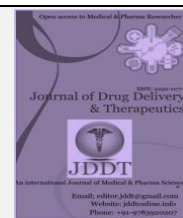
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Research Article

Formulation and Evaluation of Galantamine Hydrobromide Proniosome Gel for Alzheimer's disease

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ABSTRACT

Galantamine hydrobromide is formulated in tablets and capsules prescribed through oral delivery for the treatment of Alzheimer's disease. However, oral delivery of drugs can cause severe side effects such as nausea, vomiting, and gastrointestinal disturbance. In the present research work, Galantamine hydrobromide is formulated as proniosome gel by Coacervation phase separation method using different surfactants such as Tweens and Spans. Overall eight formulations were developed and evaluated for various parameters. The prepared gels were viewed by naked eye to observe the colour of gel. Microscopical observations of the gels showed vesicles of optimum size from 3.030 μm (P2) - 3.735 μm (P5). The gel also showed optimum rate of spontaneity in the range 9.60 $\text{mm}^3 \times 1000$ (P7) to 11.80 $\text{mm}^3 \times 1000$ (P4) and entrapment efficiency of vesicles in the range 66.15% (P5) to 86.92% (P3). The gels had pH in suitable range of skin (5.92-6.9). The in vitro drug diffusion studies revealed that the drug diffusion was affected by the various surfactants used. The rank order of surfactant effect on in-vitro drug diffusion was Tween 80 > Tween 60 > Tween 40 > Tween 20 > Span 80 > Span 60 > Span 40 > Span 20. The proniosomal gel containing Tween 80 showed maximum drug diffusion (99.24%) and the gel containing Span 20 showed minimum drug diffusion (71.74%). FT-IR studies of optimized proniosome gel P8 revealed the absence of any chemical interactions between drug and carriers used.

Keywords: Galantamine hydrobromide, Proniosome gel, Coacervation phase separation method, Surfactants, in vitro drug diffusion studies.

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INTRODUCTION

Alzheimer's disease is a progressive disorder that causes brain cells to waste away (degenerate) and die. Alzheimer's disease is the most common cause of dementia—a continuous decline in thinking, behavioral and social skills that disrupts a person's ability to function independently. The early signs of the disease may be forgetting recent events or conversations [1]. While there are currently no treatments available to slow or stop the brain damage caused by Alzheimer's disease, several medications can temporarily help improve the symptoms of dementia for some people. These medications work by increasing neurotransmitters in the brain [2]. The U.S. Food and Drug Administration (FDA) has approved two types of medications — cholinesterase inhibitors (Aricept®, Exelon®, Razadyne®) and memantine (Namenda®).

Galantamine hydrobromide is a cholinesterase inhibitor that has been used to reverse the muscular effects of gallamine triethiodide and tubocurarine, and has been studied as a treatment for Alzheimer's disease and other central nervous

system disorders. Galantamine hydrobromide has advantages such as lower muscarinic side effects and higher speed of recovery from respiratory depression, can penetrate through the blood-brain barrier, can bind to nicotinic acetylcholine receptors, and enhanced microglial amyloid-beta peptides phagocytosis. It is formulated in tablets and capsules, prescribed through oral delivery for the treatment of Alzheimer's disease. However, oral delivery of drugs can cause severe side effects such as nausea, vomiting, and gastrointestinal disturbance [3]. To avoid these side effects various novel drug delivery systems have been developed, which include niosomes, proniosomes, liposomes, nanoparticles, microspheres, micro-emulsions, implantable pumps and magnetic microcapsules[4].

Proniosomes are vesicular systems, in which the vesicles are made up of non-ionic based surfactants, cholesterol and other additives. Semisolid liquid crystal gel (proniosomes) prepared by dissolving the surfactant in a minimal amount of an acceptable solvent, namely ethanol and then hydration with least amount of water to form a gel. These structures are liquid crystalline compact niosomes hybrids that can be

converted into niosomes immediately upon hydration or used as such in the topical/transdermal applications. Proniosomal gels are generally present in transparent, translucent or white semisolid gel texture, which makes them physically stable during storage and transport [5].

OBJECTIVE

The objective of present study is to formulate Galantamine hydrobromide proniosome gel using various surfactants and evaluate the prepared gels for topical delivery.

