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Size exclusion chromatography with evaporative light scattering detection as a method for speciation analysis of polydimethylsiloxanes. I: Influence of selected factors on the signal intensity of the detector

Krystyna Mojsiewicz-Pieńkowska*

Medical University of Gdańsk, Faculty of Pharmacy, Department of Physical Chemistry, 80-416 Gdańsk, Al. Gen. Hallera 107, Poland

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ABSTRACT

Evaporative light scattering detector (ELSD) is widely recognized as a universal tool in chromatography. In this paper, the characteristics of the ELSD detector response and the influence of different factors on the signal intensity are described. Further, results are presented on the influence of some selected factors on the signal intensity and repeatability of results for linear structure polydimethylosiloxanes (PDMS), differing in molecular weight and viscosity. The following factors were studied: (i) the flow velocity of the nebulising gas, (ii) the temperature of the drift tube and the detection cell, and (iii) the flow velocity of the mobile phase, as they all constitute important parameters of the detector. Based on such studies, the optimal parameters of detector indications can be selected for a specific analysis. The results confirmed the possibility to select one set of values for those parameters that allow for analysis of linear PDMS molecules with viscosities ranging from 10 to 60,000 cSt. The following optimal and common parameter values were specified: temperature drift tube 50 °C, carrier gas pressure (for nebulisation) 140 kPa, and mobile phase flow rate 0.7 ml/min. A high repeatability of the results was demonstrated as the relative standard deviation was less than 2.5%. This type of tests for polydimethylosiloxanes has not been presented in any previous publication.

1. Introduction

For many years, the phenomenon of light scattering has been used in various measurements, e.g. for characterisation of biologically relevant particles, such as macromolecules, suspended solids and even microorganisms [1]. The light scattering phenomenon has allowed for development of the evaporative light scattering detector (ELSD). This detector enables detection of practically all relatively non-volatile analytes, and can therefore be classified as a universal device. The operational principle is based on measurement of the intensity of light scattered on particles of the analyte in aerosols. The light source may be different, such as: He–Ne laser with wavelength 635 nm or 670 nm, high efficiency blue Light Emitting Diode (LED) with wavelength 470 nm or 480 nm, high intensity halogen (polychromatic) lamp and another. Eluate from the chromatographic column is atomised in a nebuliser, by means of an inert carrier gas e.g. carbon dioxide, nitrogen, argon, air or helium. Thanks to this, evaporation of the solvent runs efficiently in the heated drift tube, and the remaining small droplets of non-volatile analyte are transferred to a detection cell, through which the laser beam is transmitted [2,3].

ELSD is generally viewed as a mass detector [4–6]. This means that the detector response depends on the size of the molecule of analyte, and the signal intensity is proportional to the mass. A common opinion is that the detector response is independent of the chemical structure of the analysed compound [4–7]. Based on such observations, it has been proposed that the detector may be useful in determining concentrations of chemical compounds, in situation when an analyst has not a standard of analyte, which is studied. Another suggested conclusion is that if the response of the ELSD detector depends only of the analyte mass, a universal calibration could be used too. The analyst could prepare one common calibration curve for different analytes. However, bearing in mind that different researchers have made different observations regarding the general applicability of one calibration curve, and have come to different conclusions, it appears that it is a large simplification to say that the ELSD detector is a mass detector. Although the intensity of the signal depends on the analyte mass, many other factors also have an impact. As the detector is increasingly used for various analyses, therefore a more detailed knowledge is needed about the mechanisms behind the formation of the signal (detector response). As has been pointed out by many authors [2,3,6–12],

* Tel.: +48 58 349 31 56; fax: +48 58 349 31 52.
E-mail address: kpienk@gumed.edu.pl.

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the most urgent problems to address are the detector response and how various factors affect the detector intensity.

It is important to recognize that the formation of the signal depends of the following two complex processes:

(a) the nebulisation and evaporation of the mobile phase and the size of the aerosol particles formed, and
(b) the scattering of monochromatic light on the particles generated.

Well-known theories are used to describe the scattering phenomenon, i.e. Rayleigh scattering, Mie scattering, reflection and refraction [4,8,13,14]. Rayleigh and Mie theories prove that the scattering intensity depends on the particle size, and therefore on the radius and the electromagnetic wavelength. Rayleigh scattering occurs when particles have a size smaller than the light wavelength and assumes that the particles are spherical. Mie scattering is applicable when particles are comparable to, or larger than, the electromagnetic wavelength, with a spherical or non-spherical shape.

