under%
4.19	0.02	4.88	0.02	120.67	4.92	140.58	25.49
4.88	0.03	5.69	0.06	140.58	5.64	163.77	31.13
5.69	0.04	6.63	0.10	163.77	6.33	190.80	37.46
6.63	0.05	7.72	0.15	190.80	6.94	222.28	44.40
7.72	0.04	9.00	0.19	222.28	7.40	258.95	51.80
9.00	0.04	10.48	0.22	258.95	7.70	301.68	59.50
10.48	0.03	12.21	0.25	301.68	7.89	351.46	67.40
12.21	0.03	14.22	0.28	351.46	7.36	409.45	74.76
14.22	0.04	16.57	0.32	409.45	6.49	477.01	81.25
16.57	0.07	19.31	0.38	477.01	5.40	555.71	86.65
19.31	0.12	22.49	0.51	555.71	4.29	647.41	90.94
22.49	0.22	26.20	0.73	647.41	3.28	754.23	94.22
26.20	0.36	30.53	1.08	754.23	2.41	878.67	96.63
30.53	0.56	35.56	1.65	878.67	1.71	1023.66	98.34
35.56	0.83	41.43	2.48	1023.66	1.12	1192.56	99.46
41.43	1.17	48.27	3.64	1192.56	0.54	1389.33	100.00
48.27	1.56	56.23	5.20	1389.33	0.00	1618.57	100.00
56.23	2.00	65.51	7.20	1618.57	0.00	1885.64	100.00
65.51	2.49	76.32	9.69	1885.64	0.00	2196.77	100.00
76.32	3.03	88.91	12.72	2196.77	0.00	2559.23	100.00
88.91	3.61	103.58	16.33	2559.23	0.00	2981.51	100.00
103.58	4.24	120.67	20.57	2981.51	0.00	3473.45	100.00

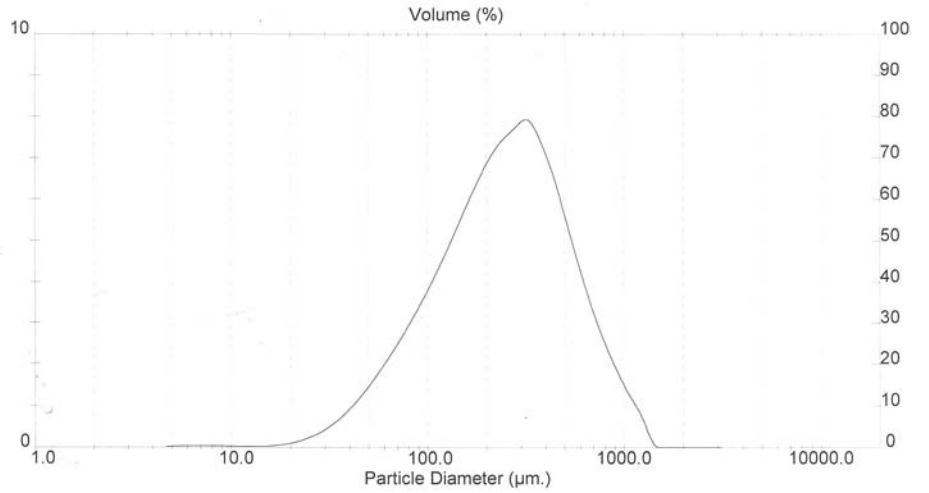

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Figure E2 Particle size distribution data of Formulation F1.

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อาคารสถาบัน 2 จุฬาลงกรณ์ ซอย 62 ถ.พญาไท ปทุมวัน กรุงเทพฯ 10330 โทร 2188029-32, 2188101 โทรสาร 2540211
Scientific and Technological Research Equipment Centre Chulalongkorn University
Building 2-3 Chula Soi 62 Phaya-Thai Rd. Phatumwan Bangkok 10330 Tel. 2188029-32, 2188101 Fax. 2540211

Result: Analysis Report

Sample Details

Sample ID: 2.5EC-1%SP80
Sample File: CHULA6
Sample Path: C:\SIZERS\DATA\
Sample Notes: Wet Analysis System
Dispersing Medium : Water
Ultrasonic : No
Additive : 0.1% Nonidet P40

Run Number: 8
Record Number: 1357

Measured: 21 Feb 2008 13:07PM
Analysed: 21 Feb 2008 13:07PM
Result Source: Analysed

System Details

Range Lens: 1000 mm
Presentation: 3_ECL
Analysis Model: Polydisperse
Modifications: None

Beam Length: 2.40 mm
[Particle R.I. = (1.4790, 0.1000); Dispersant R.I. = 1.3300]

Sampler: MS1

Obscuration: 6.0 %
Residual: 0.596 %

Result Statistics

Distribution Type: Volume
Mean Diameters:
D [4, 3] = 299.25 um

Concentration = 0.1414 %Vol
D (v, 0.1) = 78.03 um
D [3, 2] = 164.69 um

Density = 1.000 g / cub. cm
D (v, 0.5) = 249.51 um
Span = 2.068E+00

Specific S.A. = 0.0364 sq. m / g
D (v, 0.9) = 594.11 um
Uniformity = 6.400E-01

Size Low (um)	In %	Size High (um)	Under%	Size Low (um)	In %	Size High (um)	Under%
4.19	0.00	4.88	0.00	120.67	4.90	140.58	25.31
4.88	0.00	5.69	0.00	140.58	5.63	163.77	30.94
5.69	0.00	6.63	0.00	163.77	6.36	190.80	37.30
6.63	0.00	7.72	0.00	190.80	7.02	222.28	44.32
7.72	0.00	9.00	0.00	222.28	7.55	258.95	51.87
9.00	0.00	10.48	0.00	258.95	7.93	301.68	59.81
10.48	0.00	12.21	0.00	301.68	8.19	351.46	68.00
12.21	0.00	14.22	0.00	351.46	7.66	409.45	75.66
14.22	0.05	16.57	0.05	409.45	6.73	477.01	82.39
16.57	0.08	19.31	0.12	477.01	5.56	555.71	87.95
19.31	0.14	22.49	0.26	555.71	4.37	647.41	92.32
22.49	0.24	26.20	0.49	647.41	3.29	754.23	95.61
26.20	0.38	30.53	0.88	754.23	2.38	878.67	97.98
30.53	0.59	35.56	1.47	878.67	1.46	1023.66	99.45
35.56	0.86	41.43	2.33	1023.66	0.55	1192.56	100.00
41.43	1.19	48.27	3.52	1192.56	0.00	1389.33	100.00
48.27	1.58	56.23	5.09	1389.33	0.00	1618.57	100.00
56.23	2.01	65.51	7.10	1618.57	0.00	1885.64	100.00
65.51	2.49	76.32	9.60	1885.64	0.00	2196.77	100.00
76.32	3.01	88.91	12.61	2196.77	0.00	2559.23	100.00
88.91	3.59	103.58	16.20	2559.23	0.00	2981.51	100.00
103.58	4.21	120.67	20.41	2981.51	0.00	3473.45	100.00

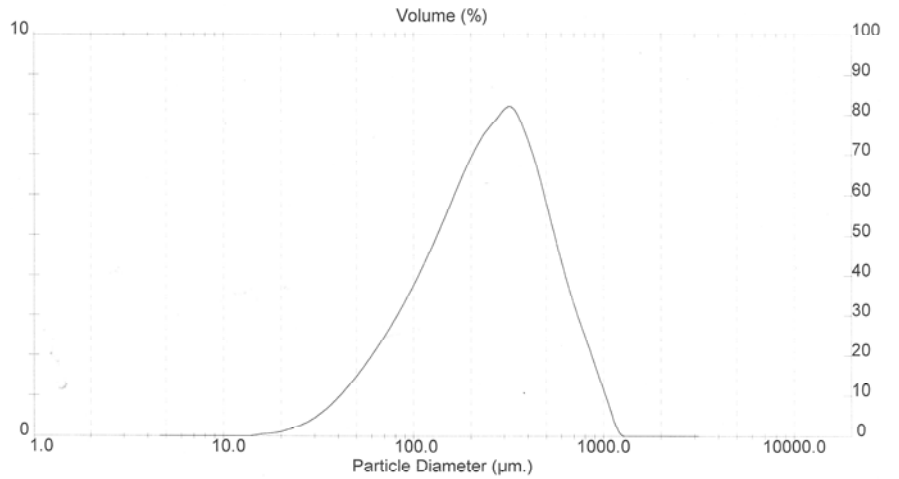

Figure E3 Particle size distribution data of Formulation F1.

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Building 2-3 Chula Soi 62 Phaya-Thai Rd. Phatumwan Bangkok 10330 Tel. 2188029-32, 2188101 Fax. 2540211

Result: Analysis Report

Sample Details

Sample ID: 5%EC-1%SP80
Sample File: CHULA6
Sample Path: C:\SIZERS\DATA\
Sample Notes: Wet Analysis System
Dispersing Medium : Water
Ultrasonic : No
Additive : 0.1% Nonidet P40

Run Number: 3
Record Number: 1362

Measured: 21 Feb 2008 13:14PM
Analysed: 21 Feb 2008 13:14PM
Result Source: Analysed

System Details

Range Lens: 1000 mm
Presentation: 3_ECL
Analysis Model: Polydisperse
Modifications: None

Beam Length: 2.40 mm
[Particle R.I. = (1.4790, 0.1000); Dispersant R.I. = 1.3300]

Sampler: MS1

Obscuration: 3.6 %
Residual: 0.811 %

Result Statistics

Distribution Type: Volume
Mean Diameters:
D [4, 3] = 391.90 um

Concentration = 0.1379 %Vol
D (v, 0.1) = 151.78 um
D [3, 2] = 273.93 um

Density = 1.000 g / cub. cm
D (v, 0.5) = 319.90 um
Span = 1.864E+00

Specific S.A. = 0.0219 sq. m / g
D (v, 0.9) = 748.02 um
Uniformity = 5.709E-01

Size Low (um)	In %	Size High (um)	Under%	Size Low (um)	In %	Size High (um)	Under%
4.19	0.00	4.88	0.00	120.67	3.50	140.58	7.55
4.88	0.00	5.69	0.00	140.58	5.36	163.77	12.91
5.69	0.00	6.63	0.00	163.77	7.19	190.80	20.10
6.63	0.00	7.72	0.00	190.80	8.42	222.28	28.52
7.72	0.00	9.00	0.00	222.28	8.98	258.95	37.50
9.00	0.00	10.48	0.00	258.95	9.06	301.68	46.56
10.48	0.00	12.21	0.00	301.68	8.86	351.46	55.43
12.21	0.00	14.22	0.00	351.46	8.50	409.45	63.93
14.22	0.00	16.57	0.00	409.45	8.05	477.01	71.98
16.57	0.00	19.31	0.00	477.01	7.13	555.71	79.11
19.31	0.00	22.49	0.00	555.71	6.09	647.41	85.20
22.49	0.00	26.20	0.00	647.41	5.05	754.23	90.25
26.20	0.00	30.53	0.00	754.23	4.00	878.67	94.25
30.53	0.00	35.56	0.00	878.67	2.96	1023.66	97.21
35.56	0.00	41.43	0.00	1023.66	1.92	1192.56	99.13
41.43	0.00	48.27	0.00	1192.56	0.87	1389.33	100.00
48.27	0.03	56.23	0.03	1389.33	0.00	1618.57	100.00
56.23	0.09	65.51	0.13	1618.57	0.00	1885.64	100.00
65.51	0.24	76.32	0.37	1885.64	0.00	2196.77	100.00
76.32	0.54	88.91	0.91	2196.77	0.00	2559.23	100.00
88.91	1.10	103.58	2.01	2559.23	0.00	2981.51	100.00
103.58	2.05	120.67	4.06	2981.51	0.00	3473.45	100.00

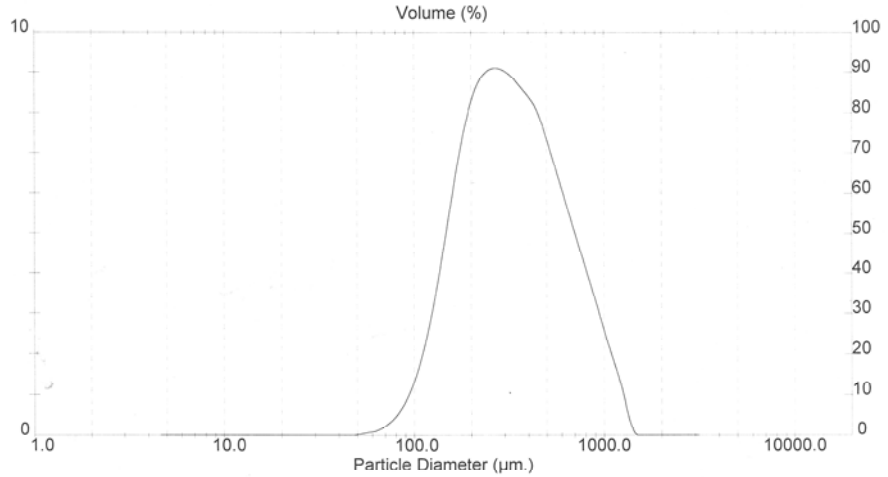

Figure E4 Particle size distribution data of Formulation F2.

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Building 2-3 Chula Soi 62 Phaya-Thai Rd. Phatumwan Bangkok 10330 Tel. 2188029-32, 2188101 Fax. 2540211

Result: Analysis Report

Sample Details

Sample ID: 5%EC-1%SP80
Sample File: CHULA6
Sample Path: C:\SIZERS\DATA\
Sample Notes: Wet Analysis System
Dispersing Medium : Water
Ultrasonic : No
Additive : 0.1% Nonidet P40

Run Number: 5
Record Number: 1364

Measured: 21 Feb 2008 13:15PM
Analysed: 21 Feb 2008 13:15PM
Result Source: Analysed

System Details

Range Lens: 1000 mm
Presentation: 3_ECL
Analysis Model: Polydisperse
Modifications: None

Beam Length: 2.40 mm
[Particle R.I. = (1.4790, 0.1000); Dispersant R.I. = 1.3300]

Sampler: MS1

Obscuration: 3.5 %
Residual: 1.276 %

Result Statistics

Distribution Type: Volume
Mean Diameters:
D [4, 3] = 393.36 um

Concentration = 0.1355 %Vol
D (v, 0.1) = 149.53 um
D [3, 2] = 272.36 um

Density = 1.000 g / cub. cm
D (v, 0.5) = 319.72 um
Span = 1.899E+00

Specific S.A. = 0.0220 sq. m / g
D (v, 0.9) = 756.63 um
Uniformity = 5.800E-01

Size Low (um)	In %	Size High (um)	Under%	Size Low (um)	In %	Size High (um)	Under%
4.19	0.00	4.88	0.00	120.67	3.62	140.58	8.03
4.88	0.00	5.69	0.00	140.58	5.40	163.77	13.43
5.69	0.00	6.63	0.00	163.77	7.09	190.80	20.52
6.63	0.00	7.72	0.00	190.80	8.28	222.28	28.80
7.72	0.00	9.00	0.00	222.28	8.86	258.95	37.66
9.00	0.00	10.48	0.00	258.95	8.97	301.68	46.63
10.48	0.00	12.21	0.00	301.68	8.75	351.46	55.38
12.21	0.00	14.22	0.00	351.46	8.32	409.45	63.70
14.22	0.00	16.57	0.00	409.45	7.81	477.01	71.51
16.57	0.00	19.31	0.00	477.01	7.01	555.71	78.52
19.31	0.00	22.49	0.00	555.71	6.16	647.41	84.68
22.49	0.00	26.20	0.00	647.41	5.22	754.23	89.90
26.20	0.00	30.53	0.00	754.23	4.20	878.67	94.10
30.53	0.00	35.56	0.00	878.67	3.08	1023.66	97.19
35.56	0.00	41.43	0.00	1023.66	1.97	1192.56	99.15
41.43	0.00	48.27	0.00	1192.56	0.85	1389.33	100.00
48.27	0.04	56.23	0.04	1389.33	0.00	1618.57	100.00
56.23	0.11	65.51	0.15	1618.57	0.00	1885.64	100.00
65.51	0.27	76.32	0.42	1885.64	0.00	2196.77	100.00
76.32	0.60	88.91	1.02	2196.77	0.00	2559.23	100.00
88.91	1.20	103.58	2.22	2559.23	0.00	2981.51	100.00
103.58	2.18	120.67	4.41	2981.51	0.00	3473.45	100.00

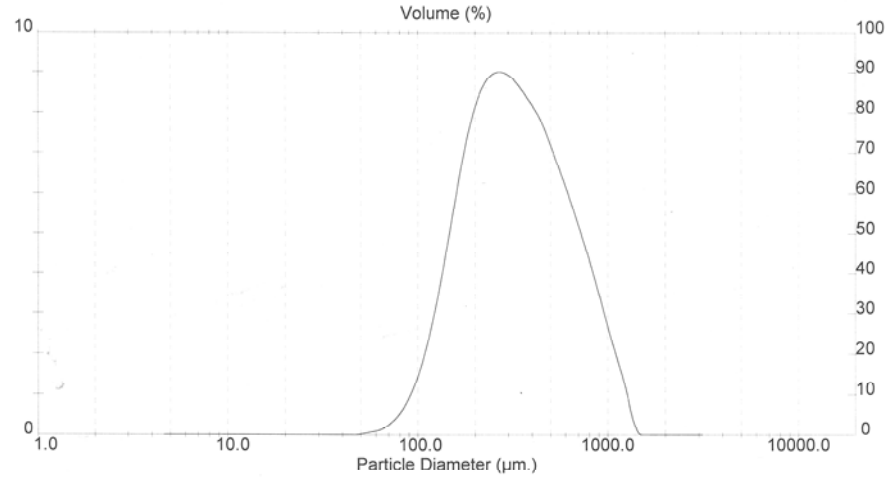

Figure E5 Particle size distribution data of Formulation F2.