MATERIAL AND METHODS

Galantamine hydrobromide was obtained as a gift sample from Caplin Point Labs, Chennai. Span 20, 40, 60, 80 was purchased from Central Drug House, Mumbai and Tween 20, 40, 60, 80, and cholesterol from S.D Fine Chemicals Pvt Ltd, Mumbai. Soya Lecithin was obtained from Hi Media Lab, Mumbai. All other chemicals used were of analytical grade.

Drug Characterisation

Identification

A 1000 µg/ml solution of Galantamine hydrobromide in phosphate buffer of pH 7.4 was scanned in UV range between 200 to 400 nm to estimate the maximum absorbance of the drug and was noted. Standard Calibration Curve was prepared by preparing drug solutions of suitable concentrations in respective medium [6].

Solubility studies

Excess amount of the selected drug was taken and dissolved in a measured amount of distilled water and phosphate buffer of pH 7.4 separately in a glass beaker to get a saturated solution. The solution was shaken intermittently to assist the attainment of equilibrium with the undissolved drug particles. Then measured quantity of the filtered drug solution was withdrawn after 24 hrs and successively diluted with respective solvents and the concentration was measured spectrophotometrically [7,8].

Partition coefficient

A drug solution of 1mg/ml was prepared in n-octanol. 25 ml of this solution was taken in a separating funnel and shaken with an equal volume of phosphate buffer of pH 7.4 (aqueous phase) for 10 min and allowed to stand for two hrs. Then aqueous phase and organic phase were collected separately. Both the phases were analyzed for the drug concentration using U.V. spectrophotometer. Partition coefficient was calculated by taking the ratio of the drug concentration in n-octanol to drug concentration in aqueous phase [9,10].

Permeability coefficient

The permeability coefficient of drug was calculated by "Potts and Guy equation" [11],

$$\text{LogKp} = -2.7 + 0.71 \times \log \frac{K_o}{w} - 0.0061 \times \text{Mol. wt} \quad \text{---(1)}$$

Where, LogKp = Permeability coefficient

K_o/w = Partition coefficient

Formulation of Proniosome gel

Proniosome gel was prepared by a coacervation-phase separation method (Fig.1). Precisely weighed amounts of surfactant, lecithin, cholesterol and drug were taken in a clean and dry wide mouthed glass vial of 5.0 ml capacity and alcohol (0.25 ml) was added to it. After warming, all the ingredients were mixed well with a glass rod; the open end of the glass bottle was covered with a lid to prevent the loss of solvent from it and warmed over water bath at 60-70°C for about 5 min until the surfactant mixture was dissolved completely. Then the aqueous phase (pH 7.4 phosphate buffer solution) was added and warmed on a water bath till a clear solution was formed which was converted into proniosome gel on cooling. The gel so obtained was preserved in the same glass bottle in dark conditions for characterization [12]. The composition of various proniosome gels in given in Table 1.

