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Research Article

Development, Characterization and Permeability Assessment Based on Caco-2 Monolayers of Self-Microemulsifying Floating Tablets of Tetrahydrocurcumin

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Abstract. Novel self-microemulsifying floating tablets were developed to enhance the dissolution and oral absorption of the poorly water-soluble tetrahydrocurcumin (THC). Their physicochemical properties and THC permeability across Caco-2 cell monolayers were assessed. The self-microemulsifying liquid containing THC was adsorbed onto colloidal silicon dioxide, mixed with HPMC, gas-generating agents (sodium bicarbonate and tartaric acid), lactose and silicified-microcrystalline cellulose and transformed into tablets by direct compression. The use of different types/concentrations of HPMC and sodium bicarbonate in tablet formulations had different effects on the floating characteristics and *in vitro* THC release. The optimum tablet formulation (F2) provided a short floating lag time (~23 s) together with a prolonged buoyancy (>12 h). About 72% of THC was released in 12 h with an emulsion droplet size in aqueous media of 33.9±1.0 nm while that of a self-microemulsifying liquid was 29.9±0.3 nm. The tablet formulation was stable under intermediate and accelerated storage conditions for up to 6 months. The THC released from the self-microemulsifying liquid and tablet formulations provided an approximately three- to fivefold greater permeability across the Caco-2 cell monolayers than the unformulated THC and indicated an enhanced absorption of THC by the formulations. The self-microemulsifying floating tablet could provide a dosage form with the potential to improve the oral bioavailability of THC and other hydrophobic compounds.

KEY WORDS: Caco-2 cells; controlled release; permeability; self-microemulsifying floating tablets; tetrahydrocurcumin.

INTRODUCTION

Tetrahydrocurcumin (THC), one of the major metabolites of curcumin, has been reported to exhibit anti-oxidative (1), anti-inflammatory (2) and anti-carcinogenic (3) activities that were comparable to curcumin. Moreover, other pharmacological activities have been demonstrated such as an anti-diabetic effect (4) and hypertension alleviative activity (5). Nevertheless, the oral absorption of THC is limited due to its low aqueous solubility. In addition, a relatively short gastric residence time can result in an incomplete release of THC from its dosage form at the site of absorption and lead to a diminished efficacy of the administered dose.

Self-emulsifying drug delivery systems are isotropic mixtures of oils, surfactants, solvents and co-solvents/co-surfactants. These systems form an oil in water (*o*/*w*) emulsion when diluted in an aqueous medium such as GI fluid under mild agitation provided by gastrointestinal motion. The droplet size formed is between 100 and 300 nm while self-microemulsifying drug delivery systems (SMEDDS) form transparent microemulsions have a droplet size of less than 50 nm (6). The SMEDDS are physically stable, easy to manufacture and can be filled into soft gelatin capsules. The already dissolved form of the drug in the SMEDDS formulation is beneficial to enhance drug absorption (7). However, the normally prepared liquid SMEDDS show some disadvantages as the incorporation of liquid into the soft or hard gelatin capsules can lead to drug leakage and precipitation as well as to problems with capsule aging (8). Moreover, the cost of the manufacturing process is high. Recently, several research groups have successfully developed solid SMEDDS by incorporating SMEDDS into pharmaceutical excipients to produce different solid dosage forms, such as microcapsules (9), pellets (10,11) and tablets (12). The advantages of SMEDDS in tablet formulations are their low cost of manufacture, and ease of production on a large scale. Furthermore, tablets provide an inexpensive way to pack and ship.

A prolonged contact time of the dosage form in the upper gastrointestinal tract has also been found to enhance drug absorption. Floating drug delivery systems (FDDS) are such dosage forms, developed to provide a continuous release of drug at a desired rate while in the stomach, with a bulk density less than that of the gastric fluid (~1.004 g/cm^3) (13). These

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systems exhibit three main advantages. First, an enhanced drug absorption can be achieved because of the extended period of time before entering the small intestine (14). In addition, the fluctuations of plasma drug concentration can be better controlled (15). Finally, the residual systems are emptied from the stomach after the release of the drug (16). It has been shown that a floating single unit dosage form of losartan developed by Chen *et al.* and given to healthy volunteers demonstrated the advantages of improving the systemic bioavailability (17).

Caco-2 cells are the most frequently used as *in vitro* models for the studies of the absorption of compounds in the human small intestine (18,19). However, few studies have been reported on the assessment of the permeability of SMEDDS formulations based on cell monolayers (20,21). This paper presents the first *in vitro* permeability studies of THC and the THC in self-microemulsifying formulations across Caco-2 cells.

The objectives of this study were to develop and characterize a self-microemulsifying floating tablets (SMEDDS floating tablets) of THC, and to investigate the absorption of solubilized THC released from the SMEDDS floating tablets using Caco-2 cell monolayers.

MATERIALS AND METHODS

Materials

THC (white to off-white powder, 99.52% purity, lot no. C61260) was from Sabinsa Corporation (Piscataway, NJ, USA). Capryol® 90 (propylene glycol monocaprylate), Labrafac® PG (propylene glycol caprylate/caprate), Labrasol® (caprylocaproyl macrogol-8 glycerides) were from Gattefossé (Saint-Priest, France). Cremophor® EL (polyoxyethylene castor oil derivatives) was from BASF (Ludwigshafen, Germany). Aerosil® 200 (colloidal silicon dioxide) was obtained from Degussa-Hüls AG (Hanau, Germany). Sodium bicarbonate and tartaric acid were from P.C. Drug Center Co., Ltd. (Bangkok, Thailand). Prosolv® SMCC 90 (silicified-microcrystalline cellulose) was from JRS Pharma (Rosenburg, Germany). Methocel® K4M Premium CR, Methocel® K15M Premium CR, Methocel® K100M Premium CR (hydroxypropyl methylcellulose; HPMC) were kindly donated by Colorcon, Inc. (Midland, MI, USA). Tablettose® 80 (lactose) was from Mggel GmbH (Wasserburg, Germany). Magnesium stearate was from Peter Greven Nederland C.V. (Venlo, Netherlands). Hard gelatin capsules (size 00) were from Capsugel (Bangkok, Thailand). Acetonitrile and methanol (HPLC grade) were obtained from RCI Labscan (Bangkok, Thailand). All other chemicals were of analytical grade. Minimum Essential Medium (MEM) powder, Fetal Bovine Serum (FBS) and Hanks' balanced Salt Solution (HBSS, 1X) were obtained from Gibco, Invitrogen (Grand Island, NY, USA). 3,(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was from Molecular Probes, Invitrogen (Eugene, OR, USA), 0.5% Trypsin-EDTA 10X was from Gibco, Invitrogen (Berlington, ON, Canada), Phosphate buffered saline, pH7.4 was obtained from Sigma (Saint Louis, MO, USA). Penicillin-Streptomycin (Pen-Strep) was from Gibco, Invitrogen (Grand Island, NY, USA). 2-(*N*-Morpholino) ethanesulfonic acid (MES sodium salt) and 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES) were from Sigma (Saint Louis, MO, USA). Dimethylsulfoxide (DMSO) was obtained from Amresco (Solon, OH, USA). Transwell® permeable filter inserts (polycarbonate filters of 3 μm pore size, 24 mm Diameter Inserts, Transwell Type 3414) were from Corning Incorporated, Costar® (Corning, NY, USA).

