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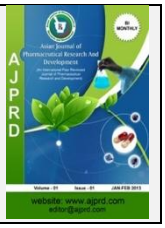

Open Access **Research Article**

Formulation and Evaluation of Liposomal Based Nanocochleate

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A B S T R A C T

Rivastigmine is a parasympathomimetics and a reversible cholinesterase inhibitor. It inhibits both acetylcholinesterase and butylcholinesterase. It is used to treat mild to moderate dementia caused by Alzheimer's or Parkinson's disease. However, its bioavailability is about 36% due to hepatic first-pass metabolism. The plasma half-life of rivastigmine is approximately 1.5 hours. Rivastigmine tartrate-loaded nanococlets (RN) were developed using a trapping method by addition of calcium ions to preformed negatively charged liposomes (RL) prepared by thin film hydration method. Liposomes were optimized by varying the concentrations of disteroylphosphodylcholine (DSPC) and cholesterol. The optimized liposomal batch (RL) containing DSPC (40mg) and cholesterol (10mg) showed mean particle size, zeta potential and entrapment of 112 ± 4 nm, -51.70 ± 7.3 mV and 99.6 ± 4 % for the final form, 51.70 ± 7.3 mV. Observed with efficiency. RN3 which was developed from RL showed 631.4 ± 4 nm, -17 ± 6.5 mV and 99 ± 4.20 %. Encapsulation of RT in nanocochleates is to protect the drug from rapid metabolism, thereby improving bioavailability and ensuring controlled release. In vitro drug release studies showed extended release up to 24 hours, reduced dosing frequency compared to plain drug formulation, nearly 100% of drug was released within 8 hours from pure RT solution, while powder nanococlets released 69% of drug. 8 hours respectively. Stability studies also concluded that nanocochleates are more stable than liposomes.

Key words: Liposome, Nanocochleate, Disteroylphosphatidylcholine, Bioavalability, Dementia, Alzhimer Disease, Parkinsons, Cholineesterase Inhibitors, Parasympathomimetics

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INTRODUCTION

Improving bioavailability and formulation techniques are always at the forefront of the development of new formulations using nanotechnology, where researchers focus on changes in drug delivery systems. Essentially, liposomes are vesicles containing at least one lipid bilayer composed of phospholipids and cholesterol, which are packaged to deliver nutrients or drugs in the desired form. Among these lipid-based nanocarriers, liposomes, cochleates, and new multilayer nanocarrier systems emerged as hydrophilic and hydrophobic drugs with better and improved stability, efficacy, increased drug permeability, and decreased drug dosage. except special drugs that produce fewer side effects.[1] Nanocochleate drug delivery is based on encapsulating the drug in a multi-layered, lipid crystalline matrix, which can safely and effectively deliver the drug to the target site. Various lipid-based nanocarrier systems such as lipoproteins, lipid nanoparticles, lipid nanocapsules, and liposomes are available, but they show limitations due to stability, oxidation, and incompatibility for the delivery of proteins and peptides. Cochleates have been developed as an alternative to lipid-based drug delivery systems. Many therapeutic agents, especially biological molecules, are not absorbed in the gut due to their inability to penetrate tissue membranes and undergo enzymatic degradation in the GIT wall.[2] Cochleate consists of a special structure, phospholipid bilayer, and hydrophobic and hydrophilic drugs are added to prevent oxidation, increase permeability, and reduce drug dosage. Thus, it provides a potential delivery system for various drugs. This novel nanocarrier system approach is applicable to macromolecules as well as hydrophobic small molecule drugs and drugs with poor oral bioavailability.[3]

The alternative structure of lipids in nanocochleates encapsulates drugs without chemical binding. The hydrophilic and hydrophobic parts of phospholipids enable the transport of hydrophobic, hydrophilic and amphiphilic drugs, presenting a wide range of applications. They have the flexibility to change the direction of administration if necessary, as nanocochleates are administered sublingually, parenterally, orally and topically. This versatility makes it

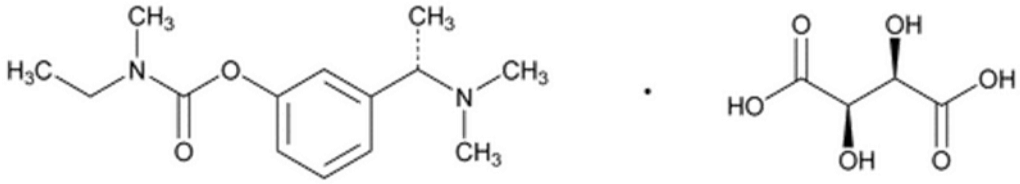
ideal for any treatment you need. Widespread use of nanocochleates technology will provide better outcomes for patients. This research describes the fundamentals and potential applications of nanocochleates.[4]

ALZHEIMER DISEASE

Alzheimer's disease is a brain disorder that worsens over time. It is characterized by changes in the brain that lead to deposits of certain proteins. Alzheimer's disease causes the brain to shrink and brain cells eventually die. Alzheimer's disease is the most common cause of dementia characterized by a gradual decline in memory, thinking, behavior and social skills. These changes affect a person's ability to function. About 6.5 million people age 65 and older in the United States are living with Alzheimer's disease. Of these, more than 70% are aged 75 years and above. Of the approximately 55 million people worldwide with dementia, 60% to 70% are estimated to have Alzheimer's disease. Alzheimer's disease is a brain condition that causes a progressive decline in memory, thinking, learning and organizing skills. It eventually affects a person's ability to carry out basic daily activities. Dementia describes the state of a person's mental function. It is not a specific disease. It is a decline in mental function from a previously high level that is severe enough to interfere with daily life. [5]

Rivastigmine Tartrate:

Rivastigmine Tartrate is a parasympathomimetic and reversible cholinesterase inhibitor. Rivastigmine inhibits both butylcholinesterase and acetylcholinesterase. It is used to treat mild to moderate dementia caused by Alzheimer's or Parkinson's disease. Rivastigmine has shown therapeutic effects on cognitive (thinking and memory), functional (activity of daily living), behavioral problems commonly associated with Alzheimer's and Parkinson's disease dementia. Rivastigmine tartrate is available under the brand names Exelon, Rivadem, etc. No apparent mechanism of action of rivastigmine tartrate has yet been reported.[6]



Chemical Structure of Rivastigmine Tartrate

MATERIALSANDMETHODS

Materials

Rivastigmine Tartrate was gifted from sun pharmaceutical Industries Limited, Mumbai, Disteroylphosphotidylcholine was gifted from Vav Lipid Pvt. Ltd., Mumbai, Cholesterol was Procured from Loba Chemie Laboratory, Mumbai, Calcium Chloride and Mannitol were procured from S.D. Fine Chem Limited, Mumbai, Chloroform was procured from S.D. Fine Chem Limited, Mumbai, Methanol was procured from Loba Chemie Laboratory, Mumbai, Ethanol was purchased from Dr. Vitthal Vikhe Patil SakharKarkhana, Ahmadnagar, Pottasium Dihydrogen Phosphate was procured from Loba Chemie Laboratory, Mumbai, Sodium Hydroxide was procured from S.D. Fine Chem Limited, Mumbai, Pottasium Bromide was procured from S.D. Fine Chem Limited, Mumbai.

Methods

Selection of Drug

Rivastigmine Tartrate: Rivastigmine is a parasympathomimetics and a reversible cholinesterase inhibitor. Rivastigmine inhibits both acetylcholinesterase and butylcholinesterase. It is used to treat mild to moderate dementia caused by Alzheimer's or Parkinson's disease, rivastigmine has been shown to treat cognitive (thinking and memory), functional (activities of daily living), behavioral problems commonly associated with Alzheimer's and Parkinson's disease dementia. However, its bioavailability is about 36% (average 30) due to hepatic first pass metabolism. The plasma half-life of rivastigmine is approximately 1.5 hours. Hence it was selected as the active drug for the preparation of liposomal based nanocochleate with sustained release action.(7)

Preformulation studies

A preformulation study is the first step in the rational development of a dosage form. It is an investigation of the physical and chemical properties of the drug substance alone and when combined with excipients. Preformulation checks are designed to identify physicochemical properties and excipients that may influence the formulation design, manufacturing method, and pharmacokinetic parameters of the resulting product.However an understanding of physicochemical properties may ultimately provide a rationale for formulation design or support the need for molecular modification or simply confirm that there is no significant barrier to compound inhibition. Following are the preformed tests for preformulation studies.

