A Nanobiotechnological Approach in the Bating Process

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Abstract

During the traditional bating processes, in order to remove this residuals commonly it's used trypsin enzyme, being isolated from the pancreas glands of swine or cattle. Generally at the end of the bating process, the water of containing enzyme don't used repeatedly, is released to the waste water panels and because of making these mistakes, bating cause extra cost waste water treatment.

Nanoparticles have large surface area and high binding capacity up to hundreds of times their own weight. Also they can use adsorbent of non-fibrillar proteins via dye attachment. In this way, we investigated the dye affinity nanoparticles as an alternative instead of enzyme in bating process. In this study, p(HEMA) (2-hydroxy ethyl methacrylate) nanoparticles were produced by surfactant free emulsion polymerization. P(HEMA) nanoparticles were characterized by Fourier transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM). The triazine dye Reactive Red 120 was chemically attached to the p(HEMA) nanoparticles. These dye incorporated p(HEMA)-Reactive Red 120 affinity nanoparticles was used adsorption of non-fibrillar proteins in aqueous solutions. In addition optimization of experimental conditions were studied in batch reactor and adsorption capacity of p(HEMA)-RR 120 was determined. It was evaluated the reusability of the nanoparticles and efficient of bating agent by results. As a result, p(HEMA)-Reactive Red 120 nanoparticles have a potential of ecofriendly, cheap and reusable material than enzyme in bating process.

Keywords: Nano Sized Particle, p(HEMA), Reactive Red 120, Leather, Bating, Waste Water, Non-fibrilar Proteins

1. Introduction

Leather industry is an age-old industry and which has been serving the society industry (Aravindhan et al. 2006). There are supplies various methods for can benefit from pelt skin, which is convert incorruptible, availed forms. This one of the methods is tanning process. Tanning is more durable and less susceptible to decomposition. In addition to advantage of tanning, this prevents thermal and microbial degradations (Krishnamoorthy et al. 2013). Several methods is helping to tanning process, one of those is bating process. Bating is the most important tannage processes. Main constituent of raw skin are moisture %65, proteins %33, mineral matter %0,5 and fatty %2-6 (Arunachalam and Saritha. 2009). Animal skin

contains proteins %90-95, constituting globular proteins (albumin, globulin) %3,5 (Vijayalakshmi et al. 2009). The purpose of bating process is loosing of the non-collagenous skin structure through the removal of the residues of the interfibrillary proteins (Foroughi et al. 2006). This makes them soft and supple and to prepare them for tanning. Also this process, non-remove the collagenous proteins causes a cementing together of the fibris when the leather is dried.

Nowadays, classical bating process in the alkaline condition makes us of proteolytic enzyme such, trypsin, chymotrypsin, and various peptidases (Kamini et al. 1999). Which are of pancreating or bacterial origin (Choudhary et al. 2004; Deselnicu et al. 1994). Bating process depends on the enzyme concentration as well as temperature, pH, time. These conditions affect to the leather quality. Generally at the end of the bating process, the water of containing enzyme don't used repeatedly, is released to the waste water panels and because of making these mistakes, bating cause extra cost waste water treatment.

Recently, dye-ligand affinity interactions have gained a considerable attention in the separation and purification of proteins. Assuring higher specificity, purity and recovery in a single separation step as well as cost efficiency, safety and adsorbent capacity. Dyes are known to bind many types of proteins showing different levels of affinity and specificity. Moreover they can easily be immobilized on matrices especially carrying hydroxyl groups (Yavuz et al. 2006; Karakoç et al.2009). P(HEMA) nanopolymers are hydrophilic, nontoxic, and biocompatible polymers. Also, nanopolymers have large surface area due to their nano-structure (Moradi et al. 2009).

In this study, p(HEMA) (2-hydroxyethyl methacrylate) nanopolymers were prepared by surfactant emulsion polymerization. Reactive Red 120 was then covalently attached to the p(HEMA) nanopolymers as a dye-ligand in order to removal of the non-fibrillar proteins in leather.

