DEVELOPMENT AND EVALUATION OF CARBAMAZEPINE LOADED TRANSFERSOMAL IN-SITU GEL FOR NOSE TO BRAIN DELIVERY

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ABSTRACT

The aim of this study is to deliver a drug into the brain via intranasal route using transfersomal in situ gel. For this purpose Carbamazepine (CBZ) which is used for the management of epilepsy was selected as a model drug. Transfersomes were formulated by thin film hydration method using Lipoid S100 and sodium cholate in 85:15% (W/W) concentration. Formed transfersomes were found to show the entrapment efficiency of 96.13±0.16%, 2.12±0.47µ particle size and cumulative CBZ release of 98.45% after 12 hours. Developed transfersomes were further incorporated into in situ gel. The transfersomal in situ gel was formulated with plain Carbopol 934P and characterized for gelling ability and gelling strength. Formulation F2 with ++ gelling ability and 22±0.56 sec gelling strength was selected for further modification with HPMC K4M. Furthermore formulation F6 with +++ gelling ability, 31.87±1.21 sec gelling strength, 278±2cps viscosity for solution and 1120±20 cps for gel, 93.38% in vitro CBZ release and good mucoadhesive strength of 55.38±0.69 dyne/cm² was optimized. From the results it can be concluded that transfersomal in situ gel of CBZ can be used to reduce the frequency of administration and increase patient compliance in the treatment of epilepsy.

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INTRODUCTION

Epilepsy is troublesome physiological condition characterized by transient alteration of behavior, results in periodic and unpredictable occurrence of seizures. Epileptic seizure requires quick management in order to avoid brain damage. However the blood brain barrier (BBB) acts as a primary obstacle for penetration of most substances including various drugs moiety into the central nervous system (CNS) from the blood stream. In order to circumvent the BBB various methods have been attempted like invasive approach (disruption of BBB, intracerebral and intrathecal implants), physiological approach (pseudonutrients, chimeric peptides), pharmacological approach including particulate drug delivery system (liposomes, nanoparticles) are used for targeting drug molecules to brain [1].

In the last decade, intranasal (IN) administration has attracted considerable interest. A major advantage of IN drug delivery over other methods of administration is that, it provides a non-invasive method for bypassing the BBB and delivers therapeutic drug directly to the CNS. Drugs administered to the nasal cavity can transfer through the olfactory mucosa and subsequently through olfactory bulb and trigeminal nerves to reach many regions within the CNS. Therefore, olfactory mucosal drug delivery via the nasal route provides potential for brain targeting. Unfortunately poor drug uptake via olfactory mucosa was found in some cases, because of their low mucosal permeability, rapid mucociliary clearance from the nasal cavity and susceptibility to degradation by nasal enzymes in the nasal cavity or during passage across the epithelial barrier. To overcome the mucociliary clearance and nasal degradation, drugs need to be encapsulated into an appropriate carrier system like liposomes, niosomes, transfersomes, microspheres or nanoparticles [2].

Carbamazepine (CBZ) is an antiepileptic drug effective for all most all types of seizures. However it belongs to class II of the biopharmaceutical classification system. Drugs in this category have high permeability and low water solubility. Consequently, bioavailability of such drugs is limited by their solubility in water. Differences in bioavailability have been observed among various commercial formulations of CBZ as it is absorbed slowly and erratically after oral administration. Peak concentration in plasma is usually attained 4–8 h after oral ingestion but may be delayed by as much as 24 h. Reportedly, it has an oral bioavailability of less than 50% [3].

Therefore, an attempt was made to develop CBZ transfersomes and incorporation of same in “in situ gel” containing hydroxyl propyl methyl cellulose (HPMC) as a mucoadhesive polymer in order to help in administration and overcome the mucocilliary clearance [4, 5].

MATERIALS AND METHODS

Materials

CBZ was provided as a gift sample from Ajanta pharma limited (Mumbai, India). Lipoid S100 was kindly supplied by Lipoid KG (Germany). Sodium cholate was procured from Ozone pharma (Mumbai, India). Carbopol 934 P and HPMC K4M were obtained from Colorcon Asia Pvt. Ltd., Singapore. Methyl paraben was procured from S. D. fine chemical limited, Mumbai, India. Dialysis membrane with molecular weight cut off of 12000 – 14000 kDa was purchased from Himedia (Mumbai, India). All other chemicals were of analytical grade and obtained commercially. For all the experiments double distilled water was used.

