A Study Of Hollow Colloidosomes Using Poly(methyl methacrylate-co-methacrylic acid), PMMA-co-MAA Via Two Step Solvent Evaporation Method

by

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ABSTRACT

In the past few decades, the dependence of the people towards the biomaterial based products such as scaffolds and hydrogels are rapidly rises in the urbanization of industrial, cosmetics, denture and pharmaceutical activities. Biomaterials are the ideal alternative compared with diesel-based materials and it is arising up recently due to its attribute as environment friendly and biocompatible to human being. Poly(methyl methacrylate-co-methacrylic acid), PMMA-co-MAA is still a newly biomaterial where has not been fully utilized in colloidosomes production. In this study, a total of 9 samples which made up from 6 samples of Polycaprolactone, PCL with two different surfacants and 3 samples of PMMA-co-MAA produced were determined by their different parameters which are concentration of structural polymer, concentration of polymer surfactant and types of surfactants and physical characteristic (partical size and diameter). The results of proximate analysis of PCL showed a significant outcome in different polymer surfactants within the six samples whereas all of the parameters are remarkable different in the particle sizes within the 9 samples. For physical characteristics analysis, both of the factors were showed obviously different in concentration of structural polymer, concentration of polymer surfactant and types of surfactants. FESEM image proved the ability of PMMA-co-MAA as hollow colloidosomes where small colloids were present and partially fused on the surface of PMMA-co-MAA colloidosome surface. PMMA-co-MAA also a birefringent material as it gives interference colours when placed between crossed polarizers. The average diameter is 30.46 μm, 32.74 μm and 33.35 μm by the samples from PCL/PVA system which the concentration of PVA were 1.20%, 0.50% and 0.25% repectively. Whereas for the average diameter of hollow colloidosomes in PCL/SE system on concentration of sugar ester 1.20%, 0.50% and 0.25% were 2.663 μm, 4.700 μm and 7.940 μm. The average hollow colloidosome' diameter for PMMA-co-MAA/PVA system was 31.68 μm with the structural polymer concentration of 3.00 wt% and 1.20% PVA as polymer surfactant. The concentration of PVA surfactant 1.20% in 1.50 wt% WT%PMMA-co-MAA/PVA systems that produced hollow colloidosomes with average diameter of 19.20 μm and the 0.25% PVA as polymer surfactant did not give any positive results. Based on the overall results, the average diameter and particle sizes of PMMA-co-MAA proved that it is suitable in synthesis hollow colloidosomes.

Satu Kajian Berkaitan Koloidosome Berongga Menggunakan Poli(metil metakrilat asid-co-metakrilik), PMMA-co-MAA Melalui Kaedah Dua Langkah penyejatan larutan

ABSTRAK

Dalam beberapa dekad yang lalu, kebergantungan manusia terhadap produk berasaskan biobahan seperti perancah dan hidrogel telah menunjukkan peningkatan mendadak dalam sektor perindustrian, kosmetik, perubatan gigi dan juga aktiviti farmaseutikal. Biobahan adalah salah satu alternatif yang sesuai berbanding dengan bahan-bahan berasaskan diesel dan kemunculan biobahan baru-baru ini disebabkan oleh sifat mesra alam dan biokompatibel dengan manusia. Poli (metil metakrilat asid-co-metakrilik), PMMA-co-MAA merupakan suatu biobahan yang baru diperkenalkan dalam bidang farmaseutikal dan masih tidak digunakan untuk penghasilan koloidosome berongga. Dalam kajian ini, sebanyak 9 sampel yang terdiri dari 6 sampel Polycaprolactone (PCL) dengan dua surfaktan yang berbeza dan 3 sampel PMMA-co-MAA dihasilkan ditentukan oleh parameter kaitan kepekatan strukur polimer, kepekatan surfaktan polimer dan jenis surfaktan yang berbeza dan ciri-ciri fizikal (saiz partical dan diameter). Keputusan analisis proksimat PCL menunjukkan keputusan yang ketara terhadap surfaktan polimer yang berbeza dalam enam 6 sampel manakala semua parameter lain dapat keputusan yang berbeza dalam saiz zarah daripada 9 sampel. Analisis dari segi ciri-ciri fizikal, kedua-dua faktor telah menunjukkan perbezaan saiz zarah yang drastik dalam kepekatan polimer struktur, kepekatan polimer surfaktan dan jenis surfaktan. Imej FESEM membuktikan keupayaan PMMA-co-MAA sebagai koloidosome berongga kerana koloid kecil berlekat dan sebahagiannya bersatu dengan permukaan koloidosome. PMMA-co-MAA juga merupakan bahan birefringent kerana mempamerkan warna apabila diletakkan antara dua polarizer berenjang. Diameter purata adalah 30.46 μm, 32.74 μm and 33.35 μm kepada sampel dalam sistem PCL / PVA dengan kepekatan PVA 1.20%, 0.50% dan 0.25%. Manakala bagi diameter purata koloidosome berongga dalam sistem PCL / SE pada kepekatan gula ester 1.20%, 0.50% dan 0.25% adalah 2.663 μm, 4.700 μm and 7.940 μm. Diameter purata koloidosome berongga untuk sistem PMMA-co-MAA / PVA adalah 31.68 μm dengan kepekatan struktur polimer 3.00 wt%, 19.20 μm bagi sistem dengan kepekatan struktur polimer 1.50 wt% dan 1.20% surfaktan polimer kepada kedua-dua sistem. Kepekatan surfaktan polimer 0.25% surfaktan polimer dalam sistem 1.5 wt% PMMA-co-MAA / PVA tidak menghasilkan keputusan positif. Keseluruhannya, diameter purata dan saiz zarah PMMA-co-MAA esperimen kali ini membuktikan bahawa PMMA-co-MAA sesuai untuk sintesis koloidosome berongga.

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LIST OF ABBREVIATIONS

AIBN	Azobisisobutyronitile
CMC	Critical Micelle Concentration
DCM	Dichloromethane
GDP	Gross Domestic Product
MAA	Methacrylic Acid
MMA	Methyl Methacrylate
O/W	Oil-in-water
PCL	Polycaprolactone
PLGA	Poly(lactic-co-glycolic acid)
PMMA	Poly(methyl methacrylate)
PMMA-co-MAA	Poly(methyl methacrylate-co-methacrylic acid)
PVA	Poly(vinyl alcohol)
SEM	Scanning Electron Microscope
THF	Tetrahydrofuran

LIST OF SYMBOLS

%	Percentage
°C	Degree Celsius
g	Gram
Hz	Hertz
kg	Kilogram
M	Molar mass
mL	Millilitre
M_n	Relative molecular mass
mol	Mole
rpm	Revolutions per minute
µm	Nanometer
$µm^2$	Area of nanometer
wt%	Weight percent
w/v%	Weight/Volume percent

Chapter 1

Introduction

1.1 Background of Study

Poly(methyl methacrylate-co-methacrylic acid), PMMA-co-MAA is chemically synthesized copolymers made from 2 monomers: methyl methacrylate (MMA) and methacrylic acid (MAA) in different ratio via free radical polymerization (Seyyed Hosseinzadeh et al., 2013).

Poly(methyl methacrylate), PMMA is one of the most widely used material for many developed countries such as Germany, Japan, United States Of America (US) and France (Kobayashi, 2011). PMMA was developed in 1901 by Otto Röhm whom successfully chemical synthesis PMMA on industrial size in the 1920s. The dough like PMMA can be slowly harden into a glassy polymer with mixing the MMA monomer with benzoyl peroxide initiator with pre-polymerized PMMA powder. On 1930s, Walter Wright applied PMMA material as the dentures base and removable partial dentures prosthetic (Alla, Swamy, Vyas, & Konakanchi, 2015).

The discovery dough moulding technique enable the PMMA to be polymerized and harden at room temperature through cold-curing process. As for this breakthrough, PMMA has been used on orthopaedics which used on synthesis femoral implants and acrylic joints cast in alginate moulds until the next 50 years. Sir John Charnley found that the addition of PMMA in acrylic cement enables the medullary canal be fully filled and adapting on the bone interface, facilitating stress transfer and anchoring the prosthesis (Seyyed Hosseinzadeh et al., 2013).

Nowadays, PMMA were used on many applications such as scaffoldings in retinal tissue engineering with ultra-thin thickness (6μm) that increase the delivery process and reduced the trauma risk and enhancing the potential of interaction with the host. PMMA has an disadvantage here as it cannot be degraded and remains in the retinal area permanently until it is removed but may interfere the retinal reattachment or risking of inflammatory response (Yao, Tao, & Young, 2011).

PMMA also used for encapsulation that controlling the encapsulated material's releasing rate and aids on long term processes. Potassium permanganate, $KMnO_4$, which is a strong and reactive oxidant that encapsulated in the PMMA that is prevented direct contact between the oxidant and the aqueous medium (Ighere & Chawla, 2014).

PMMA-co-MAA is used on treating diabetic mice that the PMMA-co-MAA promotes vascularization and wound repair (Martin, Semple, & Sefton, 2010).

Encapsulation is the method that applying a coating to the substrates to control their interaction with the environment encountered. Moreover, encapsulation also applied in drug release which is controlled by several factors such as temperature, pH and moisture. Encapsulation usually entraps nutritional compounds such as vitamins A or D, minerals and antioxidants that protects them from harmful environment's compounds (Karsa & Stephenson, 2005).

The aim of this study is a further study from the establish method (Shahidan *et al.*, 2013) using PMMA-co-MAA as a new potential material used as the shell of encapsulation and to study the factors affecting the morphology of the encapsulated shell formed.

1.2 Problem Statement

PMMA-co-MAA has a potential to be applied on encapsulation as the drug delivering agent via encapsulation. In recent research, PMMA-co-MAA is produced as hollow particles that have the potential on drug delivering and encapsulation in cosmetics (Bird, Freemont, & Saunders, 2011) . In drug delivery application, PMMA-co-MAA is used to deliver a colon-specific drug, naproxen (Mahkam & Asrin, 2012). In encapsulation in cosmetics application, PMMA-co-MAA which produce micro-meter hollow particles that is high in concentration but with low modulus values due to the low surface area-to-volume ratios of the particles (Kyriaki *et al.*, 2015)

PMMA-co-MAA has a great affinity to form hollow colloidosomes that opens a new substitute on synthetic polymer based hollow colloidosomes which is more biocompatible, low cost and environmental friendly. Therefore, in this study, the use of PMMA-co-MAA to encapsulate on targeted release drug delivery system. Furthermore, this study mainly to investigate the potential of using PMMA-co-MAA to prepare hollow colloidosome using an established method earlier in Shahidan *et al.* (2013).

1.3 Objectives

This study will focus on three objectives.

i) To investigate the potential of PMMA-co-MAA as hollow colloidosomes polymer structure.

ii) To prepare PMMA-co-MAA via two step solvent evaporation method.

iii) To characterize and analyse the morphology and size of PMMA-co-MAA shell hollow colloidosomes.

1.4 Expected Outcomes

In the end of the study, the expected few outcomes from the formation of PMMA-co-MAA hollow colloidosomes:

i) Able to obtain PMMA-co-MAA shell hollow colloidosomes structure using two step accelerated solvent evaporation.

ii) Identify the factors that affect the size and morphology of the PMMA-co-MAA shell hollow colloidosomes which are: polymer surfactant concentration, type of surfactants and concentration of structural polymer.

iii) Able to characterize the PMMA-co-MAA shell hollow colloidosomes size and morphology using scanning electron microscope (SEM), optical microscope and polarized light microscope.

