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Journal of Chromatography B, 865 (2008) 7–12
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Size exclusion chromatography with evaporative light scattering detection: Method for the determination of polydimethylsiloxanes II. Application of TSK-GEL $H_{HR}GMH_{HR}$-M column to determine and separate molecular weight of linear polydimethylsiloxanes

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Received 8 August 2007; accepted 11 December 2007
Available online 17 February 2008

Abstract

Issues concerned with molecular weight distribution analysis of linear polydimethylsiloxanes have not been extensively investigated and mastered, yet. Current publications do not provide detailed research data on the evaluation of the polymerization degree of polydimethylsiloxanes (PDMS) present in variable matrices: e.g. pharmaceuticals, cosmetics, foodstuffs nor indicate molecular weights of the polymer used. However, the information on molecular weight, i.e. viscosity, is of primary importance as it directly affects PDMS toxicity, absorption and migration in the living organism. The vast majority of currently applied methods prove to be insufficiently specific for PDMS of a particular molecular weight and therefore alternative analytical methods have to be further researched. In this paper the results of determination of molecular weights in linear polydimethylsiloxanes, using size exclusion chromatography with the evaporative light scattering detector are described. The column calibration curve obtained from low-dispersion standard polystyrene of molecular weights ranging 376–2,570,000 Da was used to determine PDMS molecular weights. Precision and accuracy of determination was obtained. For the mobile phase flow-rate of 0.3 ml/min relative standard deviation RSD ranged to 0.45% and the accuracy of measurement amounted to −0.42%, whereas for flow-rate of 1.0 ml/min RSD ranged to 0.38% and accuracy to +2.15%.

Keywords: Polydimethylsiloxanes; Size exclusion chromatography; Gel permeation chromatography; Evaporative light scattering detection

1. Introduction

The review of literature indicates that the problems of molecular weight distribution analysis of linear polydimethylsiloxanes (PDMSs) have not been well researched to date. There are few publications illustrating detailed investigations related to the polymerization degree of PDMS, which are present in various matrices such as pharmaceuticals or foodstuffs. However, data referring to molecular weight, and at the same time viscosity, are vital, as viscosity directly influences PDMS toxicity, absorption and migration in the living organism [1–14]. The majority of the methods applied to date absorption atomic spectrometry (AAS), emission atomic spectrometry (EAS), infrared spectroscopy (IR), Fourier transform infrared spectroscopy (FTIR), proton nuclear magnetic resonance spectroscopy (^{1}H NMR), gas chromatography (GC), reversed-phase high-performance liquid chromatography (RP-HPLC) are not adequately molecular weight distribution analytical to determine PDMS with a given molecular weight [15]. Lack of speciation methods, suitable for the analysis of PDMS, calls for searching alternative analytical methods. Bearing in mind the superior aim of investigations, owing to which the speciation analysis of PDMS in variable matrices (e.g. pharmaceuticals, foodstuffs or biological samples) will be possible the advantages of size exclusion chromatography are just conspicuous. The literature contains very few examples that describe exclusion chromatography for the purposes of the analysis of PDMS, refer to environmental investigations. The problem tackled by several

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doi:10.1016/j.jchromb.2007.12.029

authors was related to the assessment of pollution of the environment by the silicaorganic compounds that differ in molecular weights. Molecular weights affect the solubility of these polymers in the ecosystem, as well as their volatility [16–21].

Fendinger et al. [16] using exclusion chromatography with inductively charged plasma (GPC-ICP) and high-performance liquid chromatography, also with inductively charged plasma (HPLC-ICP) carried out tests for the:

(a) determination of PDMS as a wastewater pollutant,
(b) observation of PDMS "behavior" in the water environment, and
(c) evaluation of PDMS influence on the environment.

Soil samples were extracted with tetrahydrofuran, which also fulfilled the role of the mobile phase. The purpose of exclusion chromatography was only to separate PDMS from the soil matrix, and the expected products of PDMS hydrolysis (low-molecular fractions which were generated as a result of polymer decomposition) were separated using HPLC-ICP with reverse-phase C_{18}. PDMS were extracted from sediment, amended soil, sludge and influent and their retention times were compared with the retention time of standard PDMS of viscosity 350 cSt.

