Basic Evaluation and Preparation of Bovine Serum Albumin Nanoparticles for Advance Targeted Therapy

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Abstract

In current research work first of all we perform the preformulation study to assure the safety and efficacy of the drug which is to be used for the preparation of nanoparticles then after we perform the preparation and evaluation of the nano-formulation after all we also perform the release kinetic study and the electron microscopy study of the prepared formulation for targeted drug delivery of the prepared nanoparticles.

Key Words: *BSA, Celecoxib, Rheumatoid Arthritis, Nanoparticle.*

Introduction

Although extensive study has been done in the past to determine the cause of RA, there is still no definitive proof of this. But a lot has been discovered about the pathogenic processes that start and continue the disease process, both in the joints and in other bodily systems. The majority of current research supports the idea that the pathogenesis of RA is rooted in an immune system-mediated inflammatory response; some research also suggests that autoimmune processes may be involved. [1] There have been many additional etiologic theories proposed for RA. There is no convincing evidence that endocrine, metabolic, or dietary factors contribute in any way to disease manifestations, despite the fact that these factors have been assessed and may influence disease manifestations in some way.

Similar results have been found when demographic variables such as occupation, location, and others have been analysed. Research on potential infectious agents that may start the immunologic process involved in disease symptoms as well as host genetic variables that play a crucial part in the process are among the more promising areas of study. Numerous viruses or viral-like entities, as well as a number of bacteria, including mycobacteria and mycoplasma, have been proposed as potential causal agents over the years. Because they are challenging to culture, viruses have always been viewed as interesting etiologic possibilities. Another encouraging breakthrough was the recent discovery of a tiny virus that, when injected into neonatal mice, generated a state of arthritis. This virus was isolated from the synovial tissue of a patient with RA. Genetic variables are also taken into consideration as a potential contributor to RA susceptibility. The use of histocompatibility typing for tissue transplant by using cell surface markers on white blood cells (human leukocyte antigens, or HLA) has led to the discovery of associations between certain HLA types and several diseases. In the case of RA, the occurrence

of HLA-Dw4 or HLA-DR4 is much more common in RA patients than in persons without RA within several ethnic population groups. Among Caucasians, about 25% of the general population is positive for these antigens, whereas 56% of patients with RA are positive.

Materials

Bovine serum albumin procured from CDH chemicals india. Celecoxib was kind gift sample from Sun pharma limited, N dicyclohexyl carbodiimide purchased from Merck India, N-hydroxy succinimide also from Merck India, Acetone and Methanol of synthetic grade is purchased from CDH chemical limited.

Preformulation study profile

Physiochemical properties

There are different parameters of the drug which were observed like physical appearance, melting point, partition coefficient and solubility in different solvents.

Physical appearance

The drug was obtained as powder and it was poured in light and dark on different colours of slab and it was observed that it is white to off white powder **(Table 1)**.

Melting point

The capillary method was used to determine the melting point of CELECOXIB. Three bottom-sealed capillaries containing CELECOXIB were filled, and three observations were made (Buchi Melting Point M-500): the first when the powder began to melt, the second when half the drug had melted, and the third when the entire amount had. The melting point of the CELECOXIB was determined by average of the three measurements, and it was extremely near to the reported value. The reported melting point of the drug was 151 to 153 ^{0}C and observed was at 152.6 °C **(Table 1)**. This proved that the supplied drug is pure CELECOXIB.

Table 1: Physiochemical properties of CELECOXIB

Study	Observed	Reported
Solubility	0.0018 mg/mL	0.0028 mg/mL
Melting Point	153.2 °C	151 °C
LogP	3.91	4.27

Solubility

The shake flask method was used to assess the drug's solubility in ethanol, methanol, dimethyl sulfoxide, water, and buffer (pH 7.4). 100 mg of the medication in excess was added to 5 mL of solvents, and the mixture was stirred for 24 to 72 hours. Undissolved drugs or a muddy appearance in the solution served as indicators of saturation. After the slurry had been filtered, a sample was collected for the analysis. Finally, solubility was determined using ultraviolet/visible spectroscopy which was observed to be 122.2 mg/mL in ethanol, 132.2 mg/mL in methanol, 215 mg/mL in DMSO, 0.0013 mg/mL in water and 0.0018 mg/mL in phosphate buffer **(Table 2)**.

