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Displacement chromatography with on-column isomerization

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Abstract

Displacement chromatography was simulated for the separation of two feed components interconverting by a reversible first order reaction and with Langmuirian adsorption behavior. The study was prompted by recent interest in the isolation of *cis* and *trans* forms of peptides containing one or more peptidyl-proline residues when the isomerization reaction interferes with the separation. The parameter values used in the simulations are similar to those found experimentally by reversed-phase chromatography and capillary electrophoresis of phenylalanine–proline dipeptide. From the concentration profiles computed by the finite difference scheme, the dependence of both the yield and production rate on the temperature, column length, flow velocity and displacer concentration was evaluated. The most important operational variable of the system is the temperature as it affects both the kinetic and adsorption parameters. The yield and production rate of the component of interest were evaluated as a function of the column length and displacer concentration under conditions that facilitate its efficient separation and the plots show an optimum. Nonetheless, optimal conditions for yield and production rate were considerably different. In the temperature range from 2 to 42°C, the yield always decreases with increasing temperatures and for all the cases, optimum yield by displacement mandates the use of conditions such as pH, solvent and temperature under which the rate of interconversion is reduced to a level where it does not palpably interfere with the separation. On the other hand, under certain conditions optimal production rate can be obtained at higher temperatures.
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1. Introduction

The displacement mode of chromatography, introduced by Tiselius [1] in 1943, may offer several advantages over the elution mode in process scale separations [2–14] that include the recovery of products at relatively high concentrations, low solvent requirements and an easy control of the speed and efficiency of process with the displacer serving as an additional separating agent.

This work was prompted by the recent interest shown in the separation of various interconverting species, particularly the *cis* and *trans* conformations of various peptides containing peptidyl-proline bond [15–19]. The relatively slow rate of interconversion is believed to be the rate-limiting step in the folding process of several proteins [20–23] and to affect the action of proline specific enzymes such as prolyl *cis–trans* isomerase (PPI), prolidase, aminopeptidase P and HIV-proteinase [24–27].

Analytical separation of *cis* and *trans* proline dipeptides is carried out traditionally by reversed-

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phase chromatography in the elution mode [28–31]. Recently, capillary zone electrophoresis at subambient temperatures [15,16] when the rate of interconversion does not interfere with the separation or at rapid speed of separation [17] has proven to be an eminently suitable technique for the analytical separation of these compounds due to the high peak efficiencies of the technique. However, for preparative separations of the conformers, chromatography still remains the choice due to its relatively high throughput. Recent work from our laboratory has demonstrated that the *cis–trans* conformers of various dipeptides and oligopeptides can be isolated by preparative RP-HPLC [32] in the elution mode at temperatures as low as −25°C. However, displacement chromatography has only been used for the preparative separations of non-interconverting *cis–trans* and other isomers [33–35].

Mathematical modelling of displacement chromatography and numerical solution of the resulting differential equations has been carried out by several authors [5,11,12,36–38]. Here, we examine the potential of displacement chromatography for the separation of such isomers by simulating the separation of interconverting species under various conditions as far as the temperature, displacer concentration, column length and flow velocity are concerned. The results are presented here by illustrating the effect of the operational parameters on the yield and throughput of one of the two feed components having desired purity. They are expected to aid the optimization and scale-up of the displacement chromatographic process for the separation of such interconverting species at large.

2. Interplay of reaction and separation in linear elution chromatography and electrophoresis

Earlier treatments of the on-column reaction and migration in linear elution chromatography and capillary zone electrophoresis from our laboratory [16,29,30] used the dimensionless Damköhler number, *Da*, which expresses the relative magnitude of the migration time and the relaxation time for the reaction, to characterize the interference of the reaction with the separation in both differential migration processes. For a reversible first order reaction in the chromatographic column, the Damköhler number has been defined as [29]

$$Da = L(k_{sf}k' + k_{mf})(1 + 1/K_m)/u_0 \quad (1)$$

where k_{mf} and k_{sf} are the overall forward rate constants in the mobile and stationary phases, K_m is the equilibrium constant representing the ratio of the overall forward and backward rate constants for the *cis–trans* isomerization reaction, L is the column length, u_0 is the velocity of the mobile phase and k' is the retention factor of one of the eluites. In capillary zone electrophoresis Eq. (1) reduces to [16]

$$Da = Lk_f(1 + 1/K_m)/u_0 \quad (2)$$

where k_f is the forward rate constant.