Drug identification and characterization [8, 9]

The received drug sample i.e. Rivastimine tartrate was standardized by carrying out the following tests,

Description: A rivastigmine tartrate sample was visually evaluated for appearance and color.

Determination of solubility: The solubility of rivastigmine tartrate was carried out using the saturation method.

Determination of Melting Point:

The melting point of rivastigmine tartrate was determined by taking a small amount of the sample in a capillary tube closed at one end and placing it in a Thilles tube apparatus with a

thermometer. Heat was supplied to the Thiels tube with the help of a burner. Temperatures were obtained with a rinse thermometer. The temperature at which the drug just started to melt was recorded.

Spectrophotometric Method for Estimation of Rivastigmine Tartrate [10, 11]

Determination of Lambda Max: To ascertain the wavelength of maximum absorption of the drug, a drug solution (10µg/ml) in 0.1 N HCl was scanned against 0.1 N HCl using a spectrophotometer in the wavelength region of 400-200 nm as the blank spectra were shown in the graph. No. 1.

Infrared Spectroscopy (FT-IR) Analysis: FTIR analysis of drug sample rivastigmine tartrate was carried out to evaluate its purity. The infrared spectrum of rivastigmine tartrate was taken on an FTIR spectrophotometer. Weigh accurately 5 mg of dry IR grade potassium bromide(KBr) in an agate mortar. Grind the KBr in a mortar until there is no evidence of crystallinity and uniform distribution throughout the KBr. The sample was analyzed in the range of 400 cm-1 to 4000 cm-1. The FTIR spectrum was then studied and compared with the standard. The spectrum was shown in graph N0. 2.

Standard calibration curve of rivastigmine tartrate in 0.1 N HCL (12)

0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8 and 2 ml of the solution having a concentration of 100µg/ml were pipetted into 10 ml volumetric flasks. The volume was titrated with 0.1 N HCL to obtain final concentrations of 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 µg/ml, respectively. The absorbance of each concentration was measured at 263 nm using a UV spectrophotometer. Result Table no. was shown. A graph of absorbance vs Conc was plotted and is shown in graph no.3.

Selection of Polymers and Excipients: As per literature, DSPC, Cholesterol, CaCl2, Mannitol were used to enhance the encapsulation efficiency and stability as well as controlled release of the formulation. Polymer used in different concentrations. In the present work these polymers were selected for enhanced encapsulation efficiency and controlled release formulation.

IDENTIFICATION OF POLYMERS

A. DSPC Identification: The emulsion test is a simple method used in academic settings to determine the presence of lipids using liquid chemistry. The procedure is to suspend the sample in ethanol, which allows the lipids present to dissolve (lipids are soluble in alcohol). The liquid (alcohol with dissolved fat) is then decanted into water. Lipids do not dissolve in water while ethanol does, when ethanol is diluted, it falls out of solution to give a cloudy white liquid. [13]

B. Cholesterol Identification: The melting point of cholesterol was determined by taking a small amount of the sample in a capillary tube closed at one end and placing it in a Thiels tube apparatus with a thermometer. Heat was supplied to the Thiels tube with the help of a burner. Temperatures were obtained with a rinse thermometer. The temperature at which the drug just started to melt was recorded. [14]

C. CaCl2 Identification: Dissolve calcium chloride in dilute sulfuric acid and then add sodium carbonate solution. The formation of a white precipitate of calcium carbonate is the identification of calcium.[15]

D. Drug Excipient Interaction Studies: Drug excipients interaction study was carried out using FTIR spectrophotometer. FTIR spectrum of drug and polymer was recorded on infrared spectrophotometer. The FTIR spectrum was recorded in the frequency range 400 - 4000 cm-1. Graph No. 4 shows the spectrum of rivastigmine tartrate and polymers (DSPC and cholesterol).[16]

Preparation of Liposomal based Nanocochleate [17,18,19,20]

A. Selection of method: For preparation of liposomal based nanocochleate, liposomes were prepared by thin film hydration method. The prepared liposomes were then converted into nanocochleates using a trapping method. The method was to potentially prepare nanocochleate formulations for safeand effective drug delivery.

B. Selection of Controlled Release Polymers: Variable amounts of natural polymers are available for the preparation of liposomal-based nanocochleates. Among those polymers, phospholipid i.e. DSPC was used. These polymers increase the drug encapsulation efficiency and provide a controlled release action. They have high stability at harsh environmental conditions, which is a crucial requirement for various nanoparticulate systems, making them more advantageous than others.

C. Permeation Enhancer: For preparation of liposomal based nanocochleate, permeation enhancer i.e. cholesterol was added to improve fluidity of liposomes, alter bilayer rigidity and stability of liposomes. The rigidity of the liposomal membrane decreases with the addition of cholesterol to the liposomes.

D. Divalent Cation Salts: Divalent metal ions such as Ca+2 are used in liposomes for conversion to nanocochleates. When added to prepared liposomes it leads to the formation of nanocochleates. The divalent ion leads to destabilization of the outer bilayer of the phospholipid, and thus the divalent ion has easier access to the inner bilayer for interaction. The liposomal bilayers then fold, forming stacked sheets of cochleate cylinders.

E. Cryoprotectant: Mannitol was added to the nanocochleates suspension as a cryoprotectant to avoid lysis of the nanocochleates.

FORMULATION OF LIPOSOMAL BASED NANOCOCHLEATES

Preliminary studies were preformed to determine factors that would affect drug retention efficiency and particle size. Their proper concentration was also determined and some of them were fixed for further optimization of nanocochleite preparation and production.

Preparation of Rivastigmine Tartrate-Loaded Liposome (Priliminary Batch)[21]

A thin film hydration method with slight modification was used to prepare RT-loaded liposomes. in short,

- Different ratios of DSPC and cholesterol were dissolved in chloroform:methanol solvent mixture in the ratio of 2 : 1 v/v and then rivastigmine tartrate (RT) was added to this

mixture.

- The above mixture was introduced into a 250ml round bottom flask with a ground glass neck. The flask is then connected to a rotary evaporator and rotated at 60 rpm. The organic solvents were evaporated at about 40ºC.
- The pressure at the cylinder head was gradually increased until there was no difference between the pressure inside and outside the flask. After 15 to 30 minutes a thin film was formed on the inner surface of the flake.
- The flask was then flushed with 10 ml of distilled water and reattached to a rotary evaporator and rotated at room temperature and pressure at a speed of 60 rpm. The flask was then left to rotate for 60 min.
- After which a uniform suspension was formed. This suspension was allowed to stand overnight at 4ºC. Due to hydration of lipids, spherical vesicles were formed.
- Different batches were prepared by varying the concentration of DSPC and cholesterol.
- The effect of DSPC and cholesterol concentration on particle size and entrapment efficiency was evaluated. The initial batch was shown in Table No.1.

