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# Release kinetics of bovine serum albumin from pH-sensitive poly(vinyl ether) based hydrogels

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**Abstract**

pH-Sensitive amphiphilic hydrogels were synthesized by radiation copolymerization of ethylene glycol vinyl ether (EGVE), butyl vinyl ether (BVE) and acrylic acid (AA) in the presence of crosslinking agent, diethylene glycol divinyl ether (DEGDVE). The results of the swelling experiments indicated that the hydrogel which has 60:40:5 comonomer ratio (mol% of EGVE:BVE:AA in monomeric mixture) is pH-sensitive. While the hydrogel is in a fully hydrated form at pH > 6, it extensively dehydrates below pH 6. A two-stage volume phase transition was observed in the range of pH 6.0–7.0 and 7.5–8.0. In addition, the equilibrium water contents of the gels decreased with increasing temperature from 4 to 37 °C by following the non-Fickian diffusion mechanism. The utility of pH-sensitive gels in delivery of protein based drugs was investigated. Bovine serum albumin (BSA) loaded gels were prepared and protein release was examined by fluorescence spectroscopy in the range of pH 2–9. While the protein release was very low at pH 2, readily high amount of BSA were released at pH > 6. It was concluded that the pH-sensitive EGVE–BVE–AA terpolymers may be suitable for the oral administration of protein based drugs as a carrier through gastrointestinal (GI) tract.
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*Keywords:* pH-sensitive hydrogels; Vinyl-ether hydrogels; Protein release; Site-specific delivery

## 1. Introduction

Protein-based drugs are leading as an important class of treatment agents. The common strategy for the delivery of these drugs is frequent injections (Torres-Lugo and Peppas, 1999). However, the high molecular weights of most proteins and their limited stability have created a demand for novel methods of delivering these drugs and necessity for new drug release strategies has arised (Mitragotri et al., 1995; Yeh et al., 1995). In one of these strategies, the object is to use the drugs orally and for this purpose hydrogel based release systems preloaded with proteins have been prepared (Reddy et al., 1999; Yang et al., 2002).

Although the oral administration of protein drugs is the most attractive route clinically, it is very difficult to achieve because of high susceptibility of proteins to

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hydrolysis and digestion by the acid and enzymes in the gastrointestinal (GI) tract. Other difficulties of this system may include low bioavailability due to poor membrane permeability and low drug loading efficiency due to the solvent sorption technique (Lee, 1990; Kim et al., 1992).

To overcome these drawbacks stimuli-sensitive polymers, especially temperature-sensitive and pH-sensitive hydrogels have been investigated as candidates for the oral protein delivery. The ability of these hydrogels to respond to their environment may enhance drug loading and provide protection from GI tract conditions (Qiu and Park, 2001; Peppas, 1991; Dong et al., 1992).

In the presented study, we investigated the use of pH-sensitive poly(vinyl ether) based amphiphilic hydrogels for the oral delivery of protein drugs. In our previous study, poly(vinyl ether) based hydrogels were synthesized by radiation copolymerization of ethylene glycol vinyl ether (EGVE), butyl vinyl ether (BVE) and acrylic acid (AA) in the presence of crosslinking agent, diethylene glycol divinyl ether (DEGDVE) (Gümüşderelioğlu and Topal, in press) by following the similar procedure of Mun et al. (1999). While amphiphilic structure of EGVE–BVE is leading to the thermosensitive copolymer, poly(acrylic acid) (PAA) backbone with carboxylic pendent groups (–COOH), supplies pH-sensitivity to the terpolymer. The release characteristics of the hydrogelic system were studied by using bovine serum albumin (BSA) as a model protein drug.

## 2. Materials and methods

### 2.1. Preparation of hydrogels

The monomers used were ethylene glycol vinyl ether, butyl vinyl ether and acrylic acid. All of them and crosslinking agent, diethylene glycol divinyl ether were obtained from Aldrich (Germany) and they were used without purification.

