INTRODUCTION

Alzheimer's disease (AD) is an untreatable neurodegenerative condition marked by a lack of acetylcholine in the brain that causes degradation of neurons and synapses, memory impairment, and pathological alterations such as the production of aberrant protein aggregates known as senile neurofibrillary tangles and plaques [1].

The medication delivery technology that can enhance drug release at the desired AD is solid lipid nanoparticles. The proposed study's objective is to develop solid lipid nanoparticles (SLNs) based on glyceryl monostearate (GMS) for the regulated administration of rivastigmine tartrate (RT), which is used for the treatment of Alzheimer's disease (AD). Solid lipid nanoparticles (SLNs) are attracting importance for drug developers and therapeutic response. The available conventional treatment strategy is associated with several limitations, like a short half-life, poor solubility, bioavailability, and a dissolution issue. The outcome of the research will lead to improvements in drug release and solubility of RT for better therapeutic effect.

Materials

Rivastigmine Tartrate (RT) was obtained as a complimentary sample from Cedia Pharmaceuticals Ltd. (Ahmadabad, India). Compritol 888 ATO was a complimentary sample from Cedila Pharmaceuticals Ltd. (Ahmadabad, India). Tween 80 and Span 40 Loba chemise was provided them, and Glyceryl Monostearate (GMS) was provided from Loba chemise (Mumbai, India). Compritol 888 ATO was a complimentary sample from Cedila Pharmaceuticals Ltd. (Ahmadabad, India).
Microemulsion technique

The technique of microemulsion in the production of SLNs, a stage called microemulsion formulation, was used. In this method, the microemulsion was spontaneously formed because of the high surfactant/lipid ratio; the proportions of the excipients were essential and described the areas of microemulsion formation [11]. This method was very simple and consisted of a few common steps. A desired amount of GMS (lipid) was weighted and heated to 70 °C in Compritol 888 (surfactant) until the lipid was totally melted. For rivastigmine tartrate SLNs, rivastigmine tartrate was added to the melted lipids following vigorous mechanical stirring. A magnetic stirrer with a hot plate was used to stir the mixture. The aqueous phase was made by a 1:1 mixture of tween 80 and span 40 (surfactants) in distilled water and heating it to 70 °C in a water bath. Melted lipids were mixed with a hot surfactant solution. Gentle homogenization was applied for 2 h until the microemulsion was formed. In the second stage, the hot microemulsion was dispersed in a high volume of cold water (2-3 °C) under moderate stirring. The liquid droplets to solidify. The SLNs obtained with this technique had a spherical shape and a narrow size distribution. The microemulsion technique used to prepare the drug-loaded SLNs [F-1] [12].

Microemulsion cooling technique

In this technique, GMS (lipid) was melted at 70 °C and water was also heated on the other side to 70 °C. Water which was heated to same temperature was added to oil phase with minimal stirring to form a homogenous milky slurry. Thereafter, a suitable amount of tween 80 and span 40 (surfactant) was added to form stable and clear water in oil emulsion. The resulting transparent microemulsion was further cooled to 4 °C to precipitate SLNs from it. Microemulsion cooling technique was used to prepare drug-loaded SLNs [F-2] [13].

Solvent injection technique

When it refers to the solvent injection method, emulsifying wax was dissolved in methanol (water-miscible solvent) and tween 80 and span 40 (surfactants) were dissolved in distilled water and boiled in a water bath at 70 °C to make an aqueous surfactant solution. An injection needle was used to quickly inject a water-miscible solvent mixture into an aqueous surfactant solution. Two simultaneous effects contribute to the effective formation of SLNs. Gradual solvent diffusion out of lipid-solvent droplets into water reduced droplet size while increasing lipid concentration at the same time. Diffusion of pure solvent from a lipid-solvent droplet caused local variations in interfacial tension at the droplet surface, resulting in droplet size reduction. In this process, the particle size of SLNs could be influenced and controlled by variations in process parameters such as the injected solvent, the lipid concentration, the injected volume of solvent, the lipid concentration in the solvent phase, and the viscosity of the aqueous phase. The formulation of drug-loaded SLNs prepared by the Solvent Injection Technique [F-3] [14].

