PREPARATION AND CHARACTERIZATION OF CURCUMINOIDS NIOSOMES

Miss Nittaya Rungphanichkul

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Pharmacy
Pharmaceutical Technology
Faculty of Pharmaceutical Sciences
Chulalongkorn University
Academic Year 2004

ISBN 974-53-2092-7

การเตรียมและการตรวจสอบลักษณะของเคอคูมินอยด์นิโอโซม

นางสาว นิตยา รุ้งพาณิชย์กุล

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต

สาขาวิชาเทคโนโลยีเภสัชกรรม

คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

ปีการศึกษา 2547

ISBN 974-53-2092-7

Thesis Title	PREPARATION AND CHARACTERIZATION OF CURCUMINOIDS NIOSOMES
By	Miss Nittaya Rungphanichkul
Field of study	Pharmaceutical Technology
Thesis Advisor	Pornchai Rojsitthisak, Ph.D.
Thesis Co-advisor	Associate Professor Ubonthip Nimmannit, Ph.D.

Accepted by the Faculty of Pharmaceutical Sciences, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master's Degree

……. Dean of the Faculty of Pharmaceutical Sciences
(Associate Professor Boonyong Tantisira, Ph.D.)

THESIS COMMITTEE

……... Chairman
(Associate Professor Boonyong Tantisira, Ph.D.)

……... Thesis Advisor
(Pornchai Rojsitthisak, Ph.D.)

……... Thesis Co-advisor
(Associate Professor Ubonthip Nimmannit, Ph.D.)

……... Member
(Assistant Professor Warangkana Warisnoicharoen, Ph.D.)

……... Member
(Walaisiri Muangsiri, Ph.D.)

นิตยา รุ้งพาณิชย์กุล : การเตรียมและการตรวจสอบลักษณะของเคอคูมินอยด์นิโอโซม (PREPARATION AND CHARACTERIZATION OF CURCUMINOIDS NIOSOMES) อ.ที่ปรึกษา: อ.ดร. พรชัย โรจน์สิทธิศักดิ์, อ.ที่ปรึกษาร่วม:รศ.ดร. อุบลทิพย์ นิมมานนิตย์, 70หน้า. ISBN 974-53-2092-7

สูตรตำรับนิโอโซม ซึ่งใช้สารลดแรงตึงผิวชนิดไม่มีประจุเป็นส่วนประกอบของเวสิเคิล ได้รับการพัฒนาสำหรับกักเก็บสารสกัดเคอร์คูมินอยด์ โดยสารสกัดเคอร์คูมินอยด์ประกอบด้วยสารเคอร์คูมิน, เดสเมทอกซี่เคอร์คูมิน และบิสเดสเมทอกซี่เคอร์คูมิน ซึ่งมีฤทธิ์ที่มีประโยชน์ทางด้านยาและเครื่องสำอาง ยกตัวอย่างเช่น ฤทธิ์ในการเป็นสารต้านอนุมูลอิสระ และฤทธิ์ในการลดการอักเสบ ในการศึกษานี้ เคอร์คูมินอยด์นิโอโซมที่เหมาะสม ประกอบด้วย 150 ไมโครโมล ซอบิแทนโมโนโอลิเอท (สแปน 80), คอเลสเตอรอลและโซลูแลนซี-24 ในอัตราส่วนโดยโมลเท่ากับ 47.5: 47.5: 5 บรรจุด้วยสารเคอร์คูมินอยด์ 11 ไมโครโมล สำหรับการหาปริมาณเคอร์คูมินอยด์ที่ถูกกักเก็บและที่ไม่ถูกกักเก็บไว้ในนิโอโซม ทำด้วยวิธีไฮเปอร์เพอร์ฟอร์แมนลิควิดโครมาโทกราฟฟี่ ซึ่งจากการทดลองพบว่า นิโอโซมนี้มีประสิทธิภาพในการกักเก็บสารเคอร์คูมินอยด์ได้มากกว่า 90 เปอร์เซ็นต์ โดยมีขนาดเฉลี่ยเส้นผ่าศูนย์กลางอนุภาคประมาณ 20 ไมโครเมตร และประมาณ 74 เปอร์เซ็นต์ของอนุภาคมีขนาดอนุภาคที่เล็กกว่า 25 ไมโครเมตร จากการศึกษาเปรียบเทียบการซึมผ่านหนังงูของเคอร์คูมินอยด์จากเคอร์คูมินอยด์นิโอโซม และจากสารละลายเคอร์คูมินอยด์ในเมทานอล โดยใช้ฟรานซ์ดิฟฟิวชั่นเซลล์ พบว่า เคอร์คูมินอยด์นิโอโซมให้อัตราการซึมผ่านของเคอร์คูมินอยด์ที่ 72 ชั่วโมง เท่ากับ 0.41 ไมโครกรัมต่อชั่วโมงต่อตารางเซนติเมตร และไม่พบการซึมผ่านของสารสกัดจากสารละลายเคอร์คิวมินอยด์ จากผลการทดลองสามารถสรุปได้ว่า เคอร์คูมินอยด์นิโอโซมที่พัฒนาขึ้นสามารถช่วยเพิ่มการซึมผ่านของสารเคอร์คูมินอยด์เข้าสู่ผิวหนัง และมีความเหมาะสมสำหรับใช้ในผลิตภัณฑ์ภายนอกสำหรับผิวหนัง

สาขาวิชาเทคโนโลยีเภสัชกรรมนานาชาติ ลายมือชื่อนิสิต นิตยา รุ้งพาณิชย์กุล

ปีการศึกษา 2547 ลายมือชื่ออาจารย์ที่ปรึกษา พรชัย โรจน์สิทธิศักดิ์

ลายมือชื่ออาจารย์ที่ปรึกษาร่วม

##457685233: MAJOR PHARMACEUTICAL TECHNOLOGY (INTERNATIONAL) PROGRAM
KEY WORD: CURCUMINOIDS / NIOSOMES / ENTRAPMENT / SNAKE SKIN /
NITTAYA RUNGPHANICHKUL: PREPARATION AND CHARACTERIZATION OF CURCUMINOIDS NIOSOMES. THESIS ADVISOR: PORNCHAI ROJSITTHISAK, Ph.D. THESIS COADVISOR: ASSOC. PROF. UBOONTHIP NIMMANNIT, Ph.D. 70 pp. ISBN 974-53-2092-7.

Non-ionic surfactants based vesicles, the so called niosomes, were formulated to entrap the curcuminoids extract. Curcuminoids extract, consisting of curcumin, desmethoxycurcumin and bisdesmethoxycurcumin, have been known to possess various activities such as antioxidant and anti-inflammatory, resulting in widely used as pharmaceuticals and cosmetics. In this study, the suitable curcuminoids niosome formulation was composed of 150 µmole of sorbitan monooleate (Span 80), cholesterol and solulan C-24 (47.5: 47.5: 5 by mole) and loaded with 11 µmole of curcuminoids. Determination for the amounts of unentrapped and entrapped curcuminoids in the niosomes suspension was performed by HPLC method. The %entrapment efficiency of total curcuminoids was greater than 90%. The mean particle size of the curcuminoids niosome was approximately 20 µm in diameter, and about 74% of the particles were less than 25 µm. The permeation of curcuminoids from the curcuminoids niosome was also investigated in comparison with the curcuminoids methanolic solution, using the cobra skin as a model membrane in Franz diffusion cell. It was found the curcuminoids niosome provided the flux of curcuminoids at 0.41 µg/hr/cm^2 at 72 hour. However, curcuminoids was not detected in the receptor compartment when the curcuminoids solution was used. It can be concluded that the developed curcuminoids niosome significantly enhances the permeation of curcuminoids through the skin, and it is therefore suitable to use in the topical skin preparations.

Field of study Pharmaceutical Technology Student's signature……..
Academic year 2004 Advisor's signature…….
Co-advisor's signature……

ACKNOWLEDGEMENTS

I am deeply indebted to many people who have made their kind contributions to my study. First of all, I would like to express my profound gratitude and appreciation to my advisor, Dr. Pornchai Rojsitthisak for valuable comments and suggestions, kindness and constant encouragement throughout my study. My sincere appreciation also goes to Associate Professor Dr. Uboonthip Nimmannit, my co-advisor for her helpful and valuable advice. I am very much obliged to the members of my thesis committee for their scrutiny and discussion. I would like to thank the Scientific and Technological Research Equipment Center of Chulalongkorn University for the supply of its useful data to my thesis.

Also, I am most grateful to the Pharmaceutical Technology (International) Program, Faculty of Pharmaceutical Sciences, Chulalongkorn University for granting partly financial support to conduct my investigation. I would also like to thank all the faculty members in the Pharmaceutical Technology (International) Program for their help and encouragement.

Above all, I would like to express my sincerest and deepest gratitude to my parents for their love, understanding and encouragement throughout my life.

Finally, my deep appreciation goes to my friends and other people, whose names have not been mentioned, for helping me in anyway during the time of my study.

CONTENTS

PAGES

LIST OF TABLES

Table Page

LIST OF FIGURES

LIST OF ABBREVIATIONS

µg	=	microgram
µl	=	microliter
µm	=	micrometer
µmole	=	micromole
CV	=	coefficient of variation
g	=	gram
LUVs	=	large unilamellar vesicles
mg	=	milligram
min	=	minutes
ml	=	milliliter
MLVs	=	multilamellar vesicles
mm	=	millimeter
nm	=	nanometer
POE	=	polyoxyethylene
r^2	=	coefficient of determination
rpm	=	rovolution per minute
SUVs	=	small unilamellar vesicles

CHAPTER I

INTRODUCTION

Nowadays, the use of phytochemicals (bioactive non-nutrients plant chemicals which are presented in varying levels of different plants) e.g. flavonoids, isoflavones and phenolic compounds, becomes more interesting. Several fruits, vegetables, herbs and plants such as soybean, green tea and turmeric are rich sources of phytochemicals (Bidlack *et al.*, 1998). In traditional medicine, usage of medicinal plants and their active principles in the prevention and treatment of diseases is based on the experience from different ethic societies. In contrast, the use of medicinal plants in modern medicine have attracted the interest of scientists and been investigated for their biological activities and toxicities. One plant that has been widely investigated is *Curcuma longa* Linn.(Wang *et al*., 1997).

Curcuma longa Linn (Turmeric) is a tropical plant native to south and southeast tropical Asia. It is a member of the ginger or *Zingiberaceae* family. Turmeric is widely consumed in the countries of origin for various purposes, including dietary spice, dietary pigment and Indian folk medicine for the treatment of various illnesses. It is also used in Hindu ceremonies in one form or another as a part of the religious rites. As cosmetics, it has been used as a skin caring agent for women.

In order to accomplish their actions in the skin, curcuminoids must be delivered to the target cells in the deeper epidermis layer. The most important barrier of the skin is stratum corneum. In human, the stratum corneum consists of corneocytes, embedded in a highly organized matrix of lipid lamellae (Honeywell-Nquyen *et al.*, 2002). One standing delivery system that can overcome the barrier of skin to deliver substances into the deeper skin is lipid vesicle delivery systems such as liposomes and niosomes.

Liposomes are submicron spherical vesicles. The center of liposome consists of an aqueous cavity that is encapsulated by one or more bimolecular phospholipid sheets, each separated from the other by aqueous layers. This compartmentalized structure lends itself to the incorporation of a variety of hydrophilic substances in the aqueous core. Similarly, lipophilic substances can be incorporated in the lipid lamellar sheets, and thus are protected from the surrounding medium (Simonnet, 1994). Unfortunately, there are some disadvantages of the phospholipid in the liposomal system, which are the lack of stability and the high cost of the phospholipids. The low cost, greater stability and resultant ease of storage of non-ionic surfactants have lead to the exploitation of non-ionic surfactants as alternatives to phospholipids in liposomal system (Florence, 1993). This non-ionic surfactant based vesicles have been named niosomes.

Basically, niosomes consist of non-ionic surfactants and membrane additives. Since the structure of niosomes is analogous to that of liposomes, an entrapment of curcuminoids in niosomes could be served as an alternative choice to enhance the stability and skin permeation of curcuminoids. Hence, in this study niosomes will be used as the transdermal delivery system for curcuminoids.

This study investigated the preparation of niosomes containing curcuminoids and also characterized the obtained curcuminoids niosomes. The purposes of this study were as follows:

1. To prepare various curcuminoids niosomes with different types of surfactants and various amounts of curcuminoids loaded in the niosomes.
2. To optimize the curcuminoids niosomes formulation.
3. To characterize the curcuminoids niosomes by evaluating their morphology, particle size and particle size distribution, entrapment efficiency and skin permeation.

CHAPTER II

LITERATURE REVIEWS

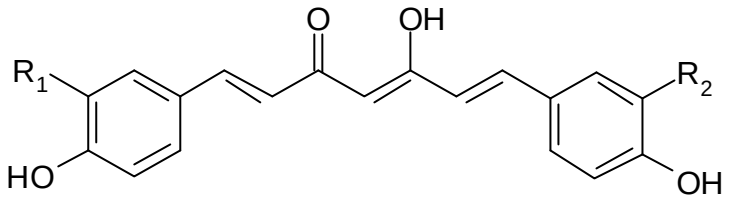
1. Curcuminoids

1.1 *Curcuma longa* (turmeric)

Curcuma longa Linn. is a herbaceous plant in Zingiberaceae family. It originates in India and Southeast Asian countries and has been grown in many countries in this region, including Thailand. *Curcuma longa* Linn. has been used in a form of turmeric powder, which has yellowish orange color and has pleasant aroma. Turmeric powder is widely used as a spice and a natural coloring agent in several foods, such as curry, mustard and potato chip, as well as in drugs and cosmetics. Curcuminoids are polyphenolic pigments found in the spice turmeric. The term "turmeric" is used both for the plant *Curcuma longa* L. and the spice derived from the rhizomes of the plant.

