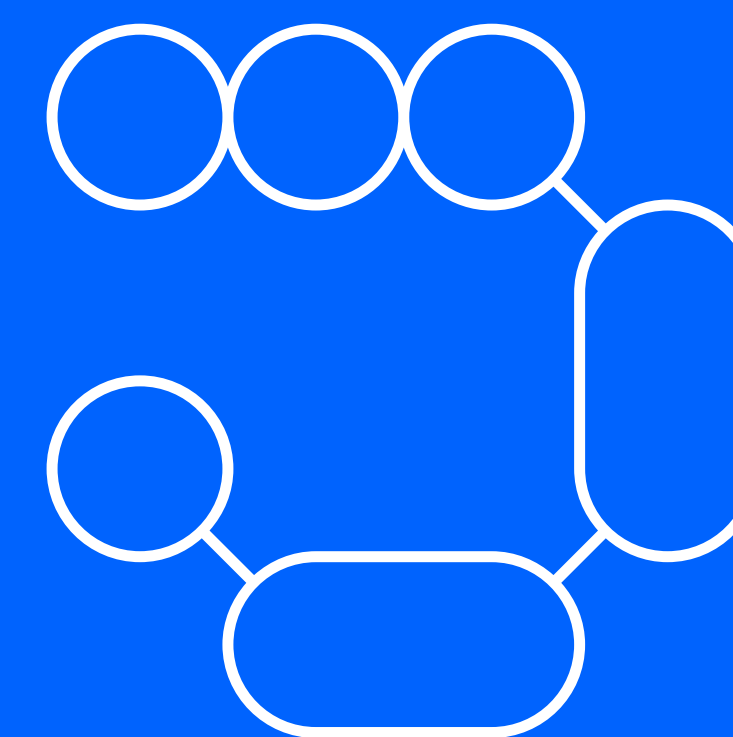


Desalting and buffer exchange of protein solutions

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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 GoBio™ Mini Dsalt 1 mL and GoBio Dsalt 5 mL are pre packed columns designed for the rapid and efficient desalting and buffer exchange of protein solutions GoBio 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 GoBio Mini A 1 mL using three GoBio Mini 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 GoBio Mini Dsalt 1 mL and 5 mL columns. Separations on GoBio Mini Dsalt 1 mL showed excellent salt removal from samples of 20 to 100 μ L (Figure 1). A sample volume of up to 300 μ L allowed removal 82 of the salt GoBio Mini 5 mL gave excellent salt removal from the 100 μ L and 750 μ L samples, with 93 of the salt removed from the 1.5 mL sample (Figure 2).

