FINAL YEAR PROJECT

A STUDY ON TWO STEP PREPARATION OF PCL80 HOLLOW COLLOIDOSOMES AT DIFFERENT OIL TO WATER PHASE RATIO

by

NUR ANIS NABILA BT MOKHTAR

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DECLARATION

I declare that this thesis entitled "A Study On Two Step Preparation Of Pcl80 Hollow Colloidosomes At Different Oil To Water Phase Ratio "is the result of my own research except as cited in the references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

Signature : .....

Name : Nur Anis Nabila Bt Mokhtar

Date : _____

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LIST OF ABBREVIATIONS

Abbreviation	Description
DCM	Dichloromethane
MS	Microsphere
MSs	Microspheres
O/W	Oil-in-water
W/O	Water-in-oil
W/O/W	Water-in-oil-in-water
PVA	Polyvinyl alcohol
PCL	Polycaprolactone
PCL80	Polycaprolactone (Mn= 80,000 kg/mol)
Mw	Molecular weight
D_n	Number average diameter
V_o	Volume of oil phase
C_{pol}	Concentration of polymer
V_w	Volume of water phase
C_{surf}	Concentration of surfactant
S1670	Sugar ester(sucrose stearate)
Wt%	mass fraction/ percentage by weight
FESEM	Field Effect Scanning Electron Micrscope

LIST OF UNITS

UNITS	Description
ºC	Degree celcius

ABSTRACT

This thesis presents a study of PCL80 potential in the formation of microcapsules. The PCL80 microcapsules are solid or colloidosomes (hollow). Colloidosomes are one of the microcapsules used for microencapsulation. This study aims towards various microencapsulation including encapsulated cells for development of tissue engineering, encapsulated drug in medicine and fragrance for personal care products (Thompson et al., 2012; Williams et al., 1999). The PCL80 was referred to Polycaprolactone with Mn values of 80kg/moles were used. The heterogeneous solution containing microcapsules were formed using special instrument. After fully rotary evaporation, the emulsion was formed. This study was carried out using PCL80 as structural polymer, PVA and Sugar ester as surfactant. Previous study by Shahidan et al., 2013 demonstrated the two step solvent evaporation method. . The PCL80 colloidosomes were prepared using solvent evaporation method. Similar optimum condition such as varied oil to water phase ratio and different surfactant concentration of 1.5wt% PCL/ 1.2wt% PVA were applied to prepare PCL80/PVA and PCL80/S1670 and the result showed PCL80 highly potential for colloidosomes formation. The formation of hollow colloidosomes using sugar ester as the surfactants has distributed the smaller number average size. Meanwhile, PVA has showed larger number average size. As expected, the PCL80 colloidosomes were birefringence under cross polarised light. It occurred due to the stress applied during solvent evaporation (Shahidan et al., 2014).

ABSTRAK

Tesis ini membentangkan satu kajian potensi PCL80 dalam pembentukan mikrokapsule. (PCL80 mikrokapsul adalah pepejal atau colloidosomes (berongga)). Colloidosomes ialah salah satu mikrokapsul yang digunakan untuk pemikrokapsulan. Kajian ini menyasarkan ke arah pelbagai pemikrokapsulan termasuk memerangkap sel untuk pembangunan kejuruteraan tisu, memerangkap dadah dalam ubat dan keharuman untuk produk-produk penjagaan peribadi(Thompson dll., 2012; Williams dll., 1999). PCL80 merujuk kepada Polycaprolactone dengan nilai-nilai berat molekul 80kg/mol telah digunakan. PCL80 colloidosomes disediakan menggunakan kaedah penyejatan pelarut. Penyelesaian heterogen mengandungi mikrokapsules ditubuhkan menggunakan alatan khas. Selepas penyejatan yang sepenuhnya berputar, emulsi dihasilkan. Kajian ini dijalankan menggunakan PCL80 sebagai struktur polimer, alkohol polivinil dan gula ester sebagai surfaktan. Pembentukan berongga colloidosomes menggunakan gula ester sebagai surfaktans telah membuktikan hasil menunjukkan saiz zarah lebih kecil.
Pengkaji terdahulu oleh Shahidan dll., 2013 menunjukkan dua langkah kaedah penyejatan pelarut. Keadaan optimum serupa seperti mengubah fasa minyak kepada nisbah fasa air dan tumpuan bahan surfaktan yang berbeza 1.5wt% PCL / PVA 1.2wt% digunakan ke atas menyediakan PCL80 / PVA dan PCL80 / S1670 dan keputusan menunjukkan PCL80 amat berpotensi untuk pembentukan colloidosomes. Seperti yang dijangkakan, PCL80 colloidosomes ialah dwirefringens di bawah silang cahaya terkutup. Ia berlaku disebabkan oleh tekanan yang dikenakan semasa penyejatan pelarut (Shahidan dll., 2014).

CHAPTER 1

INTRODUCTION

1.1 Background of study

Microcapsule is a small capsule used to contain drugs, dyes, or other substances and provide them temporarily inactive. Microcapsules have attracted features and highly potential in drug delivery. Microcapsule enables the controlled release of active ingredients in various industrial sectors such as medicine, food, and delivery drugs (Thompson et al., 2014). Microencapsulation is a process in which tiny particles or droplets are surrounded by a coating to give small capsules, of many useful properties. Microencapsulation methods would be characterized by obtaining particles with micrometric size, which is 1-1000 μm (Rodríguez et al., 2016).

Colloidosomes are an important subgroup of microcapsules whose shells consist of coagulated or solidify colloid particles (Shahidan et al., 2013). In this study, hollow colloidosomes formation would be explored the possibility of using polycaprolactone (PCL). Hollow colloidosomes would be prepared using PCL as structural polymer solvent evaporation method via two steps which are initial emulsification of polymer solution in a volatile organic solvent (oil phase) added to water phase. Then, followed by internal phase solvent evaporation or extract that result in hardening and precipitation of microparticles.

In this solvent evaporation method, polyvinyl alcohol (PVA) and sugar ester S1670 were used as polymer surfactant in water phase solution to stabilize the

mixture solution. The oil phase solutions were prepared using dichloromethane as solutes to PCL.

1.2 Problem Statement

Polycaprolactone is one of biopolymer used extensively in biomaterial field especially during the resorbable-polymer-boom in the 1970s and 1980s. However, inability of PCL that cannot being applied in high load bearing application cause less of PCL used in medical device and drug-delivery (Woodruff & Hutmacher., 2010). Thus, further studies using PCL with various molecular weight and application of advance material in the PCL could be improved its properties for return the importance of PCL.

There were many methods to control the colloidosomes particles from Coacervation, fluid extrusion, electrostatic deposition, microfabrication until sintering (Dinsmore et al., 2002). In this study, the two step solvent evaporation method would be employed. The solvent evaporation method was first presented by (Shahidan et al., 2013). However, the study of size control from previous study using two step solvent evaporation methods has not reported.

In this study, the main proposed are continuing the previous study by proposing controlling the ratio to control the size particle, the effect of using different surfactant to control size produced and study the effect different type surfactant on the size of colloidosomes.

PCL would be proven to be one of the suitable candidates as the structural polymer. To produce hollow colloidosomes, several parameters would be control such as oil to water phase ratio, surfactant concentration, and surfactant type in order to control the size. It is important to control of size since it allows flexibility in application and choice of encapsulated materials (Dinsmore et al., 2002).

The advance PCL production would be opened a wide range of opportunity to further evaluation and development for PCL application especially in medical application. This study area would be focus on commercially available PCL80.

1.3 Objectives

In this study, the objectives are:

i. To use PCL as potential structural polymer in preparation of hollow colloidosomes microsphere at different oil to water phase ratio.
ii. To investigate the effect of different surfactant and type.
iii. To observe the birefringence of PCL hollow colloidosomes.

1.4 Expected Outcome

In this study, the expected outcomes are able to explore the PCL potential as structural polymer of hollow colloidosomes microspheres, the effect of different surfactant and type to control the size. Besides, the birefringence effect of PCL hollow colloidosomes.

CHAPTER2

LITERATURE REVIEW

2.1 Tissue engineering

Tissue engineering is defined as understanding the tissue regeneration with the ultimate goal to regenerate cells and repair or replace the damage or lost tissue for clinical use (Li et al., 2015). Tissue engineering is rise from the field of biomaterials development and refers to the practice of combining scaffold, cells, and biologically active molecules into functional tissues (Williams et al., 1999). Tissue engineering scaffold would be designed to provide a support structure for the engineered tissue and also designed in a closed manner to protect implanted cells from the body's immune system or in open manner for the new cells to incorporate into the body (Williams et al., 1999). Microcarrier culture technology would be employ for producing great quantities of cells and microtissue for tissue engineering (Li et al., 2015).

2.2 Microencapsulation

Microencapsulation is a method of matrix wall to entrap a material in a microcapsule (Rodríguez et al., 2016). This matrix would release the active component when exposed to the condition such as pH, pressure, and environment (Rodríguez et al., 2016).

