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Drug release mechanisms of chemically cross-linked albumin microparticles: Effect of the matrix erosion

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ABSTRACT

Albumin (BSA) microparticles were developed as a biotechnological alternative for drug delivery. Vitamin B_{12} (Vit-B_{12}) was used as a model drug. The microparticles were obtained from maleic anhydride-functionalized BSA and *N′*,*N′*-dimethylacrylamide (DMAAm) in a W/O emulsion without and with PVA. The microparticles produced at 15 min of stirring without PVA showed the best results in terms of size, homogeneity, and sphericity. In such a case, BSA played a role as a surface active agent, replacing PVA. For longer stirring times, BSA was unable to act as an emulsifier.

These microparticles showed an uncommon release profile, consisting of a two-step release mechanism, at the pH range studied. Considering that a two-step release mechanism is occurring, the experimental data were adjusted by applying modified power law and Weibull equations in order to describe release mechanism *n* and release rate constant *k*, respectively. Each one of the release stages was related to a specific value of *n* and *k*. The second stage was driven by a super case II transport mechanism, as a result of diffusion, macromolecular relaxation, and erosion. A third model, described by Hixson–Crowell, confirmed the erosion mechanism.

Vit-B_{12} diffusion kinetics in aqueous solutions (i.e., without the microparticles) follows a one-step process, being *k* dependent on the pH, confirming that the two-step release mechanism is a characteristic profile of the developed microparticles. The microparticles released only 2.70% of their initial drug load at pH 2, and 58.53% at pH 10.

1. Introduction

One of the most promising areas of particles is the research and development of new pharmaceutical formulations, being that the main application is aimed toward the drug delivery. Among the various materials used in the production of nano and microparticles, there has been a considerable interest in the proteins as a starting substance for the synthesis of more sophisticated release systems that may preserve the molecular structure of more potent and specific drugs [1]. This is because the protein-based materials are biodegradable, nonantigenic, and susceptible to metabolization, and also are able to be chemically modified by surface reaction for further covalent fixation of drugs and/or ligands [2].

Serum albumins are the most studied and applied proteins owing to their wide availability, low cost, good structural stability, binding properties with unusual ligands, biodegradability, and non-antigenicity [3–6]. There is a great deal of interest both from a large segment of the pharmaceutical industry and from researchers in the area of biotechnology in the application of albumin as a drug carrier [7]. A remarkable property of albumin is its ability to reversibly bond to a wide variety of molecules [8], which is an interesting characteristic for drug delivery systems. Both bovine serum albumin (BSA) and human serum albumin (HSA) are used in the production of nano/microparticles [1–3,9–12] in view of their structural similarity that corresponds to 75% of the homologous sequence. BSA is more suitable for *in vivo* tests due to its lower cost.

To synthetize BSA particles, a radical cross-linking/polymerization approach in a hydroalcoholic emulsion was used, owing its advantages for ease and quick preparation [13]. The stability of an emulsion is related both to chemical nature of

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interfacial film and to attraction/repulsion force balance occurring among the particles suspended in the liquid, which are important in the prevention of the coalescence [14].

Surfactants (also called emulsifying agents) adsorb on interface reducing the interfacial tension. Many amphiphilic substances that form films are stabilizers for emulsions. Some exemples are proteins (BSA, casein), glucosides, lipids, steroids, calcium carbonate, glycerol, mercuric iodide [14], and polymers such as poly(vinyl alcohol) (PVA). The advantages of the polymerization in the emulsion include: (i) quick polymerization, (ii) products with a high molecular weight [13], and (iii) well-defined stages and simple preparation. Thus, the polymerization in emulsion is an attractive approach to produce microparticles from hydrophilic natural materials [15].

This work aimed at producing protein microparticles from BSA using a hydroalcoholic emulsion for use in drug delivery systems. Vitamin B_{12} (Vit-B_{12}) was used as the model drug. To obtain such a system, BSA was modified with maleic anhydride (MAy) in water, because only a small number of proteins may sustain dissolution in an organic medium without their molecular recognition properties being lost [16]. The nucleophilic groups in BSA, which could react with MA, are thiols of cysteine, hydroxyls of serine and tyrosine, and amines in the side chains of lysine [17]. However, the thiol groups (17 of them) are involved in disulfide bridges that aid to support the tertiary structure of the protein, except cys-34, because its hydroxyls are less nucleophilic and do not react under mild experimental conditions. In the present work, the reaction was processed at temperature of 37 °C and at pH 5.5. In such a case, only a small number of accessible sterically amine groups in the side chains of lysine react under such conditions [17]. In the light of this assessment, it could be said that MAy bonds to amine groups of lysine in BSA by nucleophilic reaction introducing its functional groups to the protein structure. The bindings between the functionalized BSA and DMAAm are of covalent nature. The advantage of covalent bindings is that they are stable with time. The idea was to use the vinyl bonds in functionalized BSA (BSA^{MAy}) as a radical cross-linking/polymerization approach for reaction with *N*′,*N*′-dimethylacrylamide (DMAAm) in the emulsion. Fig. 1 shows a schema of the chemical reactions for the cross-linking of BSA. Inside particle, DMAAm serves as a network support, owing to its good gelling capacity, and do not affect the original properties of the protein such as biocompatibility and biodegradability. Furthermore, DMAAm is a safe material for use in drug delivery systems and is more indicated for such applications than acrylamide that has a great potential to cause cancer. Furthermore, DMAAm has been widely used in the preparation of biomaterials [18–20].

