

Novel Solid Lipid Nanoparticles for Oral Delivery of Oxyresveratrol: Effect of the Formulation Parameters on the Physicochemical Properties and *in vitro* Release

Y. Sangsen, K. Likhitwitayawuid, B. Sritularak, K. Wiwattanawongsa, R. Wiwattanapatapee

Abstract—Novel solid lipid nanoparticles (SLNs) were developed to improve oral bioavailability of oxyresveratrol (OXY). The SLNs were prepared by a high speed homogenization technique, at an effective speed and time, using Compritol® 888 ATO (5% w/w) as the solid lipid. The appropriate weight proportions (0.3% w/w) of OXY affected the physicochemical properties of blank SLNs. The effects of surfactant types on the properties of the formulations such as particle size and entrapment efficacy were also investigated. Conclusively, Tween 80 combined with soy lecithin was the most appropriate surfactant to stabilize OXY-loaded SLNs. The mean particle size of the optimized formulation was 134.40 ± 0.57 nm. *In vitro* drug release study, the selected S2 formulation showed a retarded release profile for OXY with no initial burst release compared to OXY suspension in the simulated gastrointestinal fluids. Therefore, these SLNs could provide a suitable system to develop for the oral OXY delivery.

Keywords—Solid lipid nanoparticles, Physicochemical properties, *in vitro* drug release, Oxyresveratrol.

I. INTRODUCTION

OXYRESVERATROL (OXY) (*Trans*-2,4,3',5'-tetrahydroxystilbene), is a polyphenolic stilbene purified from the heartwood of a Thai traditional plant, *Artocarpus lakoocha* Roxburgh (Moraceae) [1]. It has been reported to exhibit tyrosinase inhibitory activity, acts as a potent antioxidant, is anti-inflammatory and has strong neuroprotective activity [2]-[5]. Recently, its antiviral activities have been established with activities against several types of herpes simplex virus (HSV-1 and HSV-2), various varicella zoster viruses (VZV), influenza virus as well as human immunodeficiency virus type 1 (HIV-1) [1], [6]-[9].

Y. Sangsen is with the Department of Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Songkhla 90112 Thailand (e-mail: s.yaowaporn@gmail.com).

K. Likhitwitayawuid and B. Sritularak are with the Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10330 Thailand (e-mails: Kittisak.L@chula.ac.th, Boonchoo.sr@chula.ac.th, respectively).

K. Wiwattanawongsa is with the Department of Clinical Pharmacy, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Songkhla 90112 Thailand (e-mail: kamonthip.w@psu.ac.th).

R. Wiwattanapatapee is with the Department of Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Songkhla 90112 Thailand (phone: 667-428-8915; fax: 6674 428 148; e-mail: ruedeekorn.w@psu.ac.th).

These pharmacological activities have triggered efforts to transform OXY into therapeutic formulations. However, there are some limitations for the oral delivery of OXY such as poor absorption due to intermediate permeability and active efflux mediated mechanisms, extensive hepatic metabolism, and rapid elimination from the body results in a low oral bioavailability and limits the clinical use of OXY [10]-[12]. To overcome these difficulties, it will be necessary to design a formulation for OXY that will improve its oral bioavailability.

Lipid-based drug delivery systems, especially solid lipid nanoparticles (SLNs), have been a focus in the last few years for use as alternative colloidal drug carriers. SLNs are produced by replacing the liquid lipid (oil) component of an oil-in-water (O/W) emulsion with lipids that are solid at both room and body temperatures. The systems possess many advantages such as their physical stability, protection of labile drugs from chemical or enzyme degradation, controlled release, biodegradability, and biocompatibility all derived from physiologically accepted excipients that are generally recognized as safe (GRAS) status [13]-[15]. Moreover, many particle synthetic methods can be easily scaled up it to commercial products [16]. Until recently, SLNs have been investigated successfully as oral drug delivery systems for several drugs [17]-[22]. Nonetheless, there have been no reports from investigations using such systems as carriers for the oral delivery of OXY. Predominantly, the proposed mechanisms for the enhanced absorption of SLNs that have been documented involve direct uptake through the intestine, a decreased degradation in the gastrointestinal (GI) tract and reduced hepatic metabolism with a retarded clearance from the body because of its nano-size and the lipid used in the SLNs [23]. Apart from the effect on the stability of the formulations, surfactants also increase the intestinal permeability of SLNs containing a drug by paracellular transport and restraining efflux systems thus increasing transcellular pathways [23]-[25]. Therefore, the preparation processes and composition of such SLN formulations that affect their physicochemical properties as well as their release properties should be investigated to obtain effective SLNs for oral delivery of OXY.

The aim of this study was to evaluate the effects of the preparation conditions and formulated ingredients on their appearance, physicochemical characteristics, including

particle size, size distribution, total drug content, entrapment efficacy, drug loading capacity, and *in vitro* drug release properties of developed SLNs for consideration as potential carriers for the oral delivery of OXY.

