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Computer simulation of electroinjection analysis and electrophoretically mediated microanalysis Commensurable concentrations of sample and reagent

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Abstract

The mathematical model of electroinjection analysis and electrophoretically mediated microanalysis is presented. The evolution of sample, reagent and product concentrations is described by the set of three diffusion–convection equations that are solved with the help of computer simulation. The influence of commensurable initial concentrations of sample and reagent and of reverse reaction are studied.

Keywords: Computer simulation; Mathematical modelling; Electroinjection analysis; Electrophoretically mediated microanalysis; Kinematic focusing; Diffusion; Convection

1. Introduction

Electroinjection analysis (EIA) and electrophoretically mediated microanalysis (EMMA) are a pair of mutually complementary methods of chemical analysis that can be realized with the help of capillary electrophoresis instrumentation. In both methods mixing of sample and reagent is due to the difference of the velocities of their movement (the sum of electroosmotic velocity of the flow and electrophoretic velocities of the ions) in the applied electric field. Fig. 1 displays schematic representation of EIA and EMMA. According to EIA [1–3] sample and reagent are simultaneously injected from the opposite ends of the capillary electrokinetically. According to EMMA [4–6] sample and reagent are injected from the same end of the capillary, the slower one (having the smaller velocity) first and then the faster one. In both methods the zones of sample and reagent are going through one another, in EIA they are moving

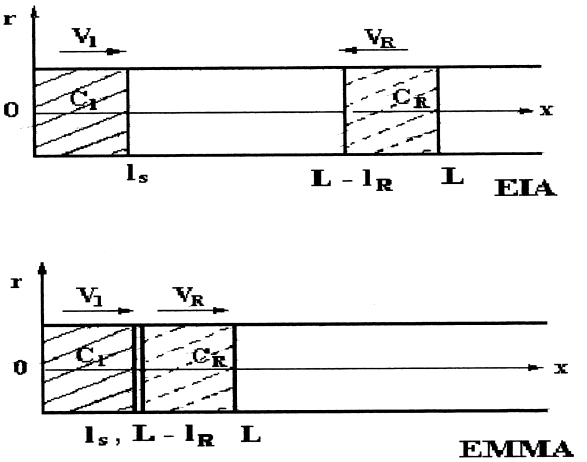

Fig. 1. Schematic representation of EIA and EMMA, V_1, V_R are sample and reagent velocities.

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in the opposite directions, in EMMA they are moving in one direction but with different velocities. Sample and reagent react and the product of chemical reaction is carried by the electric field to the on-line detector. Due to the high quality of mixing of sample and reagent the sensitivity of EIA and EMMA may be much higher than the sensitivity of flow injection analysis (FIA). Comparison of the possibilities and advantages of EIA, EMMA and FIA, together with the examples of experimental realizations of EIA and EMMA are presented in Refs. [2,3].

The mathematical model of EIA and EMMA was presented in Ref. [3] and was solved analytically for the case when the initial concentration of sample was much lower than the initial concentration of reagent. The effects of molecular diffusion and reverse reaction were ignored in Ref. [3]. Analysis performed in Ref. [3] enabled one to predict theoretically the effect of kinematic focusing that occurred when the velocities of product and reagent had the same sign and close absolute values. The high and narrow product peak is then formed, and so additional gain in sensitivity is realized. The effect of kinematic focusing was verified and proved experimentally in Refs. [7,8].

The mathematical model that is presented in this paper is free of the above mentioned assumptions of low sample concentration, negligible effects of diffusion and reverse reaction. Special attention is given to the study of the process of kinematic focusing in EIA and EMMA.

2. Theory

Consider the sample and reagent zones that are forced to move by a longitudinal electric field, E, with different velocities along a cylindrical tube of infinite length and radius, a. We will use the first group of assumptions of the model of Ref. [3] that enables one to consider the problem to be one-dimensional and linear (electrical field strength E independent of reactant concentrations and constant along the tube): (1) the concentrations of sample, reagent and product are considered to be much smaller than the concentration of background electrolyte or buffer, and so conductivity of solution is independent of coordinate and time, and E is constant. (2) Interactions between sample, reagent and product ions and capillary walls are ignored. (3) Buffer concentration is considered to be higher than 10^{-3} M, so Debye layer thickness is much smaller than capillary radius. The zeta-potential of the wall is considered to be constant along the length of the capillary, so the electroosmotic velocity is independent of transversal and longitudinal coordinates. (4) The influence of temperature effects on buffer viscosity and reactants' electrophoretic mobilities is ignored (the validity of this assumption is discussed in Ref. [9]).

Assumptions 1, 3 and 4 enable one to consider the velocities of movement of sample, reagent and product V_1, V_R and V_2 to be independent of transversal and longitudinal coordinates and together with assumption 2 reduce the problem to the one-dimensional, described by the following set of diffusion–convection equations:

$$\frac{\partial C_1}{\partial t} + V_1\frac{\partial C_1}{\partial x} = D_1\frac{\partial^2 C_1}{\partial x^2} - k_+ C_1 C_R + k_- C_2 \qquad (1)$$

$$\frac{\partial C_R}{\partial t} + V_R\frac{\partial C_R}{\partial x} = D_R\frac{\partial^2 C_R}{\partial x^2} - k_+ C_1 C_R + k_- C_2 \qquad (1a)$$

