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Transverse Diffusion of Laminar Flow Profiles To Produce Capillary Nanoreactors

Victor Okhonin, Xin Liu, and Sergey N. Krylov*

Department of Chemistry, York University, Toronto, Ontario M3J 1P3, Canada

We introduce transverse diffusion of laminar flow profiles (TDLFP), the first generic method for mixing two or more reactants inside capillaries. Conceptually, solutions of reactants are injected inside the capillary by pressure as a series of consecutive plugs. Due to the laminar nature of flow inside the capillary, the nondiffused plugs have parabolic profiles with predominantly longitudinal interfaces between them. After injection, the plugs are mixed by transverse diffusion; longitudinal diffusion does not contribute to mixing. To prove the principle, we used TDLFP to mix two reactants—an enzyme and its substrate. After mixing the reactants by TDLFP, we incubated reaction mixtures for different periods of time and measured the reaction kinetics. We found that the reaction proceeded in time- and concentration-dependent fashion, thus confirming that the reactants were mixed by TDLFP. Remarkably, the experimental reaction kinetics were not only in qualitative agreement but also in good quantitative agreement with theoretically predicted ones. TDLFP has a number of enabling features. By facilitating the preparation of reaction mixtures inside the capillary, TDLFP lowers reagent consumption to nanoliters (microliters are required for conventionally mixing reagents in a vial). The reaction products can be then analyzed "on-line" by capillary separation coupled with optical, electrochemical, or mass spectrometric detection. The combination of TDLFP with capillary separation will be an indispensable tool in screening large combinatorial libraries for affinity probes and drug candidates: a few microliters of a target protein will be sufficient to screen thousands of compounds. The new method paves the road to a wide use of capillary nanoreactors in different areas of physical and life sciences.

A capillary is a highly attractive chemical nanoreactor for a range of applications including kinetic measurements,[1,2] selection of binding ligands from large combinatorial libraries,[3] high-throughput screening,[4] analyses of biomolecules,[5,6] and analyses of single cells.[7−10] The most obvious advantages of capillary nanoreactors are the following. First, reactions in the capillary can be carried out in nanoliter volumes. Second, the products can be separated from the reactants inside the same capillary using capillary chromatography or electrophoresis. Third, the capillary can be easily interfaced with optical, electrochemical, and mass spectrometric detectors, thus, offering ultimate analytical capabilities.

The fundamental problem, which has so far retarded the use of capillary nanoreactors, was the lack of a generic method for mixing reactants inside an ordinary capillary—a round tube with a small diameter. Two methods have been used so far for mixing solutions inside a capillary. The first method relies on differences in electrophoretic mobilities of the mixed solutes.[11,12] This method requires knowledge of electrophoretic mobilities of all mixed solutes and becomes nonpractical when more than two components should be mixed or if the background electrolyte is different from the buffers of the mixed solutions. The second method uses longitudinal diffusion through the transverse interface between the plugs of different solutions.[13,14] Mixing by longitudinal diffusion through relatively long plugs is a very slow process, which cannot be applied to more than two separately injected solutions.

Here we introduce transverse diffusion of laminar flow profiles (TDLFP), the first generic method for mixing reactants inside the capillary. Conceptually, solutions of reactants are injected inside the capillary by pressure as a series of consecutive plugs. Due to the laminar nature of flow inside the capillary, the nondiffused plugs have parabolic profiles with predominantly longitudinal interfaces between the plugs. After the injection, the reactants are mixed by transverse diffusion; the contribution of longitudinal diffusion to mixing is negligible. In this work, we prove the concept of TDLFP both theoretically and experimentally. We develop a simple mathematical model of TDLFP and use it to

* To whom correspondence should be addressed. E-mail: skrylov@yorku.ca.

