DEVELOPMENT OF MULTIPLE EMULSIONS FOR DRUG STABILITY ENHANCEMENT

By

Nuntachai Hanpramukkun

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

Program of Pharmaceutical Technology

Graduate School

SILPAKORN UNIVERSITY

2009

DEVELOPMENT OF MULTIPLE EMULSIONS FOR DRUG STABILITY ENHANCEMENT

By

Nuntachai Hanpramukkun

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

Program of Pharmaceutical Technology

Graduate School

SILPAKORN UNIVERSITY

2009

การพัฒนาพหุอิมัลชันเพื่อเพิ่มเสถียรภาพของยา

โดย

นาย นันทชัย หาญประมุขกุล

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรดุษฎีบัณฑิต
สาขาวิชา เทคโนโลยีเภสัชกรรม
บัณฑิตวิทยาลัย มหาวิทยาลัยศิลปากร
ปีการศึกษา 2552

The Graduate School, Silpakorn University has approved and accredited the thesis title of "Development of Multiple Emulsions for Drug Stability Enhancement" submitted by Nuntachai Hanpramukkun as a partial fulfillment of the requirements for the degree of Doctor of Philosophy in Pharmaceutical Technology.

……..
(Assoc. Prof. Sirichai Chinatangkul, Ph.D.)
Dean of Graduate School
...../...../.....

The Thesis Advisors

1. Assoc. Prof. Gaysorn Chansiri, Ph.D.
2. Assoc. Prof. Somlak Kongmuang, Ph.D.

The Thesis Examination Committee

……..…….. Chairman
(Assoc. Prof. Thawatchai Phaechamud, Ph.D.)
...../...../.....

…….…… Member
(Narueporn Sutanthavibul, Ph.D.)
...../...../.....

…….…… Member
(Assoc. Prof. Sontaya Limmatvapirat, Ph.D.)
...../...../.....

…….…… Member
(Assoc. Prof. Gaysorn Chansiri, Ph.D.)
...../...../..... .....

…….…… Member
(Assoc. Prof. Somlak Kongmuang, Ph.D.)
...../...../.....

48353902 : MAJOR : PHARMACEUTICAL TECHNOLOGY
KEY WORDS : MULTIPLE EMULSION / STABILITY / DIFFUSION
NUNTACHAI HANPRAMUKKUN : DEVELOPMENT OF MULTIPLE EMULSIONS FOR DRUG STABILITY ENHANCEMENT . THESIS ADVISORS : ASSOC. PROF. GAYSORN CHANSIRI, Ph.D. AND ASSOC. PROF. SOMLAK KONGMUANG, Ph.D. 159 pp.

Multiple emulsions have significant potential in many pharmaceutical applications. The objective of this research is to investigate whether multiple emulsion can be used to improve stability of water soluble drug. Clindamycin phosphate was used as a model drug. The stability of clindamycin phosphate was studied in solution and water-in-oil-in-water (w/o/w) multiple emulsions at 40 °C and 4 °C for 3 months. Analysis of clindamycin phosphate remained at various periods was performed using HPLC. It was found that the stability of clindamycin phosphate in w/o/w multiple emulsions was better than in solution. The degradation rate of clindamycin phosphate in w/o/w multiple emulsion was significantly lower than that in the solution (p value < 0.05). This might be due to the effect of multiple emulsion to protect clindamycin phosphate from hydrolysis in external water phase. The multiple emulsions were found to serve as an entrapping reservoir for clindamycin phosphate that could be released from the inner aqueous phase to the outer aqueous phase via diffusion mechanism. Increasing viscosity of oil middle phase by addition of petrolatum in w/o/w multiple emulsion was found to improve clindamycin phosphate stability and increase clindamycin phosphate released from inner aqueous phase to outer aqueous phase compared to w/o/w multiple emulsions without petrolatum. The w/o/w multiple emulsions system containing petrolatum in oil middle phase resulted in droplets size increasing and the apparent viscosity decreasing. Apparent viscosity of external water phase did not affect clindamycin phosphate stability and clindamycin phosphate released profile in w/o/w multiple emulsion.

Program of Pharmaceutical Technology Graduate School, Silpakorn University Academic year 2009
Student's signature ……..……..…
Thesis Advisors' signature 1……..……..……..... 2……..……..……..

48353902 : สาขาวิชาเทคโนโลยีเภสัชกรรม
คำสำคัญ : พหุอิมัลชัน / เสถียรภาพของยา / กลไกการปลดปล่อยยาแบบแพร่

นันทชัย หาญประมุขกุล : การพัฒนาพหุอิมัลชันเพื่อเพิ่มเสถียรภาพของยา. อาจารย์ที่ปรึกษาวิทยานิพนธ์ : รศ.ดร.เกษร จันทร์ศิริ และ รศ.ดร.สมลักษณ์ คงเมือง. 159 หน้า.

พหุอิมัลชันถูกนำมาประยุกต์ใช้ประโยชน์ได้หลากหลายทางเภสัชกรรม งานวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาประสิทธิภาพของยาเตรียมรูปแบบพหุอิมัลชัน ชนิดน้ำในน้ำมันในน้ำในการเพิ่มเสภียรภาพของยาที่ละลายน้ำได้ โดยใช้คลินดามัยซินฟอสเฟตเป็นยาตัวอย่าง การทดลองทำโดยศึกษาเสถียรภาพของคลินดามัยซินฟอสเฟต ในสารละลาย และพหุอิมัลชัน ชนิดน้ำในน้ำมันในน้ำ เป็นเวลา 3 เดือน และวิเคราะห์ปริมาณคลินดามัยซินฟอสเฟต ที่เหลืออยู่ที่เวลาต่างๆ ในภาวะเร่งที่อุณหภูมิ 40 และ 4 องศาเซลเซียสโดยใช้โครมาโทกราฟฟีชนิดของเหลวสมรรถนะสูง พบว่ายาคลินดามัยซินฟอสเฟตในรูปแบบพหุอิมัลชันมีเสถียรภาพดีกว่าสารละลาย โดยพบว่าอัตราเร็วของการสลายตัวของยาคลินดามัยซินฟอสเฟต ในยาเตรียมรูปแบบพหุอิมัลชันต่ำกว่าสารละลายอย่างมีนัยสำคัญ ($p < 0.05$) ซึ่งเชื่อว่าเป็นผลมาจากการที่พหุอิมัลชันช่วยป้องกันการสลายตัวคลินดามัยซินฟอสเฟต จากกลไกไฮโดรไลซีสโดยน้ำในวัตภาคภายนอก และเมื่อทำการศึกษาการปลดปล่อยคลินดามัยซินฟอสเฟต ออกจากพหุอิมัลชันพบว่ามีการปลดปล่อยโดยใช้กลไกการแพร่ การเพิ่มความหนืดของชั้นน้ำมันโดยการเพิ่มปิโตรลาตุ้มลงในชั้นน้ำมันของพหุอิมัลชัน สามารถเพิ่มเสถียรภาพของคลินดามัยซินฟอสเฟต และเพิ่มการปลดปล่อยของคลินดามัยซินฟอสเฟต จากพหุอิมัลชัน เมื่อเปรียบเทียบกับตำรับพหุอิมัลชันที่ไม่มีการเพิ่มความหนืดของชั้นน้ำมัน การเพิ่มปิโตรลาตุ้มในตำรับพหุอิมัลชันทำให้ขนาดของหยดน้ำมันใหญ่ขึ้น และความหนืดของพหุอิมัลชันลดลง ส่วนความหนืดของวัตภาคน้ำภายนอกไม่มีผลกับเสถียรภาพของคลินดามัยซินฟอสเฟต และการปลดปล่อยของคลินดามัยซินฟอสเฟตออกจากพหุอิมัลชัน

สาขาวิชาเทคโนโลยีเภสัชกรรม บัณฑิตวิทยาลัย มหาวิทยาลัยศิลปากร ปีการศึกษา 2552
ลายมือชื่อนักศึกษา .....
ลายมือชื่ออาจารย์ที่ปรึกษาวิทยานิพนธ์ 1..... 2.....

ACKNOWLEDGEMENTS

Graduate study seems to be a burdensome task for me until I knew how many people have been supporting and inspiring me.

To my advisor, Associate Professor Dr. Gaysorn Chansiri. I would like to thank for her guidance and advice on both technical and financial support, for her helpful suggestion of the problem, especially her assistance in the preparation of my thesis and research manuscript.

To my co-advisor, Associate Professor Dr. Somlak Kongmuang. I would like to thank for his guidance and meaningful advice, and for his help in making all contacts possible and successful. For Dr. Narueporn Sutanthavibul, I would like to thank for her criticism on my thesis manuscript.

To Department of Pharmaceutical Technology, Faculty of Pharmacy, Silpakorn University, and Department of Manufacturing Pharmacy, Department of Pharmaceutical Technology, Faculty of Pharmacy, Rangsit University, I would like to thank for facilitating for all tools, equipment, chemical and many more, including all of people whom my thesis concerned with, for their assistance during my study.

To K.B. Pharmaceutical Co., Ltd., Newcharoen Pharmaceutical L.P. and Unison Laboratories Co., Ltd., thank for providing equipment in making this thesis possible, including all people whom my thesis concerned with for their assistance, especially Mr. Alongkorn Nimsuwan, Mr. Chaiwut Chinsriwongkul for their help, kindness and consult.

To my laboratory brother, sisters and friends, thanks for their assistance and kindness, and to my friends, I would like to thank for their assisting and entertaining me through the years.

To my family, I would like to thanks for their kind understanding, warmth and wonderful job as both financial and emotional backing. Moreover, thanks for taking care of me physically and encouraging me though all the obstacles.

Nuntachai Hanpramukkun

CONTENTS

Page

LIST OF TABLES

Table Page

LIST OF FIGURES

CHAPTER 1
INTRODUCTION

Emulsions are dispersed, multiphase systems consisting of at least two insoluble liquids. The dispersed phase is present in the form of droplets in a continuous phase. Depending on the emulsification process, the diameter of the droplets lies between 0.1 μm and 100 μm. Emulsions of this kind are thermodynamically unstable, which means that there is tendency to reduce the interface (as a result of a relatively high interfacial tension), causing the droplets to coalesce and therewith decreasing the total amount of interface.

Multiple emulsion or double emulsion is an emulsion in an emulsion. Two main types of multiple emulsions can be distinguished : water-in-oil-in-water (w/o/w) multiple emulsion, in which a water-in-oil (w/o) emulsion is dispersed as droplets in an aqueous phase, and oil-in-water-in-oil (o/w/o) multiple emulsion, in which an oil-in-water (o/w) emulsion is dispersed in an oil phase. Water-in-oil-in-water (w/o/w) multiple emulsions are more common than oil-in-water-in-oil (o/w/o) multiple emulsions. Multiple emulsions contain more interfaces and are even more thermodynamically unstable than single emulsions.

The most common multiple emulsions are of w/o/w but in some specific applications o/w/o emulsions can also be prepared. Many potential applications for multiple emulsions are aimed for slow and sustained release of active matter from an

internal reservoir into the continuous phase (mostly water). Multiple emulsions can serve also as an internal reservoir to entrap matter from the outer diluted continuous phase into the inner confined space. In other applications, multiple emulsions are reservoirs for improved dissolution or solubilization of insoluble materials. The active matter will dissolve in part in the inner phase, in part at the internal and occasionally at the external interface. Protection of sensitive and active molecules from oxidation in the external phase can also be observed (Benichou, Aserin and Garti 2004 : 29). Multiple emulsions system has been previously used to enhance the stability of ascorbic acid (Gallarate et al. 1999 : 241) and vitamin A (Yoshida et al. 1999 : 5).

A number of factors have been identified as affecting the stability of w/o/w multiple emulsions. These include the method of preparation, the composition of the emulsion, i.e. the nature of oil phase, type of emulsifiers and nature of entrapped materials as well as the presence of electrolytes. Other parameters such as phase volume, concentration of components also need to be considered (Benichou, Aserin and Garti 2004 : 32-33).

Clindamycin is a semisynthetic antibiotic which is a derivative of lincomycin. Clindamycin generally is used for the treatment of serious infections caused by susceptible gram-positive bacteria and anaerobic bacteria (McEvoy et al. 2007 : 3437 -3442). The drug has a solubility of approximately 400 mg/mL in water at 25 °C (Lund 1994 : 809). In this study, clindamycin phosphate was used as a model water soluble drug and was dissolved in the inner aqueous phase of water-in-oil-in-water multiple emulsions. Clindamycin phosphate was reported to show

degradation by hydrolysis mechanism (Lund 1994 : 810). In this research multiple emulsions were expected to improve stability of this drug. The aim of this study was also to determine the effect of formulation factors and producing process on the physical stability of multiple emulsion. Drug release profile and release kinetics of clindamycin phosphate loaded multiple emulsion were investigated as well.

CHAPTER 2

LITERATURE REVIEWS

An emulsion is heterogeneous preparation composed of two immiscible liquids (by conventional described as oil and water), one of which is dispersed as fine droplets uniformly throughout the other. Emulsions are thermodynamically unstable and reverse back to separate oil and water phase by fusion or coalescence of droplets unless kinetically stabilized by third component, the emulsifying agent or emulsifier. The phase presented as small droplets is called the discontinuous, dispersed, or internal phase and the supporting liquid is known as the continuous or external phase. Droplet diameters vary enormously, but in pharmaceutical emulsions they are typically polydispersed with diameters ranging from approximately 0.1 to 100 μm. Emulsions are conveniently classified as oil-in-water (o/w) or water-in-oil (w/o), depending on whether the continuous phase is aqueous or oily. Figure 1A and 1B show diagrams of simple o/w system and w/o system. Multiple emulsions, which are prepared from oil and water by the emulsification of an existing emulsion so as to provide two dispersed phases, are also of pharmaceutical interest. Multiple emulsion of the oil-in-water-in-oil (o/w/o) type is w/o emulsions in which the water globules themselves contain dispersed oil globules; conversely, water-in-oil-in-water (w/o/w) emulsions are those where the internal and external aqueous phases are separated by oil (Figure 2) (Eccleston, in Swarbrick, eds. 2002 : 1066). These complex emulsions are covered by the Broader International Union of Pure and Applied Chemistry

(IUPAC) definitionof emulsions. This extends the classical definition to include "liquid droplets and/or liquid crystals dispersed in a liquid".

Multiple emulsion or double emulsions consists of large and polydispersed droplets that are thermodynamically unstable with a strong tendency for coalescence, flocculation and creaming. The most common multiple emulsions are of w/o/w but in some specific applications o/w/o emulsions can also be prepared. Multiple emulsion promising application in the food industry (low calorie product, improved sensory characteristics, taste masking), cosmetic industry (easily spreadable cream with encapsulated ingredients in both water and oil phase), pharmaceutical industry (drug delivery systems) and other fields like agriculture and the production of multicompartment microspheres. Many potential pharmaceutical applications for multiple emulsions are aimed for slow and sustained release of active matter from an internal reservoir into the continuous phase (mostly water). Multiple emulsions can serve also as an internal reservoir to entrap matter from the outer diluted continuous phase into the inner confined space. In other applications, multiple emulsions are reservoirs for improved dissolution or solubilization of insoluble materials. The active matter will dissolve in part in the inner phase, in part at the internal and occasionally at the external interface. Protection of sensitive and active molecules from oxidation in the external phase can also be observed. (Benichou, Aserin and Garti 2004 : 29)

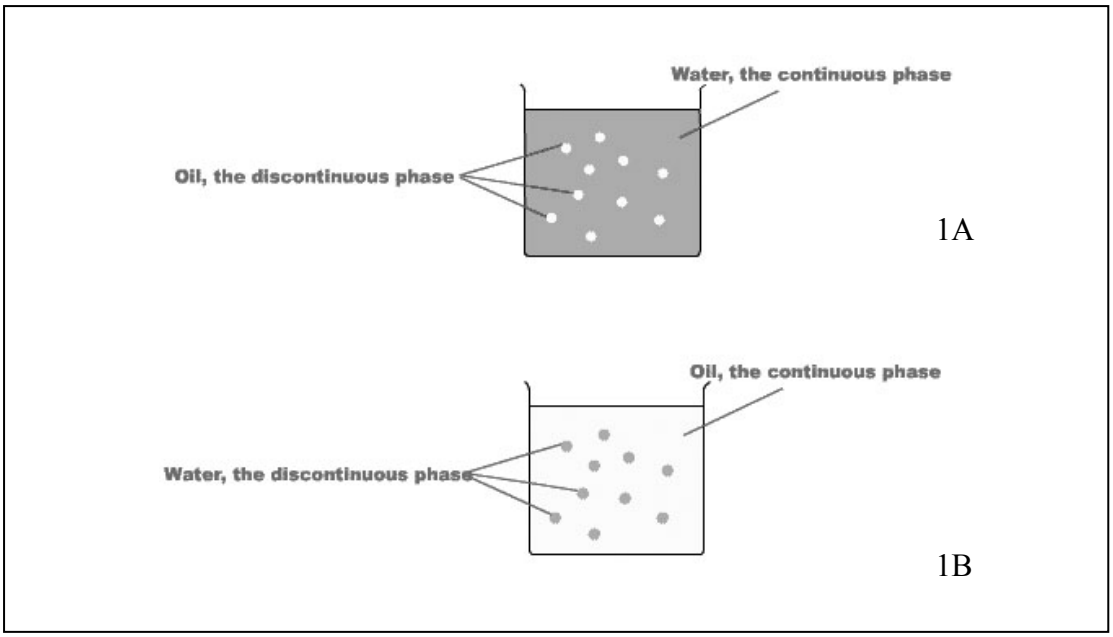


Figure 1 Simple emulsions

Keys : (1A) oil-in-water emulsions, (1B) water-in-oil emulsions

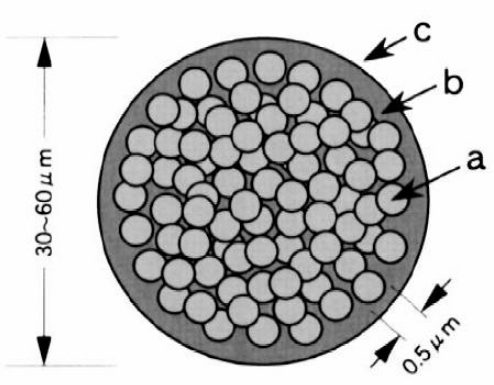


Figure 2 Ideal structure of w/o/w emulsions particle.

Keys : (a) Inner water phase; (b) middle oil phase; (c) Outer water phase

Source : Tadao Nakashima, Masataka Shimizu, and Masato Kukizaki, "Particle control of emulsion by membrane emulsification and its applications," Advanced drug delivery reviews 45 (2000) : 53.

Preparation of multiple emulsions

1. Two-step emulsification

Multiple emulsions may be prepared in the laboratory by the re-emulsification of primary emulsion. A two-step procedure is therefore, necessary (Figure 3). The first stage involves the preparation of the primary emulsion which, in the preparation of a w/o/w emulsion, is a w/o emulsion. In the second step, the primary emulsion is further emulsified in water to form the multiple emulsions. The primary emulsion may be prepared in the usual manner; for example, with a laboratory mixer, by ultrasonication, etc. In this case, a lipophilic surfactant is used to promote the formation of a w/o emulsion. This emulsion is then poured into a solution or dispersion of the secondary surfactant in water. The secondary surfactant in this case, is hydrophilic to promote o/w emulsification in which the "oil" phase is the w/o emulsion. The second emulsification step is critical, as excess mixing can fracture the drops, resulting in simple oil in water emulsion. The small internal water droplets are lost and mix with the external aqueous phase as the oil drops are separated apart (Florence and Whitehill 1982 : 281). Multiple emulsions system has been previously used to enhance the stability of ascorbic acid (Gallarate et al. 1999 : 241) and vitamin A (Yoshida et al. 1999 : 2).

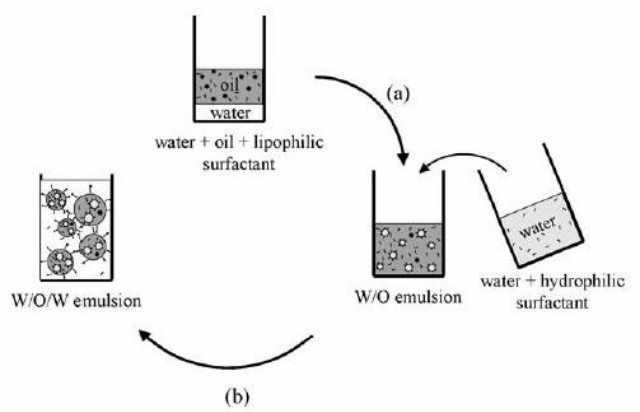


Figure 3 Preparation of a w/o/w emulsions in two steps : a high-shear emulsification step with lipophilic surfactants for the w/o emulsion (a) and a low shear emulsification step with hydrophilic surfactants for the w/o/w emulsion (b).

Source : S. Van der graaf, C.G.P.H. Schroën, and R.M. Boom, "Preparation of double emulsions by membreane emulsification-a review," Journal of membrane science 251 (2005) : 8.

2. Membrane emulsification

Water-in-oil-in-water (w/o/w) multiple emulsion are usually prepared in a two step emulsification process using two surfactants; a hydrophobic one designed to stabilize the internal droplets and a hydrophilic one for the external interface of the oil globules. The primary emulsion (w/o) is prepared under high shear conditions to obtain small droplets. So w/o/w multiple emulsion can be made by passing a w/o emulsion through a hydrophilic membrane. Membrane emulsification, in which the pressurized dispersed phase permeates a microporous membrane and form emulsion droplets. The mean size of external droplets can be precisely controlled by the membrane mean pore size. (Vladisavljević and Williams, in Aserin, eds. 2008 : 137)

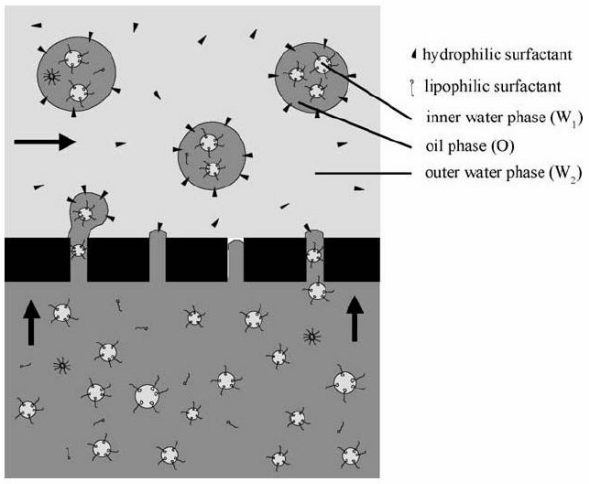


Figure 4 Schematic drawing of the production of a multiple emulsion (w/o/w) by membrane emulsification with a simple emulsion as dispersed phase. The arrows represent the direction of the fluid flow.

Source : S. van der Graaf, C.G.P.H. Schroën, and R.M. Boom, "Preparation of double emulsions by membrane emulsification-a review," Journal of membrane science 251 (2005) : 10.

3. Cross-flow membrane emulsification

Membrane emulsification, the emulsion to be dispersed phase is pressed through a microporous membrane while the continuous phase flows along the membrane surface. Droplets grow at pores and detach at a certain size, which is determined by the balance between the force acting on the droplet. The main forces are the drag force and interfacial tension force. With pores that are not cylindrical, another force is important, and can be even dominant is same cases. This is the force resulting from deformation of the dispersed growing from non-cylindrical pore will form a droplet radius that is larger than the internal smallest radius in the pore (Van der Graaf, Schroën and Boom 2005 : 9). w/o/w multiple emulsion were produced my

membrane emulsification with Shirasu porous glass (SPG) membrane. Microfluidizer was used for the primary emulsion (w/o) and SPG membranes was used for second emulsification step. It was found that the membrane had to be hydrophilic and needed to have an average pore size of at least twice the diameter of the primary water droplets of w/o emulsion, otherwise these droplets will be rejected by the membrane. The concentration of internal water droplets for w/o/w multiple emulsion should be between 30 and 50%.

4. Microchannel

The first step emulsification consists of preparing pre-mixed polydisperse w/o emulsions. The pre-mixed w/o emulsion is prepared using a homogenizer. The homogenizer rotation speed and mixing time are controlled, as the pre-mixed w/o emulsion has droplets with diameters from several to 100 μm. Microchannel emulsification is used for the second step, the apparatus for microchannel emulsification consists of a silicon microchannel plate, a microchannel module and liquid chambers supplying continuous and dispersed phase. Figure 6 depicts the experiment setup and schematic flow of the pre-mixed w/o emulsion phase through the silicon microchannel. The emulsification is observed through the glass plate using a microscope. The microchannel module is initially filled with the external water phase. The w/o emulsion, which is pressurized by the head difference of the liquid chamber, entered the space between the silicon microchannel plate and the glass plate, and w/o/w multiple emulsion droplets are formed from the microchannel. The prepared emulsion is recovered by an external water phase flow (Sugiura et al. 2004 : 223).

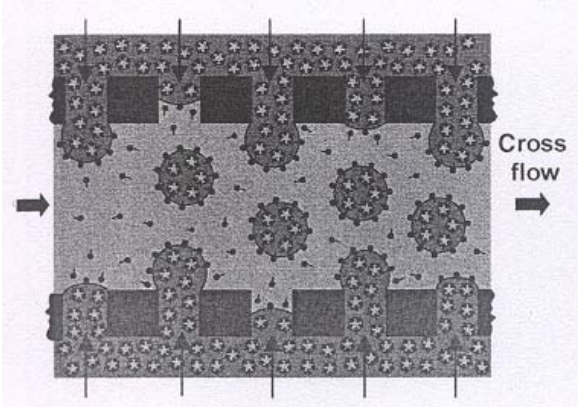


Figure 5 Production of multiple emulsion using cross-flow membrane emulsification.

Source : Abraham Aserin, Multiple emulsion technology and applications (New jersey : John wiley & sons, Inc., 2008), 137.

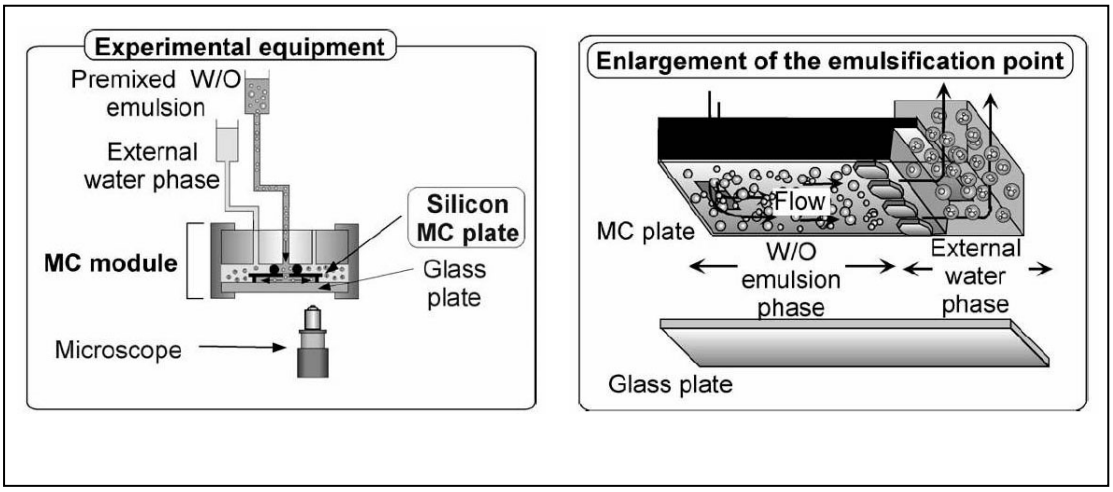
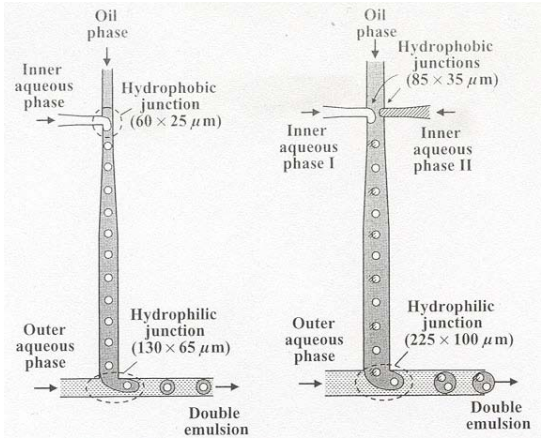


Figure 6 Schematic flow of premixed w/o emulsion phase through the microchannel.

Source : Shinji Sugiura et al., "Preparation characteristics of water-in-oil-in-water multiple emulsions using microchannel emulsification," Journal of colloid and interface science 270 (2004) : 223.

5. Microfluidic devices

Another way to prepare multiple emulsion is through the use of a microcapillary device with which single droplet with a single internal droplet is fabricated. This device consists of cylindrical glass capillary tubes nested within a square glass tube. The innermost fluid is pumped through a tapered cylindrical capillary tube and the middle fluid is pumped through the outer coaxial region. Such a device can generate multiple emulsion dispersed in hydrophobic or hydrophilic fluids (Muschiolik 2007 : 215).



(a) (b)

Figure 7 Production of multiple emulsion using microfluidic with opposite surface wettabilities.

Keys: (a) Production of w/o/w multiple emulsion in which each outer drop contains a single inner droplet

(b) Producution of w/o/w multiple emulsion in which each outer drop contains two inner droplets consisting of different aqueous phase I and II

Source : Abraham Aserin, Multiple emulsion technology and applications (New jersey : John wiley & sons, Inc., 2008), 146.

Stability of multiple emulsions

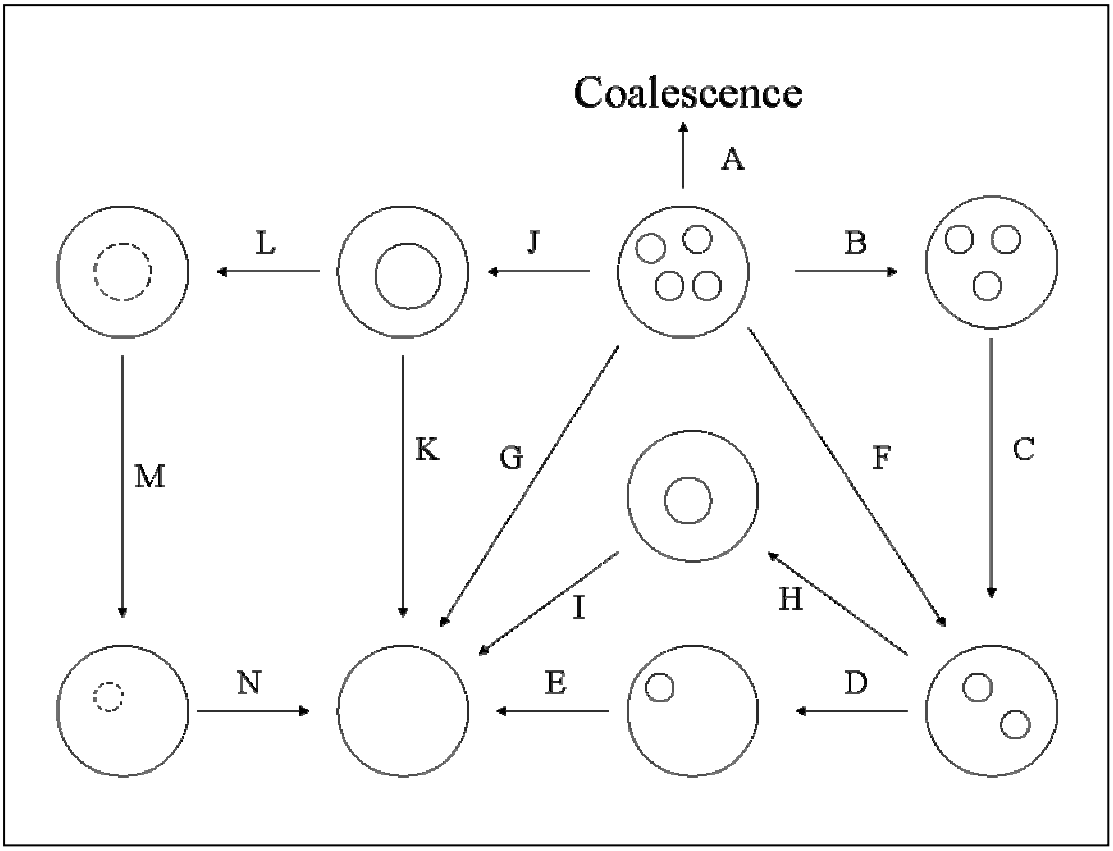


Figure 8 Representation of the possible breakdown pathways which may occur in w/o/w system.

Source : A.T. Florence and D. Whitehill., "The formulation and stability of multiple emulsions," International journal of pharmaceutics 11 (1982) : 304.

Some of the possible instability mechanisms which are possible in a w/o/w system are shown in Figure 8. The multiple (oil) drop may coalesce with other oil drops, simple or multiple (A); the internal aqueous droplets may be expelled individually (B, C, D, E) or more than one may be expelled (F); or they may be less frequently expelled in one step (G); the internal droplets may coalesce before being expelled (H, I), (J, K); or water may pass by diffusion through the oil phase gradually

resulting in shrinkage of the internal droplets (L, M, N). Whether all these mechanisms occur in all systems is not clear; neither is the relative importance of each mechanism in different w/o/w systems. On the other hand, this may be oversimplified and a combination of the above events may take place. (Florence and Whitehill 1982 : 303-304)

Factors affecting stability of multiple emulsions

The factors affecting stability of w/o/w multiple emulsions are as follows:

1. Properties of primary emulsion
    1.1 Effect of electrolyte
        1.1.1 Effect of electrolyte on osmotic pressure
        1.1.2 Effect of electrolyte on interfacial film
    1.2 Effect of emulsifier concentration
    1.3 Effect of phase volume ratio
2. Properties of membrane phase
3. Properties of interfacial film
    3.1 Effect of emulsifier film strength
    3.2 Effect of polymer addition
        3.2.1 Effect of polymer adding on internal water phase
        3.2.2 Effect of polymer adding on external water phase
            3.2.2.1 Polymer
            3.2.2.2 Protein-polysaccharide

1. Properties of primary emulsion

Since primary emulsion is the first step of multiple emulsion preparation. The stability of primary emulsion is very important. Good stability of primary emulsion leads to good stability of multiple emulsions.

