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Research paper

Solid self-nanoemulsifying drug delivery system (S-SNEDDS) containing phosphatidylcholine for enhanced bioavailability of highly lipophilic bioactive carotenoid lutein

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ABSTRACT

The objectives of this study was to prepare solid self-nanoemulsifying drug delivery system (S-SNEDDS) containing phosphatidylcholine (PC), an endogenous phospholipid with excellent in vivo solubilization capacity, as oil phase for the delivery of bioactive carotenoid lutein, by spray drying the SNEDDS (liquid system) containing PC using colloidal silica (Aerosil® 200 VV Pharma) as the inert solid carrier, and to evaluate the enhanced bioavailability (BA) of lutein from S-SNEDDS. The droplet size analyses revealed droplet size of less than 100 nm. The solid state characterization of S-SNEDDS by SEM, DSC, and XRPD revealed the absence of crystalline lutein in the S-SNEDDS. The bioavailability study performed in rabbits resulted in enhanced values of C_{max} and AUC for S-SNEDDS. The enhancement of C_{max} for S-SNEDDS was about 21-folds and 8-folds compared with lutein powder (LP) and commercial product (CP), respectively. The relative BA of S-SNEDDS compared with CP or LP was 2.74-folds or 11.79-folds, respectively. These results demonstrated excellent ability of S-SNEDDS containing PC as oil phase to enhance the BA of lutein in rabbits. Thus, S-SNEDDS containing PC as oil phase could be a useful lipid drug delivery system for enhancing the BA of lutein in vivo.

1. Introduction

Lutein (Fig. 1), a non-provitamin A carotenoid found in dark green and leafy vegetables, is a poorly aqueous soluble lipophilic drug (logP 7.8) that plays an important role in the prevention of age-related macular degeneration (AMD), cataracts, and other blinding disorders by functioning as a potent antioxidant and effective screener of high-energy blue light [1]. Lutein is highly concentrated in the macula, a small area of the retina responsible for central vision and high visual acuity [2]. Clinical and epidemiological studies have indicated that increased intake of lutein was inversely related to the risk of AMD and cataract [3–6]. Lutein has been recognized as an important supplement for the prevention of ocular diseases and has been the focus of much attention lately.

Although lutein is a vital macular component, it is not synthesized in the body and therefore, dietary ingestion is the only source for the supplementation. However, the bioavailability (BA) of lutein is extremely variable due to the inherently poor solubility [7]. Recent reports indicate that egg yolk is a highly bioavailable source of lutein, although the amounts are considerably less than other lutein-rich vegetable sources [8,9]. Currently, commercially available formulations of lutein are vegetable oil-based suspensions filled in soft gelatin capsules that demonstrate very low BA and huge inter-individual variations due to variability in solubilization of lutein in vivo [10].

The maximum advantage from a lipid formulation could only be drawn if the drug remains in lipid solution throughout its residence in the gastrointestinal (GI) tract [11]. However, the performance of lipid formulations and the fate of the drug in the GI tract depend on 3Ds that occur simultaneously viz., dispersion, dilution, and digestion of the formulation. Due to these 3Ds, lipid formulations often result in change in their composition, structure, and potential loss of their solvent capacity. Altogether, these processes may cause precipitation of drug to occur, and thus, the advantage of a lipid formulation is lost [11–13]. For this reason, selection of oil phase that has high solubilization capacity for the drug is crucial in any lipid formulation. Besides, oil (consists of various types of triglycerides) is digested in the gastrointestinal tract and may play a major role in determining rate and extent of dissolution and also uptake of the drugs into enterocytes [10–12]. Eventually, phosphatidylcholine (Phosal 53 MCT, 53% phosphatidylcholine in

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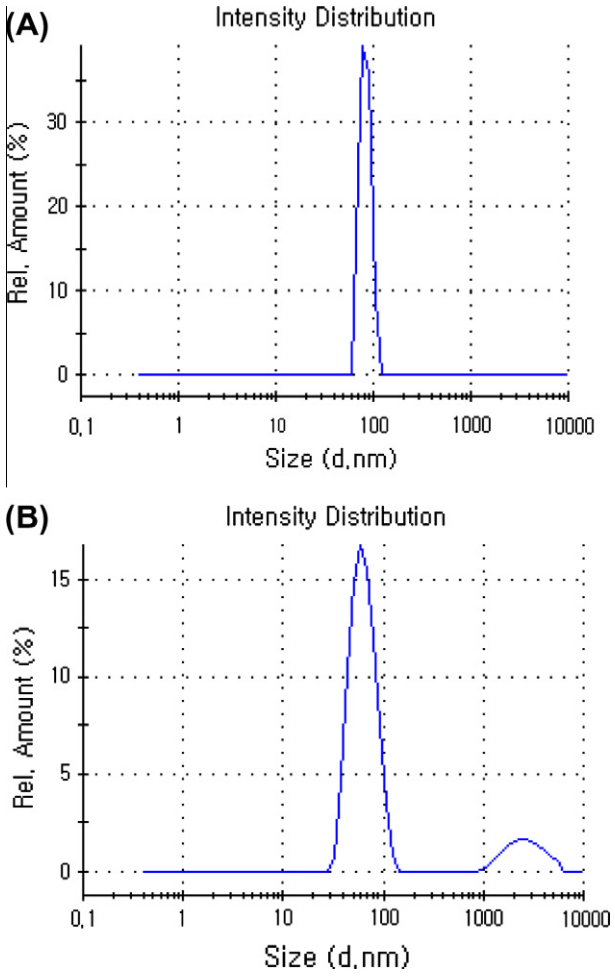

