Preparation of BSA Nanoparticles by Desolvation Technique Using Acetone as Desolvating Agent

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Received December 28, 2011; accepted March 17, 2012

ABSTRACT
In order to see the functionality and toxicity of nanoparticles in various food and drug applications, it is important to establish procedures to prepare nanoparticles of a controlled size. Desolvation is a thermodynamically driven self-assembly process for polymeric materials. In this study, we prepared BSA nanoparticles using the desolvation technique using acetone as desolvating agent. Acetone was added intermittently into 1% BSA solution at different pH under stirring at 700 rpm. Amount of acetone added, intermittent timeline of acetone addition, and pH of solution were considered as process parameters to be optimized. The effect of the process parameters on size of the nanoparticles was studied. The results indicated that the size control of BSA nanoparticles was achieved by adding acetone intermittently. The standard deviation of average size of BSA nanoparticles at each preparation condition was minimized by adding acetone intermittently. The intermittent addition in polymeric aqueous solution can be useful for size control for food or drug applications.

KEYWORDS: Desolvating agent; particle size; drug interaction; aspirin; polymeric nanoparticles.

Introduction
Nanoparticles are sub-nanosized colloidal structures composed of synthetic or semisynthetic polymers (Jahanshahi et al., 2008; Najafpour et al., 2008). Nanospheres are solid core spherical particulates which are nanometric in size. They contain the drug embedded within the matrix or adsorbed onto the surface. Nanocapsules are vesicular system in which drug is essentially encapsulated within the central volume surrounded by an embryonic polymeric sheath (Rahimejad et al., 2006). In nanocrystals the drug is mainly encapsulated in the solution system. Albumin, gelatin legumin, polysaccharides like alginates or agarose is natural polymers. Natural hydrophilic polymers are studied because of their intrinsic biodegradability and biocompatibility (Müller et al., 1996). Natural polymers are classified as proteins and polysaccharides. Proteins are gelatin, albumin, lecitin, legumin and vicillin. Polysaccharids are alginate, dextran, chitosan and pollulan (Mohanraj et al., 2006).

Synthetic polymers in nanoparticles preparation are those which are used in the preparation of microspheres. Polylactic acid, methylmethacrylate, polyacrylamide are synthetic polymers.

In order to see the functionality and toxicity of nanoparticles in various food and drug applications, it is important to establish procedures to prepare nanoparticles of a controlled size. Amphiphilic macromolecular cross linking, polymerization based methods and polymer precipitation methods are the different methods used for the preparation of nanoparticles. The amphiphilic macromolecular cross-linking desolavation technique is mainly used for the preparation of nanoparticles for proteins and polysaccharides (Azarmi et al., 2006). Desolvation is a thermodynamically driven self-assembly process for polymeric materials to prepare nanoparticles (Weber et al., 2000). The polymeric molecules form particles of different sizes depending on the preparation conditions such as protein content, pH, ionic strength, concentration of cross-linking agent, agitation speed, amount of desolvating agent etc (Coester et al., 2000). The protein or polysaccharide from an aqueous phase can be desolvated by pH change or change in temperature by adding appropriate amount of counter ions. Cross-linking may be affected simultaneously or subsequently to the desolvation step. It contains three steps: protein dissolution, protein aggregation and protein deaggregation. With the appropriate levels of desolvation and resolvation, the aggregate size could be maintained and finally these aggregated nanoparticles are cross linked using glutaraldehyde. Sodium sulphate is the main desolvating agent. Alcohol, isopropanol and ethanol are added as desolvating agents. The addition can be optimized turbidometrically using a nephelometer. Only desolvation can give the final product as nanosphere. Desolvation deaggregates the protein and turns the...
suspension colloidal and hence milky in appearance. Both lipophilic and hydrophilic drugs can be entrapped in nanoparticles using this technique.

The main purpose of this study was to prepare aspirin loaded BSA nanoparticles using the desolvation technique using continuous and intermittent addition of acetone as desolvating agent. The effect of continuous and intermittent addition of acetone on resultant nanoparticle size was studied.

