



Q: I have injected a sample and I don't see a signal. What can be the reason for this?

Comment

To answer this question it is important to know that many different detectors and detector combinations are used in GPC/SEC. Understanding the basic detector principles is vital to solve problems with missing peaks.

A: If you inject a sample and don't see a signal this might be related to a problem with the system (mainly injection system) or to the combination sample/detector.

1. If you do not see a sample peak, but the expected system peaks it is unlikely that the injection system causes the problem. If the system peaks are not present either, it might be that the seal in your injection system needs to be replaced or there is leakage in the system.

In case of a manual injector make sure that you have flushed the loop carefully. If possible, flush at least with a 5-fold excess of the loop volume.

2. If you do see system peaks but no signal peaks it is most likely that the combination sample/detector is not ideal. Fortunately any GPC/SEC detector signal can be understood using the equation in the green box below.

with $x = 0$ for RI, UV and ELS detectors (no molar mass dependence!) typical concentration detectors)

$x = 1$ for MALLS, RALLS and LALLS detectors ($x = \text{Mark-Houwink alpha}$ for viscometers) typical molar mass sensitive detectors

K_{Detector} is a detector constant and can be disregarded for our problem here.

$$\text{Signal Intensity} = K_{\text{Detector}} \cdot k_{\text{sample}} \cdot \text{Injected Mass} \cdot \text{Molar Mass}^x$$

k_{sample} is a sample-related constant and is very important here.

Depending on the detector, k_{sample} is dn/dc for refractive index and light scattering detectors dA/dc for UV detectors

To measure a signal, k_{sample} needs to be different from 0. The higher k_{sample} , the higher the signal (for the same load (molar mass), in the same solvent).

However, k_{sample} depends not only on the sample, but also on the solvent and other parameters (e.g., T , λ). A very famous example is poly(dimethylsiloxane) (PDMS). In THF $k_{\text{sample}} = 0$ meaning that the sample is isorefractive and can not be detected. Therefore, PDMS needs either to be characterized in toluene (where $k_{\text{sample}} < 0$) or an ELS detector has to be used instead of the RI.

If k_{sample} is not the problem then you need to look at the concentration and injection volume and (only in cases of light scattering, viscometry or triple) on the molar mass. If one of these experimental parameters is too low, you might not see a signal. For molar mass detectors look at the molar mass detection limit and do not forget the samples with a low dn/dc need high molar masses to be detected.

Verify also the concentration and injection volume recommended for GPC/SEC.

As a rule of thumb use for:

- Samples with a narrow PDI/molar mass distribution: 1-2 mg/mL
 - Samples with a high PDI/broad molar mass distribution: 4-5 mg/mL
- Injection volume:
- 1 column (8 x 300 mm): 20 μL
 - 2 columns (8 x 300 mm each): 50 μL
 - 3 columns (8 x 300 mm each): 100 μL
 - 4-5 columns (8 x 300 mm each): 200 μL

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