(1) Okhonin, V.; Berezovski, M.; Krylov, S. N. *J. Am. Chem. Soc.* **2004**, *126*, 7166−7167.
(2) Berezovski, M.; Krylov, S. N. *J. Am. Chem. Soc.* **2002**, *124*, 13674−13675.
(3) Berezovski, M.; Drabovich, A.; Krylova, S. M.; Musheev, M.; Okhonin, V.; Petrov, A.; Krylov, S. N. *J. Am. Chem. Soc.* **2005**, *127*, 3165−3171.
(4) Zheng, B.; Ismagilov, R. F. *Angew. Chem., Int. Ed.* **2005**, *44*, 2520−2523.
(5) Berezovski, M.; Krylov, S. N. *J. Am. Chem. Soc.* **2003**, *125*, 13451−13454.
(6) Burns, M. A.; Johnson, B. N.; Brahmasandra, S. N.; Handique, K.; Webster, J. R.; Krishnan, M.; Sammarco, T. S.; Man, P. M.; Jones, D.; Heldsinger, D.; Mastrangelo, C. H.; Burke, D. T. *Science* **1998**, *282*, 484−487.
(7) Arkhipov, S. N.; Berezovski, M.; Jitkova, J.; Krylov, S. N. *Cytometry* **2005**, *63A*, 41−47.
(8) Davis, B. *Scientist* **2005**, *19* (4), 27−29.
(9) Krylov, S. N.; Arriaga, E.; Zhang, Z.; Chan, N. W. C.; Palcic, M. M.; Dovichi, N. J. *Cytometry* **1999**, *37*, 14−20.
(10) Kennedy, R. T.; Oates, M. D.; Cooper, B. R.; Nickerson, B.; Jorgenson, J. *Science* **1989**, *246*, 57−63.
(11) Novakova, S.; Van Dyck, S.; Van Schepdael, A.; Hoogmartens, J.; Glatz, Z. *J. Chromatogr., A* **2004**, *1032*, 173−184.
(12) Sanders, B. D.; Slotcavage, R. L.; Scheerbaum, D. L.; Kochansky, C. J.; Strein, T. G. *Anal. Chem.* **2005**, *77*, 2332−2337.
(13) Van Dyck, S.; Vissers, S.; Van Schepdael, A.; Hoogmartens, J. *J. Chromatogr., A* **2003**, *986*, 303−311.
(14) Taga, A.; Honda, S. *J. Chromatogr., A* **1996**, *742*, 243−250.

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simulate the mixing of two solutions. Finally, we confirm the results of the simulation experimentally by mixing and reacting an enzyme and its substrate inside the capillary. Being a generic method, TDLFP allows mixing multiple (two or more) reactants without knowledge of their physical−chemical properties. The new method opens the opportunity for a wide use of capillary nanoreactors in different areas of physical and life sciences. The combination of TDLFP with capillary chromatography and electrophoresis will be an indispensable tool in screening large combinatorial libraries for affinity probes and drug candidates—a few microliters of a target protein will be sufficient to screen thousands of compounds. In addition, TDLFP promises to revolutionize detailed chemical analyses of single cells,[7−10] by providing a universal tool for cell lysis and labeling intracellular components with affinity probes, hybridization probes, etc.

EXPERIMENTAL SECTION

Chemicals and Materials. *Escherichia coli* β-galactosidase (β-Gal), fluorescein mono-β-D-galactopyranoside (FMG), and fluorescein sodium salt were purchased from Sigma-Aldrich (Oakville, ON, Canada). All other chemicals were from Sigma-Aldrich, Caledon (Toronto, ON, Canada), or BDH (Toronto, ON, Canada). Stock solutions of 4.4 mM FMG and 5 nM β-Gal were prepared in a reaction buffer and stored at −80 °C. The reaction and electrophoresis run buffers were identical: 10 mM phosphate at pH 7.2 supplemented with 1 mM $MgCl_2$. All solutions were made using Milli-Q-quality deionized water filtered through a 0.22-μm filter (Millipore, Nepean, ON, Canada).

Instrumentation. An uncoated fused-silica capillary (Polymicro, Phoenix, AZ) with a length of 50 cm and inner and outer diameters of 50 and 365 μm, respectively, was used in all TDLFP experiments. The capillary was mounted on a capillary electrophoresis (CE) instrument (P/ACE MDQ, Beckman-Coulter; Fullerton, CA), which could facilitate automated injection of solutions by pressure. Advantageously, the same instrument could be used to separate the reaction product from the unreacted substrate and quantify both of them with a laser-induced fluorescent (LIF) detection (fluorescence excitation at 488 nm and fluorescence detection at 520 nm).

