Journal of Advanced Zoology

ISSN: 0253-7214

Volume 44 Special Issue-2 Year 2023 Page 4644:4654

Synthesis and Optimization of Sodium Alginate-Tween 80 nanocarrier for Berberine delivery

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Article History

Received: 06 Aug 2023

Revised: 05 September 2023

Accepted:11 November 2023

Abstract

The present study focuses at the synthesis of berberine loaded sodium alginate nanoparticles using tween 80 (BSAT) as a surfactant. Berberine has poor solubility and bioavailability because of its hydrophobic nature, hence modified ionic complexation method was applied to enhance its efficacy. The synthesized nanoformulation was optimized by using Central Composite Design. The BSAT nanoparticles were experimentally characterized for particle size distribution, morphology of nanoparticles and drug encapsulation potential. It was experimentally found that BSAT nanoparticles possessed size within the limits of 50-100 nm with good encapsulation efficiency of 96.00 %. Moreover, sodium alginate at 0.05 %, w/v and tween 80 at 1 %, v/v was capable to produce isolated and free flowing nanoparticles. The nanoparticles of BSAT showed enhanced antimicrobial activity compared to pure berberine by agar well diffusion method against *Klebsiella pneumonia*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus subtilis*. The current investigation points that sodium alginate-tween 80 nanocarrier can prove to be a promising nanocarrier for hydrophobic drugs.

Keywords: Berberine, tween 80, sodium alginate, particle size, morphology.

Introduction

Berberine is an isoquinoline alkaloid found in root, rhizome and stem bark of *Berberis vulgaris* plant (Iqbal et al., 2021). It is extensively present in a number of botanical families, such as *Papaveraceae, Berberidaceae, Fumaraceae* etc and commonly known as 'Daru haldi' in Urdu (Sahibjada et al., 2018). It exhibits a wide range of pharmacological actions viz antidiarrhoeal, cardiovascular, antidiabetic, anticancer, antioxidant, anti-inflammatory, and immuno enhancing properties, etc (Singh et al., 2010). Berberine also shown activities like antioxidant and anti-inflammatory in the treatment of diabetes mellitus in animals (Li et al., 2014). It has been used by folk people in Ayurvedic and Chinese medicine since ancient times for treating many diseases (Imenshahidi et al., 2016; Rauf et al., 2021). Earlier reports indicate worldwide use of berberine as a natural product with consumption of more than 25 billion pills annually in Asia and African countries (Iqbal et al., 2021). However, for a very long time, the use of berberine was hindered by its hydrophobic characteristics, in addition to low stability and bioavailability. Advanced nanoparticulate delivery technologies have led to increase in clinical application prospects for berberine. Polymeric materials are most widely used in nanomedicine (Mirhadi et al., 2018).

Nanoformulations can be synthesized using different methods (Reis et al., 2006). The most common methods employed by different researchers for the design of nanoformulations include emulsion solvent evaporation (Sahoo et al., 2002), nanoprecipitation (Govender et al., 1999), salting out procedure (Konan et al., 2002), ionic complexation (Bakshi et al., 2023) and solid lipid nanoparticles (Li et al., 2006) with various types of solvents such as ethanol, chloroform, DMSO and methanol etc. (Khan et al., 2022).

Some natural polymers, such as chitosan and sodium alginate has been widely used in nano drug delivery systems owing to their attractive properties such as biodegradability, biocompatibility etc. (Kohli et al., 2021). Chitosan (carbohydrate biopolymer), is obtained from deacetylation of chitin, a leading component of crustacean shells like crab, shrimp, and crawfish. It is a positively charged polymer with major abundance in nature which has biocompatible and biodegradable properties (Wang et al., 2018). Other natural polymers, like sodium alginate is a linear and negatively charged copolymer belongs to one of the polysaccharides extracted from sea weeds. Sodium alginate has good biodegradability and biocompatibility which may act as a binder or help in the controlled release of drug at the targeted site. Earlier reports suggest improved bioactivity of berberine after encapsulation in polymeric formulation (Dash et al., 2020)

In the present study, berberine-sodium alginate nanoparticles were formulated using tween 80 as surfactant for preventing agglomeration which leads to improved water solubility, effectiveness as well as the enhanced bioavailability of the drug molecules.

