Introduction

Although the delivery of therapeutic compounds through an oral route is the most convenient route of administration exhibiting high patient compliance (1), in some cases, there are several challenges for the development of an oral drug delivery system which exerts appropriate bioavailability, pharmacokinetic profile, and suitable therapeutic outcomes (2-4). Poor aqueous solubility and low dissolution rates are among the major problems that are associated with the preparation of an oral drug delivery system containing a lipophilic drug (5). Moreover, due to high tendency for liver uptake and consequent metabolism, some drugs exhibit high first-pass metabolism which can lead to the inactivation of drugs immediately after absorption from intestinal epithelia into the portal vein. Another challenge for oral drug delivery is the expression and distribution of P-glycoproteins (P-gp) across the intestinal epithelium, causing the development of multi-drug resistance and low bioavailability due to the intestinal efflux of the absorbed drugs (6).

Self-nanoemulsifying drug delivery systems (SNEDDS) are the isotropic mixtures of the oil, surfactant, and co-surfactant incorporating therapeutic compounds. After the dilution of SNEDDS with gastro-intestinal (GI) fluids, thermodynamically stable oil-in-water (o/w) nano-droplets can be formed spontaneously following gentle agitation provided from GI motility (7). Forgiarini et al (8) reported that stable nano-emulsions with a droplet size of approximately 50 nm can be prepared by the addition of appropriate amounts of water into the mixture of the oil and surfactant under gentle agitation.

Lipophilic compounds, representing low aqueous solubility, have high potency to incorporate to the internal oily phase of nano-droplets. Recently, researchers have...
shown interest in SNEDDS as an alternating approach for the oral delivery of poorly water-soluble drugs. These formulations dilute with GI fluids and, upon agitation provided by gastric motility, can be self-emulsified and form nano-droplets with a size range of 100-300 nm. In contrast with emulsions which are metastable and have tendency to aggregate, SNEDDS formulations are thermodynamically stable because of easiness in manufacturing and high potency for commercialization. There are several studies reporting the development of self nano/micro-emulsifying drug delivery systems for enhancing the oral bioavailability of lipophilic compounds such as co-enzyme Q-10 (9), timolol (10), saquinavir (11), progesterone (12), ontazolast (13), cefpodoxime proxetil (14), and matrine (15). Moreover, the incorporation of lipophilic drugs to the internal oil phase of nano-droplets can reduce their exposure to hydrolytic enzymes which are active in the GI lumen, and therefore, increase the stability of therapeutic compounds (16).

Budesonide is a steroidal glucocorticoid that reduces inflammation in the body and is typically used to treat asthma and rhinitis through inhalation (17). It is also used for the treatment of mild to moderate Crohn’s disease. The drug exhibits low aqueous solubility (0.0457 mg/mL) while a high first-pass effect and thus its oral bioavailability is less than 10% (18,19). The chemical structure of budesonide is shown in Figure 1.

The main aim of this study was to prepare an SNEDDS containing budesonide in order to improve the bioavailability. To the best of our knowledge, there is no report regarding the development of an SNEDDS for improving the oral bioavailability of budesonide as a lipophilic drug which exhibits low aqueous solubility while high first-pass effects.

Materials and Methods

Materials

Budesonide USP (>99% purity) was provided as a gift from Jaber–Ben-Hayyan Pharmaceutical Company (Tehran, Iran). Liquid paraffin (medium viscosity), Tween 80, propylene glycol, sodium hydroxide, hydrochloric acid, potassium dihydrogen phosphate (KH₂PO₄), Lichrosolv® acetonitrile, and methanol for analysis were obtained from Merck (Darmstadt, Germany). In addition, the dialysing tube with a molecular cut-off of 12,000 Da was purchased from Sigma-Aldrich (St. Louis, United States). Further, double distilled water was freshly prepared as needed by the Milli-Q® millipore lab water purification system (Billericia, United States). All other chemicals were of pharmaceutical grade and used freshly.