Materials and Methods

Chemicals

BSA (Bovine serum albumin) was commercially supplied from Sigma Aldrich (St. Louis MO, USA). Analytical Grade high purity acetone was supplied from Fisher Scientifics.

Methodology

BSA nanoparticles were prepared using the desolvation method (Langer K et al., 2003). BSA powder was added to distilled water. Acetone was added continuously or intermittently into 1% BSA solution at pH 7 under stirring at 700 rpm at room temperature until the solution became just turbid. Here BSA nanoparticles were prepared by two methods: 1) Continuous addition and 2) Intermittent addition. In continuous addition method acetone was added continuously in the solution with rate addition about 1.0 to 2.0 ml per minute and for intermittent method 2 ml of acetone was added for every 5 minutes interval. Then a few ml of 6% glutaraldehyde solution was added drop wise and kept for stirring for 12 hours. Then the solvent was removed in rotary flash vacuum evaporator. The nanoparticles so obtained are kept for air drying (Dongmei Zhao et al., 2010).

Results and Discussion

Determining the Size of Nanoparticles

The size of the gelatin nanoparticles was determined by a scanning electron microscope (Maghsoudi et al., 2008). In order to perform the SEM observation, nanoparticle suspension was first diluted with ultrapure water (1/5), and then a drop of the diluted nanoparticle suspension was then directly deposited on a polished aluminum sample holder. Samples were dried in a vacuum. The morphology of nanoparticles was observed at 15 kV using a scanning electron microscope (SEM; S-3700 N, Hitachi, Japan) (Truong-Le et al., 1999). The images of nanoparticles prepared by the continuous addition method are illustrated in Figure 1 and Figure 2. The images of the nanoparticles prepared by the intermittent addition method were illustrated in Figure 3 and Figure 4.
Fourier Transforms Infrared Spectroscopy (FT-IR)
The FT-IR spectra acquired were taken for the dried samples (Figure 5 and Figure 6). An FT-IR (7000) spectrometer was used for the analysis in the frequency range between 4000-400 cm\(^{-1}\). It is made as described below. About 1 mg of the substance was triturated with approximately 300 mg of dry and finely powdered potassium bromide IR. These quantities are usually suitable for a disc 13 mm in diameter. The mixture was thoroughly ground, spread out uniformly in a suitable die and compressed under vacuum at a pressure of about 800 Mpa. Commercial dies were available and the manufacturer’s instructions were strictly followed. The resultant disc was mounted in a suitable holder in the spectrophotometer. The IR spectra of the sample were determined from 600-4400 cm\(^{-1}\). Several factors, such as inadequate or excessive grinding, moisture or other impurities in the halide carrier, may have given rise to unsatisfactory discs. A disc should be rejected if visual inspection shows a lack of uniformity or transmittance at about 2000 cm\(^{-1}\) (5 µm) in the absence of a specific absorption band is less than 75% without compensation.

**Fig. 2.** SEM images of BSA nanoparticles prepared by continuous addition method.

**Fig. 3.** SEM images of BSA nanoparticles prepared by intermittent addition method.
Fig. 4. SEM images of BSA nanoparticles prepared by intermittent addition method.

Fig. 5. FTIR spectra of aspirin, BSA and formulation (continuous addition method).
Conclusions

BSA nanoparticles are prepared by the continuous or intermittent addition of acetone as desolvating agent. Effect of the process parameters such as amount of acetone added, intermittent timeline of acetone addition, and \( \text{pH} \) of solution on the size of the nanoparticles was studied. The results indicated that the size control of BSA nanoparticle was achieved by adding acetone intermittently. The standard deviation of the average size of BSA nanoparticles at each preparation condition was minimized by adding acetone intermittently. FTIR spectra indicated that there is no polymer drug interaction. Further studies can be performed on drug encapsulation efficiency and in vitro release of drug from polymeric nanoparticles.

Acknowledgements

This work has been financially supported by UGC (University Grants commission), New Delhi, India.

References


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