Poly(EGVE–BVE–AA) terpolymers were synthesized by radiation polymerization. The comonomers EGVE and BVE were mixed in a mole ratio of 60:40. AA and DEGDVE were incorporated as 5% and 4% moles of the EGVE–BVE mixture, respectively. The monomeric mixture was placed into the Pyrex test tube, degassed and sealed in vacuum. Then the radiation copolymerization was carried out on a $^{60}$Co γ-source at irradiation dose of 2.48 kGy h$^{-1}$ during 40 h. After the polymerization the gels were removed, cut into disks of approximately 1.0 cm in diameter and 1.5 mm thickness. Then the gels were washed in distilled water for approximately 2 weeks to remove the any unreacted monomer. The disks were dried under vacuum at 25 °C until they obtained a constant weight.

### 2.2. Swelling studies

The dried hydrogels were weighted and placed into 50 mL of phosphate buffer saline (PBS) at 4 and 37 °C. The pH range studied varied from 2.0 to 9.0. At certain time intervals polymer samples were taken out of the buffer solution and the excess of buffer was removed by blotting with filter paper. The weight of the wet polymer was then measured. The water content was calculated by the ratio of the (wet weight–dry weight) to the weight of the dried polymer. The experiments were repeated three times and the results were reported as average values.

### 2.3. BSA assay

The model protein used in these experiments was bovine serum albumin (molecular weight = 65,000 Da, Fraction V, Sigma). BSA assay was performed using fluorescence spectrophotometer (Shimadzu RF 1501, Japan). All measurements were made with the freshly prepared solutions of BSA in PBS, using 1 cm$^2$ quartz cells. The intrinsic tryptophan fluorescence observed from protein solutions at 280 nm excitation and 332 nm emission wavelengths. The calibration results showed that, concentrations up to 3 μg/mL are linear curve fit and minimum detectable concentration is 0.1 μg/mL.

### 2.4. Gel loading

Solutions of approximately 0.1 and 1.0 mg/mL of BSA were prepared using phosphate buffer at different pHs i.e. pH 3, 5, 7 and 8. Previously dried hydrogel samples were placed in 20 mL of BSA solution. The sample vials were placed in a temperature-regulated incubator at 4 °C. After approximately 24 h, the samples were taken out of the solution, and the concentration of the supernatant was analyzed using fluorescence spec-

troscopy to determine the amount of loaded BSA to each hydrogel disk. Then the loaded gels were dried under vacuum at 25 °C until they obtained a constant weight.

### *2.5. Release studies*

Dried BSA loaded disks were taken and placed in a 25 mL sealed Erlenmayer containing buffer solutions at different pHs i.e. pH 2, 6, 7, 8 and 9. All release studies were conducted in a shaker agitating at 50 rpm at 37 ± 0.5 °C. At certain time intervals the releasing medium was withdrawn from the Erlenmayer and the medium was replaced with fresh buffer solution. The medium was then analyzed using the fluorimetry. Then cumulative BSA release was calculated.

## 3. Results and discussion

### *3.1. Preparation and properties of the hydrogels*

In this work, we examine the feasibility of use of a new pH-sensitive hydrogel as a possible carrier for the oral administration of the model protein, bovine serum albumin. This new type of pH-sensitive hydrogel is composed of ethylene glycol vinyl ether, butyl vinyl ether, and acrylic acid. The copolymer of EGVE and BVE containing both hydrophilic and hydrophobic groups should exhibit thermosensitivity owing to the strengthening of the hydrophobic interactions with increasing temperature. An appropriate balance of hydrophilicity and hydrophobicity in the molecular structure of the polymer chain is the key component in demonstrating the volume phase transition. In our study the balance was supplied in the 60:40 mole ratio of EGVE to BVE in the feed solution. pH-Sensitivity was obtained by the incorporation of small amount of anionic comonomer, AA, in the EGVE–BVE monomeric mixture. PAA is a well-known bioadhesive polymer, which sticks to the mucosal surfaces (Chen and Hoffman, 1995). Therefore, in order to prolong the residence time of a drug delivery vehicle in the target area, PAA is often incorporated into a delivery formulation (Bures et al., 2001). On the other hand, Bai et al. (1995) found that PAA was able to protect the some protein drugs from lumenal degradation by inhibiting the hydrolytic activity of gastrointestinal enzymes and this inhibition was dependent on the polymer concentration. Thus, AA-containing drug delivery systems have the ability to improve an in situ colonic absorption of various protein drugs.