High shear homogenization and/or ultrasonication technique

Lipid nanoparticles dispersion was made by high-shear homogenization followed by ultrasonication of melted GMS (lipid) in a warm aqueous phase (distilled water), including surfactants (span 40). This approach involves heating a solid lipid to around 5-10 °C above its melting point. To form an emulsion at the same temperature, the lipid melt was dispersed in an aqueous surfactant solution while being stirred at high speed. The emulsion’s droplet size was reduced after sonication. Lipid nanoparticle dispersion was produced by gradually cooling the warm emulsion below the lipid crystallization temperature. Ultra centrifugation could be used to generate a concentrated lipid nanoparticles dispersion formulation of drug-loaded SLNs prepared by ultrasonication method [F-4] [15].

Characterization of solid lipid nanoparticles

Solubility study of rivastigmine tartrate in different solid lipids

Since the various lipids used in the study were solid by nature, it was impossible to evaluate the solubility of RT using the equilibrium approach. As a result, a new approach was used, in which the drug and lipids were combined in two separate drug: lipid ratios (D: L), namely 2:4 and 2:6, as indicated in table 2. Solubility of RT in lipids was the main criteria for the selection and screening of lipid. Each test tube holding a medication and lipid combination was heated in a water bath to a temperature that was 10 °C over the lipid’s melting point, and then it was combined. Test tubes were visually examined for miscibility and clarity after 10 min of heating and mixing. The solubility of rivastigmine tartrate was tested using the equilibrium approach, which has been mentioned in the literature because the different lipids used in the study were solid by nature. Five different lipids, including Apilil, Compritol, GMS, Precirol ATO 5 (PA), and stearic acid, were used to assess the drug’s solubility [16].

Optimization of SLNs of rivastigmine tartrate

SLNs of Rivastigmine Tartrate was prepared by four different methods viz. Microemulsion technique, solvent injection technique, microemulsion cooling technique and high shear homogenization/ultrasonication technique. The amount of drug RT used was 2 mg as reported in the literature. Further, Glyceric monostearate was used as solid lipid in range from 100 mg to 500 mg for all the four methods. But on the basis of the solubility of the drug in GMS; 500 mg was considered as optimized for F-1, F-2 and F-4 as the method of preparation affects the size and zeta of nanoparticles. In F-3; GMS was not used because it contains emulsifying wax for the preparation of nanoparticles. The amount of Compritol 888, span 40, and tween 80 used as surfactant and co-surfactant; distilled water were also optimized by trial and error experimentation and the optimized amounts were utilized for the preparation of continuous aqueous phase and nanoparticles.

Particle size, zeta potential and polydispersity index (PDI)

Zetasizer (Anton Paar’s Litesizer TM 500) was used to determine particle size, PDI, and zeta potential by photon correlation spectroscopy. RT-SLNs were diluted ten times with double distilled water prior to assessing size, PDI, and zeta potential. By placing 1 ml of the diluted formulation in a disposable locking capillary cell for zeta potential measurements at 25 °C and a polystyrene omega cuvette, respectively, particle size and PDI measurements were carried out. At a wavelength of 263 nm and a scattering angle of 90°,
the dynamic light scattering measurements were performed using a helium-neon laser as the light source. Brownian motion causes particle diffusion, which is transferred into particle size. When an electric field is applied, particles with potential zeta move at a velocity corresponding to their zeta potential. This zeta potential is detected using a methodology named phase analysis light scattering and is transformed by Lenovo software [17].

Scanning electron microscopy (SEM)

SEM (Hitachi S3400, Tokyo, Japan) was used to analyze the surface morphology of nanoparticles. This is performed to describe how dilution affects the nanoparticles' surface morphology. An aluminum stub (plate) was used to collect the nanoparticles sample. The instrument's stub was inserted after being wrapped in carbon tape for insulation. SEM images were acquired after a vacuum was applied [18].

Fourier transform infrared spectroscopy (FTIR)

FT-IR spectroscopy was used for interactivity between drug and excipients (FTIR-8400S from shimadzu spectrophotometer). The specimens were prepared by KBr disk technique, in this analysis of specimens which were dispersed in KBr power and disk [19].

