IJPSR (2020), Volume 11, Issue 1 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 13 November 2019; received in revised form, 22 December 2019; accepted, 25 December 2019; published 01 January 2020

FORMULATION AND EVALUATION OF SOLID LIPID NANOPARTICLES LOADED WITH BACOSIDE RICH EXTRACT

Rajesh Kumar [1, 2] and Rajeev Garg [* 3]

IKG Punjab Technical University [1], Kapurthala - 144603, Punjab, India.
School of Pharmaceutical Sciences, Lovely Professional University [2], Phagwara - 144411, Punjab, India.
Amar Shaheed Baba Ajit Singh Jujhar Singh Memorial (ASBASJSM) College of Pharmacy [3], Bela, Ropar - 140111, Punjab, India.

Keywords:

Solid Lipid Nanoparticles, Stearic acid, Homogenization, Blood-Brain Barrier, Brahmi

Correspondence to Author:
Dr. Rajeev Garg

Associate Professor in Pharmaceutics, Amar Shaheed Baba Ajit Singh Jujhar Singh Memorial (ASBASJSM) College of Pharmacy, Bela, Ropar - 140111, Punjab, India.

E-mail: rajksach09@gmail.com

ABSTRACT: The treatment of neurodegenerative disorders such as Alzheimer's disease (AD) becomes much more difficult due to the presence of the blood-brain barrier (BBB). Various synthetic drugs are used for the treatment of Alzheimer's disease, but the use of herbal products has increased tremendously nowadays. An important medicinal plant widely used therapeutically is *Bacopa monnieri* (Brahmi), a well-known nootropic herb of family Scrophulariaceae. The bacosides present in Brahmi aid in the repair of damaged neurons and ultimately improve nerve impulse transmission. However, the delivery of every such drug is limited due to the failure of the drug to overcome the blood-brain barrier and reach CNS. Solid lipid nanoparticles (SLNs) are the drug carriers that have been able to penetrate the blood-brain barrier and improve the therapeutic efficacy of drugs. The current study focuses on the treatment strategy for AD using bacoside rich extract loaded SLNs. The bacoside rich concentrate was extracted from the aerial part of the plant and was characterized by melting point determination, HPTLC, UV-spectroscopy and FTIR. SLNs were prepared with a simple lipid stearic acid using hot homogenization technique and were characterized for particle size, zeta potential, drug entrapment efficiency, TEM, *in-vitro* drug release studies, release kinetics and stability studies. The formulation was found to exhibit prolonged drug release for 24 h and showed appreciable stability for 3 months during stability studies which confirmed the efficacy of formulated solid lipid nanoparticles.

INTRODUCTION: Alzheimer's disease is the major cause of mental disability in elder people. Psychiatric symptoms associated with Alzheimer's disease, although common, have not received much attention yet. Alzheimer's disease (AD) is the advanced form of dementia which mainly shows its symptoms from middle age to old age [1].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.11(1).371-77

The article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.11(1).371-77

After myocardial infarction, stroke, and cancer, it is the most leading cause of death amongst 10% of 70 years aged people. Alzheimer's disease, categorized as a chronic neurodegenerative disease, is characterized by a progressive impairment of cognitive functions and memory loss, emotional distress and behavioral symptoms [2].

Age acts as a major risk factor for the development of AD, with the prevalence doubling every 5 years after the age of 65 [3]. The main clinical feature of this disease is the impairment of memory, short term memory, and cognitive disability. As the condition progresses, additional cognitive abilities are impaired such as the ability to calculate,

visuospatial skills and ideomotor apraxia [4]. In patients of Alzheimer's disease, loss of personal capability progresses to such an extent that they even cannot memorize where they have kept their valuables or other necessary household things and have to search for hours to locate them; or cannot recall the names of their children or grandchildren. Many Alzheimer's disease patients have concurrent psychiatric symptoms during the course of illness. Alois Alzheimer, in his original report regarding research of AD, described a 51-year older woman with memory impairment together with suspiciousness, paranoid delusions, and auditory hallucination [5]. It is predicted that if no efficient therapeutic and/or early-diagnosis approaches become available in the next few decades, AD will exert a huge societal and economic impact. Therefore, strategies for early detection as well as treatment of AD are amongst the most challenging and timely areas in modern medicine.