Materials and Methods

Materials

Berberine was purchased from MP Biomedicals Pvt Ltd. (Mumbai, India). Sodium alginate and methanol used in the study were obtained from SRL Ltd. (Mumbai, India). Tween 80 was taken

from S.D. Fine-Chem. Ltd. (Mumbai, India). All other different chemicals and materials used for the laboratory work were of scientific grade.

The test bacteria used for antibacterial assay i.e., *Klebsiella pneumonia* (NCDC 138), *Escherichia coli* (NCDC 249) and *Pseudomonas aeruginosa* (NCDC 105) were taken from the National Collection of Dairy Cultures (NCDC), NDRI, Karnal, (India) while *Bacillus subtilis* (MTCC 441) was acquired from Microbial Type Culture Collection, Chandigarh, (India). The cultures of bacteria were cultured on slants of agar media at 4^{o}C by standard protocol.

Preparation of nanoformulation

BSAT nanoparticles were prepared by ionic complexation (Bakshi et al., 2023) and nanoprecipitation (Sahibjada et al., 2018) method with some modifications using sodium alginate and tween 80. Firstly, in the nanoprecipitation method, an organic solvent (methanol) was used for berberine (0.2 %, w/v) to prepare a saturated solution and mixed with sodium alginate (0.05 %, w/v) under magnetic stirring at a rate of 500 rpm for 10 min at ambient temperature. Sodium alginate mainly contains hydroxyl and carboxyl group of negative charge (Fernando et al., 2019) that interacts with the positive quaternary ammonium group of berberine that results in ionic complexation. This complex was then added dropwise to (1%) tween 80 aqueous solution with subsequent stirring of 2 h for evaporation of methanol. Then, the solution was centrifuged at 10,000 rpm. The optimized nanoformulation was then freeze dried and lyophilized in freeze dryer (Alpha 2-4 LD Plus, Martin Christ, Germany) and stored for further characterization. The blank nanoparticles were similarly prepared without adding berberine.

Optimization of BSAT nanoparticles

The optimization of BSAT nanoparticles was conducted using a central composite design (version 8.0.4.1, State-Ease Inc., Minneapolis, MN) at different levels (-1, 0, +1) for obtaining small size of particles and enhanced percentage of encapsulating nanoparticles.

Characterization of BSAT nanoparticles

The optimized berberine-sodium alginate nanoparticles were characterized by particle size, zeta potential and encapsulation efficiency. Morphology of BSAT nanoparticles were analyzed by SEM and AFM.

Particle size, Zeta potential and Encapsulation efficiency

The mean size of the optimized BSAT nanoparticles were evaluated by using a particle size analyzer or dynamic light scattering method using Zetasizer (Nano ZS-90, Malvern Instruments, UK) at an angle of 90^{o} at 25^{o}C in an automatic mode. The optimized nanoparticles were studied in zeta cuvette at constant temperature of 25^{o}C for stability studies of BSAT nanoparticles.

The entrapment efficiency of optimized BSAT nanoparticles was evaluated at a wavelength of 345 nm using equation as follows:

Entrapment efficiency = (Total drug – Unentrapped Drug) × 100/Total Drug

SEM and AFM study

The optimized nanoparticles morphology was analyzed using scanning electron microscope (SEM) (Zeiss available at All India Institute of Medical Sciences [AIIMS], New Delhi). For the SEM study, the nanoformulated particles were fixed on a clean adequate support as stubs. The stubs were dried and by using gold sputtering in a high vacuum evaporator, gold particles were coated. Observations were analysed at different magnifications at 20 kV accelerating voltage.

Atomic force microscope (AFM) (NX10 Park SYSTEMS available at Bio & Nano Technology Department, Guru Jambheshwar University of Science & Technology, Hisar, Haryana) was used for studying the surface characteristics of the nanoformulation. The physical scanning of sample surfaces at a submicron level was performed with the help of scanning probe microscope. For the AFM study, the nanoformulation drop was fixed on clean glass slide and spin-coated using spin coater (Spin Coating unit SCU2007A, Apex Instruments Co. Pvt. Ltd) to bring about adhesion of nanoparticles on the slide at room temperature. Under different magnifications, the particles were studied in non-contact mode with a scanning speed corresponding to a 1 Hz line frequency.