High-Performance Liquid Chromatography Analysis

A Shimadzu liquid chromatography system equipped with the 1200 AD binary pump, a Rheodyne injector fitted with a 20 µL injection loop, and a 120 D diode array detector set at 240 nm was used for budesonide determination. Furthermore, the Shimpack® ODS (250×4.6 mm, 5 µm) column was used for chromatography, and data were acquired using Chemsolution® software provided by Shimadzu corporation (Kyoto, Japan). The mobile phase was composed of acetonitrile, namely, previously filtered, mixed, and degassed methanol (40:60). Then, the analysis was performed at ambient temperature with a constant flow rate of 1 mL/min (20) and the retention time for budesonide as the working standard was recorded as 3.6 minutes. Moreover, the analytical method was partially validated by determining linearity, intra- and inter-day accuracy, and precision in the range of 0.5 µg/mL to 50 µg/mL. Additionally, the square regression coefficient (R²) of the obtained calibration curve was 0.9947, indicating the linearity of the analytical method in the range of analysis. The calculated error% was less than 2.0% and 5.0% for intra-day and inter-day assays, respectively, demonstrating appropriate accuracy. Similarly, the relative standard deviation for intra- and inter-day assays was less than 1.5% and 6.0%, respectively, which represented the suitable precision of the method. The limit of detection and limit of quantification were also determined according to ICH (The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use) guidelines using the signal to noise ratio and reported as 0.2 µg/mL and 0.3 µg/mL, respectively. Figure 2 displays a sample chromatogram related to the concentration of 50 µg/mL that was used for calculating the calibration curve.

Preparation and Characterization of Self-nanoemulsifying Drug Delivery Systems

Budesonide (20 mg) was completely dissolved in various amounts of liquid paraffin. To ascertain dissolution, the mixture was heated at 50°C for 5 minutes using Benmarry provided from Memmert® (Schwabach, Germany). After dissolution completion, SNEDDS prototypes were prepared by mixing budesonide containing liquid paraffin...
as the oily phase with different amounts of Tween 80 as the surfactant and propylene glycol as the co-surfactant. Further, the prototypes were magnetically agitated for complete mixing. A homogenous mixture with no sign of precipitation was obtained indicating the complete dissolution of budesonide in the components. To prepare SNEDDS, the prepared prototypes were diluted in the ratio of 1:200 using freshly prepared simulated intestinal fluid (SIF) and gently agitated for 5 minutes. Then, the SIF without any enzyme was prepared according to USP 33-28 NF using potassium dihydrogen phosphate (0.05 M) and sodium hydroxide (0.2 M) and then the pH was adjusted to 6.8. After dilution, the prepared o/w nano-emulsion was visualized for the evaluation of macroscopic appearance (i.e., clear, opalescent, and milky) and the appropriate physico-chemical properties including size, polydispersity index (PdI), and zeta potential of nano-droplets were determined using a Zetasizer 3000 HS (Malvern instrument, Worcestershire, UK). To determine entrapment efficiency (EE %) of the budesonide in SNEDDS, nano-emulsions were centrifuged at 15 000 rpm for 20 minutes using Beckman ultracentrifuge (Germany). After centrifugation, nano-droplets, settling at the top of centrifuge tubes, was discarded and the transparent aqueous phase was analyzed by high-performance liquid chromatography (HPLC) in order to determine un-entrapped budesonide. Then, the eosinophilic esophagitis (EE%) of the budesonide in SNEDDS preparations were calculated by Eq. (1).

\[
EE\% = \frac{\text{Total amount of budesonide in supernatant phase} \times 100}{\text{Total amount of budesonide}}
\]

