The Purification of Crown Ether Samples by a Quaternary Solvent Flash Chromatography System

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Crown ethers are heterocyclic chemical compounds that consist of a ring containing several ether groups. The most common crown ethers are oligomers of ethylene oxide, the repeating unit being ethyleneoxy (-CH_2CH_2O-). Important members of this series are the tetramer (n = 4, or four ethyleneoxy units), the pentamer (n = 5), and the hexamer (n = 6). The term 'crown' refers to the resemblance between the structure of a crown ether bound to a cation, and a jewelled regal crown. The first number in the name of a crown ether refers to the number of atoms in the cycle, and the second number refers to the number of those atoms that are oxygen. Crown ethers are much broader than the oligomers of ethylene oxide; an important group is derived from catechol.

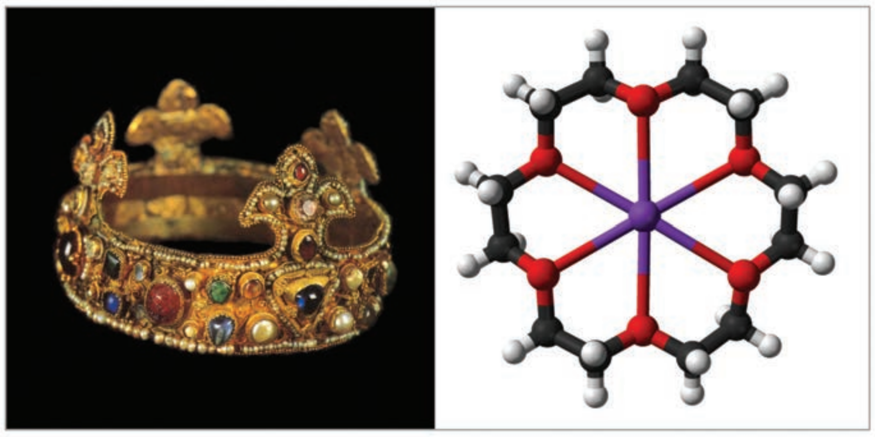

Figure 1. Jewelled crown (left) and Crown ether (right).

In 1967, Charles Pedersen, a chemist working at DuPont, discovered a simple method of synthesising crown ethers whilst trying to prepare a complexing agent for divalent cations [1]. Citing earlier work on the dissolution of potassium in 16-crown-4, he realised that the cyclic polyethers represented a new class of complexing agents that were capable of binding alkali metal cations. He proceeded to report systematic studies of the synthesis and binding properties of crown ethers in a seminal series of papers [2]. The fields of organic synthesis, phase transfer catalysts, and other emerging disciplines benefited from the discovery of crown ethers. Pedersen shared the 1987 Nobel Prize in Chemistry for the discovery of the synthetic routes to and binding properties of crown ethers.

The special properties of crown ethers are their strong coordination ability for alkali metal and alkaline earth metal cations. Depending on the size of the ether ring opening, the type of heteroatom and the number of atoms, this coordination effect shows distinct selectivity for specific metal ions. As important compounds in many fields such as coordination chemistry, extraction chemistry, phase transfer catalysis, ion-selective carriers, etc. crown ethers have attracted more and more attention [3-8]. The different properties of the substituent groups on the crown ether compound will affect its complexing strength with metal ions, selectivity and solubility. Compared with unsubstituted crown ethers, benzocrown ethers have attracted more attention in research due to their better lipophilicity [9,10]. However, benzocrown ethers exhibit poor solubility in solvents with low polarity, making them difficult to be used directly. In order to improve the properties of benzocrown ethers, in recent years many researchers have focused their work on the research and development of benzocrown ether derivatives.

In this application, the sample to be purified is a benzocrown ether derivative obtained by organic synthesis. The sample is composed of multiple components with diverse polarities, making it impossible to separate each component by conventional binary solvent gradient. Considering the sample properties, a quaternary solvent system was utilised employing a SepaBean machine T combined with SepaFlash normal phase silica cartridge (Santai Technologies) for the purification of the sample. By online switching between solvents of different polarity, the target products meeting the purity requirements were successfully obtained, suggesting a feasible solution for the purification of complex sample composed of multiple components with diverse polarity.

