pH-Sensitive Hydrogels Based on Bovine Serum Albumin for Anticancer Drug Delivery

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ABSTRACT: Protein conjugates consisting of hydroxyethyl methacrylate and acrylic acid monomers in the presence of bovine serum albumin (BSA) were prepared by gamma irradiation to examine the potential use of these hydrogels in the controlled drug release systems. The study parameter was the BSA content in the as-prepared conjugates. Polymers were characterized with FTIR, scanning electron microscopy (SEM), and swelling studies. The polymerization reaction caused the rearrangement of the BSA carbonyl hydrogen bonding and finally led to the modification of the BSA secondary structure as proved by FTIR. SEM proved that the prepared conjugates matrices are porous, with a three-dimensional interconnected microstructure. The swelling kinetics of the hydrogels and the release dynamics of an anticancer model drug (flutamide) have been studied. High equilibrium swelling values, up to 1550%, could be observed and were correlated with the increase in pH, temperature, and BSA content. The mechanism of swelling changed from Fickian to non-Fickian by reducing the acidity of the medium. This study proved that there is a direct relationship between the protein content in the conjugates and both the loaded and the released drug. These pH responsive conjugates may be exploited for the delivery of flutamide. © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 115: 2050–2059, 2010

Key words: polymeric conjugate; radiation-induced crosslinking; albumin; drug delivery; flutamide

INTRODUCTION

Biomaterials play a key role in most approaches for engineering tissues as substitutes for functional replacement, components of devices related to therapy and diagnosis, supportive scaffolds, and drug delivery systems. Modern biomaterials could be composed of various components, for example, metals, ceramics, natural tissues, and polymers.[1] Hydrogels were the first biomaterials developed for human use.[2] The unique physical properties of hydrogels have sparked a particular interest in their use in the drug delivery applications. Their highly porous structure can easily be tuned by controlling the density of crosslinks in the gel matrix and the affinity of the hydrogels for the aqueous environment in which they are swollen. Their porosity also permits loading of drugs into the gel matrix and subsequent drug release at a rate dependent on the diffusion coefficient of the small molecule or the macromolecule through the gel network.[3,4] Biodegradability may be designed into hydrogels via enzymatic, hydrolytic, or environmental (e.g., pH, temperature, or electric field) pathways; however, degradation is not always desirable depending on the time scale and location of the drug delivery device. Hydrogels are also relatively deformable. Despite these many advantageous properties, hydrogels also have several limitations.[4] The low tensile strength of many hydrogels limits their use in load-bearing applications and can result in the premature dissolution or flow away of the hydrogel from a targeted local site.[5] This limitation may not be important in many typical drug delivery applications (e.g., subcutaneous injection). More important, perhaps, are problems relating to the drug delivery properties of hydrogels. The quantity and homogeneity of a drug loading into hydrogels may be limited, particularly in the case of hydrophobic drugs. The high water content and large pore sizes of most hydrogels often result in a relatively rapid drug release.[4]

The design of new biodegradable and biocompatible stimuli-sensitive polymeric systems plays a key role in the development of stimuli-responsive biomaterials and represents an interesting incentive for several researchers.[4,6] Considerable interest in recent years has been shown in the use of natural proteins as carriers for drug delivery[7] because they are biodegradable, biocompatible, relatively nontoxic, and nonimmunogenic. In particular, albumin is an

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attractive macromolecular carrier used to prepare microspheres for the sustained delivery of therapeutic agents and as drug carriers. Albumin is the most abundant protein in plasma and has a high affinity for a wide range of materials including metals such as Cu^{2+} and Zn^{2+}, fatty acids, amino acids, and many drug compounds; it acts as a carrier for such materials in the blood stream.[8,9] Therefore, if albumin is used as a hydrogel constituent, a drug having affinity for albumin is expected to be preferably loaded on the hydrogel and to be released slowly, when compared with that in the case of a hydrogel without albumin. In addition, recent recombinant technology development has enabled the production of a large quantity of human albumin without any risk of pathogen contamination, which guarantees the safety for the use of albumin in a drug delivery system.[8]

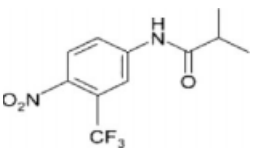
However, the traditional methods of biomaterials synthesis that include crosslinking copolymerization, crosslinking of reactive polymer precursors, and crosslinking via polymer–polymer reaction were limited in the control of their detailed structure because of side reactions, unreacted pendant groups, and entanglements. Other inadequacies of traditional hydrogels are poor mechanical properties and slow or delayed response times to external stimuli.[2] Accordingly, the natural clay (Genipin) was used to crosslink gelatin and bovine serum albumin (BSA) hydrogels with dissolution rates tailorable from 3 min to more than 100 days[4] to avoid the poisonous character of the organic crosslinking agent. Also, radiation techniques, because of the additive-free initiation and easy processability, are suitable alternative tools for the synthesis of the hydrogels used in biomedical applications, which include wound dressings and local drug delivery in cancer therapy.[1,10,11]

Therefore, this study describes the synthesis of protein–polymer conjugates consisting of BSA and 2-hydroxyethyl methacrylate (HEMA) and acrylic acid (AA) by γ-radiation-induced crosslinked polymerization, and thereafter use of these hydrogels as drug delivery devices especially for flutamide as an anticancer model drug. The synthesized hydrogels have been characterized with FTIR and scanning electron microscopy (SEM). The swelling kinetics of the hydrogels has been studied, as a function of pH and temperature of the swelling medium, and correlated with the protein content in the protein–polymer conjugates. The drug release profiles and dynamics, at different pH and at different amount of drug loaded, were also be evaluated to specify and optimize the application conditions of the aforementioned hydrogels as flutamide delivery systems.

