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วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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POLYMERIC PARTICLES FOR VITAMIN C ENCAPSULATION

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A Thesis Submitted in Partial Fulfillment of the Requirements

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Thesis Advisor	Associate Professor Supason Wanichwecharungruang, Ph.D.

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งานวิจัยนี้ศึกษาหาพอลิเมอร์ที่เหมาะสมสำหรับเตรียมอนุภาคกลับเฟสเพื่อใช้กักเก็บวิตามินซี พบว่าสามารถเตรียมอนุภาคกลับเฟสได้จากพอลิไวนิลแอลกอฮอล์ด้วยวิธีการแทนที่ด้วยตัวทำละลายและวิธีการตกตะกอนด้วยตัวทำละลาย โดยพบว่าอนุภาคกลับเฟสของพอลิไวนิลแอลกอฮอล์ที่ได้จากการเตรียมด้วยวิธีการแทนที่ด้วยตัวทำละลายมีขนาดใหญ่กว่าอนุภาคที่ได้จากการเตรียมด้วยวิธีการตกตะกอนด้วยตัวทำละลาย จากงานวิจัยยังพบว่าชนิดของโซลเวนท์และแอนตี้โซลเวนท์ที่ใช้ในการเตรียมอนุภาคด้วยวิธีการตกตะกอนด้วยตัวทำละลาย ไม่มีผลต่อขนาดอนุภาคที่เตรียมได้ เมื่อทำการกักเก็บวิตามินซีลงในอนุภาคกลับเฟสพบว่าสามารถกักเก็บวิตามินซีลงในอนุภาคกลับเฟสที่เตรียมได้จากทั้งสองวิธี โดยอนุภาคกลับเฟสที่เตรียมได้ด้วยวิธีการตกตะกอนด้วยตัวทำละลายมีประสิทธิภาพการกักเก็บวิตามินซีที่ดีกว่า (ประสิทธิภาพการกักเก็บ 32.29 เปอร์เซ็นต์และได้อนุภาคที่มีวิตามินซี 16.14 เปอร์เซ็นต์โดยน้ำหนัก) อนุภาคที่เตรียมจากวิธีการแทนที่ด้วยตัวทำละลาย (ประสิทธิภาพการกักเก็บ 7.72 เปอร์เซ็นต์และได้อนุภาคที่มีวิตามินซี 3.86 เปอร์เซ็นต์โดยน้ำหนัก)

สาขาวิชา ปิโตรเคมีและวิทยาศาสตร์พอลิเมอร์ ลายมือชื่อนิสิต.....

ปีการศึกษา 2552 ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก.....

4973425023: MAJOR PETROCHEMISTRY AND POLYMER SCIENCE
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PATSARA CHINWATVANICH: POLYMERIC PARTICLES FOR VITAMIN C ENCAPSULATION. THESIS ADVISOR: ASSOC. PROF. SUPASON WANICHWECHARUNGRUANG, Ph.D., 77pp.

In this work, appropriate polymers for vitamin C-encapsulated reverse micellar particles were sought. It was found that poly(vinyl alcohol) was able to self-assemble into reverse micellar particles through either solvent displacement or solvent coagulation processes. The reverse micellar particles obtained from solvent displacement were larger than those obtained from solvent coagulation process. Type of solvent and anti-solvent used in the solvent coagulation process did not significantly affect the particle size. Vitamin C was loaded into both reverse micellar particles. The results indicated that particles obtained from solvent coagulation process could encapsulate vitamin C more effectively (32.29 % encapsulation efficiency at 16.14 % loading) than those from solvent displacement (7.72 % encapsulation efficiency at 3.86 % loading).

Field of Study : Petrochemistry and Polymer Student's Signature .....
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CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure Page

LIST OF ABBREVIATIONS

°C	Degree Celsius
DLS	dynamic light scattering
DMSO	dimethyl sulfoxide
DMSO-d_6	deuterated dimethyl sulfoxide
h	hour
HPLC	High performance liquid chromatography
mg	milligram
min	minute
mL	milliliter
mV	millivoltage
MW	molecular weight
nm	nanometer
NMR	nuclear magnetic resonance
PDI	Polydisperse index
ppm	parts per million
SEM	scanning electron microscope
TEM	transmission electron microscope
μm	micrometer
%	Percent
UV	ultraviolet

CHAPTER I

INTRODUCTION

1.1 Ascorbic acid (Vitamin C)

Vitamins are substances that play an essential part in animal metabolic processes, but which the animals cannot synthesis. In their absence the animal develops certain deficiency diseases or other abnormal conditions. Vitamins are chemicals other than proteins, carbohydrates, fats and mineral salts that are essential constituents of the food of animals.

Ascorbic acid or vitamin C, a water soluble vitamin, is the enolic form of 3-oxo-L-gulofuranolactone and a water-soluble vitamin. Unlike most mammals, humans do not have the ability to make their own vitamin C. Therefore, we must obtain vitamin C through our diet. Vitamin C can be synthesized from glucose, or extracted from plant sources such as rose hips, blackcurrants or citrus fruits. Vitamin C is required for the synthesis of collagen, an important structural component of blood vessels, tendons, ligaments, and bone. Vitamin C also plays an important role in the synthesis of the neurotransmitter and norepinephrine. Neurotransmitters are critical to brain function and are known to affect mood. In addition, vitamin C is required for the synthesis of carnitine, a small molecule that is essential for the transport of fat to cellular organelles called mitochondria, for conversion to energy [1]. Recent research also suggests that vitamin C is involved in the metabolism of cholesterol to bile acids, which may have implications for blood cholesterol levels and the incidence of gallstones [2].

Vitamin C is also a highly effective antioxidant. Even in small amounts vitamin C can protect indispensable molecules in the body, such as proteins, lipids (fats), carbohydrates, and nucleic acids (DNA and RNA) from damage by free radicals and reactive oxygen species that can be generated during normal metabolism as well as

through exposure to toxins and pollutants (e.g. smoking). Vitamin C may also be able to regenerate other antioxidants such as vitamin E [1]. Vitamin C helps maintain elasticity of the skin aids, the absorption of iron and improves resistance to infection. Vitamin C is used in the treatment of scurvy [3, 4].

In the U.S., the recommended dietary allowance (RDA) for vitamin C was recently revised upward from 60 mg daily to 90 mg daily for men and women. The RDA continues to be based primarily on the prevention of deficiency disease, rather than the prevention of chronic disease and the promotion of optimum health. The recommended intake for smokers is 35 mg/day, higher than for nonsmokers, because smokers are under increased oxidative stress from the toxins in cigarette smoke and generally have lower blood levels of vitamin C [5].

Good sources of vitamin C are broccoli, brussels sprouts, cauliflower, cabbage, mangetout, green leafy vegetables, red peppers, chilies, watercress, parsley, blackcurrants, strawberries, kiwi fruit, guavas and citrus fruit. Vitamin C (L-ascorbic acid) is available in many forms such as ascorbates, ascorbyl palmitate and conjugate with bioflavonoids, but there is little scientific evidence that any one form is better absorbed or more effective than another.

Vitamin C is very unstable when exposed to oxygen, light, and moisture. The compound is sensitive to high temperatures and metal ions. It can easily decompose into biologically inactive compounds such as 2,3-diketo-L-gulonic acid, oxalic acid, L-threonic acid, L-xylonic acid, and L-lyxonic acid [6-8]. Degradation of vitamin C can occure both aerobically and anaerobically [9-11].

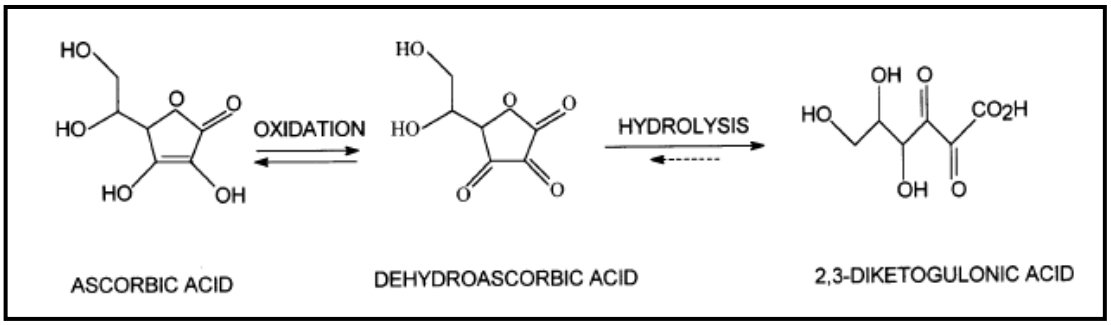

Figure 1.1 Structure of ascorbic acid and its degradation products [12].

Influence of light: Vitamin C is known to be susceptible to degradation by light. This was studied by Iwase et al. [13]. The effect of the natural light and of UV light (265 nm) on the stability of vitamin C in solution was examined in order to optimize the choice of glassware. Periodic changes of vitamin C stored in a brown flask and in a plug-free, transparent flask, which was exposed to UV light, were compared at room temperature. The results demonstrated that degradation of vitamin C was affected by both natural and UV light. After 1 h, the initial concentration of vitamin C decreased to 79.7 % under the influence of UV light. Under the influence of natural light, the initial concentration decreased to 84.2 % in the transparent flask and to 95.6 % in the brown flask. Thus, brown volumetric appeared to protect vitamin C from natural light. Some approaches also recommended protection using aluminum foil. Generally, most articles have highlighted the importance of protecting vitamin C solution from natural light.

Influence of temperature: Temperature has been described as one of the key factors, which significantly influences the stability of vitamin. The effect of the temperature on the stability of vitamin C has been studied by many research groups [12-15]. Stability studies at higher temperatures confirmed a large degree of degradation of vitamin C. The concentrations of vitamin C at 60°C and 80°C were

decreased within 1 h to less than 20% of initial concentration; at 40°C, the concentration decreased to 75% of initial concentration. The solution of vitamin C at laboratory temperature (25°C) was stable for 1 h [13].

Influence of pH: Vitamin C exhibits higher stability in solution under acidic conditions. At these conditions, the formation of dehydroascorbic acid, the main degradation product, is not favored. Some studies involved a comparison of extraction agents of various pH and their impact to vitamin C stability [13-15].

Influence of concentration: Concentration of vitamin C in solution could also influence stability. Rumelin et al. [16], Iwase et al. [13] and Novakova et al. [14] studied the stability of vitamin C in solutions of different concentrations. It was shown that the higher the concentration, the better the stability. The stability was found to decrease significantly at concentration below 0.1 mg/L [17].

Influence of metal ions: The presence of metal ions has also been described as one of the factors that could decrease the stability of vitamin C in solutions. For this reason, EDTA, as a chelating agent, can improve stability of vitamin C. Iwase et al. [13] examined the effect of Cu^{2+} and Fe^{3+} on the stability of vitamin C in standard solution. Müller et al. [18] examined the influence of Cu^{2+}, Fe^{2+}, Mg^{2+}, Ca^{2+}, Mn^{2+}, Zn^{2+} metal ions, where the amount of each element corresponded to the amount added to multivitamin preparations. Only Cu^{2+} was found to influence the content of vitamin C significantly.

The degradation of vitamin C is a major cause of quality and color changes during processing and storage of many products. By encapsulating vitamin C into carrier, it is assumed that its chemical instability can be overcome as well as higher, more efficient and equally distributed concentration throughout extended period of time can be achieved.

1.2 Poly(vinyl alcohol)

Poly(vinyl alcohol) (synonyms: vinyl alcohol polymer, PV(OH), ethenol homopolymer) is highly hydrophilic synthetic polymers used since the early 1930s. PV(OH) has excellent film forming, emulsifying, and adhesive properties. It is also resistant to oil, grease and solvent. It is odorless and nontoxic. It has high tensile strength and flexibility, as well as high oxygen and aroma barrier properties. However these properties are dependent on humidity, in other words, with higher humidity more water is absorbed. The water, which acts as a plasticizer, will then reduce its tensile strength, but increase its elongation and tear strength. PV(OH) is fully degradable and is a quick dissolver. PV(OH) has a melting point of 230°C and 180–190°C for the fully hydrolysed and partially hydrolysed grades. It decomposes rapidly above 200°C as it can undergo pyrolysis at high temperatures.

PV(OH) is an atactic material but exhibits crystallinity as the hydroxyl groups are small enough to fit into the lattice without disrupting it. PV(OH) is prepared by the polymerization of vinyl acetate, followed by partial or complete catalysed hydrolysis. The primary raw material used in the manufacture of PV(OH) is vinyl acetate monomer dissolved in methanol. The polymerization involves the presence of two proprietary catalytic agents. After polymerization, the material undergoes controlled hydrolysis with aqueous sodium hydroxide, during which the ester groups in the vinyl acetate are replaced with hydroxyl groups. PV(OH) is precipitated, washed and dried to form an odourless, tasteless, white or cream-coloured granular powder. The number of acetate groups in PV(OH) is determined by the degree of hydrolysis (86.5–89.0 % hydrolysis for this food additive specification).

The physical characteristics of PV(OH) are dependent on its method of preparation. PV(OH) is obtained from the hydrolysis or partial hydrolysis of polyvinylacetate which is made by the polymerization of vinyl acetate monomer (Figure

1.2). PV(OH) is generally classified into two groups, fully hydrolyzed (A) and partially hydrolyzed (B).