Fig. 1. Droplet size distribution of (A) SNEDDS, and (B) S-SNEDDS after mixing 100 μl or 100 mg of SNEDDS or S-SNEDDS formulations, respectively into 100 ml of distilled water under slight agitation.

lipid base of medium-chain triglycerides (caprylic/capric)), which may easily become digested by physiological lipases in the gastrointestinal tract without losing its solubilization capacity, has been proposed as a potential oil phase in lipid-based drug delivery system for enhanced dissolution and BA of lutein.

Solid self-nanoemulsifying delivery system (S-SNEDDS), one of the lipid-based drug delivery systems prepared by incorporation of liquid excipients into powders by solidification, is a promising drug delivery system for poorly water-soluble compounds as it combines the advantages of SNEDDS (solubility and bioavailability enhancement) with those of solid dosage forms (high stability with various dosage form options) [12–14]. S-SNEDDS produces oil-in-water nanoemulsions of droplet size less than 200 nm upon mild agitation in aqueous media (such as gastrointestinal fluids) [13,14]. These fine droplets of nanoemulsions have the advantage of presenting the drug in dissolved form with a large interfacial surface area for drug absorption, which results in enhanced more uniform and reproducible bioavailability [15].

In our earlier work, we showed the enhanced solubility and dissolution of lutein from SNEDDS (liquid system) containing PC [10]. In the present study, we prepared S-SNEDDS containing PC for the delivery of lutein from the established composition of SNEDDS by spray drying with Aerosil 200 VV Pharma as the inert solid carrier and evaluated its BA in rabbits. The solid state characteristics of the prepared S-SNEDDS were investigated using scanning electron microscopy (SEM), differential scanning calorimetry, (DSC) and X-ray powder diffraction (XRPD). Besides, reconstitution properties of the S-SNEEDS were investigated to assess its functionality using emulsification parameters such as emulsification time, droplet size, and charge. Finally, in vivo oral BA study in rabbits was performed to assess the enhanced BA of lutein from S-SNEDDS in comparison with LP or CP and to investigate the performance (absorption characteristics) of S-SNEDDS in comparison with SNEDDS.

2. Materials and methods

2.1. Materials

Saponified marigold extract was kindly donated by Korea Arlico Pharm (Seoul, Korea), and β-carotene was purchased from Sigma–Aldrich (St. Louis, USA). Phosphatidylcholine (Phosal® 53 MCT) was purchased from Phospholipid GmBH (Cologne, Germany). Labrasol and Transcutol-HP were obtained from Gattefosse (Saint-Priest Cedex, France). Colloidal silica (Aerosil® 200 VV Pharma) was purchased from Degussa (Frankfurt, Germany). Methanol, ethanol, acetonitrile, and methylene chloride were of HPLC grade. All other chemicals were of analytical grade and used without further purification. Purification of lutein from saponified marigold extract was done according to the previously proposed method [16], and the purity of lutein measured by HPLC was more than 98.00 ± 1%.

2.2. Methods

2.2.1. Preparation of SNEDDS

The composition of SNEDDS was established in our earlier study [17], which consists of Phosal 53® MCT/ labrasol/transcutol HP, oil/surfactant/co-surfactant, (25/60/15, w/w/w) respectively, with 4% of lutein. The SNEDDS formulations were prepared by a previously reported method [10]. Briefly, lutein (40 mg) was dissolved into the mixture (1 ml) of oil, surfactant, and co-surfactant at 60 °C in an isothermal water bath to facilitate solubilization. The resultant mixture was vortexed until a clear solution was obtained. It was then equilibrated at ambient temperature for at least 48 h and examined for signs of turbidity or phase separation prior to self-emulsification and emulsion droplet size studies.

2.2.2. Preparation of S-SNEDDS

S-SNEDDS of lutein was prepared by previously reported method using spray dry technology [17]. Briefly, colloidal silica suspension was prepared by suspending Aerosil 200 VV Pharma (500 mg) in 100 ml ethanol by magnetic stirring. One milliliter of SNEDDS containing lutein (4% w/w) was then added into the polymer suspension with constant stirring, and homogenous suspension was obtained by stirring the above mixture at room temperature for 15 min. The resultant suspension was then spray dried in a laboratory mini spray B-190 apparatus (Buchi, Switzerland) utilizing inlet temperature of 60 °C, outlet temperature of 35 °C, and aspiration of about 85%. The feeding rate of the suspension was set to 5 ml/min. The resultant powder was collected from the apparatus and measured for the final drug content using a validated HPLC method.

