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Temperature influence on the dynamic binding capacity of a monolithic ion-exchange column

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Abstract

This work investigates the influence of temperature on the binding capacity of bovine serum albumin (BSA), soybean trypsin inhibitor and L-glutamic acid to a CIM® (DEAE) weak anion-exchange disk monolithic column. The binding capacity was determined experimentally under dynamic conditions using frontal analysis. The effect on the dynamic binding capacity of dimers present in the BSA solution has been evaluated and a closed-loop frontal analysis was used to determine the equilibrium binding capacities. The binding capacity for both BSA and soybean trypsin inhibitor increased with increasing temperature. In the case of L-glutamic acid, an increase in the binding capacity was observed with temperature up to 20 °C. A further increase in temperature caused a decrease of the dynamic binding capacity.

Keywords: Temperature effects; Binding studies; Monolithic columns; Anion-exchanges; Glutamic acid; Trypsin inhibitors; Albumin

1. Introduction

CIM Convective Interaction Media® is a methacrylate based monolithic material used as a stationary phase in liquid chromatography [1]. Monolithic media are characterized by a single monolithic unit with a network of highly interconnected pores forming flow-through channels. This results in a convective flow, which enhances the mass transfer between the stationary and the mobile phase. CIM monoliths are used for the separation of various biomolecules. Their flow-unaffected characteristics [1–4] are especially advantageous for the fast separation of macromolecules such as proteins or DNA [5]. With further development, large monolithic columns appear to be a promising tool for fast preparative purification where the dynamic capacity is of significant importance [6].

In the past, the influence of temperature on ion-exchange equilibrium has been regarded as insignificant [7]. But recently, there has been increased interest in examining the temperature affect in ion-exchange chromatography [8–10] and other processes employing ion-exchange [11–16]. The influence of temperature on the ion-exchange process is a very complex phenomenon, since it can affect the physical, chemical and electrochemical properties of the solute and the adsorbent e.g. the dissociation constants of weak electrolytes, conformational changes of proteins and ligands, activity coefficients etc. The ion-exchange equilibrium is thermody-

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namically determined by the Gibbs free energy change for adsorption (ΔG_{ads}), which is a function of the enthalpy change for adsorption (ΔH_{ads}). Protein sorption on the ion-exchanger can be either endothermic or exothermic depending on the protein, the adsorbent, and the process conditions [17,18]. For the protein adsorption on the ion-exchange column, the exothermic heat of adsorption is usually described as an attractive electrostatic interaction, while the endothermic heat of adsorption is characterized as protein reconfigurations and repulsive interactions between adsorbed proteins. Repulsive interactions are usually present at high surface concentrations. The overall enthalpy change for adsorption indicates the temperature influence on the ion-exchange equilibrium and is dictated by the Van't Hoff relationship. If the enthalpy of adsorption is endothermic, increased temperature shifts the equilibrium towards a higher equilibrium capacity. Although the majority of the published results regarding the temperature influence on adsorption and ion-exchange equilibrium follow the Van't Hoff relationship [9,19], some exceptions exist [17,20]. In these cases, the anomalous behavior is explained by different types of interactions occurring at different temperatures. However, the Gibbs free energy also incorporates entropic contribution (ΔS_{ads}) in addition to enthalpic. When the adsorption enthalpy change is endothermic, the adsorption process must be entropically driven. A possible source for the entropic driving force could be a release of counter ions on adsorption, a release of water from the protein surface, the reorientations of the proteins on the surface, or conformational changes in the proteins [21].

In this work the influence of temperature on the dynamic binding capacity of two proteins and an amino acid on a commercially available weak anion-exchanger is presented.

2. Materials and methods

Experiments were performed using CIM® disk monolithic columns, from BIA Separations (Ljubljana, Slovenia), bearing diethylaminoethyl (DEAE) weak anion-exchange groups. These columns are 3 mm thick and 12 mm in diameter having a volume of 0.34 ml. Tested compounds were bovine serum albumin (BSA) (Fraction V Powder≥96%) from Fluka (Buchs, Switzerland), soybean trypsin inhibitor Type II-S from Sigma (St Louis, MO, USA), and L-glutamic acid from Sigma were used. Analytical grade NaCl from Fluka and Tris (99%+) from Aldrich (Steinheim, Germany) were also applied. The loading buffers for BSA and soybean trypsin inhibitor adsorption were 20 m*M* Tris–HCl, pH 7.4 and the elution buffer was 1 *M* NaCl in the loading buffer. The loading phase for L-glutamic acid adsorption was water at pH 5.5 made with the addition of 0.5 *M* NaOH and the elution phase was 1 *M* NaCl in the loading phase. All solutions were prepared from bidistillated water and were degassed and filtered through a 0.45-μm filter prior to use. The pH of the solutions was measured with an ISKRA MA 5741 (Slovenia) pH meter. Experiments were performed on a gradient HPLC system equipped with UV detector from Knauer (Berlin, Germany). Flow-rates were measured with a liquid flow meter GJC Instruments 5025000 (UK). The temperature of the column, which was placed into a special stainless steel housing for better heat conduction, was regulated with a thermostated water bath with an accuracy of ±1 °C. The binding capacity was determined using two different frontal analysis methods: open-loop frontal analysis and closed-loop frontal analysis. Closed-loop frontal analysis is similar to open-loop frontal analysis, but the effluent solution is transported back into the inlet vessel instead of being collected. (For closed loop experiments, 20 ml of the feed solution was circulated during the experiment.)

