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# Size-exclusion chromatography with evaporative light scattering detection: Method for determination of polydimethylsiloxanes I. Testing dependence of molecular weight of polydimethylsiloxanes and injected mass upon the detector signal

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**Abstract**

In recent years the evaporative light scattering detector has become a promising device in the analysis of variable chemical compounds using liquid chromatography. Due to the detection specificity, based on the scattering of the laser light on non-volatile analyte particles, this detector is considered a most universal one. Many authors consider detector signal as a mass signal and subsequently, evaporative light scattering detector has been regarded as a mass detector. Although the scientists pinpoint to many advantages of this device, many of its drawbacks were also noticed. Due to variable examinations carried out some scientist characterised the detector response as a non-linear, seeing in fact a significant limitation of this detector for the purposes of quantitative tests. The author of the present study researched, in many ways, for the solution to this problem, by carrying out tests on polydimethylsiloxanes (PDMS) of a linear structure. The aim of this study was to test the dependence of the evaporative light scattering detector signal upon the molecular weight of PDMS of a linear structure and viscosity ranging from 10 to 60 000 cSt and the injected mass. The evaluation of function monotonicity of the detector response and determination of the function for particular analytes referred to the mass ranges of 8.9–149.0 μg. In order to find the dependence of the integrated signal value of the detector signal intensity, expressed as a surface area in μg, upon analyte mass for particular PDMS, several analytical functions and formulas were used. Parameters of regression equations were calculated for linear and non-linear functions as well as their logarithmic transformations. The aim of the research for the optimal regression equation could mean increased reliability of results obtained from analyses of PDMS.


*Keywords:* Polydimethylsiloxanes; Evaporative light scattering detection; Linearity

## 1. Introduction

The review of literature allows to state that since mid 90s there has been a significant increase in the use of the evaporative light scattering detector to determine variable analytes. Due to the detection specificity, which involves scattering of laser light on the non-volatile analyte particles, this detector has been treated as a universal device. Moreover, many authors treat the detector signal as a mass signal and due to that opinion, laser detector is widely considered as a mass detector, i.e. whose response depends only upon the analyte mass. On this ground it could be inferred that there exists, e.g., one universal calibration curve, characterising the dependence of signal intensity upon analyte mass reaching the detector, irrespectively of the chemical constitution, molecular weight or properties. Still, analysing experimental data of other scientists, many other dependencies could be observed, in spite of the fact that there are opinions about the existence of the "universal calibration curve". Another aspect that occurred during tests, carried out by many different authors, was the frequency of non-linear detector response. Many scientists consider this as a serious limitation in quantitative analysis. In their examinations calibration curves in the form of linear regression were characterised, among others, by: Morera Pons et al. [1], Avery et al. [2], Juanéda et al. [3], or Silversand and Haux [4]. Majority of authors report about a non-linear dependence of signal intensity upon analyte concentration

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or mass [5-8]. Non-linear dependence, expressed as a exponential equation, for the examined compounds was obtained by: Charlesworth [9], Stołyhwo et al. [5,6], Trathnigg and Kollroser [10], Bünger et al. [11], Toussaint et al. [12], and many others. Scientists also described the detector response, i.e. dependence as expressed the peak surface area upon analyte mass, as a second-order polynominal equation (e.g., Toussaint et al. [12], Niemi et al. [13], Manoj Babu [14]), or the equations of many logarithmically transformed [1,12,14–16]. Linearity was one of the chief parameters characterising each detector. Still, in reality no detector is ideally linear and when we speak about detector linearity we mean only a certain range of linear response. Apart from the above, in some detectors, e.g., in a UV detector, non-linearity of the measuring converter itself is "corrected" electronically by means of logarithmic amplifiers. Moreover, it happens that the model of a regular linear regression cannot be used. Graphic representation of pairs may suggest the use of a different model than the linear one. In majority of cases linearisation of models using simple transformations of variables is possible. Therefore, e.g., such models can be linearised:

- Exponential function model—by a logarithmic transformations.
- Non-linear function model—by a logarithmic transformations.
- Inverse function model: $y' = 1/y$ or $x' = 1/x$.

