INTERNATIONAL JOURNAL OF ADVANCES IN PHARMACY MEDICINE AND BIOALLIED SCIENCES
An International, Multi-Disciplinary, Peer-Reviewed, Indexed, Open Access Journal
www.biomedjournal.com
ISSN 2348-2109

Development and *In vitro* characterization of mucoadhesive nanoemulgel (MNEG) for enhanced delivery of carbamazepine

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ORIGINAL RESEARCH ARTICLE

ABSTRACT

Background: 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 delay brain uptake with consequent peripheral side actions. The main objective of this study was to assess mucoadhesive CBZ-loaded o/w nanoemulgel (MNEG) as a nasal delivery system with the aim of improving the solubility and enhancing the landing of drug in brain, to attain rapid onset of action with good efficacy at lower doses.

Materials and methods: Preliminary screening was carried out to select proper ingredient combinations. Ternary phase diagrams were then constructed and an optimum system was designated.

Results: An optimum nano emulsion system composed of oil (15%), S_{mix} (44%) and water (41%). It possessed a mean globule size and polydispersity index of 58.3 and 0.152, respectively. 0.1% xanthan gum was found to be suitable for mucoadhesive nanoemulgel formulation exerted high bioadhesion strength to bovine nasal mucosa.

Conclusion: The findings of the study demonstrate that CBZ-loaded mucoadhesive nanoemulgel for intranasal use could be a promising approach for successful landing of CBZ to the brain.

Keywords: Nanoemulgel, carbamazepine, ternary phase diagram, mucoadhesive, nasal delivery.

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Article ID 132

INTRODUCTION

Mucoadhesion is commonly defined as the adhesion between two materials, at least one of which is a mucosal surface. Mucoadhesive systems are formulations used to slow down the mucociliary movement, decrease mucosal enzymatic activity and open the tight junctions to enhance permeation of drug through the epithelial tissue (Smith et al., 2004; Chaturvedi et al., 2011). Mucoadhesive systems thus improve therapeutic outcomes by keeping the drug at the site of action for extended time, target the drug to the specific tissue and control the drug release, resulting in decreased frequency of drug administration and improve patient compliance (Carvalhoet al., 2010; Shaikh et al., 2011). Over the past few decades, mucosal drug delivery has received a great deal of attention. Mucoadhesive dosage forms may be designed to enable prolonged retention at the site of application, providing a controlled rate of drug release for improved therapeutic outcome.

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). 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 (Voudriset 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. 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 aslow and irregular absorption (Kobayashi et al., 2000). Lipid 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 (Constantinideset al., 2000). Furthermore, nanoemulsions 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 less than 100 nm. It also offer increased absorption and improved clinical potency, which allow the total dose to be reduced and thus minimizing side effects (Sintov et al., 2006).

The main objective of this study was toassess CBZ-loaded mucoadhesive o/w nanoemulgel (MNEG) as nasaldrug delivery system with the aim of improving the solubilityand enhancing the brain uptake of the drug, to attainrapid onset of action with good efficacy at lower doses.

MATERIAL AND METHODS

Material: Carbamazepine (CBZ) was received as gift sample from Jubilant Organosys, Noida, India. Tween 80 was purchased from Merck India Ltd. Lauroglycol™ 90 and Maisine™ were obtained from Gattefosse Corp. (France). Other chemicals were of analytical grade.

METHODS

Solubility study: The solubility of CBZ in various oils were determined by adding an excess amount of drug in 2 mL of selected oils and distilled water separately in 5 mL capacity stopper vials, and mixed using a vortex mixer. The vials were then kept at 25 ± 1.0 °C in an isothermal shaker (Nirmal International, Delhi, India) for 72 hours to reach to equilibrium. The equilibrated samples were removed from shaker and centrifuged at 3000 rpm for 15 min. The supernatant was taken and filtered through a 0.45 µm membrane filter. The concentration of CBZ was determined in various oils and water using developed HPTLC method after appropriate dilution with methanol. The mobile phase was Ethyl acetate : Toluene : methanol (5.0: 4.0 : 1.0 v/v/v) with densitometric analysis at 266 nm in absorption mode with CAMAG TLC scanner III, using tungsten lamp as a radiation source and operated by win CATS software (Version 1.2.0). Solubility of CBZ in surfactants and co-surfactants were also determined so that the number of surfactants and co-surfactants used in the study can be reduced.

