Research Article

Optimization, Characterization and *In Vitro* Evaluation of Buprenorphine Microemulsion

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ABSTRACT

The aim of the present study was to develop an o/w microemulsion for poorly solubilized potent semi-synthetic opiate analgesic drug buprenorphine for sublingual administration. The oil phase, surfactant and co-surfactant were selected on the basis of their drug solubility and their efficiency to form ME. Pseudo-ternary phase diagrams were constructed and on the basis of ME existence ranges various formulations of BU were developed. The influence of surfactant and co-surfactant mass ratio (S_{mix}) on the ME formation and in vitro permeation of ME through cellophane membrane was studied respectively. The optimized formulations (ME A2 & ME C2) consisting of 0.2.44% & 2.75% (w/w) BU, 5.67% & 5.60% (w/w) Capmul® MCM C8 and 23.82% (w/w) S_{mix} (1:1) & 23.86 % (w/w) S_{mix} (2:1) respectively, has shown a globule size of 59.7 ± 0.5 nm & 12.0 ± 0.8 , a polydispersity index of 0.242 ± 0.01 & 0.328 ± 0.004 , pH 4.93 ± 0.005 & 5.15 ± 0.005, viscosity 15.4 ± 0.10 cPs & 20.4 ± 0.20 cPs, a zeta potential of -0.2 ± 0.1 & 1.4 ± 0.1 and conductance of 105.1 ± 0.50 µS & 131.7 ± 0.42 µS respectively for ME A2 & ME C2 formulation. ME A2 & ME C2 exhibited a steady state flux of about 705.226 ± 0.99 & 445.993 ± 0.47 respectively thus exhibiting higher drug permeation through ME formulations. Besides this, the formulation was also evaluated for drug content, centrifugation and stability study. Stability analysis of the microemulsion indicated that they were stable upon storage for at least 3 months. The results indicate that, the investigated ME may be used as a promising alternative for BU therapy.

Keywords: Buprenorphine HCl, Microemulsion, Sublingual permeation.

INTRODUCTION

Poor aqueous solubility of drug entities is today considered as a formidable challenge for pharmaceutical scientist, which is considered as an area of prime importance in the field of biomedical research. To overcome the solubility problems, different formulation approaches have been undertaken to improve oral, buccal & sublingual bioavailability including surfactants[1, 2], cyclodextrin complexes[3], micronization and nanosizing[4], permeation enhancers[5], nanosuspensions[6], microemulsions[7] and lately lipid based formulations[8].

Buprenorphine hydrochloride (BU) is a partial agonist at mu and kappa opioid receptor and antagonist at delta receptors used for the treatment of moderate to severe pain as well as chronic pain[9]. Buprenorphine is a derivative of the opioid alkaloid thebaine which is a more potent (25-40 times) and having longer lasting analgesic activity than morphine.

However oral delivery of BU is suffering with low bioavailability (31%) because of high hydrophobicity (log P=4.98) which is the main cause for the low water solubility and it also undergoes extensive first pass metabolism by hepatic cytochrome P-450 3A4 isozyme. Hence oral formulations of buprenorphine are not available in the market whereas parentral, buccal & sublingual formulations are available[10-12]. With respect to buccal formulations, buprenorphine's buccal film Bunavail® is available in the market. This bilayered film increases the total bioavailability of buprenorphine to more than 40% in healthy subjects[28]. Bai *et al.*, carried out the pharmacokinetic study of buprenorphine buccal film formulation in healthy volunteers and the study revealed that bioavailability of buprenorphine was about 46 to 51%[29]. This indicates further research in the enhancement of the buprenorphine is to be carried out for better bioavailability.

Hence lipid based formulations were chosen to overcome the above barriers and among them microemulsion as drug delivery systems have recently gained wide acceptance due to robust formulations perspectives, ease of production and practical enhancement of drug permeability[13]. These are clear, thermodynamically stable, isotropic liquid mixture of oil, water and surfactant, frequently in combination with a co-surfactant[14]. This o/w microemulsion formulation enhances the sublingual & buccal bioavailability of buprenorphine by facilitating transcellular (across the cell) & paracellular (between the cells) absorption. Buprenorphine being lipophillic drug transported transcellularly by a concentration dependent passive diffusion process and also being formulated into o/w type of microemulsion, therefore it is also subjected to transport via the intercellular porous route i.e. paracellularly.

