RheoSense
Simply Precise™

m-VROC™: Intrinsic Viscosity

Objective

*To demonstrate m-*VROC™ *ability to determine the molecular size of proteins, polymers and other macromolecules by measuring intrinsic viscosity.*

Introduction

Intrinsic viscosity is an increase in viscosity as a result of adding an infinitesimal amount of solute to a solvent. It is essential to measure the viscosity of the solution at different concentrations to determine the intrinsic viscosity. The intrinsic viscosity is defined as the value of $\frac{\eta_{sp}}{C}$ at zero concentration:

$$[\eta] = \lim_{C \to 0} \frac{\eta_{sp}}{C} \quad (1)$$

where $\eta_{sp} = \eta_r - 1$ and $\eta_r = \eta/\eta_s$ is the relative viscosity of the solution with respect to the solvent. In Eq. (1) C is the concentration in mg/ml or mg/dl.

Intrinsic viscosity is a means to understand molecular structure and its interaction in solution. Measurement of this parameter is considered to be a more reliable method than light scattering. Applications pertaining to intrinsic viscosity measurement include:

- Size or weight of molecules
- Polymerization
- Degradation
- Interaction of molecules
- Stability of molecules – aggregation, denaturation or conformational change
- Sensitive detection of low molecular weight
- Protein structure

Using the empirical model Mark-Houwink-Sakurada relation, intrinsic viscosity correlates to the size of the macromolecules:

$$[\eta] = KM_w^a \quad (2)$$

where K and a are constants for a given combination of macromolecule and solvent varying

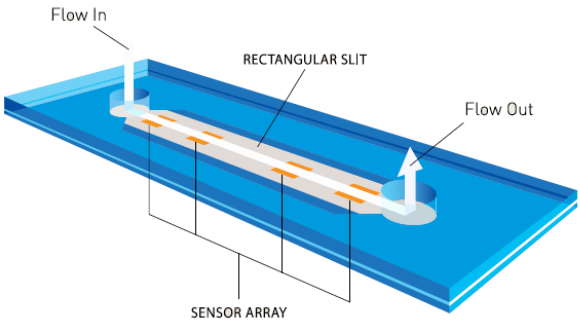
BENEFITS OF *m*-VROC™ SOLUTION

m-VROC™ offers precise shear viscosity measurements with small sample volume requirements and a wide dynamic operating range. High accuracy and repeatability makes it ideal for R&D and QC applications.

Features include:

- Accuracy: 2% of reading.
- Repeatability: 0.5% of reading.
- Smallest sample volume.
- Shear Viscosity range: 0.2 – 100,000 mPa-s.
- Shear Rate range: 0.5 -1,400,000 s^{-1}
- Temperature control: 4-70°C

VROC® Technology and Principle of Operation



RheoSense's *Viscometer-Rheometer-on-a-Chip* (VROC®) combines a microfluidic channel with a MEMS pressure sensor array to measure viscosity. As the test fluid flows through the channel the sensor array captures the pressure drop, which is proportional to the shear stress at the wall. The shear rate is calculated from the flow rate and the channel dimensions. The viscosity of the test fluid is obtained as the ratio of shear stress to shear rate.

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with temperature and molecular weight range. Using Eq. (2) molecular weight or size (M_w) can be calculated from the intrinsic viscosity value. The value of a also indicates the conformation of the molecules:

- a = 0 for compact sphere shaped molecules
- a = 0.5 ~ 0.8 for random coiled shapes
- a = 1.8 for rigid rod shapes

m-VROC™ offers exceptional repeatability (0.5% of reading) which is imperative for this kind of test. Glass capillary viscometers are conventionally used to measure intrinsic viscosity. However, their operation is laborious and time consuming. Additionally, most prevalent viscometry tools are not suitable for this application due to their poor repeatability.

Figure 1. m-VROC™ system. From left to right: Temperature control water bath, m-VROC unit and computer laptop with software.

Experimental

Samples and Test Set-Up: For this test, standard Polystyrene (PS) of three different molecular weights, from Supelco (item #: 48937), is dissolved in Toluene. Using an m-VROC™ and an A05 chip, the PS solutions are loaded into a 1 ml syringe and mounted into the m-VROC™ system. All tests are conducted at 25°C. Temperature equilibrium is usually reached within three to four minutes.

Testing procedure: Viscosity values are taken by measuring the pure solvent and the PS solutions at five different concentrations. There is no need to clean the chip in between solutions since they are compatible. For this test, each sample is measured four subsequent times and averaged (each measurement took 14.1 seconds).