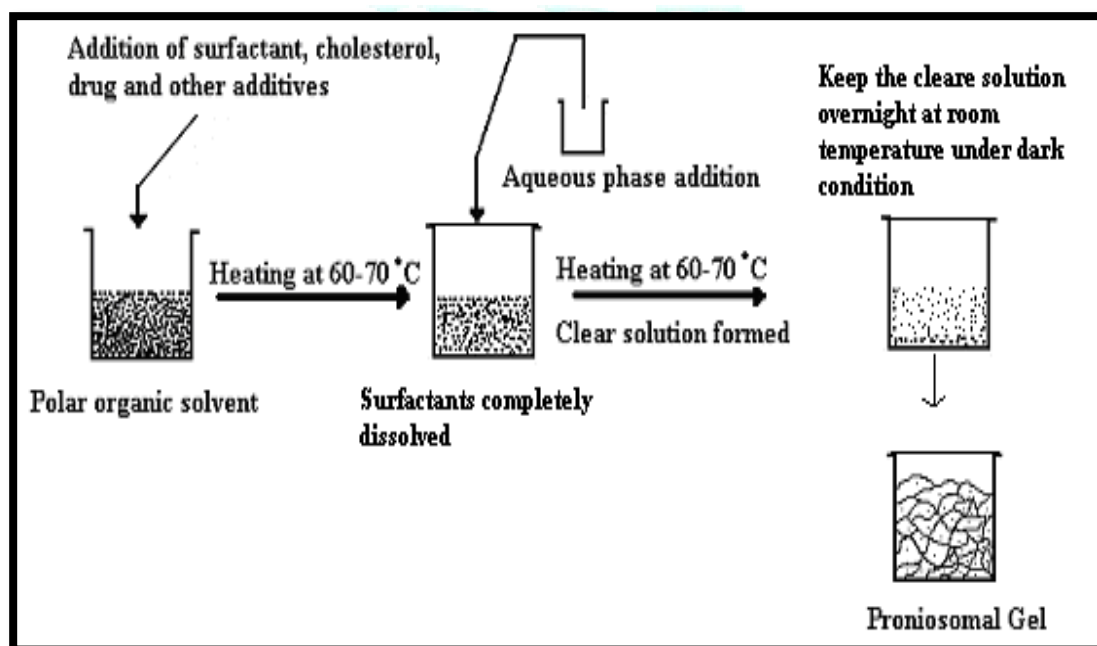


Figure 1: Diagrammatic representation of Proniosome gel preparation

Evaluation of proniosome gel

The Galantamine hydrobromide proniosome gel formulations were evaluated for the following parameters:

Morphological evaluation

The prepared gels were viewed by naked eye to observe the colour of gel and appearance.

Vesicle Size analysis: Hydration of proniosome gel (100 mg) was done by adding saline solution (0.9% solution) in a small glass vial with occasional shaking for 10 min. The dispersion was observed under optical microscope at 45 x magnification. The sizes of 200 - 300 vesicles were measured using a calibrated ocular and stage micrometer fitted in the optical microscope.

Table 1: Composition of Galantamine hydrobromide proniosome gel formulations

| Formulation code | Drug (mg) | Surfactant | Weight (mg) | Lecithin (mg) | Cholesterol (mg) | Alcohol (ml) | pH 7.4 phosphate buffer (ml) |
|------------------|-----------|----------------|-------------|---------------|------------------|--------------|------------------------------|
| P1 | 10 | Span 20 (S20) | 180 | 180 | 20 | 0.25 | 0.16 |
| P2 | 10 | Span 40 (S40) | 180 | 180 | 20 | 0.25 | 0.16 |
| P3 | 10 | Span 60 (S60) | 180 | 180 | 20 | 0.25 | 0.16 |
| P4 | 10 | Span 80 (S80) | 180 | 180 | 20 | 0.25 | 0.16 |
| P5 | 10 | Tween 20 (T20) | 180 | 180 | 20 | 0.25 | 0.16 |
| P6 | 10 | Tween 40 (T40) | 180 | 180 | 20 | 0.25 | 0.16 |
| P7 | 10 | Tween 60 (T60) | 180 | 180 | 20 | 0.25 | 0.16 |
| P8 | 10 | Tween 80 (T80) | 180 | 180 | 20 | 0.25 | 0.16 |

Rate of spontaneity: 10 to 20 mg of proniosome gel was transferred to the bottom of a clean stopper glass bottle and spread uniformly around the wall of the glass bottle with the help of a glass rod. At room temperature, 2 ml of phosphate saline (0.154 M Sodium chloride) was added carefully along the walls of the glass bottle and left in a test-tube stand. After 20 min, a drop of this saline solution was withdrawn and placed on Neubauer's Chamber to count the number of vesicles. The number of niosomes eluted from proniosomes was counted^[13].

% Entrapment efficiency: To evaluate the loading capacity of proniosome systems proniosome gel (100 mg) was dispersed in phosphate buffer pH 7.4 and warmed a little for the formation of niosomes. Then the dispersion was centrifuged at 9000 rpm for 30 min at 20°C. The clear fraction was used for the determination of free drug at 289 nm spectrophotometrically^[14]. The percentage entrapment efficiency was calculated from following Equation.

% Entrapment efficiency = $\left[\frac{\text{Total drug} - \text{Free drug}}{\text{Total drug}} \right] \times 100$ ----- (2)

pH determination: The pH of each Proniosome gel was determined using pH meter. The electrode was first calibrated with pH 4.0 and pH 7.0 solutions then sample readings were recorded on pH meter^[15].

