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# ROBUST VISCOSITY MEASUREMENT ADVANCING BIOLOGIC FORMULATION WITH FIDA TECHNOLOGY

VERSION 1.1

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This application note showcases the usage of Flow Induced Dispersion Analysis (FIDA) technology in biologics production workflows, where it enables precise, low-volume viscosity measurements. With accurate thermal control, automated sampling, and minimal material consumption, FIDA provides a fully autonomous, high-efficiency approach to viscosity analysis, supporting reliable formulation development and process optimization across the biologics pipeline.


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# INTRODUCTION

Accurate viscosity measurement is a critical parameter in the development and formulation of protein- and antibody-based therapeutics, as viscosity directly impacts manufacturability, stability, injectability, and ultimately patient experience. As biologics progress toward higher concentrations to achieve therapeutic efficacy, viscosity tends to increase sharply, creating challenges in formulation design and delivery. However, viscosity testing in this space is often complicated by the limited availability of precious protein samples during early research and development. Traditional rheological methods typically require large volumes, are time-intensive, and may not provide the sensitivity needed for highly concentrated solutions. This has driven the demand for innovative low-volume, high-precision viscosity measurement technologies that can deliver reliable insights while conserving valuable material.

# TECHNICAL BACKGROUND

FIDA viscosity measurements rely on the controlled flow of a sample through a capillary with constant inner diameter under precisely regulated temperature and pressure. For viscosity analysis, a fluorescent tracer (indicator) is injected as a small plug, enclosed within the analyte solution. As this Analyte–Indicator–Analyte segment flows through the capillary under constant pressure, the fluorescent signal is detected with a time delay relative to the start of the run.

From the point of injection to the fixed detection window (effective length: 84 cm), the analyte drives the indicator plug forward. The time taken for the maximum fluorescence intensity (highest point on the peak) to reach the detection window is termed the residence time ($t_R$) (Figure 1).

Shifts in $t_R$ directly reflect changes in sample viscosity. A larger deviation in $t_R$ compared to a reference (e.g., water or aqueous buffer) corresponds to higher viscosity of the analyte. The FIDA software then converts the $t_R$ readout into an absolute viscosity value.

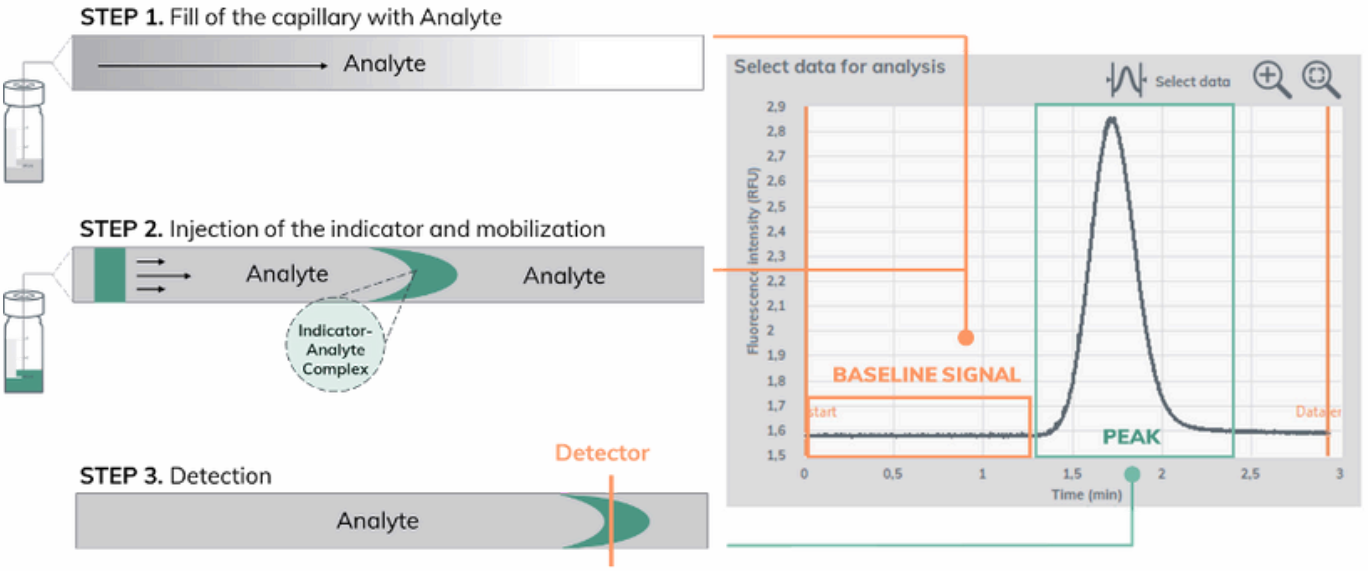


Figure 1: Representation of detection of fluorescent indicator in Fida neo. The lag time between the measurement start and appearance of fluorescence signal at fixed detection point corresponds to the viscosity of analyte sample.