2.2.3. Solid state characterization of S-SNEDDS

2.2.3.1. Scanning electron microscopy (SEM). The outer macroscopic structure of S-SNEDDS was investigated by S-4100 scanning electron microscope (Hitachi, Japan). The analyses were performed by placing the samples on a brass stub using double-sided adhesive tape and were made electrically conductive by coating in vacuum (6 Pa) with platinum (6 nm/min) using Hitachi Ion Sputter (E-1030) for 240 s at 15 mA. The SEM images were analyzed with

an image analysis system (ImageInside Ver. 2.32) for particle size analysis.

2.2.3.2. Differential scanning calorimetry (DSC). The thermal properties of pure lutein, Aerosil® 200 VV Pharma, physical mixture, and S-SNEDDS were assessed by thermograms obtained using differential scanning calorimetry (DSC Q200 v24.2 build 107, TA Instruments, USA). The samples of about 3.00 mg were placed in standard aluminum pans, and dry nitrogen was used as effluent gas. All samples were scanned at a temperature ramp speed of 5 °C/min, and the heat flow was set from 30 °C to 180 °C. Before the experiment, the DSC was calibrated using pure Indium and heat of fusion (H_{fusion}).

2.2.3.3. X-ray powder diffraction (XRPD). XRPD measurements were carried out with an X'Pert PRO diffractometer (PAN analytical, The Netherlands) at room temperature using monochromatic CuKa-radiation (k = 1.5406 Å) at 30 mA and at 40 kV over a range of 2θ angles from 10° to 90° with an angular increment of 0.02° per second. The diffractograms of lutein, Aerosil® 200 VV Pharma, physical mixture, and S-SNEDDS were obtained for analysis.

2.2.4. Reconstitution properties of S-SNEDDS

2.2.4.1. Emulsification time determination. The emulsification time of the SNEDDS or S-SNEDDS formulations was evaluated according to United State Pharmacopeia (USP) XXIII, dissolution apparatus II. In brief, either SNEDDS (250 μl) or S-SNEDDS (500 mg) was introduced into 500 ml of distilled water at 37 °C under gentle agitation by a standard stainless steel dissolution paddle rotating at 50 rpm. The emulsification time was assessed visually as reported previously [18]. All experiments were carried out in triplicates.

2.2.4.2. Emulsion droplet size and charge determination. Droplet size of the nanoemulsion formed by the addition of 50 μl of SNEDDS, or 100 mg of S-SNEDDS into 50 ml of distilled water was determined by Zetasizer Nano ZS (Malvern Instruments, UK) with dynamic light scattering particle size analyzer at a wavelength of 635 nm and at a scattering angle of 90° at 25 °C. All studies were repeated three times, and the values of z-average diameters were used. The z-average diameter, also referred to as the harmonic intensity-weighted average hydrodynamic diameter, of the nanoemulsions was derived from cumulated analysis by the Automeasure software (Malvern Instruments, UK). Zeta potential of the nanoemulsion formed after addition of L-SNEDDS or S-SNEDDS into 0.1 N HCl solution was measured using Zetasizer Nano ZS (Malvern Instruments, UK).

2.2.5. Drug release studies

Lutein release from SNEDDS and S-SNEDDS formulations were performed using USP XXIII, dissolution apparatus II with 900 ml of distilled water as dissolution medium at 37 ± 5 °C with paddle speed at 100 rpm. SNEDDS and S-SNEDDS formulation equivalent to 20 mg of lutein was introduced into the dissolution tester (Shinseang Instrument Co., Korea). LP and CP were also tested simultaneously. At predetermined time intervals, an aliquot of 5 ml was collected, filtered, and analyzed for the content of lutein by HPLC. An equivalent volume (5 ml) of fresh dissolution medium was replaced to compensate the loss due to sampling.

2.2.6. In vivo bioavailability study

2.2.6.1. Animals. All animal treatment protocols were in accordance with National Institute of Health (NIH) guidelines, Korea. Healthy, male rabbits weighing about 2.5 ± 0.2 kg were supplied by Orient Bio (Seoul, Korea) and quarantined for one week prior to use. Animals were maintained on sawdust bedding free of any known chemical contaminants in a 12 h photoperiod (light on at 08:00 and off at 20:00) in our animal facility at 23 ± 2 °C and 50–80% relative humidity (RH). The animals had free access to food and water. The animals were fasted for 10 h before the experiment day.

2.2.6.2. In vivo protocol. The pharmacokinetic (PK) profile of lutein from SNEDDS, S-SNEDDS, LP, or CP was investigated in rabbits with a dose equivalent to 5 mg/kg of lutein. LP and other formulations were orally administered to the rabbits after dispersion in 10 ml of water. Following oral administration, 2.5 ml of blood was collected from either right or left marginal ear vein using 3-ml needle at predetermined time intervals, and 1 ml of plasma was separated by centrifuging blood samples at 3000 g for 15 min. Plasma samples were stored at -20 °C until further analysis.

