ENCAPSULATION OF *Acalypha Indica* EXTRACT IN CHITOSAN-POLYCAPROLACTONE BLEND

BY

MAIZATUL AKMAL BINTI JOHARI

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Kulliyyah of Engineering
International Islamic University Malaysia

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ABSTRACT

Polymer encapsulation is commonly adopted in drug delivery systems to form encapsulation that can assist in delivering active compound to the targeted area. *Acalypha indica* (AI) crude extract was obtained from AI plants through ultrasound assisted extraction, is naturally unstable in external environment, thus need to be encapsulated to protect against volatility. Chitosan has promising properties good for drug carriers. Physically blending of chitosan with PCL via physical during encapsulation process can benefit to immobilize the AI extracts against any interactions with the external environment through encapsulation. Herein, this study emphasized the development of the encapsulations of AI extracts using chitosan-polycaprolactone (PCL) blend by emulsion-solvent evaporation and then freeze-dried methods. In the beginning, the sonication time was studied to find the best time. Later, 5 minutes time was chosen and being used throughout the study. Three (3) parameters (ratio of chitosan: PCL concentration, PVA concentration , and Concentration of chitosan-PCL blend) for Al encapsulation were studied by fixing a parameter at a time (OFAT). The percentage of encapsulation efficiency (EE%) was recorded as a response for each parameter. To study the interactions between the factors, the study proceeded with central composite design (CCD) as the optimization tools of response surface methodology (RSM). Central points were taken from the preliminary data obtained in one-parameter experiments. The validation was carried out with two data of highest and lowest EE% suggested by CCD. Fourier Transform Infrared Spectroscopy (FTIR), scanning electron microscopy (SEM), particle size analyzer, and zeta potential were used to analyze the properties of selected microencapsulated samples. The highest EE% recorded was 98.70% and the lowest EE% was 87.80%. The results showed the difference of predicted and experimental values at percentage lower than 7.5%. The SEM images revealed the formation of smooth sphrerical shapes. The zeta potential for the highest and lowst EE% recorded were not so significant difference (-24.0 and -26 mV). Whereas the particle size obtained were 2.631 ± 0.14 and 3.568 ± 1.35 respectively. Overall, the encapsulation of (AI) extracts was successful and has the potential to be applied in drug delivery.

ملخص البحث

يتم اعتماد التغليف البوليميري الحيوي بشكل شائع في أنظمة توصيل الأدوية لتشكيل تغليف يمنع انحلال المركبات النشطة قبل الوصول إلى موقع معين. على الرغم من وجود اهتمام بحثي كبير بالعقاقير الاصطناعية ، إلا أن تغليفها بمركبات نشطة طبيعية يبدو أقل دراسة بشكل ملحوظ. نظرًا لأن المركبات النشطة بيولوجيًا *Acalypha indica* غير مستقرة في البيئة الخارجية وأثناء النقل ، فإن التغليف ضروري كحامل لتقليل فقد الأدوية. Chitosan لها خصائص واعدة جيدة لشركات الأدوية.يمكن أن يفيد المزج الفيزيائي Chitosan مع PCL عبر عملية التغليف الفيزيائية في شل حركة مقتطفات AI ضد أي تفاعلات مع البيئة الخارجية من خلال التغليف. تركز هذه الدراسة على تطوير تغليفات مستخلص *Acalypha indica* لتوصيل الدواء. في هذا البحث ، تم مزج Chitosan مع polycaprolactone (PCL) بطريقة تبخير المستحلب والمذيب والتجفيف بالتجميد كأسلوب تجفيف لتقوية تشكيل كبسولات دقيقة. في البداية ، تمت دراسة وقت الصوتنة للعثور على أفضل وقت. اولا اجريت التجربة لايجاد افضل زمن صوتنة والذي تراوح من 3، 5، 7 الى 10 دقائق. تمت دراسة ثلاثة (3) معلمات (نسبة Chitosan: تركيز PCL ، تركيز PVA ، وتركيز مزيج PCL -Chitosan) لتغليف Al عن طريق تثبيت معامل في وقت واحد (OFAT). تم تسجيل النسبة المئوية لكفاءة التغليف (EE٪) كاستجابة لكل متغير. شرعت الدراسة في التصميم المركب المركزي (CCD) كأداة لتحسين منهجية سطح الاستجابة (RSM) ، باستخدام البيانات الأولية التي تم الحصول عليها من OFAT كنقاط مركزية. تم إجراء المصادقة وتم اختيار معطيات من أعلى نسبة (98.70٪) وأدنى (87.80٪) من كفاءة الطاقة (EE٪) مقترحة من قبل وزارة الطاقة. تم استخدام مطيافية فوريه لتحويل الأشعة تحت الحمراء (FTIR) وحجم الجسيمات وإمكانات زيتا لتحليل عينات الكبسلة الدقيقة المختارة. أظهرت النتائج اختلاف القيم المتوقعة والتجريبية بنسبة أقل من 7.5٪. كشفت صور SEM عن تشكيل أشكال كروية ناعمة. لم تكن إمكانات زيتا لأعلى وأدنى نسبة EE% مسجلة فرقًا كبيرًا (24.0- و 26- mV). بينما كان حجم الحبيبات التي تم الحصول عليها 2.631 ± 0.14 و 3.568 ± 1.35 على التوالي. بشكل عام ، كان تغليف مستخلصات (AI) ناجحًا ولديه إمكانية تطبيقه في توصيل الدواء.

APPROVAL PAGE

I certify that I have supervised and read this study and that in my opinion, it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master of Science in Engineering

……..
Fathilah binti Ali
Supervisor

……..
Azlin Suhaida Azmi
Co-Supervisor

…… ……..
Jamarosliza binti Jamaluddin
Co-Supervisor

This thesis was submitted to the Department of Chemical Engineering and Sustainability is accepted as a fulfilment of the requirement for the degree of Master of Science in Engineering

……..
Mohammed Saedi Jami
Head, Department of Chemical Engineering and Sustainability

This thesis was submitted to the Kulliyyah of Engineering and is accepted as a fulfilment of the requirement for the degree of Master of Science in Engineering

……..
Sany Izan Ihsan
Dean, Kulliyyah of Engineering

DECLARATION

I hereby declare that this thesis is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

Maizatul Akmal Johari

Signature ..... Date 01/09/2023

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Signature Date

This thesis is dedicated to my parents for turning on my dream into reality and my fiancé for his endless support.

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At first glance, my utmost pleasure to dedicate this work to my dear parents and my family, who granted me the gift of their unwavering belief in my ability to accomplish this goal. Thank you for your support and patience.

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LIST OF ABBREVIATIONS

AI	*Acalypha indica* Linn
CCD	Central composite design
Ch	Chitosan
DOE	Design of Experiment
EE	Encapsulation efficiency
FTIR	Fourier Transform Infrared Spectroscopy
OFAT	One-factor-at-a-time
PCL	Polycaprolactone
PEG	Poly(ethylene) glycol
PLGA	Poly Lactic-co-Glycolic Acid
PVA	Polyvinyl alcohol
RSM	Response surface methodology
SEM	Scanning electron microscope
UV-Vis	Ultraviolet-visible

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Over the past decades, a lot of research and invention has been done and keeps growing to enhance the current drug delivery system (Yusuf et al., 2023). In developing a good controlled-release drug delivery system, selecting a good drug carrier is the most challenging in the sense that the side effects of the drugs and the drug carriers on human can be minimized (Adepu & Ramakrishna, 2021). Recently, encapsulation studies are focusing more on synthetic pharmaceutical drugs. However, encapsulations of certain plant extracts somehow are less explored. The challenges of maintaining stable *Acalypha indica* (AI) extracts are related to the fact that the active compounds are naturally volatile, light and temperature sensitive and susceptible to degradation (Bazana et al., 2019). AI is a herbaceous species plant. Yet it gets less attention due to its habitat mostly grow in the yards and often treated as weed. However, AI can be found only at in certain geographical regions (Zahidin et al., 2017). Interestingly, there is a fascinating fact of this plant and highly potential for commercialization. Previously, it was reported that the leaf extracts of AI have several bioactive compounds that can treat respiratory problems such as bronchitis, asthma, and pneumonia (Martin, & Ashokkumar, 2017; Taurozzi, Hackley, & Wiesner, 2012).

Chitosan has been proven as an effective polymeric drug carrier. Chitosan is found naturally from aquatic shell wastes and exists as one of the most abundant polymers occurring in nature, thus providing a vast potential of commercial value. Due to good mucoadhesive properties and biodegradability, chitosan is safe for consumption (Roy & Sahoo, 2016; Szymańska & Winnicka, 2015). However, chitosan needs modification to improve its stability and solubility to form an excellent polymer matrix for microencapsulation. By blending chitosan with other polymers during encapsulation process, the limitations of homopolymer encapsulation can be improved while maintaining the structure of the chitosan and preserves the good physicochemical properties. Physically blending with another polymer is one of many ways to improve the characteristics of chitosan.

Poly-ε-caprolactone (PCL) is a biodegradable aliphatic polyester that has better viscoelastic and allowing a large range of structures such as microspheres. Due to its property, PCL can be easily blended with other polymers such as chitosan for microencapsulations (Christen & Vercesi, 2020). Physically blending of chitosan with PCL via physical during encapsulation process can benefit to immobilize the AI extracts against any interactions with the external environment through encapsulation (Rivas et al., 2019). Encapsulation is very vital as without encapsulation, the uncontrolled environmental condition has tendency to breakdown certain types of beneficial bioactive compounds of the AI extracts.

The emulsion-solvent evaporation method is identified as the most suitable method to encapsulate the ethanolic AI extracts. This method is suitable for the mixing of insoluble materials and polymer assisted by different phase of surfactants (Iqbal et al., 2015). This method followed by hardening and finally produced a sphere shaped particles with the active materials enclosed inside the sphere (Luo et al., 2023). Encapsulation efficiency is one of the most potential indicators of accomplishment in encapsulation. The encapsulation efficiency through this method is affected by several factors, including sonication time, the ratio of chitosan:PCL PVA concentration, and the concentration of chitosan-PCL blends.

The purpose of identifying the best sonication time is the most crucial as the microtip of the ultrasonic homogenization device is sensitive towards erosion (Taurozzi et al., 2012). Considering each brand and size of the microtips have different time of sonication depending on the input power, finding the different sonication time is the most important parameter in this study.

So, the change of these factors gave valuable findings, and this knowledge contributes to the next encapsulation studies.

1.2 STATEMENT OF THE PROBLEM

It is known that chitosan is a weak base, and insoluble in water and organic solvents which limits the encapsulation (Garg et al., 2019). Physically blending of chitosan with other polymers during encapsulation process are promising. To the best of author's knowledge, stabilization study of AI extracts through encapsulation are still less explored. In 2013, an encapsulation study of AI extract done by Amarnath et al. need improvement in terms of the microparticles stability. In conjunction of that, a study of AI extract loaded into the biodegradable chitosan-PCL was proposed to preserve the active compounds against degradations due to the interactions with the environment (Bilia et al., 2018). This blended material is very promising due to non-toxic thus safe for the human body system.

Besides, chitosan-PCL copolymer was capable to microencapsulate the hydrophobic behaviour of AI extracts by emulsion-solvent evaporation method using ultrasonic homogenizer modified by El Hady et al. (2019) methods. The ultrasonic homogenization has several parameters need to be optimized. One of the most important parameters are homogenization time since each ultrasonic homogenization devices possessed different output. Concerning of finding the applicable homogenization time, the sonication time was proposed in the beginning of this research by identifying the highest encapsulation efficiency (EE%) and the morphological characteristic of the microencapsulations. Anwar et al. (2022) reported the optimized sonication time for the encapsulation of lycopene was 6 minutes.

Other than that, the previous reported parameters are less applicable for different types of active compounds used and the polymer matrix due to the behaviour of the drugs and the polymer (El Hady et al., 2019). To overcome this drawbacks, pre-optimizations have been done by one-factor-at-a-time (OFAT) method and followed central composite design (CCD) model to study the interactions among the parameters. There are numerous studies on the chitosan copolymer encapsulation on the hydrophobic and hydrophilic drugs (Kim et al., 2019). Yet to the best of our knowledge, no studies reported on the chitosan-PCL modification encapsulated AI extract. The previous study only focusing the encapsulation for wound healing (Nezhad-Mokhtari et al., 2023). Combining two types of polymers create a new blended biomaterials

give synergistic effects for the better encapsulation. Hence, this study is motivated to produce a stable microencapsulation of AI extracts through the chitosan modification as a polymer matrix to maximize the amount of extract being encapsulated. This is because of the challenges of maintaining a stable *Acalypha indica* (AI) extract are related to the fact that the active compounds are volatile, light and temperature sensitive and degradations (Bazana et al., 2019).

1.3 PURPOSE OF THE STUDY

The *Acalypha indica* (AI) has been acknowledged as medicinal plants traditionally and scientifically proven even though they are often classified as weeds. AI has several beneficial active compounds that can be found abundantly in this plant such as quercetin, gallic acid and rosmarinic acid (Zahidin et al., 2017).

Encapsulation is highly necessary due to the instability of the bioactive compounds in external environment. According to the previous study, chitosan is a promising natural biopolymer obtained by partial deacetylation of chitin which can be naturally found in marine wastes (Kurita, 2006). Chitosan is one of the excellent types of a versatile polymer as it has numerous advantages including non-toxic. Thus, chitosan is safe for oral consumption. Additionally, chitosan is also a biomaterial which can be blended easily to encapsulate the active compound and applied in drug delivery (Wang et al., 2011). The study on the chitosan modification is a topic of interest as it is not altering the fundamental backbone of chitosan and preserve its physicochemical properties. The encapsulation process was successfully done by emulsion-solvent evaporation method with Poly(ε-caprolactone) (PCL). Towards the end of the steps, the microemulsion AI extracts were dried by freeze drying technique and turned into microparticles which in powder form.

1.4 RESEARCH OBJECTIVES

This study aims to achieve the following objectives:

1- To investigate the best sonication time for the formation of chitosan-PCL microencapsulation.

2- To analyse the optimum encapsulation parameter of *Acalypha indica* in the selected chitosan modification for maximum encapsulation efficiency.
3- To examine the characterizations of the chitosan-PCL microencapsulation.

1.5 RESEARCH SCOPES

This study is focusing on the encapsulation of *Acalypha indica* (AI) extracts by emulsion-solvent evaporation method. To be specific, the scopes of this study were further explained according to the objectives as follows:

- Sonication time was the first parameter studied in objective 1 to find the best time to form encapsulations. The time chosen was 3, 5, 7 and 10 minutes. The best sonication time was chosen based on the highest percentage of encapsulation efficiency (EE%) and supported by surface morphology characterization using scanning electron microscope (SEM).

- In objective 2, the ratio of chitosan and PCL as a polymer matrix, PVA concentrations and the polymer matrix concentrations were the other factors optimized by OFAT. Polyvinyl alcohol (PVA) was selected as the non-ionic surfactant for further improving dispersion stability. The chitosan solution with concentrations ranging 0.2-1.0 %w/v were properly diluted in 0.2% w/v acetic acid, PCL solution (0.2-1.0 %w/v) were diluted in dichloromethane (DCM), PVA solution (0.05-1.0 %w/v) were diluted in deionized water under 60 °C and the AI extracts (0.02% w/v) were diluted in ethanol absolute. These solutions were blended ultrasonically later forming the emulsions. The emulsions were stirred gently for 24 hours. The microparticles were dispersed in the deionized water and keep in the -81°C before freeze dried.

- In objective 3, the encapsulated chitosan-PCL loaded AI extract was analysed using FTIR and the morphological structure was observed under SEM. Ultraviolet-visible (UV-Vis) spectrophotometer was used to determine the percentage of encapsulation efficiency (EE%). The microparticles were further characterized with Fourier Transform Infrared (FTIR), particle size analysis and zeta potential.

1.6 THESIS ORGANIZATION

This thesis consists of five chapters which covers all the introduction, literature reviews on the chitosan biopolymers and the selection of methods, methodology, results, and discussion and finally conclusion and recommendations. The abstract briefly summarizes the whole study in this thesis.

Chapter 1 introduced the background of study which is the importance of chitosan blended with synthetic polymer for better encapsulation towards the potential applications of AI extracts. This chapter also briefly introduced the importance of finding the best sonication time and optimized the other three parameters with OFAT and RSM. Then followed by the problem statements, purpose of study, research objectives, and the research scopes.

Chapter 2 discussed details on the introduction of chitosan and its origins. Previous studies of chitosan and the need of modifications were further explained. Numerous types of chitosan copolymer that has been studied in past 10 years and the selection of methodology are also well explained in this chapter. Next, another interesting subchapter is the exploration of encapsulation technology as well as to rediscover the lack of knowledge. The study on encapsulation for drug delivery has been extensively studied not only for the modern medical drugs but also the encapsulation of plant extract. End of this chapter, briefly description of *Acalypha indica* plant and its benefits were reported.

Chapter 3 reports in detail the methodological approach in this study illustrated by flowchart of methodology to give a clear figure. The method of microencapsulation, the optimizations by OFAT and RSM, and analysis has been discussed thoroughly in this chapter. The design of experiment (DOE) of RSM were assisted by Design-Expert v.12. The zeta potential, particle size analysis and SEM are the analysis conducted in this study.

Chapter 4 discusses all the results and analysis obtained from this study. The results from OFAT were used in RSM. Two (2) data of validations suggested by DOE were selected

for the analysis in objective 3. Thus, the importance and the selection of each data were discussed thoroughly.

Chapter 5 concludes all the numerical data obtained from this research work and answered all the objectives. This chapter also includes the future outlook as well as suggestions to improve this research for the next continuation.

CHAPTER TWO

LITERATURE REVIEW

2.1 INTRODUCTION

Encapsulation is a great topic to be explored in the polymer science, and the applications are most promising in pharmaceutical and medicinal fields. Encapsulation is defined as the process of enclosing the plant extracts or drugs in a sphere of capsules to improve its stability (Castro-Enríquez et al., 2020). Encapsulation is very important for every bioactive material to protect from the external environment until reaching the targeted sites in the body system. Besides, encapsulation is important to mask the odour and taste during consuming. In conjunction of that, selection of the carriers (polymer matrix) is promising as to maximise the quantity of drugs being encapsulated and maintain stable while delivery. Moreover, encapsulation can optimize the use of raw materials as well as minimize the production cost.

