GREENER GPC/SEC

WHITE PAPER

Gel permeation chromatography/size-exclusion chromatography (GPC/SEC) as a liquid chromatography (LC) technique 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.

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WHITE PAPER

SCOPE OF WORK

Sustainable and green chemistry is a different way of thinking about how chemistry can be done.

Three of the well-known 12 principles of green chemistry[1] are central requirements for 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"

Many scientists are currently concerned with the question of how to implement these principles in the analytical laboratory.

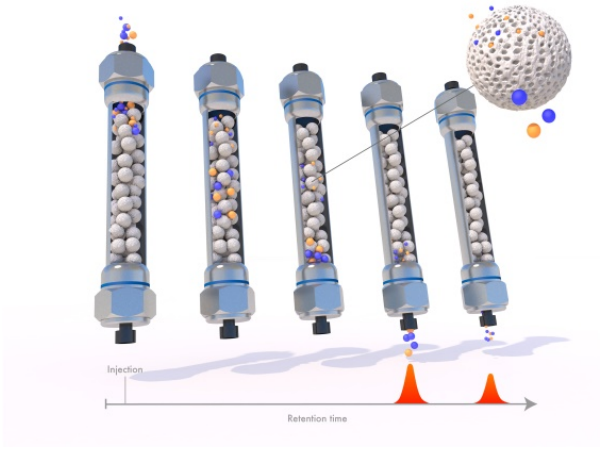
Scope of this White Paper and the work behind is to

1. Discuss less hazardous solvent alternatives from renewable resources which can be successfully applied for size separation of macromolecules
2. Describe options for users of GPC/SEC/GFC to establish solutions more in coherence with the 12 principles of green chemistry

SCOPE OF WORK

Identification of environmentally- and health-friendly solvents and solutions for users of organic GPC/SEC

INTRODUCTION TO GPC/SEC



GPC/SEC (gel permeation chromatography/size exclusion chromatography) 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 and
- 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 which are larger than pores of the stationary phase particles are excluded from the pores and remain in the flowing eluent stream thus eluting first from the column. Molecules which are smaller than the pores can diffuse in and out of the pores and thus elute later with decreasing size. Interaction of sample with the stationary phases needs to be avoided.

Both, aqueous and organic, mobile phases are used in GPC/SEC. The organic mobile phases are particular challenging from an environmental and health point of view.

A further complication is that the quality of the separation in the GPC/SEC depends on the available pore volume. GPC/SEC uses traditionally long columns with larger inner diameter. Often columns are combined to column banks to increase the resolution or the molar mass separation range.[3] As a result, GPC/SEC is classically 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 8mm inner diameter. Approx. 15 mL of solvent are required per injection.

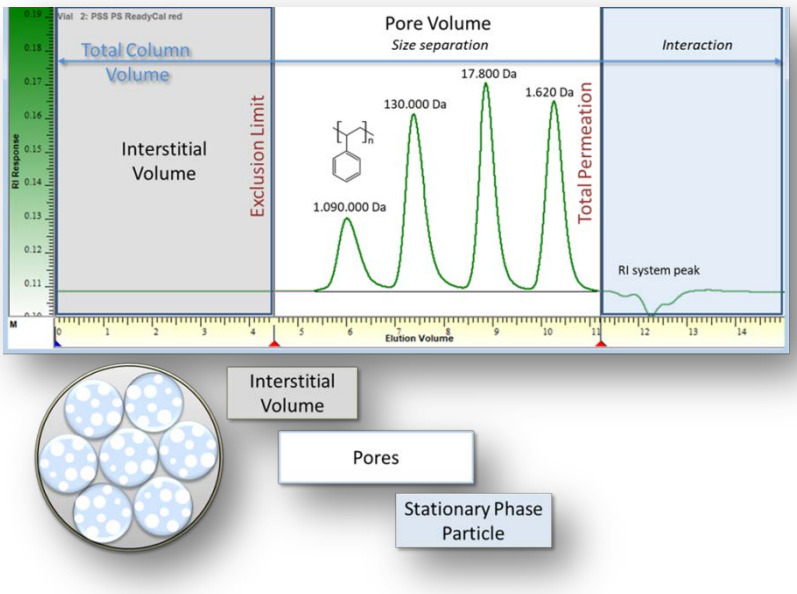


Figure 1: Graphical representation of total column volume, the interstitial volume and the pore volume of a typical analytical GPC/SEC column of the dimensions ID 8mm, length 300mm