Microcapsule is a small particle with size in micrometer range (in 1-500µm) (Whateley, 2001). They are comprised of core (internal phase) and shell (outer layer). Core material is referring to entrapped materials such as active ingredients. Shell is referring to outermost layer that can be fabricating for the efficient release mechanism of active ingredients.

Encapsulation can be observed through the naturally occurring encapsulation systems for example are plant seeds and egg shells (Yow & Routh, 2006). In the plant seed process, seed would be move away from the parent plant since the plant required intermediate to transport their spores. These intermediate would be either abiotic component (such as water and air) or biotic components (such as animals or microorganism).

2.3 Microencapsulation Preparation Method

Over the years, there are too many method of encapsulation in producing microcapsules where colloidal particles used as templates for core-shell morphology. The other preparation method of microencapsulation including polymer precipitation by phase separation, Polycondensation interfacial polymerisation, layer-by-layer polyelectrolyte deposition, polymer growth by surface polymerisation, and copolymer vesicle formation (Yow & Routh, 2006).

Polymer precipitation by phase separation was involved two steps which are polymerization induced phase separation and followed with solvent evaporation. Polycondensation interfacial polymerisation is used two monomers which dissolved

in two different phase meet at the interface. It was applied in O/W and W/O emulsions. This method is focusing on using nylon and polyurethane in the shell formation (Yow & Routh, 2006).

Layer-by-layer polyelectrolyte deposition is a method that used electrostatic deposition to build layers of polyelectrolytes. Layers of charged molecule particle or polymer was used for the fabrication of nanoporous shell (Dinsmore et al., 2002). Polymer growth by surface polymerisation is a method that placed polymer shell in the colloidal template which modify the template surface and used as seed. The core would be removed using chemical etching for the purpose of hollow microcapsule formation (Yow & Routh, 2006).

Otherwise, copolymer vesicles is an amphiphilic block copolymer formed a thin membrane through self-assembly arrangement which turns into various ordered mesosphere (Fuji et al., 2012). Amphiphilic block copolymer would be formed when low molecular weight macromolecular surfactant is dispersed in water at low concentration while created water core (Fuji et al., 2012). For example, polymersome (micron-sized copolymer vesicles) is formed through electroformation method. This method was applied using electric field for the self-assembly occurrence (Yow & Routh, 2006).

2.3.1 Solvent Evaporation Method

The solvent evaporation is one of the methods used in produced microparticles from one to five hundred micrometer in range in the preparation, particularly for drug encapsulation and other therapeutic agents (Whateley, 2001). This method involve two steps, which are initially dissolved polymer in volatile organic solvent (oil phase) added into surfactant (water phase). Then, it would be followed by internal phase solvent evaporation that results in hardening and precipitation of core-shell microparticles to produce oil-in-water emulsion (Ito & Kawakami, 2015).

In this study, the methods of solvent evaporation would be used is to prepare the colloidosomes. By observed the benefits, it could be formed by self-assembly. Thus, it is better compared to other method because this method is simple, scalable and easy to conducted (Shahidan.N.N et al., 2013). For example, sintering used for binder the colloidosomes together (Dinsmore et al., 2002).

In other preparation of solid microsphere, it requires pre preparation method that absolutely required more time production (Thompson et al., 2012). Compared with this study, colloidosomes would be formed is required less time consuming. It also could be saves many chemical solution and energy efficiency related to hardening the shell.

2.4 Release mechanisms for microencapsulation

Release mechanism depends on the application of microcapsule that involved. Some release mechanisms needed to consider for producing good encapsulation. These consideration including diffusion/osmosis dissolution/dissolve, swelling, erosion and degradation, and external forces (Rodríguez et al., 2016; Yow & Routh, 2006).

The colloidosomes shell made up of Pluronic L31 was able to release a sulforhodamine B dye when response to temperature due to swelling process (Thompson et al., 2014). Another release is colloidosomes of amylase encapsulated CaC03 shell which was released due to enzymatic core material that erode the shell (Thompson et al., 2014).

The PCL microspheres controlled released shell was first demonstrated using acetazolamide drug. This drug was released due to high rate of dissolution in neutral pH when compared to acidic pH. However, the faster dissolution rate was undesirable due to not promote the contact membrane shell with the drug (Singh.N et al., 2011).

The release mechanism of our colloidosomes would be simple diffusion. It would be occurred due to different concentration inside colloidosomes and its environment. The drug comprising the shell would diffuse out down the concentration gradient. Water from the environment is diffuse inside the shell to replace the drug that had been diffuse out. However, the effective release mechanism of drug in this study has not yet demonstrated.

2.4.1 Diffusion

In the diffusion process, the molecules would be moves caused by a concentration gradient from the higher concentrations region to lower concentration region. It involves a shell having pores membrane that allows material from the core to moves out and from the environment to moves into the core depends on their concentration. The ability for the material to cross the porous membrane is known as diffusivity (Rodríguez et al., 2016). Large molecules have lower diffusivity than small molecules.

2.4.2 Dissolution

The dissolution release mechanism is the process of attraction and association of molecules of a solvent with molecules or ions of a solute. The dissolution rate in the surrounding medium is the one factor controlling this mechanism (Rodríguez et al., 2016). These caused by melting, solvent action, enzyme attack, hydrolysis, slow disintegration, chemical reaction (Yow & Routh, 2006). In order to achieve the total

release of the shell material, disintegration should be chosen especially for the release of flavour from dry products like cake mixes when water is added (Yow & Routh, 2006).

2.4.3 Swelling

Swelling is the process of water taking up whereby the polymer matrix increases its volume (Rodríguez et al., 2016). This process occurred when surrounding medium is diffusing into the swelling shells. Swellable shell is formed by using responsive materials as core materials (Yow & Routh, 2006). This material is response when exposed to external conditions such as pH, light and temperature.

2.4.4 Erosion and degradation

In this case, Erosion is the processes for shell materials become erode. The active agent is physically moving in the core which causes surface erosion. Erosion and degradation are the combination process, which material loss from the polymer bulk and splitting of polymer chains into oligomers and monomers (Rodríguez et al., 2016).

2.4.5 External forces

This mechanisms involved applied shear or pressure that caused shell ruptured. This force is needed to break the shell which determined by the shell material and thickness and could be controlled through the making processes (Yow

& Routh, 2006). Drug could be released depends on the changes in the external environment like pH, temperature, humidity for drug delivery system (Rodríguez et al., 2016).

2.5. Application of microencapsulation

Microcapsules are used in different application including food industry (Rodríguez et al., 2016) and oral drug delivery (Whateley, 2001). One of the advantages of using microcapsules is protecting core material from environment. For example, minerals or fats and oils in the food is protected from oxidation (Rodríguez et al., 2016). Another advantage is encapsulated drugs. For example, the unpleasant taste of an anti-malaria drug is masked by microencapsulation followed by tabletting (Whateley, 2001). Microencapsulated drug is used to improving the therapeutic effect, prolonging the biological activity and controlling the drug release rate (Kim et al., 2005).

2.6 Polycaprolactone

Polycaprolactone is a hydrophobic, semi-crystalline polymer; its crystallinity tends to decrease with increasing molecular weight but this type of structure enables easy formability at low temperatures. In terms of synthesis and physiochemical properties of polycaprolactone, Polycaprolactone is very soluble in certain solvents at room temperature including dichloromethane. However, PCL was not soluble in alcohol, petroleum ether and diethyl ether. It was very low solubility in acetone, 2-butanone, ethyl acetate, dimethyl formamide and acetonitrile. It can be blended with

other certain polymers in order to provide good properties such as stress crack resistance, dye-ability and adhesion for manipulating the rate of drug release from microcapsules (Woodruff & Hutmacher., 2010). The chemical structure of polycaprolactone was showed in figure 2.1.

Figure 2.1: Polycaprolactone (Woodruff & Hutmacher, 2010)

2.7 Surfactants

Surfactants are also known as Surface Active Agents are amphipathic molecules, which have a hydrophilic part and a hydrophobic part. Surface active agents are a substance that reduces the surface tension of a liquid by improves its spreading properties once they are dissolved in liquid. Poly (vinyl alcohol) (PVA) and Sugar ester (S1670) would be used as commercial polymer surfactant. The chemical structure of amphiphilic molecule was showed in the figure 2.2.

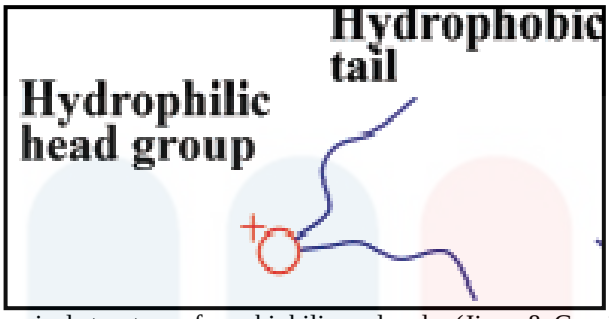


Figure 2.2: The chemical structure of amphiphilic molecule (Jiang & Granick., 2008)

2.7.1 PVA

The effect of the oil phase on control of the interfacial tension between the oil and water phases would be able to investigate in order to control the size of PCL80 particles. The addition of a hydrophilic surfactant or solvent into the water phases would reduce interfacial tension, which allows easy control of the interfacial tension between the oil and water phases (Ito & Kawakami, 2015).

