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Chitosan and cyclodextrin in intranasal microemulsion for improved brain buspirone hydrochloride pharmacokinetics in rats

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ABSTRACT

The aim of this study was to develop buspirone hydrochloride microemulsion formulations for intranasal administration to improve the drug bioavailability and provide high drug brain levels. For the purpose, chitosan aspartate, and hydroxypropyl-β-cyclodextrin were incorporated in the microemulsions. The prepared formulations were characterized. Biological investigations including pharmacokinetic studies, brain drug targeting efficiency determinations and histopathological examinations were performed on rats. The results showed that safe and stable mucoadhesive microemulsion suitable for nasal administration were successfully prepared. Ex vivo drug permeation revealed high drug permeation from microemulsions. Absolute bioavailability after intranasal administration of buspirone mucoadhesive microemulsion increased significantly and plasma concentration peaked at 15 min. The $AUC_{0-360(brain)}$ was 3 times that obtained after intravenous administration. A high brain targeting efficiency (86.6%) and a direct nose to brain transport (88%) confirmed the direct nose to brain transport of buspirone following nasal administration of the microemulsions.

1. Introduction

Site specific targeted drug delivery systems aim at localizing drugs at their desired site of action, reducing toxicity and increasing treatment efficiency. In spite of its relatively high blood flow, brain targeting remains one of the most challenging research areas in pharmaceutical sciences due to the efficient brain protection (Bisht, 2011). Among the major obstacles are the blood–brain barrier (BBB) and the blood–cerebrospinal fluid barrier (BCSFB) (Alam et al., 2010). The former is a semi permeable selective membrane that does not only function as a physical barrier, but also as a biochemical barrier expressing certain enzymes and efflux p-glycoprotein (Hawkins & Davis, 2005). Thus, the BBB is often the rate-limiting factor in determining permeation of therapeutic drugs into the brain. With the help of BCSFB and meninges, it also controls the brain internal environment. Strategies relying on manipulating BBB, bypassing it or using carrier systems can be used for drug delivery to the brain. The choice of a particular system depends not only on the drug but also on its ability to access relevant target site within CNS.

Microemulsions (MEs) are isotropic, thermodynamically stable colloidal dispersion of oil, water and surfactants with a droplet size of 20–200 nanometers (Talegaonkar et al., 2008). They are used as liquid membrane carriers to transport lipophilic substances through an aqueous medium or to carry hydrophilic substances across lipoidal membranes (Vyas & Khar, 2002). Intranasal administration (i.n.) of a microemulsion offers a practical, non-invasive, convenient and cost effective route of administration allowing direct transport to the brain by virtue of their small globule size and lipophilic nature. Increasing the nasal residence time, overcoming the mucociliary clearance (MCC) and enhancing the membrane permeability can all affect positively the absorption of drugs from the nasal cavity (Lin et al., 2007a,b; Ugwoke, Agu, Verbeke, & Kinget, 2005). Numerous studies have demonstrated that cyclodextrins are efficient well established absorption enhancers in nasal drug delivery (Hermens, Deurloo, Romeyn, Verhoef, & Merkus, 1990; Matsubara, Abe, Irie, & Uekama, 1995; Van den Berg, Verhoef, Romeijn, & Merkus, 2004). Chitosan absorbs water from the mucus layer in the nasal cavity, swells and forms a gel like layer prolonging drug residence time at the site of absorption and improving its bioavailability (Shinha et al., 2004). However, chitosan should be dissolved in dilute acids, a matter which can compromise both ME stability and safety on the nasal mucosa. Using a chitosan salt, soluble at physiological pH can overcome these problems.

Buspirone hydrochloride (BH) is a polar, poor permeability Class 3 non-sedating psychotropic drug with selective anxiolytic properties. It is currently available in the market, only, as oral conventional tablets. However, the drug suffers from poor oral bioavailability (4%) due to extensive first-pass effect as well as poor absorption

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through the lower gastrointestinal tract (Tsai et al., 2011). A strategy, which not only improves the bioavailability by preventing extensive first-pass metabolism but also provides targeting to the receptor sites in the brain, is thereby desirable (Khan, Patil, Yeole, & Gaikwad, 2009; Kumar, Misra, Babbar, et al., 2008; Kumar, Misra, Mishra, Mishra, & Pathak, 2008).

The ultimate goal of this study was to develop stable mucoadhesive BH-ME for i.n. administration to improve bioavailability of this hydrophilic drug and provide high drug brain levels. Achieving the above goal should widen the therapeutic benefit of the drug and overcome some of the shortcomings in the current delivery method. This will result in a great reduction of manufacturing cost, offering chances for easy industrialization. Excipients were selected based on their ability to target the brain and improve absorption of polar drugs. The pharmacokinetics of the drug, brain targeting efficiency and the safety of the delivery systems were the major target attributes of this study.

2. Materials and methods

2.1. Materials

Buspirone hydrochloride (BH) was kindly supplied by Bristol Myers Squibb (Cairo, Egypt). Chitosan aspartate (Cs-Asp) was synthesized in our lab from chitosan, viscosity 9 cP (Primex, Iceland) using a reported spray drying method (Orienti et al., 2002). Hydroxypropyl-β-cyclodextrin (HP-β-CD): Mw 1447, degree of substitution 5.4% purchased from Cargill Incorporation (Japan). Isopropyl myristate (IPM), acetonitrile, methanol, porcine gastric mucin were purchased from Sigma–Aldrich (USA). Diltiazem (DILT) was kindly supplied by E.P.I.C.O (Cairo, Egypt). Tween 80®, isopropyl alcohol, and propylene glycol, were purchased from Adwic (Cairo, Egypt).

2.2. Methods

2.2.1. Microemulsions preparation

Oil-in-water MEs were prepared using the water titration method (Chen et al., 2004). BH, alone or in combination with Cs-Asp ± HP-β-CD, was dissolved in water. The resultant solution was added dropwise to the selected mixture of oil, surfactant and co-surfactant with continuous stirring on a magnetic stirrer (yellow line, MGA HS 7, IKA, Germany) for 20 min at 25 °C.

2.2.2. Microemulsions characterization

2.2.2.1. Polarized light microscopy. A drop of ME was observed under the cross-polarized light microscope coupled with a camera (*Axioskop*, *Zeiss*, *Jena*, Germany).

2.2.2.2. Percent transmittance. Transparency of ME formulae was determined by measuring % transmittance through UV–vis spectrophotometer (Shimadzu, model UV-1601 PC, Kyoto, Japan) at 633 nm using purified water as blank (Kawtikwar, Kulkarni, Yadav, & Sakarkar, 2009).

2.2.2.3. Refractive index (RI). An Abbe refractometer (*Analytik Jena, GmbH*, Germany) was used to determine RI of each ME in presence of visible light as light source at ambient temperature.

2.2.2.4. Drug content. About one gram of each BH-ME was weighed, dissolved in isopropyl alcohol and the volume was adjusted to 10 ml in a volumetric flask. BH content was determined spectrophotometrically at 239 nm after appropriate dilution using similarly treated plain ME as blank (Patel, Patel, Parikh, Bhatt, & Solanki, 2010). Drug content was expressed as percentage of initially added amount (drug incorporation efficiency).