In case of the evaporative light scattering detector a non-linear character of response is definitely connected with various mechanisms of signal generation. However, with the cited works of many authors in mind, we may conclude that no linearity constitutes a detector's disqualifying feature, that rules out its suitability for quantitative determinations, because linearisation of dependencies of signal; upon the analyte mass can be carried out. It is important that the function of this dependence was monotonic and precisely defined, i.e. described by such an equation, where the determination coefficient, specifying strength of signal dependence upon the analyte mass was close to 1. Cited authors reached their goal, by describing the dependence of detector response upon the analyte mass using variable equations, using which they obtained precise analysis results. The findings testify to the fact that non-linearity is not a drawback but an inherent property of the detector. This thesis is confirmed by numerous analytical applications of the detector and great deal of attention is paid to it. From the review of the literature many compatible opinions of variable authors concerning a general strategy of the quantitative tests can be noticed. The papers referred to confirm that a linear dependence of the detector response upon the analysed mass (although also described) is rather an exception than the rule for this type of the detector. Actually a linear range, if observed, was very narrow.

This publication presents results concerning the examination of the dependence of the evaporative light scattering detector signal upon the molecular weight of polydimethylsiloxanes (PDMS) of linear structure, and viscosity ranging 10–60 000 cSt, and the injected mass. Tests were supposed to determine this dependence and assess the possibility of preparing a mutual calibration curve for variable types of PDMS. These examinations constituted a major step in the elaboration of the PDMS determination method, used in the exclusion chromatography by means of the evaporative light scattering detector. PDMS are the multi-molecular compounds of silicaorganic polymers, commonly used in pharmaceuticals, medical preparations, foodstuff and cosmetics, which due to chemical constitution and properties have limited possibilities to develop analytical workshop, which would prove useful for the purpose of the speciation analysis.

## 2. Experimental

### 2.1. Instrumentation and chemicals

Test equipment comprised: ELSD/evaporative light scattering detector/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-divinylobenzen 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.

### 2.2. Materials for tests

Tests were carried out for the following kinds of PDMS: linear polymers with low level of polymerisation (PDMS of viscosity of 10 cSt), linear polymers with medium level of polymerisation (PDMS of viscosity of 50, 300 and 350 cSt), high-molecular linear polymers (PDMS of viscosity of 1000 and 60 000 cSt). PDMS were manufactured by Aldrich Chemical Company, Ins. USA.

### 2.3. Preparation of samples

For examinations linear PDMS of viscosities ranging from 10 to 60 000 cSt solutions were prepared. 3725 g of PDMS of suitable viscosity (10, 50, 300, 350, 1000 and 60 000 cSt) was accurately weighed and dissolved in 50 ml chloroform. The standard solutions of 5% concentration were received. For each PDMS of a given viscosity 12 solutions of concentrations ranging 0.03–0.50% were prepared.

## 3. Results and discussion

Experiments were carried out in the conditions considered optimal. During each examination cycle ego constant measurement conditions were maintained: mobile phase: chloroform, mobile phase flow rate: 0.7 ml/min, temperature of the drift

Table 1
Results showing values of mean peak surface areas for selected PDMS of viscosities 10–60 000 cSt

