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Experimental studies on affinity chromatography in an electric field

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Abstract

A multicompartment electrolyzer, which has been used for preparative electrophoresis [Z. Liu, Z. Huang, J.-Y. Cong, et al., Sep. Sci. Technol. 31 (1996) 427], is applied for carrying out affinity chromatography in an alternating electric field. The effect of electric field strength on the adsorption and desorption characteristics is experimentally examined with human serum albumin and Blue Sepharose Fast Flow as a model system. It is shown that the existence of an electric field leads to a significant change in the adsorption capacity of the blue dye, which may be used for establishing a preferential adsorption to achieve a high resolution. The adsorption speed increases slightly with respect to the increase of electric field strength, while adsorption capacity in the presence of an electric field is independent of the electric field strength. Different elution behavior is observed as function of adsorption condition and a high recovery of the adsorbed protein is obtained when the adsorption is carried out in the presence of an electric field.

Keywords: Affinity chromatography; Proteins; Albumin

1. Introduction

Particularly high resolution and improved technical maturity make affinity chromatography a powerful tool in the purification of biological molecules at different scales [2]. In addition to the continuous efforts in developing novel ligands to meet the demand of high selectivity and good versatility, study on novel ways to increase separation speed has attracted growing attention. One way is to develop novel chromatography media of superior mass transfer characteristics, as exemplified by Poros [3] and Superporous agarose [4], both of which are characterized by a bimodal pore network. The use of the so-called convective pores leads to a convective transport of liquid through the beads at a speed an order of magnitude faster than that of the diffusive transport. An alternative approach to accelerate separation speed is to operate the chromatography in an electric field, as shown by capillary electrochromatography, in which electroosmosis flux within particles and between particles greatly increases the liquid flow rate [5]. However, this method is basically designed for analytical purposes and is not suitable for large-scale separations.

Based on our previous work on multichannel flow electrophoresis [1], we attempted a new combination of electrophoresis with affinity chromatography, namely electrophoretic affinity chromatography (EAC). The separation by EAC is carried out in a six-compartment electrolyzer separated by membranes, in which one of the two central compartments is packed with affinity gel media and the other is used for sample loading. Next to the central compartments are the elution compartments and the electrode compartments, respectively. During a run, the target component is transmitted from the central compartment into the media compartment and binds to the affinity media. The impurities are transmitted

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by electrophoresis or electroosmosis into elution compartments and washed out by carrier flow. After the adsorption step, an elution solution is introduced into the sample loading compartment. The product is desorbed, transmitted into the elution compartment and washed out, subsequently. The previous work of purifying human serum albumin (HSA) from human serum demonstrated the workability of this method [7]. The present work is focused on the effects of pH, buffer composition and the electric field strength on the adsorption and desorption kinetics in order to establish a comprehensive understanding of the process characteristics of the electrophoretic affinity chromatography.

2. Experimental

2.1. Apparatus and procedures

The experimental system is illustrated in Fig. 1. The heart of this system is the patented six-compartment electrolyzer partitioned by membranes [8]. In this work, HT Tuffryn membrane was used between the central compartments and the elution compartments. The elution compartments and the electrode compartments were separated by gel membranes. Each compartment was connected to a pump. All compartments were 12.0 cm in length and 1.0 cm in width. The depths of the electrode compartment and the elution compartment were 0.4 and 0.3 cm, respectively. The depths of the sample compartment and gel compartments were 0.3 and 0.45 cm, respectively.

During a run, the system was first equilibrated with starting buffer. In case of electrophoretic adsorption, the equilibration was conducted under an electric field. Sample solution was the applied for adsorption and the system was washed with the same buffer after adsorption. Afterwards, elution buffer was introduced into each compartment and products from the elution compartments were collected with fraction collectors. Finally, the gel was regenerated with 6 *M* urea.

To avoid the disadvantages of bubbles in the electrode compartments for the determination of the electric field strength, the apparatus was operated at a constant current density mode in all experiments.