As the interference by the reaction manifests itself in additional band spreading, the efficiency of separation in such a system depends on the magnitude of *Da*. It has been shown both in the chromatographic and electrophoretic practice that the most convenient and effective way to obtain satisfactory separations is to change the column temperature as opposed to changing the column length and/or the flow velocity [15,16,32]. Fig. 1 schematically illustrates the dependence on the Damköhler number of the interference by the reaction on the separation process. The level of interference is greatest when $Da > Da_H$ and so the characteristic time of the reaction is short with respect to the residence time in the column due to the use of relatively long column and/or low flow

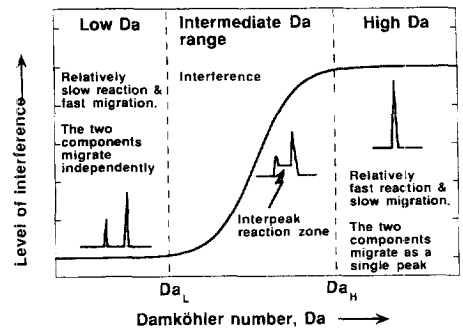


Fig. 1. Graph illustrating the level of interference by a reversible first order isomerization reaction on the separation of the interconverting species as a function of the Damköhler number. The peaks in the three domains of *Da* are representative for the results in HPLC and CZE. The lower and higher bounds on the zone of interference are at Da_L and Da_H, respectively.

velocity. At high enough values of *Da*, the two reacting components interconvert so fast that they appear as a single peak on the chromatogram. In contradistinction, when *Da* is small ($Da < Da_L$) the migration time of the two components is much smaller than the relaxation time of the reaction, i.e. the reaction kinetics is relatively slow and/or the column is short and/or the flow velocity is high. At sufficiently low *Da* values the two components migrate independently as if no reaction had taken place and at sufficiently high efficiency and selectivity appear as two separate peaks.

For the *cis–trans* isomerization of peptidyl-proline containing dipeptides, the migration times are commensurate with the characteristic time of the reaction at intermediate Damköhler numbers, $0.2 < Da < 10$ for linear elution chromatography [29], and $0.1 < Da < 0.5$ for CZE [16]. In this case the interference by the reaction leads to formation of peaks having "unchromatographic shape" and more than often manifests as an interpeak reaction zone which has an untoward effect on the separation. The magnitude of *Da* which reflects this interference depends on all operating conditions that determine the migration and kinetics of the process such as the column length, the flow velocity and the temperature.

In HPLC and CZE, the separation is expected to be a linear process so that the concentrations of the two interconverting species are coupled only via the first order reaction. In such cases, the Damköhler number is a useful parameter to express the level of interference. However, displacement chromatography is a non-linear process and the Damköhler number is not expected to be a suitable measure of interference. For this reason the Damköhler number is not used in the present work.

3. Mathematical model

3.1. Adsorption isotherms

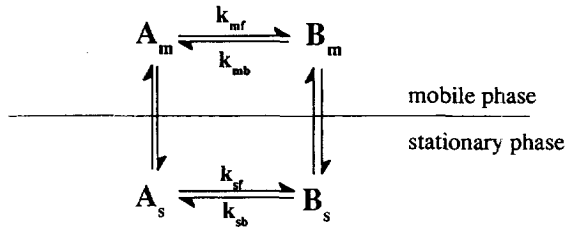
The chromatographic system under consideration consists of the two interconverting feed components, A and B, and the displacer, C, that are denoted by subscripts 1, 2 and 3, respectively. The adsorption of the three components by the stationary phase under conditions employed in displacement chromatography is represented by the competitive Langmuir isotherm given by

