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RESEARCH ARTICLE

Design and development of a self-microemulsifying drug delivery system of olmesartan medoxomil for enhanced bioavailability

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ABSTRACT

Olmesartan medoxomil (OM) is a hydrophobic antihypertensive drug with low bioavailability (26%) and is known to have adverse effects such as celiac disease and enteropathy. The purpose of this study was to develop SMEDDS to increase bioavailability and decrease potential side effects of OM. Hydrophilic lipophilic balance was calculated by testing solubility of OM in different oils, surfactants, and cosurfactants to obtain the most suitable combination of SMEDDS. Pseudoternary phase diagram was used to select the better oil/water formulation of SMEDDS. After a test for 3-month stability, dissolution tests and parallel artificial membrane permeability assay (PAMPA) were conducted to investigate drug solubility and permeability. Biodistribution of fluorescent marked SMEDDS was observed by using *in vivo* imaging system. The pharmacodynamics of the drug were determined by measuring blood pressure from tails of rats. At the end of the experiment, intestines were examined for adverse effects of OM. Compared with tablet formulation according to the dissolution study, SMEDDS formulation showed 1.67 times improvement in solubility of OM. PAMPA studies suggested a much faster permeability rate for OM SMEDDS compared to the suspension form. Labeled SMEDDS gave 3.96 times stronger fluorescent emission than control dye administered mice in *in vivo* imaging system (IVIS®) studies, indicating an increased bioavailability. Treating effect of SMEDDS was 3.1 times more efficient compared to suspension in hypertensive rats. It caused neither celiac-like enteropathy nor diarrhea, during 21-day noninvasive blood pressure system (NIBP) assay. Our results suggest that SMEEDS formulation improves dissolution and oral bioavailability of OM while reducing its adverse effects.

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Olmesartan; celiac; SMEDDS; PAMPA; IVIS®; NIBP; bioavailability

Introduction

The World Health Organization described hypertension as the most common cause of death due to the high frequency of the illness [1].

Olmesartan medoxomil (OM) is the insuperable antagonist of AT1 subtype of angiotensin-II receptors. It selectively binds to the angiotensin I receptors in the endothelial smooth muscle, thereby blocking the vasoconstrictor effects of angiotensin II and lowers high blood pressure [2–4]. OM is 2,3-dihydroxy-2-butenyl4-(1-hydroxy-1-methylethyl)-2-propyl-1-[p-(o-1Htetrazol-5-ylphenyl)benzyl]imidazole-5-carboxylate, cyclic2,3-carbonate chemically and is a prodrug that is converted into OM during its absorption from the gastrointestinal tract [5]. The absolute bioavailability of OM is approximately 28.6% because of high hepatic first-pass effect and efflux by P-glycoprotein (P-gp) transporters and its half-life is 10–15 h [6,7]. It is a Biopharmaceutics Classification System (BCS) class II drug [8].

At present, only 10, 20, and 40 mg tablet forms of the active substance are available on the market. While 2.5–20 mg per day of OM dose is recommended for children between 20 and 35 kg, 5 and 40 mg per day of OM dose is recommended for children over 35 kg and well tolerated in children and adolescents aged 6–16. It is not possible to administer the drug at low doses with tablet to children and elderly patients. Hence, it is suggested in the prescription of the drug that it is powdered and suspended in the mixture obtained by mixing the two standard carriers [9]. In addition, oral administration of drugs can often cause the therapeutic response to be non-optimal due to the poor solubility of the drug in the gastrointestinal fluids, insufficient penetration of the gastrointestinal membrane, and first pass effect. A variety of drug delivery strategies have been used to improve oral bioavailability of pure soluble drugs, such as cubosomes [10], nanoparticles [11], nanosuspensions [12], dendrimers [13], permeation enhancers [14], inclusion complexes [15], co-crystals [16,17], carbon nanotubes [18], floating tablets [19], solid dispersions [20], micronization, and nanosizing [21]. However, these formulations are limited because they only increase the solubility [22].

SMEDDS (self-microemulsifying drug delivery system) increases oral bioavailability of hydrophobic drugs by producing high surface area with nano-sized globules and incorporates hydrophobic drugs into the oil phase. This system also enhances lymphatic transport and gastrointestinal permeability, inhibits P-gp flux [23] and decreases pharmacokinetic variability [7,24,25]. The aim of work was to enhance low bioavailability of OM with SMEDDS formulation and constitute a new design that can be easily administered. For these reasons, creating a new drug form that can be

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given in appropriate doses for children and elderly patients is needed, and a new oil/water (o/w) SMEDDS formulation was developed. The quality efficiency of formulation was investigated with *in vitro* dissolution, diffusion, *in vivo* fluorescent imaging and noninvasive blood pressure measurement studies [26,27]. To predict passive gastrointestinal absorption through permeability coefficient log Pe, we performed the hexadecane membrane parallel artificial membrane assay (HDM-PAMPA) [28–31].

One of the aims of this study is to eliminate the adverse effects of OM which are celiac-like enteropathy, weight loss and severe diarrhea, as stated by the FDA safety announcement (UCM359496), by means of this lipophilic formulation [32,33]. To evaluate intestine findings for the exposure of OM at the end of the one-month noninvasive blood pressure system (NIBP) experiment, histopathology studies were performed in rats.

Materials and methods

Materials

OM was supplied by Alembic Pharmaceuticals (Gujarat, India). Transcutol, Labrafil, Capyrol 90, and Labrasol were obtained from Gattefosse (Saint Priest, France); Tween 80, Tween 20, potassium dihydrogen phosphate, dimethylsulfoxide, hexadecane, and N-hexane were provided by Merck (Kenilworth, NJ). Acetonitrile, Span 80, castor oil, PEG 400, oleic acid, and L-name were purchased from Sigma-Aldrich (Darmstadt, Germany). Phosphate buffer solution was supplied from Lonza (Morristown, NJ). Ninety-six-well plate MScn permeability donor and acceptor part were procured from Millipore (Billerica, MA). Vivotag®680 XL dye was procured from PerkinElmer (Waltham, MA). All other chemicals, solvents, and reagents used in the studies were analytical grade.

Animals

Pharmacokinetic and pharmacodynamic studies were performed on 18 adult male albino Wistar rats (weighing 160–180 g) and 12 male Swiss mice (aged 8–10 weeks and weighing 18–20 g) [28,34]. The animals were kept in polypropylene cages at 25 ± 2 °C and 55 ± 5% relative humidity. They had free access to standard diet and water, in a12 h light/dark cycle. The animals were provided by the Central Animal Laboratory of Ege University (Izmir, Turkey) and were treated in accordance with NIH guidelines. All animal experiments were reviewed by the Ethics Committee of Ege University. The assay protocol was approved by the Ethics Committee Report of Ege University Animal Experiments, Permit Issue: 2014-073/25.2.2015.

Development of SMEDDS formulation

We have created a new combination of SMEDDS by selecting suitable surfactants and cosurfactants specific to OM and calculating hydrophilic lipophilic balance (HLB) for the o/w type microemulsion. An excess amount of OM (0.5 g) was dissolved in 5 ml of different oils, surfactants and co-surfactants (Tween 80, Tween 20, Span 80, Labrasol, Labrafil, Capyrol 90, castor oil, Transcutol P, Peg 400, and oleic acid) for identifying the solubility of the drug [7]. This study aimed for an o/w type emulsion with an HLB value between 8 and 20. To construct the ideal microemulsion, the determined surfactants (Tween 80, Span 80) and cosurfactant (Transcutol) were prepared in 1:1, 2:1 surf/cosurf ratios and titrated with measured water until the moment of blurred image by mixing at 300 rpm with a magnetic stirrer (25 ± 0.5 °C) [35]. Using four different combinations of surfactant/cosurfactant and five different combinations of oil, 20 different formulations of SMEDDS were investigated by triangular phase diagram. Since formula 4 gave the largest microemulsion area, studies were continued with the combination of formula 4. SMEDDS combination values were evaluated by using a pseudoternary phase diagram software program [36] ($n = 3$). OM was dissolved in selected combination of excipients to prepare SMEDDS.

