Indian Journal of Novel Drug Delivery

IJNDD

An Official Publication of Karnataka Education and Scientific Society

Research Article

# Development and Characterisation of Donepezil Nanoparticles

P V KAMALA KUMARI[1*], Y SRINIVASA RAO[1], A NAGA RAJU

[1]Vignan Institute of Pharmaceutical Technology, Besides VSEZ, Near Kapujaggarajupeta Village, Duvvada, Visakhapatnam, Andhra Pradesh, India- 530049

**ARTICLE DETAILS**

*Article history:*
Received on 17 August 2018
Modified on 20 September 2018
Accepted on 24 September 2018

*Keywords:*
Nanoparticle,
Diffusion study,
Donepezil,
Higuchi,
Stability study

**ABSTRACT**

The purpose of the present study was to formulate and evaluate donepezil loaded nanoparticles for effective treatment of Alzheimer's disease. Nanoparticles were prepared by ionic gelation of chitosan with poloxamer. Size and morphology of nanoparticles were investigated. Scanning electron microscopy (SEM) revealed that the donepezil nanoparticles were smooth and spherical without any aggregation. F6 formulation which had drug polymer ratio of chitosan and poloxamer was decided to be the optimized formulation based on *in vitro* drug release studies. Dissolution data based on two different methods was fitted in zero order, first order and Higuchi equations. The mechanism of drug release was determined by using Higuchi equation. There was no significant change in physical and chemical properties of formulation F-6 even after three months.



## INTRODUCTION

Alzheimer's is an irreversible, progressive brain disease that slowly destroys memory and thinking skills, and eventually even the ability to carry out the simplest tasks [1]. Alzheimer's disease (AD) is rapidly becoming a major public health concern. An estimated 20% of individuals aged >80 years are believed to be affected [2].The annual incidence of AD increases with age, from about 1% in those aged 65 to 75 years to more than 8% in those aged >85 years [3,4]. A sharp increase in the number of persons afflicted by this debilitating disease is anticipated as the proportion of the population >65 years continues to rise in Western countries [5]. Diseases of the Central Nervous System (CNS) such as Alzheimer's disease require delivery of the drug to the brain for treatment. However such transport remains problematic, especially for hydrophilic drugs and large molecular weight drugs, due to the impervious nature of the endothelial membrane separating the systemic circulation and central interstitial fluid, the Blood–Brain Barrier (BBB) [6]. It is estimated that more than 98% of the small new molecules do not cross the BBB, and hence fail to achieve the therapeutic concentration within the brain parenchyma cells [7].

***Author for Correspondence:***
*Email:* kamalaparavastu@gmail.com

Donepezil is a specific and reversible inhibitor of the enzyme acetyl cholinesterase. Acetyl cholinesterase is an enzyme which breaks down acetylcholine. Donepezil may allow a greater concentration of acetylcholine in the brain, thereby improving cholinergic function.

## EXPERIMENTAL

### Method of Preparation of Donepezil Loaded Nanoparticles

Nanoparticles were prepared according to the procedure based on the ionic gelation of chitosan with poloxamer. Chitosan nanoparticles were prepared in the presence of Span 80 as a re-suspending agent to prevent aggregation, at ambient temperature while stirring. Required quantity of chitosan was taken and dissolved in 5 mL of lactic acid under stirring at 1000 rpm for 10 min. Fifty mg of the drug was dissolved in 85 mL of 0.5% v/v Span 80 solution (0.5 mL Span-80 in 100 mL of double distilled water). Drug solution was then added to chitosan solution and stirred for 20 min at 1000 rpm using magnetic stirrer. Poloxamer was dissolved in 10 mL of 0.5% Span 80 (v/v) solution and added drop wise using syringe under stirring. The suspension was then sonicated for 20 min at 80% amplitude and one second pulse for particle size reduction. The final suspension was then frozen and lyophilized at (-40 °C) for two days. A

total of eight formulations of nanoparticles were developed as shown in Table 1.