2. Material and Method

2.1 Materials

2-hydroxyethylmethacrylate (HEMA), ethylene glycol dimethacrylate (EGDMA), poly-vinyl alcohol (PVA, Mw: 85000-130000), potassium per sulfate (KPS) and other chemicals were supplied by Sigma. Sheep skins supplied from slaughter house.

2.2 Preparation of p(HEMA) Nanopolymers

To obtain polymeric nanopolymers, the following experimental recipe was used. 0.5 g of PVA (poly-vinyl alcohol Mw: 85000-130000) used stabilizer was solved in 45 mL of distilled water by heating in a glass polymerization reactor (100 mL) to obtain globe form. After it had been cool; 0.6 mL HEMA and 0.3 mL EGDMA added to the reaction mixture and then 19.8 mg KPS with 45 ml distilled water added to the mixture to start polymerization. The reactor was purged with nitrogen for 5 minutes in order to remove oxygen in the reactor and then the mixture was reacted at 70 °C for 2 h in a thermostatic water bath. After polymerization, p(HEMA) nanopolymers were centrifuged at 14000 rpm and 20 minutes and solid phase was taken. After separation the solid phase washed sequentially with water and ethyl alcohol.

2.3 Dye Incorporation to p(HEMA) Nanopolymers

Reactive Red 120 was selected as the affinity dye ligand, and covalently bonded to the p(HEMA) films. In order to prepare 0.086 μmol Reactive Red 120 /mg p(HEMA) nanopolymers, the following procedure was applied: 10 mL of solution containing 60 mg Reactive Red 120 was poured into 90 mL of the solution of the p(HEMA) nanopolymers in distilled water, and then 4.0 g of NaOH (pH 10-11) was added. These were then heated in a sealed reactor at 80°C for 4 h at a stirring rate of 400 rpm. The Reactive Red 120-attached p(HEMA) nanopolymers were filtered, and washed with distilled water and methanol several times until all the physically adsorbed dye molecules were removed. In Figure 1, schematic representation of Reactive red 120 attached p(HEMA) nanopolymers is shown.

2.4 Characterization of Dye Attached p(HEMA) Nanopolymers

FT-IR spectra of the p(HEMA) and Reactive Red 120-attached p(HEMA) nanopolymers were obtained by using a FT-IR spectrophotometer (Perkin-Elmer Spectrum 100 device with ATR). SEM photographs of p(HEMA) and Reactive Red 120-attached p(HEMA) nanopolymers were taken by Quanta 250 S FEG.

2.5 Removal Studies of non-fibrillar proteins

In the first group of experiments adsorption of albumin from aqueous solutions, this was investigated in batch adsorption–equilibrium experiments. The effects of concentration of albumin and globulin, pH of the medium and time on the adsorption rate and capacity were studied. Aqueous solutions (500 μL) containing different albumin and globulin amounts were treated with the adsorbents [the dye-attached p(HEMA)] at different pHs (3.0, 4.0, 5.0, 6.0, 7.0 and 8.0) in eppendorf tube at room temperature. And also different adsorption times (5, 15, 30, 45, 60, 90 and 120 minutes) of albumin and globulin on the Reactive Red 120-attached p(HEMA) nanopolymers were investigated. To observe the effects of ion strength, NaCl solution was used at ion strength values of 0 to 0.5 (0, 0.02, 0.05, 0.1, 0.5). Ion strength was treated in the pH 5 and room temperature conditions, it was treated 25 μL p(HEMA) and 475 μL albumin and globulin containing NaCl in eppendorf tube and retention time was 1 hour. The final albumin and globulin concentration in the aqueous phase was determined by using an UV spectrophotometer after each solution had been centrifuged 14500 g and 20 minutes.

The adsorption ratio was calculated from the initial amount of albumin on the polymer and the final albumin and globulin concentration in the adsorption medium, by using the following equation.

