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Connie D. Dunn[a]; Matthew G. Hankins[a]; Kiumars Ghowsi[a]
[a] DEPARTMENT OF CHEMISTRY AND BIOCHEMISTRY, TEXAS TECH UNIVERSITY, LUBBOCK, TEXAS

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# Reverse Direction Anion Capillary Electrophoresis: Theory and Application

CONNIE D. DUNN, MATTHEW G. HANKINS, and KIUMARS GHOWSI*
DEPARTMENT OF CHEMISTRY AND BIOCHEMISTRY
TEXAS TECH UNIVERSITY
LUBBOCK, TEXAS 79409-1061



## ABSTRACT

A new mode for operating capillary electrophoresis for separation of anions without using buffer modifiers has been demonstrated. *Reverse direction anion capillary electrophoresis*, as the new mode is designated, is performed on two anions, nitrate and nitrite, with similar electrophoretic mobilities at various buffer pH values. Since electroosmotic flow increases as buffer pH is increased, it is shown that resolution is poor at low pH and enhanced at neutral to high pH. Model equations are derived for predicting the resolution and number of theoretical plates for reverse direction anion capillary electrophoresis. From these equations, system efficiency ($N$) and resolution are plotted as a function of electroosmotic mobility to illustrate how performance can be improved by an increase in electroosmotic flow.

## INTRODUCTION

A prevalent technique for separation of low molecular weight anions is ion chromatography (1–3). Weak exchange resins are generally utilized. However, ion chromatography of anions can involve sophisticated system requirements such as specialty columns, gradient elution, or specially coupled separation hardware.

In contrast with ion chromatography, facile separation of anions might be achieved by using capillary electrophoresis, but frequently ion analysis

* To whom correspondence should be addressed.

in capillary electrophoresis involves the separation of cations (4, 5). Consequently, electrophoretic systems are arranged such that the detector is near the cathode end of the capillary (Fig. 1a). As a proposed mode of operation that can make capillary electrophoresis useful for detection of anions, the detector is positioned near the anode (Fig. 1b).

Electroosmotic flow moves from the anode to the cathode for untreated capillary surfaces. At pH values greater than 2 or 3 the capillary surface acquires a net negative charge, inducing the formation of a compact positively charged layer on the solution side of the interface. Solution bulk moves toward the cathode as this compact layer of cations is pulled in that direction. In conventional electrophoresis, anions are electrophoretically pulled in the opposite direction to electroosmotic flow, but they may still be detected at the cathode end of the capillary if their electrophoretic velocity is less than their electroosmotic velocity (Fig. 1a). If their electrophoretic velocity is greater than the electroosmotic velocity, then detection of anions is only possible on the anode side (Fig. 1b).

In 1991, Jones and Jandik demonstrated that the separation of anions using capillary electrophoresis could be performed with better efficiency and predictability than published ion chromatography methods (1, 4). Their method incorporated the use of an ion modifier in the buffer to reverse the charge on the capillary wall. Positively charged modifier was used to coat the capillary wall, resulting in formation of a compact layer of anions on the solution side of the interface. With electroosmotic flow reversed and the detector positioned next to the anode, both the electrophoretic and electroosmotic velocity vectors were aligned toward the anode (Fig. 1c).

In the present study, capillary electrophoresis is performed without a modifier, but the detector is placed next to the anode (Fig. 1b). Electrophoretic and electroosmotic velocity vectors are in opposite directions, but since the electrophoretic velocity is greater than the electroosmotic velocity, anions can be detected at the anode side. Separation can be achieved in capillaries shorter than were utilized by Jones and Jandik. Reducing the capillary length, it was surmised, would not effect efficiency or resolution since the effective capillary length is much greater than the substantive capillary length.*

* Since anions are moving in a direction opposite to bulk flow, the effective distance the anion travels is greater than the capillary length. The same scenario happens when a person paddles a canoe upstream. Though a great deal of energy may be expended in paddling, the shoreline distance traveled depends on the downstream velocity. The effective distance the canoe travels is equivalent to the shoreline distance plus the distance the stream travels during the time the canoe is in the water.