**Experimental Design Studies**

Many studies for the development of drug delivery systems are performed by changing one separate factor at the time. This method requires many experiments and therefore is costly and time-consuming. Moreover, evaluating the effects of interactions between two or more independent variables is difficult. To overcome these problems, the design of experiment (DoE) approach was evolved in pharmaceutical sciences (21). In this study, the preparation of SNEDDS was investigated using the response surface methodology, and the Box-Behnken experimental design technique was used for the optimization of SNEDDS. Furthermore, independent variables (factors) were the amounts of liquid paraffin (A), Tween 80 (B), and propylene glycol (C) while dependent variables (responses) were identified as the size \((Y_1)\) and PdI \((Y_2)\) of nano-droplets. The ranges and constrains of independent variables are shown in Table 1. Preliminary studies were used for determining the levels of independent variables (Data are not provided). Then, Design-Expert® software (version 7.0.0, Stat -Ease, Inc., Minneapolis, Unites States) was used for mathematical modeling and response optimization. According to the software, performing a total of 15 experiments was required for mathematical and statistical modeling. The Box-Behnken DoE is summarized in Table 2. Each run was experimentally prepared for mathematical and statistical model interpretation and a mathematical model was proposed for each response. The model was explained by second-order polynomial functions as Eq. (2).

\[
Y = \beta_0 + \beta_1 A + \beta_2 B + \beta_{11} A^2 + \beta_{22} B^2 + \beta_{12} AB
\]

Eq. (2)

where \(Y\): Predicted Responses; \(\beta_0\): Intercept; \(\beta_1, \beta_2\): Linear coefficient; \(\beta_{11}, \beta_{22}\): Square coefficient; \(\beta_{12}\): Interaction coefficient; \(A, B\): Independent variables.

For model validation, the statistically optimized formulation, predicted by the software, was experimentally prepared for 5 times and characterized for size, PdI, zeta potential, and EE%. The data were reported as mean ± SD. To determine the stability of SNEDDS against phase separation, the optimized formulation was centrifuged at 5000 rpm for 5 minutes and SNEDDS formulation was evaluated macroscopically for phase separation.

**Morphological Studies**

The statistically optimized SNEDDS preparation was studied morphologically using the transmission electron microscopy (TEM). A drop of prepared SNEDDS in SIF was spread on a 300-mesh copper grid, stained with 2% (w/v) phosphotungstic acid, and allowed to dry for 5

**Table 1. Defined Ranges and Constraints**

| Independent Variables (Factors) | Levels |
|---------------------------------|--------|
| Liquid Paraffin (g) (A)         | 0.2    | 0.4    |
| Tween 80 (g) (B)                | 0.05   | 0.2    |
| Propylene Glycol (g) (C)        | 0.01   | 0.05   |

| Dependent Variables (Responses) | Constrains |
|---------------------------------|------------|
| \(Y_1\) = Size (nm)            | Minimize   |
| \(Y_2\) = PdI                  | Minimize   |

Note. PdI: Polydispersity index.
minutes at room temperature. Then, the size and shape of nano-droplets were examined by an EM10C (Zeiss, Oberkochen, Germany).

**In Vitro Release Study**

The *in vitro* release of budesonide from optimized SNEDDS preparation was determined in this study. The optimized SNEDDS prototype equivalent to 5 mg of budesonide was diluted in the ratio of 1:200 in the SIF (pH=6.8) and placed in a dialyzing tube with a molecular cut-off of 12 000 Da. The content was then dialyzed against freshly prepared SIF as the release medium while incubating at 37±2 °C and gently agitating (100 rpm) using a Heidolph® Benmarry shaker (Schwabach, Germany). The volume of the release medium was adjusted to ascertain the establishment of the sink condition. In the predetermined time intervals, 1 mL of the medium was collected and immediately replaced with the equal volume of freshly prepared and pre-heated SIF. Finally, the amount of budesonide in the samples was determined using HPLC.

