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FORMULATION AND EVALUATION OF NASAL MUCOADHESIVE MICROSPHERS BY SOLVENT EVAPORATION TECHNIQUE

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ABSTARACT

The purpose of present research work was to develop mucoadhesive microspheres for nasal delivery with the aim to avoid hepatic first-pass metabolism, improve therapeutic efficacy and enhance residence time. For the treatment of migraine, hydroxypropyl methylcellulose (HPMC) K4M and K15M based microspheres containing Gellan Gum(GG) were prepared by cross-linking technique. The microspheres were evaluated with respect to the yield, particle size, entrapment efficiency, swelling property, in vitro drug release, production yield and drug loading. Microspheres were characterized by fourier Transfer Infrared spectroscopy(FTIR). It was found that the particle size, swelling ability and entrapment efficiency of microspheres increases with increasing drug-to-polymer ratio. HPMC-based microspheres show adequate mucoadhesion and do not have any destructive effect on nasal mucosa. On the basis of these results, Gellan gum microspheres based on HPMC may be considered as a promising nasal delivery system.

KEYWORDS: Gellan Gum, Microsphere, hydroxypropyl methylcellulose (HPMC), K4M, K15M, Nasal drug delivery.

1. INTRODUCTION

Nasal drug delivery is a better option and an alternative route for administration of drugs and biomolecules that are susceptible to acidic or enzymatic degradation and gastrointestinal and hepatic presystemic metabolism. The nasal route of delivery has been used for administration of drugs in the treatment of local diseases such as nasal allergy, nasal congestion and nasal infection. Also, it has been reported as a best possible portal route for the delivery of CNS

acting drug. Nasal route together with pulmonary route has been explored an alternative for systemic drug delivery for decades. This is because rapid absorption is possible in the nasal cavity due to its large surface area, relatively high blood flow while being able to avoid hepatic first pass metabolism. The drug are administered intranasally is absorbed by the highly vascular mucous membrane of the nose, the onset of action is thus considerably faster than in the case of oral administration.[1]

1.1 Mucocillary clearance[2]

The mucociliary clearance mechanism provides human with a very efficient defense system protecting the lungs against inhaled microorganisms, particles, and droplets. These material deposits on the nasal mucosa after inhalation, stick to the mucous layer, and be carried posteriorly through the nasal cavity and down the esophagus to the stomach. The transport of the mucus in the nasal cavity is closely related to the beating pattern of the cilia. These coordinated stroke result in the forward movement of the mucus layer with a flow rate of 5mm/min.

1.2 CNS Delivery through Nasal Route[3]

The drug transport from nose to CNS may occur via olfactory neuroepithelium and may involve paracellular or transcellular and/or neuronal transport. Transport may occur via trigeminal nerve system also.

1.3 Pathways for Nasal Absorption[3]

Absorption through the olfactory neurons - transneuronal absorption. Olfactory epithelium is considered as a portal for substances to enter CNS. Absorption through the supporting cells and the surrounding capillary bed - venous drainage. Absorption into the cerebrospinal fluid.

1.4 Nose to brain pathway[4]

The olfactory mucosa (smelling area in nose) is in direct contact with the brain and CSF. Medications absorbed across the olfactory mucosa directly enter the brain. This area is termed as nose to brain pathway and offers a rapid, direct route for drug delivery to the brain.

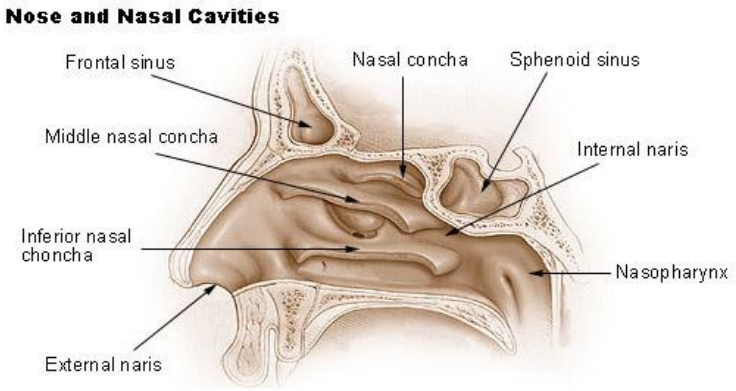


Figure No. 1: Nasal Drug Delivery System.