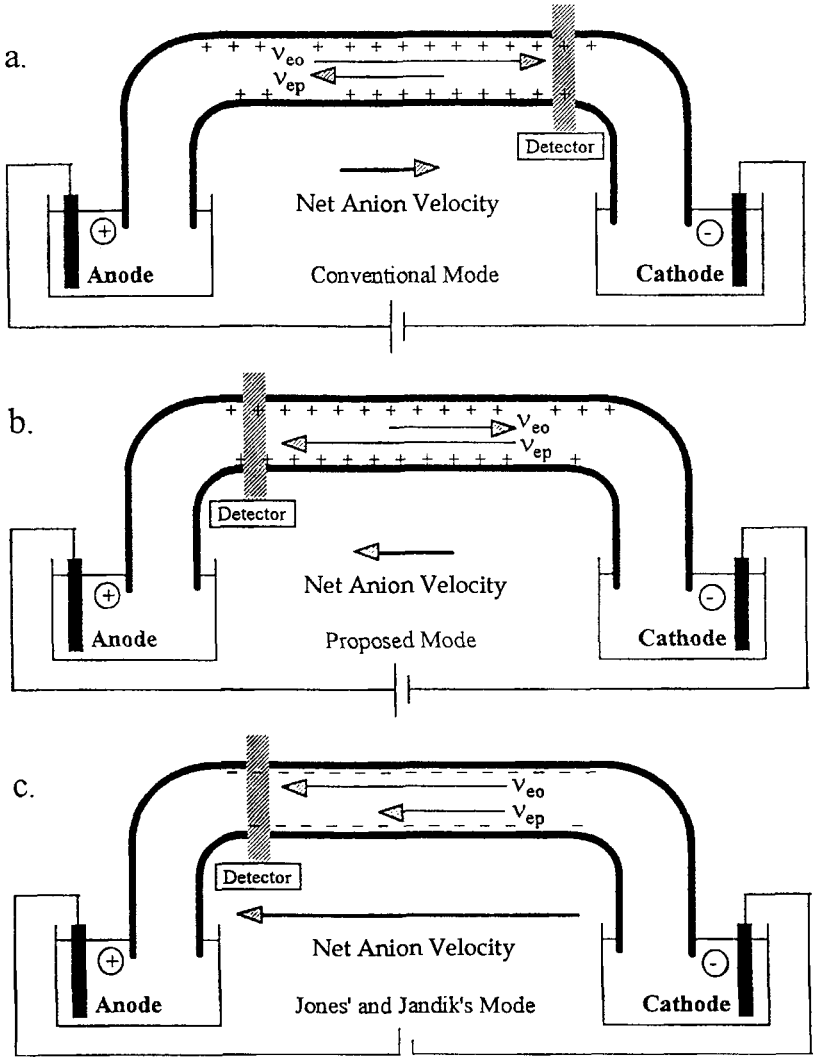


FIG. 1 Electrophoretic configurations for detection of anions. (a) Conventional anion capillary electrophoresis. (b) Reverse direction anion capillary electrophoresis without a modifier to coat the capillary surface. (c) Reverse direction anion capillary electrophoresis using a modifier to coat the capillary surface.

For the proposed mode to be completely useful, there must be a method to control electroosmotic flow so that anions will elute at their most efficient time. Adjusting the buffer pH is presently the easiest way to vary electroosmotic velocity, or an alternative is to use a coating to modify the capillary surface charge (5). Another promising technique for controlling electroosmotic velocity entails changing the capillary surface potential by other than chemical means. This method is predicated on the change caused to electroosmotic flow when a high voltage is applied to the capillary surface (6). Refinements are being made to make such instrumentation available.

Equations derived to describe *reverse direction anion capillary electrophoresis* reveal that electroosmotic flow serves to extend retention times. Thus, to a practical limit, electroosmotic flow increases both efficiency and resolution in the same way that using longer columns will improve efficiency and resolution in chromatography.



