OPTIMIZING FORMATION OF FATTY ACID ESTER NANOEMULSION SYSTEMS FOR NON-STEROIDAL ANTI-INFLAMMATORY DRUG DELIVERY

NURSYAMSYILA MAT HADZIR

FS 2012 105

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By

NURSYAMSYILA MAT HADZIR

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

November 2012

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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Chairman: Professor Mahiran bt. Basri, PhD

Faculty: Science

Pseudo-ternary phase diagrams for oleyl laurate, oleyl stearate and oleyl oleate with surfactants (Pluronic F68 and Span 20) and piroxicam were constructed. In each pseudo-ternary phase diagram, a one-phase region was located along the apex line of water and mixed surfactants. A multi-phase region was also formed and found to dominate the three pseudo-ternary phase diagrams. The formation of large multi-phase regions was believed to be due to less or no synergistic effects between the Pluronic F68 and Span 20 in facilitating the formation of nanoemulsions. Even so, a composition from the multi-phase region from each pseudo-ternary phase diagram was chosen for preparing the nanoemulsions systems containing piroxicam via low energy emulsification methods.

The incorporation of a rheology modifier (xanthan gum) into the nanoemulsions systems containing piroxicam successfully facilitated the formation of nanoemulsions systems. The results from the preliminary study via 'One-At-A-Time Approach' showed that the optimum amount (w/w) of oil for oleyl laurate nanoemulsions was 30 g (w/w) and 20 g (w/w) for oleyl stearate nanoemulsions and oleyl oleate nanoemulsions. For each nanoemulsions system, the mixed surfactants (Pluronic F68:Span 20, 8:2) and rheology modifier needed for the emulsification to take place was found to be 10 g (w/w) and 0.5 g (w/w), respectively. However, the emulsification process at optimum amount of the three variables for each nanoemulsions system showed that the low energy emulsification method was unable to form emulsions in the nano-size range.

Thus, further investigation of the emulsification process was carried out using a high shear emulsification method by employing Artificial Neural Network (ANN) and Response Surface Methodology (RSM). ANN and RSM were used to predict the optimum amount (w/w) of oil, mixed surfactants and rheology modifier in order to produce nanoemulsions systems having 'nano'-sized particles with high physical stability. The results showed that RSM gave a better prediction than ANN whereby a comparison between the predicted and experimental values showed good correspondence between them, with R^2 values ≥ 0.9. The good correspondence of predicted and experimental values indicated that the empirical models derived from RSM can be used to describe the relationship between the variables and responses for the emulsification process of palm-based nanoemulsions systems.

As a result, the optimization of the emulsification process via a high shear emulsification method was performed by RSM based on Central Composite Design (CCD). The optimal amounts (w/w) of oleyl laurate, oleyl stearate and oleyl oleate as the oil phase for the oleyl laurate nanoemulsions (OL-Opt), oleyl stearate nanoemulsions (OS-Opt) and oleyl oleate nanoemulsions (OO-Opt) were found to be 33.92 g, 17.74 g and 17.95 g, respectively. As for the mixed surfactants (Pluronic F68:Span 20, 8:2) and rheology modifier, the optimal amounts (w/w) were found to be 4.03 g (OL-Opt), 9.97 g (OS-Opt), 7.59 g (OO-Opt) and 0.71 g (OL-Opt), 0.57 g (OS-Opt) and 1.02 g (OO-Opt), respectively. The emulsification process via high shear emulsification method at optimal amounts of the three variables has produced emulsions in 'nano'-sized particles with surface charge values more negative than -30 mV at pH around 5, which suggest high physical stability of the emulsions.