EXPERIMENTAL TECHNIQUES

Materials and methods

HEMA and AA were obtained from Merck-Schuchardt, Germany; all monomers were used as received. BSA was obtained from Sigma (St. Louis, MO). Flutamide of MW 276.2 was obtained from Sigma-Aldrich, Germany, and its structure is presented later:



Synthesis of BSA conjugates

Based on preformulation studies, HEMA and AA monomers of 6 : 1 molar ratio were dissolved in BSA solutions of different concentration to achieve different weight ratio of BSA/monomers. The synthesized conjugates were designated as A*n* where *n* denoted to the aforementioned weight ratio and ranged from 1 to 5. For example, A3 means that the weight ratio of BSA to the monomer mixture was 3 : 1. The volume of the solution was adjusted to 10 mL with distilled water. Then, the solution was degassed to remove dissolved oxygen. Further, the solution was poured into glass test tubes (internal diameter: 12 mm), that were then sealed tightly with PARAFILM (Pechiney Plastic Packaging, Menasha, WI) to eliminate any contact between the solution and the ambient air. The reaction mixture was irradiated with gamma rays from ^{60}Co gamma chamber under ambient conditions, at a dose rate of 1.7 kGy s^{-1}, to an absorbed dose of 2 kGy (our preliminary results confirmed that this is the optimal irradiation dose for the crosslinking reaction to occur). The obtained cylindrical hydrogel was cut into disks of 10 mm height with weight termed "W_e" and immersed in distilled water for 3 days to remove unreacted monomers. Finally, the hydrogel was dried to a constant weight "W_d" for 1 week in a desiccator.

For the gel determination, the gel percentage of the hydrogel was determined by the following equation:

$$\text{Gel } (\%) = \frac{W_e}{W_d} \times 100. \quad (1)$$

Characterization

Polymers were characterized by FTIR spectroscopy, SEM, and swelling studies. FTIR spectra of the conjugates were recorded in KBr pellets on Nicolet 5700FTIR THERMO. For the morphological characterization, swollen hydrogels were freeze-dried

using a freeze drier (Christ, Germany, Alpha 1–2) at −52°C for 6 h. Transverse sections were cut from freeze-dried film samples using a cold knife. Samples were then examined with a Jeol JSM-5400 SEM (JEOL, Tokyo, Japan).

Swelling kinetics

Swelling kinetics of the polymeric networks was carried out in triplicate by the gravimetric method. The swelling percentage (%S) was determined at 30°C by immersion of dry hydrogel disks in double distilled water. The water absorbed was calculated by weighing the samples after wiping superficially, at various time intervals. Swollen gels were weighed by an electronic balance (Sartorius, BP 210S, $d = 0.1$ mg).

Percentage swelling (%S), the most important parameter in swelling studies, was calculated from the following equation:

$$\%S = \frac{M_t - M_o}{M_o} \times 100, \quad (2)$$

where M_t is the mass uptake of the swelling medium at time t and M_o is the mass of the dry hydrogel. On the other hand, the water retention (WR) was calculated according to eq. (3) by measuring the weight loss for fully hydrated samples at different time intervals at 30°C.

$$\mathrm{WR} = \frac{M_\infty - M_t}{M_\infty} \times 100, \quad (3)$$

where M_∞ is the mass of hydrogel at equilibrium and M_t has the same meaning as defined earlier.

To study the swelling behavior of the hydrogels at different pHs, three pieces of preweighed hydrogels were placed in buffer solutions of the required pH and allowed to equilibrate. In each case, (%S) was determined for the three pieces of hydrogels. Good agreement was found in the degree of swelling for the pieces, each having the same volume and history, and the average of the three determinations was used for calculations. The total ionic strength of the swelling medium was kept constant by addition of a suitable amount of KCl powder.

Release dynamics of flutamide from BSA conjugates

Preparation of calibration curves

In this procedure, the absorbance, of many standard solutions of flutamide at concentrations encompassing the sample concentrations, was measured on UV–visible spectrophotometer (UNICAM UV–vis Spectrometer. 1000 Model) at λ_{max} 365 nm and, consequently, calibration graph was constructed. The concentration of the drug in the sample solution was read from the graph as the concentration corresponding to the absorbance of the solution. Three calibration graphs were made to determine the amount of the drug released from the drug-loaded polymeric matrix at pH 1.5, 5, and 8 at 37°C.

Drug loading to BSA conjugates

The loading of the drug onto the hydrogels was carried out by the swelling equilibrium method. The hydrogels were allowed to swell in the drug solution of a known concentration for 24 h at pH 8 and 37°C and then dried to obtain the release device. The concentration of the rejected solution was measured to calculate the drug percentage adsorbed in the polymer matrix.

Drug release from BSA conjugates

The amount of flutamide released was measured spectrophotometrically by placing the dried loaded samples in a definite volume of the releasing medium at pH 1.5 for 3 h and then transferred to pH 8 for 18 h at 37°C. After the complete release, the hydrogels were immersed in pH 3.0 buffer solutions and then 0.1M HCl for 2 days to remove any remaining drug that may be loaded in the gel system.