There are numerous encapsulation materials studied in past years. Mostly focuses on the biopolymer-based in order to maintain stability and bioavailability. Chitosan is one of the encapsulating materials used to encapsulate various kinds of hydrophobic drugs and some bioactive compounds which obtained from plant constituents. Chitosan is not only having biocompatibility properties but also has antimicrobial properties that may kill some harmful microbes in the body system. Due to some limitations on the chitosan, it is needed to blend with other biopolymers or synthetic polymers. Instead of blending with other polymers, recent studies reported by modifying the chitosan with fatty acid-based materials for instance oleic acid (Méndez et al., 2017).

Drying is the most important steps to turn the emulsions into particles. The method of drying also depends on the solubility, sensitivity of the bioactive materials and applications. Spray drying and freeze drying are among the common methods of drying. Spray drying was used mainly for emulsion encapsulation like the encapsulations of *Boswellia carterii* essential oil (Barre et al., 2020) whereas freeze drying usually for drying of insoluble encapsulations like encapsulation of diosmin with chitosan-PLGA (El Hady et al., 2019).

Acalypha indica (AI) somehow was known as cat attractants by some rural regions due to the fresh roots attract cats for their remediation. A study done by Zahidin et al (2018) found out the potential benefits of AI. Interestingly, research also found that AI extracts have numerous good properties of antibacterial (Nikmah et al., 2019). Research and development keep on going from the scratch until ways to stabilize the active compounds of the AI. Further elaborations are given in the next subchapter of the literature review.

2.2 THE POLYMER MATRICES FOR ENCAPSULATIONS

Polymer matrix is defined as the layers consisting of multiple polymer chains (homopolymer or copolymer) which capable to entrap the hydrophilic or hydrophobic drugs. In other words, polymer matrix is the outer layer of the encapsulations (Uhrich & Abdelhamid, 2016). There are many types of polymers which classifies according to the nature of the polymers and chitosan is one of the natural polymers.

2.2.1 Chitosan

Chitosan is a biopolymer derives from chitin. Chitin is the second most important natural polymer of carbohydrates (polysaccharides) in the world. Chitin undergoes deacetylation to form a chitosan biopolymer. Chitosan or linear (1-4) linked 2-amino-2-deoxy-β-d-glucan (i.e., β-d-glucosamine) is a natural polymer which is a linear polysaccharide derived from partial deacetylation of chitins that soluble in acidic aqueous media but insoluble in higher pH media (Rinaudo, 2006). Each unit of chitosan monomer consists of two functional groups which are OH- and NH_3- as illustrates in Figure 2.1

The source of chitosan can be found abundantly from shrimp and crab shell containing chitin which is an N-acetyl glucosamine polymer (Ahmed & Ikram, 2015). Other than that, chitosan also can be obtained from the extraction of chitin from fungi, algae, Echiruda, Annelida, Mollusca, Cnidaria, Aschelminthes, Entoprocta, Bryozoa, Brachiopoda, Arthropoda, Ponogophora and the epidermal cuticle of the vertebrates (Ho et al., 2015).

2.2.2 Chemical structure of chitosan

Chitosan comprises of three reactive groups, which are primary (C-6) and secondary (C-3) hydroxyl groups on each repeat unit and amino (C-2) group on each deacetylated unit. Structurally, chitosan is a linear-chain of copolymer that composed of D-glucosamine and N-acetyl-D-glucosamine that has been synthesized by the partial deacetylation (removal of acetyl group) of chitin (Figure 2. 1). The presence of -NH_2 and -OH groups allow chitosan to interact with other polymers as well as improving the drug properties. Drug solubility, adsorption and biocompatibility are among the improvements in drug properties (Kaur et al., 2023).

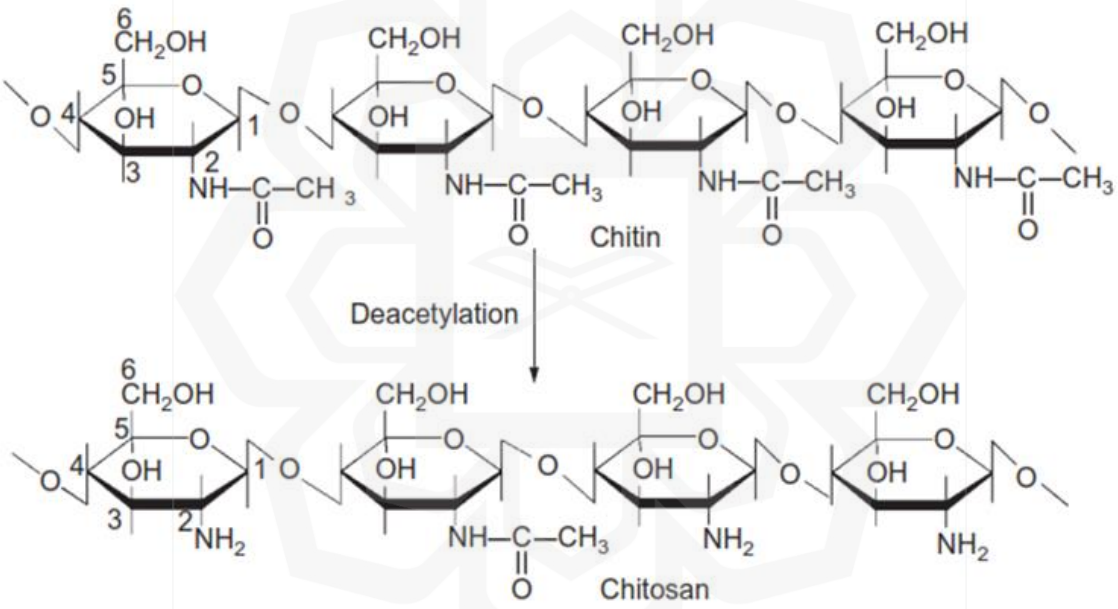

Figure 2.1 Chitin and chitosan's (linear (1-4) linked 2-amino-2-deoxy-β-d-glucan) chemical structure (Lv, 2016)

Chitosan is a non-toxic and biocompatible polymer. Hence, the viscosity of chitosan can influence the biological properties such as wound-healing properties as well as biodegradation by lysozyme. This is because chitosan also has antibacterial properties (El-Hefian et al., 2014). Next, the swelling property of the chitosan decreases with an increase in the concentration of

the cross-linking agent. The summary of physical, chemical and biological properties as tabulated in Table 2.1.

Table 2.1 Physical, chemical, and biological properties of chitosan (El-Hefian et al., 2014)

Physical properties	**Chemical properties**	**Biological properties**
White-yellow in colour	Degree of deacetylation range 70-95%	Biocompatibility
Flakes, bead or powder	Cationic polyamine	Antibacterial
High molecular weight (1.2 X 10^5 $gmol^{-1}$)	High charge density at pHs < 6.5	Safe and non-toxic
High to low viscosity	Forms gels with polyanions	Haemostatic
Intermolecular hydrogen bonding	Linear weak polyelectrolyte	Biodegradable to normal body constituents
Amorphous solid	Adheres to negatively charged surfaces	Bacteriostatic/ Fungistatic
Density range 0.18 to 0.33 g cm^{-3}	Chelates certain transitional metals	Spermicidal
Soluble in diluted aqueous acid solution (i.e.,acetic acid)	Amiable to chemical modification	Anticancerogen
Insoluble in water	Reactive amino/hydroxyl groups	Anticholestermic

According to the Table 2.1 above by El-Hefian et al. (2014), the biodegradability of the chitosan catches more attention among drug delivery researchers as the polymer matrix. Besides, chitosan only needs the diluted aqueous acid (0.1-0.2 %w/v) to dissolve thus promote better degradations in the body digestive system (El Hady et al., 2019).

2.3 ENCAPSULATION

Encapsulation is a process in which the tiny particles of drugs or bioactive chemicals are being capped by coating or surrounded in a homogenous or heterogeneous environment giving small capsules with vast beneficial properties (Deshmukh et al., 2016). Recently, encapsulation plays an important role to protect the drugs or substrate to target delivery as it involves the isolation of a compound (i.e., drug and bioactive). Encapsulation of drugs with polymers also enables the engineering of releasing rate of that drug from the polymer matrix depending on the properties of both drug and the polymer (Bayat & Nasri, 2019). Figure 2.4 shows the common pathway for the drugs or active compounds administration orally into the drug delivery system (Subramanian et al., 2016). This technology is very best suited to apply on the less stable materials such as the easily volatile phytochemical extracts. Thus the chitosan has good inclination to associate with other polymers to encapsulate the *Acalypha indic*a extract.

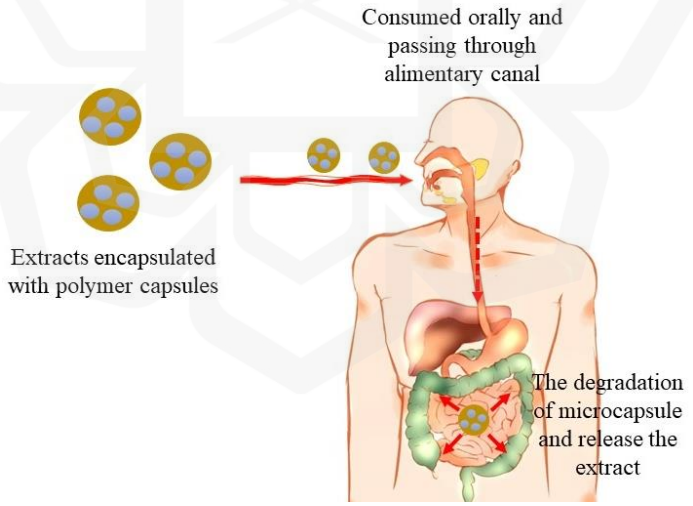

Figure 2.2 The design of common route of active compounds administration (Johari et al., 2021).

2.3.1 Methods of microencapsulation

Microencapsulation can also be known as one of the method of preservation specifically to the bioactive compounds which is highly sensitive and instable resulting more stable

products (Jyothi et al., 2010a). The product of the microencapsulation technique is known as microparticles, microcapsules and microspheres depend on the surface morphologies and size. Microencapsulation consists of several kinds of techniques depending on the nature of the drugs to be encapsulated. In this section, the microencapsulation techniques only focus on the blending methods.

2.3.1.1 Emulsions- diffusion method

This emulsion-diffusion method involves the moderately water-miscible solvent such as benzyl alcohol, propylene carbonates to dissolve the encapsulating polymer and the solvents are nontoxic (Iqbal et al., 2015). Water and solvent are the two equally saturated phases in which the saturated water includes the 8- 10 % of solvent and the saturated solvent includes the 2- 3 % of water. The stabilizer is then added and dissolved into the saturated water at ambient temperature. Then the prepared organic solution is poured into the saturated water in which the stabilizer was added. The resultant solution is stirred with a rotor-stator device in a cylindrical vessel. The oil-water (O/W) emulsion forms at room temperature. The dispersed droplets are converted into nanocapsules upon addition of a large volume of water (approximate four times from the emulsion volume) to induce the solvent diffusion. After the additions of water on the emulsion and mixing at 300 rpm, nanocapsules are formed. The solvent and the part of the water are being removed by evaporation under a reduced pressure to get a purified and concentrated suspension (Pathak et al., 2019).

A study was done by Souguir et al., 2013 to encapsulate curcumin in polyurethane and polyurea shells by emulsion-diffusion method. The encapsulation of curcumin was found in amorphous phase by thermal analysis. The nanoencapsulation was done to preserve the properties of curcumin and avoid from photodegradation (Souguir et al., 2013).

2.3.1.2 Emulsions- solvent evaporation method

Emulsions- solvent evaporation method is the most popular method frequently used to synthesis the polymer encapsulation. This method consists of two phases which are aqueous and organic phase. This method involves the dissolving of a poorly soluble drug into the polymer/solvent solution. Then followed by the emulsification of the solutions into an aqueous phase. Then, the solutions will be evaporated and the polymers with the drug are precipitated as the microspheres (Pathak et al., 2019). There are two types of single emulsions; water-oil (W/O) and oil-in-water (O/W) and it was illustrated in Figure 2.6. The advantage of this method is that the particle size formed can be adjustable by changing the homogenization speed and the amount of stabilizer.

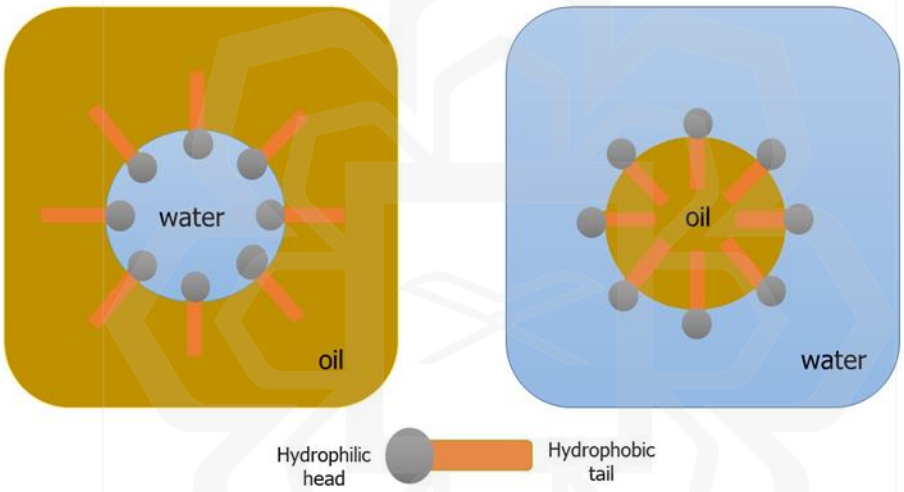

Figure 2.3 a) water-oil (W/O) emulsion, b) oil-water (O/W) emulsion

However, this method is only suitable to encapsulate the poorly water-soluble drugs such as 5-fluorouracil (Wang et al., 2013), paclitaxel (Lu et al., 2019), and celecoxib (Méndez et al., 2017). This method was improved by double emulsions method which is water-in-oil (W/O/W) emulsion.

2.3.1.3 Double emulsion and evaporation method

Double emulsion is more complex system because of the droplets of first phase is dispersed into the second phase. To improve the emulsion-solvent evaporation method

as this method is only suitable to be applied on hydrophobic drug, double emulsion method was applied to encapsulate the water-soluble drug. Long time ago, double emulsion droplets were identified three types (Figure 2. 7) by (Florence & Whitehill, 1981). In Figure 2.7, three (3) W/O/W emulsion system illustrated; Type A is the simplest system, Type B is the larger emulsion system composted of small droplets, and Type C is the most complex emulsion system due to the relative largest size encapsulates most droplets and shows slow release. But these droplets usually did not exist in one system but the droplets are predominant (Iqbal et al., 2015).

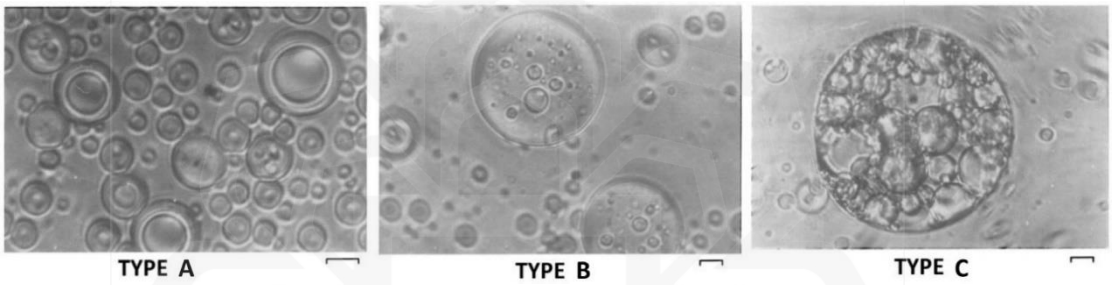

Figure 2.4 Images of the types of double emulsion droplets of Type A, Type B and Type C (Florence & Whitehill, 1981)

After that, there are two common types of double emulsion often discussed which are water-oil-water (W/O/W) and oil-water-oil (O/W/O). These methods are similar but different in sequence of mixing and depend on the types of active compounds that are encapsulated. The mechanism of double emulsion illustrates the W/O/W as the example in Figure 2.8. The double emulsion involves two steps as shown in Figure 2. 8. In the first step of W/O/W double emulsion preparation, the inner aqueous phase (W1) is dispersed in the oil phase containing the lipophilic emulsifier. Then followed by the dispersion of the primary emulsion into the outer aqueous phase (W2) containing hydrophilic emulsifier.

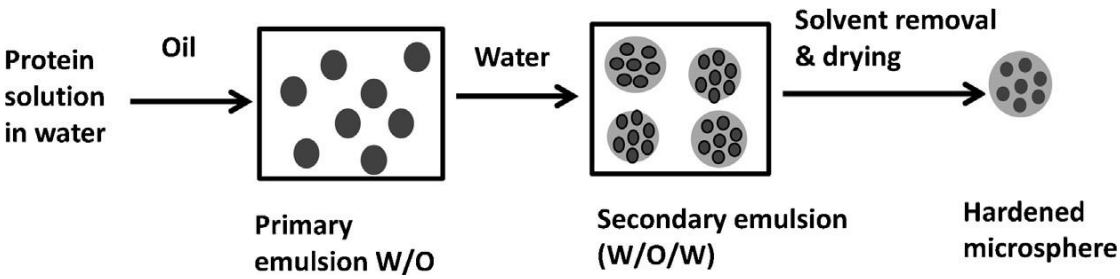

Figure 2.5 Double emulsion solvent evaporation method for microencapsulation of proteins (Iqbal et al., 2015; Yeo et al., 2001)

Many journals reported the method of double emulsion solvent used to encapsulate the hydrophilic drugs. A study done by Prado et al. (2017) applied this method PVA was used as the non-ionic surfactant and the highest percentage encapsulation efficiency (EE%) obtained was 99.04%.

2.3.1.4 Solvent displacement/ Precipitation method

This method is also suitable for encapsulation of hydrophobic and lipophilic drugs. In this method, the polymer is precipitated from an organic solution and the organic solvent diffuses in the aqueous medium in the presence or absence of surfactant. The semipolar water-miscible solvent (i.e: acetone and ethanol) is used to dissolve polymers, drugs, and lipophilic surfactant. Then under magnetic stirrer conditions, the prepared solution is added into an aqueous solution containing stabilizer. By rapid solvent diffusion, the particles are formed instantly. The solvent is removed from the suspension under the reduced pressure (Pathak et al., 2019). This method is simple and fast and does not involve the use of highly toxic solvents. The particles obtained could be monodisperse (Iqbal et al., 2015).