Experimental

The sample used in this application was a benzocrown ether derivative which was kindly provided by a university chemistry lab. The chemical structure of the sample molecule is shown in *Figure 2*. The sample has poor solubility in weak polar organic solvents since the components of the sample have diverse polarities. Therefore dichloromethane (DCM) was used as the solvent of choice for complete dissolution of the raw sample.

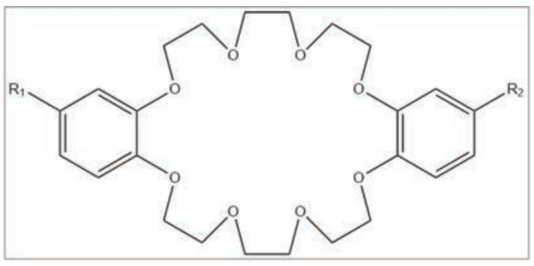

Figure 2. The chemical structure of benzocrown ether derivative sample.

To prepare the sample solution, 4.9 g of the raw sample was dissolved in small amount of DCM and absorbed onto 3.5 g of silica gel 200-400 mesh. The DCM was removed by vacuum and the absorbed sample was then placed in a 4g SepaFlash iLOK empty cartridge (order number: SD-0000-004) for solid sample loading. The sample was then eluted automatically by a SepaBean T Flash chromatography system employing the parameters shown in *Table 1*.

Results and Discussion

Considering the sample property, conventional binary solvent system DCM/methanol was employed as the mobile phase for the elution of the sample. The Flash chromatogram of the sample in this condition was shown in *Figure 3*.

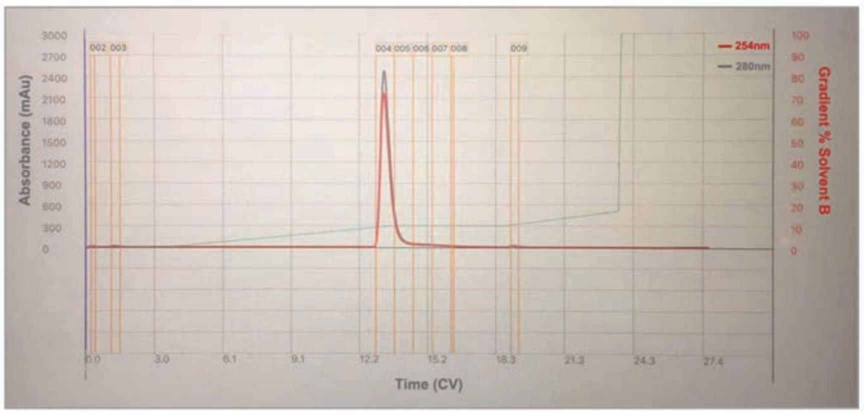

Figure 3. The Flash chromatogram of the sample by binary solvent gradient.

Table 1. The experimental setup for Flash purification.

Instrument SepaBean machine T					
Flash cartridge	12 g SepaFlash silica cartridge (irregular silica, 40 - 63 µm, 60 Å, Order number: S-5101-0012)		25 g SepaFlash silica cartridge (irregular silica, 40 - 63 µm, 60 Å, Order number: S-5101-0025)		
Wavelength	254 nm, 280 nm				
Mobile phase	Solvent A: DCM Solvent B: Methanol		Solvent A: N-hexane; Solvent B: Ethyl acetate; Solvent C: DCM; Solvent D: Methanol		
Flow rate	25 mL/min		30 mL/min		
Sample loading	500 mg (including 250 mg of the sample)		3.0 g (including 1.5 g of the sample)		
Gradient	Time (CV)	Solvent B (%)	Time (CV)	Solvent B (%)	Solvent D (%)
	0	0	0	0	/
	4.0	0	3.0	0	/
	13.5	10	6.0	16	/
	19.0	10	10.0	16	/
	23.5	16	20.0	50	/
	23.8	100	30.0	50	/
	28	100	44.0	80	/
			44.5	100	/
			63.0	100	/
			63.1	/	0
			85.0	/	4.0
			110	/	4.0