II. MATERIALS

OXY (>95% purity) was kindly obtained from the Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University (Bangkok, Thailand). Compritol® 888 ATO (glyceryl behenate; Com888) were from Gattefosse (Saint-Priest, France). Cremophor® EL (polyoxyethylene castor oil derivatives; Cre EL), and Poloxamer® 188 (polyoxyethylene esters of 12-hydroxystearic acid; P188) were from BASF (Ludwigshafen, Germany). Soy lecithin was from Rama Production Co., Ltd. (Bangkok, Thailand). Glycerol monostearate (GMS) and Propylene glycol (PG) were from PC Drug Center Co., Ltd. (Bangkok, Thailand). Tween80 (polyoxyethylene (20) sorbitan monooleate) was from S.Tong Chemicals Co., Ltd (Bangkok, Thailand). Acetonitrile and methanol (High Performance Liquid Chromatography; HPLC grade) were from RCI Labscan (Bangkok, Thailand). All other chemicals were of analytical grade.

III. METHOD

A. Formulations and Preparations of the OXY-Loaded SLNs

The SLNs containing with OXY were formulated by a high speed homogenization method. Briefly, the lipid phase: OXY and solid lipid - and the aqueous phase: surfactants combined with co-surfactant in deionized (DI) water were heated to 85°C, separately. Then, the hot aqueous phase was added to the lipid phase at 85°C under a magnetic stirrer at 600 rpm. An emulsion was obtained using a T 25 digital ULTRA-TURRAX® high speed homogenizer (IKA®, Germany) with a S25N-8G disperser. Subsequently the final dispersion was cooled to room temperature to solidify the nanoparticles. The formulations were stored in tightly sealed glass bottle until used. Before loading the drug into the SLN formulations, the homogenization speeds and times were varied to optimize the homogenization conditions (Table I). To select the most suitable components in the formulations, the degree (Table II) and types of solid lipid (Table IV), the amount of OXY (Table IV), and the types of surfactant (Table III) were investigated.

B. Particle Size and Polydispersity Index Analysis

The mean particle size (PS) and polydispersity index (PDI) of the lipid nanoparticles formed with each of the formulations were measured by photon correlation spectroscopy (Zetasizer Nano ZS®, Malvern Instruments, UK) using the dynamic light scattering technique. The samples were diluted with DI water to produce the most suitable concentration to allow for accurate measurements. Aliquots of these lipid nanoparticles were loaded into cuvettes and their size was measured. Light scattering was monitored at a 173° angle at a temperature of 25°C. All measurements were

performed in triplicate and the data are presented as a mean ± standard deviations (S.D.).

TABLE I
VARIATION OF HOMOGENIZATION CONDITIONS ON THE BLANK SLNS

Compositions	Formulations (% w/w)					
	T1	T2	T3	T4	T5	T6
<i>Lipid phase</i>						
Com888			1.2			
<i>Aqueous phase</i>						
Tween80			0.90			
Soy lecithin			0.45			
DI water			97.450			
Speed (rpm)	10,000	15,000	20,000	24,000	24,000	24,000
Time (min)	5	5	5	5	10	15

TABLE II
DIFFERENT WEIGHT PROPORTIONS OF SOLID LIPID OF THE BLANK SLNS

Compositions	Formulations (% w/w)				
	C1	C2	C3	C4	C5
<i>Lipid phase</i>					
Com888	1.2	5	7.5	9	10
<i>Aqueous phase</i>					
P188:Tween80(1:1)	0.90	3.75	5.625	6.75	7.50
Soy lecithin	0.450	1.875	2.812	3.375	3.750
DI water	97.450	89.375	84.062	80.875	78.750

C. Determination of the Total Drug Content

The total drug content (TDC) in the developed OXY-loaded SLNs was determined. Briefly, aliquots of 1mL of the OXY-loaded SLN dispersion were dissolved in methanol and the mixture was blended using a mixer (Vortex-gene 2, Becthai Bangkok Equipment & Chemical, Thailand) at a maximum speed for 15min in order to facilitate complete dissolution. Then, the obtained suspension was allowed to filter through a 0.45µm membrane filter and diluted appropriately with the HPLC mobile phase. The resulting solution was analyzed by the HPLC method. The percentage of TDC was calculated by using (1) where OXY_{cal} was the total content of OXY calculated from the experiment and the OXY_{ther} was the theoretical drug content.

$$TDC (\%) = \frac{OXY_{cal}}{OXY_{ther}} \times 100 \quad (1)$$

D. Drug Entrapment Efficiency and Drug Loading Capacity

The encapsulation efficiency (EE) and loading capacity (LC) of each of the developed OXY-loaded SLNs were evaluated. The EE of the formulations was calculated by determining the amount of free drug that was not entrapped in the formulations. The drug loading content was the ratio of incorporated drug to lipid (w/w). One mL of the OXY-loaded SLN dispersion was placed in the dialysis bag (molecular weight cut off; MWCO 12–14 kDa). These bags were then placed in a centrifuge tube and the tube was filled with methanol and centrifuged at 13,500 rpm for 15min. The mixture of solvent containing the non-entrapped drug was

then analyzed by the HPLC method. The percentage of EE and LC was calculated by using (2) and (3), respectively, where OXY_{cal} was the total amount of OXY, the OXY_f was the amount of non entrapped OXY in the solvent, and the T_{lipid} was the total weight of lipid in the formulation.

$$EE (\%) = \frac{(OXY_{cal} - OXY_f)}{OXY_{cal}} \times 100 \quad (2)$$

$$LC (\%) = \frac{(OXY_{cal} - OXY_f)}{T_{lipid}} \times 100 \quad (3)$$

TABLE III
DIFFERENT SURFACTANT TYPES OF THE OXY-LOADED SLNS

Compositions	Formulations (% w/w)						
	S1	S2	S3	S4	S5	S6	S7
OXY				0.3			
<i>Lipid phase</i>							
Com888				5			
<i>Aqueous phase</i>							
<i>Surfactants</i>							
P188	3.75						
Tween80		3.75					
Cre EL			3.75				
PG				3.75			
P188:Tween80(1:1)					3.75		
P188:Cre EL(1:1)						3.75	
P188:PG(1:1)							3.75
Soy lecithin				1.875			
DI water				89.075			