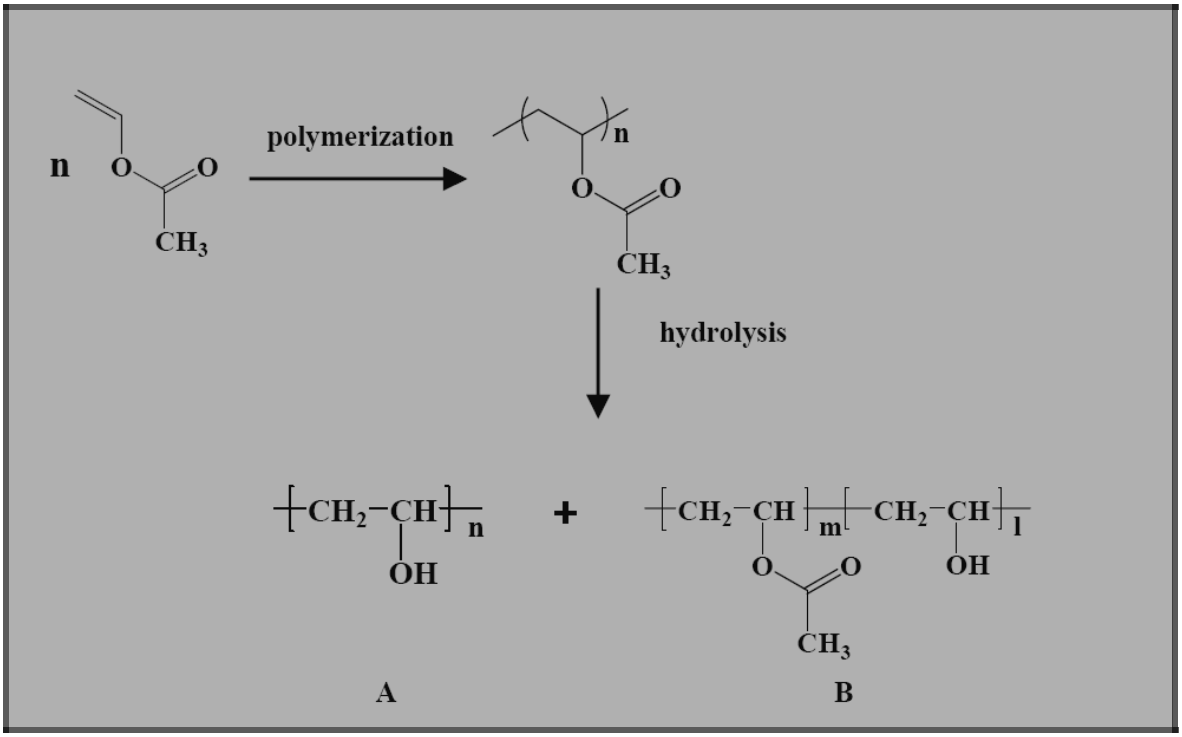

Figure 1.2 Synthesis of poly(vinyl alcohol)

Varying the length of the initial vinyl acetate polymer and the degree of hydrolysis under alkaline or acidic conditions, yields PV(OH) products of different molecular weights (20,000-400,000), solubility, flexibility, tensile strength and adhesiveness. Various properties are measured to characterize PV(OH). They include pH, viscosity, loss on drying, melting point, refractive index, heavy metals and residue on ignition. These properties vary with molecular weight and % hydrolysis and they are used for differentiating various grades of PV(OH).

PV(OH) was selected as a starting material in this work due to its biocompatibility, biodegradability non-toxicity, non-carcinogenicity and the possibility for

chemical modification at the hydroxyl groups [19, 20]. PV(OH) is also well accepted pharmaceutically safe polymer to both human and the environment [21]. The United States Food and Drug Administration (FDA) have approved that poly(vinyl alcohol) is Substances Generally Recognized as Safe (GRAS), through scientific procedures, for use in aqueous film coating formulations applied to dietary supplement products (i.e., tablets or capsules), where the coating formulation is up to four percent (by weight) of the tablet or capsule, and polyvinyl alcohol is up to 45 percent (by weight) of the coating formulation. In addition, it is used as an indirect food additive in products that are in contact with food, as diluents in color additive mixtures for coloring shell eggs, and for use in several medical applications, including in coatings applied to pharmaceutical tablets.

The safety of PV(OH) makes this polymer very popular on various applications worldwide. It was applied in pharmaceutical, medical, cosmetic and food products. Examples of products containing PV(OH) are resins, lacquers, surgical threads and contact lens fluids.

PV(OH) is used in the textile industries as a sizing and finishing agent. PV(OH) can also be incorporated into a water-soluble fabric in the manufacture of degradable protective apparel, laundry bags for hospitals, rags, sponges, sheets, covers, as well as physiological hygiene products.

PV(OH) is used as an indirect food additive in products which are in contact with food and has also been approved for use in packaging meat products by the Meat Inspection Division of the USDA and approved for use in packaging poultry products by the Poultry Division of the USDA.

As an industrial and commercial product, PV(OH) is valued for its solubility and biodegradability, which contributes to its very low environmental impact. Several microorganisms ubiquitous to artificial and natural environments such as septic systems,

landfills, compost and soil have been identified which are able to degrade PV(OH) through enzymatic processes. A combination of oxidase and hydrolase enzyme activities degrade PV(OH) into acetic acid but both the percent hydrolysis and its solubility affect the rate of PV(OH) biodegradation.

PV(OH) is safe as used in cosmetic formulations. The emulsifying binding and thickening properties of PV(OH) make it useful for cosmetic applications. Cold creams, cleansing creams, shaving creams, eye make-up and facial masks may be formulated using PV(OH).

PV(OH) is approved for use in several medical applications including transdermal patches, the preparation of jellies that dry rapidly when applied to the skin and in immediate and sustained release tablet formulations. Cross-linked polyvinyl alcohol microspheres are also used for controlled release of oral drugs. Ophthalmic solutions, such as synthetic tears, may also contain PV(OH) because it provides good dispersion and coating properties [22]. PV(OH) is included in the FDA Inactive Ingredient Guide for ophthalmic preparations and oral tablets. In the drug delivery area, most researchers are exploring the potential use of PV(OH) as an emulsifier to prepare nanoparticles with the solvent extraction/evaporation method [23] and dialysis method [24]. Usually PV(OH) is viewed as a steric stabilizer in emulsion polymerization. Recently, PV(OH) was also used as a starting material to prepare hydrogel [25] or amphiphilic polymeric micro/ nanopartilces [23, 26].

1.3 Delivery system

Well-known carrier systems for topical administration of cosmetics and pharmaceuticals include liposomes, microemulsions, multiple emulsions and solid lipid particles. Liposomes prepared from lipids, have been used as potential drug carriers because of the protection they can offer drugs contained in their core. However,

liposomes possess a low encapsulation efficiency, poor storage stability, and rapid leakage of water-soluble drugs [27-29]. Recently, biodegradable polymeric particles have shown their advantage over liposomes by their increased stability and the unique ability to create a controlled release. Polymeric particles are fabricated in order to entrap and deliver specific pharmaceutical agents to various specific locations [30-35]. Polymeric particles for use as cosmetics and drug carriers can be made from various polymeric materials. Their loading capacity and controlled release properties depend on the type of drug and the type of polymer used [36-38]. The most widely used polymers for particles have been poly(lactic acid) (PLA) [39-42]. poly(glycolic acid) (PGA) [43], poly(vinyl alcohol) (PV(OH)) [25, 44, 45], co-polymers, poly(lactide-co-glycolide) (PLGA) [46-48], poly(vinyl alcohol)-graft-poly(lactide-co-glycolide) (PV(OH)-g-PLGA) [49], ethyl cellulose [50] and chitosan [51]. These polymers have shown good biocompatibility and resorbability through natural pathways. Amphiphilic polymer, consisting of both hydrophilic and hydrophobic parts, is able to self-assemble into micro/nanostructures when placed in a solvent that is selective for either the hydrophilic or hydrophobic part. It has been proposed that the sustained release of active compounds is improved when carriers are entrapped in the intercorneocyte spaces of the skin surface [26, 52]. This, therefore, prolongs the bioavailability of various topical drugs and cosmetic actives such as vitamin A [26, 52, 53], coenzyme Q10 [38, 54], ibuprofen [55-57], betamethasone phosphate [58], 5-fluorouracil [59], mixnoxidil [60], triptorelin [61], organic UV filters [62], β-carotene [41], dexamethasone [48], norcantharidin [63], praziquantel [64], quinine [65] and α-tocopherol acetate [66], from each application. The loading of cosmetic and pharmaceutical compounds into carrier systems also protects the incorporated materials against various environmental threats [67-70], thus increasing their stability.

1.4 Encapsulation

Encapsulation of drugs or actives into carriers can be carried out using a variety of materials such as proteins, polysaccharides and synthetic polymers. The selection of matrix materials is dependent on many factors including: (a) size of encapsulate-particles required; (b) inherent properties of the drug, e.g., aqueous solubility and stability; (c) surface characteristics such as charge and permeability; (d) degree of biodegradability, biocompatibility and toxicity; (e) drug release profile desired; and (f) antigenicity of the final product [71].

Encapsulate-particles have been prepared most frequently by four methods:

1.4.1 Dispersion of preformed polymers

1.4.2 Polymerization of monomers

1.4.3 Ionic gelation or coacervation of hydrophilic polymers.

1.4.4 Production of particles using supercritical fluid technology

1.4.1 Dispersion of preformed polymers

Dispersion of preformed polymers is a common technique used to prepare biodegradable particles from poly (lactic acid) (PLA); poly (D, L-glycolide), PLG; poly (D, L-lactide-co-glycolide) (PLGA) and poly (cyanoacrylate) (PCA) [72-76]. This technique can be used in various ways as described below:

Solvent evaporation method: In this method, the polymer is dissolved in an organic solvent such as dichloromethane, chloroform or ethyl acetate which is also used as the solvent for dissolving the hydrophobic drug. The mixture of polymer and drug solution is then emulsified in an aqueous solution containing a surfactant or emulsifying agent to form oil in water (o/w) emulsion. After the formation of stable emulsion, the organic solvent is evaporated either by reducing the pressure or by continuous stirring. Particle size was found to be influenced by the type and concentrations of stabilizer,

homogenizer speed and polymer concentration [77, 78]. In order to produce small particle size, often a high-speed homogenization or ultrasonication may be employed [79].

Spontaneous emulsification or solvent diffusion method: This is a modified version of solvent evaporation method [80, 81]. In this method, the water miscible solvent along with a small amount of the water immiscible organic solvent is used as an oil phase. Due to the spontaneous diffusion of solvents an interfacial turbulence is created between the two phases leading to the formation of small particles. As the concentration of water miscible solvent increases, a decrease in the size of particle can be achieved. Both solvent evaporation and solvent diffusion methods can be used for hydrophobic or hydrophilic drugs. In the case of hydrophilic drug, a multiple w/o/w emulsion needs to be formed with the drug dissolved in the internal aqueous phase.

1.4.2 Polymerization of monomers

In this method, monomers are polymerized to form particles in an aqueous solution. Drug is incorporated either by being dissolved in the polymerization medium or by adsorption onto the particles after polymerization completed. The particle suspension is then purified to remove various stabilizers and surfactants employed for polymerization by ultracentrifugation and re-suspending the particles in an isotonic surfactant-free medium. This technique has been reported for making polybutylcyano acrylate or poly (alkylcyanoacrylate) nanoparticles [82, 83]. Nanocapsule formation and their particle size depend on the concentration of the surfactants and stabilizers used [84, 85].

1.4.3 Coacervation or ionic gelation method

Much research has been focused on the preparation of particles using biodegradable hydrophilic polymers such as chitosan, gelatin and sodium alginate [86, 87]. Calvo and coworkers developed a method for preparing hydrophilic chitosan nanoparticles by ionic gelation [88, 89]. The method involves a mixture of two aqueous phases, of which one is the chitosan polymer or copolymer of chitosan and a di-block copolymer ethylene oxide or propylene oxide (PEO-PPO) and the other is a polyanion sodium tripolyphosphate. In this method, positively charged amino group of chitosan interacts with negative charged tripolyphosphate to form coacervates with a size in the range of nanometer. Coacervates are formed as a result of electrostatic interaction between two aqueous phases, whereas, ionic gelation involves the material undergoing transition from liquid to gel due to ionic interaction conditions at room temperature.

1.4.4 Production of particles using supercritical fluid technology

Conventional methods such as solvent extraction-evaporation, solvent diffusion and organic phase separation methods require the use of organic solvents which are hazardous to the environment as well as to physiological systems. Therefore, the supercritical fluid technology has been investigated as an alternative to prepare biodegradable micro- and nanoparticles because supercritical fluids are environmentally safe [42, 83].

A supercritical fluid can be generally defined as a solvent at a temperature above its critical temperature, at which the fluid remains a single phase regardless of pressure. Supercritical CO_2 (SC CO_2) is the most widely used supercritical fluid because of its mild critical conditions (Tc = 31.1°C, Pc = 73.8 bars), nontoxicity, non-flammability, and low price. The most common processing techniques involving supercritical fluids are supercritical anti-solvent (SAS) and rapid expansion of critical solution (RESS). The

process of SAS employs a liquid solvent, e.g., methanol, which is completely miscible with the supercritical fluid (SC CO_2), to dissolve the solute to be micronized; at the process conditions, because the solute is insoluble in the supercritical fluid, the extract of the liquid solvent by supercritical fluid leads to the instantaneous precipitation of the solute, resulting the formation of nanoparticles. Thote and Gupta reported the use of a modified SAS method for formation of hydrophilic drug dexamethasone phosphate drug nanoparticles for microencapsulation purpose [90]. RESS differs from the SAS process in that its solute is dissolved in a supercritical fluid (such as supercritical methanol) and then the solution is rapidly expanded through a small nozzle into a region lower pressure [91]. Thus the solvent power of supercritical fluids dramatically decreases and the solute eventually precipitates. This technique is clean because the precipitate is basically solvent free. RESS and its modified process have been used for the product of polymeric nanoparticles [92]. Supercritical fluid technology technique, although environmentally friendly and suitable for mass production, requires specially designed equipment and is more expensive.

1.5 Hydrophilic active

Well-known active ingredients for topical administration of cosmetics and pharmaceuticals include hydrophobic and hydrophilic actives. However, many active ingredients easily decompose into biologically inactive compounds making their use very limited in the field of pharmaceuticals, dermatological and cosmetics. By encapsulating the active ingredients into carriers, actives can be protected from light, oxygen and other stimuli, thus their chemical instability can be overcome. The vesicles carriers systems are today the most widely used for hydrophilic active. The class of vesicles that have been used in drug delivery applications are liposomes and niosomes.

1.5.1 Liposomes

Liposome is a microscopic vesicle consisting of an aqueous core enclosed in one or more phospholipid layers, used to convey vaccines, drugs, enzymes, or other substances to target cells or organs. Liposomes are bilayered structures made of amphipathic (both hydrophobic and hydrophilic) phospholipids/cholesterol that spontaneously form closed structures when hydrated in aqueous solutions (Figure 1.3 and Figure 1.4).

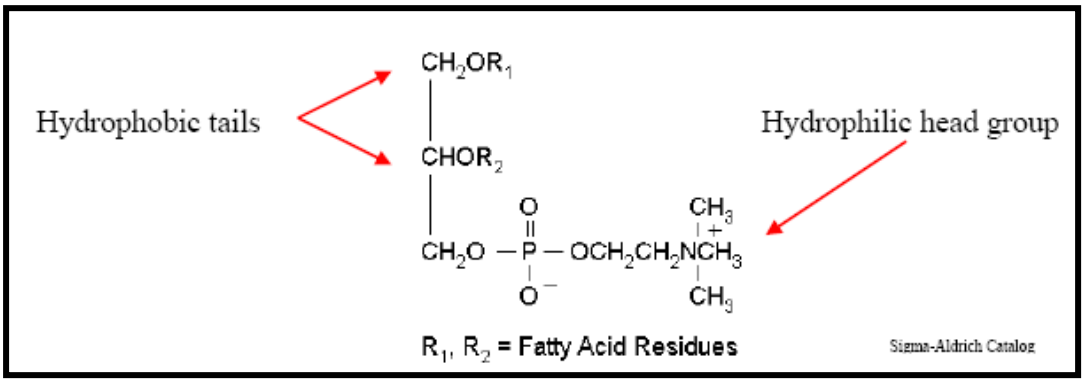

Figure 1.3 Chemical structure of Phosphatidyl-choline (PC) [93].

