Capillary Handling in the HP Capillary Electrophoresis Instrument

Capillaries are encased in cassettes for easy replacement and connections are made automatically when a cassette is installed. Air cooling of the capillary eliminates leak problems and lowers costs. Vials containing samples and electrolyte are automatically lifted from a tray to either end of the capillary.

by Hans-Peter Zimmermann

As the name capillary electrophoresis (CE) indicates, the separation capillary is a fundamental element of a CE instrument. Depending on the application, different types of capillaries are used. Capillaries can differ in length (from 25 cm to 100 cm), internal diameter (25 μm to 150 μm), or coating of the inner walls. Thus the user must have the ability to exchange the capillary easily and quickly.

In the HP CE instrument, this ability is provided by a capillary cartridge system. The capillary is encased in a cassette. By keeping several cassettes on hand, each with a different type of capillary, the user can exchange the capillary in the instrument and be ready for a different application within a few seconds. The cartridge system makes it possible to change the capillary in the cassette in a few minutes should the capillary be blocked or break, or should its surface be deactivated. Fig. 1 shows the cassette setup.

Using the Cassette

The cassette is sealed with a quick lock mechanism. The user only needs a rollerball pen to open the cassette (Fig. 1a). The capillary is threaded through the detector interface (Fig. 1b), which later, upon placement of the cassette, adjusts the capillary to the detector. The capillary can now be inserted in the cassette and reeled up on the mandrel. A clamp mechanism fixes the inlet capillary end in the cassette. The two feet of the cassette facilitate aligning both capillary ends to the same length while at the same time protecting them. When needed, the cassette is inserted into the holding fixture provided in the instrument and tipped back. Fig. 2 shows the cassette location in the instrument.

When the user inserts the cassette into the instrument, the following interfaces are established automatically:

- To the high-voltage power supply for generating the electric field within the capillary
- To the sample and electrolyte handling system (liquid handling system) to provide the capillary with sample and electrolyte for automatic operation
- To the pressure system to be able to push liquid into the capillary for injection or flushing purposes
- To the detector for measuring the absorption of the substances separated in the capillary
- To the capillary cooling system for thermostatically controlling the separation capillary temperature.

Connection to the High-Voltage Power Supply, Pressure System, and Liquid Handling System. Upon insertion of the cassette, the capillary ends are automatically threaded through the

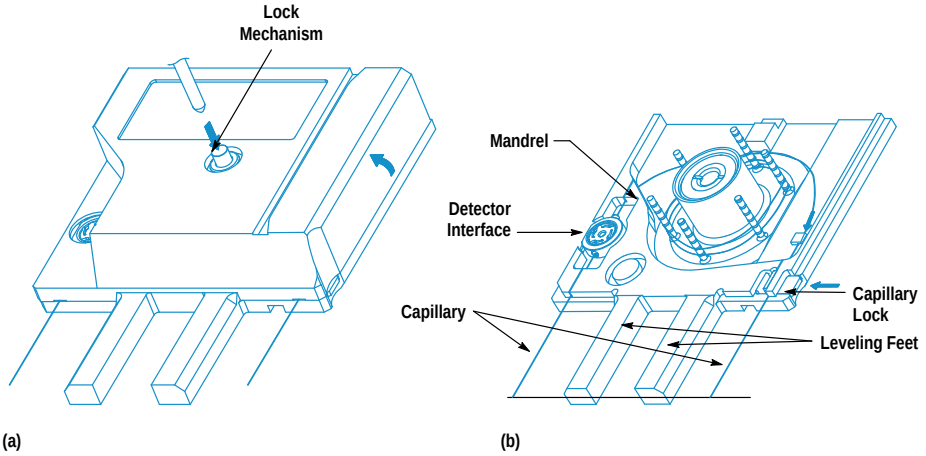

Fig. 1. Capillary cassette. (a) Opening the cassette. (b) Inserting the capillary.

