Published in final edited form as:

Electrophoresis. 2011 June ; 32(12): 1492–1499. doi:10.1002/elps.201000451.

Investigating the Effects of Conductivity on Zone Overlap with EMMA: Computer Simulation and Experiment

John W. Stahl¹, Adam D. Catherman², Ranasinghe K. Sampath², Aravinda C. Seneviratne², and Timothy G. Strein^{2,*}

¹Department of Chemistry, Geneva College, Beaver Falls, PA 15010

²Department of Chemistry, Bucknell University, Lewisburg, PA 17837

Abstract

In this paper we demonstrate, using both experiment and simulation, how sample zone conductivity can affect plug-plug mixing in small molecule applications of electrophoretically mediated microanalysis (EMMA). The effectiveness of in-line mixing, which is driven by potential, can vary widely with experimental conditions. Using two small molecule systems, the effects of local conductivity differences between analyte plugs, reagent plugs and the background electrolyte on EMMA analyses are examined. Simul 5.0, a dynamic simulation program for capillary electrophoresis (CE) systems, is used to understand the ionic boundaries and profiles that give rise to the experimentally obtained data for EMMA analyses for (i) creatinine determination via the Jaffe reaction, a reaction involving a neutral and an anion, and (ii) the redox reaction between gallate and dichloroindophenol (DCIP), two anions. Low sample conductivity, which is widely used in CE analyses, can be detrimental for in-line reactions involving a neutral reactant, as rapid migration of the ionic component across a low conductivity neutral zone results in poor reagent plug overlap, and low reaction efficiency. Conversely, with two similarly charged reagents, a low conductivity sample plug is advantageous, as it allows field-amplified stacking of the reagents into a tight reaction zone. In addition, the complexity of simultaneously overlapping three reagent zones is considered, and experimental results validate the predictions made by the simulation. The simulations, however, do not appear to predict all of the observed experimental behavior. Overall, by combining experiment with simulation, an enhanced appreciation for the local field effects in EMMA is realized, and general guidelines for an advantageous sample matrix can be established for categories of EMMA analyses.

Keywords

Capillary Electrophoresis; Conductivity; Electrokinetic; Simulation; Stacking

1 Introduction

Capillary electrophoresis (CE) has been widely developed as an analytical tool, and is used to best advantage for determinations requiring small sample size and relatively rapid analysis. In situations where the analyte must be reacted or derivatized to be detectable, or where the analyte is itself a catalyst such as an enzyme, considerable enhancement in sensitivity can be achieved by carrying out a chemical reaction involving the analyte before

Corresponding Author: Timothy G. Strein, Professor of Chemistry Department of Chemistry Bucknell University Lewisburg, PA 17837. * Author to whom correspondence should be addressed: strein@bucknell.edu FAX (570) 577-1739 .

The authors have no financial/commercial conflicts of interest.

CE separation and determination. Electrophoretically mediated microanalysis (EMMA), first introduced by Bao and Regnier has been used widely to carry out chemical and biochemical reactions on the nanoliter scale directly in the capillary itself [1, 2]. A recent review [3] presents the various modes of EMMA as well as a wide array of systems to which EMMA has been applied. In the plug-plug mode of EMMA[2], separate zones of analyte solution and reagent solution are injected into a CE capillary and voltage is applied to cause mixing of these species to initiate reaction. In this paper, we report new insights into the factors influencing optimal plug-plug mixing in small molecule applications of EMMA.

The original mathematical treatments used to predict zone overlap [1, 4] were applied to enzyme-catalyzed reactions where the plugs of analyte and reactants were dissolved in background electrolyte buffer to maintain enzyme activity. In these situations, the electric field across injected plugs is the same as the average electric field in the entire capillary. The resulting equations are well-known and are summarized below. The optimal time (t_{ol}) for the application of the overlap potential (E) is predicted from equations (1), (2) and (3).

$$v_{diff} = \Delta\mu_{ep} \cdot E \quad 1$$

where v_{diff} (cm/s) is the difference in the zone velocities of the reagent/analyte species, $\Delta\mu_{ep}$ (cm²/V·s) is the difference in the reactant/analyte electrophoretic mobilities, and E (V/cm) is the electric field experienced by the migrating species. If discrete reagent zones are loaded immediately adjacent to one another and the effects of zone broadening are negligible, the time required for two discrete zones to fully overlap, t_{ol} , is given by

$$t_{ol} = \frac{L_i}{v_{diff}} \quad 2$$

where L_i is the plug length. For a hydrodynamic injection the plug length is given by

$$L_i = \frac{\Delta P \cdot d \cdot t_i}{32 \cdot \eta \cdot L_t} \quad 3$$

where ΔP (g/cm·s²) is the injection pressure, d (cm) is the inner diameter of the capillary, t_i (s) is the time of injection, η (g/cm·s) is the viscosity of the solution being injected and L_t (cm) is the total length of the capillary being used.