2.2. Materials

The main chemicals used in this study were Tris

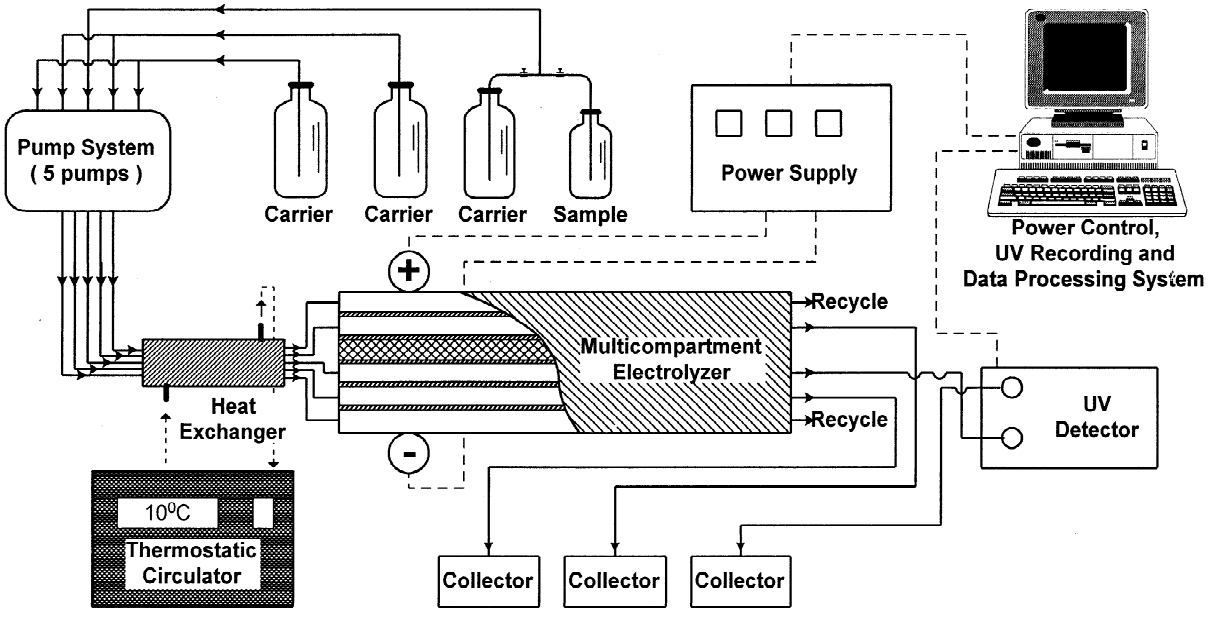

Fig. 1. Experimental system of electrophoretic affinity chromatography.

(Boehringer Mannheim, Germany) and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) (Sigma, USA). Blue Sepharose Fast Flow gel and HSA were kindly supplied by Dr. J.-C. Janson at Biomedical Center, Uppsala University, Sweden. HT Tuffryn membrane (media: hydrophilic polysulfone, pore size: 0.45 mm in diameter) was purchased from Gelman Sciences, USA. HSA concentration was spectrophotometrically determined at 280 nm.

3. Results and discussion

3.1. Effects of pH and buffer composition on the adsorption of HSA

In order to find the suitable conditions for electrophoretic adsorption and desorption, a set of batch adsorption experiments was conducted at different pH and buffer compositions. In each experiment, 1 ml wet gel media was soaked in 10 ml HSA solution containing 50 mg HSA in a shaking bath at 20°C for 2 h. The results are shown in Table 1.

It is illustrated by Table 1 that the adsorption capacity varies significantly against pH. This suggests that the electrostatic force dominates the molecular interaction between HSA and the blue dye. The different adsorption capacity obtained at pH 8.5 and 8.8 indicates that the buffering ions also have a significant effect on the adsorption capacity.

Table 1
Adsorption capacity of Blue Sepharose Fast Flow with different buffers[a]

Buffer	pH	Capacity (mg HSA/ml wet gel)
0.01 *M* HAc–NaAc	4.2	35.4
0.01 *M* HAc–NaAc	4.5	35.9
0.01 *M* Tris–HAc	4.9	32.9
0.01 *M* Tris–HAc	5.9	22.4
0.01 *M* Tris–HAc	7.5	12.9
0.002 *M* Tris–HEPES	7.5	9.4
0.01 *M* Tris–HAc	8.5	6.5
0.01 *M* Tris–Gly	8.8	1.8
0.01 *M* $NaHCO_3$–Na_2CO_3	10.0	6.4
0.01 *M* $NaHCO_3$–Na_2CO_3	11.2	4.5

[a] $Ac=CH_2COO^-$.

