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Kinetic Study of Bovine Serum Albumin (BSA) Released from Alginate-Ca^{2+}/PNIPAAm Hydrogels

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Summary: In this work the releasing of Bovine Serum Albumin (BSA) from thermosensitive hydrogels of alginate-Ca^{2+}/PNIPAAm was investigated. The hydrogels are constituted of PNPAAm network interpenetrated in alginate-Ca^{2+} network, so the hydrogels are IPN-typed. The PNIPAAm network was synthesized in the first step in which the sodium alginate remained soluble. The alginate-Ca^{2+} network was formed in the second step by immersion the membrane obtained on the first step in aqueous calcium chloride. It was changed the amount of NIPAAm in the feed solution of the first step. The fractions of BSA released as a function of time were treated according to the mathematical model recently published by our lab [*J. Coll. Interf. Sci.* **2007**, *310*, 128] that allows predicting the whole profile of solute released from polymer networks. This mathematical model is based in the partition phenomena. The amount of BSA released from alginate-Ca^{2+}/PNIPAAm hydrogels changes inversely to both amount of PNIPAAm and temperature. Thus, the IPN-typed matrixes of alginate-Ca^{2+}/PNIPAAm may be considered as smart hydrogels for release the BSA because the amount and rate of released BSA can be tailored by the amount of PNIPAAm in the hydrogel and by the control of temperature. Finally, the whole profile of released BSA can be adequately fitted by the model based in the partition phenomenon. From that model the kinetic parameter $t_{1/2}$ and rate constant of releasing, k_R, were calculated for the different hydrogels investigated in this work.

Keywords: controlled release of BSA; drug delivery systems; hydrogels; smart hydrogels; thermosensitive hydrogels

Introduction

Aqueous solution of PNIPAAm presents typically a LCST (Lower Critical Solution Temperature) phase diagram.[1] In this way, a transparent solution of PNIPAAm-water becomes opalescent when warmed above 32–33 °C because the PNIPAAm chains collapses forming a PNIPAAm-rich phase.[2,3] In this transition, the hydrophilic structured PNIPAAm (below 32 °C) changes to a hydrophobic one (above 33 °C) where the hydrogen interactions PNIPAAm-water are broken and the isopropyl groups are more exposed.[4] Due to this transition, hydrogels of PNIPAAm presented significant change in volume.[5–8] The hydrophilic-hydrophobic transition of PNIPAAm is well documented in the literature.[9,10] In the 80's years Hoffman et al.[11] proposed the application of hydrogels made of PNIPAAm (homo and or copolymers) on drug delivery field. The value of LCST can be tailored, by copolymerization, to occur close to the body temperature.[12] Initially, the drug homogenously distributed in thermosensitive hydrogel below the LCST leaves after the contraction of the matrix at above the LCST. Consequently, the drug may diffuse from the shrunken hydrogel to the body if the hydrogel matrix is placed within the body (as an implant, etc.). The interest in use of thermosensitive hydrogels to control

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the delivery of chemicals, such as drug in medicine and pharmacology,[13] nutrients in agriculture,[14] etc., is increasing in the last two decades. Hydrogels that can trigger the release, by the change in temperature (or other external properties), are classified as smart hydrogels.[13]

The alginate is a polysaccharide extracted from the brown algae *Phaeophyceae*. It presents linear chains with high molecular weight constituted by β-D-manuronic acid (M) and α-L-glucuronic acid (G) monomers linked by α-(1-4)-glycoside bonding and contains three different block structures: M-block, G-block and MG-block.[15] As the alginate presents excellent biocompatibility, hydrophilicity, porosity and biodegradability, this biopolymer is widely used in biomedical field, mainly as drug carrier.[16–18] The use of biodegradable polymers such as collagen, cellulose, chitosan, chondroitin sulphate, and others, to synthesize hydrogels for drug delivery is very important.[19] Combining the properties of alginate and those of PNIPAAm it is possible to obtain hydrogels with biocompatibility and good mechanical properties[20] that can be used as drug delivery devices. The aim of this contribution is to investigate the effect of temperature and amount of PNIPAAm in releasing the protein Bovine Serum Albumin (BSA) from alginate-Ca^{2+}/PNIPAAm hydrogels. The hydrogels are constituted by PNIPAAm network interpenetrated in alginate-Ca^{2+} network, i.e., IPN-typed hydrogels.