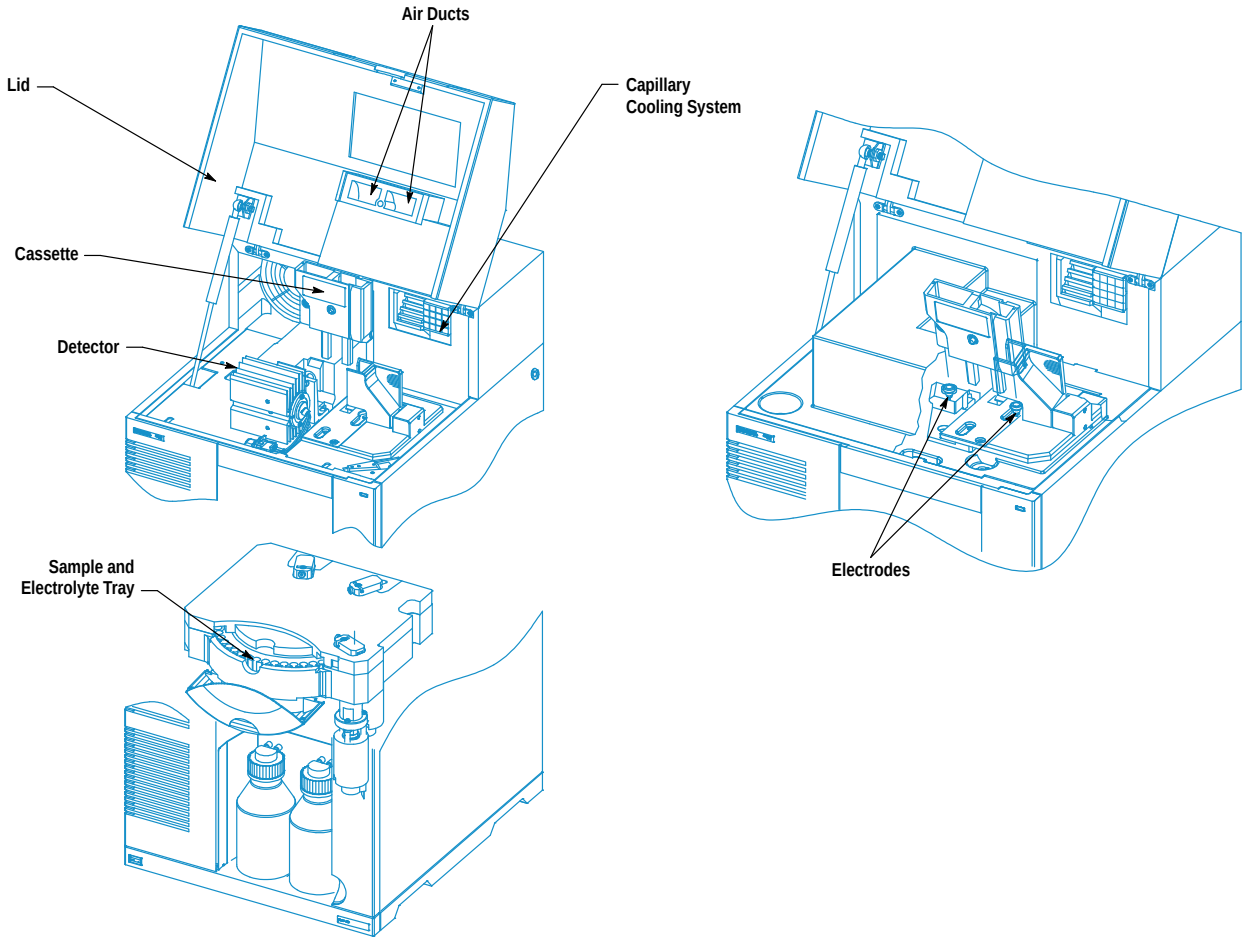

Fig. 2. Inserting the capillary cassette.

two electrodes located in the instrument. The electrodes establish the links to the high-voltage power supply, the pressure system and the liquid handling system.

Fig. 3 shows the setup of an electrode. The funnel threads the capillary through the silicone rubber seal, the housing, and then through the platinum tube electrode. This electrode is connected to the high-voltage power supply and makes the electrical connection to the two electrolyte vials during analysis to apply the electric field for separation to the capillary. To avoid corrosion of the electrodes caused by the electric field and corrosive buffers, the electrode tubes are made of platinum, which has extreme chemical stability. For sample analysis, vials containing the sample or the electrolyte buffer are lifted up to the two electrodes so that the capillary end and the enclosing platinum tube dip into the liquid.

To flush the capillary or to inject sample, it is necessary to seal the separation capillary against the vial, so that pressure can be applied inside the vial. If the lid of the instrument is closed, a spring-loaded pin inside the lid presses the cassette down on the electrodes. This compresses the silicone rubber plug inside the electrode housing so that the capillary is sealed against the electrode. During flushing, a vial is sealed to the cone at the bottom of the electrode

