Sample Injection in HP CE

For flushing or conditioning the capillary or injecting a sample, air pressures or different values and durations are applied. The injection system provides precise closed-loop control of the integral of the air pressure over time for either direction of fluid flow. The replenishment system automates the exchange of used electrolytes for fresh ones, using a special double-needle design.

by Werner Schneider

When analyzing samples with an electrophoretic instrument, there are times when the liquid contents of the separation capillary have to be moved, such as for flushing the capillary with different fluids for cleaning purposes, for conditioning it for another separation run, or for introducing sample in a quantifiable and reproducible manner. In the HP CE instrument, these tasks are performed by applying air pressures of different values and durations. How this is done is the subject of the first part of this article.

The second part of this article describes the replenishment system. During a separation run, the chemical properties of the working electrolytes change as a result of electrochemical reactions. This is typically not a problem for a single run, but for multiple runs the run-to-run stability or repeatability will be lost. The replenishment system provides the user with an automatable function for exchanging the used electrolytes with fresh ones.

Overview

Fig. 1 shows a schematic diagram of the functions mentioned above. The pressure source provides working pressures. The EMI board handles the output of the four pressure sensors (indicated by the arrows out of the other blocks) and drives the solenoid valves (the two, three, or four arrows shown going into the other blocks). For more details about the relationship of these functions to the other functional areas of the instrument, especially to the system control, see the article on page 36.

Pressure Source

Flushing, injection, and replenishment are driven by gas pressure. To avoid the need for external gas supplies, the pressure source is built into the instrument. It consists of a small dual-acting membrane pump, two valves (intake and outlet), an intake air filter, and two pressure sensors (for pressure and vacuum). Dual-acting means that the pump provides both overpressure (+p, around 930 hectopascals or millibars) and vacuum (–p, around 420 mbar below ambient). The outputs of the valves are connected to the two large bottles, which serve as pressure "capacitors" and as containers for the fresh electrolyte and the waste. Thus the containers are also parts of the replenishment system. The firmware controls the pressure values with two-point closed-loop control, with only one of the two valves active at a time.

Flushing and Injection

Driving a liquid through a tube is in general fairly straightforward: immerse the ends of the tube in liquid, apply a pressure at the inlet end, and you get a flow. According to the Hagen-Poiseuille law, the flow rate is proportional to the pressure and to the fourth power of the internal diameter of

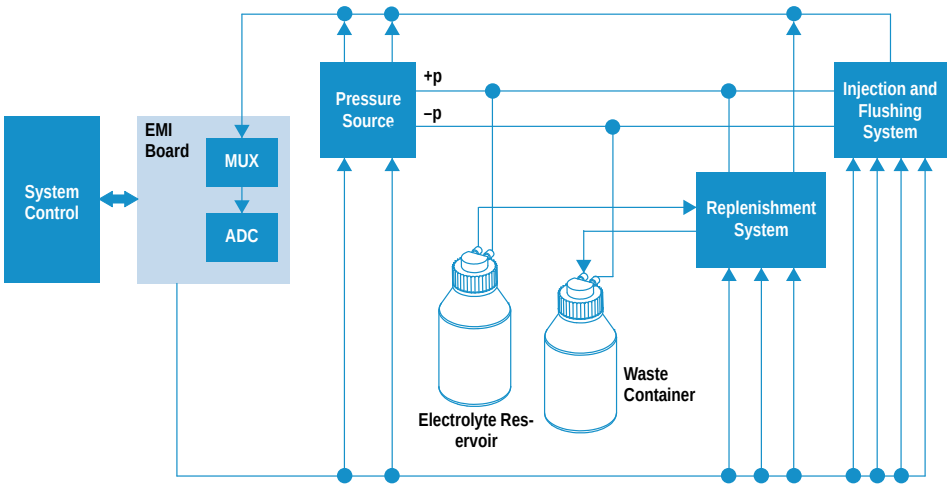

Fig. 1. Overview of the injection and replenishment systems.

the tube, and inversely proportional to the length of the tube and the viscosity of the liquid.

Flushing a capillary means applying a relatively high pressure to achieve acceptably high flow rates. As a rule of thumb, at least three to five times the liquid contents of the capillary have to be pumped through to achieve a thorough flush. A typical flush time is two minutes for a capillary with a 50-μm inside diameter and a length of 48.5 cm.