The characterization of oleyl laurate nanoemulsions (OL-Opt), oleyl stearate nanoemulsions (OS-Opt) and oleyl oleate nanoemulsions (OO-Opt) showed that the particle sizes were in the nano-range (in between 50 and 200 nm), with surface charge values and pH of -32.7 to -40.6 mV and 5.08 to 5.14, respectively. From observations, the three nanoemulsions systems were also found to be stable at various storage temperatures, which were 3 °C, 25 °C and 45 °C, with no phase separations. The physically stable nanoemulsions systems were also found to exhibit non-Newtonian flow behaviour by displaying a pseudoplastic behavior and shear-thinning properties. The conductivity values of OL-Opt (310.0 μS cm^{-1}), OS-Opt (281.0 μS cm^{-1}) and OO-Opt (413.0 μS cm^{-1}) also confirmed that oil-in-water nanoemulsions have been successfully produced. They were also found to be non-irritant to the skin.

The oleyl laurate nanoemulsions (OL-Opt), oleyl stearate nanoemulsions (OS-Opt) and oleyl oleate nanoemulsions (OO-Opt) were found to be stable for three months at various storage temperatures, which were 3 °C, 25 °C and 45 °C; and passed the Freeze-thaw cycle with no phase failures. The particle size analyses showed that there were no significant differences during the three months storage period especially at temperatures of 3 °C and 25 °C, which indicated that Ostwald ripening could be prevented from occurring by incorporating polymeric surfactants and rheology modifiers into the nanoemulsions systems. At storage temperature of 45 °C, the particle sizes for the three nanoemulsions systems were found to increase, which was probably due to the loss of water from the system, thus allowing the particles to combine and finally forming larger particles.

The in-vitro study of OL-Opt, OS-Opt and OO-Opt was carried out by investigating their penetration through the cellulose synthetic membrane and Wistar male rat skin. It was found that the highest penetration of piroxicam at the 8^{th} h was given by OS-Opt (31.12%) followed by OO-Opt (25.46%) and OL-Opt (21.55%). The addition of 1% menthol (which was labeled as WE) to each nanoemulsions system has increased the amount of piroxicam passing through the cellulose synthetic membrane as oleyl stearate nanoemulsions with menthol (OS-OptWE) showed the highest penetration of piroxicam (35.65%) followed by oleyl oleate nanoemulsions with menthol (OO-OptWE) (30.54%) and oleyl laurate nanoemulsions with menthol (OL-OptWE) (25.15%). Finally, the release of piroxicam from OL-OptWE, OS-OptWE and OO-OptWE was carried out via the rat skin. OS-OptWE was found to give the highest penetration of piroxicam (41.44%), followed by OO-OptWE (29.01%) and OL-OptWE (21.10%).

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

MENGOPTIMUM PEMBENTUKKAN SISTEM NANOEMULSI ASID LEMAK ESTER UNTUK PENGHANTARAN UBAT ANTI-RADANG BUKAN STEROID

Oleh

NURSYAMSYILA MAT HADZIR

November 2012

Pengerusi: Profesor Mahiran bt. Basri, PhD

Fakulti: Sains

Tiga sistem nanoemulsi mengandungi surfaktan (Pluronic F68 dan Span 20) dan piroksikam telah disediakan bagi tujuan membina gambarajah fasa pseudo-tiga untuk oleil laurat, oleil stearat dan oleil oleat. Setiap gambarajah fasa pseudo-tiga menunjukkan bahawa kawasan satu fasa terbentuk di kawasan jumlah air dan campuran surfaktan tertinggi. Kawasan pelbagai fasa juga terbentuk, dan didapati mendominasi hampir keseluruhan gambarajah fasa pseudo-tiga. Pembentukan kawasan pelbagai fasa yang luas dipercayai kerana kurang atau tiada kesan sinergi di antara Pluronic F68 dan Span 20 yang dapat membantu pembentukan nanoemulsi. Walaupun demikian, satu komposisi dari kawasan pelbagai fasa bagi setiap gambarajah fasa pseudo-tiga telah dipilih untuk menyediakan nanoemulsi mengandungi piroksikam melalui kaedah pengemulsian tenaga rendah.