Antibacterial assay

Antibacterial activity of control, berberine, blank and BSAT nanoparticles were checked using the agar well-diffusion method. The bacterial cultures of *Bacillus subtilis*, *Klebsiella pneumonia, Pseudomonas aeruginosa*, and *Escherichia coli* were cultured on nutrient broth for 24 hours at 30°C. Nutrient agar media was freshly prepared and poured onto the plates for 15- 20 min to solidify agar media completely. A volume of 0.1 ml bacterial culture was homogeneously spread on agar plates using a spreader and kept for 15 min. Solidified media was gently punctured with the help of a borer to form wells. The wells were poured with control (distilled water), berberine, blank and BSAT nanoparticles. The petri dish plates were then incubated at 37 ± 1°C for nearly 24 hours. Then with the scale zone was measured, and experiments were done in triplicates for each bacterial strain.

Results and Discussion

Synthesis and optimization

The concentration of sodium alginate and tween 80 were selected as independent variables while as dependent variables, particle size and encapsulation efficiency were taken and different experimental runs were carried out (**Table 1)**.

Table 1: Different runs from central composite design for optimization of berberine loaded sodium alginate nanoparticles with Tween 80.

Runs	Sodium alginate conc. (%, w/v) (A)	Tween 80 conc. (%, v/v) (B)	Particle Size (nm) (X)	Encapsulation efficiency (%) (Y)
1	0.3	1.5	228.1	94.27
2	0.05	1	54.96	96.00

3	0.05	1.5	78.98	95.04
4	0.5	1	148.20	94.67
5	0.3	1.5	257.90	93.03
6	0.3	1.5	252.80	93.18
7	0.3	1	178.18	95.00
8	0.5	1.5	159.60	94.94
9	0.3	2	259.60	93.06
10	0.5	2	178.63	95.42
11	0.05	2	98.28	95.01
12	0.3	1.5	249.28	93.65
13	0.3	1.5	238.20	93.96

It was interpreted from the results that best fitted 'model' for particle size and encapsulation efficiency responses was 'quadratic' with F- value 36.05 and 5.50 respectively, that implies the model is significant. In ANOVA, analysis of 'lack of fit' was not significant for particle size and encapsulation efficiency with F-value 3.68 and 1.43 respectively relative to the pure error. It was shown in the results that lack of fit is good when it is not significant and we need only model to be fit.

Table 2: Statistical data of the response variables.

Model (Quadratic)									Lack-of-fit	
Response factor	**F value**	**p value**	**R^2**	**Adjus. R^2**	**Pred. R^2**	**Adeq. Prec.**	**C.V %**	**Std. Dev.**	**F value**	**p value**
X	36.05	0.0001	0.9626	0.9359	0.7448	16.386	9.54	17.61	3.68	0.1200
Y	5.50	0.0226	0.7972	0.6523	0.2079	6.877	0.60	0.57	1.43	0.3585

Adjus. R^2: Adjusted R^2, Pred. R^2: Predicted R^2, Adeq. Prec.: Adequate Precision.

According to the experimental and statistical data, a ratio greater than 4 is desirable for 'Adeq. Prec.' and in the **Table 2**, the resulted value for particle size and encapsulation efficiency was 16.386 and 6.877 for adequate precision that indicates an adequate signal. The quadratic equations generated in terms of coded factors for responses X (particle size) and Y (encapsulation efficiency) are as follows:

$X = 240.96 + 39.66A + 23.16B + 0.83AB – 110.93A^2 – 11.33B^2$.

$Y = 93.64 – 0.17A – 0.36B + 0.43AB + 1.31A^2 + 0.35B^2$.

Whereas A is sodium alginate and B is tween 80.

According to the equation, positive and negative value indicates synergistic and antagonistic effects respectively, on the response factors. Design expert software revealed 3D surface plots effects of different concentrations of independent variables on particle size and encapsulation efficiency were shown in **Fig. 1**. The **Fig. 1a** plot shows that by increasing the polymer concentration, particle size also increases. But by increasing the surfactant concentration at a

certain limit, particle size decreases. Sometimes at higher concentration of polymer, particles agglomerates but with the addition of tween 80, the problem of agglomeration of particles was solved as given in studies of Scolari et al., 2019.

