SYNTHESIS, CHARACTERISATION AND APPLICATION OF AMPHIPHILIC CHITOSAN DERIVATIVES FOR WATER-INSOLUBLE PESTICIDE FORMULATIONS

NURUL FARHANA BINTI AHMAD ALJAFREE

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ABSTRACT

This study investigates the feasibility of amphiphilic chitosan derivatives, namely oleoyl carboxymethyl chitosan (OCMCs), *N*,*N*-dimethylhexadecyl carboxymethyl chitosan (DCMCs) and deoxycholic acid carboxymethyl chitosan (DACMCs) as carrier agents for rotenone in water-insoluble pesticide formulations. This research is divided into three parts, such as characterisation studies, performance studies and pot experiments. The characterisation studies were carried out using Fourier Transform Infrared (FTIR) Spectrometer, Proton Nuclear Magnetic Resonance (^{1}H NMR) Spectrometer, CHN-O Elemental Analyser (CHN-O), Transmission Electron Microscope (TEM), Differential Scanning Calorimeter (DSC) and Thermogravimetric Analyser (TGA). The critical micelle concentration (CMC) of amphiphilic chitosan derivatives was determined using a Fluorescence Spectrometer. The ability of OCMCs, DCMCs and DACMCs to load and release rotenone *in vitro* system was determined using a High Performance Liquid Chromatography (HPLC). The pot experiments were conducted for 12 weeks to evaluate the effectiveness of pesticide formulations. Chilli (*Capcisum annuum)* was used as an indicator to monitor the effects of aphids and thrips infestation. Based on TEM analysis, findings have shown that amphiphilic chitosan derivatives formed self-assembly and exhibited spherical shape. The critical micelle concentration (CMC) for OCMCs, DCMCs and DACMCs were determined as 0.093, 0.098 and 0.468 mg/mL, respectively. The encapsulation efficiency (EE) values were within the range of 60.3 to 98.7%, meanwhile the loading capacity (LC) values were within the range of 0.97 to 7.90%. OCMCs, DCMCs and DACMCs micelles exhibited an excellent ability to control the release of rotenone, of which 90.0% of rotenone was released within 40 to 52 h. Based on pot experiments, the application of OCMCs as a carrier agent and polyvinyl alcohol (PVA) as an emulsifier was proven to be the most effective formulation to treat aphids and trips infected plants. In conclusion, OCMCs, DCMCs and DACMCs possess several key features to act as effective carrier agents for pesticide formulations. The implication of this study is the utilisation of amphiphilic chitosan derivatives could reduce the application of organic solvents in agrochemicals production by 33.3%, creating a greener and safer environment.

SINTESIS, PENCIRIAN DAN PENGGUNAAN TERBITAN KITOSAN AMFIFILIK UNTUK FORMULASI RACUN PEROSAK TAK TERLARUTKAN AIR

ABSTRAK

Kajian ini menyelidik kebolehlaksanaan terbitan kitosan amfifilik, iaitu kitosan karboksimetil oleoil (OCMCs), kitosan karboksimetil *N,N*-dimetilheksadesil (DCMCs) dan kitosan karboksimetil asid deoksikolik (DACMCs) sebagai ejen pembawa untuk rotenon dalam formulasi racun perosak tak terlarutkan air. Penyelidikan ini dibahagikan kepada tiga bahagian, iaitu kajian pencirian, kajian prestasi dan eksperimen pasu. Kajian pencirian telah dilakukan menggunakan Spektrometer Inframerah Transformasi Fourier (FTIR), Spektrometer Resonans Magnet Nukleus Proton (^{1}H NMR), Penganalisis Unsur CHN-O (CHN-O), Mikroskop Pancaran Elektron (TEM), Kalorimeter Pengimbasan Pembezaan (DSC) dan Penganalisis Termogravimetri (TGA). Kepekatan Misel Kritikal (CMC) bagi terbitan kitosan amfifilik telah ditentukan menggunakan Spektrometer Pendarfluor. Keupayaan OCMCs, DCMCs dan DACMCs untuk memuatkan dan melepaskan rotenon dalam sistem *in vitro* telah ditentukan menggunakan Kromatografi Cecair Prestasi Tinggi (HPLC). Eksperimen pasu telah dijalankan selama 12 minggu untuk menilai keberkesanan formulasi racun perosak. Cili (*Capcisum annuum*) telah digunakan sebagai penunjuk untuk mengawasi kesan serangan afid dan trip. Berdasarkan analisis TEM, dapatan menunjukkan bahawa terbitan-terbitan kitosan amfifilik membentuk penswabentukan dan mempamerkan bentuk sfera. Kepekatan Misel Kritikal (CMC) untuk OCMCs, DCMCs and DACMCs telah ditentukan dengan masing-masing sebagai 0.093, 0.098 dan 0.468 mg/mL. Nilai-nilai kecekapan pengkapsulan (EE) adalah dalam lingkungan julat 60.3 hingga 98.7%, manakala nilai-nilai muatan pembebanan (LC) adalah dalam lingkungan julat 0.97 hingga 7.90%. Misel OCMCs, DCMCs and DACMCs mempamerkan keupayaan yang sangat baik untuk mengawal pelepasan rotenon, yang mana 90.0% rotenon telah dilepaskan dalam lingkungan 40 hingga 52 jam. Berdasarkan eksperimen pasu, penggunaan OCMCs sebagai ejen pembawa dan polivinil alkohol (PVA) sebagai pengemulsi telah terbukti menjadi formulasi paling berkesan untuk merawat tumbuhan yang dijangkiti afid dan trip. Kesimpulannya, OCMCs, DCMCs dan DACMCs mempunyai beberapa ciri-ciri utama untuk bertindak sebagai ejen pembawa yang berkesan untuk formulasi racun perosak. Implikasi kajian ini ialah penggunaan terbitan kitosan amfifilik mampu menurunkan penggunaan pelarut organik dalam pengeluaran agrokimia sebanyak 33.3%, mewujudkan sekitaran yang lebih hijau dan selamat.

