TOSOH

Application Note

MIXED ELECTROLYTES IN HYDROPHOBIC INTERACTION CHROMATOGRAPHY

INTRODUCTION

While process productivity of ion exchange chromatography (IEX) has been improved due to advanced surface modifications of newly developed resins (such as the TOYOPEARL GigaCap series), hydrophobic interaction chromatography (HIC) with proteins has experienced comparably small performance increases. For HIC, additional parameters besides the resin surface modification need to be tuned. Especially capacities and recoveries of HIC applications cannot compete with those of modern IEX. The general method complexity allows various strategies to approach this goal, as for many applications, selectivity of HIC is striking. This application note addresses the electrolyte composition of the liquid phase as one parameter responsible for protein adsorption and desorption. One standard salt used for HIC is ammonium sulfate. Its salting-out potential is well-known and also applicable for non-chromatography based purification methods, such as protein precipitation. Apart from sodium citrate and sodium sulfate, which also show great salting-out potentials for many proteins, salts like sodium acetate and sodium chloride belong to the manufacturing scale relevant (buffer-) salts. The latter are not typically related to HIC as their salting-out potential is weaker. The herein presented results illustrate the benefit of often neglected salts in HIC and mixtures of them, regarding capacity and selectivity.

DYNAMIC BINDING CAPACITY

The impact of the electrolyte composition of the liquid phase in HIC on the dynamic binding capacity for lysozyme and a monoclonal antibody (mAb) was investigated. The solubility limiting concentration of ammonium sulfate was optically determined to be 2.2 M for lysozyme and 1.1 M for the mAb. Hence, concentrations of 2 M and 1 M ammonium sulfate were chosen, respectively, as a reference value for the capacity comparison. Table 1a & b list exemplary dynamic binding capacities that could be achieved using ammonium sulfate and mixtures of ammonium sulfate, sodium citrate, sodium sulfate, sodium acetate and sodium chloride for protein adsorption onto TOYOPEARL PPG-600M, TOYOPEARL Phenyl-600M and TOYOPEARL Butyl-600M. Capacities were measured at 10 % breakthrough, pH 7.0, and a protein concentration of 1 g/L. The feed stream was set to 150 cm/h. The corresponding resins were packed to a total column volume of 1 mL with an inner diameter of 6.6 mm.

The dynamic binding capacities for the two presented proteins can be increased for certain salt mixtures and resins. Improvements up to roughly 50 % are possible.

SELECTIVITY

The point of elution of a certain protein does not only depend on the applied salt molarity. Factors like pH and temperature are well-known to influence protein desorption. Further, the standard protein separations presented in Figure 1 – 3 illustrate that the protein elution does not correlate linearly with the decreasing salt concentration in the liquid phase for ammonium sulfate and the exemplary presented salt mixtures. The different figures refer each to one commonly used TOYOPEARL HIC ligand: PPG, Phenyl and Butyl. For selectivity testing, cytochrome C (1), ribonuclease A (2) and lysozyme (3) were separated. 1 mL columns with an inner diameter of 6.6 mm were used. 10 mg/mL resin of each protein were loaded. A constant flow of 150 cm/h was applied.

Figure 1 illustrates the chromatograms using TOYOPEARL PPG-600M. Lysozyme, which is represented by peak 3 elutes the latest for the mixture of sodium sulfate and sodium chloride. For the same mixture, ribonuclease A (2) elutes right after the gradient starts. For ammonium sulfate as a single salt, the very same proteins elute in between. This means, that this mixture provides increased resolution for the proteins, compared to ammonium sulfate. The same behaviour is observed for TOYOPEARL Phenyl-600M (Figure 2) and TOYOPEARL Butyl-600M (Figure 3).

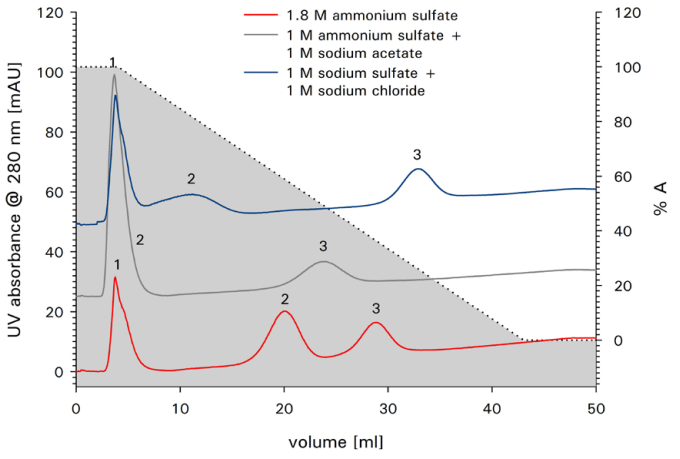

Figure 1

Separation of cytochrome C (1), ribonuclease A (2) and lysozyme (3) on TOYOPEARL PPG-600M. Ribonuclease A is hardly retained for the mixtures. Lysozyme is further retained for the sodium sulfate + sodium chloride mixture than for ammonium sulfate as a single salt.

