HP CE Separation Control Electronics and Firmware

The HP CE instrument consists of a PC and a base unit consisting of detection and separation subunits. Methods are developed on the PC and downloaded to the base unit for independent execution. The control electronics and firmware of the separation subunit takes care of tray and vial movement, capillary voltage, current, and power control, capillary temperature control, diagnostics, and related data capture.

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The HP capillary electrophoresis instrument is built of two hardware units. One is a personal computer and the other is the base unit, which contains all of the hardware needed to perform the separation and detection. The electronics and the instrument software (firmware) of the base unit are the subjects of this article.

The software running on the PC is the only interface to the user. There is no local keyboard on the base unit. A three-color LED is used as a status indicator on the front of the instrument.

The PC (an HP Vectra computer) is connected via the HP-IB (IEEE 488, IEC 625) to the base unit and performs all data handling and user interface tasks. The base unit is built as two subunits—the separation subunit and the detection subunit—and therefore has two HP-IB addresses.

There is no logical connection between the subunits. This allows the separation subunit to be compatible with future advances in detection technology and makes it possible to implement other detector types without changing the separation unit.

The detection subunit (see article, page 20) is very similar to the multiple wavelength HPLC detector of the HP 1050 LC system.1 From the control point of view it is identical to the HPLC detector and electronically it is exactly the same. The cardcage, power supply, and printed circuit boards are unchanged. The local user interface is deleted.

Like the detection subunit, the separation subunit has a cardcage in the rear that holds the various printed circuit boards and the power supplies. Most of the printed circuit boards are connected to a motherboard on the backplane of this cardcage, except for the temperature driver board, the status indicator board, and the electromechanical interface board, which includes pressure sensors, connections to Hall sensors, and the power outlets for the various solenoid valves. The electromechanical interface board extends from the front of the subunit to the motherboard. The mechanical hardware for liquid handling is connected by cables to this board or the motherboard.

Controlling a Method

A method is the sum of all of the parameter settings and commands that control a complete analysis. A choice of activities can be programmed to:

- Refill liquid into working vials (replenishment)
- Prepare the separation capillary (preconditioning)
- Inject a certain amount of liquid from the sample vial (injection)
- Perform the separation and collect data (run time)
- Evaluate data and generate reports (data analysis)
- Clean up the system (postrun and postconditioning).

During electrophoresis, electrolytic byproducts are formed at the electrodes in the inlet and outlet vials, and the sample compounds move to either end of the capillary. Therefore, the running buffer will be spoiled after use. To prevent influencing later analysis results, replenishment is used to prepare several vials with fresh buffer. Any existing liquid in the vials can be drawn into a waste bottle. The vials then can be filled to a specified level with new buffer from the reservoir bottle. During subsequent activities the refilled vials can be used as inlet or outlet vials, or as flush and waste vials.

Preconditioning consists of a set of commands to specify a treatment of the inner surface of the separation capillary and to fill it with a reproducible composition of running buffer. A list of up to 100 entries can be specified with actions like “flush with pressure,” “apply high voltage,” “wait” a specified time, or “change” inlet or outlet vials to clean the electrodes of residual liquid.

During the injection a liquid amount of typically five nanoliters is placed into one end of the capillary. This can be done by applying high voltage for a certain amount of time or by forcing a pressure drop across the length of the capillary. While the voltage mode forms a plug flow profile, the higher-mobility species are injected preferentially, which has to be considered when quantitative measurements are needed. The pressure mode does not change the quantitative composition, but the hydrodynamic flow profile reduces separation efficiency. For more complex injection tasks a
table of serial actions can be programmed, for example a sample plug followed by buffer liquid.

During run time the electric field is applied to the capillary, which causes the different species to separate. The result is measured by monitoring the optical absorbance of the eluting liquid at up to five different wavelengths. For more complex tasks a full set of optical spectra can be acquired. For defect tracking and further evaluation, up to five other signals can be stored during the analysis. A timetable can be programmed to change parameter settings during the run in synchronism with the sample plug injection. Electric field, differential pressure across the capillary, and temperature in the air around it can be changed as easily as changing vials on both ends of the capillary.