Apart from the signal formation mechanism, it is also important to consider factors influencing on the nebulisation. A good understanding of all those aspects will facilitate optimisation of the signal. The signal intensity is a critical parameter in quantitative studies, especially when they involve the analysis of trace amounts. By combining the various theories that form the basis for the ELSD technique it is possible to identify the parameters that may influence the signal intensity. Based on published data [2,4,8,13], and my own observations, those factors can be divided in four groups:

(I) Parameters that determine the separation quality, and therefore cannot be freely changed. They include the flow velocity and composition of the mobile phase, injection volumes and sample concentration.
(II) Parameters that may be specified by the analyst. Those include the gas pressure in the nebuliser, temperature of the drift tube and detection cell, and the gain factor of the photo-multiplier.
(III) Parameters related to the physical and chemical properties of the analyte itself, i.e. state of matter, particle shape, molecular size and weight, degree of unsaturation, volatility, viscosity, density, surface tension, and refraction index.
(IV) Factors related to the kind of nebulisation gas used, e.g. carbon dioxide, nitrogen, helium or air, and the plausible influence of their heat conduction on the signal.

Researchers investigating the effect of nebulisation gas pressure, temperature and speed of the mobile phase on the signal have come to different results [2,3,6,7,13,15–23]. Many argue that the temperature is a critical parameter of the detector. The optimum temperature depends on the particular analyte, i.e. its volatility, stability, and the possibility to create uniform droplets, as well as the physical properties of the mobile phase (e.g. the boiling point). It was also noted that the mobile phase composition has influence on the particle size, which is critical for the intensity of the signal. The influence of the viscosity, density and surface tension of the mobile phase has been taken into account [15]. In most cases, for various compounds, different authors have reported the following general observations [2–4,7,13,16,18]:

- The slower the flow of the carrier gas (reduced pressure), the larger the aerosol droplets reach to the detection cell. As a result, the larger scattering of the laser rays, and a higher signal intensity can be observed.
- The higher the temperature of drift tube, the lower the intensity of the signal.
- The larger the flow rate of the mobile phase, the lower the signal intensity, as smaller particles are formed in the nebuliser prior to the laser beam. As a result, the lower scattering of the laser rays, and a lower signal intensity can be observed.

The purpose of this study was to assess the impact of a few selected factors on the signal intensity and the reproducibility of results for analysis of polydimethylsiloxanes (PDMS). The chosen factors were:

(I) the flow velocity of the nebuliser gas CO_2,
(II) the temperature in the diffusion tube and the detection cell, and
(III) the flow rate of the mobile phase.

The optimalization of these parameters is necessary for the concrete analysis. It should also exercise caution in deciding on the application of universal calibration curve before verifying the impact of factors, which are listed in four groups. No similar study has been made for the analysis of polydimethylosiloxanes (PDMS). The structure and properties of PDMS limit the analytical possibilities, which would be useful for these polymers in speciation analysis. The most optimal technique seems to be size exclusion chromatography. However, in the case of PDMS there are significant limitations when selecting a suitable detector. PDMS are widely used in pharmacy, medicine and the food and cosmetic industries, therefore a new detection methods are sought. In previous studies, the author noted that ELSD detector was a useful as detector for the analysis of PDMS of linear structure and viscosities range 10–60,000 cSt [24,25].

2. Experimental

2.1. Instrumentation

In this study, an evaporative light scattering detector manufactured by BBT Automatyka Sp. z o. o. Polska (model 030195) was used. The light source consisted of a laser diode, Toshiba 10 mV 635 nm, Japan. The ELSD detector set-up was as follows: a signal measurement range of 0–200 nA; a temperature range in the drift tube and the detection cell of 25–120 °C. The nebulising gas (carrier gas) was CO_2 of industrial purity grade. Chromatographic separations were carried out using a mini Star K 500 (Knauer, Germany) double piston pump, a manual sample injector (Knauer, Germany) equipped with a 20 μl loop, and a TSK – GEL $H_{HR}GMH_{HR}$–M column with polystyrene-divinylobenzen packing (5 μm, 300 mm × 7.8 mm) from the Tosoh Biosep company (Poznań, Poland). The Eurochrom 2000 (Knauer, Germany) data processing software was used to record and integrate the chromatograms.

2.2. Materials for test and chemicals

All reagents and chemicals used were of analytical grade and purchased from Sigma–Aldrich (Poznań, Poland). The following kinds of PDMS were analysed: polymers with a linear structure and low level of polymerisation (viscosity 10 cSt), polymers with a linear structure and medium level of polymerisation (viscosity 50, 300 and 350 cSt), and high-molecular polymers with a linear structure (viscosity 1000 and 60,000 cSt). Chloroform was used as the mobile phase.

