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Original Article

# Spray Dried Mucoadhesive Microparticles of Donepezil with Chitosan and Carbopol in Alzheimer's Disease

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## ABSTRACT

**Background:** The traditional oral formulation for Alzheimer's disease treatment has the drawbacks of first-pass metabolism, plasma protein binding, and poor blood-brain barrier penetration. This study was conducted to establish the nasal route of administration for donepezil formulations in Alzheimer's disease. **Materials and Methods:** Donepezil mucoadhesive microparticles synthesized by spray-drying and evaluated for Infrared spectroscopy, differential scanning calorimetry, and scanning electron microscopy. The *ex vivo* study was done with Franz's diffusion cell using goat nasal mucosa. The *in vivo* study was performed on the Albino rat's nasal route for determining drug concentration by HPLC analysis in brain tissue at single-point evaluation. **Results:** The microparticles were with optimum size with no drug-polymer interaction in Infrared spectroscopy and Differential scanning calorimetry. Scanning electron microscopy exhibited morphology of spherical or ellipsoid microparticles with efficient drug entrapment. The percentage drug release for chitosan microparticles was 66.57 to 85.74 and for carbopol microparticles was 69.54 to 91.53 in the *ex vivo* permeability study. *In vivo* studies showed that drug concentrations of 110.48% to 114.92% for chitosan batches and 111.87% to 142.08% for carbopol batches were superior to controls. **Conclusion:** *Ex vivo* permeability study revealed drug release patterns with as high as 85.74% ±0.02 for DCH2 formulation and 91.53±0.3% for DC3 formulation. In *in vivo* study formulation DCH2 displayed drug concentration 110.87±6.87% and DC3 shown 129.51±9.82% over the control batch which is conclusive for improved drug delivery of donepezil through mucoadhesive microparticles for the nose to brain targeting in Alzheimer's disease.

**Keywords:** Microparticles, Nose to brain drug delivery, Spray-drying, Mucoadhesive, Alzheimer's Disease.

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## INTRODUCTION

Alzheimer's disease is the utmost common form of dementia and probably contributes to 60 to70% of cases.[1] Alzheimer's disease is considered a progressive decay in cognitive function. Alzheimer's disease is considerably amplified among people aged 65 years or more, with a progressive decline in memory, thinking, language, and learning capacity.[2] The conventional oral formulation has the disadvantage of first-pass metabolism, plasma protein binding, and limited penetration through the blood-brain barrier Figure 1.

Various routes of administration have been tried for CNS disorders but the major hurdle is the passing blood-brain barrier (BBB). We have selected the nasal route of administration through the olfactory pathway as it can penetrate drugs in the brain as no blood-brain barrier is involved.[3] Donepezil is prominently in the treatment of Alzheimer's disease. Donepezil selectively and reversibly inhibits the acetylcholinesterase enzyme, which normally breaks down acetylcholine which relieves the symptoms of Alzheimer's disease.[4] Donepezil is having 91% of plasma protein binding which make very few free drugs available for penetration through BBB, hence limited drug reaches the site of action.

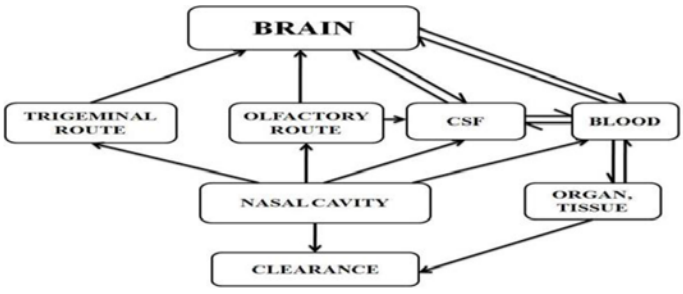


**Figure 1:** Drug distribution through various routes in the brain.

The advantage of establishing a nasal route over other routes is to avoid first-pass metabolism and also it is noninvasive compared to oral and intravenous routes respectively.[5] In this research work, we have formulated spray-dried microparticles of donepezil using mucoadhesive polymers chitosan and Carbopol 934.[6] The prepared formulations were evaluated for preliminary properties as well as some specific parameters to establish the nasal route of administration and advantage of formulation over other conventional formulations.