1.1 Effect of electrolyte

Electrolyte appears to be one of the most important factors in determining the stability and release of materials from the internal droplets. Electrolyte in the internal water phase has an influence on 2 properties of primary emulsion: (a) osmotic pressure (b) interfacial film. The former is peculiar to multiple systems. The effects of electrolytes on electrical double layers and other properties have not been considered as they are not specific to multiple systems. (Florence and Whitehill 1982 : 293)

1.1.1 Effect of electrolyte on osmotic pressure

Under the influence of osmotic gradient, oil phase of w/o/w emulsion are acting as a semipermeable membrane between the two aqueous phases, resulting in the passage of water across the oil phase. This leads to either swelling or shrinking of the internal droplets, depending on the direction of the osmotic gradient. Higher osmotic pressure in the external environment, compared to that in the internal phase, leads to shrinkage of the internal aqueous droplets, and/or rapture of the oil layer.

If the osmotic pressure is higher in the internal aqueous phase, water may pass to this phase resulting in swelling of the internal droplets, which eventually burst to release the contents. The reverse is true if the osmotic pressure is higher in the external aqueous phase and this causes shrinkage of the internal droplets. If the osmotic pressure difference across the oil layer is extreme, then passage of water is so

rapid that almost immediate rupture of the oil drops occurs with expulsion of the internal droplets. Material other than electrolytes, such as proteins and sugars and of course drugs, in the either aqueous phase can also exert this effect. The problem can be partially solved by the addition of small amounts of sodium chloride to the internal aqueous phase so that this phase is isotonic with the final external phase. The osmolarity may also be adjusted by the addition of other materials such as glucose or glycerol. Wen and Papadopoulus (2001 : 398-404) observed single w/o/w globule through capillary videomicroscopy. They find that water migration in multiple emulsions ie affected by the osmotic pressure between the two aqueous phases. In all examined cases, salt such as NaCl in the aqueous phase could not bulk-diffuse across the oil layer and be transported from one aqueous phase to the other. When internal aqueous droplets (W_1) are pure water and external aqueous phase (W_2) is NaCl solution, the entire W_1 drops would enter W_2 and disappeared. If both W_1 and W_2 initially contains salt in same concentration the system would get equilibrated at some asymptotic drop size for W_1. Wen and Papadopoulus (2000 : 161-167) The observation of water migration through capillary video microscopy technique is also used to quantitative study of the mass transfer in $W_1/O/W_2$ emulsion globules. A significant difference of water transport roles is observed between visually contacting and non-contacting W_1/O and O/W_2 interfaces. The mechanism of water transport between visually contacting W_1 and W_2 phases are realized mainly by the diffusion of hydrated surfactants, whereas at no contact spontaneously emulsified droplets and reverse micelles are nearly constant despite variations in the oil-layer thickness. Since transport via hydrated surfactants is significantly faster than via spontaneously

emulsified droplets and reverse micelles. It may be the primary way for water migration in $W_1/O/W_2$ emulsions.

1.1.2 Effect of electrolyte on interfacial film

The addition electrolyte such as sodium chloride into w/o/w multiple emulsion results in competition of sodium chloride and surfactant for water molecules at the inner w-o interface. This would result in a rigid interfacial layer which is a more effective mechanical barrier to drug transfer. (Omotosho 1990 : 83)

1.2 Effect of emulsifier concentration

Multiple emulsions can be prepared by re-emulsification of primary emulsion. First step, preparation of primary emulsion uses low HLB emulsifiers as primary surfactant to obtain water-in-oil emulsion. The primary emulsion is poured into a solution or dispersion of the high HLB emulsion in water. Second step, the high HLB emulsifier is used to promote a formation of an oil-in-water emulsion in which the “oil” phase is the water-in-oil emulsion. That relationship between primary emulsifier concentration and stability of multiple emulsions was observed. It is found that the stability of multiple emulsion increased with increasing primary emulsifier concentration. (Cróka and Erõs 1997 : 122)

1.3 Effect of phase volume ratio

Phase volume ratio of primary emulsion (water-in-oil emulsion) in multiple emulsions and phase volume ratio of water in primary emulsion (water-in-oil emulsion) have some effects on stability of multiple emulsions. Csóka and Erõs (1997 : 122) found that increasing ratio of primary emulsion (water-in-oil emulsion) in water-in-oil-in-water emulsion, the stability decreased and the multiple drop

breakdown became faster. The number of the multiple drops and specific surface area increases with increasing water-in-oil emulsion ratio which led to instability of multiple emulsions.

2. Properties of membrane phase

The properties of the membrane phase are of prime importance in determining the stability of the system. The most importance is viscosity. The stability of the membrane toward rupture and leakage of entrapped materials, however, decreased with decreasing viscosity. The thickness of the membrane phase and the type of surfactant used may also be important in determining stability.

3. Properties of interfacial film

In a second-order multiple emulsion system, there are two separate interfacial films to be considered. The nature of each film depends on the nature of the primary and secondary surfactants used to prepare the primary and multiple emulsions, respectively, and also on the presence of materials in the internal and continuous phase. The viscosity and elasticity of the oil-in-water and water-in-oil interfacial films are particularly important in determining the stability of both the primary and secondary emulsion by, for example, hindering the class approach of drops (by surfactant chain effects) and preventing coalescence (by acting as a mechanical barrier). Since the oil-in-water and water-in-oil interfaces are in close proximity to each other, diffusion of the emulsifier between the interfacial films may take place, thereby changing the composition and thickness of absorbed multilayer, presumably until equilibrium is reached. (Rosano, Gandolfo and Hidrot 1998 : 116-120)

3.1 Effect of emulsifier film strength

Emulsifiers stabilize simple emulsions by reduction of interfacial tension or formation of a mechanical or electrical interfacial barrier, or both. Effective reduction of interfacial tension aids dispersability and enhances the formation of smaller droplets. Smaller internal droplet size usually results in enhancing kinetic stability of simple emulsions to creaming. However, the strength of the mechanical barrier might be more important than interfacial tension to long-term emulsion stability. This non-ionic surfactant substantially reduces interfacial tension. It has been speculated that the film strength of both the primary and secondary interfaces are major factors influencing multiple emulsion stability. The primary interface is composed of lipophilic surfactants whereas both lipophilic and hydrophilic surfactants are present at the secondary interface. (Opawale and Burgess 1998 : 967-973; Jiao, Rhodes and Burgess 2002 : 446-450; Hou and Papadopoulus 1997 : 183-187)

3.2 Effect of polymer addition

A major problem associated with w/o/w emulsions is creaming, which is probably due to the large size of the multiple drops. Creaming may be reduced by increasing the concentration of secondary surfactant but drug release may be significantly retarded. In any case, high surfactant concentrations are not desirable in pharmaceutical systems from the point of view of toxicity. The use of a thickening agent in the external aqueous phase may also reduce creaming but one must be certain that the emulsions retain the characteristics of pourability. Polymer is used for improving the stability of multiple emulsion and controlling release of the active material. Polymer can be added to internal and external aqueous phase.

3.2.1 Effect of polymer added to internal aqueous phase

Omotosho (1989 : 83-84) finds that adding polymer or macromolecules such as acacia, gelatin, polyvinyl pyrolidone to internal aqueous phase increased stability of w/o/w emulsion. The stability enhancement of w/o/w emulsion is resulted from interfacial complexation of non-ionic surfactant and macromolecules. Complex formation at inner water-oil interface improved stability of the multiple emulsions by improving the rigidity of the interface surfactant film around the internal interface.

3.2.2 Effect of polymer added to external aqueous phase

Following emulsification, emulsion stabilization can be achieved through interference with creaming, droplet flocculation or coalescence. Thus, stability can be improved by increasing viscosity of the external aqueous phase or increasing the concentration of secondary emulsifier. Disadvantages of increasing the concentration of secondary emulsifier are high toxicity and lead to instability of multiple emulsion. The viscosity of multiple emulsions can be increased by addition of polymer or protein-polysaccharide to external aqueous phase.

3.2.2.1 Polymer

Kamouni et al. (2002 : 245-247) finds that all w/o/w emulsions prepared without a thickener separated over time. They use polymer with appropriate thixotropic properties to prevent separation. However, the polymer used does not interact destructively with other ingredients. Polymer in external aqueous phase increased viscosity of multiple emulsion which lead to decreasing creaming or coalescence.

3.2.2.2 Protein-Polysaccharide

The use of macromolecular amphiphiles and stabilizers, such as proteins and polysaccharides, is long adopted by scientists exploring stability of multiple emulsions. The proteins such as gelatin, whey protein, bovine serum albumin, caseins and other proteins are used usually in combination with other monomeric emulsifiers. A significant improvement in the stability of the emulsion is shown when these macromolecules were encapsulated into the external interface. Protein, polysaccharides and their blends are natural surface active biopolymers. Under specific conditions, the proteins and polysaccharides form complexes with enhanced functional properties in comparison to the proteins and polysaccharides alone. The complexes form with electrostatic complexation between oppositely charged proteins and polysaccharides allows better anchoring of the new-formed macromolecular amphiphile onto oil-water interfaces. Complexation between proteins and polysaccharides at the emulsion droplet surface improves steric stabilization by forming thick multilayered coating on the droplets. (Benichou, Aserin and Garti 2007 : 31)

Creaming phenomenon

Other instabilities in w/o/w multiple emulsion are related to the creaming phenomenon. The creaming phenomenon depends on oil droplet size, continuous aqueous phase viscosity and density difference according to stoke's law. For monodispersed droplets, creaming rate is depicted by

$$v = \frac{2\, g\, r^2 \left(\rho_1 - \rho_2\right)}{9\,\eta} \qquad (1)$$

and for polydispersed droplets

$$v = \left[\frac{\Sigma 8\pi}{27\,\eta\, v}\right]\left[g\, n_i\, r^2 \left(\rho_1 - \rho_2\right)\right] \qquad (2)$$

Where g is the gravimetric constant, r is the radius of the droplets, $\rho_1 - \rho_2$ is the density difference between the dispersed phase and the continuous phase, and η is the viscosity of the continuous (dispersion) phase. Reducing the oil globule size, increasing the continuous aqueous phase viscosity, and reducing density difference will minimize the creaming phenomenon. The most common solution for creaming is the use of polymeric nonadsorbing compounds that increase the viscosity of the external aqueous phase and retard (or slow down) creaming of the w/o globules (Lutz and Aserin, in Aserin, eds. 2008 : 95).

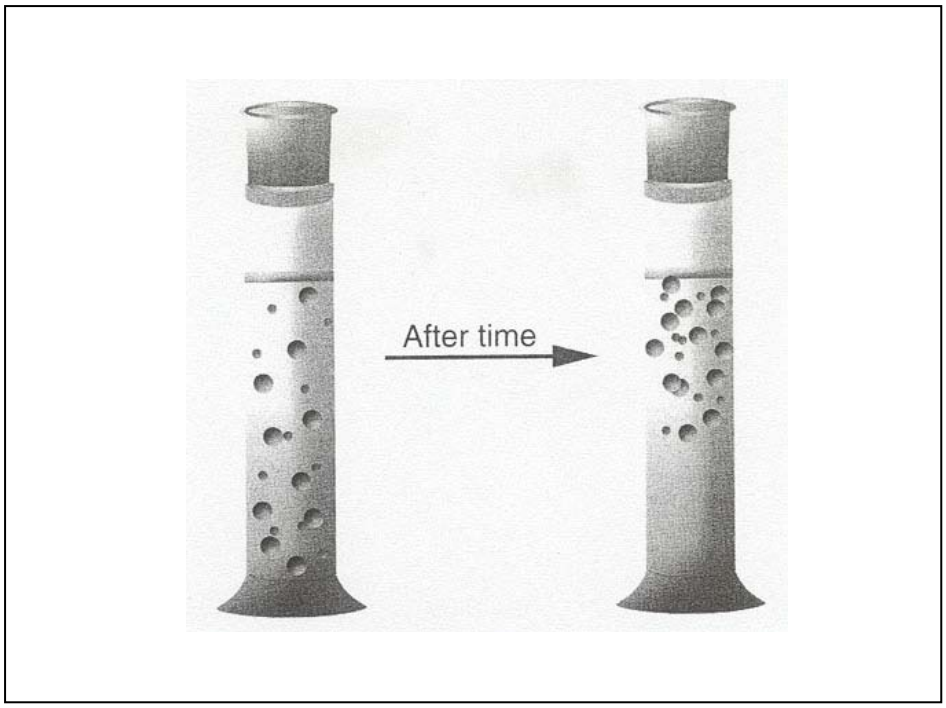


Figure 9 Illustration of creaming phenomenon

Source : Abraham Aserin, Multiple emulsion technology and applications (New jersey : John wiley & sons, Inc., 2008), 95.

Stabilization of multiple emulsion

Stability of w/o/w multiple emulsion is generally understood as the resistance of the individual globules to coalescence. The breakdown of a w/o/w multiple emulsion is described through several possible mechanisms, including (i) coalescence of the internal aqueous droplets into larger internal droplets; (ii) coalescence of the oil droplets suspended in the external aqueous phase; (iii) expulsion of the internal droplets following rupture of the thin oil films during the interaction of the internal and external aqueous phase; and (iv) swelling or shrinking due to water permeation through the oil membrane by diffusion.

1. Stabilization by monomeric emulsifiers

Most of the researches are studied for the proper monomeric emulsifier blend or combination (hydrophilic and hydrophobic) to be used at the two interfaces and the proper ratio between the two. Monomeric surfactant can migrate from w/o interface to the oil phase and hydrophilic surfactant can be transported to the inner interface and other external emulsifiers required calculation of an effective HLB value of emulsifiers to optimize the stabilization of the emulsion. In most cases the internal emulsifier is used in great excess relative to the external emulsifier. The nature of the emulsifiers also dictated the number of compartments and the internal volume that the inner phase occupies (Garti 1998 : 84).

2. Stabilization by polymerizable emulsifiers (Lutz, in Aserin, eds. 2008 : 88)

Polymeric amphiphiles are known to be multi-anchoring amphiphiles with irreversible adsorption capabilities that can improve the droplets interfacial coverage during emulsification since they provide strong steric stabilization capabilities (Figure 10). The gain in free energy by such adsorption is much greater than that of the absorbed monomeric surfactants. The polymeric amphiphiles form thick and flexible film that is strongly anchored into the oil-water interface in most cases. There are three mechanisms of stabilization with polymeric amphiphiles :

1. Steric stabilization resulting from hydrophobic interactions among adsorbed polymers.

2. Depletion stabilization by nonadsorbing macromolecules that prevent collision between droplets and provide elasticity to the system.

3. Electrostatic repulsion between two droplets carrying the same charge.

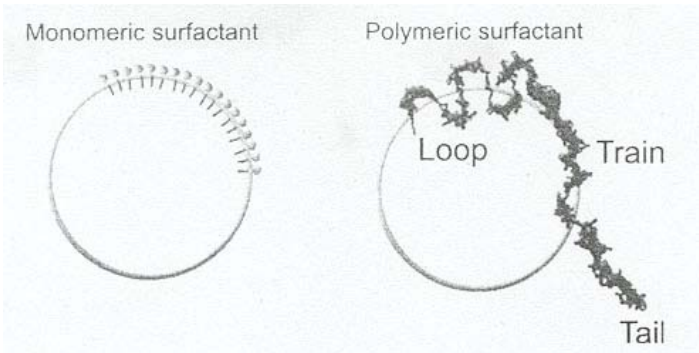


Figure 10 Schematic representation of monomeric and polymeric surfactant adsorption on interface.

Source : Abraham Aserin, Multiple emulsion technology and applications (New jersey : John wiley & sons, Inc., 2008), 88.

3. Stabilization by proteins and polysaccharides

Steric stabilization mechanism by macromolecules (adsorbing onto the interface and forming full coverage of thick flexible and well-anchored moieties) proved to be good solution for food colloid stability problem and for some food oil-in-water emulsions. The use of macromolecular amphiphiles and stabilizers such as protein and polysaccharides, is adopted by scientists exploring stability of multiple emulsion. Gelatin (Omotosho 1990 : 82), Whey proteins (Lizarraga et al. 2008 : 869), Whey protein-xanthan gum complex (Benichou, Aserin and Garti 2004 : 37; Benichou, Aserin and Garti 2007 : 22), Bovine serum albumin (BSA) (Garti, Aserin and Cohen 1994 : 42), Sodium alginate (Weiss, Scherzo and Muschiolik 2005 : 607), Sodium caseinate (Su et al. 2006 : 262; Regan and Mulvihill 2009 : 2340), Carageenan, Locust bean gum (Benna-Zayani et al. 2008 : 47), Gum arabic (Su, Flanagan and Singh 2008 : 113) and Human serum albumin (HAS) are useful as

macromolecular stabilizers. The protein is used usually in combination with other monomeric emulsifiers. A significant improvement in the stability of the emulsion is shown when these macromolecules were encapsulated onto the external interface. In most cases the macromolecule is used at low concentrations (maximum 0.2 % w/w) and in combination with a large excess of nonionic monomeric emulsifiers.

Garti, Aserin and Cohan (1994 : 42) found that bovine serum albumin (BSA) with monomeric emulsifiers are envisaged both in the inner and the outer interfaces, and found significant improvement both in the stability and in the release of markers as compared to the use of the protein in the external phase only. It is postulated that while the BSA has not shown stability effect at the inner phase, there is a strong effect on the release of the markers (mechanical film barrier). However, BSA together with small amount of monomeric emulsifiers (or hydrocolloids), serves as good steric stabilizers and improve stability and shelf-life. BSA plays double roles in the emulsions which are film former and steric stabilizers at the external interface.

Cyclodextrin (alpha, beta and gamma) is shown to be potential stabilizers for o/w/o emulsion (Yu et al. 2003 : 3; Duchêne et al. 2003 : 88). The advantages of the cyclodextrins is there ability to complex formation with camphor at the oil/water interface resulting in the unnecessity for surfactant addition. It appear that the stabilizer efficacy depend on the nature of the oil and the type of the cyclodextrin (alpha > beta > gamma). The o/w/o stabilized by camphor-cyclodextrin complex as an emulsifier was not perfectly stable. The conditions of emulsion stability in the presence of a lipophilic active ingredient or any addition, depend on possible competition between the lipophilic molecule and the fatty acid residues of triglycerides to enter the cyclodextrin cavity.

4. Stabilization by solid particles

Stabilization of multiple emulsion is achieved by adsorption of solid fat particle onto the water-oil interface bridged by monomeric hydrophobic emulsifiers. In addition, it is clearly demonstrated that colloidal microcrystalline cellulose can be adsorbed as solid particle onto water/oil emulsion interface and thus improves their stability by mechanical action.

Garti et al. (1999 : 384) use the submicron crystalline fat particle α form of triglycerides as hydrophilic emulsifiers in the outer interface of w/o/w multiple emulsion in combination with other surfactants and achieved a significant improvement in stability over systems that contained just the emulsifiers.

Midmore and Herrington (1999 : 116) use the hydrophobic silica as hydrophobic emulsifiers in the oil phase to prepare the w/o/w and o/w/o multiple emulsions. The resulting multiple emulsion both w/o/w and o/w/o multiple emulsions prepared with hydrophobic silica are proved to be highly stable and showed no variation in multiple emulsion droplet size over a 6 months period.

5. Stabilization by increasing viscosity

Stabilization by increasing viscosity is obviously shown that restrict the mobility of the active matter in the different compartment of the multiple emulsion slow down coalescence and creaming. Attempts to increase the viscosity of (1) the internal aqueous phase (adding gums or hydrocolloids), (2) the oil phase (fatty acid salts) and (3) the external water (gums) are efficient only in applications that limited the uses of these systems to cosmetic preparations, in which a semi-solid emulsions have potential application (topical skin care, cream and body lotion uses).

Diffusion (Li and Jasti, in Ghosh and Jasti, eds. 2005 : 198-204)

Diffusion is defined as a process by which molecules transfer spontaneously from a region of higher concentration to a region of lower concentration. Diffusion is a result of random molecular motion. Although diffusion of molecules with a wide spectrum of physicochemical properties occurs in various conditions and situations, the diffusion process can be abstracted to a simple system involving the molecules of interest, a diffusional barrier, and a concentration gradient within the also called permeants and penetrants. The medium in which the diffusant migrates is called diffusional barrier. The concentration gradient is the concentration profile of the difusant in the diffusional barrier. The concentration gradient is the driving force for diffusion. Molecules move from high concentration region to a low concentration region in all directions. The number of molecules that diffuse through a unit area of the diffusional barrier in a given time is termed flux, J. Flux is the measurement of the rate of molecular diffusion through the diffusional barrier.

The diffusion process can be studied by using various methods, such as the permeation method, sorption and desorption kinetics, determination of concentration profile, etc. The most commonly used method in pharmaceutical research is the permeation method. The experimental set-up for this method consist of two chambers separated by a diffusional barrier. Two types of diffusion cells used in the permeation studies are shown in figure 11. A drug solution is changed to the donor chamber. The solution of the receiver chamber is removed partially or replenished with solvent or buffer solution at predetermined time intervals. When the receiver chamber is replenished with a solvent or a solution without a drug, the concentration of drug in the receiver chamber is maintained at minimum level. This is called sink condition.

To maintain sink condition, the concentration of a permeant in the receiver chamber is generally kept below 10% of its concentration in the donor chamber. In sink condition, Fick's first law can be simplified as

$$J = \frac{D\,K}{h} C_1 \qquad (3)$$

or

$$J = PC_1 \qquad (4)$$

The amount of diffusant permeating the diffusional barrier (the membrane in this case) is determined quantitatively by chemical analysis. Mathematically, the amount of cumulative permeation of diffusant (Q) can be derived from integration of equation 6 over the time of diffusion.

$$J = \frac{dQ}{dt} \cdot \frac{1}{A} = \frac{DK}{h} C_1 \qquad (5)$$

$$Q = PAC_1t \qquad (6)$$

Since the cumulative amount permeating the barrier (Q) at a given time (t) can be quantified and the concentration of donor chamber and the diffusional area are usually known, the permeation coefficient (P) can be obtained from the slope of a plot of cumulative permeation of diffusant vs. time.

It is essential to control the experimental conditions to obtain a reproducible result in the permeation studies. Factors that should be maintained

constant or kept consistent are medium or solvent temperature, and agitation of liquid in contact with the diffusional barrier. Owing to the formation of stagnant liquid layers or diffusion layers adjacent to the diffusional barrier or membrane, the thickness of a stagnant layer can be altered by the hydrodynamic movement of liquid surrounding the membrane. The stagnant layer can be minimized or diminished by the movement of liquid contents next to the diffusional barrier. In an in vitro permeation study, this is achieved by stirring. In a biological system, such as the gastrointestinal tract, gastrointestinal movement can effectively minimize the stagnant layer in the drug absorption process.

Factor affecting the release of substance in multiple emulsions

Multiple emulsions are complex dispersion systems; known also as "emulsions of emulsions". The most common multiple emulsions are of w/o/w type, although some specific applications o/w/o emulsions can also be prepared. Water-soluble active material is entrapped in the inner aqueous phase during the emulsification. Because of the osmotic pressure gradient, the active matter tends to diffuse and migrate from the internal phase to the external interface. Generally, two main drug release mechanisms from w/o/w emulsions can be distinguished as diffusion and emulsion breakdown or membrane rupture (Vasiljevic et al. 2006 : 171).

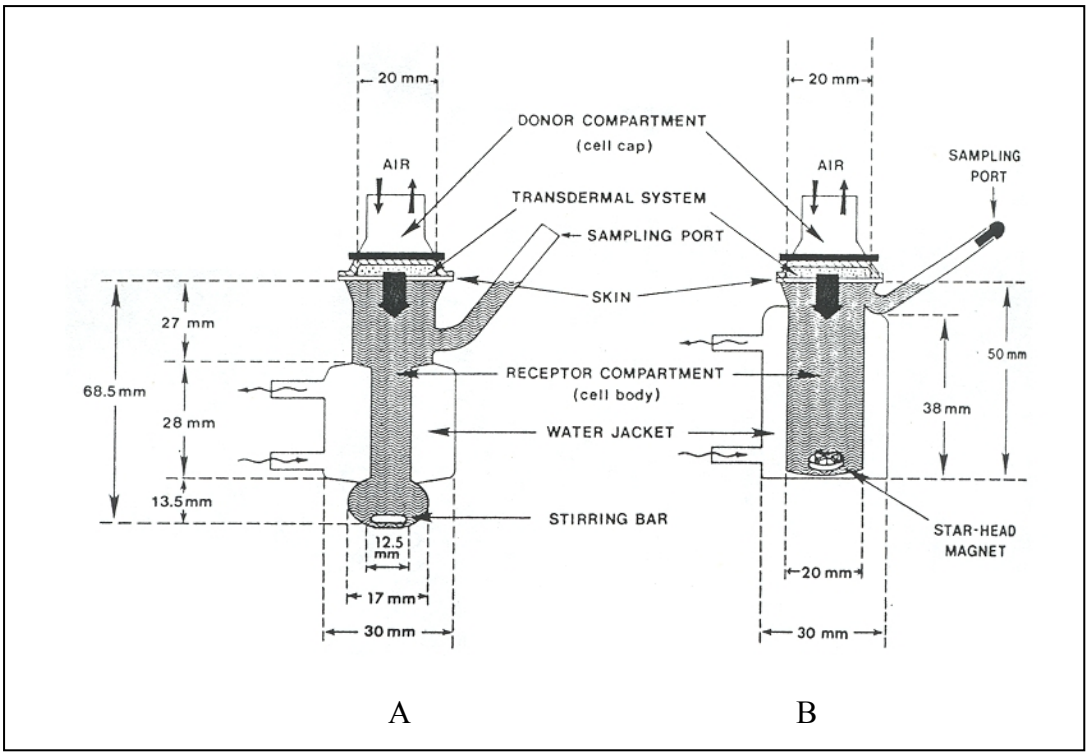


Figure 11 The vertical type in vitro skin permeation systems.

Keys : A) Franz diffusion cell, B) Keshary-Chien diffusion cell

Source : Yie W. Chien, Novel drug delivery systems 2 nd ed. (New york : Marcel dekker Inc., 1992), 338.

1. Diffusion-controlled release

Diffusion-controlled release is depended upon polarity and molecular weight of the drug and surfactant type/concentration. For multiple emulsion systems, the drug is available for absorption after a two steps partitioning phenomena. The first partitioning of the drug occurs between the internal aqueous phase and the middle oily phase and the second occurs between the middle oily phase and the outer aqueous phase. Multiple emulsion systems have lag time corresponding to the time required for partitioning through the complex barriers. Vasiljeuic D. et. al. (2006 : 175-176) find the highest concentration of the primary emulsifier resulted in the lowest droplet size and the highest apparent viscosity and sustained drug release. Drug release data is predominated diffusion drug release mechanism which would be sustained and prolonged drug release. The concentration gradient caused by all the dissolved species is effects the water flow from the external to the internal phase. This aqueous transport produces an increase of the internal microglobule size. The more the concentration of lipophilic surfactant, the more the swelling capacity of the oil globule and also the more the delay of drug release. The effect of lipophilic surfactant concentration in the swelling of the oil globule is explained by mechanism that it increased rigidity of the second interface by it is progressive migration from the first interface. During the second step of multiple emulsion preparation, lipophilic surfactant molecules can diffuse from the first to second interface. This produces a synergistic effects resulting in membrane strengthening.

The most common is the molecular diffusion controlled mechanism of oil soluble matter. Diffusion through the oil phase can be controlled in ionizable matter by controlling its dissociation. The transport rate is depended on the nature of the

entrapped material (including its dissociation constant) and on the oil, as well as on the pH of the aqueous phase. At low pH values the entrapped matter would exist almost exclusively as the unionized form and so would be readily soluble in the oil phase. The drug could, therefore, pass easily across the oil layer to the external aqueous phase containing a basic buffer, which ionizes the addenda that is now insoluble in the oil phase and becomes trapped within the internal aqueous phase. This is then be carried out with the emulsion as it is voided from the gastro-intestinal tract (Figure 12). The drug transport is found to follow the first-order kinetics according to Fick's law.

Ionized and ionizable compounds are not the only materials to be transported across the oil membrane. It has been demonstrated that both water molecules and non-electrolyte water soluble matter can easily migrate through the oil membrane without affecting the multiple emulsion stability. A mechanism based on "micellar transport" from one phase to the other has been described and determined. It is also demonstrated that the micellar transport is also diffusion controlled. One can alter the diffusion rates through the oil membrane by changing the nature of the oil, increasing its viscosity and adding various carriers. This suggests that the diffusion of the addenda through the oil is the rate determining step, and that the inner water phase does not have any effect on the determination of release rates. The two possible mechanisms of diffusion for the permeation of water and water soluble materials through the oil phase are as follows : the first being via "reverse micellar transpost" (figure 13) and the second by "diffusion across a very thin lamella" of surfactant formed where the oil layer is very thin (figure 14) (Garti 1997 : 235-236).

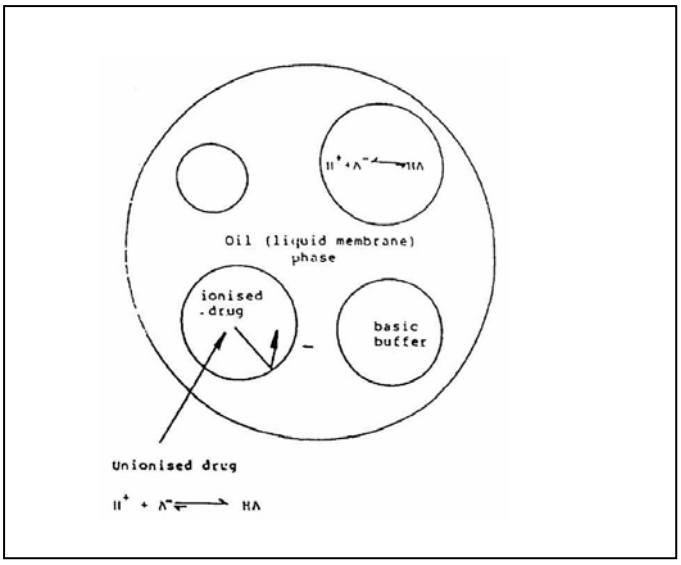


Figure 12 w/o/w "liquid membrane" system for removal of acidic drugs form an aqueous system.

Source : Nissim Garti, "Double emulsion – scope, limitations and new achievements," Colloids and surfaces A : physicochemical and engineering aspects 123-124 (1997) : 236.

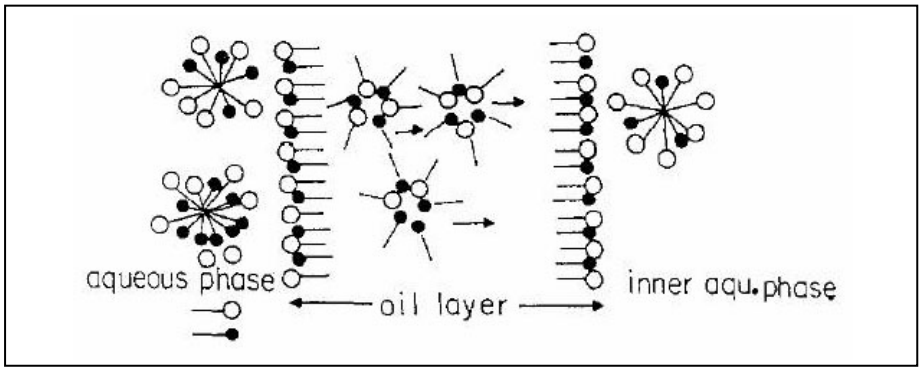


Figure 13 Schematic illustration of a model for micellar transport of water from the outer aqueous phase to the inner aqueous phase through the oil layer in w/o/w multiple emulsion.

Source : Nissim Garti, "Double emulsion – scope, limitations and new achievements," Colloids and surfaces A : physicochemical and engineering aspects 123-124 (1997) : 236.

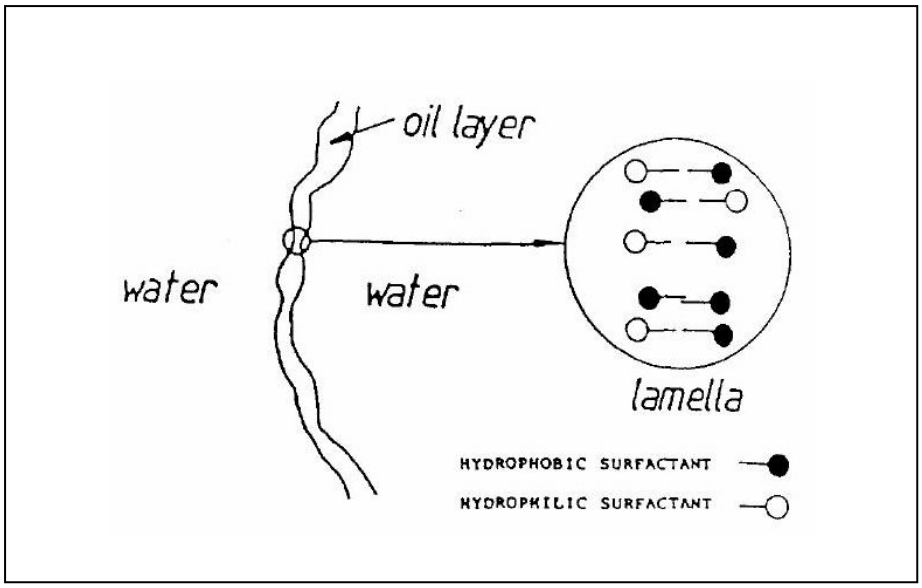


Figure 14 Schematic illustration of a model for water transport through this lamella of the surfactant due to fluctuation in the thickness of the oil layer.

Source : Nissim Garti, "Double emulsion – scope, limitations and new achievements," Colloids and surfaces A : physicochemical and engineering aspects 123-124 (1997) : 237.

2. Emulsion breakdown or membrane rupture controlled release

Emulsion breakdown or membrane rupture controlled release is depended upon the physical stability of the emulsion and surfactant type/concentration and osmoticity of drug release condition. Jager-Lezer et al. (1997 : 7-13) find that in case of the hypo-osmotic drug release condition, the concentration gradient between all dissolved species in internal phase which are higher than external phase is responsible for water flow from the external phase to the internal phase. This aqueous transport produced an increase in internal microglobule size and therefore the oil globules swell part former until a critical size is reached (the swelling step). Beyond this critical

size, the globules bursted by the breakdown of the oily membrane and released the entrapped drug (the breakdown step).