1.2 Chemistry

Chemical constituents of turmeric are curcuminoids compounds (1.8-5.4%), volatile oil (7%), and starch. Curcuminoids can be obtained by extraction of turmeric by organic solvents such as ethanol. The major curcuminoids are curcumin, desmethoxycurcumin and bisdesmethoxycurcumin. The chemical structures of curcuminoids are shown in Figure 1. Curcumin is the most studied curcuminoids. In pure form, curcumin is an orange-yellow, crystalline powder that is insoluble in water. Its melting point is 183 $^{\circ}$C. It is also known as diferuloylmethane and turmeric yellow. The molecular formula of curcumin, desmethoxycurcumin and bisdesmethoxycurcumin are $C_{21}H_{20}O_6$, $C_{20}H_{18}O_5$ and $C_{19}H_{16}O_4$ with the molecular weight of 368.39, 338.36 and 308.34, respectively.



1 : $R_1 = R_2 = OCH_3$

2 : $R_1 = H$; $R_2 = OCH_3$

3 : $R_1 = R_2 = H$

Figure 1. The chemical structures of curcumin (1),desmethoxycurcumin (2) and bisdesmethoxycurcumin (3)

1.3 Physicochemical properties

The differences in physical properties between the naturally occurring curcuminoids and their derivatives were illustrated in a study which investigated intramolecular and intermolecular hydrogen bond formation within these compounds using absorption and emission properties (Tonnesen *et al*., 2002). Toennesen et al. (1995) observed intermolecular hydrogen bond formation in both the ground state and the excited state of the curcuminoids possessing phenolic groups. The phenolic groups in curcumin possessed hydrogen bond acceptor properties, while those in bisdesmethoxycurcumin acted as hydrogen bond donors due to differences in polarity in various alcohols. It has been reported that curcuminoids is insoluble in acidic to neutral pH aqueous solution, however, it is soluble in ethanol and aqueous alkali solution (THP I, 1995; Tonnesen *et al*., 2002).

Li et al. (1996) investigated the kinetics of alkaline degradation of the curcuminoids. The results showed that bisdesmethoxycurcumin was less susceptible to degradation at pH 10.2 than curcumin or desmethoxycurcumin**,** therefore, bisdesmethoxycurcumin should be used in alkaline compositions to improve stability.

1.4 Pharmacological activities of curcuminoids

The curcuminoids are known for antioxidant (Reddy and Lokesh, 1992), anti-inflammatory, antimicrobial, antiparasitic, antimutagenic, and anticancer properties (Srimal, 1997). Other than the advantage of several biological activities of curcuminoids, it was found that curcuminoids had no toxicity in long-term administration. Advanced colorectal cancer patients ingesting *Curcuma* extract capsules containing 40 to 200 mg curcuminoids for 4 months well tolerated at all dose levels with no serve toxicity (Sharma, 2001). However, two types of adverse effects were found; nausea in one patient taking 120 mg of curcuminoids daily during the first month (Nation Cancer Institute toxicity grade 1) and diarrhea in two patients taking 80 and 200 mg of curcuminoids daily in the fourth month and first month, respectively (Nation Cancer Institute toxicity grade 2 and 1, respectively).

2. Niosomes

Non-ionic surfactant based vesicles, the so called niosomes, basically consist of non-ionic surfactants, cholesterol and stabilizer. Niosomes are analogous to phospholipid vesicles (liposomes) and served as drug carriers, which are able to encapsulate both hydrophilic and hydrophobic solutes. The lower cost, greater stability and resultant ease of storage of nonionic surfactant (Florence, 1993) have leaded to the exploitation of nonionic surfactants as alternatives to phospholipids. Hence, in this study niosomes are used as the transdermal delivery system for curcuminoids.

2.1 Formation of niosomes

Niosomes are formed from the self-assembly of nonionic ampliphiles in aqueous medium (Uchegbu and Vyas, 1998). The membrane of niosomes possesses a hydrophilic head group and a hydrophobic tail. The hydrophobic moiety may consist of one or two alkyl groups. The alkyl group chain length is usually from C_{12}-C_{18} and various types of hydrophilic group are used (Uchegbu and Vyas, 1998).

2.2 Factors governing the self-assembly of nonionic surfactants into niosomes

2.2.1 Non-ionic surfactant

Many non-ionic surfactants form vesicle with difference in toxicity. The ester bond in the alkyl ester surfactant can be hydrolyzed in the body and therefore, this kind of surfactant has less toxicity than alkyl ether surfactants. For example, sorbitan monostearate (a alkyl ester surfactant) contains 10% by mole of solulan C-24, is not haemotoxic (Uchegbu and Vyas, 1998). Moreover, the sorbitan ester surfactants are commonly used as pharmaceutical excipients. A hydrophilic lipophilic balance (HLB) is a good indicator for vesicle forming ability. A HLB value of between 4 and 8 was found to be suitable for vesicle formation (Yoshioka, 1994 and Uchegbu, 1995).

2.2.2 Membrane additives

Various additives must be included in the formulation in order to obtain stable niosomes. The most common additive found in niosomal systems is cholesterol. It is known to abolish gel to liquid through phase transition, resulting in systems that are less leaky and more stable. Cholesterol used in most formulations is a 1:1 molar ratio of cholesterol: non-ionic surfactant (Uchegbu, 1998). Addition of an optimum amount of cholesterol increases the stability of the bilayer. However, even after the addition of cholesterol, the intrinsic phase transition behavior of surfactants still influences the properties of the dispersions: notably the membrane permeability, encapsulation efficiency, bilayer rigidity.

Basically, niosomes should be stabilized by the addition of a charged molecule such as dicetyl phosphate. A steric stabilizer, solulan C-24 (poly-24-oxyethylene cholesteryl ether), must be added to the formulation to prevent niosomes aggregation (Uchegbu, 1994). The chemical structures of cholesterol and solulan C-24 are shown in Figure 2.

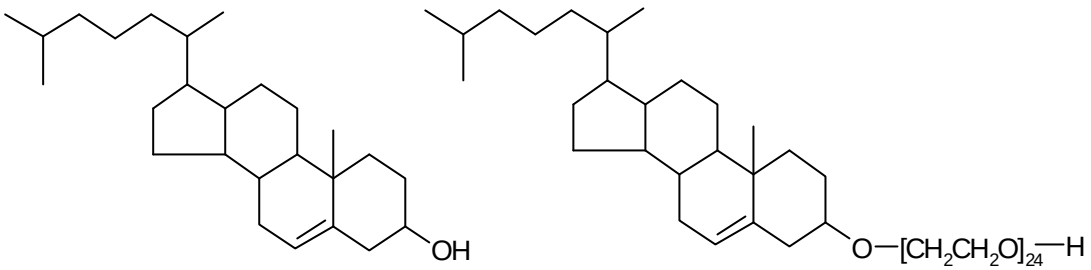


Figure 2. The chemical structures of cholesterol (left) and solulan C-24 (right)

2.3 Niosomes preparation

The formation of vesicular assemblies requires the input of energy (Lasic, 1997). Thus, all of the preparation methods consist of the hydration of a mixture of the surfactant/lipid at elevated temperature followed by an optional size reduction process to obtain a colloidal dispersion. Separation of unentrapped drug from the entrapped drug is archived by either centrifugation, gel filtration or dialysis. Similar to the liposome classifications, niosomes is largely divided into 3 classes, which are large unilamellar vesicles (LUVs), small unilamellar vesicles (SUVs) and multilamellar vesicles (MLVs) as shown in Table 1. MLVs are the simplest type of niosomes to prepare using hand-shaking method. The internal space of multilamellar vesicle is occupied by multilayers of lipid bilayers. Therefore, MLVs are suitable for entrapment of hydrophobic solutes in the lipid bilayers. SUVs are obtained from reducing the size of MLVs or LUVs using sonication or extrusion techniques. The vesicles contain only one lipid layer and one aqueous layer. LUVs possesses large aqueous compartment at the core of vesicle. Thus, LUVs are suitable for carrying hydrophilic drugs.

Table 1. The types and features of niosomes

Types of vesicle	Sizes (μm)	Features of niosomes
Multilamellar vesicles (MLVs)	0.05-10	Large retention volume, good stability
Small unilamellar vesicles (SUVs)	0.025–0.05	Uniform size and shape, small retention volume, easy refusion
Large unilamellar vesicles (LUVs)	>0.1	Large retention volume, not uniform size

3. Skin

The skin is the largest and the most heterogeneous organ in a body. It composes of tissues that grow, differentiate and renew themselves constantly. The skin structure is shown in Figure 3. The skin constructs of three major layers: epidermis, dermis and subcutaneous tissue (Mezei, 1994)

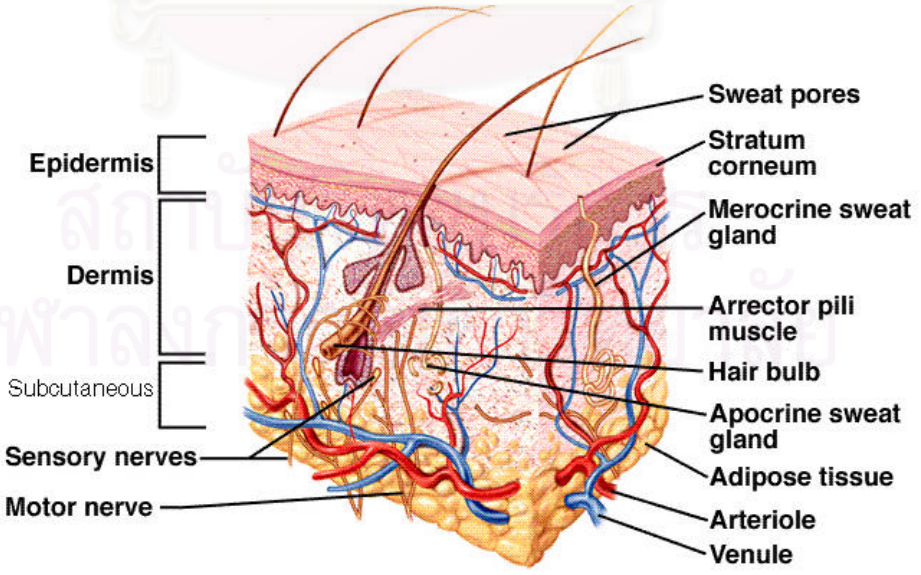


Figure 3. The cross-section of the skin structure (www.soapkitchen.com.au)

3.1 The epidermis

The multilayered structure of the epidermis varies in thickness, depending on cell size and the number of cell layer. The epidermis composes of four strata. It ascending from the proliferative layer of basal cells: the stratum germinativum, the stratum granulosum (the malpighian layer), the stratum lucidum (the granular layer) and stratum corneum (the horney layer). The epidermis changed in an ordered fashion from metabolically active and dividing cells to dense, dead and keratinized protein.

The stratum corneum is the superficial layer of the epidermis. This region is considered as a nonviable epidermis providing 10-15 layers of much flattened, keratinized dead cells, stacking up in highly organized units of vertical columns. It is approximately 10-20 micron thick. Previous studies, indicated that the stratum corneum is not uniformly homogeneous. Stratum corneum continuously evolves from below to the skin surface, and the layers represent various stages of corneocyte and intercellular lipid maturation. Each stratum corneum cell contains mainly of keratin (~70%) and lipid (~20%).

3.2 The dermis

The dermis interfaces with the epidermis at the epidermal-dermal junction. It is ten to forty times thicker than the epidermis, depending on site of the body. Dermis makes up the bulk of the skin. The dermis is metabolically less active than the epidermis; it is a matrix of loose connective tissue composed of polysaccharides and protein (collagen and elastin) that embeded in a ground substance containing a variety of lipid, protein and carbohydrate. This matrix contained nerves, blood vessels, hair follicles, sebaceous and sweat glands. The function of the dermis is to nourish the epidermis and to anchor it to the subcutaneous tissue.

3.3 The subcutaneous tissue

Subcutaneous tissue serves as a receptacle for formation and storage of fat. The subcutaneous tissue a place for dynamic lipid metabolism; it supports nerve and blood vessels passing to the dermis. The subcutaneous fat spreads all over the body. Its thickness varied with the age, sex, endocrine and nutritional status of the individual.

Consequently, the skin is an effective barrier to the penetration of substances including drugs. The skin is an important organ with respect to metabolism and immunology. Due to its heterogeneous structure and its dynamic nature, which could not be maintained in *in-vitro* conditions, only in-vivo studies could provide reliable data related to the permeation, metabolism and generally clearance of the drug within and from the skin.

4. Niosomes as topical drug carriers

The rationale for the use of vesicles as topical drug carriers. Firstly, the vesicles are serves as drug carriers to obtain the higher local drug concentrations leading to the higher thermodynamic activity. Secondly, the vesicles are served as a local depot for sustained release of drugs. Thirdly, the non-ionic surfactant vesicles serve as skin penetration enhancer. Lastly, the vesicles serve as a rate-limiting membrane for controlled transdermal drug delivery systems (Schreier, 1994).

