WYATT TECHNOLOGY

White Paper

ViscoStar III
Innovations in Online Viscometry for GPC

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Summary

Online viscometers measure the intrinsic viscosity of polymers, proteins and peptides in conjunction with GPC in order to characterize essential physico-chemical properties: the distributions of molar mass, size, conformation and branching ratio. The ViscoStar® III, representing the next generation of online viscometers, incorporates multiple technological innovations that push the limits of sensitivity and stability to new levels. This paper describes those innovations and the benefits accrued as a result of their implementation.

Introduction

Online viscometry has long been used with gel permeation chromatography (GPC) or size exclusion chromatography (SEC) for the characterization of polymers[1]. A sensitive differential viscometer utilizes a capillary bridge, shown in Figure 1, to measure the small incremental viscosity of a solution containing a polymer sample relative to the viscosity of the solvent alone, expressed as the unitless 'specific viscosity' η_{sp}. The value of η_{sp} is divided by the concentration, determined by an online concentration detector such as the Optilab T-rEX differential refractive index (RI) detector, to calculate the intrinsic polymer viscosity η.

While intrinsic viscosity (IV) is an inherently valuable property of the polymer in itself, it becomes much more useful when combined with other experimental data, in particular multi-angle light scattering (MALS) data acquired with a DAWN HELEOS II MALS detector. MALS (analyzed together with concentration) provides a first-principles analysis of molecular weight and size, independent of reference standards or column retention volume. The molar mass range that may be determined by MALS is 200 Da – 1 GDa, and the size range for root mean square (rms) radius is 10 nm – 500 nm. Figure 2 illustrates how single-detector GPC based on column calibration leads to erroneous molecular weight results, while MALS results are robust and accurate.

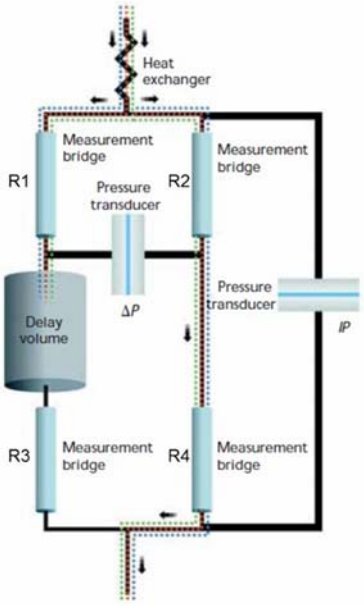

Figure 1. The capillary bridge used in a differential viscometer. IP and DP are the integral and differential pressure transducers. The bridge is balanced (so that DP=0 when pure solvent flows through all four resistive capillaries R1-R4) by adjusting the resistance ratios R1/R3=R2/R4. Sample measurement is made when sample flows through R1, R2 and R4, while the delay column passes solvent to R3. The specific viscosity is calculated as η_{sp}=2DP/(IP-4DP).

The co-analysis of MALS and IV becomes even more interesting: simultaneous MALS-IV calculations yield multiple sample properties, including

- Mark-Houwink-Sakurada parameters
- Hydrodynamic and rms radius from 1 nm and up
- Conformation (random coil, branched, globular)
- Branching ratio, branches per molecule and drainage coefficient[2]

[1] Striegel, A.M. "Viscometric Detection in Size-Exclusion Chromatography: Principles and Select Applications" *Chromatographia* **79**(15) pp 945-960 (2016)

[2] Podzimek, S. "The use of GPC coupled with a multiangle laser light scattering photometer for the characterization of polymers. On the determination of molecular weight, size and branching" *Appl. Polymer Sci.* **54**(1) pp 91-103 (1994)

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Certain polymers are not amenable to MALS analysis, especially if they are strongly fluorescent, scatter very weakly because they are index-matched to the solvent (dn/dc=0), or the solution is optically opaque. For those samples, online viscometry may be used without MALS to characterize molecular weight, albeit with higher uncertainty, via:

- Universal calibration – a technique that assumes ideal (steric) sample-column interactions; or
- Mark-Houwink-Sakurada analysis – a technique that utilizes empirical parameters determined separately for each sample/solvent combination.

