FORMULATION AND CHARACTERIZATION OF GINGER-LOADED NIOSOME

ALI BAGHERI

A dissertation submitted in partial fulfillment of the requirements for the award of the degree of Master of Engineering (Chemical)

Faculty of Chemical Engineering
Universiti Teknologi Malaysia

JULY 2013
Dedicated to my beloved parents for their support and encouragement
ACKNOWLEDGEMENT

All praise to Almighty Allah for His blessing and mercy enables me to complete my master thesis.

I am deeply indebted to my supervisor, Dr. Harisun Ya’akob, for always being by my side to encourage and help me out for every obstacle I faced throughout finishing my research.

I would like to extend my deepest thanks to Dr. Umi Aishah Asli as my Co-supervisor and Dr. Chu Boo Soong for providing very helpful advices and definitely without their help I was not able to go through challenges ahead of me. I appreciate kindness and generous cooperation of lab assistants and students in giving information and helping me throughout last year.

Last but not least, my beloved family, that never gives up on supporting me in whole of my life.
ABSTRACT

Ginger oil is well known for its potential as a bioactive phytochemical and long history of medicinal use with antioxidant, anti-obesity and cancer preventive activity. Although many herbal drugs have potential to promote health in vitro findings they have shown less or no in vivo actions due to their poor solubility or improper molecular size or both, ultimately resulting in poor bioavailability and poor absorption which are the major limiting factors of development herbal drugs. Niosome as a lipid-based carrier system with remarkable advantages over conventional drug delivery systems was used as a carrier in this study in order to get the benefits of both herb and carrier. Therefore, the main objective of this study was to formulate the ginger oil-loaded niosome. Firstly, pre-formulation studies were carried out in order to investigate the feasibility of encapsulation ginger oil as a lipophilic active compound. All niosome formulations were prepared by the film hydration method and characterized for drug entrapment efficiency (EE) and particle size (PS). Niosome formulations were optimized with using Response Surface Methodology (RSM) based on Central Composite Design (CCRD). Interaction of two formulation variables, namely amount of Span 60 (x1) and Labrasol (x2) which have great influence on particle size and entrapment efficiency were studied. Labrasol was determined as a key factor responsible for entrapment efficiency which increasing concentration of Labrasol from 0.45 (mM) to 2.5 (mM) has decreased around 10 % of entrapped drug. In conclusion, the niosomes were successfully able to encapsulate ginger oil with high entrapment efficiency which can be useful in developing more effective use of ginger oil for human health.
ABSTRAK