The shape of a peak coming from sludge and influent testified to the fact that the sample contained a wider range of molecular weights, nonetheless a suitable distribution could not be obtained. Therefore in addition, chromatography in the reverse phases was used. From the determination tests using two chromatographic methods it could be stated that PDMS present in the environment disintegrated into low-molecular compounds. Unfortunately, with chromatography in the reverse phases only polar low-molecular silanols could be identified [17], but not PDMS of a high-molecular weight occurring as an active substance in drugs or as a functional additive E-900 in food.

Many works of Lehmann and co-workers [18–21] also confirmed the ability of PDMS found in soil to degrade. Low-molecular PDMS, which originate as a result, are water-soluble and subsequently freely penetrate the environment in water. The authors used exclusion chromatography to isolate from the matrix a fraction including PDMS, and next PDMS could be separated with HPLC. It was found out that Si–CH_3 bonds are not degraded, whereas Si–O–Si are. Results showed that PDMS is unstable in the soil. This knowledge confirms the fact that PDMS is prone to transform, although it was believed to be a very stable, non-reactive, non-degradable or non-biodegradable polymer.

Dorn and Skelly Frame [17], similarly like in the test described above, joined size exclusion chromatography with the inductively charged plasma to separate and determine polydimethylsiloxanes. Samples from variable environmental matrices do not contain PDMS in high concentrations and therefore, a suitable type of detection which could allow to detect even the slightest amounts of silicaorganic compounds in water and organic solutions was searched. Chromatographic distribution of polymers of a great degree of polymerization and large molecular weights was carried out using a set of two columns: TSK-GEL GMH_{XL} with styrene–divinylbenzene packing, at the mobile phase low speed 0.7 ml/min. The mobile phases in this test were non-polar solvents: xylene and tetrahydrofuran. These solvents were suitable for the extraction of PDMS which differ in molecular weights and they did not disturb the determination using ICP detector. PDMS polymers of molecular weights 1500, 18,000 and 40,000 Da were dissolved in xylene and created a mixture of three solutions.

Andersson et al. used size exclusion chromatography with a refractometric detector [22] for the quantitative determination of polydimethylsiloxanes in pharmaceuticals. The aim of their research was to separate PDMS from smaller molecules, like carboxypolymethylene, which was the element of the pharmaceutical preparation matrix. The authors obtained one chromatogram including one peak, but they did not identify which molecular weight it responds to. It can be presumed that the extract from the pharmaceutical preparation responds quantitatively to a standard formula, known as Antifoam M manufactured by Dow Corning.

Size exclusion chromatography is also applied to the analysis of the ophthalmologic samples. The aim of tests carried out by Lakits et al. was to determine whether PDMS of viscosity 5000 cSt, used as a substitute of a vitreous body in patients, is chemically stable [23]. Earlier findings about detected presence of low-molecular products from PDMS degradation in silicone oils, and some disturbing signals of presumed toxicity of these compounds leading to chronic diseases of the eye, forced the researchers to assess the stability of the methylsilicone oil (PDMS). The material for tests was samples of the methylsilicone oil obtained from the vitreous bodies of 25 patients, in whom this material was used for ca. 9.2 months (maximum to 26 months). From the tests carried out on several columns the authors stated that using silicone materials in ophthalmology is safe, due to the fact that no peaks were observed with retention time longer than it was established for the PDMS standard of viscosity of 5000 cSt. Such a conclusion seems to be too hasty, because the authors in their paper did not include validation of the analytical method specifying, firstly and foremostly, the detection limit. It cannot be ruled out that PDMS degrades into lower PDMS molecular weights and that very small concentration may be below the method detection limit.

Exclusion chromatography was also used for the tests of implants. Liu et al. [24] tested PDMS of a linear structure which were used as implants. They were fully characterized using two methods, exclusion chromatography with refractometric detector and mass spectrometry. Tests were aimed not only to establish molecular weights of the polymers and their decomposition but also to confirm the size of polymers, and end function groups. Size exclusion chromatography was used to collect the eluate fraction including PDMS of variable molecular weights, and PDMS identification was carried out by means of mass spectrometry.

