Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 996 (2003) 45–51
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Determining and correcting ‘‘moment bias’’ in gradient polymer elution chromatography

André M. Striegel*
Solutia Inc., 730 Worcester Street, Springfield, MA 01151, USA

Received 15 November 2002; received in revised form 28 February 2003; accepted 25 March 2003

Abstract

Gradient polymer elution chromatography (GPEC) is rapidly becoming the analytical method of choice for determining the chemical composition distribution (CCD) of synthetic polymers. GPEC can be performed in traditional (strict precipitation–redissolution mechanism) or interactive (normal- and reversed-phase) modes, and results may be qualitative, semi-quantitative, or fully quantitative. Quantitative approaches have thus far relied on colligative or end group techniques for determining the values of standards used in constructing the GPEC calibration curve. While the values obtained from said methods are number-averages, they are assigned to the peak apexes of the standards (i.e. assigned as peak averages). This creates a determinate error in the quantitation, referred to herein as ‘‘moment bias’’. In this paper we determine moment bias for a series of styrene–acrylonitrile (SAN) copolymers, where the distribution and averages of the AN% have been measured using normal-phase (NP) GPEC. We also correct for the effect via statistical treatment of the chromatographic data.

Keywords: Moment bias; Gradient polymer elution chromatography; Styrene–acrylonitrile copolymers; Acrylonitrile

1. Introduction

The technique known as gradient polymer elution chromatography (GPEC) [1] is used for determining the chemical composition distribution (CCD) of copolymers (terpolymers, etc.). CCD is defined as the differential or cumulative distribution of the amount (usually in weight percent) of a particular functional group within a polymer, independent of molar mass. This is different from the chemical heterogeneity, which characterizes the average chemical composition of a polymer as a function of molar mass (i.e. in a chemical heterogeneity test only an average composition at each molar mass slice is obtained). To determine the CCD at each molar mass slice, a hyphenated SEC–GPEC experiment is needed, a non-trivial task. GPEC has quickly progressed from a qualitative to a semi-quantitative methodology, recently becoming a fully quantitative technique [2]. Problems arise, however, when attempting quantitation by GPEC, either in its traditional (precipitation–redissolution) mode [3] or when used in combination with sorptive phenomena (normal- and reversed-phase GPEC) [2,4,5]. These problems occur regardless of whether the CCD determination is semi- or fully quantitative.

As in any separation method, issues regarding chromatographic band broadening, detector response, etc. arise. Some of these, such as the response of the

*Tel.: +1-413-730-2560; fax: +1-413-730-2752.
E-mail address: amstri@solutia.com (A.M. Striegel).

0021-9673/03/$ – see front matter
doi:10.1016/S0021-9673(03)00536-3

evaporative light scattering detector (ELSD) (the most commonly used detector for GPEC) to variations in operational parameters or sample characteristics have been investigated extensively and continue to be the subject of current efforts [2,6–9]. A previous publication dealt with the linearity of the injection volume, solution concentration, and flow-rate dependences of our ELSD's response, as well as with the molar mass independence (outside of the oligomeric region) and chemical composition independence of the normal-phase (NP) GPEC methodology when quantitating the distribution of the vinyl alcohol percent in poly(vinyl butyral) [2]. Band broadening correction in GPEC is still in its infancy. Here, however, we attack a different problem.

Whenever quantitation is attempted in GPEC, the percent of one of the components of the copolymer is determined, for a set of well-characterized, relatively narrow distribution, monomodal samples (herein referred to as standards), by a known technique. These standards are used to construct a calibration curve from which percent composition and, hence, the chemical composition distribution, of unknown samples is determined. The methods used to initially characterize the standards range from titration [2,10] to near-infrared and nuclear magnetic resonance spectroscopy [8] to elemental analysis (present paper) and cloud point determination [11,12]. Though quite diverse, these techniques possess one underlying commonality: they are all colligative or end group methods and, hence, determine the number-average of the percent composition of the standards. The standards are never completely monodisperse, and the value obtained by the standard methods is then assigned to the peak apex of the chromatographic peaks. The resultant relationship between the retention times of the peak apexes and the compositional values assigned to them constitutes the basis of the GPEC calibration curve. The problem that arises, however, is that *the values assigned as peak-averages are actually number-averages* (Fig. 1A). This constitutes a source of error, which we term here ''moment bias'' (Fig. 1B), i.e. the values for the standards will be displaced by one statistical moment of the distribution.