After the measurement the separation path (capillary) can be cleaned by using a list of the same actions that were used for preconditioning. The capillary can be cleaned and filled with new liquid before the instrument is parked.

The final result of a measurement is a report that states the composition of the sample, both qualitatively and quantitatively. Using the spectral information, peak recognition and peak purity checks are possible. Calibration with previous analyses allows calculation of exact amounts. An experienced user can use the printout of an electropherogram to interpret the results.

**Control Requirements**

The electrophoretic separation works like an impulse response. The injected sample plug is the impulse introduced into the system. Because each component of the sample has a different specific mobility, the response is a series of (hopefully) separated impulses with different peak shapes: the electropherogram. This impulse response is dependent on both the input impulse (the injection plug) and the system response.

While the injection plug is mainly dependent on physical parameters, the system response is mainly dependent on chemical parameters, and both are dependent on timing relations. The injection plug depends on the geometry and length of the capillary, the viscosity and temperature of the liquid, and the value of the applied force multiplied by the time interval. While the first factors are not controlled but are more or less constant, the applied force and the time of application require good precision. If an injection of 50 mbar times 3 seconds is wanted with a precision of 1%, then a 50-mbar pressure source is required with 0.25-mbar precision and the time has to be controlled within less than 15 ms with transients taken into consideration.

The system response acts in two different ways. One is the bulk speed of the liquid (electroosmotic flow) and the other is the specific mobility of the separated compounds or molecules. These are primarily dependent on the length of the capillary and the voltage applied across it during the separation (during the run time), but are also dependent on the type of buffer and the temperature. There are also secondary dependencies, more chemical with the electroosmotic flow and more electrical with the conductivity and field distribution.

To address the electroosmotic flow dependencies the system allows activities bundled as “preconditioning.” This is a series of up to 100 steps specified by the user to pretreat the capillary’s inner wall. This treatment can include:

- Filling the separation capillary with mobile phase
- Removing gas bubbles
- Cleaning the stationary phase using extreme pH
- Exchanging the mobile phase
- Conditioning the stationary phase to the final analysis pH
- Reaching thermal equilibrium
- Removing old sample from the capillary.

While the freedom to select a number of different actions in serial combination allows the user to address various problems, the execution is reproducible and synchronized within 100 ms. This is valuable because the time constant to reach equilibrium may be days. To speed up the analysis, it is useful not to wait until equilibrium is reached, but to gain reproducibility by precision in timing. Thus, the results ride on a wave. In HPLC there is no specific timing requirement for other actions outside the run time. Different durations of injection cycles don’t show a dramatic influence on the separation results. But in HPCE preconditioning is a major aspect. Reproducible results can only be achieved when the timing of the conditioning phase for repeated runs using the same method is always the same. It may be useful to have a constant time for feeding vials to the cassette, independent of the number of vials or the move distance.

Because communication from the PC-based software to the instrument via the HP-IB is too slow, the instrument must have all of the parameters needed before the analysis is begun. Therefore, the complete method is copied to the instrument, which then executes it completely without interaction with the computer.

**Method Execution by Firmware**

The analysis consists of user-definable steps like replenishment of vials, preconditioning of the capillary, injection, running, and postconditioning of the capillary. To achieve a high reproducibility of the analysis it is essential that the durations of the steps and the intervals between them have only small variations (<100 ms). This is accomplished by generating the method on the PC and then downloading the method to the instrument, where the real-time operating system in the instrument takes care of the exact timing of the analysis. After the analysis the PC is responsible for data evaluation. The operating system in the instrument is a real-time, multitasking operating system previously used for the HP 1050 HPLC system.²

To address the conductivity and field distribution dependencies mentioned above the system allows selection of the control mode (voltage, current, or power) and the storage of the measured values of voltage, current, and power as raw data with the detector results. While voltage control is most precise when the conductivity is equally distributed across the capillary length, current control keeps the field strength in the buffer portion independent of the conductivity of the injection portion. If temperature is the major influence, power control can keep the internal temperature rise in the capillary constant to adapt to changes in overall conductivity. The stored measured voltage, current, and power data can be used for checking and diagnosis or to calculate specific behavior. For example, the mobility report uses the
voltage trace acquired during the analysis to calculate the apparent mobility of the species.

