Original Article

# Formulation and Evaluation of Bendamustine Loaded Polymeric Nanoparticle

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## ABSTRACT

**Background:** Bendamustine-loaded albumin nanoparticles were prepared using different concentrations of Bovine Serum Albumin (BSA) with the goal of delivering the medication to particular cancer cells. **Materials and Procedures:** The nanoparticles were prepared using simple coacervation technique with increasing concentrations of BSA. The nanoparticles were characterized for process yield, particle size, surface morphology, drug loading capacity (%), particle size distribution (Polydispersity index), *in-vitro* drug release. The drug release kinetics were studies using different dissolution models. The drug loading capacity of the produced nanoparticles varied from 10.4% to 19%. Formulation (F1) had a mean particle size of approximately 122.4 nm and polydispersivity index of 0.432 across the different compositions. Bendamustine nanoparticles exhibit sustained drug release with almost 51.8% bendamustine released in one day. The drug release kinetics follows Korsmeyer-Peppas model with a Fickian drug release mechanism. The Bendamustine nanoparticles were found to be stable for a month at 40±5C and 75±5% relative humidity. **Conclusion:** Bendamustine loaded BSA nanoparticles were developed which were found to exhibit a sustained drug release profile. Albumin based Bendamustine nanoparticles have the potential to be explored further for the better management of the cancer chemotherapy with reduced side effects.

**Key words:** Nanoparticles, Bendamustine, Cancer, Bovine serum albumin, Bendamustine albumin, Release kinetics, Surface morphology.

## INTRODUCTION

Cancer is a complicated disease that is associated with high mortality.[1] Cancer cells are characterized by the uncontrolled and aberrant cell proliferation. These cancerous cells spread to non-adjacent organs and tissues by invading neighboring cells and tissues.[2] Cancer cell are immortal and they continue to proliferate and produce new aberrant cells. Though there have been considerable advancements made in the field of cancer treatment modalities and extensive research is being carried out on novel therapeutic approaches. Treatment failure is due to toxicity issues or drug resistance.[3] Chemotherapy damage proliferating cells non-specifically and cause bone marrow suppression.[4] The majority of the anticancer drugs are not able to reach to the target site in therapeutic concentrations and effectively exert the pharmacological

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effect without resulting in undesirable side effects.[3] Thus, a site-specific delivery of the anticancer molecule that result in reduced side effects is needed.

Undoubtedly, the conventional cancer therapies have resulted in prolonged patients' survival, however, it has several limitations, viz., lack of site-specific targeting, drug toxicity, drug resistance and undesired side effects. Amongst various therapeutic modality, nanotechnology seems to be a promising approach which can play multiple role in cancer detection and treatment viz., biomarker identification, cancer progression and diagnostics and imaging agents.[5] Nanoparticles of cytotoxic drugs can reduce side effects and improve pharmacotherapy.[4] Nanotechnology has advanced significantly in the last few decades, and its impact is now seen in virtually every sector. Nanoparticles can be modulated for prolong circulation, site specificity, which thereby increases drug efficacy, and reduces potential side effects and chances of drug resistance.[6] Nanoparticles offer many advantages like reduced side effects, and improved tumor concentration.[6]

The current work aims to create and analyze nanoparticles containing Bendamustine for cancer treatment.

## MATERIALS AND METHODS

### Materials

Bendamustine was a kind gift from Once therapy private limited, Anekal Taluk, Bangalore, India. BSA was obtained from Central drug house private limited, New Delhi, India. Ethanol was purchased from Sigma-Aldrich. Glutaraldehyde was procured from SD fine chemicals ltd, Mumbai, India. Sodium chloride was purchased from Fisher Scientific Laboratories, India.

### Preparation of nanoparticle

A simple coacervation technique was used to make Bendamustine loaded albumin nanoparticles.[7] Prior to formulation development, compatibility of Bendamustine with BSA was evaluated using FTIR 8400S Fourier Transform Spectrophotometer (Shimadzu, India) by scanning in the range of 400–4000 $cm^{-1}$.