Differential scanning calorimetry (DSC)

Mostly Differential Scanning calorimetry was used to create data thermo-analysis on glass transition and melting endothermic objects. DuPont thermal analyzer (2010 DSC module) was used for all thermal analysis. A test sample 2 mg weight were captured and sealed hermetically in an aluminum pan having flat bottom. Test samples were balanced for a minute and heated over 10-200 °C temperature range with 20 °C/min rate in Nitrogen atmosphere. Empty aluminum pan further used as a reference. Nitrogen gas was removed at rate of 20 ml/min for all studies [20].

Determination of entrapment efficiency and drug loading

Nanoparticles having a weight 20 mg were suspended into ethanol (20 ml) and mixed with a magnetic stirrer for 1 hour. It was noticed that air bubbles were removed in 10 min with sound. 1 ml of this solution was extracted and desirable dilute solutions were made buffer solution pH 6.8. By employing the supernatant of unloaded nanoparticles with basic correction, a UV spectrophotometer operating at 263 nm was used to determine the quantity of free drug present in the clear supernatant.

The entrapment efficiency (EE %) could be achieved by the following equation.

\[
EE\% = \frac{\text{Total amount of Rivastigmine} - \text{Free Rivastigmine}}{\text{Total amount of Rivastigmine}} \times 100
\]

Loading capacity= [(Total amount of drug-Free amount of drug)/nanoparticles weight] x 100.

In vitro drug release study for SLNs

Franz diffusion cell were used to perform in vitro drug release studies of SLNs. At 37±0.5 °C, temperature observation was performed. A 20 ml of buffer solution of 6.8 pH in which receptor compartment of diffusion cell were dissolved. The magnetic stirrer was used for continuously stirred the solution at 100 rpm. Cellophane membrane (molecular weight 10,000-12,000, Hi-Media, India) was worked as a release barrier in between the receptor and donor compartment. At a definite time interval, the test samples were exerted from the sampling port of the diffusion cell and continuously added an equal volume of fresh buffer solutions. The UV-spectroscopy was operated at 263 nm for the analysis of the drug sample.

In vivo study for pure drug and solid lipid nanoparticles

The Institutional Animal Ethics Committee (IAEC), with registration number 1188/P0/Re/S/08/CPCSEA, approved the use of animals in research. Shri Rawatpura Sarkar Institute of Pharmacy, Kumhari, Durg, provided either sex/Wistar Rats (4-6 w old) or mice (C. G.). The animals were kept in an animal room at a temperature of 20±1 °C with a 12/12-hour light/dark cycle and free access to food pellets and water.

Morris water maze test

The maze was a white, circular pool with a height of 50 cm, a perimeter of 140 cm, and an inward face filled to a depth of 30 cm with water that was been 20±1 °C. There were four identical quadrants along the pool's perimeter: northwest (NW), northeast (NE), southwest (SW), and southeast (SE). A glass platform with a diameter of 22 cm was set 1 cm below the surface in the NW quadrant. The time it took for each rat to reach an escape platform after being put into the pool from the SE quadrant was timed. The animals were given various treatments over the course of 13 d. Group 1 received only saline, Group 2 received scopolamine and saline, Group 3 served as the standard, and the animals received rivastigmine tartrate solution in PBS (containing 2.5% compriol 888) (dose 2 mg/kg) with scopolamine. On the 14th day of the study, Group 4 was given the test formulation, rivastigmine tartrate SLNs (2 mg/kg) along with scopolamine. The maze activity was performed on the animals four times per day for five days, with a five-minute inter-trial rest (10th–13th days). Rats were held on the platform for 30 seconds if they couldn’t locate the escape platform within the allotted time frames, which included the first learning phase (120 s) and the scopolamine-induced amnesia trials (180 s). On the fourteenth day of the experiment, milk was added to the water to make the pool opaque, and three tests were conducted on the animals, each taking place between 30 and 45 min following the scopolamine injection. Escape delay was evaluated as a test variable for spatial learning [21].