2. Materials and methods

2.1. Materials

Bovine serum albumin, BSA ($M_w \cong 68{,}000\ g\ mol^{-1}$, ≥98.0%), maleic anhydride, MAy (≥99.0%), ammonium persulfate, APS, (≥98.0%) and vitamin B_{12} (≥98.5%) were purchased from Sigma–Aldrich. *N*′,*N*′-dimethylacrylamide, DMAAm (≥99%) and *poly(vinyl alcohol) - PVA* (87–89% hydrolyzed, $M_w \cong 31.000\ g\ mol^{-1}$) *were obtained from* Aldrich. Benzyl alcohol (99.0%) and absolute ethyl alcohol (≥99.3%) were supplied by Fmaia, whilst *N*,*N*,*N*′,*N*′-tetramethylethylenediamine, TEMED ($d = 0.780\ g\ mL^{-1}$, ≥99.0%) was obtained from Invitrogen. For buffer solutions, *sodium phosphate*, *dibasic*, *anhydrous* (98.0%) were supplied by Nuclear, and potassium phosphate, monobasic, (99.0%) from Labsynth. Buffer solutions of pH 7 and 10 were obtained from Synth. The hydrochloric acid (36.5–38.0%) and sodium chloride (Synth, 99.0%) were supplied by Chemco and Synth, respectively. All chemical were used as received.

2.2. Functionalization of BSA with MAy (BSA^{MAy})

Protein-functionalizing solution was prepared by adding 0.5 g of BSA and 0.05 g of MAy to a phosphate buffer solution (0.1 M) with pH 5.5 while stirring. After homogenization, the solution was stirred for 3 h at 37 °C. The obtained product was precipitated with cold ethanol (2 °C), subsequently separated by centrifugation at 9500 rpm, and washed with deionized–distilled water three times.

2.3. Preparation of protein microparticles using BSA^{MAy} and DMAAm

After lyophilization, 0.1 g of BSA^{MAy} was added to 5 mL of phosphate buffer solutions at pH 7.0 at 20 °C while stirring. The buffer solutions were previously prepared, but using different amounts of PVA (stabilizing agent) such as 0%, 1% and 2% (w/v). Then, 52 μL of DMAAm were incorporated under inert atmosphere of argon. After the mixture turned to a transparent, clear solution, 20 mL of benzyl alcohol were added dropwise while stirring so that a whitish emulsion was immediately formed. After 30 min, 0.02 g of ammonium persulfate, as an initiating agent, and 12.5 μL of TEMED, as a catalytic agent, were added to emulsion that was stirred at 750 rpm with the use of a propeller-shaped stirrer with 60 mm of diameter. The thus obtained product was precipitated with cold ethanol (2 °C), separated by centrifugation at 9500 rpm, and washed with deionized–distilled water three times. Other microparticles were produced in the same way but using stirring times of 15, 30, 60, and 90 min.

2.4. Loading of Vit-B_{12} onto the microparticles

Two efficient approaches may be used to load a given solute onto a polymer device: (i) *post*-loading approach (after the device processing), and (ii) *in situ* loading approach (during the device processing). The *post*-synthesis loading approach is performed by immersing the microparticles into a solution containing the drug of interest. In such a case, the drug diffusion into the microparticle occurs by absorption and/or adsorption. The efficiency of such a strategy is related to affinity of the drug for protein chains. Here, the *in situ* loading approach was used in view that a more significant amount of the drug can be loaded. In the *in situ* loading one, the drug molecules are trapped into the BSA network over the cross-linking/polymerization of the protein chains. There is no binding between Vit-B_{12} and DMAAm. The carbon–carbon double bonds of Vit-B_{12} are connected to either a carbonyl group or chemical groups, which are stabilized by resonances with steric hindrance. The initial weight of Vit-B_{12} corresponded to 10% of the total weight of BSA^{MAy} used in the microsphere-forming emulsion.

2.5. Vit-B_{12} release from the microparticles

For tests of release, 0.1 g of Vit-B_{12}-loaded microparticles was added to 30 mL of buffer solutions at pH 2, pH 6 or pH 10 at 37 °C, and subsequently introduced into a dialysis tube. The suspension-filled dialysis tube was fixed at the bottom of a glass reactor with 220 mL of the corresponding buffer solution at 37 °C. The external solution was stirred at 300 rpm using a magnetic stirrer. Then, aliquots of 5 mL were collected from the external solution at specified periods, and then absorption readings were made at 360 nm in a UV–vis spectrophotometer (Biochron, Libra S12 model). After, the aliquots were brought back into the reactor to prevent variation of volume.