1.5 Advantages of Nasal Drug Delivery System[4]

- Absorption of drug is rapid via highly vascularized mucosa.
- Onset of action is rapid.
- Unsuitable drug candidate for oral route can be successfully given via nasal route.
- Degradation of drug observed in GIT is avoided.
- Hepatic first pass metabolism is also avoided
- Improved bioavailability.
- Non-invasive, painless, needle-free administration mode.
- A self-administration is possible.
- Convenient route for the patient on long term therapy.
- Direct transport into systemic circulation and CNS is possible.
- Offers lower risk of overdose.

1.6 Limitation of Nasal drug delivery system[4]

1. Large dose drugs are not suitable candidates for nasal drug delivery.
2. Possibility of nasal irritation hence inconvenient compared with oral route

1.7 Factors Influencing Nasal Drug Absorption[4]

Physicochemical factors

- Molecular weight and size
- Lipophilicity
- Dissociation constant
- Partition coefficient
- Stability

Formulation related factors

- pH and osmolarity
- Mucosal irritancy
- Viscosity
- Drug distribution

Pharmaceutical dosage form

1.8 Strategies to Improve Nasal Absorption of Drug[4,5]

- Prodrug
- Enzymatic inhibitors
- Absorption enhancers
- Mucoadhesive drug delivery system

a) Microspheres

b) In-situ Gel

c) Powders

Novel drug delivery system

a) Microspheres

b) Liposome

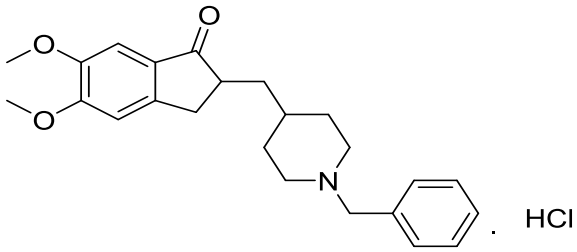
c) Nanoparticles

- **1.9 Rationale for the Nasal Delivery of Microsphere[4,3]**
- Microspheres technology has been widely applied in designing formulations for nasal drug delivery.
- Microspheres prepared by using mucoadhesive polymers e.g. chitosan, dextran, alginates, HPMC, Polyacrylic acid, etc. improves drug residence time in nasal cavity, which results in better drug absorption.
- Furthermore, microspheres may also protect the drug from enzymatic metabolism and sustain drug release, prolonging its effect.
- The microspheres used in nasal drug delivery absorb water present in the nasal mucus, results in its swelling.
- The gel formation improves the nasal residential time and hence it improves the systemic bioavailability.

- Another mechanism stated for improving nasal bioavailability is improving the nasal permeation by opening the tight junction of the nasal epithelium.

Drug and Polymer Profile

Drug Profile :



Donepezil hydrochloride

Trade Name : Aricept

Chemical formula : $C_{24}H_{29}NO3$

Molecular weight : 415.958 g/mol

IUPAC Name : 2-[(1-benzylpiperidin-4-yl)methyl]-5,6-dimethoxy-2,3-dihydro-1H-inden-1-one

Category : Anti Alzheimer

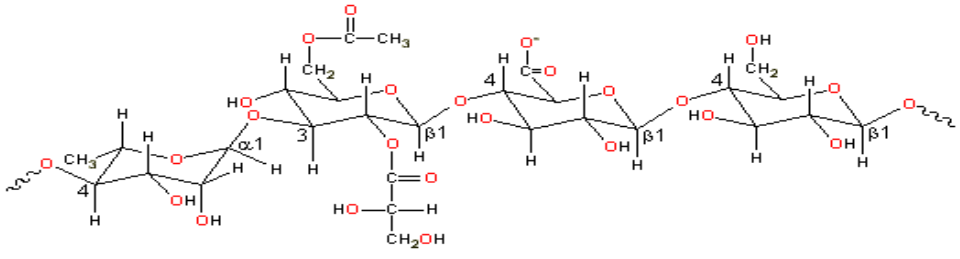
Melting point : 223˚C - 227˚C

Solubility : soluble in water

Polymer profile

Gellan gum :

Structure :