Chapter 2

Literature Review

2.1 Encapsulation

Encapsulation is a common technique that used in various field particular in drugs delivery system and food delivery systems. Figure 2.1 shows an example of encapsulation from liposome.

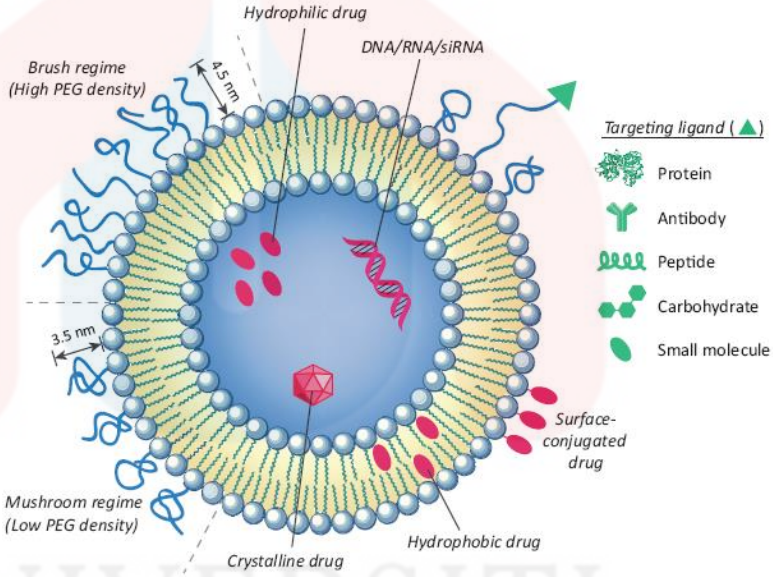

Figure 2.1: Structural and design considerations for liposomal drug delivery encapsulation (Karsa & Stephenson. 2005)

Other than two major fields in encapsulation, encapsulation also applied in washing powder, perfumes, pesticides, animal feedstuffs and adhesives. Encapsulation brings a lot of benefits to us such as to provide protection to the product from its surrounding environment that could prolonged the product's storage life of soft gel products in pharmaceutical application (Karsa & Stephenson, 2005). Moreover, it also aids in controlling the rate of release of the core material via diffusion through the

capsule's membrane. Next, encapsulation also helps in masking undesirable properties of the material such as odour, taste masking, catalytic activity in drug delivery (Shashank & Prerana, 2011). Encapsulation can achieve targeted release at specific site that usually applied in pharmaceutical field (Karsa & Stephenson, 2005).

2.1.1 Colloidosomes

Colloidosomes which consists of coagulated or fused colloid particles that form like a shell. It has been used on encapsulation widely in drugs, proteins, vitamins, flavourings and living cells in Figure 2.2 (Kim, 2007).

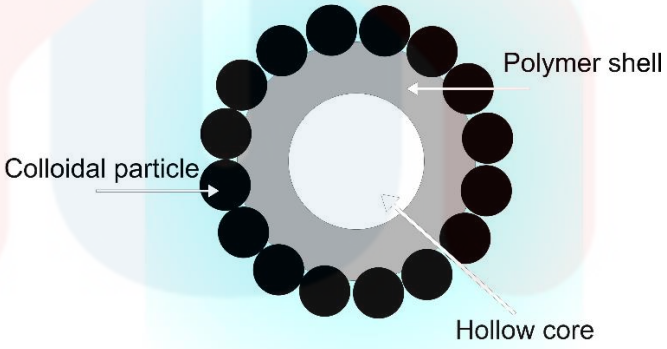

Figure 2.2: Structure of hollow colloidosomes (Kim, 2007)

Moreover, colloidosomes allow free diffusion of gases and isolate the living cells from its environment. Besides from diffusion and isolating, colloidosomes aids in facilitates tissue growth by allowing the rapid permeation of small macromolecules in forming templates (Dinsmore, 2002). Colloidosomes also allows to release content by defined shear rates through controlling the mechanical strength to enable the colloidosomes to withstand specific mechanical loads. There are three classifications of colloidosomes: water-in-oil (w/o) emulsion based colloidosomes, oil-in-water (o/w)

emulsion based colloidosomes and water-oil-water emulsion based colloidosomes (Saraf *et al.*, 2011).

2.2 Solvent Evaporation Method

Solvent evaporation is a technique to obtain solidified hollow colloidosomes in Figure 2.3.

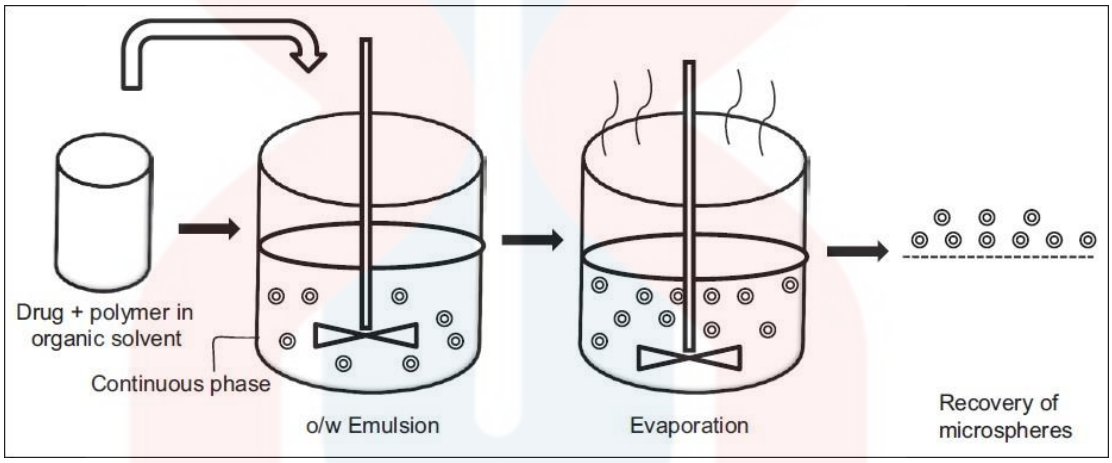

Figure 2.3: Schematic representation of solvent evaporation method. (Avinash *et al*., 2014)

The polymer/solvent system (water phase) will be added into aqueous phase (water phase) in a continuous agitating state by using a propeller type blade attached to a variable speed motor. Shear from the agitation produces emulsion. (Tiwari & Verma, 2011). The oil phase contains organic solvent that is volatile will evaporate from the surface of the dispersion at room temperature or higher temperature. The oil phase removal will create deficient cores on polymer droplets and filled with water (Shahidan *et al.*). The end product of solvent evaporation process will produce hardened microsphere.

2.3 Materials

In this study, there are two types of surfactants which are poly(Vinyl Alcohol) (PVA) that have potential to be effective with the polymer poly(methyl methacrylate-*co*-methacrylic acid), PMMA-co-MAA whereas sucrose ester (SE) that have potential to be effective with the polymer polycaprolactone (PCL). On the other hand, the nature and characteristics of Poly(methyl methacrylate-co-methacrylic acid), PMMA-co-MAA is also discussed in this study.

2.3.1 Surfactants

Surfactants have five major classifications shown in Figure 2.4.

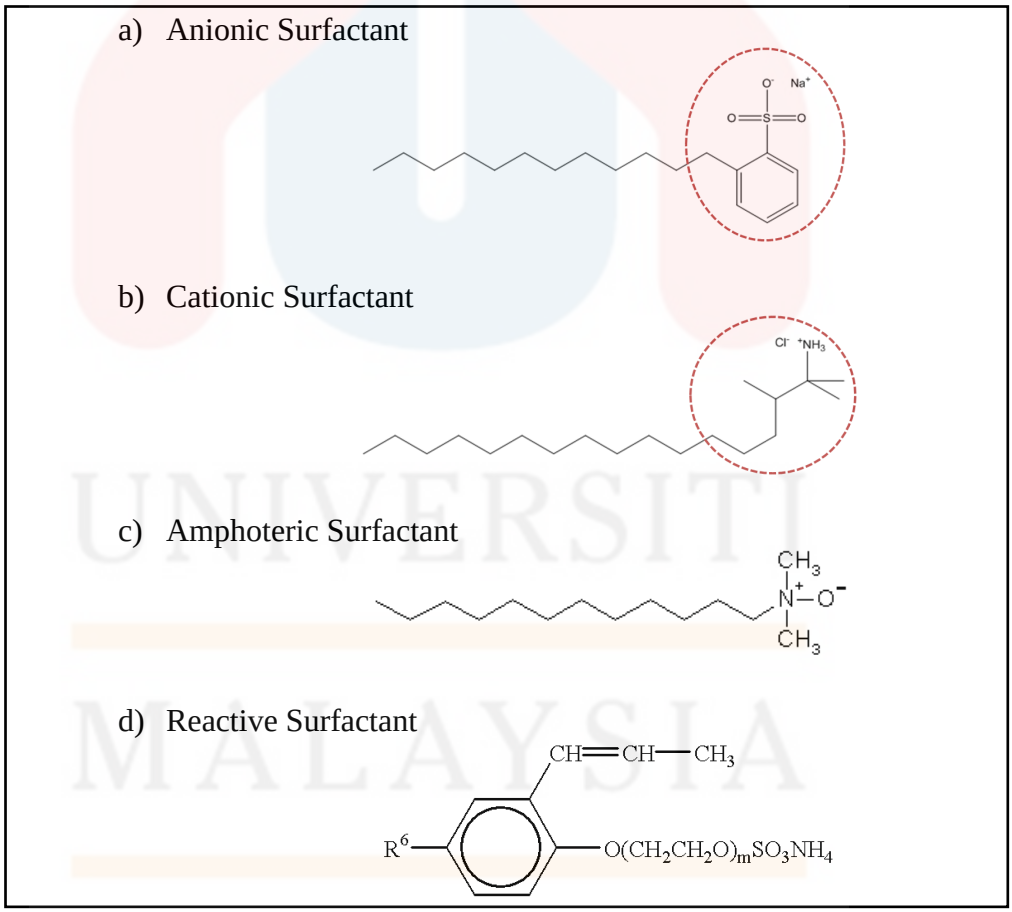

Figure 2.4: Classification of surfactants and their example. (a) Sodium dodecylbenzene sulfonate (b) Trimethylhexadecyl ammonium chloride (c) Ammonium Carboxylate (d) TREM LF-40 Sodium alkyl allyl sulfosuccinate.

The categories are according to its hydrophilic group: anionic, cationic, non-ionic, amphoteric and reactive. These major classification is shown in Figure 2.4 (Azarmi & Ashjaran, 2015). The anionic surfactants have negatively charged hydrophilic head in Figure 2.4(a) that widely used on laundering, dishwashing liquids and shampoos due to its excellent cleaning properties. The example of anionic surfactants are alkyl sulphates, soaps petroleum and carboxylates (Salager, 2002). Cationic surfactants have positively charged hydrophilic in Figure 2.4(b) that aids in reducing surface tension and as wetting agents in acid media. When alkyl halides react with primary, secondary or tertiary fatty amines, cationic surfactants are formed during the reactions (Karlberg, 1999). Non-ionic surfactants that use in textiles industry such as amine oxides, alkyl phenols and ethoxylated acids. It also can be used on controlling the percentage of the hydrophilic or hydrophobic characteristics (Denko & Tcholakova, 2010). Amphoteric surfactants have both cationic and anionic characteristics with primary, secondary or tertiary amines or quaternary ammonium cationic part and sulfonates or other anionic molecules on anionic part in Figure 2.4(c) (Vroman & Tighzert, 2009). Reactive softener is an example of reactive surfactants that usually applied in permanent surface finishing process that is resistant to washing or high wearing resistance and reacts with cellulose fibre in Figure 2.4(d) (Azarmi & Ashjaran, 2015).

