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Effects of charge density on the recognition properties of molecularly imprinted polyampholyte hydrogels

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ABSTRACT

Molecularly imprinted polymers are synthetic materials designed to selectively bind to a templated molecule. In this study, the effect of including both positive and negative charges simultaneously into the hydrogel network on the selective recognition properties of the MIP is examined. Using 3-methacrylamidopropyl trimethylammonium chloride (MAPTAC) as a cationic monomer and 2-acrylamido-2-methylpropane sulfonic acid (AMPS) as an anionic monomer, various ratios of positive to negative charges were tested. Selective recognition properties were dependant on the type of charged monomer that made up a majority of the hydrogel's mass. Additionally, polyampholyte MIPs exhibited less swelling than previously studied polyelectrolyte hydrogels.

1. Introduction

Molecularly imprinted polymers (MIPs) are synthetic materials designed to selectively bind to a templated molecule [1–3]. MIPs are synthesized by cross-linking functional monomers in the presence of the template molecule forming a polymer matrix around the template. The template is then removed leaving a cavity with a complementary shape and functionality to that of the template molecule. These polymers have been imprinted to recognize simple sugars [4], peptide sequences [5–7], and larger macromolecular complexes such as proteins [8] and viruses [9,10].

In order to increase the selective recognition properties of MIPs, monomers can be introduced into the matrix which can interact with functional groups on the template molecule. Our previous work has shown that charged monomers are effective in increasing the recognition and selectivity properties of polyacrylamide hydrogel based MIPs [8]. These polyelectrolytes were composed of acrylamide and either a positively charged monomer, 3-methacrylamidopropyl trimethylammonium chloride (MAPTAC), or a negatively charged monomer, 2-acrylamido-2-methylpropane sulfonic acid (AMPS), the structures of which can be seen in Fig. 1. We found that a concentration of 0.25% by weight of the charged monomer in the polymer matrix resulted in optimal selectivity and template recognition properties [8].

In this study, the effectiveness of polyampholytes as MIPs for the selective recognition of Bovine hemoglobin (Bhb) is explored. Polyampohlytes contain both positive and negative charges on the polymer backbone. The presence of both positively and negatively charged monomers in protein imprinted hydrogels could enable increased template molecule recognition for two reasons. First, the presence of two oppositely charged monomers in the pre-polymerization mixture could result in imprinted hydrogels with cavities that contain highly specific functional group orientation. The Bhb protein template contains a distribution of positively and negatively charged functional groups on its surface, and therefore an imprinted hydrogel containing both positively and negatively charged monomers should result in a more accurate complementary structure for Bhb recognition. Secondly, the polyampholyte hydrogels should exhibit decreased swelling when compared to their polyelectrolyte counterparts. Repulsive interactions between similarly charged monomers are shielded within polyampholyte hydrogels, resulting in decreased swelling and a lower degree of cavity deformation. The charged monomers in this study were MAPTAC for the cationic monomer and AMPS for the anionic monomer. The total concentration of charged monomers in the polymer matrix was focused around the optimal concentration of 0.25% by weight that was previously reported [8].

The goal of this work is to observe how varying concentrations of both MAPTAC and AMPS in the polymer matrix affects the recognition, selectivity and swelling properties of the hydrogels. Three types of polyampholytes, net positive, net negative and net neutral, were synthesized and their recognition properties tested.