Equation (1) is often applied with the implicit assumption of constant electric field (E) throughout the capillary. That is to say that the average field, E , is equal to the applied voltage, V , divided by the total capillary length. However, in the case where either or both of the reagent zones have significantly different conductivities compared to the background electrolyte, a non-uniform electric field will result. In such a case, the local electric field in a reagent zone will be very different from the average over the entire capillary. Thus zone velocities during the electromediated migration segment of the experiment are quite different than those during a CZE separation and overlap times predicted from the uniform field assumption will be in error.

The effects of local electric fields for field-focusing and pre-concentration have been widely recognized and oft-exploited in the literature of CE and EMMA [5]. However, such an approach is not always advantageous, and can be detrimental with EMMA. Moving electrophoretic zones adjust their concentrations in accordance with the Kohlraush regulating function, and as the system develops, zones can become concentrated (stacked) or diluted. In addition, moving zone boundaries, which can be sharp or diffuse, develop and as they move through the system, can affect the migration dynamics of other ions within the

local environment of the boundary. Accurate prediction of the transient behavior of ionic zones and the evolution of the ionic migration dynamics is, at best, difficult.

Here we present both experimental and simulated results illustrating the effects of local fields on mixing in EMMA and seek to draw systematic general conclusions. We make use of Simul 5.0, a dynamic electrophoresis simulation program to visualize the reactant migration velocities and zone shapes for two different model chemical systems. By providing a numerical solutions to the set of non-linear differential equations that account for ionic movement by both migration and diffusion, while also accounting for the requirement of electro-neutrality, acid/base chemistry of all solution species, Simul provides detailed insight into the dynamics of voltage-driven mixing [6].

2 Materials and Methods

2.1 Chemicals

Creatinine and gallic acid were purchased from Sigma Chemical Company (St. Louis, MO, USA). Picric Acid (1.2% w/v aqueous solution) was purchased from VWR Scientific (West Chester, PA, USA). Sodium 2,6-dichloroindophenol was purchased from Aldrich Chemical Company (Milwaukee, WI, USA). Buffer components were reagent grade from standard sources.

Solutions were prepared using 18 M Ω -cm water. Buffer solutions were prepared by dissolving the buffering acid-form (boric acid or monobasic sodium phosphate) in water and adjusting to the desired pH with 1 M NaOH. Alkaline sodium picrate solution was prepared by mixing 1 volume of 1.0 M NaOH solution with 9 volumes of the commercial 1.2% w/v picric acid solution, thus yielding a solution which was 47 mM in sodium picrate and 53 mM in excess sodium hydroxide. All solutions were filtered through 0.45 μ m PTFE syringe filters (JSI Scientific, Freehold, NJ, USA) directly into 1 mL polypropylene vials (Agilent Technologies, Palo Alto, CA, USA). Vials were pre-rinsed with a small portion of the solution before filling.

2.2 Instrumentation

Capillary electrophoresis experiments were conducted with an Agilent 3D CE instrument (Palo Alto, CA, USA) with photodiode array UV/vis detection. Agilent 3D-CE Chemstation software was used for all analyses. Fused silica capillaries (Polymicro, Phoenix, AZ, USA) with inner and outer diameters of 50 μ m and 360 μ m respectively were prepared with total lengths of either 33.0 or 35.0 cm and detection windows 8.5 cm from the outlet. New capillaries were conditioned by flushing with 1.0 M NaOH and ultrapure distilled water, each for 3 min at 950 mbar. Prior to injection, capillaries were flushed with BGE solution for 3 min. The capillary was maintained at 25.0 $^{\circ}$ C for all experiments. All experiments were carried out in triplicate.