Determination of OM with HPLC method

OM determinations were carried out by a previously developed and validated HPLC method [27] during solubility, *in vitro* dissolution, diffusion, and permeability studies. The HPLC separation was achieved on an Agilent 1100 HPLC device with Kromasil 100-5-C18 column (4.6 × 250 mm AkzoNobel, Bohus, Sweden) using 30 µl injection volume by a mobile phase consisting of 10 mM KH_2PO_4 (pH 3.5)/acetonitrile 55/45 v/v at a flow rate of 1.0 ml min^{-1} and at 250 nm UV detection. The calibration curve was plotted with a concentration range 5–150 µg/ml. Recovery studies OM from the SMEDDS were conducted using the same HPLC method. Limits of determination and quantification were calculated [37].

Characterization studies of SMEDDS

All studies were performed in three series and at 25 ± 0.5 °C.

Droplet size, zeta potential, and polydispersity

The mean droplet size, zeta potential (charge of surface), and polydispersity index (width of size distribution) were directly measured using Laser Light Scattering Particle Size Analysis Technique with ZetaSizer Nano ZSP Malvern device [37]. Samples were filled in 1 cm^3 cuvettes.

Refractive index

The refractive index of each formula of SMEDDS was measured with a refractometer (Shimadzu, Kyoto, Japan) by using a drop of undiluted liquid SMEDDS [37].

Self-emulsification time

The self-emulsification time properties of SMEDDS were evaluated by adding one gram each formula of SMEDDS into 250 ml pH 1.2 gastric fluid under stirring conditions 50 rpm with USP Type II dissolution apparatus (Sotax AT 7 U.S.). Dispersion time of SMEDDS was recorded as self-microemulsifying time [7].

pH

Measurements of SMEDDS pH were performed using pH meter NEL Mod.821 (NEL, TR) [26].

Electrical conductivity

Electrical conductivity was evaluated by using a conductivity meter (4071 Jenway, Staffordshire, UK) to determine the o/w type of microemulsion.

Viscosity

The viscosity was determined using a Brookfield viscometer (Brookfield ULA Viscometer, U.S.). Ten milliliters of SMEDDS was put in the jacketed sample cup that was linked to the circulating water bath. The device was balanced for 5 min beforehand. Measurement was implemented using a spindle at 30–200 rpm. The spindle speed was increased successively and the corresponding dial readings were recorded [37].

Stability studies of SMEDDS

The stability studies of SMEDDS were performed in three series and at 25 ± 2 °C/75%±5% RH for 3 months [38]. Physical appearance, droplet size, and drug content of SMEDDS were monitored.

In vitro *dissolution studies*

The dissolution studies were performed in 900 ml simulated gastric fluid (0.1 N HCl medium, pH 1.2) using USP Type II dissolution apparatus (Sotax AT 7 U.S.) at 50 rpm paddle speed. Temperature was 37 ± 0.5 °C. The test was implemented to compare the release of 10 mg/ml OM containing SMEDDS and commercial tablet containing the same quantity of OM ($n = 3$) [38]. One milliliter SMEDDS was filled into size 000 hard gelatin capsules. One milliliter of sample was taken from the 900 ml of dissolution medium, vessels, filtered, and analyzed by HPLC [39]. Samples were taken at predetermined time points, and the same amount of medium liquid was added into the vessels. The determination of the drug from the samples was done by our validated HPLC method. Drug release percentages were calculated considering drug concentration in samples and vessel volume. Briefly, the concentration calculation was made assuming that 10 mg of the active substance would be 0.011 mg/ml, if 100% of the active substance was released in a 900 ml medium volume. The results were subjected to the two-way ANOVA statistical test [8,34,40].

In vitro *diffusion studies*

The diffusion of 10 mg/ml OM SMEDDS from diffusion floating tube 1 kDa MWCO (Sigma Aldrich, Darmstadt, Germany) was monitored for determining release profile of SMEDDS from low porous membrane in gastric medium (molecular weight cutoff or MWCO refers to the lowest molecular weight solute (in Da) in which 90% of the solute is retained by the membrane) [7,41]. SMEDDS was loaded in diffusion tubes and observed 6 h in glass beakers in 100 ml pH 1.2 (0.1 N HCl 37 ± 0.5 °C medium) at 50 rpm magnetic stirring ($n = 3$). The tubes were held in the same medium solution for one day before the experiment. One milliliter samples were collected at predetermined amounts and time points, filtered, 1-ml buffer was added and the samples were analyzed by HPLC. The same amount of medium liquids was added into the beakers. Percentage of releases was evaluated.

Hexadecane membrane parallel artificial membrane permeability assay

Permeability experiments were studied using 96-well filter plate (Millipore, Billerica, MA). Plates include donor and acceptor part. HDM-PAMPA was used to estimate permeability of the gastrointestinal tract in the presence of 5% dimethylsulfoxide in pH ranging from 4 to 8. Donor part filters were treated with 15 µl 5% hexadecane in hexane to form lipid layer [29]. 1 mg/ml OM containing SMEDDS and pure drug suspension (including 0.25% w/v carboxymethylcellulose (CMC)) was prepared. 5% dimethylsulfoxide/phosphate buffer solution (pH 7.4) mixture was used as donor and receptor buffer. One hundred and fifty microliters (7.5 µl DMS + 92.5 µl PBS + 50 µl SMEDDS or CMC suspension) mixture was added into each donor compartment and 300 µl buffer was added into each acceptor compartment according to the PAMPA protocol (https://www.sigmaaldrich.com/hdm-pampa) ($n = 3$). Drug-filled donor plate was placed in the acceptor plate. Positive control series were created without hexadecane/hexane coating membrane with the same steps. The incubation time applied simultaneously in both experiments was 5 h. The quantity of the drug from both donor and acceptor sites was determined by HPLC [31]. Transit rate of OM was calculated using the equations [42,43]:

$$\log P_e = \log\left(C \times -\ln\left(1 - \frac{[\text{Drug}]_{\text{acceptor}}}{[\text{Drug}]_{\text{equilibrium}}}\right)\right) \quad (1)$$

$$C = \left(\frac{V_d \times V_a}{(V_d + V_a) \times \text{area} \times \text{time}}\right) \quad (2)$$

where P_e is the effective permeability coefficient (cm/s); V_d is the volume of donor compartment (0.15 cm^3); V_a is the volume of acceptor compartment (0.30 cm^3); area is defined as membrane area × porosity (0.24 cm^2 × 20% = 0.48 cm^2); time is the incubation time (18,000 s); [Drug] acceptor is SMEDDS or suspension drug acceptor concentration µg/ml measured by HPLC; [Drug] equilibrium is average positive control donor SMEDDS or suspension concentration measured by HPLC (the drug was transferred from the donor to the acceptor during the time when there was no membrane between the donor and the acceptor, which is positive control series. Pure drug CMC suspension, and the SMEDDS were studied in separate series and a constant divider as an average value was replaced in the formula).