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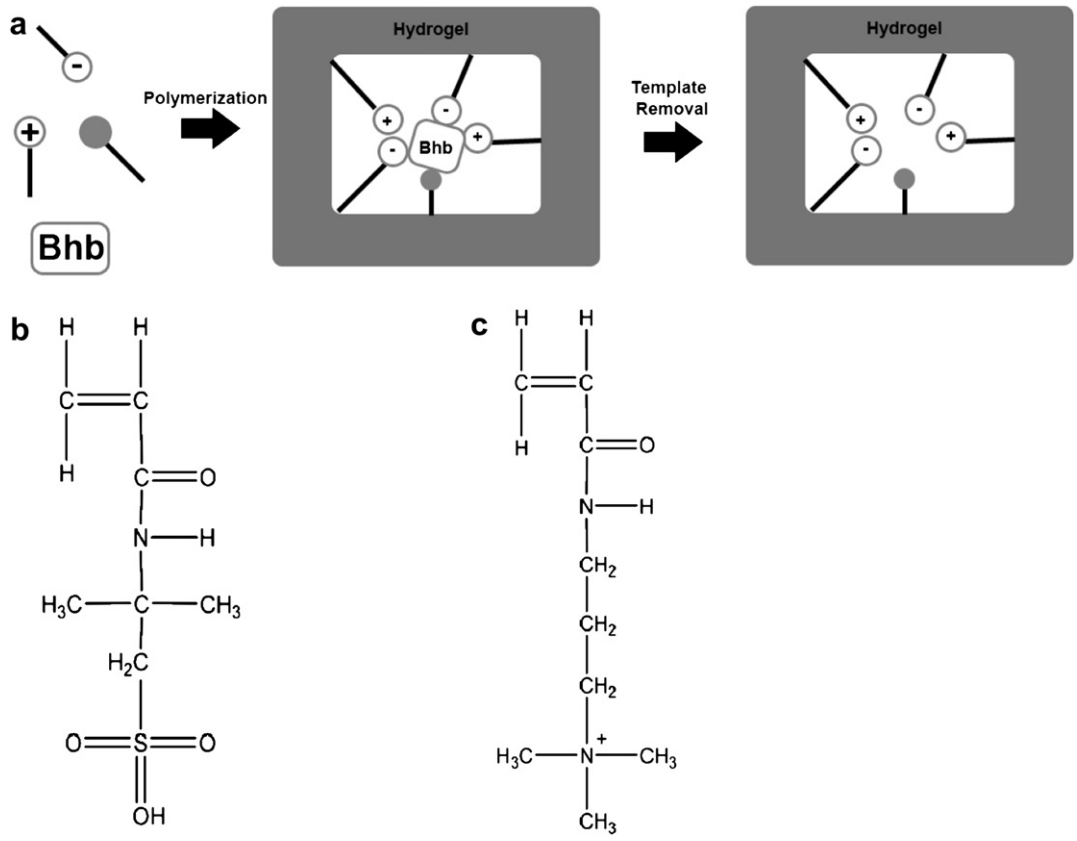

Fig. 1. (a) Schematic of the imprinting process for Bovine Hemoglobin. Molecular structures of (b) 2-acrylamido-2-methylpropane sulfonic acid and (c) 3-methacrylamidopropyl trimethylammonium chloride.

The selectivity of the polyampholyte hydrogels was measured against a competitive template, cytochrome c similarly to our previous publication with polyelectrolyte imprinted hydrogels [8]. The selectivity is defined as the ratio of bound Bhb to bound cytochrome c in the imprinted hydrogels. Cytochrome c has a lower molecular weight and higher isoelectric point than the Bhb template; therefore, it can be used to assess the recognition properties of the hydrogels in relation to template size and isoelectric point. Finally the effect that monomer charge density has on the swelling ratio of the polyampholyte hydrogels was investigated.

