Supporting Information for Adsorption of Bovine Serum Albumin on a Mixed-Mode Resin - Influence of Salts and the pH Value

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Results of the Correlation of Individual Adsorption Isotherms

The results of the batch adsorption experiments with bovine serum albumin (BSA) were correlated with the semi-empirical correlation function of Oberholzer and Lenhoff [1, 2], cf. Eq. (1) in the manuscript. The values of the parameters that were obtained in these fits are reported in Tables S.1 - S.3. Note that no dependence of the fitting parameters on the process parameters is recognizable, β and γ even oftentimes correspond to either the starting or the boundary values. Therefore, Eq. (1) in the manuscript is not suitable for extrapolations to unstudied process parameters and the prediction of equilibrium adsorption isotherms.

Table S.1: Parameters of the correlations of the individual adsorption isotherms of BSA on Toyopearl MX-Trp-650M with Eq. (1) in the manuscript for the experiments carried out at pH 4.0 and 25 °C. Some of the values correspond to the boundary values defined for the fit (0.00 and 100.00, respectively).

Salt	I / mM	K^{ads}	β	γ
None	0	33.01	0.71	7.97
NaCl	250	124.00	0.50	0.00
	500	30.51	1.86	5.26
	1000	31.86	0.90	0.00
	1500	18.23	0.85	0.00
	2000	15.71	0.91	0.00
	2500	137.04	2.12	0.00
	3000	29.84	1.22	0.00
Na_2SO_4	250	121.27	0.54	0.00
	500	90.94	1.12	2.03
	1000	25.28	100.00	17.97
	1500	50.41	2.55	4.78
	2000	104.01	0.80	0.00
	2500	169.62	0.89	0.68
	3000	100.64	0.72	1.13
NH_4Cl	250	102.91	0.52	0.00
	500	46.18	1.45	4.42
	1000	17.37	1.06	2.92
	1500	40.51	3.48	6.60
	2000	35.76	4.02	6.61
	2500	42.28	4.45	8.01
	3000	45.05	3.34	7.79
$(NH_4)_2SO_4$	250	57.91	0.33	0.00
	500	108.01	0.52	0.00
	1000	32.07	1.11	4.99
	1500	23.91	1.92	5.67
	2000	110.55	0.99	0.00
	2500	39.33	4.77	6.60
	3000	79.88	1.11	1.80

Table S.2: Parameters of the correlations of the individual adsorption isotherms of BSA on Toyopearl MX-Trp-650M with Eq. (1) in the manuscript for the experiments carried out at pH 4.7 and 25 °C. Some of the values correspond to either the starting (10.00) or the boundary values (0.00 and 100.00, respectively) defined for the fit.

Salt	I / mM	K^{ads}	β	γ
None	0	30.81	0.92	6.18
NaCl	250	13.21	0.57	0.00
	500	1.18	0.00	10.00
	1000	0.72	0.00	28.24
	1500	0.72	100.00	16.90
	2000	1.07	0.00	10.00
	2500	1.21	0.00	10.00
	3000	1.50	0.00	10.00
Na_2SO_4	250	56.14	0.79	0.00
	500	10.67	0.43	0.00
	1000	3.53	0.40	0.00
	1500	2.34	0.36	0.00
	2000	14.66	1.08	0.00
	2500	37.35	1.31	0.00
	3000	11.18	0.68	0.00
NH_4Cl	250	2038.51	2.70	0.00
	500	33.95	2.07	0.00
	1000	0.62	8.53	11.53
	1500	0.73	100.00	17.60
	2000	0.84	0.00	10.00
	2500	0.94	0.00	10.00
	3000	1.28	0.00	10.00
$(NH_4)_2SO_4$	250	193.57	0.97	0.00
	500	42.41	0.93	0.00
	1000	9.52	0.68	0.00
	1500	2.05	0.33	0.00
	2000	16.50	1.58	0.00
	2500	8.16	1.00	0.00
	3000	2.41	0.25	0.00

Table S.3: Parameters of the correlations of the individual adsorption isotherms of BSA on Toyopearl MX-Trp-650M with Eq. (1) in the manuscript for the experiments carried out at pH 7.0 and 25 °C. ome of the values correspond to either the starting (10.00) or the boundary values (0.00 and 100.00, respectively) defined for the fit.

