BSA protein adsorption and biological activity of poly(N-tert-butylacrylamide-co-AMPS IL) Hydrogels

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Abstract

Novel ionic liquid based hydrogels were prepared by using AMPS IL, N-tert-butylacrylamide monomers, methylenebisacrylamide(MBA) as crosslinker and benzoyl peroxide(BPO) as initiator via free radical polymerization in methanol at 60°C. The synthesized monomers and hydrogels were characterized by FT-IR and NMR spectral analysis to conform the monomeric units in the hydrogels. For BSA adsorption, the effect of pH, temperature and adsorption rate were studied. The maximum BSA adsorption achieved at pH 5.0 and at 10°C temperature. The synthesized hydrogels are subjected to investigate the biological activities such as antibacterial, antifungal, antioxidant, anthelmintic. These results showed that the hydrogels are active against bacterial and parasitic infections.

Keywords: Hydrogels, N-tert-butylacrylamide, ionic liquid, antioxidant, anthelmintic

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INTRODUCTION

Ionic liquids (ILs) received considerable interest because of their special properties like thermal stability, chemical stability and ionic conductivity [1-3]. Recently, ILs are used to prepare polymeric materials[4-8] which are used immobilization in solid device and as polyelectrolytes. Ionic Liquid also used to prepare BSA imprinting sensitive Hydrogels[9].

Hydrogels are usually non-toxic, watery gel having three dimensional (3D) polymeric network absorbing more amount of water and are not soluble in water. The swelling behavior and hydrolytic stability of acrylamide based hydrogels can be improved by substituting alkyl groups in acrylamide [10]. Stimuli responsive hydrogels have been used for immobilization or isolation of enzymes, proteins and cells [11-19]. Due to their absorbing capacity hydrogel surfaces in solutions led to wide applications in wastewater treatment, fine particle recovery and protein adsorption[20-22]. Hydrogels possess antibiotic resistance, antimicrobial activities and implant materials [23]. Quaternary ammonium cation based poly(ionic liquid) membranes containing Zinc ion used as antimicrobial wound healing [24]. Anbarasan et al., studied the swelling behavior of the AMPS-IL based hydrogels and noticed that the swelling behavior increased with the increasing amount of AMPS-IL [25]. Since the ionic hydrogels were used as systems of controlled drug release, protein separation and immobilization of enzymes, we focused on the synthesis of poly(N-tert-butylacrylamide-co-AMPS-IL)hydrogels in order to investigate the BSA adsorption and biological activities.

EXPERIMENTAL

Materials

Bovine serum albumin (BSA),2-acrylamido-2-methyl-1-propane sulfonic acid (AMPS), N, N'-ethylenebisacrylamide (MBA) and Benzoyl peroxide (BPO) were used as received.

Preparation of N-tert-butylacrylamide

The N-tert- butylacrylamide (NTB) monomer was prepared by the reaction of t-butylalcohol and acrylonitrile at cold condition [26]. The synthesized monomer was recrystalised using warm benzene.

Preparation of Ionic liquid monomer (AMPS IL)

The AMPS-IL monomer was synthesized by dissolving 10.35g of AMPS into 7.0 ml of trimethylamine with continuous stirring at room temperature [25].
Synthesis of poly(N-tert-butylacrylamide-co-AMPS IL) hydrogels

The NTB and AMPS IL monomer with different feed ratios, 0.1 weight % of BPO (with respect to monomers) and MBA crosslinker were dissolved in aqueous methanol. After bubbling nitrogen for 20 min, the solutions were placed in a thermostatic water bath maintained at 60\degree C for a period of 12 hours to complete the polymerization. The synthesized hydrogels were washed with aqueous methanol and dried at 40\degree C under vacuum.