***Bacopa Monnieri* in Alzheimer's Disease:** *Bacopa monnieri*, commonly known as water hyssop, is a herb often used in Ayurveda. *Bacopa monnieri* has been shown to improve cognition, by means of reducing Anxiety. It is also reliable for improving memory formation. Though the effects of this nature are usually studied in the elderly, *Bacopa monnieri* appears to affect young people as well, making it a useful Nootropic agent [6].

Bacopa's ability to enhance nerve impulse transmission can be attributed to triterpenoid saponins and their bacosides. The bacosides aid in the repair of damaged neurons by enhancing kinase activity, neuronal synthesis, and restoration of synaptic activity and ultimately nerve impulse transmission [7]. Loss of cholinergic neuronal activity in the hippocampus is the primary feature of Alzheimer's disease. Based on animal study results, bacosides have appeared to have antioxidant activity in the hippocampus, frontal cortex and striatum.

Animal research has shown bacopa extracts modulate the expression of certain enzymes involved in the generation and scavenging of reactive oxygen species in the brain. *In-vitro* research has shown that bacopa exerts a protective effect against DNA damage in astrocytes and human fibroblasts [8].

Solid Lipid Nanoparticles (SLNs): Nanocarriers are drug transport systems that have gained a great deal of attention over recent decades for their ability to facilitate site-specific drug delivery, including brain delivery [9]. Solid lipid nanoparticles, in particular, are regarded as interesting drug delivery systems. Their preparation techniques have gained a great deal of attention. In contrast to emulsion and liposome, the particulate matrix of SLNs is composed of solid lipid [10]. They are colloidal particles of submicron size, with a diameter between 50 to 1000 nm for colloid drug delivery applications.

They are made of a lipid matrix (that exists in the solid phase at room temperature), surfactants and sometimes, cosurfactants as well [11]. SLNs were developed at the beginning of the 1990s as an alternative carrier system to emulsions, liposomes, and polymeric nanoparticles. They provide advantages including stabilization of incorporated compounds, controlled release, and conclusiveness. In the current study, SLNs loaded with bacoside rich extract were prepared using lipid namely stearic acid by hot homogenization technique.

The prepared SLNs were characterized and on the basis of obtained results, the best formulation was selected amongst various formulations and further characterized for *in-vitro* performance evaluation.

MATERIALS AND METHODS:

Materials: The sample of fresh aerial part of *Bacopa monniera* was collected from Una (Himachal Pradesh) and authenticated from Central Council for Research in Ayurveda & Siddha, Tamil Naidu. The marker for Bacoside A was a generous gift from Prof (Dr.) Ikhlas A. Khan, School of Pharmacy, Mississippi University, USA. Other chemicals used during the study were of analytical grade and were used as received.

Extraction Procedure: For extraction purposes, the aerial parts of the plant were used. Collected parts were dried and crushed to powder with disintegrator. The whole process of extraction was carried out using the well-established procedure [12]. The percentage yield of bacoside rich extract was calculated by the formula:

Percentage Yield = Weight of crude extract/weight of dried plant × 100…(eq.1.)

Characterization of Extract:

Melting Point: The melting point was determined by the capillary method. The extract was filled into a capillary tube sealed at one end at a height of 3 mm from the closed end. Then this capillary was introduced into the digital melting point apparatus. The temperature at which the extract melted was noted down.

Chromatographic Analysis: The obtained extract was characterized for chromatographic analysis (TLC and HPTLC) in comparison to marker compound as a part of method development and validation and the results were published earlier [13].

FTIR Studies: FTIR spectrum of bacoside a rich extract was recorded by scanning the sample over a wavelength region of 4000 to 400 cm^{-1}. The procedure consisted of potassium bromide (KBr) pellet method. The pellet was placed in the light path and the spectrum was obtained which was further compared with the reported spectrum of marker compound [14].

