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# Enhanced bioavailability and retinal accumulation of lutein from self-emulsifying phospholipid suspension (SEPS)

Srinivasan Shanmugam[a], Jae-Hyun Park[a], Kyeong Soo Kim[a], Zong Zhu Piao[a], Chul Soon Yong[b], Han-Gon Choi[c,**], Jong Soo Woo[a,*]

[a] Pharm. R&D Institute, Hanmi Pharm. Co., Ltd., Hwasung, Gyeonggi, 445-913, Republic of Korea
[b] College of Pharmacy, Yeungnam University, Gyeongsan, Gyeongbuk, 712-749, Republic of Korea
[c] College of Pharmacy, Hanyang University, Seoul, Gyeonggi, 133-791, Republic of Korea

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ABSTRACT

Ability of any formulation to keep the drug in solubilized form in vivo is essential for bioavailability (BA) enhancement rather than the solubility of drug in the formulation vehicle/matrix itself. Besides, utilization of an excess amount of surfactants/co-surfactants to solubilize the drug in the lipid formulation poses potential pharmaceutical as well as health problems. To address this problem, self-emulsifying phospholipid suspension (SEPS) consisting of high amount of phospholipid (an endogenous lipid with efficient in vivo emulsification capability) and relatively low amount of surfactant/co-surfactant has been proposed to enhance the bioavailability (BA) of lutein. In this study, the ability of SEPS formulation to enhance the BA of lutein was assessed from three SEPS formulations with various amounts of phospholipid (SEPS-0, SEPS-I, and SEPS-II with 0 mg, 250 mg, and 500 mg of Phosal® 53 MCT, respectively) in beagle dogs following a single oral administration of lutein equivalent to 100 mg, and were compared with commercial formulation (CF). In addition, the retinal accumulation of lutein in Sprague Dawley (SD) rats' eyes from SEPS-II formulation (lutein dose of 100 mg/kg/day) was investigated following single daily oral administration for a period of 14 days. CF and placebo (vegetable oil without lutein) were also administered for the same period of time and were compared with the SEPS-II formulation. In the relative BA study in beagle dogs, no significant differences were observed between the pharmacokinetic (PK) parameters of formulation SEPS-O and CF. However, the $C_{max}$ in comparison to CF was 3.70 folds and 11.76 folds higher for SEPS-I and SEPS-II, respectively. Relative BA compared to CF was 178.88% and 473.13% for SEPS-I and SEPS-II, respectively. The retinal lutein accumulation was $0.91 \pm 0.31$ ng/g, $3.45 \pm 1.63$ ng/g, and $14.72 \pm 2.02$ ng/g for placebo, CF, and SEPS-II, respectively. This enhancement was about 16.1 folds and 4.27 folds compared to placebo and CF, respectively. The relative BA study in dogs and retinal accumulation study in rats demonstrated the excellent ability of SEPS to enhance the BA of lutein. For this reason, SEPS containing lutein could be a promising lipid based delivery system for the prevention of ocular diseases.



## 1. Introduction

Lutein (Fig. 1) is one among 600 carotenoids that has been gaining huge attention lately because of its ability to prevent ocular diseases including age-related macular degeneration (AMD) and cataracts (Hankinson et al., 1992; Brown et al., 1999; Chasan-Taber et al., 1999; Lyle et al., 1999; Berendschot et al., 2000; Gale et al., 2001; Bone et al., 2001; Bone et al., 2003). 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 (Amar et al., 2003; Johnson, 2004). Bioavailability (BA) of lutein is extremely variable due to the inherently poor aqueous solubility (0.732 μg/ml) and also depends on the nature of vehicle (Handelman et al., 1999; Surai et al., 2000). Besides, lutein shows increased absorption in the presence of lipids and therefore is considered as a potential candidate for lipid based oral drug delivery system (LBODDS) (Chakraborty et al., 2009).

LBODDS, that utilizes lipids as a carrier for poorly water soluble compounds, is one of the promising methods for enhancing BA of highly lipophilic compounds through enhanced dissolution and selective lymphatic uptake (Pouton, 2006). One of the most popular and commercially viable formulation approaches among LBODDS is

* Corresponding author. Tel.: +82 31 356 3311; fax: +82 31 356 7139.
** Co-corresponding author. Tel.: +82 31 400 5802; fax: +82 31 400 5958.
*E-mail addresses:* hangon@hanyang.ac.kr (H.-G. Choi), jswoo@hanmi.co.kr (J.S. Woo).

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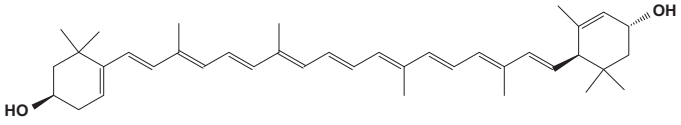


**Fig. 1.** Chemical structure of lutein.

self emulsifying drug delivery systems (SEDDS) (Nazzal et al., 2002; Wang et al., 2009). Although traditional method of preparation of SEDDS involves solubilization of drugs in oils and surfactants/co-surfactants, some compounds impose solubility problems in both water as well as lipids and are difficult to solubilize even in lipid vehicles. Solubilization of such compounds requires huge amount of surfactants/co-surfactants and presents potential drawback of possible precipitation or recrystallization upon storage, and possible gastrointestinal (GI) irritation in vivo (Nazzal et al., 2002; Pouton, 2006; Chakraborty et al., 2009; Balakrishnan et al., 2009; Wang et al., 2009).