2.3 EMMA Experiment Procedures

2.3.1 Creatinine / picrate system—A BGE of 100.0 mM sodium borate buffer at pH 9.8 was used in a 35.0 cm capillary column. The initial hydrodynamic injection sequence (used in Figures 1, 2, and 3) was as follows: (i) a 150 mbar-s plug of 47 mM sodium picrate with 53 mM NaOH was injected, (ii) a 150 mbar-s plug of 10 mM creatinine solution (in 18 M Ω -cm water or in 125 mM NaCl solution as discussed below), (iii) a 150 mbar-s plug of the BGE. The modified EMMA procedure (Figure 6) included an initial 50 mbar-s injection of 300 mM NaOH prior to the injection sequence described above. An overlap voltage of 10.0 kV was applied for a specified time. Then the electrophoretically mixed reagents were allowed to react during a zero potential “wait time” of 6.0 minutes. Finally, a separation

voltage of 15.0 kV was applied to electrophoretically separate the product and unreacted reagents. The Jaffe product was specifically detected at 485 nm.

2.3.2 Gallic Acid / 2,6-dichloroindophenol system—A BGE of 50.0 mM sodium phosphate buffer at pH 7.2 was used in a 33.0 cm capillary column. The hydrodynamic sequence of injections was as follows. (i) 150 mbar-s plug of 1.0 mM gallic acid solution (in either water or 125 mM NaCl), (ii) 150 mbar-s plug of 1.0 mM sodium 2,6-dichloroindophenol solution (in water or 125 mM NaCl), (iii) 150 mbar-s plug of the BGE. An overlap voltage of 10.0 kV was applied for a specified time. Then the electrophoretically mixed reagents were allowed to react for a “wait time” of 6.0 minutes. Finally, a separation voltage of 10.0 kV was applied to electrophoretically separate the product and unreacted reagents. The product was detected at 210 nm.

2.4 Computer Simulations

All simulations of electrophoretically-induced mixing were carried out using Simul 5.0 by Gas, et al. [6]. Simulations were carried out by actively modeling a 25 mm length of column with 2000 nodes (calculational segments) while accounting for the balance of the capillary length with a buffer-filled “fake” length of 325 mm so that the total capillary length in both simulation and experiment were the same. That is, use of the additional “fake” length results in a voltage drop across the active portion of the simulated column that is appropriately proportioned with respect to the total applied voltage. Because Simul uses an iterative numerical approach to solve non-linear differential equations, sharp boundaries can cause calculational instabilities and unrealistic numerical oscillations at the boundary. To avoid this phenomenon, lower voltages, lower time steps or an increased number of calculational nodes must be employed. In our simulation work, the applied overlap voltages programmed in Simul were reduced to one-hundredth scale (100 V) to maintain stability of the calculation, and the simulated timeframes were thus 100-fold higher than the corresponding experiment. This approach likely results in the simulated data showing more diffusional broadening than would be present in the experimental data. However, in the experiment the zones can diffuse during the zero potential reaction time and are also broadened during the pressure-driven injection sequence. While this approach is not perfect, reducing the voltage field by 1 or 2 orders of magnitude allows for what appear to be very accurate simulations of these complicated systems to be completed in a reasonable amount of time. In this paper, overlap times obtained from the Simul program have therefore been multiplied by 0.01 so that they correspond to the experimental overlap times. Within the active region of the simulated column, the initial zones of reagents were established with a width of 3.5 mm and a peak edge width of 0.6 mm in order to closely match the experimental plug widths described above. Simul 5.0 provides mobility and pKa data for all ions considered in these experiments except for two. Boric acid was modeled as a monoprotic acid having pKa of 9.22 and an anionic mobility of $50 \times 10^{-5} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$. 2,6-Dichloroindophenol was modeled as a monoprotic acid having pKa of 5.8 and anionic mobility of $20.2 \times 10^{-5} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$.

3 Results and Discussion

We have found that differences in conductivity between EMMA reactant zones and the BGE can dramatically affect the conditions for optimum reagent overlap and product formation. The original method to predict optimal overlap conditions based upon equations 1-3 assumes a uniform voltage field in all regions of the capillary, and when reagent plugs differ in conductivity from the BGE, the local voltage field will cause drastic differences in reactant overlap and subsequent product formation. We illustrate here the factors influencing local fields with two model systems.

3.1 Creatinine / picrate system

The clinically important reaction between creatinine and alkaline picrate, also known as the Jaffe reaction has been the focus of previous and on-going work in our laboratories [7, 8, 9]. In the pH range of our experiments (pH 8-12), creatinine is neutral and picrate is an anion, and therefore in-line mixing of the reactants can be accomplished with an EMMA approach. The use of the EMMA methodology to perform this reaction is attractive because both the consumption of reagents and generation of waste can be minimized, and also because analysis times can be reduced with this approach. Regardless of these practical advantages, this chemical reaction serves as interesting model system with which to study reagent overlap dynamics.