Mechanism of swelling and drug release

In a hydrogel system, the absorption of water from the environment changes the dimensions and the physicochemical properties of the system and thus the drug release kinetics. Although many reports deal with the mathematical modeling of drug release from swellable polymeric systems, no single model successfully predicts all the experimental observations. As most complex models do not yield a convenient formula and require numerical solution techniques, generalized empirical equations were widely used to describe both the water uptake through the swellable glassy polymers and the drug release from these devices. The swelling mechanism could be analyzed in swollen polymeric systems by the following equation:

$$\frac{M_t}{M_\infty} = kt^n, \quad (4)$$

where k (the swelling constant) and n (the swelling exponent) are characteristic parameters of the specific (bioactive agent/dissolution medium) system. The values of n and k were calculated from the slope and intercept of the plot of $\ln(M_t \backslash M_\infty)$ against $\ln(t)$, respectively.

This equation, is applied to the initial stages of swelling, yields straight lines up to almost 60%

increases in the mass of the hydrogel and can be used to describe the diffusional behavior of any polymer-penetrant system whatever the temperature and the penetrant activity. Equation (4) is termed the power-law model and has been used frequently in the literature, because of its utility in describing the relative importance of Fickian ($n = 0.5$) and Case II ($n = 1.0$) transport in anomalous diffusion. This equation is also capable of predicting Super Case II transport, where $n > 1.0$.[3,11] The equilibrium swelling was taken after 24 h. Ritger and Peppas[12,13] showed that the above power-law expression could be used for the evaluation of drug release from swellable systems. In this case, where M_t/M_∞ is the fractional release of the drug at time t (M_t and M_∞ are the drug released at time t and at equilibrium, respectively), k is the constant characteristic of the drug–polymer system, and n is the diffusion exponent characteristic of the release mechanism, which predicts that the fractional release of drug is exponentially related to the release time and adequately describes the release of drug from slabs, spheres, cylinders, and discs from both swellable and nonswellable matrices.

The results shown later were the average of at least three points measured independently. The weight of any swollen sample was normalized to its dry weight. The standard deviation in all measurements was close to zero. The total uncertainly for all experiments ranged from 3 to 5%.

RESULTS AND DISCUSSION

Synthesis of BSA conjugates by radiation-induced crosslinking polymerization

Radiation formation of hydrogels in aqueous solutions proceeds according to the radical mechanism. The probable mechanism of the radiation-induced crosslinking polymerization involves the irradiation of the aqueous polymer solution, which results in the formation of the radicals on the polymer chains (BSA). Radiolysis of water involves the formation of hydroxyl radicals, which also attack the polymer chains thus leading to the macroradicals formation. In the processes of polymerization and crosslinking, recombination of the macroradicals on the different polymeric chains results in the formation of covalent bonds and finally a crosslinked structure.[11] Table I illustrates the relation between the BSA content and the gel fraction in the as-prepared conjugates. Table I shows that the increase of BSA content in the feed reduced the gel fraction and increased the sol fraction, which is inversely correlated with the crosslinking index (j) (the number of crosslinks per single macromolecule).[14] One explanation might be that the formed macroradicals would react with the monomer molecules; therefore, the polymer backbone becomes busy at these positions for crosslinking reactions.[15] This is also confirmed by SEM and the obtained high values of the equilibrium swelling as the protein content increased.

Characterization of the BSA conjugates

FTIR

FTIR spectroscopy has been proven to be a powerful tool for providing conformational and structural dynamics information of proteins. Infrared spectra of proteins exhibit a number of the amide bands, which represent different vibrations of the peptide moiety. Among the amide bands of the protein, amide I band ranging from 1600 to 1700 cm^{-1} (mainly C=O stretch) and amide II band $\approx$1548 cm^{-1} (C—N stretch coupled with N—H bending mode) have been widely accepted as the typical ones to be used. They both have a relationship with the secondary structure of the protein. However, the amide I band is more sensitive to the change of protein secondary structure than amide II.[16] The band assignment for the secondary structure previously reported in the literature[17,18] is as follows: α-helix, 1658–1650 $cm^{-1;}$ β-sheets, 1637–1613 cm^{-1} turns, 1673–1666 cm^{-1}; random coil, 1645–1637 cm^{-1}; and β antiparallel, 1695–1675 cm^{-1}. Figure 1(a–c) shows the FTIR spectra of the free BSA, HEMA-AA copolymer, and the albumin conjugate "A5," respectively. As shown in Figure 1(a), the bands assigned at 1624 and 1640 cm^{-1} (amide I band C=O stretch), referring to turns and random coils configurations, were predominated in the albumin secondary structure, respectively. Also, by observing the FTIR spectra of HEMA-AA copolymer, Figure 1(b), it may be noticed that the broad absorption band at 3437 cm^{-1} was due to —OH stretching, indicating the strong association in this copolymer.[19] The infrared absorption bands at 1721 and at 1631 cm^{-1} (due to C=O stretching of the ester and the acid, respectively) while that at 1298 and 1182 cm^{-1} (due to the C—O stretching of esters and the acid, respectively) were observed. Focusing on the FTIR spectra of BSA conjugate "A5," Figure 1(c), the peak position of amide I band (1624 and1640 cm^{-1}) was shifted to (1456 and 1395 cm^{-1}) after the crosslinking reaction (γ-radiation), which may indicate that the monomers interacted with the C=O and C—N groups in the protein. This interaction caused the rearrangement of the polypeptide carbonyl groups hydrogen bonding and finally led to the modification of the BSA secondary structure.