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Building 2-3 Chula Soi 62 Phaya-Thai Rd. Phatumwan Bangkok 10330 Tel. 2188029-32, 2188101 Fax. 2540211

Result: Analysis Report

Sample Details

Sample ID: 5%EC-1%SP80
Sample File: CHULA6
Sample Path: C:\SIZERS\DATA\
Sample Notes: Wet Analysis System
Dispersing Medium : Water
Ultrasonic : No
Additive : 0.1% Nonidet P40

Run Number: 8
Record Number: 1367

Measured: 21 Feb 2008 13:15PM
Analysed: 21 Feb 2008 13:15PM
Result Source: Analysed

System Details

Range Lens: 1000 mm
Presentation: 3_ECL
Analysis Model: Polydisperse
Modifications: None

Beam Length: 2.40 mm
[Particle R.I. = (1.4790, 0.1000); Dispersant R.I. = 1.3300]

Sampler: MS1

Obscuration: 3.7 %
Residual: 0.665 %

Result Statistics

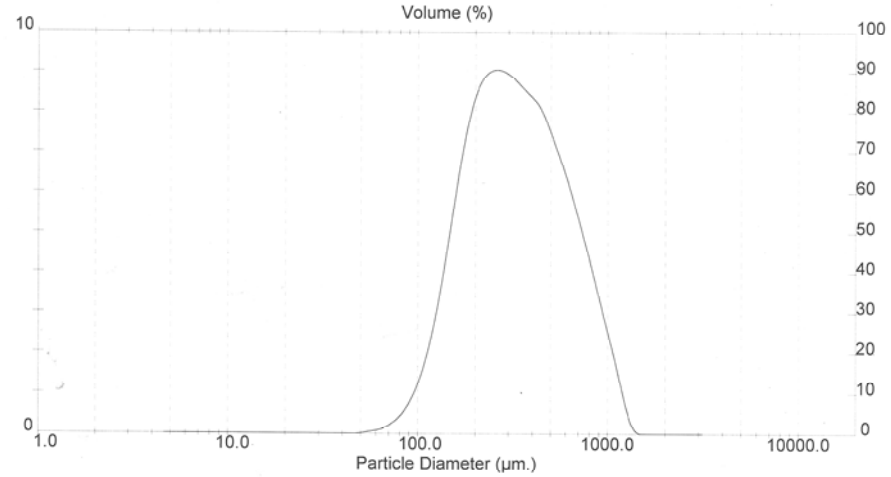
Distribution Type: Volume
Mean Diameters:
D [4, 3] = 389.57 um

Concentration = 0.1433 %Vol
D (v, 0.1) = 153.01 um
D [3, 2] = 275.17 um

Density = 1.000 g / cub. cm
D (v, 0.5) = 321.22 um
Span = 1.817E+00

Specific S.A. = 0.0218 sq. m / g
D (v, 0.9) = 736.66 um
Uniformity = 5.582E-01

Size Low (um)	In %	Size High (um)	Under%	Size Low (um)	In %	Size High (um)	Under%
4.19	0.00	4.88	0.00	120.67	3.44	140.58	7.25
4.88	0.00	5.69	0.00	140.58	5.39	163.77	12.64
5.69	0.00	6.63	0.00	163.77	7.27	190.80	19.91
6.63	0.00	7.72	0.00	190.80	8.48	222.28	28.40
7.72	0.00	9.00	0.00	222.28	8.97	258.95	37.36
9.00	0.00	10.48	0.00	258.95	9.00	301.68	46.36
10.48	0.00	12.21	0.00	301.68	8.77	351.46	55.13
12.21	0.00	14.22	0.00	351.46	8.44	409.45	63.58
14.22	0.00	16.57	0.00	409.45	8.07	477.01	71.65
16.57	0.00	19.31	0.00	477.01	7.31	555.71	78.96
19.31	0.00	22.49	0.00	555.71	6.42	647.41	85.38
22.49	0.00	26.20	0.00	647.41	5.37	754.23	90.76
26.20	0.00	30.53	0.00	754.23	4.19	878.67	94.94
30.53	0.00	35.56	0.00	878.67	2.94	1023.66	97.88
35.56	0.00	41.43	0.00	1023.66	1.69	1192.56	99.57
41.43	0.00	48.27	0.00	1192.56	0.43	1389.33	100.00
48.27	0.04	56.23	0.04	1389.33	0.00	1618.57	100.00
56.23	0.09	65.51	0.13	1618.57	0.00	1885.64	100.00
65.51	0.23	76.32	0.35	1885.64	0.00	2196.77	100.00
76.32	0.50	88.91	0.85	2196.77	0.00	2559.23	100.00
88.91	1.02	103.58	1.87	2559.23	0.00	2981.51	100.00
103.58	1.95	120.67	3.82	2981.51	0.00	3473.45	100.00



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Serial Number: 32734-89

Figure E6 Particle size distribution data of Formulation F2.

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Building 2-3 Chula Soi 62 Phaya-Thai Rd. Phatumwan Bangkok 10330 Tel. 2188029-32, 2188101 Fax. 2540211

Result: Analysis Report

Sample Details

Sample ID: 7.5%EC-1%SP80
Sample File: CHULA6
Sample Path: C:\SIZERS\DATA\
Sample Notes: Wet Analysis System
Dispersing Medium : Water
Ultrasonic : No
Additive : 0.1% Nonidet P40

Run Number: 15
Record Number: 1389

Measured: 21 Feb 2008 13:33PM
Analysed: 21 Feb 2008 13:33PM
Result Source: Analysed

System Details

Range Lens: 1000 mm
Presentation: 3_ECL
Analysis Model: Polydisperse
Modifications: None

Beam Length: 2.40 mm
[Particle R.I. = (1.4790, 0.1000); Dispersant R.I. = 1.3300]

Sampler: MS1

Obscuration: 5.9 %
Residual: 1.267 %

Result Statistics

Distribution Type: Volume
Mean Diameters:
D [4, 3] = 458.05 um

Concentration = 0.3012 %Vol
D (v, 0.1) = 220.29 um
D [3, 2] = 358.52 um

Density = 1.000 g / cub. cm
D (v, 0.5) = 392.59 um
Span = 1.466E+00

Specific S.A. = 0.0167 sq. m / g
D (v, 0.9) = 795.95 um
Uniformity = 4.514E-01

Size Low (um)	In %	Size High (um)	Under%	Size Low (um)	In %	Size High (um)	Under%
4.19	0.00	4.88	0.00	120.67	0.69	140.58	1.17
4.88	0.00	5.69	0.00	140.58	1.43	163.77	2.59
5.69	0.00	6.63	0.00	163.77	2.77	190.80	5.37
6.63	0.00	7.72	0.00	190.80	4.99	222.28	10.36
7.72	0.00	9.00	0.00	222.28	7.95	258.95	18.31
9.00	0.00	10.48	0.00	258.95	10.68	301.68	28.99
10.48	0.00	12.21	0.00	301.68	12.12	351.46	41.11
12.21	0.00	14.22	0.00	351.46	12.27	409.45	53.37
14.22	0.00	16.57	0.00	409.45	11.83	477.01	65.20
16.57	0.00	19.31	0.00	477.01	9.75	555.71	74.95
19.31	0.00	22.49	0.00	555.71	7.63	647.41	82.58
22.49	0.00	26.20	0.00	647.41	5.76	754.23	88.34
26.20	0.00	30.53	0.00	754.23	4.29	878.67	92.64
30.53	0.00	35.56	0.00	878.67	3.17	1023.66	95.81
35.56	0.00	41.43	0.00	1023.66	2.29	1192.56	98.09
41.43	0.00	48.27	0.00	1192.56	1.40	1389.33	99.49
48.27	0.00	56.23	0.00	1389.33	0.51	1618.57	100.00
56.23	0.00	65.51	0.00	1618.57	0.00	1885.64	100.00
65.51	0.00	76.32	0.00	1885.64	0.00	2196.77	100.00
76.32	0.05	88.91	0.05	2196.77	0.00	2559.23	100.00
88.91	0.12	103.58	0.17	2559.23	0.00	2981.51	100.00
103.58	0.31	120.67	0.48	2981.51	0.00	3473.45	100.00

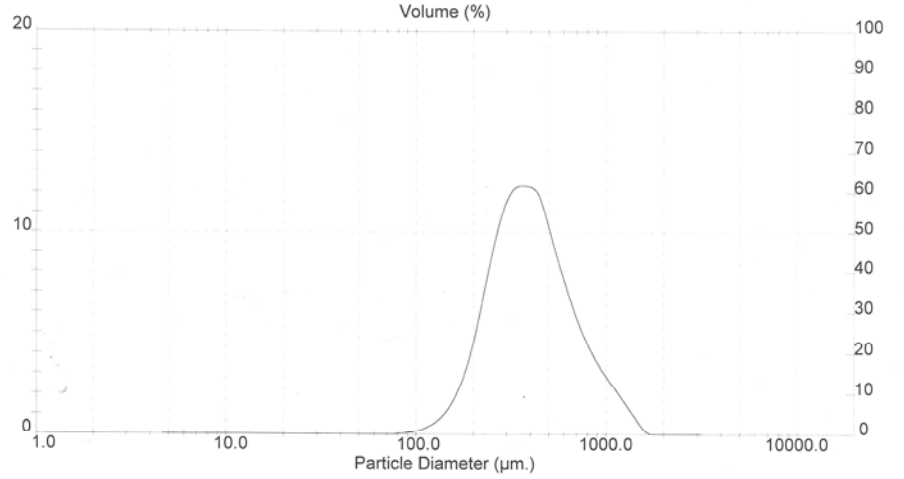
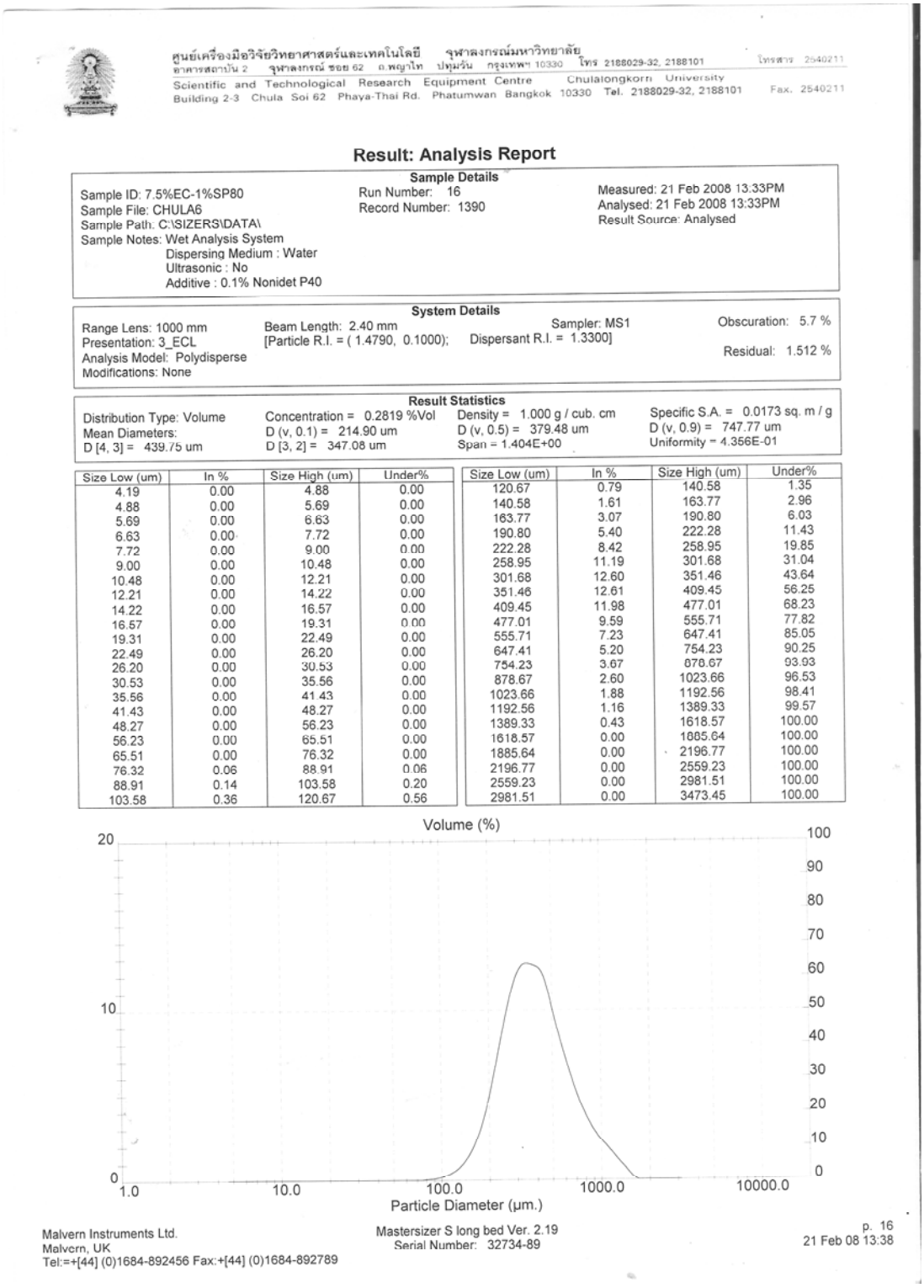


Figure E7 Particle size distribution data of Formulation F3.



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Building 2-3 Chula Soi 62 Phaya-Thai Rd. Phatumwan Bangkok 10330 Tel. 2188029-32, 2188101 Fax. 2540211

Result: Analysis Report

Sample Details

Sample ID: 7.5%EC-1%SP80
Sample File: CHULA6
Sample Path: C:\SIZERS\DATA\
Sample Notes: Wet Analysis System
Dispersing Medium : Water
Ultrasonic : No
Additive : 0.1% Nonidet P40

Run Number: 16
Record Number: 1390

Measured: 21 Feb 2008 13:33PM
Analysed: 21 Feb 2008 13:33PM
Result Source: Analysed

System Details

Range Lens: 1000 mm
Presentation: 3_ECL
Analysis Model: Polydisperse
Modifications: None

Beam Length: 2.40 mm
[Particle R.I. = (1.4790, 0.1000);

Sampler: MS1
Dispersant R.I. = 1.3300]

Obscuration: 5.7 %
Residual: 1.512 %

Result Statistics

Distribution Type: Volume
Mean Diameters:
D [4, 3] = 439.75 um

Concentration = 0.2819 %Vol
D (v, 0.1) = 214.90 um
D [3, 2] = 347.08 um

Density = 1.000 g / cub. cm
D (v, 0.5) = 379.48 um
Span = 1.404E+00

Specific S.A. = 0.0173 sq. m / g
D (v, 0.9) = 747.77 um
Uniformity = 4.356E-01

Size Low (um)	In %	Size High (um)	Under%	Size Low (um)	In %	Size High (um)	Under%
4.19	0.00	4.88	0.00	120.67	0.79	140.58	1.35
4.88	0.00	5.69	0.00	140.58	1.61	163.77	2.96
5.69	0.00	6.63	0.00	163.77	3.07	190.80	6.03
6.63	0.00	7.72	0.00	190.80	5.40	222.28	11.43
7.72	0.00	9.00	0.00	222.28	8.42	258.95	19.85
9.00	0.00	10.48	0.00	258.95	11.19	301.68	31.04
10.48	0.00	12.21	0.00	301.68	12.60	351.46	43.64
12.21	0.00	14.22	0.00	351.46	12.61	409.45	56.25
14.22	0.00	16.57	0.00	409.45	11.98	477.01	68.23
16.57	0.00	19.31	0.00	477.01	9.59	555.71	77.82
19.31	0.00	22.49	0.00	555.71	7.23	647.41	85.05
22.49	0.00	26.20	0.00	647.41	5.20	754.23	90.25
26.20	0.00	30.53	0.00	754.23	3.67	878.67	93.93
30.53	0.00	35.56	0.00	878.67	2.60	1023.66	96.53
35.56	0.00	41.43	0.00	1023.66	1.88	1192.56	98.41
41.43	0.00	48.27	0.00	1192.56	1.16	1389.33	99.57
48.27	0.00	56.23	0.00	1389.33	0.43	1618.57	100.00
56.23	0.00	65.51	0.00	1618.57	0.00	1885.64	100.00
65.51	0.00	76.32	0.00	1885.64	0.00	2196.77	100.00
76.32	0.06	88.91	0.06	2196.77	0.00	2559.23	100.00
88.91	0.14	103.58	0.20	2559.23	0.00	2981.51	100.00
103.58	0.36	120.67	0.56	2981.51	0.00	3473.45	100.00

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p. 16
21 Feb 08 13:38

Figure E8 Particle size distribution data of Formulation F3.

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Building 2-3 Chula Soi 62 Phaya-Thai Rd. Phatumwan Bangkok 10330 Tel. 2188029-32, 2188101 Fax. 2540211

Result: Analysis Report

Sample Details

Sample ID: 7.5%EC-1%SP80
Sample File: CHULA6
Sample Path: C:\SIZERS\DATA\
Sample Notes: Wet Analysis System
Dispersing Medium : Water
Ultrasonic : No
Additive : 0.1% Nonidet P40

Run Number: 21
Record Number: 1395

Measured: 21 Feb 2008 13:34PM
Analysed: 21 Feb 2008 13:34PM
Result Source: Analysed

System Details

Range Lens: 1000 mm
Presentation: 3_ECL
Analysis Model: Polydisperse
Modifications: None

Beam Length: 2.40 mm
[Particle R.I. = (1.4790, 0.1000); Dispersant R.I. = 1.3300]

Sampler: MS1

Obscuration: 6.2 %
Residual: 1.530 %

Result Statistics

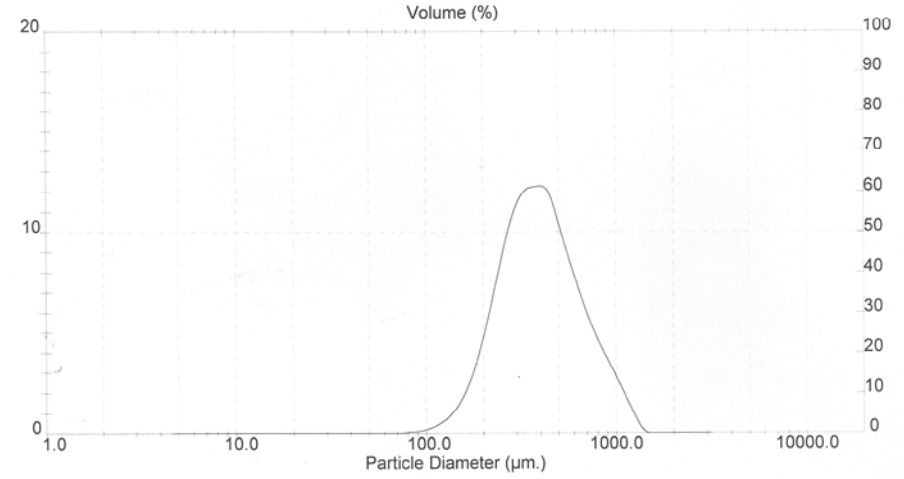
Distribution Type: Volume
Mean Diameters:
D [4, 3] = 445.93 um

