UNIVERSITI PUTRA MALAYSIA

FORMULATION OF NANOEMULSIONS ENCAPSULATED WITH POTENTIAL ANTICANCER DRUG, BETULINIC ACID

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FS 2014 7

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By

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Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

January 2014

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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January 2014

Chairman : Assoc. Prof. Intan Safinar Ismail, PhD

Faculty : Science

The betulinic acid provided was recrystallized in order to obtain high purity compound and was confirmed by spectroscopic analysis. Betulinic acid was incorporated in the oil phase prior to the construction of ternary phase diagram. Phase behaviours of soybean oil and non-ionic surfactants were determined through the construction of ternary phase diagrams. The phase behaviours were affected by hydrophilic-lipophilic balance (HLB) value of surfactants. Higher HLB values produced larger one-phase regions: homogenous and isotropic, in ternary phase diagrams of soybean oil/non-ionic surfactant/deionized water and soybean oil/non-ionic surfactant-co-surfactant/deionized water. The largest one-phase regions were formed by soybean oil/Cremophor EL-Span 20/deionized water formulation.

A few compositions with 70% water content were selected on the ternary phase diagram of soybean oil/Cremophor EL/deionized water system as the formulation of emulsions. The selected compositions were 15:15:70, 18:12:70, 21:9:70 and 24:6:70. The first set of emulsions was prepared via low-energy emulsification method, while the other set was formulated via high-energy emulsification method using a high-pressure homogenizer with homogenizing cycle of 2, 4, 6 and 8. Characteristics of emulsions were studied. The average particle size of low-energy formulated emulsions was larger than 130 nm at week 1 and the size increased rapidly throughout 12-weeks of study while for emulsions formulated via 8 homogenizing cycles, the average particle size was below 57 nm at week 1 and remained below 100 nm after 12-weeks. Formulation of 24:6:70 produced the smallest average size which was 59 nm.

The surface charge values for all formulations with betulinic acid were more negative than -26.7 mV which indicates moderate stability of the emulsions. The stability of

emulsions was also studied via visual observation for 6 months. All high-energy formulated emulsions were still in one phase without any separation of layers observed. The pH values were between 3.9 to 4.1 for all formulations. Betulinic acid can still be detected by HPLC-RI detector in the selected 24:6:70 formulation even after 6 months of storage.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENGHASILAN NANOEMULSI YANG MENGANDUNGI UBAT ANTI KANSER YANG BERPOTENSI, ASID BETULINIK

Oleh

NUR NADIAH BINTI ABDUL RASHID

Januari 2014

Pengerusi : Prof. Madya Intan Safinar Ismail, PhD

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Asid betulinik yang disediakan telah melalui proses pengkristalan semula bagi mendapatkan asid betulinik yang lebih tulen. Ia dianalisis menggunakan FTIR dan NMR bagi memastikan ianya adalah asid betulinik. Asid betulinik dilarutkan ke dalam fasa minyak sebelum penghasilan rajah tiga fasa. Ciri-ciri fasa minyak kacang soya dan surfaktan tidak berion dikaji dan ditentukan dengan menggunakan rajah tiga fasa. Ciri-ciri ini dipengaruhi oleh nilai keseimbangan komponen hidrofilik dan lipofilik (HLB) surfaktan. Nilai HLB yang lebih tinggi menghasilkan kawasan satu fasa iaitu homogen dan isotropik yang lebih besar pada rajah tiga fasa formulasi minyak kacang soya/surfaktan tidak berion/air dinyah ion. Rajah tiga fasa formulasi minyak kacang soya/Cremophor EL-Span20/air dinyah ion menghasilkan fasa homogen dan isotropik terbesar.

