Carbamazepine Mucoadhesive Nanoemulgel (MNEG) as brain targeting delivery system via the olfactory mucosa

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Abstract
Carbamazepine (CBZ) is an antiepileptic orally administered drug, but due to its low solubility in water, its gastrointestinal absorption is slow and irregular, leading to delayed brain uptake with consequent peripheral side actions. The objective of this study was the brain targeting of CBZ via the olfactory mucosa in the form of an intranasal mucoadhesive o/w nanoemulgel (MNEG). CBZ was formulated in a nanoemulgel system containing oleic acid/labrasol in a ratio of 1:5 as oil/surfactant and 0.1% xanthan gum as anionic mucoadhesive polymer. The prepared MNEG was characterized with respect to oil droplet size, mucoadhesion, in-vitro release of the drug and CBZ uptake by phosphatidylcholine liposomes as an in-vitro model for olfactory cells. The anticonvulsant action of nasal MNEG was studied on chemically and electrically induced convulsive Swiss Albino mice. The in-vitro release of CBZ from MNEG was very low, however CBZ uptake via liposomal membrane reached 65% within 1 hr. Treatment of animals with MNEG significantly prolonged the onset times for convulsion of chemically convulsive mice and protected the animals from two electric shocks. One can thus spire and hope for the emergence of a new intranasal treatment of epilepsy with consequent decrease in the peripheral side actions of CBZ.

Keywords: Nanoemulgel, carbamazepine, brain targeting, delivery via olfactory mucosa, uptake of drug by liposomes as biomembrane model

1. Introduction
Carbamazepine (CBZ) is a widely used antiepileptic agent, which has been effective in the therapy of psychomotor seizures and trigeminal neuralgia for 40 years (Goodman et al., 2001; Sweetman, 2006). However, systemic CBZ therapy is commonly associated with dramatic side effects including “carbamazepine-hypersensitivity-syndrome” in hematologic, hepatic, renal, and pulmonary systems (Newell et al., 2009), severe skin reactions (Mansur et al., 2008) as well as hepatic abnormalities, ranging from an asymptomatic rise in liver function tests to acute liver failure (Syn et al., 2005). Furthermore, ambulatory children, who received CBZ monotherapy, suffered from early alteration in bone metabolism (Aggarwal et al., 2005), probably due to the hepatic enzyme-inducing character of CBZ (Voudris et al., 2005).

These latter problems, besides the need of a therapeutic prompt action make CBZ a good candidate for the development of a brain target formulation. Several studies have shown a direct route of transport from the olfactory region to the central nervous system (CNS) in animal models without prior absorption to the circulating blood (Dahlin et al. 2000; Illum, 2004). The dendritic processes of the olfactory neurons are directly exposed to the external environment in the upper nasal passage, while their axons project to olfactory bulb. Targeting the brain via intranasal administration of drugs has been investigated recently in many studies. Zhang et al., 2004, have developed nimodipine-loaded microemulsion system for brain. Vyas et al., 2006, showed that, intranasal delivery of sumatriptan mucoadhesive microemulsion indicated effective brain targeting with a promising role in the treatment of acute attacks of migraine.
Moreover, intranasal administration is associated with several advantages (non-invasiveness, ease of application, rapid termination of effects in the event of adverse reaction, bypasses of the blood–brain barrier and avoidance of prior absorption to the circulating blood) that encourage its study as a viable strategy for delivering CBZ into the CNS. However, nasal mucociliary clearance is one of the most important limiting factors for nasal drug delivery (Soane et al., 1999). Yet, mucoadhesive preparations have been developed to increase the contact time between the dosage form and mucosal layers of nasal cavities, thus enhancing drug absorption as well as preventing rapid nasal clearance (Edman et al. 1992).

A problem facing the delivery of CBZ is its poor water solubility (< 200 μg/ml), which generally results in a slow and irregular absorption (Kobayashi et al., 2000; Sethia and Squillante, 2002). Emulsion formulations offer an appealing alternative for the administration of poorly water soluble drugs due to their effectiveness for drug solubilization and potential for improved efficacy (Constantinides et al., 2004). Furthermore, microemulsions have drawn attention for their use as vehicles for drug delivery. They possess several interesting characteristics, namely, enhanced drug solubilization, good thermodynamic stability, ease of preparation, low viscosity, high drug loading capacity, and small droplet size usually less than 200 nm. Microemulsions also offer increased absorption and improved clinical potency, which allow the total dose to be reduced and thus minimizing side effects (Gasco, 1997; Sintov and Botner 2006). Recently, the preparation of submicron emulsions has emerged as a promising alternative for CBZ intravenous administration (Becirevic-Lacan et al., 2002; Akkar and Müller, 2003, Kelmann et al., 2007).