It has been reported that the ability of any lipid formulation to keep the drug in solubilized form after dilution in the GI tract is critical for BA enhancement rather than solubility of the drug in the formulation vehicle itself (Nazzal et al., 2002; Pouton, 2006; Chakraborty et al., 2009; Wang et al., 2009). Lipid excipients are able to solubilize hydrophobic drugs within the dosage form matrix, however their solubilization capacity might be lost due to three Ds viz., dispersion, dilution, and digestion in GI tract (Chakraborty et al., 2009). Therefore, necessity of new lipid vehicles that could withstand three Ds of GI tract for enhanced BA of highly lipophilic drugs such as lutein has been gaining much momentum lately.

Phosphatidylcholine (PC), a phospholipid primarily presents 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 (Sugawara et al., 2001; Baskaran et al., 2003; Yonekura and Nagao, 2007). Besides, PCs enhance the BA through selective lymphatic delivery by assisting in chylomicron formation. Recent reports indicate that the BA of lutein from egg yolk is relatively higher than from other lutein-rich vegetable sources even though the amount of lutein is considerably less than other sources. This enhanced BA of lutein from egg yolk was apparently due to the presence of PC in egg yolk (egg contains high amount of PC and little amount of lutein) (Handelman et al., 1999; Surai et al., 2000). Eventually, self-emulsifying phospholipid suspension (SEPS) consisting of high amount of phospholipid and relatively low amount of surfactant/co-surfactant has been proposed to enhance the bioavailability (BA) of lutein.

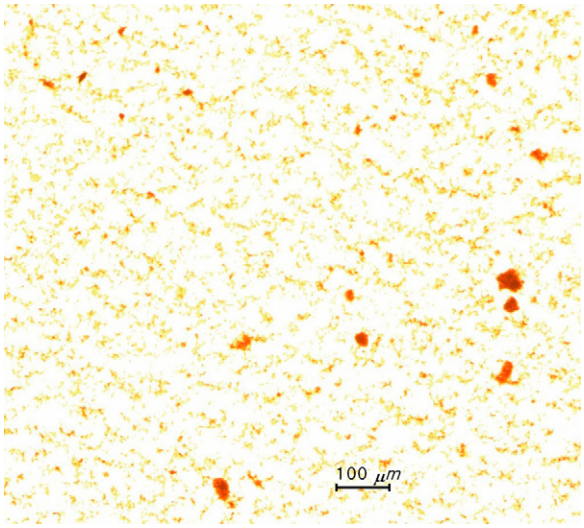


**Fig. 2.** Optical microscopic picture of SEPS-II formulation viewed with 200× magnification.

In this study, the ability of SEPS to enhance the BA of lutein was assessed from three SEPS formulations with various amounts of phospholipid (SEPS-0, SEPS-I, and SEPS-II with 0 mg, 250 mg, and 500 mg of Phosal® 53 MCT, respectively) in beagle dogs following a single oral administration of lutein equivalent to 100 mg, and were compared with commercial formulation (CF). In addition, the retinal accumulation of lutein in Sprague Dawley (SD) rats' eyes from SEPS-II formulation was investigated following a single daily oral administration of lutein equivalent to 100 mg/kg for a period of 14 days (Fig. 2). CF and placebo (vegetable oil without lutein) were also administered for the same period of time and were compared with the SEPS-II formulation.

## 2. Materials and methods

### 2.1. Materials

The following materials were purchased from various companies and then used as received. Lutein 40% extract in sunflower oil (INABATA, Japan) was purchased and used without further purification. Phosal® 53 MCT was purchased from Phospholipid GmBH (Cologne, Germany). Propylene glycol monocaprylate (Capryol™ PGMC) was purchased from Gattefossé (Saint-Priest Cedex, France). Polysorbate 80 (Tween 80), ascorbic acid, glycerin, and D-α-tocopherol were obtained from Sigma Chemicals Co. Ltd. (St. Louis, USA). Methanol, acetonitrile, chloroform, tetrahydrofuran, and all other chemicals were of HPLC grade, and used without further purification. Commercial formulation (CF), Eyelak Lutein®, a vegetable oil suspension of 20 mg of lutein in soft gelatin capsule was purchased from Korea Arlico Pharm. Co. Ltd. (Seoul, South Korea).

### 2.2. Preparation of SEPS

The composition of vehicles used for the preparation of self-emulsifying phospholipid suspension (SEPS) is shown in Table 1. SEPS was prepared by dispersing appropriate amount of 40% lutein extract (lutein equivalent to 10 mg) into a mixture of Phosal® 53 MCT, Tween 80, and Capryol™ PGMC. To the above mixture, 10 mg of D-α-tocopherol and 30 mg of glycerin were added. The final mixture was then homogenized by Ultra-Turrax homogenizer at 4000 rpm for 20 min at ambient temperature to obtain a homogeneous suspension. The formulation was then filtered through Tyler mesh #80 (pore size of 180 μm) and was filled into the body of hard gelatin capsules. The SEPS without phospholipid (SEPS-O) was prepared by the above-mentioned procedure without the addition of