$Q=[(C_o-C)\cdot V]/m$

Q is the albumin and globulin adsorption capacity of the Reactive Red 120-attached p(HEMA) nanopolymers (mg/g), Co is the concentrations of albumin and globulin in the solution initially (mg/ml), C is the concentrations of albumin and globulin in the solution after adsorption (mg/ml), V is the volume of the aqueous phase (ml); and A is adsorbent quantity (g).

Desorption of albumin and globulin was studied in 0.05 M Tris/HCl buffer containing 1 M NaSCN. Reactive Red 120-attached p(HEMA) nanopolymers loaded with albumin and globulin in this desorption medium stirred for 2 hours in eppendorf tube at room temperature. The final albumin and globulin concentration in the aqueous phase was determined by using

an UV spectrophotometer after each solution had been centrifuged 14500 g and 20 minutes. Desorption ratio was calculated from the initial amount of albumin and globulin on the polymer and the final albumin and globulin concentration in desorption medium, by using the following equation. In order to determined of reusability of Reactive Red 120-attached p(HEMA) nanopolymers adsorption-desorption cycle was repeated three times by same nanopolymers.

Desorption Ratio (%) = (amount of albumin desorbed to the elution medium / amount of albumin and globulin adsorbed on the nanopolymer) x 100

In the second group of experiments, supplied sheep leather were applied various process for the bating processing. Applied processes were showed to the table 1. Sheep leathers were cut from back area to two pieces. And then was named as one piece experiment, other one piece control group. Experiment groups were supplied with Reactive Red 120-attached p(HEMA) nanopolymers and control groups were supplied with distil water in the bating process. Other experiment groups were supplied with enzyme perpetrate and control groups with distil water in the bating process. And of the bating process was a determined albumin and globulin adsorption value in the bath by using an UV spectrophotometer. In order that desorption values, was used as a desorption agent 0.05 M Tris/HCl buffer containing 1 M NaSCN.

3. Results and Discussion

3.1 Properties of poly(HEMA) and Dye attached poly(HEMA) nanopolymers

P(HEMA) can be fairly reactive in nucleophilic substitution reaction via free alcoholic- OH groups and occurring chemical bond if substrate has a leaving group which is suitable for substitution reaction, such as chloro atoms. Thus, an adsorbent can be yielded containing aromatic groups. FTIR spectra of p(HEMA) and Reactive Red 120-attached p(HEMA) nanopolymers 120 are given in Figure 2. FTIR spectra of both p(HEMA) and Reactive Red 120-attached p(HEMA) nanopolymers have the characteristic stretching vibration band of hydrogen bonded alcohol, O-H and N-H groups around 3446 cm^{-1}. The band at 1324-1617 cm^{-1} represent symmetric stretching of aril C=C bond vibration. The band at 3466 and 2987/2955 cm^{-1} represent alcoholic O-H strain and aliphatic CH_2-CH_3 strain in p(HEMA). Blistring pic At 1727 cm^{-1} due to there is C=O. The Reactive Red 120 molecules were covalently attached into the p(HEMA) beads. And also ether linkages are formed between reactive triazine ring of the dye and the hydroxyl groups of the p(HEMA).

The surface morphology and internal structure of the p(HEMA) beads are shown by the Electron micrographs (Quanta 250 S FEG) in Figure 3. As seen in the figure, nanopolymers are show sphere shape. Particles size diameter 17 to 44 nm was determined. Nanopolymers more can absorb proteins molecules because of nanopolymers have a little size diameter. Also nanopolymers have a large surface area.

3.2 Removal of non-fibrillar proteins

3.2.1 Adsorption time

Figure 4 shows the adsorption time. As seen in the figure, with an increase at time, the adsorbed amount of albumin (BSA) in solution. The adsorption amount of albumin was very high at the 60 min. The adsorption amount of albumin doesn’t change an increase time. In

addition to figure 5 shows the adsorption time. As seen in the figure, with an increase at time, the adsorbed amount of globulin (IgG) in solution. The adsorption amount of globulin was very high at the 60 min. The adsorption amount of globulin doesn't change an increase time. Thus adsorption time was selected 60 min.