Elevated plus maze test

Examining answers linked to memory was done using the Plus Maze test. Equipment for making the plus sign that has two open arms and two closed arms, each measuring 5 cm by 30 cm. All of the animals received a 7-day course of treatment. Group 1 received saline as the vehicle, Group 2 received scopolamine in the vehicle, Group 3 was used as the standard, and the animals in this group received rivastigmine tartrate (2 mg/kg) solution in PBS (containing 2.5% compriol 888 ATO) with scopolamine, and Group 4 received the test formulation, rivastigmine tartrate SLNs (2 mg/kg) with scopolamine. The maze activity was performed on the animals four times per day for five days, with a five-minute inter-trial rest (10th–13th days). Rats were held on the platform for 30 seconds if they couldn’t locate the escape platform within the allotted time frames, which included the first learning phase (120 s) and the scopolamine-induced amnesia trials (180 s). On the fourteenth day of the experiment, milk was added to the water to make the pool opaque, and three tests were conducted on the animals, each taking place between 30 and 45 min following the scopolamine injection. Escape delay was evaluated as a test variable for spatial learning [21].

Stability studies

By storing F-2 SLNs stability studies in glass tubes for three months at 4±2 °C (refrigeration temperature) and 25±2 °C (room temperature), it was possible to assess their stability. After completing this course, the particle size, PDI, and zeta potential of RT-SLNs were calculated using the same methodology.

RESULTS AND DISCUSSION

Solubility study of rivastigmine tartrate in different solid lipids

The amount of drug encapsulated in solid lipid is a limiting factor in the formulation of SLNs. Because the amount of medicine that is solubilized greatly affects the drug’s %EE, solid lipid optimization is essential. The solubility investigation used five distinct solid lipids, as per the information in table 2. Because of the inclusion of mono, di, triacylglycerols, and glycerides, GMS, Compritol, and PA among them have shown better solubility than Apfil and stearic acid at D: L (2:4). Shah et. al in 2015 used five different lipids for preparation of SLNs of rivastigmine for intranasal delivery; as in his research GMS was used which is also best suited solid lipid in our study as well for preliminary optimization [1]. The lipid screening was done as per the method reported by Chauhan et al, 2019 and the results were also suitable as per the reported findings [21]. GMS contains mono, di, triacylglycerols and glycerides which makes it suitable for preparation of SLNs.
were evaluated. Fig. 2 graphically represented the size of the particle size and PDI of SLNs loaded with rivastigmine tartrate. The first formulation system is shown in the below table 3. 

Required to evaluate every possible combination of excipients in the formulation system is shown in the below table 3. In our study, we have followed the procedure of optimization as reported by Ravi et al in 2017 [4]. The results of optimization with 3² factorial designs were compatible with the data obtained as in research done by soma et al in 2017 [22]. The surface plot of parameters on size, entrapment efficiency and drug release is shown in fig. 1.

The regression equations were produced by using a factorial design

Particle size = +418.12487+3.02154 *lipid–751.02148 *surfactant

Entrapment efficiency (%) = 64.215487–3.02154 *lipid+54.02154 *surfactant

Drug release = 75.02154+36.2154–0.2351 *lipid+85.2314 *surfactant

*Data are expressed as mean±SD, n=3

Optimization with full factorial design

A scientific technique used to make evident how the input impacts the answer and to more clearly comprehend the process is the design of experiments. In the current study, a 3² factorial design was utilized to evaluate how various factors impacted the replies that were selected. Depending on how the product will be used and processed, various excipients are added. In our study, we have followed the procedure of optimization as reported by Ravi et al in 2017 [4]. The results of optimization with 3² factorial designs were compatible with the data obtained as in research done by soma et al in 2017 [4]. The results of optimization with 3² factorial designs were compatible with the data obtained as in research done by soma et al in 2017 [4].

The results indicate that particle size and surfactant concentration had an indirect relationship and that surfactant concentration affected the particle size of nanoparticles. The results indicated that the particle size of nanoparticles was also influenced by zeta potential. The higher the zeta potential, the more stable the dispersion. Zeta potential was measured by using zeta potential analyser. Our research focused on investigating best method with optimum size, zeta and PDI. The presence of a negative charge on the interface of SLNs will cause double-layer repulsion between the formed droplets and prevent their agglomeration by strong repulsive forces during storage. Our findings were similar to the results reported by Arora et al, 2022 [24].