**Temperature**

The temperature of the capillary in the cassette has to be stabilized because it influences the electrophoretic results. One can compare this with the thermostabilization of an HPLC column. Electromobilities depend on solvent viscosity, which changes at a rate of about 2%/°C for typical buffer solvents. Chemical retention, if it occurs, is also influenced by temperature. In general, cooling is required because the current through the capillary heats the solvent and would boil the liquid with the sample in it. The temperature must be stable within at least ±0.1°C and perhaps as low as 10°C below ambient.

For cassette cooling a Peltier element is used instead of a chiller. This element is made of semiconductor material, and with electrical current applied, one end is heated while the other end is cooled. The direction of current flow defines which end is cooled. Thus the unit can be used for both heating and cooling by reversing the excitation voltage.

During automated sequences the samples to be analyzed are kept in the liquid handling tray. If temperature-sensitive samples like biosamples are being analyzed, the sample storage area has to be cooled to as low as 5°C to ensure that the samples are not spoiled while they are stored in the instrument. While capillary cooling is always required, the tray cooling is an optional feature. The user can connect a chiller to control the tray temperature and the HP CE instrument will measure and display the tray’s temperature.

Forced-air cooling is used. The forced-air circulation has to be fast enough to ensure that water condenses only on the surface of the cooling heat exchanger. This is important because a wetted surface is able to conduct electrical current. With 30 kV in a sample bottle this can lead to unexpected high voltages in other areas of the instrument.

An analog-to-digital converter (ADC) resolution of 12 bits is sufficient for all of the signals that need to be converted. The highest resolution is required for temperature control of the capillary and for raw data generation. A 12-bit ADC allows a resolution of 0.075 μA in current and 0.025°C in temperature measurements.

One ADC is multiplexed to measure the analog signals for voltage, current, return current, pressures, and temperature. Multiplexing requires more firmware support. It is a load to the interrupt level of the system processor and is therefore time-sensitive, but it saves hardware cost.

**Separation Subunit**

Fig. 1 shows the functional blocks of the separation subunit, which are:

- Operating system
- Vial and liquid handling
- High-voltage control and safety functions
- Thermal control
- Replenishment and injection.

These functions are separated onto different printed circuit boards. The separation subunit contains the following boards (see Fig. 2):

- Main processor board (CMP)
- PC interface board (CRB)
- Electromechanical interface board (EMI)

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**Fig. 1.** Functional block diagram of the separation subunit.
Fig. 2. Electronic block diagram of the separation subunit. CRB = PC interface board. CMP = main processor board. EMI = electromechanical interface board. PDV = servo boards. HVT = high-voltage and temperature control board. TDR = temperature driver board.

- Four servo boards (PDV)
- High voltage and temperature control board (HVT)
- Temperature driver board (TDR) for Peltier current control.

**Position Control Servos**

The central functionality of the separation subunit is vial handling. Up to 50 vials can be selected to be placed at either the inlet or the outlet end of the separation capillary. In addition to taking a vial from the tray and placing it at a capillary end, the vial handling system has to place the vials at a defined height. Depending on the liquid level in the vial and the necessary depth of immersion for the electrode, a positioning offset can be set. A position servo system is used to drive the lift spindle, which is able to provide position and force feedback.

The same type of position servo is used for the tray movement. The servos are initialized to a special position and all vial positions are in reference to that. For the tray this special position is a fixed distance from the mechanical stop that prevents the tray from being turned more than one revolution.

Every movement using the servo positioning is controlled by a smooth harmonic function. Both the speed and the acceleration are ramped. This generates a stress-free moving profile that is easily calculated by the microprocessor at an 80-Hz interrupt rate.

