AMPHIPHILIC CHITOSAN DERIVATIVES AS ENVIRONMENTAL FRIENDLY MEDIA FOR WATER-INSOLUBLE PESTICIDE FORMULATIONS

SITI NAJIAH BINTI MOHD YUSOFF

UNIVERSITI PENDIDIKAN SULTAN IDRIS

2019

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05-4506832 pustaka.upsi.edu.my Perpustakaan Tuanku Bainun Kampus Sultan Abdul Jalil Shah PustakaTBainun ptbupsi

THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENT FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

FACULTY OF SCIENCE AND MATHEMATICS
UNIVERSITI PENDIDIKAN SULTAN IDRIS

2019

05-4506832 pustaka.upsi.edu.my Perpustakaan Tuanku Bainun Kampus Sultan Abdul Jalil Shah PustakaTBainun ptbupsi

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ACKNOWLEDGEMENTS

All praise to Allah S.W.T, for blessing me with strength and health, I was able to finish this Doctor of Philosophy (PhD) research work.

First of all, I would like to express my deepest gratitude to my main supervisor, Associate Professor Dr. Azlan Kamari for his dedication, patience, encouragement, perceptive comment and guidance throughout this study. Without his support, it might be tougher for me in to walk in this journey. Special thanks also to my co-supervisor, Associate Professor Dr. Norhayati Hashim for generous help towards my study. I owe a great thank to Associate Professor Dr. Fatimah Mohamed, from Department of Biology, Faculty Science and Mathematics, Universiti Pendidikan Sultan Idris for her expert advices. I convey my warmest gratitude to Professor Wan Saime Wan Ngah for his kindness and advices.

I am grateful to Universiti Pendidikan Sultan Idris (UPSI) and Islamic Educational, Scientific and Cultural Organization (ISESCO) for financial support and facilities. I also would like to thank Ministry of Education Malaysia (Higher Education) for granting me a scholarship under programme MyBrain15 (MyPhD).

I extend my sincere thanks to staffs and laboratory assistants of Department of Chemistry, Faculty Science and Mathematics, UPSI;- Mr. Mohd Hashimi Ma'ani, Mr. Ahmad Supian Abdullah, Mr. Zurin Mahmud, Mrs. Johana Jumiran, Mr. Muhammad Hazim Baharudin, Mr. Ahmad Isa Hamizi, Mr. Mohd Farhan, Mr Ibrahim Saidin, Mrs. Radiah Idris and Ms. Siti Noor Farina Mohd Fuad Ooi for their assistance.

I also grateful to my friends and collages;- Ms. Tretya Ardyani, Ms. Nurul Farhana Aljafree, Mrs. Syukriyah Ishak, Mrs. Mimi Syakila Samsi, Ms. Nur Indah Wardani, Mrs. Mariah Rihan Fasyir, Mr. Wiwid Pranata Putra, Mr. Ridwan Shamsudin, Mr. Al Luqman Abdul Halim, Mr. Argo Khoirul Anas, Ms. Syarifah Norain, Mrs. Zuhailimuna, Ms. Syarifah Nazirah, Mrs. Anis Husna, Ms. Hidayani Ahmad, Mr. Sahli Muda, Ms. Juliana Jumadi and Ms. Susana Wong for their support and help.

Last but not least, my heartfelt gratitude to my parents;- Mr. Mohd Yusoff Salleh and Mrs. Faridah Yahaya, and my family for unconditional moral support through this tough journey.