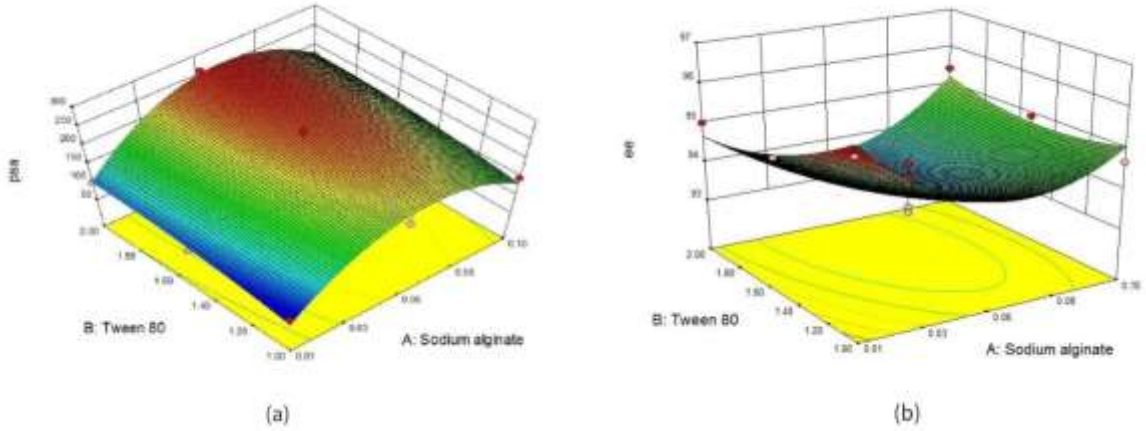

Fig. 1: 3D surface graph showing the concentration effect of polymer and surfactant on (a) particle size and (b) encapsulation efficiency.

Fig. 1b depicts the effect of independent variables concentration on encapsulation efficiency. The effect shows that by increasing the concentration of sodium alginate, encapsulation efficiency also increases. Increase in the encapsulation efficiency with the increase in sodium alginate concentration was also described by Baghbani et al., 2016. The graph also shows that with the increase in concentration of tween 80 with nanoparticles, encapsulation efficiency also increases due to better retention of concentration of surfactant tween 80 with the drug (Das et al., 2012).

The batch with minimum percentage error was considered as optimized batch for final concentration. The final optimized batch concentration of BSAT nanoparticles was obtained by numerical optimization with desirability approach with criteria of minimum particle size and maximum encapsulation efficiency. The final concentration for the optimized batch of BSAT nanoparticles were 0.05 % and 1% of sodium alginate and tween 80 respectively. The predicted size of nanoparticles (66.90 nm) and encapsulation efficiency (96.13 %) was obtained from design expert software.

Characterization of BSAT nanoparticles

Particle size, Zeta potential and encapsulation efficiency

The particle size of various BSAT nanoparticles was found to be in the range of 54.96 – 259.6 nm. The resultant experimental value of particle size was 54.96 nm and encapsulation efficiency (96.00 %), indicating the formulation was suitable for the nanoparticles (**Fig. 2a**). The size of nanoparticles was analyzed with the help of particle size analyzer.

The physical stability of the BSAT nanoparticles was confirmed by zeta potential value by using zetasizer ZS- 90. Zeta potential of optimized BSAT nanoparticles was -21.4 mV (**Fig. 2b**), indicating the anionic nature due to negative charge on BSAT. Presence of negative zeta value was due to sodium alginate that is anionic in nature with hydroxyl and carboxyl group (Fernando et al., 2019).