Regardless of whether the technique of choice is SEC-MALS-IV or SEC-IV, clean measurements of intrinsic viscosity require sophisticated technology.

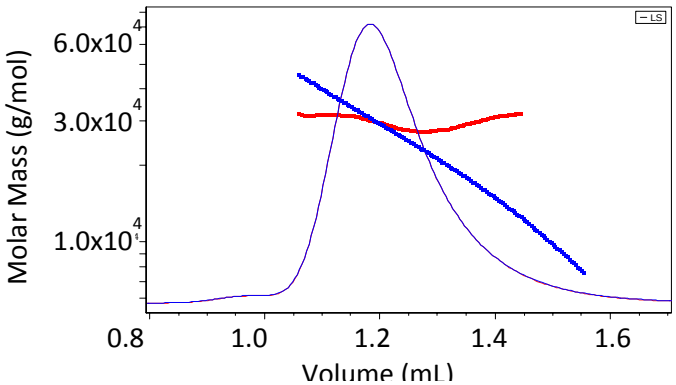


Figure 2. Comparison of single-detector GPC (blue) and MALS analysis (red) of molecular weight for a 29 kDa polystyrene standard. While MALS shows a narrow molecular weight range around 29-30 kDa, column calibration by definition implies that the peak is polydisperse from 20-40 kDa.

Overcoming the challenges of online viscometry

The magnitude of η_{sp} for a low-concentration polymer following elution from the GPC column may be on the order of just a few parts per million (ppm) so it is easy to understand why a differential viscometer must be highly sensitive. Yet simultaneously it must present immunity to pressure and temperature fluctuations that occur as a result of HPLC pump pulses or room temperature variations, and which could produce differential pressure signals comparable to—or even greater than—those from the sample. Technological advances in differential viscometry generally aim to improve sensitivity to the sample while overcoming these environmental noise signals.

Pump pulse immunity

For anyone working with HPLC pumps, periodic pressure pulses are a fact of life. They are often the ultimate limiting factor to quantitation for refractive index (RI) HPLC concentration detectors, though a well-designed instrument will overcome such pulses and suppress the optical response to well below ppm levels.

On the other hand, differential viscometers are actually *supposed* to measure pressure changes, so overcoming these pulses is less straightforward than in other types of detectors.

There are typically two layers of technology that address pulse-less IV measurements, bridge balancing and electronic smoothing.

Bridge balancing

An ideally-balanced capillary bridge, with all four arms presenting equal flow resistance and volume, should in principle cancel out completely any flow rate fluctuations. That is typically the condition of a new instrument. In practice, the pulse-cancelling effect due to bridge symmetry is significant but not complete, and may vary with solvent viscosity and flow rate. Moreover, bridges tend to go out of balance over time due to aging and accumulation of contaminants on the capillary walls.

How it's been done

One of the avenues engineers have explored in an attempt to overcome loss of balance is automated mechanical adjustment of the length of capillary R3 by means of a sliding seal. The bridge is rebalanced when R1/R3=R2/R4. Unfortunately, sliding seals represent potential weak links in the reliability chain, as they are particularly prone to degradation and leaks under aggressive organic solvents,

In the ViscoStar III

Thermal tuning of the bridge, as described in US Patent 7,213,439, takes advantage of a non-mechanical means of balancing the capillary bridge, by adjusting the temperature of a tuning capillary, which in turn adjusts its resistance. The tuning element is adjacent to R3 as shown in Figure 3. During tuning, the desired mobile phase is run through the entire instrument at the same flow rate as will be used during the GPC measurement. Figure 4 illustrates the tuning process, wherein the temperature of the tuning capillary is automatically adjusted so as to bring the DP signal to zero, to within 0.001 psi.

As seen in the graph, regulation to within roughly 0.03°C is required to maintain bridge balance to within 0.0001 psi (0.7 Pa). This requirement is not at all stringent, considering that the instrument's entire thermal enclosure is regulated to within 0.005°C. The entire process takes just 10 minutes, making it entirely feasible to rebalance the bridge daily, even though this is typically overkill; a more likely scenario would be rebalancing with each change of solvent or flow rate, or once per week if the same solvent and flow rate are maintained, to ensure top performance. With the advantages of speed and robustness against leaks, thermal tuning firmly establishes the ViscoStar III as a next-generation instrument.