Table 1: Preliminary batch of RT-Loaded Liposomes

Formulation	DSPC(mg)	Cholesterol(mg)	RT(mg)
RL1	10	10	3
RL2	20	10	3
RL3	30	10	3
RL4	40	10	3
RL5	10	20	3
RL6	10	30	3
RL7	10	40	3

OPTIMIZATION OF RT-LOADED LIPOSOME

Determination of Entrapment Efficiency (EE) of RL [22]

Entrapment efficiency was calculated by separating non-encapsulated RT from a vesicular suspension of liposomes (100 µL) by centrifugation at 12,000 rpm for 2 h. The sediment vesicles were dissolved in 1 ml of ethanol to release the entrapped drug. This was suitably diluted with phosphate buffer (pH 7.4). (concentrations of 1 to 6 µg/mL in PBS) at 263 nm. EE (%) was obtained using the equation.

$$\text{Entrapment Efficienct (\%)} = \frac{\text{(Amount of drug entrapped in Liposome)}}{\text{(Total amount of drug present)}} \times 100$$

Particle Size Analysis [23]

The particle size of RT-loaded liposomes (RL) was studied by particle size analyzer. This technique is based on the principle of photon cross-correlation spectroscopy. The sample was diluted with distilled water, filled into a transparent cuvette and placed in a thermostated water bath, maintained at 25 ◦C. A laser beam was incident on the sample at a scattering angle of 90◦ and the particles in the sample underwent Brownian motion. The measured particle speed is converted to hydrodynamic diameter using the Stokes-Einstein equation. The average particle size was measured in triplicate. Result Table no. 7 is indicated.

Zeta Potential Measuremets [23]

The surface charge of RL was determined using a zeta potential analyzer. Analysis time was kept for 1 min and average zeta potential and charge and RL were determined. The temperature was set at 25.2 ◦C and 3 runs were performed. The result is shown in Table No. 7.

Preparation of RT-Loaded Nanocochleates [24]

Nanocochleates were prepared from preformed liposomes using a Tarpping method.

- Optimized batches of liposomes were selected for formulation of nanocochleates. For the formation of nanocochlites.
- 50 µL of calcium chloride (CaCl2) solution 1.0 molar was added dropwise to the RT-loaded liposomal vesicles under vortexing using a probe sonicator.
- The vesicle phase immediately became turbid, indicating the formation of nanocochlites.
- Precipitated nanococchlites were refrigerated at 2-8ºC. Optimized batch shown in Table No.2.

Table 2: Formulation of Nanocochleates from optimized liposomal formulation

Formulation	DSPC (mg)	Cholesterol (mg)	RT (mg)
RN1	20	10	3
RN2	30	10	3
RN3	40	10	3

Evaluation of Nanocochleate Formulation :

1. Particle size: The particle size of RT-loaded nanocochlites was studied by particle size analyzer. This technique is based on the principle of photon cross-correlation spectroscopy. The sample was diluted with distilled water, filled into a transparent cuvette and placed in a thermostated water bath, maintained at 25 ◦C. A laser beam was incident on the sample at a scattering angle of 90◦ and the particles in the sample underwent Brownian motion. The measured particle speed is converted to hydrodynamic diameter using the Stokes-Einstein equation. Average particle size was measured in triplicate. The result is shown in Table No. 8

2. Zeta potential: The charge on the surface of RN was determined using a zeta potential analyzer. Analysis time was kept for 1 min and average zeta potential and charge and RN were determined. The temperature was set at 25.2ºC and 3 runs were performed. The result is shown in Table No. 8.

3. Drug content of RT-loaded nanocochleites: The redispersed nanocochleites suspension is centrifuged at 15000 rpm for 40 min to separate the free drug content in the supernatant. Dilute the free drug supernatant with distilled water, then dilute to a concentration of 1 to 6 µg/ml in distilled water. They can be determined by ultraviolet (UV-visible) spectrophotometer. Absorbance was recorded at 263 nm. The % drug content was obtained using Eq. The results were discussed in Table no. 9. [25, 26]

$$\% \text{ Drug Content} = \frac{\text{Absorbance of Test sample}}{\text{Absorbance of Standard}} \times 100$$

4. Entrapment efficiency of RT-loaded nanocochlites: RN (100 µL) was added to a centrifuge tube, centrifuged at 6000 rpm for 20 min, and the supernatant and sediment were separated. In total, 60 µL of EDTA (pH 9.5) was added to the RN sediment to release rivastigmine tartrate from the cochleates. Ethanol (1 mL) was added to the above mixture for further extraction of the drug. The resulting clear solution was suitably diluted with phosphate buffer pH 7.4. 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 ml of the solution having concentration of 100µg/ml were taken out in 10 ml volumetric flask. Volume was titrated with PBS to obtain final concentrations of 1, 2, 3, 4, 5, 6 µg/ml, respectively. The absorbance of each concentration was measured at 263 nm using a UV spectrophotometer. The result was shown in Table No. 8. [27]

5. In-vitro drug release of RT from nanocochleates: In-vitro release studies of RT from nanocochleates were carried out in phosphate buffered saline (pH 7.4) using dialysis bag diffusion method and the results were compared with pure solution of rivastigmine tartrate (3 mg). /mL in 30% w/w polyethylene glycol 400 and water). Formulations RN3 and RL4 equivalent to 3 mg of RT were placed in dialysis bags (cellulose membrane, molecular weight cut off 120,000 Da), completely sealed and immersed in 100 mL of dissolution medium. The assembly was maintained at 37 ± 0.5 ◦C with constant stirring by a magnetic stirrer at 100 rpm. At predetermined intervals, samples were withdrawn and an equal volume of fresh medium was added to achieve sink conditions. The absorbance of RT in solution was determined using a UV-Vis spectrophotometer and compared with the plain drug. The result is shown in Table No.10. [28, 29].

6. Stability study of RT loaded nanocochlite: Freshly prepared RT-loaded nanocochlite batch was stored in amber colored vials at 5 ◦C ± 3 ◦C for two months and effect on various parameters like particle size, zeta potential and entrapment efficiency. . The result was shown in Table No.11 [30]

Preparation of Optimized Rt-Loaded Nanocochleatesinto Solid

- The optimized nanocochleates suspension was further lyophilized.
- Mannitol (5% w/v solution) was added to the RT-loaded nanocochleates suspension as a cryoprotectant to avoid lysis of the nanocochleates.
- Then this solution was placed under lyophilizer at -55ºC for 24 hours and filled into 0 size capsule under vacuum pressure below 100 Pa to obtain freeze-dried nanocochleate powder drug.
- Amount of powdered medicine filled into capsules using manual method. The amount of powdered drug filled into the capsule was calculated from the amount of drug in the powder and the minimum amount of rivastigmine tartrate. [31]

Evaluation of RT-loaded Solid Nanocochleate Formulation [32]

1. Drug content: The solid nanocochleate was dissolved in distilled water and then centrifuged at 15000 rpm for 40 min at 25ºC to separate the free drug content in the supernatant. Dilute the free drug supernatant with distilled water, then dilute to a concentration of 1 to 6 µg/ml in distilled water. They can be determined by ultraviolet (UV-visible) spectrophotometer absorbance was recorded at 263nm. The % drug content was obtained using the above equation. The results were discussed in Table no. 12.

2. In-vitro drug release: In vitro release studies of rivastigmine tartrate from solid nanococclets were carried out in phosphate buffered saline (pH 7.4) using dialysis bag diffusion method and the results were compared with pure solution of rivastigmine tartrate (3 mg). /mL in 30% w/w polyethylene glycol 400 and water). Solid RN3 equivalent to 3 mg RT was placed in a dialysis bag (cellulose membrane, molecular weight cut off 120,000 Da), completely sealed, and immersed in 100 mL dissolution medium. The assembly was maintained at 37 ± 0.5 ◦C with constant stirring by a magnetic stirrer at 100 rpm. At predetermined intervals, samples were withdrawn and an equal volume of fresh medium was added to achieve sink conditions. The absorbance of RT in the solution was determined using a UV-Vis spectrophotometer. The result was shown in Table No. 13. [33]

3. Stability study of RT loaded liposomes and optimized solid nanocochleates: Freshly prepared RT-loaded solid nanocochleates and liposomes were stored in amber colored vials at 5ºC ± 3 ◦C for two months and effect on parameters

like particle size and entrapment efficiency was studied. The result was shown in Table No. 14 [34]

RESULTS AND DISCUSSION

SELECTION OF DRUG

Rivastigmine Tartrate: For development of liposomal based Nanocochleates, Rivastigmine Tartrate was selected as an Active Pharmaceutical Drug.

PREFORMULATION STUDIES

The result of prefomulation studies are given below.