Preparation of Liquid THC-SMEDDS

The formulations of liquid THC-SMEDDS (mixtures of Labrasol, Cremophor EL, Capryol 90 and Labrafac PG) were optimized by solubility assay and pseudo-ternary phase diagram analysis according to our previous study (11). Briefly, the solubility of THC in various vehicles, including oils, surfactants and co-surfactants was determined by the shake-flask method. The pseudo-ternary phase diagrams of the selected oil, surfactant–co-surfactant, and water were developed to identify the most efficient self-emulsification region using the water titration method. An optimum formulation contained 2 g of THC, 7.84 g of Cremophor EL, 7.84 g of Labrasol, 3.36 g of Capryol 90, and 3.36 g of Labrafac PG. These components were accurately weighed and mixed using a magnetic stirrer until a solution (THC-SMEDDS) was obtained. The liquid formulation after mixing was left for 24 h at room temperature.

Formulation of THC-SMEDDS Floating Tablets

The SMEDDS floating tablet formulations based on an effervescent system were prepared by direct compression. The relative humidity during tablet manufacturing was controlled in a small partitioned room equipped with the air-conditioner and portable dehumidifier to reduce the humidity lower as much as possible.

All the solid ingredients were accurately weighed and passed through sieve No. 40. The liquid THC-SMEDDS was adsorbed onto colloidal silicon dioxide by mixing in the mortar. The uniform powder obtained was continuously mixed with HPMC, gas-generating agents (sodium bicarbonate and tartaric acid), lactose, and silicified-microcrystalline cellulose by a geometric dilution method to receive the homogeneously mixed powder. The mixture was then blended further with magnesium stearate as a lubricant in the mortar for 3 min. The blend was directly compressed on a single-punch tablet machine (Charatchai Machinery, Thailand) with a 13-mm concave punch to produce biconvex tablets weighing 750 mg. The compression force was applied to produce tablets with the hardness of 5 kg measured with the Monsanto hardness tester. The different formulations of the tablets were manufactured as shown in Tables I, II, and III.

Tablet Friability

Friability of the optimum tablet formulation was determined with 20 tablets in a Roche friabilator for 4 min at 25 rpm. The percentage loss in weights were calculated and taken as a measure of friability (22).

Table I. Compositions of the SMEDDS Floating Tablets of THC with Different Amounts of HPMC K4M

Ingredients (mg/tablet)	F1	F2	F3
THC	10.0	10.0	10.0
Cremophor EL	38.4	38.4	38.4
Labrasol	38.4	38.4	38.4
Capryol 90	16.6	16.6	16.6
Labrafac PG	16.6	16.6	16.6
Silicon dioxide	72.0	72.0	72.0
HPMC K4M	97.5	142.5	187.5
Sodium bicarbonate	60.0	60.0	60.0
Tartaric acid	20.0	20.0	20.0
Lactose	45.0	45.0	45.0
Silicified-microcrystalline cellulose	331.7	286.7	286.7
Magnesium stearate	3.8	3.8	3.8
Floating lag time	25.3 s	23.0 s	12.3 min
Floating duration	>12 h	>12 h	>12 h

Table III. Compositions of the SMEDDS Floating Tablets of THC with Different Amounts of Sodium Bicarbonate

Ingredients (mg/tablet)	F7	F8[a]	F9
THC	10.0	10.0	10.0
Cremophor EL	38.4	38.4	38.4
Labrasol	38.4	38.4	38.4
Capryol 90	16.6	16.6	16.6
Labrafac PG	16.6	16.6	16.6
Silicon dioxide	72.0	72.0	72.0
HPMC K4M	142.5	142.5	142.5
Sodium bicarbonate	45	60	75
Tartaric acid	20	20	20
Lactose	45	45	45
Silicified-microcrystalline cellulose	301.7	286.7	271.7
Magnesium stearate	3.8	3.8	3.8
Floating lag time	36.2 s	23.0 s	21.7 s
Floating duration	6 h	>12 h	>12 h

[a] The composition of formulation F8 is identical to that of formulation F2 (Table I)

Monitoring of Floating Characteristics

The *in vitro* buoyancy studies were carried out using Dissolution testing apparatus 2; paddle method (Vankel® dissolution apparatus; VK 7000, USA). The medium was 450 mL of simulated gastric fluid (SGF, pH1.2) without pepsin. The studies were performed at 50 rpm and 37.0±0.5°C. The tablets were monitored for floating lag time and their duration of floating. The floating lag time was defined as the time taken by the tablet to reach the top of the medium, and the floating duration as the time period during which the tablet constantly floated on the surface of the medium (23).

Emulsion Droplet Size Analysis

The droplet size and distribution of the microemulsions from SMEDDS were determined by photon correlation spectroscopy (Zeta potential analyzer, Model ZetaPALS, Brookhaven, USA). SMEDDS floating tablets and liquid SMEDDS containing the same dose of THC (equivalent to 10 mg of THC) were separately immersed into 200 mL of purified water. The microemulsions were prepared by stirring at 50 rpm with a magnetic stirrer for 5 min and 12 h for the liquid SMEDDS and SMEDDS floating tablets, respectively. The insoluble substances were filtered with filter paper Whatman No.1. Then, the filtrate was filtered through the 0.45 μm syringe filter and the droplet sized measurement was performed. The sample viscosity and the water refractive index were factored into the program for the measurement of droplet sizes. Light scattering was monitored at a 90º angle and at a temperature of 25°C.

