Self-Nanoemulsifying Drug Delivery System of Lutein: Physicochemical Properties and Effect on Bioavailability of Warfarin

Juno Yoo¹, Rengarajan Baskaran² and Bong-Kyu Yoo²,*

¹Department of Diagnostics, MediFuture, Seoul 150-835,
²College of Pharmacy, Gachon University, Incheon 406-799, Republic of Korea

Abstract
Objective of present study was to prepare and characterize self-nanoemulsifying drug delivery system (SNEDDS) of lutein and to evaluate its effect on bioavailability of warfarin. The SNEDDS was prepared using an oil, a surfactant, and co-surfactants with optimal composition based on pseudo-ternary phase diagram. Effect of the SNEDDS on the bioavailability of warfarin was performed using Sprague Dawley rats. Lutein was successfully formulated as SNEDDS for immediate self-emulsification and dissolution by using combination of Peceol as oil, Labrasol as surfactant, and Transcutol-HP or Lutrol-E400 as co-surfactant. Almost complete dissolution was achieved after 15 min while lutein was not detectable from the lutein powder or intra-capsule content of a commercial formulation. SNEDDS formulation of lutein affected bioavailability of warfarin, showing about 10% increase in Cmax and AUC of the drug in rats while lutein as non-SNEDDS did not alter these parameters. Although exact mechanism is not yet elucidated, it appears that surfactant and co-surfactant used for SNEDDS formulation caused disturbance in the anatomy of small intestinal microvilli, leading to permeability change of the mucosal membrane. Based on this finding, it is suggested that drugs with narrow therapeutic range such as warfarin be administered with caution to avoid undesirable drug interaction due to large amount of surfactants contained in SNEDDS.

Key Words: Self-nanoemulsifying drug delivery systems, Lutein, Warfarin, Dissolution, Bioavailability

INTRODUCTION
Carotenoids, poorly soluble lipophilic compounds, are a group of widely distributed plant pigments that have various physiological activities in human and animals. Lutein is one among several carotenoids that has been gaining attention lately because of its ability to prevent ocular diseases including age-related macular degeneration (AMD) and cataracts caused by complications of metabolic disorders such as diabetes (Hankinson et al., 1992; Seddon et al., 1994; Brown et al., 1999; Chasan-Taber et al., 1999; Lyle et al., 1999; Mares-Perlman et al., 2001; Gale et al., 2001). Lutein possesses antioxidant activity due to its conjugated double bonds that are highly effective in quenching reactive oxygen species that is involved in the pathogenesis of ocular diseases such as AMD and cataracts. Although lutein is a vital macular pigment with many beneficial activities, human is not capable of synthesizing lutein de novo and thus its presence in human tissues is entirely of dietary origin (Granado et al., 1996; Landrum and Bone, 2001; O'Neill et al., 2001; Johnson, 2004). Furthermore, bioavailability of lutein is reported to be very low (Chung et al., 2004; Granado-Lorencio et al., 2010). There have been many studies reported to improve its bioavailability by using various strategies such as mixed micelle and suspension formulations (Cha et al., 2011; Mamatha and Baskaran, 2011; Mitri et al., 2011; Shammugam et al., 2011).

