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Non-uniform electrical field effect caused by different concentrations of electrolyte in capillary zone electrophoresis

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ABSTRACT

Capillary zone electrophoresis is usually performed with uniform electrolyte concentrations in the electrolyte reservoirs and the capillary and a resultant uniform electrical field. Thus, the sample species migrates as the algebraic sum of electroosmotic flow mobility and ionic mobilities. A non-uniform electrical field can be formed by filling the capillary and outlet reservoir with electrolyte at a given concentration (initial electrolyte) and the inlet reservoir with a different electrolyte concentration of the same chemical species (replacement electrolyte). Application of an electrical field causes continuous entrance of the replacement electrolyte changing the conductivity consequent to electrolyte concentration. Therefore, the electrical field strength is not uniform along the capillary. In this paper we will discuss the effect of this non-uniform electrical field on migration rates of different sample ions as well as its application to model samples.

INTRODUCTION

Capillary electrophoresis (CE) methodology has developed rapidly[1–3]. In capillary zone electrophoresis (CZE), generally, the same electrolyte solution is used in the inlet reservoir, outlet reservoir and capillary tube. When voltage is applied to this system, an electroosmotic flow is produced and moves from capillary inlet (usually at the anode) to the outlet (usually at the cathode). In other words, the electrolyte solution in the inlet reservoir (replacement electrolyte) will move continuously into the capillary and the electrolyte solution initially inside the capillary (initial electrolyte) will be replaced continuously by the replacement electrolyte. Since there is no difference in composition and concentration between replacement electrolyte and initial electrolyte, there are no differences in electrolytic conductivity. If it is assumed that the electrical disturbance from sample zone can be neglected, a uniform electrical field will be established along the capillary. The value of this uniform electrical field strength is constant along the capillary length (spatially uniform) and with time (temporally uniform). In some cases, different kinds of electrolyte solutions are employed in the capillary and electrolyte reservoirs such as in capillary isotachophoresis[4], isoelectric focusing[5] and CZE with gradient electrolyte[6]. Either the composition or con-

centration of one or both of replacement and initial electrolytes may be different. Under these conditions, the electrical field strength along the capillary is not uniform. In this paper, a simplified equation is presented to describe this non-uniform electrical field.

EXPERIMENTAL

All experiments were performed with a P/ACE™ 2000 instrument (Beckman, Palo Alto, CA, U.S.A.). For these studies the capillary was 75 μm in diameter, 66 cm in total length, 61 cm from inlet to detector with an applied voltage of 20 kV. UV detection was at 254 nm. Temperature was controlled at 20°C. Phosphate buffer (pH 6.8) was made at different concentration levels from a stock solution of 100 m*M*. Pyridoxamine, nucleosides and nucleotides were purchased from Sigma (St. Louis, MO, U.S.A.) and used without further purification. Samples were diluted from stock solution (20 m*M*) to 0.2 m*M* with phosphate buffers.

RESULTS AND DISCUSSION

Preparation and simplified description of non-uniform electrical field

The formation of a non-uniform electrical field along the capillary can be explained by a simple model (see Fig. 1). Before applying voltage, the capillary was filled with initial electrolyte which has the unit resistance r(i) (where unit resistance is defined as total resistance divided by capillary length). Since only initial electrolyte exists at this time, a uniform electrical field is established along the capillary. This is true for most situations in zone electrophoresis which use uniform electrolyte and samples which are not overloaded. Assuming that electroosmotic flow moves toward the cathode, the replacement electrolyte in the anode reservoir will continuously enter the inlet of the capillary. If the unit resistance of the replacement electrolyte is differ-

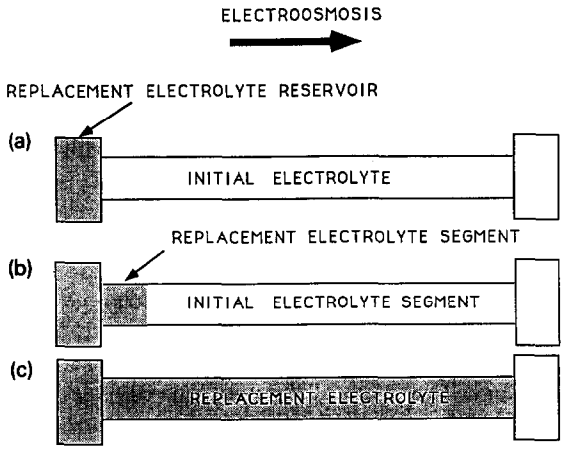

Fig. 1. Schematic model of non-uniform electrical field. (a) Uniform electrical field at the start of electrophoresis. (b) Non-uniform electrical field when the replacement electrolyte replaces some of the initial electrolyte. (c) Uniform electrical field after replacement electrolyte occupies the whole capillary.

ent from the initial electrolyte, for example at higher unit resistance, the electrical field along the capilary will not remain uniform.