Poly (vinyl alcohol) (PVA) is one of the most commonly used stabilizers in the encapsulation of different active molecules because it is well-known as hydrophilic polymer, biocompatible polymer, have good mechanical strength, low fouling potential and lasting temperature stability and pH stability (Iqbal et al., 2015). This stabilizer would be able to maintain the stability state of an emulsion system making it a suitable candidate to be used in encapsulation. The chemical structural of PVA with the linear formula is shown in the Figure 2.3..

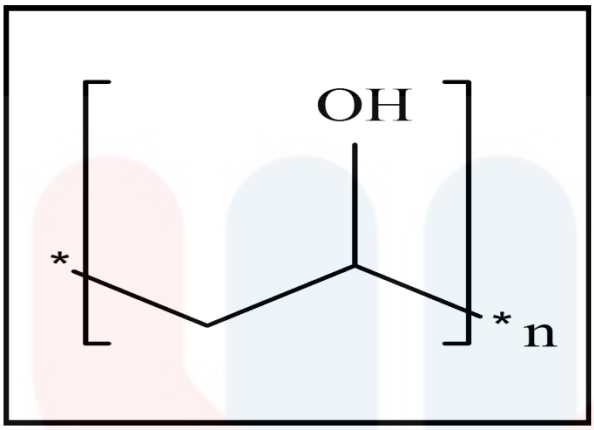


Figure 2.3: The chemical structural of polyvinyl alcohol with the linear formula (Sigma-Aldrich, 2016)

2.7.2 Sugar Ester

Another famous surfactant is sugar ester. Sugar ester is able to reduce surface tension (Sampaio et al., 2012; Szuts et al., 2012). When sugar ester surfactant was added, its molecule would oriented between two faces with non-polar end in non-polar phases while polar end in polar phases, which reduce interfacial tension. Sugar ester is non-ionic biosurfactant. It was produced from renewable resources, for instance fructose monoesters synthesize by lipase. It would reduce chemical side effects absolutely (Sampaio et al., 2012). Moreover, it would be employ high emulsifying, stabilizing and detergency effect (Neta et al., 2013). Sugar ester surfactant is widely used due to their therapeutic potential with antitumor activity, plant growth inhibition and antibiotic activities (Neta et al., 2013).

2.8 Hollow Colloidosomes

Colloidosomes are microcapsules which colloidal particles formed the shell layer (Thompson et al., 2014). Figure 2.4 show the microparticles which particles are differ between hollow particles and hollow colloidosomes particle.

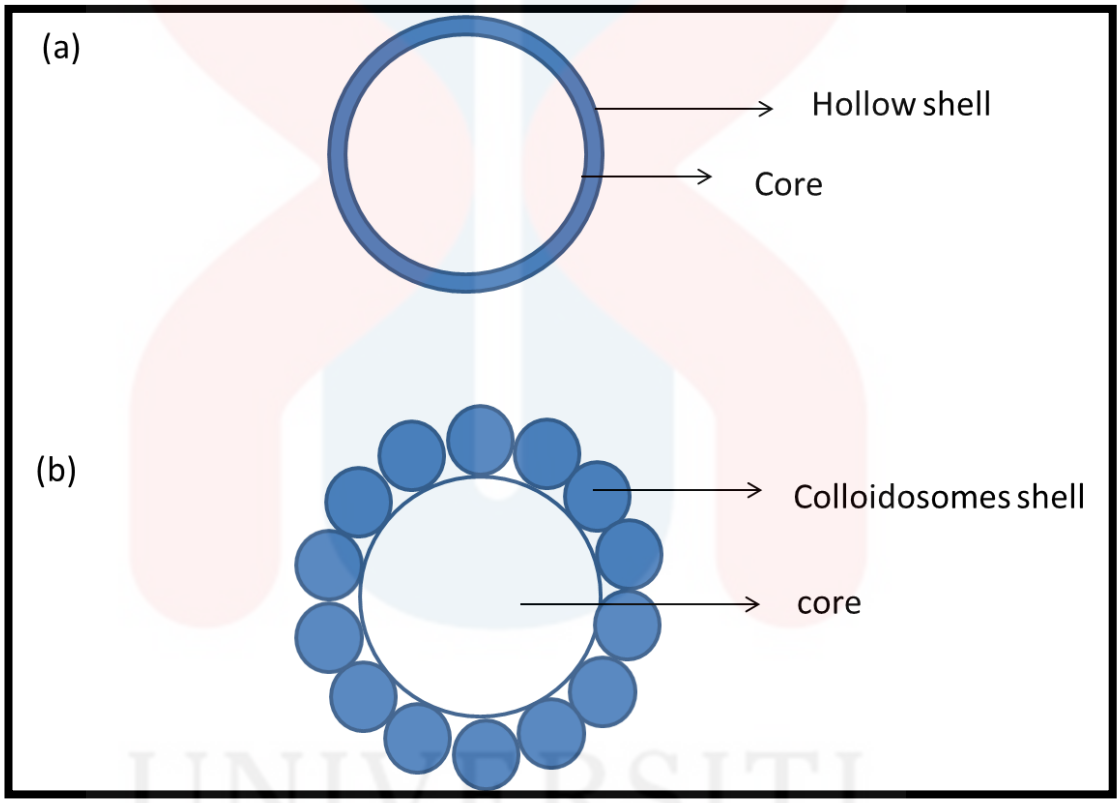


Figure 2.4: (a) hollow particle. (b) Hollow colloidosomes.

According to figure 2.4(a), hollow particle is a powder contained hollow structure which is a solid shell (Fuji et al., 2012). Hollow particle have special properties of low densities, thermal insulation and optical activity that potentially applied in the field of drug delivery, catalysis and as contrast-enhancing reagent in optical imaging (Fuji et al., 2012). This hollow particle was prepared by coated desired materials on the surface of shell followed by removal of the core once the

coating process of hollow shell was completed. The core materials included organic spheres, inorganic particles and metal crystals.

According to the figure 2.4(b), colloidosomes are defined as hollow, elastic shells which permeability and elasticity can be precisely controlled. Colloidosomes can be produced using precise control of size, permeability, mechanical strength, and compatibility (Dinsmore et al., 2002). Therefore, colloidosomes are able to provide efficient encapsulation in structures based on that controllable parameter (Singh. et al., 2011).

The purpose of controlling size would be able allowing either flexibility or compatibility. The flexibility and compatible shell employ speciality encapsulated core material such as biomolecules and cells. Meanwhile, the controlling permeability would be allows vary of selective material core and time release (Dinsmore et al., 2002). Reducing the rate of permeability would employ the contact between drug and the shells (Singh et al., 2011).

2.9 Classification of colloidosomes

Colloidosomes are produced from the controlled self-assembly of colloidal particles onto the emulsion droplets. They can be classified into three classes, which are water-in-oil emulsion based colloidosomes, oil-in-water emulsion based colloidosomes, and water-oil-water emulsion based colloidosomes (Singh., et al., 2011).

2.9.1 Water-in-oil emulsion based colloidosomes

Water in oil(w/o) is an emulsion having oil continuous phase with water droplets which occurred due to high weight ratio oil (Fuji et al., 2012). Aqueous solution is added into oil could be emulsified in presence of colloidal particles to produce water-in–oil emulsion (Singh et al., 2011).

For example, w/o involved controlled self-assembly which involves three steps. Firstly, a suspension would be emulsified in an immiscible fluid containing colloidal particles that able adsorb on the surface of the emulsion droplets. Secondly, the droplet surface would be completely covered the particles when formed an elastic shell by locking the particles together. This particle is called as colloidosomes which observing the interstices between the particles form holes in optical microscope (Dinsmore et al., 2002).

The capsules are transferred by centrifugation into a solvent that is typically same as the internal phase. This eliminates the interface between the internal and external fluids and would be allows the interstitial holes to control the colloidosomes permeability reported by (Dinsmore et al., 2002). Figure 2.5 shows the visualization of the self-assembly for colloidosomes.

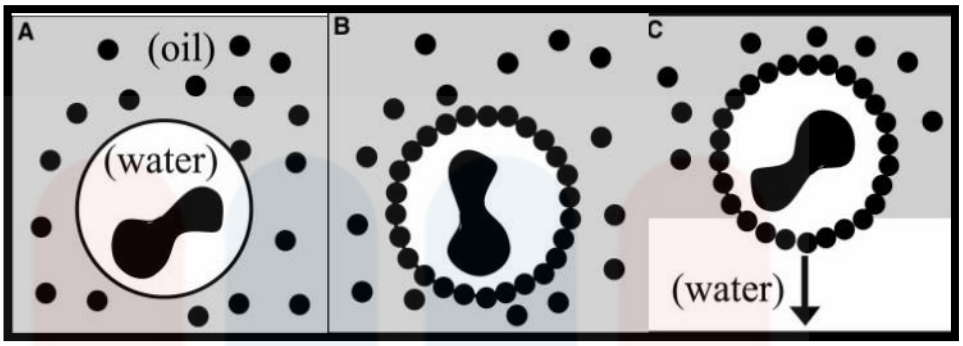


Figure 2.5: (A) water in oil phase solution is prepared. (B) Particle adsorb onto the surface. (c) Apply centrifugation to encapsulate oil droplets (as core) with the shell of particles from water phase, which produce emulsion (Dinsmore et al., 2002).

