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Full Length Research Paper

Production of biological nanoparticles from α-lactalbumin for drug delivery and food science application

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In recent years, the concept of controlled release of encapsulated ingredients at the right place and the right time has become of more interest to the food and pharmaceutical industry. Whey proteins are valuable by-products from the cheese industry. The physicochemical properties of the whey proteins suggest that they may be suitable for novel food and drug delivery system. The objective of this study was to characterize the two step desolvation process of α-lactalbumin for preparation of its nanoparticles. Following the desolvation of the protein with acetone, the produced nanoparticles were stabilized and cross linked by addition of glutaraldehyde. Nanoparticle sample was subsequently purified by 3 cycle's centrifugation (15000 ×g, 20 min). Three process parameters were examined to achieve a suitable size of nanoparticles including the pH value, temperature and desolvating agent type. In addition, fabricated nanoparticles were analyzed by photon correlation spectroscopy (PCS) as well as scanning electron microscopy (SEM). The smallest size of the nanoparticles achieved was 102 nm while the largest size was 454 nm.

Key words: α-lactalbumin, nanoparticles, food proteins, drug delivery, two-step desolvtion method.

INTRODUCTION

Over the past three decades, considerable research interest has risen worldwide in the development of new colloidal drug delivery systems. The ideal colloidal delivery system would transport the associated drug to its desired site of action and then release it at an optimum rate. The carrier itself should be non-toxic and able to be degraded *in vivo* so that it dose not accumulate indefinitely in the tissues (Panyam and Labhasetwar, 2003). These delivery systems offer numerous advantages compared to conventional dosage forms which include improved efficiency, reduced toxicity and improved patient compliance and convenience.

To convey a sufficient dose of drug to the lesion, suitable carrier of drug is needed (Majeti et al., 2000). Nanoparticles have emerged as versatile systems for the specific delivery of drugs to organs and tissues (Langer et al., 2008). Nanoparticles are colloidal particles which are less than 1 µm in diameter. In colloidal system, the size of particle is very important in distribution of drug in human body (Rahimnejad et al., 2006). Nanoparticles have the unique property to accumulate at the site of inflammation and therefore, are very suitable for targeted drug delivery. The selection of matrix materials is dependent on many factors including: (a) Size of nanoparticles required; (b) inherent properties of the drug, for example, aqueous solubility and stability; (c) surface characteristics such as charge and permeability; (d) degree of biodegradability, biocompatibility and toxicity; (e) drug release profile desired; and (f) antigenicity of the final product (Jahanshahi and Babaei, 2008).

Nonoparticles are normally made from synthetic or natural polymers. The polymers used may also be either biodegradable or nonbiodegradable. An ideal polymer should be biocompatible, biodegradable, sterile, non-

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pyrogenic and with minimum or no toxicity. The polymer should also have a high capacity to accommodate the drugs and protect them from degradation until they are delivered to the target site. Natural polymers have the distinct advantages of biodegradability and lack of toxicity, but suffer from reproducibility and well defined physico-chemical properties.

Polymeric nanoparticles have attractive physicochemical properties such as size, surface potential, hydrophilic-hydrophobic balance etc, and for this reason they have been recognizes as potential drug carrier for bioactive ingredient such as anticancer drugs, vaccines, oligonucleotides, peptides, etc (Jahanshahi et al., 2008b).

The major advantage of colloidal drug carrier systems is the possibility of drug targeting by a modified body distribution as well as the enhancement of the cellular uptake of a number of substances. Among these colloidal systems those based on proteins may be very promising, since they are biodegradable and non-antigenic, relatively easy to prepare and their size distribution can be monitored easily (Weber et al., 2000).

Nanomaterials derived from proteins, especially protein nanoparticles are biodegradable, non-antigenic, metabolizable and can also be easily amenable for surface modification and covalent attachment of drugs and ligands. Because of the defined primary structure of proteins, the protein-based nanoparticles may suggest various possibilities for surface alteration and covalent drug attachment (Weber et al., 2000). In fact protein is biopolymer which is commonly used for preparation of nanostructured molecules for drug delivery.