Based on extensive review of literature, it revealed that controlled delivery buccal patches of buprenorphine has been developed using polyisobutylene, polyisoprene and carbopol 934P as bioadhesive polymer. Nearly 75% of the buprenorphine released after *in vitro* evaluation studies

from the buccal patches following 24 hrs incubation period [30]. Also bilayed buccal film of buprenorphine is available with about bioavailability of buprenorphine to more than 40% in healthy subjects[28]. Thus literature review reveals lack of information about the bioavailability enhancement of poorly water soluble buprenorphine using microemulsion as drug delivery systems. Thus the current study was aimed to develop an o/w type of buprenorphine microemulsion to enhance its sublingual bioavailability. To achieve this, buprenorphine solubility was tested in various vehicles and vehicles with highest solubility for buprenorphine were selected as components (oils, surfactants and co-surfactants) of microemulsion. The developed buprenorphine loaded o/w microemulsion was investigated for their physiochemical characteristics. Afterwards, the optimized buprenorphine microemulsion was evaluated by means of in vitro diffusion study using modified Franz diffusion cell. The stability of the prepared microemulsion was also investigated.

MATERIALS AND METHODS

Materials

Buprenorphine Hydrochloride was purchased from Sun Pharmaceutical Industries Ltd. Labrasol® (Caprylocaproyl macrogol-8 glycerides) was obtained as a gift sample from Gattefosse Saint Priest (Lyon, France). Monebat® -20 (Polyoxyethylene 20 sorbitan monolaurate) was obtained as a gift sample from Mohini Organics Pvt. Ltd. Malad (West), Mumbai. Monoolein® (Glycerol Mono-oleate) was purchased from Tokyo Chemical Industries Co., Ltd. Tokyo, Japan. Oleic acid pure was purchased from Merk Specialities Pvt., Worli, Mumbai. Propylene Glycol was purchased from Shell Chemicals, Singapore. Glycerin was purchased from KL-Kepong Oleomas, Malaysia. Sesame oil was obtained as a gift sample from Global Merchants. Capmul® MCM C8 (Mono/diglycerides of caprylic acid), Capmul® PG 8 (Propylene glycol monocaprylate) & Acconon® MC8-2 (Polyoxyethylene 8 caprylic/capric glycerides) was obtained as a gift sample from ABITEC Corporation, Columbus, USA.

Screening of oils, surfactants and co-surfactants for ME

The solubility of BU in various oils, surfactants and co-surfactants was determined to find out the appropriate oils, surfactants and co-surfactants with good solubilizing capacity for BU in ME. Oils employed were Oleic acid, Monoolein®, Sesame oil, Capmul® MCM C8 & Capmul® PG 8. Surfactants and co-surfactants employed were tween 20, labrasol, Acconon® MC8-2, Propylene glycol & Glycerin. An excess amount of BU was added into 5 ml of each oil, surfactants and co-surfactants and the resultant mixtures were sonicated at 37°C for 2 h followed by centrifugation for 10 min at 3000 rpm. The supernatant was filtered through a membrane filter (0.45 µm) and BU was determined spectrophotometrically at 289 nm by appropriate dilution of filtrate with methanol. Solubility results of BU in various oils, surfactants and co-surfactants are given in Table 1.

Construction of pseudo-ternary phase diagrams

To find out the concentration range for ME components, pseudoternary phase diagrams were constructed using water titration method at ambient temperature. Three phase diagrams were prepared with the 1:1, 1:2 and 2:1 weight ratios of polysorbate 20 to propylene glycol, respectively. For each phase diagram at a specific surfactant (S)/cosurfactant (CoS) mixing ratio (Km), the ratios of oil to the mixture of S/CoS were varied from 1:9 to 9:1. Each mixture of oil and S/CoS was diluted with water, added drop wise, under moderate shaking. After being equilibrated at ambient temperature for 24 h, the mixtures were assessed visually for the clarity of mixture. Phase diagrams were constructed using CHEMIX Ver.3.60 Ternary diagram software. From this ternary phase diagrams, ME compositions were selected that existed into o/w region of ternary phase system.

Preparation of Microemulsion

The ME for BU was prepared by the water titration method. Based on the ME areas in the phase diagrams, different BU ME formulations were prepared by varying the ratios between S/CoS and at Km = 1:1, 1:2 & 2:1 as given in Table 2. BU added in the range of 2.0 – 3.0 % (w/w) as per solubility into the capmul ® MCM C8, propylene glycol and tween 20. The oil and Smix mixture was then titrated with drop wise addition of double distilled water with continuous shaking to produce a clear mixture. MEs were optimized with respect to Smix ratio and its concentration effect on ex vivo permeation characteristics. Double distilled water was used in order to avoid surface active impurities.