Zeta potential

Zeta potential higher than ±28 mV indicates the stability of the nanoparticulate system. Nanoparticles with a zeta potential above ±30 mV have been shown to be stable, as the surface charge prevents aggregation of the particles. Zeta potential of Rivastigmine tartrate-loaded SLNs were evaluated. Physical stability of solid lipid nanoparticles was also influenced by zeta potential. The higher the zeta potential, the more stable the dispersion. Zeta potential was negative i.e. -24±0.01mV for F-2 and +28±0.01mV for F-2. The formed droplets and prevent their agglomeration by strong repulsive forces during storage. Our findings were similar to the results reported by Arora et al, 2022 [24].

Nanoparticles were prepared by modified solvent emulsification technique using lipid, surfactant, permeation enhancer and other solvents. Their research contributed size in range from 300 to 420 nm, which was due to the excipients and method of preparation used. Our research focused on investigating best method with optimum size, zeta and PDI. The presence of a negative charge on the interface of SLNs will cause double-layer repulsion between the formed droplets and prevent their agglomeration by strong repulsive forces during storage. Our findings were similar to the results reported by Arora et al, 2022 [24].

Particle size and polydispersity index (PDI)

A full factorial design solid lipid nanoparticles of experiments is required to evaluate every possible combination of excipients in the first formulation system is shown in the below table 3.

The particle size and PDI of SLNs loaded with rivastigmine tartrate were evaluated. Fig. 2 graphically represented the size of nanoparticles. The results indicate that particle size and surfactant concentration had an indirect relationship and that surfactant concentration affected the particle size of nanoparticles. The nanoparticles particle size was found to be 152.40+0.21 nm, 138.22+0.01 nm, 163.80+0.25 nm and 172.79 +0.23 nm for F-1, F-2, F-3 and F-4 formulations, respectively. Results are shown in table 3. All measurements were carried out by first diluting them with distilled water and removing the air bubbles by sonication. The readings were taken three times, and data were presented as mean±SD from the data obtained; it is clear that the minimum particle size was obtained in F-2 due to its method of preparation. Further, the nanoparticles were characterized, and cell viability study was conducted. The size was in range from 170±0.16 to 708±0.301 nm, while change in polymer and surfactant led to a decrease in particle size; our research led to the optimum size required for nanoparticles delivery through oral route. Ravi et al. in the year 2017 prepared solid lipid nanoparticles of rivastigmine tartrate and optimized it by using full factorial design [3].

Table 2: Solubility study of rivastigmine tartrate

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Name of lipids</th>
<th>Lipids point</th>
<th>Solubility</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Apifil</td>
<td>62-65±0.23</td>
<td>Not clear</td>
</tr>
<tr>
<td>2.</td>
<td>Compritol</td>
<td>65-77±0.02</td>
<td>Turbid</td>
</tr>
<tr>
<td>3.</td>
<td>GMS</td>
<td>55-60±0.22</td>
<td>Soluble and clear</td>
</tr>
<tr>
<td>4.</td>
<td>Precirol ATO 5 (PA)</td>
<td>52-55±0.55</td>
<td>Turbid</td>
</tr>
<tr>
<td>5.</td>
<td>Stearic acid</td>
<td>69-70±0.11</td>
<td>Not clear</td>
</tr>
</tbody>
</table>
potential. The particle sizes of all the formulations are similar; however, the zeta potential of the F-2 formulation was slightly different from the others, making it more stable than the others. The zeta potential of formulation F-2 is shown in fig. 3 and table 3. Our result of optimized formulation supports the findings similar to that of Kulkarni et. al, 2018 [23].