The binding capacity during the open-loop experiments was determined by the breakthrough area integration method [22] and by the measurement of the quantity of the eluted component. Whereas in the closed-loop experiments, the binding capacity was determined by the difference between the initial and the final concentration of the solution and by measuring the quantity of the eluted component from the elution step. The concentration of the eluted solution was measured on UV spectrophotometer (Varian Cary 50 Probe, Varian Australia).

3. Results and discussion

3.1. Bovine serum albumin

It is important when estimating the influence of temperature on ion-exchange equilibrium that the employed experimental method is sensitive enough to detect slight changes in the equilibrium binding capacity with the temperature variation. The accuracy of the measurement of equilibrium binding capacity is essential for the construction of adsorption isotherms at different temperatures. It is well known that water solutions prepared from commercial BSA contain BSA monomers and higher aggregates, such as BSA dimers and others [23–25]. These aggregates have a pronounced influence on the tailing of breakthrough curves in the open-loop frontal analysis [24] and prolong the time required for equilibration. This fact limits the applicability of this commonly used technique for the determination of a column's capacity.

In order to avoid these problems batch experiments can be used for capacity estimation. However, a large diffusional resistance due to the monolith's dimensions affects the mass transfer to such an extent that even after 80 h less than half of the capacity obtained under dynamic conditions is achieved. The comparison between capacities obtained from batch experiments at different residence times is shown in Fig. 1. These results are also important for immobilization procedures, where batch methods are usually applicable. The dynamic immobilization method seems to be more suitable for immobilizations performed on monolithic columns.

As previously mentioned, the presence of BSA dimers causes extensive tailing of the breakthrough curve in the open-loop frontal analysis which prevents equilibration in a reasonable time. For this reason, the dynamic binding capacity of an ion-exchanger is usually presented at 90 or 95% of the breakthrough, yet, equilibrium has not been achieved. The lower part of Fig. 2 presents the results from an open-loop frontal analysis of a solution prepared by dissolving the commercial BSA and of the partially dimer-free solution. While both curves have a typical S shape, there is a pronounced difference in tailing behavior. The breakthrough

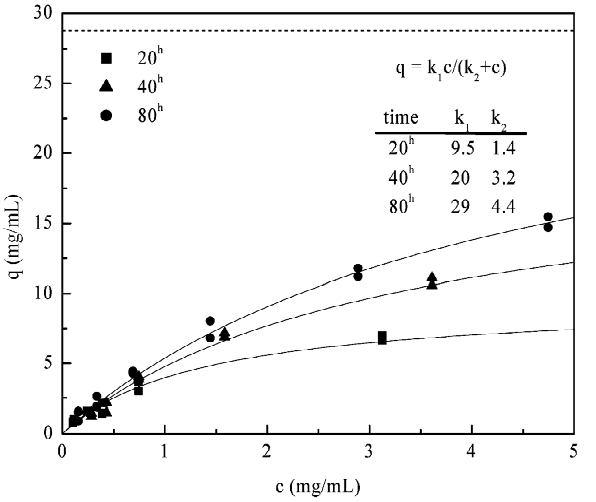

Fig. 1. Batch adsorption isotherms at different residence times. Solid lines represent Langmuir fits and dashed line stands for adsorption isotherm obtained under dynamic conditions [4]. Experimental conditions: loading phase: BSA solution in 20 m*M* Tris–HCl pH 7.4 loading buffer; stationary phase: CIM® DEAE disk monolithic column; temperature: 20 °C; detection: UV at 280 nm. Parameters of the Langmuir fits are given in the inserted table.