Salt	I / mM	K^{ads}	β	γ
NaCl	250	0.96	0.00	10.00
	3000	0.80	0.00	10.00
Na_2SO_4	250	0.20	1.72	41.06
	3000	1.04	0.00	9.98
NH_4Cl	250	2.22	3.28	0.00
	3000	0.89	100.00	13.11
$(NH_4)_2SO_4$	250	30.00	6.07	0.00
	3000	0.92	0.00	10.00

The individual correlations, cf. Eq. (1) in the manuscript, are shown in Figures S.1 - S.3 together with the corresponding experimental data. For improved clarity, data for sodium sulfate and ammonium chloride at pH 4.0 are displayed in separate diagrams for low and high ionic strengths. The BSA concentration in the liquid phase $c_{\mathrm{BSA}} = 0.09$ mM, which was used as an example for the description of the model development, is marked with vertical dashed lines in Figures S.1 and S.2.

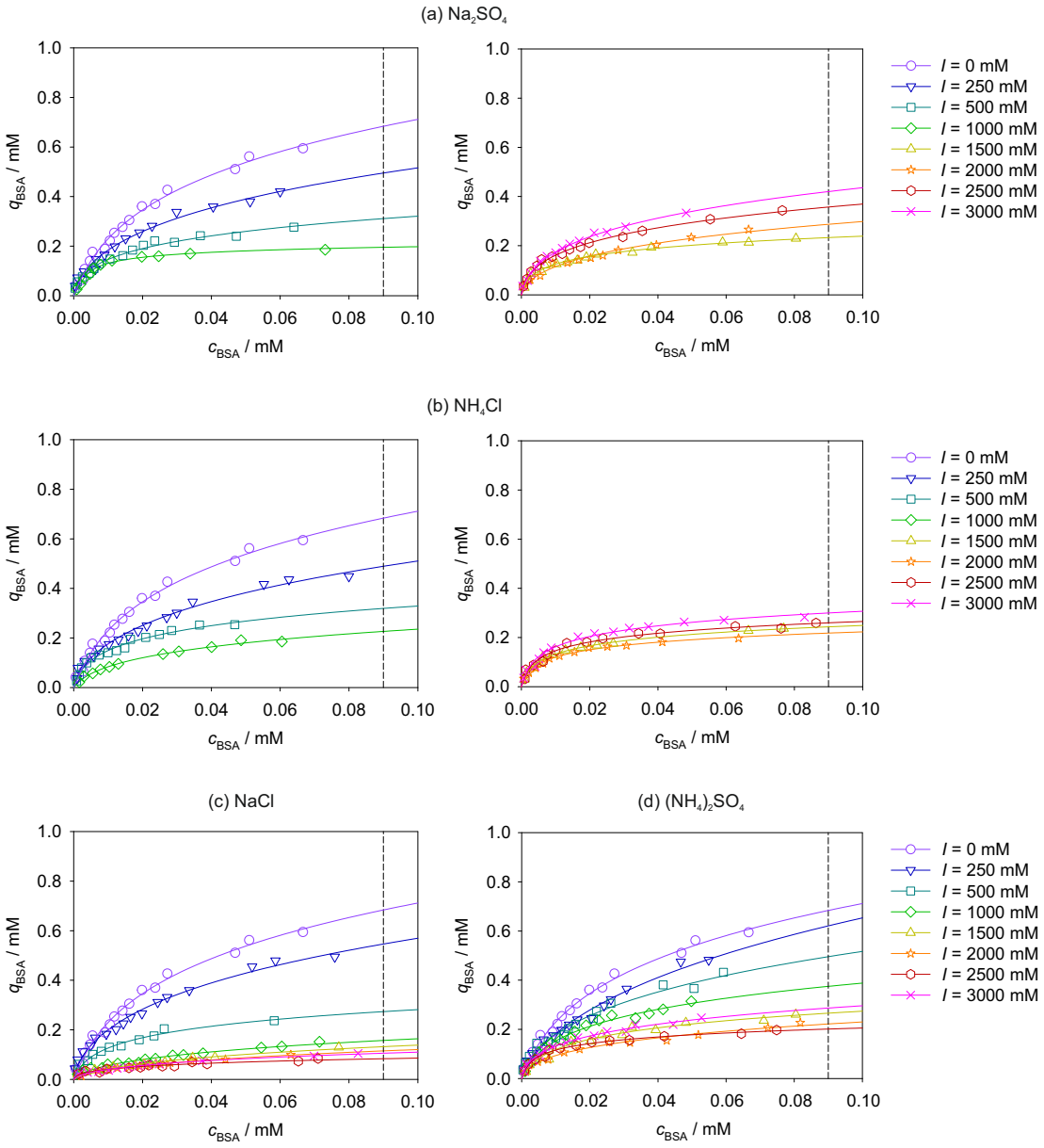

Figure S.1: Equilibrium adsorption isotherms of BSA on Toyopearl MX-Trp-650M at pH 4.0 and 25 °C for four salts at different ionic strengths I. Symbols: experimental data; lines: individual correlations, cf. Eq. (1) in the manuscript.

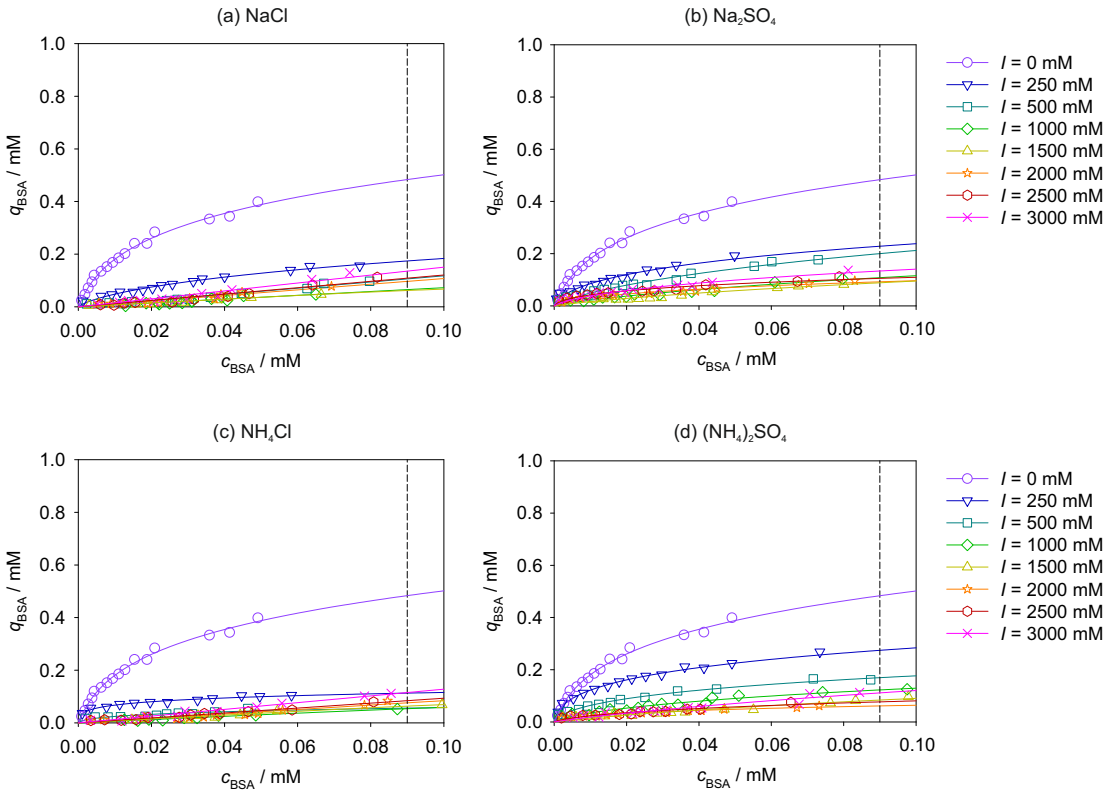

Figure S.2: Equilibrium adsorption isotherms of BSA on Toyopearl MX-Trp-650M at pH 4.7 and 25 °C for four salts at different ionic strengths I. Symbols: experimental data; lines: individual correlations, cf. Eq. (1) in the manuscript.

