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Lysozyme Imprinted poly(hydroxyethyl methacrylate) Cryogel Membrane with the Assistance of polydopamine

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ABSTRACT

The surface imprinted technique was utilized in creating the synthetic lysozyme receptors on the poly(hydroxyethyl methacrylate) [p(HEMA)] cryogel membrane with the assistance of polydopamine under alkaline conditions. Following the design of the artificial receptors, Fourier-transform infrared spectroscopy (FTIR), scanning electron microscope (SEM) and the swelling test were used for the characterization studies and the lysozyme adsorption capability of the adsorbent was evaluated in the aqueous solution.

The maximum adsorption capacity (Qmax) of lysozyme was found as 108.9 mg/g and the adsorption of the target protein on the adsorbent was monolayer and showed a good fit to the Langmuir isotherm model. The IF factor was 3.26 and the adsorbent was highly selective against creatinine and bovine serum albumin (BSA). Furthermore, the adsorbent maintained its stability after 5 adsorption, desorption, and regeneration cycles.

Keywords:
Lysozyme; Adsorption; Molecular imprinting; p(HEMA); Dopamine; Cryogel

INTRODUCTION

Molecular imprinting technology (MIT) is a versatile technique to design or create specific recognition cavities that recognized the template molecules with high specificity and selectivity like natural antibodies [1,2]. Before the design of tailor-made receptors, the functional monomer/s are pre-organized with the template to be recognized and the polymerization has occurred with the suitable polymer precourses. After the polymerization, the molecule to be recognized is extracted from the polymeric material to design the specific binding cavities, which recognize the template molecule with its shape, size, and 3D structure [3].

The surface molecular imprinting technique is a branch of MIT and the recognition of cavities has been conducted on the surface of the adsorbent by using the same method during the MIT [4]. However, this approach with high separation capability reduces the embedding process as compared with MIT [4], so, these key features make the surface imprinted technology to be utilized for many purposes e.g., sensor technology [5], biomedical areas, separation, and purification studies [4]. Furthermore, by using the surface imprinting technique, it is possible to eliminate the template removal problem and increase mass transfer during the adsorption process [6].

Cryogels with interconnected macropores materials are synthesized under semi-frozen conditions [7] and these materials have potentially been used in separation, and purification studies [8-10] due to the superior features like high swelling kinetic [11], high mass transfer capability [12]. In addition to these superior features, the surface modification of these materials including p(HEMA) based cryogels make them favorable adsorbents for purification and separation studies of various analytes [13-15].

Dopamine is a natural molecule that contains catechol and amine functional groups [16] and is self-polymerized on the surface of metal oxides, polymers, and ceramics [17,18] under mild alkali conditions. By using the polymerization of dopamine on the adsorbents or the sensing surfaces, various molecules such as hormones [19], proteins [20], clinic analytes [21], and antibiotics [22] could be imprinted via the surface imprinting technique.

In this research article, dopamine was polymerized on the p(HEMA) based cryogel membrane under alkali conditions [20] via the surface imprinting approach to creating the artificial lysozyme recognition cavities on the p(HEMA) based cryogel membrane surface. After the removal of lysozyme using 5% sodium dodecyl sulfate (SDS) solution, the prepared adsorbent was optimized with the adjusting of pH of the adsorption medium and the lysozyme concentrations in the aqueous solution. Following the optimization studies, the selectivity of the p(HEMA) adsorbent towards lysozyme was evaluated against the BSA and creatinine.

MATERIALS AND METHODS

Materials

The basic monomer of the p(HEMA) cryogel, 2-hydroxyethyl methacrylate (HEMA), dopamine, lysozyme, ammonium persulfate (APS), N,N,N′,N′-Tetramethyl ethylenediamine (Temed), BSA, and creatinine were purchased from Merck. During the whole experimental studies, deionized water (DW) was obtained from Barnstead (Dubuque, IA, USA) ROpure LP® reverse osmosis unit system.