Construction of ternary phase diagram: On the basis of the solubility study Maisine 35-1 was selected as the oil phase. Tween 80 was used as a surfactant and Lauroglycol 90 as co-surfactant. Distilled water was used as an aqueous phase. Surfactant and co-surfactant (S_{mix}) were mixed in different volume ratios (1:0, 1:1, 1:2, 1:3, 2:1, 3:1, 4:1). These S_{mix} ratios were chosen in increasing concentration of cosurfactant with respect to surfactant and increasing concentration of surfactant with respect to cosurfactant for detailed study of the phase diagrams in nanoemulsion formation. For each phase diagram, oil and specific S_{mix} ratio was mixed thoroughly in different volume ratios from 1:9 to 9:1 in different glass vials. Sixteen different combinations of oil and S_{mix}, 1:9, 1:8, 1:7, 1:6, 1:5, 2:8 (1:4), 1:3.5, 1:3, 3:7 (1:2.3), 1:2, 4:6 (1:1.5), 5:5 (1:1), 6:4 (1:0.7), 7:3 (1:0.43), 8:2(1:0.25), 9:1 (1:0.1), were made so that maximum ratios were covered for the study to delineate the boundaries of phases precisely formed in the phase diagrams.

Pseudo ternary phase diagrams were developed using aqueous titration method. Slow titration with aqueous phase was done to each combination of oil and S_{mix} separately. The amount of water added was varied to give water concentration in the range of 5-95 % of total volume at 5 % intervals. After every 5 % addition of the water to the oil and S_{mix} mixture, visual observation was made and recorded. The physical state of the nanoemulsion was marked on a pseudo-three-component phase diagrams with one axis representing aqueous phase, the other representing oil and the third representing a mixture of surfactant and cosurfactant at fixed volume ratios (S_{mix} ratio). These observations were made for each S_{mix} ratio separately and for each S_{mix} ratio phase diagram was constructed separately.

Thermodynamic stability studies

Heating cooling cycle: Six cycles between refrigerator temperature (4 °C) and 40 °C with storage at each temperature of not less than 48 hours was done. Those formulations, which were stable at these temperatures, were subjected to centrifugation test.

Centrifugation: Passed formulations were centrifuged at 3500 rpm for 30 min. Those formulations that did not show any phase separation were taken for the freeze thaw stress test.

Freeze thaw cycle: Three freeze-thaw cycles were done for the formulation between -21 °C and +25 °C. Those formulations, which passed these thermodynamic stress tests, were further taken for the dispersebility test for assessing the efficiency of self-emulsification.

Dispersibility test: Dispersibility study were carried out by adding 1 mL of each formulation to 500 mL of water , 0.1N HCland 6.8 phosphate buffer saline at 37 ± 0.5 °C.Agitation was provided by standard stainless steel dissolution paddle rotating at 50 rpm. The *in vitro* performance of the formulations was visually assessed using the following grading system:Grade I: Rapidly forming (within 1 min) nanoemulsion, having a clear or bluish appearance; Grade II: Rapidly forming, slightly less clear emulsion, having a bluish white appearance; Grade III: Bright white emulsion (similar to mill in appearance) formed within 2 minutes; Grade IV: Dull, grayish white emulsion having slightly oily appearance that is slow to emulsify (longer than 2 min); Grade V: Formulation, exhibiting either poor or minimal emulsification with large oil globules present on the surface

Incorporation of drug in placebo formulation: Formulations that passed the thermodynamic stability and dispersebility test were selected from each percent of oil (10, 15, 20 and 25 %), having the least S_{mix} concentration irrespective of S_{mix} ratio used. CBZ was incorporated in each placebo formulation of oil mixture and respective S_{mix} ratio on the vortex mixer.

Particle size analysis of oil droplets: Nanoemulsion formulation was diluted to 100 mL with distilled water in a flask and was mixed gently by inverting the flask. The droplet size of the nanoemulsion was determined by photon correlation spectroscopy. Measurement was done using a Zetasizer 1000 HS (Malvern Instruments, Worcestershire, UK). Light scattering was monitored at 25 °C at a 90° angle.