In-vitro diffusion studies: In vitro diffusion studies on proniosome gel were performed using Franz-diffusion cell. The capacity of receptor compartment was 40 ml. The dialysis cellophane membrane was mounted between the donor and receptor compartment. A weighed amount of proniosome gel equivalent to 4 mg (152 mg of gel) was placed on the donor compartment side. The receptor medium was phosphate saline buffer pH 7.4^[16]. Heat was provided using a thermostatic hot plate with a magnetic stirrer. The receptor fluid was stirred by a Teflon-coated magnetic bead fitted to a magnetic stirrer. At each sampling

interval i.e., every hour, 1 ml of sample was withdrawn and replaced with 1 ml fresh buffer to maintain sink condition. Samples withdrawn were analyzed spectrophotometrically at 289 nm.

Fourier transformer infrared spectroscopy (FTIR) study

For establishing compatibility of drug and excipients several methods are being used. Most commonly used are spectroscopic studies like IR, HNMR, Mass, differential scanning calorimetry (DSC), X-ray diffraction etc. In the present study we have utilized values of IR spectra.

RESULTS AND DISCUSSION

Drug characterisation

Identification

The absorption maximum was found to be 289 nm in phosphate buffer of pH 7.4 which was in accordance with the literature reports (Table 2).

Solubility

The available literature on solubility profile of Galantamine hydrobromide indicated that the drug is sparingly soluble in water, and very slightly soluble in anhydrous ethanol. However, to mimic the physiological conditions of the skin, phosphate buffer pH 7.4 was used as the diffusion medium. The solubility was found to be 2.1 mg/ml in water with neutral pH and 2.2 mg/ml in phosphate buffer pH 7.4. The results of solubility studies are given in Table 2.

Partition coefficient

Partition coefficient is a measure of distribution of molecules between two phases. For transdermal delivery studies of partition coefficient between n-octanol and water is often used as a guide as to how well a molecule will distribute between stratum corneum lipids and water. n-octanol gives a partition coefficient range consistent with the physical properties of

stratum corneum compared to other solvents. Phosphate buffer pH 7.4 was used to get absolute K^o_w . The partition coefficient of drug Galantamine hydrobromide (1 mg/ml) between n-octanol and phosphate buffer pH 7.4 (aqueous phase) was found to be $K^o_w = 1.44$ (Table 2). Since K^o_w value is less than 3, the drug will traverse the stratum corneum exclusively by intercellular pathway^(9,10).

Permeability coefficient

Permeability coefficient describes the rate of drug transport per unit concentration (cm/hr). The permeability coefficient of drug according to "Potts and Guy equation" was found to be 4.835 cm/hr (Table 2).

Table 2: Characterisation of Galantamine hydrobromide pure drug

| Studies | Identification λ_{max} (UV) | Solubility (mg/ml) | | Partition co-efficient | Permeability co-efficient |
|----------------------------|--|--------------------|----------------------------|---------------------------|------------------------------|
| | | Water | Phosphate buffer pH 7.4 | | |
| Results | 289 | 2.1 | 2.2 | 1.44 | 4.835 |
| Reported ^[4,17] | 289 | 1.7 | - | 1.39 | - |

Evaluation of proniosome gel

Morphological evaluation

The colour of the prepared proniosome gel formulations was observed by naked eye and the results are given in Table 3. The proniosome gel formulations showed brown and yellow colour depending on their chemical compositions. Proniosome gels P1 and P2 when viewed by optical microscope at 45 × magnification, showed spherical and discrete shaped vesicles with sharp boundaries. The observation is given in Fig. 2.

Vesicle size analysis

Determination of vesicle size is important for the topical application of vesicles. Brain et al demonstrated that vesicle more than 10 micrometer remains on skin surface and the vesicle of 3-10 micrometer concentrates in follicle and less than 3 micrometer penetrates the stratum corneum⁽¹⁸⁾. Results of Vesicle size of Galantamine hydrobromide proniosome gel formulations are presented in Table 3. The Vesicle size of different formulations ranged from 3.030 μm (P2) to 3.735 μm (P5) indicating that vesicles formed could easily penetrate the stratum corneum. It was observed that Vesicles formed with Span as surfactant (P1 - P4) were smaller in size than vesicles formed with Tweens (P5 - P8); this was attributed to the fact that Spans have greater hydrophobicity than Tweens. It is indicated that increase in hydrophobicity decreases surface energy of surfactants resulting in smaller vesicle size.