Experimental Part

Materials

The sodium alginate (SA, 18,094-7, $M_v = 3.0 \times 10^5$ g mol^{-1} with M/G ratio 1.56), N-isopropyl acrylamide (NIPAAm, 97%, 41,532-4), N,N,N′,N′-tetramethylethylenediamine (TEMED, 99%, T22500) and sodium persulfate (SP, 98%, 216232) were supplied by Aldrich. N,N′-methylene-bisacrylamide (MBAAm, 17-1304-02) was supplied by Plusone; calcium chloride (C1042.01.AG, was from Synth, Brazil). The Bovine Serum Albumin (BSA, A-9056, $M_w = 66$ kDa) was supplied by Sigma. All chemicals were used as received.

Synthesis of Hydrogels

The synthesis of the hydrogels was published elsewhere.[21] Briefly, aqueous solution of SA 1% (w/v) conc. were prepared. NIPPAm was added to this solution to obtain the conc. 2.5%, 5.0% or 10.0 (w/v) in this monomer. Also, the conc. of MBAAm, used as cross-linker, was fixed in 1% (molar) relative to NIPAAm. After stirring to complete homogenization, the system was deoxygenated by N_2 bubbling for 15 min. After, 1.0 mL of aqueous solution of sodium persulfate ($Na_2S_2O_8$, conc. 20 mg mL^{-1}) was added. The mixture was quickly inserted between two glass plaques separated by a rubber gasket of 3 mm thickness. The system was kept closed by the use of metallic straps for 24 h at ambient temperature (ca. 25 °C). At this stage the complete copolymerization/cross-linking of NIPAAm/MBAAm occurred and the alginate chains remained entrapped in the PNIPPAm network. The top plaque was removed and the system was immersed in an aqueous $CaCl_2$ solution (1% w/v) and the network of alginate-Ca^{2+} was formed. Such immersion was maintained for 24 h. At this stage the PNIPAAm network is interpenetrated in the alginate-Ca^{2+} network. Thus, the membrane of alginate-Ca^{2+}/PNIPAAm IPN hydrogel was transferred to a recipient containing 500 mL of distilled/de-ionized water to remove the excess of Ca^{2+} ions. The water was changed every day for a week. To identify the different formulations, the hydrogels are labeled as (A-C-P), in that A, C and P indicate, respectively, the concentration (in %-w/v) of SA (=1), $CaCl_2$ (=1) and NIPAAm (2.5, 5.0 or 10.0).

Loading of Bovine Serum Albumin (BSA) into Hydrogels and the Release at Different Temperatures

A BSA solution, conc. 0.19% (m/v), in buffer Tris-hydroxymethylamine-methane (Tris-Cl, 0.1 M, pH = 7.4) was prepared.

For loading the BSA, each hydrogel was dried and soaked in the BSA solution, keeping immersed for two days at 22 °C. After, the swollen BSA-loaded hydrogel was removed and the amount of BSA remained in the solution was determined by the UV measurements at $\lambda = 277$ nm based in a previously build analytical curve. The amount of BSA loaded in each hydrogel was determined through the equation

$$\text{Amount of BSA loaded} = [\text{BSA}]_{\text{before loading}} - [\text{BSA}]_{\text{after loading}} \quad (1)$$

where $[\text{BSA}]_{\text{before loading}}$ and $[\text{BSA}]_{\text{after loading}}$ are the BSA conc. in the solution before and after the loading process. The experiments for releasing the BSA from the hydrogel were done *in vitro* at 22.0 and 37.0 °C, using distilled/de-ionized water. As these experiments were done below and above the LCST of PNIPAAm, it was possible to observe the influence of hydrophilic-hydrophobic transition on the BSA releasing process. The value of fraction of BSA released from the hydrogel (F_R) at the desired time t was obtained from the equation

$$F_R = (M_t/M_\infty) \quad (2)$$

where M_t is the cumulative amount of BSA released up to the time t and M_∞ is the amount of BSA loaded into hydrogel. The results were presented as curves of F_R as a function of time (F_R *vs.* t).