2.2.2.5. Viscosity. The viscosity of ME was measured using a Brookfield Viscometer (*Brookfield HADV II+ CP Programmable viscometer*, USA) at 25 °C using a CP40 spindle at 5 rpm with 30 s intervals.

2.2.2.6. Measurement of mucoadhesive strength. The mucoadhesive potential of ME was determined by measuring the force required to detach the formula from a 50 mg compressed mucin disk using a modified balance developed in our lab based on previously reported methods (see Fig. 1S supplement) (Yong et al., 2001). Hydrated mucin discs were horizontally attached to the upper stage of the balance by cyanoacrylate adhesive. A sample of ME (0.5 g) was placed on the lower vertically movable stage which was then elevated till the surface of the sample became in contact with the disk. A constant preload downward force of 10 g weight was applied for 30 s. The sample was left for 4 min in contact with the mucin disk then water was dropped into a plastic jar placed on the pan at a rate of 13–15 drops per minute. The minimum weight of water required to detach the sample from the mucin disk was used to calculate the detachment stress (N/m^2) applying the following equation (Ch'ng, Park, Kelly, & Robinson, 1985).

$$\text{Detachment stress } (N/m^2) = mg/A \quad (1)$$

where m is the weight of water in grams, g is the acceleration due to gravity (9.81 m/s^2) and A is the area of the mucin disk.

2.2.2.7. Fourier transform infrared spectroscopy (FTIR). FTIR spectra of ME formulations were recorded on an FTIR spectrometer (*Nicolet 6700, Thermoscientific*, USA), using KBr plates, in the frequency range 4000–400 cm^{-1} with spectral resolution of 4 cm^{-1} (Mehta, Kaur, & Bhasin, 2007).

2.2.2.8. Determination of the droplet size, polydispersity index (PDI) and zeta potential. The size, PDI and zeta potential of ME droplets were determined by means of dynamic light scattering using a zetasizer (Zetasizer nanoseries, Malvern instruments, Malvern, UK). The samples were loaded into 1 cm^3 cuvettes in a thermostated chamber at 25 °C. The measurements were performed after diluting samples 100-folds with the continuous phase (Rasal, Mahajan, Shaikh, & Surana, 2010).

2.2.2.9. Transmission electron microscopy (TEM). Morphology and size of ME droplets were studied using TEM (*Jeol JEM 2100F*, Japan). A drop of ME was diluted with distilled water (1:100), stained with 2% phosphotungstic acid and applied on carbon-coated grid.

2.2.2.10. Ex vivo permeation studies. Fresh nasal tissues were carefully removed from the nasal cavity of sheep obtained from local slaughterhouse. Tissue samples were inserted in a modified Franz diffusion cell containing 23 ml of phosphate buffer saline (PBS), pH 6.8 at 34 °C, in the acceptor chamber. To ensure oxygenation and agitation, a mixture of 95% O_2 and 5% CO_2 was bubbled through the system. After a pre-incubation time of 20 min, pure drug solution or ME containing 10 mg of BH was placed in the donor chamber. Aliquots of one ml sample were withdrawn from the acceptor compartment at different time intervals: 5, 10, 15, 30, 45, 60, 120, 180, and 360 min. All samples were replaced by same volume of fresh PBS, pH 6.8. The withdrawn samples were filtered and used for the determination of the amount of permeated drug using a UV–vis spectrophotometer (Rasal et al., 2010).

2.2.2.11. Stability study. Stability of ME was carried according to ICH guidelines as follow: selected ME was stored for 6 months at

refrigerator condition (4 °C), room temperature (30 ± 2 °C) and elevated temperature (50 ± 2 °C). The stored ME were evaluated by: visual inspection (phase separation), % transmittance, particle size and drug content. ME formulations were also centrifuged at different speeds: 5000, 10,000 and 15,000 rpm for 30 min and observed for any change in homogeneity.

2.2.3. Pharmacokinetic studies and determination of brain targeting efficiency for buspirone hydrochloride microemulsions

The pharmacokinetic and brain distribution studies of BH were determined for drug solution (BHS) as well as for intranasally administered selected ME formulae. The data were compared to those obtained after i.v. administration.

2.2.3.1. Animal handling and drug administration. The experimental procedures conformed to the ethical principles of Faculty of Pharmacy, Ain Shams University. Two hundred and seventy Wister albino rats, weighing 180–220 g each, were selected and divided into 5 groups each consisting of 54 rats. Each group received the assigned treatment containing BH equivalent to 1 μg/g. For i.n. administration, conscious rats were held from the back in slanted position and 10 μl was administered in each nostril using a micropipette attached with low density polyethylene tube having 0.1 mm internal diameter. For i.v. administration, a volume of 0.5 ml of BH solution was injected through the rat tail vein. At each of the following time points: 5, 10, 15, 30, 45, 60, 120, 180 and 360 min, blood samples were collected from the animals tail veins into heparinized tubes. The animals were then sacrificed and brains were separated.

2.2.3.2. Sample preparation for analysis.

2.2.3.2.1. Plasma treatment. Each blood sample was centrifuged (*Cooling centrifuge*, *Hermie Labortechnik GmbH*, *type Z216MK*, Germany) at 4000 rpm for 10 min and the plasma was separated and kept immediately at −80 °C. For analysis, 500 μl of each plasma sample was mixed with 100 μl of DILT solution (1000 ng/ml), used as internal standard, and one ml of acetonitrile. The mixture was vortex-mixed, (*Vortex*, *IKA MS3 Digital*, Germany) for 30 s and centrifuged at 6000 rpm for 15 min. The supernatant was analyzed for BH content using HPLC.

2.2.3.2.2. Brain treatment. Each collected brain tissue was homogenized (*Heidolph Diax 900*, Germany) with three-folds its volume of normal saline at 25,000 rpm for one minute. Brain homogenates were treated as mentioned above for plasma samples and assayed for BH content.

2.2.3.3. HPLC analysis of buspirone hydrochloride. An HPLC system (*Agilent Technologies 1200 series*, Germany) consisting of an LC-G 1311A solvent delivery pump equipped with a 20-μl loop and rheodyne sample injector and G1315D diode array detector was used. An Agilent® TC-C_{18} column (250 mm × 4.6 mm I.D, particle size 5 μm) at 30 °C and a mobile phase consisting of acetonitrile, potassium phosphate buffer (10 mM) 35:65 (v/v) adjusted to pH 4.6 with ortho phosphoric acid were used. The elute was monitored at 235 nm at a flow rate of 1 ml/min and the injection volume was 50 μl.