Niosomes are microscopic vesicles composed of membrane-like lipid layers surrounded by aqueous compartments (Mezei, 1994). Hofland *et al*. (1994) demonstrated niosomes structural changes when niosomes penetrated deeper in the stratum corneum, resembling multilamellar vesicular structures. Schreier *et al*. (1994) speculated that either intact niosomes migrated into the stratum corneum, or that molecularly dispersed high local concentrations of nonionic surfactants could form curved lamellar structures within the intercellular lipid of the stratum corneum. Some common mechanisms that had been reported for niosomes were fusion of vesicles on the surface of the skin that might lead to high thermodynamic activity of drug that is the driving force for the permeation of lipophilic drugs (Fang, 2001).

Direct contact between vesicles and skin is essential for penetration and drug delivery. If direct contact is obstructed, drug transport is decreases. Komatsu *et al*. (1986) showed that a penetration enhancement of the molecular components could not fully account for the increase in transport of drugs encapsulated in vesicles. Moreover, it appeared that vesicles had a more pronounce effect on transport of lipophilic drugs than on that of hydrophilic drugs (Scherier, 1994).

5. Snake skin

Typically, shed snake skin penetration studies provide conservative estimation for human skin penetration since it is less permeable than human skin for most compounds (Itoh, 1990). Recently, the use of shed snake skin as membrane model for *in vitro* diffusion studies catch attractive.

It is particularly difficult to obtain human skin for *in vitro* experiments therefore, it is important to have alternate biological or synthetic membranes that mimic human skin membranes for diffusion experiments. Snake skin is shed as a large intact sheet, thus, a single snakes skin could provide multiple samples. Since snakes skin lacks of hair follicles, the problems associated with the transfollicular route of penetration, which might be significant in mammalian skins, is overlooked.

However shed snake skin consists of three distinctive layers, beta-keratin-rich outermost beta layer, alpha-keratin and lipid-rich innermost alpha layer. Furthermore, the mesos layer shows three to five layers of cornified cells surrounding by intercellular lipids. Therefore, the mesos layer is similar to human stratum corneum. This mesos layer is also a major depot of lipids. The mesos layer and alpha layer are considered to be the main barrier to water penetration through the skin. Furthermore, water permeabilities of shed snake skins from normal snakes and scaleless skin are determined and showed that the existence of scales might not affect significantly the permeability of compounds through shed snake skin.

Different snake species display different permeation characteristics. It is important that if shed snake skin is used as a membrane, the species and skin site should be reported. The integrity of shed snake skin, as verified by electron microscopy, indicated that it behaved as a diffusion membrane. It appeared that shed snake skin might be a useful membrane for studying diffusion of specific drugs, determining effects of different formulations or different enhancers on drug penetration. However care must be exercised when permeability values through shed snake skin is extrapolated to in vivo situation (Haigh, 1998).

Ponjanyakul et al. (2000) compared the permeation of nicotine through cobra skin with human skin. Permeation values from both specimens showed good correlation. Moreover, the value of permeation fluxes obtained with the cobra skin gave lower %CV than those obtained with the human skin. Thus, cobra skin could be used as a potential membrane to evaluate drug permeation since it demonstrated low intra- and interspecimen variation. Hence, in this study cobra skin (*Naja naja* Khaotia), a snake that is available in Thailand, was selected as a model membrane.

CHAPTER III

MATERIALS AND METHODS

MATERIALS

1. SPAN 40 , Sorbitan monopalmitate, SP (The East Asiatic) (MW = 403)
2. SPAN 60 , Sorbitan monostearate, SS (The East Asiatic) (MW = 431)
3. SPAN 80 , Sorbitan monooleate, SO (The East Asiatic) (MW = 429)
4. Cholesterol (Sigma) (MW = 373)
5. Solulan C-24 ,Poly(24)oxyethylene cholesteryl ether (Amerchol) (MW = 1249)
6. Methanol AR grade (Lab-Scan)
7. Methanol HPLC grade (Lab-Scan)
8. Acetonitrile HPLC grade (Lab-Scan)
9. Ethanol AR grade (Lab-Scan)
10. Acetic acid AR grade (Lab-Scan)
11. Curcuminoids (Thai-Chinese Flavors and Fragrances Industry Co., Ltd., %purity of curcumin, desmethoxycurcumin and bisdesmethoxycurcumin = 79.24, 20.16 and 2.57, respectively) (MW of curcumin, desmethoxycurcumin and bisdesmethoxycurcumin = 368.39, 338.36 and 308.34, respectively)

MEMBRANES

Cobra shed snake skin (*Naja Naja Khoatia*) was donated by Thai Red Cross Society, Bangkok, Thailand.

EQUIPMENTS

1. Rotary evaporator (Buchi)
2. High performance liquid chromatography system (Shimadzu)
   Liquid chromatography : LC-10ADvp
   UV-VIS detector : SPD-10Avp
   System controller : SCL-10Avp
3. Modified Franz diffusion cell
4. Optical light microscope with an attached camera (Olympus)
5. Scanning electron microscope (JSM-5410LV)
6. Ion sputter (Balzers)
7. Critical point drier (Tousimis)
8. Mastersizer S long bed version 2.11 (Malvern)
9. Ultracentrifugation (Beckman)
10. Hot air oven (Binder)
11. Ultrasonic bath (Elma)
12. Vortex mixer (Vortex- genic)

METHODS

1. Analysis of the amount of curcuminoids using HPLC

1.1 Niosomes placebo suspension

Niosomes placebo solution (without curcuminoids) was prepared by mixing non-ionic surfactant (Sorbitan monooleate, SO, Span 80, 0.61 g), cholesterol (0.53 g) and solulan C-24 (0.21 g) in 400 ml of methanol in a 250-ml round bottom flask. The mixture was evaporated using a rotary evaporator at 60°C until a thin film was developed. The dried lipid film was subsequently hydrated with 125 ml of distilled water and the mixture was shaken in a water bath at 80°C for 1 hour. The niosomes vesicles were subsequently formed.

1.2 Preparation of standard and sample solutions

Stock standard curcuminoids solution (1.0 mg/ml) was prepared by dissolving standard curcuminoids in methanol. The solution was subsequently diluted with methanol to obtain working standard curcuminoids solution (0.1 mg/ml). Sample solutions for validation were prepared by mixing various amounts of stock standard curcuminoids solution with 5.0 ml of niosomes placebo suspensions in a 25-ml volumetric flask. The final concentrations of curcuminoids were about 2, 4, 6, 8, 10 and 12 µg/ml. All solutions were filtered through 0.45 µm nylon membrane prior to HPLC analysis.

1.3 HPLC Assay

A reversed-phase HPLC assay was developed and validated for the determination of curcumin, desmethoxycurcumin and bisdesmethoxycurcumin. Samples were separated on a HiQ-Sil C18 column (4.6 mm x 150 mm, 5 µm particle size) and eluted with acetonitrile and 2% acetic acid (40:60). The mobile phase solution was filtered through 0.45 µm nylon membrane and degassed before use. The injection volume was 20 µl, and the elution was isocratic with a flow rate 1.5 ml/min. The detection was carried out at 425 nm. The total chromatographic analysis time per sample was 30 min with retention time of curcumin, desmethoxycurcumin and bisdesmethoxycurcumin eluting about 16.2, 18.1 and 20.2 min, respectively (Figure 6-8, Appendix B).

1.4 Assay validation

Accuracy was performed by triplicated injections of working standard solution and sample solutions. The theoretical concentration, the measured concentration and %recovery of curcumin, desmethoxycurcumin and bisdesmethoxycurcumin were determined. Peak area was used to calculate the measured concentration. The method linearity was obtained by plotting the measured concentration versus the theoretical concentration, and the least squares regression equation was calculated. The intra-day precisions were performed by duplicated injections of six replicated sample solutions, and the %CV of peak area of curcumin,

desmethoxycurcumin and bisdesmethoxycurcumin were determined. The LOD and LOQ were calculated from the results of analysis of sample solutions for validation by plotting the concentration against peak area. The equation for LOD is 3.3(SD/S) where, SD is the residual standard deviation of the regression line and S is the slope. The equation for LOQ is 10(SD/S) where, SD is the residual standard deviation of the regression line and S is the slope.

2. Preparation of curcuminoids niosomes

The preparation process of curcuminoids niosomes was described as follows. Briefly, the appropriate amount of non-ionic surfactant, cholesterol and solulan C-24 (Table 2) were dissolved in 20 ml of methanol in a 100-ml round bottom flask. After that, one milliliter of curcuminoids solution (1 mg/ml) was added into the solution. The mixture was evaporated using a rotary evaporator at 60°C until a thin film on the flask wall was formed. The dried lipid film was subsequently hydrated with 5.0 ml of distilled water, and the mixture was shaken in a water bath at 80°C for 1 hour. The niosomes vesicles containing curcuminoids were subsequently formed.

3. Effect of non-ionic surfactants on the preparation of curcuminoids niosomes

In this study, the mole ratio of non-ionic surfactant, cholesterol and solulan C-24 were kept constant at 47.5: 47.5: 5. Three different non-ionic surfactants used in the preparation of niosomes in this study were Span 40 (sorbitan monopalmitate, SP), Span 60 (sorbitan monostearate, SS) and Span 80 (sorbitan monooleate, SO). One milligram of curcuminoids (2.82 µmol) was mixed with 150 µmol of niosomes. The preparation process was similar to previously described. The details of each formulation were summarized in Table 2. The obtained niosomes suspension was subsequently characterized on the morphology of the vesicle using optical and scanning electron microscope as described below.

Table 2. Curcuminiods niosomes formulations consisting of curcuminoids (1mg, 2.82μmol) and niosomes (150μmol) formed by different surfactants

			Niosomes*							
	Curcuminoids		Surfactant***		Cholesterol		Solulan C-24		Total	
No	mg	μmol**	mg	μmol	mg	μmol	mg	μmol	mg	μmol
A	1	2.82	28.7	71.25	26.6	71.25	10.7	7.5	66.0	150
B	1	2.82	30.7	71.25	26.6	71.25	10.7	7.5	68.0	150
C	1	2.82	30.7	71.25	26.6	71.25	10.7	7.5	68.0	150

*Surfactant: Cholesterol: Solulan C-24 = 47.5: 47.5: 5 by mole

**2.82 μmol of curcuminoids consists of curcumin, desmethoxycurcumin and bisdesmethoxycurcumin at 0.215, 0.059 and 0.008 μmol, respectively

***A= Sorbitan monopalmitate, B= Sorbitan monostearate, C= Sorbitan monooleate

4. **Determination of morphology of niosomes**

4.1 Optical microscopy

Normally, the ordinary microscope is used for particle size measurement in the range of 0.2 μm to about 100 μm (Martin, 1969). Based on this study, the vesicle formation and its shape can be examined with an optical microscope with an attached camera. One drop of niosomes suspensions was mounted on a slide and covered with cover slide and placed on the stage. The pictures were taken and the shape of the vesicles was recorded.

4.2 Scanning electron microscopy

The morphology of vesicles was investigated by scanning electron microscopy. One drop of niosomes suspensions was air-dried on the cover slide. The dried sample was prefixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer at pH 7.2 for two hours in the refrigerator. After that, the dried sample was washed with phosphate buffer for three times, ten to fifteen minutes each, followed by the post-fixed process. The sample was fixed with 1% osmium tetroxide in 0.1 M phosphate buffer at pH 7.2, washed with phosphate buffer and then washed again twice with distilled water for ten to fifteen minutes each. The dried and fixed sample was dehydrated through a graded series of alcohol (30%, 50%, 70%, 90% and 100%) for ten to fifteen minutes each. For 100% alcohol, three-times washing were performed. The sample was dried under CO_2, mounted on the stub under a stereomicroscope, and then it was coated with gold for four to five minutes with ion sputter. Finally, the sample was observed under scanning electron microscopy.

5. Effect of curcuminoids concentration on preparation of curcuminoids niosomes

After three prepared curcuminoids niosomes were determined on their morphology, the preparation with high amount of vesicles and round-shaped vesicles was selected for further investigation on the effect of curcuminoids concentration on the niosomes preparation. The constant amount of niosomes at 150 µmol was mixed with various amounts of curcuminoids ranging from 2 to 12 mg (5.64-33.84 µmol). The details of each formulation were summarized in Table 3. The six formulations of prepared curcuminoids niosomes were then evaluated on the entrapment efficiency as described below. The niosomes formulation with highest entrapment efficiency was subjected to the determination of particle size and particle size distribution. In addition, the permeation study of the selected formulation was also investigated using modified Franz cell diffusion technique as further described.