Table 1
pH-dependent equilibrium water contents (EWC) of poly(EGVE–BVE–AA) hydrogel at 37 °C (equilibrium period is 24 h)

| pH of buffer | EWC (%) |
|---|---|
| 2.1 | 5.3 ± 0.5 |
| 3.0 | 6.8 ± 0.3 |
| 5.1 | 8.1 ± 0.7 |
| 6.1 | 16.5 ± 1.2 |
| 7.0 | 80.1 ± 2.0 |
| 7.5 | 82.1 ± 2.1 |
| 8.0 | 229.5 ± 4.5 |
| 9.0 | 249.7 ± 3.8 |

The hydrogel consisting of comonomers in 60:40:5 mole ratio (EGVE:BVE:AA) in feed was used in this study. The total radiation dose for gel formation is 100.0 kGy and the mole ratio of crosslinker is 4. The details of synthesis and characterization of the hydrogels were given in our previous publication (Gümüşderelioğlu and Topal, in press). The temperature decrease from 37 to 4 °C at pH 7.2 enhances the equilibrium water content (EWC) approximately 31% (EWC values at 4 and 37 °C are 114.5% and 83.7%, respectively). The water content change is not sufficient to exhibit a sharp volume phase transition behavior between 4 and 37 °C. However, the protein loading procedure can be carried out at 4 °C in order to increase the amount of loaded protein.

Table 1 shows the pH-dependent water contents of the EGVE–BVE–AA hydrogel. There is no remarkable change of EWC with pH below 6, however, above pH 6 the hydrogel exhibits increased equilibrium swelling. Since AA is a weak acid, its ionization in gels occurring at a pH, i.e. pH > 6, higher than gel p$K_a$ led to an increase in the hydrophilicity and charge repulsion and as a consequence, in water uptake of hydrogels. We observed two steps volume phase transition firstly between pH values of 6.0 and 7.0; secondly between pH 7.5 and 8.0 in response to pH change. This is why, at low pH, this hydrogel remains collapsed, and the protein drug will be protected from the acidic environment of the stomach due to its limited release. However, as the device passes from the stomach to the upper small intestine, the drastic pH change will cause the network

to swell, and the protein will be released. It was also found that the pH-sensitive hydrogel shows a reversible swelling pattern with a faster response in deswelling than swelling (Gümüşderelioğlu and Topal, in press). In addition, kinetic swelling data showed that at the end of 10 h swelling period 80% of EWC was achieved for the gels in 1.5 mm thickness.

### 3.2. BSA loading to the hydrogels

The most common drug loading methods are known as solvent sorption (or embedding) technique and loading during polymerization/crosslinking method (Ramkissoon-Ganorkar et al., 1999). In this study, BSA loading into crosslinked hydrogel was performed by a solvent (water) sorption. By taking into account the temperature-dependent water sorption property of the hydrogel, BSA was loaded at low temperature, i.e. at 4 °C, which allows high swelling and thus high loading capacity. Since swelling is also pH-dependent (Table 1), loading procedure was realized at four different pHs, i.e. pH 3, 5, 7, and 8, in order to adjust the amount of loaded protein. The amount of loaded protein to each hydrogel disk was calculated from the difference between original and final reservoir concentrations and it was represented as "milligram protein per gram of dry hydrogel". Experiments in which the loading time was varied showed that 24 h was sufficient for complete loading. The studies showed that, highest amount of BSA was loaded at pH 8 (5 mg BSA/g dry gel) when the initial BSA concentration of reservoir was 1.0 mg/mL. The amount of loaded BSA at pH 3, 5 and 7 was 3.1, 4.3 and 4.6 mg/g dry gel, respectively. Although the water sorption values of hydrogels are very close to each other at pH 3 and 5 (Table 1), the difference between the loading capacities at pH 3 and 5 can be explained by the interactions between the hydrogel and the protein. The isoelectric point (p*I*) of BSA is 4.7, which implies that above pH 4.7 the net charge of the molecule is negative. Therefore, electrostatic interactions occur between the polymer and the protein.