***In vitro* drug release:** The release of CBZ from nanoemulsion formulation (NE1-NE5) and plain CBZ were evaluated using the dialysis technique. Dialysis bags (MWCO 12,000 g/mole; Sigma, USA) were soaked in diffusion medium (phosphate buffer saline). Nanoemulgel formulation (equivalent to 10 mg of CBZ) and plain CBZ was placed in each dialysis bag (n = 3), then sealed at both ends with medicell clips (Spectrum, USA), and placed at the bottom of dissolution vessels containing 500 ml phosphate buffer saline. The study was carried out in a USP dissolution apparatus (USP XXIV method (Dissolution apparatus # 2) at 37 ±0.5 °C using an agitation speed of 75 rpm. Aliquots of 2 ml were withdrawn from the dissolution medium at regular time intervals 1, 2, 4, 8, 12, 16, 20h) and replaced by fresh phosphate buffer saline. The samples were analyzed for drug content using developed HPLTC method. The experiment was performed in triplicate.

Development of mucoadhesive nanoemulgel (MNEG): Nasal formulation should have required mucoadhesive property. Hence, CBZ was formulated in a nanoemulgel system (MNEG) containing xanthan gum as anionic mucoadhesive polymer. From the phase diagram, the nanoemulsion formulation optimized (NE3) were chosen and 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, surfactant and co-surfactant mixture under stirring till a transparent gel was formed.

Transmission electron microscopy: Morphology 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 nanoemulgel formulation was diluted with water (1:100). 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.

Bioadhesion strength of MNEG: A modified balance method 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). 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 cm^2 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.

RESULS AND DISCUSSION

Solubility study: The solubility of CBZ was found to be highest in Maisine 35-1 (158± 1.17 mg/mL) as compared to other oils. This may be attributed to the polarity of the poorly water soluble drugs that favour their solubilisation in small/medium molecular volume oils such as medium chain mono/di/triglycerides. Thus, Maisine 35-1 was selected as the oil phase for the development of the formulation. The higher solubility of the drug in the oil phase is important for the nanoemulsion to maintain the drug in solubilized form.

The process is thermodynamically driven by the requirement of the surfactant to maintain an aqueous phase concentration equivalent to its CMC under the prevailing conditions of temperature, pH and ionic strength (Kawakami et al., 2002). In the present study, surfactant Tween 80 having HLB value of 15 and Lauroglycol 90 (HLB 5) has been used as co-surfactant due to its good solubility.

Construction of ternary phase diagram: Constructing phase diagrams is time consuming, particularly when the aim is to accurately delineate a phase boundary(Lawrence and Rees, 2002). Care was taken to ensure that observations are not made on metastable systems, although the free energy required to form an emulsion is very low, the formation is thermodynamically spontaneous(Craig et al., 1995). The relationship between the phase behaviour of a mixture and its composition can be captured with the aid of a phase diagram. Maisine 35-1 as oil phase, Tween 80 as surfactant and lauroglycol 90 (cosurfactant), were used to study the phase diagrams.

When surfactant was used alone (S_{mix} ratio-1:0; Fig. 1A), large nanoemulsion gel area (not shown in figure) was obtained while small o/w nanoemulsion region was found towards aqueous rich apex and S_{mix} rich apex. The maximum concentration of oil that could be solubilized was 26% v/v by using 40% v/v of S_{mix}. When cosurfactant was added along with surfactant in equal ratio (S_{mix} ratio- 1:1; Fig. 1B), the whole area which was nanoemulsion gel in S_{mix} 1:0 changed to easily flowable o/w nanoemulsion area. This may be attributed to the fact that the addition of cosurfactant may lead to greater penetration of the oil phase in the hydrophobic region of the surfactant monomers thereby further decreasing the interfacial tension, which will lead to increase in the fluidity of the interface thus increasing the entropy of the system. (Warisnoicharoen et al., 2000).The maximum oil that could be solubilized was 26% v/v using 35% v/v of S_{mix}. When cosurfactant concentration was increased, (S_{mix} ratio-1:2, 1:3), there was phase separation after 24 hours. In contrast, when surfactant concentration was increased as compared to cosurfactant, (S_{mix} ratio-2:1; Fig. 1C), the concentration of oil that could be solubilized was increased up to 30% v/v using S_{mix} concentration 36% v/v only, also nanoemulsion region increased as compared to 1:1. A small nanoemulsion gel area was also observed which may be due to increased character of surfactant. When further surfactant concentration was increased to (S_{mix} ratio-3:1; Fig. 1D), and (S_{mix} ratio-4:1; Fig. 1E), nanoemulsion area in the phase diagrams slowly decreased with increase in nanoemulsion gel area. The maximum concentration of oil that could be solubilized in S_{mix} ratio 4:1 was 22% v/v with a very high S_{mix} concentration of 61% v/v.

