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Superporous polyacrylate/chitosan IPN hydrogels for protein delivery

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Abstract In this study, poly(acrylamide), poly(AAm), and poly(acrylamide-co-acrylic acid), poly(AAm-co-AA) superporous hydrogels (SPHs) were synthesized by radical polymerization in the presence of gas blowing agent, sodium bicarbonate. In addition, ionically crosslinked chitosan (CH) superporous hydrogels were synthesized to form interpenetrating superporous hydrogels, i.e. poly (AAm)-CH and poly(AAm-co-AA)-CH SPH-IPNs. The hydrogels have a structure of interconnected pores with pore sizes of approximately 100–150 μm. Although the extent of swelling increased when AA were incorporated to the poly(AAm) structure, the time to reach the equilibrium swelling (~30 s) was not affected so much. In the presence of chitosan network mechanical properties significantly improved when compared with SPHs, however, equilibrium swelling time (~30 min) was prolonged significantly as due to the lower porosities and pore sizes of SPH-IPNs than that of SPHs. Model protein bovine serum albumin (BSA) was loaded into SPHs and SPH-IPNs by solvent sorption in very short time (<1 h) and very high capacities (~30–300 mg BSA/g dry gel) when compared to conventional hydrogels. BSA release profiles from SPHs and SPH-IPNs were characterized by an initial burst of protein during the first 20 min followed by a completed release within 1 h. However, total releasable amount of BSA from SPH-IPNs was lower than that of SPHs as due to the electrostatic interactions between chitosan and BSA.

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1 Introduction

Protein-based drugs i.e. peptides and proteins are increasingly being used for the treatment of various diseases. However, effective application of these drugs requires special delivery methods. Although the oral administration of protein drugs is the most attractive route clinically, it is very difficult to achieve this because of high susceptibility of proteins to hydrolysis and digestion by the acid and enzymes in the gastrointestinal (GI) tract, and their low bioavailability due to poor membrane permeability [1, 2].

To overcome these drawbacks numerous efforts have been undertaken in the development of appropriate oral dosage forms for protein-based drugs [3]. There are a number of vital issues for designing an effective protein-based drug delivery system, e.g. site-specific drug delivery to achieve a predictable and reproducible absorption in therapeutic doses, improvement of the poor oral bioavailability of these drugs, and overcoming both transmucosal transport and metabolic barriers to achieve a desirable therapeutic effect [4, 5]. Up to now, several approaches have been utilized for site-specific peptide drug delivery such as magnetic systems, unfoldable or expandable systems and muco-adhesive systems [6]. A muco-adhesive delivery system helps to provide a close contact with the absorbing membrane and an extended residence time in the intestine [7, 8]. Hydrogels having muco-adhesive properties such as poly(acrylic acid) and its derivatives, collagen, alginate and chitosan have attracted the attention due to their biocompatibility and high water affinity. Since these non-porous hydrogels have low swelling rate and low loading capacities, their utilization in effective drug delivery have been restricted [9].

Superporous hydrogels (SPHs) and SPH composites (SPHCs) are new generations of hydrogels, which are

capable of swell within a couple of minutes by absorbing a large amount of water owing to their highly porous structure [10, 11]. Dorkoosh et al. [6] developed a SPH-based peroral peptide delivery system which absorbs the intestinal fluids and swells rapidly due to high porosity of polymer when it is administered to the GI tract. Thus, the system adheres to the intestinal epithelium mechanically and opens tight junctions by mechanical pressure. It was shown that this system enhanced the intestinal absorption of a number of protein-based drugs, e.g. buserelin, octreotide, insulin and desmopressin [12].

However, both of SPHs and SPHCs are much brittle and so development of their mechanical properties is needed crucially. Incorporation of the second polymer into the SPH structure to form an interpenetrating network (IPN) improves the mechanical strength and elasticity of the polymer [13]. Yin et al. [9] developed SPH-IPNs containing poly(acrylic acid-co-acrylamide)/O-carboxymethyl chitosan and they investigated delivery of insulin from this system. However, until now the release of high molecular weight proteins from SPHs and SPH-IPNs has not been investigated.

In the presented study, a novel group of superporous hydrogels containing poly(acrylamide)/chitosan and poly(acrylamide-co-acrylic acid)/chitosan interpenetrating polymer networks (SPH-IPNs) with improved mechanical strength were synthesized and investigated for delivery of model protein, bovine serum albumine (BSA). Acrylic acid (AA) and acrylamide (AAm) were preferred as the vinyl monomers due to their high water affinity and fast co-polymerization rate while chitosan (CH) was choosen as the second polymer component for its biocompatibility and bioadhesion. Besides their different compositions from the similar hydrogels reported in the related literature, SPH-IPNs synthesized here were ionically crosslinked and relatively high molecular weight protein, BSA, was loaded into the SPHs and SPH-IPNs by soaking method before or after the IPN formation. The in vitro release characteristics of the delivery system were studied in simulated intestinal medium.