2. Experimental section

2.1. Synthesis

The synthesis of protein imprinted polyampholyte hydrogels was conducted in a similar manner to the synthesis of polyelectrolyte hydrogels as discussed in previous studies [8,11]. The total charge density of all polyampholyte hydrogels was fixed at 0.25%. A typical synthesis for a net neutral polyampholyte hydrogel containing 50% AMPS monomer and 50% MAPTAC monomers was performed as follows. Stock solutions of the AMPS and MAPTAC monomers were prepared by dissolving a specified amount of monomer into deionized water and titrating with 1 M NaOH or 1 M HCl to pH 7. 53.86 mg acrylamide (monomer), 0.07 mg AMPS (monomer), 0.07 mg MAPTAC (monomer), 6 mg *N*,*N*-methylenebisacrylamide (cross-linker), 10 μl of 5% (v/v) *N*,*N*,*N*,*N*-tetramethylethylenediamine (TEMED, catalyst) and 12 mg Bhb (purchased from Sigma Aldrich) template were dissolved in 1 ml of deionized water in a microcentrifuge tube. The cross-linker was not varied in any hydrogels synthesized. It was attempted to crosslink the hydrogels as much as possible to create specific cavities for the protein. The maximum amount of cross-linker was dissolved in the hydrogel solution without the Bhb precipitating out of solution. Nitrogen was bubbled through the solution for 5 min to purge any oxygen that is capable of inhibiting the formation of free radicals. Subsequent to nitrogen bubbling, 10 μl of 10% (w/v) ammonium persulfate (APS, initiator) was added to the solution. Free radical cross-linking copolymerization occurred overnight, producing bulk material hydrogels that were then removed from the microcentrifuge tubes. The tube leaves the hydrogel as a cylinder with a height of 1″ and a diameter of ¼″. The hydrogels were then granulated by passing through a 75 μm sieve (Fisher Scientific, Pittsburgh, PA) prior to washing. Non-imprinted, neutral hydrogels were prepared in the same manner, in the absence of the template protein Bhb. Net neutral hydrogels were prepared using the procedure described above and adjusting the amount of positively charged MAPTAC monomer and negatively charged AMPS monomer accordingly. The synthesis parameters for all hydrogels prepared in this study are given in Table 1.

2.2. Bhb template extraction

Template extraction experiments were identical to the modified protocol experiments performed on protein-imprinted polyelectrolyte hydrogels discussed in the previous study [8]. The effects of the template removal protocol on the binding properties of protein imprinted and non-imprinted polyampholyte hydrogels were studied in order to determine whether or not these gels behave similarly to their polyelectrolyte counterparts. Specifically,

Table 1
Synthesis parameters for neutral, positively (MAPTAC) and negatively charged (AMPS) hydrogels. All values are in mg.

AMPS/MAPTAC	Acrylamide	AMPS	MAPTAC	BAAm	Bhb
50%/50%					
Imprinted	53.86	0.07	0.07	6.0	12.0
Non-Imprinted	53.86	0.07	0.07	6.0	0.0
25%/75%					
Imprinted	53.86	0.04	0.1	6.0	12.0
Non-Imprinted	53.86	0.04	0.1	6.0	0.0
75%/25%					
Imprinted	53.86	0.1	0.04	6.0	12.0
Non-Imprinted	53.86	0.1	0.04	6.0	0.0

a study was performed to determine the effect of SDS on the binding affinity of the polyampholyte gels. The study was conducted in the following manner. A number of non-imprinted gels, each containing 50% MAPTAC and 50% AMPS were synthesized according to the experimental procedure outlined in the section above. The resultant gels were washed with either a 10% SDS solution, a 10% HOAc solution, or the SDS–HOAc solution normally used for template extraction. Subsequent to washing the gels, a typical rebinding experiment was conducted where the gels were exposed to a 2 ml solution containing 6 mg Bhb and allowed to associate for 10 min. The gels were then washed five times with water and aliquots were taken from each wash and analyzed using UV–Vis at 404 nm to determine the concentration of Bhb within each wash. Using the three components of the template removal solution separately, the effect that each of the components has on the binding properties of the gels can be determined. A dependence (increase or decrease) of low-affinity binding on the composition of the template removal solution would be indicative of a binding affinity that is subject to modulation through simple variation of the template removal protocol.

2.3. Template rebinding

In a typical protein template rebinding experiment, washed and granulated Bhb-imprinted and non-imprinted hydrogels were loaded with 2 ml of a deionized water solution containing 3 mg/ml of Bhb. The gels were placed on a Labquake mixer (Barnstead International, Dubuque, IA) and allowed to associate with the template for 10 min. Following template association, gels were removed from the mixer and subjected to the same extraction experiments described in the Bhb template extraction section.

