Desalting and buffer exchange of protein solutions

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Introduction

There is often a need for removing unwanted salt or low molecular weight compounds, or to exchange the buffer in protein purification and in subsequent studies. BabyBio™ Dsalt 1 ml and BabyBio Dsalt 5 ml are pre-packed columns designed for the rapid and efficient desalting and buffer exchange of protein solutions. BabyBio columns can be directly connected, without adaptors, to most chromatography systems. The effect of sample volume on the separation between protein and unwanted salt was investigated. The scale-up enabled by connecting columns in series were evaluated. Furthermore, rapid buffer exchange after elution at low pH of antibodies purified on BabyBio A 1 ml using three BabyBio Dsalt 5 ml connected in series was investigated.

Results

The effect of sample volume on the separation between bovine serum albumin (BSA) and salt was investigated for BabyBio Dsalt 1 ml and 5 ml columns. Separations on BabyBio Dsalt 1 ml showed excellent salt removal from samples of 20 to 100 µl (Figure 1). A sample volume of up to 300 µl allowed removal 82 % of the salt. BabyBio 5 ml gave excellent salt removal from the 100-µl and 750-µl samples, with 93 % of the salt removed from the 1.5-ml sample (Figure 2).

Column: BabyBio Dsalt 1 ml
Sample: 2 mg/ml BSA in 25 mM Na-phosphate, 500 mM NaCl, pH 7.0
Buffer: 25 mM Na-phosphate, 150 mM NaCl, pH 7.0

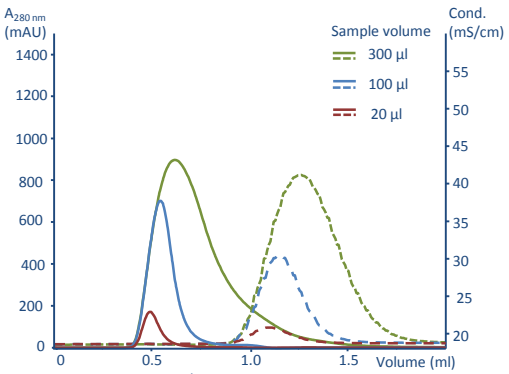

Figure 1. Desalting of 2 mg/ml BSA in 25 mM Na-phosphate, 500 mM NaCl on a BabyBio 1 ml. Sample volume range was 20-300 µl. The solid lines correspond to the protein ($A_{280\ nm}$) and the dashed lines correspond to the salt (Cond.).

Column: BabyBio Dsalt 5 ml
Sample: 2 mg/ml BSA in 25 mM Na-phosphate, 500 mM NaCl, pH 7.0
Buffer: 25 mM Na-phosphate, 150 mM NaCl, pH 7.0

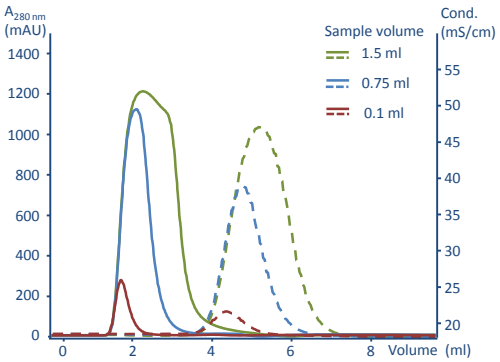

Figure 2. Desalting of a sample containing 2 mg/ml BSA in 25 mM Na-phosphate, 500 mM NaCl, pH 7.0 using BabyBio 5 ml. The sample volume range was 0.1-1.5 ml. The solid lines correspond to the protein peak ($A_{280\ nm}$) and the dashed lines correspond to the salt (Cond.).

BabyBio columns are designed to be easily connected together easily in series without accessories. The scale-up effects of connecting BabyBio Dsalt in series was investigated by the comparison of one BabyBio Dsalt 5 ml with five BabyBio Dsalt 1 ml connected together (Figure 3).

Column: BabyBio Dsalt 5 ml and BabyBio Dsalt 1 ml (5 columns in series)
Sample: 2 mg/ml BSA in 25 mM Na-phosphate, 500 mM NaCl, pH 7.0
Sample volume: 1 ml
Buffer: 25 mM Na-phosphate, 150 mM NaCl pH 7.0

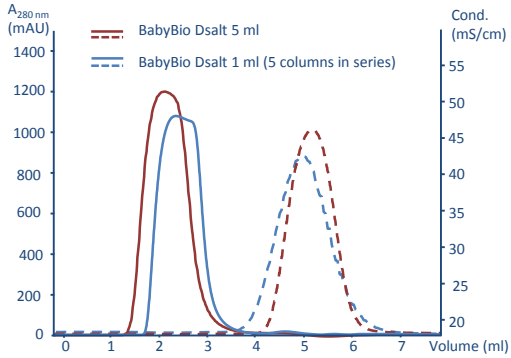

Figure 3. Scale-up comparison of desalting using BabyBio Dsalt 5 ml and five BabyBio Dsalt 1 ml connected in series. Sample volume of 1 ml 2 mg/ml BSA in 25 mM, 500 mM NaCl, pH 7.0. The solid lines correspond to the protein peak ($A_{280\ nm}$) and the dashed lines correspond to the salt (Cond.).

Purification of IgG usually involves elution using low pH. Acidic pH may induce aggregation of the antibodies. This can be avoided by rapid buffer exchange of the purified antibodies. Three BabyBio Dsalt 5 ml columns were connected in series to accommodate the material eluted from the BabyBio A 1ml column (Figure 4).

Column A: BabyBio A 1 ml
Sample: 20 ml 1 mg/ml human, polyclonal IgG in PBS, pH 7.4
Binding Buffer: PBS, pH 7.4
Elution Buffer: 100 mM Gly-HCl, pH 2.7

Column B: BabyBio Dsalt 5 ml (3 columns connected in series)
Sample: 3 ml of elution pool from BabyBio A 1 ml
Buffer: 25 mM Na-phosphate, 150 mM NaCl pH 7.0

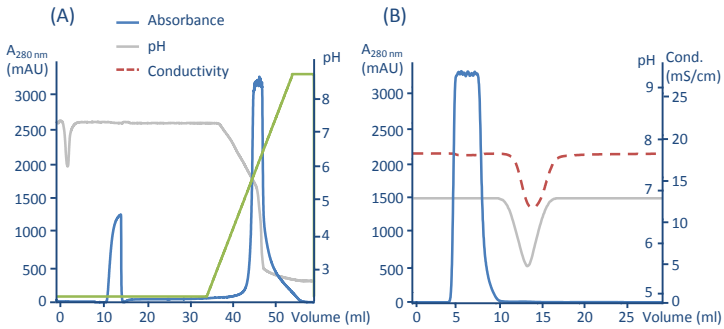

Figure 4. Human polyclonal IgG was adsorbed on a BabyBio A 1 ml column and desorbed by low pH (A), and the collected 3-ml of IgG solution was immediately applied on a set of three BabyBio Dsalt 5 ml connected in series to restore the pH by buffer exchange.

The BabyBio Dsalt 1 ml and 5 ml columns are designed for efficient desalting or buffer exchange of proteins. Rapid buffer exchange can rescue antibodies following low-pH elution from a Protein A column.

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