2.3.1.1 Poly(Vinyl Alcohol)

Poly(Vinyl Alcohol) (PVA) is a non-ionic surfactant. It has the following characteristics which are odourless, tasteless, translucent, white or cream in colour granular powder. It majorly used on food supplement tablets as moisture barrier film. It is a hydrophilic compound which soluble in water, slightly soluble in ethanol but

insoluble in other organic solvents and its chemical structure is shown in Figure 2.5 (Gaaz *et al.*, 2015).

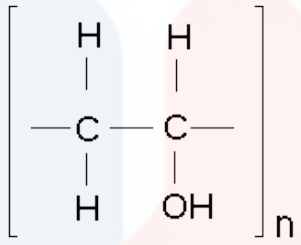

Figure 2.5: Chemical structure of poly(Vinyl Alcohol), PVA (Gaaz *et al*., 2015)

Moreover, it has a melting point from the range of 180 to 190°C. PVA acts as a functional surfactant that tends an interconnected network with the polymer at the surface and it is difficult to removed (Mu *et al.*, 2003).

2.3.1.2 Sucrose Ester

Sucrose ester is a non-ionic biosurfactant that has the following properties: biodegradability, emulsion forming, antiadhesive and antimicrobial activities. Its properties promote its usage as antibacterial agents applicable to food additives (Sabeder *et al.*, 2005), cosmetic oil gels (Tulay & Linhardt, 2001) and usage of sugar ester in preparing the avocado oil nano-emulsion in pharmaceutical industry (Eid *et al.*). The sucrose ester has few advantage over the commercial synthetic surfactants such as: tasteless, excellent biodegradability and do not cause environmental pollution (Neta *et al.*). Sucrose ester has high critical micelle concentration (CMC) which is the value represents the concentration of surfactant required to solubilize hydrophobic molecules in water (Tulay, 2001).

2.3.2 Synthetic Polymer

Synthetic polymers are formed with polymerization which adding in repeating small molecules to form a long and continuous chain of polymer. The small molecule in the polymers is called monomers. The chemists begin to replicate natural polymers and create synthetic polymer which is nylon that mimics silk's strength and flexibility (Shakhashiri, 2012). Synthetic polymers are mainly used in daily life and industry. Moreover, synthetic polymers usually produced on a very large scale and have wider range properties and uses compared to natural polymers. The examples of synthetic polymers are plastic (polythene), synthetic rubbers (Buna – S) and synthetic fibres (nylon 6,6) (Syamal, 2016).

2.3.2.1 Poly(methyl methacrylate), PMMA

Poly(methyl methacrylate), PMMA is the most common in the esters of methacrylic acids. PMMA has high mechanical strength, high Young's modulus and low elongation at breaking point. It also represents one of the most hardest, highest scratch resistance thermoplastic, clear, colourless and does not shatter on rupture. At the same time, PMMA also exhibits low moisture and water absorbing capacity that both of these characteristics will increase as the temperature rises (Koleva, 1982).

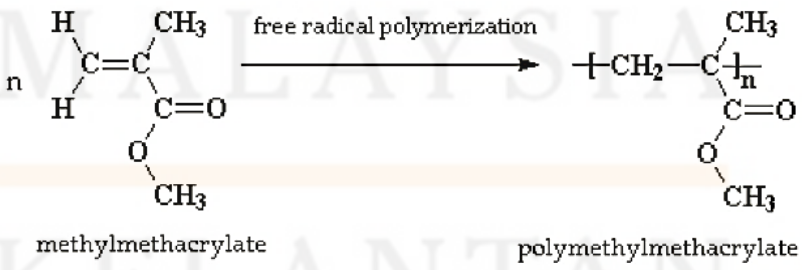

Figure 2.6: Chemical structure of Poly(methyl methacrylate), PMMA (Seyyed Hosseinzadeh *et al.*, 2013)

Figure 2.6 showed the methyl methacrylate (MMA) monomer has two covalently bounded carbon atoms which one of the carbon atom bonds covalently to two hydrogen atoms while the another carbon atom covalently bonded to a methyl and an acrylic group. The free radical polymerization of MMA produces Poly(methyl methacrylate) or poly(metylpropanoate) (Seyyed Hosseinzadeh *et al.*, 2013).

2.3.2.2 Poly(methyl methacrylate-*co*-methacrylic acid), PMMA-co-MAA

PMMA-co-MAA is the product from methyl methacrylate (MMA) and methacrylic acid (MAA) by using free radical polymerization reaction in Tetrahydrofuran (THF) using free radical initiator Azobisisobutyronitile (AIBN) on Figure 2.7 (Seyyed Hosseinzadeh et al., 2013).

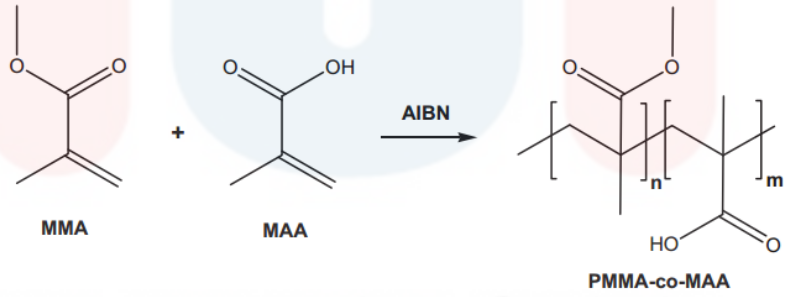

Figure 2.7: Chemical structure of Poly(methyl methacrylate-co-methacrylic acid), PMMA-co-MAA (Hosseini, Ibrahim, Djordjevic, & Koole, 2014)

Carboxyl group (-COOH) on the surface of PMMA-co-MAA which derived from MAA monomers will cause the surface functional groups in the polymeric matrix would not be deactivated over the time and could not be affected by aging effect or reorientation phenomena (Hosseini & Ibrahim, 2016).

2.4 Instrumentations

In this study, the instruments that been used on observation for the size and morphology of the PMMA-co-MAA shell hollow colloidosomes.

2.4.1 Optical Microscope

The optical microscope is a common instrument that is used to magnify the object in the range of 10x to 100x. An optical microscope was made up of several important features such as objective lens, eyepiece lens, illuminator in Figure 2.8 (Carlsson, 2007).

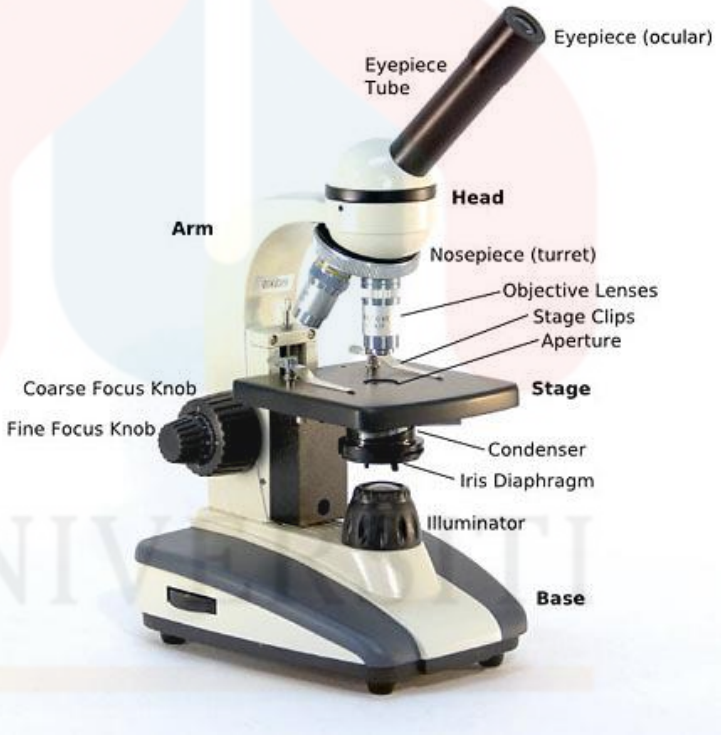

Figure 2.8: Component of Optical Microscope (Carlsson, 2007)

The mechanism of the optical microscope is mainly depending on light, where the light is generated through a light source or it comes from the reflection of sunlight. The light beam will pass through the condenser lens and through the light absorbing specimen. There are two types of lights which are the undeviated light and deviated

light. Undeviated light is the lights that pass both around and does not pass through the specimen on its path whereas deviated light is the light passing through the specimen on its path. Both of these lights will arrive at an image plane located at the specific position on diaphragm of the eyepiece. The eyepiece lens magnifies the image that projected onto the eye's retina or the camera's film plane. If the sample is metal, the metal should embed in a polymer, undergoes sectioning and polishing. Optical microscope also being modified to improve its performances such as dark field microscopy, phase contrast microscopy and fluorescence. Optical microscope also being modified to improve its performances such as dark field microscopy, phase contrast microscopy and fluorescence (Carlsson, 2007). In this study, optical microscope will be used to observe the formation of hollow colloidosomes with the high-powered lens.

2.4.2 Scanning Electron Microscopy

Scanning Electron Microscopy (SEM) is made up of electron gun, condenser lens, scanning coil, objective lens, specimen stage, secondary electron detector and display unit as depicted in Figure 2.9 (Cheney, 2009).

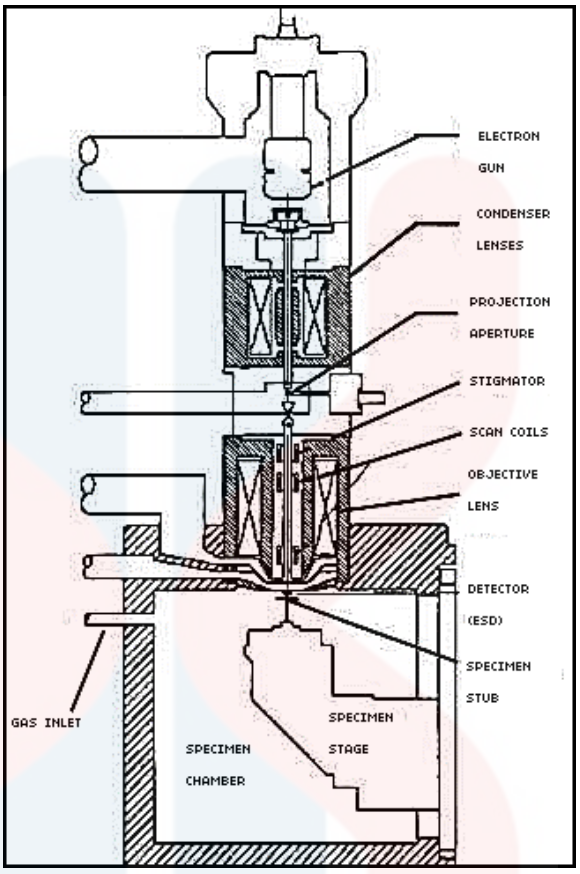

Figure 2.9: Component of Scanning Electron Microscope (Cheney, 2009)

SEM is used on topography analysis that the two-dimensional image which produced from the detection of the secondary electrons. The secondary electrons are emitted from the specimen surface as the specimen is irradiated with a fine beam of electron which produced from the electron gun. The electron is scanned by using an electron probe on the specimen surface and the image signal is collected. The image will be formed on cathode ray screen and displayed after suitable amplification and processing on computers (JEOL Ltd., 2013). SEM also obtained high magnification image in a vacuum system to prevent loss on electrons because they cannot penetrate very deep. The magnification of SEM is 10000 times which is more efficient than optical microscope. In this study, SEM used to characterize the PMMA-co-MAA shell size and determine the hollow colloidosomes size.