2.2.6.3. HPLC analyses of lutein in rabbit plasma. The lutein content of rabbit plasma samples was analyzed by HPLC system equipped with Class VP computer software, LC 10 AD VP pump, and SPD 10A UV–VIS detector at 445 nm using β-carotene as internal standard. The column used was Inertsil ODS-3 (4.6 × 150 mm, GL Science Inc, Japan) and mobile phase consisted of a mixture of acetonitrile/methanol/methylene chloride (4/4/2, v/v/v). Injection volume was 50 μl, and flow rate was 1 ml/min. Validation of the HPLC assay was performed by repeating five times a day for five consecutive days using exactly same condition in the range of 0.05–100 μg/ml concentration. The intra- and inter-day accuracy of lutein ranged from 97.8–103.9% and 98.3–105.4%, respectively, with their respective% coefficient of variation (CV) being less than 6.4% and 7.1%, respectively.

To 1 ml of plasma, 100 μl of internal standard (50 μg/ml of β-carotene in ethanol) and 9 ml of ethanol were added, and vortex-mixed for 2 min followed by centrifugation at 12,000 rpm for 2 min to precipitate the proteins. The supernatant was evaporated in a rotary centrifugal vacuum evaporator. The residue obtained was reconstituted with 200 μl ethanol, and 50 μl of the resulting solution was analyzed by HPLC as mentioned earlier.

2.2.6.4. PK parameter analyses. The plasma concentration of lutein versus time profile was analyzed by a non-compartmental method using WinNonlin Professional Version 2.1 program for windows (Pharsight, Cary, NC, USA). The comparative bioavailability (BA) of lutein was calculated using the following equation;

$$\text{Relative BA} = \frac{AUC_{\text{test}}}{AUC_{\text{reference}}} \times \frac{Dose_{\text{reference}}}{Dose_{\text{test}}}$$

where AUC is the area under plasma drug concentration curve from time zero to the last sampling time.

2.2.7. Statistical analyses

Statistical analyses were carried out using SPSS statistical software (SPSS Statistics, Ver. 17.0). Multiple comparisons between different formulation groups and their statistical significance were analyzed using ANOVA followed by Tukey HD post hoc test. Confidence interval of 90% was used to calculate the statistical significance in all analyses performed.

3. Results and discussion

The composition of lipid excipients that constitutes the ternary phase of optimized SNEDDS was shown in Table 1. The spray dried particles of S-SNEDDS had good flowability properties due to the presence of Aerosil® 200 VV Pharma which is regarded as a suitable excipient for the solid dosage forms. The final lutein content of the prepared SNEDDS and S-SNEDDS measured by HPLC was 3.85% and 2.15%, respectively.

Table 1
Composition of optimized SNEDDS and S-SNEDDS formulations for the delivery of lutein.

Vehicle type	Name	HLB value	Composition (mg)	
			SNEDDS	S-SNEDDS
Oil	Phosal® 53 MCT	NA	250	250
Surfactant	Labrasol	14	600	600
Co-surfactant	Transcutol-HP	4.3	150	150
Lutein	API	NA	40	40
Aerosil® 200 VV Pharma	Colloidal SiO_2	NA	–	500

NA not applicable; Aerosil® 200 VV Pharma is a high purity amorphous anhydrous colloidal silicon dioxide has a specific surface area of 200 ± 25 m^2/g and tapped density of approximately 120 g/L; Phosal®53 MCT is 53% phosphatidylcholine solubilized in medium-chain triglycerides.

3.1. Solid state characterization of S-SNEDDS

The SEM pictures of Aerosil® 200 VV Pharma, LP, and S-SNEDDS are shown in Fig. 2. Aerosil® 200 VV Pharma (Fig. 2A) appeared as a rough surface with porous particles. Lutein powder (Fig. 2B) appeared as smooth-surfaced, irregularly shaped, flat crystals in shape. However, the S-SNEDDS (Fig. 2C) appeared as smooth-surfaced particles without any crystalline shape, indicating complete adsorption of SNEDDS containing amorphous lutein inside the pores of Aerosil® 200 VV Pharma.

Since the physical state of lutein in the S-SNEDDS would have an important influence on the in vitro and in vivo release characteristics, the DSC thermograms of Aerosil® 200 VV Pharma, lutein, physical mixture, and S-SNEDDS were obtained. The DSC thermograms were shown in Fig. 3. Homogenous physical mixture was prepared by mixing Aerosil® 200 VV Pharma and lutein (1/1, w/w ratio) using mortar and pestle. Pure crystalline lutein showed two small endothermic peaks at about 60 °C (curve C). This endothermic peak of lutein is in compliance with previous report [18]. The physical mixture exhibited a relatively two small endothermic peaks for lutein (curve D). Aerosil® 200 VV Pharma did not show any peak over the entire range of the tested temperatures (curve A). No obvious peak for lutein was found for the S-SNEDDS (curve B), indicating that the drug must be present in amorphous or molecularly dissolved state in solid SEDDS [12,13,17].

