

# polymer reports

## Analytical ultracentrifugation with the new Optima XL-A and its digital u.v./vis. detector: determination of molar mass distribution of polymers from sedimentation velocity

J.-H. Kehrhahn and M. D. Lechner\*

*Physikalische Chemie, Universität Osnabrück, Barbarastrasse 7, D-4500 Osnabrück, Germany*

and W. Mächtle

*Kunststofflaboratorium, Polymerphysik, BASF AG, D-6700 Ludwigshafen, Germany*

*(Received 26 October 1992; revised 20 January 1993)*

The determination of average molar masses and molar mass distributions (MMDs) of polymers and polyelectrolytes is demonstrated with the new analytical ultracentrifuge Optima XL-A and its very sensitive digital u.v./vis. detector (180–800 nm continuously) from Beckman Instruments, Inc. The machine was tested with the narrow distribution polystyrenes PS 106 000 and 300 000 (and a 50:50 mixture), sodium poly(styrene sulfonate) NaPSS 100 000 and the broad distribution polystyrene NBS 706. Full details of the calculation of average molar masses and MMDs of the polymers from the experimental values are given. The results show that the new absorption optics enable the above-mentioned quantities to be determined as the absorption optics are able to measure at very low concentrations, which allows either a precise extrapolation to zero concentration or averaging of the data. Owing to the fact that a sedimentation run is a real fractionation process (with a higher resolution than size exclusion chromatography) one measures the real MMD. There is no need to presume a special model MMD to analyse the measurement data. The data from the Optima XL-A are accessible as ASCII-files. As the original Beckman computer programs are not suitable for our purposes, we have developed our own programs, which are available on request.

(Keywords: ultracentrifugation; molar mass distribution; sedimentation velocity)

### INTRODUCTION

In recent years the analytical ultracentrifuge (AUC) has become of major interest for certain applications. For complicated polymers and biopolymers such as polyelectrolytes, polymers with high molar masses and aggregated biopolymers the AUC has many advantages compared with scattering methods, osmotic pressure and size exclusion chromatography (s.e.c.). Beckman Instruments, Inc. now offers a new modern AUC with highly sensitive digital u.v./vis. absorption optics (180–800 nm continuously); this instrument, the Optima XL-A, follows the famous Spinco model E. Compared to the model E the most evident advantages of the new Optima XL-A are that all distances from the centre of rotation are obtained absolutely, very low concentrations (a factor of 10 or smaller) may be used, and control of the machine, data acquisition and handling is done by a computer. Nevertheless, it should be kept in mind that the polymer and solvent must be suitable for u.v. absorption in the wavelength region used. In many cases it is advantageous to register complete u.v./vis. spectra of dissolved polymer inside the measuring cell of a spinning AUC rotor.

The purpose of this paper is to present some initial sedimentation velocity measurements with the new machine, and to demonstrate that the AUC is a powerful tool for the determination of molar masses

and molar mass distributions (MMDs) of polymers, especially polyelectrolytes and bipolymers. Sedimentation equilibrium measurements with the Optima XL-A (where we need a model MMD to calculate the MMD) are presented in a previous paper<sup>1</sup>.

### BASIC CONSIDERATIONS

The variation of the concentration  $c_i$  of component  $i$  with time  $t$  at distance  $r$  and angular velocity  $\omega$  may be expressed by Lamm's differential equation<sup>2,3</sup>:

$$\left(\frac{\partial c_i}{\partial t}\right)_r = \frac{1}{r} \frac{\partial}{\partial r} \left[ r \sum_{j=1}^q D_{ij} \frac{\partial c_j}{\partial r} - s_i \omega^2 r^2 c_i \right] \quad i = 1, 2, \dots, q \quad (1)$$

Equation (1) holds for one component  $i$  of the polymer in a multicomponent system consisting of a solvent and polydisperse polymer, where  $D_{ij}(\partial c_j / \partial r)$  must be summed over all components  $j = 1, 2, \dots, q$ . Therefore one has to solve a set of  $q$  differential equations for polydisperse polymers. Further restrictions of Lamm's equation are that the solvent must be incompressible and at thermal equilibrium. The variables in equation (1) are:  $c_i$  = volume concentration of component  $i$  in mass/volume;  $t$  = time;  $r$  = distance of component  $i$  from centre of rotation;  $D_{ij}$  = cross term diffusion coefficients ( $i \neq j$ );  $D_{ij}$  = diffusion coefficient of component  $i$  ( $i = j$ );  $s_i$  = sedimentation coefficient of component  $i$ ;  $\omega$  = angular velocity.

\* To whom correspondence should be addressed

The sedimentation coefficient  $s$  is defined as:

$$s = \frac{dr/dt}{\omega^2 r} = \frac{\ln(r/r_m)}{\int_0^t \omega^2 dt} \quad (2)$$

where  $r_m$  is the radial distance of the meniscus and  $r$  is the radial distance of a specified point in the ultracentrifuge cell.