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AZA	Azadirachtin
CMCs	Carboxymethyl Chitosan
DACMCs	Deoxycholic Acid Carboxymethyl Chitosan
DCMCs	*N*,*N*-Dimethylhexadecyl Carboxymethyl Chitosan
DOCA	deoxycholic acid
DSC	Differential Scanning Calorimeter
EDC	1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride
FAOSTAT	Food and Agriculture Organization of the United Nations Statistics Division
FTIR	Fourier Transform Infrared
HPLC	High Performance Liquid Chromatography
MANCID	Malaysian National Committee of ICID
MCPA	Methylphenoxyacetic acid
NHS	*N*-Hydroxysuccinimide
NMR	Nuclear Magnetic Resonance
OCMCs	Oleoyl Carboxymethyl Chitosan
PCA	Polycitric acid
PVA	Polyvinyl alcohol
TEM	Transmission Electron Microscope
TGA	Thermogravimetric Analyser
TMTD	Tetramethylthiuram disulphide
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

1.1 Research Background

Food is an essential necessity for the survival of each living organism. Agriculture is one of the main food source for human population that contribute around 99.7% of the world food production (Pimentel, 2009). According to United Nation, around one billion of people were undernourished in 2010 (Odegard & van der Voet, 2014). Hence, the increment in agricultural productivity to meet the necessity for food demands of human population is a matter of great concern for all countries (Sachdev & Cameotra, 2013).

According to the United Nations (2015), the world population already reached 7.30 billion in 2015 and will continue to increase to about 9.70 billion by the year

2050. Of all the continents, Asia has the highest human population with the percentage of 60.31% (4.30 billion) followed by Africa with 15% (1.00 billion) of the human population. Third is Europe with 11% (733.00 million) of world's population whereas Latin American and Caribbean regions both consist of 9% (600.00 million) of human population. Meanwhile, United States of America and Canada both are estimated with 5% (352.00 million) human population. Oceania, has the least human population with 0.50% (35.00 million) (Msangi, 2014). The rapid escalation in human population has forced food production to be increased in order to cope with the global population growth.

Table 1.1 presents the human population of several countries around the world. Based on the data by WHO (2014), China has the highest human population throughout the years followed by India where the human population were more than 1.20 billion in each country. According to Msangi (2014), both human populations in China and India contribute around 37% of the world's population. Meanwhile, United States, Indonesia and Brazil are in the top five countries that have high human population in the world with more than 1.90 million people. Although Brunei has the least human population as compared to other countries, the population growth shows a steady increment over the years. Overall, each country showed a continuous increase in human population from 2010 to 2013.

The increase pattern of human population observed in every country has upsurge the demand for food. Although food supply has generally afford human with safe and healthy food, the food production is still outrun the population growth (Chen, Shi, Sivakumar, & Peart, 2016). Several problems such as hunger and malnutrition could occur and people could also predispose themselves to contagious diseases (Pimentel, 2009). Furthermore, the reduction in manufacture of food in agricultural sector could force other food industry to produce canned and junk food. Consequently, people could undergo health complication such as undernutrition, overweight and chronic diseases due to high amount of saturated fat, free sugar and sodium that the canned and junk food possess (Herforth & Ahmed, 2015). Therefore, it is necessary to increase the current levels of food production to provide human with a better quality of food.