Accordingly, the main objective of this study was to assess CBZ-loaded mucoadhesive o/w nanoemulgel (MNEG) as brain targeting delivery system via the olfactory mucosa with the aim of improving the solubility and enhancing the brain uptake of the drug, to attain rapid onset of action with good efficacy at lower doses.

2. Materials and Methods

2.1. Materials

Carbamazepine was kindly supplied by Novartis, Switzerland. Phosphatidylcholine, cholesterol and pentylene tetrazole were obtained from Sigma-Aldrich, Germany. The other materials used in the study were oleic acid (Adwic, Egypt), labrasol (caprylocaproyl polyoxyyl-8 glycerides [NF]) (Gattefossé, France), xanthan gum (Kahira Pharmaceuticals & Chemical Industries Company, Egypt). All other reagents were of analytical grade.

2.2. Methods

2.2.1. Preparation of nanoemulsion

Nanoemulsion was prepared by spontaneous emulsification following water titration method (Zhang et al., 2004; Shafiq et al., 2007) using oleic acid as the oil phase, labrasol as surfactant and double distilled water was used as the aqueous phase. The ratio of oil to surfactant (S) varied as 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:5, 1:6, 1:7, 1:8, 1:9 (w/w). Water was added dropwise to each oil-S mixture under moderate magnetic stirring at room temperature (25°C). After equilibrium, the samples were visually checked and determined as being clear, transparent and easily flowable o/w nanoemulsions or emulsions. Pseudo-ternary phase diagram of oil, surfactant and water was constructed to delineate the boundaries of phases precisely formed and thus obtain the concentration ranges that can result in large existence area of nanoemulsion.

Once the nanoemulsion region was identified, the nanoemulsion formulations at desired component ratios were prepared with or without CBZ. The preparation of CBZ-loaded nanoemulsion (0.1%) was performed by dissolving CBZ into the oil-S mixture, adding the required weight of water, and stirring to form a clear and transparent liquid. The resulting nanoemulsions were tightly sealed and stored at ambient temperature, and their physical stability was measured by observing periodically the occurrence of phase separation.

2.2.2. Preparation of mucoadhesive nanoemulgel (MNEG)

For nasal application, CBZ was formulated in a nanoemulgel system (NEG) containing xanthan gum as anionic mucoadhesive polymer. From the phase diagram, the nanoemulsion formulation chosen for the study was used to prepare the nanoemulgel. Xanthan gum as selected mucoadhesive polymer was dissolved in the aqueous phase to form 0.1% w/w. The polymer solution was added to the oil-S mixture under stirring till a transparent gel was formed. The formulation was stored at 4°C and was subjected to characterization.

2.2.3. Characterization of CBZ loaded MNEG

2.2.3.1. Microscopic examination: Morphology and droplet size of the CBZ nanoemulsion were characterized using transmission electron microscopy (TEM); (JOEL JEM-1230, Japan) operating at 200 kV capable of point-to-point resolution. Combination of bright field imaging at increasing magnification and of diffraction modes was used to reveal the form and size of the nanoemulsion.

In order to perform the TEM observations, the nanoemulsion formulation was diluted with water (1:10). A drop of the diluted nanoemulsion was then directly deposited on the holey film grid, stained by 1% aqueous solution of phosphotungestic acid and observed after drying.