One of the major advantages of the Optima XL-A compared to the model E is the precise integration of  $\int \omega^2 dt$  and determination of  $s$  with respect to equation (2). Most theories ignore the fact that the machine drive of the ultracentrifuge needs a certain time to accelerate the rotor from zero to the operational speed. In practice this was previously taken into account by shifting the time  $t$  to a time  $t-t_0$ , where  $t$  is the time when the rotor is set in motion and  $t_0$  is the zero time correction; the angular velocity  $\omega$  is then taken to be constant:  $s = \ln(r/r_m)/[\omega^2(t-t_0)]$ . The exact integration of  $\int \omega^2 dt$  with variable  $\omega$  in the start-up phase allows a precise sedimentation coefficient to be calculated. Auxiliary equations which include a zero time correction  $t_0$  (e.g. the method of Blair and Williams<sup>4</sup> and Stickler<sup>5</sup>) are no longer necessary.

Furthermore, ultracentrifugation involves a pressure gradient from 0.1 MPa at the meniscus to 20.0 MPa at the bottom (depending on the angular velocity of the rotor) of the cell. Therefore changes of viscosity, density, concentration and partial specific volume of the polymer system along the cell have to be taken into account. The pressure dependence of  $s$  may be recognized by the fact that the plateau zone exhibits a positive slope (see Figures 1a, 3a and 4a). In most cases it is sufficient to assume a linear dependence of the sedimentation coefficient  $s$  with pressure<sup>6</sup>:

$$s = s_0(1 - \mu p) \quad (3)$$

with  $p = 1/2\omega^2 r_m^2 \rho_0^0 [(r/r_m)^2 - 1]$ , where  $s_0$  is the sedimentation coefficient at zero pressure,  $\mu$  is a constant depending on the polymer-solvent system,  $p$  is the pressure and  $\rho_0^0$  is the density of the solvent at  $p=0.1$  MPa.

The determination of the complete molar mass distribution from sedimentation measurements requires the dependence of  $s$  from molar mass,  $M$ :

$$s(M) = K_s M^{a_s} \quad (4)$$

As the absorption optics of the Optima XL-A measure directly quantities proportional to the polymer concentration of the sedimentation boundary  $c$  at distance  $r$  and time  $t$ , the apparent integral distribution of  $s$  and  $D$ ,  $G''(s, D, c)$ , is directly available:

$$G''(s, D, c) = \frac{c}{c_0} \left( \frac{r}{r_m} \right)^2 \quad (5)$$

The term  $r/r_m$  is due to the dilution rule:

$$\frac{c}{c_0} = \left( \frac{r_m}{r} \right)^2 \quad (6)$$

where  $c_0$  denotes the initial concentration, and  $c$  the concentration at the sedimentation boundary, at radius position  $r$ .

Extrapolation of  $G''(s, D, c)$  to  $t \rightarrow \infty$  at constant  $s$  values

eliminates the influence of diffusion<sup>7</sup>:

$$\lim_{t \rightarrow \infty} [G''(s, D, c)]_s = G'(s, c) \quad (7)$$

It should be noted that extrapolation to infinite time is usually done<sup>7</sup> by extrapolating  $1/r^2 t \rightarrow 0$  or  $1/t \rightarrow 0$  (ref. 8). According to an idea of Ortlepp and Stickler<sup>9</sup>, we performed this extrapolation by plotting  $G''(s, D, c)$  at constant  $s$  values versus  $1/t^2$ .

For concentrations far from infinite dilution an extrapolation of  $G'(s, c)$  to  $c \rightarrow 0$  eliminates the influence of concentration:

$$\lim_{c \rightarrow 0} G'(s, c) = G(s) \quad (8)$$

For polymers whose  $M$  and  $s$  are related (i.e. by equation (4)) the differential molar mass distribution  $w(M)$  is connected with the differential sedimentation coefficient distribution  $g(s)$  via  $w(M)dM = g(s)ds$ . Integration leads to:

$$W(M) = G(s) \quad (9)$$

with  $w(M) = dW(M)/dM$ ,  $g(s) = dG(s)/ds$  and the average molar masses<sup>10</sup>:

$$M_\beta = \frac{\left[ \int_0^\infty w(M) M^\beta dM \right]}{\left[ \int_0^\infty w(M) M^{\beta-1} dM \right]} \quad \beta = 0, 1, 2, \dots (n, w, z, \dots) \quad (10)$$