Description: Colour- White

Odour- Odourless

Taste- Bitter Taste

Procured drug Rivastigmine Tartrate had same physical description as mentioned in reference research paper.

Solubility Study: The solubility of sample Rivastigmine Tartrate was found to be,

Table 3: Solubility Profile of Rivastigmine Tartrate

Sr. No.	Solvent	Solubility (mg / ml)
1	Water	80 mg/ml
2	Methanol	16 mg/ml
3	DMSO	25 mg/ml
4	Benzene	Practically Insoluble
5	Ethanol	16 mg/ml

The procured drug was shown same solubility behavior as described in various referred reference research articles.

Melting Point Determination

Melting point was measured using capillary tube method. It was found to be 124ºC which is within the reported range of 123ºC to 125ºC. It complies with the purity of the drug sample.

Rivastigmine Tartrate has same melting point range as mentioned in referred reference research articles and books

Spectrophotometric Method for the Estimation of Rivastigmine Tartrate

Determination of Lambda Max:

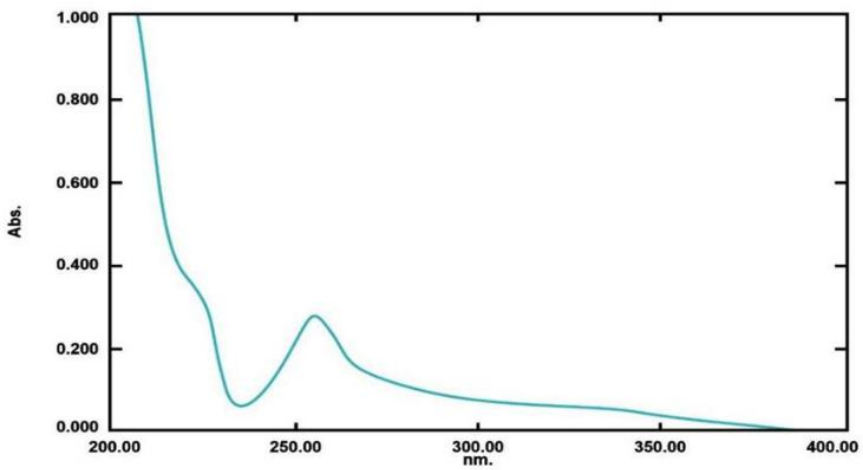


Figure 1: UV Spectrum of Rivastigmine Tartrate

After scanning 10 µg/ml solution, only one peak at 263 nm was observed and considered as Lambda max. The UV spectrum was as shown in **Figure: 1**

This isconfirmatory analytical test for drug, showing UV spectrum as described in reference books and the absorbance curve showed characteristics absorption maximum at 263 nm for Rivastigmine Tartrate.

FTIR Spectroscopy Studies:

The spectrum Rivastigmine Tartrate showed the following functional groups at their frequencies mentioned in **Table 12**. The FTIR of drug shown in **Figure: 2** was matched with the standard FTIR spectrum of Rivastigmine Tartrate.

Table 4: Functional Group and Their Frequencies of Rivastigmine Tartrate

Sr. No.	Functional Group	Observed Peak	Reference Peak
1	-C-H (Aliphatic)	2883.70	3000-2850
2	C=C (Aromatic)	1429.31	1600-1475
3	C=O (Ketone)	1718.65	1725-1705
4	N-H (3º amide)	3434.40	3500-3100
5	C-N (amine)	1248.96	1350-1000

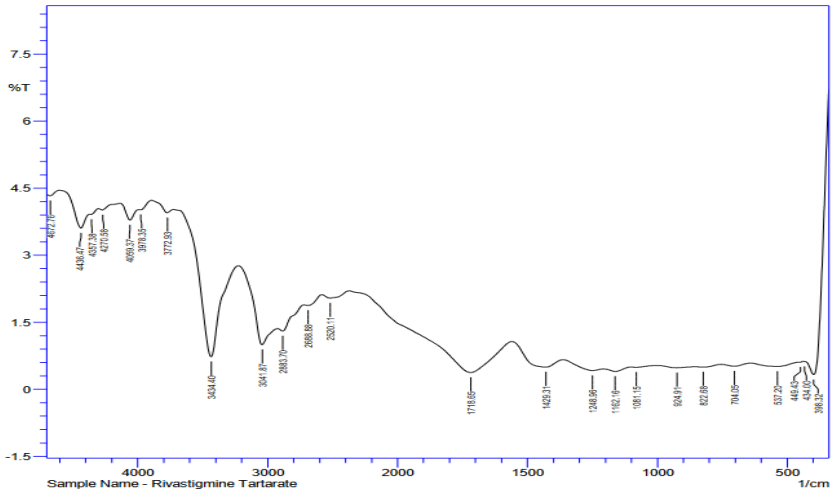


Figure 2: FTIR Spectrum of Rivastigmine Tartrate

The spectra studied at 4000 to 400 cm-1. The spectrum of Rivastigmine Tartrate showed the above groups at their frequencies mention in Table No. 12. The FTIR of drug was found to be similar to the standard FTIR spectrum of Rivastigmine Tartrate.

Standard Calibration Curve of Rivastigmine Tartrate in 0.1 N HCL

The absorbance of the Rivastigmine Tartrate in 0.1 N HCL (pH .2), was measuredat awavelength of 263 nm. The result are given in **Table No. 13** and **Figure 3.**

Table 4: Std Calibration Curve of Rivastigmine Tartrate in 0.1 N HCL

Sr. No.	Concentration (µg/ml)	Absorbance at 263 nm
1	0	0 ± 0
2	2	0.087 ± 0.0011
3	4	0.149 ± 0.0008
4	6	0.221 ± 0.0013
5	8	0.286 ± 0.0017
6	10	0.349 ± 0.0006
7	12	0.421 ± 0.0009
8	14	0.491 ± 0.0012
9	16	0.570 ± 0.0011

n = 3

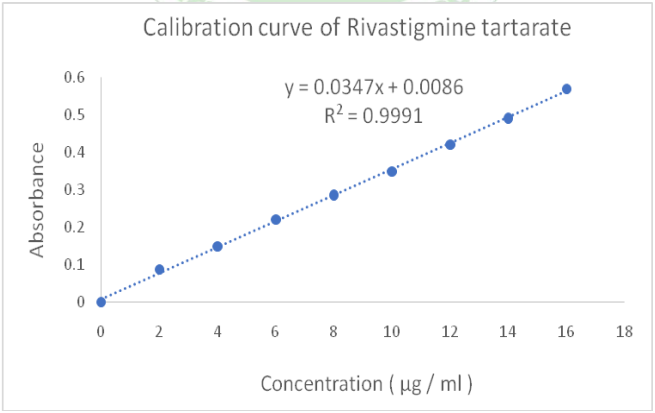


Figure 3: Standard Calibration Curve Of Rivastigmine Tartrate

From the standard curve it was observed thet, the drug obeys beers law in concentration range of 2.0 to 16 µg/ml in 0.1 N HCL. Drug has shown good linearity with regression of coefficient $\mathbf{R^2 = 0.9991}$ and equation for line was found to be **y = 0.0347x + 0.0086** which is used for calculation drug release study.

SELECTION OF POLYMERS

Identification of Polymers

DSPC Identification: Since lipids do not dissolve in water while ethanol does, when the ethanol is diluted, it falls out of the solution to give a cloudy white emulsion. Test was complies for the Di Steroyl Phosphotidylcholine.

Identification Test for DSPC as a polymer was passes as per reference no.66.

Cholesterol: The Cholesterol was start to convert into liquid at the temperature 149ºC. The test was complies to the Cholesterol as per IP, the standard range of M.P. of cholesterol is 148ºC to 150ºC.

Identification test for Cholesterol as a polymer was passes as per IP.

Calcium Chloride: A white precipited of calcium carbonate was produced therefore the identification test was complies for calcium chloride.

Identification of calcium chloride as a excipient passes as per reference no. 68.

