SEC Absolute Molar Mass Detection by Online Membrane Osmometry

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ABSTRACT: A membrane osmometer with a short response time based on a previously developed concentric design with a capillary-shaped membrane has been applied as an online SEC detector for absolute molar mass detection in combination with a concentration detector. The flow cell volume is 12.2 μL, the response time approximately 15 s, and the molar mass cutoff is below 5 kg/mol. The performance has been tested with polystyrene standards in toluene under realistic SEC conditions.

Introduction

Size exclusion chromatography (SEC) is—among other liquid chromatography techniques, like high-performance liquid chromatography or the various field flow fractionation methods[1]—probably the most widely employed tool for routine polymer characterization.

Usually, concentration sensitive detectors are employed and a calibration curve is constructed from suitable polymer standards, which are, however, only available for a limited number of polymers. One way to work around this limitation is to measure the viscosity and the concentration simultaneously and to rely on universal calibration,[2] which assumes a fractionation strictly according to the hydrodynamic volume of the polymer molecules. Especially for polar polymers, adsorptive interaction with the column material cannot be ruled out and the reliability of the universal calibration technique becomes questionable.

To avoid this problem absolute molar mass sensitive detectors are very desirable, and static light scattering has successfully been employed together with a conventional concentration detector, based on optical absorption or refractive index (RI) changes.[3] Since the light scattering signal is proportional cM, c being the concentration and M the molar mass, the method is best suited for high molar masses and becomes impractical below $M \approx 10$ kg/mol.

Colligative properties, like the osmotic pressure, are only sensitive to the number of molecules in solution, $n \propto c/M$, without any other a priori assumptions. As compared to light scattering, an absolute SEC calibration can, in principle, be obtained from a simultaneous measurement of concentration and some colligative property. In terms of sensitivity, membrane osmometry is the most promising one.

Commercially available membrane osmometers are designed for static measurement, and the cell design with a flat membrane is not suited for continuous flow operation. The equilibration time constant, which is largely due to the balloon effect of the flat membrane, is typically of the order of 10 min, whereas SEC online detection under realistic conditions requires a time constant of the order of a few seconds to avoid peak distortion.

Yau et al. tried to overcome this limitation and developed a detector which measures the flow resistance of a column caused by osmotic swelling and deswelling of soft gel particles used for packing.[4,5] While a number sensitive detector with a fast response was obtained, the absolute calibration was lost, since the change in flow resistance cannot easily be related to the osmotic pressure of the solution.

Recently, an osmometer based on a capillary-shaped membrane has been developed in our laboratory,[6] which avoids many problems associated with flat membranes. The solution chamber inside the capillary can easily be flushed and is ideally suited as a flow cell. The balloon effect of the membrane is completely negligible and a response time of the order of seconds is achieved, which is mainly determined by the compressibility of the pressure sensor itself. In this article the application of the design, when equipped with a suitable membrane, as an absolute SEC online detector is demonstrated.

Experimental Section

A standard size exclusion chromatography (SEC) setup consisting of a solvent reservoir, a pump (Waters 590), two pulsation dampers (Shodex DP-1), an injection valve (Rheodyne 7125), columns (PSS Gel SDV 500 Å, 5 μm, PL Gel 10^4, both 8×300 mm), a refractive index (RI) detector (ERMA ERC 7522), a waste reservoir, and a personal computer for data collection was used (Figure 1). For the measurements with osmometric detection the RI detector was replaced by the osmometric flow cell. The flow rate was 0.5 mL/min, and the injection volume, 0.1 mL. For static measurements the inlet of the osmometer was connected to an injection valve and a syringe was used to inject approximately 0.75 mL of solution.

Narrow polystyrene (PS) calibration standards (PSS, Mainz) with nominal number average molar masses of $M_n = 5250$ and 47 400 g/mol were used. The solvent was toluene (p.a., Riedel de Hãen).