E. In Vitro Drug Release Study

The *in vitro* release study of OXY from the developed formulations was carried out by the dialysis bag diffusion technique [23]. Briefly, the OXY-loaded SLN dispersion equivalent to 10mg OXY was filled into the dialysis bag (MWCO 12–14 kDa). The bag was immersed in 200 mL of release medium in a chamber of the USP30 dissolution apparatus 2 (Hanson Research Corporation, USA), stirred at 100rpm and maintained at a temperature $37 \pm 0.5^\circ\text{C}$. The dissolution medium was either simulated gastric fluid (SGF, pH 1.2) without pepsin or simulated intestinal fluid (SIF, pH 6.8) without pancreatin, respectively. Five mL aliquots of were withdrawn at various time intervals and replaced with fresh dissolution medium. Samples were filtered using a 0.45

um filter and analyzed using the HPLC method. Three separate replicate studies were conducted for each of the formulations, and the data are presented as a mean \pm S.D. ($n = 3$). The release profiles of the OXY from the SLNs were compared to an unformulated OXY.

F. Kinetic Analysis of In Vitro Drug Release Study

The Ritger–Peppas model has been widely used in many studies for analysis of the drug release kinetics in the matrix [26]. The Ritger–Peppas kinetic model is described by using (4) where M_t/M_∞ is the fraction of the drug released at time t , K is the constant for incorporating the structural and geometrical characteristics of the dosage form, and n is the release exponent that indicates the drug release mechanism. For the Fickian diffusion from the spheres, $n = 0.43$ while for the anomalous transport, n is between 0.43 and 0.85 and for a case II transport (zero-order release), $n = 0.85$.

$$\frac{M_t}{M_\infty} = Kt^n \quad (4)$$

G. High Performance Liquid Chromatography (HPLC) Analysis of OXY

The quantitative determination of OXY was performed using an Agilent HPLC system (HP 1100, Agilent, USA) with a C18 column (VertiSep™ pHendure 4.6 x 250mm, 5- μm , Ligand Scientific, Bangkok, Thailand), and a UV detector set at the wavelength of 320nm. Chromatographic conditions: the eluent was an isocratic solvent system at ambient temperature with a flow rate and injection volume of 1.0mL/min and 20 μL , respectively. The mobile phase consisted of acetonitrile and 0.5% v/v aqueous acetic acid in the ratio of 27:73 v/v. The retention time of OXY was about 7 min. The calibration curve for OXY was constructed by plotting the concentrations *versus* the corresponding mean peak areas that were calculated from three determinations. A good linearity was achieved with a correlation coefficient (r^2) of 0.9994 over the concentration range of 0.2–10 $\mu\text{g/mL}$. The intra-day precision was obtained by three repeated injections of each concentration of samples that showed the percent relative standard deviation (% RSD) of 0.14 to 0.86. The inter-day precision of the method gave a %RSD that ranged from 0.45 to 1.26. The recovery percentage of the method was between 95.50 ± 4.40 and 101.87 ± 1.61 .

TABLE IV
DIFFERENT LIPID TYPES AND WEIGHT PROPORTIONS OF OXY ON THE SLNS

Compositions	Formulations (% w/w)							
	F1	F2	F3	F4	F5	F6	F7	F8
OXY	0.1	0.2	0.3	0.4	0.5	1	2	0.3
<i>Lipid phase</i>								
Com888	5	5	5	5	5	5	5	
GMS								5
<i>Aqueous phase</i>								
P188:Tween80(1:1)				3.75				
Soy lecithin				1.875				
DI water	89.275	89.175	89.075	88.975	88.875	88.375	87.375	89.075

H. Statistical Analysis

All results were expressed as a mean \pm S.D. Statistical comparisons were performed by the Student's *T*-test or one-way ANOVA. Differences were considered significant at a *p*-value ($P < 0.05$).

IV. RESULTS AND DISCUSSION

A. Effect of the Homogenization Conditions on the Physical Properties of the Blank SLNs

SLNs can be prepared by various methods including hot and cold homogenization, high speed homogenization, ultrasonication, solvent emulsification and evaporation, and microemulsion [16], [21], [22], [27]-[29]. For this study, the hot high speed homogenization method was used due to it being simple and quick. The homogenization conditions, in term of speeds and times were optimized to obtain particles that were nano-sized ($< 200\text{nm}$) and had a narrow size distribution (PDI ~ 0.2 - 0.4) so that the reduced particle size improved the surface area of the SLNs. One of the fundamental principles for the design of nanoparticles systems is the increase of the interfacial energy because of the high curvatures of small sized particles as this result in an increase of the solubility of a given substance [30]. Moreover, this small size allows for an efficient uptake in the intestine particularly in the lymphoid tissue thus bypassing the first pass metabolism and resulting in an improvement of drug absorption [23]. The effects of the homogenization speed and time on the physical properties of blank SLN formulation are summarized in Table V. The homogenizer speed was varied from 10,000 to 24,000 rpm (T1-T4) with a fixed time of 5 min. When the speed rates increased, the particle size was reduced and the size distribution narrowed. The T4 appeared as a milky suspension with $< 200\text{ nm}$ particle size, thus 24,000 rpm was fixed as the optimum homogenization speed. The time was varied from 5 to 15 min (T4-T6). The increased time for mixing reduced the particle size of the homogeneous T6 suspension and produced the smallest size and narrowest distribution of particles. Therefore, the speed rate of 24,000 rpm and a time of 15min were selected as the standard homogenization step for all formulations.