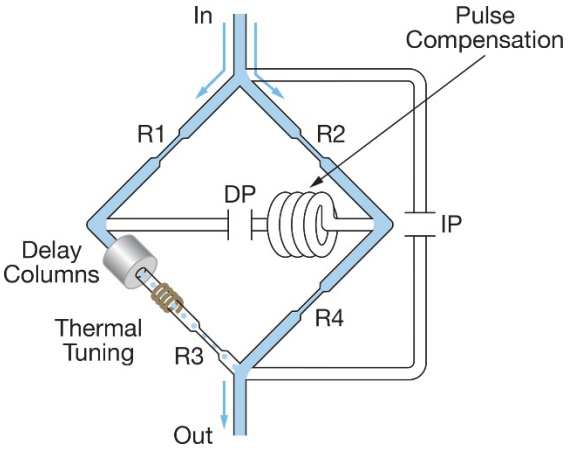


Figure 3. Capillary bridge in the ViscoStar III, implementing thermal tuning of R3 and a pulse compensation element for full impedance matching.

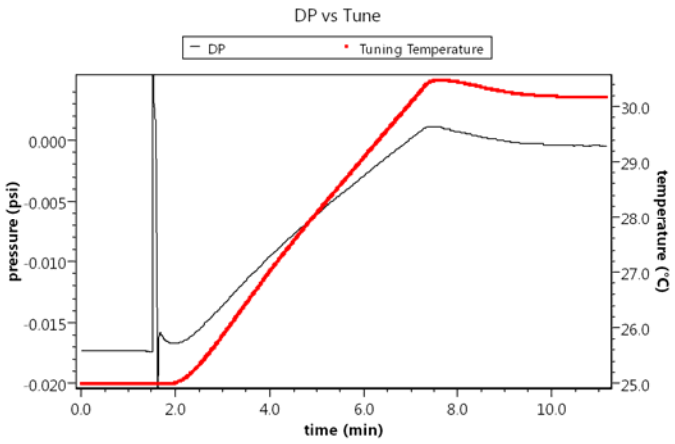


Figure 4. Thermal bridge tuning takes just ten minutes and does not require excessively difficult temperature regulation. The black graph shows the change in DP signal vs. temperature of the tuning capillary.

Pulse compensation

In real instruments, no capillary bridge is ideal. What's more, the delay column in the 3rd arm of the bridge *inherently* creates a volume imbalance, even if the four arms are perfectly balanced for resistance. This volume imbalance does not present a problem as long as the flow rate and pressure are constant, but the propagation of a pressure pulse through unequal volumes means that it arrives at R3 after the other capillaries and manifests as a *dynamic* imbalance. Pump pulses show up in the IP signal and especially in signals from the super-sensitive DP.

How it's been done

While pulses appear in the IP signal, the impact on overall specific viscosity is small. More critical are the pulses in the DP signal.

The DP transducers that have been in use for the last couple of decades are *membrane transducers*. The sensing membrane in these transducers has a relaxation time of several seconds, which serves (as a side effect) to smear out and hide pump pulses. At the same time, the membrane's long time constant also broadens the trailing edges of chromatographic peaks and contributes to loss of resolution. Hence previous generation differential viscometers tend to have mediocre chromatographic resolution.

In the ViscoStar III

A multi-layered approach is brought to bear in the ViscoStar III for true pulse *compensation and filtering*, rather than coarse pulse smearing with its associated peak broadening.

Impedance matching

The first advance in pulse compensation, full impedance matching, is introduced at the hardware (fluidics) level. In order to compensate for pulse propagation imbalance through the delay column, a second delay volume is added on the opposite side of the bridge (patent pending). With an appropriate matching volume, pulses propagating on the left and right sides of the bridge reach the DP transducer at the same time and cancel out.