2 Materials and methods

2.1 Materials

The comonomers, acrylamide (AAm) and acrylic acid (AA) were obtained from Acros Organics (Germany). *N*,*N*′-methylenebisacrylamide (Bis), Pluronic® F127 (PF127), ammonium persulfate (APS), *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED) and sodium bicarbonate ($NaHCO_3$) were obtained from Sigma-Aldrich Chemical Company (Germany). Chitosan (CH) derived from crab shell with a deacetylation degree of minimum 85% (Cat.No: C3646), glycerol phosphate disodium hydrate salt (GP) and phosphate buffer saline tablets (PBS, pH = 7.4) were purchased from Sigma (Germany). Bovine serum albumine (BSA, MW = 65 000 Da, Fraction V, Sigma) was used as a model protein. The solvent used was deionized, distilled water (DDW). All other materials were of analytical grade.

2.2 Preparation of hydrogels

2.2.1 Synthesis of superporous hydrogels (SPHs)

In this study, poly(AAm-co-AA) and poly(AAm) superporous hydrogels (SPHs) were synthesized as described previously [14, 15]. For the synthesis of poly(AAm-co-AA) SPH the following components were added sequentially to a test tube (15 mm outer diameter and 110 mm length): 300 μl of 50% AAm and 200 μl of 50% AA as comonomers, 100 μl of 2.5% Bis as crosslinker, 330 μl DDW, 30 μl of 10% PF127 as foam stabilizer, 20 μl of 20% APS and 20 μl of 20% TEMED as a pair of redox initiator. The test tube was shaken to mix the solution after each ingredient was added. The pH of the monomer solution was adjusted to 5 using 50% NaOH. Finally, 120 mg of $NaHCO_3$ was added and the mixture was immediately stirred vigorously using a vortex mixer (Heidolp Reax Top, Germany) for 10 s. As a result, pores were generated within the structure by gas foaming method. Polymerization was allowed to continue for 1 h at room temperature. The synthesized SPHs were removed from the test tube by adding a small amount of absolute ethanol and allowed to swell in water. The SPHs were then dried in an oven (Nuve ES 500, Turkey) at 60°C for a day. The same procedure was used for the synthesis of poly(AAm) SPHs without pH adjustment. The synthesis recipe for poly(AAm) SPHs includes the following ingredients: 1000 μl of 50% AAm, 200 μl of 2.5% Bis, 460 μl DDW, 100 μl of 10% PF127, 45 μl of AA as a catalyst, 40 μl of 20% APS, 40 μl of 20% TEMED and 90 mg $NaHCO_3$.

2.2.2 Synthesis of superporous IPN hydrogels (SPH-IPNs)

The fully dried poly(AAm) and poly(AAm-co-AA) SPHs were cut into disk shape (diameter:8 mm, thickness:4 mm) by a razor blade. The chitosan (CH) solution was prepared by dissolving 100 mg chitosan in 9.5 ml of 1% acetic acid solution by using a magnetic stirrer. Then, 0.5 ml of a glycerol phosphate disodium hydrate salt (GP) solution (0.56 g salt/ml DDW) as an ionic crosslinker was slowly added to the CH solution. Finally, 1 ml of CH/GP mixture (pH–7.2 at 25°C) was embedded into the SPH disks and then the disks were put in an oven at 37°C for a day to complete the formation of SPH-IPNs.

2.3 Characterization of SPHs and SPH-IPNs

2.3.1 Swelling behaviour

The dried SPHs and SPH-IPNs were weighed and immersed into 25 ml of phosphate buffer saline (PBS) at 37°C. The selected pHs were 3.2, 4.7 and 7.4. At predetermined time intervals, polymer samples were taken out of the buffer solution and the excess buffer was removed by using a paper towel. The weight of the wet polymer was measured. Then swelling ratios (Q) of SPHs and SPH-IPNs were calculated by means of the following equation.

$$Q = (W_s - W_d)/W_d \quad (2.1)$$

where W_d is the weight of dried hydrogel and W_s is the weight of swollen hydrogel at certain time intervals.

2.3.2 Morphological studies

The dried hydrogel samples were examined by Scanning Electron Microscope (SEM). The inner surfaces of the SPHs and SPH-IPNs were coated with a thin layer of gold and visualized in a SEM (JEOL, JSM-840) using 15 kV voltage. The pore size and size range were determined from the SEM photographs.

2.3.3 Thermal analysis

Thermal behaviours of SPHs and SPH-IPNs were examined by thermogravimetric analyzer (TGA, SETARAM TG-DTA92, France). TGA studies were carried out in air atmosphere and 25–600°C temperature range by 20°C/min heating rate.