$$\frac{\partial C_2}{\partial t} + V_2\frac{\partial C_2}{\partial x} = D_2\frac{\partial^2 C_2}{\partial x^2} + k_+ C_1 C_R - k_- C_2 \qquad (1b)$$

with initial conditions

$$\begin{array}{ll} C_1 = C_{10}, & \text{for} \quad 0 < x < l_s \\ C_1 = 0, & \text{for} \quad x < 0, x > l_s \end{array}, \quad t = 0 \qquad (2)$$

$$\begin{array}{ll} C_R = C_{R0} & \text{for} \quad L - l_R < x < L \\ C_R = 0 & \text{for} \quad x < L - l_R, x > L \end{array}, \quad t = 0 \qquad (3)$$

$$C_2 = 0, t = 0 \qquad (4)$$

where D_1, D_R, D_2 are sample, reagent and product diffusion coefficients, and k_+, k_- are direct and reverse reaction rates, respectively. The model describes both EIA and EMMA; in the case of EMMA V_R is positive, in the case of EIA V_R is negative. Unlike Ref. [3], no other assumptions were used in this study.

In contrast to Ref. [3] instead of solving the set of Eqs. (1–1b) (analytically or numerically) in this investigation an algorithm of computer simulation of the physico–chemical processes, which are described

by the set of Eqs. (1–1b) was constructed. This algorithm is based on a combination of the method of weighted great particles introduced by Bird [11] and modified here to imitate motion of reactants and change of their local concentrations in process of reaction, and the method which can be called ''cells scanning'' and which has much in common with that used in Ref. [6]. The details of this method are presented in Ref. [10]. In order to test the algorithm we used it to solve the problem for the same case that was treated in Ref. [3] analytically (see Figs. 2–8 of Ref. [3]) and got the perfect coincidence. The results of this test are presented in Ref. [10] and prove the high precision of the algorithm.

3. Results and discussion

In the following figures the dependences of product concentration divided by the initial concentration of sample C_2/C_{10} versus the non-dimensional coordinate $Z=xk_+C_{R0}/V_R$ are presented. The logic of the study was as follows. The cases studied in Ref. [3] were taken and then the factors that were ignored in Ref. [3] were consequently added. So Fig. 2 corresponds to Fig. 2 of Ref. [3] (the case of EMMA). Fig. 2a presents the case where diffusion is added but the concentration of sample is still much smaller than the concentration of reagent ($C_{10}=0.01C_{R0}$), Fig. 2b reverse reaction is added ($\gamma=0.1$, where $\gamma=k_-/k_+C_{R0}$), and Fig. 2c sample and reagent initial concentrations are commensurable ($C_{10}=0.5C_{R0}$) and in Fig. 2d initial concentrations of sample and reagent are equal ($C_{10}=C_{R0}$). The same logic is presented in Fig. 3a–d, Fig. 4a–d, which correspond to the cases presented in Figs. 3 and 4 of Ref. [3] (EIA with counterdirected motion of reagent and product and codirected motion of reagent and product, correspondingly).

The values of diffusion coefficient used were a kind of higher estimates $\alpha_m=0.00224$ ($\alpha_m=D_mk_+C_{R0}/V_R^2$) than for the values of dimensional parameters used in Ref. [3] $V_2=0.25$ cm/s and $(k_+C_{R0})^{-1}=1$ s corresponds to $D_m=1.4\cdot10^{-4}$cm^2/s, that is higher than the diffusion coefficients of any ions in water at room temperatures. As can be seen from Figs. 2a and 3a in comparison with Figs. 2 and 3 of Ref. [3] even these high diffusivity values have not influenced the results. There is some small decrease of peak amplitudes (2%) for the case presented in Fig. 4a in comparison with Fig. 4 of Ref. [3]. The influence of reverse reaction is much more significant and is leading to the decrease of the amplitude of the highest peak (15%, 9% and 15.5% decrease for Figs. 2b, 3b and 4b, correspondingly). Reverse reaction also leads to the very substantial decrease of the amplitudes of the product peaks for the following time values, when the sample and reagent zones have already passed through one another and the main stage of product production is finished (some small amount of product can be still produced due to the secondary reaction between the products of the reverse reaction). The results show that it is rather important to choose the right position for the detector if the rate of the reverse reaction is not too small. For example, if the detector is placed in the point where reactants zones have passed through one another, then the product peak amplitude will be lower than the maximum possible, and so the sensitivity will be lower also.

Figs. 2c, d; 3c, d; 4c, d illustrate the influence of the commensurability of sample and reagent concentrations. For the case of EMMA presented in Fig. 2c, d the maximum peak amplitude decreases by 25% for $C_{10}/C_{R0}=0.5$ and by 25% more for $C_{10}/C_{R0}=1$. For the case of EIA with counterdirected movement of reagent and product, presented in Fig. 3c, d, commensurability of sample and reagent initial concentrations leads to the very insignificant change of the peak amplitudes, while the forms of the peaks are changing substantially. With the growth of C_{10}/C_{R0} the largest peak and the following peaks become sharper and thinner. For the case of EIA with codirected movement of product and reagent, presented in Fig. 4c, d, the growth of C_{10}/C_{R0} leads as in the case of EMMA to the substantial decrease of peak amplitudes, the peaks become more smooth and wide with the growth of C_{10}/C_{R0}. Here it is interesting to note that in the case where $C_{10}/C_{R0}=1$, there is no more difference between sample and reagent because the set of Eqs. (1–1b) is invariant to the change of indexes $1\leftrightarrow R$. The results presented in Fig. 3d and Fig. 4d are in full correspondence with these observations as the curves presented in Figs. 3d and 4d are mirror symmetrical to each other. This