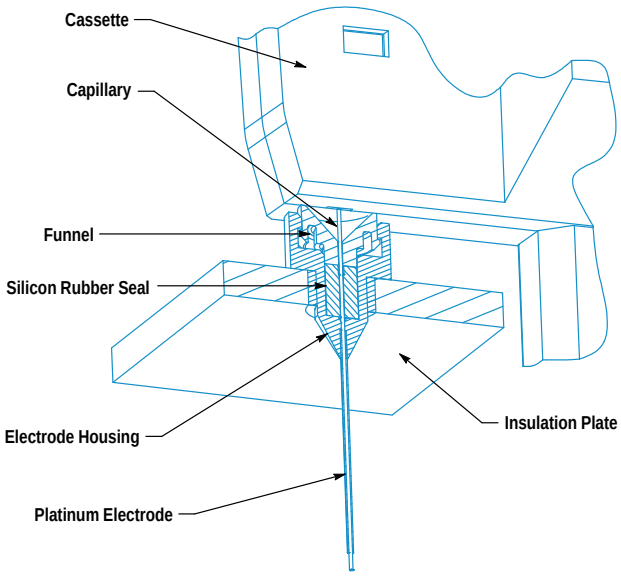

Fig. 3. Electrode details.

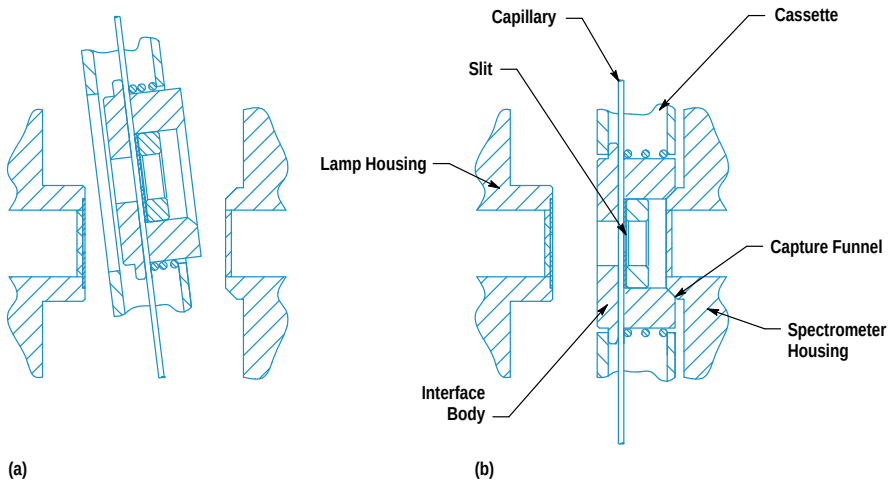

Fig. 4. Detector interface. (a) Sliding in the cassette. (b) Final position.

housing so that the pressure inside the vials presses liquid into the capillary.

Connection to the Diode Array Detector. When the capillary is threaded through the detector interface, it is aligned radially by a V-shaped slot to the slit in the detector interface, which shades the capillary and leaves only the detection window open. The correct axial position of the capillary relative to the slit is achieved by a small stopper affixed to the capillary. The size of the slit depends on the internal diameter of the capillary, so different detector interfaces must be used for the different capillary types. The correct choice of interface for each type of capillary is indicated by a color code.

The detector interface floats in the cassette with a clearance of ±1 mm. When the cassette is tipped back, the detector interface is automatically adjusted to the detection slit by a capture funnel (see Fig. 4).

Connection to the Capillary Cooling System. For cooling and temperature control of the separation capillary, air is blown into the capillary cassette at high speed. The capillary cooling system is located in the upper back part of the instrument (see Fig. 2). When the lid is closed, air ducts in the lid automatically link the cooling system to the cassette. The function of the capillary cooling system is described in more detail in the next section.

Capillary Cooling System

Depending on the applied field strength and conductivity of the buffer located in the capillary, there is an energy throughput of up to 6W in the capillary during analysis. If the capillary were cooled only by natural convection, the liquid core in the capillary would rise to more than 80°C above room temperature in a 1-m capillary at maximum power. This might cause destruction of sensitive samples or even boiling of the buffer. Fig. 5 shows the setup and the different capillary and fluid temperatures T_c, T_1, T_2, T_3, and T_f.

The temperatures in the capillary can be calculated according to Newton's Law: For the temperature difference in the core, the following is true:[1]

$$\Delta T = T_c - T_3 = \frac{P}{2\pi L}\left(\frac{1}{2k_b} + \frac{1}{k_s}\ln\frac{r_2}{r_1} + \frac{1}{k_p}\ln\frac{r_3}{r_2}\right), \quad (1)$$

where r_1, r_2, r_3, and L are the dimensions of the capillary (see Fig. 5), k_b, k_s, and k_p are the thermal conductivities of the buffer, the quartz wall, and the polymer coating, and P is the power level inside the capillary.

