Setting the power coefficient and the baseline to linearise the signal of the evaporative light scattering detector

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The theories of light scattering (LS), originally introduced by Lord Rayleigh in 1871 [1], may seem complex at first glance. However, these theories were effectively summarised in a paper that explored the practical application of LS in measuring beer haze [2]. When these principles are applied to the evaporative light scattering detector (ELSD) for liquid chromatography (LC), specific equations can be derived to calculate the intensity of scattered light caused by the presence of a solute. When the eluted mass W(t) is distributed among N nebulised particles, there are three key scenarios to consider in function of the ratio between the radius of particles (r), and the wavelength of the light source (λ):

(a) Intensity is directly proportional to $W(t)^2$ when $r << \lambda$ (Rayleigh scattering).
(b) Intensity is proportional to $W(t)^{4/3}$ when the $r \sim \lambda$ (Mie scattering).
(c) Intensity is proportional to $W(t)^{2/3}$ when the $r >> \lambda$ (reflection-refraction).

The first patent for an evaporative light scattering detector (ELSD) for LC was granted in 1968 [3]. Unfortunately, in seventies, only integrators were available for LC to correlate the injected concentration to the chromatogram peak, so the first works using ELSD as LC detector reached the wrong conclusion that the response factor of this detector varied even for solutions of the same polymer but with different polydispersity. This led to ELSD being deemed unsuitable for gel permeation chromatography (GPC), an LC technique introduced by Moore in 1964 [4]. During the last 60 years, GPC has emerged as the common technique for separating polymers that are dissolved in a compatible solvent, according to their hydrodynamic volume. This is accomplished by passing the polymer solution through a stationary phase consisting of porous beads. Larger molecules can only partially penetrate the pores, causing them to elute before smaller molecules, which can fully enter the pores and therefore have longer elution times.

To address the challenges associated with high evaporation temperatures that can result in the loss of low molecular weight fractions in polydisperse polymers, Polymer Laboratories (now Agilent Technologies) developed and patented [5, 6] an enhanced nebuliser that utilised a secondary nitrogen jet. Initial tests demonstrated the potential to achieve molecular weight distributions comparable to those obtained with traditional concentration detectors such as differential refractive index for polymers, by linearising the ELSD signal for each point of chromatogram using the following equation [7, 8]:

$$\text{ELSD signal intensity} = k_{ELSD} \cdot \text{concentration}^{\alpha_{ELSD}}$$

A simplified method to measure the power coefficient using only one injection of a mixture of 4 standards is explained here in detail using the chromatogram shown in *Figure 1*. As a first step, the minimum ELSD intensity value is subtracted from each point of the chromatogram to ensure all values are positive. Subsequently, the baseline and the four peaks are identified in the chromatogram, following standard LC procedures.

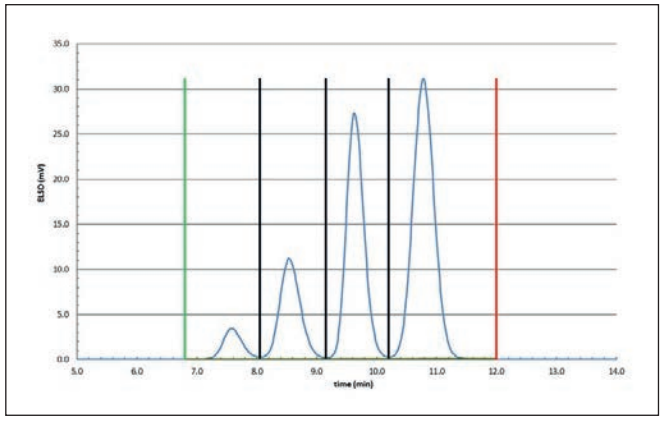

Figure 1: Method to calculate the power coefficient: the chromatogram illustrates the process of selecting both the baseline and the peaks.

An inverse power law is applied on the intensity of the measured signal in each point of the chromatogram with the purpose of obtaining a linearised signal. The power coefficient is adjusted, and its correct value is achieved when the areas of the 4 peaks have the following expected percentages: 10%, 20%, 30%, and 40%. The resulting chromatogram for the optimal power coefficient of 1.75 as well as the plot of the measured concentrations versus the expected values of peak areas are given in the next *Figure 2a and 2b*:

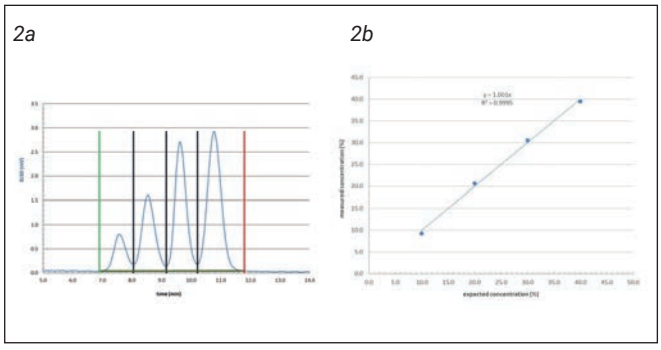

Figure 2: Method to calculate the power coefficient: a) Chromatogram after applying the linearisation. b) The plot of the measured concentration versus expected concentration after linearisation.

