Polyethylene glycol (PEG) (Figure 1) is a material widely used in the industry: thickening agent, conservative agent, solvent, formulation component in cosmetic preparations, and even pharmaceutical active ingredient as a laxative agent (for example colorectal examination preparation agent).

This compound is a polymer obtained from Ethylene glycol (Figure 2) monomers that are polymerized into mixtures of polyethylene glycol which degree of polymerization is controlled via chromatography. PEG is generally labelled with a number corresponding to its average molecular mass which is directly related to the degree of polymerization of the mixture.

PEGs are easily dissolved in water, as well as alcohols, acetone and other solvents. Low molecular mass PEG (<600g.mol\(^{-1}\)) are generally found as liquids at room temperature and solid for higher molecular mass mixtures.

**Analysis Conditions**

While Steric Exclusion Chromatography (SEC) appears as an obvious technique to check the quality of PEG batches, it is known that PEG are rather easy to analyse in reverse phase chromatography\(^{[1,2]}\). Using HPLC, or UHPLC allows generally faster results than traditional SEC. PEG do not contain chromophore, so the detection cannot rely on ultra-violet or visible spectroscopy. Both Refractive Index Detector (RID) or Evaporative Light Scattering Detector (ELSD) could allow an easy PEG detection in chromatography.

Evaporative Light Scattering Detection (ELSD) is preferred to Refractive Index Detector (RID) for several reasons\(^{[3]}\):

- ELSD is more sensitive than RID.
- ELSD is non sensitive to room temperature, while RID is very dependant to environmental temperature (baseline can drift if the laboratory is not well temperature controlled). With ELSD the user is sure to run the samples and do not have to wait for long periods of time for temperature stabilization.
- RID is incompatible with gradients: this detector monitors the refractive index of the output of the column, and during a gradient, the refractive index changes as the proportion of the solvents change during the slopes of the gradient, generating a huge baseline drift. This compromise the detectability of the solutes. ELSD evaporate the mobile phase before detection, so whatever is the composition of the mobile phase, the baseline will remain flat.

For this study, we will use SEDEX LT-ELSD 100 and SEDEX LT-ELSD LC (Figure 3) which are
ELSDs providing high sensitivity for both HPLC and UHPLC conditions. Those ELSD are Low Temperature ELSD, this important feature will be highlighted in this study by the evidence of semi-volatile solutes in the mixture. Those ELSD provide also 'SAGA', a gain management feature that allow the ELSD to provide a direct dynamic range of more than 5 decades. Thanks to SAGA, the ELSD provides high sensitivity and very low signal saturation risk, enhancing ease- of-use and quantitative data generation.

In this study, it has been found that both detectors are compatible with the conditions optimized and can be swapped without compromising the results. However, thanks to a higher data rate of acquisition (up to 100Hz), SEDEX LT-ELSD will be the preferred ELSD for high throughput analysis. This detector is also more sensitive and should be providing a better quantitation of less abundant moieties.

Checking the literature about reported PEG analysis methods in reverse phase, most of those methods are based on C18 column, with a mobile phase composed of water-methanol or water-acetonitrile (+ 0.1% formic acid in some case). However, most methods found are based on HPLC methods (particle sizes of 5µm) and are long cycle time. Here, we use an ELSD which allows with gradient and better stability than RID a shorter cycle time, i.e. a higher throughput of analysis, in parallel to high sensitivity analysis. In addition, ELSD is coupled easily with UHPLC systems and preserves low peak widths generally obtained in UHPLC. The system with the use of the right HPLC column should be able to analyse PEG batches within a short time compared to standard HPLC or SEC.

As a demonstration we have prepared a mix composed of several commercial batches of PEG labelled MW200, MW300, MW400, MW600, MW1000, MW2000. This mix is used as illustration purposes and has been used for the method optimization in order to provide a method that will be compatible with each of those commercial batch individually.

The column is an ACE Excel C-18 (100x3.0 mm; 1.7 µm) available in the laboratory. This column is a sub-2µm particle size that will allow the flowrate to be increased for shortening the cycle time.

For this synthetic and complex mixture of PEG (Figure 4), the baseline separation of each PEG is obtained within 11 minutes. The gradient is a simple two slope gradient with slope decreased after elution of the lower
polymerization degree PEG. The baseline separation is obtained starting with Ethylene glycol monomer (first peak) to a polymer composed of 60 monomers of ethylene glycol! This two-slope gradient can be easily adapted to focus on a batch type for quality control purposes.

For higher polymerization level mixtures, the slope can be increased as well as the acetonitrile content at the end of the gradient.

Since the goal of this study is to provide the fastest analysis of the mixture with the column we have, we tried to change the gradient in order to reduce the time between each peak. However, we focused on keeping the resolution high enough to keep a baseline separation between each peak.

By increasing the slopes, we managed keeping baseline separation (the loss of resolution is seen mainly on first peaks that remain well resolved). The total run time is 8.5min (Figure 5) instead of 11min, which means a >20% decrease of run time. For users focusing on PEG with MW<1000, the run time will be approximatively 5min or so.

**Low Temperature analysis**

During the optimization, we have kept the evaporative tube temperature as low as possible in order to increase the universality of the ELSD. Generally, the ELSD is considered as a nearly universal detector. The principle of detection is to evaporate the mobile phase before detection of light diffusion by solid remains from the mobile phase (solute). In the case of volatile or semi-volatile solutes, if the temperature is too high during the evaporation process, the solutes will not be detected since transferred into gas and not anymore diffusing solid particle.