In vivo *imaging studies (IVIS®)*

Absorption of SMEDDS was monitored by using florescent dye with 3D-fluorescence imaging computed tomography technique IVIS® [28]. Pharmacokinetic studies were performed on 12 male Swiss mice (aged 8–10 weeks and weighing 18–20 g) [44]. Generally, *in vivo* imaging is based on illuminating the targeted tissue. *In vivo* events containing fluorescent markers are displayed in an animal under anesthesia under a two-photon microscope [45]. For this purpose, fluorescence labeled SMEDDS and control dye solution were orally administered to two groups of six mice and were imaged and compared at predetermined minutes with the device of *In vivo* Imaging IVIS® Caliper (PerkinElmer, Waltham, MA) [46]. The dye selected for the study was the VivoTag®680 XL (PerkinElmer, Waltham, MA) which was bound strongly to small molecules. The stock solution of dye was prepared with dissolving 5 mg VivoTag®680 XL in 500 µl dimethylsulfoxide (VivoTag®680 XL protocol) [28]. Control dye mixture was prepared with 270 µl stock dye, 150 µl buffer (50 mM $NaHCO_3$) solution, and 480 µl water for six mice. Nine hundred microliters of PBS (pH 7) was added, vortexed, and centrifuged at 15,300 rpm for 10 min [47,48]. This washing step was repeated three times and supernatant was removed. The remaining washed portion was administered with 150 µl of oral gavage to the mice. The mice were anesthetized with 5% isoflurane via an integrated anesthesia system just before getting an image and were kept in motion and conscious during the whole study. Another mixture was prepared by adding 480 µl SMEDDS instead of one water in the same order and applied the same processes. The washed SMEDDS and control dye solution was

given to mice 150 µl with oral gavage. The distribution of VivoTag®680 XL was examined on an IVIS® instrument at 640/700 nm excitation/emission wavelength. Imaging was performed at 2nd, 3rd, 4th, 5th, and 6th hours after the application [49]. During imaging, the light absorbed by the SMEDDS was observed simultaneously with the control group, and the concentration of fluorescent dye in the tissues (region of interest ROI) was noted as average radiance [50]. After the fluorescent imaging studies were completed, mice were sacrificed and their organs (kidneys, lung, liver, spleen, intestine, stomach, heart) were removed to measure the concentration of fluorescent dye in the tissues. Living Image® 4.0 software (PerkinElmer, Waltham, MA, US) was used for imaging (see Supplementary Material).

In vivo *pharmacodynamic efficiency studies (NIBP)*

The aim of the study was to reveal antihypertensive response of the OM SMEDDS against the artificial hypertension (systolic blood pressure) produced by L-name (N-ω-nitro-L-arginine methyl ester) in experimental animals [51]. Pharmacodynamic studies were performed on 18 albino adult male Wistar rats (160–180 g). L-name was administered twice a day intraperitoneally to the rats at a dose of 185 µmol/kg for seven days to induce hypertension. Rat blood pressure measurements were obtained with small animal tail NIBP by using a tail-cuff. The rats were divided into three groups ($n = 6$). The rats of group 1 were designated as the control group and the drug was not administered. Group 2 and group 3 rats were orally given 1.3 mg/kg OM containing OM SMEDDS and OM suspension (including 0.25% w/v CMC) respectively once a day, taking into consideration previous pharmacokinetic parameters of OM administered rat blood results [34,52]. L-name was administered at 185 µmol/kg dose for 14 days concurrently with the OM treatment. Blood pressure was measured at 1st and 12th hours following administration of SMEDDS and suspension. The results were evaluated, and blood pressure findings were examined in all three groups for 14 days.

Tissue isolation and histopathological examination in rats

At the end of the one-month NIBP experiment, histopathology of intestinal tissue was investigated to determine whether the developed SMEDDS and pure drug suspension had side effects versus control group. Intestinal specimens were taken from suspension, SMEDDS and only L-name administered control group rats. The intestinal epithelium was fixed in 10% formol for histochemical examinations and then was washed under the stream for one night to remove the fixative. For dehydration of the samples, the intestinal epithelium was kept for 20 min in the series of 70%, 80%, and 96% ethyl alcohol respectively; and for 20 min in four different acetone batches. Two different xylenes were applied for 30 min for transparency. After immersing with soft paraffin two times, each for 1 h, the tissues were buried in hard paraffin blocks. Sections with a thickness of 5 µm were taken by means of a Rotari microtome (RM2255, Leica, Wetzlar, Germany), and were conducted in accordance with hematoxylin–eosin protocol [53]. Histopathological images were evaluated for the presence of celiac-like enteropathy. Body weights of each rat were measured at the beginning of the treatment and once a week during the NIBP experiment. The diarrhea findings were observed in each group.

Statistical evaluation

Inter-experimental precision studies were evaluated in terms of 95% confidence intervals and mean values. The results of stability studies were statistically evaluated with one-way analysis of variance (ANOVA) statistical test. *In vitro* dissolution results were subjected to ANOVA statistical test and the percentages of releases were evaluated to examine statistical change. Statistical significance of the difference between treatment efficacy of the control, SMEDDS, and suspension in the artificial tension model with L-name (NIBP) was determined using the two-way ANOVA test with GraphPad Prism 5.0 software (La Jolla, CA) [34].

Results and discussion

Development of SMEDDS

Oleic acid (fatty acid) which has lipophilic properties showed better solubility for the active ingredient OM with 51.6 µg/ml. Tween 80 (HLB > 12) as a water soluble surfactant exhibited enhanced solubility with 100 µg/ml because of its polar and hydrophilic specifications such as diglycerides with medium chain length [25]. On the other hand, transcutol containing ethylene glycol gave 99.1 µg/ml solubility and increased the microemulsion area with its surface wetting properties [7]. Span 80 (sorbitan monooleate), which is a nonionic sugar ester surfactant, showed 90 µg/ml solubility. The combination of ionic and nonionic surfactant enhanced the width of the microemulsion area [54]. Oleic acid as oil, Tween 80, Span 80 as surfactant, and Transcutol P as cosurfactant were selected for this study due to high solubility of OM, and distilled water was used as water phase (Figure 1(A)). Four different formulas of SMEDDS were prepared at various surf/cosurf ratios according to o/w HLB calculation (Table 1(A)). Twenty different combinations of surf/cosurf/oil ratio were studied in proportion as 1:1 and 2:1 surf/cosurf while the surfactants (Tween 80/Span 80) were at ratios of 2:3 and 1:1. The ratio of surfactant and cosurfactants is very important because of their stability effects, and it increases the membrane permeability of the active substance. In addition, the concentration of the usual surfactant in the self-emulsifying formulations required to form and maintain an emulsion state in the gastrointestinal path is between 30 and 60% by weight of the formulation. A large amount of surfactant can irritate the gastrointestinal pathway. The high HLB of the surfactants maintains hydrophilicity and the immediate formation of o/w droplets. This allows rapid dispersion and self-emulsification in aqueous media. Surfactants are amphiphilic and can dissolve high amounts of hydrophobic drugs. They prevent sedimentation in the gastrointestinal lumen and provide effective absorption by keeping molecules soluble. The self-emulsifying characteristics of SMEDDS were evaluated by water titration and identified by creating pseudo-ternary phase diagrams. It was determined that the formula giving the best microemulsion area (the most water carrying area by the components such as oil, surfactant and cosurfactants) was the formula 4 SMEDDS formulation at a ratio of 2:1 surfactant/cosurfactant (Figure 1(B)). The findings of SMEDDS microemulsion areas in phase diagrams are given in Table 1(B). All studies were performed at 25 ± 0.5 °C. Formula 4 SMEDDS formulation was selected for further studies.

