Theory and Practice of GPC

Troubleshooting

Introduction

Purpose

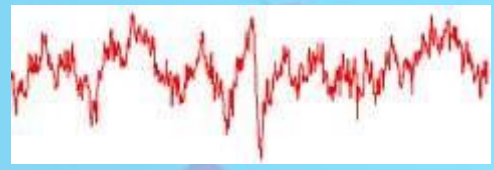
- To briefly examine common sources of problems in GPC/SEC
- To highlight possible causes
- To propose possible solutions to the problems

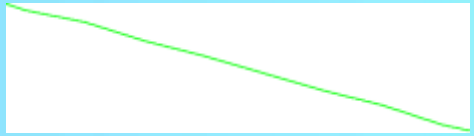
Aim

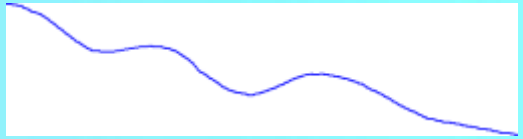
- To increase understanding of GPC
- To help solve problems in your laboratory

Baseline Noise and Drift

Random noise is usually a result of the build-up of contamination in the column or in the detector cell, steady baseline drift usually results from the build up of contaminations







- Flush the column and the detector cell to waste
- Make sure the samples are clean – filter with 0.45µm filters
- Use high quality solvents for HPLC or GPC

Spikes are usually due to bubbles in detector

- Make sure you have degassed mobile phase before use

Random drift can also be cause by temperature changes

- If thermostatting, make sure you insulate the column and tubing

Baseline Drift at Start of Operation

- Usually caused by the column settling down
- Make sure you allow sufficient time for column to equilibrate
- Can be caused by the detector equilibrating
- Allow time to reach stability - very common for RI detectors
- Ensure detector is not in a draught or direct sunlight
- Baseline variations can also be cause by RI Reference cell contents decaying or degrading, especially at temperature
- Regularly flush the reference cell with mobile phase

Leaks in the System

Most common cause is loose connections between columns and detectors

- Check all the connectors and tighten if necessary
- If the leak persists, disassemble and replace the leaking connector

Internal Detector Leak can be seen in the detector, injection valve or pump

- Often due to solvent spillage near the instruments solvent sensor
- Can be due to failed detector seal or cracked cell – these must be replaced
- Leaks are sometimes seen from worn rotor seal in the injection valve
- Injection valve siphoning can draw solution from the waste - lower waste bottle
- Pump purge valve failure will cause leaks – tighten the valve or replace
- Pump seal and gasket failure will result in leaks - these must be replaced

Leaking can be seen in from the column end-fittings

- The end-fitting may be loose - tighten as necessary
- The frit & spreader in the column may need to be replaced

Ghost Peaks

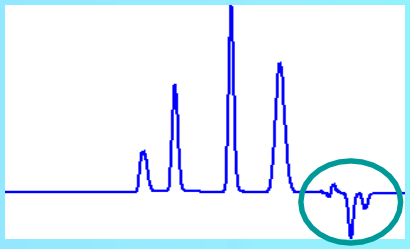
Ghost peaks are often peaks which come from the previous injection

- Make sure you do not inject next sample until previous one has fully eluted!
- If there is absorption, some material may elutes after the total permeation limit
- If there is absorption, make sure you flush the column completely
- During injection, ensure that injection loop is completely filled and flushed

Negative Peaks

- On RI detectors can occur is the dn/dc is less than the solvent
- Reversing signal polarity gives a positive peak
- On UV detectors can occur is the solute absorbs less than the eluent
- Need to change eluents to get a positive peak

Negative peaks and baseline disturbance at total permeation due to differences in refractive indices of injection solvent and eluent



- Cannot be avoided, but it helps if the samples are prepared in the mobile phase

Split Peaks

Often seen if the sample loading on the column is too large

- Reduce the size of the injection loop or the concentration

Can also be caused by a blocked or partially blocked frit

- Need to replace the frit in the column
- Stop the frit clogging by using an in-line solvent filter of about 2µm

A void or channel in the column will also cause split peaks

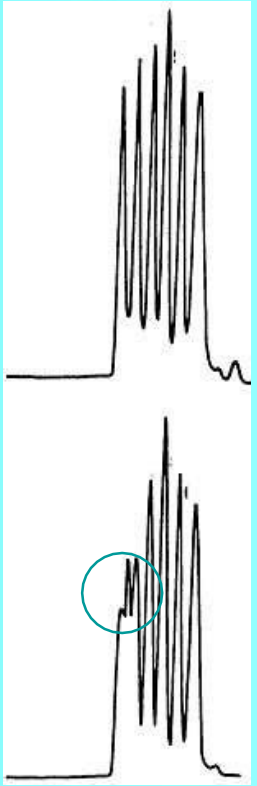
- Unfortunately you will need to replace column!