GREENER MOBILE PHASE ALTERNATIVES

Most typical organic GPC/SEC solvents such as THF (tetrahydrofuran), trichloromethane, toluene or DMF (dimethylformamide)/DMAc (dimethylacetamide) 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: Exemplary 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 Trichlormethane	Ethyl lactate	no major health risks
	Ethyl acetate	lower health risks
DMF, DMAc	**DMSO**	no major health risks, but penetration enhancer

Whether these potential alternatives are basically suitable for GPC/SEC separations has been evaluated in more detail under the following aspects:

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

REPLACING THF WITH 2-METHYL-THF

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

However, in direct comparison to THF there are some application limitations:

- when using 2-methyl-THF with a UV/VIS detector, a wavelength greater than 230 nm has to be used. This is slightly higher than the cut-off 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, DI/TRICHLOROMETHANE WITH ETHYL ACETATE

Ethyl acetate is a very promising alternative that meets many of the above criteria and can be used with some limitations.

Ethyl acetate is suitable for use with *PSS SDV columns* and is a good solvent for various types of polymers such as polystyrene (PS) and derivatives, poly(meth)acrylates and polydimethylsiloxane (PDMS).

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. Thus, for such analytes, ethyl acetate represents a potential solvent alternative with fewer health risks.

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

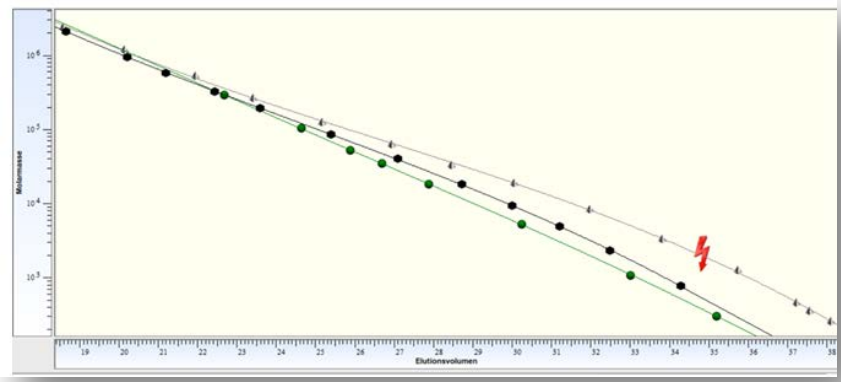


Figure 2: Overlay of different calibration curves (PS gray, PMMA black, PDMS green), measured in ethyl acetate, flow rate 1ml/min, room temperature, sample concentration 0.5mg/mL to 1mg/mL, injection volume 20µL, RI detection

REPLACING DMF/DMAC WITH DMSO

For medium-polar, viscous eluents, such as DMF or DMAc, DMSO is a solvent alternative 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 penetrate the organism.

In addition, due to the relatively high viscosity (even higher than DMF/DMAc), it is recommended to work at a higher temperature (typically 60-80 °C). 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 a limited resolution. The dependence of GPC/SEC column characteristics and experimental parameters on the resolution is quite complex. Column material particle size and packing quality and many other factors influence the mass transfer and therefore the resolution.[4]

Traditionally GPC/SEC columns with a length of 300 mm and approx. 8 mm inner diameter have been used. As shown in Figure 1 the amount of solvent required per injection for such a columns is approximately 15 mL. To achieve the required resolution as requested by standards, such as ISO 13885, columns have been combined to 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 quite often comprise 2-3 analytical columns and thus require 30 to 45 mL of mobile phase per injection.