TABLE V
EFFECT OF THE HOMOGENIZATION CONDITIONS ON THE PHYSICAL PROPERTIES OF THE BLANK SLNs

Compositions	Formulations (% w/w)					
	T1	T2	T3	T4	T5	T6
Appearance	Milky cream	Viscous and milky suspension	Milky suspension	White suspension		
Aggregation ^a	/	/	/	-	-	-
PS (nm)	-	563.20 ± 69.83	251.60 ± 1.57	174.20 ± 0.06	137.70 ± 0.17	130.20 ± 0.40
PDI	-	0.57 \pm 0.07	0.39 \pm 0.01	0.28 \pm 0.01	0.24 \pm 0.01	0.17 \pm 0.01

^a Visual observations on particle aggregation in the formulations: (/) denoting the observed aggregates of particles. Data represent a mean \pm S.D. (n = 3).

B. Effect of the Weight Proportions of the Solid Lipid on the Physical Properties of the Blank SLNs

The amount of the lipid matrix also had an important effect on the particle size, size distribution, drug loading, and the stability of SLNs, and this should be taken into consideration during the screening and development of the formulations [14], [19]. The composition of the blank SLN formulations that had different weight proportions of solid lipid and the effect of them on the physical properties of such formulations are summarized in Tables II and VI, respectively. When the weight proportions of the solid lipid increased, the particle size and size distribution of SLNs increased, so it was difficult to obtain a small and uniform size distribution when the solid lipid in the formulations was increased because the Com888 had a high viscosity. A good appearance was obtained with the non flocculated C1 and C2 formulations. The particle sizes and size distribution of both these formulations were not significantly different ($P > 0.05$) as they were each around 100-120nm in PS and 0.2-0.3 in PDI. Therefore, the formulation containing a high weight proportion of solid lipid (5% w/w; C2) that was expected to achieve a high drug loading was chosen for further development of the OXY-loaded SLNs.

C. The Effect of the Weight Proportions of OXY on the Physicochemical Properties of the SLNs

To investigate the effects of the amount of the incorporated OXY on the selected C2 formulation, the OXY loaded into the formulations was varied from 0.1 to 2 % w/w as shown in Table IV (F1-F7). The effects of the loaded OXY on the physicochemical properties of the SLNs are summarized in Table VII. A larger size of the nanoparticles was obtained by increasing the amount of the OXY in the SLN formulations and the PDI detected were greater. Until the weight proportion of the OXY was higher than 0.5% w/w, a cream-like appearance was observed (F6 and F7). This result was thought to be due to an insufficient amount of the lipid to encapsulate the drug. The particle size and the PDI of the F1-F3 formulations were less than 200nm and 0.4, respectively, and followed the expected values. The OXY had high entrapment efficiency in all of the three formulations ($> 80\%$). However, the highest capacity for drug loading ($4.85 \pm 0.01\%$ w/w) was obtained in the F3 formulation. Moreover, the PS of the F3 was significantly larger than the blank SLNs (C2) ($P < 0.05$), thus the presence of the OXY did significantly affect the PS of the blank SLNs. Therefore, the optimum OXY loaded for the SLN formulation was determined to be the F3 that contained 0.3% w/w of the OXY with good characteristics.

TABLE VI
EFFECT OF THE WEIGHT PROPORTIONS OF SOLID LIPID ON THE PHYSICAL PROPERTIES OF THE BLANK SLNS

Physical properties	Formulations				
	C1	C2	C3	C4	C5
Appearance	White suspension		Viscous and milky suspension		Milky suspension
Aggregation ^a	-	-	/	/	/
PS (nm)	108.10 ± 0.74	110.10 ± 0.95	312.70 ± 4.13	377.10 ± 10.97	196.60 ± 0.89
PDI	0.27± 0.01	0.24± 0.01	0.44± 0.02	0.45± 0.03	0.28± 0.00

^a Visual observations on particle aggregation in the formulations: (/) denoting observed aggregates of particles. Data represents a mean ± S.D. (n = 3).

D. Effect of the Lipid Types on the Physical Properties of the OXY Loaded-SLNs

Apart from the degree of the solid lipid, the type of lipid matrix had another important effect on the physicochemical properties and the drug release properties of the SLN formulations [14], [19]. Two types of solid lipid including glyceryl behenate (Com888) and glyceryl monostearate (GMS) were used as the lipid phase (5% w/w) and compared as shown in Table VII. After incorporation of OXY (0.3% w/w) into both types of the formulations, the Com888-based (F3) and GMS-based (F8) SLNs were observed to be a homogenous suspensions and yellowish cream, respectively. The Com888 was chosen as the solid lipid matrix in this study. This was associated with having an amphiphilic characteristic with a high chemical stability that resulted in a good ability to encapsulate a lipophilic and/or hydrophilic drug [30]. Reference [19] showed that SLNs prepared from such solid lipid displayed favorable physicochemical characteristics such as particle size, size distribution and morphology.