For these experiments, capillary electrophoresis was performed on two anions, nitrite and nitrate, having similar electrophoretic mobilities, at various pH values. Conventional equations of chromatography for calculating efficiency, $N$, and resolution, $R$, were used to treat data (7). Efficiency can be calculated from migration time, $t_R$, and the estimated peak width at the baseline, $W$:

$$N = 16\left(\frac{t_R}{W}\right)^2 \tag{1}$$

The equation of resolution makes use of migration time for each species and peak width at the baseline:

$$R = \frac{2[(t_R)_A - (t_R)_B]}{W_A + W_B} \tag{2}$$

In what follows, a theoretical model has been developed for examining the electroosmotic effect on electrophoretic separation. A short discussion concerns the comparison of empirical data and theoretical calculations regarding efficiency and resolution. It is also our purpose to show that reverse direction anion capillary electrophoresis is potentially useful for anion separation.

## EXPERIMENTAL

### Instrumentation

A Bertran power supply (Bertran Associates Inc., Hicksville, New York) running at 30 kV was used for the capillary electrophoresis system.

The current was monitored at the various buffer pH's using an ECG digital multimeter (ECG Multimeter, Taiwan). Fused silica capillaries (61 cm long by 363 μm o.d.) were obtained from Polymicro Technologies Inc. (Phoenex, Arizona). An ISCO $CV^4$ capillary electrophoresis absorbance detector was used. Detection was fixed at 214 nm using the following settings: sensitivity, 0.01; rise, 0.8 second; time constant, 0.36 second. Data were collected with a Linear Scientific recorder (Reno, Nevada) set at 5 V.

### Buffers and Reagents

Phosphate buffers were prepared in the pH range 5.8 to 8.0 with $KH_2PO_4$ and NaOH according to literature (8). $KH_2PO_4$ was purchased from MCB Reagents and NaOH was purchased from Fisher Scientific. Analytes were purchased as sodium salts from Fisher Scientific ($NO_2^-$ and $NO_3^-$) and prepared to 0.0010 M in a buffer matrix.



### System Operation

Samples were injected hydrostatically. The capillary tip was submerged in the sample container, then raised 5 cm above the level of the detector window and held for 30 seconds. Injections were made at the cathode end of the capillary, 30.1 cm from the point of detection. After injection, the capillary tip was wiped clean and placed in buffer solution. The potential (30 kV) was then applied to effectuate the separation. Before and between each injection, the capillary was rinsed for from 2 to 5 minutes with buffer solution at the appropriate pH value. A series of electropherograms were recorded for nitrate and nitrite ions at buffer pH values between 5.8 and 8.0.

## THEORY

Over two decades ago, Giddings (9) displayed that efficiency ($N$) could be related to the change in potential energy experienced by the analyte during separation ($-\Delta\mu^{ext}$) and the thermal energy of the system ($RT$):

$$N = -\Delta\mu^{ext}/2\theta RT \tag{3}$$

The term θ augments the molecular diffusion coefficient and is present when other processes in addition to molecular diffusion contribute to total diffusion. In the present work, we assume only molecular diffusion is occurring and θ is unity. As one observes from Eq. (3), the larger the change in potential energy (or chemical potential change), the more efficient the separation. However, the terms in the denominator are responsi-

ble for diffusion and an increase in these parameters will result in greater band broadening. The potential energy term in Eq. (3) is more fully described by the product of the electric field, $E$, the displacement of the analyte, $X$, and the Faraday constant, $F$:

$$-\Delta\mu^{ext} = FEX \tag{4}$$

## Equations for Efficiency in Absence of Electrophoretic Flow

By substituting Eq. (4) into Eq. (3), an expression for efficiency is derived which reveals the dependence of efficiency on analyte displacement, $X$ (or capillary length). As shown, efficiency improves with increased capillary length:

$$N = \left(\frac{F}{2RT}\right)(EX) \tag{5}$$

The dependence of efficiency on time of separation is seen when a second substitution is made. By definition, the product of the electrophoretic velocity, $\nu_{ep}$, and retention time, $t$, is equivalent to analyte displacement. Furthermore, electrophoretic velocity is related to electrophoretic mobility as expressed here:

$$X = \nu_{ep}t = \mu_{ep}Et \tag{6}$$

Thus, with the suitable substitution, the equation for efficiency can be written as shown below. And as one might expect, efficiency is directly proportional to retention time. It is also evident from the ensuing expression that an improvement in efficiency is effectuated by an increase in electrophoretic mobility.