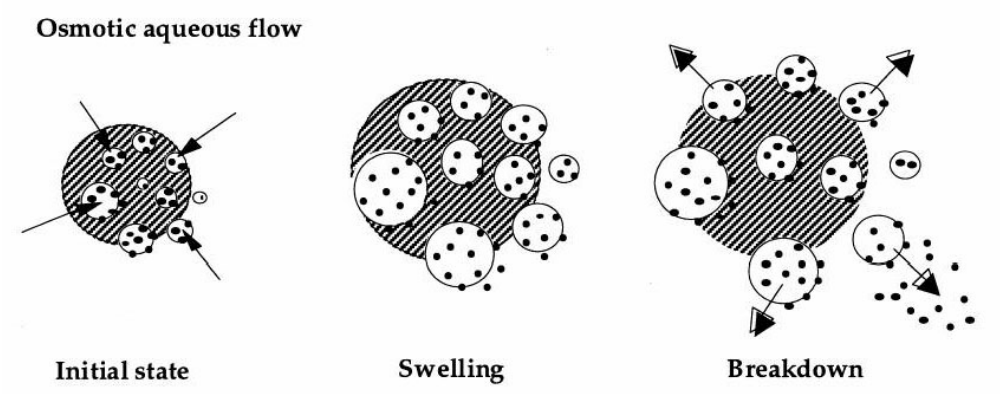


Figure 15 A diagram of the release mechanism by swelling-breakdown.
Source : S. Geiger et al, "Kinetics of swelling-breakdown of a w/o/w multiple emulsion: possible mechanisms for the lipophilic surfactant effect," Journal of controlled release 52 (1998) : 105.

They find that formulation containing lipophilic surfactant at low concentration (without surfactant excess) is leading to lower swelling, but higher release. The formulation containing more percentage of lipophilic surfactant lead to increasing swelling capacity of oil globules and the more the delay in drug release. Geiger et al. (1998 : 103-107) explains the influence of the lipophilic surfactant concentration on the swelling breakdown of the oily globules and proposed two difference mechanisms. Both mechanisms implied the migration of the lipophilic surfactant and take place successively when the concentration of the lipophilic surfactant in the formulation is increased. The first mechanism involved in an

increase of the rigidity of the second interface by the progressive migration of the lipophilic surfactant. During the second step of multiple emulsion preparation, lipophilic surfactant molecules diffuse from the first to the second interface, where they produced a synergistic effect resulting in membrane strengthening. The second mechanism involved a delay in the aqueous droplet coalescence. In the course of swelling of the oily globules, the lipophilic surfactant molecules, which excess in the oily phase, could diffuse to the first interface to fill up free spaces caused by the swelling.

Clindamycin phosphate (McEvoy et al. 2007 : 3437 -3442)

Clindamycin is a semisynthetic antibiotic which is a derivative of lincomycin. Clindamycin generally is used for the treatment of serious infections caused by susceptible gram-positive bacteria and anaerobic bacteria.

Uses

Acne Vulgaris : Clindamycin phosphate is used topically alone or in conjunction with benzyl peroxide in the treatment of inflammatory acne vulgaris. In weighing the potential benefits of topical clindamycin therapy, the possibility of serious adverse GI effects associated with the drug should be considered. The drug is not indicated in the treatment of noninflammatory acne. Therapy of acne vulgaris must be individualized and frequently modified depending on the types of acne lesions which predominate and the response to therapy. Topical anti-infectives, including clindamycin, generally are effective in the treatment of mild to moderate inflammatory acne and are particularly useful in the treatment of mild, papular acne of puberty and early adolescence and papular-pustular acne in adult women.

Bacterial vaginosis : Clindamycin is used intravaginally as a vaginal cream or suppository or orally for the treatment of bacterial vaginosis (formerly called *Haemophilus vaginitis*, *Gardnerella vaginitis*, nonspecific vaginitis, *Corynebacterium vaginitis*, or anaerobic vaginosis)

Dosage and administration

Administration

Clindamycin phosphate is applied topically to the skin or intravaginally in appropriate formulations.

Topical administration

Clindamycin phosphate is applied topically to the skin as a gel, lotion, or solution containing clindamycin 1%. Clindamycin phosphate also is applied topically to the skin in the form of a gel containing clindamycin 1 % in combination with benzyl peroxide 5%. Topical preparation containing clindamycin phosphate are for external use only and should not be used orally or intravaginally or use near or in the eyes, nose, mouth or mucous membranes.

Intravaginal administration

Clindamycin phosphate is administered intravaginally as a vaginal cream containing 2% clindamycin or as a vaginal suppository containing 100 mg of the drug. Patients should be instructed for the usage of the vaginal applicator and should be given a copy of the instructions provided by the manufacturer.

Dosage

Acne vulgaris : For the topical treatment of acne vulgaris, a thin film of the commercially available gel, lotion, or solution containing clindamycin 1% should be applied to the cleansed affected area twice daily. Prolonged therapy (several

months or years) may be necessary for the treatment of acne vulgaris. Therapy usually is continued as long as a satisfactory response is obtained and no severe or intolerable toxicity occurs.

Bacterial vaginosis : Clindamycin vaginal cream is used for the treatment of bacterial vaginosis in nonpregnant women. One applicatorful (approximately 5 g) of clindamycin phosphate (2% clindamycin) vaginal cream (100 mg of clindamycin) is administered intravaginally once daily, preferably at bedtime, for 3 or 7 consecutive days.

Mechanism of action

Clindamycin may be bacteriostatic or bactericidal in action, depending on the concentration of the drug attained at the site of infection and the susceptibility of the infecting organism. Clindamycin phosphate is inactive until hydrolyzed to free clindamycin; phosphatases on the skin rapidly hydrolyze the drug following topical application.

Clindamycin appears to inhibit protein synthesis in susceptible organisms by binding to 50S ribosomal subunits; the primary effect is inhibition of peptide-bond formation. The binding sites of clindamycin appear to be the same as or to overlap those of chloramphenicol and erythromycin.

The exact mechanisms by which clindamycin reduces lesions of acne vulgaris have not been fully elucidated; however, the effect appears to partly result from the antibacterial activity of the drug. Following topical application to the skin of 1% hydroalcoholic solution of clindamycin as the hydrochloride or the phosphate, the drug inhibits the growth of susceptible organisms (primarily *Propionibacterium acnes*) on the surface of the skin and reduces the concentration of free fatty acids in

sebum. The reduction in free fatty acids in sebum may be an indirect result of the inhibition of lipase-producing organisms that convert triglycerides into free fatty acid or may be direct result of interference with lipase production in these organisms. Free fatty acids are comedogenic and are believed to be a possible cause of the inflammatory lesions (e.g. papules, pustules, nodules, cysts) of acne. However, other mechanisms also appear to be involved because clinical improvement of acne vulgaris with topical clindamycin therapy does not necessarily correspond with a reduction in the bacterial flora of the skin or a decrease in the free fatty acid content of sebum. Topical application of a 1% hydroalcoholic solution of clidamycin as the base or as the phosphate also resulted in inhibition of *P.acnes* within open comedones.

Spectrum

In general, clindamycin is active in vitro and in vivo against most aerobic gram-positive cocci and several anaerobic and microaerophillic gram-negative and gram-positive organisms. The drug is inactive against *Enterobacteriaceae*, fungi, and viruses. In vitro, clindamycin at a concentration of 0.04-0.4 mcg/mL inhibits most susceptible strains of staphylococci, streptococci, pneumococci, *Actinomyces*, *Arachnia propionica*, *Corynebacterium diphtheriae* and *Propionibacterium acnes*. Clindamycin at concentrations of 0.1-4 mcg/mL inhibits most susceptible strains of *Clostridium*, *Fusobacterium*, *Moraxella*, *Mycoplasma* and *Neisseria gonorrhoeae* in vitro. Clindamycin is active in vitro and in vivo against *Gardnerella vaginalis* (formerly *Haemophilus vaginalis*). The drug also is active in vitro against *Mycoplasma hominis* and anaerobic organisms including *Bacteroides*, *Prevotella* and *Porphyromona* (both formerly classified as *Bacteroides*), *Peptostreptococcus* and *Mobiluncus*.

Chemistry and Stability

Chemistry

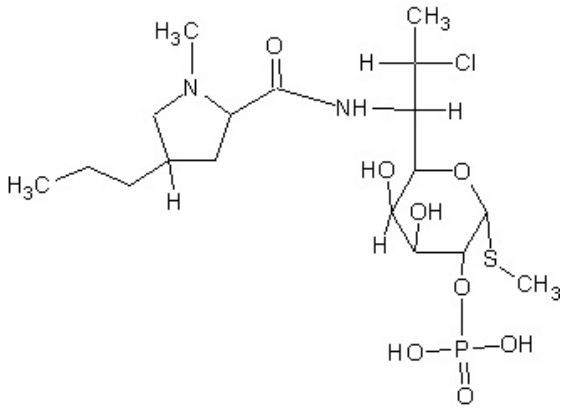


Figure 16 Structure of clindamycin phosphate

Source : T.O. Oesterling and E.L. Rowe., "Hydrolysis of lincomycin-2-phosphate and Clindamycin-2-phosphate," Journal of pharmaceutical sciences 59 (1970) : 176.

Clindamycin is a semisynthetic derivative of lincomycin, an antibiotic obtained from cultures of *Streptomyces lincolnensis*. Clindamycin differs structurally from lincomycin in the substitution of a chloride atom for the 7-hydroxyl group and the inversion of the involved 7-carbon. Clindamycin phosphate ($C_{18}H_{34}ClN_2O_8PS$, molecular weight 504.97) occurs as a white to off-white, hygroscopic, crystalline powder that is odorless or practically odorless and has a bitter taste. The drug has a solubility of approximately 400 mg/mL in water at 25 °C and is slightly soluble in dehydrated alcohol.

Stability

Commercially available clindamycin phosphate 1% topical gel, lotion and solution should be stored in tight containers at 20-25 °C. Freezing has to be avoided. Croubles, Baere and Backer (2003 : 424) studies the stability of clindamycin hydrochloride in water and stores in the dark at 2-8 °C. It is found that solution of clindamycin hydrochloride in water degraded to below -10% of their initial amount in 17 days after storage. Clindamycin phosphate in aqueous solution is shown to degrade by two major routes: apparent first-order hydrolyses of the thioglycoside and phosphate ester. Clindamycin phosphate is not stable in solution.

CHAPTER 3
MATERIALS AND METHODS

1. Materials

1. Clindamycin phosphate (Lot number P-006-2007013, Zhejiang hisoar pharmaceutical co., ltd, Zhejiang, China)

2. Polyoxyethylene sorbitan fatty acid esters (Polysorbate 80, NOF Corporation, Japan)

3. Sorbitan fatty acid esters (Span® 80, NOF Corporation, Japan)

4. Poloxamer 188 (Lutrol® F68, Lot number WPNA544C, Ludwigshafen, Germany)

5. Poloxamer 407 (Lutrol® F127, Lot number 52-0113, Ludwigshafen, Germany)

6. PEG 30-dipolyhydroxystearate (Arlacel® P135, Lot number 55439, Uniqema, U.S.A.)

7. Sodium chloride (Lot number 3M135274A, Carlo erba, Italy)

8. Monobasic potassium phosphate (Lot number AF501339, Ajax chemicals, Australia)

9. Dibasic sodium phosphate (Lot number AF405300, Ajax chemicals, Australia)

10. Citric acid monohydrate (Fluka chemie AG, Switzerland)

11. Acetonitrile HPLC grade (Lab-scan®, Thailand)

12. Sodium azide (Sigma®, Germany)

13. Xanthan gum (Fluka chemie AG, Switzerland)

14. Isopropyl myristate (Uniqema, United kingdom)

15. Dextrose anhydrous (Lot number NRE1631, Roquette freres, France)

2. Equipments

1. Electric precision balance (Mettler® BB-300, Greifensee, Germany)

2. Heater and Magnetic stirrer (Heidolph®, Germany)

3. Homogenizer (Janke & Kunkel IKA® Ultra-turrax T25, Staufen, Germany)

4. Impeller (CAT® R-17, Staufen, Germany)

5. Water Bath thermostat circulator (Heto® HMT 200, Gydevang, Denmark)

6. Viscometer (Brookfield® model DV-II$^+$, Maryland, U.S.A.)

7. Incubator (Gallenkamp plus series, England)

8. High performance liquid chromatography (HPLC)

- High pressure pump (Model P4000, Thermo separation product, U.S.A.)

- Auto sampler (Model AS3000, Thermo separation product, U.S.A.)

- Variable UV-detector (Model UV1000, Thermo separation product, U.S.A.)

9. Chromatographic column (Capcellpak® C8DD, 150 mm x 4.6 i.d., 5 μ, Shiseido, Japan)

10. Analytical balance (A&D® Model GR-200, Tokyo, Japan)

11. pH meter (Schott® Model CG840, Germany)

12. Auto pipette (Labopette®, Eberstadt, Germany)

13. Optical microscope (Nikon® Model TS100F, Tokyo, Japan)

14. Digital camera (Nikon® Model 6300 and 5700, Tokyo, Japan)

15. Laser light scattering particle analyzer (Mastersizer® 2000S, Worcestershire, UK)

16. Confocal laser scanning microscopy (Olympus® Model Fluoview FV1000, Tokyo, Japan)

17. Cellulose acetate membrane 0.45 μm (Sartorius AG, Goettingen, Germany)

18. Franze diffusion cell (Hanson research, Chatsworth, CA, USA)

3. Methods

1. Preparation of multiple emulsions (Florence and Whitehill 1982 : 281)

The general formulation (by weight) of the w/o/w multiple emulsion studied as below. The multiple emulsions were obtained by two steps process. In the first step, the primary w/o emulsion (PE) was prepared by slowly adding water phase (containing the drug) preheated to 65 ± 2 °C to the oil phase (containing the lipophilic surfactant) preheated to 60 ± 2 °C. The stirring was performed by laboratory homogenizer at 9,800 rpm for 30 minutes. The primary emulsion was cooled down to ambient temperature.

In the second step, the aqueous phase contained hydrophilic surfactant and dispersed polymer. The primary emulsion was slowly added while the system was stirred at 1,000 rpm for 5 minutes. The composition of multiple emulsions are as follows,

Primary emulsion	% w/w
Citrate-phosphate buffer pH 5.5	50
Lipophilic surfactant	X
Isopropyl myristate qs to	100
Multiple emulsion	% w/w
Primary emulsion	50
Hydrophilic surfactant	Y
Sodium azide	0.01
Xanthan gum	0.25
Sodium chloride	0.2
De-ionized water qs to	100

2. Effect of formulation factors on the stability of multiple emulsions

This study was performed to investigate the effect of formulation factors on the stability of multiple emulsions. These factors were as follows;

2.1 Polymeric thickener

The polymeric thickeners used were hydroxy propyl cellulose (HPC), hydroxypropyl methylcellulose (HPMC) and xanthan gum. The concentrations of all polymeric thickener were 0.5, 1, 1.5, 2 and 3 % w/w.

2.2 Emulsifier

The lipophilic emulsifiers used for the preparation of w/o emulsions were as below;

- Span® 80 (a sorbitan fatty acid esters as lipophilic nonionic surfactant) at 5 %w/w.

- Arlacel® P135 (a PEG 30-dipolyhydroxystearate as polymeric surfactant) at 0.5, 1, 1.5 and 2 %w/w

The hydrophilic emulsifiers used for the preparation of w/o/w emulsion were as follow;

- Tween® 80 (a polyoxyethylene sorbitan fatty acid esters as hydrophilic nonionic surfactant) at 2.5 %w/w.

- Poloxamer 188 (a polyoxyethylene-polyoxypropylene block copolymer as polymeric surfactant) at 0.5, 1, 1.5, 2, 2.5 and 3 %w/w.

- Poloxamer 407 (an ethoxylated propylene oxide copolymer as polymeric surfactant) at 0.5, 1, 1.5, 2, 2.5 and 3 %w/w.

2.3 Type and concentration of stiffening agent in oil middle phase

A variety of waxes was used to increase viscosity of oil phase. These waxes included stearic acid, glyceryl monostearate (GMS), petrolatum, cetyl alcohol and colloidal silicon dioxide. The concentration of stiffening agents were as follow;

- Stearic acid at 0.5, 1 and 2 %w/w.

- Glyceryl monostearate at 0.5, 1, 2 and 8 %w/w.

- Petrolatum at 1, 3, 5, 8, 10, 15 and 20 %w/w.

- Cetyl alcohol at 1, 3 and 5 %w/w.

- Colloidal silicon dioxide at 1, 2 and 3 %w/w.

2.4 Process of producing

A variety of stirrer speeds in the second step of multiple emulsions preparation was used to investigate the effect on droplet size of multiple emulsions. The speed was set at 800, 1000 and 1200 rpm.

2.5 Osmolality adjusting agent

The osmolality adjusting agents were used to adjust osmolality of multiple emulsion sodium chloride and dextrose.

2. 6 Phase volume ratio

Both primary and multiple emulsions prepared at different content of inner phase are given in table 1.

Table 1 Phase volume ratio of the primary and multiple emulsions

		Phase volume ratio of primary emulsion in multiple emulsion		
		0.4	0.5	0.6
Phase volume ratio of water inner phase in primary emulsion	0.4	0.4 : 0.4	0.4 : 0.5	0.4 : 0.6
	0.5	0.5 : 0.4	0.5 : 0.5	0.5 : 0.6
	0.6	0.6 : 0.4	0.6 : 0.5	0.6 : 0.6

All the prepared multiple emulsions were evaluated by observing the characteristics of multiple emulsions, microscope evaluation, droplet size and viscosity measurement.

3. Preparation of clindamycin phosphate multiple emulsion

A stable multiple emulsions obtained from previous study were selected to produce clindamycin phosphate multiple emulsion. Preparation process was the same as the previous study. The concentration of clindamycin phosphate in the final multiple emulsion was 1 % w/w. Prepared sample was kept at room temperature. Clindamycin phosphate multiple emulsions sample was evaluated for the content of cindamycin phosphate, stability of clindamycin phosphate multiple emulsions and physical properties.

4. Determination of clindamycin phosphate content

High performance liquid chromatography (HPLC) was used to determine the content of clidamycin phosphate in multiple emulsions.

4.1 Chromatographic condition and instrumental setting

The chromatographic column was a C8 column and the mobile phase contained 22.5 %(v/v) acetonitrile and 77.5 %(v/v) monobasic potassium phosphate buffer (pH 2.5) solution in water. The flow rate was 1.0 mL/min. The detector was set at 210 nm. The analyses were performed at room temperature with an injection volume of 20 μl.

4.2 Preparation of standard solution and standard curve

Accurately weighed about 22 mg of clindamycin phosphate to 100 mL-volumetric flask, dissolved and diluted with mobile phase to volume. Appropriate dilutions were then made to obtained standard solution of 0.022, 0.044, 0.088, 0.132, 0.176 and 0.220 mg/mL in mobile phase. The solution was filtered through 0.45 μm membrane filter. Twenty micro liter of each standard solution was

injected into the column and the ultraviolet absorption at the wavelength of 210 nm was determined to obtain standard curve. The peak area of clindamycin phosphate from the chromatogram was calculated and plotted versus the concentration of clindamycin phosphate.

4.3 System suitability tests

The system suitability was evaluated by making six replicate injections of standard preparation. Peak and responds were recorded and compared. The system was deemed to be suitable for used if the coefficient of variation was not more than 2.5%. The system suitability was tested before the injection of sample.

4.4 Determination of the content of clindamycin phosphate in the multiple emulsions

An accurately weight portion of the multiple emulsion, equivalent to about 2 mg of clindamycin phosphate (0.2 g of clindamycin phosphate multiple emulsion), was added to a 10-mL volumetric flask then 7 mL of ethanol was added. The mixture was dissolved used by sonicator for 30 minutes. The volume was adjusted with mobile phase. A few mL of mixture was filtered through 0.45 μm membrane filter. The filtrate was used to determine for clindamycin phosphate by using HPLC. Twenty microliters was injected into column. The chromatographic system used to analyze the content of clindamycin phosphate was as described in 4.1. The amount of clindamycin phosphate was calculated from the standard calibration graph. The drug content was reported as mean percentage drug content of three determinations.

5. Stability study

Both physical and chemical stability studied of multiple emulsions and clindamycin phosphate multiple emulsion were conducted.

5.1 Physical stability of multiple emulsions

Physical stability of multiple emulsions was evaluated both before and after temperature cycling method (TCM). The heat-cool cycle testing contains five cooling/heating cycles. One cycle consisted of storage at 4 °C for 24 hours and switch to 40 °C for 24 hours. Multiple emulsions were inspected for appearance, % creaming, cracking, viscosity and droplet size both before and after temperature heat-cool cycle test.

5.2 Chemical stability

1 % clindamycin phosphate multiple emulsions and 1% clindamycin phosphate solution in citrate-phosphate buffer were stored in light-protection amber glass containers and were placed over the saturated solution of sodium chloride in the glass desiccators. The desiccators were placed in an incubator set at 40 °C and 75% relative humidity, 4 °C and 75% relative humidity (Block, in Lieberman, eds. 1996 : 94). The drug content was determined at 1, 2, 3, 4, 6, 8, 10 and 12 weeks using high performance liquid chromatography. Chemical stability test of multiple emulsions were studied after modification of type and concentration of stiffening agent in oil phase, concentration of lipophilic surfactant and type of electrolyte for osmolality adjusting agent.

6. Determination of drug release from clindamycin phosphate multiple emulsions

Clindamycin phosphate release rate from multiple emulsions was measured through 0.45 μm cellulose acetate membrane using Franz diffusion cells with a diffusion area of 2.01 cm^2. Cellulose acetate membrane was sandwiched between the upper donor compartment and the lower compartment. Two grams of the formulation containing 1 % of clidamycin phosphate was placed on the membrane surface in the donor compartment while the receptor compartment was filled with 11 mL of receptor medium containing phosphate buffer isotonic solution (pH 5.5), with 100 rpm and kept at 33.5 ± 1 °C. The 1 mL of solution was sampled from receptor chamber at 5, 10, 20, 30, 60, 90, 120, 180, 240, 360, 540, 720, 900, 1080, 1260 and 1440 minutes and fresh receptor medium was replaced. The amount of clindamycin phosphate released was analyzed by HPLC. Zero point six milliliter of filtered sample was mixed with 0.6 mL of mobile phase (see section 4.1). The final solution was filtered through 0.45 μm membrane filter and determined using HPLC.

7. Evaluations of the physical properties of multiple emulsions

7.1 Characteristics of the multiple emulsions

Characterizations of multiple emulsions was performed by visualization. Multiple emulsions were observed for their appearance, homogeneity, consistency and sedimentation and separation. The sedimentation or creaming is reversible separation of the emulsion into dilute and concentrated regions. The separation or cracking is irreversible separation. The sedimentation or creaming was

defined as the ratio of the final volume of emulsion (V_u) to the total volume of emulsion before setting (V_0) as follow

$$\% \text{ Creaming} = \frac{V_u}{V_0} \times 100$$

The higher percentage value of creaming, the more acceptable of the product.

7.2 Microscopic analysis

Microscopic analysis of the investigated samples was conducted in order to gain information about the multiple character of the emulsion. Optical microscopic with camera was used throughout the study. Photomicrographs of the multiple emulsions were taken at various times, after diluting sample with 0.5% erythrosine iso-osmotic solution.

7.3 Confocal laser microscopy

Confocal scanning laser microscopy was used to observe the w/o/w multiple emulsions samples both before and after accelerated stability test. Samples (1 mL) of multiple emulsions was stained with 0.1 mL of 1 %w/w nile red solution, and stained samples were dropped on concave glass slides and examined with 40X magnification lens using an He/Ne green laser with an excitation line of 543 nm.

7.4 Droplet size analysis of w/o/w multiple emulsions

Droplet size distributions and average droplet sizes were determined immediately after preparation using a laser light scattering particle analyzer. Droplet size measurements of w/o/w were preformed in 0.9% w/v sodium chloride solution (refractive index is 1.370). The droplet size was reported as volume

weight mean droplet size (d_{43}) and standard deviation of the mean of three determinations.

7.5 Viscosity measurement

Viscosity measurement was performed using rotating spindle viscometer, coupled with spindle measuring device and small sample cup, at 30 ± 0.5 °C. The viscosity was reported as mean and standard deviation of the mean of ten determinations. The % viscosity change was defined as the ratio of the final, viscosity of emulsion after TCM (η_u) to the viscosity of emulsion before TCM (η_0) as follow

$$\% \text{ Viscosity change} = \left(\frac{\eta_u - \eta_0}{\eta_0} \right) \times 100$$

8. Statistical analyses

The results were analyzed using ANOVA and paired *t*-test for significance ($p<0.05$) using data analysis tool in Origin® Pro 7.5 software.

CHAPTER 4
RESULTS AND DISCUSSION

1. Preparation of multiple emulsion

In this study, multiple emulsions were prepared by two-step process. Citrate-phosphate buffer pH 5.5 was used as internal water phase. Isopropyl myristate and sodium azide served as oil middle phase and preservative respectively. Sodium chloride or dextrose were used as osmolality adjusting agent and de-ionized water as an external water phase. The resulted multiple emulsion appeared as milky white and viscous liquids. But immediately after preparation, this multiple emulsion showed creaming. A major problem associated with w/o/w multiple emulsion was creaming. Creaming could be reduced by increasing the concentration of secondary emulsifier, however, high emulsifier concentration was not desirable in pharmaceutical systems due to toxicity. The use of a thickening agent in the external aqueous phase might also reduce creaming but it should be certain that the emulsion retained the characteristics of pourability (Florence and Whitehill 1982 : 306).

2. Effect of formulation factors on the stability of multiple emulsion

2.1 Polymer thickener

The polymeric thickeners tested were hydroxyl propyl cellulose (HPC), hydroxylpropyl methylcellulose (HPMC) and xanthan gum. The compositions of primary and multiple emulsions are presented in table 2. The polymeric thickener were added in the external aqueous phase of multiple emulsions. The ability of polymers to stabilize w/o/w multiple emulsion was investigated.

Multiple emulsions were inspected for appearance, % creaming, cracking and viscosity both before and after temperature cycling test (TCM) (table 3). Immediately after preparation, all formula of multiple emulsion containing thickening agent were apparently white and homogeneous liquid except the multiple emulsion prepared without thickening agent that exhibited creaming after preparation. It was seen that the viscosity of multiple emulsion increased with increasing concentration of HPMC or HPC from 0.5 – 3 %w/w. However, HPMC and HPC were not suitable to be thickener for multiple emulsion preparation since the multiple emulsion cracked after TCM. Increasing the concentration of xanthan gum in external water phase of multiple emulsion from 0.5 – 1.0 %w/w resulted in the increased viscosity of multiple emulsion from 11781.49 (± 223.22) cp to 35808.36 (± 565.62) cp. However, it was found that multiple emulsions containing 1.25 % w/w of xanthan gum was unstable due to separation or cracking after preparation. The viscosity of multiple emulsion containing 0.5 – 1 %w/w xanthan gum significantly decreased to 7254.45 (± 297.00) and 16900.39 (± 160.19) cp, respectively which should be owing to some degree of coalescence during TCM. However, the physical appearance of those multiple emulsions were quite good since they provided high percentage of creaming (88 % and 96%). Actually, all thickeners (HPMC, HPC and xanthan gum) in this experiment could be used for multiple emulsion preparation, but more experiment should be performed to optimize the formula for each thickener. However, in this study, xanthan gum at 0.5 – 1 %w/w were chosen as thickener in multiple emulsion for the next experiment.

Table 2 Compositions of primary and multiple emulsions (%w/w of ingredients)

Primary emulsion	% w/w			
Citrate-phosphate buffer pH 5.5	50			
Span® 80	5			
Isopropyl myristate, qs to	100			
Multiple emulsion (ME)	ME	ME HPMC	ME HPC	ME XA
Primary emulsion	50	50	50	50
Tween® 80	2.5	2.5	2.5	2.5
Sodium chloride	0.4	0.4	0.4	0.4
Sodium azide	0.01	0.01	0.01	0.01
HPMC	-	0.5 – 3	-	-
HPC	-	-	0.5 – 3	-
Xanthan gum	-	-	-	0.5-1.25
De-ionized water, qs to	100	100	100	100

Remark : The concentrations of HPMC used were 0.5, 1, 1.5, 2, 2.5, 3 % w/w

The concentrations of HPC used were 0.5, 1, 1.5, 2, 2.5, 3 % w/w

The concentrations of xanthan gum used were 0.5, 1, 1.25 % w/w

Table 3 Characteristic of multiple emulsion prepared with various polymer thickeners

%w/w	Before TCM		After TCM	
	Appearance % Creaming	Viscosity, cp (SD) at 30 °C	Appearance % Creaming	Viscosity, cp (SD) at 30 °C
Without thickener				
-	52 %	-	Crack	-
Hydroxylpropyl methylcellulose (HPMC)				
0.5 %	100 %	367.92 (131.69)	Crack	-
1 %	100 %	1231.74 (136.98)	Crack	-
1.5 %	100 %	2151.54 (166.28)	Crack	-
2 %	100 %	4039.14 (151.10)	Crack	-
3 %	100 %	11381.57 (203.22)	Crack	-
hydroxyl propyl cellulose (HPC)				
0.5 %	100 %	159.97 (108.61)	Crack	-
1 %	100 %	495.89 (154.54)	Crack	-
1.5 %	100 %	1383.70 (151.05)	Crack	-
2 %	100 %	1711.63 (177.65)	Crack	-
3 %	100 %	3567.24 (185.48)	Crack	-
Xanthan gum				
0.5 %	100 %	11781.49 (223.22)	88 %	7254.45 (297.00)
1 %	100 %	35808.36 (565.62)	96 %	16900.39 (160.19)
1.25 %	Crack	-	Crack	-

2.2 Emulsifier

Emulsifiers also have an important role in the process of emulsification. Surfactant emulsifiers reduce interfacial tensions during emulsification, making droplets easier to break up as well as reducing the tendency for recombination. In multiple emulsion, the first step is to prepare primary emulsion using low HLB emulsifiers (lipophilic surfactant) as primary surfactant to obtain water-in-oil (w/o) emulsion. In the second step, the high HLB emulsifier is used to promote a formation of an oil-in-water emulsion in which the "oil" phase is the water-in-oil-in-water (Florence and Whitehill 1982 : 281). In this study, the lipophilic emulsifiers used for the w/o emulsion preparation were nonionic surfactant (sorbitan fatty acid ester, Span® 80) and polymeric surfactant (PEG 30-dipolyhydroxystearate, Arlacel® P135). The hydrophilic surfactants used for w/o/w multiple emulsion preparation were nonionic surfactants (polyoxyethylene sorbitan fatty acid esters, Tween® 80) and polymeric surfactants (polyoxyethylene-polyoxypropylene block copolymer, Poloxamer 188, and ethoxylated propylene propylene oxide copolymer, Poloxamer 407).

2.2.1 Span® 80 and Tween® 80

This experiment used Span® 80 as lipophilic surfactant and Tween® 80 as hydrophilic surfactant. It was found from section 2.1 that the primary emulsion prepared with 5 % w/w Span® 80 was apparently white, homogeneous and viscous. It showed good stability over the temperature cycling method (table 4). This primary emulsion was then gradually dispersed into external water phase consisting of 2.5 % w/w Tween® 80 with various concentrations of HPMC, HPC and xanthan gum as thickener (table 2). Immediately after preparation, the multiple emulsions obtained

were apparently white and homogeneous liquid. After temperature cycling method (TCM), these multiple emulsions were not stable due to creaming (table 3).

2.2.2 Lipophilic nonionic and Hydrophilic polymeric surfactant

This experiment used 5%w/w of Span® 80 as lipophilic surfactant and Poloxamer 188 and 407 at 0.5 – 3 %w/w as hydrophilic surfactants. Xanthan gum at various concentrations (0, 0.25, 0.5, and 0.8 %w/w) was also added to external aqueous phase.

2.2.2.1 Span® 80 and Poloxamer 188

Formula of multiple emulsion consisting of xanthan gum was prepared with various Polxamer 188 concentrations as shown in table 5. All formula of multiple emulsion were apparently white and homogeneous liquid except the formula prepared without xanthan gum that exhibited creaming at 5 minutes after preparation. After TCM, formula containing 0.25% and 0.8% w/w of xanthan gum were not stable and showed phase separation. But the formula containing 0.5% w/w xanthan gum was stable and was apparently white and homogeneous liquid with significant degree of viscosity decrease (table 6 and figure 17). The viscosity of multiple emulsions containing 0.25 %w/w xanthan gum were too low to prevent oil droplets coalescence. The formula consisting of 0.8 %w/w xanthan gum showed phase separation after preparation since the viscosity of the external aqueous phase was too high to be properly mixed with primary emulsion at stirrer speed of 1000 rpm.

From the previous experiment, the viscosity of multiple emulsion consisting of 2.5 %w/w Tween® 80 and 0.5 %w/w xanthan gum in external water phase was 11781.49 ± 223.22 cp. Changing secondary emulsifier from Tween® 80 to

poloxamer 188 exhibited high viscosity increase of multiple emulsions. This might be owing to the effect of polymeric surfactant (poloxamer) adsorption on the oil-water interface. Polymeric surfactants were known to be multi-anchoring amphiphiles with irreversible adsorption capabilities that could improve the droplets interfacial coverage during emulsification and provided strong steric stabilization (Lutz and Aserin, in Aserin, eds. 2008 : 88).