Penambahan bahan pengubahsuai reologi (xanthan gam) ke dalam sistem nanoemulsi yang mengandungi piroksikam telah berjaya menghasilkan nanoemulsi yang stabil. Oleh itu, satu kajian awal telah dijalankan melalui kaedah konvensional iaitu memvariasikan satu parameter pada-satu-masa. Keputusan yang diperolehi menunjukkan bahawa jumlah optimum (w/w) minyak untuk nanoemulsi oleil laurat adalah 30 g (w/w) dan 20 g (w/w) untuk kedua-dua nanoemulsi oleil stearat dan oleil oleat. Bagi setiap sistem nanoemulsi, campuran surfaktan dan pengubahsuai reologi yang diperlukan untuk proses pengemulsian adalah 10 g (w/w) dan 0.5 g (w/w). Walau bagaimanapun, keputusan eksperimen menunjukkan bahawa proses pengemulsian pada jumlah optimum bagi ketiga-tiga pembolehubah menunjukkan bahawa kaedah pengemulsian tenaga rendah tidak dapat membentuk emulsi dalam julat saiz nano.

Oleh itu, ujikaji lanjutan terhadap proses emulsifikasi telah dijalankan menggunakan Rangkaian Saraf Tiruan (ANN) dan Kaedah Permukaan Respons (RSM). ANN dan RSM telah digunakan untuk meramal jumlah optimum bagi fasa minyak, campuran surfaktan dan pengubahsuai reologi untuk menghasilkan emulsi dengan zarah bersaiz 'nano' dan stabil. Hasil kajian menunjukkan bahawa RSM memberikan keputusan yang lebih baik berbanding ANN, di mana perbandingan di antara nilai ramalan dan nilai sebenar ujikaji menunjukkan jalinan yang baik diantara mereka, dengan nilai $R^2 \geq 0.9$. Hubungkait yang baik di antara keputusan ramalan dan keputusan sebenar menunjukkan bahawa model empirik yang diperolehi dari RSM boleh digunakan untuk menerangkan hubungkait antara pembolehubah dan maklumbalas bagi proses pengemulsian nanoemulsi.

Proses mengoptimum emulsi telah dijalankan menggunakan RSM berdasarkan kepada Pusat Komposit Berputar (CCD). Jumlah optimum (w/w) fasa minyak (oleil laurat) untuk nanoemulsi oleil laurat (OL-Opt) adalah 33.92 g, manakala 17.74 g oleil stearat dan 17.95 g oleil oleat diperlukan untuk menghasilkan nanoemulsi oleil stearat (OS-Opt) dan nanoemulsi oleil oleat (OO-Opt). Keputusan ujikaji juga menunjukkan bahawa jumlah optimum bagi campuran surfaktan adalah 4.03 g (OL-Opt), 9.97 g (OS-Opt), 7.59 g (OO-Opt) dan jumlah optimum bagi pengubahsuai reologi adalah 0.71 g (OL-Opt), 0.57 g (OS-Opt) dan 1.02 g (OO-Opt). Penghasilan emulsi menggunakan kaedah pengemulsi ricih tinggi pada nilai optimum bagi ketiga-tiga pembolehubah telah menghasilkan nanoemulsi dengan nilai cas permukaan melebihi -30 mV pada pH sekitar 5 telah mencadangkan bahawa ketiga-tiga nanoemulsi yang dihasilkan mempunyai kestabilan fizikal yang tinggi.