Injecting a sample means applying pressure and driving the liquid into the capillary. Here only small amounts of liquid are brought into the capillary, and the repeatability of the sample volume must be high. The run-to-run variation must be in the range of 3% or better.

Small amounts of liquid means 1% to 2% of the capillary volume (or, in more common CE terms, 1% to 2% of the capillary length). Since all the factors of the Hagen-Poiseuille law can be considered constant in the short term, the flow rate is directly proportional to the pressure difference across the capillary. This means that the integral of the pressure over time is an acceptably precise measure for the amount of liquid forced into the capillary. Since the user wants to select the injection amount as a parameter of the method, the instrument needs to generate the time integral of the pressure. For example, with a 50-μm inside diameter, 48.5-cm-long capillary, a pressure of 40 mbar for 4.6 s achieves a sample plug approximately 3 mm long.

Fig. 2 shows the schematic diagram of the injection and flushing system, together with a view of the inlet lift holding a sample vial. The lift is part of the liquid handling system described in the article on page 25. Valve 1 and valve 2 deliver air flows for injection, and valve 3 is for flushing. Valve 4 is used for controlling the venting port. Venting is active during all movements of the components to avoid the buildup of any spurious pressures. For example, some compression takes place when the O-ring between the lift head and the electrode holder has begun to seal but has not yet reached its final sealing position.

Flushing. Flushing is straightforward. After the vial is fetched and brought to the sealing position, both valve 3 and valve 4 are activated. Valve 3 directly connects the vial to the system pressure +p, pushing the liquid into the capillary. When the user-defined time has elapsed, both valves are switched off again, and the lift head is released from the sealing position to its default height for the analysis run.

Injection. As indicated above, injection requires much smaller pressures than flushing. For avoiding detrimental effects like splashing, the pressure is not applied instantaneously but in a smooth manner. The manifold that carries the valves also has an internal chamber. When switching valve 1 on, for example, air from +p flows through restriction 1 into this chamber. With the sample vial in the sealing position and valve 4 activated, the air flow causes a rise in the pressure in this closed system (phase 1 in Fig. 3), which is measured by the injection pressure sensor. This is analogous to an electrical resistor-capacitor arrangement, that is, the pressure rise follows an exponential curve. Since the maximum value of 50 mbar is small compared to that of the pressure source, the rise is almost linear. The same mechanism is applied to generate the downslope. Through valve 2 and restriction 2, the closed system is connected to −p, thus drawing the air out of the closed system (phase 2 in Fig. 3).

The timing of the valves is controlled by a successive approximation algorithm implemented in the instrument firmware. Actively controlling both the upslope and the

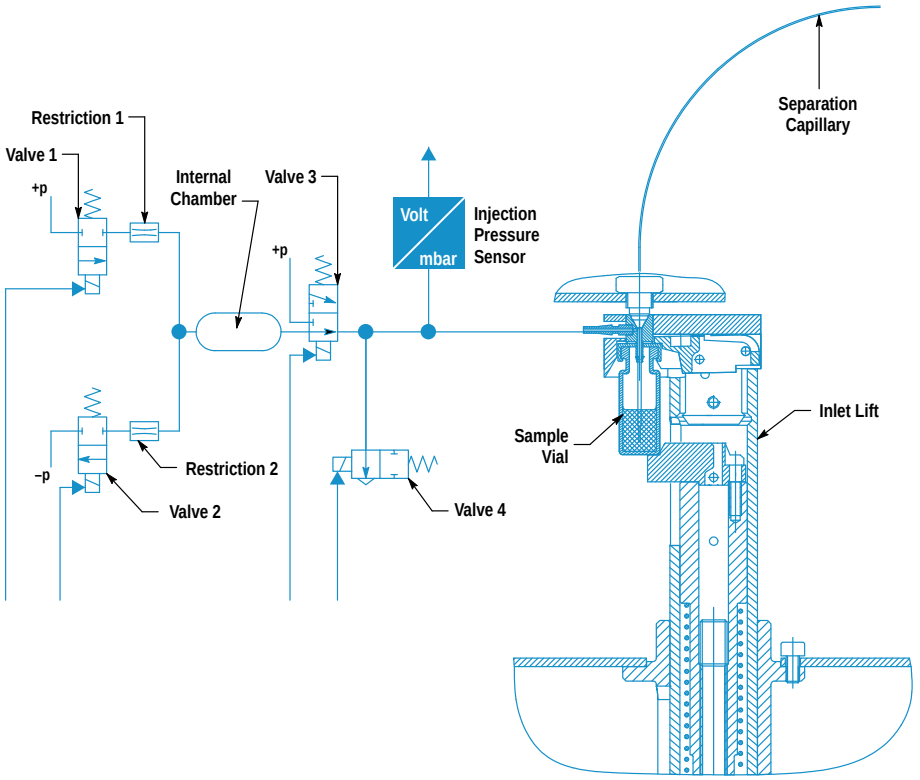

Fig. 2. Injection and flushing system.