Using typical substance values and measures, equation 1 predicts a temperature difference between the core and the outside of the capillary of about 2.0 degrees with a power of 4.5W and a 1-m capillary length. The temperature gradient within the capillary only plays a minor part. The main contributor to the temperature rise in the core is the heat transfer to the ambient air at the capillary outer surface. This temperature difference can be calculated according to the following equation:[1]

$$\Delta T = T_3 - T_f = \frac{P}{2\pi L r_3 h}, \quad (2)$$

where h is the heat transfer coefficient at the capillary surface.

Unfortunately, the temperature drop at the capillary surface is not as easily calculated as equation 2 suggests, since the heat transfer coefficient h has a nonlinear dependence on the flow velocity and the temperature of the surrounding coolant. Fig. 6 shows the temperature rise in the core (T_c–T_f) as a function of flow velocity for air and for a typical cooling liquid (fluorocarbon FC77 from 3M) with a power dissipation of 4.5 W/m in the capillary.

Fig. 6 shows that when cooling the capillary with forced air, above a flow velocity of about 10 m/s, improvements in cooling performance cannot be achieved. For liquid cooling, the temperature rise inside the capillary is about 3.6 times lower than for air cooling at equal velocity, according to equation 2. Flow velocities as high as with air, however, can hardly be

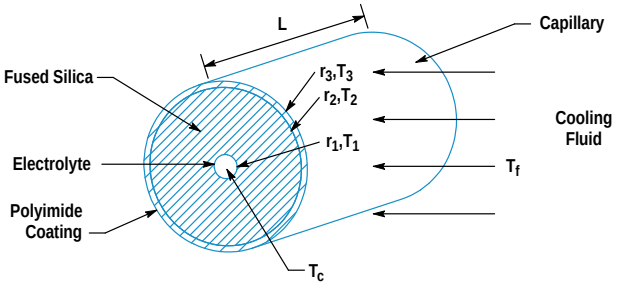

Fig. 5. Temperatures and radii of fused silica capillaries.

Rapid Prototyping for the HP CE Project

In the past few years, methods offering the possibility of transforming 3D CAD data directly into a ready-to-install part have experienced a steep upturn. These methods, which have been commercially available for about ten years now, are generally known under the catchword "rapid prototyping." Some of these methods and their combination with different replication techniques have reached the real aim of rapid prototyping, namely the reduction of time to market. Within the HP capillary electrophoresis (CE) project, rapid prototyping methods became indispensable, not only because of the ambitious project schedule, but also because of the fact that some parts, because of their complexity, could not have been produced in the traditional way without substantial compromises.

At the Waldbronn Analytical Division, these methods were used for the first time, involving significant risk because none of the people concerned could rely on personal experience. Another reason for using rapid prototyping methods certainly was the cost aspect. However, it proved necessary to balance the requirements of the individual development steps, the time available, and the complexity of the individual parts against one another. In accordance with part requirements, three methods were pursued in the HP CE project, two ways to get a resin-plastic part and a third method to get an alumina part:

- 3D data → stereolithography process → vacuum casting → plastic model
- 3D data → Solider process → vacuum casting → plastic model
- 3D data → STL file (Fig.1) → stereolithography process →STL model (Fig. 2) → vacuum casting with silicone → wax model (Fig. 3) →ceramic shell for traditional investment casting for alumina parts (Fig.4).

The real rapid prototyping step results in directly translating the 3D construction data into a solid part. The principle of all three methods mentioned above is similar: a 3D computer model is split into layers of a certain thickness by software.

Various methods are applied to copy the section in question to materials such as photopolymers or powdery thermoplastics by using different reproduction techniques. This procedure is repeated until the whole part, composed of hundreds of layers, is complete (see Fig. 5).

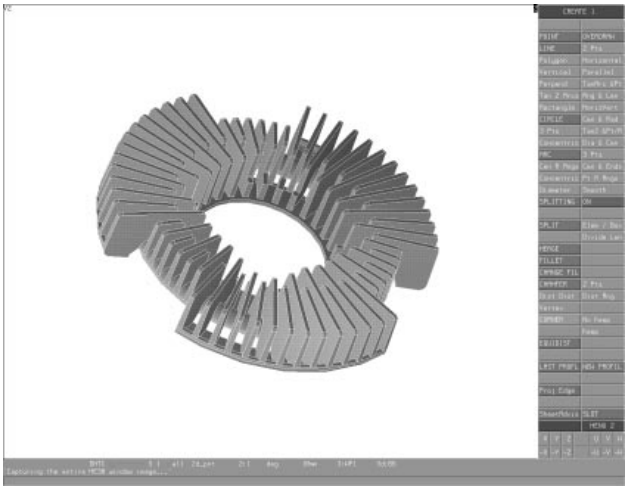

Fig. 1. STL file created with a CAD application.