Minyak halia terkenal mempunyai potensi bioaktif fitokimia dan mempunyai sejarah penggunaan perubatan seperti antioksida, anti-obesiti, dan pencegahan aktiviti kanser. Melalui kaedah in vitro, pelbagai herba menunjukkan potensi memberansangkan dalam menggalakkan kesihatan, namun ia tidak dapat ditemui melalui kaedah in vivo yang disebabkan oleh kelarutan yang rendah atau kelemahan saiz molekul atau kedua-duanya sekali. Keadaan ini menyebabkan bioavailibiti dan penyerapan yang lemah seterusnya menjadi faktor penghalang utama dalam pembangunan ubatan herba. Niosome yang mempunyai kelebihan berbanding sistem penghantaran ubatan konvensional telah digunakan sebagai agen pembawa dalam kajian ini untuk mengekplotiasi kelebihan bagi herba dan pembawa. Oleh itu, objektif utama dalam kajian ini adalah untuk memformulakan niosome yang menyelaputi minyak halia. Kajian pra-formulasi dijalankan untuk menyiapkan kebolehan menyelidik keboleh kebolehkan penyeludupan minyak halia sebagai komponen aktif yang bersifat lipophilik. Semua formula niosome telah disediakan dengan kaedah hidrasi filem dan dikategorikan untuk keberkesanan pemerangkapan herba (EE) dan saiz partikel (PS). Formula niosome telah dioptimumkan dengan menggunakan Response Surface Methodology (RSM) berdasarkan Central Composite Design (CCRD). Interaksi antara dua formula pemboleh hubabai iaitu jumlah Span 60 (X1) dan Labrasol (X2) yang mana mempengaruhi saiz partikel dan keberkesanan pemerangkapan telah dikaji. Labrasol ditemui sebagai faktor utama yang memberi kesan terhadap keberkesanan pemerangkapan yang mana peningkatan dalam kepekatan Labrasol dari pada 0.45 (mM) kepada 2.5 (mM) akan mengurangkan herba yang terperangkap sebanyak 10 %. Kesimpulannya, niosome berjaya menyelaputi minyak halia dengan pemerangkapan herba yang tinggi yang mana hasil kajian ini boleh digunakan dalam membangunkan pengunaan halia secara efektif dan berkesan untuk kesihatan..
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>CHAPTER</th>
<th>TITLE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>DECLARATION</td>
<td>i</td>
<td>ii</td>
</tr>
<tr>
<td>DEDICATION</td>
<td>iii</td>
<td></td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>iv</td>
<td></td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>v</td>
<td></td>
</tr>
<tr>
<td>ABSTRAK</td>
<td>vi</td>
<td></td>
</tr>
<tr>
<td>TABLE OF CONTENTS</td>
<td>vii</td>
<td></td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>xii</td>
<td></td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xiii</td>
<td></td>
</tr>
<tr>
<td>LIST OF ABBREVIATIONS</td>
<td>xv</td>
<td></td>
</tr>
<tr>
<td>LIST OF APPENDIX</td>
<td>xvi</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>1.1</td>
<td>Introduction</td>
<td>1</td>
</tr>
<tr>
<td>1.2</td>
<td>Research Background</td>
<td>2</td>
</tr>
<tr>
<td>1.3</td>
<td>Problem Statement</td>
<td>3</td>
</tr>
<tr>
<td>1.4</td>
<td>Hypothesis</td>
<td>4</td>
</tr>
<tr>
<td>1.5</td>
<td>Objectives of the study</td>
<td>5</td>
</tr>
</tbody>
</table>
1.6 Scopes of the study 5

2 LITERATURE REVIEW 6

2.1 Phytochemicals and Potential Benefits for Human Health 6

2.2 Limitations of Using Phytochemicals for Medical Purposes 7

2.3 Importance of Novel Drug Delivery Systems for Phytochemicals 8

2.4 Ginger and Pharmaceutical Properties 9

2.5 Novel Drug Delivery Systems 11

2.5.1 Novel Drug Delivery System for Herbal Formulations 12

2.5.2 Lipid–Based Drug Delivery Systems 14

2.6 Vesicular Systems 15

2.6.1 Classification of Vesicular Systems 16

2.7 Niosomes 17

2.7.1 Potential of Niosome as a Novel Drug Delivery System for Herbal Formulations 18

2.7.2 Structure of Niosomes 20

2.7.3 Significant Features and Advantages of Niosomes as a Drug Delivery System 21

2.7.4 Niosomes Formulation; Components and Their Effects 24

2.7.4.1 Nonionic Surfactants 25

2.7.4.2 Nonionic Surfactants Classification and Advantages of Span 60 26

2.7.4.3 Nonionic Surfactant Properties Influencing Niosome Formulation 27
2.7.4.4 Hydration Medium 28
2.7.4.5 Use of Cholesterol or Other Lipids and Their Impact 29
2.7.4.6 Surfactant and lipid amount 30
2.7.4.7 Effect of encapsulated drug 30

2.8 Methods of Preparation 31
  2.8.1 Ether Injection 31
  2.8.2 Lipid Layer Hydration 32

2.9 Characterization of Niosomes 32
  2.9.1 Vesicle structure and shape 32
  2.9.2 Vesicle Size, Size Distribution and Surface Charge 32
  2.9.3 Entrapment Efficiency 33

2.10 Design Expert 34
  2.10.1 Response Surface Method (RSM) Overview 35
  2.10.2 Central Composite Rotatable Design 36

3 RESEARCH METHODOLOGY 37

  3.1 Introduction 37
  3.2 Research activities 38
  3.3 Chemicals 39
  3.4 Preparation of Niosome 39
  3.5 Experimental designs 42
  3.6 Determination of Entrapment Efficiency %EE 45
  3.7 Determination of Vesicle Diameter 46
RESULTS AND DISCUSSION