As shown in *Figure 3*, the sample had a good retention on the silica cartridge when eluted by the DCM/methanol binary solvent system. However, there was no resolution for the components in the sample mixture. Reviewing the synthetic route of the sample and referring to the classic synthetic route of dibenzo-18-crown-6 *(as shown in Figure 4)*, shows that along with the non-polar (lipophilic) target product in the sample mixture, there are excess/unreacted starting materials with strong polarities (such as catechol) or other by-products, resulting in multiple components of diverse polarities. When a simple binary solvent gradient is utilised, the DCM/methanol system is suitable for polar components. However, the selectivity for less polar components when employing the DCM/methanol solvent system is not as good as that of those solvent systems such as n-hexane/ethyl acetate(EA). Therefore, as an improvement to the simple binary solvent gradient, switching between binary solvent systems of different selectivities and continuously eluting the column may offer better resolution for the components.

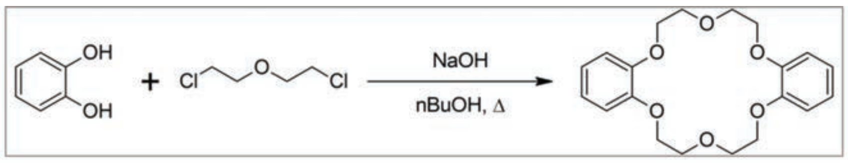

Figure 4. The classic synthetic route of dibenzo-18-crown-6.

Referring to Snyder's Solvent Selectivity Triangle *(as shown in Figure 5)*, commonly used solvents can be roughly divided into eight groups according to their different acidic, basic or dipole properties. Each group of solvents occupies a certain position in this triangle. According to the rule of 'like dissolves like', different groups of solvents have different selectivities for the same sample. Therefore, better selectivity and improved resolution for the components in the sample mixture could be obtained by optimising the solvent combination.

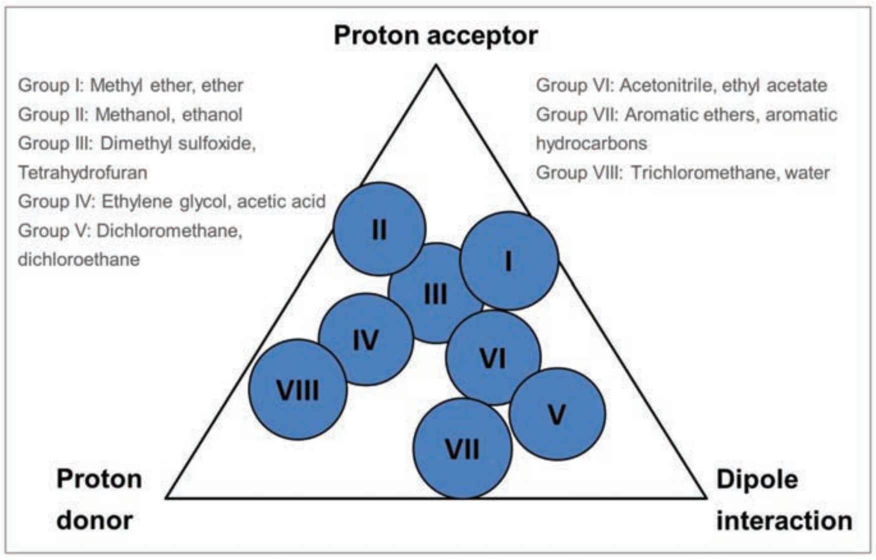

Figure 5. The schematic diagram of Snyder's Solvent Selectivity Triangle.

Looking at the conventional binary solvent system used in the previous experiment: DCM/methanol system. The solvent polarity table, *(as shown in Table 2)*, shows the polarities of DCM and methanol are 3.1 and 5.1, respectively. As a comparison, the polarities of n-hexane and EA are 0.1 and 4.4, respectively. Comparing these two normal phase solvent systems, it can be speculated that for a complex sample mixture composed of multiple components of diverse polarities, the best could be obtained if one solvent system could be replaced by another one for continuous elution.

Table 2. The polarity parameter P' of commonly used solvents.