3.2.2 Effect of albumin and globulin concentration

Figure 6 shows the effects of concentration of albumin adsorbed. As seen in this figure, with an increase in albumin concentration in solution, the adsorbed amount of albumin per unit mass of beads increases until about 10 mg/mL, and then approaches saturation. It is clear that this increase in adsorption amount is because of specific interaction between attached dye molecules (i.e., Reactive Red 120) and albumin molecules which promote the adsorption of albumin (Akgöl et al. 2007).

3.2.3 Effect of pH

Figure 7 and figure 8 shows the effect of pH. As seen the figure, in all the investigated cases, the maximum adsorption of albumin and globulin are observed at pH 6.0. With the increase of pH above and below pH 6.0, albumin and globulin adsorption amount decreased. Experiments were supplied pH 3-8 between and every experiment was prepared with different buffers. However the adsorption capacity of Reactive Red 120 attached p(HEMA) nanopolymers has increased between pH 3-6 values, has reduced between pH 6-8 values. Interactions between dye and protein molecules, in this way may explain amino acid chains in albumin and globulin structures and the ionization states of several groups on both the ligands.

3.2.3 Effect of ionic strength

The effect of ionic strength (adjusted by adding NaCl) on albumin and globulin adsorption is presented in Figure 9 and figure 10, which shows that beginning, the adsorption amount increases bitingly with increasing ionic strength. And then the adsorption amount decreases with increasing ion strength. In addition to the adsorption amount increases with increasing ionic strength. The NaCl concentration changes from 0 to 0.5 M. The decrease in the adsorption amount as the ionic strength increases can be attributed to the repulsive electrostatic interactions between the dye-attached beads and protein molecules.

3.3 Desorption and Repeated use of Reactive Red 120 attached nano p(HEMA)

Desorption of the adsorbed albumin and globulin from the Reactive Red 120 attached p(HEMA) beads is studied in a batch experimental setup. Adsorbed of albumin and globulin are removed when 0.05 M Tris/HCl buffer containing 1 M NaSCN, and retention time was 2 hours. Desorption resulting shows that the nanopolymer can be desorbed respective % 55 and % 67. In addition to it hasn't seen any dye leakage in the nanopolymer solution during the desorption process. Because dye molecules are attached strongly to p(HEMA) beads. The Reactive Red 120 attached p(HEMA) nanopolymer can be used repeatedly and adsorption-desorption cycle are repeated 3 times in a batch experimental set-up.

4. Conclusion

From this study, as p(HEMA) Reactive Red 120 nanopolymer is ability protein containment (adsorption), alternative a studying was performed to enzyme studies in the bating process.

Nano-sized p(HEMA) were prepared by polymerization technique, Reactive Red 120 was covalently attached to the polymer. Optimum adsorption conditions of albumin and globulin were determined.

This study showed that Nano-sized p(HEMA) can be used as a bating material that it supported some environmental protection. However, further studies need to be conducted to develop new leather products using Nano-sized p(HEMA).

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Table 1: The preparing of sheep skins for bating process.

Process	**Prop. %**	**Material**	**Temp. °C**	**Time**	**pH**
Soaking	500	water	25		
	0,2	bactericid		1 day	
Unhairing	16 Be	Na2S	25	4 hour	
	10 Be	Ca(OH)2			
Liming	250	water	25	1 night	12
	2	Na2S			
	5	Ca(OH)2			
Washing					
Deliming	300	water	25	1 hour	8.2
	3	Amonium Sulphate			
Bating	100	water	37	1 hour	
	1	Enzyme (tyrpsin)			
Washing					