RP-HPLC analysis of Buprenorphine

A validated method was used for the analysis of BU in all formulations[15]. Details of the method are as below:

Instrument: HPLC (LC-2010C HT liquid chromatography Shimadzu, Kyoto, Japan) instrument was equipped with a quaternary pump, online degasser, column heater, autosampler and UV detector

- Column: PrincetonSPHER -100 C18 HPLC column (250 mm × 4.6 mm, 5 µm)
- Mobile phase: Acetonitrile and 10 $mmolL^{-1}$ potassium phosphate buffer adjusted to pH 6.0 with triethanolamine (83:17, v/v)
- Flow rate: 1.0 $mLmin^{-1}$
- Column temperature: 30°C
- Wavelength: 284 nm
- Injection volume: 20 µL

The method developed was validated for linearity, precision and accuracy.

Characterization of ME

According to the regions obtained for o/w ME in the phase diagrams, five ME formulations were selected and evaluated for following parameters:

Measurement of pH

The pH of the prepared MEs was measured by direct immersion of pH meter electrode (Contech Instruments Ltd., Navi Mumbai, India) in the formulations at room temperature and all the measurements were carried out in triplicates. The pH meter was standardized using pH 4 and 7 buffers before use.

Measurement of electrical conductivity

Electrical conductivity of the formulations was measured using a conductivity meter (CYBERSCAN CON 510, EUTECH Instruments Ltd., Singapore) and based on the electrical conductivity, the phase systems of the MEs were determined. The electrode was dipped in the ME sample until equilibrium was reached. Before conductivity measurement, the conductivity cell was calibrated using standard KCl solution[16,] 17.

Measurement of viscosity

The ME formulations were evaluated for their viscosity at 25 ± 2°C using Brookfield viscometer model LV DV-II + PRO [2000 Series] equipped with spindle number S00. For better accuracy, brookfield ULA – EY – UL adapter was used with standard brookfield viscometer to make accurate and reproducible measurements of viscosity.

Droplet size and polydispersity index (PDI)

The average droplet size and its distribution (characterized by polydispersity index, PDI) in MEs were measured using dynamic light scattering zetasizer (DLS) (Malvern Zetasizer ZEN3500, UK). All measurements were performed with a scattering angle of 90° at 25.0°C after diluting the dispersion to an appropriate volume and having dispersion medium viscosity 0.894 mPa.s.

Zeta potential measurements

The charge on the surface of particles was measured characterized by the nanopartica SZ-100 (Horiba Scientific Ltd., Japan) by measuring the zeta potential of MEs. Electrophoretic mobility (µm/s) was measured using small-volume disposable zeta cell and converted to zeta potential by in-built software using the Helmholtz-Smoluchowski equation. Zeta potential determinations were carried out in triplicate.

Drug content

The quantity of ME containing about 4.0 mg of BU, was taken in a 100.0 mL volumetric flask. The samples were mixed gently with nearly 15 mL of methanol & sonicated (Leela sonic Sonicator, Leele Electronics, Mumbai, Maharashtra) to extract BU completely & then volume was made with mobile phase. This solution was then filtered through 0.45 µm Polytetrafluorethylene (PTFE) filter and analysed using validated HPLC method.

***In vitro* diffusion study**

Franz diffusion cell with an effective diffusion area of 2.009 cm^2 was used for in vitro release studies. The dialysis membrane with average flat width 29.31 mm, average diameter 17.5 mm (Himedia Laboratories Pvt. Ltd.) were mounted carefully in between donor and receptor compartment of diffusion cell. Donor compartment was applied with 0.2 g of test microemulsion and the receiver compartment was filled with 27.0 ml distilled water pH 6.5. Temperature of receptor medium was maintained at 37± 0.5°C with magnetic stirring at 100 rpm throughout the experiment. For each experiment, 1ml sample of the receiver medium was withdrawn at predetermined time and then the volume was made up with the equal volume of fresh receiver medium. All samples were filtered through a 0.45 µm pore size cellulose membrane filter and analyzed by HPLC. The mean cumulative values for % drug diffused through the dialysis membrane into the receptor fluid were plotted versus time. The cumulative amount of BU in the receptor fluid per unit area of dialysis membrane, Qt/A (A = 2.009 cm^2), was plotted against time (t). The steady-state fluxes (J_{SS}) were calculated from using following formula:

$$Jss = Q / (A \bullet t)$$

Where, Q is the quantity of compound transported through the membrane in time t

A is the area of exposed membrane in cm^2.