Table 3. Niosomes containing curcuminoids formulation consisting of constant amount of noisome (150 µmol) and various amounts of curcuminoids

No	SO: Chol: Solulan C24* (47.5: 47.5: 5) (µmol)	Curcuminoids	
		mg	µmol
D	150	2	5.64
E	150	4	11.28
F	150	6	16.92
G	150	8	22.56
H	150	10	28.20
I	150	12	33.84

*SO = sorbitan monooleate and Chol = cholesterol

6. Determination of entrapment efficiency

The niosomes suspension was centrifuged at 50000 rpm at 4°C for 30 minutes. The vesicle was then precipitated and the supernatant was clear with yellow color. The supernatant was further completely removed by pipetting. The yellow precipitate was lysed with methanol. The amount of curcumin, desmethoxycurcumin and bisdesmethoxycurcumin in the supernatant and in the vesicle (precipitate) were determined, and the amounts found were considered as the un-entrapped and entrapped curcuminoids, respectively. The %entrapment efficiency was calculated as the percentage of the ratio between the entrapped curcuminoids and total curcuminoids added in the formulation. The %recovery of curcuminoids was calculated as the ratio of sum of unentrapped and entrapped curcuminoids against total curcuminoids loaded in the formulation.

7. Determination of particle size and particle size distribution

The curcuminoids niosomes suspension was subjected to determine on particle size and particle size distribution using Mastersizer S long bed version 2.11. Triplicate measurement was performed and the mean particle size was calculated. The particle distribution was examined by plotting the number of particles against particle size within a range of 0.05-878.7.

8. *In vitro* permeation study

The most useful apparatus for drug permeation studies was Franz diffusion cell. *In vitro* permeation measurement was made with two chambers, the donor and receptor chambers. The dorsal region of the skin was faced to the donor chamber and curcuminoids niosomes suspension (0.8 mg of curcuminoids/ml, 2.0 ml) was applied on this region. The diameter of the Franz cell was 1.6 cm corresponding to an effectively permeable area of 2.01 cm^2. The receptor chambers contained 10 ml of 50% methanol as the receptor fluid. The receptor compartment was equipped with a magnetic stirring bar and the temperature was kept at 32°C by circulating water through a jacket surrounding the cell body throughout the experiment. Four sets of the niosomes were prepared for permeation studies. Curcuminoids solution in methanol (0.8 mg/ml, 2.0ml) was used as control. Two milliliters of receptor fluid was collected at 48, 60 and 72 hours. The fresh fluid was replaced after each collection. The collected solutions were subjected to HPLC analysis of the amount of curcumin, desmethoxycurcumin and bisdesmethoxycurcumin. The standard curcuminoids solution for HPLC analysis was prepared at 2 µg/ml. The cumulative amount of curcuminoids and the flux of curcuminoids from curcuminoids niosomes in the receptor compartment were calculated in µg and µg/hr/cm^2, respectively.

CHAPTER IV

RESULTS AND DISCUSSION

1. Analysis of the amount of curcuminoids using HPLC

1.1 Assay validation

The %recovery of each concentration of curcumin, desmethoxycurcumin and bisdesmethoxycurcumin in the sample solutions for validation were shown in Table 4. The accuracy of triplicated determination across six concentrations gave the mean %recoveries of 97.18-100.93, 97.93-100.45 and 98.07-100.42 for curcumin, desmethoxycurcumin and bisdesmethoxycurcumin, respectively (Table 4). The intra-day precision of assay for quantification of curcumin, desmethoxycurcumin and bisdesmethoxycurcumin gave coefficient of variation (%CV) of 0.12-0.88, 0.11-0.45 and 0.10-1.00%, respectively (Table 4). The precision of six determinations of curcumin. desmethoxycurcumin and bisdesmethoxycurcumin at 63 µg/ml, 16 µg/ml and 2 µg/ml provided mean %recoveries and %CVs of 100.25 (0.42), 100.27 (0.26) and 99.88 (0.76), respectively (Table 5). The obtained %recoveries (between 97-101) and %CVs (<1) indicated satisfactory accuracy and precision. The linearity curves were constructed by plotting the theoretical concentration and the measured concentrations. The curves were linear, ranging from 15.86-95.18, 4.04-24.22 and 0.51-3.09 µg/ml for curcumin, desmethoxycurcumin and bisdesmethoxycurcumin, respectively. The regression analysis parameters of the calibration curves were summarized in Table 6. The high coefficients of determination (r^2) indicated high degree of correlations. The LODs of curcumin, desmethoxycurcumin and bisdesmethoxycurcumin were 3.36, 0.90 and 0.25 µg/ml, respectively (See details in Table 20, Appendix B). The LOQs of curcumin, desmethoxycurcumin and bisdesmethoxycurcumin were 10.18, 2.73 and 0.75 µg/ml, respectively (See details in Table 20, Appendix B). The method was also proved to be specific without interference from the constituents in the preparation (See details in Figure 6-9, Appendix B). The validation results demonstrated good accuracy, precision, linearity, range and specificity of the method.

Table 4. Accuracy (%recovery) and intra-day precision (%CV) of assay for quantification of curcumin, desmethoxycurcumin and bisdesmethoxycurcumin (*n* = 3) (See details in Tables 11-13, Appendix B)

Curcuminoids	Theoretical Conc. (µg/ml)	Measured Conc. (µg/ml)	Recovery (%)	CV (%)
Curcumin	15.86	15.87	100.04	0.88
	31.73	32.02	100.93	0.34
	47.59	47.80	100.44	0.36
	63.46	63.70	100.39	0.39
	79.32	77.71	97.97	0.12
	95.18	93.47	97.18	0.46
Desmethoxycurcumin	4.04	4.02	99.72	0.29
	8.07	8.06	99.83	0.11
	12.11	11.94	98.58	0.37
	16.14	16.22	100.45	0.13
	20.18	19.76	97.93	0.18
	24.22	23.82	98.35	0.45
Bisdesmethoxycurcumin	0.51	0.50	98.07	1.00
	1.03	1.02	98.65	0.81
	1.54	1.52	98.72	0.76
	2.06	2.05	99.65	0.96
	2.57	2.58	100.42	0.10
	3.09	3.08	99.67	0.33

Table 5. Precision (%CV) of assay for quantification of curcumin, desmethoxycurcumin and bisdesmethoxycurcumin (n = 6) (See details in Tables 14-16, Appendix B)

Curcuminoids	Theoretical Conc. (µg/ml)	Measured Conc. (µg/ml)	Recovery (%)	CV (%)
Curcumin	63.46	63.94	100.77	0.42
	63.46	63.72	100.42	
	63.46	63.45	99.99	
	63.39	63.16	99.64	
	63.39	63.77	100.60	
	63.39	63.43	100.06	
Desmethoxycurcumin	16.14	16.21	100.40	0.26
	16.14	16.20	100.35	
	16.14	16.25	100.60	
	16.13	16.11	99.88	
	16.13	16.18	100.35	
	16.13	16.14	100.06	
Bisdesmethoxycurcumin	2.06	2.07	100.75	0.76
	2.06	2.04	99.09	
	2.06	2.04	99.12	
	2.06	2.05	99.75	
	2.06	2.0	100.80	
	2.06	2.05	99.75	

Table 6. Linear regression analysis parameters for quantification of curcumin, desmethoxycurcumin and bisdesmethoxycurcumin (See details in Tables 17-19 and Figures 10-12, Appendix B)

Curcuminoids	Theoretical Concentration range (μg/ml)	Intra-day		
		slope	y-intercept	r^2
Curcumin	15.86-95.18	0.9744	0.9975	0.9996
Desmethoxycurcumin	4.04-24.22	0.9794	0.1340	0.9996
Bisdesmethoxycurcumin	0.51-3.09	1.0047	0.0169	0.9999

2. Effect of non-ionic surfactant on preparation of curcuminoids niosomes

Many non-ionic surfactants formed vesicle with difference in toxicity. Since the ester bond in the alkyl ester surfactant can be hydrolyzed in the body, this kind of surfactant has less toxicity than alkyl ether surfactants. Moreover, the sorbitan ester surfactants have already been established as pharmaceutical excipients. Sorbitan monoesters were therefore used as non-ionic surfactants in this study. Three different non-ionic surfactants used in the preparation of niosomes were sorbitan monopalmitate (SP, Span 40), sorbitan monostearate (SS, Span 60) and sorbitan monooleate (SO, Span 80). A parameter like the HLB has been used as an indicator of the vesicle forming ability of any surfactants. The HLB number between 4 and 8 was found to be compatible with vesicle formation (Yoshioka *et al*, 1994 and Uchegbu, 1995). Therefore, SP, SS and SO with the HLB numbers of 4.7, 6.7 and 4.3, respectively, were chosen. The details of each sorbitan monoester used in this study were summarized in appendix A).

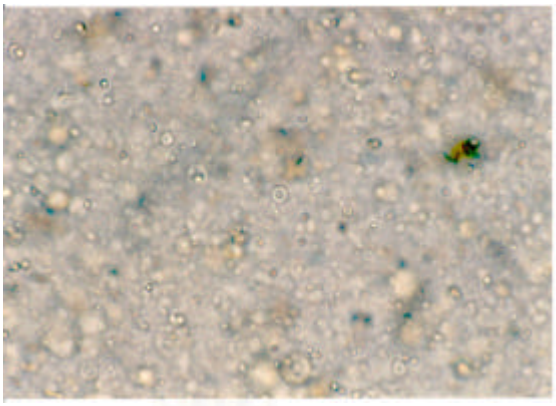
Uchegbu and Vyas (1998) suggested that the addition of an optimum amount of cholesterol in the formulation increased the stability of bilayer. The molar ratio of cholesterol and surfactant was also recommended to be 1:1. The level of surfactant/lipid used to make niosomal dispersions is generally 10-30 mM (Uchegbu and Vyas, 1998). In addition, it was found that sorbitan monosterate containing

solulan C-24 more than 10% by mole was found to be haemotoxic (Uchegbu and Vyas, 1998).

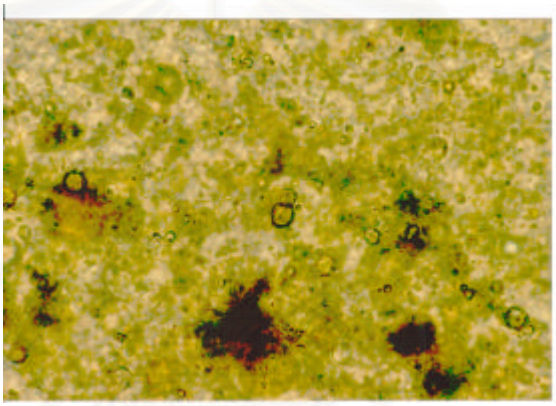
According the studies described above, three formulations with three different surfactants, which are SP, SS and SO, were investigated. The mole ratio of non-ionic surfactant, and cholesterol was set at 1:1 and solulan C-24 was added at 5% by mole to avoid toxicities. Consequently, the mole ratio of non-ionic surfactant, cholesterol and solulan C-24 was kept constant at 47.5: 47.5: 5. Niosomes was prepared at 150 μ mol of the total amount of surfactant, cholesterol and solulan C-24, and purified water of 5.0 ml was used to form the vesicles. The total concentration of niosomes ingredients was about 30 mM. The amount of curcuminoids loaded in the formulation was 1 mg (2.82μmol).

It was found that obtained niosomes from the three formulations could be formed. Since curcuminoids are hydrophobic molecules, they could be entrapped mainly in the hydrophobic region of niosomes membranes. The formation of vesicle was proved by the use of an optical microscope (Figure 4) and scanning electron microscope (Figure 5). Figure 4 showed that prepared curcuminoids niosomes using non-ionic surfactants (SP, SS, and SO) provided spherical vesicles with lamellae. The vesicles were found to be varied in size. Not only the spherical shape but also tubular shape was formed in the preparation. The bigger in size of spherical shape, the more layers of membrane was generated in niosomes vesicles.

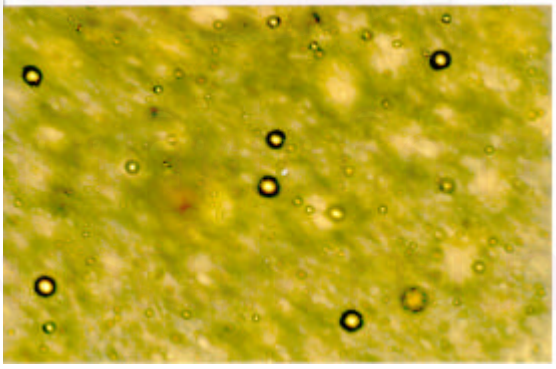
Figure 5 showed that curcuminoids niosomes were spherical shape with smooth surface. The size of the vesicles was varied, which was consistent with the results demonstrated in Figure 4. It was noticed that aggregation of formulation ingredients was occurred when SP and SS. However, this phenomena was not found with formulation using SO. In Figure 5C, the number of vesicles formed by SO was higher than those formed by SP and SS. In addition, the vesicles formed by SO was round-shaped. According to these results, SO would be used as non-ionic surfactant in niosomes preparation for further studies.