This paper describes test results which testify to the possibility of using size exclusion chromatography with the evaporative light scattering detector to separate and determine molecular weights of polydimethylsiloxanes of linear structure.

2. Experimental

2.1. Instrumentation and chemicals

Test equipment comprised: evaporative light scattering detector (ELSD) manufactured by BBT Automatyka Sp. z o. o. Polska-model 030195 (radiation source: laser diode Toshiba 10 mV 635 nm, Japan; photodetector: photoelectric multiplier Hammamtsu K-372 HA; signal measurement range: 0–200 nA; temperature range—drift tube: 25–120 °C, measurement cell 25–120 °C; evaporation gas: CO_2), TSK-GEL $H_{HR}GMH_{HR}$-M column, with polystyrene–divinylbenzene packing of Tosoh Biosep Company (5 μm, 300 mm × 7.8 mm) (Poznań, Poland); Mini Star K 500-pumping device manufactured by Knauer, Germany; injection loop of volume 20 μl manufactured by Knauer. Reagents used: chloroform was HPLC grade and purchased from Sigma–Aldrich (Poznań, Poland). Data storage was carried out by means of computer program Eurochrom 2000 by Knauer Germany. During studies constant measurement conditions were maintained. The samples were eluted with chloroform at a flow-rate of 0.3 ml/min during 15 min and 1.0 ml/min during 40 min. The temperature of drift tube and measurement cell amount to 60 °C and pressure CO_2 130 kPa.

2.2. Materials

Three mixtures of polystyrene standards were used to prepare column calibration curve:

(I) mixture of polystyrenes of molecular weights ranging 3420–2,570,000 Da,
(II) mixture of polystyrenes of molecular weights ranging 1620–1,090,000 Da, and
(III) mixture of polystyrenes of molecular weights ranging 376–702,000 Da.

Polystyrene standards were purchased from Sigma–Aldrich (Poznań, Poland) and were certified.

Tests were carried out for the following types of PDMS: polymers of linear structure of a low degree of polymerization (PDMS of viscosity 10 cSt), polymers of linear structure and of medium degree of polymerization (PDMS of viscosity 50 and 350 cSt), high-molecular polymers of linear structure (PDMS of viscosity 1000, 5000 and 60,000 cSt). PDMS purchased from Sigma–Aldrich (Poznań, Poland).

In order to assess the accuracy of determination of molecular weight standard PDMS of molecular weight 93,700 Da purchased from Aldrich Chemical Company, Inc. USA (Poznań, Poland) were used.

2.3. Preparation of samples

75 ml of chloroform was added to each of three tubes including four polystyrenes of various molecular weights and thus polystyrene of concentration ranging 0.067–0.134% was obtained. The solutions were left for 2 h for polystyrene mixtures to dissolve. Polydispersion coefficient for all polystyrene standards ranged from 1.02 to 1.11. This testified to the fact that applied standards characterized by low-molecular weight dispersion distribution.

PDMS standards were prepared as chloroform standards. For each PDMS standard of 10, 50, 350, 1000, 5000, 60,000 cSt solutions of concentration 0.5% were prepared.

3. Results and discussion

Applying exclusion chromatography to specify molecular weights of tested polymers or chemical compounds requires previous determination of a specific type calibration curve. These tests are necessary for every specific chromatographic column to determine its retention characteristic. Between the so-called total exclusion and total penetration there is an operating range, characteristic of a column in question. It is operating range that proves so useful to determine the analytes molecular weights.

3.1. Determination of dependence between molecular weight and retention time of standard polystyrenes

In order to specify dependence between molecular weight and retention time three mixtures of certified standard polystyrenes of different molecular weights were used.