In comparison to conventional oral and other formulations, donepezil nasal mucoadhesive microparticles will be a promising dosage form because it provides advantages such as avoiding first-pass metabolism, no issue with plasma protein binding, and importantly, penetration into the brain via the olfactory route, which avoids the BBB.

## MATERIALS AND METHODS

### Chemicals and Reagents

Donepezil hydrochloride was purchased from Lab India Ltd, Chitosan was procured from India sea Foods, Cochin, Carbopol 934 obtained from Lab India Ltd, Ethanol purchased from Loba Chemie Pvt ltd, HPLC

**Table 1: Formulation Design.**

| Polymer | Chitosan | | | | Carbopol 934 | | | |
|---|---|---|---|---|---|---|---|---|
| Drug | Donepezil | | | | Donepezil | | | |
| Formulation code | DCH1 | DCH2 | DCH3 | DCH4 | DC1 | DC2 | DC3 | DC4 |
| Drug-Polymer ratio | 0.5:1 | 1:1 | 1:1.5 | 1:0.5 | 0.5:1 | 1:1 | 1:1.5 | 1:0.5 |
| Drug (mg) | 500 | 1000 | 1000 | 1000 | 500 | 1000 | 1000 | 1000 |
| Polymer (mg) | 1000 | 1000 | 1500 | 500 | 1000 | 1000 | 1500 | 500 |

grade methanol purchased from Merck Pvt ltd Mumbai. The remaining reagents used were of analytical grade.

## Formulations

Formations were created using the following ratios of donepezil to the polymers chitosan and carbolpol 934, with a unique formulation code assigned to each batch Table 1.

## Preparation of spray-dried microparticles

The spray-drying process was used to create the formulations.[7] To produce batches with varying drug-polymer ratios, a spray dryer from techno search instruments, model SPD-D-111, was used.[8] For all batches, the flow rate was kept constant at 1 ml/min, and the temperature range was kept between 70°C and 100°C. The suspensions of drug and polymer were prepared in a 1:1 ratio of solvent water and ethanol with nozzle adjustment to get particle size range between 5µm to 50µm.

## Characterization of microparticles

**Appearance:** All the batches of formulation were characterized for some primary parameter like color and results were recorded for different batches of formulation

**Particle size analysis:** All the batches of formulation were characterized for particle size using optical microscopy and average particle size was determined for all the batches.[9]

**Percentage yield:** All the formulations were used to find out the percentage yield after spray drying.

**Drug content determination:** Drug content determination was done using UV spectroscopy on Shimadzu UV-vis spectrophotometer model UV-3600i.[10] The formulations of donepezil hydrochloride were dissolved in methanol and dilutions were made using distilled water. The readings for formulation were taken at $\lambda_{max}$ 231nm.[11] The drug content was calculated using a calibration curve of donepezil hydrochloride in the lambert bear range of 5-40µg/ml concentration.

**Infrared Spectroscopy:** Infrared spectroscopy was carried out on the Bruker alpha ATR instrument. 1mg for sample from the formulation batches were taken for analysis. The selected formulations DCH1 and DC3 were used to study IR spectroscopy.[12]

**Differential Scanning Calorimetry:** For Differential Scanning Calorimetry, the Mettler Toledo instrument was utilized. Analysis was performed using an aluminum sample holder with a temperature range of 25°C to 300°C. The selected formulations DCH1 and DC3 were analyzed for Differential Scanning Calorimetry.[13]