2.2.3.4. Pharmacokinetic analysis. Pharmacokinetic parameters (C_{max}, T_{max}, AUC_{0-360}, $t_{1/2}$, mean residence time (MRT)) in both plasma and brain were calculated for each treatment using the WinNonlin® program (*Version 2*, *Pharsight Co.*, *Mountain View*, CA, USA). Absolute and relative bioavailabilities (BAV) of BH following i.n. administration were calculated using Eqs. (2) and (3) respectively:

$$\text{Abs BAV} = [AUC_{0-360\ min}(\text{i.n.})/AUC_{0-360\ min}(\text{i.v.})] \times 100 \quad (2)$$

$$\text{Rel BAV} = [AUC_{0-360\ min}(\text{i.n. ME})/AUC_{0-360\ min}(\text{i.n. solution})] \times 100 \quad (3)$$

To evaluate brain targeting efficiency, two indices, namely drug targeting efficiency (DTE%) and brain drug direct transport percentage (DTP%), were calculated using Eqs. (4)–(6) (Haque et al., 2012; Zhang et al., 2004):

$$\text{DTE\%} = \left[\frac{(AUC_{brain}/AUC_{blood})\text{i.n.}}{(AUC_{brain}/AUC_{blood})\text{i.v.}}\right] \times 100 \quad (4)$$

$$\text{DTP\%} = \left[\frac{B_{\text{i.n.}} - B_X}{B_{\text{i.n.}}}\right] \times 100 \quad (5)$$

$$B_X = \left(\frac{B\text{i.v.}}{P\text{i.v.}}\right) \times P_{\text{i.n.}} \quad (6)$$

where B_x is the brain AUC fraction contributed by systemic circulation through BBB following i.n. administration, $B_{\text{i.v.}}$ the AUC_{0-360} (brain) following i.v. administration, $P_{\text{i.v.}}$ the AUC_{0-360} (blood) following IV administration, $B_{\text{i.n.}}$ the AUC_{0-360} (brain) following nasal administration, $P_{\text{i.n.}}$ the AUC_{0-360} (blood) following nasal administration.

2.2.4. Histopathological studies

Nasal histopathological examinations were carried out on male Wister albino rats weighing 250 ± 50 g. A volume of 10 μl from each tested formula containing a dose of BH equivalent to 1 μg/g was administered daily for 7 days in each nostril. The nasal mucosa from the bottom of inferior meatus was dissected from sacrificed rats after 7 days. The tissues were separated, fixed, cut vertically, dehydrated, cleared, impregnated in soft and hard paraffin, sectioned at 5 mm thickness with the microtone and stained with hematoxylin and eosin. The sections were examined and photographed using *Nikon Eclipse TE2000-S* inverted microscope (*Nikon Corp*., Japan).

2.2.5. Data analysis

At least three replicates of each experiment in the study were performed and the data were expressed as mean ± standard deviation (S.D.) or mean ± standard error (S.E.). One way ANOVA was used to test the differences between treatments followed by Newman Keuls test for multiple comparisons. Differences were considered significant at $p < 0.05$.

3. Results and discussion

Before starting to prepare the ME, an initial study was conducted to select the most appropriate oil and surfactant system. Based on this study, IPM was selected due to its ability to solubilize a higher amount of BH compared to the other investigated oils (Bshara, Ahmed, Holayel, & El-Shamy, 2012). By constructing pseudo-ternary phase diagrams, a 2:1 surfactant ratio of Tween 80/propylene glycol exhibited the largest ME domain among the tested systems and was therefore used in this work. From the ME region, the system composed of IPM (5%), Tween 80 (30%), propylene glycol (15%) and water (50%) was chosen because of its stability and low surfactant concentration. BH was loaded in the selected system in a concentration of 1% (w/w). Cs-Asp was used in order to achieve a more intimate contact between the formulation and the nasal mucosa and decrease chances for the MCC of the formulation. HP-β-CD was used as absorption enhancer to overcome the low permeability of BH. Table 1 shows the composition of the prepared ME formulations.

Table 1
Composition of BH loaded microemulsion formulations.

Components (%, w/w)	Formulations		
	F1	F2	F3
Isopropyl myristate	5	5	5
Tween 80	30	30	30
Propylene glycol	15	15	15
Water	50	50	50
Buspirone HCl	1	1	1
Chitosan aspartate	–	0.1	0.1
Hydroxypropyl-β-cyclodextrin	–	–	1

3.1. Optical clarity, drug incorporation efficiency and viscosity of ME formulae

All investigated ME did not interfere with polarized light and the fields remained dark confirming their isotropic nature. Table 2 shows that the percentage transmittance with all ME formulae was more than 99% and the RI was about 1.44. BH was efficiently incorporated in all ME formulae as demonstrated by the high incorporation efficiency exceeding 98%. The presence of both Cs-Asp and HP-β-CD did not compete with BH. Results of the viscosity study revealed that the use of Cs-Asp in the aqueous phase increased significantly the system viscosity while the addition of HP-β-CD to the ME did not affect it. The impact of the viscosity on intranasally administered drug solutions is critical: although, a high viscosity is required to overcome MCC, yet it can impede drug spreading in the nasal cavity and its penetration through mucus. An optimum viscosity should always be compromised. That is why we did not increase further the concentration of Cs-Asp.

3.2. Mucoadhesive strength

Results, shown in Table 2, reveal that a detachment stress of 783 dynes/cm^2 was found with F1. The presence of Cs-Asp caused more than 1.3 fold increase in this value and addition of HP-β-CD caused a 1.7 increase in this value as seen in F3. The electronic theory, one of the theories adapted for investigation of mucoadhesion, assumes that electron transfer occurs upon contact of adhering surfaces due to differences in their electronic structure. This results in the formation of an electrical double layer at the interface, with subsequent adhesion. Several studies have shown that mucoadhesive properties of polymers containing ionizable groups are affected by pH of surrounding media (Peppas & Sahlin, 1996). For cationic polymers like chitosan, the positive charge will favor binding to negatively charged groups (such as carboxyl or sulphate) on the mucin or cell surface (Fiebrig, Harding, Rowe, Hyman, & Davis, 1995). Thus, at nasal pH (5.5–6.5), cationic polymers will exist in ionized form, thus NH_3^+ groups on deacetylated N-acetyl groups will allow electrostatic interaction with negatively charged mucus (Harding, 2006). F3 had higher detachment stress than F2 probably due to presence of HP-β-CD which possesses polar outer surface that are able to form hydrogen bonds between hydroxyl groups on the sugar and other O- and N-containing groups on the protein found in mucus (Marttin, Verhoef, & Merkus, 1998).

3.3. Fourier transform infrared spectroscopy (FTIR)

The results in Table 2 show that O—H bond of F1 absorbs at a lower frequency (3392.4 cm^{-1}) than that reported for pure water (3400 cm^{-1}) (see Fig. 2S, supplement). Incorporation of Cs-Asp and HP-β-CD showed a negligible effect on O—H bond frequency denoting that these additives did not influence water–surfactant interaction to a large extent.

3.4. Droplet size, PDI, zeta potential and transmission electron microscopy (TEM)

The particle size of the tested ME show unimodal distribution (Fig. 3S) with average droplet size of about 35 nm (Table 2). Incorporation of both Cs-Asp and HP-β-CD did not affect the droplets size as confirmed by TEM micrographs (Fig. 1). The small PI of 0.2–0.3 indicated the narrow size distribution of the globule size approaching thus a monodisperse system. This uniform small particle size was expected to be suitable for our purpose. Drugs are released at appropriate tailored rate from nanosized particles delivered nasally and are also able to reach specific sites in brain if their size is less than 100 nm (Sharma, Mali, Baboota, & Ali, 2007). This, in turn, prevents the availability of the drug at non-targeting sites leading to enhancement in therapeutic effect, decrease in dose and frequency of dosing, reduction in toxicity, side effects and perhaps even in the cost of therapy (Kumar, Misra, Babbar, et al., 2008; Kumar, Misra, Mishra, et al., 2008). This is in addition to the expected high stability of small ME nanodroplets and possible sterilization by filtration. In spite of the difficulty of using scanning electron microscopy to investigate the ME morphology, some trials had been made and are presented in Fig. 4S. ME-F1 droplets exhibited a slightly negative zeta potential probably due to the combination of non-ionic surfactants. The addition of CS conferred a slight positive potential to the globules. This value was slightly but significantly lowered from 5.36 in F2 to 3.83 after the addition of HP-β-CD in F3. These small zeta potential values are expected to confer a high physical stability to the ME with low tendency to globules flocculation (Sabale & Vora, 2012). The positive zeta potential probably contributed to the higher detachment stress seen with F2 and F3 compared to F1.