For developing formulation, Bendamustine was accurately weighed and added to a 2% w/v BSA ne serum albumin solution. Nanoparticles were prepared with varying concentrations of BSA viz., 1:1 (F1), 1:2 (F2), 1:3 (F3), 1:4 (F4), and 1:5 (F5). Ethanol was slowly injected into the drug and albumin solution at a rate of 1 ml/min with constant stirring for 3hr at room temperature. Finally, Cryoprotectant (glucose) is added as a fine powder while stirring. The nanoparticle suspension was then centrifuged for 20 min and freeze dried at -30°C for 5hr after being centrifuged for 20 min at 10,000rpm. Bendamustine loaded albumin nanoparticles were then characterized.

### Particle size and its morphological analysis

Malvern Zetasizer Nano ZS 90 was used to measure mean diameter and polydispersity index (PDI) (Malvern Instruments, UK). Bendamustine nanoparticles were diluted with double distilled water to weaken opalescence before particle size analysis. All measurements were carried out in triplicate at a temperature of 25°C.[8]

Morphological analysis of nanoparticles was carried out using Scanning electron microscopy (SEM) using JSM 6100 JEOL. Using a sputter coater unit (JEOL JFM-1100, Tokyo, Japan), samples were coated with a thin gold–palladium layer and examined microscopically at an accelerating voltage of 10.0 kV.[9]

### Process yield (PY) and Loading Efficiency (LE)

Using a phosphate buffer of pH 7.4, PY (%) and LE (%) were calculated by determining the amount of non-encapsulated Bendamustine in the solution as described by Chalikwar *et al.*, 2012 and the following equations.[10]

$$\% \text{ PY} = \left( \frac{\text{Weight of nanoparticles recovered}}{\text{Weight of (polymer + Drug)}} \right) \times 100 \qquad (1)$$

$$\% \text{ LE} = \left( \frac{W_a - W_s}{W_L} \right) \times 100 \qquad (2)$$

Where Wa = amount of bendamustine added to formulation, Ws = amount of free bendamustine, $W_L$ = weight of nanoparticles

### *In-vitro* Drug Release

The drug release from Bendamustine nanoparticle was studied in phosphate buffer (pH 7.4), using the dialysis-bag method.[11] A presoaked dialysis membrane having a molecular weight cut off of100 kD and pore size of 2.4 nm was used. The dialysis bag filled with nanoparticles was then placed in 250 mL phosphate buffer (pH 7.4) which was maintained at 37 ± 2°C and continuously stirred at 100 rpm. The sink condition was maintained by changing the samples with new pre-warmed dissolving medium held at the same temperature for one day. Bendamustine content was measured in the samples using a UV spectrophotometer (1800, Shimadzu, Japan) set to max 231.5 nm.

### Drug release kinetics

Various kinetic models were used to fit the kinetics and mechanism of Bendamustine release from nanoparticles during an *in vitro* drug release investigation. The following kinetic models were investigated: The zero order model (equation 3), the first order model (equation 4), the Higuchi model (equation 5), the Hixson-Crowell model (equation 6) and the Korsmeyer-Peppas model (equation 7).[10]

$$Q = K_0 t \quad (3)$$

$$\log Q_{10} - \log Qt = K_1 t/2.303 \quad (4)$$

$$Q = K_2 t^{1/2} \quad (5)$$

$$Q_0^{1/3} - Q_t^{1/3} = k_{HC}.t \quad (6)$$

$$Q_t = K_{kp} .t^n \quad (7)$$

### Stability studies

Bendamustine nanoparticle formulations were subjected to stability tests. The experiments were carried out by keeping the bendamustine-loaded BSA nanoparticles formulations in air tight low density polyethylene containers for 30 days at 40±5°C and 75±5%relative humidity.[12] These samples were taken at 10th, 20th and 30th day intervals and examined for any changes in physical appearance or drug content.

## RESULTS

### Compatibility studies

Bendamustine-Albumin compatibility was evaluated using FTIR to determine any possible interaction between them. Any incompatibility would be indicated by a shift in the position or absence of any of the distinctive drug peak.[13] Bendamustine, BSA, and drug-loaded BSA nanoparticles have their FTIR spectra recorded. (Figure 1, 2 and 3). The major peaks produced from the mixture were comparable to those obtained from the pure drug.