The relation between the absorption  $A = \lg(I_0/I)$  and the concentration is given by Lambert-Beer's law:

$$A = \lg\left(\frac{I_0}{I}\right) = \varepsilon lc \quad (11)$$

Here  $\varepsilon$  denotes the specific decadic absorption coefficient and  $l$  the thickness of the cell.  $\varepsilon$  is either given in literature or may be calculated with the help of the law of conservation of mass:

$$\int_{r_m}^{r_b} 2rc(r)dr = c_0(r_b^2 - r_m^2) \quad (12)$$

Combination of equations (11) and (12) gives:

$$\varepsilon = \left[ \frac{1}{lc_0(r_b^2 - r_m^2)} \right] \int_{r_m}^{r_b} 2rA dr \quad (13)$$

As  $c_0$ ,  $l$  and  $\int_{r_m}^{r_b} 2rA dr$  are given experimentally,  $\varepsilon$  may be determined from sedimentation runs with absorption optics. The absorption optics of the Optima XL-A are not able to measure in the vicinity of the meniscus and bottom; therefore the determination of  $\varepsilon$  according to equation (13) is not very exact. Another possibility for the calculation of  $\varepsilon$  is the relation between the concentration and distance of the sedimentation boundary at the plateau region  $c_p$  and  $r_p$  and the initial concentration  $c_0$  (equation (6)):

$$c_p = c_0 \left( \frac{r_m}{r_p} \right)^2 \quad (14)$$

Introduction of equation (11) into equation (14) gives:

$$\varepsilon = \frac{A(r_p)}{lc_0(r_m/r_p)^2} \quad (15)$$

$\varepsilon$  depends strongly on the selected u.v. wavelength  $\lambda$ .

The procedure for determination of the MMD with the digital u.v./vis. absorption optics is in principle as follows: the primary experimental values of  $A=f(r)$  measured at different times and concentrations give the apparent sedimentation coefficient distribution  $G''(s, D, c)$  according to equations (2), (5) and (11). In the case of cyclohexane as solvent the  $s$  values are pressure-corrected according to equation (3) using a  $\mu$  parameter from literature<sup>11</sup>. Extrapolation of  $G''(s, D, c)$  to  $1/t^2 \rightarrow 0$  and  $c_0 \rightarrow 0$  according to equations (7) and (8) results in obtaining  $G(s)$ . From the  $sM$  relation (equation (4)) the integral MMD  $W(M)$  is directly obtained according to equation (9). Differentiation of  $W(M)$  with respect to  $M$  leads to  $w(M)$  and the average molar masses  $M_n$ ,  $M_w$  and  $M_z$  according to equation (10).

As the absorption optics of the Optima XL-A are able to measure at very small concentrations compared with the schlieren and interference optics, the absorption optics give a much better opportunity to determine the MMD of polymers, because solute-solute interactions are diminished.

## EXPERIMENTAL

The following well characterized nearly monodisperse and broad distribution polymer samples were used ( $1 \text{ sved} = 1 \times 10^{-13} \text{ s}^{-1}$ ):

1. Polystyrene PS 106 000 (Polymer Standard Service, Mainz, Germany, lot no. PS 61126);  $M_w = 106\,000 \text{ g mol}^{-1}$ ,  $M_w/M_n \leq 1.06$ . The  $sM$  relation is given in literature<sup>12</sup>:  $s_0/\text{sved} = 0.01343 \times M^{0.5}$  (25°C, cyclohexane).
2. Polystyrene PS 300 000 (Polymer Standard Service, Mainz, Germany, lot no. 00507);  $M_w = 300\,000 \text{ g mol}^{-1}$ ,  $M_w/M_n \leq 1.06$ .
3. Polystyrene NBS 706 (National Bureau of Standards, Washington, DC, USA);  $M_n = 136\,000 \text{ g mol}^{-1}$ ,  $M_w = 258\,000\text{--}288\,000 \text{ g mol}^{-1}$ ,  $M_z = 355\,000\text{--}400\,000 \text{ g mol}^{-1}$ ,  $M_w/M_n = 1.9\text{--}2.1$ .
4. Sodium poly(styrene sulfonate) NaPSS 100 000 (Polymer Standard Service, Mainz, Germany, lot PSS 25, dialysed);  $M_w = 100\,000 \text{ g mol}^{-1}$ ,  $M_w/M_n \leq 1.10$ . The  $sM$  relationship was determined by own measurements:  $s_0/\text{sved} = 0.024 \times M^{0.46}$  (25°C, 0.5 M NaCl).

For the first three samples (and a 50:50 mixture of PS 100 000 and 300 000) cyclohexane (pro analysis) was used as solvent; the last sample was dissolved in 0.5 M NaCl.

All experiments were executed with the new analytical ultracentrifuge Optima XL-A. The instrument, which has been operating in our laboratory since November 1991, has a xenon flash light source and a holographic grating monochromator with a wavelength range  $\lambda = 180\text{--}800 \text{ nm}$ . The flash lamp illuminates only the selected sample during scanning. The analytical rotor can receive up to three cells and a counterbalance. Double-sector cells (12 mm) are used for the measurements reported, with solvent in one sector and solution in the other sector. The light passing through the cells is detected by a shiftable slit and a photomultiplier in the form of two pulses  $I$  and  $I_0$ , which are immediately digitized and given on the computer screen as absorption  $A = \lg(I_0/I)$  as function of  $r$ . The temperature of the rotor is controlled by an i.r. sensor within 0.5°C. Control of the entire process and data input/output is done by a computer.