**Statistical Analysis**

In this study, each experiment was done on triplicate (unless otherwise stated) and the data were reported as the mean ± SD. Two-sample independent t-test was used for statistical comparison between two groups using SPSS® software (version 19.0.0, IBM Statistics, New York, USA). For a comparison between several groups, one-way analysis variance was performed using the same software. In addition, Design-Expert® software (version 7.0.0, Stat-Ease, Inc., Minneapolis, USA) was used for performing a central composite response surface DoE. The level of significance was considered as 0.05 in all studies.

**Results**

**Preparation and Characterization of SNEDDS**

The Box-Behnken response surface methodology was applied to determine the effects of independent variables including the amount of liquid paraffin, Tween 80, and propylene glycol on the size and PdI of the prepared SNEDDS. The experimental results are summarized in Table 2.

**Size of Nano-droplets**

The size of the droplets exhibited a significant role in mucus permeability and absorption across the intestinal epithelium and drug release. According to data (Table 2), the size of nano-droplets varied in the range of 128±51 nm to 3018±432 nm. Using the step-wise method, the statistical and regression analysis of the obtained data indicated that the proposed two-factor interaction (2 FI) model was significant ($P<0.05$). The linear coefficients of independent factor A (the amount of liquid paraffin), factor B (the amount of Tween 80), and the interaction coefficient of AB demonstrated significant influences concerning the size of SNEDDS ($P<0.05$). On the other hand, the data revealed the insignificancy of the linear coefficient of factor C (the amount of propylene glycol) for the size of SNEDDS ($P>0.05$). The summary of the fitted model is presented in Table 3 and is mathematically explained by Eq. (3).

$$Y_1=4.67178-4.50321*A-3.84364*B-3.00708A.B$$

**Figure 3** depicts the 3-D response surface plots of the

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**Table 2. Box-Behnken Experimental Design (n=3)**

| Independent Variables (Factors) | Dependent Variable (Responses) |
|---------------------------------|--------------------------------|
| A: Liquid Paraffin (g) | B: Tween 80 (g) | C: Propylene Glycol (g) | Y1: Size (nm) (Mean ± SD) | Y2: PdI (Mean ± SD) |
| 0.2 | 0.125 | 0.05 | 2280±136 | 0.4320±06 |
| 0.4 | 0.125 | 0.01 | 1842±2 | 0.9050±52 |
| 0.4 | 0.125 | 0.05 | 1285±1 | 0.3606±22 |
| 0.3 | 0.125 | 0.03 | 5893±38 | 0.7870±88 |
| 0.2 | 0.125 | 0.01 | 30184±12 | 1.000 |
| 0.2 | 0.05 | 0.03 | 8784±7 | 0.4820±48 |
| 0.4 | 0.05 | 0.03 | 6198±2 | 0.3340±02 |
| 0.3 | 0.05 | 0.05 | 213510±6 | 0.1750±38 |
| 0.3 | 0.2 | 0.01 | 2431±8 | 0.7420±58 |
| 0.3 | 0.2 | 0.05 | 242±56 | 0.2630±02 |
| 0.2 | 0.2 | 0.03 | 1240±36 | 0.7820±06 |
| 0.3 | 0.125 | 0.03 | 3675±8 | 0.6540±04 |
| 0.3 | 0.05 | 0.01 | 1625±92 | 0.685±03 |
| 0.4 | 0.2 | 0.03 | 127±14 | 0.47±0.01 |
| 0.3 | 0.125 | 0.03 | 2286±164 | 0.559±0.05 |

Note: SD: Standard deviation; PdI: Polydispersity index.
alteration in the size of nano-droplets due to changes in independent factors. As shown, although, in the lowest amount of Tween 80 (i.e., 0.05 g), the size of nano-droplets sharply decreased by increasing the amount of liquid paraffin from 0.2 g to 0.4 g, in the highest amount of Tween 80 (i.e., 0.2 g), increasing the amount of liquid paraffin exhibited a slight decrease in the size of nano-droplets. Based on the data in Figure 3, an increase in the amount of Tween 80 from 0.05 g to 0.2 g caused a sharp decrease in the size of nano-droplets in the lowest amount of liquid paraffin (i.e., 0.2 g) while, in the highest amount of liquid paraffin (0.4 g), the size of nano-droplets represented no significant change by increasing the amount of Tween 80.

