Purification of crownethers containing molecules by Centrifugal Partition Chromatography (CPC)

Isabelle Lachaise[1], Sandrine Peyrat[2], Valessa Barbier[2]
[1]Plateforme Chromatographie Analytique et semi preparative, [2] Equipe Système Polymères Complexes
Institut de Chimie et des Matériaux(ICMPE), 2 rue Henri Dunant , 94320 Thiais, France

Introduction

At ICMPE (Thiais, France), researchers have developed new crownethers containing macromolecules displaying membrane permeabilization properties. These molecules are currently of interest in cancer research. They are synthesised from the polymerisation of crownether containing monomers (see Figure 1). Monomers A and B are routinely purified by extraction in hot pentane. 10 to 15 extraction cycles are required to obtain a suitable yield (45-60%). In this application note we illustrate a new purification method for these monomers using Centrifuge Partition Chromatography (CPC).

Samples

Synthesised samples of crownether containing monomers A and B have been chosen for purification by CPC (Figure 1). Crownether molecules are synthesized according a standard procedure as described in *RSC Advances*, **2012**, 2, 8606–8609. Previous analysis of samples A and B by HPLC are shown in figure 1. For both samples, HPLC chromatograms show the major compounds, (53 % for monomer A and 44% for monomer B), and 4 additional molecules.

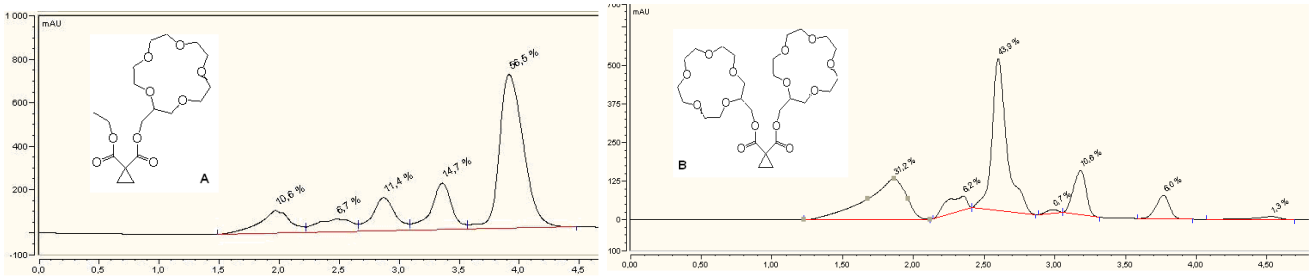
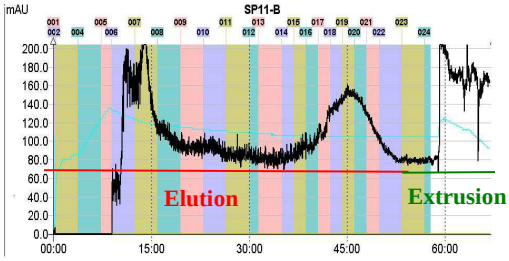


Figure 1 : *Analytical HPLC chromatograms of samples; 0.8ml/min, Injection volume : 10µl, 205 nm*
(left) Sample A : MeOH/H_2O (70/30), Purosphere RP18 column (250mmx4.6mmx5µm); (right) Sample B : MeOH/H_2O (85/15) betabasic-C18 column (150mmx4.6mmx3µm). Insert : monomer chemical structure.

CPC experiments

CPC is a separation process that involves two immiscible solvent phases. One remains the stationary phase while the other is the mobile phase. The aim is to find a suitable solvent system which provides both a good separation and a high yield.
Several Arizona biphasic systems have been tested for the purification of both samples. Sample A has been successfully purified using the L Arizona system Hept/EtOAc/MeOH/H_2O (2/3/2/3) with a partition coefficient equal to 1.13 in ascending mode.
CPC runs have been performed with 1.58 to 3.6 g of sample A. Subsequent HPLC analysis shows high purity fractions were collected with a recovery of over 90%, (see table 2, and Figure 3).



Injected mass	Run time	Solvent consumption	Purified product mass	Purity
1,58 g	45 mn	750 ml	1,17 g	92,36%
2,01 g			1,49 g	93,46%
3,6 g			2,78 g	94,62%

***Table 2** : CPC of sample A ; CPC column volume:250 ml, 8ml/mn, 1600 rpm: Hept/EtOAc/MeOH/H_2O (2 /3 /2 3), isocratic mode, UV detection : 205, 245 nm*

Due to its higher polarity, sample B could not be purified using an Arizona system Hept/EtOAc/MeOH/H_2O as no satisfying partition coefficient could be obtained between upper and lower phase. Nevertheless, purification was successfully performed using a ternary polar system, BuOH/MeOH/H_2O (45.8%/4.16%/50%). The partition coefficient was 1.39 in descending isocratic mode and 1.07g of pure B (94,6% purity by HPLC) has been recovered (1.84 g injected) (Figure 3).

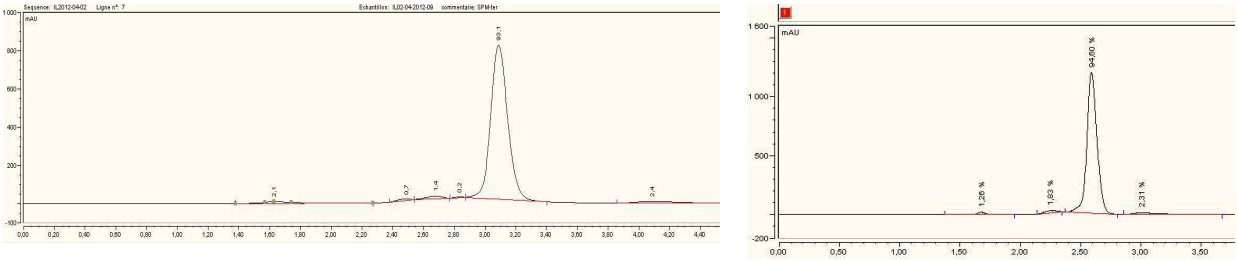


***Figure 3**: HPLC analysis after purification by CPC (left) : sample A and (right): sample B (for conditions see figure1 sample B)*

Conclusion

We have developed a new methodology by CPC for purification of crownethers containing molecules. CPC allows for the quick purification of several grams of sample with a good yield and a high purity of collected fractions. Further purifications involving larger, and thus more polar, crownether macrocyles are currently under investigation in our laboratory.