Determination of OM with HPLC method

Based on a review of the literature, new SMEDDS formulation was developed and the validation method of OM was adapted and optimized. First, in the linearity studies, a six-point calibration line

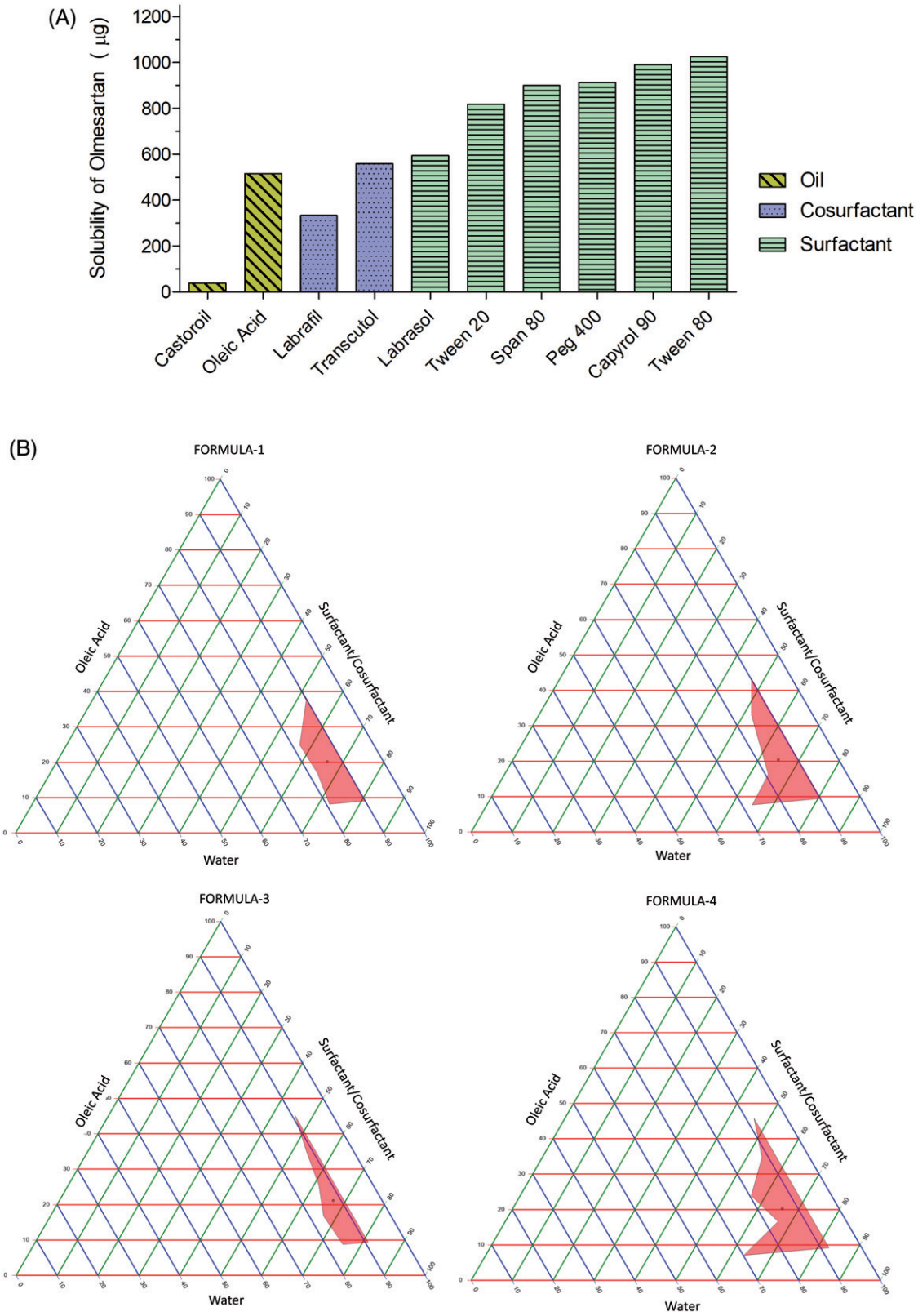

Figure 1. (A) The solubility of olmesartan medoxomil in different surf/cosurf and oil at 37 ± 1 °C. Based on the data of this study, Span 80 and Tween 80 were chosen as surfactant, oleic acid as oil and transcutol as cosurfactant. (B) Triangular phase diagrams for different ratios of oil/surfactant/cosurfactant. Surf/cosurf ratios were studied in proportion as 1:1 and 2:1 ratio while the surfactants (Tween 80/Span 80) were are 2:3 and 1:1 in itself shown as formula 1, 2, 3, and 4. Formula 4 which gave the largest area of o/w microemulsion (the most water carrying area by the components such as oil, surfactant, and cosurfactants) was selected our microemulsion formulation.

between 5 and 150 µg/ml concentrations was generated in three replicates, and determination coefficient r^2 was found to be 0.9999 as the manifestation of the linearity of the calibration curve. Specificity and selectivity were proved by observing the difference between chromatogram of empty buffer without OM and chromatogram of buffer injected with OM. No peak was observed

Table 1. (A) Surfactant, cosurfactant and oil ratios SMEDDS formulations (according to oil/water HLB calculations).

Surf/cosurf ratio	Oil (oleic acid)	Surf (tween80/span80)	Cosurf (transcutol)	Surf/cosurf ratio	Oil (oleic acid)	Surf (tween80/span80)	Cosurf (transcutol)
Formula 1		2:3		Formula 3		2:3	
1:1	1	1.8/2.7	4.5	2:1	1	2.4/3.6	3
1:1	2	1.6/2.4	4	2:1	2	2.13/3.2	2.67
1:1	3	1.4/2.1	3.5	2:1	3	1.86/2.81	2.33
1:1	4	1.2/1.8	3	2:1	4	1.6/2.4	2
1:1	5	1/1.5	2.5	2:1	5	1.33/2	1.67
Formula 2		1:1		Formula 4		1:1	
1:1	1	2.25/2.25	4.5	2:1	1	3/3	3
1:1	2	2/2	4	2:1	2	2.66/2.67	2.67
1:1	3	1.75/1.75	3.5	2:1	3	2.33/2.33	2.33
1:1	4	1.5/1.5	3	2:1	4	2/2	2
1:1	5	1.25/1.25	2.5	2:1	5	1.66/1.67	1.67

(B) Pseudoternary phase diagram findings.

Formula	A (oil)	B (surf/cosurf)	C (water)	X	Y	Microemulsion area
1	13.75	66.09	20.16	0.76	0.17	158.89
2	14.72	64.87	20.41	0.75	0.18	215.27
3	11.94	66.92	21.15	0.77	0.18	124.51
4	13.9	65.88	20.23	0.76	0.18	259.44

in the first 6 min, but the OM peak relative standard deviation (RSD) percentage values related with validation studies met FDA requirements, which are 20% for low concentrations and 15% for medium and high concentrations. The accuracy values provided requirement greater than 80% according to FDA criteria. Determination limit (LOD) and quantification limit (LOQ) were determined as 0.167 μg/ml and 0.506 μg/ml, respectively. These values provide sufficient precision for formulation development and quantitation studies. The data of inter-experimental precision studies were within the lower and upper bound with a 95% confidence interval [55]. In the next process, validation studies were carried out using the method previously developed for quantification of OM from the selected SMEDDS by HPLC. Based on all these validation findings, the HPLC method developed for OM was found to be accurate, precise, selective, sensitive, and stable, according to the criteria specified by the FDA [56]. RSD was calculated as 0.433% at 50 μg/ml concentration of active ingredient recovery.

Characterization of SMEDDS

Droplet size

Measured droplet size of SMEDDS with and without OM was found to be 196 nm and 200 nm, respectively. The droplet sizes of the 4 formula of SMEDDS are shown in Table 2. Stable microemulsions have a size between 20 and 200 nm [40]. This decrease in droplet size can be explained by the production of hydrogen bonds which are released during the loading of the active substance into the SMEDDS [57]. In addition, as the oil content of o/w microemulsions decreases, the particle size decreases [37].

Zeta potential

Formula 4 SMEDDS formulation showed −33 mV zeta potential, and this may indicate polar phospholipid and fatty acid groups zeta potential values of SMEDDS are shown in Table 2. In general, zeta potential above ±30 mV could form a physically stable dispersion. This means that particles with zeta potentials more positive than +30 mV or more negative than −30 mV could be considered stable [58–62]. This negative charge maintains homogenous microemulsion and prohibits gathering of vesicles.

Polydispersity

Formula 4 showed the highest (0.299) polydispersity among our o/w SMEDDSs (Table 2). The average of polydispersity index (0.3) indicates that the SMEDDS was homogenous and polydisperse. The polydispersity index determines the size range of particles in the system [59]. The relatively high polydispersity index shows the existence of bicontinuous structures and the dynamic structure of the system [63].

