DEVELOPMENT, CHARACTERIZATION AND EVALUATION OF RALOXIFENE NANOEMULGEL FOR TOPICAL DELIVERY

BY

EBRAHIM MOHAMMED HASAN ALI

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Kulliyyah of Pharmacy
International Islamic University Malaysia

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ABSTRACT

Raloxifene is a second-generation selective oestrogen receptor modulator (SERM) used in treatment of osteoporosis in postmenopausal women as well as in the prevention of invasive breast cancer. This drug has a poor bioavailability, which is limited to only 2%. The aim of this study was to develop, characterize and evaluate of raloxifene nanoemulgel for topical delivery. Four nanoemulsion formulations (NE13, NE14, NE15 and NE16), containing sunflower oil as oil phase, distilled water, Tween 20 as surfactant and Transcutol as co-surfactant were prepared at different ratios. The nanoemulsion formulations were prepared by using a sonicator. The formulations were characterized such as pH, transmittance, refractive index, viscosity, particle size, zeta potential, thermodynamic stability and morphology structure (TEM). NE15 was selected as the most stable formulation that did not show any phase separation, which passed the thermodynamic stability. In addition, it had a high transmittance (%T) at 98.4%, refractive index at 1.38, zeta potential value at 33.80±1.35 mV with a low particle size at 153.10± 4.47 nm and polydispersion index of 0.259±0.03. The viscosity of NE15 was recorded at 12.80±0.33. The TEM image showed that, nanoemulsion globules less than 200 nm, which agreement with the result in nano size range that obtained by the Malvern Zetasizer. The optimized nanoemulsion was incorporated into different ratio of gel Carbopol® 940 3% (gelling agent) to fabricate nanoemulgel (NG1, NG2, NG3, NG4 and NG5). The nanoemulgel formulations were subjected for different evaluations, namely, stability study, drug content, texture analysis (hardness and cohesiveness), rheological properties, spreadability, assessment of local toxicity on rat skin and permeation study using abdominal skin rat. The permeation of raloxifene from nanoemulgel was measured using Frenz diffusion cell and the data were analysed by HPLC. The ex-vivo permeation of nanoemulgel formulations were compared with the suspension raloxifene (control). NG3 was selected as the best formulation as it has the highest drug loading at 99.07 %±1.25 with a higher maximum cumulative release at 76.72 (μg/cm^2) and flux (J) was found to be at 11.84 μg/cm^2/h. The drug release of the nanoemulgel formulations were found to be higher than suspension (control). The rheological properties exhibited that, the formulation has a viscoelastic behaviour. The viscosity, hardness and cohesiveness of NG3 recorded at 60.13±10 Pas, 94.0±4.62 g and -0.26±0.05 respectively. The result of the assessment of local toxicity on skin showed that, the skin structure remained intact after applying nanoemulgel. The stability study of nanoemulgel (NG3) was done for one month at room temperature to evaluate drug content (drug degradation) and physical changes such as colour, pH and separation phase. Only 1 % of the drug in nanoemulgel formulation was degraded after one month. There was no physical change in the formulation. Hence, it can be concluded that, nanoemulgel formulation is a suitable and safe for application as a topical delivery of raloxifene.