Table II. Compositions of the SMEDDS Floating Tablets of THC with Different Viscosity Grades of HPMC

Ingredients (mg)	F4[a]	F5	F6
THC	10.0	10.0	10.0
Cremophor EL	38.4	38.4	38.4
Labrasol	38.4	38.4	38.4
Capryol 90	16.6	16.6	16.6
Labrafac PG	16.6	16.6	16.6
Silicon dioxide	72.0	72.0	72.0
HPMC K4M	142.5	–	–
HPMC K15M	–	142.5	–
HPMC K100M	–	–	142.5
Sodium bicarbonate	60.0	60.0	60.0
Tartaric acid	20.0	20.0	20.0
Lactose	45.0	45.0	45.0
Silicified-microcrystalline cellulose	286.7	286.7	286.7
Magnesium stearate	3.8	3.8	3.8
Floating lag time	23.0 s	15.3 s	13.0 s
Floating duration	> 12 h	> 12 h	> 12 h

[a] The composition of formulation F4 is identical to that of formulation F2 (Table I)

In Vitro Drug Release Experiments

The release profiles of THC from SMEDDS floating tablets were determined according to the specifications of the Dissolution testing apparatus 2. The medium was 450 mL simulated gastric fluid (SGF, pH1.2) without pepsin. The studies were performed at 50 rpm and 37.0±0.5°C. The samples were taken at 10, 20, 30, 45, 60, 90, 120, 180, 240, 300, 360, 420, 480, 600 and 720 min, diluted with methanol, filtered through a 0.45 μm membrane filter, and assayed by HPLC. A plot of the cumulative % released of THC against time was constructed to illustrate the drug release profiles.

High-Performance Liquid Chromatography Analysis of THC

The high-performance liquid chromatography (HPLC) method was performed on an Agilent HPLC system (HP 1100, Agilent, USA) with a C18 column (VertiSep™ UPS C18 column 4.6×250 mm, 5 μm, Ligand Scientific, Bangkok, Thailand) as the stationary phase, and a UV detector set at the wavelength of 282 nm. The flow rate and injection volume was 1 mL/min, and 50 μL, respectively. The mobile phase was composed of 2% *v/v* acetic acid and acetonitrile in the ratio of 30:70 *v/v*. The retention time of THC and indomethacin used as an internal standard was about 6 and 7 min, respectively. The mean peak areas for each concentration

were calculated from three determinations, and the standard curve was constructed by plotting concentrations against peak areas. The concentration range with good linearity of 0.1–20 μg/mL showed a correlation coefficient (r^2) of 0.9998. The intraday precision obtained by repeated three replicates per each concentration of samples showed the percent relative standard deviation (% RSD) of 0.85 to 2.91. The interday precision of the method gave the %RSD ranging from 1.25 to 3.84. The recovery percentage of the method was between 92.33±0.23 and 101.62±2.03.

Scanning Electron Microscopy

A representative of the optimum formulation was selected to illustrate the scanning electron microscopy (SEM) images. The tablet was mounted on the stub. This specimen was then sputter-coated with gold particles and observed with a LV-SEM 5800 (JEOL, Japan) at an accelerating voltage of 10 kV. The surface and cross-sectional morphologies of the tablet before the *in vitro* release test and 120, 300, 480, and 720 min after *in vitro* release test were compared.

Kinetic Analysis of the *In Vitro* Drug Release

The Korsmeyer–Peppas model has been widely used in many studies for analysis of drug release kinetics in the matrix containing HPMC (24–26). The Korsmeyer–Peppas kinetic model was shown as the following equation.

$$M_t/M_\infty = Kt^n \quad (1)$$

where M_t/M_∞ is the fraction of drug released at time t, K is the constant incorporating structural and geometrical characteristics of the dosage form, and n is the release exponent that indicates the drug release mechanism. For the Fickian diffusion from the cylinder, n=0.45 while for the Anomalous transport, n is between 0.45 and 0.89 and for a case II transport (zero-order release), n=0.89.

Stability Studies of Tablets

The stability testing was carried out according to the ICH guidelines (2003) on the topic of Q 1 A (R2): stability testing of new drug substances and products. The floating tablets (n=100) were stored in air-tight glass containers and protected from light. The stability under intermediate conditions (30°C±2°C/65% RH±5% RH, 6 months) and accelerated conditions (45°C±2°C/75% RH±5% RH, 6 months) was determined. The optimum floating tablet formulation was evaluated for its appearance, the emulsion droplet size, and the drug content.

Cell Culture

The Caco-2 cell line was obtained from the American Type Culture Collection (ATCC, Virginia, USA). Cells were grown on plastic culture flask in MEM supplemented with 15% *v/v* of FBS and 1% *v/v* of 0.5% Pen-Strep. Cell cultures were maintained at 37°C in an atmosphere of 5% CO_2 and 90% relative humidity. The cells were split at 80% confluency. For transport studies, cells were trypsinized with trypsin-EDTA prior to seeding on Transwell® at a seeding density of 60,000 cells/cm^2. The completely differentiated cells, 21–23 days after seeding were used. Cells from passage number 28 to 32 were used for the transport experiments. The medium was changed every alternate day in both the donor and the acceptor compartment.

Cell Viability Evaluation

Toxicity of the THC and THC formulated as SMEDDS formulations was studied with the MTT toxicity assay. THC powder was solubilized in a DMSO/HBSS mixture to obtain THC concentration of between 25 and 200 μM. THC liquid SMEDDS was dispersed in water and finally diluted with HBSS. To prepare THC microemulsions from THC-SMEDDS floating tablets, the tablet was placed into 50 mL of water and stirred for 12 h. The obtained microemulsion was diluted with HBSS to obtain various concentrations of THC. Caco-2 cells were seeded to 96-well plates at a density of 2×10^4 cells/well. Two hundred microliters of suspended cells in complete MEM culture medium were added to each well. Cells were cultured for 96 h under the conditions previously described in this study prior to the toxicity experiments. The experiment was started by removal of the culture medium and cells were washed with PBS pH7.4. One hundred microliters of the sample solutions at different concentrations was added to each well. After 3 h treatment, samples were removed, and cells were rinsed with PBS. Fifty microliters of 5 mg/mL MTT solution in PBS was added to each well. The cells were further incubated under the same conditions for 4 h. The MTT solution was discarded carefully, and 100 μL DMSO was subsequently added to each well to dissolve the formed formazan. The UV absorbance at 570 nm was subsequent measured. Eight wells were used for each sample. Wells incubated with HBSS were used as a negative control, and 0.1% sodium lauryl sulfate was used as a positive control. The Percentage cell viability was calculated relative to the measured absorbance of the negative control that represented 100% cell viability.