Results and Discussion

The hydrogels of alginate-Ca^{2+}/PNIPAAm are thermosensitive because they shrink at ca. 32–35 °C as presented elsewhere[21] where the dependences of swelling degree for these hydrogels on the temperature are described. The hydrogels presented high loading BSA capability. The hydrogels loaded up to 35% of BSA in relation to the initial BSA solution. The profiles of BSA released from the (1-1-P) hydrogel at 22.0 °C are shown in Figure 1. It is possible to see that the BSA is faster released from (1-1-2.5) hydrogel than from the (1-1-5.0) and (1-1-10.0) ones. This was attributed to the lower compaction of the (1-1-2.5) hydrogel matrix that allows higher swelling degree.[21] In this way, the releasing of BSA occurs faster and in a larger extension from hydrogels constituted of lower amount of PNIPAAm. In the first hours, there is a steep increase in the F_R that was attributed to the release of BSA placed at or close to the surface.[22] In spite of this, the equilibrium of releasing was achieved at ca. 20-25 h for the three BSA-loaded hydrogels presented in Figure 1. In addition, the hydrogels did not release the total loaded BSA. This was assigned to the strong interaction BSA-hydrogel matrix.

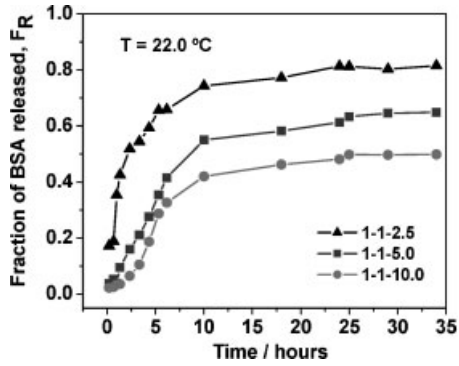

Figure 1.
Fraction of BSA released for three different hydrogels of alginate-Ca^{2+}/PNIPAAm, at 22.0 °C, as a function of time.

The dependences of F_R to the time for (1-1-2.5), (1-1-5.0) and (1-1-10.0) hydrogels are presented in Figures 2a, 2b and 2c, respectively, at 22.0 and 37.0 °C. At the beginning, the profiles of F_R vs. time for the three hydrogels are comparable in the two temperatures. However, as the time is elapsed, F_R gradually increases at 37 °C but lesser than at 22 °C. It can be also observed in Figures 2a, 2b and 2c that the value of F_R for each hydrogel after 22–25 h (after the equilibrium is reached or $F_R = F_{max}$) at 22.0 or 37.0 °C is lower for higher-amounted PNIPAAm hydrogels, as already mentioned. Because the differences on F_{max} between 22 and 37 °C increased with the amount of PNIPAAm, it can be

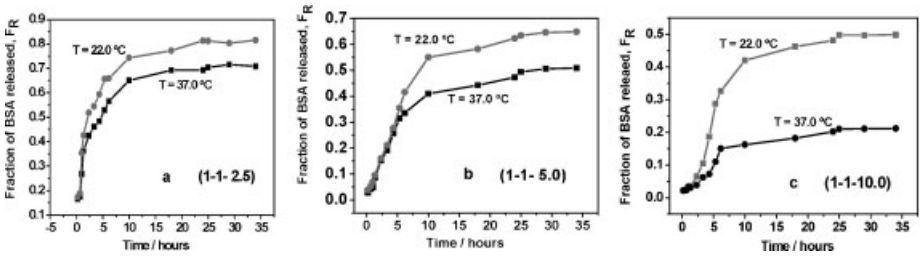

Figure 2.
Fraction of BSA released (F_R) from (1-1-2.5) (a), (1-1-5.0) (b); and (1-1-10.0) (c) hydrogels, at 22.0 °C and 37.0 °C, as a function of time.

