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PROTEINACEOUS NANOPARTICLES FOR CONTROLLED RELEASE

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Proteinaceous nanoparticles, sonochemical method.

ABSTRACT

The worldwide increased interest in nanotechnology to delivery pharmacologically and cosmetically active ingredients was made developing several user-controlled nanoparticles systems. The specific goal of this work was the synthesis of protein nanoparticles applying high intensity ultrasound and study the ultrasonic effects on nanoparticles formation. The importance of different protein concentrations and ratios of aqueous/organic phase was investigated. The developed products were analyzed and physicochemical characterized in terms of particle size, polydispersity index, surface electrical charge, morphology and yield of nanoparticles formation.

INTRODUCTION

In the last decade, new directions of modern research, broadly defined as ‘‘nanoscale science and technology’’, have emerged. Nanoparticles based on polymers as well as on natural materials have attracted great research interest in the field of drug delivery due to their ability to deliver many kinds of drugs to targeted areas of the body (Ahlin, Kristl et al. 2002). Among these colloidal systems, the one based on proteins may be rather promising, since it is biodegradable and non-antigenic. One technique proposed to prepare proteinaceous nanoparticles is the sonochemical method that has been discussed previously, by Suslick and co-workers, and is a direct result of the chemical effects of ultrasound radiation on an aqueous medium. They purpose that the nanoparticles are held together by disulfide bonds between protein Cys residues and that superoxide, sonochemically produced by acoustic cavitation, is the cross link agent (Suslick and Grinstaff 1990). However, other studies shown that it is possible the formation of nanoparticles, with the proteins that did not have the Cys amino acids (a.a.) in their structure (Avivi and Gedanken 2002). Nevertheless, the mechanism for this nanoparticles formation is not completly understood. In this work it was used the ultrasonic method described by Suslick (Suslick and Grinstaff 1990) for nanoparticles preparation. For that, it were used two proteins with different contents of amino acids residues, albumin serum bovine (BSA) and silk fibroin (SF).

RESULTS

The efficiency of nanoparticles formation was obtained by Lowry assay. The effects of protein concentrations and the different aqueous/organic phase ratios used on nanoparticles formation is shown in Figure 1 for BSA and Figure 2 for SF. From these figures, it is concluded that as the concentration of protein increases, the nanoparticles yield increases. It was reached the maximum yield near to 100% at concentration of 5 $g.L^{-1}$ and 1 $g.L^{-1}$ for BSA and SF, respectively.

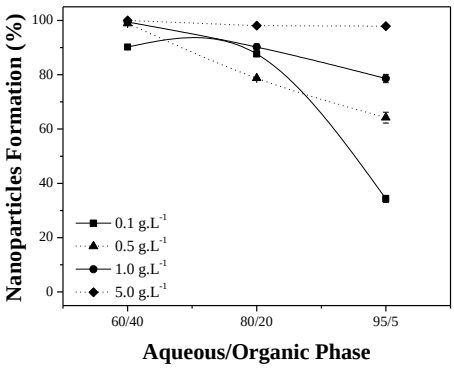

Figure 1: Yield of BSA nanoparticles.

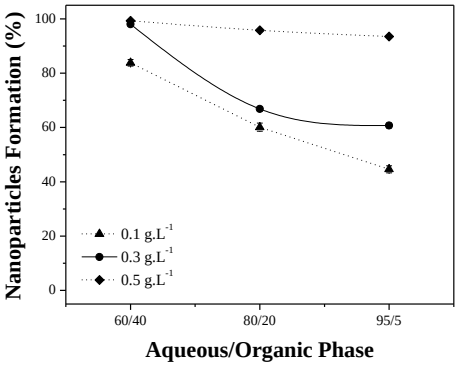

Figure 2: Yield of SF nanoparticles.

In terms of size distribution, when are used lower quantity of organic phase (5%), and higher protein concentration it is possible to obtain a narrower size distribution of proteinaceous nanoparticles with a smaller size. However, when compared the size for BSA (≈350-500 nm) and SF (≈650-950 nm) nanoparticles it was verified that smaller sizes are obtained for BSA nanoparticles. The results obtained are mainly dependent of chemical structure of the protein considered. It is well known that the SF has hydrophobic

and hydrophilic segments, in which 73% of the amino acid residues are hydrophobic, but there are still a lot of amino acids with polar side groups, that have strong affinity for water (Tanaka, Inoue et al. 1999; Kim, Park et al. 2005). Hence, SF contains both hydrophobic and hydrophilic segments and could self-assemble in aqueous solution to form nanoparticles. This is largely due to the hydrophobic effect, which drives the non-polar region of each polymer molecule away from water and towards one another. In a SF aqueous solution the hydrophobic segments and hydrophilic segments are supposed to disperse randomly. Therefore, the SF molecules are easy to aggregate by physical or chemical stimuli, such as the use of ultrasound systems. On the other hand, the presence of Cys of BSA can led to form disulfide bonds in the presence of high ultrasonic energy promoting the nanoparticles formation. The measurement of the surface charge of nanoparticles, on a zeta-potential analyzer, showed that the proteinaceous nanoparticles were negatively charged (≈ -40 mV). The morphology studies showed that the particles have a spherical shape (Figure 3). This shape would offer the highest potential for controlled release and protection of incorporated drugs, as they provide minimum contact with the aqueous environment, as well as the longest diffusion pathways.

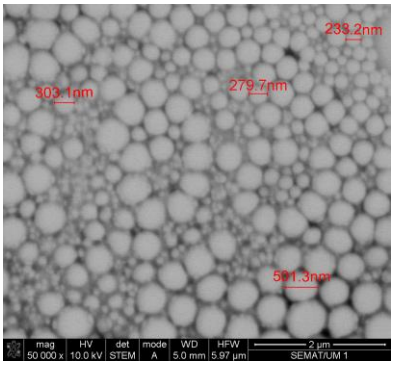

Figure 3: STEM photograph for BSA nanoparticles.

This line of work exemplifies the enormous potential of ultrasound for the production of well defined nanostructures by controlling the protein concentration and the ratios of aqueous/organic phase. Further studies are underway in order to use these nanoparticles as delivery systems that can be applied in different areas, as medicine and cosmetic.

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