Osmometer Design. The design of the osmometer flow cell is shown in Figure 2. The main part has cylinder symmetry and comprises the semipermeable hollow fiber membrane and the outer glass tube. The polymer solution flows through the bore of the membrane capillary. The reference cell, which is filled with pure solvent, is the volume between the membrane and the supporting glass tube. It is sealed on both ends with epoxy resin, which also serves to hold the membrane in place. The length of the cell is 43 mm, the inner diameter of the glass tube is 2.0 mm, the inner diameter of the membrane is 0.6 mm, and the wall thickness is 0.2 mm. The volume of the solution cell is 12.2 μL. Stainless steel caps with Teflon gaskets are pressed against both ends of the glass

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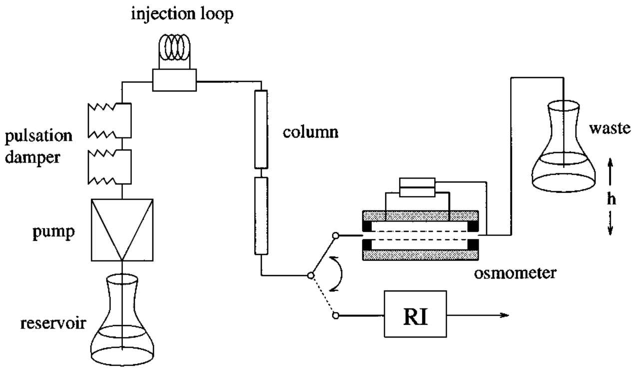

Figure 1. Sketch of the SEC setup. Either the refractive index detector or the osmometer is used as the detector.

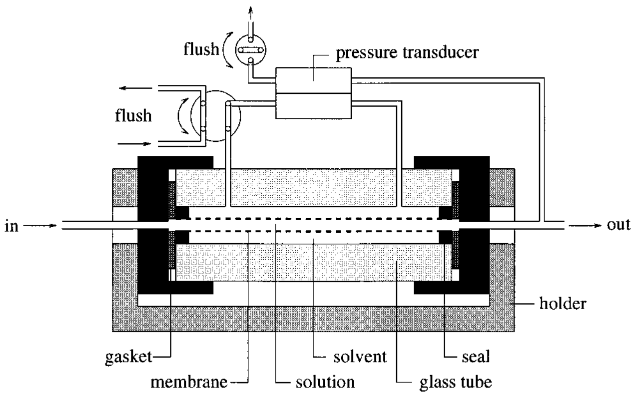

Figure 2. Design of the osmometric flow cell.

tube and connect the inlet and outlet of the osmometer with steel capillaries to the SEC setup.

The pressure between the solvent and solution chamber is measured with a differential pressure sensor taken from a Knauer differential refractometer–viscometer. Its high-pressure side senses the pressure close to the outlet of the osmometric flow cell. The low-pressure side is connected to the solvent chamber with two stainless steel capillaries which are glued into holes drilled into the glass tube. A valve (Rheodyne 7125) allows for flushing of the solvent chamber and the low-pressure side of the pressure sensor. The transparent glass tube allows detection of air bubbles in the solvent chamber, whose compressibility would slow down the osmometer response and cause baseline noise. The solution side of the pressure sensor can be flushed by means of a similar valve. All components shown in Figure 2 are housed inside a massive aluminum box to dampen temperature fluctuations and pressure noise from the ambient laboratory.

The outlet of the osmometer is connected with a short (15 cm) thick (1 mm) capillary to a reservoir for waste collection. The pressure inside the solution chamber is determined by the nearly constant hydrostatic pressure of the waste reservoir. The immersed end of the steel capillary avoids pressure fluctuations caused by the varying surface tension during droplet formation. Care has been taken to minimize back pressure due to the flow resistance of the outlet capillary, which would lead to pressure noise from pulsation of the pump. To minimize back pressure, the osmometer must be the last detector in a multidetector SEC setup.

During operation eluent flows continuously through the flow cell. The solvent side of the osmometer is refreshed from time to time to remove permeated solute. Furthermore, since the osmotic pressure is measured with respect to a baseline established by injection of pure solvent, small amounts of slowly permeating solute within the solvent chamber do not lead to wrong results.