Table 4 Composition and physical stability of primary emulsion prepared with nonionic surfactant (Span® 80)

Primary emulsion	% w/w		
Citrate-phosphate buffer pH 5.5	50		
Span® 80	5		
Isopropyl myristate, qs to	100		
Physical stability			
Before TCM		After TCM	
%Cream	Viscosity. cp (SD) at 30 °C	%Cream	Viscosity. cp (SD) at 30 °C
100%	14220.97 (703.16)	100%	13877.04 (794.70)

Table 5 Compositions of primary and multiple emulsions prepared with Span® 80 and Poloxamer 188

Primary emulsion	% w/w
Citrate-phosphate buffer pH 5.5	50
Span® 80	5
Isopropyl myristate, qs to	100

Multiple emulsion	% w/w	% w/w	% w/w	% w/w
Primary emulsion	50	50	50	50
Poloxamer 188	0.5 – 3	0.5 – 3	0.5 – 3	0.5 – 3
Sodium chloride	0.2	0.2	0.2	0.2
Sodium azide	0.01	0.01	0.01	0.01
Xanthan gum	-	0.25	0.5	0.8
De-ionized water, qs to	100	100	100	100

Remark :

The concentrations of poloxamer 188 used were 0.5, 1, 1.5, 2, 2.5 and 3 % w/w

Table 6 Characteristic of multiple emulsion prepared with Span® 80 and Poloxamer 188

% w/w poloxamer 188	Before TCM		After TCM		% Viscosity Change
	%Cream	Viscosity, cp (SD) at 30 °C	%Cream	Viscosity, cp (SD) at 30 °C	
Xanthan gum 0 % and various poloxamer 188 concentrations					
0.5 %	66	-	crack	-	-
1 %	66	-	crack	-	-
1.5 %	62	-	crack	-	-
2 %	58	-	crack	-	-
2.5 %	60	-	crack	-	-
3 %	60	-	crack	-	-
Xanthan gum 0.25% and various poloxamer 188 concentrations					
0.5 %	100	24914.68 (591.67)	Crack	-	-
1 %	100	22739.15 (507.33)	crack	-	-
1.5 %	100	24682.73 (840.73)	crack	-	-
2 %	100	28034.02 (938.26)	crack	-	-
2.5 %	100	24946.68 (532.48)	crack	-	-
3 %	100	24082.86 (582.22)	crack	-	-

Table 6 Characteristic of multiple emulsion prepared with Span® 80 and Poloxamer 188 (continue)

% w/w poloxamer 188	Before TCM		After TCM		% Viscosity Change
	%Cream	Viscosity, cp (SD) at 30 °C	%Cream	Viscosity, cp (SD) at 30 °C	
Xanthan gum 0.5% and various poloxamer 188 concentrations					
0.5 %	100	63746.40 (1689.14)	100	31073.37 (339.37)	-51.25
1 %	100	65602.00 (2058.88)	100	30817.42 (434.91)	-53.02
1.5 %	100	65194.09 (1914.56)	100	38359.81 (1953.30)	-41.16
2 %	100	61618.85 (1732.84)	100	37655.97 (1870.38)	-38.89
2.5 %	100	62794.60 (2244.42)	100	34456.65 (1498.62)	-45.13
3 %	100	63554.44 (2200.20)	100	35504.42 (2144.99)	-44.13
Xanthan gum 0.8% and various poloxamer 188 concentrations					
0.5 %	crack	-	-	-	-
1 %	crack	-	-	-	-
1.5 %	crack	-	-	-	-
2 %	crack	-	-	-	-
2.5 %	crack	-	-	-	-
3 %	crack	-	-	-	-

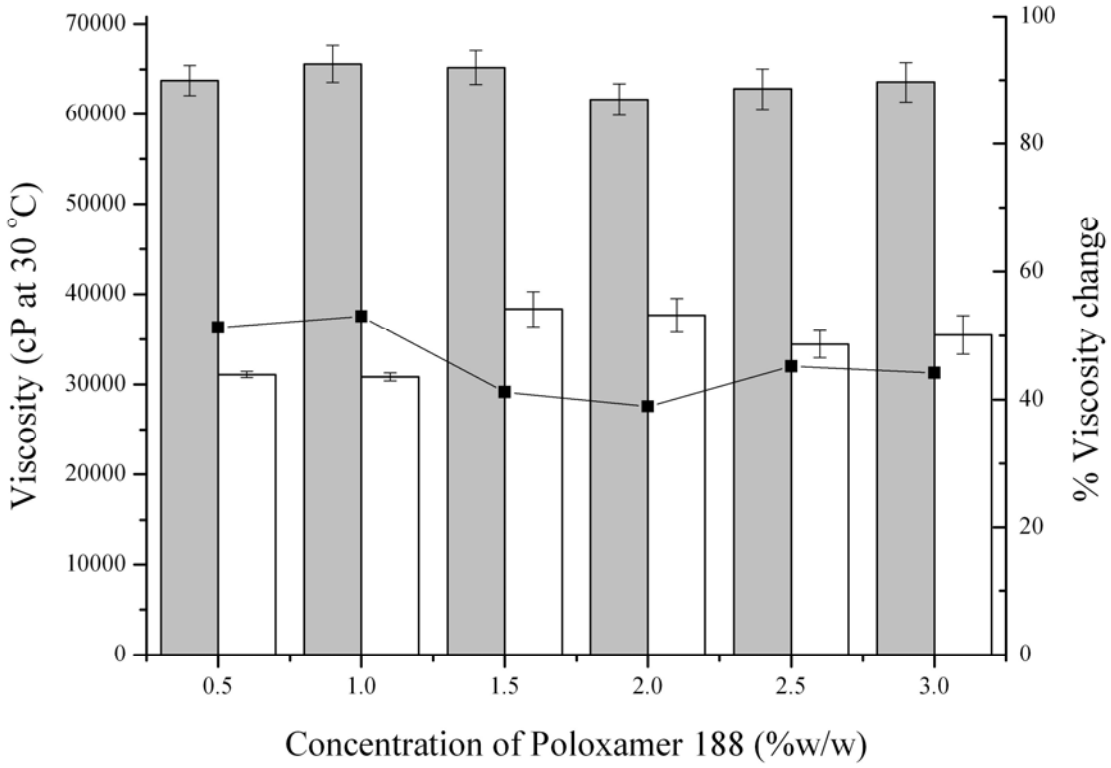


Figure 17 Effect of concentration of secondary emulsifier (Poloxamer 188) on the viscosity of multiple emulsion prepared with 5%w/w span® 80 in oil phase and 0.5 % xanthan gum in external phase. (n = 10)

Keys : Before temperature cycling method ; Gray columns

After temperature cycling method ; Blank columns

% Viscosity change ; closed square and line

2.2.2.2 Span® 80 and Poloxamer 407

Multiple emulsions containing 5%w/w of span® 80 and poloxamer 407 at 0.5 – 3 %w/w were prepared with various concentrations of xanthan gum (0, 0.25 and 0.5% w/w). The formula of those multiple emulsions are presented in table 7. All formula of multiple emulsion were milky white and homogeneous liquid except the formula containing 0 % w/w xanthan gum that were creaming after preparation. It was also found that multiple emulsions using poloxamer 188 as secondary emulsifier were more viscous than those using poloxamer 407. After TCM, formula containing 0.25% w/w xanthan gum exhibited phase separation. But the formula containing 0.5% w/w xanthan gum in the external phase showed good stability after TCM (table 8 and figure 18). It could be seen from table 8 that the viscosity of multiple emulsion containing 0.5%w/w xanthan gum at all concentrations of poloxamer 407 was largely increased compared to those containing 0.25%w/w xanthan gum. There might be some interaction between poloxamer 407 and xanthan gum at 0.5%w/w. This should be due to the fact that xanthan gum increased the viscosity of multiple emulsion and reduced the coalescence rate between oil droplets in multiple emulsion leading to the improvement of emulsion stability. However, the relationship between this secondary emulsifier in multiple emulsion and 0.5% w/w xanthan gum was not fully known. It was also found that the percentage of viscosity decrease of multiple emulsion after TCM increased with increasing secondary emulsifier concentration. It should be that the formation of mixed micelles of excess hydrophilic surfactant in aqueous phase increased solubility of lipophilic surfactant in aqueous phase and increased migration of lipophilic surfactant from oil phase to

external aqueous phase (Opawale and Burgess 1998 : 967). This phenomenon might result in coalescence of multiple emulsion and led to viscosity decrease.

Table 7 Compositions of primary and multiple emulsions prepared with Span® 80 and Poloxamer 407

Primary emulsion	% w/w		
Citrate-phosphate buffer pH 5.5	50		
Span® 80	5		
Isopropyl myristate, qs to	100		
Multiple emulsion	% w/w	% w/w	% w/w
Primary emulsion	50	50	50
Poloxamer 407	0.5 – 3	0.5 – 3	0.5 – 3
Sodium chloride	0.2	0.2	0.2
Sodium azide	0.01	0.01	0.01
Xanthan gum	0	0.25	0.5
De-ionized water, qs to	100	100	100

Remark :

The concentrations of poloxamer 407 used were 0.5, 1, 1.5, 2, 2.5 and 3 % w/w

Table 8 Characteristic of multiple emulsion prepared with Span® 80 and Poloxamer 407

% w/w poloxamer 407	Before TCM		After TCM		% Viscosity Change
	%Cream	Viscosity, cp (SD) at 30 °C	%Cream	Viscosity, cp (SD) at 30 °C	
Xanthan gum 0 % and various poloxamer 407 concentrations					
0.5 %	66	-	crack	-	
1 %	66	-	crack	-	
1.5 %	66	-	crack	-	
2 %	60	-	crack	-	
2.5 %	66	-	crack	-	
3 %	72	-	crack	-	
Xanthan gum 0.25% and various poloxamer 407 concentrations					
0.5 %	100	16612.46 (106.98)	crack	-	
1 %	100	17068.36 (285.16)	crack	-	
1.5 %	100	17300.31 (168.83)	crack	-	
2 %	100	5950.73 (120.42)	crack	-	
2.5 %	100	11637.52 (101.52)	crack	-	
3 %	100	9829.90 (166.28)	crack	-	

Table 8 Characteristic of multiple emulsion prepared with Span® 80 and Poloxamer 407 (continue).

% w/w poloxamer 407	Before TCM		After TCM		% Viscosity Change
	%Cream	Viscosity, cp (SD) at 30 °C	%Cream	Viscosity, cp (SD) at 30 °C	
Xanthan gum 0.5% and various poloxamer 407 concentrations					
0.5 %	100	62314.70 (513.46)	100	55324.19 (1126.75)	-11.22
1 %	100	59771.25 (1112.15)	100	46941.98 (506.5)	-21.50
1.5 %	100	56939.85 (2002.04)	100	44326.54 (921.87)	-22.15
2 %	100	50589.20 (1743.50)	100	30097.58 (357.79)	-40.50
2.5 %	100	56539.94 (2428.50)	100	30865.41 (1012.73)	-45.41
3 %	100	52428.81 (1455.04)	100	25122.64 (600.26)	-52.08

2.2.3 Lipophilic and Hydrophilic polymeric surfactant

In this study, lipophilic polymeric surfactant (PEG 30-dipolyhydroxystearate) at 0.5 – 2 %w/w was used as primary emulsifier to obtain water-in-oil (w/o) emulsion. The secondary surfactant used to promote a formation of water-in-oil-in-water (w/o/w) multiple emulsion was hydrophilic surfactant (Poloxamer 188 and 407). Formula and physical stability data of primary emulsion are shown in table 9. Primary emulsion was apparently white and homogeneous liquid. Primary emulsion prepared with 1-2 % w/w emulsifier showed good stability after TCM. So primary emulsions containing 1 and 2 % w/w PEG 30-

dipolyhydroxystearate were selected to prepare multiple emulsion in the next experiment.

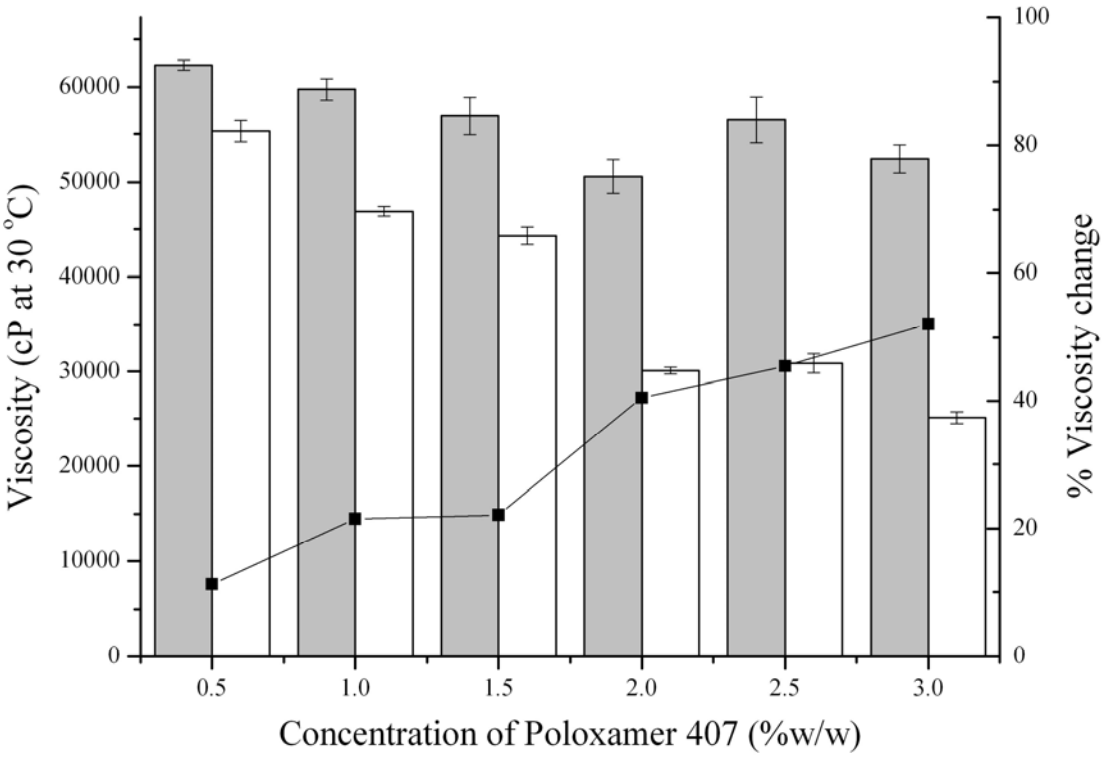


Figure 18 Effect of concentration of secondary emulsifier (Poloxamer 407) on the viscosity of multiple emulsion prepared with 5%w/w span® 80 in oil phase and 0.5 % xanthan gum in external phase. (n = 10)

Keys : Before temperature cycling method ; Gray columns

After temperature cycling method ; Blank columns

% Viscosity change ; closed square and line

Multiple emulsions using PEG 30-dipolyhydroxystearate as primary emulsifier were prepared with various concentrations of xanthan gum (0.25, 0.5 and 0.8 % w/w) and various concentrations of hydrophilic surfactant (0.5, 1, 1.5,

2, 2.5 and 3 % w/w) in external water phase. The formula consisting of 0.5 and 0.8% w/w xanthan gum could not be prepared since primary emulsion was separated from external water phase during preparation. This might be due to high viscosity difference between primary emulsion and external water phase. Formula of multiple emulsion consisting of 0.25% w/w xanthan gum and poloxamer 188 is shown in table 10. Immediately after processing, all formulas were apparently white and homogeneous. The samples were stable after TCM since there was no separation and the percentage of viscosity change was quite low. However, the multiple emulsion looked non-homogeneous after TCM.

Hydrophilic surfactant was then changed from poloxamer 188 to poloxamer 407 in the w/o/w multiple emulsion prepared with 2% PEG 30-dipolyhydroxystearate in oil phase and 0.25 % w/w xanthan gum in external water phase (table 10). The concentration of poloxamer 407 was varied from 0.5 – 3 %w/w. The resulting emulsions were white, homogeneous and viscous except that the formula containing 0.5 %w/w poloxamer 407 exhibited 60% creaming immediately after preparation. The samples did not show any change in appearance, homogeneity and consistency over the TCM (table 12 and figure 19) except the formula containing 0.5%w/w poloxamer 407 which exhibited high percentage of viscosity decrease after TCM.

Multiple emulsion prepared with both Span®80 (nonionic surfactant) and PEG 30-dipolyhydroxystearate (polymeric surfactant) as primary emulsifier showed good stability. But the percentage of viscosity change of formula consisting of polymeric surfactant was less than those containing nonionic surfactant. This should be owing to the steric stabilization mechanisms by polymeric surfactant

adsorption on the water-oil interface and forming full coverage of thick flexible and well-anchored moieties (Garti 1998 : 85). Therefore, multiple emulsion containing 2%w/w PEG 30-dipolyhydroxystearate as primary emulsifier, 0.25%w/w xanthan gum as thickener in external water phase and poloxamer 188 or 407 as secondary emulsifier was selected for the next experiment.

Table 9 Composition and physical stability of primary emulsion prepared with polymeric surfactant (PEG 30-dipolyhydroxystearate, Arlacel® P135)

	PEA135 05 %w/w	PEA135 1 %w/w	PEA135 15 %w/w	PEA135 2 %w/w
Citrate-phosphate buffer pH 5.5	50	50	50	50
PEG 30-dipolyhydroxystearate	0.5	1	1.5	2
Isopropyl myristate, qs to	100	100	100	100
Before TCM				
Type of emulsion	w/o	w/o	w/o	w/o
% Cream	100	100	100	100
Viscosity, cp (SD) at 30 °C	215.95 (141.33)	223.95 (145.05)	239.95 (75.41)	255.95 (98.32)
After TCM				
Type of emulsion	-	w/o	w/o	w/o
% Cream	crack	100	100	100
Viscosity, cp (SD) at 30 °C	-	151.97 (157.50)	239.95 (119.23)	207.96 (136.99)

Table 10 Compositions of primary and multiple emulsions prepared with PEG 30-dipolyhydroxystearate and various types of poloxamer (188 and 407)

	PEA135 1 (%w/w)	PEA135 2 (%w/w)	
Citrate-phosphate buffer pH 5.5	50	50	
PEG 30-dipolyhydroxystearate	1	2	
Isopropyl myristate, qs to	100	100	
Multiple emulsion	%w/w		
Primary emulsion	50 (PEA135 1)	50 (PEA135 2)	50 (PEA135 2)
Poloxamer 188	0.5 – 3	0.5 – 3	-
Poloxamer 407	-	-	0.5 – 3
Sodium chloride	0.2	0.2	0.2
Sodium azide	0.01	0.01	0.01
Xanthan gum	0.25	0.25	0.25
De-ionized water, qs to	100	100	100

Remark :

The concentrations of poloxamer 188 used were 0.5, 1, 1.5, 2, 2.5 and 3 % w/w

The concentrations of poloxamer 407 used were 0.5, 1, 1.5, 2, 2.5 and 3 % w/w

Table 11 Characteristic of multiple emulsion prepared with PEG 30-dipolyhydroxystearate and Poloxamer 188

% poloxamer 188	Before TCM		After TCM		% Viscosity Change
	%Cream	Viscosity, cp (SD) at 30 °C	%Cream	Viscosity, cp (SD) at 30 °C	
1 % PEG 30-dipolyhydroxystearate and various poloxamer188 concentrations					
0.5 %	100	22723.15 (541.75)	100	19899.75 (243.77)	-12.42
1 %	100	23994.88 (99.76)	100	22475.20 (258.49)	-6.33
1.5 %	100	24386.80 (442.04)	100	22491.20 (391.47)	-7.77
2 %	100	27682.09 (417.23)	100	27378.16 (125.33)	-1.09
2.5 %	100	32233.12 (308.62)	100	31081.37 (632.26)	-3.57
3 %	100	26146.42 (450.01)	100	27202.20 (95.76)	+4.04
2 % PEG 30-dipolyhydroxystearate and various poloxamer 188 concentrations					
0.5 %	100	21955.32 (371.82)	100	23882.90 (213.95)	+8.78
1 %	100	26058.44 (753.90)	100	28665.88 (562.03)	+10.00
1.5 %	100	30713.45 (303.98)	100	31729.23 (464.93)	+3.31
2 %	100	29761.65 (338.18)	100	28473.92 (578.00)	-4.33
2.5 %	100	34160.71 (477.60)	100	33680.81 (774.04)	-1.40
3 %	100	30273.54 (421.97)	100	30401.51 (446.84)	+0.42

Table 12 Characteristic of multiple emulsion prepared with PEG 30-dipolyhydroxystearate and poloxamer 407

% w/w poloxamer 407	Before TCM		After TCM		% Viscosity Change
	%Cream	Viscosity, cp (SD) at 30 °C	%Cream	Viscosity, cp (SD) at 30 °C	
2 % PEG 30-dipolyhydroxystearate and various poloxamer407 concentrations					
0.5 %	100	-	60	-	-
1 %	100	21971.31 (834.60)	100	583.88 (184.90)	-97.34
1.5 %	100	29625.68 (165.21)	100	27442.14 (682.28)	-7.37
2 %	100	31393.30 (1319.25)	100	28289.96 (164.56)	-9.88
2.5 %	100	42103.02 (302.10)	100	38127.86 (898.64)	-9.44
3 %	100	51165.08 (1699.65)	100	38607.76 (279.75)	-24.54

2.3 Type and concentration of stiffening agent in oil middle phase

The two mechanisms of multiple emulsion instability included flocculation and coalescence of outer droplets or inner water droplets. The destabilization of multiple emulsion was the result of water transport across the oil layer from the inner phase to the outer phase or in the opposite direction. Highly viscous oil middle phase was used to prevent diffusion of water / surfactant / active ingredient from the inner phase. In this study, a variety of waxes was used to increase viscosity of oil phase. These waxes included glyceryl monostearate (GMS), stearic acid, cetyl alcohol, colloidal silicon dioxide and petrolatum. Formula of primary emulsion (w/o emulsion) containing 2%w/w PEG 30-dipolyhydroxystearate as

emulsifier and various types and concentrations of stiffening agent are given in table 13.

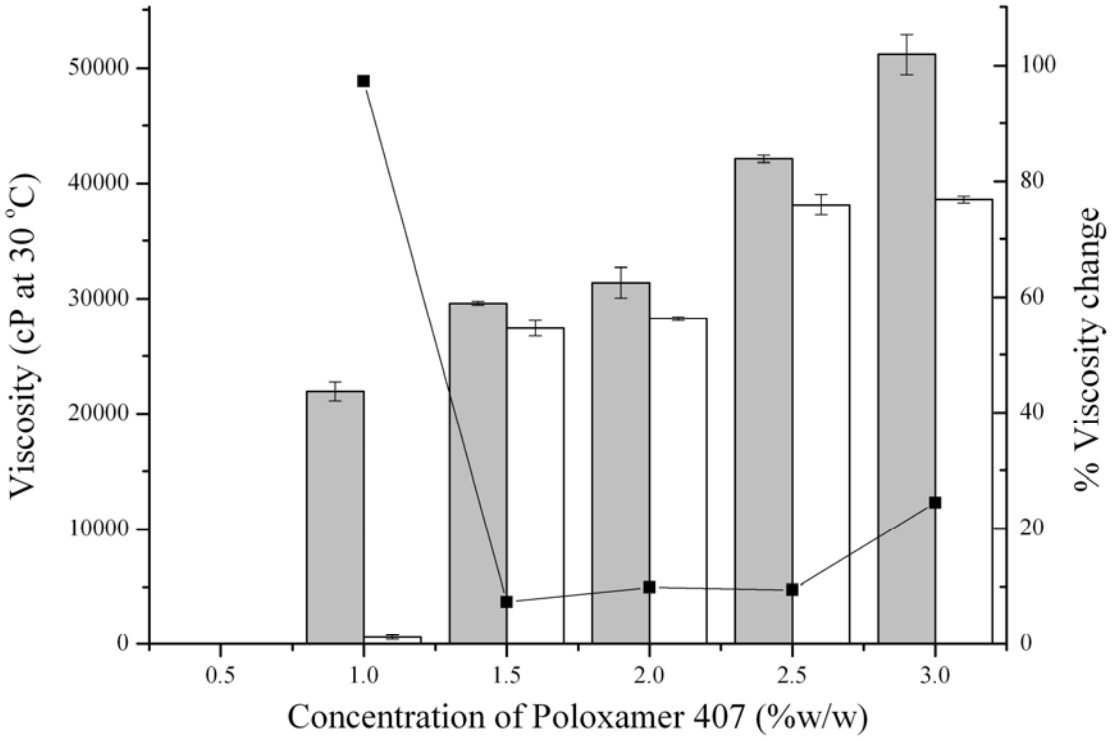


Figure 19 Effect of concentration of secondary emulsifier (Poloxamer 407) on the viscosity of multiple emulsion prepared with 2 % PEG 30-dipolyhydroxystearate in oil phase and 0.25 % xanthan gum in external phase. (n = 10)

Keys : Before temperature cycling method ; Gray columns

After temperature cycling method ; Blank columns

% Viscosity change ; closed square and line

Table 13 Compositions of primary emulsions prepared with various oil viscosity increasing agent. (%w/w of ingredients)

Composition (%w/w)	ST05	ST1	ST2
Citrate-phosphate buffer pH 5.5	50	50	50
PEG 30-dipolyhydroxystearate	2	2	2
Stearic acid	0.5	1	2
Isopropyl myristate, qs to	100	100	100

Composition (%w/w)	control	GMS05	GMS1	GMS2	GMS8
Citrate-phosphate buffer pH 5.5	50	50	50	50	50
PEG 30-dipolyhydroxystearate	0	2	2	2	2
Glyceryl monostearate (GMS)	2	0.5	1	2	8
Isopropyl myristate, qs to	100	100	100	100	100

Composition (%w/w)	PT1	PT3	PT5	PT8	PT10	PT15	PT20
Citrate-phosphate buffer pH 5.5	50	50	50	50	50	50	50
PEG 30-dipolyhydroxystearate	2	2	2	2	2	2	2
Petrolatum	1	3	5	8	10	15	20
Isopropyl myristate, qs to	100	100	100	100	100	100	100

Table 13 Compositions of primary emulsions prepared with various oil viscosity increasing agent. (%w/w of ingredients) (continue)

Composition (%w/w)	Control	CA1	CA3	CA5
Citrate-phosphate buffer pH 5.5	50	50	50	50
PEG 30-dipolyhydroxystearate	0	2	2	2
Cetyl alcohol	3	1	3	5
Isopropyl myristate, qs to	100	100	100	100

Composition (%w/w)	CS1	CS2	CS3
Citrate-phosphate buffer pH 5.5	50	50	50
PEG 30-dipolyhydroxystearate	2	2	2
Colloidal silicon dioxide	1	2	3
Isopropyl myristate, qs to	100	100	100

Primary emulsions with all concentrations of stearic acid and GMS were apparently white, homogeneous and viscous. After TCM, all formula cracked (table 14) and showed some wax particles precipitated at the bottom of the container. Primary emulsions prepared with all concentrations of petrolatum were apparently white, homogeneous and viscous. Viscosity of primary emulsion prepared with petrolatum increased with increasing petrolatum concentration. The samples were stable over the TCM. Primary emulsion prepared with cetyl alcohol and colloidal silicon dioxide were apparently white, homogeneous and viscous. After TCM, all formula cracked except the primary emulsion containing 3% w/w colloidal silicone

dioxide which was very viscous and could not flow. Formula consisting of GMS or cetyl alcohol were prepared without emulsifier as control formula to investigate the potential of these stiffening agent to be used as emulsifier. It was found that GMS and cetyl alcohol could be served as w/o emulsifier since emulsion could be obtained after preparation but they were not stable after TCM.

In order to develop a stable multiple emulsion for drug loading, the previous factors investigated such as thickening agent, hydrophilic emulsifier, lipophilic emulsifier and stiffening agent, were considered and chosen. Xanthan gum at 0.25 and 0.5 %w/w were chosen as thickening agent in water phase. Poloxamer 188 and poloxamer 407 at 0.5 – 3 %w/w were selected for hydrophilic emulsifier. PEG 30-dipolyhydroxystearate at 2 %w/w was used as lipophilic emulsifier. Petrolatum at 10 %w/w was selected as stiffening agent in oil phase. The compositions of primary and multiple emulsions are shown in table 15.

Immediately after preparation, all multiple emulsions prepared with 0.25 % w/w xanthan gum were apparently milky white and homogeneous. After TCM, multiple emulsion consisting of 0.25% w/w xanthan gum and poloxamer 188 in external phase were still stable but the emulsion mass looked non-homogeneous (table 16 and figure 20). Multiple emulsion containing 0.25% w/w xanthan gum and poloxamer 407 also showed good appearance and good stability over TCM (table 16 and figure 21).

Table 14 Physical appearance and viscosity of primary emulsion prepared with various types and concentrations of stiffening agent.

Concentration of stiffening agent (%w/w)	Before TCM		After TCM	
	% Cream	Viscosity, cp (SD) at 30 °C	% Cream	Viscosity, cp (SD) at 30 °C
0% Stiffening agent	100	255.95 (98.32)	100	207.96 (136.99)
Stearic acid				
0.5, 1, 2 %	100	-	crack	-
GMS				
2 % without EA.	100	-	crack	-
0.5, 1, 2, 8 %	100	-	crack	-
Petrolatum				
1 %	100	89.98 (121.88)	100	103.98 (136.21)
3 %	100	119.97 (142.33)	100	127.97 (142.08)
5 %	100	231.95 (161.95)	100	159.97 (172.78)
8 %	100	631.87 (127.58)	100	263.94 (184.90)
10 %	100	2497.47 (212.62)	100	1137.76 (202.34)
15 %	100	6790.00 (329.67)	100	8134.26 (458.77)
20 %	100	11709.50 (855.81)	100	15012.80 (960.32)

Remark : EA. = emulsifier

Table 14 Physical appearance and viscosity of primary emulsion prepared with various types and concentrations of stiffening agent. (continue)

Concentration of stiffening agent (%w/w)	Before TCM		After TCM	
	% Cream	Viscosity, cp (SD) at 30 °C	% Cream	Viscosity, cp (SD) at 30 °C
Cetyl alcohol				
3 % without EA.	crack	-	crack	-
1, 3, 5, 8 %	100	-	crack	-
Colloidal silicon dioxide				
1, 2 %	100	-	crack	-
3 %	100	High viscosity	100	High viscosity

Remark : EA. = emulsifier

Table 15 Compositions of primary and multiple emulsions prepared with PEG 30-dipolyhydroxystearate and 10% petrolatum in oil phase

Primary emulsion	PEA135 2 (% w/w)			
Citrate-phosphate buffer pH 5.5 PEG 30-dipolyhydroxystearate Petrolatum Isopropyl myristate, qs to	50 2 10 100			
Multiple emulsion	X025P8 % w/w	X05P8 % w/w	X025P4 % w/w	X05P4 % w/w
Primary emulsion (PEA135 2) Poloxamer 188 Poloxamer 407 Sodium chloride Sodium azide Xanthan gum De-ionized water, qs to	50 0.5 – 3 - 0.2 0.01 0.25 100	50 0.5 – 3 - 0.2 0.01 0.5 100	50 - 0.5 – 3 0.2 0.01 0.25 100	50 - 0.5 – 3 0.2 0.01 0.5 100

Remark :

The concentrations of poloxamer 188 used were 0.5, 1, 1.5, 2, 2.5 and 3 % w/w

The concentrations of poloxamer 407 used were 0.5, 1, 1.5, 2, 2.5 and 3 % w/w

Table 16 Characteristic of multiple emulsion prepared with PEG 30-dipolyhydroxystearate and 10% petrolatum in oil phase

% w/w poloxamer	Before TCM		After TCM		% Viscosity Change
	%Cream	Viscosity, cp (SD) at 30 °C	%Cream	Viscosity, cp (SD) at 30 °C	
0.25 % w/w xanthan gum and various poloxamer188 concentrations					
0.5 %	100	15140.77 (591.43)	100	8070.28 (427.32)	-46.70
1 %	100	12757.28 (114.67)	100	7230.46 (147.00)	-43.32
1.5 %	100	12981.23 (367.59)	100	11397.57 (354.20)	-12.20
2 %	100	14172.98 (134.90)	100	12005.44 (166.28)	-15.29
2.5 %	100	15124.77 (573.61)	100	12469.34 (613.14)	-17.56
3 %	100	19243.89 (247.82)	100	14380.93 (154.54)	-25.27
0.25 % w/w xanthan gum and various poloxamer407 concentrations					
0.5 %	100	12757.28 (428.55)	100	11141.62 (359.77)	-12.66
1 %	100	11389.57 (136.99)	100	9885.89 (126.18)	-13.20
1.5 %	100	10453.77 (414.83)	100	9693.93 (393.28)	-7.27
2 %	100	9310.01 (165.21)	100	8110.27 (173.60)	-12.89
2.5 %	100	10621.73 (940.15)	100	8742.13 (720.39)	-17.69
3 %	100	6174.68 (205.82)	100	6382.64 (212.62)	+3.37

It was found that viscosity of multiple emulsion decreased after TCM. This might be owing to water transporation across the oil layer from the inner phase to the outer phase which led to instability of multiple emulsion.

Immediately after preparation, multiple emulsion prepared with both poloxamer and 0.5 % w/w xanthan gum were apparently milky white with non-homogeneous texture. After the TCM, all formula were quite stable but showed apparently rough texture except formula containing 2, 2.5 and 3% w/w poloxamer 407 in external phase showed phase separation (cracking). This might be due to a large viscosity difference between primary emulsion and external water phase which could not be properly mixed with a stirrer speed of 1000 rpm. Therefore, xanthan gum at 0.25% w/w and poloxamer 407 were used for next experiment.

The concentration of petrolatum was increased from 10 % w/w to 20 %w/w in primary emulsion. The multiple emulsion contained 0.25% w/w xanthan gum and 0.5 – 3 %w/w poloxamer 407 in external phase. Composition of primary emulsion and multiple emulsion are shown in table 17.

