Gel permeation chromatographic analysis of *N*-trifluoroacetylated poly(ε-caprolactam): Calibration procedures

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A calibration procedure for the gel permeation chromatography (g.p.c.) of *N*-trifluoroacetylated poly(ε-caprolactam) (*N*-TFA PCL) is proposed. The method requires only that $\bar{M}_n$ and $\bar{M}_w$ be determined independently without any preliminary condition on the distribution broadness. Four samples covering a range of molecular masses typical of commercial PCL materials are analysed and very good agreement between the measured average molar masses and the g.p.c. computed ones is found. The Mark–Houwink relationship in methylene chloride is derived from the molar mass distribution data and the intrinsic viscosity measurements on the unfractionated samples. The universal calibration curve based on polystyrene hydrodynamic volume is found to be inapplicable to *N*-TFA PCL, which shows a very peculiar retention behaviour.

(Keywords: *N*-trifluoroacetylated poly(ε-caprolactam); polydisperse samples; gel permeation chromatography calibration; intrinsic viscosity; Mark–Houwink constant; abnormal retention behaviour)

INTRODUCTION

Aim of the work

Until very recently, the molar mass distribution of aliphatic polyamides by gel permeation chromatography (g.p.c.) was possible only under rather drastic conditions, involving the use of highly polar solvents such as *m*-cresol, benzyl alcohol, hexafluoro-2-propanol, hexamethylphosphorotriamide, etc., often associated with high temperatures[1,2].

Recent developments based on the trifluoroacetylation reaction of the amide group have provided a simple method for the functionalization of several aliphatic polyamides[3], making them soluble in a variety of common organic solvents as well as easily analysable by the g.p.c. technique[4–7].

Thus, new opportunities for a full molar mass characterization of this important class of polymers have become available at present.

The above papers[3–7], dealing with the chemistry and the kinetics of the *N*-trifluoroacetylation (*N*-TFA) reaction, have shown the reproducibility and reliability of this method, so that classical measurements such as osmometry and light scattering could be successfully applied for the evaluation of average molar masses, as in the case of poly(ε-caprolactam) (PCL)[8].

Routine g.p.c. analyses of *N*-trifluoroacetylated polyamides (mainly PCL) are commonly performed at present in several academic and industrial laboratories, but most of the results are nevertheless purely qualitative, because the problem of proper g.p.c. calibration has not yet been satisfactorily solved for this type of polymer. Only very recently has Weisskopf[9] proposed for polydisperse polymers, obeying the Schulz–Zimm distribution, a simple method based on the direct calibration of weight average molar mass vs. the elution volume at g.p.c. peak maximum.

The aim of the present work is to propose a suitable calibration procedure for the functionalized PCL (*N*-TFA PCL) and to evaluate the hydrodynamic behaviour of this polymer in our g.p.c. solvent (methylene dichloride) as compared to that predicted by the universal calibration method of Benoit and coworkers[10].

Gel permeation chromatography calibration methods

As is well known, g.p.c. is a relative method, which requires a preliminary calibration. When samples of monodisperse polymer standards are available, a calibration curve representing the molar mass of the monodisperse fraction as a function of the elution volume V can be obtained. A large, central portion of this curve usually shows a linear dependence of $\log M$ on V, as given by:

$$\log M = A - BV \tag{1}$$

Unfortunately, only very few polymers (e.g. polystyrene) are produced as narrow fraction standards. The use of a polystyrene-based calibration curve for other polymers is not advisable and can lead to erroneous results.

The most widely used calibration technique is the universal calibration method introduced by Grubisic *et al.*[10] This method is based on the concept of hydrodynamic volume (a quantity proportional to the product of the limiting viscosity number and the molar mass, $[\eta]M$), assuming that the separation process is controlled only by this parameter. The combination of viscosity and g.p.c. measurements on monodisperse

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polystyrene standards allows one to construct the universal calibration curve. In the linear region, the following equation applies:

$$\log([\eta]M) = C - DV \tag{2}$$

which is assumed to be valid for every polymer.

This approach has been successfully applied to many linear polymers and is now generally accepted, even if some significant exceptions have been found[11]. Some refined treatments of the theory have been proposed by Rudin and Hoegy[12]. However, in the case of no information regarding Mark–Houwink coefficients for the specific polymer under evaluation, or no apparatus for performing on-line intrinsic viscosity measurements on the g.p.c. eluant, the universal calibration is obviously not easily applicable.

