FORMULATION ANDCHARACTERIZATION 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 mengekploitasi 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 menyelidik kebolehan penyalutan 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 pembolehubah 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 daripada 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

LIST OF TABLES

LIST OF FIGURE

LIST OF ABBREVIATIONS

PS	-	Particle Size
EE	-	Entrapment efficiency
Span 60	-	Sorbitan monostearate
CCRD	-	Central composite rotational design
RSM	-	Response surface methodology
PBS	-	Phosphate buffer saline
DOE	-	Design of experiment
LUV	-	Unilammelar vesicles

LIST OF APPENDICES

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, nanoemulsionsand 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

Abdelbary, G. and El-Gendy, N. (2008). Niosome-encapsulated Gentamicin for Ophthalmic Controlled Delivery. *AAPS PharmSciTech*. 9(3), 7–17.

Abhinav, K. and Lal, P. (2011). Review on Niosomes as Novel Drug Delivery System. *International Research Journal of Pharmacy*. 2(5), 61–65.

Ajazuddin and Saraf, S. (2010). Applications of Novel Drug Delivery System for Herbal Formulations. *Fitoterapia*. 81(7), 680–9.

Aqil, F., Munagala, R., Jeyabalan, J. and Vadhanam, M. V (2013). Bioavailability of Phytochemicals and its Enhancement by Drug Delivery Systems. *Cancer letters*. 334(1), 133–141.

Atashak, S., Peeri, M., Jafari, A. and Azarbayjani, M. (2011). Effects of Ginger Supplementation and Resistance Training on Lipid Profiles and Body Composition in Obese Men. *Journal of Medicinal Plants Research*. 5(16), 3827–3832.

Barratt, G. (2000). Therapeutic Applications of Colloidal Drug Carriers. *Pharmaceutical Science & Technology Today*. 3(5), 163–171.

Bezerra, M., Santelli, R., Oliveira, E., Villar, L. and Escaleira, L. (2008). Response Surface Methodology (RSM) As a Tool for Optimization in Analytical Chemistry. *Talanta*. 76(5), 965–977.

Chen, C.-C., Tsai, T.-H., Huang, Z.-R. and Fang, J.-Y. (2010). Effects of Lipophilic Emulsifiers on the Oral Administration of Lovastatinfrom Nanostructured Lipid Carriers: Physicochemical Characterization and Pharmacokinetics. *European Journal of Pharmaceutics and Biopharmaceutics*. 74(3), 474–82.

Chennam, J.V., Velchuri, S., Chandu, B.R., Ala, A., Bosco, D., College, P.G., Vatticherukuru, M. and Pradesh, A. (2012). Niosomes – A Novel Drug Carrier Approach. *International Journal of Research in Pharmaceutical and Biomedical Sciences*. 3(2), 722–729.

Colon-Gonzalez, F., Kim, G.W., Lin, J.E., Valentino, M. a and Waldman, S. a (2012). Obesity Pharmacotherapy: What Is Next? *Molecular Aspects of Medicine*. 34(1), 1–13.

Dressman, J.B. and Reppas, C. (2000). *In vitro-In vivo* Correlations for Lipophilic, Poorly Water-soluble Drugs. *European Journal of Pharmaceutical Sciences*. 2, 73–80.

El-ridy, M., Badawi, A., Safar, M. and Mohsen, A. (2012). Niosomes as a Novel Pharmaceutical Formulation Encapsulating the Hepatoprotective Drug Silymarin. *International Journal of Pharmacy and Pharmaceutical Sciences*. 4(1).

Finer, N. (2002). Pharmacotherapy of Obesity. *Best Practice & Research Clinical Endocrinology & Metabolism*. 16(4), 717–742.

Gadhiya, P., Shukla, S., Modi, D. and Bharadia, P. (2012). Niosomes in Targeted Drug Delivery – A Review. *International Journal for Pharmaceutical Research Scholars*. 1(2).

Gangwar, M., Singh, R., Goel, R. and Nath, G. (2012). Recent Advances in Various Emerging Vescicular Systems: An Overview. *Asian Pacific Journal of Tropical Biomedicine*. 2(2), S1176–S1188.

