Alzheimer’s disease (AD) is currently one of the biggest healthcare concerns especially in the developed countries. The available treatment strategies fail to impart any significant impact since the drug doesn’t reach in required amount to central nervous system (CNS) owing to presence of blood-brain barrier (BBB). The present study includes extraction of bacside rich extract from a well-known nootropic herb, *Bacopa monnieri* (Brahmi), and its loading to solid lipid nanoparticles (SLNs). Solid lipid nanoparticles are well known for their BBB permeability and controlled drug release characteristics. For preparation of SLNs, glyceryl monostearate (GMS) was used. The extract was characterized for various parameters like melting point, UV-spectroscopy and Fourier transform infra red (FTIR) spectroscopy. SLNs were prepared by hot homogenization followed by sonication method. The drug loaded SLNs were characterized for their particle size, zeta potential and poly dispersity index (PDI) initially to select the best formulation which was further characterize for drug entrapment efficiency, morphological study using transmission electron microscopy (TEM) and *in vitro* drug release profile. Further, the mechanism of drug release was found out by applying various kinetic models and the formulation was evaluated for its storage stability profile. The formulation was found to possess nanometric size and was able to control the release of drug up to 24 hrs. It followed Hixson-Crowell performance evaluation to establish its true potential.

**Keywords**: Alzheimer’s disease, Central Nervous System (CNS), Bacosides, Solid Lipid Nanoparticles, Glyceryl monostearate.

**Introduction**

Alzheimer’s Disease (AD) is one of the most significant causes of memory loss or dementia (Alzheimer’s Association, 2019) which mainly affects the elder people. AD is a central nervous system related disorder that involves progressive deterioration of neurons resulting in progressive loss of cognitive behaviour, memory impairment etc. eventually leading to mental illness (Folch et al., 2016). Out of the 47 million people reported worldwide for being suffering from dementia, around 37 million are reported with AD (Prince et al., 2016). Currently, AD is considered as the 6th leading cause of death of elder patiens which is assumed to get doubled every 20 years. The average expense of its treatment is very high that makes it difficult for middle class people to afford with (Alzheimer’s Association, 2017).

**Role of Bacopa monnieri in Alzheimer’s disease**

Herbal medications have gained increased acceptance being safer than the synthetic drugs (Kahol et al., 2004). Herbs like Mandukaparni, Shankhpushpi, Guduchi, and Yastimadhu have been traditionally mentioned to have a memory improving effects. Some other herbs like Brahmi, Jatamansi, and Vacha have also been known for their efficacy in such situations (Kulkarni et al., 2012). *Bacopa monnieri* (Brahmi or water hyssop) is recognized for its role as a Medhya Rasayana or a nootropic plant in Ayurveda (Singh et al., 1997). Brahmi is seen distributed in the plains of Southeast Asia, tropical Asia, sub-tropical United States, tropical Africa, and Australia (Russo et al., 2005). Including roots, the entire plant can be used for its medicinal uses (Aguier et al., 2013). Many researchers have proved that *Bacopa monnieri* extract and isolated bacside (the major active principles present in Brahmi) have got beneficial effects in the treatment of Alzheimer’s disease.

Animal studies of *Bacopa monnieri* whole plant alcohol extracts have been reported to have cognition-enhancing effects including improved motor learning and acquisition, consolidation, and retention of memory in rats. The memory-enhancing effects have been attributed to saponins (bacosides, bacopasides, or bacopasaponins). Bacopa extracts have also reduced β-amyloid levels in the brain of a doubly transgenic mouse model of rapid amyloid deposition (PSAPP mice), suggesting mechanisms of action relevant to Alzheimer’s disease (Carlo et al., 2008).

The sulfhydryl and polyphenol components of *Bacopa monniera* extract have also been shown to affect the oxidative stress cascade by scavenging reactive oxygen species, inhibiting lipoxigenase activity and reducing divalent metals. This mechanism of action explains the effect of *Bacopa monniera* extract in reducing beta-amyloid deposits in mice with Alzheimer's disease (Dhanasekaran et al., 2007). The results of clinical trial were also highly encouraging which showed improvement of various components of cognitive functions in geriatric patients suffering from Alzheimer’s disease who consumed *Bacopa monnieri* for six months (Shishir et al., 2001).

**Solid Lipid Nanoparticles (SLNs)**

Delivery of drugs to CNS is a tough task owing to presence of BBB. It asks for drug carriers which are capable
of taking drug molecules across the barrier to CNS. Solid lipid nanoparticles (SLNs) by virtue of their surface functionalization (Luo et al., 2006) neutral lipid character and nanoscale particle size, can effectively transport a delivery package across the BBB and into the brain tissue. SLNs have shown a great promise for reaching the goal of controlled and site-specific drug delivery and hence have attracted wide attention of researchers around the globe (Xu et al., 2009). In addition to that, they offer drug targeting, avoidance of carrier associated biotoxicity, biodegradation and easy large-scale production (Piazzinia et al., 2019).

