White Paper

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Towards Greener GPC/SEC

Abstract

As a liquid chromatography (LC) technique, gel permeation chromatography/size-exclusion chromatography (GPC/SEC) requires the use of a mobile phase. The growing awareness of the need for more sustainable (greener) solutions has focused attention on environmentally- and health-friendly solvents and solutions.

Three of the well-known 12 principles of green chemistry[1] are central requirements for liquid chromatography: waste prevention, safer solvents and auxiliaries, and use of renewable feedstocks. Many scientists are currently concerned with the question of how to implement these principles in the analytical laboratory.

This white paper discusses less hazardous solvent alternatives from renewable resources that can be successfully applied for size separation of macromolecules. Options are also presented for GPC/SEC users to establish solutions that align with the 12 principles of green chemistry.

Introduction

GPC/SEC is an established liquid chromatography technique for characterizing macromolecules in solution.[2] A typical GPC/SEC system comprises:

- An isocratic pump, to transport the mobile phase
- An injection system (manual or automated) to introduce the sample, which is dissolved in the mobile phase
- One or more separation columns filled with a stationary phase of macroporous particles
- One or more detectors

In a diffusion-controlled process, the dissolved macromolecules are separated by their hydrodynamic volume in the pores of the macroporous stationary phase. Molecules that are larger than the pores of the stationary phase particles are excluded from the pores and remain in the flowing eluent stream, and elute first from the column. Molecules that are smaller than the pores can diffuse in and out of the pores and elute later with decreasing size. Interaction of sample with the stationary phases must be avoided.

Both aqueous and organic mobile phases are used in GPC/SEC. The organic mobile phases are particularly challenging in terms of their impact on the environment and human health.

A further complication is that the quality of the separation in GPC/SEC depends on the available pore volume. Traditionally, GPC/SEC uses long columns with large inner diameters. Columns are often combined to form column banks, increasing the resolution or the molar mass separation range.[3] As a result, GPC/SEC is typically a slow method with high solvent consumption.

Figure 1 shows a typical chromatogram of a separation on an analytical GPC/SEC column of 300 mm length and 8 mm inner diameter. Approximately 15 mL of solvent is required per injection.

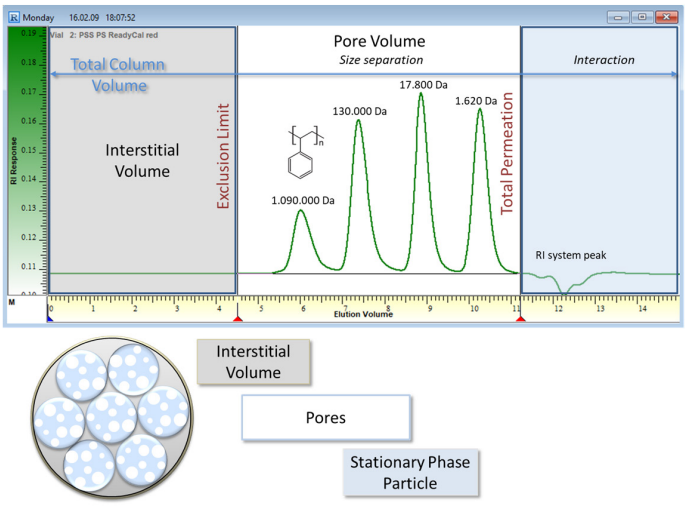

Figure 1. Graphical representation of the total column volume, interstitial volume, and pore volume of a typical analytical GPC/SEC column with an inner diameter of 8 mm and length of 300 mm.

Given these challenges, it is in the interests of GPC/SEC users to turn to green chemistry solutions to reduce the negative impact of the technique. Of the so-called 12 principles of green chemistry[1], three are especially applicable to liquid chromatography:

- **Prevention:** "It is better to prevent waste than to treat or clean up waste after it has been created."
- **Safer solvents and auxiliaries:** "The use of auxiliary substances (e.g., solvents, separation agents, etc.) should be made unnecessary wherever possible and innocuous when used."
- **Use of renewable feedstocks:** "A raw material or feedstock should be renewable rather than depleting whenever technically and economically practicable."

