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

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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.

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/H2O (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).
Due to its higher polarity, sample B could not be purified using an Arizona system Hept/EtOAc/MeOH/H$_2$O 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$_2$O (45.8%/4.16%/50%). The partition coefficient was 1.39 in descending isocratic mode and 1.07 g of pure B (94.6% purity by HPLC) has been recovered (1.84 g injected) (Figure 3).

**Table 2**: CPC of sample A ; CPC column volume:250 ml, 8ml/mn, 1600 rpm: Hept/EtOAc/MeOH/H$_2$O (2/3/2/3), isocratic mode, UV detection : 205, 245 nm

<table>
<thead>
<tr>
<th>Injected mass</th>
<th>Run time</th>
<th>Solvent consumption</th>
<th>Purified product mass</th>
<th>Purity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.58 g</td>
<td>45 mn</td>
<td>750 ml</td>
<td>1.17 g</td>
<td>92.36%</td>
</tr>
<tr>
<td>2.01 g</td>
<td></td>
<td></td>
<td>1.49 g</td>
<td>93.46%</td>
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<tr>
<td>3.6 g</td>
<td></td>
<td></td>
<td>2.78 g</td>
<td>94.62%</td>
</tr>
</tbody>
</table>

**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 macrocycles are currently under investigation in our laboratory.