In order to obtain the permeability coefficient Kp (cm/h), the following equation was used;

$$Kp = Q / [A \bullet t \bullet (Co - Ci)]$$

Where, Q is the quantity of compound transported through the membrane in time t (min)

Co and Ci are the concentrations of the compound on the outer side (donor side) and the inner side (receptor side) of the membrane respectively

A is the area of exposed membrane in cm^2

Usually Co can be simplified as the donor concentration and Ci as 0.

Stability of ME

MEs were analysed visually for transparency, phase separation by keeping at 40°C & 75% RH and at room temperature for a period of 3 months. The centrifugation (Laboratory Centrifuge Remi R-8C, India), of formulations at 3,000 rpm for 30 min was carried out to assess the physical stability of ME.

Clarity, phase separation were investigated to judge the optimal stability of ME formulation.

RESULTS AND DISCUSSION

Screening of oils, surfactants and co-surfactants for ME

Solubility of BU in various oils and non-ionic surfactants is shown in Table 1. The solubility of BU was highest in Capmul® MCM C8, followed by Capmul® PG 8, Monoolein, Sesame oil and Oleic acid. Besides that, the drug has a relative high solubility in Capmul® MCM C8 compared to other oils; it was also selected for the preparation of MEs due to its well-known bioavailability and permeation enhancing property and biocompatibility[18-20]. Choice of the surfactant is critical in formulation of MEs, as it helps in the reduction of the interfacial tension by forming a film at the oil–water interface resulting in the spontaneous formation of MEs[21]. There are literature reports regarding the selection of surfactant on the basis of drug solubility. However, the solubilization of oil with the surfactant is also an important factor. It is not necessary that, the surfactant having good solubilizing property for drug would also have equally good affinity for the selected oil phase. Non-ionic surfactants were included in the screening of surfactants since they are well-known for their non-irritant nature. They are less affected by changes in pH and ionic strength and are generally regarded as safe and biocompatible. Polysorbate 20 was non-ionic surfactant and had high solubility than other surfactants, so Polysorbate 20 was used to prepare MEs. Co-surfactants are also added to achieve ME systems at low surfactant concentration. Amphiphilic nature, hydrophobic chain and terminal hydroxyl groups of co-surfactants enable them to intermingle with surfactant monolayer at the interface resulting into changes in their packing arrangement which in turn can affect the curvature of the interface and interfacial energy. The incorporation of co-surfactant enhanced the penetration of the oil phase in the hydrophobic zone of the surfactant monomers, which in turn reduced the interfacial tension and increased the flexibility and fluidity of the interface, ultimately leading to increased entropy of the system[22]. The presence of co-surfactant decreases the bending stress of the interface and imparts the interfacial film sufficient flexibility to take up different curvatures required to form ME over a wide range of composition. PG showed high solubility than other co-surfactants, so it was used for further study. So in this study Capmul® MCM C8, Polysorbate 20 and PG were selected as the oil phase, surfactants and co-surfactants respectively for the formulations of ME containing BU.

Table 1: Saturation solubility of BU in different oils, surfactants and co-surfactants at 37°C (mean ± SD; n = 3).

Sr. no	Components	Solubility (mg/ml)
1	Capmul® MCM C8	3.058 ± 0.06
2	Capmul® PG 8	2.482 ± 0.001
3	Monoolein	0.403 ± 0.02
4	Sesame oil	0.116 ± 0.01
5	Oleic acid	0.048 ± 0.005
6	Polysorbate 20	2.517 ± 0.02
7	Acconon® MC 8-2	1.372 ± 0.04
8	Labrasol®	1.398 ± 0.05
9	Propylene glycol	3.912 ± 0.004
10	Glycerin	1.142 ± 0.009

Construction of pseudo-ternary phase diagrams

The pseudoternary phase diagrams were constructed to determine the concentration range of components in the existence range of ME. The pseudoternary phase diagrams were constructed by titration of homogeneous liquid mixtures of oil, surfactant and co-surfactant with water at room temperature as shown in Fig. 1.[23] At Km (S: CoS) values of 1:1, 1:2 and 2:1, mixture of oil, surfactant and co-surfactant blend was varied from 9:1 to 1:9 and vortexed. Each mixture was then slowly titrated with aliquots of distilled water and stirred at room temperature to attain equilibrium. The mixture was visually examined for transparency. All the components were converted to percent weight before constructing the phase diagram.[24] The marked area represent all formulations that could self-emulsify in seconds and be infinitely diluted by distilled water indicating that the ME formed are capable of keeping BU solubilized. The shaded areas of phase diagrams shows the ME regions, whereas the non-shaded area display the turbid region. Then within this shaded area that particular ME's are selected which formed oil in water type of ME's as mentioned in the Table 2. The formed ME's are clear, isotropic, transparent and of low viscosity determined by visual inspection.

