Get Insight into the SepaBean Machine with Engineer: The Impact of Detector Flow Cell on Flash Chromatography

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Chromatography Application Note
AN034

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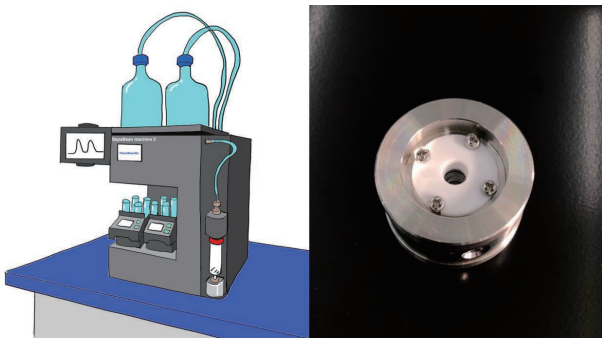

The detector module of the liquid chromatography system includes components such as light source, flow cell, photoelectric conversion device and the corresponding control circuits. As we all know, the light source determines the applicable detection range of the detector. The photoelectric conversion device determines the efficiency of converting optical signals into electrical signals. And the flow cell is a key component that determines the sensitivity and dynamic range of the detector. In this post, we will focus on how to choose the right flow cell depending on experimental purpose.

Take the diode array detector (DAD) used in SepaBean machine as an example. The working principle of DAD is shown in Figure 1.

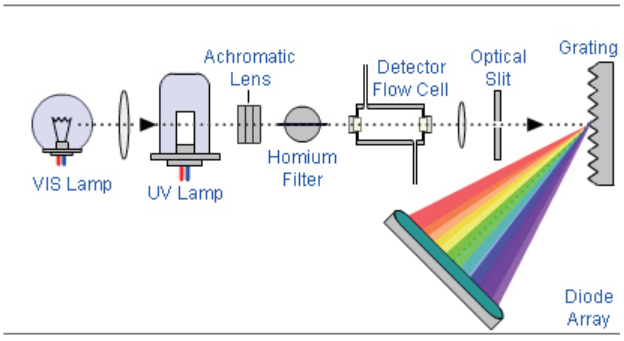

Figure 1. The schematic diagram of the working principle of DAD.

As shown in Figure 1, the continuous light from the light source pass through the flow cell and the slit, then dispersed into monochromatic lights by the holographic grating. Afterwards, the monochromatic lights are irradiated onto the photodiodes for detection by photoelectric conversion. Due to the different fractions eluted from the chromatography column that have their unique absorption of the incident light, the intensity of the transmitted light irradiated onto the photodiodes will be different in the full spectrum, therefore we could see the peaks with various sizes in the chromatogram. The structure of a universal flow cell is shown in Figure 2.

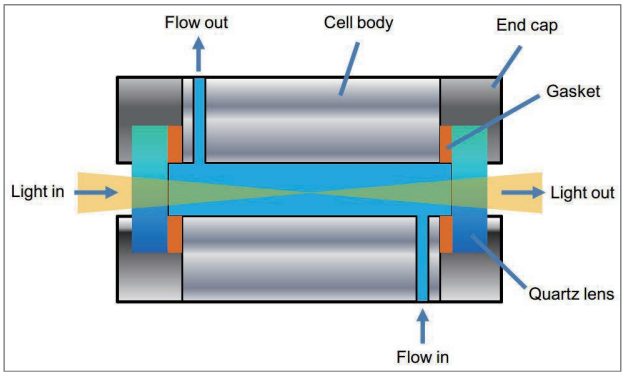

Figure 2. The schematic diagram of the structure of a universal flow cell.

A typical flow cell body is usually made of stainless steel. A round hole with a certain diameter is drilled in the flow direction of the mobile phase. The round hole is covered by a quartz window with good light transmission and sealed with a polymer gasket. Two tiny round holes are drilled at each end of the flow cell in a direction perpendicular to the optical path for the introduction and outflow of the mobile phase. The zigzag type design of the mobile phase flow path inside the flow cell ensure that no air bubbles are introduced into the light path of the flow cell.

In the following part, we will discuss the key parameters affecting the performance of the flow cell. First, we will consider the effect of the flow cell volume on the detection limit and resolution. When a large-volume flow cell is used, meaning large luminous flux and optical path, a relatively higher optical intensity will be obtained on the photo sensor when the sample flow through the flow cell. Therefore higher sensitivity will be obtained, which is beneficial to achieve a better detection limit. However, the diffusion effect will increase when a sample flows through a larger flow cell, resulting compromised resolution on chromatographic peaks. Therefore, this contradiction makes the volume of flow cell need to be comprehensively considered and determined according to actual application

needs.