Each encapsulation techniques have pro and cons depending on the materials to be encapsulated and aims. Here are the summary of encapsulation methods simplify in Table 2.2.

Table 2.2 Methods of blending encapsulation

Methods	**Advantage**	**Disadvantages**	**References**
Emulsions- diffusion method	-Facile reaction without agitation through sonication. -Easy to scale up the process.	Less suitable for water soluble drugs as water soluble drugs easily leached.	(Tahara et al., 2008)
Emulsions- solvent evaporation method	-Obtaining small size of particles and monodisperse. -Reproducibility	- The probe of the ultrasonic homogenizer device easily wear and affect the reaction - Quite hard to scale up	(Rençber et al., 2020)
Double emulsion and evaporation method	-Suitable to encapsulate hydrophilic molecules and biopharmaceutical compounds	Shear force from homogenization may damage the molecules and affect the biological activity.	(Iqbal et al., 2015)
Solvent displacement/ Precipitation method	Absence of shear stress	Can be used only for water miscible solvent.	(Bashir et al., 2022)

2.3.2 Chitosan as a blended polymer for encapsulation

Chitosan has been blended to improve the properties microparticle encapsulation such as biostability, viscosity and surface tension (Doğan et al., 2013). The blending can be done by mixing either both with the natural polymers or synthetic polymers. In pharmaceutical, chitosan blends are widely used to control release of drugs. Various

physical forms of chitosan blends are microparticles, tablets, films, beads and gels depending on the mechanism of reactions (El-Hefian et al., 2014). There are two major categories of polymers blended chitosan which are natural and synthetic polymers.

The blending of chitosan with other natural polymer becomes a promising method as it brings about a new biomaterial. Previous study was done by combinations of natural polymer (i.e. collagen, gelatine, and starch). Starch blended with chitosan with certain ratios can improve the solubility of chitosan as it may be due to the amino group of chitosan creating a bond with hydroxyl groups of fibre and starch (Bhattarai et al., 2010). Usually, the natural polymers have been employed in biomedical applications especially drug delivery systems and tissue engineering matrices (Kausar, 2017).

Next, another type of copolymer blended chitosan is blending with a synthetic polymer. There are some combinations of synthetic polymer which are; polycaprolactone (PCL) (Zhang et al., 2016), Polysulfone-chitosan blend, polycarbonate/chitosan blend, polystyrene-chitosan blend, polyaniline-chitosan blend, epoxyl-chitosan blended, polyvinyl alcohol (PVA) (Mulchandani et al., 2017) and polymethylmethacrylate (PMMA)-chitosan blended (Kausar, 2017). Polyurethane also has been blended with chitosan using a solvent casting method homoeopathic and the mechanical properties have been evaluated (El-Hefian et al., 2014). These kinds of polymer employed with chitosan depend on the applications.

Another successful study was done by Paula et al. (2017) reported that the modification of chitosan-oleic acid (OA) by emulsion/solvent evaporation method can encapsulate the hydrophobic drug which is celecoxib resulting in promising encapsulation efficiency (EE %) obtained was 75. 8 %. Besides, Xie reported by grafting of chitosan with stearic acid resulting high loading capacity of doxorubicin and posed a slow-released behaviour. This is good since a high amount of the drug accumulated in the brain and less amount in the heart (Xie et al., 2012).

2.3.2.1 Chitosan blends with natural polymers

Natural polymers are naturally occurring polymers through the extractions of living organisms such as plant constituents, marine shells, and fungi. Usually, natural polymers are basically polysaccharides and no side effects due to biocompatibility properties.

In plants, the sources of biopolymers are from the cell wall which consists of mainly cellulose, hemicellulose and pectin. Cellulose is the most abundant natural polymer. Whereas in animals, the sources of biopolymers are mainly derived from proteins, polysaccharides, and lipids (Wankhade, 2020). The example of natural polymers origin from plants and animals are simplified as in Table 2.2 below.

Table 2.3 Example of polymers from plant origin and animal origin

Polymers from plant origin	**Polymers from animal origin**
Cellulose	Chitin
Hemicellulose	
Glucomannan	Carageenans
Agar	Psyllium
starch	Xanthan gum
Pectin	
Inulin	
Rosin	
Guar gum	
Arabic gum	

Specifically, chitosan modification has been extensively study (Mourya & Inamdar, 2008). Chitosan has been reported in various applications such as in nanofiltration, tissue engineering, and coating materials for targeting drug delivery transportations (Varsha, 2020). Table 2.3 summarizes the modification of chitosan with among natural biopolymers used to coat the active materials. The mixing ratio of the

chitosan and the blended polymer were studied by Dadou et al. (2018). All the formation of encapsulations were confirmed by SEM and FTIR.

Table 2.4 Some examples of the chitosan blends with natural polymer encapsulation

Chitosan with natural polymers	**Solubility in water**	**Forms**	**Applications**	**References**
Alginate	Insoluble	Powder	Antibacterial	(Kumar et al., 2021)
Xanthan gum	soluble	Bead hydrogels	Drug delivery systems	(Dadou et al., 2018)
Arabic gum	Soluble	powder	Drug delivery systems	(Sakloetsakun et al., 2015)

2.3.2.2 Chitosan blends with synthetic polymer

Synthetic polymers are generally derived from petroleum or lab synthesized and has been used to modify with chitosan which is approved by FDA. Commonly there are various synthetic polymers has been used in the present studies which are polycaprolactone (PCL), polylactic acid (PLA), polyethylene glycol (PEG), and poly(lactic-co-glycolic acid) (PLGA). Blending with synthetic polymer has a good advantages during preparation in terms of reproducibility. This is because synthetic polymers are more homogenous and higher purity than natural polymers (Lai et al., 2014). Table 2.4 sum up the various modification of chitosan with synthetic polymers.

Table 2.5 Chitosan blends with synthetic polymer

Chitosan-blended polymer	**Encapsulation method**	**Forms**	**Applications**	**Research gaps**	**References**
Chitosan-PLGA	double emulsion solvent evaporation	Spherical nanoparticle	Colon cancer treatment	Optimal reading of zeta potential (-5.40mV) indicated less stable particle	(Mostafa et al., 2023)
chitosan-coated PLGA/PCL	double emulsion (W/O/W) solvent evaporation	Spherical nanoparticle	Antitumour	1% surfactant (PVA) resulted low zeta potential and low encapsulation efficiency	(Badran et al., 2018)

2.3.3 Encapsulation of pharmaceutical drugs

Pharmaceutical drugs are the well establish drugs and approved by Food and Drug Administration (FDA) and US Pharmacopeia. These drugs are safe to use as medicine according to the approved administration dose. With advancement of drug delivery invention, the conventional method of drug delivery has been improved through encapsulation technology.

The method of encapsulations and the drugs used are summarized in Table 2.6. The trend of encapsulation methods are depending on the nature of the drugs and the application. According to El Hady et al. (2019), the encapsulation of hydrophobic drug

(diosmin) by O/W emulsion-solvent evaporation method resulted the highest EE% was 75.20 ± 2.60%. The morphological structure was confirmed through SEM. The SEM image displayed that nearly spherical with smooth surface for the chitosan coated PLGA nanoencapsulation with 2% PVA. However, increasing chitosan concentration also increased the size of nanoparticles. Nevertheless, it is found that the coating nanoparticles were more stable than uncoated through the surface charge by the zeta potential (El Hady et al., 2019). Thus, the combination of chitosan with other synthetic polymer is highly necessary to improve the properties of chitosan and give synergistic effects. Another studies are summarized in Table 2.5.

Table 2.6 Summarized of the drugs encapsulation by the modification of chitosan

Chitosan blends & solubility		**Drugs & solubility**		**Encapsulation Techniques**	**References**
PLGA	Hydrophobic	Exendin 14	Soluble	W/O/W double emulsion-solvent evaporation method	(M. Wang et al., 2013)
PLGA	Hydrophobic	Diosmin	Insoluble	O/W emulsion-solvent evaporation method	(El Hady et al., 2019)
PLGA	Hydrophobic	Paclitaxel	Insoluble	High-gravity rotating packed bed (RPB)	(Lu et al., 2019)
PLGA	Hydrophobic	5-fluorouracil	Insoluble	1.Physical adsorption: W/O/W double emulsion and solvent evaporation. 2.Chemical binding	(Y. Wang et al., 2013)
PLGA	Hydrophobic	dexamethasone	Insoluble	emulsification/solvent evaporation method	(Rençber et al., 2020)
Oleic acid (unsaturated fatty acid)	Hydrophobic	Celecoxib	Insoluble	Emulsion/solvent evaporation method	(Méndez et al., 2017)
PEG	Hydrophilic	Insulin	Soluble	Ionotropic gelation	(Ho et al., 2015)

PEG	Hydrophilic	Resveratrol & quercetin	Insoluble	Ionotropic gelation of TPP	(Natesan et al., 2017)
PEG	Hydrophilic	Salmon calcitonin	Soluble	Solvent displacement	(Prego et al., 2006)

2.3.4 Encapsulation of plant extracts

The encapsulation on plant extracts also gets good attention among researchers. The problems faced by plant extracts are lack of shelf life due to the sensitivity towards light and heat can be solved by encapsulations. In addition, some of the plant extracts tasted bitter and astringent as well as lack of water solubility. So the encapsulation is very promising (Munin & Edwards-Lévy, 2011). Each types of bioactive compounds have their specific kinds of extraction including the selection of solvents depending on the polarity and the steps of extractions must be considered (Jun et al., 2014; Kaewseejan & Siriamornpun, 2015). Thus, the encapsulation techniques should depend on the nature of plant extracts so that the bioactive compounds and the encapsulation will be compatible.

This literature review more focus on the plant from Euphorbiaceae family as this family plants are rich in medicinal compounds (Mwine & van Damme, 2011). Euphorbiaceae family plants also used for respiratory problems such as asthma, bronchitis, pneumonia and rheumatism (Chekole et al., 2015). The main characteristic of Euphorbiaceae family is the presence of white milky latex and less toxic (Kumar et al., 2010).

However, less study on the immobilization and the method of encapsulations from the Euphorbiaceae family plants. Table 2.7 summarized some of the reported encapsulation studies on plant extracts from Euphorbiaceae family. The method of encapsulation is dependent on the nature of the active compounds. The double emulsion of water-in-oil-water- (W/O/W) method was used for water soluble extracts and the single emulsion of oil-in-water encapsulation method was designated for hydrophobic active compounds (Chaudhary et al., 2020; Muhaimin et al., 2020).

The encapsulation of the active compounds from Euphorbiaceae family plants were confirmed through zeta potential and particle size analysis. The encapsulation of *Emblica officinalis* found that the zeta potential was -58.93 mV indicated the stable microencapsulation. The results confirmed that the hydrophilic active compounds were successfully encapsulated through double emulsion-solvent evaporation method.

Table 2.7 Previous study of encapsulation on various Euphorbiaceae family plants

Plants	**Active compounds**	**Method of encapsulations**	**Applications**	**Remarks**	**References**
Emblica officinalis	Polyphenols and gallic acid (Water-soluble extracts)	Water-in-oil-water (W/O/W) double emulsion	Drug delivery	The method used is suitable for water-soluble extracts	(Chaudhary et al., 2020)
Euphorbia hirta L.	Flavonoid, Phenolic	Extract mixed with 20% maltodextrin with ratio 1:10 and spray drying	Antidiabetic	Study more focus on the encapsulation of different extracts	(Tran et, 2020)
Macaranga gigantea	Ethanolic crude extracts-Alkaloids, Flavonoids, tanins, phenolic, and steroids (less water soluble)	Oil-in- water (O/W) Solvent evaporation method	Antitumor, anticancer, antimalaria, antimicrobes, antioxidant	Higher concentration of polymer used the higher the EE%	(Muhaimin et al., 2020)

Acalypha indica L.	Polyphenols	Colloidal coacervation technique	human prostate cancer cell line	-Low zeta potential	(Amarnath et al., 2013)

2.4 THE SELECTED PLANT EXTRACT - *Acalypha indica* Linn.

Acalypha indica Linn. (AI) belongs to the Acalypha genus which classified under Euphorbiaceae family is an annual herb weed plant that grows in moist soil, and fertile loamy soils (Zahidin et al., 2017). Another name for AI which commonly known by Malay people is "Kucing Galak". In Indian culture, this plant is known as "kuppameni" which means rubbish plant because this plant is a common shrub in Indian gardens, growing in the backyard of houses and waste place (Murugan & Saranraj, 2011).

AI often classified as a herbaceous plant because of the average height is 60 cm and it is a non-woody plant. Since a long time ago in Indian culture, Acalypha indica was used for antiulcer, anthelmintic, diuretic and expectorant-based illness such as bronchitis, pneumonia and rheumatism. Nowadays modern research has been done to prove the effectiveness as well as come out with the precise formulation according to the standard dosage for ideal consumption and targeting the specific disease (Kalimuthu et al., 2010). Based on Ayurvedic knowledge and from indigenous people, this plant further investigated using modern technology and the identified active ingredients were extracted.

The use of AI for ethnomedicinal purposes can be divided into three main parts; whole plants, leaves, and roots (Figure 2.11). The whole parts in Acalypha indica possess beneficial herbal activities. This plant has a long history of medicinally used to treat against pneumonia and asthma. In some regions in India, the whole plants can be consumed to cure bronchitis by blending the plant to get fresh juice (Senthilkumar et al., 2006). In Malaysian culture, this plant has been used and good for oral disease such as mouth ulcer (Zahidin et al., 2017). Besides, it has been used as a homoeopathic medications diuretic, carminative, expectorant, emetic properties, laxative, tooth problems and earache (Kalimuthu et al., 2010).

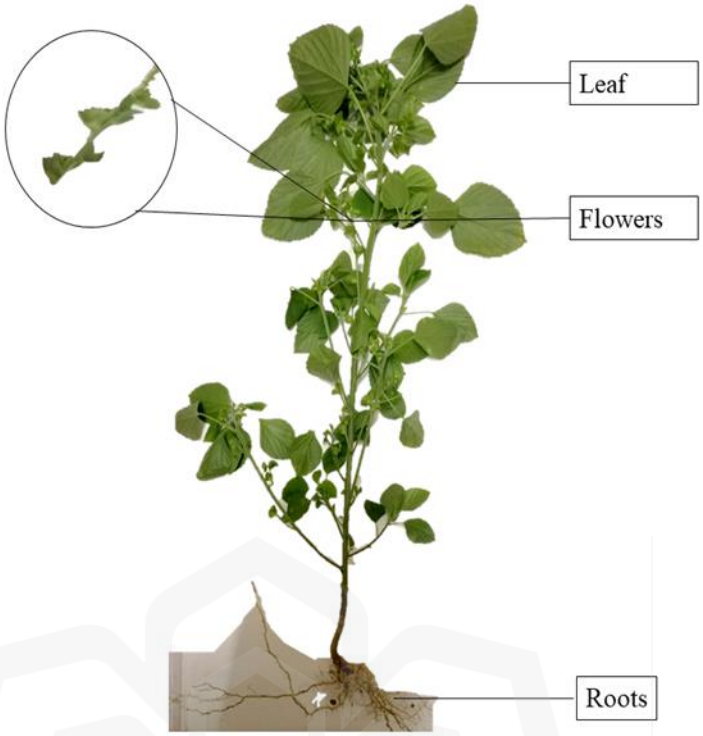

Figure 2.6 *Acalypha indica* Linn. whole plant

AI leaf contains a good antibacterial property. This statement supported by the study on the antibacterial screening which was done against nosocomial infection causing bacterial pathogens due to the presence of alkaloids and tannins (Murugan & Saranraj, 2011). Another study reported that the extracts from the leaves could reduce the E.coli mutagenesis. The leaves of Acalypha indica is very good for anthelmintic (Mohan et al., 2014; Zahidin et al., 2017). The early cases of ringworm can be cured with AI leaf paste (Mohan et al., 2014). In the 1930s, people used the dried leaves of AI to treat bedsores and wounds and making juice to treat a variety of skin problems. At some region in Malaysia, people consume the leaves as one of the leafy green vegetables by raw or cook as this plant can induce weight loss. Malay people also dried the AI leaves and selling to the public as a healthy drink (Zahidin et al., 2018).

Besides, the ethanolic extract from root bark is good for emollient. On the other hand, the roots of this plant are known to have drug-like effects in cats as shows in Figure 2.12 (Source: Personal collection). The presence of natural volatile compounds which are isodihydronepetalactone and isoiridomyrmecin emitted by the plant roots causes a drug-like effect on the behavioural activity in cats. The similar compound

(isodihydronepetalactone) is also found in the catnip plants (*Nepeta cataria*) (Scaffidi et al., 2016). Scaffidi et al. (2016) found that the effect of the roots towards the cat lost after few days exposed to the atmosphere proved that these bioactive compounds are volatile.

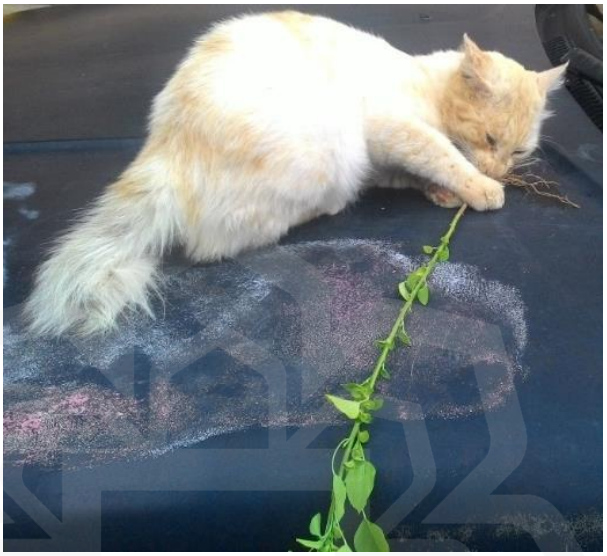

Figure 2.7 Root of the *Acalypha indica* plant attracts cat (self collection)

2.4.1 Phytochemicals in AI

Each indigenous medicinal plants have their specific phytochemical properties which are abundant. Phytochemical derives from the word, “Phyto” usually called as “plant natural products” that refers to the bioactive compounds produced by secondary plant products and have been related to the medicinal properties of diverse plants (Nadia et al., 2016). Every part of the plant has its own phytochemicals properties and different yield of phytochemicals. Even though AI is a shrub plant, all parts of this plant (i.e: leaf, roots and stem) posses numerous herbal activities. Generally, plant phytochemicals can be classified into 5 major classes (Figure 2.13).