2.3.4 Mechanical properties

The mechanical properties of swollen SPHs and SPH-IPNs were investigated using mechanical test apparatus (LLOYD Instruments, LR5K, England). The hydrogel samples were cut into certain length of disk shape (diameter: 2 cm, thickness: 1 cm) and swollen in PBS to reach the equilibrium. The swollen samples were mounted on the apparatus for compression test and the compression moduli (E_c) of the hydrogels were determined. The test speed was 5 mm/min and the trigger force was 500 N (ASTM D882-97). All measurements were made in triplicate.

2.3.5 Void fraction measurement

The pore volume fractions inside SPHs and SPH-IPNs were determined [16]. Dry hydrogels were immersed into the buffer (PBS) solution. The void fraction was calculated from the following equation:

$$\text{Void fraction} = (\pi D^2/4)L/V\rho \quad (2.2)$$

where D and L are the diameter and the height of the swollen hydrogel, respectively. V is the volume of absorbed buffer by the hydrogel and ρ is the density of buffer.

2.3.6 Density measurement

Densities of the dried SPHs and SPH-IPNs were determined from direct mass measurements. The density (d) of a dried SPH was calculated by dividing the weight of a dried SPH (W_d) with the volume of the dried SPH (V_d). The volume (V_d) was calculated by a solvent displacing method [14]. Briefly, by the use of tweezers, a dried SPH was immersed into the n-hexane in a graduated cylinder and then it was quickly removed from the n-hexane. The volume change read from the graduated cylinder before and after the removal was indicated as the volume of the dried superporous hydrogel. In this procedure n-hexane was used since it is very hydrophobic and does not absorbed by hydrogels.

2.4 Protein release studies

2.4.1 BSA loading

Protein solutions of 1, 3 and 5 mg/ml of BSA were prepared using PBS at pH 7.4. BSA solution was embedded into the previously prepared poly(AAm-co-AA) or poly (AAm) SPH disk samples (8 mm in diameter and 4 mm in thickness) by means of a micro pipet in very short time ($\sim$1 min) and in high drug loading capacities compared to conventional hydrogels. The amount of loaded BSA ($W = CV$, mg) to each hydrogel disk was determined by utilizing the loaded solution volume (V, ml) and the solution concentration (C, mg/ml). Then the BSA loaded SPH disks were dried under vacuum at −85°C for 6 days in a freeze-drier (Christ, Germany). A part of these dry and loaded SPH disks were used for preparing the loaded SPH-IPN disks as described previously in Sect. 2.2.2. These samples were indicated as "1st group of BSA loaded SPH-IPNs". In the second approach, IPN formation was realized before the embedding procedure. Hence, "2nd group of BSA-loaded SPH-IPNs" were obtained.

2.4.2 In vitro release kinetics

BSA loaded poly(AAm-co-AA) SPH (control group) and 1st and 2nd groups of SPH-IPN disks were placed into 25 ml sealed Erlenmayers containing PBS (7.4). 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 fluorescence spectrophotometer (VARIAN Cary Eclipse, Australia). The intrinsic tryptophan fluorescence observed from protein solutions at 280 nm excitation (λ_{ex}) and 332 nm emission (λ_{em}) wavelengths. Then cumulative BSA release was calculated.

All experiments were realized in the BSA-precoated vessels in order to prevent the adsorption of BSA onto vessel walls and they were repeated at least three times. The results were reported as average values.

3 Results

3.1 Preparation of hydrogels

3.1.1 Synthesis of superporous hydrogels (SPHs)

In the presented study, acrylamide-based superporous hydrogels, poly(AAm) and poly(AAm-co-AA) SPHs, were prepared by solution polymerization technique using APS and TEMED as an initiator system. Superporosity was achieved by means of carbon dioxide gas bubbles generated from the reaction of sodium bicarbonate and acrylic acid. While *N*,*N*′-methylenebisacrylamide (Bis) was using as crosslinker, Pluronic F127 was used as a foam stabilizer. To prepare superporous hydrogels with homogeneously distributed gas bubbles, polymerization and foaming process must be realized simultaneously. The role of crosslinker, initiator system, foam stabilizer and foaming agent in the polymerization reaction were discussed in detail in previous studies [10, 14, 17]. By taking into account these studies, the recipes including the optimum amounts of ingredients were formed and reported in Sect. 2.2.1.

3.1.2 Synthesis of superporous IPN hydrogels (SPH-IPNs)

Chitosan molecules in SPHs are expected to form a network by ionic crosslinkings, resulting in full-interpenetrating network (IPN). In this study, glycerol phosphate (GP) crosslinked chitosan SPHs were synthesized to form SPH-IPNs. The addition of GP, which leads to the formation of a hydrated gel-like precipitate, provided ionic crosslinking. This precipitation, or gel formation, is due to the neutralization of chitosan amine groups and consequent removal of repulsive interchain electrostatic forces which allows for extensive hydrogen bonding and hydrophobic interactions between chains [18]. Moreover, ionically crosslinked chitosan hydrogels are generally thought to be well-tolerated and their potential medical and pharmaceutical applications are numerous since ionic crosslinkers are often biocompatible.

