Preparation and Primary Characterization of Bovine Serum Albumin-Based Nanoparticle for Drug Delivery

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Abstract
In current research work, we prepared a nano-based system for targeted drug delivery in which the drug is encapsulated into activated folic acid attached bovine serum albumin nanoparticles the drug for choice is celecoxib which is useful in rheumatoid arthritis disease. In this work, we first prepared the nanoparticle which is used for further study and we found that the nanoparticle is successfully prepared and has having very suggestive size range and zeta potential The mean size of BSA-CELECOXIB and FA-BSA-CELECOXIB nanoparticles was observed to be 105±6 and 148.7±9 nm along with ζ-potential value of -15±1.3 and -10.4±1.7 mV and pdi1±0.2 and 1±0.5, respectively it is also have very good stability profile percentage residual drug in nanoparticles formulation BSA-CELECOXIB was found to be 85.37 when stored at 4±2 °C and 70.54% when stored at 37±2 °C and 74.23% which suggest the research work for further studies.

Keywords: Encapsulated, Bovine serum, Nanoparticles, Zeta potential, Stability.

Introduction
Since 1939, there has been no any idea about rheumatoid arthritis but, Davidson (1943) [1] remarks, something about the research in this area. During the war years much has been written on the subject of rheumatoid arthritis and our ideas have crystallized in several directions [2]. As mentioned above, it is unknown what causes rheumatoid arthritis. However, British opinion is expressed in the statement that "suggestive features favour the view that infections play some etiological role," but that the majority of evidence "points to the fact that it is due to the abnormal immunological reaction of the host to infection" rather than the response to a specific infection. [3] comes to the conclusion that the essential factor is an abnormal immune body response, while both [4] in separate series of blood and synovial fluid cultures, found no evidence to support a specific infective aetiology. Slater (1943) analyses the case histories of 388 patients and finds that exhaustion and emotional crises play a significant role, but that the impact of heredity, the endocrine system, pregnancy, menopause, body build, vitamins, and food cannot be demonstrated to be causal variables. He discovered that the average age of onset was 40 and that women made up 70% of the cases. [5].

Material and Methods
Bovine serum albumin procured from CDH chemicals india. Celecoxib was kind gift sample from Sun Pharma Limited, N-dicyclohexyl

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carbodiimide purchased from Merck India, N-hydroxysuccinimide also from Merck India, and Acetone and Methanol of synthetic grade was purchased from CDH Chemical Limited.

**Preparation of CELECOXIB-loaded BSA nanoparticles**

With a small modification, Merodio and colleagues' desolvation cross-linking method was used to create BSA nanoparticles. A different glutaraldehyde concentration was applied, and particle size was optimised. First, distilled water (20 mL) was added, followed by 300 mg of BSA, and swirled for 1.5 hours in a round bottom flask (RBF). It then received an additional 25 mg of CELECOXIB and was continuously stirred for a further 4 hours after the addition of ethanol at 1 mL/min as a desolvating agent. Three RBFs were equally divided with the solution mixture. BSA-CELECOXIB-1, BSA-CELECOXIB-2, and BSA-CELECOXIB-3 were used to create the RBF. BSA-CELECOXIB-1 received 0.25% glutaraldehyde solution; BSA-CELECOXIB-2 received 0.8% glutaraldehyde solution; and BSA-CELECOXIB-3 received 0.5% glutaraldehyde solution. [6]. Scheme of steps involved are mentioned in Fig.1.

5.3 Preparation of NHS ester of folic acid

The published approach [43] was used to create the folic acid NHS ester (folic-NHS). Dimethyl sulfoxide (10 mL) was used to dissolve the folic acid (500 mg), then triethylamine (0.2 mL) was added. With stirring, 4.8 g of N-dicyclohexylcarbodiimide and 2.6 g of NHS were added to the folic acid-dimethyl sulfoxide solution. Overnight, the reaction was allowed to run at room temperature [7,8,9]. Filtration was used to get rid of the byproduct of making folic-NHS, dicyclohexyl urea. (Fig. 2).
5.4 Preparation of FA-BSA-CELECOXIB nanoparticles
NHS-folate (50 mg) was dissolved in 1.0 mL of dimethyl sulfoxide and gently added with stirring to the BSA-NPs suspension (2 mL), with the pH being adjusted to 10 using 1.0 M carbonate/bicarbonate buffer. BSA-CELECOXIB solution was then taken into an RBF. The dispersion was passed down a sephadex G-50 column to separate the folate-conjugated BSA-NPs from unreacted folic acid and other byproducts after stirring for three hours at room temperature. The folate-conjugated BSA-NPs were eluted from the column in the void fraction after the suspension was centrifuged at 1900 g for 35 min. PBS (pH 7.4) was used to re-disperse the pellets. Finally, the FA-BSA-CELECOXIB nanoparticle loaded drug was obtained. [10]

5.5 Percent drug loading and encapsulation efficiency
After the conjugation to NHS folate with BSA-CELECOXIB the solution was centrifuged at 1900 g for 35 min continuously and then the supernatant collected[11]. It was analysed through UV-visible spectrophotometer for the measurement of unbounded drug. Further the obtained solid product(FA-BSA-CELECOXIB) was dried with vacuum drying and the calculation of percent encapsulation and drug loading by given formulae.

\[
DL \text{ (\% w/w)} = \frac{\text{Mass of drug in nanoparticles}}{\text{Mass of nanoparticle drug}} \times 100
\]

\[
EE \text{ (\% w/w)} = \frac{\text{Mass of drug in nanoparticles}}{\text{Mass of total feed drug}} \times 100
\]

Characterization
Various characterization studies were carried out for physiochemical properties of the prepared sample such as FT-IR, size, zeta potential, polydispersity index (pdi), scanning electron microscopy (SEM) and nuclear magnetic resonance (NMR).