ABSTRACT

This research aimed to synthesis and investigate the potential of amphiphilic chitosan derivatives, namely *N*-octyl-*O*-sulfate chitosan (NOOSC), *N*-octyl-*N*-succinyl chitosan (NONSC), *N*-octyl-*O*-glycol chitosan (NOOGC), *N*-deoxycholic acid-*O*-glycol chitosan (DAGC), *N*-hexanoyl-*O*-glycol chitosan (HGC) and *N*-lauryl-*O*-glycol chitosan (LGC) as environmental friendly media for atrazine, rotenone and thymol formulations. The amphiphilic chitosan derivatives were characterised using proton nuclear magnetic resonance (^{1}H NMR) spectrometer, Fourier transform infrared (FTIR) spectrometer, CHNS-O elemental analyser, fluorescence spectrometer and scanning transmission electron microscope (STEM). Encapsulation efficiency of pesticide active ingredients by amphiphilic chitosan derivatives was determined using a high performance liquid chromatography (HPLC). The release mechanism of atrazine, rotenone and thymol from the amphiphilic chitosan derivatives micelles was fitted to four kinetic models. Pot experiments were carried out to monitor the effectiveness of each pesticide formulation on weed (*Cyperus kyllingia*) and chilli (*Capcisum annuum*) plant infested by aphids, thrips and white fly. Research findings found that the amphiphilic chitosan derivatives have formed self-aggregates with a spherical shape. The critical micelles concentration (CMC) values of the amphiphilic chitosan derivatives were between 0.008 and 0.089 mg/mL. The encapsulation efficiency values for the amphiphilic chitosan were higher than 50%. All amphiphilic chitosan derivatives enhanced pesticides release performance as compared to pure pesticides solution. The constant (n) values obtained from Korsmeyer-Peppas kinetic model suggest that the release of pesticide active ingredients from the chitosan derivatives micelle was controlled by relaxation of polymer chains. Based on pot experiments, the amphiphilic chitosan derivatives formulation effectively treat the target species. In conclusion, the amphiphilic chitosan derivatives are potential as carrier agents for pesticide active ingredients. In implication, the amphiphilic chitosan derivatives as environmental friendly media able to reduce the use of organic solvents in pesticide formulations more than 60%.

TERBITAN KITOSAN AMFIFILIK SEBAGAI MEDIA MESRA ALAM UNTUK FORMULASI RACUN PEROSAK TAK TERLARUTKAN AIR

ABSTRAK

Kajian ini bertujuan untuk mensintesis dan menyelidik potensi terbitan kitosan amfifilik, iaitu kitosan *N*-oktil-*O*-sulfat (KNOOS), kitosan *N*-oktil-*N*-suksinil (KNONS), kitosan *N*-oktil-*O*-glikol (KNOOG), kitosan *N*-asid deoksikolik-*O*-glikol (KADG), kitosan *N*-heksanoil-*O*-glikol (KHG) dan kitosan *N*-lauril-*O*-glikol (KLG) sebagai media mesra alam untuk formulasi atrazin, rotenon dan timol. Terbitan kitosan amfifilik dicirikan menggunakan spektrometer resonans magnet nukleus proton (^{1}H NMR), spektrometer inframerah transformasi Fourier (FTIR), penganalisis unsur CHNS-O, spektrometer pendarfluor dan mikroskop pengimbas penghantaran elektron (STEM). Kecekapan pengkapsulan bahan aktif racun perosak oleh terbitan kitosan amfifilik ditentukan menggunakan kromatografi cecair prestasi tinggi (HPLC). Mekanisma pelepasan atrazin, rotenon dan timol dipadankan kepada empat model kinetik. Eksperimen pasu telah dijalankan untuk memantau keberkesanan setiap formulasi racun perosak ke atas rumput (*Cyperus kyllingia*) dan pokok cili (*Capcisum annuum*) yang telah diserang afid, trip dan lalat putih. Dapatan kajian mendapati bahawa terbitan kitosan amfifilik telah membentuk penswabentukan dengan bentuk sfera. Nilai-nilai kepekatan misel kritikal (KMK) terbitan kitosan amfifilik adalah di antara 0.008 dan 0.089 mg/mL. Nilai-nilai kecekapan pengkapsulan untuk terbitan kitosan amfifilik adalah lebih besar daripada 50%. Semua terbitan kitosan amfifilik meningkatkan prestasi pelepasan racun perosak berbanding larutan bahan aktif racun perosak tulen. Nilai-nilai pemalar (n) dari model kinetik Korsmeyer-Peppas mencadangkan bahawa pelepasan bahan aktif racun perosak dari misel terbitan kitosan amfifilik telah dikawal oleh pengenduran rantai polimer. Berdasarkan eksperimen pasu, formulasi terbitan kitosan amfifilik merawat secara berkesan spesies sasaran. Kesimpulannya, terbitan kitosan amfifilik berpotensi sebagai agen pembawa untuk bahan aktif racun perosak. Implikasinya, terbitan kitosan amfifilik sebagai media mesra alam mampu mengurangkan penggunaan pelarut organik dalam formulasi racun perosak melebihi 60%.