curve of the commercial BSA solution exhibits a more pronounced tailing, where the effluent concentration remains below 95% of the feed value at even 1000 s. This is in accordance with the published results in the literature [4]. The same experiment was repeated with a partially dimer-free BSA solution. The partial separation of BSA monomers and dimers was achieved by collecting the effluent BSA solution from the previous experiment. According to the literature [24], the effluent BSA solution should be partially dimer-free shortly after the breakthrough, because BSA dimers are more strongly bound on the ion-exchanger than monomers. Gradient separations of the commercial BSA solution and of the partially dimer-free effluent BSA solution are presented in Fig. 3. It can be seen that the effluent BSA solution contains a lower concentration of dimers than the feed solution. The frontal analysis using this partially dimer-free BSA solution (see lower part of Fig. 2) exhibits a less pronounced tailing. The effluent concentration reaches 99% of inlet value at 1000 s and it can be assumed that the

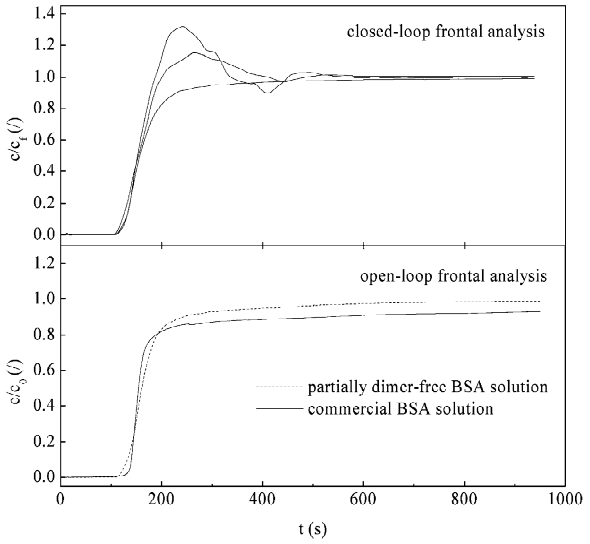

Fig. 2. Comparison between breakthrough curves obtained using two different methods. The lower part of the figure represents results from the open-loop frontal analysis and the upper part represents results from closed-loop frontal analysis. Experimental conditions: loading phase for open loop experiments: 1 mg/ml BSA in loading buffer; stationary phase: CIM® DEAE disk monolithic column; inlet volume of the BSA solution with initial concentration of 1 mg/ml BSA in loading buffer used for closed-loop experiments: 20 ml; flow-rate: 3.0 ml/min; detection: UV at 280 nm; temperature open-loop: 22 °C; temperature closed-loop: 2 °C.

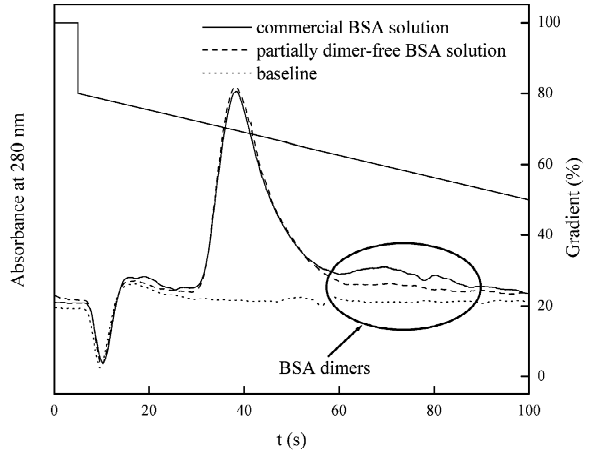

Fig. 3. Gradient separations of commercial BSA solution and partially dimer-free BSA solution on CIM® DEAE disk monolithic column. Experimental conditions: flow-rate: 3.0 ml/min; detection: UV at 280 nm; injection loop: 20 μl; loading buffer: 20 m*M* Tris–HCl, pH 7.4; elution buffer: 1 *M* NaCl in loading buffer; temperature: 22 °C.