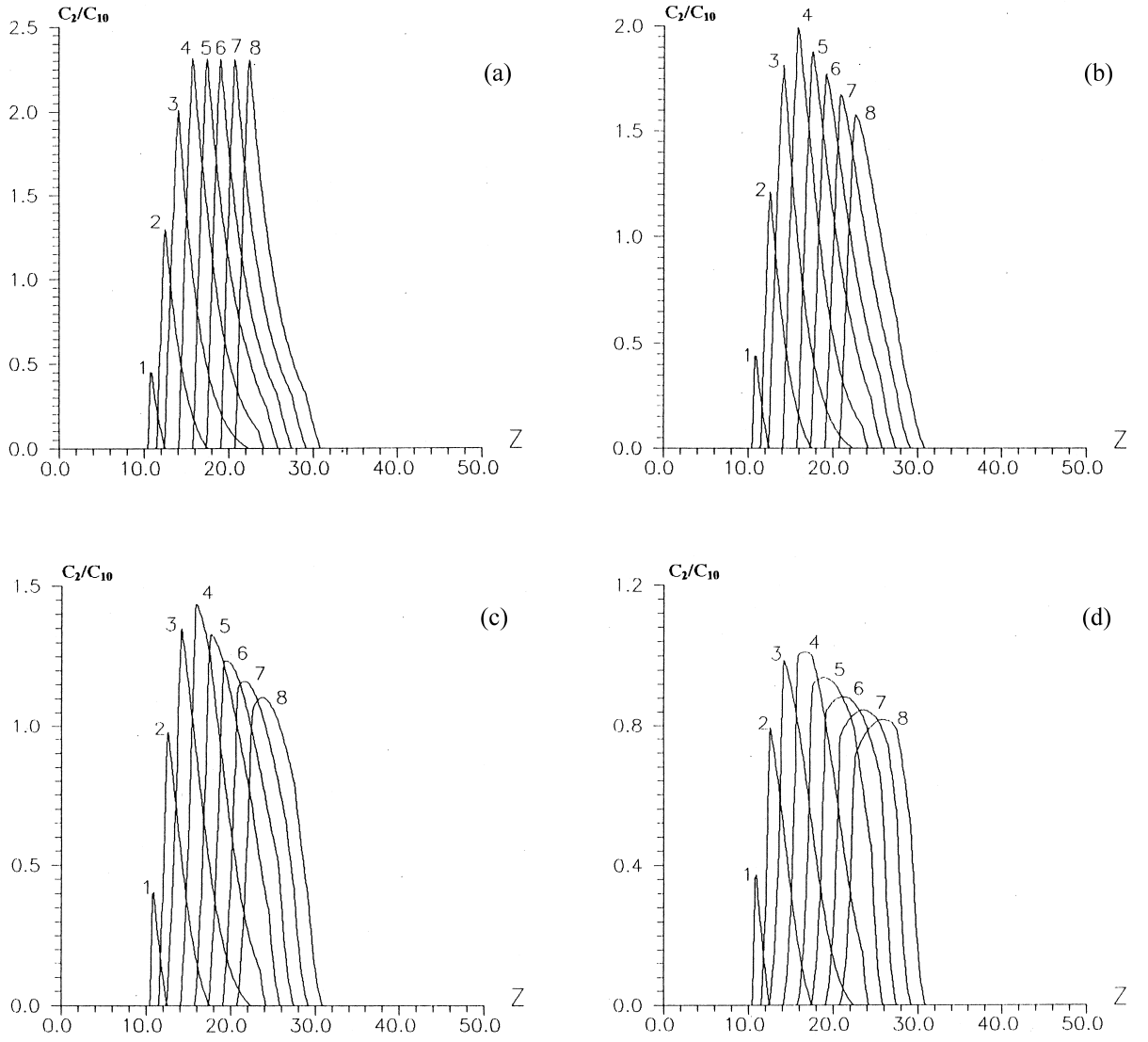

Fig. 2. (a) Product concentration versus coordinate for EMMA; $(k_{+}C_{R0})^{-1}=1$ s; no reverse reaction $\gamma=0$; $V_1=0.5$ cm/s; $V_R=0.1$ cm/s; $V_2=0.2$ cm/s; $C_{10}/C_{R0}=0.01$; $\alpha_m=0.00224$; $L=5$ cm; $l_s=1$ cm; $l_R=1$ cm. Peaks 1–8 are for time moments separated by 1 s. (b) Product concentration versus coordinate for EMMA; influence of reverse reaction $\gamma=0.1$. All other parameters are the same as in (a). (c) Product concentration versus coordinate for EMMA; influence of reverse reaction $\gamma=0.1$ and commensurability of initial concentrations of sample and reagent $C_{10}/C_{R0}=0.5$. All other parameters are the same as in (a). (d) Product concentration versus coordinate for EMMA; influence of reverse reaction $\gamma=0.1$, and equality of initial concentrations of sample and reagent $C_{10}/C_{R0}=1$. All other parameters are the same as in (a).

result also proves the very high precision of the numerical algorithm used.