Characterization of poly (N-tert-butylacrylamide-co-AMPS IL) hydrogels

The IR spectra of prepared hydrogels were recorded on the IR Affinity 1. The 1H-NMR spectrum of hydrogels were recorded on the GSX-400 spectrometer (JEOL, Tokyo, Japan) operating at 400 MHz in CDCl₃. Thermal Analysis (TG-DTA) was carried out using TGA SDT Q600 V20.9 Build 20 under nitrogen atmosphere.

BSA adsorption behavior of hydrogels

Approximately 0.1g of dried hydrogel placed in 20 ml of solution containing 4.0mg/ml of BSA in B-R buffers (with various pH levels) and allowed to equilibrium swelling at 30\degree C. A portion of the protein solution was taken from the beaker containing hydrogels and measured their concentration at 279nm using UV-Visible spectrophotometer. The amount of BSA adsorbed by the hydrogel (qₑ) was calculated using the following equation.

\[ qₑ = \frac{(Cᵢ - Cₑ)Vₑ}{m} \]

where qₑ is the amount of BSA adsorbed in mg, Cᵢ is the initial concentration, Cₑ is the equilibrium concentration of solution, Vₑ is the volume of solution taken and m is the mass of the dry hydrogel. To study the effect of temperature on the adsorption behavior the experiments carried out from 10\degree C to 60\degree C. For determine the effect of pH on the adsorption the experiments were carried out using the same procedure at various pH level (pH = 2.0-9.0).

Biological activities of the synthesized hydrogels

Antimicrobial activity

To investigate antimicrobial studies of hydrogels, the hydrogels were dissolved in dimethyl formamide (DMF). The antibacterial activities were determined by disc diffusion method (using ciprofloxacin as standard drug). Sample discs were soaked in test solution and standard Ciprofloxacin and placed in the plates maintained at 37\degree C for 24 hrs. After 24hrs of incubation, the bacterial growth (gram +ve and gram –ve) zone of inhibition was noticed and is given in mm. Similarly, the antifungal activity also determined using Fluconazole as standard drug.

Anthelmintic (antiparasitic) activity

The in-vitro anthelmintic screening of hydrogel solutions were tested against adult earthworm. Earthworms were placed in 50 mL formulations containing three different concentrations (25, 50 and 100 mg/mL in normal saline) of prepared hydrogel with composition 0.5:0.5. The time taken for paralysis and death of the worm was noted. Normal saline was used as control and Albendazole (10 mg/mL) as reference standard.
Antioxidant activity

The antioxidant property of hydrogels was studied spectrophotometrically using DPPH radical scavenging method and absorption was measured at 517nm.

RESULTS AND DISCUSSION

The schematic representation of poly (N-tert-butylacrylamide-co-AMPS IL) hydrogel is given as scheme 1. The feed composition of monomers was given in Table 1.

Scheme 1. Synthesis of poly (N-tert-butylacrylamide-co-AMPS IL) hydrogel
Table 1. Copolymerization of N-tert-butylacrylamide and AMPS IL

<table>
<thead>
<tr>
<th>Mole fraction of NTB in feed, $M_1$</th>
<th>Mole fraction of AMPS-IL in feed, $M_2$</th>
<th>Reaction time (hrs)</th>
<th>Weight of hydrogel (g)</th>
<th>Hydrogel conversion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>0.9</td>
<td>12</td>
<td>0.2954</td>
<td>9.59</td>
</tr>
<tr>
<td>0.3</td>
<td>0.7</td>
<td>12</td>
<td>0.2895</td>
<td>9.39</td>
</tr>
<tr>
<td>0.5</td>
<td>0.5</td>
<td>12</td>
<td>0.2845</td>
<td>9.23</td>
</tr>
<tr>
<td>0.7</td>
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<td>12</td>
<td>0.2812</td>
<td>9.12</td>
</tr>
<tr>
<td>0.9</td>
<td>0.1</td>
<td>12</td>
<td>0.2799</td>
<td>9.08</td>
</tr>
</tbody>
</table>

