Comparison of Air and Liquid Cooling in Capillary Electrophoresis

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Summary

Data presented in this article demonstrate the advantages of a liquid-cooled capillary electrophoresis system when compared with air-cooled instruments. Liquid cooling will accommodate a wide variety of common experimental conditions (moderate to high ionic strengths; low electroosmotic flow) without the need to compromise on experimental design.

I. Introduction

Current Status

Although capillary electrophoresis (CE) has gained acceptance in the competitive separations market, the reproducibility of the technique has at times been questioned. Compared to HPLC (CVs <1%), CE can have values which are greater than 5%. The larger-than-expected variances have been attributed to inadequate system equilibration, variable electroosmotic flow (EOF), and/or inefficient cooling systems. In most cases, the equilibration and EOF issues have been addressed and remedied. This bulletin will address the relationship between cooling methods and system performance.

In the following sections, we will define an important experimental criterion to evaluate cooling effectiveness, review the principles of heat generation and transfer, analyze the relationship between system parameters and heat generation, and examine the effect of temperature on system performance. In the process we will demonstrate that:

1) Experimental compensations for heating effects can be limiting;
2) One method of cooling is not just as effective as another;
3) Air-cooled systems tend to run hotter than the corresponding liquid cooled systems; and
4) Temperature fluctuations encountered in air-cooled systems can compromise system validation.

II. Criteria for Cooling Effectiveness

A. General Features

A viable cooling system must be able to accommodate typical capillary electrophoresis applications without compromising experimental conditions. The requirements of the sample, not the hardware,
should define the experimental parameters. To accommodate the needs of the pharmaceutical and biotech companies, the cooling systems must be validatable, i.e., ensure reliability and reproducible performance.

**B. Evaluation Conditions**

As we examine the effects of different cooling systems in the following sections, it will be important to evaluate the conditions under which the comparisons were made (buffer pH, ionic strength, EOF, and temperature). Along the way, we will point out the limitations of the low ionic strength “Designer Buffers” systems and include examples from high ionic strength buffers (>100 mM) since they are integral to a number of CE techniques. Such conditions are required to prevent analyte adsorption, to facilitate sample stacking, to control analyte/analyte interactions, to optimize the MEKC method, or to increase buffering capacity of the system.

**III. Fundamental Principles**

**A. Joule Heating and System Parameters**

Before considering the effects of different cooling modes, it is appropriate to examine the theoretical basis for heat generation and cooling during capillary electrophoresis.

When current passes through a solution, electrical energy is partially converted into Joule heating (Figure 1). Depending on the application/electrophoresis conditions, temperature changes may range from a few degrees above the surroundings to boiling/outgassing. Unless this heat is removed (Figure 1), it can have an impact upon the electrophoresis results primarily though changes in the migration time (viscosity, net charge, EOF) and changes in the resolution/profile (diffusion coefficient and stability). In some cases, quantitation is affected from losses due to thermal expansion.

It is important to keep in mind that changes in Joule heat may also occur randomly. These factors can be induced by changes in the buffer (ion depletion, ionization) and/or the EOF (low buffer capacity, analyte adsorption, hydrostatic flow). It is imperative to control Joule heating since this parameter is directly linked to analyte mobility, resolution, and stability, as well as system reproducibility.

During electrophoresis, thermal gradients develop radially and transversely (the long capillary axis). This occurs because heat is not removed rapidly or uniformly enough from the capillary via conduction, convection, radiation, or displacement (EOF). These processes are not uniformly distributed even in the best of systems. In addition, the heat transfer efficiency differs throughout the system. A diagram of this process is shown in Figure 2A. Since the temperature is highest at the center of the capillary and only somewhat lower at the capillary wall, most of the temperature drop occurs between the outside wall and the cooling medium.

In the body of the capillary, heat is transferred by conduction across the capillary wall, and by convection with respect to the coolant which may be air or liquid (see Figure 2B). The buffer vial functions as a component of the cooling system, i.e., it acts as a heat sink for the respective ends of the capillary and serves as a source of cooled buffer for the “pumping” action of EO flow.