Pencirian sistem nanoemulsi pada amaun optimum (w/w) menunjukkan bahawa saiz zarah berada dalam julat nano (di antara 50 dan 200 nm), dengan nilai cas permukaan di antara 32.7 mV dan 40.6 mV dengan nilai pH di antara 5.05 dan 5.14. Pemerhatian juga menunjukkan bahawa ketiga-tiga sistem nanoemulsi berada dalam keadaan stabil pada suhu penyimpanan 3 °C, 25 °C dan 45 °C. Sistem-sistem nanoemulsi tersebut juga mempamerkan sifat bukan Newtonian, atau dalam erti kata yang lain bermaksud sistem tersebut mempamerkan sifat pseudoplastik dan ricih-penipisan. Nilai kekonduksian bagi sistem nanoemulsi oleil laurat (310.0 $\mu S\ cm^{-1}$), nanoemulsi oleil stearat (281.0 $\mu S\ cm^{-1}$) dan nanoemulsi oleil oleat (413.0 $\mu S\ cm^{-1}$) telah mengesahkan bahawa nanoemulsi dari jenis minyak-di dalam-air telah berjaya dihasilkan. Ketiga-tiga nanoemulsi juga didapati tidak meyebabkan kerengsaan kepada kulit.

Seterusnya, sistem nanoemulsi juga didapati stabil untuk tempoh 3 bulan apabila disimpan pada suhu 3 °C, 25 °C dan 45 °C, serta lulus ujian kitaran beku-cair. Sepanjang tempoh 3 bulan penyimpanan, saiz zarah nanoemulsi di dapati tidak menunjukkan sebarang perubahan yang signifikan terutama pada suhu 3 °C dan 25 °C, yang mana ianya menunjukkan bahawa proses pematangan Ostwald boleh dihalang apabila menggunapakai surfaktan polimer dan pengubahsuai reologi di dalam sistem nanoemulsi. Pada suhu penyimpanan 45 °C, saiz zarah bagi ketiga-tiga sistem nanoemulsi telah didapati meningkat, yang mana ianya mungkin disebabkan oleh kehilangan air daripada sistem nanoemulsi, dan seterusnya menyebabkan zarah-zarah bergabung, dan membentuk partikel yang lebih besar.

Kajian *in vitro* terhadap sistem nanoemulsi telah dijalankan untuk mengetahui kadar penembusan piroksikam melalui membran selulosa sintetik dan kulit tikus jantan Wistar. Hasil kajian menunjukkan bahawa penembusan tertinggi piroksikam pada jam ke-8 diperolehi dari OS-Opt (31.12%), diikuti oleh OO-Opt (25.46%) dan OL-Opt (21.55%). Penambahan 1% menthol (dilabelkan sebagai WE) kepada sistem nanoemulsi berjaya meningkatkan jumlah penembusan piroksikam melalui membran selulosa sintetik. Keputusan menunjukkan bahawa nanoemulsi oleil stearat mengandungi menthol (OS-OptWE) memberikan penembusan tertinggi piroksikam (35.65%) diikuti oleh nanoemulsi oleil oleat mengandungi menthol (OO-OptWE) (30.54%) dan nanoemulsi oleil laurat mengandungi menthol (OL-OptWE) (25.15%). Ujikaji terakhir adalah mencari peratusan pembebasan piroksikam dari setiap sistem nanoemulsi melalui kulit tikus. Keputusan ujikaji menunjukkan bahawa OS-OptWE telah memberikan pembebasan tertinggi piroksikam (41.44%) diikuti oleh OO-OptWE (29.01%) dan OL-OptWE (21.10%).

ACKNOWLEDGEMENTS

First and foremost, I thank Allah the Almighty for giving me the strength and showing me the light to complete this thesis.

To complete this thesis, I am indebted to many- too many for me to include all in this little acknowledgement.

My heartfelt gratitude and greatest appreciation goes to my supervisor, Professor Dr. Mahiran Basri, for her countless time, tireless advice, patience, guidance, encouragement, support and warmth which gave me the confidence and strength in the preparation and completion of this arduous task and for making this thesis a reality despite her busy schedule as the Director of Centre of Foundation Studies For Agricultural Science, UPM. All her assistance and kindness helped to make this academic journey an invaluable experience. There are no words to describe my gratefulness to her.

My special thanks and sincere appreciation also is extended to my co-supervisors, Professor Dato' Dr. Abu Bakar Salleh and Professor Dr. Mohd Basyaruddin Abd Rahman for their help, encouraging support and assistance.

