Original Article

Nanotechnology Based Drug Delivery System Of Rivastigmine For The Treatment Of Alzheimer's Disease

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Abstract

Rivastigmine is the important drug used in the treatment of Alzheimer's disease and it is a reversible cholinesterase inhibitor [1]. Rivastigmine is very poor bioavailability through oral route, to overcome these problems Rivastigmine nanoparticles were prepared and administered through route of intranasal passage. Nasal drug delivery systems are having many advantages like rapid onset of action, less side effects to cross the blood-brain barrier Nano formulations plays an important role and great potential to analyse the limitations of the neurodegenerative diseases and many other diseases. Novel Nano formulations used in this method were developed and successfully optimized with many parameters for the treatment of Alzheimer's disease [2]. At a specific concentration of 1% P80 mixed with chitosan, drug was dissolved within this study. There is no toxic effect for this formulation. Rivastigmine-loaded chitosan nanoparticles were successfully optimized using at a specific concentration of 1% P80 mixed with chitosan. Optimization was done by taking formulation variables such as taking various concentrations of polymer, drug, cross linking agent, surfactant, cross linking time, stirring speed. Coated nanoparticles showed particle size is less than 200nm and rivastigmine permeation is more in invitro drug permeation indicating nasal sheep. Rivastigmine nanoparticles are prepared to avoid the poor bioavailability and to increase the bioavailability, safety and efficacy

Chitosan is very important in its nature as polycation [3] and its charge density depends on the degree of acetylation and pH of the media. acetylation degree and molecular weight decides the polymer solubility. EUDRAGIT NE 30 D is the aqueous dispersion of a neutral polymer based on ethyl acrylate and methyl methacrylate. Important features to choose EUDRAGIT NE 30 D is no need to add plasticizer, highly flexible and Suitable for matrix structure. Eudragit NE 30 D contains α-(4-nonylphenyl) ω-hydroxypoly-(oxy-1, 2-ethanediyl), namely nonoxynol 100 (1.5%). Talc addition is required to avoid tackiness and agglomeration of the coated pellets. Melting point (~ 60°C) is high, due to this nature leads to increase in drug release. Due to the excellent mucoadhesive properties [4] of EUDRAGIT NE 30 D extends the contact time between drug and mucus layer, disruption of tight junctions, permeation increases and prolong the duration of action, respectively. Current studies are investigating to develop mucoadhesive rivastigmine loaded in chitosan and coated with EUDRAGIT NE 30 D for intranasal delivery, which is useful for the first-pass metabolism of drugs, help to achieve a sustained drug release over a prolonged period. Based on the review of literature this is the first study by coating with EUDRAGIT NE 30 D where rivastigmine was loaded with chitosan. By taking many different types of formulation and parameters, method was optimized to obtain chitosan nanoparticles with achieving the quality. other polymer also used for nose to brain delivery is poly (lactic -co-glycolic acid)

These developed Nano formulations showed significant difference when compared with free drug. This novel method of approach has enhanced the Nano formulations. stability. Finally, these formulations may hold the potential to contribute to develop an effective treatment of AD [5]. If intranasally administered, drugs survive the mucociliary clearance in the vestibular region, move to the posterior regions of nasal cavity and contact with respiratory epithelium in the respiratory region. Some of them are absorbed through the epithelium into the blood or the lymphatic system, being subsequently transported into the systemic circulation.[6].

Keywords: Eudragit NE 30 D, Rivastigmine, Alzheimer's disease, Nano formulation

INTRODUCTION

Alzheimer's Disease (AD) is one of the most common forms of chronic neurodegenerative disease affecting over 46 million people, according to AD International leads to brain damage [7]. From the last few decades, there has been considerable effective in nanomedicines development. Using nanocarriers A medicine is only effective if it reaches its target. Some of the active molecules can be demonstrated very efficiently in vitro. nanocarriers decreases the side effects. Nanotechnology has had a great contribution in Drug Delivery Systems (DDS) development [8]. These DDS could function as reservoirs for sustained drug release or control the pharmacokinetics and biodistribution of the drugs. In the current view, using nanotechnology as DDS for the clinically used cholinesterase inhibitor drugs Rivastigmine),[9] used for AD treatment Regarding DDS used for Rivastigmine, our research work dealt with polymeric nanoparticles (NPs) including poly (lactic -co-glycolic acid), EUDRAGIT NE 30 D and chitosan The highest application of NPs was related to polymeric NPs and modified LPs.