4.1 Introduction

4.2 Screening the Process Conditions in Ginger Oil-Loaded Niosome

4.2.1 Solvent System

4.2.2 Hydration Type, Volume and Time

4.2.3 Hydration and Thin Layer Formation

4.2.4 Rotational Speed of Evaporator Flask in Thin Layer Formation Step

4.2.5 Rotational Speed of Evaporator Flask in Hydration Step

4.3 Size Reduction Process

4.4 HPLC Analysis for Ginger Oil

4.5 Effects of Ingredients on Ginger Oil Loaded Niosome

4.6 Formulation conditions optimization

4.6.1 Experimental Design and Actual Responses the Experiments

4.6.2 Analysis and Fitting the Model for Two Actual Responses

4.6.3 Effects of Variables and Their Interactions on Particle Size (PS)

4.6.4 Effects of Variables and Their Interactions on the EE %

4.7 Impact of Total Lipid Concentration and Labrasol Content

4.8 Effect of Labrasol Content

4.9 Formulation Optimization
5 CONCLUSION 68

5.1 Project Achievements 68

5.2 Limitation of the Study and Justifications 69

5.3 Recommendations for Future Research 69

REFERENCES 71

Appendix 77
## LIST OF TABLES

<table>
<thead>
<tr>
<th>TABLE NO.</th>
<th>TITLE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Different vesicular systems and their principal components</td>
<td>16</td>
</tr>
<tr>
<td>2.2</td>
<td>HLB value Impact on formulation</td>
<td>28</td>
</tr>
<tr>
<td>2.3</td>
<td>Methods of separation of entrapped drug</td>
<td>34</td>
</tr>
<tr>
<td>3.1</td>
<td>Values of independent variables at different levels of the CCRD design</td>
<td>44</td>
</tr>
<tr>
<td>3.2</td>
<td>Experimental design of central composite rotatable design</td>
<td>44</td>
</tr>
<tr>
<td>4.1</td>
<td>Process Parameters For Thin Layer Hydration Technique</td>
<td>50</td>
</tr>
<tr>
<td>4.2</td>
<td>Niosomes pre-formulation composition</td>
<td>54</td>
</tr>
<tr>
<td>4.3</td>
<td>Entrapment efficiency of prepared ginger oil niosome</td>
<td>55</td>
</tr>
<tr>
<td>4.4</td>
<td>Values of independent variables at different levels of the CCRD design</td>
<td>56</td>
</tr>
<tr>
<td>4.5</td>
<td>ANOVA Based on the Quadratic Model for the PS Response</td>
<td>57</td>
</tr>
<tr>
<td>4.6</td>
<td>ANOVA Based on the Quadratic Model for the EE Response</td>
<td>57</td>
</tr>
<tr>
<td>4.7</td>
<td>Observed and predicted values of PS and EE%</td>
<td>58</td>
</tr>
<tr>
<td>4.8</td>
<td>Constraints of each variable for the numerical optimization of the EE%</td>
<td>66</td>
</tr>
<tr>
<td>4.9</td>
<td>Optimal conditions for maximal EE%</td>
<td>67</td>
</tr>
</tbody>
</table>
# LIST OF FIGURE