Calibration Curve: The calibration curve of the bacoside rich extract was prepared according to the reported procedure with slight modifications [15]. For the preparation of the stock solution, 100 mg of extract was dissolved in 100 ml of methanol with the addition of 0.1 ml of polyethylene glycol (PEG). Using the stock solution, different dilutions were made ranging from 50-500 µg/ml. The solution was scanned on a UV spectrophotometer and λ_{max} was noted down.

Preparation of Solid Lipid Nanoparticles:

Preparation by Hot Homogenization: Solid lipid nanoparticles of bacoside A rich extract were prepared by using lipid (stearic acid), surfactant (Phospholipon 80-H) and stabilizer (tween 80) by hot homogenization technique using the high-speed homogenizer. The organic phase was first prepared by dissolving the drug and surfactant in dichloromethane and then mixing it with the stearic acid which was previously melted. Then it was further mixed with aqueous tween 80 solutions, maintained at the same temperature as that of the organic phase and stirred at 12,000 rpm for 30 minutes. This formulation was removed from the water bath and the dispersion of SLNs was mixed gently by slow magnetic stirring for one hour at room temperature until cooling [16]. The composition of formulations is shown in **Table 1** below.

TABLE 1: COMPOSITION OF PREPARED FORMULATIONS

Chemicals	Formulations			
	SA1	SA2	SA3	SA4
Bacoside A rich extract (mg)	40	40	40	40
Stearic acid (mg)	600	300	300	600
Phospholipon 80H (mg)	600	600	300	300
Tween 80 (ml)	1.5	1.5	2	1.5
Dichloromethane (ml)	20	20	20	20
Water (ml)	30	30	30	30

Characterization of Nanoparticles:

Particle Size and Zeta Potential Determination: The particle size was measured by photon correlation spectroscopy based on dynamic light scattering technique using Malvern Zeta sizer. Zeta potential of core particles was also measured using the principle of electrophoretic mobility under an electric field. Based on the observed results for particle size analysis, one formulation was selected as an optimized formulation 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 and sonicated. Few drops of the diluted nanoparticles were placed on a Cu grid, which was then placed in the sample holder to capture the images of formulated particles.

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 min, the 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 was calculated using the below-given formula [17].

Entrapment Efficiency (EE%) = Total drug - Free drug in supernatant / Total drug × 100 (eq.3.)

***In-vitro* Drug Release Study:** *In-vitro* drug release study was carried out for 24 h 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 cellophane membranes 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 [18] and on the basis of obtained results, optimized formulation from each batch 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 [19]. The correlation coefficient (R^2) for each rate order was calculated.

Stability Studies: The optimized SLN formulation was stored in a refrigerator (*i.e.* at 2-8 °C) and at 25 °C / 65% RH alternatively to assess the storage stability of optimized formulation and ascertain the storage conditions. The samples were periodically withdrawn at monthly intervals for 3 months and examined for its particle size and drug entrapment efficiency [20].

RESULTS AND DISCUSSION:

Yield of Bacoside Rich Extract:

Percentage Yield = Weight of crude extract / Weight of dried plant × 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 [12].

Charmatographic Analysis (HPTLC Method Development and Validation): Bacoside rich extract was found to show a peak at 225 nm in HPTLC chromatogram similar to marker compound.

The observed R_f value (0.44) was also found to be in agreement with the reported value for marker compound (R_f = 0.43) [13].

FTIR Studies: The FTIR spectrum of bacoside A rich extract is shown in **Fig. 1** below. The functional groups were found to be comparable with those present in the reported spectrum of bacoside Amarker [14].