2.4.3 Polarizing Microscope

Polarizing microscope has the following important component: Observation tube prism, eyepiece with crosshair, image formation lens, Bertrand lens, analyser, test plate, compensator, centerable revolver, strain-free objective, rotating stage, specimen stage, polarizing condenser, polarizer and transmitted light illuminator as shows in Figure 2.10 (Olympus, 2003).

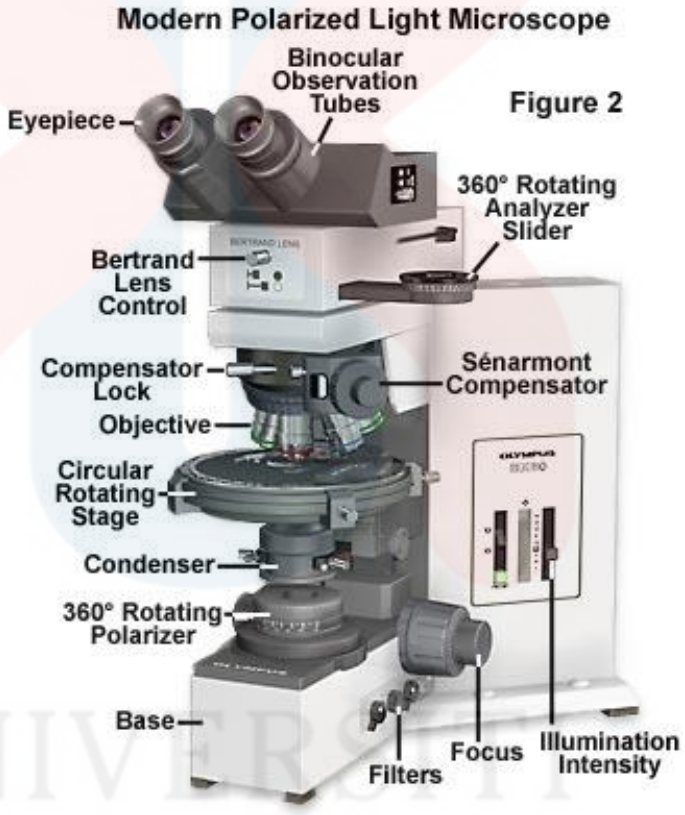

Figure 2.10: Component of Polarizing Microscope (Olympus, 2003)

The light will be polarized to move in one direction when it moves upwards from the light source through the polarizer. Two types of specimen which is anisotropic specimen that reflected the light where make it appear bright in contrast and isotropic specimen can allow light to travel through that makes it dark (Carlton, 2016). In this study, polarizing microscopy will be used to aid the determination of anisotropic PMMA-co-MAA hollow colloidosomes shell.

Chapter 3

Materials and Methods

3.1 Materials

Dichloromethane, CH_2Cl_2 (98%) with a molar mass, M= 84.93 g/mol had purchased from EMSURE, Chloroform for analysis with M= 119.38 g/mol was purchased from EMSURE, Poly(vinylalcohol), PVA (98% hydrolysed, M_n = 13-23 kg/mol) purchased from Aldrich and used as received. Polycaprolactone, PCL with a number-average molecular weight (M_n) of 13-28 kg/mol was brought from Aldrich and used as received. Whereas the Poly(methyl methacrylate-co-methacrylic acid), PMMA-co-MAA (M_w =~34kg/mol, M_n=~15 kg/mol by GPC) brought from Aldrich and used as received. Ryoto sucrose ester (SE-1670) purchased from Mitsubishi. Water is distilled water from Biology Lab. The polymer surfactants for this experiment is poly(vinylalcohol), PVA and sucrose ester.

3.2 Reagent Preparation

The polymer surfactants concentration in water phase and the structural polymer concentration in oil phase were prepared in 250 mL of Schott bottle.

3.2.1 Water Phase Preparation

The PVA solution is prepared by dissolving 1.20g, 0.50g and 0.25g of solid PVA powder respectively into 100 mL of distilled water to produce 1.20%, 0.50% and 0.25% PVA solution. The solution is dissolved in 70°C water bath for 3 hours. Whereas

the sugar ester follows the same procedure but it needs to be dried in oven at 80°C to remove moisture content before dissolving in distilled water.

3.2.2 Oil Phase Preparation

The structural polymer PMMA-co-MAA with 1.50g and 3.00g are dissolved in 100 mL of chloroform to produce 1.50 wt% and 3.00 wt% of feed and waited until it is fully dissolved in the organic solvent with time approximately 20 minutes. On the other hand, PCL with 1.50g is dissolved in 100mL of CH_2CL_2 to produce 1.50 wt% of feed. Both of these procedures must work in a fume hood as the organic solvent is volatile, wear goggle and gloves as the organic solvent will irritates human skin if directly contacted.

3.3 Colloidosomes Preparation

There are three different factors in preparation of colloidosomes to manipulate in order for comparison. The following factors to manipulate are concentration of polymer surfactants, concentration of structural polymer and type of the polymer surfactant (PVA and sucrose ester). This study followed Shahidan *et al*., 2013 method. Table 3.1 shows the overall conditions of all hollow colloidosome samples.

Table 3.1 Hollow Colloidosome Preparation Conditions Employed

Entry	Systems	V_w [a]/mL	C_{Surf} [b]/wt.%	V_o [c]/mL	C_{Pol} [d]/w/v%
1	PCL/PVA	50	1.20	75	1.50
2	PCL/PVA	50	0.50	75	1.50
3	PCL/PVA	50	0.25	75	1.50
4	PCL/SE	50	1.20	75	1.50
5	PCL/SE	50	0.50	75	1.50
6	PCL/SE	50	0.25	75	1.50
7	PMMA-co-MAA/PVA	50	1.20	75	1.50
8	PMMA-co-MAA/PVA	50	0.25	75	1.50
9	PMMA-co-MAA/PVA	50	1.20	75	3.00

[a]Volume of water. [b]Polymer surfactant concentration. [c]Volume of oil phase. [d]Concentration of structural polymer

The oil phase is prepared by dissolving different concentration of structural polymer (PCL and PMMA-co-MAA) into organic solvent (Dichloromethane, CH_2Cl_2). The dissolved structural polymer/solvent solution is injected into the beaker that contains water phase which has different concentration of poly(vinylalcohol) PVA and water. The solution will be stirred with high sheer homogenizer which is shown in Figure 3.1.

Figure 3.1: IKA T18 digital Ultra Turrax homogenizer

The oil phase, CH_2Cl_2 inside the shell was evaporated by using rotatory evaporator and the water diffuse into the hollow shell. After the CH_2Cl_2 has evaporated, the PCL or PMMA-co-MAA shell hollow colloidosomes were formed. Further observation on the size, optical properties and morphology of the PCL or PMMA-co-MAA shell hollow colloidosomes were obtained can used by optical microscope, polarized light microscope and scanning electron microscope respectively. To prepare sample for optical microscope observation, a drop of given as-prepared dispersion of sample was placed on a microscope slide and was view under a microscope immediately. A drop or more drop of distilled water was dropped on the microscope slide to dilute the dispersed sample and prevents drying of sample while under observing.

3.4 Solvent Evaporation

The homogenized emulsion was transferred to a round-bottom flask and immediately placed to a rotary evaporator shown in Figure 3.2.

Figure 3.2: Rotary Evaporator with 1 stage vacuum pump

The emulsion is evaporated under vacuum system using TW-2A model (Voltage= 220V-240V/50Hz) 1 stage vacuum pump by giving ultimate pressure of 2 Pascals (Pa), water bath at room temperature ~ 25°C and with a rotation speed of 95 rpm for approximately 10 minutes.

3.5 Materials Characterization

The PCL or PMMA-co-MAA shell hollow colloidosomes were observed and the analysed under optical microscope, polarized light microscope and scanning electron microscope. The image that obtained from the all three types of microscopes were analysed in graphs and tabulated.

1) Optical Microscope

Microscope with model MT8100 from Meiji Techno shown in Figure 3.3 was used to obtain optical images using transmitted light.

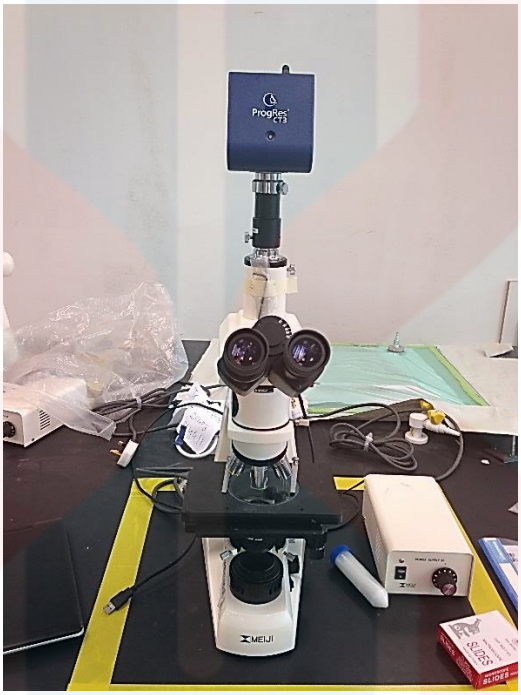

Figure 3.3: Optical microscope from Meiji Techno with model MT8100

The objective lenses used with magnification of X10 and X20 and the sample number average sizes (D_m) were determined by counting manually at least 100 colloidosomes. The images that obtained was analysed and sizing through software and manually to obtain the size of the hollow colloidosomes.

2) Polarized Light Microscope

A polarizer is placed between the eyepiece and the sample with a 90 degrees' orientation to block the direct transmitted light and generating contrast on the birefringent hollow colloidosomes with the background. The objective lenses used with

magnification of X10, X40, X60 and X100 and the sample number average sizes (D_m) were determined by counting manually at least 100 colloidosomes. The images that obtained was analysed and sizing through software and manually to obtain the size of the hollow colloidosomes.

3) Scanning Electron Microscope

Field Emission Scanning Electron Microscope (FESEM) from Center for Research and Instrumentation (CRIM), University Kebangsaan Malaysia, (UKM) was used to observed more detailed on morphology of hollow colloidosomes. The sample was coated with a thin film of carbon for electric conductivity to avoid charging effect. Negatively charged sample will reject electrons causing them to fly distorted paths and show distorted image.