<table>
<thead>
<tr>
<th>FIGURE NO.</th>
<th>TITLE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Ginger and Ginger Oil</td>
<td>11</td>
</tr>
<tr>
<td>2.2</td>
<td>Structure of Niosomes</td>
<td>20</td>
</tr>
<tr>
<td>2.3</td>
<td>Entrapment of drugs in the structure of niosome according to its nature</td>
<td>21</td>
</tr>
<tr>
<td>2.4</td>
<td>Targeted drug delivery</td>
<td>22</td>
</tr>
<tr>
<td>2.5</td>
<td>Increased bioavailability</td>
<td>22</td>
</tr>
<tr>
<td>2.6</td>
<td>Sustained release</td>
<td>23</td>
</tr>
<tr>
<td>2.7</td>
<td>Protection of Drug</td>
<td>24</td>
</tr>
<tr>
<td>4.1</td>
<td>The photomicrograph (×40) of sample after downsizing</td>
<td>51</td>
</tr>
<tr>
<td>4.2</td>
<td>Results from laser particle analysis (a) Sample before size reduction (b) After downsizing</td>
<td>5</td>
</tr>
<tr>
<td>4.3</td>
<td>HPLC data of Ginger Oil</td>
<td>53</td>
</tr>
<tr>
<td>4.4</td>
<td>Mean vesicle size versus molar ratio</td>
<td>54</td>
</tr>
<tr>
<td>4.5</td>
<td>The photomicrographs (×40) of ginger oil loaded niosome F2</td>
<td>55</td>
</tr>
<tr>
<td>4.6</td>
<td>Comparison between the predicted and actual response of PS</td>
<td>59</td>
</tr>
<tr>
<td>4.7</td>
<td>Comparison between the predicted and actual response of EE%</td>
<td>59</td>
</tr>
<tr>
<td>4.8</td>
<td>Normal Probability Plot of the Residuals for PS</td>
<td>60</td>
</tr>
<tr>
<td>Section</td>
<td>Description</td>
<td>Page</td>
</tr>
<tr>
<td>---------</td>
<td>-----------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>4.9</td>
<td>Normal Probability Plot of the Residuals for EE</td>
<td>61</td>
</tr>
<tr>
<td>4.10</td>
<td>Outlier T Plot for PS</td>
<td>61</td>
</tr>
<tr>
<td>4.11</td>
<td>Outlier T Plot for EE%</td>
<td>62</td>
</tr>
<tr>
<td>4.12</td>
<td>Three-dimensional response surface plot representing the effect of the interaction between Span 60 and Labrasol amount on particle size</td>
<td>63</td>
</tr>
<tr>
<td>4.13</td>
<td>Three-dimensional response surface plot representing the effect of the interaction between the Span 60 and Labrasol amount on entrapment efficiency.</td>
<td>64</td>
</tr>
</tbody>
</table>
LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>PS</td>
<td>Particle Size</td>
</tr>
<tr>
<td>EE</td>
<td>Entrapment efficiency</td>
</tr>
<tr>
<td>Span 60</td>
<td>Sorbitan monostearate</td>
</tr>
<tr>
<td>CCRD</td>
<td>Central composite rotational design</td>
</tr>
<tr>
<td>RSM</td>
<td>Response surface methodology</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffer saline</td>
</tr>
<tr>
<td>DOE</td>
<td>Design of experiment</td>
</tr>
<tr>
<td>LUV</td>
<td>Unilamellar vesicles</td>
</tr>
</tbody>
</table>
# LIST OF APPENDICES

<table>
<thead>
<tr>
<th>APPENDIX</th>
<th>TITLE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>The Sample Result of HPLC for Entrapment Efficiency Determination</td>
<td>77</td>
</tr>
<tr>
<td>B</td>
<td>Standard Curve (Peak versus Concentration)</td>
<td>78</td>
</tr>
</tbody>
</table>
CHAPTER 1

INTRODUCTION

1.1 Introduction

In recent years, drug development programs focus on the natural materials, including plant bioactive due to dissatisfaction of synthetic chemical drugs for treatment of chronic diseases (Kusum et al., 2010). Use of herbal medicines has been increased due to their therapeutic effects and fewer side effects as compared to the modern medicines (Goyal et al., 2011). Ginger and its identified bioactive compounds have a long history of medicinal use. It has been known to have a strong anti-inflammatory, antioxidant and cancer preventive activities while in other literatures anti-obesity activity of ginger has been studied (Mansour et al., 2012). Therefore, ginger with various therapeutic values for human health has been investigated in recent years (Atashak et al., 2011).

Since many natural substances were discovered, substantial attention has been focused on the development of novel drug delivery system especially for bioactive compounds and herbal drugs in order to find scientific approach to deliver the components and overcoming problems associated with plant medicines. Several bioactive compounds have poor solubility and insufficient bioavailability like ginger
oil, which are common problems of new herbal drug molecules. Molecular size is another major limiting factor for herbal drug molecules to pass the biological membrane to be absorbed systematically following oral or topical administration.

Nowadays with the development in the technology, novel carrier systems opens different approach towards the development of herbal drug delivery systems since they offer remarkable advantages over conventional formulations of plant actives. Using novel delivery systems in herbal formulations has been reported with advantages such as enhancement of solubility, protection from toxicity, enhancement of pharmacological activity, enhancement of stability, bioavailability, improved tissue macrophages distribution, sustained delivery, and protection from physical and chemical degradation (Goyal et al., 2011; Kusum et al., 2010). Increasing the efficacy and reducing the side effects of herbal compounds and herbs is the basic idea behind incorporating novel method of drug delivery in herbal medicines (Kusum et al., 2010). Among different novel carriers, niosome exhibits some remarkable features that make this kind of delivery system preferable over other kind of delivery systems (Kumarn and Rajeshwarrao, 2011).