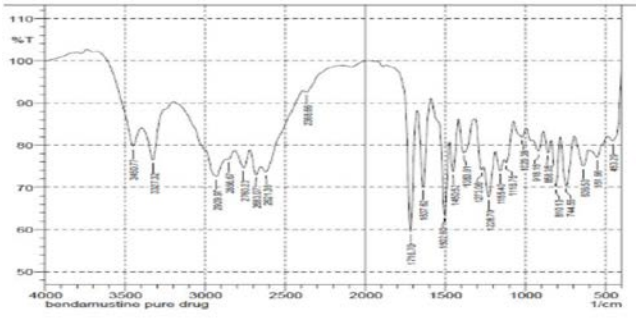


**Figure 1: FT-IR spectrum of Bendamustine.**

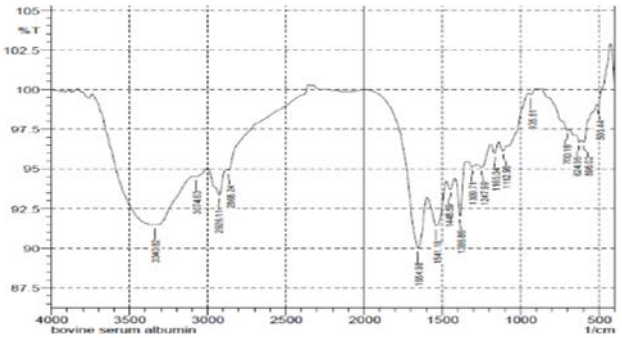


**Figure 2: FT-IR spectrum of BSA.**

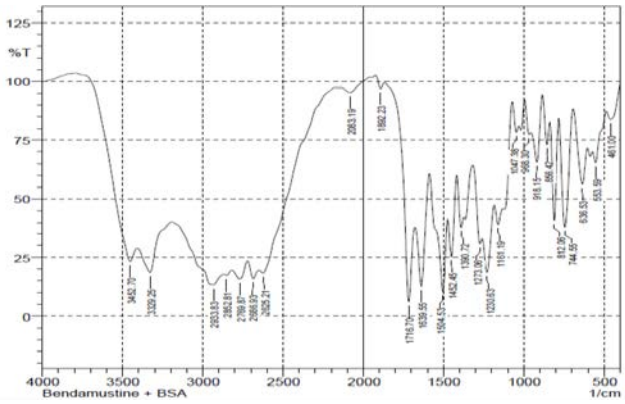


**Figure 3: FT-IR spectra of Bendamustine-loaded BSA nanoparticles.**

### Yield and Morphological studies of Bendamustine nanoparticles

Bendamustine nanoparticles were prepared with varying concentrations of BSA viz., 1:1 (F1), 1:2 (F2), 1:3 (F3), 1:4 (F4), and 1:5 (F5) using simple coacervation technique. The process yield and drug loading efficiency of all the batches are enlisted in Table 1. Based on the drug polymer ratio, the process yield ranging from 14% to 63%. The medication loading capacity varied from 10.4% and 19 % w/w.

The morphology of the Bendamustine nanoparticles was studied using SEM as shown in Figure 4. Microscopic evaluation revealed formation of relatively distinct spherical nanoparticles with particle size less than 150nm. The particle size was in compliance with particle size determined using Malvern zeta sizer (~122.4 nm and PDI= 0.432) (Figure 5).

### Bendamustine release

The drug release from Bendamustine nanoparticles was studied using dialysis bag method. Figure 6 depicts the

**Table 1: Process yield and drug loading efficiency of Bendamustine nanoparticles prepared with varying concentration of BSA (F1to F5).**

| Batch code | Process yield (%) | Drug loading (%) |
|---|---|---|
| F1 | 60 | 12 |
| F2 | 63 | 19 |
| F3 | 26 | 10.4 |
| F4 | 26.87 | 13.4 |
| F5 | 14 | 15 |