Table 1.1

Human population for selected countries (2010-2013)

	Total population (in thousands)			
Country	2010	2011	2012	2013
China	1,370,000	1,380,000	1,390,000	1,393,337
India	1,210,000	1,220,000	1,240,000	1,252,140
United States	312,000	315,000	318,000	320,051
Indonesia	241,000	244,000	247,000	249,866
Brazil	195,000	197,000	199,000	200,362
Thailand	66,402	66,576	66,785	67,010
United Kingdom	62,066	62,427	62,783	63,136
Malaysia	28,276	28,759	29,240	29,717
Singapore	5,079	5,192	5,303	5,412
Brunei	401	407	412	418

Note. Adapted from *World Health Organization (WHO)*, 2014.

Agriculture industry possess many challenges due to climate change, bio-energy and land degradation (Msangi, 2014). The restraint in fertile land, water, and other resources as well as to harvest good quality of crop yield have caused an immense pressure on the agriculture sector (Specht et al., 2013). Furthermore, around 35% of crop yield worldwide has lost due to pre-harvest pests. In this context, the use of pesticide in agriculture to meet the dietary demand of public is absolutely unavoidable. The evidence of utilisation of pesticide as a crucial tool to retain and enhance the crop production was inevitable (Popp, Peto, & Nagy, 2013).

1.1.1 Definition of Pesticides

Pesticides work by killing the targeted organisms, interfered using a specific variation of biochemical and physiological procedures found in the large scope of living organisms (Pretty, 2005). Pesticides names are originated from the Latin or scientific name which depend on their function against the target organisms. Most of the pesticides have the ending or suffix *–cide* which represent kill or killer. However, some of the pesticides have different names rather than end with *–cide*. For instance, growth regulators (activate or hinder the development of pests) and defoliants (stimulate plants to drop off its leaves), pesticides encompasses a diverse type which consist of insecticides, herbicides, fungicides, nematocides, rodenticides and many others that are being classified by their targeted pests. Pesticides could also be classified by their chemical identity which share mutual class of compounds (The University of Arizona, 2000).

World Health Organization (WHO) was first to develop a simple classification system for pesticides, and sanctioned by its 28th World Health Assembly in 1975. In 1978, after the guidelines were early issued, these guidelines were being reviewed followed by reissued within the intervals of two-years since then. Table 1.2 shows classification of pesticides by WHO. The classification was based on acute risks to human health including the possible outcome of single or morefold exposures at a period of time. The tests were done on rats in order to measure the acute oral and dermal toxicity of the products as this is a standard procedure in toxicology. These are measured by the LD_{50} value, a statistic approximation on the amounts of mg of toxicant per kg of bodyweight needed to destroy 50 percent of a large species on the animal evaluation (Pretty, 2005).

WHO classified pesticides into four risk categories, namely Class Ia (extremely hazardous), Class Ib (highly hazardous), Class II (moderately hazardous) and Class III (slightly hazardous). For example, the active ingredients of cholinesterase-inhibiting pesticides often categorised by WHO as Class Ia, Ib or II. In several developed countries, Class Ia and Ib are either banned or restricted.

Table 1.2

Classification of pesticides by WHO

Class of pesticide	LD$_{50}$ for rat (mg/kg body weight)			
	Oral		Dermal	
	Solids[1]	Liquids	Solids	Liquids
Ia–extremely hazardous	<5	<20	<10	<40
Ib – highly hazardous	5-50	20-200	10-100	40-400
II–moderately hazardous	50-500	200-2000	100-1000	400-4000
III– slightly hazardous	>500	>2000	>1000	>4000

Note. [1] This refers to the physical state of the active ingredient. Adapted from *Pretty*, 2005; *IPCS*, 2002.

WHO does not provide specific symbols to aid farmers or other pesticide users

to determine these classes in its recommendations. However, it does state Class Ia and Ib types must possess a symbol to indicate the pesticides are highly hazardous which commonly symbolised as a skull and cross-boned with a keyword, such as poison or toxic.

Even though the organic sector has established many effective agricultural systems across the world that are not reliable to synthetic pesticides most of the farmers are still utilised several pesticides to their crops. However, the majority of pesticides are categorised as Class Ia, Ib and II which have significant effect on human and environment.

1.1.2 History of Pesticides

Pesticides have already been used for such a long time to regulate pests and ailments in the agricultural industry (Pretty, 2005). In 2500 BC sulphur compounds were applied by Sumerians for pest regulation, followed by plenty of natural organic materials by Chinese farmers to prevent against any types of pests. Inorganic mercury and arsenic compounds were also be used to regulate body lice (Pretty, 2005).

In the 1600s, nicotine was utilised as a natural pesticide followed by the discovery of the wood preservative properties of mercuric oxide in the early 1700s, and the fungicidal properties of copper sulphate in the early 1800s. In mid-19th century, rotenone derived from the roots of derris and pyrethrum from chrysanthemum flowers had been explored, and were associated by a rapid growth in the use of inorganic products, especially arsenic (Pretty, 2005).

