Original Article

Development of Donepezil Hydrochloride Loaded Gellan Gum Based Nasal Mucoadhesive Microspheres by Spray Drying Method

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ABSTRACT

Objectives: The aim of present study was the formulation of donepezil hydrochloride loaded polymeric mucoadhesive microspheres for delivery via nasal route to increase the residence time and absorption of drug from the nasal mucosa. **Methodologies:** The microspheres were formulated by conventional spray drying technique by using gellan gum as a mucoadhesive polymer. A 3^2 full factorial design was utilized with polymer concentration (X_1) and liquid feed flow rate (X_2) as independent variables in formulation of the microspheres and their effect was studied on entrapment efficiency, particle size and drug loading. **Results:** The results specified that the X_1-X_2 interaction had significant effect on drug loading while X_2 alone effect on entrapment efficiency and particle size. The optimized microspheres were further evaluated for drug release kinetic study, histological examination, *ex vivo* permeation study and stability study. **Conclusion:** It was concluded that the gellan gum containing microspheres of donepezil hydrochloride with mucoadhesive property are suitable for nasal delivery.

Key words: Mucoadhesion, Intranasal, Microsphere, Donepezil hydrochloride, Spray drying.

INTRODUCTION

Donepezil hydrochloride is a centrally acting reversible acetylcholinesterase inhibitor. It is mainly used in the treatment of Alzheimer's disease where it increases cortical acetylcholine. Donepezil hydrochloride showed its therapeutic effect in schizophrenia, mild cognitive impairment and attention deficit disorder. Nasal drug delivery protects the therapeutic drug from gastrointestinal environment, hepatic presystemic metabolism or enzymatic degradation. Therefore, nasal drug delivery has been viewed as the suitable route to deliver the drugs that are susceptible to acidic/basic pH or enzymatic hydrolysis.[1-3] In addition, it also provides several advantages over the other conventional route of administration. It is non-invasive, rapid and comfortable mode of drug administration, it also bypasses the blood brain barrier (BBB) and targets the central nervous system (CNS) and thus reduces systemic side effects.[4,5]

Spray drying is a mostly used technique to prepare solid particles, dry powders, granules and microparticles etc. It is a rapid single-step technique of drying with high reproducibility of production of a spherical particle in the micro size range.

The main aim of this study involves development of a mucoadhesive microsphere formulation of donepezil hydrochloride by spray drying method.

MATERIALS AND METHODS

Materials

Donepezil hydrochloride was procured as a gift sample from Cipla, Mumbai. Gellan gum was also obtained from Cipla, Mumbai. All other chemicals, solvents and additives used were of analytical grade.

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Microspheres preparation by spray drying method

The formulations of donepezil hydrochloride were prepared using gellan gum in three different ratios (Table 1). The feed solutions in definite proportion were formed by dissolving the polymer in distilled water with gentle heating and mixing on a magnetic stirrer to augment the dissolution and the drug was dissolved in this polymeric solution. The microspheres were developed by spraying the feed with a spray dryer (LU222, Labultima, India). The feed solution was atomized under 0.7 mm nozzle size and inlet air temperature was set at 120°C. The outlet temperature was ranged between 80 to 90°C. The feed rate was 3-7 ml/min, atomizing air flow rate and aspiration rate was kept at 357 L/h and 45% respectively.[6,7]

Experimental Design

Response Surface Methodology (RSM) was used to recognize the combined effect of two or more factors and also to designate whether or not interaction occurs between the factors and thereby influence the magnitude of the response. The data were interpreted by means of response surface methodology (Design Expert Software Version 11.0.1.0, Stat-Ease, Inc.).

Validation was performed on the basis of results obtained for drug loading, entrapment efficiency and particle size for microspheres of donepezil hydrochloride using Design Expert Software (Version 11.0.1.0, Stat-Ease, Inc.). Overall desirability value is an indicator of the optimum formulation as it is calculated from the individual values which in turn and the same are calculated based on the desired target response of independent variables.[8]

Drug –polymer interactions studies

A weighed quantity of the donepezil hydrochloride *i.e.* 1 mg was mixed methodically with potassium bromide (which was previously dried at 40°-50°C) and compressed under 10-ton pressure using the hydraulic press to form a pellet. This pellet was then scanned from 4000–400 cm^{-1} using FTIR spectrophotometer.[9-11] FTIR absorption spectra of pure drugs, all the polymers used and the combination of drugs and polymers were taken to corroborate the identity for both the drugs and to detect the interaction of the drug with the excipients.

