Polymer Bulletin 6, 501-508 (1982)

Polymer Bulletin

© Springer-Verlag 1982

Size Exclusion Chromatography (GPC) of Cellulose Nitrate Using Modified Silicagel as Stationary Phase

M. Marx-Figini and O. Soubelet

Div. Macromoléculas, Instituto de Investigaciones Fisicoquímicas Teoricas y Aplicadas (INIFTA), C. Correo 16, Sucursal 4, 1900 La Plata, Argentina

SUMMARY

It had been studied the applicability of coated silicagel as stationary phase in size exclusion chromatography of cellulose nitrate. Sample concentration and injection volume had been standarized. The elution volumes corresponding to discrete molecular weights resulted in a high reproducibility provided the application of the standarized conditions. There does not occur any adsorption of the cellulose nitrate on the packing material. It could be confirmed that separation takes place by a size exclusion mechanism. Separation power of the system had been proved by comparing the distribution curves obtained by means of GPC and fractionation precipitation respectively on the same sample.

I INTRODUCTION

In the last decade, determination of molecular weight distribution by means of Size Exclusion Chromatography (HPSEC,GPC) had been developed to high performance especially for vinyl polymers. Its application on cellulose, however, is still problematic due to difficulties arising by the molecular characteristics of the cellulose itself. Among others, there may be mentioned the following obstacles: the solvents for unsubstituted cellulose are strongly colored, and cellulose suffers in most of them a considerable degradation when oxygen is not completely excluded. On the other hand, those solvents in which unsubstituted cellulose shows a greater stability do not dissolve it molecular dispersely, at least not in the range of DP>3000 (GRUBER and GRUBER, 1978; MARX-FIGINI, 1978). As a consequence, for most investigations on its molecular properties, cellulose must be converted firstly in one of its derivatives solubles in organic solvents. The derivative mostly used for this purpose is the nitrate, the polymer analogous and homogeneous preparation which had been elaborated and proved recently (MARX-FIGINI, 1961; 1962). In GPC experiments of former authors arose, however, that cellulose nitrate remains partially adsorbed in the columns when styragel or not passivated silicagel are applied as column packing material (MEYERHOFF, 1970; SELLEN et al., 1978). Therefore, it was proposed to use tricarbanilate as cellulose derivative instead of cellulose nitrate (VALTASAARI and SAARELA, 1975; KOSSLER et al., 1974, 1976). In contrary to the nitrate, however, there do not yet exist exact investigations

0170-0839/82/0006/0501/$01.60

on the homogeneity and reproducibility of the degree of substitution and the influence of these parameters on the molecular properties and the measurements of them respectively. Because of these reasons, the nitrate seems to be the more appropiate derivative for purposes of GPC. Therefore, a detailed comparative study on the adaptability of this method on the special characteristics of the cellulose nitrate was projected, covering an ample range of molecular weight and applying different types of column packing material. In the present paper are dealt with the first results, referring to a range of $250<DP<2500$, using as stationary phase a silicagel coated with C-8 chains.

II EXPERIMENTAL

Samples: Fractions of hydrolytically degraded cotton cellulose and of technologically treated cellulose, both nitrated before fractionation.
Solvent: Peroxydefree THF, Merck p.a., freshly destilled in a nitrogene atmosphere the day before, and degased immediately before its use.
Apparatus: Waters HPLC, Model 244.
Columns: A set of three prepacked columns filled with Merck Lichrospher CH-8, 10 μm, types 4000-4000-1000.
Detector: UV-Spectrophotometer, WATERS, Model 440, λ = 254 nm.
Elution velocity: 0.3 ml/min. In the calculation of the elution volumes from the position of the maximum of the peaks, possible slight irregularities in the flow rate had been taken into account by use of an "internal standard" (1,2,4,-trichlorobenzene) (ANDREETTA and FIGINI, 1981).

