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Original Article

LIPID MICROEMULSION-BASED HYDROGELS FOR EFFECTIVE TOPICAL DELIVERY OF PHENYTOIN

SIANG YIN LEE[1,2*], CHIA CHIN TEO[1], WEI JEN TAN[1], HUI YAN LIM[1], HWEI HWEI LIM[1], SIEW YONG TEO[3]

[1]Department of Pharmaceutical Chemistry, School of Pharmacy, International Medical University, No. 126, Jalan Jalil Perkasa 19, Bukit Jalil, 57000 Kuala Lumpur, Malaysia, [2]Unit Colloids and Interface Science (CISC), Centre of Excellence (COE), RRIM Sungai Buloh Research Station, Malaysian Rubber Board (MRB), 47000 Sungai Buloh, Selangor, Malaysia, [3]School of Postgraduate Studies and Research, International Medical University, No. 126, Jalan Jalil Perkasa 19, Bukit Jalil, 57000 Kuala Lumpur, Malaysia
Email: leesiangyin@lgm.gov.my

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ABSTRACT

Objective: Microemulsion is a promising drug delivery vehicle for lipophilic drugs but its acceptability for topical application is limited to its very low viscosity. The aim of the present study was to develop and characterize lipid microemulsion hydrogel as a topical drug carrier for phenytoin.

Methods: Lipid oil-in-water (O/W) emulsions were formulated from palm kernel oil (PKO), coconut kernel oil (CKO) and soybean oil (SBO), and their blends using phase inversion temperature method. Stable nano-sized microemulsions were identified and formulated into phenytoin loaded hydrogels. The physicochemical properties of the formulations were evaluated in term of emulsion stability index, droplet size, zeta potential, pH, and rheological properties. The efficacy of *in vitro* drug release of phenytoin was further evaluated using Franz diffusion cells.

Results: Stability study revealed that ten lipid emulsions mixing with surfactant Tween 80 at an oil-to-surfactant ratio of 1:9 having 100% emulsion stability indices. Among these, two emulsions (F6 and F21) were identified as the most stable nano-sized microemulsions with clear and transparent appearances; mean droplet size maintained within 100 nm (11–16 nm) as per stability study. Rheological data showed that all phenytoin is loaded hydrogels exhibited non-Newtonian and shear-thinning flow behavior, with high yield stress of a 10.3–18.8 Pa. The *in vitro* release profiles followed the first-order kinetic model, with R^2>0.95, where F21 demonstrated the highest release rate, with 93.12% drug released in 12 h.

Conclusion: These findings concluded that CKO/SBO blend microemulsion hydrogel has the highest potential for topical phenytoin delivery.

Keywords: Lipid, Microemulsion, Hydrogel, Topical delivery, Phenytoin

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INTRODUCTION

There is a growing interest in employing transparent, thermo-dynamically stable lipid-based nanoemulsion formulations, with droplet size ranged 1–100 nm, for delivering lipophilic drugs [1-7]. These formulations were reported to exhibit superior solubilization properties, ease of preparation and reduction of stratum corneum's diffusion barrier over traditional creams, gels, and solutions. Lately, the utilization of lipid nanoemulsions as topical drug delivery vehicle has also shown very promising effects [2, 8, 9]. Results indicated that the lipid emulsion-based topical dosage forms enhance drug solubilization and bioavailability, as well as facilitate topical delivery of hydrophilic drugs.

Microemulsions are single optically isotropic, thermodynamically stable colloidal dispersion systems which are formed by mixtures of oil, water, and surfactant at appropriate ratios [10-12]. These dispersion systems exhibited several desirable physical properties, such as the nanometer droplet size range (10–100 nm), low interfacial tension, enhanced drug solubilization, and higher transdermal permeability over conventional formulations, making them a promising topical drug delivery vehicle. Despite many advantages of microemulsions, the major limitation of this formulation is its inconvenience to apply on skin.