**Characterization of Nanoparticles**

**Particles Size Distribution and Zeta Potential**

The particle size and polydispersity index were performed by dynamic light scattering (DLS) [8]. The size measurement was performed at 250 cat. 900 scattering angle, and it was recorded for 180 s for each measurement. Nanoparticles were characterized with zeta potential ($\zeta$) using a zetasizer 4 (Malvern Instruments Ltd., Malvern, UK) [9]. The zeta potential was measured by an aqueous dip cell in an automatic mode. Samples were diluted in ultra purified water and placed in a capillary measurement cell, with the cell position adjusted.

**Scanning Electron Microscopy**

Measurement of particle size and information about shape of the particle were obtained using FEG-SEM (JSM-7600 F, Jeol, Tokyo). The samples for SEM were prepared by sprinkling the nanoparticle powder on a double adhesive tape that stucks to an aluminium stub. They were then vaccum-coated for 45 seconds with platinum mixture. The samples were than randomly scanned and photographs were taken randomly [10].

**Drug Content**

A quantity of drug-loaded nanospheres from each batch equivalent to 1.0 mg was added to 50 mL of normal saline and stirred continuously for 2.0 hrs and then the final colloidal suspensions were centrifuged at 2000 rpm at 22 ± 2°C for 0.5 h. The supernatant was analyzed for drug content by measuring the absorbance at 268 nm using UV spectrophotometer [11].

***In Vitro* Drug Release Studies**

Thirty mg of drug-loaded nanoparticles was placed in an USP dissolution test apparatus having basket type stirring element. The basket was covered with cellophane membrane. 900 mL of phosphate buffer solution (pH 7.4) was used as dissolution medium and kept at 37°C. The basket was rotated at a speed of 100 rpm. 5.0 mL of medium was withdrawn at various time intervals of 1.0 hr, 2.0 hrs, 3.0 hrs, 4.0 hrs, 5.0 hrs, 6.0 hrs, 7.0 hrs and 8.0 hrs with the help of 5.0 mL pipette and replaced by 5.0 mL of phosphate buffer solution (pH 7.4). The drug content was estimated by UV spectrophotometer at 268 nm [12].

**Stability Studies**

Stability studies of prepared nanoparticles were carried out, by storing formulation F5 at 4°C ±1°C and 30°C ± 2°C in stability chamber for 90 days. The samples were analyzed for drug content (ICH Q1A (*R*2) 2003) [13].

**Encapsulation Efficiency**

The amount of donepezil entrapped in the nanoparticles was determined by the separation of donepezil-loaded nanoparticles from the suspension containing free donepezil by centrifugation. The suspension obtained after solvent evaporation was centrifuged, and the amount of free donepezil in the supernatant was measured by ultraviolet (UV) spectrophotometer at 268 nm. The amount of drug entrapped into nanoparticles was calculated as the difference between the drugs used for the formulation and the amount of drug in the supernatant. The percent of entrapment efficiency was calculated by the following formula [14]

$$\text{EE \%} = \frac{\text{Total amount of drug added} - \text{Non bound drug}}{\text{Total amount of drug}} \times 100$$

**RESULTS**

**Drug - excipient compatibility studies (FT-IR)**

The compatibility between the drug and the selected lipid and other excipients was evaluated using FTIR peak matching method [15]. There was no appearance or disappearance of peaks in the drug-lipid mixture, which confirmed the absence of any chemical interaction between the drug, lipid and other chemicals as shown in Fig. 1 and Fig. 2. Particle size of NPs was found to be increased on increasing the concentration of polymer. Nanoparticles with smaller size have valuable characteristics such as improved drug delivery, longer circulation in blood, and lower toxicity as shown in Table 2.