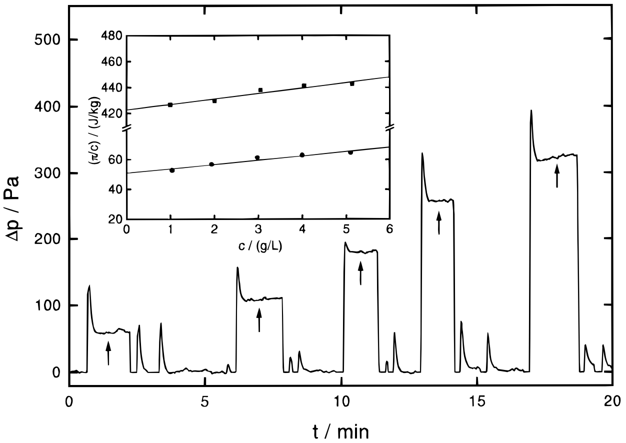

Figure 3. Static measurement: Concentration series with multiple injection of PS (5250 g/mol) in toluene. Concentrations: 1.07, 1.91, 2.94, 4.11, and 5.03 g/L. Multiple solvent injection to establish the baseline. Insert: data evaluation for concentration series of PS (5250) and PS (47400) in toluene.

Membrane. The semipermeable membrane is a highly asymmetric poly(acrylonitrile) hollow fiber as the support membrane with an inner separating layer. The inner side (solution side) of the support membrane has been coated with a dilute solution of poly(dimethylsiloxane), which was later dried and cross-linked. Such a composite membrane is best suited to meet the contradictory requirements for online detection, which are mechanical stiffness, high solvent permeability, and a low molar mass cutoff. Details about the membrane will be reported in a forthcoming publication.[7]

Results and Discussion

Static Measurements. For conventional static measurements the solution chamber was filled with solution and sufficient time was given for pressure equilibration. Alternating with the solutions, pure solvent was injected to establish the baseline. The data were evaluated according to[8]

$$\left(\frac{\pi}{c}\right)_{c\to 0} = RT\left(\frac{1}{M_n} + A_2 c\right) \tag{1}$$

c is the concentration, π is the osmotic pressure measured as the pressure difference between the solvent and solution chamber, R is the gas constant, T is the temperature, M_n is the number average molar mass, and A_2 is the second virial coefficient. Figure 3 shows the pressure as a function of time during a static measurement. The narrow spikes are caused by the pressure perturbation during solution and solvent injection.

The data evaluation according to eq 1 is plotted in the insert in Figure 3 for the two PS samples. From the extrapolation $M_n = 5800$ (5250) g/mol and $M_n = 48\,300$ (47 400) g/mol have been found. The numbers in parentheses are the nominal values supplied by the vendor. The corresponding second virial coefficients are $A_2 = 1.7 \times 10^{-3}$ and $A_2 = 1.2 \times 10^{-3}$ mol kg^{-2} m^3, respectively. The numbers are comparable to literature values, which show, however, a substantial scatter.[9]

No significant membrane permeation of the two PS samples was observed within 20 min. The low molar mass is, however, close to the lower cutoff of the membrane. For $M_n = 3100$ g/mol a 50% pressure drop was observed after 12 min. Care must be taken when permeating polymers are measured, since even an infinitely fast osmometer does not allow the measurement of the correct osmotic pressure due to the Staverman effect.[10]

SEC Online Detection. To test the suitability of the osmometer as an online flow detector for SEC, a concentration series of PS (5250) with $c_i = 0.99$, 2.54, 5.00, and 10.32 g/L was prepared and $V_i = 0.1$ mL of