The measurements were performed at three concentrations and a rotor speed of  $40\,000 \text{ rev min}^{-1}$ . The

polymer concentrations were very small, i.e.  $c = 0.1, 0.2$  and  $0.3 \text{ g l}^{-1}$ . Measurements were made at 25°C.

The data from the Optima XL-A are accessible as ASCII-files. As the original Beckman programs are not suitable for our purposes we developed our own programs. These programs are available on request. All data evaluation was performed by computer program in the following way.

1. The experimental values  $A(r)$ , obtained from the Optima XL-A as ASCII-files, are smoothed by a special regression spline procedure and the sedimentation boundaries and the plateau zone are interactively determined.
2. The  $A$  values at the beginning of the sedimentation boundary are set to zero because excess absorption sometimes occurs.
3. The dilution rule is applied to the data.
4.  $\varepsilon$  is calculated from  $A(r_p)$  according to equation (15).
5. With the previously determined  $\varepsilon$ ,  $c(r)$  is calculated according to equation (11).
6. The integral distribution function  $G''(s, D, c)$  is calculated according to equation (5).
7. Extrapolation to infinite time according to equation (7) is done by plotting  $G''(s, D, c)$  at constant  $s$  values versus  $1/t^2$  to obtain  $G'(s, c)$  by linear regression.
8. Instead of extrapolation to  $c=0$  according to equation (8), the  $G'(s, c)$  curves are averaged to obtain  $G(s)$ . Extrapolation to  $c=0$  is avoided for three reasons: (a) due to the experimental errors, three points (corresponding to three concentrations) contain too little information to perform a reliable extrapolation; (b) because of the low concentrations, the solute interactions should be very small and the extrapolation distance is short, therefore  $G(s)$  is expected to be very near to  $G'(s, c)$ ; (c) polystyrene samples were studied in cyclohexane at 25°C — under these conditions the solute is solved monomolecularly and the sedimentation coefficient is independent of concentration<sup>12,13</sup>.
9. The integral and differential MMDs  $W(M)$ ,  $w(M)$  and the average molar masses are calculated and plotted according to equations (4), (9) and (10) using known  $sM$  relationships.

## RESULTS AND DISCUSSION

Figures 1 and 2 show the data acquisition and evaluation of the Optima XL-A with the polymer system PS 106 000/cyclohexane at 25°C for three concentrations (0.2, 0.4 and  $0.6 \text{ g l}^{-1}$ ), measured simultaneously in one run to obtain the MMD. Figure 1a shows the absorption  $A$  at  $\lambda = 257 \text{ nm}$  as a function of the distance  $r$  at concentration  $c = 0.2 \text{ g l}^{-1}$  and at different scanning times; the small points are the experimental values and the dashed lines represent the smoothed curves obtained by a special spline procedure. In Figure 1b the solid lines represent the apparent  $G''(s, D, c)$ , obtained by equation (5) for different scanning times. To eliminate the time-dependent diffusion broadening from each curve the  $G''(s, D, c)$  values — at constant  $s$  values between  $s_{\min}$  and  $s_{\max}$  in 100  $\Delta s$  substeps — are taken and plotted versus  $1/t^2$  (Figure 2). The diffusion corrected distribution curve  $G'(s, c)$  is obtained by linear extrapolation to infinite time ( $1/t^2 \rightarrow 0$ ). The dashed curve in Figure 1b shows the result for one concentration ( $c = 0.2 \text{ g l}^{-1}$ ) and Figure 1c demonstrates all  $G'(s, c)$  curves at concentrations 0.2, 0.4

