Development and Application of a Fast and Simple Method for Multiparallel Chromatography Parameter Testing

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Introduction

In recent years downstream processing has seen a trend to multi-parallel and high-throughput screenings for performance optimisation of purification processes. This is mainly due to the large number of commercially available chromatography resins and the need to investigate the properties of many resins to find the best candidate for the purification of a given product. Another pressing aspect is the demand of being first on the market. The combination of which even deepens the problem of finding the best chromatography resin and the optimal process parameters out of a plethora of possibilities in a short time.

The aim of this work was to develop a simple method for multiparallel chromatography parameter testing without automated liquid-handling or sophisticated laboratory equipment. Furthermore, its applicability had to be proven by comparability experiments on parameter testing in batch and column chromatography and by methods for the investigation into the chromatographic background of both systems.

Methods

Parameter Testing

Microwell plate experiments were conducted with 50 µL gel in 200 µL total volume per well. A stepwise approach was taken to resemble the column chromatography.

First equilibration was done with three steps of 100 µL binding buffer. Then 100 µL protein solution were applied. The gel was washed three times with 100 µL binding buffer and afterwards elution was done with two steps of 100 µL elution buffer.

For each single step first 100 µL supernatant were removed from the wells. Then 100 µL appropriate buffer or sample solution were added. Mixing of buffer and gel was achieved by overhead shaking for 5 minutes. Afterwards the gel was either sedimented by gravity for 10 minutes or by centrifugation for 1 minute at 44xg. In order to reduce further binding activity during the 10 minutes of sedimentation, the plates were stored in a refrigerator.

The supernatant of the sample application step was mixed with the supernatans of the washing steps, thus generating a single fraction to be analysed. The supernatants from the elution steps were pooled, accordingly.

Column Chromatography

Column chromatography experiments were carried out using an ÄKTA Purifier 100 (GE Healthcare) with K9-columns (GE Healthcare) containing 2.5 mL of gel. For the equilibration and washing step 15 mL of loading buffer were used each. The sample solution was applied with a 5 mL loop and 10 mL of elution buffer were used for desorption of bound protein. The volumetric flow velocity was 1.2 mL/min to result in a contact time of 5 min. Fractions were collected according to the batch chromatography process and analysed for protein content.

For immuno-affinity experiments the anti-erythropoietin antibody was adsorbed to the protein A matrix MabSelect (GE Healthcare) and crosslinked using dimethylpimelimidate-hydrochloride. Further information on this topic is provided on the poster by Siwiora-Brenke et al.

The resulting immuno-affinity matrix was filled into a K9-column to a final volume of 1.15 mL, thereby generating a packing of 1.8 cm bed height and 0.9 cm diameter. The chromatographic runs were performed with an ÄKTA Purifier 100.

Analytics

The protein amount in the fractions was determined by BCA (BC Assay UP40840A, Uptima) or Bradford (Coo Assay UPF86400, Uptima) assay as applicable. The BCA assay was used for experiments with SP-Sepharose FF and Q-Sepharose FF. Due to interferences of buffer substances with the BCA assay for experminents with Butyl-Sepharose FF the protein quantification was done using the Bradford assay. The assays were performed according to the maufacturers instructions.

Recombinant human erythropoietin (rhEPO) was measured using a reversed phase HPLC method.

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Results

Development and Applicability

To determine whether experiments in microwell plates are suitable to predict binding and elution efficiencies in columns, tests were conducted with multiple binding parameters studied in both systems.

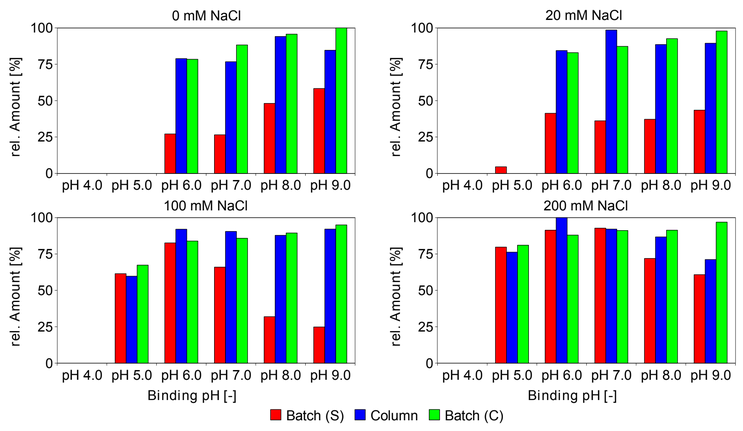

*Fig. 1: Non-bound protein after application of **bovine albumin** to **SP-Sepharose FF** in column chromatography and batch experiments with sedimentation (S) and centrifugation (C)*

Binding of bovine albumin to SP-Sepharose FF in batch and column experiments under different conditions resulted in the same optimal binding parameters at low pH and salt concentration. Due to the nature of the cation exchanger this was expected. The batch experiment with sedimentation for solid liquid separation showed a somewhat better binding. This is likely due to the longer contact time which is caused by the slow sedimentation of the gel.