**Field Emission Scanning Electron Microscopy:** Particle size and Morphology of microparticles were analyzed by Field Emission Scanning Electron Microscopy which was performed on the instrument FEI Nova NanoSEM 450. The instrument was having ultra-high resolution 1.0 nm at 15kV, 1.4 nm at 1kV and 1.8 nm at 3kV, and 30 Pa. The 1mg sample was first plated with gold particles and then the sample was mounted on carbon conductive adhesive tape on the sample holder. The analysis was carried out at various resolutions to observe microparticle size and morphology.[14]

***Ex vivo* Permeability Study:** Permeability study is the important evaluation to justify drug absorption through nasal membrane hence using Franz's diffusion cell study was carried out. The Franz's diffusion cell with a capacity of 50 ml was utilized and for the membrane, the fresh nasal mucosa of a goat was obtained from the wet market. The optimized two formulations from each polymer chitosan and carbopol 934 were taken for further study of diffusion.[15]

Phosphate buffer with pH 6.5 was prepared as a solvent in the receptor compartment using potassium dihydrogen phosphate and Dipotassium hydrogen phosphate.[16] Sample equivalent to 4mg of drug donepezil hydrochloride was taken from each formulation and used in donor compartment placed on the nasal mucosal membrane of goat.[17] After placing the sample the diffusion study was initiated and 1ml aliquots were taken from each sample at time intervals 5,10,15,30,45,60,90 min. The diluted samples were analyzed with a UV spectrophotometer using 231nm as $\lambda_{max}$.[18]

## *In vivo* Studies

**Ethical Statement:** The *in vivo* studies were performed as per the CPCSEA guidelines.

The animal study protocol was duly approved by the CPCSEA Approved drug testing Laboratory with registration 1410/c/11/CPCSEA. The CPCSEA/IAEC number given for the *in vivo* study is CPCSEA/IAEC/0220/156

**Animal model method:** In-bred Albino Rats were used for the experiment. The animals were housed in individually ventilated cage systems in noise and temperature-controlled rooms. Animals were fed on a standard pellet diet and water *ad libitum*.[19]

Test samples were diluted in PBS at a concentration of 50mg/ml. 20 ul solutions were instilled in each nostril of the animal and allowed to stand for 15 min. The animals were sacrificed 15 min post administration and brains were dissected out and kept on ice. The brains were homogenized (Remi) in PBS at a concentration of 100mg/ml.[20] Homogenates were centrifuged at 5000 rpm (Remi) and the supernatant was collected. The supernatants were precipitated by methanol (500ul homogenate +50ul methanol). The samples were centrifuged and the supernatant was used for HPLC analysis.

**HPLC Analysis:** The HPLC analysis was carried out on Shimadzu HPLC model LC-2050. The column utilized was $C_{18}$ column of 250 mm length, 4.6 mm internal diameter, and 5 µm particle sizes were used in the analysis.[21-22] For Donepezil Hydrochloride isocratic mode with Sodium dihydrogen orthophosphate as a buffer (pH 3.5): Acetonitrile (80:20), a flow rate of 1.0 ml/min at ambient temperature. Quantification was attained with UV detection at a wavelength of 231 nm.[23]

**Table 2: Preliminary Characterization of formulations.**

| Batch | DCH1 | DCH2 | DCH3 | DCH4 | DC1 | DC2 | DC3 | DC4 |
|---|---|---|---|---|---|---|---|---|
| **Color** | Yellowish | Yellowish | Yellowish | Yellowish | White | White | White | White |
| **Particle size range (µm)** | 12-35 | 14-38 | 11-33 | 15-39 | 7-45 | 8-42 | 10-35 | 11-47 |
| **Mean size (µm)** | 18.3 | 20.1 | 21.4 | 19.4 | 14.7 | 16.2 | 15.9 | 18.3 |
| **% yield** | 68.12 | 54.65 | 56.58 | 43.96 | 56.46 | 63.23 | 58.48 | 59.47 |
| **Drug content** | 87.32 | 96.05 | 95.65 | 89.87 | 85.07 | 93.15 | 91.5 | 88.35 |