In order to explain, why the growth of C_{10}/C_{R0} leads to the decrease of peak amplitude in the first and third case and does not lead to the change of the peak amplitude in the second case, one must take into consideration the important difference between these cases. As it was shown in Ref. [3], when the product and reagent are moving in the same direction and the values of their velocities are close enough, the effect of kinematic focusing takes place. It means that the portions of the product formed at different

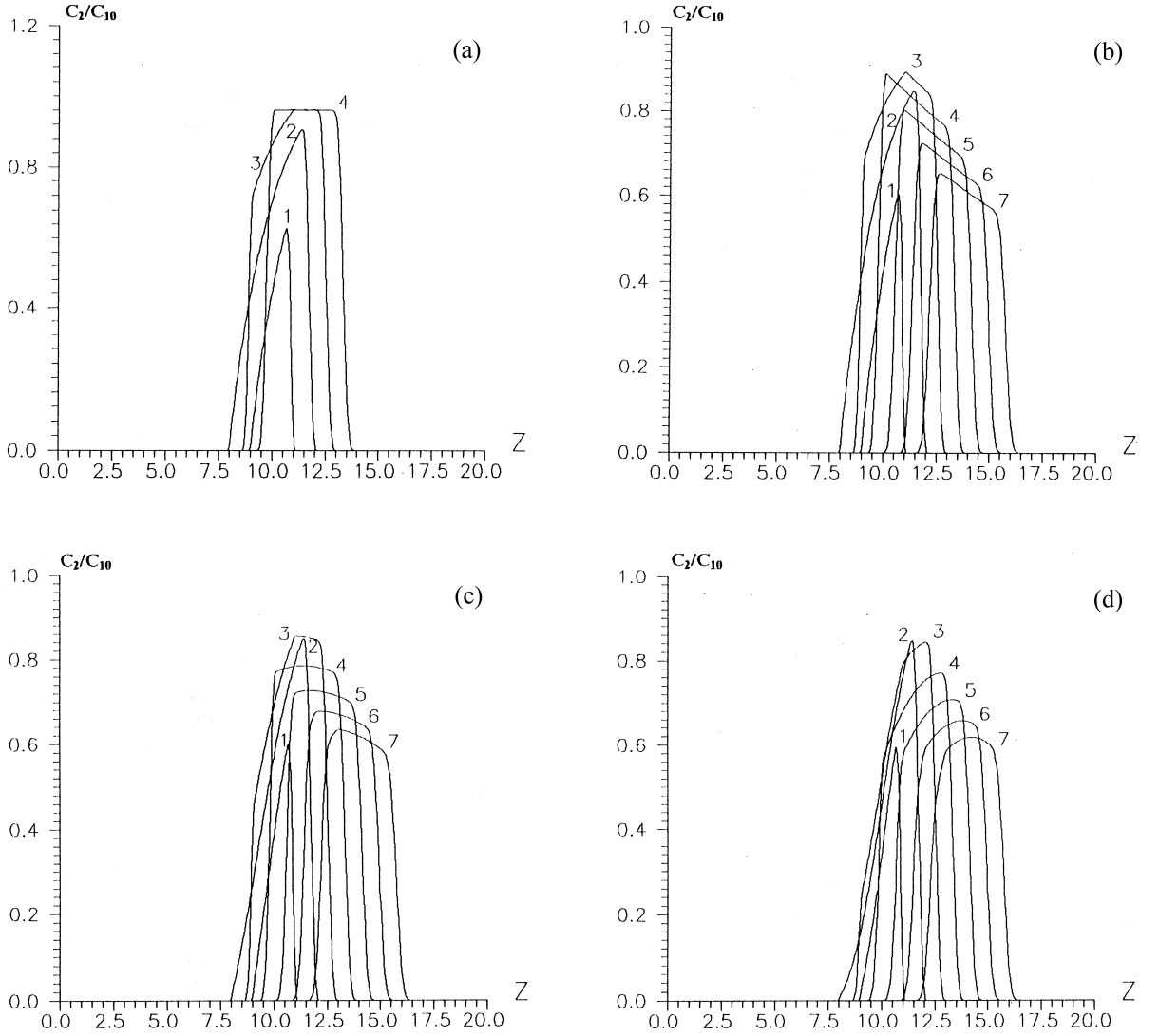

Fig. 3. (a) Product concentration versus coordinate for EIA with counterdirected motion of reagent and product; $(k_{+}C_{R0})^{-1}=1$ s;. no reverse reaction $\gamma=0$; $V_1=0.25$ cm/s; $V_R=-0.25$ cm/s; $V_2=0.2$ cm/s; $C_{10}/C_{R0}=0.01$; $\alpha_m=0.00224$; $L=5$ cm; $l_s=1$ cm; $l_R=1$ cm. Peaks 1–4 are for time moments separated by 1 s. (b) Product concentration versus coordinate for EIA with counterdirected motion of reagent and product; influence of reverse reaction $\gamma=0.1$. All other parameters are the same as in (a). (c) Product concentration versus coordinate for EIA with counterdirected motion of reagent and product; influence of reverse reaction $\gamma=0.1$ and commensurability of initial concentrations of sample and reagent $C_{10}/C_{R0}=0.5$. All other parameters are the same as in (a). (d) Product concentration versus coordinate for EIA with counterdirected motion of reagent and product; influence of reverse reaction $\gamma=0.1$ and equality of initial concentrations of sample and reagent $C_{10}/C_{R0}=1$. All other parameters are the same as in (a).

moments of time gather in these cases in the same spatial domain. It is most easily understood for the case where the concentration of reagent is much higher than the concentration of sample. Then all the events are happening at the front of reagent. Sample is reacting with reagent at its front and is practically all consumed at the front, so that it does not penetrate into the reagent zone. If the velocities of product V_2 and reagent V_R are different, then there is a spatial shift between the portions of the product