Table 1
Retention time test results for separated polystyrene standard mixtures I–III at mobile phase flow-rate of 0.3 ml/min ($n = 7$)

Peak molecular weight (Da)	$\log M_p$	Retention time average value (min)	Standard deviation (s)	Relative standard deviation RSD (%)
2,570,000	6.4099	16.91	0.0756	0.45
1,090,000	6.0374	17.97	0.0391	0.22
702,000	5.8463	18.70	0.0270	0.14
250,000	5.3979	20.41	0.0462	0.23
130,000	5.1139	21.55	0.0337	0.16
67,500	4.8293	22.92	0.0318	0.14
34,700	4.5403	24.18	0.0454	0.19
17,800	4.2504	25.42	0.0438	0.17
8,400	3.9243	26.65	0.0489	0.18
3,420	3.5340	28.13	0.0496	0.18
1,620	3.2095	29.34	0.0604	0.21
376	2.5752	31.51	0.0559	0.18

Table 2
Retention time test results for separated polystyrene standard mixtures I–III at mobile phase flow-rate of 1.0 ml/min ($n = 7$)

Peak molecular weight (Da)	log M_p	Retention time average value (min)	Standard deviation (s)	Relative standard deviation RSD (%)
2,570,000	6.4099	5.07	0.0170	0.34
1,090,000	6.0374	5.40	0.0207	0.38
702,000	5.8463	5.60	0.0100	0.18
250,000	5.3979	6.07	0.0163	0.27
130,000	5.1139	6.46	0.0237	0.37
67,500	4.8293	6.84	0.0098	0.14
34,700	4.5403	7.20	0.0053	0.07
17,800	4.2504	7.59	0.0098	0.13
8,400	3.9243	7.95	0.0151	0.19
3,420	3.5340	8.38	0.0238	0.28
1,620	3.2095	8.76	0.0181	0.21
376	2.5752	9.43	0.0243	0.26

Since mixtures I–III of polystyrene standard had three different molecular weight ranges, for the purposes of clarity of this paper they were shown in one table, using mobile phase flow-rate. Table 1 shows results obtained at the flow-rate of 0.3 ml/min, whereas Table 2 shows results obtained at 1.0 ml/min. Superimposed chromatograms from the distribution of standard polystyrene mixtures I–III were also shown. Fig. 1a and b shows model chromatograms obtained at selected flow-rate of mobile phase.

From the measurements of retention times it was observed that the greater polymer molecular weight the shorter retention time. At each chromatographic process, polystyrene molecules of the highest molecular weights were fastest eluted from the column, whereas the lowest molecular weight particles were eluted slowest, due to the fact that they penetrated deepest bed pores. Results obtained from these tests also substantiate the major mechanism of separation in size exclusion chromatography. Superimposed chromatograms (Fig. 1a and b) confirm suitable separation abilities of a given column, though total separation of the mixture was obtained for polymers which differed in molecular weights irrespectively of the chosen mobile phase flow-rate. High precision of determination was also obtained, testifying to the results repeatability. In the mobile phase flow-rate of 0.3 ml/min relative standard deviation RSD ranged from 0.14 to 0.45%, whereas for flow-rate of 1.0 ml/min 0.07–0.38%. At the chloroform flow-rate of 1.0 ml/min noises increased slightly. The noises generation was most possibly concerned with the decrease in the efficiency of mobile phase evaporation in the detector drift tube and the solvent in the analyte molecules aerosol. However, the noises did not affect the repeatability of retention times. Values of relative standard deviation testify to the high precision in polystyrene separation in the range of molecular weights 2,570,000–8400 Da for a given column. Results were used to determine the dependence between mass logarithm M_p and the retention time t_r. The dependence of these parameters is called calibration curve, and their course was shown in Fig. 2a and b.