Refractive index

Formula 4 SMEDDS with the highest water content showed the lowest refractive index (1.327 ± 0.001) (Table 2). SMEDDS formulation with high water content shows low refractive index value because of its o/w structure [37].

Self-microemulsifying time

The self-emulsification time T emul values of all formulations ranged between 28 s and 44 s (Table 2). The time of formula 4 self-microemulsifying was 28 s, which indicates its rapid emulsification. The present study showed that the lower emulsification time values were related to spontaneous microemulsion production with a transparent bluish color [40]. Emulsification time depends on the amount of lipid in the formulation and emulsifying excipients. In this regard, formulations containing higher amounts of surfactant exhibited faster emulsification efficiency due to the complete mixing of the micellar solubility of the lipids in the aqueous phase with the micelle solubilization [25,64].

pH

pH measurement of our formula 4 SMEDDS was 4.711. The slight acidity of pH indicates that the gastrointestinal tract will not be irritated [26]. The pH of the analyzed microemulsion formulations is shown in Table 2. At low and medium water concentrations, the pH of the microemulsion shows a complex trend due to dilution and ranges from 3.0 to 3.8 (20–74% by weight). As the water concentration increases, the pH decreases from 3.8 to 3.0. Water content above 74% by weight changes the pH value and increases to 3.3. This can be explained by two opposite factors controlling pH along the dilution line. These factors are the ionization of

Table 2. Characterization of SMEDDS.

SMEDDS formulation	Drug (olmesartan) mg/ml	Surfactant tween80/span80 (g)	Cosurfactant transcutol (g)	Oil (g)	Droplet size (nm ± SD)	Polydispersity index (mean ± SD)	Zeta potential (mV ± SD)	Self emulsification time (s)	Refractive index	Electrical conductivity (μScm^{-1}±SD)	pH	Viscosity (cps)
Formula 1	1	2.203	1.1015	0.6875	295.667 ± 1.14	0.309 ± 0.003	(−)23.1 ± 0.23	44 ± 0.266	1.388 ± 0.38	259.7 ± 2.5	4.71 ± 0.009	104.6 ± 0.005
Formula 2	1	2.162	1.081	0.736	266.876 ± 1.49	0.308 ± 0.0005	(−)26.22 ± 0.16	36 ± 0.17	1.396 ± 0.002	258 ± 1.73	4.732 ± 0.005	103.7 ± 0.008
Formula 3	1	2.23	1.115	0.597	200.714 ± 1.206	0.319 ± 0.001	(−)27.55 ± 0.23	37 ± 0.105	1.375 ± 0.003	257.7 ± 4.61	4.707 ± 0.015	105.7 ± 0.0021
Formula 4	1	2.196	1.098	0.695	195.53 ± 1.386	0.299 ± 0.001	(−)33.44 ± 0.33	28 ± 0.201	1.327 ± 0.001	260 ± 2.01	4.711 ± 0.011	101.0 ± 0.005

organic acid and water dilution factor. When the water content is above 74%, the entire acid is in the continuous water phase and there is no additional release of H^+ ions. Thus, the pH of the microemulsion increases with the effect of the dilution factor and is governed by the pH dilution factor. This explains the significant reduction in pH and degree of ionization by the increased water content in microemulsion formulations [65].

Electrical conductivity

The electrical conductivity of formula 4 SMEDDS was 260 μS cm^{-1} at 25 ± 0.5 °C (Table 2). The high value 260 μS cm^{-1} of electrical conductivity is related to the formation of water in the outer phase. This increase in conductivity is due to the increase in NaCl ions which are not trapped in the oil core and present in the outer water phase. In addition, the presence of propylene glycol with the water in the external phase may reduce conductivity. It can be used to measure the electrical conductivity of a SMEDDS to determine its colloidal microstructure, strength, and their formation [37].

Viscosity

The viscosity of formula 4 SMEDDS formulation was 101.0 cps (Table 2). The viscosity decreases with increasing water phase concentration [66]. Viscosity measurements also give hints about the reverse micelles in the microemulsion (rod-like or worm-like) and o/w microemulsions exist when viscosity is greater than 100 cps. The graph of shear stress versus shear rate showed that Newtonian flow was determined as the finding of SMEDDS formulation viscosity. This was the proof of small and spherical droplets.

Formula 4 SMEDDS formulation provided sufficient qualification and was selected for further study among other o/w SMEDDS (Table 2).

Stability studies

The results of the formulations were suitable according to FDA Guidance for Industry Q1A (R2). No significant difference in physical properties (like color, odor, and transparency), drug content and droplet size was observed for 3 months, at 25 °C/75% RH and 5 ± 3 °C. This indicates the stability of the selected SMEDDS formulation in storage at room temperature and refrigeration [40,67]. The results of the formulations were not changed significantly during 3 months ($p > .05$) (Table 3).

In vitro *dissolution studies*

Dissolution rate results of SMEDDS in hard gelatin capsule and commercial tablet were compared [58]. SMEDDS dissolution ratios were 1.67 times higher than the tablet formulation. The release of olmesartan from the SMEDDS formulation was 88% at the 30th minute (Figure 2(A)). If at least 85% of the written dose on the label is dissolved within 30 min, it is considered to be fast soluble and no bioequivalence study is required [68]. The difference between the SMEDDS and tablet groups was statistically significant ($p < .001$) according to two-way ANOVA (statistical test) (Figure 2(C)) [39]. Observed power value is 100% and partial eta squared value was also high. (Partial) eta squared is an effect size measure for one-way or factorial ANOVA. This was the manifestation of increased release.

Table 3. SMEDDS stability values.*

	Droplet size (nm)		Drug content	
Months	(25 °C/60% RH) SD, RSD%	(5 ± 3 °C)SD, RSD%	(25 °C/60% RH) SD, RSD%	(5 ± 3 °C) SD, RSD%
Initial value	193.657 ± 1.19 0.614%		99.96 ± 0.44 0.440%	
1st month	194.933 ± 1.41 0.723%	196.533 ± 1.55 0.789%	99.87 ± 0.47 0.471%	99.75 ± 0.26 0.261%
2nd month	194.971 ± 1.21 0.621%	198.437 ± 1.35 0.680%	99.99 ± 0.43 0.430%	99.81 ± 0.50 0.501%
3rd month	195.532 ± 1.38 0.706%	198.939 ± 1.07 0.538%	99.89 ± 0.41 0.411%	99.61 ± 0.45 0.502%

*$p > .05$.

