SFC meets SEC – 3 Modes in one Application

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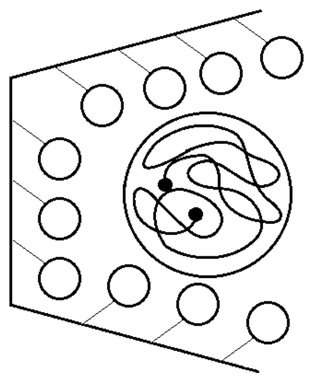
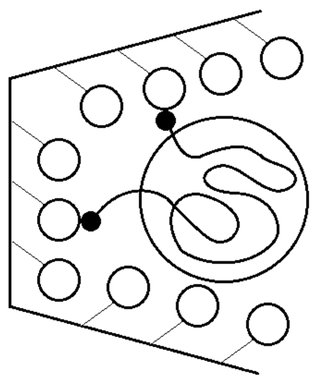
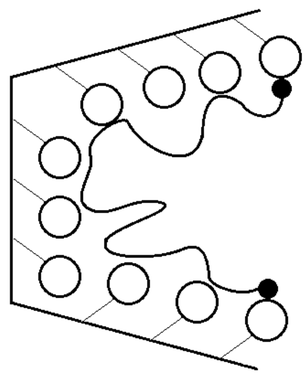
Introduction

The predominant modes for the chromatography of polymers are size-exclusion chromatography (SEC) and liquid adsorption chromatography (LAC). On the transition between these two modes, there exists the so called liquid adsorption chromatography at critical conditions (LACCC). All these modes are used to separate polymers based on specific molecular attributes. There are just a few, older investigations on the use of these three modes with supercritical CO_2. We want to reinvestigate on these modes with the recent technological progress in the area of SFC. In the present work, we showed all three modes on Polyethylene glycol (PEG) as a model substance. All modes were successfully used with the same chromatographic system and the same column by varying the ratio of CO_2 and Methanol as eluent. To determine the parameters for the chromatography at critical conditions a Quality-by-Design approach was used to minimize the experiments needed. As a practical usage, one developed method was transferred to preparative scale. PEGs of different molecular weights were separated, purified and uniform polymers were obtained.

Theory on the Chromatography of Polymers

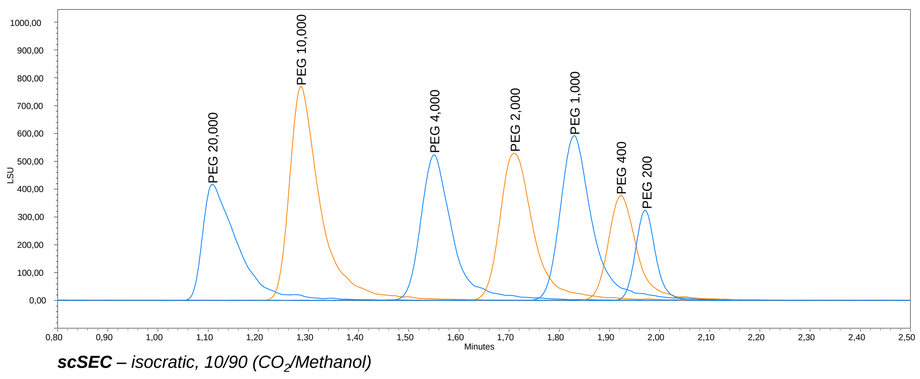
Supercritical Size Exclusion Chromatography **scSEC**	Supercritical Fluid Adsorption Chromatography at critical conditions **SFACCC**	Supercritical Fluid Adsorption Chromatography **SFAC**
• High amount of modifier • strong solvent • no interaction enthalpic between polymer and stationary phase	• mixture of strong and weak solvent • enthalpic and entropic effects equal each other	• High amounts of CO_2 (weak solvent) • ad- and desorption of the polymer to the stationary phase
		
• separation based on the hydrodynamic volume • higher MW elute first	• coelution of polymers independent of the MW • separation based on the end groups	• Separation based on the strength of interaction • Higher MW elute later

Comparison of the different separation modes for polymers with supercritical fluid chromatography. Which mode is dominant is controlled by chromatographic conditions, mainly the solvent strength.

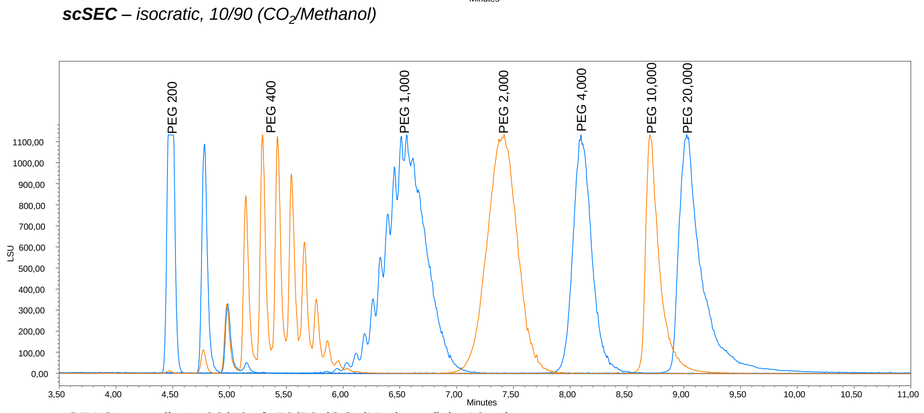
SEC and Adsorption for PEG on the same System

scSEC:
- Isocratic analysis with high amount of strong solvent
- Higher MW PEGs elute earlier than lower MW PEGs
- Conditions in subcritical region