In this paper we describe a method to both determine and correct moment bias in GPEC, illustrated for a series of styrene–acrylonitrile copolymers (SAN, Fig. 2) with varying AN composition as studied by NP-GPEC. The moment bias investigation undertaken here is independent of sample composition or detector response, as the statistical calculations were applied to the same dissolutions/injections of each sample. It will also be shown that the NP-GPEC method is independent of molar mass effects.

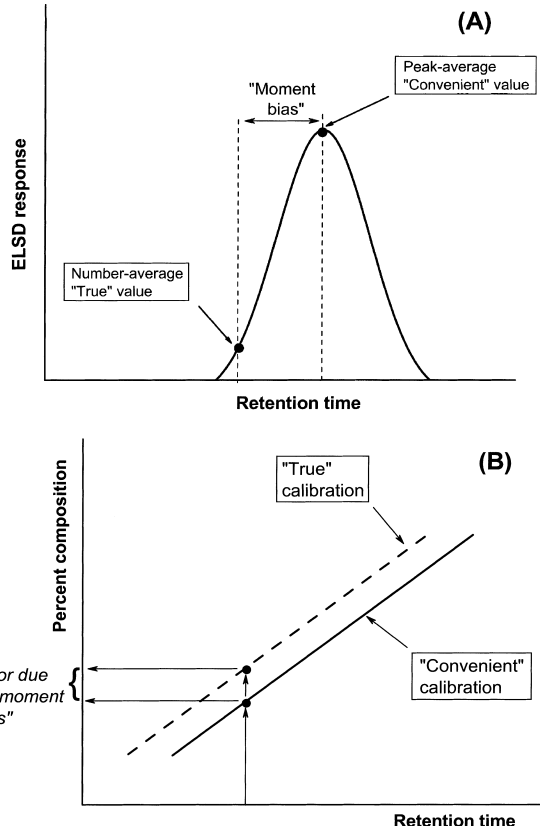

Fig. 1. (A) ''Moment bias'' resultant from assigning the number-average (determined via colligative or end group methods) to the peak-average of the GPEC peak of a calibration standard. (B) Consequences of moment bias for the GPEC calibration curve and the compositional percentages derived therefrom.

2. Experimental

2.1. Materials

The SAN samples used in this study were kindly provided by Bayer. Polystyrene-relative molar mass

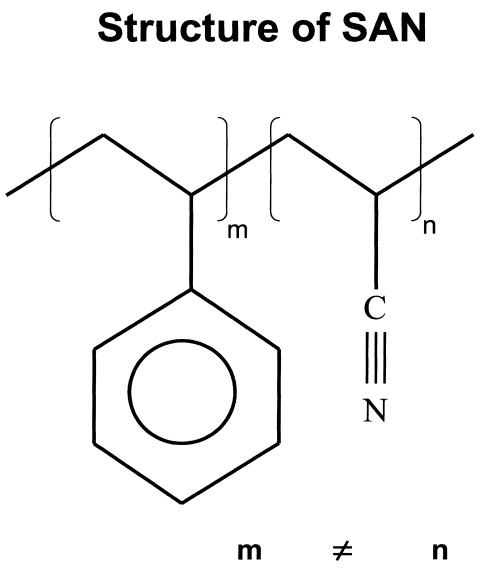

Fig. 2. Structure of SAN, poly(styrene-*co*-acrylonitrile).

averages and polydispersities (obtained by size-exclusion chromatography in tetrahydrofuran at 35 °C) are as follows: SAN1: $M_w = 152{,}000$, $M_w/M_n = 2.75$; SAN2: $M_w = 168{,}000$, $M_w/M_n = 2.39$; SAN3: $M_w = 197{,}000$, $M_w/M_n = 2.12$. Acrylonitrile content (in weight percent) is given in Table 1. The solvents used were purchased from Fisher (Pittsburgh, PA, USA).