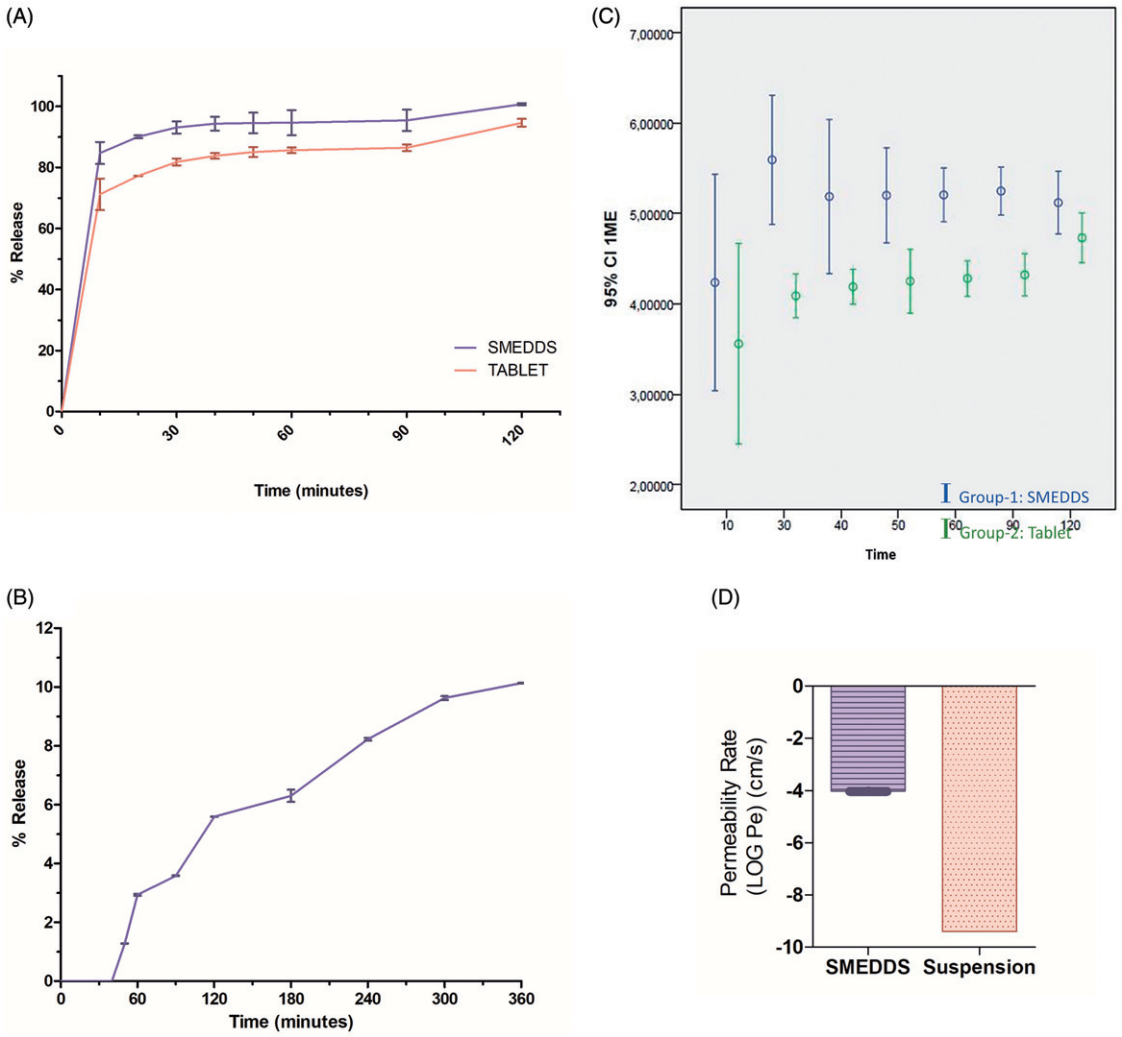

Figure 2. (A) Dissolution graphs of olmesartan from SMEDDS in hard gelatin capsule versus tablet in pH 1.2 medium, using USP apparatus type 2 at 50 rpm, analyzed by the HPLC. SMEDDS dissolution values were 1.67 times higher than the market formulation. 5 °C, USP apparatus 2 at 50 rpm, analyzed by the LC-UV and UV methods. (B) Diffusion graph profile of olmesartan from SMEDDS by using 1 kDa MWCO floating tube in pH 1.2 medium. (C) ANOVA statistical findings of microemulsion in hard gelatin capsule versus tablet dissolution. $p < .001$. 'observed power' value is 100% and partial eta squared value is high. (D) Calculated permeability rates of SMEDDS and CMC suspension. In accordance with the formula, calculated permeability rate of the SMEDDS formulation was –4.014 ($Pe = 9.895 \times 10^{-5}$). As CMC suspension permeability rate can be calculated only if there is detectable transition, CMC suspension transition rate was calculated by using the value 0.05 μg which is the detection limit of CMC suspension, and CMC suspension permeability rate was calculated as –6.395 ($Pe = 4.030 \times 10^{-7}$). The antilogarithm of microemulsion permeability was 100 times faster than suspension.

In vitro *diffusion studies*

To simulate release profile of SMEDDS in gastric medium (0.1 N HCl, pH 1.2), three series SMEDDS experiments were performed with 1 kDa MWCO diffusion floating tube at 50 rpm (molecular weight cutoff or MWCO refers to the lowest molecular weight solute (in Da) in which 90% of the solute is retained by the membrane. Results were found on average to be 10% at the end of 6 h (Figure 2(B)). The dialysis membrane illuminated the diffusion of drug-loaded SMEDDS globules. The decreased drug release from the tube was due to the less porous structure of the tube membrane, which only allows passage of the free molecular state of OM (average mass 0.558 kDa) from the formulation. SMEDDS

Table 4. (A) Experimental findings of PAMPA transition studies when CMC suspension permeability value is taken equal to LOD value.

	SMEDDS acceptor	Log Pe (cm/s)	CMC suspension acceptor	Log Pe (cm/s)
AVG	0.610506366	–4.028388509	0.00005	–6.39468602
STDEV	0.060403697	0.066511258	4.09429E–20	8.94411E–15
RSD	0.894032287	–1.651063634	8.18858E–14	–1.3987E–13

(B) Experimental findings of PAMPA positive control well transition studies.

	Positive control Me	Acceptor Me (μg/ml)	Positive control CMC suspension	Acceptor CMC (μg/ml)
Pc1	1	1.101	1	13.795
	2	1.095	2	13.763
	3	1.117	3	14.853
	4	1.101	4	14.82
	5	1.095	5	14.751
	6	1.095	6	14.725
Pc2	1	1.063	1	13.699
	2	1.062	2	13.679
	3	1.052	3	13.749
	4	1.051	4	13.718
	5	1.146	5	14.674
	6	1.145	6	14.672
Pc3	1	1.121	1	12.971
	2	1.119	2	13
	3	1.07	3	14.459
	4	1.07	4	14.461
	5	1.139	5	14.557
	6	1.135	6	14.576
AVG		1.099		14.160
SD±		0.032		0.617
RSD		2.90		4.36

containing large hydrocarbon chain and micelles failed to pass through 1 kDa MWCO dialysis membrane [7]. This decreased release profile was similar to the decreased release behavior of SMEDDS in our PAMPA assay's positive control wells (which refers to wells uncoated with lipidic hexadecane membrane and low pore size) when compared to the CMC suspension of drug (Table 4(B)).

Drug release properties of SNEDDS from 1 kDa and 12 kDa MWCO of diffusion floating tubes were previously investigated in different pH environments by Beg et al. [7] and Jain et al. [41]. They obtained 10% drug transition from 1 kDa MWCO tube, and 90% drug transition from 12 kDa MWCO tube, respectively.

Permeability assay values

In PAMPA studies, the transition velocities of SMEDDS and CMC suspension of drug from parallel artificial membranes were compared. The results of PAMPA transition from inert hexane/hexadecane coated membrane (HDM) findings are shown in Table 4(A) and the results of uncoated membrane (positive control series) are shown in Table 4(B). Positive control compartments represented transition in the absence of membrane. Since the positive control wells were not coated with hexane/hexadecane lipid bilayer, the lipophilicity of SMEDDS could not be utilized and the passage of the SMEDDS was slower than pure drug CMC suspension. After the 5-h transition, there was no detectable permeation in suspension wells coated hexadecane. The transition rate was calculated according to the formula of HDM-PAMPA. The amounts of OM from solutions were determined by HPLC. Calculated transition rate of the SMEDDS formulation according to the formula was Log Pe = –4.014 ($Pe = 9.895 \times 10^{-5}$). As there was no detectable transition in the CMC suspension well or it was below the detectable limit, it was not possible to calculate the CMC suspension Log Pe. If there were a transition at the detection limit (0.05 μg), CMC suspension Log Pe would be calculated as –6.395 ($Pe = 4.030 \times 10^{-7}$). Since minimum detection limit was less than 0.05 μg, the antilogarithm of microemulsion permeability rate should be calculated at least 100 times faster than suspension (Figure 2(D)) if the transition of CMC suspension value was assumed to be equal to or less than LOD value. It can be said that drugs with a Pe value lower than 10^{-7} have a low permeability rate, and drugs with a Pe value higher than this value have a high rate [30,69]. The computed value –4.014 Pe from Equations (1) and (2) and Table 4(A,B) is greater than –7. For this reason, our formula provided a very permeable condition because of the lipophilic characteristic of SMEDDS. Low standard deviation of permeability values indicated better reproducibility of PAMPA system. Olmesartan permeability was examined with *in situ* and Caco-2 methods by Beg et al. and Gorain et al. previously [7,34]. By imitating the intestinal barrier, these models are useful tools for permeability screening purposes and are useful to examine the permeability of less soluble drugs containing drug delivery systems [70]. PAMPA may also provide information about permeability of a compound from membranes through passive diffusion, lipophilicity, ionization status, solubility, and bioavailability [31,71].

