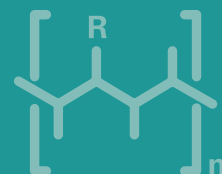
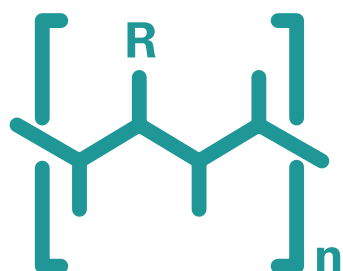




TOSOH



Greener solvents for GPC/SEC and light scattering of polymers



Sustainable polymer analysis

Your Challenge

- ▶ You want to replace current solvents with safer alternatives that are less toxic to the environment.
- ▶ You want to use greener solvents that have similar performance to solvents that you already use.

Our Solution

EcoSEC Elite™ GPC system and LenS™₃ MALS detector

- ▶ An optimized chromatography method for greener laboratory solutions

What was done?

- ▶ A comparative study on 2-methyl-THF for GPC/SEC analysis to assess performance compared to THF

What was the result?

- ▶ 2-methyl-THF works effectively as a green solvent without compromising any results

Switching your GPC/SEC solvent from THF to 2-methyl-THF provides an easy swap to provide a greener, safer, and less toxic option.

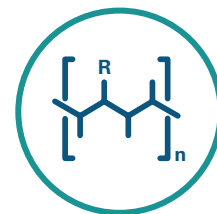
Your Benefit

Comprehensive polymer characterization using greener laboratory solvents with no compromises

TOSOH BIOSCIENCE

SEPARATION & PURIFICATION

CONNECTING MINDS.
TOUCHING LIVES.



Greener Solvent Alternatives in GPC/SEC and Light Scattering Analyses of Polymers

A crucial method in examining macromolecules like polymers and proteins is gel permeation chromatography (GPC), also known as size exclusion chromatography (SEC). This is a method that allows for the characterization of polymers and biomaterials to assess many properties like size and molecular weight. Choosing the right solvent for GPC/SEC is essential to obtaining reliable findings. Due to the hydrophobic nature of many polymers, the use of non-polar, organic solvents as a mobile phase is necessary. However, many of these solvents are derived from petrochemical sources and their disposal and usage can pose safety and environmental concerns.

Due to its solvating abilities, tetrahydrofuran (THF) is a widely used solvent in GPC/SEC despite several inherent disadvantages, such as human and environmental toxicity, and difficult handling requirements. Herein, a THF alternative is investigated, to examine the potential of 2-methyltetrahydrofuran (2-methyl-THF) as a greener solvent substitute for GPC. 2-methyl-THF is an interesting alternative for multiple reasons. It can be produced by catalytic hydrogenation of furfural, a molecule that can be obtained from various sustainable biomass sources rich with cellulose. Additionally, it presents less toxicity and environmental concerns than THF¹.

Experimental Conditions

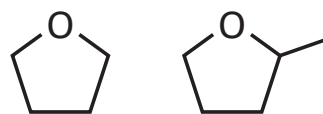
Column: TSKgel® SuperMultipore HZ-M (4.6 mm ID x 15 cm, 4 μm)
 Mobile Phase: THF; 2-methyl-THF
 Flow rate: 0.35 mL/min
 Detection: Refractive Index (RI) and MALS
 Temperature: 40 °C
 Injection vol.: 20 μL
 Samples: Tosoh polystyrene standards: F-4 (41.1 kDa); standard mixes PSTQuick B and C
 Instrument: EcoSEC Elite™ (HLC-8420) GPC System equipped with LenS™₃ MALS

Results and Discussion

THF and 2-methyl-THF (*Figure 1*) were investigated as solvents for the analytical separation and analysis of polystyrene.

Ultimately, it is of interest to determine if 2-methyl-THF can provide comparable chromatography results by investigating the quality of the separation, as well as the sensitivity, repeatability, and accuracy of molecular weight measurements determined during GPC/SEC experiments. Polystyrene was chosen as the polymer to characterize as it often serves as a universal standard for GPC/SEC quality tests.

Figure 1. Structural formulas of tetrahydrofuran (left) and 2-methyl-tetrahydrofuran (right)

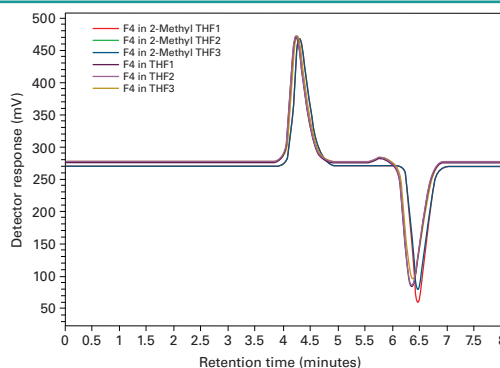


All polystyrene samples were weighed on a calibrated balance and dissolved in the respective solvent for one hour each with gentle swirling to prevent any shear degradation that can occur with rapid mixing.