Lipid-based oral drug delivery system has been gaining attention recently with increasing application of lipid as a carrier for the delivery of poorly soluble lipophilic drugs (Pouton, 2006; Chakraborty et al., 2009). The unique properties of lipids, namely, their physicochemical diversity, biocompatibility, and ability to enhance oral bioavailability of poorly water soluble lipophilic drugs through selective lymphatic uptake have made them attractive candidates as carriers for oral formulations. Among those, self-nanoemulsifying drug delivery system (SNEDDS) is considered as a promising approach to improve solubility and absorption of poorly water soluble lipophilic drugs (Shao et al., 2010; Wu et al., 2011; Ma et al.,...
resulting in a fine milky emulsion, and it was judged ‘bad’ when the droplets spread easily in distilled water (Yoo et al., 2010). Tendency to form an emulsion was judged as ‘good’ when the droplets spread easily in distilled water (Yoo et al., 2010). Tendency to form an emulsion was judged as ‘good’ when the droplets spread easily in distilled water (Yoo et al., 2010). Tendency to form an emulsion was judged as ‘good’ when the droplets spread easily in distilled water (Yoo et al., 2010). Tendency to form an emulsion was judged as ‘good’ when the droplets spread easily in distilled water (Yoo et al., 2010). Tendency to form an emulsion was judged as ‘good’ when the droplets spread easily in distilled water (Yoo et al., 2010). Tendency to form an emulsion was judged as ‘good’ when the droplets spread easily in distilled water (Yoo et al., 2010). Tendency to form an emulsion was judged as ‘good’ when the droplets spread easily in distilled water (Yoo et al., 2010). Tendency to form an emulsion was judged as ‘good’ when the droplets spread easily in distilled water (Yoo et al., 2010). Tendency to form an emulsion was judged as ‘good’ when the droplets spread easily in distilled water (Yoo et al., 2010). Tendency to form an emulsion was judged as ‘good’ when the droplets spread easily in distilled water (Yoo et al., 2010). Tendency to form an emulsion was judged as ‘good’ when the droplets spread easily in distilled water (Yoo et al., 2010). Tendency to form an emulsion was judged as ‘good’ when the droplets spread easily in distilled water (Yoo et al., 2010). Tendency to form an emulsion was judged as ‘good’ when the droplets spread easily in distilled water (Yoo et al., 2010). Tendency to form an emulsion was judged as ‘good’ when the droplets spread easily in distilled water (Yoo et al., 2010). Tendency to form an emulsion was judged as ‘good’ when the droplets spread easily in distilled water (Yoo et al., 2010). Tendency to form an emulsion was judged as ‘good’ when the droplets spread easily in distilled water (Yoo et al., 2010). Tendency to form an emulsion was judged as ‘good’ when the droplets spread easily in distilled water (Yoo et al., 2010). Tendency to form an emulsion was judged as ‘good’ when the droplets spread easily in distilled water (Yoo et al., 2010). Tendency to form an emulsion was judged as ‘good’ when the droplets spread easily in distilled water (Yoo et al., 2010). Tendency to form an emulsion was judged as ‘good’ when the droplets spread easily in distilled water (Yoo et al., 2010). Tendency to form an emulsion was judged as ‘good’ when the droplets spread easily in distilled water (Yoo et al., 2010). Tendency to form an emulsion was judged as ‘good’ when the droplets spread easily in distilled water (Yoo et al., 2010). Tendency to form an emulsion was judged as ‘good’ when the droplets spread easily in distilled water (Yoo et al., 2010). Tendency to form an emulsion was judged as ‘good’ when the droplets spread easily in distilled water (Yoo et al., 2010). Tendency to form an emulsion was judged as ‘good’ when the droplets spread easily in distilled water (Yoo et al., 2010). Tendency to form an emulsion was judged as ‘good’ when the droplets spread easily in distilled water (Yoo et al., 2010). Tendency to form an emulsion was judged as ‘good’ when the droplets spread easily in distilled water (Yoo et al., 2010). Tendency to form an emulsion was judged as ‘good’ when the droplets spread easily in distilled water (Yoo et al., 2010). Tendency to form an emulsion was judged as ‘good’ when the droplets spread easily in distilled water (Yoo et al., 2010). Tendency to form an emulsion was judged as ‘good’ when the droplets spread easily in distilled water (Yoo et al., 2010). Tendency to form an emulsion was judged as ‘good’ when the droplets spread easily in distilled water (Yoo et al., 2010). Tendency to form an emulsion was judged as ‘good’ when the droplets spread easily in distilled water (Yoo et al., 2010). Tendency to form an emulsion was judged as ‘good’ when the droplets spread easily in distilled water (Yoo et al., 2010). Tendency to form an emulsion was judged as ‘good’ when the droplets spread easily in distilled water (Yoo et al., 2010).
ed for deproteination of the plasma sample followed by centrifugation for 1 min at 13,000 g to precipitate the proteins, and the clear supernatant was evaporated in a centrifugal vacuum evaporator. The residue obtained was reconstituted with 200 μl of mobile phase and injected to HPLC.