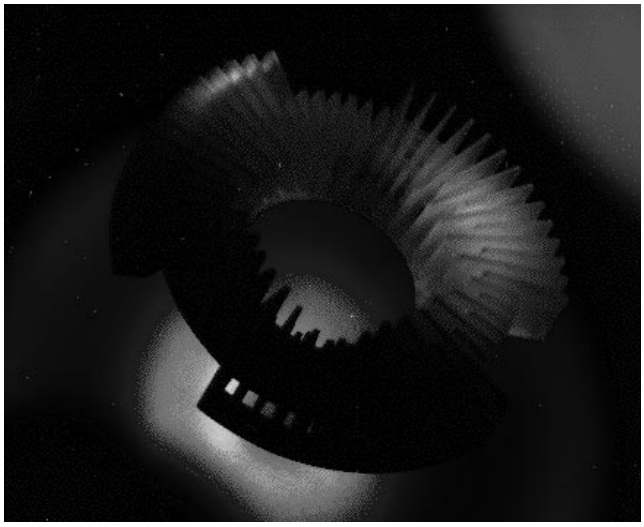

Fig. 2. STL model.

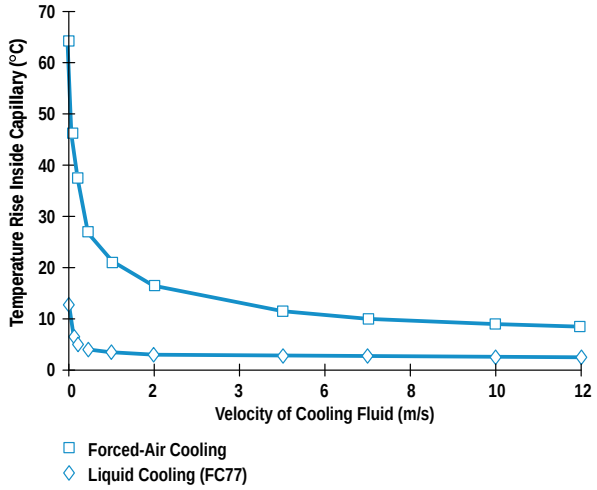

Fig. 6. Temperature rise inside capillary.

realized with liquids. At typical velocities of about 0.5 m/s with liquid cooling, the difference from high-speed forced-air cooling only is about 5°C (8.8° for air; 3.9° for liquid) with a 4.5 W/m energy throughput in the capillary. The advantage of air cooling versus liquid cooling is that capillary handling is significantly easier for the user. When exchanging the capillary, no liquid has to be drained and no costly sealing of the cassette is needed. With air as the coolant, low leakage has no disturbing influence. Also, no expensive cooling fluids are necessary, which lowers the cost of ownership.

Because of these advantages of air cooling, we decided that an excess temperature of five degrees in the capillary core could be accepted. In the HP CE instrument, forced-air capillary cooling is used with air velocities of about 10 m/s at the capillary.

Because the mobility of the substances in the capillary changes by about 2% per degree, another important requirement of the cooling system is the ability to adjust the temperature of the cooling air with a precision of ±0.1 degrees to suppress variations in room temperature. This is achieved by a Peltier heat exchanger element regulated by three temperature sensors. This element makes it possible to adjust

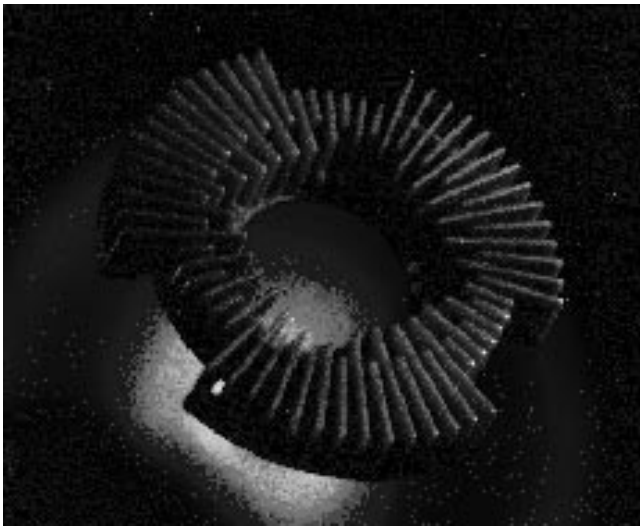

Fig. 3. Wax model.