The internal physical state of lutein in the S-SNEDDS was further verified using XRPD diffractograms (Fig. 4). Pure lutein powder, and physical mixture showed prominent diffraction peaks in

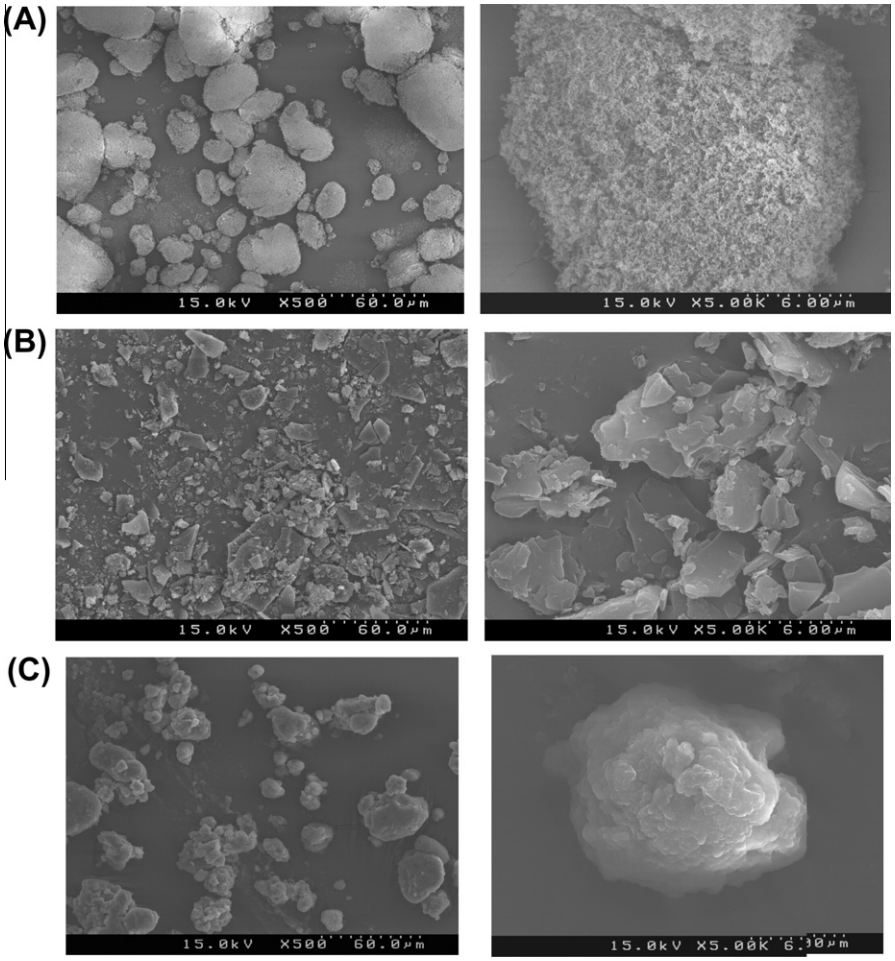

Fig. 2. Scanning electron microscope (SEM) pictures of (A) Aerosil 200 VV Pharma, (B) Lutein powder (LP), and (C) S-SNEDDS formulations under 500× and 5000× magnification, respectively.

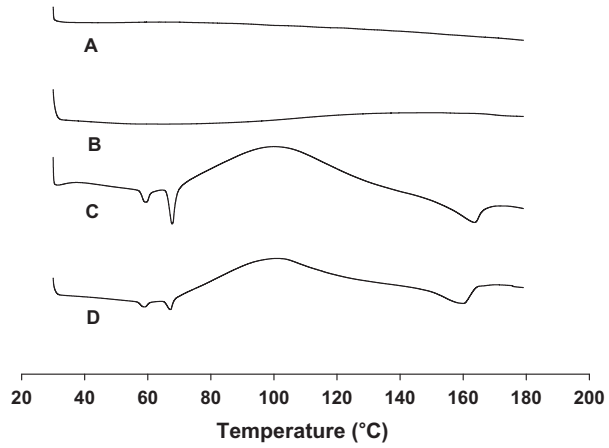

Fig. 3. Differential scanning calorimetric thermograms of (A) Aerosil® 200 VV Pharma, (B) S-SNEDDS, (C) lutein powder (LP), and (D) physical mixture of Aerosil® 200 VV Pharma and lutein powder (1:1, w/w) prepared using mortar and pestle.

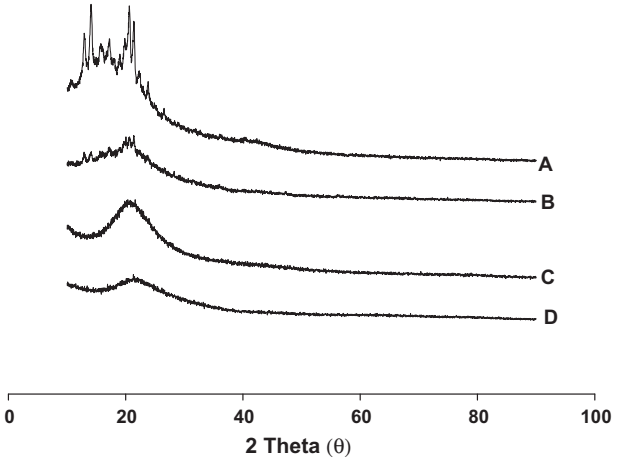

Fig. 4. X-ray powder diffractograms of (A) lutein powder (LP), (B) physical mixture of Aerosil® 200 VV Pharma and lutein powder (1:1, w/w) prepared using mortar and pestle, (C) Aerosil® 200 VV Pharma, and (D) S-SNEDDS.

the range of 5–25° 2θ (curve A and curve B). However, no obvious peaks representing crystals of lutein were seen for the S-SNEDDS, indicating the absence of crystalline structure of lutein in the formulation (curve D).