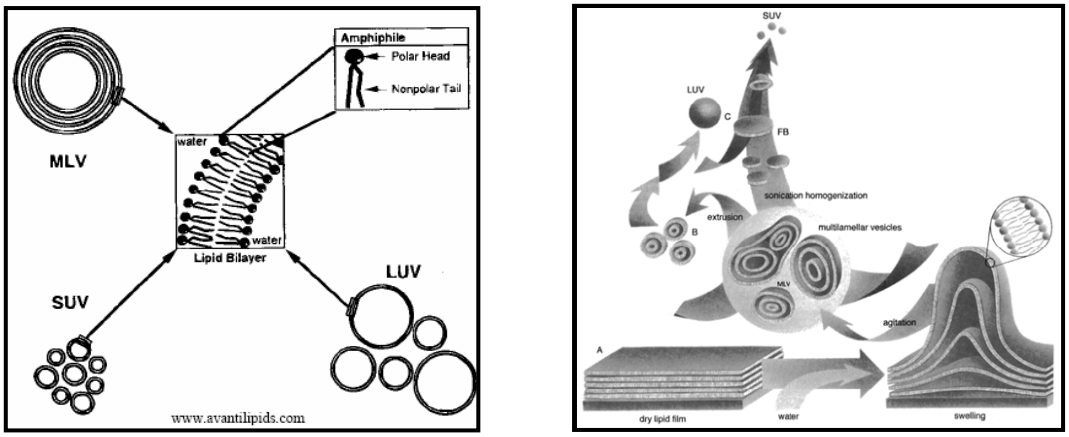

Figure 1.4 Possible formations of liposome vesicles when hydrating in aqueous solution [93].

Depending on the number of bilayers (Figure 1.4), liposomes are classified as multilamellar (MLV), large unilamellar (LUVs) or small unilamellar (SUVs) and range in size from 0.025~20 μm in diameter. The size and morphology of liposomes are regulated by the method of preparation and composition. Mainly, factors that contribute to the overall vesicle formation are phase transition temperature, stability and cholesterol. The phase transition temperature is defined as the temperature required to induce a change in the lipid physical state from the ordered gel phase (hydrocarbon chains are fully extended and closely packed) to the disordered liquid crystalline phase (hydrocarbon chains are randomly oriented and fluid) [94]. There are several factors which directly affect the phase transition temperature; however, the length of the hydrocarbon chain provides a major contribution to the overall transition temperature. The stability of the final structure depends on the type of emulsion which is created (oil in water or water in oil). The larger the hydrophobic chains, the greater the intermolecular force on the hydrophilic part of the molecule. This forms a structure which extends the hydrophilic end (head) into the water substrate while the hydrophobic chains (tails) are separated from this substrate by the intrabilayer spaces of the sphere. In general, high transition lipids provide a stable (non-leaky) delivery system [95].

Today, liposomes are used in drug delivery systems [96-98]. For example, liposomal systems integrated with receptor protein conjugates are being studied for cardiovascular treatments targeting activated platelets [99]. Acoustic liposomes have been created for echocardiographic enhancement of pathological components of atherosclerotic lesions [99]. Modified liposome has been shown to attach to early atheroma in animal model when targeted to vascular cell adhesion molecule type 1 (VCAM-1), or intercellular adhesion molecule-1 (ICAM-1) on atherosclerotic lesion [99-101]. Liposome has been used to encapsulate hydrophilic active e.g. soduim diclofenac [102], fluoroquinolone [103] and gentamicin [104].

At present, vitamin C encapsulated liposome has been on the market, e.g. Liposomal Vitamin C (Natural Health Resources Inc, San Diego, California, USA) and Lypo-Spheric Vitamin C (Livon Laboratories, Henderson, Nevada, USA). However, demonstration of the stability of vitamin C in these products have never been presented. Plus, the life-time and stability of both the liposome itself together with the encapsulated vitamin C, are not clearly defined in there products.

Drug encapsulation of liposomes can be prepared from the dehydration-rehydration method (see Figure 1.5). After the thin film is prepared, the aqueous phase is introduced. Upon agitation, liposomes form multilamellar vesicles (MLV), large unilamellar vesicles (LUVs) or small unilamellar vesicles (SUVs) upon rehydration. While in the assembly mode, there are two primary mechanisms such as encapsulation (formation of liposomes passively entrapped water soluble drug in the interlamellar spaces) and partitioning (formation of liposomes passively entrapped organic soluble drug in the intrabilayer spaces).

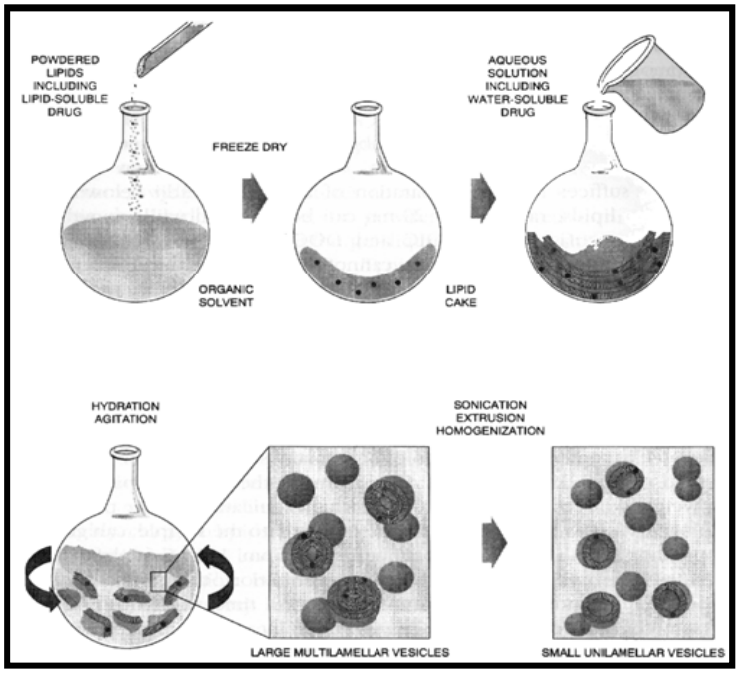

Figure 1.5 Preparation of liposome using dehydration/rehydration technique [93].

1.5.2 Niosomes

Because of a liposome's instability, alternative nonionic surfactants have been investigated [95]. Using similar techniques, nonionic surfactant vesicles or "niosomes" have been synthesized [105]. The ability of nonionic surfactant to form bilayer vesicles instead of micelles is dependent on the hydrophilic-lipophilic balance values (HLB) of the surfactant, the chemical structure of the components and the critical packing parameter [106]. The general form of a single bilayer vesicle is shown in Figure 1.7

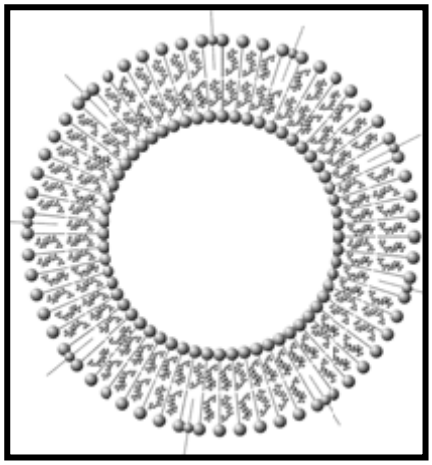

Figure 1.6 Bilayer membrane structure [93].

Niosomes has been used to encapsulate hydrophilic active e.g. colchicines [107], glucose [108], daunorubicin hydrochloride [109] and hydrophobic active e.g. estradiol [110], tretinoin [111, 112], dithranol [113, 114], enoxacin [115] for applications such as anticancer, anti-tubercular, anti-leishmanial, anti-inflammatory, hormonal drugs and oral vaccine [116-123].

1.6 Literature reviews

Poly(vinyl alcohol) as carriers

In 2007, Luadthong and coworker synthesized poly(vinyl alcohol-co-vinylcinnamate) to prepared nanoparticle by dialysis method and studied the effect of concentration and solvent to the morphology of particles. The particle sizes increased with increasing polymer concentration during particle formation. Changing solvent from DMF to DMSO did not affect particle morphology. Self-assembly of the obtained poly(vinylalcohol-co-vinylcinnamate) derivatives gave both spherical micelle nanoparticles and hollow reverse micellar microparticles of uniform sizes [20].

In 2002, Luppi and coworker prepared nanocarriers from poly(vinylalcohol-co-vinyloleate), at a substitution degree of 4.8 %. The particles size were in the range of 200-300 nm. The nanocarriers have contained a hydrophobic core able to host lipophilic drugs such as retinyl palmitate and enhanced retinyl palmitate transcutaneous permeation [26].

In 1998, Wu and coworker prepared poly(vinyl alcohol) hydrogel nanoparticles (675 $\pm$ 42.7 nm diameter) by using a water-in-oil emulsion technology plus cyclic freezing-thawing process (Figure 1.7). Encapsulation of the bovine serum albumin (BSA) into the particles was also carried out. It was demonstrated that BSA is stable in the PV(OH) hydrogel nanoparticles. The particles also showed prolonged released characteristics [25].

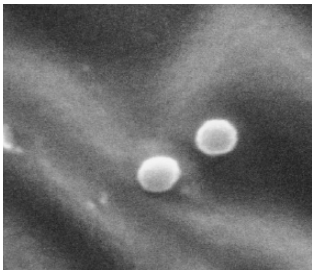

Figure 1.7 Scanning electron micrograph of the PV(OH) hydrogel nanoparticles (this figure is taken from reference 25).

In 1999, Wu and coworker loaded the bovine serum albumin (BSA) into the PV(OH) nanoparticles, then incorporated the obtained BSA-containing nanoparticles into PLGA microspheres. The PLGA-PV(OH) composite microspheres are spherical in shape (Figure 1.8) with nonporous surface and possess prolonged BSA release characteristic (two months). The average particle size is ~71.5-282.7 μm [124].

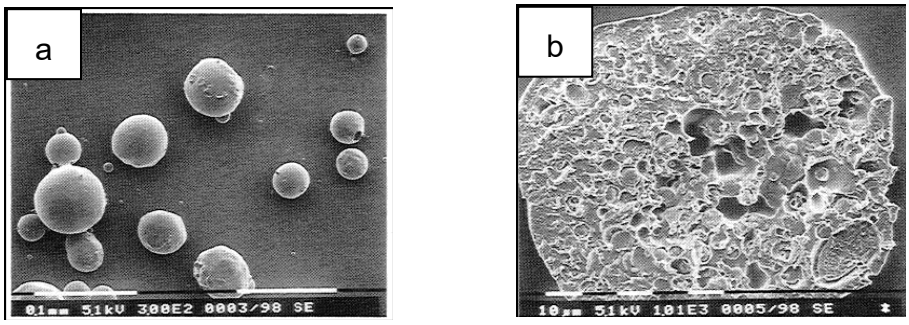

Figure 1.8 Scanning electron micrograph of PLGA-PV(OH) composite microshere (a) and the internal structure and distribution of the PV(OH) nanoparticle inside (b) (this figure is taken from reference124).

In 2007, Kissel and coworker prepared PV(OH)-graft-poly(lactide-co-glycolide) with varying PLGA chain length. The obtained polymers were induced into nanoparticles by solvent displacement method (Figure 1.9). The average diameters of nanoparticles were < 180 nm in diameter. The paclitaxel (anticancer) which is a hydrophobic drug could be loaded into nanoparticles without affecting the nanoparticle size [49].

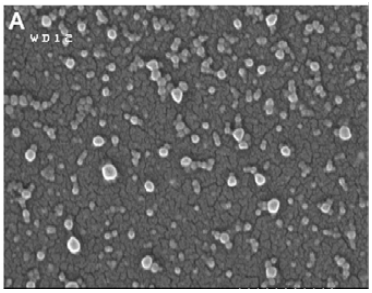

Figure 1.9 Scanning electron micrograph of PV(OH)-graft-poly(lactide-co-glycolide) nanoparticles (this figure is taken from reference 49).

In 2005, Orienti and coworker grafted oleyl amine onto PV(OH) at a substitution degree of 1.5% and prepared polymeric micelles from the grafted product. Polymeric micelle able to complex hydrophobic drugs by entrapment in their hydrophobic inner core. Entrapment of hydrophobic drug such as all-*trans*-retinoic acid (ATRA) was carried out by spray-dying method. The average diameters of nanoparticles was ~100-500 nm. [23].

In 2002, Cascone and coworker prepared biodegradable poly(lactide-coglycolide) (PLGA) nanoparticles loaded with dexamethasone (hydrophobic drug). The obtained particles could be incorporated into dextran-PV(OH) hydrogel (Figure 1.10) [125].

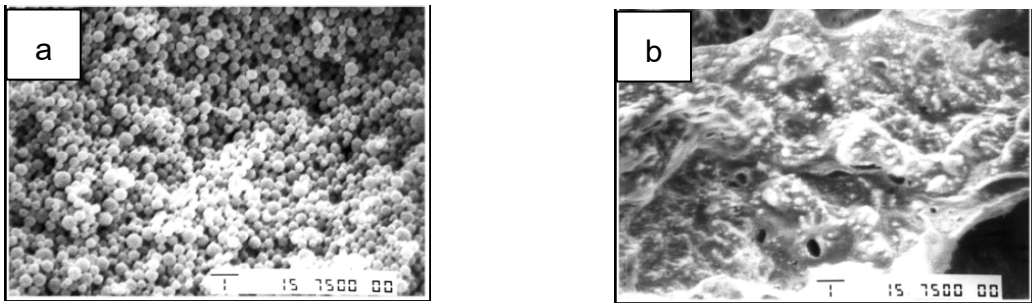

Figure 1.10 Scanning electron micrograph of free PLGA nanoparticles loaded with dexamethasone (a). PLGA nanoparticles entrapped into the dextran-PV(OH) hydrogel (b) (this figure is taken from reference125).

Encapsulation of Ascorbic acid (vitamin c)

In 2007, Magdalena and coworker produced ascorbic acid-encapsulated poly(D,L-lactide-co-glycolide)(DLPLG) nanospheres using solvent/non-solvent coagulation system with the aid of centrifugation and polyvinyl pyrrolidone dispersing agent. Particles of the DLPLG with different content of ascorbic acid have different morphology. Results showed encapsulation and loading efficiency of 98.2 % and

14.7 %, respectively. The authors demonstrated that DLPLG nanospheres completely degraded in sodium chloride solution within 39 days and fully released all the encapsulated ascorbic acid [126].