2.9.2 Oil-in-water emulsion based colloidosomes

This emulsion occurred when oil phase is added into aqueous solution (water phase), which emulsified with the presence of surfactant. Surfactant is used to stabilize the emulsion (Singh et al., 2011). For example, Hollow solid $CaCO_3$ of vaterite spheres was obtained through o/w interface reaction between carbonate ions in aqueous phase with diffuse Ca^{2+} (Fuji et al., 2012). Hollow bead titanate particles was formed via o/w interface through condensation reaction of titanium butoxide (Fuji et al., 2012). Latest, PCL colloidosomes formation through the accelerated solvent evaporation by using o/w template (Shahidan et al., 2013).

2.9.3 Water-oil-water emulsion based colloidosomes

Water-oil-water emulsion is the multiples emulsion, which addition drop of an aqueous suspension of particles is formed in an oil phase (Singh et al., 2011). For instance, latex colloidosomes was formed by addition drop solution containing latex particles in an oil phase. The addition water drop in oil is coated with adsorbed

particles through oil in water interface, which produce spherical water in oil in water film supported by latex particles (Singh et al., 2011). Besides, according to w/o/w emulsion preparation, PHB microspheres was prepared using solvent evaporation method is founded to be a good candidates for drug carrier (Goreva et al., 2008)

2.10 Instrumentations

2.10.1 Optical Microscope.

In this study, the size would be determined using optical microscope. Generally, optical microscope is required for the examination of the size and it's hollow of the sample. Optical microscope is used for resolutions down to roughly the wavelength of light (about half a micron). By using this instrument, we would also be able to identify whether they are hollow or solid particles.

2.10.2 Polarized Microscope

Polarizing microscope would be used polarized light to investigate the optical properties of the sample. Polarized light give the information about magnetic fields, chemical interaction, crystal structures, quality variations and mechanical stress can all affect the polarization of a beam of light. The image of optical microscopy would be obtained by using light transmission. The light would be passing through a polarizer and an analyser. The polarizer between the object and the observer's eye is

called the analyser. The analyser must always be removable to permit special method of observation with only one polarizer in polarizing microscope (Patzelt., 1985).

The application of polarized-light microscopy is used increasingly in the industrial laboratory. For instance, strain measurements in glasses, glass to metal seals and plastics, measurement of the birefringence of fibres or orientation axis of crystals used in the industry. The formation of the birefringence might be caused by fibrous or laminar particles embedded in a liquid or synthetic resin. Polarization microscopy is the only available method for studying the structure, formation and examining the effects of chemicals, drugs, or environmental conditions on cellular structures in vivo (Patzelt., 1985).

Polarized light is a contrast enhancing technique that improves the quality of the image obtained with birefringent materials when compared to other techniques such as dark field and bright field illumination, differential interference contrast, phase contrast, Hoffman modulation contrast, and fluorescence (Patzelt., 1985).

Polarized light microscopes have a high degree of sensitivity and can be utilized for both quantitative and qualitative studies targeted at a wide range of anisotropic specimens. Advances studies made over the past few years have enabled biologists to study the birefringent character of many anisotropic subcellular assemblies (Patzelt., 1985).

2.10.3 Scanning Electron Microscope

Scanning electron microscope (SEM) in this study will be used for observation of specimen surfaces. When the specimen is irradiated with a fine electron beam (called an electron probe), secondary electrons are emitted from the specimen surface. Topography of the surface can be observed by two-dimensional scanning of electron probe over the surface and acquisition of an image from the detected secondary electrons. SEM is one of the major characterization techniques used routinely to study topography of solid sample. SEM will be produced image by probing the specimen with a focused electron beam that is scanned across the specimen. SEM will be used scanning the electron probe over surface and collect image signal-display. SEM images will be demonstrate that the wall thickness of the hollow spheres that can be readily controlled by varying the number of nanoparticle-polymer deposition cycles, and the size and shape will be determined by the morphology of the templating colloid (Caruso., 1998).

CHAPTER 3

MATERIAL AND METHOD

3.1 Chemical and reagent

Polycaprolactone with M_n values of 80kg/mol (PCL80, $M_{n=}$ 80kg/mol) and Dichloromethane, CH_2Cl_2 (98%) were purchased from Sigma Aldrich, United State. Sugar ester S1670 were purchased from Mitsubishi-Kagaku Foods Corporation, Japan. Polyvinyl alcohol, PVA (98% hydrolysed Mn=13-28kg/mole) were also purchased from Aldrich and used as received.

3.2 Two Step Solvent Evaporation Method

Firstly, The water phase was prepared using polyvinyl alcohol dissolve in distilled water shaking in shaker water bath at 75ºC until it was fully dissolved. This water phase was prepared at concentration 1.2wt% which referred to 6g of polyvinyl alcohol dissolved in 500ml distilled water. The experimental of this study were conducted once the water phase cooled to room temperature. Secondly, oil phase was prepared using the same preparation method in water phase.

In order to conduct this study, the oil phase solution was added into water phase solution by using syringe pump over 30min at feeding rate 2.5ml/min. The oil in water (O/W) emulsion was homogenized at 9000rpm using IKA homogenizer. Immediately, the emulsion was rotary evaporation at room temperature to remove volatile solvent and trigger the formation of the hollow colloidosomes particle. The

emulsion was rotary-evaporated approximately 10 minutes at 95rpm. The figure of this method can be observed using figure 3.1 below.

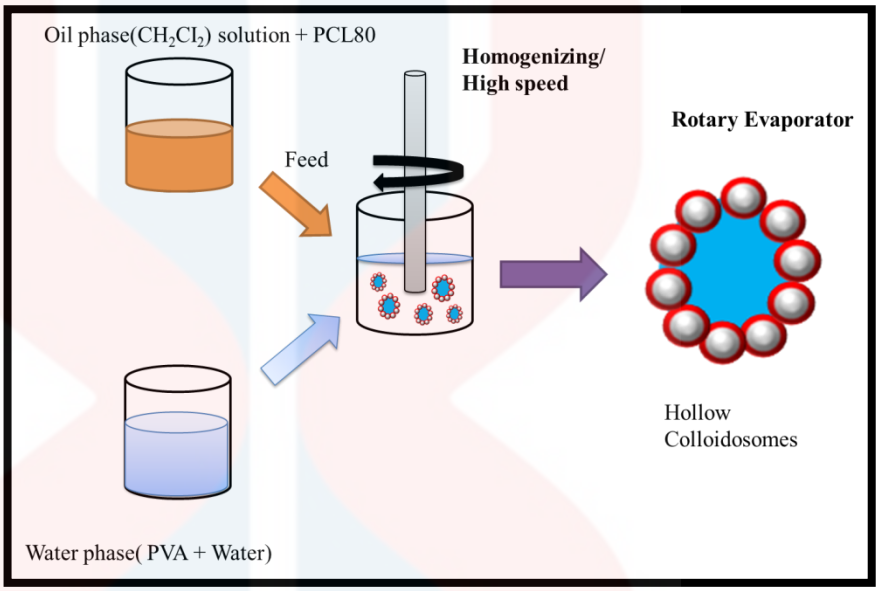


Figure 3.1: Solvent evaporation method

In this study, the solvent evaporation preparation method is employed from prior studies that produce hollow colloidosomes (Shahidan et al., 2013). Solvent evaporation would be started with the preparation of concentrated oil in water emulsion using a feed of CH_2Cl_2 or structural polymer solution (Shahidan et al., 2013).

The preparations of colloidosomes using accelerated solvent evaporation, which colloidosomes would be prepared containing shells of partially fused PCL particles. PCL is stabilized by using polymer surfactant which is PVA. The shell was produced. Volatile solvent which is CH_2Cl_2 was partially evaporated once evaporation was carried out immediately. The volatile solvent was fully evaporated that caused complete shell formation. The polymer cores of the droplets filled with

water due to the CH_2Cl_2 removal and the shell was formed completely. The figure preparation of colloidosomes using accelerated solvent evaporation was showed in the figure 3.2.