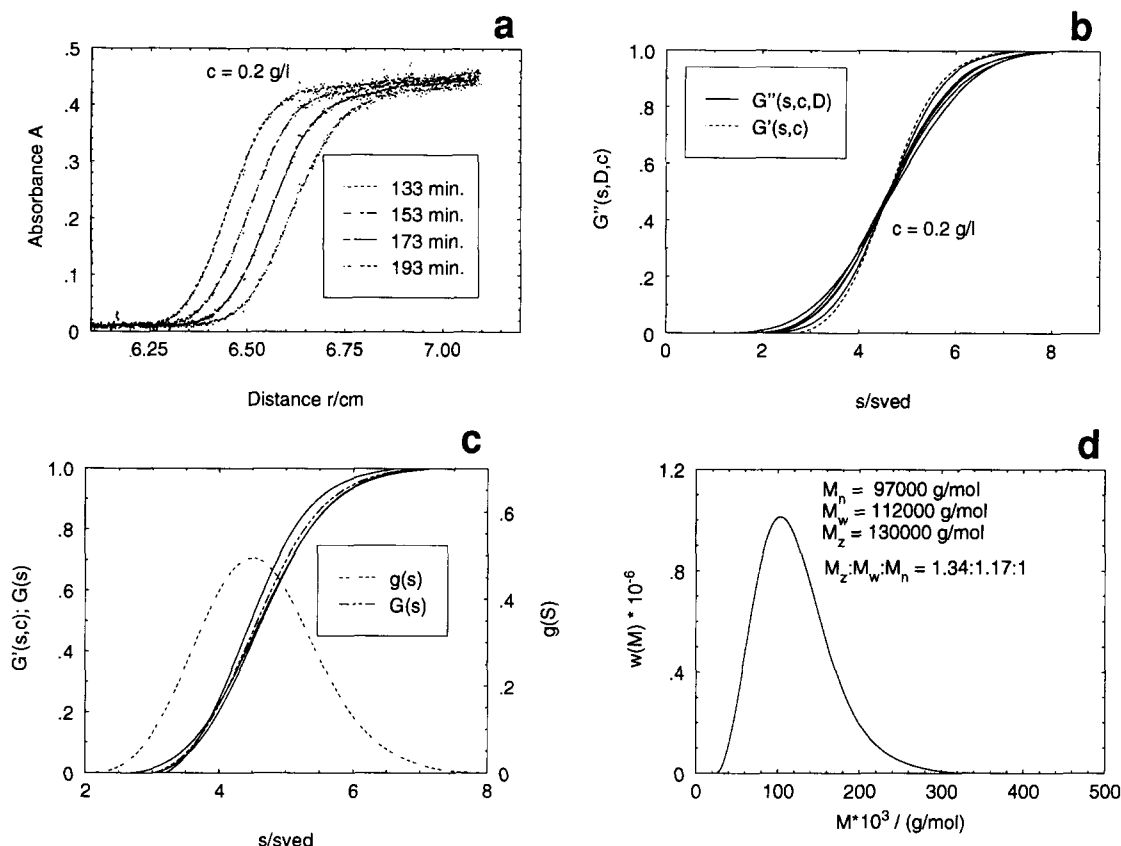


Figure 1 Plots of (a)  $A-r$ ; (b)  $G''(s, c, D)$ ,  $G'(s, c)$ ; (c)  $G'(s, c)$ ,  $G(s)$ ,  $g(s)$ ; (d)  $w(M)=f(M)$ , for PS 106 000/cyclohexane.  $\omega = 40\,000 \text{ min}^{-1}$ ,  $\lambda = 257 \text{ nm}$

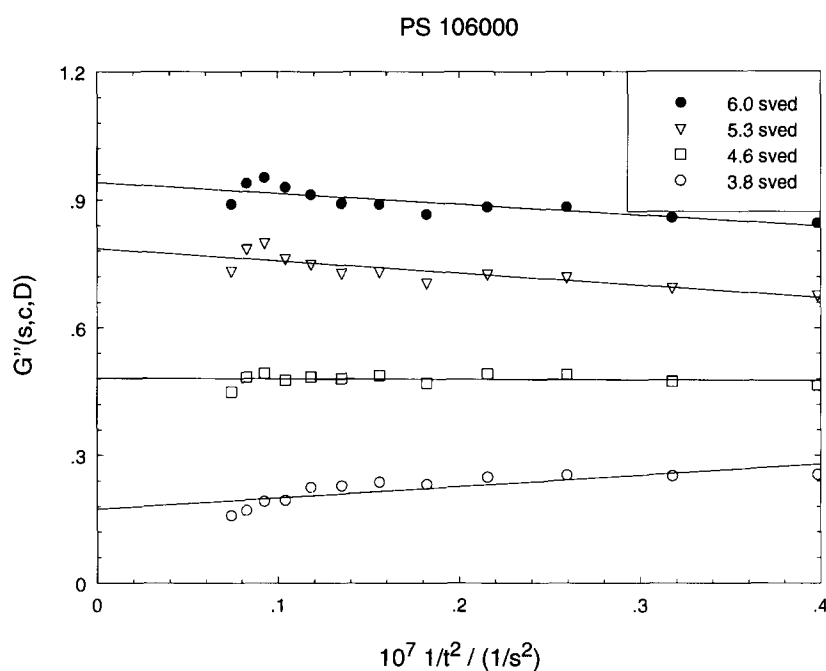
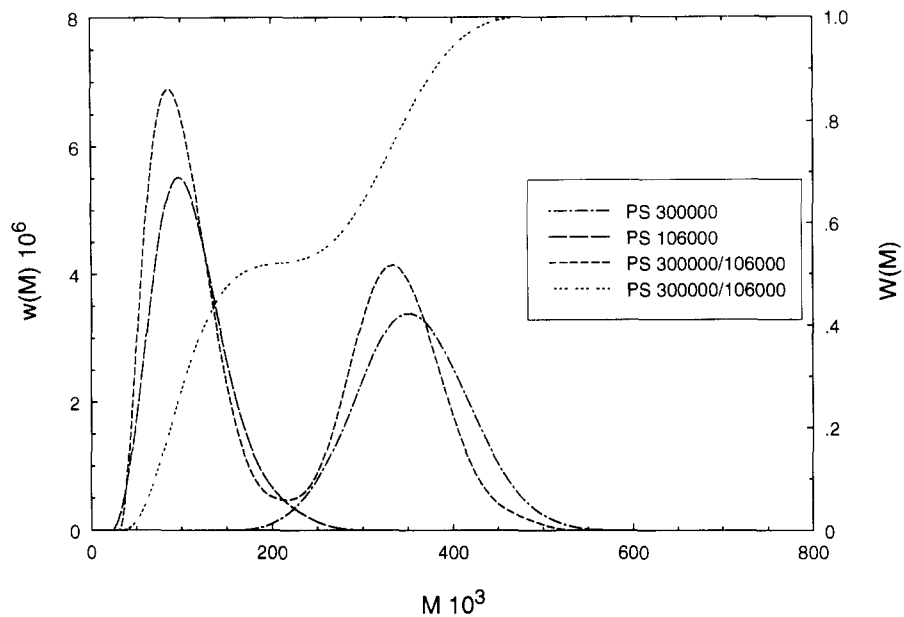