Spectral Characterization of poly (N-tert-butylacrylamide-co-AMPS IL) hydrogel

FT-IR spectroscopy

The synthesized hydrogel was characterized by FT-IR spectroscopy (Figure 1). In the FT-IR spectra, the absorption band at 3429 cm$^{-1}$ revealed the presence of $\text{–NH}$ group. The peak observed at 2972 cm$^{-1}$ corresponds to CH stretching. Peak at 1659 cm$^{-1}$ confirmed the presence of C=O group. The characteristic peak at 1561 cm$^{-1}$ showed absorption of $\text{–C\text{-N}}$ bond. The peaks at 1224 cm$^{-1}$ and 1039 cm$^{-1}$ were attributed to $\text{–S=O}$ and S-O bond in AMPS IL respectively. Peak 626 cm$^{-1}$ is absorption of the C-S bond. The FT-IR analysis conforms the incorporation of all monomeric units in the crosslinked hydrogel network.

Figure 1. FT-IR spectrum of poly (N-tert-butylacrylamide-co-AMPS IL) hydrogel
**1H- NMR spectral studies**

The 1H-NMR spectrum of the synthesized hydrogel is shown in Figure 2. The characteristic group peak assignments of hydrogels by 1H-NMR can be made as follows: 1.25-1.93ppm showed the presence of tert-butyl proton of NTB and dimethyl protons present in comonomer AMPS-IL, 3.07-3.18ppm for CH₂ and 7.25-9.88ppm for NH protons.

![1H-NMR spectrum of poly (N-tert-butylacrylamide-co-AMPS IL) hydrogel](image)

**Figure 2.** 1H-NMR spectrum of poly (N-tert-butylacrylamide-co-AMPS IL) hydrogel

**Thermal Analysis**

Thermogram for the varying feed compositions of poly (NTB-co- AMPS IL) hydrogels are shown in Figure 3. The thermal degradation of poly (NTB-co- AMPS IL) hydrogels occurred in three stages: evaporation of residual water until 130°C, decomposition of amide groups in the range of 282-306°C, degradation of sulfonic groups and breakdown of polymer backbone from 410 - 420°C. The residual weight % for various compositions 0.3:0.7, 0.5:0.5, 0.7:0.3 are 2.435, 1.241 and 1.43 respectively.
Figure 3. TGA of poly (NTB-co- AMPS- IL) hydrogels (a) 0.3:0.7 (b) 0.5:0.5   (c) 0.7:0.3

Effect of pH and Temperature on swelling of hydrogels

The effect of pH and Temperature on swelling of hydrogels is shown in Figure 4 and Figure 5 respectively. The influences of pH on the adsorption of protein (BSA) onto poly (NTB-co-AMPS- IL) hydrogels were inspected by varying the pH of the protein buffer solution between 2 and 9. The initial concentration of BSA was fixed at 4mgmL⁻¹. For all the hydrogels examined, the BSA adsorption capacity increased with increasing pH value and attained a maximum value and then decreased. As a consequence of electrostatic repulsion between similar charge protein molecules on hydrogels, the BSA adsorption ability of the hydrogel get reduced in more acidic and alkaline pH regions. Even though, the dissociation of AMPS IL unit increases at high pH buffer solutions, alteration of the charge on protein molecules and conformational variations produces a decrease in the amount of adsorbed BSA. The maximum adsorption hydrogels with different monomer feed compositions were found at pH 5.0 buffer solution, close to the isoelectronic point of BSA(4.8). This may be attributed to the ionic interactions between the negative (SO₃⁻ unit of hydrogels) and positive charges (lysyl or arginyl unit of BSA) available on BSA adsorbed hydrogel[27]. The conformation adapted by protein molecules was another factor that contributed to the variation of BSA adsorption capacity on polymeric hydrogels.