Preparation of Samples for Permeability Tests

THC solution was prepared by dilution of the stock THC solution in DMSO in either, transport medium pH6.5 (for apical side loading), or pH7.4 (for basolateral side loading) to obtain a THC solution at a concentration of 134 μM. The SMEDDS formulations were also diluted to obtain THC concentration of 134 μM by a 200-fold dilution. This concentration was tested to be safe according to a cytotoxicity test determined by the MTT assay. The THC microemulsion was prepared by diluting the THC-SMEDDS in distilled water. The microemulsion was diluted with either transport medium pH6.5 or pH7.4. The microemulsion released from the THC-SMEDDS floating tablets was obtained by placing the tablet in distilled water for 12 h with continuous stirring. The obtained mixture was filtered through Whatman No. 1 filter to yield a microemulsion that was also further diluted with transport medium.

Transport Across the Caco-2 Monolayers

The Caco-2 cell system was used in this study as an *in vitro* model for the evaluation of the permeability of THC and THC prepared in various formulations. The bidirectional assay was able to determine whether the drug compound did undergo active efflux.

The permeability of THC in the formulations including the THC liquid SMEDDS and THC-SMEDDS floating tablets was determined and compared with that of THC solubilized in DMSO. Cell monolayers cultured for 21–23 days were used. The integrity of the monolayers was regularly monitored by measuring the transepithelial electrical resistance (TEER), using a Millipore ERS voltmeter (Millipore, Bedford, MA). Monolayers with TEER of more than 300 Ωcm^2 were used in the transport experiments. A mixture of 39.2 mL HBSS (1X), 0.8 mL of 1 M MES or 1 M HEPES buffer solution (HBSS containing 20 mM buffer solution) were used as the transport medium at pH6.5 and pH7.4, respectively. THC at a concentration of 134 μM in the transport medium was added to the cell monolayer**.** The viability of Caco-2 cells observed by MTT assay was higher than 90% for up to 3 h. Thus, 3 h transport studies were conducted. Each experiment was performed in triplicate. Transport studies were performed in a shaking incubator maintained at 37°C and 50 rpm.

Prior to the experiments, the cell culture medium was removed, and the monolayers were washed with phosphate buffered saline pH7.4. The culture medium was replaced with prewarmed (37°C) transport medium pH6.5 for the apical compartment and at pH7.4 for the basolateral compartment. The TEER values of the monolayers were determined. For the absorptive transport (AP→BL) experiments, 1.5 mL of samples in transport medium pH6.5 was added to the apical (donor) compartment, and 2.6 mL of transport medium pH7.4 was added to the basolateral (receiver) compartment. For secretive transport (BL→AP) studies, 2.6 mL of samples in transport medium pH 7.4 was loaded into the basolateral (donor) compartment, and 1.5 mL of transport medium pH6.5 was added to apical (receiver) compartment. A sample volume of 400 and 100 μL was removed from the receiver and donor side, respectively, at various times (*i.e.*, 5, 15, 30, 45, 60, 90, 120, 150 and 180 min). The withdrawn volume from the receiver compartment was replaced with the same volume of prewarmed fresh transport medium. The cumulative amount of the drug that permeated through the monolayers measured in the receiver compartment was plotted against the sampling time. The apparent permeability coefficient was calculated according to the following equation (27).

$$P_{app} = (dQ/dt)/(AC_0) \quad (2)$$

where *dQ/dt* is the slope obtained by linear regression of the cumulative transported amount (in microgram) as a function of time. *A* is the surface area of the filters (4.67 cm^2 in 6-wells). C_0 is the initial concentration of THC on the donor compartment (in microgram per milliliter).

The efflux ratio (*ER*) was calculated from the following equation (28)

$$ER = P_{app(BA)} \Big/ P_{app(AB)} \quad (3)$$

where $P_{app\ (BA)}$ is the apparent permeability coefficient for the secretory transport, and $P_{app\ (AB)}$ is the apparent permeability coefficient for the absorptive transport.

Statistical Analysis

All results are expressed as mean±S.D. Differences between two related parameters were performed by student's *T* test or one-way ANOVA. Differences were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

Design and Development of the THC-SMEDDS Floating Tablets

It was necessary to find suitable excipients for processing the active substance into a self-microemulsifying floating solid dosage form with the desired properties. The solidification technique was used in this study to transform liquid SMEDDS to solid SMEDDS by adsorption onto the solid carrier. Colloidal silicon dioxide (Aerosil® 200) was suitable for use as a sorbent for the liquid SMEDDS because of its high specific surface area (200±25 m^2/g) and low density (0.05 g/cm^3) (29). Sodium bicarbonate and tartaric acid were incorporated as the gas-generating agents. HPMC was used for gas-entrapment, and a sustained-release polymer. Furthermore, lactose (agglomerated form) and silicified-microcrystalline cellulose, designed to be direct compression fillers, were used to improve the powder flow property and compressibility (30,31). The powder mixtures could then be readily compressed into tablets by direct compression. The obtained tablets were white and biconvex in shape. The diameter of the tablets was 12.85±0.14 mm, and the thickness was 5.75± 0.03 mm. The average weight of tablets was 751.2±0.7 mg. The friability of the tablets in all formulations was 0.74± 0.05%. The drug content was between 91.5 and 103.3%.

Floating Properties

Carbon dioxide gas was generated when the tablet contacted the simulated gastric fluid and was trapped within the HPMC polymer matrix. This produced an upward motion of the dosage form and maintained its buoyancy. The influence of the HPMC and sodium bicarbonate on the floating characteristics was therefore investigated.

The gel layer formed by HPMC trapped the generated carbon dioxide gas with possible effects from its concentrations and viscosity grades. The amount of HPMC was therefore varied as shown in Table I. F1 and F2 were found to give a similar short floating lag time (~23–25 s), probably because the amount of HPMC within this range (97.5–142.5 mg/tablet) was able to strongly trap the generated gas within the matrix so it could reach a density of below 1 g/cm^3 rapidly. However, a further increase of HPMC to 187.5 mg/tablet (F3) provided a longer floating lag time (~12 min). This could be due to the greater swelling property (water uptake), resulting in an increased weight and longer time to reach the density below 1 g/cm^3. Tablets containing various HPMC viscosity grades of 142.5 mg/tablet (Table II) were found to have no significant effect on the floating lag time and stayed afloat for longer than 12 h.