Separation performance

In the first series of experiments, the separation profile of polystyrene was assessed using both mobile phases (THF and 2-methyl-THF) with all other parameters and experimental conditions kept constant. The repeatability, retention time, and elution profile were investigated to understand if both solvents offer the same level of performance. *Figure 2* illustrates the chromatography of a single concentration (0.5 mg/mL) of polystyrene standard F-4 (41.1 kDa) analyzed in triplicate in both THF and 2-methyl-THF.

Figure 2. Comparison of separation for a 41.1 kDa polystyrene standard



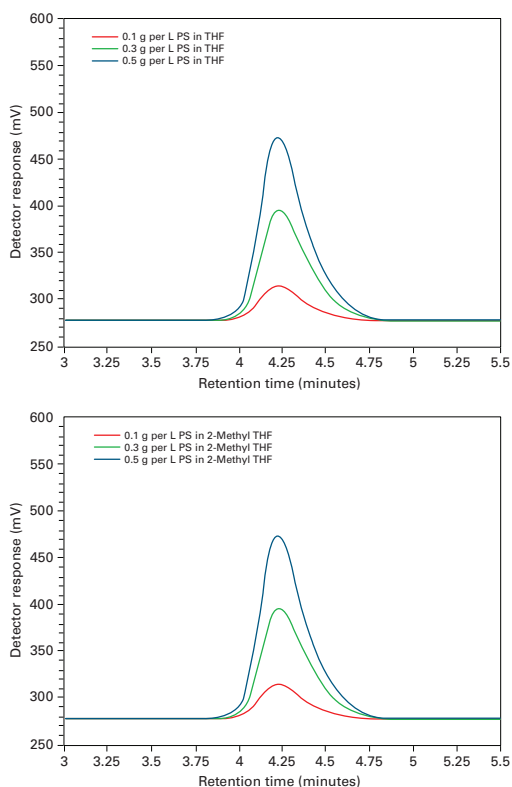
As demonstrated in *Figure 2*, for each set of three injections, the elution profile, peak shape, and peak intensity are almost identical. Notably, 2-methyl-THF has a slightly higher retention time for both the polystyrene sample and the solvent peak. This might be related to the different dynamic viscosity of both mobile phases. THF has a lower viscosity (0.3750 mPa.s at 40° C²) compared to 2-methyl-THF (0.41 mPa.s at 40° C³), which may impact the actual flow rate of the mobile phase delivered by the chromatography system pumps. Although the peak position differs marginally in 2-methyl-THF, the performance of the separation remains as good as in THF. Peak shapes and peak intensities are identical in both mobile phases.

Sensitivity comparison

Second, it is important to assess whether 2-methyl-THF has a comparable sensitivity to THF to be able to work with comparable injection loads. Due to their similar structures, it is expected that both solvents should show similar solubilization abilities, refractive indices, and consequently similar specific refractive index increments (dn/dc) of polystyrene.

The data in *Figure 3* was generated by running 41.1 kDa polystyrene solutions at varying concentrations (0.1, 0.3 and 0.5 mg/mL) while holding injection volume constant at 20 μ L.

Figure 3. Comparison of sensitivity for a 41.1 kDa polystyrene standard



The results of the sensitivity measurements on the 41.1 kDa PS samples at different concentrations in *Figure 3* illustrate identical elution profiles with similar responses in RI detection.

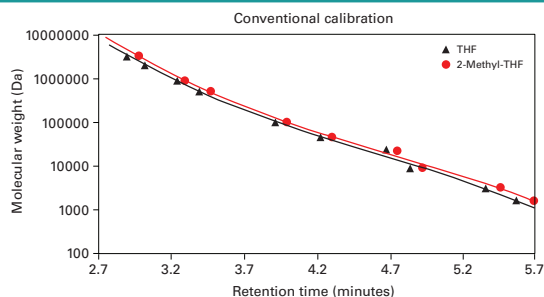
Molecular weight determination

To complete the assessment of 2-methyl-THF as a potential replacement for THF, the molecular weight (MW) determination of a polystyrene standard was conducted by conventional column calibration and multi-angle light scattering (MALS) in both solvents.

