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The role of phospholipid as a solubility- and permeability-enhancing excipient for the improved delivery of the bioactive phytoconstituents of *Bacopa monnieri*

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ABSTRACT

In an attempt to improve the solubility and permeability of Standardized Bacopa Extract (SBE), a complexation approach based on phospholipid was employed. A solvent evaporation method was used to prepare the SBE-phospholipid complex (Bacopa Naturosome, BN). The formulation and process variables were optimized using a central-composite design. The formation of BN was confirmed by photomicroscopy, Scanning Electron Microscopy (SEM), Fourier Transform Infrared Spectroscopy (FTIR), Differential Scanning Calorimetry (DSC), and Powder X-ray Diffraction (PXRD). The saturation solubility, the in-vitro dissolution, and the ex-vivo permeability studies were used for the functional evaluation of the prepared complex. BN exhibited a significantly higher aqueous solubility compared to the pure SBE (20-fold), or the physical mixture of SBE and the phospholipid (13-fold). Similarly, the in-vitro dissolution revealed a significantly higher efficiency of the prepared complex (BN) in releasing the SBE (>97%) in comparison to the pure SCE (~42%), or the physical mixture (~47%). The ex-vivo permeation studies showed that the prepared BN significantly improved the permeation of SBE (>90%), compared to the pure SBE (~21%), or the physical mixture (~24%). Drug-phospholipid complexation may thus be a promising strategy for solubility enhancement of bioactive phytoconstituents.

1. Introduction

Recent years have seen a dramatic increase in the popularity of natural products in the management of several ailments (Sikarwar et al., 2008). Among other challenges, the poor bioavailability of the bioactive phytoconstituents is accepted as a major limitation to the use of natural products in mainstream pharmacotherapy. Attributes such as high molecular size, poor aqueous solubility, and lower plasma membrane permeability of pharmacologically active phytoconstituents are known to result in overall poor bioavailability of these components, thus limiting their pharmaceutical applications (Husch et al., 2013; Manach et al., 2004).

A major rate-limiting factor in the development of pharmaceutical drug products from the bioactive phytoconstituents is improving their solubility and permeability, thereby optimizing their bioavailability. Preparing drug-phospholipid complexes is among the several recently explored, and promising approaches aimed at improving the bioavailability of drugs. Several studies have reported an improvement in the solubility, permeability, and subsequently the systemic absorption and bioavailability of active phytoconstituents by forming their aggregates with phospholipid molecules (forming *Naturosomes*, *Phytosomes*, or *Herbosomes*) (Husch et al., 2013; Khan et al., 2013). The pharmacological profiles have also been shown to successfully improve by using drug-phospholipid complexation approaches (Freag et al., 2013; Kennedy et al., 2007; Mukherjee et al., 2011; Pathan and Bhandari, 2011; Zaidi et al., 2011). Additionally, fundamental advantages and significant breakthroughs for clinical use have been observed for several phytoconstituents by improving their oral availability, owing to the amphiphilic characteristics of *Naturosomes* (Fricker et al., 2010; Kidd, 2009). Naturosome technology have been successfully used to deliver herbal extracts as well as phytochemicals with significant improvements in pharmacokinetic as well as in pharmacodynamic functionalities (Kennedy et al., 2007; Kidd and Head, 2005; Maiti et al., 2006; Maiti et al., 2009).

Bacopa monnieri Linn is a plant native to the Indian subcontinent, and has been described in ancient text as being used to sharpen intellect and attenuate several mental deficits (Aguiar and Borowski, 2013; Sairam et al., 2002). Current literature also reports its usefulness in the treatment of anxiety, improving cognitive functions,

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memory enhancement, neuroprotection, and hepato-protection (Calabrese et al., 2008; Janani et al., 2009; Rastogi et al., 2012). The major pharmacologically active constituents reported in *Bacopa monnieri* are bacopasides (triterpenoid saponins) such as bacoside A3, bacopaside I, bacopaside II, bacoside A (bacopasaponine C), and a jujubogenin isomer of bacopasaponine C (Sivaramakrishna et al., 2005; Zhou et al., 2007).

Safety, efficacy, and quality are the cornerstones of any pharmaceutical product application. In recent years, the goal of developing a quality product, and minimizing its functional variability have been successfully achieved via adopting a Quality by Design (QbD) principles. QbD has been described earlier as a scientific, risk-based, holistic, and proactive approach to pharmaceutical development (Dave et al., 2015). This approach includes a sound science-based understanding of the predefined objectives, formulation and process variables, and a thorough assessment and management of risks with respect to the product quality.

Several pharmaceutical and nutraceutical products containing extracts of *Bacopa monnieri* are commercially available (Pravina et al., 2007). However, the current literature lacks studies on the issue of the low aqueous solubility of its extract, and any approaches to improve the solubility. In a recent study, the authors have demonstrated success in improving the solubility and permeability of a standardized extract of *Centella asiatica* via preparation of its complex with a hydrogenated soy phosphatidylcholine (Phospholipon® 90H)(Saoji et al., 2015a). In the present study, this approach was used to assess the feasibility of improving the aqueous solubility of Standardized Bacopa Extract (SBE).