In 2008, Keum-Il and Hyeon study the stability and characteristics of L-ascorbic acid (AA)-load chitosan (CS) nanoparticle during heat processing in aqueous solutions. AA-loaded-CS nanoparticles were prepared by ionic gelation of CS with tripolyphosphate (TPP) anions. The smallest CS nanoparticles (170 nm) were obtained. As the concentration of AA increased, the particle size increased, while the zeta potential decreased. The encapsulation efficiency of AA-loaded-CS nanoparticles was 10-12 % at loading of only 0.5-1.3 %. During heat processing at various temperatures, the size and zeta potential of the particles decreased rapidly in the first 5 min and then slowly fell to the regular range. It was confirmed that the stability of AA-loaded CS nanoparticles was affected by temperature but that the internal stability was greater than the surface stability. AA in aqueous solution rapidly decreased to about 75.5 % for 15 min, whereas AA release from CS nanoparticles was shown to be over 40 % for 4 days at 100 $^{\circ}$C. This result suggests that the stability of AA with CS nanoparticles was higher than the one of AA without CS nanoparticles [127].

In 2008, Juno and coworker prepared transdermal formulation of L-ascorbic acid 2-phosphate magnesium salt (A2P) using multilamellar vesicles (MLV). A2P was either physically mixed with or entrapped into three different MLVs of neutral, cationic and anionic liposome vesicles. The preparation of neutral MLVs was based on phosphatidyl-choline (PC) and cholesterol (CH). For cationic and anionic MLVs, dioleoyl-trimethylammonium-propane and dimyristoyl glycerophosphate were added as surface charge inducers, respectively. Particle size of the three A2P-loaded MLVs was submicron. Encapsulation efficiency (EE) of A2P in the neutral MLV was the highest (11.30 %). The EE of 6.92 % and 1.67 % were obtained in the cationic and anionic MLV,

respectively. Skin penetration study with hairless mouse skin showed that both physical mixtures of A2P with empty MLVs and A2P loaded MLVs increased penetration of the drug compared to aqueous A2P solution. During the penetration, the significant amount of the drug was methabolized into L-ascorbic acid [128].

Reverse micellar nanoparticle

In 2004, Ugo and coworker investigated the versatility of nanoprecipitation technique for encapsulation of hydrophilic drug. Various parameters in the nanoprecipitation method, such as the solvent and the non-solvent nature, solvent/non solvent volume ratio and the polymer concentration were study. They obtained nanoparticles of size 85-560 nm. The result showed that mean particle size was closely dependent on the type of non-solvent selected. Changing the solvent/non-solvent volume ratio did not significantly affect the particle size [129].

In 2000, Florence and coworker study two methods for encapsulation of a 25-mer-phosphorothioate oligonucleotide (ODN) into poly(D,L-lactic acid) (PLA) particles. First, a double emulsion technique was used to prepare nano- and microparticles. Secondly, the ODN was combined with a quaternary ammonium, the cethyltrimethyl-ammonium bromide (CTAB), to enhance the hydrophobicity of the molecule before entrapment by the emulsification-diffusion method. The results showed that the similar entrapment efficiencies were obtained for the nanoparticles of both method (approx. 27 %) whereas 45 % of entrapment efficiency were observed for microparticles. Seventy five percent of the ODN were released in 60 min with the particles prepared by the emulsification-diffusion method, whereas only 7 % were released in 60 h when using the double emulsion technique. Particles containing CTAB appeared more toxic than the ones obtained by the double emulsion technique, however, these particles could still be used for antisense activity since high oligonucleotide loading could be achieved [130].

1.7 Research goal

The objectives of this research can be summarized as follows:

1. To fabricate reverse micellar particles from amphiphilic polymer.

2. To encapsulate the ascorbic acid (Vitamin C) into reverse micellar particles.

CHAPTER II

EXPERIMENTAL

2.1 Materials and Chemicals

Poly(vinyl alcohol) (MW of 31,000 – 50,000, 98-99 % hydrolyzed), poly(vinyl alcohol–*co*-ethylene) (ethylene content 44 mole %) and ethyl cellulose (MW of 175,323) was purchased from Aldrich Chemical Company (Steinheim, Germany). *N,N'*-Dimethyl sulfoxide (DMSO), Dimethyl formamide (DMF) and methanol used in syntheses and spectroscopic works were reagent or analytical grades purchased from Labscan (Bangkok, Thailand). Hexane, iso-propanol, ethanol, acetone and ethyl acetate were purified from commercial grade solvent prior to use by distillation. Ascorbic acid was obtained from Institute of Beauty and Health Sciences Co.,Ltd. (Samutprakarn, Thailand). Membranes used for dialysis experiments were polyethylene bag, 100 mm flat width, 40 μm film thickness, 10 mL/cm volume capacity, purchased from Unique Plastics Inc (Samutprakarn, Thailand). Centrifugal filtering devices with molecular weight cut-off 100,000 Dalton was purchased from Millipore (County cork, Ireland).

2.2 Instrument and Equipments

The ^{1}H-NMR analyses was carried out in deuterated dimethylsulfoxide (DMSO-d_6) with an internal reference using Varian Mercury spectrometer which operated at 400.00 MHz (Variance Company, Palo Alto, CA, USA). Centrifugation was carried out on a Beckman Coulter Instrument (Allegra 64R high speed centrifuge Beckman Coulter, Beckman Coulter, Inc, Tokyo, Japan). Transmission electron micrographs (TEM) and scanning electron micrographs (SEM) were obtained from JEM-2100 (JEOL, Tokyo, Japan) and JSM-6400 (JEOL, Tokyo, Japan), respectively. The particle size analyses were carried out on Mastersizer S (Mulvern Instruments, Worcestershire, UK). High

performance liquid chromatography (HPLC) was performed with a ThermoFinnigan P4000 (pump), connected to a UV6000LP (UV/VIS detector). Sorbents for the HPLC column was Hypersil BSD C-18 reversed-phase (Thermo Fisher Scientific Inc, Waltham, Massachusetts, USA), with a column size of 100 x 4.6 mm.

2.3 Preparation of reverse micellar particles

2.3.1 Solvent displacement by dialysis

The formation of reverse micellar particles was performed at room temperature by dialysis. Twenty four milligrams of poly(vinyl alcohol)(PV(OH)) were dissolved in 40 mL of DMSO, yielding polymeric solution at concentration of 600 ppm. The resulting solution was transferred into the polyethylene bag which was previously soaked and rinsed several times with distilled hexane and dried under room temperature [131]. The distance between the two clipped ends of the dialysis bag was 8.0 cm (length of the flat bag) which accounted for approximately 75–80 mL volume, thus leaving approximately 35–40 mL void volume inside the dialysis bag at the beginning of the dialysis process. The solution in the dialysis bag was placed in a beaker containing 400 mL of the solvent mixture (hexane: ethyl acetate at the volume ratio of 3:2). The solution was dialyzed against solvent mixture (5 x 400 mL, 3 days). After dialysis, a volume of the obtained colloidal suspension inside the dialysis bag was adjusted to 75 mL. The obtained colloidal particle suspension in the dialysis bag was directly subjected to morphological analyses.

Similar process of dialysis was also carried out with various types of solvents and anti-solvents (Table 2.1).

Table 2.1 Type of solvent and anti-solvent experimented in the fabrication of PV(OH) reverse micellar particles using dialysis method.

Method	Solvent	Anti-solvent
Dialysis	DMSO	hexane-ethyl acetate mixture (3:2)
Dialysis	DMSO	hexane
Dialysis	DMSO	ethyl acetate
Dialysis	DMF	hexane-ethyl acetate mixture (3:2)
Dialysis	DMF	hexane
Dialysis	DMF	ethyl acetate

2.3.2 Solvent coagulation

Twelve milligrams of PV(OH) were dissolved in 20 mL of DMSO then 70 mL of ethyl acetate were slowly dropped into the obtained solution at the rate of 1.5 mL/min while the solution was being stirred at 500 rpm. The obtained colloidal particle suspension was directly subjected to morphological analyses.

Similar process of solvent coagulation was also carried out with various types of solvents and anti-solvents (Table 2.2).

Table 2.2 Type of solvent and anti-solvent experimented in the PV(OH) reverse micellar particles preparation using solvent coagulation method.

Method	Solvent	Anti-solvent
solvent coagulation	DMSO	hexane-ethyl acetate mixture (3:2)
solvent coagulation	DMSO	hexane
solvent coagulation	DMSO	ethyl acetate
solvent coagulation	DMF	hexane-ethyl acetate mixture (3:2)
solvent coagulation	DMF	hexane
solvent coagulation	DMF	ethyl acetate
solvent coagulation	water	Iso-propanol
solvent coagulation	water	ethanol
solvent coagulation	water	acetone
solvent coagulation	water	hexane

Preparation of reverse micellar particles through both methods (solvent displacement by dialysis and solvent coagulation) were repeated with poly(vinyl alcohol–*co*-ethylene) and ethyl cellulose.

2.4 Encapsulation of ascorbic acid into the reverse micellar particles

2.4.1 Solvent displacement by dialysis

Encapsulation of ascorbic acid was carried out by the same procedure described in 2.3.1 except that 12 mg of ascorbic acid were added into the DMSO together with the 24 mg of PV(OH). The obtained ascorbic acid-encapsulated particle suspension was directly subjected to morphological analyses.

2.4.2 Solvent coagulation

Encapsulation of ascorbic acid was carried out by the same procedure in 2.3.2 except that 6 mg of ascorbic acid were added into the DMSO together with the 12 mg of PV(OH). The obtained ascorbic acid-encapsulated particle suspension was directly subjected to morphological analyses.

2.5 Encapsulation efficiency

The amount of ascorbic acid loaded into the reverse micellar particles was analyzed using HPLC. Five milliliters of ascorbic acid-encapsulated particles suspension was centrifugally filtered using centrifugal-filtering devices with MWCO 100,000 (Amicon Ultra-15,Millipore, Ireland) at 15,778 x g for 15 min. The solid product was very quickly rinsed with deionized water (Milli-Q) to remove the ascorbic acid adhered at the outside of particles. The encapsulated ascorbic acid was extracted by sonicating the filtered particles with 10 mL water in the ultrasonic bath at 4°C for 30 min. The sonicated mixture was then centrifugally filtered at 15,778 x g for 20 min. The solid product obtained after filtering centrifugation was also subjected to ascorbic acid extraction again and the liquid extract was quantitated for ascorbic acid using similar procedure. The amounts of ascorbic acid in the filtrate were determined using HPLC with the aid of a calibration curve.

The encapsulation efficiency and loading was calculated according to the following equation (1) and (2), respectively.

$$\% \text{ encapsulation efficiency (\%EE)} = \frac{\text{weight of encapsulated ascorbic acid}}{\text{weight of ascorbic acid used}} \times 100 \quad (1)$$

$$\% \text{ loading} = \frac{\text{weight of encapsulated ascorbic acid}}{\text{weight of polymer used}} \times 100 \quad (2)$$

HPLC was performed with a ThermoFinnigan P4000 (pump), connected to a UV6000LP (UV/VIS detector). Sorbents for the HPLC column was C-18 reversed-phase, with a column size of 100 x 4.6 mm. The mobile phase was 25 mM potassium dihydrogen phosphate and adjusted to pH 3.0 with phosphoric acid. The flow rate was 1.0 mL/min, and the detection wavelength of the UV detector was set at 245 nm. An injection volume of 5 µL was delivered. Calibration curve was constructed from a series of ascorbic acid solutions prepared in water at concentration of 2.5, 5, 10, 15 and 20 ppm.

2.6 Particle size, zeta potential, SEM and TEM analyses

TEM photographs were acquired on a transmission electron microscope (JEM-2100, JEOL, Japan) with an accelerating voltage of 100–120 kV in conjunction with selected area electron diffraction (SAED).

SEM photographs were obtained using a scanning electron microscope (JSM-6400, JEOL, Japan). A drop of the particle suspension was placed on a glass slide and dried overnight. After mounting the slide on aluminum pin, the sample was coated with a

gold layer under vacuum at 15 kV for 90 s. The coated sample was then mounted on an SEM stud for visualization. The accelerating voltage used was 15 kV.

The particle sizes of unloaded and ascorbic acid-loaded particles were acquired with Mastersizer S (model (Malvern Instruments, Worcestershire, UK). Analysis was performed using 0.1 mg/mL suspension. Each measurement was repeated five times and an average value was reported.

2.7 Stability of encapsulated ascorbic acid

The suspension of 24-h.-kept-ascorbic acid-encapsulated PV(OH) particles prepared by solvent coagulation method (sphere D, see p.47) were subjected to ascorbic acid extraction and quantitation. Five mL of the suspension (sphere D in iso-propanol; concentration of ascorbic acid = 10 ppm) was centrifuged (15,778 x g, 15 min) quickly washed with 12 mL water. The washed pellet was sonicated in water (12 mL) at 4°C for 30 min. The sample was centrifugally filtered and the supernatant was subjected to ascorbic acid analysis by HPLC. Analysis of the 24-h.-kept-free ascorbic acid solution (10 ppm solution) was also carried out.

CHAPTER III

RESULT AND DISCUSSION

In this work, novel fabrication methods for the reverse micellar polymeric particles were experimented. Encapsulation of water-soluble ascorbic acid into the obtained carriers was also carried out.

3.1 Preparation of reverse micellar particles

Three types of polymer screened for reverse micellar particle preparation included PV(OH), poly(vinyl alcohol–*co*-ethylene)(PV(OH)-*co*-PE) and ethyl cellulose. The particles were prepared by solvent displacement (dialysis) and solvent coagulation method. It was found that PV(OH) was able to self-assemble into reverse micellar particles through either solvent displacement or solvent coagulation processes. Under various concentrations and with many trials on various solvents and anti-solvents, it was found that PV(OH)-*co*-PE and ethyl cellulose could not self-assemble into particles. Therefore, this study has focused on the relationship between fabricating technique and morphology of the obtained particles from PV(OH) (Table 3.1).

Table 3.1 Type of method, solvent and anti-solvent screened for PV(OH) reverse micellar particles.