| Concentration of PDMS (%) | PDMS mass (μg) | Viscosity of PDMS (cSt) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 10 | 50 | 300 | 350 | 1000 | 60 000 |
| | | Mean values[a] of peak surface areas (mV min) | | | | | |
| 0.03 | 8.94 | 9.02 ± 0.23 | 11.28 ± 0.12 | 11.67 ± 0.20 | 11.53 ± 0.28 | 11.35 ± 0.23 | 11.13 ± 0.12 |
| 0.04 | 11.92 | 12.36 ± 0.21 | 15.05 ± 0.18 | 15.46 ± 0.31 | 15.48 ± 0.33 | 15.73 ± 0.33 | 14.89 ± 0.19 |
| 0.05 | 14.90 | 17.00 ± 0.45 | 19.76 ± 0.35 | 20.42 ± 0.35 | 20.60 ± 0.30 | 20.23 ± 0.38 | 19.44 ± 0.38 |
| 0.07 | 20.86 | 22.74 ± 0.53 | 27.40 ± 0.51 | 29.71 ± 0.52 | 28.72 ± 0.38 | 28.94 ± 0.41 | 27.80 ± 0.39 |
| 0.10 | 29.80 | 40.87 ± 0.78 | 44.81 ± 0.96 | 46.36 ± 0.86 | 45.99 ± 0.43 | 44.85 ± 0.67 | 44.62 ± 0.79 |
| 0.15 | 44.70 | 58.23 ± 1.23 | 69.39 ± 1.21 | 66.79 ± 0.83 | 71.00 ± 0.78 | 70.34 ± 0.59 | 72.50 ± 1.24 |
| 0.20 | 59.60 | 71.35 ± 0.71 | 83.50 ± 0.65 | 85.59 ± 1.04 | 83.84 ± 0.95 | 81.74 ± 1.03 | 82.62 ± 1.03 |
| 0.30 | 89.40 | 117.23 ± 1.50 | 128.89 ± 1.68 | 132.74 ± 1.54 | 131.72 ± 1.19 | 127.39 ± 1.83 | 125.52 ± 1.74 |
| 0.35 | 104.30 | 135.34 ± 2.59 | 150.45 ± 1.80 | 153.10 ± 2.35 | 154.62 ± 1.76 | 151.07 ± 1.35 | 146.84 ± 2.15 |
| 0.40 | 119.20 | 155.25 ± 2.07 | 177.11 ± 1.40 | 178.78 ± 1.73 | 179.44 ± 2.20 | 176.82 ± 2.32 | 175.47 ± 2.53 |
| 0.45 | 134.10 | 172.70 ± 2.01 | 193.78 ± 1.78 | 203.34 ± 3.16 | 192.85 ± 2.04 | 195.22 ± 2.21 | 180.50 ± 2.38 |
| 0.50 | 149.00 | 189.77 ± 2.78 | 226.41 ± 2.66 | 221.13 ± 3.50 | 226.14 ± 3.24 | 228.10 ± 1.85 | 210.26 ± 2.69 |

[a] ±Standard deviation ($n = 7$).

tube and measurement cell: 50 °C, pressure $CO_2$: 140 kPa. Test results referring to the dependence of the signal intensity upon the molecular weight and the injected mass for the tested analytes were shown in Table 1, where mean values of peak surface area obtained from seven independent measurements were presented. Therefore, in Table 1 standard deviation was shown.

In order to find the dependence of the evaporative light scattering detector (expressed as the peak surface area, $p$) upon mass, $m$ in (μg) of the analyte for particular PDMS (Table 1) the parameters of regression equations were calculated:

(a) Linear model, where, $y = p$, $x = m$.
(b) Log-transformed linear model, where, $y = \log p$, $x = \log m$.
(c) Exponential model, where, $y = p$, $x = m$.
(d) Log-transformed exponential model, where, $y = \log p$, $x = \log m$.
(e) Second-order polynominal model, where, $y = p$, $x = m$
(f) Log-transformed second-order polynominal model, where, $y = \log p$, $x = \log m$.

Data were collected in Tables 2–4, and calibration curves prepared from them were shown in Figs. 1–6.

Searching for the optimal regression equation is aimed to make the results more reliable in the result analyses. The criterion of selecting the best equation to describe this dependence for particular PDMS was coefficient of determination $R^2$, which is the measure of the strength of the variables relationship and the possible simplest form of the regression equation. It must be remembered that the simpler form of the equation, the more efficient further quantitative analysis of the analyte in samples, and subsequently the lesser possibility of making mistakes in the analysis.