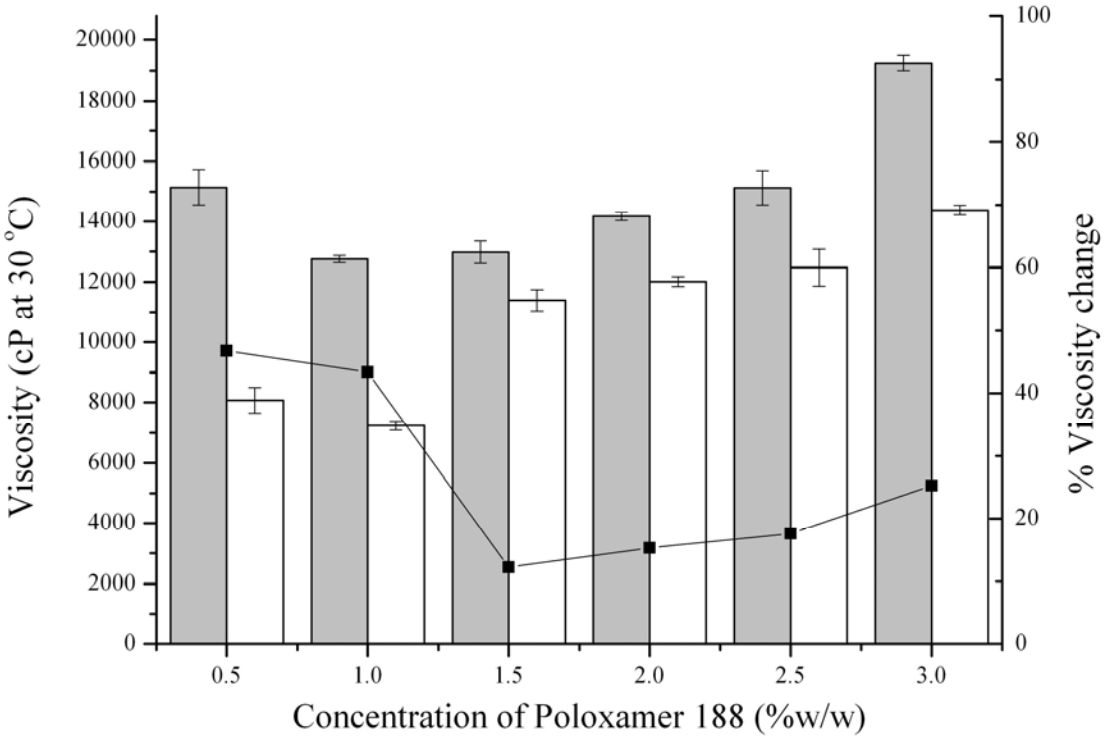


Figure 20 Effect of concentration of secondary emulsifier (Poloxamer 188) on the viscosity of multiple emulsion prepared with 2 % PEG 30-dipolyhydroxystearate, 10% petrolatum in oil phase and 0.25 % xanthan gum in external phase. (n = 10)

Keys : Before temperature cycling method ; Gray columns

After temperature cycling method ; Blank columns

% Viscosity change ; closed square and line

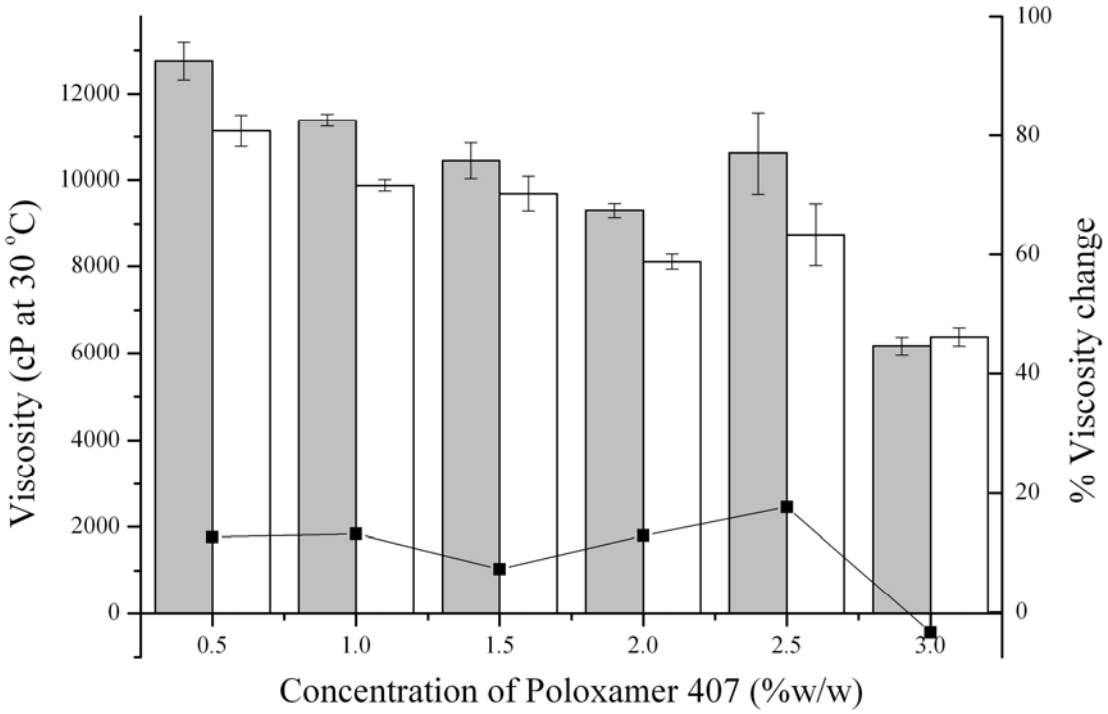


Figure 21 Effect of concentration of secondary emulsifier (Poloxamer 407) on the viscosity of multiple emulsion prepared with 2 % PEG 30-dipolyhydroxystearate, 10% petrolatum in oil phase and 0.25 % xanthan gum in external phase. (n = 10)

Keys : Before temperature cycling method ; Gray columns

After temperature cycling method ; Blank columns

% Viscosity change ; closed square and line

Table 17 Compositions of primary emulsion and multiple emulsions prepared with PEG 30-dipolyhydroxystearate and 20% petrolatum in oil phase

Primary emulsion	%w/w
Citrate-phosphate buffer pH 5.5	50
PEG 30-dipolyhydroxystearate	2
Petrolatum	20
Isopropyl myristate, qs to	100
Multiple emulsion	**%w/w**
Primary emulsion	50
Poloxamer 407	0.5, 1, 1.5, 2, 2.5, 3
Sodium chloride	0.2
Sodium azide	0.01
Xanthan gum	0.25
De-ionized water, qs to	100

Table 18 Characteristic of multiple emulsion prepared with PEG 30-dipolyhydroxystearate and 20% petrolatum in oil phase

% w/w poloxamer 407	Before TCM		After TCM		% Viscosity Change
	%Cream	Viscosity, cp (SD) at 30 °C	%Cream	Viscosity, cp (SD) at 30 °C	
0.25 % w/w xanthan gum and various poloxamer407 concentrations					
0.5 %	100	12005.44 (387.18)	100	10245.81 (658.96)	-14.66
1 %	100	9150.05 (77.27)	100	7854.32 (140.67)	-14.16
1.5 %	100	10165.83 (342.36)	100	9853.90 (491.31)	-3.07
2 %	100	7614.38 (171.96)	100	6470.62 (109.60)	-15.02
2.5 %	100	9214.03 (685.76)	100	8566.17 (524.42)	-7.03
3 %	100	5350.86 (161.95)	100	8214.25 (709.45)	+53.51

All the multiple emulsion consisting of 20 % w/w petrolatum in oil phase and various poloxamer 407 concentrations were stable over the TCM (table 18). The viscosity differences between before and after TCM were plotted as shown in figure 22. It could be seen that the viscosity of multiple emulsion containing 10 % w/w and 20 %w/w petrolatum was not significantly difference, eventhough the viscosity of related primary emulsions was largely different. Therefore, petrolatum at both (10 % and 20 % w/w) concentrations could be used to prepare stables multiple emulsions.

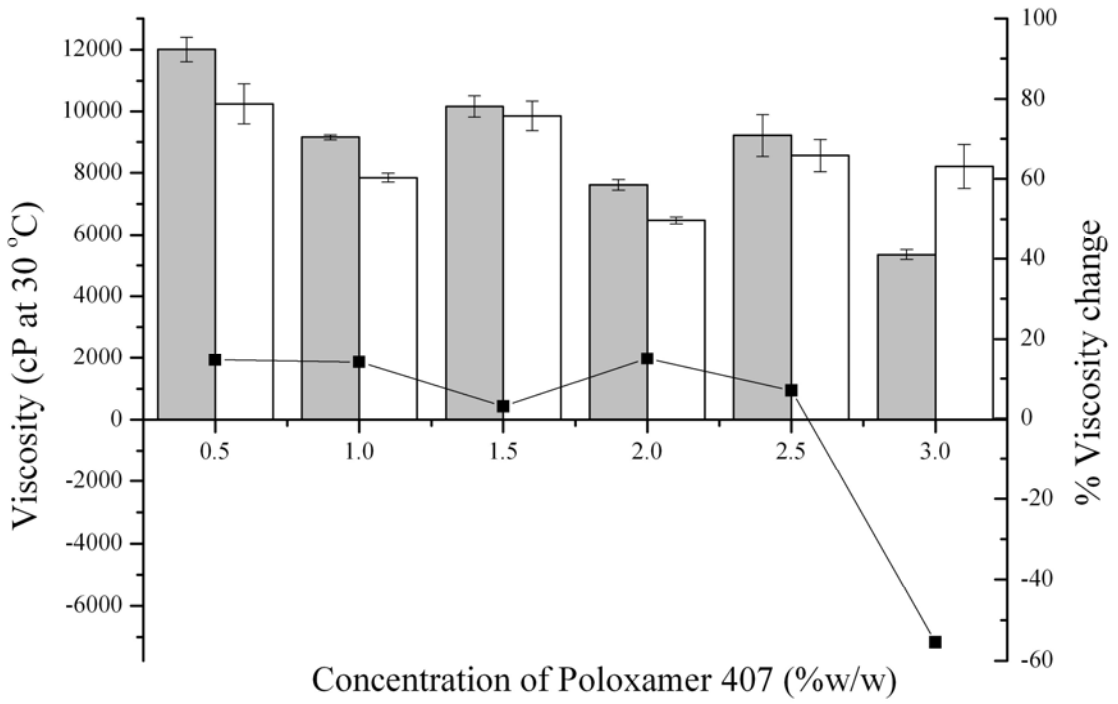


Figure 22 Effect of concentration of secondary emulsifier (Poloxamer 407) on the viscosity of multiple emulsion prepared with 2 % PEG 30-dipolyhydroxystearate, 20% petrolatum in oil phase and 0.25 % xanthan gum in external phase. (n = 10)

Keys : Before temperature cycling method ; Gray columns

After temperature cycling method ; Blank columns

% Viscosity change ; closed square and line

2.4 Osmolality adjusting agent

From previous study, the formula of multiple emulsion consisting of 20 % w/w petrolatum in oil phase and 0.25 % w/w xanthan gum in external water phase were selected to study the effect of osmolality adjusting agent on physical stability of multiple emulsion. In this study, osmolality adjusting agent was change from sodium chloride to dextrose. The w/o/w multiple emulsions were also prepared with various poloxamer 407 concentrations (0.5 – 3%w/w). Composition of primary emulsion and multiple emulsions are shown in table 19.

Water-in-oil-in-water multiple emulsion prepared with dextrose showed good stability both before and after TCM (table 20 and figure 23) except some degree of viscosity decrease after TCM. Changing osmolality adjusting agent from sodium chloride to dextrose exhibited viscosity increase of multiple emulsion. This should be owing to the effect of sodium chloride on the conformation of xanthan gum which was used as viscosity inducing agent in external water phase. With the existing of sodium chloride, xanthan gum conformation changed from disorder (random coil) to order (helix) conformation which the backbone was taken on a helical conformation and the charged trisaccharide side chains collapsed down on to the backbone. This may be led to the decrease in viscosity of multiple emulsion containing sodium chloride (Viturawong 2008 : 108).

However, it was shown that sodium chloride and dextrose could be used to adjust osmolality of multiple emulsion and both of them produced good stability multiple emulsion.

Table 19 Compositions of multiple emulsions prepared with dextrose as osmolality adjusting agent

Primary emulsion	%w/w
Citrate-phosphate buffer pH 5.5	50
PEG 30-dipolyhydroxystearate	2
Petrolatum	20
Isopropyl myristate, qs to	100
Multiple emulsion	**%w/w**
Primary emulsion	50
Poloxamer 407	0.5, 1, 1.5, 2, 2.5, 3
Dextrose	2.7
Sodium azide	0.01
Xanthan gum	0.25
De-ionized water, qs to	100

Table 20 Characteristic of multiple emulsion prepared with 20% petrolatum in oil phase and dextrose as osmolality adjusting agent

% w/w poloxamer 407	Before TCM		After TCM		% Viscosity Change
	%Cream	Viscosity, cp (SD) at 30 °C	%Cream	Viscosity, cp (SD) at 30 °C	
0.25 % w/w xanthan gum and various poloxamer 407 concentrations					
0.5 %	100	42350.96 (395.89)	100	37679.96 (1787.06)	-11.03
1 %	100	46054.17 (547.43)	100	34816.57 (2395.35)	-24.40
1.5 %	100	49173.51 (742.50)	100	35472.43 (2005.52)	-27.86
2 %	100	55140.23 (901.13)	100	43758.66 (1193.77)	-20.64
2.5 %	100	62634.64 (930.04)	100	46430.09 (341.95)	-25.87
3 %	100	62394.69 (645.89)	100	43606.70 (552.60)	-30.11

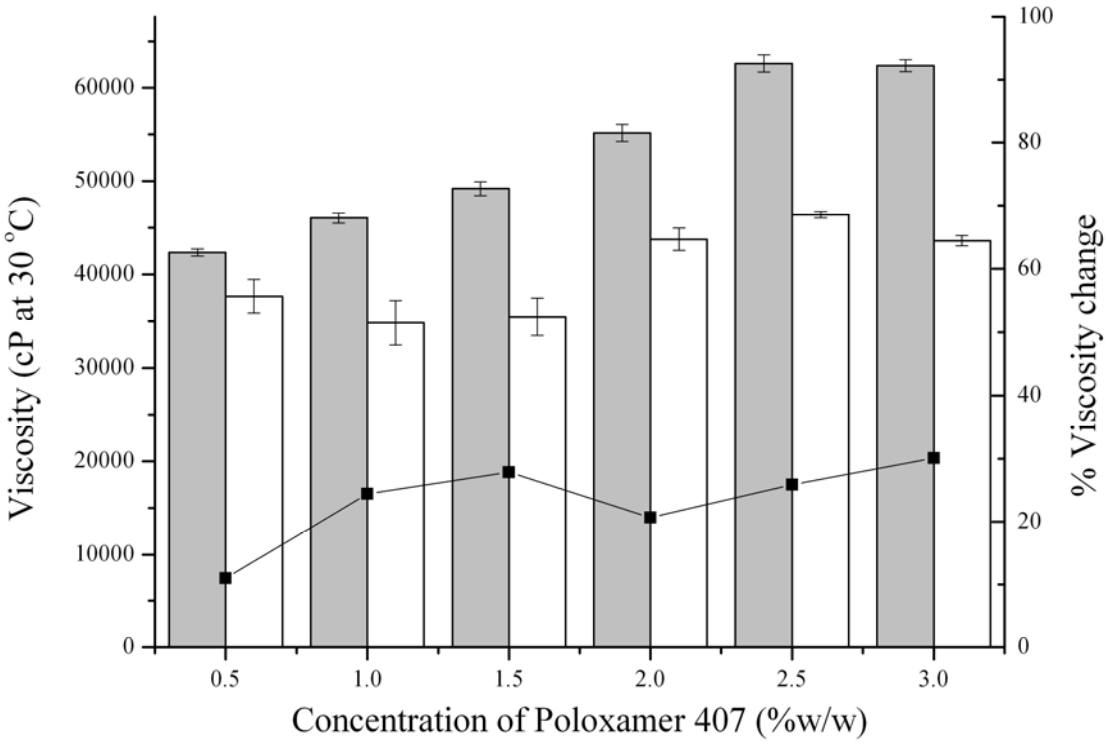


Figure 23 Effect of concentration of secondary emulsifier (Poloxamer 407) on the viscosity of multiple emulsion prepared with 2 % PEG 30-dipolyhydroxystearate, 20% petrolatum in oil phase and 0.25 % xanthan gum and dextrose in external phase.(n = 10)

Keys : Before temperature cycling method ; Gray columns

After temperature cycling method ; Blank columns

% Viscosity change ; closed square and line

2.5 Rotating process

The selected formula of multiple emulsion for this study are shown in table 21. In this study, a variety of stirrer speeds in the second step of multiple emulsion preparation was used to investigate the effect on physical stability of multiple emulsion. The speed was set at 800, 1000 and 1200 rpm.

Table 21 Compositions of multiple emulsions prepared with sodium chloride or dextrose as osmolality adjusting agent (%w/w of ingredients)

Primary emulsion	%w/w	
Citrate-phosphate buffer pH 5.5	50	
PEG 30-dipolyhydroxystearate	2	
Petrolatum	10	
Isopropyl myristate, qs to	100	
Multiple emulsion	%w/w	%w/w
Primary emulsion	50	50
Poloxamer 407	1.5	1.5
Sodium chloride	0.2	-
Dextrose	-	2.7
Sodium azide	0.01	0.01
Xanthan gum	0.25	0.25
De-ionized water, qs to	100	100

Immediately after preparation, all formula of multiple emulsion were apparently white and homogeneous liquid. They were stable and showed no significant change in appearance, homogeneity and consistency after TCM (table 22 and figure 24, 25). However, the particle size of formula containing sodium chloride at 800 rpm increased significantly after TCM. It was found that the droplet size of multiple emulsion decreased with increasing stirrer speeds since high speed increased stirring energy to break up oil droplet. Stirring speed at 1000 rpm and 1200 rpm exhibited no significant difference in droplet size. Therefore, stirring speed at 1000 rpm should be used for multiple emulsion preparation. It was also observed that the particle size in multiple emulsion containing dextrose was significantly smaller than those containing sodium chloride at all stirring speed. Since emulsification was a process in which the breaking and coalescence steps were in dynamic equilibrium. It should be that the higher viscosity produced in formula containing dextrose helped decrease rate of coalescence of oil droplet (Salager, in Nielloud and Marti-Mestres, eds. 2000 : 76).

Table 22 Characteristic of multiple emulsion prepared with various stirrer speeds

Osmolality adjusting agent	Speed (rpm)	% Cream	Viscosity, cp (SD) at 30 °C	Droplet size, μm (SD)
Before TCM				
Sodium chloride	800	100	14620.88 (205.83)	57.62 (2.95)
Sodium chloride	1000	100	14037.00 (478.79)	60.50 (0.48)
Sodium chloride	1200	100	14013.01 (419.52)	50.39 (0.91)
Dextrose	800	100	45668.66 (278.22)	47.36 (0.30)
Dextrose	1000	100	50667.59 (313.65)	34.78 (0.17)
Dextrose	1200	100	47788.20 (177.65)	30.92 (0.15)
After TCM				
Sodium chloride	800	100	13237.18 (245.08)	86.00 (4.39)
Sodium chloride	1000	100	12573.32 (353.30)	60.56 (1.31)
Sodium chloride	1200	100	13061.21 (345.67)	51.94 (0.43)
Dextrose	800	100	35992.32 (150.82)	48.22 (0.36)
Dextrose	1000	100	50181.29 (607.03)	33.76 (0.12)
Dextrose	1200	100	45102.35 (476.11)	32.21 (0.12)

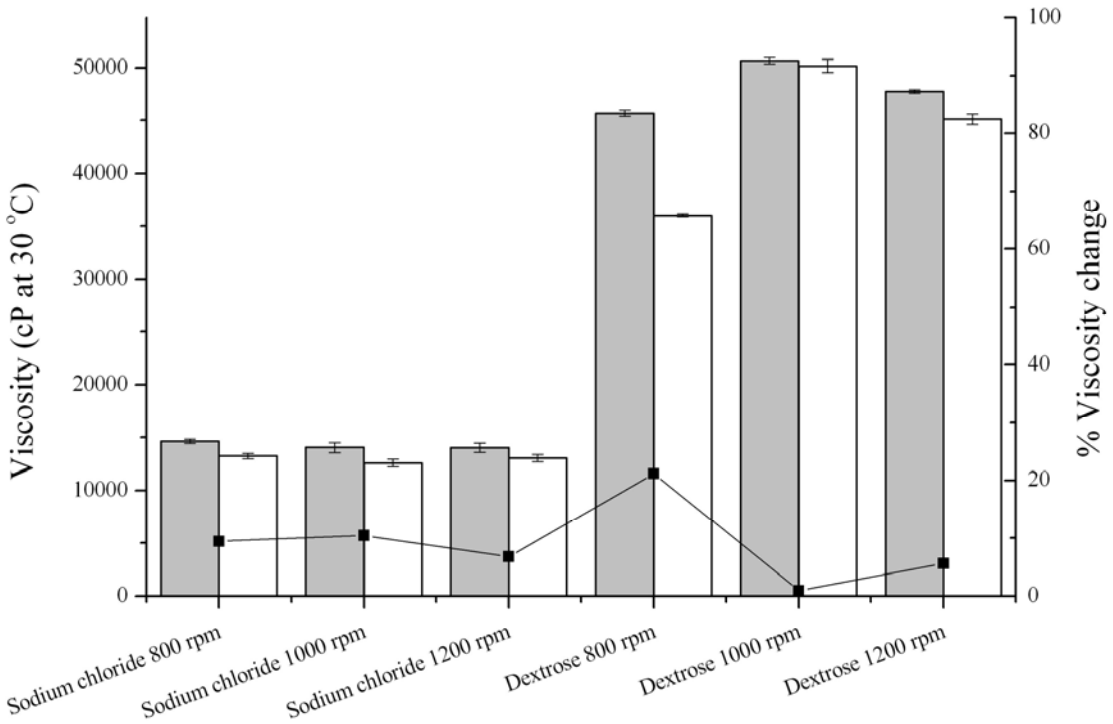


Figure 24 Effect of stirrer speeds on viscosity of multiple emulsion prepared with various osmolality adjusting agent (sodium chloride and dextrose) in external phase. (n = 10)

Keys : Before temperature cycling method ; Gray columns

After temperature cycling method ; Blank columns

% Viscosity change ; closed square and line

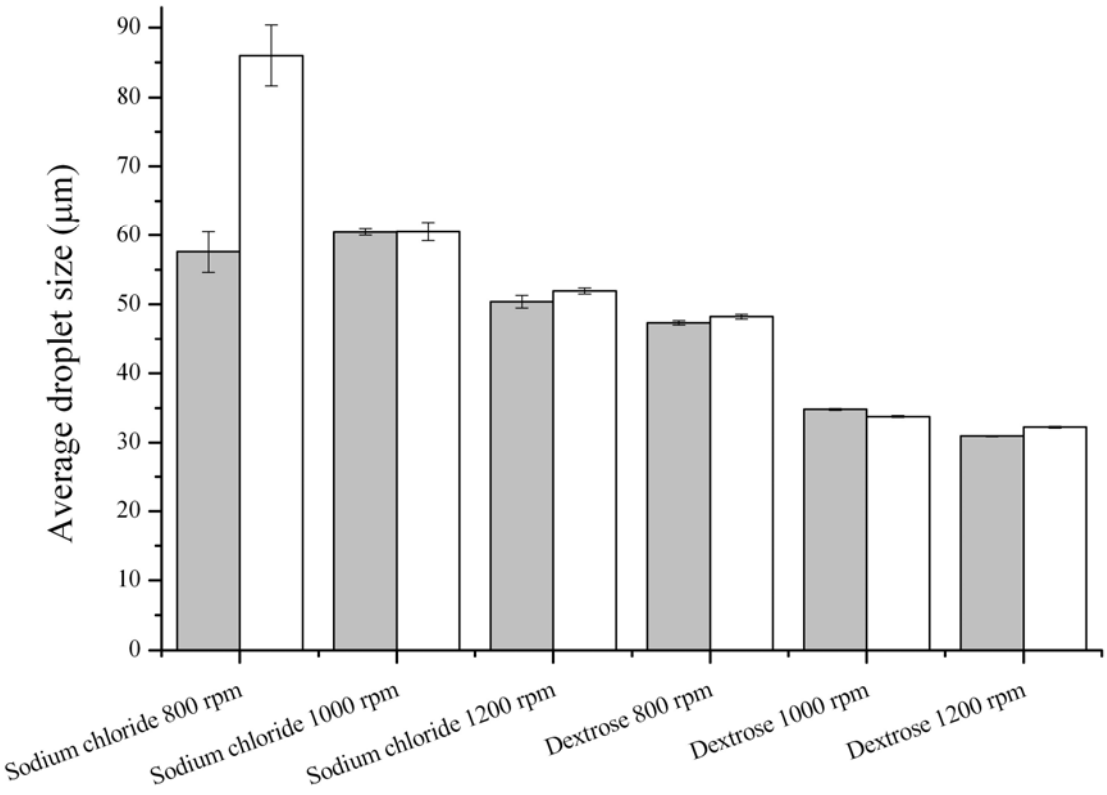


Figure 25 Effect of stirrer speeds on average droplet size of multiple emulsion prepared with various osmolality adjusting agent (sodium chloride and dextrose) in external phase. (n = 3)

Keys : Before temperature cycling method ; Gray columns

After temperature cycling method ; Blank columns

2.6 Phase volume ratio

The selected formula of multiple emulsion for this study are shown in table 23. The stirrer speed in the second step of multiple emulsion preparation was set at 1000 rpm. The phase volume ratio of primary and multiple emulsion was set according to table 24. The phase volume ratio of water in primary emulsion was varied from 0.4 – 0.6. The phase volume ratio of primary emulsion in multiple emulsion was also varied from 0.4 – 0.6.

Table 23 Compositions of multiple emulsions (%w/w of ingredients)

Primary emulsion	%w/w
Citrate-phosphate buffer pH 5.5	50
PEG 30-dipolyhydroxystearate	2
Petrolatum	10
Isopropyl myristate, qs to	100
Multiple emulsion	**%w/w**
Primary emulsion	50
Poloxamer 407	1.5
Sodium chloride	0.2
Sodium azide	0.01
Xanthan gum	0.25
De-ionized water, qs to	100

Table 24 Phase volume ratio of the primary and multiple emulsions

		Phase volume ratio of primary emulsion in multiple emulsion		
		0.4	0.5	0.6
Phase volume ratio of water phase in primary emulsion	0.4	0.4 : 0.4	0.4 : 0.5	0.4 : 0.6
	0.5	0.5 : 0.4	0.5 : 0.5	0.5 : 0.6
	0.6	0.6 : 0.4	0.6 : 0.5	0.6 : 0.6

It was found that phase volume ratio of water phase in primary emulsion had no effect on viscosity of multiple emulsion (table 25 and figure 26). The viscosity of multiple emulsion increased with increasing phase volume ratio of primary emulsion in multiple emulsion (ϕ_2). Immediately after preparation, all formula of multiple emulsions were apparently white and homogeneous liquid. With no significant change in appearance, homogeneity and consistency after TCM except multiple emulsion prepared at 0.4 phase volume ratio of water phase in primary emulsion (ϕ_1) and 0.6 phase volume ratio of primary emulsion in multiple emulsion (ϕ_2) which showed phase separation after TCM. It seemed that multiple emulsion prepared with the highest oil volume in formula ($\phi_1 : \phi_2 = 0.4 : 0.6$) was not stable due to phase separation after TCM. Figure 26 examined the effect of phase volume ratio on viscosity of multiple emulsion.

Table 25 Characteristic of multiple emulsion prepared with various phase volume ratios of the primary and multiple emulsions

$\phi_1 : \phi_2$	% Cream	Viscosity, cp (SD)at 30 °C	Droplet size, μm (SD)
Before TCM			
0.4 : 0.4	100	6150.69 (211.45)	59.72 (4.46)
0.4 : 0.5	100	10957.66 (363.61)	43.50 (2.32)
0.4 : 0.6	100	9910.79 (126.18)	-
0.5 : 0.4	100	6398.63 (172.78)	95.26 (7.28)
0.5 : 0.5	100	9725.92 (367.88)	63.59 (1.66)
0.5 : 0.6	100	18907.97 (724.47)	33.95 (2.43)
0.6 : 0.4	100	4663.01 (177.05)	164.80 (18.65)
0.6 : 0.5	100	9134.05 (276.55)	76.83 (1.58)
0.6 : 0.6	100	20587.61 (789.27)	39.90 (1.16)
After TCM			
0.4 : 0.4	100	5966.73 (242.01)	65.50 (4.72)
0.4 : 0.5	100	9302.02 (337.34)	42.53 (0.99)
0.4 : 0.6	crack	-	-
0.5 : 0.4	100	6094.70 (234.86)	97.87 (4.36)
0.5 : 0.5	100	9965.87 (250.67)	65.46 (1.23)
0.5 : 0.6	100	15404.71 (409.92)	31.15 (1.50)
0.6 : 0.4	100	5926.74 (267.81)	169.27 (3.50)
0.6 : 0.5	100	9613.95 (222.42)	75.96 (3.24)
0.6 : 0.6	100	17556.25 (578.30)	39.87 (1.17)

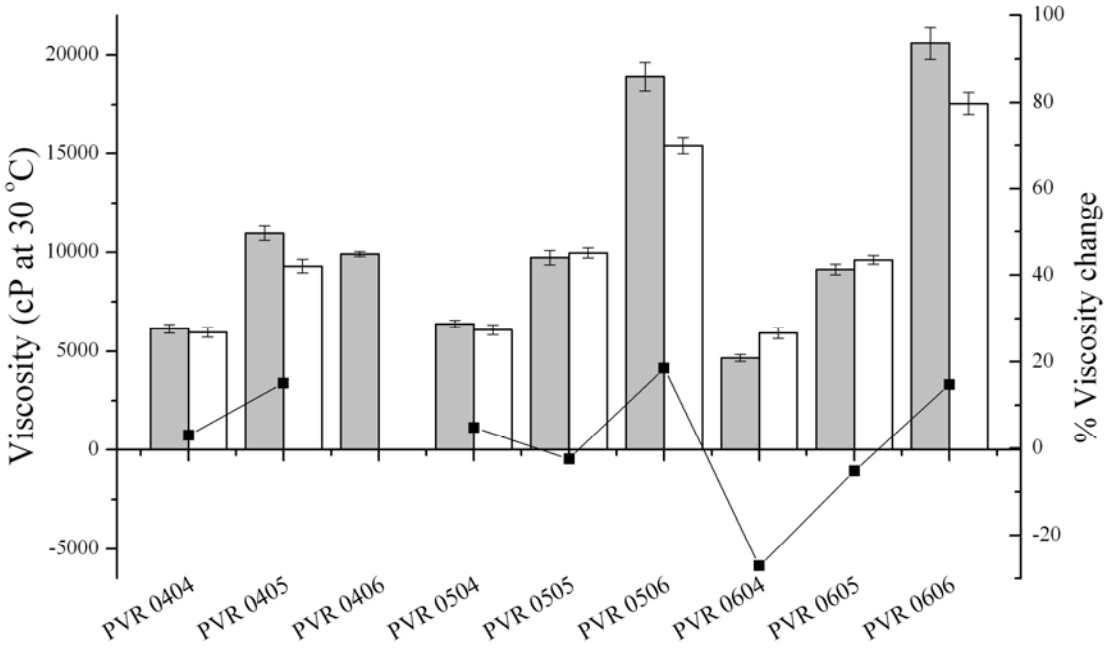


Figure 26 Effect of phase volume ratio of the primary and multiple emulsions on viscosity of multiple emulsion. (n = 10)

Keys : Before temperature cycling method ; Gray columns

After temperature cycling method ; Blank columns

% Viscosity change ; closed square and line

It was found that droplet size of multiple emulsion increased with increasing phase volume ratio of water phase in primary emulsion (ϕ_1) (figure 27 and 28). This might be due to the swelling of oil droplet from the volume increase of water phase inside. The droplet size of multiple emulsion decreased with increasing phase volume ratio of primary emulsion in multiple emulsion (ϕ_2). This might be due to the viscosity of multiple emulsion which increased with increasing phase volume ratio of primary emulsion in multiple emulsion (ϕ_2). This high viscosity helped decrease rate of coalescence of oil droplet during emulsification.

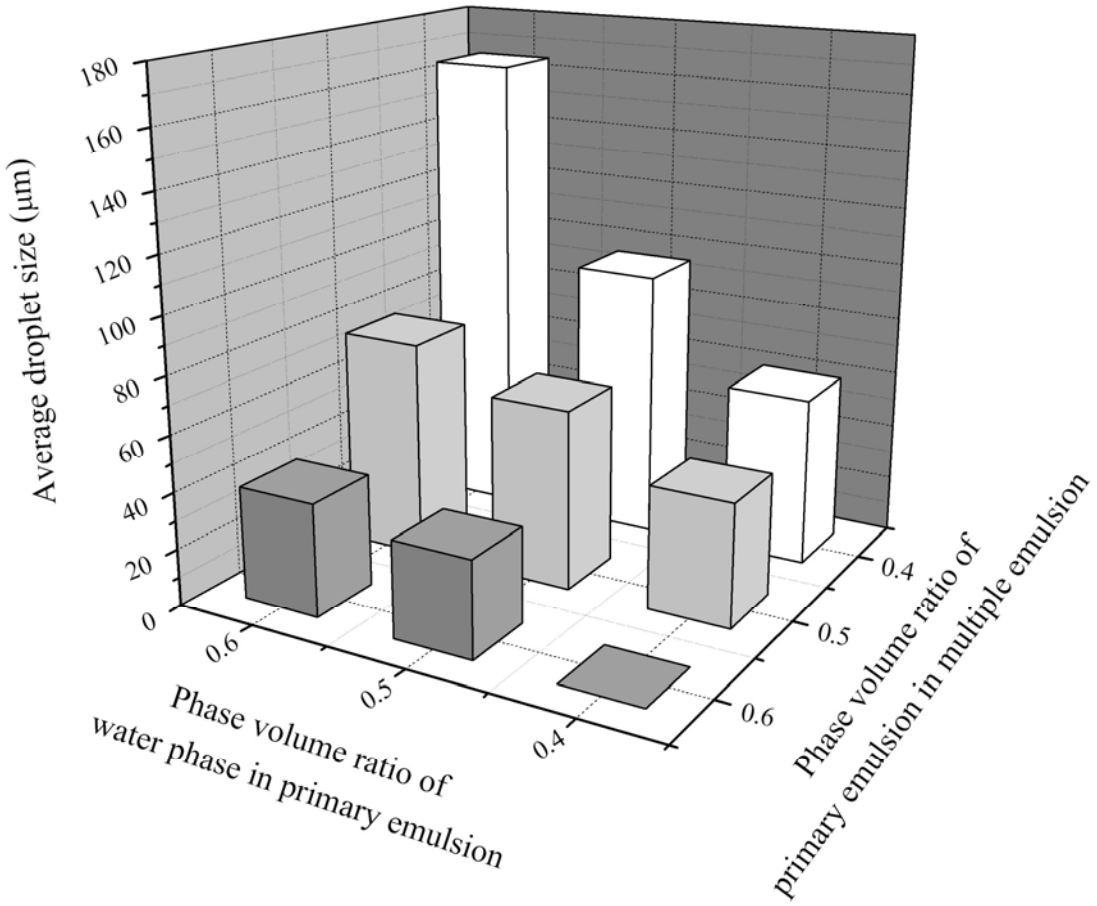


Figure 27 Effect of phase volume ratio of the primary and multiple emulsions on average droplet size of multiple emulsion before temperature cycling method. (n = 3)

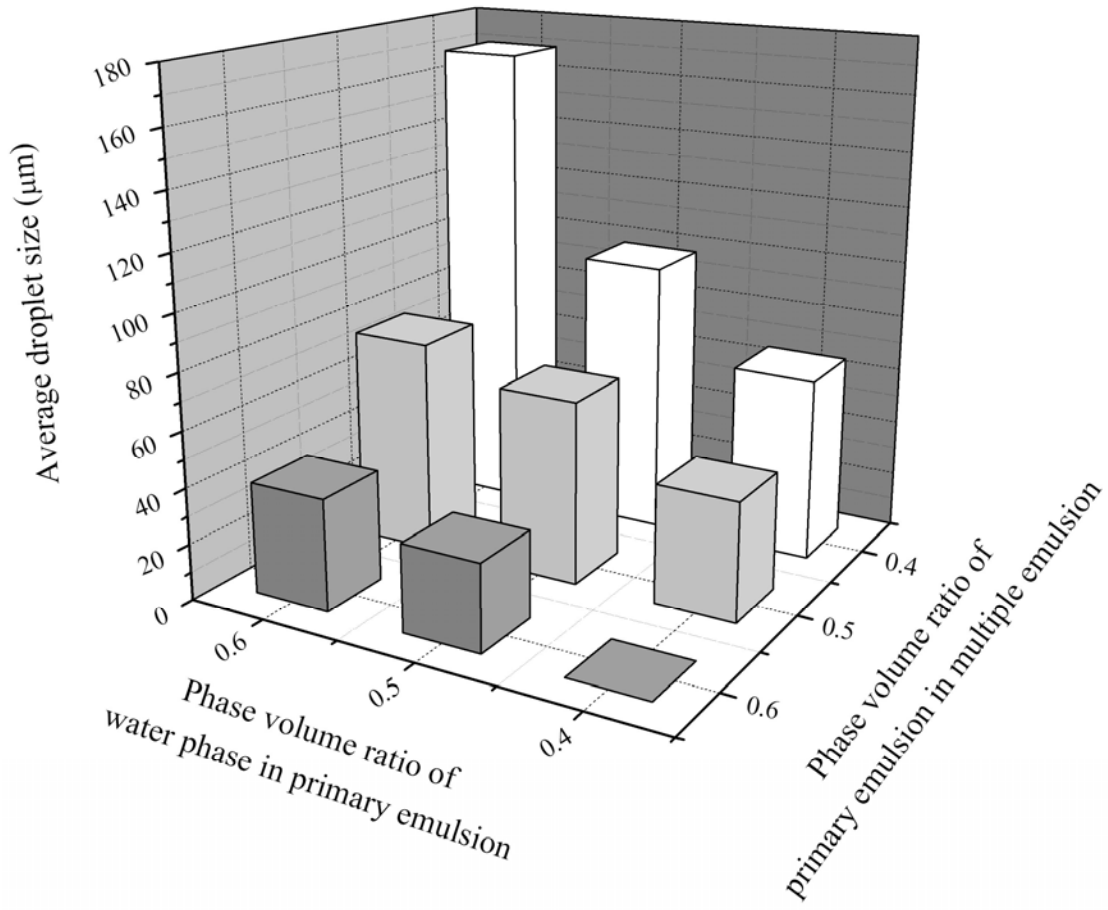


Figure 28 Effect of phase volume ratio of the primary and multiple emulsions on average droplet size of multiple emulsion after temperature cycling method. (n = 3)

3. Preparation of clindamycin phosphate multiple emulsion

A stable multiple emulsion obtained from previous study was selected to produce clindamycin phosphate multiple emulsion. Preparation process was the same as the previous study. The concentration of clindamycin phosphate in the final multiple emulsion was 1 % w/w. The formula of clindamycin phosphate multiple emulsion are shown in table 26.