Methods

Preparation of p(HEMA) cryogel membranes and creation of imprinted cavities

The p(HEMA) based cryogel membranes were fabricated via free-radical polymerization using APS and TEMED pairs. For the preparation of p(HEMA), 1.3 mL of HEMA and 3.7 mL of DW were mixed in a beaker at room temperature (RT) during the homogenized solution occurred. In the other beaker, the crosslinker, MBAAm, (283 mg) was dissolved at RT and the two solutions were mixed and stirred again at RT during the homogenous solution occurred. After that, APS (20 mg), the initiator, was added to the polymer solution that was stirred for 5 min in the ice bath. Following that, TEMED (25 µL) was added to the solution and the polymer solution was stirred again for 1 min in the ice bath again, finally, the adsorbents were prepared between two glass plates under -14°C for 24 h. After the polymerization, the p(HEMA) membranes were cut using a perforator and were washed with DW during the washing color of the solution turned white.

The lysozyme imprinted cavities on the p(HEMA) membrane surfaces were designed with the previous study [20]. Before the formation of artificial recognition lysozyme cavities, the p(HEMA) cryogel membranes were washed with DW and the adsorbents were swelled with pH 8.5 (25 mM) Tris-HCI buffer solution for 30 min at RT using an orbital rotator at 10 rpm. After that, 20 mg of lysozyme was added to the polymer solution containing p(HEMA) cryogel and the solution containing the target protein was stirred for 5 h at RT using an orbital rotator at 10 rpm. Following that, 20 mL of dopamine (100 mg) was added to the polymer solution and stirred overnight at RT using an orbital rotator at the same rpm value. Subsequently, the adsorbents were obtained from the polymer solution and were washed with DW and finally, 5 mL of SDS solution (5%) was used to remove the lysozyme from the adsorbent surface [s(HEMA)] to create the artificial recognition cavities. The control polymer [d(HEMA)] was prepared with the same procedure without the addition of the protein.

In this work, p(HEMA) was not used during the adsorption process as a control polymer because the aim of the study was based on the surface imprinting approach and to create the recognition cavities on the p(HEMA) adsorbent. So, during the whole adsorption process especially, the selectivity studies and the calculation of the imprinting factor value, the use of s(HEMA) and d(HEMA) were more suitable than p(HEMA) adsorbent results.

Characterization Studies

FTIR was used to analyze the functional groups p(HEMA), d(HEMA), and s(HEMA) and before the FTIR analysis, the dry adsorbents were layer on a crystal and their characteristic peaks were recorded with Thermo Scientific, Nicolet IS5 in the range of 4000-400 cm^{-1} wave numbers.

The surface differences of d(HEMA) and s(HEMA) were determined using a SEM, but, the images of p(HEMA) were not taken with a SEM because of not using the adsorption process. Before the taking of SEM images, the cryogels were dried at RT and their surfaces were coated with gold and after that, the images of d(HEMA) and s(HEMA) were taken (Philips/FEI, Quanta 400F) with different magnification values.

The swelling degrees of p(HEMA), d(HEMA), and s(HEMA) were determined to examine the effect of surface modification on the swelling behavior of the adsorbents. For this purpose, the dry cryogel to be investigated was weighted (mdry) and the dry cryogel was immersed in DW for 2 h at RT [23]. After 2 h, the weight of swollen cryogel was recorded (mwet) and the swelling rate of the desired cryogel was calculated with equation (1).

$$Swelling\ degree\ (\%):\ \left[\left(W_{wet}-W_{dry}\right)/\ W_{wet}\right] x\ 100 \qquad (1)$$

Adsorption studies

During the whole adsorption studies, the rpm of the or-

bital rotator was fixed at 9 rpm and the adsorption time was fixed for 2h at RT. Prior to the experimental studies, the s(HEMA) was firstly equilibrated with the desired buffer solutions (pH 4-7) for 30 min at room RT. After that 1 mg/mL protein solution was prepared using the same equilibrium buffer and the solution was interacted with s(HEMA) for 2 h at RT using an orbital rotator. After 2 h, lysozyme was removed from the surface using SDS solution for 15 min and the s(HEMA) was washed with DW for 30 min.