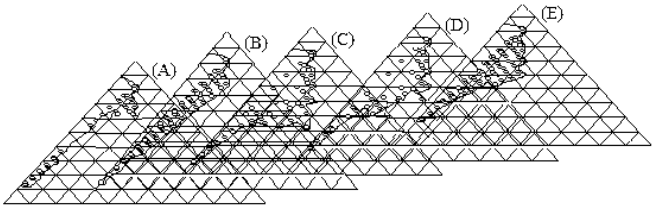

Figure 1. Pseudo-ternary phase diagrams (S_{mix} ratio = 1:0 (A), 1:1 (B), 2:1 (C), 3:1 (D), 4:1 (E)).

Thermodynamic stability studies: Nanoemulsion formulation should have stability such that it does not undergo precipitation, creaming or cracking. It is the thermostability which differentiates nanoemulsion from emulsions that have kinetic stability and will eventually phase separate. Therefore to check the stability, formulation was exposed to centrifugation study, heating and cooling cycle and freeze thawing cycle. The formulations that passed these tests were selected for the dispersibility study in order to estimate the efficiency of dispersibility. No formulations selected having S_{mix} ratio 4:1 passes the thermodynamic test. Formulations selected from each phase diagram with their thermodynamic stability testare given in Table 1.

Dispersibility studies: It is important that formed nanoemulsion does not undergo precipitation following phase separation with dilution. It is observed more prominently with drugs having poor aqueous solubility or nanoemulsion which undergoes phase transition. To avoid such a situation, dispersibility studies were vital. Formulations passing the dispersibility test in all the media in grade I and II were considered to pass the dispersibility test. Since these formulations were certain to form nanoemulsion upon dilution in the aqueous environment, these were selected for further study. Formulations selected after thermodynamic study with their dispersibility test are given in Table 2.

Droplet size analysis and polydispersity index: The droplet size of the nanoemulsion is important factor in nanoemulsion formulation, as this determines the rate and extent of drug release as well as absorption. The droplet size distribution and polydispersity index (PDI) of various formulations selected after dispersibility test having minimum surfactant concentration is given in Table 3.

***In-vitro* release study:** Dissolution studies were performed to compare the release of drug from different formulations, and plain CBZ, having same quantity (10 mg) of CBZ. The release was quantified by developed HPTLC method. The release of drug from nanoemulsion formulations were highly significant ($p < 0.01$) when compared to plain CBZ. The highest release i.e., 98 ± 1.5 % was obtained in case of formulation NE3. Approximately, 50% drug release was obtained in first 15 minutes of the study itself compared to suspension, which released less than 6 % of the drug. This is because of small globule size, and eventually higher surface area in case of nanoemulsions, which permit faster rate of drug release.

Table 1. Different nanoemulsion formulations selected from phase diagram.