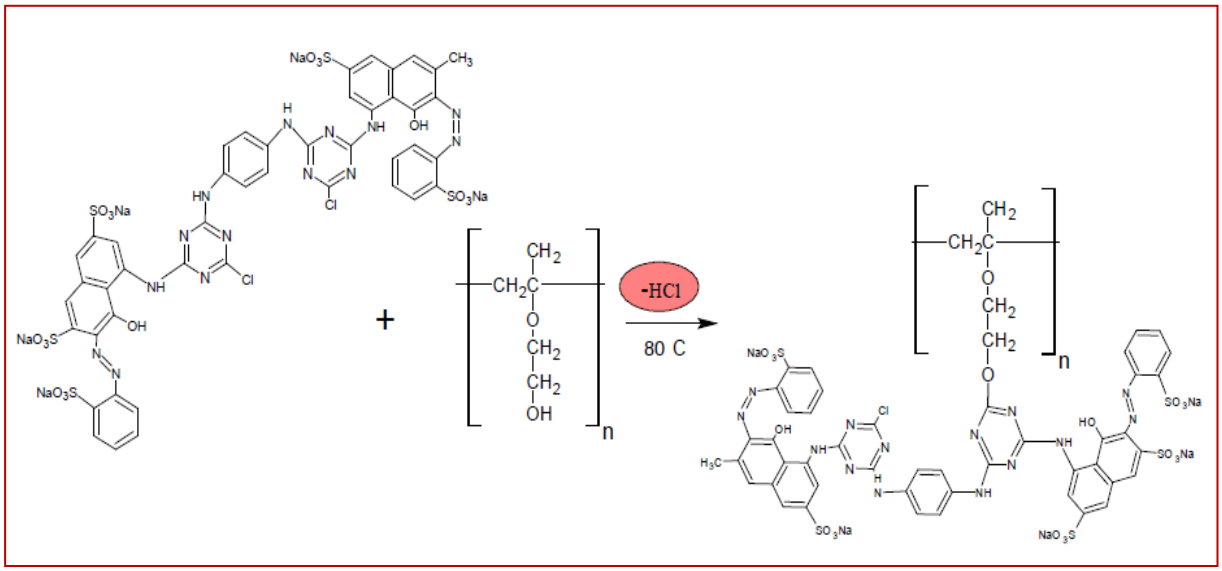

Figure 1: Schematic representation of Reactive Red 120 attached p(HEMA) nanopolymers.