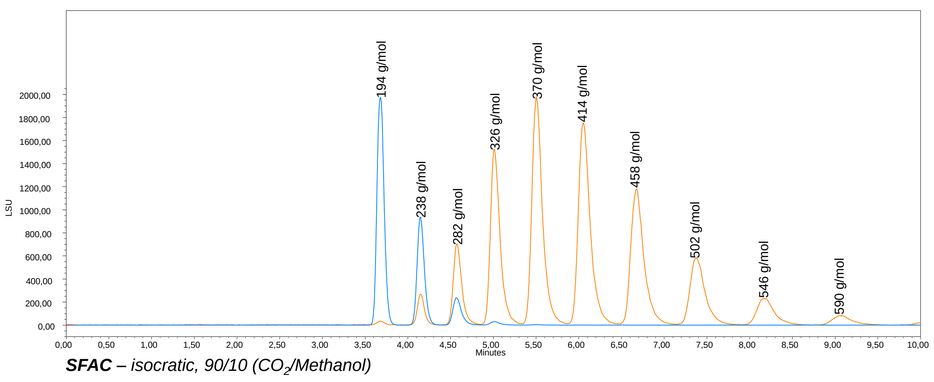
SFAC:
- Gradient to elute polydispers PEGs with MW from 200 Da – 20 kDa
- Isocratic composition with low amounts of modifier lead to a breakdown of polydispers PEG 200 and PEG 400 samples to their monodisperse components
- Molecular masses were confirmed by SFC-MS (ESI+) coupling



***scSEC** – isocratic, 10/90 (CO_2/Methanol)*



***SFAC** – gradient, 90/10 → 50/50 (CO_2/Methanol) in 10 min*



***SFAC** – isocratic, 90/10 (CO_2/Methanol)*

On the same system:

Column:
200 Å; 1.9 µm

Backpressure:
1800 psi (124 bar)

Eluent:
CO_2 (A), Methanol (B)

Flow:
1 mL/min

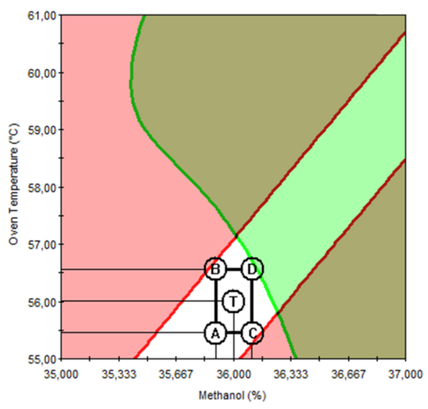
Temperature:
40°C

Detection:
ELSD

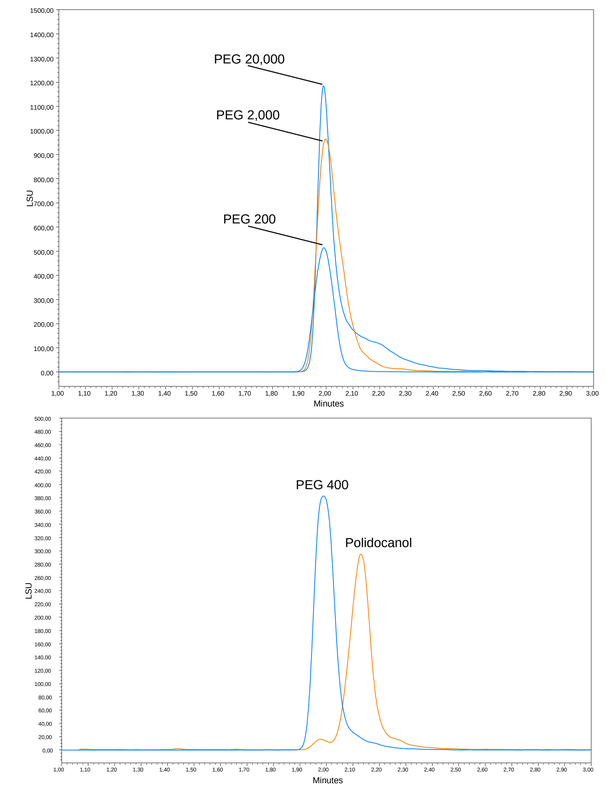
QbD for SFACCC

Determination of the critical chromatographical conditions by QbD:
- After a first screening, a QbD approach was used to determine the critical chromatographical conditions with a minimum of experiments
- The small region of conditions, where a coelution of all PEGs is achieved, is shown in white
- The point was verified by experiment
- Separation of PEG 400 and Polidocanol (PEG 400 with C12-alkyl end group) was demonstrated under these conditions