TDLFP Mixing and "In-Capillary" Enzymatic Reaction. The injection sequence for two-step mixing was as follows: FMG solution (0.5 psi × 3 s) and β-Gal solution (0.5 psi × 3 s) followed by 1-min mixing. For three-step mixing, it was as follows: FMG solution (0.5 psi × 3 s), β-Gal solution (0.5 psi × 3 s), and FMG solution (0.5 psi × 3 s) followed by 1-min mixing. For four-step mixing, the sequence was as follows: FMG solution (0.5 psi × 3 s), β-Gal solution (0.5 psi × 3 s), FMG solution (0.5 psi × 3 s), and the reaction buffer (0.5 psi × 9 s) followed by a 1-min mixing. After each injection of FMG or β-Gal, the capillary and electrode were dipped in the reaction buffer in order to prevent the contamination of solutions, from which the next plugs were to be injected. To allow the enzymatic reaction to proceed, the reaction mixtures were incubated for periods of time ranging from 3 to 40 min. An electric filed of 500 V/cm was then applied to (i) stop the enzymatic reaction by separating the enzyme from the substrate, (ii) separate the product from the unreacted substrate by CE, and (iii) quantify both the product and the unreacted substrate with LIF detection. The reaction buffer was used as a CE run buffer.

"In-Vial" Enzymatic Reaction. Six microliters of 1 μM FMG and 3 μL of 1 nM β-Gal were mixed with 9 μL of the reaction buffer in a 200-μL reservoir. The mixture was capped, mixed, centrifuged briefly at 8000*g*, and allowed to incubate at 25 °C for a period of time ranging from 5 to 65 min. To quantify the amount of the product, a small sample of the reaction mixture was injected into the capillary by a 0.5 psi × 3 s pressure pulse; the product was then separated from the unreacted substrate by CE at 500 V/cm and quantified with LIF. The reaction buffer was used as a CE run buffer. To determine an absolute amount of the product, we used fluorescein, a known amount of which was added to the reaction mixture, as an internal reference.

Determination of K_m and k_{cat}. The K_m and k_{cat} values were determined using an approach previously described elsewhere.[15] Briefly, a series of solutions with different concentrations of FMG were used in the in-vial enzyme reaction with an incubation time of 35 min. Other conditions were similar to those described above. The values of K_m and k_{cat} were calculated using the Michaelis−Menten equation.

RESULTS AND DISCUSSION

First, we explain the physical bases of TDLFP and its mathematical model. The inner diameter of the capillary is assumed to be 50 μm and the length of every solution plug is assumed to be at least 30 times greater than the diameter. A few-millimeter-long plug of a solution can be injected into the capillary by high pressure within a fraction of a second without disturbing the laminar nature of the flow.[16] Characteristic times of transverse diffusion in the capillary are ∼1 s for small biomolecules, such as amino acids.[17,18] For biopolymers, such as proteins, transverse diffusion can take as long as 1 min. This allows us to make the first simplifying assumption: diffusion can be neglected during fast injection of the solution plug inside the capillary. Crucial to TDLFP, the profile of a nondiffused plug has a universal parabolic shape.[19,20] Injecting the second reactant requires replacing the reservoir with a solution, which takes longer than 1 s. The first plug may diffuse significantly in the transverse direction while the reservoir is replaced. We make the second simplifying assumption: after every injection, the plugs inside the capillary diffuse and eliminate concentration gradients in the transverse direction; the concentration gradients in the longitudinal direction remain virtually undisturbed since the diffusion across long plugs can take from several hours to several days. A simple mathematical model of TDLFP was developed, based on the two assumptions (see the Mathematical Appendix for details). The nonnumerical nature of the model advantageously allowed us to use Excel-type software for modeling TDLFP.