Pdi of Nano-droplets

The results also demonstrated that the Pdi of nano-droplets varied in the range of 0.175±0.03 to 1.000. Using the step-wise method, the statistical and regression analysis of the obtained data indicated that the proposed two-factor interaction (2 FI) model was significant ($P<0.05$). Although the linear coefficient of independent factor A (the amount of liquid paraffin) was not significant ($P>0.05$), the linear co-efficient of independent factor C (the amount of propylene glycol) and the interaction coefficient of AB exhibited significant influences regarding the size of SNEDDS ($P<0.05$). The summary of the fitted model is provided in Table 3 and mathematically explained by Eq. (4).

\[
Y_2=1.23043-0.84750*A-13.47500*C+3.65421*A*C \quad \text{Eq. (4)}
\]

where $Y_2$: Predicted response for the Pdi of nano-droplets; $A$: Amount of liquid paraffin (g); $C$: Amount of propylene glycol (g); $A*C$: Interaction co-efficient of $A$ and $C$.

The 3-D response surface plots of alterations in Pdi due to changes in independent factors are illustrated in Figure 4. As shown, the Pdi of nano-droplets sharply decreased by increasing the amount of propylene glycol as the co-surfactant from 0.01 g to 0.2 g. This trend was observed in both the highest and lowest amounts of liquid paraffin. Based on the data in Figure 3, the Pdi of nano-droplets reduced by increasing the amount of liquid paraffin from 0.2 g to 0.4 g in the lowest amount of propylene glycol (i.e., 0.02 g) while increasing the amount of liquid paraffin did not significantly change the Pdi in the highest amount of propylene glycol (i.e., 0.2 g).

**Optimization and Model Validation**

The values of independent variables for optimized SNEDDS preparation that exhibit the smallest size and lowest Pdi were predicted by the software using mathematical calculations. The predicted optimized formulation is summarized in Table 4. For model validation, the optimized formulation was experimentally prepared in the laboratory and repeated 5 times, and appropriate physico-chemical properties were determined, including size, Pdi, zeta potential, and EE%. The data designated as the observed values were reported as mean ± SD (Table 5). The error% for predicted physico-chemical properties was calculated by Eq. (5).

![Figure 3. 3-D Response Surface Plot for the Size of Nano-droplets](image)

![Figure 4. 3-D Response Surface Plot for Polydispersity Index of Nano-droplets](image)

### Table 3. Characterization of Best Fitted Mathematical Models for the Prediction of Responses

| Responses | Best Fitted Model | Lack of Fit | R-Squared | Adjusted R-Squared | Predicted R-Squared | Adequate Precision |
|-----------|------------------|-------------|-----------|--------------------|---------------------|-------------------|
| $Y_1$: Size | 2FI | Not significant ($P>0.1$) | 0.6971 | 0.6145 | 0.4611 | 9.510 |
| $Y_2$: Pdi | 2FI | Not significant ($P>0.1$) | 0.8004 | 0.7460 | 0.6147 | 11.042 |

**Note:** 2FI: Two-factor interaction; Pdi: Polydispersity index.
As shown in Table 5, the calculated error% was less than 10% in all cases, indicating the proper accuracy and reliability of the proposed models.

To determine the stability of the optimized formulation against phase separation, the SNEDDS was prepared and centrifuged at 5000 rpm for 5 minutes. After centrifugation, nano-emulsion was visually evaluated and no macroscopic sign of phase separation and/or creaming was observable.