It is commonplace in CE to use analyte solutions that have lower ionic strength than the background electrolyte as this arrangement sets up a field focusing effect, an approach that is referred to as field-amplified or intrinsic stacking. This stacking concentrates the charged analyte into a narrow zone and correspondingly higher peak efficiencies are obtained [10]. However, when low ionic strength solutions of creatinine (a neutral reactant) are used to perform the EMMA-based Jaffe analysis, low product peak areas are obtained, as shown in Figure 1A. The classic uniform field theory would predict that applying a potential of 10 kV for a period of 3.7 sec would result in complete overlap of the creatinine and picrate plugs. That is, after 3.7 sec of overlap time, the reactants should be well-mixed and the subsequent zero-potential reaction should produce the maximal amount of product. Yet, the experimentally determined optimum overlap time in Figure 1A is much shorter, being about 1.0 second. Modifying the creatinine matrix by the addition of 125 mM sodium chloride (Figure 1B) was found to yield an optimum overlap time of about 3 seconds and also nearly double the yield of Jaffe product, as indicated by the larger peak area.

In an attempt to gain deeper understanding of the dynamic effects, both temporal and spatial, computer simulations of the in-line electrophoretic mixing event for the Jaffe chemistry were performed with Simul 5.0. Shown in Figures 2 and 3 are the simulation results for the creatinine/picrate overlap at various times with and without the addition of sodium chloride to the creatinine zone. It is clear from the simulations that conceptualizing zone overlap in the linear manner assumed in the classic uniform field equations greatly oversimplifies reality. An over-arching factor apparent in the simulation data in Figures 2 and 3 is that the rate of instantaneous migration of any ion can be predicted by the local field experienced by that ion; the local field is governed by the conductivity of the solution in the immediate region surrounding the ion. In a conducting electrolyte, the current density (i/A) at any point is given by: $i/A = \kappa E$, where κ is the total conductivity of all electrolytes present at a given point, and E is the electric field at that point. In the one-dimensional situation of a capillary, the current must be equal at all points, and cross-sectional area is a constant, so that conductivity and local field are inversely proportional. Therefore, the conductivity displayed in simulation figures provides information about the local fields prevailing within various zones during the overlap step in the EMMA experiment.

Figure 2 depicts the simulated zone profiles that develop for the situation of neutral creatinine dissolved in water; this zone has very low conductivity. Consequently the local electric field within the injected creatinine zone is extremely large. Both hydroxide and picrate anions migrate very rapidly across this zone, with hydroxide migrating faster due to its much larger mobility value. Neither anion accumulates to any large extent in the creatinine zone, which is the cause of the overall poor reaction yield. In Figure 2B, the simulation depicts the situation at the experimentally determined overlap time of 1.0 second, which gave rise to the maximum amount of product for this system; while some overlap of picrate and creatinine zones occurs at the edges of the watery creatinine zone, the zones are clearly not well mixed. The experimental decline in Jaffe product peak area at overlap times

greater than 1.0 second in Figure 1A can be understood as due to both this “zipping” of picrate across the watery creatinine zone and the concurrent rapid loss of hydroxide from the reaction zone. Figure 2C depicts this situation when both reagents have migrated well past the creatinine zone.

In Figure 3, the simulation zone profile results are shown for the Jaffe EMMA experiment with creatinine dissolved in 125 mM sodium chloride. The NaCl in the creatinine zone creates a high conductivity zone with a low local electric field. Therefore, both hydroxide and picrate slow down upon entering the creatinine zone, yielding better spatial overlap and better reaction yield. Since the Jaffe chemical reaction requires all three species [11, 12], the experimentally observed optimum overlap time of 3.0 sec represents a compromise between picrate entrance into the creatinine zone and hydroxide exit from the creatinine zone. We have considered a multizone injection method to improve the overlap of all three reagent (vide infra, Figure 6). Regardless, it is clear that when performing an EMMA analysis involving a reaction between an anion and a neutral, it is advantageous for the zone in which the neutral reactant resides to have conductivity equal to or higher than both the anion reagent zone and the BGE.