Synonym : Chaplin

Trade Name : Gelrite

pH : 5 -7

State : powder

Color : off white

Solubility : soluble in water

2. MATERIAL AND METHOD

2.1 List of materials and supplier

MATERIAL	Supplier
1) Donepezil HCl	Cipla Mumbai
2) Gellan Gum	Cipla Mumbai
3) N-Octanol	Loba Chemical
4) 2% Span 80	Loba Chemical
5) Calcium Chloride	Loba Chemical
6) Distilled Water	Loba Chemical

2.2 Preparation of gellan gum microsphers

A. Gellan gum was dissolved in double distilled water by heating at 90˚c.Donepezil HCl was uniformly dispersed in Gellan gum solution with constant agitation (500) at 40˚c than weighed amount of drug was added to the Gellan gum homogeneous solution.

B. Then the suspension was quickly injected using a 5ml syringe into 100ml of n-Octanol water system (20: 1 ratio) containing 2% w/v span-80 in a 500 ml beaker, with constant agitation at 1800 rpm, using a mechanical stirrer.

C. The w/o emulsion was stirred for 30 min. 4% $CaCl_2$ solution was than added drop wise and than dispersion was agitated for 5min.

D. The formed microspheres were filtered through Whatman filter paper. The residue was washed 2-3 times with isopropyl alcohol.

E. The product was then dried in a hot air oven at 50˚c and stored in a desiccator at room temperature.

TABLE NO. 1: Formulation design.

Formulation code	Drug (mg)	Gellan gum	Polymer ratio	Stirring speed (rpm)
N1	200	200	1 : 1	1700
N2	200	400	1 : 2	1700
N3	200	600	1 : 3	1700
N4	200	800	1 : 4	1700

3 PREFORMULATION STUDY

3.1 Identification study

Identification of donepezil Hcl was carried out by melting point determination, UV spectroscopy, infrared spectroscopy.

3.2 Melting point

Melting point of the drug determination by taking small amount of drug in a capillary tube closed at one end. The capillary tube was placed in melting point apparatus and the temperature at which drug melt was recorded this procedure thrice and average value was noted.

3.3 Drug and polymer interaction study(FTIR)

The study was conducted with an intention to check the compatibility of polymer gellan gum with donepezil hydrochloride. Also, it helps to check the suitability of polymer for the preparation of microsphere. FTIR spectrum were studied using a shimadzu FTIR spectrometer (IR affinity 1model, japan) spectrometer. The samples of pure drug and physical mixture such as donepezil hydrochloride and gellan gum were prepared into kbr disks. The scanning range was kept from 4000 to 500 cm^{-1}

Determination of λmax and plotting calibration curve of Donepezil Hcl:

a) In Distilled Water

Accurately weight about 10mg of donepezil hcl was dissolved in 100ml of water to obtain 100µg/ml concentration of drug (stock solution) from stock solution were dilute to obtain concentration of 5,10,15,20 and 25µg/ml of donepezil hcl. All dilutions were scanned from 400 to 200nm against water as a blank. The spectrum of the drug was studied to verify λmax and calibration curve was plotted with absorbance versus concentration.

b) In phosphate buffer pH 6.4

Accurately weight about 10mg of donepezil hcl was dissolved in 100ml of water to obtain 100µg/ml concentration of drug (stock solution) from stock solution were dilute to obtain concentration of 5,10,15,20 and 25µg/ml of donepezil hcl. All dilutions were scanned from 400 to 200nm against phosphate buffer 6.4 as a blank. The spectrum of the drug was studied to verify λmax and calibration curve was plotted with absorbance versus concentration.

4. PREPARATION OF MUCOADHESIVE MICROSPHERE

4.1 w/o emulsification cross linking technique

W/o emulsification cross linking method is one of the preparation techniques widely used for microspheres. Donepezil HCl is soluble in water. So, in the present investigation, water solvent was used as to form w/o type of system. Mucoadhesive polymers gellan gum crosslink with $CaCl_2$ for preparation of intranasal microspheres. These polymer were

employed for the fact that they possess good biocompatibility; and are non-irritant and non-toxic. Gellan gum can prolong the residence time of drugs at the absorption site due to their desirable mucoadhesive property.