3.2 Main characteristics of SPHs and SPH-IPNs

SEM photographs in Fig. 1 show the morphological structures of SPHs and SPH-IPNs in dry state. It can be concluded that, interconnected pores forming capillary channels are existent for all types of hydrogels. However, higher porosity was observed for SPHs (Fig. 1a, b) than that of SPH-IPNs (Fig. 1c, d). This is in accordance with the results obtained from density measurements and swelling characteristics. On the other hand, the presence of comonomer AA in the SPH caused increased pore sizes when was compared with poly(AAm) SPH (Fig. 1a, b). While the average pore size of poly(AAm) SPH is 100 ± 60 μm, it is 148 ± 90 μm for poly(AAm-co-AA) SPH (Table 1). Unlike to the SPHs, many collapsed fibrous structures were observed in the sections of SPH-IPN samples, which make the IPN sample coarser in appearence (Fig. 1c, d). The average pore sizes of SPH-IPNs were also significantly decreased due to the presence of chitosan network within the SPHs (Table 1).

Figure 2 shows the photographs of poly(AAm-co-AA) SPH and poly(AAm-co-AA)-CH SPH-IPN samples at dried and swollen states. Swelling causes approximately 8-fold increase in volume of SPHs (Fig. 2a), however, about 2-fold increase in volume was observed for SPH-IPNs (Fig. 2b). While the dried SPH is opaque, swelling causes transparency. When the chitosan network was formed inside an original SPH, the hydrogel becomes opaque in both dry and swollen forms as shown in Fig. 2b.

The swelling profiles of poly(AAm) and poly(AAm-co-AA) SPHs and poly(AAm)-CH and poly(AAm-co-AA)-CH SPH-IPNs at pH = 7.4 and 37°C are shown in Fig. 3. The extent of swelling was decreased significantly in the case of SPH-IPNs (Fig. 3b). The reason for the observed decrease in swelling ratio (Q) can be explained by greater intermolecular attraction between macromolecular chains. Thus the chains become compact and this obviously restrains the penetration of water molecules into the hydrogel. Also Q was decreased in the absence of AA within the hydrogel. The swelling of dried SPHs took about 30 s to reach their equilibrium values. While the extent of swelling decreased when chitosan was incorporated to the structure, the time to reach the equilibrium swelling increased significantly i.e. increased from 30 s to 30 min. This result indicates that the interconnected pore structure was not maintained completely in the case of SPH-IPNs.

The main structural characteristics of SPHs and SPH-IPNs synthesized in this study are summarized in Table 1. As seen from this table, the density of SPHs was increased when chitosan is present in SPHs. Additionally, the void fraction of SPH-IPNs is lower than that of SPHs as a result of ionically crosslinked chitosan structure. These results are consistent with the decrease in swelling ratio (Q).