The addition of suitable gelling agent into the emulsion system for the formation of the microemulsion-based hydrogel may serve as a better alternative to overcome this limitation [13-16]. These gelling polymers demonstrate complex functions such as thickeners and emulsifiers to facilitate the formation of the stable microemulsion-based hydrogel by further decreasing the interfacial tension and simultaneously increasing the viscosity of the resultant system. It was noteworthy that these microemulsion-based hydrogels for dermatological use have various advantages like thixotropic, easily spreadable, emollient, long shelf life, greaseless, easily removable, non-staining, and bio-friendly.

One of the primary aims of this study was the formulation of stable oil-in-water (O/W) lipid microemulsions using low-energy input emulsification technique. The chosen lipids in this study were coconut kernel oil (CKO), palm kernel oil (PKO) and soya bean oil (SBO). CKO is extracted from the kernel of coconuts harvested from the coconut palm (*Cocos nucifera*); PKO is obtained from the kernel of the fruits of oil palm tree (*Elaeis guineensis*); while, SBO is predominantly obtained from soybeans (*Glycina maxima*) [17, 18]. Both CKO and PKO are saturated lipids with medium-chain triglycerides (MCTs) ranged from 6 to 12 carbon chains; whereas SBO is long-chain triglycerides (LCTs) composed of unsaturated fatty acids with 18 to 24 carbon chains. For preparing lipid-based emulsions, the selection of oil phase plays an important role in determining the stability of an emulsion.

Phenytoin was introduced in 1938 as an antiepileptic agent as the first-line therapeutic approach for various generalized convulsive disorders [19-23]. The apparent stimulatory effect of phenytoin on connective tissue suggested the potential use of phenytoin as a promising healing agent in the treatment of various cutaneous wounds and disorders. However, its widespread use in clinical settings was restricted by the fact that the best formulation to deliver the drug is not known. Topical phenytoin powder dressing and topical phenytoin sodium suspension (2 and 4% w/w) had been using to apply directly to the wounds. However, the efficiencies of these formulations remained controversial due to the low contact period of phenytoin over the wounds, difficulty for even application and lack of a randomized controlled trial to access its efficacy.

For these reasons, the aim of this present work was to describe the potential of gel formulated from PKO, CKO, SBO, and the blends as the effective drug carriers for topical application. The rheological behavior and storage stability of the resultant gels were investigated to evaluate their suitability as drug delivery vehicles. The efficacy of *in vitro* drug release of these gel formulations was evaluated using the hydrophobic drug, phenytoin.

MATERIALS AND METHODS

Chemicals

PKO, CKO, and SBO gifted from MOI Foods Malaysia Sdn. Bhd. were used without further purification as the oil phase. Ammonium acryloyldimethyltaurate/VP copolymer also referred as Aristoflex® AVC, was purchased from Comatrix Sdn. Bhd., Selangor, Malaysia. Tween 80 and sodium benzoate were purchased from Fisher Scientific Sdn. Bhd., Selangor, Malaysia. Phenytoin was purchased from Sigma-Aldrich Sdn. Bhd., Kuala Lumpur, Malaysia. All chemicals were of reagent grade and used as received.

Formulation of lipid microemulsions

Lipid microemulsions were formulated from lipid (PKO, CKO, and SBO, resp.) as an oil phase, Tween 80 as the non-ionic surfactant and distilled water containing 0.1% w/w sodium benzoate using the phase inversion temperature (PIT) method described by Shinoda [24]. The mixture was weighed into a round bottom flask equipped with a condenser and a conductivity electrode. A condenser was used to prevent evaporation. The change of conductivity around the phase inversion point was indicated by a conductivity meter (Oakton Con II conductivity meter). The mixture was stirred continuously with a magnetic stirrer and warmed up using a water bath. The conductivity of the emulsion was measured as a function of temperature. After the phase inversion point had reached, the emulsion was rapidly cooled by immersion in an ice bath. During the cooling, the emulsions were continuously stirred. Two series of lipid emulsions were prepared, i.e. optimization of surfactant concentration in specific oil-to-surfactant (O/S) ratios and selection of MCT, LCT or its blend as an oil phase in specific oil-to-oil (O/O) ratios. Subsequently, the emulsion stability of these series was investigated.