2.2.3.2. Bioadhesion Strength of MNEG: A modified balance method (Parodi et al., 1996) was used to determine the bioadhesive performance of the MNEG by measuring the force required to detach the gel from a mucosal surface. The instrument is broadly
composed of a modified two arms physical balance in which the right pan had been replaced by a glass plate (4 × 4 cm). Bovine nasal mucosa was dissected, washed then placed in isotonic buffer at pH 5.5–6.5 (simulated nasal medium) (Harding, 2006). A piece of nasal mucosa was glued to the lower side of the glass plate with α-cyanoacrylate glue. This was followed by tarring the balance. The gel was spread on an area of 1 cm² on another piece of mucosa, which was then adhered to a moving platform. The platform was slowly raised until the gel touched the upper mucosa. The gel and mucosa were left in contact for 2 minutes, after which weights were added to the left pan. Addition of weights was stopped upon detachment of the gel from the mucosa. The weight of detachment was recorded and bioadhesive force of CBZ-MNEG per unit area of mucosa (N) was calculated after the equation stated by Ch'ng et al., 1985. The experiment was performed in triplicate.

2.2.3.3. In vitro drug release: The release of CBZ from MNEG was evaluated using the dialysis technique. Dialysis bags (Spectra/Por® membrane MWCO 100,000 Spectrum, USA) were soaked in diffusion medium (phosphate buffer pH 6). One gram of nanoemulgel formulation (equivalent to 1 mg of CBZ) was placed in each dialysis bag (n = 3), then sealed at both ends with medicial clips (Spectrum, USA), and placed at the bottom of dissolution vessels containing 500 ml phosphate buffer pH 6. The study was carried out in a USP dissolution apparatus (Pharma Test, Germany), at 37 ± 0.5°C using an agitation speed of 50 rpm. Aliquots of 2 ml were withdrawn from the dissolution medium at regular time intervals (15, 30, 45 and 60 min) and replaced by fresh phosphate buffer. The samples were analyzed for drug content using HPLC at 286 nm against blank nanoemulgel treated by the same method as CBZ-loaded MNEG. The experiment was performed in triplicate.

2.2.3.4. CBZ uptake study by phosphatidylcoline liposomes: CBZ uptake in the olfactory bulbs from CBZ-loaded MNEG was evaluated via phosphatidylcoline liposomes as an in-vitro model for olfactory cells.

2.2.3.4.1. Preparation of phosphatidylcoline unloaded liposomes Large unilamellar vesicles were prepared by the conventional thin film hydration method. The lipid phase composed of phosphatidylcoline (150 mg) and cholesterol (4 mg) in a molar ratio of 20:1 respectively were dissolved in 5 ml chloroform. The lipid mixture was added to a 50 ml rounded bottom flask and the solvent was evaporated under nitrogen in reduced pressure by a rotary evaporator (Heidolph-Elektro, type VV2000, Germany) until a thin lipid film was deposited on the wall of the flask. The aqueous phase was phosphate buffer adjusted at pH 6. The lipid film was hydrated with 5 ml of the aqueous phase. The hydration was continued for 30 min while the flask was kept rotating at 55°C (a temperature above the transition temperature of the lipid). Hydration was carried out under nitrogen gas and in reduced pressure to avoid oxidation of the phospholipid. The liposomal suspension was allowed to stand for further 2 h at 55°C in order to complete the swelling process. The hydrated liposomes were sonicated at 55°C for 15–20 min in a bath type sonicator with a temperature control system (Retsch GmbH & Co., Type USI, Germany), then centrifuged (18000 rpm; –10°C) for 10 min. The residue (liposomes) was washed by buffer and recentrifuged. The liposomal fraction was diluted with 10% lactose solution as cryoprotectant to maintain liposomes’ stability during freezing (–4°C) until use.

2.2.3.4.2. CBZ uptake by phosphatidylcoline liposomes Samples each of 10 mg of nanoemulgel (representing 10 µg CBZ) were mixed with 10 ml liposomal dispersion. The mixtures were incubated in a shaker water bath (50 rpm) at 37°C for different time periods (15, 30, 45 and 60 min). Samples were centrifuged at 4°C for 5 min. Aliquots of 0.2 ml were withdrawn from the supernatants and diluted with equivalent volume of methanol, filtered through Millipore filter (0.22 µm) and analyzed for the CBZ content using HPLC at 285 nm. Each experiment was done in triplicate. For comparison purposes, blank solutions were prepared by diluting 10 g blank nanoemulgel with 10 ml of 10% lactose solution and treated with the same manner as CBZ containing samples.