2.3. Preparation of samples

For the experiments, 3.725 g PDMS with the viscosities 10, 50, 300, 350, 1000 and 60,000 cSt, respectively, were accurately

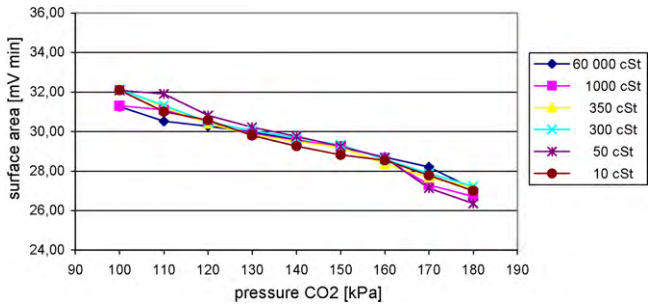

Fig. 1. Dependence of ELSD detector signal intensity on different pressure of nebulising gas (CO_2) for linear PDMS molecules with viscosities 10–60,000 cSt.

weighed and dissolved in 50 ml chloroform. Standard solutions with the concentration 5% were prepared. For each PDMS of a given viscosity, a 0.1% solution was also prepared.

3. Results and discussion

3.1. Influence of the carrier gas pressure on the detector signal

The flow rate of the carrier gas, CO_2, and hence its pressure, has a direct influence on the important process a forming a detector signal. From the flow rate of the carrier gas depends the nebulisation process of eluate (i.e. atomisation an aerosol). During this process homogeneous droplets of a specified size are formed. Flow rate of the nebulising gas may be regulated, within ranges that depend on the detector type. In the detector used in this study, the CO_2 pressure varied between 100 and 180 kPa, the temperature of the drift tube and detection cell was 50 °C, and the mobile phase flow rate was 0.7 ml/min. Peak areas were evaluated for 12 independent measurements for each PDMS (Fig. 1).

For PDMS with a linear structure and viscosities ranging from 10 to 60,000 cSt, the ELSD signal intensity (expressed as peak area) was clearly inversely dependent on the nebulising gas pressure (Fig. 1.), i.e. a higher CO_2 pressure resulted in, a lower signal intensity. In the CO_2 pressure range 120–160 kPa, the response of the signal to increases in gas pressure was similar irrespective of the PDMS viscosity. In this pressure range, the relative standard deviation (RSD), which is a measure of the repeatability of the peak areas, varied between 0.9% and 2.0% for a each PDMS.

At pressures below 120 kPa and above 160 kPa, however, the signals intensity differed more between PDMS. Those phenomena may have been caused by the nebulisation performance. At lower CO_2 pressures (100–110 kPa) large drops are formed, which may lead to incomplete evaporation of the mobile phase. This would influence the noise in the detector signal. Moreover, a slight deterioration of the repeatability of the signal intensity (a peak area) was also noticed for all analytes at low pressures, as the RSD increased to 3.8%. When larger drops are formed, the size is less homogeneous and differences in the light scattering may occur.

At lower pressures, problems with the capacity of the nebulising device were observed. It required frequent cleaning, which considerably prolonged the time required for the analyses. Increasing the CO_2 flow rate with a pressure above 160 kPa also resulted in an increase in RSD to 4%. In summary, CO_2 pressures in the range 120–160 kPa seemed optimal for the analysis of PDMS with viscosities between 10 and 60,000 cSt. From this range a one value of pressure CO_2 for specific analysis can be choose.

3.2. Statistical analysis of the influence of carrier gas pressure on the detector signal

A statistical analysis was made to establish whether there were significant differences in the detector's response (signal intensity) between analytes that differed in molecular weights and viscosities. Based on the experimental results presented above, the CO_2 pressure range with the most homogeneous response, i.e. 120–160 kPa, was selected and a one-way analysis of variance (ANOVA test) [26] performed for the different PDMS viscosities at two extreme pressure values within this range (Table 1).

Before using the one-way ANOVA to test the zero hypothesis that there was no difference between the mean values, the homogeneity of the variance (S^2) in the individual measurement series had to be tested. For this purpose an auxiliary zero hypothesis was formulated: H_0: $S1^2 = S2^2 = S3^2 = S4^2 = S5^2 = S6^2$ and verified with Hartley's test [25]. This test is used for verification of equal variance when dealing with more than 2 populations with equal number of observations in each population. In this case there were 6 pop-

Table 1
Integrated peak areas for ELDS detection of linear PDMS molecules with viscosities between 10 and 60,000 cSt at 120 and 160 kPa CO_2 nebulisation pressure.