خلاصة البحث

رالوكسيفين هو الجيل الثاني من مستقبلات الاستروجين الانتقائية (SERM) المستخدم في علاج هشاشة العظام في النساء بعد سن اليأس وكذلك في الوقاية من سرطان الثدي. التوافر البيولوجي للدواء هو 2 ٪ فقط. وتهدف هذه الدراسة الى تطوير وتقييم Nanoemulgel لإيصال الرالوكسيفين عبر الجلد. تم تحضير سبعة عشر تركيبه من Nanoemulsion. أربع تركيبات من Nanoemulsion(NE13-NE16) تم اختيارها من بين سبعة عشر تركيبه. هذه التركيبات تتكون من نسب مختلفة من زيت دوار الشمس كطور زيتي، ماء مقطر، Tween 20 كعامل استحلال وTranscutol كعامل استحلال مساعد. تم تحضير Nanoemulsion والذي يتركب من زيت دوار الشمس، Tween 20 وTranscutol اضافة الى الرالوكسيفين بواسطة استخدام sonicator. النفاذية، معامل الانكسار، اللزوجة، pH، حجم الجسيمات، PDI، TEM، Zeta potential ، Thermodynamic stability تم قياسهم لجميع التركيبات. تم اختيار NE15 كأكثر التركيبات استقرارًا والتي لم تظهر أي فصل طوري. حيث سجلت اعلى قيمة Zeta potential 33.80، وحجم الجسيمات عند 153.10 نانومتر وPDI 0.259. تم تحضير Nanoemulgel بإذابة (3%) Carbopol 940في ماء مقطر. تم دمج NE15 في الجل في نسب مختلفة. خمسة تركيبات تم تحضيرها (NG1-NG5). تم تقييم هذه التركيبات لعدة دراسات مثل اللزوجة،Drug content ، الصلابة والتماسك، وخصائص الريولوجيا، قابلية الانتشار، وتقييم سمية الدواء باستخدام جلد الفأر ودراسة تدفق الدواء عبر الجلد. تم قياس تتدفق الدواء عبر الجلد باستخدام Frenz cell diffusionوتم تحليل البيانات بواسطة HPLC. وتمت مقارنة تدفق nanoemulgel عبر الجلد مع محلول الرالوكسيفين(RLX) (Suspension) . تم اختيار NG3 كأفضل تركيبة بين جميع تركيبات حيث سجلت اعلى قيمة Drug content 1.25±% 99.07. وجد ان تدفق الدواء خلال الجسم كان اعلى في التركيبات (NG1-NG5) من محلول الرالوكسيفين حيث NG3 سجلت اقصى تدفق للدواء خلال الجسم مقارنة بالتركيبات الاخرى. أظهرت نتائج تقييم سميه الدواء على الجلد ان Stratum corneum، epidermis and dermis للجلد ظلت سليمة بعد تطبيق nanoemulgel. أجريتStability study للعينه(NG3) بعد شهر في درجة حرارة الغرفة لتقييم انحلال تركيز الدواء واللون، pH ومراحل الفصل. وجد ان تركيز الدواء انحل بمقدار 1 ٪ بعد شهر واحد. ايضا لم يكن اي فصل طوري او تغيير في لون وpH التركيبة. يمكن الاستنتاج من ذلك، RLX nanoemulgel هو أفضل بديل لإيصال الدواء. كما انه امن ومناسب للتطبيق الموضعي.

APPROVAL PAGE

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……..
Bappaditya Chatterjee
Supervisor

……..
Pinaki Sengupta
Co-Supervisor

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master in Pharmaceutical Sciences (Pharmaceutical Technology)

.....……
Muhammed Taher Bin Bakhtiar
Internal Examiner

.....……
Shariza Sahudin
External Examiner

This thesis was submitted to the Department of Pharmaceutical Technology and is accepted as a fulfilment of the requirement for the degree of Master in Pharmaceutical Sciences (Pharmaceutical Technology)

……..
Muhammed Taher Bin Bakhtiar
Head, Department of
Pharmaceutical Technology

This thesis was submitted to the Kulliyyah of Pharmacy and is accepted as a fulfilment of the requirement for the degree of Master in Pharmaceutical Sciences (Pharmaceutical Technology)

……..
Juliana Bt. Md. Jaffri
Dean, Kulliyyah of Pharmacy

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LIST OF ABBREVIATIONS

RLX	Raloxifene
SC	Stratum corneum
NE	Nanoemulsion
NG	Nanoemulgel
T%	Transmittance
TRc	Transcutol
CADP	Cumulative drug release
GRAS	General regard as safe
PDI	Polydispersity index
CR	Controlled rate
CS	Controlled stress
TEM	Transmission electron microscopy
SER	Selective estrogen receptor modulator
ER	Enhancement ratio
SLNs	Solid lipid nanoparticles
CP	Crospovidone
CCs	Croscarmellose sodium
SSG	Sodium starch glycolate
NLs	Nanostructured lipid carrier
SECosomes	Surfactant-ethanol-cholestrol-osome
CPE	Chemical penetration enhancers
PG	Propylene glycol
HLP	Hydrophilic-lipophilic Balance
CMC	Critical micelle concentration