**Table 1**
Composition of self-emulsifying phospholipid suspension (SEPS).

| Component | Composition | Amount (mg) | | |
|---|---|---|---|---|
| | | SEPS-O | SEPS-I | SEPS-II |
| Lutein 40% extract | API | 25[b] | 25[b] | 25[b] |
| Phosal® 53 MCT [a] | Phospholipid | – | 250 | 500 |
| Tween 80 | Polyoxyethylene sorbitan monooleate | 75 | 75 | 75 |
| Capryol™ PGMC | Propylene glycol caprylate | 50 | 50 | 50 |
| D-α-Tocopherol | Antioxidant | 10 | 10 | 10 |
| Glycerin | Humectant | 30 | 30 | 30 |
| Total | | 190 | 440 | 690 |

[a] Phosal® 53 MCT is 53% phosphatidylcholine in medium chain fatty acid triglycerides; SEPS self-emulsifying phospholipid suspension.
[b] Lutein 10 mg in 25 mg of 40% extract in sunflower oil.

Phosal® 53 MCT. The three final formulations SEPS-O, SEPS-I, and SEPS-II containing 0 mg, 250 mg, and 500 mg of Phosal® 53 MCT, respectively, were stored appropriately in a closed container.

### 2.3. Characterization of SEPS

The particle size and shape of the SEPS formulations were assessed using Olympus BX51 optical microscope (Olympus corporation, Japan) with ImagePartner™ 3.5 software for calibration purpose. The viscosity of the SEPS formulations was measured using Brookfield Programmable DV-II+ Viscometer (Brookfield Engineering Laboratories, Inc., USA). The pH of the SEPS formulations was measured using Orion 3 Star pH meter (Thermo Electron Corporation, USA). Particle size, polydispersity index (PI), and zeta potential ($\zeta$) of the emulsion formed by the addition of 200 μl of SEPS into 200 ml of distilled water under slight agitation was determined by Photal ELSZ zeta potential & particle size analyzer (Otsuka Electronics Co. Ltd., Japan). All measurements were performed in triplicates.

### 2.4. Physicochemical stability of lutein in SEPS

In order to investigate the chemical stability of lutein in the prepared SEPS formulations, stability studies were performed at accelerated condition (40 °C and 75 ± 5% relative humidity (RH)) and at room temperature (25 ± 2 °C), for a period of eight weeks in stability chamber (FTL-600, Fine Scientific Instruments, Korea). The samples were analyzed at 0, 1, 2, 4, and 8 weeks. The amount of lutein was analyzed by a validated HPLC method mentioned below. All the analyses were performed in triplicates. The physical stability in terms of phase separation was also assessed for the same period of time (eight weeks) at the same storage conditions (accelerated and room temperature).

The amount of lutein in the stability samples was measured using Hitachi HPLC system equipped with L-2130 pump, L-2200 autosampler, and L-2400 UV-VIS detector at 446 nm. The column used was Inertsil ODS-4 (4.6 mm × 250 mm, GLScience Inc., Japan) with a column temperature of 25 °C, and mobile phase consisted of a mixture of methanol: acetonitrile: tetrahydrofuran (10:9:1 (v/v/v)). Injection volume was 5 μl and flow rate was 1.2 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 lutein concentration.

### 2.5. Lutein release study

Lutein release from the three SEPS formulations was investigated using USP XXIII, dissolution apparatus II with 900 ml of distilled water as dissolution medium at 37 ± 0.5 °C with paddle speed of 100 rpm. The capsules containing formulations (10 mg as of lutein) were introduced into DST-810 dissolution tester (Labfine, Korea). At predetermined time intervals, an aliquot of 5 ml was collected, filtered, and analyzed for the content of lutein by the above-mentioned HPLC method. An equivalent volume (5 ml) of fresh dissolution medium was replaced to compensate the loss due to sampling, and the sink condition was maintained throughout the release study. Commercial formulation (CF) was also tested by the same dissolution condition for comparison of release profile.

### 2.6. Relative BA and retinal accumulation study

#### 2.6.1. Animals

All animal treatment protocols were in accordance with National Institute of Health (NIH) guidelines, South Korea. Male Sprague-Dawley (SD) rats weighing about 275–315 g and male beagle dogs weighing about 10–12 kg were purchased from Oriental Bio (Seoul, Korea). The rats were kept in plastic cages with free access to Purina Certified Rodent Chow No. 5002 meal (Ralston Purina, St. Louis, MO) and water. Dogs were housed individually with free access to Golden-pet dog diet (Agribrands, Seoul, Korea) and water. Animals were maintained on 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% RH (TECNIPLAST, Italy).

#### 2.6.2. In vivo protocol

To evaluate the relative BA of lutein from the three SEPS formulations in beagle dogs, animals were weighed and divided into 4 groups with 4 animals in each group. The animals were fasted for 12 h prior to study and weighed before dosing. On the experiment day, capsules containing CF, SEPS-O, SEPS-I, or SEPS-II with lutein equivalent to 100 mg were orally administered with 40 ml of water. Blood samples (1.5 ml) were collected prior to and at 1, 2, 3, 4, 6, 8, 10, and 24 h following administration. Plasma samples were harvested by centrifugation at 1500 *g* for 10 min and stored at −20 °C until analysis.