4.2 Evaluation of mucoadhesive microsphere

- **Production yield (%)**

The production yields were calculated as the weight percentage of the final product after drying, with respect to the initial total amount of donepezil hydrochloride and gellan gum used for the preparations.

$$\text{Production yield \%} = \frac{\text{Practical mass (microspheres)}}{\text{Theoretical mass (polymer + drug)}} \times 100$$

- **Drug loading (%)**

Donepezil hydrochloride in microsphere of each formulation was extracted in water on mechanical shaker for 24 hours in order to extract the entrapped drug completely. The solution was filtered through whatmann filter paper. 1 ml of this solution was withdrawn and diluted to 10 ml with water. This solution was assayed for drug content by UV spectrophotometer at 314 nm.

$$\text{Drug loading(\%)} = \frac{\text{actual amount of drug loaded in microspheres}}{\text{Weighed quantity of microspheres}} \times 100$$

- **Entrapment Efficiency (%)**

Donepezil hydrochloride in microsphere of each formulation was extracted in water on mechanical shaker for 24 hours in order to extract the entrapped drug completely. The solution was filtered through whatmann filter paper. 1 ml of this solution was withdrawn and dilute to 10ml with water. This solution was assayed for drug content by UV spectrophotometer at 314nm

$$\text{Entrapment efficiency(\%)} = \frac{\text{actual amount of drug entrapped in microspheres}}{\text{Amount of drug added}} \times 100$$

- **Degree of swelling**

The swelling of microspheres in nasal simulated fluid. Accurately weight amount of microsphere placed on a millipore filter paper (NY 11 0.22µm) using franz diffusion cell with

phosphate buffer 6.4 and kept for 3.5min. The degree of swelling was calculated using following formula:

$$\text{Degree of swelling} = (Ws - Wo) / Ws$$

Where, Wo- Initial weight of microspheres and

Ws- Weight of microspheres after swelling.

- **Fourier Transformation Infrared Spectroscopy (FTIR)**

The study was conducted with an intention to check the compatibility of polymer Gellan gum, and donepezil hydrochloride. Also, it helps to check the suitability of polymer for the preparation of microsphere. FTIR spectra were obtained using a Shimadzu FTIR spectrometer (IR Affinity 1Model, Japan) spectrometer. The samples of drug loaded microspheres were prepared into KBr disks. The scanning range was kept from 4000 to 500 cm^{-1}.

- **In vitro drug diffusion studies**

In vitro drug release test of the microsphere was performed using franz diffusion cell with dialysis membrane in which the donor compartment contained the microsphere while the receptor compartment was filled with phosphate buffer solution of pH 6.4 That was within the pH rang in the nasal cavity. the donor chamber was placed in a such way that it just touched of diffusion medium in the receptor chamber. The temperature was maintained constant at 37˚C with the help of a circulating water bath. Samples were periodically withdrawn from the receptor compartment and assayed using a UV spectrophotometer at 314nm.

5. RESULT AND DISCUSSION

5.1Preformulation study

- **Drug name :** donepezil HCl.
- **Organoleptic property:** off white color
- **Melting point:** 223-227 ^{O}C
- **Solubility:** Soluble in water

Determination of λmax and calibration curve of donepezil Hcl in phosphate buffer 6.6

5.2 FT-IR SPECTROSCOPIC METHOD

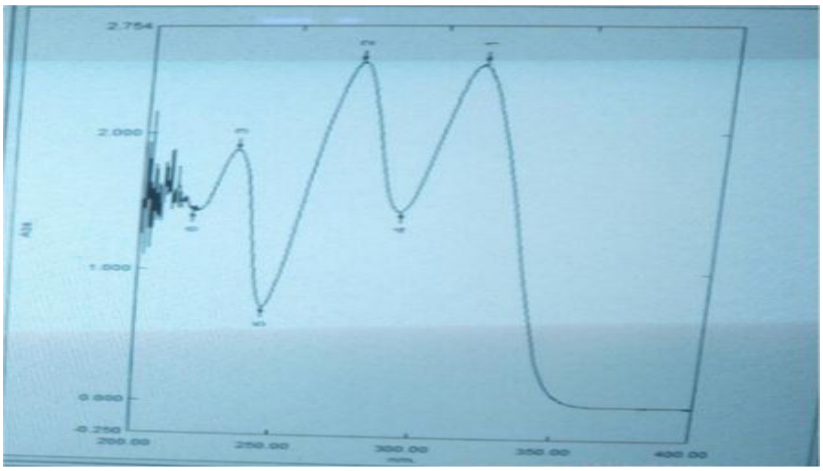


Figure no. 2: Donepezil Hcl Spectra.

