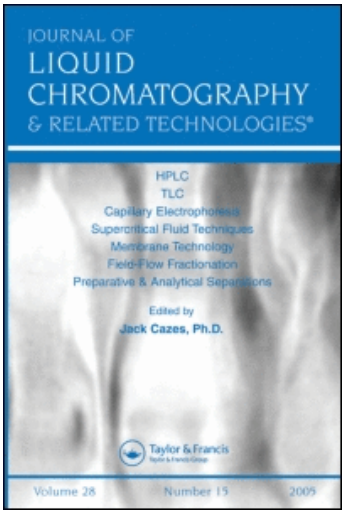

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SIMULATION OF SIZE EXCLUSION CHROMATOGRAMS FROM VISCOMETRY AND REFRACTOMETRY DETECTORS: AN ANALYSIS OF THE INFLUENCE OF CONCENTRATION ERRORS ON RELIABILITY OF ESTIMATED PARAMETERS

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ABSTRACT

Size exclusion chromatograms of polymer samples of known molecular weight distribution (MWD), in solutions of known Mark–Houwink constants, as obtained from viscometry and refractometry detectors, have been simulated to check the influence of some errors in the solution concentration on the evaluation of the sample molecular characteristics and of the solution properties. It has been found that concentration errors arising from uncorrect estimate of the weight of injected polymer yield proportional errors on the average molecular weights and the Mark–Houwink K constant, but do not affect the MWD and the a exponent. Concentration errors arising from uncorrect estimate of the difference between the times occurring to identical macromolecules to reach the detectors strongly modify the MWD, with consequences on the evaluation of both molecular characteristics and Mark–Houwink constants, and give rise to curvatures in the Mark–Houwink plots, thus suggesting a check, during the treatment of the chromatograms, of the reliability of the time difference estimate.

INTRODUCTION

The diffusion of molecular weight sensitive detectors, such as those base on low-angle-laser-light-scattering (LALLS) and on differential viscometry (DV combined with a concentration detector, is one of the most important developments c SEC in recent years. The LALLS detectors give an absolute measure of the molecula weight at each retention volume, whereas the DV ones allow to evaluate the intrinsi viscosity [η] at each retention volume, from which the molecular weight can b obtained if the universal calibration procedure(1) can be applied. Obviously, this la condition seems to suggest a superiority of the LALLS detectors. However, the D detectors are more useful for copolymers, are many times cheaper, and make use of th same relation, the Mark-Houwink equation, on which the universal calibration procedu is based. Furthermore, as it has been reported(2,3) and will be shown later in this pape the treatment of the data from a DV detector allows, in principle, to evaluate K and a they are not known, or to check them if they come from Literature.

Both LALLS and DV detectors, as well as any other type of detectors, hav inherent drawbaks which must be taken into account in order to get correct averag molecular weights and molecular weight distributions (MWDs). However, the need c combining them with a concentration sensitive detector, frequently one based on th measure of the differential refractive index (DRI), introduces new sources of possibl errors.

In order to get either molecular weights or intrinsic viscosities at each retentio volume, it is necessary to compare the signals from DRI detector with those from eith LALLS or DV detectors *at corresponding elution times*, i.e. the times required fc identical macromolecules to reach the different detectors. If such transport times a uncorrectly estimated, concentration errors will result, with likely consequences on th evaluation of both molecular weights and MWDs. The estimate of the shift time, that

the difference between the transport times, can be performed through the procedure suggested by Lesec[4], based on the elution of a narrow MWD standard excluded from the SEC pore volume. It will be shown later that the shift time can be directly checked in the treatment of the chromatograms from DV and DRI detectors.

Another type of concentration errors arises if the weight of injected macromolecules is not exactly known. When a concentration detector is used by alone in a SEC run, the ordinates of the chromatogram are proportional to the polymer concentration in the solutions eluting at each abscissa value, but the knowledge of such a concentration is unnecessary for the data treatment. On the other hand, when the chromatogram from a concentration detector must be combined with that from either LALLS or DV detector, the concentration of the solution at each elution volume must be known, through the precise knowledge of the concentration and the volume of the injected solution.

