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Analysis

Size Exclusion Chromatography of Cellulose Nitrate

Molecular Weight Averages and Molecular Weight Distributions

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SUMMARY

There had been studied the calibration conditions on the system cellulose nitrate (sharp fractions) -THF-µ-Styragel by comparing the viscosity averages of DP obtained from the elution curves of GPC with the corresponding viscosimetrically determined ones. Deriving the elution volumes from the integral elution curves and applying adjustment of the calibration by means of adequate iterations, a very good coincidence between $DP_{\eta}(visc.)$ and $DP_{\eta}(GPC)$ can be reached. The molecular weight distribution curves obtained by GPC and by precipitation fractionation agree within the limits of error of both methods. It appears, however, that if in the calibration only one kind of average value of the "master" samples had been involved, only the same type of average can be determined with satisfying exactness.

I. INTRODUCTION

As it had been pointed out just recently, the application of size exclusion chromatography on cellulose exhibits still a lot of problematic features. A detailed adaptation of GPC on the proper characteristics of cellulose had been started, therefore, laying emphasis in a minute study and appropiate improvement of the experimental as well as the calibration conditions. Taking into account that nitration (a) takes place without any degradation (MARX-FIGINI, 1961) when observing the necessary precautions, and (b) results in a homogeneous, reproducible and well defined substitution (MARX-FIGINI, 1962), the nitrate was considered to be the most adequate cellulose derivative for the realization of GPC on cellulose. The usefulness of the nitrate is corroborated by the existence of a very sure relationship between $[\eta]$ and DP (and M respectively) derived from more than 60 individual pairs of $[\eta]$-M-data reduced to standard conditions (MARX-FIGINI and SCHULZ, 1962; MARX-FIGINI, 1978).

According to the suspicion expressed by some authors that cellulose nitrate may remain partially adsorbed when styragel is used as stationary phase, the first part of the study treated the suitability of passivated silicagel for this purpose (MARX-FIGINI and SOUBELET, 1982). It resulted, however, that in spite of the very good resolution power exhibited by this kind of stationary phase in the range $200 < DP < 1500$, the exclusion limit of the actually obtainable passivated silicagel packing materials limits their applicability to a relative small range of molecular weight. Considering that cellulose samples may have a DP up to 14000 (MARX-FIGINI and PENZEL, 1965), the study had been continued investigating the chromatographic conditions on μ-Styragel as stationary phase controlling thoroughly whether adsorption of the cellulose nitrate on it could take place or not. The respective findings in a range of DP up to 6000 are dealt with in the present paper.

II. EXPERIMENTAL

The calibration samples were prepared from a high molecular weight cellulose, which was hydrolytically degraded for different length of time, and afterwards nitrated and fractionated by precipitation fractionation (MARX-FIGINI and PENZEL, 1965).

The solute concentrations applied in the chromatographic procedures had been adjusted for each sample to give a specific viscosity between $0.3 < \eta_{sp} < 0.6$. As solvent as well as eluent, fresh or recovered THF had been used which was distilled over $NaBH_4$ in a nitrogene atmosphere the day before, and degassed immediately before its use.

The chromatographic equipment was a WATERS HPLC-apparatus, Model 244, combined with a set of four columns packed with μ-Styragel 10^6-10^5-10^4-10^3. An UV-Spectrophotometer WATERS, Model 440 had been used as detector. The applied elution velocity amounted to 0.6 ml/min. Before injection, the solutions were relieved from dust and possible microgels by centrifugation at 28000 rpm and subsequent filtration through Teflon-membrane filters of 0.45 μm pore diameter.

According to the above mentioned adjustment of the solute concentration to a standarized specific viscosity, the first one must be drastically reduced when using samples of higher molecular weights. In order to produce nevertheless well evaluavable elution curves, the injection volume had to be increased to 200 μl (compared with 75 μl in the previous investigation), together with a corresponding increase of the sensibility of the UV-detector.

The molecular weight of the calibration samples had been determined viscosimetrically under standard conditions (MARX-FIGINI and SCHULZ, 1962; MARX-FIGINI, 1978). Taking into account the findings discussed later on it must be emphasized here that the relationship between log $[\eta]$ and log DP (or log M respectively) exhibits a certain decrease of its slope when DP > 1000. The curved relationship can be expressed by two straight lines showing the following parameters of the equation of Staudinger-Mark-Howink: for $DP_\eta < 1000$: $k = 0.82$ $a = 1.0$; for $DP_\eta > 1000$: $k = 4.46$ $a = 0.76$, when $[\eta]$ is given in ml/g^{-1}.