Viscosity of PDMS [cSt]												
Number of measurement	10		50		300		350		1000		60,000	
	Pressure of CO_2 [kPa]											
	120	160	120	160	120	160	120	160	120	160	120	160
	Integrated peak area [mV min]											
1.	30.08	28.85	31.43	29.75	30.48	28.66	30.87	28.70	30.47	28.41	30.87	27.99
2.	29.74	29.01	30.63	29.28	30.23	28.14	29.85	28.23	29.95	27.77	29.68	28.45
3.	31.48	29.53	30.57	28.73	30.99	29.52	30.26	28.41	30.74	28.60	30.25	29.04
4.	31.49	27.78	30.53	27.83	31.02	29.20	30.14	28.99	31.25	28.63	30.02	28.19
5.	30.26	28.63	30.91	28.86	31.21	29.04	31.12	29.11	31.21	29.04	30.36	28.66
6.	30.28	28.41	30.78	28.68	30.21	28.87	30.13	27.87	30.97	28.50	30.14	29.06
7.	30.45	28.87	31.02	28.08	29.99	28.35	30.35	28.11	29.88	28.57	30.65	28.25
8.	30.62	28.58	30.15	28.92	30.10	27.99	31.32	28.02	30.26	28.64	29.99	29.20
9.	31.24	28.04	30.59	29.00	30.26	28.36	29.74	28.44	30.41	29.00	30.55	28.29
10.	30.61	28.31	31.68	28.59	30.47	28.41	30.20	28.35	30.52	28.72	30.31	29.10
11.	30.74	28.03	31.47	27.78	30.52	28.33	30.02	28.03	30.33	29.18	30.28	29.31
12.	30.02	28.55	30.06	28.69	30.01	28.25	30.87	28.22	29.58	28.71	30.17	29.00
Mean value	30.58	28.55	30.82	28.68	30.46	28.59	30.41	28.37	30.46	28.65	30.27	28.71
Standard deviation S	0.57	0.48	0.51	0.57	0.41	0.47	0.51	0.39	0.52	0.36	0.32	0.46
Variance S^2	0.32	0.23	0.26	0.33	0.17	0.22	0.26	0.15	0.27	0.13	0.10	0.21
Relative standard deviation RSD [%]	1.9	1.7	1.7	2.0	1.4	1.6	1.7	1.4	1.7	1.3	1.0	1.6

Number of objects (columns 10–60,000 cSt)—$k = 6$.
Number of observations (repeated measurements)—$n = 12$.
Overall number of observations—$n \times k = 72$.

Table 2
Results from a one-way analysis of variance of ELDS detector peak areas for PDMS molecules with viscosities between 10 and 60,000 cSt at CO_2 gas pressures of 120 and 160 kPa.

Variation	Sum of square, SS		Degrees of freedom, df		Mean squares, MS		Critical value calculated, F_{cal}		Probability, p-values		Critical value from the table, F_{tab}	
	Pressure of CO_2 [kPa]											
	120	160	120	160	120	160	120	160	120	160	120	160
Between groups	2.066	0.903	5	5	0.413	0.180	1.435	0.853	0.223	0.517	2.354	
Within groups	18.994	13.972	66	66	0.288	0.212						
Total	21.060	14.875	71	71								

ulations (number of objects – columns) with 12 observations in each (number of observations – repeated measurements) and the resulting F-values are given below:

$$CO_2 = 120\,\text{kPa}: \quad F_{\max} = \frac{S^2_{1\,\max}}{S^2_{2\,\min}} = \frac{0.32}{0.10} = 3.20$$

$$CO_2 = 160\,\text{kPa}: \quad F_{\max} = \frac{S^2_{1\,\max}}{S^2_{2\,\min}} = \frac{0.33}{0.13} = 2.54$$

At the level of significance $\alpha = 0.05$, the critical $F_{\max}$ was equal to 6.32 for $k = 6$ and the degrees of freedom $n - 1 = 11$, which is higher than the calculated F-values. It was thus concluded that there was no reason to reject the zero hypothesis of the homogeneous variance. Next, it was necessary to verify basic hypothesis of the homogeneity of the mean values. Data of one-way analysis of variance (test ANOVA) for $\alpha = 0.05$ for PDMS of linear structure and viscosities ranging from 10 to 60,000 cSt and CO_2 pressure 120 and 160 kPa have been presented in Table 2.

Due to the fact that on the accepted significance level $\alpha = 0.05$, F_{calc} were lesser than the value $F_{tab} = 2.354$, no basis for rejection of the hypothesis of the equality of average values was found.

The experimental results indicated that in the pressure range 120–160 kPa, there were no significant differences in the detector response for PDMS with viscosities of 10–60,000 cSt.

It was noticed that for CO_2 pressures ranging from 120 to 160 kPa, the signal strength was an inverse linear function of the gas pressure (Fig. 1). Therefore, the parameters of the rectilinear regression equation for the analytes were determined (Table 3).

The correlation coefficients for all PDMS tested confirmed the linear dependency (Table 3). Therefore, for practical purposes a general regression equation was calculated for all PDMS with viscosities 10–60,000 cSt. The value of the slope (a) amounted to −0.0463, the intercept (b) was 36.038 and the coefficient of determination (R^2) 0.9976 for the common dependency of the signal intensity on CO_2 pressure, valid in the range 120–160 kPa. It is worth noting that the parameter values of the common linear regression equation are similar to the ones in the equations for each specific PDMS type. This confirms the validity, and motivates the future use, of one common regression equation. The statistical analysis confirmed that the influence of CO_2 pressure on the signal intensity was the same for the tested PDSM compounds. Hence, the laser light scattering detector is neither influenced by the molecular weights of the analytes nor by viscosities ranging from 10 to 60,000 cSt.

