Get Insight into the SepaBean™ Machine with Engineer: Pumps and Mixing Valves

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Chromatography Application Note
AN025

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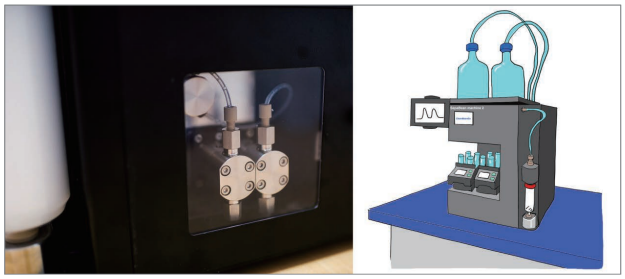

In the parameter list of the flash chromatography system, one key parameter is the number of solvent lines, which means how much solvent lines can be controlled at the same time. Generally speaking, the commonly used solvent lines are two or four, which are respectively called binary solvent or quaternary solvent. The corresponding system pumps are called binary pumps or quaternary pumps. According to the different pump configuration, the mixing mode of multiple solvent lines in the chromatography system is also different, which can be divided into two types: pre-pump mixing and post-pump mixing. The pre-pump mixing is also called high-pressure mixing. The high-pressure mixing pump is composed of multiple pumps, where each pump corresponding to its related solvent line. Therefore the number of pumps corresponds to the number of solvent lines. In the product line of SepaBean™ machine series launched by Santai Technologies, SepaBean™ machine standard version employs a binary high-pressure gradient pump, which is composed of two independent pumps and a post-pump mixer. The post-pump mixing is also called low-pressure mixing, where single pump and multiple solenoid valves are employed. The number of solenoid valves corresponds to the number of solvent lines. For instance, SepaBean™ machine T employs a quaternary low-pressure gradient pump, which is composed of a single pump and a pre-pump quaternary mixing valve.

In next part, we will focus on the detailed differences between high-pressure gradient and low-pressure gradient. First, we will introduce the binary high-pressure gradient pump from SepaBean™ machine standard version.

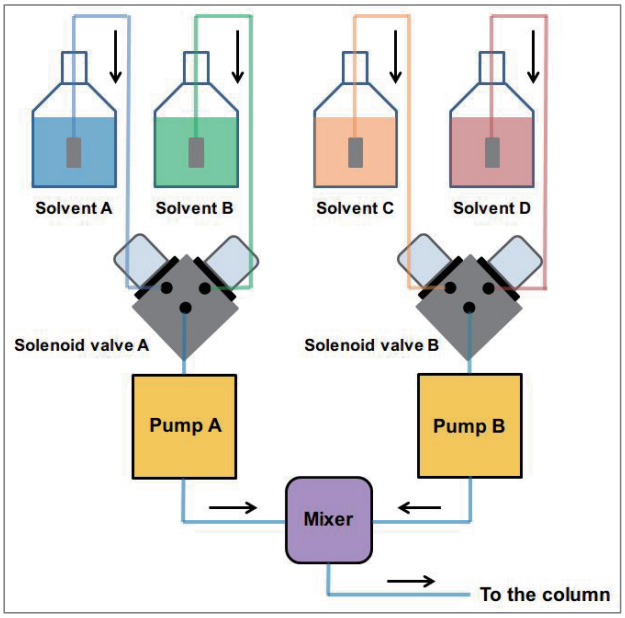

Figure 1. The schematic diagram of system configuration of binary high-pressure gradient pumps.

As show in Figure 1, two independently operated pumps are configured in binary high-pressure gradient system. Each pump is connected with a two-way solenoid valve to switch between different solvent lines. A mixer is configured after these two pumps for solvent mixing. During working procedure, Solenoid valve A controls the priming of Solvent A or Solvent B into Pump A, while Solenoid valve B controls the priming of Solvent C or Solvent D into Pump B. The solvents from two lines are mixed in the post-pump mixer after passing through the pumps and then go to the flash column for elution. Since the mixing is under higher pressure after the pump, bubbles are less likely to be generated due to higher solubility for the liquid under high pressure.

In the following part, we will introduce low-pressure gradient system using the quaternary low-pressure gradient pump from SepaBean™ machine T as an example.