At pH 4.0 and 4.7, higher BSA loadings are observed in the ion exchange (IEC) region at low ionic strengths than in the hydrophobic interaction (HIC) region at high ionic strengths for all studied salts. The highest BSA loadings are found at $I = 0$ mM; the addition of salts leads to a decrease of the BSA loading with increasing ionic strength in the IEC region. By contrast, increasing ionic strengths lead to an increase of the BSA loading in the HIC region. The adsorption at pH 4.0 is generally higher than at pH 4.7 for all considered salts, especially in the IEC region. At pH 7.0, the adsorption is generally at a low level. Here, the adsorption at $I = 3000$ mM is higher than at $I = 250$ mM (except for sodium chloride).

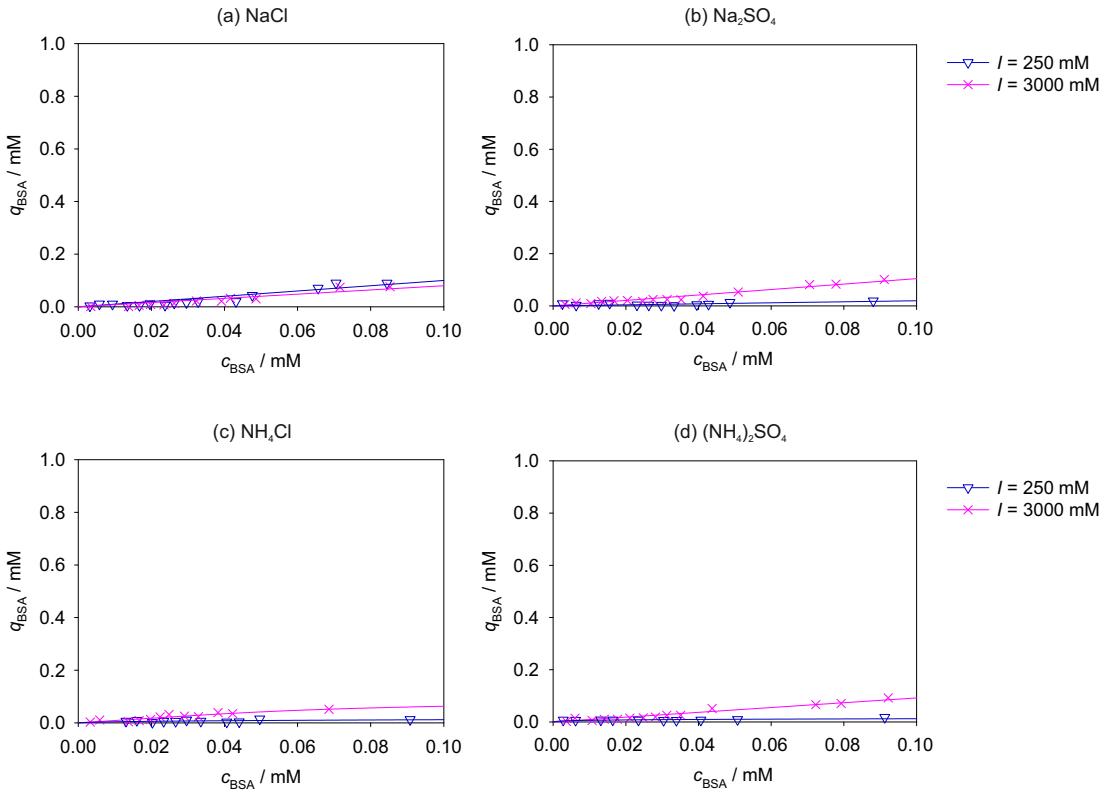

Figure S.3: Equilibrium adsorption isotherms of BSA on Toyopearl MX-Trp-650M at pH 7.0 and 25 ℃ for four salts at different ionic strengths I. Symbols: experimental data; lines: individual correlations, cf. Eq. (1) in the manuscript.