Rate of spontaneity studies

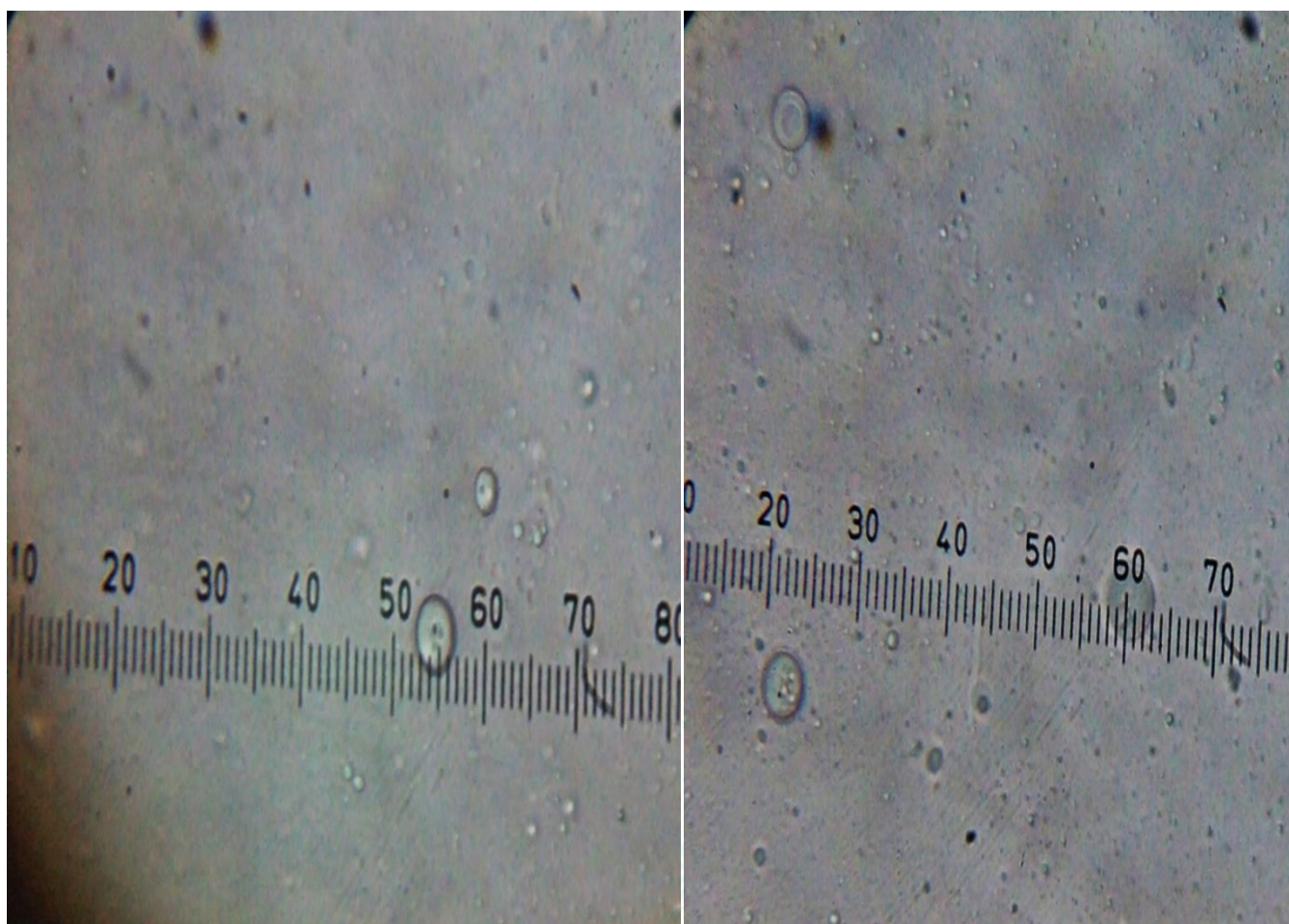
Rate of spontaneity is the number of niosomes formed after hydration of proniosomes for 20 mins. The results of study are given in Table 3. Rate of spontaneity for different proniosome gel formulations were varied between 9.60 $\text{mm}^3 \times 1000$ (P7) to 11.80 $\text{mm}^3 \times 1000$ (P4). Proniosome formulations prepared with Spans formed niosomes more spontaneously than Tweens. It was also observed that as the vesicle size decreased the rate of spontaneity increased.