In spite of successful elaboration of many synthetic polymers as delivery systems, these cannot be used in food applications that require compounds generally recognized as safe (GRAS). Food biopolymers, specifically food proteins are widely used in formulate food because they have high nutritional value and are generally recognized as safe. Food proteins show great promise for developing and engineering a range of new GRAS matrices with the potential to incorporate nutraceutical compound and provide controlled release via the oral route. Clear advantages of food protein matrices include their high nutritional value, abundant renewable sources and acceptability as naturally occurring food components degradable by digestive enzymes.

The ability to control the particle size of proteinaceous materials is of primary importance not only for determining food products properties such as taste, aroma, texture and appearance, but also for determining the release rates of the carried bioactive compounds and ultimately how much is absorbed into the body and hence the overall efficacy of the compounds (Chen et al., 2006). Protein based nanoparticles have found wide and rapidly increasing application in the food industry because they can be precisely designed for use in many food formulations and virtually any ingredient can be encapsulated whether hydrophobic, hydrophilic or even microbial.

Due to their sub-cellular size, nanoparticles offer promising means of improving the bioavailability of nutraceutical compounds, especially poorly soluble substance such as functional lipids (carotenoids, phytosterols, ώ-3 fatty acids), natural antioxidants and numerous other compounds that are widely used as active ingredients in various food products. They can dramatically prolong compound residence time in the gastro-intestinal (GI) tract by decreasing the influence of intestinal clearance mechanisms and increasing the surface available to interact with the biological support (Kavashim, 2001; Peppas, 1995; Arbos et al., 2002). They can also penetrate deeply into tissues through fine capillaries, cross the epithelial lining fenestration (e. g. in the liver) and are generally taken up efficiency by cells, thus, allowing efficient delivery of active compounds to target sites in the body (Chen et al., 2006).

There are two categories of milk protein in the milk - whey and casein proteins. Whey proteins (WP) are important food ingredients that are used in a number of food products that include dairy products, confectionary and desserts. WP composes about 20% of bovine milk and is generally produced as a co-product of the cheese industry. The utilization of WP has been an important research focus over the past few decades because of its abundance and excellent nutritional value (DeWit et al., 1984; Tziboula et al., 1998; Muir et al., 1984).

α-Lactalbumin is the major whey protein found in milk. α-Lactalbumin is a protein present in the milk of all mammals. The molecular weight is 14176 Da and the isoelectric point is between 4.2 and 4.5. In addition, α-lactalbumin has significant nutritional properties and is associated with some positive health effects upon consumption. α-Lactalbumin has an important function in mammary secretary cells: it is one of the two components of the lactose biosynthesis in the lactating mammary gland. Its amino acid composition seems to be optimal for the requirements of the infant and it has a high digestibility. It is relatively rich in tryptophan (four residues per molecule) and α-lactalbumin consumption increase plasma tryptophan which is known to have a positive effect on satiety and mood. Evening consumption of α-lactalbumin was shown to increase plasma tryptophan availability and improved morning alertness and brain-sustained attention processes.

In other studies, α-lactalbumin consumption was shown to improve mood and cognitive performance in vulnerable subjects. α-lactalbumin itself (or its fragments) possesses bacterial or antitumor activity (Graveland-Bikker and de Kruif 2006). It can be used as a basis for design of antitumour agent, acting through disorganization of chromatin structure due to electrostatic interaction between α-lactalbumin and histone proteins (Permyakov et al., 2004).

In addition, synthesis of nanoparticles has been attempted using various methods based on physical and chemical processes. Basically three different preparation of such nanoparticles (protein nanoparticles) have been

described based on emulsion formation, desolvation or coacervation (Jahanshahi et al., 2008a). The disadvantage of the emulsion methods for particle preparation is the need for applying organic solvents for the removal both of the oily residues of the preparation process and of surfactants required for emulsion stabilization.