Drug -Excipients Interaction Studies

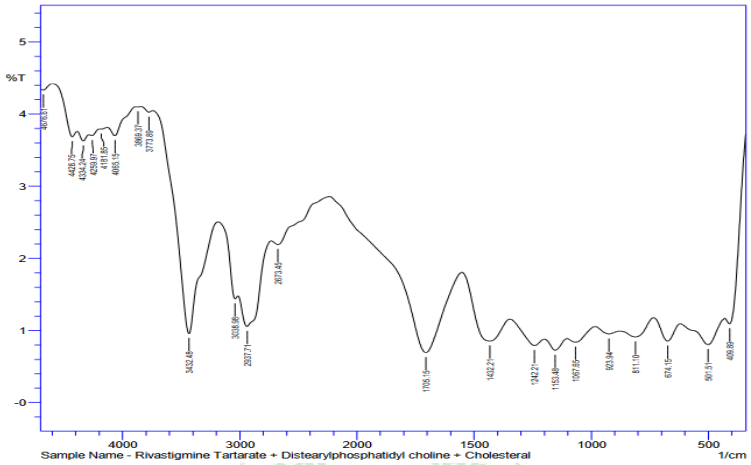


Figure 4: FTIR Spectra of Drug And Polymers Mixture

Table 6: Functional Group and frequencies of RT

Sr. No.	Functional Group	Observed Peak	Reference Peak
1	-C-H (Aliphatic)	2937.71	3000-2850
2	C=C (Aromatic)	1432.21	1600-1475
3	C=O (Ketone)	1705.15	1725-1705
4	N-H (3º amide)	3432.48	3500-3100
5	C-N (amine)	1242.21	1350-1000

Drug excipient interaction study by FTIR spectroscopy was carried out as per standard procedure. FTIR spectra of physical mixture of API and Polymers i.e., DSPC and Cholesterol was shown in **Figure 4**. It was observed that principle peaks of Rivastigmine Tartrate was found in FTIR spectra of physical mixture of drug and excipients. It was suggested that, there was no physical and chemical interaction between drug and polymers.

FORMULATION OF LIPOSOMAL BASED NANOCOCHLEATES

Preparation of Rivastigmine Tartrate-Loaded Liposome (Priliminary Batch)

Evaluation of Priliminary Batch of Different Concentration of Polymer and Drug

Table 7: Evaluation of RT-Loaded Liposomes

Formulation	Entrapment Efficiency	Particle Size (nm)	Zeta Potential mV)	Appearance
RL1	66.5 ± 5.23	345 ± 3	-42.32 ± 9.3	Spherical
RL2	93 ± 3.23	145 ± 2	- 40.25 ± 6.1	Spherical
RL3	98 ± 4.35	150 ± 2	- 48.66 ± 9.3	Spherical
RL4	99.6 ± 5.34	112 ± 4	- 51.70 ± 7.3	Spherical
RL5	75 ± 2.34	233 ± 5	- 41.32 ± 5.5	Spherical
RL6	70 ± 1.35	210 ± 3	- 53.65 ± 8.2	Spherical
RL7	80.4 ± 2.34	212± 3	- 40.34 ± 8.5	Spherical

EE and Particle size parameters readings were taken in triplicate (Mean ± SD, n = 3)

Thin-film hydration method was used for the preparation of liposomes as this methodhad been widely used due to the ease of handling. The different batches were preparedby varying the concentration of DSPC and cholesterol as shown in **Table 9**. For thepreparation of liposomes, the ratio of DSPC and Cholesterol was taken as 1:1, 2:1, 3:1, 4:1, 1:2, 1:3, and 1:4 w/w respectively. The size of the vesicles increased asthe amount ofthe drug increased; on the other hand, as thedrug concentration increased, the EE reduced.These results might be attributed to an insufficient amount of phospholipid to entrap thedrug. Hence, decided to use the lowest amount of the drug (3 mg) for thepreparation of nanocochleates. In the study, the formulations of RL2, RL3 and RL4 containing 3 mg of Rivastigmine Tartrate had better entrapment efficiency,

zeta potential, particle sizeand sphericity of particles, as shown in **Table No.14**.Therefore, these were considered optimized batches ofliposomes and were converted to Nanocochleates.

Formulation of Nanocochleates from optimized liposomal formulation

Preparation of RT-Loaded Nanocochleate Formulations

The trapping method was used for the preparation of nanocochleates. Optimizedbatches of liposomes were selected for the formulation of nanocochleates as shown inTable 10. Liposomes with a 3 mg loading capacity were converted into nanocochleates by the addition of CaCl2 solution (1 M). The effect of change in the volume of calcium chloridesolution (10 µL, 50 µL and 100 µL). CaCl2 (10 µL, 1 M) was notsufficient to convert all liposomes into nanocochleates. No difference was observed when50 µL and 100 µL of CaCl2 were added into prepared liposomes; thus, 50 µL CaCl2 (1 M) was fixed for the formation of nanocochleates.

Evaluation of RT-Loaded Nanocochleate Formulations

Table 8: Evaluation of Nanocochleate Loaded with Rivastigmine Tartrate

Formulation	EE %	Particle Size (nm)	Zeta Potential (mV)
RN1	96 ± 4.23	644 ± 3	-26 ± 9.38
RN2	97 ± 3.25	670 ± 2	-19 ± 8.8
RN3	99 ± 4.20	631.4 ± 4	-17 ± 6.5

n=3

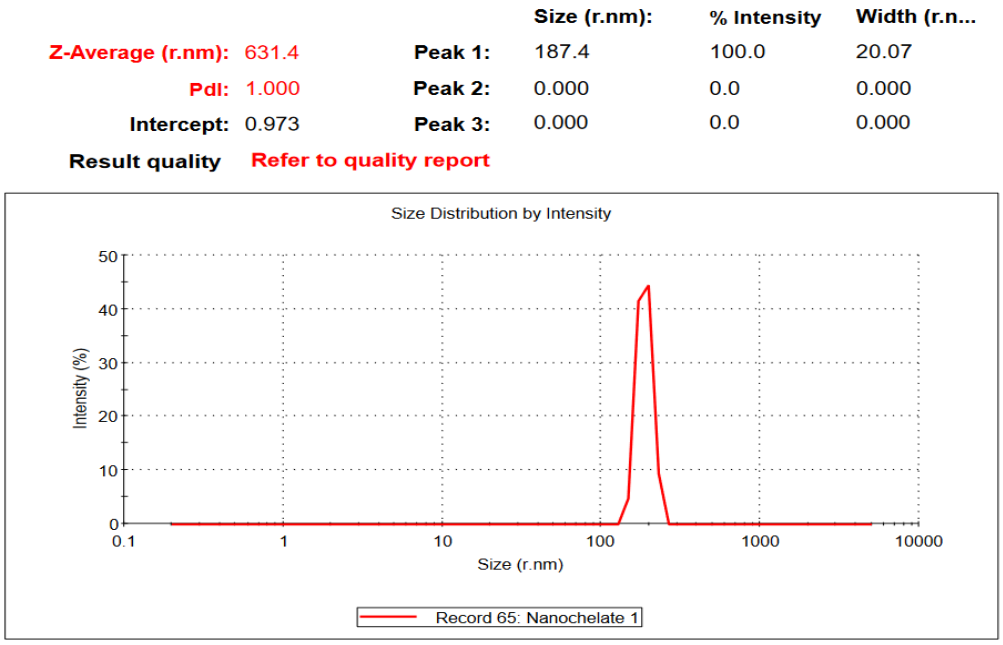


Figure 5: Particle Size and PDI of RT-Loaded Nanocochleate

Particle Size and PDI Determination: Particle size and polydispersity index were of crucial importance during the formulation of lipid-based nanocarriers as these majorly affect theappearance, process ability,performance and stability of the final product. The mean particle size of formulation RL4 and RN3 was found to be 112 ± 2 nm and 631 ± 4 nm **(Figure 5).** It wasobserved that liposomes were smaller in size than nanocochleates, as nanocochleates wererod-shaped.Polydispersibility index (PdI) ranging from 0 to 1 reflects the polydispersityindex of the suspension with the lower value in the range of 0 to 1.0, indicating a highlymonodispersed suspension. PdI of RN3 was found to be 1.0 indicating the formation ofmonodispersed suspension. Observed in **Figure 5**