The primary objective of this study was to prepare and optimize the SBE-Phospholipon® 90H complex by a modified solvent evaporation method. The formulation and process variables were optimized by the means of a central composite design. The prepared complex was characterized for its physicochemical properties using photo microscopy, Scanning Electron Microscopy (SEM), particle size distribution and zeta potential, Fourier Transform Infrared Spectroscopy (FTIR), Differential Scanning Calorimetry (DSC), Thermogravimetric Analysis (TGA), and Powder X-ray Diffractometry (PXRD). The functional properties of the prepared complex were evaluated by saturation solubility studies, in-vitro dissolution studies, and ex-vivo permeation studies. These properties of the prepared complex were compared to that of the pure SBE and a physical mixture (1:1) of SBE and Phospholipon® 90H.

2. Materials and methods

2.1. Materials

The Standardized Bacopa Extract (SBE), containing approximately 40% w/w bacopasides was obtained from Arjuna Natural Extracts Ltd., Kerala, India. The identity and purity of the SBE composition were confirmed by HPLC analysis. The hydrogenated soy phosphatidylcholine i.e. Phospholipon® 90H obtained from Lipoid, Ludwigshafen, Germany. All other chemicals and reagents used were of analytical grade.

2.2. Analytical method for the triterpenes present in SBE

The concentrations of the triterpene saponins present in SBE i.e. bacoside A (a mixture of bacoside A3, bacopaside II, jujubogenin isomer of bacopasaponine C, and bacopasaponine C) were determined using a modified, reverse-phase high performance liquid chromatography (RP-HPLC) method previously described by Phrompittayarat et al. (Phrompittayarat et al., 2007). Briefly, the HPLC system (Model: Prominence, Shimadzu Corporation, Kyoto, Japan) with LC solution software, equipped with a LC-20AD HPLC pump, a manual rheodyne sample injector, and a SPD-M20A detector were utilized for the separation. The mobile phase composed of phosphoric acid (0.2%): acetonitrile (65:35 v/v), pH adjusted to 2.8 with 3 M Sodium hydroxide, and a flow rate of 1.5 mL/min. A Micra- NPS RP18 column (33 × 8.0 × 4.6 mm, 1.5 μm) was used as a stationary phase, and the detector wavelength was 205 nm at room temperature. The trailing/asymmetry factor, theoretical plates, and the relative standard deviation (RSD) were calculated to monitor the system suitability of the chromatographic setup. The calibration curves were obtained by plotting the peak areas versus concentration of the standard solution. For each component of the extract (bacopaside), seven concentrations of standard solution were analyzed. The implemented chromatographic method was validated by analyzing several relevant validation characteristics such as linearity, accuracy, and precision.

2.3. Preparation of Bacopa Naturosome (BN)

A slightly modified solvent evaporation method, previously described by Bhattacharyya et al. was used to prepare the BN (Bhattacharyya et al., 2014a). Briefly, different ratios of Phospholipon® 90H and SBE, i.e. 0.5:1, 1:1, 1.75:1, 2.5:1, or 3:1 were added to a 100 mL round bottom flask containing 40 mL ethanol. The reactions were carried out at various controlled temperatures, i.e. 40 °C, 44 °C, 50 °C, 56 °C, or 60 °C, and for different durations, i.e. 1 h, 1.4 h, 2 h, 2.6 h or 3 h. The resultant clear solution was evaporated to about 2–3 mL. Excess amount of n-hexane was added to this mixture with continuous stirring. The formed BN was then precipitated, filtered, and dried under a vacuum to remove any traces of the solvents. The prepared BN was stored at room temperature in amber colored bottles, flushed with nitrogen.

2.4. Quality by design (QbD) based design of experiments

The Critical Quality Attributes (CQA) of the product, i.e. the drug entrapment efficiency of the BN was studied systematically for the joint influence of the formulation and the process variables such as, phospholipid-to-drug ratio (X_1, w:w), reaction temperature (X_2, °C), and the reaction time (X_3, h) by applying a QbD-based approach using central composite design. Using this design, 20 possible combinations of experimental trials were carried out to analyze the influence of the above three factors (Yue et al., 2010; Yue et al., 2008). The responses were evaluated employing a statistical model incorporating the interactive and polynomial terms using the Eq. (1) below:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{12}X_1X_2 + b_{23}X_2X_3 + b_{13}X_1X_3 \quad (1)$$

where, Y is the dependent variable, b_0 is the arithmetic mean response of the 20 runs, and b_i is the estimated coefficient for the factor X_i. The average response due to a change in the level of one factor at a time is represented by the main effects (X_1, X_2, and X_3). The changes in the response when all factors were simultaneously changed are represented by the interaction terms (X_1X_2, X_2X_3, and X_1X_3). The on-linearity was evaluated by including the polynomial terms (X_1^2, X_2^2, and X_3^2). The Tables 1 and 2 shows the levels of the evaluated factors, and the composition of the trial batches, respectively.

Table 1
Coded levels and "Real" values for each factor under study.

Variables	Levels				
	−1.7	−1	0	+1	+1.7
Independent	Real values				
Phospholipid: drug ratio (X_1, w:w)	0.5	1.0	1.75	2.5	3.0
Reaction temperature (X_2, °C)	40.0	44.0	50.0	56.0	60.0
Reaction time (X_3, h)	1.0	1.4	2.0	2.6	3.0
Dependent					
Entrapment efficiency (Y, % w/w)					