Emulsion stability index

All emulsions were subjected to heating/cooling test for seven cycles, where storage temperature was alternated between 4 °C and 40 °C in an incubator (Heraeus, Hanau, Germany) in every 24 h, for the duration of 14 d. Emulsion stability index (ESI) of each emulsion was measured to evaluate the degree of phase separation as shown in equation 1.

$$ESI = \frac{HE\text{-}HC}{HE} \times 100 \rightarrow \text{Eq. 1}$$

Where HC is the height of cream layer and HE is the initial emulsion height.

Physico-chemical evaluation of emulsion

All formulations were subjected to visual inspection, pH, zeta potential, and droplet size determinations. The measurements of zeta potential and droplet size were performed using a Zetasizer ZEN 3600 (Malvern, Worcestershire, UK) at room temperature. The instrument was calibrated using zeta potential standard and polystyrene latex standard, respectively. Zeta potential measurements were determined using electrophoretic light scattering (ELS). Droplet size measurements were determined using dynamic light scattering (DLS). The pH of all prepared formulations was assessed using Mettler Toledo S220 Seven Compact pH meter. All measurements were performed in triplicate.

Formulation of phenytoin-loaded hydrogels

Phenytoin-loaded hydrogels were formulated by first mixing phenytoin into the mixture of oil and surfactant at room temperature. The mixture was vortexed thoroughly at 1400 rpm until a clear dispersion was formed, indicating the completion of drug solubilization. The mixture was then subjected to the subsequent steps to formulate microemulsion as described above. Aristoflex® AVC was added into the resultant drug-loaded emulsions as a gelling agent. The mixture was homogenized at 10,000 rpm for 10 min to obtain the phenytoin-loaded hydrogel.

Rheological study

The rheological properties of hydrogels were evaluated using an Anton Paar Rheolab MC1 rheometer with a concentric cylinder system operating in a rotational mode. Samples were tested at a controlled temperature of 25±2°C under shear rate control conditions within the range 0 s^{-1} to 100 s^{-1}. Two rheograms were plotted from apparent viscosity against shear rate and shear stress against shear rate. The data obtained were fitted to the power law model, as shown in equation 2.

$$\tau = K\dot{\gamma}^n \rightarrow \text{Eq. 2}$$

Where τ is the shear stress, $\dot{\gamma}$ is the shear rate, K is the consistency index (Pa. s^n) and n is the flow behavior index.

Drug loading and entrapment efficiency

The drug loading and entrapment efficiency of phenytoin in lipid microemulsions were determined using a high-performance liquid chromatography (HPLC) method [25]. The method employed a test facility of a reverse phase HPLC system (1200 series, Agilent Technologies) equipped with a pump, injector valve with 20 µl sample loop, ZORBAX Eclipse Plus C-18 analytical column (250 mm × 4.6 mm, 5µm particles), and UV detector with data processor (Chem Station Software). Aliquots of samples were dispersed in the mobile phase of acetonitrile and ultrapure water (50:50) mixture and the injected sample volume was 20 µl with the flow rate of 1.0 ml/min. A standard curve of peak area against the concentration of phenytoin was plotted, and the drug content of each sample was determined by comparison with the standard curve. The percentage drug loading and encapsulation efficiency of the microemulsions were calculated using the following equations.

$$\text{Drug loading (\%)} = \frac{\text{Total weight of drug}}{\text{Total weight of sample}} \text{ X } 100\% \rightarrow \text{Eq. 3}$$

$$\text{Entrapment efficiency (\%)} = \frac{\text{Actual drug loading}}{\text{Theoretical drug loading}} \text{ X } 100\% \rightarrow \text{Eq. 4}$$

The stability of phenytoin in the nanoemulsions was determined using the stability-indicating HPLC assay described above. Measurements were carried out using freshly prepared samples and samples stored for three months at 5 °C, 25 °C and 45 °C. Significant differences in drug content between the two groups were assessed using one-way analysis of variance.

In vitro drug release study

The *in vitro* drug release profiles of phenytoin from formulations were studied using Franz diffusion cells (PermeGear, USA). A calibration curve was first generated using the freshly prepared standard solutions of phenytoin with concentrations of 5, 10, 15, 20, 25 and 30 µg/ml. Approximately 1 g of gel formulation was placed on the cellulose acetate membrane with pore size 45 µm in the donor chamber. Ethanol 96% was filled into the 14 ml receptor chamber as the receiving medium which was constantly stirred at 32±0.5 °C. Aliquots of the receiving medium were withdrawn at time intervals of 1, 2, 4, 6, 8, and 12 h. The volume sampled was replaced with fresh receiving medium.