Table 3
Slope a, intercept b and correlation coefficient r, for the progresses of intensities dependences of PDMS of viscosities 10–60,000 cSt on CO_2 pressure ranging from 120 to 160 kPa.

Viscosity of PDMS [cSt]	Parameters of regression equation		
	a	b	r
10	−0.050	36.45	0.9835
50	−0.053	37.12	0.9990
300	−0.045	35.93	0.9881
350	−0.047	36.09	0.9838
1000	−0.042	35.51	0.9915
60,000	−0.039	35.51	0.9975

The results also suggested that the optimal CO_2 pressure for determination of linear PDMS compounds with viscosities ranging from 10 to 60,000 cSt was 140 kPa. At this pressure, the signal intensity was quite significant and the nebulisation process proceeded correctly, which was confirmed by the lack of noise. Furthermore, the proper size of the generated aerosol droplets enabled complete vaporisation of the mobile phase, chloroform, and no clogging of the nebulizer was observed during the whole course of the experiment (such problems occurred at CO_2 pressures below 120 kPa). The relative standard deviation (RSD) for measurements of individual PDSM compounds with viscosities 10–60,000 cSt were 1.0–1.4% at 140 kPa, and no significant differences in detector response between the compounds were observed. This further confirmed the conclusion that the mechanisms underlying the signal generation are independent of molecular weight and viscosity of the analyte.

3.3. Influence of drift tube and detection cell temperature on the detector signal

An important step in the detection process is the evaporation of the mobile phase in the carrier gas stream, as this makes it possible to detect the laser light scattering by the "dry" analyte particles. An efficient evaporation of the mobile phase depends on the nebulisation of the eluate and an appropriate temperature. Therefore, the temperature of the drift tube and detection cell is an important parameter for an accurate detection process. The temperature should be chosen high enough for a complete vaporisation of the solvent (eluent), while not causing evaporation of the analyte. Because of this, the ELSD detector (considered to be a universal detector) is useful for detection of all non-volatile substances, or those that are considerably less volatile than the mobile phase. Taking into consideration the boiling points of solvents that typically constitute the mobile phase, e.g. chloroform, hexane, acetone, acetonitrile, methylene chloride, temperatures ranging between 45 and 60 °C in the drift tube and detection cell are sufficient for solvent evaporation. Increased temperatures in the drift tube and detection cell are needed to increase the flow of the eluate through the nebuliser. However, excessive temperatures may result in boiling of the mobile phase, which subsequently will lead to an increase in the noise level. Moreover, too high temperature may also negatively influence the analyte itself by increasing its volatility or even lead to degradation. Therefore, selection of the vaporisation temperature for a specific type of analysis is based on the character of the analyte and the mobile phase. For the detector used in the present study, temperatures could be selected in the range of 30–110 °C. Experiments were carried out at a CO_2 pressure of 140 kPa and a mobile phase velocity of 0.7 ml/min (Fig. 2).

It was noticed that for linear PDMS with viscosities ranging between 10 and 60,000 cSt, the ELSD signal intensity (expressed as peak area; mean of 12 independent measurements) depended on

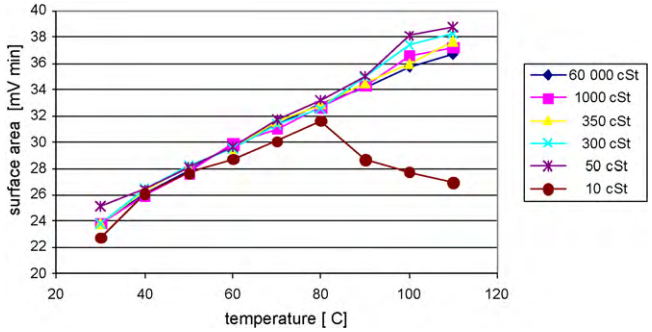

Fig. 2. Dependence of the detector signal intensity on the temperature of the drift tube for PDMS of viscosities 10–60,000 cSt.

the temperature in the drift tube and detection cell (Fig. 2). For viscosities between 50 and 60,000 cSt, higher temperatures resulted in larger peak areas, i.e. a higher signal intensity. For PDMS with a viscosity of 10 cSt, a decrease in the signal intensity was observed at temperatures above 80 °C. In the temperature range between 40 and 80 °C, the linear response was similar for all PDMS compounds analysed, independent of viscosity. The relative standard deviation RSD, for individual PDMS compounds, amounted to 1.3–3.0% for this temperature range. At extreme temperatures – below 40 °C and above 90 °C – the signal intensities differed between the different analytes. Furthermore, peaks separation was often observed, and both peaks shape and height were not repeatable in sequential measurements. The larger spread in the results was shown by the higher RSD value of 6%. For PDMS with the viscosity 10 cSt, a linear dependency of the signal intensity on temperature was only observed for temperatures in the range of 40–80 °C. Above 80 °C, the signal intensity decreased which might have been related to the analyte volatility, or caused by admixture of different volatile forms which used during generation of PDMS with the viscosity 10 cSt.