Characterization of mucoadhesive microsphere

Production yield

The production yield of microspheres was calculated from final product weight after drying with respect to the initial total weight of the drug and polymer. The following formula was used to calculate the production yield.[12]

$$\% \text{ Production Yield} = \frac{\text{Practical Mass (Microspheres)}}{\text{Theoretical Mass (Polymer+Drug)}} \times 100 \quad (1)$$

Drug loading

The total quantity of the drug within the microspheres was determined by addition of weighed quantity of microspheres in distilled water and kept overnight. Then solution was filtered through microfilter paper and quantified using U.V Spectrophotometer (UV, Shimadzu, 1800) to determine the drug loading (DL) using the following equation.[13]

$$\text{Drug Loading } \% = \frac{\text{wt. of drug loaded in Microspheres}}{\text{Total weight of Microspheres}} \times 100 \quad (2)$$

Particle size analysis

The particle sizes and morphology were verified in a Motic, B1 series microscope. The sample of microspheres was taken on a microscope slide. A microscopical field was scanned by a video camera. The average particle diameter (particle size) was determined by using the following equation.[14]

$$\text{Average diameter} = \frac{\Sigma nd}{\Sigma n} \quad (3)$$

Where n = number of microspheres observed, d =Mean size range.

Zeta potential

The surface charge of the microspheres was determined with Zetasizer Nano ZS, Malvern instruments. The measurements were carried out in an aqueous solution of KCl 0.1 N. Immediately before the determinations microspheres were diluted with KCl solution. The measured values were corrected to a standard reference

Table 1: Formulation batches (Donepezil HCl: Gellan Gum).

Batch code (Drug: Polymer)	Feed rate (ml/min)	Donepezil HCl (mg)	Gellan gum (mg)
G1 (1:2)	3	200	400
G2 (1:2)	5	200	400
G3 (1:2)	7	200	400
G4 (1:3)	3	200	600
G5 (1:3)	5	200	600
G6 (1:3)	7	200	600
G7 (1:4)	3	200	800
G8 (1:4)	5	200	800
G9 (1:4)	7	200	800

for temperature of 20°C. The results are the means of triplicate experiments.

Entrapment efficiency

A weighed quantity of microspheres of donepezil hydrochloride was separately dissolved in distilled water and kept overnight, then vortexed for 1 min to extract the entrapped drug. The solution was then filtered through microfilter paper and quantified using a UV spectrophotometer at 231 nm.[15]

$$\text{Entrapment efficiency \%} = \frac{\text{Practical drug content}}{\text{Theoretical drug content}} \text{ X } 100 \quad (4)$$

Swelling Property

The experiment was performed by dispersing microsphere formulations (10 mg) on Millipore filter (0.22 µm) placed on a Franz diffusion cell (16 ml capacity) filled with buffer solution and kept for 3.5 min to ensure complete equilibrium of swelling. The degree of swelling was determined as per the following equation.[16]

$$\alpha = \frac{\text{Ws-Wo}}{\text{Wo}} \text{ x } 100 \quad (5)$$

Where Wo is the weight of microspheres before swelling, α is a degree of swelling and Ws is the weight of microspheres after swelling.

Scanning Electron Microscopy (SEM)

The surface morphology of microspheres was identified with the help of scanning electron microscopy (SEM, JSM 6390, JEOL DATUM Ltd., Tokyo, Japan).[17]

In vitro mucoadhesion study

A freshly cut portion of sheep nasal mucosa (2 cm^2) was obtained and cleaned by washing with an isotonic saline solution. Mucosal surface was fixed over polyethylene support at an angle of 45° with respect to the horizontal plane and measured quantity of microspheres was placed on it. The mucosa was washed with pre-warmed (37°C) phosphate buffer at pH 6.6 at the rate of 5 ml/min. The amount of drug in collected solution was determined spectrophotometrically after one hour of the administration of microspheres. The quantity of microsphere corresponding to the drug quantity in solution was determined. The disparity between the quantity of microspheres and the applied microspheres present in perfusate was estimated from quantity of adhered microspheres to mucosa.[18]

$$\textit{In vitro} \text{ mucoadhesion (\%)} = \frac{\text{Amount of drug in washout liquid}}{\text{Actual amount of drug in applied microspheres}} \text{ x } 100 \quad (6)$$

In vitro drug diffusion studies

An *in vitro* drug release test of the microspheres was executed using Franz diffusion cells with a dialysis membrane (cut-off Mol. Weight. 12000). The receptor compartment was loaded with phosphate buffer solution of pH 6.6 and microspheres were placed in donor compartment. The donor chamber was adjusted in such a manner that it just touched the diffusion medium in the receptor chamber. The temperature was constantly upholded at 37 ± 0.5°C with the help of a circulating water bath. The microspheres equivalent to 15 mg of drug was dispersed on donor compartment and 3 ml of simulated nasal fluid was added on it. From the receptor compartment, samples were withdrawn periodically and restored with the identical quantity of unsullied pre-warmed buffer solution and assayed using a UV spectrophotometer (UV 1800, Shimadzu, Japan).[19]

Differential scanning colorimetry

Differential scanning colorimetry (DSC) was performed using Mettler Toledo equipment. This technique determines the thermal change events of given microspheres by measuring the enthalpy, onset of the thermal event, or by determining the melting point of the crystalline material and glass transition temperature of the amorphous material. A weight between 3.5 to 4 mg of sample was placed into an aluminum pan (40 µl) and the pan was sealed using the sealing press apparatus. The samples were heated at a rate of 10°C/min and the scanning was performed between 35-300°C.[20]