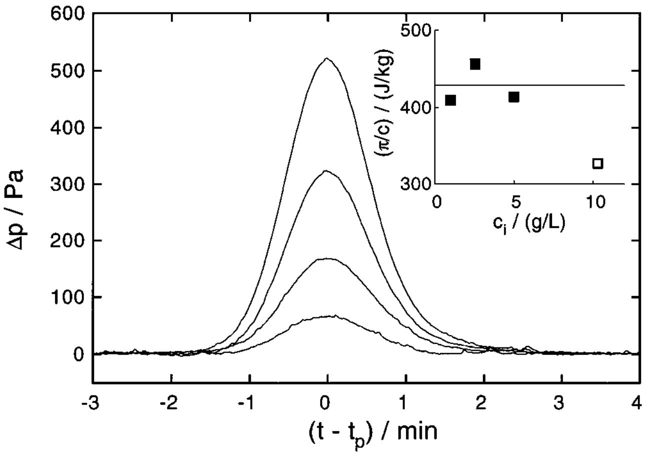

Figure 4. Osmometric detection of SEC peaks of PS (5250 g/mol) in toluene for different injected concentrations c_i. The insert shows the reduced osmotic pressure according to eq 1 plotted as a function of c_i.

the solutions were injected into the SEC apparatus. The index i at the concentration indicates the injected concentration, which is different from the concentration within the osmometric cell due to dilution within the columns. The pressure signals Δp are plotted in Figure 4 as a function of $t - t_p$, where $t_p \approx 32$ min is the peak retention time. Since the total injected mass $m_i = c_iV_i$ and the flow rate ϕ are known, M_n can be calculated from the peak area according to eq 1 when A_2 is neglected:

$$\frac{RT}{M_n} = \frac{\pi}{c} = \frac{\phi}{c_iV_i}\int_{\text{peak}} \mathrm{d}t\, \Delta p(t) \tag{2}$$

The peak width (fwhm) is approximately 1.25 min (Figure 4), corresponding to a volume of 0.63 mL. Hence, the solutions are diluted by roughly a factor of 5 and A_2 can safely be neglected.

The insert of Figure 4 shows π/c according to eq 2 plotted as a function of the injection concentration c_i. From the average of the three lower concentrations a value of $M_n = 5735$ g/mol is obtained, which compares well with the value of 5800 g/mol from the static measurements. The scatter of the data is noticeably larger than that in the static measurement. The reason for the low π/c value for the highest concentration is unclear and needs further investigation.

Figure 5 shows the osmotic pressure signals together with the RI signals from a 1:1 mixture of PS (5250) and PS (47 400). The total concentration was $c_i = 5.02$ g/L. Osmotic pressure and concentration (RI) were measured in two separate runs and the time offset of the RI detector was compensated by adjusting the maximum of the peaks with the longer retention time. Also shown in Figure 5 are the integrals over the two signals (cumulative distributions). The difference between both detectors is evident. The ratio between the areas of the two RI peaks is almost exactly unity, whereas the ratio between the two osmotic pressure peaks is 8.2, which is close to 9.0 as expected from the two molar masses.

M_n can be calculated for every slice ranging from t_1 to t_2 from the combination of the osmotic pressure, the concentration signal and the injected concentration without any a priori knowledge of the SEC calibration curve:

$$M_n = RT\frac{c_iV_i}{\phi}\int_{t_1}^{t_2} \mathrm{d}t\, C(t)\Big[\int_{t_1}^{t_2} \mathrm{d}t\, \Delta p(t)\Big]^{-1} \tag{3}$$

$C(t)$ is the normalized concentration signal. The results for the two peaks are $M_n = 5630$ (5250) g/mol and $M_n = 45\,900$ (47 400) g/mol, in reasonable agreement with the nominal values (in parentheses) and the ones found from the static measurements.