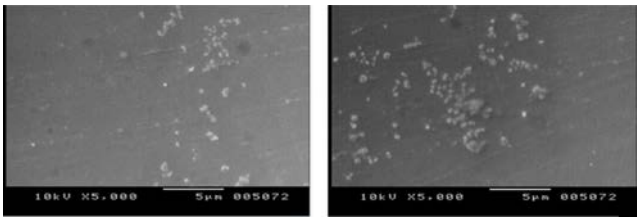

**Figure 4: Scanning electron microphotographs of Bendamustine nanoparticles.**

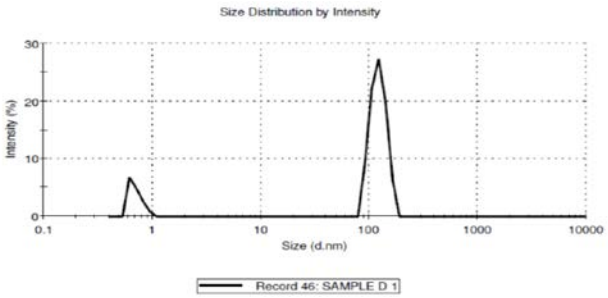


**Figure 5: Particle size distribution Bendamustine nanoparticles (F1).**

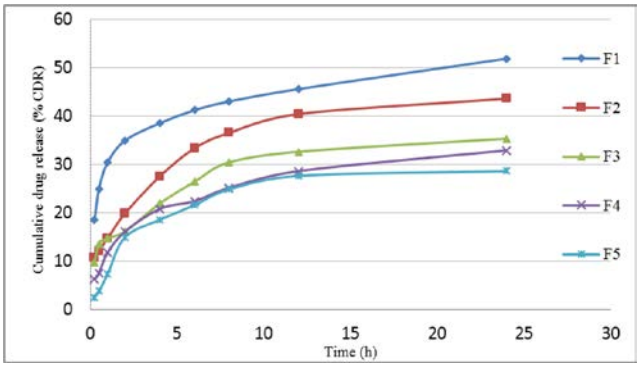


**Figure 6: *In vitro* drug release profile of Bendamustine nanoparticle in phosphate buffer pH7.8.**

drug release profile of Bendamustine nanoparticles for various formulations. The Figure depicts the drug release profile for Bendamustine nanoparticle formulations with varying concentration of albumin; F1(<50% drug release in 24 hr) and F5(>30% drug release at the end of 24hr) exhibit highest and lowest drug release respectively (Figure 6). All formulations demonstrated biphasic release pattern. Surface adsorbed drug gets dissolved faster showing rapid initial release. Encasulated drug comes out slowly showing sustained release. Similar results have been reported by Liu *et al.* by genistein nanoparticls.[14] For kinetic study following plots were made as described in methods section. Figure 6 depicts the drug release profile of Bendamustine nanoparticles for various formulations. Figure 7 shows the best fit with the highest correlation ($R^2$) value, the drug release pattern follows Korsmeyer-Peppas with $R^2$=0.96 and $n$>0.45, suggesting a non-fickian drug release pattern.[15]

### Stability studies of Bendamustine nanoparticles

Stability studies were performed on the preferred Bendamustine nanoparticles formulation, F1. The nanoparticles were subjected to 40±5°C and 75±5% RH. These samples were collected at regular intervals of 10-, 20- and 30 day intervals and examined for any changes in physical appearance or drug content; the results are depicted in Table 2. Bendamustine nanoparticles were shown to be stable at 40±5°C and 7±5% relative humidity, with no change in physical appearance or drug content detected.

## DISCUSSION

Preformulation studies of Bendamustine with BSA indicates that Bendamustine is compatible with BSA.

