Donepezil loaded PLGA Nanoparticles, from Modified Nano-Precipitation, an Advanced Drug Delivery System to treat Alzheimer Disease

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ABSTRACT- In present works we synthesized Donepezil loaded PLGA nanoparticles (NPs). The approach of our research group was to prove the improvement of drug transport through the Blood Brain Barrier when donepezil was loaded with PLGA. It promoted the uptake of drug into brain endothelium compared with the free drug and play a significant role in the treatment of Alzheimer’s disease (AD). The NPs were synthesized by modified Nano precipitation method. These synthesized polymeric nanoparticles were characterized for particle size, Polydispersity index (PDI) and Zeta potential. The average size and PDI of drug loaded polymeric nanoparticle for preferred formulation were found to be 40.8 nm and 0.188 respectively. The Entrapment Efficiency was 74% and Process yield was 78%. The electron microscopic images of polymeric nanoparticles suggested that the particles were spherical in shape. The pharmacokinetics showed that the release behavior of NPs were very much similar to sustained release and follow Hixson Crowell model.

Keywords- Alzheimer, Blood Brain Barrier, Donepezil, Nanoparticles, Pharmacokinetics

Introduction- Alzheimer’s Disease (AD) is a neurodegenerative disorder1. It is associated with cognitive and behavioral impairment. AD is a common type of dementia2. As per “World Alzheimer Report 2019” there were more than 50 million people living with dementia in all over world, a figure set to increase to 152 million people by 2050. The Conventional treatments for AD in currents are acetyl cholinesterase inhibitor drugs which only suppressed the degradation of Acetylcholine but failed to cure AD because of their poor solubility and lower bioavailability3. These drugs are also unable to cross the Blood Brain Barrier (BBB)4. But nanotechnology provides design, Characterization and medicinal applications for Nano scale drug delivery system. The nanotechnological treatments promising the formation of polymeric NPs and advance delivery of therapeutic devices to the brain via various administrative routes5-7.

In the modern era, AD will spread out over world. A large number of drug modifications have been designed, which have focused on ‘how to cross the BBB’. The symptoms in AD consist of three stages as mild, moderate and severe. Donepezil, a piperdine derivative approved by USFDA in 1986, is the only drug which is used for the treatment of all stages of AD. Donepezil is an acetyl cholinesterase inhibitor drug used for the treatment of AD8-9. Although the therapies have been used presently for treatment of AD still lack of efficacy because of higher concentration of donepezil is prescribed to target the brain. This leads to various side-effects in gastrointestinal alteration. In treatment of AD, BBB is the major issue. The continuous growth in
the number of patient of AD, Anti-Alzheimer’s treatment is rapidly going on. Therefore the drug delivery by polymeric NPs have used to enhance the localized therapeutic effects of the drug\textsuperscript{10-12}.

The polymeric NPs used for the drug delivery have been providing an alternate path for the treatment of AD through the systematic route of administration and reached to any human organs without any enzymatic degradation of drug. The smaller size of polymeric NPs can cross BBB easily and increase the pharmacological activity of drug at Central nervous system. These polymeric NPs can be prepared easily. Poly(lactic-co-glycolic acid) (PLGA) is USFDA approved polymer used as a host for therapeutic devices due to its Bioavailability and biocompatibility. Among the diverse methodologies, we were using modified nanoprecipitation method for the preparation of polymeric NPs\textsuperscript{13-14}.

In this context, the objective of present work was to prepare the Donepezil loaded polymeric NPs by modified Nanoprecipitation method as advanced drug delivery system to treat AD. Polymeric NPs were prepared by using biodegradable and biocompatible PLGA as a polymer and surfactant used in this method was Poly vinyl alcohol (PVA). Donepezil used as a drug because of their activity in all stages of treatment of AD\textsuperscript{15-17}.

Materials

Donepezil hydrochloride was obtained from Alembic pharma limited as a gift sample. PLGA (molecular weight in between 30,000 - 60,000, dl-lactide:glycolide of 50:50), Acetone and PVA were purchased from Sigma Aldrich. Dialysis bag (molecular weight in between 12000 - 14000 Dalton and pore size 2.4 nm) was purchased from Hi Media. And all the other chemicals of AR grade were used in the experiment. In entire experimental work purified Milli-Q quality purified water was used.

Method

Modified Nano precipitation method was used for the synthesis of Donepezil loaded polymeric NPs. In which PLGA and Donepezil hydrochloride taken in organic solvent as organic phase and the Surfactant with water taken as aqueous phase. Sonicate both Organic and aqueous phases for 30 seconds under bath sonicator (Bronson, Delhi, India). Then Organic phase was continuously added into aqueous phase (with continue stirring on magnetic stirrer, Remi India) drop by drop with the help of syringe. The formation of precipitate was immediately appeared. After that for the evaporation of solvent, the mixture was stirred for four hours under atmospheric condition at 300 rpm. These prepared polymeric NPs solution was analysed for particle size, Polydispersity index and surface charge by using instrument (Zeta/Particle size analyzer, Nanoplus).