Method	Solvent	Anti solvent	Physical appearance	SEM results
Dialysis	**DMSO**	**hexane-ethyl acetate mixture (3:2)**	**cloudy**	**nano/micro-particles**
Dialysis	DMSO	hexane	clear	non-nano/micro-structured particulates
Dialysis	DMSO	ethyl acetate	cloudy and precipitate	non-nano/micro-structured particulates
Dialysis	DMF	hexane-ethyl acetate mixture (3:2)	cloudy and precipitate	undetectable
Dialysis	DMF	hexane	clear	undetectable
Dialysis	DMF	ethyl acetate	cloudy and precipitate	undetectable
Solvent coagulation	DMSO	hexane-ethyl acetate mixture (3:2)	cloudy	non-nano/micro-structured particulates
Solvent coagulation	**DMSO**	**hexane**	**cloudy**	**nano/micro-particles**
Solvent coagulation	**DMSO**	**ethyl acetate**	**cloudy**	**nano/micro-particles**
Solvent coagulation	DMF	hexane-ethyl acetate mixture (3:2)	cloudy	Undetectable
Solvent coagulation	DMF	hexane	clear	Undetectable
Solvent coagulation	DMF	ethyl acetate	cloudy	non-nano/micro-structured particulates
Solvent coagulation	**water**	**iso-propanol**	**cloudy**	**nano/micro-spherical particles**
Solvent coagulation	water	ethanol	cloudy	non-nano/micro-structured particulates
Solvent coagulation	water	acetone	cloudy and precipitate	Undetectable
Solvent coagulation	water	hexane	clear	Undetectable

3.1.1 Solvent displacement by dialysis

SEM and TEM analyses of PV(OH) particles obtained from dialysis process (displacing DMSO with hexane-ethyl acetate mixture) at the starting polymer concentration of 600 ppm indicate spherical particles with diameter of 1.24 ± 0.43 μm. (Figure 3.1 and 3.2). The obtained particles appear as stable colloidal suspension in the hexane-ethyl acetate solvent mixture (Figure 3.3 a). TEM image also confirmed that the obtained particles were not vesicle-like (Figure 3.2).

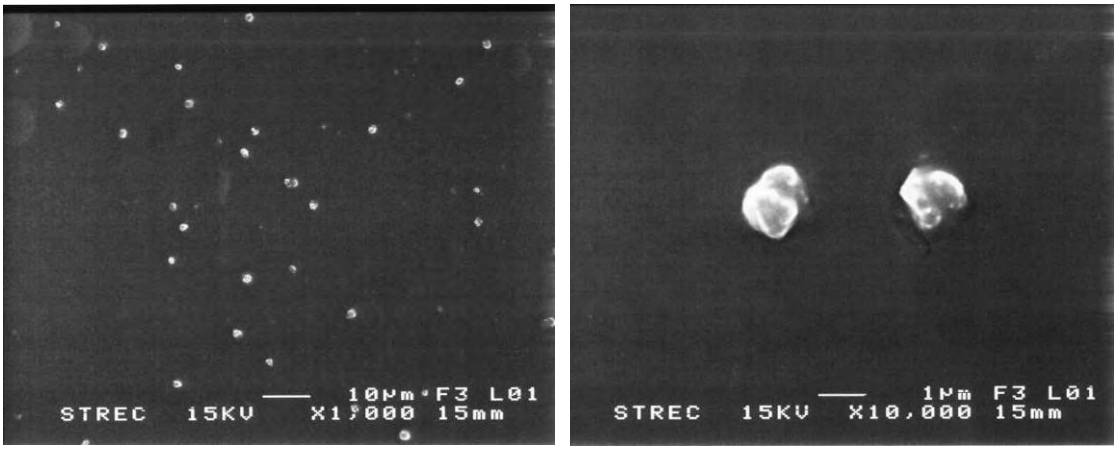

Figure 3.1 SEM photographs of PV(OH) reverse micellar particles prepared by dialysis (displacing DMSO with hexane-ethyl acetate mixture) at the starting polymer concentration of 600 ppm (left at 1,000x magnification, right at 10,000x magnification)

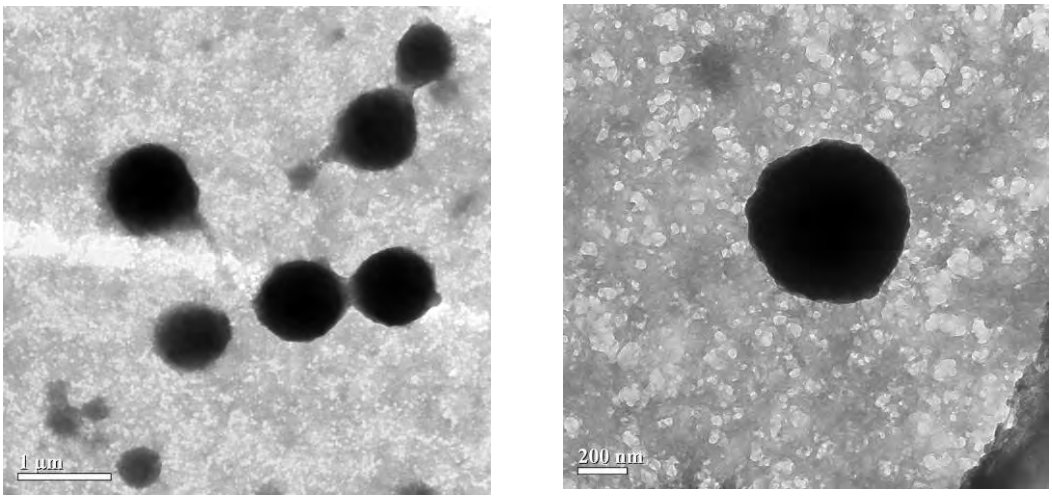

Figure 3.2 TEM photographs of PV(OH) reverse micellar particles prepared by dialysis (displacing DMSO with hexane-ethyl acetate mixture) at the starting polymer concentration of 600 ppm (left at 4,000x magnification, right at 20,000x magnification)

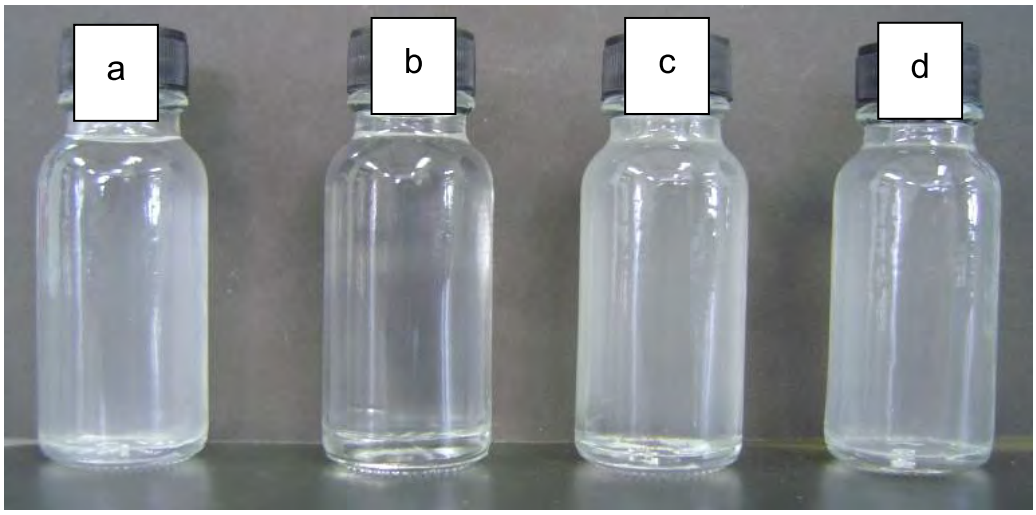

Figure 3.3 Pictures of colloidal suspension of PV(OH) reverse micellar particles: (a) prepared by dialysis (displacing DMSO with hexane-ethyl acetate mixture), (b) prepared by solvent coagulation method (DMSO = solvent, ethyl acetate = anti-solvent), (c) prepared by solvent coagulation method (DMSO = solvent, hexane = anti-solvent) and (d) prepared by solvent coagulation method (water = solvent, iso-propanol = anti-solvent). All particles were prepared at the starting polymer concentration of 600 ppm.

We speculate that when DMSO was displaced by the solvent mixture (hexane: ethyl acetate at the volume ratio of 3:2), the hydrophilic groups (hydroxyl) of the polymer were directed inwards while the methylene moieties of the polymer backbone arranged themselves to give maximum interaction with the hydrophobic solvent, leading to spontaneous particle formation.

Since under normal condition, PV(OH) cannot disperse well in hexane-ethyl acetate medium, the excellent dispersibility of the obtained spheres implied self-organization in the way that directed the hydrophobic methylene moieties to have maximum interaction with solvent molecules. This explained why the obtained particles could form stable colloidal suspension in the hydrophobic medium. Thus it could be concluded that under the condition used, self-assembly at starting PV(OH) concentration of 600 ppm could lead to the formation of reverse micellar particles.

3.1.2 Solvent coagulation

3.1.2.1 DMSO as solvent, ethyl acetate as anti-solvent

SEM analyses of the particles obtained from solvent coagulation process (DMSO = solvent, ethyl acetate = anti-solvent) at the starting polymer concentration of 600 ppm indicate spherical particles with diameter of 0.40 $\pm$ 0.15 μm. (Figure 3.4).

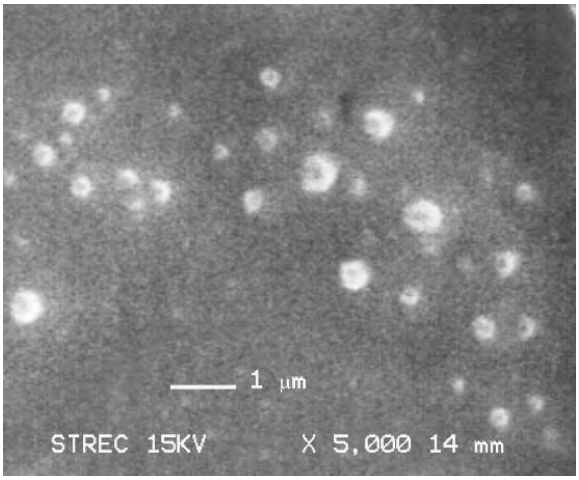

Figure 3.4 SEM photograph of PV(OH) reverse micellar particles prepared by solvent coagulation (DMSO = solvent, ethyl acetate = anti-solvent) at the starting polymer concentration of 600 ppm

When ethyl acetate was dropped into the polymer solution, particles were spontaneously formed. This appeared as the formation of colloidal suspension (Figure 3.3 b). Again, we expected that the hydrophilic groups (hydroxyl) of the polymer were directed inwards while the methylene moieties of the polymer backbone arranged themselves to give maximum interaction with the hydrophobic solvent, leading to spontaneous particle formation.

3.1.2.2 DMSO as solvent, hexane as anti-solvent

Changing the anti-solvent from ethyl acetate to hexane did not significantly affect the particle morphology. The shape and size of the obtained PV(OH) reverse micellar particles (Figure 3.5) were similar to those obtained when ethyl acetate was used as anti-solvent. The obtained particles appear as stable colloidal suspension in the hexane solvent (Figure 3.3 c). SEM analyses of the particles indicate spherical particles with diameter of 0.38 $\pm$ 0.16 μm. (Figure 3.5).

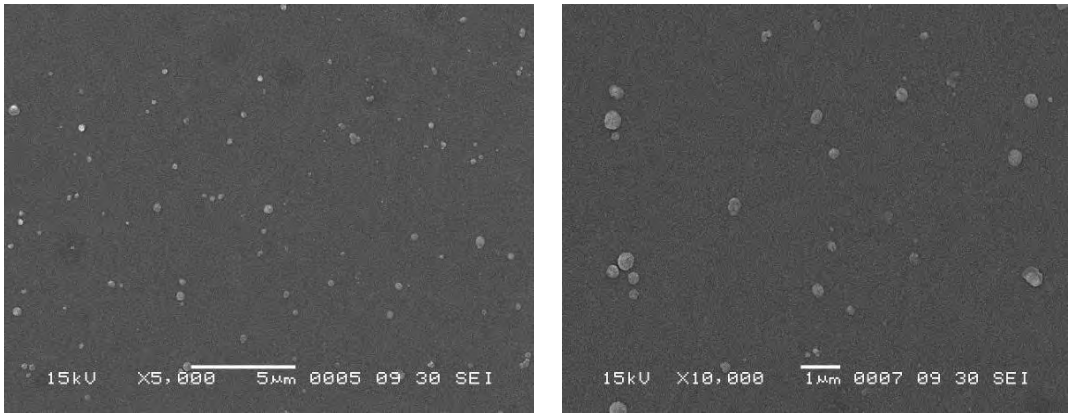

Figure 3.5 SEM photographs of PV(OH) reverse micellar particles prepared by solvent coagulation (DMSO = solvent, hexane = anti-solvent) at the starting polymer concentration of 600 ppm (left at 5000x magnification, right at 10,000x magnification)

3.1.2.3 Water as solvent, iso-propanol as anti-solvent

Self-assembly by solvent coagulation was also performed with the starting PV(OH) aqueous solution using iso-propanol as the anti-solvent. When iso-propanol was dropped into the polymer solution, particles were spontaneously formed. This appeared as the formation of colloidal suspension (Figure 3.3 d). SEM analyses of the particles obtained from solvent coagulation process (water = solvent, iso-propanol = anti-solvent) at the starting polymer concentration of 600 ppm indicate spherical particles with diameter of 0.45 $\pm$ 0.14 µm. (Figure 3.6).

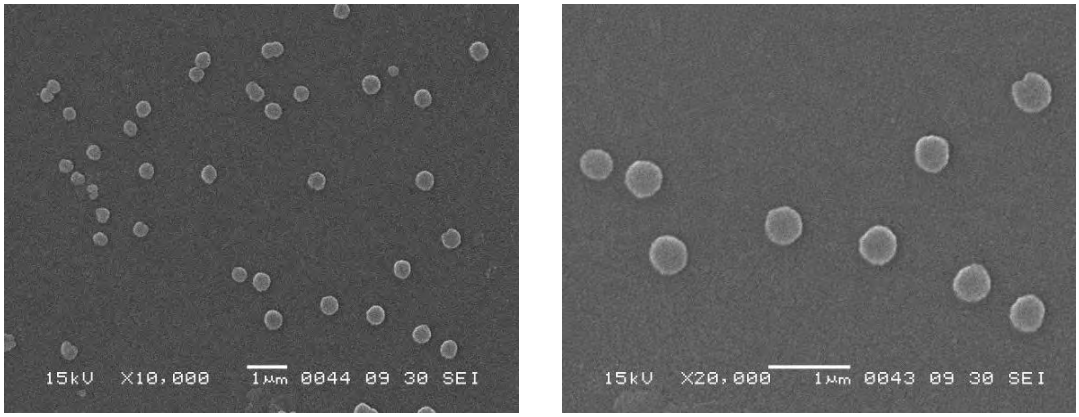

Figure 3.6 SEM photographs of PV(OH) reverse micellar particles prepared by solvent coagulation (water = solvent, iso-propanol = anti-solvent) at the starting polymer concentration of 600 ppm (left at 10,000x magnification, right at 20,000x magnification)

All colloidal suspensions of particles (in hexane, in ethyl acetate and in iso-propanol) were very stable and no precipitate was observed even after being left for 6 months (Figure 3.7). This indicates that the surfaces of the particles should have good interactions with the organic solvent molecules around them. This implied that most hydroxyl groups should be directed inwards leaving most hydrophobic methylene groups at the surfaces. Thus the particles must be reverse micelles.