Scanning electron microscope

The SEM images were obtained to characterize the microstructure of the freeze-dried albumin

TABLE I
The Gelation Percentage of BSA Conjugates

Conjugate	A1	A2	A3	A4	A5
BSA/monomers[a] weight ratio	1 : 1	2 : 1	3 : 1	4 : 1	5 : 1
Gelation (%)	97.5	96.2	95.97	93.41	89.6

[a] Monomers: HEMA/AA of molar ratio 6 : 1.

conjugates "A1" and "A5" and are presented in Figure 2(a,b), respectively. It is clear that the prepared conjugates matrices were porous, with a three-dimensional interconnected microstructure by virtue of the freeze-drying step with the pores being the result of ice crystal formation, resembling other natural macromolecular hydrogel system structure. The interconnection between pores could be assigned to the crosslinking network formation in gels. Also, it may be noticed that, by comparing between Figure 2(a,b), the pore dimensions were reduced in accordance with the decrease of BSA content bringing about an increase of network densities.

Evaluation of BSA conjugates as flutamide controlled release devices

Swelling kinetics

When a dry hydrogel is immersed in a thermodynamically compatible solvent, the solvent movement into the hydrogel polymer chains leads to a considerable volume expansion and a macromolecular rearrangement depending on the extent of crosslinking within the network.[20] The rate at which a polymer expands or swells depends on two coupled processes, the relative rates of polymer-chain relaxation and solvent penetration into the network. Swelling equilibrium is reached when these two forces are equal.[21] Not only the swelling-controlled release systems (i.e., pH-sensitive hydrogels) but also the degradation-controlled systems are shown to be related to the swelling behavior of hydrogels,[22] which is further dependent on the composition of the hydrogels and pH of the swelling medium. Molecular weight of the drug also affects its release profile from the hydrogels.[11] Therefore, the study of swelling behavior of hydrogels is of considerable importance for the development of carriers for site-specific delivery of drugs.[22]

Generally, during the initial stages of the polymer swelling, polymer chains relaxation begins that has started the opening of the networks and is responsible for the slow rate of diffusion of water in the polymer networks. Formation of a thin layer of a swollen gel occurs on the polymer surface in which the polymer chains are slowly hydrating and relaxing. On the other hand, in the later stages of swelling, all the polymer chins are completely relaxed and equilibrium swelling is about to be established. Therefore, in the initial and later stages, the rates of diffusion of water molecules in the polymer networks are slow.[11]

The swelling of the synthesized conjugates versus time at 25°C and at pH 3, 5, and 8 is presented in Figure 3. A5 conjugate had the highest swelling percentage at the pH range of the study (its equilibrium swelling was 1555% at pH 8). Accordingly, the content of BSA played an important role in improving

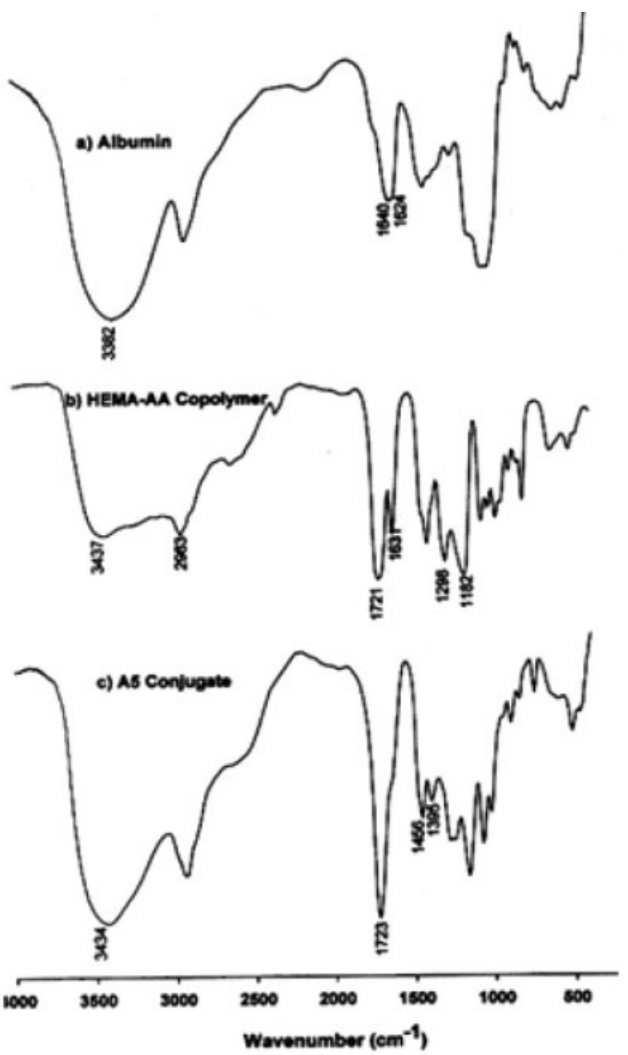


Figure 1 FTIR of (a) albumin, (b) HEMA-AA copolymer, and (c) A5 conjugate.