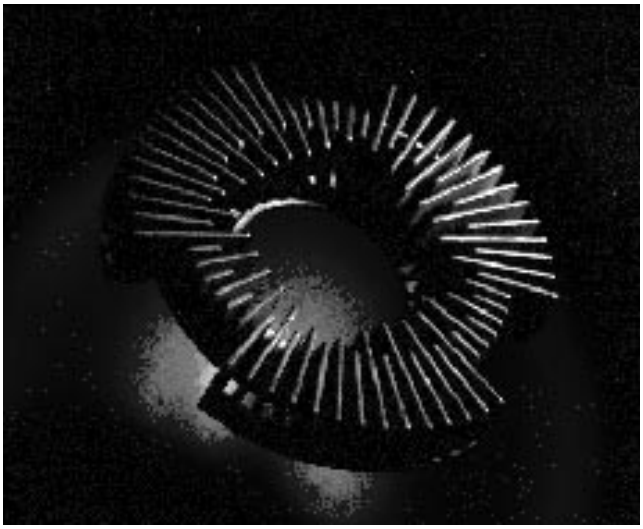

Fig. 4. Production part.

Depending on the method applied, the 3D data has to be prepared in different ways (e.g., support framework). Regarding the actual formation of the part, however, each method has its process-specific advantages and disadvantages.

In qualification of these rapid prototyping methods, however, it must be pointed out that the parts cannot be used without costly subsequent treatment and replication—preferably with silicone—if specific requirements on surface quality, accuracy, and stability are to be met.

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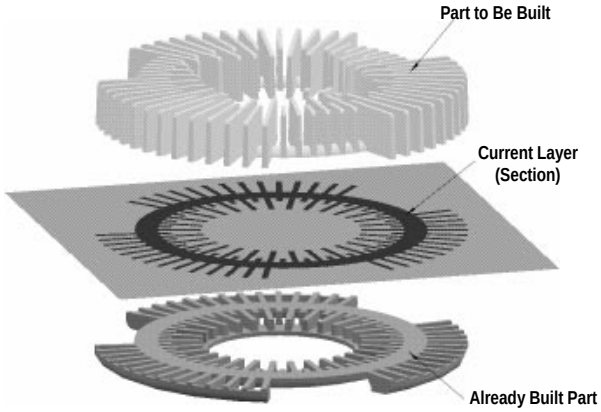


Fig. 5. The solid models are built up in layers.

the air temperature from ten degrees below ambient to 60°C. Fig. 7 shows the setup of the cooling system.

A radial blower draws air past the heat exchanger of the Peltier module and blows the cooled or warmed air towards the cassette through the air ducts in the lid. From there, the air is fed back to the heat exchanger. The Peltier element is able to deliver up to 50W of cooling power and up to 100W of heating power to the capillary cooling circuit. Because of the relatively low efficiency of a Peltier element, there is a power dissipation of up to 150W at the heat sink of the Peltier module. To remove this heat, the heat sink is forced-air cooled by an axial ventilator. Ventilator, Peltier, heat exchanger, and driver electronics are located in the cooling system, embedded in two isolating foam shells.

Sample and Electrolyte Handling

To achieve a high sample throughput in a CE instrument, the supply of samples and electrolytes to the two capillary ends has to be automated. In the HP CE instrument, this task is performed by the liquid handling system shown in Fig. 8.

Samples and electrolytes are in small vials of 2 ml or 0.3 ml volume in a circular tray ring. The tray ring can accept a maximum of 48 vials. A heat exchanger in the center of the tray ring can be connected to an external cooling bath for adjusting the vial temperature. The heat exchanger is an example of several parts of the HP CE instrument that were designed using rapid prototyping, as explained above. The tray ring rests on three rollers at its perimeter and is driven by a servo motor via a pulley.

Samples and electrolytes in the tray have to be positioned at both the capillary inlet and the capillary outlet. Because the tray ring must be free to rotate, the vials must be lifted from the tray to the capillary ends. This task is performed by the lift modules. Two lift modules lift the vials up to the capillary ends, and a third lift module moves vials to a needle