TOYOPEARL Butyl-600M, as the most hydrophobic resin among the tested ones, allows binding of cytochrome C (1) for some salts or salt mixtures. Only the mixture of ammonium sulfate and sodium acetate does not cause cytochrome C binding, while lysozyme is almost as much retained as for ammonium sulfate as a single salt. Ribonuclease A is only weakly bound to the resin using this mixture. If lysozyme would represent the target molecule, the almost exclusive binding of lysozyme would allow higher binding capacities as for the other two liquid phase compositions, where parts of the resin capacity are occupied by the other sample components.

It can be concluded that every target protein and its related impurities require distinct salts or salt mixtures to achieve highest resolution, purity and capacity. On the one hand, this enlarges the set of parameters in HIC, but on the other hand, this allows selectivity modulation. Higher resolution of target substances and impurities can be achieved. Capacities can also benefit from salt mixtures. Besides a more sophisticated binding of the target component, general capacities up to 150 % of the ammonium sulfate capacity can be reached. Using mixed electrolytes in HIC opens up an additional opportunity to improve HIC separations as an alternative to traditional HIC applications using ammonium sulfate.

Salt	Capacity [mg/mL] PPG-600M	Capacity [mg/mL] Phenyl-600M	Capacity [mg/mL] Butyl-600M
2 M ammonium sulfate	30	46	15
1 M sodium sulfate + 1 M sodium acetate	39	63	18
1 M ammonium sulfate + 1 M sodium chloride	31	54	10
0.9 M trisodium citrate + 0.9 M sodium chloride	38	43	20

Table 1a

Dynamic binding capacities for lysozyme and salt mixtures on TOYOPEARL PPG-600M, TOYOPEARL Phenyl-600M and TOYOPEARL-Butyl-600M. The capacity determined applying ammonium sulfate serves as a reference standard.

Salt	Capacity [mg/mL] PPG-600M	Capacity [mg/mL] Phenyl-600M	Capacity [mg/mL] Butyl-600M
1 M ammonium sulfate	18	20	18
1.7 M sodium chloride + 0.7 M ammonium sulfate	30	25	15
1.3 M sodium chloride + 0.6 M ammonium sulfate	25	20	12

Table 1b

Capacities at 10 % breakthrough for mAb and representative mixtures on TOYOPEARL PPG-600M, TOYOPEARL Phenyl-600M and TOYOPEARL-Butyl-600M. The capacity for ammonium sulfate may be regarded as a reference standard.

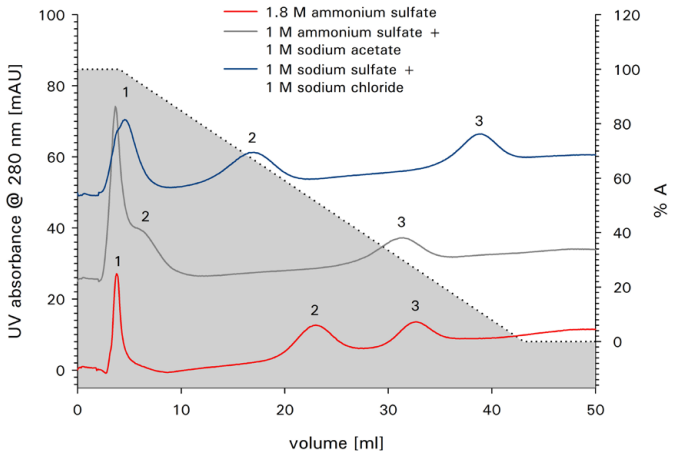

Figure 2

The three standard molecules cytochrome C (1), ribonuclease A (2) and lysozyme (3) are separated using TOYOPEARL Phenyl-600M. Retention of lysozyme is as high or higher for the two mixtures, compared to ammonium sulfate. For ribonuclease A and the two mixtures, a significant decrease in retention, compared to ammonium sulfate, can be observed.

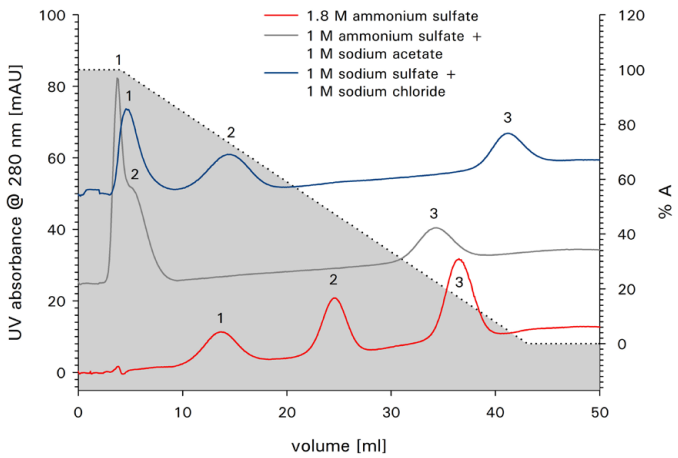

Figure 3

Separation of cytochrome C (1), ribonuclease A (2) and lysozyme (3) on TOYOPEARL Butyl-600M. Ammonium sulfate is compared to mixtures of ammonium sulfate + sodium acetate and sodium sulfate + sodium chloride. Resolution of ribonuclease A and lysozyme is increased for the mixtures. Binding of cytochrome C cannot be achieved for the mixtures, although in total, higher molarities are applied.