In this study, SLNs were prepared using a lipid glyceryl monostearate and then loaded with bacoside-A rich extract. SLNs were prepared using hot homogenization technique followed by sonication. The prepared SLNs were characterized and on the basis of preliminary results of particle size and zeta potential, the best formulation was selected for further characterization to evaluate its in-vitro performance.

![Fig. 1: Extraction procedure for Bacopa monnieri](image)

Characterization of extract

Melting point

The melting point was determined by filling the extract into capillary tube sealed at one end at a height of 3 mm from the closed end. The capillary was then introduced into the digital melting point apparatus. The temperature at which the extract melted was noted down.

FTIR studies

FTIR spectrum of obtained bacoside-A rich extract was recorded by scanning the sample over a wavelength region of 4000 to 400 cm\(^{-1}\). The procedure consisted potassium bromide (KBr) pellet method. The spectrum so obtained was compared with reported spectrum of marker compound (Gohel et al., 2014).

Determination of Saponin content

Saponin content was determined by double solvent extraction gravimetric method (Mbagwu et al., 2010).

Materials and Methods

Materials

Fresh aerial parts of Bacopa monniera plant were collected from Una (Himachal Pradesh) and got authenticated from Central Council for Research in Ayurveda & Siddha, Tamil Naidu. The Bacoside A was a generous gift from Prof (Dr) Ikhlas A. Khan, School of Pharmacy, Mississippi University, USA. All the other chemicals were of analytical grade.

Extraction Procedure

The aerial parts of the plant were used for extraction. Collected plant parts were dried and crushed to make powder. The extraction process was carried out using a reported procedure (Kahol et al., 2004) The percentage yield was calculated using given formula:

\[
\text{Percentage yield} = \frac{\text{Weight of crude extract} \times 100}{\text{Weight of dried plant}}
\]

Characterization of extract

The % saponin content was calculated using the formula given below:

\[
\% \text{Saponin} = \left(1 - \frac{W_2}{W_1}\right) \times 100 \times 1
\]

where \(W_1\) = Weight of evaporating dish; \(W_2\) = Weight of dish + sample

Calibration curve

A reported procedure with slight modifications was followed for the calibration curve of the bacoside rich extract (Deshpande SG et al., 2014). Methanol was used for the calibration curve and dilutions ranging from 50-500 µg/ml were made. The solution was scanned on UV spectrophotometer and \(\lambda_{max}\) was noted down.
Preparation of Solid Lipid Nanoparticles

Preparation by hot homogenization followed by sonication

Bacoside-A rich extract (methanolic), glyceryl mono stearate and soya lecithin were dissolved in 20 mL mixture of chloroform and methanol. Organic solvents were completely removed using a rotaevaporator and drug embedded lipid layer was melted by heating at 5°C above melting point of the lipid. Poloxamer 188 was dissolved in distilled water heated to the same temperature as that of oil phase to prepare an aqueous solution. Aqueous phase was added to the oil phase and then the homogenization was carried out at 11,000 rpm for 3 minutes. This hot oil in water emulsion was ultrasonicated for 2 minutes. Solid lipid nanoparticles were obtained by allowing hot nanoemulsion to cool to room temperature (Thatipamula R et al., 2012).

Table 1: Composition of prepared SLN formulations

<table>
<thead>
<tr>
<th>Chemicals</th>
<th>Formulations</th>
<th>GMS-1</th>
<th>GMS-2</th>
<th>GMS-3</th>
<th>GMS-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacoside A rich extract (mg)</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Glyceryl monostearate (mg)</td>
<td>450</td>
<td>600</td>
<td>300</td>
<td>450</td>
<td>300</td>
</tr>
<tr>
<td>Soya lecithin (mg)</td>
<td>150</td>
<td>300</td>
<td>300</td>
<td>300</td>
<td>300</td>
</tr>
<tr>
<td>Poloxomer F-68 (mg)</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1.5</td>
<td>1</td>
</tr>
<tr>
<td>Chloroform: methanol (ml)</td>
<td>20:5</td>
<td>20:5</td>
<td>20:5</td>
<td>20:5</td>
<td>20:5</td>
</tr>
<tr>
<td>Water (ml)</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
</tr>
</tbody>
</table>

Characterization of nanoparticles

Particle size and zeta potential determination

The prepared nanoparticles were evaluated for particle size using photon correlation spectroscopy based on dynamic light scattering technique using Malvern Zeta sizer. Zeta potential was also measured with Zeta sizer using the principle of electrophoretic mobility under an electric field. The best formulation was selected based on the observed particle size analysis and was subjected to further characterization.