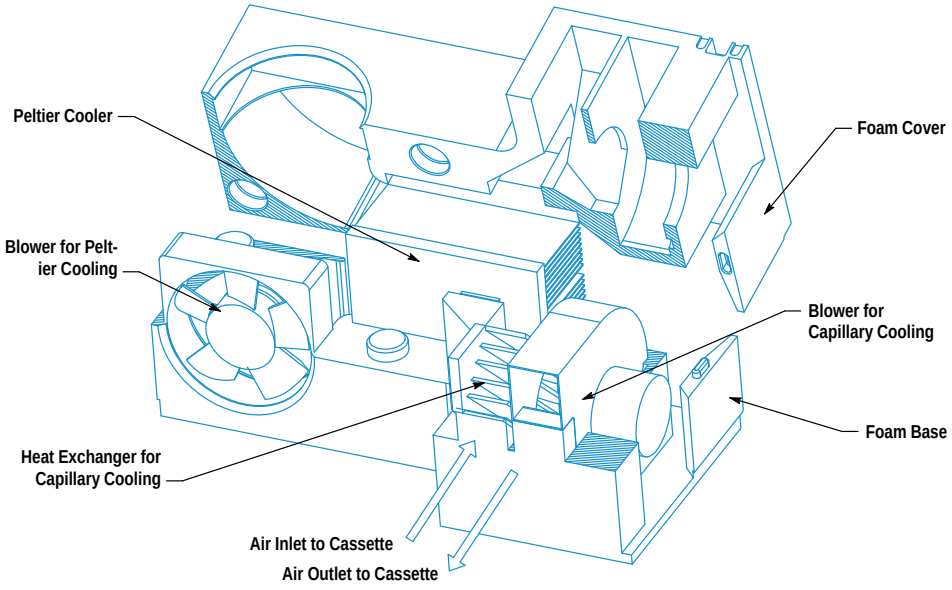
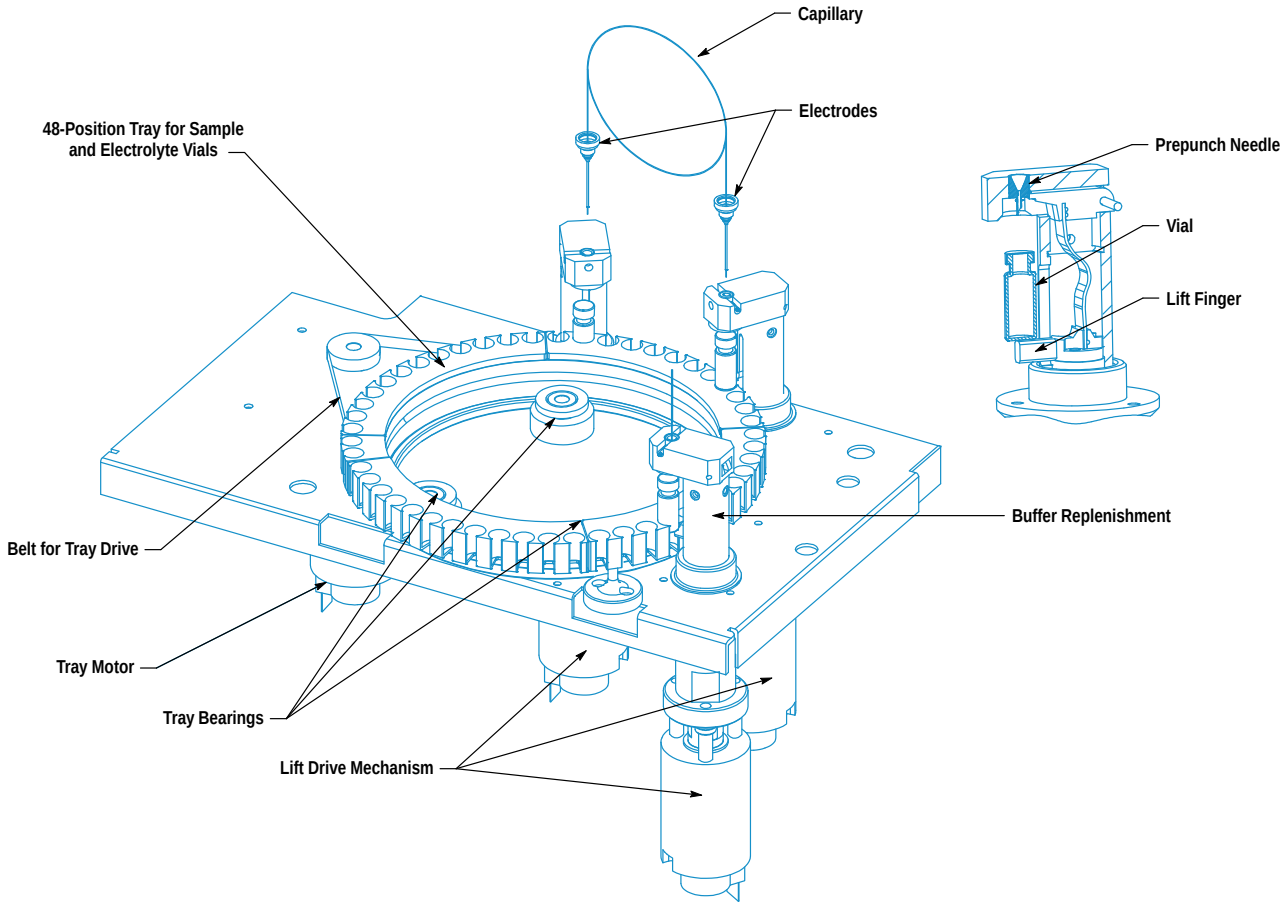