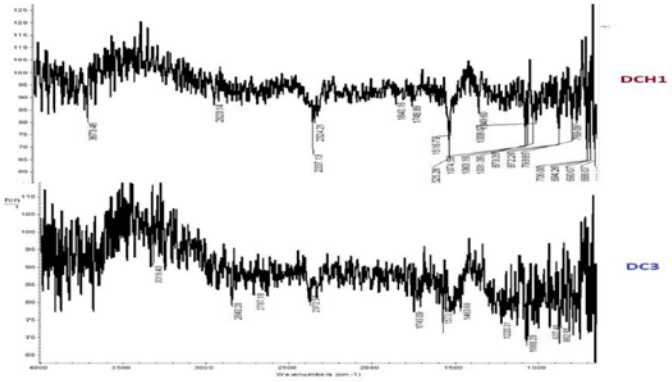


**Figure 2:** Infrared Spectroscopy of DCH1 and DC3 formulations.

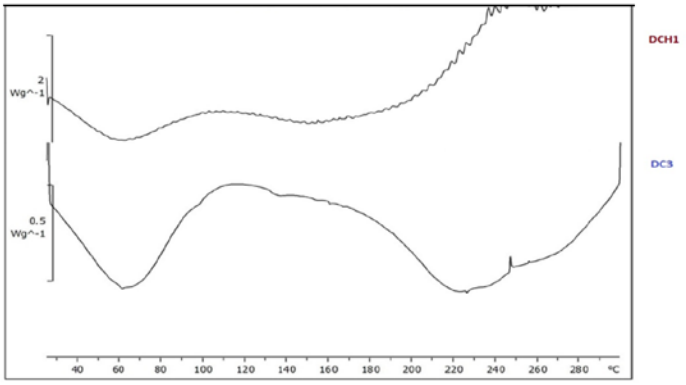


**Figure 3:** Differential Scanning Calorimetry of DCH1 and DC3 formulations.

## RESULTS

### Appearance, Particle size, % yield and drug content

The donepezil and chitosan batches observed to be yellowish in appearance while donepezil and carbopol 934 were appeared to be white. The particle size range of chitosan formulations was found between 11µm to39µm while carbopol 394 batches were between 7µm to 47µm. The mean particle size for chitosan formulations was observed between 18.3µm to 21.4µm and for carbopol 14.7µm to 18.3µm. The percentage yield for chitosan formulations was ranging between 43.96% to 68.12% % 56.46% to 63.23% for carbopol 934. The drug content was also calculated using UV spectrophotometry and it was observed between 89.87% to 96.05% for chitosan formulations and 91.5% to 95.07% for carbopol 934 formulations. (Table 2).

### Infrared Spectroscopy

The formulations DCH1 and DC3 were used to study IR spectroscopy. After coinciding both spectra it was observed that that the most of characteristic peaks of the drug donepezil hydrochloride functional group were intact. In DCH1 formulation the peaks were observed at 3673cm$^{-1}$,2920 cm$^{-1}$,2337 cm$^{-1}$,2324 cm$^{-1}$,1840 cm$^{-1}$,1748 cm$^{-1}$,1525 cm$^{-1}$,1516 cm$^{-1}$ and 1074 cm$^{-1}$ . In DC3 formulations peaks were observed at 3319 cm$^{-1}$, 2840 cm$^{-1}$, 2707 cm$^{-1}$, 2372 cm$^{-1}$, 1745 cm$^{-1}$, 1573 cm$^{-1}$, 1463 cm$^{-1}$, 1220 cm$^{-1}$ and 1068 cm$^{-1}$ . (Figure 2).