Material Used:

Rivastigmine tartrate (purity > 98%) was procured from Sigma Aldrich Bangalore India. Low-molecular-weight chitosan (50,000 Da; 75–85% deacetylated) was purchased from Acros Organics Ltd Eudragit NE 30D and poly (lactic -co-glycolic acid) was received from Evonik India, Mumbai, India. P 80, poly (lactic -co-glycolic acid) and glutaraldehyde were obtained from Industrial Grade, Indore, India. Glacial acetic acid was procured from High Purity Lab Chemicals, Mumbai, India. Light and heavy liquid paraffin were obtained from Central Drug House, New Delhi, India.

Preliminary Optimization:

Preliminary Optimization has done for the different Parameters for Blank Nanoparticle Preparation
Blank Nanoparticles preparation is important to choose stirrer position and the external phase. Stirrer position is optimized to know the position of stirrer if it sets in the top, in the middle or in the bottom to know the nanoparticles formation. As well we need to choose the external phase by doing the experiment with different phases first one is with Light liquid Paraffin, second one is with heavy liquid paraffin. a stable emulsion was obtained with heavy liquid paraffin, whereas the flakes were formed with the combination of light and heavy paraffin. On the other hand, light liquid paraffin was found to be suitable for the creation of nanoparticles. Therefore, it was selected as an external phase for further investigations. It was shown that at middle position of the stirrer was set, it produced better and desirable results when compared to the other positions. Therefore, the middle position of the stirrer was selected for further examination

Optimization of Parameters to Produce Drug-Loaded Nanoparticles

First step is to optimize the chitosan, different batches of nanoparticles are prepared in the ratio of 1%, 2% and 3%respectively other components should fix and add the amount of drug in batches wise to know the influence of drugs on the characteristics of formulations. In the same way volume percentage of cross-linking agent, stirrer speed in different speeds, cross linking time also in a batch wise to get the desired quality product**.**

Design of the Rivastigmine-Loaded Nanoparticles

Here discussing about independent variables and Dependent variables. In this we are keeping three Independent variables such as drug-to-polymer ratio, stirrer speed, and cross linking time.
Dependent Variables are the variable that is being measured or tested in an experiment. Two Independent variables are % drug release and % EE

Preparation of Nanoparticles

Weigh accurately amount of drug and chitosan were added to 10ml of concentration of 3 mg/ml in 0.5% acetic acid solution, then raised to pH 4.6 with 10 N NaOH and filtered the solution using 0.45 filter. The emulsion cross linking agent was chosen for preparation of chitosan nanoparticles containing rivastigmine tartrate by a method described. Chitosan should be dissolved completely so the sonication process continued still formed a transparent gel. Then prepare mixture of P 80 and liquid paraffin in a beaker. And stirred properly to get a uniform solution. So that drug-polymer solution was added drop wise to the to the external oil phase with constant stirring at rpm for 10-15min. Then add glutaraldehyde solution drop wise to the emulsion to facilitate cross linking and stirring continued for 3–5 h. The suspension was left to stand for 20 min to allow nanoparticles to sediment under the force of gravity. The supernatant was discarded, and the remaining portion constituting nanoparticles with small amounts of oil was separated employing a vacuum filter The nanoparticles were then washed 4–5 times using petroleum ether to remove the traces of oil from the surface of nanoparticles and freeze-dried at −60 °C for 24 h. Finally, it was air-dried for 24 h at room temperature (25 ± 1 °C) and stored in a cool and dry place.

Chitosan

Chitosan-based formulation with different concentrations of polymer, drug, surfactant, cross linking agent, and processing variables, such as stirring speed and cross-linking time on the quality of the product [10]. All the samples were subjected to HPLC assay and concentration was measured using the standard curve plotted for Rivastigmine in PBS. Accumulated concentration in the receiver chamber was measured against free drug used as a control in the plate.

Chitosan – it is defined as a decomposable natural polymer. It has many uses like nontoxicity, bio compatibility, and biodegradability.

Applications are as follows:

1)Medicine.[11] As a delivery carrier, it has great potential and cannot be compared with other polymers.