Table 2: Compositions of selected BU ME formulation

Formulation code	Composition [Capmul® MCM C8: Smix (1:1) (Tween 20:propylene glycol): water: BU]	Composition [Capmul® MCM C8: Smix (1:2) (Tween 20:propylene glycol): water: BU]	Composition [Capmul® MCM C8: Smix (2:1) (Tween 20:propylene glycol): water: BU]
ME A2	5.67:23.82:68.06:2.44	-	-
ME A3	13.01:31.87:52.04:3.06	-	-
ME B2	-	6.64:27.44:63.82:2.08	-
ME C2	-	-	5.60:23.86:67.77:2.75
ME C3	-	-	11.91:29.60:55.58:2.89

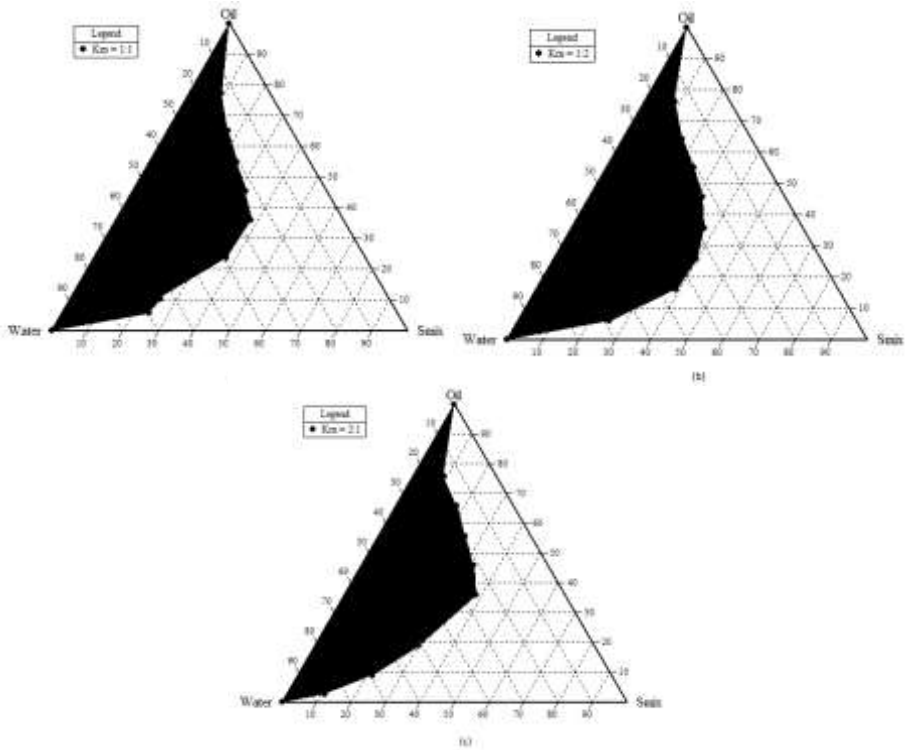


Figure 1: Pseudo-ternary phase diagrams of the region of existence of the ME systems obtained with three different tween 20/propylene glycol blends 1:1 (a), 1:2 (b) and 2:1(c).

Table 3: Characterization of selected BU ME formulation

Formulation code	Visual Observation	pH at 25°C	Centrifugation	Drug content (%)	Viscosity (cPs)	Conductance (µS)	Droplet size (nm)	Polydispersity index (PDI)	Zeta potential (mV)
ME A2	Clear solution	4.93± 0.005	No phase separation	99.1 ± 0.1	15.4± 0.10	105.1±0.51	59.7± 0.5	0.242±0.01	-0.2±0.1
ME A3	Clear solution	5.05± 0.005	No phase separation	100.4 ± 0.12	34.6± 0.15	75±0.38	718.9± 1.4	0.452±0.01	6.3±0.2
ME B2	Clear solution	4.65± 0.011	No phase separation	99.2 ± 0.8	18.3± 0.15	63.6±0.25	166.4± 1.5	0.431±0.012	5.1±0.3
ME C2	Clear solution	5.15± 0.005	No phase separation	99.4 ± 0.5	20.4± 0.20	131.7±0.42	12.0± 0.8	0.328±0.004	1.4±0.1
ME C3	Clear solution	5.04± 0.005	No phase separation	99.9 ± 1.1	44.2± 0.31	106.8±0.30	33.0± 1.2	0.412±0.019	1.3±0.1

Characterization

ME formulations existing in o/w region of the developed three different ternary phase diagrams with 1:1, 1:2 and 2:1 weight ratios (Km) of polysorbate 20 to PG were characterized for different parameters and the results are given in Table 3. It was observed that the disperse system of five formulations of ME were macroscopically identical, i.e., homogeneous, transparent without any precipitates and optically isotropic. BU addition to the originally obtained ME did not have an effect on the viscosity of the disperse system.

pH, viscosity and conductivity

The pH value of MEs was in the range of 4.65 to 5.15 (Table 3). This lower pH range is suitable for absorption of BU from the sublingual formulation across the sublingual mucosa[25].