Concentration = 0.3157 %Vol
D (v, 0.1) = 216.64 um
D [3, 2] = 353.51 um

Density = 1.000 g / cub. cm
D (v, 0.5) = 391.48 um
Span = 1.397E+00

Specific S.A. = 0.0170 sq. m / g
D (v, 0.9) = 763.45 um
Uniformity = 4.276E-01

Size Low (um)	In %	Size High (um)	Under%	Size Low (um)	In %	Size High (um)	Under%
4.19	0.00	4.88	0.00	120.67	0.79	140.58	1.37
4.88	0.00	5.69	0.00	140.58	1.58	163.77	2.95
5.69	0.00	6.63	0.00	163.77	2.97	190.80	5.92
6.63	0.00	7.72	0.00	190.80	5.13	222.28	11.05
7.72	0.00	9.00	0.00	222.28	7.91	258.95	18.96
9.00	0.00	10.48	0.00	258.95	10.49	301.68	29.44
10.48	0.00	12.21	0.00	301.68	11.94	351.46	41.38
12.21	0.00	14.22	0.00	351.46	12.22	409.45	53.60
14.22	0.00	16.57	0.00	409.45	11.96	477.01	65.56
16.57	0.00	19.31	0.00	477.01	10.01	555.71	75.58
19.31	0.00	22.49	0.00	555.71	7.96	647.41	83.53
22.49	0.00	26.20	0.00	647.41	6.06	754.23	89.59
26.20	0.00	30.53	0.00	754.23	4.51	878.67	94.10
30.53	0.00	35.56	0.00	878.67	3.24	1023.66	97.34
35.56	0.00	41.43	0.00	1023.66	1.97	1192.56	99.30
41.43	0.00	48.27	0.00	1192.56	0.70	1389.33	100.00
48.27	0.00	56.23	0.00	1389.33	0.00	1618.57	100.00
56.23	0.00	65.51	0.00	1618.57	0.00	1885.64	100.00
65.51	0.00	76.32	0.00	1885.64	0.00	2196.77	100.00
76.32	0.07	88.91	0.07	2196.77	0.00	2559.23	100.00
88.91	0.15	103.58	0.22	2559.23	0.00	2981.51	100.00
103.58	0.36	120.67	0.58	2981.51	0.00	3473.45	100.00



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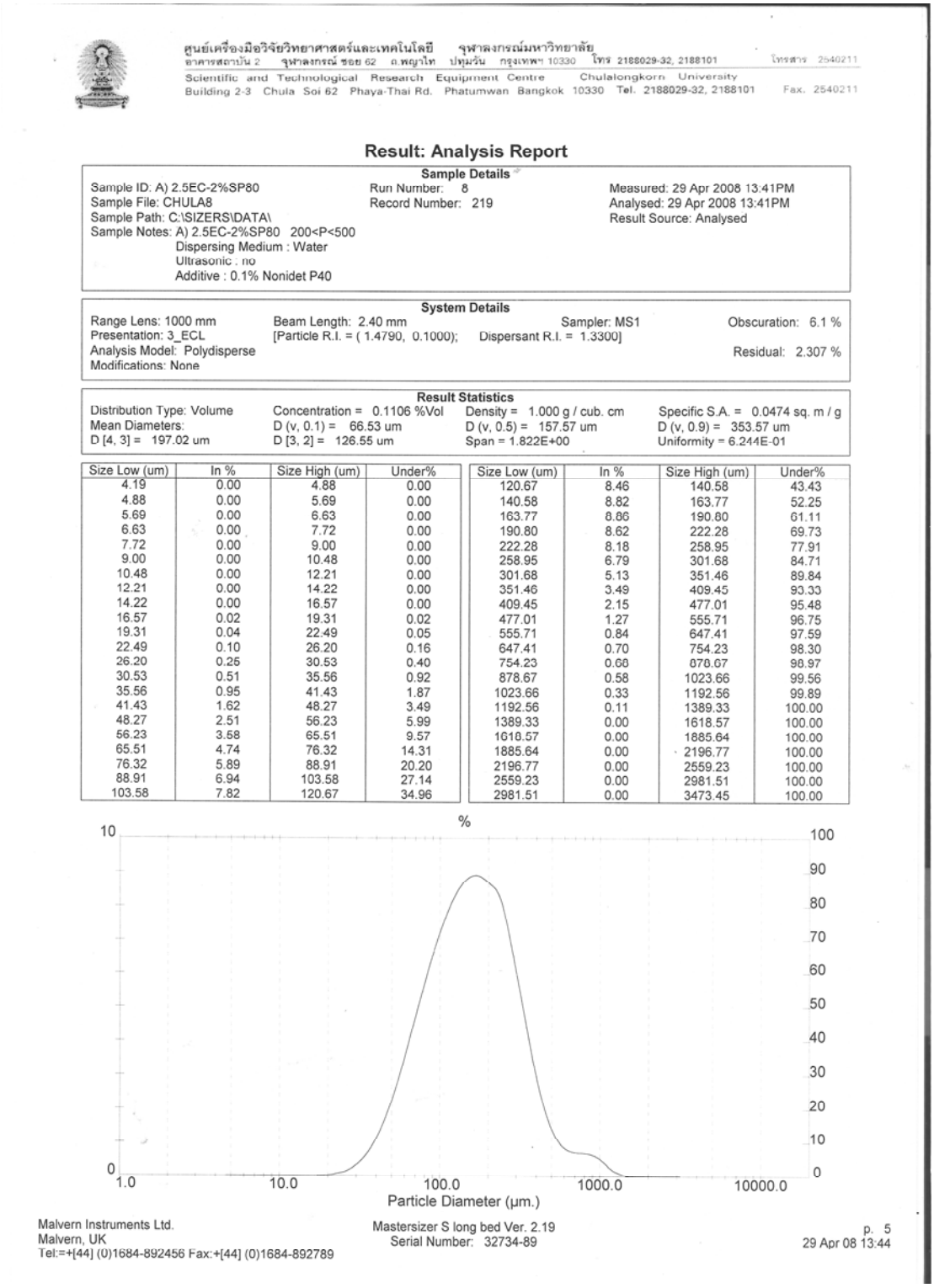
p. 15
21 Feb 08 13:37

Figure E9 Particle size distribution data of Formulation F3.



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Result: Analysis Report

Sample Details

Sample ID: A) 2.5EC-2%SP80
Sample File: CHULA8
Sample Path: C:\SIZERS\DATA\
Sample Notes: A) 2.5EC-2%SP80 200<P<500
Dispersing Medium : Water
Ultrasonic : no
Additive : 0.1% Nonidet P40

Run Number: 8
Record Number: 219

Measured: 29 Apr 2008 13:41PM
Analysed: 29 Apr 2008 13:41PM
Result Source: Analysed

System Details

Range Lens: 1000 mm
Presentation: 3_ECL
Analysis Model: Polydisperse
Modifications: None

Beam Length: 2.40 mm
[Particle R.I. = (1.4790, 0.1000); Dispersant R.I. = 1.3300]

Sampler: MS1

Obscuration: 6.1 %
Residual: 2.307 %

Result Statistics

Distribution Type: Volume
Mean Diameters:
D [4, 3] = 197.02 um

Concentration = 0.1106 %Vol
D (v, 0.1) = 66.53 um
D [3, 2] = 126.55 um

Density = 1.000 g / cub. cm
D (v, 0.5) = 157.57 um
Span = 1.822E+00

Specific S.A. = 0.0474 sq. m / g
D (v, 0.9) = 353.57 um
Uniformity = 6.244E-01

Size Low (um)	In %	Size High (um)	Under%	Size Low (um)	In %	Size High (um)	Under%
4.19	0.00	4.88	0.00	120.67	8.46	140.58	43.43
4.88	0.00	5.69	0.00	140.58	8.82	163.77	52.25
5.69	0.00	6.63	0.00	163.77	8.66	190.80	61.11
6.63	0.00	7.72	0.00	190.80	8.62	222.28	69.73
7.72	0.00	9.00	0.00	222.28	8.18	258.95	77.91
9.00	0.00	10.48	0.00	258.95	6.79	301.68	84.71
10.48	0.00	12.21	0.00	301.68	5.13	351.46	89.84
12.21	0.00	14.22	0.00	351.46	3.49	409.45	93.33
14.22	0.00	16.57	0.00	409.45	2.15	477.01	95.48
16.57	0.02	19.31	0.02	477.01	1.27	555.71	96.75
19.31	0.04	22.49	0.05	555.71	0.84	647.41	97.59
22.49	0.10	26.20	0.16	647.41	0.70	754.23	98.30
26.20	0.25	30.53	0.40	754.23	0.66	878.67	98.97
30.53	0.51	35.56	0.92	878.67	0.58	1023.66	99.56
35.56	0.95	41.43	1.87	1023.66	0.33	1192.56	99.89
41.43	1.62	48.27	3.49	1192.56	0.11	1389.33	100.00
48.27	2.51	56.23	5.99	1389.33	0.00	1618.57	100.00
56.23	3.58	65.51	9.57	1618.57	0.00	1885.64	100.00
65.51	4.74	76.32	14.31	1885.64	0.00	2196.77	100.00
76.32	5.89	88.91	20.20	2196.77	0.00	2559.23	100.00
88.91	6.94	103.58	27.14	2559.23	0.00	2981.51	100.00
103.58	7.82	120.67	34.96	2981.51	0.00	3473.45	100.00

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p. 5
29 Apr 08 13:44

Figure E10 Particle size distribution data of Formulation F4.

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Result: Analysis Report

Sample Details

Sample ID. A) 2.5EC-2%SP80
Sample File: CHULA8
Sample Path: C:\SIZERS\DATA\
Sample Notes: A) 2.5EC-2%SP80 200<P<500
Dispersing Medium : Water
Ultrasonic : no
Additive : 0.1% Nonidet P40

Run Number: 10
Record Number: 221

Measured: 29 Apr 2008 13:42PM
Analysed: 29 Apr 2008 13:42PM
Result Source: Analysed

System Details

Range Lens: 1000 mm
Presentation: 3_ECL
Analysis Model: Polydisperse
Modifications: None

Beam Length: 2.40 mm
[Particle R.I. = (1.4790, 0.1000); Dispersant R.I. = 1.3300]

Sampler: MS1

Obscuration: 6.2 %
Residual: 2.155 %

Result Statistics

Distribution Type: Volume
Mean Diameters:
D [4, 3] = 190.58 um

Concentration = 0.1113 %Vol
D (v, 0.1) = 66.93 um
D [3, 2] = 125.72 um

Density = 1.000 g / cub. cm
D (v, 0.5) = 155.64 um
Span = 1.745E+00

Specific S.A. = 0.0477 sq. m / g
D (v, 0.9) = 338.59 um
Uniformity = 5.918E-01

Size Low (um)	In %	Size High (um)	Under%	Size Low (um)	In %	Size High (um)	Under%
4.19	0.00	4.88	0.00	120.67	8.72	140.58	43.94
4.88	0.00	5.69	0.00	140.58	9.13	163.77	53.07
5.69	0.00	6.63	0.00	163.77	9.27	190.80	62.34
6.63	0.00	7.72	0.00	190.80	9.21	222.28	71.55
7.72	0.00	9.00	0.00	222.28	8.11	258.95	79.66
9.00	0.00	10.48	0.00	258.95	6.55	301.68	86.21
10.48	0.00	12.21	0.00	301.68	4.81	351.46	91.02
12.21	0.00	14.22	0.00	351.46	3.17	409.45	94.19
14.22	0.00	16.57	0.00	409.45	1.92	477.01	96.11
16.57	0.02	19.31	0.02	477.01	1.13	555.71	97.24
19.31	0.04	22.49	0.05	555.71	0.77	647.41	98.01
22.49	0.10	26.20	0.15	647.41	0.65	754.23	98.66
26.20	0.24	30.53	0.39	754.23	0.61	878.67	99.27
30.53	0.49	35.56	0.89	878.67	0.48	1023.66	99.75
35.56	0.93	41.43	1.81	1023.66	0.23	1192.56	99.98
41.43	1.58	48.27	3.39	1192.56	0.02	1389.33	100.00
48.27	2.46	56.23	5.85	1389.33	0.00	1618.57	100.00
56.23	3.55	65.51	9.40	1618.57	0.00	1885.64	100.00
65.51	4.75	76.32	14.15	1885.64	0.00	2196.77	100.00
76.32	5.96	88.91	20.11	2196.77	0.00	2559.23	100.00
88.91	7.08	103.58	27.19	2559.23	0.00	2981.51	100.00
103.58	8.03	120.67	35.22	2981.51	0.00	3473.45	100.00

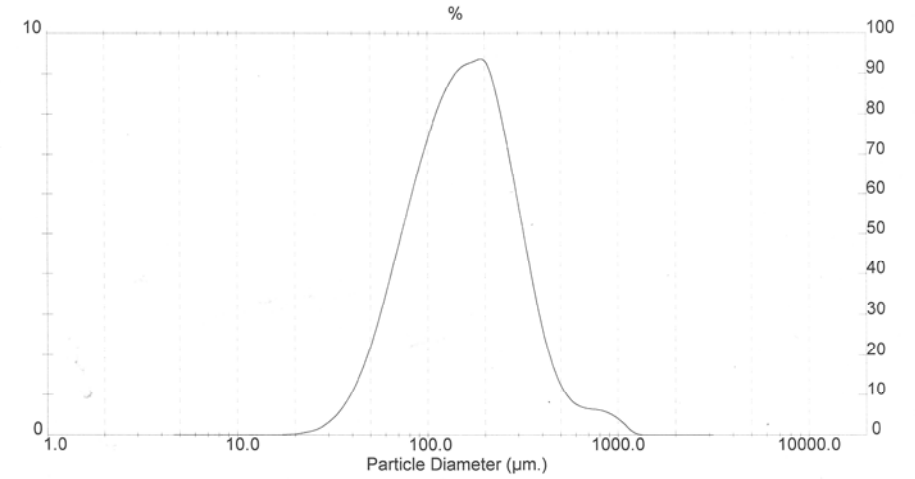

Figure E11 Particle size distribution data of Formulation F4.

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Result: Analysis Report

Sample Details

Sample ID: A) 2.5EC-2%SP80
Sample File: CHULA8
Sample Path: C:\SIZERS\DATA\
Sample Notes: A) 2.5EC-2%SP80 200<P<500
Dispersing Medium : Water
Ultrasonic : no
Additive : 0.1% Nonidet P40

Run Number: 13
Record Number: 224

Measured: 29 Apr 2008 13:42PM
Analysed: 29 Apr 2008 13:42PM
Result Source: Analysed

System Details

Range Lens: 1000 mm
Presentation: 3_ECL
Analysis Model: Polydisperse
Modifications: None

Beam Length: 2.40 mm
[Particle R.I. = (1.4790, 0.1000); Dispersant R.I. = 1.3300]

Sampler: MS1

Obscuration: 6.1 %
Residual: 2.200 %

Result Statistics

Distribution Type: Volume
Mean Diameters:
D [4, 3] = 192.26 um

Concentration = 0.1115 %Vol
D (v, 0.1) = 67.16 um
D [3, 2] = 126.85 um

Density = 1.000 g / cub. cm
D (v, 0.5) = 157.89 um
Span = 1.762E+00

Specific S.A. = 0.0473 sq. m / g
D (v, 0.9) = 345.29 um
Uniformity = 5.893E-01

Size Low (um)	In %	Size High (um)	Under%	Size Low (um)	In %	Size High (um)	Under%
4.19	0.00	4.88	0.00	120.67	8.53	140.58	43.21
4.88	0.00	5.69	0.00	140.58	8.96	163.77	52.17
5.69	0.00	6.63	0.00	163.77	9.14	190.80	61.31
6.63	0.00	7.72	0.00	190.80	9.15	222.28	70.46
7.72	0.00	9.00	0.00	222.28	8.19	258.95	78.65
9.00	0.00	10.48	0.00	258.95	6.77	301.68	85.42
10.48	0.00	12.21	0.00	301.68	5.09	351.46	90.51
12.21	0.00	14.22	0.00	351.46	3.46	409.45	93.97
14.22	0.00	16.57	0.00	409.45	2.14	477.01	96.11
16.57	0.02	19.31	0.02	477.01	1.27	555.71	97.38
19.31	0.04	22.49	0.05	555.71	0.82	647.41	98.20
22.49	0.10	26.20	0.15	647.41	0.64	754.23	98.83
26.20	0.23	30.53	0.38	754.23	0.56	878.67	99.39
30.53	0.49	35.56	0.87	878.67	0.42	1023.66	99.81
35.56	0.92	41.43	1.78	1023.66	0.18	1192.56	100.00
41.43	1.56	48.27	3.35	1192.56	0.00	1389.33	100.00
48.27	2.44	56.23	5.79	1389.33	0.00	1618.57	100.00
56.23	3.52	65.51	9.31	1618.57	0.00	1885.64	100.00
65.51	4.69	76.32	14.01	1885.64	0.00	2196.77	100.00
76.32	5.86	88.91	19.87	2196.77	0.00	2559.23	100.00
88.91	6.95	103.58	26.82	2559.23	0.00	2981.51	100.00
103.58	7.86	120.67	34.68	2981.51	0.00	3473.45	100.00

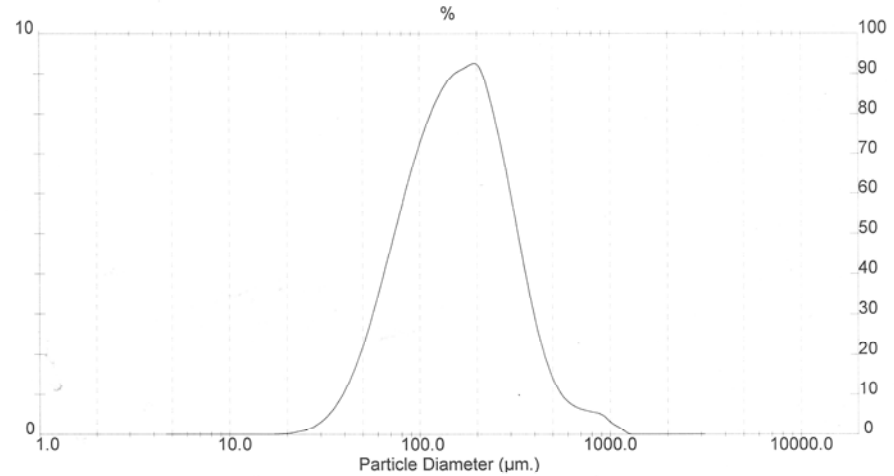

Figure E12 Particle size distribution data of Formulation F4.