Chitosan is very difficult to solubilize in water,

It can be solubilized in acidic solution. Its insolubility in water is a major limitation for its use in medical applications. Many techniques as acylation, alkylation, sulfation, hydroxylation, quaternization, esterification, graft copolymerization, and etherification. These techniques are useful for synthesis of Chitosan derivatives chemical modification. Chitosan has chemical properties superior to unmodified chitosan [12]. For example, nanoparticles produced from chitosan derivatives can be used to deliver drugs due to their stability and bio compatibility. This review mainly focuses on the properties of chitosan, chitosan derivatives, and the origin of chitosan-based nanoparticles. In addition, applications of chitosan-based nanoparticles in drug delivery, vaccine delivery, antimicrobial applications,[13] and callus and tissue regeneration are also presented. In summary, nanoparticles based on chitosan have great potential for research and development of new nano vaccines and nano drugs in the future.

In Vitro Release of Rivastigmine from the Designed Batches:

For the In Vitro Release of Rivastigmine from the Designed Batches studies, we are using dialysis tube method. Weigh accurately 6 mg of drug dispersed in 2 ml of nasal fluid with PH 6.4 and placed in receptor compartment. Receiver fluid temperature was maintained at 37 ± 0.5 °C. 3 ml of sample solution was taken from the receptor compartment at different time intervals, Samples was measured at 220nm using beer-Lambert's Law equation. It is used for the calculation of % drug release at different times. Kinetics and possible mechanisms of drug release from formulations were evaluated by fitting the data into various mathematical models.

% Entrapment Efficiency:

It is defined as percentage of the content was estimated as the difference between the initial drug quantity and the free or unentrapped quantity of drug in the supernatant with respect to the total quantity incorporated in the nanocarrier preparation

It is given by the formula

$$\%EE = \frac{\text{Amount Of Drug In Nanoparticles}}{\text{Amount Of Drug used}} \text{x } 100$$

Weigh accurately 50mg of nanoparticles and dissolve in 50 ml of acetonitrile and shake with magnetic stirrer then kept aside for some time between 2-4 hours followed by filtration then concentration of drug in acetonitrile and measured at 220 nm by using UV Spectrophotometer to know the amount of drug in the nanoparticles.

Drug loading: It is also same method as above It is given with following formula

$$\%EE = \frac{\text{Amount Of Drug In Nanoparticles}}{\text{Amount Of Nanoparticles formed}} \text{x } 100$$

Coating of the Optimized Nanoparticles:

The emulsion solvent evaporation technique is used for the Nanoparticles coating. Eudragit NE 30D is soluble in acetone. Different ratios of nanoparticles are prepared to know the drug release effect.

Light liquid paraffin was selected as the external phase and P 80 was used as an emulsifier for the coating. Optimized batch D6 was selected as the core and the coating was done by the method the required quantity of polymer was dissolved in acetone and previously prepared chitosan nanoparticles were added to the polymeric solution with constant stirring. The mixture was then added drop wise to light liquid paraffin and P 80 solution with magnetic stirring at constant speed for 2 h to evaporate acetone completely. The hardened nanoparticles were recovered by centrifugation and washed three times in petroleum ether to remove the excess of oil. Nanoparticles were then lyophilized and stored in a cool and dry place. Different ratios of nanoparticles and the coating polymer-eudragit (1:1 (batch C1), 1:3 (batch C2), and 1:5 (batch C3) was tested to fabricate the coated nanoparticles.

Different ratios of nanoparticles and the coating polymer-poly (lactic -co-glycolic acid) were tested to fabricate the coated nanoparticles

Polydispersity Index(PDI)

Particle Size Distribution refers to size distribution in a homogenous population of nanocarriers [14]. It describes the range of nanocarriers and degree of non uniformity of size in the system. It depends on number of factors like composition of nanocarriers as well as characteristics of the detergents used in them. It's analyzed within a scale between0.03 to0.7 as it suggests that PDI value below 0.03 (fig 2) are largely monodispersed norms and if its over0.7, it suggests that the sample has broad size distribution that it'll not be suitable to assay by DLS. These measures limit refers to ISO standard documents. PDI value of 0.0 suggests that the sample has invariant size distribution and if its1.0, it suggests that the sample has multiple size distribution. In case of polymer grounded nanocarriers, the most common respectable value is0.2 PDI whereas in case of lipid grounded nanocarriers, PDI value is most respectable and considerable.