The appropriate sodium bicarbonate concentrations were found to be in the range of 45–75 mg/tablet due to the fact that the tablets were either unable to float at lower concentration

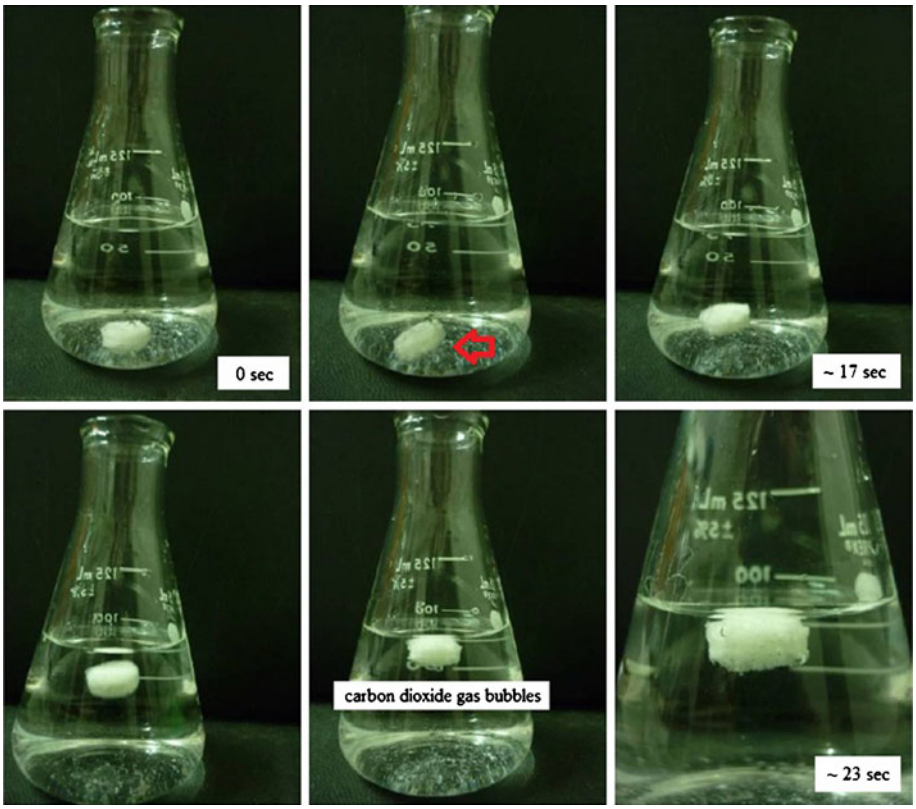

Fig. 1. Photographs taken while the tablet was showing an upward motion at different time interval in simulated gastric fluid (SGF, pH1.2)

or disintegrated with ones higher than 75 mg/tablet. The concentrations within this range provided a satisfactory floating lag time (~21–36 s) (Table III). In the case of the floating duration, F7 containing 45 mg/tablet of sodium bicarbonate was found to sink after 6 h which was due to the inadequate amount of gas to maintain a longer floatation whereas tablets containing 60 and 75 mg/tablet of sodium bicarbonate could float longer than 12 h. This indicated that higher amount of sodium bicarbonate allowed a longer floating duration and would facilitate the prolonged and complete release of the THC. The photographs taken while the tablet was showing an upward motion in the SGF are shown in Fig. 1.

Measurement of the Droplet Size of the Resultant Microemulsion

The droplet size of the microemulsion from the self-microemulsifying floating tablets (the optimum formulation; F2) and liquid SMEDDS was 33.9±1.0 nm and 29.9±0.3 nm, respectively. The polydispersity index of the microemulsion of the SMEDDS floating tablets and liquid SMEDDS was 0.224±0.01 and 0.192±0.013, respectively. The obtained oil droplet size of less than 50 nm indicated that this drug delivery system provided a microemulsion with good emulsifying property. Furthermore, incorporating the liquid SMEDDS into a solid dosage form did not have any remarkable effect on the emulsion droplet size.

In Vitro Drug Release

The THC release rate could be adjusted by varying the concentration and the viscosity grade of the HPMC that played an important role in the retardation of drug release. An increase in the concentration of HPMC K4M resulted in a

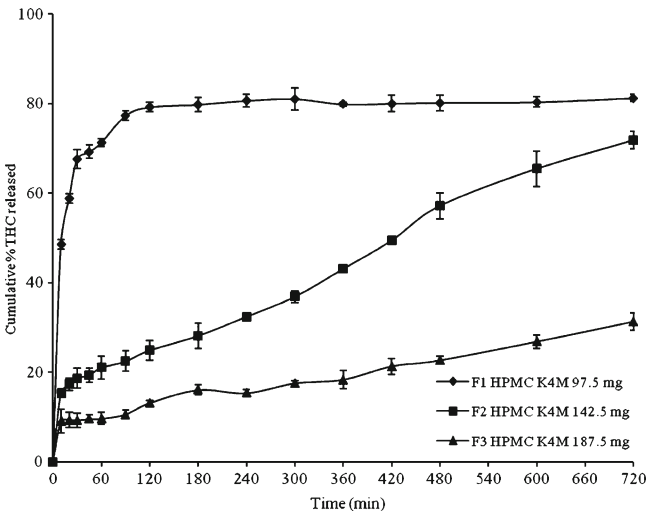

Fig. 2. Release profiles of THC from the SMEDDS floating tablets with different amounts of HPMC K4M (*F1* HPMC K4M 97.5 mg; *F2* HPMC K4M 142.5 mg; *F3* HPMC K4M 187.5 mg) in simulated gastric fluid (SGF, pH1.2) without pepsin. Data represents the mean±S.D. (n=3)

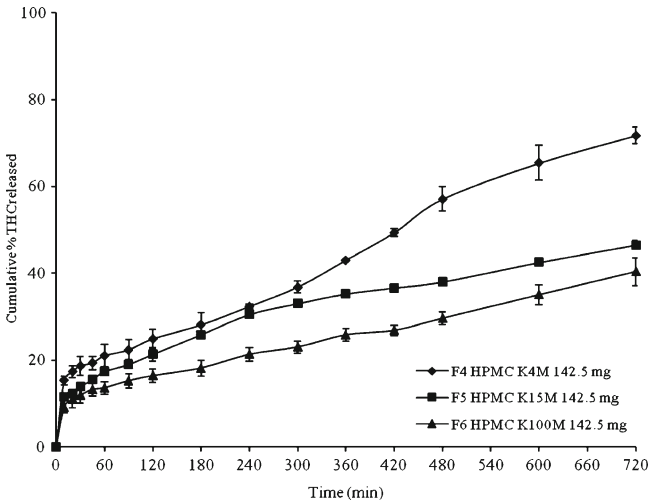

Fig. 3. Release profiles of THC from the SMEDDS tablets with different viscosity grades of HPMC (*F4* HPMC K4M 142.5 mg; *F5* HPMC K15M 142.5 mg; *F6* HPMC K100M 142.5 mg) in simulated gastric fluid (SGF, pH1.2) without pepsin. Data represents the mean± S.D. (n=3)