Research Flow Chart

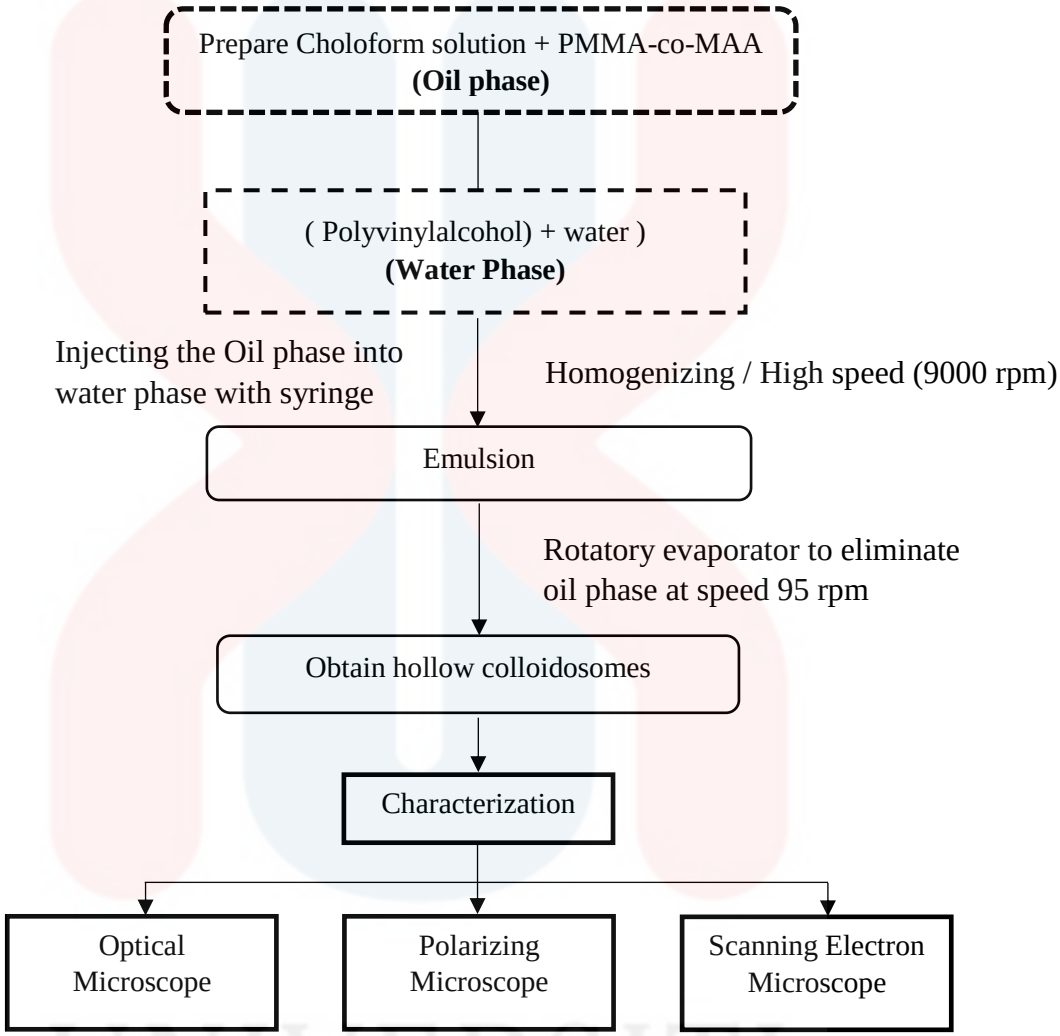

Figure 3.4: Flow chart of the experiment.

Chapter 4

Results and Discussion

4.1 Introduction

All 3 parameters that have 9 samples in total of hollow colloidosomes are analysed and the overall information is shown in Table 4.1.

Table 4.1 Hollow Colloidosome Preparation Conditions Employed and Data

Entry	Systems	V_w [a]/ mL	C_{Surf} [b]/ wt.%	V_o [c]/ mL	C_{Pol} [d]/ w/v%	D_n [e]/ μm
1	PCL/PVA	50	1.20	75	1.50	30.46
2	PCL/PVA	50	0.50	75	1.50	32.74
3	PCL/PVA	50	0.25	75	1.50	33.35
4	PCL/SE	50	1.20	75	1.50	2.663
5	PCL/SE	50	0.50	75	1.50	4.700
6	PCL/SE	50	0.25	75	1.50	7.940
7	PMMA-co-MAA/PVA	50	1.20	75	1.50	19.20
8	PMMA-co-MAA/PVA	50	0.25	75	1.50	-
9	PMMA-co-MAA/PVA	50	1.20	75	3.00	18.22

[a]Volume of water. [b]Polymer surfactant concentration. [c]Volume of oil phase. [d]Concentration of structural polymer. [e]Number-average diameter.

In Table 4.1, all results are tabulated and organized in order. The weight percent, wt.% of PCL was fixed variable in this experiment so the result that obtained was manipulated by the type of polymer surfactants and concentration of the polymer surfactants in the PCL polymer-based system.

4.2 Effect of polymer surfactant concentration

From the experiment data, all three different PVA concentration will produced the different size of hollow colloidosomes.

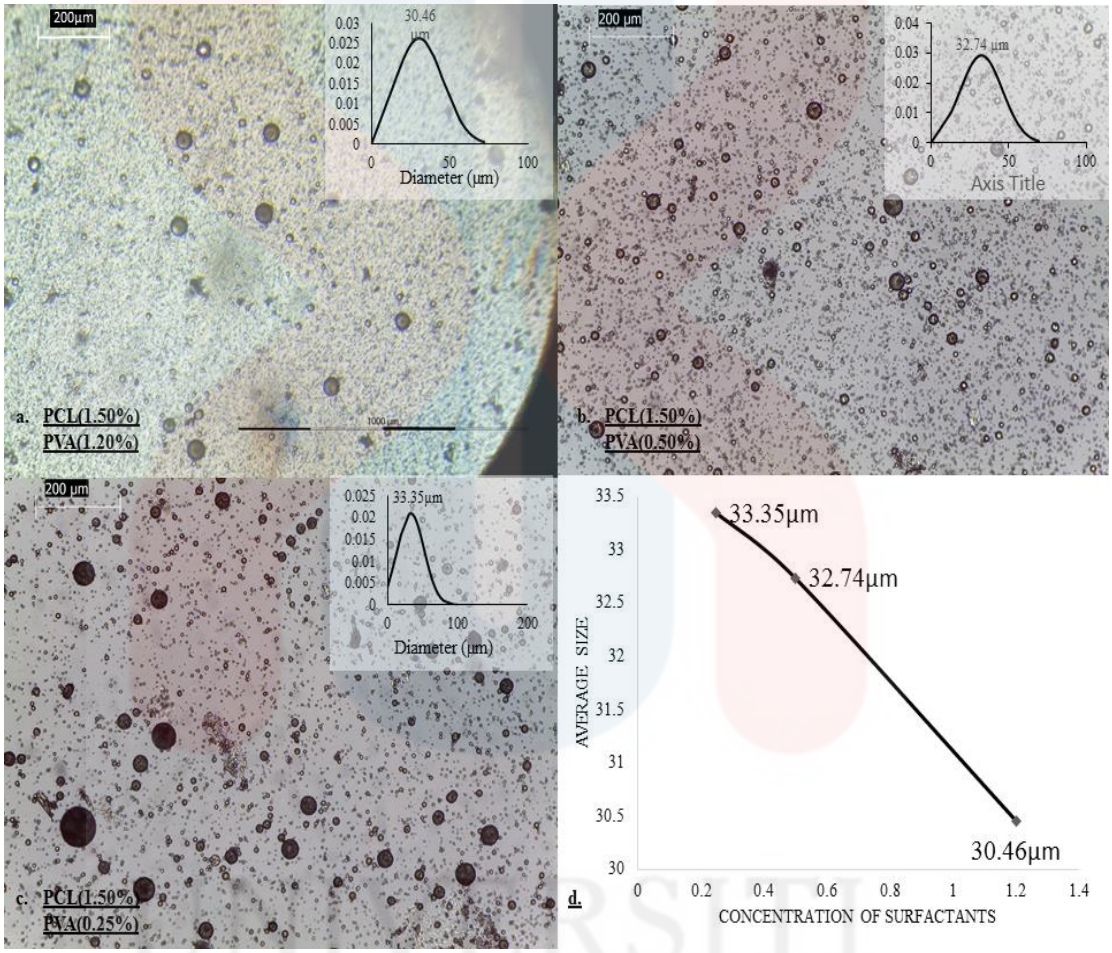

Figure 4.1: Size distribution of 1.50 wt% PCL and their optical microscope image with their respectively surfactant concentration of PVA. (a) PCL (1.50%), PVA (1.20%) (b) PCL (1.50%), PVA (0.50%) (c) PCL (1.50%), PVA (0.25%) (d) Comparison on hollow colloidosomes size between three different concentrations of surfactant PVA.

Figure 4.1 (a) showed that with surfactant concentration of 1.20% produced an average diameter of 30.45µm. Figure 4.1 (b) showed that an average diameter of 32.74 µm for 0.50% of PVA concentration and its size is placed between 1.20% and 0.25% of PVA

concentration average diameter. Figure 4.1 (c) showed that an average diameter of 33.35 µm in hollow colloidosomes obtained. The comparison between the size of PCL hollow colloidosomes with three different surfactant concentration is shown in Figure 4.1 (d). The size distribution of PCL hollow colloidosome decreases as increasing the surfactant concentration where the size of PCL hollow colloidosomes descend in order of 0.25% > 0.50% > 1.20%.

Figure 4.2 show that the size distribution of hollow colloidosomes obtained by using 1.20%, 0.50% and 0.25% of SE as surfactants.

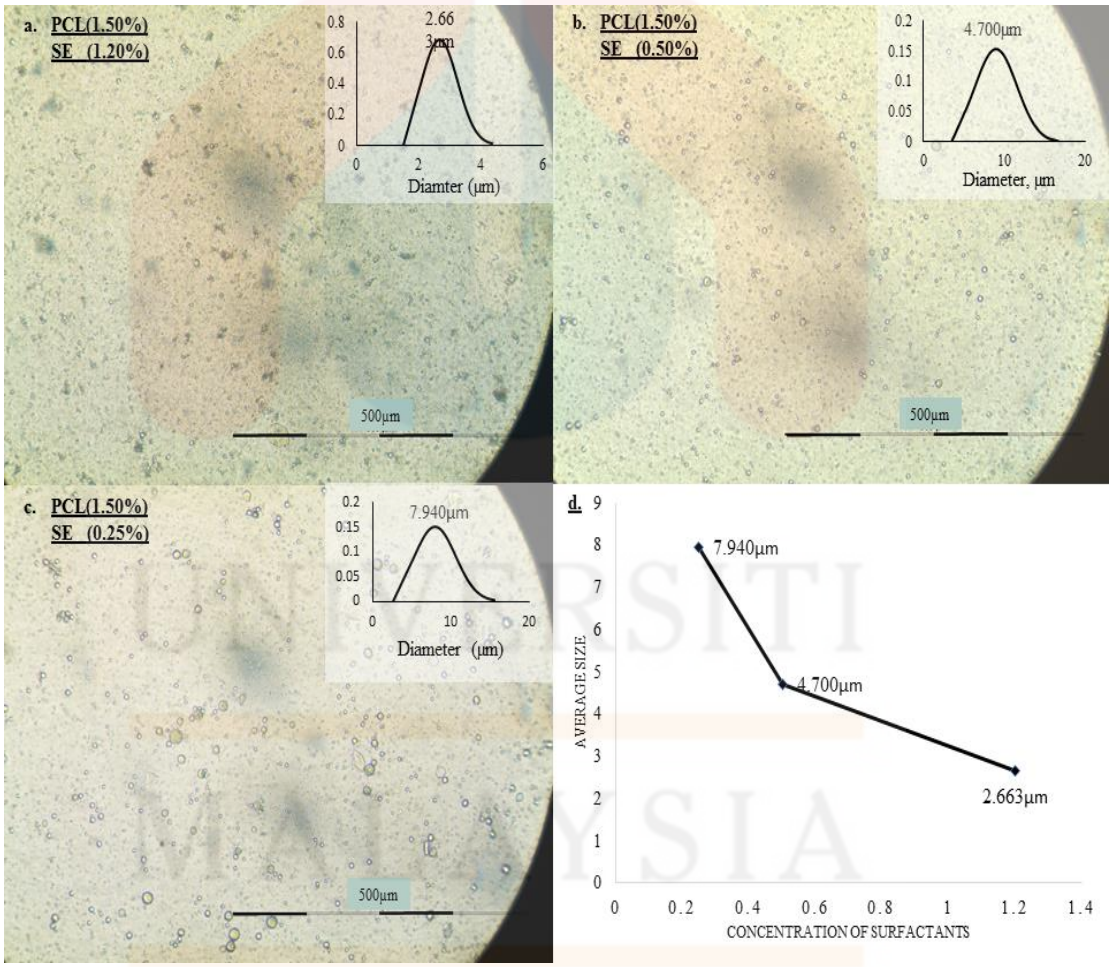

Figure 4.2: Size distribution of 1.50 wt% PCL and their optical microscope image with their respectively surfactant concentration of sugar ester (SE). (a) 1.20% of SE. (b) 0.50% of SE. (c) 0.25% of SE. (d) Comparison on hollow colloidosomes size between three different concentrations of surfactant sugar ester.