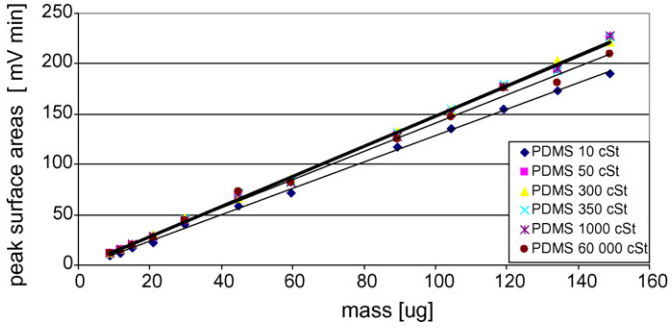


Fig. 1. Dependence of the detector signal upon mass PDMS of viscosities 10–60 000 cSt as a linear model ($n = 7$).

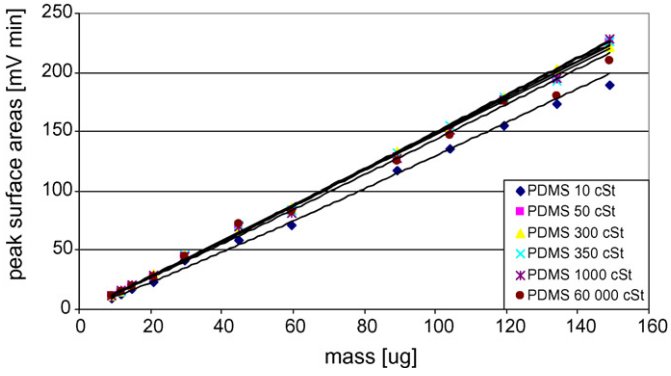


Fig. 2. Dependence of the detector signal upon mass PDMS of viscosities 10–60 000 cSt as a non-linear function (exponential model) ($n = 7$).

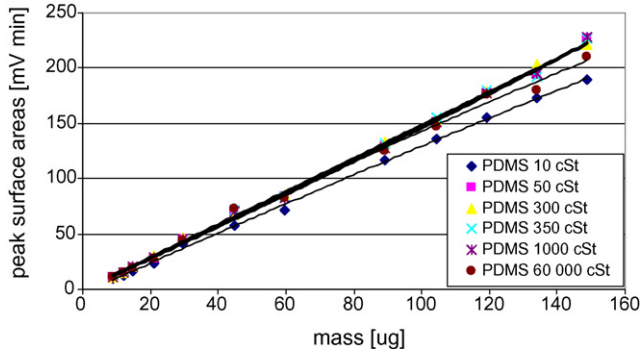


Fig. 3. Dependence of the detector signal upon mass PDMS of viscosities 10–60 000 cSt as a non-linear function (second-order polynominal model) ($n = 7$).

Table 2
Parameters of equations of regression $a$, $b$ and values of the coefficient of determination $R^2$ for the dependences of the detector signal upon the mass of PDMS, linear model and log-transformed linear model

| Regression equations parameters | | | | | | |
|---|---|---|---|---|---|---|
| Viscosity of PDMS (cSt) | Linear model $y = ax + b$ | | | Log-transformed linear model | | |
| | $a$ | $b$ | $R^2$ | $a$ | $b$ | $R^2$ |
| 10 | 1.3062 | −2.1454 | 0.9987 | 1.0823 | -0.0523 | 0.9966 |
| 50 | 1.4948 | −2.3491 | 0.9982 | 1.0536 | 0.0617 | 0.9982 |
| 300 | 1.5053 | −1.5986 | 0.9994 | 1.0399 | 0.0927 | 0.9984 |
| 350 | 1.4966 | −1.2897 | 0.9981 | 1.0397 | 0.0921 | 0.9979 |
| 1000 | 1.4972 | −2.1749 | 0.9976 | 1.0434 | 0.0827 | 0.9978 |
| 60 000 | 1.4051 | 0.5156 | 0.9959 | 1.0390 | 0.0782 | 0.9961 |