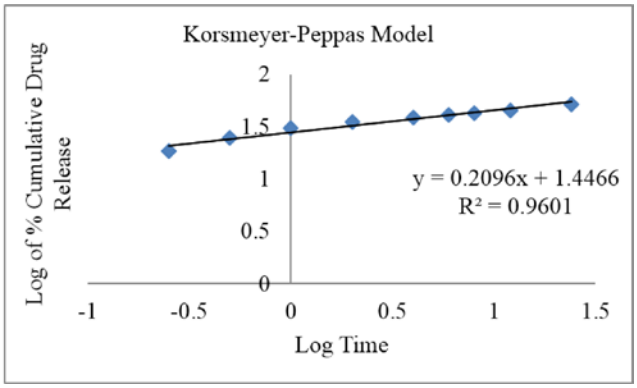


**Figure 7: Bendamustine loaded BSA nanoparticles release kinetics according to the Korsmeyer-Peppas model.**

**Table 2: Stability studies of Bendamustine nanoparticles at 40±5°C and 75±5% RH.**

| Evaluation parameters | Initial | Observation (days) | | |
|---|---|---|---|---|
| | | 10 | 20 | 30 |
| | N.S.C | N.S.C | N.S.C | N.S.C |
| Drug content (%w/w) | 85.7% | 83.7±0.2 | 79.94±0.15 | 75.9±0.25 |

N.S.C- No significant change

The drug-polymer interaction was investigated using FT-IR analysis, which revealed that there were no changes in the IR spectra of pure drug Bendamustine in the presence of albumin (Figures 1 to 3), indicating that the polymer does not interact with the drug, indicating drug-excipient compatibility.

Albumin nanoparticles containing Bendamustine were prepared using a simple coacervation technique with varying amount of serum albumin. Incubation of Bendamustine with 2%w/w BSA resulted in formation of excellent nanoparticles. The addition of ethanol was found to aid the production of nanoparticles.[16]

Bendamustine nanoparticles were found to have a mean particle size of 122.4 nm with narrow particle size distribution (Figure 5). Nanoparticles' potential to modify medication biodistribution and pharmacokinetics has sufficient *in vivo* therapeutic implications.[17] The size and surface characteristics of nanoparticles are critical in this regard. Nanoparticles with a hydrophilic surface and smaller size circulate in the bloodstream for longer. Such methods improve the efficiency of medication targeting to particular locations while also extending the duration of pharmacological action.[18]

The polydispersity index is a measurement of nanoparticles dispersion and Bendamustine nanoparticles were found to have narrow size distribution of >1 (PDI- 0.432), indicating that the particle size distribution is uniform.[17]

To reduce the amount of delivery system required per ml of solvent, the drug payload for any carrier system should be high. The drug loading capacity of the produced nanoparticles varied from 10.4 to 19% w/w (Table 1). The drug release profile of Bedamustine nanoparticles revealed that cumulative drug release was in the range of 28.58% to 51.8 % at the end of 24hr (Figure 7).

The formulation F1 was chosen as the optimum formulation for further studies such as release kinetics and stability study, based on it mean particle size, higher drug loading capacity, and sustained drug release pattern in 24 hr.

The kinetics of drug release were investigated by fitting the drug release data to several kinetic models. Bendamustine nanoparticles were found to follow Korsmeyer-Peppas model with n>0.45 indicating Fickian drug release mechanism.[19] Bendamustine nanoparticles were stable at for 30 days when stored at 40±5°C and 75±5% RH, with no significant change in the drug content or physical appearance.[20]

## CONCLUSION

The purpose of the present work was to develop nanoparticle for the water-soluble drug Bendamustine. A simple coacervation technique was employed to make albumin nanoparticles with Bendamustine. Bendamustine was found to be compatible with serum albumin. The resultant nanoparticles were homogenous and spherical in shape and size (particle size ~122.4 nm and PDI>1). The Bendamustine nanoparticles exhibit a Fickian drug release pattern with sustained drug release profile. The developed Bendamustine nanoparticles were stable for a month at accelerated stability conditions (40±5°C and 75±5% RH). Thus, the developed Bendamustine nanoparticles prepared using biodegradable and biologically safe polymers, albumin, are stable nanoparticles that have potential for reduced frequency of drug administration, lower risk of dose related adverse effects, improved bioavailability and efficacy.

## ACKNOWLEDGEMENT

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## SUMMARY

A simple coacervation technique was employed to make albumin nanoparticles with Bendamustine. The resultant nanoparticles were homogenous and spherical in shape and size (particle size ~122.4 nm and PDI>1) they exhibit a Fickian drug release pattern with sustained drug release profile.

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