![Particle size distribution](image1)

**Fig. 2: Particle sizes of F-2 solid lipid nanoparticles**

![Zeta potential distribution](image2)

**Fig. 3: Zeta potential of F-2 solid lipid nanoparticles**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Formulation code</th>
<th>Lipid (mg)</th>
<th>Surfactant (%)</th>
<th>Zeta potential (mV)</th>
<th>Particle size (nm)</th>
<th>PDI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F-1</td>
<td>750</td>
<td>5%</td>
<td>-12.3±0.12</td>
<td>152.4±0.21</td>
<td>0.18±0.25</td>
</tr>
<tr>
<td>2</td>
<td>F-2</td>
<td>500</td>
<td>4%</td>
<td>-24.8±0.01</td>
<td>138.2±0.01</td>
<td>0.24±0.15</td>
</tr>
<tr>
<td>3</td>
<td>F-3</td>
<td>250</td>
<td>3%</td>
<td>-17.4±0.25</td>
<td>163.8±0.25</td>
<td>0.16±0.02</td>
</tr>
<tr>
<td>4</td>
<td>F-4</td>
<td>100</td>
<td>2%</td>
<td>-11.6±0.21</td>
<td>172.7±0.23</td>
<td>0.12±0.14</td>
</tr>
</tbody>
</table>

Parameters are expressed as mean±SD, n=3.
Scanning electron microscopy (SEM)

More information regarding particle size and shape of F2-SLNs dispersion was obtained using scanning electron microscopy. Nanoparticles photographed for SEM is shown in fig. 4. The nanoparticles were uneven in shape, as evidenced by SEM images. Drug loading and release behavior of nanoparticles might be affected by particle shape. SEM image describes that the particles have uniform loose aggregates, spherical in shape with a smooth surface and they are uniformly distributed. Solid lipid nanoparticles of rivastigmine tartrate were found to be spherical and irregular and their surface was smooth and devoid of cracks giving them good appearance. These results were similar to research supported by Kulkarni et al, 2018 [23].

Fourier transform infrared spectroscopy (FTIR)

FTIR analysis of pure drug and optimized formulation containing rivastigmine tartrate, GMS, Tween80 and Span 40 were shown in the fig. 5 (a), (b) and (c), respectively. The spectrum of pure drug rivastigmine tartrate showed an intense, well-defined peak, infrared band at around 1558.55 cm⁻¹ (-C=C-) and 3373.64 cm⁻¹ (-COOH) carboxylic acid peak and 3430.55 cm⁻¹ (-NH amine) peaks were observed. Infrared spectra of the optimized formulation showed the characteristic peaks of the pure drug rivastigmine tartrate. From the above interpretation, it was found that there was no shifting in the frequencies of above said, functional groups. Hence, above result conclude that no drug and excipients interaction were found. The data of FTIR were similar to results obtained in work done by Ravi et al 2017 and Kulkarni 2018 [4, 23].
Fig. 5: (C) FTIR spectra of rivastigmine SLNs-[F-2]. All values shown in the graph are measured as mean±SD, n=3

Differential scanning calorimetry (DSC)

Fig. 6 illustrates the DSC thermo grams of pure rivastigmine tartrate and rivastigmine tartrate SLNs F-2. The endotherm of pure rivastigmine tartrate peaked at 181 °C, which corresponds to its melting point or transition temperature. When the drug was formed into SLNs, the melting point of the drug changed, suggesting lower crystallinity. Table 4 contains the information gained from the dynamic DSC scans. The temperature T0, Tm and Tc are, respectively the onset of melt, the melting point and the completion of the compound. The results were similar to the findings reported by Ravi et. al 2017 and Dhawan et. al 2011 [4, 14].

Table 4: DSC thermo gram data for rivastigmine tartrate and optimized formulation

<table>
<thead>
<tr>
<th>Drug and formulation</th>
<th>To (°C)</th>
<th>Tm (°C)</th>
<th>Tc (°C)</th>
<th>Melting range (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rivastigmine tartrate</td>
<td>121.4±0.12</td>
<td>127.3±0.34</td>
<td>130.8±0.85</td>
<td>9.4±0.52</td>
</tr>
<tr>
<td>SLNs</td>
<td>118.7±0.10</td>
<td>124.4±0.25</td>
<td>131.2±0.56</td>
<td>12.5±0.21</td>
</tr>
</tbody>
</table>

Each value is represented as mean±SD of n=3 observations.