Transmission electron microscopy (TEM)

Morphology of the prepared nanoparticles was observed by Transmission Electron Microscopy (TEM). Drug loaded SLNs (optimized) were diluted with distilled water, sonicated and a few drops were placed on Cu grid to place it in sample holder for capturing the images of formulated nanoparticles.

Drug Entrapment Efficiency

The percentage of entrapped bacoside A rich extract was determined spectrophotometrically at detected wavelength. After centrifugation of the aqueous suspension at 15000 rpm for 15 minutes, amount of the free drug was detected in the supernatant and the amount of entrapped drug was determined as a result of initial drug minus free drug. The entrapment efficiency can be calculated using the given formula (Raina et al., 2017).

\[
\text{Entrapment Efficiency (EE\%)} = \frac{\text{Total drug} - \text{Free drug in supernatant}}{\text{Total drug}} \times 100
\]

In vitro drug release study

In vitro drug release study was carried out for 24 hours using phosphate buffer pH 7.4 as dissolution medium. The study was performed by incubating 10 ml of formulation (placed in a small cylinder fitted with 12000 Da cellulose membrane at the bottom) in 50 ml of aqueous buffer pH 7.4 at 37°C with continuous stirring on magnetic stirrer. Samples (2 mL) were withdrawn periodically. Equal volume of medium was replaced after each withdrawal. The withdrawn samples were then analyzed for the amount of drug released by measuring absorbance using UV spectrophotometer. The study was carried out in triplicate (Makwana et al., 2015) and on the basis of obtained results, optimized formulation from was selected and characterized further.

Drug Release Kinetics

An appropriate drug release test is required to characterize the drug product and ensure batch-to-batch reproducibility and consistent pharmacological/biological activity. The dissolution data were analyzed on the basis of zero-order model (cumulative amount of drug released vs time), first-order rate (log cumulative amount of drug remaining vs time), Higuchi model (cumulative amount of drug released vs square root of time), Korsmeyer-Peppas model (log cumulative amount of drug released vs log of time) and Hixon-Crowell. The correlation coefficient ($R^2$) for each rate order was calculated (Perge et al., 2012).

Stability studies

The optimized SLN formulation was divided into 2 parts and stored in a refrigerator (i.e. at 2-8°C) and at 25°C/65% RH respectively to assess the storage stability of optimized formulation and ascertain the required storage conditions. Samples were periodically withdrawn for 3 months and examined for their particle size and drug entrapment efficiency (Dhawan et al., 2011).

Results and Discussion

Bacoside rich extract yield

\[
\text{Percentage Yield} = \frac{\text{Weight of crude extract}}{\text{Weight of dried plant}} \times 100
\]

where,

Weight of crude extract = 3.3 g Weight of dried plant powder = 40 g

So, percentage yield was found to be 8.25%

Characterization of bacoside rich extract

Melting point: The melting point of Bacoside A rich extract was found to be 254°C in comparison to reported melting point of 250°C (Kahol et al., 2004).

FTIR studies: The FTIR spectrum of bacoside A rich extract showed bands at 3365 cm⁻¹ (hydroxy group), 2935.16 (alkane), 1614.7 (ketone), 1429.15 (double bond), 1281.17 (ether), 1094.7 (ether linkage) respectively, which were
almost identical with the bands of functional groups present in reported spectrum of bacoside A marker (Gohel et al., 2016).

**Determination of Saponin content**

\[
\% \text{Saponins} = \frac{W_2 - W_1}{\text{Weight of Sample}} \times 100
\]

where \( W_1 \) = Weight of evaporating dish; \( W_2 \) = Weight of dish + sample

**Saponins in Crude Drug**

\[
\% \text{Saponins} = \frac{66.195 - 66.050}{2} \times 100
\]

\[
\% \text{Saponins} = \frac{0.145}{2} \times 100
\]

\[
\% \text{Saponins} = 0.0725 \times 100 = 7.25\%
\]

**Saponins in bacoside A rich extract**

\[
\% \text{Saponins} = \frac{66.367 - 66.072}{2} \times 100
\]

\[
\% \text{Saponins} = \frac{0.295}{2} \times 100
\]

\[
\% \text{Saponins} = 0.1475 \times 100 = 14.75\%
\]

The percentage yield obtained for the saponin content in crude drug and bacoside A rich extract were 7.25% and 14.75% respectively. This difference was almost double due to concentration of saponins in bacoside rich extract.

**Calibration curve**: Calibration curve of bacoside A rich extract was prepared to know the straight-line equation which was further used for estimating the drug release from prepared formulations. The calibration curve along with straight line equation are given in Fig. 2 below.