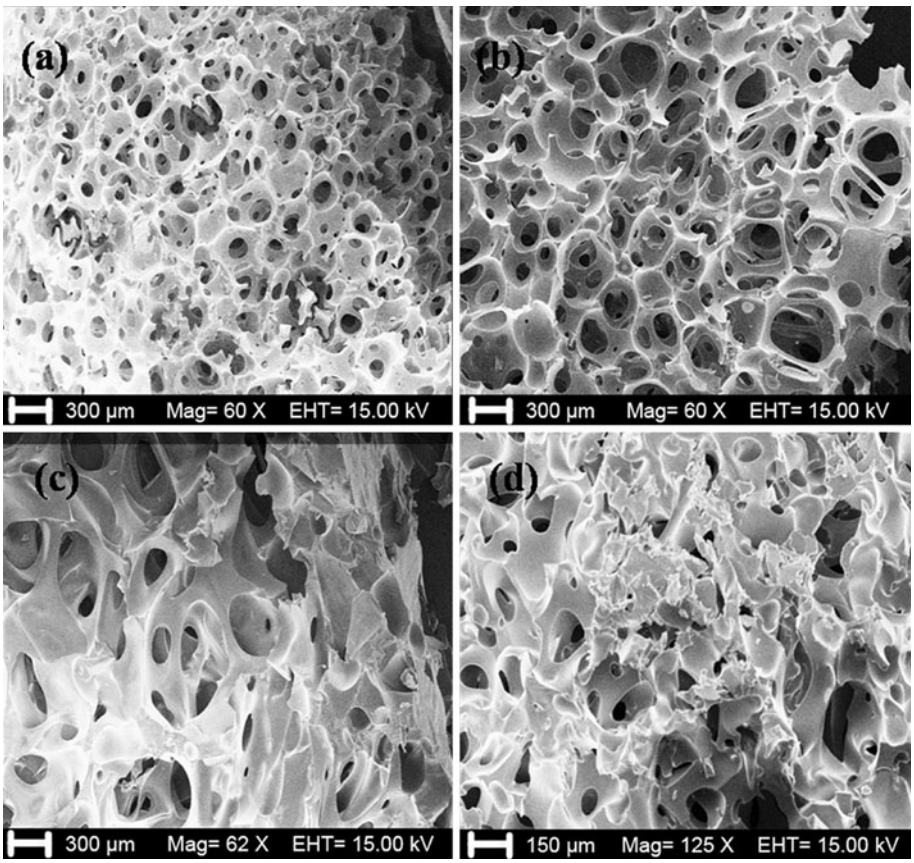

Fig. 1 SEM micrographs of SPHs and SPH-IPNs synthesized in this study. **a** poly(AAm) SPH, **b** poly(AAm-co-AA) SPH, **c** poly(AAm)-CH SPH-IPN, **d** poly(AAm-co-AA)-CH SPH-IPN

Table 1 Main characteristics of SPHs and SPH-IPNs synthesized in this study

Hydrogel	Pore size (µm)	Pore size range (µm)	Void fraction (ml/g)	E_c (kPa)	Density (g/ml)	Onset of thermal degradation (°C)
Poly(AAm) SPH	100 ± 60	27–200	1.28 ± 0.25	22 ± 3	0.16 ± 0.04	190.0
Poly(AAm-co-AA) SPH	148 ± 90	30–212	1.16 ± 0.04	17 ± 4	0.16 ± 0.00	219.0
Poly(AAm)-CH SPH-IPN	65 ± 21	19–130	0.98 ± 0.14	821 ± 84	0.31 ± 0.12	155.0
Poly(AAm-co-AA)-CH SPH-IPN	87 ± 53	38–338	1.08 ± 0.08	312 ± 241	0.32 ± 0.01	178.0

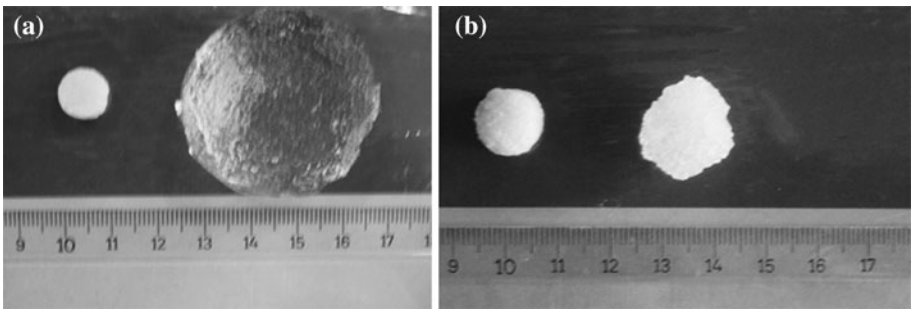

Fig. 2 Photographs of **a** poly(AAm-co-AA) SPH in dried (*left*) and swollen (*right*) state. **b** poly(AAm-co-AA)-CH SPH-IPN in dried (*left*) and swollen (*right*) state

The decrease in void volume leaded to decreased amount of of water uptake into the structure, causing the decreased swelling ratio of SPH-IPNs.

The compression moduli (E_c) of SPHs and SPH-IPNs were compared in Table 1. While the presence of AA is decreasing compression moduli, the formation of chitosan

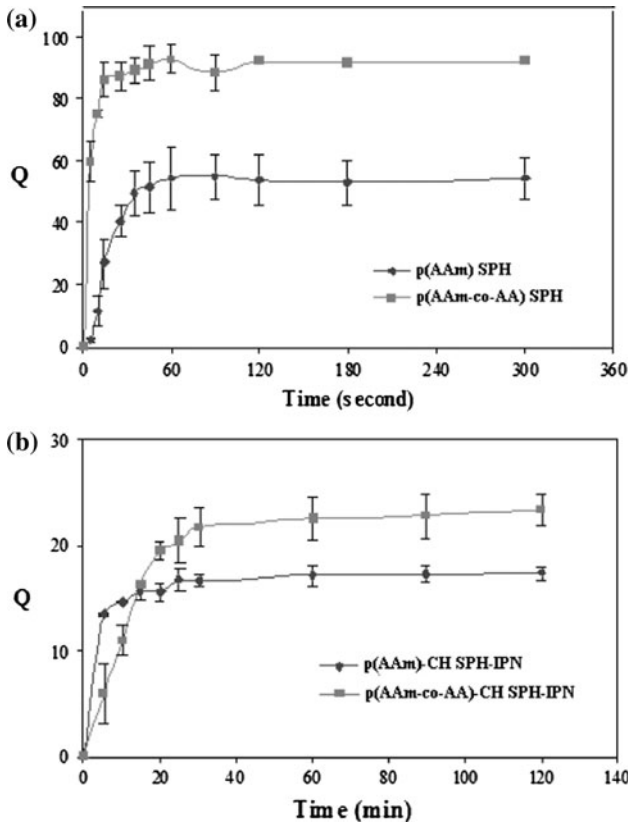

Fig. 3 Swelling kinetics of **a** SPHs and **b** SPH-IPNs in PBS (at pH 7.4 and 37°C) $n = 3$, mean ± SD