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LIST OF ABBREVIATIONS

ACD	Amphiphilic Chitosan Derivatives
CMC	Critical Micelle Concentration
CS	Microcapsules
DAGC	*N*-deoxycholic acid-*O*-glycol Chitosan
DOA	Department of Agriculture Malaysia
DOE	Department of Environment Malaysia
DOSM	Department of Statistics Malaysia
DP	Dust
DSC	Differential Scanning Calorimetry
EC	Emulsifiable Concentrate
EDC	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
EE	Encapsulation Efficiency
EW	Oil-in-water Emulsion
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FTIR	Fourier Transform Infrared
GC	*O*-glycol Chitosan
GRAS	Generally Recognised as Safe
Ha	Hectare
HGC	*N*-hexanoyl-*O*-gycol Chitosan
HPLC	High Performance Liquid Chromatography
IUPAC	International Union of Pure and Applied Chemistry

LD_{50}	Lethal Dermal of Half Population
LGC	*N*-lauryl-*O*-glycol Chitosan
LOD	Limit of Detection
LOQ	Limit of Quantification
ME	Microemulsion
MOA	Ministry of Agriculture and Agro-based Industry Malaysia
MRL	Maximum Residue Limit
MWCO	Molecular Weight Cut-off
NHS	*N*-hydroxysuccimide
NMP	*N*-methyl-2-pyrrolidone
NMR	Nuclear Magnetic Resonance
NOC	*N*-octyl Chitosan
NONSC	*N*-octyl-*N*-succinyl Chitosan
NOOGC	*N*-octyl-*O*-glycol Chitosan
NOOSC	*N*-octyl-*O*-sulfate Chitosan
PBS	Phosphate Buffer Saline
SC	Suspension Concentrates
SEM	Scanning Electron Microscopy
STEM	Scanning Transmission Electron Microscopy
TEM	Transmission Electron Microscopy
TGA	Thermogravimetric Analysis
UN	United Nations
USA	United States of America
USD	United States Dollar
USEPA	United States Environment Protection Agency
UV	Ultraviolet

VOCs	Volatile Organic Compounds
WG	Water Dispersible Granule
WHO	World Health Organization
WP	Wettable powders

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CHAPTER 1

INTRODUCTION

1.1 Research Background

Food is one the basic necessities for human in life. In order to achieve a sustainable life and good health, the access towards sufficient amount of safe and nutritious foods is indeed important (World Health Organization [WHO], 2017). Agriculture plays an important role as a major food contributor, as it provides more than 99.7% of world's food supply (Ballantyne & Marrs, 2004). It was reported that around 500 million of small farms worldwide has contributed to 80% of the food supply for the large part of the developing country (United Nations [UN], 2018).

According to Food and Agriculture Organization (FAO) of the United Nations, the global population is expected to grow and reach around 9.8 billion of people by 2050 (FAO, 2014). One of the main goals for global agriculture is to provide sufficient amount of food to feed the current population (Sadowski & Baer-Nawrocka, 2018). Therefore, in order to feed the continuous growth of human population, the global production for agricultural product is expected to grow for about 1.1% per year from 2005 to 2050 (Alexandratos & Bruinsma, 2012). Consequently, it becomes a great challenge for key players of agriculture sector to ensure a sustainable food production to meet the high demand of the public (de Oliveira, Campos, Bakshi, Abhilash, & Fraceto, 2014).

The major problems faced by agriculture sector are the attack from pest, diseases and weed which has caused significant loss in quantity and quality of agricultural production (de Oliveira et al., 2014; E. I. Pereira et al., 2015). It was estimated that there are around 9,000 species of insects and mites, 50,000 species of plant pathogens and 8,000 species of weed that are accountable for the damage of agriculture crops worldwide (W. Zhang, 2018). For instance, it was reported that around 45% of the world annual food production has lost due to pest attack (Odukkathil & Vasudevan, 2013). In addition, without application of pesticide agriculture sector could face serious loss in fruits (78%), vegetables (54%) and cereals production (32%) (W. Zhang, 2018). J Erik Fyrwald, the Head of the Syngenta, one of the world biggest pesticides makers in the interview with The Guardian has stated that the world may face food famines due to elimination of pesticides (Carrington, 2018). For that reason, in order to protect the crops, to maximise the agricultural activity and to ensure food sustainability, the use of pesticide is necessary (de Oliveira et al., 2014; E. I. Pereira et al., 2015).