In order to see the effects of different errors on the parameters which can be evaluated by SEC with combined DRI and DV detectors, chromatograms were simulated for polymers of known MWDs in solutions of known Mark-Houwink constants, and then used to recalculate both MWDs and Mark-Houwink constants.

SIMULATIONS

The weight fractions of polymer samples were calculated according to the Schulz-Zimm MWD:

$$w(M) = \frac{(k/M_n)(k+1)}{\Gamma(k+1)} M^k \exp\left(-k\frac{M}{M_n}\right) \qquad (1)$$

where w(M) is the weight fraction of molecules with molecular weight M, M_n is the number average molecular weight and k is related to the breath of the distribution: two sets of w(M) were calculated, with either k = 10 (narrow distribution) or k = 1 (most

probable distribution). The M values were obtained, in a suitable time interval Δt at tim increments of 0.05 min, from the hypothetical universal calibration curve:

$$\log([\eta]M) = \log(K M^{a+1}) = 14.1 - 0.408\ t \quad (2$$

where K and a are the Mark–Houwink constants: for each distribution, two pairs c constants, namely:

$$K = 2.68 \cdot 10^{-2} \qquad a = 0.5$$

$$K = 1 \cdot 10^{-2} \qquad a = 0.7$$

where employed, typical of ideal and good solvent systems respectively.

The characteristics of the polymer samples are collected in Table I. From thes samples chromatograms, as obtained from DRI detector, were simulated by assuming constant flow rate during the SEC run (it is supposed that flow rate fluctuations as thos described by Lesec(5) have been eliminated), by converting at each time $t_i = t_0 + 0.05$ (where t_0 is the time at which the chromatographic peak begins and n is the number c time increments) the weight fractions into the weight of polymer eluting at t_i, an multiplying the weight by 0.05. Therefore, the detector signal was expressed i conventional surface units, in such a way that $5 \cdot 10^{-4}$ g of polymer (the total weigl injected), multiplied by Δt, gave a peak area of $7 \cdot 10^6$ conventional units.

The chromatograms, as obtained from DV detector, were simulated by converting th specific viscosity (i.e. the product of the intrinsic viscosity by the polymer weight) in th

Table I – Average molecular weights, dispersity index and intrinsic viscosity of th polymer samples in ideal solutions (S5) and in the good solvent (S6).

Sample	M_n	M_w	M_z	M_w/M_n	$[\eta]$ ml/g
S5.NRW	98458	108304	118134	1.10	8.72
S5.BRD	98626	196951	295418	2.00	11.18
S7.NRW	98638	108492	118328	1.10	33.16
S7.BRD	98820	197297	295912	2.00	48.39