3.5. Ex-vivo permeation studies

As shown in Fig. 2, BH solution showed 29.59% of cumulative drug permeation after 6 h. Percentage drug permeation increased reaching 65.15% after 6 h for ME-F1. Addition of Cs-Asp in ME-F2 caused a significant enhancement in drug permeation where

Table 2
Physicochemical characterization of BH microemulsion formulations.

Parameters[a]	Formulations		
	F1	F2	F3
% transmittance	99.52 ± 0.43%	99.32 ± 0.29%	99.23 ± 0.18%
Refractive index	1.445 ± 0.011	1.446 ± 0.015	1.447 ± 0.020
Drug incorporation efficiency (%)	98.19 ± 0.12	99.16 ± 0.48	99.11 ± 0.15
Viscosity (cP)	286.9 ± 5.3	343 ± 4.7	350 ± 6.1
Detachment stress (dyne/cm^2)	783 ± 7.2	1056 ± 12.4	1340 ± 8.6
OH band frequency (cm^{-1})	3392.4	3389.3	3391.3
Droplet size (nm)	35.70 ± 2.91	35.88 ± 4.75	36.2 ± 3.86
Polydispersity index (PDI)	0.131 ± 0.02	0.139 ± 0.03	0.142 ± 0.02
Zeta potential	−2.43 ± 0.184	5.36 ± 0.24	3.83 ± 0.36

[a] Values are mean ± S.D., $n = 3$.

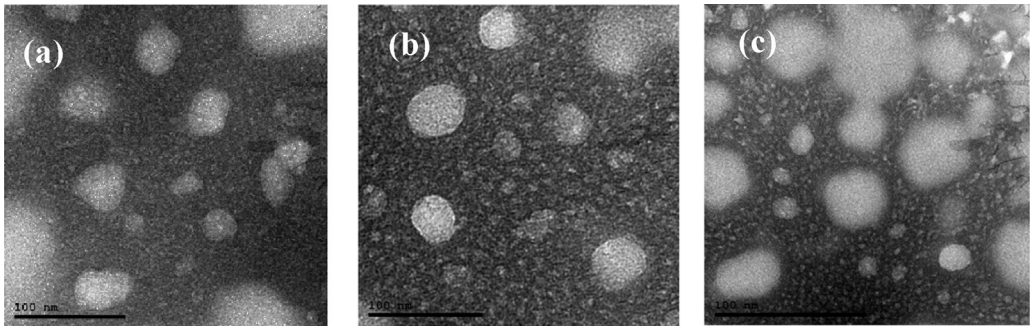

Fig. 1. TEM of buspirone hydrochloride microemulsion formulae (a) F1, (b) F2 and (c) F3.

its percentage reached 75.5% after 6 h. A marked improvement in drug permeation after addition of HP-β-CD was also noticed where 100% of BH permeation could be seen after 6 h in F3. The improvement of BH permeation in case of ME compared to solution could be attributed to surfactants effect.

3.6. Stability study

Results of the stability studies of ME-F1 (Table 1S, supplement) indicated that BH loaded ME was stable up to six months as it showed no change in globules size, % transmittance and drug content. The viscosity of the system with the globules zeta potential, added to the effect of surfactants contributed in preventing droplets aggregation and hence high system stability. Neither change in clarity, nor phase separation was observed in the tested formula after centrifugation proving its stability against gravitation effect. The ability of the system to withstand high temperature used (50 °C) without phase separation indicates its high phase inversion temperature contributing to the high stability seen.

3.7. Pharmacokinetic studies and determination of brain targeting efficiency

A simple and validated modified HPLC method was used for determination of BH in rat plasma and brain. Retention times for BH were found to be 6.6 and 6.9 min and for the internal standard, DILT, 10.3 and 10.5 for plasma and brain respectively with a total run time of less than 15 min. BH and DILT peaks were separated and resolved with good symmetry. No endogenous interfering peaks were observed in individual blank serum at BH and DILT retention times confirming analytical method specificity. Standard plots for plasma and brain samples were linear in the concentration range 1–800 ng/ml with correlation coefficient values of 0.9987 and 0.9977 for rat plasma and brain respectively. The percentage recovery values were in the range 92.289–98.981 for BH-spiked plasma samples and 88.984–97.633 for spiked brain samples ensuring consistency and efficiency. Inter-day accuracy and precision for rat plasma and brain samples were determined on three different days and the percentage C.V. was determined by analysis of three replicates of quality control (QC) samples at three different concentrations. Inter-day and intra-day precisions of the QC samples were satisfactory with coefficient of variation (C.V.) less than 15%. Limit of quantitation (LOQ) of the method was found to be 1 ng/ml for BH in rat plasma and brain with C.V. less than 20% and an accuracy of 85–110%. Limit of detection (LOD) was determined to be 0.5 ng/ml based on signal-to-noise (s/n) ratio of 3:1.

Mean plasma and brain concentration versus time profiles of BH after i.n. delivery and i.v. injection are displayed in Fig. 3a and b respectively. The brain/plasma concentration ratios at the various time intervals are displayed in Table 3. It is to be noted that for the i.n. solution, BH was not detected till 5 min in plasma and 10 min in brain achieving also the lowest C_{max} and AUC_{0-360} values in both plasma and brain as seen in Table 4. On the other hand and as expected, the i.v. solution showed the highest plasma BH concentration at all time points and the fastest C_{max} among all tested formulae. However, ME formulae gave significantly higher C_{max} values in brain achieving 2.5, 3.7 and 4.3 folds increases in C_{max} with F1, F2 and F3 when compared respectively to i.v. solution. The maximum drug concentration in brain was attained after 30 and 60 min for both i.v and i.n solutions respectively and these values were higher than those observed in plasma. Furthermore, a faster absorption was obtained with ME formulae compared to the less

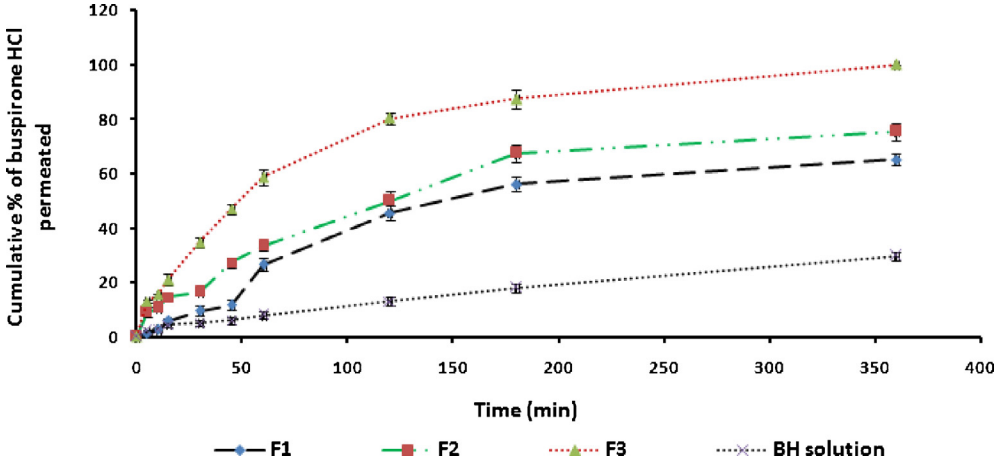

Fig. 2. Ex vivo buspirone hydrochloride release profile in PBS (pH 6.8).