This thesis is also dedicated to my husband, my parents and parents-in-law, my lovely Misha and Amin, and my siblings, for their patience and never-ending support

throughout the time I undertook this PhD programme. They have been a constant source of motivation and inspiration in moments of dismay.

Special thanks to Puan Norizan Yusof, friends in Lab 401 and staffs of the Faculty of Science UPM, The Animal Care and Use Committee of the Faculty of Medicine and Health Sciences UPM and colleagues in UiTM Perlis who directly and indirectly helped me through during the PhD stages, especially the writing phases: Dr Zeti Zuryani and Dr Norizul Azida, thank you for the kind words of encouragement and for never saying no to any help needed by me and for being there whenever I need help; and to all- for being understanding in times of distress and stressfulness.

To all, thank you for being there for me.

I certify that an Examination Committee has met on 29 November 2012 to conduct the final examination of Nursyamsyila binti Mat Hadzir on her Doctor of Philosophy thesis entitled "Optimizing The Formation Of Fatty Acid Ester Nanoemulsions System For Non-Steroidal Anti-Inflammatory Drugs Delivery" in accordance with the Universities and University College 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 Doctor of Philosophy degree.

Members of the Examination Committee were as follows:

Mansor bin Haji Ahmad, PhD
Associate Professor
Faculty of Science
Universiti Putra Malaysia
(Chairman)

Mohd Zobir Hussein, PhD
Professor
Faculty of Science
Universiti Putra Malaysia
(Internal Examiner)

Mohd Zaizi Desa, PhD
Lecturer
Centre of Foundation Studies For Agricultural Science
Universiti Putra Malaysia
(Internal Examiner)

Sanjula Baboota, PhD
Assistant Professor
Faculty of Pharmacy
Hamdard University
(External Examiner)

SEOW HENG KONG, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia
Date:

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

Mahiran Basri, PhD
Professor
Faculty of Science
Universiti Putra Malaysia
(Chairman)

Dato' Abu Bakar Salleh, PhD
Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

Mohd. Basyaruddin Abdul Rahman, PhD
Professor
Faculty of Science
Universiti Putra Malaysia
(Member)

Azmin Mohd Noor, PhD
Associate Professor
School of Pharmaceutical Sciences
Universiti Sains Malaysia
(Member)

BUJANG BIN KIM HUAT, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia
Date:

DECLARATION

I declare that the thesis is my original work except for the quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

NURSYAMSYILA MAT HADZIR
Date: 29 November 2012

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CHAPTER 1

INTRODUCTION

1.1 Background Of The Study

The pharmaceutical industry is an important component of the healthcare sector in Malaysia. Its development is driven by rising wealth, increased longevity of the population, greater awareness of healthcare and better access to medicines. In addition to that, there are opportunities to benefit from the diversified natural resources (such as form Malaysia's flora and fauna) for the development of various types of resource-based and bio-generic drugs. For instance, the utilization of palm-based raw materials in the pharmaceutical industry (as drug delivery agent) is expected to maintain Malaysia's position as a global producer and exporter of oil palm products.

Palm oil consists of triglycerides with the combination of glycerol and different fatty acids. The fatty acids can be converted to wax esters by synthesizing the fatty acids with long chain alcohols using lipases at mild reaction conditions and an environmentally friendly process, as described by Mat Radzi *et al*. (2005a). Wax esters are preferable as the oil phase for a nanoemulsions system over other types of oil phases such as triglycerides. This is due to the novel characteristics exhibited by wax esters, such as superb wetting behaviour at interfaces, without the 'oily feeling' when applied on skin surfaces and able to form nanoemulsions with selected surfactants.