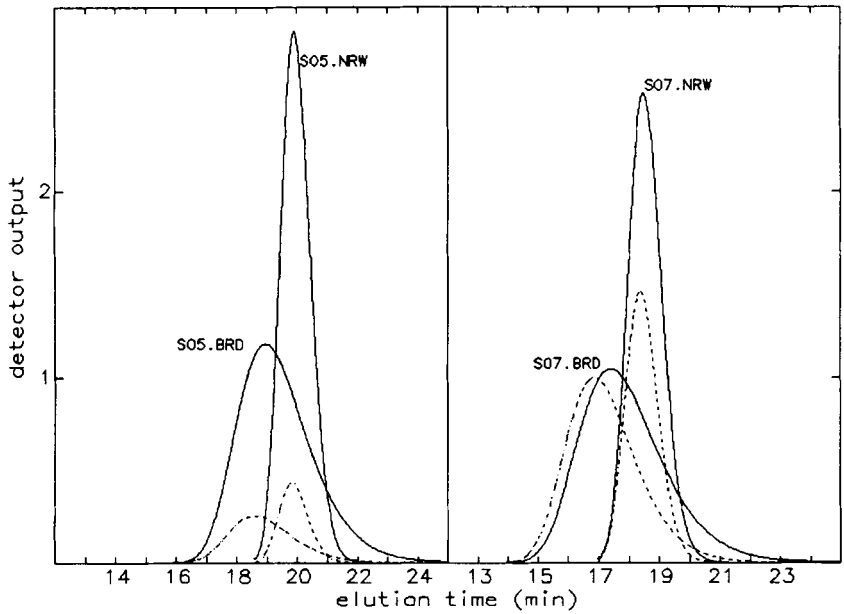

Figure 1 – Simulated chromatograms of the samples of Table I, as obtained from a DRI detector (solid lines) and a DV detector (broken lines). On the left the polymer solutions are ideal; on the right the polymers are in a good solvent.

fraction eluting at each t_i into the difference between the pressures exerted on the capillary walls by the polymer solution and the pure solvent. Also these pressures were measured in conventional surface units, corresponding to peak sections 0.05 min wide. These sections were then shifted back on the time axis of 0.15 min, to simulate that identical polymer molecules require this time to move from the DV detector to the DRI one, in a serial arrangement.

In Figure 1 are shown the simulated chromatograms for samples of Table I. It is readily evident that solutions at the same concentration of polymers with the same M_n give strongly different DV peaks, due to changes of molecular dimensions, when the thermodynamic quality of the solvent is changed. This points out that the DV detector sensitivity increases for polymer solutions in good solvents.

DISCUSSION

The chromatograms of Figure 1 were used to evaluate by usual procedures the MWD distribution of the polymer samples and the Mark–Houwink constants. They were divided in slices 0.05 min wide, and the slice surfaces were measured. For DRI chromatograms the ratio between these surfaces and the total chromatogram surface is the weight fraction w_i of the polymer eluting at each t_i; from the weight fraction, knowing the total weight of injected polymer, the concentration c_i is calculated.

The slice surfaces of the DV chromatograms are proportional to the difference $P_i - P_0$ between the pressures exerted on the capillary walls by the polymer solution eluting at each t_i and the pure solvent. Such a difference is equal to the difference $\eta_i - \eta_0$ between the viscosities of the polymer solution and of the pure solvent and can be converted into the specific viscosity $[\eta]_i c_i$ of the polymer in the solution, by applying the single point intrinsic viscosity determination suggested by Solomon[6,7]:

$$[\eta]_i c_i = \left[2\left(\frac{\eta_i - \eta_0}{\eta_0} - \ln\frac{\eta_i}{\eta_0}\right)\right]^{\frac{1}{2}} \qquad (3)$$

were η_0 is obtained from the detector output at the base line of the chromatogram.

The ratio between $[\eta]_i c_i$ from the DV chromatogram and c_i from the DRI chromatogram allows the evaluation of $[\eta]_i$ at each t_i. However, account must be taken of the time difference at which identical macromolecules reach the two detectors, and this is done by shifting the time scale of the DV chromatograms in order to sumperimpose them on the DRI chromatograms, which are supposed to have been used to evaluate the universal calibration curve eq. (2).

By introducing into eq. (2) $[\eta]_i$ and t_i one calculates M_i and by plotting M_i vs w_i the MWD is obtained, from which the average molecular weights can be evaluated Furthermore, by the Mark–Houwink plot of $\log[\eta]_i$ vs. $\log M_i$ the constants K and a can be computed. Finally, it is possible to evaluate for the original samples $[\eta] = \Sigma_i w_i[\eta]_i$.

Table II – Average molecular weights, dispersity index and intrinsic viscosity of polymer samples, as obtained from the chromatograms of Figure 1, by assuming a shift time between the detectors of 0.15 min and a weight of injected sample of $5 \cdot 10^{-4}$ g.