2.2.3.5. Kinetic analysis of in vitro release data and CBZ liposomal uptake: In order to describe the release model which describes the pattern of drug release the data were analyzed according to zero-order (Mt = M∞ + k1t), first-order (∞ = ln M∞ – ln M0 – k t) and diffusion controlled according to the simplified Higuchi model (Mt = k2t½/2), where M is the amount of drug released at time t, M0 is the initial amount of drug and k1, k, k2 are the release rate constants of zero order, first order and Higuchi equation, respectively. The preference of a certain mechanism was based on the determination coefficient (r²) for the parameters studied, where the highest determination coefficient is preferred for the selection of the order of release (Dangprasirt and Pongwai, 1998). To analyze the release mechanism of the drug from the nanoemulgel, the release data obtained were fit to the power law (Peppas, 1985):

\[ M_t / M_\infty = K t^n \]

Where, \( M_t / M_\infty \) is the fraction of drug release at time t and k is the rate constant and n is the diffusion exponent related to the mechanism of the drug release. For non-Fickian release, the value of n falls between 0.5 and 1.0; while in case of Fickian diffusion, n = 0.5; and for zero-order, n = 1.

2.2.3.6. Anticonvulsant action evaluation: The anticonvulsant action of nasal CBZ (MNEG) was studied on chemically and electrically induced convulsions in Swiss Albino mice.
2.2.3.6.1. Animal housing and handling. Swiss Albino mice (25 g body weight) were provided by experimental animal care centre, College of Pharmacy, King Saud University. They were kept in rodents’ cages at a temperature of 22 ± 1°C and relative humidity of 55 ± 5%. Water and rodents’ chow were provided ad lib. The animals were kept in a dark:light cycle of 12 hours each.

2.2.3.6.2. Chemical induction of convulsions. Clonic convulsions were induced in mice by the intraperitoneal administration of pentylene tetrozole (100 µg/kg) following a modification of the methods described by Banziger and Hane (1967); El Tahir and Abdel Kader (2008). Mice were divided into two groups of 10 animals each (a control and a test group). Each animal was anaesthetized using diethyl ether. In each animal of the test group, a polyethylene cannula (0.8 mm internal diameter, Kit Kath i.v. cannula, Hindustan Syringes and medical device LTD, India) strengthened by a jacketed non-protruding needle (22G x 1) was introduced into one nostril, the needle was withdrawn and 0.1 ml of 0.1% w/v CBZ-loaded MNEG was administered equivalent to 25 mg/kg body weight of CBZ (therapeutic 70 kg human dose of carbamazepine (200 mg) was converted to 25 g mouse dose (0.625 mg), by using multiplication factors for dose conversion between different species (Paget and Barnes, 1964). Care was taken to cater for the gel that remained in the cannula. Each animal was then allowed to rest for 5 min to recover from the anesthesia completely and to allow time for the drug to reach the brain. Five minutes after the administration of CBZ-loaded MNEG, each animal was injected intraperitoneally with pentylene tetrozole in a dose of 100 mg/kg and immediately placed under a large glass funnel (internal diameter 30 cm) and then observed continuously both visually and via a video-camera. For comparison with intravenous administration, a control group (n = 5) was injected intravenously via the tail vein with 0.1 ml propylene glycol, while the treated group (n = 5) was injected intravenously with 0.1 ml of 0.625% w/v carbamazepine solution in propylene glycol containing an equivalent amount of the drug to that administered nostrily. Five minutes later, each animal in the two groups was injected intraperitoneally (i.p.) with pentylene tetrozole at a dose of 100 mg/kg. For each animal, the following parameters were recorded: (a) onset time of the first clonic convolution, (b) frequency of convulsions per unit time and (c) time of death or (d) protection from death if any. Each animal in the control group was sham-treated with no drug administered and the four parameters were registered following administration of pentylene tetrozole.