### Differential Scanning Calorimetry

Differential Scanning Calorimetry was performed on DCH1 and DC3 formulations. The curves obtained exhibit characteristic endothermic peaks of donepezil hydrochloride in both formulations DCH1 and DC3. The initial broad peak was first observed at 60°C in both the formulations DCH1 and DC3 this peak was more prominent in carbopol 934 formulation DC3. The characteristic peak of the melting point of donepezil hydrochloride was observed as a broad peak at 223°C in DC3 i.e. carbopol 934 formulations. The melting point peak of donepezil hydrochloride was not found prominently seen in chitosan formulation DCH1. (Figure 3).

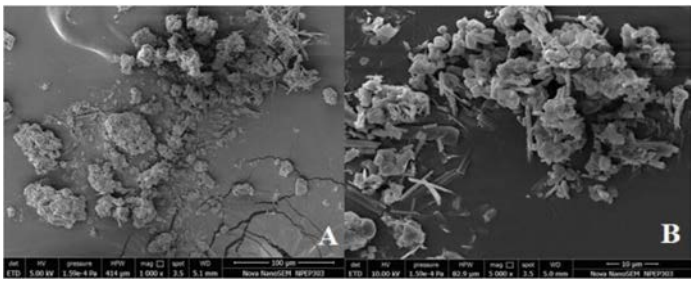


**Figure 4:** Field Emission Scanning Electron Microscopy of DCH1 formulation. a) Particles with size range 5-50µm range b) Drug found to be embedded in the polymer after spray drying.

### Field Emission Scanning Electron Microscopy

The analysis was carried out at various resolutions to observe microparticle size and external morphology. It was observed that the particle size was in the range of 5µm-50µm which was needful for the nasal drug delivery of microparticles as more than 50µm size will not allow the particle to adhere to the mucosal surface while less than 5µm size may take microparticles to the lung. The morphology was also observed as spherical or ellipsoid particles. With higher resolutions, we can observe the drug entrapped within the polymers. Figure 4

***Ex vivo* permeability study:** Permeability study is the important evaluation to justify drug absorption through nasal membrane hence using Franz's diffusion cell study was carried out. The formulations from each polymer chitosan and carbopol 934 were taken for this study.

The absorbance using a UV spectrophotometer was taken to find out the percentage of drug release from each formulation. Release kinetic equations were studied to find out possible drug release models. The % drug release for chitosan batches was found to be between 66.57 - 85.74

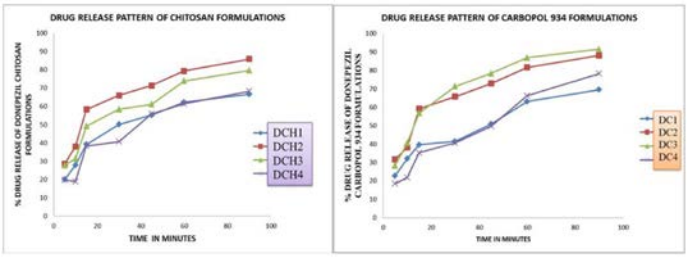


**Figure 5:** The drug release pattern of chitosan formulations and carbopol 934 formulations.

**Table 3: Release Kinetics for all formulations.**

| Formulation | Release curve equation | $R^2$ |
|---|---|---|
| DCH1 | y=0.0035x+0.1797 | 0.8495 |
| DCH2 | y=0.0041x+0.2598 | 0.8075 |
| DCH3 | y=0.004x + 0.2197 | 0.8687 |
| DCH4 | y=0.004x + 0.1459 | 0.8766 |
| DC1 | y=0.0035x+0.1795 | 0.9322 |
| DC2 | y=0.0042x+0.2671 | 0.8332 |
| DC3 | y=0.0047x+0.2648 | 0.8184 |
| DC4 | y=0.0047x+0.1262 | 0.9626 |