TABLE VII
EFFECT OF LIPID TYPES AND THE WEIGHT PROPORTIONS OF OXY ON THE PHYSICO-CHEMICAL PROPERTIES OF THE SLNS

Properties	Formulations				
	F1	F2	F3	F4	F5
Appearance	Yellowish suspension			Viscous and yellowish suspension	
Aggregation ^a	-	-	-	/	/
PS (nm)	95.90 ± 0.04	126.60 ± 0.31	158.00 ± 0.76	389.30 ± 5.77	620.70 ± 15.62
PDI	0.27± 0.01	0.20± 0.02	0.23± 0.01	0.31± 0.04	0.49± 0.00
TDC (%)	108.82 ± 2.13	91.98 ± 6.38	93.79 ± 4.70	94.80 ± 9.18	96.38 ± 8.83
EE (%)	90.87±	83.54±	86.14±	86.01±	87.45±
	0.08	0.13	0.17	0.05	0.10
DL (%)	1.98±	3.07±	4.85±	6.52±	8.43±
	0.00	0.00	0.01	0.00	0.01

^a Visual observations on particle aggregation in the formulations: (/) denoting the observed aggregates of particles. Data represents a mean ± S.D. (n = 3).

^bF6-F8 appeared as a yellowish cream.

E. Effect of Surfactants Types on the Physicochemical Properties of the OXY Loaded-SLNs

The surfactants used in the SLNs as permeability enhancers also contributed to both the particle stability and an improved bioavailability of the SLNs. There have been many data reported that the soy lecithin, used as a co-surfactant in this study, was an important factor for drug-loaded SLNs without the drug readily separating from the formulations, irrespective of the lipid used [17], [19]. The lecithin was mainly distributed at the interface of the oil and the aqueous phase. In our study, the soy lecithin (hydrophilic-lipophilic balance; HLB of 5) was added to the system to adjust the HLB of other surfactants so as to enhance the ability to emulsify the lipid and stabilize the system. The OXY-loaded SLN formulations with seven different types of a single or a combination of surfactants and the effect of them on the physicochemical properties of the formulations are summarized in Table III and VIII, respectively. All the surfactants used in this experiment formed particles. However, the formulations containing Cre EL (S3 and S6) and PG (S4 and S7), that provided a high solubility for OXY (data was not shown), showed undesirable physical characteristics such as having a large particle size (> 200nm) and a non-uniform size distribution. Whereas, for the formulations (S1, S2, and S5) containing P188 (HLB of 29) and/or Tween80 (HLB of 15) they had a good appearance and did not flocculate. The mean particle sizes obtained from these formulations was less than 200 nm (144.20 ± 1.10, 134.40 ± 0.57, 158.00 ± 0.76 nm for S1, S2, S5, respectively) and they also had a narrow size distribution (PDI 0.22-0.27). As in previous studies, the non-toxic and non-ionic P188 and Tween 80 were selected as surfactants for the Com888-based formulations due to their compatibility. The SLNs that were prepared using sufficient P188 and/or Tween80 as surfactants had a small particle size, uniform size distribution, and also good stability [17], [19], [21]. An important issue with respect to the use of nanoparticles as drug carriers is their capacity for drug loading and their entrapment efficiency on the basis of similar total drug content (about 100% TDC). It is clear that the entrapment efficiency of the S1, S2, and S5 formulations was satisfactorily high compared to such values of the SLNs loaded with other drugs in the previous reports [21]-[23]. The average drug loading of the three OXY-loaded SLNs was about 4-5% w/w of the lipid phase.

TABLE VIII
EFFECT OF SURFACTANT TYPES ON THE PHYSICO-CHEMICAL PROPERTIES OF THE OXY-LOADED SLNS

Physicochemical properties	Formulations						
	S1	S2	S3	S4	S5	S6	S7
Appearance	Yellowish suspension	Milky suspension	Milky suspension	Milky suspension	Yellowish suspension	Milky cream	Milky suspension
Aggregation ^a	-	-	/	/	-	/	/
PS (nm)	144.20 ± 1.10	134.40 ± 0.57	288.70 ± 3.86	297.50 ± 1.79	158.00 ± 0.76	630.50 ± 36.08	384.30 ± 25.68
PDI	0.27 ± 0.00	0.25 ± 0.00	0.25 ± 0.01	0.40 ± 0.01	0.23 ± 0.01	0.58 ± 0.09	0.51 ± 0.08
TDC (%)	92.00 ± 5.43	102.05 ± 1.97	85.59 ± 2.64	73.73 ± 6.48	93.79 ± 4.70	87.25 ± 7.81	68.51 ± 6.48
EE (%)	78.64 ± 0.46	90.55 ± 0.28	85.21 ± 0.69	82.76 ± 0.95	86.14 ± 0.17	89.77 ± 0.39	86.93 ± 1.10
DL (%)	4.34 ± 0.02	5.54 ± 0.02	4.38 ± 0.03	3.66 ± 0.04	4.85 ± 0.01	4.70 ± 0.02	3.57 ± 0.04

^aVisual observations on particle aggregation in the formulations: (/) denoting observed aggregates of particles. Data represents a mean ± S.D. (n = 3)