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Figure 2 illustrates a batch analysis of viscosity where a set of target samples were loaded and measured in a walk-away mode. PBS was used as reference for viscosity (black peak in Fig 2). Individual peak corresponds to each sample measured and the shift or delay in time (on x-axis) relates to the viscosity of each.

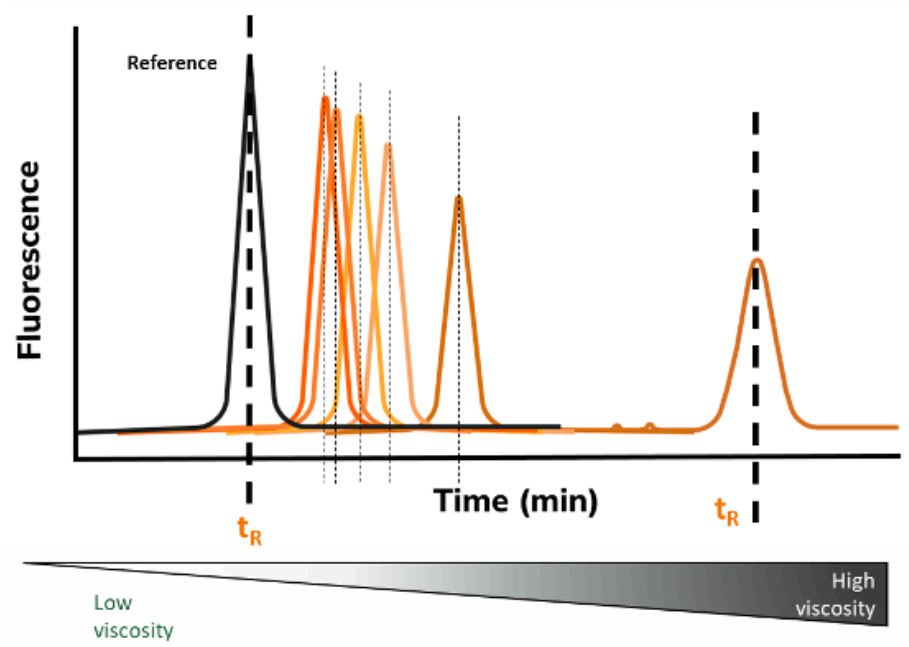


Figure 2: Representation of batch analysis of viscosity. Every peak is measured for the right shift/delay in residence time, with respect to a reference (in black) which is typically an aqueous buffer solution.

# MATERIALS AND METHODS

Viscosity measurements were performed on the Fida Neo instrument equipped with a 480 nm LED fluorescence detector. A dynamically coated capillary (length: 100 cm, inner diameter: 75 µm, effective length: 84 cm) prepared with the HS coating reagent (Fida Biosystems) was used for all experiments.

Chicken egg white lysozyme (sigma) was prepared by dissolving the lyophilized powder in 0.1 M $H_3PO_4$ at a concentration of 400 g/L. The protein was then subsequently diluted in 100 mM $H_3PO_4/H_2NaPO_4$ (NaPi buffer) pH 3.0. Fluorescein was prepared by dissolving the powder in PBS at 1 mM and diluted in NaPi buffer to the target concentration.

A 1000 nM fluorescein dye solution in sample dilution buffer served as the fluorescent tracer (indicator). The assay was conducted as a capillary mixing experiment, where the capillary was first filled with the test sample (analyte) - followed by injection of a 40 nL indicator plug- mobilization was carried out using the analyte at 2500 mbar, with capillary maintained at 25ºC. Sample loading tray was maintained at 25ºC throughout the run. The resulting data were processed and analyzed using the viscosity module in Fida software (version 3.2).