Table 3
Parameters of equations of regression $a$, $b$ and values of the coefficient of determination $R^2$ for the dependence of the detector signal upon the mass of PDMS, exponential model and log-transformed exponential model

| Regression equations parameters | | | | | | |
|---|---|---|---|---|---|---|
| Viscosity of PDMS (cSt) | Exponential model $y = ax^b$ | | | Log-transformed exponential model | | |
| | $a$ | $b$ | $R^2$ | $a$ | $b$ | $R^2$ |
| 10 | 0.8925 | 1.0808 | 0.9968 | 1.0236 | 1.0491 | 0.9958 |
| 50 | 1.1420 | 1.0555 | 0.9982 | 1.1091 | 0.9694 | 0.9983 |
| 300 | 1.2186 | 1.0445 | 0.9988 | 1.1257 | 0.9501 | 0.9985 |
| 350 | 1.2131 | 1.0450 | 0.9980 | 1.1239 | 0.9523 | 0.9983 |
| 1000 | 1.2064 | 1.0439 | 0.9982 | 1.1190 | 0.9570 | 0.9981 |
| 60 000 | 1.1917 | 1.0399 | 0.9962 | 1.1078 | 0.9638 | 0.9967 |

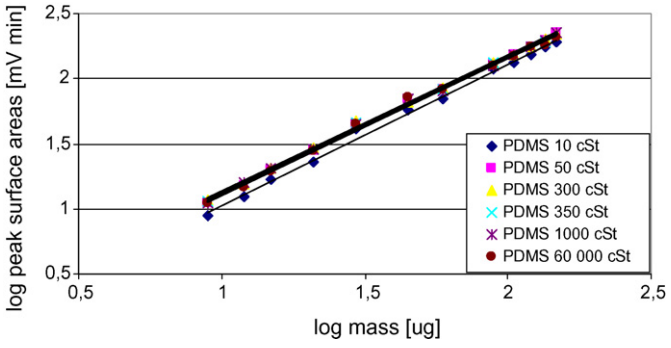


Fig. 4. Dependence of the detector signal upon mass PDMS of viscosities 10–60 000 cSt as a linear model in the log–log scale ($n = 7$).

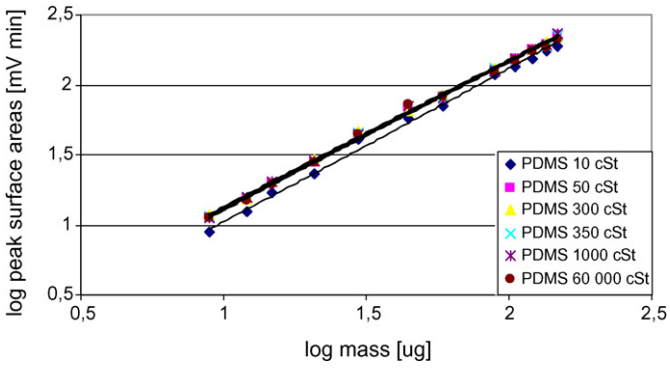


Fig. 5. Dependence of the detector signal upon mass PDMS of viscosities 10–60 000 cSt as a non-linear function (exponential model) in the log–log scale ($n = 7$).

Table 4
Parameters of equations of regression $a$, $b$, $c$ and values of the coefficient of determination $R^2$ for the dependence of the detector signal upon the mass of PDMS, second-order polynominal model and log-transformed second-order polynominal model

| Regression equations parameters | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Viscosity of PDMS (cSt) | Second-order polynominal model $y = ax^2 + bx + c$ | | | | Log-transformed second-order polynominal model | | | |
| | $a$ | $b$ | $c$ | $R^2$ | $a$ | $b$ | $c$ | $R^2$ |
| 10 | −0.0005 | 1.3802 | −3.6617 | 0.9988 | 0.1068 | 0.5690 | 0.3206 | 0.9981 |
| 50 | 0.0006 | 1.4000 | −0.4052 | 0.9984 | 0.0741 | 0.6921 | 0.1495 | 0.9988 |
| 300 | 0.00008 | 1.4935 | −1.3562 | 0.9994 | 0.0743 | 0.7027 | 0.1220 | 0.9990 |
| 350 | 0.0001 | 1.4814 | −0.9772 | 0.9981 | 0.0893 | 0.6512 | 0.1642 | 0.9988 |
| 1000 | 0.0110 | 1.3397 | 1.0530 | 0.9982 | 0.0651 | 0.7313 | 0.1061 | 0.9983 |
| 60 000 | −0.0010 | 1.5479 | −2.4121 | 0.9964 | 0.1348 | 0.4985 | 0.2976 | 0.998 |