Entrapment efficiency (%)

Vesicle entrapment efficiency mainly depends on the type of surfactant used, amount of surfactant required to form the bilayer and intrinsic properties of surfactants like HLB value, chemical structure, lipophilicity, phase transition temperature and alkyl chain length. It was found that surfactants which had low HLB value, higher lipophilicity, higher phase transition temperature and longer alkyl chain length showed higher entrapment. The results obtained were quite similar to the above discussion. The entrapment efficiency of all formulations was in the range of 66.15% (P6) to 86.92% (P3) as depicted in Table 3. The proniosome gels prepared with Spans (P1 -P4) as surfactants gave higher entrapment efficiency than Tweens (P5 - P8) because of smaller size of vesicles and higher lipophilic nature of spans than tweens.

Table 3: Evaluation of Galantamine hydrobromide proniosome gel formulations

| Sl. No. | Formulation code | Colour | Vesicle size(μm) | Rate of spontaneity ($\text{mm}^3 \times 1000$) | Entrapment efficiency (%) | pH |
|---------|------------------|--------|-------------------------------|---|---------------------------|------|
| 1 | P1 | Brown | 3.315 | 11.75 | 78.00 | 6.9 |
| 2 | P2 | Yellow | 3.030 | 10.70 | 80.00 | 6.84 |
| 3 | P3 | Yellow | 3.233 | 10.90 | 86.92 | 6.32 |
| 4 | P4 | Yellow | 3.195 | 11.80 | 77.69 | 6.43 |
| 5 | P5 | Brown | 3.735 | 9.91 | 66.15 | 6.70 |
| 6 | P6 | Brown | 3.712 | 9.72 | 69.23 | 6.61 |
| 7 | P7 | Brown | 3.630 | 9.60 | 76.53 | 5.92 |
| 8 | P8 | Brown | 3.495 | 10.52 | 76.92 | 6.52 |



Proniosome gel P1

Proniosome gel P2

Fig. 2: Optical photomicrograph of Galantamine hydrobromide proniosome gel P1 and P2.

Among the proniosome gels prepared with Spans the entrapment efficiency was in the order Span 60 (P3) > Span 40 (P2) > Span 20 (P1) > Span 80 (P4). Span 60 showed higher entrapment efficiency than others. The entrapment efficiency of Span 80 (P4 -77.69%) formulation was less than that of Span60 (P3-86.92%). This was due to the reason that Spans 60 and 80 have the same head group, but Span 80 has an unsaturated alkyl chain that is responsible for low drug entrapment.

pH determination

The pH of each Proniosome gel was determined using pH meter. The pH was determined in order to investigate the possibility of any side effects in-vivo due to acidic or alkaline pH which may irritate the skin. The pH was found in between 5.92 (P7) to 6.9 (P1) which was well within the physiological skin surface pH⁽¹⁹⁾ (Table 3). Changes in pH are reported to play an important role in the pathogenesis of skin diseases. Maintaining the skin pH helps maintain a proper balance of the “acid mantle” which aids in protecting the body from bacteria and helps prevent moisture loss⁽²⁰⁾.

In- vitro diffusion study

The in-vitro diffusion studies were conducted for all the 8 formulations prepared. All proniosome gels delivered the drug for a period of 360 mins. The proniosome gels prepared with different Spans as surfactants i.e., P1(S20), P2 (S40), P3 (S60) and P4 (S80) released 71.74%, 73.30%, 74.55% and 77.85% of drug respectively at the end of 360 mins. The proniosome gels prepared with different Tweens as surfactants i.e., P5 (T20), P6 (T40), P7 (T60) and P8 (T80) released 78.55%, 81.35%, 90.77% and 99.24% of drug at the end of 360 mins. The results are given in Fig. 3 and 4. It was evident from the results that proniosome gels prepared with Tweens gave a higher release than Spans. This is because Tweens are more hydrophilic, having higher HLB value, shorter alkyl chain length and low phase transition temperature than Spans. The drug being lipophilic favors more partition in the proniosome gel in case of spans and hence less drug is released from span formulations than tweens.