inferred that at 37 °C the PNIPAAm chains collapse pulling the alginate network and the whole hydrogel shrinks. As result, the BSA molecules remain entrapped in the shrunken hydrogel. So the releasing at 37 °C does not occur as fast and in the same extent as do at 22.0 °C. So, as more hydrophobic are these hydrogels lower is the amount of BSA released. Other thermosensitive hydrogels prepared in our lab, those of polyacrylamide (PAAM) matrix with PNIPAAm entangled, do not shrink when warmed above the LCST of PNIPAAm because the PAAm mechanically supports the collapsing of PNIPAAm chains.[8] In this case the diffusion of solute (orange II) is enhanced as the temperature is increased.

In this work the release of BSA was treated as a partition phenomenon using a mathematical model recently published in our lab.[23] That model allows predicting the whole profile of solute release from hydrogel networks. The model was applied to the data presented in the Figures 1 and 2. According to that model, the parameter that determines the partition activity, α, may be calculated from the equation

$$\alpha = \frac{F_{\max}}{1 - F_{\max}} \tag{3}$$

In fact, the term α quantifies the ratio of physical-chemistry affinity of solute (in this case, the BSA) for the solvent/hydrogel matrix. The value of α depends of large numbers of factors such as temperature, pressure, pH, ionic strength, hydrogel composition and its hydrophobic aspects, swelling degree, chemical nature of solute and solvent.[23] Higher value of α indicates that the diffusion of solute from the hydrogel into the solution is more favorable.

From the values of $F_{\max}$ extracted from the curves shown in Figures 1 and 2, the respective values of α were obtained and are presented in Table 1. The values of $F_{\max}$ and α were treated by the use of Equation 4 (details for obtaining this equation can be found in reference[23]). The rate constant for BSA releasing (k_R) for each hydrogel and temperature was obtained by plotting the

Table 1.
Values of F_{max}, α, k_R and R^2 for BSA released from different hydrogels constituted of PNIPAAm/alginate-Ca^{2+} (IPN) hydrogel at 22.0 and 37.0 °C.

Hydrogel	Temperature, °C	F_{max}	α	K_R (h^{-1})	R^2
(1-1-2.5)	22.0	0.82	4.41	0.46	0.992
(1-1-2.5)	37.0	0.72	2.52	0.19	0.998
(1-1-5.0)	22.0	0.65	1.84	0.11	0.998
(1-1-5.0)	37.0	0.51	1.03	0.075	0.958
(1-1-10.0)	22.0	0.50	0.99	0.074	0.989
(1-1-10.0)	37.0	0.21	0.27	0.019	0.991

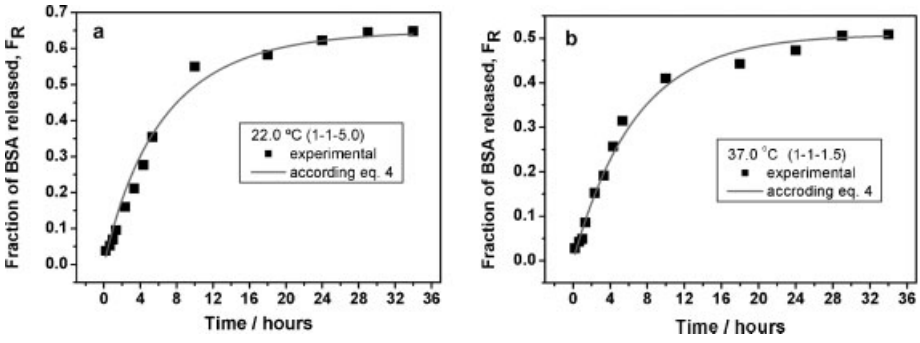

Figure 3.
Experimental values of fraction BSA released (F_R) and curve of F_R, treated according to Equation 4, as a function of time (1-1-5.0) hydrogel: 22.0 °C (a); and 37.0 °C (b).