Next, experiments were performed to investigate the influence of the detection cell temperature on the ELSD signal intensity. The tests involved a constant carrier gas (CO_2) pressure of 140 kPa, and a constant temperature in the drift tube (60 °C). Three different temperatures of the detection cell were tested: 45, 60, and 75 °C, and three selected PDMS with viscosities 10, 350 and 60,000 cSt were analysed at each temperature.

It was observed that for the three linear PDMS the signal intensity did not depend on the detection cell temperature (Table 4). Therefore, the ELSD signal intensity was solely influenced by the drift tube temperature.

3.4. Statistical analysis of the influence of drift tube temperature on the ELSD signal intensity

To establish whether there were significant differences in the detector response (signal intensity) between specific PDMS analytes at the selected temperatures of 40, 50, and 60 °C, the data were subject to a statistical analysis. In this case, the crucial issue

Table 4
Testing the influence of detection cell temperature on signal intensity for PDMS of viscosities 10, 350, 60,000 cSt.

PDMS viscosity [cSt]	Temperature of detection cell and drift tube		
	45 °C, 60 °C	60 °C, 60 °C	75 °C, 60 °C
	Mean value of integrated peak area [mV min]		
10	28.59	28.69	28.53
350	29.96	29.84	29.91
60,000	30.37	30.35	30.29

Table 5
Results integrated peak area for PDMS viscosities 10–60,000 cSt at the temperature 40, 50 and 60 °C.

Viscosity of PDMS [cSt]																				
Number of measurement	10			50			300			350			1000			60,000				
	Temperature [°C]																			
	40	50	60	40	50	60	40	50	60	40	50	60	40	50	60	40	50	60		
	Integrated peak area [mV min]																			
1.	24.67	26.62	28.75	24.82	28.36	29.93	26.30	28.39	29.63	26.87	28.53	29.32	26.48	28.56	29.87	26.85	28.21	30.56		
2.	26.59	28.04	29.44	26.60	28.55	29.66	26.98	28.13	30.12	25.97	28.78	28.97	25.87	27.14	30.48	25.16	26.89	29.77		
3.	26.53	28.62	29.47	26.59	28.36	29.15	27.28	27.46	28.78	26.65	27.65	29.21	25.13	26.56	29.05	25.78	28.56	29.57		
4.	26.36	27.49	27.57	26.14	27.73	30.15	25.98	27.17	29.12	26.54	27.84	30.02	25.24	28.32	29.47	25.88	28.41	30.42		
5.	25.64	28.59	28.93	25.84	28.43	29.85	26.07	28.23	29.45	25.94	28.97	29.58	25.68	27.12	30.02	26.11	27.56	29.89		
6.	25.67	27.59	28.38	26.99	27.98	29.03	26.94	28.48	30.01	26.31	27.52	29.15	26.48	28.54	30.51	25.46	27.14	30.19		
7.	26.75	28.15	29.01	27.37	26.79	30.11	26.33	28.98	30.25	26.75	26.98	29.74	25.88	27.48	29.36	25.78	28.01	29.41		
8.	26.50	27.18	27.31	26.48	28.94	29.15	27.01	28.02	28.98	27.48	27.95	28.87	27.56	28.00	29.58	26.31	27.05	30.11		
9.	26.12	27.39	28.27	26.11	27.78	29.02	26.88	28.74	29.32	26.41	28.35	28.95	26.01	27.16	29.99	26.19	28.77	29.77		
10.	26.06	27.53	28.48	26.86	28.65	28.67	26.23	26.89	29.84	24.74	28.35	29.63	24.98	27.35	30.78	25.47	28.32	28.99		
11.	26.15	27.09	29.62	26.75	27.80	30.25	24.77	28.69	29.77	27.01	27.76	30.11	25.76	28.22	30.12	27.39	27.21	29.31		
12.	25.93	27.53	29.42	26.61	27.96	30.18	26.91	28.89	29.32	26.32	28.79	29.86	26.33	27.45	29.14	26.28	27.65	29.27		
Mean value x	26.08	27.65	28.72	26.43	28.11	29.60	26.47	28.17	29.55	26.42	28.12	29.45	25.95	27.66	29.86	26.08	27.82	29.77		
Standard deviation s	0.57	0.60	0.75	0.65	0.57	0.56	0.69	0.68	0.46	0.68	0.60	0.43	0.71	0.65	0.56	0.64	0.65	0.48		
Variance s^2	0.32	0.36	0.56	0.42	0.32	0.31	0.47	0.46	0.22	0.46	0.36	0.19	0.50	0.42	0.31	0.41	0.42	0.24		
Relative standard deviation RSD [%]	2.2	2.2	2.6	2.5	2.0	1.8	2.6	2.4	1.5	2.6	2.1	1.4	2.7	2.3	1.8	2.5	2.3	1.6		

Number of objects (columns 10–60,000 cSt)—$k = 6$.
Number of observations (repeats)—$n = 12$.
Total number of observations—$n \times k = 72$.