$$N = \left(\frac{F}{2RT}\right)(\mu_{ep}E^2t) \tag{7}$$

The correlation between plate height and efficiency is well known; that is, theoretical plate height is the ratio of electrophoretic analyte displacement to efficiency:

$$H = X/N \tag{8}$$

Any one of the expressions for efficiency, Eq. (5) for example, can be used to derive equations for plate height by combining with Eq. (2):

$$H = 2RT/FE \tag{9}$$

## Equations for Efficiency That Include Electroosmotic Flow

Equation (5) is valid for the case when only electrophoretic displacement occurs. When both electrophoretic displacement and electroosmotic flow are present, efficiency, $N$, has slightly different dependencies. For electroosmotic flow in the opposite direction to electrophoretic displacement, the effective distance the analyte travels is greater than the capillary length. If the effective length is defined as $X'$, then a simple substitution into Eq. (5) yields an expression for efficiency which includes electroosmotic flow:

$$N = \left(\frac{F}{2RT}\right) EX' \tag{10}$$

By definition, the effective distance the analyte travels under the influence of an electric field, $X'$, divided by the retention time, $t$, is equivalent to the electrophoretic velocity ($\nu_{ep}$):

$$\nu_{ep} = X'/t \tag{11}$$

Now, Eq. (10) can be rewritten to include electrophoretic velocity:

$$N = \left(\frac{F}{2RT}\right)(\nu_{ep}Et) \tag{12}$$

Replacing the electrophoretic velocity variable with the product of electrophoretic mobility and electric field ($\nu_{ep} = \mu_{ep}E$) yields the following expression:

$$N = \left(\frac{F}{2RT}\right)(\mu_{ep}E^2t) \tag{13}$$

One observes that Eqs. (7) and (13) are identical. Whether the system involves electroosmotic flow or not, the exact same dependencies on retention time and electrophoretic mobility are observed. However, one must consider that additional time is required to complete a separation when electroosmotic flow is operating in reverse to electrophoretic displacement.

In order to observe the dependence efficiency has on capillary length and electroosmosis, a substitution for the time variable in Eq. (13) is made. Net displacement of the analyte or capillary length, $X$, is related to the retention time, $t$, as shown:

$$X = t(\nu_{ep} - \nu_{eo}) \tag{14}$$

Rearrangement of Eq. (14) gives Eq. (15):

$$t = \frac{X}{(\nu_{ep} - \nu_{eo})} \tag{15}$$

Substituting Eq. (15) into Eq. (12) yields the ensuing expression:

$$N = \left(\frac{F}{2RT}\right)\left(\frac{\nu_{ep}}{\nu_{ep} - \nu_{eo}}\right)XE \tag{16}$$

By making an additional substitution for electrophoretic velocity, ($\nu = \mu E$), a final equation for efficiency is produced that reveals the dependence the number of theoretical plates has on electroosmotic mobility:

$$N = \left(\frac{F}{2RT}\right)\left(\frac{\mu_{ep}}{\mu_{ep} - \mu_{eo}}\right)XE \tag{17}$$

The curve generated by plotting the efficiency, $N$, as a function of electroosmotic flow is presented in Fig. 2 for a typical range of electroosmotic mobilities. As depicted, electroosmotic flow helps to improve the number of theoretical plates and, according to Eq. (17), if the electroosmotic mobility approaches the electrophoretic mobility ($\mu_{eo} = \mu_{ep}$), the number of theoretical plates will approach infinity. When this happens, the retention time also approaches infinity according to Eq. (15).