The use of columns with smaller column dimensions (micro-columns), such as e.g. 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, empirically a decreasing number of plates has been found with smaller diameters.[5] In addition the resolution decreases with column length.

Thus, when trying to replace columns of an existing application, the resulting loss of resolution should be at least partially compensated. This 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 3µm can be applied for oligomers in low viscous solvents and for proteins. It is still under investigation if 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 8x300 mm SDV column with 5µm particles and a 4.6x250 mm semi-micro column with 3µm particles. Analytical conditions (injected mass, flow-rate, etc.) and instrumentation (RI-detector) have been set to recommended standard conditions.

Table II: 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	4.6x250	0.33	10	3.5
analytical	8x300	1.00	12.5	12.5

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

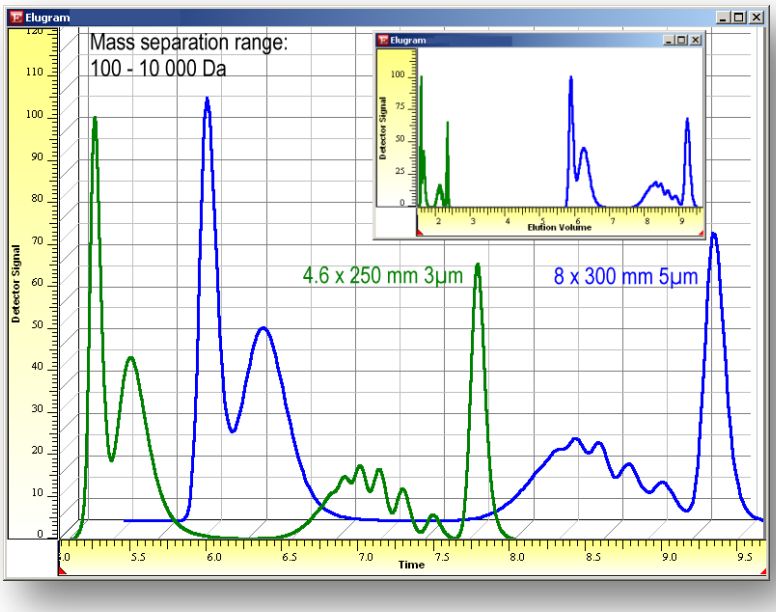


Figure 3:

Overlay of 2 Chromatograms obtained on an analytical SDV column with 5µm particles (blue) and a semi-micro column with 3µm particles (green). For easier visual comparison the large figure shows the required time to obtain the chromatograms. The inset shows the amount of required solvent which is significantly less for the semi-micro column.

PREVENTION - OVERLAID INJECTION

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. Normally during the sample analysis cycle, this pure solvent is 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 again being refilled with pure solvent. Typical GPC/SEC columns filled with polymer gel stationary phases comprise an interstitial volume of approx. 30%. For an analytical column with a length of 300 mm and a diameter of 8 mm this corresponds to 5 mL of mobile phase. The emptying and refilling of the interstitial volume with pure solvent contributes nothing to the separation, it just consumes both time and solvent.
Thus by injecting a sample before the previous sample has completely eluted time and solvent can be saved providing the software incorporates this feature.

Figure 4 shows how this feature can be implemented.[6] It shows the PSS WinGPC UniChrom raw data window displaying 2 (+1) different injections, one after the other. Each inject is marked 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 approx. 23 ml. Data evaluation for sample “Vial 5: Sample 3” is not affected by that; baseline limits (compare the 2 red triangles) and integration limits can still be set as required by national and international GPC standards, e.g. ISO 13885.[7]

The green area or respectively the red area show the total required volume for “Vial 5: Sample 3” and “Vial 6 Sample 4”. Approx. 8 mL mobile phase are saved for every injection in this example. An even further reduction is possible, as there is more than sufficient baseline area to set the baseline limits properly.