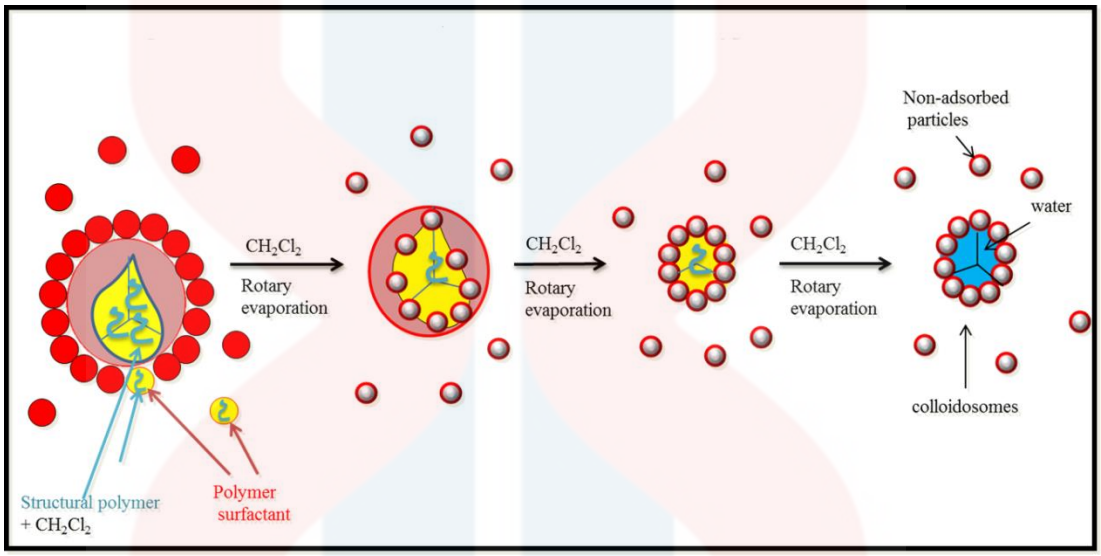


Figure 3.2: Preparation of Colloidosomes Using Accelerated Solvent Evaporation (Shahidan et al., 2013)

3.3 Characterization

For characterization, the hollow colloidosomes was characterized by using Meiji MT800 optical microscope, DM750 Leica polarized microscope and also the morphology structure was observed by using FESEM Scanning Electron Microscope (SEM). The samples were analysed and identified using Meiji MT800 optical microscope with an attached camera and objective lens of 5x , 10x, 20x for the purpose of particle sizing. The Progres Capture Pro 2.8.8 software was used to obtain the distribution and size of the particles in the samples. Particle size distribution of colloidosomes at different ratios, were determined by optical imaging and droplet

size analysis using an optical microscope. One drop of the sample and one drop of distilled water were transferred to the slide.

DM750 Leica Polarized microscope was used in Science Nuclear Department, Faculty Science and Technology, UKM. This microscope was used to observe the size and optical properties on PCL80 hollow colloidosomes.

FESEM was used to characterize the morphology of the PCL hollow colloidosomes. The sample had been put on the carbon tape and dried overnight at room temperature. Then, the sample would be coated with carbon coating prior to characterization.

CHAPTER 4

RESULT AND DISCUSSION

4.1 Introduction

This study was conducted at different system and oil to water phase ratio. We have used system abbreviation as (polymer/surfactant) which is PCL80/PVA and PCL80/S1670. Same concentration for PCL80 and different concentration surfactant were employed which is PCL80 at 1.5wt% while S1670 at 1.2wt% and 0.1wt%.The concentration polymer 1.5wt% for entry 1 which referred to 0.75g mass of PCL80 was dissolved in 50ml DCM. Otherwise, the other concentration polymer was followed. The concentration surfactant 1.2wt% which referred to 6g of polyvinyl alcohol was dissolved in 500ml distilled water. The colloidosomes preparation conditions provided and size distribution data was showed in table 4.1.

Table 4.1: Colloidosomes preparation conditions provided and size distribution data. [a] volume of oil, [b] volume of water , [c] concentration of polymer, [d] concentration of surfactant, [e] number average diameter.

Entry	System	V_o [a]/ml	V_w [b]/ ml	Ratio	C_{Pol} [c]/ wt%	C_{Surf} [d]/ wt%	D_n [e]/ um
1	PCL80 (1.5wt %) / PVA (1.2wt %)	25	50	0.5:1	1.5	1.2	2.590
2	PCL80 (1.5wt %) / PVA (1.2wt %)	50	50	1:1	1.5	1.2	3.720
3	PCL80 (1.5wt %) / PVA (1.2wt %)	75	50	1.5:1	1.5	1.2	3.830
4	PCL80 (1.5wt %) / PVA (1.2wt %)	100	50	2.0:1	1.5	1.2	4.060
5	PCL80 (1.5wt %) / PVA (1.2wt %)	125	50	2.5:1	1.5	1.2	4.120
6	PCL80 (1.5wt %) / S1670 (1.2wt %)	25	50	0.5:1	1.5	1.2	0.753

7	PCL80 (1.5wt %) / S1670 (1.2wt %)	75	50	1.5:1	1.5	1.2	0.815
8	PCL80 (1.5wt %) / S1670 (1.2wt %)	125	50	2.5:1	1.5	1.2	1.079
9	PCL80 (1.5wt %) / S1670 (0.1wt %)	75	50	1.5:1	1.5	0.1	1.096
10	PCL80 (1.5wt %) / S1670 (0.1wt %)	125	50	2.5:1	1.5	0.1	1.434
11	PCL80 (1.5wt %) / S1670 (0.1wt %)	25	50	0.5:1	1.5	0.1	0.869

4.2 Effect ratio of water phase and oil phase on Colloidosomes preparation.

The different volume ratio of oil to water phase have affect the size of colloidosomes particle. In this study, we have employed different oil phase volume only and remain used the same volume of water phase. We have used oil phase at volume 25ml, 50ml, 75ml, 100ml, and 125ml. According to our conducted experiment, we have observed that increased the volume of oil phase has increased the size of the particles in which the number average diameter increased.

Large amount of volume oil phase used would be increased the particle diameter (Achouri et al., 2012; Singh et al., 2011). We have observed that the average particle sizing increased when increased the volume of oil phase (Table 4.1, entry 1-5 and entry 6-8).

According to the Table 4.1, entry 10 and 11 were not followed the same condition in which normally we have used vacuum system at room temperature but entry 10 and 11 were used 10º C that's why it does not followed the trend. By lowering the temperature might decrease the evaporation rate.

In this study, we found that increased the oil phase volume had increase the yield of colloidosomes. The yield of colloidosomes was influenced by the coagulum formation (Shahidan et al., 2013). Figure 4.1 shows different particles size was obtained from high volume of oil phase to lower volume of oil phase, from (a) to (e) using surfactant PVA.

