미세소자에서 고체상 추출을 이용한 BSA제거

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Removal of BSA using solid-phase extraction in microfluidic device

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1. Introduction

In the last several years, there has been raised interest in the proteome of both serum and plasma. The composition of the serum protein can reflect the physiological and pathophysiological state of the status of the human body. But albumin, immunoglobulin which constitute about 60~97% of the total serum protein usually hinder the detection of other proteins which are diagnostically significant and present at far lower concentrations. Removal of abundant serum protein will increase intensity of informative disease markers which exists in low concentration.

There have been many attempts to separate serum albumin from human plasma for more accurate analysis for biomarkers by using affinity chromatography, iso-electric trapping and ultrafiltration. In this study solid phase extraction (SPE) using in-situ polymerized stationary phase was selected.

SPE is widely used for the isolation and concentration of target analytes, and the clean-up of samples in pharmaceutical, clinical, environmental and food chemistry. SPE was initially developed to replace the liquid-liquid extraction (LLE). LLE system consumes relatively large amount of high-purity solvents with expensive disposal requirements. In addition, it is difficult to automate and labor intensive. Contrastively, SPE benefits from low intrinsic costs, shorter processing times, low solvent consumption and simpler processing procedures.

2. Experimental

2.1 Materials

Stationary phase in SPE system Ethylene glycol dimethacrylate (EGDMA) and Butyl methacylate (BMA) were purchased from Sigma-Aldrich Chemical. 2-Acrylamido-2-methyl-1-propanesulfonic

acid (AMPS) was obtained from Aldrich. EGDMA and BMA were purified with alumina powder. 2,2-Dimethoxy-2-phenyl-acetophenone (DPA, Aldrich) was used as a initiator for the polymerization of the stationary phase. Ethanol and methanol (Merck) were used as a porogenic solevent.

Model protein Bovine serum albumin (BSA, Sigma) was used as a model protein. γ-Globulin from bovine was purchased from Sigma.

Buffer An 0.1M acetic buffer, a mixture of sodium acetate (Sigma-Aldrich) and acetic acid (Junsei), was used.

2.2 Preparation of polymerized stationary phase in microchannel

The monomer solutions were mixed with the adjusted composition and monomer solutions were pumped into channel and exposed to UV light source for 15min. After the polymerization reaction, the channel was washed with methanol to remove the unreacted monomers and porogenic solvent.

2.3 Analysis of protein concentration

Protein concentration of samples was determined by Bradford method. BSA concentration was determined on the basis of this calibration.

3. Results and discussion

3.1 Characterization of polymerized adsorbent

Pore size/ BET. EGDMA, BMA and AMPS were polymerized in various ratio with constant amount of porogenic solvents responsible for the pore formation. In spite of the composition change in monomer solution, the pore size was in the range from 90 to 100nm. Specific surface area was in the range from 3 to 4 m^2/g.

SEM. SEM images of polymerized monolith were shown in Figure 1. As shown in the Figure 1, the monolith was polymerized as spherical shape. It is confirmed that the particle size is about 2 ㎛.

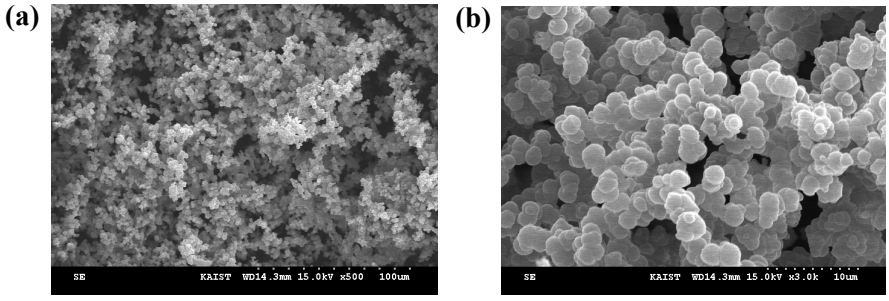

Figure 1. Scanning electron microscope images of polymerized adsorbent (a) x500 (b) x3000.

IR. In the case of polymerized adsorbent with AMPS, the polymer has the functional group of SO_2 and NH_2. As shown in Figure 2, S=O was confirmed at the $1000cm^{-1}$.