There are two characteristics. First, at any moment prior to the front of replacement electrolyte reaching the capillary outlet, two different electrical field strengths exist along the capillary. The front of the replacement electrolyte acts as the boundary of these two levels of the electrical field. Under this condition, the replacement electrolyte at higher unit resistance has a greater electrical field and the initial electrolyte has lesser field. Second, this electrical field strength will continuously change with the rate of replacement electrolyte entering the capillary inlet and will establish a uniform electrical field strength after replacement electrolyte completely occupies the capillary.

A simple mathematical expression can be derived to describe the non-uniform electrical field along the capillary. In the beginning, the capillary is filled only with the initial electrolyte and the electrical field strength is constant:

$$E = V/L \tag{1}$$

Here, the E is electrical field strength (V/cm), V is applied voltage and L is total length of the capillary. Then, the replacement electrolyte moves into the capillary inlet and starts to replace the initial electrolyte inside the capillary. At time t the replacement electrolyte enters into the capillary to length l, then the total resistance R across the capillary is:

$$R = lr(\mathrm{r}) + (L - l)r(\mathrm{i}) \tag{2}$$

where r(i) is the unit resistance of the initial electrolyte; r(r) is the unit resistance of the replacement electrolyte; l is the length of segment of replacement electrolyte.

The voltage drop on l is:

$$V(l) = \frac{Vlr(\mathrm{r})}{lr(\mathrm{r}) + (L - l)r(\mathrm{i})} \tag{3}$$

The electrical field strength on l is

$$E(l) = \frac{V(l)}{l} = \frac{Vr(\mathrm{r})}{lr(\mathrm{r}) + (L - l)r(\mathrm{i})}$$

$$\frac{V}{l + (L - l)(r(\mathrm{i})/r(\mathrm{r})} \tag{4}$$

Letting $K = \dfrac{r(\mathrm{i})}{r(\mathrm{r})}$, eqn. 4 can be simplified to

$$E(l) = \frac{V}{l + (L - l)K} \tag{5}$$

Likewise, we can calculate electrical field strength on the initial electrolyte segment, $E(L-l)$

$$E(L-l) = \frac{V}{l/K + (L-l)} \tag{6}$$

From eqn. 5 we can see that:

(1) When $K = 1$, *i.e.*, there is no difference between initial and replacement electrolyte, the equation is simplified to $E(l) = V/L$, which is the same as the uniform electrical field situation.

(2) When $K \neq 1$, the electrical field strength along the capillary will not stay the same but will change with time. Fig. 2a shows the relationship between the electrical field strength and the length of replacement electrolyte at $K = 0.8$. Fig. 2b shows the relationship between electrical field strength and the length of initial electrolyte at $K = 1.25$.

(3) When the absolute value of K is close to 1, the difference between uniform and non-uniform electrical field is decreasing. Fig. 3 shows the relationship between K values and the changes of non-uniform fields.

Theoretically, K is the ratio of resistances of initial and replacement electrolyte. Since in CZE the ionic strength of electrolytes is quite small, we can probably use the ratio of concentrations without introducing significant errors in most cases.

Effect of non-uniform electrical field on the migration rates of different species

From Fig. 2, we can see that there are two different electrical field strengths along the capillary. For simplicity, we only discuss the $K < 1$ case (Fig. 2a). *i.e.*, the

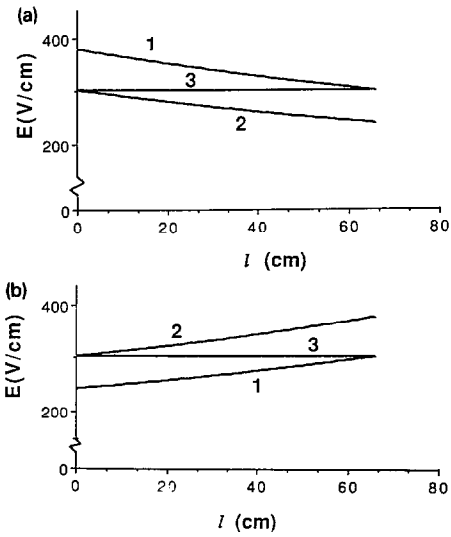

Fig. 2. Calculated electrical field strength in initial and replacement electrolyte segments. Parameters: Capillary length 66 cm. Applied voltage 20 kV. (a) $K = 0.8$; (b) $K = 1.25$. Curves: 1 = electrical field in replacement electrolyte segment; 2 = electrical field in initial electrolyte segment; 3 = in uniform electrical field.