Table No 2: Absorbance of Donepezil Hcl.

Sr.no	Conc. (µg/ml)	Absorbance
1.	5	0.181
2.	10	0.274
3.	15	0.369
4.	20	0.453
5.	25	0.556

Table no 3: Drug and polymer interaction study(FTIR).

Sr. no.	Parameters	Drug (Donepezil Hcl)
1.	Detection Wavelength (λmax)	314
2.	Regression Equation	y = 0.0186x +0.0879
3.	Correlation Coefficient	R^2 = 0.9993

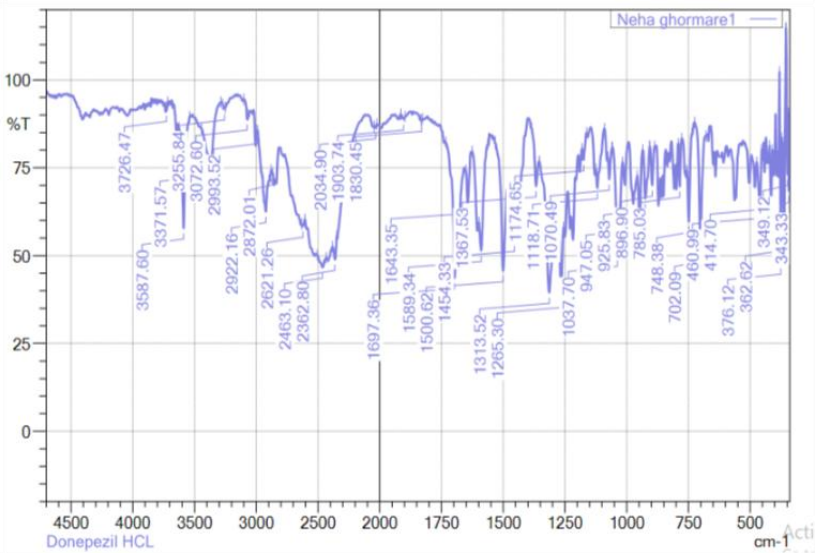


Figure no 3: FTIR spectra of donepezil HCl.

Table no. 4: Function group and IR region.

Material	Peaks $(cm)^{-1}$	Characteristics functional group
Donepezil Hcl	3726	O - H stretching
	3526	O – H stretching
	2362	C = C bond
	1697	Carbonyl C = O bond
	1265	C-N bond
	1454	Halogen compound
	1174	Carbonyl C = O

DONEPEZIL HCL

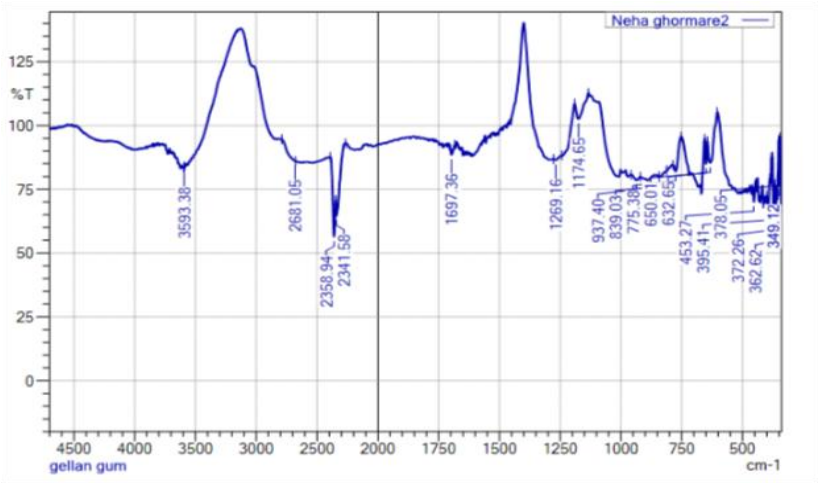


Figure no. 4- FTIR spectra of gellan gum.

