The Measurement of Temperature Inside Capillaries for Electrophoresis Using Thermochromic Solutions

H. Wätzig
Institute of Pharmacy and Food Chemistry, University of Würzburg, Am Hubland, 8700 Würzburg, Germany

Key Words
Capillary electrophoresis
Temperature inside capillaries
Thermochromic solution
Cobalt(II)chloride

Summary
Capillary temperature is one of the most important parameters in capillary electrophoresis (CE). Its control is essential. The efficiency of different methods of thermostatic control are difficult to compare because the measurement of temperature inside the capillary has previously only been possible using indirect methods. Direct measurement of the temperature is now possible using the thermochromic effect. Cobalt(II)chloride solutions change their absorption spectrum distinctively with changes in temperature. The results obtained by this method show an increase in temperature of several degrees Kelvin despite thermostatic control. This is in good agreement with theoretical calculations [1, 13].

Introduction
Many parameters in capillary electrophoresis (CE) depend on temperature: electroosmotic flow, viscosity, conductivity and the coefficient of diffusion. All these parameters influence the main estimates of CE: the migration time and quantitative results. Therefore temperature should be kept constant during CE measurements. This is hardly possible because the temperature is unavoidably increased by Joule heating caused by the electrical power. Joule heating causes a decrease in the buffer resistance. If the voltage is kept constant, the current will increase and hence the power as well, leading to additional heating.

Two possibilities for avoiding this problem are to use a buffer with a relatively high resistance and effective temperature control. Thermostats work either using a liquid (e.g. PACE 2100, Beckman; BioFocus 3000, Biorad) or streaming gas (e.g. 270A-HT, Applied Biosystems Instruments; CES, Dionex; SpectraPHORESIS 500/1000, Spectraphysics; Model 3140, Isco). Both methods manage to maintain constant temperature in the compartment, in which the capillary is placed. If high electric power is used (e.g. 5 W) only a small increase in temperature (about 0.1 °C) can be measured. This is compensated within a few minutes.

However, these temperatures are always measured at a certain distance from the capillary. A clear statement about the effectiveness of a thermostat is only possible by measuring the temperature within the capillary. This measurement would include the effects of heat transport through the capillary wall. However, the placement of thermistors to measure temperatures inside the capillary is not possible because of its small diameter, therefore, an indirect method of temperature measurement must be used. Terabe, Otsuka and Ando [2] tried a measurement of this kind using equilibrium constants but achieved insufficient precision using this measuring principle. Bergstrom, Goodall, Survay and Williams used conductivity and ion mobility measurements [1] and achieved consistent results. Burgi, Salomon and Chien investigated the internal temperature of capillaries by changes in the electroosmotic flow [12] but it is only possible to measure an average temperature over the whole capillary.

A new possibility is the use of the thermochromic effect. Some substances change their uv/visible absorption spectrum with temperature. If these changes are large enough and reproducible, a temperature measurement can easily be obtained: the uv/visible-detector, a component of almost all CE-instruments, can be used as the thermometer.

Thermochromic Behaviour
A change of absorption spectra caused by changes in temperature can often be found [3, 4]. For inorganic substances in solution, a change of ligands or the coordination number of complexes can be responsible for this. Thermochromic effects of organic substances depend on temperature-induced shifts in equilibria between, for example, differently coloured conformers. To use such an equilibrium for temperature measurement, all other parameters such as concentrations of substances that take part in the equilibrium, especially the pH, must be kept constant.

Although there are a lot of substances that show a thermochromic effect, there are only few that do in...
aqueous solution in a temperature range 20–40 °C. According to the literature, model substances which should be useful include: copper(II)xylenol orange [5], copper(II)methylthymol blue [6], cobalt(II)chloride [7] and 3,5-dinitrosalicylic acid [8]. Of these the cobalt(II)chloride proved to be the best suited for thermochromic measurements in CE; the necessary chemicals are available in most laboratories. Figure 1 shows the absorption spectrum of this complex at different temperatures. The thermochromic behavior of cobalt(II)chloride is even more dramatic using lower pH, but the pH of 5 used in these experiments is more convenient under usual CE-conditions.

Experimental

The temperature-dependent spectra of cobalt(II)chloride in Figure 1 were performed with a PE 320 instrument (Perkin-Elmer). The diameter of the cuvettes was 1 cm. The temperature was kept constant by a self-made metal cuvette holder which is irrigated with thermostatted water. Before and after measuring the spectra the temperature within the cuvette was controlled by a thermometer. The temperature measurement within capillaries was performed with a P/ACE 2100 (Beckman) and a fused silica capillary (57 cm × 75 μm i.d., Polymicro Inc.); absorbance was detected at 495 nm and the temperature was calculated from these measurements. Therefore only the temperature within the uncoated capillary within the detector cell is estimated; this part of the capillary is properly thermostatted. There are necessarily parts of the capillary outside the thermostat system, leading from the buffer vials to the thermostatted cartridge. In these parts the temperature should be higher than in the thermostatted section.

All chemicals used were of analytical grade. The water was highly purified (Millipore). For the preparation of the thermochromic solution used, 664.0 mg hexaquacobalt(II)nitrate and 133.3 mg (CE-experiments) or 2133.4 mg (spectra in Figure 1) sodium chloride are dissolved in 100.0 ml 50 mM potassium phthalate buffer, pH 5.0. This solution was degassed (sonicated for 10 min) and filtered through a 0.45 μm membrane filter prior to use.

Temperature Measurement by Thermochromic Solutions

First a static temperature measurement was carried out with the solution described above. The capillary was filled with thermochromic solution, then the temperature was increased using the thermostat of the CE apparatus in 1 °C steps (Figure 2). There is always an overshoot in the temperature. Heating and cooling of the thermostat system and the thermometer that controls the set values are apart from each other hence the temperature increase is detected after some delay and heating continues during that time. In fact when the set temperature is measured by the thermometer, the real temperature is higher, cooling becomes necessary, and another overshoot in the opposite direction occurs. After half an hour equilibrium is nearly reached. All these temperature changes can be observed by the thermochromic measurements. An almost linear change of absorbance (2.5 mAU °C⁻¹ at 495 nm; see Figure 3) with temperature can be stated.

![Figure 1](link)