To experimentally validate the bimodal picrate zone behavior predicted by simulation with a low conductivity creatinine sample as depicted in the simulation shown in Figure 2B, the following experiment was performed. A picrate plug and a low conductivity plug (1 mM NaCl) were sequentially introduced onto the short end of the capillary, an overlap potential of -10 kV was applied for varying amounts of time, and all zones were then immediately pushed past the detector with a pressure of -20 mbar, while the absorbance at 210 nm was monitored. The watery neutral reactant zone did not contain creatinine, analogous to a blank sample, so only picrate was detected. The raw data, shown in Figure 4, confirm the rapid anion migration behavior predicted by the simulations. An additional simulation of this blank experiment is shown in Figure 5 with good agreement to the experiment. It should be noted that some laminar dispersion and also diffusion is no doubt occurring during the experiment with pressure-driven mobilization of the zones, but this does not mask the bimodal distribution.

The insights gained from simulations can lead to rational design of a more complicated injection sequence for three reactants of the Jaffe chemistry that will result in a better overlap of all three reactant zones. In this direction, an overlap simulation that includes an additional small plug of concentrated NaOH prior to the picrate plug is shown in Figure 6. The simulation predicts that significantly better overlap of the picrate and hydroxide anions can be achieved within the hi-conductivity zone of neutral creatinine, compared to that achieved in Figure 3C. Experimental data indicated that the optimum overlap time with this additional NaOH plug is 4.6 seconds. At this overlap time, the experimental peak area of the Jaffe reaction product for 11 mM creatinine was found to be 630 mAU-mm without an extra hydroxide plug, but increased to 840 mAU-mm with a 50 mbar-sec plug of 300 mM NaOH, an increase of 33% which validates the prediction of the simulation. However, due to the complex kinetics of the formation and degradation of the Jaffe reaction product, further increases in the size of the extra hydroxide zone, or placement of the hydroxide zone further up field from the picrate zone, do not uniformly result in further improvements. These conditions are the subject of further study. However, it is clear that Simul can be very helpful in designing rational improvements in experimental work with EMMA systems.

3.2 Gallic Acid / 2,6-dichloroindophenol system

When each reactant in the EMMA system carries a charge, the reactant overlap dynamics are quite different from those seen in the prior case where one reactant was neutral, and a correspondingly different pattern of optimization behavior is found. This effect is illustrated

with the redox reaction between the anions of gallic acid and 2,6-dichloroindophenol (DCIP). Gallic acid is a model antioxidant and DCIP is an oxidizing agent that has been used in our laboratories for determination of antioxidant capacity in foods [13]. In the pH range near 7, both of these molecules are (-1) anions. Figure 7 depicts experimental results when comparable EMMA experiments are performed with the two reagents placed in water versus dissolved in 60 mM sodium chloride solution. The classic uniform field equations 1-3 can be used to predict an optimum overlap time of about 24 seconds for these conditions. As can be seen in Figure 7A, the optimum time is 5 seconds or less in water with a drastic loss in product peak area at longer times. Figure 7B demonstrates that in salt solution, the product peak area is a weak function of overlap time with a broad maximum at about 10 seconds. Also, in this case, dissolving the reactants in water results in a much higher yield of product than when the reactants are dissolved in salt solution, the reverse of the trend in the Jaffe reaction.

Once again, simulation provides key insights into this behavior, as depicted in Figures 8 and 9 which compare simulated zone dynamics for anionic analytes dissolved in water and salt solution, respectively. When both reactants are anions, they migrate rapidly through the high-field watery zone, accumulating in a narrow zone of high concentration at the zone edge via field-amplified stacking as shown in Figure 8B. Using the simulation after-the-fact, it could be determined that the short overlap time of 0.40 seconds provided the best spatial overlap for these conditions, consistent with the experimental result that the shortest time was best. When the reactants are dissolved in salt as depicted in Figure 9, the local field is approximately the same as in the BGE, and both anions migrate slowly. Gallate has a larger mobility value than DCIP, but requires a time of at approximately 10 seconds to overtake the DCIP zone, as depicted in Figure 9C. In this more uniform field situation, the two reactants have lower concentrations in the reaction zone and therefore react slower, resulting in less product formed within the fixed 6.0 minute wait time of the experiment.