To evaluate the amount of lutein accumulated in the retina of the eyes of SD rats, animals were randomly divided into three groups ($n = 6$). Two groups of animals received single oral dose of either CF or SEPS-II, daily for 14 days. The dose of lutein was fixed at 100 mg/kg/day for each animal. The third group animals received same volume of placebo formulation containing vegetable oil without lutein for the same period of time. On day 15, pair of eyeballs from each animal was extracted after sacrificing by bleeding the abdominal aorta under diethyl ether anesthesia. The eyes were kept in a container and stored in −80 °C until analysis.

#### 2.6.3. HPLC analyses of lutein in dog plasma and rat eye

The analyses of lutein levels in all plasma and eye samples were measured by Hitachi HPLC system equipped with L-2130 pump, L-2200 Autosampler, and L-2400 UV-VIS detector at 446 nm was used. The column used was Inertsil ODS-4 (4.6 mm × 250 mm, GLScience Inc., Japan) with a column temperature of 25 °C, and mobile phase consisted of a mixture of methanol: acetonitrile: chloroform (47:47:6 (v/v/v)). Injection volume was 100 μl and flow rate was 1.0 ml/min. Linearity of lutein was investigated separately in dog plasma and rat eye homogenate by constructing six-point calibration curves at concentration range of 0.01–5 μg/ml. Calibration curves showed excellent linearity with satisfactory coefficients of determination for dog plasma ($R^2 > 0.9996$) and rat eye homogenate ($R^2 > 0.9994$) samples. The method was precise and accurate with coefficient of variations of less than 10% for both analyses.

Prior to extraction, frozen plasma samples were thawed in a water bath at ambient temperature, and an aliquot of 500 μl of plasma was used for extraction of lutein. To the 500 μl plasma, 50 μl of internal standard (2 μg/ml of β-Apo-8′-carotenal (trans) in ethanol) was added and vortexed for 5 s. To this, 3 ml of isopropyl alcohol/ethyl acetate solution (1/1 (v/v)) containing 0.0001% butylated hydroxytoluene was added, and vortexed again for 5 min followed by centrifugation at 800 × *g* for 5 min. The supernatant organic layer (3 ml) was evaporated under nitrogen gas. The residue was then reconstituted with 200 μl of mobile phase using vortex mixer followed by centrifugation at 12,000 × *g* for 3 min, and 100 μl of this solution was injected into HPLC for lutein measurement.

The retinal lutein content was analyzed by combining both eyes of each rat. The eyes were homogenized in a 0.6 ml of methanol containing 0.0001% (w/v) of butylated hydroxytoluene using Ultra-Turrax homogenizer at 21,000 rpm for 15 min under ice bath. The homogenizer was then washed with 0.6 ml of phosphate buffered saline containing 1% ascorbic acid and mixed with the homogenate using vortex. To 1 ml of the homogenate 10 μl of internal standard (2 μg/ml of β-Apo-8′-carotenal (trans) dissolved in ethanol) was added and vortexed for 5 s. About 0.8 ml of ethyl acetate solution

containing 0.0001% butylated hydroxytoluene was added and vortexed again for 30 min. The resultant mixture was then centrifuged at 4000 × g for 10 min. The organic phase was extracted and evaporated under nitrogen. The residue was reconstituted with addition of 200 μl of mobile phase using vortex mixer followed by centrifugation at 12,000 × g for 3 min and 100 μl of this solution was injected into HPLC for lutein content measurement.

### 2.7. PK parameter analyses

The plasma concentration of lutein vs. time profile was analyzed by a non-compartmental method using WinNonlin Professional Version 2.1 program for windows (Pharsight, Cary, NC, USA). The relative bioavailability (BA) of lutein to reference drug, CF was calculated using the following equation:

$$\text{Relative BA}\,(\%) = \frac{\text{AUC}_{\text{test}}}{\text{AUC}_{\text{reference}}} \times \frac{\text{Dose}_{\text{reference}}}{\text{Dose}_{\text{test}}} \times 100$$

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

### 2.8. 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

One of the primary challenges to any oral formulation is maintaining drug solubility within the gastrointestinal tract (O'Driscoll and Griffin, 2008; Kohli et al., 2010). For poorly water soluble lipophilic compounds that exhibit dissolution-rate-limited absorption, SEDDS can offer an improvement in rate and extent of absorption. However, poorly water-soluble compounds, which are generally classified as lipophilic, behave differently in similar kind of vehicles and therefore, demanding the need to assess compounds on individual basis for successful formulation (Tang et al., 2008; Kohli et al., 2010). Besides, it has been reported that lipid excipients capable of being digested in the GI tract play a major role in determining the rate and extent of absorption of drugs from the GI tract. Even the lipid excipients capable of solubilizing the high lipophilic drug in formulation matrix with good in vitro dissolution profile would result in reduction of their solubilization capacity in vivo, due to the breakdown of lipid vehicle in the presence of gastric lipase (Chen, 2008; Jannin et al., 2008; Pouton and Porter, 2008; Chakraborty et al., 2009). For this reason, ability of lipid excipients to sustain in vivo solubility rather than formulation solubility is of prime importance in the development of LBODDS formulation. Eventually, considering the endogenous emulsification capability of phospholipid, SEPS has been expected to enhance the BA of lutein.