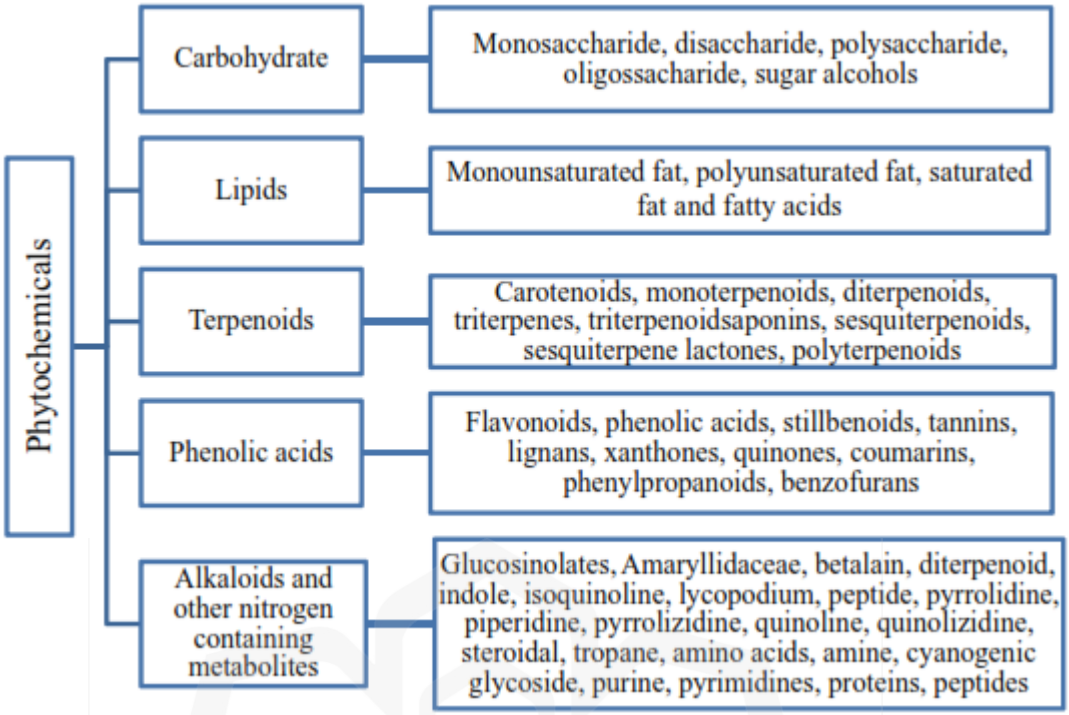

Figure 2.8 Classifications of phytochemicals in most plants (Huang et al., 2016)

There are several phytochemicals which are abundantly distributed in AI such as alkaloid, phenolic compound, flavonoids, triterpenes, steroids and acalyphamide. According to Paindla & Mamidala (2014), the phytochemical screening of AI leaves revealed abundantly the presence of alkaloids, carbohydrates, starch, glycosides and protein but slightly amount of phenol and tannin present and negative results for saponins. Details of the phytochemical existed in the AI leaves are shown in Table 2.8 and the whole plants are in Table 2.9. Based on these tables, it shows that flavonoids exist most abundant in the AI plants but alkaloids does not exist in the AI leaves.

Table 2.8 Phytochemical screenings on AI leaves

Phytochemicals	**Test conducted**	**Extracts**				
		(H)	(C)	(EA)	(A)	(M)
Alkaloids	Mayer's test	++	+	-	-	-
	Hager's test	+++	+++	+++	+++	+++
Carbohydrates	Fehling's test	+++	+++	+++	+++	+++
	Benedict's test	-	+	++	+++	+++
Starch	Iodine test	+++	+++	+++	+++	+++
Glycosides	Borntrager's test	+++	+++	+++	+++	+++
	Brown ring test	+++	+++	+++	+++	+++
Saponins	Saponification test	-	-	-	-	-
Proteins	Millon's test	++	+	-	-	-
	Biuret test	+++	++	+	-	-
	Nin hydrin test	+++	+++	+++	+++	+++
Phenols	Lead acetate test	+++	++	-	+	-

slightly presence, ++ = moderately presence, +++ = highly presence, - = AbsenceH= Hexane, C= Chloroform, EA= Ethyl acetate, A= Acetone, M= Methanol

Table 2.9 Phytochemical screenings at the whole part in AI plant

	Dried root	**Dried stem**	**Dried leaves**	**Dried whole plant**
Alkaloids	-	-	-	+
Saponins	-	-	-	-
Flavonoids	++	+	+	+
Tannins	-	-	+	+
triterpenes	+	++	+++	+++
Steroid	+	++	+++	+++

2.4.2 The prospects of *Acalypha indica* extracts

Acalypha indica (AI) is a common traditional plant that highly rich in nutritional and nutraceutical properties (Umate & Marathe, 2018). Quercetin derivatives of the phenolic compound can reduce the lipid accumulation as well as improving reverse cholesterol transport (RCT) which is one of the crucial processes in the human body system. Recent scientists have found out many ways to extract the beneficial phytochemicals and transforming into new products such as functional foods. However, most recent technology is more focusing on the phytochemicals in medical applications.

AI also has good potential for the treatment of any skin infections. According to Seebaluk et al., the whole plant decoction of AI is useful to against scabies, dermatitis and other skin infections. Other than that, AI also antiparasite that can get rid of tapeworm from the stomach by applying externally with salt or quicklime (Seebaluck et al., 2015).

However, commercialization through the original stage of plant is impossible as AI is perishable. The bioactive compound from the extraction also faces several difficulties to maintain the stabilization in which the thermal processing can degrade these compounds through oxidation and cleavage of covalent bonds. By developing the method of immobilization as well as stabilizing the bioactive compound through encapsulation, the bioactive compounds can further be produced for consumption and last longer shelf life. Exceptionally, less study reported on encapsulation of AI extract.

2.5 OPTIMIZATION

In polymer engineering research, optimization is the most crucial to evaluate the best formulation of successful encapsulations. Without optimization study, it is impossible to evaluate the best parameter values and the study will take more lengthy process. Another advantage of optimization is to reduce cost of running experiments as well as reduce the production costs. Other than that, the budget can be predicted more

accurately thus reduce the waste of the raw materials. There are several common optimizations tool used in the polymer research which are one- factor-at-a-time (OFAT) and response surface methodology (RSM).

2.5.1 One- factor-at-a-time optimization

One-factor-at-a- time is an optimization method performed by varying only one factor and keep the other factors fixed. Basically, the OFAT is carried out in the beginning of research to evaluate the central points and to narrow down the range of factor values. Since OFAT only tested by varying one factor at one time, the interactions between the parameters limit the validity of this optimization. However, some studies still use the OFAT for certain reason. Table 2.10 summarizes the recent optimization studies using OFAT.

Table 2.10 Previous study using OFAT

Summarization of experiment	**References**
This study aims to synthesize the chitosan-alginate core-shell nanoparticles and loaded the rifaximin. (Rif@CS-NPs). By varying the concentration of Rif@CS-NPs the cell viability were recorded.	(D. Kumar et al., 2021)
To improve the solubility and bioavailability of diosmin by coating of PLGA with chitosan. The research was conducted by varying the drug: PLGA ratio and selected the best ratio to coat with chitosan with three difference concentration.	(El Hady et al., 2019)
This study used the established range of gum Arabic (GA) (0.8, 1.6, 2.0, and 2.2 mg/mL)	(Kim et al., 2019)

The experiment was conducted to encapsulate the doxorubicin hydrochloride (DOX ·HCL), which is a water-soluble anticancer drug. One factor study was varied the preparation condition to obtain the controlled size of $CaCO_3$/CMC hybrid.	(Wang et al., 2010)

Although there are several limitations in OFAT, however OFAT is very important in the beginning of study to find the range of optimum factor. Without range, the further optimization study may be hard and impossible to get the most optimum parameters. Here are several advantages and limitations of OFAT (Table 2.10).

Table 2.11 The advantages and limitations of OFAT

Advantages	**Disadvantages**	**References**
Can predict the trends of parameter effects on the responds.	Inefficient to explain the interactions between the parameters towards the responses.	(Anani et al., 2022)
Convenience for small set of experiments with one variable.	Unmanageable of dealing with the large number of variables	(Suryawanshi & Eswari, 2022)
Time and cost effective of dealing with one variable of experiments.	Time and cost consuming when dealing with more than one variable to study.	(Suryawanshi & Eswari, 2022)

Due to this research is less explored from the previous study using active compounds of *Acalypha indica* extract, the literature review table was done and compared in Table 2.11 to decide the possible range of the parameters for OFAT and adjusted to find the highest value of response based on the percentage of encapsulation efficiency (EE%). The highest EE% which is 99.04% was recorded by Prado et al.

(2017) used PVA as the surfactant less than 1% w/v concentration. So this research started to study with 1% w/v concentration of PVA.

Table 2.12 Concentration of the polymers used from previous research

Polymer used for encapsulation	**Method**	**Polymer concentrations**	**PVA concentrations**	**Encapsulation Efficiency (EE%)**	**References**
PLGA	Solvent displacement	1 % w/v	5 %	79 %	(Pool et al., 2012)
Chitosan-PLGA	Emulsion-solvent-evaporation	Chitosan: 0. 1-0. 3 % w/v PLGA: 7. 5 % w/v	2 %	75. 30 ±2. 60	(El Hady et al., 2019)
Chitosan-PCL	Double emulsion (W/O/W)	Chitosan: 0. 2 % w/v PCL: 0. 6 %w/v	First step emulsion: 0. 5 % w/v Second step emulsion: 1 % w/v	99. 04 ± 0. 001%,	(Prado et al., 2017)
Chitosan-PLGA	Multiple emul-sion and solvent evaporation (W/O/W)	Chitosan: 0. 4 PLGA: 1	-	25. 3	(M. Wang et al., 2013)
Chitosan-PLGA	Single emulsion O/W	Chitosan: 10 % w/v PLGA: 10 % w/v	1, 5, 10 % w/v	According to size: 277. 4 ± 6. 4	(Choksawad et al., 2018)

2.5.2 Response Surface Methodology

Response surface methodology (RSM) is a design of experiment by applying the mathematical and statistical technique for the optimization of variable parameters that providing interactions and analyse by an empirical model. Empirical model is an approximating model that is based on the experimental data (Sarabia & Ortiz, 2009). The empirical model can accurately estimate the response of values even no experiments were done. First-order surface response model is one of the example of empirical model which describes the interaction between y and X_i. The formula as in [1]. Another example is second-order model if the first-order model is inadequate and the formula as described in [2]

$$y = ß_0 + ß_1X_1 + ß_2X_2 + \cdots ß_kX_k + \varepsilon \quad [1]$$

$$y = ß_0 + ß_1X_1 + ß_2X_2 + \cdots ß_kX_k + ß_{12}X_1X_2 + ß_{13}X_1X_3 + \cdots + ß_{1k}X_1X_k + ß_{23}X_2X_3 + ß_{24}X_2X_4 + \cdots ß_{2k}X_2X_k + \cdots + ß_{k-1k}X_{k-1}X_k + ß_{11}X_1^2 + ß_{22}X_2^2 + \cdots + ß_{kk}X_k^2 + \varepsilon \quad [2]$$

Another aim of RSM is to make a decision more confident under uncertainty conditions as well as minimized the ambiguity (Sarabia & Ortiz, 2009). RSM also provides the fitted model by the equation and 3D graphs. The analysis of variance (ANOVA) provides the best significancy of the experiments conducted. The $p < 0.01$ implies that the model is significance.

Till now, RSM is among frequented used methods of optimization in engineering. Li et al. (2017) presented an optimization study of *Hohenbuehelia serotina* polysaccharides nanoemulsions using RSM. Four (4) independent variables were studied; X1=PVA content,X2= *H.serotina* polysaccharides concentration, X3= stirring speed, and X4=stirring time. The equations were obtained as follows:

$$Y1 = 74.78 + 3.34X_1 - 4.08X_2 - 4.19X_3 + 1.92X_4 - 2.34X_1X_2 - 0.94X_1X_3 4.63X_1X_4 - 3.13X_2X_3 - 2.96X_2X_4 + 2.80X_3X_4 - 5.73X^2_1 - 9.32X^2_2 - 7.60X^2_3 - 1.42X^2_4$$

$$Y2 = 413.80 + 9.75X_1 + 9.33X_2 - 11.25X_3 - 9.50X_4 + 7.50X_1X_2 - 5.50X_1X_3 - 0.75X_1X_4 - 5.50X_2X_3 - 5.50X_2X_4 + 6.25X_3X_4 + 9.64X^2_1 + 8.77X^2_2 + 3.64X^2_3 + 6.52X^2_4$$

2.5.2.1 Lack of fit test

Lack of fit test explains any regression model is a poor model for the data. There are several ways to test the lack-of-fit. Goodness of fit and the lack-of-fit F test are the examples of the tests of statistical models. The goodness of fit test is suitable for the experiment dealing with populations. Lack of fit F test can be the analysis of variance (ANOVA) is summarized in ANOVA table. Details of table and the formula outlines in Table 2.13 (Sarabia & Ortiz, 2009).

Table 2.13 ANOVA table for regression and the lack of fit formulas

Source of variation	**Degree of freedom (DF)**	**Sum of squares**	**Mean square (MS)**	**F_{Calc}**
Regression	1	$SS_R = \sum_{i=1}^{c}\sum_{j=1}^{ni}(\hat{y}_{ij} - \bar{y})^2$	$MS_R = \frac{SSR}{1}$	$F = \frac{MSR}{MSE}$
Residual error	n-2	$SS_E = \sum_{i=1}^{c}\sum_{j=1}^{ni}(y_{ij} - \hat{y}_{ij})^2$	$MS_E = \frac{SSE}{n-2}$	
Lack of fit	c-2	$SS_{LOF} = \sum_{i=1}^{c}\sum_{j=1}^{ni}(\bar{y}_i - \hat{y}_{ij})^2$	$MS_{LOF} = \frac{SSLF}{c-2}$	$F^* = \frac{MS_{LOF}}{MS_{PE}}$
Pure error	n-c	$SS_{PE} = \sum_{i=1}^{c}\sum_{j=1}^{ni}(y_{ij} - \bar{y}_i)^2$	$MS_{PE} = \frac{SSPE}{n-c}$	
Total	n-1	$SS_{TO} = \sum_{i=1}^{c}\sum_{j=1}^{ni}(y_{ij} - \bar{y})^2$		

2.6 CHAPTER SUMMARY

Recent years, the research on polymers for the applications in drug delivery system has developed tremendously to improve the conventional drug system. Chitosan is a fascinating natural polymer derivative from natural sources (i.e: chitin of crustacean and fungi) as it is biocompatible and biodegradable thus safe for consumptions. Due to its biocompatibility properties, chitosan is used as a drug carrier for transportation in the body system. The limitations on chitosan can be improved by several methods of modification. Encapsulations have been done with many types of drugs according to the targeted delivery. However, less study has been done to encapsulate the plant extracts especially AI extract. By identifying the most suitable methods according to the literature review, the emulsion-solvent evaporation method is chosen to encapsulate the AI ethanolic extracts.

CHAPTER THREE

RESEARCH METHODOLOGY

3.1 FLOW CHART OF METHODOLOGY

The workflow of this study was summarized in the flowchart below:

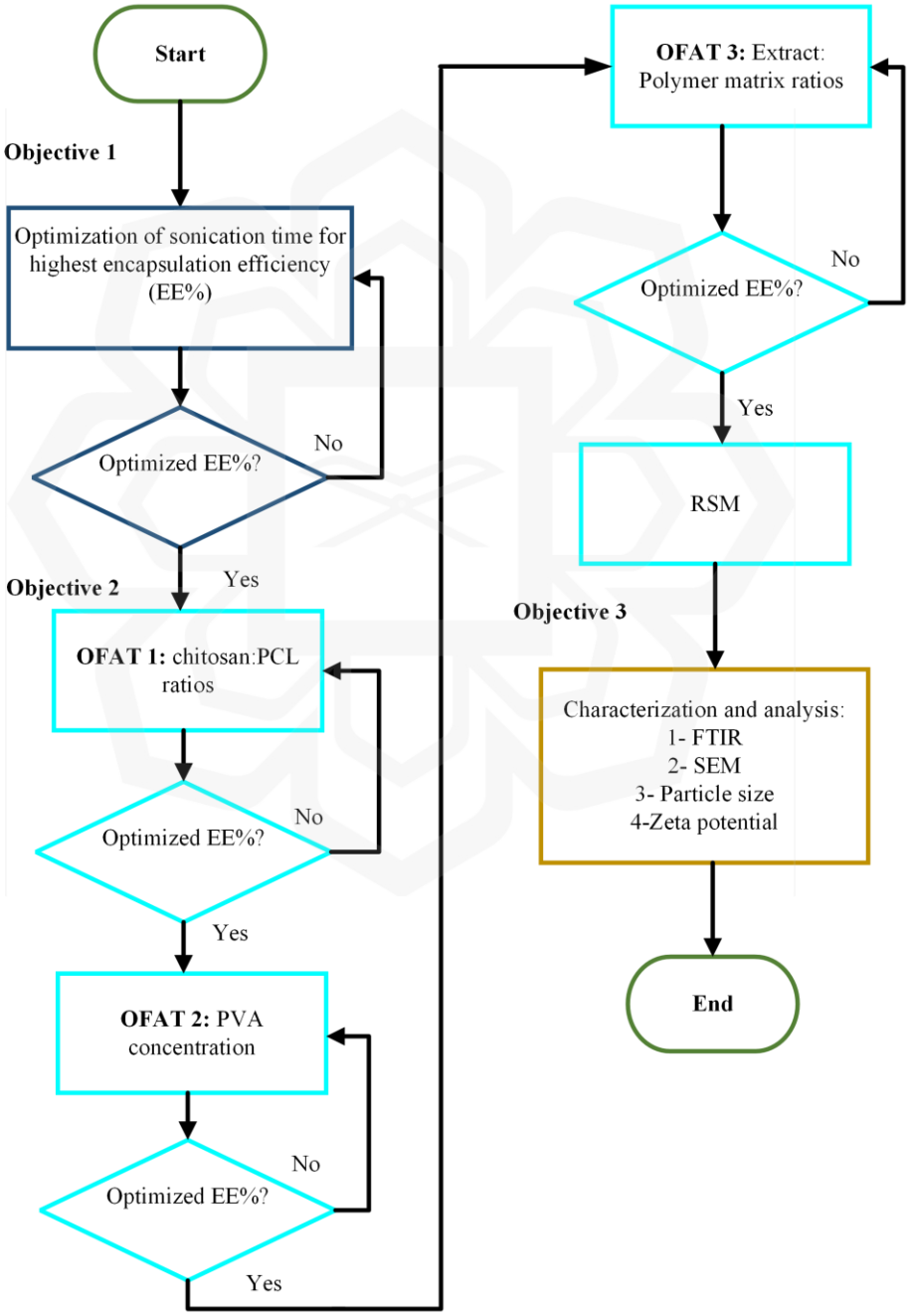

Figure 3.1 Flow chart of methodology

3.2 MATERIALS

Chitosan (medium molecular weight= 190,000-310,000 Da, ~85% deacetylated), poly-(ε-caprolactone)(PCL) (molecular weight= 45,000 Da), and poly(vinyl alcohol) (PVA) (molecular weight= 31,000- 50,000 Da, 98-99% hydrolyzed), were purchased from Sigma Aldrich. Methylene chloride (DCM) (84.93 g/mol, reagent grade) was purchased from Merck Millipore®. Acetic acid glacial (60.05 g/mol, analytical grade) was purchased from Bendosen Laboratory Chemicals. *Acalypha indica* (AI) extracts was obtained from Universiti Teknologi Malaysia (UTM) through ultrasound assisted extraction using water bath sonicator and ethanol as the solvent. Anhydrous ethanol, 99. 8% v/v absolute denatured grade was purchased from HmbG® Chemicals.