Although there is no complete data on the overall total pesticides production worldwide, the United States Environmental Protection Agency (USEPA) has reported that the world expenditure on the pesticide at producer level has reached nearly USD 56 billion in 2012 (Atwood & Paisley-Jones, 2017). In addition, it was estimated that United States has produced around 1.2 billion pounds of pesticide (in amount of active ingredient) which is nearly 544,310 tonnes in 2006 and 2007 (Grube, Donaldson, Kiely, & Wu, 2011).

The amount of pesticide used on agriculture crop was estimated to be around 1.0 to 2.5 million tonnes annually (Fenner, Canonica, Wackett, & Elsner, 2013; Odukkathil & Vasudevan, 2013). Based on statistics data released by the FAO of the UN, Asian countries has used the highest amount of pesticides with a total pesticides usage of around 2.0 million tonnes in 2014, followed by American countries (north and south) with around 900,000 tonnes and European countries with around 400,000 tonnes of pesticide in the same year. Herbicides (869,566.71 tonnes) were accounted largest portion of global pesticide usage in 2014, followed by fungicides and bactericides (417,968.17 tonnes), insecticides (259,901.61 tonnes) and plant growth regulators (42,899.92 tonnes).

Table 1.1 shows the list of populations, agriculture areas and pesticides usage (active ingredients) for selected countries in 2014. Malaysia which has around 30 million of population and 7.8 million hectare of agriculture areas has consumed more than 49,000 tonnes of pesticide (in terms of active ingredients) in 2014. Meanwhile, China (mainland, Macao SAR, Hong Kong SAR, and Taiwan Province), whose now supply for about 25% world's food was the major consumer of pesticides among

Asian countries with a total of 1,815,706.68 tonnes of pesticides used in 2014 (FAO, 2018a; Y. Xu, Li, & Wan, 2017).

Table 1.1

Populations, Agriculture Areas and Pesticides Usage for Selected Countries in 2014.

Country	Population	Agriculture area (ha)	Pesticides used (Tonnes)
Argentina	42,981,515	148,700,000	207,706
Brazil	204,213,133	282,589,000	352,336
China	1,421,307,384	515,357,700	1,815,706.68
Egypt	91,812,566	3,745,000	11,363
France	64,190,638	28,766,500	74,909.60
Germany	81,489,660	16,725,000	45,836.29
Italy	59,585,668	13,162,000	59,422
Japan	128,162,873	4,519,000	53,543.7
Malaysia	30,228,017	7,839,000	49,199.43
Mexico	124,221,600	106,705,000	53,196.66
Myanmar	51,924,182	12,645,000	5,417.80
Spain	46,521,827	26,578,000	61,067
Thailand	68,416,772	22,110,000	21,800
United Kingdom	65,015,686	17,232,000	18,392.45

Adapted from *Food and Agriculture Organization of the United Nations* (FAO), 2018a.

1.2 Problem Statement

The pesticide formulations are normally comprised of inert and active ingredients, where the latter is the chemical that is responsible to kill, repel or control the target pest. The active ingredients can be divided into two types, namely synthetic (inorganic and organic) and biological pesticides (Isman, 2006). The synthetic pesticides are often referred as man-made pesticides. The best examples of synthetic pesticides are organochlorines, organophosphates and carbamates that have been extensively applied in agriculture. Whereas, the example of the biological pesticides are azadirachtin, rotenone, ryanodine and thymol, which are synthesised from small organic molecules produced by plants that called secondary metabolites (Cavoski, Caboni, & Miano, 2011). These secondary metabolites have some pesticidal properties that are effective to defend and protect plants from pest attack (de Oliveira et al., 2014).