Second, we applied the mathematical model to simulate TDLFP-based mixing. Figure 1 illustrates the simulated mixing of two reactants, blue and red, in several steps, with gradually increasing spatial overlap of the reactants. In step 1, a parabolic

(15) Berezovski, M.; Li, W.-P.; Poulter, C. D.; Krylov, S. N. *Electrophoresis* **2002**, *23*, 3398−3403.
(16) Sharp, K. V.; Adrian, R. J. *Exp. Fluids* **2004**, *36*, 741−747.
(17) Wu, Y.; Ma, P.; Liu, Y.; Li, S. *Fluid Phase Equilib.* **2001**, *186*, 27−38.
(18) Sharma, U.; Gleason, N. J.; Carbeck, J. D. *Anal. Chem.* **2005**, *77*, 806−813.
(19) Gosch, M.; Blom, H.; Holm, J.; Heino, T.; Rigler, R. *Anal. Chem.* **2000**, *72*, 3260−3265.
(20) Culbertson, C. T.; Jorgenson, J. W. *Anal. Chem.* **1994**, *66*, 955−962.

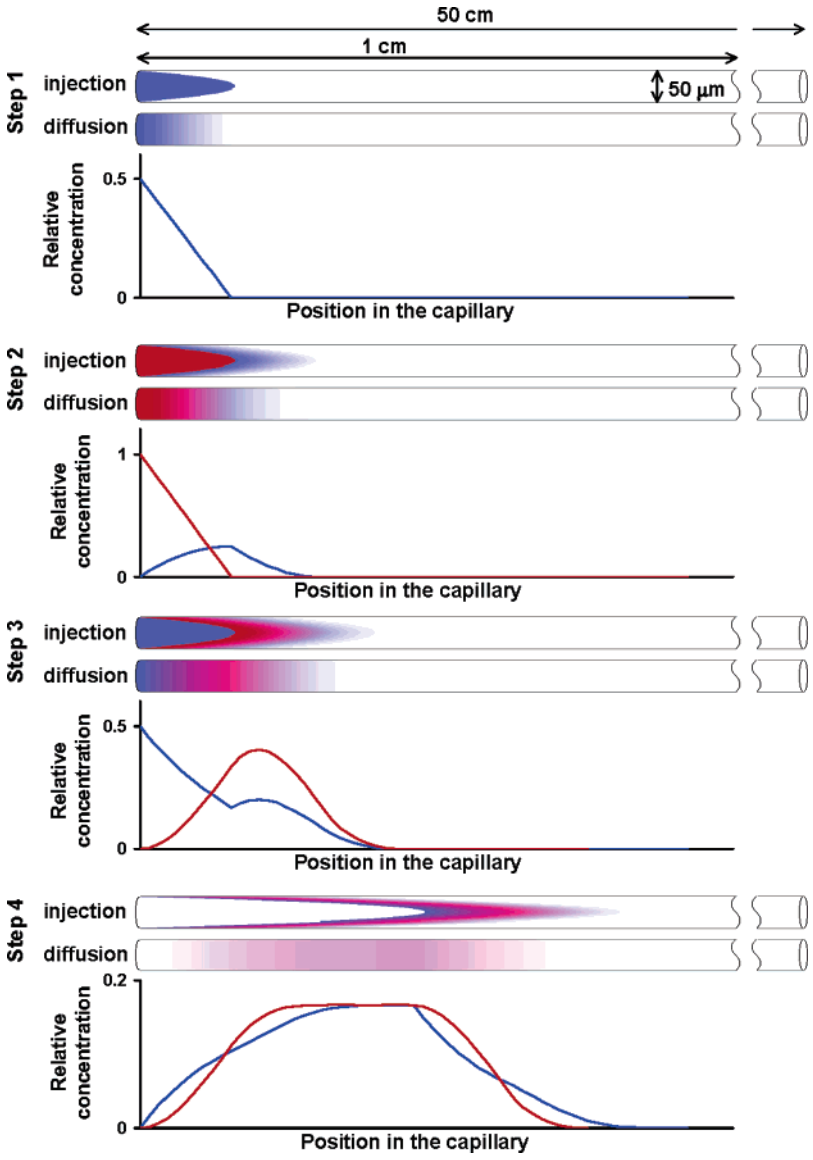

Figure 1. Simulated mixing of two components, blue and red, inside the capillary by transverse diffusion of laminar flow profiles. White color inside the capillary represents the blank buffer. The graphs show concentration profiles of the two components inside the capillary after every step. The colored plugs have identical volumes; the volume of the white plug in step 4 is equal to the sum volume of the three colored plugs.