equilibrium conditions would be achieved even sooner if BSA dimers were completely absent in the feed solution. However, the problem with producing a sufficient quantity of dimer-free BSA solution limits the applicability of this method for the determination of the adsorption equilibrium. It should be stressed that the position and the shape of the breakthrough curves determines the column's capacity, which has to satisfy an overall material balance. Since the position of both curves is almost identical, but their tailing is significantly different, it can be inferred that the ion-exchange column has a smaller dynamic binding capacity for the partially dimer-free BSA solution. The tailing of the breakthrough curve results from the displacement of monomers by the stronger bound dimers. Thus, an excessive supply of dimers present during an open-loop frontal analysis would eventually lead to an equilibrium state dominated by dimers adsorption. However, such an equilibrium capacity of the ion exchanger would not represent the capacity of the feed BSA solution but would be higher due to BSA dimer adsorption. The experimental determination of the equilibrium capacity from the open-system frontal analysis for a commercial BSA solution is difficult, because the increased capacity presumably causes agglomeration of the BSA leading to increased backpressure, which can then damage the monolithic column. The increasing of the dynamic binding capacity by using commercial BSA solution was experimentally tested by the open-loop frontal analysis. The breakthrough experiment, presented in Fig. 2, was repeated with various duration times. The binding capacities determined from the integration of the breakthrough areas and from the measurements of eluted BSA, confirmed that the dynamic binding capacity increases with the duration of the experiment. Between 1000 and 2000 s, the dynamic binding capacity increased for approximately 5%, but still did not reach the full saturation of the column.

To avoid these problems, a closed-loop frontal analysis with the circulation of a finite volume (20 ml) of the feed BSA solution was used. This method prevents an excessive supply of BSA dimers to the ion-exchange column that substantially influence the binding capacity. According to the literature [24], additional dimers probably do not form from BSA monomers in the solution over the time frame of the

experiment implying that only a relatively small amount of dimers are present in the system. The upper part of Fig. 2 presents the breakthrough curves obtained from the closed-loop frontal analysis with the circulation of a 20-ml BSA solution. These curves can not be compared with the breakthrough curves from the open-loop frontal analysis, because the inlet concentration is changing with time. Therefore, the breakthrough curves can have very different shapes even though obtained under similar experimental conditions (see the upper part of Fig. 2). This is due to different mixing and concentration profiles present in the inlet vessel. It should be stressed that the breakthrough curves in a closed loop system are presented as c/c_f vs. time, where $c_f \neq c_0$ (c_f is the final concentration of BSA in the steady-state). It is obvious from Fig. 2 that by using closed-loop frontal analysis, steady state is achieved in less than 1000 s in all three cases. Three different breakthrough curves are presented to show that the time required to obtain steady state is independent of the course of the breakthrough curve. Therefore, this method can be considered as very robust. The achievement of steady-state also proves that additional dimers do not form during the experiment. If additional dimers did form, it would change the composition of the solution and prevent system equilibration. The equilibrium binding capacity obtained from the closed-loop frontal analysis is approximately 30% lower than the dynamic binding capacity determined from the open-loop frontal analysis at 95% breakthrough [4]. This confirms the influence of BSA dimers on the dynamic binding capacity.

Application of closed-loop frontal analysis has another advantage: capacity determination is independent of molecular mobility. It is well known that molecules have increased mobility with increasing temperature. Because of this, molecules may reach the active surface faster resulting in an apparent increase in the dynamic binding capacity. This is especially true for the open-loop breakthrough experiments. In the case of closed-loop experiments, however, capacity is determined after steady-state condition is achieved. Because of that any changes in capacity cannot be attributed to differences in molecular mobility.

The closed-loop frontal analysis, presented in Fig. 4, enabled the determination of the equilibrium capacity of the ion-exchanger as a function of temperature. Here it can be seen that the capacity increases with temperature in the range from 2 to 30 °C. The confirmation of the temperature effect is presented in Fig. 5, where the temperature of the previously equilibrated column at 20 °C was being altered during the experiment. In this experiment, the

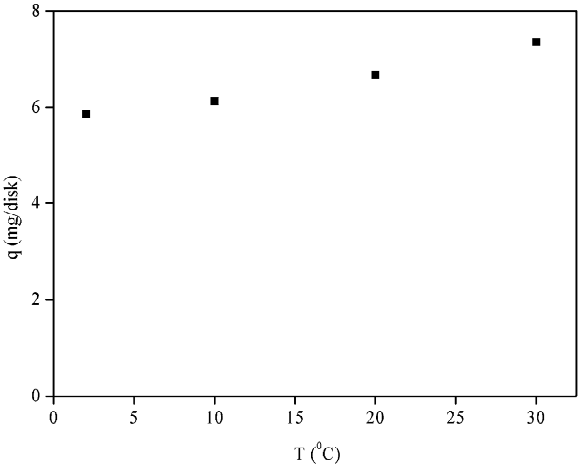

Fig. 4. Equilibrium CIM® DEAE disk column capacity as a function of temperature. Experimental conditions: approximate equilibrium liquid phase concentration of BSA: 0.6 mg/ml; flow-rate: 3.0 ml/min; volume of the circulating BSA solution: 20 ml.