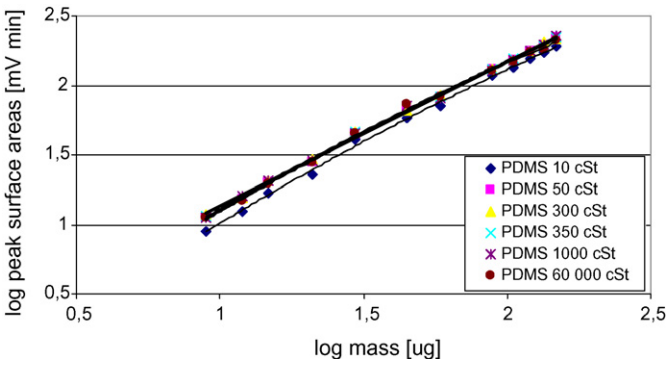


Fig. 6. Dependence of the detector signal upon mass PDMS of viscosities 10–60 000 cSt as a non-linear function (second-order polynominal model) in the log–log scale ($n = 7$).

Values of all coefficients of determination (Tables 2–4) are close to one, which mean that there is a strong correlation between the detector signal and the analyte mass. Moreover, all values obtained were similar and this piece of information testifies to the fact that no regression equation distinguishes itself and therefore all might be useful for the determination of this dependence. However, from the previous experience it comes that the selection of the regression equation for the representation of the linear function is the best for quantitative tests. So much so, it can be decided that this one is a suitable equation to describe the dependence of the ELSD signal upon the mass of polydimethylosiloxanes, within the ranges of examined masses. Moreover, the value of parameter $a$ and the coefficient of determination $R^2$ of the regression equation represented as a second-order polynominal equation (Table 4), as well as the sign change in these values suggest that the linear regression may be accepted as a correct one, for the purposes of determination of this dependence. Figs. 2–6 also do not show significant changes which would justify the choice of other equation, than the linear one to describe the dependence in question.

Another aim of the examinations was the evaluation of the mutual detector calibration for linear polydimethylosiloxanes, which differed in viscosity, and at the same time with molecular weight. Such a solution would be important from the practical point of view, as the samples of pharmaceutical preparations and foodstuffs, to be tested in the future may include PDMS of different viscosity, including the undesirable one. From the analysis of curves in Fig. 1 it has been incurred that the courses of dependencies of signal intensity upon injected mass are similar to PDMS of linear structure and viscosity ranging 50–60 000 cSt. Actually the curves almost overlap, i.e. the slope, determined by the value of the coefficient $a$, is quite similar and the point of intersection with the $OY$ axis, also determined by the coefficient $b$ (intercept). However, the figure showing the calibration curve differs noticeably at the calibration curve for PDMS of viscosity 10 cSt. The point of intersection with the $OY$ axis is similar to other curves, but the slope of calibration curve is lesser.