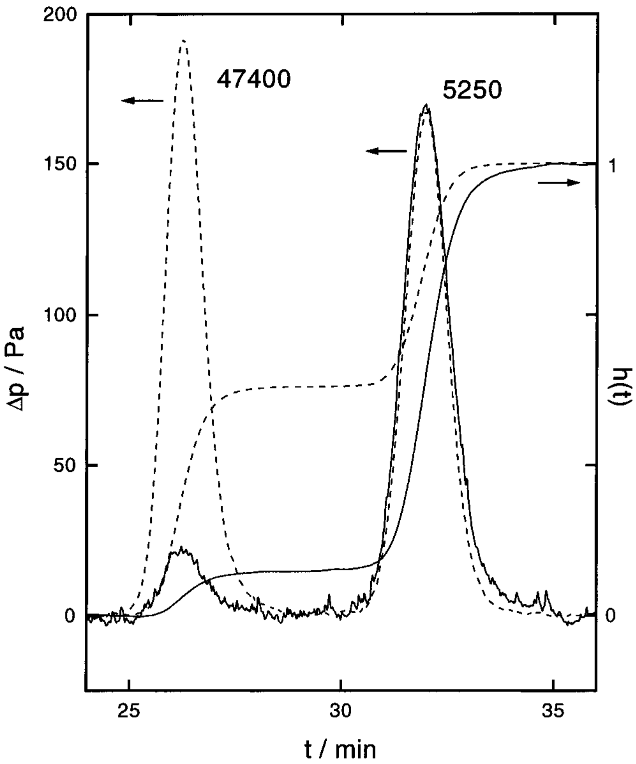

Figure 5. SEC elugrams for mixture of two PS as measured with osmotic pressure (solid line) and RI (dashed line) detectors. Also shown are the normalized cumulative distributions $h(t)$. The numbers are the nominal M_n values. The RI curve is arbitrarily normalized to the osmotic pressure signal for the low molar mass peak.

Figure 5 shows a good measurement, but in general the baseline stability of the osmometer is not yet satisfactory for routine online detection, and occasional baseline drifts may cause problems for the evaluation of broad peaks.

Time Resolution. The response time of the osmometer is determined by both the solvent permeability of the membrane and the balloon effect of the membrane and the solvent chamber. It must be short enough to avoid peak distortion due to memory effects. Contrary to flat membranes, which unavoidably show significant ballooning, the balloon effect of the capillary membrane tube is negligible. The response time can be measured by applying a pressure jump at one side of the membrane, e.g. by rapidly changing the height of the waste reservoir and removing the connection between the waste reservoir and the solution side of the pressure sensor (Figure 1). After such a perturbation the pressure approaches exponentially its new equilibrium value. The result of such a pressure jump is shown in Figure 6 superimposed to a SEC peak. The pressure jump is applied at the time of the peak maximum ($t - t_p = 0$) and its amplitude is normalized to the peak height. The decay time of $\tau = 15$ s is significantly shorter than the half-width of the peak, but not completely negligible. Since the time needed by the solvent to flow through the pores of the membrane is proportional to the solvent viscosity, the response time of the osmometer is expected to show the same dependence. The detection cell volume of $V_d = 12.2$ μL translates to

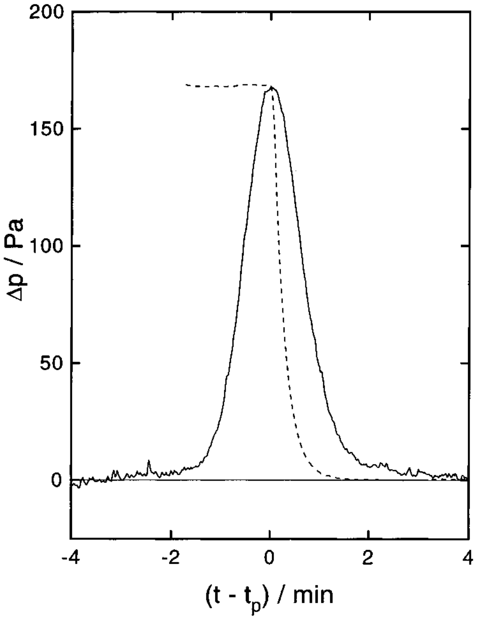

Figure 6. Osmometer time response to steplike pressure perturbation (dashed line) as compared to the SEC peak.

a time resolution of $\Delta t = V_d/\phi = 1.5$ s, which is well below the osmometer response time.