Pesticide active ingredients are generally having a wide range of solubility in solvent. Some of them are readily soluble in water, while some only dissolve in organic solvent or sometimes in neither one (Chin, Wu, & Wang, 2011; El Jay, 1996). For example, biologically active ingredients, derived from plants such as capsaicin and rotenone, known for their poor solubility in water which are around 0.06 mg/mL and 0.002 mg/mL, respectively (L. Hu, Xia, Zhan, Huang, & Xu, 2006; C. Shen, Yang, Wang, Zhou, & Chen, 2012; Turgut, Newby, & Cutright, 2004). Meanwhile, herbicides metalachor and prochloraz have solubilities of 0.53 and 0.34 g/L in water, respectively (Fan, Wu, & Peng, 2014).

Therefore, in order to dissolve these active ingredients, large amount of organic solvents is often required in the current pesticide formulations (Fan et al., 2014; Katagi, 2008). Nonetheless, there are various negative side effects upon the usage of organic solvents in pesticide production (Kah, Beulke, Tiede, & Hofmann, 2012; L. G. Pan, Tao, & Zhang, 2005). The organic solvents are often toxic, flammable and volatile (García, Alfaro, Calero, & Muñoz, 2014; Tominack, 2000). Organic solvents can be a source for volatile organic compounds (VOCs) emission and can contaminate the environment, and consequently cause a health hazard to humans and animals (Capello, Wernet, Sutter, Hellweg, & Hungerbühler, 2009; Jessop et al., 2015; R. S. S. Kumar et al., 2013; Pérez-Martínez, Ginés, Morillo, González-Rodríguez, & Moyano Méndez, 2000; Tominack, 2000).

For example, polychloromethanes and polychloroethanes are chlorinated VOCs and carcinogen, and are frequently used as solvents in pesticide industry. USEPA, European Commission (EC) and Ministry of Environmental Protection (MEP) of China have listed these compounds as priority pollutants (Huang, Lei, Wei, & Zeng, 2014). According to W. Zhu, Schmehl, Mullin, and Frazier (2014), the utilisation of *N*-methyl-2-pyrrolidone (NMP) as a solvent in pesticide formulation can heighten the toxicity effect to nervous, cardio-vascular, respiratory and hormonal systems. In Malaysia, both Department of Agriculture and Department of Environment Malaysia have highlighted environmental issues arising from the release of organic solvent to the environment from pesticides application.

Moreover, although organic solvents have their own toxicity effect, they can interact with pesticide active ingredients in the formulation thus increase their toxicity properties (Cox & Surgan, 2006). Brand and Muller (2002) noted that dermal penetration for herbicide formulation is notably greater than pure active ingredients (Atrazine, Alachlor and Trifluralin). Meanwhile, the role of cyclohexanone as a coformulator in the formulation of commercial insecticide, dimethoate EC40 has been explored by Eddleston et al. in 2012. Even though there was no significant toxicity effect reported on guinea pig for cyclohexanone and dimethoate after applied them separately, the presence of both chemicals in pesticide formulation has caused severe toxicity effects on guinea pig, as cyclohexanone was assumed has interacted and altered the actual toxicity of dimethoate.

The uncontrolled release of pesticides into the environment has received great concern from environmental scientists. In many cases, farmers are often applied pesticides on crops at a higher rate than permitted maximum dosage. This situation can be related to the fact that most (60 to 70%) of the pesticide loss and does not reach the target surface when applied on agricultural field (E. I. Pereira et al., 2015). These occur as the pesticides typically settle in the environment due to the leaching, volatilisation, immobilisation and also erosion (Al-Rajab & Hakami, 2014; E. I. Pereira et al., 2015). For instance, herbicides alcahor and insecticide imidacloprid have always been applied at a higher threshold concentration in agricultural field to counter the losses (Armbrust & Peeler, 2002; Fernandez-Urrusuno, Gines, & Morillo, 2000). Consequently, the pesticides particularly for highly soluble ones can be the sources for some environmental issues as they can enter the soil (J. Li, Yao, Li, & Shao, 2012). As the pesticides leach into the soil, they can leach and settle into the groundwater system and thus potentially be the sources of pesticide contamination. If persist, they may accumulate in the plants and animals, and can cause a serious health threat (A. Roy, Singh, Bajpai, & Bajpai, 2014).