The adsorbed amount of lysozyme was calculated with the following equation (2) using a UV spectrophotometer (Shimadzu, Model 1601, Tokyo, Japan) at 279 nm.

$$q = \left[\left(C_i - C_f\right)\right] x\ V / M \qquad (2)$$

q; the adsorbed amount of lysozyme, Ci; and Cf; the initial concentration and final concentrations of lysozyme (mg/mL), respectively, V; the volume of the solution (mL) and M; the weight of the adsorbent (g).

The concentration effects of lysozyme were investigated between 0.25 mg/mL-1.25 mg/mL and before the investigation of concentration effects on lysozyme adsorption, the same procedure was applied abovementioned. After the protein adsorption studies, the adsorbents were washed with DW and kept at 4°C until used.

RESULTS AND DISCUSSION

The FTIR results of p(HEMA), d(HEMA), and s(PEMA) cryogels were figured out in Fig. 1 and the characteristic peaks of p(HEMA) stemmed from 3292 cm^{-1} (-OH), 2934 cm^{-1} (aliphatic -C-H), 1723 cm^{-1} (-C=O functional groups), 1158 cm^{-1} (-C-O stretching), 1650 cm^{-1} (amide I, C=O), and 1528 cm^{-1} (amide II, C=O) [24,25]. The other common peaks of d(HEMA) and s(HEMA) were observed at around 1640 cm^{-1}, 1397 cm^{-1} (aromatic-C-C stretching), 1162 cm^{-1} (aromatic -COH out of plane), and around 3305 cm^{-1} (-OH and -NH_2 groups for polydopamine) [24,26]. The observation of the same peaks of d(HEMA) and s(HEMA) showed that the surface modification and lysozyme recognition cavities have successfully occurred on the p(HEMA) surface with the assistance of polydopamine.

The optical and SEM images of p(HEMA), d(HEMA), and s(HEMA) were illustrated in Fig. 2. The p(HEMA) cryogel membrane was opaque in color, but, after the surface modification, the color of d(HEMA) and s(HEMA) cryogel membranes turned brownish; so, these results revealed that dopamine was successfully polymerized on the p(HEMA) surfaces.

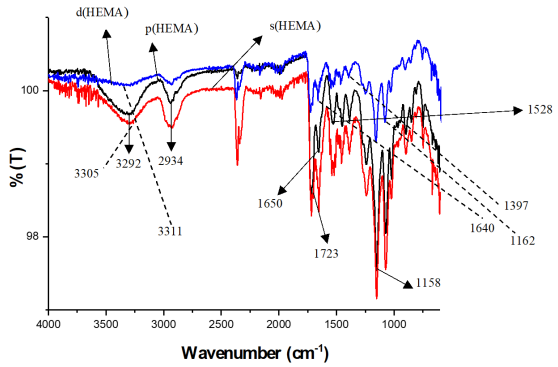

Figure 1. The FTIR results of both cryogel membranes.

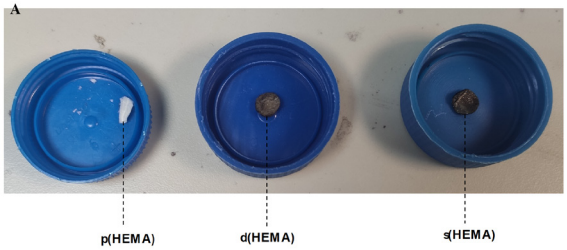

Figure 2. A; the optical images of p(HEMA), d(HEMA), and s(HEMA) cryogel membranes.

The swelling ratios of p(HEMA), d(HEMA), and s(HEMA) were calculated as 88,5%, 90,8%, and 88.1%, respectively. The swelling ratio of d(HEMA) was higher than p(HEMA) due to the addition of hydrophilic -NH2 groups on the surface of p(HEMA) cryogel, however, after the design of lysozyme recognition cavities could decrease the swelling ratio of s(HEMA) as compared to p(HEMA).