Conventional column calibration

The molecular weight of the polystyrene standard via column calibration was obtained by creating a calibration curve that plots the molecular weight of known standards as a function of their elution time. In *Figure 4*, two calibrations using the polystyrene mixtures PSTQuick B and C were performed in THF (black) and 2-methyl-THF (red).

Figure 4. Conventional calibration curves



Both mobile phases show a similar slope in the linear section of their calibration curve. The primary difference is a small shift between the two curves due to the retention time difference discussed earlier in *Figure 2*. To confirm these minor differences in molecular weight determination between the two solvents, polystyrene standard F-4 was analyzed by determining its relative molecular weight using the respective elution time and conventional calibration curve with each solvent. *Table 1* below illustrates that the determined relative molecular weight is effectively the same.

As can be seen from the data, there is a slight difference to the specified nominal value of the F-4 standard (41.1 kDa). This is because the calibration is based on a cubic regression curve, which can result in slight deviations from the actual value. In this work, the small shift in retention time is accounted for by performing a new column calibration after any change in the experimental conditions, even when switching to a new solvent with seemingly comparable properties.

Table 1. Molecular weight determination of the PS standard F-4 by conventional calibration

Concentration (mg/mL)	M_w by conventional calibration (Da)	
	THF	2-methyl-THF
0.1	45,988	45,581
0.3	45,482	46,736
0.5	46,507	46,491

Light scattering

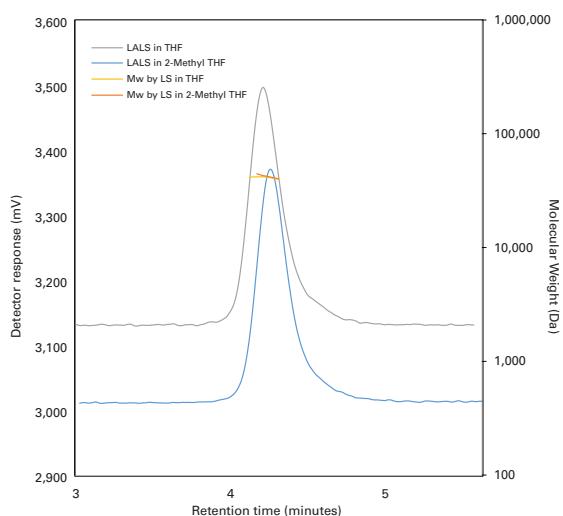
Multi-angle light scattering (MALS) was used for direct molecular weight measurements of polystyrene samples in the two solvents. Polystyrene standard F-4 was tested in three concentrations in both mobile phases.

Table 2. Molecular weight determination of the PS standard F-4 by MALS

Concentration (mg/mL)	M _w by MALS (Da)	
	THF	2-methyl-THF
0.1	40,949	41,397
0.3	42,650	41,250
0.5	41,211	41,290

Table 2 shows the weight-average molecular weight (M_w) results obtained by MALS. For MALS analysis, the refractive index of the solvent and the dn/dc of the polymer are required. A refractive index of 1.407 was used for both solvents⁴, while polystyrene has a well-established dn/dc value of 0.185 mL/g in THF. In **Figure 3** we have already shown that the RI peak area against the injected mass of PS sample is identical for both solvents. This confirms that the dn/dc value of PS is the same in 2-methyl-THF as in THF.

Figure 5. Comparison of MALS analysis



As seen in **Figure 5**, both mobile phases show a similar detector response in the low-angle light scattering (LALS) signal at the same concentration of the polystyrene standard F4 in THF and 2-methyl-THF. We also can see that the core baseline noise of the detector is also highly comparable for both mobile phases, thus providing identical signal-to-noise ratio.

As a result, the MW reported in **Table 2** are in very good agreement between the two solvents and with the nominal value of 41,100 Da. In addition, the molecular weight traces in **Figure 5** overlay perfectly, illustrating that the use of the greener solvent alternative has no influence on the performance of the molecular weight determination by MALS.

Conclusion

2-methyl-THF was assessed against THF as a potential, more environmentally friendly solvent for GPC/SEC applications, due to its greener synthesis and safer properties. It was found to deliver similar performance by providing as good separation, sensitivity, and molecular weight determination accuracy as THF, by conventional calibration and MALS. 2-methyl-THF thus offers a greener alternative to THF. However, the cost of 2-methyl-THF remains higher in current market conditions. A higher demand and increased production capacity in the near future would help chromatographers effectively replace THF in their daily work.

References

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- ⁴ CRC Handbook of Chemistry and Physics, David R.L., 2010

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