2.2. NP-GPEC

The differential and cumulative distributions of the acrylonitrile percent (AN%) in the SAN samples, along with the concomitant number- and weight-averages and polydispersity in AN%, were determined by a normal-phase gradient polymer elution chromatography (NP-GPEC) method developed in our laboratory. Firstly, 50 mg of sample or standard were dissolved in 20 ml of *N*,*N*-dimethyl formamide (DMF) by shaking the solution in a laboratory shaker, at room temperature, for ~1 h. With extremely rare exceptions, this provided for both complete dissolution and complete solvation of the polymers, as observed using size-exclusion chromatography (data not shown). After dissolution, 20 μl of unfiltered solution were injected into a system consisting of a 600E System Controller (Waters, Milford, MA, USA), a 717+ WISP autosampler (Waters), and a PL-EMD 960 evaporative light scattering detector (Polymer Laboratories, Amherst, MA, USA). The mobile phase profile used in all measurements was: 0–7 min with 100% cyclohexane, 7–37 min using a linear gradient of cyclohexane–acetone (80:20, v/v), 37–47 min using a linear gradient of cyclohexane–acetone (30:70, v/v), 47–55 min using a linear gradient of acetone–acetonitrile (70:30, v/v), then back to 100% acetonitrile for 10 min. DMF allows for ready dissolution of the polymer regardless of AN%, while the cyclohexane–acetone gradient allows for both precipitation–redissolution as well as for selective displacement of acrylonitrile groups locally adsorbed onto the cyano packing of the column. Acetonitrile is used as a ''flush'' solvent to recondition the column at the end of each run, with the acetone–acetonitrile portion of the gradient allowing for a gentle transition into the flush solvent (this last step was found to extend column lifetime substantially). Mobile phase flow-rate was 1.0 ml/min. Compressed air flow in the ELSD system was maintained at 4.5–4.6 l/min and temperature at 55 °C. Separation occurred on a 250×4.6 mm IB-Sil 5-μm Cyano (CN) column (Phenomenex, Torrance, CA, USA) maintained at room temperature. Stan-

Table 1
Effect of ''moment bias'' on statistical moments and polydispersity of the chemical composition distribution

Sample	$(AN\%)_{EA}$	$(AN\%)_n$ uncorrected	$(AN\%)_n$ corrected	$(AN\%)_w$ uncorrected	$(AN\%)_w$ corrected	$PDI_{AN\%}$ uncorrected	$PDI_{AN\%}$ corrected
SAN1	40.4	39.9	40.6	41.2	41.9	1.03	1.03
SAN2	22.5	21.9	22.3	24.1	24.5	1.10	1.10
SAN3	59.0	57.0	58.3	60.7	62.2	1.06	1.07

Results constitute averages from two injections each from two separate dissolutions of sample. In all cases, standard deviations ≤0.1. $(AN\%)_{EA}$ corresponds to results of elemental analysis.

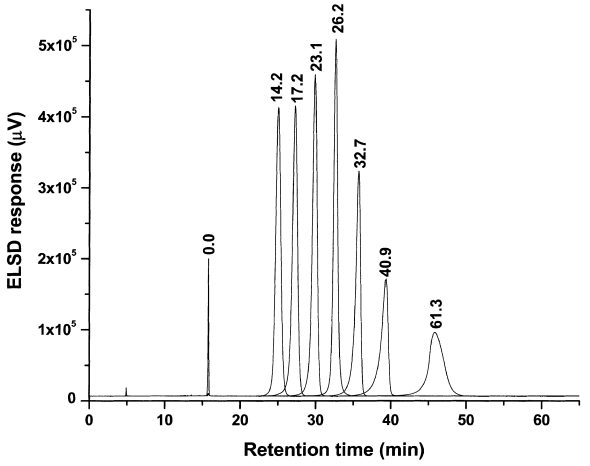

Fig. 3. Elution profiles of GPEC calibration standards. Numbers above peaks represent percent acrylonitrile (AN%) of each standard. ''0.0'' peak corresponds to polystyrene used for copolymerization of SAN.