Figure 2 Extrapolation of  $G''(s, c, D)$  to  $t \rightarrow \infty$  by plotting  $G''(s, c, D)$  versus  $1/t^2$  for PS 106 000/cyclohexane

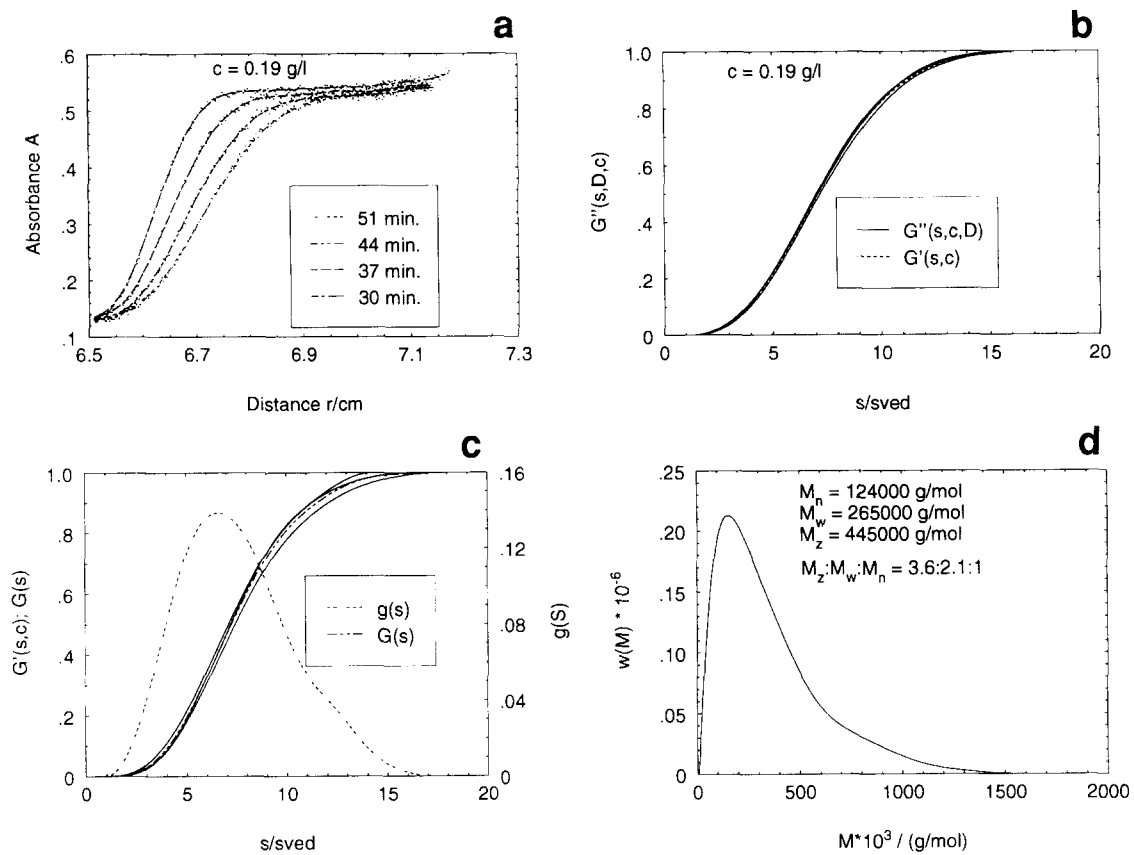
and  $0.6 \text{ g l}^{-1}$  as solid lines. From these curves,  $G(s)$  and their derivatives  $g(s)$  are calculated according to equations (8) and (9). Finally Figure 1d demonstrates the differential MMD function. The measurement exhibits the following values:  $M_z = 130\,000 \text{ g mol}^{-1}$ ,  $M_w = 112\,000 \text{ g mol}^{-1}$  and  $M_n = 97\,000 \text{ g mol}^{-1}$ ;  $M_z:M_w:M_n = 1.34:1.17:1$ . These values are in reasonable agreement with the values given by Polymer Standard Service for PS 106 000.