Drug delivery is the method or process of administering a pharmaceutical compound to achieve a therapeutic effect in humans or animals (Friedman, 2008). Investigation on drug delivery technologies is carried out for the benefit of improving product efficacy and safety, as well as patient convenience and compliance. Most common routes of administration include the preferred non-invasive peroral (through the mouth), topical (skin), transmucosal (nasal, buccal/sublingual, vaginal, ocular and rectal) and inhalation routes. However, many medications may not be delivered using these routes because they might be susceptible to enzymatic degradation or cannot be absorbed into the systemic circulation efficiently due to its molecular size.

1.2 Problem Statements

Non-steroidal anti-inflammatory drugs (NSAIDs) are one of the most widely prescribed medications in the world (Sostres *et al*., 2010). They are known to have prominent anti-inflammatory, analgesic and antipyretic properties. In spite of these properties, some of the drugs are unable to be marketed due to difficulties in delivery. Some of the obstacles in drug delivery are poor solubility of the drugs, low bioavailability, short in-vitro and in-vivo stability, adverse side effects (such as irritation and ulceration to the gastro-intestinal mucosa) especially when taken orally, and regulatory issues. Therefore, such drugs need to be administered through a delivery system (such as topical delivery) that can make them marketable and acceptable for treatment of patients.

1.3 Significance Of The Study

Topical application of a pharmacologically active compound onto the skin offers several advantages over other methods of administration such as oral and parenteral. The advantages of topical application are reduction in first pass metabolism by the liver, non-invasiveness, avoidance of the gastric route, reducing the potential for both degradation of the drug and gastric irritation, improved owner compliance with drug administration as well as elimination of pain and other complications of parenteral administration (Wosicka and Cal, 2010). In terms of the clinical use, topical administration will be the most preferred way of drug delivery due to limited or no side effects, especially for elderly patients who cannot tolerate oral dosage form and prolonged therapy.

Hence, a new carrier-system for NSAIDs which is more efficient than the existing one has to be found, especially if it is able to reduce the adverse effects and solubility problem of the drug, thus overcoming the permeability problem of the drug. Therefore, an emulsions system, which is in nanometric size, mainly covering a size range of 50–200 nm, (Kong and Park, 2011) appears to be a potential carrier for transdermal delivery because the penetration through rough skin is easier, which also enhances the penetration of the actives (Tadros *et al*., 2004). Nanoemulsions are believed to be independent of the molecular size of the actives. Using oil-in-water nanoemulsions, the drug can be solubilized in the lipophilic phase, and the surfactant and co-surfactant in the nanoemulsions system may function as permeation enhancers by reducing the diffusional barrier of the stratum corneum.

1.4 Objectives

Consequently, there are specific objectives, which have been listed below, that need to be carried out to develop a nanoemulsions system as a potential carrier for a transdermal delivery for the prototype NSAIDs, piroxicam:

1. To prepare and construct pseudo-ternary phase diagrams of single fatty acid esters.
2. To prepare nanoemulsions for piroxicam via spontaneous and high shear emulsifications methods.
3. To predict and optimize the conditions for preparing nanoemulsions with nano-size particle and good physical stability using Response Surface Methodology (RSM) and Artificial Neural Network (ANN).
4. To characterize the rheological and physicochemical properties of the nanoemulsions containing piroxicam.
5. To evaluate the stability of the nanoemulsions system containing piroxicam.
6. To study the delivery potential of the nanoemulsions system containing piroxicam.