Table 6
One-way analysis of variance for PDMS of viscosities 10–60,000 cSt at the temperature of 60 °C.

Variation	Sum of square, SS	Degrees of freedom, df	Mean squares, MS	Critical value calculated, F_{cal}	Probability, p-values	Critical value from the table, F_{tab}
Between groups	9.925	5	1.985	6.531	5.435×10^{-5}	2.353
Within groups	20.061	66	0.303			
Total	29.897	71				

Table 7
One-way analysis of variance for PDMS of viscosities 50–60,000 cSt at the temperature of 60 °C.

Variation	Sum of square, SS	Degrees of freedom, df	Mean squares, MS	Critical value calculated, F_{cal}	Probability, p-values	Critical value from the table, F_{tab}
Between groups	1.360	4	0.340	1.349	0.263	2.540
Within groups	13.865	55	0.252			
Total	15.226	59				

Table 8
One-way analysis of variance for PDMS of viscosities 10–60,000 cSt at temperature of 40 °C and 50 °C.

Variation	Sum of square, SS		Degrees of freedom, df		Mean squares, MS		Critical value calculated, F_{cal}		Probability, p-values		Critical value from the table, F_{tab}	
	Temperature [°C]											
	40	50	40	50	40	50	40	50	40	50	40	50
Between groups	3.179	3.51	5	5	0.636	0.70	1.481	1.794	0.208	0.126	2.354	
Within groups	28.330	25.8	66	66	0.429	0.39						
Total	31.509	29.3	71	71								

was confined to a comparison of 6 mean values out of the total data set, i.e. the peak areas for PDMS with the specified viscosities at the temperature of 60 °C (Table 5), and a one-way analysis of variance (ANOVA) was chosen [25].

The results from Hartley's test for temperature of 60 °C showed that the calculated F-value was 2.94, which is lower than the critical F-value 6.32 ($k = 6$ and degrees of freedom $n - 1 = 11$) for the level of significance $\alpha = 0.05$, hence the variance was homogeneous enough for the ANOVA analysis.

Results from the one-way analysis of variance, assuming $\alpha = 0.05$, showed that the calculated F-value, $F_{calc} = 6.531$, was higher than the critical F-value, $F_{tab} = 2.353$ (Table 6). Therefore, the zero hypothesis could be discarded. Given there was a large difference between the mean peak area for PDMS with the viscosity 10 cSt, and the PDMS molecules with viscosities ranging from 50 to 60,000 cSt (Fig. 2 and Table 5), the ANOVA was repeated excluding the data for PDMS 10 cSt (Table 7). The results ($F_{calc} = 1.349$ and $F_{tab} = 2.540$) showed that there was no significant difference between the mean values of peak areas for the remaining PDMS compounds.

Fig. 2 indicates that there may be some temperature ranges in which there are no differences in the signal intensity for PDMS with viscosities between 50 and 60,000 cSt, and which could also be suitable for detection of PDMS with a viscosity of 10 cSt. This was statistically verified by a one-way analysis of variance ($\alpha = 0.05$) for data from the tests with temperature 50 and 40 °C (Tables 5 and 8), where the calculated F-values were 1.794 and 1.481, respectively. As the critical value for significant difference was 2.354, the hypothesis that the mean values were equal could not be rejected.

In summary, the results indicate that there is a narrow range of temperatures, 40–50 °C, in which there are no significant differences in the detector response for PDMS with viscosities ranging from 10 to 60,000 cSt. If only PDMS with viscosities ranging from 50 to 60,000 cSt were included, the temperature range widens up to 90 °C (Fig. 2). The statistical analysis showed that the ELSD detector signal was not significantly influenced by the molecular weights of the analytes or by viscosities of 50–60,000 cSt. As for the purposes of tests PDSM of various viscosities but the same concentration of 0.1% were used, the mass signal was confirmed.

The temperature of 50 °C was selected as the best for determination of linear PDMS compounds with viscosities 10–60,000 cSt. At this temperature, chloroform as the mobile phase was totally vaporised (boiling point of chloroform is 61.2 °C) and the intensity of the signal was satisfactory with no observable noise or peaks division (the latter phenomena occurred at temperatures of 100 and 110 °C). The relative standard deviation (RSD) for measurements of specific PDMS compounds with viscosities 10–60,000 cSt, ranged from 2.0% to 2.4%. At this temperature, no significant differences in the detector's response between PDMS with different viscosities in the range 10–60,000 cSt were observed. This further confirmed the conclusion that the underlying mechanism for signal generation was independent of molecular weight and viscosity of the analyte.