Greener mobile phase alternatives

Most typical organic GPC/SEC solvents, such as tetrahydrofuran (THF), trichloromethane, toluene, or dimethylformamide/dimethylacetamide (DMF/DMAc), pose significant health and environmental hazards.

For many of these eluents, alternatives are available that meet at least one of the 12 principles of green chemistry. Typical organic solvents and their potential alternatives are summarized in Table 1.

Table 1. Example summary of organic GPC/SEC solvents and their alternatives.

Mobile Phase	Potential Alternative	Comment
THF	Cyclopentyl methyl ether	Less peroxide formation
	2-methyl-THF	Can be obtained from renewable raw materials
THF, Toluene, Di-, and Trichloromethane	Ethyl lactate	No major health risks
	Ethyl acetate	Lower health risks
DMF, DMAc	**DMSO**	No major health risks, but penetration enhancer

The suitability of these alternatives for GPC/SEC separations has been evaluated in more detail using the following criteria:

- Are the alternatives compatible with typical stationary GPC/SEC phases?
- Can the mobile phases be used under preventive sustainable (practicable) conditions (e.g., pressure, temperature)?
- Are there suitable GPC/SEC calibration standards available and can they be used for calibration?
- Are suitable detection methods available?

Replacing THF with 2-methyl-THF

For many applications, THF can be replaced by 2-methyl-THF, a solvent that is obtained from renewable resources. Agilent SDV columns (polystyrene-divinylbenzene copolymer particles) are fully and directly compatible with 2-methyl-THF.

However, in direct comparison with THF, 2-methyl-THF has certain limitations:

When using 2-methyl-THF with a UV-Vis detector, a wavelength greater than 230 nm must be used. This value is slightly higher than the cutoff for THF, which is typically at 212 nm.

In addition, PEG/PEO cannot be measured in 2-methyl-THF. While PEG/PEO is soluble when heated, it precipitates on cooling down to room temperature.

Replacing THF, toluene, and di/trichloromethane with ethyl acetate

Ethyl acetate is a promising alternative that meets many of the previously mentioned criteria and can be used with some limitations. Ethyl acetate is suitable for use with SDV columns and is a good solvent for various types of polymers such as polystyrene (PS) and derivatives, poly(meth)acrylates, and polydimethylsiloxane (PDMS).

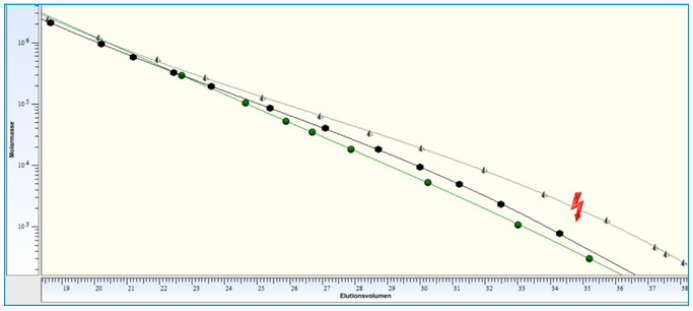

Figure 2. Overlay of different calibration curves in WinGPC Software (PS: gray; PMMA: black; PDMS: green), measured in ethyl acetate, flow rate 1 mL/min, at room temperature, sample concentration 0.5 to 1 mg/mL, injection volume 20 µL, with RI detection.

However, interaction-free chromatography and pure size separation cannot be achieved for all soluble polymers. PS and its derivatives show delayed elution and are probably not separated by size only. Thus, ethyl acetate cannot be used as an alternative for these types of polymers.

Polyacrylates and polymethacrylates, on the other hand, can be easily separated according to size with ethyl acetate. For this reason, for these analytes, ethyl acetate represents a potential alternative solvent with fewer health risks.

PDMS reference materials also show typical GPC/SEC behavior in ethyl acetate. Since PDMS must be routinely chromatographed in toluene or trichloromethane to be detectable when using a refractive index detector, ethyl acetate is a possible alternative. In Figure 2, calibration curves of different polymer types are superimposed. Here, the potential problem of PS is clearly visible.