2.4. Hydrogel swelling

Swelling experiments were designed to determine the swollen state of the hydrogels in conditions that mimic those present during template rebinding. All swelling experiments were performed using non-imprinted hydrogels. Hydrogels of varying charge density were synthesized, granulated, and weighed to determine the initial mass of the gels. The gels were then washed using the template extraction protocol previously described. Subsequent to washing, the gels were then placed in 15 ml centrifuge tubes along with 2 ml of deionized water. The gels were swollen in these tubes for 10 min and then centrifuged for 5 min at 3000 rpm. The deionized water supernatant was removed and the gels were weighed again to determine the final mass. The swelling ratio (SR) was determined by dividing the final mass by the initial mass.

3. Results and discussion

Fig. 2 shows the results of a template rebind experiment performed on a non-imprinted, net neutral polyampholyte hydrogel containing 50% AMPS and 50% MAPTAC that has been washed solely with HOAc. The data shows that a large fraction (4.6 mg) of the Bhb template is removed after five washes with deionized water have been completed. These results indicate that HOAc itself has little effect on the template recognition properties of the polyampholyte hydrogels. The result of a Bhb rebind experiment performed on a non-imprinted, net neutral polyampholyte hydrogel containing 50% AMPS and 50% MAPTAC that was washed solely with SDS is shown in Fig. 2. The gel washed only with SDS exhibits a much lower unbound Bhb fraction (1.6 mg) compared to the unbound fraction (2.8 mg) of a similar gel that was washed only with HOAc. The decrease in the unbound fraction of Bhb in the supernatant of the gel washed with SDS indicates that, after template association occurs, a large fraction of the 6 mg Bhb available resides within the gel. Therefore, similar to their polyelectrolyte counterparts, SDS molecules diffuse into the polyampholyte hydrogel matrix during the template extraction step and remain trapped within the matrix, acting as a high-affinity binding sink for the Bhb template. Fig. 2 contains the results of a Bhb template rebinding experiment

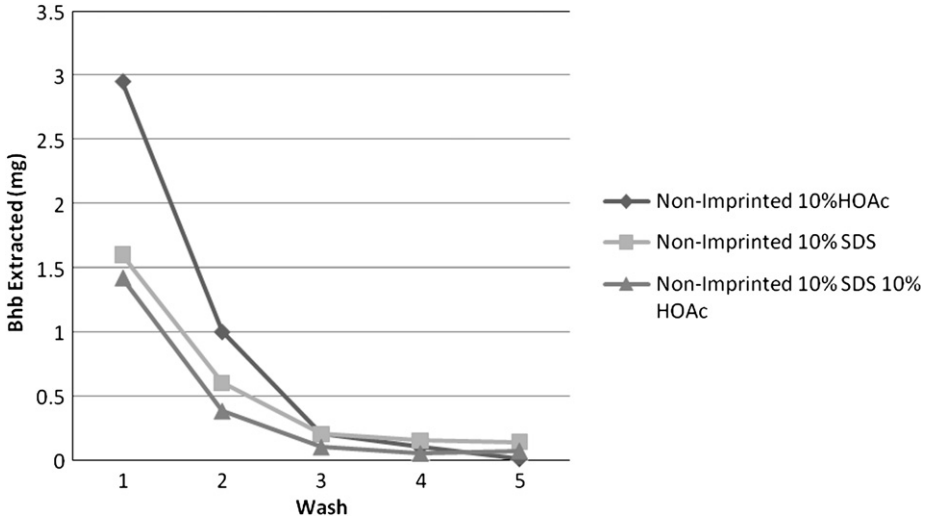

Fig. 2. Binding experiment performed on a net neutral (50% AMPS–50% MAPTAC), non-imprinted polyampholyte hydrogel washed with either a 10% HOAc solution, 10% SDS solution or a 10% SDS–10% HOAC solution. Washes (1–5) were performed using deionized water. The hydrogels were loaded with 6 mg of Bhb.