**B. Experimental Compensation**

A common empirical approach has been to compensate for heating effects by modification of the experimental parameters. “First Principles” have been used to justify modification of one or more of the experimental conditions, i.e., the buffer ionic strength, the capillary i.d., or the run voltage, in order to reduce the effects of Joule heating. While these changes result in a reduction in heat, they do not necessarily produce the most efficient or desired results and can represent an unsatisfactory compromise (see Table 1).
IV. Cooling Effectiveness and System Parameters

A. Ohm’s Law Relationship

Ohm’s Law can be used to evaluate the relative effectiveness of different cooling methods.\(^\text{(11)}\)

The voltage (V) is linearly related to the current (I) as long as the resistance remains constant (V = IR). If the temperature changes as a result of the electrophoretic process, corresponding changes will occur in the resistance and the current will deviate from linearity. This condition typically prevails as a consequence of excess Joule heating.

It is possible to use this relationship to define a limiting value for the field strength (E, volts/cm) for a given capillary, buffer, temperature, and cooling combination. A plot of the potential (kV) vs. current (µamps) will be linear as long as the temperature is controlled. As the voltage increases, excess heat will not be completely removed; the internal temperature will rise, viscosity will decrease, and the current will increase. A comparison of both air and liquid cooling is shown in Figure 3.\(^\text{(12)}\)

It is obvious that liquid cooling allows significantly higher field strength (E, volts/cm) to be employed which means faster run times and better efficiency. In addition, the higher allowable “running currents” (linear portion) observed with the forced-air system correspond to higher internal temperatures for a chosen value of E. This difference which is subject to experimental variation can be expected to affect the reproducibility and reliability of the system (see Figure 1 for the interrelationship of the various experimental parameters).

Table 1. Typical Adverse System Effects Caused by Varying Experimental Parameters to Reduce Joule Heating

<table>
<thead>
<tr>
<th>Options</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Lower voltage</td>
<td>• Longer run times</td>
</tr>
<tr>
<td></td>
<td>• Increased band broadening</td>
</tr>
<tr>
<td></td>
<td>• Decreased throughput</td>
</tr>
<tr>
<td>2. Smaller i.d. capillary</td>
<td>• Decreased sensitivity</td>
</tr>
<tr>
<td></td>
<td>• Manipulation problems</td>
</tr>
<tr>
<td></td>
<td>• Reduced fraction collection capability</td>
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<tr>
<td>3. Decreased ionic strength</td>
<td>• Faster EOFs</td>
</tr>
<tr>
<td></td>
<td>• Lower sensitivity</td>
</tr>
<tr>
<td></td>
<td>• Increased adsorption</td>
</tr>
<tr>
<td></td>
<td>• Decreased resolution</td>
</tr>
<tr>
<td></td>
<td>• Longer regeneration times</td>
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While all three options result in lower overall current, choices #1 and #3 can have a direct impact on the separation, while the second option adds further restrictions to a technique that is already sample-limited for preparative studies. Obviously, a more efficient cooling system is the solution of choice over experimental modifications.
While changes in current are indicative of temperature changes for this set of moderate buffers, *i.e.*, medium ionic strengths and low to medium EOFs, other more taxing conditions are also employed which cause even larger current changes. What then is the relationship between the current change and the internal temperature?

**B. Run Buffers and Capillary Temperature**

In this section, we will examine the impact of various buffers on the internal temperature of the capillary vs. a fixed external set temperature for an air-cooled system.

The Joule heat generated by a given buffer can be evaluated in system terms, *i.e.*, the difference in temperature between the center of the capillary and the capillary wall or between the center of the capillary and the surroundings. Somewhat unexpectedly, the system can “efficiently” transfer heat from the central lumen to the outside capillary wall under a variety of conditions (Figure 4A and Equation 1). At most, the maximum temperature changes represent only a few degrees.

By contrast, the difference in temperature between the central lumen and the surrounding “fluid” is rather large (Figure 4B and Equations 2 and 3). Using typical buffer conditions, the internal temperatures ranged from 5°C to greater than 40°C above the set temperature when a field strength of 300 V/cm was used! For 100 mM sodium phosphate, pH 7.0, these changes were so large that one or more of the experimental conditions must be changed before continuing (Section III B).

For “typical” buffer conditions (curves 2 and 3, Figure 4), temperature changes in the 5 to 10°C range might be expected when operating at 300 V/cm. How significant are these changes and what impact do they have on system performance, *i.e.*, on migration time or on mobility? These questions will be addressed for both types of cooling systems in the next section.

**V. Cooling Effectiveness and System Performance**

**Critical Factors**

The effect(s) of temperature on the system parameters (migration time, EOF, and analyte mobility) will be considered under two different sets of conditions, *i.e.*, moderate pH and low-moderate ionic strength or under low pH and moderate-high ionic strength. In the latter case, the influence of EOF has been removed so that analyte mobility must be used to evaluate the relative effectiveness of the cooling systems. It will be readily apparent that buffer EOF along with ionic strength must be taken into consideration when drawing any conclusions from this comparison.