While the symmetry is not perfect, as shown in Figure 5, impedance matching reduces the DP pulse by ~100x *with no loss of chromatographic resolution*. Additionally, the position of the compensation volume adjacent to the transducer means that it does not affect the static bridge

balance, sample does not pass through it, and it does not adversely impact specific viscosity measurements.

Pulse filtering

The second advance in pulse compensation is matched filtering of the residual DP pulses in software. Since these pulses arrive at fixed intervals, it is straightforward to identify their frequency and remove them via Fourier filtering. Figure 5 shows that this algorithm eliminates entirely residual pulses.

Proprietary calculation

After the pulses have been eliminated from the DP signal, small fluctuations in the IP remain to affect specific viscosity calculation. The third advance eliminates the contribution of these fluctuations by means of a proprietary algorithm (Us Patent 7,331,218) that utilizes a baseline IP value and calculates η_{sp} without reference to instantaneous IP signals.

Multi-layered pulse compensation technology incorporated in the ViscoStar III takes it to the next level of sensitivity, limited primarily by quality of the chromatography and sample rather than system properties.

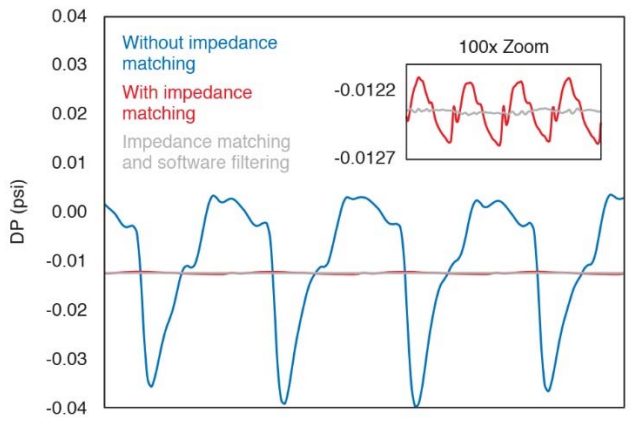


Figure 5. Advanced pulse compensation in the DP transducer signal. The first layer, impedance matching by means of a compensation volume, reduces the amplitude of the pulse by ~100-fold. The second layer, filtering at the pump frequency in software, eliminates residual pulses.

Thermal stability

The basic elements of thermal stability are not a secret: good passive isolation, and an active thermal element to make small adjustments and keep the temperature stable. In a differential viscometer, the bridge capillaries should be maintained at a constant temperature to within less than 0.01°C, even when room temperature varies by several degrees C, so active thermal management is required even when passive isolation is at a high level.

Passive isolation

In a well-designed viscometer the capillaries are in good thermal contact with each other via a massive thermal sink, and kept well away from room-temperature variations or even from temperature changes within the instrument due to the power supply heating up. The former is accomplished by squarely attaching the capillaries to a single metallic block, while the latter involves building a well-insulated box around all fluidic and transducer components.

Departure of any component, especially the bridge or delay column, from the thermal box and from good thermal grounding opens the system to thermal drift and corresponding baseline drift of the transducer signals. The ViscoStar III was designed from the ground up with optimal passive isolation in mind, implementing optimal engineering practices.

Active regulation

Passive isolation is generally insufficient for high sensitivity and so an active element with feedback is needed.

How it's been done

Some instruments make do with simple heating elements; this typically limits the range of thermal stabilization to 10°C above room temperature and higher. With heating alone, the instrument cannot be stabilized at room temperature. More advanced instruments such as the ViscoStar II utilize thermoelectric Peltier-based thermal regulation, which means that the instrument can be heated *or* cooled. Peltier regulation provides stability at room temperature as well as lower temperatures which might be needed for proteins, peptides or other sensitive samples.

Another aspect of active thermal regulation is the number of active elements and temperature sensors as well as their positioning around the thermal box. Typically the design will include only one active element and one or two sensors. The laws of physics dictate that a thermal gradient will develop in the instrument, radiating out from the single active element. This gradient is responsible for

some degree of drift. To a large extent the gradient can be managed by rigorous engineering, but it cannot be completely overcome without an additional active element.