- Flow rate is set to 600 µl/min.
- The first 200 µL of each new sample is used to prime* the chip and discarded during data analysis.

Results

Table 1 below summarizes the data measured for PS48k (Mw = 48 kDa) in Toluene at 25 °C. As shown by Figure 2, several solutions are prepared and tested as a function of increasing concentration to extrapolate to zero concentration.

Table 1. Viscosity of PS and Toluene solutions.

C [mg/ml]	*Viscosity, mPa-s*				
	#1	***#2***	***#3***	***#4***	***Ave.***
Toluene	*0.558*	*0.560*	*0.558*	*0.560*	*0.559*
3.855	*0.626*	*0.628*	*0.629*	*0.625*	*0.627*
8.610	*0.716*	*0.716*	*0.716*	*0.715*	*0.716*
13.455	*0.815*	*0.816*	*0.817*	*0.817*	*0.816*
21.944	*1.019*	*1.017*	*1.019*	*1.024*	*1.020*
30.98	*1.260*	*1.262*	*1.258*	*1.266*	*1.262*

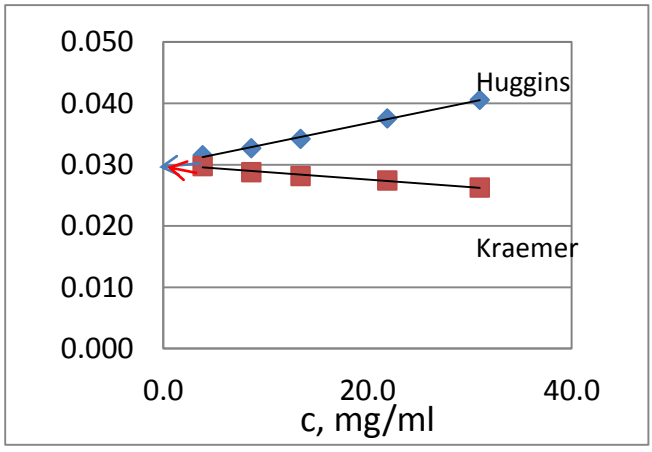

Figure 2. Graphical representation on how to obtain intrinsic viscosity using Huggins and Kraemer equations.

The Huggins and Kraemer equations are used for intrinsic viscosity calculation and summarized below.

Huggins: $\frac{\eta_{sp}}{c} = [\eta] + k_H[\eta]^2 C$ (3)

Kraemer: $\frac{\ln \eta_r}{c} = [\eta] - k_K[\eta]^2 C$ (4)

where k_H = 0.38 and k_K = 0.14 are Huggins and Kraemer's constant respectively.

Table 2. Left hand side of Eqns. (3) and (4).

	Huggins	Kraemer
C, mg/ml	η_{sp}/c	$\ln(\eta_r)/c$
3.855	0.032	0.030
8.610	0.033	0.029
13.455	0.034	0.028
21.944	0.038	0.027
30.98	0.041	0.026

The intercept of both Eqns. (3) and (4) with the y-axis is the intrinsic viscosity. The Mark-Houwink-Sakurada equation for PS in Toluene at 25°C is well known with parameters K=0.000037 ml/mg and a = 0.62. Using these constants, the average molecular weights are calculated for all three PS samples and summarized in Table 3.

Table 3. Molecular weight and hydrodynamic radius estimates.

Sample	*Molecular weight, Da*		$[\eta]$,	r_h,
	Reference	*Measured*	***ml/g***	***nm***
PS48k	*48,100*	*48,997*	*29.9*	*6.15*
PS16K	*16,600*	*12,800*	*12.9*	*2.98*
PS8k	*8,400*	*7638*	*9.5*	*2.26*
BSA*	*66,463*	-	*4.5*	*3.63*

(*BSA in PBS buffer: Bovine Serum Albumin has been reported to have a hydrodynamic radius of 3.7 nm, which is close to the measured value.)

The hydrodynamic radius r_h can be calculated from the molecular weight and the intrinsic viscosity using the well-known Einstein-Simha equation:

$$r_h = \left(\frac{3\, M_w[\eta]}{10\, \pi\, N_A}\right)^{1/3} \quad (5)$$

Where N_A is the Avogadro number. Results for the hydrodynamic radius are also summarized in Table 3.

Conclusion

- *m*-VROC™ enables highly accurate intrinsic as well as true dynamic viscosity measurements with unsurpassed degree of repeatability.
- High throughput measurement of six samples, including solvent, takes only an hour. Cleaning the chip with compatible samples eliminates the need to flush it after each test. This is in contrast with glass capillary viscometers which require cleaning between each tests.

Contact Information

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

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