S_{mix} (S:CoS)	%v/v of nanoemulsion component			Thermodynamic stability test	Infer.
	Oil	S_{mix}	Water	(H/C) (Cent) (Freez)	
1:0 (1A)	10	67	23	(X) (-) (-)	Fail
	10	70	20	(√) (√) (√)	Pass
	10	75	15	(√) (√) (√)	Pass
	15	60	25	(√) (X) (-)	Fail
	15	65	20	(√) (√) (√)	Pass
	20	49	31	(√) (√) (√)	Pass
	20	52	28	(√) (√) (√)	Pass
1:1 (1B)	10	26	64	(√) (X) (-)	Fail
	10	30	60	(X) (-) (-)	Fail
	10	50	40	(√) (√) (√)	Pass
	15	30	55	(√) (√) (√)	Pass
	15	35	50	(√) (√) (√)	Pass
	15	45	40	(√) (√) (√)	Pass
	20	33	47	(√) (√) (X)	Fail
	20	36	44	(√) (√) (√)	Pass
	20	45	35	(√) (√) (√)	Pass
	25	33	42	(X) (-) (-)	Fail
	25	35	40	(√) (√) (√)	Pass
	25	37	38	(√) (√) (√)	Pass
2:1 (1C)	10	35	55	(√) (√) (X)	Fail
	10	40	50	(√) (X) (-)	Fail
	10	50	40	(√) (√) (√)	Pass
	15	41	44	(√) (√) (√)	Pass
	15	44	41	(√) (√) (√)	Pass
	15	50	35	(X) (-) (-)	Fail
	20	38	42	(√) (√) (√)	Pass
	20	43	37	(√) (√) (√)	Pass
	20	50	30	(√) (√) (√)	Pass
	25	37	38	(√) (√) (√)	Pass
	25	40	35	(√) (√) (√)	Pass
	25	45	30	(√) (X) (-)	Fail
	30	36	34	(X) (-) (-)	Fail
3:1 (1D)	10	36	54	(X) (-) (-)	Fail
	10	40	50	(X) (-) (-)	Fail
	10	55	35	(√) (√) (√)	Pass
	15	35	50	(X) (-) (-)	Fail
	15	40	45	(√) (√) (√)	Pass
	15	50	35	(√) (√) (√)	Pass
	20	41	39	(√) (√) (√)	Pass
	20	45	35	(√) (√) (√)	Pass
	20	50	30	(√) (√) (√)	Pass
	25	33	42	(√) (X) (-)	Fail
	25	36	39	(X) (-) (-)	Fail
	25	40	35	(√) (√) (√)	Pass
	30	30	40	(X) (-) (-)	Fail
	30	35	35	(√) (X) (-)	Fail

Heating cooling cycle (H/C), Centrifugation (Cent.), Freeze thaw cycle (Freez), Dispersebility test (Disp).

Table 2. Different nanoemulsion formulations selected after thermodynamic stability test.

S_{mix} Ratio (S:CoS)	% v/v of nanoemulsion component			Dispersebility test	Infer.
	Oil	S_{mix}	Water	(D.W) (0.1N HCl) (6.8 PB)	
1:0	10	70	20	(I) (I) (III)	Fail
	10	75	15	(II/III) (III) (-)	Fail
	15	65	20	(II) (II) (III)	Fail
	20	49	31	(I) (III) (I)	Fail
	20	52	28	(III) (-) (-)	Fail
1:1	10	50	40	(I) (I/II) (III)	Fail
	15	30	55	(II) (II/III) (III)	Fail
	15	35	50	(II) (III) (-)	Fail
	15	45	40	(I) (I) (I)	Pass*
	20	36	44	(II) (IV) (-)	Fail
	20	45	35	(I) (I) (I)	Pass*
	25	35	40	(III) (-) (-)	Fail
	25	37	38	(I) (II/II) (III)	Fail
2:1	10	50	40	(I) (III) (-)	Fail
	15	41	44	(III) (-) (-)	Fail
	15	44	41	(I) (I) (I)	Pass*
	20	38	42	(I) (I) (I)	Pass*
	20	43	37	(II) (I) ((I/II)	Pass
	20	50	30	(I) (I) (I)	Pass
	25	37	38	(II) (II/III) (III)	Fail
	25	40	35	(I) (I) (I)	Pass*
3:1	10	55	35	(I) (I) (III)	Fail
	15	40	45	(IV) (-) (-)	Fail
	15	50	35	(I) (I) (I)	Pass*
	20	41	39	(I) (I) (III)	Fail
	20	45	35	(I) (I) (I)	Pass*
	20	50	30	(II) (I) (I)	Pass
	25	40	35	(II/III) (III) (-)	Fail