**1. Temperature and Migration Time**

From a theoretical consideration, electrophoretic-mobility changes exponentially with the temperature (Equation 5). However, over a narrow range, the mobility increases approximately 1-2% per degree Kelvin. The experimental results in Figure 5 illustrate this relationship. As the temperature increases, there is an approximately linear decrease in the migration time. It is apparent that the relatively small changes in temperature (± 5°C) cannot be neglected since they correspond to significant changes in migration time.
into the system from the buffer vial. Localized “hot spots” do not develop since these sections are continuously moving. Thus, higher fields can be employed without experiencing failure. However, when EOF is eliminated, and more robust buffers are employed, greater system differences are apparent.

3. Effects of Temperature on Analyte Mobility in the Absence of EOF

To compare the cooling efficiencies of different systems, low EOF and moderate to high ionic strengths conditions (>100 mM) should also be employed since they challenge the system. These conditions are representative of a number of key CE applications (see Section II. B). To monitor the differences, analyte rather than EOF mobilities will be used in these studies since the EOF is low.

2. Temperature and EOF

Comparisons of cooling systems are often made with moderate EOFs (pH 6-7) and moderate-low ionic strengths (< 50 mM) [see Figure 6, and reference 15]. Under these conditions, there seems to be no particular advantage to either cooling system since they behave similarly. However, the actual differences have been minimized due to EOF cooling. With this process, cool buffer is continually pumped...
Figure 7. Effect of field strength on mobility and peak width using either (A) a liquid or (B) an air-cooled system. For these analyses, the buffer was 150 mM Phosphate, pH = 2.8, detection was at 214 nm. Run conditions (V/cm) for panel A, top to bottom, were 175, 263, 350 and 439; conditions for panel B, top to bottom, were 153, 255, 357 and 459. The capillary (liquid-cooled, Beckman) was 75 µm i.d., fused-silica, with an effective length of 50 cm x 57 cm while the air cooled system was 75 µm i.d. which was 40 cm x 49 cm in length. In each case, aliquots of a peptide mixture (50 µg/mL each of angiotensin I and II, bradykinin, and neurotensin in 0.1% TFA) were injected for 5 sec. at 0.5 psi. For both instruments, the cooling systems were thermostated at 25°C while the buffer vials were kept at ambient temperature. Buffers were changed after three runs in order to avoid “Buffer Depletion effects.”

Figure 8. (A) Comparison of analyte mobility at different field strengths using either a liquid- or an air-cooled system. Conditions are as in Figure 7. In each case, the apparent mobility was calculated for peak 3 and plotted against the field strength. (B) Comparison of analyte mobility at different power settings using either an air- or liquid-cooled system. The conditions for this experiment are the same as Figure 7. This method of plotting the data allows us to include the current along with the voltage in our consideration.

All analyses were conducted with a four-component “standard” peptide mix which included angiotensin I and II, bradykinin, and neurotensin. The buffer was chosen for its low EOF and moderate-high ionic strength (150 mM sodium phosphate, pH 2.8) (Figure 7A & B). The successive electropherograms were obtained by increasing the run voltage by 5 kV between runs. Only a portion of the analysis could be completed at approximately 400 V/cm (Figure 7B) since the air-cooled system crashed after a few minutes.

While both cooling systems demonstrated field strength dependence, the liquid-cooled unit clearly outperformed the air-cooled system (Figure 8A).
In each case, the apparent mobilities were plotted against the field strength. The mobilities from the air-cooled system were approximately 20% and 30% higher at 300 V/cm and 400 V/cm respectively! More significantly, the initial portion of the current traces tended to be irregular throughout the experiments with the air-cooled system. This condition finally led this system to fail during the analysis at 400 V/cm. By contrast, no current irregularities were observed with the liquid-cooled unit. This unit continued to operate until the limiting current of 250 μamps was reached.

It is informative to consider the effect of power (watts/meter) on cooling system performance. This is shown in Figure 8B where the mobility is plotted against power. As expected, both curves show a linear correlation but the slopes are significantly different. This difference is reflected in higher internal temperatures for the air-cooled system, resulting in shorter migration times, higher mobilities, broader peaks, and poorer resolution for the same analyte with the same buffer using the same conditions.