**Tray and Lift Initialization**

After power-up of the instrument the lifts and the tray must be initialized to a known state before the firmware can use them. The initialization procedure first interprets the current state of the mechanics, then finds the home positions for the servos, and then calibrates the lift positions relative to the segments of the tray. The sequence allows precise positioning without adjustments of the mechanical assembly. The need for absolute position sensors is avoided by using the position and torque information from the servo drivers. This lowers the cost, since even inexpensive sensors such as light switches and Hall sensors cost money and require cabling, connectors, and often adjustments. Furthermore, the presence of sensors in the high-voltage area could lead to reliability problems.

The mechanism is designed to allow random access by the lifts to the vials in the tray. To achieve this the lift finger is lifted through slits in the tray segments and the movement of the tray is blocked when the lift finger is within a slit. This means that the system needs to know the exact position of the tray segments relative to all lifts. All servos need absolute reference points for proper positioning. To bring the vials in the lift stations back to their correct locations after a power failure, the location of the vials is held in continuous memory.

To find the current state of the system the initialization procedure first tries to initialize the tray. In most cases this is successful because the lifts have normally reached their upper or lower positions before the instrument is switched off. If the tray is blocked by one or more lifts the next step is to resolve the blockage. The lifts are moved down with reduced torque. Because the orientation of the tray relative to the lifts is unknown in this phase, it can happen that the lift finger is blocked by a vial or by a tray segment. In this case the lift is moved upward again out of the tray. Discrimination between hitting a vial or a segment and a correct initialization of a lift is based on the characteristics of the torque increase when the lift finger hits something hard. The real endstop is much stiffer, so the torque increase as a function of distance is steeper compared to the situation when the lift hits a vial, for example. After this step no lift should be blocking the tray.

Now the initialization and find home process starts. First the tray starts to initialize again, checking that its complete path is free. It does a full turn while monitoring the force to detect blockages. The lifts are now initialized and the vials in the lifts are brought into the corresponding tray locations. If there is any vial conflict, for example the user put a vial in location 20 of the tray while a vial with the same number is already in a lift station, the system sends an error message to the user to remove this vial first, and the instrument turns the tray so that the corresponding location moves to the front access region. Because of problems like vial conflicts, the initialization process needs some user interaction. It is not recommended to start operation automatically at turn-on of the instrument.

The next step is to calibrate the position of the lifts relative to the tray. Mechanical tolerances in mounting the lifts and segments are compensated by this calibration procedure. The lift finger of a lift is brought into the slit of segment 0 and the tray is moved with constant torque to the left and to the right. The average of the positions reached gives the exact tray position for that lift. With the same procedure the position of every tray segment relative to the lifts is measured and stored in the continuous RAM. Every access to a vial uses the stored values to eliminate mechanical tolerances.

**Servo Control**

An application-specific servo chip, optimized for velocity control, is used in this instrument. Special firmware routines generate the velocity profile to achieve a gentle movement of the servos. The servo chip uses current control to drive
the motor, and the control error can be used as a signal proportional to the required servo torque. This allows full control over the forces in the system. It also makes it possible to detect that a servo is blocked and switch to the error recovery routine to resolve the blockage. To achieve a good dynamic response of the servo, the motor driver can generate a maximum current that will destroy the motor with just eight seconds of continuous operation. Therefore, to avoid damage we implemented a firmware fuse. The firmware calculates a simulated temperature of the motor according to the heat generated by the current through the winding, subtracting expected thermal losses. If this simulated temperature becomes too high the servo is switched off and an error message is generated.

**Movement Control**

The movement control firmware translates high-level move instructions into low-level move commands for the servos and a signal to indicate when an instruction has been executed. The high-level instructions include:

- Find the home position for a servo
- Remove the vial from a specified lift
- Find a vial, put it in the lift, and raise it to a specified position
- Set torque (move a servo with a specified torque)
- Put the two home vials in their lifts and raise them to their normal positions.

The low-level move commands include:

- `restartservo`. Find the orientation of the rotor and stator for a servo.
- `initservo`. Find the zero position and search index.
- `segmentcheck`. Measure the position of a tray segment relative to a lift.
- `move`. Move a servo to a given position with a velocity profile.
- `findvial`. Find the location of a specified vial.
- `findvialinlift`. Find which vial, if any, is in a specified lift.
- `storevial`. Store the location of a vial in continuous RAM.
- Signal the end of the high-level command.