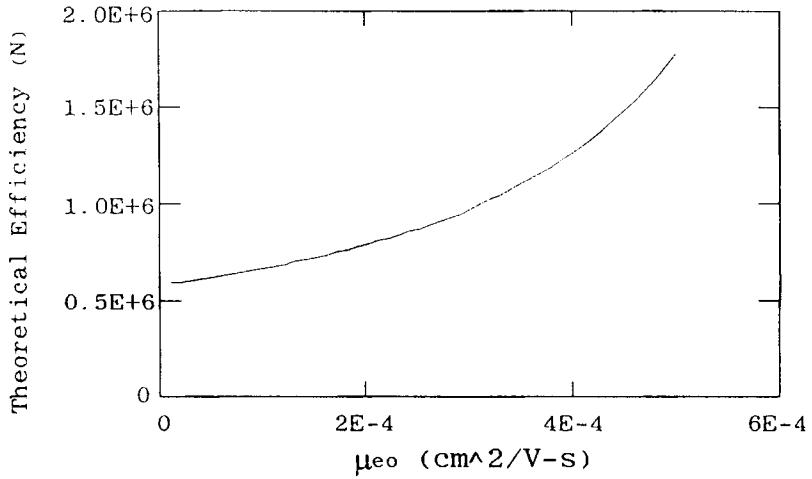


FIG. 2 Theoretical efficiency ($N$) as a function of electroosmotic mobility based on derived Eq. (17). The calculation was made using a typical mobility value for nitrate ion (7.40 $cm^2 \cdot V^{-1} \cdot s^{-1}$), 30 kV, and room temperature (298 K).

Thus, there is some theoretical advantage to developing an electrophoretic system for which $\mu_{ep}$ and $\mu_{eo}$ are nearly identical, but practically speaking, peaks would be short, perhaps unrecognizable if the separation required a long time. Therefore, the goal is to model a system that will produce the maximum number of theoretical plates in the minimum amount of time. The parameter $N/t$ is useful for this purpose.

An expression for $N/t$ can be written by combining Eqs. (5) and (6) or (15) and (16). This same equation is valid for either systems which contain electroosmotic flow or those which do not:

$$\frac{N}{t} = \frac{\nu_{ep} EF}{2RT} \tag{18}$$

And electrophoretic velocity can be replace with electrophoretic mobility ($\nu_{ep} = \mu_{ep}E$) to give the following expression:

$$\frac{N}{t} = \frac{\mu_{ep} E^2 F}{2RT} \tag{19}$$

As Eq. (19) reveals, the rate of generation of theoretical plates does not vary with electroosmotic mobility, $\mu_{eo}$, but it is proportional to the electrophoretic mobility, $\mu_{ep}$, and to the square of the electric field. The arguments presented above serve as the theoretical basis for the statement: The only fundamental effect on efficiency caused by the occurrence of electroosmosis in the proposed mode of electrophoretic operation is by lengthening retention times and thereby increasing efficiency. But there should be no actual improvement or deterioration to the rate at which theoretical plates are generated ($N/t$) when electroosmotic flow is operating in reverse to electrophoretic displacement.

In a like manner, equations for plate height are identical whether electroosmosis is in operation or not. Plate height is the ratio of effective length ($X'$) to efficiency ($N$) when electroosmosis is present:

$$H = X'/N \tag{20}$$

Substituting Eq. (10) into Eq. (20) yields an expression for plate height matching the one derived when electroosmosis is absent.

$$H = 2RT/FE \tag{21}$$

This interesting result shows that theoretical plate height is independent of electroosmotic flow when it is based on the effective distance the analyte travels rather than the capillary length. Instead, plate height has a simple inverse relation with the electric field strength.