Another limiting factor in development of pesticide formulation is the application of non-selective pesticide. Due to their toxicity effects, the non-selective pesticides can cause adverse toxic effects on wide range of non-target organisms (Arias-Estévez et al., 2008; Saxena & Pandey, 2001). The leach, drift, runoff and volatilisation of the pesticide could reach the non-target species in adjacent habitats and can change the species richness and diversity (Arias-Estévez et al., 2008; Boutin, Strandberg, Carpenter, Mathiassen, & Thomas, 2014). For example, thiocarbamates, an organosulfur compound that is applied extensively in agricultural field as a pesticide can affect non-target pests including mammals (Mathieu, Duval, Xu, Rodrigues-Lima, & Dupret, 2015). In addition, herbicide paraquat has an acute and non-selective behaviour that can kill any green plant tissues upon contact (Brigante & Avena, 2014). Permethrin also has wide range of toxicity and severe unintended effects on non-target arthropods such as spiders, damsels and bugs.

1.3 Research Significances

Despite the benefit of the application of agrochemical products to protect the crops, the environmental issues arise from their application brought so much concern to the public. Great efforts have been directed to solve environmental issues relating to the pesticide applications, worldwide. New trends of pesticides formulation has lean towards reducing the application of organic solvent and improve the activity of active ingredients. Most of the efforts have been focused on technical and engineering aspects especially during pesticide formulations. From fundamental aspect, introduction of good carrier or media system can reduce the amount of organic solvents used in the production of pesticides by enhancing solubility of the hydrophobic pesticide active ingredients and reduce its environmental risk by controlled-release technique.

This research combines the recent advancement in materials science and the application of polymeric micelle in production of agrochemical products. In this research, amphiphilic chitosan derivatives which are extensively studied in medical and pharmaceutical field are utilised as green media or carrier agents in pesticide formulation due to their unique properties. Chitosan is chosen due to its outstanding biological properties and functions such as biodegradability, biocompatibility, insecticidal and antibacterial activity, and importantly easy to modify (Larsson et al., 2013). The amphiphilic chitosan can be developed by introducing the hydrophobic and hydrophilic moieties to the chitosan backbone. When come in contacts with aqueous solvent, the amphiphilic molecule chains tend to reorganise and form micelles aggregate via intra- or intermolecular association between moieties (core-shell structure) (Larsson et al., 2013). The hydrophobic core will provide loading space for hydrophobic pesticide active ingredients whereas the hydrophilic shell will protect the encapsulated pesticide active ingredients, thus enhance its the solubility in water. Figure 1.1 shows the schematic diagram of encapsulation of hydrophobic pesticides active ingredient by amphiphilic chitosan micelles.

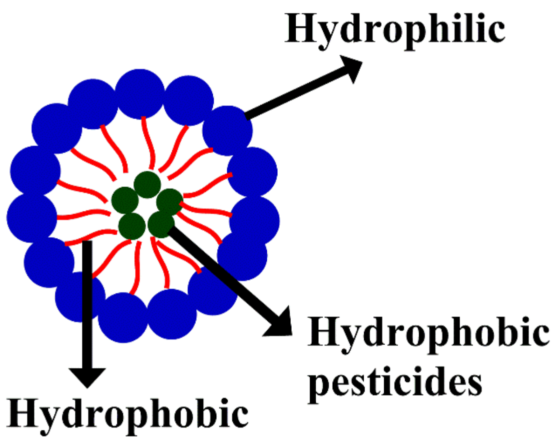

Figure 1.1. The Schematic Diagram of the Encapsulation of the Hydrophobic Pesticides by Amphiphilic Chitosan Micelles.

In addition, although there are many research focused on chitosan-based materials for agricultural practices, there are only few comprehensive studies that have been currently conducted on application amphiphilic chitosan derivatives in agriculture, particularly in pesticide formulations. This research proposed a formulation for production of agrochemicals products which useful to overcome the environmental issues relating to the use of organic solvents and uncontrolled release of pesticides. This research is relevance to the National Agro-Food Policy set by Ministry of Agriculture and Agro-based Industry Malaysia (MOA) which focused on ensuring food security for Malaysian citizen by promoting sustainable development in agriculture (MOA, 2011). This research is also significance to the National Policy on the Environment set by Department of Environment Malaysia (DOE) particularly on Continuous Improvement in the Quality of the Environment that directed towards achieving clean, safe and healthy, and productive environment for present and future generation (DOE, 2002; Economic Planning Unit, 2015).