Therefore, as an alternative method for the preparation of nanoparticles as desolvation process derived from coacervation method of micro encapsulation was developed. Protein nanoparticle has been extensively studied in our previous work and gradually fabrication process have been developed (Rahimnejad et al., 2006; Jahanshahi et al., 2008a, b).

In this work, simple coacervation method is used for manufacturing α-lactalbumin nanoparticles as a drug and food delivery systems and the essential parameters are considered. Investigations are focused on the influence of pH value, temperature and desolvating agent upon the produced nanoparticle size.

MATERIALS AND METHODS

Material

α-Lactalbumin (purity > 85%) and glutaraldehyde (25% solution) were commercially supplied by Sigma Aldrich. Acetone and all other chemicals were supplied from Merck (Germany).

Preparation of α-lactalbumin nanoparticles

Two-step desolvation technique was implemented for preparation of α-lactalbumin nanoparticles. 25 $mg.ml^{-1}$ aqueous solution of α-lactalbumin was stirred on 500 rpm magnetic stirrer at 50°C for 10 min. and then 4 ml acetone (desolvating agent), all at once was added to cause sedimentation of high molecular weight (HMW) component of α-lactalbumin. The first step is performed to discard low molecular weight (LMW) component of α-lactalbumin which would make the production of stable nanoparticles with a uni-model size distribution impossible.

After 24 h incubation at room temperature and resolvation of sediment in 1 ml purified water and pH-adjustment (pH 2.5), acetone was added drop-wise until the solution become just turbid. After desolvation process, 30 µl of 25% glutaraldehyde was added for cross linking and stirred continuously at room temperature for 12 h. The formed nanoparticles were purified by 3 cycles of centrifugation. For each centrifugation step, supernatant was centrifuged at 15000 g for 20 min. Shape and morphology of α-lactalbumin nanoparticles were determined by scanning electron microscopy (SEM).

Determination of nanoparticle size and distribution

The size distribution of the prepared α-lactalbumin nanoparticle was analyzed by photon correlation spectroscopy (PCS), (SALD-2101, Japan). PCS is industrially preferred method of sub-micron particle size analysis. The sample analyzed in the PCS device should consist of well dispersed particles in liquid medium. In such condition the particles are in constant random motion, referred to as Brownian motion and PCS measures the speed of this motion by passing a laser. PCS determines the average particle size and polydispersity Index (PI) which is a range of measurement of the particle size within measured samples. The accurate measurement of particle size must be below 0.7 (70%).

Scanning electronic microscopic (SEM)

For electronic microscopic scanning micrographs, samples were taken from nanoparticles that were experimentally obtained. The samples were dipped into liquid nitrojen for 10 min, then freeze dried for 7 h in the freeze drier, EMITECH, model IK750, Cambridg, UK. The sample was fixed on the aluminium stub and coated with gold palladium by Polaron machin model SD515, EMITECH, Cambridg, UK, at 20 nm coating thickness. Finally the sample was examined under SEM using Stereoscan model S360 brand SEM – Leica Cambridg, Cambridg, UK.

RESULTS AND DISCUSSION

The nanoparticle sample was analyzed by scanning electron microscopy (SEM) and photon correlation spectroscopy (PCS). The morphology and size distribution of prepared nanoparticles from α-lactalbumin were examined with SEM. Figure 1 shows the particle size with magnification of 10000. The shape of the nanoparticles demonstrated in SEM is semi-spherical.

In our experiments, we studied the effects of varying production parameters on the nanoparticle size. Different synthesis parameters were changed, including pH value, temperature and desolvating agent type. The goal was to prepare small nanoparticles with a narrow size distribution. It has been shown that, particle size has a great impact on the uptake of nanoparticles. Desai and coworkers (Amidon, 1997) showed that 100 nm size nanoparticles had 2.5 fold greater uptakes compared to 1 µm and 6 fold higher uptakes compared to 10 µm microparticles in a Caco-2 cell line.