dards were run in triplicate, samples in quadruplicate, the latter comprising two injections each from two separate dissolutions. Calibration of the system was performed using a series of eight SANs with acrylonitrile percentages varying from 0.0 to 61.3% (the 0.0% standard is composed solely of the polystyrene used in the copolymerization of SAN) (Fig. 3). AN content for the standards was determined by elemental analysis (C, H, and N, assuming all the nitrogen present is from acrylonitrile). The resulting second-order curve (each point representing the average of three injections) had a correlation coefficient (r^2) of 0.996 (see Fig. 4). Consistency of the calibration was checked with the 23.1, 40.9, and 61.3% standards. Data were acquired using Turbochrom Navigator (V. 6.1.2.0.1:D19, Perkin-Elmer, San Jose, CA, USA). Data processing and calculations were accomplished using a custom-designed Origin (OriginLab, Northampton, MA, USA) program, the details of which have been outlined in a previous publication [13].

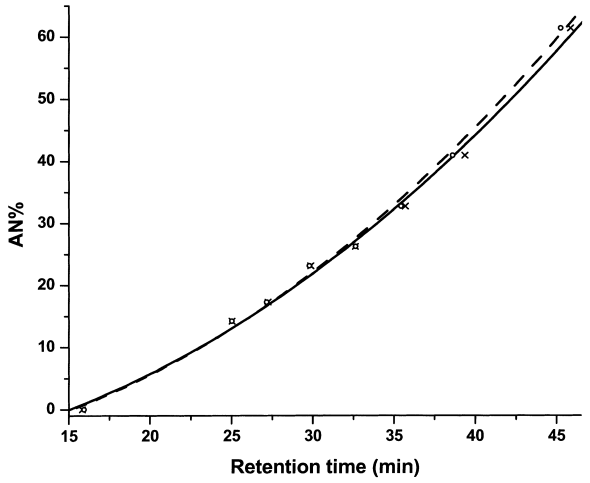

Fig. 4. NP-GPEC calibration curve and effect of moment bias. (×) correspond to values for the standards using ''convenient'' assignment, (○) to moment bias-corrected (''true'') values. Solid line is ''convenient'' calibration curve, dashed line is the corrected curve. Both curves are second-order. Data points represent averages of triplicate determinations, with standard deviations substantially smaller than data points and, therefore, not shown.

3. Results and discussion

In order to determine and correct ''moment bias'' in GPEC, we must first do one of two things: either determine the correct compositional percent of the sample eluting at the peak apex of the standards, or find the location on the peak (i.e. the retention time) of the number-average, as the latter is the value we obtain when analyzing the standards using colligative or end group methods. Here, we have opted for the latter, as we found it to be the more manageable approach. The number-average of any distribution is statistically defined as the first-moment about the center of mass of the distribution. In general, the kth moment of a distribution about a fixed point, x_0, is given by Eq. (1) [14]:

$$k^{\text{th}}\ \text{moment} = \sum_i f_i (x_i - x_0)^k \quad (1)$$

where

$$f_i = n_i / \sum_i n_i \quad (2)$$

and n_i is defined as the number of particles in each class. In the present case, x_0 corresponds to the retention time of the peak apex, x_i to the retention time of each data slice, n_i to the response of the ELSD detector at each slice and, for the number-average $k=1$. It was brought to this author's atten-

tion during review that a similar procedure was developed by Teremachi et al. over a decade ago [15]. In those experiments, involving PMMA-*g*-PS, the average styrene content was determined by ^{1}H NMR and the approach given above was iteratively combined with the equation for the calibration curve. The average compositions calculated by those authors' method were found to be within 0.1–3.2% of the experimental values. It appears that the method of Teremachi et al. is slightly more accurate than ours, though our approach is both more precise and easier to implement.

We applied the above formalism of Eqs. (1) and (2) to each injection of each of the calibration standards for the NP-GPEC method used for studying the distribution of the AN% in SAN. The resulting moment bias-corrected calibration curve is overlaid upon the uncorrected curve in Fig. 4. For illustrative purposes the effect is shown, in exaggerated fashion, in Fig. 1B for a generic calibration curve, where the curve obtained by assigning the values from colligative or end group methods to the peak apexes is described as a ''Convenient'' calibration, whereas the curve constructed from assigning the same values to the first moment of the peaks is referred to as the ''True'' calibration.