Additional Results

Comparison of Mixed-Mode and Single-Mode Hydrophobic Adsorption

In Figure S.4, the results for the BSA adsorption on the mixed-mode resin Toyopearl MX-Trp-650M, predicted with our model, cf. Sections 3.2 - 3.3, are compared to results for the adsorption of BSA on the single-mode HIC resin Toyopearl PPG-600M [3]. All results are for 25 ℃, isotherms for all studied salts are shown for two combinations of pH value and ionic strength: pH 4.0 and $I = 1500$ mM, which is in the transition region between the IEC and the HIC region, and pH 4.7 and $I = 2700$ mM, which is in the

HIC region.

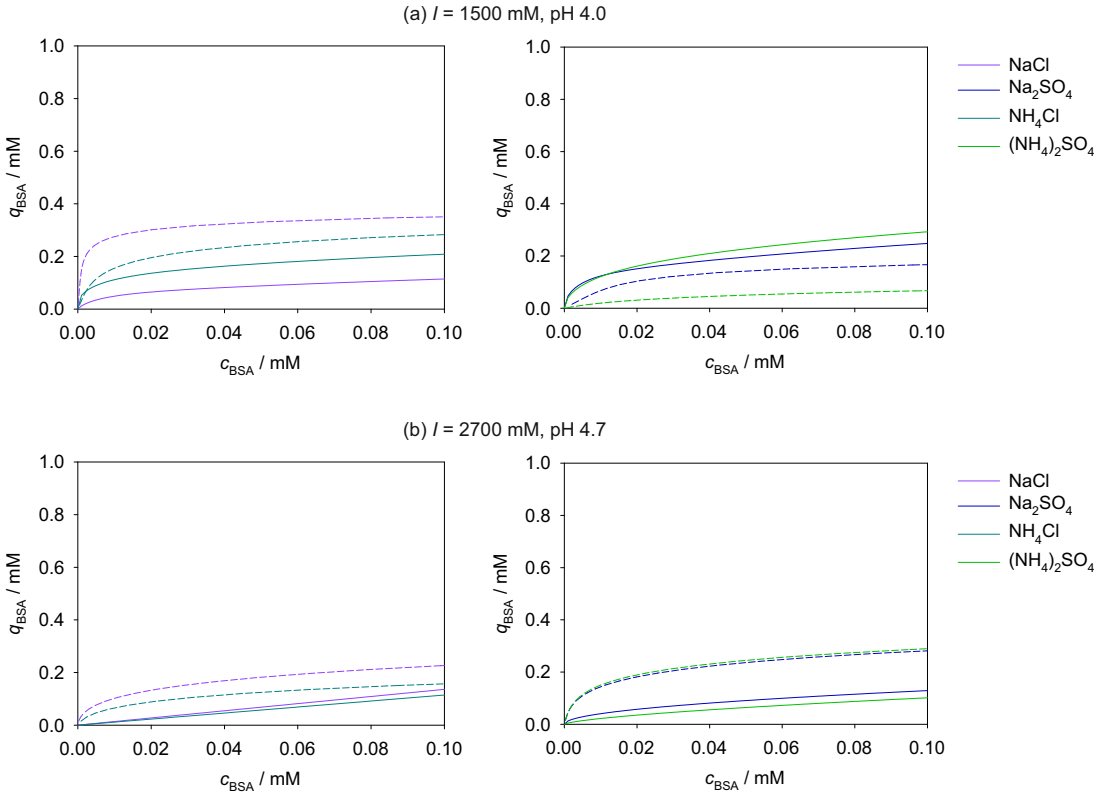

Figure S.4: Comparison of equilibrium adsorption isotherms of BSA on different resins at 25 °C for the studied salts at (a) $I = 1500$ mM, pH 4.0 and (b) $I = 2700$ mM, pH 4.7. Solid lines: mixed-mode resin Toyopearl MX-Trp-650M; dashed lines: HIC resin Toyopearl PPG-600M [3].

At $I = 2700$ mM, adsorption on the single-mode HIC resin is stronger than on the mixed-mode resin for all salts, which can be attributed to the different hydrophobic ligands of the two resins and differences in the occupancy of the surface of the resins with hydrophobic ligands.