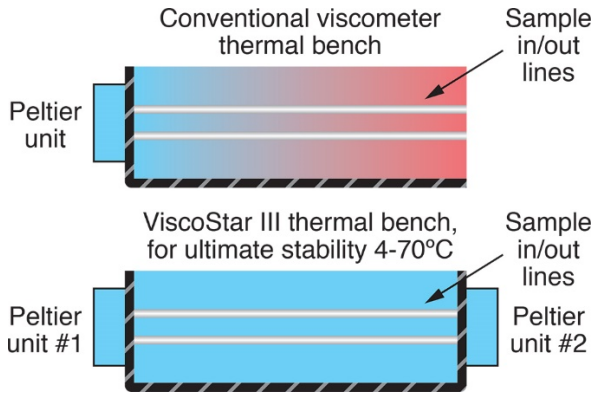


Figure 6. Thermal regulation of the ViscoStar III bench utilizes two Peltier units to eliminate thermal gradients and concomitant signal drift.

In the ViscoStar III

Dual-Peltier distributed regulation of the thermal bench has been implemented in the ViscoStar III. As shown in Figure 6, this design not only allows for active control across a wide temperature range from 4-70°C, but eliminates the thermal gradients that are inherent to a single-element design, providing faster thermal equilibration, better isolation from room temperature fluctuations and reduced thermal drift.

Transducer reliability

The DP transducers used in previous generation viscometers are very sensitive but also fragile. The two sources of failure are overpressure and corrosion.

Overpressure

Excessive pressure could be caused by any combination of high flow rate, high solvent viscosity and clogged tubing. Various schemes have been implemented to protect DP transducers from damaging pressure levels which could rupture the membrane.

How it's been done

Passive protection, implemented in the ViscoStar II, incorporates a standard HPLC backpressure regulator to ensure that system pressure exceeding the limit is safely diverted to a waste line. While this method is quite reliable, it does not do a good job of notifying the instrument user that an overpressure event has occurred. If sample was diverted to waste then potentially the measurement was corrupted but no message conveyed this to the user.

Additional protection can be provided by monitoring the DP signal and, should it exceed the permitted value, relieving the excess pressure by opening a transducer purge valve. However, this is only a partial solution since it does not respond to high system pressure that does not produce a differential pressure signal. It protects the DP transducer but not necessarily other sensitive components.

In the ViscoStar III

Active transducer & system protection means that all components of the instrument are monitored and protected from overpressure. In the ViscoStar III, the passive protection device was replaced by an internal sensor and active relief valve. When system backpressure exceeds the safe limit, the relief valve is activated electronically to divert solvent flow, bypassing the bridge including the DP *and* IP transducers.

The ViscoStar III's system pressure sensor provides a continuous pressure reading that can be logged and monitored over time to determine if a clog is building or maintenance required. If the valve is activated due to overpressure, the user is notified and can take remedial action including aborting a measurement.

Since this system relies on internal software, the design includes a 'normally open' valve that diverts solvent flow to bypass the bridge when the instrument is powered down. Therefore the sensitive components are fully protected even if the instrument is not in use.

Corrosion

Highly sensitive magnetic differential pressure transducers used in differential viscometers are often constructed of 410 stainless steel with welded Inconel® caps. The 410 stainless material, and in particular the weld joints between it and Inconel, are subject to corrosion by salt-containing aqueous solutions. This susceptibility limits the use of online viscometers in protein applications and others that require moderate to high salt, or extreme pH.

How it's been done

One solution to reduce susceptibility to corrosion has been to machine the entire transducer from a single material with no weld joints. However, magnetic materials generally are susceptible to corrosion by high-salt buffers. Another solution has been to coat the wetted parts of the transducer with a corrosion-resistant material such as PTFE[3]. This works well as long as the coating does not degrade. While both of these provide pretty good protection against corrosion, there was certainly room for improvement.