### 3.1. Preparation of SEPS

The solubility of lutein in most of the tested lipid excipients such as oils, surfactants/co-surfactants is limited and is less than 7 mg/ml (data not shown). The compositions of SEPS with different amounts of phospholipid for the evaluation of relative BA in beagle dogs are summarized in Table 1. A relatively low amount of Tween-80 and Capryol™ PGMC were included for better miscibility of lutein 40% extract in the mixture of Phosal® 53 MCT, and for uniform dispersion of lipid components upon dilution into aqueous media. D-α-Tocopherol was added to prevent any possible oxidation of lutein during storage. All formulations of SEPS were prepared by simple mixing and homogenization method. The prepared SEPS formulations were filtered to ensure the homogeneity of suspensions.

**Table 2**
Properties of SEPS formulations expressed in terms of particle size, viscosity, pH, and polydispersity index.

| Properties | SEPS-O | SEPS-I | SEPS-II |
|---|---|---|---|
| *Suspension characteristics* | | | |
| Particle size (μm) | 64.13 ± 22.44 | 37.95 ± 12.64 | 16.72 ± 6.82 |
| Viscosity (cP) | 354.90 ± 21.50 | 472.40 ± 39.20 | 517.40 ± 27.80 |
| pH | 6.84 ± 0.43 | 6.45 ± 0.37 | 6.34 ± 0.46 |
| *Emulsion characteristics*[a] | | | |
| Particle size (μm) | 2.02 ± 0.74 | 1.49 ± 0.67 | 1.67 ± 0.48 |
| PI | 0.75 ± 0.21 | 0.48 ± 0.23 | 0.59 ± 0.17 |
| Zeta potential (ζ) | 7.98 ± 2.43 | −22.25 ± 3.87 | −24.05 ± 1.46 |

All values are mean of three values with standard deviation; cP centipoises; PI polydispersity index.
[a] Emulsion formed after addition of 200 μl of SEPS into 200 ml of distilled water under slight agitation was used for the measurement of particle size and polydispersity index (PI).

### 3.2. SEPS characterization

The particle size, viscosity, and pH of the prepared SEPS formulations are shown in Table 2. The mean particle size of all three SEPS formulations was 64.13 ± 22.44 μm, 37.95 ± 12.64 μm, and 16.72 ± 6.82 μm for SEPS-O, SEPS-I, and SEPS-II, respectively. Decreased particle size with increasing amount of PC might be due to its enhanced capability to disperse the drug effectively within the formulation vehicle. Since high viscosity ensures suspension stability from the point of view of desired rheological properties, viscosity measurements of the suspensions were carried out and the values were 354.90 ± 21.50 cP, 472.40 ± 39.20 cP, and 517.40 ± 27.80 cP for SEPS-O, SEPS-I, and SEPS-II, respectively. Relatively higher viscosity of SEPS-I and SEPS-II compared to SEPS-O suggested possible higher physical stability of these phospholipid suspensions. The pH was 6.84 ± 0.43, 6.45 ± 0.37, 6.34 ± 0.46 for SEPS-O, SEPS-I, and SEPS-II, respectively. Addition of Phosal® 53 MCT did not seem to alter the pH of SEPS formulations significantly. The particle size of the emulsion formed by adding 200 μl of all three SEPS-I and SEPS-II formulations into 200 ml of distilled water under mild agitation was found to be less than 2.0 μm (Table 2). The polydispersity index of formulations SEPS-O, SEPS-I, and SEPS-II were found to be 0.75 ± 0.21, 0.48 ± 0.23, and 0.59 ± 0.17, respectively suggesting reasonable homogeneity of the resultant emulsion. The ζ potential of SEPS-O was positive with a value of 7.98 ± 2.43 mV, while it was −22.25 ± 3.87 and −24.05 ± 1.46 for SEPS-I and SEPS-II, respectively. The value of more than ±20.0 of SEPS-I and SEPS-II formulations suggested good stability of the prepared formulation.

### 3.3. Physicochemical stability

Lutein is an isoprenoid polymer containing many conjugated double bonds (Fig. 1), which can be readily isomerized, oxidized, and degraded (Shi and Chen, 1997; Henry et al., 1998). For this reason, chemical stability study was performed and the % lutein remaining in SEPS formulations stored at room temperature (25 ± 2 °C) and accelerated condition (40 °C and 75 ± 5% RH) for a period of eight weeks is presented in Fig. 3A and B, respectively. The chemical stability profile of lutein at the end of eight weeks was not significantly different from the initial stability, and no significant degradation of the active from the initial value was seen. The % of lutein remaining after eight weeks of storage for SEPS-O, SEPS-