Niosome used as a carrier in this study in order to encapsulate ginger oil to obtain higher efficacy, performance and bioavailability of bioactive compounds.

1.2 Research Background

Potential features of natural products including higher reliance and safety, producing better results of natural products over synthetic drugs or surgery, high costs and potentially hazardous side effects of chemical drugs, development of natural products is one the areas of focus during last few decades. Furthermore, it has been demonstrated that the compounds derived from botanicals instead of
chemicals are more easily metabolized by the body (Kusum et al., 2010). For reasons mentioned above, a variety of natural resources, including crude extracts and isolated compounds from plants as an excellent alternative strategy for developing future cost-effective and safe drugs have been widely investigated (Löbenberg and Amidon, 2000; Ajazuddin and Saraf, 2010).

Over the past several years, novel drug delivery systems for plant actives and extracts were one of the areas of phyto-formulation research which presented remarkable advantages over conventional formulations of plant actives and extracts (Ajazuddin and Saraf, 2010). Among various novel formulation including nanocapsules, liposomes, polymeric nanoparticles, phytosomes, transferosomes microsphere, nanoemulsions and ethosomes has been reported using bioactive and plant extracts, niosomes are attracting major attention as lipid based, nano-sized and novel colloidal carrier system in past few years due to their potential features and advantages (Gangwar et al., 2012; Ramadan, 2010).

In almost all of the niosome formulations that have been studied so far, cholesterol was used as a principal component for lipid part while, Labrasol as a well-known lipid-based excipient used in different drug formulations and showed key features for oral, (trans)dermal and ocular drug delivery systems (Koga et al., 2006); (Mura et al., 2009). As a result, Labrasol can be a good alternative for cholesterol in niosome formulation since it is lipid based excipient with remarkable features.

1.3 Problem Statement

Nowadays due to dissatisfaction with high costs and potentially hazardous side-effects of available chemical drugs on the market, development of natural products especially various plant bioactive is under investigation as an alternative
approach for developing future effective and safe drugs (Moro and Basile, 2000; Finer, 2002; Colon-Gonzalez et al., 2012).

Conventional drug delivery systems for plant bioactive have been reported with some limitations. These drawbacks could be eliminated with using novel drug carrier systems such as niosomes which have been showed remarkable advantages for both herbal and chemical drugs (Ajazuddin and Saraf, 2010).

In summary, dissatisfaction of available chemical drugs for treatment of chronic diseases and drawbacks of conventional drug delivery systems are two major problems in current scientific world. Ginger oil was used as a model in encapsulation of a hydrophobic compound which has poor water solubility, poor absorption and bioavailability through skin permeation. This study was carried out to find a scientific approach to encapsulate ginger oil in niosome for the first time in order to have better therapeutic effect from ginger oil.

1.4 Hypothesis

Ginger oil which has been reported with various pharmaceutical values can be encapsulated in niosomes, as lipid based, nano-sized and colloidal herbal drug carrier system to enhance the efficacy and performance of bioactive. In this study, for the first time, based on the successes and the advantages of niosomes, encapsulation of ginger oil in niosome was carried out. Enhancement of bioavailability, stability, therapeutic values and some other features of niosome, as a novel formulation, can be achieved. It is expected to use niosome for delivery of ginger oil either for oral or topical administration in future research, so using Labrasol may leads to achieve higher intestinal absorption and bioavailability or promote drug penetration and permeation respectively.
1.5 Objectives of the study

The main objective of this study is to formulate and evaluate ginger oil-loaded niosome which was prepared by lipid layer hydration method.

1.6 Scopes of the study

In order to achieve the objective, three major scopes will be involved in this study:

1. Formulation of the ginger oil-loaded niosome.
2. Determination of main effects and interactions of variables.
3. Optimization of the formulation parameters that affect two dependent parameters, entrapment efficiency (EE %) and particle size (PS) of ginger oil-loaded niosome using Respond Surface Methodology (RSM).
LIST OF REFERENCES