Table 2: Solubility of CELECOXIB in different solvents

S. No.	Solvent	Results
1.	Methanol	+++
2.	Chloroform	+++
3.	Water	---
4.	Buffer (phosphate)	+++
5.	DMSO	++

+++: Freely soluble, ++: Soluble, ---: Practically insoluble

Partition co-efficient

Lipophilicity of any drug is usually expressed in terms of partition between water and n-octanol. CELECOXIB was shaken with a mixture of water and n-octanol and concentration in each layer was determined.

$$P = \frac{[\text{Drug}]\text{octanol}}{[\text{Drug}]\text{water}}$$

The partition co-efficient of the given drug was observed to be 3.91 and the reported value is 4.27.

UV-visible spectroscopy and absorption maxima

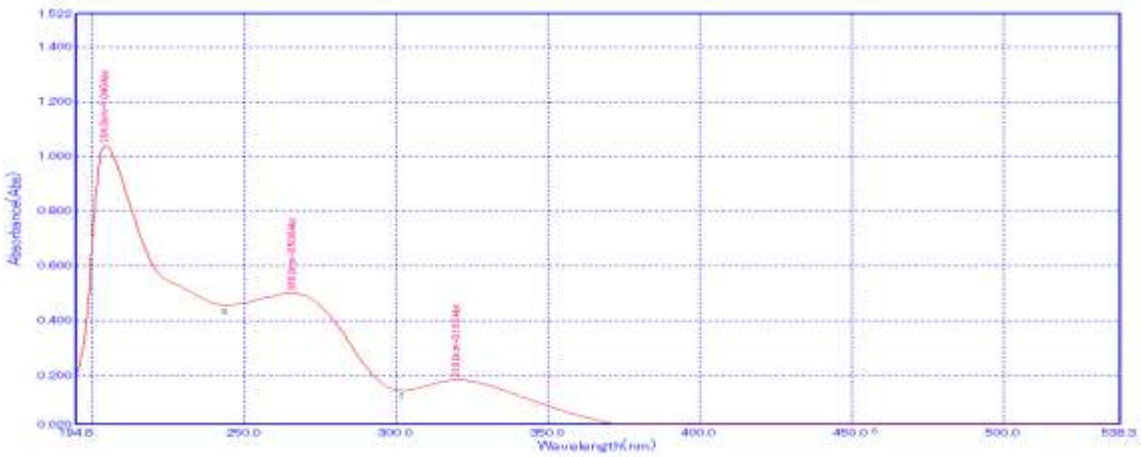


Fig. 1 Spectrum of celecoxib showing absorption maxima

Preparation of CELECOXIB-loaded BSA nanoparticles

BSA nanoparticles were prepared by the de-solvation cross-linking technique as reported earlier by Merodio and co-workers with slight modification. Different concentration of glutaraldehyde was added and optimized for particle size. Firstly, 300 mg of BSA was taken and transferred to the round bottom flask (RBF) and then distilled water (20 mL) was added to it and stirred for 1.5 hrs. Further 25 mg of the CELECOXIB was added to it and was further stirred continuously up to 4 hrs following ethanol addition at 1 mL/min as desolvating agent. Solution mixture was divided into three RBF equally. Named the RBF as BSA-CELECOXIB-1, BSA-CELECOXIB-2, BSA-CELECOXIB-3 and 0.25% glutaraldehyde solution was added to the BSA-CELECOXIB-

1, 0.8% glutaraldehyde solution was added to the flask containing BSA-CELECOXIB-2 and 0.5% glutaraldehyde solution was added to the third RBF named as BSA-CELECOXIB-3 until the crosslinking to the amine group in the nanoparticles and it was performed by continuous stirring the solution up to 16 hrs. Then the mixtures were cold centrifuged at 12000 rpm for 35 min and then the supernatant was removed and taken for the ultraviolet spectroscopy for calculation of the encapsulation efficiency and nanoparticle stored [1]. Scheme of steps involved are mentioned in **(Fig. 2)**.