Next, let us take a look at the effect of flow cell optical path on sensitivity and S/N (signal-to-noise) ratio. When the volume of the flow cell is defined, the appropriate optical path length should be determined next. According to Lambert-Beer's Law:

$$A = \varepsilon_\lambda c d$$

Where A is the absorbance, ε_λ is the molar extinction coefficient, c is the sample concentration (mol/L) and d is the optical path length (cm). When the sample type and concentration are determined, the absorbance A is proportional to the optical path length d. Therefore, choosing a flow cell with a longer optical path will help to increasing absorbance and thus improving detection sensitivity. However, the optical path length should not be too large since it will lead to increased optical loss, reduced luminous flux and increased interference absorption from mobile phase.

The following part will introduce a sample purification as the example to illustrate the influence of flow cells with different optical path length on detection sensitivity. The sample used in the experiment was from a customer, which had weak UV absorbance and the maximum absorption wavelength was around 210 nm. The experimental setup for Flash purification of the sample was shown in Table 1.

Instrument	SepaBean machine T	
Flash cartridge	80g SepaFlash® C18 RP cartridge (irregular C18 bonded silica, 40 – 63 μm, 60 Å, Order number: SW-5201-080-IR)	120g SepaFlash® C18 RP cartridge (irregular C18 bonded silica, 40 – 63 μm, 60 Å, Order number: SW-5201-120-IR)
Wavelength	210 nm; 254 nm	
Mobile phase	Solvent A: Water; Solvent B: Acetonitrile	
Flow rate	30 mL/min	
Flow cell optical path length	0.3 mm	2.4 mm

Table 1. The experimental setup for Flash purification.

In the first run, the default flow cell which has a 0.3 mm optical path length was used. The Flash chromatogram of the sample was shown in Figure 3. The target product was collected and analyzed by HPLC. However, the purity of the collected fractions cannot meet the requirement. After detailed inspection on the Flash chromatogram, the peak height of the target product was not ideal. Some impurities may not be detected by the detector, which may cause the purity of the target product to be substandard.

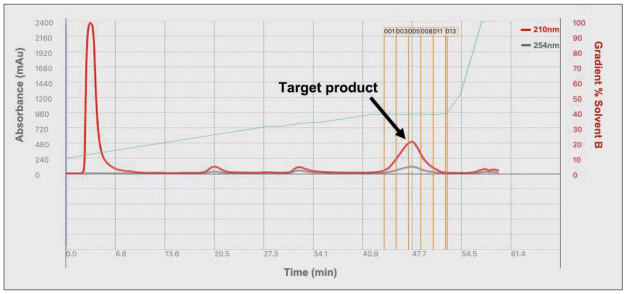

Figure 3. The Flash chromatogram of the sample with the detector equipped with the flow cell that has a 0.3 mm optical path length.

To increase the detector response signal to the sample, a flow cell that has a 2.4 mm optical path length was used. The Flash chromatogram of the sample was shown in Figure 4.

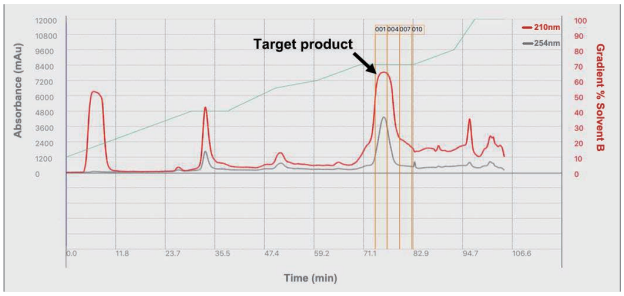

Figure 4. The Flash chromatogram of the sample with the detector equipped with the flow cell that has a 2.4 mm optical path length.

As shown in Figure 4, the absorption signals of the target product and impurities in the sample had increased significantly. Though there are some very close impurity peaks near the elution peak of the target product, we managed to avoid these impurity peaks by tube switching and finally the target product meeting purity requirements was obtained.

Through the above mentioned case, we can get some tips: when the detector response signal to the sample is weak, in order to improve the resolution of the sample components, we can consider using a flow cell with a longer optical path to improve the S/N ratio of the detection and thus get a better result. The Flash chromatography system SepaBean machine series presented by

Santai Technologies can be equipped with flow cells with different optical path length, providing flexible options for those applications with diverse detection sensitivity requirements.

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

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