3.3 MICROENCAPSULATION METHOD

Generally, objective 1 was initiated by the selection of the best sonication time towards the highest encapsulation efficiency (EE%) as the main response. The modification of chitosan by copolymerization with PCL was done by blending method which applying the emulsion-solvent evaporation technique. Figure 3.2 simplifies the mechanism of AI extracts encapsulation.

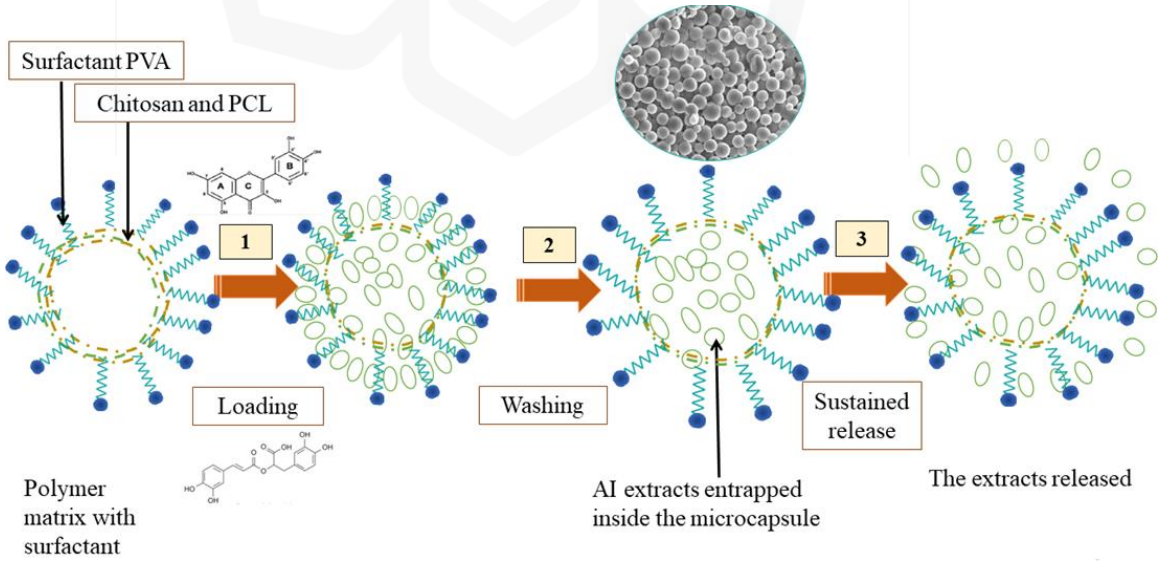

Figure 3.2 The overview process of encapsulation of AI extracts and the extract release

3.3.1 Preparation of chitosan-PCL microencapsulation

Firstly, the experiment starts with the preparation of polymer solutions. PCL was emulsified in an organic phase of DCM (0. 5 % w/v), PVA was dissolved in deionized water (2 % w/v), and chitosan was dissolved in aqueous 0. 2 % v/v acetic acid (0. 5 % w/v). Stirring was done for 24 hours under vigorous stirring without applying heat except PVA were heated under 60°C. The preparation process of polymer solutions is done in a fume hood to ensure the environmental conditions under controlled.

Next, the preparation of microparticles with and without *Acalypha indica* (AI) extract was performed by emulsion-solvent evaporation method according to El. Hadi et al. (2019) with slight modification. 5 mL emulsions of PCL was transferred into an empty 20 mL-sized vial followed by 5 mL of AI extracts, 5 mL of chitosan and 5 mL of PVA. The solutions were homogenized under ultrasonic homogenizer (Fisher Scientific FB705,700 watts) with amplitude 90 % and varying the time of homogenizations as a parameter in this objective (3, 5, 7 and 10 minutes) to form a water-in-oil (W/O) emulsion. After that, the solutions were stirred moderately on the magnetic stirrer for 24 hours under room temperature in a controlled environment. During stirring, the particles start to ageing and forming microencapsulation.

After 24 hours, the solutions were centrifuged under 13,000 rpm for 1 hour using a mini centrifuge (Eppendorf, speed x 1,000). After centrifuge, two layers of supernatant and precipitate were formed. The particles were recovered by separating from the solvents. The solvent solutions were removed and were replaced by deionized water. Then the precipitates and the deionized water were mixed by vortexing. Again, the solutions were centrifuged under 13,000 rpm for 15 minutes. After that, the centrifugation steps were repeated several times until the solutions turn the pH to neutral using a blue litmus paper as an indicator. Then the samples were ready for lyophilization. The blank microparticles encapsulation were prepared using similar procedure without the addition of AI extracts. The summarized of the process illustrates in Figure 3. 3.

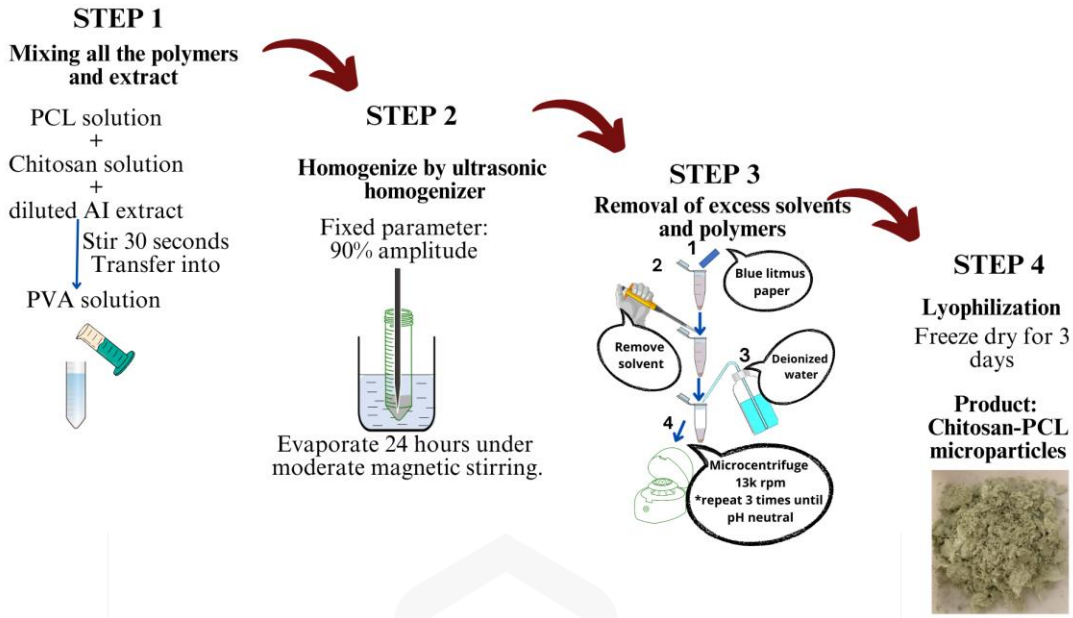

Figure 3.3 Diagram of the emulsion-solvent evaporation method

3.3.2 Encapsulation efficiency of chitosan-PCL determination

Three major steps involved obtaining the EE% described in section 3.3.2.1 to 3.3.2.3.

3.3.2.1 Preparation of standard sample

A series of wavelength screenings was done under ultraviolet-visible (UV-VIS) spectrophotometers from 200-1000 nm on the crude AI extract samples. The highest peak was detected at 285nm. Ethanol absolute was used as the blank solution. After the wavelength of the maximum absorbance obtained, five standard samples of known concentrations (0.02, 0.05, 0.1, 0.15 and 0.2 % w/v) were tested according to the previous wavelength. The linear relationship and the UV absorbance (y) was plotted according to the standard curve of spectrophotometry and the equation was obtained and was used to find the unknown concentration of the extracts being encapsulated in the microcapsules.

3.3.2.2 Releasing the Acalypha indica extract

The encapsulated AI extracts were released from its chitosan-PCL microcapsules by dissolving in the 1.0 mL of 99. 98% ethanol determine the percentage of encapsulation of AI extract that was entrapped in the microcapsule. The samples were centrifuged using a mini centrifuge (Eppendorf, speed x 1,000) under 13,000 rpm and 1 hour to release the *Acalypha indica* extracts from the microcapsule suspension into the ethanol. After centrifuge, the supernatant was taken and transfer into a cuvette for testing. The UV absorbance (*y*) of the free *Acalypha indica* extracts was tested by UV-vis spectrophotometer under wavelength of 285nm. To obtain the concentration (*x*) of entrapped extract, the Y value was substituted into the linear equation from the standard curve.

3.3.2.3 Determination of encapsulation efficiency

Finally, the value of EE% was calculated using Equation 3 and the data was tabulated.

$$EE\% = \frac{amount\ of\ extract\ entrapped\ in\ the\ microcapsules}{Total\ extract\ added} \text{ x } 100\% \qquad [3]$$

3.4 OPTIMIZATION OF MICROENCAPSULATION PROCESS

3.4.1 One-Factor-at-a-time

The experiment was continued with a series of OFAT to find the the preferred range for each parameter and selected the desired range and levels for response surface methodology (RSM). In this experiment, only one parameter was manipulated at a time while the other parameters remained constant. Each run was repeated thrice.

3.4.1.1 Ratio of Chitosan:PCL concentration (OFAT 1)

With the homogenization duration fixed at 5 minutes, the second OFAT of Chitosan:PCL concentration ratio was conducted, where the values were varied as shown in Table 2. The ratio which gave the highest EE% was chosen for the next OFAT step.

Table 3.1 Various ratio concentration of Chitosan:PCL using OFAT (Factor 1)

F1: Chitosan:PCL concentration ratio (%w/v)	**F2: PVA concentration as a surfactant (%w/v)**	**F3: Concentration of chitosan-PCL blends (%w/v)**
0.2: 0.8	2	1
0.4: 0.6	2	1
0.5:0.5	2	1
0.6:0.4	2	1
0.8:0.2	2	1

3.4.1.2 PVA concentration

With the ratio of Chitosan:PCL concentration fixed at 0.6:0.4, the third OFAT of PVA concentration ratio was conducted, where the values were varied as shown in Table 3. Similarly, highest EE% as the response was recorded and carried out for the next step.

Table 3.2 Various PVA concentration using OFAT (Factor 2)

F1: Chitosan:PCL concentration ratio (%w/v)	**F2: PVA concentration as a surfactant (%w/v)**	**F3: Concentration of chitosan-PCL blends (%w/v)**
0.6:0.4	0.01	1
0.6:0.4	0.05	1
0.6:0.4	0.1	1
0.6:0.4	0.3	1
0.6:0.4	0.5	1
0.6:0.4	1	1
0.6:0.4	2	1

3.4.1.3 Concentration of chitosan-PCL blends (OFAT 3)

With the PVA concentration fixed at 0.05%, the fourth OFAT of Chitosan-PCL blends concentration was conducted, where the values were varied as shown in Table 4 and EE% was recorded.

Table 3.3 Various concentration of Chitosan-PCL blends using OFAT (Factor 3)

F1: Chitosan:PCL concentration ratio (%w/v))	**F2: PVA concentration as a surfactant (%w/v))**	**F3: Concentration of chitosan-PCL blends (%w/v)**
0.6:0.4	0.05	0.33
0.6:0.4	0.05	0.67
0.6:0.4	0.05	1.00
0.6:0.4	0.05	1.33
0.6:0.4	0.05	1.67

3.4.2 Study on interaction between variables by central composite design (CCD) for surface response methodology (RSM)

The experiment was continued with the interaction study by response surface methodology (RSM). Central composite design (CCD) was chosen as the response surface model. The design of experiment was aided by Design-Expert version12 software using central composite design (CCD) to study the impact and interactions of independent variables.

Since the DOE software is unable to process the ratio values, the values used were only integer numbers. For instance, in the first factor which is the ratio of chitosan to PCL, the value 4 means the ratio of chitosan to PCL was 0.4:0.6. Table 3.4 illustrates the values for the coded levels used in the DOE. For the fourth factor, the range of concentration of Chitosan-PCL blends (%w/v) in this study was limited according to the maximum concentration of chitosan that reached the gelation point for dissolution in 0.2% v/v of acetic acid. A preliminary experiment was done before to find the

maximum concentration reaching the gelation point. It was found that the chitosan reached the gelation point at a 1% g/mL concentration.

Thus, there (3) factors were used to study the interactions. The independence variables of chitosan:PCL concentration ratios (X1), PVA concentration (X2), and the polymer matrix concentration (X3) were selected with the specific range obtaining from the single experiments, OFAT. Details of the value of codified levels were tabulated in Table 3.4.

Table 3.4 Codified level for independent variables used in CCD for encapsulation optimization

Independent variables	**Units**	**Coded levels**		
		-1	0	+1
X1: Ratio of Chitosan (n) :PCL (10-n)	N chitosan % w/v	4	6	8
X2: PVA concentration	%w/v	0.01	0.05	0.09
X3 Concentration of chitosan-PCL blends (%w/v)	%w/v	0.6	1	1.4

In CCD, three (3) factors and one (1) response were decided and to predict the response at any points, codified units is the cube with the side is [-1,1]. The design of CCD was proposed in Table 3.5 and simplified with the value of the coded units.

Table 3.5 Design matrix of codified variables for central composite design

	Code			**Experimental**		
Run	Factor 1: Chitosan:PCL ratio	Factor 2: PVA concentration	Factor 3: Drug:polymer ratio	Factor 1: Chitosan:PCL ratio (%w/v)	Factor 2: PVA concentration	Factor 3: Concentration of chitosan-PCL blends (%w/v)
1	-1	1	-1	4	0.09	0.6
2	1	1	-1	8	0.09	0.6
3	-1	1	1	4	0.09	1.4
4	1	-1	1	8	0.01	1.4
5	1	1	1	8	0.09	1.4
6	1	-1	-1	8	0.01	0.6
7	1	0	0	8	0.05	1
8	-1	-1	1	4	0.01	1.4
9	0	-1	0	6	0.01	1
10	0	1	0	6	0.09	1
11	-1	-1	-1	4	0.01	0.6
12	0	0	-1	6	0.05	0.6
13	0	0	0	6	0.05	1
14	0	0	1	6	0.05	1.4
15	0	0	0	6	0.05	1
16	0	0	0	6	0.05	1
17	-1	0	0	4	0.05	1

3.4.2.1 Lack of fit test

The lack of fit test table was obtained from Design-Expert v12. The table template as below:

Table 3.6 ANOVA table

Source of variation	**Degree of freedom (DF)**	**Sum of squares**	**Mean square (MS)**	**F_{Calc}**
Regression	1	SS_R	MS_R	$\frac{MSR}{MSE}$
Residual error	n-2	SS_E	MS_E	
Lack of fit	c-2	SS_{LOF}	MS_{LOF}	$\frac{MS_{LOF}}{MS_{PE}}$
Pure error	n-c	SS_{PE}	MS_{PE}	
Total	n-1	SS_{TO}		

3.4.3 Model validation

Validation study is an additional set of experiments that has been carried out to validate and reproduce the output from the model. In this study, two additional experiments were conducted referring to the new optimized parameters suggested by the DOE software. These two experiments were selected according to the highest and lowest EE% response predicted by DOE software. Subsequently, these two optimum samples were used for further characterizations.

3.5 CHARACTERIZATION

3.5.1 Fourier-transform infrared spectroscopy

The Fourier-transform infrared spectroscopy (FTIR) examination of the selected AI extract microencapsulation was determined using Nicolet iS50 FT-IR spectrophotometer (Thermo Scientific, Massachusetts, US). The samples in powder form were scanned under the regions of 4000- 400 cm^{-1} wavenumber.

3.5.2 Scanning electron microscope examination

A scanning electron microscope (SEM) (JEOL JSM-IT 100 InTouchScope™) accelerated at 5 kV was used to examine the morphological surface of the selected microparticles. The samples were coated with sputter coating (QC7620, Quorum Ltd, London) by placing the samples on the Nisshin EM conductive carbon tape that acted as a sample holder. The prepared samples were placed on a brass holder under the vacuum holder.

3.5.3 Particle size analysis and zeta potential

The average particle size was quantified by dynamic light scattering (DLS) technique by zetasizer Nano (Malvern instruments Inc., UK). The system temperature was 25°C, the repetition was thrice, Viscosity (cP) was 1.2, and the count rate (kcps): 139.2. The refractive index of the material was 1.59. The microparticles were suspended in ethanol.