Apart from pH, viscosity of all the five o/w MEs was in the range of 15.4 to 44.2 cPs (Table 3). This lower viscosity is suitable for easy incorporation of MEs into the polymer dispersion of film casting solution.

Apart from the results of pseudoternary phase diagram obtained for three different ratios of tween 20/propylene glycol with Km 1:1, 1:2 & 2:1 showed that all the five shortlisted MEs are o/w type. This further was confirmed by conductivity test. Conductivity of all the five o/w MEs as shown in table 3, ranged from 63.6 to 131.7 µS. Such higher conductivity values confirmed the existence of o/w MEs.

Droplet size and polydispersity index (PDI)

Droplet size of ME's with varying K_m i.e. 1:1, 1:2 & 2:1 was shown in the Table 3. It was observed that globule size reduces on increasing surfactant concentration. As the

concentration of surfactant in the S_{mix} ratio increases to 2:1 in C2 & C3 formulation, subsequent particle size reduction has been observed with respect to formulation A2, A3 & B2. Thus surfactant had played a major role in the formation of microemulsion by lowering the interfacial tension and reducing Laplace pressure P; that means the pressure required to break a drop was reduced & thus resulted into formation of smaller size droplets. As a generalization, the droplet size is inversely proportional to emulsion stability. Thus, smaller particle size i.e. 12.0 nm, 33.0 nm & 59.7 nm for formulations C2, C3 & A2 shown in the Fig.2. a, b & c, respectively would be more stable.

The polydispersity index (PDI) of all the MEs was found below 0.45 which confirmed narrow size distribution of oil droplets. Generally, for narrow distribution PDI ranges from 0.01 to 0.5 while samples with very broad size distribution have PDI > 0.7. [26,27] Thus PDI of A2 & C2 was 0.242±0.01 and 0.328±0.004 shown in Table 3, confirmed narrow size distribution of oil droplets.