Fig. 6: DSC thermo grams of pure rivastigmine tartrate and RT-SLNs

Determination of entrapment efficiency and drug loading

It was shown that the percentage of entrapment might range from 34.59±0.01% to 69.27±0.23%. From 34.59±0.56% to 42.56±0.58% (i.e., formulations batches F-1, F-3 and F-4), the concentration of lipid (GMS) was seen to rise with the percentage entrapment efficiency. However, the entrapment efficiency did not significantly increase as the concentration of lipid rose over 50.96±0.45%. In comparison to other formulations, rivastigmine tartrate SLNs F-2 demonstrated a greater percentage of entrapment or 69.27±0.22%. The results were similar to the findings reported by Ravi et. al 2017 and Kulkarni et al., 2018 [4, 23].

In vitro drug release study for SLNs

Percentage of drug diffused over the duration (in hours) during in vitro diffusion between rivastigmine tartrate solid lipid nanoparticles and rivastigmine tartrate aqueous solution. With RT-SLNs, the percentage of RT diffused up to 24 h was 97.11±0.44%, while with rivastigmine tartrate, it was 28.36±0.22%. Because of rivastigmine, tartrate increased aqueous solubility in an aqueous medium; there is a modest increase in drug diffusion when using the drug solution at the beginning time points. In contrast, the initial time needed for the drug to leach out of the lipid core in RT-SLNs was longer than it was for the drug solution. The composition of the lipid matrix and the quantity of surfactants may have an impact on how quickly drugs are released from SLNs. The amphiphilic nature of GMS, which gives RT good solubility and leads to homogeneous distribution of RT within the lipid matrix, could explain why SLNs release more RT. Lowering of enthalpy and intensity, respectively, with lipid in RT-SLNs verified a flaw in the crystal structure of GMS, as demonstrated in DSC. As is common knowledge, melting less perfect crystal material needs less energy than melting a less ordered and/or...
flawed crystal lattice. Since the drug diffused out through defective crystal lattices on the lipid surface of RT-SLNs, this may be one of the causes of greater diffusion in the case of SLNs. Table 5 and fig. 7 contains a list of the information. The Rivastigmine tartrate SLNs particle size was inversely proportional to the drug release i.e. as the size of the nanoparticles decreases; there was an increase in the drug release. Lower concentration of lipid and lower concentration of surfactant retarded the drug release, whereas higher concentration of surfactant showed faster drug release. The release pattern supports the findings reported by Ravi et. al 2017 [3].

Table 5: In vitro drug diffusion (%) studies data

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Drug release (in %) F-1</th>
<th>Drug release (in %) F-2</th>
<th>Drug release (in %) F-3</th>
<th>Drug release (in %) F-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>22.67±0.01</td>
<td>100.43±0.26</td>
<td>35.48±0.65</td>
<td>38.6±1.02</td>
</tr>
<tr>
<td>4</td>
<td>49.41±0.21</td>
<td>30.78±0.85</td>
<td>57.55±0.36</td>
<td>55.4±0.59</td>
</tr>
<tr>
<td>8</td>
<td>65.88±0.55</td>
<td>49.86±0.54</td>
<td>79.87±0.58</td>
<td>79.3±1.03</td>
</tr>
<tr>
<td>16</td>
<td>78.14±0.02</td>
<td>68.13±0.56</td>
<td>91.86±0.55</td>
<td>93.6±0.56</td>
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<tr>
<td>20</td>
<td>92.12±0.21</td>
<td>83.36±0.26</td>
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<td>-</td>
</tr>
<tr>
<td>24</td>
<td>-</td>
<td>97.11±0.44</td>
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<td>-</td>
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</tbody>
</table>

Parameters are expressed as mean±SD, n=3.