The drug content in the withdrawn aliquots was analyzed using ultraviolet-visible (UV/Vis) spectrophotometer (Perkin-Elmer, Malaysia) at 240 nm. Triplicate experiments were conducted for each formulation, and the results were presented as the average cumulative percentage of drug release as a function of time.

In vitro drug release kinetics

The drug release kinetics of gel formulations were determined by fitting the release data into the kinetic models of zero-order, first-order, and Higuchi which are given by equations 5, 6, and 7, respectively.

$$Q = k_0 t \rightarrow \text{Eq. 5}$$

$$\ln (Q_0 - Q) = \ln Q_0 - k_1 t \rightarrow \text{Eq. 6}$$

$$Q = k_2 \sqrt{t} \rightarrow \text{Eq. 7}$$

Where Q_0 is the initial amount of drug, Q is the amount of drug released at time *t*, k_0, k_1, and k_2 are the rate constants for zero-order, first-order and Higuchi models respectively.

RESULTS AND DISCUSSION

Formulation and characterization of lipid microemulsions

A total of 21 emulsions were prepared using low energy input PIT method with the formulation shown in table 1 and ESI is shown in table 2. Ten formulations (F1, F6, F7, F11, F16, F17, F18, F19, F20, and F21) indicated thermodynamically stable with 100% ESI and no sign of phase separation throughout the heating/cooling test for seven cycles. This suggests that stable lipid emulsions could be formulated utilizing Tween 80 in the absence of co-surfactants. This is an advantage to avoid the possibility of partitioning of the co-surfactant out of the interfacial region into the continuous phase during phase inversion emulsification.

From the visual observation, only nine emulsions with 100% ESI exhibited clear and transparent appearances, except F7, demonstrated milky appearance. The particle size measurement showed that only lipid emulsions formulated with an oil-to-surfactant (O/S) ratio of 1:9 satisfied the size criterion of nanoemulsions (droplets<100 nm) and exhibited a very narrow size ranged between 11 nm and 16 nm at 0 cycles. This indicates that 18% w/w of Tween 80 in the continuous phase is the optimal surfactant concentration to promote a complete solubilization of oil phase during the phase inversion and thus highly stabilized the microdroplets. This optimal surfactant concentration can render long-term stability for F1, F6, F20, and F21 to maintain the nano-droplet size throughout the 7 heating/cooling cycles. This was in agreement with Vasiliki *et al.* that optimal surfactant concentration was a key factor during formulation of microemulsion as it lowered the interfacial tension and stabilized the oil droplets against creaming and coalescence [26]. The zeta potential values of all emulsions ranged between-1 mV and-7mV. The small negative charge may due to the steric stabilization exerted by the non-ionic surfactant, leading to the salvation of oil droplet and polyoxyethylene groups of Tween 80 at the oil-water interface. Overall, the lipid microemulsions have almost neutral electrical charge since the zeta potential values were in the range between −10mV and+10mV [27]. Furthermore, all lipid emulsions exhibited almost neutral pH values in the range of 6.0 and 7.5, which was close to that of skin pH, suggesting they are suitable for topical application [28]. In addition, no significant change of pH values was detected indicating all emulsions were stable towards hydrolysis and ionization over the storage period.

Table 1: Formulations of lipid emulsions

Emulsion	Percentage weight per weight (% w/w)					O/S ratio	O/O ratio
	PKO	CKO	SBO	T80	Water		
F1	2	-	-	18	80	1:9	-
F2	6	-	-	14	80	3:7	-
F3	10	-	-	10	80	5:5	-
F4	14	-	-	6	80	7:3	-
F5	18	-	-	2	80	9:1	-
F6	-	2	-	18	80	1:9	-
F7	-	6	-	14	80	3:7	-
F8	-	10	-	10	80	5:5	-
F9	-	14	-	6	80	7:3	-
F10	-	18	-	2	80	9:1	-
F11	-	-	2	18	80	1:9	-
F12	-	-	6	14	80	3:7	-
F13	-	-	10	10	80	5:5	-
F14	-	-	14	6	80	7:3	-
F15	-	-	18	2	80	9:1	-
F16	0.6	-	1.4	18	80	1:9	30:70
F17	1.0	-	1.0	18	80	1:9	50:50
F18	1.4	-	0.6	18	80	1:9	70:30
F19	-	0.6	1.4	18	80	1:9	30:70
F20	-	1.0	1.0	18	80	1:9	50:50
F21	-	1.4	0.6	18	80	1:9	70:30