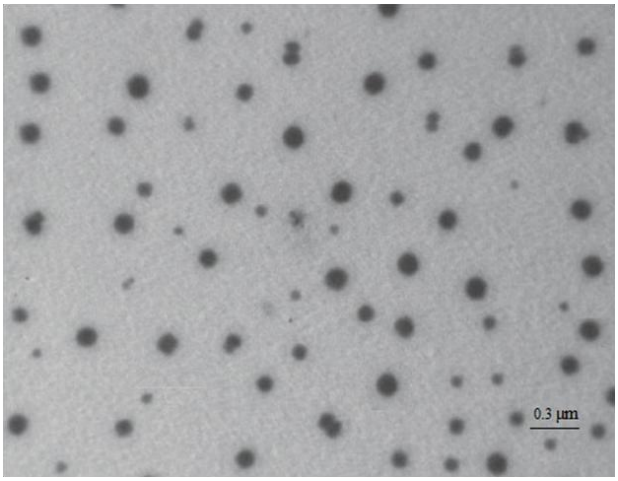

Figure 2. Transmission electron microscopy image of nanoemulsion after dilution of MNEG with water (1:100).

Microscopic examination

Photomicrograph shows transmission electron microscopy of CBZ nanoemulsion obtained after dilution of nanoemulgel with water (Fig. 2). The figure reveals that, the oil droplets of the dispersed phase were almost spherical in shape and were present in the nanometer range with no significant droplet size change during a storage period of 12 months.

Table 3. Formulations selected with their droplet size, polydispersity index.

Code	Components in nanoemulsion (% v/v) containing CBZ			Precipit-ation (PPT)	Mean Droplet Size ±SD (nm)	PDI
	Oil	S_{mix}	Water			
NE1	15	45	40	Stable	64.42±0.91	0.432
NE2	20	45	35	Stable	71.6±0.46	0.312
NE3	15	44	41	Stable	58.3±1.05	0.152
NE4	20	38	42	Stable	65.4±0.86	0.233
NE5	25	40	35	Stable	68.8±0.83	0.328
NE6	15	50	35	PPT	-	-
NE7	20	45	35	PPT	-	-

Bioadhesion potential of MNEG

MNEG exerted high bioadhesion strength (0.146 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 (Eftaihaet al., 2010). Similarly, Needleman et al., 1997 reported superior mucoadhesive properties of xanthan gum among other studied polymers (Needleman and Smales, 1995). 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 (Peppas and Buri, 1985). 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).

***In vitro* drug release**

In-vitro release study was performed to compare the release of CBZ from mucoadhesive nanoemulgel (MNEG) and nanoemulsion formulation. Previous *in vitro* method was adopted for the study. The mean cumulative % of CBZ released from nanoemulsion versus time plot is presented in Fig. 3 which illustrated a very slow release of the drug (less than 10 % in 60 min) from MNEG? A sustained delivery from nanoemulsion systems is also observed in other studies (Abrol et al., 2005). 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).

It was reported that, nanoemulsions show an increased drug uptake by living tissues. 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.

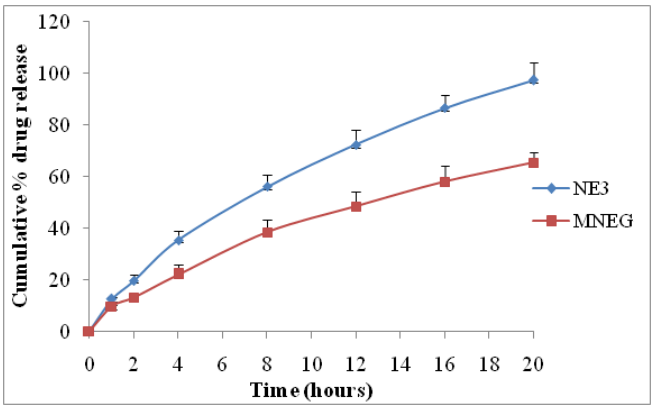

Figure 3: Cumulative % release of CBZ (mean percent release ± S.D., n=3) NE3 and MNEG. ($p < 0.05$)and plain drug.

CONCLUSIONS

Nanoemulsion formulations were successfully prepared by phase titration method. The differences in the droplet size between the formulations selected from the phase diagram was not statistically significant, although the polydispersity was at a minimum for the formulation containing 15% oil, 44% S_{mix} and 41%, v/v of water. 0.1 % xanthan gum was found to be suitable for mucoadhesive

nanoemulgel formulation exerted high bioadhesion strength to bovine nasal mucosa. The findings of our study demonstrate that CBZ-loaded mucoadhesive nanoemulgel for intranasal use could be a promising new brain targeting delivery system.

CONFLICT OF INTEREST

None declared.

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