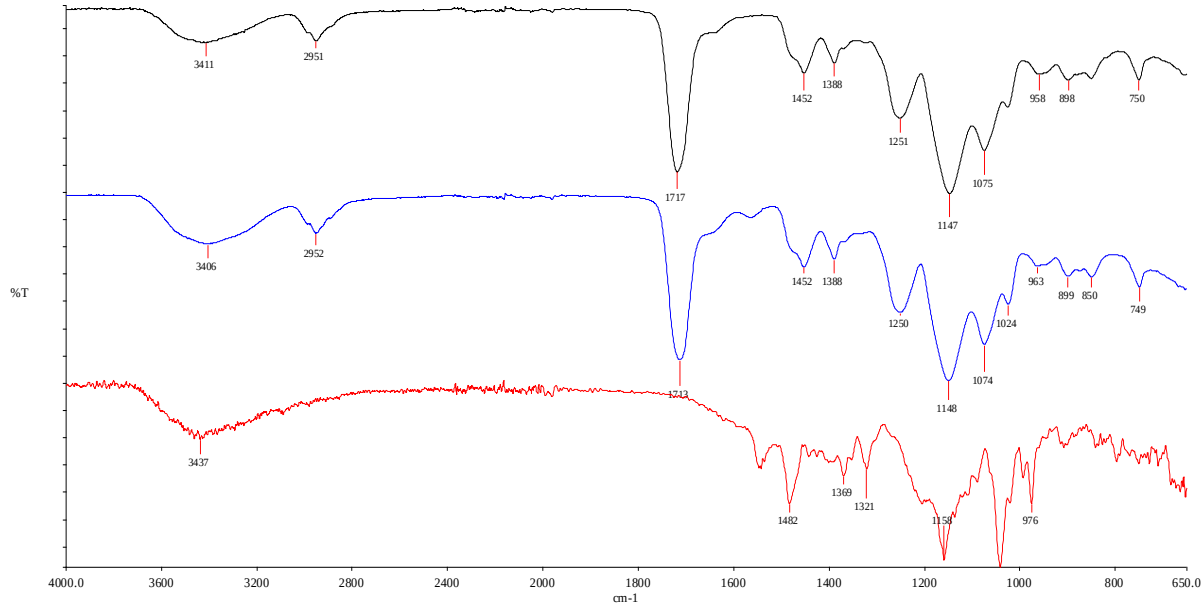

Figure 2: FTIR Spectra of: (a) p (HEMA), (b) Reactive Red 120 attached p(HEMA) and (c) Reactive Red 120.

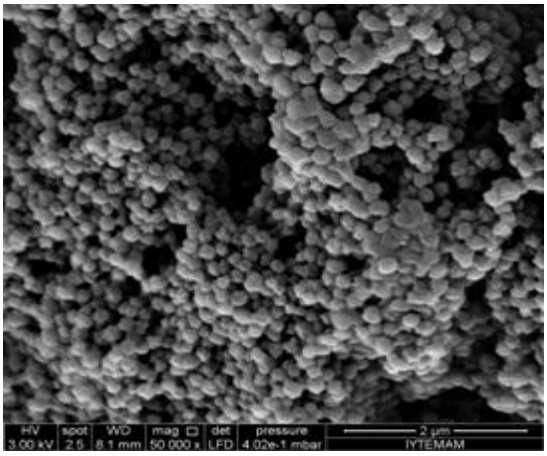

Figure 3: The surface morphology and internal structure of the p(HEMA) beads.

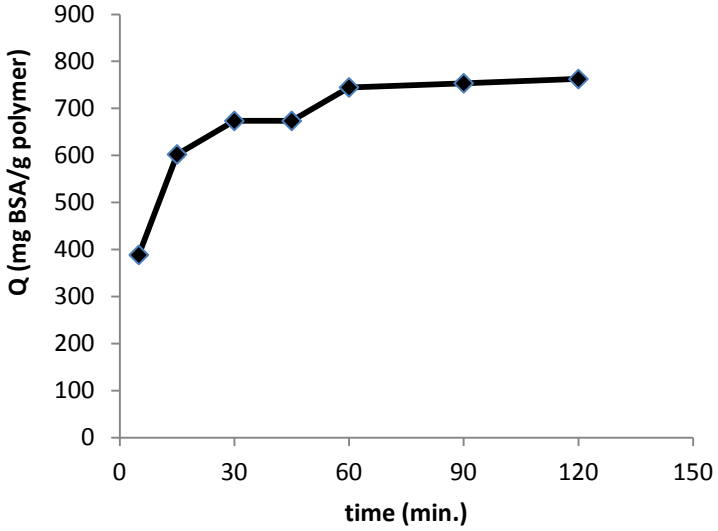

Figure:4 The adsorbed amount of albumin (BSA) as a function of time ($C_{BSA:}$ 5mg/mL, pH 6, T:25˚C)