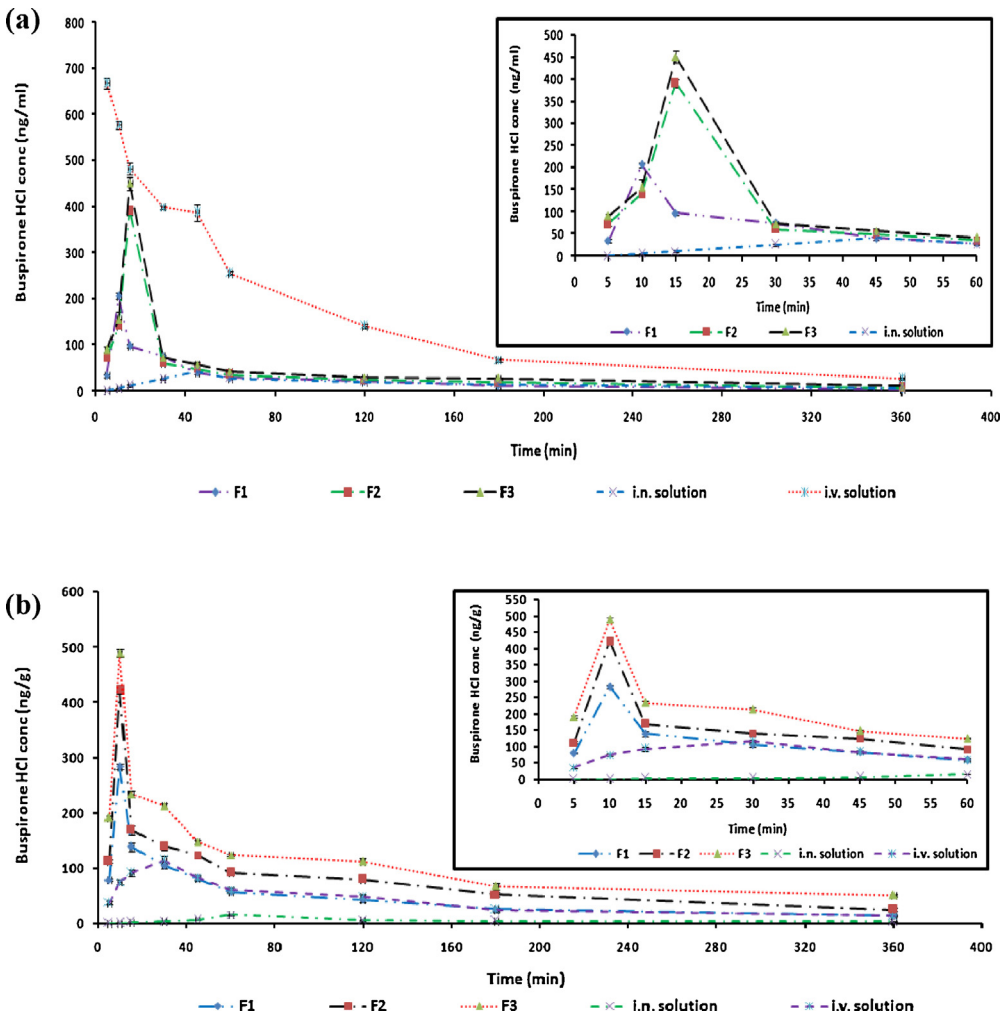

Fig. 3. Mean buspirone hydrochloride concentrations in (a) rat plasma and (b) rat brain.

viscous i.n. solution as seen by their shorter T_{max}. The presence of Cs-Asp with or without HP-β-CD did not affect the absorption rate, although it increased C_{max}. Moreover, AUC_{0-360} values for nasal formulae show similar ranking to their C_{max} which was also in accordance with the amount of drug permeated in ex vivo study.

Significant differences in absolute bioavailability, at $p < 0.05$, were found between various formulae. Delivery of the drug via the nasal route raised its BAV to 11% exceeding the reported oral BAV (4%) which could not be evaluated in this study as the drug concentrations were below LOD even after increasing the oral dose up to two folds. Avoidance of first-pass effect accounted for such an improvement. Similarly, previous studies reported an improvement in BH bioavailability to 61% after delivery as a nasal solution with chitosan and cyclodextrin (Khan et al., 2009). Compared to nasal solution, the rel BAV of BH showed a significant increase of 43% after formulation in ME. A further considerable increase was seen after inclusion of Cs-Asp and the rel BAV almost doubled, followed by another 50% increase after addition of HP-β-CD (Table 4). The increase in BAV of BH after formulation in ME could be attributed to the nature of ME especially its surfactant content, low surface tension, high lipophilic character and small globule size. It was demonstrated in a previous study that ME was a promising carrier for the transdermal delivery of BH where a high drug permeation through the skin, depending on the surfactants

Table 3
Brain/plasma concentrations ratios at various time intervals.

Time (min)	Formulation (route of administration)				
	F1 (i.n.)	F2 (i.n.)	F3 (i.n.)	Solution (i.n.)	Solution (i.v.)
5	2.331	1.529	2.125	[a]	0.055
10	1.372	2.988	3.134	[b]	0.129
15	1.424	0.432	0.521	0.251	0.192
30	1.409	2.264	2.905	0.118	0.287
45	2.010	2.534	2.555	0.156	0.213
60	1.961	2.564	2.933	0.576	0.238
120	2.013	3.330	3.931	0.262	0.340
180	2.141	2.898	2.491	0.284	0.366
360	3.023	3.599	4.913	0.464	0.493

[a] Plasma and brain concentrations were below limit of quantitation of assay.
[b] Brain concentration was below limit of quantitation of assay.

Table 4
Pharmacokinetic parameters, brain targeting efficiency, and direct nose-to-brain transport following administration of BH formulations.