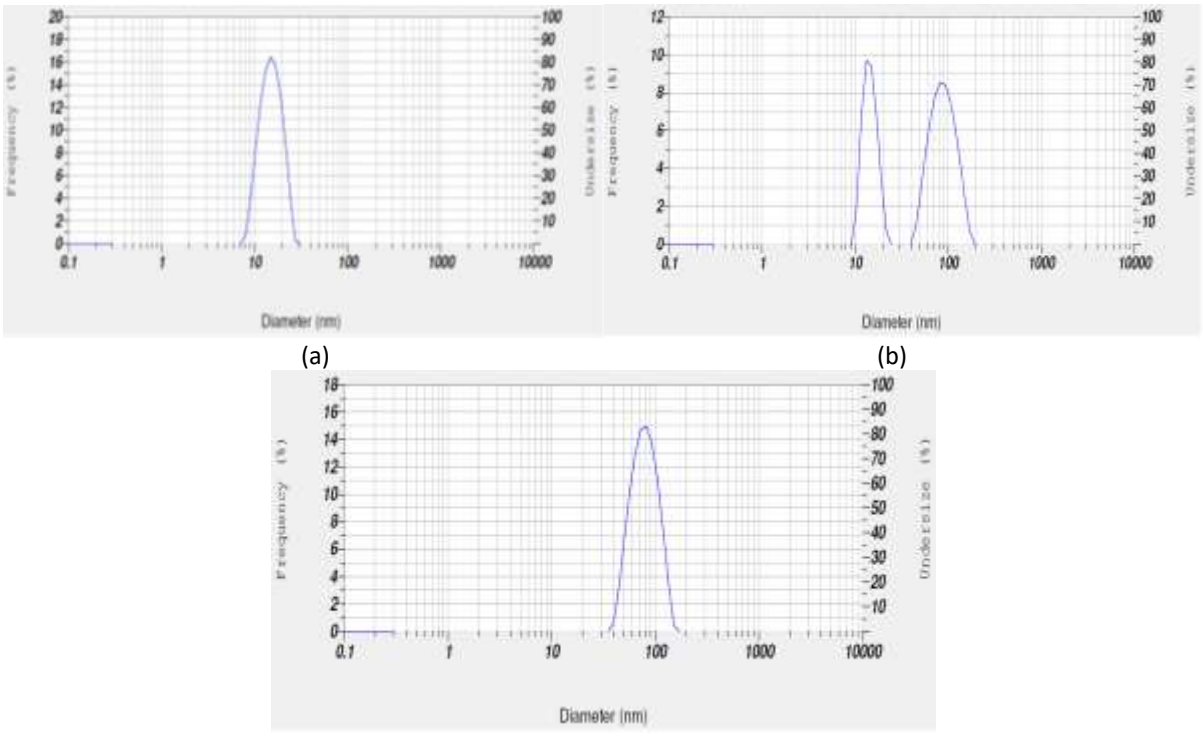
Zeta potential (ZP) measurements

ZP is an indicator of the stability of ME. This ZP is the charge present on the dispersed phase (oil globule) at the shear plane of the electric double layer in the aqueous solution as shown in the Fig.3. As per the principle of o/w ME, a uniform layer of tween 20 (surfactant) has formed surrounding the oil globule of Capmul MCM C8. As the literature study reveals that absorbed layer of large molecules shifts the shear plane to a farer distance from the particle surface & this leads to a reduction of the measured ZP. That means in case of highly charged particle surface, a relatively low ZP will be measured & despite the low ZP the system will be stable. Thus the tween 20 (mol wt 1128) had shifted the shear plane to a farer distance from the surface of oil globule & resulted into low ZP value as observed in all the formulations i.e. A2, A3, B2, C2 & C3. The ZP measurement for ME's A2 & C2 was shown in the table 3 and graph of intensity (a.u.) vs ZP (mV) was exhibited in the Fig.4. a & b.

***In vitro* diffusion study**

In vitro diffusion studies of optimized BU ME formulations shows successful diffusion through dialysis membrane (Himedia Laboratories Pvt. Ltd.) and the results obtained are presented in Fig.5., and the calculated steady state flux (J_{ss}) are tabulated in table 4, along with the regression coefficient (r^2) for Zero order, Matrix, Peppas, Hix. crow modeling of the diffusion profiles for each formulation.

BU o/w ME crosses the dialysis membrane mimicking the sublingual mucosa using two different pathways: transcellularly (across the cell) and paracellularly (between the cells). BU being the lipophillic drug transported transcellularly by a concentration dependent passive diffusion process, by facilitated diffusion using a receptor or carrier molecule, or by vesicular transport mechanism. BU being formulated into oil in water type of ME, therefore it is also subjected to transport via the intercellular porous route (paracellular route), across the sublingual route. The presence of oil droplets containing BU along with external aqueous phase appeared in favor of BU permeability. It might be stated that ME could act as drug reservoirs where loaded drug is released from the internal phase to the external phase and finally onto the mucosa.



(a) (b)

(c)

Figure 2: Particle size distribution plot of microemulsion (a) C2 ME formulation (b) C3 ME formulation (c) A2 ME formulation

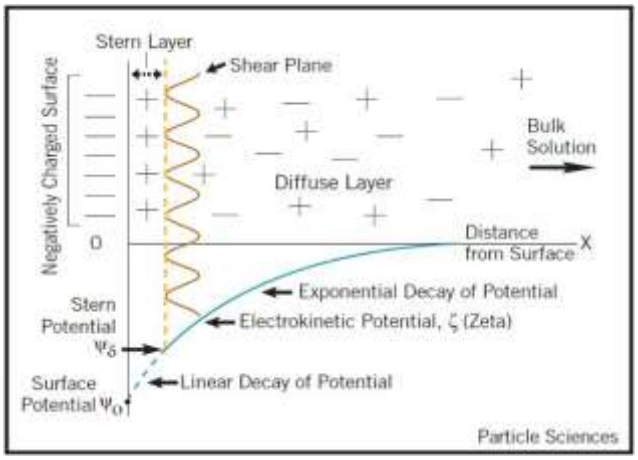
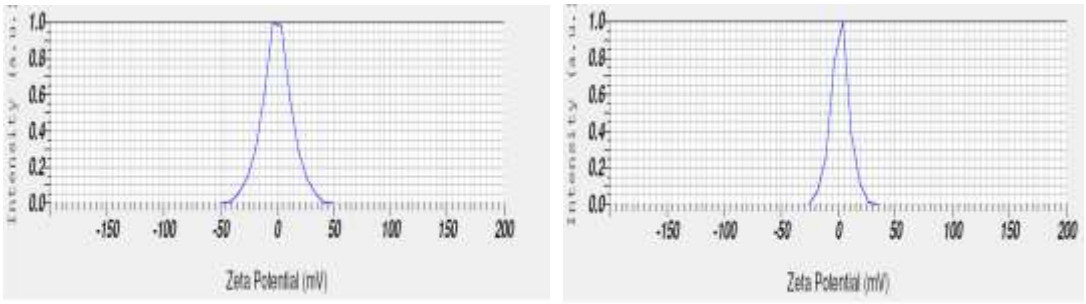