2.2.3.6.3. Electrical induction of convulsions. Electrical convulsions were induced in mice following a modification of the method described by El Tahir and Abdel Kader (2008); White et al., (1995). Mice were divided into two groups (a control and a test group) of 10 animals each. Each animal was anaesthetized with ether. Test animals received CBZ-loaded MNEG intranostally, as described before, whereas each animal in the control group was sham-operated. For comparison with intravenous administration, two groups (a control and a test group) of 5 animals each were treated as explained under 2.2.3.6.2. Five minutes after administration of the gel or the sham-operation of the control mice, the two clip ear electrodes of the convulsive unit (ECT unit 7801, Ugo Basile) were attached to the ears of each mouse in both test and control groups. The mice were then placed under a large glass funnel and an electric shock was applied using the following parameters: 99 mA, frequency 95 Hz, pulse with width 5ms and shock duration of 11 s. Each mouse was observed visually and by video camera, for the full clonic convolution with the characteristic full extension of the hind limbs and occurrence of death. In case of control animals, death is an ultimate. All mice die from the first shock. If a certain animal as in the treated group tolerated the first shock and recovered with full consciousness, another shock was applied using the same parameters mentioned above. Shocks were then repeated until death of the animal. The number of tolerated electrical shocks before death was then recorded. Index of tolerance was then calculated as percentage by multiplying the number of the tolerated electrical shocks by 100%.

2.2.3.6.4. Statistical analysis. Results were reported as mean ± SE. Significant differences between the control and treated groups were calculated using non-paired “t” test in case of chemically-induced convulsion and non-parametric Wilcoxon test in case of electrically-induced convulsions, p≤0.05 was taken as significant.

2.2.4. High Performance Liquid Chromatography (HPLC)
A modified method for HPLC analysis of CBZ previously reported by Kelmann et al., 2007 was adopted. A Shimadzu LC-10A system (Kyoto, Japan) was used. Separation was performed on a 4.6mm × 150mm, 5 µm, Thermo BDS Hypersil C18 column (Electron CO., UK). Mobile phase used was a mixture of methanol: water (50:50 v/v) at a flow rate of 1 ml/min. CBZ was detected at 286 nm (La Chrom, UV/VIS detector, L-7420, La Chrom and interface module, D-7000, Merk Hitachi, Germany).

3. Results and Discussion
3.1. Preparation of nanoemulsion
The pseudo-ternary phase diagram (Figure 1) with various weight ratios of oil (oleic acid): surfactant (labrasol): water was constructed to delineate the boundaries of different phases, with the shaded region highlighting the translucent o/w nanoemulsion region. The rest of the phase diagram represents the conventional emulsion. Based on visual observation, the area of the isotropic nanoemulsion region increased with increasing the surfactant concentration. This increase was towards the oil-water axis,
indicating that by increasing the ratio of labrasol to oleic acid, the water incorporation capacity improved with an increase in size of the nanoemulsion region.

The selection of the optimum formulation from the phase diagram should be based on the characteristics required for stable and safe medicated nanoemulsion. Mustafa et al., 2009 suggested that, the oil concentration of the selected formulation should be such that it dissolved the drug and for each percentage of oil selected, only the formulation was chosen which contained minimum concentration of surfactant. Minimum concentration of surfactant as a requirement for superior formulations was also supported by Shafiq et al., 2007. For that, from the phase diagram for nanoemulgel formulation, the ratio of oil to surfactant (S) (oleic acid/labrasol) in a ratio of 1:5 was selected to prepare the nanoemulgel. Selection of suitable oil and surfactant is very critical in developing an appropriate nanoemulsion system. The study was based upon using labrasol as a non-ionic surfactant and oleic acid as the oil phase without the incorporation of a co-surfactant. It was reported by Kawakami et al, 2002 that, the use of non-ionic surfactants generally leads to less toxicity along with lower critical micellar concentration (CMC) as compared to their ionic counterparts. Furthermore, o/w nanoemulsions based on nonionic surfactants are likely to offer better in vivo stability. But, transient negative interfacial tension and fluid interfacial film are rarely achieved by the use of single surfactant; it usually necessitates the addition of a co-surfactant. The presence of co-surfactant decreases the bending stress of interface and provides the interfacial film sufficient flexibility to take up different curvatures required to form nanoemulsion over a wide range of composition. The idea of not using a co-surfactant in the present work and choosing oleic acid as the oil phase was based on previous studies done by Yamaguchi et al., 1995, Kelmann et al., 2007 and Kuminek et al., 2009 in which CBZ was formulated as nanoemulsion using castor oil as the oil phase. Moreover, Yamaguchi et al., 1995, have suggested that, free fatty acids (85–87%), which are contained in the castor oil can act as co-surfactant. It has been denoted that, oleic acid (about 30 g kg⁻¹) is the most important fatty acid in castor oil (Rojas-Barros et al., 2004). Free fatty acids may act as co-surfactant, as they reduce the ζ-potential (zeta potential), and thereby suppressing the coalescence and providing an additional stabilizing factor (Yamaguchi et al., 1995). Moreover, hydrogen bonding between amino groups (-NH₂) on CBZ surface and functional group of other materials was previously detected (Punyapalakul and Sitthisorn, 2010). Consequently, bonding between oleic acid and CBZ was expected. This could explain the higher solubility of CBZ in oleic acid (>20 mg/g) than castor oil (7mg/g) (Kelmann et al., 2007). Furthermore, oleic acid was previously selected as oil phase in preparing oil in water atorvastatin nanoemulsion (Mustafa et al., 2009). This was attributed to the favored solubilization of the poor water soluble drugs in small/medium molecular weight oils such as oleic acid (Lawrence and Rees, 2000).