Goyal, A., Kumar, S., Nagpal, M., Singh, I. and Arora, S. (2011). Potential of Novel Drug Delivery Systems for Herbal Drugs. *Indian Journal of Pharmaceutical Education and Research*. 45(3), 225–235.

Hanu, P. and Harmanpreet, S. (2012). Formulation and Evaluation of Niosomes Containing Punicalagin from Peels of Punica Granatum. *Journal of Drug Delivery & Therapeutics*. 2(6), 56–67.

Hao, Y.-M. and Li, K. (2011). Entrapment and Release Difference Resulting from Hydrogen Bonding Interactions in Niosome. *International Journal of Pharmaceutics*. 403(2), 245–253.

Issa, A.Y., Volate, S.R. and Wargovich, M.J. (2006). The Role of Phytochemicals in Inhibition of Cancer and Inflammation: New Directions and Perspectives. *Journal of Food Composition and Analysis*. 19(5), 405–419.

Jia-You Fanga, B., Yua, S.-Y., Wua, P.-C., Huanga, Y.-B. and Tsai, Y.-H. (2001). *In vitro* skin permeation of estradiol from various proniosome formulations. *International Journal of Pharmaceutics*. 215(1-2), 91–99.

Jin, Y., Wen, J., Garg, S., Liu, D., Zhou, Y., Teng, L. and Zhang, W. (2013). Development of a Novel Niosomal ystem for Oral Delivery of Ginkgo Biloba Extract. *International Journal of Nanomedicine*. 8, 421–430.

Kapil, K. and Rai, A.. (2012). Proniosomes as a Drug Carrier for Transdermal Delivery of Herbal Drug. *Journal of Pharmacy Research*. 5(2).

Kesarwani, K., Gupta, R. and Mukerjee, A. (2013). Bioavailability Enhancers of Herbal Origin: An overview. *Asian Pacific Journal of Tropical Biomedicine*. 3(4), 253–266.

Koga, K., Kusawake, Y., Ito, Y., Sugioka, N., Shibata, N. and Takada, K. (2006). Enhancing mechanism of Labrasol on intestinal membrane permeability of the hydrophilic drug gentamicin sulfate. *European journal of pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft für Pharmazeutische Verfahrenstechnik e.V*. 64(1), 82–91.

Kumar, B.N. (2011). Formulation, Characterization, Stability and Invitro Evaluation of Nimesulide Niosomes. *pharmacophore Journal*. 2(3), 168–185.

Kumarn, G. and Rajeshwarrao, P. (2011). Nonionic surfactant vesicular systems for effective drug delivery—an overview. *Acta Pharmaceutica Sinica B*. 1(4), 208–219.

Kushal, A.M. and Pragna, K.S. (2012). Formulation and Development of Topical Niosomal Gel Of Bcs Class-Iii Anti-Viral Drug for Better Efficacy as Herpes Treatment. *International Research Journal of Pharmacy*. 3(3), 271–276.

Kusum, D., Jain, N. and Valli, K. s (2010). Importance of Novel Drug Delivery Systems in Herbal Medicines. *Pharmacognosy Reviews*. 7(4), 27–31.

Langin, D. (2006). Adipose TissueLipolysis as a Metabolic Pathway to Define Pharmacological Strategies against Obesity and the Metabolic Syndrome. *Journal of Pharmacy Research*. 5(1), 485–491.

Leiherer, A., Mündlein, A. and Drexel, H. (2012). Phytochemicals and their impact on adipose tissue inflammation and diabetes. *Vascular pharmacology*.

Lingan, A., Sathali, A.H., Vijaya kumur, M.R. and Gokila, A. (2011). Formulation and Evaluation of Topical Drug Delivery System Containing Clobetasol Propionate Niosomes. *Sci entific Reviews and Chemical Communications …*. 1(1), 7–17.

Löbenberg, R. and Amidon, G. (2000). Modern Bioavailability , Bioequivalence and Biopharmaceutics Classification System . New Scientific Approaches to International Regulatory Standards. *European Journal of Pharmaceutics and Bopharmaceutics*. 50, 3–12.

Mahale, N.B., Thakkar, P.D., Mali, R.G., Walunj, D.R. and Chaudhari, S.R. (2012). Niosomes: Novel Sustained Release Nonionic Stable Vesicular SOystems--an verview. *Advances in Colloid and iIterface Science*. 183-184, 46–54.