Based on these measurements, we can conclude that in order to linearise the signal of ELSD, it is necessary to set a power coefficient value of 1.75. This is true for the post-linearisation process, which can be integrated into the software, but may restrict the use of the ELSD to LC programs that have this capability. Therefore, to expand the detector's applicability, the firmware has been adjusted to conduct the linearisation in real-time during analysis by applying an inverse power law to the signal intensity. To utilise previously determined linearisation parameters, it is essential to set the baseline as close to zero as possible using the Zero function in the firmware, as well as the complete absence of the drift. In most cases this is not possible, so for practical reasons, the baseline must be set at a sufficiently high level to prevent noise and drifting from causing negative values during the analysis.

An Excel template was created to simulate how Gaussian peaks are transformed by an inverse power law when the baseline is set to different values. Unexpectedly, for a baseline set at 1 mV it was found advantageous to also set the power coefficient to 2 instead of 1.75, as shown by the Excel template snippet in *Figure 3*. The program simulates a chromatogram containing 8 Gaussian peaks, which is first scaled using a power function with the exponent 1.75. Then the peaks are shifted up by adding the Zero value (here 1 mV). Further, the resulting chromatogram is linearised by using an inverse power law (here the linearisation factor was set to 2). The first two peaks simulate the detection and quantification limits, respectively. The next 4 peaks correspond to the 4 PS standards found in EasiVial Red having peak areas in the ratio of 10:20:30:40. The last two peaks simulate higher concentrations, for which some peak broadening is expected.

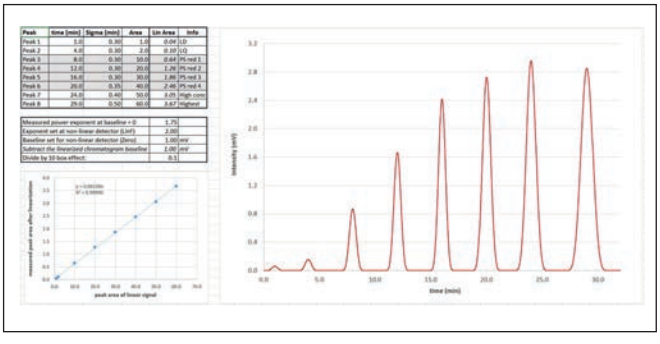

Figure 3: Excel template to simulate the combined effect of the values set for power coefficient and Zero

Real tests were done by injecting the solution of a mixture of 4 PS standards while at ELSD the linearisation factor was set to 2 and the Zero parameter was set to 1 mV. As shown in *Figure 4*, the intensity of the ELSD signal increases with the Zero parameter. As expected, as the parameter value increases, the baseline is shifted farther away from the mathematically challenging values near zero.

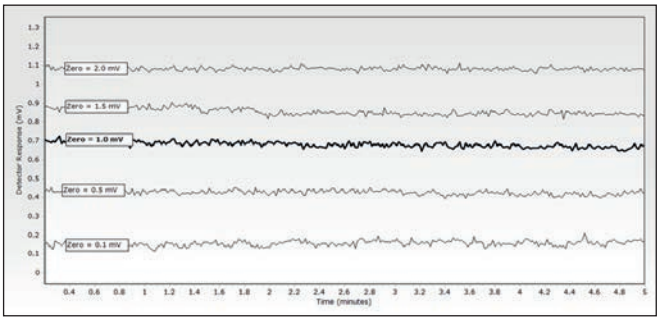

Figure 4: Output ELSD signal for Zero parameter with the following settings: 0.1, 0.5, 1.0, 1.5 and 2.0 mV.

Important to note is that by setting the linearisation coefficient (power exponent) to 2, similar chromatograms are obtained in the range of Zero between 0.5 and 2 mV, as presented by the overlay in *Figure 5*.

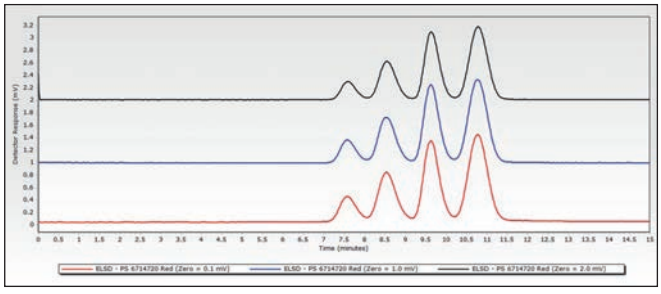

Figure 5: Overlay of the chromatograms of a mixture of 4 PS standards recorded for Zero parameter having the following settings: 0.1, 1.0, and 2.0 mV.

The measured 4 peak areas (in percentages) at different settings of Zero parameter are compared with their expected values in *Table 1*.