Since internal temperature is a system parameter, validation becomes increasingly difficult to assign since the actual temperature is unknown and subject to experimental fluctuations from changes in capillary diameter, EOF, ionic strength, and hydrostatic head pressure.

VI. Conclusions

From a comparison of the air- and liquid-cooled systems, it is possible to show that:

• Heat is more efficiently transferred to a liquid than to air no matter how fast the air is moving;
• Higher internal temperatures result in shorter migration times, broader peaks, and variable results;
• Using so-called “Designer Buffers” for comparative studies can be misleading since the EOF cooling tends to minimize actual system differences;
• Comparison studies should be performed with a broad range of buffers that include low EOF and moderate to high ionic strength buffers which can challenge the cooling system;
• Liquid-cooled systems possess the distinct advantage in cooling efficiency since they accommodate a broader range of CE application requirements.

Equations

**Equation 1.** Temperature differences can be expressed in terms of watts/volume generated, capillary radii (inside & outside), and the thermal conductivity of the buffer. The temperature difference within the buffer is of concern since its potential parabolic profile would tend to promote decreased efficiency. This relationship is shown as:

\[ T_0 - T_1 = \frac{P_m R_1^2}{4k_b} \]

where

- \( P_m \) = Watts/capillary volume
- \( R_1 \) = Capillary radius (inside)
- \( T_0 \) = Temperature at capillary center
- \( T_1 \) = Temperature at capillary wall (inside)
- \( k_b \) = Thermal conductivity of buffer

**Equation 2.** The temperature change between the center of the capillary (\( T_0 \)) and the surrounding coolant (\( T_a \)) can be approximated from the following relationship:

\[ T_0 - T_a = \frac{P_m R_1^2}{R_3 h} \]

where

- \( T_a \) = Temperature of surroundings
- \( R_3 \) = The outside radius
- \( h \) = The heat transfer coefficient
- \( T_0 \) = Temperature at capillary center

It can be difficult to determine the \( \Delta T \) with this equation since the value of “\( h \)” is a function of the particular cooling mode, configuration of the capillary, and the net EO flow. It is possible, however, to estimate \( \Delta T \) from another relationship (Equation 3) and then derive a value for “\( h \)” which should be instrument/capillary specific.

**Equation 3.** The internal temperature can be calculated from a measurement of the electroosmotic mobility at two different voltages according to the equation:

\[ T_2 = \frac{A}{\ln(\mu_{eo1}) - \ln(\mu_{eo2})} + B \]

where \( T_2 \) is the internal temperature, \( \mu_{eo1} \) and \( \mu_{eo2} \) are the EO mobilities at voltages 1 and 2, and \( A = \frac{E_a}{R} = 1820K \) and \( B = \frac{E_a}{RT_1} \) [\( B = 6.11 \) when \( T_1 \) is measured at room temperature, or 298K] are constants which were derived from the temperature dependence of the mobility.\(^{(14)}\) The first voltage must be chosen well below the Ohm’s Law break so that the internal and set temperature are identical.
Note: This expression should also be used for estimating the internal temperature parameter for various methods (MEKC, inclusion CE, etc.) and for system validation. It is not reasonable to rely on the external setting of the temperature.

**Equation 4.** Mobility is related to temperature through the viscosity ($\mu = \frac{Q}{6\pi\eta r}$) while resolution is directly proportional to the applied voltage ($V$) and inversely proportional to the diffusion coefficient ($D$) according to the following equation:

$$R_s = C_1 \left[ \frac{V}{D (m_{av} + m_{eo})} \right]^{1/2}$$
$$Rs = C_2 \left[ \frac{Vh}{T (m_{av} + m_{eo})} \right]^{1/2}$$

where “C” is a constant, and $\mu_{av}$, $\mu_{eo}$ are the average mobility and EO mobility respectively. Temperature is incorporated into this expression from Fick’s law, i.e., $D = \frac{kT}{6\pi\eta r}$ ($k$ = Boltzman constant, $T$ = absolute temperature, $\eta$ = viscosity, and $r$ = hydrodynamic radius).

**Equation 5.** The influence of temperature on mobility is attributed to changes in the viscosity. The dependence of viscosity on temperature is expressed by the relationship:

$$\eta = C e^{E_a/RT}$$

where $C$ is a constant, $E_a$ is the activation energy for viscous flow, $R$ is the gas constant, and $T$ is the temperature in Kelvin.$^{(15)}$ Since the mobility is inversely related to the viscosity, it is apparent that it will increase exponentially with increases in temperature.

**References**


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