plug of the blue solution is injected, and transverse diffusion of the parabolic profile eliminates the concentration gradient in the transverse direction. The resulting concentration profile of the blue component is linear. In step 2, the same volume of the red solution is injected; parabolic profiles of both plugs are established with the interface between them being predominantly in the longitudinal direction. Transverse diffusion of the parabolic profiles, which follows the injection, eliminates the concentration gradients in the transverse direction and mixes the blue and the red solutions. The mixing zone has a length equal to that of the red plug. The concentration profiles of the blue and red components are not similar, but overall, 100% of the red component and ~50% of the blue component are mixed. In step 3, the second blue plug of the same volume is injected and mixed with the two previously injected plugs. The concentration profiles are still not similar, but ~100% of the two components are mixed. In step 4, a plug of the buffer of a volume equal to the total volume of the first three plugs is injected and mixed with the first three plugs. Notably, the resulting concentration profiles are almost identical. In the resulting reaction mixture, the blue and red components are diluted by factors of ~3 and ~6, respectively. More than two components can be mixed in a similar way.

Third, we examined the results of the simulated mixing experimentally. In a manner similar to that illustrated in Figure 1, we mixed an enzyme, β-galactosidase, with its fluorescently labeled substrate, fluorescein mono-β-D-galactopyranoside. The order of the four plugs was substrate, enzyme, substrate, and buffer. After mixing, the enzymatic reaction was allowed to proceed for varying periods of time. The reaction was stopped by electrophoretically separating the enzyme from the substrate; the product was separated from the unreacted substrate, also electrophoretically. Note, due to high efficiency of capillary electrophoresis, this separation takes no longer than a few seconds. This time is much shorter than the time required for TDLFP-based mixing; thus, it can be ignored in the overall consideration. The quantity of the product was measured with a fluorescence detector

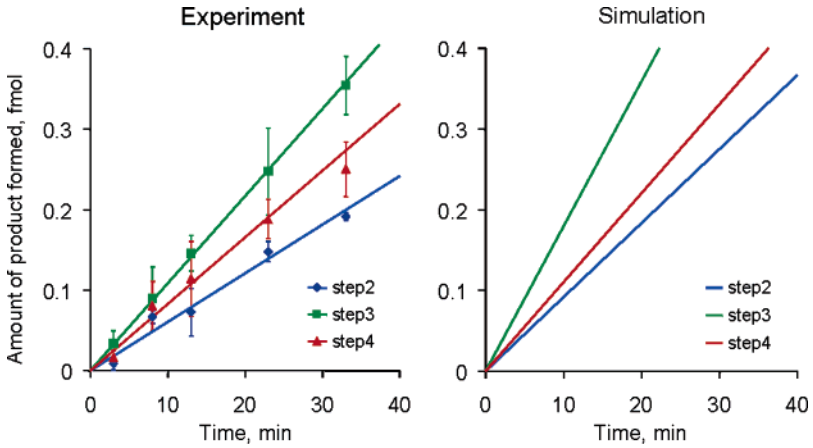

Figure 2. Experimental (left graph) and simulated (right graph) kinetics of enzymatic glycosylation of fluorescein mono-β-D-galactopyranoside. The enzyme and the substrate were mixed by TDLFP using the general procedure depicted in Figure 1. Experimental conditions are described in the Experimental Section. The simulated kinetics was calculated by integrating product formation along the capillary length using experimental values of K_m and k_{cat}.