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

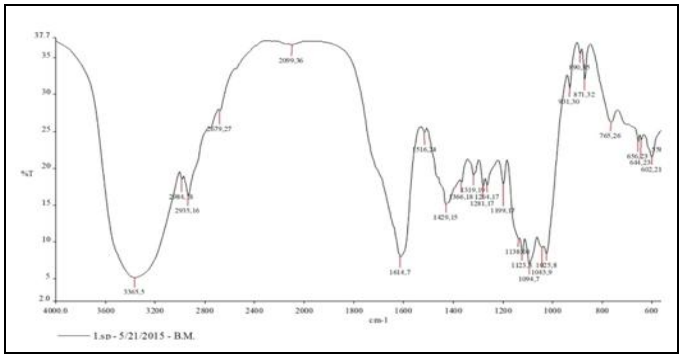

FIG. 1: FTIR SPECTRUM OF BACOSIDE A RICH EXTRACT

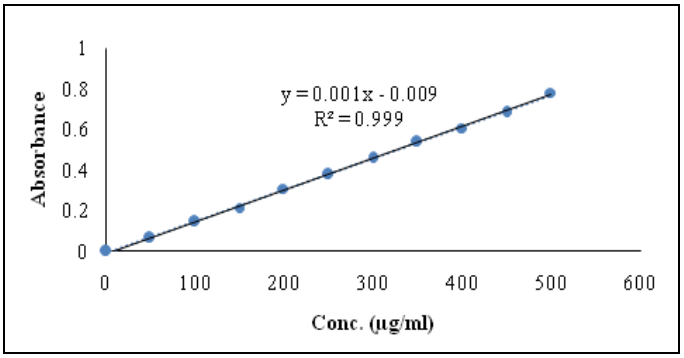

FIG. 2: CALIBRATION CURVE OF BACOSIDE A RICH EXTRACT

Characterization of Nanoparticles:

Particle Size and Zeta Potential: Prepared formulations were analyzed to determine their particle size distribution, zeta potential, and PDI values. The results are represented in **Table 2**. The average particle size of formulation SA2 was found

to be 240.3 while remaining formulations were existing in the micrometer range. Increasing lipid content was found to exhibit greater particle size probably due to a reduction in homogenization efficiency with increasing dispersed lipid phase. Based on the nanometric size, formulation SA2 was selected as an optimized one which possessed a zeta potential of -16.6.

Other formulations were showing lower values for zeta potential and higher value for DPI whereas the selected formulation was showing PDI as 0.337.

TABLE 2: PARTICLE SIZE, PDI AND ZETA POTENTIAL OF SELECTED FORMULATION

Sr. no.	Formulation	Mean particle diameter (nm)	Zeta potential (mV)	Polydispersity index (PDI)
1	SA2	240.3	-16.6	0.337

Transmission Electron Microscopy (TEM): The TEM images indicated that the Bacoside rich extract loaded solid lipid nanoparticles were in nanometric range (below 250 nm) and spherical and elongated in shape. The TEM image of individual particles clearly revealed the shape with irregular surfaces.