The early years of the 20th century seen an increment in the utilisation of many hazardous products derived from arsenic, cyanide and mercury. This has brought public concern regarding the residues of these products on fruits and vegetables. However, over the time, pesticide products have tended to become less broad-ranging in their effects and are focusing toward the targeted pests, weeds or ailments (Pretty, 2005).

1.1.3 Amount of Pesticides Used

Around two million tonnes of pesticides are used worldwide annually, of which 45% in Europe, 24% in USA and the remaining 25% are used in other countries (Yadav et al., 2015). Most of the developed countries such as North America, Western Europe and Japan consumed three quarters of the total pesticide utilised worldwide.

The consumption of pesticides in three consecutive years (2010-2012) in several countries is given in Table 1.3. As compared to eight other countries, Brazil used the highest amount of pesticides with more than 300,000 tonnes. De Siqueira et al. (2010) stated that almost 50% of the agricultural pesticides used in Latin America was from Brazil. In Brazil, pesticides such as deltamethrin, fenitrothion, fipronil and sulfluramid were used to combat leaf-cutting ants and termites (Zanuncio et al., 2016). Meanwhile, Mexico was in the second place with 110,00 tonnes of pesticides used within the three consecutive years. Mexico was ranked in sixth place worldwide for the utilisation of dichlorodiphenyltrichloroethane (DDT) in agricultural sector (Li & Macdonald, 2005). Chlordane and Lindane have been used for a long time in Mexico as agriculture insecticides to control termite and ectoparasite on cattle and other animals (Alegria et al., 2008).

In Malaysia, around 6% of the entire Malaysian annual export comes from the chemicals industry (Lee, Mokhtar, Goh, Singh, & Chan, 2015). Majority of the farmers in Malaysia used herbicides to combat weed problems, which has been regarded as one of the most severe issues encountered in agriculture field in Malaysia (Sapari & Ismail, 2012). For instance, herbicides such as 2,4-dichlorophenoxy(2,4-D), 2-methyl-4-chlorophenoxyacetic acid (MCPA), pyrazosulfuron, bensulfuron, and metsulfuron were applied by the farmers in the rice fields of the Alor Setar district, Kedah (Ismail, Prayitno, & Tayeb, 2015). Meanwhile, Netherlands consumed least amount of pesticides than other countries with less than 8,000 tonnes. Similar to Malaysia, the usage of herbicides was found highest in Netherlands. According to Pan, Jiang, and Kuil (2012) about 92% of herbicides were used on the arable fields in Netherlands. Although the usage of pesticides worldwide was still considered high, other alternatives have also been taken into measure in order to reduce the pesticide impacts.

Table 1.3

Domestic consumption of pesticides in selected countries (2010-2012)

Country	Amount pesticides used (tonnes)		
	2010	2011	2012
Brazil	311,591	305,882	308,882
Mexico	113,880	118,649	116,478
Thailand	67,581	85,259	69,418
Japan	55,042	51,284	54,157
Malaysia	59,944	41,369	49,674
Germany	27,585	29,109	29,735
United Kingdom	13,876	13,507	14,293
China	7,769	8,198	9,332
Netherlands	7,890	7,566	7,995

Note. Adapted from *Food and Agriculture Organization of the United Nations Statistics Division (FAOSTAT)*, 2015.

In the past 50 years, the global utilisation of pesticides in agriculture had increased rapidly to about 2.56 billion kg annually which the market value cost around US$25-30 billion in the 1990s and 2000s. Around US$3 billion of sales are from the developing countries (CropLife, 2003) which include herbicides account for 37% of sales, insecticides 25% fungicides 22% and other 28% between 1998 and 1999 (Pretty, 2005).

Pesticides can be categorised as herbicides, insecticides, fungicides, bactericides, nematicides, molluscicides, rodenticides, and plant growth hormones (Mahmood, Bilal, & Jan, 2014). Each type of pesticide works differently. For instance, insecticides are used to control pests and insects (Pang, 2014). Meanwhile, herbicide and fungicide are applied in order to kill weeds and inhibit fungi or fungi spores, respectively (Mahmood et al., 2014; Leadbeater, 2014). Without pesticides usage the productivity of the world crop could decrease as infestation of agriculture pests or diseases would be much more severe (Willers, DeFauw, English, & Jenkins, 2014). Such problems would cause an economic loss estimated around $ 500 billion per annum and the reduce in the world's food supply (Willers et al., 2014).

Table 1.4 shows the usage of several type of pesticides in three consecutive years from 2010 to 2012. According to FAO, the highest type of pesticides used in 2010 were fungicides and bactericides. Meanwhile in 2011 and 2012, herbicides were highly consumed. The amount of each type of pesticide utilised worldwide exhibited a decrease pattern during the three years. This primarily due to diverse development in agricultural systems in recent years (Willers et al., 2014). For instance, current practices such as biotechnology and bioengineering expose farmers to latest