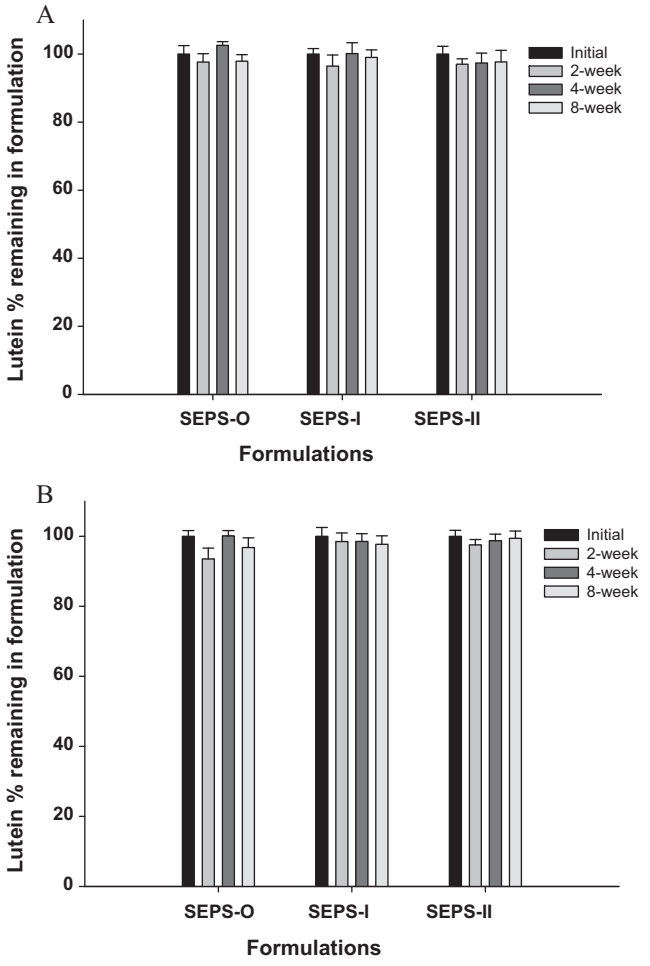


**Fig. 3.** Chemical stability of lutein in self-emulsifying phospholipid suspension (SEPS). Formulations stored at (A) 25 ± 2 °C, (B) 40 ± 2 °C/75 ± 5 RH for a period of eight weeks.

I and SEPS-II was 97.92 ± 1.90%, 99.02 ± 2.20%, and 97.70 ± 3.4% at 25 ± 2 °C, and 96.80 ± 2.71%, 97.69 ± 2.43%, and 99.40 ± 2.08% at 40 ± 2 °C, respectively. The percentage change of lutein at the end of eight weeks from the initial period was less than 3.00% for all formulations at both storage conditions, suggesting good chemical compatibility of lutein with the formulated excipients. In terms of physical stability, all three SEPS formulations showed good physical stability without any kind of phase separation at both storage temperatures for a period of eight weeks.

### 3.4. In vitro drug release study

Release profiles of lutein from SEPS formulations containing 10 mg of lutein are presented in Fig. 4. No lutein was released from the CF capsules until the end of study (up to 6 h). SEPS showed enhanced release of lutein from the capsules compared to CF. The release of lutein from SEPS-O, SEPS-I, and SEPS-II was 0.66 ± 0.01 mg, 2.38 ± 0.03 mg, and 5.46 ± 0.14 mg, respectively at the end of release study. Lutein release was highest for SEPS-II, the formulation containing highest phospholipid followed by SEPS-I, and SEPS-O. Among the SEPS, increased phospholipid resulted in increased drug release, and lutein release from SEPS-II was about 2.3 times higher than SEPS-I. The enhanced drug release from the phospholipid suspensions could be due to better emulsification properties of phospholipids.

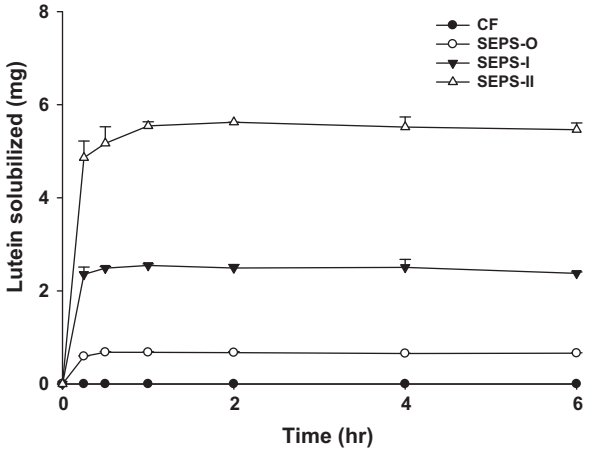


**Fig. 4.** Lutein release profile from self-emulsifying phospholipid suspension (SEPS). Formulations and commercial formulation (CF) expressed as mg of lutein dissolved in 900 ml of water.

### 3.5. Bioavailability study in beagle dogs

The plasma concentration time profiles of lutein from CF and three SEPS formulations in beagle dogs following a single oral administration of lutein equivalent to 100 mg is shown in Fig. 5. The non-compartmental PK parameters of lutein following 100 mg oral dose of lutein from capsules containing CF, SEPS-O, SEPS-I, or SEPS-II are summarized in Table 3.

There was no significant difference between the $C_{max}$ of CF and SEPS-O formulations. Relatively similar $C_{max}$ of SEPS-O compared to CF despite the presence of surfactant/co-surfactant (Tween 80/Capryol™ PGMC) could be due to the possible loss of solvent capacity upon dilution in vivo. Among the three SEPS formulations tested, it could be seen from Table 3 that $C_{max}$ of lutein was higher for suspensions with phospholipid (SEPS-I and SEPS-II) compared to suspension without phospholipid (SEPS-O). The highest $C_{max}$ of 277.93 ± 92.67 ng/ml was observed for SEPS-II that contains the highest amount of phospholipid (500 mg), and is significantly higher compared to all other formulations tested ($p < 0.001$ vs. CF, $p < 0.001$ vs. SEPS-O, and $p < 0.004$ vs. SEPS-I). The $C_{max}$ in comparison to both CF and SEPS-O formulations was about 4 folds and 11 folds higher for SEPS-I and SEPS-II formulations, respectively. This 11-fold increase in $C_{max}$ of lutein from SEPS-II clearly indicates the