Manosroi, A., Chuputrapat, R., Abe, M. and Manosroi, W. (2012). Anti-aging Efficacy of Topical Formulations Containing Niosomes Entrapped With Rice Bran Bioactive Compounds. *Pharmaceutical Biology*. 50(2), 208–224.

Manosroi, A. and Jantrawut, P. (2011). Transdermal absorption enhancement of gel containing elastic niosomes loaded with gallic acid from Terminalia chebula galls. *Pharmaceutical …*. 49(6), 553–562.

Manosroi, A., Wongtrakul, P., Manosroi, J., Sakai, H., Sugawara, F., Yuasa, M. and Abe, M. (2003). Characterization of Vesicles Prepared With Various Non-ionic Surfactants Mixed with Cholesterol. *Colloids and Surfaces B: Biointerfaces*. 30(1-2), 129–138.

Mansour, M.S., Ni, Y.-M., Roberts, A.L., Kelleman, M., Roychoudhury, A. and St-Onge, M.-P. (2012). Ginger Consumption Enhances the Thermic Effect of Food and Promotes Feelings of Satiety without Affecting Metabolic and Hormonal Parameters in Overweight Men: a Pilot Study. *Metabolism: clinical and experimental*. 61(10), 1347–52.

Marianecci, C., Paolino, D., Celia, C., Fresta, M., Carafa, M. and Alhaique, F. (2010). Non-Ionic Surfactant Vesicles in Pulmonary Glucocorticoid Delivery: Characterization and Interaction with Human Lung Fibroblasts. *Journal of Controlled Release : official Journal of the Controlled Release Society*. 147(1), 127–135.

Mokhtar, M., Sammour, O. a, Hammad, M. a and Megrab, N. a (2008). Effect of Some Formulation Parameters on Flurbiprofen Encapsulation and Release Rates of Niosomes Prepared from Proniosomes. *International Journal of Pharmaceutics*. 361, 104–111.

Moro, C.O. and Basile, G. (2000). Obesity and Medicinal Plants. *Fitoterapia*. 71 Suppl 1, S73–82.

Mujoria, R. and Bodlab, R. (2011). Niosomes–Challenge In Preparation for Pharmaceutical Scientist. *International Journal of applied Pharmaceutics*. 3(3).

Muller, R.H. and Keck, C.M. (2004). Challenges and solutions for the delivery of biotech drugs--a review of drug nanocrystal technology and lipid nanoparticles. *Journal of biotechnology*. 113(1-3), 151–70.

Mura, S., Manconi, M., Sinico, C., Valenti, D. and Fadda, A.M. (2009). Penetration enhancer-containing vesicles (PEVs) as carriers for cutaneous delivery of minoxidil. *International journal of pharmaceutics*. 380(1-2), 72–9.

Ochekpe, N.A., Olorunfemi, P.O. and Ndidi, C. (2009). Nanotechnology and Drug Delivery Part 2 : Nanostructures for Drug Delivery. *Tropical Journal of Pharmaceutical Research*. 8(June), 275–287.

Özgüney, I. (2011). Conventional and Novel Pharmaceutical Dosage Forms on Prevention of Gastric Ulcers. In *Peptic Ulcer Disease*. pp. 1–18.

Panchal, D., Patel, U., Bhimani, B., Daslaniya, D. and Patel, G. (2012). Nasal In-Situ Gel : A Novel Drug Delivery System. *International Journal for Pharmaceutical Research Scholars*. 1(2), 457–473.

Panesar, P.S. (2008). Application of Response sSurface Methodology in the Permeabilization of Yyeast Cells for Lactose Hydrolysis. *Biochemical Engineering Journal*. 39(1), 91–96.

Pardakhty, A., Varshosaz, J. and Rouholamini, A. (2007). *In vitro* Study of Polyoxyethylene Alkyl Ether Niosomes for Delivery of Insulin. *International Journal of Pharmaceutics*. 328(2), 130–41.

Patel, R., Patel, K., Modi, K. and Pathak, C. (2011). Novel Anti-Acne Drug Delivery System of Tretinoin. *International Research Journal of Pharmaceutical s*. 01(02), 65–71.