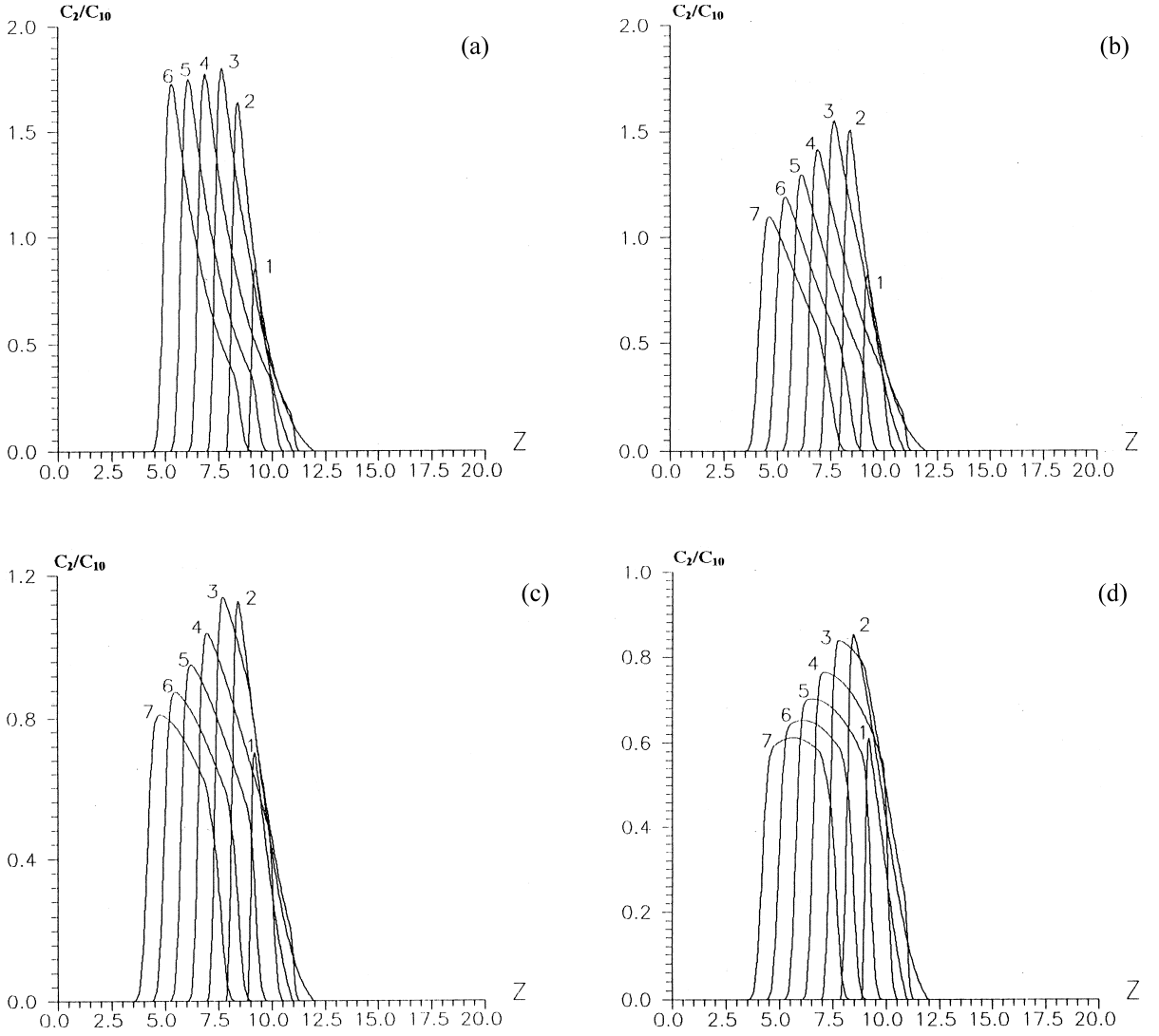

Fig. 4. (a) Product concentration versus coordinate for EIA with codirected motion of reagent and product; $(k_+C_{R0})^{-1}=1$ s; no reverse reaction $\gamma=0$; $V_1=0.25$ cm/s; $V_R=-0.25$ cm/s; $V_2=-0.2$ cm/s; $C_{10}/C_{R0}=0.01$; $\alpha_m=0.00224$; $L=5$ cm; $l_s=1$ cm; $l_R=1$ cm. Peaks 1–4 are for time moments separated by 1 s. (b) Product concentration versus coordinate for EIA with codirected motion of reagent and product; influence of reverse reaction $\gamma=0.1$. All other parameters are the same as in (a). (c) Product concentration versus coordinate for EIA with codirected motion of reagent and product; influence of reverse reaction $\gamma=0.1$ and commensurability of initial concentrations of sample and reagent $C_{10}/C_{R0}=0.5$. All other parameters are the same as in (a). (d) Product concentration versus coordinate for EIA with codirected motion of reagent and product; influence of reverse reaction $\gamma=0.1$ and equality of initial concentrations of sample and reagent $C_{10}/C_{R0}=1$. All other parameters are the same as in (a).