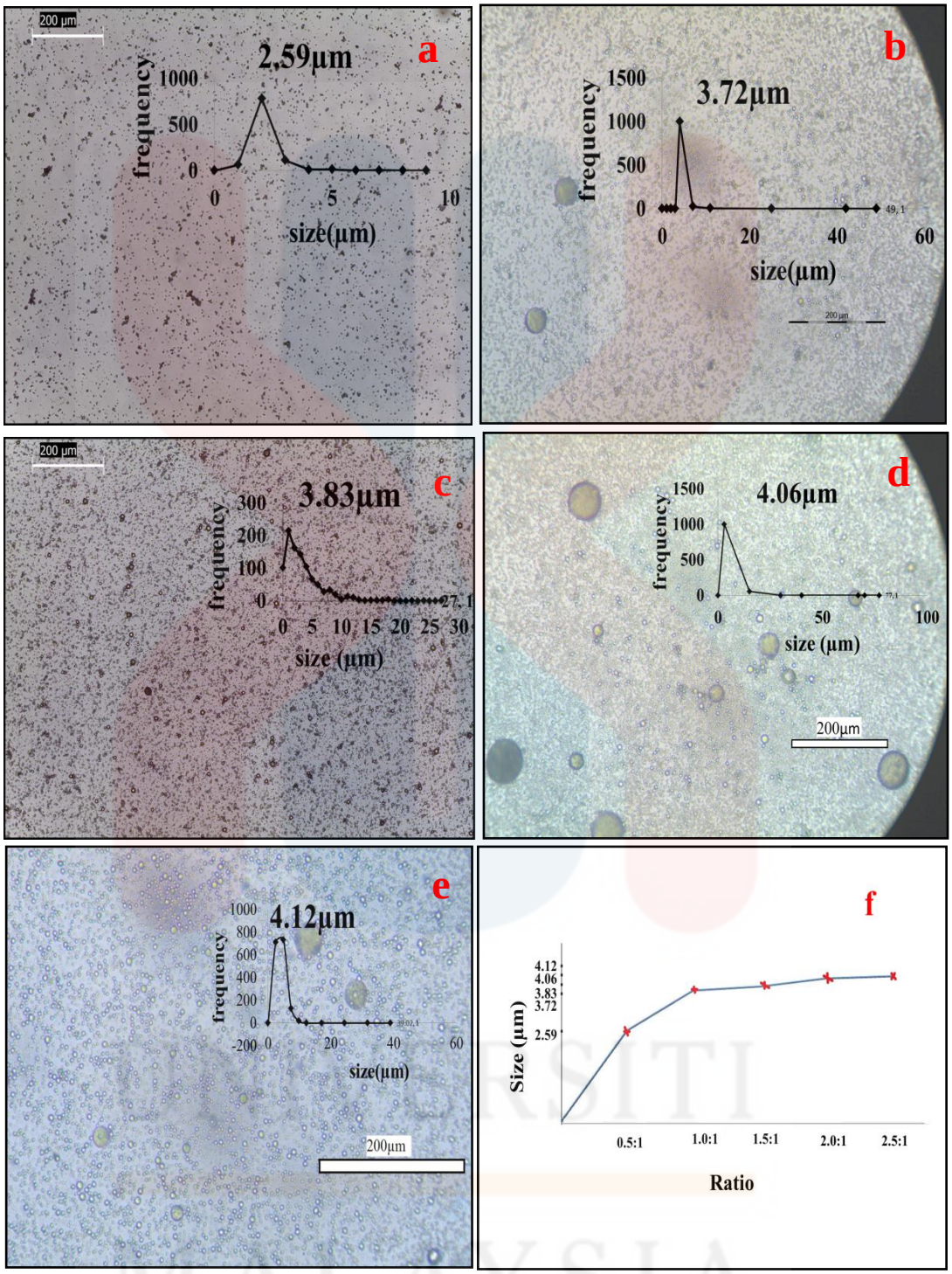


Figure 4.1: The colloidosomes prepared using different volume of oil phase (a)-(e) was referred to entry 1-5. (f) Showed the graph of number average diameter against ratio using surfactant PVA.

Figure 4.2 showed the colloidosomes particles using Sugar ester surfactant. The number average diameter increase from (a)-(c). The graph provided below (d) shown the increase number average diameter from different concentration of sugar ester perceptively.

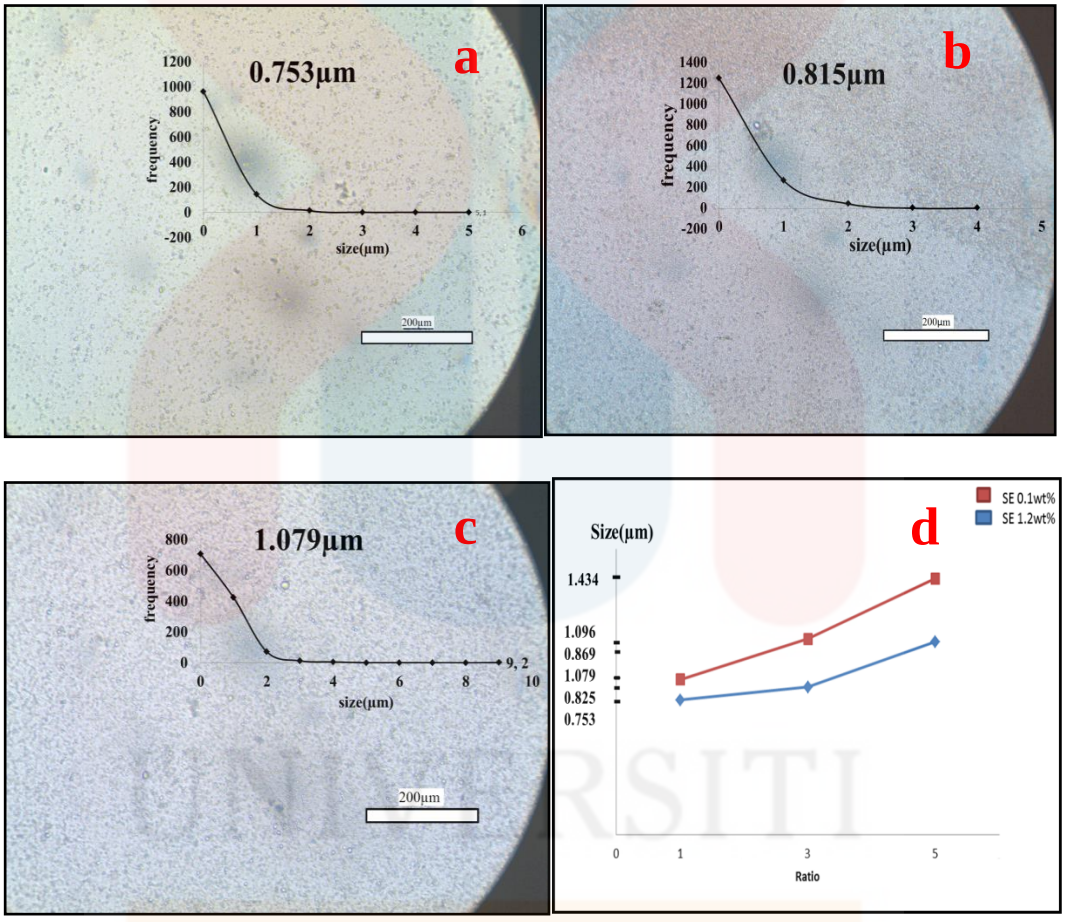


Figure 4.2: colloidosomes prepared using sugar ester surfactant (a)-(c) referred to entry 6-7. (d) The graph above showed the trend comparison between sugar ester surfactant at concentration 1.2wt% and 0.1wt%.

4.3 Effects of different surfactant type and concentration

The surfactant concentrations influence the colloidosomes formation. Based on the figure 4.1, using entry 1,3 and 5 shows average particle size of colloidosomes PCL80 in PVA surfactant systems. Figure 4.2 have showed the average particle size for sugar ester surfactant which was prepared using same polymer surfactant concentration (C_{Surf}) at 1.2wt% and 0.1wt%. This information was very important to the effect of sugar ester on the stability of PCL80 emulsion. Large droplet of emulsion tends to have reduction on the particle size distribution when small-molecule surfactant was added (Sampaio et al., 2012) It was occurred because of the surface tension have reduced and the emulsion stability had improved (Sampaio et al., 2012). In our study, the surfactant sugar ester was added that reduced the average particles sizing. Thus, the colloidosomes particles were larger in PVA surfactant compared to S1670 surfactant. Figure 4.3 showed the colloidosomes particle between different concentration of surfactant PVA and S1670 at 1.2wt% and S1670 at 0.1wt%.

We have observed that reduced the surfactant concentration had increase the size of the particles. Absolutely, it was been proven that the particle decrease as the surfactant concentration increase (Kung, 1997). The increase in surfactant concentration indicates increase in aggregation but not show any drastic changes in the size or shape of micelles (Glatter et al., 2001). Figure 4.3 below showed the colloidosomes particle from high magnification view and low magnification view. From a-b (entry 9 on table 4.1), the average particle size was smaller than c-d, (entry 7 on table 4.1).

