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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

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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],

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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