at the distal end of the capillary. We found that the enzymatic reaction proceeded in time- and concentration-dependent fashion, thus confirming that the reactants were mixed. Next, we compared experimental reaction kinetics with kinetics predicted by the mathematical model of TDLFP. The simulated kinetics was calculated by integrating product formation along the capillary length. The model used the values of the catalytic rate constant, $k_{cat} = 0.54\ s^{-1}$, and the Michaelis constant, $K_m = 3.3\ \mu M$, which were determined in a separate experiment by mixing the reaction components in a vial. Figure 2 compares experimental and simulated reaction kinetics. To generate comprehensive data, this comparison was done for two-, three-, and four-step mixing procedures described in Figure 1. Remarkably, the experimental initial reaction rates were not only in qualitative agreement but also in good quantitative agreement. The simulated reaction rates for steps 2−4 were greater than the experimental ones by a factor of ~1.5. This relatively small difference between experimental and simulated rates is due to the model not taking into consideration transverse diffusion during plug injection. This result demonstrates that not only can TDLFP mix reactants inside the capillary but it also can mix them in a predictable fashion.

Finally, we outline major features of TDLFP-based mixing. Classical mixing in a vial relies on creating vortexes, complex hydrodynamic structures, which can hardly be modeled. As a result, classical mixing is largely unpredictable. TDLFP, in contrast, relies on diffusion of laminar flows, which can be accurately modeled using simple mathematics. The mathematical model of TDLFP relies on two easily achievable assumptions: (i) no diffusion during injections and (ii) fast transverse diffusion but no longitudinal diffusion after injections. The first assumption is achieved by fast injections; the second assumption is achieved by prolonging the period of time between injections to the characteristic diffusion time for the largest reactant injected in the capillary. The two assumptions play different roles in TDLFP. Satisfying the first assumption is essential for efficient mixing. The second assumption is used solely to simplify the model. The major limitation of the method is the relatively long time required for diffusion. Diffusion is a slow process—TDLFP-based mixing is possible only due to the small diameter of the capillary. Mixing time can range from seconds to minutes, depending on the size of mixed molecules and the number of steps in mixing. Because of this limitation, when TDLFP-based mixing is used to study kinetics, the concentrations of reactants should be chosen so that mixing kinetics is faster than reaction kinetics. Another limitation is the increasing time of mixing with the increasing number of reactants to be mixed.

To conclude, TDLFP represents the first generic method for efficient and controlled mixing of reactants inside the capillary. The method overcomes the long-standing limitation of capillary nanoreactors and paves the road for their wide practical use. TDLFP becomes especially attractive when it is combined with capillary separation tools. We foresee that the combination of TDLFP with capillary chromatography and electrophoresis will be an indispensable tool in screening large combinatorial libraries for affinity probes and drug candidates—a few microliters of a target protein will be sufficient to screen thousands of compounds. We also predict that TDLFP will revolutionize detailed chemical analyses of single cells,[7−10] by providing a universal tool for cell lysis and labeling intracellular components with affinity probes, hybridization probes, etc.

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MATHEMATICAL APPENDIX

The general model of mass transfer aims at calculating concentrations of substances mixed by TDLFP as functions of the position in the capillary and time passed since the beginning of mixing. If the solution is injected in the capillary by differential pressure, mass transfer is described by the following equation:

$$\frac{\partial n}{\partial t} = -v(r)\frac{\partial n}{\partial x} + \mu\left(\frac{\partial^2 n}{\partial x^2} + \frac{1}{r}\frac{\partial}{\partial r}r\frac{\partial n}{\partial r}\right)$$

$$v(r) = v_0(1 - (r/r_0)^2)$$

$$\mu\frac{\partial n}{\partial r}\Big|_{r=r_0} = 0 \qquad (1)$$

where n is the concentration of the substance, μ is its diffusion

coefficient, v is its velocity along the axis of the capillary, r is the distance from an axis of a capillary, x is the distance from the injection end of the capillary, t is time from the beginning of injection, r_0 is the radius of the capillary, and v_0 is the velocity of the substance along the axis of the capillary for $r = 0$.