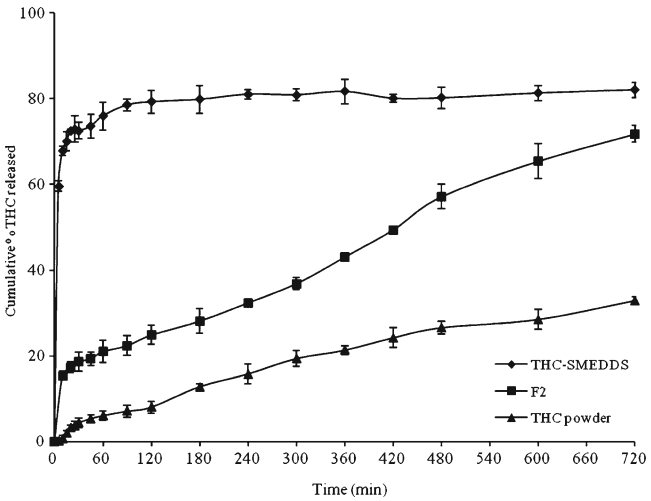

Fig. 5. Release profiles of THC from the THC-SMEDDS floating tablets (*F2*) compared with the release from the liquid THC-SMEDDS and dissolution of the unformulated THC powder in simulated gastric fluid (SGF, pH1.2) without pepsin. Data represents the mean±S.D. (n=3)

decrease in the release rate of THC from the tablets ($p<0.05$) (Fig. 2). This finding was consistent with the floating tablets developed by Arza *et al.* (32). A high HPMC K4M content resulted in a greater amount of gel being formed. After 12 h, the THC released was about 74% for F2 whereas that for F3 was only 32%. The tablets containing the lowest concentration of the polymer (F1) exhibited the poor tablet integrity and showed the signs of tablet disintegration immediately after the administration of the tablets into the medium that also resulted in an initial burst release.

Tablets containing a lower viscosity grade of HPMC (K4M) showed a faster drug release ($p<0.05$) compared to those containing higher viscosity grade of HPMC (K15M and K100M) (Fig. 3). The higher viscosity of HPMC formed a higher viscous gel that appeared to be more capable of retarding the ingress of the medium and the consequent drug release.

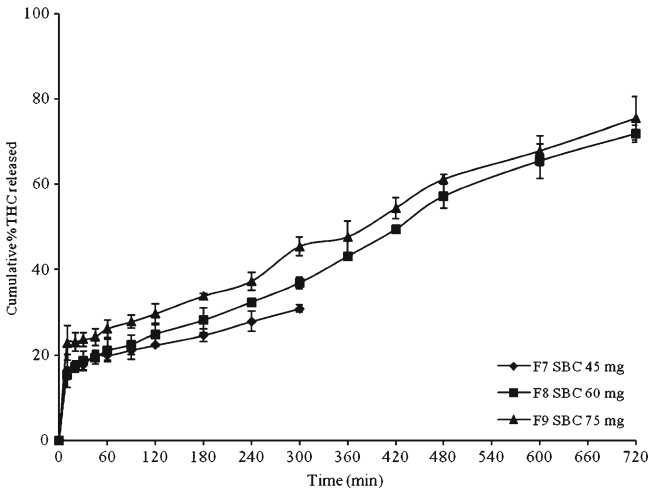

Fig. 4. Release profiles of THC from the SMEDDS floating tablets with different amounts of sodium bicarbonate (SBC) (*F7* SBC 45 mg; *F8* SBC 60 mg; *F9* SBC 75 mg) in simulated gastric fluid (SGF, pH1.2) without pepsin. Data represents the mean±S.D. (n=3)

The influence of the sodium bicarbonate level on the THC release is shown in Fig. 4. As the concentration of the gas-forming agent increased, the drug release rate also increased ($p<0.05$). Tadros (33) has reported that an increase in the gas-generating agent concentration produced a larger amount of effervescence and led to an increase in the rate of pore formation; therefore, a faster drug release rate was observed. Martínez *et al.* (34) explained that the carbon dioxide bubbles moving from the matrix inside to its periphery decreased the matrix coherence and resulted in matrix relaxation. F9 containing the highest amount of sodium bicarbonate (75 mg) showed an initial burst release of 24% in the first 30 min. This burst release might be attributed to the rapid liberation of carbon dioxide gas that created the formation of more pores. This phenomenon led to a greater release of the drug residing in the gel layer. F7 with the lowest content of sodium bicarbonate (45 mg) was found to sink after 6 h and indicated that insufficient gas was produced in the tablets.

The Optimum Formulation

According to the floating properties and the THC release profiles, F2 was selected as the optimum formulation. F2 exhibited a short floating lag time (23 s) and long floating duration (>12 h). The drug was also released over a period of time in a controlled release manner. The release rate and

Table IV. Linear Regression Analyses of the *In Vitro* Drug Release Data of the F2 SMEDDS Floating Tablets Using the Korsmeyer–Peppas Kinetic Model