The suspension of polymeric solution was centrifuged at 20000 RPM for 15 minutes to disparate the polymeric NPs from free Donepezil and excess amount of PVA. There after the obtained polymeric NPs were washed with Milli-Q quality purified water and remains suspended NPs
were lyophilized for future preservation by instrument (LAMBCONCO, GNCIIM, Freezing at -43°C and 0.2-1.0 mPa) for future preservation.

Results and Discussion

1. Particle size, PDI and Zeta potential-

The average size, size distribution and PDI of Donepezil loaded PLGA NPs were studied by using instrument (Zeta/ Nano particle size analyzer, Nanoplus). The prepared donepezil loaded polymeric NPs were discrete, average particle size was 40.8 nm and PDI was 0.188. The negative zeta potential value of polymeric NPs prevented the aggregation of the particles because of electrostatic repulsion between them.
2. Surface morphology

Morphological study of prepared polymeric NPs was done by using Transmission Electron microscopy (TEM) and Scanning Electron Microscopy (SEM). TEM analysis was done by using an instrument (TECNA) for the study of size and shape of polymeric NPs and SEM analysis was done for the surface morphology by using instrument (NOVA, NANO FESEM 450). SEM and TEM image of polymeric particles showed the particles were smooth and spherical in shape.

![TEM Image](image1.png) ![SEM Image](image2.png)

Figure 2: Images of (a) TEM and (b) SEM

3. Entrapment Efficiency (EE) and Process yield

The prepared polymeric NPs were disparate from the dispersed solution by centrifugation at 20000 RPM for 15 minutes. These obtained supernatant was diluted properly and used for the analysis of free Donepezil by using UV-Visible spectrophotometer (Labindia).

The percentage EE and Process yield were calculated by using following formulas-

\[
\text{\% EE} = \frac{(\text{Total amount of drug} - \text{free amount of drug}) \times 100}{\text{Total amount of drug}}
\]

\[
\text{\% Process yield} = \frac{(\text{Total amount of NPs recovered}) \times 100}{\text{Amounts of (Polymer + Drug + Surfactant)}}
\]
The percentage EE and process yield for the preferred formulation were found to be 74% and 78% respectively.

4. In-vitro drug release study

In-Vitro drug release study of prepared polymeric NPs was done by Dialysis bag method. In which 5 ml of sample was put into the dialysis bag and tied it from both side. There after the dialysis bag was immersed in compartment of receptor which contains 100 ml phosphate buffer solution (pH 7.4), stirring at 100 RPM. The temperature was maintained 37±1℃. 5 ml of aliquots withdrawn from the receptor compartment at different time intervals and replaced it with freshly prepared phosphate Buffer solution (pH 7.4). The concentration of drug was studied by using UV-Visible spectrophotometer. These exercise performed three times. The 100% drug release of the formulation was completed within 24 hour and release behavior of prepared NPs was very similar to sustain release.

5. In-Vitro drug release kinetics

The kinetic study of polymeric NPs were studied by fitted drug release data with different mathematical models such as-

| S.N. | Kinetic models                    | Mathematical Expression          |
|------|----------------------------------|----------------------------------|
| 1    | Zero order model                 | \( Q_t = k_0 t \)                |
| 2    | First order model                | \( \log Q_0 - \log Q_t = k_1 t/2.303 \) |
| 3    | Huguchi model                    | \( Q_t = k_0 t^{1/2} \)          |
| 4    | Hixson-Crowell model             | \( Q_0^{1/3} - Q_t^{1/3} = k_{HC} t \) |
| 5    | Korsemeyer peppas model          | \( Q_t = k_{KP} t^n \)           |

In-Vitro drug release kinetics of prepared polymeric NPs was analyzed by plotted graph for different mathematical models. The best fitted Correlation \( (R^2) \) value showed that Hixson-Crowell model was followed by prepared polymeric NPs with \( R^2 = 0.9735 \). It revealed that NPs were sustained in nature. It also supported that the dissolution rate was not occurred by diffusion rather it occurred through polymer matrix. Hixson-Crowell model also supported the SEM and TEM images that the drug particles were spherical and smooth.
Figure (3): Plots for (a) Zero order model, (b) First order model, (c) Huguchi Model, (d) Hoxson-Crowell model and (e) Korsemeyer peppas model

Conclusion

The current study demonstrates the role of Nanotechnology in the field of biomedical science. Donepezil loaded PLGA NPs were prepared by modified Nano precipitation method. As described in result section, the prepared Donepezil loaded PLGA NPs have average size around 40.8 nm and negative surface charge. These prepared small NPs will show advantageous while considering in vivo therapies in future by decreasing side effects, crossing Blood Brain Barrier (BBB) and enhanced therapeutic effect. Due the presence of surfactant as a stabilizer these NPs were stable for at least 3 months. The preferred formulation of polymeric NPs were found to be spherical in shape. The results showed that the formulations have great EE and process yield. We could forecast the size of particle and EE for different combination of the preparation variable. The pharmacokinetics study showed sustained release of drug from NPs and follow Hixson Crowell model. The longer term clinical trial and attempts to increase their impact in treatment of anti-Alzheimer drug are highly needed in order to support the findings.

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