**Morphological Studies**

Optimized SNEDDS were morphologically studied using the TEM. Microscopic images are illustrated in Figure 5. As shown, spherical nano-globules with no sign of aggregation were observed in TEM images. Further, the sizes of nano-droplets determined by TEM were in accordance with the data obtained from photon correlation spectroscopy (Figure 6).

**In Vitro Release Studies**

Figure 7 displays the in vitro release profile of budesonide from nano-emulsions in the SIF (pH adjusted to 6.8). Based on the data, SNEDDS formulation exhibited sustained drug release and 33.81±1.67% of the drug was released in SIF 360 minutes post-incubation.

**Discussion**

SNEDDS are categorized as mucus penetrating drug delivery systems which can deliver the drug intactly across the mucus layer of the GI epithelium. Previous studies revealed that using long-chain oils in the structure of SNEDDS can enhance the lymphatic transport of the drug delivery system mediated by M-cells rather than portal vein epithelial absorption. On the other hand, the lymphatic mechanism of absorption reduces the hepatic uptake and the hepatic metabolism of the administered drug and, therefore, can increase the oral bioavailability of compounds exhibiting high first-pass effects. For example, Holm et al (22) demonstrated that SNEEDS prepared from long-chain oily phases with more than 12 carbon chains can cause a 4-fold increase in the lymphatic accumulation of halofantrine as a lipophilic drug compared to SNEDDS prepared from the medium or low-chain oil phase. In another study, Ichihashi et al (23) found that first-pass metabolism can be avoided by lymphatic absorption. Moreover, other studies revealed that SNEDDS can inhibit the drug efflux mediated by P-gp, leading to an increase in the oral bioavailability of some therapeutic compounds (24,25).

The ratio of surfactant to co-surfactant is considered as a dominant factor which influences the size of the droplets. Shahnaz et al (26) reported a decrease in the size of SNEDDS nano-droplets by increasing the ratio of

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**Table 4. Statistically Optimized Formulation**

| Optimized Independent Variables | Predicted Dependent Variables (Responses) | Desirability |
|--------------------------------|------------------------------------------|--------------|
| Liquid paraffin (A) (g)         | Tween 80 (B) (g)                        | Propylene Glycol (C) (g) | Y<sub>i</sub> = Size (nm) | Y<sub>i</sub> = Pdl |          |
| 0.4                             | 0.2                                      | 0.05          | 139.5                        | 0.218               | 0.784     |

Note: Pdl: Polydispersity index.

**Table 5. The Observed Responses for Predicted Optimized Formulations (n = 5)**

| Size (nm) | PdI | Zeta (mV) | EE (%) | Appearance |
|-----------|-----|-----------|--------|------------|
| Observed response (Mean ± SD) | Prediction error (%) | Observed response (Mean ± SD) | Prediction error (%) | Observed response (Mean ± SD) | Observed response (Mean ± SD) | |
| 146±37 | +4.45% | 0.211±0.06 | -3.31% | +3.6±0.84 | 94.3±6.58 | Transparent |

Note: SD: Standard deviation; PdI: Polydispersity index; EE: Eosinophilic esophagitis.

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Equation (5)

\[
\text{Error}\% = \frac{\text{observed value} - \text{predicted value}}{\text{observed value}} \times 100
\]
surfactant to co-surfactant. In this study, in the constant amount of surfactant (i.e., 0.05 g), the size of nano-droplets decreased by increasing the amount of liquid paraffin. The observed phenomenon can be justified by considering that increasing the amount of the oil in the constant amount of the surfactant causes simultaneous increase in the ratio of surfactant to co-surfactant and thus a sharp decrease can be expected in the size of the droplets. It is believed that in nano-emulsions, increasing the ratio of surfactant to co-surfactant causes the interfacial film to condense and therefore the size of nano-droplets represents a decrease. On the other hand, decreasing the ratio causes the expansion of the interfacial film and consequently increases the size of the droplets. In the highest amount of the surfactant (i.e., 0.2 g), increasing the amount of liquid paraffin exhibited a small effect on the ratio of the surfactant to co-surfactant and therefore the size of the globules decreased slightly compared to SNEDDS formulations containing the lowest amount of surfactant.