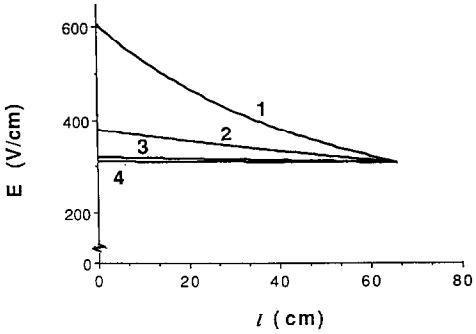

Fig. 3. The influence of K values to electrical field strength in replacement electrolyte segment. Parameters as in Fig. 2. Curves: (1) $K = 0.5$; (2) $K = 0.8$; (3) $K = 0.95$; (4) $K = 0.98$.

unit resistance of replacement electrolyte is higher than that of the initial electrolyte, in this case, the electrical field of the initial electrolyte is lower than the electrical field of the replacement electrolyte. For cations, the direction of electrophoretic mobility is the same as the electroosmotic flow. The total migration rate of a cation is:

$$v(\text{total}) = v(\text{eos}) + v(\text{i}) = v(\text{eos}) + E\mu(\text{i}) \tag{7}$$

were v(i) and μ(i) are the migration rate and the mobility of the cation, respectively and v(eos) is the electroosmotic velocity. It should be noted that with a non-uniform field, the electroosmotic flow rate will not stay the same as it would under a uniform field conditions. For simplicity, here we use an overall electroosmotic velocity.

Since cations have greater total migration rate, they will migrate ahead of the front of the replacement electrolyte. In other words, they will migrate in the region of initial electrolyte. Since the electrical field is lower in initial electrolyte (when $K<1$), the migration rate of cations will be lower than in uniform electrical field conditions. On the other hand, the direction of electrophoretic mobility of an anion is opposite to the electroosmotic flow. In this case the total migration rate is:

$$v(\text{total}) = v(\text{cos}) - v(\text{i}) = v(\text{eos}) - E\mu(\text{i}) \tag{8}$$

Since anions move slower than the electroosmotic flow, they migrate behind the front of replacement electrolyte, *i.e.*, they first migrate with a lower total velocity under a non-uniform electrical field and then migrate with a constant velocity under a newly-formed uniform electrical field after replacement electrolyte completely occupies the capillary. For less negatively charged anions, they will stay a shorter time in the later uniform electrical field. More negatively charged anions will stay longer in the later electrical field. Neutral molecules will migrate with the electroosmotic flow, *i.e.*, they will stay between the initial and replacement electrolyte. The difference of ionic strength and viscosity between the initial and replacement electrolyte will determine the migration rate of these neutral molecules. Fig. 4 is an example of the effects of a non-uniform electrical field on different species at different K values. Tables I and II give the calculated data of this effect. The ADP peak disappeared at $K = 0.5$.