Since fluorescence emission is much more sensitive to changes in the environment of the chromophore than its light absorption, fluorescence spectroscopy is an excellent method to investigate conformational changes of proteins (Schmid, 1989). In this study protein fluorescence was followed from its tryptophan residue. In proteins that contain tryptophan, both shifts in wavelength and changes in intensity are generally observed upon unfolding. The fluorescence excitation and emission spectra of BSA before and after the loading procedure showed that there was no significant change in the conformation of the loaded BSA. These results further indicate that the activity of BSA was retained after the loading procedure despite prolonged contact of the BSA with polymer and aqueous media which has different pHs i.e. pH 3, 5, 7 and 8.

### 3.3. Release behaviour of BSA from the hydrogels

#### 3.3.1. Effect of solute loading concentration on the release kinetics

The release behaviour of BSA was studied in vitro. Fig. 1 shows the effect of solute loading concentration (at pH 5) on the release kinetics of BSA. The rate of BSA released at pH 7 medium dramatically increased as the concentration of loading solution increased from 0.1 to 1.0 mg/mL. This result was attributed to the increasing driving force, i.e. concentration difference, for drug diffusion. Since the higher concentrations of BSA than 1.0 mg/mL did not affect the amount of loaded BSA, all release studies were done with the hydrogels loaded from 1.0 mg/mL BSA solution.

#### 3.3.2. Effect of releasing medium pH on BSA release

While orally administered tablet passes through the GI tract, it faces with media having pH values between 1 and 9. That is why it was decided that in vitro release studies should have done in media having different pH-values, i.e. pH 2, 6, 7, 8 and 9.

Results of release studies were summarized in Table 2. As due to the lowest swelling ability of the hydrogel at pH 2, BSA release occurs slowly from the matrix. During the 31 h of releasing period total released BSA was less than 10%. It was shown that, as expected, total released protein increases with increasing pH from 2 to 8.

Amount of released BSA from the hydrogel as a function of time at pH 7 and at pH 9 are shown in Figs. 2 and 3, respectively, for hydrogels loaded with BSA at pH 3, 5, 7 and 8. While the hydrogels loaded at pH 3, 5 and 7 are showing the similar release profile with linear release kinetics up to 300 h at pH 7, the hydrogel loaded at pH 8 represents different pro-

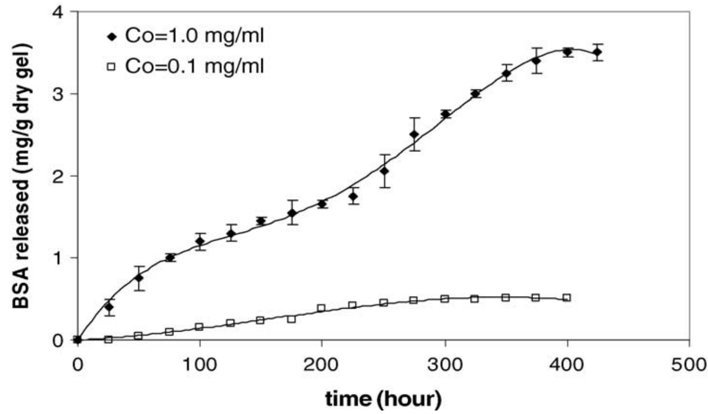


Fig. 1. Effect of initial BSA concentration of loading medium (pH 5) on the BSA release kinetics from the hydrogels at pH 7.