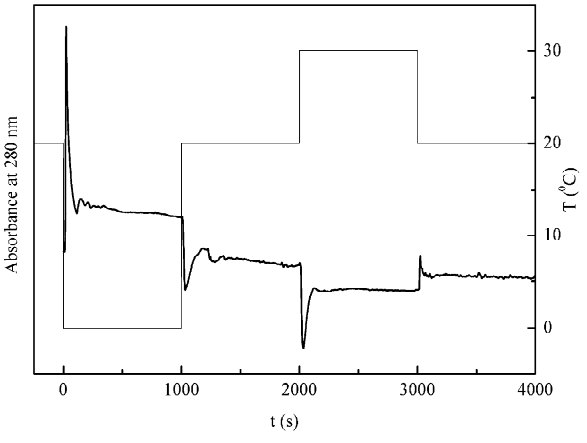

Fig. 5. Thermo-induced BSA concentration profiles at closed-loop frontal analysis using pre-equilibrated CIM® DEAE disk monolithic column at 20 °C. Temperature profile is presented in the figure. Volume of the BSA solution: 20 ml; flow-rate: 3 ml/min; detection: UV at 280 nm.

pH of BSA solution was adjusted to 7.4 at 20 °C. As it is well known, the pH of the Tris buffer is a function of temperature, and for this reason the pH of the solution varied during the experiment. The pH changes of the 20 m*M* Tris–HCl (pH 7.4 at 20 °C) buffer with temperature can be described by the following equation [26]:

$$\mathrm{pH} = 8.0 - 0.03T\ (^{\circ}\mathrm{C})$$

Since the pH influences the net charge of the BSA [27], it also significantly affects the binding capacity. The increased pH causes the BSA to have a higher net charge leading to a higher binding capacity. Fig. 6 presents a comparison between open-loop frontal analysis breakthrough curves at different pH values of BSA solution. A higher pH causes a shifting of the breakthrough curve indicating an increase in the dynamic binding capacity. This behavior was confirmed by measuring the quantity of the eluted protein. The dependence of the dynamic binding capacity at 95% breakthrough on the pH is shown as an insertion in Fig. 6.

Considering the above, it would be expected that increase in temperature would cause a decrease in the buffer pH resulting in a decreased net charge of BSA. In this case, a lower dynamic binding capacity would be expected and this would lead to an elution peak of BSA. Yet, a quite different effect can be observed from Fig. 5. Here a decrease of temperature results in an elution peak of BSA and shifts the equilibrium absorbance. Such behavior confirms that the dependence of the dynamic binding capacity on the temperature is more pronounced than its dependence on the pH change of the solution for the presented experimental conditions. This experiment also confirms that the equilibrium column capacity decreases with the lowering of the temperature, and a contrary behavior is evident when the column is immersed in a bath at a higher temperature. It should be stressed that this behavior is reversible and can be repeated in the temperature range from 2 to 30 °C.

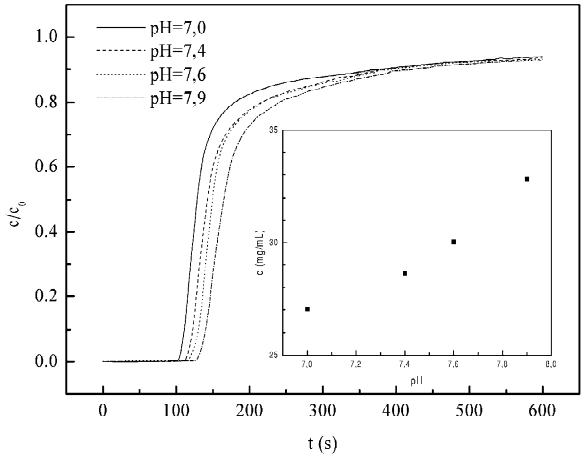

Fig. 6. Open-loop frontal analysis breakthrough curves at various pH values of loading phase. Inserted figure shows dependence of the dynamic binding capacity at 95% breakthrough on the pH of the BSA solution. Experimental conditions: loading phase: 1 mg/ml BSA in 20 m*M* Tris–HCl loading buffer; stationary phase: CIM® DEAE disk monolithic column; flow-rate: 3.9 ml/min; detection: UV at 280 nm; temperature: 22 °C.

At a temperature of 40 °C, a substantial increase in the binding capacity was observed. In the subsequent elution step only a partial elution of the bound BSA occurred. At the next loading, the capacity dropped significantly because of the irreversible binding of BSA. The reason for this could be a conformational change of the BSA or the agglomeration of the protein within the pores of the ion exchanger caused by a higher capacity.