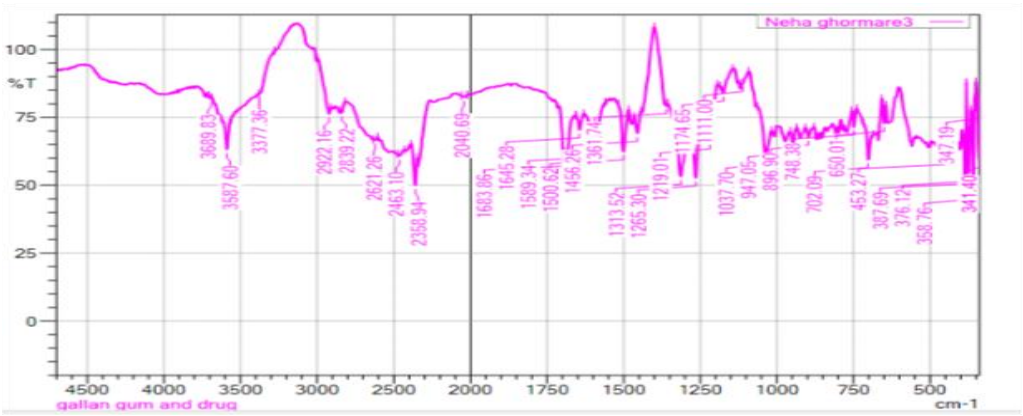


Figure no 5: FTIR spectra of drug & polymer physical mix.

Table no. 6.

Material	Peaks$(cm)^{-1}$	Characteristics functional group
Gellan gum	3593	O –H stretching
	2358	C = C Bond
	1697	Carbonyl C = O Stretching
	1269	C –N Stretching
	1174	Carbonyl C = O Stretching

Table no. 6: Function group and ir region of physical mixture.

Material	Peaks(cm)$^{-1}$	Characteristics functional group
Physical mixture	3587	O – H Stretching
	2358	C = C Bond
	1683	Carbonyl C =O Stretching
	1645	N –H Bonding
	1265	C –N stretching
	1174	Carbonyl C = O Stretching

5.3 EVALUATION PARAMETER OF NASAL MUCOADHESIVE MICROSPHERE

5.3.1 Practical yield (%)

Production yield of mucoadhesive microsphere was found to be between 44.26% to 55.93%. It was found that production yield of microspheres prepared by Gellan gum.

5.3.2 Drug laoding

Formulation code	Production yield %
NI	40.26%
N2	44.83%
N3	50.45%
N4	55.93%

Drug loading of mucoadhesive microsphere was found in the range between 3.45% to 5.23%. It was found that drug loading of mucoadhesive microspheres prepared by Gellan gum.

Table no 8: Drug loading of mucoadhesive microsphere.

Formulation code	Drug loading* (% ± SD)
N1	5.23 ± 0.01
N2	4.23 ± 0.01
N3	4.05 ± 0.02
N4	3.45 ± 0.03

*Values expressed as Mean ± SD, n=3

5.3.3 Particle size analysis

Average particle size of microspheres ranged from 100 to 400 µm, such particles are considered to be suitable for nasal administration. Average particle size of microspheres ranged from 157.65 µm to 373.87 µm. Particle size mainly depends on the stirring rate; hence, as the stirring rate increased, the particle size decreased irrespective of the concentration of mucoadhesive polymer.

Table no 9: Partical Size of Mucoadhesive Microsphere.

Formulation	Particle size (µm)
N1	373.87µm
N2	157.65µm
N3	245.68µm
N4	267.87µm

5.3.4. Entrapment efficiency (%)

Entrapment efficiency of mucoadhesive microsphere was found in the range between 34.5 to 53%. As the concentration of mucoadhesive polymer increased, entrapment efficiency increased both at higher and lower stirring rate. It was found that mucoadhesive microspheres prepared by Gellan gum had higher effect on entrapment efficiency.

Table no 10: Entrapment efficiency of mucoadhesive microsphere.

Formulation code	Entrapment efficiency* (% ± SD)
N1	53 ± 1
N2	42.3 ± 0.15
N3	40.5 ± 0.15
N4	34.5 ± 0.2

*Values expressed as Mean ± SD, n=3

5.3.5 Swelling property

Swelling index of mucoadhesive microsphere ranged from 0.82% to 0.91% The degree of swelling increases marginally as the concentration of mucoadhesive polymer increases. Marginal decrease in swelling at lower level of mucoadhesive polymer may be due to the higher level of film forming polymer in those formulations which allows lesser penetration of water inside the polymer matrix. Higher degree of swelling of Gellan gum microsphere.