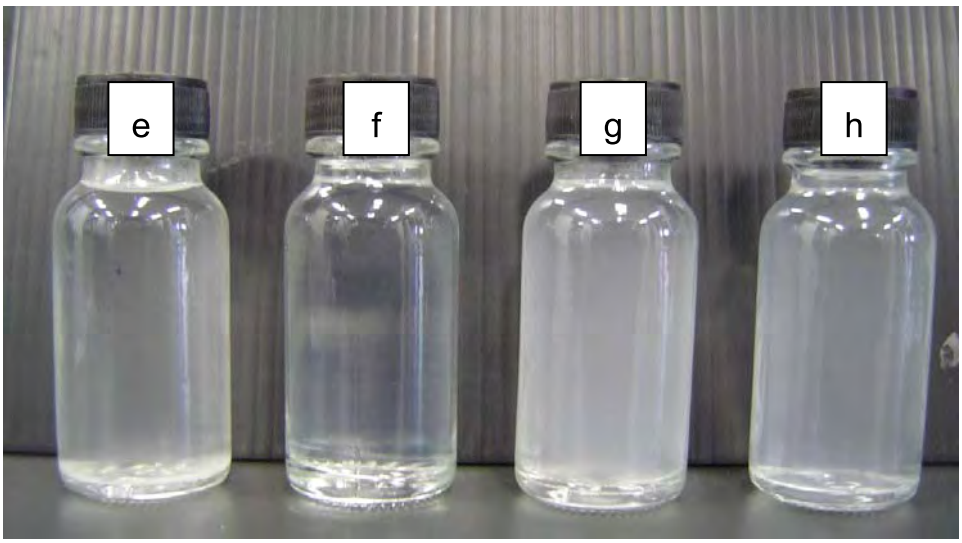

Figure 3.7 Pictures of colloidal suspension of PV(OH) reverse micellar particles after 4 months: (e) prepared by dialysis (displacing DMSO with hexane-ethyl acetate mixture), (f) prepared by solvent coagulation method (DMSO = solvent, ethyl acetate = anti-solvent), (g) prepared by solvent coagulation method (DMSO = solvent, hexane = anti-solvent) and (h) prepared by solvent coagulation method (water = solvent, iso-propanol = anti-solvent). All particles were prepared at the starting polymer concentration of 600 ppm.

The average hydrodynamic diameter of PV(OH) reverse micellar particles determined by dynamic light scattering (DLS) method are shown in Table 3.2. The numbers agree well with sizes visualized by SEM and TEM. The averages diameters of particles from solvent displacement (dialysis) were bigger than those from solvent coagulation. In fact, this agrees well with their appearance of the suspensions, i.e., suspension from dialysis was more opaque than that from solvent coagulation (Figure 3.3 a VS 3.3 b and 3.7 e VS 3.7 f). Type of solvent-anti solvent used did not significantly affect the particle size. The reason that particles obtained from solvent coagulation were smaller than those from solvent displacement probably involves the shear force presence only in the solvent coagulation.

Table 3.2 The hydrodynamic diameters determined from dynamic light scattering technique of PV(OH) reverse micellar particles suspensions prepared from solvent displacement (dialysis) and solvent coagulation.

Method	Hydrodynamic diameters (nm) (Average size ± SD)	Polydisperse Index (PDI)
Dialysis (displacing DMSO with hexane-ethyl acetate mixture)	1629 ± 74	0.547
Solvent coagulation (DMSO = solvent, ethyl acetate = anti-solvent)	790 ± 73	0.387
Solvent coagulation (DMSO = solvent, hexane = anti-solvent)	774 ± 25	0.307
Solvent coagulation (water = solvent, iso-prapanol = anti-solvent)	743 ± 9	0.389

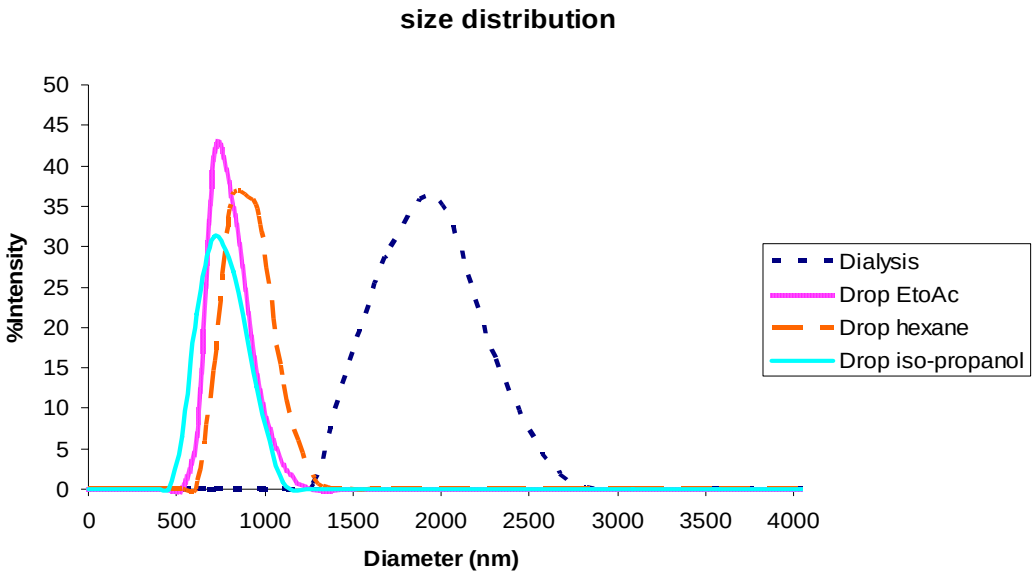

Figure 3.8 Size distributions of PV(OH) reverse micellar particles prepared from dialysis and solvent coagulation.

All the dry particles obtained also showed good dispersibility in cream-base media and hydrophobic cyclo-methicone. This demonstrates that they can be readily used in cream-base or oil-base cosmetic formulations.

3.2 Encapsulation of ascorbic acid into reverse micellar particles

3.2.1 Solvent displacement by dialysis

Encapsulation of ascorbic acid was carried out by dialyzing 40 ml of solution containing twenty four milligrams of PV(OH) and twelve milligrams of ascorbic acid in DMSO. As shown by SEM analysis (Figure 3.9), ascorbic acid-encapsulate-particles are spherical with diameter of 1.40 $\pm$ 0.43 μm.

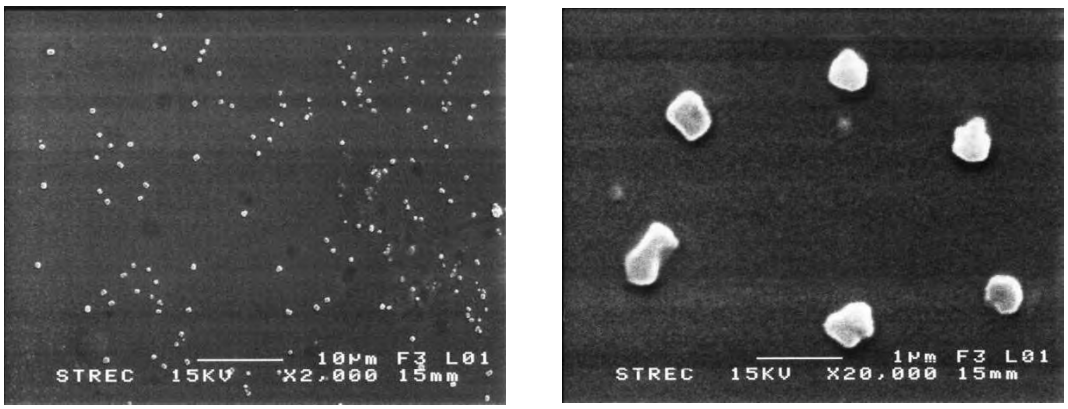

Figure 3.9 SEM photographs of ascorbic acid-encapsulate-particles prepared by dialysis (displacing DMSO with hexane: ethyl acetate mixture) at the starting polymer concentration of 600 ppm (left at 2,000x magnification, right at 20,000x magnification)

As can be seen in the TEM photographs (Figure 3.10), the ascorbic acid-encapsulated particles are spherical with obvious distinct cores. This obvious core appearance is probably a result of ascorbic acid encapsulation. Dark core indicates a

presence of ascorbic acid. Particles with no ascorbic acid encapsulation show no obvious dark core (Figure 3.2).

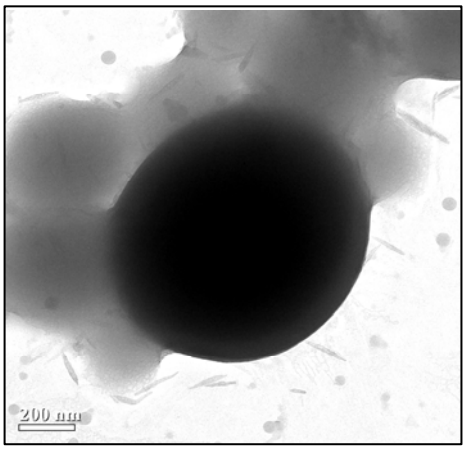

Figure 3.10 TEM photographs of ascorbic acid-encapsulate-particles prepared by dialysis (displacing DMSO with hexane: ethyl acetate mixture) at the starting polymer concentration of 600 ppm

3.2.2 Solvent coagulation

3.2.2.1 DMSO as solvent, ethyl acetate as anti-solvent

Encapsulation of ascorbic acid was carried out by solvent coagulating 20 ml of solution containing twelve milligrams of PV(OH) and six milligrams of ascorbic acid in DMSO with 70 mL ethyl acetate. SEM analyses of ascorbic acid-encapsulated particles indicate spherical particles with diameter of 0.60 $\pm$ 0.19 μm. (Figure 3.11).

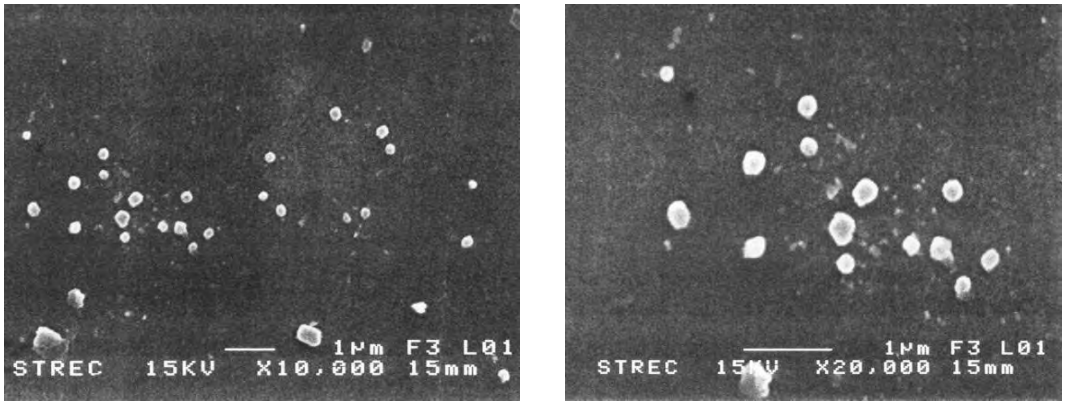

Figure 3.11 SEM photographs of ascorbic acid-encapsulate-particles prepared by solvent coagulation (DMSO = solvent, ethyl acetate = anti-solvent) at the starting polymer concentration of 600 ppm (left at 10,000x magnification, right at 20,000x magnification)

3.2.2.2 DMSO as solvent, hexane as anti-solvent

Changing anti-solvent from ethyl acetate to hexane did not significantly affect the particle morphology. SEM analyses of ascorbic acid-encapsulate-particles indicate spherical particles with diameter of 0.59 $\pm$ 0.19 µm. (Figure 3.12).

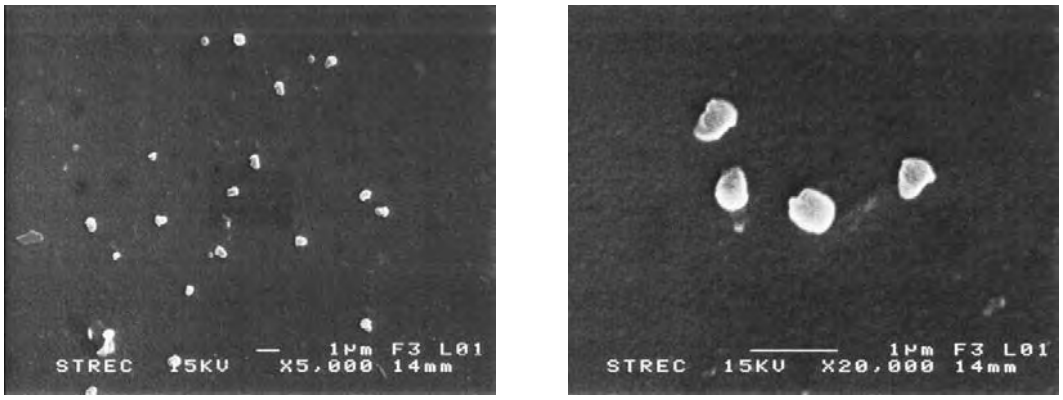

Figure 3.12 SEM photographs of ascorbic acid-encapsulate-particles prepared by solvent coagulation method (DMSO = solvent, hexane = anti-solvent) at the starting polymer concentration of 600 ppm. (left at 5,000x magnification, right at 20,000x magnification)

3.2.2.3 Water as solvent, iso-propanal as anti-solvent

Changing solvent from DMSO to water and anti-solvent from ethyl acetate to iso-propanol did not significantly affect the particle morphology. SEM and TEM analyses of ascorbic acid-encapsulate-particles also indicate spherical particles with diameter of 0.61 ± 0.19 μm. (Figure 3.13 and 3.14).