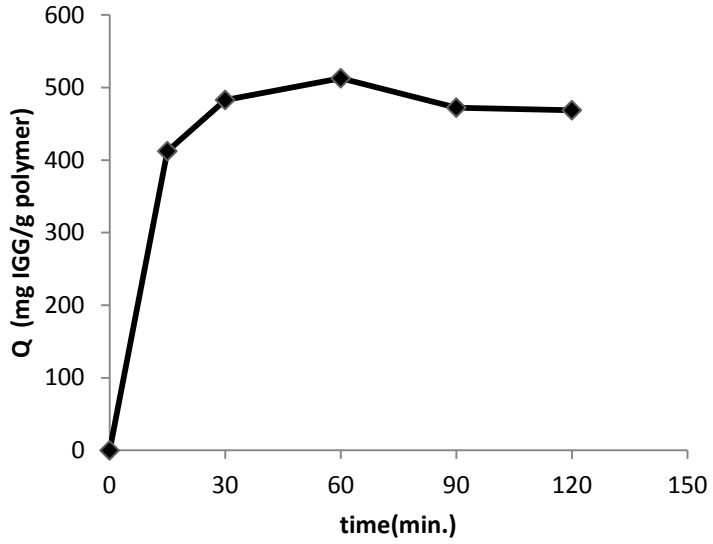

Figure:5 The adsorbed amount of globulin (IgG) as a function of time ($C_{IgG:}$ 5mg/mL, pH 6, T:25˚C)

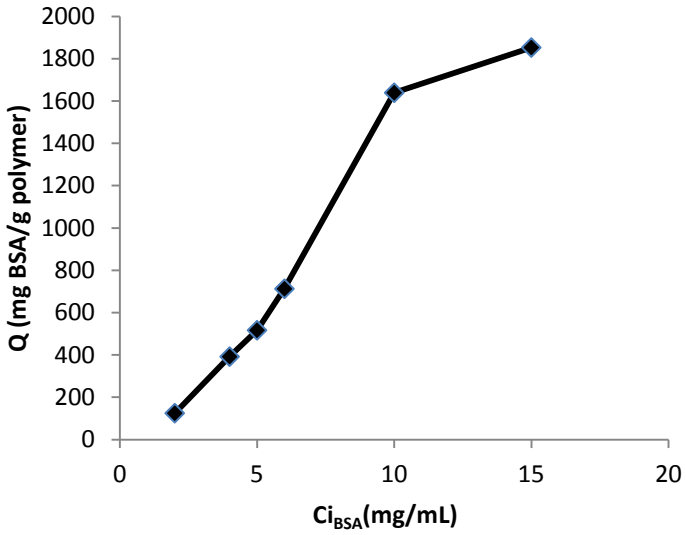

Figure 6: The adsorbed amount of albumin (BSA) as a function of initial albumin concentration (pH 6, T:25˚C)

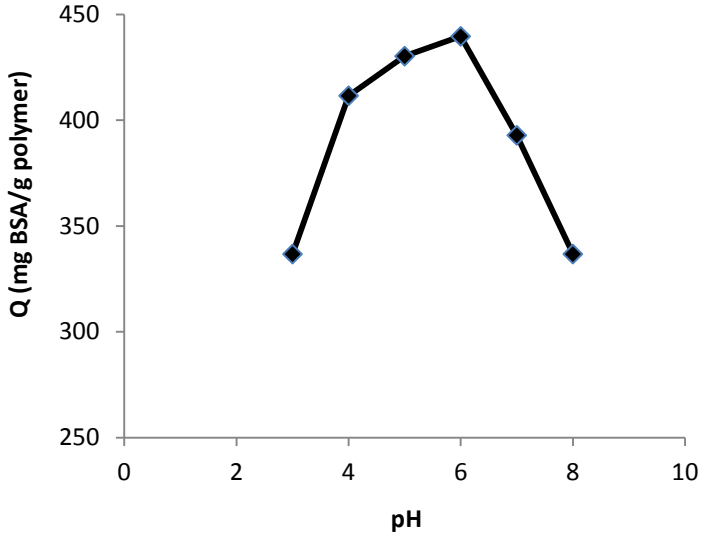

Figure 7: pH effect of albumin (BSA) adsorption (C_{BSA}: 5mg/mL, T:25˚C)

