**Equilibrium properties of multimodal particle and membrane chromatographic adsorbents**

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**Highlights**

* Adsorption properties of multimodal particle and membrane adsorbents were investigated.
* Interesting influence of Hofmeister ions on the binding capacity was observed.
* Single- and bi-component adsorption of proteins and DNA was studied.
* Desorption experiments revealed problems with reversibility of binding.

**1. Introduction**

Multimodal chromatographic adsorbents are novel materials with promising properties for downstream processing of therapeutic glycoproteins. This presentation summarizes the results of our research dealing with the properties of the salt-tolerant chromatographic membrane Sartobind STIC a several particle multimodal adsorbents with both cation- and anion-exchange ligands. The equilibrium properties of these materials were investigated for a number of model proteins and DNA. Moreover, their performance in the purification of recombinant human erythropoietin (rhEPO) from a cultivation broth produced by human embryonic kidney cells was examined.

**2. Methods**

The investigated adsorbents were MEP HyperCel, HEA HyperCel, and PPA HyperCel from Pall Corporation, Capto MMC, Capto MMC Impress from GE Healthcare, Eshmuno HCX from Merck, and Sartobind STIC from Sartorius Stedim Biotech. The compounds used for adsorption were bovine serum albumin, human serum albumin, ovalbumin, α-lactalbumin, cytoglobin, fetuin, lysozyme, and salmon DNA. rhEPO was produced by cultivation of human embryonic kidney cells 293 (HEK 293) [1]. Static adsorption experiments were carried out either in 96-well plates or in conventional shaken vessels. Flow experiments were carried out using the FPLC system ÄKTA Purifier (GE Healthcare, Uppsala, Sweden). The adsorbents were packed either in small laboratory columns or placed in a membrane module.

**3. Results and discussion**

One of the investigated aspects was the effect of buffer type on binding on multimodal adsorbents. It was found that no BSA could be bound onto Sartobind STIC from a polyvalent phosphate buffer at all. On the contrary, salmon DNA binding was not affected by the buffer valency.

The effect of ions of Hofmeister series on the binding capacity was investigated for several adsorbents but most focus was on the performance of the salt-tolerant anion-exchange membrane Sartobind STIC and particle adsorbent with cation-exchange ligands Capto MMC. Eight different salts were examined ranging from strongly kosmotropic ones such as (NH4)2SO4 to salts with strongly chaotropic ions such as Mg2+ or SCN-. A typical upper limit of salt concentration was 2.5 M.

Very different behavior was observed. Kosmotropic salts had adverse effect on the adsorption capacity of BSA on Sartobind STIC. The protein was not adsorbed at all at the concentrations of about 0.1 M. On the contrary, the adsorption capacity of several proteins for Capto MMC was essentially unaffected up to the salt concentrations of 1-1.5 M. The binding of DNA on Sartobind STIC had however a different pattern. The binding capacity was more-or-less constant up to 0.6 M kosmotropic salts concentration and then gradually declined to zero at about 1.5 M.

Chaotropic salts had a rather negative effect on the adsorption capacity for Sartobind STIC and Capto MMC. In the case of Mg2+ ions, the polyvalent effect of this cation has been manifested. The effect of NaCl concentration was studied most carefully. All adsorbents exhibited salt tolerance when the adsorption capacity often did not change very much if the NaCl concentration was in the range 0–0.5 M. A rather high capacity was observed in many cases at the salt concentrations as high as 2–2.5 M. Differences in the salt concentration effect were observed among individual proteins were observed with respect to pH. This was more emphasized for the multimodal adsorbents with cation-exchange ligands where the electrostatic interactions become dominant for positively charged proteins.

The adsorption capacity of DNA on Sartobind STIC exhibited typically a maximum with the salt concentration. Bi-component binding of DNA and BSA showed that DNA was bound preferentially so the equilibrium adsorbed amounts of BSA were smaller than for single component isotherms. Multicomponent adsorption was studied also for the separation of rhEPO produced by cultivation of human embryonic kidney cells. Cation exchangers, both conventional and salt tolerant, were found to have the best selectivity for this separation which was also verified in column experiments.

**4. Conclusions**

This work presents the overview of our research activities on the equilibrium and performance properties of multimodal adsorbents.

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**References [Calibri 10]**

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