The correction shown in Fig. 4 appears to be quite minor; so much so that one may legitimately ask whether there is any effect whatsoever. To illustrate the validity of the moment bias effect and its consequences, Table 1 gives the number- and weight-averages of the AN% distribution, with the corresponding polydispersities ($PDI_{AN\%} = (AN\%)_w / (AN\%)_n$), for three SAN samples located in low, middle, and high AN% regions of the calibration curve. In all cases there is a noticeable change in the averages post-correction. The adjustment is more marked for the sample located in the high AN% region, which is where the corrected and uncorrected calibration curves differ most. This is due to the fact that the standards with higher AN% are also broader (Fig. 3) and, hence, the moment bias as illustrated in Fig. 1A will also be greater. It is worth noting that moment bias does not appear to affect the polydispersity of the chemical composition distribution in the present cases. However, when/if broader standards are used for a calibration, the difference between the peak- and number-averages will be greater, moment bias will be more pronounced, and the correction of the weight-average may be greater (or smaller) than that of the number-average, such that changes in the CCD's polydispersity will be observed.

Figs. 5 and 6 show both the differential and cumulative CCDs of SAN1 and SAN3, respectively (SAN2 is a monomodal sample, i.e. its distributions are similar in shape to those of SAN3). The figures show the uncorrected and the moment bias-corrected distributions and display graphical evidence that the effect is both real and measurable. In the case of SAN3, the $(AN\%)_n$ has shifted by more than 1.2%, highly exceeding the precision of the determinations

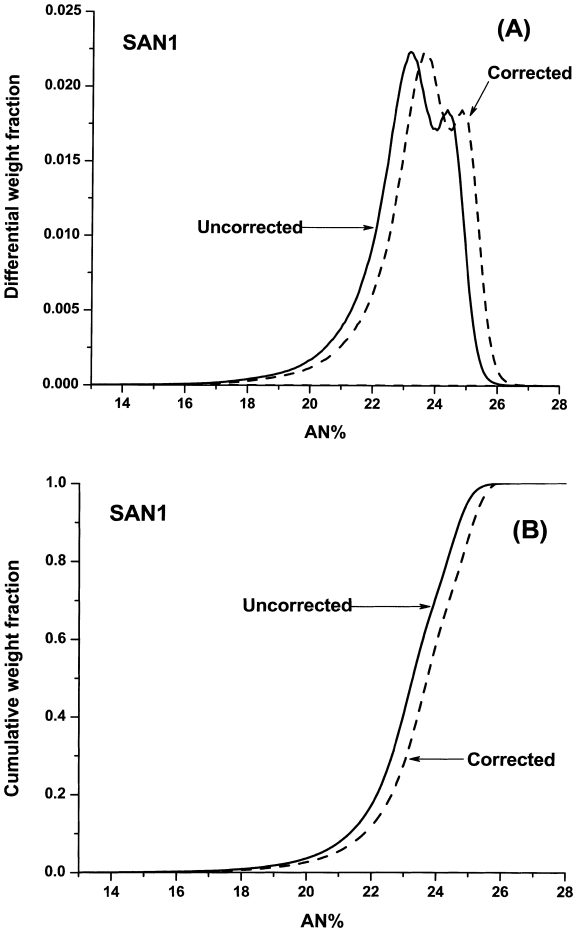

Fig. 5. Moment bias-corrected and -uncorrected AN% distributions for SAN1. (A) Differential distribution. (B) Cumulative distribution.