RESULTS

The solubility of lutein in various surfactants, co-surfactants and oils was presented in Table 1. Among surfactants and co-surfactants tested, lutein has the highest solubility in Labrasol with 56.54 ± 4.24 mg/ml followed by Lutrol-E400 (31.53 ± 3.27 mg/ml) and Transcutol-HP (16.27 ± 2.35 mg/ml). The lowest solubility of lutein among surfactants and co-surfactants was observed with Labrafil-2125 (3.77 ± 1.22 mg/ml). Pecceol showed highest solubility of lutein (11.76 ± 1.38 mg/ml) among the oils screened, and solubility of lutein in all vegetable oils tested was less than 6 mg/ml. Therefore, Labrasol and Pecceol were chosen as surfactant and oil, respectively, and Transcutol-HP and Lutrol-E400 were chosen as co-surfactants for the preparation of two different SNEDDS.

Table 1. Composition, HLB value, and solubility profile of vehicles screened for selection of SNEDDS

<table>
<thead>
<tr>
<th>Vehicles</th>
<th>Composition</th>
<th>HLB</th>
<th>Solubility (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surfactants/Co-surfactants:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Labrasol</td>
<td>Capryliccaproyl macrogolglycerides</td>
<td>14</td>
<td>56.54 ± 4.24</td>
</tr>
<tr>
<td>Lauroglycol-FCC</td>
<td>Propyleneglycol caprylate</td>
<td>5</td>
<td>7.09 ± 1.18</td>
</tr>
<tr>
<td>Transcutol-HP</td>
<td>Diethylene glycol monoethyl ether</td>
<td>4.2</td>
<td>16.27 ± 2.35</td>
</tr>
<tr>
<td>Lutrol-E400</td>
<td>Polyethylene glycol; polyalkylene glycol; polyol</td>
<td>4</td>
<td>31.53 ± 3.27</td>
</tr>
<tr>
<td>Labrafil-M1944</td>
<td>Oleoyl macrogolglycerides</td>
<td>4</td>
<td>4.35 ± 1.02</td>
</tr>
<tr>
<td>Labrafil-2125</td>
<td>Linoleoyl macrogolglycerides</td>
<td>4</td>
<td>3.77 ± 1.22</td>
</tr>
<tr>
<td>Oils:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Labrafac CC</td>
<td>Capryliccapric triglycerides</td>
<td>1</td>
<td>7.83 ± 0.29</td>
</tr>
<tr>
<td>Pecceol</td>
<td>Glycerol monooleate</td>
<td>3</td>
<td>11.76 ± 1.38</td>
</tr>
<tr>
<td>Corn oil</td>
<td>Linoleic acid 58%; oleic acid 28%; palmitic acid 11%</td>
<td>-</td>
<td>1.86 ± 0.25</td>
</tr>
<tr>
<td>Cotton seed oil</td>
<td>Linoleic acid 54%; oleic acid 19%; palmitic acid 22%</td>
<td>-</td>
<td>2.01 ± 0.33</td>
</tr>
<tr>
<td>Peanut oil</td>
<td>Linoleic acid 32%; oleic acid 48%; palmitic acid 11%</td>
<td>-</td>
<td>1.46 ± 0.11</td>
</tr>
<tr>
<td>Castor oil</td>
<td>Ricinoleic acid 95%; oleic acid 2%; linoleic acid 1%</td>
<td>-</td>
<td>5.55 ± 0.36</td>
</tr>
</tbody>
</table>

Fig. 1. Pseudo-ternary phase diagrams of SNEDDS. (A) System A. (B) System B. Ternary mixtures inside the solid line exhibited self-emulsification, and the self-emulsification efficiency was good when sum of the surfactant and co-surfactant concentration was more than 80% of SNEDDS formulation.
**Table 2.** Vehicle composition, zeta potential, droplet size, and emulsification time of the two SNEDDS formulations

<table>
<thead>
<tr>
<th>Vehicle</th>
<th>System A</th>
<th>System B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peceol (%)</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Labrasol (%)</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>Transcutol-HP (%)</td>
<td>25</td>
<td>-</td>
</tr>
<tr>
<td>Lutrol-E400 (%)</td>
<td>-</td>
<td>25</td>
</tr>
<tr>
<td>Zeta potential (mV)</td>
<td>-3.02</td>
<td>-2.17</td>
</tr>
<tr>
<td>Droplet size (nm)</td>
<td>172.8 ± 14.8</td>
<td>93.2 ± 4.6*</td>
</tr>
<tr>
<td>Emulsification time (sec)</td>
<td>15 ± 3</td>
<td>18 ± 2</td>
</tr>
</tbody>
</table>

*p<0.01 compared to System A.

