The Purification of Porphyrins by SepaBean™ machine

Chromatography Application Note
AN009

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Introduction

The porphyrins are a group of macromolecular heterocyclic compounds which are composed of four pyrrole subunits connected by methylene bridges (=CH-). The parent compound of porphyrin is porphin (C20H14N4). The porphyrin ring has 26 π electrons, which is a highly conjugated system and therefore the porphyrin appears in dark color. The English name of porphyrin is derived from Greek words, meaning purple. So porphyrins are also called purpura. Since porphyrins can easily form 1:1 coordination compounds with metal ions, therefore porphyrins are widely used in the biophotosynthesis simulation, solar cells, organic electroluminescence, photoconductive materials as well as research and development for anti-tumor drugs.

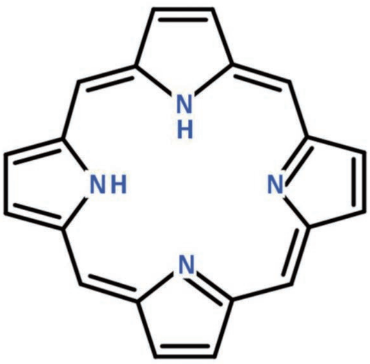

Figure.1 The chemical structure of porphin, the simplest porphyrin.

The total synthesis process of these compounds is complex with low yield and large amount of by-products which are generated after the reaction. Therefore the purification of these compounds should be very careful to avoid large sample losses. For the purification of the reaction products, researchers usually take the manual chromatography method which is accomplished by a long glass column packed with silica gels and using gravity as the driving force for the purification of the sample (as shown in Figure 2). The drawbacks of the manual method are obvious. For one thing, the operation of the long glass column is complicated and unsafe. For another thing, the deep colored sample turns the whole column bed to a dark color, making it difficult to determine the position of the target fraction.

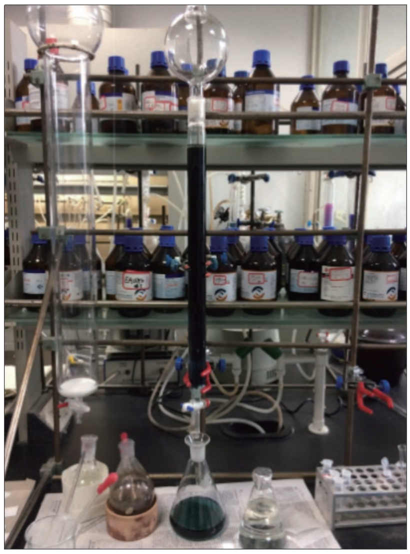

Figure 2. The experimental setup used in manual method for the purification of porphyrins.

To overcome the disadvantages of manual chromatography method, researchers from Santai Technologies have done some researches in the purification of porphyrin samples by an automated preparative liquid chromatography system, SepaBean™ machine, combined with flash cartridges pre-packed with high efficiency silica gels. The results showed that the automated purification method for the porphyrin samples could not only deliver good separation results but also save lot of time and thus enhance work efficiency.

Experimental

The sample used in the experiment was donated by an organic synthesis lab from a university. The reaction formula of the sample was shown in Figure 3.

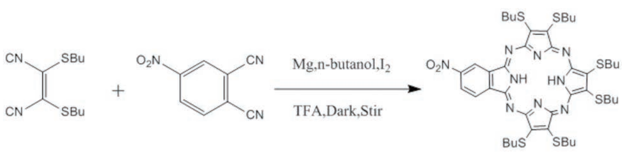

Figure 3. The reaction formula of the porphyrin sample.

When the synthetic reaction was completed, the flash purification experiment for the synthetic products was performed and the experimental setup was listed in Table 1.

Instrument	SepaBean™ machine	
Cartridges	40g SepaFlash™ HP Series silica (25 - 40 µm, 60 Å, Order number: SW-5102-040)	
Wavelength	254 nm (detection), 365 nm (monitoring)	
Mobile phase	Solvent A: N-hexane Solvent B: Dichloromethane	
Flow rate	25 ml/min	
Loading capacity	200 mg	
Gradient	Column volume (CV)	Solvent B (%)
	0.0	12
	3.0	12
	6.3	37
	12.0	40
	22.0	95

Table 1. The experimental setup for flash purification.

Results and Discussion

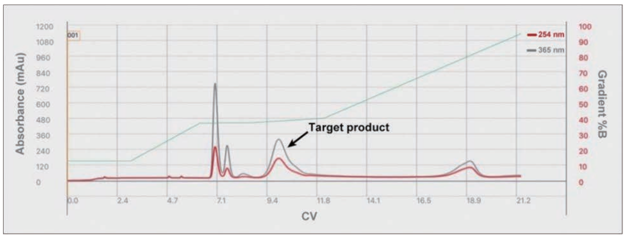

Figure 4. The flash chromatogram of the porphyrin sample.

In the flash purification experiment, a 40 g sized SepaFlash™ HP Series silica cartridge was used and the whole experiment was run for about 22 colume volumes (CV). The target product was automatically collected and the flash chromatogram was shown in Figure 4. By analyzing the TLC information as well as the flash chromatogram, the separation efficiency for the complex porphyrin sample was greatly improved by using flash cartridges pre-packed with small particle sized irregular silica gels (25 - 40 µm). The target product was completely separated with the impurities at baseline level. Furthermore, the function of elution gradient recommendation according to the Rf value of the sample, which is integrated in the control software of the SepaBean™ machine, makes the whole flash purification much more simple and efficient.

The user just need to input the Rf values of each component in the sample (acquired from TLC experiment) and choose the proper eluting solvents, the software will automatically generate an optimized elution gradient. There is no need to manually set or repeatedly optimize the elution gradient. Satisfying separation results was achieved in just one experiment. Compared with the manual chromatography method, approximately 2/3 of the separation time was saved in the automated method, greatly improving the work efficiency.

About the SepaFlash™ HP Series silica cartridges

There are a series of the SepaFlash™ HP Series silica cartridges with different specifications from Santai Technology (as shown in Table 2).

Item Number	Column Size	Flow Rate (mL/min)	Max.Pressure (psi/bar)
SW-5102-0004	4 g	15-30	400/27.5
SW-5102-0012	12 g	25-50	400/27.5
SW-5102-0025	25 g	25-50	400/27.5
SW-5102-0040	40 g	30-60	400/27.5
SW-5102-0080	80 g	40-80	350/24.0
SW-5102-0120	120 g	45-90	300/20.7
SW-5102-0220	220 g	60-120	300/20.7
SW-5102-0330	330 g	60-120	250/17.2

Table 2. SepaFlash HP Series silica cartridges. Packing materials: High-efficiency irregular silica, 25 - 40 µm, 60 Å.

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