In the ViscoStar III

An ideal transducer would contain no wetted materials or joints that are susceptible to corrosion. The ViscoStar III replaces the magnetic membrane transducer with a piezoresistive transducer that does not require a magnetic metal membrane. In the new DP transducer, *all* wetted surfaces are 316 stainless steel (a material found in most HPLC systems which has excellent resistance to corrosion, even at high salt concentration) and PTFE.

In fact, the entire fluidics path in the ViscoStar III has been engineered to eliminate all materials susceptible to corrosion or damage from harsh organic solvents. This instrument exhibits excellent solvent compatibility across the widest range of aqueous *and* organic solvents.

More benefits

The new DP transducer is not only superior to previous generation components in terms of corrosion, it also has a much faster response time than the magnetic-membrane transducer. That means that the benefits of pump pulse compensation mentioned earlier can be fully realized in terms of reduced peak broadening.

The advantage of the piezoresistive DP transducers is seen in Figure 7, where the DP peaks resulting from SEC separation of BSA monomer and dimer are significantly narrower in the ViscoStar III than in previous-generation instruments. Thanks to advanced pulse-suppression technologies, the faster DP transducer response does not result in signal fluctuations arising from pump pulses.

[3] Trainoff, S. P. Corrosion resistant pressure transducer PCT/US2013/072428

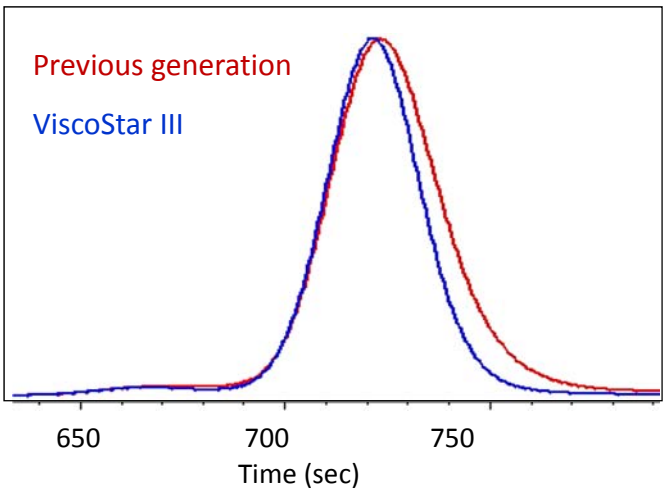


Figure 7. Chromatogram of BSA monomer and dimer, shown in the DP signals of the previous generation of viscometer (red) and the ViscoStar III (blue). The ViscoStar III greatly reduces artificial band broadening induced by the slow DP transducer of previous generations, allowing for resolution of the BSA dimer at 670 sec.

The next generation viscometer

In differential viscometers of previous generations, engineers have found ways to address some of the key challenges, and conventional instruments in fact do quite a commendable job. With the ViscoStar III, we set forth to do a *spectacular* job of addressing and eliminating the technical pain points, in order to produce the next-generation instrument with enhanced sensitivity, stability and solvent compatibility.

Dynamic range, sensitivity and resolution

Dynamic range is the ratio of the largest signal that can be measured, to the smallest, and is indicative of the range of samples and conditions that can be analyzed. The ViscoStar II had a dynamic range of over 35,000:1, which exceeds that of competing instruments fourfold. The ViscoStar III extends that large range to over 135,000:1—by far the best available. Some of that large dynamic range is presented in Figure 8.

The improvements in sensitivity and resolution are shown in Figure 9 for epoxy resin at different concentrations. The intrinsic viscosity measurements for both previous generation (ViscoStar II) and next generation (ViscoStar III) are of good quality where sample concentration is relatively high, across the main

peaks. However, where the concentration is low (e.g. at the leading edge near 18.0 minutes, or around 24.0 minutes) the greatly improved ViscoStar III measurements provide more robust analyses. In fact, the sensitivity of the ViscoStar III, at just 0.05 Pa, means that it can measure with good fidelity an injection of just 0.1 µg of 100 kDa polystyrene[4]. This is 3-fold better than the ViscoStar II and 10-fold better than competing viscometers.