Name	Goal	Lower Bound	Upper Bound	Color
Max RT Difference of all PEGs	Minimize	0.030	---	Red
RT difference PD & PEG400	Maximize	---	0.100	Green

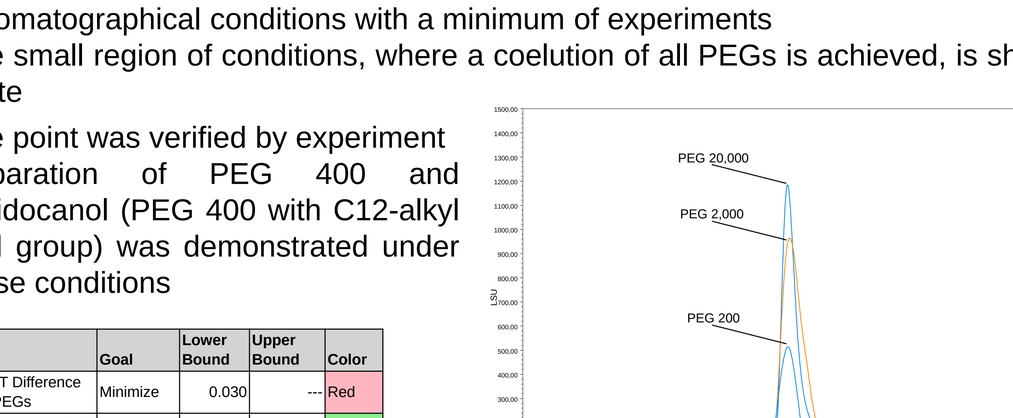


Design space of the QbD approach, generated by Fusion QbD software; critical chromatographical conditions at Ⓣ



Coelution of different PEGs (top) and separation of PEG 400 and Polidocanol (bottom) at critical chromatographical conditions – 36% Methanol and 56°C

Preparative Isolation of Uniform Polymers

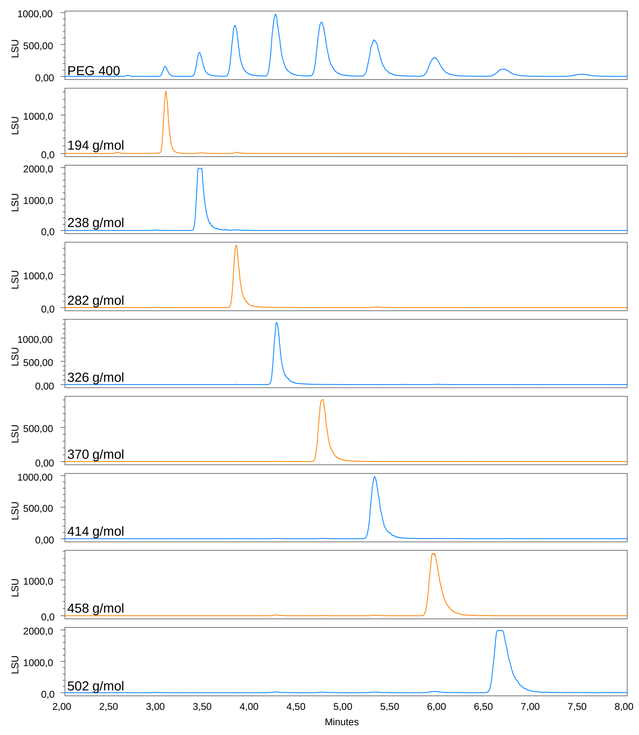


SFAC-mode was used to isolate uniform polymers form PEG 400

Isolation (Sepiatec SFC-250):

Column: 200 Å; 5 µm
Eluent: CO_2 (A), Methanol (B)
Flow: 60 mL/min; 93/7
Temperature: 40°C
Detection: ELSD

Correct MW [g/mol]	MW „classical“ calibration [g/mol]	Deviation [%]	MW „uniform“ calibration [g/mol]	Deavaition [%]
194	232	16.42	198	1.84
238	254	6.26	237	-0.60
282	297	5.03	278	-1.30
326	345	5.55	328	0.67
370	380	2.70	372	0.56
414	431	3.91	420	1.54
458	486	5.73	465	1.41
502	530	5.27	498	-0.89
Mean		**6.47**		**1.10**



SEC calibration:

A calibration of the derived uniform polymers was compare to a conventional calibration with polydispers PEGs to demonstrate a possible usage of uniform polymers

Purity of the Polymers:

The purity of the uniform polymers was checked with the developed SFAC on analytical scale; A purity of > 99% was achieved for all polymers without optimizing the isolation

Instruments

Column – Reprosil SEC 200Å (Dr. Maisch, Germany)
150 mm x 2.1 mm, 1.9 µm (analytical)
250 mm x 20 mm, 5 µm (preparative)
Analytical – Waters UPC² with Acquity ELSD (Waters)
Preparative – Sepiatec SFC-250 with ELSD (Sepiatec)
Software – Fusion QbD software (S-Matrix Corp.)

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