III RESULTS

Standarization: Because of the little difference between the R_i of cellulose nitrate and that of THF, UV-Spectrophotometry had been chosen as detection method. Its application on cellulose nitrate requires, however, solutions of sufficient high sample concentration in order to garantize exact detection. On the other hand, the applied sample concentration must be sufficiently low to avoid undesired effects as "viscous fingering", presence of microgels, dangerous increase of the pressure, etc. In order to find out the optical concentration range, there had been carried out under the same conditions a serie of chromatographic runs on solutions of the same fractions having different concentrations. It appeared that concentrations giving a specific viscosity of $\eta_{sp} > 1.0$ provoke very poor reproducibility of the corresponding elution volumes. In contrary to this, concentrations corresponding to values of $\eta_{sp}<0.6$ result in very good reproducible elution volumes, as well as in a throughout constant pressure. Concentration had been, therefore, standarized in such a way that the specific viscosity of the respective solution amounts $0.3<\eta_{sp}<0.6$, independently from the degree of polymerization of the respective sample. It is interesting to note that this range of η_{sp} coincides with that established formerly for the determination of the intrinsic viscosity of cellulose nitrates (MARX-FIGINI and SCHULZ, 1959). Variation of the injection volume from 50 up to 100 μl showed a slight increase in the corresponding elution volume. The

injection volume was, therefore, standarized at a 75 μl quantity which revealed an optimum behaviour with respect to the applied concentrations and to the resulting elution times.
After the elaboration of the mentioned standarizations, reproducibility of the system had been proved by injecting at different days and at different external conditions solutions of the same fraction. The reproducibility resulted to be excellent, also after intervals of two months, when the equipment was allowed to stabilize each day at least during two hours before injection.

Calibration

Calibration had been carried out using fractions, the molecular weights of them had been derived from the respective intrinsic viscosities in acetone by means of the equs. (1a), (1b), (2a) and (2b), according as $[\eta] > 800$ ml.g^{-1} or $[\eta] < 800$ ml.g^{-1} respectively (MARX-FIGINI and SCHULZ, 1962). The respective dates are compiled in Table 1.

$$[\eta] = 4.46 \cdot DP^{0.76} \quad (1a) \qquad [\eta] = 5.9 \times 10^{-2} \cdot M^{0.76} \quad (2a)$$

$$[\eta] = 0.82 \cdot DP \quad (1b) \qquad [\eta] = 2.8 \times 10^{-3} \cdot M \quad (2b)$$

TABLE 1

Molecular Parameters of the Calibration Samples, and Corresponding Elution Volume V_e

Sample	$[\eta]_{Aceton}$ (ml.g^{-1})	DP	M	V_e ml
1	1660	2470	726770	5.73
2	1376	1931	567714	5.76
3	1069	1384	406994	5.94
4	929	1124	338100	6.12
5	846	1016	298700	6.33
6	724	884	259749	6.51
7	600	730	168476	6.75
8	493	600	176700	6.99
9	410	500	147000	7.26
10	288	351	103194	7.71
11	198	240	70854	7.95
12	163	198	58712	8.07

Plotting the logarithm of the molecular weight expressed as lg DP as a function of the elution volume (fig. 1) resulted in the range of $300 < DP < 1500$ a straight line which obeys to the general equs. 3a and 3b,

$$\lg_{10} DP = A - B.V_e \quad (3a)$$

$$\ln\ DP = A' - B'.V_e \quad (3b)$$

where A, A', B and B' possess the following values:

$$A = 5.22 \qquad B = -0.348$$

$$A' = 12.02 \qquad B' = -0.8062$$

with a standard deviation in B and B' of less than 0.5%.