Rama Prasad, Y.V., Minamimoto, T., Yoshikawa, Y., Shibata, N., Mori, S., Matsuura, A. and Takada, K. (2004). In Situ Intestinal Absorption Studies on Low Molecular Weight Heparin in Rats Using Labrasol as Absorption Enhancer. *International Journal of Pharmaceutics*. 271(1-2), 225–232.

Ramadan, A.A. (2010). *Study of Some Lipid-based Drug Delivery Systems*.

Rangasamy, M.B.A. (2008). Formulation and *In vitro* Evaluation of Niosome Encapsulated Acyclovir. *Journal of Pharmacy Research*. 1(2), 163–166.

Ruckmani, K. and Sankar, V. (2010). Formulation and optimization of zidovudine niosomes. *AAPS PharmSciTech*. 11(3), 1119–1127.

Ruenraroengsak, P., Cook, J.M. and Florence, A.T. (2010). Nanosystem Drug Targeting: Facing up to Complex Realities. *Journal of Controlled Release*. 141(3), 265–276.

Sahin, N.O. (2007). Niosomes as Nanocarriers Systems. In *Nanomaterials and Nanosystems for Biomedical Applications*. pp. 67–81.

Sankhyan, A. and Pawar, P. (2012). Recent Trends in Niosome as Vesicular Drug Delivery System. *Journal of Applied Pharmaceutical Science*. 02(06), 20–32.

Sharma, A. and Sharma, U.S. (1997). Liposomes in Drug Delivery: Progress and Limitations. *International Journal of Pharmaceutics*. 154, 123–140.

Shukla, Y. and Singh, M. (2007). Cancer Preventive Properties of Ginger: a Brief Review. *Food and Chemical Toxicology*. 45, 683–690.

Sosnik, A., Carcaboso, A.M., Glisoni, R.J., Moretton, M. a and Chiappetta, D. a (2010). New Old Challenges in Tuberculosis: Potentially Effective Nanotechnologies in Drug Delivery. *Advanced Drug Delivery Reviews*. 62(4-5), 547–59.

Srivastava Ankar, N. kumarverma; (2012). Niosome: an Study on Novel Drug Delivery System -A review. *International Journal for Pharmaceutical Research and developement*. 3(12), 100–106.

Tan, I., Ahmad, a and Hameed, B. (2008). Optimization of preparation conditions for activated carbons from coconut husk using response surface methodology. *Chemical Engineering Journal*. 137(3), 462–470.

Tsao, R. and Deng, Z. (2004). Separation Procedures for Naturally Occurring Antioxidant Phytochemicals. *Journal of Chromatography. B, Analytical Technologies in the Biomedical and Lfe Sciences*. 812(1-2), 85–99.

Uchegbu, I.F. and Vyas, S.P. (1998). Non-ionic Surfactant Based Vesicles (niosomes) in Drug Delivery. *International Journal of Pharmaceutics*. 172, 33–70.

Vargas, A.M.M., Garcia, C. a., Reis, E.M., Lenzi, E., Costa, W.F. and Almeida, V.C. (2010). Naoh-Activated Carbon from Flamboyant (Delonix Regia) Pods: Optimization of Preparation Conditions Using Central Composite Rotatable Design. *Chemical Engineering Journal*. 162(1), 43–50.

Westerterp-Plantenga, M., Diepvens, K., Joosen, A.M.C.P., Bérubé-Parent, S. and Tremblay, A. (2006). Metabolic Effects of Spices,Teas, and Caffeine. *Physiology & Behavior*. 89(1), 85–91.

Williams, D.J., Edwards, D., Hamernig, I., Jian, L., James, A.P., Johnson, S.K. and Tapsell, L.C. (2013). Vegetables Containing Phytochemicals with Potential Anti-obesity Properties: A review. *Food Research International*. 52(1), 323–333.

Wissing, S. a, Kayser, O. and Müller, R.H. (2004). Solid Lipid Nanoparticles for Parenteral Drug Delivery. *Advanced Drug Delivery Reviews*. 56(9), 1257–72.

Xiong, Y., Guo, D., Wang, L., Zheng, X., Zhang, Y. and Chen, J. (2009). Development of Nobiliside a Loaded Liposomal Formulation Using Response Surface Methodology. *International Journal of Pharmaceutics*. 371(1-2), 197–203.