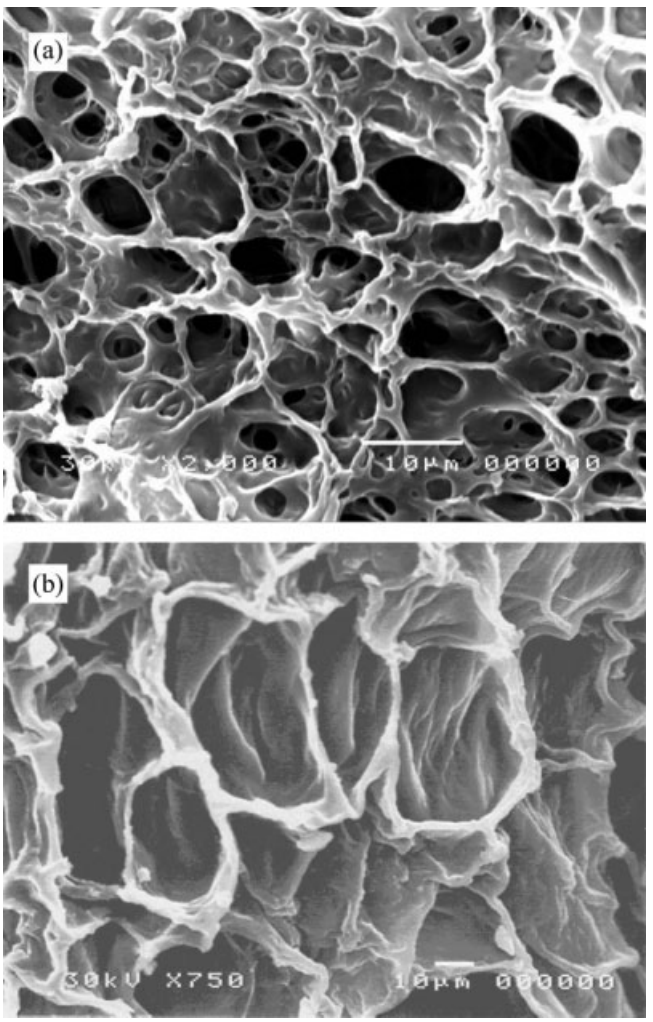


Figure 2 SEM micrographs of freeze-dried BSA conjugates after equilibrium swelling (a: A1 and b: A5).

the swelling of these hydrogels. Fifty percent of the total swelling occurred at 438, 393, 243, 187, and 142 min for A1, A2, A3, A4, and A5 at pH 8, respectively, indicating that the rate of swelling increased by increasing the amount of BSA. It is worth mentioning that the total amount of water taken up by any synthesized conjugates A1–A5 was at least 8 g g^{-1} gel at pH 8, which is higher than that obtained (2.26–3.74 g g^{-1} gel) for molecularly imprinted poly(HEMA-*co*-AA) crosslinked chemically with methylene bisacrylamide.[19]

The values of diffusion exponent and gel characteristics constant k were evaluated and the results are presented in Table II. The hydrogels exhibited a fair pH-dependent swelling behavior with transition from Fickian ($n = 0.41$) to non-Fickian ($n = 0.76$) as the pH of the swelling medium varied from 3.0 to 8.0. Thus, the swelling mechanism was probably a non-Fickian with n values approaching Fickian (diffusion controlled). In conclusion, the mechanism of transport mainly depended on diffusion of water rather than polymer relaxation. Generally, two factors, namely osmotic swelling pressure and chain relaxation process, are responsible for the gel to exhibit non-Fickian type of swelling behavior.[22] This dramatic change of swelling mechanism was observed by other authors[19,22,23] for different polymeric systems.

BSA conjugates swelling as a function of the temperature. Table III represents the effect of temperature of the swelling medium on the equilibrium swelling of the synthesized BSA conjugates. The elevated equilibrium swelling values can be observed and correlated with the increase in both temperature and protein content. This may be due to an increase in both the kinetic energy of solvent molecules and the rate of diffusion of water molecules as a result of the increase in temperature of the swelling medium.[11] On the other hand, this is in contrary to thermosensitive polymers that contain a relatively hydrophobic monomer, so most of the swelling ratios with different compositions decreased with the increase of temperature due to the enhanced hydrophobic interactions between hydrophobic groups.[24]

Swelling as a function of pH of the swelling medium. Figure 4 illuminates the effect of conjugates ionization (pH of the medium) on their swelling. In general, the swelling values increased gradually, as the pH increased, to attain an equilibrium value ranged from 800 to 1400% based on the protein content in the synthesized conjugates.

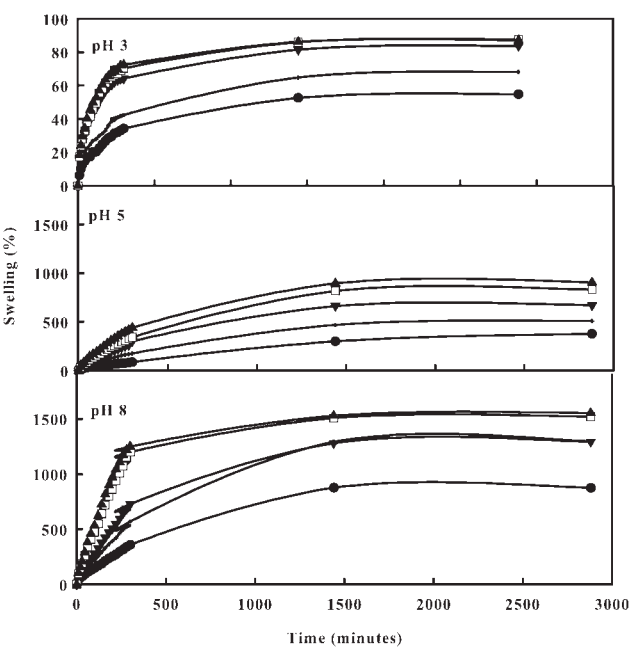


Figure 3 The swelling of BSA conjugates against time at 25°C and pH 3, 5, and 8. The figure parameter is the BSA content (●: A1, •: A2, ▼: A3, □: A4, and ▲: A5).