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FIDA method used:

| Inlet tray | Inlet vial pos | Pressure | Time (s) | Outlet vial pos | Measure | Temperature (°C) Tray 1 | Tray 2 | Capillary | Comments |
|---|---|---|---|---|---|---|---|---|---|
| Tray 2 | 2 | 3500 | 60 | Variable | No | 25 | 25 | 25 | Assay buffer rinse and eq |
| Tray 1 | Analyte | 3500 | 20 | Variable | No | 25 | 25 | 25 | Fill with analyte sample |
| Tray 1 | Indicator | 50 | 10 | Variable | No | 25 | 25 | 25 | Inject indicator |
| Tray 1 | Analyte | 2500 | 50 | Variable | Yes | 25 | 25 | 25 | Mobilize and measure |
| | | | | | | | | | |

# RESULTS

Overall, 34 metal binding proteins were tested. A signal change of 5 % in fluorescence area or fluorescence ratio as well as a size difference bigger than 0.1 nm (1 Å) was enough to call the protein as “interacting with the metal ion”. Only one out of the three signals had to change. Out of 34 tested proteins, 26 showed binding (24 size changes, 26 fluorescence area changes, 24 fluorescence ratio changes), 7 did not show binding and for 1 it was inconclusive. Figure 3 highlights the results from the screening approach and the various metals. From the 26 proteins, 4 were selected and a metal concentration titration assay was performed with the same batch of proteins.

## Reference measurement

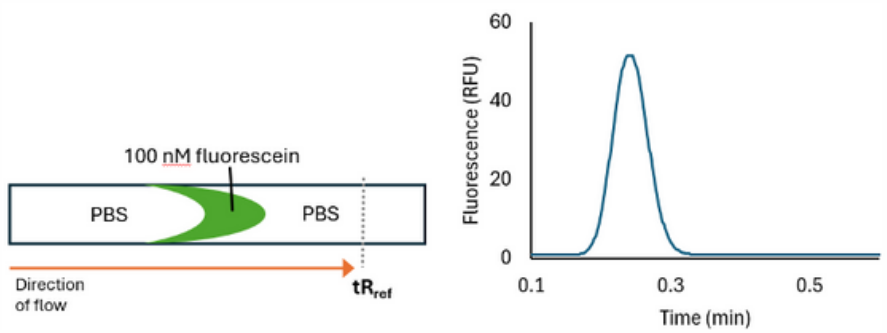


Figure 3: Reference measurement performed at 2500 mbar mobilization pressure.

The $t_R$ reference measurement was performed using the indicator molecule, i.e., 100 nM fluorescein in NaPi. The measurement was performed at 2500 mbar mobilization pressure in the capillary mixing format, PBS-Fluorescein-PBS (Figure 3). The **reference tR** value of 0.24 mins was recorded.

## Test measurement

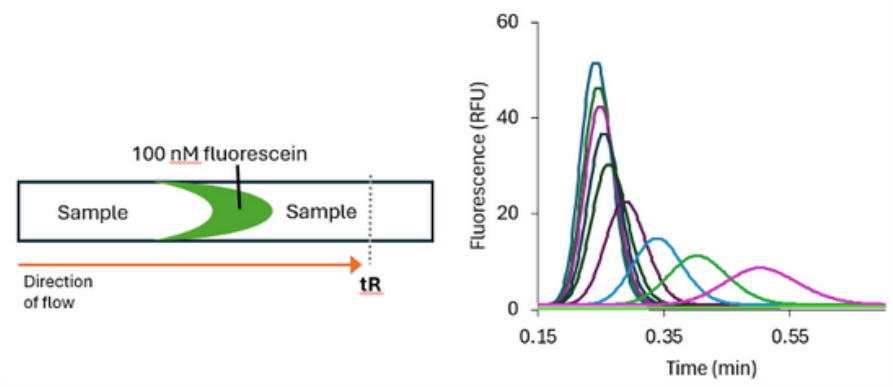


Figure 4: Test measurements were performed at a mobilization pressure of 2500 mbar. The plot shows an overlay of mobilization profiles for different lysozyme concentration points.


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Viscosity measurements were carried out using lysozyme as the model protein. Samples were prepared at concentrations up to 355 mg/mL, with serial dilutions measured across this range. For each concentration point, 50 µL of sample was dispensed into a 96-well plate (FidaBio, catalog no. 220-002), and measurements were performed in triplicate.

## Data Analysis Using Fida software

To obtain the reference tR, the corresponding data point was loaded into the software interface. A single-species Gaussian fit was applied to the curve, and the resulting $tR_(ref)$ value was displayed in the "Residence Time" readout box (Figure 5–I). The obtained $tR_(ref)$ was then entered into the "tR Reference for Viscosity" field (Figure 5–II) and applied to the test data points. The calculated sample viscosity values for each data point were subsequently reported under the Generic Parameters section.