The above problems have generated a lot of different calibration procedures, independent of the universal calibration. These methods, recently reviewed by Dawkins[11], are based on polydisperse standards having the same structure as the polymer under investigation and well characterized in terms of number average ($\bar{M}_n$), weight average ($\bar{M}_w$) and/or viscosity average ($\bar{M}_v$) molar mass. The computing procedure is usually a trial-and-error loop in which the calibration curve is adjusted until the calculated and experimental average molar mass values agree at the desired level of accuracy.

Among the various approaches, we have adopted a method based on the average retention volume concept, proposed by Szewczyk[13,14]. This method, specially suitable for materials having a unimodal distribution over a wide polydispersity range, requires at least one standard sample whose $\bar{M}_n$ and $\bar{M}_w$ are precisely known.

EXPERIMENTAL

Four PCL samples (B_1, B_2, B_3 and B_4) carefully characterized in terms of $\bar{M}_w$ (light scattering, LS), $\bar{M}_n$ (g.p.c.) and $[\eta]$ in sulphuric acid have been kindly supplied by Bayer AG[15,16]. Independent confirmatory evaluations of $\bar{M}_w$ (from $[\eta]$ in trifluoroethanol at 25°C) and $\bar{M}_n$ (from $[\eta]$ in sulphuric acid at 25°C) have been carried out in our laboratory. The *N*-trifluoroacetylated PCL samples have been analysed in a standard g.p.c. apparatus (methylene chloride as eluant) directly interfaced with an Apple Computer model IIE. The experimental instrumentation and conditions are the same as those described in ref. 6. The elution data have been processed by using a g.p.c. evaluation program devised for the purpose.

Polystyrene standards (Pressure Chemicals Inc. and Polymer Labs) have been analysed under the same experimental conditions as for *N*-TFA PCL samples. Viscosity measurements of functionalized polyamides and polystyrene standards have been performed in methylene chloride at 25°C using an automatic Schott-Geräte viscometer.

RESULTS AND DISCUSSION

Sample characterization

Table 1 $\bar{M}_w$ and $\bar{M}_n$ values for the Bayer standards

	$\bar{M}_w \times 10^{-3}$		$\bar{M}_n \times 10^{-3}$	
Sample	LS[a]	Viscosity[b]	G.p.c.[a]	Viscosity[a,b]
B_1	27.4	29.9	11.5	14.0
B_2	34.5	37.2	15.3	16.8
B_3	51.0	52.6	21.2	23.1
B_4	57.1	59.5	24.3	26.3

[a] Data from Bayer AG
[b] Data from our laboratory

Table 2 $\bar{M}_w$ and $\bar{M}_n$ values of the *N*-TFA derivatives (average values)

Sample	$\bar{M}_w \times 10^{-3}$	$\bar{M}_n \times 10^{-3}$
B_1	53.1	25.9
B_2	66.4	31.0
B_3	96.2	42.7
B_4	107.9	48.7

The molar mass characterization data for the four samples are given in *Table 1*. $\bar{M}_w$ data in column 3, obtained from $[\eta]$ values as already mentioned in the 'Experimental' section, were evaluated according to the Mark–Houwink (MH) relationship recently proposed by Aharoni *et al.*[17] $\bar{M}_w$ values from LS and $[\eta]$ show very good mutual agreement; thus, straightforward averaging has been used to provide input data. $\bar{M}_n$ values from $[\eta]$ data have been obtained on the basis of the MH relationship found by Šebenda *et al.*[18] As compared to the corresponding data derived from g.p.c., the above values show an excellent agreement. Owing to the lack of information on the calibration procedure in order to obtain $\bar{M}_n$ from g.p.c., we have based all further calculations on our own data.

The average values of $\bar{M}_n$ and $\bar{M}_w$ for the corresponding *N*-TFA derivatives of the four samples are given in *Table 2*. These values have been used to feed the computer according to the method described in the next paragraph.