Can be caused by a partially blocked or damaged flowpath in the injector

- Need to replace the rotor seal in the injector

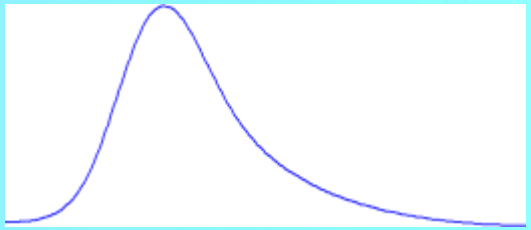
Split peak may be due to a single peak with interfering components

- Need to prepare a fresh solution!



Peak Tailing

Tailing can result from excessive dead volumes



- Make sure the tubing length is minimised,
- Make sure the injection seal is tight and there are no leaks
- Ensure that the connector fittings are properly seated

Tailing can result from degradation of column

- Repair or replace the column!

Interaction of sample with surface of stationary phase can cause tailing

- Overcome with using mobile phase additives
- Amines or salts to can be used in organic GPC

Peak Broadening

Large dead volumes will contribute significantly to peak broadening

- Always use LDV end fittings and connectors
- Minimise lengths and diameters of tubing wherever possible

Broadening will result if the eluent is too viscous

- May need to increase operational temperature

Broadening may result if the detector cell volume too large

- If possible, use a smaller cell volume

Broadening will result if the column is not performing

- Repair or replace the column

Variable Retention Time

Lab temperature changes may result in retention time changes

- Overcome by thermostatting the columns

Insufficient equilibration time for the column may give unstable retention behavior

- Allow at least 2 GPC column volumes through the column(s)

Decreasing Retention Times

Usually a result of the flow rate speeding up

- Check the pump and reset the flow if necessary

Increasing Retention Times

Usually a result of the flow rate slowing down

- Check for the presence of bubbles in pump head

Retention beyond total permeation volume will be observed if there are specific interactions between the sample and the with stationary phase

- Interactions and be Inhibited by adding modifiers to mobile phase

Adsorption of sample can occur if you are using a poor solvent, for instance analyzing polystyrenes in DMF

- Change eluent so that samples, standards and solvent are of similar polarity

Pressure Increasing

Can be caused by build-up of particulates in the sample

- Can be avoided by filtering the samples and mobile phase

In certain cases, solvent freezing in GPC tubing can cause pressure problems

- For these solvents eg TCB and DMSO, elevate the temperature of the solvent resevoir

Pressure Falling

Falling pressure can be caused by pump cavitation

- Make sure you thoroughly degas solvents

If the pressure is low it could be due to insufficient flow to column

- Clear any blocked solvent lines
- Loosen cap of eluent reservoir to prevent pressure problems

High Pressure

A high pressure will result if the flow rate is too high

- Check pump flow rate independently by measuring with flow with stopwatch

High pressure will also result if the column has a blockage

- Filter samples to avoid this problem
- Use a guard column to improve the column lifetime

High pressure may be due to a blocked inlet frit on the column

- Reverse flow through column to clear any blockage
- Replace frit to repair the column

Pressure Fluctuation

Fluctuation will be caused by a Leaky check valve or pump seal

- Replace or clean the check valve

A bubble in pump head will also cause fluctuations

- Remove the bubble by purging the pump head
- Degas solvents thoroughly to avoid bubble build-up

Insufficient liquid flow to pump will cause pressure problems

- Mobile phase inlet may be blocked - remove and clean it
- Elevate reservoir above pump head to help siphoning

Poor Detector Sensitivity

The sample will not be observed if it is injected at a concentration below the minimum detectable level

- Increase concentration **or** sample volume to improve response

Sometimes a small peak will be observed for the first few sample injections due to adsorption of sample onto the column

- Condition column with concentrated sample will reduce effect

Injecting an under filled injection loop will give small peaks

- Overfill the sample loop by a factor of x3, prior to injection

Poor Column Lifetime

Packing media can be degraded by aggressive or impurities in mobile phase

- Use stabilised THF, TCB with antioxidant, etc

Shorter lifetime are observed with high temperature

- Unfortunately there is not much that can be done

Shorter lifetimes are observed when using small particle columns

- Switch to larger particle size to reduce problem

Deterioration can also occur due to contaminant build-up on the column

- This can be avoided by using a “sacrificial” guard column

Conclusions

It is important to be familiar with your system to know when it is operating at maximum effic

- Keep records of NORMAL operating conditions and chromatograms. Use a sample of your prod a “control” sample.

Classify symptoms eg:

- Pressure
- Leaks
- Quality of data - unstable, drift, noise - long or short term random etc

Conclusions

- Refer to manufacturers' handbooks
- Call supplier for advice
- Discuss problem(s) with other LC users