![Calibration curve](image)

**Fig. 2**: Calibration curve of Bacoside-A rich extract

**Characterization of nanoparticles**

All the prepared formulations were characterized for their particle size analysis. However, only one formulation was having particle size in nanometric range whereas the remaining formulations possessed μm range. The average particle size of GMS-3 was found to be less than 200 nm (180.2 nm). Increase in lipid content was found to exhibit greater particle size probably due to reduction in homogenization efficiency with increasing dispersed lipid phase. So, the best formulation (GMS-3) was selected for further studies.

**Zeta Potential and PDI**

The results of zeta potential and PDI are given in table below. The optimized formulation possessed a zeta potential of -10.4 mV and PDI of 0.350 which was quite convincing.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Formulation</th>
<th>Mean particle diameter (nm)</th>
<th>Zeta potential (mV)</th>
<th>Polydispersity index (PDI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>GMS-3</td>
<td>180.2</td>
<td>-10.4</td>
<td>0.350</td>
</tr>
</tbody>
</table>

**Transmission electron microscopy (TEM)**

The TEM images indicated that the Bacoside A loaded solid lipid nanoparticles were in nanometric range (below 200 nm) and spherical and elongated in shape. TEM image of individual particles clearly revealed the shape with irregular surfaces.
Drug entrapment efficiency

The drug entrapment efficiency of solid lipid nanoparticles was found to be 81.9 ± 2.74 for formulation GMS-3.

In vitro drug release study

Cumulative amount of drug release was plotted against time in order to construct release profile (formulation GMS-3). An initial rapid release was observed followed by slower release rate. The initial burst rate may be due to desorption of drug associated with the surface of nanoparticles and the slow release in the later stage was attributed to the fact that solubilized drug can only be released slowly from the lipid matrices due to dissolution and diffusion. The formulation was able to release the drug up to 24 hrs at a sustained rate.

Drug release kinetics

The in vitro dissolution data of selected formulation was subjected to goodness of fit test by linear regression analysis according to zero-order, first-order kinetic equations, Higuchi model, Korsmeyer-Peppas and Hixson-Crowell models to assess the mechanism of drug release. The formulations GMS-3 followed Hixson-Crowell dissolution model which meant that the release of drug from the formulation was significantly affected by change in surface area during the process of dissolution (Perge L et al., 2012).

![Fig. 3: TEM images showing particles](image)

![Fig. 4: Cumulative % drug release profile of formulation GMS-3](image)

![Fig. 5. Drug release kinetics for formulation GMS-3](image)

Stability studies

The formulation stored in refrigerated conditions didn’t exhibit any significant change in their particle size and drug entrapment efficiency after 3 months of storage, however, the formulation stored at higher temperature and humidity (25°C+65% RH) showed a significant increase in particle size from nanometer range to micrometer range in association with a significant decrease in drug entrapment efficiency which indicated the optimum conditions of prepared formulations. The results of the stability studies are summarized in table 3 below.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Stability time points in months</th>
<th>Stability time points in months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Refrigerated conditions</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Particle size (nm)</td>
<td>180</td>
<td>184</td>
</tr>
<tr>
<td>Entrapment efficiency (%)</td>
<td>81.90</td>
<td>79.02</td>
</tr>
<tr>
<td>25°C + 65% RH</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3: Various parameters of the optimized formulation analysed during stability studies
Conclusion

In the present study, Bacoside-A rich concentrate was extracted from the aerial part of the nootropic/cognitive enhancer drug Bacopa monnieri (Scrophulariaceae) and loaded to solid lipid nanoparticles composed of glyceryl monostearate.

The extract was found to be in compliance with the reported parameters. The UV-scan of bacoside-A rich extract in methanol showed absorption maxima at 226.3 nm which was in accordance with the reported value (i.e. 225 nm). The FTIR study revealed the comparable characteristics bands in bacoside-A rich extract as that of marker. A total of 4 formulations were prepared using glyceryl monostearate by hot homogenization followed by sonication technique. On the basis of particle size analysis, the best formulation was selected and further characterized for drug entrapment efficiency, zeta potential measurement, in vitro drug release study. The formulation showed a zeta potential of -10.4mV with 0.350 PDI. The drug entrapment efficiency was found to be 81.9% for glyceryl monostearate nanoparticles. TEM images of the solid lipid nanoparticles showed almost spherical shape with irregular surfaces. The solid lipid nanoparticle formulation was able to prolong the drug release upto 24 hours (90% drug release) and followed Hixson-Crowell drug release model. Furthermore, the formulation was found to be stable over refrigerated temp for a period of 3 months. The in vitro performance of formulated solid lipid nanoparticles was satisfactory and demands evaluation of its in vivo performance to establish the potential of developed delivery system with respect to CNS targeting and sustained nootropic effect.

Conflict of Interest

Authors have none to declare

Acknowledgement

The authors are grateful to IKG Punjab Technical University for providing access to literature available online. Authors also wish to thank Prof (Dr) Ikhlas A. Khan, School of Pharmacy, Mississippi University, USA for a generous gift of Bacoside A marker.

References