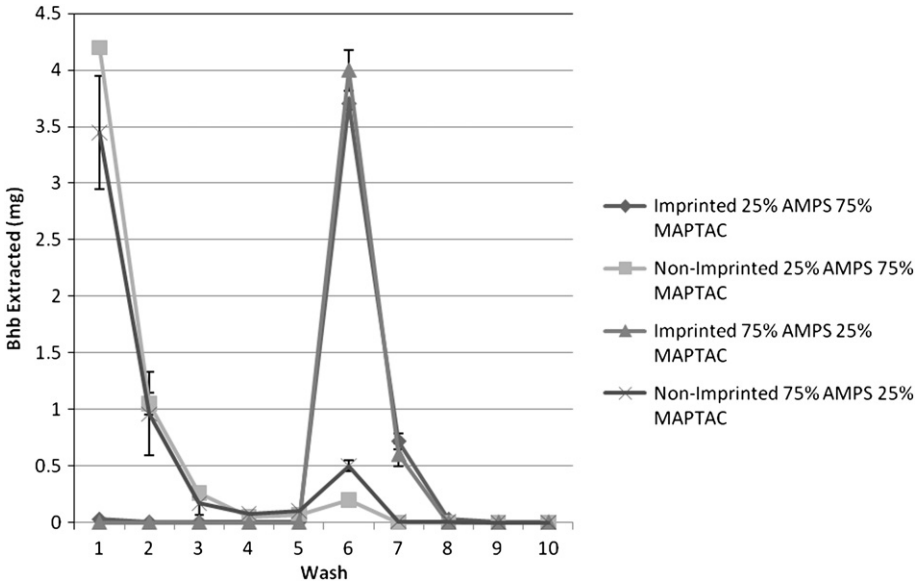

Fig. 3. Bhb template rebinding data for various hydrogels. Positively charged, Bhb-imprinted and non-imprinted polyampholyte hydrogels containing 25% AMPS and 75% MAPTAC. Negatively charged, Bhb-imprinted and non-imprinted polyampholyte hydrogels containing 25% MAPTAC and 75% AMPS. All hydrogels were loaded with 6 mg of Bhb.

performed on a non-imprinted polyampholyte hydrogel containing 50% AMPS and 50% MAPTAC that was washed with the SDS–HOAc solution. Approximately 1.4 mg Bhb was unbound following the template association. Therefore, the polyampholyte gels washed with SDS and SDS–HOAc behaved in a similar fashion to the polyelectrolyte gels, absorbing a considerable fraction of Bhb with relatively high affinity when compared to the gel washed with HOAc only. These results indicate that SDS alone, not HOAc or the SDS–HOAc combination, is responsible for the high-affinity binding exhibited by both protein imprinted and non-imprinted polyampholyte hydrogels. In an effort to remove any excess SDS and prevent non-specific binding caused by SDS entrapped within the polymer matrix, all protein imprinted and non-imprinted hydrogels were washed using a modified protocol. Specifically, an additional wash with 3 M NaCl was added subsequent to the SDS–HOAc wash.

3.1. Positively charged polyampholytes

Fig. 3 shows the results of a Bhb template rebinding experiment performed on an imprinted polyampholyte hydrogel containing 25% AMPS and 75% MAPTAC. The gel shows good recognition properties, as approximately 4.6 mg (77%) of the Bhb template resided in high-affinity binding sites subsequent to the template association step. In comparison, an insignificant amount (1%) of the Bhb template remained in low-affinity binding sites. The results indicate that the inclusion of both positive and negatively charged monomers in the hydrogel matrix results in an increase of the recognition properites of the MIP hydrogel. Fig. 3 shows the Bhb template rebinding for an identical, non-imprinted polyampholyte hydrogel. Notice that this rebinding profile is nearly the opposite of the rebinding profile for an imprinted polyampholyte hydrogel.