Microcalorimetric studies [18] of BSA adsorption in overloaded ion-exchange columns have shown that the enthalpy of adsorption is endothermic. This is primarily due to the repulsive interactions between adsorbed BSA molecules. The increase in the equilibrium capacity with increased temperature observed in this study is therefore probably influenced by thermodynamic principles.

3.2. Soybean trypsin inhibitor

Open-loop frontal analysis experiments were performed for determination of the temperature influence on the dynamic binding capacity of trypsin inhibitor. In this work, two different inlet concentrations, 0.1 and 1.0 mg/ml, were used. In Fig. 7, breakthrough curves at the inlet concentration of 0.1 mg/ml at different temperatures are presented. From the shape of these breakthrough curves one could speculate that the sample contained several components, some impurities and the trypsin inhibitor. The presence of impurities was confirmed after gradient separation of the feed solution, shown in

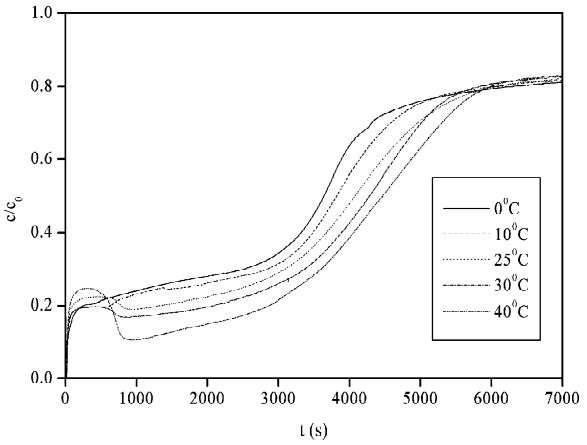

Fig. 7. Breakthrough curves of soybean trypsin inhibitor at different temperatures. Stationary phase: CIM® DEAE disk monolithic column; loading phase: 0.1 mg/ml soybean trypsin inhibitor in loading buffer; flow-rate: 3 ml/min; detection: UV at 280 nm.

Fig. 8. As expected, the separation confirmed the presence of several impurities of which some were retained and others were not. These impurities considerably influenced the shape of the breakthrough curves. Non-retained impurities lead to instantaneous breakthrough, while strongly retained impurities prevented c/c_0 from equaling 1. However, the impurities have little effect on the dynamic binding capacity as they can be easily displaced by

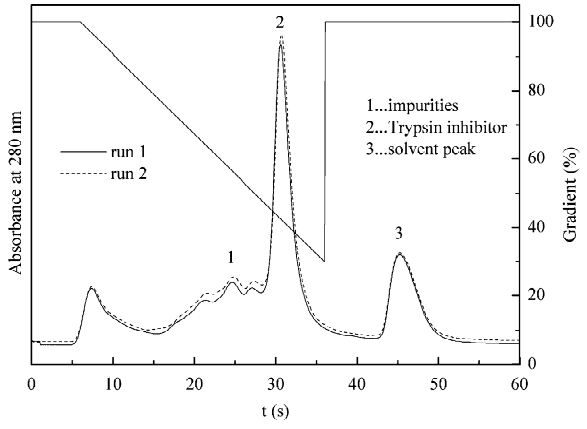

Fig. 8. Gradient separation of soybean trypsin inhibitor. Experimental conditions: loading buffer: 20 m*M* Tris–HCl, pH 7.4; stationary phase: CIM® DEAE disk monolithic column; elution buffer: 1 *M* NaCl in loading buffer; injection loop: 20 μl; detection: UV at 280 nm; temperature: 21 °C.

the trypsin inhibitor on the ion-exchange column. Although the impurities demonstrate competitive adsorption resulting in a lower dynamic binding capacity, they do not change the trend of temperature influence on the adsorption capacity.

The breakthrough curves presented in Fig. 7 are similar in shape but their position varies with temperature. The higher the temperature the later the breakthrough occurs indicating that the capacity of the ion-exchanger increases with temperature. This trend was also confirmed by measuring the quantity of the eluted protein at 80% breakthrough. These values are presented in Table 1. In contrast to BSA, the trypsin inhibitor binds reversibly and obtains a maximum capacity of 20 mg/disk at 40 °C.

Temperature influence on the dynamic binding capacity for soybean trypsin inhibitor is similar to BSA. To confirm the temperature influence on the binding capacity of the trypsin inhibitor, a closed-loop frontal analysis with a changing column temperature was performed. Thermo-induced concentration profiles are presented in Fig. 9. The DEAE column was pre-equilibrated at 10 °C. It can be seen that decreasing temperature causes an elution of the protein and a contrary effect occurs when the temperature is increased. This experiment confirmed that the capacity increases with temperature.

