

# Tips & Tricks: GPC/SEC

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## How to find the ideal stationary GPC/SEC separation phase.

### What are the driving forces for separation in GPC/SEC and how do they influence method development?

Gel permeation chromatography/size exclusion chromatography (GPC/SEC) is a powerful and well-established method for the analysis of macromolecules in terms of molar masses (MMs), molar mass distributions (MMDs) and molecule dimensions. It is a liquid chromatography (LC) technique using the same or comparable instrumentation as other LC methods, such as liquid adsorption chromatography (LAC). The major difference to other LC set-ups is the use of dedicated GPC/SEC columns with stationary phases that allow an interaction-free separation according to molecular size. Any chromatographic separation process can be described by the distribution coefficient ( $K$ ), the ratio of the sample concentration between stationary ( $a_s$ ) and mobile phase ( $a_m$ ).

$$K = \frac{a_s}{a_m} = \exp \left[ -\frac{\Delta G}{RT} \right] = \exp \left[ -\frac{\Delta H - T\Delta S}{RT} \right] \quad [1]$$

This equation can be divided into two parts, related to LAC, based on enthalpic attractive interactions, and SEC, based on an entropic-driven process without any interaction between sample and stationary phase (column material). This is illustrated in Figure 1.

$$K_{LAC} = \exp \left[ \frac{-\Delta H}{RT} \right] \text{ and } K_{SEC} = \exp \left[ \frac{\Delta S}{RT} \right] \quad [2]$$

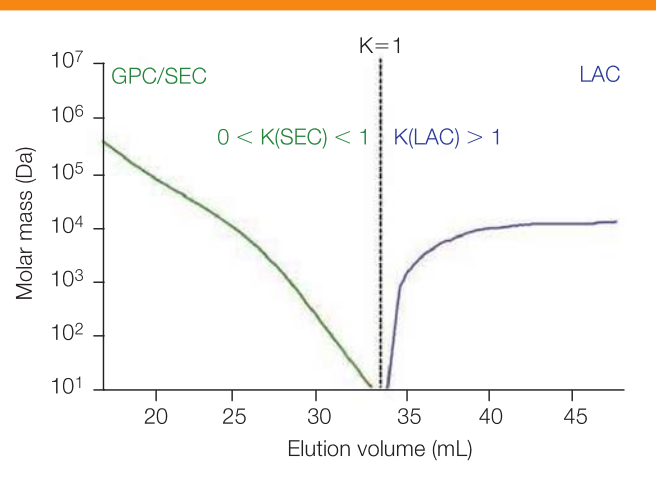
with  $0 < K_{SEC} < 1$  and  $\Delta H = 0$  and  $K_{LAC} > 1$  and  $\Delta S \approx 0$ . In order to analyse a sample by GPC/SEC and to have a

“true” GPC/SEC separation mechanism (which is also required for advanced characterization with MM-sensitive detectors such as on-line viscometers and light scattering detectors), interactions between sample and stationary phase have to be avoided or suppressed.

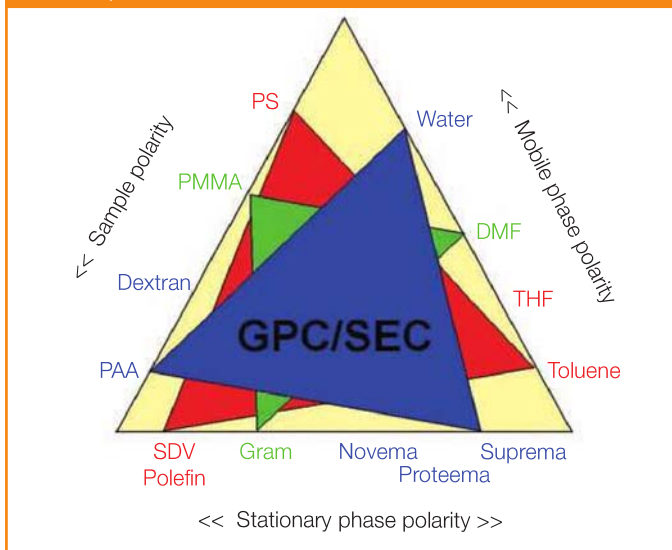
Interaction can be either attractive or repulsive. Attractive interaction leads to adsorption because the affinity of the sample to the stationary phase is larger than the affinity to remain in the mobile phase (solvent). This kind of interaction is successfully used in polymer LAC to separate, for example, copolymers according to chemical composition.<sup>1</sup>

Repulsive interaction between sample and stationary phase means that the sample does not see the true pore size but

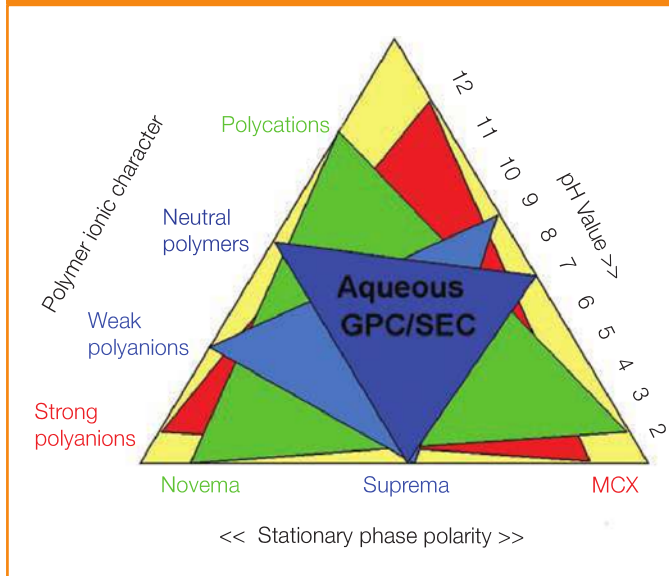
**Figure 1:** Molar mass versus elution volume in GPC/SEC and LAC.