F. In Vitro Drug Release Study

In order to develop a controlled release system, it is vital to understand the release pattern, the mechanism and the kinetics. In this study, the conventional dialysis bag diffusion technique that is widely used for measuring the release of drugs from colloidal solutions was used. The *in vitro* OXY release profiles of the S1, S2, S5, and OXY suspension in the SGF pH 1.2 and SIF pH 6.8 are shown in Figs. 1 and 2, respectively. The cumulative amount of the OXY released from each formulation was plotted as a function of time and it was evident that the OXY suspension showed a cumulative release of OXY of 30% within 4 h in the SGF pH 1.2 and 80% within 48 h in the SIF pH 6.8, respectively. Comparing the release curves of the three OXY-loaded SLN formulations in pH 1.2 and pH 6.8, we concluded that these formulations showed no initial burst release in either medium. On the other hand the release of OXY from the SLNs slowed down considerably and it varied from 4.94% to 9.69% after 4 h in the SGF pH 1.2 depending on the types of surfactant present. Likewise, a retarded release profile from the three SLN formulations was demonstrated compared with that of the OXY suspension in the SIF pH 6.8. From the experimental data, the release rate of S2 formulated using Tween80 was slower than for S5 (P188:Tween80) and S1 (P188) during the study times. The difference of the % cumulative release was significant at 48 h with S2 (24.24 ± 0.67 %), S5 (37.30 ± 1.39 %), and S1 (48.46 ± 7.64 %), respectively ($P < 0.05$). In addition the Com888 used in this study had previously been utilized as a retardant material for a sustained release dosage form, and this may be due to the stronger solubilization of P188 compared to Tween 80 [19], [25], [31]-[32]. Moreover, there was more non-entrapped drug in the particles S1 than in S5 and S2 that exhibited %EE of 78.64 ± 0.46%, 86.14 ± 0.17%, and 90.55 ± 0.28%, respectively. From these results, we concluded that the surfactants (Tween80 and P188) made an important contribution to the differences between the release from the three SLN formulations and their diffusion from the OXY suspension. The results indicated that the OXY was entrapped in the SLNs and was protected from the strong acidic environment of the stomach and then the SLNs containing the OXY subsequently reached the small intestine. Possibly, the major content of OXY in the SLNs could be

uptake by the intestinal cells and enter the body circulation to perform a sustained release *in vivo*.

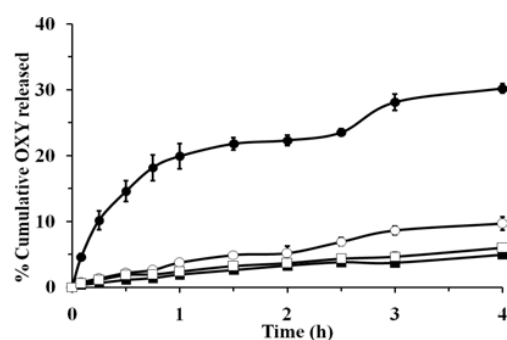


Fig. 1 The *in vitro* OXY release profiles from suspension (●), S1 (○), S2 (■), and S5 (□) in simulated gastric fluid (SGF, pH 1.2) without pepsin

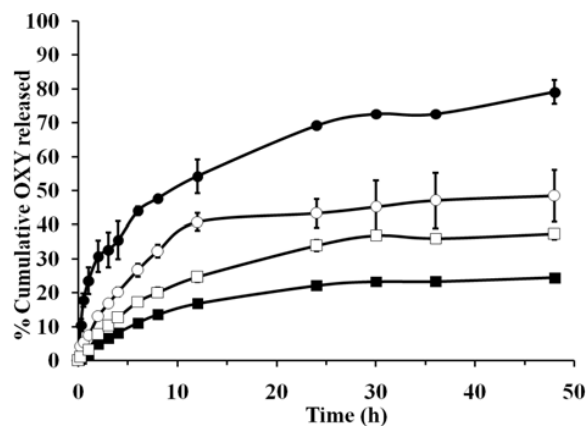


Fig. 2 The *in vitro* OXY release profiles from suspension (●), S1 (○), S2 (■), and S5 (□) in simulated intestinal fluid (SIF, pH 6.8) without pancreatin

G. Kinetic Analysis of *in vitro* Drug Release Study

To explain the release kinetics of OXY from the SLN formulations, the mean data for the fraction of OXY released in the SIF pH 6.8 was subjected to linear regression analysis using conventional mathematical models [33] and a Ritger-Peppas kinetics model [26]. The data for the drug release from

different types of surfactant (S1, S2, and S5) was mostly fitted to a Ritger–Peppas kinetics model and gave an R^2 close to 1 for all the formulations as shown in Table IX. Based on the fitting result of the Ritger–Peppas model, the value of n was 0.5155, 0.7679, 0.6985 in the case of S1, S2, S5, respectively ($0.43 < n < 0.85$), and this indicated that the mechanism of OXY release from the SLNs was by a mixing of the drug diffusion and the erosion of the lipid matrix. The n value was close to 0.85 in the case of the S2 formulation. This indicated that the effect of the erosion of the lipid matrix was predominant and the major amount of drug was enriched in the core of SLNs. The minor amount of drug in the shell can diffuse into the medium. In contrast, the release of the OXY from the S1 formulation appeared to be predominantly by a diffusion-controlled mechanism. OXY was mainly released by diffusion through the channels formed in the matrix into the medium.