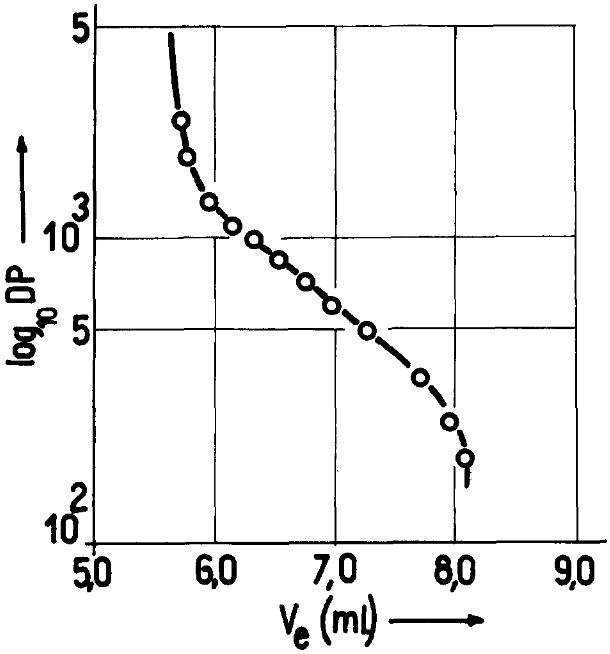

Fig. 1 - Log DP as a function of V_e.
Cellulose nitrate in THF, degree of substitution S = 2.90 ± 0.02

In order to examine if the elaborated calibration curve would follow the so called universal calibration (BENOIT et al., 1967) there had been carried out HPSEC-experiments on five samples of nearly monodisperse polystyrenes (Pressure Chemical Company). It had been taken care that the corresponding hydrodynamic volumes $[\eta]\,M$ cover the same range as those of the used cellulose nitrates. For this purpose, the intrinsic viscosities of the polystyrene samples had firstly been determined in THF and converted into the corresponding molecular weights by use of equ. (4) (COLL and GILDING, 1970)

$$[\eta]_{THF} = 1.60 \times 10^{-4} \cdot M_w^{0.706} \qquad (4)$$

The concentrations of polystyrene applied in the HPSEC experiments had been adjusted to the standarized specific viscosity range. The intrinsic viscosity of the cellulose nitrates in THF had been derived from the corresponding intrinsic viscosities in acetone by means of the intrinsic viscosity ration $[\eta]_{THF}/[\eta]_{Ac} = 1.31$, determined previously.
The resulting hydrodynamic volumes $[\eta] \cdot M$, corresponding to the cellulose nitrate and polystyrene samples respectively are plotted in fig. 2 as a function of the corresponding elution volumes V_e.

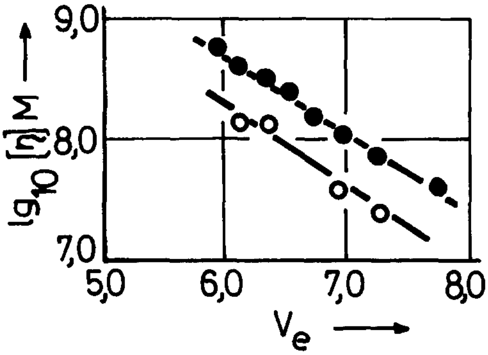

Fig. 2 - log [η]M as a function of V_e

● cellulose nitrate
o polystyrene

The fig. 2 seems to indicate that the cellulose nitrate may not follow to the universal calibration, although it must be emphasized the parallelity of the two straight lines. Taking into account that the molecular weights of different cellulose derivatives are governed by the molecular weights of the respective substituents, and that, therefore, in the case of substituted polymers rather the degree of polymerization can be considered as the proper characteristic feature, in fig. 3 [η]xDP of both, cellulose nitrate and polystyrene, was plotted as a function of the elution volume. As the fig. 3 shows, cellulose calibration fits now very well with the universal calibration.

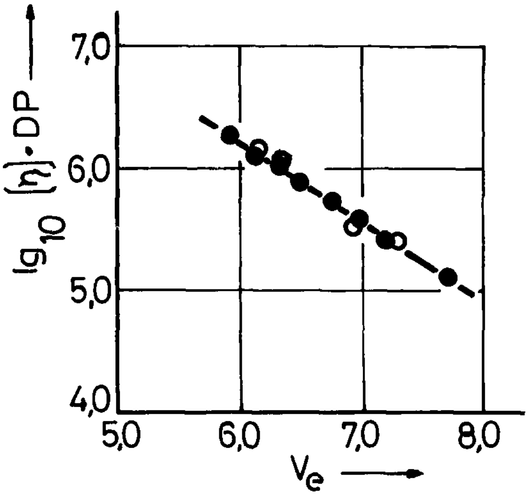

Fig. 3 - log [η]DP as a function of V_e

● cellulose nitrate
o polystyrene

Control of performance: In order to examine the performance of the system, the molecular weight distribution of a corresponding sample was determined by GPC as well as by precipitation fractionation. GPC had been carried out under the standarized conditions described in the foregoing chapters, using the elaborated calibration in order to convert V_e in DP. Precipitation fractionation was conduced according to a technique published elsewhere (MARX-FIGINI, 1959; MARX-FIGINI and SCHULZ, 1963) with a 4:1 acetone-water mixture as precipitant.