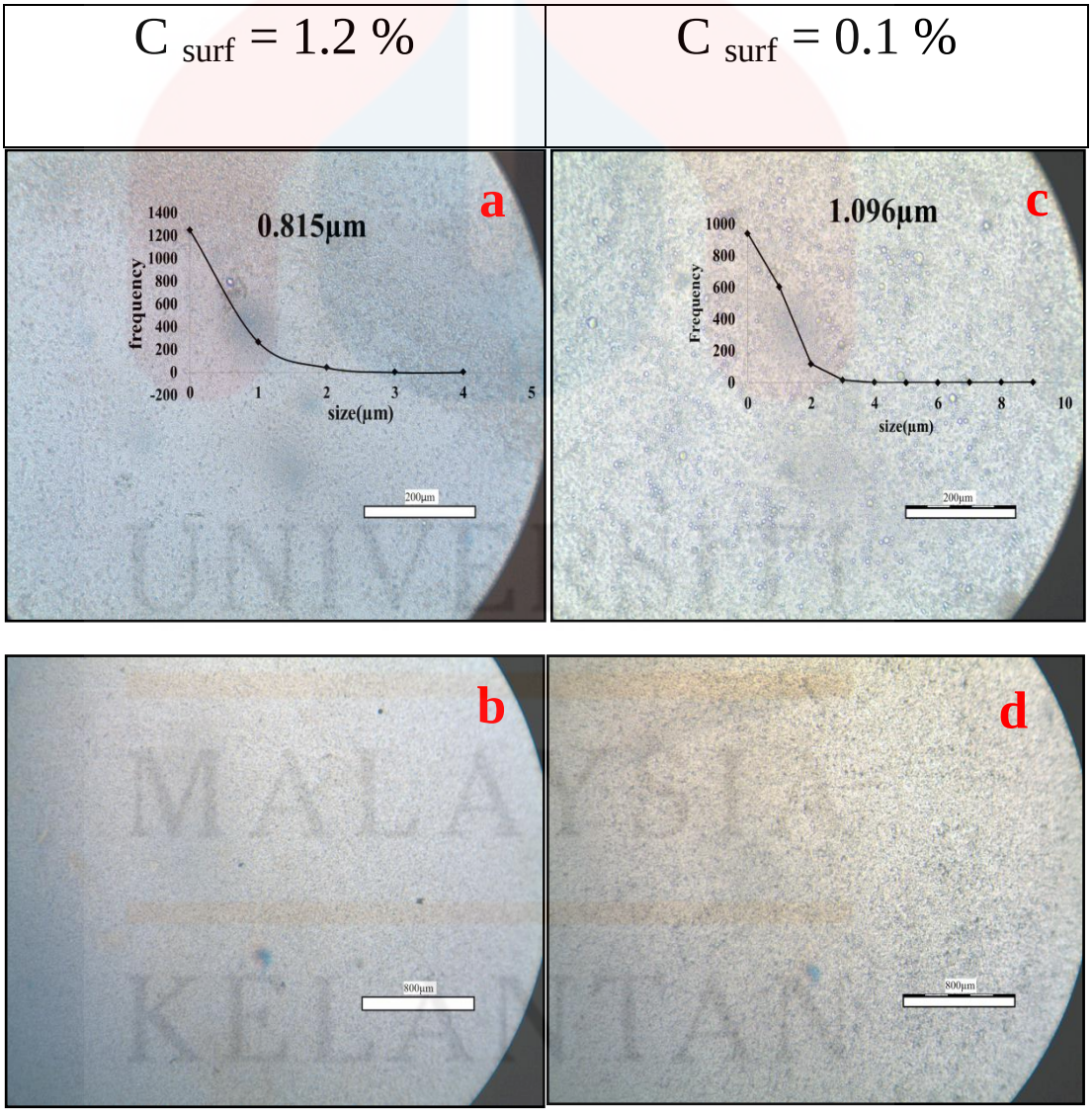


Figure 4.3: The colloidosomes particle and size distribution of PCL80/S1670 (a) and (b) using C_{surf} of 1.2wt% (entry 7, table 4.1). (c) and (d) using C_{surf} of 0.1wt% (entry 9, table 4.1)

The main system was observed that showed the colloidosomes particle based on the different type of surfactant which is system used ratio 1.5:1 volume oil to water phase. These particle size distributions were mentioned in figure 4.4.

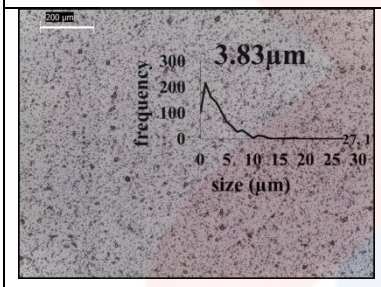
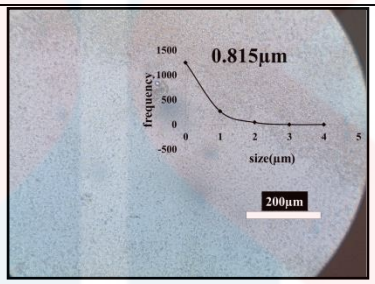
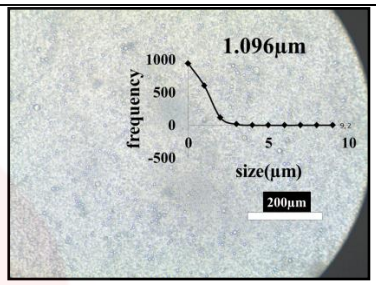
PCL80/PVA (1.2wt %)	PCL80/S1670 (1.2wt %)	PCL80/S1670 (0.1 wt %)
		

Figure 4.4: colloidosomes particles from the same ratio 1.5:1 volume oil to water phase respectively

4.4 The Birefringent Effect and the Morphology Study of Polycaprolactone Hollow Colloidosomes.

PCL is one of the semi crystalline polymer (Woodruff & Hutmacher., 2010). Therefore, this polymer showed birefringent. This birefringent was observed based on the interference colour exhibited by the particle. These interference colour exhibited due to (1) the birefringence of the material, (2) the thickness and (3) the orientation (Ingham., 2013). The Michel-Levy Interference Colour Chart can be used to identify the thickness of the hollow particle since it shows certain colours at certain thickness. The birefringent image showed green, red, blue coloured shell or violet coloured shell. The birefringent was occurred because of the anisotropic and crystalline arrays under cross-polarised light conditions (Lopéz et al., 2013). The crossed polarised light was referred to first plane was 90º to second plane. The birefringent images colloidosomes particle under polarised microscope by the PVA surfactant was showed in figure 4.5. Image (a) referred to without polarised (b) referred to polarised image and (c) showed the magnified images of colloidosomes particle.

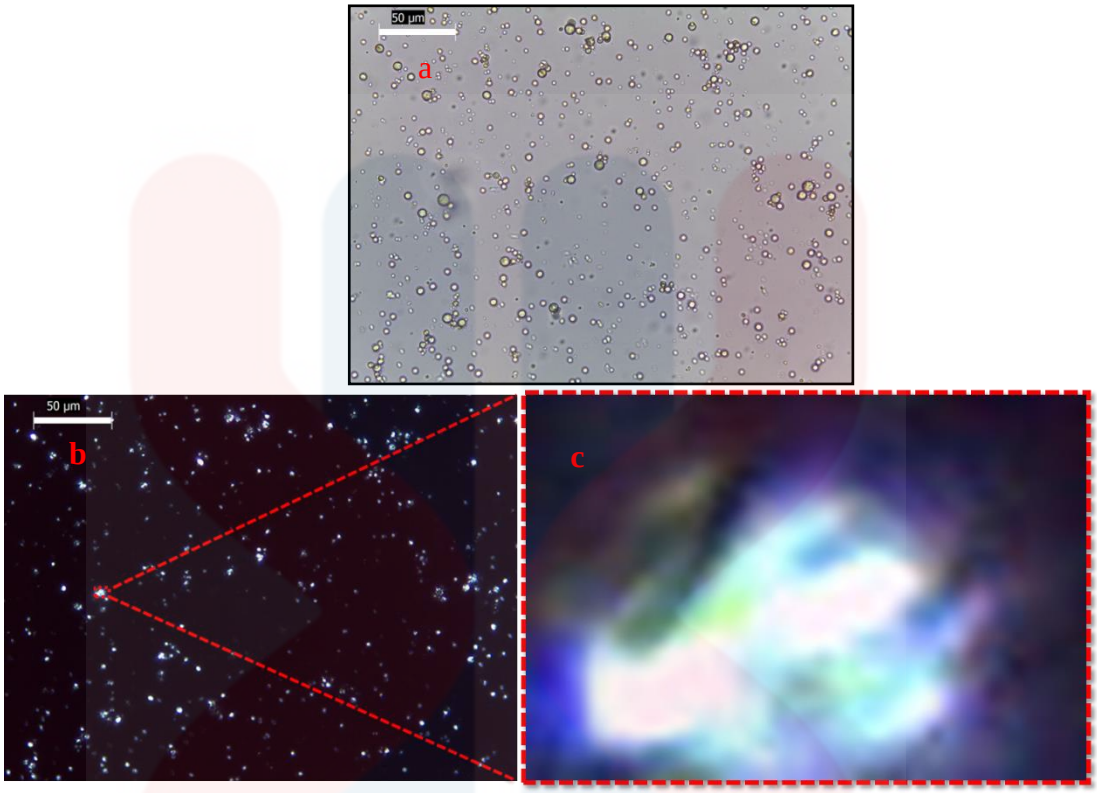


Figure 4.5: the birefringent images using PCL80/PVA system (entry1, table 4.1)

The birefringent images colloidosomes particle under polarised microscope by the sugar ester surfactant was showed in figure 4.6. Images (a) showed the particle under without polarised light (b) showed the particle under polarised light microscope and image (c) showed the magnified images of colloidosomes particle. These particles showed the green and violet coloured shell.