Emulsification time for both Systems was within 20 sec, showing relatively faster in the System A compared to System B (15 ± 3 vs 18 ± 2 sec). Dissolution profile of the two SNEDDS formulations was shown in Fig. 3. The dissolution of lutein was more than 90% accomplished within 10 min for the System B. Almost complete dissolution was achieved after 15 min while lutein was not detectable from the lutein powder or intra-capsule content of a commercial product (20 mg as of lutein in soft gelatin capsule) even after 4 h. No precipitation or aggregation was found for more than a week after dissolution study of the two SNEDDS formulations.

Table 3 summarized the pharmacokinetic parameters of warfarin in rats following a single oral dose of 1.5 mg/kg with lutein as non-SNEDDS (lutein powder) or SNEDDS formulations. Time to reach maximum plasma concentration (*T*<sub>max</sub>) of warfarin was 6 h when the drug was administered with or without 5 mg/kg of lutein as non-SNEDDS while it was 4 h when administered with lutein as SNEDDS formulations.

**Fig. 2.** Effect of co-surfactant concentration on droplet size of SNEDDS containing fixed surfactant concentration of 60%. Droplet size was decreased as concentration of Transcutol-HP and Lutrol-E400 increased up to 25%. However, when the concentration of the co-surfactants was beyond 25%, the size was increased. The lowest size was observed when the concentrations of Labrasol and Lutrol-E400 were 60% and 25%, respectively.

**Fig. 3.** Dissolution profile of lutein from SNEDDS. Each value represents the mean of three samples ± standard deviation. Dissolution of lutein from SNEDDS was very quick and accomplished within 10 min. System B exhibited faster and more complete dissolution than System A because of its smaller droplet size compared to System A. Undissolved amount of lutein is attributed to lutein in the emulsion droplets trapped by syringe filter (0.2 μm). In contrast, commercial formulation (Eyelac® soft gelatin capsule) and lutein powder did not dissolve lutein even at the end of the dissolution study.

Maximum plasma concentration (*C*<sub>max</sub>) of warfarin was significantly increased when the drug was administered with lutein as SNEDDS (*p*<0.01). Area under the curve (AUC) was also increased, but the increase was significant only for the System B (*p*<0.05). Elimination half life (*t*<sub>1/2</sub>) of warfarin was slightly shortened with concomitant administration with the SNEDDS formulations. Fig. 4 shows plasma level of warfarin as a function of time.

**DISCUSSION**

Lutein is a very hydrophobic substance which follows same intestinal absorption path as dietary fat. When lutein is administered orally, the drug is emulsified in gastrointestinal tract and incorporated into mixed micelles in the presence of bile salts and biliary phospholipids (Baskaran et al., 2003; Lakshminarayana et al., 2006; Yonekura and Nagao, 2007). Therefore, absorption of lutein is affected by inappropriate bile juice secretion in patients with biliary diseases. For this reason, an alternative delivery system that relies on its own self-emulsification ability rather than the aid of bile juice would offer benefit to the elderly or patients who cannot eat food appropriately and have altered digestive functions. Hence, SNEDDS may be an ideal delivery system to improve solubilization and absorption of lutein because it spontaneously forms nanosized droplets of oil in water emulsion in aqueous environment without the aid of bile juice secretion.

There have been several strategies reported to improve bioavailability of lutein. Most widely used are mixed micelles and emulsion formulations with or without dietary fats (Mariiddaiah and Baskaran, 2009; Mamatha and Baskaran, 2011). Among the emulsion formulations, SNEDDS is considered promising approach because it distributes readily in the gastrointestinal tract, and digestive motility of the stomach and intestine provides sufficient agitation for spontaneous formation of emulsion. Furthermore, it does not require costly equipments in the manufacturing process and the emulsion droplet size is far less than a micron which is advantageous for gastrointestinal absorption.