4 Concluding Remarks

Overall, with the aid of Simul, the complex dynamics of reagent zone overlap in EMMA with small molecules in zones of non-BGE solution can be visualized and understood. While each individual EMMA system exhibits a complicated set of dynamic ionic migrational processes, some very useful general trends in migration behavior with local conductivity can be drawn. Often with EMMA the local electric field in the reactant zone(s) is vastly different from the average electric field across the entire column. When one reactant is a neutral species, the addition of an inert salt to the neutral zone can boost reaction yield by slowing the migration velocity of charged reactants and thus maximizing local concentration of the ionic reactant within the reaction zone defined by the neutral reactant. However, the addition of salt to EMMA reagent samples will not uniformly boost performance. Indeed, when both reactants carry charge, adding salt can be detrimental. In fact, with anion/anion EMMA systems, it is preferable to have reagent zones with lower conductivity than the BGE, as electrophoretic stacking of the reagents is possible, and stacking results in higher local concentrations, increased reaction rates to form the reaction product, and a narrower CE peak for that product. By understanding the local conductivities and voltage fields within individual reagent zones, and how these parameters effect the migration of EMMA reactants, in-line reactions schemes can be more appropriately designed and implemented. It is also obvious that in developing EMMA-CE schemes for real-life samples, knowledge of the conductivity of the sample matrix is essential to design of the method.

Acknowledgments

The authors wish to thank Bohuslav Gaš and Vlastik Hruska for sharing the Simul program and for their expert help with using it correctly. We also thank Bill Napoli for performing simulations that guided parts of this work. This work was supported by NIH grant number R15EB003854 from the National Institute of Biomedical Imaging and Bioengineering. The content is solely the responsibility of the authors and does not represent the views of the National Institute of Biomedical Imaging And Bioengineering of the National Institutes of Health.

5 References

1. Bao J, Regnier FE. J. Chromatography A. 1992; 608:217–224.
2. Avila LZ, Whitesides GM. J. Org. Chem. 1993; 58:5508–5512.
3. Zhang J, Hoogmartens J, Van Schepdael A. Electrophoresis. 2008; 29:56–65. [PubMed: 18040992]
4. Harmon BJ, Patterson DH, Regnier FE. Anal. Chem. 1993; 65:2655–2662. [PubMed: 8238944]
5. Ptolemy AS, Britz-McKibbin P. J. Chromatography A. 2006; 1106:7–18.
6. Hruška V, Jaroš M, Gaš B. Electrophoresis. 2006; 27:984–991. [PubMed: 16523464]
7. Kochansky CJ, Koziol S, Strein TG. Electrophoresis. 2001; 22:2518–2525. [PubMed: 11519956]
8. Sanders BD, Slotcavage RL, Scheerbaum DL, Kochansky CJ, Strein TG. Anal. Chem. 2005; 77:2332–2337. [PubMed: 15828765]
9. Mason PE, Schildt DC, Strein TG. J. Chromatography A. 2009; 1216:154–158.
10. Urbánek M, Křivánková L, Boček P. Electrophoresis. 2003; 24:466–485. [PubMed: 12569538]
11. Vasiliades J. Clin.Chem. 1976; 22:1664–1671. [PubMed: 10095]
12. Pardue HL, Bacon BL, Nevius MG, Skoug JW. Clin. Chem. 1987; 33:278–285. [PubMed: 3802512]
13. Merola ET, Catherman AD, Yehl JB, Strein TG. J. Ag and Food Chem. 2009; 57(15):6518–6523.

Figure 1. Product peak area obtained upon in-line EMMA Jaffe reaction between creatinine (C) and picrate (P⁻) as a function of the time of applied overlap voltage during the reactant mixing step

Overlap time (x-axis) is the time 10 kV is applied prior to allowing the reaction to proceed at 0 potential. Top: schematic of capillary showing initial plug arrangement; Panel A: product peak area obtained with creatinine dissolved in 18 MΩ-cm water; Panel B: creatinine dissolved in 125 mM sodium chloride. All other EMMA conditions were as follows: injection sequence: 150 mbar-s of 47 mM sodium picrate in 53 mM NaOH, 150 mbar-s of 10 mM creatinine solution, 150 mbar-s of BGE; BGE = 100 mM sodium borate buffer at pH 9.8; total capillary length = 35.0 cm, length to detector = 26.5 cm; overlap potential = 10.0 kV; o potential reaction “wait” time = 6.0 minutes; separation potential = 15.0 kV; detection of Jaffe product at 485 nm.