For example, say that vial 30 is in the replenish lift and vial 25 is in the inlet lift and the task is composed of the high-level commands, "Find vial 30, put it in the inlet lift, and raise it to the default position." This is translated into the following low-level command sequence:

- `findvial` of vial 30. This will find vial 30 in the replenish lift.
- `move` vial position 30 of the tray under the replenish lift.
- `move` the replenish lift downwards until the vial is in the tray.
- `storevial` of vial 30 in the tray.
- `findvialinlift` in the inlet lift. This will find vial 25 in the inlet lift.
- `move` vial position 25 of the tray under the inlet lift.
- `move` the inlet lift downwards until the vial is in the tray.
- `storevial` of vial 25 in the tray.
- `move` vial position 30 of the tray under the inlet lift.
- `storevial` of vial 30 in the inlet lift.
- `move` the inlet lift upwards until the default position is reached.
- Signal the end of the command.

These low-level move commands are put in a queue. The servo control firmware executes one element after the other from this queue. When a new high-level command arrives before the queue is empty the routine that translates the high-level commands to low-level commands can't use the actual vial locations because the commands that are still in the queue may alter these locations. To follow the movement of the vials, every entry in the queue creates a virtual vial location which represents the vial location after this entry is executed. When a movement error occurs the queue is cleared. Depending on the type of error, the error recovery routine generates a new queue to bring the mechanism into a controlled state and gives a description of what caused the error.

**High-Voltage Control and Analog Signals**

The high-voltage power source is an encapsulated unit that contains two cascades, one for each polarity. A high-voltage vacuum relay is used to switch polarity according to the method settings. To interface the high-voltage power source to the microprocessor, some hardware is used to generate analog control signals and some monitoring signals are fed to the ADC. As mentioned above, the ADC is multiplexed to measure the analog feedback from the high-voltage power source, the pressure and vacuum signals, and the temperatures. Digital status information from the high-voltage source is fed to the microprocessor for control and error detection.

A double-contact relay is implemented for safety purposes. In any emergency condition this relay breaks the 24V supply for the high-voltage power source. The double contact (two contacts in series) ensures the required safety level for this type of functionality.

**Voltage, Current, or Power Control**

Historically, voltage control has been used for electrophoresis. This keeps the field strength constant during the analysis. However, even the best cooling and thermostating of the capillary’s outer wall cannot prevent a temperature rise inside in the liquid, especially when using a high salt concentration. A temperature rise changes the viscosity and thus the mobility of the various species. This makes the different peaks elute faster, even when the field strength is kept constant. Because the conductivity of the buffer liquid also increases with rising temperature and thus the joule heating is increased, this effect is self-amplifying.

In such a situation, current control is advantageous. With current control, the feedback loop causes the voltage across the capillary to decrease when the conductivity increases as a result of an internal temperature rise. Thus, the effect is more self-limiting. However, measuring very small currents at high voltage is difficult, so the precision of current control is not as good as voltage control: 1% compared to 0.1%.

In thermally sensitive applications it may be advantageous to use power control. Variations in conductivity are then compensated, leading to a more reproducible internal temperature, which in turn stabilizes the analysis results.

**Voltage Measurement**

The high-voltage supply outputs a signal proportional to the high voltage at the output. This signal is used for monitoring, power control, electromigration injection, raw data to the PC for data evaluation, and limit sensing.
The output voltage is controlled by the high-voltage source internally. This means that voltage measurement is not required for voltage control. The major performance requirement is the precision and stability of the output voltage; absolute accuracy is secondary. The HP CE design yields a precision of 0.1%, which means only 10V fluctuations on a 10-kV output.

**Current Measurements**

Two current measurements are made on the high-voltage source. One is the current monitor signal from the high-voltage power supply and the other is the current at the capillary outlet next to the detector. For the latter, there is a current sense resistor between the vial ground electrode and the power supply's grounding pin. This leads to the requirement that the ground electrode be insulated. Even when there is no voltage difference, the current must be confined until it is measured. This measurement detects current from the high-voltage supply that is not flowing through the capillary. Such leakage current can be caused by wet surfaces or carbon tracks in plastic parts. It is a good safety and diagnostic feature to detect such leakage traces in the high-voltage path.