3.6 STATISTICAL ANALYSIS

All the result of encapsulation efficiency (EE %) were analyzed and tabulated as mean ± standard deviation.

3.7 CHAPTER SUMMARY

The preliminary study has been done prior to decide the compatible materials for this encapsulation fabrication. The study has been done in determining the compatible molecular weight of PVA and the PVA with molecular weight 31,000-50,000 Da. Another preliminary study has been done for chitosan to determine the highest concentration before the gelation point by using the diluted acetic acid (0.2% w/v) as the solvent. Next, the experiments were proceeded with one factor characterization (OFAT) and RSM. The objective 3 were more on characterization and analysis. The selection of parameters value was based on suggested by Design- Expert software under RSM.

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 INTRODUCTION

After all, the results obtained from this study were reported and discussed. The discussion flows were organized according to the objectives. In 4.2 subchapter, the data reported regarding to objective 1 which is the sonication time. In 4.3 subchapter, the data of objective 2 were discussed as well as in 4.4 which is the data of analysis in objective 3.

In objective 1, one (1) set of standard samples of known AI extract concentration was prepared to obtain an equation to be used in finding the concentration of encapsulated AI extract. Next, the main experiment was done to find the optimum sonication time for encapsulation. five (5) durations of time were selected which are; 3, 5, 7, and 10 minutes. The data of encapsulation efficiency (EE%) were taken and the best time chosen according to the highest EE%. The effect of sonication time towards the EE% were discussed.

Objective 2 discussed on the result of one-factor-at-a-time (OFAT). The study was done with 3 set of OFAT which are: a) OFAT 1:chitosan:PCL ratios; b) OFAT 2:surfactant (PVA) concentrations; c) OFAT 3:polymer matrix concentrations. The effect of the amount of chitosan and PCL were discussed in this section.

Objective 3 discussed the analysis of chitosan-PCL loaded *Acalypha indica* (AI) extracts based on the three (3) data from Objective 2 that giving the highest, medium and lowest EE%. Scanning electron microscope (SEM) images, Fourier-transform infrared spectroscopy (FTIR), particle size and zeta potential are the analysis reported in this section.

4.2 PREPARATION OF STANDARD SAMPLE

The experiment began by identifying the highest peak of the crude AI extract which indicated the most abundant active compound presented. The AI extract were analysed using UV-Vis spectroscopy (Secomam Uviline 9400) ranged wavelength from 200-1000 nm (Figure 4.1). The highest absorbance was detected at wavelength 285nm.

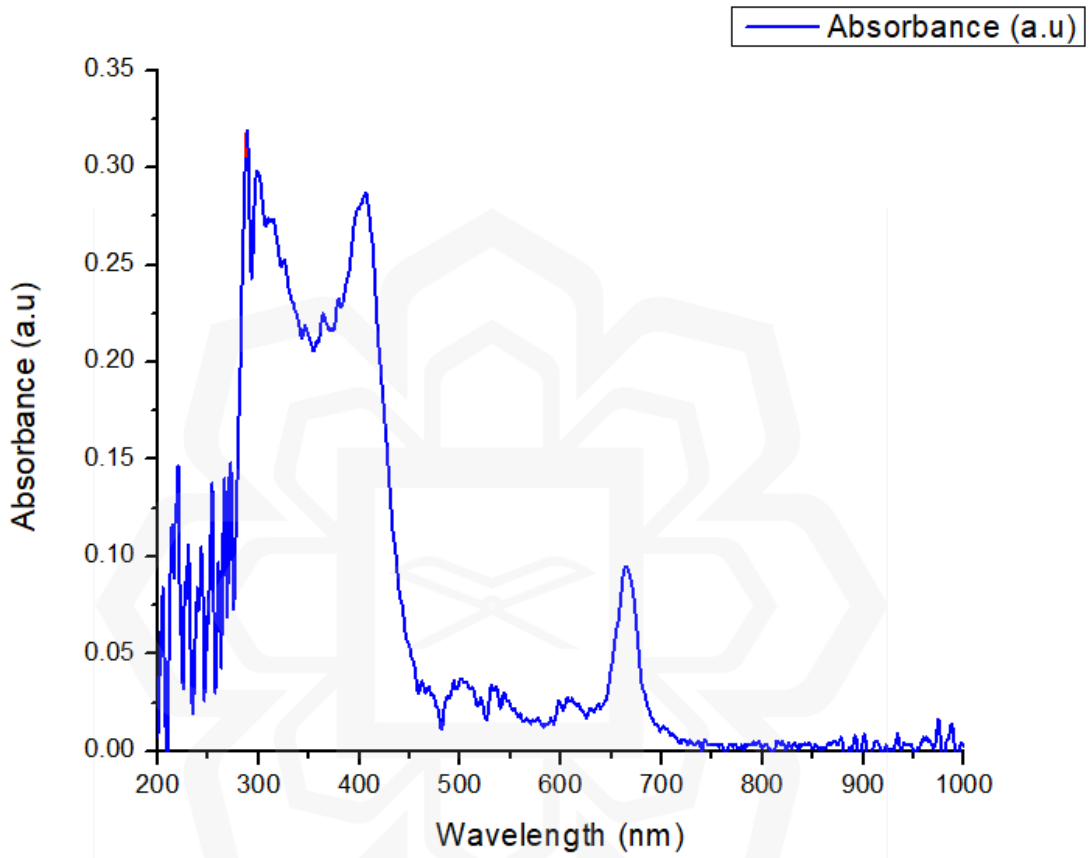

Figure 4.1 Screening of AI extract

Once the wavelength of the most abundant active compound was detected at 285nm, a series of known concentrations (0.05, 0.1, 0.15, and 0.2 %w/v) were run through Uv-Vis spectrometer. From the absorbance data, a standard curve was obtained as shown in Figure 4.2 with the equation y = 3.7883x + 1.2192.

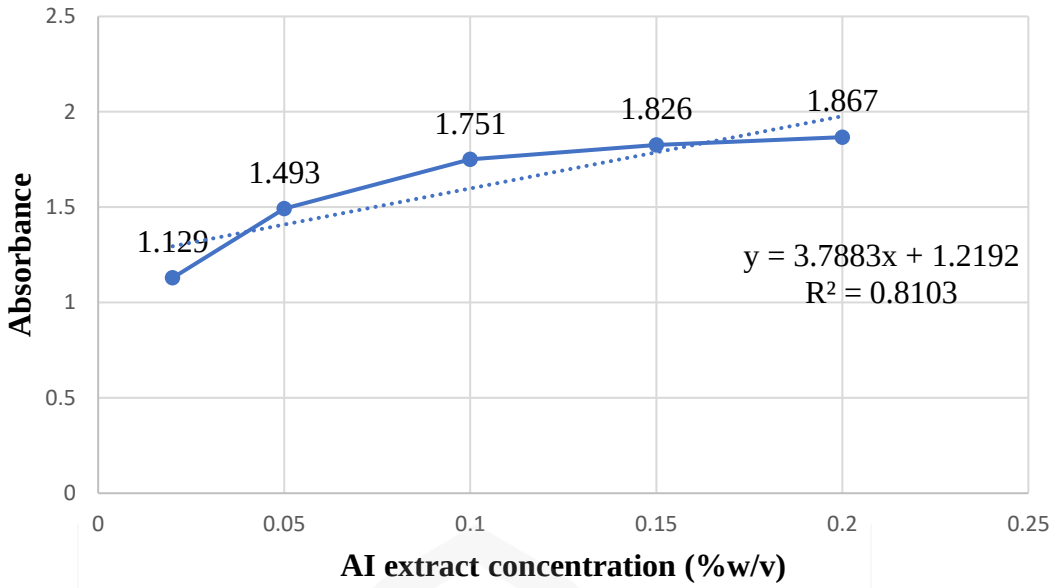

Figure 4.2 Standard curve for AI extract

4.3 SELECTION OF THE BEST TIME OF SONICATION FOR THE FORMATION OF CHITOSAN-PCL MICROENCAPSULATION

In Figure 4.3, encapsulation efficiency exhibited an upward trend from 3 minutes up to 5 minutes of homogenization. During 3 minutes of duration homogenization, the oil/water phases were not completely break into smaller droplets. The physical observations were made from the solutions after solvent removal steps through 130 rpm/60 minutes centrifugations. Through observation in Figure 4, the solutions turned into more translucent as the sonication time increased and more precipitates deposited. The microparticles recovered from centrifugation similarly reported by Leong et al., (2017). From centrifugation, less amount of microparticles were deposited and the supernatant was slightly cloudy. During this time, the emulsification occurred incomplete.

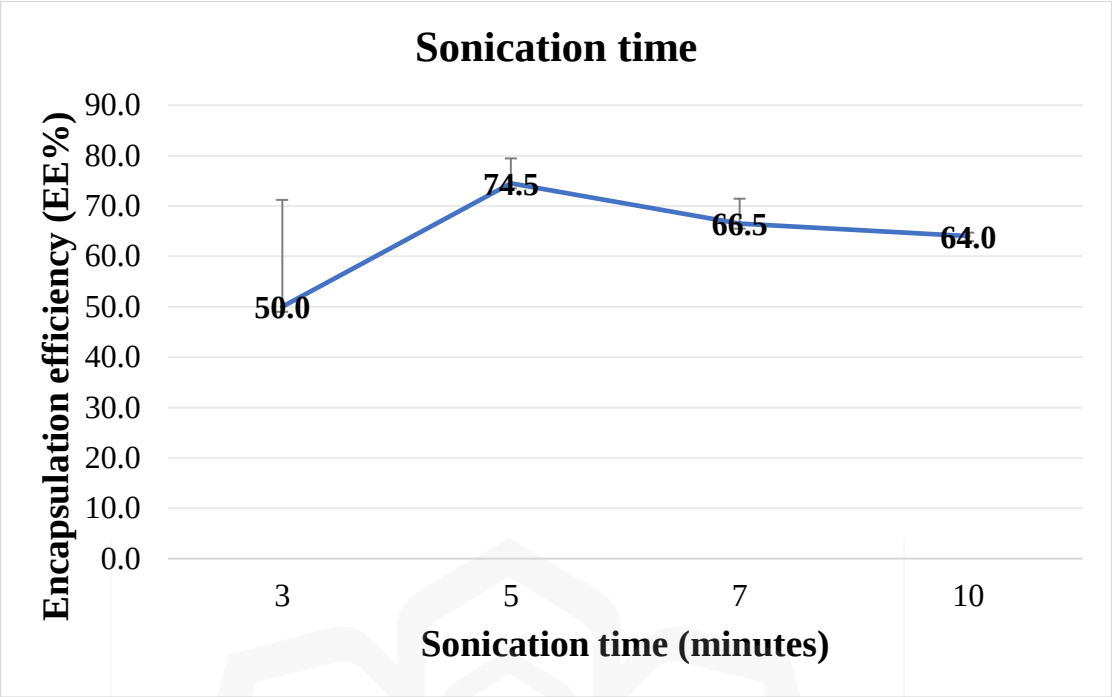

Figure 4.3 The effect of sonication time towards the encapsulation efficiency of chitosan: PCL encapsulation .

After 5 minutes of homogenization, it is unlikely to result in any significant changes in encapsulation efficiency and the SEM images, but the trend of encapsulation efficiency declined. As the duration of homogenization increased, the input energy also increased thus boosting the shear force due to acoustic cavitation. Due to this phenomenon, as time passes, more energy is dissipated to break down the larger oil/water molecules into smaller droplets (Abriata et al., 2019; Taha et al., 2020). According to Eric et al. (2017), this short homogenization duration time may also result in the formation of coarse emulsion (Figure 4.4 b), which is larger diameter of emulsion droplets. 10 minutes duration somehow observed a good encapsulation image, but the EE% was lower than 5 and 7 minutes. Longer exposure time of the samples to homogenization may reduce the cavitation efficiency of the microtips and eroded (Taha et al., 2020). Through this experiment, 5 minutes of homogenization duration was selected and used in the next experiments.

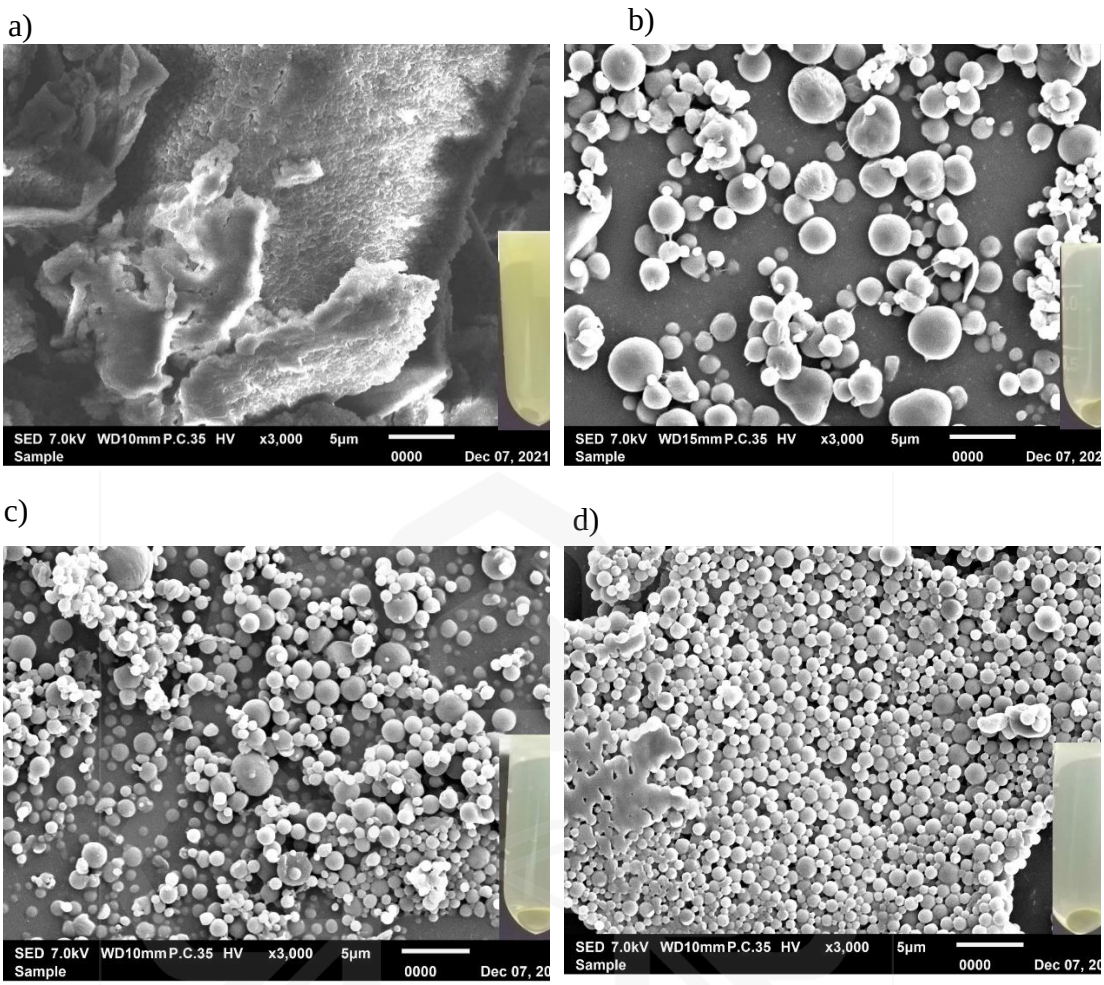

Figure 4.4 SEM images and visual register of the suspensions of samples at different homogenization duration : (a) 3 minutes; (b) 5 minutes; (c) 7 minutes; and (d) 10 minutes

Finding the optimized sonication time is important not only can save the reaction time, but also prolonged the life span of the microtips against corrosion. Objective 1 has successfully achieved. Through this experiment, 5 minutes of homogenization duration was selected and used in the next experiments.

4.4 OPTIMIZATION OF MICROENCAPSULATION PROCESS

The microencapsulation of AI extracts has been successfully encapsulated by blending of chitosan with poly-ε-caprolactone (PCL) and the surface morphology is confirmed

by scanning electron microscope (SEM). From objective 1, the spherical shapes were obtained. Optimization is further continued to obtain the optimum condition each of the parameters. The first optimization to obtain the center point is done by OFAT with 3 factors which are the ratio of chitosan:PCL, the concentration of the surfactant (PVA) and the ratio of the polymer to *Acalypha indica*. Further optimization were done using the design of experiments (DOE) by central composite design (CCD).

4.4.1 ONE-FACTOR-AT-A-TIME (OFAT)

In the preliminary experiment to find the range and the central points, one-factor-at-a-time (OFAT) experiments were carried out. Three (3) factors were studied; the ratio of chitosan: PCL, PVA concentrations and the polymer matrix concentrations.

4.4.1.1 OFAT 1 : The ratio of chitosan: PCL concentration

In the sequence of OFAT, the composition of chitosan and PCL is the most prioritized factor that makes up the concentration of chitosan-PCL blend as the carrier matrix. Figure 4.5 shows the combination of higher amount of PCL (0.2:0.8) resulted in the lowest EE% (33.80%). The EE% increased as the concentration of chitosan and PCL increased until the concentration of chitosan more than PCL (0.6:0.4). Thus, the optimum concentration of chitosan and PCL obtained was 0.6 and 0.4 %w/v respectively with 70.10% EE%. The decreasing of EE% after the 0.6 %w/v concentration of chitosan possibility related to the viscosity of chitosan. In this case, the 0.8%w/v concentration of chitosan dissolved in 0.2%w/v acetic acid started to reach the gelation point. Thus, during the homogenization process, the chitosan molecules unable to break well into smaller size and the entrapment due to the chitosan not fully dissolved.

This is because the higher viscosity will reduce the intensity of the acoustic cavitation (Taurozzi et al., 2012).