Zeta potential

Another important parameter for an indirect evaluation of stability of the nanoparticles is the surface charge on these particles [15]. This is often referred as zeta potential. It is an electric potential at the shear plane. Particles moving in a medium are surrounded by a liquid layer attached to them carry this charge on their surroundings. It is testified that the particle with zeta potential value of 30.20 have less stability whereas zeta potential value below 5mV will result in fast aggregation of the particles. It has been reported that the particles with zetapotential between -30mVand30mV are most likely to be agglomerated. In contrast to these, zeta potential over 60mV are found to be most stable particles. This can be influenced by number of reasons such as the nature and ratio of surfactant. If an anionic surfactant is used, then the zeta potential tends to be higher in turn. Presence of ethanol in the particles also impact zeta potential It also determines the interaction of nanocarriers with bio-active compounds and biological environment. This can be used to evaluate hydrophobicity at the surface and the nature of the material coated on the surface or encapsulated inside

In this thesis, it was aimed to keep the selection of the formulations within this range from -30mV to +30mV in

order to achieve stable particles

Drug entrapment efficiency

This is another parameter to test the efficiency of the Nano formulations [16]. It measures the capacity of a nanoparticle to entrap a drug. This can be calculated using formula % drug entrapment= W-w/W x 100.

Where W represents the amount of the drug added and w represents the amount of drug present in the supernatant.

Toxicity

After the administration, these formulations get distributed to different part of the body, which are then excreted by the kidney. Thus, it is important to determine the toxicity of these Nano formulations. Surface charge of the Nano formulations play a crucial role in toxicity of the formulations as the cationic formulations can cause instability of the cell membrane or cell lysis. In this work, cytotoxicity of each formulation was tested using MTT assay.[17]

Freeze-drying for the preparation of samples for various techniques

It is the process of removal of frozen solvents or ice from the material through the process of sublimation and elimination of water molecules via desorption. It involves the removal of water contents from the product in a frozen state at a very low pressure. In the previous studies, it was indicated that freeze dried liposomes retained as much as 100% of their original substances [18]

It maintains the low temperature conditions during the process so that it does not affect appearance or features of the product. It can be applied to various materials such proteins, microbes, tissues, plasma as well as pharmaceuticals. This is also known as lyophilization depending on the location or industry it is being used in

Sublimation is the state when ice in solid phase changes directly to its vapour phase without going into liquid phase. It consists of three stages. Freeze, vacuum and dry. At freeze stage, the product is frozen in a tray, flask or vial. At vacuum stage, the product is placed in a vacuum and at dry stage, heat energy is provided to the product causing ice to sublime

Most commonly freeze-drying equipment consists of vacuum system, product chamber and condenser. The vacuum system includes separate vacuum pump connected to an airtight condenser and attached product

chamber. Product chamber includes either mini folds where flasks or tubes can be attached or a larger chamber with few shelves where product can be placed on. The condenser is the part of the equipment, which attracts vapours being sublimed of the products during the process. These vapours condense back to solid forms or ice due to low energy levels maintained in the condensers. Shelf freeze dryer could have condenser located inside the product chamber connected internally. In the shelf freeze dryers, heat is transferred into the samples via conduction. This it is important to maximize the contact of the sample vials to the surface of the shelves

In this thesis, Each type of formulation was freeze dried to be able to perform FTIR. 1ml of each formulation was kept in a-20°C freezer overnight and once the samples were fully frozen, these samples were subjected to 2 shelved freeze dried in a glass vial overnight. Samples were prepared by freeze drying each sample using the method described above. After freeze-drying, each formulation resulted in Powdered samples.

Stability of nanoformulations

Once the nanoparticles are prepared, it is important to measure the stability of the particles prepared. This can be achieved by measuring particle size, PDI and Zeta potential at different times such as in days, weeks and months.

Conclusion:

Nano formulations plays an important role and great potential to analyze the limitations of the neurodegenerative diseases and many other diseases. Novel Nano formulations used in this method were developed and successfully optimized with many parameters for the treatment of Alzheimer's Disease. At a specific concentration of 1% P80 mixed with chitosan, drug was dissolved within this study. There is no toxic effect for this formulation. Rivastigmine-loaded chitosan nanoparticles were successfully optimized using at a specific concentration of 1% P80 mixed with chitosan. Optimization was done by taking formulation variables such as taking various concentrations of polymer, drug, cross linking agent, surfactant, cross linking time, stirring speed. Coated nanoparticles showed particle size is less than 200nm and rivastigmine permeation is more in invitro drug permeation indicating nasal sheep. Rivastigmine nanoparticles are prepared to avoid the poor bioavailability and to increase the bioavailability, safety and efficacy

Table 1:

Drug %	**Chitosan %**	**Appearance**
1%	1%	**Clear**
0.5%	1%	**less cloudy**
0.1%	1%	**Very cloudy**

1%	0.5%	**Less clear**
1%	0.1%	**Very cloudy**
0.5%	0.1%	**clear**
0.1%	0.5%	**Less Clear**
0.1%	0.1%	**Less Clear**

Table 2 Formulation composition and characterization parameters of batch 7 to fabricate nanoparticles containing rivastigmine.