Preparation of transfersomes

Transfersomes were prepared by thin film hydration method. Briefly Lipoid S100 and sodium cholate were taken in 85:15% (W/W) concentration and dissolved in 10ml of ethanol along with 100 mg of drug in dry round bottom flask. Ethanol was later removed by rotary vacuum evaporator (Eqitron rota evaporator, Medica instrument, India) above the lipid transition temperature 50°C at 65 rpm. Traces of ethanol were removed from the deposited lipid films under vacuum overnight. Lipid film was then hydrated with 10 ml deionised water with 65 rpm rotation (30 min) at the room temperature [6, 7].

Preparation of transfersomal in situ gel

1% of CBZ in situ gels with varying concentrations of carbopol 934P were prepared by dispersing required quantity of corbopol in prepared transfersomal stock solution with continuous stirring and kept overnight for complete hydration. Further appropriate quantities of sodium chloride, sodium metabisulphite and methyl paraben were added to previous polymeric mixture. Final pH of the preparation was adjusted to 4.5 with 0.5 M sodium hydroxide solution [3, 8]. In situ gel was then further modified by addition of varying proportion of HPMC K4M (Table 1).

Table 1: Formulation of transfersomal gel

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
<th>F6</th>
<th>F7</th>
<th>F8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transfersomal dispersion (ml)</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Carbopol 934P (% W/V)</td>
<td>0.2</td>
<td>0.3</td>
<td>0.4</td>
<td>0.5</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Sodium metabisulphite (% W/V)</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>HPMC K4M (% W/V)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.5</td>
<td>0.1</td>
<td>0.15</td>
<td>0.2</td>
</tr>
<tr>
<td>Sodium chloride (% W/V)</td>
<td>0.9</td>
<td>0.9</td>
<td>0.9</td>
<td>0.9</td>
<td>0.9</td>
<td>0.9</td>
<td>0.9</td>
<td>0.9</td>
</tr>
<tr>
<td>Methyl paraben (% W/V)</td>
<td>0.033</td>
<td>0.033</td>
<td>0.033</td>
<td>0.033</td>
<td>0.033</td>
<td>0.033</td>
<td>0.033</td>
<td>0.033</td>
</tr>
</tbody>
</table>

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Evaluation of transfersomes

Entrapment efficiency

Prepared transfersomes were separated from unentrapped drug by ultracentrifugation at 4 °C with 25,000g relative centrifugal force (rcf) for 30 min. Further vesicles were disrupted by sonication (Pci Analytics JIJ 158) for 15 minutes within 5 ml ethanol. Then the solution was filtered and 1 ml of filtrate was further diluted with phosphate buffer of pH 6 up to 10 ml and the concentration of drug was analyzed spectrophotometrically at 284 nm. All the analysis was carried out in triplicate and the values were averaged. Entrapment efficiency was illustrated in % [9].

Vesicle size analysis

Vesicle size analysis was done by optical microscopy with 400 X magnifications power by using future winjoe projection microscope (MEM 1300). For observation 1 ml transfersomal suspension was diluted with 5 ml phosphate buffer pH 6 with manual shaking for 5 min. Then dispersion was observed under the microscope. Each measurement was done in triplicate [10].

In vitro CBZ release

Prepared transfersomes were further characterized for in vitro release study of CBZ using Franz diffusion cell with effective surface area 3.14 cm². Cellophane membrane (Himedia molecular weight12000 – 14000 kDa) was preincubated in phosphate buffer of pH 6 for 24 hrs and was mounted between the donor and receptor compartments. 15 ml of phosphate buffer of pH 6 was used as a receptor medium and donor medium consisted of 1ml suspension of transfersomes. The receptor content was maintained at temperature 37°C. 1 ml of samples were withdrawn at specific time interval of 0.15, 0.30, 0.45, 1, 2, 4, 6, 8, 10, 12 hrs and replaced with the same amount of fresh phosphate buffer solution, and CBZ content was assayed by a spectrophotometer at 284 nm[11]. This experiment was carried out in triplicate.