Column: GoBio Mini 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

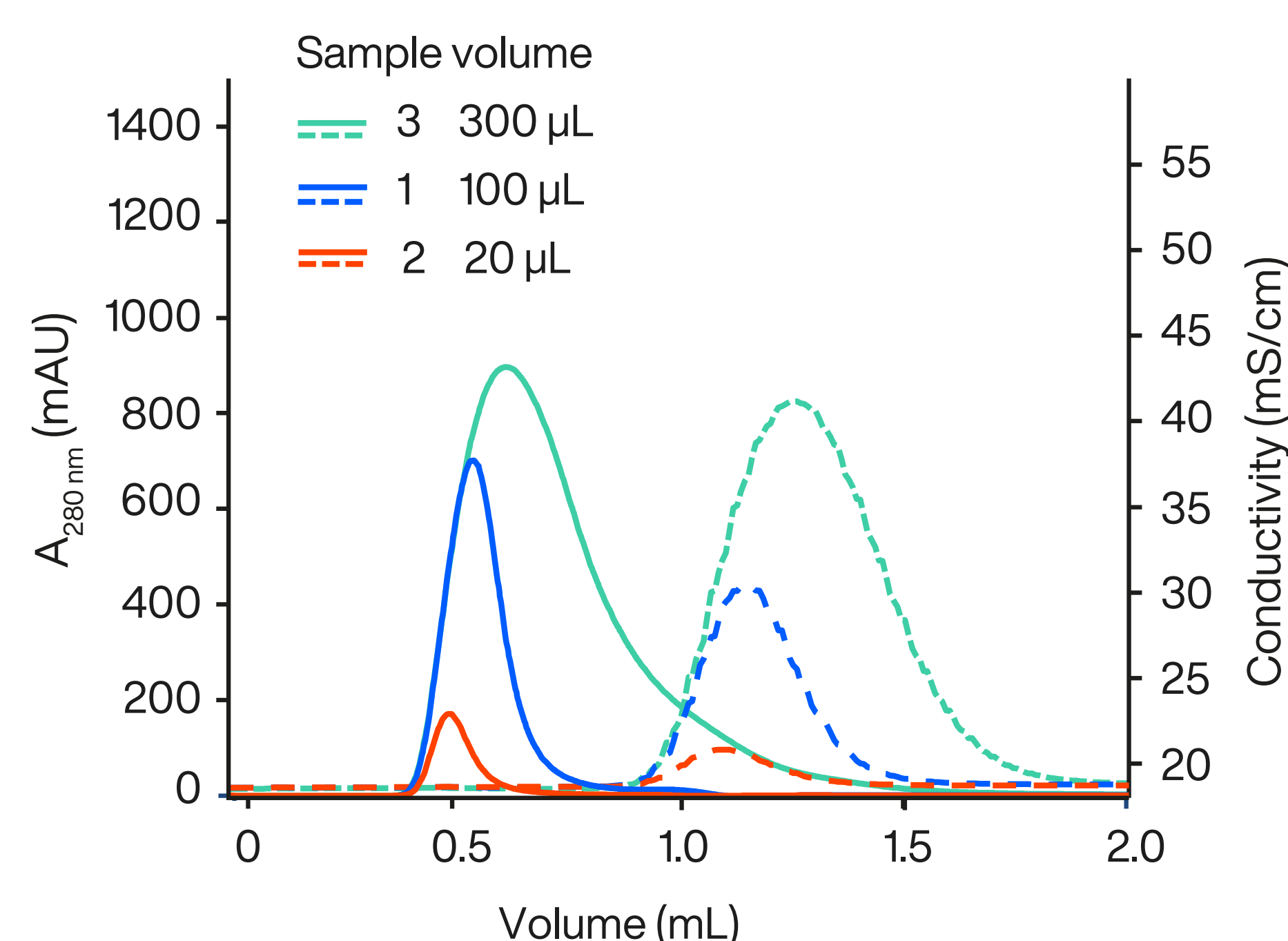


Figure 1. Desalting of 2 mg/mL BSA in 25 mM Na phosphate 500 mM NaCl on a GoBio Mini 1 mL. Sample volume range was 20–300 μ L. The solid lines correspond to the protein $A_{280\text{ nm}}$ and the dashed lines correspond to the salt (Cond.).