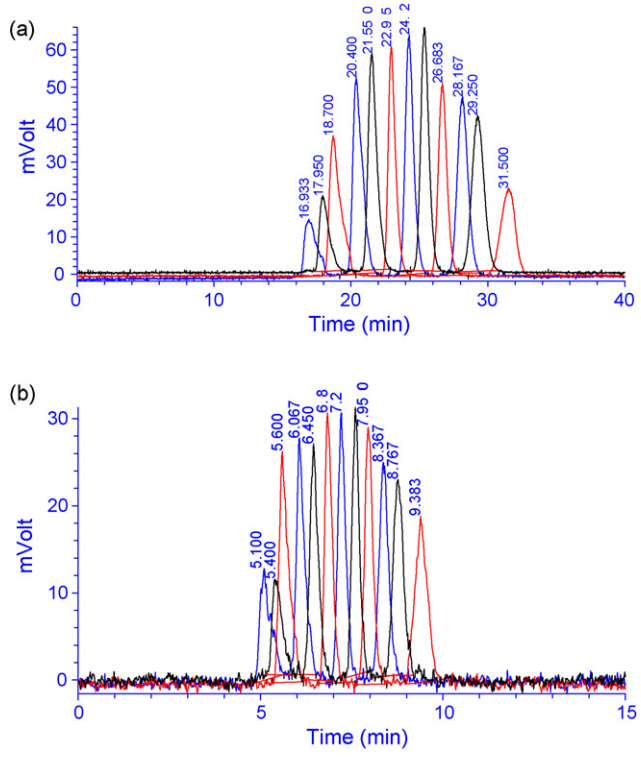

Fig. 1. Superimposed chromatograms from the distribution of standard polystyrene mixtures I–III at mobile phase flow-rate of (a) 0.3 ml/min ($n = 7$) and (b) 1.0 ml/min ($n = 7$).

Both diagrams (Fig. 2a and b) are of linear course, though inversely proportional. Parameters of linear regression equations were also calculated and shown in the figures. In the cases a high-determination coefficient R^2 (close to 1) was obtained. Moreover, it was confirmed that for the column TSK-GEL $H_{HR}GMH_{HR}$-M there exists a wide operating range which, irrespectively of the mobile phase flow velocity, amounts to 2,570,000–376 Da. Obtained regression equations served to determine PDMS molecular weights.

3.2. Determining PDMS molecular weights

Standard PDMS of viscosities ranging 10–60,000 cSt were tested. For each solution the measurements of retention times were being carried out: at the chloroform flow-rate of 0.3 ml/min for 40 min and at the flow-rate of 1.0 ml/min for 15 min. The table below shows mean values of retention times obtained

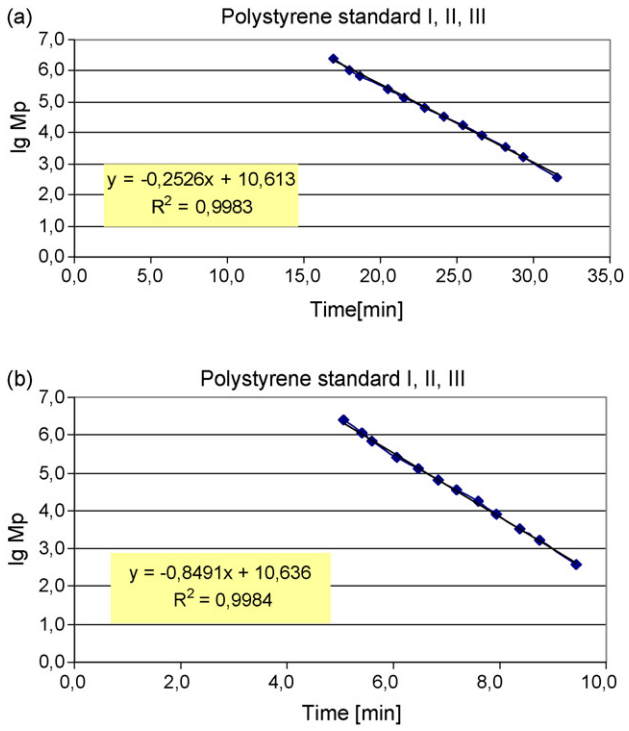

Fig. 2. Dependences between the logarithm and the molecular weight (M_p) of polystyrene standards and the retention time at the mobile phase flow-rate of (a) 0.3 ml/min and (b) 1.0 ml/min.