Korsmeyer–Peppas $M_t/M_\infty = Kt^n$		
K	n	r^2
2.6872	0.4723	0.9914

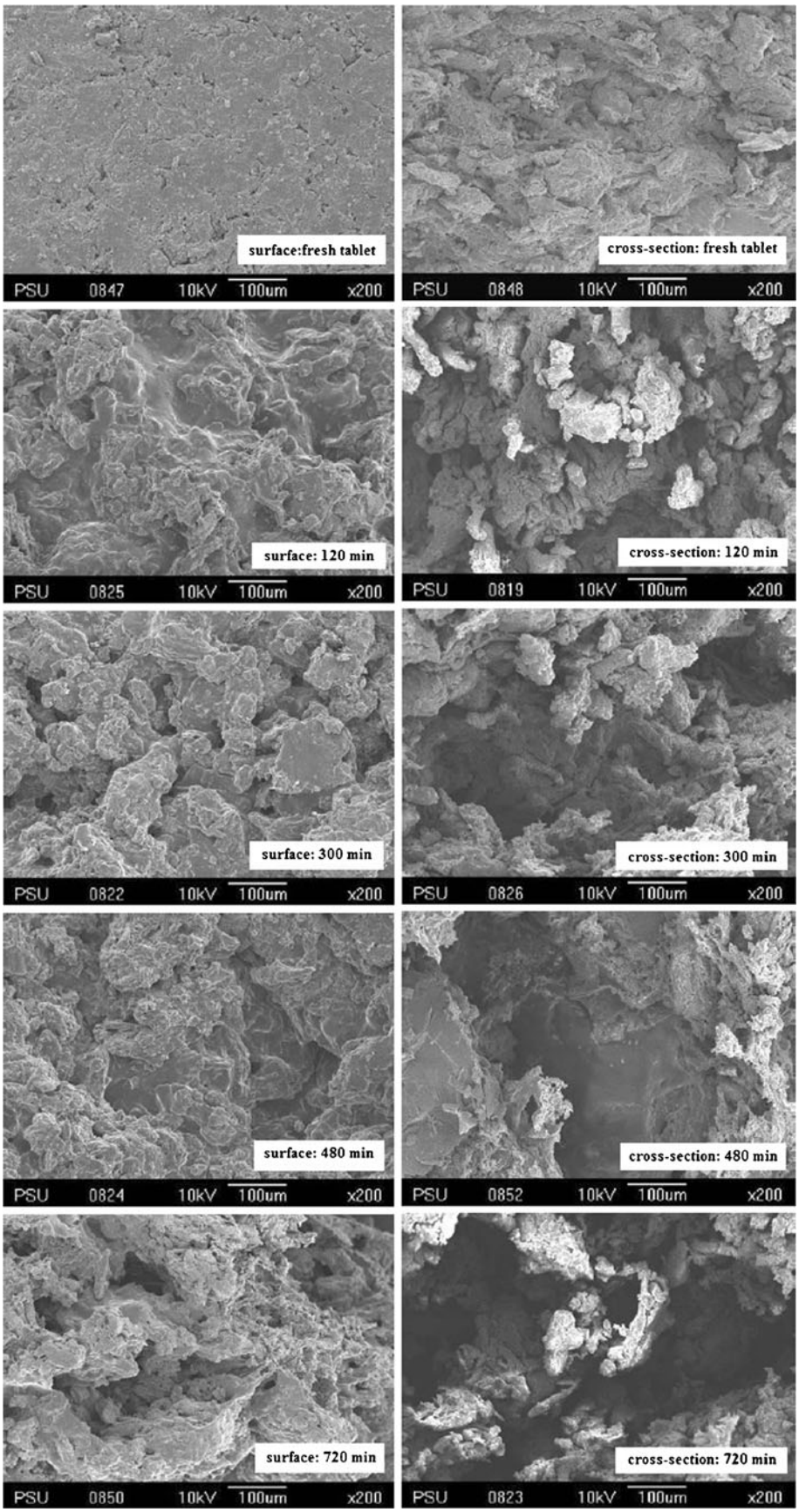

Fig. 6. *Left column* surface morphologies of the fresh tablet, and the tablets taken at 120, 300, 480 and 720 min after the *in vitro* release test, respectively. *Right column* cross-sectional morphologies of the fresh tablet, and the tablets taken at 120, 300, 480 and 720 min after the *in vitro* release test, respectively (×200)

extent of release of THC liquid SMEDDS (80% within 2 h) and the self-microemulsifying floating tablet formulation (72% within 12 h) were significantly higher than that of unformulated THC (only 30% within 12 h) ($p<0.05$) (Fig. 5).

Kinetic Analysis of *In Vitro* Drug Release

The THC release data from formulation F2 was fitted according to Korsmeyer–Peppas model and exhibited the best fit when n value was 0.47, indicating the contribution of both diffusion and polymer relaxation/erosion controlled the rate of drug release (anomalous transport) (Table IV). When in contact with water, the HPMC polymer forms a hydrated gel layer which then controlled the diffusion of water into the matrix and retarded the release of the dissolved THC to the dissolution medium. For the second mechanism, the outer hydrated gel that was eroded or dissolved in aqueous media allowed for the drug to be released. The results were correlated with the morphological analysis and erosion studies that showed that matrix tablets underwent erosion during the drug release (data not shown).

SEM Observation of SMEDDS Floating Tablets

The surface and cross-sectional SEM images of the tablets (F2) are shown in Fig. 6. SEM micrographs of the surface of the fresh tablet showed no pores, and the cross-sectional image showed a firm structure. In contrast, both the surface and the cross-sectional images of the tablets after the *in vitro* release test revealed a porous structure with increasing width and depth. Consequently, THC might be released *via* diffusion through the expanded pores. In addition, larger pore structures were observed with increasing hydration time.

Stability Studies

There were no significant changes in both the appearance and emulsion droplet size for up to 6 months under both intermediate storage condition (30±2°C, 65±5%RH) and accelerated storage condition (45±2°C, 75±5%RH). However, the THC content was found to be slightly decreased under both storage conditions (96.23±2.12% and 92.11±1.49%, respectively) (Table V). It is possible that the moisture absorption property of the formulation might affect the chemical stability of THC. In addition, the tablets in this study had not been stored in any moisture proof packs. Therefore, the proper storage condition and special packaging for this type of formulation should be concerned.

Table V. Stability Data of the THC-SMEDDS Floating Tablets in Intermediate (30°C±2°C/65% RH±5% RH, 6 months) and Accelerated Conditions (45°C±2°C/75% RH±5% RH, 6 months)

Sampling time	Emulsion droplet size (nm)	PDI	Drug content (%)
Intermediate conditions			
0 month	33.9±1.0	0.224±0.01	103.02±1.41
3 months	35.2±0.3	0.087±0.007	98.83±1.28
6 months	35.5±0.2	0.173±0.02	96.23±2.12
Accelerated conditions			
0 month	33.9±1.0	0.224±0.01	103.02±1.41
3 months	36.4±0.3	0.171±0.04	96.06±1.46
6 months	35.7±0.2	0.133±0.03	92.11±1.49

Cell Viability Evaluation

It is important to determine the toxicity of compounds used in the transport experiments as high permeability values can result from the loss of cells or cell death. Thus, assessment of cell viability ensured that the high permeability results did not arise from the loss of cells. The viability of Caco-2 cells against each sample was observed by the 3-(4, 5-dimethylthiazoyl-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The succinate dehydrogenase enzyme in the mitochondria of the viable cells reduced the yellow MTT through cleavage of the tetrazolium ring to produce a water-insoluble purple-blue formazan that precipitated in the cellular cytosol and was dissolved in DMSO after cell lysis, whereas dead cells could not transform MTT as they had no dehydrogenase activity (35).

The cytotoxicity of THC to Caco-2 cells is presented as a percentage of cell viability relative to those treated with HBSS (negative control), which was taken as 100% viability. Over the concentration range tested (25–200 μM), greater than 90% of the cells survived after a 3-h incubation with either liquid SMEDDS or tablet SMEDDS (Fig. 7). Likewise, THC (134 μM) exhibited nearly 100% cell viability. Monolayers treated with 0.1% *w/v* SDS showed only 3% viability.