Replacing DMF/DMAc with DMSO

For medium-polar, viscous eluents, such as DMF or DMAc, DMSO is an alternative solvent with a significantly lower health risk. However, it should be noted that DMSO is a penetration enhancer and dissolved substances can easily overcome the human skin barrier and enter the body.

Due to the relatively high viscosity of DMSO (even higher than DMF/DMAc), working at a higher temperature (typically 60 to 80 °C) is recommended. Reducing the solvent viscosity by increasing temperature will result in lower backpressure and higher resolution. Currently, no alternative solvent with lower viscosity has been found for this polarity range. Thus, there is no alternative that can be used at room temperature to fulfill the green chemistry requirement of low energy consumption. Nevertheless, DMSO is an interesting alternative to replace DMF or DMAc.

Prevention: columns with smaller dimensions

Compared to other chromatographic techniques, GPC/SEC suffers from limited resolution. The dependence of GPC/SEC column characteristics and experimental parameters on resolution is complex. Column material particle size, packing quality, and many other factors influence mass transfer and therefore resolution.[4]

Traditionally, GPC/SEC columns with a length of 300 mm and approximately 8 mm inner diameter have been used. As shown in Figure 1, the amount of solvent required per injection for these columns is approximately 15 mL. To achieve the resolution required by standards such as ISO 13885, columns have been combined into column sets or column banks. The disadvantage of this concept is that solvent consumption and waste increase linearly with the number of columns. Column banks often comprise two to three analytical columns and thus require 30 to 45 mL of mobile phase per injection.

The use of columns with smaller column dimensions, such as an id of 0.46 mm or less and a length of 150 or 250 mm or less, leads to a significantly lower solvent consumption. However, a decreasing number of plates has been observed with smaller diameters.[5] Resolution also decreases with column length.

As a result, when trying to replace columns of an existing application, the resulting loss of resolution should be at least partially compensated. This compensation can be achieved, for example, by using smaller particles. Smaller particles can be packed with a smaller interstitial volume and thus improve the resolution. It should be emphasized that this approach requires optimized hardware with minimized dead volume and small detector cells.[4]

A disadvantage of smaller particles is that the pressure increases with decreasing particle size. A potential threat when using smaller particle sizes, especially when discussing larger macromolecules, is shear degradation. According to current scientific investigation, small particle sizes down to 3 µm can be applied for oligomers in low viscous solvents and for proteins. It is still under investigation whether higher molar masses or more rigid structures can be measured on small particle size columns with smaller porosity frits without the danger of chain scission and without chromatographic artifacts.

Figure 3 compares chromatograms of an analytical 8 × 300 mm SDV column with 5 µm particles and a 4.6 × 250 mm semi-microcolumn with 3 µm particles. Analytical conditions (injected mass, flow rate, etc.) and instrumentation (RI detector) have been set to recommended standard conditions.

Table 2. Comparison of semi-micro and analytical GPC/SEC columns.

Analysis Type	Typical Column Dimensions (mm)	Ideal Operating Flow Rate (mL/min)	Analysis Time/ Column (min)	Eluent Consumption/ Column (mL)
Semi-Micro	4.6 × 250	0.33	10	3.5
Analytical	8 × 300	1.00	12.5	12.5

To allow for an easier visual comparison, Figure 3 uses a time-based axis so that the chromatograms can be easily compared. The inset shows the consumed solvent on the X-axis, demonstrating the significant amount of savings possible, while the resolution is slightly increased.