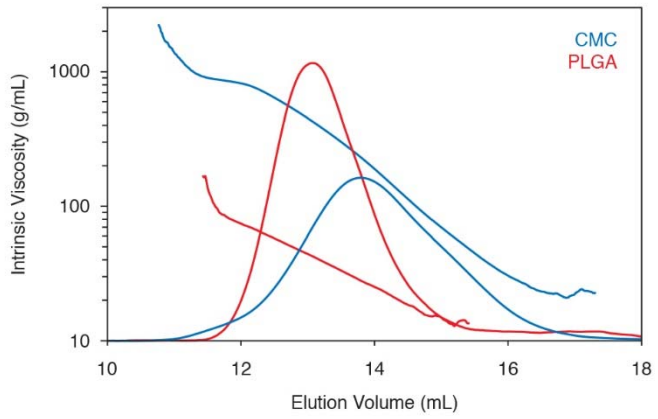


Figure 8. Intrinsic viscosity measurements of poly(lactic co-glycolic acid (PLGA) and carboxymethyl cellulose, covering a large range. Intrinsic viscosity in the single digits is readily measured as well.

Solvent Compatibility

Versatility to the widest range of conditions and robustness to resist corrosion are essential for an analytical instrument. The ViscoStar III has been shown to resist difficult solvents such as:

- THF with 10% formic acid
- 100 mM acetate buffer, pH 3.5
- DMF with 0.1% LiBr
- 1% acetic acid + 500 mM NaCl
- 800 ppm SDS + 250 ppm NaN_3
- 100 mM Na_2SO4 + 250 ppm NaN_3

Stability

The excellent intrinsic viscosity results shown in Figure 9 are a good indicator of the stability of the ViscoStar III in the course of a run. Other tests indicate superior isolation from room temperature fluctuations which may result from air conditioner cycling. In fact the baseline drift is 50x better than that of competing instruments, guaranteeing excellent performance over the course of extended runs or multi-injection sequences. With the ability of Wyatt's ASTRA chromatography software to control 3rd-party HPLC pumps, the implications for processing many samples unattended, over the course of a night or even several days using top-of-the-line GPC equipment and detectors, is clear.