We assume that the characteristic length of the injected plugs, L, is much greater than the diameter of the capillary. In this case, the time required for transverse diffusion, t_r, is much shorter than the time required for longitudinal diffusion, t_x:

$$t_r \sim r_0^2/\mu,\ t_x \sim L^2/\mu$$

$$t_x/t_r \sim L^2/r_0^2 \tag{2}$$

This allows us to neglect mixing by longitudinal diffusion and simplify the top equation in system 1:

$$\frac{\partial n}{\partial t} = -v(r)\frac{\partial n}{\partial x} + \mu\frac{1}{r}\frac{\partial}{\partial r}r\frac{\partial n}{\partial r}$$

$$v(r) = v_0(1 - (r/r_0)^2)$$

$$\mu\frac{\partial n}{\partial r}\bigg|_{r=r_0} = 0 \tag{3}$$

Although system 3 provides the general basis for modeling plug formation, its analytical solution is difficult. Solving this system analytically becomes feasible, however, if either of the two terms at the right-hand side of the top equation is negligible with respect to the other one. In other words, the analytical solution of system 3 can be found if either the coefficient of diffusion or the velocity can be assumed to be zero. We use both assumptions in our modeling: we assume that diffusion is negligible during injection, and we assume that velocity is zero during mixing. Turning off the differential pressure easily satisfies the second assumption in experiment. This assumption simplifies system 3 to

$$\frac{\partial n}{\partial t} = -v(r)\frac{\partial n}{\partial x}$$

$$v(r) = v_0(1 - (r/r_0)^2) \tag{4}$$

System 4 has the following general solution:

$$n = F(x - tv(r)) \tag{5}$$

If the plug is injected from a vial, in which the concentration of the substance is n_0, the distribution of the substance concentration inside the capillary is described by the following equation:

$$n(t, x, r) = n_0\theta(x - tv_0(1 - (r/r_0)^2)) \tag{6}$$

Here $\theta(x)$ is a function, which equals to 1 if $x > 0$ and equals to 0 otherwise. This function allows us to describe the distribution of the substance not only after the injection but also prior to it ($t < 0$). According to eq 6, if $t < 0$, the concentration of the substance inside the capillary ($x > 0$) is 0. On the interface between the capillary and the solution in the vial ($x = 0$), the concentration of the substance is equal to that in the vial. According to eq 6, the profile of the injected plug is parabolic.

Inside the capillary, the concentration equals to n_0 within the plug and equals to 0 outside the plug. Knowing the form of the plug allows us to calculate the average concentration for each section of a capillary:

$$N(x) = \frac{2}{r_0^2}\int_0^{r_0} n(t, x, r)r\,dr \tag{7}$$

In particular, for the parabolic distribution described by eq 6, the average "per-section" concentration is

$$N(x) = n_0\theta(x - tv_0)(x/tv_0 - 1) \tag{8}$$

The x profile of this distribution for the fixed t is linear.

Generally, multiple plugs of different substances are injected. After every injection, we allow the injected substances to diffuse into each other for a period of time that is longer than t_r and shorter than t_x. As a result, gradients in the direction perpendicular to the capillary axis are eliminated for all injected plugs. Let us assume that the resulting x profile of plug number j is described by a function $N_j(x)$. After injecting plug number $j + 1$, with time of injection t, the distribution of plug j will become nonuniform:

$$n(t, x, r) = N_j(x - v_0(1 - (r/r_0)^2)t) \tag{9}$$

where the last equation is obtained from the general solution represented by eq 5.

If we substitute eq 9 into eq 7, we can obtain the x distribution of the concentration for plug $j + 1$ after time of injection t_{j+1}:

$$N_{j+1}(x) = 2\int_0^{r_0} N_j(x - v_0(1 - (r/r_0)^2)t_{j+1})rdr/r_0^2 \tag{10}$$

Equation 11 can be transformed to

$$N_{j+1}(x) = \int_{x - t_{j+1}v_0}^{x} N_j(z)dz/(t_{j+1}v_0) \tag{11}$$

where

$$z = x - v_0(1 - (r/r_0)^2)t_{j+1} \tag{12}$$

Equation 11 sets the iterative procedure, which allows us to determine final distributions of solutes in all injected plugs by means of repetitive integration. In particular, if we use distribution 8 as N_1, we will replace N_j in the right part of eq 11 with expression 8. This allows us to calculate analytically the corresponding integral and obtain N_2. Then, in a similar way, we can calculate N_3, N_4, and so on.

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