network within the SPHs increased their compression strength significantly. The presence of chitosan network in SPHs results in improved mechanical properties and polymer networks with higher modulus can be achieved when compared to conventional SPHs.

According to the TGA data given in Table 1, thermal degradation of poly(AAm) SPH and poly(AAm-co-AA) SPH starts at 190°C and 219°C, respectively whereas poly(AAm)-CH SPH-IPN and poly(AAm-co-AA)-CH SPH-IPN start to degrade at 155°C and 178°C, respectively. TGA curves showed that while the SPHs are indicating more thermostable behavior up to onset of degradation, in the case of SPH-IPNs weight loss occur continuously by temperature.

In this study, pH-sensitivity of the SPHs and SPH-IPNs was also investigated. Since the pH-sensitive hydrogels are based on polymeric or copolymeric systems of ionizable polyelectrolytes, such as acrylic or methacrylic acids or ammonium salts, the hydrogels consisting AA comonomer within their structure show pH-sensitivity. The parameters that affect the degree of swelling of ionic polymers are the properties of the polymer and properties of the swelling medium. This is why, experiments to determine the pH-dependent swelling characteristics of SPHs and SPH-IPNs were performed in PBS at three different pHs, i.e. pH: 3.2, 4.7 and 7.4. Table 2 represents the variation of the equilibrium swelling ratios of all hydrogels synthesized in this study as a function of the pH of the swelling medium at 37°C. Owing to the nonionic structure, poly(AAm) SPHs was not affected by pH variations. But, incorporation of AA within the SPH structure causes increase of Q by increasing pH. This may be explained by depending upon the ionization of AA which is a weak acid at lower pHs. However, we did not observe a sharp pH-dependent transition due to the presence of AA. It was reported that, a sharp phase transition of hydrogels in response to pH change requires incorporating hydrophobic monomers in gels [19].

3.3 Loading of BSA into the SPHs and SPH-IPNs

In this study, BSA loading into SPHs was performed by solvent (water) sorption i.e. embedding method at room temperature. The loading procedure was realized at pH = 7.4. Since the formation of chitosan IPN was performed in two ways, i.e. before or after the embedding procedure, two groups of BSA-loaded SPH-IPNs were obtained in each composition, poly(AAm) or poly(AAm-co-AA).

The amount of loaded protein (BSA) to each hydrogel disk was calculated from the difference between original and final reservoir concentrations of the loading solution and it was represented as "milligram protein per gram of dry hydrogel". Experiments in which the loading time was varied showed that 1 h was sufficient for complete loading. There is a linear relationship between the initial BSA concentration and the loading capacity of poly(AAm-co-AA) SPHs. The variation of initial BSA concentration in the embedding solution as 1, 3 and 5 mg/ml, leads to the different loading amounts as 50, 166 and 294 mg BSA/g dry gel, respectively. By using this information, it is possible to load the desired amount of protein to the hydrogel. The loading amounts from 5 mg/ml of BSA solutions to the SPHs and SPH-IPNs were listed in Table 3. While the 1st group of SPH-IPNs (IPN formation was realized after embedding procedure) has the same amounts of BSA with their SPH analogs, 2nd group of SPH-IPNs (IPN formation was realized before embedding procedure) has lowest amounts of BSA as due to their relatively low swelling abilities.

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 [20]. 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.

SEM photographs in Fig. 4 clearly show the cross-sectional morphology of SPHs and SPH-IPNs after BSA

Table 2 Swelling ratios of SPHs and SPH-IPNs at different pHs

Hydrogel	Q (Swelling ratio)[a]		
	pH 3.2	pH 4.7	pH 7.4
Poly(AAm) SPH	51.78 ± 2.20	57.94 ± 3.20	57.26 ± 5.50
Poly(AAm-co-AA) SPH	78.22 ± 7.50	86.92 ± 2.40	91.59 ± 0.80
Poly(AAm)-CH SPH-IPN	24.15 ± 1.20	29.53 ± 3.90	17.45 ± 0.40
Poly(AAm-co-AA)-CH SPH-IPN	17.63 ± 1.20	19.25 ± 2.50	23.39 ± 2.40