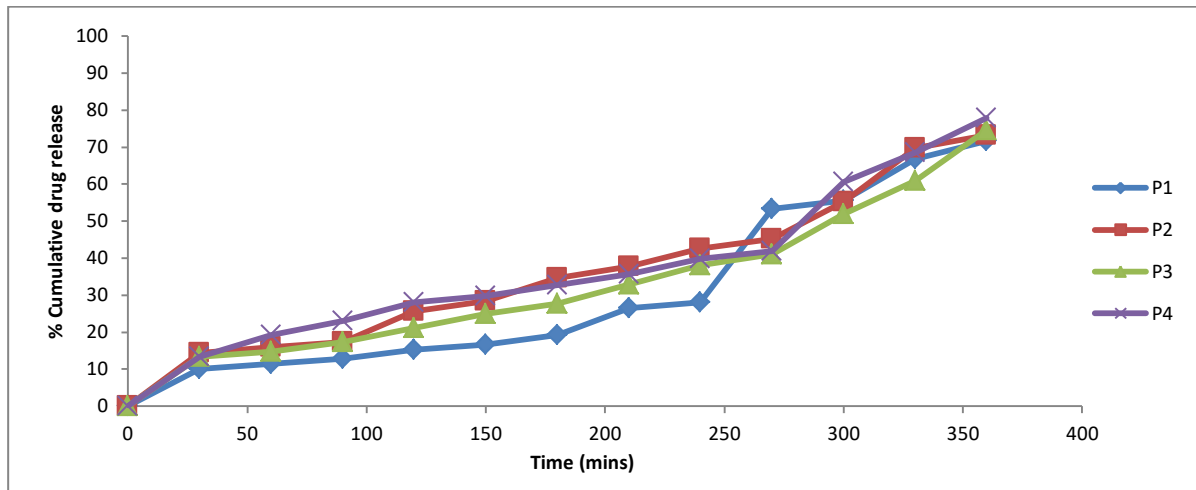


Fig. 3: In-vitro diffusion profiles of proniosome gel formulations (P1 to P4)

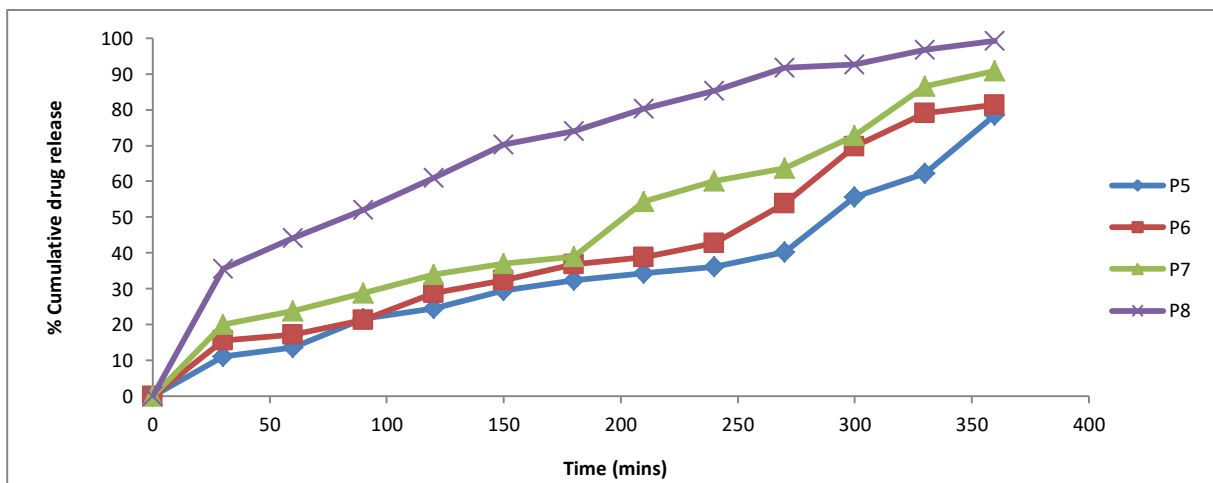


Fig. 4: In-vitro diffusion profiles of proniosome gel formulations (P5 to P8)

Fourier transformer infrared spectroscopy (FTIR) study

For establishing compatibility of drug and excipients IR spectra was used. It was evident from the values of IR spectra of pure drug and its formulations (P8) Tween 80 (Fig. 5 & 6) that there is much resemblance in the spectra of drug and its formulation and it is almost identical in nature without showing much shift in the positions of characteristic

absorption bands. This is possible only when drug remains in its normal form even after it is used for preparing formulations with carriers and other excipients. Since there is no appreciable change in the positions of characteristic absorption bands of different groups and bonds present in the drug molecule and its formulation it was concluded that drug has not reacted with the carriers and other excipients suggesting that there is no interaction of the drug.