In contrast, at $I = 1500$ mM, the behaviour strongly depends on the salts present in solution. If salts containing chloride ions are present, the adsorption on the single-mode resin is stronger, while stronger adsorption on the mixed-mode resin is found if salts containing sulfate ions are present. Taking into account that the hydrophobic interactions are weaker for the mixed-mode than for the single-mode resin for all studied

salts, cf. above, we conclude that at $I = 1500$ mM the adsorption on the mixed-mode resin is dominated by hydrophobic interactions if chloride ions are present, whereas it is still dominated by ionic interactions if sulfate ions are present. Sodium chloride seems to have a strong shielding effect combined with a rather weak salting-out effect, thus leading to an overall low adsorption at $I = 1500$ mM, i.e., in the transition region between IEC and HIC adsorption, which agrees well with our observations described in Section 3.1 in the manuscript. In contrast, the shielding effect of ammonium sulfate is significantly weaker leading to rather strong ionic interactions between BSA and the IEC ligands of the mixed-mode resin and therefore to a stronger adsorption on the mixed-mode compared to the single-mode HIC resin at $I = 1500$ mM. For sodium sulfate and ammonium chloride only minor differences between the adsorption on the mixed-mode resin and the single-mode HIC resin at $I = 1500$ mM can be found. We can attribute this to a shielding effect of these two salts that lies in between that of sodium chloride and ammonium sulfate. As a result, at $I = 1500$ mM of sodium sulfate or ammonium chloride, the ionic interactions for the mixed-mode resin roughly balance out the weaker hydrophobic interactions compared to the single-mode HIC resin.

Influence of the pH Value

A comparison of the experimental adsorption isotherm data at pH 4.0, 4.7, and 7.0 is depicted in Figure S.5 for solutions containing sodium chloride and ammonium sulfate at the lowest and highest studied ionic strengths, i.e., at $I = 250$ mM and $I = 3000$ mM. At pH 4.0 and 4.7, modeled equilibrium adsorption isotherms are included, whereas at pH 7.0, the corresponding individual correlations, cf. Eq. (1) in the manuscript, are shown.