X-Ray Diffraction study

The physical states of donepezil hydrochloride microspheres were found out by X-ray diffractometry (Brucker AXS D8 Advance). X-ray diffractograms of pure drug, blank microspheres and drug loaded microspheres were observed. The powder samples were spread on metal sample holders; and glass slide was used to press and smooth the powder surfaces. The diffraction intensity was recorded at 2-theta and the run time was 20 min. The current and voltage generator was set at 35 mA and 40 kV, respectively.[21,22]

Ex vivo permeation study

Franz diffusion cell was employed to study optimized formulation for drug permeation through the nasal mucosa. The sheep nasal mucosa was fixed between the receptor and donor compartment. In a donor compartment weighed quantities of microspheres were placed and 3 ml of SNF was added to it. Phosphate buffer solution (pH 6.6) was filled in receptor compartment and temperature was upholded at

37±0.5°C. Samples were withdrawn periodically for 4 hr from the receptor compartment and replaced with the same quantity of fresh pre-warmed buffer solution and assayed by means of a UV spectrophotometer (UV 1800, Shimadzu, Japan).[23,24]

Drug release kinetics

The drug release data was analyzed with the following mathematical models and interaction of diffusion release mechanism to investigate the drug release mechanism from microspheres.

Zero order kinetics $Q = Q_o - K_o t$ (7)

First order kinetics $Q = Q_o (1 - e^{-K_1 t})$ (8)

Higuchi square root model $Qt = K_H t^{1/2}$ (9)

Hixson-Crowell cube root model $\sqrt[3]{Qo} - \sqrt[3]{Qt} = K_{HC} t$ (10)

Korsmeyer- peppas model $Qt/ Q_\infty = K_k t^n$ (11)

Where, Qt = amount of drug release d at time t.

Qo = initial amount of drug.

And KH, K_1, K_{HC}, Ko and K_K are the coefficients of equations. The most appropriate model was selected on the basis of regression values (r^2) and diffusion release exponent (n). Where the value n is the release exponent characterizes the release mechanism of the drug. The release exponent n = 0.45 indicates a Fickian diffusion mechanism, 0.45 < n < 0.89 to non- Fickian transport, n = 0.89 to case II (relaxational) transport and n > 0.89 to super case II transport. Drug release kinetics and best fit model for all the selected batches was found out with the help of Microsoft Excel.[25-27]

Stability Studies

The optimized batch was also subjected to stability studies for six months. The vials filled with microspheres were sealed with rubber caps and kept at moisture conditions 40°C and 75% RH for duration of 6 months in a stability chamber (CHM-10S, Remi Instruments, Mumbai, India). The samples were tested for particle size (μm), swelling % and mucoadhesion potential % at the 1-month interval, as per methods mentioned earlier.[28]

Histological study

The freshly isolated nasal mucosa was taken immediately after sacrificing a healthy sheep from the local slaughter house. The nasal mucosa was sectioned into pieces and washed with a normal saline solution and treated for 8 hr with drug loaded-microspheres. Untreated piece of sheep nasal mucosa was kept as a control. Samples were taken out and washed with sodium chloride solution (0.9%) and the tissue was fixed in 10% buffered formalin and embedded in paraffin wax for 4 hr. Paraffin sections 7-5 mm were cut onto glass slides and stained with hematoxylin and eosin. Sections were inspected by light microscopy to detect any damage during incubation.[29]

Statistical analysis

All experiments were carried out in a triplicate manner. The obtained data were expressed as mean ± standard deviation (SD). For statistical calculations, Minitab® ver. 17 was employed. For pharmacological activities, the unpaired Student t-test (two-tailed) was used to determine the difference between control and tested groups.

RESULTS AND DISCUSSION

Response Surface Analysis

The quadratic model obtained from the regression analysis was employed to build a 3-D graph in which the responses were signified by curvature surface as a function of independent variables. Three dimensional (3-D) surface plots (Figure 4) were drawn based on the model polynomial functions to assess the change of the response surface. The relationship between the response and independent variables can be expressed visualized from the response surface plots are presented in Figure 5. These plots explain the relationship between the independent variables and dependent variables; i.e. the effects of two factors on the response at one time. The response surface analysis for polymer concentration (X_1) and feed rate (X_2) was studied which showed significant results (Figure 6). The model f-value of drug loading (213.71), entrapment efficiency (108.65) and particle size (40.22) for polymer concentration (X_1) and feed rate (X_2) implies the model is significant. Values of "P" less than 0.0500 indicate model terms are resignificant. The "Pred R-Squared" of 0.997 for drug loading, 0.995 entrapment efficiencies for and 0.990 for particle size is in reasonable agreement with the "Adj R-Squared" of 0.973 for drug loading, 0.999 entrapment efficiencies and 0.998 for particle size. The probability value; i.e. also P-value was found less than 0.0500. This model can be used to develop the design (Table 2).