$$q_i = \frac{a_i c_i}{1 + \sum_{j=1}^{3} b_j c_j} \qquad \text{for } i = 1 \text{ to } 3 \tag{3}$$

where c_i and q_i are the respective mobile and stationary phase concentrations of species i and a_i and b_i are their Langmuir isotherm parameters. The ease of mathematical handling of the Langmuir isotherms makes them an attractive choice for mathematical analysis [5,39,40] and since *cis* and *trans* peptides are very similar in size, structure, charge and mass [16,28,30] it is expected that the selected isotherms will be applicable for this system.

3.2. Reaction kinetics

The reversible interconversion of the two feed components is assumed to be of first order and taking place both in the stationary and mobile phases. It is schematically represented as [29]



The forward rate constants are k_{sf} and k_{mf} in the stationary and mobile phases, respectively, and k_{sb} and k_{mb} are the corresponding backward rate constants. The overall reaction scheme is

$$A \underset{k_b}{\overset{k_f}{\Leftrightarrow}} B$$

where k_f and k_b are the rate constants of the overall forward and backward reaction and can be expressed in terms of the individual rate constants as [29]

$$k_f = k_{sf}\phi\left(\frac{q_1}{c_1}\right) + k_{mf} \tag{4}$$

and

$$k_b = k_{sb}\phi\left(\frac{q_2}{c_2}\right) + k_{mb}. \tag{5}$$

The phase ratio, ϕ, in the above equations is defined as

$$\phi = \frac{1-\varepsilon}{\varepsilon} \tag{6}$$

where ε is the total porosity of the column packing.

3.3. Material balance equations and boundary conditions

The mathematical modelling of the above system involves two steps. First we consider the chromatographic system with no reaction and then we take into account on-column reaction also.

Material balance equation for the non-reacting system is written as

$$\frac{\partial c_i}{\partial t} + u_0 \frac{\partial c_i}{\partial z} + \left(\frac{1-\varepsilon}{\varepsilon}\right) \frac{\partial q_i}{\partial t} - \mathscr{D}_i \frac{\partial^2 c_i}{\partial z^2} = 0$$

for $i = 1$ to 3 (7)

where $\mathscr{D}_i$ is the axial dispersion coefficient, u_0 is the mobile phase velocity and t and z are the temporal and spatial coordinates, respectively. We assume that the mass transfer between the phases and adsorption kinetics are adequately fast so that mass transfer resistances in both the phases and kinetic resistances in the stationary phase can be ignored so that band broadening is assumed to take place solely due to longitudinal diffusion. In practice this translates to the use of highly efficient columns to compensate for the untoward effect of the on-column reaction on the yield.

The equations represented by Eq. (7) for the three components are coupled by the multicomponent Langmuir isotherms, given in Eq. (3). In light of Eqs. (3)–(7), displacement chromatography with the first order reversible isomerization reaction is described by the following three equations

For feed component A

$$\frac{\partial c_1}{\partial t} + u_0 \frac{\partial c_1}{\partial z} + \left(\frac{1-\varepsilon}{\varepsilon}\right) \frac{\partial q_1}{\partial t} - \mathscr{D}_1 \frac{\partial^2 c_1}{\partial z^2} + k_f c_1 - k_b c_2 = 0 \tag{8}$$

for feed component B

$$\frac{\partial c_2}{\partial t} + u_0 \frac{\partial c_2}{\partial z} + \left(\frac{1-\varepsilon}{\varepsilon}\right) \frac{\partial q_2}{\partial t} - \mathscr{D}_2 \frac{\partial^2 c_2}{\partial z^2} + k_b c_2 - k_f c_1 = 0 \tag{9}$$

and for the displacer

$$\frac{\partial c_3}{\partial t} + u_0 \frac{\partial c_3}{\partial z} + \left(\frac{1-\varepsilon}{\varepsilon}\right) \frac{\partial q_3}{\partial t} - \mathscr{D}_3 \frac{\partial^2 c_3}{\partial z^2} = 0. \tag{10}$$

It is seen that Eq. (8) and Eq. (9) are coupled also by the term expressing the isomerization reaction.