Sample	M_n	M_w	M_z	M_w/M_n	$[\eta]$ ml/g
S5.NRW	98469	108312	118142	1.10	8.72
S5.BRD	99534	196942	295386	1.98	11.18
S7.NRW	98664	108519	118356	1.10	33.16
S7.BRD	99593	197305	295902	1.98	48.39

The chromatograms of Figure 1 were treated according to the procedure outlined above by first assuming that $5 \cdot 10^{-4}$ g of the samples were injected in the system, and that the time required for identical macromolecules to shift from DV to DRI detectors was 0.15 min. MWDs indistinguishible from the original ones were obtained, from which the average molecular weights, the dispersity indexes and the intrinsic viscosities collected in Table II were calculated. The agreement between the data in Tables I and II simply shows the consistency of the procedure followed to simulate the chromatograms from the MWDs of the samples, and to recalculate the MWDs from the chromatograms. The slightly higher M_n values calculated for the broad S5.BRD and S7.BRD samples depend on the simulation procedure: the choice was made that the weight fractions calculated from (1) summed up to 1.000, and consequently in the simulated broad samples there is a fraction of very low molecular weight molecules which are accounted for in obtaining the M_n values in Table I, but are not detected by the differential viscometer.

By plotting $\log [\eta]_i$ vs. $\log M_i$ according to the Mark–Houwink equation, again assuming that the injected polymer weight was $5 \cdot 10^{-4}$ g and the shift time between the detectors was 0.15 min, the constants K and a collected in Table III were computed, in very good agreement with those introduced into eq. (1) to simulate the samples. This shows that the Mark–Houwink constants can be directly estimated in SEC

Table III – Mark–Houwink constants calculated from the chromatograms of Figure 1 under the same experimental assumptions made for obtaining the data of Table II.

Sample	$K \cdot 10^2$	a
S5.NRW	2.666	0.500
S5.BRD	2.683	0.500
S7.NRW	1.003	0.700
S7.BRD	0.999	0.700

experiments, provided the universal calibration method can be applied and the level of the experimental errors is low. In the following, the influence of some concentration errors in the evaluation of both K and a and MWD will be shown.

One kind of concentration errors arises when the weight of injected polymer is uncorrectly evaluated (because either the concentration of the polymer solution or the injected volume are uncorrect). It is easy to see by considering eqs. (2) and (3), that an overestimation of the injected weight, which is equal to the same overestimation of c_i, implies an underestimation of $[\eta]_i$ and an overestimation of M_i. As a consequence, the MWD should be shifted toward higher molecular weights, but its shape should not change: the average molecular weights should be more or less altered, but the dispersity index should not. As to the Mark–Houwink plot, the data points should be downshifted because of $[\eta]_i$ underestimate and rightshifted because of M_i overestimate: the slope of the curve (the exponent a) should be scarcely affected by the concentration error, whereas both $[\eta]_i$ underestimate and M_i overestimate play a role in decreasing the intercept (the K constant).

The above predictions are well supported by the results shown in Figure 2 and in Table IV, obtained by assuming that the weight of the injected polymer samples was overestimated or underestimated by 10% ($5.5 \cdot 10^{-4}$ g and $4.5 \cdot 10^{-4}$ g, respectively, instead of the true value $5 \cdot 10^{-4}$ g): no errors are introduced in the evaluation of the