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CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Raloxifene (RLX) is a second-generation selective oestrogen receptor modulator, generally used to treat osteoporosis and invasive breast cancer in postmenopausal women (Salazar, Codevilla, Meneghini, & Bergold, 2015). The poor bioavailability (only 2%) owing to its low solubility and higher first pass metabolism, has limited the effectiveness of current oral therapy with RLX (Burra et al., 2013). RLX is generally supplied at a daily dose of 60 mg as oral tablet. The commonly reported adverse effects connected with this high oral dose of RLX warned its wide use. Administration of such high dose increases the risk of developing blood clot in the body of the patients.

The advantages of the topical route in avoiding the first-pass metabolism is well known (Jepps, Dancik, Anissimov, & Roberts, 2013). To make the topical route of drug delivery more effective and useful, drug penetration through stratum corneum layer can be improved by employing novel approaches. Different studies suggested that drug delivery through the skin have gained considerable interest as reduction in size to nanoscale provides deeper skin penetration (Khurana, Jain, & Bedi, 2013; Severino et al., 2013). Nanoemulsions has been reported as a useful alternative formulation to increase the permeability and bioavailability of lipophilic drugs by enhancing their absorption through skin (Abolmaali, Tamaddon, Farvadi, Daneshamuz, & Moghimi, 2011). Nanoemulsion, a multipurpose technology, can be exploited in drug delivery for poorly soluble drugs like RLX. Nanoemulsions have a higher solubilization capacity than simple micellar solutions. Their thermodynamic stability offers advantages over unstable dispersions, such as emulsions and suspensions, as they can be manufactured

with little energy input (heat or mixing) and have a longer shelf life (Shafiq-un-Nabi et al., 2007). The ability to improve the penetration and permeation of active ingredients through the skin, without incorporating penetration enhancer in the formulation, is one of the main advantages of using nanoemulsions topically (Schmidts, Dobler, Nissing, & Runkel, 2009; Shah, Bhalodia, & Shelat, 2010; Shakeel, Ramadan, & Ahmed, 2009).

But, topical delivery though nanoemulsion cream or lotion may not be so effective due to its low viscosity and therefore, less skin retention time. If the cream is prepared as oil base then it may last longer on skin but that will eventually cause less user acceptance due to greasiness. The oleaginous base cream also retards drug release (Ajazuddin et al., 2013). Therefore, the modification of nanoemulsion physical state by incorporating gelling agent in order to increase the viscosity could be a better transdermal delivery system. Nanoemulgel formulation is a combined approach, where the nanoemulsions are incorporated into a gel matrix for additional therapeutic and application related improvement. High surface area due to nanosize provides bioadhesivity and formation of a film which enhances drug penetration to blood via transdermal route owing to its occlusive and hydrating properties (Pund, Pawar, Gangurde, & Divate, 2015).

Despite of the development of different modified dosage forms and techniques for enhancement of the bioavailability of RLX (Burra et al., 2013; Jagadish et al., 2010; Lu, Liu, Wang, & Li, 2015; Tran et al., 2013), it is yet to reach a satisfactory level. Attempts to improve the bioavailability of the drug can be treated as a thirst area of research to minimize the common unfavourable adverse events associated with its use. An alternative route is required to be designed to minimize the dose of RLX without affecting its therapeutic efficacy. To the best of our knowledge, there is no reported research on development of nanoemulgel formulation for RLX. The primary objective

of this research was to come up with a model aqueous gel based nanoemulsion formulation, which will serve as a systemic delivery platform through topical route for the lipophilic drug RLX.