3.2. Reconstitution properties of S-SNEDDS

S-SNEDDS formulation should disperse quickly and completely when subjected to aqueous environment under mild agitation. The efficiency of self-emulsification can be estimated by measuring the rate of emulsification and the droplet size distribution. The rate of emulsification of S-SNEDDS formulations is measured by visual observation as reported previously [10,19,20]. It was observed that emulsification time of SNEDDS was 17 ± 2 s for SNEDDS, while it was 33 ± 5 s for the S-SNEDDS formulations (Table 2). The efficiency of self-emulsification of surfactant and co-surfactant is much related to their hydrophilic–lipophilic balance (HLB) value. Generally, surfactants with HLB 12-15 are regarded as being of good efficiency for self-emulsification. Thus, labrasol with HLB value of 14 (known to possess good self-emulsification ability) was selected as primary surfactant in SNEDDS for proper self-emulsification.

Table 2
Self-emulsification properties of SNEDDS and S-SNEDDS expressed in terms of emulsification time, zeta potential, droplet size, and polydispersity index.

Vehicle	SNEDDS	S-SNEDDS
Emulsification time (in seconds)	17 ± 2	33 ± 5
Zeta potential (ζ)	+1.40 ± 0.22	+1.16 ± 0.35
Droplet size (nm)	88.12 ± 3.56	92.24 ± 4.27
Polydispersity index (PDI)	0.167 ± 0.019	0.208 ± 0.033

Mean values of three samples ± standard deviation.

It has been reported that the nature of the oil affects the emulsion droplet size. Variation in penetration of oil molecules into the surfactant alkyl chain region affects interfacial film composition and flexibility. Any change in interfacial film influences the surface curvature of the drop let leading to differences in the droplet size [21,22]. The emulsion droplet size and polydispersity index (PDI) of SNEDDS and S-SNEDDS are shown in Table 2. The droplet size and PDI were 88.12 ± 3.56 nm and 0.167 ± 0.019 for SNEDDS, and 92.24 ± 4.27 nm and 0.208 ± 0.033 for S-SNEDDS, respectively. Both formulations showed reasonable homogeneity. No significant difference between droplet sizes of SNEDDS and S-SNEDDS suggested the capability of the lipid components of S-SNEDDS to retain its emulsification properties irrespective of physical form change. The results suggested that the oil phase used in this study positively influenced the formation of relatively nano-sized droplets. Both labrasol and phosphatidylcholine are widely used in the pharmaceutical and food industries due to their excellent safety profile. These lipid-based components were expected to help the emulsion formed in the stomach be readily restructured into mixed micelles even in the absence of biliary phospholipid, thereby facilitating the uptake of lutein by enterocytes [10,12,17].

The results from zeta potential analyses of both formulations were shown in Table 2. The zeta potential of SNEDDS and S-SNEDDS were +1.40 ± 0.22 and +1.16 ± 0.35, respectively. There was no significant difference between the charges of the two formulations. It is reported that in addition to particle size, zeta potential also plays an important role in the interactions with mucus of the gastrointestinal tract [21,22]. In our study, the surface charge of the formed emulsion was positive for the droplets in 0.1 N HCl, suggesting the formulated SNEDDS would reach a positive zeta potential at physiological pH. It was reported earlier that the positively charged droplets could have better interaction with the mucus of the gastrointestinal tract as the intestinal cells carry negative charges with the presence of mucosal fluid [12,17]. Because of this reason, it is likely that the SNEDDS would enhance the intestinal absorption of lutein.

3.3. In vitro release study

Lutein release profile from the S-SNEDDS, SNEDDS, LP, or CP was presented in Fig. 5. The lutein dissolution from both SNEDDS and S-SNEDDS formulations took place immediately. The percentage dissolution of lutein at 30 min was 80.72 ± 1.05% and 79.45 ± 1.03% from SNEDDS and S-SNEDDS, respectively. The solid carrier used (Aerosil 200 VV Pharma) in the present study did not interfere the dissolution of lutein from the S-SNEDDS. At the end of the study, the dissolution was around 90% for both formulations. Once lutein was dissolved from the SNEDDS, the drug did not form precipitation or aggregation. No lutein was detected in the dissolution medium from LP or CP even up to 4 h. This phenomenon was in compliance with early report [10].The results of the release profile suggested that the S-SNEDDS preserved the enhancement of