Parameters[a]	Tissue/organ	Formulation (route of administration)				
		F1 (i.n.)	F2 (i.n.)	F3 (i.n.)	Solution (i.n.)	Solution (i.v.)
C_{max}(ng/ml)/(ng/g)	Plasma	206.69 ± 13.95	391.14 ± 24.48	450.85 ± 33.06	44.49 ± 13.68	668.07 ± 27.85
	Brain	283.66 ± 13.43	421.55 ± 16.92	489.48 ± 15.44	16.02 ± 2.06	114.81 ± 10.15
T_{max} (min)	Plasma	10	15	15	45	–
	Brain	10	10	10	60	30
$AUC_{0-360\,min}$	Plasma	8167.51 ± 532.11	12,225.95 ± 1171.56	15,220.16 ± 1205.49	5677.16 ± 291.25	51,606.27 ± 1617.20
(ng/ml min)/(ng/g min)	Brain	15,137.31 ± 692.38	25,113.87 ± 1128.14	35,342.95 ± 1122.14	1839.14 ± 71.00	13,835.45 ± 541.55
AUC ratio	$AUC_{0-360brain}/AUC_{0-360Plasma}$	1.85	2.05	2.32	0.32	0.26
Abs BAV %)	Plasma	15.83 ± 0.42	23.69 ± 0.93	29.49 ± 0.95	11.00 ± 0.23	100
Rel BAV (%)	Plasma	143.87 ± 3.83	215.35 ± 8.42	268.09 ± 8.67	100	–
$t_{1/2}$ (min)	Plasma	110.46 ± 5.58	117.53 ± 5.78	155.35 ± 11.95	151.62 ± 9.30	95.28 ± 7.58
	Brain	156.78 ± 26.0	143.61 ± 12.95	195.45 ± 2.45	227.33 ± 34.1	129.80 ± 7.87
MRT (min)	Plasma	123.14 ± 4.25	118.61 ± 5.64	158.52 ± 10.23	224.06 ± 12.85	113.05 ± 5.70
	Brain	191.55 ± 28.81	192.71 ± 16.83	265.61 ± 15.24	324.30 ± 41.43	177.13 ± 8.27
DTE (%)	Brain/plasma	691.3026	766.1966	866.1493	120.8350	–
DTP (%)	Brain/plasma	85.5346	86.9482	88.4546	17.2425	–

[a] Values are mean ± SE, $n = 6$.

used, was achieved (Tsai et al., 2011). The increase in BAV for other drugs after administration in nasal ME was also demonstrated in various studies (Cho et al., 2012). The increase in rel BAV noted after inclusion of Cs-Asp and HP-β-CD could be explained as follows: the MCC under normal circumstances rapidly clears any instilled formulation including MEs. The mucoadhesive agent (Cs-Asp) was able to prolong ME contact time with nasal mucosa causing significant increases in both plasma and brain C_{max} and AUC_{0-360}. Interaction of positively charged amino group on C-2 position of Cs with negatively charged sites on mucosal epithelial cell membranes and tight junctions probably allowed opening of the latter. This transient effect of Cs on tight junctions had been previously demonstrated in various studies (Borchard et al., 1996; Dodane, Khan, & Merwin, 1999). F3 showed the highest rel BAV, C_{max} and AUC_{0-360} values among all tested ME formulae probably due to a synergistic effect of CD and Cs on the permeation of drug (Zerrouk et al., 2006). The chitosan salt opened up the tight junctions while cyclodextrin extracted the phospholipids and proteins from membrane forming new inclusion compartment in the aqueous phase enhancing BH permeation as evidenced by the increase in C_{max} and AUC_{0-360}.

It could be seen that i.n. solution gave the lowest C_{max} and AUC_{0-360} with the longest T_{max} in brain compared to both i.v. solution and ME-F1. The presence of BH as monoprotonated polar drug, at nasal pH, accounts for its inability to pass via the transcellular route and its relatively high molecular weight hindered its paracellular translocation. That is why its direct transport to brain via the olfactory region was not possible. Moreover, the poor localization of the solution in the nasal cavity and its drainage or more probably its translocation to the nasopharynx and then its passage to the gastrointestinal tract (GIT) also contributed to the long T_{max}. The highest MRT seen with this formula support this explanation.

Data show also that F2 and F3 gave significantly higher C_{max} and AUC_{0-360} value compared to all the other tested formulae with the superiority of F3. For F1 and F2, although slight increases in C_{max} were seen in brain compared to plasma, yet the $AUC_{0-360(brain)}$ showed more than two folds increase in brain compared to plasma suggesting a direct nose-to brain uptake of the drug (Zhang et al., 2004). The low $AUC_{0-360(brain)}/AUC_{0-360(plasma)}$ seen with i.v. solution suggests poor brain targeting efficiency of because of BBB and rapid clearance from the extracellular fluid. It is obvious that, with all formulations and irrespective of administration route, drug $t_{1/2}$ and MRT are higher in brain than in plasma. ME formulae showed increase in both parameters compared to i.v. solution with a maximum increase corresponding to 1.6–1.5 and 1.4–1.3 in MRT in plasma and brain respectively in F3.

Values for DTE %, shown in Table 4, indicate that formulating BH in ME for i.n. administration raised its efficiency for rat brain drug targeting increased by about 7 folds compared to that of i.n. solution. The increase in viscosity and possible opening of tight junction caused by inclusion of Cs-Asp raised this amount to 7.6 folds and addition of cyclodextrin lead to further increase in DTE reaching 8.6 folds. This increase in DTE might be due to: Firstly: Size of MEs droplets was less than that of the axons in the *filia olfactoria* (Mistry, Stolnik, & Illum, 2009), hence they were probably taken into the neurons and supporting cells by endocytic mechanisms, via the intraaxonal route. Secondly: penetration enhancement effect of surfactants used (Lin et al., 2007a,b) notably Tween 80® with its special role in brain targeting and its effect as p-glycoprotein efflux inhibitor (Nerurkar et al., 1997; Ramge et al., 2002; Rasal et al., 2010) providing a better chance for drugs which are normally effluxed by cells to remain in the cell or to be transported to its target. Thirdly: IPM, used as oily phase, also worked as permeation enhancer in intranasal delivery for brain targeting (Vyas et al., 2006).

Drug uptake from the nasal mucosa into the brain occurs mainly via two different pathways. One is the systemic pathway by which some of the drug is absorbed into the systemic circulation and subsequently reaches the brain by crossing the BBB. The other is the olfactory pathway by which the drug partly travels from the nasal cavity to CSF and/or brain tissue. It is therefore known that the amount of the drug in the brain tissue after nasal administration is attributed to these two pathways (Illum, 2003). DTP% values obtained (more than 85%), shown in Table 4, suggest that the main drug transport of BH from ME formulations to rat brain occurs most probably via olfactory pathway For the nasal solution, the drug transport was via systemic pathway confirming the possibility for the absorption through GIT.

Although, these results support greatly our formulation strategy, yet it is worthy to say that they might differ when extending our work to humans due to differences in olfactory epithelium histological features (Thorne et al., 2004). For instance, the surface area of olfactory epithelium in rat is 56% of the total nasal epithelium whereas in human it is only about 10% (Adams, 1972; Morrison