The resulting mass distribution curves are plotted in the figs. 4a and 4b. They show a very good coincidence, at least with respect to the position of the maximum. The number and weight average values derived from the integral distribution curves as well as the values of DP_n and DP_η determined by osmotic and viscosimetric measurements on the unfractionated samples are compiled in Table 2.

TABLE 2

Molecular weight averages and polydispersity U, experimentally determined as well as derived from the integral mass distribution curves obtained by GPC and precipitation fractionation respectively.

	DP_n	DP_η	DP_w	$DP_w/DP_n - 1 = U$
Osmosis	630	-	-	0.53
Viscosimetry	-	930	-	
GPC	614	-	963	0.57
precip.fract.	675	-	905	0.34*

*Applying a correction of the apparent polydispersity U_{app} by use of the equ. $U_{real} = U_{app} + U_{Fr}(1+U_{app})$, and assuming a polydispersity of the fractions of $U_{Fr} \approx 0.15$ would result in U_{real} = 0.57

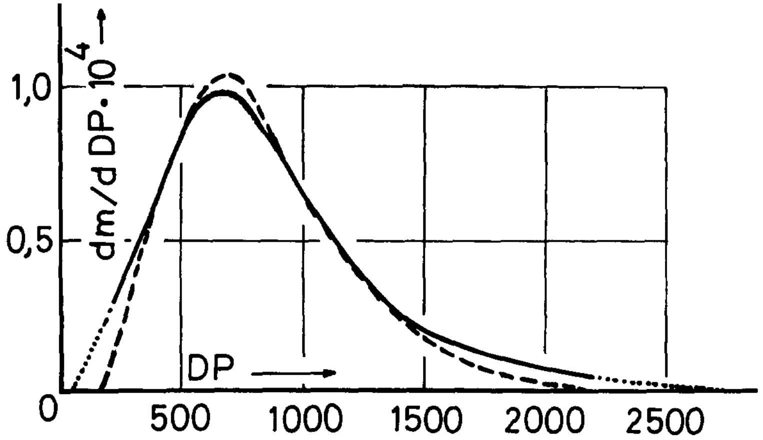

Fig. 4a - Differential mass distribution curves
——— size exclusion chromatography
– – – precipitation fractionation (derived from the respective integral curve of fig. 4b).
..... correspond to ranges of DP which are not covered by the calibration curve

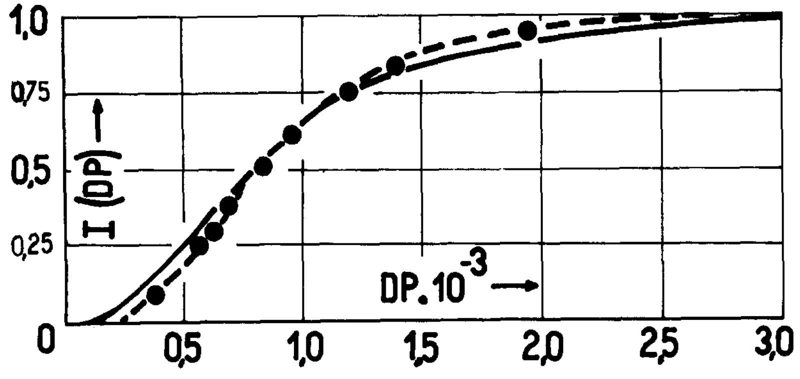

Fig. 4b - Integral mass distribution curves

—— size exclusion chromatography (derived from the respective diferential curve of fig. 4a)

—•— precipitation fractionation, experimentally obtained values

DISCUSSION

The results show that silicagel passivated with C-8 chains represents a very appropiate material for size exclusion chromatography of cellulose nitrate. The coincidence of the slope of the straight lines when plotting the hydrodynamic volumes $[\eta]$ M of cellulose nitrate and polystyrene solutions in THF respectively as a function of V_e confirms that separation occurs by a size exclusion mechanism. The reason why cellulose nitrate fits completely with the universal calibration when the hydrodynamic volume is expressed in terms of $[\eta]$ DP instead of $[\eta]$ M is not yet clear. Exacter knowledge on the kind of molecular parameters involved in size exclusion chromatography calibration is necessary. This will be the aim of a future investigation.