Figure 4.2 show that the size distribution of hollow colloidosomes obtained by using 1.20% SE as surfactants. The average diameter is 2.663 µm for the 1.20% SE surfactants in PCL/SE system in Figure 4.2 (a) which was the smallest in size among three results of different surfactants' concentration. Next, the average size distribution of 1.50 wt% PCL with 0.5% SE as surfactant that shown in Figure 4.2 (b) which is 4.700 µm. The average size for PCL/SE with 0.25% SE surfactant concentration is the largest for the PCL/SE system which is 7.94 µm shown in Figure 4.2 (c). Figure 4.2 (d) showed the comparison of the size of PCL hollow colloidosomes formed with different concentration of surfactants. 0.25% SE had produced the largest size of PCL colloidosomes where the 1.20% SE produced the smallest size of PCL colloidosomes. The size of PCL colloidosomes formed is arranged in descending order of 0.25% > 0.50% > 1.20%.

High surfactant concentration decreased the surface tension of the polymer and stabilizes newly developed polymer surfaces during homogenizing. In contrary, low and insufficient amount of surfactant leads to instability and recrystallization. The instability of the newly polymer surface will cause hydrophobic interactions that lead to aggregation on the emulsion. Hence, smaller size of hollow colloidosomes particles is produced with higher surfactant concentration (Maryam & Akram, 2015). In PCL/PVA and PCL/SE system, both of these system follows the trend and results displayed in Maryam & Akram. Moreover, low concentration of surfactant caused the energy of the surface lowered and provokes agglomeration. Therefore, the system tends to reduce the total surface area. Non-polar surfaces which are hydrophobic polymer has low surface energy which required surfactants in aqueous phase to aid in wetting by lowering the surface tension (Prozorov *et al.*, 1999).

4.3 Effects of Polymer Surfactant Type

The polymer surfactant was a joint for the whole experiment, its type decides the final size of the hollow colloidosomes formed. In Figure 4.3 and Figure 4.4 showed the comparison on types of surfactant between sugar ester, SE and poly(vinylalcohol) were carry out to determine that which has better yield and larger size in hollow colloidosomes. The difference of hollow colloidosomes size is obvious when comparing PCL/PVA (Red dots) and PCL/SE (Blue dots).

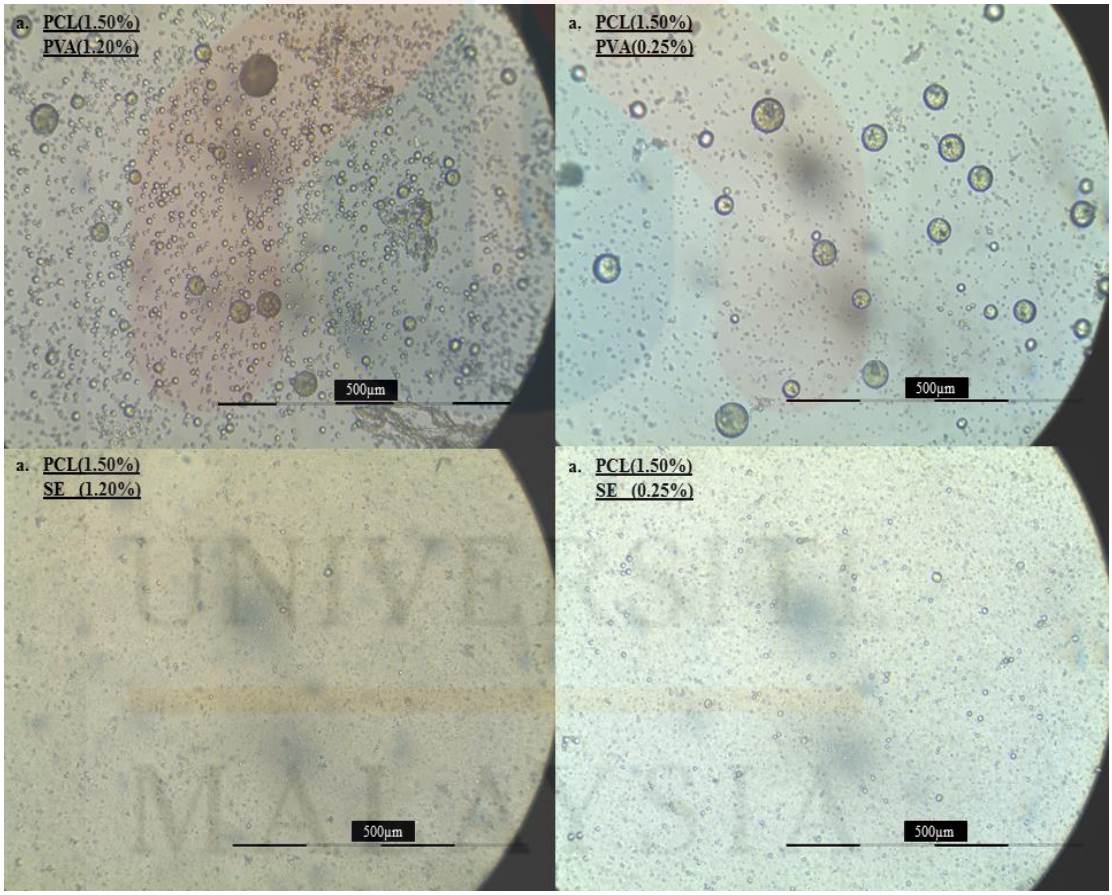

Figure 4.3: Optical images of 1.50% PCL hollow colloidosomes for two different surfactants: PVA and SE (a) PVA (1.20%) (b) PVA (0.25%) (c) SE (1.20%) (d) SE (0.25%)

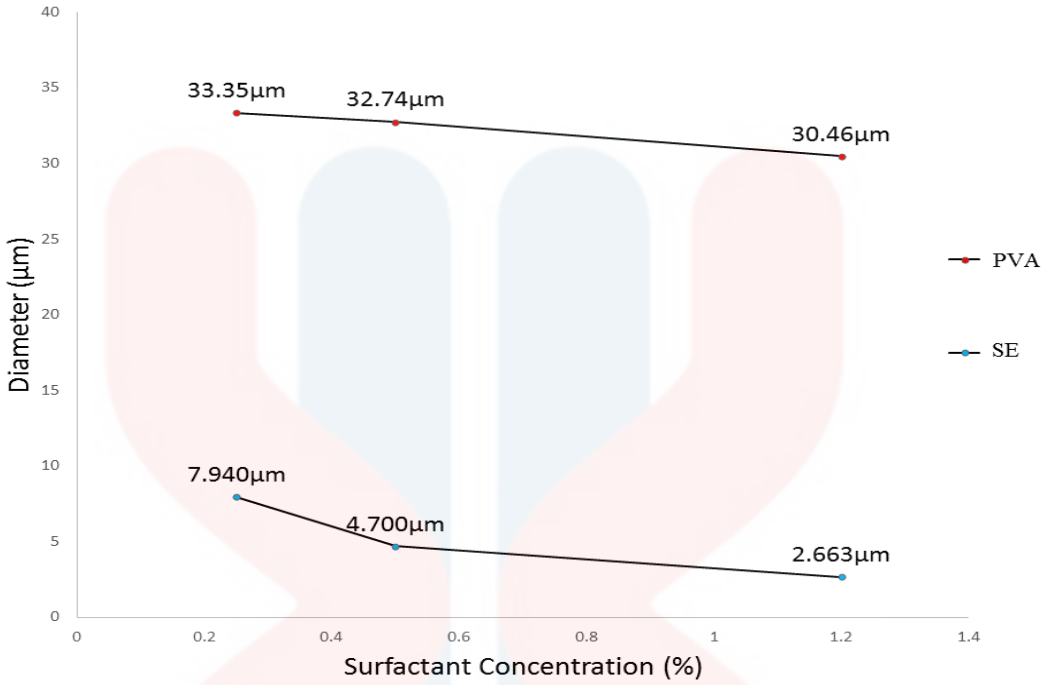

Figure 4.4: Comparison of the size of hollow colloidosomes between two surfactants: PVA and SE in 1.50 wt% of PCL.

PVA was less interface active than the SE (Lalduhsanga & Bhaskar, 2009). The PVA tends to stops further dispersion of hydrophobic interactions during emulsion than SE. With lesser dispersion, PVA was able to hold more polymer on forming a micelle which results in larger size of hollow colloidosomes produced. Whereas the SE has greater affinity in dispersing the polymer during emulsion that leaded to smaller micelles produced and smaller size of hollow colloidosomes produced (Maryam & Akram, 2016).

4.4 Effects of structural polymer concentration

The PMMA-co-MAA/PVA system in Figure 4.5 shows a very positive result in forming hollow colloidosomes with an overall diameter of 19.20 µm in 1.50 wt%

PMMA-co-MAA with 1.20% PVA and 31.68 µm in 3.00 wt% PMMA-co-MAA with 1.20% PVA.

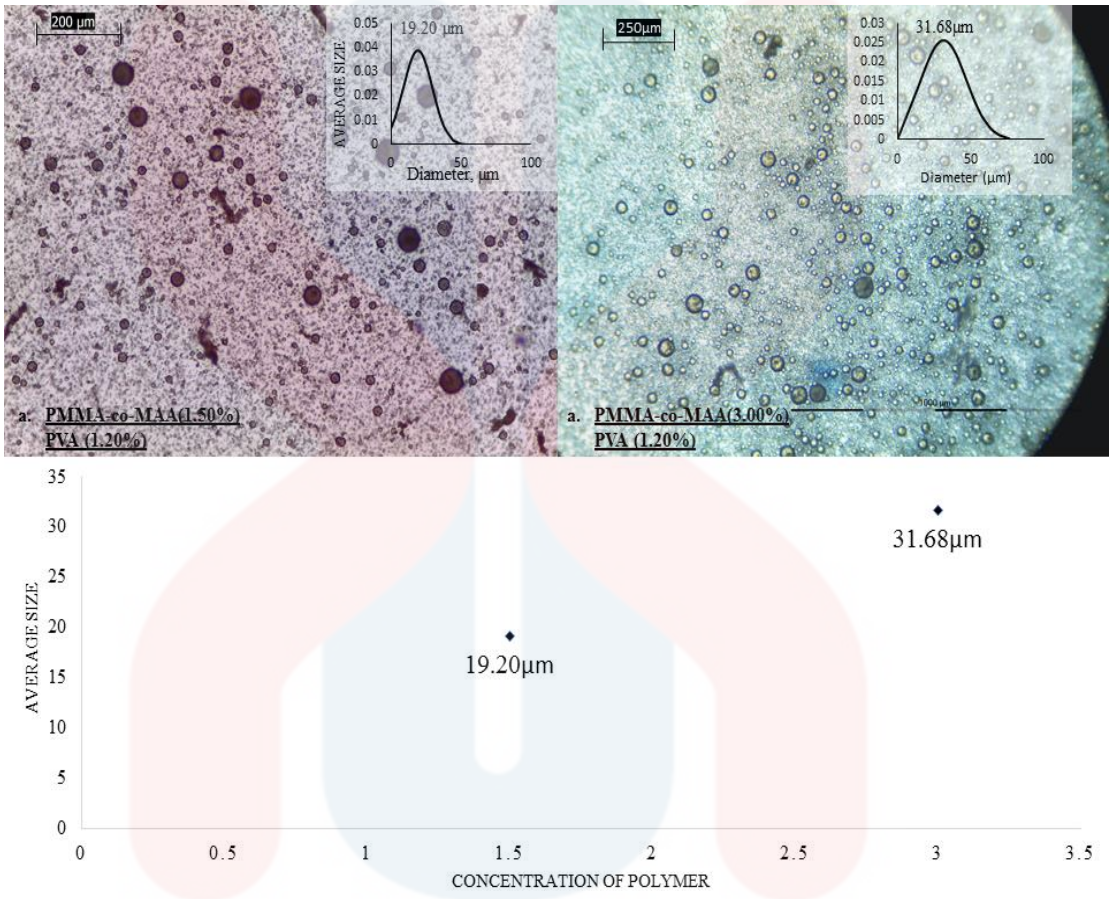

Figure 4.5: Size distribution of 1.50 wt% and 3.00wt% PMMA-co-MAA and their optical microscope image with 1.20% surfactant concentration of PVA. (a) 1.50% of PMMA-co-MAA. (b) 3.00% of PMMA-co-MAA. (c) Comparison on hollow colloidosomes size between two different concentrations of structural polymer.