Figure 3 shows the differential MMDs of two single-component measurements (PS 106 000 and PS 300 000), multiplied by the factor 0.5 and the differential and integral MMD of a 50:50 mixture of both components.

Figures 4 and 5 demonstrate the same procedure for the systems polystyrene NBS 706/cyclohexane and NaPSS 100 000/0.5 M NaCl. In spite of measuring with



**Figure 3** Plots of the differential MMDs of PS 106000 and PS 300000 (normalized to 0.5) and the differential and integral MMD of a 50:50 mixture of both components.  $\omega = 40\,000 \text{ min}^{-1}$ ,  $\lambda = 257 \text{ nm}$



**Figure 4** Plots of (a)  $A-r$ ; (b)  $G''(s, c, D)$ ,  $G'(s, c)$ ; (c)  $G'(s, c)$ ,  $G(s)$ ,  $g(s)$ ; (d)  $w(M)$ , for NBS 706/cyclohexane.  $\omega = 40\,000 \text{ min}^{-1}$ ,  $\lambda = 260 \text{ nm}$

a double-sector cell, *Figure 4* exhibits excess absorptions; further work by the manufacturer and users of the machine is required to overcome these difficulties. Nevertheless the results in *Figures 4* and *5* again show a reasonable agreement between our AUC  $M$  values and the values given by Polymer Standard Service.

As mentioned previously, very small concentrations of the polymer may be used with u.v./vis. optics in comparison to schlieren or interference optics.

Several experiments demonstrate that the Optima XL-A needs concentrations which are a factor of 10 or smaller than needed by the model E.

## CONCLUSIONS

A few remarks on the new XL-A machine should be made.