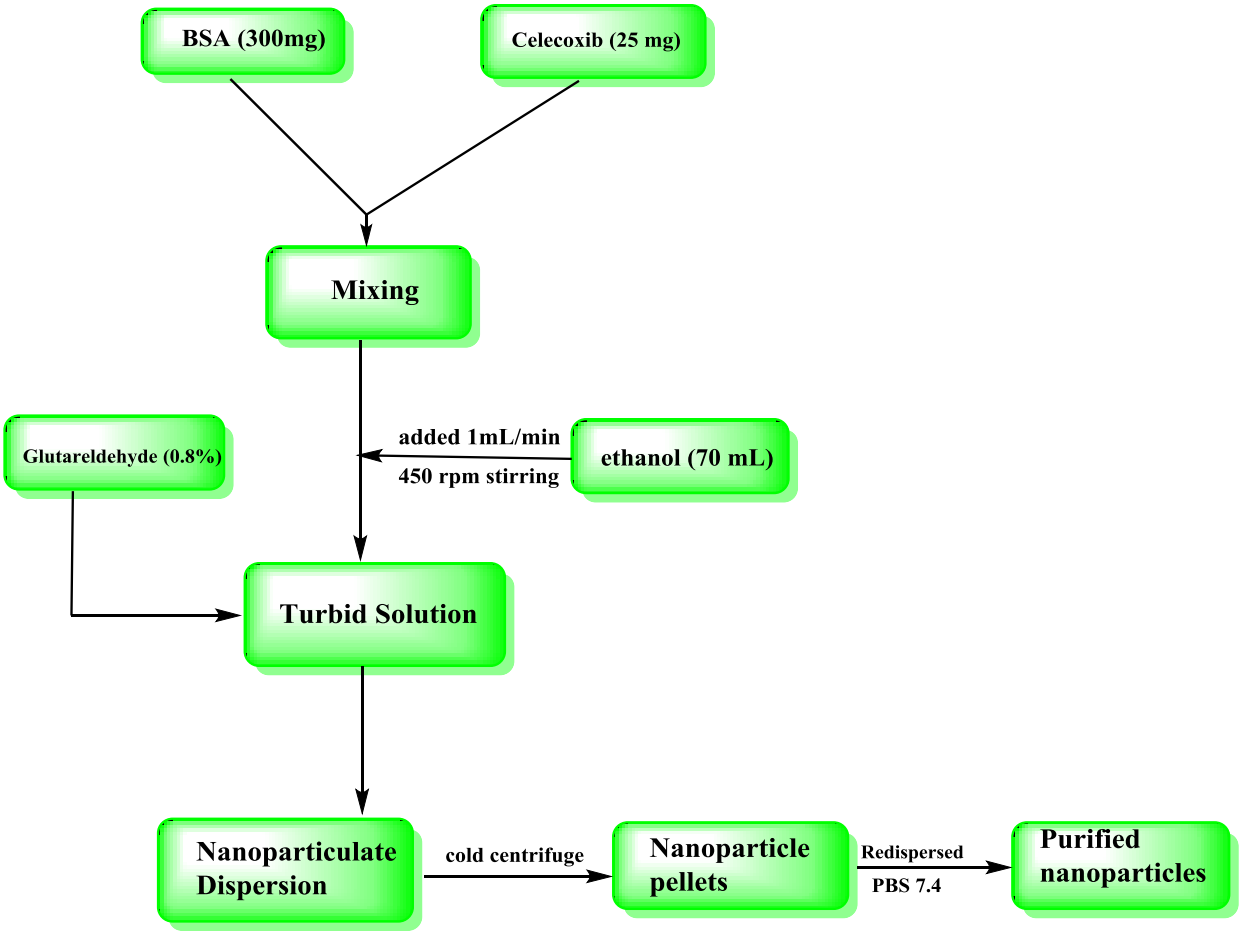


Fig. 2: Steps involved in the preparation of BSA nanoparticles.