Final equation in terms of coded factors for particle size, drug loading and entrapment efficiency conveyed that R^2 was high pointing out towards the sufficient fitting of the Quadratic Model. As all the three factors showed (+ coefficient) it indicated the optimum entrapment efficiency, drug loading and particle size. Based on the obtained results from various evaluation parameters and surface response analysis batch G1 was shown significant results and was observed to be optimized.

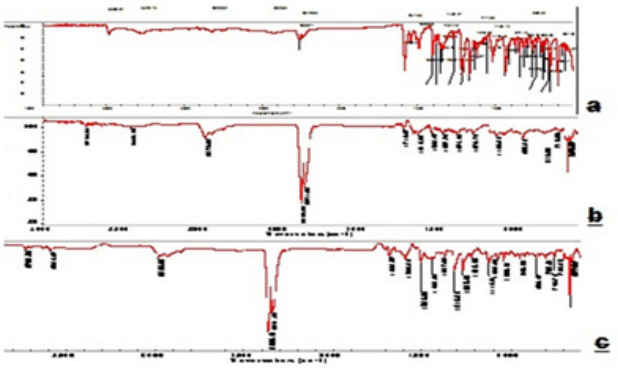

Figure 1: Infrared spectroscopy of a) donepezil hydrochloride b) gellan gum and c) physical mixture.

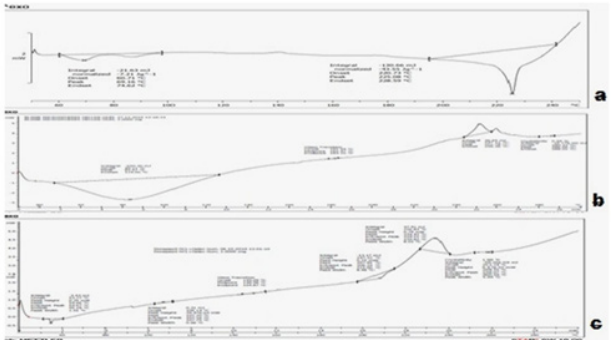

Figure 2: DSC of a) donepezil hydrochloride b) gellan gum and c) drug loaded microspheres.

Table 2: ANOVA for surface responses.

Responses	Source	Sum of Squares	Df	Mean Square	F-value	*p*-value
Drug loading	Model	213.71	5	42.74	267.58	<0.0004
	A-Polymer concentration	153.19	1	153.19	959.02	< 0.0001
	B-Feed Rate	3.38	1	3.38	21.13	0.0194
	AB	0.6724	1	0.6724	4.21	0.1325
	A^2	3.23	1	3.23	20.19	0.0206
	B^2	0.0000	1	0.0000	0.0000	1.0000
	Residual	0.4792	3	0.1597		
	Cor Total	214.19	8			
Entrapment efficiency	Model	108.65	5	21.73	59.43	<0.0034
	A-Polymer concentration	7.48	1	7.48	20.47	<0.0202
	B-Feed rate	8.45	1	8.45	23.11	<0.0171
	AB	1.70	1	1.70	4.66	<0.1198
	A^2	30.94	1	30.94	84.62	<0.0027
	B^2	1.99	1	1.99	5.45	<0.1017
	Residual	1.10	3	0.3657		
	Cor Total	109.75	8			
Particle size	Model	40.22	5	8.04	549.90	<0.0001
	A-Polymer concentration	12.88	1	12.88	880.33	<0.0001
	B-Feed rate	3.39	1	3.39	231.74	<0.0006
	AB	0.1640	1	0.1640	11.21	<0.0441
	A^2	0.3990	1	0.3990	27.28	<0.0137
	B^2	0.4544	1	0.4544	31.06	<0.0114
	Residual	0.0439	3	0.0146		
	Cor Total	40.27	8			

Drug –polymer interactions studies

FTIR spectra of donepezil hydrochloride, gellan gum and their physical mixture were compared. It was observed that there was no functional group inter-conversion when donepezil hydrochloride reacts with the excipients (gellan gum). Donepezil was observed to be compatible with the excipients (Figure 1). The subsequent bands were detected in the FTIR spectrum of physical mixture; *i.e.,* C=O (1680 cm^{-1}), C-N (1264 cm^{-1}), C-O (1036 cm^{-1}), C-H bending aromatic (868 cm^{-1}), C=C Aromatic (1588 cm^{-1}), C-H stretching in CH_3 (2920 cm^{-1}).

Characterization of mucoadhesive microspheres

Production yield

The yield of the microspheres varied from 11.8 - 23.75 %. Increasing the rate of the feed from 3 to 7 ml/min

resulted in decreased production yield. Such tendency was observed with all three concentrations of gellan gum. When increasing the concentrations of polymer from 1:2 to 1:3 and 1:4 at low pump rate of 3 ml/min; an increase in the yield was observed. However, such a tendency was not observed at the feed rate of 5 ml/min and high 7 ml/min. The highest production yield (23.75 %) was obtained at low feed rate from 3 ml/min gellan gum (G1 Batch) (Table 3).