Nuclear magnetic resonance
Activated folic acid with NHS was scanned for\(^1\)H NMR spectrum and \(^{13}\)C using nuclear magnetic resonance spectrometer(Bruker Ascend-500.3 MHz, Bruker Bio Spin Corporation, Switzerland). Folic acid ester was used for
recording the NMR spectrum in deuterated dimethyl sulfoxide.

5.5.1 Particle size, zeta potential and polydispersity index (pdi)

By using photon correlation spectroscopy, produced NPs' mean particle size, zeta potential, and size distribution were measured (Nano ZS, Malvern, UK). Room temperature was maintained throughout the analysis and all measurements were made in triplicate at a 90° angle. The material was diluted with deionized water prior to testing [12]. The zeta potential of the prepared formulation FA-BSA-CELECOXIB was determined with the aid of an electrophoretic cell with an electric field using the apparatus as stated above, and the average particle size obtained as hydrodynamic diameter was reported with the aid of intensity distribution by cumulated analysis. Following three separate experiments, zeta potential measurements were carried out in duplicate in automatic mode with an average of 10 measurements being used for each sample within the duplicates.

Stability study

Study involves evaluating the physical, chemical, and microbiological characteristics of a product over a defined period under controlled conditions. The purpose of such a study is to determine the shelf life and storage conditions required to maintain the quality, efficacy, and safety of the product. Collect representative samples of the product to be tested. Use appropriate sample sizes and packaging materials as per regulatory guidelines or industry standards. Storage conditions: Place the samples in containers suitable for maintaining the desired storage conditions. Ensure that the storage conditions are closely monitored and controlled throughout the study.

Results and Discussion

The NMR spectrum confirmed the successful conjugation of NHS with the folic acid. The confirmation results were recorded by the dilution of the FA-NHS through deuterated dimethyl sulfoxide. The observed peaks for 1H NMR spectra, δ 8.7 states the free carboxylic acid proton of the given conjugated structure.

Fig. 3 NMR study of prepared final formulation

Percent drug loading (DL) and entrapment efficiency (EE)

Entrapment efficiency and drug loading were observed 81.13 ± 0.22 and 14.1 ± 0.05 %, respectively (Table 1). On the contrary, BSA-
based systems were reported to offer poor drug loading. It has been established to enhance the drugentrapment of both hydrophobic and hydrophilic drugs.

**Particle size, zeta potential and polydispersity index**
The observed particle size, polydispersity index (pdi) and ζ-potential are shown in Table 1. Results showed that BSA-CELECOXIB and FA-BSA-CELECOXIB were spherical with the same structure. The mean size of BSA-CELECOXIB and FA-BSA-CELECOXIB nanoparticles was observed to be 105±6 and 148.7±9 nm along with ζ-potential value of -15±1.3 and -10.4±1.7 mV and pdi1±0.2 and 1±0.5, respectively. The smaller size of the blank and higher size of the loaded NPs indirectly confirmed the drug loading. The size and morphology were further evaluated using electron microscopy.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Particle size (nm)</th>
<th>Zeta potential (mV)</th>
<th>Polydispersity index (Pdi)</th>
<th>Entrapment efficiency (%)</th>
<th>Drug loading (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSA-CELECOXIB</td>
<td>105.7±6</td>
<td>-15 ± 1.3</td>
<td>1±0.2</td>
<td>74.23±0.65</td>
<td>13.23±0.08</td>
</tr>
<tr>
<td>FA-BSA-CELECOXIB</td>
<td>148.7±9</td>
<td>-10.4±1.7</td>
<td>1±0.5</td>
<td>81.13±0.22</td>
<td>14.1±0.05</td>
</tr>
</tbody>
</table>

**6.6 Stability studies**
BSA-CELECOXIB and FA-BSA-CELECOXIBformulation were subjected to stability studies. The formulation was stored at refrigerated temperature 4±1, 37±2 °C and at room temperature in dark. Change in the particle size and residual drug content after thetime interval of 10, 20, 30, 45 and 60 days was determined. The average particle size of the nanoparticleformulations was found to be increased at higher temperature which could be due to aggregation of particles (Fig. 16). This effect was encountered less in case of formulation when stored at 4±1 °C. By keeping the initial drug content 100%, the determination of percentage residual drug in nanoparticles formulation BSA-CELECOXIB was found to be 85.37 when stored at 4±2 °C and 70.54% when stored at 37±2 °C and 74.23% when stored in dark at room temperature after 60 days.
Further, the formulation FA-BSA-CELECOXIB was found to be 83.5 and 72.81% of indomethacin when stored at 4±2 and 37±2 °C after 60 days, respectively and it was 76.34% when stored in dark room (Fig. 3). This could be due to comparatively more leaching of the drug at high temperature. These results suggested that nanoparticle formulation was more stable when stored at refrigerator temperature.

**Conclusion**

The successful synthesis of stable and well-sized BSA nanoparticles represents a significant achievement in the field of nanotechnology. The meticulous preparation process, along with the incorporation of stabilizers and control over size, ensures their suitability for a wide range of applications. The continued exploration and refinement of BSA nanoparticles hold promise for further advancements in the field of biomedicine and beyond.

**References**