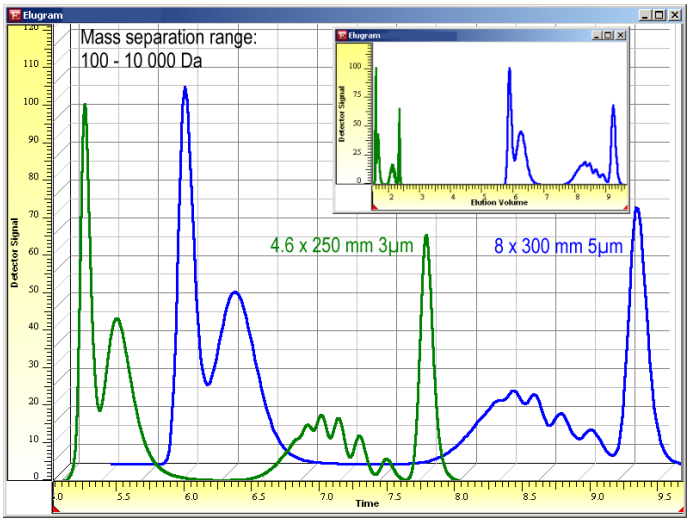

Figure 3. Overlay of two chromatograms obtained on an analytical SDV column with 5 µm particles (blue) and a semi-microcolumn with 3 µm particles (green). For easier visual comparison, the large figure shows the required time to obtain the chromatograms. The inset shows the amount of solvent required, which is significantly less for the semi-microcolumn.

Prevention: overlapping injections

Another option to prevent waste and save solvent is a data acquisition-based software feature that reduces the redundant time between injections due to the column interstitial volume.

Figure 1 introduced the interstitial volume of a GPC/SEC column, which is normally filled with pure solvent before any injection. During the sample analysis cycle, this pure solvent is typically first emptied from the column and only then can the earliest eluting components emerge. While the late eluting compounds are emerging, the interstitial volume is refilled with pure solvent.

Typical GPC/SEC columns filled with polymer gel stationary phases have an interstitial volume of around 30%. For an analytical column with a length of 300 mm and a diameter of 8 mm, this volume corresponds to 5 mL of mobile phase. The emptying and refilling of the interstitial volume with pure solvent does not influence the separation; it simply consumes both time and solvent. Thus, by injecting a sample before the previous sample has completely eluted, time and solvent can be saved if the software includes this feature.

Figure 4 shows how this feature can be implemented[6], displaying the Agilent WinGPC raw data window with two injections, one after the other (and a further injection that is not considered here). Each injection is indicated by an injection mark, a blue triangle at the bottom, and the sample name at the top. Before the system peaks of Vial 5: Sample 3 are eluted, the next sample, Vial 6: Sample 4, is already injected at approximately 23 mL. Data evaluation for sample Vial 5: Sample 3 is unaffected by the second injection; baseline limits (compare the two red triangles) and integration limits can still be set as required by national and international GPC standards (e.g., ISO 13885).[7]

The green and red areas show the total required volume for Vial 5: Sample 3 and Vial 6: Sample 4, respectively. In this example, approximately 8 mL mobile phase is saved for every injection. Further solvent savings are possible, as there is more than sufficient baseline area to set the baseline limits properly.

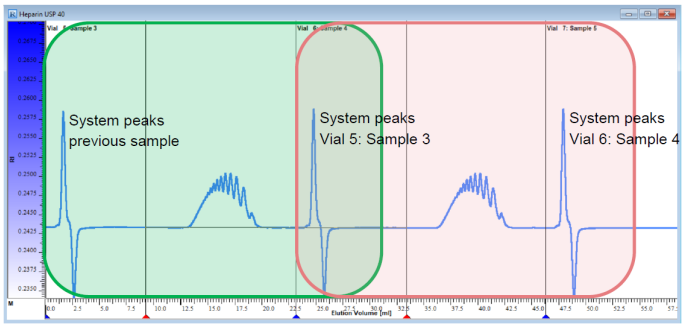

Figure 4. Overlapping injections: Vial 6: Sample 4 is injected before Vial 5: Sample 3 is completely eluted. The displayed elution volume is valid for Vial 5: Sample 3. Approximately 30% solvent can be saved with this software feature.

Overlapping injections (Figure 4) is a feature that can be applied with all types of columns, independent of length and diameter. The only action required is to shorten the injection interval. Resolution and analytical conditions, such as flow rate or column loading, are unaffected.

Conclusion

There are various options for scientists to reduce the environmental and health impact of their GPC/SEC analysis by aligning with the established principles of green chemistry. These guidelines encourage the prevention of waste, as well as the use of safer solvents, auxiliaries, and renewable feedstocks. This white paper has presented strategies to follow these principles, proposing alternative solvents, smaller columns, or overlapping injections in GPC/SEC, all of which reduce or prevent hazardous waste.

References

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