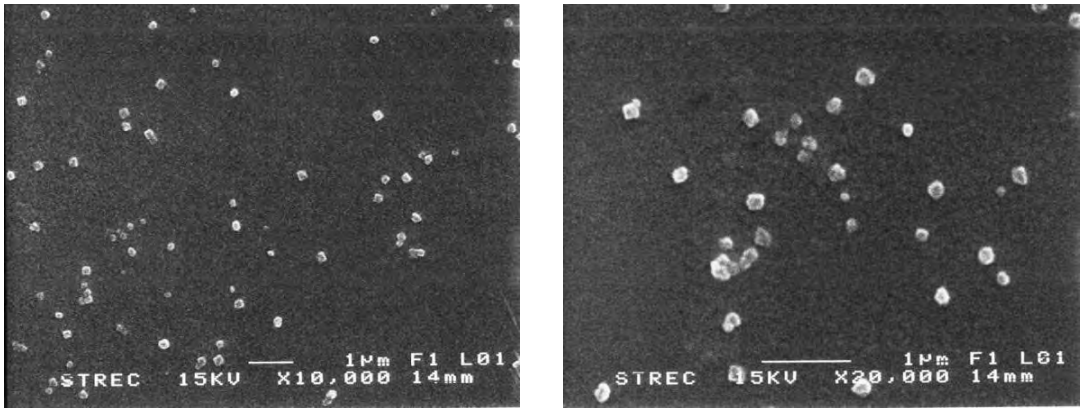

Figure 3.13 SEM photographs of ascorbic acid-encapsulate-particles prepared by solvent coagulation (water = solvent, iso-propanol = anti-solvent) at the starting polymer concentration of 600 ppm. (left at 10,000x magnification, right at 20,000x magnification)

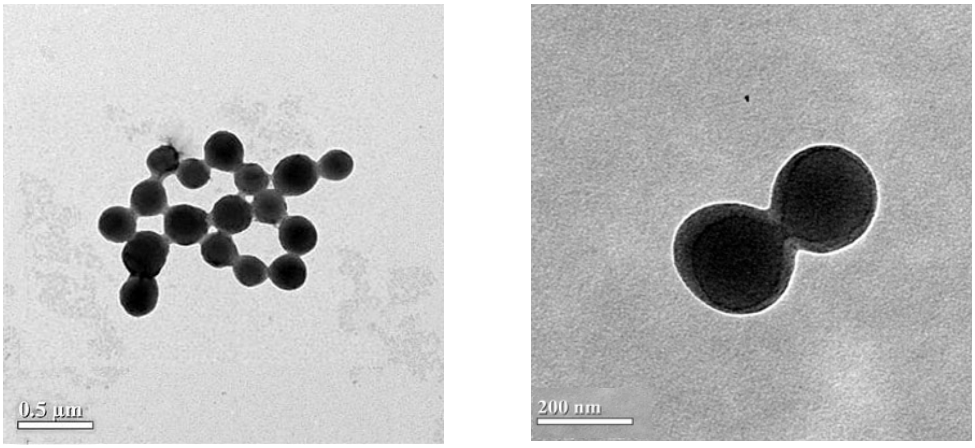

Figure 3.14 TEM photographs of ascorbic acid-encapsulate-particles prepared by solvent coagulation (water = solvent, iso-propanol = anti-solvent) at the starting polymer concentration of 600 ppm (left at 8,000x magnification, right at 20,000x magnification)

As can be seen in the TEM photographs (Figure 3.14), the ascorbic acid-encapsulated particles are spherical with obvious distinct core. This obvious core

appearance is probably a result of ascorbic acid encapsulation. Dark core indicates a presence of ascorbic acid.

The average hydrodynamic diameter of ascorbic acid-encapsulated particles was also determined by DLS methods (Table 3.3). The diameter of the particles increased upon ascorbic acid encapsulation. The averages diameters of particles from dialysis were bigger than those from solvent coagulation. Type of solvent-anti solvent used did not significantly affect the particle size. The reason that particles obtained from solvent coagulation were smaller than those from solvent displacement may involve the shear force presence only in the solvent coagulation. The sizes of particles prepared from different stirring rates, e.g., 300 rpm, 500 rpm and 700 rpm were compared. The results indicated that stirring rate of 300 rpm gave maximum particles size (600 - 800 nm) while the stirring rate of 500 rpm and 700 rpm gave particles with diameter of 200 - 300 nm and 100 - 150 nm, respectively (Figure 3.15).

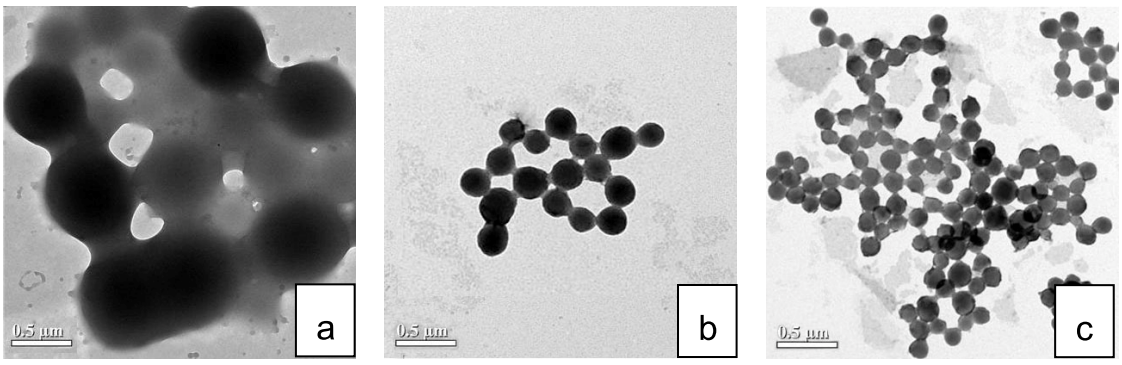

Figure 3.15 TEM photographs of ascorbic acid-encapsulate particles at various stirring rate (a) stirring rate of 300 rpm, (b) stirring rate of 500 rpm and (c) stirring rate of 700 rpm

Table 3.3 The hydrodynamic diameters determined from dynamic light scattering technique of ascorbic acid-encapsulated and un-encapsulated particle suspensions prepared from solvent displace (dialysis) and solvent coagulation.

Method	Hydrodynamic diameters (nm) (Average size ± SD)	
	un-encapsulated particle	Ascorbic acid-encapsulated particle
Dialysis (displacing DMSO with hexane-ethyl acetate mixture)	1629 ± 74	1805 ± 20
Solvent coagulation (DMSO = solvent, ethyl acetate = anti-solvent)	790 ± 73	1441 ± 91
Solvent coagulation (DMSO = solvent, hexane = anti-solvent)	774 ± 25	1102 ± 50
Solvent coagulation (water = solvent, iso-prapanol = anti-solvent)	743 ± 9	1028 ± 31

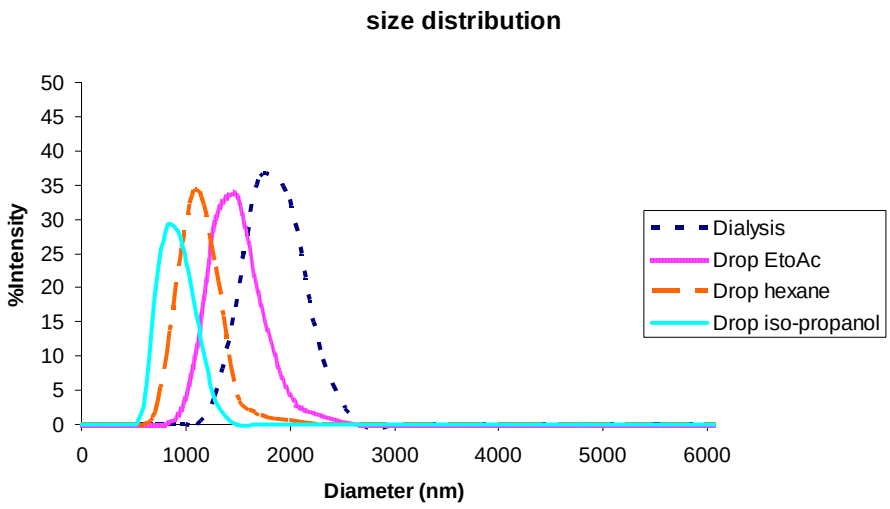

Figure 3.16 Size distributions of ascorbic acid-encapsulated particle prepared from dialysis and solvent coagulation.

3.3 Encapsulation efficiency

Encapsulation efficiency was determined by quantitating amounts of ascorbic acid in the particles, using HPLC equipped with UV detector. The encapsulation efficiency and loading was calculated according to the equation (1) and (2):

$$\% \text{ encapsulation efficiency (\%EE)} = \frac{\text{weight of encapsulated ascorbic acid}}{\text{weight of ascorbic acid used}} \times 100 \quad (1)$$

$$\% \text{ loading} = \frac{\text{weight of encapsulated ascorbic acid}}{\text{weight of polymer used}} \times 100 \quad (2)$$

Quantitation of ascorbic acid could be achieved by HPLC using column C-18 reversed-phase, the mobile phase 25 mM potassium dihydrogen phosphate adjusted to pH 3.0 with phosphoric acid. The flow rate was 1.0 mL/min and UV detection at 245 nm. Under such condition, ascorbic acid was evaluated at retention time of 1.6 – 1.8 min (Figure A2 – A6, Appendix A). Calibration curve was constructed from a series of ascorbic acid solutions freshly prepared in water at concentration of 2.5, 5, 10, 15 and 20 ppm. The obtained calibration curve was linear (Figure A1 and A4, Appendix A). Encapsulation efficiency and loading are shown in Table 3.4 (see Appendix A for calculation).

Table 3.4 % encapsulation efficiency (%EE) and % loading of 600 ppm ascorbic acid-encapsulate particles.

Samples	Method	% EE (± SD)	% loading (± SD)
Sphere A	Solvent displacement by dialysis (DMSO = solvent, hexane:ethyl acetate = anti-solvent)	7.72 ± 0.32	3.86 ± 0.16
Sphere B	Solvent coagulation (DMSO = solvent, ethyl acetate = anti-solvent)	21.39 ± 0.23	10.69 ± 0.11
Sphere C	Solvent coagulation (DMSO = solvent, hexane = anti-solvent)	23.90 ± 0.83	11.95 ± 0.41
Sphere D	Solvent coagulation (water = solvent, iso-propanol = anti-solvent)	32.29 ± 0.57	16.14 ± 0.28

The results indicated that solvent coagulation (water = solvent, iso-propanol = anti-solvent) gave maximum encapsulation efficiency and maximum loading. We speculate that solvent coagulation process might trapped ascorbic acid into the quickly formed spheres in a more effective manner comparing to the entrapment into the slowly formed spheres from dialysis process. The processes resemble a more entrapment of impurities into precipitate comparing to very low entrapment of impurities into the slowly form crystal.

This encapsulation process has been repeated 3 times and the shape of the ascorbic acid-encapsulated particles (Figure 3.17) together with encapsulation efficiency and loading were very repeatable. Thus we have concluded that the process of solvent coagulation with PV(OH) polymer can be used to encapsulate the water soluble ascorbic acid with the loading of ~16 % at the encapsulation efficiency of ~32 %. Although in this research the obtained ascorbic acid-encapsulated particles were less encapsulation and loading. But the obtained encapsulation efficiency and loading was more efficient than another research which encapsulated ascorbic acid [126-127].

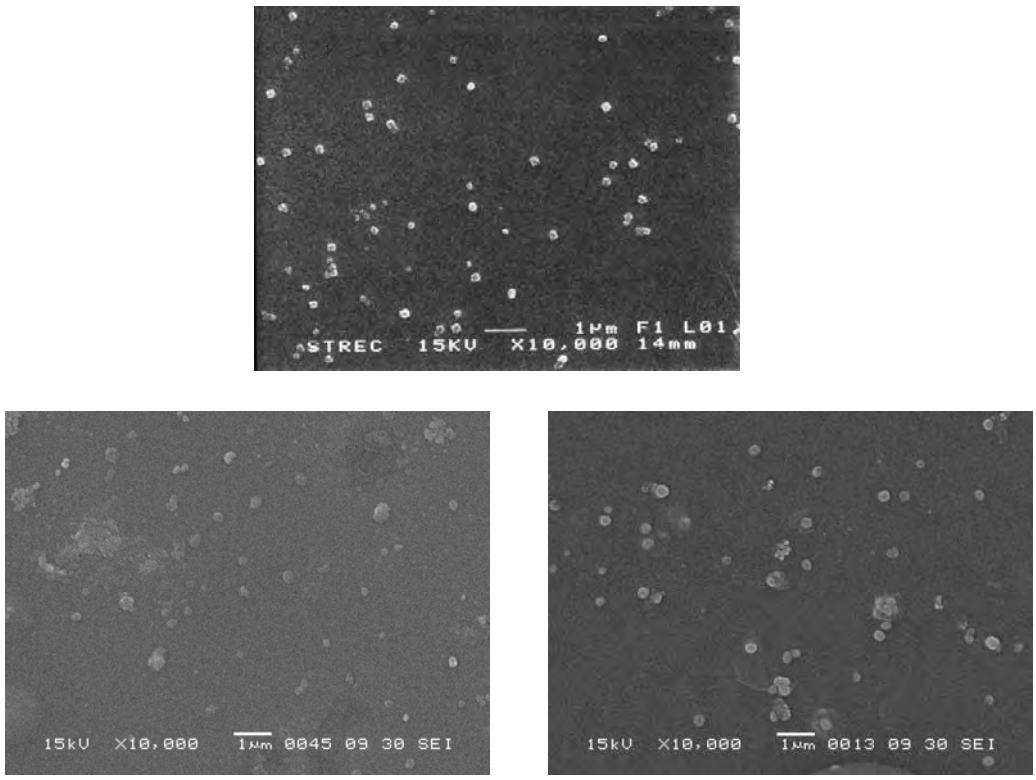

Figure 3.17 SEM photographs of ascorbic acid-encapsulate-particles (a) batch 1, (b) batch 2 and (c) batch 3 prepared by solvent coagulation (water = solvent, iso-propanol = anti-solvent) at the starting polymer concentration of 600 ppm

Table 3.5 Stability of free ascorbic acid and encapsulated ascorbic acid. (Sphere D)

Time (h)	Amount of ascorbic acid detected	
	% Free ascorbic acid	% Encapsulated ascorbic acid
0	100	100
24	66 ± 1.77	98 ± 1.20

3.4 Stability of the encapsulated ascorbic acid

Quantitative analysis of the encapsulated ascorbic acid (sphere D) indicated that they degraded significantly more slowly than unencapsulated ascorbic acid, (table 3.5), i.e., after being kept for 24 h about 34 % decrease of ascorbic acid was found in free ascorbic acid sample while only about 2 % decrease was witnessed in the encapsulated sample.

Thus we have concluded that the reverse micellar particle from PV(OH) polymer from the solvent coagulation process can enhance the stability of encapsulated ascorbic acid.

CHAPTER IV

CONCLUSION

In this research, reveres micellar particles could be fabricated successfully using PV(OH). The polymer was induced into particles by either solvent displacement or solvent coagulation processes. Particles obtained from solvent displacement were bigger than particles obtained from solvent coagulation. Type of solvent and anti-solvent used in the solvent coagulation process did not significantly affect the particle size. Ascorbic acid could be encapsulated into both reveres micellar particles. The results indicated that particles obtained from solvent coagulation process could encapsulate ascorbic acid more effectively (32.29 % encapsulation efficiency at 16.14 % loading) than those from solvent displacement (7.72 % encapsulation efficiency at 3.86 % loading). Encapsulated ascorbic acid was significantly more stable than un-encapsulated ascorbic acid.

With this first-time demonstrated reverse micellar particles from PV(OH), further study and encapsulation of other hydrophilic compounds should be carried out. In addition, release characteristic and compatibility in formulation should also be further investigated.