**Figure 2:** The “magic” triangle to balance the polarities of the components.



**Figure 3:** The “magic” triangle for aqueous applications.



an apparent smaller pore size. This is the reason why a polyelectrolyte (PE) such as poly(styrene sulphonate) (PSS) analysed on a stationary phase with a negative-charged surface (e.g., MCX) elutes much earlier than a neutral polysaccharide of comparable size such as Pullulan. A separation according to the size of the molecules is still possible but the universal calibration, the transformation from one calibration curve into another, will fail because of the different interactions for the samples.

**What are the consequences for GPC/SEC method development?**

The goal for method development in GPC/SEC is to balance the polarity of sample, mobile phase (eluent), and stationary phase (column material), so that any kind of interaction is eliminated. Since sample polarity and solvent polarity are often given parameters for an application, this can be done by selection of a stationary phase with matching polarity. This leads to robust SEC/GPC methods with high reproducibility and guaranteed long-term stability.

**How can the matching stationary phase be selected?**

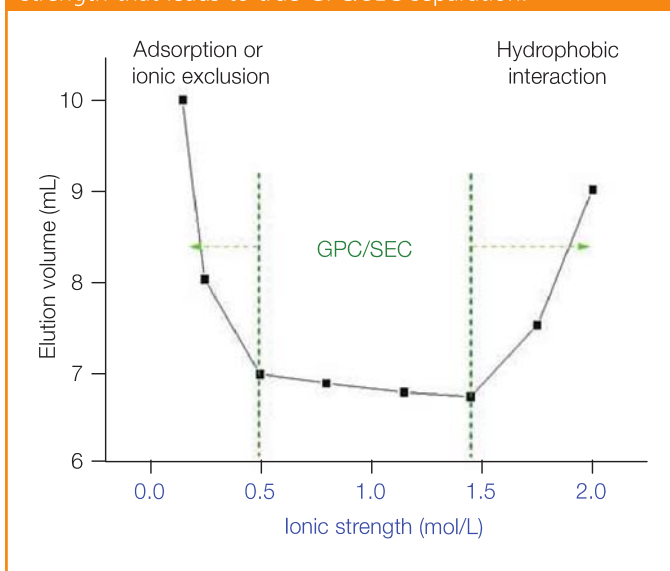
A visual guide for column material selection can be a triangle, where the polarities of the three components are

related (the “magic” triangle). Figure 2 shows the isosceles magic triangle. Each side represents one of the components: sample, mobile phase and stationary phase. The given examples for sample, solvent and column material are sorted according to their polarity. It can be shown that isosceles inner triangles can be drawn to find the best choice of solvent (mobile phase) and column material (stationary phase) for each sample.<sup>2</sup> For a given water-soluble sample, such as poly(acrylic acid) (PA) a hydrophilic polar matrix has to be chosen.

For aqueous applications the magic triangle concept can be expanded to describe the column material selection for neutral, cationic and anionic samples. In aqueous GPC/SEC the samples have to be shifted into the fully dissociated state (salt form) to suppress any kind of hydrophobic interaction. This can be done by varying the pH value of the mobile phase water (Figure 3). At a pH of 9 PA — a weak organic acid — is fully dissociated and no hydrophobic interaction (hydrogen bonding) occurs. It can be measured on a hydroxylated methacrylate(MA)-based material, such as Suprema. Strong polyanions such as poly(styrene sulphonates) (PSSs) will be measured at a high pH value and this corresponds perfectly to highly polar charged stationary phase (e.g., MCX).

**Table 1:** Balanced polarity for different chromatographic systems.

Polarity	Sample	Solvent (mobile phase)	Column material (stationary phase)
Unpolar	Polystyrene	Toluene	Styrene-divinyl benzene (e.g., SDV)
Medium polar	Poly(methyl methacrylate)	Dimehtylacetamide (DMAc)	Polyester (e.g., Gram)
Polar	Dextran	Water	Hydroxylated methacrylate (e.g., Suprema)

**Figure 4:** GPC/SEC window for a salt concentration/ionic strength that leads to true GPC/SEC separation.**Is any additional method development required?**

For samples with many functional or ionic groups, such as polyelectrolytes (PEs), a balanced polarity is the minimum prerequisite but not sufficient for interaction-free GPC/SEC. In order to suppress ion-exclusion the addition of low-molecular-weight neutral salts or the use of buffer solutions is required. These salts can reduce the interactions with the stationary phase and can suppress aggregation of the sample. However, the concentration of the salt should be selected carefully.<sup>3</sup> Only within a small concentration window can both hydrophilic and hydrophobic interactions be avoided. Figure 4 shows the elution volume dependence of a sample as the function of increasing ionic strength. A

very low ionic strength leads to an increased elution volume because of adsorption or ion exclusion. Increasing the ionic strength to 0.5–1.5 ensures GPC/SEC separation. Above an ionic strength of 1.5, hydrophobic interactions occur and the elution volume increases again.

Depending on the polarity of the given stationary phase, the GPC/SEC separation window can be shifted to higher or lower ionic strengths, even down to zero for many organic mobile phases.

**References**

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