3.2. Electrophoretic adsorption of HSA on Blue Sepharose Fast Flow

Adsorption of HSA in the presence of an electric field was buffered with 0.0025 *M* Tris–HEPES buffer and conducted in constant current density mode. The reason for selecting Tris–HEPES is that it possesses a higher buffering capacity at a lower ionic strength. This is suitable for reducing Joule heating when conducting adsorption at a higher applied potential. The HSA concentration in the sample was maintained at 1.0 mg/ml and the sample loading flow rate was 2 ml/min in each experiment. The buffer flow rates for elution compartments and electrode compartments were 3 and 6 ml/min, respectively. Samples from the central and elution compartments were taken every 5 or 10 min. An alternating electric field was applied to reduce the concentration polarization and membrane fouling during the adsorption process [6]. The alternation pattern was 3 min of positive electric field and 4 s of negative electric field. In each experiment, washing of the system after adsorption was operated in the presence of an electric field and could be finished in 10 min. In this set of experiments, elution of HSA was conducted with 0.02 *M* pH 7.2 phosphate buffer, containing 2 *M* NaCl in the absence of an electric field.

The time course of HSA adsorption under different current densities are shown in Fig. 2. It can be

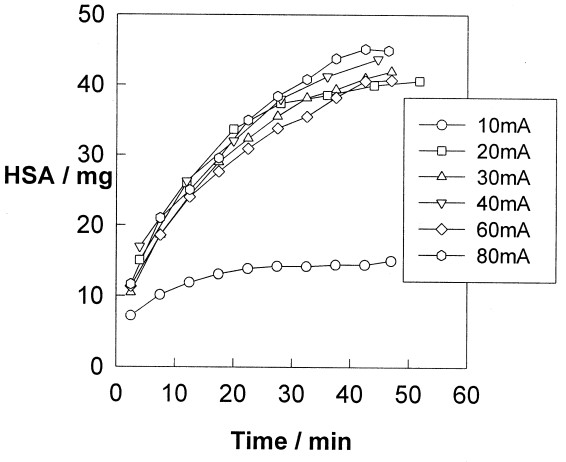

Fig. 2. Electrophoretic adsorption of HSA at different electric field strength indicated by current density.

seen that in all cases the accumulation of HSA in gel grows rapidly at the beginning stage of adsorption and approaches a certain value gradually. It takes about 60 min for HSA adsorption to get to equilibrium.

The amount of adsorbed HSA is shown in Fig. 3. It is shown that the amount of HSA adsorbed in the presence of an electric field is almost the same in all experiments. This may due to the reason that under the adsorption condition, the molecular interaction between ligand and ligate is much stronger than the electrostatic force, as described by Yarmush and Olson [10]. Therefore, the adsorption capacity is independent of the electric field strength.

One important fact that should be noted here is the significant reduction in adsorption capacity caused by applying an electric field. The average adsorption capacity in the above experiments, except the one conducted with 10 mA that was too low to accomplish a sufficient mass transfer, was 6.1 mg per ml wet gel. This was only 65% as much as that obtained in the absence of an electric field when using the same buffer. The change of adsorption capacity, though which is negative for the case of adsorption of HSA on Blue Sepharose Fast Flow gel, may be applied for obtaining a high resolution.

Electrosmotic flux was observed in the experiments described in Figs. 2 and 3, which convectively transmits proteins from mobile phase into gel matrix. In the above experiment, the electroosmosis flux was eliminated by applying a back pressure using the valves set on the line of the liquid flow. Therefore, the results described above only reflect the effects of electrophoretic migration on the binding characteristics.

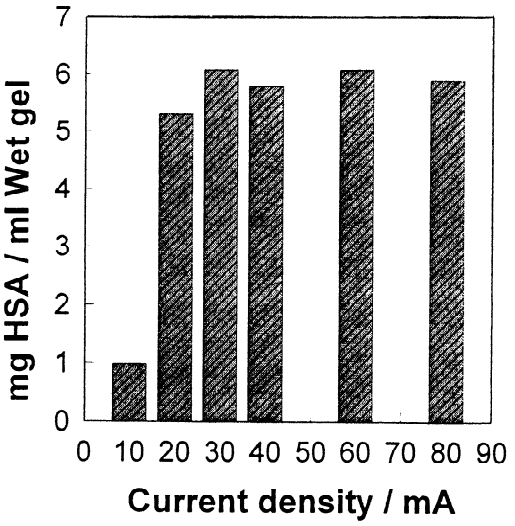

Fig. 3. Adsorption capacity as a function of electric field strength indicated by current density.