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Scientific and Technological Research Equipment Centre Chulalongkorn University
Building 2-3 Chula Soi 62 Phaya-Thai Rd. Phatumwan Bangkok 10330 Tel. 2188029-32, 2188101 Fax. 2540211

Result: Analysis Report

Sample Details

Sample ID: B) 2.5EC-4%SP80
Sample File: CHULA8
Sample Path: C:\SIZERS\DATA\
Sample Notes: B) 2.5EC-4%SP80 200<P<500
Dispersing Medium : Water
Ultrasonic : no
Additive : 0.1% Nonidet P40

Run Number: 12
Record Number: 236

Measured: 29 Apr 2008 13:51PM
Analysed: 29 Apr 2008 13:52PM
Result Source: Analysed

System Details

Range Lens: 1000 mm
Presentation: 3_ECL
Analysis Model: Polydisperse
Modifications: None

Beam Length: 2.40 mm
[Particle R.I. = (1.4790, 0.1000); Dispersant R.I. = 1.3300]

Sampler: MS1

Obscuration: 8.8 %
Residual: 1.664 %

Result Statistics

Distribution Type: Volume
Mean Diameters:
D [4, 3] = 141.20 um

Concentration = 0.1217 %Vol
D (v, 0.1) = 56.59 um
D [3, 2] = 95.79 um

Density = 1.000 g / cub. cm
D (v, 0.5) = 105.59 um
Span = 1.653E+00

Specific S.A. = 0.0626 sq. m / g
D (v, 0.9) = 231.09 um
Uniformity = 6.382E-01

Size Low (um)	In %	Size High (um)	Under%	Size Low (um)	In %	Size High (um)	Under%
4.19	0.00	4.88	0.00	120.67	10.50	140.58	70.28
4.88	0.00	5.69	0.00	140.58	8.37	163.77	78.65
5.69	0.00	6.63	0.00	163.77	6.20	190.80	84.85
6.63	0.00	7.72	0.00	190.80	4.31	222.28	89.16
7.72	0.00	9.00	0.00	222.28	2.84	258.95	92.00
9.00	0.00	10.48	0.00	258.95	1.83	301.68	93.83
10.48	0.00	12.21	0.00	301.68	1.20	351.46	95.02
12.21	0.00	14.22	0.00	351.46	0.86	409.45	95.88
14.22	0.00	16.57	0.00	409.45	0.70	477.01	96.59
16.57	0.00	19.31	0.00	477.01	0.67	555.71	97.25
19.31	0.04	22.49	0.04	555.71	0.69	647.41	97.94
22.49	0.10	26.20	0.14	647.41	0.70	754.23	98.63
26.20	0.28	30.53	0.42	754.23	0.64	878.67	99.28
30.53	0.67	35.56	1.09	878.67	0.48	1023.66	99.76
35.56	1.42	41.43	2.51	1023.66	0.24	1192.56	100.00
41.43	2.69	48.27	5.20	1192.56	0.00	1389.33	100.00
48.27	4.56	56.23	9.75	1389.33	0.00	1618.57	100.00
56.23	6.96	65.51	16.71	1618.57	0.00	1885.64	100.00
65.51	9.34	76.32	26.05	1885.64	0.00	2196.77	100.00
76.32	10.98	88.91	37.03	2196.77	0.00	2559.23	100.00
88.91	11.53	103.58	48.56	2559.23	0.00	2981.51	100.00
103.58	11.23	120.67	59.79	2981.51	0.00	3473.45	100.00

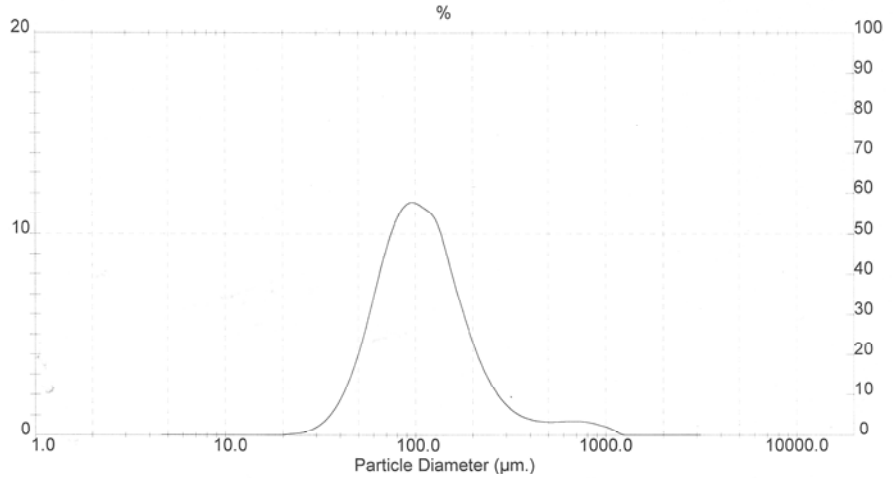

Figure E13 Particle size distribution data of Formulation F5.

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Result: Analysis Report

Sample Details

Sample ID: B) 2.5EC-4%SP80
Sample File: CHULA8
Sample Path: C:\SIZERS\DATA\
Sample Notes: B) 2.5EC-4%SP80 200<P<500
Dispersing Medium : Water
Ultrasonic : no
Additive : 0.1% Nonidet P40

Run Number: 14
Record Number: 238

Measured: 29 Apr 2008 13:52PM
Analysed: 29 Apr 2008 13:52PM
Result Source: Analysed

System Details

Range Lens: 1000 mm
Presentation: 3_ECL
Analysis Model: Polydisperse
Modifications: None

Beam Length: 2.40 mm
[Particle R.I. = (1.4790, 0.1000); Dispersant R.I. = 1.3300]

Sampler: MS1

Obscuration: 8.6 %
Residual: 1.592 %

Result Statistics

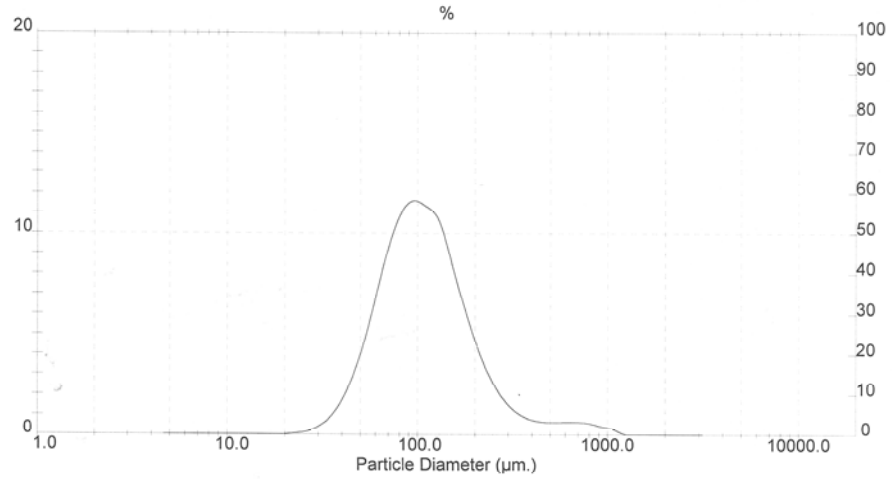
Distribution Type: Volume
Mean Diameters:
D [4, 3] = 138.65 um

Concentration = 0.1195 %Vol
D (v, 0.1) = 56.66 um
D [3, 2] = 95.60 um

Density = 1.000 g / cub. cm
D (v, 0.5) = 105.48 um
Span = 1.608E+00

Specific S.A. = 0.0628 sq. m / g
D (v, 0.9) = 226.31 um
Uniformity = 6.146E-01

Size Low (um)	In %	Size High (um)	Under%	Size Low (um)	In %	Size High (um)	Under%
4.19	0.00	4.88	0.00	120.67	10.56	140.58	70.49
4.88	0.00	5.69	0.00	140.58	8.44	163.77	78.93
5.69	0.00	6.63	0.00	163.77	6.28	190.80	85.21
6.63	0.00	7.72	0.00	190.80	4.38	222.28	89.60
7.72	0.00	9.00	0.00	222.28	2.89	258.95	92.49
9.00	0.00	10.48	0.00	258.95	1.84	301.68	94.32
10.48	0.00	12.21	0.00	301.68	1.18	351.46	95.50
12.21	0.00	14.22	0.00	351.46	0.81	409.45	96.31
14.22	0.00	16.57	0.00	409.45	0.65	477.01	96.96
16.57	0.00	19.31	0.00	477.01	0.61	555.71	97.58
19.31	0.04	22.49	0.04	555.71	0.63	647.41	98.21
22.49	0.10	26.20	0.14	647.41	0.63	754.23	98.83
26.20	0.27	30.53	0.41	754.23	0.55	878.67	99.38
30.53	0.67	35.56	1.08	878.67	0.39	1023.66	99.77
35.56	1.41	41.43	2.49	1023.66	0.23	1192.56	100.00
41.43	2.67	48.27	5.16	1192.56	0.00	1389.33	100.00
48.27	4.54	56.23	9.71	1389.33	0.00	1618.57	100.00
56.23	6.96	65.51	16.66	1618.57	0.00	1885.64	100.00
65.51	9.36	76.32	26.03	1885.64	0.00	2196.77	100.00
76.32	11.02	88.91	37.04	2196.77	0.00	2559.23	100.00
88.91	11.59	103.58	48.63	2559.23	0.00	2981.51	100.00
103.58	11.29	120.67	59.92	2981.51	0.00	3473.45	100.00



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p. 9
29 Apr 08 13:53

Figure E14 Particle size distribution data of Formulation F5.

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Result: Analysis Report

Sample Details

Sample ID: B) 2.5EC-4%SP80
Sample File: CHULA8
Sample Path: C:\SIZERS\DATA\
Sample Notes: B) 2.5EC-4%SP80 200<P<500
Dispersing Medium : Water
Ultrasonic : no
Additive : 0.1% Nonidet P40

Run Number: 16
Record Number: 240

Measured: 29 Apr 2008 13:52PM
Analysed: 29 Apr 2008 13:52PM
Result Source: Analysed

System Details

Range Lens: 1000 mm
Presentation: 3_ECL
Analysis Model: Polydisperse
Modifications: None

Beam Length: 2.40 mm
[Particle R.I. = (1.4790, 0.1000); Dispersant R.I. = 1.3300]

Sampler: MS1

Obscuration: 8.7 %
Residual: 1.604 %

Result Statistics

Distribution Type: Volume
Mean Diameters:
D [4, 3] = 142.15 um

Concentration = 0.1218 %Vol
D (v, 0.1) = 57.14 um
D [3, 2] = 96.94 um

Density = 1.000 g / cub. cm
D (v, 0.5) = 106.99 um
Span = 1.682E+00

Specific S.A. = 0.0619 sq. m / g
D (v, 0.9) = 237.08 um
Uniformity = 6.304E-01

Size Low (um)	In %	Size High (um)	Under%	Size Low (um)	In %	Size High (um)	Under%
4.19	0.00	4.88	0.00	120.67	10.53	140.58	69.32
4.88	0.00	5.69	0.00	140.58	8.46	163.77	77.77
5.69	0.00	6.63	0.00	163.77	6.34	190.80	84.11
6.63	0.00	7.72	0.00	190.80	4.47	222.28	88.58
7.72	0.00	9.00	0.00	222.28	3.01	258.95	91.59
9.00	0.00	10.48	0.00	258.95	1.98	301.68	93.57
10.48	0.00	12.21	0.00	301.68	1.34	351.46	94.91
12.21	0.00	14.22	0.00	351.46	0.98	409.45	95.89
14.22	0.00	16.57	0.00	409.45	0.81	477.01	96.70
16.57	0.00	19.31	0.00	477.01	0.74	555.71	97.43
19.31	0.04	22.49	0.04	555.71	0.71	647.41	98.15
22.49	0.09	26.20	0.13	647.41	0.68	754.23	98.82
26.20	0.26	30.53	0.39	754.23	0.58	878.67	99.40
30.53	0.64	35.56	1.03	878.67	0.39	1023.66	99.79
35.56	1.36	41.43	2.40	1023.66	0.21	1192.56	100.00
41.43	2.59	48.27	4.99	1192.56	0.00	1389.33	100.00
48.27	4.41	56.23	9.40	1389.33	0.00	1618.57	100.00
56.23	6.77	65.51	16.17	1618.57	0.00	1885.64	100.00
65.51	9.16	76.32	25.32	1885.64	0.00	2196.77	100.00
76.32	10.83	88.91	36.15	2196.77	0.00	2559.23	100.00
88.91	11.44	103.58	47.59	2559.23	0.00	2981.51	100.00
103.58	11.20	120.67	58.79	2981.51	0.00	3473.45	100.00

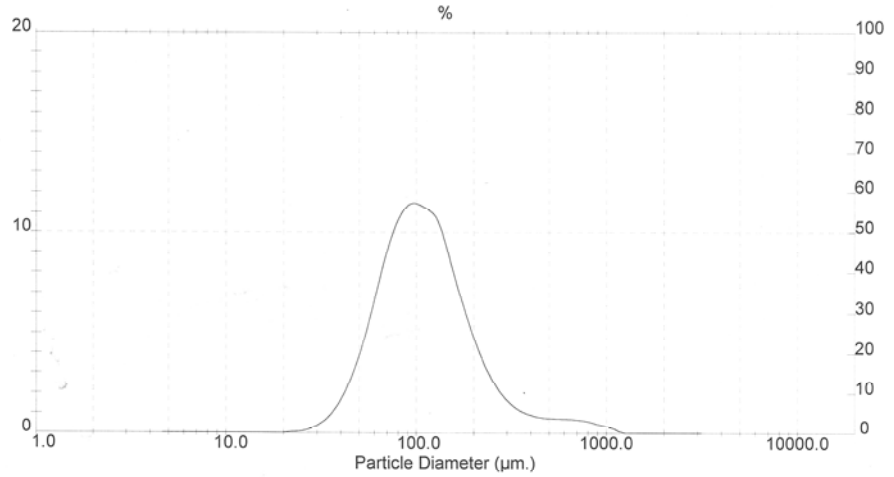

Figure E15 Particle size distribution data of Formulation F5.

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Result: Analysis Report

Sample Details

Sample ID: 2.5EC-1%SP80-2.5%SU
Sample File: CHULA8
Sample Path: C:\SIZERS\DATA\
Sample Notes: 2.5EC-1%SP80-2.5%SU
Dispersing Medium : Water
Ultrasonic : no
Additive : 0.1% Nonidet P40

Run Number: 6
Record Number: 316

Measured: 29 Apr 2008 14:57PM
Analysed: 29 Apr 2008 14:57PM
Result Source: Analysed

System Details

Range Lens: 1000 mm
Presentation: 3_ECL
Analysis Model: Polydisperse
Modifications: None

Beam Length: 2.40 mm
[Particle R.I. = (1.4790, 0.1000); Dispersant R.I. = 1.3300]

Sampler: MS1

Obscuration: 4.7 %
Residual: 1.731 %

Result Statistics

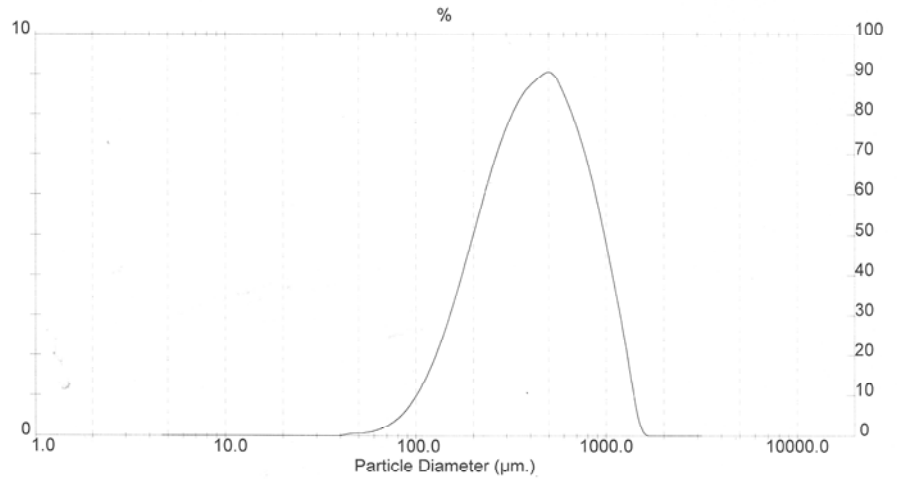
Distribution Type: Volume
Mean Diameters:
D [4, 3] = 489.87 um

Concentration = 0.2233 %Vol
D (v, 0.1) = 174.97 um
D [3, 2] = 331.84 um

Density = 1.000 g / cub. cm
D (v, 0.5) = 425.19 um
Span = 1.733E+00

Specific S.A. = 0.0181 sq. m / g
D (v, 0.9) = 911.62 um
Uniformity = 5.326E-01

Size Low (um)	In %	Size High (um)	Under%	Size Low (um)	In %	Size High (um)	Under%
4.19	0.00	4.88	0.00	120.67	2.11	140.58	5.33
4.88	0.00	5.69	0.00	140.58	3.03	163.77	8.36
5.69	0.00	6.63	0.00	163.77	4.09	190.80	12.45
6.63	0.00	7.72	0.00	190.80	5.21	222.28	17.66
7.72	0.00	9.00	0.00	222.28	6.29	258.95	23.95
9.00	0.00	10.48	0.00	258.95	7.26	301.68	31.21
10.48	0.00	12.21	0.00	301.68	8.04	351.46	39.25
12.21	0.00	14.22	0.00	351.46	8.58	409.45	47.83
14.22	0.00	16.57	0.00	409.45	8.88	477.01	56.71
16.57	0.00	19.31	0.00	477.01	9.02	555.71	65.73
19.31	0.00	22.49	0.00	555.71	8.51	647.41	74.24
22.49	0.00	26.20	0.00	647.41	7.71	754.23	81.95
26.20	0.00	30.53	0.00	754.23	6.64	878.67	88.59
30.53	0.00	35.56	0.00	878.67	5.31	1023.66	93.90
35.56	0.00	41.43	0.00	1023.66	3.74	1192.56	97.64
41.43	0.06	48.27	0.06	1192.56	2.03	1389.33	99.68
48.27	0.08	56.23	0.13	1389.33	0.32	1618.57	100.00
56.23	0.13	65.51	0.26	1618.57	0.00	1885.64	100.00
65.51	0.26	76.32	0.52	1885.64	0.00	2196.77	100.00
76.32	0.48	88.91	1.00	2196.77	0.00	2559.23	100.00
88.91	0.84	103.58	1.85	2559.23	0.00	2981.51	100.00
103.58	1.38	120.67	3.22	2981.51	0.00	3473.45	100.00



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p. 20
29 Apr 08 15:00

Figure E16 Particle size distribution data of Formulation F6.