TABLE IX
LINEAR REGRESSION ANALYSES OF THE IN VITRO DRUG RELEASE DATA OF THE FORMULATIONS USING THE RITGER–PEPPAS KINETIC MODEL

Formulations	Ritger–Peppas model ($Mt/M\infty = Kt^n$)		
	K	n	r^2
S1	0.0889	0.5155	0.9587
S2	0.0204	0.7679	0.9423
S5	0.0376	0.6985	0.9585

V. CONCLUSION

In conclusion, the OXY-loaded SLN formulation (S2) comprising Compritol® 888 ATO with Tween80 combined with soy-lecithin had a good appearance, an ability to entrap the OXY drug, displayed the most favorable physicochemical properties, and had a retarded release of OXY in a SGF pH 1.2 and a SIF pH 6.8. Based on the data obtained in this work, the formulation S2 was judged to be the optimum formulation, and will be further for *in vitro* permeability, and *in vivo* absorption studies to ensure it was a potential carrier for the oral delivery of OXY.

TABLE X
SUMMARY OF HOMOGENIZATION CONDITIONS COMPOSITIONS AND PROPERTIES OF THE OPTIMUM S2 FORMULATION

Formulation n	Homogenization conditions		Compositions (% w/w)			Physicochemical properties					
	Speed (rpm)	Time (min)	Solid lipid (5%)	Surfactant (3.75%)	Co-surfactant (1.875%)	Appearance	PS(nm)	PDI	TDC (%)	EE (%)	DL (%)
S2	24,000	15	Com888	Tween80	Soy lecithin	Milky suspension	134.40 ±0.57	0.25 ±0.00	102.05 ±1.97	90.55 ±0.28	5.54 ±0.02

ACKNOWLEDGMENT

Financial support was granted by the Thailand Research Fund (BRG 5580004) and the Faculty of Pharmaceutical Sciences, Prince of Songkla University, Thailand. We also wish to thank Dr. Brian Hodgson for assistance with the English.

REFERENCES

- [1] K. Likhitwitayawuid, *et al.*, "Phenolics with antiviral activity from *Millettiery thocalyx* and *Artocarpus lakoocha*," *Nat. Prod. Res.*, vol. 19, no. 2, pp. 177–182, Feb. 2005.
- [2] K. O. Chung, *et al.*, "In-vitro and in-vivo anti-inflammatory effect of oxyresveratrol from *Morus alba* L.," *J. Pharm. Pharmacol.*, vol. 55, no. 12, pp. 1695–1700, Dec. 2003.
- [3] P. Lorenz, S. Roychowdhury, M. Engelmann, G. Wolf, and T. F. W. Horn, "Oxyresveratrol and resveratrol are potent antioxidants and free radical scavengers: effect on nitrosative and oxidative stress derived from microglial cells," *Nitric Oxide*, vol. 9, no. 2, pp. 64–76, Sep. 2003.
- [4] P. Tengamnuay, K. Pengrungruangwong, I. Pheansri, and K. Likhitwitayawuid, "Artocarpus lakoocha heartwood extract as a novel cosmetic ingredient: evaluation of the *in vitro* anti-tyrosinase and *in vivo* skin whitening activities," *Int. J. Cosmet. Sci.*, vol. 28, no. 4, pp. 269–276, Aug. 2006.
- [5] J. T. Weber, *et al.*, "Potential neuroprotective effects of oxyresveratrol against traumatic injury," *Eur. J. Pharmacol.*, vol. 680, no. 1, pp. 55–62, Apr. 2012.
- [6] P. Sasivimolphan, *et al.*, "Inhibitory activity of oxyresveratrol on wild-type and drug-resistant varicella-zoster virus replication *in vitro*," *Antivir. Res.*, vol. 84, no. 1, pp. 95–97, Oct. 2009.
- [7] A. L. Liu, *et al.*, "In vitro anti-influenza viral activities of stilbenoids from the lianas of *Gnetum pendulum*," *Planta. Med.*, vol. 76, no. 16, pp. 1874–1876, Nov. 2010.
- [8] V. Lipipun, *et al.*, "Topical cream-based oxyresveratrol in the treatment of cutaneous HSV-1 infection in mice," *Antivir. Res.*, vol. 91, no. 2, pp. 154–160, Aug. 2011.
- [9] W. Wang, J. Qian, X. Wang, and A. Jia, "Anti-HIV-1 activities of extracts and phenolics from *Smilax China* L.," School of Environmental and Biological Engineering, Nanjing University of Science and Technology, Nanjing, private communication, 2013.
- [10] H. Huang, *et al.*, "High performance liquid chromatographic method for the determination and pharmacokinetic studies of oxyresveratrol and resveratrol in rat plasma after oral administration of Smilax china extract," *Biomed. Chromatogr.*, vol. 22, no. 4, pp. 421–427, Dec. 2007.
- [11] H. Huang, *et al.*, "Identification of seven metabolites of oxyresveratrol in rat urine and bile using liquid chromatography/tandem mass spectrometry," *Biomed. Chromatogr.*, vol. 24, no. 4, pp. 426–432, Aug. 2010.
- [12] M. Mei, *et al.*, "In vitro pharmacokinetic characterization of Mulberoside A, the main polyhydroxylated stilbene in mulberry (*Morus alba* L.), and its bacterial metabolite oxyresveratrol in traditional oral use," *J. Agr. Food Chem.*, vol. 60, no. 9, pp. 2299–2308, Dec. 2012.
- [13] R. H. Müller, K. Mäder, and S. Gohla, "Solid lipid nanoparticles (SLN) for controlled drug delivery – a review of the state of the art," *Eur. J. Pharm. Biopharm.*, vol. 50, no. 1, pp. 161–177, July 2000.
- [14] S. Chakraborty, D. Shukla, and S. Singh, "Lipid – an emerging platform for oral delivery of drugs with poor bioavailability," *Eur. J. Pharm. Biopharm.*, vol. 73, no. 1, pp. 1–15, Sep. 2009.
- [15] E. B. Souto, and R. H. Müller, "Lipid nanoparticles: effect on bioavailability and pharmacokinetic changes," in *Drug Delivery*, 1st ed. vol. 197, M. Schäfer-Korting, Ed. New York: Springer-Verlag, 2010, pp. 115–141.
- [16] W. Mehnert, and K. Mäder, "Solid lipid nanoparticles: production, characterization and applications," *Adv. Drug Deliv. Rev.*, vol. 47, no. 2, pp. 165–196, Apr. 2001.
- [17] Y. Luo, D. Chen, L. Ren, X. Zhao, and J. Qin, "Solid lipid nanoparticles for enhancing vinpocetine's oral bioavailability," *J. Control. Release*, vol. 114, no. 1, pp. 53–59, May 2006.