In vivo *imaging studies (IVIS®)*

Since there is already considerable literature on the pharmacokinetics of OM tablet or pure drug, we aimed to observe the biodistribution of the SMEDDS preparation containing OM. Hence, visualization of fluorescent labeled SMEDDS's biodistribution after oral administration is an innovative application. After fluorescence-labeled microemulsion and control dye solution with

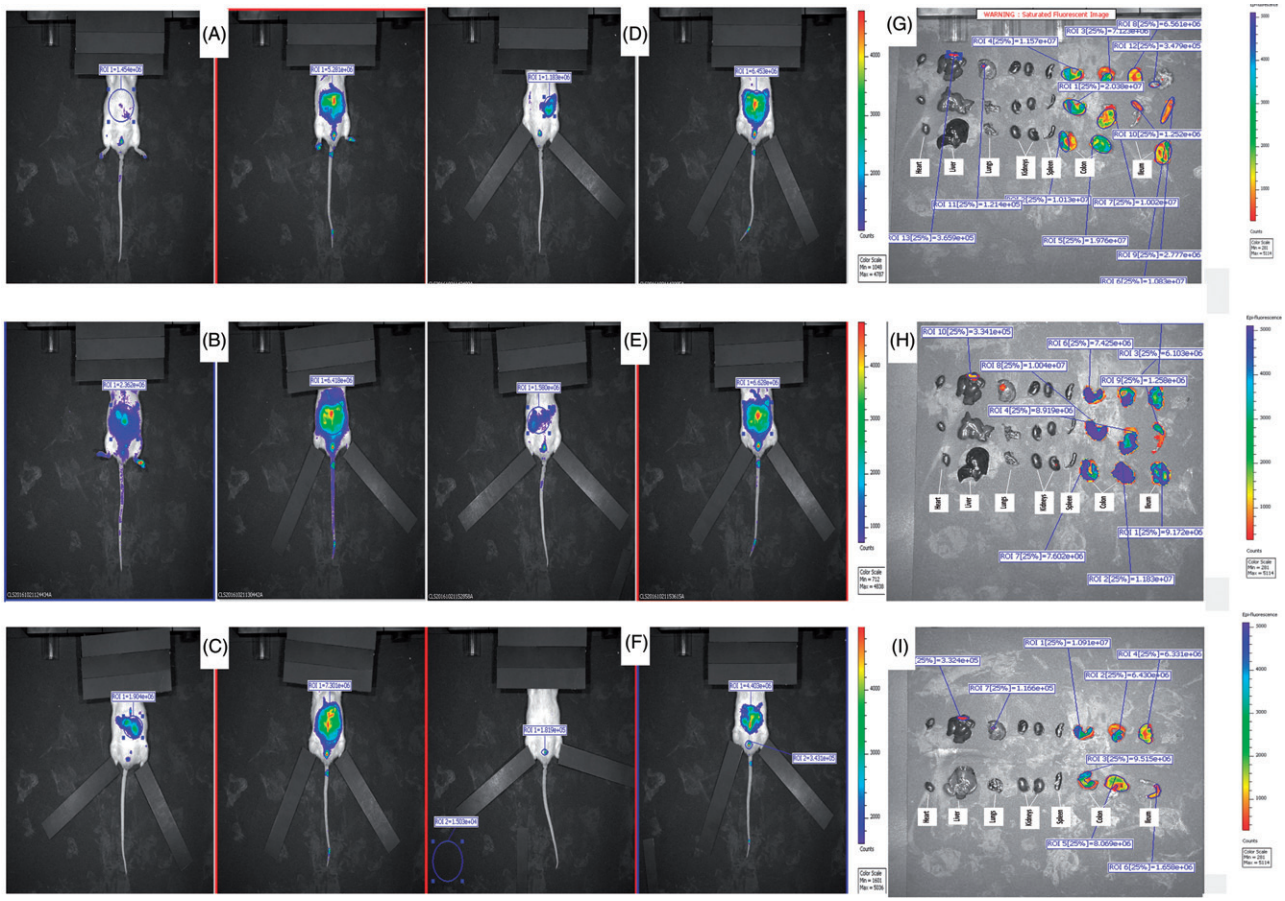

Figure 3. 3D *in vivo* and ex vivo fluorescence imaging Vivotag®680XL IVIS® images demonstrating biodistribution of formulation at 1st (A), 2nd (B), 3rd (C), 4th (D), 5th (E), and 6th (F) hours ($n = 6$). Compared to the control group (left), mice administered with fluorescent Vivotag®680 XL dye labeled microemulsion (right) emitted stronger signal. (G–I) Ex vivo IVIS® findings of organs (heart, liver, lung, kidney, spleen, stomach, colon, and ileum, respectively) after 6-h administration of Vivotag®680 XL. Microemulsion group gave stronger emission than control group.

Table 5. The comparison of *ex vivo* IVIS® ROI[a] findings of organs after 7-h administration of vivotag 680 XL between control-emulsion emission values.

VIVOTAG 680 XL	Emulsion ROI (photons/s/cm^2/sr)	Control ROI (photons/s/cm^2/sr)
Liver	1.43e + 06	3.71e + 06
Lung	346,000	0
Stomach	1.46e + 08	1850000
Colon	1.35e + 08	3.70e + 07
Ileum	7.20e + 07	4.70e + 07
Total	3.54e + 08	89,546,000
Compare	3.957923	

SMEDDS formulation gave 3.96 times more emission values than control group and indicated more absorption in tissues and organs.
[a]Region of interest (ROI) refers to signal intensity measurement (photons/s/cm^2/sr = photons per second per centimeter squared per steradian = $1.25664 \times 10 + 5$ m^{-2} s^{-1} SI_unit).

Vivotag®680 XL were orally administered to 12 mice (two groups of six mice) and were imaged with the device of *In Vivo* Imaging, images and emission values were evaluated (Figure 3). The comparison of ex vivo IVIS® findings of organs (heart, liver, lung, kidney, spleen, colon, and ileum, respectively) after 6-h administration of Vivotag®680 XL is displayed in Table 5. Labeled SMEDDS with VivoTag®680 XL gave 3.96 times stronger fluorescent emission than control dye administered mice in IVIS® studies. These results indicated increased bioavailability [28].

In vivo *pharmacodynamic efficiency studies*

In this study, systolic blood pressure was measured by tail cuff technique utilizing a noninvasive blood pressure monitor on the tail of conscious rats. During our experiment, L-name treated rats had 180 mmHg blood pressure, a constant tension of 108 mmHg was provided in the OM SMEDDS administered group. In contrast, in rats treated with OM suspension, had blood pressure reduced to 150 mmHg. This state lasted 14 days. As a result, SMEDDS formulation attenuated 3.1 times more effectively in increased tension compared to the CMC suspension in NIBP studies. The difference between the treatments of the OM medoxomil SMEDDS (OMEM) and OM CMC pure drug suspension (OMCMC) was statistically highly significant ($p < .0001$) (Figure 4). Nitric oxide modulates vascular smooth muscle tone and the inhibition of nitric oxide synthase increases arterial blood pressure. L-name is a competitive inhibitor of this enzyme and leads to hypertension artificially. Similar to this study, treatment with ANG-(1–7) of L-name

administered hypertensive rats was investigated by Benter et al. [72]. Also, Beg et al. formed an artificial hypertension model using dexamethasone [7] and L-name was used by Gorain et al. for the same purpose [34]. Rats with hypertension induced by I-NAME were treated with extract of *Lagenaria siceraria* fruit and blood pressure measured from the tail-cuff [73]. The pharmacodynamic properties of metoprolol succinate and telmisartan were investigated by Nandi et al. with the same technique [74].