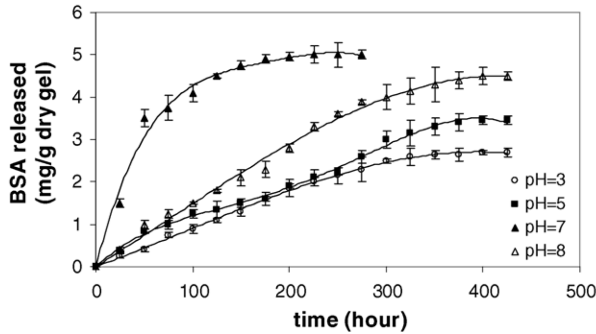


Fig. 2. Release of BSA at pH 7 from hydrogels loaded with BSA at different pHs.

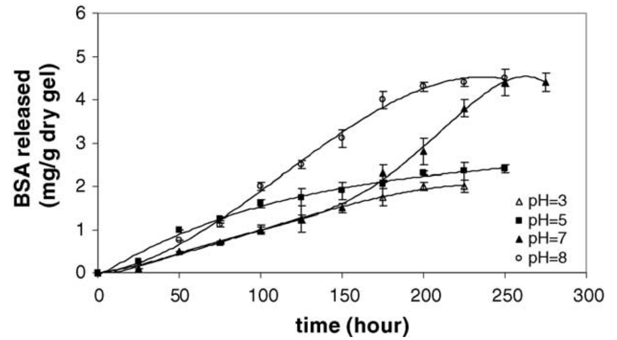


Fig. 3. Release of BSA at pH 9 from hydrogels loaded with BSA at different pHs.

file which is characterized by an initial burst of protein during the first 100 h followed by a longer period of sustained release. This may be explained by the high amount of BSA loaded into the hydrogel at pH 8. Faster release was observed at pH 9 than that of pH 7. There was a direct relationship between the total amount of BSA released and the hydrogel swelling ability.

Table 2
Amount of loaded BSA, total released BSA (mg/g dry gel) and release period as a function of loading and releasing media pH

| BSA-loaded gel[a] | Loaded BSA (mg/g dry gel) | Released BSA[b] | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | pH 2 | | pH 6 | | pH 7 | | pH 8 | |
| | | mg/g dry gel | Releasing time[c] (hour) | mg/g dry gel | Releasing time[c] (hour) | mg/g dry gel | Releasing time[c] (hour) | mg/g dry gel | Releasing time[c] (hour) |
| Gel loaded at pH 3 | 3.1 | 0.23 | 31 | 2.4 | 230 | 2.7 | 425 | 3.1 | 300 |
| Gel loaded at pH 5 | 4.3 | 0.28 | 31 | 3.5 | 255 | 3.5 | 425 | 4.3 | 228 |
| Gel loaded at pH 7 | 4.6 | 0.35 | 31 | 2.5 | 230 | 4.6 | 305 | 4.6 | 269 |
| Gel loaded at pH 8 | 5.0 | 0.58 | 31 | 2.6 | 235 | 5.0 | 250 | 5.0 | 200 |

[a] Loading was performed during 24 h from 1.0 mg/mL BSA solutions at 4 °C.
[b] Protein release was performed with PBS at 37 °C.
[c] Time to reach plateau.