In case of microemulsion systems there should be no concern about the irritating effect of oleic acid, which is known to limit its use in pharmaceutical preparations and cosmetics. It was reported by Paolino et al, 2002 that the incorporation of oleic acid in a microemulsion structure increased its tolerability with respect to oleic acid aqueous dispersions containing the same amount of oleic acid. This finding was interpreted on basis of the encapsulation of oleic acid by the surfactant system. Furthermore, Elshafeeey et al, 2009 have reported that microemulsions containing oleic acid and labrasol (in concentration higher than those used in the present study) revealed no ciliotoxicity.

Xanthan gum has been chosen to formulate the mucoadhesive nanoemulsion gel. Garcia-Ochoa et al., 2000 have previously reported that, the hydrophilic polymer xanthan gum is a high molecular weight heteropolysaccharide gum. The high charge densities owing to polyelectrolyte behavior of xanthan molecules lead to significant changes in their characteristic properties. In addition, xanthan solutions are highly viscous, even at low concentrations. These properties are useful in many pharmaceutical applications, where it was used as a thickener and stabilizer of suspensions and emulsions (Garcia-Ochoa et al., 2000). Furthermore, xanthan is a useful pharmaceutical excipient, since it is of natural origin, biocompatible and safe – it is assigned the GRAS (generally recognized as safe) label – as well as being relatively cheap (Coviello et al., 2007). Moreover, xanthan gum is known to have superior bioadhesive properties (discussed in 3.2.2).

### 3.2. Characterization of CBZ loaded MNEG

#### 3.2.1. Microscopic examination

Figure 2 shows the transmission electron photomicrograph of CBZ nanoemulsion. The figure reveals that, the oil droplets of the dispersed phase were almost

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spherical in shape and were present in the nanometer range (45–146 nm) with no significant droplet size change during a storage period of 12 months.

3.2.2. Bioadhesion potential of MNEG

MNEG exerted high bioadhesion strength (0.142 N) to bovine nasal mucosa. This finding is in accordance with various studies. Eftaiha et al., 2010 have found that xanthan gum had the highest weight of detachment compared to that of chitosan and PEG 10,000. Similarly, Needleman et al., 1997 reported superior mucoadhesive properties of xanthan gum among other studied polymers. A variety of factors affect the mucoadhesive properties of polymers, such as molecular weight, flexibility, hydrogen bonding capacity, cross-linking density, charge, concentration and hydration of a polymer. Xanthan gum is known to be a high molecular weight anionic hydrophilic polymer. The presence of charged functional groups in the polymer chain is known to have a marked effect on the strength of the bioadhesion. Peppas and Buri (1985) have demonstrated that strong anionic charge on the polymer is one of the required characteristics for mucoadhesion. Furthermore, anionic polyelectrolytes are believed to exhibit strong hydrogen bonding with the mucin present in the mucosal layer (Andrew et al., 2009). Moreover, the xanthan polymer is present in the formulation as a swollen hydrated gel. Hydration induces mobility in the polymer chains thus enhances the interpenetration process between polymer and mucin. Polymer swelling permits a mechanical entanglement by exposing the bioadhesive sites for hydrogen bonding and/or interaction between the polymer and the mucous network (Gu et al., 1998).