The effect of temperature on adsorption of BSA molecule was investigated in the temperature range of 10-60°C for hydrogels of varying feed compositions. From Figure 6, it is clear that the maximum adsorption of BSA was observed at 10°C. The equilibrium adsorption of BSA onto hydrogels considerably decreased with increasing temperature. This may be credited to the chemical interaction between the polymer chains and protein with increase in temperature. Since adsorption is an exothermic phenomenon, the protein adsorption ability got decreased with temperature[28].
Figure 4. Effect of pH on BSA adsorption

Figure 5. Effect of temperature on BSA Adsorption
Biological activity of poly (NTB-co-AMPS IL) hydrogel

The zone of inhibition of the hydrogel against microorganisms such as *Staphylococcus aureus, Bacillus subtilis, Micrococcus luteus* (Gram +ve), *Escherichia coli, Pseudomonas aeruginosa, Salmonella paratyphi* (Gram -ve) are shown in Figure 6. The zone of inhibition revealed that the antibacterial activities of the test compounds are specific to the microorganisms examined. The hydrogel is more active against *Pseudomonas aeruginosa*, which is a Gram -ve bacteria. The data also points out that all hydrogels with varying feed composition shows good inhibition values. It leads to the conclusion that the hydrogel possessing N, O, S groups exhibits good antibacterial properties.

The zone of inhibition of the hydrogel against fungi such as *Candida albicans, Aspergillus niger* and *Aspergillus fumigatus* are shown in Figure 7. The zone of inhibition indicated that the antifungal properties of hydrogels were different against various fungi employed. All feed compositions of hydrogel shows good inhibition value against the fungi *Aspergillus niger*. The hydrogel exhibited good activity even in low concentrations of NTB. The efficacy of copolymeric hydrogels to resist the growth of microorganisms increases with increase in NTB content in the hydrogel.

Figure 6. Antibacterial activity of poly (NTB-co-AMPS- IL)hydrogel

Figure 7. Antifungal activity of poly (NTB-co-AMPS- IL) hydrogel
Anthelmintic activity

The anthelmintic activity of hydrogel (0.5:0.5) was examined using earthworms. The time of paralysis and time of death were studied and the activity was compared with Albendazole as standard (Figure 8). The hydrogel exhibited significant anthelmintic activity as evidenced by decreased paralyzing time and death time. It is observed that the higher concentration of extract produced paralytic effect much earlier and the time taken for death was shorter for the worm studied. The results thus support the use of hydrogel as an anthelmintic agent. The wormicidal activity of the hydrogel against earthworms suggests that it is effective against parasitic infections of humans.

Antioxidant Activity

2,2-diphenyl-1-picrylhydrazyl (DPPH) assay characterized as free radical assay with maximum absorption at 517 nm is an antioxidant assay rooted on electron transfer. The DPPH assay provided an easy and rapid way to determine the antioxidant activity of the prepared hydrogels. The free radical scavenging activity of different compositions of hydrogels was investigated by taking quercetin as standard. The Percentage inhibition of DPPH radical Scavenging assay of hydrogel is shown in Figure 9. The data indicates that there is an increase in DPPH scavenging activity with increase in the concentration of the standard. DPPH scavenging activity of hydrogel showed an increase in activity with increase in sample concentration. Ionic hydrogel with composition 0.7:0.3 showed maximum scavenging activity of 93.76% at sample concentration 100mg/mL.

![Figure 8. Anthelmintic activity of poly (NTB-co-AMPS-IL) hydrogel](image-url)
CONCLUSION

In this study, various feed compositions of poly (NTB-co-AMPS IL) hydrogels were prepared by free radical copolymerization in methanol at 60°C. Characterizations were made using FT-IR, 1H-NMR and TG-DTA analytical techniques. The swelling results of hydrogels showed maximum protein adsorption at pH 5.0 and at temperature 10°C. The presence of sulphur and nitrogen groups in the hydrogels had a noticeable advantage in its antimicrobial property. The anthelmintic activity of hydrogels was effective against parasitic infections of humans. The DPPH scavenging assay results supporting the use of ionic liquid hydrogel as an antioxidant agent.

REFERENCES