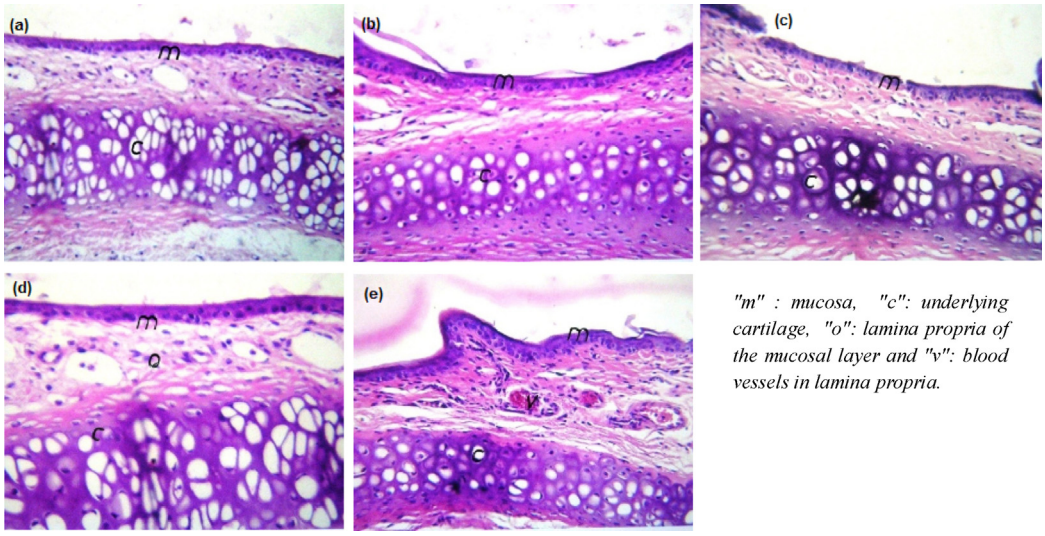

Fig. 4. Light photomicrograph of (a) untreated rat epithelium and rat epithelium treated with (b) normal saline pH 6.8, (c) F1, (d) F2 and (e) F3. "m", mucosa; "c", underlying cartilage; "o", lamina propria of the mucosal layer; "v", blood vessels in lamina propria.

& Costanzo, 1990). Therefore, proportionally more drug molecules may be transported to the rat brain via the olfactory route rather than systemic pathway compared to humans. In addition, clearance half-life of mucus from the nasal cavity is 1 min in mice compared to 15 min in humans (Gizurarson, 1990). Furthermore, the average weight of the human brain (1500 g) is larger than that of rat (2 g) making penetration of drug molecules deep into the rat brain more than in humans.

3.8. Histopathological studies

Fig. 4 shows light photomicrographs taken from anterior cross sections of rat nasal cavity following 7 days exposure to BH-ME formulae. The micrographs show normal histological structure of the mucosa and underlying cartilage. No severe signs of necrosis, sloughing of epithelial cells or hemorrhage were detected in any of the rats. However, only mild edema in lamina propria of mucosal layer was noticed in rat treated with F2 and also mild congestion of blood vessels in lamina propria in rat treated with F3. The results obtained were in agreement with other reports in the literature (Cho et al., 2012) and elect these formulations for further clinical studies.

4. Conclusions

The study demonstrated that ME formulations can be employed to improve BH bioavailability and provide high drug brain levels. Enhanced rate and extent of transport of BH following intranasal administration of microemulsion, mucoadhesive microemulsion, and mucoadhesive microemulsion with HP-β-CD may help decreasing the dose and frequency of dosing and possibly maximize the therapeutic index. Calculated pharmacokinetic data indicate that BH-ME offer greater therapeutic effect than conventional oral dosage forms by increasing brain. The higher DTE% and DTP% obtained suggest that ME formulations have better brain targeting efficiency mainly because of substantial direct nose-to-brain transport.

Conflict of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2013.08.027.

References

Adams, D. R. (1972). Olfactory and non-olfactory epithelia in nasal cavity of mouse, *Peromyscus*. *American Journal of Anatomy*, *133*, 37–49.
Alam, I. M., Beg, S., Samad, A., Baboota, S., Kohli, K., Ali, J., et al. (2010). Strategy for effective brain drug delivery. *European Journal of Pharmaceutical Sciences*, *40*, 385–403.
Bisht, R. (2011). Brain drug delivery system: A comprehensive review on recent experimental and clinical findings. *International Journal of Pharmaceutical Sciences and Research*, *2*, 792–806.
Borchard, G., Luessen, H. L., deBoer, A. G., Verhoef, J. C., Lehr, C. M., & Junginger, H. E. (1996). The potential of mucoadhesive polymers in enhancing intestinal peptide drug absorption. III. Effects of chitosan-glutamate and carbomer on epithelial tight junctions in-vitro. *Journal of Controlled Release*, *39*, 131–138.
Bshara, H. N., Ahmed, R. O., Holayel, S. M., & El-Shamy, A. A. (2012). Improvement of the bioavailability of buspirone HCl using intranasal delivery systems. *Azhar Journal of Pharmaceutical Sciences*, *45*, 86–102.
Ch'ng, H. S., Park, H., Kelly, P., & Robinson, J. R. (1985). Bioadhesive polymers as platforms for oral controlled drug delivery. Part 2. Synthesis and evaluation of some swelling, water insoluble bioadhesive polymers. *Journal of Pharmaceutical Sciences*, *74*, 399–411.
Chen, H., Chang, X., Weng, T., Zhao, X., Gao, Z., Yang, Y., et al. (2004). A study of microemulsion systems for transdermal delivery of triptolide. *Journal of Controlled Release*, *98*, 427–436.
Cho, H.-J., Ku, W.-S., Termsarasab, U., Yoon, I., Chung, C.-W., Moon, H. T., et al. (2012). Development of udenafil-loaded microemulsions for intranasal delivery: In vitro and in vivo evaluations. *International Journal of Pharmaceutics*, *423*, 153–160.
Dodane, V., Khan, M. A., & Merwin, J. R. (1999). Effect of chitosan on epithelial permeability and structure. *International Journal of Pharmaceutics*, *182*, 21–32.
Fiebrig, G., Harding, S. E., Rowe, A. J., Hyman, S. C., & Davis, S. S. (1995). Transmission electron microscope studies on pig gastric mucin and its interactions with chitosan. *Carbohydrate Polymers*, *28*, 239–244.
Gizurarson, S. (1990). Animal-models for intranasal drug delivery studies: A review. *Acta Pharmaceutica Nordica*, *2*, 105–122.
Haque, S., Md, S., Fazil, M., Kumar, M., Sahni, J. K., Ali, J., et al. (2012). Venlafaxine loaded chitosan NPs for brain targeting: Pharmacokinetic and pharmacodynamic evaluation. *Carbohydrate Polymers*, *89*, 72–79.