- [18] A. J. Almeida, and E. Souto, "Solid lipid nanoparticles as a drug delivery system for peptides and proteins," *Adv. Drug Deliv. Rev.*, vol. 59, no. 6, pp. 478-490, July 2007.
- [19] L. Hu, Q. Xing, J. Meng, and C. Shang, "Preparation and enhanced oral bioavailability of cryptotanshinone loaded solid lipid nanoparticles," *AAPS Pharm. Sci. Tech.*, vol. 11, no. 2, pp. 582-587, June 2010.
- [20] V. Kakkar, S. Singh, D. Singla, and I. P. Kaur, "Exploring solid lipid nanoparticles to enhance the oral bioavailability of curcumin," *Mol. Nutr. Food Res.*, vol. 55, no. 3, pp. 495-503, Aug. 2010.
- [21] E. H. Gokce, *et al.*, "Resveratrol-loaded solid lipid nanoparticles versus nanostructured lipid carriers: evaluation of antioxidant potential for dermal applications," *Int. J. Nanomedicine*, vol. 7, pp. 1841-1850, Apr. 2012
- [22] A. R. Neves, M. Lúcio, S. Martins, J. L. C. Lima, and S. Reis, "Novel resveratrol nanodelivery systems based on lipid nanoparticles to enhance its oral bioavailability," *Int. J. Nanomedicine*, vol. 8, pp. 177-187, Jan. 2013.
- [23] R. Tiwari, and K. Pathak, "Nanostructured lipid carrier versus solid lipid nanoparticles of simvastatin: comparative analysis of characteristics, pharmacokinetics and tissue uptake," *Int. J. Pharm.*, vol. 415, no. 1, pp. 232-243, Aug. 2011.
- [24] M. Gibaldi, and S. Feldman, "Mechanisms of surfactant effects on drug absorption," *J. Pharm. Sci.*, vol. 59, no. 5, pp. 579-89, May 1970.
- [25] G. Wu, and K. Y. C. Lee, "Effects of poloxamer 188 on phospholipid monolayer morphology: an atomic force microscopy study," *Langmuir*, vol. 25, no. 4, pp. 2133-2139, Jan. 2009.
- [26] P. L. Ritger, and N. A. Peppas, "A simple equation for description of solute release I. Fickian and non-fickian release from non-swelling devices in the form of slabs, spheres, cylinders or discs," *J. Control. Rel.*, vol. 5, no. 1, pp. 23-36, June 1987.
- [27] C. Y. Zhuang, *et al.*, "Preparation and characterization of vinpocetine loaded nanostructured lipid carriers (NLC) for improved oral bioavailability," *Int. J. Pharm.*, vol. 394, no. 1, pp. 179-185, July 2010.
- [28] M. Trotta, F. Debernardi, and O. Caputo, "Preparation of solid lipid nanoparticles by a solvent emulsification-diffusion technique," *Int. J. Pharm.*, vol. 257, no. 1, pp. 153-160, May 2003.
- [29] F. Q. Hu, *et al.*, "Preparation and characterization of stearic acid nanostructured lipid carriers by solvent diffusion method in an aqueous system," *Colloids Surf. B Biointerfaces*, vol.45, no. 3, pp. 167-173, Nov. 2005.
- [30] J. E. Kipp, "The role of solid nanoparticle technology in the parenteral delivery of poorly water-soluble drugs," *Int. J. Pharm.*, vol. 284, no. 1-2, pp. 109-22, Oct. 2004.
- [31] E. Souto, W. Mehnert, R. Müller, "Polymorphic behaviour of Compritol® 888 ATO as bulk lipid and as SLN and NLC," *J. microencapsul.*, vol. 23, no. 4, pp. 417-433, June 2006.
- [32] W. Sutananta, D. Q. M. Craig, and J. M. Newton, "An evaluation of the mechanisms of drug release from glyceride bases," *J. Pharm. Pharmacol.*, vol. 47, no. 3, pp. 182-187, March 1995.
- [33] P. Costa, and J. M. Sousa Lobo, "Modeling and comparison of dissolution profiles," *Eur. J. Pharm. Sci.*, vol. 13, no. 2, pp. 123-33, Dec. 2000.