By comparing the size between PMMA-co-MAA and PCL colloidosomes, the samples' average diameter from PMMA-co-MAA are slightly smaller than the samples at PCL/PVA which PMMA-co-MAA is 19.27 µm and PCL is 30.45 µm that differ in 11.18 µm. In addition, PMMA-co-MAA/PVA system does not gives a positive result in 0.25% of PVA surfactants that will discuss later. Figure 4.6 shows the distribution of PCL/PVA and PMMA-co-MAA with both 1.20% PVA as surfactants.

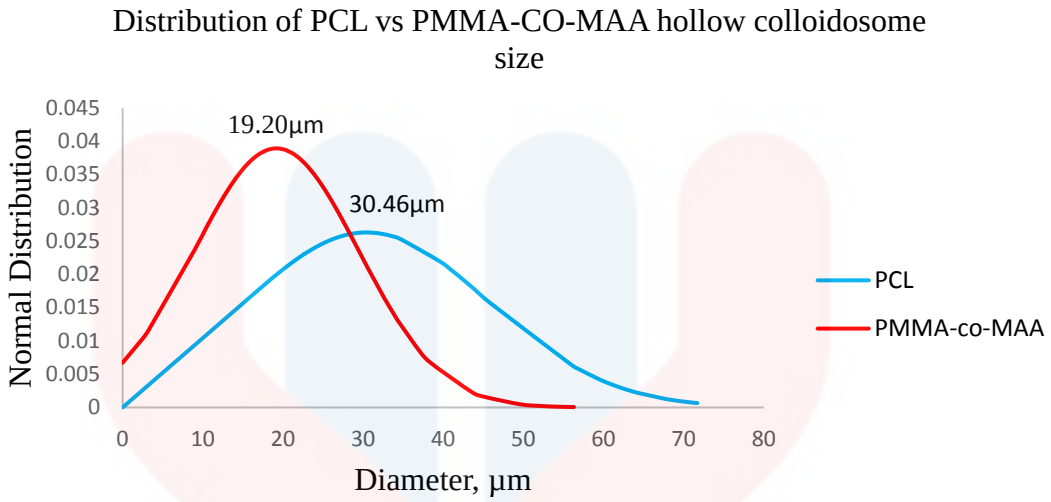

Figure 4.6: Comparison between the size distribution of 1.50 wt% PCL and 3.00 wt% PMMA-co-MAA with 1.20% PVA as surfactant.

Figure 4.5 and Figure 4.6 is a confirmation of the potential of PMMA-co-MAA to form hollow colloidosomes with a size of ~20µm. The colloidosome size that formed is slightly smaller than PCL colloidosomes and through the experiment found that by changing the structural polymer weight percent does not affect much in the particle size. The M_w of both structural polymer does not differ much with PCL (13-28 kg/mol) and PMMA-co-MAA (~34kg/mol). Two polymer should produce same size of hollow colloidosomes but the outcome was the size of colloidosome gained for PCL is larger compared to PMMA-co-MAA. This was affected by the chemical structure of PMMA-co-MAA which consist of the hydrophilic part on the MAA monomer that decrease the hydrophobicity of the polymer. Hence, the polymer M_w of PMMA-co-MAA was actually lower stated due to the hydrophilic part of PMMA-co-MAA.

4.5 Effects of Time Prior to Rotary Evaporation

Rotary evaporation is an evaporation method used to trigger formation, remove the organic solvent in emulsion and accelerated the phase separation. The duration of rotary evaporation is crucial for the hollow colloidosomes formation. 10 mins was the ideal time for rotary evaporation for all system in this experiment. Moreover, rotary evaporation has greater effect when on vacuum state which the emulsion solution's boiling point will dropped due to lower pressure. Moreover, the solution may have boiled at room temperature if under vacuum.

In the experiment, 6 mins, 8 mins and 10 mins' rotary evaporation is tried to test the optimal evaporation duration in gaining highest yield of hollow colloidosomes. For 6 mins evaporation time, the yield or the hollow colloidosomes obtained is large but there is still has organic solvent remains inside the emulsion where the polymer surface is not fully dispersed. There are still have a portion of polymer still did not form colloidosomes but formed in long, thin sheets as shown in Figure 4.7.

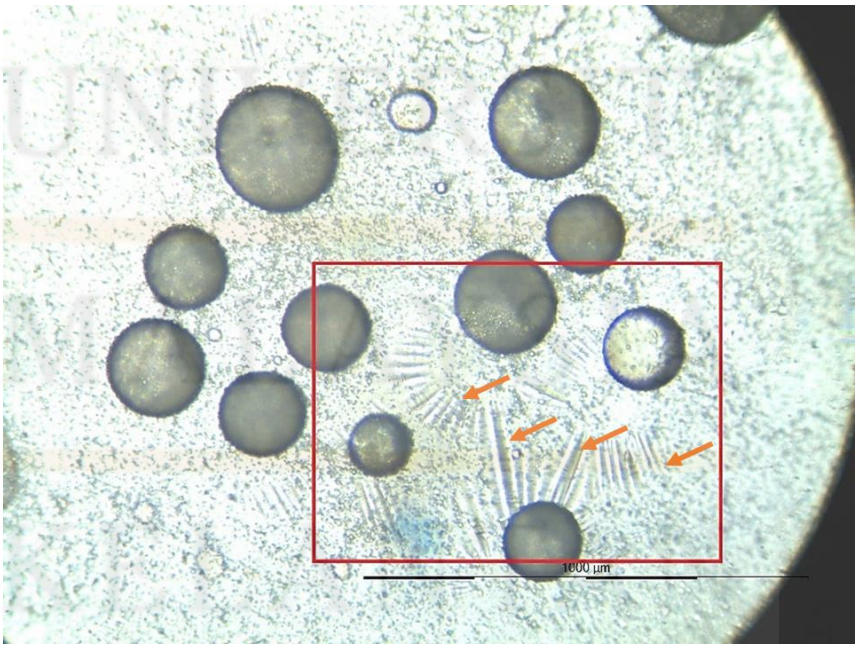

Figure 4.7: Organic solvent residues on the PMMA-co-MAA sample in 6 mins' rotary evaporation.

A strong smell from the emulsion indicated the sample still has unevaporated remaining organic solvent when observed under microscope. The unevaporated organic solvent will appear as film cover on top of the surface of the sample. On 10 mins' rotary evaporating, the sample may have the chance being aggregated to form a layer of polymer sheet as shown at Figure 4.8.

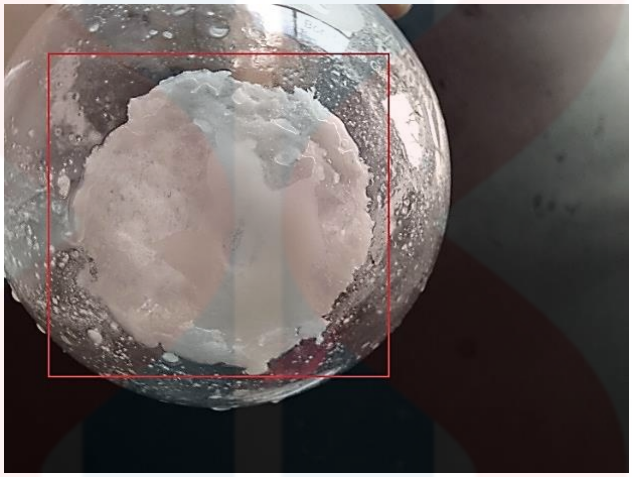

Figure 4.8: PMMA-co-MAA sample aggregated in 10 mins' rotary evaporation

Therefore, 8 mins' rotary evaporation is the most ideal time for PMMA-co-MAA hollow colloidosome to form with most perfect hollow colloidosomes which all organic solvent has been evaporated and the polymer was not aggregated together that shown in Figure 4.9.

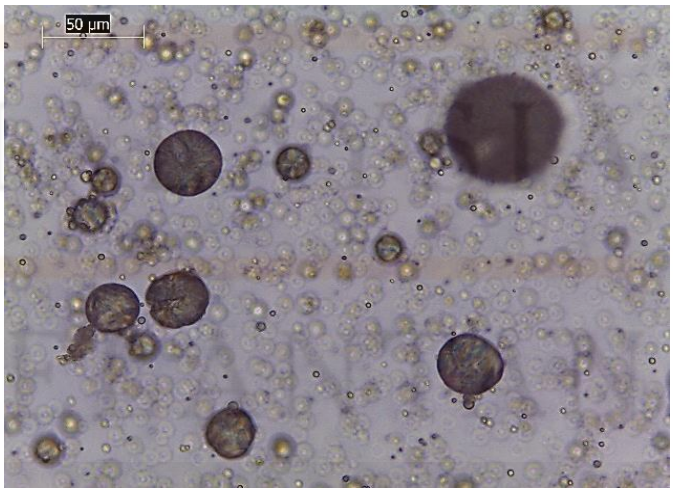

Figure 4.9: PMMA-co-MAA hollow colloidosomes rotary evaporated at 8 mins Leica image

4.6 Birefringence images of PMMA-co-MAA hollow colloidosomes

When light is passed through a polarizer to produced linearly polarized light and that light is then passed through the PMMA-co-MAA hollow colloidosomes, the light is broken up into two components. Since the index of refraction of each small colloid different orientation and position, that component will lagged in phase. Then if the light is passed through a crossed polarizer, only that part of each of the components which is in the transmission plane emerged.