term in the left of the equation

$$\frac{\alpha}{2} \times \ln\left(\frac{F_L - 2F_L F_{\max} + F_{\max}}{F_{\max} - F_L}\right) = k_R t \quad (4)$$

as a function of time, t. It should be pointed out that straight lines were obtained in all cases and the respective values of R^2 are presented in Table 1 as well as the values of k_R. The values of α and k_R can be correlated to the amount of PNIPAAm in the hydrogels and also to the temperature. From the data of Table 1, it was possible to point out that the values of α diminished as the amount of PNIPAAm and the temperature of hydrogel are increased. According to the Equation 3, values of α higher than unity indicate that the releasing of BSA to the solution is more favorable.[23] In this work, the highest α value was found for (1-1-2.5) hydrogel at 22.0 °C. Furthermore, higher amount of PNIPAAm in the hydrogels leads to a decrease in k_R. Two main factors might contribute to this behavior: the compaction of hydrogels and the entrapping of BSA due to the shrinking of hydrogel, as already discussed.

According to the Equation 4, the value of F_R at time t can be calculated for a system with a defined α (or F_{max}) parameter. Based on this statement and on the respective values of F_{max}, α and k_R for a respective hydrogel and temperature, values of F_R were generated by the use of Equation 4. Curve of F_R as a function of time was plotted for each hydrogel and temperature. Figure 3 shows the experimental data of F_R *vs.* t and the curves obtained by application of Equation 4 for the (1-1-5.0) hydrogel at 22.0 °C (a) and 37.0 °C (b). The data generated from the model (Equation 4) fit very well the experimental data in the whole releasing profile.

Taking into account that when $F_R = 0.5\ F_{max}$, the released BSA half-time, $t_{1/2}$, can be calculated. In this condition the Equation 4 can be expressed as

$$t_{1/2} = \frac{\alpha}{2k_R} \times \ln(3 - 2F_{\max}) \quad (5)$$

Table 2 shows the values of $t_{1/2}$ for the BSA released from hydrogels of alginate-Ca^{2+}/PNIPAAm studied in this work. The

Table 2.
Values of $t_{1/2}$ for BSA released from different hydrogels, at 22.0 and 37.0 °C.

Hydrogel	(1-1-2.5) 22.0 °C	(1-1-2.5) 37.0 °C	(1-1-5.0) 22.0 °C	(1-1-5.0) 37.0 °C	(1-1-10.0) 22.0 °C	(1-1-10.0) 37.0 °C
$t_{1/2}$ (h)	1.50	2.94	4.49	4.70	4.67	6.72

hydrogel (1-1-2.5) releases 50% of the loaded BSA ($F_R = 0.5\ F_{max}$) in 1.5 h at 22.0 °C and at 37.0 °C the $t_{1/2}$ is ca. 2.9 h. For the (1-1-10.0) hydrogel at 22.0 and 37.0 °C the respective values of $t_{1/2}$ are 4.7 h and 6.7 h. It should be highlighted that F_{max} do not represents the releasing of total BSA loaded in the hydrogel. It is possible to confirm that the rate of BSA releasing from hydrogels is inversely to both amount of PNIPAAm and temperature. The amount and rate of BSA released may be tailored by the amount of PNIPAAm in the hydrogel and by the control of temperature. Finally, the whole profile of BSA releasing of these hydrogels is fitted by the mathematical model based in the partition phenomena.[23]

Conclusions

The hydrogels of alginate-Ca^{2+}/PNIPAAm IPN-typed synthesized in this work may be considered as smart hydrogel for releasing of BSA because the amount and rate of BSA released can be tailored by the amount of PNIPAAm in the hydrogel and by the control of temperature. Using the mathematical model based in the partition phenomena,[23] it was possible to fit the whole profile of released BSA and to calculate the values of α, $t_{1/2}$ and k_R for these hydrogels at 22.0 and 37.0 °C as well. The IPN hydrogels made of alginate-Ca^{2+}/PNIPAAm may have applications in biotechnology for controlled release of BSA and other drugs.

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