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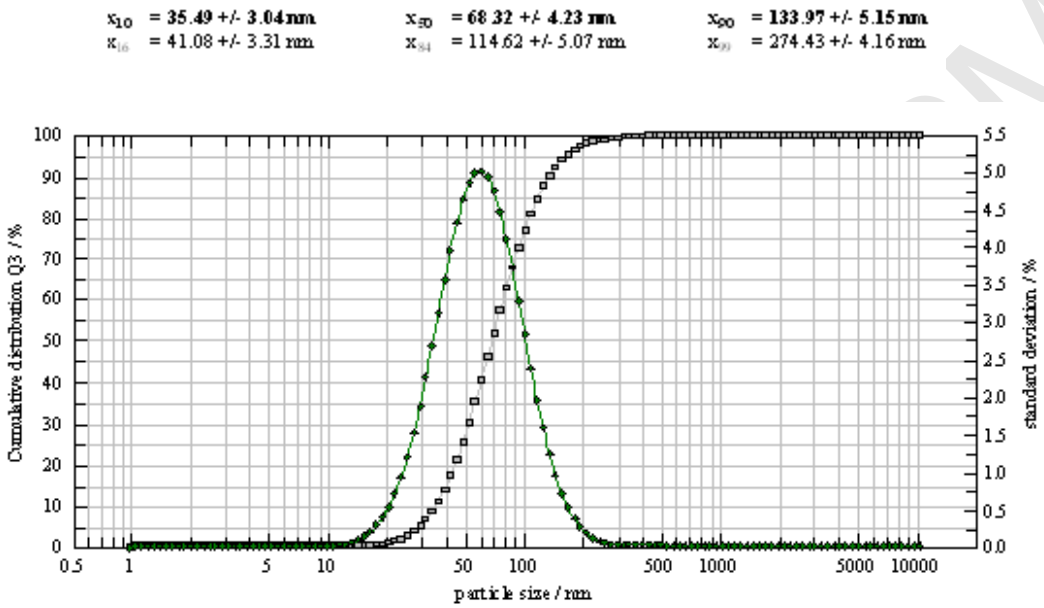
APPENDIX

An example of a typical output of particle size distribution using Nanophox at 25.0 ± 0.5 °C.



LIST OF PUBLICATIONS

1. Mat Hadzir, N., Basri, M., Salleh, A. B., Rahman, M. B. A., Basri, H. and Rahman, R. N. Z., (2013). Phase Behaviour and Formation of Fatty Acid Esters Nanoemulsions for Piroxicam Delivery. *AAPS Pharmaceutical Science and Technology*, 14(1): 456-463

2. Mat Hadzir, N., Basri, M., Salleh, A. B., Rahman, M. B. A., Basri, H. and Rahman, R. N. Z.
   Comparison of the optimizing ability of RSM and ANN on the stability of oleyl oleate nanoemulsions. (submitted)

3. Mat Hadzir, N., Basri, M., Salleh, A. B., Rahman, M. B. A., Basri, H. and Rahman, R. N. Z.
   Application of RSM in determining the factors that affecting the particles size of fatty acid esters nanoemulsions. (In preparation)

4. Mat Hadzir, N., Basri, M., Salleh, A. B., Rahman, M. B. A., Basri, H. and Rahman, R. N. Z.
   Formation and characterizations of palm-based transdermal nanodelivery of piroxicam. (In preparation)

5. Mat Hadzir, N., Basri, M., Salleh, A. B., Rahman, M. B. A., Basri, H. and Rahman, R. N. Z.
   Effect of xanthan gum on the formation of oleyl oleate nanoemulsions system. (In preparation)

BIODATA OF STUDENT

Nursyamsyila Mat Hadzir was born in Kangar, Perlis on 26th December 1974. She received her primary education at Sekolah Kebangsaan Jejawi, Kangar, Perlis. She continued her secondary education at Sekolah Menengah Derma, Kangar, Perlis. In 1994, she completed her matriculation study from Universiti Putra Malaysia. Then, she was offered to pursue her studies at UPM and four years later in 1998, she obtained her first degree in Bachelor Of Science (Hons.) majoring in Industrial Chemistry. Starting on July 1998, she enrolled in Master of Science programme at Department of Chemistry, Faculty of Science, UPM under the supervision of Prof. Dr. Mahiran Basri. In January 2002, she started her career as a lecturer in Universiti Teknologi MARA Perlis until present. In 2007, she was offered a scholarship from the Ministry of Higher Education (MOHE) for her PhD's programme.