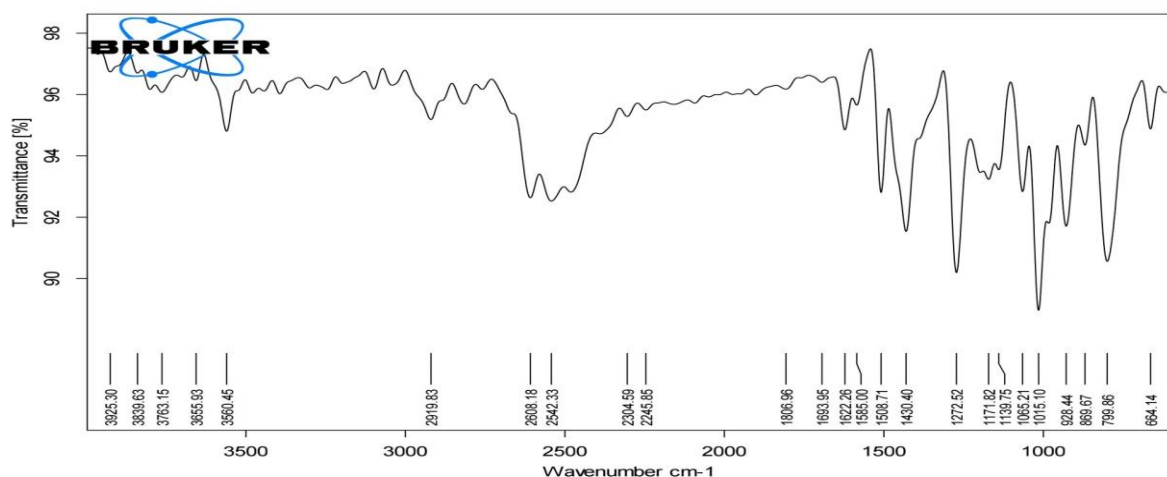


Fig. 5: FTIR spectra of pure drug Galantamine hydrobromide

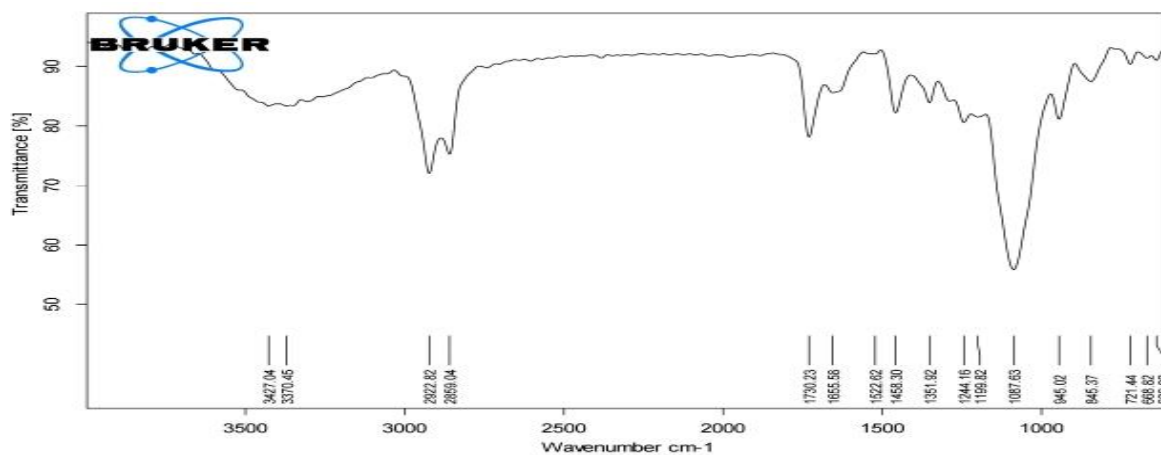


Fig. 6: FTIR spectra of optimized Galantamine hydrobromide proniosome gel- (P8)

CONCLUSION

In conclusion it can be stated that Galantamine hydrobromide was successfully developed in to proniosome gel for transdermal delivery. The adopted method i.e., coacervation-phase separation method yielded uniform and reproducible proniosome gel with the surfactants used. The surface morphology, vesicle size, rate of spontaneity, entrapment efficiency, and pH and in-vitro diffusion studies were uniform. Proniosome gel has the potential as an alternative dosage form in treating Alzheimer's disease. Further studies are needed to investigate their in vivo performance on suitable models.

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CONFLICT OF INTEREST

None declared.

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