3.2. Negatively charged polyampholytes

The negatively charged polyampholytes, containing 25% MAPTAC and 75% AMPS, displayed similar behavior to their positively charged counterparts. Fig. 3 shows data from Bhb template rebinding studies performed on negatively charged, Bhb-imprinted polyampholyte hydrogels. The imprinted hydrogels displayed template recognition properties that were nearly identical to those displayed by the positively charged polyampholyte hydrogels. Approximately 4.8 mg (80%) of the Bhb template was bound in high-affinity binding sites subsequent to the template association

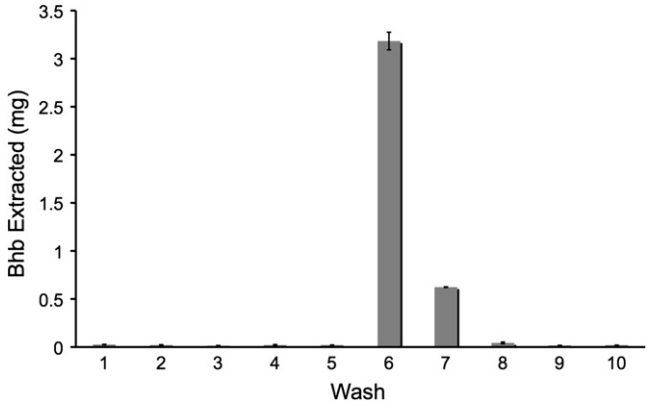

Fig. 4. Bhb template rebinding data for net neutral, Bhb-imprinted polyampholyte hydrogels containing 50% MAPTAC and 50% AMPS. The hydrogel was loaded with 6 mg Bhb.

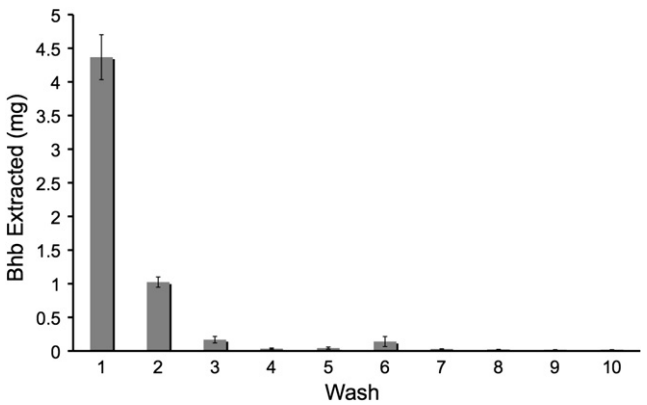

Fig. 5. Bhb template rebinding data for net neutral, non-imprinted polyampholyte hydrogels containing 50% MAPTAC and 50% AMPS. The hydrogel was loaded with 6 mg Bhb.

Table 2
Selectivity factor (α) for polyampholyte gels washed under the modified protocol.

Gel	(α)
25% MAPTAC–75% AMPS	1.3±0.1
25% AMPS–75% MAPTAC	1.2±0.1
50% AMPS–50% MAPTAC	0.8±0.1

step. In addition, a small amount (1%) of the Bhb template was present in low-affinity binding sites, indicating a stronger preference for the Bhb template by the imprinted gels.

3.3. Neutral polyampholytes

Net neutral polyampholytes, containing equal amounts of positive and negative charge, are expected to undergo only a minimal amount of cavity deformation. The equal number of positive and negatively charged monomers should effectively screen one another and therefore limit swelling. The results of Bhb template rebinding experiments performed on net neutral, Bhb-imprinted polyampholyte hydrogels containing 50% AMPS and 50% MAPTAC are shown in Fig. 4. These gels exhibit excellent template recognition properties, with 3.6 mg Bhb bound in high-affinity sites and only 0.4 mg Bhb bound in low affinity sites. The binding experiment performed on non-imprinted gels, shown in Fig. 5, revealed that non-imprinted net neutral hydrogels exhibited template affinity that was nearly opposite of their imprinted counterparts. Nearly 5.2 mg of Bhb was either unbound or bound in low affinity sites subsequent to the template association step, while only 0.2 mg was bound in high affinity sites.