Drug Loading

The drug loading was observed to be 20.39 - 35.28 %. The drug loading goes on lessening as the polymer concentration augments from G1 batch to G9 batch. As the feed rate was increased from 3 - 7 ml/min, the drug loading decreased (Table 3).

Particle Size Analysis

The mean particle size of microspheres ranges from 14.36 µm to 21.63 µm, which is ideal for intranasal absorption (Table 3). The lower concentrations of gellan gum (1:2) causes clumping of microspheres, whereas the high concentration of gellan gum (1:3 and 1:4) resulted in formation of disjoined microspheres with a mean particle size >21.63 µm. This could be accredited to an augment in the relative viscosity at higher polymer concentration and formation of larger particles. Hence, an optimum gellan gum concentration of 1:2 was chosen.

Zeta potential

Microparticle formulations were characterized also in term of zeta potential because, as well known, it showed positive values in range of 10.4-14.2 which concluded that electrostatic repulsion between particles with the same electric charge prevents the aggregation of the spheres.

Entrapment Efficiency

The entrapment efficiency ranged from 75-86.48 %. Total drug content of microsphere was estimated by spectrophotometric method. The consequence of polymer concentration on drug entrapment efficiency was studied. No drastic effect was found by increasing the polymer concentration on drug entrapment. In batch G1, the drug entrapment was observed to be highest and having the polymer concentration 1:2 (Table 3).

Swelling property

The degree of swelling of all the formulations has been shown in (Table 3). Gellan gum has been reported to show good swelling properties. It was shown that with an augment in the quantity of gellan gum, the degree of swelling also increases in the range of 0.42±0.03 to 0.66±0.15. It is suggested that when the microspheres are in contact with mucus layer, they absorbs the liquid from the mucus layer and swell rapidly, which results in the prolongation of residence time of microspheres in nasal cavity.

In vitro mucoadhesion study

All formulations were tested for *in vitro* mucoadhesion studies. It was found that with increasing amount of gellan gum from batch G1 to G9, the mucoadhesive strength of microparticles was increased. The results cleared that the microparticles remain adhered for a prolonged period. The mucoadhesion was in the range of 80.30 –94.43 % (Table 3).

ANOVA of Particle Size, Drug Loading and Entrapment Efficiency

Evaluation and interpretation of research findings are important and the *P*-value serves a valuable purpose in these findings. ANOVA for the dependent variables polymer concentration (X_1) and feed rate (X_2) was carried out. The coefficients of X_1 and X_2 were found

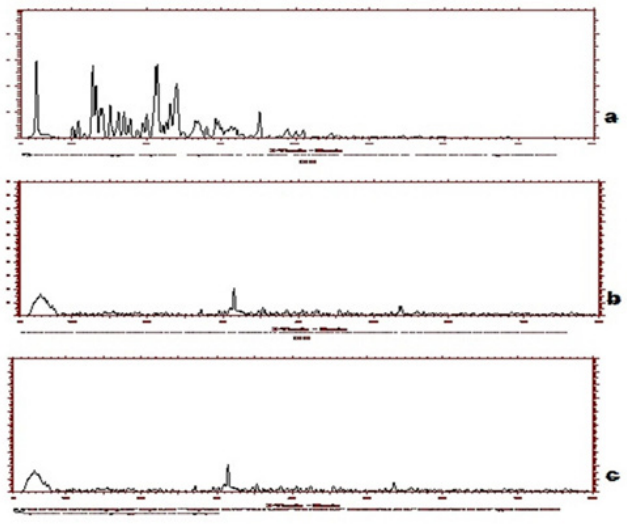

Figure 3: X-Ray diffractogram of a) donepezil hydrochloride b) gellan gum and c) drug loaded microspheres.

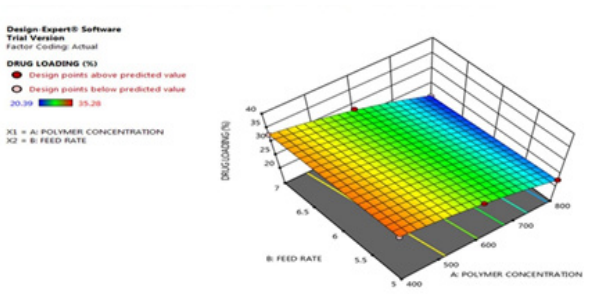

Figure 4: Response 1: (R1) Drug loading 3D graph.

to be considerable at $p<0.05$, hence it confirmed that both the variables have a noteworthy effect on the selected responses. Overall both the variables caused a significant alteration in the responses.