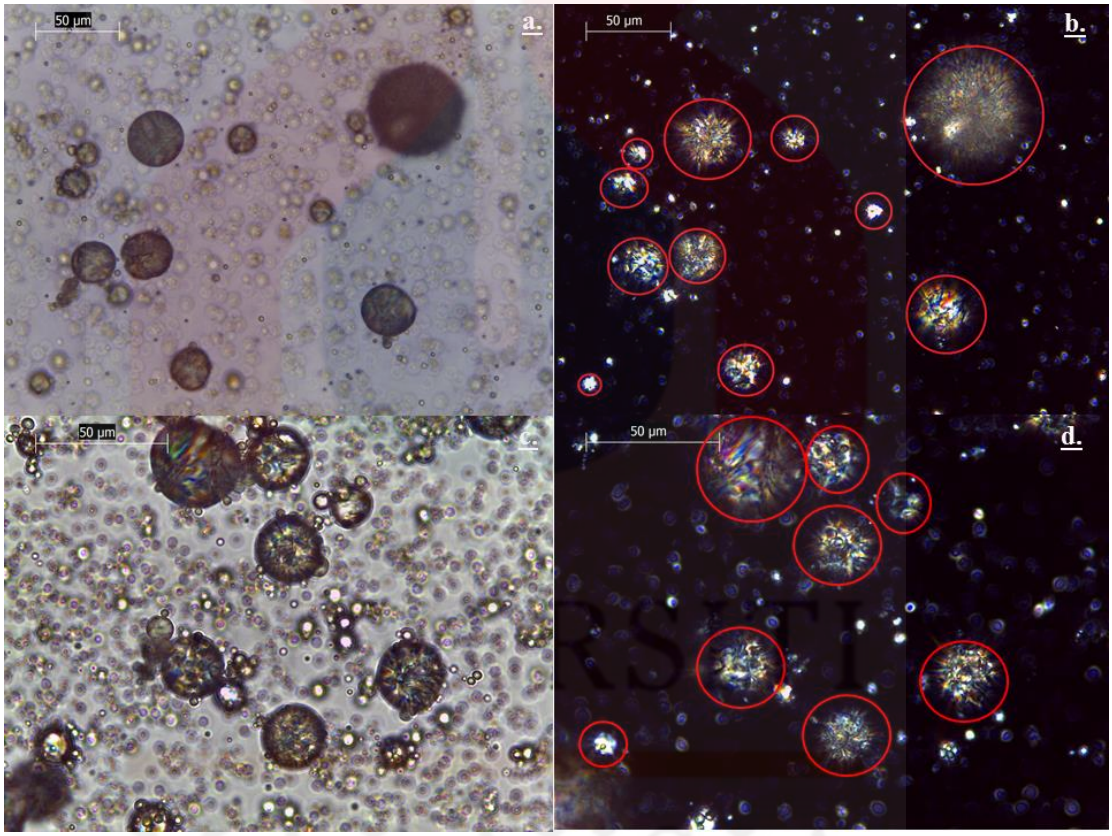

Figure 4.10: Birefringence of 1.50 wt% PMMA-co-MAA with 1.20% PVA.

The thickness of each PMMA-co-MAA colloids varies due to its shell thickness that caused some colours under cross polarizer light. The light that pass through the polarizer and shell will undergoes destructive interference and some constructive interference that gave an interference pattern of varying colours shown in Figure 4.10.

4.7 Morphology of PMMA-co-MAA shell hollow colloidosomes

Hollow colloidosomes that had a shell that formed up by one or more layer of small colloids. PMMA-co-MAA shell hollow colloidosomes with a diameter approximately 106 μm shown in Figure 4.11 and 4.12.

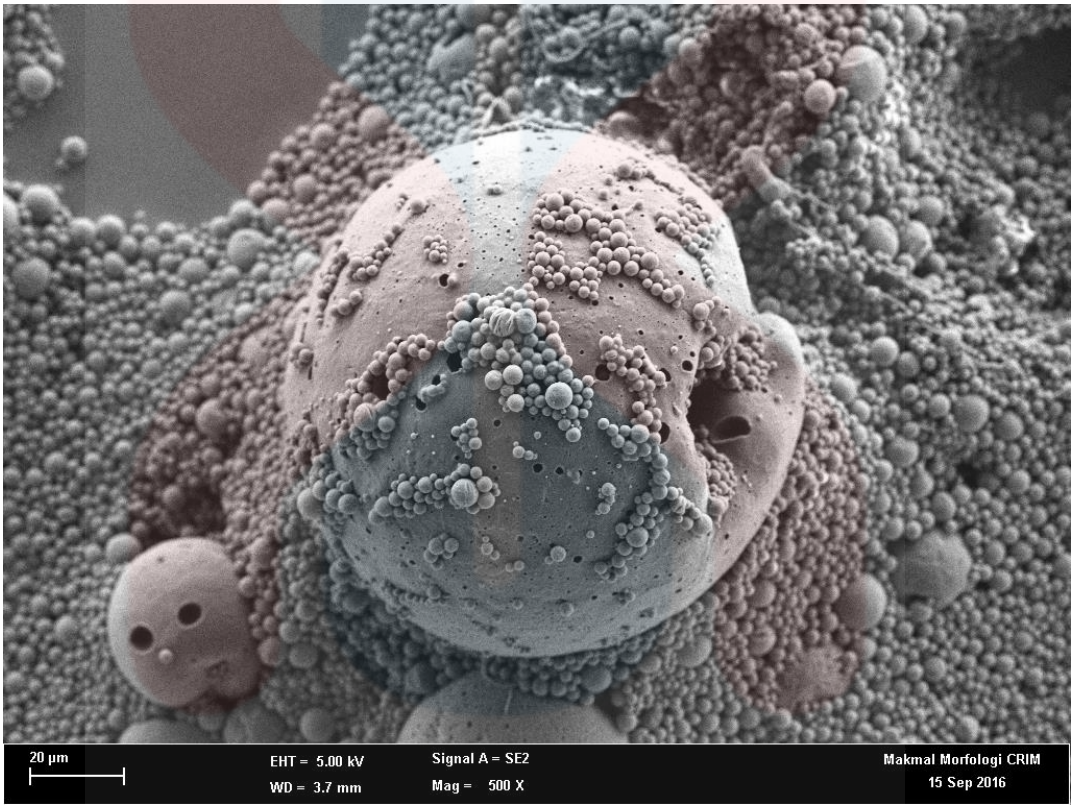

Figure 4.11: FESEM of 1.50 wt% PMMA-co-MAA with 1.20% PVA with 500X magnification.

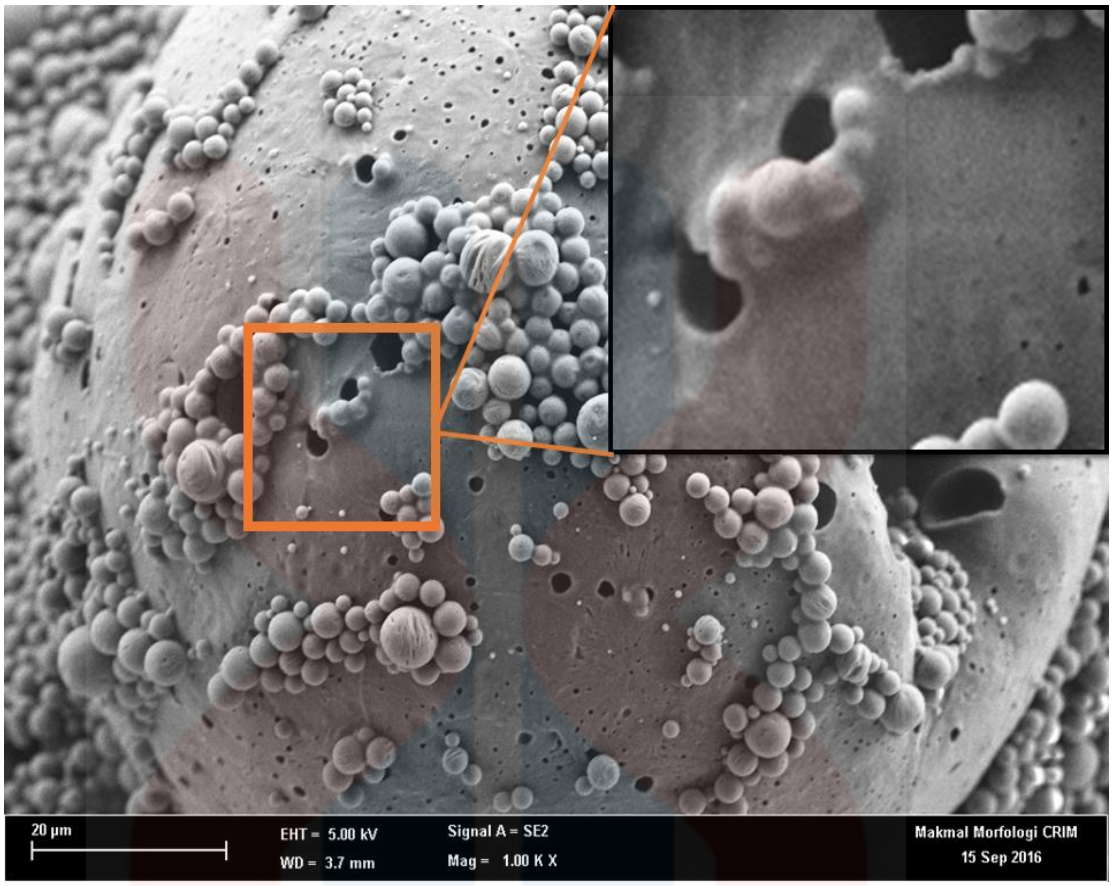

Figure 4.12: FESEM of 1.50 wt% PMMA-co-MAA with 1.20% PVA with 1000X magnification.

The higher magnification images in Figure 4.12 showed that small particles were present and partially fused on the surface of PMMA-co-MAA colloidosome surface. The FESEM image that obtained had a lot of small colloids at the surrounding that can be eliminated by further washing that would remove the small colloids.

Chapter 5

Conclusion and Recommendations

5.1 Conclusion

The PMMA-co-MAA hollow colloidosomes was able to obtained via two step solvent evaporation method. PMMA-co-MAA is a new material that able to produce hollow colloidosomes polymer structure with an average size of 19.20 μm. The size of the PMMA-co-MAA hollow colloidosome was affected by the concentration of surfactant. The higher the concentration of surfactant used will produce smaller size of colloidosome and vice versa. Moreover, type of surfactant used will decide the size of colloidosome produced. Poly(Vinyl Alcohol) (PVA) had produced larger size of colloidosomes compared to the size of colloidosome produced by sucrose ester (SE). On the other hand, the change in structural polymer concentration did affect the size of colloidosomes produced for both weight percent, wt.% of 1.50 and 3.00. The higher the structural polymer concentration produced larger size of colloidosomes. The PCL does produced a larger size of hollow colloidosome than PMMA-co-MAA. The accelerated solvent evaporation is used to gain partially fused colloids or particles which had an average size of 20.00 μm on the hollow colloidosome shells when viewed by FESEM. Optical microscope and polarizing light microscope showed the PMMA-co-MAA colloidosomes were birefringent, which the image showed interference pattern of varying colours on the spherical hollow colloidosomes. In conclusion, PMMA-co-MAA has potential as hollow colloidosomes and should be published to the

public on PMMA-co-MAA as new materials to form hollow colloidosomes that could be an alternative polymer to suit certain application.

5.1 Recommendations

There are several recommendations at the end of this study to improve for the further research purpose. First of all, different pressure during solvent evaporation could have different sizes of colloidosomes formed and should be carried out on further studies to investigate the effect of pressure on rotary evaporator. The increase in pressure will resulted strong emulsion and collision that promotes in the generation of tiny dropets (Nan *et al*., 2014)

Furthermore, investigating the rotary speed that affects the evaporation rate of emulsion and the size of the colloidosomes. In the study of Manga, 2012, the emulsion droplets produced decreased in droplet size as the rotational speed was increased. As the droplet size decreased, the diameter of shell that formed the colloidosome also decreased. Hence, the colloidosome produced will have smaller size as the rotary speed increased.

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