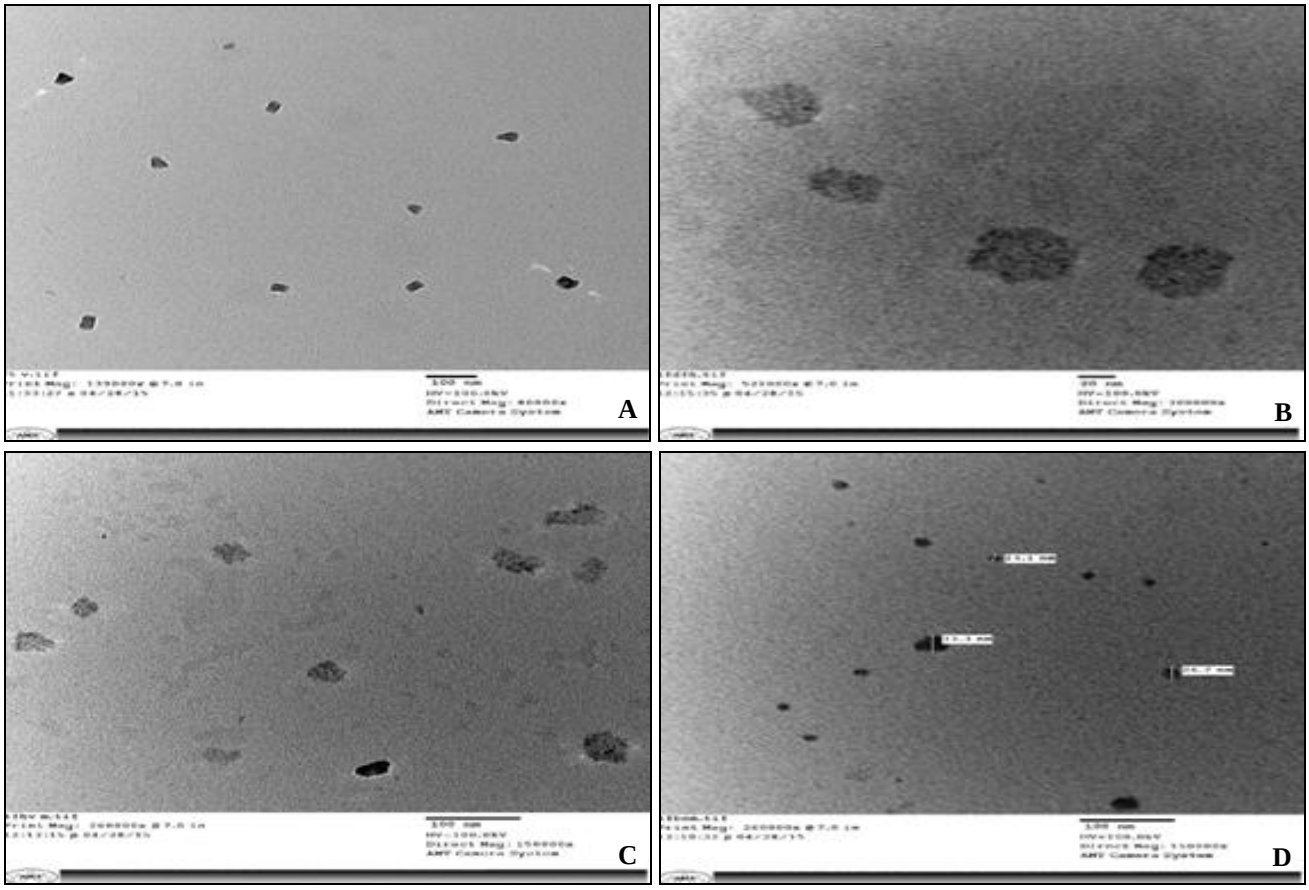

FIG. 3: TEM IMAGES SHOWING PARTICLE SIZE AND SHAPE OF THE FORMULATION SA2

Drug Entrapment Efficiency: The drug entrapment efficiency of selected solid lipid nanoparticle formulation (SA2) was found to be 76.73 ± 0.4.

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

Drug Release Kinetics: The *in-vitro* dissolution data of selected formulation was subjected to the 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 formulation SA2 was best fitted to the Hixson-Crowell dissolution model which meant that that change in surface area during the process of dissolution had a significant effect on drug release [19].