Choosing the right combination of oil base, surfactant,
Table 3. Pharmacokinetic parameters of warfarin in rats following a single oral dose of 1.5 mg/kg with lutein as non-SNEDDS or SNEDDS formulations (n=6)

<table>
<thead>
<tr>
<th></th>
<th>Warfarin only</th>
<th>With lutein as non-SNEDDS</th>
<th>With lutein as system A</th>
<th>With lutein as system B</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_{max}$ (h)</td>
<td>6.00</td>
<td>6.00</td>
<td>4.00</td>
<td>4.00</td>
</tr>
<tr>
<td>$C_{max}$ (μg/ml)</td>
<td>5.62 ± 0.11</td>
<td>5.65 ± 0.15</td>
<td>6.14 ± 0.36**</td>
<td>6.34 ± 0.22**</td>
</tr>
<tr>
<td>AUC (μg/ml×h)</td>
<td>305.70 ± 9.42</td>
<td>308.89 ± 10.23</td>
<td>313.57 ± 12.24</td>
<td>324.10 ± 10.96*</td>
</tr>
<tr>
<td>$t_{1/2}$ (h)</td>
<td>22.91 ± 1.26</td>
<td>23.89 ± 1.74</td>
<td>21.12 ± 1.86</td>
<td>22.70 ± 1.67</td>
</tr>
</tbody>
</table>

$T_{max}$: time to reach maximum plasma concentration; $C_{max}$: maximum plasma concentration; AUC: area under the curve; $t_{1/2}$: elimination half life; *p<0.05 and **p<0.01 compared to warfarin only.

![Graph showing plasma level of warfarin in rats following a single oral dose of 1.5 mg/kg with lutein as non-SNEDDS or SNEDDS formulations](image-url)

Fig. 4. Plasma level of warfarin in rats following a single oral dose of 1.5 mg/kg with lutein as non-SNEDDS or SNEDDS formulations (n=6). $T_{max}$ of warfarin was reached at 4 h when the drug was administered with lutein as SNEDDS while it was 6 h when administered with lutein as non-SNEDDS. $C_{max}$ and AUC of warfarin was significantly increased when the drug was administered with lutein as SNEDDS (p<0.01). Reason for the enhanced bioavailability appears that SNEDDS formulation affected permeability of warfarin through the mucus membrane of gastrointestinal tract.

and co-surfactant is one of the important points in designing SNEDDS formulations. Surfactant and co-surfactant molecules get preferentially absorbed at the liquid interface during the process of emulsion formation, reducing the interfacial energy of the system. This helps spontaneous emulsification without high energy input. Thus, well-designed SNEDDS formulation can ensure efficient self-emulsification as well as high solubilization capacity for the drug in the resultant dispersion.

In this study, we used Peceol, a glyceride-rich excipient mainly consisted of glyceryl monooleate, as oil base. Rationale for choosing Peceol was based on previous researcher’s finding that lipids consisted of monounsaturated fatty acid trended toward better absorption of lutein and other carotenoids in healthy subjects (Goltz et al., 2012). We used Labrasol as surfactant because it showed highest solubility for lutein (56.54 ± 4.24 mg/ml) among the vehicles screened and its HLB value was 14 (Table 1). Generally, surfactants with HLB 12-15 are regarded as being of good efficiency for self-emulsification (Singh et al., 2008; Thi et al., 2009; Shanmugam et al., 2011).

Also, this vehicle is widely used in the pharmaceutical and food industries due to its excellent safety profile (Kommuru et al., 2001; Yan et al., 2011) Labrasol is composed of fatty acid esters of polyethylene glycol and medium chain fatty acids of caproic acid (C6:0) and caprylic acid (C8:0) (mixture of mono-, di-, and tri-glycerides). We used two different co-surfactants in System A and System B (Transcutol-HP and Lutrol-E400, respectively). Reason for choosing Transcutol-HP and Lutrol-E400 was that they have similar HLB values (4.2 versus 4.0) but different solubility profile for lutein (16.27 versus 31.53 mg/ml, Table 1).