Figure 2. Simulation results for the overlap step of the EMMA experiment with the Jaffe reaction between anionic picrate (P^-) and neutral creatinine (C) with creatinine dissolved in water

Panel A: initial configuration of reagent zones; Panel B: simulation results at 1.0 sec of 10 kV overlap potential; Panel C: simulation results at 3.7 sec of 10 kV overlap potential. Key: Creatinine = C (---); Picrate Anion = P^- (—); Hydroxide ion = OH^- (-...-); Conductivity (....). Initial reactant zones were set up to simulate the experimental conditions given in Figure 1A.

Figure 3. Simulation results for the overlap step of the EMMA experiment with the Jaffe reaction between anionic picrate (P^-) and neutral creatinine (C) with creatinine dissolved in 125 mM saline solution

A: initial configuration of reagent zones; B : simulation results at 1.0 sec of 10 kV overlap potential; C: simulation results at 3.7 sec of 10 kV overlap potential. Key: Creatinine = C (---); Picrate Anion = P^- (—); Hydroxide ion = OH^- (-.-.-); Conductivity (....). Initial reactant zones and BGE (100 mM borate, pH 9.8) were set up to simulate the experimental conditions given in Figure 1B.

Figure 4. Experimental CE data demonstrating rapid picrate anion migration through a low conductivity “watery” zone

Top: schematic of capillary showing initial plug arrangement. Panels top to bottom show picrate profiles after application of the indicated duration of -10 kV overlap potential. Injection sequence (on short end): 150 mbar-s of 47 mM sodium picrate in 53 mM NaOH, 250 mbar-s of 1 mM NaCl solution, and 150 mbar-s of BGE. BGE = 100 mM sodium borate buffer at pH 9.8. Total capillary length = 37.0 cm, length to detector = 8.50 cm. Overlap potential = -10.0 kV for the indicated period of time. Picrate zones were moved to the detector by application of -20 mbar pressure. Detection at 210 nm.

Figure 5. Simulation results for rapid picrate migration through a watery zone
Simulation zones and BGE were set to match the experimental conditions in Figure 4.

Figure 6. Simulation results for the overlap step of a modified Jaffe reaction EMMA experiment with an extra plug of hydroxide

A: initial configuration of zone profiles; B: simulation results after 4.6 sec of 10 kV overlap potential. Key: Creatinine = C (---); Picrate Anion = P⁻ (—); Hydroxide ion = OH⁻ (-.-.); Conductivity (....). Initial reactant zones and BGE were set up identically to those in Figure 3, with the addition of a 1.1 mm width zone of 300 mM NaOH to the right (i.e. toward the positive negative electrode) of the picrate zone.

Figure 7. Product peak area obtained upon in-line EMMA reaction of 2,6-dichloroindophenol anion (DCIP⁻) with gallate (GA⁻) anion as a function of applied overlap voltage time

Top: Initial zone arrangement. Panel A: Curve obtained with both reactants dissolved in water; Panel B: curve obtained with both reactants dissolved in 60 mM sodium chloride. All other conditions identical. Injection sequence: 150 mbar-s of 1.00 mM gallic acid solution, 150 mbar-s of 1.00 mM sodium 2,6-dichloroindophenol solution, 150 mbar-s of BGE. BGE = 50 mM sodium phosphate buffer at pH 7.2. Total capillary length = 33.0 cm, length to detector = 24.5 cm. Overlap potential = 10.0 kV. Wait time of 6.0 minutes. Separation potential = 10 kV. Detection of product at 210 nm.

Figure 8. Simulation results for the overlap step of a 2,6-dichloroindophenol (DCIP⁻) and gallate (GA⁻) EMMA experiment with reactants dissolved in water

A: initial configuration of reagent zones; B: simulation results at 0.40 sec of 10.0 kV overlap potential. Initial reactant zones and BGE (50 mM sodium phosphate at pH 7.2) were set up to simulate the experimental conditions given in Figure 7A.

Figure 9. Simulation results for the overlap step of a 2,6-dichloroindophenol (DCIP⁻) and gallate (GA⁻) EMMA experiment with reactants dissolved in 60 mM saline

A: initial configuration of reagent zones; B: simulation results at 0.40 sec of 10.0 kV overlap potential; C: simulation results at 10.0 sec of 10.0 kV overlap potential, which is approximately the optimum time determined by experiment. Initial reactant zones and BGE (50 mM sodium phosphate at pH 7.2) were set up to simulate the experimental conditions given in Figure 7B.