(A)



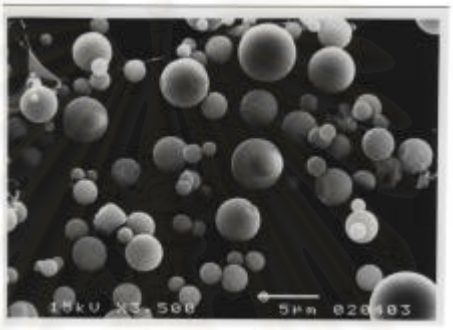
(B)



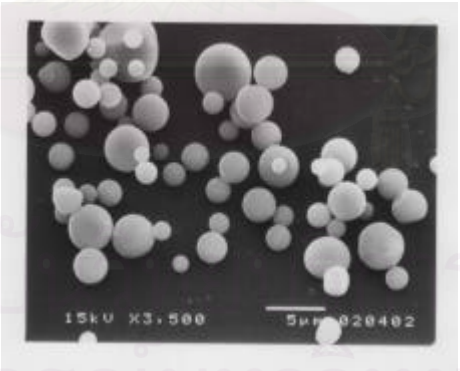
(C)

Figure 4. Photographs under optical microscope of curcuminods niosomes using different non-ionic surfactant: cholesterol: solulan C-24 (47.5: 47.5: 5); (A) SS (B) SP and (C) SO

(A)



(B)



(C)

Figure 5. Photographs under scanning electron microscope of curcuminods niosomes using different non-ionic surfactant: cholesterol: solulan C-24 (47.5: 47.5: 5); (A) SS (B) SP and (C) SO

3. Effect of curcuminoids concentration on preparation of curcuminoids niosomes

According to the morphology study of niosomes vesicles as described above, it was shown that the use of SO in the formulation together with cholesterol and solulan C-24 exhibited satisfactory formulation. The mole ratio of SO, cholesterol and solulan C-24 of such formulation was 47.5: 47.5: 5 with the loaded-curcuminoids of 1 mg (2.82 µmol) in 150 µmol of niosomes. It is therefore interested in determination of the maximum amount of curcuminoids that can be loaded in the formulation.

The experiment was set by varying the amount of curcuminoids (2-12 mg or 5.64 µmol-33.84 µmol) added in niosomes suspensions (150 µmol). The results showed that curcuminoids added at 6 mg, 8 mg, 10 mg and 12 mg in niosomes preparation caused the precipitation of curcuminoids. This implied that are excess amount of curcuminoids could not be entrapped in the niosomes preparation. As mentioned earlier, it was likely that curcuminoids were entrapped in the vesicle membrane in stead of the cavity of the vesicle due to the hydrophobicity of the molecules. Therefore, it was possible that the volume of vesicle membranes limited the amount of curcuminoids that could be loaded in the niosomes.

The precipitation of curcuminoids in the niosomes formulation with loaded-curcuminoids of 2 and 4 mg was not observed. The formulation was determined on the amounts of curcumin, desmethoxycurcumin and bisdesmethoxycurcumin in the vesicles (entrapped curcuminoids) and in the supernatant (unentrapped curcuminoids). In addition, the %entrapment efficiency and the %recovery were calculated. The results were demonstrated in Table 7. It was clearly shown that the amounts of curcumin, desmethoxycurcumin and bisdesmethoxycurcumin added in niosomes at 4 mg provided higher value of %entrapment efficiency, compared with 2 mg loading. The results were consistent with previously reported by Yoshioka *et al.* (1994), suggesting that the increment of adding drug in niosomes suspensions resulted in more vesicle entrapment. It was also noticed that the order of entrapment efficiency was curcumin > desmethoxycurcumin > bisdesmethoxycurcumin. This result correlated with the order of hydrophobicity of the molecules, which is

curcumin > desmethoxycurcumin > bisdesmethoxycurcumin. The correlation supported that the entrapment of the molecules was occurred in the membrane of the vesicles. The niosomes preparation provided high %recovery of total curcuminoids (>80%), suggesting that the method was suitable for preparing niosomes containing curcuminoids. It was also shown that curcuminoids loaded at 4 mg gave slightly higher %recovery (about 10% higher) than 2 mg loading. This may due to the loss of curcuminoids during the transfer of niosomes vesicles for the curcuminoids analysis, which affected more on the formulation with less curcuminiods loading (2 mg).

Table 7. Determination of the amount of entrapped curcuminoids, unentrapped curcuminoids, % entrapment efficiency and % recovery of niosomes formulation loaded with 2 and 4 mg curcuminoids (See details in Tables 21-24, Appendix C)

	Amount of curcuminoids (mg)				% Entrapment efficiency	% Recovery
	Loaded*	Unentrapped	Entrapped	Total**		
Curcumin						
	1.58	0.82	0.48	1.30	30.38	82.28
	3.17	0.15	2.84	2.99	89.59	94.32
Desmethoxycurcumin						
	0.40	0.20	0.10	0.30	25.00	75.00
	0.81	0.16	0.55	0.71	67.90	87.65
Bisdesmethoxycurcumin						
	0.05	0.02	0.01	0.03	20.00	60.00
	0.10	0.04	0.03	0.07	30.00	70.00
Total Curcuminoids						
	2.04	1.05	0.58	1.63	28.43	79.90
	4.08	0.35	3.42	3.77	83.82	92.40

*%purity of curcumin, desmethoxycurcumin and bisdesmethoxycurcumin were 79.24, 20.16 and 2.57, respectively

**Total = the sum of unentrapped and entrapped

4. Characterization of optimized curcuminoids niosomes formulation

According to the above results, the optimized niosomes preparations were developed as the following formulation. The niosomes consisted of SO, cholesterol and solulan C-24 at the mole ratio of 47.5: 47.5: 5. The niosomes formulation was loaded with curcuminoids at 4 mg. The optimized formulation was prepared and subsequently characterized on the particle size and particle size distribution including the *in vitro* permeation study, which were described below.

4.1 Determination of particle size and particle size distribution

The mean of particle size of curcuminoids niosomes prepared by SO: cholesterol: solulan C-24 (47.5: 47.5: 5) loaded with curcuminoids (4 mg) was approximately 20 µm. The prepared niosomes with this size could be considered as multilamellar vesicle type. It was known that the size of the vesicles prepared by mechanical shaking is dependent on the hydrophile-lipophile balance (HLB) of the surfactants; the lower the HLB the smaller the size of the vesicles (Yoshioka *et al*, 1994). The HLB of SO is 4.3 which should provided small size of vesicle. However, the particle size of the prepared niosomes was quite large. It was previously suggested that a further reduction of the vesicle size could be achieved by sonication (Sentjurc *et al*., 1999). However, in this preparation of niosomes, the hand-shaking method was used without sonication causing the size of the vesicles larger than 10 µ m. It is therefore recommended that the sonication of the niosomes should be done if the smaller size is preferred. In addition, according to the particle size distribution as shown in Table 8, about 74% of the numbers of particles possessed less than 25 µm diameter with slightly widely distributed in size. Yoshioka *et al*. (1994) suggested that the size distribution of vesicles trended to be fairly wide depended on the hydration time and degree of shaking. However, the sonication of niosomes vesicles can also lower the particle size distribution as well as the size reduction as mentioned earlier.

Table 8. The particle size distribution of curcuminoids niosomes (See details in Figure 13 and Table 25, Appendix D)

Particle size (μm)	% of particles
0.00 - 5.00	30.45
5.01 - 25.00	43.53
25.01-50.00	18.44
50.01-75.00	4.25
75.01-100.00	3.33

4.2 Permeation study of curcuminoids niosomes

Typically, niosomes prepared by SO: cholesterol: solulan C-24 (47.5:47.5:5) with curcuminoids (4 mg) was investigated on the permeation study. The permeation study was performed in four replications. The receptor fluid was collected at 48, 60 and 72 hour. The cumulative amount of curcuminoids found in the receptor compartment was investigated. The permeation of curcuminoids niosomes was compared with curcuminoids solution. The permeation results of curcuminoids niosomes was shown in Table 9 and Table 10. Table 9 demonstrated the cumulative amounts of curcumin, desmethoxycurcumin and bisdesmethoxycurcumin including total curcuminoids in the receptor compartment. In Table 10, the flux of curcumin, desmethoxycurcumin and bisdesmethoxycurcumin including total curcuminoids passing through the skin were shown. For the permeation study of curcuminoids solution, it was found that curcuminoids could not be detected in the receptor compartment. However, curcuminoids in niosome formulation could penetrate the skin and approximately 60 μg of the total amount of curcuminoids at 72 hours was detected in the receptor fluid. The flux of total curcuminoids from niosomes was found to be about 0.41 μg/hr/cm^2 at 72 hr. Therefore, it could be concluded that the niosomes formulation significantly enhanced the permeation of curcuminoids through the skin compared to the curcuminoids solution. However, since the total amount of curcuminoids loaded onto the skin was approximately 1600 μg, the %permeation was calculated to be only 4%. It might be due to the hydrophobicity of

curcuminoids, causing in the high entrapment of curcuminoids in lipid barrier of shed snake skin.

Table 9. The cumulative amount of curcuminoids from niosomes prepared by SO: cholesterol: solulan C-24 (47.5: 47.5: 5) (*n*=4) (See details in Tables 26-28, Appendix E)

Samples*	Cumulative amount of curcuminoids in receptor compartment (µg)		
	48Hr	60Hr	72Hr
C	20.03	27.90	46.84
D	4.24	8.49	10.57
B	0.57	1.44	1.95
T	24.84	37.83	59.36

*C = curcumin; D = desmethoxycurcumin; B = bisdesmethoxycurcumin
T = total amount of curcuminoids

Table 10. The flux of curcuminoids through snake skin from niosomes prepared by SO: cholesterol: solulan C-24 (47.5: 47.5: 5) (*n*=4)

Samples*	Flux (µg/hr/cm^2)		
	48Hr	60Hr	72Hr
C	0.21	0.23	0.32
D	0.04	0.07	0.07
B	0.01	0.01	0.01
T	0.26	0.31	0.41

*C = curcumin; D = desmethoxycurcumin; B = bisdesmethoxycurcumin
T = total amount of curcuminoids

CHAPTER V

CONCLUSIONS

In this study, the optimized niosomes preparation was developed to entrap curcuminoids. The niosomes formulation was composed of non-ionic surfactant, cholesterol and solualn C-24 at the mole ratio of 47.5: 47.5: 5. The total niosomes ingredients were prepared at 150 micromoles in 5 ml (30 mM). The hand-shaking method was chosen to prepare the curcuminoids niosomes. Three different non-ionic surfactants used in the preparation of niosomes were sorbitan monopalmitate (SP, Span 40), sorbitan monostearate (SS, Span 60) and sorbitan monooleate (SO, Span 80). It was found that curcuminoids niosomes formed by SO with curcuminoids loading at 1 mg provided high amount of vesicles and round-shaped vesicles. Therefore, SO was selected as the suitable non-ionic surfactant under the condition used.

The niosomes formed by SO was subsequently investigated for the appropriate amount of curcuminoids that could be loaded in the preparation. It was found that such niosomes could be loaded with curcuminoids up to 4 mg, providing the %entrapment efficiency of curcuminoids greater than 90%.

The niosomes formulation with SO and 4 mg loading of curcuminoids was prepared and characterized on the mean particle size and particle distribution. The mean particle size of approximately 20 μm diameter was found using the laser diffraction techniques. The permeation of curcuminoids from curcuminoids niosomes was also determined using of cobra skin as a model membrane in Franz diffusion cell in comparison with curcuminoids solution. It was found that curcuminoids niosomes demonstrated high penetration enhancement activity, compared with curcuminoids solution. Therefore, it can be concluded that the curcuminoids developed in this study was successful in enhancing the shin permeation of curcuminoids.

This thesis research would provide suitable preparations for curcuminoids niosomes. The obtained curcuminoids niosomes preparations could be served as raw materials in the formulation of topical dosage forms to enhance the skin permeation of curcuminoids. The developed niosomes formulation might be of benefit for developing niosomes containing other drugs that possess similar physicochemical properties to curcuminoids.

REFERENCES

Baillie, A.J., Florence, A.T., Hume, L.R., Muirhead, G.T., Rogerson, A. 1985. The preparation and properties of niosomes-non-ionic surfactant vesicles. J. Pharm. 37:863-868.

Bidlack, W.R., Omaye, S.T., Meskin, M.S., Jahner, D. 1998. Phytochemicals: A new paradigm. Technomic. USA.

Fang, J.Y., Yu, S.Y., Wu, P.C., Huang, Y.B., Tsai, Y.H. 2001. In vitro skin permeation of estradiol from various proniosome formulations. Int. J. of Pharma. 215:91-99.

Florence, A.T. 1993. New drug delivery systems. Chemistry and Industry. Dec, 20:1000-1004.

Haigh, J.M., Beyssac, E., Chanet, L., Aiache, J.M. 1998. In vitro permeation of progesterone from a gel through the shed skin of three different snake species. Int. of Pharma. 170:151-156.

Hoftland, H.E.J., Junger, H.E. and Bouwstra, J.A. 1994. Estradiol permeation from nonionic surfactant veicles through human skin *in-vitro*. Pharm. Res. 11(5): 659-654.

Honeywell-Nguyen, P.L., Graff, A.M., Groenink, H.W.W. and Bouwstra, J.A. 2002. The *in vivo* and *in vitro* interactions of elastic and rigid vesicles with human skin. Biochimica et Biophysica Acta. 1573:130-140.

Itoh, M., Xia, J., Magavi, R., Nishihata, T., Rytting, J.H. 1990. Use of shed snake skin as a model membrane for *in vitro* percutaneous penetration studies: comparison with human skin. Pharm. Res. 7, 10:1042-1047.

Komatsu, H., Okamoto, H., Miyagawa, K., Higgaki, K., Hashida, M., Sezaki, H. 1986. Preservative activity and *in vivo* percutaneous penetration of butyl paraben entrapped in liposomes. Chem. Pharm. Bull. 34: 3415-3422.

Lasic, D.D. 1997. Liposome. Liposome in gene delivery. 67-112.