III. RESULTS

In the fig. 1 are graphed the degrees of polymerization of the different calibration samples as a function of the corresponding elution volumes. Since calibration was carried out with nearly homogeneous fractions, the values of V_e were derived from only the maximum of the corresponding elugramms, as it had been done also in the foregoing paper.

The dashed line of the fig. 1 shows that in the investigated range of $DP_\eta < 6000$, the μ-Styragel exhibits a very good resolution power. It seems that at $DP_\eta \approx 6000$ the exclusion limit is still not reached. We think, however, that for a range $DP_\eta >> 6000$, a μ-Styragel column set with other characteristics (e.g. $2x10^6$, $2x10^5$, 10^4) would be more adequate.

The dashed line of fig. 1 shows furthermore that the calibration relationship exhibits two linear regions, each of which can be described by either of the common equations:

$$\ln DP = A - B.V_e \tag{1a}$$

$$\log DP = A - B.V_e \tag{1b}$$

The parameters of the two regions differ remarkably. There had been derived by use of the method of least squares the following value pairs corresponding to the eq. 1a and corresponding to the ranges $200 < DP < 1000$ and $1000 < DP < 5000$ respectively:

$A_1 = 15.78 \quad B_1 = 0.342 \quad ; \quad A_2 = 26.81 \quad B_2 = 0.784$

Using these parameters in order to calculate the viscosity averages from the corresponding elution curves applying an adequate computer program, it turned out, however, that the so obtained DP deviate remarkably from those determined by viscosimetric measurements, especially in the range of higher molecular weights (see Table 1, column 2). One can suppose that in the present case the elaboration of the elution volumes from the maximum of the elution curves only is insufficient, in spite of the use of sharp fractions as calibration samples. This may be a consequence of the considerably higher actual elution volumes (22-30 ml) compared to those occurring in the previous experiments (5.5 - 7.5 ml), accompanied by a no longer negligible instrumental spreading. In order to overcome this incorrectness, the integral elution volume had been used instead of V_e at the maximum only. Furthermore it had been carried out an adjustment of the calibration parameters according to a method proposed for the use of calibration samples with broad molecular weight distributions (VRIBERGEN et al., 1978). This method involves repeated iterations until a minimum had been reached for the deviations of the calculated DP_η from the viscosimetrically determined ones. The adjusted calibration function is represented in fig. 1 (continuous line). The respective parameters show now the following values:

$A_1^* = 14.72 \quad B_1^* = 0.3044$ for the range $200 < DP < 1000$

$A_2^* = 23.33 \quad B_2^* = 0.6468$ for the range $1000 < DP < 5000$

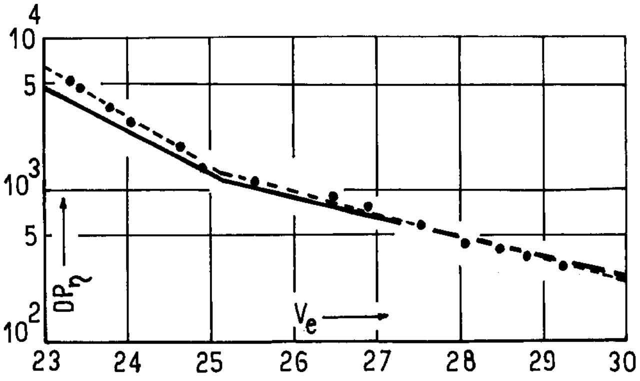

Fig. 1 - DP_η as a function of V_e according to eq. 1b
-●-●- V_e derived from the maximum only
(● = measured dates)
——— V_e derived from the integral elution curve and adjusted according to VRIBERGEN et al.

The values of DP_η calculated from the respective elugrams by means of the parameters A_1^*, A_2^*, B_1^* and B_2^* fit now very much better with the viscosimetrically determined ones as it can be seen from the Table 1, column 4. The small differences still existing can be attributed to the instrumental spreading. It is to expect that they disappear, within the limits of error of the respective methods, with a still more detailed optimization of the calibration (ANDREETTA and FIGINI, 1981). In order to realize this refinement, the knowledge of at least two distinct molecular weight averages of the calibration samples determined by use of the proper methods is necessary. Unfortunately, the available quantities of most of the fractions prepared for calibration purposes were too small to carry out reliable osmotic measurements which demand greater concentrations, especially in the range of higher molecular weights. This will be done in a future study using unfractionated cellulose nitrates as "master" samples.

TABLE 1: Viscosity average of degree of polymerization determined viscosimetrically and by GPC using not adjusted and adjusted calibration.