[a] The equilibrium swelling times of SPHs and SPH-IPNs are 1 and 30 min, respectively ($n = 3$)

Table 3 Amount of loaded BSA to SPHs and SPH-IPNs

Superporous hydrogels	Amount of BSA[a] loaded (mg BSA/g dry gel)
poly(AAm) SPH	183.5 ± 7.2
poly(AAm-co-AA) SPH	294.3 ± 5.0
1st group of poly(AAm)-CH SPH-IPN	183.5 ± 7.2
1st group of poly(AAm-co-AA)-CH SPH-IPN	294.3 ± 5.0
2nd group of poly(AAm)-CH SPH-IPN	33.6 ± 5.5
2nd group of poly(AAm-co-AA)-CH SPH-IPN	53.5 ± 4.3

[a] Loading conditions: pH = 7.4, 5 mg/ml BSA solution

loading. The presence of BSA molecules within the structure causes morphological differences due to the localization of protein molecules in the bulk phase of hydrogels.

3.4 Release Behaviour of BSA from the SPHs and SPH-IPNs

The release behaviour of BSA was studied in vitro. The released percentages of BSA from the poly(AAm-co-AA) SPH and 1st group of poly(AAm-co-AA)-CH SPH-IPN as a function of time at pH 7.4 and 37°C are shown in Fig. 5. The hydrogels contain the same amount of BSA, i.e. 294.3 mg BSA/g dry gel. Both of the release profiles are characterized by an initial burst of protein during the first 20 min followed by a completed release within 1 h.

As due to the relatively lower swelling ability of the SPH-IPN than that of SPH, BSA release occurs relatively slow from the SPH-IPN matrix. During the releasing period total releasable BSA from SPH-IPNs is less than 60%. However, about 80% of loaded BSA is released from the SPHs.

4 Discussion

Formation of interpenetrating polymer network (IPN) is one of the approaches to improve the mechanical strength of SPHs. Kim and Park [20] have synthesized poly(AA-co-AAm)/polyethyleneimine (PEI) SPH-semi IPNs. However, due to the linear structure of PEI, no remarkably improved mechanical strength was obtained. Poly(AA-co-AAm)/O-carboxymethyl chitosan (O-CMC) full-IPN SPH synthesized by Yin et al. [9] showed improved mechanical strength and muco-adhesive properties. The formation of full-IPN was realized by crosslinking of O-CMC with a toxic chemical, glutaraldehyde. In our investigation, considering the drawbacks of previous studies, poly(AAm)-chitosan and poly(AAm-co-AA)-chitosan full-IPN SPHs were synthesized. Chitosan network was formed by ionic crosslinking with glycerol phosphate. Since ionic crosslinking is an extremely simple and mild method to prepare hydrogels, ionically crosslinked chitosan in SPHs favors biocompatibility and offer more possibilities as drug delivery systems compared to covalently crosslinked systems [21].

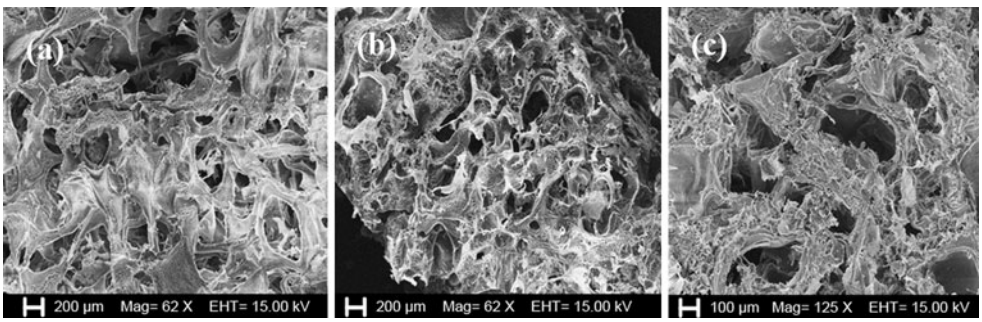

Fig. 4 SEM photographs of BSA loaded **a** poly(AAm) SPH, **b** 2nd group of poly(AAm)-CH SPH-IPN, **c** 2nd group of poly(AAm-co-AA)-CH SPH-IPN

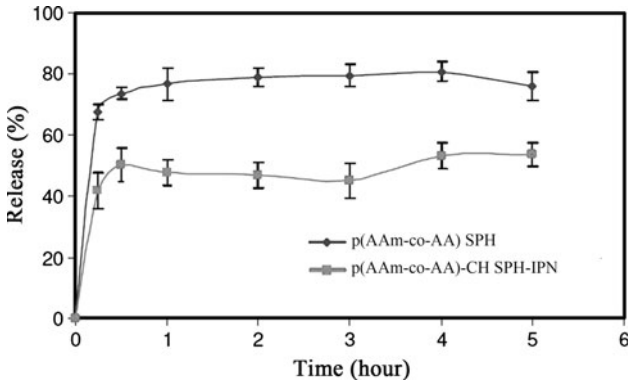

Fig. 5 BSA release kinetics from poly(AAm-co-AA) SPH and 1st group of poly(AAm-co-AA)-CH SPH-IPN at pH = 7.4 and 37°C