3.2.3. In vitro drug release

The mean cumulative % of CBZ released from nanoemulsion versus time plot is presented in Figure 3, which illustrated, a very slow release of the drug (less than 10% in 60 min) from MNEG. A sustained delivery from microemulsion systems is also observed in other studies (Schultz et al., 1997; Constantides et al., 2000; Abrol et al., 2005). Kelmann et al., 2007 reported a sustained release of CBZ from nanoemulsion (17.9% in 60 min) compared to aqueous suspension (31% in 60 min). The prolonged drug release observed in vitro can be explained by the fact that CBZ diffusion from the oily core and interface is hindered by the aqueous medium, which acts as a barrier to drug transport due to its very low solubility in water. In the case of CBZ nanoemulgel the increased viscosity of the preparation acts as an additional factor in lessening drug release. Xanthan solutions are known to have high intrinsic viscosity and a pronounced pseudoplastic flow at relatively low concentrations (Ughini et al., 2004), and are widely used for their drug retarding ability (Talukdar et al., 1996). However, many authors observed lack of correlation between in vivo conditions and in vitro release studies (Henriksen et al., 1995; D’Souza and DeLuca, 2006). It was reported that, nanoemulsions show an increased drug uptake by living tissues (Grassi et al., 2000; Abrol et al., 2005).

Consequently, an ordinary in vitro drug release experiment fails to correlate in vivo findings. Using an oily phase for drug release in an attempt to mimic the lipophilic biological membrane would be also not suitable, owing to the dissimilarity between the isotropic oily solvent and the anisotropic biological membrane. Kelmann et al., 2007 advised that further test conditions for evaluating CBZ release should be investigated.

3.2.4. Kinetic analysis of in vitro release data

In vitro release kinetics appeared to be square root time dependent. \( r^2 = 0.992 \) (Figure 3). The n value was close to 0.5, suggesting that CBZ was released from MNEG through aqueous channels of the gel matrix by Fickian diffusion release model. The results were conformed to the release kinetics of CBZ nanoemulsion in water shown by Kelmann et al., 2007.
3.2.5. **CBZ uptake by phosphatidylinositol liposomes**

Recently, the construction of biomimetic physicochemical tools to evaluate transfer of drugs through membranes has been investigated (Sugano, 2007). An approach of using liposomes as model membranes was studied. The lipid chains within biological membranes – despite being in the fluid-like state – are orientated in an ordered manner and thus render the bilayer anisotropic behaviour. Liposomes are anisotropic media, very similar in structure and composition to natural membranes, and are thus considered as good biomembrane models in the effort to reproduce drug–membrane interactions which are involved in both pharmacokinetic and pharmacodynamic processes (Herbette et al., 1991; Van Ballen et al., 2004). It is valuable to note that, phospholipid accounted for 81% (by weight) of the total lipid of rat olfactory mucosa and phosphatidylinositol was the predominant phospholipid (46% of total phospholipids) (Russell et al., 1989).

Consequently, in the present study the uptake of CBZ by phosphatidylinositol liposomes as an in-vitro model for olfactory cells uptake was investigated. Unlike the very slow drug release from CBZ MNEG shown in the in vitro release study (less than 10% in 1 hr), liposomal membrane uptake reached about 65% of nanoemulsion drug content within 1 hr (Figure 3). A great similarity was observed between the transfer of a drug between lipophilic membranes and its transfer from the internal oily phase of an o/w microemulsion to lipophilic membranes of the olfactory cells. The olfactory epithelium is moistened by secretions produced by Bowman’s glands, found in the lamina propria of the olfactory region (Belinsky et al., 1987). For olfactory uptake, the drug should thus be slightly water-soluble to dissolve in the aqueous environment that covers the olfactory epithelium, and it has to be fat-soluble to enter the cilia of the receptor (Graziadei, 1973). Thus, for a lipophilic drug to be transferred from one lipophilic membrane to the other it has to pass through the aqueous phase separating these membranes.

One would therefore expect for a highly lipophilic drug to be highly immobilized with respect to leaving the oil phase and exchanging with the aqueous environment. These considerations are, however, not at all correlated with the experimentally observed kinetics of the transfer process. For example, despite its high lipophilicity, cyclosporin A exhibits remarkably high exchange rates between different lipid layers (Fahr and Seelig, 2001) and paclitaxel (Fahr et al., 1996) behaves in a similar manner. Therefore, it is believed that in case of highly lipophilic drugs to pass through membranes (especially those with high phospholipid concentrations) the main transferring mechanism is the collision between the lipid vesicles. The drug thus moves directly along its concentration gradient from the donor lipid domain to the acceptor lipid domain (possibly through an aqueous boundary layer) at the moment of collision (Fahr et al., 2005).