3.3. L-Glutamic acid

L-Glutamic acid was used in order to examine the effect of temperature on the dynamic binding capacity of a small molecule, where conformational

Table 1
The dependence of the CIM® DEAE disk capacity for the soybean trypsin inhibitor adsorption on monolithic column at different temperatures

T (°C)	*c* (mg/ml)	*q* (mg/disk)
0	0.1	9.6
10	0.1	15.1
20	0.1	16.5
30	0.1	17.1
40	0.1	17.7
0	1.0	13.0
10	1.0	16.5
20	1.0	18.4
30	1.0	19.2
40	1.0	19.6

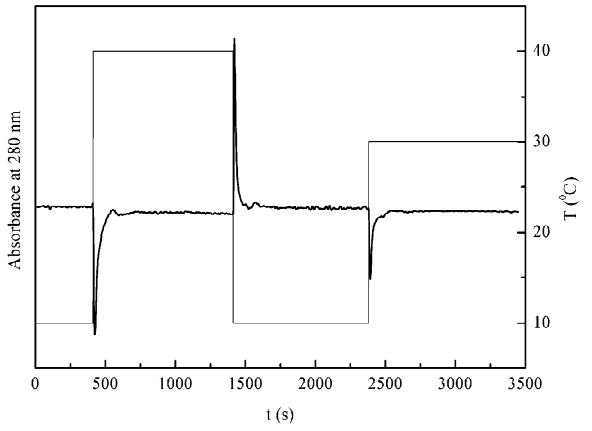

Fig. 9. Thermo-induced concentration profiles of the soybean trypsin inhibitor at closed-loop experiments using a pre-equilibrated DEAE column. Temperature profile is presented in the figure. Experimental conditions: feed volume of soybean trypsin inhibitor solution at 1 mg/ml in 20 m*M* Tris–HCl pH 7.4 buffer: 20 ml; flow-rate: 3.0 ml/min; detection: UV at 280 nm.

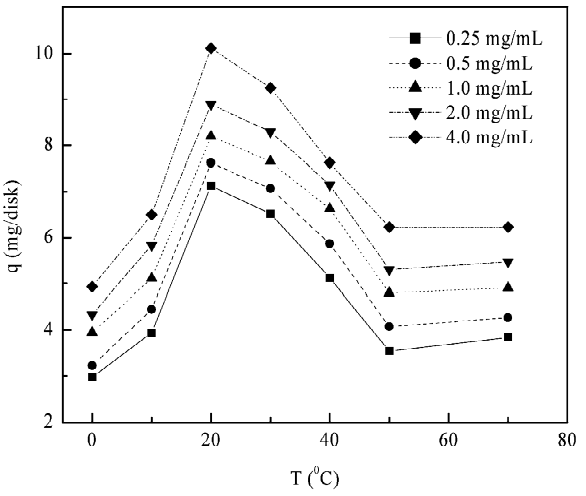

Fig. 11. Dependence of the dynamic binding capacity of L-glutamic acid on temperature at different feed concentrations.

changes could not influence the ion-exchange equilibrium. The open-loop frontal analysis was used for binding of the amino acid. The dynamic binding capacity was determined only by measuring the quantity of the eluted L-glutamic acid at 90% breakthrough. Five different feed concentrations and seven different column temperatures were used for the construction of the adsorption isotherms. In Fig. 10, all measured adsorption isotherms and their Langmuir fits are presented. In Fig. 11, the dynamic binding capacity as a function of temperature is presented. The fact that the maximum capacity is achieved at 20 °C is somewhat surprising, although, a similar behavior has already been observed for HEWL (hen egg white lysozyme) adsorption on an ion exchanger [20] and BSA adsorption on a reversed-phase C_4 support [17]. A decreasing capacity with increasing temperature above 20 °C was confirmed by using the closed-loop frontal analysis experiment. The temperature of the equilibrated column at 20 °C was changed to 80 °C without stopping the pump. This led to an extensive elution peak of L-glutamic acid indicating a decrease in capacity, (see Fig. 12). It can be also seen from Fig. 11 that the capacity at 70 °C is slightly higher than at 50 °C. Since the difference between the two is very small and within experimental error it cannot be concluded that the trend is changing.