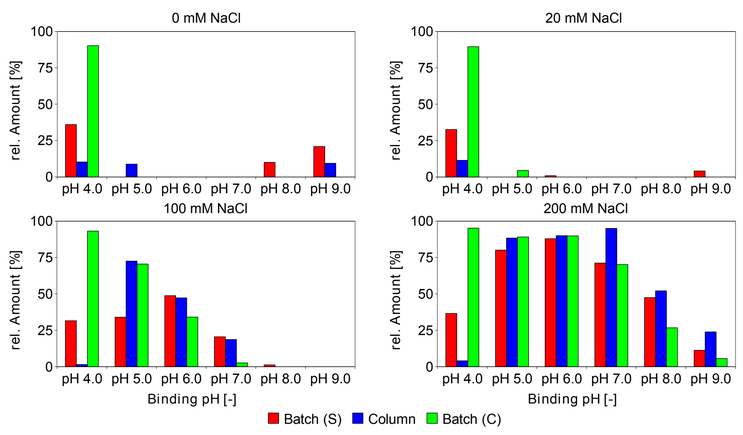

*Fig. 2: Non-bound protein after application of **bovine albumin** to **Q-Sepharose FF** in column chromatography and batch experiments with sedimentation (S) and centrifugation (C)*

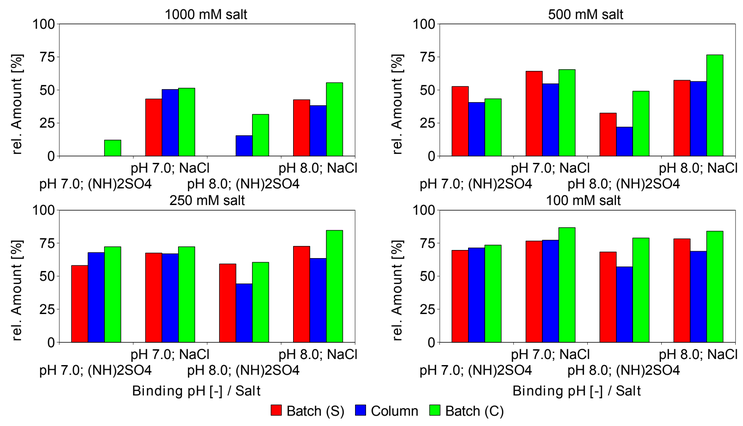

*Fig. 3: Non-bound protein after application of **bovine albumin** to **Butyl-Sepharose 4FF** in column chromatography and batch experiments with sedimentation (S) and centrifugation (C)*

The experiments with Q-Sepharose FF and Butyl-Sepharose 4FF also showed a good comparability between column and batch experiments. Only the data for Q-Sepharose FF at pH 4 are not comparable. An explanation for this binding under unlikely conditions could not be found. Apart from that the experiments support the findings from the first experiment.

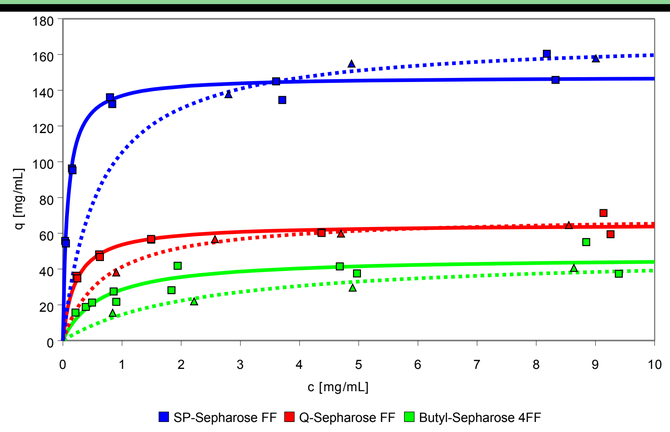

*Fig. 4: **Langmuir adsorption isotherms** for bovine albumin binding to SP-, Q-Sepharose FF and Butyl-Sepharose 4FF*

For all three tested resins the adsorption isotherms show higher binding constants in batch (squares/solid lines) than in the column (triangles/dashed lines). This is likely due to the flow along the beads in column chromatograhy. It might facilitate a higher binding efficiency in batch experiments.