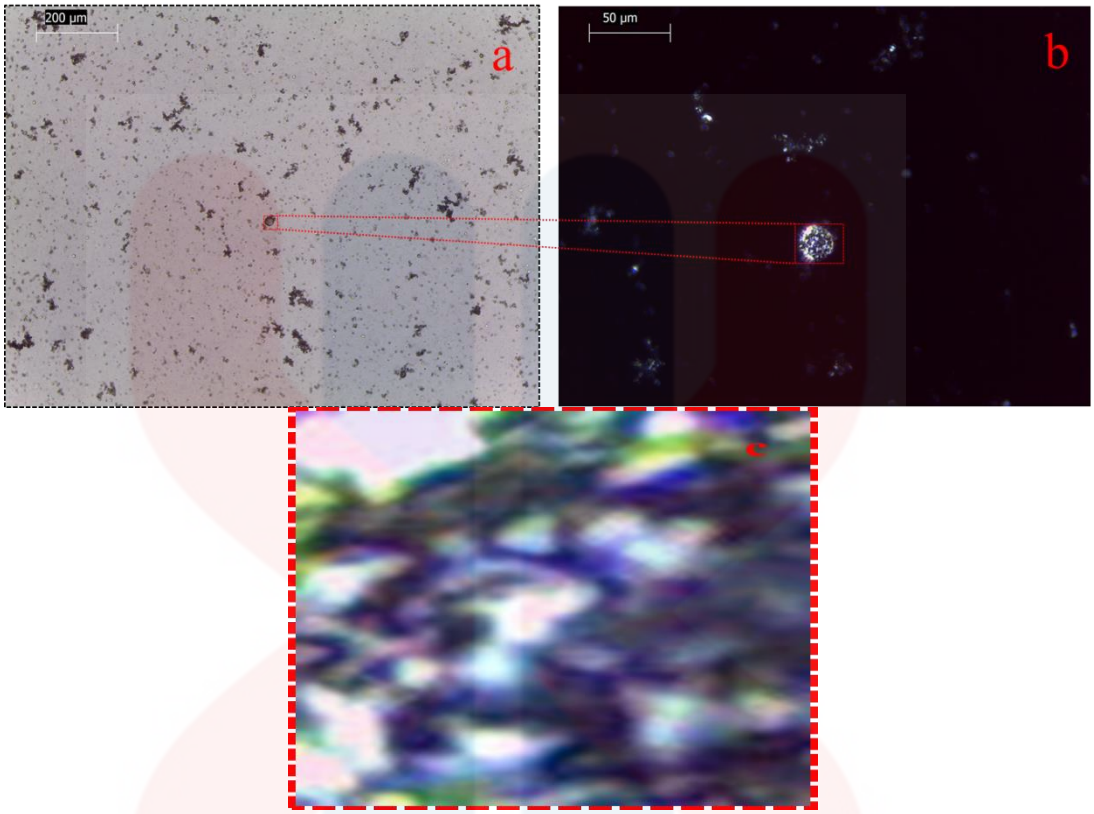


Figure 4.6: The birefringent images using PCL80/SE system (entry 6, table 4.1). The dotted red boxes represent the area of the magnified image.

However, the colloidosomes particle also showed coloured shell under polarised condition. The coloured shell of colloidosomes under polarised microscope was showed in figure 4.7.

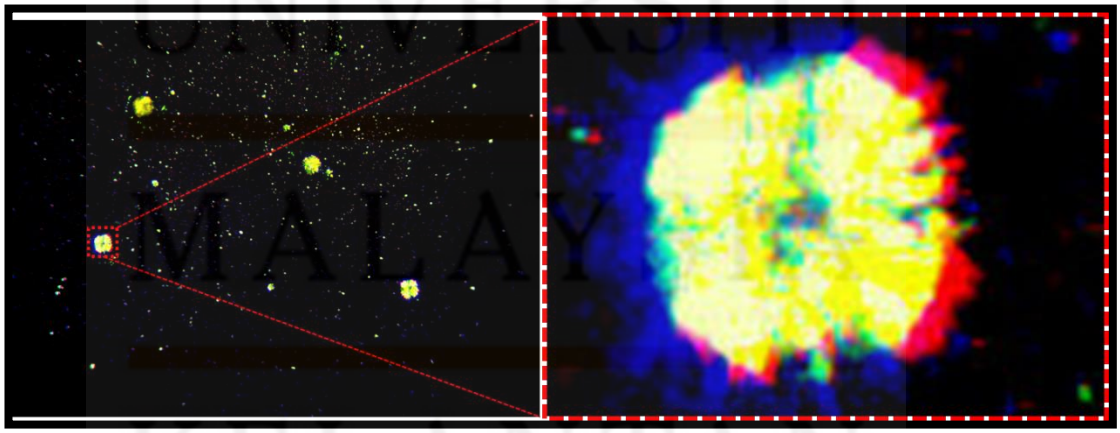


Figure 4.7: The birefringent images using PCL80/PVA system (entry 5, table 4.1). The dotted red boxes represent the area of the magnified image.

The morphology of the colloidosomes prepared using PCL80 as the structural polymer was observed using SEM. Figure 4.8 shown that the colloidosomes shells were composed of small particles for PCL80/PVA system. These images show further evidence of shell consisted of small partially fused small particle by observed the magnified images.

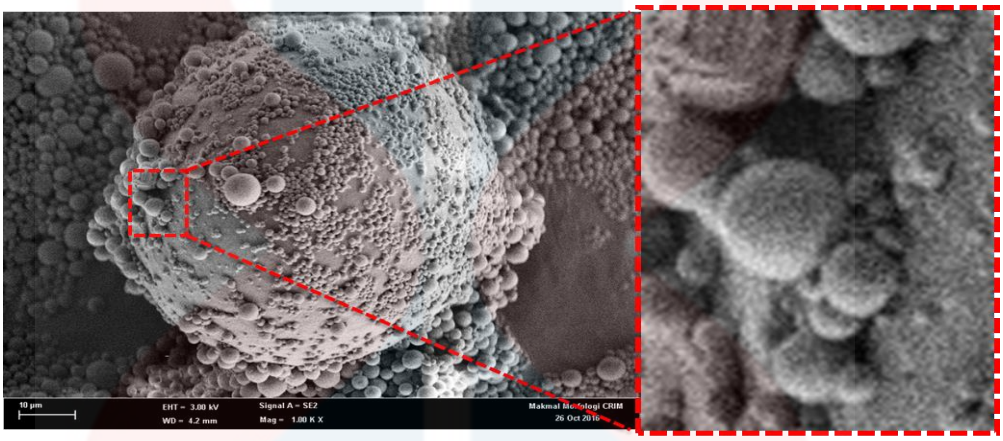


Figure 4.8: The morphology of colloidosomes prepared using (a)-(b) PCL80/PVA (entry 5, table 4.1). The dotted red boxes represent the area of the magnified images in (b).

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

This research was conducted to use PCL as potential structural polymer in the preparation of hollow colloidosomes microsphere at different oil to water phase ratio. We have conducted preparation of hollow colloidosomes by accelerated solvent evaporation method. During the homogenization process, we have used different volume of oil phase to water phase to produce the colloidosomes particle. We have been used PCL80 only for the formation of hollow colloidosomes particles.

We have investigated the effect of different surfactant concentration and type. The surfactant S1670 and PVA were used. Specifically, surfactant S1670 was used at concentration 1.2wt% and 0.1wt% to differentiate the effect of concentration. By the surfactant S1670 at concentration 1.2wt% showed smaller in size compared to 0.1wt% which is larger size of colloidosomes particle. Meanwhile, surfactant PVA was used at concentration 1.2wt% to differ with surfactant S1670 for the hollow colloidosomes formation.

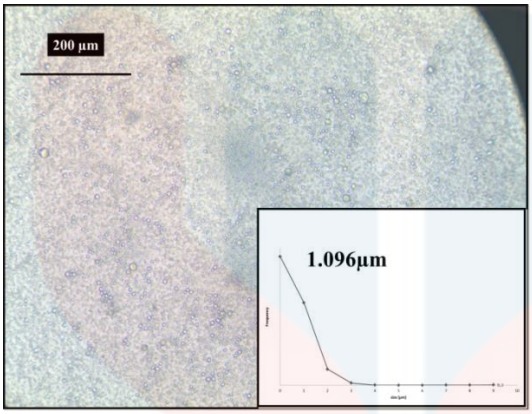
The birefringent effect and the morphology of PCL hollow colloidosomes were observed. We have demonstrated the birefringent by optical microscope using polarised light condition. Leica polarised microscope was also used for the best image birefringent observation. The morphology of hollow colloidosomes particle

was obtained by the FESEM. The morphology showed many small particles were embedded on the large particle showed that the particle was hollow colloidosomes.

5.2 RECOMMENDATION

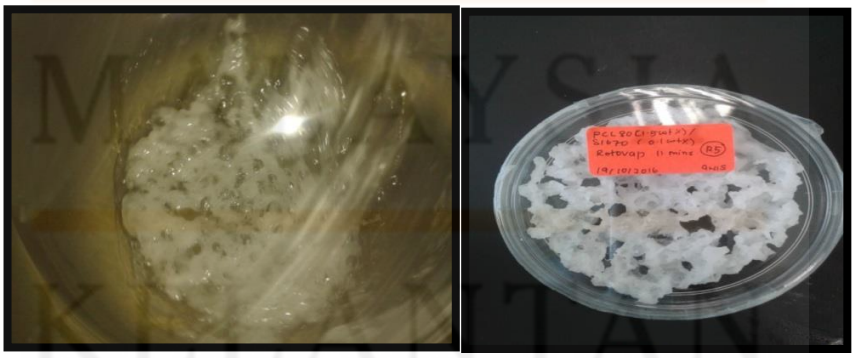
For the future research of this study, we would like to suggest the particle size in diameter was influence by the thickness of the structural polymer, PCL. PCL thickness should be varied using different molecular weight of PCL to show the interesting part of effect molecular weight. The larger size would be obtained using higher molecular weight of PCL (Jeong et al., 2003). The thickness might be influence the release mechanism of drug inside the shell. Thus, by thinner the shell might be boosting the release rate in certain application.

APPENDIX



The images showed the colloidosomes particles
at 0.1wt% ratio 1.5:1 of volume oil to water phase

Homogenization process which involve syringe pump and IKA homogenizer



Flakes produced in round bottom flask during rotary evaporation

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