Calibration method

As previously mentioned, we have adopted a calibration procedure, developed by Szewczyk[13,14]. This method is based on the concept of average retention volume, $\bar{V}$. Assuming that the linear calibration holds and rewriting equation (1) as follows:

$$V = C_1 - C_2 \log M \tag{1a}$$

one can derive the following equations:

$$\bar{V}_1 = C_1 - C_2 \log \bar{M}_n \tag{3}$$

$$\bar{V}_2 = C_1 - C_2 \log \bar{M}_w \tag{4}$$

These equations can be solved for C_1 and C_2, giving:

$$C_1 = \bar{V}_1 + C_2 \log \bar{M}_n \tag{5}$$

$$C_2 = (\bar{V}_1 - \bar{V}_2)/\log(\bar{M}_w/\bar{M}_n) \tag{6}$$

Applying the definitions of average molar mass, the following equations can be obtained:

$$\bar{V}_1 = C_2 \log\left(\sum_i w_i 10^{V_i/C_2}\right) \tag{7}$$

$$\bar{V}_2 = -C_2 \log\left(\sum_i w_i 10^{-V_i/C_2}\right) \tag{8}$$

In equations (7) and (8) w_i and V_i are the weight fraction and the elution volume of the *i*th fraction, respectively.

For an assumed arbitrary C_2 value, used as the iterative variable, the values of $\bar{V}_1$ and $\bar{V}_2$ are calculated from the experimental chromatogram on the basis of equations (7) and (8). As a consequence, a corrected C_2 value can be obtained from equation (6). This value is introduced into equations (7) and (8) and the iterative process is repeated until the last-but-one C_2 and the last value agree at the desired level of accuracy: in our systems, a difference $\leqslant 10^{-3}$ has been adopted as the final level of precision. Then, from equation (5) the corresponding C_1 value is obtained. On the basis of the calculated values of C_1 and C_2, a calibration curve for the specific polymer under examination is finally drawn from equation (1a).

Applying the above procedure to our standards (B_1, B_2, B_3 and B_4), we have obtained the results given in *Table* 3. It is evident that a single calibration curve is not provided by the experimental data. However, reasonably good agreement, both in terms of slope and intercept, can be found among the four samples.

As shown in *Tables 1* and *2*, the four samples cover rather similar ranges of molar masses; therefore, the above differences could not be specifically attributed to some different hydrodynamic behaviour. As a reasonable hypothesis, uncertainties in the correct $\bar{M}_w$ and $\bar{M}_n$ values as input data, as well as small but uncontrollable experimental errors during functionalization and g.p.c. analysis, may be mainly responsible for these fluctuations.

As a consequence, an average calibration curve, based on the four sets of data given in *Table 3*, has been evaluated. The following relationship, valid for *N*-TFA PCL under our experimental conditions, has been obtained:

$$\log M = 8.132 - 0.1257V \qquad (9)$$

It is assumed that the validity range of the above relationship goes from *V* values of 18.5 ml to 36.5 ml, which corresponds to the overall elution range of the four samples.

On the basis of equation (9) and the proper conversion factor (1.848) for mass differences on the repeat units, $\bar{M}_n$ and $\bar{M}_w$ values for the four samples of PCL have been calculated and compared with the experimental values of *Table 1* (average values). The full set of data are listed in *Table 4*. The differences are within 10% for $\bar{M}_w$ and 14% for $\bar{M}_n$. Taking into account not only all errors typical of a g.p.c. analysis but also the experimental problems connected with the specific functionalization reaction, the agreement can be regarded as rather satisfactory.

Mark–Houwink relationship of N-*TFA PCL in methylene chloride*

The limiting viscosity numbers of the four samples after *N*-trifluoroacetylation have been measured in methylene chloride at 20°C with the aim of determining the relationship between the hydrodynamic volume of *N*-TFA PCL and the g.p.c. elution time in the above eluant. Indeed, the limiting viscosity number of a polydisperse sample is related to that of the *i*th molar mass through the following equation:

Table 3 Slope and intercept of equation (1) (Szewczyk's method)

Sample	Slope, B (ml^{-1})	Intercept, A
B_1	0.1294	8.190
B_2	0.1321	8.278
B_3	0.1251	8.158
B_4	0.1163	7.901

Table 4 Comparison between average molar masses calculated from g.p.c. on the basis of equation (9) and experimentally determined

	$\bar{M}_w \times 10^{-3}$		$\bar{M}_n \times 10^{-3}$	
Sample	G.p.c.	Meas.	G.p.c.	Meas.
B_1	31.4	28.7	16.0	14.0
B_2	37.3	35.9	18.9	16.8
B_3	47.4	51.8	21.0	23.1
B_4	59.5	58.3	23.2	26.3

Table 5 Intrinsic viscosity and viscosity average molar mass of PCL samples and *N*-TFA derivatives