1. The measurements often exhibit noisy absorption values. We recognized that often only  $I_0$  is noisy while

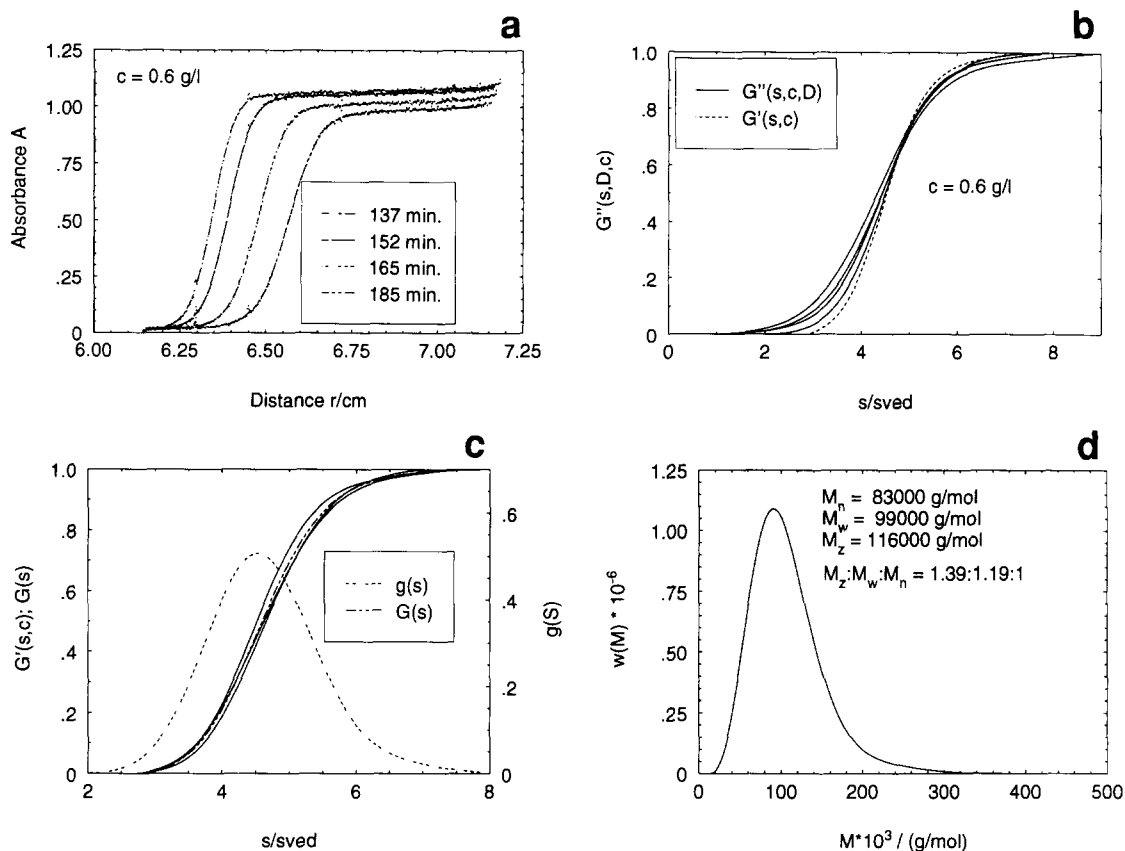


Figure 5 Plots of (a)  $A-r$ ; (b)  $G''(s, c, D)$ ,  $G'(s, c)$ ; (c)  $G'(s, c)$ ,  $G(s)$ ,  $g(s)$ ; (d)  $w(M) = f(M)$ , for NaPSS 100 000/0.5 M NaCl.  $\omega = 40\,000\text{ min}^{-1}$ ,  $\lambda = 260\text{ nm}$

$I$  is available in much better quality (the machine enables separate values of  $I$  and  $I_0$  to be obtained only from the last mapping of a sedimentation experiment). Obviously, data smoothing of  $I_0$  would be less likely to introduce error than smoothing of  $\lg(I_0/I)$ .

- The regions near  $r_m$  and  $r_b$  often show physically meaningless absorption traces. Extrapolation to  $r_m$  and  $r_b$  and calculation of  $\varepsilon$  by using the law of conservation of mass is not sufficiently precise.
- To obtain enough information within the sedimentation boundary it is necessary to choose maximum resolution. The data acquisition requires 3–10 min for each scan. Since  $t$  and  $\int \omega^2 dt$  are only captured by the machine at the beginning of the measurement, and since polymer sedimentation proceeds while data acquisition is running (especially in the case of high  $M$  values), the calculated  $M_w/M_n$  values are, in almost all cases, a little too broad. The scan time needs to be decreased considerably.
- The programming of the rotor acceleration is only possible in steps of  $100\text{ rev min}^{-1}$ . We would prefer a continuous smooth increase of the rotor speed up to the final speed.
- The XL-A four-hole rotor allows the measurement of only three samples simultaneously. For economic reasons we would prefer eight-hole rotors, which are standard since 1979<sup>14,15</sup>.

A great advantage of the Optima XL-A and its u.v./vis. scanner is that they are fully computerized. In comparison to the old model E, an MMD measurement with the XL-A is very quick. In particular there is no tedious evaluation of photoplates or scanner charts. The complete

MMD is ready and plotted a few minutes after the XL-A run is finished.

This first study of synthetic polyelectrolytes and polymers with the Optima XL-A demonstrates that one is able to determine average molar masses and MMDs of polymers and polyelectrolytes in solution. The highly sensitive u.v./vis. detector allows absorption measurements at very low concentrations.

#### ACKNOWLEDGEMENT

Support of this work by BASF AG, Ludwigshafen and by Fonds der Chemischen Industrie is gratefully acknowledged.

#### REFERENCES

- Lechner, M. D. and Mächtle, W. *Makromol. Chem., Rapid Commun.* 1992, **13**, 555
- Fujita, H. 'Mathematical Theory of Sedimentation Analysis', Academic Press, New York, 1962
- Fujita, H. 'Foundations of Ultracentrifugal Analysis', Wiley, New York, 1975
- Blair, J. E. and Williams, J. W. *J. Phys. Chem.* 1964, **68**, 161
- Stückler, M. *Angew. Makromol. Chem.* 1984, **123/124**, 85
- Oth, J. and Desreux, V. *Bull. Soc. Chim. Belges* 1954, **63**, 133
- Schachmann, H. K. 'Ultracentrifugation in Biochemistry', Academic Press, New York, 1959
- Scholtan, W. *Makromol. Chem.* 1960, **36**, 162
- Ortlepp, B. and Stückler, M. Lecture at Dechema meeting 'Polyreaktionen', 19 January 1982
- Brandrup, J. and Immergut, E. H. (Eds) 'Polymer Handbook', Wiley, New York, 1989
- Tung, L. H. and Runyon, J. R. *J. Appl. Polym. Sci.* 1973, **17**, 1589
- Scholte, Th. G. *Eur. Polym. J.* 1970, **6**, 51
- Cantow, H. J. *Makromol. Chem.* 1959, **30**, 170
- Mächtle, W. and Klodwig, U. *Makromol. Chem.* 1979, **180**, 2507
- Mächtle, W. *Prog. Colloid Polym. Sci.* 1991, **86**, 111