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Building 2-3 Chula Soi 62 Phaya-Thai Rd. Phatumwan Bangkok 10330 Tel. 2188029-32, 2188101 Fax. 2540211

Result: Analysis Report

Sample Details

Sample ID: 2.5EC-1%SP80-2.5%SU
Sample File: CHULA8
Sample Path: C:\SIZERS\DATA\
Sample Notes: 2.5EC-1%SP80-2.5%SU
Dispersing Medium : Water
Ultrasonic : no
Additive : 0.1% Nonidet P40

Run Number: 7
Record Number: 317

Measured: 29 Apr 2008 14:57PM
Analysed: 29 Apr 2008 14:57PM
Result Source: Analysed

System Details

Range Lens: 1000 mm
Presentation: 3_ECL
Analysis Model: Polydisperse
Modifications: None

Beam Length: 2.40 mm
[Particle R.I. = (1.4790, 0.1000); Dispersant R.I. = 1.3300]

Sampler: MS1

Obscuration: 4.7 %
Residual: 1.974 %

Result Statistics

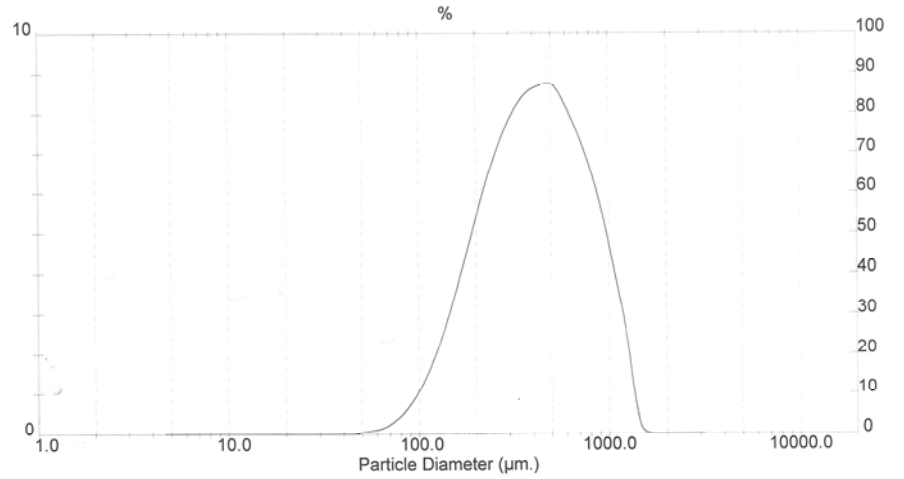
Distribution Type: Volume
Mean Diameters:
D [4, 3] = 479.65 um

Concentration = 0.2191 %Vol
D (v, 0.1) = 170.58 um
D [3, 2] = 325.12 um

Density = 1.000 g / cub. cm
D (v, 0.5) = 412.20 um
Span = 1.775E+00

Specific S.A. = 0.0185 sq. m / g
D (v, 0.9) = 902.13 um
Uniformity = 5.448E-01

Size Low (um)	In %	Size High (um)	Under%	Size Low (um)	In %	Size High (um)	Under%
4.19	0.00	4.88	0.00	120.67	2.30	140.58	5.63
4.88	0.00	5.69	0.00	140.58	3.30	163.77	8.93
5.69	0.00	6.63	0.00	163.77	4.42	190.80	13.35
6.63	0.00	7.72	0.00	190.80	5.55	222.28	18.90
7.72	0.00	9.00	0.00	222.28	6.60	258.95	25.50
9.00	0.00	10.48	0.00	258.95	7.48	301.68	32.97
10.48	0.00	12.21	0.00	301.68	8.13	351.46	41.10
12.21	0.00	14.22	0.00	351.46	8.52	409.45	49.62
14.22	0.00	16.57	0.00	409.45	8.67	477.01	58.29
16.57	0.00	19.31	0.00	477.01	8.67	555.71	66.97
19.31	0.00	22.49	0.00	555.71	8.15	647.41	75.11
22.49	0.00	26.20	0.00	647.41	7.41	754.23	82.53
26.20	0.00	30.53	0.00	754.23	6.47	878.67	89.00
30.53	0.00	35.56	0.00	878.67	5.25	1023.66	94.25
35.56	0.00	41.43	0.00	1023.66	3.71	1192.56	97.96
41.43	0.00	48.27	0.00	1192.56	1.92	1389.33	99.88
48.27	0.06	56.23	0.06	1389.33	0.12	1618.57	100.00
56.23	0.12	65.51	0.17	1618.57	0.00	1885.64	100.00
65.51	0.25	76.32	0.42	1885.64	0.00	2196.77	100.00
76.32	0.50	88.91	0.92	2196.77	0.00	2559.23	100.00
88.91	0.90	103.58	1.82	2559.23	0.00	2981.51	100.00
103.58	1.50	120.67	3.32	2981.51	0.00	3473.45	100.00



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p. 21
29 Apr 08 15:00

Figure E17 Particle size distribution data of Formulation F6.

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Building 2-3 Chula Soi 62 Phaya-Thai Rd. Phatumwan Bangkok 10330 Tel. 2188029-32, 2188101 Fax. 2540211

Result: Analysis Report

Sample Details

Sample ID: 2.5EC-1%SP80-2.5%SU
Sample File: CHULA8
Sample Path: C:\SIZERS\DATA\
Sample Notes: 2.5EC-1%SP80-2.5%SU
Dispersing Medium : Water
Ultrasonic : no
Additive : 0.1% Nonidet P40

Run Number: 14
Record Number: 324

Measured: 29 Apr 2008 14:58PM
Analysed: 29 Apr 2008 14:58PM
Result Source: Analysed

System Details

Range Lens: 1000 mm
Presentation: 3_ECL
Analysis Model: Polydisperse
Modifications: None

Beam Length: 2.40 mm
[Particle R.I. = (1.4790, 0.1000); Dispersant R.I. = 1.3300]

Sampler: MS1

Obscuration: 4.9 %
Residual: 1.959 %

Result Statistics

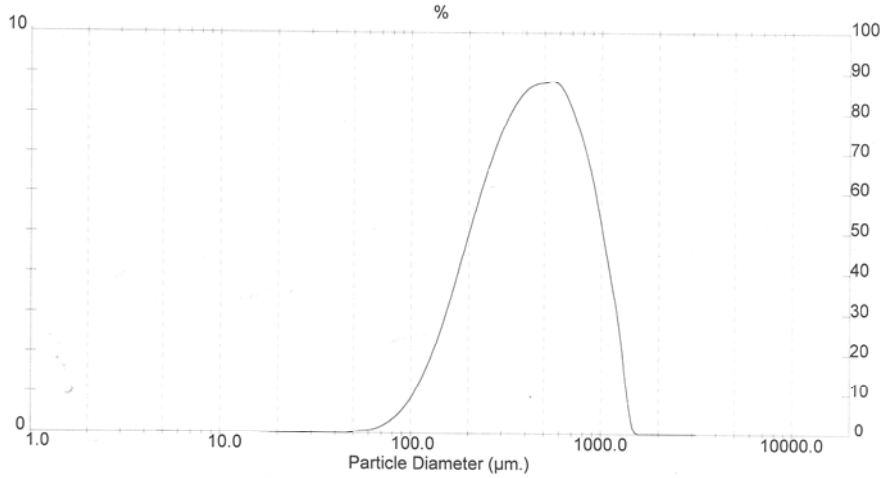
Distribution Type: Volume
Mean Diameters:
D [4, 3] = 497.59 um

Concentration = 0.2367 %Vol
D (v, 0.1) = 178.87 um
D [3, 2] = 340.57 um

Density = 1.000 g / cub. cm
D (v, 0.5) = 434.44 um
Span = 1.707E+00

Specific S.A. = 0.0176 sq. m / g
D (v, 0.9) = 920.64 um
Uniformity = 5.254E-01

Size Low (um)	In %	Size High (um)	Under%	Size Low (um)	In %	Size High (um)	Under%
4.19	0.00	4.88	0.00	120.67	2.03	140.58	4.85
4.88	0.00	5.69	0.00	140.58	2.96	163.77	7.81
5.69	0.00	6.63	0.00	163.77	4.03	190.80	11.83
6.63	0.00	7.72	0.00	190.80	5.14	222.28	16.98
7.72	0.00	9.00	0.00	222.28	6.21	258.95	23.19
9.00	0.00	10.48	0.00	258.95	7.15	301.68	30.34
10.48	0.00	12.21	0.00	301.68	7.90	351.46	38.24
12.21	0.00	14.22	0.00	351.46	8.41	409.45	46.65
14.22	0.00	16.57	0.00	409.45	8.68	477.01	55.33
16.57	0.00	19.31	0.00	477.01	8.74	555.71	64.06
19.31	0.00	22.49	0.00	555.71	8.70	647.41	72.76
22.49	0.00	26.20	0.00	647.41	8.12	754.23	80.88
26.20	0.00	30.53	0.00	754.23	7.18	878.67	88.06
30.53	0.00	35.56	0.00	878.67	5.80	1023.66	93.86
35.56	0.00	41.43	0.00	1023.66	4.04	1192.56	97.90
41.43	0.00	48.27	0.00	1192.56	2.05	1389.33	99.94
48.27	0.05	56.23	0.05	1389.33	0.06	1618.57	100.00
56.23	0.09	65.51	0.14	1618.57	0.00	1885.64	100.00
65.51	0.20	76.32	0.34	1885.64	0.00	2196.77	100.00
76.32	0.41	88.91	0.76	2196.77	0.00	2559.23	100.00
88.91	0.77	103.58	1.53	2559.23	0.00	2981.51	100.00
103.58	1.30	120.67	2.82	2981.51	0.00	3473.45	100.00



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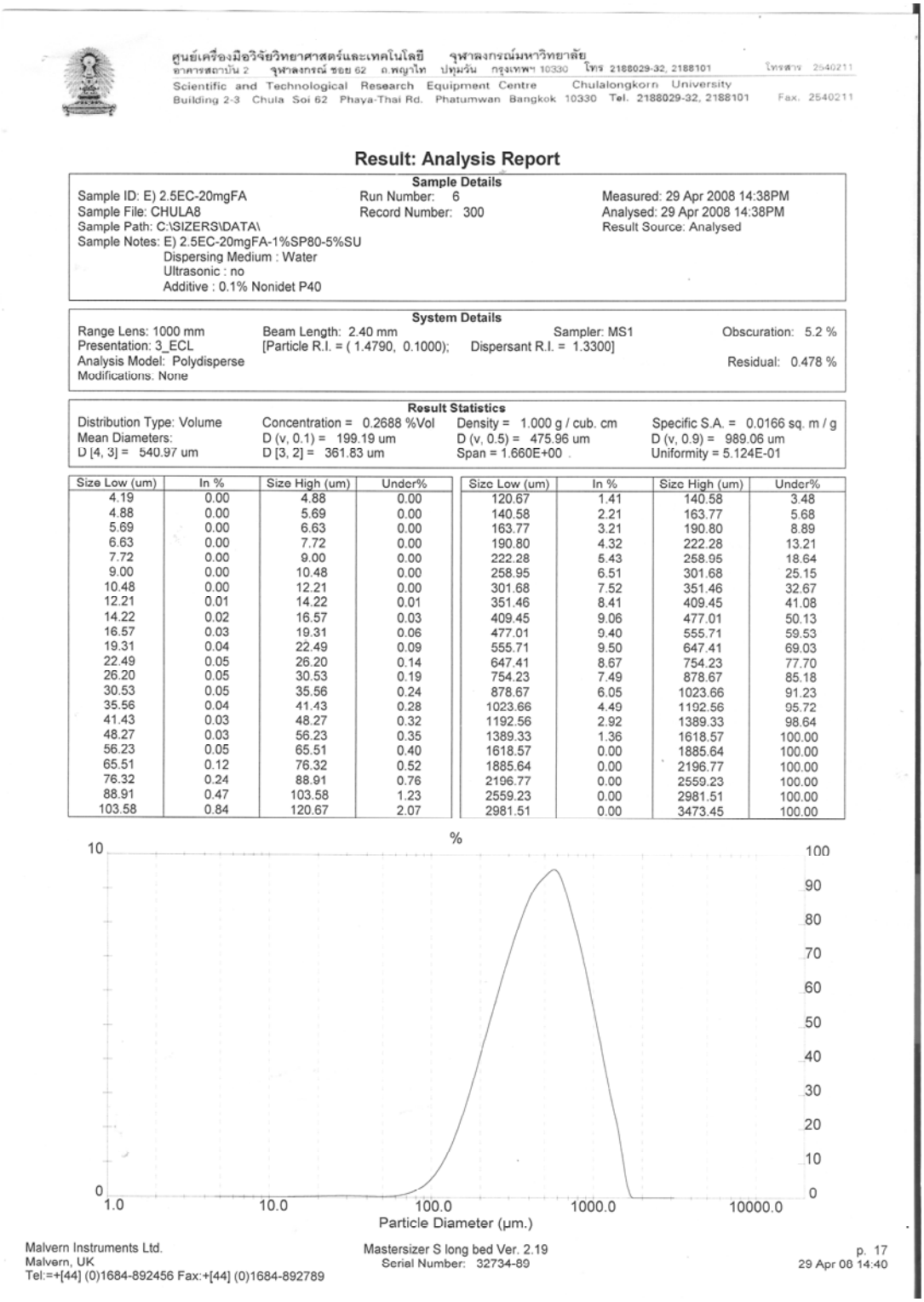
p. 22
29 Apr 08 15:00

Figure E18 Particle size distribution data of Formulation F6.



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Result: Analysis Report

Sample Details

Sample ID: E) 2.5EC-20mgFA
Sample File: CHULA8
Sample Path: C:\SIZERS\DATA\
Sample Notes: E) 2.5EC-20mgFA-1%SP80-5%SU
Dispersing Medium : Water
Ultrasonic : no
Additive : 0.1% Nonidet P40

Run Number: 6
Record Number: 300

Measured: 29 Apr 2008 14:38PM
Analysed: 29 Apr 2008 14:38PM
Result Source: Analysed

System Details

Range Lens: 1000 mm
Presentation: 3_ECL
Analysis Model: Polydisperse
Modifications. None

Beam Length: 2.40 mm
[Particle R.I. = (1.4790, 0.1000); Dispersant R.I. = 1.3300]

Sampler: MS1

Obscuration: 5.2 %
Residual: 0.478 %

Result Statistics

Distribution Type: Volume
Mean Diameters:
D [4, 3] = 540.97 um

Concentration = 0.2688 %Vol
D (v, 0.1) = 199.19 um
D [3, 2] = 361.83 um

Density = 1.000 g / cub. cm
D (v, 0.5) = 475.96 um
Span = 1.660E+00

Specific S.A. = 0.0166 sq. m / g
D (v, 0.9) = 989.06 um
Uniformity = 5.124E-01

Size Low (um)	In %	Size High (um)	Under%	Size Low (um)	In %	Size High (um)	Under%
4.19	0.00	4.88	0.00	120.67	1.41	140.58	3.48
4.88	0.00	5.69	0.00	140.58	2.21	163.77	5.68
5.69	0.00	6.63	0.00	163.77	3.21	190.80	8.89
6.63	0.00	7.72	0.00	190.80	4.32	222.28	13.21
7.72	0.00	9.00	0.00	222.28	5.43	258.95	18.64
9.00	0.00	10.48	0.00	258.95	6.51	301.68	25.15
10.48	0.00	12.21	0.00	301.68	7.52	351.46	32.67
12.21	0.01	14.22	0.01	351.46	8.41	409.45	41.08
14.22	0.02	16.57	0.03	409.45	9.06	477.01	50.13
16.57	0.03	19.31	0.06	477.01	9.40	555.71	59.53
19.31	0.04	22.49	0.09	555.71	9.50	647.41	69.03
22.49	0.05	26.20	0.14	647.41	8.67	754.23	77.70
26.20	0.05	30.53	0.19	754.23	7.49	878.67	85.18
30.53	0.05	35.56	0.24	878.67	6.05	1023.66	91.23
35.56	0.04	41.43	0.28	1023.66	4.49	1192.56	95.72
41.43	0.03	48.27	0.32	1192.56	2.92	1389.33	98.64
48.27	0.03	56.23	0.35	1389.33	1.36	1618.57	100.00
56.23	0.05	65.51	0.40	1618.57	0.00	1885.64	100.00
65.51	0.12	76.32	0.52	1885.64	0.00	2196.77	100.00
76.32	0.24	88.91	0.76	2196.77	0.00	2559.23	100.00
88.91	0.47	103.58	1.23	2559.23	0.00	2981.51	100.00
103.58	0.84	120.67	2.07	2981.51	0.00	3473.45	100.00

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p. 17
29 Apr 08 14:40

Figure E19 Particle size distribution data of Formulation F7.

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Result: Analysis Report

Sample Details

Sample ID: E) 2.5EC-20mgFA
Sample File: CHULA8
Sample Path: C:\SIZERS\DATA\
Sample Notes: E) 2.5EC-20mgFA-1%SP80-5%SU
Dispersing Medium : Water
Ultrasonic : no
Additive : 0.1% Nonidet P40

Run Number: 10
Record Number: 304

Measured: 29 Apr 2008 14:39PM
Analysed: 29 Apr 2008 14:39PM
Result Source: Analysed

System Details

Range Lens: 1000 mm
Presentation: 3_ECL
Analysis Model: Polydisperse
Modifications: None

Beam Length: 2.40 mm
[Particle R.I. = (1.4790, 0.1000); Dispersant R.I. = 1.3300]

Sampler: MS1

Obscuration: 5.3 %
Residual: 0.336 %

Result Statistics

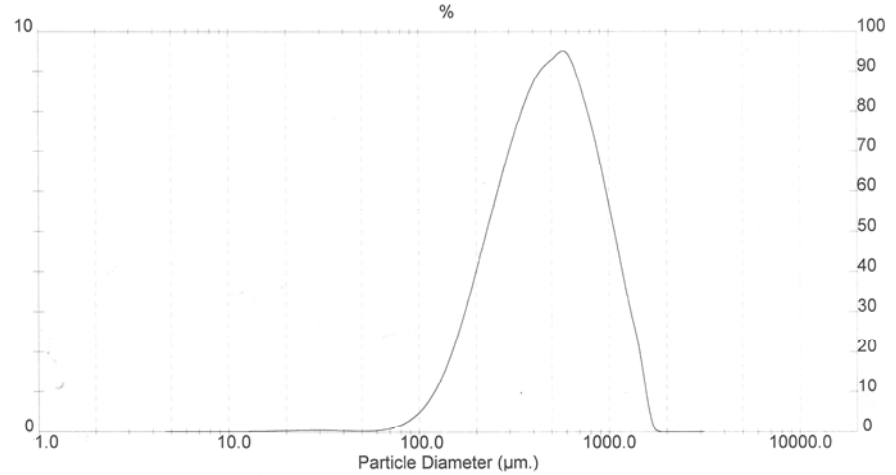
Distribution Type: Volume
Mean Diameters:
D [4, 3] = 550.00 um

Concentration = 0.2777 %Vol
D (v, 0.1) = 203.92 um
D [3, 2] = 368.02 um

Density = 1.000 g / cub. cm
D (v, 0.5) = 482.16 um
Span = 1.665E+00

Specific S.A. = 0.0163 sq. m / g
D (v, 0.9) = 1006.48 um
Uniformity = 5.143E-01

Size Low (um)	In %	Size High (um)	Under%	Size Low (um)	In %	Size High (um)	Under%
4.19	0.00	4.88	0.00	120.67	1.29	140.58	3.15
4.88	0.00	5.69	0.00	140.58	2.07	163.77	5.22
5.69	0.00	6.63	0.00	163.77	3.08	190.80	8.30
6.63	0.00	7.72	0.00	190.80	4.22	222.28	12.52
7.72	0.00	9.00	0.00	222.28	5.37	258.95	17.89
9.00	0.00	10.48	0.00	258.95	6.49	301.68	24.38
10.48	0.00	12.21	0.00	301.68	7.55	351.46	31.93
12.21	0.02	14.22	0.02	351.46	8.42	409.45	40.35
14.22	0.02	16.57	0.04	409.45	9.01	477.01	49.35
16.57	0.03	19.31	0.06	477.01	9.30	555.71	58.66
19.31	0.04	22.49	0.10	555.71	9.44	647.41	68.10
22.49	0.05	26.20	0.15	647.41	8.72	754.23	76.82
26.20	0.05	30.53	0.20	754.23	7.61	878.67	84.43
30.53	0.05	35.56	0.25	878.67	6.19	1023.66	90.62
35.56	0.04	41.43	0.29	1023.66	4.63	1192.56	95.25
41.43	0.03	48.27	0.32	1192.56	3.11	1389.33	98.36
48.27	0.03	56.23	0.35	1389.33	1.58	1618.57	99.94
56.23	0.04	65.51	0.39	1618.57	0.06	1885.64	100.00
65.51	0.10	76.32	0.49	1885.64	0.00	2196.77	100.00
76.32	0.21	88.91	0.70	2196.77	0.00	2559.23	100.00
88.91	0.41	103.58	1.11	2559.23	0.00	2981.51	100.00
103.58	0.75	120.67	1.86	2981.51	0.00	3473.45	100.00



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p. 18
29 Apr 08 14:40

Figure E20 Particle size distribution data of Formulation F7.