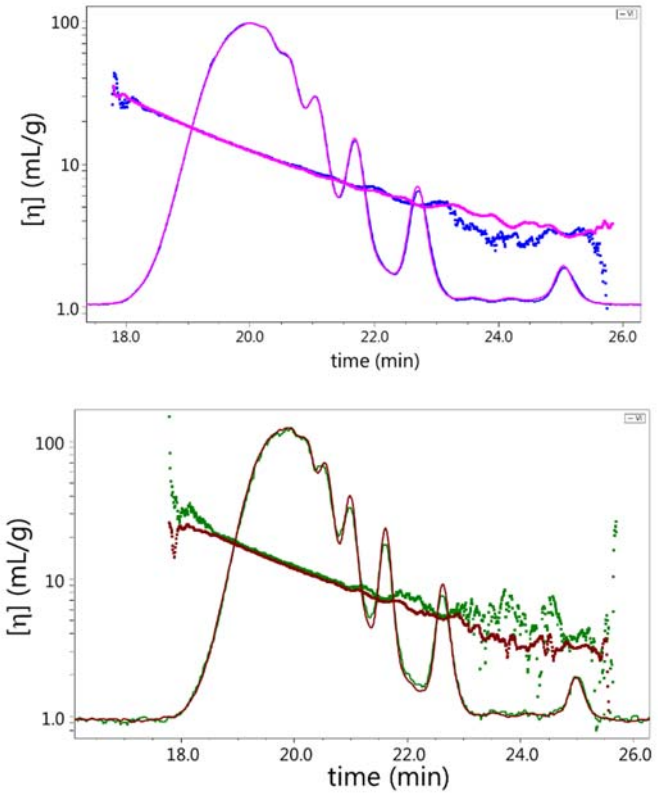
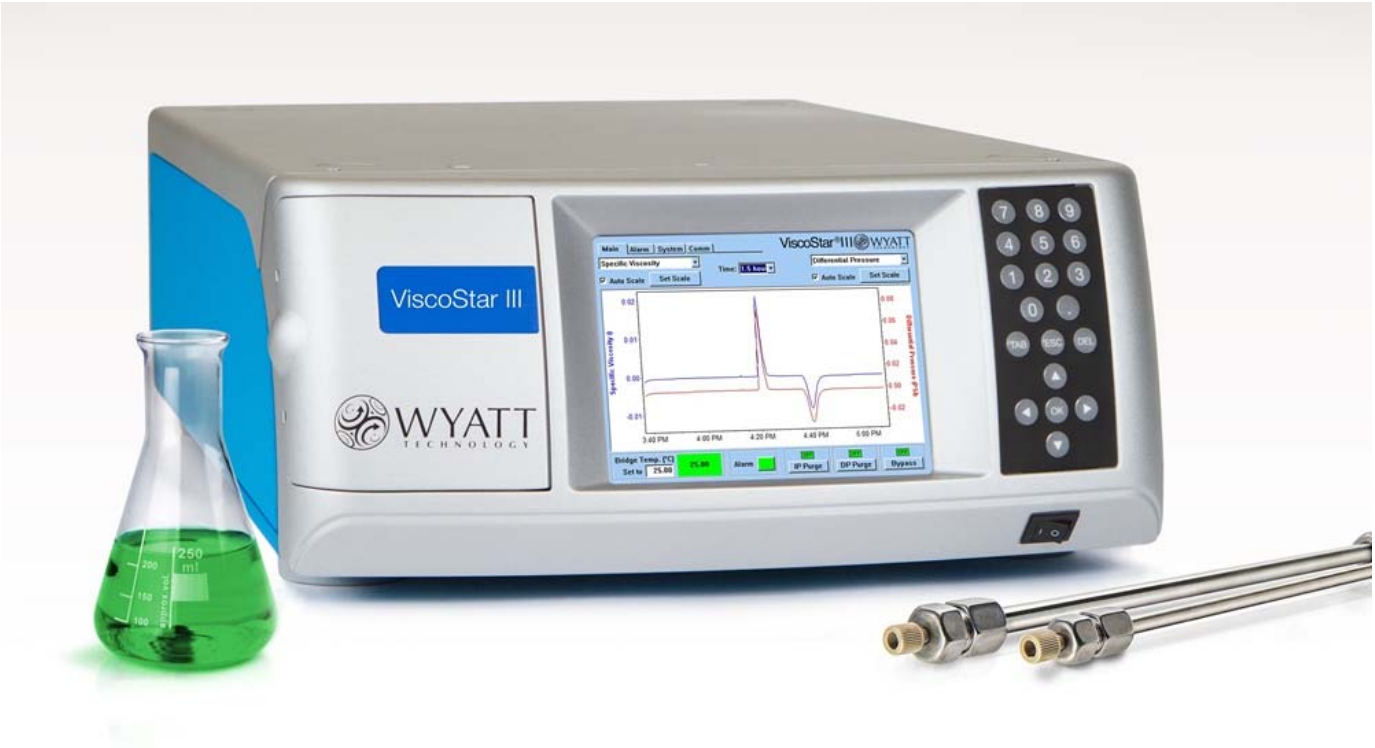


Figure 9. Intrinsic viscosity of epoxy resin. Top: 100 µL injection, ViscoStar II (blue) vs. ViscoStar III (magenta); bottom: 12.5 µL injection, ViscoStar II (green) vs. ViscoStar III (brown). While the intrinsic viscosity results are of good quality across the primary peaks for both the previous generation viscometer (ViscoStar II) and the next generation (ViscoStar III), where the sample concentration is low the higher signal-to-noise ratio and measurement quality of the new instrument is obvious.

[4] in THF on a standard GPC column running at 1 mL/min

Conclusions

Representing the next generation of online viscometers, the ViscoStar III exhibits a major leap forward in performance and versatility. In combination with ASTRA and the industry-leading performance of the DAWN® HELEOS® II MALS detector and Optilab® T-rEX refractive index detector, the ViscoStar III is the viscometer of choice for the most demanding GPC analyses of polymers, peptides, proteins and more.



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