Formulasi emulsi berkomposisi 15:15:70, 18:12:70, 21:9:70 dan 24:6:70 telah dipilih pada rajah tiga fasa minyak kacang soya/Cremophor EL/air dinyah ion bagi kajian lanjutan. Emulsi disediakan melalui dua kaedah: pengemulsian bertenaga rendah dan pengemulsian bertenaga tinggi yang menggunakan instrumen penghomogenan bertekanan tinggi dengan kitaran penghomogenan sebanyak 2, 4, 6 dan 8. Purata saiz partikel bagi emulsi yang desediakan dengan menggunakan kaedah pengemulsian bertenaga rendah adalah lebih besar dari 130 nm pada minggu pertama dan meningkat secara mendadak sepanjang kajian selama 12 minggu. Bagi emulsi yang dihasilkan melalui kaedah bertenaga tinggi, purata saiz partikel adalah lebih kecil dari 57 nm pada minggu pertama dan kekal di bawah saiz 100 nm selepas 12 minggu kajian. Formulasi 24:6:70 dengan 8 kitaran penghomogenan menghasilkan purata saiz partikel paling rendah iaitu 59 nm selepas 12 minggu.

Bacaan cas permukaan bagi semua formulasi adalah lebih negatif dari -26.7 mV, di mana ia membuktikan kestabilan formulasi yang sederhana. Kestabilan emulsi juga dikaji melalui pemerhatian fizikalselama 6 bulan. Kesemua emulsi yang dihasilkan melalui kaedah bertenaga tinggi masih berada dalam satu fasa selepas 6 bulan dan tiada pengasingan lapisan. Nilai pH bagi kesemua formulasi adalah di antara 3.9 hingga 4.1. Asid betulinik di dalam emulsi 24:6:70 masih dapat dikesan dengan menggunakan HPLC-RI selepas penyimpanan selama 6 bulan.

ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to Allah S.W.T for the opportunity and strength given in completing this study. Million appreciations to my helpful supervisor, Assoc. Prof. Dr. Intan Safinar Ismail for all the guidance and knowledge given throughout this hard-earned success but yet beautiful journey. Thank you for all the patience and advice. My appreciation also goes to my former supervisor, Prof. Dr. Faujan bin Ahmad who has joined Universiti Teknologi Mara recently for initiating the ideas and giving full support in starting the whole research. Not to be missed, I would also like to thank my co-supervisors, Dr. Siti Salwa Abd Gani and Assoc. Prof. Dr. Latifah Saiful Yazan for sparing their time for discussions, encouragement and moral supports.

Special thanks to the staffs of the Department of Chemistry, Faculty of Science and Institute of Bioscience (IBS), Universiti Putra Malaysia for providing the facilities and lending their hands in this research. I would like to thank Ms. Nur Diyana Syamim, research assistant from Faculty of Food Science and Technology for analyzing my samples and providing information on my chromatograms. To my housemates, labmates and other master and PhD students who have helped me directly or indirectly, thank you.

My endless appreciation to my family especially my parents; Dr. Abdul Rashid bin Baba and Mrs. Halilah binti Midon for supporting me this whole time with patience. To my dearest husband, Dr. Hijaz bin Hj Ridzwan, my true supporter, who picked me up when I was down; and to my twin boys, my little cheerers: Aiman Yusuf and Adam Mikail, I cannot thank all of you enough.

Last but not least, I would like to acknowledge Universiti Putra Malaysia and Ministry of Higher Education (MOHE) for the financial support via GRF scholarship and MyMaster under MyBrain15 program respectively.

I certify that a Thesis Examination Committee has met on 28 January 2014 to conduct the final examination of Nur Nadiah binti Abdul Rashid on her thesis entitled "Formulation of Nanoemulsions Encapsulated with Potential Anticancer Drug Betulinic Acid" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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LIST OF ABBREVIATIONS

BA	betulinic acid
DLS	dynamic light scattering
FTIR	Fourier transform infrared
HIV	human immunodeficiency virus
HLB	hydrophilic-lipophilic balance
IR	infrared
MOPI	Malaysian Organization of Pharmaceutical Industries
o/w	oil-in-water
o/w/o	oil-in-water-in-oil
PCCS	Photon cross correlation spectroscopy
PIT	phase inversion temperature
RI	refractive index
Span 20	sorbitan mono-laurate
Tween 80	polyoxyethylene(20) sorbitan mono-oleate
w/o	water-in-oil
w/o/w	water-in-oil-in-water