Permeability Studies

The obtained microemulsions after dilution of THC-SMEDDS with transport media (pH6.8 and 7.4) were transparent. The average droplet size of microemulsion was in the range of 32.13 to 35.97 nm. The SMEDDS exposed to both transport media revealed no precipitation or phase separation indicating the formulations were stable toward different media. The average TEER values observed in this study before the transport experiments were 629±94 Ωcm^2, and this indicated that the monolayers were intact. The permeability of THC was significantly increased when formulated in SMEDDS (both liquid and tablets). The P_{app} values of THC in the DMSO solution, SMEDDS liquid and floating tablets

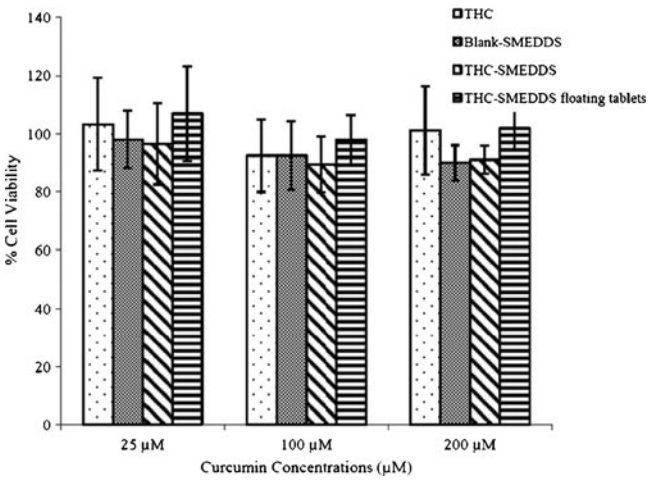

Fig. 7. Effect of THC, Blank-SMEDDS, THC-SMEDDS and THC-SMEDDS floating tablets on the intestinal cell monolayer viability

were 2.01, 8.14 and 6.84 ($\times 10^{-5}$) cm/s for AP→BL transport, respectively and 2.30, 10.17 and 8.90 ($\times 10^{-5}$) cm/s for BL→AP transport, respectively. The P_{app} for the THC loaded SMEDDS was significantly higher than that of the THC alone ($p<0.05$). The SMEDDS formulated THC exhibited approximately a three- to fivefold greater permeability than the unformulated THC (Fig. 8). THC released from the liquid SMEDDS formulation had a slightly higher permeability than the THC from the SMEDDS floating tablets. Moreover, the efflux ratio of 1.14–1.30 from the formulations examined, as shown in Table VI, implied that THC permeated through the Caco-2 monolayers without interactions with a cellular efflux pump. The enhanced permeability effect of the drug by self-microemulsifying formulations was also found by Zvonar *et al.* The formed microemulsions could enhance the absorptive permeability of the drug because of the close contact between the apical membrane and microemulsion droplets, and this creates altered membrane fluidity (21).

According to Kogan *et al.*, compounds with P_{app} values below 1×10^{-5}cm/s are medium permeability substances, and high permeability substances show P_{app} values of more than 1×10^{-5}cm/s. THC is a poorly soluble but highly permeable drug, and could be classified as a BCS class II according to the Biopharmaceutical Drug Classification System (BCS). The permeation of the microemulsion may occur by various mechanisms such as (1) collision and possible adsorption of the microemulsion and the extracellular release of its contents and transport into the cells; (2) endocytotic internalization of the microemulsion followed by intracellular release of the drug (36). The resultant microemulsion significantly enhanced the permeation of THC across the Caco-2 monolayers. More studies are required to identify the mechanism of absorption of this developed formulation. In addition, the effects of surfactants in the formulation as permeation enhancers might also improve the absorption of THC.

Caco-2 monolayer model, the primary tool to study the oral absorption of drugs has emerged as a viable alternative because use of this model can decrease the number of animals needed for experimental studies. The correlation between the *in vitro* P_{app} across Caco-2 monolayers and the *in vivo* fraction absorbed was well established (37). The result of this study

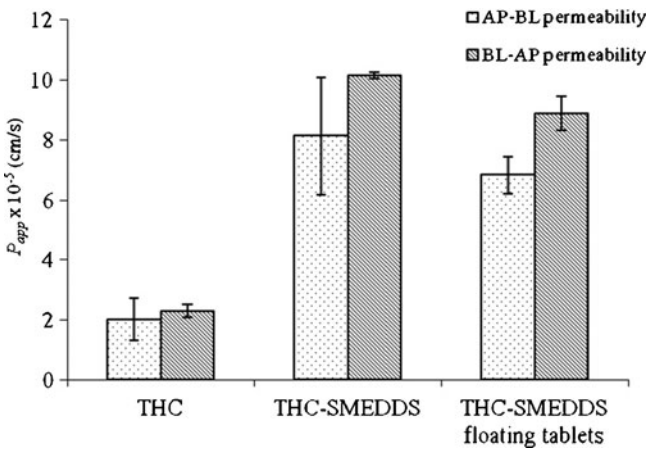

Fig. 8. Bidirectional transport across the Caco-2 monolayers of THC, liquid THC-SMEDDS and THC-SMEDDS floating tablets**.** The results are shown as the apparent permeability coefficient (P_{app}) in the absorptive (AP→BL) and the secretory directions (BL→AB)

Table VI. The Efflux Ratio of THC, THC in SMEDDS and SMEDDS Floating Tablet Formulations

Formulation	Efflux ratio $P_{app\ (BA)}/P_{app\ (AB)}$
THC solubilized in DMSO	1.14
THC-SMEDDS	1.25
THC-SMEDDS floating tablets	1.30

indicated that the permeability of THC was clearly enhanced by self-microemulsifying formulations. However, other pharmacokinetic parameters such as clearance, volume of distribution and elimination half life could not be predicted by this experimental approach. Therefore, in order to obtain the pharmacokinetic data of the developed formulations, further *in vivo* studies are required.

CONCLUSIONS

Self-microemulsifying floating tablets of THC using colloidal silicon dioxide, HPMC K4M, sodium bicarbonate, tartaric acid, lactose and silicified-microcrystalline cellulose were developed. The optimum formulation F2 showed good floating behavior along with a better controlled drug release in comparison to other prepared formulations. About 72% of the drug was released in 12 h, compared with 80% in 90 min from the liquid SMEDDS and the only 30% in 12 h from the unformulated THC. According to the Caco-2 permeation studies, both liquid and solid SMEDDS formulations showed a comparatively higher (three- to fivefold) transport of THC than the unformulated drug. Our studies illustrated the potential use of a new solid self-microemulsifying system for oral delivery of poorly water-soluble drug such as THC.

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