The results of other researchers also showed that particle size significantly affects cellular and tissue uptake and in some cell lines, only the submicron size particles are taken up efficiently in lieu of the larger size microparticles (Desai et al., 1996; Zauner et al., 2001). We investigated the effect of these different parameters on the particle size and the polydispersity index where the polydispersity index measures the second moment of the size distribution of the nanoparticle population. A lower polydispersity index indicates a narrower size distribution.

In order to evaluate the effects of desolvating agent type, acetone and ethanol were employed. In these experiments the temperature was kept constant at 50°C and 30 µL of glutaraldehyde was added as cross-linking agent. The results are shown in Table 1. The results show that acetone was the preferred desolvating agent as the nanoparticles prepared with acetone were generally smaller compared to those prepared with ethanol.

The pH value of the α-lactalbumin influenced the resulting particle size. The effect of pH on nanoparticle size was conducted and shown in Figure 2. Decreasing pH will also decrease on the particle size. Therefore, the particle size is defined in the range of 102 to 454 nm. The results showed that pH 2.5 was the optimum pH for

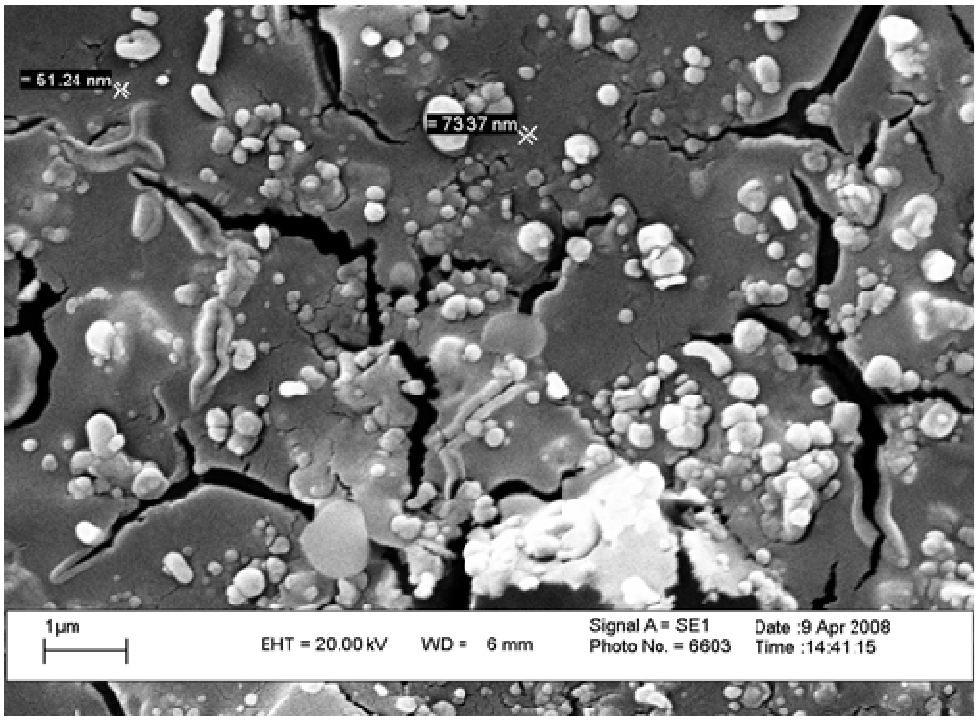

Figure 1. Scanning electron microscopy of the outer surface of α- lactalbumin nanoparticles.

Table 1. Influence of type of desolvating agent on the particle size. (30 µL of glutaraldehyde was used as cross-linking agent at 50℃ temperatures).