## Equations for Resolution That Include Electroosmotic Flow

Resolution is the quantitative measure of ability to separate two analytes. For two adjacent peaks with similar elution times, peak base width should be nearly identical:

$$W_A \approx W_B \tag{22}$$

Assuming Eq. (22) is true, resolution for species A and B is expressed in terms of their retention times and the peak base width for either species (10):

$$R_S = \frac{(t_R)_B - (t_R)_A}{W_B} \tag{23}$$

The conventional expression for separation efficiency can be written with parameters related to either species A or B, shown here using the retention time and peak base width for species B:

$$N = 16\left(\frac{(t_R)_B}{W_B}\right)^2 \tag{24}$$

By combining Eqs. (23) and (24), an equation of chromatography is produced that expresses resolution in terms of efficiency and retention times (10):

$$R_S = \left(\frac{\sqrt{N}}{4}\right)\left(\frac{(t_R)_B - (t_R)_A}{(t_R)_B}\right) \tag{25}$$

The retention time variable, $t_R$, and the efficiency term, $N$, are eliminated by inserting Eqs. (15) and (16) into Eq. (25):

$$R_S = \left(\left(\frac{F}{32RT}\right)\left(\frac{(\nu_{ep})_B}{(\nu_{ep})_B - \nu_{eo}}\right) XE\right)^{1/2} \left(\frac{(\nu_{ep})_A - (\nu_{ep})_B}{(\nu_{ep})_A - \nu_{eo}}\right) \tag{26}$$

In the derivation of Eq. (25), the assumption was made that peak base widths of species A and B are nearly identical. The small error that results from this assumption can be partially corrected by making another assumption. In Eq. (25), the electrophoretic velocity of either species can be replaced by the average electrophoretic velocity of both species:

$$(\nu_{ep})_{AB} = \frac{(\nu_{ep})_A + (\nu_{ep})_B}{2} \tag{27}$$

This substitution is made into Eq. (26) for each place the variable, $(\nu_{ep})_A$

or $(\nu_{ep})_B$, appears, except in the numerator of the right term:

$$R_S = \left(\left(\frac{F}{32RT}\right)\left(\frac{(\nu_{ep})_{AB}}{(\nu_{ep})_{AB} - \nu_{eo}}\right) XE\right)^{1/2} \left(\frac{(\nu_{ep})_A - (\nu_{ep})_B}{(\nu_{ep})_{AB} - \nu_{eo}}\right) \quad (28)$$

As a good approximation, one may assume the majority of the voltage drop for capillary electrophoresis is between the point of injection and the detector. When electrophoretic velocity, $\nu_{ep}$, is replaced with $\mu_{ep}E$, and applied potential, $V$, is substituted for $XE$, one obtains the following:

$$R_S = \left(\frac{FV(\mu_{ep})_{AB}}{32RT}\right)^{1/2} \left(\frac{(\mu_{ep})_A - (\mu_{ep})_B}{((\mu_{ep})_{AB} - \mu_{eo})^{3/2}}\right) \quad (29)$$

One test to the validity of Eq. (29) is to considers how the equation is transformed in the absence of electroosmotic flow ($\mu_{eo} = 0$). The result is identical to the expression that is derived for a system with only electrophoretic displacement:

$$R_S = \left(\frac{FV}{32RT}\right)^{1/2} \left(\frac{(\mu_{ep})_A - (\mu_{ep})_B}{(\mu_{ep})_{AB}}\right) \quad (30)$$

According to Eq. (29) improved resolution is predicted for higher electroosmotic mobility values. For ease of visualization, the theoretical resolution has been plotted as a function of electroosmotic flow (Fig. 3).

## RESULTS AND DISCUSSION

Ideally, between 500,000 and 1,500,000 theoretical plates (shown in Fig. 2) can be achieved with electrophoresis operating within a normal electroosmotic flow range. As usual, experimentally calculated efficiencies are much less than theory predicts (Fig. 4). Efficiency hovers around 3500 plates at buffer pH values between 6.5 and 8.0. Of course, much higher efficiencies have been achieved with more costly electrophoretic equipment (11). The plateau-shaped curve results from the sample injection method, not the mode of electrophoretic operation. For hydrostatically injected samples, the initial sample zone width is the main contributor to the bandwidth from which the efficiency is calculated. Depositing sample on a capillary column is similar to sucking water into a thin straw from a large reservoir. Obtaining a thin and reproducible initial bandwidth requires precise timing and pressure regulation. Thus, normal diffusion processes contribute only slightly to band broadening. In this study, the final