Evaluation of transfersomal in situ gel

pH Study

pH of all the batches was evaluated with the help of calibrated digital pH meter. The studies were conducted in triplicate.

Gelation studies

In situ gelation is the process by which the liquid phase (sol) goes under transition into gel. For gelation study 5ml transfersomal in situ gel of each formulation was mixed with 0.5 ml of 0.5 M NaOH in 10 ml measuring cylinder according to the report [12]. Gelation was assessed by visual examination. The consistency of formed gel was checked and graded, as follows.

<table>
<thead>
<tr>
<th>Sign</th>
<th>Gradation</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>No gelation occurred</td>
</tr>
<tr>
<td>+</td>
<td>Gelation occurred within few minutes and remained for few hrs</td>
</tr>
<tr>
<td>++</td>
<td>Gelation occurred immediately and remained for few hrs</td>
</tr>
<tr>
<td>+++</td>
<td>Gelation occurred immediately and remained for extended period</td>
</tr>
<tr>
<td>++++</td>
<td>Very stiff gel was formed</td>
</tr>
</tbody>
</table>

Gelling strength

The gel strength is an indication for the viscosity of the nasal gel at physiological condition. Gel strength was determined by taking 50 gm of formulation in 100 ml of measuring cylinder and gelled by neutralization with 0.5 M NaOH. A weight of 1g was placed onto the gelled solution and gel strength was determined by the time in seconds required by the weight to move 5 cm into the gel [13].The studies were conducted in triplicate.

Viscosity

Viscosity of the formulation in sol and gel state was determined in triplicate at room temperature using suitable spindle of Brookfield viscometer (RVT 230, USA) at 100rpm for 5 minutes and values were averaged. For studying the viscosity, transfersomal in situ gel solution was taken into the beaker and spindle number 2 was lowered perpendicularly into the in situ solution [13]. After noting the viscosity of in situ solution it was gelled with 0.5M NaOH and the viscosity was further measured using spindle number 5.

Content uniformity

All formulations were tested for content uniformity. Vials containing formulation were shaken for 2-3 min. 1 ml of in situ gel formulation was withdrawn and dissolved in 100 ml phosphate buffer of pH 6. The solution was filtered and analyzed at 284 nm by UV spectrophotometry. The studies were conducted in triplicate.

In vitro release of CBZ from prepared transfersomal gel

Drug release from in situ gel was tested with diffusion cell, using dialysis membrane12000-14000 dalton (High media) with permeation area of 3.14cm². 15 ml of phosphate buffer of pH 6 was added into the acceptor chamber. 1ml of in situ gel was put in donor chamber & gelled by using 0.5M NaOH. At predetermined time points, 1 ml sample were withdrawn from the acceptor compartment, replacing the sampled volume with same quantity of fresh phosphate buffer solution of pH 6 and measured spectrophotometrically at 284 nm. The studies were conducted in triplicate.
Kinetic analysis of the release data
The release data were analyzed by best fit linear regression according to:
Zero-order equation: \( Q = Q^0 + Kt \)
First-order equation: \( Q = Q^0 e^{-kt} \)
Higuchi’s square root model: \( Q = K\sqrt{t} \)
and also by
Korsmeyer-Peppas power equation: \( \frac{M_t}{M_\infty} = Kt^n \)
The correlation coefficient \((r)\) was found out in each case [14].

Determination of mucoadhesive force
Mucoadhesive force was measured according to the reported method [13] with slight modifications. The mucoadhesive potential of each formulation is force required to detach the formulation from goat nasal olfactory mucosa. A section of sheep olfactory nasal mucosa was fixed using thread and rubber bands on the bottom of two glass vial with mucosal side facing upward. 50mg of gel was applied to the mucosa attached on first vial and this vial was fixed in an inverted position between the jaw of digital mucoadhesive strength tester (Lami Coat Equipments, Mumbai), while another vial was fixed on the first vial. Both the vials were adjusted in such a way that gel formulation between them held in contact with mucosal surface. Then the second vial was attached with the hook of instrument using tread and instrument was started. The mucoadhesive force expressed as the detachment strength in dynes/cm², was determined from the minimal weight that detached the mucosal tissue from surface of formulation by using following formula. The studies were conducted in triplicate.