formed at the different moments. The shift is proportional to the difference in product and reagent velocities, and the larger is the difference the broader is the peak. If the velocities of product and reagent zones are close or equal, then all portions of product, formed at the different moments will be situated in the same spatial domain and a sharp product peak will be formed. In the case of the counterdirected motion of product and reagent the portions of the product are also formed at the reagent's front, but

they are moving in the opposite direction to the reagent front, and so the low and wide product peak is produced. When the concentrations of the sample and reagent are commensurable the significant part of the reagent at the front of the zone is consumed due to the reaction with the sample, and the sample is able to penetrate the reagent zone. The first fact leads to the decrease of the amplitude of the product portions formed at the following moments, while the second fact leads to the spatial shift between the portions of the product and together they lead to the decrease of focusing and production of much lower and wider peak than in the ideal case of ($C_{10} << C_{R0}$). For the case of counterdirected V_2 and V_R (case 2) the fact that the significant part of the reagent is consumed leads to the decrease of the amplitude of the portions of the product formed at the following moments. These portions already have the time shift, so the decrease of their amplitude cannot lead to the decrease of product peak height but leads to the decrease of its width.

Note that in every figure the ratio of C_2/C_{10} is presented, so the constant value of product peak height for different C_{10}/C_{R0} means the linearity of calibration curve C_{2max} versus C_{10}. For the second case the range of $C_{2max}(C_{10})$ linearity is very broad. So one must conclude from these results that the case of codirected V_2 and V_R leads to higher sensitivity of analysis, while the case of counterdirected V_2 and V_R leads to the larger range of linearity of calibration curves.

As it was already shown in Ref. [3] the effect of kinematic focusing is more pronounced for the case of fast chemical reactions. For $C_{10} << C_{R0}$ and $V_2 = V_R$, one has:

$$C_{2max}/C_{10} = \frac{l_s}{V_1 - V_R} k_+ C_{R0} = \frac{t_{int}}{t_c} \quad (5)$$

so that the gain in sensitivity due to kinematic focusing is equal to ratio of the time of zones interaction (t_{int}) and the characteristic time of chemical reaction (t_c). Fig. 5a–d and Fig. 6a–d present the cases of EMMA and EIA with kinematic focusing ($V_2 = V_R$) for the 10-times faster chemical reaction ($k_+ C_{R0} = 10\ s^{-1}$). Here the velocity of sample in the EMMA case is taken to be equal to $V_{1EMMA} = 0.75$ cm/s in order to make the values of time of zones interaction equal for the EIA and EMMA cases:

$$t_{int} = [l_s/(|V_1| - |V_R|)]_{EMMA} = [l_s/(|V_1| + |V_R|)]_{EIA}$$

According to Eq. (5) this choice of velocities gives the possibility to compare the properties of kinematic focusing in EIA and EMMA. Figs. 5a and 6a correspond to the ideal case ($C_{10} = 0.01 C_{R0}$ and no reverse reaction, the effect of diffusion is taken into consideration but is practically negligible). As can be seen the peak heights and peak widths are practically the same for both cases (note that in the case of EMMA product peak is moving in the positive direction, while in EIA case it is moving in negative direction). Figs. 5b and 6b illustrate the influence of reverse reaction, that in both cases leads to the two-times decrease of the amplitude of the largest peaks, with even more significant decrease of the amplitudes of peaks, following the largest ones, and increase of their widths. So the influence of reverse reaction on the peak shape is practically the same for EMMA and EIA cases. It can be explained as follows. The product is formed and focused at the reagent front. The sample that is produced due to the reverse reaction is also formed at the reagent front. During the characteristic time of chemical reaction t_c it penetrates inside the reagent zone, and the mean distance it travels is proportional to $t_c(|V_1| - |V_R|)$ for EMMA and $t_c(|V_1| + |V_R|)$ for EIA, then it is again consumed and the product is formed at the above mentioned distance from the reagent front. As the times of interaction are chosen to be equal for the studied examples of EIA and EMMA then the peak widths are also equal for both cases. Figs. 5c and 6c are for the cases where $C_{10} = 0.1 C_{R0}$. Commensurability of sample and reagent concentrations leads to more than 30% decrease of the amplitudes of the peaks. Another important feature that can be seen from these figures and from Figs. 5d and 6d ($C_{10} = 0.5 C_{R0}$) is that the distance between the peak maximums for EMMA and EIA cases are not equal now. Note that the product velocities and the time intervals are equal for both cases, and the distances between the peaks are equal for the case of small sample concentrations (Figs. 5a and 6a). The explanation of this result is rather evident. When $C_{10} << C_{R0}$, then the reagent is practically not consumed during the reaction so that product peaks are formed at the reagent zone front, and are moving with its velocity that is equal to the velocity of reagent