Protein	Desolvating agent	Particle size (nm)
α-Lactalbumin	Acetone	213
α-Lactalbumin	Ethanol	390

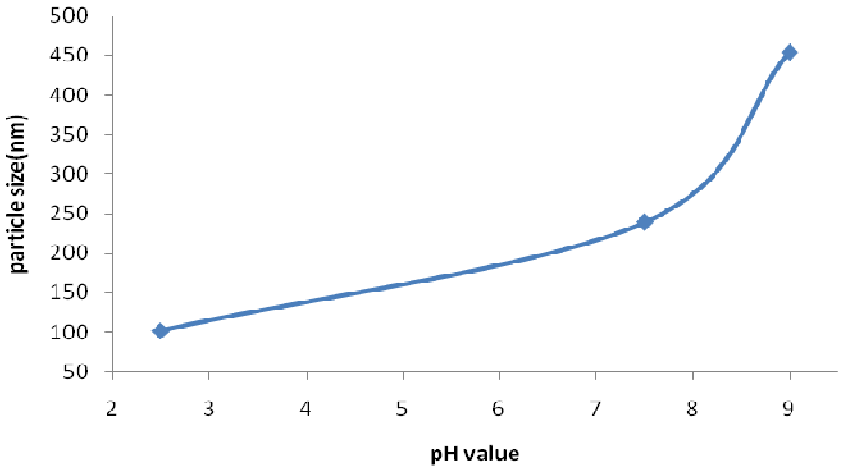

Figure 2. Influence of pH value on the diameter of α-lactalbumin nanoparticles.

preparing the nanoparticles. Figure 3 show the effect of temperature on nanoparticle size. It can be concluded that as the temperature increased the particle size was decreased and preparation at 50°C produced significantly

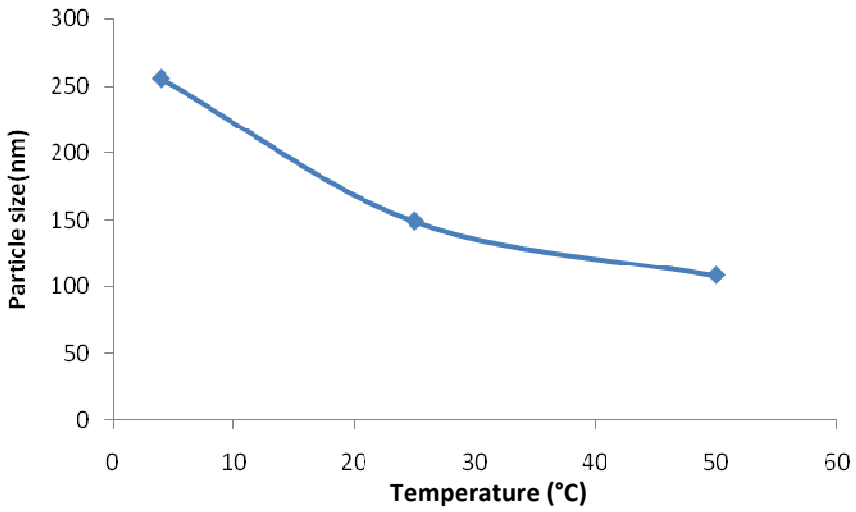

Figure 3. Influence of temperature on the diameter of α-lactalbumin nanoparticles.

smaller particles. The particle sizes of 256, 149 and 108 nm were fabricated with temperature of 4, 25 and 50°C, respectively.

Conclusion

Controlled drug delivery technology represents one of the frontier areas of science, which involves multidisciplinary scientific approach, contributing to human health care. Whey proteins have interesting functional property and can be used as nanoparticles systems for encapsulation and controlled delivery application. Since α-lactalbumin is a milk protein, it will be fairly easy to apply in foods and pharmaceutics. It can be anticipated that α-lactalbumin the second most abundant protein in the whey fraction of bovine milk, can be induced to form nanoparticles.

Therefore, fabrication of α-lactalbumin nanoparticles as a drug and food delivery system was investigated herein. The nanoparticles size prepared from α-lactalbumin was influenced by several process variables including pH, agitation speed, temperature, etc. In predetermined conditions (protein concentration of 25 $mg.ml^{-1}$), the particle was prepared at 50°C, pH 2.5 and agitation rate of 500 rpm. To the best of our knowledge, the current paper is the first discussing potential of α-lactalbumin nanoparticles as delivery system and deserves further study. Optimization of this fabrication method for manufacturing α-lactalbumin nanoparticles will be the subject of our next publication.

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