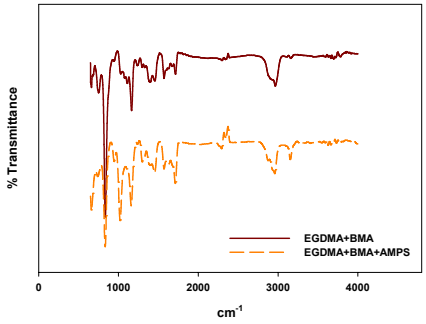

Figure 2. IR spectra of polymerized adsorbent with AMPS.

3.2 Adsorption of BSA onto polymerized adsorbent

3.2.1 Effect of pH on BSA adsorption

Effect of pH on BSA adsorption was shown in Figure 3. The isoelectric point of BSA is about 4.9. As deviating from the isoelectric point, the adsorbed amount of protein was decreased drastically. This observation accorded with some other researchers where the maximum adsorption from aqueous protein solutions was usually observed at isoelectric point.

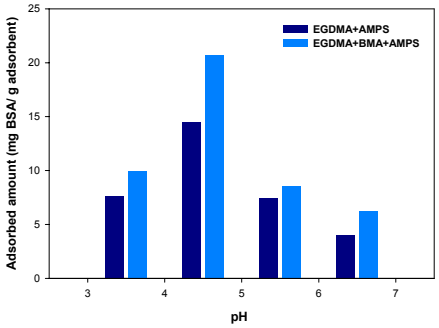

Figure 3. Effect of pH on BSA adsorption.

3.2.2 Effect of hydrophilic and hydrophobic interaction on BSA adsorption

Figure 4(a) shows the effect of hydrophilic interaction on BSA adsorption with different amount of hydrophilic AMPS in adsorbent. As shown in the Figure 4(a), adsorbed amount of BSA increased with the increase of AMPS and AAm. It could be concluded that the hydrophilic interaction was important on BSA adsorption. As shown in Figure 4 (b), adsorbed amount of adsorbent which is co-polymerized with hydrophobic BMA increased slightly compared to that on hydrophilic adsorbent.

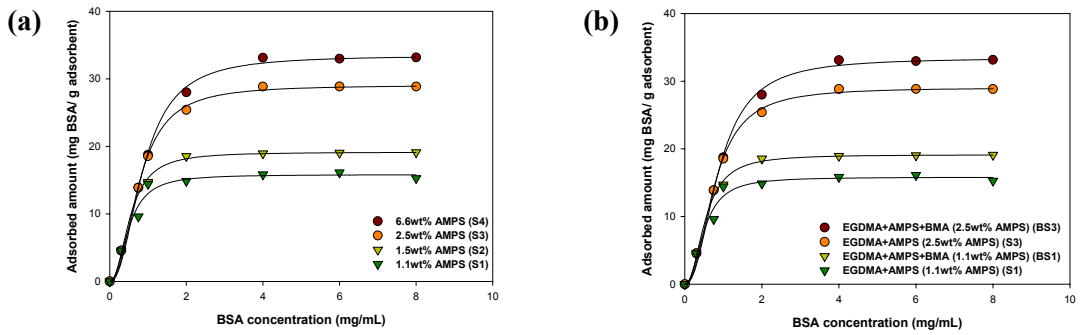

Figure 4. Effect of hydrophilic and hydrophobic interaction on BSA adsorption with AMPS adsorbent.

3.3 Selective removal of BSA

The maximum adsorbed amount of protein is usually observed around the isoelectric point. The isoelectric point of BSA is 4.8 and that of γ-Globulin is 6.5. Using these characters, when the SPE was performed at pH 4.5, the selective removal could be possible.

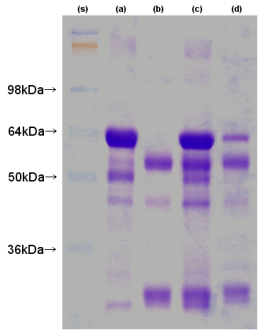

Figure 5. SDS-PAGE of selective removal of BSA (s) standard marker (a) 1mg/ml BSA (b) 1mg/ml BGG (c) starting sample: BSA+BGG (d) selective removal of BSA using SPE in microfluidic device4.

4. Conclusions

In this study, BSA was removed using sulfonated adsorbent which was in-situ polymerized in microchannel. The maximum capacity of adsorbent was 36mg BSA/g adsorbent and that was distinguished with other adsorbent. Also, selective removal of BSA was possible as adjusting the pH. Finally, it could be concluded that the SPE was successfully performed in microfluidic device.

5. References

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