Column: GoBio Mini 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

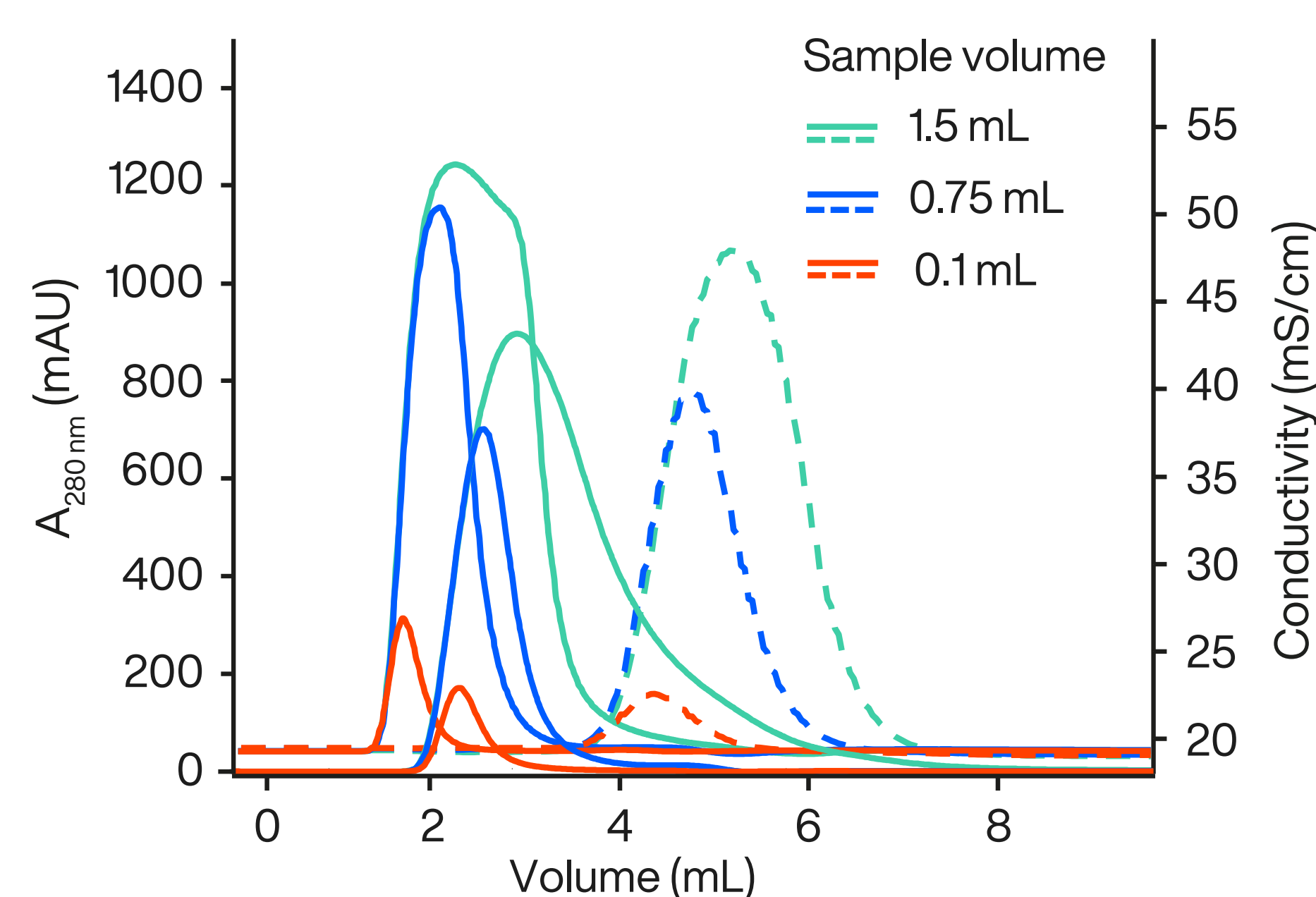


Figure 2. Desalting of a sample containing 2 mg/mL BSA in 25 mM Na phosphate, 500 mM NaCl, pH 7.0 using GoBio Mini 5 mL. The sample volume range was 0.1–1.5 mL. The solid lines correspond to the protein peak $A_{280\text{ nm}}$ and the dashed lines correspond to the salt (Cond.).

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

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 GoBio Mini Dsalt 5 mL columns were connected in series to accommodate the material eluted from the GoBio Mini A 1 mL column (Figure 4).