TABLE II
Diffusion Exponent and Gel Characteristics Constant *k* for the Synthesized BSA Conjugates at pH 3, 5, and 8 at 25°C

	pH 3			pH 5			pH 8		
Conjugate	n	k	R^2	n	$k \times 10^{-3}$	R^2	n	$k \times 10^{-3}$	R^2
A1	0.4684	0.0429	0.9887	0.5556	9.269	0.9966	0.6568	8.897	0.9922
A2	0.4195	0.0607	0.9951	0.6069	11	0.9990	0.7178	6.758	0.9927
A3	0.4176	0.0774	0.9956	0.6636	9.279	0.9988	0.7572	7.147	0.9950
A4	0.4178	0.0786	0.9968	0.7003	7.55	0.9985	0.7698	10.4	0.9982
A5	0.4134	0.0839	0.9958	0.7005	8.906	0.9985	0.7604	10	0.9972

BSA Conjugates Sweling as a function of the temperature.

This may be due to the difference in the concentration of mobile ions in the hydrogel interior relative to the external solution (osmotic pressure), with the changes in the solvent pH, which drives the volume change.[25] As the pH of the swelling media approached neutral, the carboxylic groups of AA underwent ionization, thus resulting in an increased osmotic swelling pressure [$\pi_{ion} = RT\ (C_i^g - C_i^s)$, where C_i^g and C_i^s are molar concentrations of mobile ions in the gel and the solution phase, respectively] in accordance with the Donnan equilibrium, which ultimately imposed the extensive gel swelling. In addition to this, the electrostatic force of the repulsion among the similarly charged $-COO^-$ groups along the macromolecular chains also enforced the polymeric segments to become unfolded or relaxed, followed by an enhanced degree of swelling.[22]

Water retention. The relation between the WR and the time for the BSA conjugates at 25°C is shown in Figure 5. This figure illustrates that the WR was composition dependent. As the protein content of the synthesized conjugates increased, the relative hydrophilicity and the affinity of the conjugates toward water were improved through dominant hydrogen bonding interactions, and consequently, the deswelling rate decreased and the WR increased. It is worth mentioning that the decrease of WRs would be especially beneficial to improve the efficiencies of applications in drug delivery, concentration, and separation processes.[24]

TABLE III
Effect of Temperature on the Equilibrium Swelling (%) for BSA Conjugates at pH 8

	Temperature (°C)		
	25	32	45
Conjugate	Equilibrium swelling (%)		
A1	777.50	877.04	1009.20
A2	418.70	1294.03	1416.80
A3	748.14	1281.12	1324.00
A4	868.90	1529.26	1654.70
A5	630.00	1509.28	1664.50

Loading of flutamide on the synthesized conjugates

The efficacy of drugs is known to be much influenced by the binding ability of drugs to albumin in circulation.[9] Two schemes are used to load therapeutics into hydrogels: either to produce the gel in the presence of a drug or synthesize the gel and then load a drug into the gel via equilibrium partitioning. The value of hydrogels in drug delivery systems comes from their ability to control the diffusion behavior of molecules in or through them, and the ability to amplify the microscopic events occurring at the mesh chain level into macroscopic phenomena.[21]

Figure 6 clarifies the role of the BSA content on the adsorbed amount of "flutamide" at three drug concentration, namely, 0.27, 0.5, and 0.8 mg mL^{-1} and at pH 8 and at 37°C. Two remarks may be stressed here; the first one is the amount of the-loaded drug had a direct proportionality with the drug concentration for the same conjugate and the second one is that the increase in BSA content in the conjugates caused an increase in loaded flutamide at constant drug concentration, indicating the high affinity of the drug to BSA.[9] This affinity may

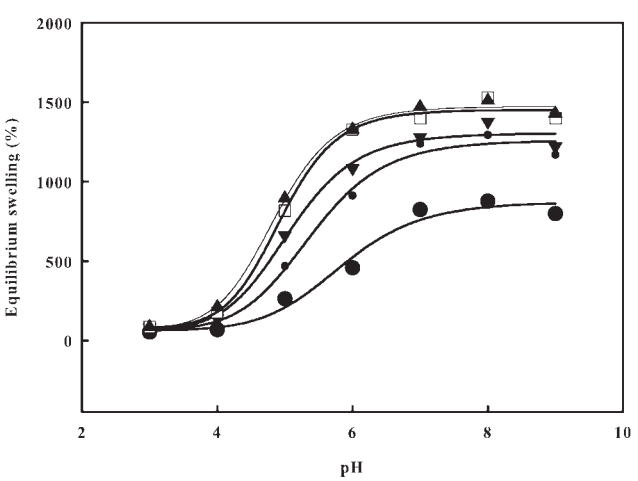


Figure 4 Effect of pH on the equilibrium swelling of the BSA conjugates at 25°C (●: A1, •: A2, ▼: A3, □: A4, and ▲: A5).