Mucoadhesive strength (dynes/cm²) = Weight in dynes/A

Where,
A-Area of the tissue exposed and is equal to \( \pi r^2 \)

RESULT AND DISCUSSION
Evaluation of transfersomes
Entrapment efficiency, Vesicle size analysis, in vitro CBZ release
Entrapment efficiency of the prepared transfersome was found to be 96.13±0.16%. The particle size of developed transfersomal batch was found to be 2.12±0.47µ. Cumulative CBZ release was found to be 98.45% after 12hours.

Evaluation of transfersomal in situ gel
pH Study
pH of all formulations were found to be in the range of nasal physiological pH between 4.50±0.05 to 4.58±0.06 Table 2.

Gelation studies
From the result of gelation studies (Table 2) it showed that formulation F1 containing 0.2% concentration of carbapol 934P could form weak gel, while formulations F2 and F3 showed good gelations. Formulation F4 with 0.5% carbapol 934 P concentration formed stiff gel at physiological pH. Formulations F5, F6 and F7 showed good gelation, while stiff gelation was observed in F8 batch.

Table 2: Evaluation data of transfersomal gel.

<table>
<thead>
<tr>
<th>Formula</th>
<th>pH</th>
<th>Gelation studies</th>
<th>Gel strength (Sec)</th>
<th>Viscosity (cPs)</th>
<th>Mucoadhesive force (dyne/cm²)</th>
<th>Drug content (%W/V)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Solution</td>
<td>Gel</td>
<td></td>
</tr>
<tr>
<td>F1</td>
<td>4.56±0.05</td>
<td>+</td>
<td>14.65±1.34</td>
<td>190±2</td>
<td>980±10</td>
<td>14.12±1.43</td>
</tr>
<tr>
<td>F2</td>
<td>4.52±0.03</td>
<td>++</td>
<td>22.00±0.56</td>
<td>226±4</td>
<td>1020±20</td>
<td>19.32±0.87</td>
</tr>
<tr>
<td>F3</td>
<td>4.58±0.06</td>
<td>+++</td>
<td>38.43±1.42</td>
<td>276±2</td>
<td>1153±15</td>
<td>23.54±1.23</td>
</tr>
<tr>
<td>F4</td>
<td>4.50±0.05</td>
<td>++++</td>
<td>51.38±2.63</td>
<td>306±2</td>
<td>1380±10</td>
<td>27.83±0.78</td>
</tr>
<tr>
<td>F5</td>
<td>4.56±0.06</td>
<td>+++</td>
<td>26.49±1.49</td>
<td>234±4</td>
<td>1080±10</td>
<td>35.39±1.63</td>
</tr>
<tr>
<td>F6</td>
<td>4.51±0.04</td>
<td>+++</td>
<td>31.87±1.21</td>
<td>278±2</td>
<td>1120±20</td>
<td>55.38±0.69</td>
</tr>
<tr>
<td>F7</td>
<td>4.50±0.05</td>
<td>+++</td>
<td>36.41±2.36</td>
<td>308±4</td>
<td>1187±20</td>
<td>64.31±0.44</td>
</tr>
<tr>
<td>F8</td>
<td>4.57±0.03</td>
<td>++++</td>
<td>40.47±1.94</td>
<td>386±2</td>
<td>1286±10</td>
<td>76.59±1.65</td>
</tr>
</tbody>
</table>

Values expressed as mean±S.D, n=3
Gelling strength

Appropriate gel strength of the formulation is important task in the development of nasal in situ gel so that the in situ gel must be administered easily as drops and after administration will get convert into gel to prevent anterior leakage. Upon comparing gelation strengths it was observed that, it was affected by the concentration of carbopol and HPMC K4M. Increasing the concentration of these polymers also increases the gelling strength. Formulations F1 had gel strength less than 25 sec, erode rapidly and could not withstand gel structure. Formulation F4 showed gel strength more than 50 sec, formed stiff gel which may cause discomfort. The formulations F2, F3 with optimum gelling strength were considered for further modification but formulation F3 was not optimized further as addition of mucoadhesive polymer may also increases gelling ability and gelling strength. Furthermore comparing the results of gelling ability and gelling strength, formulations F1 and F4 were excluded from further modifications. Formulation F2 was considered for further modification using HPMC K4 M and results of F5, F6, F7 and F8 are depicted in Table 2.