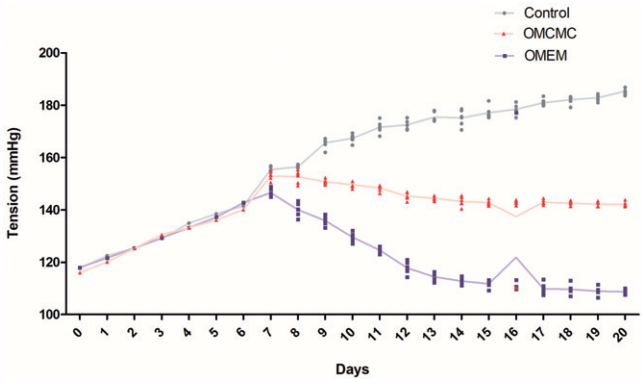

Figure 4. Statistical display of blood pressure measurements graphs after application drug on test animals. SMEDDS formulation was 3.1 times more effective in increased tension compared to the CMC suspension ($p<.0001$).

SMEDDS does not cause side effects

After 21 days of experiment, histochemical findings of the intestinal samples were taken from control group, SMEDDS and CMC suspension administered rats. The duodenum was used as the intestinal segment [75]. Histological examinations of rat intestine indicated that SMEDDS-treated rats and control group had no enteropathy findings while the CMC suspension-treated group showed enteropathy findings with increased mononuclear cell infiltration (Figure 5). We believe our transport system reduced the contact of OM with the intestines because of its lipophilic characteristics. This effect of SMEDDS can be explained by increased bile secretion in the gastrointestinal tract, dividing into mixed micelles, increasing lymphatic transport, and modulating

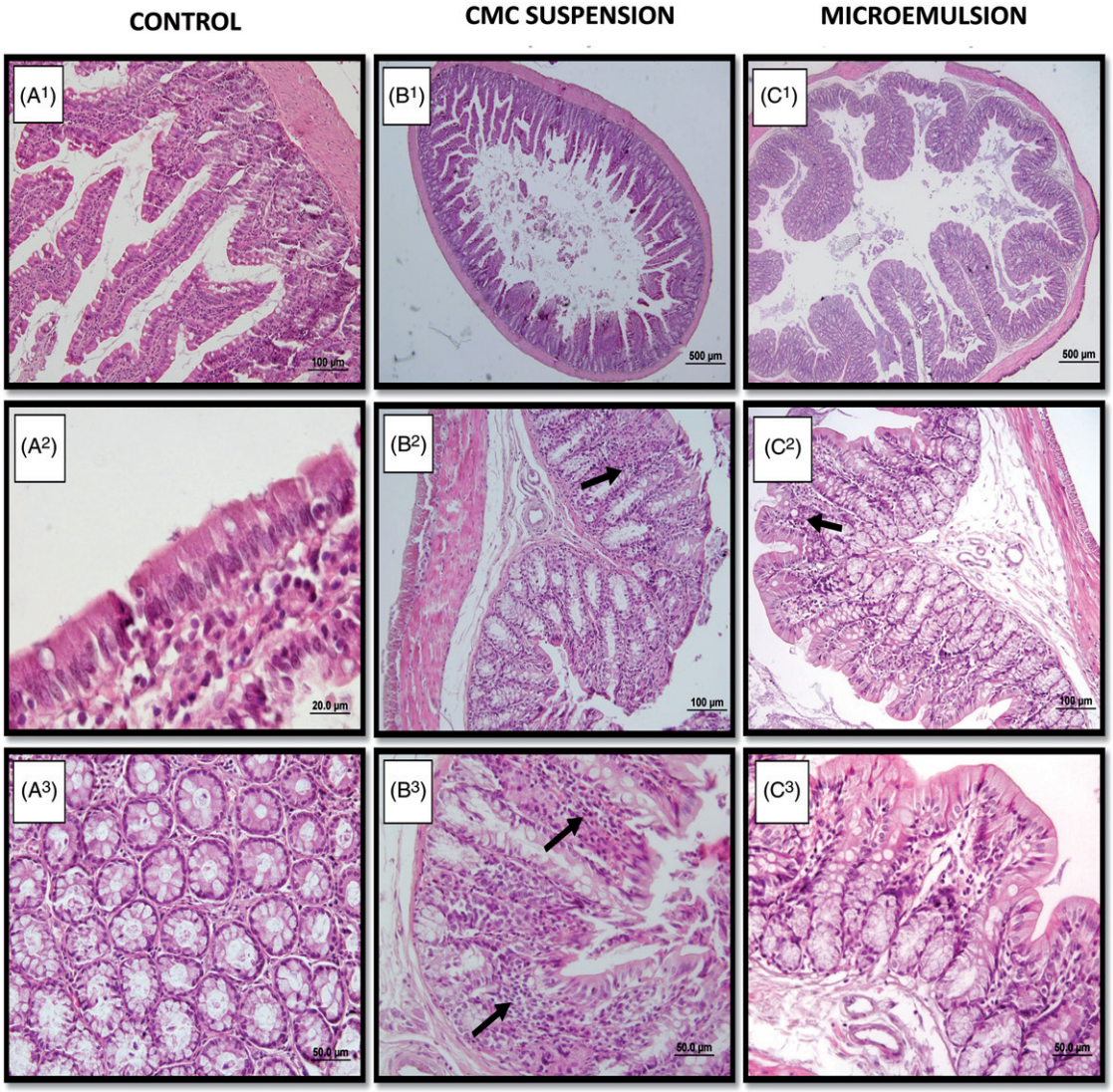

Figure 5. Images of histopathological examinations in rat duodenum after treatment with microemulsion or suspension. Formulation of olmesartan microemulsion administered (C_1, C_2, C_3) and control group (A_1, A_2, A_3) intestinal imaging did not indicate enteropathic findings such as celiac in contrast to the suspension administered rats. Duodenum biopsy showed olmesartan-associated enteropathy findings in suspension administered group of rats (B_1, B_2, B_3). Arrows indicate increased mononuclear cell infiltration and scale was 20 µm in A_2, 50 µm in A_3, B_3, C_3, 100 µm in A_1, B_2, C_2 and 500 µm in B_1, C_1.

enterocyte-based enzyme and carrier systems [25]. Throughout the NIBP experiment, SMEDDS did not cause diarrhea or weight loss compared to suspension. This finding suggests that the SMEDDS will prevent celiac-like enteropathy.

Conclusions

The stability tests of developed OM SMEDDS demonstrated that it is stable, transparent and suitable for drug delivery, and is a self-emulsifying stable system. Enhanced dissolution rates are observed and PAMPA studies suggested much faster permeability rate of OM SMEDDS compared to the pure drug suspension form. Labeled SMEDDS gave stronger fluorescent emission than control dye administered to mice in IVIS® studies, which indicates increased bioavailability. Treating effect of SMEDDS was more efficient than suspension in hypertensive rats according to NIBP findings. SMEDDS formulation caused neither enteropathy nor diarrhea and celiac-like enteropathy, during 21-day NIBP assay by reducing the contact of OM with the intestines. The remarkable results indicate that SMEEDS formulation improves dissolution and oral bioavailability of hydrophobic OM while reducing the adverse effects of celiac-like enteropathy. Since the formulation can be administered to adults and children both in gelatin capsule and liquid form in measured doses, it may be recommended to the drug industry.

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Disclosure statement

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