Abbreviation**:** PKO, palm kernel oil; CKO, coconut kernel oil; SBO, soybean oil; T80, Tween 80; Water, distilled water containing 0.1% w/w sodium benzoate; O/S, oil-to-surfactant ratio; O/O, oil-to-oil ratio. Note: F1 to F5 were PKO emulsions; F6 to F10 were CKO emulsions; F11 to F15 were SBO emulsions; F16 to F18 were PKO/SBO blend emulsions; F19 to F21 were CKO/SBO blend emulsions.

Fig. 1 shows the droplet size of single lipid phase microemulsions measured at different heating/cooling cycles when subjected to stability testing. It was notable that SBO microemulsion (F11) exhibited the greatest increase in droplet size, ranged from 13.44 nm to 196.67 nm, indicating its highly thermodynamic instability as compared to PKO and CKO microemulsions. This could be due to higher interfacial tension exerted by SBO long hydrocarbon chain length against the aqueous phase, leading to droplet coalescence. PKO and CKO are the two commonly known MCT which comprises of medium-chain fatty acid (MCFA) molecules while SBO is rich in polyunsaturated fatty acid (PUFA) mainly linoleic acid (table 3) [29]. Highly unsaturated SBO has oxidative stability index (OSI) of 1.95 h and PUFA of 59.3% whereas highly saturated PKO and CKO have very little PUFA (2% and 1.5% respectively). Thus, PKO and CKO have higher OSI than SBO (9.88 h and 9.94 h respectively) [30]. Since OSI is inversely proportional to PUFA content, stable LCT emulsions could be formulated by an inclusion of highly saturated MCT into LCT.

Fig. 2 displays the droplet size measurement of MCT/lCT lipid blends microemulsions at different heating/cooling cycles in the stability test. As CKO has a higher degree of saturation (90.69%) than PKO (80.34%), CKO/SBO blends (F19, F20, F21) show higher emulsion stability than that of PKO/SBO blend (F16, F17, F18) emulsions. In general, the result also indicates that enhanced emulsion stability can be improved by increasing the MCT ratio in the oil phase for both PKO/SBO and CKO/SBO blends, particularly with MCT up to 70% in the blends. In this case, SBO may function as co-surfactant and greatly decreases the interfacial tension and simultaneously increases the viscosity of MCT, and thus highly stabilized the microdroplets. Overall, two microemulsions that are exhibiting the greatest stability with the most stable nano-sized droplets, F6 of the single lipid phase microemulsion and F21 of the lipid blend microemulsion, were selected for the subsequent formulations of microemulsion-based hydrogels.

Table 2: Emulsion stability indices of lipid emulsions

Emulsion	ESI (%) per cycle							
	0	1	2	3	4	5	6	7
F1	100	100	100	100	100	100	100	100
F2	100	100	98.5	98.5	98.5	98.4	98.4	98.4
F3	100	94.7	94.7	94.5	94.5	94.5	94.5	94.5
F4	100	91.4	90.1	90.0	90.0	90.0	90.0	88.6
F5	90.0	90.0	89.8	89.8	89.2	89.0	89.0	89.0
F6	100	100	100	100	100	100	100	100
F7	100	100	100	100	100	100	100	100
F8	99.0	99.0	99.0	98.0	98.0	98.0	98.0	98.0
F9	98.0	98.0	97.0	95.0	95.0	95.0	95.0	95.0
F10	96.0	96.0	96.0	93.0	93.0	93.0	93.0	93.0
F11	100	100	100	100	100	100	100	100
F12	97.5	97.5	97.4	96.3	96.3	96.2	96.2	93.8
F13	94.7	93.4	93.4	93.4	93.2	92.7	92.0	91.9
F14	87.8	86.7	86.7	86.7	86.7	86.7	86.5	85.7
F15	82.0	81.4	81.0	80.6	80.3	80.1	80.0	80.0
F16	100	100	100	100	100	100	100	100
F17	100	100	100	100	100	100	100	100
F18	100	100	100	100	100	100	100	100
F19	100	100	100	100	100	100	100	100
F20	100	100	100	100	100	100	100	100
F21	100	100	100	100	100	100	100	100