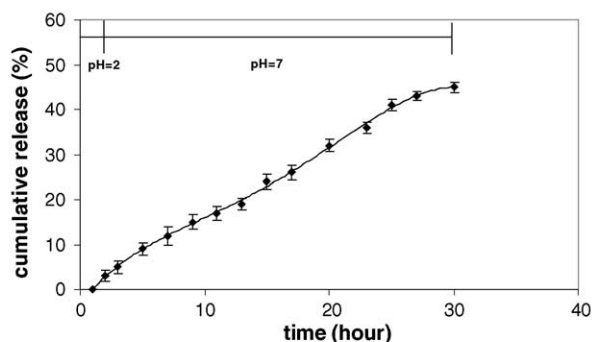


Fig. 4. Fractional BSA release initially at pH 2 for 2 h, then at pH 7 up to 30 h.

The release profile for polymeric discs at 37 °C placed for 2 h at pH 2.0 and then at pH 7.0 is shown in Fig. 4. At pH 2.0 and 37 °C, the hydrogels did not swell, under these release conditions, a negligible amount of BSA (less than 1%) was released from the hydrogels. At pH 7.0 and body temperature, the hydrogels swelled and released BSA slowly. Fifty percent of BSA was released during a period of 30 h.

### 3.4. Mathematical analysis

The kinetic data obtained for in vitro release studies were evaluated using the equation given below (Ritger and Peppas, 1987).

$$\frac{M_t}{M_\infty} = kt^n$$

Where $M_t/M_\infty$ is the fraction of drug released; $t$, the release time; $k$, a constant characteristic of the system; and $n$ is an exponent which characterizes the diffusional release kinetic mechanism.

The $n$ and $k$ values determined from the initial portion of ($M_t/M_\infty \leq 0.6$) log–log plots of $M_t/M_\infty$ versus time are presented in Table 3 together with regression coefficients. In the case of cylinder geometry, $n$-values between 0.45 and 0.89 show non-Fickian diffusion, while $n$ is equal to 0.45 for Fickian diffusion. As could be seen in Table 3, calculated $n$-values ranging between 0.46 and 0.84 indicate that the release deviates from Fickian mode. In this study, BSA loaded hydrogels were stored in the dry state before release experiments. Thus, the absorption of water and release of drug via a swelling-controlled diffusion mechanism occurs simultaneously. The existence of some molecular relaxation process in addition to diffusion is believed to be responsible for the observed non-Fickian behavior.

Table 3
The results of mathematical analysis of BSA release kinetics

| Sample | $n$ | $k$ | $r^2$ |
|---|---|---|---|
| Loaded gel at pH 3 | | | |
| Reservoir pH | | | |
| 7.0 | 0.46 | 0.131 | 0.98 |
| 8.0 | 0.84 | 0.104 | 0.93 |
| 9.0 | 0.73 | 0.149 | 0.99 |
| Loaded gel at pH 5 | | | |
| Reservoir pH | | | |
| 7.0 | 0.60 | 0.190 | 0.98 |
| 8.0 | 0.77 | 0.148 | 0.99 |
| 9.0 | 0.67 | 0.167 | 1.00 |
| Loaded gel at pH 7 | | | |
| Reservoir pH | | | |
| 7.0 | 0.72 | 0.285 | 0.98 |
| 8.0 | 0.77 | 0.111 | 0.91 |
| 9.0 | 0.57 | 0.167 | 0.90 |
| Loaded gel at pH 8 | | | |
| Reservoir pH | | | |
| 7.0 | 0.54 | 0.216 | 0.99 |
| 8.0 | 0.73 | 0.142 | 0.93 |
| 9.0 | 0.63 | 0.167 | 0.91 |

## 4. Conclusion

This study shows that pH-sensitive vinyl ether-based hydrogels prepared by radiation polymerization have attractive properties as protein releasing matrices. It is possible to load maximum 5.0 mg BSA per gram of disk at 4 °C and pH 8 without causing denaturation of protein molecules. It was found that, a negligible amount of BSA was released at pH 2.0, while 80–100% of BSA initially present was releasing within the time period up to 300–400 h at pHs 7.0, 8.0 and 9.0. Therefore, this hydrogel can be considered as a suitable system for the colon-specific delivery of bioactive compounds.

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