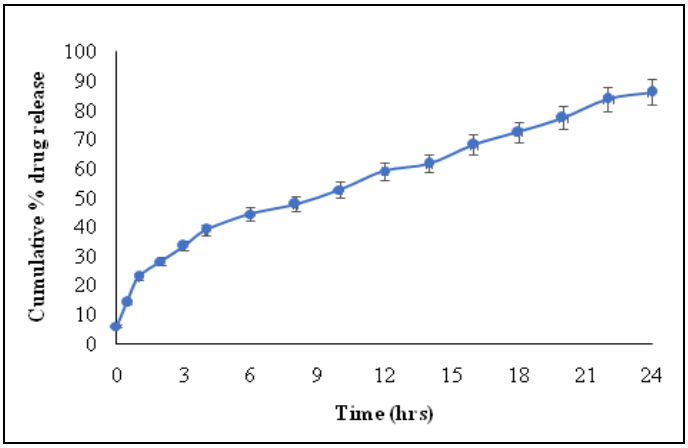

FIG. 4: CUMULATIVE % DRUG RELEASE PROFILE OF FORMULATIONS A2

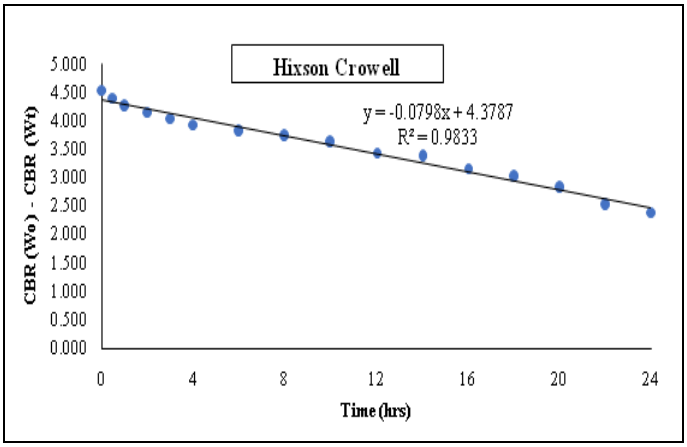

FIG. 5: DRUG RELEASE KINETICS OF FORMULATION SA2

Stability Studies: The formulation stored in refrigerated conditions didn't exhibit any significant change in its particle size and drug entrapment efficiency after 3 months of storage; however, the formulation stored at higher temperature and humidity (25 °C + 65% RH) exhibited a significant increase in particle size from nanometer range to micrometer range coupled 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 3: VARIOUS PARAMETERS OF THE OPTIMIZED FORMULATION ANALYSED DURING STABILITY STUDIES

Parameters	Stability time points in months							
	0	1	2	3	0	1	2	3
	Formulation SA2							
	Refrigerated conditions				25 °C + 65% RH			
Particle size (nm)	240	246	252	254	240	1018	1341	1638
Entrapment efficiency (%)	76.73	75.30	74.21	73.54	76.73	64.02	52.18	40.29

CONCLUSION: Nanoparticles have been known for their potential to deliver the drug across the blood-brain barrier for the treatment of various CNS disorders like brain tumors, Parkinsonism and Alzheimer's disease *etc*. In the present work, Bacoside A (saponin) rich concentrate was extracted from the aerial part of the nootropic / cognitive enhancer drug *Bacopa monnieri* (Scrophulariaceae) and was loaded to solid lipid nanoparticles. The extract was characterized using various techniques such as melting point, HPTLC, UV scan analysis and FTIR analysis and the results were found to be in accordance with the available literature. A total of 4 formulations were prepared with stearic acid using hot homogenization technique. On the basis of particle size analysis, one best formulation was selected for further characterization of drug entrapment efficiency, *in-vitro* drug release study, drug release kinetics and stability studies. The drug entrapment efficiency was found to be 76.73% with less than 250 nm particle size and -16.6 mV zeta potential.

TEM images of the solid lipid nanoparticles showed almost spherical shape with irregular surfaces. The selected formulation was found to have a prolonged drug release up to 24 h (approximately 86%) following the Hixson-Crowell model of drug release. Also, the formulation showed appreciable stability over a period of 3 months when stored at refrigerated temperature during stability studies. The successful incorporation of Bacoside A rich extract into solid lipid nanoparticles opens a wide scope of study of

the delivery system with respect to CNS targeting and sustained nootropic effect. The *in-vivo* performance of nootropic drug-loaded SLNs is currently being evaluated to establish its actual potential.

ACKNOWLEDGEMENT: The authors are grateful to IKG Punjab Technical University for providing access to the literature available online. The authors would like to thank Dr. Chander Mohan, Director-Principal, RBIP for providing us the required facilities to carry out this work. The authors are also thankful to Prof (Dr) Ikhlas A. Khan, School of Pharmacy, Mississippi University, USA for a generous gift of Bacoside A marker.

CONFLICTS OF INTEREST: The authors declare no conflicts of interest.

REFERENCES:

1. Burke AD, Goldfarb D, Bollam P and Khokher S: Diagnosing and treating depression in patients with Alzheimer's disease. Neuro and Therapy 2019; 1: 1-26.
2. Apostolova LG: Alzheimer disease. continuum (Minneap Minn) 2016; 22(2): 419-34.
3. Williams JW, Plassman BL, Burke J, Holsinger T and Benjamin S: Preventing Alzheimer's disease and cognitive decline. Evidence Report/Technology Assess 2010; 193.
4. Cummings JL: Dementia in the failing brain Lancet 1995; 345: 1481-84.
5. Vani R and Lyketsos G: Delusions in Alzheimer's disease: a review. Journal of Neuropsychiatry and Clinical Neurosciences 1998; 10: 373-82.
6. Kashmira J and Jagruti A: A review on *Bacopa monniera*: current research and future prospects. International Journal of Green Pharmacy 2010; 5: 54-58.
7. Enz A, Amstutz R and Boddeke H: Brain selective inhibition of acetylcholinesterase: a novel approach to therapy for Alzheimer's disease. Progress in Brain Research 1993; 98: 431-38.
8. Russo A, Izzo A and Borrelli F: Free radical scavenging capacity and protective effect of *Bacopa monniera* L. on DNA damage. Phytotherapy Research 2003; 17: 870-75.
9. Muntoni E, Martina K, Marini E, Giorgis M, Lazzarato L, Salaroglio IC, Riganti C, Lanotte M and Battaglia L: Methotrexate-loaded solid lipid nanoparticles: protein functionalization to improve brain bio distribution. Pharmaceutics 2019; 11(2): 1-18.
10. Naseri N, Valizadeh H and Zakeri-Milani P: Solid lipid nanoparticles and nanostructured lipid carriers: structure, preparation and application. Advanced Pharmaceutical Bulletin 2015; 5(3): 305.
11. Bayón-Cordero L, Alkorta I and Arana L: Application of solid lipid nanoparticles to improve the efficiency of anticancer drugs. Nanomaterials (Basel) 2019; 9(3): 1-20.
12. Kahol, AP, Singh T, Tandon S, Gupta MM and Khanuja SPS: Process for the preparation of the extract rich in bacosides from the herb *Bacopa monnieri*. US Patent 2004; US 6833143B1.
13. Kumar R, Singh T, Kumar D, Singh M, Kaur S, Garg R: Estimation of Bacoside-A in *Bacopa monnieri* aerial parts using TLC Densitometry. International Journal of Pharmacy and Pharmaceutical Sciences 2015; 7: 293-95.
14. Gohel M, Patel A, Patel A and Hingorani L: Optimization of Bacoside, a loaded SNEDDS, using D-optimal mixture design for enhancement in solubility and bioavailability. Int Jl of Phar and Pharma Sciences 2016; 8(12): 213-20.
15. Anand T, Jalarama Reddy K, Ramya S and Farhath K: Optimization of conditions for nanoencapsulation of bacoside rich extract by RSM technique and its characterization. Frontiers in Nanoscience and Nanotechnology 2018; 4(1): 1-7.
16. Gupta S, Kesarla R, Chotai N, Misra A and Omri A: Systematic approach for the formulation and optimization of solid lipid nanoparticles of efavirenz by high-pressure homogenization using design of experiments for brain targeting and enhanced bioavailability. Bio-Med Research International 2017; 2017: 1-19.
17. Raina H, Kaur S and Jindal AB: Development of efavirenz loaded solid lipid nanoparticles: Risk assessment, quality-by-design (QbD) based optimisation and physicochemical characterization. Journal of Drug Delivery Science and Technology 2017; 39: 180-91.
18. Makwana V, Jain R, Patel K, Nivsarkar M and Joshi A: Solid lipid nanoparticles (SLN) of Efavirenz as lymph targeting drug delivery system: elucidation of mechanism of uptake using chylomicron flow blocking approach. International Journal of Pharmaceutics 2015; 495: 439-46.
19. Mircioiu C, Voicu V, Anuta V, Tudose A, Celia C, Paolino D, Fresta M, Sandulovici R and Mircioiu I: Mathematical modeling of release kinetics from supramolecular drug delivery systems. Pharmaceutics 2019; 11(3): 140.
20. Makoni PA, WaKasongo K and Walker RB: Short term stability testing of efavirenz-loaded solid lipid nanoparticle (SLN) and nanostructured lipid carrier (NLC) dispersions. Pharmaceutics 2019; 11(8): 397.

How to cite this article:
Kumar R and Garg R: Formulation and evaluation of solid lipid nanoparticles loaded with bacoside rich extract. Int J Pharm Sci & Res 2020; 11(1): 371-77. doi: 10.13040/IJPSR.0975-8232.11(1).371-77.