Dissolution profile of lutein from System B was faster and more complete than System A because of significantly smaller mean droplet size of System B. Although both Systems instantaneously formed nanoemulsion after introduction to dissolution medium (distilled water), the mean droplet size of System B was about half size of System A. Difference in the solubility of lutein to co-surfactants used in the two Systems might have also contributed to dissolution profile. Lutrol-E400 used as co-surfactant in System B showed about two-folds higher solubility for lutein compared to Transcutol-HP used in System A. Undissolved amount of lutein in the dissolution study is attributed to lutein in the emulsion droplets trapped by syringe filter (0.2 μm).

In contrast to SNEDDS, commercial formulation (Eyelac® soft gelatin capsule) and lutein powder did not dissolve lutein even at the end of the study. Inability of the commercial formulation to dissolve lutein may be due to oily nature of the vehicles that were used. Usually, such commercial product should be taken right after meal so that lutein can be emulsified by physiological emulsifiers such as bile juice. However, the SNEDDS that we have formulated does not need to be taken after meal as was evidenced by very rapid dissolution due to its self-emulsifying ability. This especially offers benefit to the elderly and patients who cannot eat food appropriately.

Previously, we reported that gastrointestinal absorption of lutein was significantly enhanced when administered to rabbits as SNEDDS formulation (Shanmugam et al., 2011). $C_{max}$ and AUC were enhanced as much as 21-fold and 12-fold compared to lutein powder, respectively. Compared to a commercial product (Eyelac®), relative bioavailability of the SNEDDS formulation was also significantly improved, showing about 3-folds increase based on AUC. Although this result was encouraging, there remains a concern that the SNEDDS formulation may affect gastrointestinal absorption of other drugs administered together. Especially if therapeutic index of the concomitantly administered drug is narrow, undesirable drug interactions could occur.

We hypothesized that surfactant and co-surfactant may affect absorption of concomitantly administered drugs because they are usually more than 80% of the SNEDDS formulation in weight basis. In this study, we investigated the effect of SNEDDS formulation of lutein on the pharmacokinetic param-
eters of warfarin using rats. Although there are many research conducted regarding the drug interaction issue of warfarin, interaction between warfarin and surfactants used in the SNEDDS has not been published so far (Graefe-Mody et al., 2011; Malhotra et al., 2011; Zhou et al., 2012). Since warfarin requires close therapeutic monitoring, bioavailability of the drug is of great concern. In the dispensing practice of warfarin prescription, even generic substitution is discouraged due to the bioavailability difference between commercially available brands (Ghate et al., 2011; Haines, 2011). Our result showed that $C_{\text{max}}$ and AUC of warfarin were increased about 10% when administered concomitantly with SNEDDS containing lutein. $T_{\text{max}}$ was also prolonged from 6 h to 4 h. These results clearly identified that SNEDDS formulation affected the absorption of warfarin, leading to bioavailability difference. Exact mechanism for this difference is not yet elucidated, but it appears that SNEDDS formulation affected permeability of warfarin through the mucosal membrane of gastrointestinal tract. This speculation is backed up by the lack of bioavailability change in rats administered with warfarin and lutein as non-SNEDDS. Limitation of this study may be that total amount of blood taken for bioavailability measurement was slightly more than 10% of total blood of the rats tested.

Finally, we hypothesized that surfactants in the SNEDDS formulation of lutein may affect bioavailability of warfarin and identified that the surfactants increased $C_{\text{max}}$ and AUC of warfarin. Based on this finding, it is suggested that SNEDDS formulations be administered before or after appropriate time interval when used together with narrow therapeutic range drugs.

Lutein was successfully formulated as SNEDDS for immediate self-emulsification and dissolution by using combination of oil base, surfactant, and co-surfactant. However, surfactants used in the SNEDDS affected bioavailability of warfarin, showing about 10% increase in $C_{\text{max}}$ and AUC of the drug in rats. $T_{\text{max}}$ was also changed from 6 h to 4 h. Although there is no strict consensus about how much bioavailability change is tolerable, concomitant administration of SNEDDS formulation and drugs with narrow therapeutic range appears to require attention for drug interaction.

ACKNOWLEDGMENTS

This work was supported by the Gachon University research fund of 2012 (GCU 2012-M107).

REFERENCES


http://dx.doi.org/10.4062/biomolther.2013.011