Differences visible for the calibration curve of PDMS of viscosity 10 cSt, may be correlated with greater volatility of this analyte, or contamination in the form of low-molecular PDMS. It is known that a given viscosity may be obtained as a result of mixing several PDMS of different viscosity. Comparing values of the mean peak surface areas (Table 1), as well as values of the coefficient $a$ in the equation of linear regression for all tested PDMS (Table 2) show that PDMS of viscosity 10 cSt may not belong to the same group. In order to verify the possibility of creating a mutual calibration curve for PDMS of viscosity ranging 10–60 000 cSt, or exclude from this group PDMS of viscosity of 10 cSt, a common linear regression equation, and the linear regression equation excluding PDMS of viscosity equal to 10 cSt were calculated. Value of the slope ($a$) amounted 1.4509, intercept ($b$) –1.506 and coefficient of determination ($R^2$) 0.9989 for the common dependence of signal intensity of PDMS of viscosities 10–60 000 cSt. For the common dependence of signal intensity of PDMS of viscosities 50–60 000 cSt value of the slope ($a$) amounted 1.4798, intercept ($b$) –1.3807 and coefficient of determination ($R^2$) 0.9986. Comparing parameters $a$, $b$, $R^2$ from received linear regression equations above and Table 2 a similarity of values may be noticed. Moreover, the value of the coefficient of determination equal to 0.9989 for the common dependence of signal intensity of PDMS of viscosity ranging 10–60 000 cSt upon the mass does not exclude the affiliation of PDMS of the viscosity of 10 cSt to the same group. This confirms the sense of calculating, and applying a common regression equation in the form of a linear function for the PDMS of linear structure and the viscosity ranging 10–60 000 cSt. It is very important from the practical point of view, as this is going to simplify the quantitative analysis and may prove suitable when a detailed separation of various PDMS found in the samples will be complicated. This will allow to evaluate a total amount of linear PDMS and viscosity ranging from 10 to 60 000 cSt. On the grounds of the tests carried out it was found out that the evaporative light scattering detector signal does not depend on the chain length, analyte viscosity and subsequently molecular weights.

Many authors [10,17] suggested, that the differences occurring between the signals for many different analytes may be concerned with the value of the refractive index (refraction). Pinpointing to this physical property seems justified for two reasons. Firstly, the refractive index is present in the Rayleigh's formula, describing the phenomenon of light scattering. Moreover, selection of the mass ranges (8.94–149.0 μg) and resultant sizes of the particles generated during the nebulisation process may suggest that scattering is most probably and predominantly caused by reflection and refraction. Therefore, the values of refractive indexes for the selected analytes were measured. Detailed measurements are shown in Table 5.

Table 5
Values of the refractive index

| Item | Viscosity of PDMS (cSt) | Index of refraction $n_D^{20}$ |
|---|---|---|
| 1 | 60 000 | 1.406 |
| 2 | 1000 | 1.406 |
| 3 | 350 | 1.406 |
| 4 | 300 | 1.406 |
| 5 | 50 | 1.405 |
| 6 | 10 | 1.401 |

No differences in the detector responses for determined PDMS may result from the lack of significant differences in the values of the refractive index. Tests for selected analytes confirm obtaining the mass signal.

Authors cited in this paper notice that evaporative light scattering detector has become a very promising detector for the purposes of chromatographic analysis. Tests carried out indicate that this detector may also be suitable for the analysis of linear polydimethylosiloxanes of viscosities 10–60 000 cSt. However, a correct use of this type of detector requires from the analyst understanding the mechanism of signal generation, and subsequently the necessity to control constant values at the analysis of various parameters upon which the detector response depends.

## 4. Conclusions

Experiments carried out allowed to state that the length of the chain (polymerisation degree), viscosity, and at the same time molecular weights of selected PDMS do not influence the strength of laser light scattering, i.e. signal intensity. Lack of significant differences in the course of dependencies of ELSD signal intensity on the mass for selected analytes confirms gaining mass signal. The possibility of drawing a common calibration curve for various PDMS of a linear structure and viscosities 10–60 000 cSt, strictly maintaining specified conditions (mobile phase flow rate, drift tube temperature, nebulising gas pressure) was confirmed. This finding is quite important from the practical reason, as it is foreseen to facilitate quantitative analysis, and simplify method validation problem. It has been conformed that for the ranges of examined masses the regression equation in the form of a linear function is the best to describe the dependence of the ELSD detector signal upon mass of linear PDMS of viscosities 10–60 000 cSt.

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