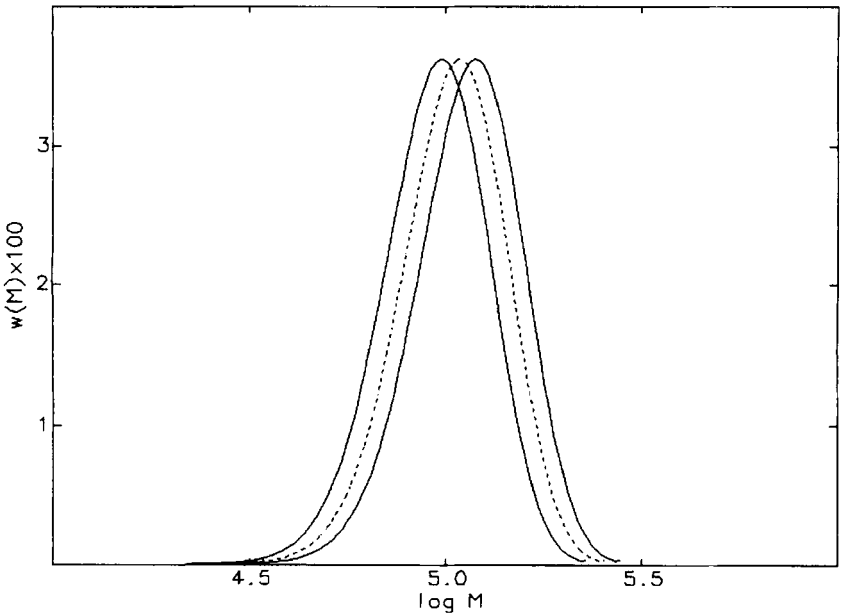

Figure 2 – MWD of the simulated sample S7.NRW (broken curve) and MWDs calculated from the chromatogram by 10% overestimating (solid curve on the right) and underestimating (solid curve on the left) the weight of injected polymer.

Table IV – Average molecular weights, dispersity index, intrinsic viscosity and Mark–Houwink constants of the polymer samples in a good solvent, as obtained by overestimating and uderestimating the weight of injected polymer.

	S7.BRD	S7.NRW
estimated injected weight:	$5.5 \cdot 10^{-4}$ g	
M_n	109552	108530
M_w	217036	119371
M_z	325492	130192
M_w/M_n	1.98	1.10
$[\eta]$ (ml/g)	43.99	30.14
$K \cdot 10^2$	0.851	0.853
a	0.700	0.700
estimated injected weight:	$4.5 \cdot 10^{-4}$ g	
M_n	89634	88797
M_w	177575	97667
M_z	266312	106521
M_w/M_n	1.98	1.10
$[\eta]$ (ml/g)	53.76	36.84
$K \cdot 10^2$	1.192	1.201
a	0.700	0.700

dispersity index and the exponent a, 10% errors are found in the average values, and 15–20% errors are introduced in the evaluation of K.

Practically identical results were obtained for the polymer samples in ideal solution.

Another source of concentration errors is the uncorrect evaluation of the time required by identical macromolecules to shift from one detector to the second one. In this case the viscosity of each slice of the DV chromatogram is combined with the concentration of a wrong slice of the DRI chromatogram. It is likely that a distortion of the MWD results, with more or less heavy consequences on all the parameters to be determined.

In Figure 3 and in Table V are shown the results obtained from the chromatograms of Figure 1, by assuming that the shift time between DV and DRI detectors was 0.05 min shorter or longer than the true 0.15 min shift time. Because of the route followed to simulate the chromatograms, a difference of ±0.05 min is the minimum error which can be done in the evaluation of the shift time. In a real SEC experiment such a minimum error would correspond to the sampling time in data acquisition.

Figure 3 shows that MWDs are higly disturbed by small errors in the evaluation of the shift time, which is reflected in the dispersity indexes M_W/M_n (Table V). The effects are stronger for the narrow MWD samples, as a consequence of the greater sharpness of the chromatograms, but are by no means negligible even for the broad MWD samples. Of course, the MWD distortion is manifested also in the average parameters, but where it gives spectacularly wrong results is in the evaluation of both K and a constants. In this last case, however, a clear departure from linearity in the Mark–Houwink plot can be observed when uncorrect shift times are employed. This is shown in Figures 4 and 5, for narrow and broad MWD respectively.