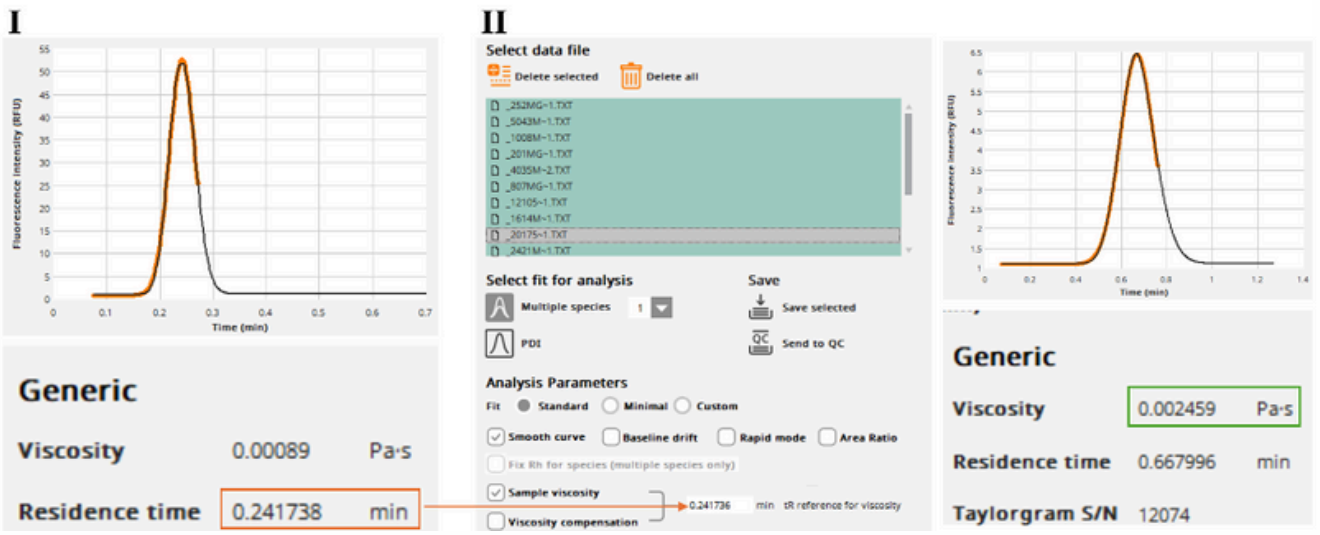


Figure 5: Processing of the reference tR (I) to obtain the sample viscosity (II) on the Fida software.

Batch processing of datasets was performed using the 'Autoanalysis' mode in the Fida software. The resulting analyses were published on the built-in QC dashboard, where users can configure export options as needed (Figure 6). In this case, species information and generic parameters were exported in both PDF and CSV formats.

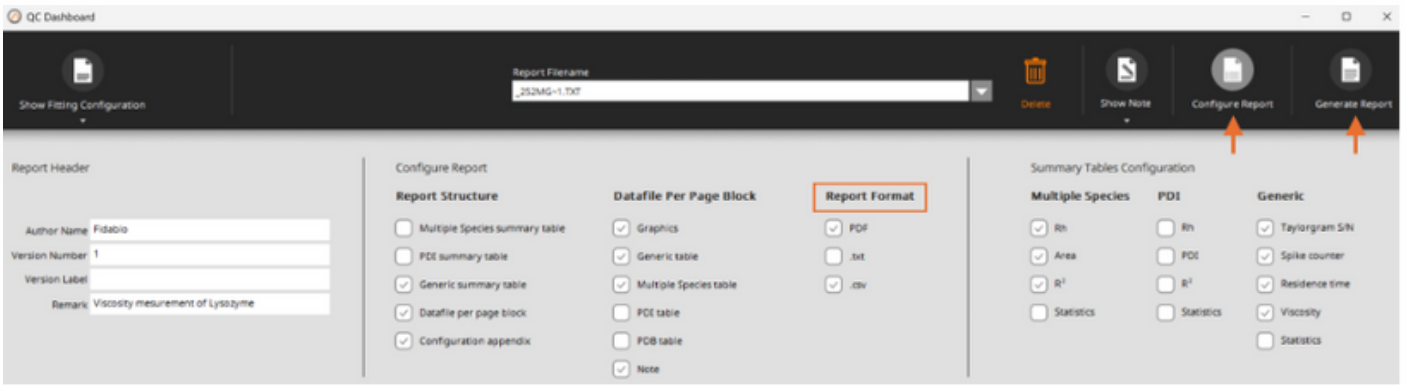


Figure 6: Screenshot of QC dashboard which enables exporting of required parameters in different formats (pdf, txt and csv).