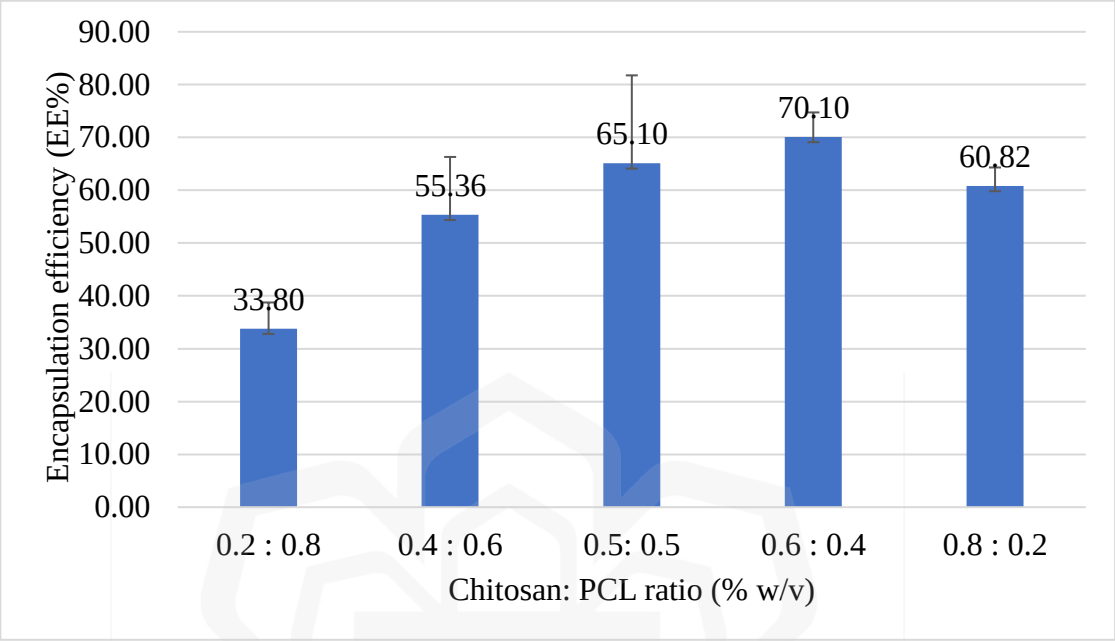

Figure 4.5 Chitosan-PCL copolymer encapsulation with AI extract with various ratio of chitosan:PCL

Concurrently, the experiment was also done on the homopolymer (PCL only and Chitosan only). However, the result was omitted as the encapsulation does not form. This might be due to during opening or pore creation, the monomer droplets formed are very porous, unable to hold the extract and the extract has been leaked out during cavitation process before they start forming microemulsion (Leong et al., 2017b). Unlike the heteropolymer encapsulation, while depolymerization occurs, both monomer of PCL and chitosan incorporate together thus entrap the AI extract inside. Medium molecular weight chitosan has a molecular weight of 190–310 kDa, deacetylation degree of 75–85%, and a viscosity of 200–800 cPs. Less viscous liquid(eg:water) undergoes cavitation more easily and becomes emulsion phase (O/W). Next OFAT experiment was identifying the optimum concentration of surfactant,PVA.

4.4.1.2 OFAT 2: The surfactant (PVA) concentration

Surface active agent commonly known as a surfactant used in this study is polyvinyl alcohol (PVA). PVA is a non-ionic surfactant which is a nonelectrolyte. The emulsified droplets of chitosan-PCL loaded AI extract microencapsulation need a surfactant to preserve the stability as well as prevent continuous phase separation by coalescence (Leong et al., 2009).

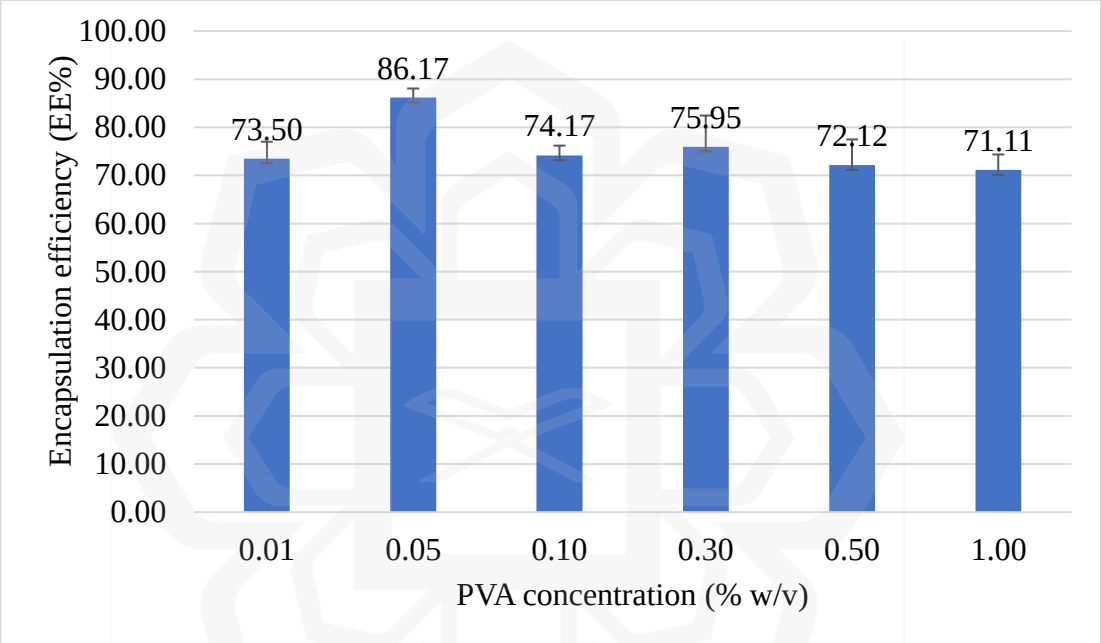

Figure 4.6 Effect of PVA concentrations on encapsulation efficiency

As a stabilizer, the presence of PVA at the outer space of microparticles significantly affect the percentage of encapsulation efficiency. The maximum encapsulation efficiency was found when the concentration of PVA was 0.05% which is 86.17% supported by the uniformly arranged morphological structure as in Figure 4.7. According to trend graph in Figure 4.6, the trend of EE% started to decrease after 0.1 % w/v from 74.17 to 71.11%. Thus, only 0.05% PVA needed to reach the maximum encapsulation efficiency which is 86.17%. The more PVA used, the higher the viscosity of the microemulsions resulting low encapsulation efficiency (Li et al., 2017).

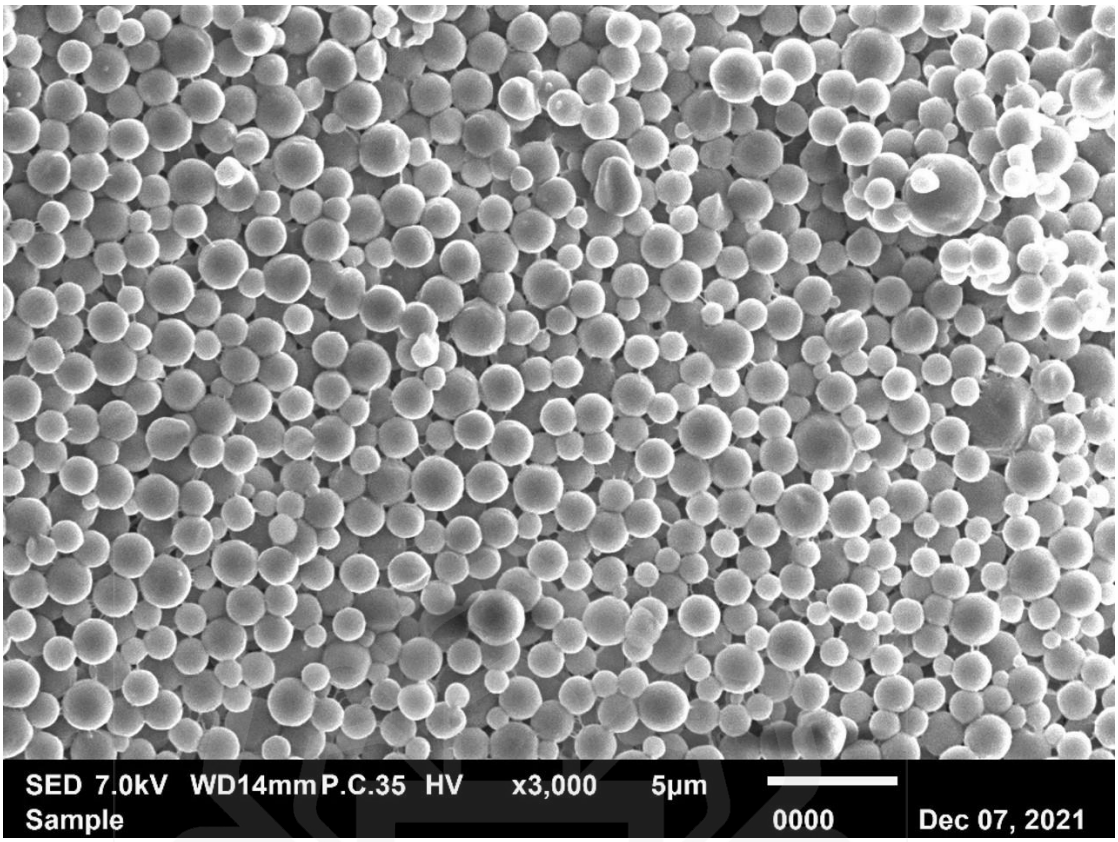

Figure 4.7 SEM image for 0.05% PVA

4.4.1.3 OFAT 3: Concentration of Chitosan-PCL blend

Chitosan-PCL blend concentration is a part of the important factor to determine the best amounts of polymer matrix that meet the encapsulation of the AI extracts. The amount of AI extracts used was 0.2% w/v and kept constant in this study. The amounts of polymer matrix which consisted of 0.6% w/v of chitosan and 0.4% w/v of PCL and the ratios of chitosan-PCL was 6:4. The ratio composition was selected from the highest EE% in OFAT 1. The polymer matrix concentration study was limited according to the maximum concentration of chitosan that reach the gelation point when dissolved in 0.2% v/v acetic acid glacial.

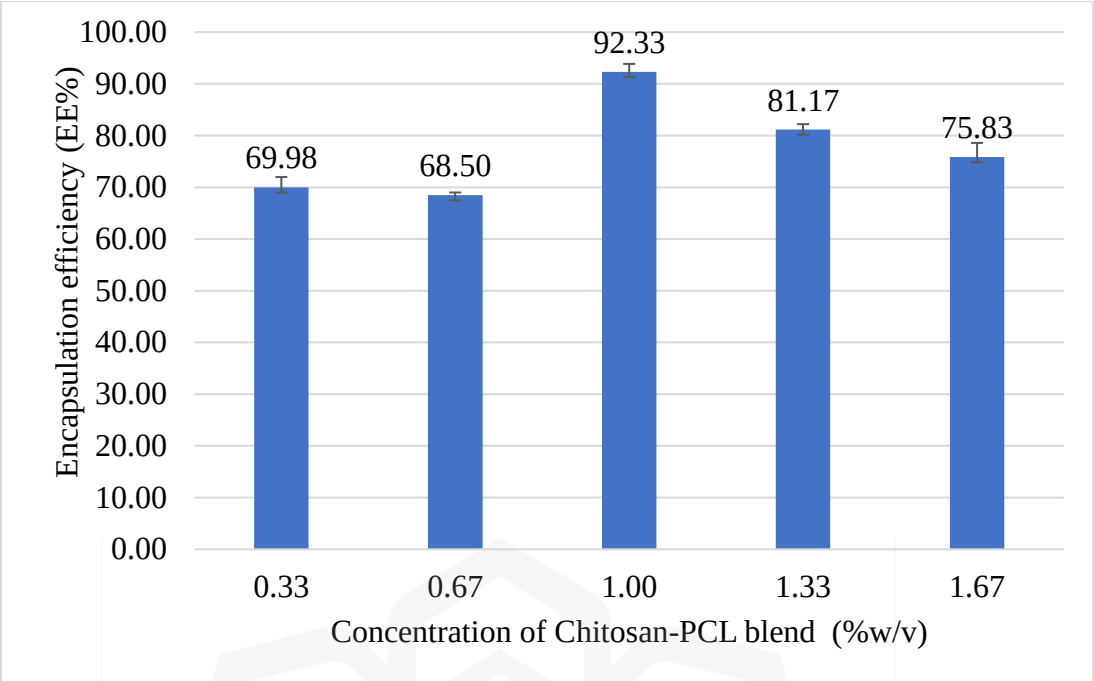

Figure 4.8 Concentration of Chitosan-PCL blend

Figure 4.8 shows the maximum Chitosan-PCL blend concentration was obtained at a concentration of 1.0 % which resulted in 92.33% of EE. Beyond 1% polymer concentration resulted of decrease in EE. This trend of EE% was also observed by Iqbal et al., (2015) and El. Hady et al. (2019). This was due to the viscosity of the polymer which caused the AI extract entrapment became less efficient. The increasing of polymer concentration also increased the thickness of the polymer matrix.

4.4.2 Response surface methodology (RSM)

The central composite design (CCD) model is one of the types of response surface methodology (RSM) usually used in polymer research experiment to study the interaction between more than two factors. The central point was often augmented with star points ±α for each factors. The star points represented the lower and higher values and each factors have different central points and star points values.

Table 4.2 shows ANOVA analysis for three factors which are chitosan:PCL, PVA concentration and polymer matrix concentration. The analysis shows that, the model *F*-value is 14.25 which implied that the model was significant which determined by a *p*-value <0.001. The regression coefficient (R^2) recorded was higher than 0.9 which is

0.9344, indicating that the experimental data was relatively strong. The difference between the adjusted coefficient of determination (R^2) and predicted R^2 was less than 0.2 considered reasonable agreement. Whilst, the lack of fit was reported as not significant, which means that the polynomial model was fitted well and statistically accurate (Li et al., 2017).

Table 4.1 ANOVA table

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	5.562×10^{11}	8	6.952×10^{10}	14.25	0.0006	significant
A-Chitosan (n) :PCL (10-n) ratio	1.935×10^{11}	1	1.935×10^{11}	39.66	0.0002	
B-PVA concentration	8.209×10^{9}	1	8.209×10^{9}	1.68	0.2308	
C-Concentration of Chitosan-PCL blend	2.259×10^{11}	1	2.259×10^{11}	46.31	0.0001	
AC	2.973×10^{10}	1	2.973×10^{10}	6.09	0.0388	
BC	3.241×10^{9}	1	3.241×10^{9}	0.6643	0.4386	
A^2	3.100×10^{9}	1	3.100×10^{8}	0.0635	0.8074	
B^2	3.314×10^{10}	1	3.314×10^{10}	6.79	0.0313	
C^2	7.568×10^{9}	1	7.568×10^{9}	1.55	0.2482	
Residual	3.903×10^{10}	8	4.879×10^{9}			
Lack of Fit	2.72×10^{10}	6	4.536×10^{9}	0.7676	0.6611	not significant
Pure Error	1.182×10^{10}					

Cor Total	5.952×10^{11}
R^2	0.9344
Adjusted R^2	0.86890
Predicted R^2	0.7105
Adeq. Precision	13.0331
Std. Dev	69848.92
Mean	4.874×10^5

The 3D contour plot graphs predicted the interaction between the parameters as shown in Figures 4.9 and 4.10. This study revealed that having some difficulties to find the optimum conditions as the contour lines increment continuously and shape became slightly sharp. This could be due to some limitation of the ultrasonic homogenization device contributed the external factors such as the condition of the microtips of ultrasonic homogenizer getting less efficient after many times usage. Another external factor is the temperature of the solutions during encapsulation process. The heat dissipated during homogenization unavoidable may led to dichloromethane evaporation and the volume of the reacted solution decreased (Taurozzi et al., 2012).

Figure 4.9 shows the linear interaction between chitosan: PCL ratio and the polymer matrix concentration. As one increased the ratio of the chitosan:PCL the EE% kept increasing and while the polymer matrix concentration increased, the EE% decreased. This is because practically, as the concentration of polymer matrix increased, the concentration of chitosan and PCL also increased while the ratio was maintained. This case happened may due to the increasing concentration of chitosan increased the viscosity thus hindered the ultrasonic homogenizer to break apart the chitosan molecules to form encapsulation (Taha et al., 2020). Whereas Figure 10 shows the 3D graph of the relationship between PVA concentration and concentration of chitosan-PCL blend. The EE% started to increase and decreased as the chitosan-PCL blend and PVA concentration increased. In this case, only a small amount of PVA is needed to form a good encapsulation.