Validation of results of drug loading, entrapment efficiency and particle size

Overall desirability value is an indicator of the optimum formulation as it is calculated from the individual values which in turn and the same are calculated based on the desired target response. From Figure 7 for drug loading, entrapment efficiency and particle size; it is clear that the result of drug loading, entrapment efficiency and particle size which was obtained from formulation has optimum concentration of polymers. (Design expert software version 11.0.1.0, Stat-Ease, Inc). The desirability found was 1 and hence it can also be concluded that the acquired results matches with the software prediction and hence the formulation is also validated.

Differential Scanning Calorimetry

The thermogram of the pure drug showed an endothermic peak at 225.8°C. The drug loaded microspheres shows a peak at 226.83°C. Thus it was concluded that there was no major interaction between drug and drug loaded microspheres (Figure 2).

X-Ray Diffraction (XRD) Study

The diffractogram for the pure drug, blank microspheres and drug-loaded microspheres were determined (Figure 3). The diffraction spectrum of pure donepezil hydrochloride showed that the drug is crystalline in nature as demonstrated by numerous distinctive peaks. The prominent drug peak of high intensity at a 2θ value of 21.251 and peaks of lower intensities at 2θ values of 12.656, 18.594, 23.951 and 35.186 are in agreement with x-ray data found for donepezil hydrochloride. Diffraction pattern of blank gellan gum microspheres show no characteristic peaks indicating that it is amorphous in nature. The XRD profile of the drug loaded microspheres (Figure 3) has shown lower intensity peak demonstrated the amorphous peaks for donepezil HCl corresponding to 2 θ value of 21.251 and peaks of lower intensities of 2 θ values at 12.656, 18.594 and 23.951. This data lead to the fact that the drug turned into amorphous form in microspheres but there was no change in lower intensity peaks which means that drug and polymer are compatible.

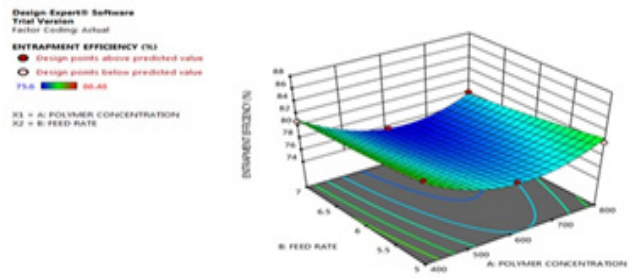

Figure 5: Response 2: (R2) Entrapment efficiency 3D graph.

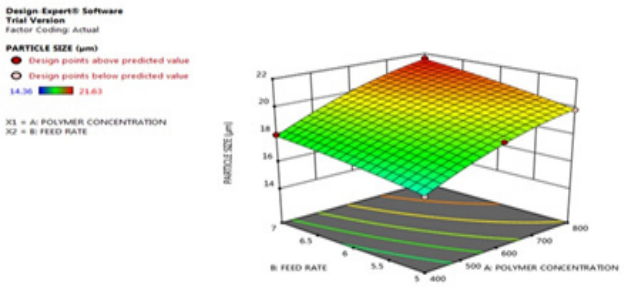

Figure 6: Response 3: (R3) Particle size 3D graph.

Table 3: Table showing results of average particle size, % production yield, entrapment efficiency (%), % drug loading, mucoadhesion and swelling index.

Sr. No.	Formulation Code	Average Particle Size* (Mm±SD)	% Production Yield * (±SD)	Entrapment Efficiency (%)* (±SD)	% Drug Loading * (±SD)	Mucoadhesion* (%±SD)	Swelling Index* (% ± SD)
1	G1	14.36 ± 0.25	23.75± 0.23	86.48± 2.13	35.28± 2.50	80.30 ± 2.10	0.42±0.03
2	G2	16.57± 0.15	19.83± 0.45	83.24± 1.56	33.85± 1.10	85.85 ± 0.90	0.46±0.06
3	G3	18.00± 0.51	18.16± 0.39	80.87± 1.29	32.71± 1.50	83.31 ± 1.10	0.51±0.10
4	G4	16.27± 0.27	22.50± 0.24	82.00± 1.25	30.57± 1.25	89.64 ± 2.46	0.54±0.13
5	G5	18.79± 0.82	18.50± 0.21	78.24± 1.63	29.57± 0.82	90.21 ± 0.79	0.62±0.12
6	G6	20.06± 0.22	15.58± 0.12	75.60± 1.72	28.35± 1.22	90.11 ± 1.69	0.56±0.09
7	G7	17.18± 0.03	22.50± 0.15	86.34± 1.28	24.60± 0.03	92.88 ± 2.09	0.63±0.07
8	G8	19.82± 0.42	18.40± 0.19	80.23± 1.45	22.53± 1.45	91.54 ± 1.69	0.54±0.15
9	G9	21.63± 0.40	11.80± 0.31	78.12± 1.40	20.39± 0.45	94.43 ± 1.83	0.66±0.15

*Values expressed as Mean ± SD, $n=3$

Table 4: Drug release kinetics for best fit model for batch G1.