Table no. 11: Swelling index of mucoadhesive microsphere.

Formulation code	Swelling property* (% ± SD)
N1	0.82 ± 0.01
N2	0.83 ± 0.02
N3	0.88 ± 0.01
N4	0.91 ± 0.01

*Values expressed as Mean ± SD, n=3

5.4 Fourier Transform Infrared Spectroscopy (Ftir)

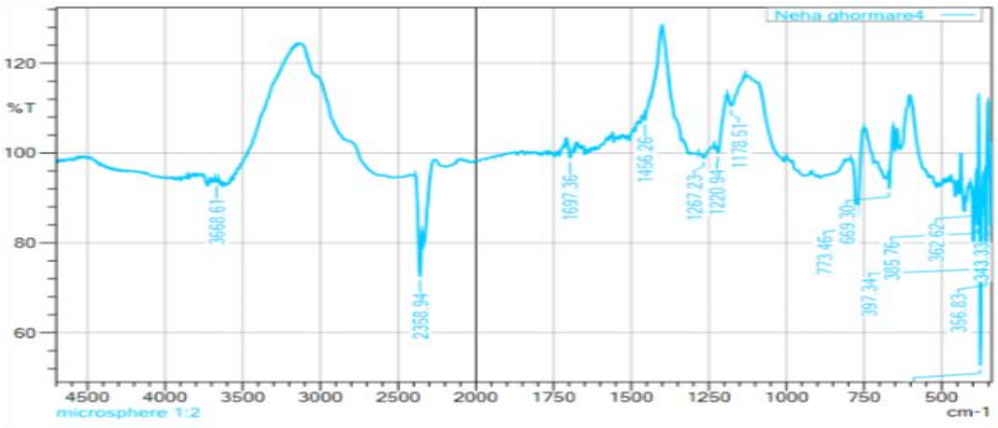


Figure no 6: FTIR spectrum of mucoadhesive microspheres N_2.

Table no 12: FTIR spectrum of mucoadhesive microspheres N_2 of function group and IR region.

Material	Peak(cm)$^{-1}$	Characteristics functional group	Formulation N1
Donepezil Hcl	2362	C = C Bond	2358
	1697	Carbonyl C = O Bond	1697
	1454	Halogen compound	1456
	1265	C – N Stretching	1267
	1154	Carbonyl C = O Bond	1178

5.4 IN VITRO DRUG RELEASE STUDY

The release profile of Donepezil Hcl and Gellan gum microspheres at pH 6.6 phosphate buffer. Cumulative percent drug release.

Table no. 13: In vitro drug release study.

Time	N1(% ± SD)	N2(% ± SD)	N3(% ± SD)	N4(% ± SD)
0	0	0	0	0
15 min	15.52 ± 0.01	19.690 ±0.01	9.78 ± 0.03	12.07 ± 0.02
30 min	31.93± 0.01	41.200 ±0.01	25.67 ± 0.02	25.55 ±0.01
60 min	38.07± 0.02	47.920 ± 0.03	37.71 ± 0.01	31.29 ± 0.01
90 min	44.43± 0.01	54.480 ± 0.01	44.16 ± 0.02	36.39 ± 0.05
120min	50.39± 0.01	62.940 ± 0.01	49.11 ± 0.01	44.05 ± 0.01
150 min	53.72± 0.02	67.940 ± 0.02	53.93 ±0.04	50.09 ±0.02
180min	57.81± 0.02	72.860 ± 0.01	55.93 ±0.04	53.61 ± 0.01
s210 min	63.06± 0.02	79.030 ± 0.01	59.58 ±0.05	59.27 ± 1.15
240 min	66.41± 0.01	85.070 ± 0.02	64.79 ± 0.01	65.31 ± 0.01
270 min	72.62± 0.02	92.870 ±0.03	71.97 ±0.02	68.16 ± 0.02
300 min	78.24±0.01	98.260 ±0.01	77.21 ±0.01	76.92 ± 0.01