Entrapment Efficiency measurement: Encapsulation efficiency was important for enhancing thebioavailability of the drug. It was found that the entrapment efficiency increased with anincreased amount of DSPC and increased cholesterol concentration decrease fluidity and enhance rigidity. Theseresults might be attributed to the complete encapsulation of the drug within the bilayersat higher concentrations of DSPC and lower conc. of cholesterol. The large size of thenanocochleates meant that they could incorporate a largerdrug amount as seen from theincreased entrapment efficiency of RN3. It was found that an increasing the lipid concentration EE of the formulation increases. The % EE of formulation RL4 and RN3 was found to be 99.6% ± 5.34 and 99% ± 4.20 **(Table No.14 and 15).**

Zeta Potential Measurements

Zeta Potential (mV):	-17.0	**Peak 1:**	-17.0	100.0	9.38
Zeta Deviation (mV):	9.38	**Peak 2:**	0.00	0.0	0.00
Conductivity (mS/cm):	0.0199	**Peak 3:**	0.00	0.0	0.00
Result quality	Good				

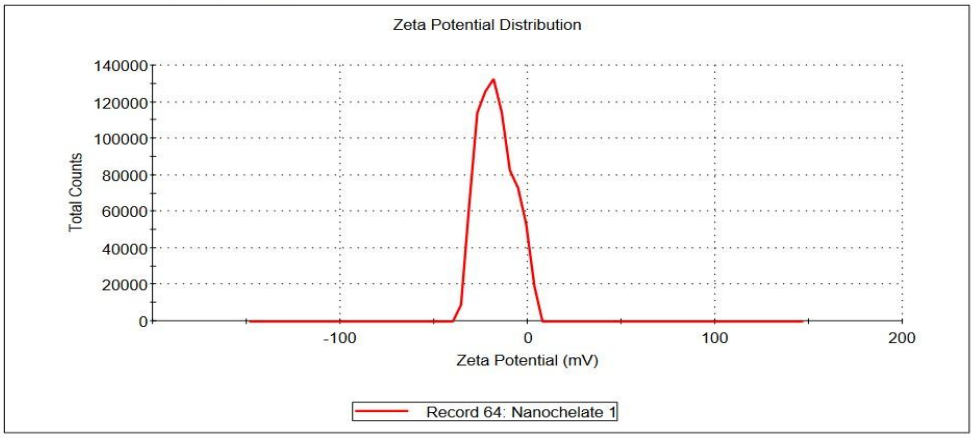


Figure 6: Zeta Potential of Nanocochleate Formulation

Zeta potential measurement is a marker of stability of a colloidal system.The measurement indicates the overall surface charge of a particle and provides information as towhether the system may remain stable or consequently undergo aggregation or flocculation. As depicted in Graph 5, the zeta potential ofthe nanocochleates (RN3) was found to be − 17 mV, respectively. Theseresults indicated that prepared nanocochleates exhibited sufficient chargeto avoid the aggregation of the vesicle. The negative zeta potential value was probablydue to the anionic nature of the lipid (DSPC).Theincorporation of Ca2+ to liposomesencourages the fusion of the lipidmembrane, resulting in thegeneration of planar sheets,which ultimately coil near theinitial point of folding to generate rod-shaped cochleates andthus the alteration of zeta potential was observed. Thus, based on the results of Particle Size, Zeta Potential and Entrapment Efficiency,**(Table 15)**, Formulation RN3 was consideredan optimized batch and as evaluated further.

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Drug content of optimize Batches of RT-loaded Nanocochleates Formulations

The drug content of RT-loaded nanocochleate formulations was calculated using standard formula. The % drug content of the Nanocochleates formulations which is optimized from the preliminary batch, are given as

Table 9: Percentages of drug in RN Solution

Formulation	% Drug Content
RN1	85.33 ± 3.5
RN2	91.57 ± 2.2
RN3	92.72 ± 4.6

n = 3

The RT-loaded nanocochleates was assayed by UV spectrophotometric method. As seen in **Table No.16.** Percent drug content for optimize batch is 92.72 % That concluded the sufficient amount of drug were loaded into the formulation.

In-Vitro Drug Release

In-Vitro Drug Release of Nanocochleate and Liposome Compared with Plane Drug

Table 10: *In-Vitro* Drug Release from plane drug, RN, RL

Time (hr)	Cumulative Percentage of Drug Release (%)		
	RN	RL	Plane Drug
0	0	0	0
1	8 ± 0.49	15 ± 0.87	45 ± 0.95
2	12 ± 0.85	20 ± 1.21	56 ± 0.75
3	18 ± 0.73	28 ± 1.41	60 ± 1.02
4	27 ± 1.06	38 ± 0.95	69 ± 1.28
5	36 ± 0.98	46 ± 1.13	75± 1.43
6	46 ± 1.12	52 ± 1.41	82± 0.92
7	55 ± 0.62	66 ± 2.12	91 ± 0.22
8	70 ± 2.22	75 ± 1.30	100 ± 1.25

Mean ± Standard Deviation, n = 3

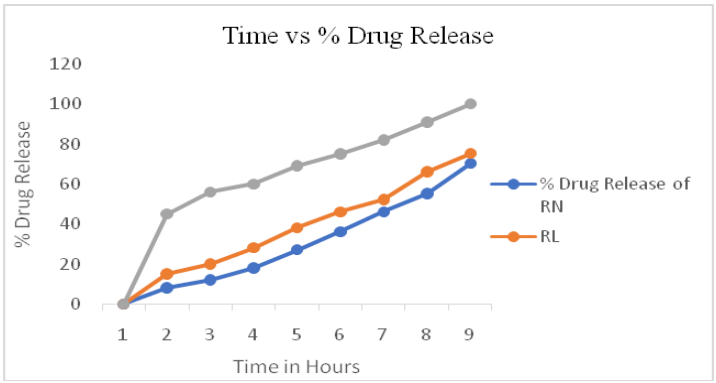

Figure 7: *In-Vitro* Drug Release Plane Drug, RN, RL

In vitro release of Rivastigmine Tartrate was performed by the dialysis bag diffusion method in PBS saline solution (pH 7.4).The release of Rivastigmine Tartrate from liposomes and nanocochleates in trial RL4 and RN3, respectively, was compared with the pure RT solution. As observed from **Figure 7**, the burst release of RT from RL4 and RN3was noted within 1 h, which might be the result of the trapped drug being released onthe surface of the formulations. Almost 100% of the drug was released from the pure RT solution within 8 hours, whereas RL and RN released 70% and 75% of the drug in 8 hours, respectively. Compared to liposomes, nanocochleates showed more controlled releasedue to their coiled structure.

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Stability Study of RT-Loaded Nanocochleates.

Table 11: Stability study of RT-Loaded Nanocochleate

Optimized batch	Sampling Point	Zeta Potential	Particle Size	% EE
RN1	0 Day	-26± 8.7	644 ± 3	96 ± 4.23
	30 Days	-26± 9.6	646.6 ± 2	82 ± 3.20
	60 Days	-27± 5.5	650 ± 4	75 ± 4.20
RN2	0 Day	-19± 4.2	670 ± 2	97 ± 3.25
	30 Days	-21± 9.4	680 ± 4	85 ± 2.12
	60 Days	-23± 4.1	680 ± 2	70 ± 4.23
RN3	0 Day	-17± 5.3	631.4 ± 4	99 ± 4.20
	30 Days	-17± 8.2	632 ± 3	93 ± 2.20
	60 Days	-18.5± 6.3	632 ± 4	88 ± 4.40

n = 3

PREPARATION OF OPTIMIZED RT-LOADED NANOCOCHLEATES INTO SOLID:

Formulation RN3 was consideredan optimized batch and was converted into Solid Nanocochleates and evaluated further.