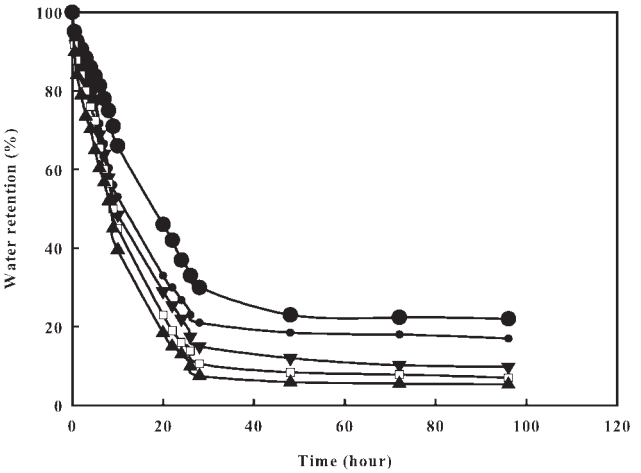


Figure 5 Water retention of BSA conjugates against time at 25°C (●: A1, •: A2, ▼: A3, □: A4, and ▲: A5).

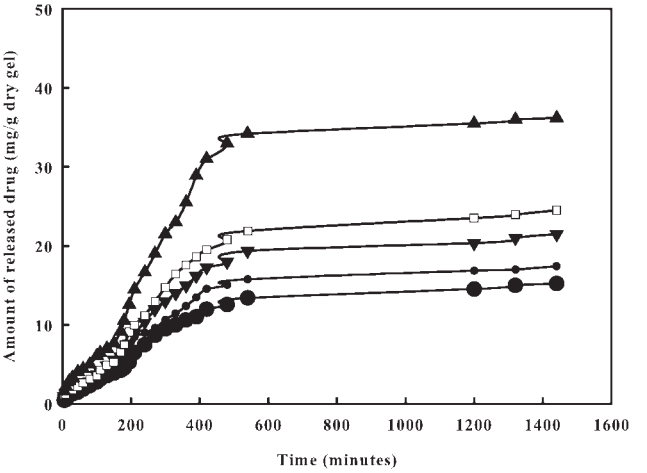


Figure 7 Amount of released drug by BSA conjugates at pH 1.5 for 180 min and then at pH 8 for 18 h at 37°C (●: A1, •: A2, ▼: A3, □: A4, and ▲: A5).

rationalize the considerable interest of using natural proteins, particularly BSA, as a carrier for drug delivery in recent years.[7]

Release of flutamide (kinetics and mechanism)

The entrapped drug in hydrogels releases only when the water penetrates into the polymer networks and dissolves the drug. A drug solubilization is followed by diffusion through a network of fluid-filled pores and channels. In such cases, the control over the internal pore size, distribution, connectivity, and tortuosity would be useful for fine tuning formulation conditions and drug release kinetics.[26]

The release kinetics often deviates from the expected one and is strongly influenced by a variety of parameters. These parameters include device geometry, the physicochemical properties of the polymer matrix (molecular weight, swelling, crystallinity, and glass transition temperature), initial drug concentration profile, drug–matrix interactions, and osmotic pressure associated with drug solubility and its loading content.[27]

Figure 7 shows the release profile of flutamide from different loaded BSA conjugates at two physiological conditions, namely, pH 1.5 and 8 for 3 and 18 h, respectively, and at 37°C. The drug concentration was 0.8 mg mL^{-1}. Many remarks can be highlighted by inspection of Figure 7. A linear increase of the released drug with the time for 9 h, followed by a plateau may be observed. A decrease in the drug release is expected over time as the drug is depleted in the matrix and the diffusion path length increases.[26] Also, a remarkable increase in flutamide release, related to the increase in BSA content in the

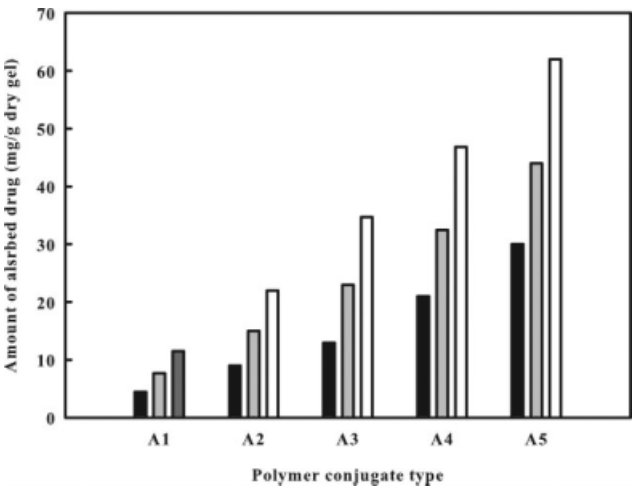


Figure 6 Amount of adsorbed drug on BSA conjugates at different drug concentration 0.27, 0.5, and 0.8 mg mL^{-1} at 37°C and pH 8 (■: 0.27 mg mL^{-1}, ▒: 0.5 mg mL^{-1}, and □: 0.8 mg mL^{-1}).

