APPLICATION NOTE

Time study of parallel mAb purification from clarified CHO cell broth at 1 mL MAGicBeads ProA scale

Introduction

Chromatography media is a very important tool for the separation and enrichment of different macromolecules. For this, the media is very commonly packed in a column, where sample and buffer flows are adjusted and controlled by an instrument. With a standard column chromatography instrument, if several samples need to be processed, the operator runs the first sample from start to finish, after which purification of the second sample can start. The third sample can then only be processed after the second purification has been finished, and so on. The use of parallel purification at small sample scale is common, e.g., in immunoprecipitations, using chromatography beads in suspension, but not so often for larger sample volumes, since the available amount of column chromatography instruments easily is limited.

Herein, a parallel purification setup was performed on four 200 mL samples containing a recombinant and humanized monoclonal antibody (mAb), using magnetic agarose beads at a scale to directly mimic a pre-packed 1 mL chromatography column.

Experimental setup

POD clarified CHO cell broth was obtained from an external source. The stably transfected CHO cell line produced a mAb at low expression levels (see Table 1). 200 mL cell culture harvest was aliquoted in each of four 500 mL bottles. 4x 1 mL MAGicBeads ProA (an early, non-alkali-tolerant predecessor of the current Protein A+ ligand) were washed with PBS in 50 mL tubes,

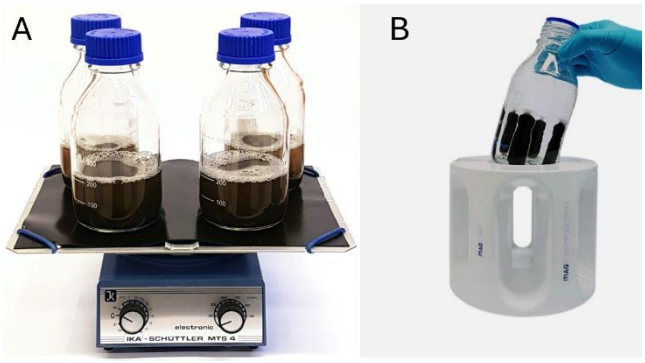

Figure 1. The adsorption step was performed (A) with beads kept in homogenous suspension through gentle mixing on an IKA Schüttler MTS 4 at 50 rpm and a 3 mm orbital motion. Separation of beads (B) in MAGicAccio PILOT 500 magnetic separator.

resuspended in 10 mL of sample and transferred to each sample container. Residual beads were resuspended in additional sample liquid and transferred to the sample-containing bottles. Adsorption was performed for 2 hours (Figure 1), whereafter the beads were separated using the MAGicAccio PILOT 500 magnetic separator. The unbound fraction was removed with a 50 mL pipette attached to an electronic pipette aid. The last 50 mL liquid sample was used to resuspend and transfer the beads to a 50 mL centrifuge tube, for further handling in the MAGicAccio PILOT 50 separator. Residual beads were washed out with PBS and pooled with beads in the centrifuge tubes. The beads in each tube were thereafter washed with 3x 35 mL PBS, bound mAb was eluted twice with citrate, and released antibodies were neutralized with the addition of high-molar Tris-HCl. The beads were regenerated two times with an additional 10 bead volumes of citrate elution buffer, washed two times with 10 bead volumes of PBS, and finally changed into

storage buffer (PBS, 20% EtOH) as a 10% bead suspension. The handheld MAGicAccio LAB cube magnet was sometimes used when withdrawing the last amounts of liquid during separations in the 50 mL tubes.

Table 1. *Purification condition*

Magnetic beads	MAGicBeads ProA (non-alkali-tolerant predecessor of Protein A+)
Sample	200 mL POD filtered CHO cell broth (0.02 – 0.025 mg IgG/mL)
Bead volume	1 mL (10 mL 10% bead solution)
Magnetic separators	MAGicAccio LAB cube, MAGicAccio PILOT 50 and MAGicAccio PILOT 500
Binding conditions	2 h, RT
Elution conditions	2x 9 mL acidic elution buffer (pH 3.0)
Neutralization	1.8 mL 2 M Tris-HCl, pH 9.0
Concentration step	MacroSep Advance Centrifugal Devices 30 K (Pall)

The elution and neutralization volumes were 2x 9 mL and 1.8 mL, respectively, and the total volumes were subsequently loaded into the MacroSep Advance ultrafiltration devices (Pall) to concentrate the eluted antibodies. Centrifugation was performed for 30 min in a Thermo Scientific SL 40R centrifuge at 22°C and 4,816x g. Remaining liquid, <1 mL, was transferred to a new tube. The sample reservoirs were washed with an additional 0.9 mL flow through to obtain residual IgG. A_{280} was measured on pooled fractions and concentrations calculated using Abs 1 mg IgG/mL at 1.44 (Figure 2). Purity of the eluates was assessed by SDS-PAGE (Figure 3).

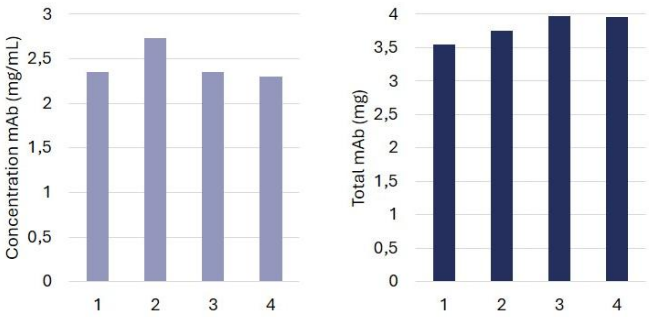

Figure 2. Concentration (left) and total amount (right) of purified and concentrated humanized mAb from 200 mL clarified CHO-cell broth, using a four-parallel purification setup with 1 mL MAGicBeads ProA each.