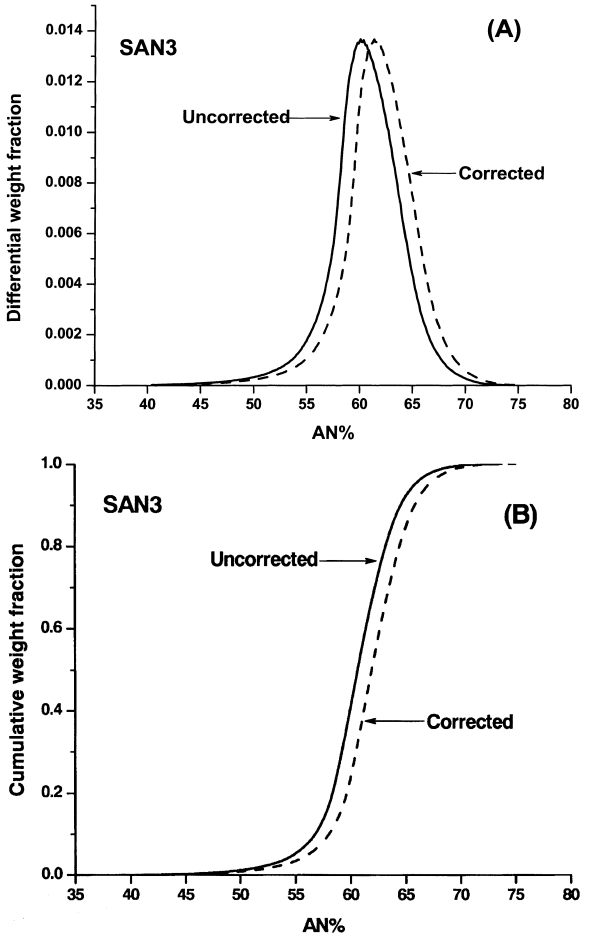

Fig. 6. Moment bias-corrected and -uncorrected AN% distributions for SAN3. (A) Differential distribution. (B) Cumulative distribution.

(±0.1%) and certainly enough to affect the polymer's performance properties [16,17].

Elution in GPEC can be adversely affected by molar mass factors, and the response of ELSD detectors has sometimes been found to be non-constant in the oligomeric region of molar mass (see Refs. [6,8] for differing views on the subject). For the present analysis, as given in the Experimental, these are relatively high molar mass polymers where even the lowest portion of the molar mass distributions (MMD) of the samples is well above the oligomeric region (~0% of the MMD is below 5 KDa), thereby obviating any detector bias with respect to molar mass. Table 2 shows the results of the NP-GPEC analysis of two SAN samples with identical AN% (as determined by elemental analysis), and with identical molar mass polydispersities, but with highly (~25%) different molar mass averages (note that the molar mass polydispersities of these two samples are, essentially, identical thereby eliminating the PDI_M as a factor). Results for AN% averages and polydispersity are observed to be virtually invariant to molar mass effects, in accord with other NP-GPEC methods developed in our laboratory [2,18].

Several points should now be made. Firstly, once the GPEC calibration curve has been corrected for moment bias, there is no need to correct the individual samples, as the correction will be automatically applied to the samples through the calibration curve. Secondly, the statistical data treatment described above is independent of sample or detector effects, as the corrected and uncorrected results in Table 1 and Figs. 4–6 correspond to the same injections of each sample/standard. This does not mean, however, that other corrections (e.g. band broadening corrections) are not necessary in order to ensure more accurate data. As a matter of fact, band broadening will make moment bias appear worse than it may actually be. At this moment, it is impossible to tell which effect, band broadening or moment bias, may be more severe as a study of the former has not yet been realized. Thirdly, as mentioned in the Introduction, it should be noted that the CCD and statistical moments and polydispersity associated with it are for a sample as a whole, i.e. no correspondence between a particular CCD slice and a

Table 2
Effect of molar mass on the determination of AN% averages and polydispersity

Sample	M_w ($\times 10^5$)	PDI_M	$(AN\%)_n$	$PDI_{AN\%}$	$(AN\%)_{EA}$
SAN4	1.91	2.27	23.2	1.09	23.0
SAN5	1.45	2.30	23.0	1.09	23.0

$PDI_M = M_w/M_n$; $PDI_{AN\%} = (AN\%)_w/(AN\%)_n$; $(AN\%)_{EA}$, acrylonitrile weight percent by elemental analysis. All AN% results are corrected for moment bias. Molar mass data are polystyrene-relative, obtained by SEC in THF at 35 °C.

particular molar mass slice can be drawn. For this, a hyphenated two-dimensional liquid chromatographic experiment is needed [19].