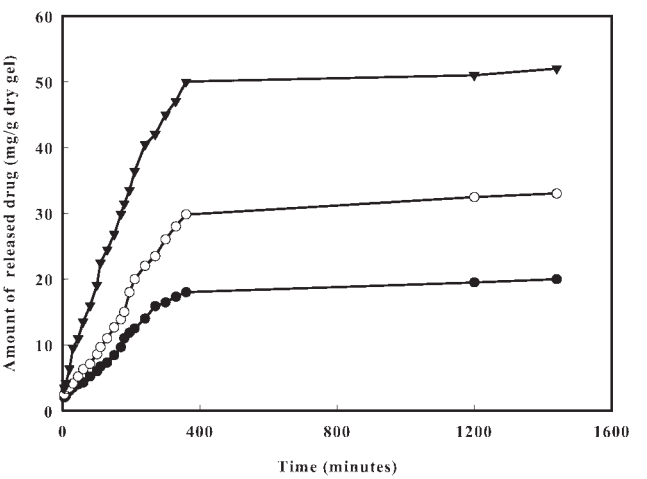


Figure 8 Amount of released drug against time by BSA conjugate "A5" at pH 1.5, 5, and 8 at 37°C for 0.8 mg mL^{-1} drug concentration (•: pH 1.5, ○: pH 5, and ▼: pH 8).

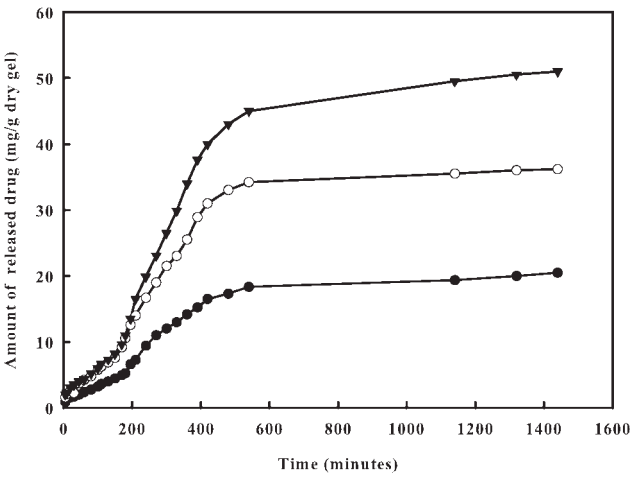


Figure 9 Amount of released drug by BSA conjugate "A5" versus time at pH 1.5 for 180 min and then at pH 8 for 0.27, 0.5, and 0.8 mg mL^{-1} drug concentration at 37°C (•: 0.27 mg mL^{-1}, ○: 0.5 mg mL^{-1}, and ▼: 0.8 mg mL^{-1}).

BSA conjugates, can be noticed. These remarks coincide with both flutamide loading studies and conjugates swelling data in this study. The highest drug release was observed for the highly swollen hydrogel "A5 conjugate." This is probably because of its highest swelling capacity, in which loading occurs mainly through simple diffusion of the drug into a hydrogel along its swelling[28] and/or by increasing binding ability of the drug through high mount of BSA content in the conjugates.[9]

The release of hydrophilic drugs from depot-type devices, in which drug particles are dispersed in a polymeric matrix, is frequently controlled by Fickian diffusion mechanisms, where the amount of the drug release shows a square root time dependency. In a Fickian model of release kinetics, the relaxation rate is high and the diffusion processes are rate-limiting, resulting in the release rate of the drug being proportional to the concentration gradient between the drug source and the surroundings.[21]

To optimize the conditions of flutamide release, the effect of the pH on the release pattern of flutamide was studied by varying the pH of the release medium (1.5 and 8) at the same drug concentration (0.8 mg mL^{-1}) and at 37°C. The release profile of flutamide of the loaded hydrogels, especially A5 conjugate, has been shown in Figure 8. It has been observed from this figure that the amount of flutamide released was related directly to the pH of the releasing medium. The released flutamide was 52 mg g^{-1} of dry hydrogel at pH 8, whereas that obtained at pH 1.5 was 20 mg g^{-1}.

Also, the release of flutamide was evaluated for A5 conjugate as a function of time for different loaded amounts of drug (Fig. 9), at two pH intervals (pH 1.5 at time up to 180 min and pH 8 at time above 180 min) and at 37°C. The maximum drug released values were 51, 36, and 20 mg g^{-1} dry hydrogel for 0.8, 0.5, and 0.27 mg mL^{-1}, respectively.

CONCLUSIONS

In hybridizing a polymeric gel with a protein motif, one can facilitate the combination of the best of multiple worlds—the polymeric gel world and the protein world. In this study, HEMA and AA were polymerized in the presence of different concentrations of BSA by γ-radiation. This reaction caused the rearrangement of the polypeptide carbonyl hydrogen bonding and finally led to the modification of BSA secondary structure. SEM proved that the prepared conjugates matrices are porous, with a three-dimensional interconnected microstructure. High equilibrium swelling values could be observed and were correlated with the increase in pH, BSA content, and temperature. In conclusion, the composition of the prepared conjugates and the nature of the swelling medium affected their swelling capability. The mechanism of swelling changed from Fickian to non-Fickian by reducing the acidity of the medium. Flutamide was loaded on the as-synthesized conjugates as an anticancer model drug. The amount of BSA in the conjugate was an essential parameter in determining the loaded amount of flutamide. Increasing the BSA content enhanced flutamide binding ability and consequently its release. Accordingly, these conjugates, especially those with high BSA content, may be good candidates as carriers for flutamide delivery.

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