The differential equations listed above are second order in space and first order in time coordinates so that each requires two boundary conditions and one initial condition to be specified as given below.

Boundary conditions for components 1 and 2

$$c_i(t, 0) = c_{0,i} \quad \text{for } i = 1, 2 \text{ and } 0 \le t \le t_0 \tag{11}$$

and

$$c_i(t, 0) = 0 \quad \text{for } i = 1, 2 \text{ and } t_0 < t$$

and for the displacer

$$c_3(t, 0) = 0 \quad 0 \le t \le t_0 \tag{12}$$

and

$$c_3(t, 0) = c_{0,3} \quad t_0 < t$$

where t_0 is the feed time and $c_{0,i}$ is the concentration of the ith component in the feed.

Initial condition for the three components is given by

$$c_i(x, 0) = 0 \quad \text{for } i = 1 \text{ to } 3 \quad x > 0 \tag{13}$$

3.4. Numerical calculations

The finite difference method was used to solve the above system of equations with there boundary and initial conditions. Discretization of the derivatives across a grid in both the temporal and spatial directions is carried out and the truncation error, frequently referred to as "numerical dispersion", is accounted for by dropping the diffusion term and selecting the space and time integration increments so that the dispersion term matches exactly the neglected axial diffusion term as follows [37,41]

$$\Delta z = \frac{2\mathscr{D}}{u_0} \tag{14}$$

The procedure used for discretization of the derivatives is well established in the literature [5,42]. The solution of Eqs. (8)–(14) yielded the concentration profiles of the three components that were plotted to illustrate the behavior of the displacement system with on-column reaction.

4. Results and discussion

The influence of the various operating conditions such as temperature, the column length, the flow velocity and the displacer concentration on separation efficiency is systematically studied here. Since the parameters are coupled, the effect of one on the displacement processes is examined while keeping all the others constant. The displacement profiles have overlapping zones and the efficiency of separation is quantified by the yield of product obtained with a specified purity [6,43], Y_i, as follows

$$Y_i = 100\left(\frac{m_{i,90}}{m_{i,0}}\right) \tag{17}$$

where $m_{i,90}$ is the amount of component recovered with 90% purity and $m_{i,0}$ is the total amount of the component i in the feed. Another measure of separation efficacy employed here is the rate of production or throughput, P_i, which quantifies the amount of product of a given purity recovered per unit time. It is defined as [4,6]

$$P_i = \frac{m_{i,90}}{t_c} \tag{18}$$

where t_c is the total cycle time that includes the time of migration of the displacement front across the column, the feed time and the time required for column regeneration and equilibration. When the feed and regeneration times are fixed then the cycle time is affected solely by the time of migration of the displacement front.

The final results are presented here in form of displacement chromatograms and plots of the yield and production rate of component A to illustrate the effect of certain operational variables on the quality of separation. Conditions have been chosen so that the reaction kinetics favors the production of A and hence under these conditions the yield of B is poor. However, if B is the component of interest, the separation should be carried out under a different set of operating conditions in order to obtain optimal yield and production rates for B.

4.1. Effect of temperature

Temperature has been found to be the most important operational variable to affect the efficiency of separation of interconverting species in HPLC and CZE [16,29,30,32]. It has been shown to have a large effect on the kinetic and a relatively modest effect on adsorption parameters. The importance of temperature as a process variable and the use of elevated temperatures to enhance the speed of separation of proteins and polypeptides has been recently proven [44,45]. Table 1 lists the values of Langmuir isotherm parameters of the three components and the forward rate constants of the two reacting species that were used in this study for the simulation of the displacement process. The parameters of the adsorption isotherms at 10°C were from the literature [5] and enthalpy for both "a_i and b_i" was taken as −2.5 and −0.704 kcal/mol, respectively [46] (1 cal=4.184 J). The forward rate constant, k_{mf}, at 10°C and the activation energy for the reaction are based on experimental data measured in our laboratory for the *cis–trans* isomerization of L-Phe–L-Pro dipeptide [15]. Arrhenius dependency of the forward rate constant was assumed and the other rate constants are expressed in terms of k_{mf} as: $k_{mb}=5k_{mf}$, $k_{sf}=0.9k_{mf}$ and $k_{sb}=2.7k_{sf}$ [16].