Li, L. et al. 1996. *Curcuma longa* L. tuber yield simulation model and its application under combined agronomic measures for good-quality, high-yield and obvious economic results. Chung Kuo Chung Yao Tsa Chih. 21(9):527-9,574.

Martin, A.N., Swarbrick, J., Cammarata, A. 1969. Micrometric. In physical Pharmacy. 467-496. (n.p.): Lea&Febiger.

Mezei, M. 1994. Liposomes as penetration promoters and localizers of topically pplied drugs. Drug permeation enhancement. 171-198. (n.p.): Marcel Dekker.

Ponjanyakul, T., Prakongpan, S. and Priprem, A. 2000. Permeation studies comparing cobra skin with human skin using nicotine transdermal patches. Drug Dev. Ind.Pharm. 26(6): 635-642.

Reddy, A.C.P., Lokesh, B.R. 1992. Studies on spice principles as antioxidants in the inhibition of lipid peroxidation of rat liver mcrosomes. Mol.Cell. Biochem. 111, 117-124.

Schreier, H., Bouwstra, J. 1994. Review liposomes and niosomes as topical drug carriers: dermal and tansdermal drug delivery. J. of controlled release. 30:1-15.

Sentjurc, M., Vrhovnik, K., Kristl, J. 1999. Liposomes as a topical delivery system: the role of size on transport studies by the EPR imaging method. J. of controlled release. 59:87-97.

Sharma, R.A., McLelland, H.R., Hill, K.A., Ireson, C.R., Euden, S.A., Manson, M.M., Pirmohamed, M., Marnett, L.J., Gescher, A.J., Steward, W.P. 2001. Pharmacodynamic and pharmacokinetic study of oral *Curcuma* extract in patients with colorectal cancer. Clin. Cancer Res. 7: 1984-1900.

Simonnet, J.T. 1994. Lipid vesicles technology and advances. Cosmetic. July, 109:45-52.

Srimal, R.C.1997. Turmeric: a brief review of medicinal properties. Fitoterapia. 68, 483-493.

Thai Herbal Pharmacopoeia. Volume I, 1995.pp. 3844, 84.

Toennesen, H.H. et al. 1995. Characterization of the spectroscopic properties of the naturally occurring curcuminoids and selected derivatives. Pharmazie. 50 (10):689-93.

Toennesen, H.H, Grinberg, L.N., Shalev, O., Rachmilewitz, E.A. 2002. Studies of curcumin and curcuminoids: Cyclodextrin complexation: solubility, chemical and photochemical stability. Int. J. Pharm. 244: 127-135.

Uchegbu, I.F. and Florence, A.T. 1994. Non-ionic surfactant vesicles (niosomes) physical and pharmaceutical chemistry. Adv. Colloid Interface Sci. 58:1-55.

Uchegbu, I.F. and Vyas, S.P. 1998. Non-ionic surfactant vesicles (niosomes) in drug delivery. Int. J.Pharm. 172:33-70.

Uchegbu, I.F., Double, J.A., Turton, J.A. and Florence, A.T. 1995. Distribution, metabolism and tumoricidal activity of doxorubicin administered in sorbitan monostearate (Span 60) niosomes in the mouse. Pharm. Res. 12:1019-1024.

Wang, Y.J., Pan, M.H., Cheng, A.L., Lin, L.I., H, Y.S., Hsieh, C.Y., Lin, J.K. 1997. Stability of curcumin in buffer solutions and characterization of its degradation products. J. Pharm. Biomed. Anal. 15:1867-1876.

Yoshioka, T. and Florence, A.T.1994. Vesicles (niosome)-in-water-in-oil (v/w/o) emulsion-an *in vitro* study. Int. J. Pharm. 108:117-123.

APPENDICES

APPENDIX A

The data of non-ionic surfactants used in niosomes preparation

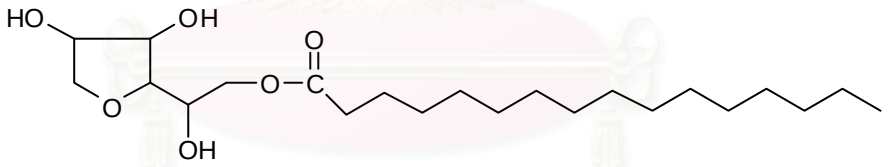
Span 40

Sorbitan monopalmitate (SP)

Sorbitan, ester, monohexadecanoate

Empirical formula	Molecular weight
$C_{22}H_{42}O_6$	403
HLB	6.7
Melting point range	44-47^{O}C
Functional category	wetting and/or solubilizing agent
	Emulsifying and/or solubilizing agent

Structure formula



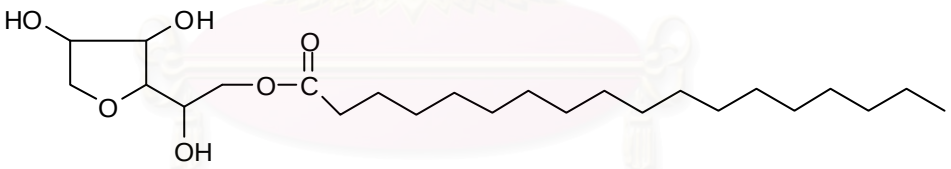
Span 60

Sorbitan monostearate (SS)

Sorbitan, ester, mono-octadecanoate

Empirical formula	Molecular weight
$C_{24}H_{46}O_6$	431
HLB	4.7
Melting point range	50-53^{O}C
Functional category	wetting and/or solubilizing agent
	Emulsifying and/or solubilizing agent

Structure formula



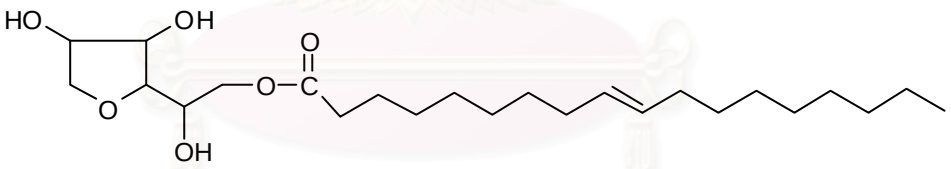
Span 80

Sorbitan monooleate (SO)

Sorbitan, ester, mono-9-octadecanoate

Empirical formula	Molecular weight
$C_{24}H_{44}O_6$	429
HLB	4.3
Melting point range	liquid
Functional category	wetting and/or solubilizing agent
	Emulsifying and/or solubilizing agent

Structure formula



APPENDIX B

The chromatograms and statistical data from analysis of the amount of curcuminoids using HPLC

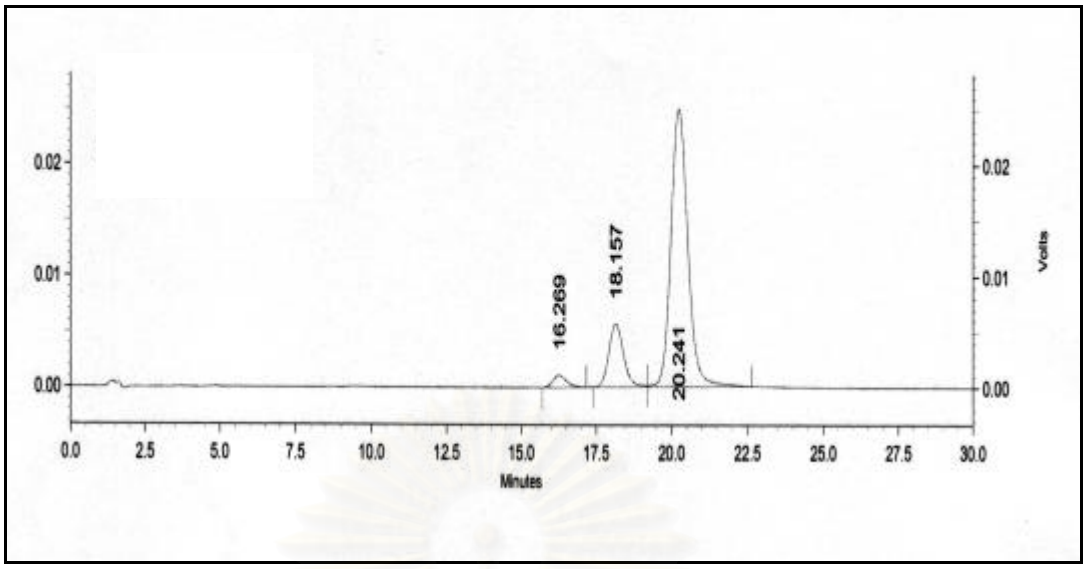


Figure 6. The chromatogram of curcumin, desmethoxycurcumin and bisdesmethoxycurcumin of curcuminoids extract in standard solution

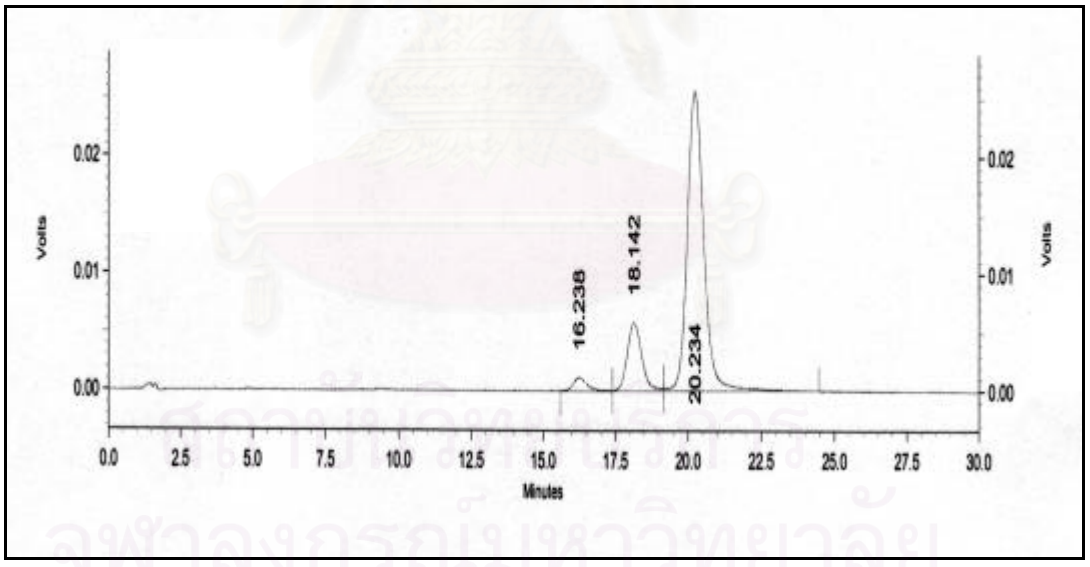


Figure 7. The chromatogram of curcumin, desmethoxycurcumin and bisdesmethoxycurcumin of curcuminoids extract in the pellet

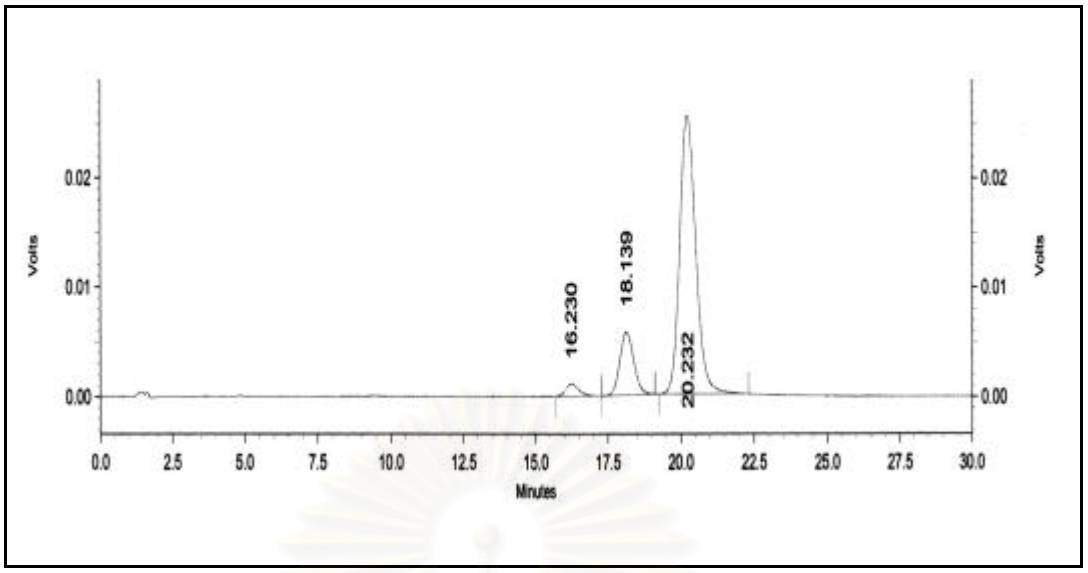


Figure 8. The chromatogram of curcumin, desmethoxycurcumin and bisdesmethoxycurcumin of unentrapped curcuminoids