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

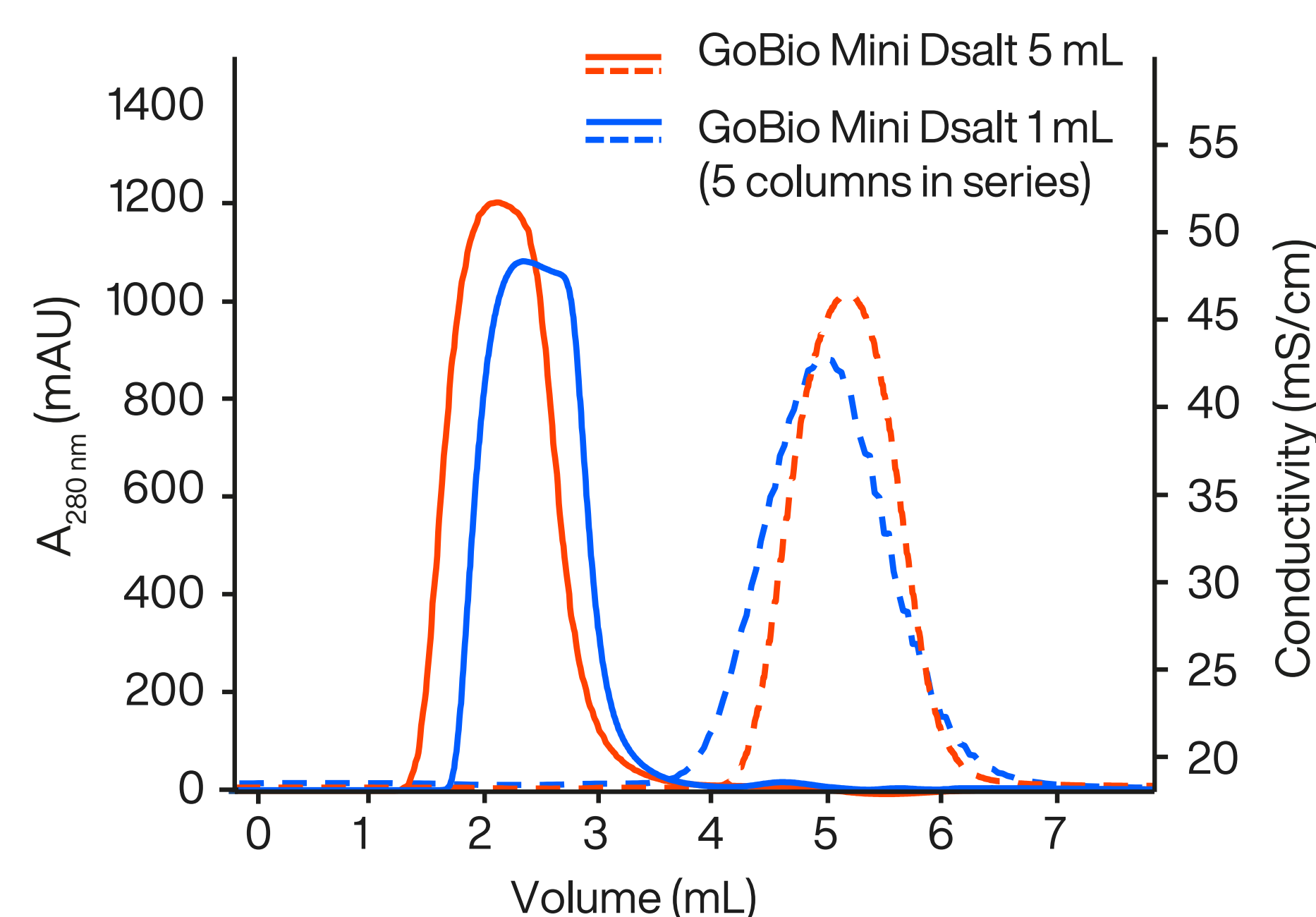


Figure 3. Scale up comparison of desalting using GoBio Dsalt 5 mL and five GoBio Mini Dsalt 1 mL connected in series. Sample volume of 1 mL 2 mg/mL BSA in 25 mM 500 mM NaCl, pH 7.0. The solid lines correspond to the protein peak $A_{280\text{ nm}}$ and the dashed lines correspond to the salt (Cond.).

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

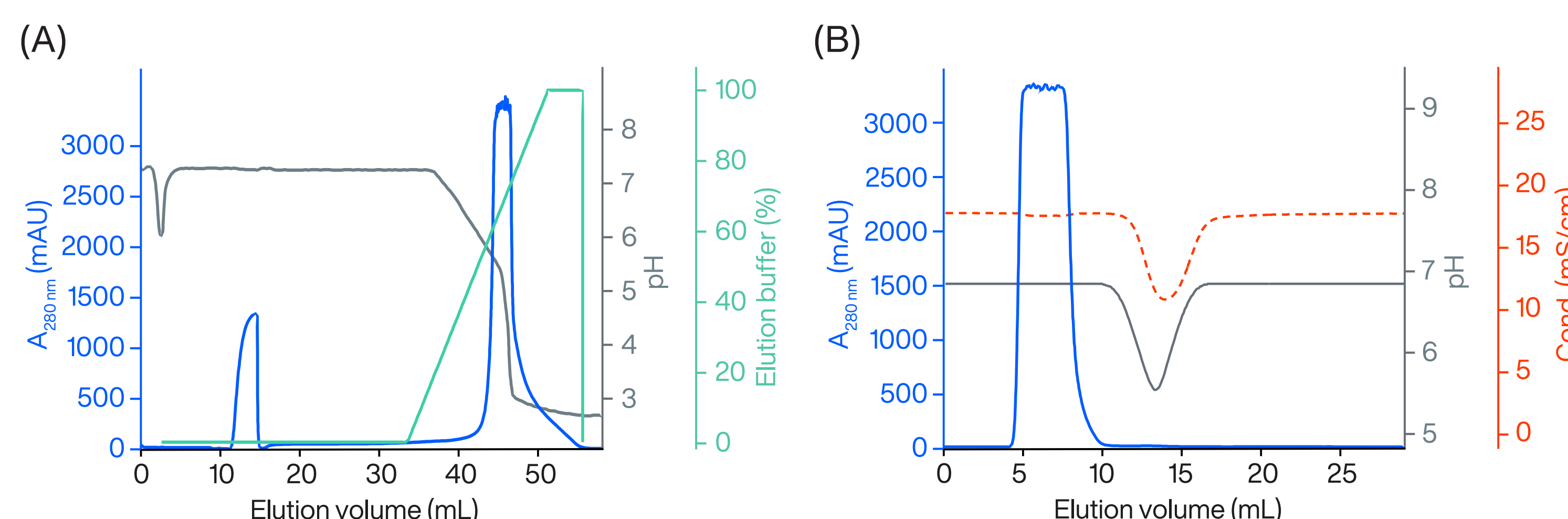


Figure 4. Human polyclonal IgG was adsorbed on a GoBio Mini A 1 mL column and desorbed by low pH (and the collected 3 mL of IgG solution was immediately applied on a set of three GoBio Mini Dsalt 5 mL connected in series to restore the pH by buffer exchange).

The GoBio Mini 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.