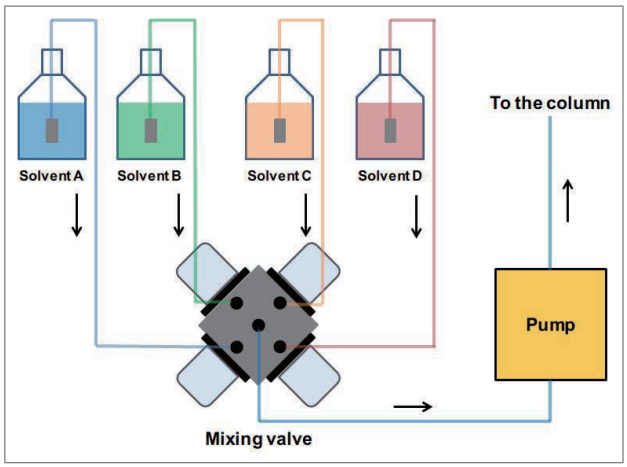

Figure 2. The schematic diagram of system configuration of a quaternary low-pressure gradient pump.

As show in Figure 2, a single system pump is configured in the quaternary low-pressure gradient system. This system pump is connected to a four-way solenoid mixing valve for switching among four solvent lines as well as the solvent mixing.

During working procedure, any combination of two solvent lines from Solvent A, Solvent B, Solvent C or Solvent D is controlled by the four-way solenoid mixing valve to enter into the system line.

Meanwhile one of the four solvent lines could be allowed to enter into the system line as a mobile phase additive and then mixed with other solvent lines in the mixing valve. Afterwards the mixed mobile phase was transported into the flash column by the system pump. It is easy to see that in this operation mode, the gradient is formed by switching the solenoid valve among different solvent lines to allow the system pump to segmentally deliver the corresponding solvents. Due to the pre-pump configuration of the mixing valve, bubbles are more likely to be generated during the mixing process since the solvents are mixed under ambient pressure. It should be noticed that for flash chromatography system, the bubbles in the solvent line is not a big problem since a small amount of bubbles will not affect the sample purification result. The only bad point is that there may be some spike peaks on the chromatogram.

Furthermore, in gradient elution, the ratio of each solvent line in the binary high-pressure gradient is controlled by separate pumps via changing the flow rate. In general, the flow rate of the pump is very accurate, therefore the generated gradient will be better in accuracy. In contrast, the ratio of each solvent line in the quaternary low-pressure gradient is controlled by solenoid valves via changing the opening and closing time for different solvent lines. Theoretically the gradient generated in this way should also be accurate. However, there is a lag time during the solenoid valve opening or closing.

It takes a certain amount of time regardless of how fast the solenoid valve responds. Therefore, for precise gradient control under extreme conditions, the quaternary low-pressure gradient may be greatly affected. As an extreme example, it is required to generate a mobile phase composed of 99% A and 1% B at the flow rate below 5 mL/min. In this case, the solenoid valve controlling solvent line A opens for 99% of the whole cycle, while the one controlling solvent line B opens for only 1% of the whole cycle. Therefore the delay of the solenoid valve will have a considerable impact. The lag time may be even longer than the working time, resulting in inaccurate gradient generation. Nevertheless, the gradient ratio close to the limit at low flow rates is rarely used in the practical application of flash chromatography system. If the customer does have such requirement, it is recommended to choose the high-pressure gradient system to meet the demand.

Table 1 lists the pros and cons for the binary high-pressure gradient pumps as well as the quaternary low-pressure gradient pump. Users can choose the most suitable system according to their actual needs.

Type	Pros	Cons
Binary high-pressure gradient pumps	1.Two system pumps can be controlled and used independently. 2.Better flow accuracy and mixing precision at low flow rates (< 5 mL/min). 3.The solvents are less likely to generate bubbles since the mixing is post-pump under high pressure.	1.No flexibility in switching solvent lines and changing ratios when compared with quaternary pump, especially when developing separation methods 2.The maintenance cost is slightly higher than quaternary pump since more pumps are employed. 3.Since each solvent is pumped separately, the compressibility and the variation in thermodynamic volume of the solvent after entering the mixer may affect the gradient ratio generated.
Quaternary low-pressure gradient pump	1.More flexible choice of different elution solvents with any combination from and switching between four solvent lines, and support for the addition of a third solvent line in the binary gradient as the mobile phase additive. 2.Simple in structure and low maintenance cost for only one system pump is employed. 3.Since the solvents are mixed under ambient pressure, the effect of solvent compressibility can be reduced and the error caused by the variation in thermodynamic volume during the solvent mixing procedure can be completely eliminated.	1.The solvents are more likely to generate bubbles since the mixing is occurred pre-pump under ambient pressure. 2.The mixing accuracy is slightly lower than the binary high-pressure gradient pump.

Table 1. The pros and cons for two types of gradient pumps.

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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