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Result: Analysis Report

Sample Details

Sample ID: E) 2.5EC-20mgFA
Sample File: CHULA8
Sample Path: C:\SIZERS\DATA\
Sample Notes: E) 2.5EC-20mgFA-1%SP80-5%SU
Dispersing Medium : Water
Ultrasonic : no
Additive : 0.1% Nonidet P40

Run Number: 14
Record Number: 308

Measured: 29 Apr 2008 14:39PM
Analysed: 29 Apr 2008 14:39PM
Result Source: Analysed

System Details

Range Lens: 1000 mm
Presentation: 3_ECL
Analysis Model: Polydisperse
Modifications: None

Beam Length: 2.40 mm
[Particle R.I. = (1.4790, 0.1000); Dispersant R.I. = 1.3300]

Sampler: MS1

Obscuration: 5.6 %
Residual: 0.533 %

Result Statistics

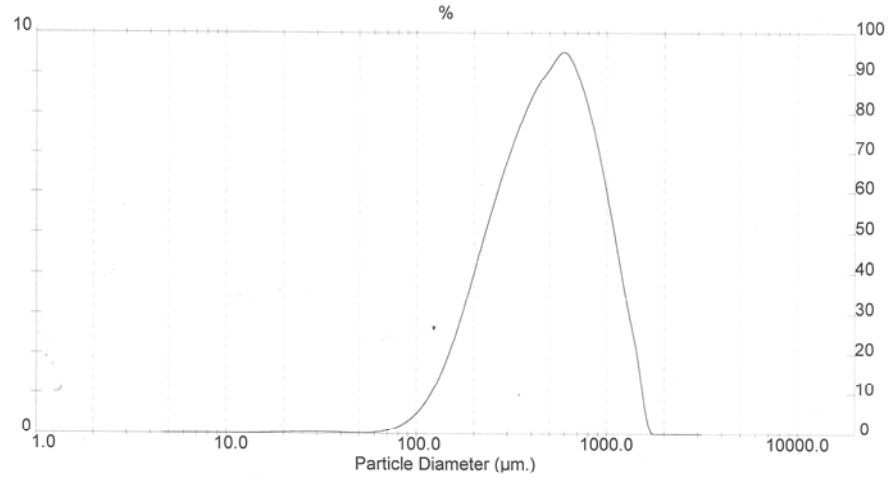
Distribution Type: Volume
Mean Diameters:
D [4, 3] = 555.05 um

Concentration = 0.2955 %Vol
D (v, 0.1) = 202.84 um
D [3, 2] = 372.04 um

Density = 1.000 g / cub. cm
D (v, 0.5) = 491.21 um
Span = 1.646E+00

Specific S.A. = 0.0161 sq. m / g
D (v, 0.9) = 1011.42 um
Uniformity = 5.099E-01

Size Low (um)	In %	Size High (um)	Under%	Size Low (um)	In %	Size High (um)	Under%
4.19	0.00	4.88	0.00	120.67	1.34	140.58	3.21
4.88	0.00	5.69	0.00	140.58	2.13	163.77	5.34
5.69	0.00	6.63	0.00	163.77	3.11	190.80	8.45
6.63	0.00	7.72	0.00	190.80	4.19	222.28	12.64
7.72	0.00	9.00	0.00	222.28	5.28	258.95	17.92
9.00	0.00	10.48	0.00	258.95	6.31	301.68	24.23
10.48	0.00	12.21	0.00	301.68	7.26	351.46	31.49
12.21	0.01	14.22	0.01	351.46	8.08	409.45	39.57
14.22	0.02	16.57	0.03	409.45	8.71	477.01	48.28
16.57	0.02	19.31	0.05	477.01	9.15	555.71	57.43
19.31	0.03	22.49	0.09	555.71	9.48	647.41	66.91
22.49	0.04	26.20	0.13	647.41	8.99	754.23	75.90
26.20	0.04	30.53	0.17	754.23	8.00	878.67	83.90
30.53	0.04	35.56	0.21	878.67	6.56	1023.66	90.46
35.56	0.03	41.43	0.25	1023.66	4.87	1192.56	95.34
41.43	0.03	48.27	0.27	1192.56	3.18	1389.33	98.51
48.27	0.02	56.23	0.30	1389.33	1.49	1618.57	100.00
56.23	0.04	65.51	0.34	1618.57	0.00	1885.64	100.00
65.51	0.10	76.32	0.43	1885.64	0.00	2196.77	100.00
76.32	0.21	88.91	0.65	2196.77	0.00	2559.23	100.00
88.91	0.43	103.58	1.08	2559.23	0.00	2981.51	100.00
103.58	0.79	120.67	1.86	2981.51	0.00	3473.45	100.00



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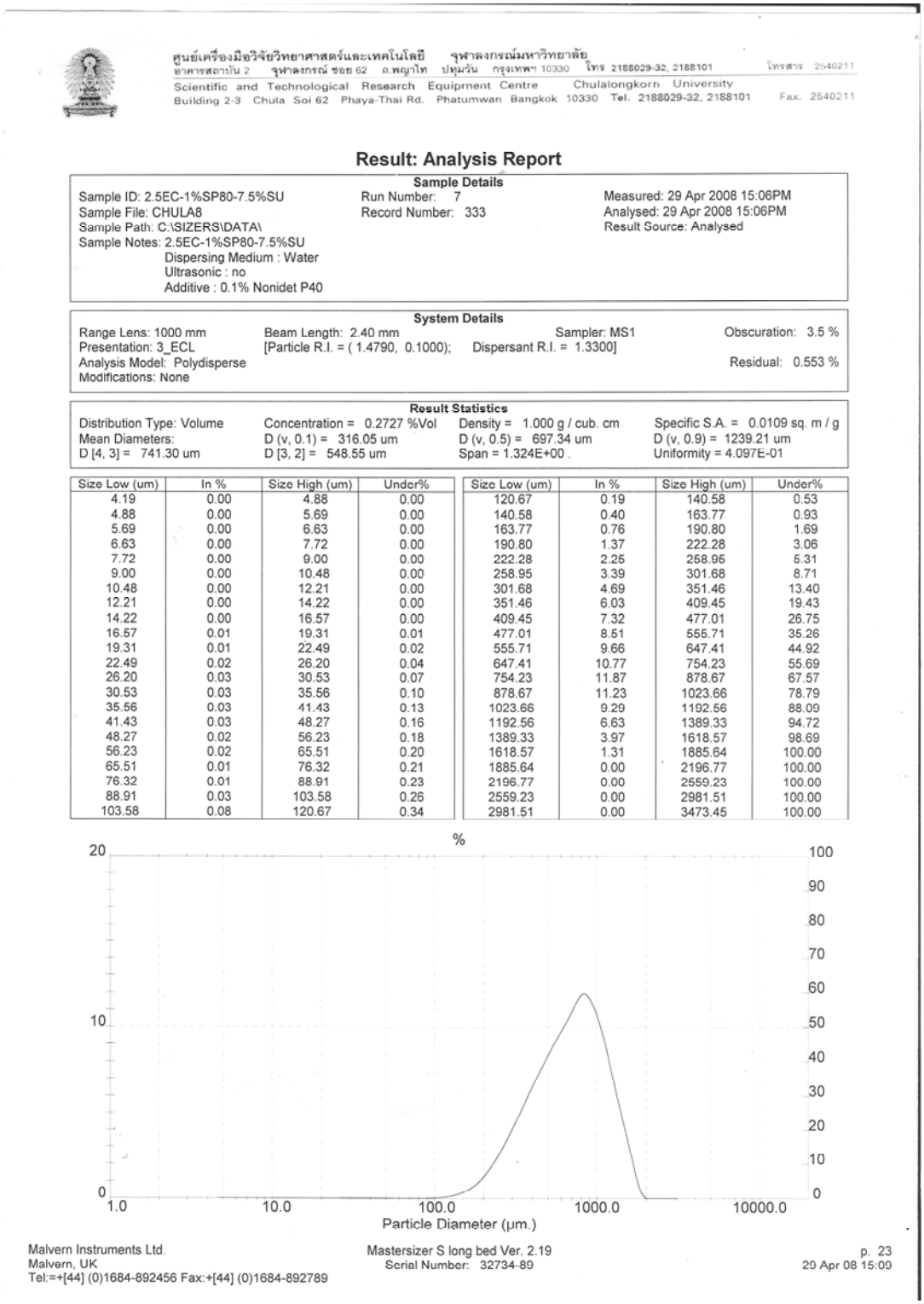
p. 19
29 Apr 08 14:40

Figure E21 Particle size distribution data of Formulation F7.



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Building 2-3 Chula Soi 62 Phaya-Thai Rd. Phatumwan Bangkok 10330 Tel. 2188029-32, 2188101 Fax. 2540211

Result: Analysis Report

Sample Details

Sample ID: 2.5EC-1%SP80-7.5%SU
Sample File: CHULA8
Sample Path: C:\SIZERS\DATA\
Sample Notes: 2.5EC-1%SP80-7.5%SU
Dispersing Medium : Water
Ultrasonic : no
Additive : 0.1% Nonidet P40

Run Number: 7
Record Number: 333

Measured: 29 Apr 2008 15:06PM
Analysed: 29 Apr 2008 15:06PM
Result Source: Analysed

System Details

Range Lens: 1000 mm
Presentation: 3_ECL
Analysis Model: Polydisperse
Modifications: None

Beam Length: 2.40 mm
[Particle R.I. = (1.4790, 0.1000); Dispersant R.I. = 1.3300]

Sampler: MS1

Obscuration: 3.5 %
Residual: 0.553 %

Result Statistics

Distribution Type: Volume
Mean Diameters:
D [4, 3] = 741.30 um

Concentration = 0.2727 %Vol
D (v, 0.1) = 316.05 um
D [3, 2] = 548.55 um

Density = 1.000 g / cub. cm
D (v, 0.5) = 697.34 um
Span = 1.324E+00

Specific S.A. = 0.0109 sq. m / g
D (v, 0.9) = 1239.21 um
Uniformity = 4.097E-01

Size Low (um)	In %	Size High (um)	Under%	Size Low (um)	In %	Size High (um)	Under%
4.19	0.00	4.88	0.00	120.67	0.19	140.58	0.53
4.88	0.00	5.69	0.00	140.58	0.40	163.77	0.93
5.69	0.00	6.63	0.00	163.77	0.76	190.80	1.69
6.63	0.00	7.72	0.00	190.80	1.37	222.28	3.06
7.72	0.00	9.00	0.00	222.28	2.25	258.95	5.31
9.00	0.00	10.48	0.00	258.95	3.39	301.68	8.71
10.48	0.00	12.21	0.00	301.68	4.69	351.46	13.40
12.21	0.00	14.22	0.00	351.46	6.03	409.45	19.43
14.22	0.00	16.57	0.00	409.45	7.32	477.01	26.75
16.57	0.01	19.31	0.01	477.01	8.51	555.71	35.26
19.31	0.01	22.49	0.02	555.71	9.66	647.41	44.92
22.49	0.02	26.20	0.04	647.41	10.77	754.23	55.69
26.20	0.03	30.53	0.07	754.23	11.87	878.67	67.57
30.53	0.03	35.56	0.10	878.67	11.23	1023.66	78.79
35.56	0.03	41.43	0.13	1023.66	9.29	1192.56	88.09
41.43	0.03	48.27	0.16	1192.56	6.63	1389.33	94.72
48.27	0.02	56.23	0.18	1389.33	3.97	1618.57	98.69
56.23	0.02	65.51	0.20	1618.57	1.31	1885.64	100.00
65.51	0.01	76.32	0.21	1885.64	0.00	2196.77	100.00
76.32	0.01	88.91	0.23	2196.77	0.00	2559.23	100.00
88.91	0.03	103.58	0.26	2559.23	0.00	2981.51	100.00
103.58	0.08	120.67	0.34	2981.51	0.00	3473.45	100.00

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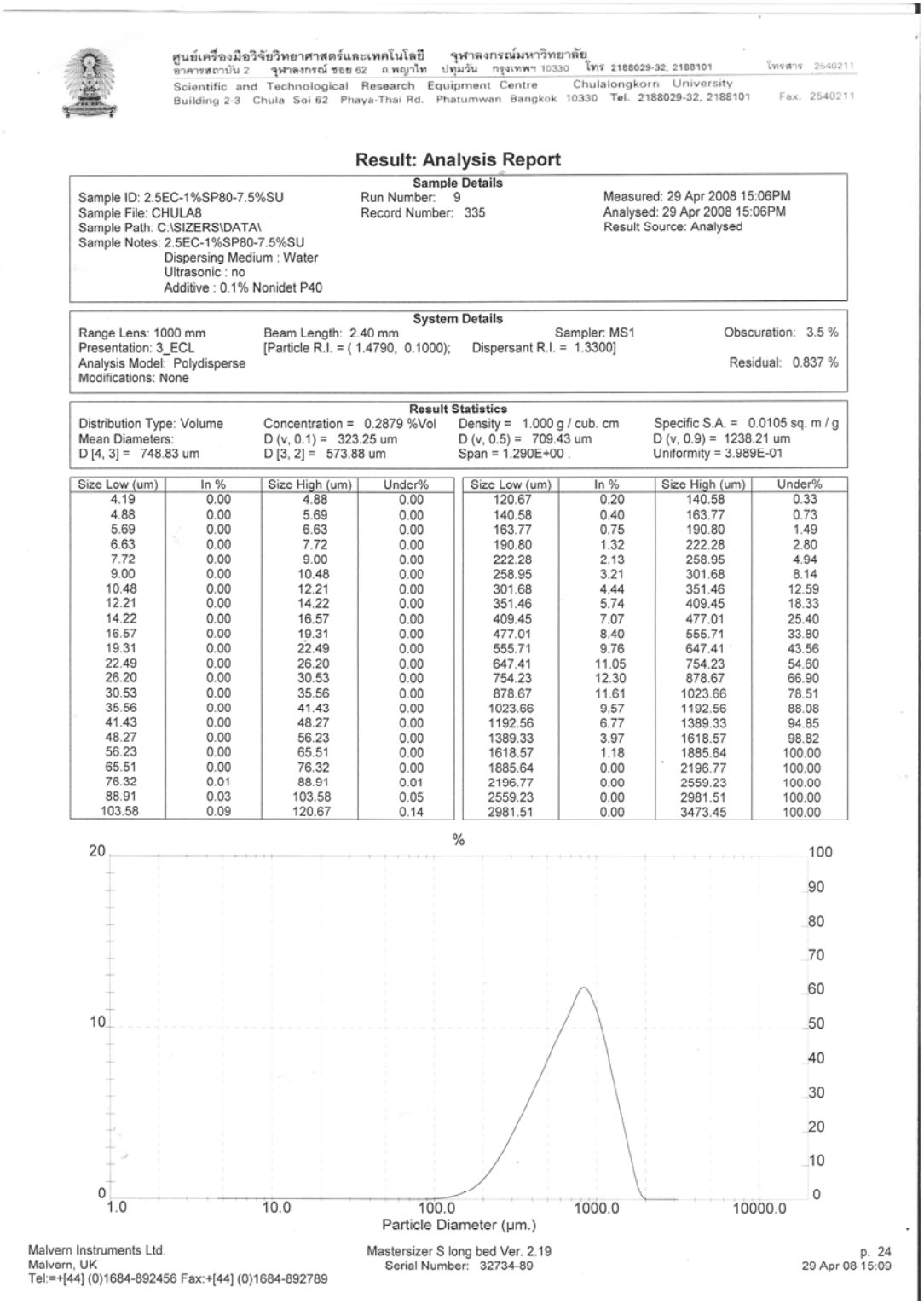
p. 23
29 Apr 08 15:09

Figure E22 Particle size distribution data of Formulation F8.