In the Fig. 3, A, B, and C images represented the d(HEMA) cryogel membrane and the d(HEMA) cryogel with macropores and polydopamine structures were illustrated with i and ii. The diameter of macropores of d(HEMA) was nearly 50 µM and the polydopamine structure was located on the macropores structures. In Figure 3, D, E, and F represented the s(HEMA) and the macroporous structure of s(HEMA) was given in D and E (i). Furthermore, ii represented the polydopamine accumulation on s(HEMA) surface. From these experimental findings, the surface modification of both cryogels with polydopamine was succesfully accomplished under alkaline conditions and the surface modification could not change the structural properties of p(HEMA) and s(HEMA).

The adsorption studies of lysozyme were examined in the aqueous solution at RT by changing the pH and the lysozyme concentration. The pH effects of lysozyme was illustrated in Fig. 4. The adsorbed amount of lysozyme was found as 106.9 mg/g polymer at pH 6 and the isoelectric point of polydopamine is around 4 [27] and at pH 6, polydopamine carries a negative charge. On the other hand, the isoelectric point of lysozyme is 11.2 [20] and at pH 6, this

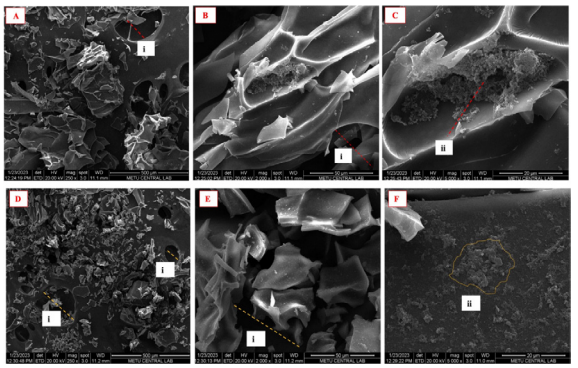

Figure 3. The SEM images of d(HEMA) (A, B, and C) and s(HEMA) (D, E, and F). i and ii represented the macropores structure and polydopamine accumulation on both cryogels, respectively.

protein holds a positive charge due to its functional groups. At pH 6, the protein with the functional groups carrying positive charges could interact with polydopamine groups carrying negative charges, so, thanks to the electrostatic interactions, the adsorbed amount of lysozyme was increased.

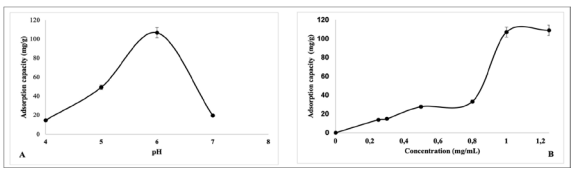

Figure 4. A; The adsorbed amount of lysozyme with different pH values (lysozyme concentration; 1 mg/mL, rpm; 8) using an orbital rotator at RT. B; the adsorption capacity of s(HEMA) cryogel membrane with the different lysozyme concentrations [pH; 6 (100 mM), rpm; 8] at RT using an orbital rotator.

The concentration effects on the lysozyme adsorption were investigated with the range of 0.25-1.25 mg/mL and the results were shown in Fig. 3. As given in Fig. 3B, the increased amount of lysozyme resulted in increasing the adsorption capacity and the Qmax of the s(HEMA) was determined as 108.9 mg/g at pH 6.

To understand the adsorption phenomena of lysozyme on the s(HEMA) surface was determined with the Langmuir, Freundlich, and Langmuir-Freundlich (L-F) isotherms and their adsorption parameters were calculated with the previous studies [28,29]. The R2 values of the Langmuir, the Freundlich, and the L-F of lysozyme adsorption were found as 0.9543, 0.8972 and 0.9668, respectively. Additionally, the RL value of Langmuir was found as 0.99 and the adsorbed amount of lysozyme was in accordance with the Qmax value of the Langmuir mathematic model; so, the lysozyme adsorption has occurred on homogenous binding sites and the protein adsorption is a favorable according to the RL value of the Langmuir isotherm model.