Table 3
Measurement results of retention times for PDMS of viscosities 10–60,000 cSt

Viscosity of PDMS (cSt)	Retention time mean value ($n = 7$) (min)	
	0.3 ml/min[a]	1.0 ml/min[a]
10	29.77	8.89
50	28.24	8.42
350	25.07	7.48
1,000	24.25	7.23
5,000	23.45	7.02
60,000	22.58	6.75

[a] Mobile phase flow-rate.

from seven independent measurements for the each viscosity of PDMS (Table 3).

PDMS molecular weights were calculated from obtained retention times results, based on linear calibration curves equations. The results were shown in Table 4.

No major differences were found between calculated masses for selected mobile phase flow velocities in these results.

Table 4
PDMS molecular weights calculated on calibration curves, with the use of polystyrene

Viscosity of PDMS (cSt)	Molecular weights (Da)		Difference ΔM (%)
	0.3 ml/min[a]	1.0 ml/min[a]	
10	1,239	1,223	1.31
50	3,017	3,066	1.60
350	19,069	19,263	1.10
1,000	30,722	31,405	2.20
5,000	48,925	47,350	3.30
60,000	81,151	80,274	1.10

[a] Mobile phase flow-rate.

3.3. Assessment of the accuracy of PDMS molecular weight determination from calibration curves with the use of polystyrene and precision of measurements

To assess the error of PDMS molecular weight determination from calibration curves with the use of polystyrene first, the accuracy of accepted methodology had to be evaluated. Accuracy was established on the basis of PDMS standard of molecular weight as declared by the manufacturer (Aldrich Chemical Company, Inc. USA) equal to 93,700 Da and concentration of 0.5%.

Tests were carried out at the mobile phase flow-rate of 0.3 and 1.0 ml/min. Method accuracy was determined on the basis of the relative error calculation. Molecular weight as stated by the manufacturer, i.e. 93,700 Da, was considered to be the real value. Table 5 shows mean value of retention time and a calculated mean value of molecular weight for PDMS standard. Mean values were obtained from seven independent measurements. The table also shows calculated value of the relative error, being the measure of molecular weight determination accuracy and precision as relative standard deviation from seven measurements of retention time.

From the results for the PDMS standard of molecular weight equal to 93,700 Da it was found out that when determining unknown PDMS molecular weights, calibration curves prepared on the basis of polystyrene prove quite applicable. Molecular weights determined by this method will be characterized by a slight error. The accuracy of the mobile phase flow-rate of 0.3 ml/min amounted to −0.42%, whereas for the flow-rate of 1.0 ml/min + 2.15%. High precision of determination was also obtained independent of flow-rate mobile phase. At flow-rate of 0.3 ml/min relative standard deviation was 0.35%, whereas for flow-rate of 1.0 ml/min 0.54%.

Table 5
Assessment of accuracy for determination of PDMS of molecular weight 93,700 Da on the basis of calibration curves for polystyrene and precision of measurements (1–7)

Mobile phase flow-rate (ml/min)	Retention time mean value (min)	Retention time (min)							Relative standard deviation RSD (%)	Calculated molecular weight (Da) (relative error)
		1	2	3	4	5	6	7		
0.3	22.34	22.35	22.42	22.41	22.40	22.20	22.35	22.28	0.35	93,307 (−0.42)
1.0	6.66	6.96	6.61	6.97	6.33	7.12	6.49	6.17	0.54	95,718 (+2.15)

4. Conclusions

Tests confirmed that size exclusion chromatography with the evaporative light scattering detector (SEC/ELSD) is a useful method to identify linear PDMSs. In order to determine PDMS molecular weight a column calibration curve obtained when using low-dispersion polystyrene standards of molecular weights ranging 376–2,570,000 Da may be applied, on the condition that the same analytical parameters at separation and determination of retention time for both polymers are maintained. Calibration curve determined by using polystyrene standards, describing the dependence between the molecular weight and retention time, proves a universal curve for PDMS. It is possible to regard the calculation of dependence for polystyrene as equivalent for PDMS. Differences in structures and properties between polystyrene and PDMS do not affect the differences between the dependence of the molecular weight and the retention time. The SEC/ELSD method developed in this work proved to be accurate and precise.

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