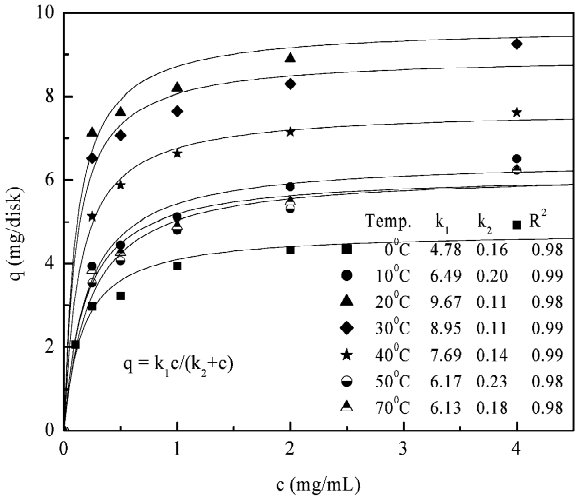

Fig. 10. Adsorption isotherms with Langmuir fits of L-glutamic acid on CIM® DEAE disk monolithic columns at different temperatures.

In the absence of conformational changes, non-specific interactions could affect the equilibrium. Since the base matrix for the DEAE columns consists of hydroxy and epoxy groups, an experiment using hydroxy and epoxy columns was performed to estimate the impact of non-specific interactions. The results, presented in Fig. 12, show that the hydroxy

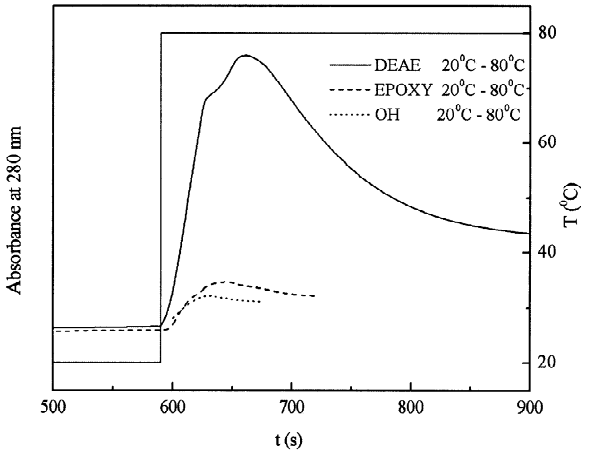

Fig. 12. Thermo-induced concentration profiles of L-glutamic acid on different monolithic disks. Temperature profile is presented in the figure. Flow-rate: 3 ml/min; detection: UV at 215 nm.

and epoxy columns are basically inert and produce a negligible effluent peak of L-glutamic acid. This experiment confirms that the capacity of L-glutamic acid changes with temperature on a DEAE column because of the presence of the ion-exchange groups and that non-specific interactions are negligible. However, since L-glutamic acid is a weak electrolyte, its ionic composition is determined by dissociation constants. It is well known that these constants vary with temperature influencing the proportion of the different ionic forms in the solution and consequently the pH. In the experiments, the pH of the amino acid solution has been adjusted to 5.5 at 20 °C. Dependence of the solution pH on the temperature was checked in the range from 5 to 50 °C and was almost constant with the deviation of ±0.1 on the pH scale. But, even such a small difference in pH could have an important effect on the ionic composition of L-glutamic acid. According to the literature [28], the capacity of a weak basic ion exchanger is highest at the isoelectric point of L-glutamic acid e.g. at pH 3.22 and it decreases significantly at higher or lower pH values. This is another reason for the complex behavior of L-glutamic acid adsorption on the weak anion-exchanger (because the adsorption mechanism has not yet been fully explained [28,29]). Since the proteins consist of amino acids also the overall effect of the temperature is difficult to predict.

4. Conclusions

The temperature effect on the ion-exchange binding capacity of BSA, soybean trypsin inhibitor and L-glutamic acid is presented and discussed. Results show that temperature is an important parameter, which can considerably influence the ion-exchange capacity. However, the influence of temperature on the capacity is very complex because it affects not only the physical properties of the solution but also the properties of the adsorbate and adsorbent. In the case of proteins bound on the methacrylate based monolithic matrix, conformational changes do not account for this behavior since the same is observed for amino acid and neither is it due to unspecific interactions with the matrix as confirmed with the test of the matrix without ion-exchange moieties. Furthermore, increased dynamic binding capacity cannot be attributed to the increased mobility of the adsorbate since closed-loop experiments were run until the steady-sate was achieved. Because of this complexity, the effect of temperature should be experimentally determined for each system of adsorbate-solution–adsorbent and it has to be taken into account during the development of chromatographic methods.

5. Symbols

c	Concentration (mg/ml)
c_0	Inlet concentration (mg/ml)
c_f	Final concentration (mg/ml)
CV	Column volume (ml)
q	Binding capacity of the column (mg/ml or mg/disk)
t	Time (s)
T	Temperature (°C)

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