Abbreviation: ESI, emulsion stability index, Note: F1 to F5 were PKO emulsions; F6 to F10 were CKO emulsions; F11 to F15 were SBO emulsions; F16 to F18 were PKO/SBO blend emulsions; F19 to F21 were CKO/SBO blend emulsions.

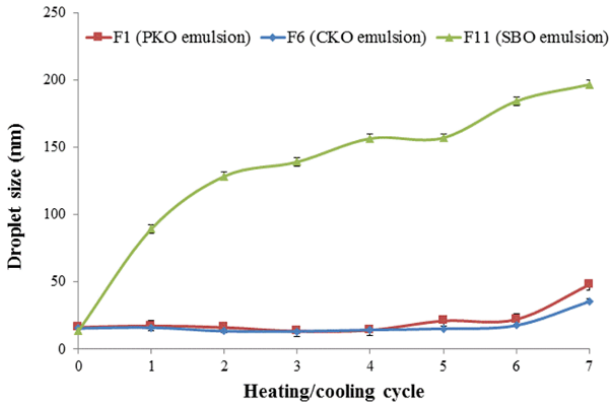

Fig. 1: Droplet size measurement of single lipid phase microemulsions subjected to stability testing at different heating/cooling cycles, Footnote: Data are reported as means from three independent replications (n = 3) for each sample. F1, F6, and F11 were PKO, CKO and SBO emulsions, respectively

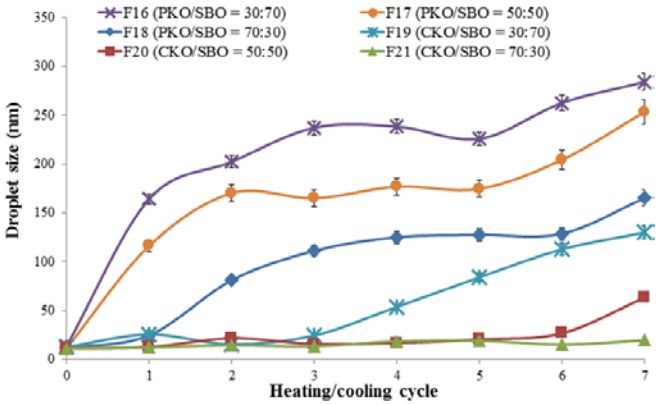

Fig. 2: Droplet size measurement of lipid blend microemulsions subjected to stability testing at different heating/cooling cycles, Footnote: Data are reported as means from three independent replications (n = 3) for each sample. F16, F17, and F18 were PKO/SBO blend emulsions and F19, F20, and F21 were CKO/SBO blend emulsions

Table 3: Fatty acids composition of PKO, CKO, and SBO [29]

Fatty acid	Empirical formula	Type of oil (%)		
		PKO	CKO	SBO
Caproic	C6:0	0.3	0.5	-
Caprylic	C8:0	3.9	8	-
Capric	C10:0	4	6.4	-
Lauric	C12:0	49.6	48.5	-
Myristic	C14:0	16	17.6	0.1
Palmitic	C16:0	8	8.4	11
Palmitoleic	C16:1	-	-	0.1
Stearic	C18:0	2.4	2.5	4
Oleic	C18:1	13.7	6.5	23.4
Linoleic	C18:2	2	1.5	53.2
Linolenic	C18:3	-	-	7.8
Arachidic	C20:0	0.1	0.1	0.3
Behenic	C22:0	-	-	0.1

Formulation and characterization of lipid microemulsion hydrogels

Practically, the low viscosity exhibited by microemulsions was usually non-desirable in the formulation of topical products as it is difficult to be applied on the skin and hence influences the performance of drug delivery system. The two nano-sized thermodynamically stable microemulsions (F6 and F21) were formulated into hydrogels using Aristoflex® AVC as the gelling agent. These hydrogels exhibited clear and transparent appearances with enhanced viscosity, rigid texture and smooth sensation. pH measurement showed no significant change (in the range of 6.3 and 6.8) on the formulations, with 100% ESI throughout the seven cycles of heating/cooling stability test (table 4), suggesting thermodynamic stable of the hydrogels.