3.5. Influence of the mobile phase flow rate on the detector signal

Because of the necessity to completely evaporate the mobile phase prior to the detection moment, it is necessary to carefully select the appropriate temperature or CO_2 pressure. Solvent vaporisation is also considerably influenced by the mobile phase flow rate and therefore the following test was made to investigate how the mobile phase flow rate influences the generation of noises, as well as the signal intensity and the repeatability of the results. The mobile phase flow rate is an important parameter as it influences the time required for the analysis. Because of the lack of significant differences between different analytes, demonstrated above, only three analytes were selected for the following investigation. Tests were carried out for linear PDMS with the viscosities 10, 350 and 60,000 cSt at the following chloroform (mobile phase) flow rates: 0.3, 0.5, 0.7, 1.0, 1.5, and 2.0 ml/min. All measurements were carried out at the temperature 50 °C, due to the foreseen higher flows of the mobile phase, and the CO_2 pressure amounted to 140 kPa (Fig. 3).

Differences were observed between signal strengths (expressed as peak areas) for the selected values of the mobile phase flow

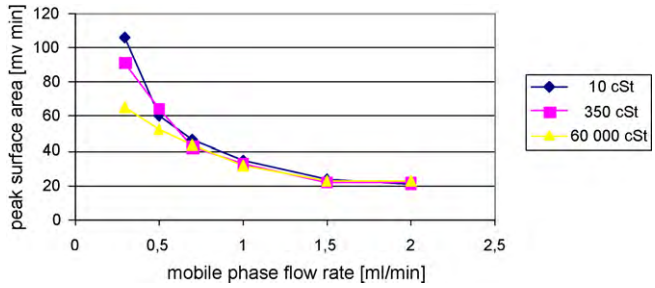

Fig. 3. Dependence of detector signal intensity on mobile phase flow rate for PDMS of viscosities 10, 350 and 60,000 cSt.

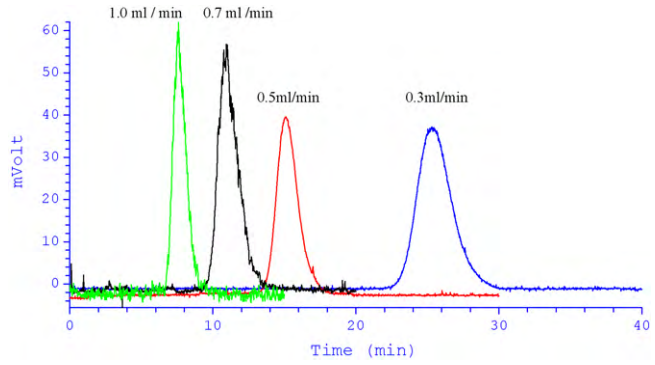

Fig. 4. Superimposed chromatograms of PDMS of viscosity 350 cSt at various mobile phase flow rate.

rate (Fig. 3). The higher the mobile phase velocity, the lower the ELSD signal intensity. The differences are probably by the drop in nebulisation capacity causing an incomplete vaporisation of the mobile phase. The test results indicated that due to the generation of noises and the considerable drop in signal intensity (Fig. 3), the flow rate of the mobile phase should not exceed 1.0 ml/min. However, considering the over-all importance of a high signal intensity, in combination with the need to minimize the time required for the analysis, the optimal flow rate is suggested to be 0.7 ml/min. The largest differences in signal intensities between specific analytes were observed at the lowest flow rate (0.3 ml/min). At the high flow rate of 1.5 ml/min, more noise was generated, and the repeatability of the results dropped (the relative standard deviation for all analytes was 4%). An even higher flow, 2.0 ml/min, additionally caused peak deformation and the RSD increased to 8%. In summary, it was shown that for the selected analytes, the formation of the signal and the signal intensity was clearly dependent on the flow rates of the mobile phase. Therefore, the nebulisation process was not influenced by neither molecular weight nor analyte viscosity.

The appearance of chromatograms obtained after optimizing the temperature drift tube 50 °C, carrier gas pressure (for nebulisation) 140 kPa and for different flow rate of the mobile phase is shown in Fig. 4.

4. Conclusions

Based on the tests results and the statistical analyses, it was established that the signal intensity was very similar for linear PDMS with viscosities between 10 and 60,000 cSt. It is a very important result, since this implies that the validation procedures can be simplified when the method is used to detect numerous analytes from the same chemical group. The parameters characterizing the method (e.g. linearity, accuracy, sensitivity, precision, and limit of detection) can be determined for one selected viscosity, but used for the analysis of linear polydimethylosiloxanes that differ with respect to their degree of polymerization, and hence in molecular weight. Hence, for this group of compounds, the signal from the ELSD detector is a mass signal, and the mechanism underlying the signal formation depends foremost on the size of the aerosol particles. Bearing in mind that different researchers have come to different results when using this detector, the results should not be generalized. Although the magnitude of the signal (intensity) is highly dependent on the analyte mass, many other factors have an impact. The ELSD detector can therefore not be used without prior examination of the universal calibration curve.

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