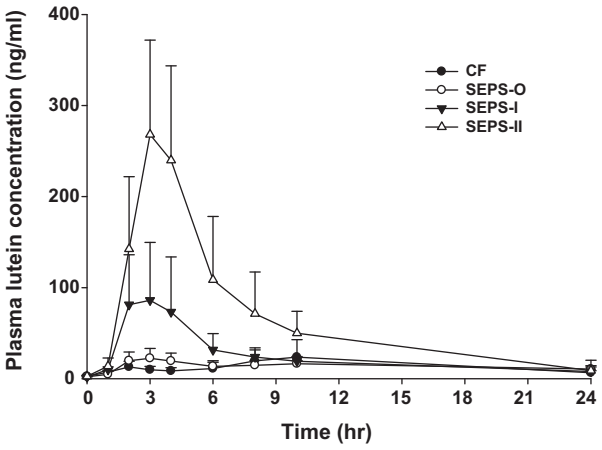


**Fig. 5.** Mean plasma concentration–time profile of lutein after oral administration of 100 mg dose of lutein from capsules of self-emulsifying phospholipid suspension (SEPS) formulations or commercial formulation (CF) in beagle dogs. Mean ± S.D. ($n = 4$).

**Table 3**
Pharmacokinetic parameters of lutein obtained after oral administration of either CF or SEPS formulations at a dose equivalent to 100 mg of lutein in male beagle dogs ($n = 4$).

| PK parameters | CF | SEPS-O | SEPS-I | SEPS-II |
|---|---|---|---|---|
| $C_{max}$ (ng/ml) | 23.63 ± 19.28 | 25.80 ± 8.11 | 87.55 ± 62.02 | 277.93 ± 92.67$^{*,\S,\Psi}$ |
| $T_{max}$ (h) | 10.00 ± 0.00 | 10 ± 2.58 | 3.00 ± 0.82$^{*,\S}$ | 3.25 ± 0.50$^{*,\S}$ |
| $AUC_{last}$ (ng h/ml) | 339.67 ± 225.70 | 338.69 ± 64.70 | 607.61 ± 311.79 | 1607.08 ± 732.43$^{*,\S,\Psi}$ |
| Relative BA (%) | – | 99.71 | 178.88 | 473.13 |

All values are expressed as mean of four samples ± standard deviation.
$^{*}$ Significant difference ($p < 0.05$) compared to CF formulation.
$^{\S}$ Significant difference ($p < 0.05$) compared between SEPS-O and SEPS-I or SEPS-II formulations.
$^{\Psi}$ Significant difference ($p < 0.05$) compared between SEPS-I and SEPS-II formulations; CF commercial formulation of lutein available as an oily suspension; SEPS self-emulsifying phospholipid suspensions of lutein; $AUC_{last}$ area under the concentration-time curve from the time of dosing to last observation; BA bioavailability; $C_{max}$ maximum measured plasma concentration; $T_{max}$ time of maximum plasma concentration.

ability of phospholipid suspensions to enhance the solubility and absorption in vivo. $AUC_{last}$ was found to be the highest for SEPS-II with a value of 1607.08 ± 732.43 and was statistically significant compared to all other formulations tested ($p < 0.012$ vs. CF, $p < 0.007$ vs. SEPS-O, and $p < 0.0031$ vs. SEPS-I). There was a five-fold increase in $AUC_{last}$ of lutein with SEPS-II formulation compared to CF. In addition, the enhanced $C_{max}$ and AUC from SEPS-I and SEPS-II in comparison to CF and SEPS-O could be due to improved lymphatic transport pathway.

Interestingly, even though there was no drug release from the commercial formulation (CF) in the in vitro drug release study, plasma concentrations of lutein in beagle dogs from the CF were measurable with $C_{max}$ of 23.63 ± 19.28 ng/ml. 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 (Clevidence and Bieri, 1993; Johnson, 2004; Yonekura and Nagao, 2007). Although the exact mechanism of absorption is not yet fully unveiled, lutein has been thought to be absorbed through 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 uptaken 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 (Yonekura and Nagao, 2007). However, any disturbance in endogenous pathway, such as absence of bile or any generalized malfunction would interfere with absorption of lutein (Clevidence and Bieri, 1993). Besides, intake of a high-fat meal (10–25 g of fat) stimulates maximal biliary and pancreatic secretions that would aid the absorption of lutein. However, the absence of high-fat meal would result in poor absorption of lutein. In this study, the minimal appearance of lutein from the CF could be due to the vegetable oil in the formulation that might have produced minimal stimulation of biliary and pancreatic juice.

Regarding the time to reach maximum plasma concentration ($T_{max}$), suspensions with phospholipid (SEPS-I and SEPS-II) reached the $C_{max}$ relatively early compared to CF and SEPS-O. The $T_{max}$ of CF (10.00 ± 0.00 h) and SEPS-O (10 ± 2.58 h) was around 10 h, while it was around 3 h for the suspensions with phospholipid (3.00 ± 0.82 h for SEPS-I and 3.25 ± 0.50 h for SEPS-II). Significant reduction in $T_{max}$ of lutein from the phospholipid suspensions could be because of the faster rate of absorption of lutein from the GI tract. Phosal® 53 MCT, the main lipid excipient used in the preparation of phospholipid suspension, is a liquid that consists mainly of phospholipids (PC 53% and LPC 6%). Digested and undigested components of PC/LPC are efficient emulsifiers in vivo that would present lutein in solubilized form as mixed micelle around the unstirred water layer for absorption by the intestinal enterocytes (Gartner et al., 1996; Castenmiller and West, 1998; Castenmiller et al., 1999). This could be the possible reason for the highest $C_{max}$ in relatively reduced time ($T_{max}$) of lutein from SEPS formulations.