1.2 STATEMENT OF THE PROBLEM

Nanoemulsion is a useful delivery system for lipophilic drugs, lacking solubility (Ganta et al., 2014). Apart from invasive and inconvenient parenteral route, nanoemulsion can be delivered orally or topically. However, oral nanoemulsion formulations are unable to overcome first pass metabolism through liver, which leads to higher dose and the surfactant content in formulation may cause gastric irritation. Topical nanoemulsion can overcome this problem by avoiding the first-pass metabolism. But lower duration of skin retention and challenge to enhance skin permeation of lipophilic molecule are the main constraints of this topical delivery. Oil- base cream may retain on skin longer, but greasiness and less penetration are major setback (Ajazuddin et al., 2013).

To deal with this problem, our approach is to develop a nano-sized aqueous base nanoemulsion, followed by its conversion to gel by incorporating nanoemulsion system into hydrogel matrix for transdermal delivery of a lipophilic drug. The present study is designed to make up these voids as much as possible by developing a model nanoemulgel platform, which could be used for different lipophilic drug. Development of nanoemulsion gel formulation for RLX, which is widely used for the treatment and prevention of breast cancer, osteoporosis and postmenopausal symptoms, is required due to its:

i. Poor and irregular absorption after oral administration
ii. Poor water solubility and extensive first pass metabolism
iii. Poor systemic exposure with only 2% absolute bioavailability

iv. Wide use for the treatment and prevention of breast cancer, osteoporosis and postmenopausal symptoms. By quantitating key formulation parameters required to optimize for such delivery system.

1.3 RESEARCH OBJECTIVES

Overall objective of the proposed research is to come up with an aqueous gel based RLX nanoemulsion, which will serve as a systemic delivery platform through topical route for lipophilic molecule. To achieve the main objective, following subsequent objectives are to be satisfied:

i. To design and develop RLX nanoemulsion.
ii. To select the most desirable nanoemulsion by in vitro characterization studies.
iii. To fabricate a suitable nanoemulgel by loading chosen nanoemulsion onto gel platform.
iv. To investigate rheology, toxicity and ex-vivo skin permeation of RLX from the nanoemulgel platform.

1.4 RESEARCH HYPOTHESIS

Based on the groundwork and preliminary studies, it is hypothesized that:

i. Minimum amount of surfactant along with proper oil ingredient will be capable to design stable and non-toxic gel based nano formulation.
ii. Physically modified aqueous base nanoemulsion or nanoemulgel will have longer skin retention.

iii. Presence of surfactant and nano sized oil entrapped globule will enhance penetration of lipophilic molecule through skin to enhance their bioavailability.

1.5 RESEARCH QUESTIONS

The proposed research will face the following challenges to satisfy the objectives

i. What will be the minimum surfactant and lipid base to incorporate a lipid molecule?
ii. How the oil entrapped globule size will correlate with skin penetration for a model delivery system?
iii. How much percentage of improvement for lipophilic molecule penetration through skin is possible from such modified nano emulsion?

1.6 SIGNIFICANT OF THE STUDY

Successful completion of this research would come out with new findings about the responsible key formulation factors for a topical gel based nanoemulsion with respect to drug penetration enhancement for improving bioavailability of poor bioavailable lipophilic drugs.

1.7 SCOPE OF THE STUDY

The scope of the study of this research is to develop RLX gel based nanoemulsion for topical delivery system by using sunflower oil, tween 20, transcutol and carbopol 940. The study focuses on the physical characterization to improve the stability and rheological properties. In addition, it aims to measure the ex-vivo drug permeation

profiles and compare the values obtained from nanoemulgel with the solution/suspension of RLX.

CHAPTER TWO

LITERATURE REVIEW

2.1 OSTEOPOROSIS

Osteoporosis is a wide spread problem reported from almost all of the social classes and economic domains of the world. Finding a suitable medication method is one of the biggest challenges for the healthcare industry (Casillas et al., 2009). Osteoporosis (silent disease) is a disease where weakening of bones leads to the risk of bone fracture, commonly observed among postmenopausal women and old people. Fractures occur most often in the hip, spine and wrist bones (Golob & Laya, 2015). Figure 2.1 showed the differences between normal bone and bone with osteoporosis.