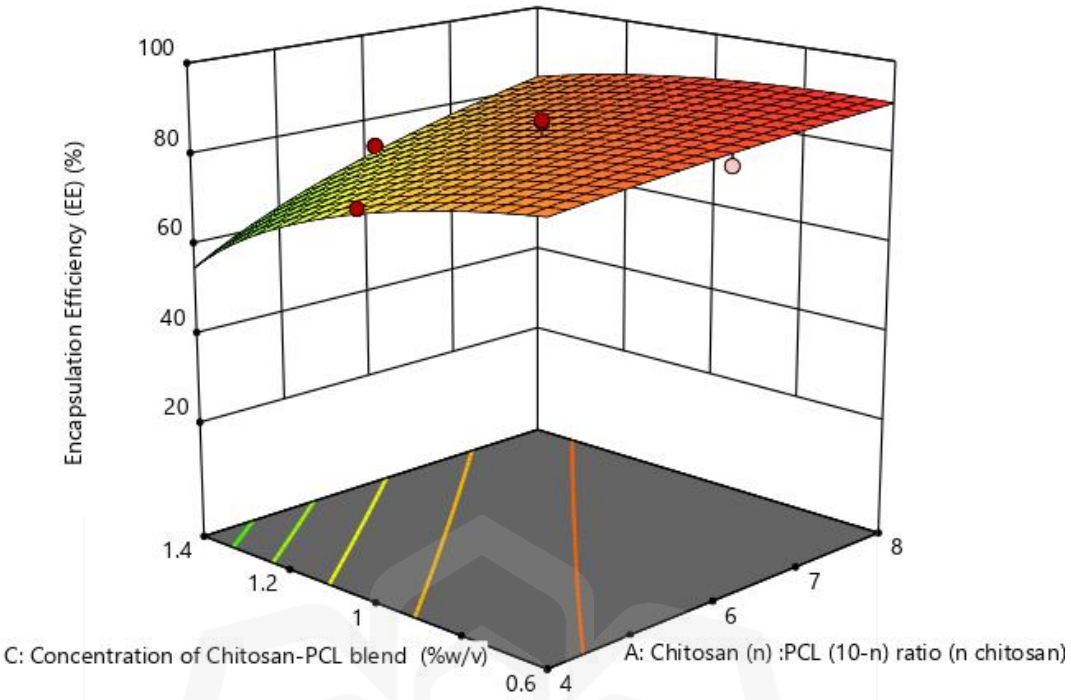

Figure 4.9 AC: 3D model surface relationship between ratio of chitosan:PCL and concentration of Chitosan-PCL blend

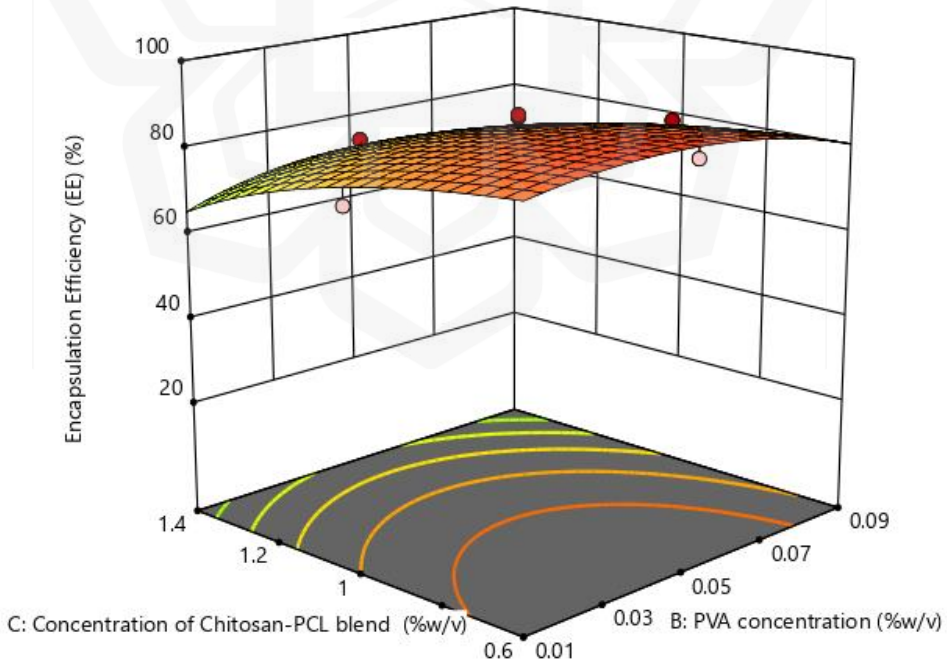

Figure 4.10 BC: 3D model surface relationship between PVA concentration and concentration of Chitosan-PCL blend

Next, based on this model, the second order polynomial equation of the encapsulation efficiency was fitted in the coded equation 2. This following equation obtained represented the quantitative effects of three factors on the encapsulation efficiency (EE%).

$$Y= 5.904 \times 10^5 + 1.391 X10^5 xA – 28650.51XB – 1.503x10^5 xC + 60960.18 xAC +20128.31 xBC – 10755.73 xA^2 – 10755.73 xB^2 -53148.54 xC^2 \quad [2]$$

4.4.3 Model validation

Validation has been done to determine the validity of the hypothesized model that was predicted by DOE software. The parameter details are tabulated in Table 4.2. The formulations with high and low EE% were chosen as used for the next analysis. It is found that the difference between the predicted value from DOE and experimental value was not much difference and reasonable. The experimental values revealed that the prediction of DOE was acceptable. The highest EE% recorded was beyond the expectation which is 98.7% as compared to the predicted was 91.30% and the error was 7.5%. However, the lowest EE% prediction value was recorded the experimental value was within the predicted range and the error was 1.2% which is acceptable.

From this optimization formulation, it shows that the best ratio of chitosan:PCL is 8:2 giving the highest EE% compared to the ratio of 5.2:4.8 chitosan:PCL. The result can be hypothesized that chitosan was needed more than PCL to give better encapsulation. The higher concentration of chitosan-PCL blend may precipitate faster thus prevent the AI extract to diffuse out of the polymer matrix (Jyothi et al., 2010b)

Table 4.2 Validation test of the model

	Factors			Encapsulation efficiency (EE %)		Error (%)
Run	Chitosan (n) :PCL (10-n) ratio	PVA concentration	Concentration of chitosan-PCL blend	Predicted value from DOE	Experimental value	
1	8	0.042	0.648	91.30	98.70	7.5
39	5.216	0.052	0.6	86.75	87.80	1.2

4.5 CHARACTERIZATION STUDY

In objective 3, the validated samples as per suggested by Design- Expert software were selected to proceed for analysis. Two (2) formulations were selected which reported the highest EE% and lowest suggested EE%. Based on the experimental values in the last objective 2, the highest EE% was 98.70% and the lowest EE% was 87.90%. The selected analysis to carry out in objective 3 are Fourier transform infrared spectroscopy (FTIR) characterization, surface morphology by scanning electron microscope (SEM), zeta potential and particle size analysis (PSA).

4.5.1 Characterization: Fourier transform infrared spectroscopy

The FTIR analysis was done to confirm the composition of the polymer matrix which consisted of chitosan and PCL, and the PVA as surfactant by the characteristics of spectra. The spectra graph of the encapsulated Chitosan-PCL loaded AI extract, the encapsulated Chitosan-PCL blank, PVA, PCL, chitosan and AI extract were obtained and compared as in Figure 11.

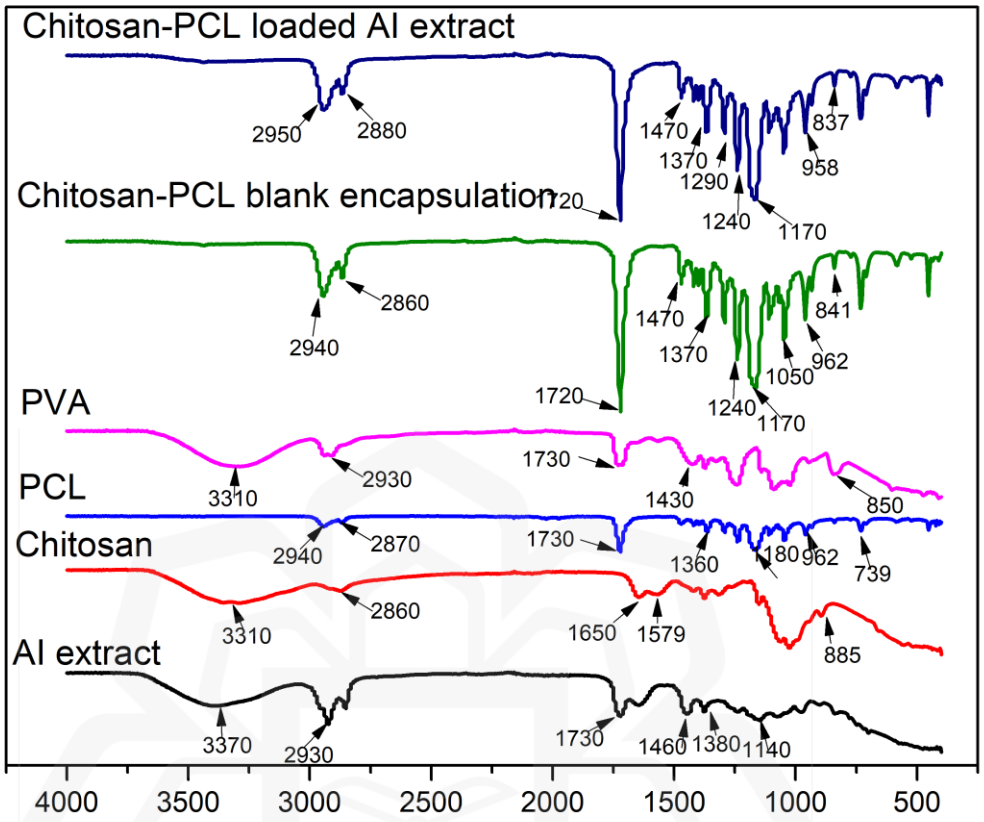

Figure 4.11 FTIR spectra for AI extract, chitosan, PCL, PVA, Chitosan-PCL blank encapsulation and Chitosan-PCL loaded AI extract

Chitosan parent chain is known to have an amino glucose structure which is linked with an amide linkage. Due to the presence of two strong functional groups, chitosan was capable of being modify to improve the limitation of chitosan. The two important functional group are two hydroxyl groups and one primary amine group. Under the IR spectrum, the chitosan in powder form was tested and a broad peak in between 2940-2860 cm^{-1} was exposed. The chitosan spectra can be detected due to the existence of amide I and amide II. Amide I was detected at 1650 cm^{-1} associated with C=O stretching vibration. Amide II was detected at 1579 cm-^{1} which is associated and recognizable by N-H stretching. The C-H stretching was detected at 2860 cm^{-1}. The ß-linked glycosidic bond was detected at 885 cm^{-1}. 1020 cm^{-1} indicated that the absorbance of O bridge stretching of glucosamine residue present. The characteristics of PCL were detected from the peak at 2940 cm^{-1} which is the asymmetric vibration stretching for -CH_3 group and 2870 cm^{-1} which is the -CH_2 (methylene) stretching. The stretching on 1730 cm^{-1}

indicated the C=O (ketone). The band stretching at 1360 cm^{-1} indicated the presence of C-O-C group and followed by more vibration bands by asymmetric stretching at 1180, 962 and 739 cm^{-1}.

The interaction of the polymer compositions showed characteristic bands at 2950-2880 cm^{-1} (C-H stretching), the spectra in PCL was switched from 1730 to 1720 cm^{-1} which indicated ketone and several inorganic ions from 1370-453 cm^{-1} in which the similar bands were existed in the single ingredients. Ultimately, similar findings were reported by Almeida et al. (2018). The encapsulation of AI extracts were successful through the presence of characteristic band of chitosan, PCL and the AI extracts. On the IR spectrum of chitosan-PCL loaded AI extracts, the presence of a sharp peak at 1470cm^{-1} indicates the characteristic of Amide II of chitosan. The presence of PCL was detected through the strong characteristic band of ketone at 1720 cm^{-1} . The characteristic band of AI extracts were expressed at 1470 and 1370 cm^{-1} showed the interactions of AI extracts occurred. Based on the FTIR spectra, the encapsulation of AI extract with chitosan-PCL blend occurred and can be suggested.

4.5.2 Surface morphology by scanning electron microscope (SEM)

The SEM images exhibited the formation of spherical shapes with smooth surface for the sample with highest EE% (98.70%) in Figure 4.12 (a) and less polydisperse. Whereas in Figure 4.12 (b), the surface morphology of the encapsulation with lower EE% (87.80%) also showed the spherical shape.

In Figure 4.12 (a), the structure of highest EE% revealed the formation of smooth spherical and no surface imperfection (cracks or burst) observed. Similar morphological structure reported by Lourenço et al. (2021). Due to the highest EE%, the agglomeration may provide more stability to encapsulate more AI extract and provided more protection towards leaching (Lourenço et al., 2021).

As in Figure 4.12 (b), the microparticles density less packed compared to (a) resulted the EE% was less than in (a). More gaps between the particles might be less intermolecular interactions.

a) b)

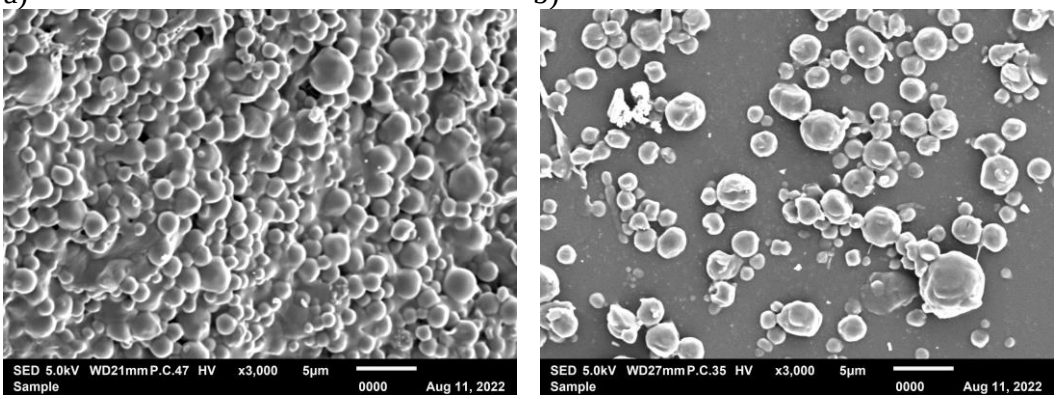

Figure 4.12 SEM image for Chitosan-PCL loaded AI extract with a) EE 98.70% and b) 87.80%

4.5.3 Zeta potential and particle size analysis

Table 4.4 summarizes the findings on the effects of zeta potential and particle size towards the EE% of the AI extract microencapsulations. In this study, the values of zeta potential were negative due to the adsorption of the surfactant on the surface of the microparticles (Abriata et al., 2019). Interestingly, most encapsulations of plant extracts studies reported found in agreement with the negative value of zeta potential obtained from this study. In 2020, the encapsulation of *Boswellia carterii* essential oil by Barre et al., (2020) found that the zeta potential was -25 to -36 mV (Barre et al., 2020). In 2021, the encapsulation of phenolic bioactive compounds found the zeta potential were -30 to -44 mV (Xue et al., 2021).

Table 4.3 The zeta potential and particle size for the validated samples

Sample validation lable	**Encapsulation efficiency (EE%)**	**Zeta potential (mV)**	**Particle size (µm)**
1	98.70	-24.0	2.631 ± 0.14
39	87.80	-26.2	3.568 ± 1.35

This result was found as an improvement from the previous AI extracts encapsulation study reported by Amarnath et al. (2014) and Muhaimin et al. (2020) in which the zeta potential obtained was -3.47 mV (Amarnath, Dhanabal, Agarwal, & Seshadry, 2014)).

The high EE% was obtained from the small size of particles. The small particle size may entrap more active ingredients of AI extract thus giving a higher EE%. The smaller size also led to the microparticles to being absorbed and released the active ingredients to the targeted parts. The particle size obtained was found to be similar as obtained by Amarnath et al. (2014) that encapsulate the AI extract with chitosan-casein which was 2µm. Another encapsulation study of *M. gigantea* leave extracts obtained 3.6-5.9 µm in size (Muhaimin et al., 2020).

4.6 CHAPTER SUMMARY

In this study, objective 1 has been successfully achieved from the formation of spherical shaped of AI extract encapsulation in chitosan-PCL and successfully optimized under 5 minutes sonication time. The EE% obtained was 74.5% and steadily decreased as increasing of sonication time. In this way, the time was less consumed thus maintained the condition of the microtips of the ultrasonic homogenization device.

Objective 2 is about optimization of three (3) important factors in the encapsulation study which are Chitosan:PCL concentration ratio (%w/v), PVA concentration as a surfactant (%w/v), and the concentration of chitosan-PCL blends mixture (%w/v). A series of OFAT experiments was done in order to get the range points for another series of RSM optimization to study the interactions between each of the factors. The most optimized ratio of Chitosan:PCL was 0.6:0.4 and the EE% was 70.10%. This study revealed that chitosan was needed more than PCL. However, without each of chitosan or PCL alone, the encapsulation would not occur. The second factor was PVA concentration is also important to study. The trend showed the reduction of EE% as increasing the concentration of PVA. 0.05% w/v of PVA resulted to the highest EE% (86.17%). Whereas the optimum concentration of Chitosan-PCL blend was 1.00%w/v with the EE% 92.33%. beyond 1.00%, the trend decreased. Based

on the OFAT data, the range value for each factors were obtained and the experiments were continued with RSM optimization. After optimization, validations were done based on the factors values suggested by DOE software. Two (2) sets of optimized factors were selected and further analysis were done.

In Objective 3, the analysis were proceed with the optimized encapsulation samples.1` The highest EE% recorded was 98.70%, the zeta potential was -24 mV and the particle size was 2.631 ± 0.14. The surfactant only needed in small amounts (0.052% w/v) to make a good encapsulation. However, slightly agglomeration may be detected from the value of zeta potential but does not affect the encapsulations.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 CONCLUSIONS

As for conclusion in Objective 1, 5 minutes resulted the best homogenization duration giving highest encapsulation efficiency (74.5%), The other three parameters, 0.6:0.4 ratio of Chitosan:PCL concentration was 70.10% w/v, 0.05% w/v concentration of PVA giving 86.17% and the 1% w/v concentration of Chitosan-PCL blend was 92.33% have been successfully find the optimized value. The values were proceed for the parameter interaction studies by applying the central composite design (CCD) using Design-Expert v.12 software. The validation results based on the optimum condition, the highest EE% obtained was 98.70% and the lowest EE% was 87.80%.

In Objective 3, surface morphology showed the spherical shape of microparticles. The zeta potential for both higher EE% and lower EE% microencapsulations resulted in small difference. Overall, the encapsulation of *Acalypha indica* (AI) extracts has been successfully done by the chitosan-PCL copolymer blends by emulsion-solvent evaporation method. This research has high potential to explore the usage of AI in pharmaceutical applications.

5.2 RECOMMENDATIONS

The study of chitosan copolymerization should be more extensive and the exploration of AI should get more attention in gaining more innovative products thus meet the market demand as AI extract sustained release supplements. As for recommendation, the next study of encapsulation can improve all the limitation from this study. The method of encapsulation using ultrasonic homogenizer can be replaced with other device as the microtips of the ultrasonic homogenizer is easily wear for heavily used.

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LIST OF PUBLICATIONS

Papers:

Maizatul, A. J et al., (2021) The Potential Encapsulation of Euphorbiaceae Plant Extracts: A Review. Journal of Polymer Science and Technology 6(2) 2021:11-19 (Manuscript submitted and published)

M A Johari et al 2021 IOP Conf. Ser.: Mater. Sci. Eng. 1192 012007 (Manuscript submitted and published)

M.A.Johari et al., (2023) Microencapsulation of Acalypha indica Linn. extracts using Chitosan-Polycaprolactone Blends. Journal of Science and Technology (Manuscript accepted)

Participations:

Kerice 2020 (Video presentation)

PG Colloquium 2021 mute poster competition

3 Minutes Thesis (3MT) competition

6th International Conference of Biotechnology Engineering 2021 (ICBioE 2021)

1st International Conference on Bioscience and Biorefinery (IC2B)

Kerice 2021 (Video presentation)

APPENDIX

Table 1 Standard curve data

Concentration (%w/v)	Absorbance
0.02	1.129
0.05	1.493
0.1	1.751
0.15	1.826
0.2	1.867