UNIVERSITI PUTRA MALAYSIA

FORMULATION OF NANOEMULSIONS ENCAPSULATED WITH POTENTIAL ANTICANCER DRUG, BETULINIC ACID

NUR NADIAH BINTI ABDUL RASHID

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FORMULATION OF NANOEMULSIONS ENCAPSULATED WITH POTENTIAL ANTICANCER DRUG, BETULINIC ACID

By

NUR NADIAH BINTI ABDUL RASHID

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

FORMULATION OF NANOEMULSIONS ENCAPSULATED WITH
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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

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Januari 2014

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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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<td>betulinic acid</td>
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<tr>
<td>DLS</td>
<td>dynamic light scattering</td>
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<td>FTIR</td>
<td>Fourier transform infrared</td>
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<td>HIV</td>
<td>human immunodeficiency virus</td>
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<td>HLB</td>
<td>hydrophilic-lipophilic balance</td>
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<td>IR</td>
<td>infrared</td>
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<td>MOPI</td>
<td>Malaysian Organization of Pharmaceutical Industries</td>
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<td>o/w</td>
<td>oil-in-water</td>
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CHAPTER 1

INTRODUCTION

Pharmaceutical products or more commonly known as medicines or drugs are 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 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 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 nanoemulsions.

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.
REFERENCES