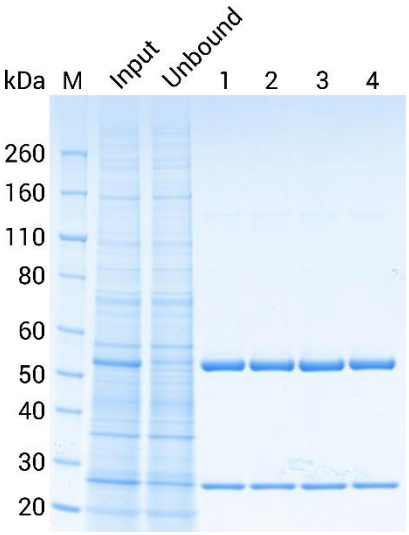

Figure 3. Visual representation of purity. Input CHO-cell harvest sample (20 µL), unbound fraction (from sample No.1; 20 µL) and eluted fractions from samples No. 1–4, were separated under reducing conditions on SDS-PAGE and stained with colloidal Coomassie.

Discussion

The availability of high-capacity magnetic agarose beads, coupled with protein A, makes them a viable, low-cost alternative to instrument controlled column chromatography. For purification and target enrichment at small sample volumes and low target concentrations, e.g., immunoprecipitations, the use of traditional agarose or synthetic beads moving freely in the sample as a homogenous suspension, during the adsorption phase, is norm. For larger sample volumes, though, chromatography media packed in columns is the norm and the use of prepacked 1 and 5 mL columns is a common way of performing a purification of choice. An up and running chromatography instrument is a convenient way of performing purifications. There is, however, a need to perform proper maintenance to take care of such an instrument for hassle-free usage over time. Besides that, something as simple as introduced air bubbles can be enough to postpone an intended purification significantly and an instrument that awaits ordered spare parts, can easily be non-functional for a week. Another inherent trait of column chromatography instruments is that purification of many similar samples, using the same column, is performed in cycles one after the other, which can tie up an instrument for a lengthy time.

Herein, we performed four parallel purifications using 1 mL MAGicBeads magnetic agarose each, to mimic the common 1 mL pre-packed columns. Our samples were 200 mL of clarified broth from a CHO-cell culture, but could easily have been

from four separate CHO-cells producing different mAbs. The start-up is simple and straightforward, aliquot beads, equilibrate, and add directly to the samples. Separation of the beads was performed with the MAGicAccio PILOT magnetic separators. These separators can also mimic 5 mL pre-packed columns, since the maximum capacity for separation in a 50 mL centrifuge tube is 5 mL of MAGicBeads magnetic agarose. MAGicAccio PILOT 500 can even handle 30 mL of MAGicBeads separation media, making this system usable for up to 1 gram of mAb.

Elution from beads in suspension, differ from elution in a column. In a column, the released antibody is washed out from the beads by the continuous flow of elution buffer. For magnetic agarose, elution takes place with continuous mixing in suspension. After a short elution of 1 min, the released antibody is homogenously present in the elution mixture. At separation, since the beads contain an intrinsic volume of the elution buffer, and thereby also released antibody, a volume equal to the bead volume will contain an antibody concentration identical to the collected eluate. The remaining antibodies will be obtained, if wished, in a second elution event. Due to this difference in elution-mechanism, the total elution volume from magnetic beads, to obtain a high yield, is significantly larger than for column chromatography. In our set-up, we eluted 2x with 9 mL buffer each. The addition of 1.8 mL neutralization buffer gave a total of 19.8 mL for each purified antibody; in comparison to around 2.5 mL eluted and neutralized material from a 1 mL column. A quick ultrafiltration step, though, was utilized to concentrate our neutralized samples to less than 2 mL.

We clocked the four parallel purifications, from 200 mL samples each, for a total of 254 min (Table 2). An estimated time for one single purification of this same 200 mL sample, using a 1 mL column in a chromatography instrument, adds up to an estimated 305 min. Time for setup might vary, which can shorten the total amount of time. However, if the operator for any reason decides to use a longer contact time for the sample in the column, e.g., a flow rate of 0.5 mL/min, that would add an extra 200 min to the total time. With a longer contact time, one single purification could easily run into a full 8-hour working day. With the latter, and performing one purification per day, handling, e.g., 18 samples of different mAbs that need to be purified on this scale, equals 18 days until the last sample is processed. If performing one run per each day and night, it still would take nine working days. With the LOABeads system, and handling six samples in parallel per day, the last samples would be finished after three days. Higher mAb concentrations in the starting material, also significantly shortens the adsorption step for the MAGicBeads/MAGicAccio system. With magnetic agarose beads in suspension, there is also the possibility to easily scale the amount of beads to use, to accommodate specific mAb concentrations and sample volumes.

Parallel purification MAGicAccio (actual times)			Single purification chromatography (estimated times)		
1.	200 mL sample into four bottles	2 min	1.	Set up instrument, equilibrate column	2 min
2.	Equilibration 4x 1 mL MAGicBeads	13 min	2.	Load 200 mL sample at 1 mL/min	200 min
3.	Add beads to bottles	2 min	3.	Wash	15 min
4.	Adsorption	120 min	4.	Elute	15 min
5.	Separate & transfer beads to 50 mL-tubes	22 min	5.	Regenerate column for storage	15 min
6.	Wash x3	26 min			
7.	Elute 2x & neutralize	20 min			
8.	Regenerate beads for storage	14 min			
9.	Concentrate through ultrafiltration	35 min			
	Σ	254 min		Σ	305 min

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