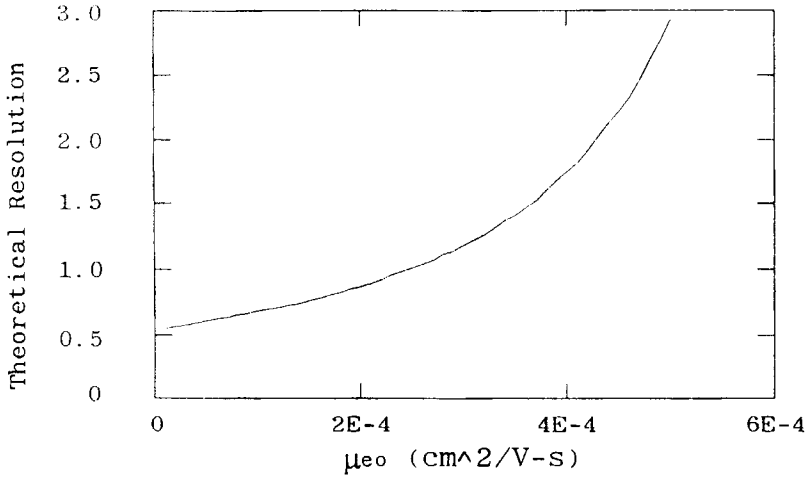


FIG. 3 Theoretical electrophoretic resolution for nitrate and nitrite ion as a function of electroosmotic flow based on derived Eq. (29). The calculation was made using typical mobility values at infinite dilution for nitrite (7.44 $cm^2 \cdot V^{-1} \cdot s^{-1}$) and nitrate (7.40 $cm^2 \cdot V^{-1} \cdot s^{-1}$) ions, 30 kV, and room temperature (298 K).



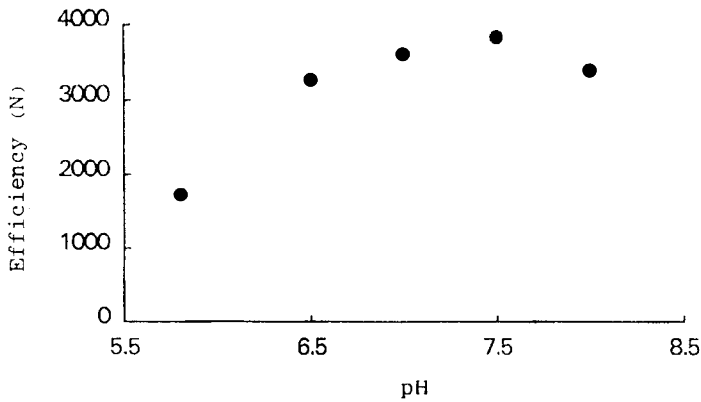


FIG. 4 Electrophoretic efficiency, $N$, of proposed method calculated from Eq. (1) for a series of separations performed at various pH values. The efficiency is based on retention times and peak base widths for the nitrate ion. The electrophoretic system setup included hydrostatic injection, UV detection, and a potential drop of 30 kV.

bandwidth is large because a small sample volume initially occupies a relatively large capillary length.

On the other hand, Eq. (17) predicts a large number of theoretical plates based on the assumption that the initial bandwidth is insignificant compared to normal diffusion processes. Although our equations do not make accurate practical predictions, basic trends can be predicted from theory, and since there is a general increase in efficiency going from low to high pH as shown by Fig. 4, theory and practice do agree somewhat.