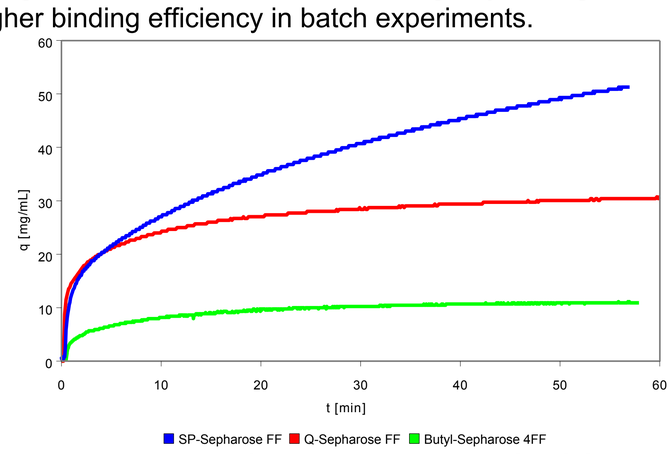

*Fig. 5: **Adsorption kinetics** of bovine albumin binding to SP-, Q-Sepharose FF and Butyl-Sepharose 4FF*

The kinetics for adsorption of bovine albumin on SP-Sepharose FF, Q-Sepharose FF and Butyl-Sepharose 4FF all show that complete saturation of the materials takes longer than 45 minutes. Such a long contact time is not suitable for screening experiments. As a large part of the available binding sites seems occupied after five minutes this is considered to be sufficient.

Application

One application of the developed method was to optimise the binding of rhEPO to an immuno-affinity matrix. The binding conditions had been optimised with a BIAcore system before, but the binding efficience was as low as 50% and the yield below 20%. Multiparallel tests of 27 binding and 19 elution conditions indicated that a binding efficiency of 100% and a yield of 90% could be achieved. A futher optimisation step in column chromatography led to a yield of 100%.

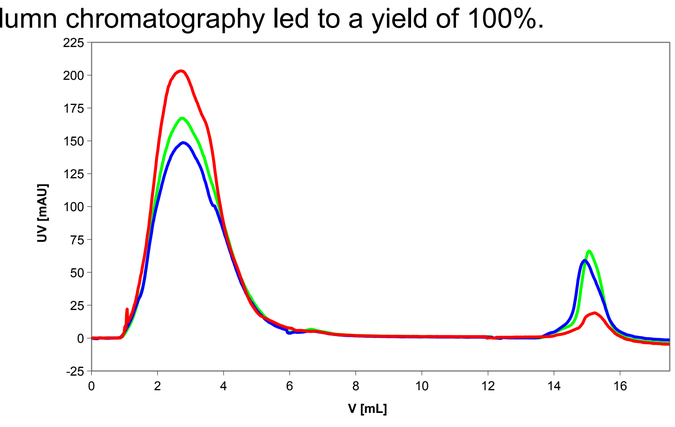

*Fig. 6/Tab. 1: **Concluding chromatography runs** to determine the effect of the binding and elution optimisation in column chromatography*

Buffer composition	Binding efficiency [%]	Yield [%]
Prior to optimisation	50,08	13,52
After multiparallel screening	100,00	89,97
Final chromatography run	100,00	100,19

Conclusions

Development and Applicability

- Experiments in batch and column chromatography result in comparable data on binding and elution (not shown) efficiency.
- The method for multiparallel chromatography parameter testing is well applicable. Attention has to be paid to systematic differences which can lead to differences in absolute results. (see batch experiments with sedimentation)
- For the task of finding the best binding or elution condition from a given set of parameters the proposed method gives the same results as column chromatography experiments in a considerably shorter time.

Application

- The first application of the illustrated method was a success.
- The expected advantages over parameter testing in column chromatography were met. Also the obtained set of parameters for the chromatographic runs were far superior to those used before the optimisation.
- Based on this data only a single optimisation step in column chromatography was neccessary to raise the process effieciency further to a yield of 100%.
- We could show that it is feasible to use this fast and simple method to boost ones efficiency in downstream processing.