**Table 4: HPLC analysis for selected formulations.**

| Formulation Code | 1 | 2 | 3 | 4 | 5 | Average | S.D. |
|---|---|---|---|---|---|---|---|
| D | 0.01023 | 0.01025 | 0.01017 | 0.01238 | 0.01097 | 0.0108 | 0.00094 |
| DCH2 | 0.01162 | 0.01178 | 0.01195 | 0.01212 | 0.01212 | 0.01192 | 0.00022 |
| DC3 | 0.01323 | 0.01356 | 0.01445 | 0.01385 | 0.01448 | 0.01392 | 0.00055 |

and DCH2 formulation was with the highest 85.74± 0.2% of drug release. The % drug release for carbopol 934 batches were found to be between 69.54 - 91.53 and DCH2 formulation was with highest 91.53±0.3% of drug release.

The drug-release kinetics when studied and applied with Korsmeyer Peppas model and it was observed that regression value was less than 1 in all the formulations of chitosan and carbopol 934.[24] Figure 5. Table 3.

### *In vivo* Studies

Inbred Albino Rats were utilized for the study. The homogenized brains in PBS were centrifuged and the supernatant was collected. The supernatants were precipitated by centrifugation and the supernatant was used for HPLC analysis.

The calibration of donepezil hydrochloride was done on HPLC using Sodium dihydrogen orthophosphate as a buffer (pH 3.5): Acetonitrile (80:20), a flow rate of 1.0 ml/min at ambient temperature and area under curve AUC of 68951 was obtained for 0.01 µg/ml concentrations which were utilized as a standard for further calculations. The AUC observed for optimized formulations DCH2 and DC3 was obtained by the HPLC method.

The homogenized supernatants obtained after centrifugation were analyzed on HPLC to find out concentrations of drug in brain homogenate. The concentration of drug in brain tissue homogenate after 15 mins were determined single point detection at 15 min was carried out as we need to study the faster onset of action through this route. Also, the mucociliary clearance time was generally observed up to 20 mins the estimations must be done within that time window. Table 4.

The concentration of donepezil hydrochloride for a pure drug as control and selected formulationDCH2 and DC3 was obtained from the *in vivo* study. The chitosan formulation DCH2 was having 110.87±6.87% more drug concentration in brain tissue than that of pure drug. Also the carbopol 934 formulation DC3 exhibited as high as 129.51±9.82% more drug concentration compared to pure drug donepezil hydrochloride. Figure 6.

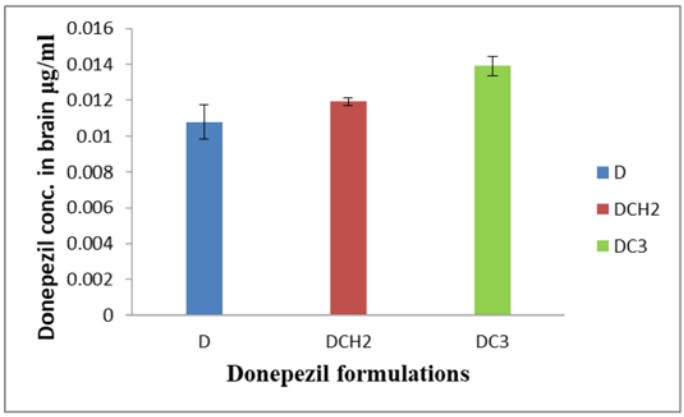


**Figure 6:** Concentrations of donepezil in brain tissue.

## DISCUSSION

CNS illnesses such as Alzheimer's disease, epilepsy, biopolar disorders, and a few of others are on the rise as a result of stress and a variety of other variables. The majority of these illnesses' therapies rely on traditional dose forms such as tablets, capsules, and, in certain cases, parenteral. Because these traditional dosage forms have drawbacks such as first-pass metabolism, protein binding, and limited penetration into the brain, they must be replaced with enhanced dosage forms utilizing novel routes of administration. This study was focused on establishing nose to brain route of administration especially through olfactory area as it is not having blood brain barrier. The nasal route has advantage of faster penetration of drug in brain tissue even to parenteral route which was concluded at the end of this study.