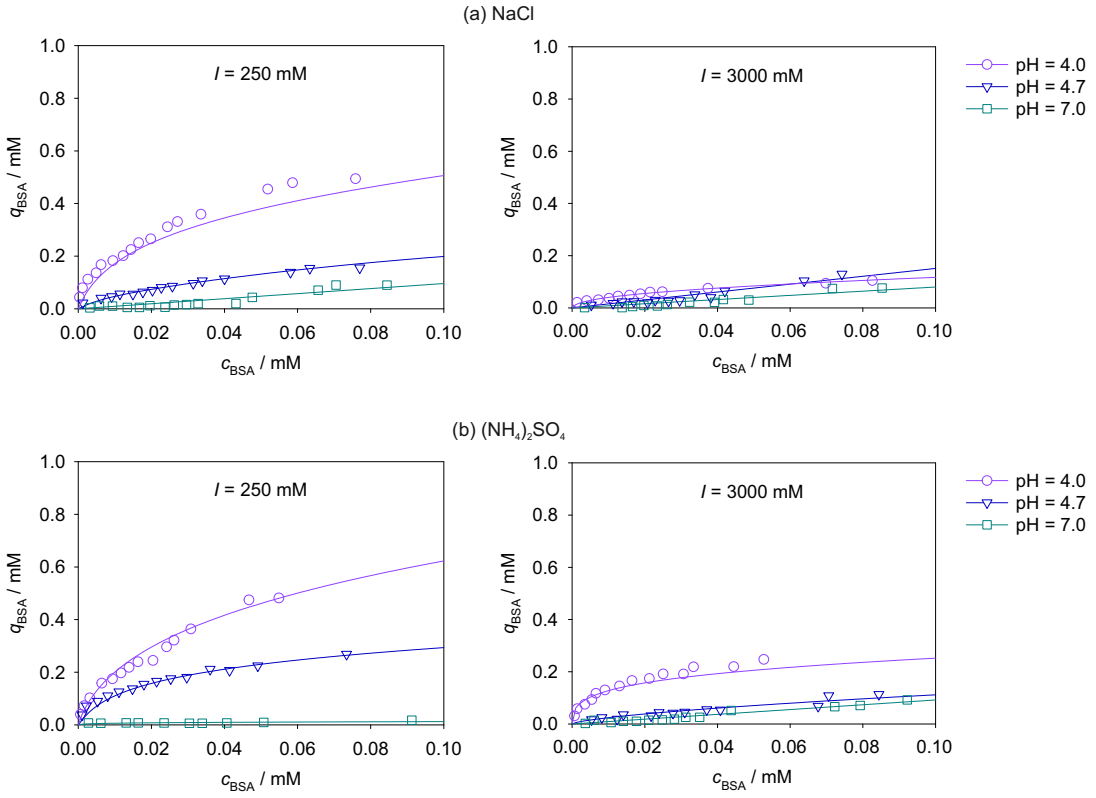

Figure S.5: Equilibrium adsorption isotherms of BSA on Toyopearl MX-Trp-650M at pH 4.0, 4.7, 7.0 and 25 °C for (a) sodium chloride and (b) ammonium sulfate. Symbols: experimental data; lines: model for pH 4.0 and 4.7; individual correlations for pH 7.0, cf. Eq. (1) in the manuscript.

Independent of the ionic strength and the considered salt, BSA loadings at pH 4.0 are generally higher than at pH 4.7, which in turn are higher than at pH 7.0. While adsorption in the IEC region is strongly influenced by the pH value, the influence of the pH value on the adsorption in the HIC region is small.

Influence of the Ionic Strength

The influence of the ionic strength on the adsorption is depicted in Figure S.6 for a fixed concentration of BSA in the liquid phase c_{BSA} for all studied salts and for pH 4.0 and 4.7. Results for $c_{\mathrm{BSA}} = 0.01$ mM are given in the left panel and for $c_{\mathrm{BSA}} = 0.1$ mM

in the right panel.