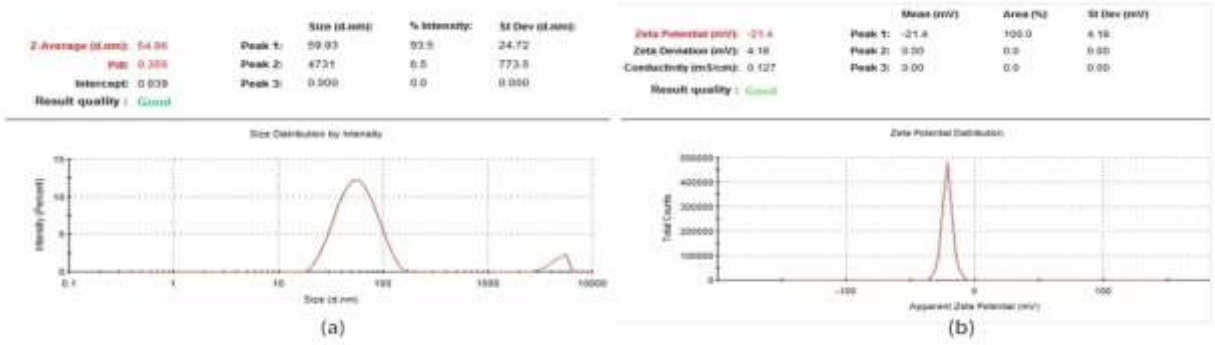

Fig. 2: (a) Particle size and (b) Zeta potential of optimized BSAT nanoparticles.

The encapsulation efficiency of various BSAT nanoparticles was in the range of 93.03 - 96.00 % whereas the encapsulation efficiency of optimized batch was found to be 96.00 % (**Fig. 1b**).

SEM and AFM

The scanning electron microscopy (SEM) of optimized batch of BSAT showed that nanoparticles were smooth and spherical **(Fig. 3a).**

The topographical 3D image of BSAT nanoparticles was carried out in non contact mode under different magnifications by (AFM) atomic force microscopy **(Fig. 3b**) with height distribution of about 40 nm in size.

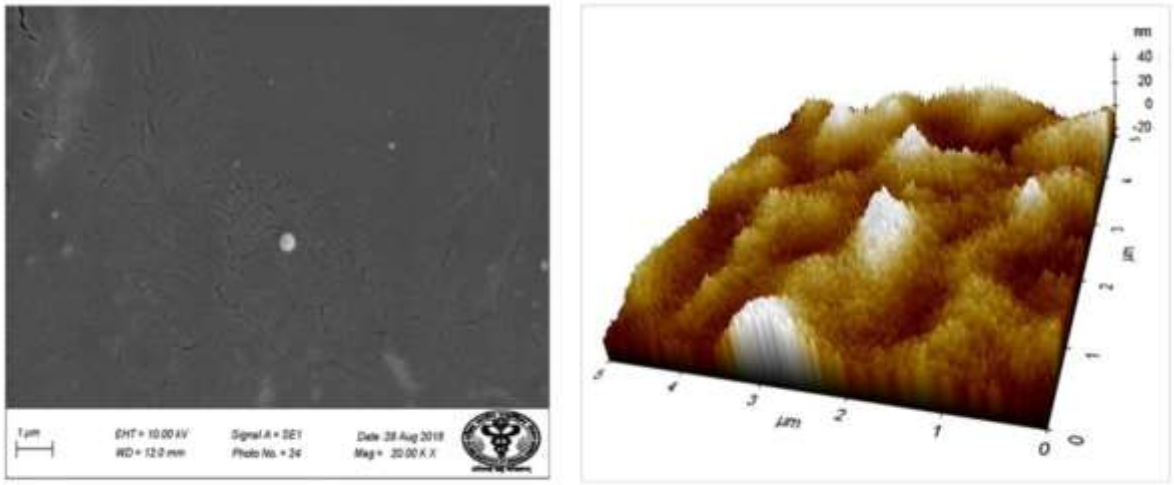

Fig. 3: (a) SEM and (b) AFM image of optimized BSAT nanoparticles.

Antibacterial activity of BSAT nanoparticles

Antimicrobial potential of (1) distilled water as control, (2) blank nanoparticles, (3) BSAT, and (4) berberine were evaluated on (A1) *Bacillus subtilis*, (B1) *Klebsiella pneumonia*, (C1) *Pseudomonas aeruginosa, and* (D1) *Escherichia coli* **(Fig. 4, Table 3)**. Berberine is reported to have antibacterial effect (Nguyen et al., 2022). In this study, BSAT nanoparticles have a potency to effectively kill these microbes in comparison to berberine and blank nanoparticles whereas distilled water was taken as control. The surface area of these nanoparticles is very high potentially raising its antimicrobial activity. The results show that BSAT nanoparticles exhibited improved antibacterial effect than berberine alone against all bacteria. The best antibacterial activity was shown against *Klebsiella pneumonia* followed by *Escherichia coli.* As studied in earlier reports, the incorporation of chitosan biopolymer with drugs or natural compounds in nanosystems is an important strategy to enhance antimicrobial potential (Dash et al., 2020). Berberine nanoformulation formed by antisolvent precipitation method showed enhanced antibacterial activity against *Staphylococcus aureus and Escherichia coli* (Sahibzada et al., 2018).