The spray-dried microparticles developed had particle sizes ranging from 5 to 50 micrometers, which were suitable for administration to the nasal cavity.[25] Most formulations had promising percentage yields and drug content, and the formulations with the best findings were picked for further testing.

Following infrared spectroscopy as shown in Figure 2. It was discovered that the donepezil peaks were either intact or slightly shifted by polymers, with no significant change in the drug's characteristics, indicating that there is no probable drug-polymer interaction in the formulations.[26]

DCH1 and DC3 were analyzed using DSC to determine the physical condition of the drug in microparticles. The results are displayed in Figure 3. The DSC thermogram showed a sharp endothermic peak at 223°C due to the melting of the drug. The drug peak for donepezil-loaded microparticles was not visible in the thermogram, indicating that donepezil was molecularly distributed inside the microparticles.[27]

Field Emission Scanning Electron microscopy photographs as shown in Figure 4 exhibited regular spherical or ellipsoid morphology for DCH1 formulation and no free drug was present. At higher resolution drug was found to be entrapped inside the polymer network with very little exception needle-like structures.[28]

When the drug-release kinetics were analyzed and used with the Korsmeyer Peppas model, it was discovered that the regression value was smaller than 1 in all chitosan and carbopol 934 formulations. The "n" value for formulations was recorded less than 0.5 which represents the quasi fickian type of release pattern. The polymer relaxation time and solvent diffusion time were observed to be closer hence non-fickian release pattern was followed.[29]

The purpose of the *in vivo* study was to establish nose to brain route of administration for donepezil hydrochloride in Alzheimer's disease. *In vivo* study was also performed to observe the absorption of spray-dried formulations of donepezil hydrochloride in comparison with pure drug donepezil hydrochloride as control.[30]

The chitosan formulation DCH2 was having 110.87±6.87% more drug concentration in brain tissue than that of pure drug. Also, the carbopol 934 formulation DC3 exhibited as high as 129.51±9.82% more drug concentration compared to pure drug donepezil hydrochloride. This represents the efficaciousness of spray-dried formulations and we can utilize these formulations for enhanced drug delivery of donepezil from nose to brain route of administration in Alzheimer's disease.

The study done establishes the usefulness of nose-to-brain microparticles which is a powder formulation to patients with Alzheimer's disease. Because few devices can give such a dosage form at the moment, device development for such formulations is the future focus of the research. More ever due to the small surface area of the nasal cavity drugs with small doses can only be utilized for this kind of formulation.

Patient compliance will be crucial for nasal formulations in the future since they are easier to administer than other dosage forms and do not require specific medical help. They are considered superior to parenteral dosage forms in terms of patient compliance.

## CONCLUSION

The morphology and drug entrapment in the polymer were further confirmed with field emission scanning electron microscopy. The particle size earlier determined by optical microscopy was reconfirmed here and found to be between 5-50 µm which is a necessary parameter for successful nasal drug delivery.

*Ex vivo* permeability study showed excellent drug release patterns with as high as 85.74% ±0.02 for DCH2 formulation and 91.53±0.3% for DC3 formulation which concludes the efficacy of the formulations in terms of drug release. The release kinetic was observed to be following quasi fickian diffusion model with an "n" value less than 0.5.

*In vivo* animal study exhibited promising results as it showed a higher concentration of drug donepezil in brain tissue for the formulations in comparison with pure drug as control after homogenization and HPLC analysis. The formulation DCH2 exhibited drug concentration 110.87±6.87% and DC3 shown 129.51±9.82% over the control batch which is conclusive for enhanced drug delivery of drugs using these prepared formulations for the nose to brain drug delivery in Alzheimer's disease.

## ACKNOWLEDGEMENT

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## ABBREVIATIONS

**BBB:** Blood Brain Barrier, **AD:** Alzheimer's Disease.

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