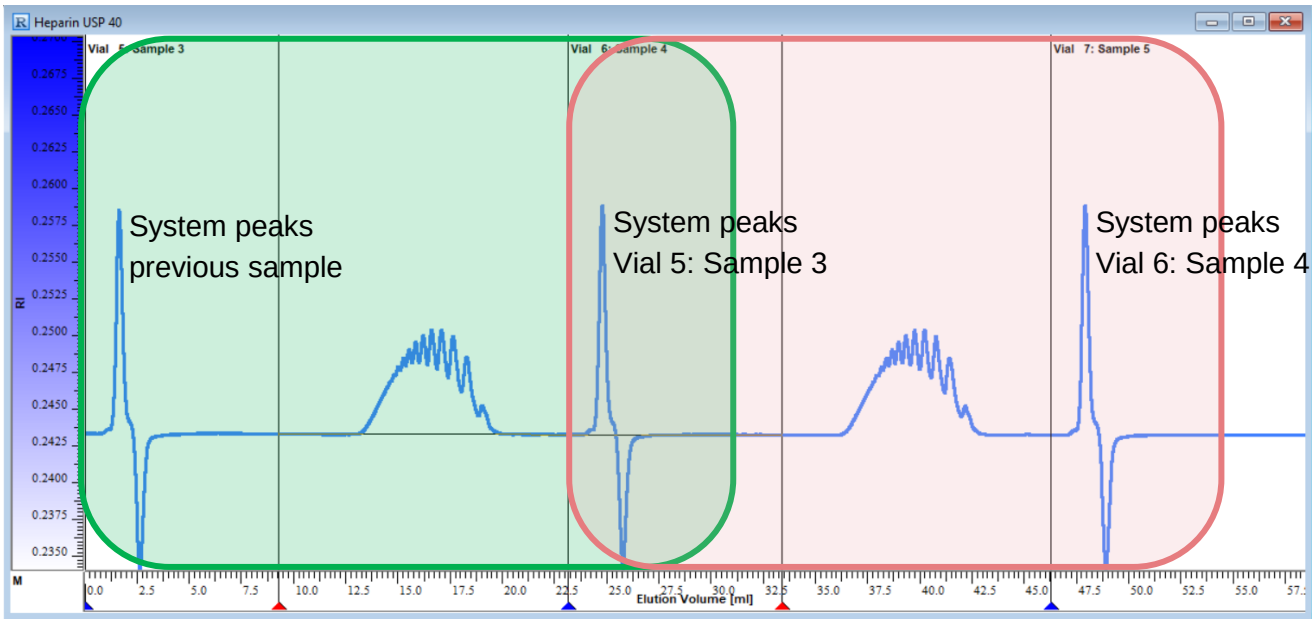


Figure 4 Overlaid injection: Vial 6 Sample 4 is injected before Vial 5: Sample 3 is completely eluted. The displayed elution volume is valid for Vial 5 sample 3, approx. 30% solvent can be saved with this software feature

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

LITERATURE AND FURTHER READING

[1] Anastas, P. T.; Warner, J. C. (1998) Green Chemistry: Theory and Practice, Oxford University Press: New York

[2] André M. Striegel Wallace W. Yau Joseph J. Kirkland Donald D. Bly, Modern Size-Exclusion Liquid Chromatography: Practice of Gel Permeation and Gel Filtration Chromatography, Second Edition, John Wiley & Sons, Inc. (2009)

[3] For more details see PSS e-book GPC/SEC Columns (2018)

[4] PSS White Paper “Resolution increase in GPC/SEC/GFC by decreasing particle size and increasing the number of columns” (2015)

[5] E. G. Franklin , T. W. Kahler, Effects of Column Inner Diameter and Packed Bed Heterogeneities on Chromatographic Performance, LCGC North America, Volume 34, Issue 6 (2016)

[6] Application #27: „Overlaid Injection“, PSS WinGPC Newsletter 01 (2005), PSS Publication Number 30102

[7] ISO 13885 Gel permeation chromatography (GPC) (2020) -1: THF, 2 -DMAc