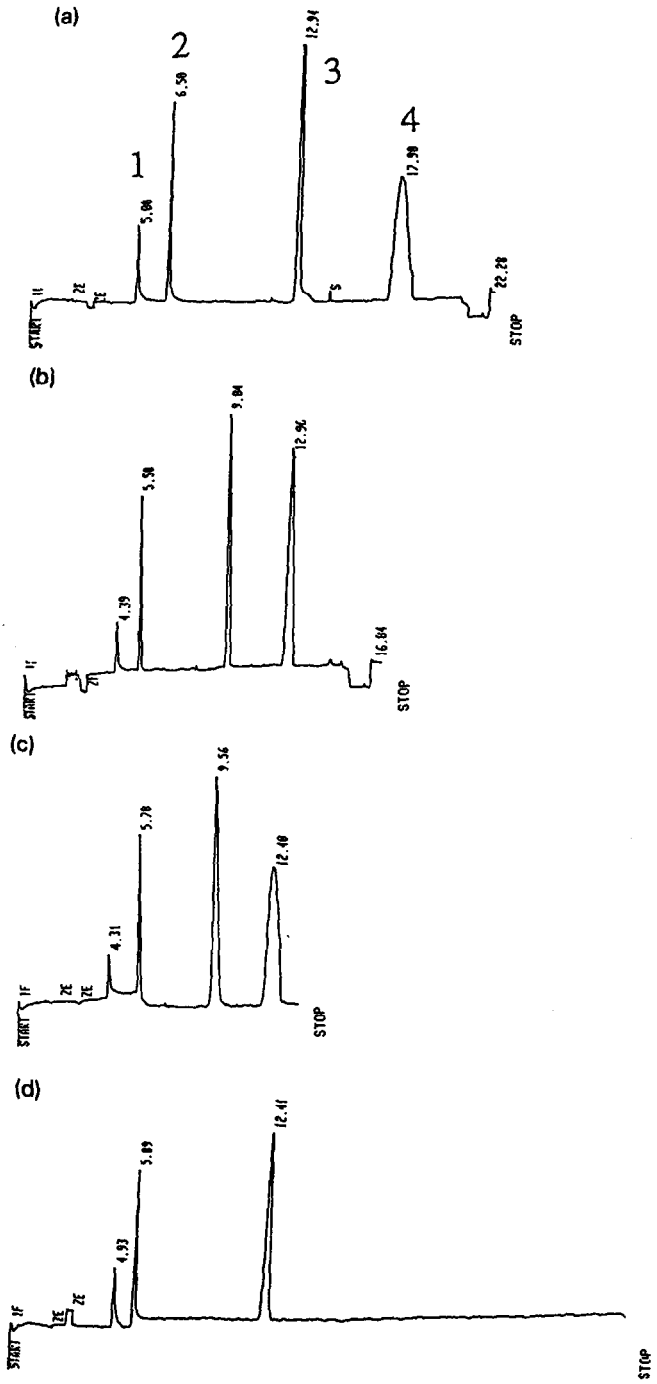

Fig. 4. Effect of non-uniform electrical field on the migration rates of different species. Capillary 75 μm I.D., 66 cm total length, 61 cm to detector. Applied voltage 20 kV. Phosphate buffer, pH 6.8. *Sample*: 1 = pyridoxamine; 2 = adenosine; 3 = AMP. 4 = ADP. (a) K = 1: Initial electrolyte 20 mM, replacement electrolyte 20 mM; (b) K = 1: Initial electrolyte 10 mM, replacement electrolyte 10 mM; (c) K = 2: Initial electrolyte 10 mM, replacement electrolyte 20 mM; (d) K = 0.5: Initial electrolyte 20 mM, replacement electrolyte 10 mM. Numbers at peaks indicate migration times in min.

TABLE I

MEAN OF MIGRATION RATE OF THREE SPECIES UNDER ELECTRICAL FIELD WITH DIFFERENT K VALUES.

Electrophoresis conditions as in Fig. 4. Pyr = pyridoxamine; Ado = adenosine; AMP = adenosine monophosphate.

K	*Migration rate (mm/s)*		
	Pyr	*Ado*	*AMP*
1.0 (20 mM)	0.47	1.54	−0.77
1.0 (10 mM)	0.47	1.83	−0.81
0.5	0.34	1.71	−0.90
2.0	0.59	1.75	−0.69

because ADP molecules have a higher negative charge. After injection the inlet of the capillary was placed in a higher unit resistance replacement electrolyte. When the replacement electrolyte enters the capillary, the ADP band will be in the replacement segment which has a higher electrical field strength. When v(eos) is less than $E\mu$(ADP), ADP will migrate backward and escape detection. As mentioned above, when K is close to 1, the effect will be reduced. At K = 0.98, this influence is insignificant.

Effects of non-uniform electrical field on sample injection

The simplified model of non-uniform electrical field can be used to explain the sampling bias in electrokinetic injection. When the conductivity of the sample solution is different from that of the running electrolyte in capillary zone electrophoresis, a non-uniform electrical field strength is formed along the capillary. This field will change the movement of the sample species in the sample solution. Here we assume that the sample concentration is at least 100 times less than the electrolyte concentration in which the sample is dissolved. Assuming the unit resistance of bulk sample solution is higher than the running electrolyte, *i.e.*, the local non-uniform electrical field of the sample zone which formed during sampling is higher than the uniform electrical field strength at the same applied voltage, the migration rate of both electroosmotic flow and ions will be altered. Thus the cation will enter the capillary inlet

TABLE II

RELATIVE CHANGE OF MIGRATION RATES SHOWN IN TABLE I

The values were obtained by comparison with the mean of these species in uniform electrical field (K = 1, in both of 10 mM and 20 mM conditions)

K	*Relative change of migration rate (%)*		
	Pyr	*Ado*	*AMP*
0.5	−28	<2	+12
2.0	+26	<4	−13

with a higher total velocity than in uniform electrical field strength conditions. Since the injection volume is proportional to the total velocity, more cations will be injected. A previous study discussed this kind of bias[7]. Anions have more complicated behavior. If the difference of electroosmotic mobility and ionic mobility does not suffer significant changes, the amount of anion injected is proportional to the local electrical field strength of the sample plug. In some cases, when non-uniform electrolyte is high enough due to high unit resistance of the sample solution, the product of E and $\mu(i)$ may be equal to or even larger than the electroosmotic flow-rate. Thus, the total velocities of anions will be equal to zero or a negative value. It means that these anions will not enter the capillary inlet during the sampling process, and as a result, these peaks will not appear in the electropherogram.

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