Table 4: pH values and emulsion stability indices of lipid microemulsion hydrogels subjected to stability testing at specific heating/cooling cycles

Hydrogel	Properties at specified heating/cooling cycles*			
	pH		ESI (%)	
	0	7th	0	7th
F6-gel	6.77±0.01	6.39±0.02	100	100
F21-gel	6.56±0.01	6.30±0.03	100	100

*Data are reported as means from three independent replications (n = 3) for each sample, Note: F6-gel was CKO microemulsion hydrogel; F21-gel were CKO/SBO microemulsion hydrogel with CKO/SBO blended at O/O ratios of 70:30.

Fig. 3 reveals the rheological behavior of the lipid microemulsion hydrogels and the data obtained was fitted into the power law model (equation 2). Good data fits were acquired with high correlation coefficients (r^2>0.99). The flow curves in fig. 3 showed no linear proportionality between shear rate and shear stress, indicating all hydrogels displayed non-Newtonian fluid behavior. It was observed that the apparent viscosities of the two formulations decreased gradually with increasing shear rate suggesting these materials were shear-thinning or pseudoplastic in nature. These findings were further supported by their flow indices with the magnitude of n<1 (table 5). The presence of long, high molecular weight gelling agent may account for this pseudoplasticity flow behavior. The presence of weak attractive forces between polymers facilitated the formation of entangling loops between the droplets and the surrounding aqueous phase within the system. As shear rate increased, deformation occurred which caused the rigid gel matrix to lose their elasticity and become disentangled, thus allowing the material to flow in the direction of shearing force [31].

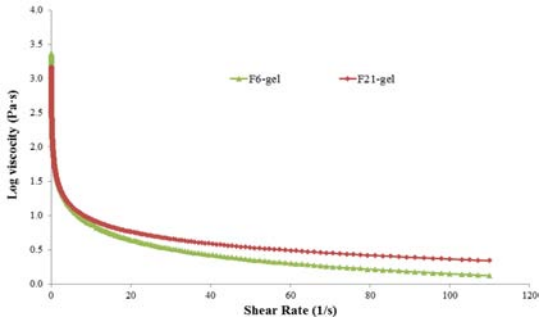

Fig. 3: Rheological behavior of lipid microemulsion hydrogels, Footnote: F6-gel was CKO microemulsion hydrogel and F21-gel was CKO/SBO microemulsion hydrogel with CKO/SBO blended at O/O ratios of 70:30. All error bars of results are too small and are hidden by written symbols

Table 5: The rheological properties of different topical hydrogel formulation

Hydrogel	Yield stress, γ (Pa)	Consistency index, K (Pa·s)	Flow index, n
F6-gel	10.3	22.00	0.44
F21-gel	18.8	35.79	0.40

Note: F6-gel was CKO microemulsion hydrogel; F21-gel was CKO/SBO microemulsion hydrogel with CKO/SBO blended at O/O ratios of 70:30, Abbreviation: Pa, pascal; Pa·s, pascal-second.

From table 5, all hydrogels demonstrated high yield stress and thus indicating high stability towards creaming. It was also observed that F6-gel showed slightly higher flow index than others, pertaining to its lower pseudo plasticity and better mobility.

The high values of consistency index (k) and yield stress (γ) of F21-gel suggested that its gel system exhibited more thinning behavior with the presence of SBO. The facts that F21-gel possessed higher yield stress than F6-gel implied that greater shearing force would be required to trigger its fluid nature. This phenomenon could be interpreted as the presence of 30% LCT as part of the oil phase in the F21 which enhanced its viscosity.