Harding, S. E. (2006). Trends in mucoadhesive analysis. *Trends in Food Science Technology*, *17*, 255–262.
Hawkins, B. T., & Davis, T. P. (2005). The blood–brain-barrier/neurovascular unit in health and disease. *Pharmacology Reviews*, *57*, 173–185.
Hermens, W. A., Deurloo, M. J., Romeyn, S. G., Verhoef, J. C., & Merkus, F. W. (1990). Nasal absorption enhancement of 17 beta-estradiol by dimethyl-beta-cyclodextrin in rabbits and rats. *Pharmaceutical Research*, *7*, 500–503.
Illum, L. (2003). Nasal drug delivery, possibilities, problem and solutions. *Journal of Controlled Release*, *87*, 187–198.
Kawtikwar, P. S., Kulkarni, N. P., Yadav, S., & Sakarkar, D. M. (2009). Formulation and evaluation of an anti-epileptic drug-loaded microemulsion for nose to brain delivery. *Asian Journal of Pharmaceutics*, *3*, 143–147.
Khan, S., Patil, K., Yeole, P., & Gaikwad, R. (2009). Brain targeting studies on buspirone hydrochloride after intranasal administration of mucoadhesive formulation in rats. *Journal of Pharmacy and Pharmacology*, *61*, 669–675.
Kumar, M., Misra, A., Babbar, A. K., Mishra, A. K., Mishra, P., & Pathak, K. (2008). Intranasal nanoemulsion based brain targeting drug delivery system of risperidone. *International Journal of Pharmaceutics*, *358*, 285–291.
Kumar, M., Misra, A., Mishra, A. K., Mishra, P., & Pathak, K. (2008). Mucoadhesive nanoemulsion-based intranasal drug delivery system of olanzapine for brain targeting. *Journal of Drug Targeting*, *16*, 806–814.
Lin, H., Gebhardt, M., Bian, S., Kwon, K. A., Shim, C. K., Chung, S. J., et al. (2007). Enhancing effect of surfactants on fexofenadine HCl transport across the human nasal epithelial cell monolayer. *International Journal of Pharmaceutics*, *330*, 23–31.
Lin, Y., Shen, Q., Katsumi, H., Okada, N., Fujita, T., Jiang, X., et al. (2007). Effects of Labrasol and other pharmaceutical excipients on the intestinal transport and absorption of rhodamine, a P-glycoprotein substrate, in rats. *Biological Pharmaceutical Bulletin*, *30*, 1301–1307.
Marttin, E., Verhoef, J. C., & Merkus, F. W. (1998). Efficacy, safety and mechanism of cyclodextrins as absorption enhancers in nasal delivery of peptide and protein drugs. *Journal of Drug Targeting*, *6*, 17–36.
Matsubara, K., Abe, K., Irie, T., & Uekama, K. (1995). Improvement of nasal bioavailability of luteinizing hormone-releasing hormone agonist, buserelin, by cyclodextrin derivatives in rats. *Journal of Pharmaceutical Sciences*, *84*, 1295–1300.
Mehta, S. K., Kaur, G., & Bhasin, K. K. (2007). Analysis of Tween based microemulsion in the presence of TB drug rifampicin. *Colloids and Surfaces B: Biointerfaces*, *60*, 95–104.
Mistry, A., Stolnik, S., & Illum, L. (2009). Nanoparticles for direct nose-to-brain delivery of drugs. *International Journal of Pharmaceutics*, *379*, 146–157.
Morrison, E. E., & Costanzo, R. M. (1990). Morphology of the human olfactory epithelium. *Journal of Comparative Neurology*, *297*, 1–13.
Nerurkar, M. M., Ho, N. F. H., Burton, P. S., Vidmar, T. J., & Borchardt, R. T. (1997). Mechanistic roles of neutral surfactants on concurrent polarized and passive membrane transport of a model peptide in Caco-2 cells. *Journal of Pharmaceutical Sciences*, *86*, 813–821.
Orienti, I., Cerchiara, T., Luppi, B., Bigucci, F., Zuccari, G., & Zecch, V. (2002). Influence of different chitosan salts on the release of sodium diclofenac in colon-specific delivery. *International Journal of Pharmaceutics*, *238*, 51–59.
Patel, M. R., Patel, R. B., Parikh, J. R., Bhatt, K. K., & Solanki, A. B. (2010). Investigating the effect of vehicle on *in-vitro* skin permeation of ketoconazole applied in o/w microemulsions. *Acta Pharmaceutica Sciencia*, *52*, 65–77.
Peppas, N. A., & Sahlin, J. J. (1996). Hydrogels as mucoadhesive and bioadhesive materials: A review. *Biomaterials*, *17*, 1553–1561.
Ramge, P., Unger, R. E., Oltrogge, J. B., Zenker, D., Begley, D., Kreuter, J., et al. (2002). Polysorbate-80 coating enhances uptake of polybutylcyanoacrylate (PBCA)-nanoparticles by human and bovine primary brain capillary endothelial cells. *European Journal of Neuroscience*, *12*, 1931–1940.
Rasal, A., Mahajan, H. S., Shaikh, H. T., & Surana, S. J. (2010). Development and characterization of nasal mucoadhesive microemulsion of sumatriptan succinate. *Indian Journal of Novel Drug Delivery*, *2*, 103–108.
Sabale, V., & Vora, S. (2012). Formulation and evaluation of microemulsion-based hydrogel for topical delivery. *International Journal of Pharmaceutical Investigation*, *2*(3), 140–149.
Sharma, B. V., Mali, S., Baboota, J., & Ali, J. (2007). Preparation and characterization of chitosan nanoparticles for nose to brain delivery of a cholinesterase inhibitor. *Indian Journal of Pharmaceutical Sciences*, *69*, 712–713.
Shinha, V. R., Singla, A. K., Wadhawan, S., Kaushik, R., Kumria, R., Bansal, K., et al. (2004). Chitosan microspheres as a potential carrier for drugs: A review. *International Journal of Pharmaceutics*, *274*, 1–33.
Talegaonkar, S., Azeem, A., Ahmad, F. J., Khar, P. K., Pathan, S. A., & Khan, Z. I. (2008). Microemulsions: A novel approach to enhanced drug delivery. *Recent Patents on Drug Delivery and Formulations*, *2*(3), 238–257.
Thorne, R. G., Pronk, G. J., Padmanabhan, V., & Frey, W. H. (2004). Delivery of insulin-like growth factor-I to the rat brain and spinal cord along olfactory and trigeminal pathways following intranasal administration. *Neuroscience*, *127*, 481–496.
Tsai, Y.-H., Chang, J. T., Chang, J.-S., Huang, C.-T., Huang, Y.-B., & Wu, P.-C. (2011). The effect of component of microemulsions on transdermal delivery of buspirone hydrochloride. *Journal of Pharmaceutical Sciences*, *100*(6), 2358–2365.
Ugwoke, M. I., Agu, R. U., Verbeke, N., & Kinget, R. (2005). Nasal mucoadhesive drug delivery: Background, applications, trends and future perspectives. *Advanced Drug Delivery Reviews*, *57*, 1640–1665.
Van den Berg, M. P., Verhoef, J. C., Romeijn, S. G., & Merkus, F. W. (2004). Uptake of estradiol or progesterone into the CSF following intranasal and intravenous delivery in rats. *European Journal of Pharmaceutics and Biopharmaceutics*, *58*, 131–135.
Vyas, T. K., Babbar, A. K., Sharma, R. K., Singh, S., & Misra, A. (2006). Preliminary brain targeting studies on intranasal mucoadhesive microemulsion of sumatriptan succinate. *AAPS Pharm SciTech*, *6*, 1–16.
Vyas, S. P., & Khar, R. K. (2002). Targeted and controlled drug delivery, novel carrier system. In S. P. Vyas, & R. K. Khar (Eds.), *Submicron emulsion*. New Delhi: CBS Publishers and Distributors.
Yong, C. S., Choi, J. S., Quan, Q. Z., Rhee, J. D., Kim, C. K., Lim, S. J., et al. (2001). Effect of sodium chloride on the gelation temperature, gel strength and bioadhesive force of poloxamer gels containing diclofenac sodium. *International Journal of Pharmaceutics*, *226*, 195–205.
Zerrouk, N., Corti, G., Ancillotti, S., Maestrelli, F., Cirri, M., & Mura, P. (2006). Influence of cyclodextrins and chitosan, separately or in combination, on glyburide solubility and permeability. *European Journal of Pharmaceutics and Biopharmaceutics*, *62*, 241–246.
Zhang, Q., Jiang, X., Jiang, W., Lu, W., Su, L., & Shi, Z. (2004). Preparation of nimodipine loaded microemulsion for intranasal delivery and evaluation on the targeting efficiency to the brain. *International Journal of Pharmaceutics*, *275*, 85–96.