In accordance with the results of previous study [9], our morphological observations indicated that formation of full-IPN would not destroy the superporous structure of hydrogels. However, extent of swelling and swelling rate decreased in the presence of chitosan within the SPH. Moreover, chitosan containing SPHs have improved mechanical strength with higher compression modulus than that of poly(AAm) and poly(AAm-co-AA) SPHs. All these results are attributed to the inter- and intra-molecular interactions between the two polymer networks, i.e., acrylamide-based network and chitosan network.

It is concluded that presence of chitosan IPN within the hydrogels causes shifting of degradation temperature to lower values compared to SPHs due to the relatively lower thermal degradation temperature of chitosan. Since almost all of the biotechnological and biomedical applications need easily sterilizable materials, a high degradation temperatures (i.e. above 120°C) of all SPHs and SPH-IPNs offer advantage such those applications.

Peroral administration is still the most convenient route for the delivery of hydrophilic macromolecular drugs including peptides, proteins and polysaccharides [22]. In order to solve the "inefficient absorption" problem of these drugs across mucosal membranes, SPH- or SPHC-based delivery systems have been developed [6]. These systems swell within a couple of minutes to achieve mechanical fixation of the drug at desirable sites of absorption in the intestine by controlled rapid swelling. A number of peptide drugs e.g. buserelin, octreotide and insulin were studied by using SPH-based delivery systems [9, 12]. Since BSA is representing the relatively high molecular weight (MW = 65,000 Da) globular proteins (hydrodynamic diameter is 7.7 nm) when compared with peptide drugs studied by others, we choosed it as a model protein. BSA was loaded into the hydrogels by embedding in two ways i.e. before IPN formation or after IPN formation. High amounts of protein (~300 mg BSA/g dry gel) was loaded into the poly(AAm-co-AA) SPHs and their IPNs (1st group) which were loaded by BSA before IPN formation.

Loading capacities of 1st group of SPH-IPNs were significantly higher than that of SPH-IPNs produced by others [12] which might be due to the loading process applied in our study, i.e. IPN formation followed embedding. On the other hand, in our previous studies, loading amounts of BSA into the poly(ethylene glycol divinyl ether)-based and dextran-based hydrogels were approximately 5 mg and 25 mg per gram of dry hydrogel, respectively [2, 23]. In the presented study, more than 10-fold increase in the loading amount of BSA was obtained by means of SPHs. In addition, fluorimetric investigations further indicate that the activity of BSA was retained after the loading procedure due to the short contact time of BSA with polymer and aqueous medium at pH = 7.4.

The release profiles of BSA from our SPHs and SPH-IPNs were found similar to the release profiles of buserelin, octreotide and insulin which were studied by the other researchers [9, 12]. The typical characteristics of these profiles is the burst release within 1 h. In oral peptide/protein delivery, a burst release of proteins is required for their effective absorption in intestinal epithelium [6].

The release studies implie that BSA did not covalently bind to the SPHs and SPH-IPNs. The isoelectric point of BSA is 4.7 which indicates that above pH = 4.7 the net charge of molecule is negative. Therefore, electrostatic interactions occur between the hydrogels and the protein. Because of the cationic character of chitosan at physiological pH (pH = 7.4), electrostatic interactions between SPH-IPNs and BSA are more stronger than that of the SPHs and BSA. This is why, the total amount of released BSA was decreased in the case of SPH-IPNs according to the SPHs.

5 Conclusion

In the presented study, superporous poly(acrylamide-co-acrylic acid) hydrogels (SPHs) were synthesized and then a full-IPN structure was introduced into SPHs with the ionic crosslinking of chitosan to form SPH-IPNs. The formation of IPN within the SPHs increased the mechanical properties of the superporous hydrogel, keeping the interconnected pore structure. This interconnected structure is useful to allow the diffusion of high molecular weight protein drugs into the matrix and from the matrix. Therefore, model protein, BSA was loaded into the SPHs and SPH-IPNs in very short time (<1 h) and very high capacities (~30–300 mg BSA/g dry gel) when compared to conventional hydrogels. The release characteristics of BSA from the hydrogels demonstrated a release profile consisting a initial burst and then completed release within 1 h. However, it is necessary to prolong the release period by some modifications, i.e. special coatings of hydrogels.

Then, the synthesized hydrogels, especially ionically crosslinked SPH-IPNs, can be evaluated as novel candidates for peroral delivery of high molecular weight protein drugs.

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