5.3 Preparation of NHS ester of folic acid

The NHS ester of folic acid (folic-NHS) was prepared by following reported method **[2,3]**. Folic acid (500 mg) was dissolved in dimethyl sulfoxide (10 mL), to which was added triethylamine (0.2 mL). N-di-cyclo hexyl carbodiimide (4.8 g) and NHS (2.6 g) was added to the folic acid-dimethyl sulfoxide solution with stirring. The reaction was continued at room temperature overnight. Dicyclohexyl urea, which is the by-product of the synthesis of folic-NHS, was removed by filtration **(Fig. 3)**.

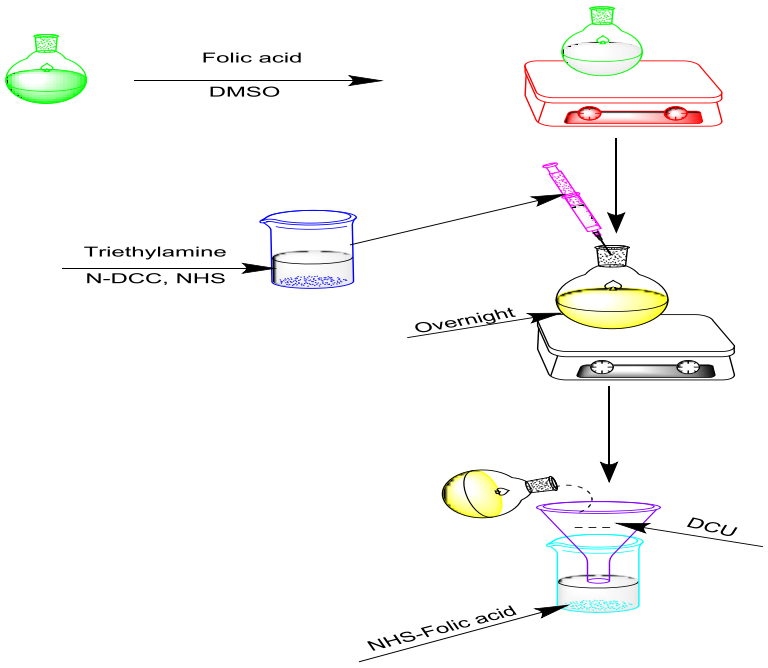


Fig. 3: Folic acid-NHS esterification process.

Preparation of FA-BSA-CELECOXIB nanoparticles

BSA-CELECOXIB solution was taken into a RBF and NHS-folate (50 mg) was dissolved in 1.0 mL of dimethyl sulfoxide and added slowly with stirring to the BSA-NPs suspension (2 mL), pH was adjusted to 10 using 1.0 M carbonate/ bicarbonate buffer. After stirring for 3 hrs at room temperature, the dispersion was passed through sephadex G-50 column to separate the folate-conjugated BSA-NPs from unreacted folic acid and other by-products. The suspension was centrifuged at 1900 g for 35 min and folate-conjugated BSA-NPs eluted in the void fraction from the column. The pellets were redispersed in PBS (pH 7.4). So, the FA-BSA-CELECOXIB nanoparticle having loaded indomethacin drug was obtained finally100 ppm solution of CELECOXIB was prepared in methanol and scan was done. 100 ppm solution was prepared by dissolving the 1 mg of CELECOXIB in 5 mL of the methanol and volume was make up to 10 mL. Before scanning the solution was filtered through syringe filter[4,5,6].

Electron microscopy

The prepared samples (FA-BSA-CELECOXIB) surface morphology and shape was analysed through the SEM (scanning electron microscope, S-3400 N, Hitachi, Japan). It was performed at SAIF, AIIMS, New Delhi, India). Therefore, the photographs are recorded and analysed [7].