4. Conclusions

We have demonstrated here the existence of an effect termed ''moment bias'', through which the chemical composition distribution of polymers, as determined via gradient polymer elution chromatography, is adversely affected. We determined the bias for the acrylonitrile percent distribution in a series of styrene–acrylonitrile copolymers, as quantitated using normal-phase GPEC. While seemingly insignificant when comparing moment bias-corrected and -uncorrected calibration curves, the effect is seen to be both real and important when examining the results of the NP-GPEC experiments. Corrections of the statistical moments of the AN% distribution reached values as high as 1.2%.

We have observed similar effects in the corrected and uncorrected moments and distributions of the vinyl alcohol percent in poly(vinyl butyral), for which we recently introduced a fully quantitative determination by NP-GPEC [2,18]. In that case, the values for the standards were determined by an ASTM titration method [10], while in the present case the standards were characterized by elemental analysis. Other methods of characterization such as nuclear magnetic resonance spectroscopy, near infrared spectroscopy, or cloud point determination are bound to suffer from the same effect, as all of these are either colligative or end group methods that measure the number-average of the CCD.

Acknowledgements

The author would like to thank Victoria F. DeSantis (Solutia) for her assistance with sample analysis, and Nancy K. Lape (Department of Chemical Engineering and Materials Science, University of Minnesota) for interesting discussions regarding statistical moments.

References

[1] C.F. Poole, The Essence of Chromatography, Elsevier, Amsterdam, 2003.
[2] A.M. Striegel, J. Chromatogr. A 971 (2002) 151.
[3] S.V. Greene, T.T. Khau, G.P. Stitcher, D.O. McCunn, D.L. Wooton, in: Paper presented at the 4th International Conference on Advanced Polymers via Macromolecular Engineering, Gatlinburg, TN, August, 2001.
[4] M.A. Quarry, M.A. Stadalius, T.H. Mourey, L.R. Snyder, J. Chromatogr. 358 (1986) 1.
[5] W.J. Staal, P. Cools, A.M. van Herk, A.L. German, J. Liq. Chromatogr. 17 (1994) 3191.
[6] R. Schultz, H. Engelhardt, Chromatographia 29 (1990) 517.
[7] B. Klumperman, P. Cools, H. Philipsen, W. Staal, Macromol. Symp. 110 (1996) 1.
[8] H.J.A. Philipsen, B. Klumperman, F.A.M. Leermakers, F.P.C. Wubbe, A.L. German, Chromatographia 55 (2002) 533.
[9] P. Schoenmakers, F. Fitzpatrick, R. Grothey, J. Chromatogr. A 965 (2002) 93.
[10] ASTM, ASTM Method D 1396-92, in: Annual Book of American Society for Testing Materials, ASTM, Philadelphia, PA, 1992, p. 286.
[11] W.J. Staal, Ph.D. Dissertation, Eindhoven University of Technology, Eindhoven, The Netherlands, 1996.
[12] V. Verhelst, P. Vandereecken, J. Chromatogr. A 871 (2000) 269.
[13] S. Goodchild, J. Hurlbut, Am. Lab. 30 (1998) 44.
[14] P.C. Hiemenz, in: 2nd ed, revised and expanded, Principles of Colloid and Surface Chemistry, Marcel Dekker, New York, 1986.
[15] S. Teremachi, A. Hasegawa, T. Matsumoto, K. Kitahara, Y. Tsukahara, Y. Yamashita, Macromolecules 25 (1992) 4025.
[16] D.W. van Krevelen, in: 2nd completely revised ed, Properties of Polymers, Elsevier, Amsterdam, 1976.
[17] J.A. Brydson, in: 5th ed, Plastics Materials, Butterworths, London, 1989.
[18] A.M. Striegel, Polym. Mater. Sci. Eng. 88 (2003) 402.
[19] D. Hunkeler, A. Bartkowiak, F. Sauzedde, Am. Lab. 32 (2000) 44.