The peak broadening caused by the finite response time can be seen in Figure 5, where the RI peak for $M_n = 5250$ g/mol is slightly narrower than the corresponding osmometric one. To account for the retardation effect the measured pressure signal $\Delta p(t)$ is written as

$$\Delta p(t) = \frac{1}{\tau}\int_{-\infty}^{t} \mathrm{d}t'\, \Delta p^{o}(t')\mathrm{e}^{-(t-t')/\tau} \qquad (4)$$

Δp^o is the true osmotic pressure, as would be measured with an infinitely fast osmometer. In Figure 7 the expected ideal pressure signal for the measurement in Figure 5 has been calculated from the concentration signal as $\Delta p^o(t) \propto C(t)/M(t)$. $M(t)$ is the SEC calibration curve. The expected pressure signal, $\Delta p(t)$, has been obtained by incorporating the memory effect according to eq 4. The result is plotted together with the measured pressure signal. Both curves coincide almost perfectly.

The insert in Figure 7 shows an enlargement of the low molar mass peak. In addition to the main part of the figure, the calculated peak prior to the convolution, $\Delta p^o(t)$, is also plotted to illustrate the peak broadening due to the memory effect.

Summary and Conclusion

A membrane osmometer based on a concentric design with a capillary-shaped membrane has successfully been applied to absolute number sensitive SEC online detection. The cylinder symmetry and stiffness of the osmometer and the favorable properties of the membrane are ideally combined to meet the requirements for online detection: low cell volume, short response time, and low molar mass cutoff. The instrument has been tested in both batch and continuous flow operation with PS standards and toluene as solvent, and a good quantitative agreement with the nominal molar masses has been found.

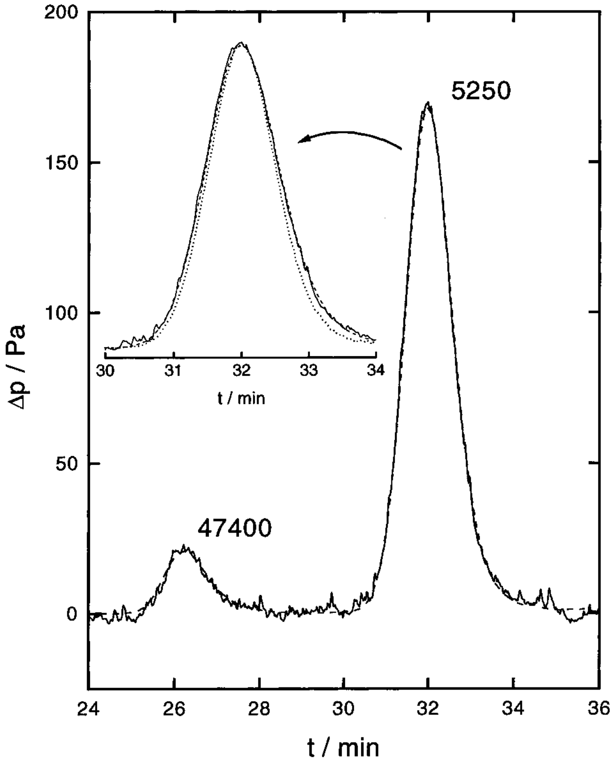

Figure 7. Osmotic pressure signal $\Delta p(t)$ as measured (solid line) and as calculated from the concentration (RI) signal, the SEC calibration curve, and the response time τ (dashed line). The insert shows an enlargement of the peak at $t = 32$ min together with the signal calculated without accounting for the memory effect (dotted line).

While the response time is very short when compared to standard membrane osmometers, it still causes a slight peak broadening, and a further reduction would require a stiffer pressure sensor. More work needs to be done for a reduction of the pressure noise level, which strongly exceeds the noise in the corresponding concentration signal, and the elimination of long term baseline drifts of unknown origin. Ideally, the polymer concentration would be measured inside the osmometer to eliminate the time offset between the two detectors. One possible way to do so would be the incorporation of the flow cell into one arm of an interferometric RI detector as used for refractive index increment measurements.[11]

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