Components	**Optimized values**
Chitosan	1%
P80	1%
Glutaraldehyde	3 mL
Stirrer speed	1500 rpm
Cross linking time	3 h
Average particle size	150 nm
% EE	65.92
% Drug loading	11.78
Aggregation	no

% Mucoadhesion	88
% Cumulative drug release (within 8 h)	83.78

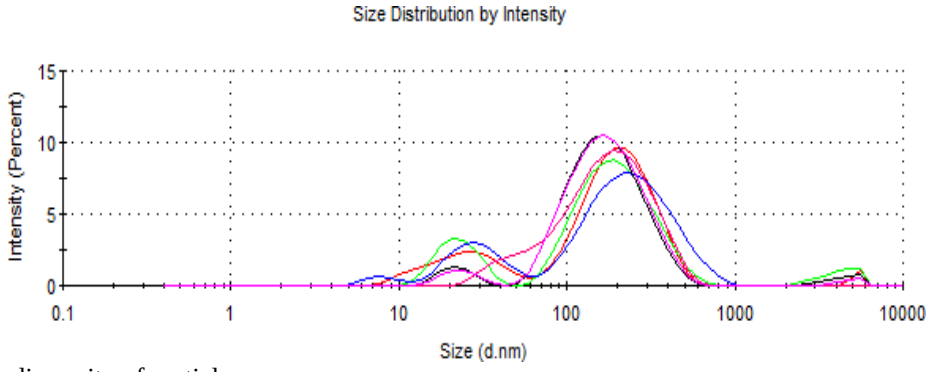
Table **3**: Particle size and zeta potential of following batches

Batches	**Particle size**	**Zeta potential**
D1	165.2 ± 33.1	**22 ± 5.8**
D2	161.4 ± 33.3	**21 ± 3.8**
D3	154.2 ± 30.7	**19 ± 2.1**
D4	151.9 ± 28.5	**18 ± 5.2**
D5	148.9 ± 29.2	**20 ± 5.8**
D6	143.2 ± 28.4	**21 ± 3.2**
C1	173.4 ± 41.1	**19 ± 3.6**
C2	182.3 ± 40.9	**18 ± 4.8**
C3	191.1 ± 43.9	**22 ± 5.2**

Table 4: dependent variables Responses for following batches.

Batches	**Cumulative Drug Release (%) after 8 h**	**% EE**
B1	78.75	43.45
B2	72.33	57.32
B3	72.43	49.32
B4	67.23	75.62
B5	83.23	40.23

B6	77.23	55.22
B7	76.34	45.23
B8	70.76	73.23



Spectrum of polydispersity of particles

Figure 1: Spectrum of polydispersity of particles

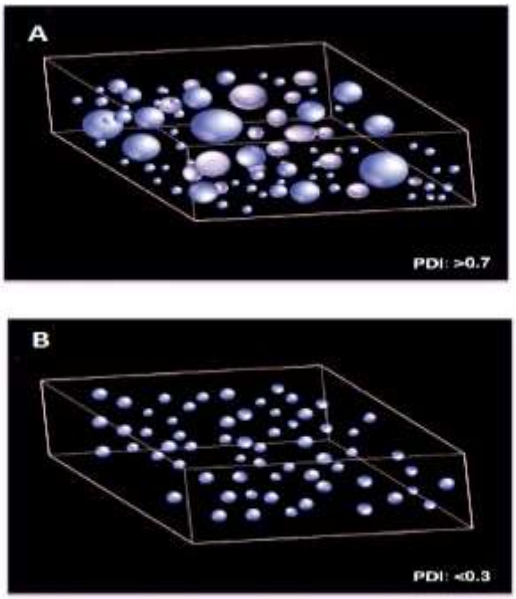


Figure 2 Representation of particle size distribution based on particle size:

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