In the absence of interconversion the major effect of an increase in temperature is to cause the velocity of the displacement front to increase and so the bands traverse the column faster. The effect of temperature on the concentration profiles is illustrated in Fig. 2a, and it is seen that in the case of no reaction at 2°C, the separation is nearly complete and it deteriorates upon increasing the temperature to 17°C. At 42°C due to the relatively high velocity of the displacement front, only very poor separation was attained with a 25 cm long column.

In a reactive system, the concentration profiles simulated at different temperatures demonstrate that the reaction kinetics has a very large effect on the extent of band overlap. As shown in Fig. 2b, the overlapping region between the separated bands,

Table 1
Forward rate constants of the on-column isomerization reaction and the isotherm parameters of the interconverting feed components A and B and of the displacer used in the simulations

Temperature (°C)	Forward rate constant, k_{mf} (l mol^{-1} s^{-1})	Langmuirian isotherm parameters					
		a_1	a_2	a_3	b_1 (l mol^{-1})	b_2 (l mol^{-1})	b_3 (l mol^{-1})
2	1.0×10^{-5}	24.2	29.1	34.9	13.4	16.1	19.4
6	8.0×10^{-5}	21.3	25.5	30.6	13.0	15.5	18.6
10	1.0×10^{-4}	18.8	22.5	27.0	12.5	15.0	18.0
17	3.3×10^{-4}	15.2	18.2	21.8	11.8	14.1	17.0
22	5.0×10^{-4}	13.1	15.7	18.8	11.3	13.6	16.3
42	5.0×10^{-3}	7.6	9.2	11.0	9.7	11.6	14.0

The adsorption isotherms at 10°C were taken from literature [5] and enthalpy changes associated with the Langmuirian parameters "a_i and b_i" were −2.5 and −0.704 kcal/mol, respectively [46]. The activation energy of 9.8 kcal/mol [32] was taken for the isomerization reaction. The relationships between the rate constants were as follows: $k_{mb}=5k_{mf}$, $k_{sf}=0.9k_{mf}$ and $k_{sb}=2.7k_{sf}$ [16]. The phase ratio was 0.25.

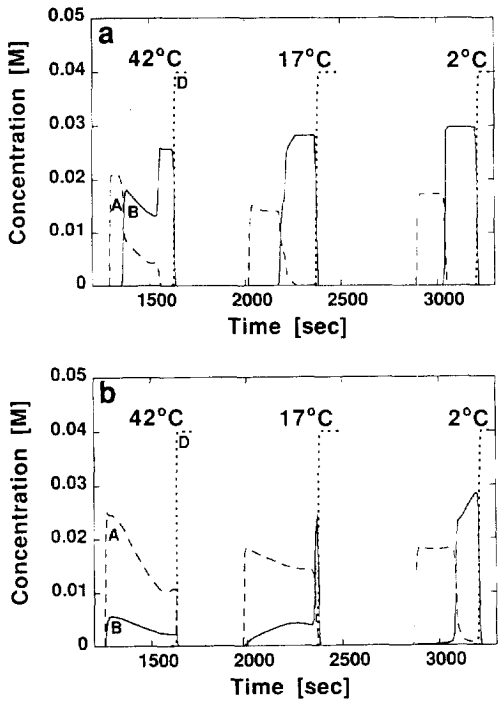


Fig. 2. Simulated concentration profiles in displacement chromatography at various column temperatures (a) without and (b) with reversible on-column isomerization reaction. Conditions: ε, 0.8; ϕ, 0.25; u_0, 5×10^{-2} cm/s; column I.D., 0.46 cm; feed time, 250 s; diffusivity of all three components of the system, D_i, 1×10^{-5} cm^2/s; feed concentrations, $c_{01}=0.01$ M, $c_{02}=0.02$ M and displacer concentration, $c_{03}=0.04$ M; column length, 25 cm. The concentration profiles of components A and B, as well as the displacer, are shown by dashed, solid and dotted lines, respectively.