Drug Content of RT-loaded Solid Nanocochleate

After conversion of optimized batch of nanocochleates into powder nanocochleate. Drug content of the Solid formulations was calculated using standard formula shown in section **4.5.4.**

Table 12: Percentages of drug in RN powder

Formulation	% Drug Content
RNF1	88.23 ± 6.5
RNF2	93.86 ± 2.4
RNF3	95.01 ± 6.6

n = 3

The percent drug content of Nanocochleates powder for optimize batch was found to be 95.01 %. As shown in Table No. 20.

In-Vitro Drug Release

In-Vitro Drug Release of Nanocochleate Powder Compared with Plane drug

Table 13: *In-Vitro* Drug Release Plane drug and RN powder

Time in Hours	Cumulative percent of drug release	
	Plane Drug	RNF Powder
0	0	0
1	40 ± 0.95	8 ± 0.44
2	55 ± 0.75	12 ± 1.52
3	63 ± 1.02	19 ± 1.28

4	72 ± 1.28	25 ± 0.92
5	80 ± 1.43	35 ± 0.24
6	88 ± 0.92	46 ±0.44
7	95 ± 1.43	55 ± 1.65
8	100 ± 0.95	69 ± 1.8

n = 3

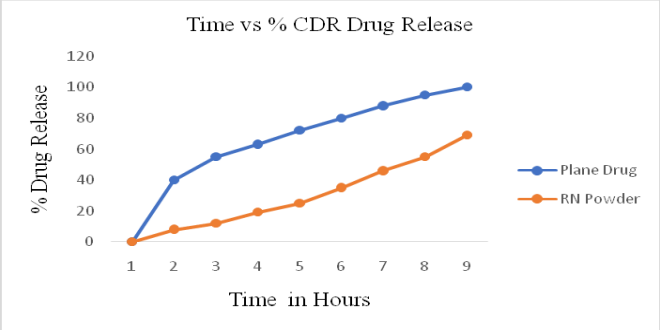


Figure 8 : *In-Vitro* Drug Release plane drug and RN powder

The release of Rivastigmine Tartrate from nanocochleates Powder was compared with the pure RT solution. As observed from **Figure 9** and **Table No.20**.The burst release of RT from optimized batch of powder nanocochleateswas noted within 1 h, which might be the result of the trapped drug being released onthe surface of the formulations. Almost 100% of the drug was released from the pure RT solution within 8 hours, whereas Powder nanocochleates release 69% of the drug in 8 hours, respectively.

Stability studies of Developed Formulations

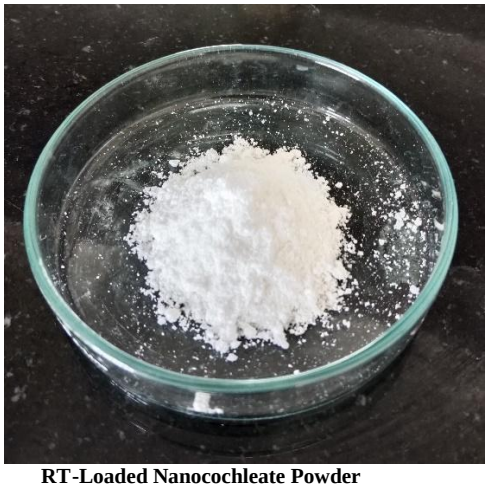
Table 14: Stability data of RN and RL

Formulations	Sampling Point	Particle Size (nm)	EE (%)
RNF3	0 Day	631.4 ± 4	97 ± 3.12
	30 Day	637 ± 2	90 ± 2.20
	60 Day	637 ± 2.8	88.20 ± 4.40
RL	0 Day	112 ± 2	99.6 ± 5.34
	30 Day	425 ± 3.33	84 ± 2.32
	60 Day	454 ± 4	70 ± 4.20

The stability of RT-loaded liposomal and Solid nanocochleate formulations was tested at 5 ± 3ºC for 2 months. It was observed that liposomalformulation was not stable, it formed agglomerates due to the fusion of lipids while Solid nanocochleates were stable, i.e., in the powder form. From Table 21, it can beseen that there was a significant reduction in the %EE of liposomes loaded with Rivastigmine Tartrate which might be due to leakage of the drug from the vesicles. An increased particle sizeof the liposomes after 60 days indicated the fusion of vesicles upon storage. RT-loaded Solid nanocochleates did not show any significant change in these parametersafter storage. Thus, it can be concluded that nanocochleates were more stable than Liposomes.

Final Formulaton of RT-Loaded Nanocochleates

RT-Loaded Nanocochleate Solution



RT-Loaded Nanocochleate Powder

Summary and Conclusion

SUMMARY

The study focused on the formulation and evaluation of Rivastigmine Tartrate (RT)-loaded nanocochleates (RN) using a trapping technique, wherein calcium ions were added to preformed negatively charged liposomes (RL) containing Disteroylphosphatidyl choline (DSPC) and cholesterol. Both DSPC and cholesterol had a significant impact on particle size and entrapment efficiency, thus emphasizing their selection for altering these parameters.

The optimized formulation of RT-loaded nanocochleates exhibited enhanced encapsulation efficiency and controlled release properties, presenting them as a potential alternative to conventional oral RT formulations. The study aimed to enhance patient compliance and ensure controlled drug release.

Compatibility studies using Fourier Transform Infrared Spectroscopy (FTIR) confirmed no interaction between the drug and polymers.

Nanocochleate formulation technology was deemed suitable for various types of drugs, including those with rapid first-pass metabolism. Encapsulation of RT in nanocochleates aimed to protect the drug from rapid metabolism, thereby improving bioavailability and ensuring controlled release.

In vitro drug release studies demonstrated extended release up to 24 hours, reducing the dosing frequency compared to plain drug formulations.

The optimized RN3 formulation showed a high drug content, indicating effective encapsulation.

The study highlighted the importance of selecting DSPC and cholesterol for stable nanocochleate formation, with DSPC chosen for its compatibility with human use and its potential to react with calcium ions to form stable cochleates. Cholesterol played a crucial role in forming drug complexes and stabilizing the phospholipid membrane, thereby enhancing liposome stability. calcium ions facilitated the formation of rod-shaped cochleates with a unique structure that allowed maximum drug encapsulation. RL2, RL3, and RL4 formulations containing 3 mg of RT were identified as optimized liposome batches converted into nanocochleates. The coil-like structure of nanocochleates provided protection from environmental conditions and enabled controlled drug release, ultimately enhancing oral bioavailability and patient compliance.

The study also provided a comprehensive review of liposomal-based nanocochleates as a drug delivery system and the properties of Rivastigmine Tartrate as the main API.

CONCLUSION

Rivastigmine Tartrate-loaded nanocochleates represent a promising drug delivery system for enhancing the therapeutic efficacy of the drug. Developed using a trapping method, these nanocochleates offer significant improvements over liposomes, including enhanced encapsulation efficiency and controlled release for up to 24 hours. Nanocochleates effectively protected RT from rapid metabolism and exhibited superior stability compared to liposomes. The final optimized formulation, RNF3, containing DSPC (40mg) and cholesterol (10mg), demonstrated effectiveness in meeting patient compliance. The study concludes that nanocochleates can act as a better alternative to liposomes for delivering drugs with poor stability and bioavailability, thus improving their therapeutic efficacy. Future research directions could explore applications in delivering various biological molecules susceptible to oxidation and first-pass metabolism, with a focus on optimizing cholesterol levels in liposome production.

Future Scope

Nanocochleate demonstrates applications in the delivery of various biological molecules which are susceptible to oxidation and undergoes first pass metabolism. There is a future scope that Liposomal based Nanocochleate is a promising method for enhancing oral bioavalability of drugs which undergoes Rapid first pass metabolism, through encochleation with intact nanocochleate, drug molecules, such as Rivastigmine Tartrate may be protected by nanocochleate by multilayered structure in the form of rolled shape. Cholesterol tend to maintain membranes fluidity and its concentration increased and decreased. However, the optimal amount of cholesterol has not yet been identified. Future field research should be based on the principle of finding the optimal amount cholesterol in liposome production.