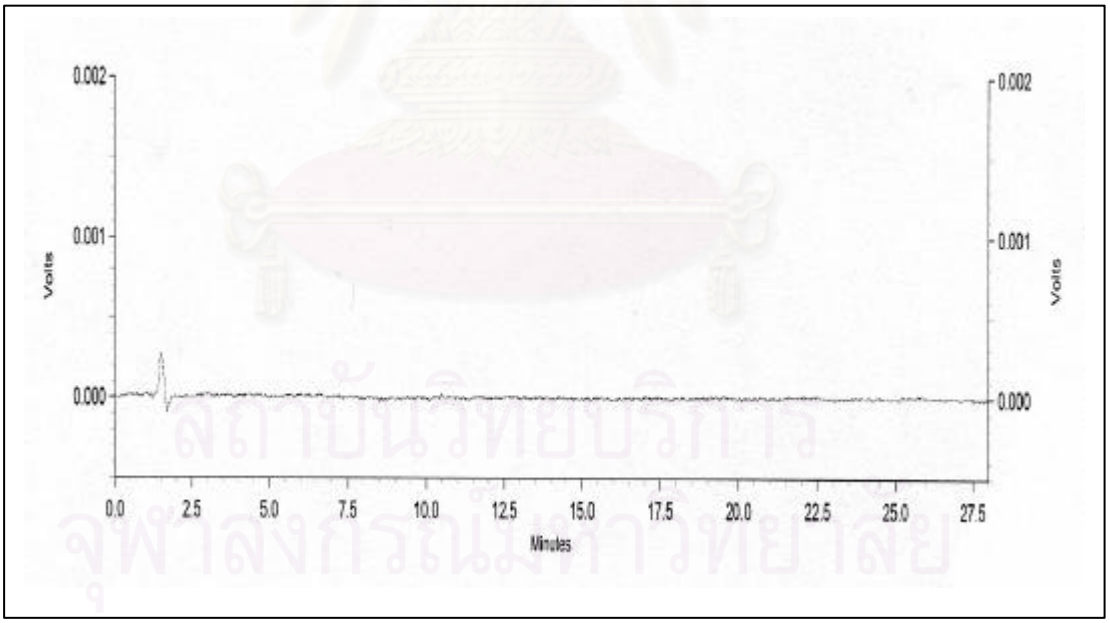


Figure 9. The chromatogram of niosomes placebo solution

Table 11. Accuracy data of curcumin assayed by the HPLC method

Samples	Theoretical Conc. (µg/ml)	Measured Conc. (µg/ml)	%Recovery	Average (Range)	%CV
1A	15.8638	15.8241	99.75		
1B	15.8638	15.7595	99.34	100.04	0.88
1C	15.8638	16.0262	101.02	(99.34-101.02)	
2A	31.7277	31.9247	100.62		
2B	31.7277	32.0019	100.86	100.93	0.34
2C	31.7277	32.1389	101.30	(100.62-101.30)	
3A	47.5915	47.9720	100.80		
3B	47.5915	47.8033	100.45	100.44	0.36
3C	47.5915	47.6265	100.07	(100.07-100.80)	
4A	63.4554	63.9446	100.77		
4B	63.4554	63.7194	100.42	100.39	0.39
4C	63.4554	63.4475	99.99	(99.99-100.77)	
5A	79.3192	77.6186	97.86		
5B	79.3192	77.8118	98.10	97.97	0.12
5C	79.3192	77.7036	97.96	(97.86-98.10)	
6A	96.1831	93.9035	97.63		
6B	96.1831	93.4767	97.19	97.18	0.46
6C	96.1831	93.0442	96.74	(96.74-97.63)	

Table 12. Accuracy data of desmethoxycurcumin assayed by the HPLC method

Samples	Theoretical Conc. (µg/ml)	Measured Conc. (µg/ml)	%Recovery	Average (Range)	%CV
1A	4.0360	4.0383	100.06		
1B	4.0360	4.0181	99.56	99.72	0.29
1C	4.0360	4.0178	99.55	(99.55-100.06)	
2A	8.0721	8.0643	99.90		
2B	8.0721	8.0482	99.70	99.83	0.11
2C	8.0721	8.0616	99.87	(99.70-99.90)	
3A	12.1081	11.9459	98.66		
3B	12.1081	11.9746	98.90	98.58	0.37
3C	12.1081	11.8882	98.18	(98.18-98.90)	
4A	16.1441	16.2089	100.40		
4B	16.1441	16.2003	100.35	100.45	0.13
4C	16.1441	16.2409	100.60	(100.35-100.60)	
5A	20.1802	19.7518	97.88		
5B	20.1802	19.7336	97.79	97.93	0.18
5C	20.1802	19.8006	98.12	(97.79-98.12)	
6A	24.2162	23.9299	98.82		
6B	24.2162	23.8009	98.29	98.35	0.45
6C	24.2162	23.7173	97.94	(97.94-98.82)	

Table 13. Accuracy data of bisdesmethoxycurcumin assayed by the HPLC method

Samples	Theoretical Conc. (µg/ml)	Measured Conc. (µg/ml)	%Recovery	Average (Range)	%CV
1A	0.5145	0.5066	98.46		
1B	0.5145	0.4988	96.95	98.07	1.00
1C	0.5145	0.5083	98.79	(96.95-98.79)	
2A	1.0290	1.0167	98.80		
2B	1.0290	1.0061	97.77	98.65	0.81
2C	1.0290	1.0224	99.36	(97.77-99.36)	
3A	1.5435	1.5183	98.37		
3B	1.5435	1.5371	99.59	98.72	0.76
3C	1.5435	1.5159	98.21	(98.21-99.59)	
4A	2.0581	2.0736	100.75		
4B	2.0581	2.0393	99.09	99.65	0.96
4C	2.0581	2.0400	99.12	(99.09-100.75)	
5A	2.5726	2.5826	100.39		
5B	2.5726	2.5813	100.34	100.42	0.10
5C	2.5726	2.5864	100.54	(100.34-100.54)	
6A	3.0871	3.0845	99.92		
6B	3.0871	3.0810	99.80	99.67	0.33
6C	3.0871	3.0652	99.29	(99.29-99.92)	

Table 14. Precision data of curcumin assayed by the HPLC method

Samples	Theoretical conc. (µg/ml)	Measured conc. (µg/ml)	%Recovery	Average (Range)	%CV
4A	63.4554	63.9446	100.77		
4B	63.4554	63.7194	100.42		
4C	63.4554	63.4475	99.99	100.25	
4D	63.3920	63.1617	99.64	(99.64-100.77)	0.42
4E	63.3920	63.7729	100.60		
4F	63.3920	63.4316	100.06		

Table 15. Precision data of desmethoxycurcumin assayed by the HPLC method

Samples	Theoretical conc. (µg/ml)	Measured conc. (µg/ml)	%Recovery	Average (Range)	%CV
4A	16.1441	16.2089	100.40		
4B	16.1441	16.2003	100.35		
4C	16.1441	16.2409	100.60	100.27	
4D	16.1280	16.1093	99.88	(99.88-100.60)	0.26
4E	16.1280	16.1849	100.35		
4F	16.1280	16.1382	100.06		

Table 16. Precision data of bisdesmethoxycurcumin assayed by the HPLC method

Samples	Theoretical conc. (µg/ml)	Measured conc. (µg/ml)	%Recovery	Average (Range)	%CV
4A	2.0581	2.0736	100.75		
4B	2.0581	2.0393	99.09		
4C	2.0581	2.0400	99.12	99.88	
4D	2.0560	2.0509	99.75	(99.09-100.80)	0.76
4E	2.0560	2.0725	100.80		
4F	2.0560	2.0508	99.75		

Table 17. Linearity data of curcumin assayed by the HPLC method

Samples	Theoretical Conc. (µg/ml)	Average Theoretical Conc. (µg/ml)	Measured Conc. (µg/ml)	Average Measured Conc. (µg/ml)
1A	15.8638		15.8241	
1B	15.8638	15.8638	15.7595	15.8699
1C	15.8638		16.0262	
2A	31.7277		31.9247	
2B	31.7277	31.7277	32.0019	32.0218
2C	31.7277		32.1389	
3A	47.5915		47.9720	
3B	47.5915	47.5915	47.8033	47.8006
3C	47.5915		47.6265	
4A	63.4554		63.9446	
4B	63.4554	63.4554	63.7194	63.7038
4C	63.4554		63.4475	
5A	79.3192		77.6186	
5B	79.3192	79.3192	77.8118	77.7113
5C	79.3192		77.7036	
6A	95.1831		93.9035	
6B	95.1831	95.1831	93.4767	93.4748
6C	95.1831		93.0442	

Table 18. Linearity data of desmethoxycurcumin assayed by the HPLC method

Samples	Theoretical Conc. (µg/ml)	Average Theoretical Conc. (µg/ml)	Measured Conc. (µg/ml)	Average Measured Conc. (µg/ml)
1A	4.0360		4.0383	
1B	4.0360	4.0360	4.0181	4.0247
1C	4.0360		4.0178	
2A	8.0721		8.0643	
2B	8.0721	8.0721	8.0482	8.0580
2C	8.0721		8.0616	
3A	12.1081		11.9459	
3B	12.1081	12.1081	11.9746	11.9362
3C	12.1081		11.8882	
4A	16.1441		16.2089	
4B	16.1441	16.1441	16.2003	16.2167
4C	16.1441		16.2409	
5A	20.1802		19.7518	
5B	20.1802	20.1802	19.7336	19.7620
5C	20.1802		19.8006	
6A	24.2162		23.9299	
6B	24.2162	24.2162	23.8009	23.8160
6C	24.2162		23.7173	

Table 19. Linearity data of bisdesmethoxycurcumin assayed by the HPLC method

Samples	Theoretical Conc. (µg/ml)	Average Theoretical Conc. (µg/ml)	Measured Conc. (µg/ml)	Average Measured Conc. (µg/ml)
1A	0.5145		0.5066	
1B	0.5145	0.5145	0.4988	0.5046
1C	0.5145		0.5083	
2A	1.0290		1.0167	
2B	1.0290	1.0290	1.0061	1.0151
2C	1.0290		1.0224	
3A	1.5435		1.5183	
3B	1.5435	1.5435	1.5371	1.5238
3C	1.5435		1.5159	
4A	2.0581		2.0736	
4B	2.0581	2.0581	2.0393	2.0510
4C	2.0581		2.0400	
5A	2.5726		2.5826	
5B	2.5726	2.5726	2.5813	2.5834
5C	2.5726		2.5864	
6A	3.0871		3.0845	
6B	3.0871	3.0871	3.0810	3.0769
6C	3.0871		3.0652	