Formulation	Kinetic Models (R^2)			
G-1	Zero order	First order	Higuchi	Best fit model
	0.988	0.91	0.975	Zero order
	Korsmeyer Peppas equation			
G-1	R^2	K value	n' value	Mechanism
	0.973	1.765	0.737	Case II diffusion

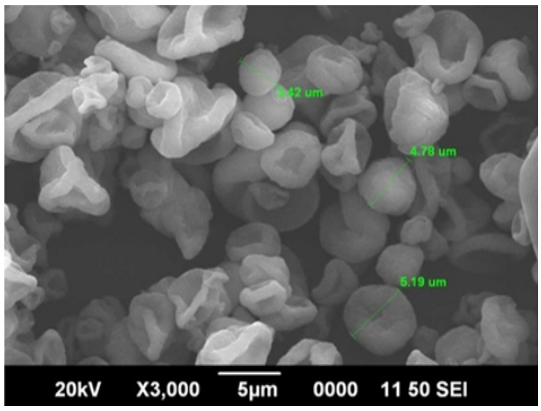

Figure 8: SEM microphotograph of formulation G1.

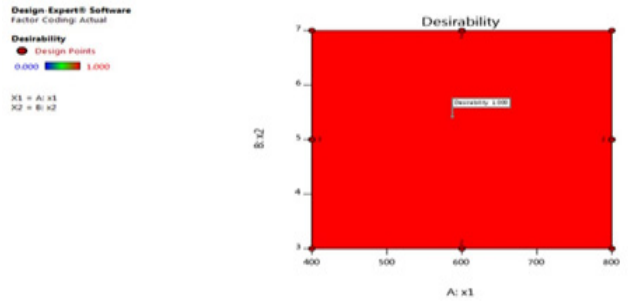

Figure 7: Desirability for all the three responses.

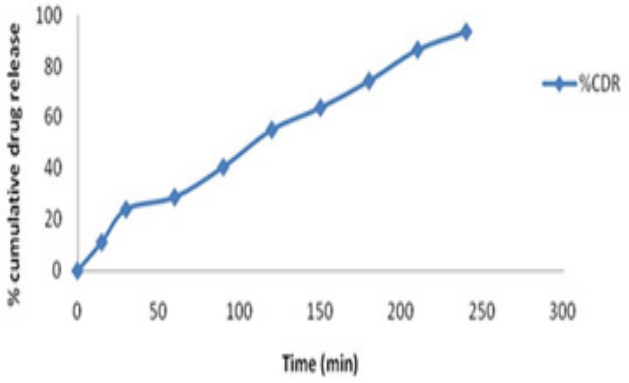

Figure 9: *In-vitro* drug release study of donepezil hydrochloride loaded microspheres.

Scanning Electron Microscopy (SEM)

SEM picture of optimized formulation (G1) is shown in (Figure 8). The microspheres have spherical morphology and smooth surface in shape. The obtained microspheres had no fractures on the surface, which would result in good deposition and cleared slowly from nasal cavity.

In vitro drug diffusion studies

Franz diffusion cell was employed for *in vitro* release study of donepezil hydrochloride microspheres with a dialysis membrane (cut-off Mol. Weight. 12000). On the basis of results of entrapment efficiency, particle size and drug loading batch G1 was only taken for *in-vitro* drug diffusion study. The release profile of donepezil hydrochloride from G1 batch of microsphere at pH 6.6 of phosphate buffer is shown in (Figure 9). Batch G1 showed zero order drug release and thus considered it as an optimized formulation. The release pattern of the optimized formulation (G1) revealed that drug released from microspheres showing consistent release with zero order, which may be attributed to the lower viscosity of gellan gum.

Drug release kinetics

The data obtained from drug release were fitted to a variety of kinetic models, to explore the kinetics of drug release. It was observed that the *in vitro* drug release data was fitted best to zero-order kinetic model, as indicated by highest linearity coefficient (R^2 0.988) when compared to first-order and Higuchi's model. The n-value indicates that the optimized formulation followed the non-Fickian or anomalous diffusion mechanism of drug release, which indicates the linear, time-dependent (zero order) drug release controlled by diffusion of drug through swollen polymer matrix into the external releasing medium (Table 4).

Ex vivo permeation study

The permeation study was performed in Franz diffusion cells, using the sheep nasal mucosa. The percentages of cumulative permeation of the donepezil hydrochloride incorporated into formulation were plotted against time in (Figure 10). The results obtained revealed more tissue permeation of microsphere. More permeation may be because of the small size of the microsphere. The permeation was found to be 84.92 % at 240 min.