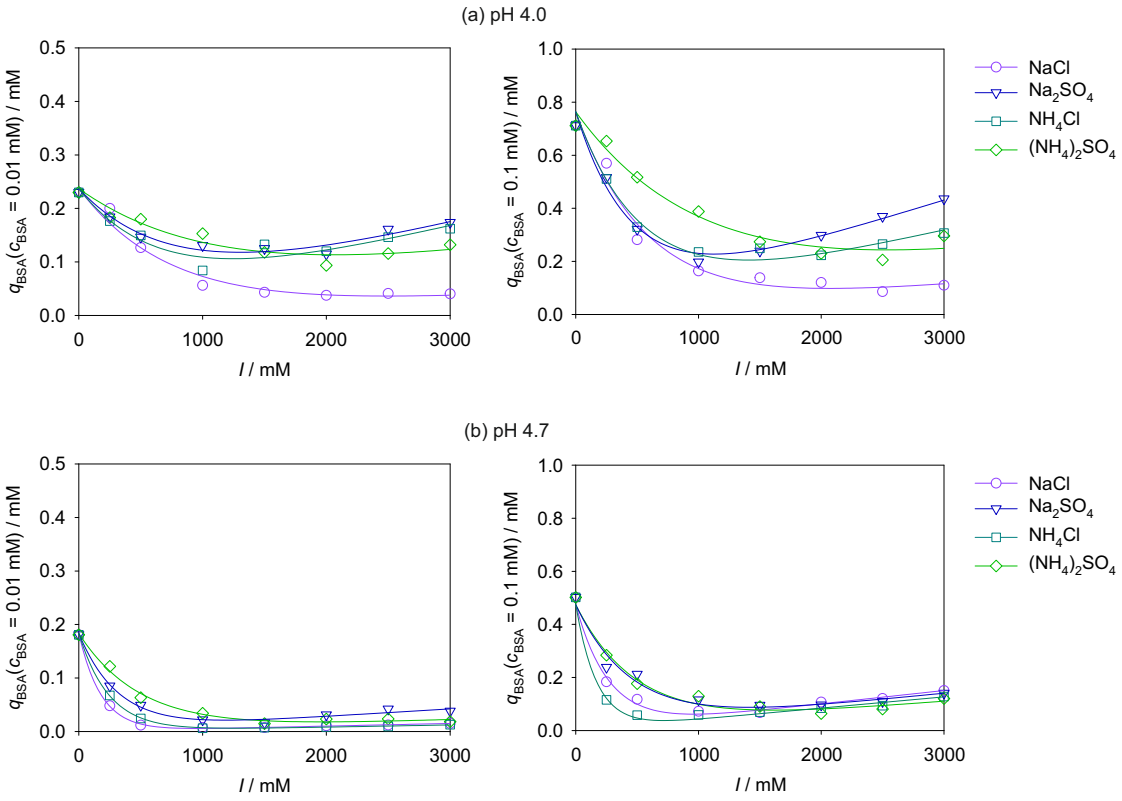

Figure S.6: BSA loading q_{BSA} of Toyopearl MX-Trp-650M at 25 °C for all studied salts as a function of the ionic strength I at (a) pH 4.0 and (b) pH 4.7. Symbols: experimental results (correlated with Eq. (1) in the manuscript); lines: model, cf. Eq. (3) in the manuscript.

The dependence of the BSA loading at $c_{\mathrm{BSA}} = 0.01$ mM and $c_{\mathrm{BSA}} = 0.1$ mM is similar to the one at $c_{\mathrm{BSA}} = 0.09$ mM discussed in detail in Figure 4 in the manuscript; for all salts and both pH values, an exponential decay of the adsorption with increasing ionic strength is observed in the IEC region. At pH 4.0, the exponential decay is weakest with the addition of ammonium sulfate. In contrast, in the HIC region, a linear increase is found for most salts and pH values, which is especially strong with the addition of sodium sulfate or ammonium chloride at pH 4.0. Overall, the model fits with Eq. (3) in the manuscript (lines) describe the data from the individual correlations (symbols) well.

Comparison of BSA and Lysozyme Loadings

A comparison of the results of BSA to those of lysozyme, cf. [4, 5], at pH 7.0 is shown in Figure S.7. The studies were carried out with the same resin and the same salts. The protein loading is reported as a function of the ionic strength for a fixed protein concentration in the solutions, which was $c_P = 0.09$ mM here. The results for BSA stem from individual correlations, cf. Eq. (1) in the manuscript, those for lysozyme were obtained by the model developed in our prior work [4, 5].

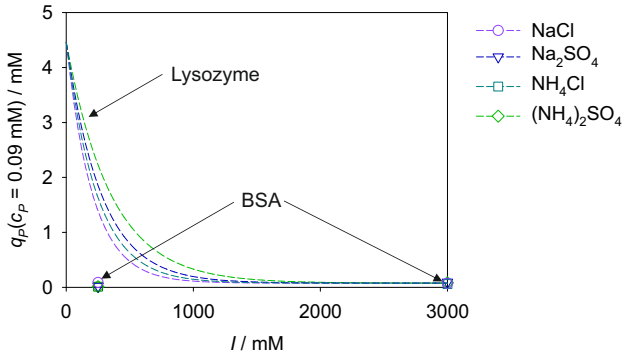

Figure S.7: Comparison of BSA and lysozyme loading of Toyopearl MX-Trp-650M at $c_P = 0.09$ mM (P ={BSA,lysozyme}) at pH 7.0 and 25 °C for all studied salts as a function of ionic strength I. Symbols: BSA loading from individual correlations, cf. Eq. (1) in the manuscript; dashed lines: lysozyme loading, cf. [4, 5].

At pH 7.0, an even more significant difference between the adsorption of the two proteins is observed than at pH 4.7, the adsorption of lysozyme is much stronger than that of BSA. Since the net charge of BSA is negative at pH 7.0, hardly any attractive ionic interactions between BSA and the resin can occur. The repulsion between the negatively charged mixed-mode resin and the negatively charged BSA molecules leads to the particularly visible difference of the loading of BSA to the one of lysozyme, which still carries a positive net charge at pH 7.0.

References

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