1.4 Research Aim

The overall aim of this research was to utilise amphiphilic chitosan derivatives as green media (carrier agents) in the formulation of environmental friendly pesticides.

1.5 Research Objectives

The objectives of this research were:

1. To synthesis and characterise the physical and chemical properties of amphiphilic chitosan derivatives.
2. To evaluate the ability of amphiphilic chitosan derivatives to encapsulate and increase water solubility of hydrophobic pesticide active ingredients in pesticide formulations.
3. To assess the ability of amphiphilic chitosan derivatives to release hydrophobic pesticide active ingredients through in vitro system.
4. To evaluate the effectiveness of proposed pesticide formulations on target species or infected plants.

1.6 Research Scopes

This research consisted of four main parts, namely synthesis, characterisation, application and effectiveness studies. In this research, six types of amphiphilic chitosan derivatives, which consist of different types of hydrophilic and hydrophobic groups, namely *N*-octyl-*O*-sulfate chitosan, *N*-octyl-*N*-succinyl chitosan, *N*-octyl-*O*-glycol chitosan, *N*-deoxycholic acid-*O*-glycol chitosan, *N*-hexanoyl-*O*-glycol chitosan and *N*-lauryl-*O*-glycol chitosan were synthesised. *N*-octyl chitosan and *O*-glycol chitosan were used as precursors to synthesis the respective amphiphilic chitosan derivatives. Several characterisation studies were carried out on each amphiphilic chitosan derivative in order to determine the chemical structure, the presence of functional groups, the elemental composition, thermal properties and internal morphology of the materials.

The amphiphilic chitosan derivatives were then applied to encapsulate three types of hydrophobic pesticides active ingredients, namely atrazine, rotenone and thymol. The ability of the amphiphilic chitosan derivatives to increase the solubility of atrazine, rotenone and thymol in water was evaluated based on its encapsulation efficiency.

The in vitro release of the atrazine, rotenone and thymol from the amphiphilic chitosan derivatives micelles was conducted in phosphate buffer saline (PBS) solution to evaluate the controlled release properties. The release mechanism of atrazine, rotenone and thymol from the amphiphilic chitosan derivatives micelles was obtained by fitting the in vitro release data to four kinetic models.

The pot experiment was performed to assess the effectiveness of the amphiphilic chitosan derivatives formulations on target species. In this study, aphids, trips and white fly were used as target species for formulations of the amphiphilic chitosan derivatives-loaded with rotenone or thymol. Meanwhile, weed (*Cyperus* *kyllingia*) was used as target species for the formulation of amphiphilic chitosan derivatives-loaded with atrazine.

1.7 Thesis Outline

This thesis is organised into 5 chapters. Each chapter is arranged as follows; Chapter 1 explained about background of the study, problem statement, research aim and objectives, and the scopes of the study. Chapter 2 discussed the literature reviews on the topics related to pesticides such as the backgrounds of the pesticides application in the world, the classification of the pesticides, the environmental effects and health hazards due to the application of pesticides and organic solvents. The agriculture trends and application of the pesticides in Malaysia was also described. The examples of the carrier agents used in the pesticides formulation and research gaps were also highlighted.

On the other hand, Chapter 3 focussed on the research methodologies. The list of the chemicals used throughout this study was provided. The details on the procedure to synthesis each of the amphiphilic chitosan derivatives, to encapsulate the pesticides active ingredients in the amphiphilic chitosan derivatives micelles and the analytical techniques to characterise the chitosan derivatives were explained. The production of the pesticides formulation for studied pesticides active ingredients and procedure to evaluate effectiveness of the pesticides formulation was also presented.

Next, Chapter 4 discussed the results obtained from the characterisation of each of the amphiphilic chitosan derivative, the application study and effectiveness of the pesticides formulation on targeted species. In chapter 5, the research findings were summarised and some suggestions for future work were presented.