Regarding the relative BA of lutein from SEPS formulations, BA of SEPS-O (without phospholipid) was similar to CF with no relative increase in BA. However, SEPS-I and SEPS-II enhanced BA of lutein enormously in comparison to CF, and the relative BA was 178.88% and 473.13% for SEPS-I and SEPS-II, respectively. A 100% increase in the amount of phospholipid from formulation SEPS-I to SEPS-II (from 250 mg to 500 mg) resulted in an increase of BA by 264.49%. The presence of surfactants/co-surfactants in SEPS-O did not seem to improve the BA of lutein compared to CF. However, SEPS-I and SEPS-II (with phospholipid) showed enhanced BA of lutein, and the enhancement increased with increasing amount of phospholipid in SEPS. Overall, this study demonstrated ability of SEPS, containing phospholipid as a main constituent, to increase the BA of lutein in beagle dogs.

### 3.6. Retinal accumulation study in SD rats

Although lutein is present in number of human tissues, the target organ for lutein oral delivery is retina of the eye, and the lutein concentrations of eye are about 500-fold higher than the concentration in other tissues (Schmitz et al., 1993). Thus, appearance of lutein in plasma always results in accumulation of lutein in retina of the eye. To assess whether or not the enhanced BA resulted in enhanced accumulation of lutein in retina of the eyes of SD rats, multiple dose study of lutein from the phospholipid suspension was performed. The phospholipid formulation that showed highest BA (SEPS-II) was chosen for this study to assess the eye accumulation.

Fig. 6 shows the retinal content of lutein in SD rats' eyes that were administered with SEPS-II or CF containing 100 mg/kg/day

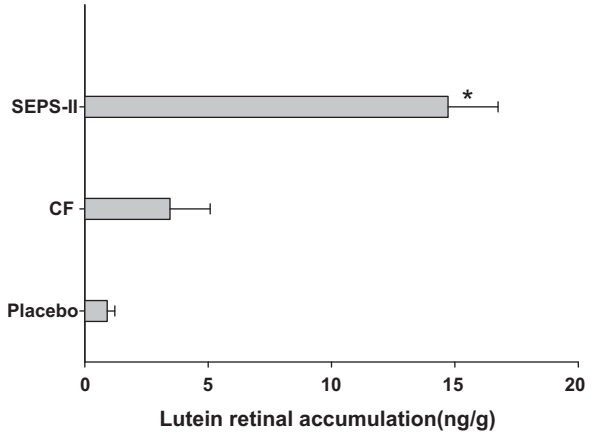


**Fig. 6.** Mean lutein content of eye in SD rats after two weeks of oral administration of 6 mg/kg dose of lutein from SEPS-II formulation or commercial formulation CF. Mean ± S.D. ($n = 6$) and * significantly different ($p < 0.05$) compared to CF or placebo.

dose of lutein daily for a period of 14 days. The results were compared by administering same volume of placebo (containing vegetable oil without lutein) for the same period of time. The retinal lutein content was $0.91 \pm 0.31$ ng/g, $3.45 \pm 1.63$ ng/g, $14.72 \pm 2.02$ ng/kg for placebo, CF, and SEPS-II, respectively. The retinal accumulation was significantly higher for SEPS-II than the placebo and CF ($p < 0.001$). The enhancement of lutein accumulation in to retina of the SD rats' eyes from SEPS-II was about 16.1 folds and 4.27 folds compared to placebo and CF, respectively.

The delivery of lutein to extrahepatic tissue is accomplished through the interaction of lipoprotein particles and lutein reaches retina of the eye through high density lipoproteins (HDL) (Yeum et al., 1995; Yeum et al., 1999; Mares-Perlman et al., 2001). One of the main compositions of HDL is phospholipid (about 29%) and ingestion of higher amount of phospholipid through SEPS formulation could possibly enhance the formation of HDL (Burgess et al., 2005). This would have enhanced the transportation and accumulation of lutein in retina of the eyes. Thus, the enhanced accumulation of lutein in the retina of eyes, the target organ, from the phospholipid suspension would be more beneficial in prevention of ocular diseases like AMD, blindness, etc.

## 4. Conclusion

Proper selection of lipid excipients is critical to an efficient lipid based drug delivery formulations. In this study, we evaluated the feasibility of SEPS as a lipid delivery system to enhance the BA of lutein, and found that SEPS enhanced the lutein BA in dogs and accumulation in retina of the eyes in rats. Both relative BA and retinal accumulation of lutein from the phospholipid formulation, SEPS-II was more than 400% in comparison to commercial formulation (CF). It could be concluded that lutein absorption into the systemic circulation (BA) and corresponding accumulation into the retina of eyes (eye content) were significantly enhanced by the self-emulsifying phospholipid suspensions (SEPS). For this reason, SEPS containing lutein could be a promising lipid based delivery system for the prevention of ocular diseases. Further development and clinical studies will be performed as a part of development process.

## Conflict of interest

Authors have no conflict of interest.

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