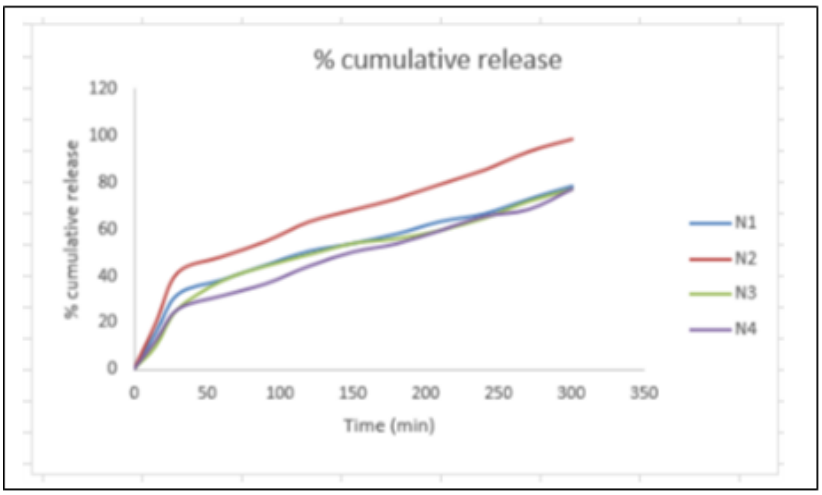


Figure no 7: % Cumulative release graph.

6. CONCLUSION

Gellan gum Microsphere of Donepezil with a smooth surface & spherical shape were prepared by emulsification cross linking method. The size of microsphere was in the range 373.87µm-157.65µm which is favorable for intra nasal absorption. The entrapment efficiency was observed in the range 34-53%. A strong interaction between mucin & gellan gum micro spheres was explain more specific adsorption process. All batch of micro spheres released around 98% of drug in 7hrs.

7. AKNOWLEDGEMENT

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8. REFERENCES

1. Illum, L. Nasal clearance in health and disease. *Journal of aerosol medicine*, 2006; *19*(1): 92-99.
2. Pardeshi, C. V., & Belgamwar, V. S. Direct nose to brain drug delivery via integrated nerve pathways bypassing the blood–brain barrier: an excellent platform for brain targeting. *Expert opinion on drug delivery*, 2013; *10*(7): 957-972.
3. Romeo, V. D., DeMeireles, J. C., Gries, W. J., Xia, W. J., Sileno, A. P., Pimplaskar, H. K., & Behl, C. R. Optimization of systemic nasal drug delivery with pharmaceutical excipients. *Advanced drug delivery reviews*, 1998; *29*(1-2): 117-133.

4. Abbas and marihal, et.al, "Intranasal delivery of almotripton microsphers," Journal of pharmacy and Bioallied Sciences, 2017; 117-211.
5. Belgamwar, V. S., Patel, H. S., Joshi, A. S., Agrawal, A., Surana, S. J., & Tekade, A. R. Design and development of nasal mucoadhesive microspheres containing tramadol HCl for CNS targeting. *Drug delivery*, 2011; *18*(5): 353-360.
6. Lim, S. T., Martin, G. P., Berry, D. J., & Brown, M. B. Preparation and evaluation of the in vitro drug release properties and mucoadhesion of novel microspheres of hyaluronic acid and chitosan. *Journal of Controlled Release*, 2000; *66*(2-3): 281-292.
7. Suryawanshi, S. R., Thakare, N. P., More, D. P., & Thombre, N. A. Bioavailability enhancement of ondansetron after nasal administration of Caesalpinia pulcherrima-based microspheres. *Drug delivery*, 2015; *22*(7): 894-902.
8. Lordi, N. G., Lachman, L., & Liberman, H. A. The theory and practice of industrial pharmacy. *LEA & FEBIGER Philadelphia, USA*, 1987; 430-456.
9. Allen, L. V., Popovich, N. G., & Ansel, H. C. Pharmaceutical dosage forms and drug delivery systems. *Delhi, India: BI Pubication*, 2005; *8:* 265.
10. Aulton, M. E. *Pharmaceutics: The science of dosage form design*. Churchill livingstone, 2002.
11. Ramington, G. A. The science and practice of pharmacy. *Delhi, India: BI publication*, 2006.