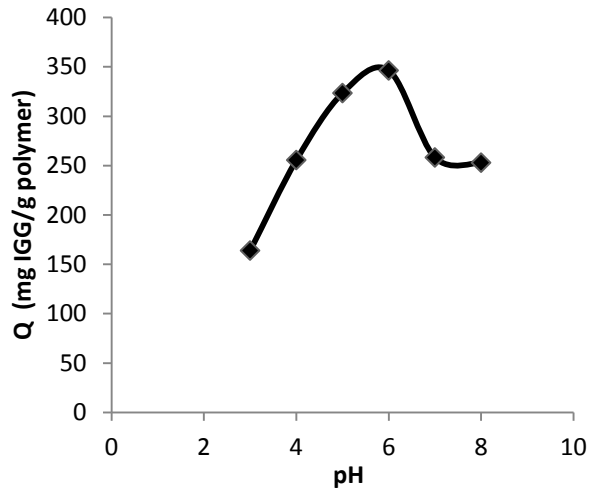

Figure 8: pH effect of globulin (IgG) adsorption on Reactive Red 120-attached p(HEMA) nanopolymers ($C_{IgG:}$ 5mg/mL, T:25˚C)

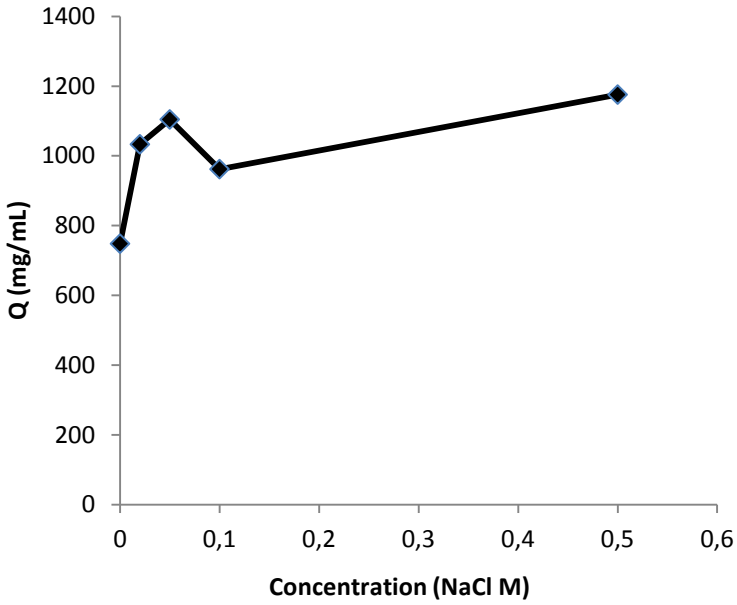

Figure 9: Ion strength of albumin (BSA) adsorption (C_{BSA}: 5mg/mL, pH 6, T:25˚C)

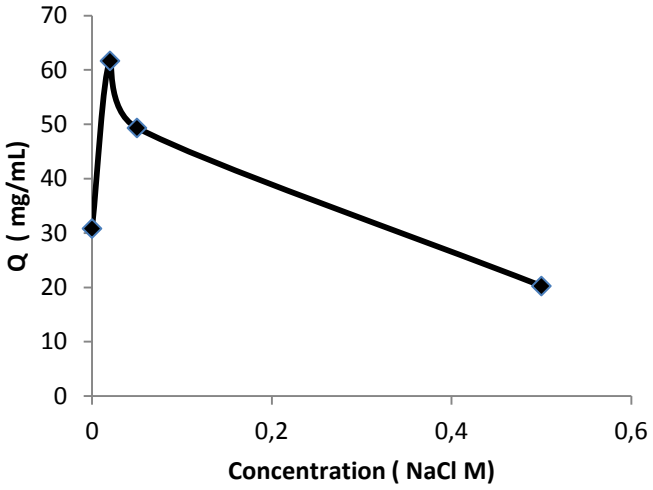

Figure 10: Ion strength of globulin (IGG) adsorption ($C_{IgG:}$ 5mg/mL, pH 6, T:25˚C)