REFERENCES

1. Pawar SF, Bothara SS, Mahaparale PR, Musale VL. Nanocochleate–An Important Drug Delivery System Offering Unique Features. World J. Pharm. Res. 2020 Jul 2; 9(9):621-37.
2. Panda P, De M, Basak S. Nanocochleates: A novel lipid-based nanocarrier system for drug delivery. InDesign and Applications of Theranostic Nanomedicines 2023; 1:143-173. Woodhead Publishing.
3. Talke S, Salunkhe K, Chavan MA, Hundiwale JC, Harwalkar M, Waykar M. Review on nanocochleates novel approach for drug delivery. World J. Pharm. Pharm. Sci. 2018; 7(7):284-94.
4. Tilawat M, Bonde S. Nanocochleates: A potential drug delivery system. Journal of Molecular Liquids. 2021 Jul 15; 334:116115.
5. Gauthreaux K, Bonnett TA, Besser LM, Brenowitz WD, Teylan M, Mock C, Chen YC, Chan KC, Keene CD, Zhou XH, Kukull WA. Concordance of clinical Alzheimer diagnosis and neuropathological features at autopsy. Journal of Neuropathology & Experimental Neurology. 2020 May 1; 79(5):465-73.
6. Rosler M. Efficacy and safety of rivastigmine in patients with Alzheimer's disease: international randomised controlled trial (vol 318, pg 633, 1999). British Medical Journal. 2001 Jun 16; 322(7300):1456-.
7. Shamsi A, Anwar S, Mohammad T, Alajmi MF, Hussain A, Rehman MT, Hasan GM, Islam A, Hassan MI. MARK4 inhibited by AChE inhibitors, donepezil and Rivastigmine tartrate: Insights into Alzheimer's disease therapy. Biomolecules. 2020 May 20;10(5):789.
8. Aulton ME, Taylor K. Pharmaceutical preformulation. Aulton's pharmaceutics: The design and manufacture of medicines.(4thedn), Elsevie r Health Sciences, Edinburgh. 2013.
9. Sakore S, Choudhari S, Chakraborty BH. Biowaiver monograph for immediate release solid oral dosage forms: ofloxacin. Int J Pharm Pharm Sci. 2010; 2(Suppl 4):156-61.
10. Tapkir N, Soni F, Sahu AK, Jadav T, Tekade RK, Sengupta P. A comprehensive review on assessment and key control strategies for impurities in drug development with a special emphasis on post-marketing surveillance. Journal of Pharmaceutical Innovation. 2021:1-20.
11. Part SN. Bachelor of Science B. Sc. Chemistry Degree Course (With effect from 2020-2021) The Course of Study and the Scheme of Examinations.
12. Sharmila SK, Srilakshimi M, Renukadevi G, Rahaman SA, Shanthakumari K. Development and validation of UV-spectrophotometric method for the estimation of rivastigmine tartrate in bulk and pharmaceutical dosage form. Indo American Journal of Pharmaceutical Research. 2013;3(10):8394-9.

13. Rothery, Mark. "Techniques: Biochemical Tests". Mark Rothery's Biology Web Site. Wikipedia.
14. Williams JH, Kuchmak M, Witter RF. Purity of cholesterol to be used as a primary standard. Journal of Lipid Research. 1965 Oct 1; 6(4):461-5.
15. H.W. Hanna and A. Dittmar; Laboratory Manual for General Chemistry, 4th ed.; Morton Publishing Company, Denver, Colorado.
16. 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.
17. Delmarre D, Lu R, Tatton N, Krause-Elsmore S, Gould-Fogerite S, Mannino RJ. Formulation of hydrophobic drugs into cochleate delivery vehicles: A simplified protocol & formulation kit. Drug delivery technology. 2004; 4(1):64-9.
18. Bothiraja C, Rajput N, Poudel I, Rajalakshmi S, Panda B, Pawar A. Development of novel biofunctionalized chitosan decorated nanocochleates as a cancer targeted drug delivery platform. Artificial cells, nanomedicine, and biotechnology. 2018 Oct 31; 46(sup1):447-61.
19. Asprea M, Leto I, Bergonzi MC, Bilia AR. Thyme essential oil loaded in nanocochleates: Encapsulation efficiency, in vitro release study and antioxidant activity. LWT. 2017 Apr 1; 77:497-502.
20. Shanmugam T, Joshi N, Kaviratna A, Ahamad N, Bhatia E, Banerjee R. Aerosol delivery of paclitaxel-containing self-assembled nanocochleates for treating pulmonary metastasis: An approach supporting pulmonary mechanics. ACS Biomaterials Science & Engineering. 2020 Dec 21;7(1):144-56.
21. Elsana H, Olusanya TO, Carr-Wilkinson J, Darby S, Faheem A, Elkordy AA. Evaluation of novel cationic gene based liposomes with cyclodextrin prepared by thin film hydration and microfluidic systems. Scientific reports. 2019 Oct 22; 9(1):15120.
22. Ghule MM, Bhoyar GS. Formulation and Evaluation of modified liposome for transdermal drug. Journal of Developing Drugs. 2018;7(1):2-3.
23. Oyungerel G, Batdemberel G, Munkhsaikhan G. Study of particle size in natural and technological water suspensions using photon cross correlation spectroscopy with Nanophox. Phys. Chem. 2020; 10:1-4.
24. Selvamani V. Stability studies on nanomaterials used in drugs. InCharacterization and biology of nanomaterials for drug delivery 2019 Jan 1 (pp. 425-444). Elsevier.
25. Ramasamy T, Khandasamy U, Hinabindhu RU, Kona K. Nanocochleate—a new drug delivery system. FABAD journal of pharmaceutical sciences. 2009 Mar; 34:91-101.
26. Çoban Ö, Değim Z, Yılmaz Ş, Altıntaş L, Arsoy T, Sözmen M. Efficacy of targeted liposomes and nanocochleates containing imatinib plus dexketoprofen against fibrosarcoma. Drug development research. 2019; 80(5):556-65.
27. Pawar AY. Nanocochleate: a novel drug delivery system. Asian Journal of Pharmaceutics (AJP). 2016;10(03).
28. Landge A, Pawar A, Shaikh K. Investigation of cochleates as carriers for topical drug delivery. Int. J. Pharm. Pharm. Sci. 2013 Mar; 5(2):314-20.
29. Perlin DS. Amphotericin B cochleates: a vehicle for oral delivery. Current Opinion in Investigational Drugs (London, England: 2000). 2004 Feb 1; 5(2):198-201.
30. Mali AJ, Joshi PA, Bothiraja C, Pawar AP. Fabrication and application of dimyristoyl phosphatidylcholine biomaterial-based nanocochleates dry powder inhaler for controlled release resveratrol delivery. Future Journal of Pharmaceutical Sciences. 2021 Dec; 7:1-2.
31. Poudel I, Ahiwale R, Pawar A, Mahadik K, Bothiraja C. Development of novel biotinylated chitosan-decorated docetaxel-loaded nanocochleates for breast cancer targeting. Artificial cells, nanomedicine, and biotechnology. 2018 Nov 5; 46(sup2):229-40.
32. Yücel Ç, Altintaş Y, Değim Z, Yılmaz Ş, Arsoy T, Altıntaş L, Çokçalışkan C, Sözmen M. Novel approach to the treatment of diabetes: Embryonic stem cell and insulin-loaded liposomes and nanocochleates. Journal of nanoscience and nanotechnology. 2019 Jul 1;19(7):3706-19.
33. Chen L, Yue B, Liu Z, Luo Y, Ni L, Zhou Z, Ge X. Study on the preparation, characterization, and stability of freeze-dried curcumin-loaded cochleates. Foods. 2022 Feb 28;11(5):710.
34. Jin T, Zarif L, Mannino R, inventors; University of Medicine, Dentistry of New Jersey, Biodelivery Sciences Inc, assignee. Nanocochleate formulations, process of preparation and method of delivery of pharmaceutical agents. United States patent US 6,153,217. 2000 Nov 28.