Low Temperature Evaporative Light Scattering Detection (LT-ELSD) is a SEDERE concept that consists in a unique design of the detector with a target of low temperature evaporation of the mobile phase. The design initiated several decades ago by SEDERE makes the low temperature evaporation

<table>
<thead>
<tr>
<th>Evaporative Tube Temperature</th>
<th>Degree of Polymerization (DP) content (% peak area for each individual DP)</th>
<th>Average Polymerization degree</th>
</tr>
</thead>
<tbody>
<tr>
<td>45°C</td>
<td>0.6 5.7 13.3 19.8 20.6 17.2 11.8 6.5 2.8 1.1 0.4</td>
<td>6.8</td>
</tr>
<tr>
<td>50°C</td>
<td>0.2 4.8 14.1 19.6 20.6 17.7 12.1 6.3 2.9 1.2 0.4</td>
<td>6.8</td>
</tr>
<tr>
<td>55°C</td>
<td>0.0 3.3 13.3 20.5 20.5 18.8 12.3 6.8 2.8 1.2 0.4</td>
<td>6.9</td>
</tr>
<tr>
<td>60°C</td>
<td>0.0 1.8 11.8 20.4 22.5 19.4 12.3 7.0 3.1 1.3 0.4</td>
<td>7.0</td>
</tr>
<tr>
<td>65°C</td>
<td>0.0 0.6 9.0 19.9 23.3 19.7 14.6 7.8 3.3 1.2 0.5</td>
<td>7.2</td>
</tr>
<tr>
<td>70°C</td>
<td>0.0 0.1 6.0 18.4 24.9 21.2 15.4 8.5 3.5 1.5 0.4</td>
<td>7.3</td>
</tr>
<tr>
<td>75°C</td>
<td>0.0 0.0 2.6 14.9 26.3 23.4 16.9 9.6 4.0 1.6 0.5</td>
<td>7.5</td>
</tr>
<tr>
<td>80°C</td>
<td>0.0 0.0 0.9 11.4 23.2 26.0 19.4 11.5 4.9 1.9 0.6</td>
<td>7.7</td>
</tr>
</tbody>
</table>

Table 1: Determination of polymerization degree of PEG as a function of the evaporative tube temperature
possible with a single temperature parameter, while some other ELSDs design require several parameters (for example nebulizer temperature in addition to evaporative tube temperature).

The use of only one temperature makes optimization, validation and daily use much simpler. In addition, it has been proven that SEDEX LT-ELSD original design is superior to alternative designs based on impactors, cooled nebulizers or nebulization chamber.

The importance of low temperature operation is shown with lower polymerization degree PEG. As an illustration, we focus the study on PEG 200 which is containing from degree of polymerisation 1 (ethylene glycol) to 12.

To highlight the effect of the evaporation tube temperature on the results, the same PEG400 sample is injected in the ELSD with evaporation tube temperature of 45°C, 60°C and 80°C (Figure 6).

On this overlay, we observe that, by increasing the temperature, there is a shift of average polymerization degree to higher polymerization degrees. This shift is caused by the volatility of lower molecular weight PEGs. The smaller is the PEG, the more volatile it is. In that case, decreasing the temperature allows the user to get the real polymerization degree profile avoiding the small PEG to be evaporated with the mobile phase in the ELSD. Remarkably, the evaporation rate of smaller PEG at one evaporation temperature do not change the distribution appearance. This confirms the need for a short study on the only relevant parameter of ELSD: evaporative tube temperature. When optimizing a method, we usually run the same chromatographic conditions at 2 or 3 evaporative tube temperatures. A change in the chromatographic profiles at the ELSD temperature setpoint emphasize the presence of a semi volatile solute in the sample.

Characterization of a PEG400 batch
As an illustration, we used the characterization of this sample of PEG400 as an illustration of the error made using high temperatures: we use the area of each individual peak and its MW to estimate the average MW of the mixture. ELSD response is known to be very homogeneous over a chemical family. Therefore, based on retention time (MW attribution of each peak), and peak area, we can calculate easily an average mixture MW or an average polymerization ratio. The results of the average MW as a function of the evaporative tube temperature is shown in Table 1. The average MW calculated on peak areas decreases with the evaporative tube temperature.

This complies with the overlay shown. For the sample studied, the results is an asymptotic curve in the direction of the low temperatures where the smallest MW PEG of the samples are totally preserved thanks to LT-ELSD technology, and provides the actual real MW of the PEG sample (420 g/Mol for this sample). As shown in Table 1, running higher evaporative tube temperature (>60°C) generates a shift that is an error on MW PEG determination.

Conclusion
We have shown in this study that ELSD is a perfect detector for non chromophoric polymers such as PEGs. We have developed a straightforward and fast chromatographic method that allow the complete profile of PEG mixtures to be characterized. This fast method is shown to be able to provide a baseline separation of polymerization degrees between single ethylene glycol and a polymer composed of 60 units of ethylene glycol. Conditions may be adapted to focus on commercially available polymers mixture.

We have also confirmed the importance of low temperature evaporation feature of the ELSD used for PEG quantitation. Using a high temperature for evaporation may generate an error on the characterization of the degree of polymerization of PEG mix. Using low temperature allows the characterization to be done unbiased and should be preferred.

References