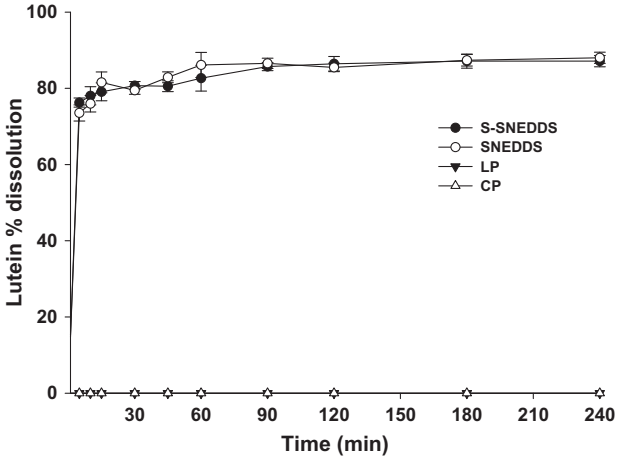

Fig. 5. Dissolution profile of lutein from SNEDDS, S-SNEDDS, lutein powder (LP), or CP in distilled water. Each value represents mean ± standard deviation ($n = 6$).

in vitro dissolution of SNEDDS and would eventually enhance the dissolution of drug in vivo.

3.4. Bioavailability in rabbits

The PK parameters of lutein following oral administration of SNEDDS, S-SNEDDS, LP, or CP in rabbits at a dose of 5 mg/kg were shown in Table 3. The plasma concentration–time profile of lutein from these formulations was shown in Fig. 6. There were no significant differences between the PK parameters of LP and CP. Interestingly, even though there was no lutein release from CP or LP in the in vitro drug release study, plasma concentrations of lutein were measurable in rabbits with C_{max} of 19.09 ± 7.66 ng/ml and 6.98 ± 4.36 ng/ml for CP and LP, respectively. This implies the involvement of endogenous emulsifiers in promoting solubilization and absorption of lutein in vivo.

It has been reported that lutein a poorly water-soluble lipophilic compound, follows the same route of absorption like lipids [23–25]. Although the exact mechanism of the absorption is not yet fully understood, lutein has been thought to be absorbed though enterocytes by simple diffusion or receptor-mediated transport. Specifically, lutein is emulsified into small lipid droplets in the stomach and further incorporated into mixed micelles by the action of bile salts and biliary phospholipids, after which mixed micelles are taken up by enterocytes with the aid of the scavenger receptor class B type I (SR-BI), a member of the ATP-binding cassette (ABC) transporter super-family [7,8,23–25]. Thus, the appearance of relatively low concentrations of lutein in rabbits' plasma was possibly due to the involvement of the aforementioned absorption mechanism.

SNEDDS formulations that rely on their own self-emulsifying capabilities showed enhanced absorption of lutein in rabbits with a C_{max} and AUC of 174.48 ± 21.48 and 1218.92 ± 312.87 ng h ml^{-1} for SNEDDS, and 148.24 ± 36.84 and 1166.84 ± 429.12 ng h ml^{-1} for S-SNEDDS, respectively. The concentration profiles of SNEDDS and S-SNEDDS were comparable and bioequivalent, suggesting that the S-SNEDDS maintained the absorption characteristics of SNEDDS. The enhancement of C_{max} compared with LP and CP was about 25-folds and 9-folds for SNEDDS and was about 21-folds and 8-folds for S-SNEDDS formulations, respectively. The relative BA of SNEDDS and S-SNEDDS was 2.86-folds and 2.74-folds compared with CP, and 12.32-folds and 11.79-folds compared with LP, respectively.

There was no significant differences between the half-life ($t_{1/2}$) and elimination rate constant (λ_z) of lutein for all tested formulations. Time required to reach the maximum plasma concentration (T_{max}) of lutein for all formulations was same with a value of 12 h (Table 3). The delay in T_{max} (12 h) suggests possible absorption and circulation of lutein though lymphatic pathway rather than direct systemic absorption. It was reported that the compounds with logP > 5 tend to be absorbed via the lymphatic route by the formation of chylomicrons, which is the usual pathway for the lipid absorption [8,25,26]. Besides, it was reported that lutein showed increased absorption in the presence of PC, indicating that chylomicron transportation to intestinal lymphatic system is involved.

Surfactants are known to increase the permeability of drugs through perturbation of the cell membrane (transcellular permeation) and/or modifying tight junction between the cells (paracellular permeation) [27–30]. However, if the surfactant or co-surfactant is contributing to the drug solubilization, there could be a risk of precipitation leading to decreased BA [31–33]. This is crucial especially for the high lipophilic compounds like lutein with logP > 5. For this reason, the solubility of the drug in oil phase is more important as the ability of the nanoemulsion to maintain the drug in solubilized form is greatly influenced by the solubility of the drug in the oil phase [34,35]. Phosal® 53 MCT contains PC, a phospholipid primarily present in bile secretion, undergoes natural process of digestion and is converted into lyso-phosphatidylcholine (LPC) by phospholipase in GI tract. Both PC and LPC are efficient emulsifiers and present the drug in solubilized form in GI tract, and eventually enhance the uptake of lutein by intestinal cells [7,8]. Besides, PCs enhance the BA through selective lymphatic delivery by assisting in chylomicron formation. For this reason, egg yolk which is rich source of phospholipid is a highly bioavailable source of lutein, although the amounts are considerably less than other lutein-rich vegetable sources [1–4,7,8]. Thus, for highly lipophilic compound like lutein (logP 7.8), incorporation of PC as one of the components would yield more satisfactory enhancement in BA as demonstrated by this study.