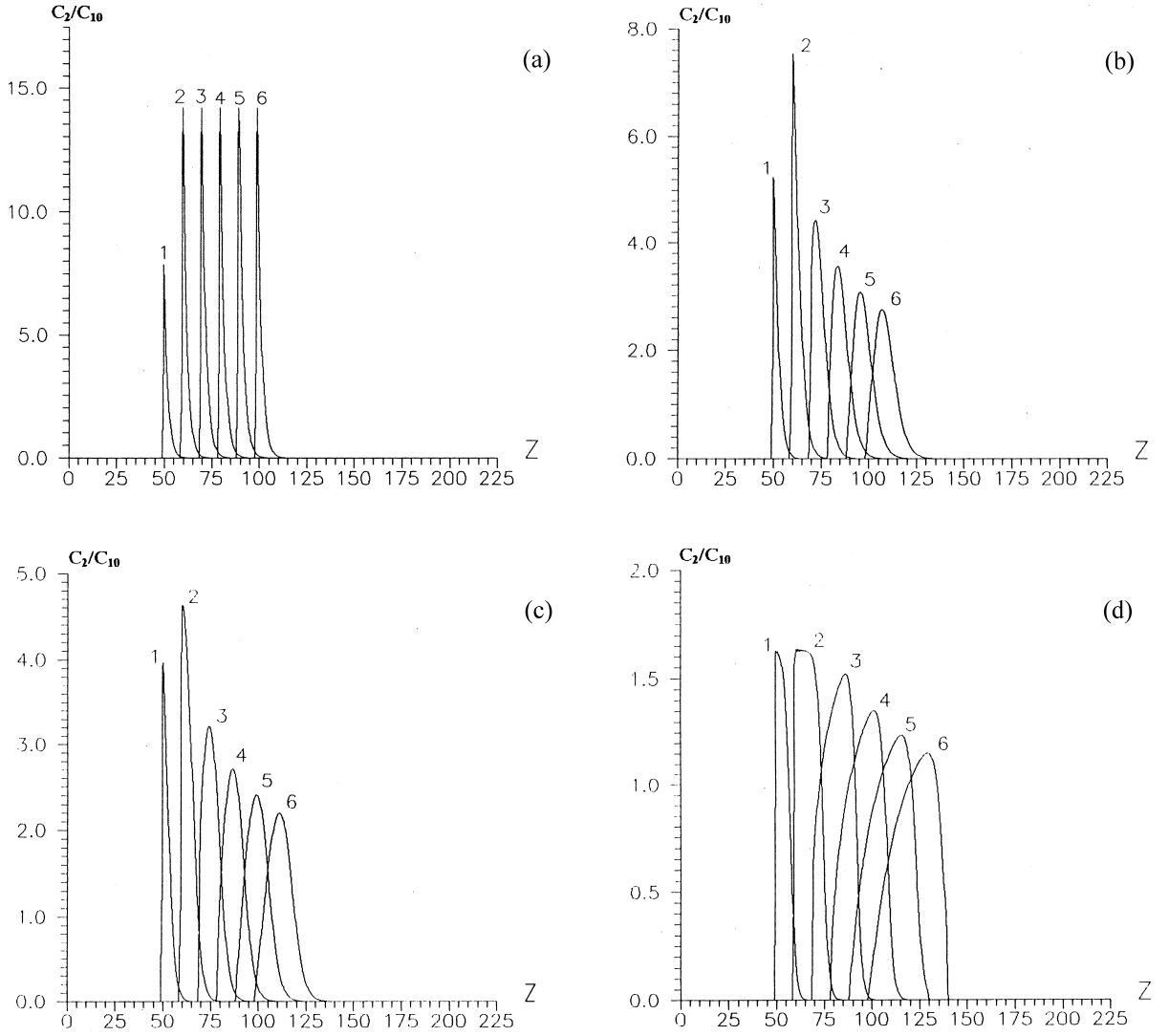

Fig. 5. (a) Product concentration versus coordinate for EMMA with kinematic focusing; fast reaction $(k_{+}C_{R0})^{-1}=0.1$ s; no reverse reaction $\gamma=0$; $\alpha_{m}=0.00224$; $C_{10}/C_{R0}=0.01$; $V_{1}=0.75$ cm/s, $V_{R}=0.25$ cm/s, $V_{2}=0.25$ cm/s; $L=5$ cm; $l_{s}=1$ cm; $l_{R}=1$ cm. Peaks 1–6 are for time moments separated by 1 s. (b) Product concentration versus coordinate for EMMA with kinematic focusing; fast reaction; influence of reverse reaction $\gamma=0.1$. All other parameters are the same as in (a). (c) Product concentration versus coordinate for EMMA with kinematic focusing; fast reaction; influence of reverse reaction $\gamma=0.1$ and commensurability of sample and reagent concentration $C_{10}/C_{R0}=0.1$. All other parameters are the same as in (a). (d) Product concentration versus coordinate for EMMA with kinematic focusing; fast reaction; influence of reverse reaction $\gamma=0.1$ and commensurability of sample and reagent concentration $C_{10}/C_{R0}=0.5$. All other parameters are the same as in (a).

molecules. When the concentrations of sample and reagent are commensurable, then the reagent is consumed during the reaction, so the velocity of the reagent zone front is now not equal to the velocity of the reagent molecules, but depends on the velocity of the sample, sample concentration and reaction rate. For EMMA, where sample and reagent are moving in the same direction, velocity of the reagent zone front is larger than the velocity of reagent molecules. For EIA, where sample and reagent are moving in