Fig. 7. Cooling system.

through which contaminated electrolyte can be sucked off and vials can be refilled with fresh electrolyte from a dispenser bottle (see article, page 32).

This setup offers several advantages. First, it provides random access to all of the vials in the autosampler. Each vial can be positioned either at the capillary inlet, at the capillary outlet, or at the replenishment module. This means that the user can decide how much of the tray capacity to use for sample vials and how much for electrolyte vials. Second, changing vials at the capillary outlet allows the different fractions of a sample to be collected separately. Third, this approach allows buffer replenishment and capillary preconditioning to proceed in parallel.

To avoid sample evaporation, the vials are sealed with caps whose thin membranes are punched upon injection. The sensitive ends of the separation capillary would break in this



ig. 8. Liquid handling system.

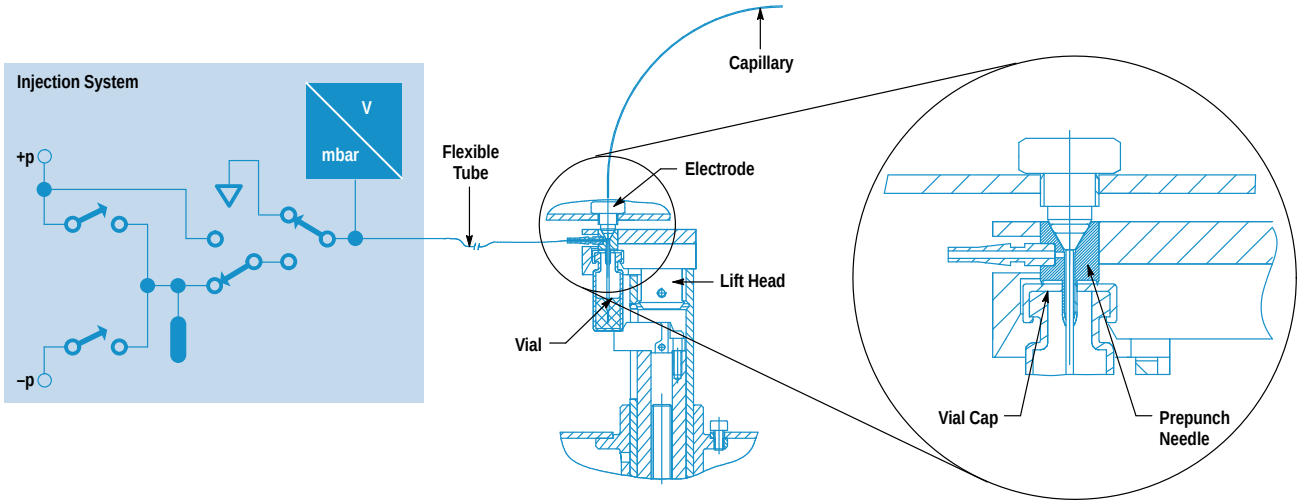


Fig. 9. Connection to the injection system.

process, so a punching needle is located in the lift head to prepunch the caps. In operation, the vial is held by a kind of pliers and is pressed onto the punching needle by a spring before being lifted to the capillary. A ring-shaped blade seals the prepunch needle to the vial cap. The lift presses the cone of the prepunch needle against the seal at the electrode housing (the seal can be seen in Fig. 3), thereby providing the seal between the vial and the capillary. Pressure for flushing or injection can then be applied to the vial through a bore in the prepunch needle. The final position of the vial is shown in Fig. 9.

Acknowledgments

I would like to acknowledge the contributions of John Christianson, who set the directions for the capillary and liquid handling techniques in the HP CE instrument. Also, special thanks to Rolf Dörrmann and Greg Wilson for their support during the project.

Reference

1. J.H. Knox and K.A. McCormack, "Temperature Effects in Capillary Electrophoresis, Part 1: Internal Capillary Temperature and Effects upon Performance," *Chromatographia*, Vol. 38, no. 3/4, February 1994, pp. 207-221.