Drug loading and entrapment efficiency of lipid microemulsions

In this study, phenytoin was incorporated in F6 and F21 microemulsions using PIT method. Since phenytoin has poor aqueous solubility and hence require the test was carried out in a mobile phase of acetonitrile and ultrapure water (50:50) using the reversed phase-HPLC system. The measured drug loading of 2 mg/ml was recorded. The encapsulation efficiency of F6 and F21 was calculated as 98.7±0.6% and 98.5±0.9%, respectively. This indicates that an efficient phenytoin encapsulation within the two lipid microemulsions was achieved.

***In vitro* drug release study**

The *in vitro* phenytoin release profiles of the two hydrogel formulations were presented as a plot of cumulative percentage of drug release as a function of time, as shown in fig. 4

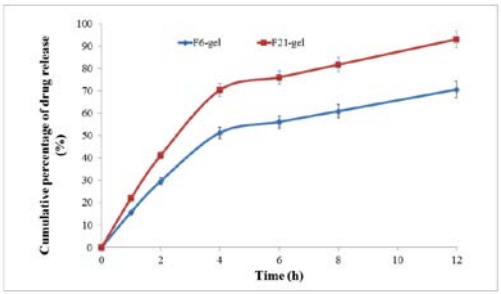

Fig. 4: Cumulative percentage of phenytoin release from lipid microemulsion hydrogels, Footnote: Data are reported as means from three independent replications (n = 3) for each sample. F6-gel was CKO microemulsion hydrogel and F21-gel was CKO/SBO microemulsion hydrogel with CKO/SBO blended at O/O ratios of 70:30

From the plot aforementioned, it was noteworthy that all hydrogel formulations were able to sustain the phenytoin release up to 12 h, with about 70% of phenytoin being released. This may be attributed to the smallest emulsion droplet size which has provided a large interfacial area for phenytoin solubilization and thus improved the drug penetration ability.

Fig. 4 shows that F21-gel demonstrated the higher release rate than F6-gel, with 93.1% of phenytoin being released in 12 h as compared to F6-gel with the amount of 70.6%. This suggested that the presence of SBO in F21-gel has reduced the rigidity of the gel matrix which might subsequently facilitate the diffusion of phenytoin into the Franz cell receptor medium more easily and hence increase the release of the drug.

The release data from the plot were then treated with different release kinetic models in order to determine their release characteristics. Table 6 shows the release profile of phenytoin from the two hydrogel formulations was best-fitted into the first-order equation, with each exhibiting R^2>0.95. This implied that that rate of release is proportional to the concentration of phenytoin in the hydrogel formulations.

Table 6: The release kinetics of different topical phenytoin-lipid microemulsion hydrogel formulations

Formulation	Zero-order kinetics		First-order kinetics		Higuchi model	
	k_0	R_0^2	k_1	R_1^2	k_H	R_H^2
F6-gel	10.21	0.83	-0.07	0.98	31.11	0.94
F21-gel	13.70	0.78	-0.16	0.99	40.57	0.94

Note: F6-gel was CKO microemulsion hydrogel; F21-gel was CKO/SBO microemulsion hydrogel with CKO/SBO blended at O/O ratios of 70:30.

CONCLUSION

Ten lipid microemulsions exhibiting 100% emulsion stability index were successfully formulated from lipids blended with Tween 80 at an O/S ratio of 1:9. F1, F6, F20, and F21 was identified as the nano-sized thermodynamically stable microemulsions that could maintain the nano-size range (<100 nm) throughout the heating/cooling test for seven cycles. The hydrogels of F6 and F21 exhibited favorable pharmaceutical characteristics, with pH values around 6.5, non-Newtonian pseudoplastic behavior, and good stability index. The *in vitro* drug release study indicated that both F6 and F21 hydrogels were able to sustain the release of phenytoin within 12 h, with F21-hydrogel showed the highest phenytoin release rate of 93.1%. Both hydrogels followed the first-order kinetic model. Hence, F21-hydrogel formulated from CKO/SBO blended in a ratio of 70:30 was the most identified as the most promising topical phenytoin-lipid microemulsion hydrogel formulation.

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CONFLICT OF INTERESTS

Authors declare that they have no conflict of interest in the research

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