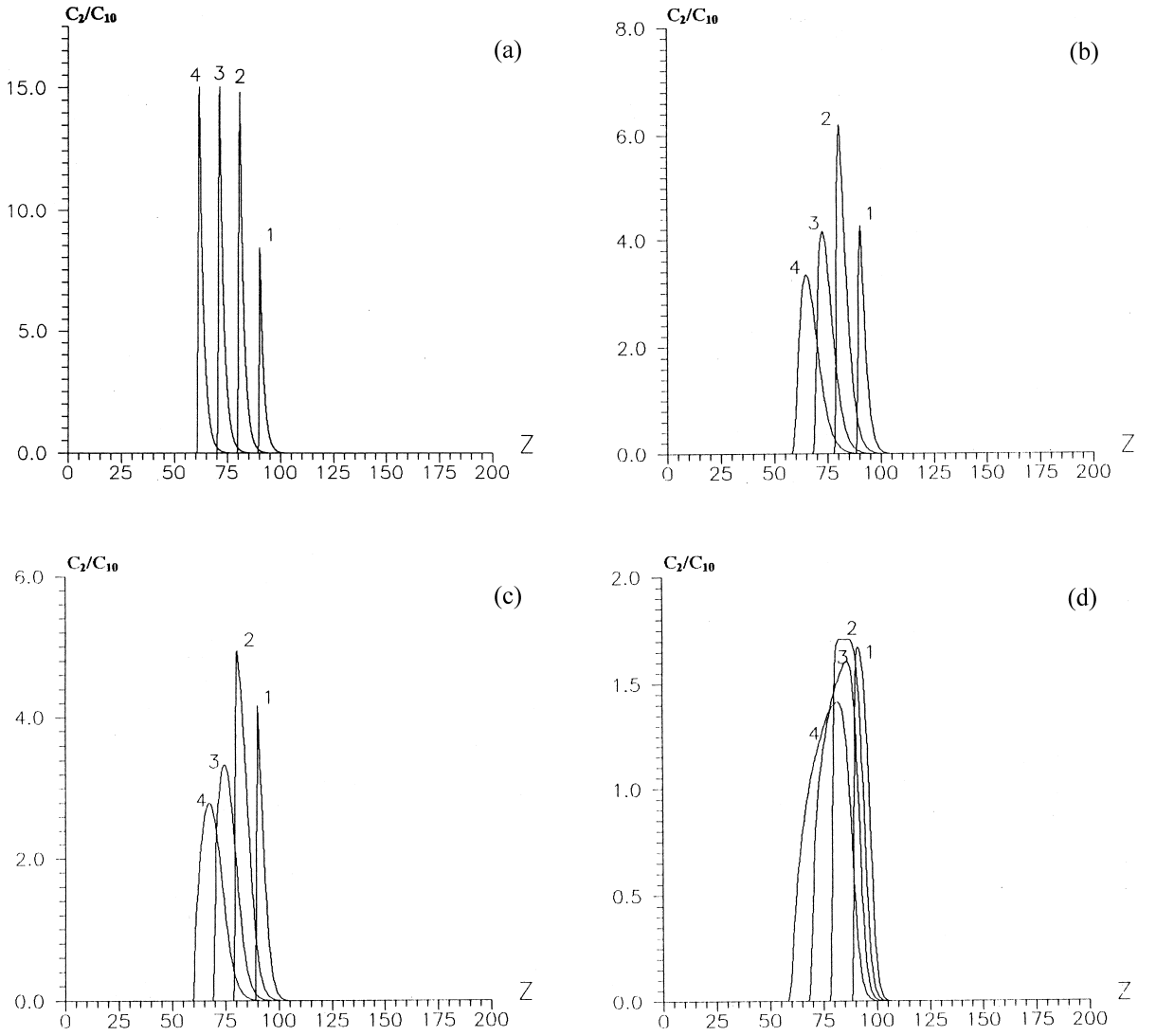

Fig. 6. (a) Product concentration versus coordinate for EIA with kinematic focusing; fast reaction $(k_{+}C_{R0})^{-1}=0.1$ s; no reverse reaction $\gamma=0$, $C_{10}/C_{R0}=0.01$, $V_1=0.25$ cm/s, $V_R=-0.25$ cm/s, $V_2=-0.25$ cm/s, $\alpha_m=0.00224$; $L=5$ cm; $l_s=1$ cm; $l_R=1$ cm. Peaks 1–4 are for time moments separated by 1 s. (b) Product concentration versus coordinate for EIA with kinematic focusing; influence of reverse reaction $\gamma=0.1$. All other parameters are the same as in (a). (c) Product concentration versus coordinate for EIA with kinematic focusing; influence of reverse reaction $\gamma=0.1$ and commensurability of initial concentrations of sample and reagent $C_{10}/C_{R0}=0.1$. All other parameters are the same as in (a). (d) Product concentration versus coordinate for EIA with kinematic focusing; influence of reverse reaction $\gamma=0.1$ and commensurability of initial concentrations of sample and reagent $C_{10}/C_{R0}=0.5$. All other parameters are the same as in (a).

the opposite directions, velocity of the reagent zone front is smaller than the velocity of the reagent molecules. That is why the distance between peak maximums is increasing with C_{10}/C_{R0} for EMMA and is decreasing with C_{10}/C_{R0} for EIA. Peak amplitudes are a little bit larger for EIA than for EMMA case (see Figs. 5d and 6d). Nevertheless from the practical point of view the properties of kinematic focusing in EIA and EMMA are nearly the same if the values of zones interaction time are equal. Note that for the case of equal zone interaction time and optimized position of the detector the

values of time of analysis will differ for EIA and EMMA only by the time of injection. The later is smaller for EIA because sample and reagent are injected simultaneously unlike EMMA, where they are injected one after another.

4. Conclusion

The mathematical model of EIA and EMMA taking into consideration the effect of diffusion, reverse reaction and commensurability of reactants concentrations is developed. The influence of all these effects are studied by computer simulation. The results of the simulation are in perfect correspondence with the results of the analytical model, thus proving the validity of both models. The effect of kinematic focusing and its susceptibility to the commensurability of reactants concentrations are studied.

Acknowledgements

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