			$\bar{M}_v \times 10^{-3}$	
Sample	$[\eta]_{\text{exp}}$ (cm^3 g^{-1})	$[\eta]_{\text{calc}}$ (cm^3 g^{-1})	*N*-TFA PCL	PCL
B_1	36.0	36.0	49.8	26.9
B_2	38.5	40.1	60.4	32.6
B_3	49.0	46.5	88.0	47.6
B_4	52.0	53.0	96.6	52.2

$$[\eta] = \sum_i w_i[\eta]_i \qquad (10)$$

or, by using the MH relationship:

$$[\eta] = \sum_i w_i K M_i^a = K \sum_i w_i M^a \qquad (11)$$

where *K* and *a* are the coefficients of the MH equation, and w_i and M_i are the weight fraction and the molar mass of the *i*th fraction, respectively.

By applying a trial-and-error procedure, it is possible to evaluate average values of *K* and *a* from the experimental data pertaining to $[\eta]$, w_i and M_i. Our method starts from an arbitrary value of *a*, chosen between the limits of 0.5 and 1, respectively, and evaluates the corresponding *K* for each sample, using the experimental $[\eta]$ values given in the second column of *Table 5*. The value of *a* is varied stepwise until the error ε, defined as:

$$\varepsilon = \sum_{n=1}^{4} \left([\eta]_{\text{exp},n} - \sum w_{i,n} K M_{i,n}^a\right)^2 \qquad (12)$$

reaches a minimum.

K and *a* values corresponding to the minimum ε are taken as MH coefficients. The derived equation is:

$$[\eta] = 3.355 \times 10^{-2} \bar{M}_v^{0.64} \quad \text{cm}^3\ \text{g}^{-1} \qquad (13)$$

The limiting viscosity numbers calculated from equation (11) and the viscosity average molar masses are quoted in *Table 5*. The excellent agreement between experimental and calculated values of $[\eta]$ is evident. As compared with the data of *Table 4*, $\bar{M}_v$ values lie, as expected, between $\bar{M}_n$ and $\bar{M}_w$ values, and very close to the latter.

Comparison with the universal calibration

Combining equation (13) with equation (9), it is possible to correlate the hydrodynamic volume of *N*-TFA PCL, expressed in terms of the product $[\eta]M$, with the g.p.c. elution volume in methylene chloride. The following relationship has been found:

$$\log([\eta]M) = 11.863 - 0.2061V \quad \text{cm}^3\,\text{g}^{-1} \qquad (14)$$

as represented in *Figure 1*.

In order to compare the above equation with the universal calibration curve, based on monodisperse polystyrene standards, we have used a set of PS samples ranging from 1.2×10^3 to 3×10^6 as molar masses. The g.p.c. data under the same experimental conditions used for *N*-TFA PCL provide the following relationship between M and V in the linear portion of the curve:

$$\log M_{\text{PS}} = 9.536 - 0.1755V \qquad (15)$$

which refers to V values between 21.5 ml and 37 ml (see inset of *Figure 1*).

The limiting viscosity numbers of all PS samples, mentioned above, have been measured. The corresponding MH equation for M values equal to or higher than 1×10^4 is as follows:

$$[\eta]_{\text{PS}} = 1.533 \times 10^{-2}\,M^{0.695} \quad \text{cm}^3\,\text{g}^{-1} \qquad (16)$$

From the experimental data of M and $[\eta]$, one gets for polystyrene samples in methylene dichloride:

$$\log([\eta]_{\text{PS}}M_{\text{PS}}) = 14.480 - 0.3040V \qquad (17)$$

which is also given in *Figure 1*. By combination of equations (15) and (16), equation (17a) is obtained:

$$\log([\eta]_{\text{PS}}M_{\text{PS}}) = 14.349 - 0.2975V \qquad (17a)$$

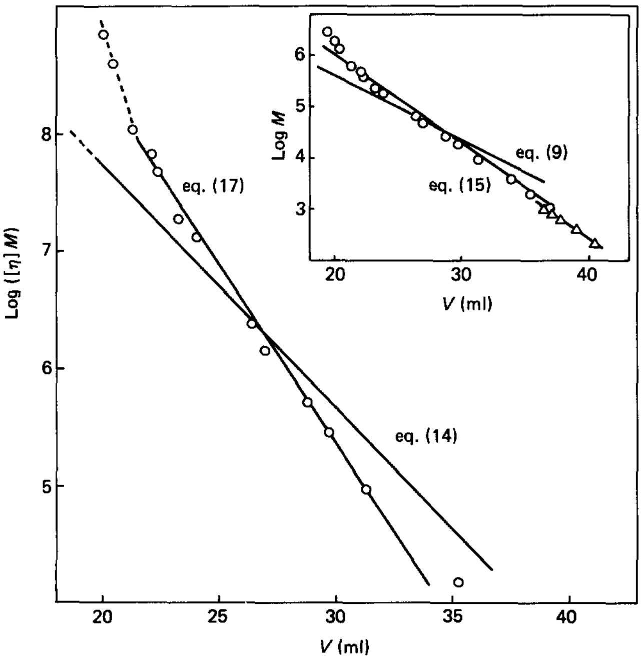

Figure 1 Log($[\eta]M$) vs. elution volume V for *N*-TFA PCL and PS (open circles) samples. The inset shows log M vs. V for *N*-TFA PCL, PS (open circles) and cyclic oligomers (open triangles)

As expected, equation (17a) is practically indistinguishable from equation (17).

A comparison between the two 'universal calibration' equations clearly shows a sharp difference in both intercept and slope, well beyond any experimental error.

A similar behaviour has already been found by Weisskopf[9] in tetrahydrofuran (THF), although with much less dramatic disagreement between the two sets of curves. Very recent results from Di Silvestro *et al.*[19] in methylene dichloride seem to reach conclusions close to ours. At variance with the findings of Weisskopf, elution volumes of the trifluoroacetylated cyclic oligomers (from monomer to pentamer) do not obey the same linear relationship valid for the linear polymer chains. From the data shown in the inset of *Figure 1* it is clear that cyclic oligomers (open triangles) are aligned much below the straight line of equation (9).

CONCLUSIONS

Our data, summarized in *Figure 1*, show earlier elution times as compared with the polystyrene standards for molar masses higher than $\sim 4 \times 10^4$, whereas higher retentions are found below this value. Corresponding behaviour is also shown by the $[\eta]M$ product.

The abnormal retention behaviour of our system reveals that, besides the primary steric exclusion mechanism, other interaction phenomena are also present. As a preliminary hypothesis, one can assume that the chosen eluant (methylene dichloride) does not entirely fulfil the requirements for minimizing secondary separation mechanisms, due to the relatively low value of a in equation (13). However, the poor solvent properties of the eluant towards *N*-TFA PCL, as compared to its relatively high a value for PS in equation (16), is unable to explain our results, provided that in similar systems solutes do not display any preferential affinity for the mobile or the stationary phase[20].

Non-exclusion interactions of the solute retention type have been interpreted in terms of solubility parameter (δ) differences; in our case, δ values are as follows: 19.3 MPa$^{1/2}$ for polystyrene, 20.2 MPa$^{1/2}$ for methylene dichloride and 20.8 MPa$^{1/2}$ for *N*-TFA PCL. The above values have been calculated from the three-component Hansen parameters and group contributions[21] and are in good agreement with the experimental data. The parameters found do not seem to support any model or theory able to explain our results. Indeed, retention due to adsorption or partition phenomena requires[20] $\delta_{\text{solvent}} > \delta_{\text{gel}}$ and $\delta_{\text{solute}} > \delta_{\text{solvent}}$, as in the case of poly(vinyl pyridine)$\leftrightarrow$($\delta = 21.4$)/chloroform$\leftrightarrow$($\delta = 19.0$/polystyrene ($\delta = 18.6$) gel[22].

A second type of non-exclusion interaction, which causes earlier elutions, is due to solute–gel incompatibility because of repulsive interactions between them. Incompatibility can be expressed in terms of Flory–Huggins interaction parameter χ and is dependent on the molar mass of the solute[23], as found for the system poly(vinyl acetate)/tetrachloroethylene. Both effects seem to be present in our systems. However, a unifying theory capable of justifying both retardation and earlier elution as functions of molar mass does not seem to be available at present, despite some recent attempts[24,25] to provide thermodynamic models for the non-exclusion phenomena in g.p.c.

The peculiar chemical structure of the functionalized poly(ε-caprolactam), characterized by strongly polar $-COCF_3$ groups pendant at regular distances along the main chain, should cause repulsive interactions with the column gel and justify, at least in part, the abnormal retention behaviour. Further studies based on quantitative thermodynamic approaches to our complex system are necessary before any definitive conclusion could be drawn.

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