***In-vitro* drug release**

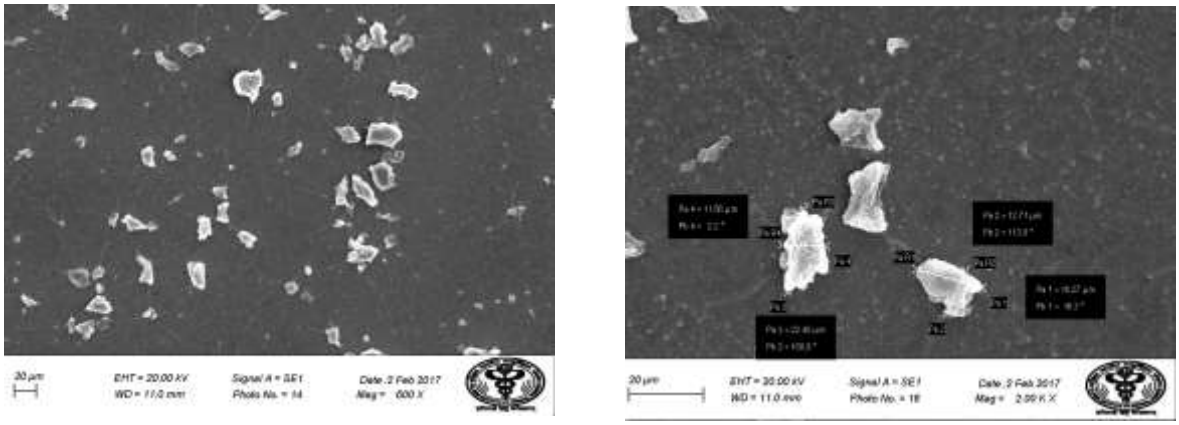
The indirect membrane dialysis method using cellulose membrane with fixed cut off (MW cut-off 5000, Hi-Media) was used to conduct the in vitro drug release investigation. Dialysis totally removes the NPs from the release medium while also allowing unrestricted diffusion of the drug molecules into the release medium. In 2 mL of 1% tween-20 phosphate buffer solution (pH 6.5), 5 mg of the drug-loaded formulation (FA-BSA-CELECOXIB) were suspended in a dialysis bag. The buffer solution's pH was raised to 7.4. The dialysis bag was subsequently submerged in 50

mL of the buffer solution at 25 °C while being magnetically stirred (sink condition). [8]. At successive time intervals, aliquots (2 mL) of the release medium was collected and replaced with a fresh 1% tween-20 buffer solution. Further the absorbance was measured at 320 nm through ultraviolet visible spectroscopy. The scan was done of all the aliquots until the absorbance of the two to three aliquots was continuously same [9].

Result and Discussion

Electron microscopy

Scanning electron microscopy of the prepared FA-BSA-CELECOXIB was observed compared to BSA-CELECOXIB nanoparticles and it was observed that the flat surface was observed with some curved images for FA-BSA-CELECOXIB when observed at higher resolutions **(Fig. 4)**.



BSA-CELECOXIB FA-BSA-CELECOXIB

Fig. 4: Scanning electron microscopy of BSA-CELECOXIB and FA-BSA-CELECOXIB.

***In-vitro* drug release**

In vitro release study of the pure drug CELECOXIB and CELECOXIB incorporated nanoparticles i.e. BSA-CELECOXIB and FA-BSA-CELECOXIB was studied. It was found that 90% pure CELECOXIB drug was released in 6 hrs and the 65% release of the drug from the BSA-CELECOXIB was observed. In case of FA-BSA-CELECOXIB 65% release was observed in 14.5 hrs which confirmed that the conjugation of FA with the BSA-CELECOXIB support the extended release of the CELECOXIB and FA does not affect the release profile and the (r^2 =0.976**) (Fig. 5)**. Further the first order kinetics of the cumulative percentage to be release (r^2 =0.8848). The analysis on the base of korsmeyer-peppas model the (r^2 =0.9625) defined in the **(Fig. 6)**.