Solvent	P'	Solvent	P'	Solvent	P'
N-pentane	0.0	N-propanol	4.0	Acetone	5.1
N-hexane	0.1	THF	4.0	Methanol	5.1
Benzene	2.7	Chloroform	4.1	Acetonitrile	5.8
Diethyl ether	2.8	Ethanol	4.3	Acetic acid	6.0
DCM	3.1	EA	4.4	Water	10.2

In a conventional configuration for a binary solvent Flash chromatography system, the mobile phase gradient is generated by a system pump in combination with a binary proportioning valve. When an instrument is used in this configuration for continuous gradient elution requiring two solvents to be replaced at the same time, the column must be removed from the instrument and then the tubing should be flushed with the solvent to be used in the subsequent procedure. Afterwards the column should be re-installed on the instrument for next elution step. These operations will undoubtedly lower the work efficiency. In this configuration a quaternary proportional valve is employed to generate a binary gradient with a combination from any two of the four solvent lines, making it possible to continuously switch online between different solvent systems during the elution procedure. Considering solvent miscibility, n-hexane/EA system was employed as the starting eluting solvent and then transitioned to the DCM/methanol system for the continuous elution of the sample. The experimental setup was listed in *Table 1*. The Flash chromatogram of the sample in this condition was shown in *Figure 6*.

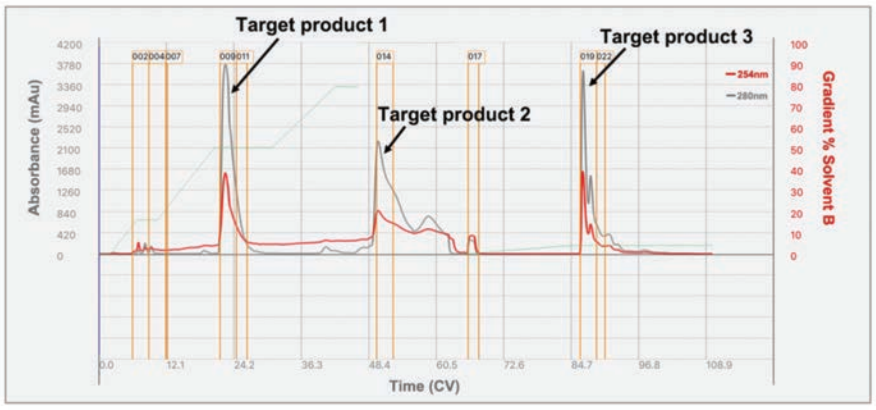

Figure 6. The Flash chromatogram of the sample by quaternary solvent gradient.

As shown in *Figure 6*, when n-hexane/EA system was employed as the eluting solvent in the first part of the separation, less polar components in the sample were eluted from the column. In the second part of the separation, a DCM/methanol system was utilised as the eluting solvent for the elution of more polar components. Therefore, multiple components of diverse polarity in the sample were separated and collected. The collected fractions were further identified by TLC. A 0.5% potassium permanganate solution was used as the colour developer for TLC analysis. After heating with a heat gun for 5 min, the TLC results show complete separation of the target compounds and byproducts *(Figure 7)*. It can be concluded that the components in the sample were effectively separated and can be used in next step research and development.

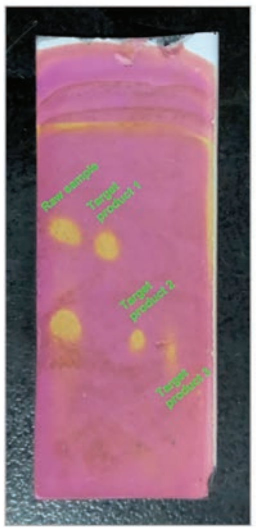

Figure 7. The TLC identification results of raw sample and collected fractions.

Conclusion

For complex sample mixtures such as benzocrown derivatives, it is impossible for a conventional binary solvent system to provide satisfactory resolutions of the components in the sample due to the wide distribution range of component polarity. Utilising a quaternary solvent Flash chromatography system for continuous online switching between solvent systems of different polarities, satisfactory purification results were obtained, suggesting a feasible and efficient solution for the preparative separation of this complex sample mixture.

For further information on detailed specifications of SepaBean machine, or the ordering information on SepaFlash series flash cartridges, please visit http://www.santaitech.com/index/.

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