3.4. Selectivity

Selectivity experiments were conducted by loading Bhb-imprinted and non-imprinted hydrogels with cytochrome c (purchased from Sigma Aldrich). Ideally, the Bhb-imprinted polyampholyte hydrogels are expected to exhibit low affinity for cytochrome c and higher affinity for the Bhb template.

As Table 2 shows, the selectivity exhibited by the polyampholyte gels for Bhb was moderate. The positively charged polyampholyte gels exhibited slightly higher selectivity than the negatively charged gels, although the difference was minor. Overall, the polyampholyte hydrogels displayed a lower selectivity than gels containing only positive or negatively charged monomers.

3.5. Swelling

To gain a better understanding of the recognition properties of the polyampholyte hydrogels, swelling experiments were performed on non-imprinted polyampholyte hydrogels. The swelling data (Fig. 6) reveals that polyampholyte hydrogels containing a net negative charge showed increased swelling compared to those gels containing a net positive charge. In comparison with many of the polyelectrolye hydrogels previously reported [8], the swelling ratio of all polyampholyte hydrogels is considerably lower. In fact, only the polyelectrolyte hydrogels containing 0.25% AMPS, and the uncharged polyelectrolyte hydrogels (100% acrylamide) exhibited lower swelling ratios. The lower swelling ratios exhibited by the polyampholyte hydrogels of this study should result in higher affinity for the Bhb template, due to the fact that lower swelling ratios generally correspond to decreased cavity deformation. The polyampholyte hydrogels did, in general, exhibit a higher binding affinity for the Bhb template as well as an increased imprinting factor.

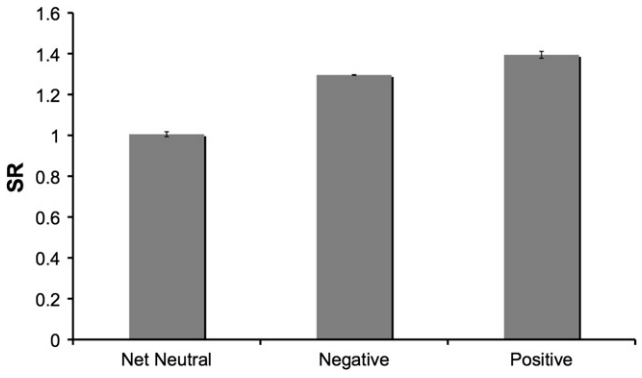

Fig. 6. Swelling ratio (SR) measurements for polyampholyte hydrogels.

4. Conclusion

The results of experiments performed on polyampholyte-imprinted hydrogels indicate that swelling can be controlled to eliminate excess cavity deformation through the simultaneous inclusion of positively and negatively charged co-monomers within the hydrogel. While decreased swelling results in an increased imprinting factor, the selectivity of the hydrogels significantly decreases when compared to gels containing only positively or negatively charged monomers. The high specificity arises from the decreased swelling of the polyampholyte hydrogels compared to their polyelectrolyte counterparts. Decreased swelling results in decreased cavity deformation, and therefore the cavity retains its size, shape, and functional group orientation upon Bhb template rebinding, resulting in higher template affinity and increased imprinting factors. The low selectivity is a result of the presence of the AMPS monomer. The positive charge of the competitive cytochrome c template at neutral pH is associated with the negatively charged AMPS monomer. The polyampholyte hydrogels all contain AMPS, and therefore, the cytochrome c is attracted to negatively charged regions of the polyampholyte hydrogels, resulting in decreased selectivity. The specificity of these polyampholyte hydrogels is comparable to polyelectrolyte hydrogels containing only positive charges [8]. Additionally, our previous work has shown that neutral hydrogels have a third of the specificity of the polyampholyte hydrogels [8]. It is also interesting to note that compared to previous studies the non-imprinted polyampholyte hydrogels bound little or no Bhb [8,11]. The polyampholyte hydrogels specificity is several orders of magnitude worse than natural antibodies but MIP hydrogels have the advantage of reusability, low cost and ease of production.

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