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The obtained viscosity values were plotted as a function of lysozyme concentration, revealing an exponential increase in viscosity up to approximately 280 mg/mL. As illustrated in Figure 7, a deviation from this exponential trend is observed at higher concentrations, where the viscosity increase becomes non-exponential.

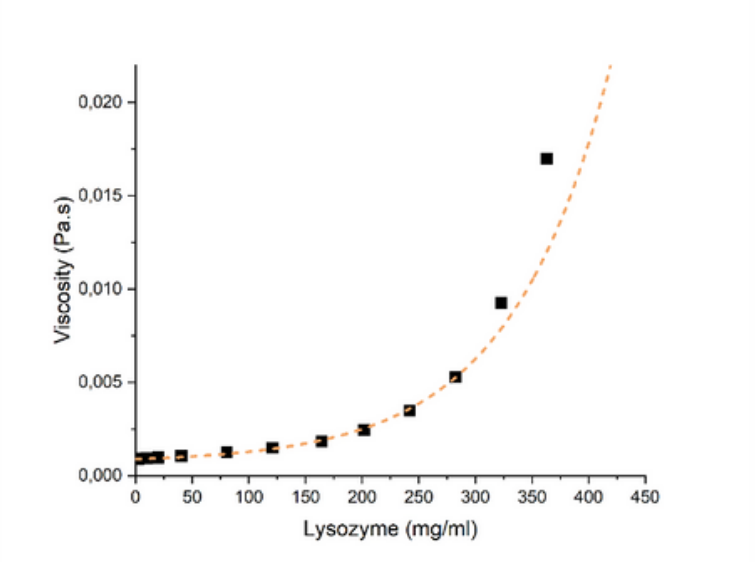


Figure 7: Plot of sample viscosity as a function of Lysozyme concentration showing deviation from exponential fit at high concentrations.

## FIDA in Biologics Production Worklow

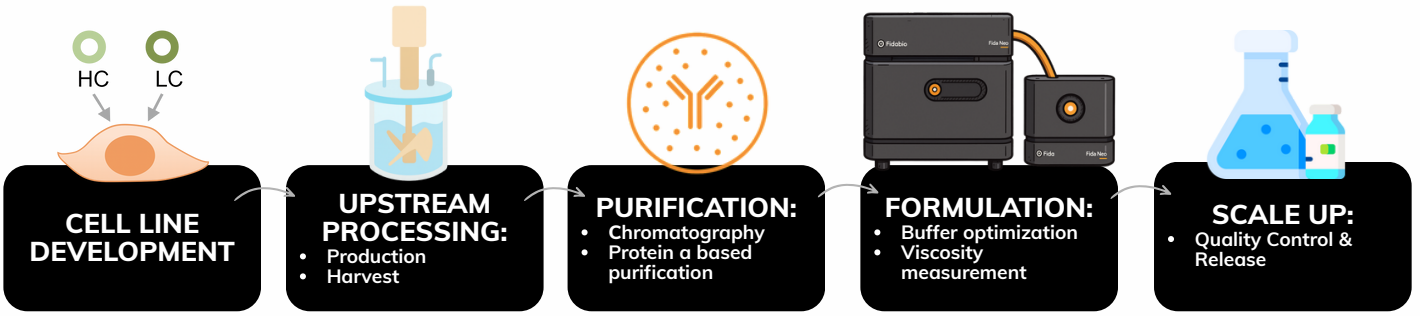


Figure 8: Workflow of biologics production involving FIDA Technology (Fida Neo model depicted).


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# CONCLUSION

In this appnote we have presented the implementation of FIDA technology in viscosity determination. The method is fully autonomous and can be used to perform batch measurements in a 96-well format. Here, Fida Instrument (Fida Neo) offers several advantages over a broad range of aspects like -

1. **Thermal control** – Accurate temperature regulation between 10–55 °C in the measurement chamber enables viscosity measurements under optimal conditions.
2. **Auto-sampling** – Samples loaded onto the FidaNeo instrument are analyzed in a fully automated, walk-away manner.
3. **Low sample consumption** – Each measurement requires only a few microliters of test sample, minimizing material use and reducing overall product development costs.
4. **High efficiency** – Automated viscosity measurements eliminate manual handling, freeing operators to focus on other tasks and improving overall productivity.

Scan the code to read more about FIDA viscosity measurement


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