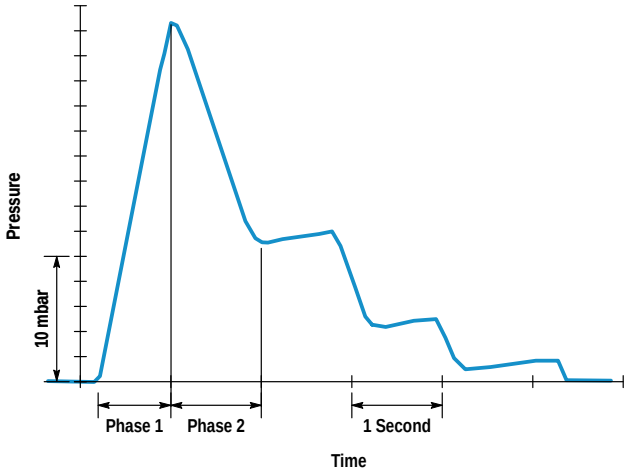

Fig. 3. Capillary pressure profile during sample injection.

downslope provides precise closed-loop control of the integral of the pressure over time. Its precision and reproducibility are determined by the timing resolution of the instrument's multitasking system and the resolution and accuracy of the pressure sensor. The system totally compensates for the variations and tolerances of the other components, including the switching characteristics of the valves, the tolerances of the restrictions, the temperature dependences of dimensions, and gas viscosities.

The total area under the pressure curve in Fig. 3 represents approximately 50 mbar·s. Values down to 20 mbar·s (this corresponds to a plug length of 0.3 mm) are achievable with less than 0.3% variation. Hence the variations of the injection scheme are significantly smaller than those caused by the so-called "zero injection effect" as described in the article on page 50.

Additional Features

The bipolar pressure source allows not only closed-loop control of the injection cycle, but also generation of a similar curve with reversed sign, that is, the flow direction can be reversed. This is advantageous for separations performed from the detection side of the capillary, such as fast screening separations.

By appropriate firmware control, this scheme also allows the application of small pressures in the range of −50 mbar to +50 mbar. Like the other method parameters, this function is time programmable and is available during the separation run.

For separation methods like isoelectric focusing, where no electroosmotic flow is present, external pressure is necessary for pushing the separated species past the detector.

Replenishment

Repeated runs for every sample are mandatory for valid analysis results. Therefore, it is necessary to keep all the conditions and parameters that influence the separation as stable as possible. In electrophoretic analyses, the electroosmotic flow is one of the major aspects, and is strongly influenced by the pH value of the run electrolyte. As a result of the voltage applied to perform the separation, electrochemical by-products are generated. One of the effects is the decomposition of water. This leads to the generation of protons at the positive electrode and hydroxide ions at the negative. More protons in a solution means that the solution gets more acidic, and more hydroxide ions do the opposite. These changes directly influence the electroosmotic flow. In general, the electrolyte solution is buffered to suppress this change, but after a number of separation runs, the buffer capacity is exhausted. In the HP CE instrument, one of the key features is automated, unattended operation. Instead of using valuable vial positions in the instrument's autosampler for a large number of run buffer vials, the replenishment system offers the ability to work with a minimum set of buffer vials, supplying fresh electrolyte into them. This provides the user with the maximum number of positions for sample vials.

The replenishment system is shown in Fig. 4. It consists of the electrolyte reservoir, the waste container for holding the used liquid, valve 2 for drawing the used liquid out of the vial into the waste container, valve 1 for dispensing fresh electrolyte into the vial, a measuring scheme for controlling the whole process, and a double needle for accessing the vial.