In Fig. 5, resolution of nitrate and nitrite ions is plotted against pH. At low pH, since electroosmotic flow is minimal, the sample is quickly eluted from the capillary, hence, the retention time is too short for adequate separation. As the pH is increased, the electroosmotic velocity increases. At pH 7, the sample resides in the column for enough time to permit complete separation. The curve produced from data takes on a linear shape, but the important point is that resolution consistently increases going from low to high pH as is predicted by Eq. (29) (Fig. 6). Within typical electroosmotic velocities the theoretical resolution is between 0.5 and 2.0, not too much different from values achieved experimentally. Theoretical equations of resolution are not affected as much by the initial zone width as are equations of efficiency. In fact, an improved injection system might easily show good agreement between theoretical values based on anion mobilities and practice so that valid predictions could be made.



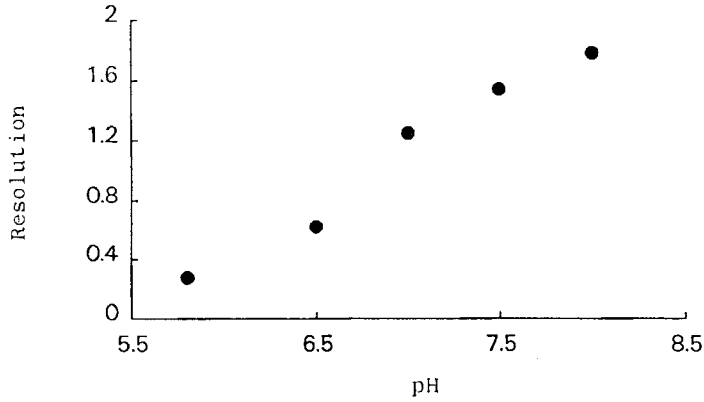


FIG. 5 Resolution of nitrate and nitrite ions for the proposed mode of electrophoretic operation calculated from Eq. (2) for a series of separations performed at various pH values. The electrophoretic system setup included hydrostatic injection, UV detection, and a potential drop of 30 kV.

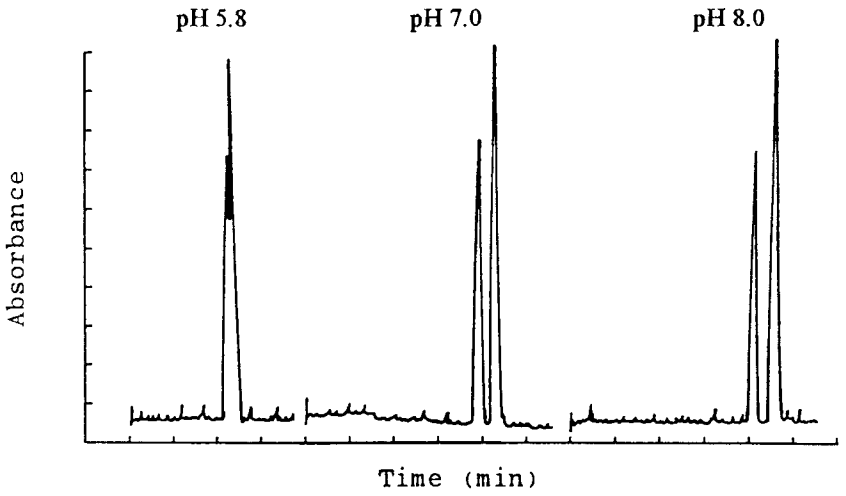


FIG. 6 Reverse direction anion capillary electropherograms depicting improved resolution of nitrate and nitrite anions as the pH is increased.



## CONCLUSIONS

Making use of uncomplicated hardware, adequate separation of anions has been demonstrated by utilizing a new mode of electrophoresis base on the principle of sample detection at the anode end of the capillary, but without the use of a buffer modifier to reverse electroosmotic flow. Furthermore, reasonable derivations for theoretical efficiency and resolution have been made which include the effect electroosmosis has on *reversed direction anion capillary electrophoresis*. These same equations are valid for conventional electrophoresis (when signs are modified slightly) and may serve as a basis for developing model equations that include other band-broadening effects such as nonmolecular diffusion and initial zone width. Finally, the electropherograms produced from this method compare favorably with theory.

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