The measured current data is used for monitoring, limit sensing, power control, electromigration injection, raw data to the PC for data evaluation, leakage current detection, and bubble detection. For generation of raw current data only the return current from the capillary is used. This signal is integrated to determine injection volumes during electromigration injection when the current and time have been specified.

Corona effects, sparking, and insulation breakdown are detected and a yellow warning flag is displayed on the PC user interface to point out these problems.

To stop the analysis when a gas bubble has formed inside the capillary, a lower current limit can be set. If the current is not over this limit half a minute after voltage is applied, the analysis will be terminated to prevent local burning of the capillary.

**Pressure Measurements**

Data from pressure measurements is used for control of the pressure source, hydrodynamic injection, liquid level sensing during refill, raw data to the PC, and diagnosis and error detection.

For various needs, the pressure source can supply up to 1 bar of pressure and 0.4 bar of vacuum. Both pressures are stored in reservoirs which are filled sequentially from a membrane pump. This bipolar source allows push-pull actions which are used for empty-refill operations, up-down injection profiles, or controlled bipolar pressure across the capillary.

**Injection Control Algorithm**

To get high reproducibility for quantitative measurements in CE it is essential to have a very precise injection process. The HP CE instrument allows either hydrodynamic or electromigration injection. In the hydrodynamic injection mode the injection volume is determined by the time integral of pressure across the capillary. This pressure/time integral can be as low as 20 mbar·s. When pressure is applied by simply opening a valve for a specified time the slope of the pressure during switching of the valve is not very reproducible.

Gas rushing through the system will cause pressure drops that may result in transient errors in the pressure sensor signal relative to the real pressure generated across the capillary. Therefore, as described earlier, the HP CE instrument uses pressure tanks as gas flow sources with high output impedance. The conditions are controlled so that the pressure increases or decreases according to a linear ramp function. With the bipolar gas sources this ramping can be performed in both directions. With this system, valve switching time, the resistance and capacitance of the tube and chambers, the signal sampling rate, and even limited leakage are no longer factors that influence the precision of the pressure integral applied across the capillary.

To finish the injection profile another valve is switched to ramp down the pressure in the inlet vial. A kind of pulse width modulation is used to achieve a predefined slope. At specific pressure values during the ramp the system checks to see if the integral has reached the appropriate value. By design, the integral will always be smaller than the expected value. The ramp is then stopped and the pressure is held passively until the measured integral is correct, and then the ramp starts again. This leads to a successive approximation of the integral until the target value is reached within ±0.5%.

For hydrodynamic injection a variable pressure can be selected in a range of ±50 mbar. For flush and clean procedures the full pressure of about 1 bar is applied to the inlet vial.

**Replenishment Control**

During the analysis the content of the run buffer will become modified by the addition of electrochemical by-products. To achieve good reproducibility of the analysis the run buffer should be exchanged after several runs. The replenishment system allows the user to empty a vial, refill a vial, or bring a vial to a desired level automatically.

To determine the liquid level in a vial the following steps are executed:

- Draw a small airflow through the short end of the replenish double needle and measure the pressure drop in the needle.
- Move the vial slowly upward against the end of the capillary and wait for a sharp increase in the pressure drop, indicating that the needle has touched the liquid surface.

The noise of the pressure sensor must be very small because the detection algorithm uses the slope of the pressure to achieve a small delay between touching the surface and detection. For a complete description of the replenishment system, see the article on page 32.

**Capillary Temperature Control**

Because a temperature change of 1°C changes the viscosity by more than 2% and viscosity has an influence on electrophoretic mobility, the capillary temperature must be controlled so that it is stable within ±0.1°C so as not to degrade the analytical reproducibility.

The capillary temperature is controlled by heating or cooling, depending on its relation to ambient. A Peltier cooler is used as a cooling element for the capillary. By reversing the current polarity the same element can be used for heating. Because its power efficiency may be as low as 20%, an electrical supply of 100 watts is required. A current source was selected to drive the Peltier element because in this type of
application the Peltier is much more linear than with voltage control. An additional benefit is that current control is independent of the supply voltage, which allows operation with an unstabilized voltage, reducing the cost of the power supply and increasing the overall efficiency.