3.2.6. **Kinetic analysis of CBZ uptake by phosphatidylinositol liposomes**

CBZ uptake data obeyed simplified Higuchi model ($r^2=0.9999$) indicating partitioning through diffusion. This is in accordence with in vitro transport studies conducted with lipophilic drugs, namely hydroxyzine and triprolidine across the bovine olfactory mucosa, which did not demonstrate polarized flux or saturable submucosal–mucosal transport, suggesting that the flux of these two compounds is predominantly driven by passive diffusion (Chou and Donovan, 1997). Similar passive transport of compounds from nose-to-brain has been previously described for lipophilic compounds such as progesterone and diazepam (Illum, 2000) and several sulfonamides (Sakane et al., 1994). The kinetic mechanism was previously explained by Fahr et al., 2005, where, an equilibrium exists between the drug dissolved in the lipophilic membrane (in the present study in oil droplets) and drug dissolved (to a much lower extent) in the aqueous phase. Upon addition of an acceptor membrane, a flux of the drug, through the water phase, from the donor to the acceptor membrane will be initiated. One would thus expect for highly lipophilic drugs, which have very little solubility in water, to face difficulty in reaching their receptor sites. The theory of lipid vesicle collision may explain the high drug uptake observed for highly lipophilic drugs from microemulsion preparations to olfactory epithelium.

3.2.7. **Anticonvulsant action evaluation**

Administration of pentylenetetrazole to sham-operated animals induced clonic convulsions with mean onset time of 1.5±0.1 minutes, that ended in death of the animals with mean death time 2.5±0.3 minutes ($n=10$). Treatment of Swiss Albino mice with 0.1% CBZ loaded MNEG in a dose of 25 mg/kg mouse pentylenetetrazole induced via the olfactory route, significantly prolonged the onset time for convolution to 7.5±0.6 min ($p<0.01$, $n=10$). The treatment also prolonged the onset times of death to 27.5±1.2 min ($p<0.01$, $n=10$). Mice treated intravenously with carbamazepine doses of 25 mg /kg delayed the onset of convulsions to 6±0.5 minutes and the time of death was prolonged to 24±1.4 minutes ($p<0.01$, $n=5$) (Figure 4).

For electrically convulsive mice, all untreated animals died within 30 s following 1st shock with immediate clonic convulsions with full extension of the hind limbs, however, in MNEG treated animals, electric shocks failed to induce convulsions following the 1st shock with 200% index of tolerance ($p<0.01$, $n=10$) equivalent to 2±0.1 tolerated shocks. Intravenously treated mice with carbamazepine tolerated 1.5±0.2 shocks with 150% index of tolerance ($p<0.01$, $n=5$).

The results clearly demonstrate that, CBZ loaded MNEG via olfactory route protected animals against chemically as well as electrically – induced convulsions and induced significant delays in the convulsions.
and prolongations of the onset of death, with successful antagonism to pentylene tetrozole. The treatment prevented also electrical current induced biochemical changes that usually terminate in convulsions and death. Puranam and McNamara (1999) have previously concluded that, electrically and pentylene tetrozole induced convulsions involve the release of the brain stimulant glutamic acid. Furthermore, inhibition of the brain depressant \( \gamma \)-aminobutyric acid (GABA) at the post synaptic level could be involved (Ren-Qi Huang et al., 2001). Thus, it can be concluded that, the protective effects of CBZ released from MNEG were probably due to its ability to inhibit the release of glutamic acid or to block its post synaptic receptors and/or to elevate GABA release or to enhance its postsynaptic actions.

**Conclusions**

The findings of our study demonstrate that CBZ-loaded mucoadhesive nanoemulgel for intranasal use could be a promising new brain targeting delivery system via the olfactory mucosa for the treatment of epilepsy at lower doses with consequent decrease in the peripheral side actions of CBZ.

**Declaration of interest**

The authors report no declarations of interest.

**References**