Immediately after preparation, all formula of multiple emulsion were apparently white and homogeneous liquid. All formula did not show any change in appearance, homogeneity and consistency after TCM (table 27).

The presence of w/o/w multiple emulsion droplets in these emulsions was confirmed by confocal microscopy in figure 29 and 30, the larger water droplets were clearly visible within the oil droplet whereas the majority of fine water droplets were only visible by the non-homogeneous appearance of the oil droplets.

Photomicrographs for multiple emulsions immediately after preparation and after TCM are given in figure 31 and 32. Multiple emulsion drops with a large number of small internal droplets could be observed. Fat globules enclosing aqueous droplets were clearly observed showing the compartmented native of the emulsion. When the concentration of petrolatum in the oil phase increased, multiple emulsion droplets size increased (table 27).

Table 26 Compositions of clindamycin phosphate multiple emulsion

Primary emulsion	PE0 %w/w		PE10 %w/w		PE20 %w/w	
Clindamycin phosphate	2		2		2	
Citrate-phosphate buffer pH 3	50		50		50	
PEG 30-dipolyhydroxystearate	2		2		2	
Petrolatum	-		10		20	
Isopropyl myristate, qs to	100		100		100	
Multiple emulsion	MED PT0 %w/w	MED PT10 %w/w	MED PT20 %w/w	DEX PT0 %w/w	DEX PT10 %w/w	DEX PT20 %w/w
Primary emulsion (PE0)	50	-	-	50	-	-
Primary emulsion (PE10)	-	50	-	-	50	-
Primary emulsion (PE20)	-	-	50	-	-	50
Poloxamer 407	1.5	1.5	1.5	1.5	1.5	1.5
Sodium chloride	0.4	0.4	0.4	-	-	-
Dextrose	-	-	-	2.4	2.4	2.4
Sodium azide	0.01	0.01	0.01	0.01	0.01	0.01
Xanthan gum	0.25	0.25	0.25	0.25	0.25	0.25
De-ionized water, qs to	100	100	100	100	100	100

Table 27 Characteristic of clindamycin phosphate multiple emulsion

	% Cream	Viscosity, cp (SD)at 30 °C	Droplet size, μm (SD)
Before TCM			
MEDPT0	100	11037.64 (478.41)	39.72 (0.24)
MEDPT10	100	8726.14 (327.51)	47.10 (0.80)
MEDPT20	100	7654.37 (507.33)	54.32 (1.90)
DEXPT0	100	371200.79 (1945.61)	29.20 (0.36)
DEXPT10	100	22659.17 (297.00)	35.41 (1.80)
DEXPT20	100	11285.59 (394.46)	45.35 (1.30)
After TCM			
MEDPT0	100	10661.73 (391.93)	34.03 (0.22)
MEDPT10	100	8302.23 (349.25)	46.58 (0.40)
MEDPT20	100	8062.28 (489.87)	53.80 (0.72)
DEXPT0	100	313333.14 (10293.43)	30.27 (0.08)
DEXPT10	100	21987.31 (457.84)	37.65 (1.05)
DEXPT20	100	12013.44 (296.40)	61.75 (5.61)

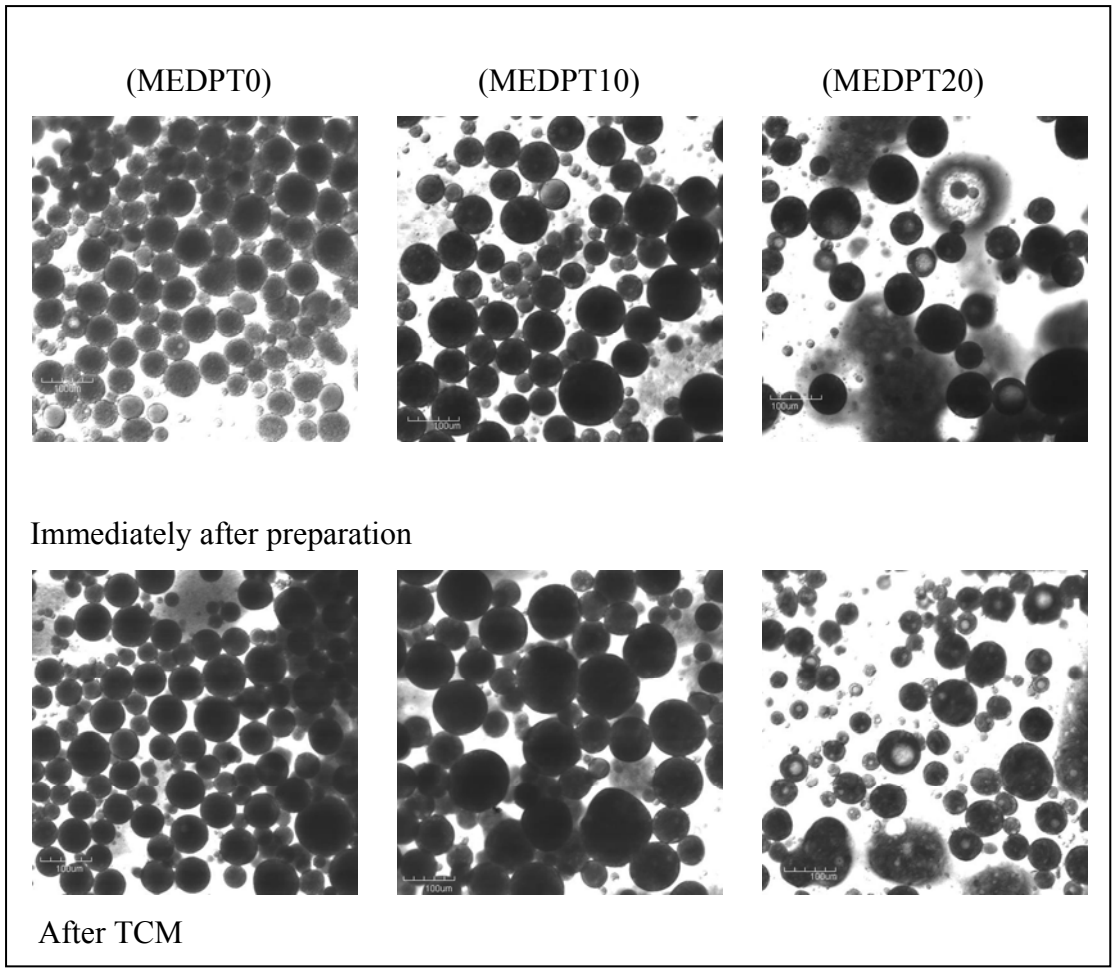


Figure 29 Confocal micrographs of w/o/w multiple emulsions prepared with 0 %, 10 % and 20 % w/w petrolatum in oil phase, citrate-phosphate buffer, pH 3 in internal phase and sodium chloride, xanthan gum in the external phase. scale bar represents 100 μm.

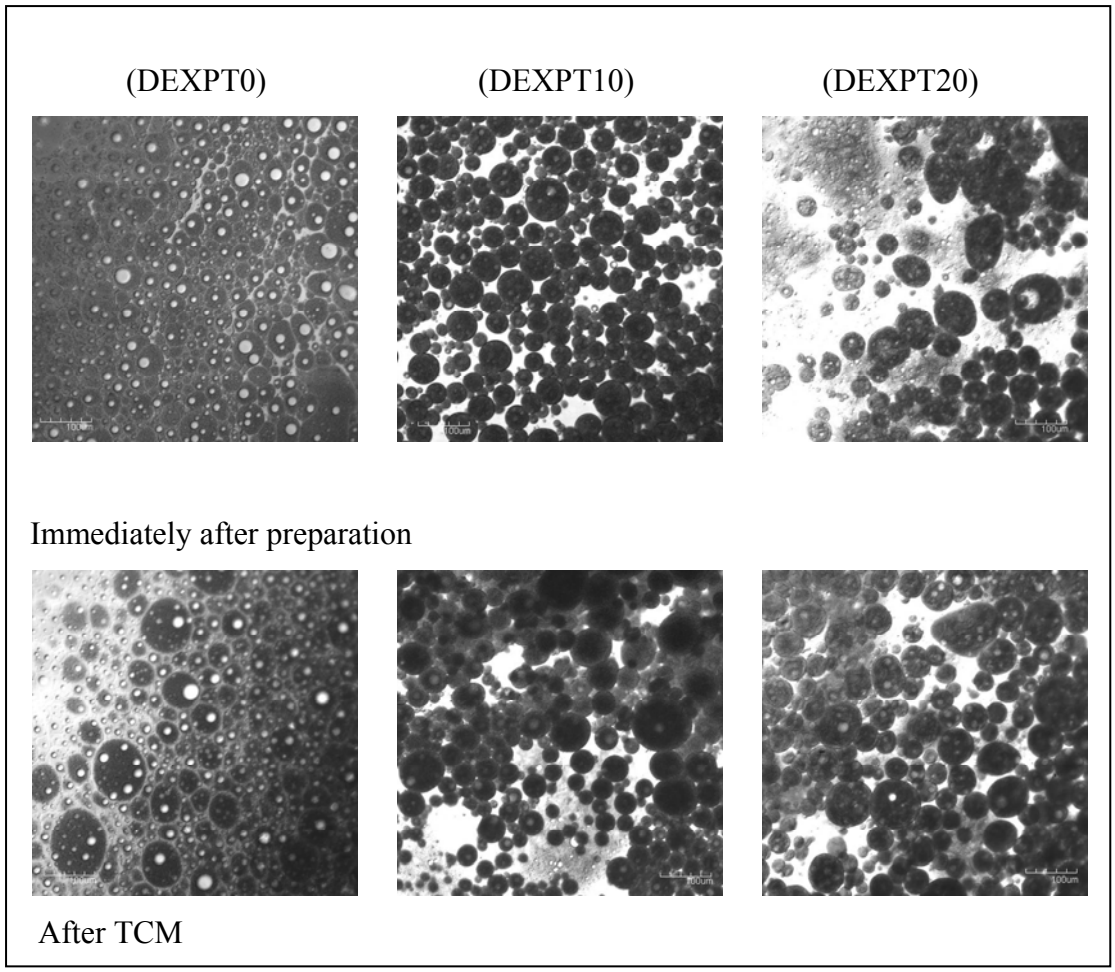


Figure 30 Confocal micrographs of w/o/w multiple emulsions prepared with 0 %, 10 % and 20 % w/w petrolatum in oil phase, citrate-phosphate buffer, pH 3 in internal phase and dextrose, xanthan gum in the external phase. scale bar represents 100 μm.

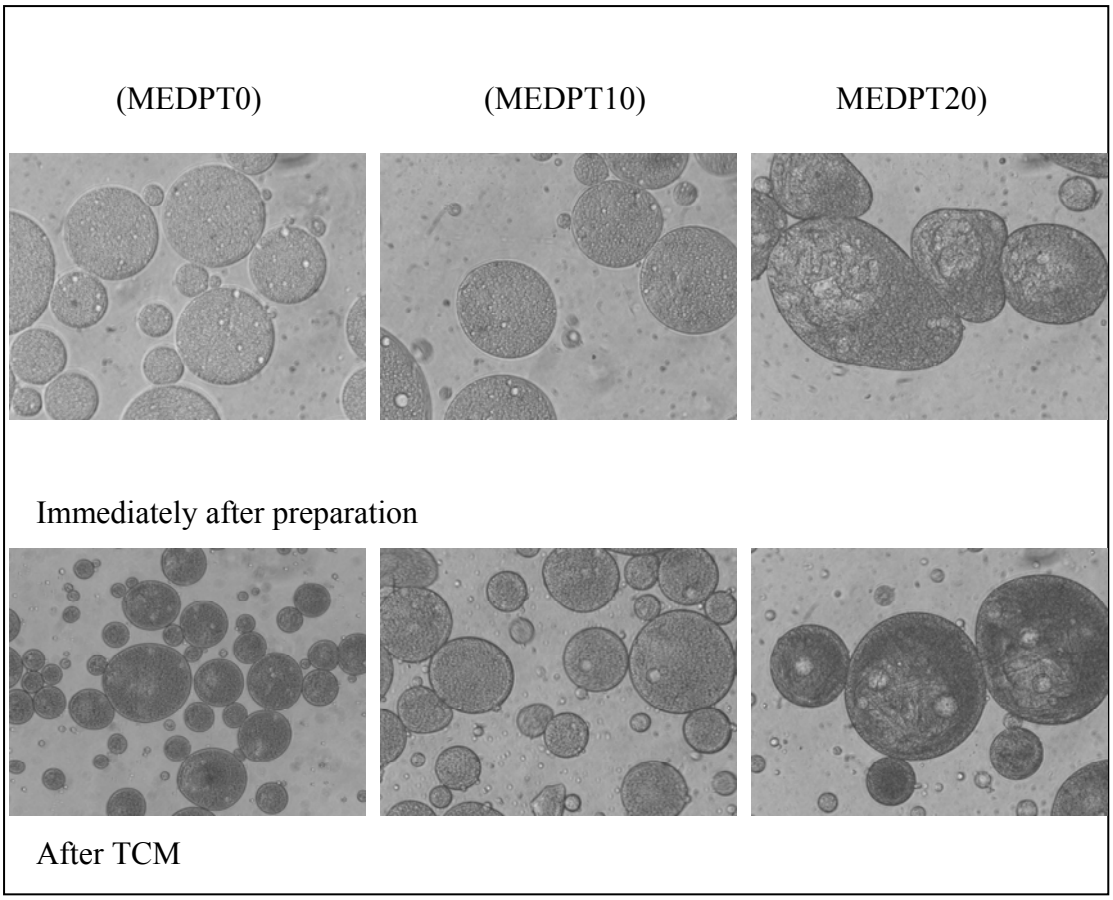


Figure 31 Photomicrographs of w/o/w multiple emulsions prepared with 0 %, 10 % and 20 % w/w petrolatum in oil phase, citrate-phosphate buffer, pH 3 in internal phase and sodium chloride, xanthan gum in the external phase.; magnification 400X.

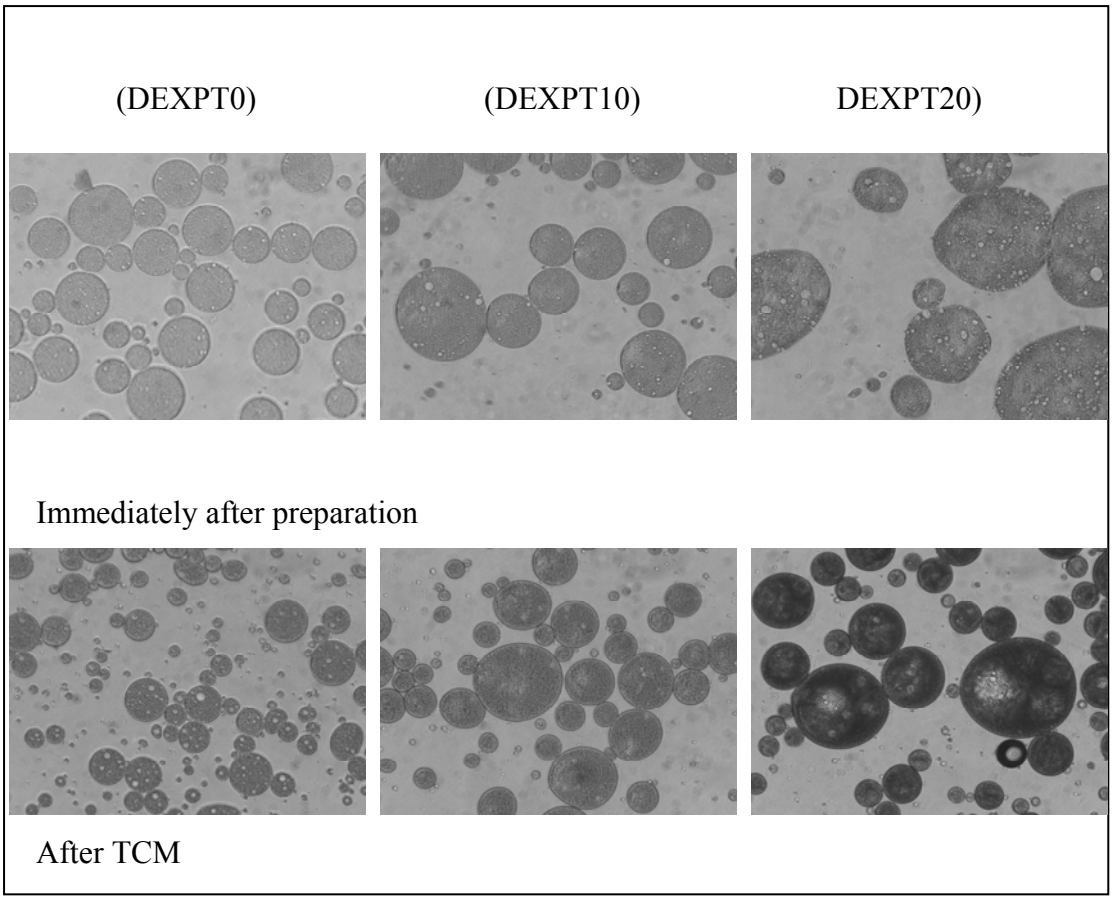


Figure 32 Photomicrographs of w/o/w multiple emulsions prepared with 0 %, 10 % and 20 % w/w petrolatum in oil phase, citrate-phosphate buffer, pH 3 in internal phase and dextrose, xanthan gum in the external phase.; magnification 400X.

4. Determination of clindamycin phosphate multiple emulsion

4.1 Calibration curve of clindamycin phosphate

The concentration of clindamycin phosphate and corresponding peak areas are tabulated in table 28. Calibration curve was obtained by plotting the concentration of clindamycin phosphate standard solution versus the peak area. The calibration curve was shown in figure 33 and found to be a straight line with coefficient of determination (R^2) of 0.9999. Beer's law equation was calculated and presented as followed :

$$A = 1292103.3\ C - 1242.51$$

Where A was the peak area and C was the concentration of clindamycin phosphate (mg/mL)

Table 28 Peak area of clindamycin phosphate assayed by HPLC method (n = 3)

Concentration (mg/mL)	Peak area ± SD.
0.022	28111 ± 404.47
0.044	59845.5 ± 724.78
0.088	115380 ± 288.50
0.132	170860 ± 1033.79
0.176	229244 ± 493.56
0.220	285229 ± 456.79

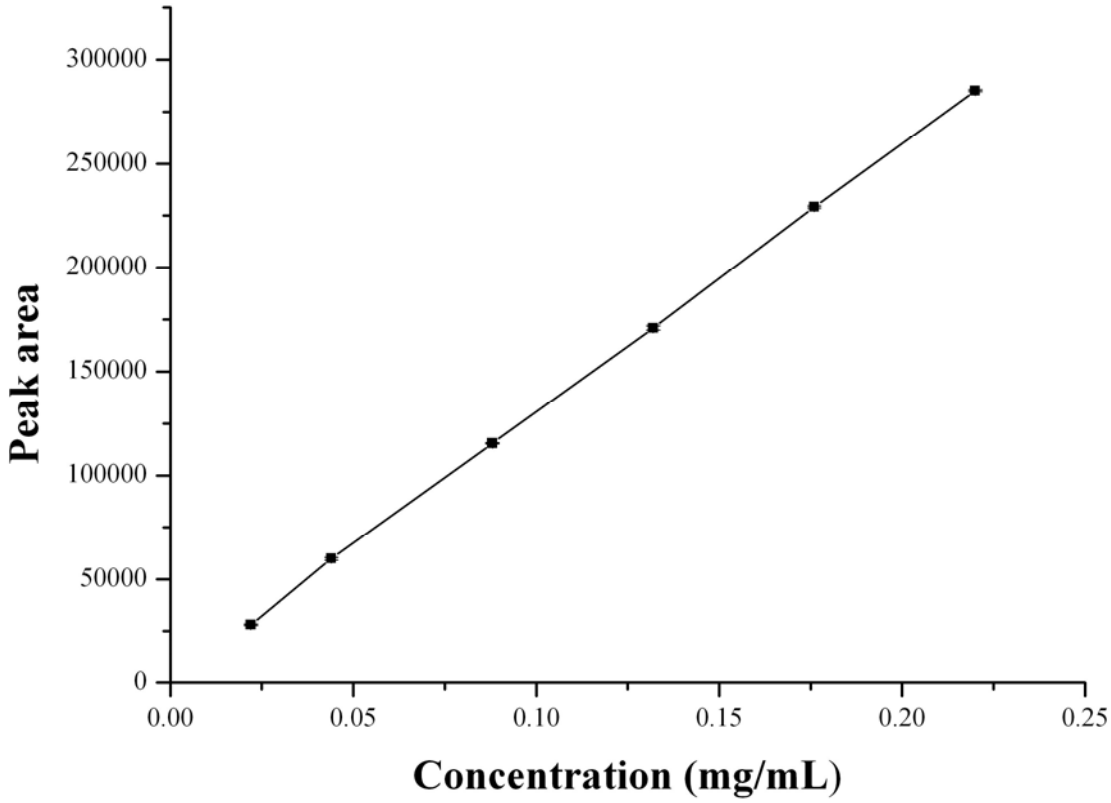


Figure 33 Calibration curve of clindamycin phosphate determined by HPLC (n = 3)

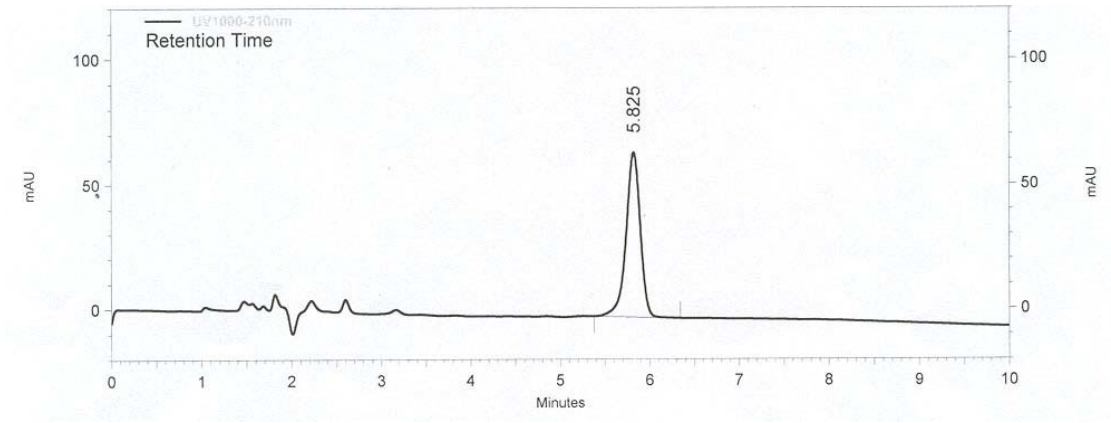


Figure 34 HPLC chromatogram of clindamycin phosphate

5. Stability study

5.1 Stability of clindamycin phosphate solution

The clindamycin phosphate was dissolved in citrate-phosphate buffer. The concentration of clindamycin phosphate was 1% w/v in the buffer pH range 2-8. Clindamycin phosphate solutions were stored in light protection amber glass container and were placed into constant temperature incubator set at 40 °C and 4 °C. The drug content was determined at 1, 2, 3, 4, 6, 8, 10 and 12 weeks using high performance liquid chromatography.

The effect of temperature on rate of clindamycin phosphate degradation was studied. It was found that clindamycin phosphate solution stored at 40 °C decomposed faster than those stored at 4 °C. Temperature was considered to be a factor to induce degradation of clindamycin phosphate. Clindamycin phosphate in aqueous solution was found to show degradation by two major routes : hydrolysis of thioglycoside and phosphate ester (Oesterling 1970 : 65). The pH-rate relationship of clindamycin phosphate in citrate phosphate buffer in pH range 2-8 at 40 °C is shown in figure 35.

The pH rate profile of clindamycin phosphate in citrate-phosphate buffer (figure 35) showed that the rate of degradation increased with decreasing pH in the pH range 3-7. Above pH 7 the rate constant of the reaction increased. At pH 3 the first-order rate constant of clindamycin phosphate in citrate-phosphate buffer seemed to be maximum and exhibited the lowest durg stability. Therefore multiple emulsion using citrate-phosphate buffer pH 3 as internal water phase was selected for accelerated stability study of clindamycin phosphate.

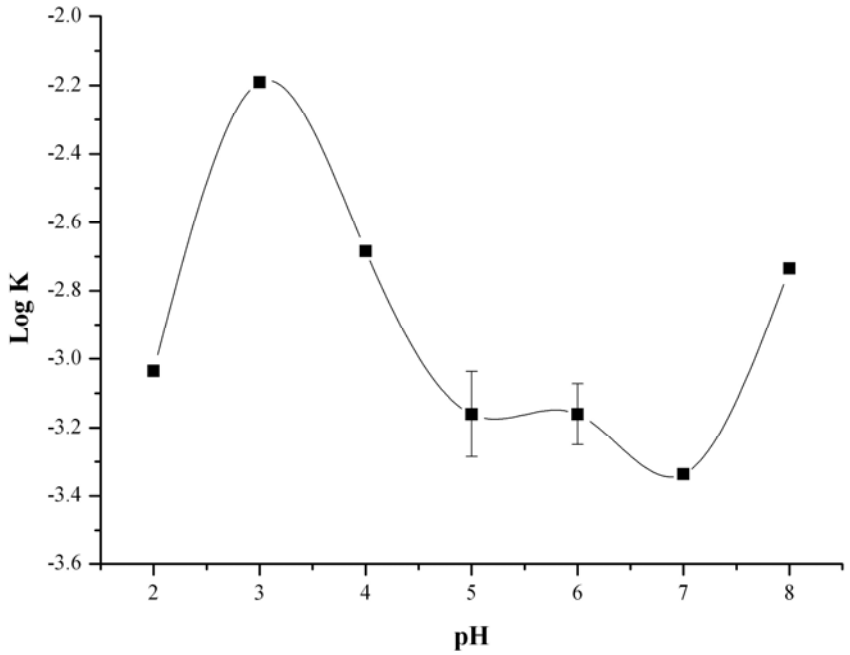


Figure 35 pH-log rate profile of clindamycin phosphate solution, 40 °C (n = 3)

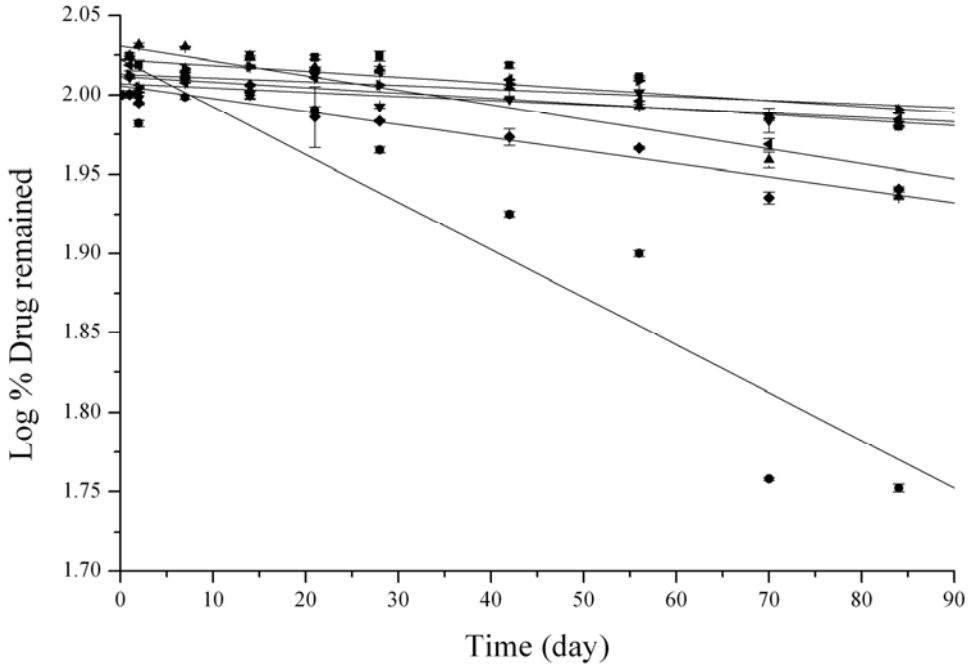


Figure 36 Degradation of clindamycin phosphate at 40 °C in citrate-phosphate buffer solution.

Keys : ■ pH2, ● pH3, ▲ pH4, ▼ pH5, ◄ pH6, ► pH7, ◆ pH8

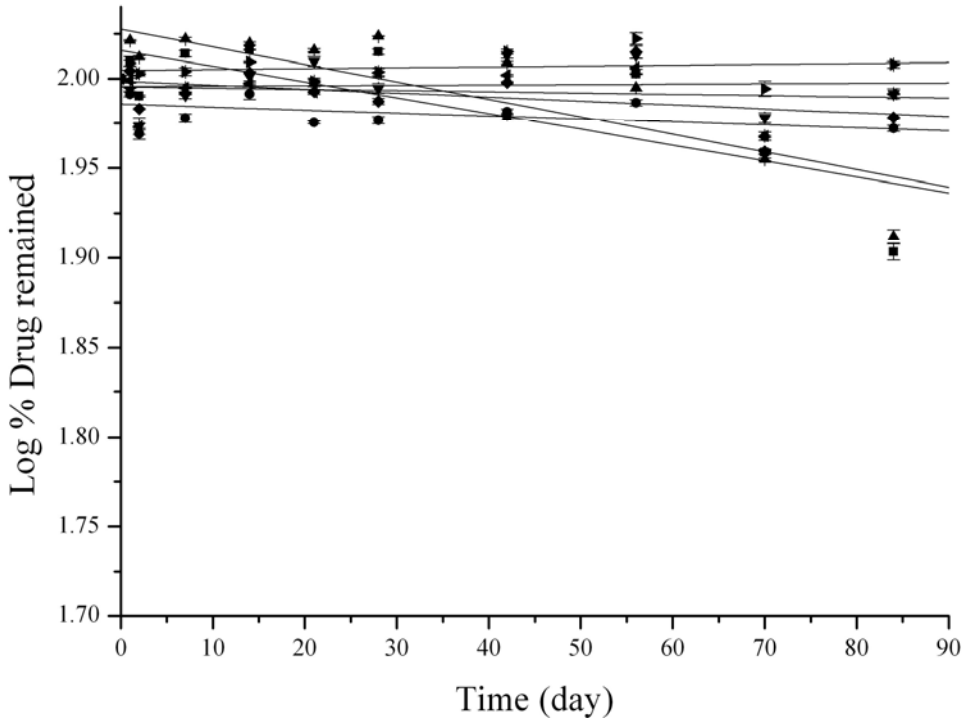


Figure 37 Degradation of clindamycin phosphate at 4 °C in citrate-phosphate buffer solution.

Keys : ■ pH2, ● pH3, ▲ pH4, ▼ pH5, ◄ pH6, ► pH7, ◆ pH8

5.2 Stability of clindamycin phosphate multiple emulsion

The composition of primary emulsion and multiple emulsion used in this study was the same as those in table 26 (see section 3) these multiple emulsion were kept at 40 °C and 75% relative humidity, 4 °C and 75% relative humidity. The percentage of drug remained was analyzed at 0, 7, 14, 21, 28, 42, 56, 70 and 84 days by high performance liquid chromatography (HPLC). Plotting of percentage of drug remained versus time (Figure 38) was zero order plots. A simple solution of clindamycin phosphate in citrate-phosphate buffer at pH 3 was also prepared as a control. It was found that clindamycin phosphate solution and multiple emulsions

stored at 40 °C decomposed faster than those stored at 4 °C (Figure 38, 39). Clindamycin phosphate in aqueous solution was found to show degradation by two major routes : hydrolysis of the thioglycoside and phosphate ester. Temperature was also a factor to induce degradation of clindamycin phosphate in multiple emulsion. The stability of clindamycin phosphate in multiple emulsions (8.152 mg/ml/day) was found to be significantly better than in citrate-phosphate buffer at pH 3 (13.160 mg/ml/day). Clindamycin phosphate was dissolved in the inner water phase of w/o/w multiple emulsion. Therefore, the multilayer of oil and water might serve as barrier to prevent clindamycin phosphate from contaction with water in the external medium. Therefore, hydrolysis of clindamycin phosphate was inhibited. The water in the inner phase of multiple emulsion might have some effect on the stability of clindamycin phosphate. However, the effect was believed to be small since the inner water phase contained less amount of active water for degradation than citrate-phosphate buffer in the bulk external phase.