The separation power of the investigated system is sufficiently high to deliver reliable distribution curves, as it could be proved by the comparison of the respective distribution curves obtained by the present method and by precipitation fractionation of the sample. The distribution delivered by GPC is somewhat broader and, therefore, the resulting polydispersity quotient somewhat higher. This was to expect considering that the chromatographic process is subjected to an instrumental spreading. On the other hand it is known, that the precipitation fractionation technique results in somewhat too low polydispersities because of the, although low, polydispersity of the proper fractions which is not taken into account. According to Table 2 it may be assumed that in the present system, broadening by the instrumental spreading plays the minor role. However, considering the limits of error of the experimental methods involved in each method of molecular weight determination as well as in the precipitation fractionation technique, the present study does not permit to draw definite conclusions.

Unfortunately, the exclusion limit of the tested columns takes place already at degrees of polymerization about DP = 1900 (M 550.000) what is relatively low compared to the highest possible degree of polymerization of cellulose of DP ≈ 14.000 (MARX-FIGINI and PENZEL, 1965). This is a consequence of the considerably higher intrinsic viscosities of the cellulose nitrate than those of the polystyrenes, considering the same molecular weights. Silicagel columns with greater pore sizes are, however, not yet available because of technical reasons. A certain alternative would be to increase the partical size and to compensate the so caused decrease in exactness by taking into account the instrumental dispersion.-In the investigated range of DP, UV-spectrophotometry is a very appropiate detection method because of its remarkably less sensibility against external factors as temperature, pressure, etc. The molar absorption coefficient of cellulose nitrates depends on their degree of substitution. This means that UV-detection requires not only a homogeneous degree of substitution of the samples in analysis, but also its complete coincidence with that of the samples used in calibration. This condition, however, does not produce problems applying the above mentioned nitration method and taking the necessary precautions to avoid inhomogeneous accesibility of the materials to the nitration mixture.

REFERENCES

GRUBER, E. and GRUBER, R., Cellulose Chemistry and Technology, 12, 345 (1978)

MARX-FIGINI, M., Angew.Makromol.Chem. 72, 154 (1978)

MARX-FIGINI, M., Makromol.Chem. 50, 196 (1961)

MARX-FIGINI, M., Makromol.Chem. 52, 133 (1962)

MEYERHOFF, G., Makromol.Chem. 134, 129 (1970)

HOLT, C., MACKIE, W. and SELLEN, D.B., Polymer (London) 19, 1421 (1978)

VALTASAARI, L. and SAARELA, K., Paperi ja Pun N° 1 , 5 (1975)

ELASHMAWY, A.E., DANELKA, J. and KOSSLER, J., Svensk. Papperstidn. 16, 603 (1974)

DAUELKA, J. and KOSSLER, J., J.Polym.Sci., Polym.Chemistry Edition 14, 287 (1976)

MARX-FIGINI, M., Makromol.Chem. 32, 233 (1959)

ANDREETTA, A.H. and FIGINI, R.V., Angew.Makromol.Chem. 93, 143 (1981)

MARX-FIGINI, M. and SCHULZ, G.V., Makromol.Chem. 31, 140 (1959)

MARX-FIGINI, M. and SCHULZ, G.V., Makromol.Chem. 54, 102 (1962)

GRUBISIC, F., REMPP, P. and BENOIT, H., Polym.Letters 5, 753 (1967)

COLL, H. and GILDING, D.K., J.Polym.Sci., Part A-2, 8, 89 (1970)

MARX-FIGINI, M. and SCHULZ, G.V., Makromol.Chem. 62, 49 (1963)

MARX-FIGINI, M. and PENZEL, E., Makromol.Chem. 87, 507 (1965)

Received January 2o, accepted January 25, 1982

C