Figure 3: Simplified model of the electric double layer at a charged interface in aqueous solution



(a) (b)

Figure 4: Zeta potential plot of microemulsion (a) A2 ME formulation (b) C2 ME formulation

BU showed better diffusion from ME A2 & ME C2 than ME B2 & ME C3 respectively. The decreasing order of steady state flux (J_{ss}) for the tested formulations was ME B2 < ME C3 < ME C2 < ME A2. For ME A2, the drug exhibited highest steady state flux, whereas it was least for ME B2. The ME A2 & ME C2 exhibited higher steady state flux due to smaller particle size and also having appropriate oil: S_{mix} proportion which facilitates the diffusion process.

Further on modeling, the diffusion of BU from ME A2 & ME C2 formulations exhibited higher r^2 values for peppas model as compared to other models. The values of n (release exponent) in the peppas model were found to be 0.899 & 1.303 for ME A2 & ME C2, respectively that confirmed super case II transport release of BU from the ME formulation. The values of n in the peppas model are used to characterize different release mechanism.

Table 4: Steady state flux and modeling parameters of optimized BU Microemulsion formulations

Formulation code	*In vitro* release study		Zero order	Matrix	Peppas	Hix. Crow.
	Steady state flux J_{ss} (µg/cm². h)	Permeability coefficient K_p (cm/hr)	r^2	r^2	r^2	r^2
ME A2	705.226±0.991	0.109±0.004	0.945	0.851	0.970	0.672
ME B2	181.839±0.817	0.043±0.001	0.799	0.695	0.909	0.644
ME C2	445.993±0.472	0.079±0.009	0.808	0.672	0.946	0.892
ME C3	262.494±0.159	0.044±0.003	0.986	0.952	0.991	0.142

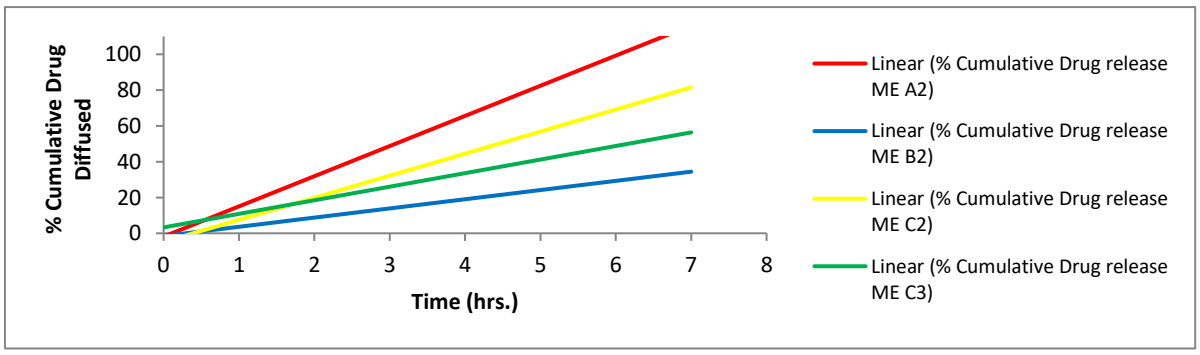


Figure 5: Percent cumulative drug diffused verses time profiles of BU through optimized A2, B2, C2 & C3 microemulsion formulations

Stability study

In stability studies, the ME exhibited no precipitation of drug, creaming, phase separation, and flocculation on visual observation and was found to be stable after centrifugation (3000 rpm for 15 min) at 40°C & 75% RH and at room temperature.

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CONCLUSION

The study has indicated that, stable microemulsion drug delivery systems of poorly permeable drug BU was successfully prepared. The selected ME formulations contain capmul MCM C8 as oil phase, tween 20 as a surfactant and propylene glycol as a co-surfactant. The prepared ME formulations exhibited enhanced permeability with a steady state flux of 705.226 and 445.993 µg/cm^2.h.

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