3.3. Electrophoretic desorption of HSA from Blue Sepharose Fast Flow

We believe that electrophoretic desorption should be carried out at such a condition that is also suitable for chemical desorption. According to the adsorption results shown in Fig. 2, electrophoretic desorption was conducted at pH 8.8 using 0.01 *M* Tris–Gly, at which the adsorption capacity of blue dye for HSA is the minimum. The adsorption was conducted in the absence of an electric field and buffered with 0.1 *M* pH 4.5 HAc–NaAc, at which a maximum adsorption capacity was obtained. In each experiment, 100 ml HSA sample (l mg/ml) was introduced into the sample application compartment and forced to pass through the gel compartment at the volume flow rate of 2 ml/min (equivalent linear flow rate of 12 cm/min) and the average adsorption capacity was 90 mg. Desorption curves obtained at different electric field strengths, as indicated by current densities, are shown in Fig. 4.

Fig. 4 shows that the electrophoretic desorption processes in above conditions have a similar characteristics that can be divided into two stages. In the initial stage, the increase of the electric field strength resulted in a relatively large increase in the amount of desorbed HSA. An exception to this was the result obtained in 60 mA, which was a bit higher than that of 80 mA. This may be mainly due to the experimental error. In the final stage of electrophoretic desorption, the desorption rates under different conditions, as can be seen from the slope of the curves, were more similar. The maximum amount of HSA recovered within 3 h was less than 70 mg. These results suggested that a multi-layer adsorption of HSA occurred at pH 4.5, at which an enhanced electrostatic adsorption existed for the adsorption of the positively charged HSA on the negatively charged blue dye. The increase in the desorbed HSA as a result of increased electric field strength in the

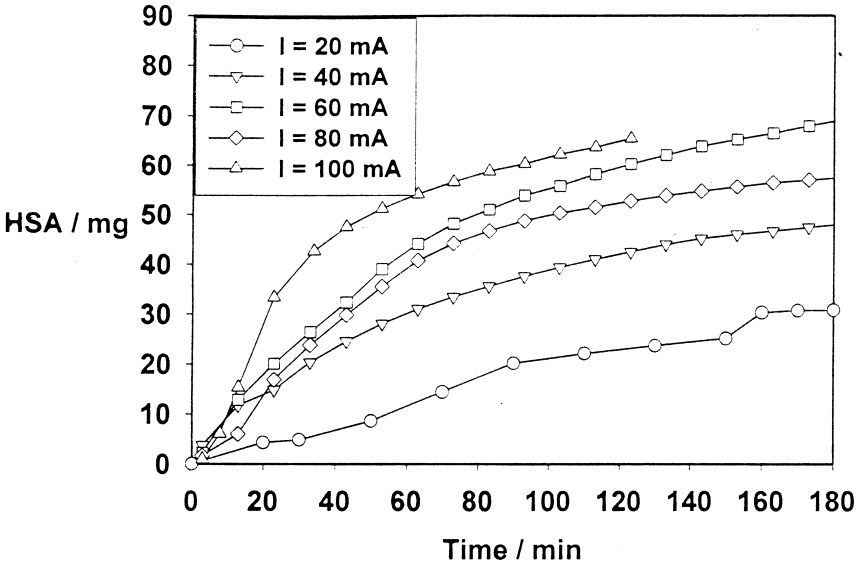

Fig. 4. Electrophoretic desorption rate as a function of electric field strength.

beginning stage may mainly due to the molecules adsorbed at the outer-layer of the gel through the long-range electrostatic forces. At the final stage where the inner layer of HSA was desorbed, the desorption rate became chemically determined by the molecular interaction of HSA and the blue dye, which, as described by Yarmush and Olson [9], is not affected by the strength of the electric field.

3.4. Electrophoretic affinity chromatography of HSA

Adsorption of HSA was buffered with 0.002 *M*, pH 7.5 Tris–HEPES and conducted at a 60 mA current density. Desorption was buffered with 0.01 *M*, pH 8.8 Tris–Gly and conducted at 40 mA and 60 mA, respectively. The amount of HSA on Blue Sepharose was shown in Fig. 5.