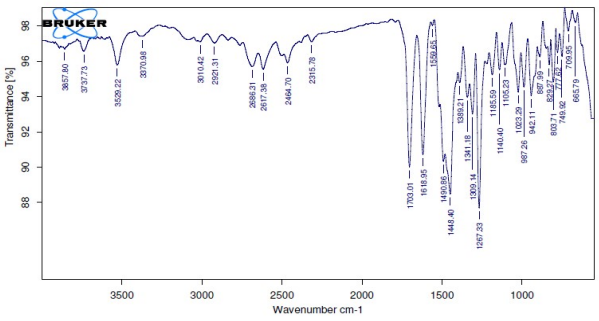


**Figure 1:** FT-IR sample for Donepezil

**Table 1:** Composition of the nanoparticles

| Ingredients | Formulation | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 |
| **Chitosan** | 25 | 50 | 75 | 100 | 25 | 50 | 75 | 100 |
| **Poloxamer** | 10 | 20 | 30 | 40 | 40 | 30 | 20 | 10 |
| **Donepezil (mg)** | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 |
| **Acetone (mL)** | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| **Water (mL)** | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |

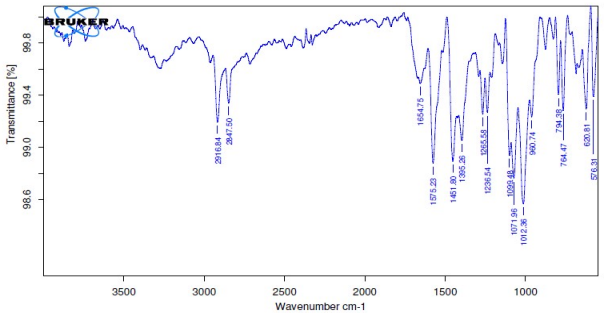


**Figure 2:** FT-IR sample for formulation F6

**Table 2:** Particle size, loaded drug, encapsulation efficiency of various formulations

| Formulation | Particle size (nm) | Drug Loaded (mg) | Entrapment Efficiency (%) |
|---|---|---|---|
| **F1** | 250.8 | 0.2 | 32±0.40 |
| **F2** | 152.5 | 0.23 | 46±0.45 |
| **F3** | 539.9 | 1.2 | 42±0.21 |
| **F4** | 102.5 | 1.05 | 37±0.20 |
| **F5** | 106.8 | 2.9 | 38±0.25 |
| **F6** | 132.3 | 4.7 | 78±0.32 |
| **F7** | 155.5 | 4.1 | 65±0.52 |
| **F8** | 122.4 | 4.0 | 71±0.62 |

## Surface morphology

Scanning electron microscopy (SEM) revealed that the donepezil nanoparticles were smooth and spherical without any aggregation as shown in Fig. 3.

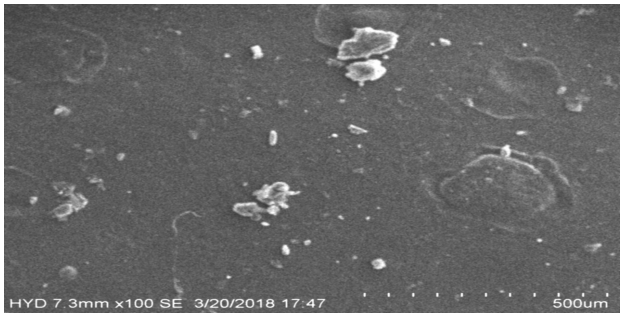


**Figure 3:** SEM analysis of formulation F6.

## Drug Release Studies

The *in vitro* diffusion studies were performed in pH 7.4 buffer using dialysis membrane for 8 hours. Initially the release of drug from all the three batches was found to be about 25-35 % in 8 hours as shown in Table 3 and Fig. 4. This was due to the release of adsorbed drug from the surface of nanoparticles. Later on a constant and slow drug release was observed for 8hrs. F6 formulation which had drug polymer ratio of chitosan and poloxamer was decided to be the optimized formulation.