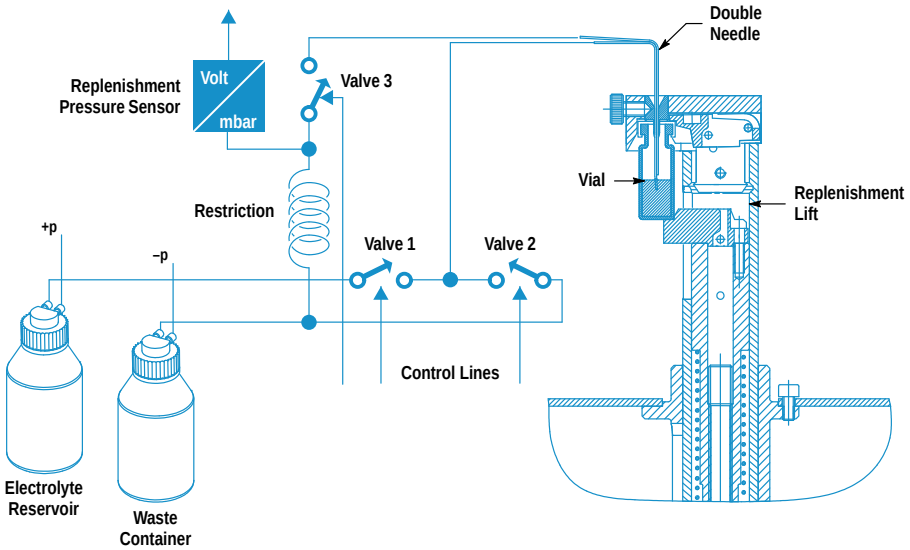

Fig. 4. Replenishment system.

The double needle is provided by the replenishment lift and serves for emptying and filling the vial and as the probe for the measuring function. It consists of two pieces of stainless steel capillary which are welded together with an axial offset of their orifices of five millimeters. The double needle is attached to the frame of the instrument. The lift moves the vial up and down and the double needle enters the vial through the prepunch needle in the lift head (see article, page 25).

The measuring scheme uses the pressure sensor, the restriction, the measuring line of the double needle, and valve 3. It detects when liquid comes in contact with the orifice of the measuring line, that is, when the shorter end of the double needle touches the liquid surface. When the measuring system is activated by switching valve 3 on, a small flow of air is drawn through the measuring line and the restriction. Like a pair of electrical resistors, these two parts of the flow path act like a voltage divider. The ratio of the restriction values is approximately 1:15. With one end of the restriction connected to −p and the open end of the measuring line at ambient pressure, the connection point is at approximately −25 mbar. This value is measured by the pressure sensor. As soon as liquid touches the orifice, the air flow is interrupted. Thus, the pressure at the connection point is drawn towards −p. The control algorithm implemented in the instrument firmware interprets this change of the pressure value as the signal for "liquid detected." The related parameters, especially the air flow and the internal volumes of the lines and the pressure sensor, are selected so that the delay between touching the liquid and issuing the signal is less than 100 milliseconds.

The vial is filled and emptied through valve 1 and valve 2, which are connected to the longer end of the double needle. Switching on valve 2 applies the vacuum. This is done before the double needle is immersed in the liquid. The lift then slowly raises the vial while the liquid is drawn into the waste container. The free end of the double needle is tilted at a slight angle to avoid blocking the orifice when the bottom of the vial is reached.

With valve 1 activated and valve 2 closed, liquid is dispensed into the vial, driven by the pressure in the electrolyte reservoir. The filling height is selectable through the positioning function of the lift. It is determined by the sensing scheme described above and is user-programmable as part of the method for every vial independently. For the highest repeatability of the results, the user might replenish the electrolyte for every run. To keep the consumption of electrolyte low, the user might select the minimum filling level (the electrodes must be immersed during the run). In separations that are chemically robust, the buffering capacity of highly filled vials might suffice for six to ten runs.

When preparing the separation capillary for the next run, in the preconditioning phase of the method, the capillary is flushed with buffer. These flushes consume liquid, so from time to time the buffer vial has to be filled up again. For this purpose, the system offers the refill function. Instead of emptying and filling, the vial is only refilled to the desired level.

It is possible to perform parallel replenishment by using two pairs of run buffer vials and replenishing the pair of vials for one separation run while another separation run is in progress. This means that after the initial setup, no additional time is required for replenishment, provided that the separation run time is not extremely short.

Acknowledgments

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