Most of the commercial products containing lutein in vegetable oil-based suspension filled in soft gelatin capsules should be taken

Table 3
Pharmacokinetic parameters of lutein obtained after oral administration of SNEDDS, S-SNEDDS, LP, or CP at a dose of 5 mg/kg of lutein in rabbits ($n = 3$).

PK Parameters	SNEDDS	S-SNEDDS	LP	CP
T_{max} (h)	12.00 ± 0.00	12.00 ± 0.00	12.00 ± 0.00	12.00 ± 0.00
C_{max} (ng/ml)	174.48 ± 21.48*,**	148.24 ± 36.84*,**	6.98 ± 4.36	19.09 ± 7.66
AUC_{0-48} (ng h/ml)	1218.92 ± 312.87*,**	1166.84 ± 429.12*,**	98.94 ± 53.87	425.51 ± 118.74
$t_{1/2}$ (h)	10.19 ± 1.50	8.26 ± 2.60	11.93 ± 2.50	13.92 ± 3.80
λ_z (h^{-1})	0.067 ± 0.014	0.084 ± 0.027	0.058 ± 0.037	0.050 ± 0.044
Relative BA	12.32	11.79	–	4.30

All values are expressed as mean of the samples ± standard deviation; BA bioavailability; CP commercial product that is a vegetable oil suspension containing lutein; LP lutein powder; S-SNEDDS solid self-nanoemulsifying drug delivery system; AUC_{0-48} area under the concentration–time curve from the time of dosing to last observation; C_{max} maximum measured plasma concentration; T_{max} time of maximum plasma concentration; $t_{1/2}$ half-life; λ_z elimination rate constant.

* Significant difference ($p < 0.05$) compared with LP.

** Significant difference ($p < 0.05$) compared with CP; SNEDDS self-nanoemulsifying drug delivery system.

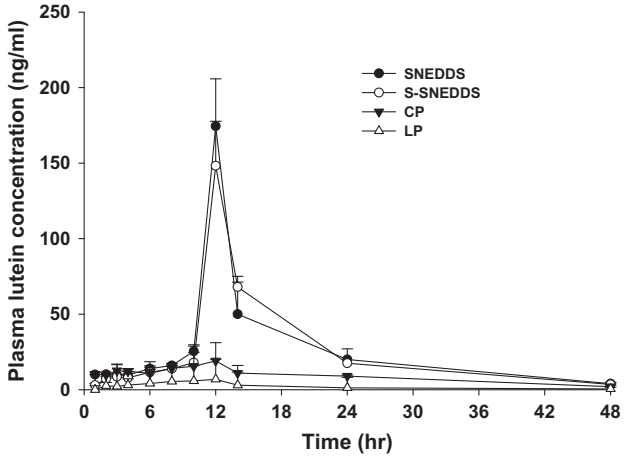

Fig. 6. Plasma concentration–time profiles of lutein following oral administration of 5 mg/kg equivalent dose of lutein from SNEDDS, S-SNEDDS, lutein powder (LP), or commercial product (CP) in rabbits. Each value represents mean ± standard deviation (n = 3).

right after meal so that lutein is emulsified by physiological emulsifiers such as bile juice. However, any disturbance in endogenous pathway, such as absence of bile or any generalized malfunction would interfere with absorption of lutein leading to ocular diseases. The S-SNEDDS that we prepared containing PC (an efficient endogenous emulsifier) does not need to be taken after meal as was evidenced by enhanced BA. This especially offers benefit to the elderly and critically ill patients with high incidence of ocular diseases who cannot eat food appropriately.

4. Conclusion

In this study, S-SNEDDS containing PC was prepared for the delivery of lutein by spray drying using Aerosil 200 VV Pharma as the inert solid carrier. The solid state characterization of S-SNEDDS by SEM, DSC, and XRPD revealed the absence of crystalline lutein in the formulation. Reconstitution evaluation of S-SNEDDS demonstrated excellent self-emulsification properties similar to SNEDDS. In vitro drug release study demonstrated faster and excellent drug release profile of S-SNEDDS and SNEDDS compared with LP and CP. The BA study in rabbits showed significantly enhanced BA of lutein from S-SNEDDS and SNEDDS compared with LP or CP. Relative BA of S-SNEDDS to that of SNEDDS was comparable and bioequivalent, indicating its ability to preserve the self-emulsification functionality and performance of SNEDDS. Therefore,
S-SNEDDS containing PC for the delivery of lutein would be a promising dosage form in the prevention of ocular diseases like AMD, blindness, etc.

5. Conflict of interest

Authors have no conflict of interest with the content of this article.

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