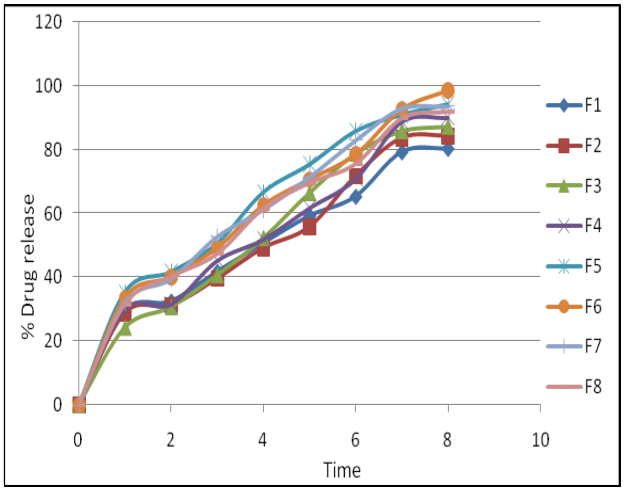


**Figure 4:** *In vitro* drug release studies for all formulations

## Kinetic Modeling of Drug Release

All the formulations of prepared of donepezil nanoparticles were subjected to *in vitro* release studies these studies were carried out using dissolution apparatus. The results obtaining *in vitro* release studies were plotted in different model of data treatment as:

1. Cumulative percent drug released vs. time (zero order rate kinetics)
2. Log cumulative percent drug retained vs. time (First Order rate Kinetics)

**Table 3:** Diffusion study profiles for all formulations

| Time (hrs) | $F_1$ | $F_2$ | $F_3$ | $F_4$ | $F_5$ | $F_6$ | $F_7$ | $F_8$ |
|---|---|---|---|---|---|---|---|---|
| **0** | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **1** | 29.43 | 28.82 | 24.29 | 29.94 | 35.32 | 33.62 | 32.10 | 31.50 |
| **2** | 32.51 | 31.28 | 30.78 | 31.52 | 41.71 | 40.19 | 39.50 | 40.19 |
| **3** | 41.78 | 39.61 | 40.76 | 45.21 | 50.69 | 49.28 | 52.69 | 47.30 |
| **4** | 50.7 | 49.20 | 52.32 | 51.87 | 66.65 | 62.62 | 61.19 | 61.50 |
| **5** | 59.2 | 55.81 | 66.49 | 61.71 | 75.37 | 70.74 | 71.40 | 69.50 |
| **6** | 65.3 | 71.76 | 78.77 | 70.86 | 85.78 | 78.56 | 82.91 | 75.47 |
| **7** | 79.2 | 83.63 | 85.84 | 88.82 | 90.9 | 92.68 | 92.9 | 89.71 |
| **8** | 80.3 | 84.32 | 87.24 | 90.12 | 94.21 | 98.62 | 93.55 | 91.80 |

**Table 4:** Drug release kinetics of Formulation F6

| S.No | Time | log T | Square root of Time | % CR | % Drug remaining | log % CR | Log % drug retained | cube root of % drug remaining |
|---|---|---|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 | 100 | 0 | 2 | 4.641589 |
| 1 | 1 | 0 | 1 | 33.62 | 81.57 | 1.265525 | 1.91153 | 4.336874 |
| 2 | 2 | 0.30103 | 1.414214 | 40.19 | 59.46 | 1.607884 | 1.774225 | 3.903088 |
| 3 | 3 | 0.60206 | 2 | 49.28 | 35.35 | 1.810569 | 1.548389 | 3.281934 |
| 4 | 4 | 0.778151 | 2.44949 | 62.62 | 21.63 | 1.89415 | 1.335057 | 2.786242 |
| 5 | 5 | 1 | 3.162278 | 70.74 | 10.53 | 1.951677 | 1.022428 | 2.191843 |
| 6 | 6 | 1.079181 | 3.12984 | 78.56 | 3.72 | 1.983536 | 1.26854 | 2.158723 |
| 7 | 7 | 1.02381 | 2.15986 | 92.68 | 2.96 | 1.19874 | 0.35874 3 | 2.135848 |
| 8 | 8 | 1.87265 | 2.46598 | 98.62 | 15.69 | 1.26987 | 0.42986 | 2.298564 |