Histological study

The histological alterations in nasal mucosa caused by formulations have been examined in order to make

Table 5: Stability parameters for donepezil hydrochloride loaded optimized formulation				
(G1).	Time (Days)	Particle Size** (µm)	Swelling* (%)	Mucoadhesion Potential* (%)
1	0	14.36±0.44	0.42±0.03	80.30 ± 2.50
2	30	14.34±0.35	0.42±0.03	80.10 ± 0.05
3	60	14.34±0.86	0.42±0.03	80.20 ± 2.01
4	90	14.34±0.64	0.42±0.04	80.20 ± 2.01
5	180	14.34±0.23	0.42±0.04	80.20 ± 2.01

*Values expressed as Mean ± SD, *n*=3, **n*=100

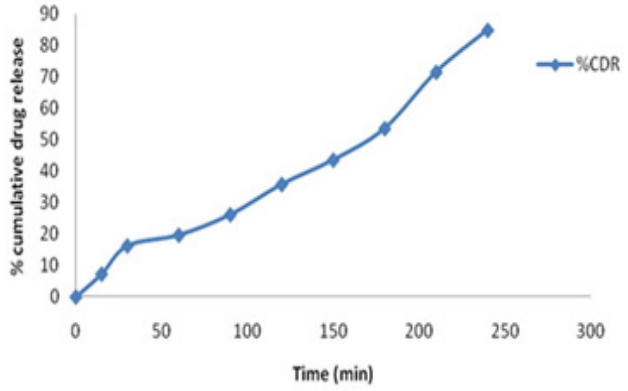

Figure 10: *Ex-vivo* drug release study of donepezil hydrochloride loaded microspheres (G1).

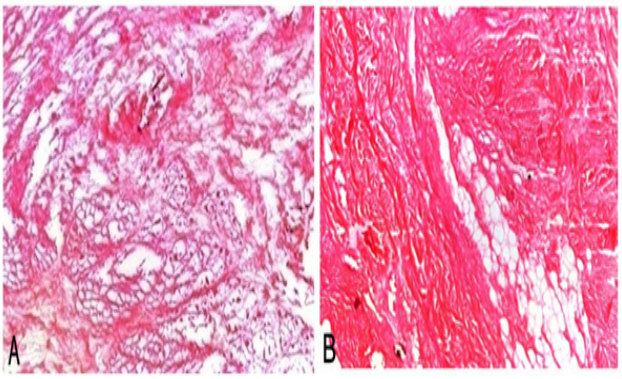

Figure 11: Histology evaluations of sections of untreated sheep nasal mucosa (A) and microspheres treated sheep nasal mucosa.

it for practical use. Untreated nasal mucosa and the microspheres treated nasal mucosa has been shown in (Figure 11 a and b). The microsphere treated mucosa did not show any deleterious response and adverse effect on the nasal mucosa.

Accelerated Stability Study

Formulation (G1) showing optimum particle size, swelling property and mucoadhesion were subjected further to the accelerated stability studies at 40°C ± 2°C/75% ± 5% RH for a period of 6 months. Estimation at each month intervals for three months and last estimation after six months revealed that the changes in particle size, swelling property and mucoadhesion were negligible to conclude that formulation remained stable right through the stability period as given in (Table 5).

CONCLUSION

From this study it was concluded that spray drying is a suitable method for the formulation of donepezil hydrochloride loaded gellan gum based mucoadhesive microspheres for intra nasal delivery. By selecting and controlling the processing parameters it is feasible to produce microspheres with desired characteristics such as entrapment efficiency, suitable particle size and drug loading. This study concludes that the microspheres based on gellan gum to be considered as an optimistic nasal delivery system for the administration of donepezil hydrochloride. This interesting formulation deserves further characterization with reference to *in vivo* studies in the future.

CONFLICT OF INTEREST

No conflict of interest is declared regarding publication of this manuscript.

ABBREVIATIONS

SNF: Simulated Nasal Fluid; **BBB:** Blood brain barrier; **CNS:** Central nervous system; **XRD:** X-Ray Diffraction; **DSC:** Differential Scanning Calorimetry; **SEM:** Scanning Electron Microscopy.

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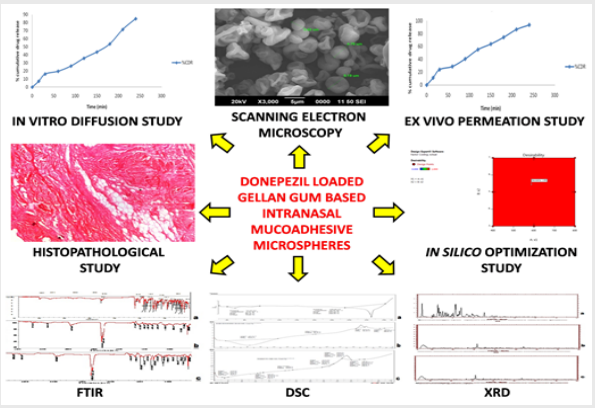
PICTORIAL ABSTRACT



SUMMARY

- Formulation of donepezil hydrochloride loaded polymeric mucoadhesive microspheres for delivery via nasal route to enhance the residence time and absorption of drug through the nasal mucosa.
- 3^2 full factorial design was employed with polymer concentration (X_1) and liquid feed flow rate (X_2) as independent variables in formulating the microspheres and their effect was studied on entrapment efficiency, particle size and drug loading.
- The optimal microspheres were evaluated for drug release kinetic study, *ex vivo* permeation study, histological examination and stability study.

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