Figure 10. The representative linearity curve for curcumin

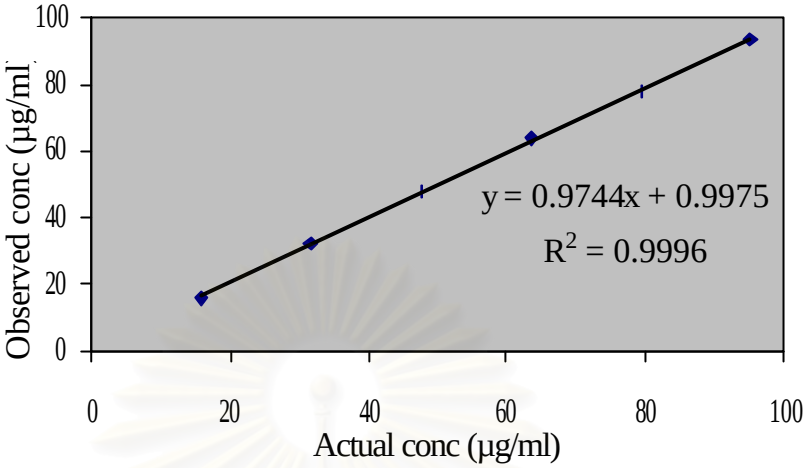


Figure 11. The representative linearity curve for desmethoxycurcumin

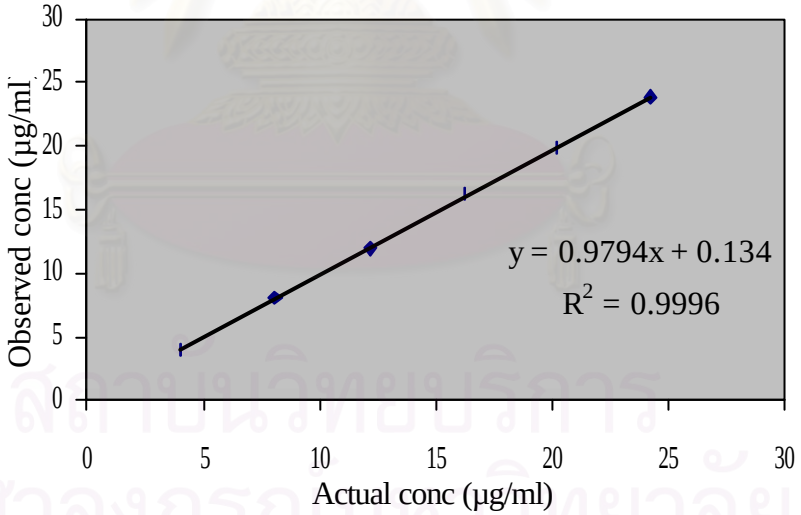


Figure 12. The representative linearity curve for bisdesmethoxycurcumin

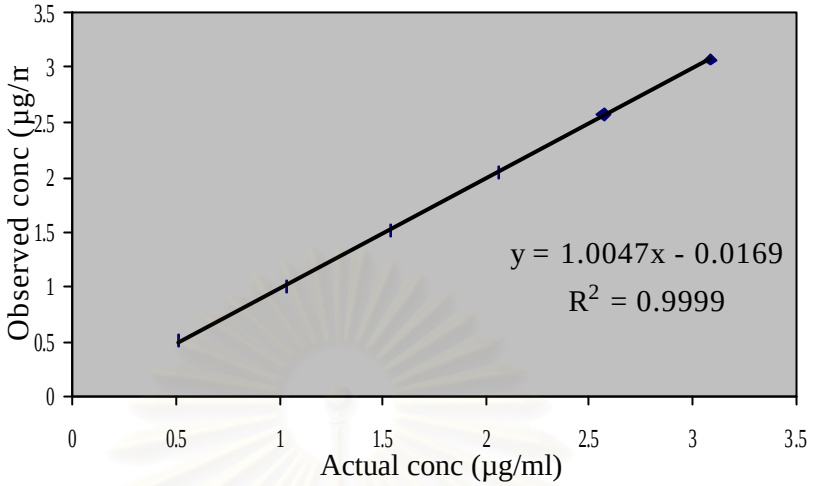


Table 20. LOD and LOQ data of curcuminoids assayed by the HPLC method

Curcuminoids*	Theoretical Conc. (µg/ml)	Peak Area	Regression Parameters*	LOD (µg/ml)	LOQ (µg/ml)
C	15.86	1894594		3.36	10.18
	31.73	3716332	Slope = 118762		
	47.59	5617058	Intercept = 55140		
	63.46	7278738	r^2 = 0.9991		
	79.32	9365887	SD = 120931		
	95.18	11360808			
D	4.04	387892		0.90	2.73
	8.07	789452	Slope = 94163		
	12.1	1135376	Intercept = 5581.3		
	16.14	1486572	r^2 = 0.9990		
	20.18	1906885	SD = 25675		
	24.22	2307658			
B	0.51	67404		0.25	0.75
	1.03	155942	Slope = 157339		
	1.54	222873	Intercept = 15321		
	2.06	291610	r^2 = 0.9952		
	2.57	386546	SD = 11787		
	3.09	482956			

*C= Curcumin, D= Desmethoxycurcumin and B= Bisdesmethoxycurcumin

** SD = Residual standard deviation of regression line

APPENDIX C

The data of effect of curcuminoids concentration on preparation of curcuminoids niosomes

Table 21. The amount of entrapped and unentrapped curcumin in niosomes suspensions prepared by SO, cholesterol and solulan C-24 with different amount of curcuminoids

	Amount of curcumin (mg)				% Entrapment	% Recovery
	Loaded	Unentrapped	Entrapped	Total		
I*	1.58	0.82	0.47	1.29	29.74	81.61
	1.58	0.83	0.47	1.30	29.95	82.02
	1.58	0.82	0.49	1.32	30.95	82.98
Mean	1.58	0.82	0.48	1.30	30.21	82.20
% CV	0.00	0.00	0.01	0.01	0.65	0.70
II**	3.17	0.14	2.83	2.97	89.30	93.82
	3.17	0.15	2.84	2.99	89.61	94.24
	3.17	0.15	2.85	3.00	89.92	94.64
Mean	3.17	0.15	2.84	2.99	89.61	94.23
% CV	0.00	0.00	0.01	0.01	0.31	0.41

*I = niosomes suspension loaded with 2 mg of curcuminoids

**II = niosomes suspension loaded with 4 mg of curcuminoids

Table 22. The amount of entrapped and unentrapped desmethoxycurcumin in niosomes suspensions prepared by SO, cholesterol and solulan C-24 with different amount of curcuminoids

	Amount of desmethoxycurcumin (mg)					
	Loaded	Unentrapped	Entrapped	Total	% Entrapment	% Recovery
I*	0.40	0.20	0.10	0.30	24.31	74.21
	0.40	0.21	0.10	0.30	24.31	75.32
Mean	0.40	0.20	0.10	0.30	24.14	74.52
% CV	0.00	0.00	0.00	0.00	0.29	0.70
II**	0.81	0.16	0.55	0.71	68.51	88.43
	0.81	0.16	0.55	0.72	68.51	88.73
	0.81	0.17	0.55	0.72	68.70	89.41
Mean	0.81	0.16	0.55	0.72	68.58	88.86
% CV	0.00	0.00	0.00	0.00	0.11	0.50

*I = niosomes suspension loaded with 2 mg of curcuminoids

**II = niosomes suspension loaded with 4 mg of curcuminoids

Table 23. The amount of entrapped and unentrapped bisdesmethoxycurcumin in niosomes suspensions prepared by SO, cholesterol and solulan C-24 with different amount of curcuminoids

	Amount of bisdesmethoxycurcumin (mg)					
	Loaded	Unentrapped	Entrapped	Total	% Entrapment	% Recovery
I*	0.05	0.02	0.01	0.03	15.37	58.95
	0.05	0.02	0.01	0.03	15.37	59.73
	0.05	0.02	0.01	0.03	13.81	58.37
Mean	0.05	0.02	0.01	0.03	14.85	59.01
% CV	0.00	0.00	0.00	0.00	0.90	0.68
II**	0.10	0.04	0.03	0.07	27.72	67.51
	0.10	0.04	0.03	0.07	27.82	66.73
	0.10	0.04	0.03	0.07	28.02	68.09
Mean	0.10	0.04	0.03	0.07	27.85	67.44
% CV	0.00	0.00	0.00	0.00	0.15	0.68

*I = niosomes suspension loaded with 2 mg of curcuminoids

**II = niosomes suspension loaded with 4 mg of curcuminoids

Table 24. The amount of entrapped and unentrapped curcuminoids in niosomes suspensions prepared by SO, cholesterol and solulan C-24 with different amount of curcuminoids

	Amount of curcuminoids (mg)					
	Loaded	Unentrapped	Entrapped	Total	% Entrapment	% Recovery
I*	2.04	1.05	0.58	1.62	28.20	79.54
	2.04	1.05	0.58	1.63	28.46	79.92
	2.04	1.05	0.60	1.65	29.20	80.84
Mean	2.04	1.05	0.58	1.63	28.62	80.10
% CV	0.00	0.00	0.01	0.01	0.52	0.67
II**	4.08	0.35	3.41	3.76	83.63	92.09
	4.08	0.35	3.42	3.77	83.88	92.46
	4.08	0.36	3.44	3.80	84.32	93.09
Mean	4.08	0.35	3.42	3.77	83.95	92.55
% CV	0.00	0.01	0.01	0.02	0.35	0.50

*I = niosomes suspension loaded with 2 mg of curcuminoids

**II = niosomes suspension loaded with 4 mg of curcuminoids

APPENDIX D

The data of particle size distribution for curcuminoids niosomes

Figure 13. Particle size distribution of curcuminoids niosomes

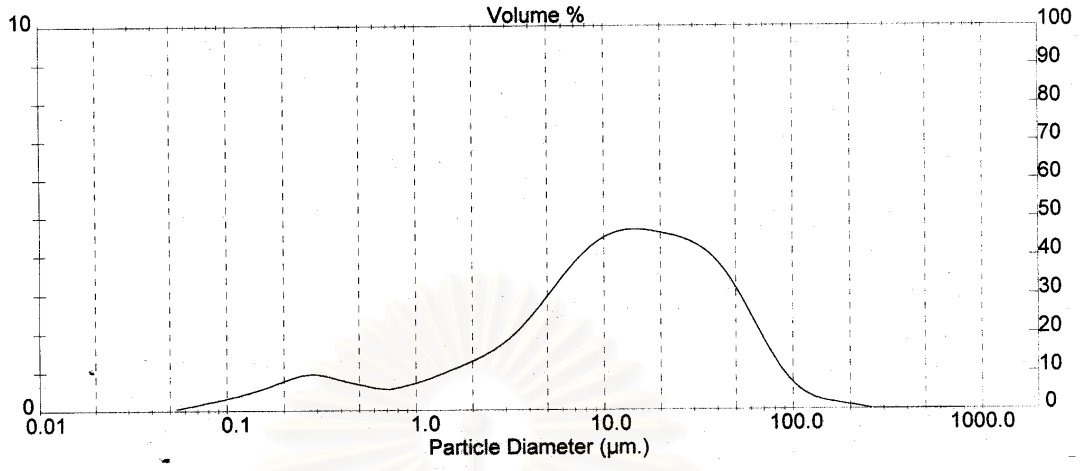


Table 25. The particle size distribution of curcuminoids niosomes

Size_low (mm)	In%	Size_high (mm)	Under%	Size_low (mm)	In%	Size_high (mm)	Under%
0.05	0.06	0.06	0.06	6.63	3.94	7.72	37.94
0.06	0.12	0.07	0.18	7.72	4.25	9.00	42.20
0.07	0.19	0.08	0.37	9.00	4.48	10.48	46.68
0.08	0.26	0.09	0.63	10.48	4.62	12.21	51.30
0.09	0.33	0.11	0.95	12.21	4.67	14.22	55.97
0.11	0.41	0.13	1.36	14.22	4.65	16.57	60.62
0.13	0.50	0.15	1.86	16.57	4.57	19.31	65.19
0.15	0.60	0.17	2.46	19.31	4.46	22.49	69.65
0.17	0.72	0.20	3.18	22.49	4.33	26.20	73.98
0.20	0.84	0.23	4.01	26.20	4.21	30.53	78.19
0.23	0.93	0.27	4.94	30.53	4.04	35.56	82.23
0.27	0.96	0.31	5.90	35.56	3.79	41.43	86.02
0.31	0.90	0.36	6.80	41.43	3.43	48.27	89.46
0.36	0.81	0.42	7.61	48.27	2.96	56.23	92.42
0.42	0.72	0.49	8.33	56.23	2.41	65.51	94.83
0.49	0.65	0.58	8.98	65.51	1.85	76.32	96.67
0.58	0.58	0.67	9.56	76.32	1.34	88.91	98.01
0.67	0.55	0.78	10.11	88.91	0.92	103.58	98.93
0.78	0.62	0.91	10.73	103.58	0.59	120.67	99.52
0.91	0.71	1.06	11.44	120.67	0.33	140.58	99.85
1.06	0.82	1.24	12.25	140.58	0.14	163.77	99.99
1.24	0.95	1.44	13.20	163.77	0.01	190.80	100.00
1.44	1.08	1.68	14.28	190.80	0.00	222.28	100.00
1.68	1.22	1.95	15.51	222.28	0.00	258.95	100.00
1.95	1.37	2.28	16.88	258.95	0.00	301.68	100.00
2.28	1.54	2.65	18.43	301.68	0.00	351.46	100.00
2.65	1.76	3.09	20.18	351.46	0.00	409.45	100.00
3.09	2.03	3.60	22.21	409.45	0.00	477.01	100.00
3.60	2.35	4.19	24.56	477.01	0.00	555.71	100.00
4.19	2.73	4.88	27.30	555.71	0.00	647.41	100.00
4.88	3.15	5.69	30.45	647.41	0.00	754.23	100.00
5.69	3.56	6.63	34.01	754.23	0.00	878.67	100.00

APPENDIX E

The data from studying curcuminoids niosomes permeation *in vitro*

Table 26. The cumulative amount of curcumin from niosomes prepared by SO: cholesterol: solulan C-24 = 47.5: 47.5: 5 (*n*=4)

Sample	Peak area	Conc. (µg/ml)	Cumulative amount (µg)
48 hr			
1	140952	2.03	20.27
2	135426	1.95	19.52
3	137516	1.98	19.80
4	142869	2.05	20.53
60 hr			
1	168453	2.40	28.05
2	165482	2.36	27.49
3	166540	2.37	27.69
4	170432	2.43	28.37
72 hr			
1	271549	3.80	46.81
2	270586	3.78	46.45
3	272355	3.81	46.78
4	274488	3.84	47.32

Table 27. The cumulative amount of desmethoxycurcumin from niosomes prepared by SO: cholesterol: solulan C-24 = 47.5: 47.5: 5 (*n*=4)

Sample	Peak area	Conc. (µg/ml)	Cumulative amount (µg)
48 hr			
1	23563	0.43	4.29
2	23390	0.43	4.26
3	22471	0.41	4.10
4	23785	0.43	4.33
60 hr			
1	43390	0.77	8.58
2	42546	0.76	8.43
3	41634	0.74	8.24
4	44008	0.78	8.70
72 hr			
1	45842	0.81	10.55
2	46089	0.82	10.56
3	45582	0.81	10.41
4	46784	0.83	10.74

Table 28. The cumulative amount of bisdesmethoxycurcumin from niosomes prepared by SO: cholesterol: solulan C-24 = 47.5: 47.5: 5 (*n*=4)

Sample	Peak area	Conc. (µg/ml)	Cumulative amount (µg)
48 hr			
1	4753	0.06	0.58
2	4544	0.06	0.56
3	4639	0.06	0.57
4	4725	0.06	0.58
60 hr			
1	11007	0.13	1.46
2	10856	0.13	1.44
3	10687	0.13	1.42
4	10943	0.13	1.45
72 hr			
1	12948	0.16	1.96
2	12890	0.16	1.95
3	12684	0.15	1.92
4	12995	0.16	1.97

VITA

Miss Nittaya Rungphanichkul was born on January 23, 1980 in Phayao, Thailand. She received her Bachelor Degree in Nursing Science from the Faculty of Nursing Sciences, Chiangmai University in 2001.