**Table 5:** Results of stability studies of optimized formulation F-6

| Formulation | Parameters | Initial | 1st Month | 2nd Month | 3rd Month | Limits as per Specifications |
|---|---|---|---|---|---|---|
| **F-6** | 25°C/60 % RH % release | 98.62 | 98.54 | 98.49 | 98.39 | Not less than 85 % |
| **F-6** | 30°C/75 % RH % release | 98.62 | 98.55 | 98.48 | 98.35 | Not less than 85 % |
| **F-6** | 40°C/75 % RH % release | 98.62 | 98.54 | 98.37 | 98.28 | Not less than 85 % |

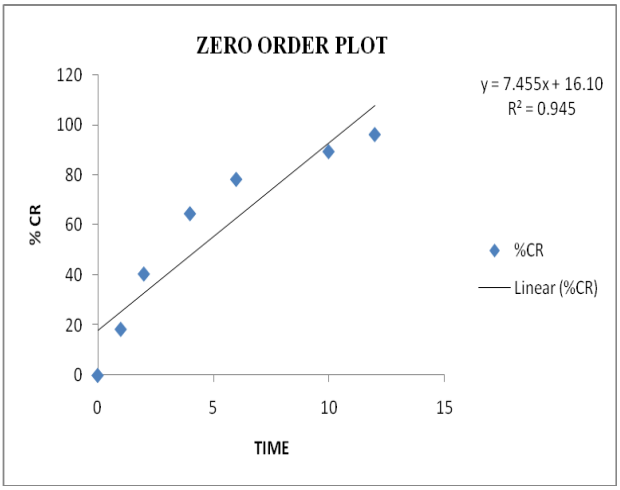


**Figure 5:** Zero order plot for optimized formula

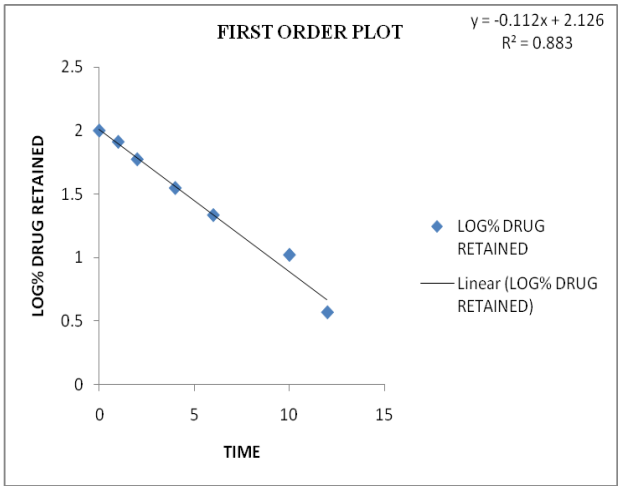


**Figure 6:** First order for optimized formula

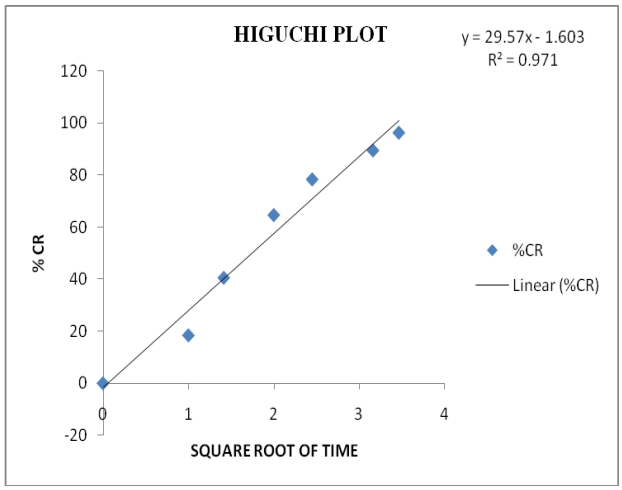


**Figure 7:** Higuchi plot for optimized formula

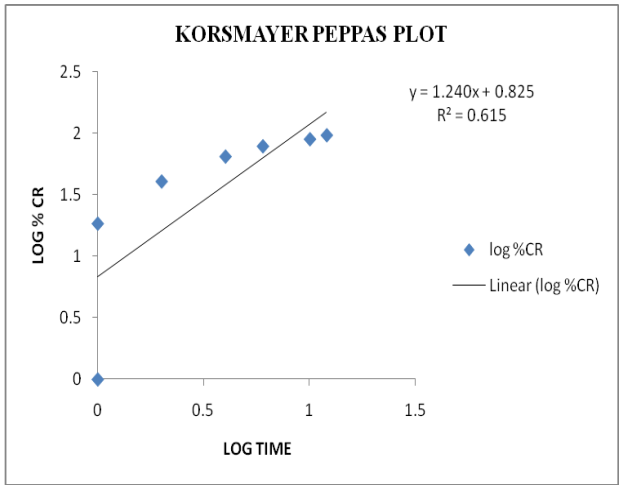


**Figure 8:** Korsmayer peppas plot for optimized formula

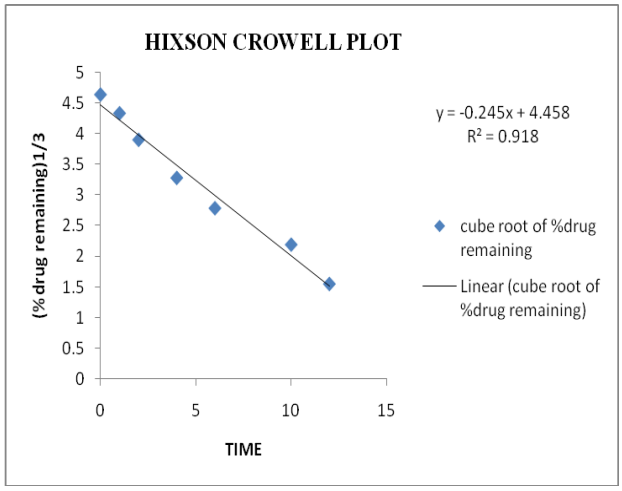


**Figure 9:** Hixson crowell plot for optimized formula

3. Cumulative percent drug released vs. square root of time (Higuchi's Classical Diffusion Equation)
4. Log of cumulative % release Vs log time (Peppas Exponential Equation)
5. (Percentage retained) $1/^3$ Vs time (Hixson – Crowell Erosion Equation)

Dissolution data of above two methods was fitted in Zero order, First order and Higuchi equations. The mechanism of drug release was determined by using Higuchi equation.

The drug release from the Nanoparticles was found to follow Zero order release based on the "r" value obtained for Zero order (0.945) and first order (0.883) for F6 formulation as shown in Table 4. Also, the drug release mechanism was found to be "Diffusion" based on the "r" value of 0.971 obtained for Higuchi's plot. Similarly, the drug release mechanism was found to be of Anomalous diffusion mechanism based on the "n" value of 0.615 obtained for Peppa's equation (Fig. 5 to 9).

## Stability Studies

There was no significant change in physical and chemical properties of the tablets of formulation F-6 after 3 months. Parameters quantified at various time intervals were shown in Table 6.

## DISCUSSION

The aim of present study was to prepare and evaluate chitosan nanoparticles for the controlled drug delivery of donepezil. Eight batches of nanoparticles were prepared using ionic gelation technique in order to study the process parameters (formulation development and *in vitro* characterization). During SEM analysis, donepezil nanoparticles were smooth and spherical without any aggregation. Drug loading capacity ranged from 0.2 to 4.7 mg. It can be inferred that, as the concentration of polymer increases loading capacity was also increased. In these studies, we found that around 78% of drug was entrapped in nanoparticles. After that, drug content was found to be decreased; this might be due to the separation capacity of the polymer. The cumulative percent release of drug for various formulations (F1–F8) was found to be from 70 to 83% for different formulations. Among all formulations, maximum drug (around 78%) was released from F6. Burst release of donepezil was resultant from nanoparticles at initial stage. It might be due to dissolution of drug crystals on the surface of nanoparticles. Electrostatic interactions between protonated amino residues on donepezil and anionic group are responsible for the surface bound

interactions involved in the burst release. On observation, we found that maximum *in vitro* release was found to be for F-6 formulation.