Selectivity studies are highly important to determine the recognition capability of the polymer against the template molecule; so, BSA and creatinine were selected to test the selectivity of the s(HEMA). Creatinine is a small molecule with 113 Da as compared with lysozyme and is the waste product of creatine metabolism. Whereas, the other competitor molecule, BSA, is one of the abundant proteins in the body and its molecular weight is 66,5 kDa with the pI value of 4.5 [29] and its size is larger than lysozyme, thereby, these two biomolecules were selected to examine the imprinted performance of the s(PEMA) depending on the shape-size performance. For this aim, the imprinting factor (IF), selectivity coefficient (k), and relative efficiency coefficient (k') of the s(HEMA) were calculated and their results were given in Fig. 4. IF was calculated as the q value of the s(HEMA)/p(HEMA) and the IF was found as 3.26. As seen in Figure 4, the s(HEMA) was capable of recognizing lysozyme towards the competitor molecules more than 2 times.

Reusability studies of the affinity adsorbent play a significant role to test the durability and stability of the adsorbent during the whole adsorption process. So, in this study, one of the s(HEMA) cryogel membranes was chosen and the reusability of the selected adsorbent was used repetitively in 5 adsorption-desorption-regeneration cycles, and its results were illustrated in Fig. 5. As seen in Fig. 5, the adsorbent protected its stability after the 5 adsorption-desorption-regeneration cycles.

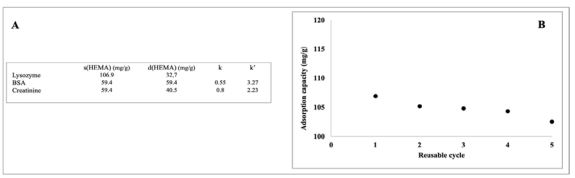

Figure 5. A; the selectivity performance s(PEMA) against the competitor biomolecules (the concentration biomolecules; 1 mg/mL, pH of the media; 6 and rpm; 8, RT) and B; the reusability of the adsorbent (concentration; 1mg/mL, pH of the media; 6, and rpm; 8, RT).

Owing to the main functions of lysozyme, many researchers used molecularly imprinted polymers to purify this protein, for instance, Xu and coworkers [30] prepared lysozyme imprinted polymers using Fe3O4 via surface imprinting technique and IF result of this study was reported as 2.02. In another study, silica-coated magnetic nanoparticles were employed in purifying lysozyme [31] and the maximum adsorption capacity, and the IF of the adsorbent were calculated as 108mg/g and 2.82, respectively. In the next study, the magnetic adsorbent was prepared via the surface imprinting method using acrylamide (AAm) as a functional monomer [32]. The adsorbent amount of the target protein was reported as 341.1 mg/g polymer and its IF was found as 2.94.

The experimental findings of that study were in accordance with the previous studies, so, the fabricated cryogel membrane via surface imprinting technique could be potentially used to purify the lysozyme from the aqueous solution.

CONCLUSION

In this work, the surface p(HEMA) cryogel was modified with the polymerization of dopamine under basic conditions design of the artificial lysozyme receptors on its surface and the adsorption capacity of the s(HEMA) was evaluated in the aqueous solution. FTIR results supported that the surface modification of the cryogels successfully was accomplished and these modifications can change not only the surface morphologies, and topologies but also the swelling ratios of cryogels. The adsorbed amount of lysozyme was calculated as nearly 107 mg/g at the optimum pH and the Qmax of s(HEMA) was found as approximately 109 mg/g, which is close to the Q max value of the Langmuir isotherm model and the protein adsorption is favorable according to the RL value of the Langmuir mathematical model. s(HEMA) cryogel membrane was recognized as 3.25 times greater than BSA and as 2.23 than creatinine due to its shape and size recognition capability. According to the experimental findings, the s(HEMA) cryogel membrane could be used as an alternative adsorbent to purify lysozyme protein from the aqueous solution.

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CONFLICT OF INTEREST

In this work, the only author is the corresponding author; so, there is no conflict of interest.

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