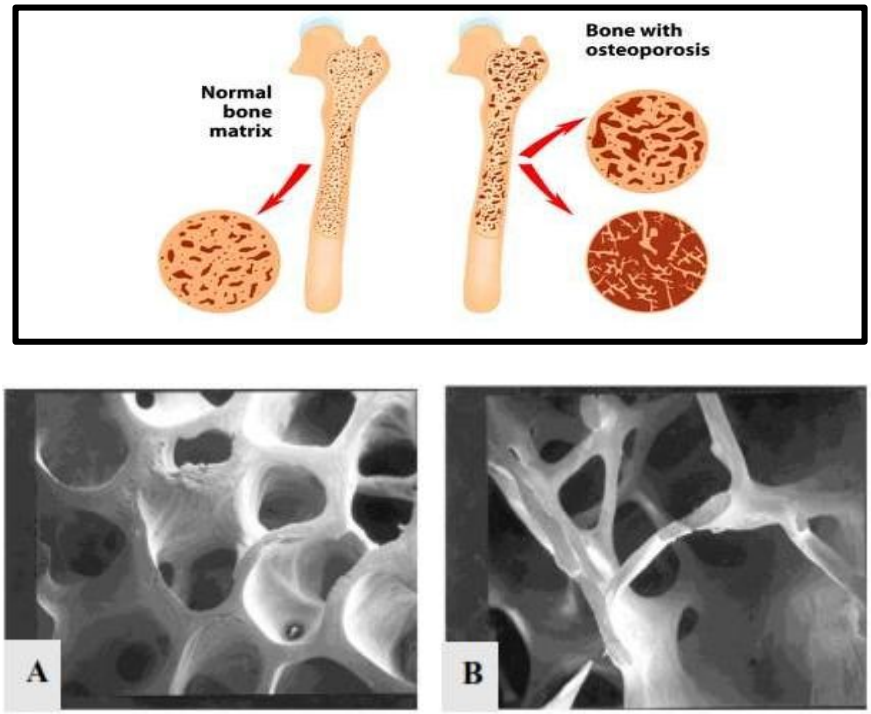

Figure 2.1 Illustrates the differences between (A) normal bone and (B) bone with osteoporosis (Cosman et al., 2014)

There are two types of osteoporosis: postmenopausal osteoporosis (type I) and senile osteoporosis (type II). Type I mostly develops in women, especially after the stage of menopause, at the age between 50 and 70 years. In this type, the amount of trabecular bone decreases and the hard cortical bone from inside becomes sponge like. The result is wrist and vertebral body fractures due to weak bone strength. The second type of osteoporosis (senile osteoporosis) happens in women who are above 70 years old. In Type II, thinning occurs in both the hard cortical bone and the trabecular bone. This causes hip and vertebral body fractures (US Department of Health and Human Services 2004). Approximately 200 million women worldwide have osteoporosis, where most of them are in the age 60 or above according to the International Osteoporosis Foundation (Maria and Witt-E nderby, 2014).

Key risk factors for osteoporosis include lack of exercise, eating disorders, lack of calcium and vitamin D intake, genetics, personal history of fracture as an adult, low body weight, nicotine intake ,excessive alcohol consumption, advanced age, history of rheumatoid arthritis and family history of osteoporosis. Patients are not usually percipient of having osteoporosis until the bone fractures occur (Golob & Laya, 2015).However, there are some signs and symptoms, like, fractures of the (hip, wrist and spine), backache due to collapsed vertebra, sleep disorders, stooped posture and loss of height.

Current pharmacologic prevention and treatment options for osteoporosis include antiresorptive therapies (alendronate, risedronate, ibandronate, raloxifene, hormone therapy, and calcitonin) and the anabolic agent teriparatide (Miller et al., 2006). For either osteoporosis treatment or prevention, supplemental calcium and/or vitamin D should be added to the diet in case of inadequate daily intake.