However, the improved stability of clindamycin phosphate might be due to the results of its ionization. The pKa of clindamycin phosphate was 7.7. At pH 3, clindamycin phosphate might present as non-ionized form and distributed more in the oil phase. Therefore, the drug was more stable from hydrolysis. However, the emulsion dosage form was still benefit to improve stability of clindamycin phosphate.

To examine the effect of oil viscosity increasing agent on chemical stability of clindymycin phosphate in w/o/w multiple emulsions, the influence of addition of petrolatum as oil viscosity increasing agent in primary emulsion used to prepare w/o/w multiple emulsions was studied. The enhanced stability of clindamycin phosphate was found in the w/o/w multiple emulsions containing 10 %

w/w petrolatum in middle oil phase (3.698 mg/ml/day) rather than in the w/o/w multiple emulsions without petrolatum (8.152 mg/ml/day). Primary emulsions consisting of 10 and 20 % w/w petrolatum were used to prepare w/o/w multiple emulsions. After aging, the clindamycin phosphate degradation profiles of w/o/w multiple emulsion prepared with various petrolatum concentrations (10, 20 %w/w) in oil phase were not significantly different ($p<0.05$) (3.698 mg/ml/day and 5.010 mg/ml/day, respectively) . The degradation rate of clindamycin phosphate in multiple emulsion is shown in table 29.

Changing osmolality adjusting agent from sodium chloride to dextrose in the w/o/w multiple emulsions prepared with 20 % w/w petrolatum in oil phase resulted in the increasing of apparent viscosity of multiple emulsion. This should be owing to the effect of sodium chloride on the conformation of xanthan gum which was used as viscosity inducing agent in external water phase. With the existing of sodium chloride, xanthan gum conformation changed from disorder (random coil) to order (helix) conformation which the backbone was taken on a helical conformation and the charged trisaccharide side chains collapsed down on to the backbone. This might be led to the decrease in apparent viscosity of multiple emulsion containing sodium chloride (Viturawong 2008 : 108). However, the clindamycin phosphate degradation profiles of w/o/w multiple emulsion prepared with sodium chloride or dextrose in external water phase were not significantly different ($p<0.05$).

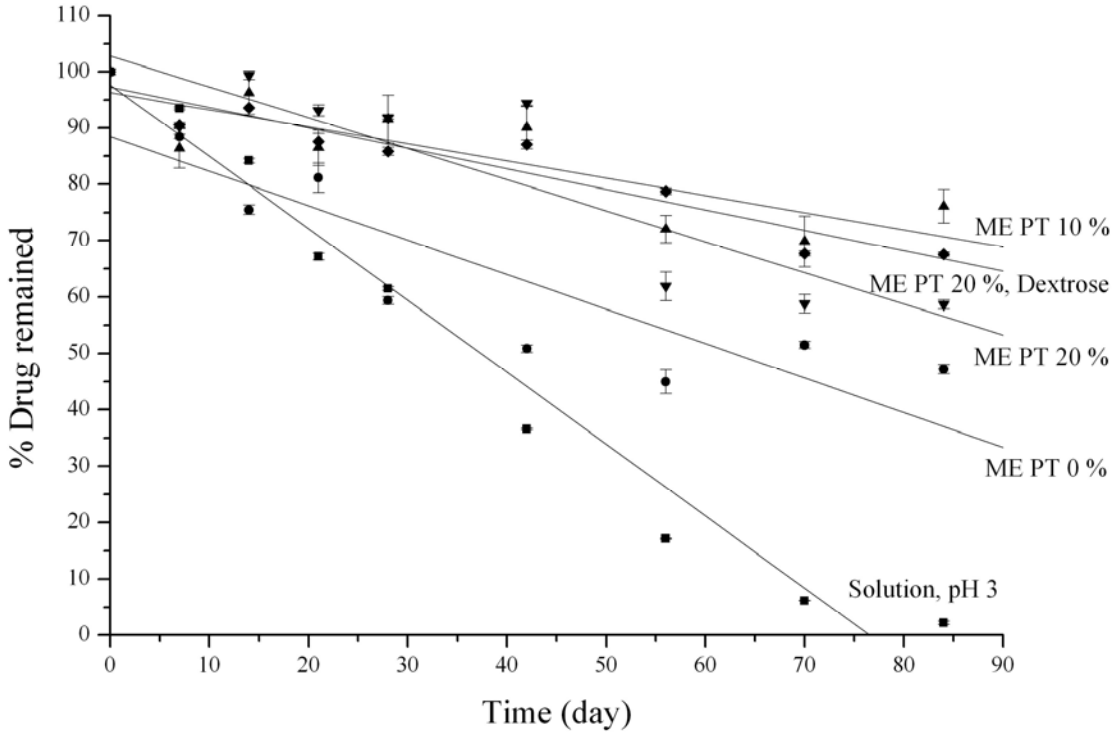


Figure 38 Degradation of clindamycin phosphate at 40 °C in buffer solution and in w/o/w multiple emulsions.

Keys : ■ Solution, pH 3

● Multiple emulsion containing petrolatum 0 %

▲ Multiple emulsion containing petrolatum 10 %

▼ Multiple emulsion containing petrolatum 20 %

◆ Multiple emulsion containing petrolatum 20 % with dextrose

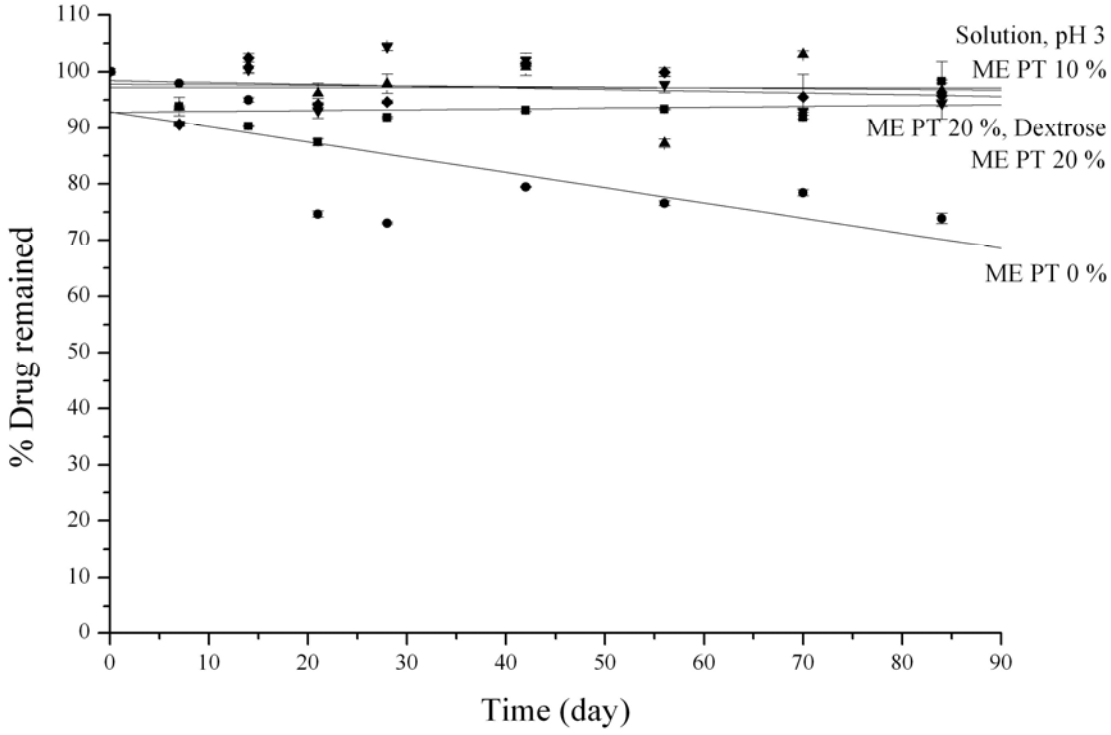


Figure 39 Degradation of clindamycin phosphate at 4 °C in buffer solution and in w/o/w multiple emulsions.

Keys : ■ Solution, pH 3

● Multiple emulsion containing petrolatum 0 %

▲ Multiple emulsion containing petrolatum 10 %

▼ Multiple emulsion containing petrolatum 20 %

◆ Multiple emulsion containing petrolatum 20 % with dextrose

Table 29 Degradation rate of clindamycin phosphate in multiple emulsion and solution

Formula	Degradation rate
40 °C	
Solution, pH 3	13.160 mg/ml/day
MEDPT0	8.152 mg/ml/day
MEDPT10	3.698 mg/ml/day
MEDPT20	5.010 mg/ml/day
DEXPT20	4.116 mg/ml/day
4 °C	
Solution, pH 3	1.112 mg/ml/day
MEDPT0	3.957 mg/ml/day
MEDPT10	0.497 mg/ml/day
MEDPT20	0.589 mg/ml/day
DEXPT20	0.500 mg/ml/day

6. Determination of drug released from clindamycin phosphate multiple emulsion

This study was planed to evaluate clindamycin phosphate released from the multiple emulsion and the effect of petrolatum and types of osmolality adjusting agent on drug release. The composition of primary emulsion and multiple emulsions used was the same as those in table 26 (see section 3). Clindamycin phosphate released profile from multiple emulsion is shown in figure 40 (zero order model) and figure 41 (first order model).

The kinetic model of drug released was analyzed. Linear regression of clindamycin phosphate released from multiple emulsion is show in table 30. It was found that the release kinetic of clindamycin phosphate mostly followed Higuchi's model rather more than zero order or first order. Since it exhibited highest regression coefficients for Higuchi's model. Linear relationship between amount of drug released and square root of time, characterized with the regression coefficients higher than 0.98, was obtained for each formulation (figure 42). The obtained results indicated that the release mechanism of clindamycin phosphate from investigated formulations could be described by the diffusional model and that rate-controlling step in the release process was diffusion of the dissolved drug through the vehicle (Vesiljevic et al. 2006 : 175). Release flux of clindamycin phosphate released from multiple emulsion is show in table 31.

Drug released profiles of the multiple emulsion prepared without petrolatum using either sodium chloride or dextrose as osmolality adjusting agent were not difference (table 42) (figure 40). Even though the viscosity of external water phase increased when dextrose was used to adjust osmolality instead of sodium chloride. However, this exhibited no effect on clindamycin phosphate released from

multiple emulsion. It was found that drug release rate from multiple emulsion prepared with petrolatum in oil phase was significantly ($p<0.05$) faster than those prepared without petrolatum (table 30). Clindamycin phosphate might diffuse from the internal aqueous phase compartment across the oil phase to the external aqueous phase of w/o/w multiple emulsion. Therefore, multiple emulsion containing petrolatum in oil middle phase exhibited faster rate of drug release. However, clindamycin phosphate released profiles from multiple emulsion prepared with various petrolatum concentrations (10, 20 %w/w) in oil phase and sodium chloride and dextrose in external water phase were not different (table 32). Diffusion rate of clindamycin phosphate through the oil phase should increase if the drug was in an unionized from due to its solubility in oil. The diffusion rate therefore depended on the nature (pKa and solubility) of the drug, the polarity of oil phase, as well as the pH of the aqueous phase. It was found that at pH lower than drug pKa, the drug would exist mostly as unionized form and was readily soluble in the oil phase. The drug could, therefore, pass easily across the oil layer to the external aqueous phase (Garti 1997 : 235). pKa of clindamycin phosphate was 7.7 at 25 °C. In citrate-phosphate buffer pH 3, clindamycin phosphate would exist exclusively as the unionized form and would be readily soluble in oil phase which its polarity was decreased by addition of petrolatum. Then the partition of drug across the oil layer to the external aqueous phase was forced by the high concentration gradient between oil phase and external phase.

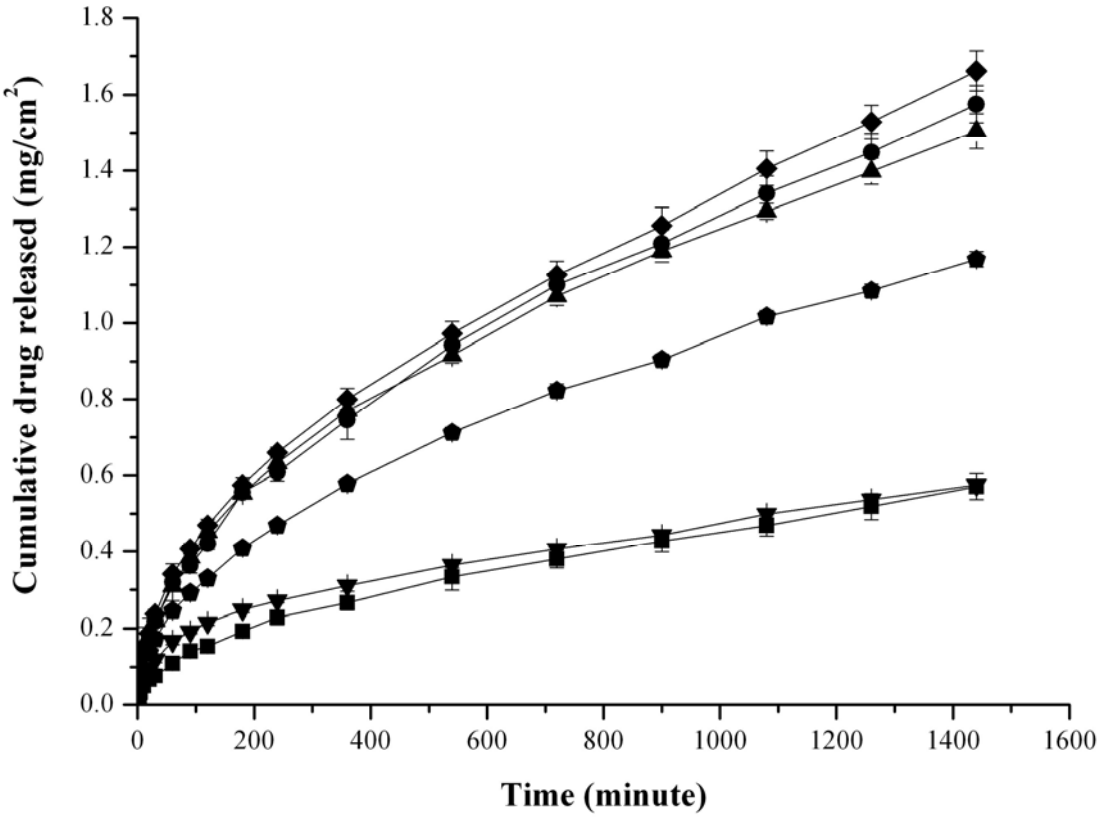


Figure 40 Release profile of clindamycin phosphate multiple emulsion

Keys : ■ ME containing petrolatum 0 % and sodium chloride,

● ME containing petrolatum 10 % and sodium chloride,

▲ ME containing petrolatum 20 % and sodium chloride,

▼ ME containing petrolatum 0 % and dextrose,

◆ ME containing petrolatum 10 % and dextrose ,

⬟ ME containing petrolatum 20 % and dextrose

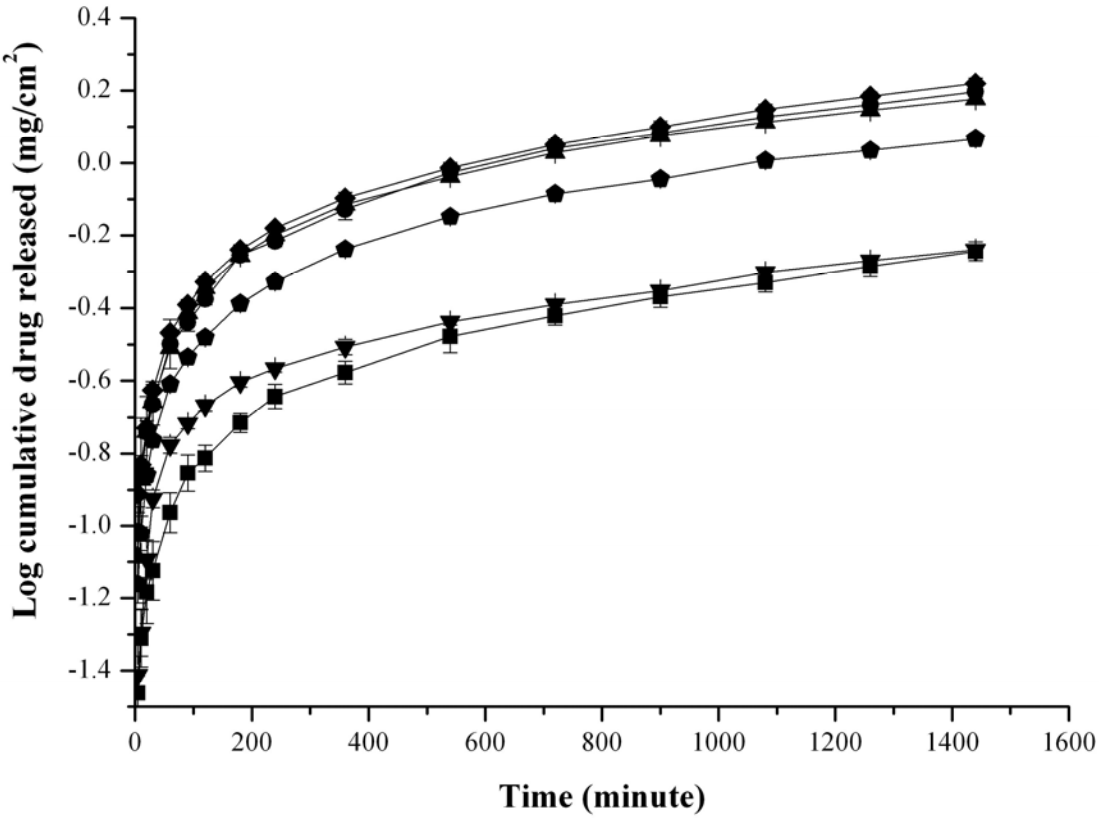


Figure 41 Release profile of clindamycin phosphate multiple emulsion (first order model)

Keys : ■ ME containing petrolatum 0 % and sodium chloride,

● ME containing petrolatum 10 % and sodium chloride,

▲ ME containing petrolatum 20 % and sodium chloride,

▼ ME containing petrolatum 0 % and dextrose,

◆ ME containing petrolatum 10 % and dextrose ,

⬟ ME containing petrolatum 20 % and dextrose

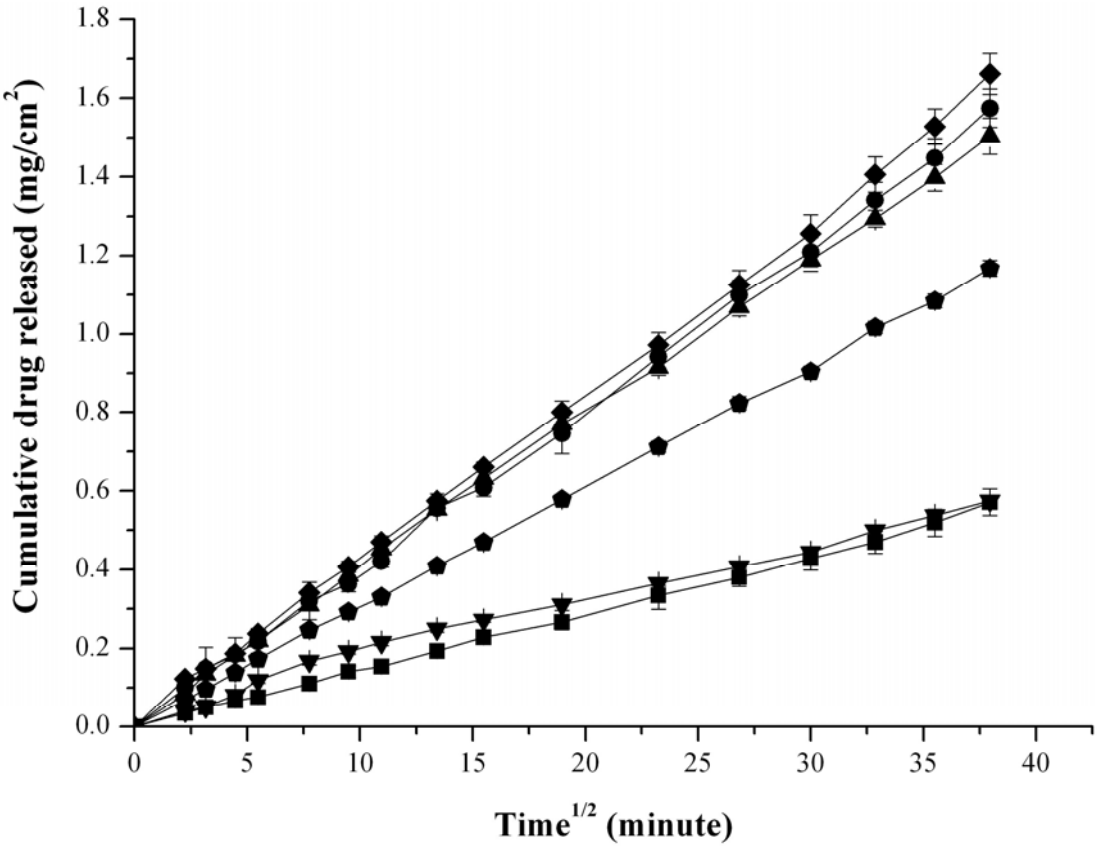


Figure 42 Release profile of clindamycin phosphate multiple emulsion (Higuchi's model)

Keys : ■ ME containing petrolatum 0 % and sodium chloride,

● ME containing petrolatum 10 % and sodium chloride,

▲ ME containing petrolatum 20 % and sodium chloride,

▼ ME containing petrolatum 0 % and dextrose,

◆ ME containing petrolatum 10 % and dextrose ,

⬟ ME containing petrolatum 20 % and dextrose

Table 30 Linear regression of clindamycin phosphate release from multiple emulsion

	Linear regression	R^2
Zero order model		
MEDPT0	Y = 0.0004X + 0.0843	0.9489
MEDPT10	Y = 0.001X + 0.2364	0.9481
MEDPT20	Y = 0.001X + 0.2446	0.9333
DEXPT0	Y = 0.0004X + 0.1189	0.8974
DEXPT10	Y = 0.0011X + 0.2546	0.9480
DEXPT20	Y = 0.0008X + 0.1821	0.9411
First order model		
MEDPT0	Y = 0.0007X – 1.0345	0.7406
MEDPT10	Y = 0.0007X – 0.5878	0.7449
MEDPT20	Y = 0.0007X – 0.5893	0.7053
DEXPT0	Y = 0.0006X – 0.9206	0.6422
DEXPT10	Y = 0.0007X – 0.5486	0.7528
DEXPT20	Y = 0.0007X – 0.7077	0.7227
Higuchi's model		
MEDPT0	Y = 0.0292X – 0.0027	0.9984
MEDPT10	Y = 0.0822X – 0.0093	0.9991
MEDPT20	Y = 0.0792X + 0.0184	0.9997
DEXPT0	Y = 0.029X + 0.0615	0.9890
DEXPT10	Y = 0.0857X + 0.0068	0.9990
DEXPT20	Y = 0.0615X + 0.0009	0.9998

Table 31 Release flux of clindamycin phosphate released from multiple emulsion (n=6)

Formula	Release flux ± SD
MEDPT0	0.0145 ± 0.0009 $mg/cm^2/min^{1/2}$
MEDPT10	0.0409 ± 0.0009 $mg/cm^2/min^{1/2}$
MEDPT20	0.0394 ± 0.0009 $mg/cm^2/min^{1/2}$
DEXPT0	0.0144 ± 0.0002 $mg/cm^2/min^{1/2}$
DEXPT10	0.0426 ± 0.0014 $mg/cm^2/min^{1/2}$
DEXPT20	0.0306 ± 0.0005 $mg/cm^2/min^{1/2}$

Table 32 Difference factor (f_1) and similarity factor (f_2) of clindamycin phosphate released profiles from multiple emulsion

Data 1	Data 2	Difference factor (f_1)	Similarity factor (f_2)
MEDPT0	MEDPT10	181	97
MEDPT0	MEDPT20	176	97
MEDPT0	DEXPT0	13	100
MEDPT0	DEXPT10	196	97
MEDPT0	DEXPT20	112	99
MEDPT10	MEDPT20	3	100
MEDPT10	DEXPT0	60	97
MEDPT10	DEXPT10	5	100
MEDPT10	DEXPT20	15	100
MEDPT20	DEXPT0	59	98
MEDPT20	DEXPT10	7	100
MEDPT20	DEXPT20	13	100
DEXPT0	DEXPT10	163	97
DEXPT0	DEXPT20	88	99
DEXPT10	DEXPT20	14	100

The difference factor (f_1) values lower than 15 (0 – 15) and similarity factor (f_2) values higher than 50 (50 – 100) showed the similarity of the dissolution profiles. (Costa and Lobo 2001 : 130; Shah et al. 1998 : 890)

CHAPTER 5
CONCLUSIONS

This study was designed to investigate whether the multiple emulsion system could be used to improve stability of water soluble drug and degraded by hydrolysis. The model drug used was clindamycin phosphate which was water soluble and degraded by hydrolysis. The w/o/w multiple emulsion was successfully prepared by two steps emulsification process using lipophilic and hydrophilic polymeric surfactant as emulsifier and xanthan gum as thickener in external phase. Clindamycin phosphate was dissolved in internal water phase (Figure 43). Water-in-oil-in-water multiple emulsion system could improve clindamycin phosphate stability from hydrolysis degradation. Since the amount of internal water phase which was directly contact with the drug was small and the diffusion rate of the external water phase across the oil phase to internal water phase was retarded due to the increased viscosity of oil phase containing petrolatum. Clindamycin phosphate might diffuse from the internal water phase compartment across the oil phase to the external water phase of w/o/w multiple emulsion. The release mechanism of clindamycin phosphate from w/o/w multiple emulsion could be described by the diffusion model.

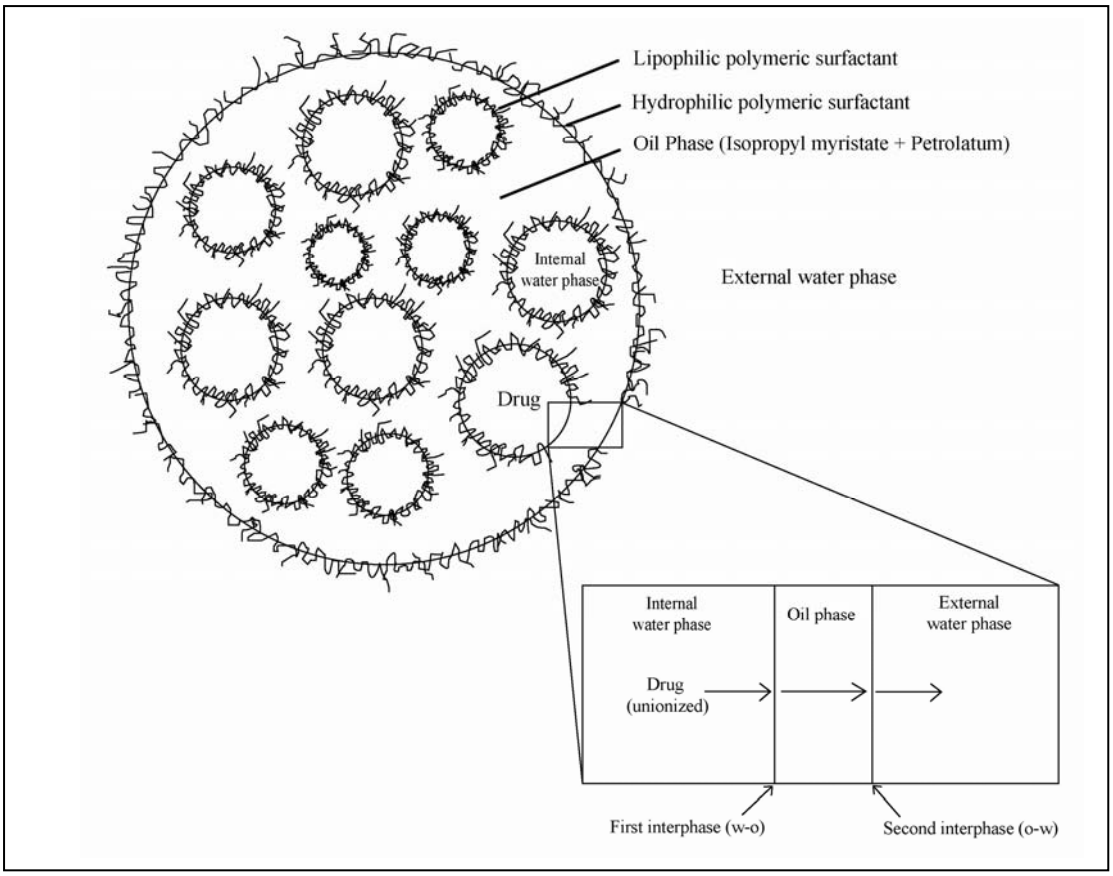


Figure 43 Schematic representation of the w/o/w multiple emulsion and possible drug diffuse occurring in w/o/w multiple emulsion

The physical and chemical properties of multiple emulsion were evaluated.The finding could be summarized as follows :

1. The formula of stable w/o/w multiple emulsion consisted of 50 %w/w primary emulsion (containing 50 %w/w citrate-phosphate buffer, 2 %w/w PEG 30-dipolyhydroxystearate and 48 % w/w isopropyl myristate), 1.5 % w/w poloxamer 407, 0.01% w/w sodium azide, 0.25 %w/w xanthan gum, 0.2% sodium chloride and 48.04 %w/w de-ionized water

2. Xanthan gum at 0.25 – 0.5 %w/w was more effective used as thickener in external water phases of w/o/w multiple emulsion.

3. The multiple emulsion containing poloxamer 188 and 407 at 1.5 %w/w (hydrophilic polymeric surfactant) in external water phases exhibited better physical stability than those tween®80 at 2.5 %w/w (hydrophilic non ionic surfactant).

4. Viscosity of primary emulsion should be nearly equal to that of the external water phases to prevent phase separation in the second emulsification step.

5. Viscosity of primary emulsion containing petrolatum increased with increasing petrolatum concentration.

6. The w/o/w multiple emulsion systems with highest concentration of petrolatum in primary emulsion showed the highest droplet size and lowest viscosity.

7. The droplets size of multiple emulsion decreased with increasing stirrer speeds from 800 – 1200 rpm.

8. Increasing phase volume ratio of water phase in primary emulsion from 0.4 – 0.6 showed no effect on the viscosity of multiple emulsion.

9. The droplet size of multiple emulsion sigificantly increased with increasing phase volume ratio of water phase in primary emulsion from 0.4 to 0.6.

10. The droplet size of multiple emulsion decreased with increasing phase volume ratio of primary emulsion in multiple emulsion from 0.4 to 0.6.

11. The rate of degradation of clindamycin phosphate in citrate-phosphate buffer increased with decreasing pH in the pH range 3-7.

12. The w/o/w multiple emulsion system containing clindamycin phosphate in the water inner phase could be used to improve clindamycin phosphate stability from hydrolysis degradation compared to clindamycin phosphate solution.

13. Increasing viscosity of oil middle phase by addition of 10 – 20 % w/w petrolatum in w/o/w multiple emulsion system was found to improve clindamycin phosphate stability in w/o/w multiple emulsion compare to that containing no petralatum.

14. Viscosity of external water phase increased when dextrose was used to adjust osmolality instead of sodium chloride and exhibited no effect on clindamycin phosphate stability in multiple emulsion.

15. Viscosity of external water phase exhibited no effect on clindamycin phosphate stability in multiple emulsion.

16. The clindamycin phosphate released from multiple emulsion could be described by the diffusional model.

17. Release rate of clidamycin phosphate from multiple emulsion prepared with petrolatum in oil phase was higher than that containing no petrolatum.

18. Viscosity of external water phase exhibited no effect on clindamycin phosphate released from multiple emulsion.

BIBLIOGRAPHY

Benichou, A., A. Aserin., and N. Garti. "Double emulsions stabilized with hybrids of natural polymers for entrapment and slow release of active matters." Advances in Colloid and Interface Science 108-109 (2004) : 29-41.

_____. "W/O/W double emulsions stabilized with WPI-polysaccharide complexes." Colloids and Surfaces A: Physicochemical and Engineering Aspects 294 (2007) : 20-32.

Benna-Zayani, M. et al. "Stabilisation of w/o/w double emulsion by polysaccharides as weak gels." Colloids and Surfaces A: Physicochemical and Engineering Aspects 316 (2008) : 46-54.

Block, L.H. "Pharmaceutical emulsions and microemulsions." In Pharmaceutical Dosage Forms Disperse Systems vol. 2, 47-109. Edited by H.A. Lieberman, M.M. Rieger., and G.S. Banker. New york : Marcel Dekker Inc., 1996.

Chien, Y.W. "Transdermal drug delivery and delivery systems." In Novel Drug Delivery Systems 301-380. Edited by Y.W. Chien. New york : Marcel Dekker Inc., 1992.

Costa, Paulo., and José Manuel Sousa Lobo. "Modeling and comparison of dissolution profiles." European Journal of Pharmaceutical Sciences 13 (2001) : 123-133.

Croubels, S., D.S. Baere., and P.D. Backer. "Practical approach for the stability testing of veterinary drugs in solutions and in biological matrices during storage." Analytica Chimica Acta 483 (2003) : 419-427.

Csóka, I., and I. Erõs. "Stability of multiple emulsions I. Determination of factors influencing multiple drop breakdown." International Journal of Pharmaceutics 156 (1997) : 119-123.

Duchêne, D. et al. "Cyclodextrins and emulsions." International Journal of Pharmaceutics 266 (2003) : 85-90.

Eccleston, G.M. "Emulsions and microemulsions." In Encyclopedia of Pharmaceutical Technology, 1066-1085. Edited by Swarbrick J. New York : Marcel Dekker Inc., 2002.

Florence, A.T., and D. Whitehill. "The formulation and stability of multiple emulsions." International Journal of Pharmaceutics 11 (1982) : 277-308.

Gallarate, M. et al. "On the stability of ascorbic acid in emulsified systems for topical and cosmetic use." International Journal of Pharmaceutics 188 (1999) : 233-241.

Garti, N., A. Aserin., and Y. Cohen. "Mechanistic considerations on the release of electrolytes from multiple emulsions stabilized by BSA and nonionic surfactants." Journal of Controlled Release 29 (1994) : 41-51.