As shown in Fig. 5, the adsorption was finished in 50 min and the adsorption capacity was 6.07 mg/ml (wet gel). For washing, 15 min was taken and 100 min was used for electrophoretic elution, in which over 90% HSA adsorbed on the blue gel was eluted and the average desorption rate obtained at 40 mA and 60 mA was 16.8 and 17.2 mg/h, respectively. The tiny difference may be due to the error in the measurement of the sample concentration, mostly in the later part of the elution step.

It is interesting to note that the rate of desorption obtained here is shown to be independent of the electric field strength and constant in the main part of the elution period. This indicates a single layer adsorption of HSA obtained in the presence of an electric field. This may be the reason that leads to the difference in electrophoretic desorption behavior obtained at different adsorption conditions.

4. Conclusions

Electrophoretic affinity chromatography is a new combination of electrophoresis with affinity chromatography, which is designed for preparative scale separations. In present study, the effect of electric field strength on binding and desorption characteristics was experimentally studied. It was shown that applying an electric field led to the changes in the adsorption mechanism and, consequently, the significant changes of adsorption capacity compared to that in the absence of an electric field. This change might be used for enhancing the selectivity of adsorption. The electrophoretic desorption was shown to be a function of the adsorption conditions. For chroma-

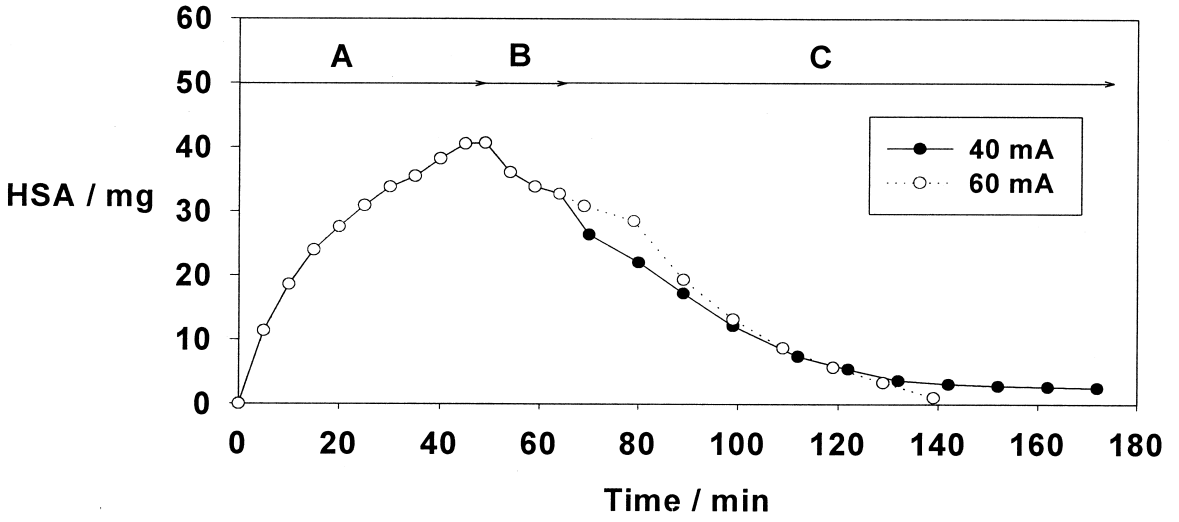

Fig. 5. Electrophoretic affinity chromatography of HAS on Blue Sepharose Fast Flow. (A)=Adsorption at 60 mA, buffer: 0.002 *M*, pH 7.5 Tris–HEPES; (B)=Washing, without electric field, buffer: 0.002 *M*, pH 7.5 Tris–HEPES; (C)=Desorption at 40/60 mA, buffer: 0.01 *M* pH 8.8 Tris–Gly.

tography of HSA on Blue Sepharose Fast Flow, when the adsorption was operated at pH 4.5 in the absence of an electric field, the electrophoretic desorption showed two-stage characteristics. The increase in the electric field strength resulted in a corresponding increase in the desorption rate in the first stage. In the second stage, the electrophoretic desorption rate varied little with respect to the electric field strength. When the adsorption was carried out at pH 7.5 in the presence of an electric field, the electrophoretic desorption rate was constant for the most part of the running period and independent of the electric field strength. The elution yield was over 90% and the desorption rate was 17 mg/h on average, which is higher than the results reported in the literature [10].

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