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Building 2-3 Chula Soi 62 Phaya-Thai Rd. Phatumwan Bangkok 10330 Tel. 2188029-32, 2188101 Fax. 2540211

Result: Analysis Report

Sample Details

Sample ID: 2.5EC-1%SP80-7.5%SU
Sample File: CHULA8
Sample Path: C:\SIZERS\DATA\
Sample Notes: 2.5EC-1%SP80-7.5%SU
Dispersing Medium : Water
Ultrasonic : no
Additive : 0.1% Nonidet P40

Run Number: 9
Record Number: 335

Measured: 29 Apr 2008 15:06PM
Analysed: 29 Apr 2008 15:06PM
Result Source: Analysed

System Details

Range Lens: 1000 mm
Presentation: 3_ECL
Analysis Model: Polydisperse
Modifications: None

Beam Length: 2.40 mm
[Particle R.I. = (1.4790, 0.1000); Dispersant R.I. = 1.3300]

Sampler: MS1

Obscuration: 3.5 %
Residual: 0.837 %

Result Statistics

Distribution Type: Volume
Mean Diameters:
D [4, 3] = 748.83 um

Concentration = 0.2879 %Vol
D (v, 0.1) = 323.25 um
D [3, 2] = 573.88 um

Density = 1.000 g / cub. cm
D (v, 0.5) = 709.43 um
Span = 1.290E+00

Specific S.A. = 0.0105 sq. m / g
D (v, 0.9) = 1238.21 um
Uniformity = 3.989E-01

Size Low (um)	In %	Size High (um)	Under%	Size Low (um)	In %	Size High (um)	Under%
4.19	0.00	4.88	0.00	120.67	0.20	140.58	0.33
4.88	0.00	5.69	0.00	140.58	0.40	163.77	0.73
5.69	0.00	6.63	0.00	163.77	0.75	190.80	1.49
6.63	0.00	7.72	0.00	190.80	1.32	222.28	2.80
7.72	0.00	9.00	0.00	222.28	2.13	258.95	4.94
9.00	0.00	10.48	0.00	258.95	3.21	301.68	8.14
10.48	0.00	12.21	0.00	301.68	4.44	351.46	12.59
12.21	0.00	14.22	0.00	351.46	5.74	409.45	18.33
14.22	0.00	16.57	0.00	409.45	7.07	477.01	25.40
16.57	0.00	19.31	0.00	477.01	8.40	555.71	33.80
19.31	0.00	22.49	0.00	555.71	9.76	647.41	43.56
22.49	0.00	26.20	0.00	647.41	11.05	754.23	54.60
26.20	0.00	30.53	0.00	754.23	12.30	878.67	66.90
30.53	0.00	35.56	0.00	878.67	11.61	1023.66	78.51
35.56	0.00	41.43	0.00	1023.66	9.57	1192.56	88.08
41.43	0.00	48.27	0.00	1192.56	6.77	1389.33	94.85
48.27	0.00	56.23	0.00	1389.33	3.97	1618.57	98.82
56.23	0.00	65.51	0.00	1618.57	1.18	1885.64	100.00
65.51	0.00	76.32	0.00	1885.64	0.00	2196.77	100.00
76.32	0.01	88.91	0.01	2196.77	0.00	2559.23	100.00
88.91	0.03	103.58	0.05	2559.23	0.00	2981.51	100.00
103.58	0.09	120.67	0.14	2981.51	0.00	3473.45	100.00

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p. 24
29 Apr 08 15:09

Figure E23 Particle size distribution data of Formulation F8.

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Scientific and Technological Research Equipment Centre Chulalongkorn University
Building 2-3 Chula Soi 62 Phaya-Thai Rd. Phatumwan Bangkok 10330 Tel. 2188029-32, 2188101 Fax. 2540211

Result: Analysis Report

Sample Details

Sample ID: 2.5EC-1%SP80-7.5%SU
Sample File: CHULA8
Sample Path: C:\SIZERS\DATA\
Sample Notes: 2.5EC-1%SP80-7.5%SU
Dispersing Medium : Water
Ultrasonic : no
Additive : 0.1% Nonidet P40

Run Number: 16
Record Number: 342

Measured: 29 Apr 2008 15:07PM
Analysed: 29 Apr 2008 15:08PM
Result Source: Analysed

System Details

Range Lens: 1000 mm
Presentation: 3_ECL
Analysis Model: Polydisperse
Modifications: None

Beam Length: 2.40 mm
[Particle R.I. = (1.4790, 0.1000);

Sampler: MS1
Dispersant R.I. = 1.3300]

Obscuration: 3.5 %
Residual: 0.562 %

Result Statistics

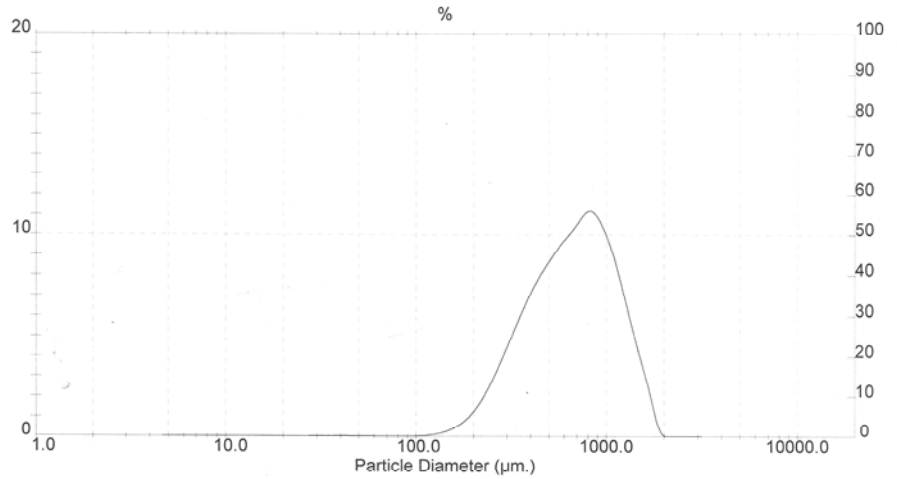
Distribution Type: Volume
Mean Diameters:
D [4, 3] = 730.50 um

Concentration = 0.2678 %Vol
D (v, 0.1) = 310.83 um
D [3, 2] = 540.65 um

Density = 1.000 g / cub. cm
D (v, 0.5) = 674.35 um
Span = 1.382E+00

Specific S.A. = 0.0111 sq. m / g
D (v, 0.9) = 1242.80 um
Uniformity = 4.311E-01

Size Low (um)	In %	Size High (um)	Under%	Size Low (um)	In %	Size High (um)	Under%
4.19	0.00	4.88	0.00	120.67	0.15	140.58	0.45
4.88	0.00	5.69	0.00	140.58	0.36	163.77	0.81
5.69	0.00	6.63	0.00	163.77	0.74	190.80	1.55
6.63	0.00	7.72	0.00	190.80	1.40	222.28	2.95
7.72	0.00	9.00	0.00	222.28	2.41	258.95	5.35
9.00	0.00	10.48	0.00	258.95	3.74	301.68	9.09
10.48	0.00	12.21	0.00	301.68	5.22	351.46	14.31
12.21	0.00	14.22	0.00	351.46	6.64	409.45	20.95
14.22	0.00	16.57	0.00	409.45	7.85	477.01	28.80
16.57	0.00	19.31	0.00	477.01	8.82	555.71	37.62
19.31	0.01	22.49	0.01	555.71	9.67	647.41	47.29
22.49	0.02	26.20	0.03	647.41	10.44	754.23	57.73
26.20	0.02	30.53	0.05	754.23	11.15	878.67	68.89
30.53	0.03	35.56	0.08	878.67	10.43	1023.66	79.32
35.56	0.03	41.43	0.11	1023.66	8.73	1192.56	88.05
41.43	0.03	48.27	0.15	1192.56	6.36	1389.33	94.40
48.27	0.03	56.23	0.17	1389.33	3.98	1618.57	98.39
56.23	0.02	65.51	0.19	1618.57	1.61	1885.64	100.00
65.51	0.01	76.32	0.21	1885.64	0.00	2196.77	100.00
76.32	0.01	88.91	0.22	2196.77	0.00	2559.23	100.00
88.91	0.02	103.58	0.24	2559.23	0.00	2981.51	100.00
103.58	0.06	120.67	0.30	2981.51	0.00	3473.45	100.00



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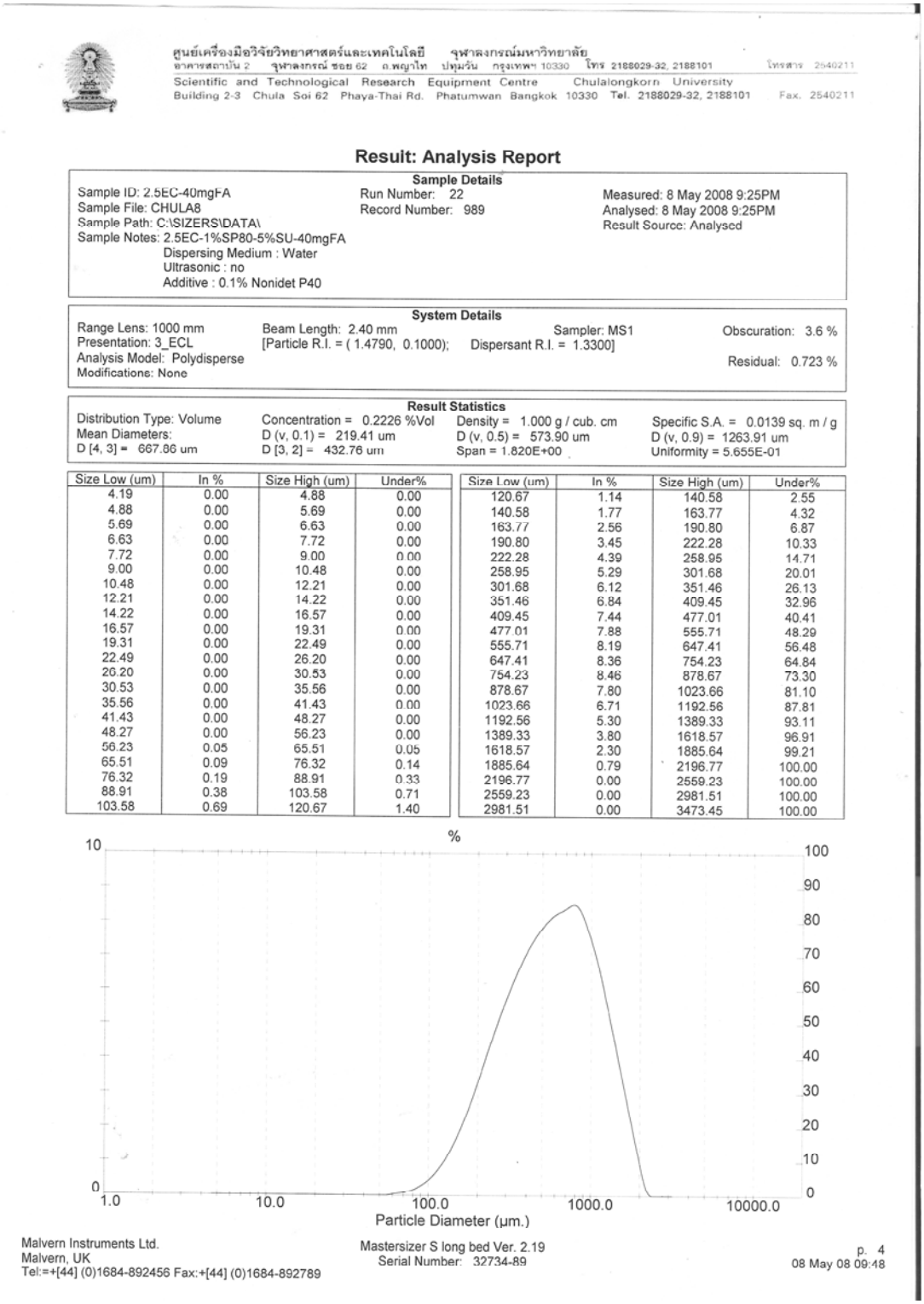
p. 25
29 Apr 08 15:09

Figure E24 Particle size distribution data of Formulation F8.



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Result: Analysis Report

Sample Details

Sample ID: 2.5EC-40mgFA
Sample File: CHULA8
Sample Path: C:\SIZERS\DATA\
Sample Notes: 2.5EC-1%SP80-5%SU-40mgFA
Dispersing Medium : Water
Ultrasonic : no
Additive : 0.1% Nonidet P40

Run Number: 22
Record Number: 989

Measured: 8 May 2008 9:25PM
Analysed: 8 May 2008 9:25PM
Result Source: Analysed

System Details

Range Lens: 1000 mm
Presentation: 3_ECL
Analysis Model: Polydisperse
Modifications: None

Beam Length: 2.40 mm
[Particle R.I. = (1.4790, 0.1000); Dispersant R.I. = 1.3300]

Sampler: MS1

Obscuration: 3.6 %
Residual: 0.723 %

Result Statistics

Distribution Type: Volume
Mean Diameters:
D [4, 3] = 667.86 um

Concentration = 0.2226 %Vol
D (v, 0.1) = 219.41 um
D [3, 2] = 432.76 um

Density = 1.000 g / cub. cm
D (v, 0.5) = 573.90 um
Span = 1.820E+00

Specific S.A. = 0.0139 sq. m / g
D (v, 0.9) = 1263.91 um
Uniformity = 5.655E-01

Size Low (um)	In %	Size High (um)	Under%	Size Low (um)	In %	Size High (um)	Under%
4.19	0.00	4.88	0.00	120.67	1.14	140.58	2.55
4.88	0.00	5.69	0.00	140.58	1.77	163.77	4.32
5.69	0.00	6.63	0.00	163.77	2.56	190.80	6.87
6.63	0.00	7.72	0.00	190.80	3.45	222.28	10.33
7.72	0.00	9.00	0.00	222.28	4.39	258.95	14.71
9.00	0.00	10.48	0.00	258.95	5.29	301.68	20.01
10.48	0.00	12.21	0.00	301.68	6.12	351.46	26.13
12.21	0.00	14.22	0.00	351.46	6.84	409.45	32.96
14.22	0.00	16.57	0.00	409.45	7.44	477.01	40.41
16.57	0.00	19.31	0.00	477.01	7.88	555.71	48.29
19.31	0.00	22.49	0.00	555.71	8.19	647.41	56.48
22.49	0.00	26.20	0.00	647.41	8.36	754.23	64.84
26.20	0.00	30.53	0.00	754.23	8.46	878.67	73.30
30.53	0.00	35.56	0.00	878.67	7.80	1023.66	81.10
35.56	0.00	41.43	0.00	1023.66	6.71	1192.56	87.81
41.43	0.00	48.27	0.00	1192.56	5.30	1389.33	93.11
48.27	0.00	56.23	0.00	1389.33	3.80	1618.57	96.91
56.23	0.05	65.51	0.05	1618.57	2.30	1885.64	99.21
65.51	0.09	76.32	0.14	1885.64	0.79	2196.77	100.00
76.32	0.19	88.91	0.33	2196.77	0.00	2559.23	100.00
88.91	0.38	103.58	0.71	2559.23	0.00	2981.51	100.00
103.58	0.69	120.67	1.40	2981.51	0.00	3473.45	100.00

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p. 4
08 May 08 09:48

Figure E25 Particle size distribution data of Formulation F9.

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Result: Analysis Report

Sample Details

Sample ID: 2.5EC-40mgFA
Sample File: CHULA8
Sample Path: C:\SIZERS\DATA\
Sample Notes: 2.5EC-1%SP80-5%SU-40mgFA
Dispersing Medium : Water
Ultrasonic : no
Additive : 0.1% Nonidet P40

Run Number: 25
Record Number: 992

Measured: 8 May 2008 9:26PM
Analysed: 8 May 2008 9:26PM
Result Source: Analysed

System Details

Range Lens: 1000 mm
Presentation: 3_ECL
Analysis Model: Polydisperse
Modifications: None

Beam Length: 2.40 mm
[Particle R.I. = (1.4790, 0.1000); Dispersant R.I. = 1.3300]

Sampler: MS1

Obscuration: 3.7 %
Residual: 0.828 %

Result Statistics

Distribution Type: Volume
Mean Diameters:
D [4, 3] = 648.66 um

Concentration = 0.2213 %Vol
D (v, 0.1) = 213.79 um
D [3, 2] = 420.80 um

Density = 1.000 g / cub. cm
D (v, 0.5) = 552.65 um
Span = 1.846E+00

Specific S.A. = 0.0143 sq. m / g
D (v, 0.9) = 1233.99 um
Uniformity = 5.758E-01

Size Low (um)	In %	Size High (um)	Under%	Size Low (um)	In %	Size High (um)	Under%
4.19	0.00	4.88	0.00	120.67	1.21	140.58	2.60
4.88	0.00	5.69	0.00	140.58	1.90	163.77	4.50
5.69	0.00	6.63	0.00	163.77	2.78	190.80	7.28
6.63	0.00	7.72	0.00	190.80	3.77	222.28	11.05
7.72	0.00	9.00	0.00	222.28	4.77	258.95	15.83
9.00	0.00	10.48	0.00	258.95	5.68	301.68	21.51
10.48	0.00	12.21	0.00	301.68	6.45	351.46	27.96
12.21	0.00	14.22	0.00	351.46	7.05	409.45	35.01
14.22	0.00	16.57	0.00	409.45	7.48	477.01	42.49
16.57	0.00	19.31	0.00	477.01	7.79	555.71	50.29
19.31	0.00	22.49	0.00	555.71	8.04	647.41	58.32
22.49	0.00	26.20	0.00	647.41	8.27	754.23	66.59
26.20	0.00	30.53	0.00	754.23	8.09	878.67	74.68
30.53	0.00	35.56	0.00	878.67	7.53	1023.66	82.21
35.56	0.00	41.43	0.00	1023.66	6.52	1192.56	88.73
41.43	0.00	48.27	0.00	1192.56	5.09	1389.33	93.82
48.27	0.00	56.23	0.00	1389.33	3.50	1618.57	97.33
56.23	0.04	65.51	0.04	1618.57	2.06	1885.64	99.38
65.51	0.08	76.32	0.12	1885.64	0.62	2196.77	100.00
76.32	0.18	88.91	0.30	2196.77	0.00	2559.23	100.00
88.91	0.38	103.58	0.68	2559.23	0.00	2981.51	100.00
103.58	0.71	120.67	1.39	2981.51	0.00	3473.45	100.00

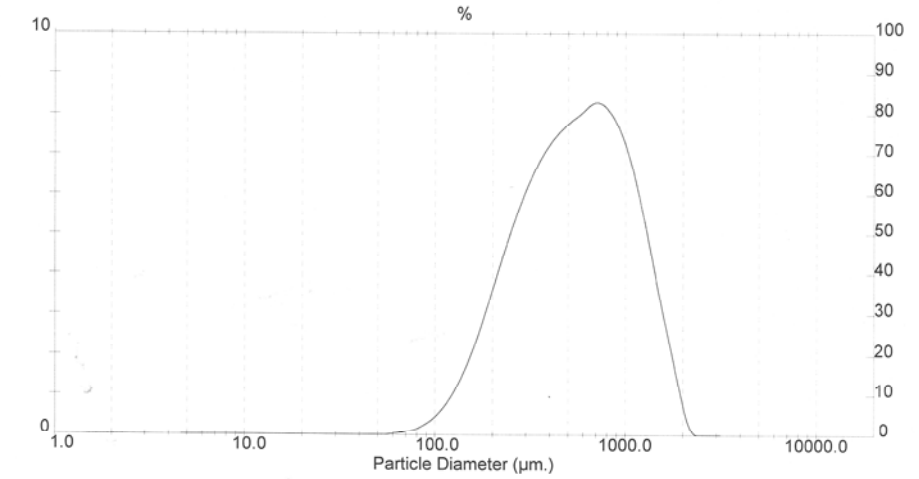

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Figure E26 Particle size distribution data of Formulation F9.