CHAPTER 1

INTRODUCTION

Pharmaceutical products or more commonly known as medicines or drugs are a fundamental components of both modern and traditional medicines. It is essential that such products are safe, effective, of good quality, and are prescribed and used rationally. The worldwide pharmaceuticals market growth is accelerating in this 21st century as the number of demand from consumer increases. This is due to increment of number of patients for all sorts of illnesses including cancer. Cancer is currently a fast recurring illness among men and women. According to World Health Organization (WHO), the global cancer rates could increase by 50% to 15 million by 2020. In many countries, more than a quarter of deaths are attributable to cancer.

One defining feature of cancer is the rapid creation of abnormal cells that grow beyond their usual boundaries, and which can then invade adjoining parts of the body and spread to other organs. Statistically, there were 7.6 million people worldwide died because of cancer. Approximately 70% of cancer deaths occur in low and middle income countries (World Health Organization, 2011). World Cancer Report provides clear evidence that action on smoking, diet and infections can prevent one third of cancers and another one third can be cured by the modern treatments.

Betulinic acid has attracted the interests of researchers due to its variety of biological and pharmacological activities. It can be easily extracted from barks of huge trees. Betulinic acid is a naturally occurring pentacyclic triterpenoid which exhibits the anticancer, anti-HIV, antibacterial, antimalarial and anthelmintic activities. In addition, it is also reported to exhibit analgesic and anti-inflammatory properties (Fulda & Debatin, 2000; Yogeeswari & Sriram, 2005).

In these modern days, pharmaceutical products in the form of emulsions have been increasing in numbers. The main concern about emulsions is regarding its stability. According to Tadros in 2005, emulsions are thermodynamically stable. Emulsions with small particle size, generally below 500 nm are called as nanoemulsions. The idea of nanoemulsions formations has caught the attentions of industries due to its small average particle size. This small particle size property contributes to the improvement of drug stability and absorption in human's body. Nanoemulsions are commonly used as drug carrier for active ingredients. It has been suggested that the encapsulation of poor-water soluble agents such as betulinic acid in nanoemulsions can improve the solubility.

Nanoemulsions, which have an average droplet size of 20 to 200 nm, have the ability to penetrate the membranes and have higher chances of reaching the targeted areas and

improve absorption of the active ingredients. The physical appearance of nanoemulsions is translucent but it depends on the materials used in the formulation. This property is due to the fact that light waves are scattered by the droplets.

The basic compositions of nanoemulsions formation are water, surfactant and oil or ester. The purpose of surfactant is to lower the surface tension of a liquid or the interfacial tension between two different liquids. Nanoemulsions can be successfully formed through high-energy emulsification method. In this research, the high-energy emulsification method used is high-pressure homogenization. Before the formulation undergoes high-pressure homogenization process, the emulsions are initially formulated through low-energy emulsification method which involves the stepwise addition of water to oil-surfactant mixture or stepwise addition of oil to water-surfactant mixture and mixed vigorously using vortex mixer.

Problem Statements

Betulinic acid has been discovered as an anticancer agent for more than a decade. The main disadvantage of betulinic acid is the poor water-solubility property. Human's body consist of more than 55 % of water, which relates to the lower efficiency of betulinic acid in one's body. In contrast, betulinic acid has higher solubility property in oil and lipid phase. To combat solubility problem, betulinic acid is solubilised in oil-phase which is soybean oil, prior to the formulation of emulsion. Emulsions with large particle size are often related to low stability. In order to form small particle size emulsions with high stability, alternative preparation methods were used.

Objectives

i. To construct the ternary phase diagram of soybean oil/non-ionic surfactant/deionized water and soybean oil/non-ionic surfactant-co-surfactant/ deionized water.
ii. To study the phase behaviour of the constructed phase diagrams and select the compositions based from the ternary phase diagram for formulation of nanoemulsion.
iii. To formulate nanoemulsion as drug carrier with encapsulation of betulinic acid based on soybean oil.
iv. To characterize the formulations through the stability study, particle size, zeta potential, pH value and drug analysis.

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