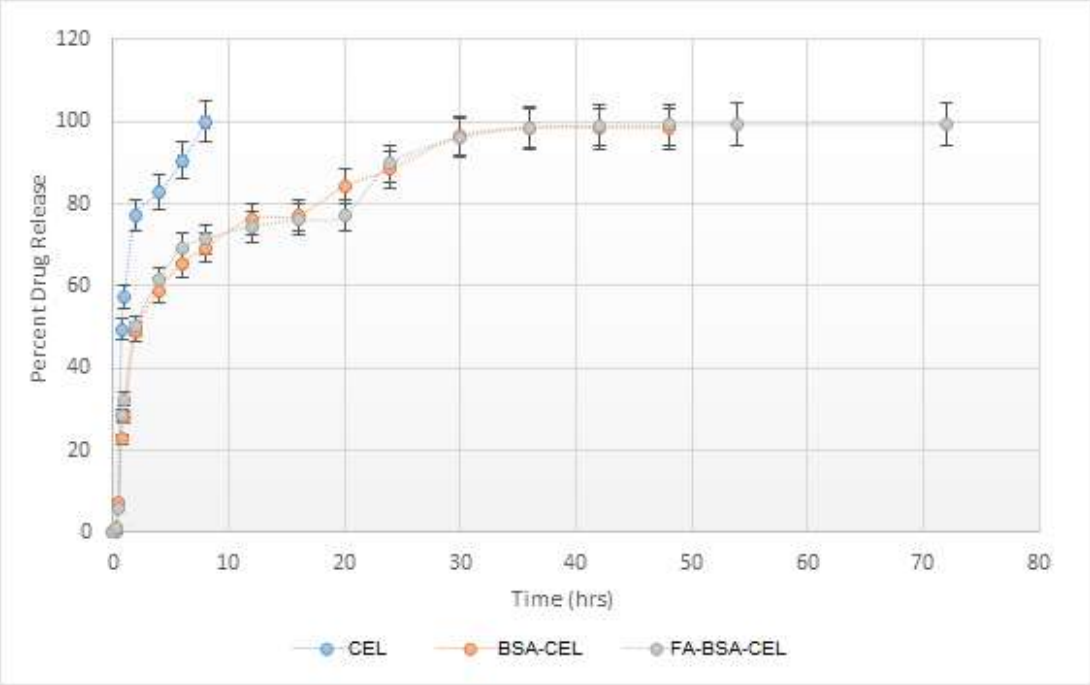


Fig. 5: *In-vitro* release of CELECOXIB, BSA-CELECOXIB, FA-BSA-CELECOXIB

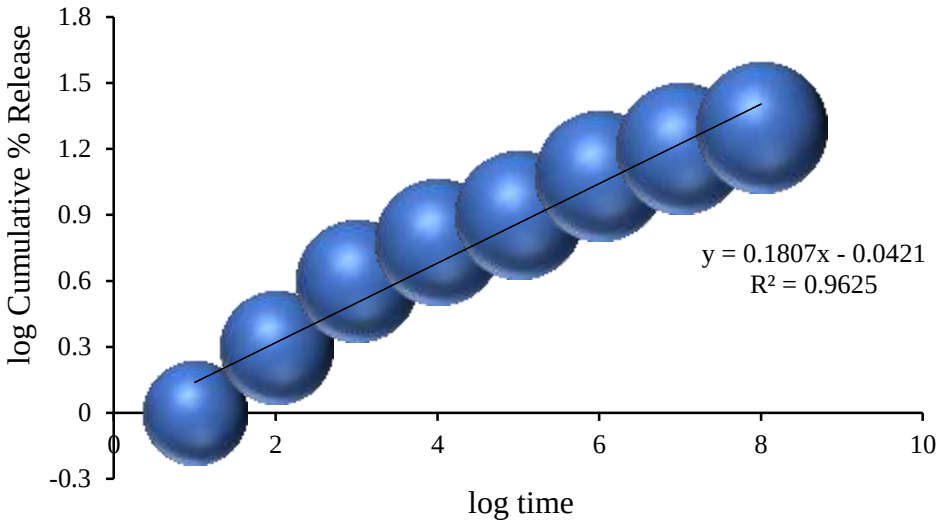


Fig. 6: Korsmeyer-Peppas model for *in-vitro* drug release from FA-BSA-CELECOXIB.

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