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Result: Analysis Report

Sample Details

Sample ID: 2.5EC-40mgFA
Sample File: CHULA8
Sample Path: C:\SIZERS\DATA\
Sample Notes: 2.5EC-1%SP80-5%SU-40mgFA
Dispersing Medium : Water
Ultrasonic : no
Additive : 0.1% Nonidet P40

Run Number: 26
Record Number: 993

Measured: 8 May 2008 9:26PM
Analysed: 8 May 2008 9:26PM
Result Source: Analysed

System Details

Range Lens: 1000 mm
Presentation: 3_ECL
Analysis Model: Polydisperse
Modifications: None

Beam Length: 2.40 mm
[Particle R.I. = (1.4790, 0.1000); Dispersant R.I. = 1.3300]

Sampler: MS1

Obscuration: 3.6 %
Residual: 0.649 %

Result Statistics

Distribution Type: Volume
Mean Diameters:
D [4, 3] = 631.47 um

Concentration = 0.2088 %Vol
D (v, 0.1) = 217.74 um
D [3, 2] = 406.10 um

Density = 1.000 g / cub. cm
D (v, 0.5) = 543.85 um
Span = 1.780E+00

Specific S.A. = 0.0148 sq. m / g
D (v, 0.9) = 1185.80 um
Uniformity = 5.533E-01

Size Low (um)	In %	Size High (um)	Under%	Size Low (um)	In %	Size High (um)	Under%
4.19	0.00	4.88	0.00	120.67	1.07	140.58	2.56
4.88	0.00	5.69	0.00	140.58	1.74	163.77	4.30
5.69	0.00	6.63	0.00	163.77	2.61	190.80	6.92
6.63	0.00	7.72	0.00	190.80	3.64	222.28	10.55
7.72	0.00	9.00	0.00	222.28	4.72	258.95	15.27
9.00	0.00	10.48	0.00	258.95	5.77	301.68	21.04
10.48	0.00	12.21	0.00	301.68	6.67	351.46	27.71
12.21	0.00	14.22	0.00	351.46	7.37	409.45	35.08
14.22	0.01	16.57	0.01	409.45	7.88	477.01	42.96
16.57	0.02	19.31	0.03	477.01	8.21	555.71	51.17
19.31	0.03	22.49	0.06	555.71	8.45	647.41	59.62
22.49	0.04	26.20	0.10	647.41	8.65	754.23	68.28
26.20	0.05	30.53	0.15	754.23	8.27	878.67	76.55
30.53	0.05	35.56	0.21	878.67	7.45	1023.66	84.00
35.56	0.05	41.43	0.26	1023.66	6.21	1192.56	90.21
41.43	0.04	48.27	0.30	1192.56	4.70	1389.33	94.91
48.27	0.03	56.23	0.33	1389.33	3.20	1618.57	98.11
56.23	0.03	65.51	0.36	1618.57	1.70	1885.64	99.81
65.51	0.06	76.32	0.42	1885.64	0.19	2196.77	100.00
76.32	0.14	88.91	0.56	2196.77	0.00	2559.23	100.00
88.91	0.31	103.58	0.88	2559.23	0.00	2981.51	100.00
103.58	0.61	120.67	1.49	2981.51	0.00	3473.45	100.00

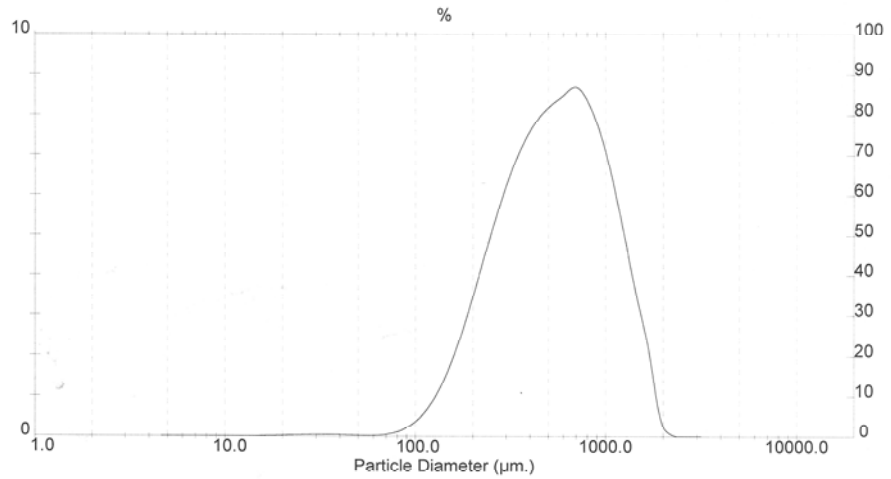

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Figure E27 Particle size distribution data of Formulation F9.

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Result: Analysis Report

Sample Details

Sample ID: 2.5EC-60mgFA
Sample File: CHULA8
Sample Path: C:\SIZERS\DATA\
Sample Notes: 2.5EC-1%SP80-5%SU-60mgFA
Dispersing Medium : Water
Ultrasonic : no
Additive : 0.1% Nonidet P40

Run Number: 6
Record Number: 1002

Measured: 8 May 2008 10:15PM
Analysed: 8 May 2008 10:15PM
Result Source: Analysed

System Details

Range Lens: 1000 mm
Presentation: 3_ECL
Analysis Model: Polydisperse
Modifications: None

Beam Length: 2.40 mm
[Particle R.I. = (1.4790, 0.1000); Dispersant R.I. = 1.3300]

Sampler: MS1

Obscuration: 4.5 %
Residual: 1.289 %

Result Statistics

Distribution Type: Volume
Mean Diameters:
D [4, 3] = 776.85 um

Concentration = 0.3236 %Vol
D (v, 0.1) = 326.58 um
D [3, 2] = 507.46 um

Density = 1.000 g / cub. cm
D (v, 0.5) = 742.45 um
Span = 1.292E+00

Specific S.A. = 0.0118 sq. m / g
D (v, 0.9) = 1285.54 um
Uniformity = 4.014E-01

Size Low (um)	In %	Size High (um)	Under%	Size Low (um)	In %	Size High (um)	Under%
4.19	0.01	4.88	0.01	120.67	0.38	140.58	1.70
4.88	0.01	5.69	0.02	140.58	0.54	163.77	2.24
5.69	0.01	6.63	0.03	163.77	0.78	190.80	3.02
6.63	0.01	7.72	0.04	190.80	1.15	222.28	4.18
7.72	0.01	9.00	0.05	222.28	1.69	258.95	5.87
9.00	0.01	10.48	0.06	258.95	2.46	301.68	8.33
10.48	0.01	12.21	0.07	301.68	3.49	351.46	11.82
12.21	0.01	14.22	0.09	351.46	4.77	409.45	16.59
14.22	0.02	16.57	0.10	409.45	6.26	477.01	22.85
16.57	0.02	19.31	0.13	477.01	7.85	555.71	30.70
19.31	0.03	22.49	0.16	555.71	9.48	647.41	40.18
22.49	0.04	26.20	0.19	647.41	11.03	754.23	51.21
26.20	0.04	30.53	0.23	754.23	12.46	878.67	63.67
30.53	0.05	35.56	0.28	878.67	11.98	1023.66	75.65
35.56	0.05	41.43	0.34	1023.66	10.25	1192.56	85.90
41.43	0.06	48.27	0.40	1192.56	7.58	1389.33	93.48
48.27	0.07	56.23	0.47	1389.33	4.58	1618.57	98.06
56.23	0.09	65.51	0.56	1618.57	1.94	1885.64	100.00
65.51	0.12	76.32	0.68	1885.64	0.00	2196.77	100.00
76.32	0.15	88.91	0.83	2196.77	0.00	2559.23	100.00
88.91	0.21	103.58	1.04	2559.23	0.00	2981.51	100.00
103.58	0.28	120.67	1.31	2981.51	0.00	3473.45	100.00

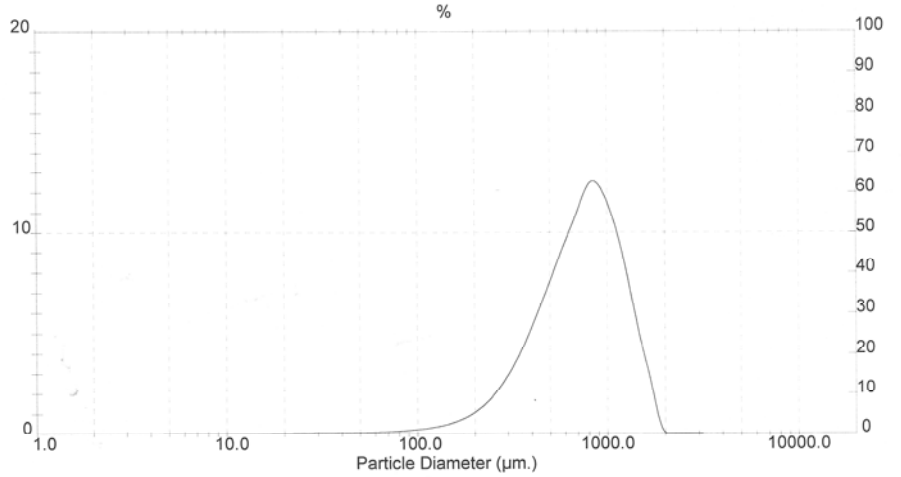


Figure E28 Particle size distribution data of Formulation F10.

ศูนย์เครื่องมือวิจัยวิทยาศาสตร์และเทคโนโลยี จุฬาลงกรณ์มหาวิทยาลัย
อาคารสถาบัน 2 จุฬาลงกรณ์ ซอย 62 ถ.พญาไท ปทุมวัน กรุงเทพฯ 10330 โทร 2188029-32, 2188101 โทรสาร 2540211
Scientific and Technological Research Equipment Centre Chulalongkorn University
Building 2-3 Chula Soi 62 Phaya-Thai Rd. Phatumwan Bangkok 10330 Tel. 2188029-32, 2188101 Fax. 2540211

Result: Analysis Report

Sample Details

Sample ID: 2.5EC-60mgFA
Sample File: CHULA8
Sample Path: C:\SIZERS\DATA\
Sample Notes: 2.5EC-1%SP80-5%SU-60mgFA
Dispersing Medium : Water
Ultrasonic : no
Additive : 0.1% Nonidet P40

Run Number: 12
Record Number: 1008

Measured: 8 May 2008 10:16PM
Analysed: 8 May 2008 10:16PM
Result Source: Analysed

System Details

Range Lens: 1000 mm
Presentation: 3_ECL
Analysis Model: Polydisperse
Modifications: None

Beam Length: 2.40 mm
[Particle R.I. = (1.4790, 0.1000); Dispersant R.I. = 1.3300]

Sampler: MS1

Obscuration: 4.6 %
Residual: 1.205 %

Result Statistics

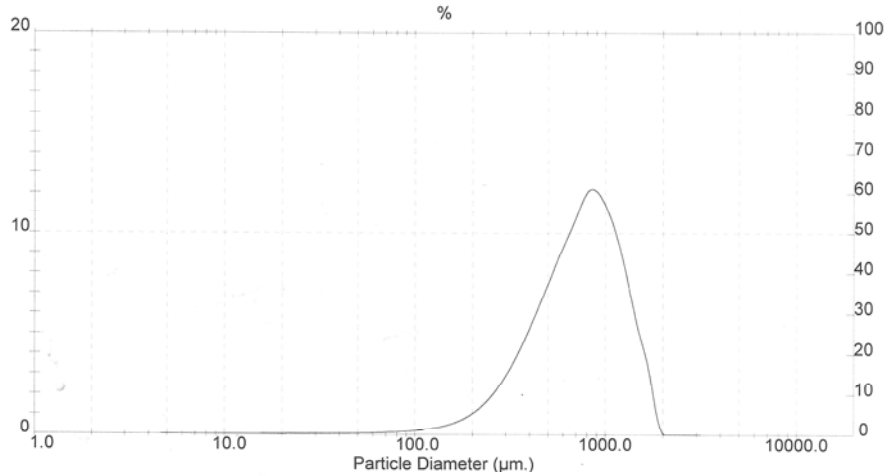
Distribution Type: Volume
Mean Diameters:
D [4, 3] = 789.56 um

Concentration = 0.3267 %Vol
D (v, 0.1) = 328.61 um
D [3, 2] = 504.46 um

Density = 1.000 g / cub. cm
D (v, 0.5) = 751.17 um
Span = 1.317E+00

Specific S.A. = 0.0119 sq. m / g
D (v, 0.9) = 1318.12 um
Uniformity = 4.090E-01

Size Low (um)	In %	Size High (um)	Under%	Size Low (um)	In %	Size High (um)	Under%
4.19	0.01	4.88	0.01	120.67	0.35	140.58	1.67
4.88	0.01	5.69	0.02	140.58	0.51	163.77	2.18
5.69	0.01	6.63	0.03	163.77	0.75	190.80	2.93
6.63	0.01	7.72	0.04	190.80	1.13	222.28	4.06
7.72	0.01	9.00	0.06	222.28	1.68	258.95	5.74
9.00	0.02	10.48	0.07	258.95	2.45	301.68	8.19
10.48	0.02	12.21	0.09	301.68	3.48	351.46	11.67
12.21	0.02	14.22	0.11	351.46	4.76	409.45	16.43
14.22	0.02	16.57	0.13	409.45	6.21	477.01	22.64
16.57	0.03	19.31	0.15	477.01	7.73	555.71	30.36
19.31	0.03	22.49	0.19	555.71	9.25	647.41	39.61
22.49	0.04	26.20	0.22	647.41	10.69	754.23	50.30
26.20	0.04	30.53	0.27	754.23	12.07	878.67	62.37
30.53	0.05	35.56	0.32	878.67	11.80	1023.66	74.17
35.56	0.06	41.43	0.37	1023.66	10.32	1192.56	84.49
41.43	0.06	48.27	0.44	1192.56	7.91	1389.33	92.40
48.27	0.08	56.23	0.51	1389.33	5.10	1618.57	97.50
56.23	0.09	65.51	0.61	1618.57	2.50	1885.64	100.00
65.51	0.11	76.32	0.72	1885.64	0.00	2196.77	100.00
76.32	0.15	88.91	0.87	2196.77	0.00	2559.23	100.00
88.91	0.19	103.58	1.06	2559.23	0.00	2981.51	100.00
103.58	0.26	120.67	1.31	2981.51	0.00	3473.45	100.00



Malvern Instruments Ltd.
Malvern, UK
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Serial Number: 32734-89

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Figure E29 Particle size distribution data of Formulation F10.

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Scientific and Technological Research Equipment Centre Chulalongkorn University
Building 2-3 Chula Soi 62 Phaya-Thai Rd. Phatumwan Bangkok 10330 Tel. 2188029-32, 2188101 Fax. 2540211

Result: Analysis Report

Sample Details

Sample ID: 2.5EC-60mgFA
Sample File: CHULA8
Sample Path: C:\SIZERS\DATA\
Sample Notes: 2.5EC-1%SP80-5%SU-60mgFA
Dispersing Medium : Water
Ultrasonic : no
Additive : 0.1% Nonidet P40

Run Number: 14
Record Number: 1010

Measured: 8 May 2008 10:17PM
Analysed: 8 May 2008 10:17PM
Result Source: Analysed

System Details

Range Lens: 1000 mm
Presentation: 3_ECL
Analysis Model: Polydisperse
Modifications: None

Beam Length: 2.40 mm
[Particle R.I. = (1.4790, 0.1000); Dispersant R.I. = 1.3300]

Sampler: MS1

Obscuration: 4.8 %
Residual: 1.530 %

Result Statistics

Distribution Type: Volume
Mean Diameters:
D [4, 3] = 803.51 um

Concentration = 0.3531 %Vol
D (v, 0.1) = 340.33 um
D [3, 2] = 519.76 um

Density = 1.000 g / cub. cm
D (v, 0.5) = 769.04 um
Span = 1.283E+00

Specific S.A. = 0.0115 sq. m / g
D (v, 0.9) = 1327.11 um
Uniformity = 3.978E-01

Size Low (um)	In %	Size High (um)	Under%	Size Low (um)	In %	Size High (um)	Under%
4.19	0.01	4.88	0.01	120.67	0.35	140.58	1.60
4.88	0.01	5.69	0.02	140.58	0.49	163.77	2.09
5.69	0.01	6.63	0.03	163.77	0.70	190.80	2.80
6.63	0.01	7.72	0.04	190.80	1.03	222.28	3.82
7.72	0.01	9.00	0.06	222.28	1.52	258.95	5.35
9.00	0.01	10.48	0.07	258.95	2.23	301.68	7.57
10.48	0.01	12.21	0.09	301.68	3.19	351.46	10.76
12.21	0.02	14.22	0.10	351.46	4.43	409.45	15.19
14.22	0.02	16.57	0.12	409.45	5.89	477.01	21.08
16.57	0.02	19.31	0.14	477.01	7.49	555.71	28.58
19.31	0.03	22.49	0.17	555.71	9.15	647.41	37.73
22.49	0.03	26.20	0.21	647.41	10.76	754.23	48.49
26.20	0.04	30.53	0.25	754.23	12.32	878.67	60.81
30.53	0.04	35.56	0.29	878.67	12.21	1023.66	73.02
35.56	0.05	41.43	0.34	1023.66	10.82	1192.56	83.84
41.43	0.06	48.27	0.40	1192.56	8.36	1389.33	92.20
48.27	0.07	56.23	0.46	1389.33	5.34	1618.57	97.53
56.23	0.08	65.51	0.55	1618.57	2.47	1885.64	100.00
65.51	0.11	76.32	0.66	1885.64	0.00	2196.77	100.00
76.32	0.14	88.91	0.80	2196.77	0.00	2559.23	100.00
88.91	0.19	103.58	0.99	2559.23	0.00	2981.51	100.00
103.58	0.26	120.67	1.25	2981.51	0.00	3473.45	100.00

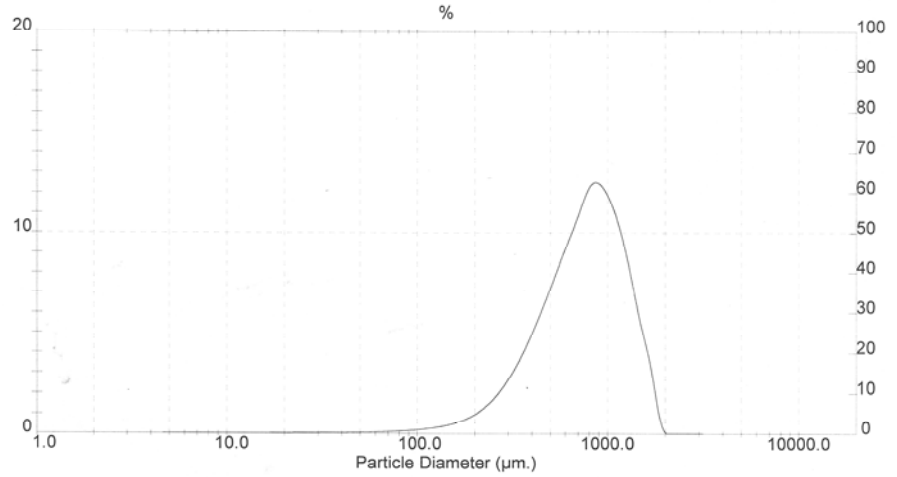

Figure E30 Particle size distribution data of Formulation F10.

VITA

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