Table 1: Relative peak areas of the four PS standards in EasiVIal Red chromatograms recorded for Zero parameter having different settings between 0.1 and 2.0 mV.

Expected/test	**Relative peak areas (%)**			
	PS 6545000	**PS 448500**	**PS 17120**	**PS 1180**
Expected Value (%)	10	20	30	40
Zero set to 0.1 mV	9.93	20.62	29.97	39.49
Zero set to 0.5 mV	9.31	20.38	30.47	39.84
Zero set to 1.0 mV	**9.20**	**20.28**	**30.57**	**39.95**
Zero set to 1.5 mV	8.91	20.12	30.53	40.44
Zero set to 2.0 mV	8.46	19.85	31.04	40.65

The repeatability and accuracy of the GPC method with a linearised ELSD for which the power coefficient was set to 2 and the baseline was set to 1 mV, were verified using the linear polyethylene NIST HDPE 1475, for which the average molecular weights are known. The chromatograms in *Figure 6* were recorded as a print screen to show that the analyses could be done in automatic mode:

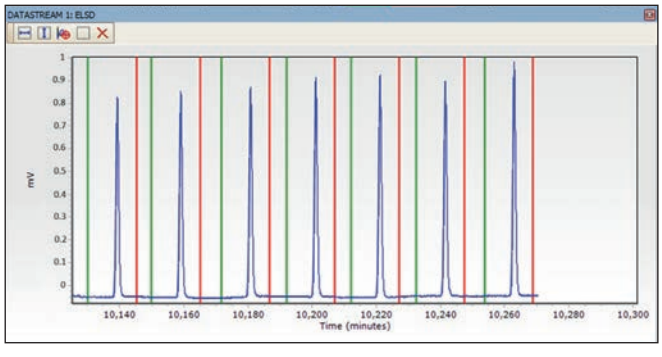

Figure 6: Recorded ELSD chromatograms of the NIST HDPE 1475.

The molecular weight distributions were measured based on a calibration with polystyrene standards. The overlay of these MWD is given in *Figure 7*.

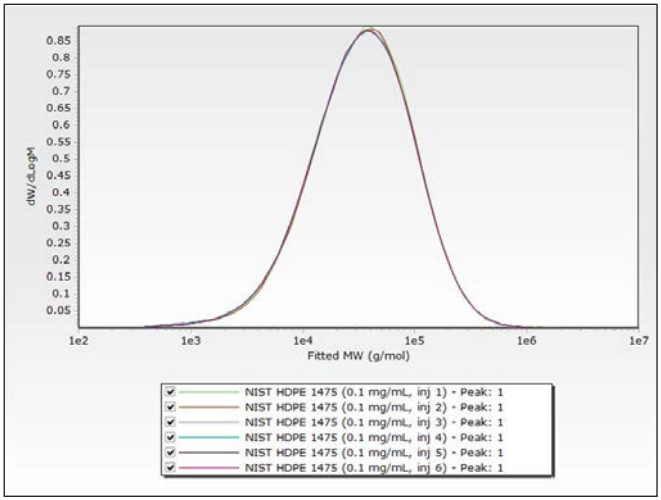

Figure 7: Overlay of the MWD obtained for 6 injections of NIST HDPE 1475.

The measured Mn, Mw, and Mz are compared with their expected values in Table 2.

Table 2: Average molecular weights of NIST HDPE 1475 compared with the expected values.

Expected/test	Mn	Mw	Mz	Đ
Expected Value (%)	18300	53100	138000	2.9
Injection 1	17772	55778	143784	3.1
Injection 2	17446	55508	137163	3.2
Injection 3	16602	54302	126975	3.3
Injection 4	16816	54948	137652	3.3
Injection 5	17400	55198	138102	3.2
Injection 6	17368	55035	135897	3.2

Conclusions and further developments

Here, we have introduced a straightforward method for determining the theoretical value of the power coefficient that correlates the intensity of scattered light with eluted concentrations. Additionally, we have outlined the necessary adjustments needed to correctly set the power coefficient ELSD parameter when the baseline is set to a value other than zero. In our opinion, setting the power coefficient to 2 offers several advantages:

- Reduces the impact of baseline fluctuations and drift by allowing to set the Zero parameter to 1 mV
- Simplifies the implementation of the inverse power function
- Provides the opportunity to implement an analogical solution.

We tested this implementation by measuring the average molecular weights of a HDPE reference, which align well with the expected values, confirming that setting the power coefficient to 2 and the zero to 1 mV enables the use of the linearised ELSD as a concentration detector for GPC. It is worth noting that the presented ELSD chromatograms of HDPE 1475 were generated using a concentration of 0.1 mg/mL, significantly lower than the concentration of 2 mg/mL typically employed in high-temperature GPC with a traditional differential refractive index detector. Moreover, the ELSD detector allowed to conduct the analyses in xylene, a less hazardous solvent compared to the commonly used trichlorobenzene (TCB), which is listed on REACH Annex XVII, entry 49.

References

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