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Every Protein is Different: Solubility Considerations to Maximize your Instruments

Key Words: proteins, solubility, cleaning, bovine gammaglobulin, bovine serum albumin, PBS, buffer, ionic strength, pH

Goal: The following application note describes how to improve cleaning of your VROC® chip after measuring protein solution samples. Protein solubility depends on the specific protein and solvent used, and choosing the correct primary solvent can extend the life and maintain the high performance of your VROC® chips.

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Introduction

Proteins exhibit a wide range of solubility based on many factors such as solvent composition, pH, and ionic strength. It has become apparent that using a single, universal cleaning solvent (1% Aquet) may not be optimal depending on the properties of the protein sample. Previous observations have shown that certain high-concentration protein solutions can leave thin residue films in the microfluidic channel of the VROC® chip, which could lead to a decrease in measurement quality. We have developed several cleaning methods for high concentration protein solutions to ensure the longevity and accuracy of your RheoSense products.

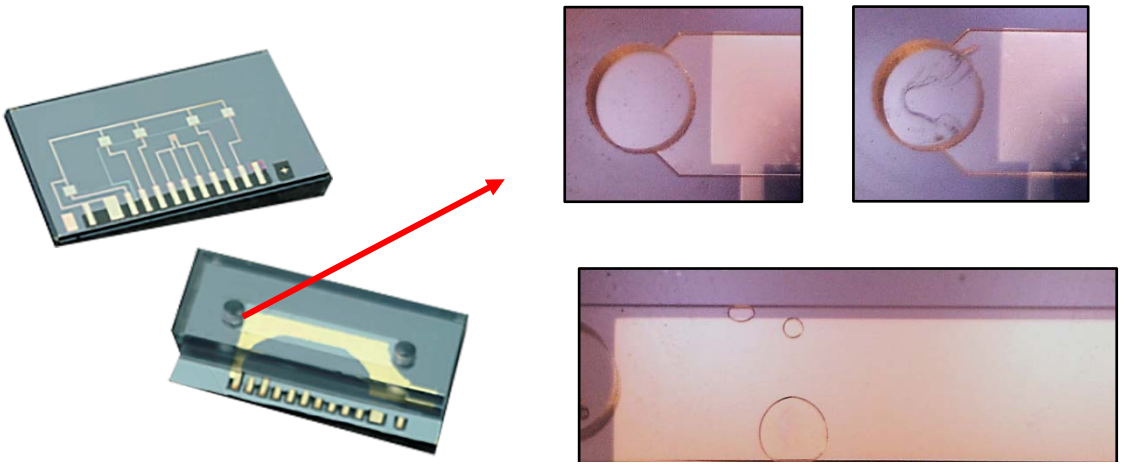

Figure 1. (Left)VROC® chips measure viscosity as fluid is pumped through the microfluidic channel. Proteins with low solubility with the primary cleaning solvent may leave trace amounts of residue behind (Top Middle – Before Measurement of BGG, Top Right – After Measurement). (Bottom Right) These surface modifications can promote the entrapment of bubbles during measurement, affecting measurement quality.

Sample and Cleaning Protocol	Avg Visc (cP)	SD (cP)	RSD (%)	n
BSA 200 mg/mL, Standard Cleaning	2.769	0.030	1.10	4
BGG 200 mg/mL, Standard Cleaning	8.729	0.297	3.40	4
BGG 200 mg/mL, Buffer Cleaning	8.381	0.058	0.69	4

Table 1. Viscosity data of BSA and BGG dissolved in PBS, measured with VROC® initium. Gradual protein buildup of BGG using standard cleaning protocols (1% Aquet as primary solvent) caused increased viscosity readings and decreased repeatability.

Buffer Solution as Primary Solvent

The VROC® initium automated viscometer currently uses 1% Aquet as its primary solvent during automatic post-sample cleaning. This solvent is sufficient in most cases for water-soluble samples. E.g., measurements of multiple samples of bovine serum albumin (BSA) dissolved in PBS showed highly repeatable results (Table 1). However, proteins with low solubility with 1% Aquet, such as Bovine Gamma Globulin (BGG) (see Fig 1), may be difficult to remove completely. Over time, protein accumulation in the microfluidic channel may decrease the accuracy (Fig 2) and repeatability of your measurements (Table 1).

To improve cleaning performance, we have developed protocols allowing users to choose the optimal primary cleaning solvent for their particular protein solutions. Cleaning BGG samples using PBS as the primary buffer showed vastly improved measurement repeatability between samples (Table 1). Viscosity did not sharply increase after the 1st sample, suggesting a clean and healthy chip (Fig 2). The buffer cleaning setup requires modifications of the solvent configuration and loading and cleaning protocols, so please contact us for more information.

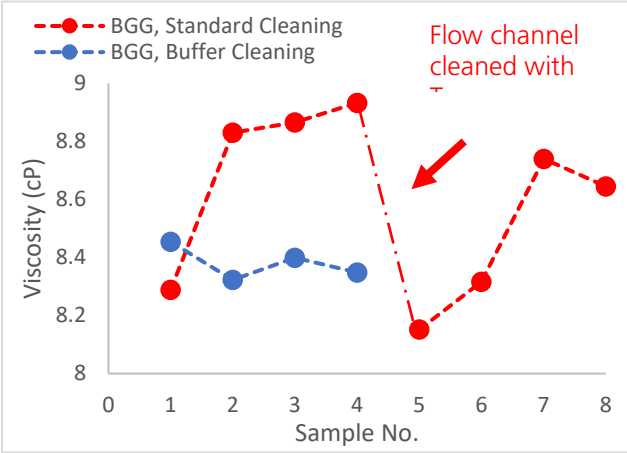

Figure 2. Viscosity measurements of BGG using standard (Aquet) or PBS buffer cleaning protocols. Viscosity increased between measurements using standard cleaning protocols, suggesting protein buildup. Cleaning the chip with 1% Tergazyme solution was effective in returning readings to their original levels, but subsequent measurements showed similar increases. Viscosity did not significantly vary between samples using the buffer cleaning protocol.

Cleaning Chips with Protein Buildup

What to do if you suspect that your chip may already be suffering from protein buildup? We have found that protein residue can be successfully removed by soaking 1% solution of Tergazyme™ Enzyme-Active Powdered Detergent (by Alconox™) in the flow channel at an elevated temperature (50°C). Tergazyme can be automatically loaded and soaked in the initium through sample loading and measurement protocols we have developed. Please ask us about cleaning and maintaining your VROC® chips!

If you have questions or need more information about this product or other applications, please contact us:

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