The efficiency of a Peltier cooler is dependent on the temperature difference between the hot side and the cold side. At a certain temperature difference there is no more cooling even when more electrical power is supplied. To avoid operation in this region both Peltier side temperatures are measured. This information is also used to prevent either side of the Peltier from becoming heated above 80°C, which would decrease the lifetime dramatically.

The temperature to be controlled is not the Peltier's, but the capillary's. While the exact temperature of the capillary is hard to measure because of the size and geometry, the air temperature around the capillary tracks it closely in value and time. Therefore, a sensor is placed where the airflow exits the capillary cartridge. This allows a fixed location for the sensor and wiring while the cartridge is easy to access. The high airflow speed of 10 m/s provides good, fast thermal contact between the capillary and the thermosensor.

**Cascaded Control**

To achieve good precision combined with steep slopes, a cascaded control concept is implemented. This requires a little more hardware and firmware, but offers better controlability along with flexibility and ease of optimization. This saved risk and speeded the development process.

As already mentioned, the efficiency of the Peltier is strongly dependent on the temperature difference between its hot and cold sides. Therefore, it is wise to keep the temperature difference between the air and the Peltier as low as possible. This leads to a heat sink large in size and volume. A good heat sink material is metal, which has a high thermal capacitance. The result is a long dead time and a high time constant between the Peltier temperature and the air temperature.

Conventional control algorithms are either slow or show long ringing or instabilities. The cascaded control loop of the HP CE instrument uses a sensor close to the Peltier’s cold side to achieve speedy measurement for steep slopes and good stability. However, the ambient rejection is not so good. Varying loads, dominated by the ambient temperature, may lead to a temperature difference of up to 8°C between the heat sink close to the Peltier and the air at the capillary.

To correct this problem, a second control loop is implemented around the control loop that stabilizes the temperature at the Peltier. This outer loop overrides the setpoint value for the first loop and adapts to ambient changes in the “minute” range while the inner loop of the cascade operates in the “second” range. As soon as the inner loop reaches a temperature close to its setpoint, the outer loop is activated to adapt the setpoint accordingly to reduce the difference between the capillary air temperature and the user-programmed value.

The Peltier element is mounted on a heat sink built into the top rear area of the instrument above the two card cages of the subunits. The switched-mode current source is on an extra printed circuit board next to the Peltier. The power transistors are connected to the same heat sink as the Peltier’s hot side and therefore can be cooled efficiently. The current source is controlled by two digital signals. One signal controls the power direction and the other defines the current through the Peltier.

The pulse width modulation for the current source has a frequency of about 30 kHz with 180-step pulse width resolution using a clock of 6.66 MHz. An 8-bit data word is needed for this, but if only 8 bits were used, the limited resolution would require a fast response to keep the resulting fluctuations small. Therefore, a 6-bit fine-tune feature first used in the HP 1050 HPLC system is used for Peltier control to keep the temperature stable within ±0.1°C or better. This gives a controllable dynamic range of about 10000:1 with the highest resolution at low power values. The control loop calculates a 14-bit control signal every three seconds. This calculation is somewhat complicated, but is not executed very often. The upper 8 bits are fed to the pulse width modulator, while the lower 6 bits are used to modulate the pulse width modulator in a 12.5-ms time slice (see Fig. 3). The benefit is a higher base frequency, which is filtered better by the thermal time constant of the system. Thus, the required temperature stability is achieved with inexpensive digital hardware, simple firmware algorithms executing at an 80-Hz rate, and complex calculations every three seconds.

**Fig. 3.** A six-bit fine-tune function is added to the basic 8-bit pulse width modulation of the switched-mode current source that drives the Peltier cooler. This increases the precision of the capillary temperature control.
Safety
To guarantee user safety, the HP CE instrument implements a number of safety features. Tray rotation is stopped after a maximum of 400 ms when the tray door is opened. Any movement that was in progress is finished after the door is closed again. The high-voltage power supply is brought to 0V and switched off when the top cover is opened or when a leak is detected. The cassette fan is switched off when the top cover is opened to avoid finger injuries. Pressure on the vials is released immediately when the top cover is opened; otherwise, the vial contents would be sprayed inside the detector compartment. Pressure is removed from the buffer bottle if a leak is detected.