It is known[8,9] that curvatures in the Mark–Houwink plot from DV chromatograms can be due either to molecular weight dependent degree of branching in

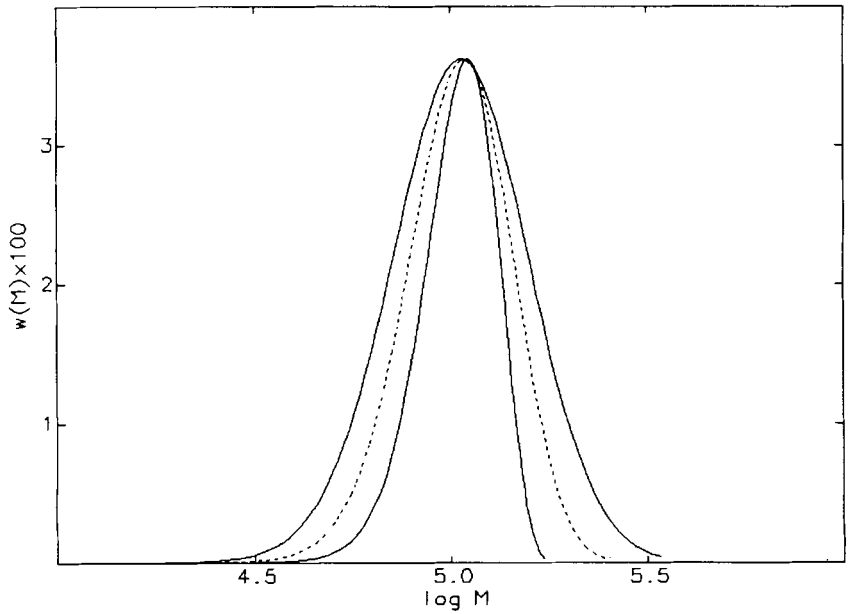

Figure 3 – MWD of the simulated sample S7.NRW (broken curve) and MWDs calculated from the chromatograms by assuming a shift time between the detectors shorter (narrower solid curve) and longer (broader solid curve) than the true value.

Table V – Average molecular weights, dispersity index, intrinsic viscosity and Mark–Houwink constants of the polymer samples in a good solvent, as obtained by assuming a shift time between the detectors either shorter or longer than the true value.

	S7.BRD	S7.NRW
shift time:	0.10 min	
M_n	104382	103420
M_w	196129	108645
M_z	279684	113452
M_w/M_n	1.88	1.05
$[\eta]$ (ml/g)	48.39	33.14
$K \cdot 10^2$	0.780	0.033
a	0.714	0.983
shift time:	0.20 min	
M_n	95022	94111
M_w	199380	110299
M_z	317222	128294
M_w/M_n	2.10	1.17
$[\eta]$ (ml/g)	48.39	33.17
$K \cdot 10^2$	1.265	14.34
a	0.687	0.477

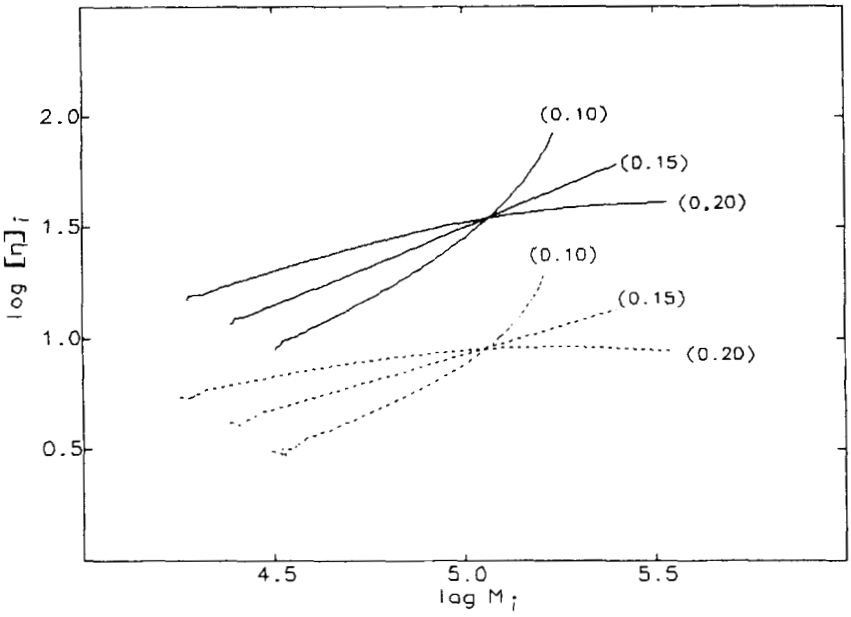

Figure 4 - Mark–Houwink plots for intrisic viscosities calculated from the chromatograms of narrow MWD sample by assuming different shift times (in parentheses). Solid curves: good solvent; broken curves: ideal solution.