Garti, N. "Double emulsions – scope, limitations and new achievements." Colloids and Surfaces A: Physicochemical and Engineering Aspects 123-124 (1997) : 233-246.

_____. "New trends in double emulsions for controlled release." Progress in Colloid and Polymer Science 108 (1998) : 83-92.

Garti, N. et al. "Double emulsions of water-in-oil-in-water stabilized by α-form fat microcrystals. part 1: selection of emulsifiers and fat microcrystalline particles." Journal of The American Oil Chemists'Society 76 (1999) : 383-389.

Geiger, S. et al. "Kinetics of swelling-breakdown of a w/o/w multiple emulsion: possible mechanisms for the lipophilic surfactant effect." Journal of Controlled Release 52 (1998) : 99-107.

Hou, W., and K.D. Papadopoulos. "$W_1/O/W_2$ and $O_1/W/O_2$ globules stabilized with span 80 and tween 80." Colloids and Surfaces A: Physicochemical and Engineering Aspects 125 (1997) : 181-187.

Jager-Lezer, N., et al. "Influence of lipophilic surfactant on the release kinetics of water-soluble molecules entrapped in a w/o/w multiple emulsion." Journal of Controlled Release 45 (1997) : 1-13.

Jiao, J., David.G. Rhodes., and Diane.J. Burgess. "Multiple emulsion stability : pressure balance and interfacial film strength." Journal of Colloid and Interface Science 250 (2002) : 444-450.

Kanouni, M., H.L. Rosano, and N. Naouli. "Preparation of a stable double emulsion ($W_1/O/W_2$): role of the interfacial films on the stability of the system." Advance in Colloid and Interface Science 99 (2002) : 229-254.

Li, X., and B.R. Jasti. "Theory and applications of diffusion and dissolution." In Theory and Practice of Contemporary Pharmaceutics, 198-204. Edited by T.K. Ghosh, and B.R. Jasti. Florida : CRC Press LLC., 2005.

Lizarraga, M. S. et al. "Stability of concentrated emulsions measured by optical and rheological methods, effect of processing conditions – I. whey protein concentrate." Food Hydrocolloids 22 (2008) 868-878.

Lutz, R., and A. Aserin. "Multiple emulsions stabilized by biopolymers." In Multiple Emulsions Technology and Applications, 85-120. Edited by A. Aserin, New Jersey : John wiley & sons Inc., 2008.

McEvoy, G. K. et al. AHFS Drug Information 2007. USA : American society of health system pharmacists, Inc., 2007.

Midmore, B. R., and T.M. Herrington. "Silica-stabilised multiple emulsions." Progress in Colloid and Polymer Science 112 (1999) : 115-120.

Muschiolik, G. "Multiple emulsion for food use." Current Opinion in Colloid & Interface Science 12 (2007) : 213-220.

Nakashima, T., M. Shimizu., and M. Kukizaki. "Particle control of emulsion by membrane emulsification and its applications." Advanced Drug Delivery Reviews 45 (2000) : 47-56.

Oesterling, T. O. "Aqueous stability of clindamycin." Journal of Pharmaceutical Science 59 (1970) : 63-67.

Oesterling, T. O., and E.L. Rowe. "Hydrolysis of lincomycin-2-phosphate and clindamycin-2-phosphate." Journal of Pharmaceutical Science 59 (1970) : 175-179.

Omotosho, J. A. " The effect of acacia, gelatin and polyvinylpyrrolidone on chloroquine transport from multiple w/o/w emulsions." International Journal of Pharmaceutics 62 (1990) : 81-84.

Opawale, Foyeke. O., and Diane. J. Burgess. "Influence of interfacial rheological properties of mixed emulsifier films on the stability of water-in-oil-in-water emulsions." Journal of Pharmacy and Pharmacology 50 (1998) : 965-973.

Regan, J. O. and D.M. Mulvihill. "Water soluble inner aqueous phase markers as indicators of the encapsulation properties of water-in-oil-in-water emulsions stabilized with sodium caseinate." Food Hydrocolloids 23 (2009) : 2339-2345.

Rosano, H. L., F.G. Gandolfo., and J.P. Hidrot. "Stability of W1/O/W2 multiple emulsions influence of ripening and interfacial interactions." Colloids and Surfaces A: Physicochemical and Engineering Aspects 138 (1998) : 109-121.

Salager, J. L. "Emulsion properties and related know-how to attain them." In Pharmaceutical Emulsion and Suspensions, 74-125. Edited by F. Nielloud, and G. Marti-Mestres. New york : Marcel Dekker Inc., 2000.

Shah, Vinod P. et al. "*In Vitro* Dissolution profile comparisom – statistics and analysis of the similarity factor, f_2." Pharmaceutical Research 15 (1998) : 889-896

Su, J. et al. "Synergistic effect of polyglycerol ester of polyricinoleic acid and sodium caseinate on the stabilization of water-oil-water emulsions." Food Hydrocolloids 20 (2006) : 261-268.

Su, J., J. Flanagan, and H. Singh. "Improving encapsulation efficiency and stability of water-in-oil-in-water emulsions using a modified gum arabic (Acacia (sen) SUPER GUMTM)." Food Hydrocolloids 22 (2008) : 112-120.

Sugiura, Shiji. et al. "Preparation characteristics of water-in-oil-in-water multiple emulsions using microchannel emulsification." Journal of Colloid and Interface Science 270 (2004) : 221-228.

Tados, T. F. "Emulsion science and technology : A general introduction." In Emulsion Science and Technology, 1-56. Edited by T.F. Tados. Germany : Wiley-vch verlag GmbH., 2009.

Van der graaf, S., C.G.P.H. Schroën., and R. M. Boom. "Preparation of double emulsiond by membrane emulsification – a review." Journal of Membrane Science 251 (2005) : 7-15.

Vanderhoff, J. W. "Theory of colloids." In Pharmaceutical Dosage Forms Disperse Systems vol. 1, 91-152. Edited by H.A. Lieberman, M.M. Rieger, and G.S. Banker. New york : Marcel Dekker Inc., 1996.

Vasiljevic, D. et al. "An investigation into the characteristics and drug release properties of multiple w/o/w emulsion systems containing low concentration of lipophilic polymeric emulsifier." International Journal of Pharmaceutics 309 (2006) : 171-177.

Viturawong, Y., P. Achayuthakan., and M. Suphantharika. "Gelatinization and rheological properties of rice starch/xanthan mixtures: effect of molecular weight of xanthan and different salts." Food Chemistry 111 (2008) : 106-114.

Vladisavljević, G. T. and R.A. Williams. "Recent development in manufacturing particulate product from double emulsion templates using membrane and microfluidic devices." In Multiple Emulsions Technology and Applications, 121-164. Edited by A. Aserin. New jersey : John wiley & sons Inc., 2008.

Weiss, J., I. Scherze, and G. Muschiolik. "Polysaccharide gel with multiple emulsion." Food Hydrocolloids 19 (2005) : 605-615.

Wen, L. and K..D. Papadopoulos. "Visualization of water transport in W1/O/W2 emulsions." Colloids and Surfaces A: Physicochemical and Engineering Aspects 174 (2000) : 159-167.

_____. "Effect of osmotic pressure on water transport in W1/O/W2 emulsions." Journal of Colloid and Interface Science 235 (2001) : 398-404.

Yoshida, Katsunori. et al. "Stability of vitamin A in oil-in-water-in-oil-type multiple emulsions." Journal of The American oil Chemists'Society 76 (1999) : 1-6

Yu, S. et al. "Effect of camphor/cyclodextrin complexation on the stability of o/w/o multiple emulsions." International Journal of Pharmaceutics 261 (2003) : 1-8.

APPENDIX

Table 33 Viscosity of clindamycin phosphate multiple emulsion containing 0, 10 and 20 % w/w petrolatum in oil phase and sodium chloride in external phase both before and after temperature cycling method (TCM)

	Before TCM Viscosity, cp at 30 °C			After TCM Viscosity, cp at 30 °C		
Sample	MEDPT0	MEDPT10	MEDPT20	MEDPT0	MEDPT10	MEDPT20
1	12237.39	9358.00	8558.17	11357.58	9038.07	8718.14
2	11357.58	9198.04	8158.26	11117.63	8478.19	8798.12
3	10957.66	8798.12	8158.26	11037.64	8478.19	8558.17
4	11037.64	8558.17	7383.33	10557.75	8558.17	8078.28
5	11117.63	8638.16	7438.41	10397.78	8238.24	7918.31
6	10717.71	8798.12	7358.43	10637.73	7998.29	8078.28
7	10637.73	8478.19	7518.40	10637.73	8158.26	7918.31
8	10797.70	8318.23	7438.41	10237.82	8238.24	7518.40
9	10877.68	8478.19	7038.50	10157.83	7998.29	7438.41
10	10637.73	8638.16	7038.50	10477.76	7838.33	7598.38
Average	11037.64	8726.14	7654.37	10661.73	8302.23	8062.28
SD	478.41	327.51	507.33	391.93	349.25	489.87

Table 34 Viscosity of clindamycin phosphate multiple emulsion containing 0, 10 and 20 % w/w petrolatum in oil phase and dextrose in external phase both before and after temperature cycling method (TCM)

	Before TCM Viscosity, cp at 30 °C			After TCM Viscosity, cp at 30 °C		
Sample	DEXPT0	DEXPT10	DEXPT20	DEXPT0	DEXPT10	DEXPT20
1	373520.30	23435.00	12077.42	285139.16	23035.08	12397.35
2	373120.38	22715.15	11837.47	309134.04	22475.20	12397.35
3	372320.55	22635.17	11357.58	316732.42	22075.29	12397.35
4	371920.64	22715.15	11197.61	317932.16	21835.34	12077.42
5	371120.81	22635.17	11277.59	318332.07	21995.31	11837.47
6	370720.90	22475.20	11277.59	318731.99	21915.32	11917.46
7	369921.07	22395.22	10877.68	318332.07	21675.37	11997.44
8	369521.15	22635.17	10877.68	317532.25	21515.41	11757.49
9	369921.07	22555.19	11037.64	315932.59	21675.37	11597.53
10	369921.07	22395.22	11037.64	315532.67	21675.37	11757.49
Average	371200.79	22659.17	11285.59	313333.14	21987.31	12013.44
SD	1445.61	297.00	394.46	10293.43	457.84	296.40

Table 35 Droplet size of clindamycin phosphate multiple emulsion containing 0, 10 and 20 % w/w petrolatum in oil phase and sodium chloride in external phase both before and after temperature cycling method (TCM)

	Before TCM Droplet size, μm			After TCM Droplet size, μm		
Sample	MEDPT0	MEDPT10	MEDPT20	MEDPT0	MEDPT10	MEDPT20
1	39.656	46.453	56.515	33.781	46.788	53.731
2	39.524	47.990	53.227	34.202	46.112	54.551
3	39.991	46.859	53.228	34.114	46.831	53.122
Average	39.724	47.101	54.323	34.032	46.577	53.801
SD	0.241	0.796	1.898	0.222	0.403	0.717

Table 36 Droplet size of clindamycin phosphate multiple emulsion containing 0, 10 and 20 % w/w petrolatum in oil phase and dextrose in external phase both before and after temperature cycling method (TCM)

	Before TCM Droplet size, μm			After TCM Droplet size, μm		
Sample	DEXPT0	DEXPT10	DEXPT20	DEXPT0	DEXPT10	DEXPT20
1	26.614	36.685	45.328	30.200	38.323	66.094
2	28.958	36.190	46.655	30.367	38.194	63.744
3	29.020	33.366	44.068	30.246	36.443	55.420
Average	29.197	35.414	45.350	30.271	37.653	61.753
SD	0.362	1.791	1.294	0.086	1.050	5.609

Table 37 Percentages of non-degraded clindamycin phosphate solution in citrate-phosphate buffer various pH at 40 °C

pH	Percentages of non-degraded clindamycin phosphate (SD), n = 3										
	Day 0	Day 1	Day 2	Day 7	Day 14	Day 21	Day 28	Day 42	Day 56	Day 70	Day 84
2	100.00(0.00)	105.80(0.41)	104.41(0.79)	103.01(0.42)	105.82(0.65)	105.60(0.32)	105.82(0.73)	104.42(0.43)	102.71(0.15)	96.81(0.26)	95.44(0.19)
3	100.00(0.00)	100.10(0.57)	95.90(0.47)	99.68(0.28)	99.99(0.64)	97.79(0.47)	92.29(0.41)	84.14(0.36)	79.44(0.37)	62.48(0.12)	56.53(0.33)
4	100.00(0.00)	105.83(0.02)	107.53(0.27)	107.19(0.08)	105.75(0.11)	104.14(0.06)	103.91(0.37)	101.08(0.31)	98.33(0.30)	90.98(1.05)	86.30(0.11)
5	100.00(0.00)	102.63(0.05)	99.53(0.96)	102.96(0.72)	100.04(0.55)	102.96(0.32)	98.29(0.25)	99.37(0.08)	100.37(0.41)	96.32(1.72)	95.79(0.37)
6	100.00(0.00)	104.51(0.12)	100.36(0.04)	101.85(0.08)	100.38(0.40)	102.56(0.03)	103.49(0.31)	102.28(0.10)	99.11(0.04)	93.06(0.74)	96.50(0.84)
7	100.00(0.00)	102.97(0.61)	101.14(0.29)	103.94(0.60)	104.22(0.34)	103.71(0.08)	101.43(0.03)	101.66(0.61)	102.13(0.09)	96.69(0.39)	97.87(0.54)
8	100.00(0.00)	100.06(0.04)	98.85(0.08)	102.58(0.30)	101.46(0.19)	96.82(4.30)	96.26(0.18)	94.01(1.15)	92.55(0.19)	86.15(0.76)	87.23(0.34)
%RSD	0.20	0.23	0.48	0.31	0.16	0.39	0.71	0.48	0.75	1.01	1.34

Table 38 Percentages of non-degraded clindamycin phosphate solution in citrate-phosphate buffer various pH at 4 °C

pH	Percentages of non-degraded clindamycin phosphate (SD), n = 3										
	Day 0	Day 1	Day 2	Day 7	Day 14	Day 21	Day 28	Day 42	Day 56	Day 70	Day 84
2	100.00(0.00)	102.23(0.67)	97.78(0.08)	103.27(0.43)	103.81(0.05)	99.57(0.53)	103.50(0.38)	95.23(0.41)	100.63(0.08)	90.92(0.24)	80.04(0.83)
3	100.00(0.00)	98.04(0.29)	93.10(0.66)	95.05(0.49)	98.06(0.70)	94.52(0.18)	94.77(0.44)	95.81(0.35)	96.95(0.37)	92.75(0.10)	93.79(0.36)
4	100.00(0.00)	105.01(0.09)	102.79(0.19)	105.20(0.28)	104.61(0.19)	103.70(0.21)	105.58(0.03)	102.04(0.18)	98.84(0.41)	90.11(0.16)	81.67(0.71)
5	100.00(0.00)	98.61(0.20)	94.04(1.03)	97.91(0.44)	100.35(0.60)	102.25(0.63)	98.77(0.61)	103.07(0.42)	103.04(0.45)	95.06(0.64)	98.09(0.63)
6	100.00(0.00)	99.79(0.32)	93.97(0.40)	98.84(0.08)	99.38(0.13)	98.28(0.01)	100.79(0.52)	100.46(0.12)	101.42(0.18)	92.83(0.50)	98.04(0.06)
7	100.00(0.00)	101.99(0.37)	100.67(0.29)	100.92(0.48)	102.16(0.09)	99.65(0.21)	100.89(0.45)	103.53(0.34)	105.26(0.77)	98.73(0.95)	101.82(0.46)
8	100.00(0.00)	101.02(0.56)	96.20(0.08)	98.13(0.13)	100.76(0.07)	98.36(0.31)	97.13(0.37)	99.53(0.14)	103.47(0.81)	90.98(0.29)	95.10(0.07)
%RSD	0.20	0.23	0.48	0.31	0.16	0.39	0.71	0.48	0.75	1.01	1.34

Table 39 Percentages of non-degraded clindamycin phosphate solution and multiple emulsion at 40 °C

Day	Percentages of non-degraded clindamycin phosphate (SD), n = 3					%RSD
	Solution, pH3	MEDPT0	MEDPT10	MEDPT20	DEXPT20	
0	100.00(0.00)	100.00(0.00)	100.00(0.00)	100.00(0.00)	100.00(0.00)	0.00
7	93.47(0.21)	88.42(0.39)	86.44(3.52)	90.20(0.30)	90.49(0.43)	0.43
14	84.14(0.39)	75.47(0.84)	96.34(3.90)	99.33(0.73)	93.57(0.09)	0.25
21	67.15(0.64)	81.11(2.59)	86.54(3.15)	93.11(0.97)	87.51(1.50)	0.30
28	61.46(0.34)	59.35(0.68)	91.66(4.32)	91.79(0.29)	85.80(0.70)	0.20
42	36.57(0.16)	50.83(0.62)	90.19(3.87)	94.40(0.55)	87.00(0.79)	0.56
52	17.12(0.06)	44.94(2.13)	72.07(2.42)	61.90(2.54)	78.72(0.53)	0.37
70	6.17(0.03)	51.48(0.62)	69.92(4.52)	58.76(1.65)	67.68(0.45)	0.35
84	2.18(0.08)	47.21(0.80)	76.15(2.99)	58.66(0.77)	67.56(0.38)	0.47

Table 40 Percentages of non-degraded clindamycin phosphate solution and multiple emulsion at 4 °C

Day	Percentages of non-degraded clindamycin phosphate (SD), n = 3					%RSD
	Solution, pH3	MEDPT0	MEDPT10	MEDPT20	DEXPT20	
0	100.00(0.00)	100.00(0.00)	100.00(0.00)	100.00(0.00)	100.00(0.00)	0.00
7	93.82(0.18)	97.95(0.16)	93.81(1.63)	90.65(0.36)	90.63(0.37)	0.43
14	90.30(0.09)	95.00(0.38)	101.13(1.42)	100.38(0.60)	102.43(0.73)	0.25
21	87.44(0.63)	74.70(0.55)	96.20(1.81)	93.13(1.42)	94.18(1.12)	0.30
28	91.89(0.16)	73.05(0.19)	97.89(1.70)	104.38(0.67)	94.64(0.28)	0.20
42	93.17(0.21)	79.46(0.09)	100.86(1.53)	102.08(1.19)	101.38(0.41)	0.56
52	93.35(0.13)	76.58(0.44)	87.26(0.76)	97.73(1.45)	99.90(0.75)	0.37
70	91.94(0.35)	78.45(0.54)	103.06(0.61)	93.05(0.22)	95.57(3.93)	0.35
84	98.28(0.29)	73.85(0.95)	96.66(5.05)	94.39(0.53)	95.79(0.62)	0.47

Table 41 Statistical analyses data of non-degraded clindamycin phosphate solution and multiple emulsion

Data 1	Data 2	Level	Result
Solution at 40 °C	MEDPT0 40 °C	0.05	the population means is significantly different
MEDPT0 40 °C	MEDPT10 40 °C	0.05	the population means is significantly different
MED PT0 40 °C	MEDPT20 40 °C	0.05	the population means is significantly different
MEDPT10 40 °C	MEDPT20 40 °C	0.05	the population means is not significantly different
MEDPT20 40 °C	DEX PT20 40 °C	0.05	the population means is not significantly different
Solution at 4 °C	MEDPT0 4 °C	0.05	the population means is significantly different
MEDPT0 4 °C	MEDPT10 4 °C	0.05	the population means is significantly different
MEDPT0 4 °C	MEDPT20 4 °C	0.05	the population means is significantly different
MEDPT 10 4 °C	MEDPT20 4 °C	0.05	the population means is not significantly different
MEDPT20 4 °C	DEXPT20 4 °C	0.05	the population means is not significantly different

Table 42 Cumulative released data of clindamycin phosphate multiple emulsion, containing 0 % w/w petrolatum in oil phase and sodium chloride in external phase. (MEDPT0)

Time (min)	Cumulative drug released, mg						Average	SD
	Sample1	Sample2	Sample3	Sample4	Sample5	Sample6		
0	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
5	0.0697	0.0675	0.0859	0.0639	0.0657	0.0659	0.0698	0.0081
10	0.1104	0.1138	0.1179	0.0964	0.0869	0.0734	0.0998	0.0174
20	0.1362	0.1471	0.1756	0.1207	0.1293	0.0963	0.1342	0.0266
30	0.1430	0.1824	0.1916	0.1358	0.1494	0.1169	0.1532	0.0285
60	0.2113	0.2455	0.2609	0.2042	0.2153	0.1846	0.2203	0.0280
90	0.3089	0.2912	0.3178	0.2728	0.2737	0.2312	0.2826	0.0310
120	0.3037	0.3357	0.3365	0.2883	0.3210	0.2745	0.3100	0.0255
180	0.3921	0.3871	0.4251	0.3530	0.3859	0.3863	0.3883	0.0229
240	0.4356	0.4390	0.5325	0.4581	0.4479	0.4382	0.4585	0.0372
360	0.5049	0.5305	0.5898	0.5200	0.5672	0.4902	0.5338	0.0379
540	0.6157	0.6876	0.7251	0.6236	0.7777	0.5990	0.6715	0.0708
720	0.7333	0.7869	0.8311	0.7277	0.7895	0.7115	0.7633	0.0462
900	0.8074	0.8697	0.9303	0.8368	0.9254	0.7928	0.8604	0.0585
1080	0.8977	0.9512	1.0261	0.9119	0.9888	0.8773	0.9422	0.0572
1260	1.0006	1.0530	1.1584	0.9885	1.0773	0.9782	1.0427	0.0686
1440	1.0747	1.1997	1.2395	1.0826	1.1850	1.1091	1.1484	0.0687
Weight	2.04 g	2.09 g	2.00 g	2.00 g	2.04 g	2.00 g		

Table 43 Cumulative released data of clindamycin phosphate multiple emulsion, containing 10 % w/w petrolatum in oil phase and sodium chloride in external phase. (MEDPT10)

Time (min)	Cumulative drug released, mg						Average	SD
	Sample1	Sample2	Sample3	Sample4	Sample5	Sample6		
0	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
5	0.2227	0.2006	0.3040	0.1919	0.1198	0.1705	0.2016	0.0611
10	0.4483	0.2508	0.4278	0.2024	0.2353	0.2210	0.2976	0.1101
20	0.3911	0.4745	0.4693	0.2903	0.3062	0.3050	0.3727	0.0846
30	0.4261	0.4482	0.5504	0.4241	0.3879	0.3882	0.4375	0.0601
60	0.5932	0.5633	0.7174	0.5184	0.7107	0.7546	0.6429	0.0969
90	0.7563	0.7560	0.7765	0.6719	0.7183	0.7029	0.7303	0.0394
120	0.8674	0.8641	0.8828	0.7982	0.8299	0.8407	0.8472	0.0307
180	0.1197	1.1042	1.1662	1.0573	1.1262	1.1297	1.1172	0.0358
240	1.2530	1.2269	1.2874	1.1652	1.2024	1.1956	1.2218	0.0437
360	1.5446	1.4874	1.6857	1.4052	1.4596	1.4154	1.4997	0.1043
540	1.9218	1.8684	1.9870	1.8469	1.8305	1.9019	1.8927	0.0573
720	2.2085	2.2769	2.3168	2.1805	2.1532	2.1245	2.2101	0.0739
900	2.4498	2.4434	2.5149	2.3012	2.4617	2.4143	2.4309	0.0716
1080	2.6872	2.6989	2.8052	2.5539	2.7788	2.6524	2.6961	0.0905
1260	2.9008	2.9087	3.0275	2.7352	2.9928	2.9019	2.9111	0.1013
1440	3.1986	3.1767	3.2812	3.0068	3.2353	3.0964	3.1658	0.0995
Weight	2.04 g	2.00 g	2.04 g	2.05 g	2.01 g	2.02 g		

Table 44 Cumulative released data of clindamycin phosphate multiple emulsion, containing 20 % w/w petrolatum in oil phase and sodium chloride in external phase. (MEDPT20)

Time (min)	Cumulative drug released, mg						Average	SD
	Sample1	Sample2	Sample3	Sample4	Sample5	Sample6		
0	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
5	0.1012	0.1333	0.2059	0.2096	0.1757	0.1920	0.1696	0.0435
10	0.2756	0.2841	0.2757	0.2999	0.2504	0.2388	0.2707	0.0224
20	0.3636	0.3820	0.3731	0.3762	0.3684	0.3371	0.3667	0.0159
30	0.4463	0.4521	0.4448	0.4694	0.4331	0.4047	0.4417	0.0217
60	0.6054	0.6285	0.6333	0.6230	0.6146	0.6296	0.6224	0.0106
90	0.7503	0.7755	0.7912	0.7880	0.7560	0.7872	0.7747	0.0176
120	0.8983	0.9339	0.9264	0.9142	0.8753	0.9062	0.9090	0.0210
180	1.0553	1.1061	1.1423	1.1174	1.1263	1.1243	1.1119	0.0302
240	1.2360	1.2952	1.3155	1.2636	1.2426	1.2722	1.2708	0.0305
360	1.4967	1.5431	1.5975	1.5497	1.5349	1.5635	1.5476	0.0332
540	1.7727	1.8385	1.8943	1.8462	1.8308	1.8583	1.8401	0.0398
720	2.0649	2.1516	2.2085	2.1605	2.1445	2.1704	2.1501	0.0474
900	2.2986	2.3870	2.4429	2.4539	2.3678	2.3905	2.3901	0.0561
1080	2.5351	2.6121	2.6420	2.6469	2.5718	2.6021	2.6017	0.0427
1260	2.6886	2.7970	2.8805	2.8590	2.8025	2.8335	2.8102	0.0676
1440	2.8688	2.9817	3.1116	3.1144	3.0095	3.0543	3.0234	0.0926
Weight	2.03 g	2.01 g	2.02 g	2.03 g	2.03 g	2.00 g		

Table 45 Cumulative released data of clindamycin phosphate multiple emulsion, containing 0 % w/w petrolatum in oil phase and dextrose in external phase. (DEXPT0)

Time (min)	Cumulative drug released, mg						Average	SD
	Sample1	Sample2	Sample3	Sample4	Sample5	Sample6		
0	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
5	0.0763	0.0874	0.0813	0.1095	0.0776	0.0484	0.0801	0.0197
10	0.0953	0.1045	0.0989	0.1336	0.0973	0.0865	0.1027	0.0162
20	0.1389	0.1477	0.1690	0.1973	0.1713	0.1562	0.1634	0.0207
30	0.2260	0.2288	0.2389	0.2585	0.2513	0.2315	0.2392	0.0131
60	0.3389	0.3162	0.3308	0.3666	0.3258	0.3392	0.3363	0.0172
90	0.3884	0.3815	0.3914	0.4008	0.3895	0.3654	0.3862	0.0119
120	0.4321	0.4166	0.4275	0.4626	0.4287	0.4225	0.4317	0.0161
180	0.5042	0.4889	0.5244	0.5097	0.4956	0.4824	0.5009	0.0152
240	0.5556	0.5295	0.5545	0.5613	0.5356	0.5416	0.5464	0.0126
360	0.5857	0.6418	0.6149	0.6744	0.6327	0.6052	0.6258	0.0310
540	0.7164	0.7268	0.7427	0.7558	0.7360	0.7226	0.7334	0.0144
720	0.7955	0.8372	0.8344	0.8370	0.8132	0.7929	0.8184	0.0208
900	0.8690	0.8889	0.9017	0.9251	0.8894	0.8836	0.8930	0.0190
1080	0.9743	1.0001	1.0011	1.0292	1.0108	0.9978	1.0022	0.0179
1260	1.0482	1.0811	1.0845	1.0978	1.0839	1.0781	1.0789	0.0165
1440	1.1332	1.1537	1.1606	1.1823	1.1564	1.1534	1.1566	0.0158
Weight	2.05 g	2.02 g	2.04 g	2.01 g	2.04 g	2.07 g		

Table 46 Cumulative released data of clindamycin phosphate multiple emulsion, containing 10 % w/w petrolatum in oil phase and dextrose in external phase. (DEXPT10)

Time (min)	Cumulative drug released, mg						Average	SD
	Sample1	Sample2	Sample3	Sample4	Sample5	Sample6		
0	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
5	0.2569	0.2781	0.2388	0.2419	0.2492	0.2175	0.2470	0.0202
10	0.3062	0.2855	0.3270	0.2930	0.2927	0.2790	0.2972	0.0172
20	0.3772	0.3959	0.3728	0.3668	0.3922	0.3502	0.3759	0.0168
30	0.4885	0.4732	0.4847	0.5053	0.4796	0.4282	0.4766	0.0261
60	0.6775	0.7009	0.6881	0.7000	0.6872	0.6544	0.6847	0.0172
90	0.7987	0.8472	0.8293	0.8492	0.7867	0.7973	0.8181	0.0273
120	0.9421	0.9974	0.9549	0.9489	0.9243	0.8997	0.9445	0.0327
180	1.1193	1.2071	1.1788	1.1763	1.1405	1.1159	1.1563	0.0367
240	1.3202	1.3532	1.3661	1.3177	1.3093	1.2979	1.3274	0.0265
360	1.5949	1.6703	1.6785	1.6011	1.5758	1.5267	1.6079	0.0578
540	1.9278	2.0294	2.0160	1.9477	1.9424	1.8555	1.9531	0.0634
720	2.2063	2.3503	2.3328	2.2855	2.2439	2.1596	2.2631	0.0739
900	2.4872	2.6463	2.6261	2.4527	2.5331	2.4047	2.5250	0.0960
1080	2.7826	2.9325	2.9224	2.8168	2.8114	2.6938	2.8266	0.0898
1260	3.0390	3.1695	3.1836	3.0112	3.0697	2.9550	3.0713	0.0899
1440	3.2999	3.4428	3.4673	3.2690	3.3669	3.1940	3.3400	0.1053
Weight	2.02 g	2.02 g	2.07 g	2.00 g	2.07 g	2.04 g		

Table 47 Cumulative released data of clindamycin phosphate multiple emulsion, containing 20 % w/w petrolatum in oil phase and dextrose in external phase. (DEXPT20)

Time (min)	Cumulative drug released, mg						Average	SD
	Sample1	Sample2	Sample3	Sample4	Sample5	Sample6		
0	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
5	0.1348	0.1332	0.1457	0.1354	0.1372	0.1472	0.1389	0.0060
10	0.1760	0.1806	0.1838	0.1856	0.2387	0.1896	0.1924	0.0232
20	0.2654	0.2796	0.2913	0.2801	0.2868	0.2662	0.2782	0.0106
30	0.3342	0.3481	0.3585	0.3510	0.3485	0.3438	0.3474	0.0081
60	0.4841	0.4996	0.5021	0.4932	0.4993	0.4899	0.4947	0.0069
90	0.5570	0.5892	0.5952	0.5942	0.5778	0.6050	0.5864	0.0169
120	0.6611	0.6550	0.6700	0.6590	0.6632	0.6722	0.6634	0.0065
180	0.7989	0.8022	0.8359	0.8311	0.8340	0.8332	0.8225	0.0171
240	0.9151	0.9469	0.9581	0.9382	0.9624	0.9406	0.9435	0.0169
360	1.1433	1.1574	1.1858	1.1707	1.1542	1.1619	1.1622	0.0146
540	1.3890	1.4182	1.4743	1.4440	1.4384	1.4195	1.4306	0.0288
720	1.6121	1.6483	1.7157	1.6531	1.6566	1.6415	1.6546	0.0339
900	1.7654	1.8201	1.8529	1.8196	1.8323	1.8142	1.8174	0.0290
1080	2.0392	2.0271	2.0935	2.0196	2.0647	2.0185	2.0438	0.0298
1260	2.1682	2.1712	2.2273	2.1655	2.2157	2.1391	2.1812	0.0335
1440	2.3107	2.3387	2.3986	2.3478	2.3889	2.2974	2.3470	0.0407
Weight	2.02 g	2.06 g	2.02 g	2.08 g	2.02 g	2.13 g		

Table 48 Release flux of clindamycin phosphate released from multiple emulsion

	MEDPT0	MEDPT10	MEDPT20	DEXPT0	DEXPT10	DEXPT20
Sample 1	0.0136	0.0406	0.0378	0.0141	0.0419	0.0303
Sample 2	0.0147	0.0410	0.0391	0.0145	0.0442	0.0305
Sample 3	0.0156	0.0415	0.0403	0.0145	0.0443	0.0314
Sample 4	0.0139	0.0394	0.0400	0.0145	0.0418	0.0305
Sample 5	0.0155	0.0422	0.0392	0.0144	0.0427	0.0309
Sample 6	0.0139	0.0406	0.0398	0.0145	0.0409	0.0301
Average	0.0145	0.0409	0.0394	0.0144	0.0426	0.0306
SD	0.0009	0.0009	0.0009	0.0002	0.0014	0.0005

Table 49 List of abbreviations

Symbol	Definition
°C	degree Celsius
>	more than
<	less than
η	viscosity
μg	microgram
μL	microliter
μm	micrometer
%	percent
%RSD	coefficient of variation

Table 48 List of abbreviations (continue)

Symbol	Definition
%v/v	percent volume by volume
%w/v	percent weight by volume
%w/w	percent weight by weight
cm^2	square centimeter
cp	centipoise
g	gram
GI	gastro intestinal
GMS	glyceryl monostearate
HLB	hydrophile-lipophile balance
HPC	hydroxypropyl cellulose
HPLC	high pressure liquid chromatography
HPMC	hydroxypropyl methylcellulose
h	hours
ME	multiple emulsion
mg	milligram
min	minute
mL	milliliter
NaCl	sodium chloride
nm	nanometer
o/w	oil in water
o/w/o	oil in water in oil

Table 48 List of abbreviations (continue)

Symbol	Definition
PE	primary emulsion
pH	The negative logarithm of the hydrogen ion concentration
qs. to	add to
R^2	coefficient of determination
rpm	revolution per minute
SD	standard deviation
TCM	Temperature cycling method
UV	ultraviolet
UV-VIS	UV visible
w/o	water in oil
w/o/w	water in oil in water

BIOGRAPHY

Name Nuntachai Hanpramukkun, Mr.

Address 50 Jomthong rd., Jomthong Bangkok Thailand

Workplace

1999-present Department of Pharmaceutical technology, Faculty of Pharmacy, Rangsit University, Pathumthani, Thailand

Institution Attended

1992-1997 Rangsit University : Bachelor of Science (Pharmacy)

1997-1999 Mahidol University : Master of Science in Pharmacy (Pharmaceutics)

2005 Education in Silpakorn University : Doctor of Philosophy (Pharmaceutical Technology)