DP (visc)	DP_η(GPC) using A_1,B_1,A_2,B_2	% deviation $DP_\eta(visc)-DP_\eta(GPC)$	DP_η(GPC) using A_1^*,B_1^*,A_2^*,B_2^*	% deviation $DP_\eta(visc)-DP_\eta(GPC)$
4785	4216	11.9	4573	4.45
3373	3313	1.8	3405	0.9
2784	3483	25.1	3074	10.4
1929	2091	8.6	1889	1.9
1384	1670	20.7	1361	1.7
1150	1428	24.2	1095	4.7
884	1002	13.3	863	2.4
449	476	6.0	470	4.7
315	304	3.5	312	0.9
278	249	10.4	264	5.0
	mean deviation:	12.5	mean deviation:	3.7

Besides of the control exhibited in Table 1, column 4, the reliability of the parameters $A^*_{(1,2)}$ and $B^*_{(1,2)}$ had been proved furthermore by comparing the differential distribution curves as well as the corresponding molecular parameters DP_η and DP_n, determined on a nonfractionated cellulose nitrate by means of GPC, precipitation fractionation, viscosimetry and osmometry. According to the obtained values given in the table 2, the coincidence between $DP_{\eta(visc.)}$ and $DP_{\eta(GPC)}$ can be considered as satisfactory. The difference of about 4% may be attributed to the failing of the refinement which in the present calibration had not yet been taken into account (see above).

TABLE 2: Values of DP_η and DP_n, determined by different methods

Method	DP_η	DP_n
Osmometry	-	630 ± 30
Viscosimetry	930	-
GPC	969	714
Precip.Fractionation	905	675

In contrary to the viscosity average of DP, the values of the number average show a greater dispersion among them. It is remarkable that the value of $DP_{n(GPC)}$ is about 10% higher than the osmotically determined one, in spite of the fact that the instrumental spreading would provoke a lowering of the DP_n. Therefore, it may be argued that the deviation of $DP_{n(GPC)}$ from $DP_{n(Osm)}$ can rather be attributed to the fact that the calibration had been made "selfconsistent" only with respect to the viscosity average but not with respect to the number average DP.

Concerning the differential distribution, the fig. 2 shows a satisfactory good coincidence of the positions of the maximum obtained by GPC and precipitation fractionation respectively.

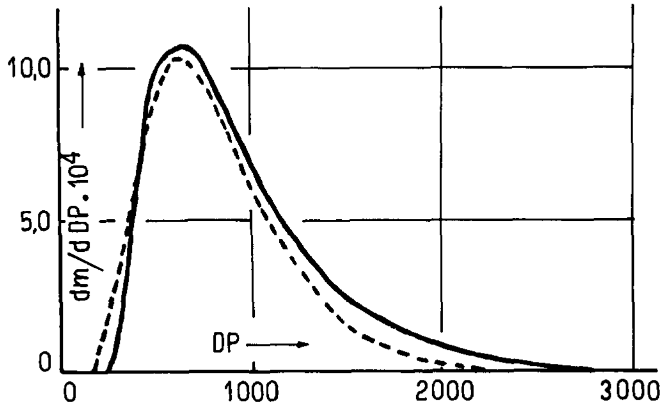

Fig. 2 - Differential mass distribution curves obtained by
——— Size exclusion chromat.;µ-Styragel 10^6-10^5-10^4-10^3
----- Precipitation fractionation

The realtive small differences in the ranges of higher and lower molecular weights may be caused besides of the difficulty in separating sharp upper fractions in the precipitation fractionation procedures, by the already discussed failing of the refinement in the calibration.

IV. DISCUSSION

μ-Styragel exhibits for cellulose nitrate excellent separation properties up to molecular weights higher than DP ≈ 5000. Basic requirements are, however, the application of appropriate conditions in the experimental as well as in the calibration procedures. Utilizing such conditions, separation occurs by a real size exclusion mechanism without involving improper processes as i.e. adsorption of cellulose nitrate on the stationary phase. This already turns out from the observation of coincidence between the crossover points of the two regions shown by the V_e - M as well as by $[\eta]$ -M relationship, corroborating by this way the conception of the hydrodynamic volume being one of the determining factors of size exclusion chromatography.

Contrary to the good coincidence of the viscosity averages, a notable difference between the number average values $DP_{n(GPC)}$ and $DP_{n(osm.)}$ is found in the control experiment on the unfractionated cellulose nitrate. This suggests that the derivation of more than one molecular weight averages from the elution curves requires the involvement of each of the respective averages in the elaboration of the calibration parameters. Apparently, calibrating with one average value of the "master" samples permits the exact derivation of only this kind of average on an unknown sample, even when instrumental spreading may be negligible.

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