## CONCLUSION

Donepezil loaded nanoparticles were successfully prepared by modified ionic gelation technique. The concentration of polaxmer used for formulating these batches of nanoparticles showed significant effect on its efficiency to entrap donepezil molecule. Donepezil loaded nanoparticles with a small size and narrow size distribution were obtained. *In-vitro* release study revealed that donepezil loaded nanoparticles were capable of releasing the drug in a slow sustained manner. It may significantly improve the ability to cross blood-brain barrier and serve as an effective tool to treat Alzheimer's disease.

## REFERENCES


[1] Berchtold NC, Cotman CW. Evolution in the conceptualization of dementia and Alzheimer's disease: Greco-Roman period to the 1960s. Neurobiology of aging. 1998 May 1; 19(3):173-89.

[2] Bachman DL, Wolf PA, Linn R, Knoefel JE, CobbS J, Belanger A, D'agostino RB, White LR. Prevalence of dementia and probable senile dementia of the Alzheimer type in the Framingham Study. Neurology. 1992 Jan 1; 42(1):115.

[3] Hebert LE, Scherr PA, Beckett LA, Albert MS, Pilgrim DM, Chown MJ, Funkenstein HH, Evans DA. Age-specific incidence of Alzheimer's disease in a community population. Jama. 1995 May 3; 273(17):1354-9.

[4] Bachman DL, Wolf PA, Linn RT, Knoefel JE, Cobb JL, Belanger AJ, White LR, D'agostino RB. Incidence of dementia and probable Alzheimer's disease in a general population the Framingham study. Neurology. 1993 Mar 1; 43(3 Part 1):515.

[5] Berchtold, Nicole C., and Carl W. Cotman. "Evolution in the conceptualization of dementia and Alzheimer's disease: Greco-Roman period to the 1960s." *Neurobiology of aging*19.3 (1998): 173-189.

[6] Ernst RL, Hay JW. The US economic and social costs of Alzheimer's disease revisited. American Journal of Public Health. 1994 Aug; 84(8):1261-4.

[7] Pardridge WM. Brain drug targeting: the future of brain drug development. Cambridge University Press; 2001 May 31.

[8] Patel AA, Patel RJ, Patel SR. Nanomedicine for Intranasal Delivery to Improve Brain Uptake. Current drug delivery. 2018 May 1; 15(4):461-9.

[9] Janes KA, Fresneau MP, Marazuela A, Fabra A, Alonso MJ. Chitosan nanoparticles as delivery systems for doxorubicin. Journal of controlled Release. 2001 Jun 15; 73(2-3):255-67.

[10] Craparo EF, Pitarresi G, Bondì ML, Casaletto MP, Licciardi M, Giammona G. A Nanoparticulate Drug-Delivery System for Rivastigmine: Physico-Chemical and in vitro Biological Characterization. Macromolecular bioscience. 2008 Mar 10; 8(3):247-59.

[11] Keum CG, Noh YW, Baek JS, Lim JH, Hwang CJ, Na YG, Shin SC, Cho CW. Practical preparation procedures for docetaxel-loaded nanoparticles using polylactic acid-co-glycolic acid. International journal of nanomedicine. 2011; 6: 2225.

[12] Sangeetha S, Deepika K, Thrishala B, Chaitanya CH, Harish G, Damodharan N. Formulation and in vitro evaluation of sodium alginate nanospheres containing ofloxacin. Int J Appl Pharm. 2010; 2(4):1-3.

[13] Khaira R, Sharma J, Saini V. Development and characterization of nanoparticles for the delivery of gemcitabine hydrochloride. The Scientific World Journal. 2014; 2014.

[14] Chopra M, Kaur P, Bernela M, Thakur R. Synthesis and optimization of streptomycin loaded chitosan-alginate nanoparticles. International Journal of Scientific & Technology Research. 2012 Nov 25; 1(10):31-4.

[15] Ramachandran R, Shanmughavel P. Preparation and characterization of biopolymeric nanoparticles used in drug delivery.