Evaluation
When a concentration dependent detector signal is integrated over time the peak area is a measure of the amount of separated sample compound. The sample area and the amount are related by the speed of the absorbing particles as they move through the detector cell.

In HPLC separations the speed of the sample is normally known. It is the flow rate of the liquid. In electrophoresis, the separating power is the difference in the mobility of the compounds, which means that the compounds have different specific speeds while moving through the detector.

To correct for speed differences in later evaluation steps, information about the moving force is needed. In a simple analysis the voltage is constant during the complete run, but temperature changes have an influence on viscosity and mobility, and a time-programmed voltage or fraction collection can have a tremendous influence on absolute-area-to-amount calculations.

To support future evaluation developments the HP CE instrument supports five raw data channels in addition to the detector signals:
- Voltage, current, and power of the high-voltage source
- Pressure across the separation capillary
- Temperature of the air from the cassette.

Diagnostics
Traditionally, diagnostic routines built into instruments are based on the perception that the failure modes are already known during the development phase. For these failure mechanisms special tests are implemented (RAM, ROM, communication, display, etc.) which generate a pass/fail indication or a value. Sometimes the knowledge of a specific failure mode in the early development phase leads to changes of the product that reduce or even eliminate the failure mode. However, there is no possibility of avoiding the learning curve. Unexpected behavior and dependencies always appear after the product is released, and may increase the list of diagnostic functions needed. Thus, the diagnostics implemented in the early phase might be of little use while those most needed are not supported. The disadvantage of the traditional concept is that the diagnosis of failure modes discovered after the development phase is not possible without product changes.

The HP CE instrument implements traditional diagnostic functions as outlined above, but the firmware also implements basic functions for diagnosis, such as read sensors, or write to hardware address. Macros on the PC can use these basic functions to construct complex diagnosis functions to address specific problems. Now if a new failure mode is discovered, we just add a diagnosis macro for it and create an updated version of the diagnostics disc to distribute it to the manufacturing and service organizations. This leads to a continuous improvement of the diagnostics for the instrument, and we spend the time to create diagnosis functions only for failures that have actually occurred in the field. A valuable side effect is that most module test routines are implemented within the product, so manufacturing does not need a large amount of special equipment. The service person at the customer's site has all the equipment needed to perform the same tests as originally used in the factory.

Diagnosis Functions
The instrument normally collects equally spaced data for voltage, current, power, vial pressure, and temperature in a buffer memory in the instrument RAM. The diagnosis firmware makes it possible to redirect other data like servo speed, servo position, servo current, sensor data, valve states, and so on to this buffer. Thus, we can store up to five configurable measured values, with a configurable data rate of up to 20 Hz. The PC software reads this buffer and generates reports and graphics.

This tool greatly improved our ability to find bugs during the development phase because we got the history of servo positions, pressure values, and other measured values when an error occurred. It also gave us much more insight (like a built-in oscilloscope for electromechanics) into what really happened in the unit than we would have obtained from single measurements.

An advantage of this firmware feature is that service personnel can use it to find problems in the field and can send the printout to the factory for a simple form of remote diagnosis. An example is the macro for checking the pressure system. It takes about five minutes and checks the power of the air pump and the tightness of the pressure and vacuum system, the injection system, and the replenish system and stores the data from all of the pressure sensors. A graph of these quantities characterizes most of the pressure system and helps diagnose the problem if there are deviations from the normal behavior.

Acknowledgments
The success of this product is a result of the exceptionally good teamwork of all the people who worked on it. We especially want to thank Gerda Biselli for her patience in writing the online help and the manual, Arno Graf for the discussions about serviceability and diagnostics, and Claudia Maschke for designing the temperature controller board. The great support of the production and marketing groups during development helped us to find and realize some of the ideas that make the instrument as good as it is.

References