Viscosity

The viscosities in all formulations from sol to gel were found to be proportionately increased (Table 2). In situ liquids exhibited viscosity from 190±2 to 386±2 cPs, while in gel state all formulations exhibited viscosity from 980±10 to 1380±10 cPs.

Content uniformity

The % drug content (Table 2) of all the prepared formulations was observed to be in the range of 98.62±0.48 - 99.75±0.84 %

In vitro release of CBZ from transfersomal gel

Fig. 1 explains comparative results of % drug release of in situ gelling system. It was found that the release profile of plain carbopol in situ gel was faster, whereas formulations having HPMC K4M in carbopol 934P were very efficient in controlling the CBZ release. The rate and extent of drug release from in situ gelling system is decreased by increasing the polymers concentrations. Release of CBZ from in situ system is inversely proportional to viscosity and gelling strength.

Kinetic analysis of the release data

The release data of CBZ from transfersomal formulations was treated mathematically according to zero, first order, Higuchi diffusion and Peppas korsmeyer models. The data of regression coefficient is illustrated in table 3. The R^2 values of all formulations were highest for Higuchi plot; hence it is clear that the release mechanism of transfersomal gel follows diffusion controlled mechanism. Also the data of Peppas exponential model shows fickian diffusion.

Table 3: Regression coefficient data of in vitro release study.

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Correlation Coefficient (R^2)</th>
<th>'n' values of Korsmeyerpeppas</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Zero order</td>
<td>First order</td>
</tr>
<tr>
<td>F1</td>
<td>0.8578</td>
<td>0.677</td>
</tr>
<tr>
<td>F2</td>
<td>0.8811</td>
<td>0.749</td>
</tr>
<tr>
<td>F3</td>
<td>0.908</td>
<td>0.867</td>
</tr>
<tr>
<td>F4</td>
<td>0.9205</td>
<td>0.833</td>
</tr>
<tr>
<td>F5</td>
<td>0.8915</td>
<td>0.7602</td>
</tr>
<tr>
<td>F6</td>
<td>0.8947</td>
<td>0.7170</td>
</tr>
<tr>
<td>F7</td>
<td>0.9163</td>
<td>0.8395</td>
</tr>
<tr>
<td>F8</td>
<td>0.9278</td>
<td>0.9122</td>
</tr>
</tbody>
</table>
Determination of mucoadhesive force

Mucoadhesive force was found to increase with increasing concentrations of polymers. Mucoadhesive force was found to be in the range of 14.12±1.43 to 76.59±1.65 dyne/cm². The stronger the mucoadhesive force is, the more it can prevent the gelled solution coming out of the nose. But if the mucoadhesive force is too excessive, the gel can damage the nasal mucosal membrane.

CONCLUSION

Initially transfersomal in situ nasal systems of plain Carbopol 934 P were optimized for gelation studies and gelling strength to ensure sol to gel conversion at physiological conditions without drop out from nose, after administration. Formulation F2 with ++ gelling ability and 22±0.56 sec gelling strength was optimized for incorporation of mucoadhesive HPMC K4M polymer. When HPMC K4M is used in combination with Carbopol 934 P for development of in situ gel, formulation F6 with +++ gelling ability, 31.87±1.21 sec gelling strength, 278±2 cps viscosity for solution and 1120±20 cps for gel, 93.38% in vitro CBZ release and good mucoadhesive strength of 55.38±0.69 dyne/cm² was considered as optimized batch. Sustained release of CBZ from the transfersomes may be used to reduce the frequency of administration and increase patient compliance. Possibilities of direct delivery of CBZ into the brain in significant quantities recommend the further research in this area.

ACKNOWLEDGEMENT

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CONFLICT OF INTEREST STATEMENT

All authors hereby declare that this is an original, self-funded research work performed using the facilities available in our laboratories and authors do not have any financial, personal, employment related, stock ownership related, honoraria or paid expert testimony related, patent related, grants or other funding related or any other conflict with any people, organizations or consultancies that could inappropriately influence (bias) our work. The authors report no declarations of interest.
REFERENCES