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APPENDICES

APPENDIX A

Encapsulation efficiency and loading of vitamin C loaded into PV(OH) reverse micellar particles

1) Solvent displacement by dialysis (DMSO as solvent, hexane-ethyl acetate as anti-solvent)

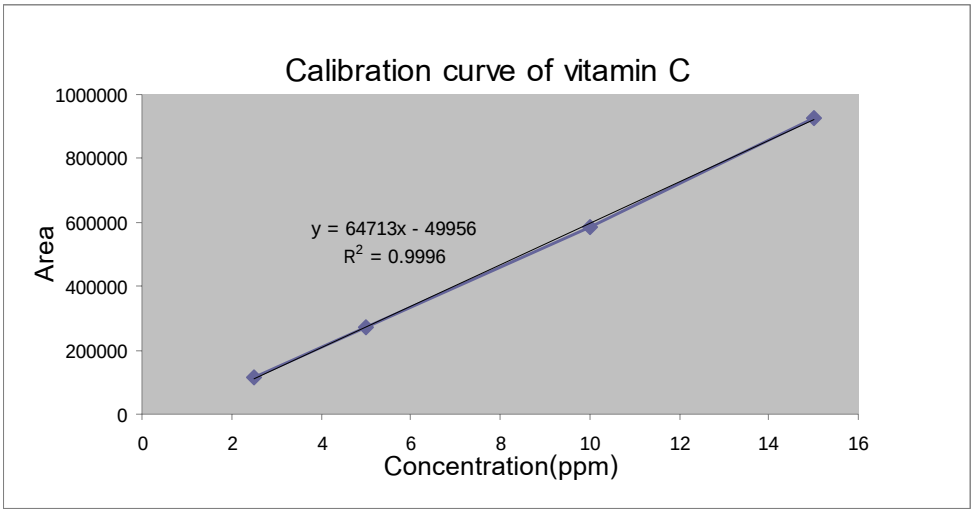

Figure A1 Calibration curve of vitamin C aqueous solution.

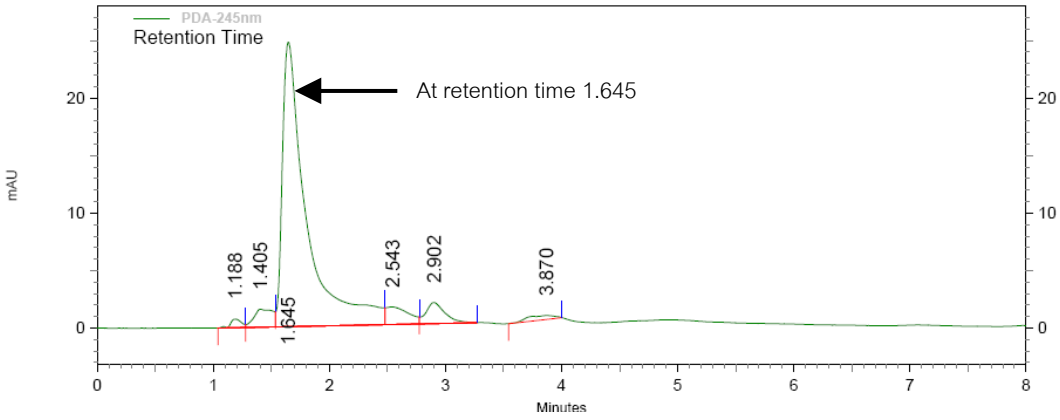

Figure A2 Retention time and area of vitamin C loaded into particles by dialysis method.

From the equation of calibration curve;

$$y = 64713X - 49956,\ R^2 = 0.9996 \quad (1)$$

The amount of vitamin C loaded into particles was calculated by equation (1);

350008 = 64713X – 49956

X = 6.1805

Volume of the product 5 mL gave content of vitamin C in reverse micellar particles 6.1805 ppm

ppm ⟶ mg/ 1000 mL ∴ 6.1805 ppm = 6.1805 mg/ 1000 mL

Product volumetric 5 mL gave content of vitamin C 0.061805 mg

∴ Product volumetric 75 mL gave content of vitamin C 0.9270 mg

Weight of vitamin C in reverse micellar particles from volumetric of the product 75 mL = 0.9270 mg

$$\% \text{ encapsulation efficiency (\%EE)} = \frac{\text{weight of encapsulated ascorbic acid}}{\text{weight of ascorbic acid used}} \times 100 \quad (2)$$

$$= (0.9270 \times 100)/ 12$$

$$= 7.72\ \%$$

$$\% \text{ loading} = \frac{\text{weight of encapsulated ascorbic acid}}{\text{weight of polymer used}} \times 100 \quad (3)$$

$$= (0.9270 \times 100)/ 24$$

$$= 3.86\ \%$$

2) Solvent coagulation

2.1) DMSO as solvent, ethyl acetate as anti-solvent

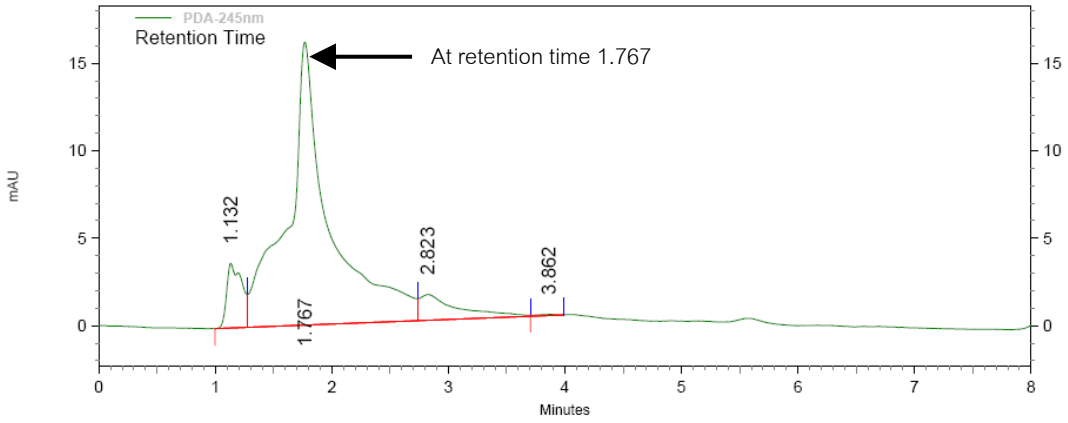

Figure A3 Retention time and area of vitamin C loaded into particles by solvent coagulation (DMSO as solvent, ethyl acetate as anti-solvent)

The amount of vitamin C loaded into particles was calculated by equation (1);

411625 = 64713X – 49956

X = 7.1327

Volume of the product 5 mL gave content of vitamin C in reverse micellar particles 7.1327 ppm

ppm ⟶ mg/ 1000 mL ∴ 7.1327 ppm = 7.1327 mg/ 1000 mL

Product volumetric 5 mL gave content of vitamin C 0.071327 mg

∴ Product volumetric 90 mL gave content of vitamin C 1.2838 mg

Weight of vitamin C in reverse micellar particles from volumetric of the product 90 mL = 1.2838 mg

% EE and loading were calculated by equations (2) and (3), respectively;

% encapsulation efficiency (%EE) = (1.2838x100)/ 6

= 21.39 %

% loading = (1.2838x100)/ 12

= 10.69 %

2.2) DMSO as solvent, hexane as anti-solvent

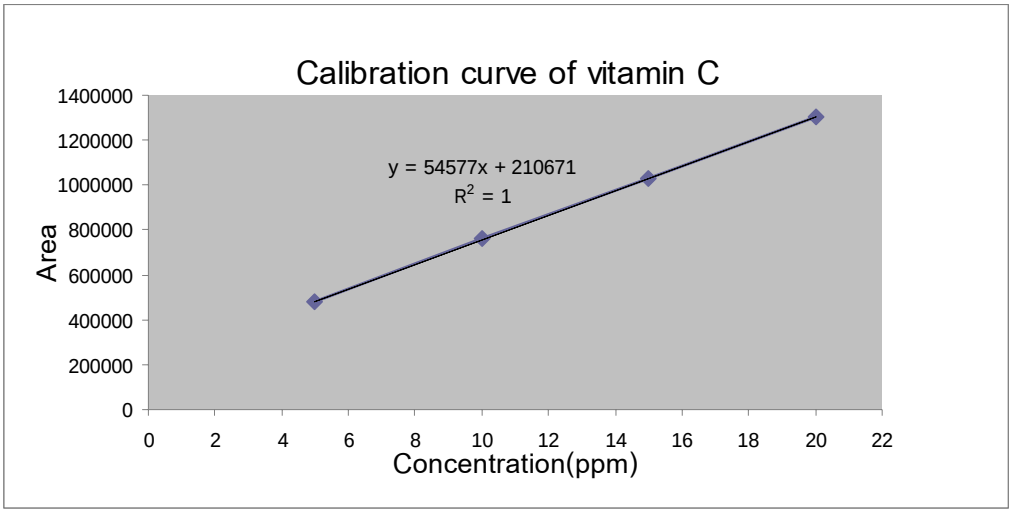

Figure A4 Calibration curve of vitamin C aqueous solution.

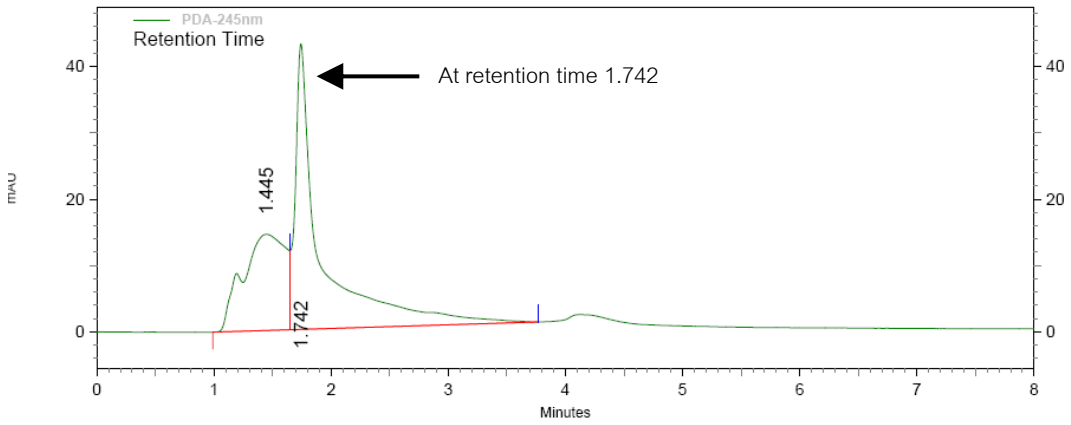

Figure A5 Retention time and area of vitamin C loaded into particles by solvent coagulation (DMSO as solvent, hexane as anti-solvent)

From the equation of calibration curve;

$y = 54577X + 210671, R^2 = 1$ (4)

The amount of vitamin C loaded into particles was calculated by equation (4);

645638 = 54557X + 210671

X = 7.9697

Volume of the product 5 mL gave content of vitamin C in reverse micellar particles 7.9697 ppm

ppm ⟶ mg/ 1000 mL ∴ 7.9697 ppm = 7.9697 mg/ 1000 mL

Product volumetric 5 mL gave content of vitamin C 0.079697 mg

∴ Product volumetric 90 mL gave content of vitamin C 1.4345 mg

Weight of vitamin C in reverse micellar particles from volumetric of the product 90 mL = 1.4345 mg

% EE and loading were calculated by equations (2) and (3), respectively;

% encapsulation efficiency (%EE) = (1.4345x100)/ 6

= 23.90 %

% loading = (1.4345x100)/ 12

= 11.95 %

2.1) Water as solvent, iso-propanol as anti-solvent

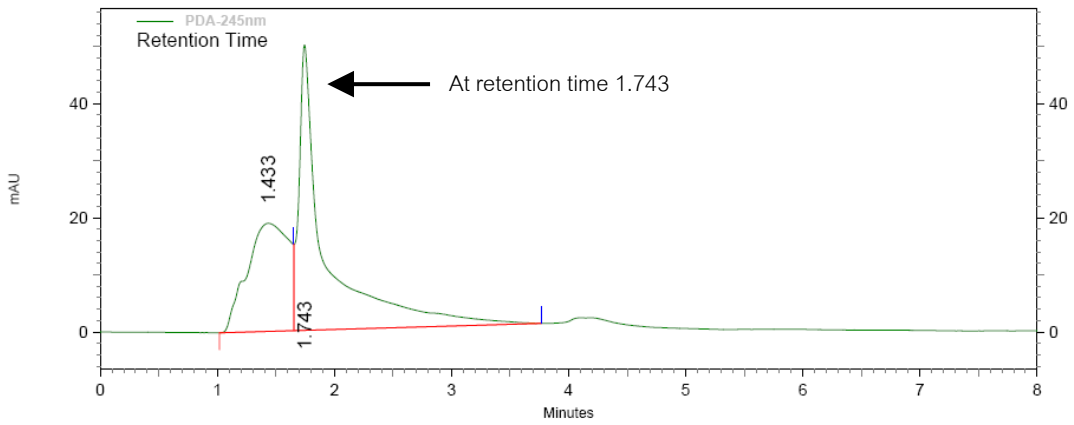

Figure A6 Retention time and area of vitamin C loaded into particles by solvent coagulation (water as solvent, iso-propanol as anti-solvent)

The amount of vitamin C loaded into particles was calculated by equation (4);

797929 = 54557X + 210671

X = 10.7641

Volume of the product 5 mL gave content of vitamin C in reverse micellar particles 10.7641 ppm

ppm ⟶ mg/ 1000 mL ∴ 10.7641 ppm = 10.7641 mg/ 1000 mL

Product volumetric 5 mL gave content of vitamin C 0.107641 mg

∴ Product volumetric 90 mL gave content of vitamin C 1.9375 mg

Weight of vitamin C in reverse micellar particles from volumetric of the product 90 mL = 1.9375 mg

% EE and loading were calculated by equations (2) and (3), respectively;

% encapsulation efficiency (%EE) = (1.9375x100)/ 6

= 32.29 %

% loading = (1.9375x100)/ 12

= 16.14 %

VITA

Ms. Patsara Chinwatvanich was born on October 5, 1982 in Ayutthaya,Thailand. She obtained a Bachelor's Degree of Engineering in Petrochemicals and Polymeric Material from Silpakorn University in 2005. After that, Miss Chinwatvanich has worked as a Packaging Analyst with S&J International Enterprises Public Company Limited. At the same time she also started her master study in the Program of Petrochemistry and Polymer Science at Chulalongkorn University, During her study Miss Chinwatvanich contributed academically to the Pure and Applied Chemistry International Conference (PACCON 2009) via a research presentation entitled "Preparation of Reverse Micellar Particles with Hydrophilic Core from Poly(Vinyl alcohol)"

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