Fig. 7: Comparative drug release profile of SLNs-F-1, F-2, F-3 and F-4. All values shown in graph are measured as mean±SD, n=3

Fig. 8: Escape latency (time taken by animal to find out the platform submerged in water) of different groups assessed by Morris water maze test. All values shown in graph are measured as mean±SD, n=3. **p<0.05 compare to control group. /Control - Negative control - Standard (RT) /Test (SLNs)
In vivo study-morris water maze test

Rats were examined for their spatial learning in the Morris water maze. When an animal finds the platform immersed in water, this is known as the "escape latency." The ability to locate the platform effectively will depend on how the signs outside the tank are arranged, as there is no proximal indicator to indicate its location. Learning is shown in the decreasing length of the path to the platform and the shorter latencies to escape. In all groups, the acquisition delay prior to therapy gradually decreased. Scopolamine, the negative control, showed a significant shift in retention latency following treatment (83.67±1.49 sec), indicating the development of dementia in rats (fig. 8). By disrupting ACh transmission in the CNS, scopolamine is known to produce amnesia. When compared to the negative control, memory retention for the standard formulation (RT solution) and test formulation (RT-SLNs) increased significantly by 27.17±0.78 sec and 25.33±1.58 sec, respectively. This demonstrated that RT protects memory by counteracting scopolamine's effects in rats. When compared to RT solution (normal formulation), the RT-SLNs demonstrated much superior memory retention, which may be explained by the lipid composition and nanostructure of the delivery system, which may lead to more drug accumulation in the brain for improved effect. Morris water maze model is suitable for in vivo study related to Alzheimer and is referred to be most effective by maximum researcher. The results were supported by the findings similar to the work done by Anand et al, 2019 [7].

Elevated plus maze test

The elevated plus maze experiment is predicated on the idea that rats prefer closed arms to open arms. Transfer latency is a measure of learning/memory based on the amount of time rats took to go from an open arm to an enclosed one (TL). If the animal has already entered the closed arms, the transfer delay is reduced. This suggests that memory may be involved in the decreased transfer delay. The animals were placed in the experiment one after the other at the end of the open arm, farther away from the centre. The same process was carried out three times for each group. The animal's travel time from the open to the closed arms was measured as TL1 during the first trial (pre-test). Rats were placed in the enclosed arm and given a further 60 seconds to explore if they did not reach the closed arm within the 90s, in which case TL2 was regarded as the 90s. After 24 h, the test was conducted in a manner identical to that of the primary trial for the second (retention) trial in order to record TL2. TL1 and TL2 did not differ significantly in the control group, which is attributable to the lack of any therapy, whereas TL2 increased dramatically in the negative control group after receiving scopolamine as a treatment (fig. 9). This can be the result of amnesia brought on by scopolamine. Both the standard and test formulation groups had a statistically significant decline in TL2, which may have been caused by the training-induced memory retention, although the impact was most pronounced in the test formulation group (RT-SLNs). Anand et al, 2019 conducted the same animal model study of SLNs for rivastigmine and the observation were similar to our results [7].

Fig. 9: Transfer latency (time consumed by rats to travel from an open arm to enclosed one) of different groups assessed by elevated plus maze test. All values shown in graph are measured as mean±SD, n=3. **p<0.05 compare to control group/Control Negative control Standard (RT)/Test (SLNs)

STABILITY STUDY

The formulation F-2 SLNs were studied for stability study as per ICH guidelines. The formulation was stable at room temperature with minor change in particle size in three months. The initial particle size at day 0 was 138.22±0.01 nm and the size after 3 mo was 139±0.4 nm. Cunha et. al in year 2020 performed an experiment on rivastigmine and followed the ICH guidelines [5]. This minor increase in particle size may be due to the weak Vander Waals force that holds the particles together, which lead to the formation soft agglomerates. Thus SLNs were stable at room temperature for 3 mo.

CONCLUSION

The solid lipid nanoparticles were successfully prepared for oral delivery of rivastigmine used for the treatment Alzheimer's disease. The present research work concludes the best method suited for stability and patient compliance with all desired characteristics parameters. SLNs prepared by microemulsion cooling technique were best of all the methods selected. This shows that method of preparation affects the release profile, size and zeta of nanoparticles. To support the above findings, higher evaluation and further experiments should be conducted.

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AUTHORS CONTRIBUTIONS

All authors have contributed equally.
CONFLICTS OF INTERESTS
Declared none

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