omopolymers or to molecular weight dependent composition in copolymers. However, in the case of samples whose molecules have a unique dependence between size and molecular structure (linear polymers, omogeneous copolymers, molecular weight independent degree of branching, etc.), the linearity of the Mark–Houwink plot is a practical check of the reliability of the shift time for the SEC system, which can be done directly in the chromatograms treatment. Of course, seeking for linearity of the Mark–Houwink plot may be a route, alternative to the procedure described by Lesec[4], to evaluate the shift time of a SEC system, without the need of resorting to an excluded narrow MWD standard.

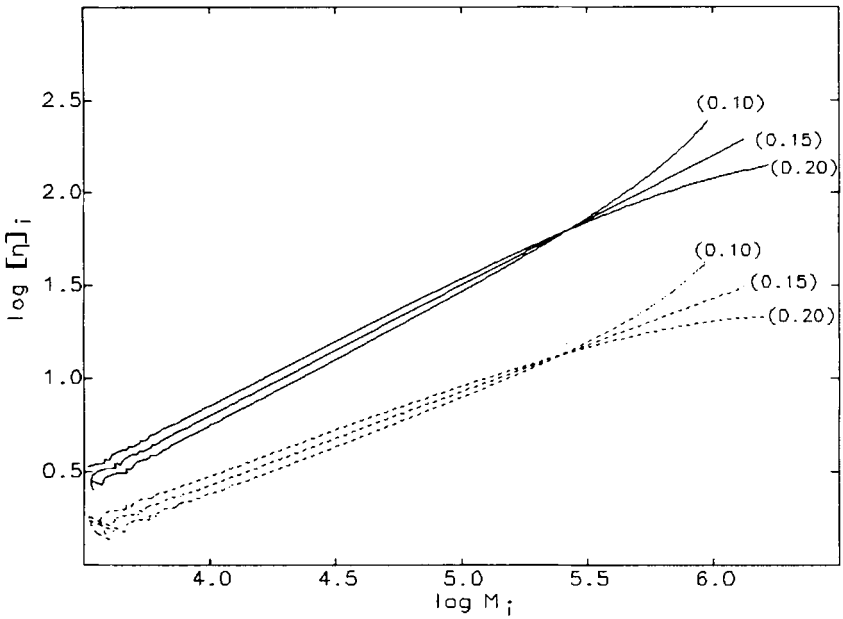

Figure 5 – Mark–Houwink plots for intrinsic viscosities calculated from the chromatograms of broad MWD sample by assuming different shift times (in parenthesis). Solid curves: good solvent; broken curves: ideal solution.

CONCLUSIONS

Working on simulated chromatograms, as obtained from DV and DRI detectors, of samples of known MWD in solutions of known Mark–Houwink costants, it has been possible to show that uncorrect estimates of the weight of injected polymer yield proportional errors on the average molecular weights and on the Mark–Houwink K costant, whereas do not affect the shape of the MWD (hence the dispersity index) and the Mark–Houwink a exponent. On the other hand, slightly uncorrect shift times modify the MWD (the more so, the narrower the distribution), with consequences on the evaluation of all the molecular characteristics, as well as of both the Mark–Houwink constants. Furthermore, the logarithmic plot of the intrinsic viscosity of the slices into which the

DV chromatograms are partitioned as a function of the molecular weight become markedly non-linear, giving the possibility of checking the reliability of the shift time (a constant of the chromatographic system) in the treatment of the chromatograms of any unknown sample. At the same time, the search for linearity of the Mark-Houwink plot can be a useful procedure to find the shift time of a new chromatographic system.

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