Table 3: Zone of inhibition (area in mm) of blank, BSAT nps and berberine against different bacteria.

Samples	***Bacillus subtilis*** **(A1)**	***Klebsiella pneumonia*** **(B1)**	***Pseudomonas aeruginosa*** **(C1)**	***Escherichia coli*** **(D1)**
(1) Control	0	0	0	0
(2) Blank nps	3	4	4	4
(3) BSAT nps	16	22	15	21
(4) Berberine	13	15	11	15

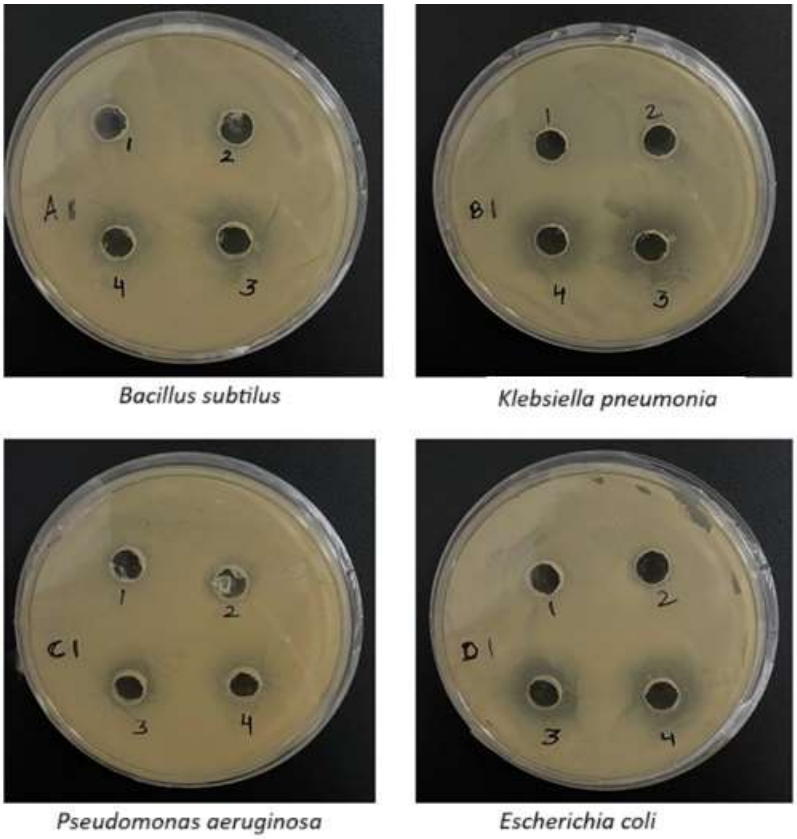

Fig. 4: Zone of inhibition of (1) distilled water, (2) blank nanoparticles, (3) BSAT nanoparticles, and (4) berberine against various bacteria.

Conclusion

This study provides the idea about the combined effect of berberine and sodium alginate against different Gram +ve and Gram –ve bacteria. The BSAT nanoparticles were prepared by a combination approach of ionic complexation and nanoprecipitation with minor modifications. The Central Composite Design was used for the optimization of nanoparticles as concentration of sodium alginate (0.05 %, w/v) and tween 80 (1%, w/v) taken as independent variables, to check the effect on dependent variables (PSA and EE). The BSAT nanoparticles possessed particle size within the range of 50-100 nm, having a good encapsulation efficiency of 96.00 % and showed enhanced antibacterial effect against *Klebsiella pneumonia,* and *Escherichia coli*. Hence it can be concluded that Sodium Alginate-Tween 80 nanocarrier enhances the bioavailability and solubility of hydrophobic drugs like berberine.

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