In this study, a sharp decrease was observed in the size of nano-droplets followed by an increase in the amount of surfactant. This finding is in well accordance with the results of Parmar et al (27). They suggested that the localization of the excess amount of surfactant molecules at the oil-water interface can stabilize the nano-emulsions and decrease the size of the droplets. Similarly, Pouton (28) indicated that reductions in the size of the droplet by increasing the amount of surfactant can be due to the excess penetration of the aqueous phase to the oil phase which cause the split of the interfacial film to small nano-droplets. Beside this mechanism, a decrease in the size of nano-droplets by increasing the surfactant can be justified by enhancements in the solubilization of the oil component following an increase in Tween 80 (29). Based on the findings of this study, although the amount of propylene glycol demonstrated no significant effect on the size of nano-droplets, Elnaggar et al (30) found that SNEDDS formulations without any co-surfactants cannot be emulsified under mild agitation.

The results of this study represented that the PdI of nano-droplets decreased by increasing the amount of co-surfactant. Accordingly, it is suggested that co-surfactants can render flexibility to the interfacial film developed by surfactants between two immiscible phases of the oil and water, facilitating the formation of stable nano-droplets and thus decreasing the PdI (31).

The obtained data from in vitro release evaluations revealed the slow and sustained release of budesonide from nano-droplets. Friedl et al (32) exhibited that SNEDDS can be considered as the mucus-penetrating drug delivery system intended for intact absorption through the mucus layer of the intestinal epithelium. The evidence suggests that the slow release of the drug incorporated in the oil phase of nano-droplets to the intestinal fluid leads to an increase in bioavailability due to an increase in the amount of the intact drug in nano-droplet penetration to the mucus layer in addition to the prevention of the incorporated drug from pre-systemic degradation mechanisms including hepatic first-pass effects. Therefore, the observed slow-release rate of budesonide from SNEDDS preparation is favourable. The obtained in vitro release profile is in accordance with the findings of Mahjub et al (33). Based on their results, SNEDDS prepared from liquid paraffin as the oil phase exhibited more stability against lipase activity in the GI and was considered as the lipase-undegradable SNEDDS that poses a slower release rate of octreotide compared to SNEDDS prepared from the olive oil as the oil phase. Due to the lipase-resistant feature of liquid paraffin, the simulated intestinal medium without any enzyme is used as the release medium. On the other hand, it is assumed that liquid paraffin (medium viscosity) with 18 carbon chains can be considered as the long-chain oil which enhances lymphatic absorption.

**Conclusion**

In this study, the preparation of SNEDDS incorporating budesonide as a hydrophobic compound was statistically optimized by the Box-Behnken response surface methodology using Design-Expert® software. The optimized nano-droplets were morphologically studied by TEM, and the images revealed spherical globules with no sign of aggregation. The in vitro release study of heparin from nano-droplets showed a slow release rate of the drug in the SIF.

As a mucus-penetrating drug delivery system, SNEDDS requires further investigation. It is still unknown by which mechanism this system can penetrate the mucus layer of the intestinal epithelium. Thus, it is necessary to evaluate the effects of physico-chemical properties such as size and zeta potential on the mucus permeability of nano-droplets, as well as the effects of SNEDDS on the lymphatic absorption of the drugs. It is interesting to investigate the extent of reductions in first-pass effects using SNEEDS. It is also necessary to study the effects of SNEDDS on...
trans-epithelial electrical resistance on the Caco-2 cell monolayer and consequently to determine the effects of this drug delivery system on the tight junctions of the intestinal epithelium.

Conflict of Interests
The authors report no conflict of interests.

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Conflict of Interests
The authors report no conflict of interests.

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