increases with temperature. The overlap is due to the development of reaction zone with a concomitant loss of separation efficiency. The separation is best at 2°C where the reaction kinetics is slow enough to reduce and almost eliminate interference. As the temperature in increased to 17°C, a broad reaction zone is formed leaving narrow bands of the pure components. Finally at 42°C, the reaction rate is so high that the two components form a single band and thus no separation is achieved. Also, increasing temperature decreases the adsorption parameters with the result that velocity of the displacer front increases.

An important difference between the concentration profiles in Fig. 2a,b should be noted at this point. Since the feed concentration of component B was twice that of component A, the ratio of the areas under the bands of these components reflects this in the case without reaction as shown in Fig. 2a. However, when the reaction interferes with the separation, the amount of A in the final displacement profile is nearly three times higher than that of B as shown in Fig. 2b although the feed concentrations are the same as in Fig. 2a. This shows that the product composition is determined by the equilibrium constant of the isomerization reaction.

Fig. 3 shows the yield of component A plotted against the temperature for a 25 cm column with no reaction and for 5, 10, 25 and 60 cm long columns with reaction. For all cases, a region of relatively

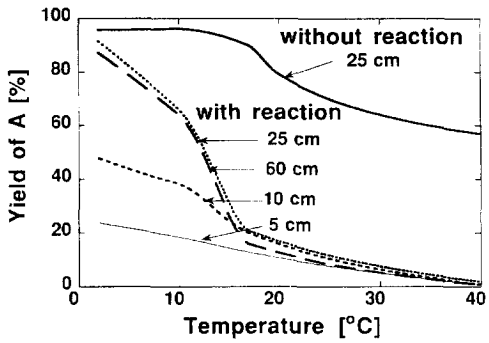


Fig. 3. Graph illustrating the dependence of the yield on the column temperature in the separation of the interconverting species by displacement chromatography with the column length as the parameter. For comparison, the plot for the case of no reaction is also shown. Conditions the same as in Fig. 2.

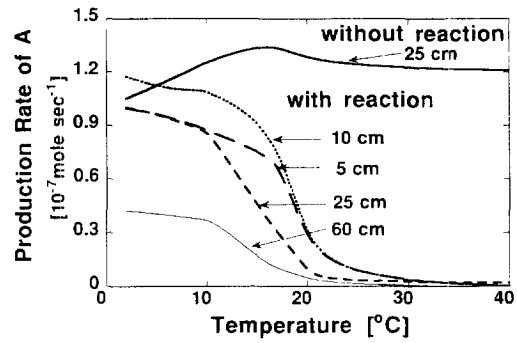


Fig. 4. Graph illustrating the dependence of the rate of production on the column temperature in the separation of the interconverting species by displacement chromatography with the column length as the parameter. For comparison, the plot for the case of no reaction is also shown. Conditions the same as in Fig. 2.

high yield obtained at low temperatures is followed by a rapid deterioration in the yield with increasing temperature. As expected from the discussion above, the yield decreases monotonically with increasing temperature irrespective of the column length. The fall occurs in a comparatively small temperature range from 10 to 16°C which appears to be a characteristic of the separation system under consideration. At temperatures higher than 16°C the slope of the yield vs. temperature plots is much smaller than in the critical temperature range. At temperatures above 35°C, the yield obtained with all four columns approaches to zero because at high temperatures the reaction time is much smaller than the time it takes the displacement front to traverse the column.

In Fig. 4 the production rate is plotted against the temperature for the cases just discussed. In the case of no reaction, the production rate initially increases with the temperature and then slightly decreases and plateaus. The initial increase in the production rate is due to the increasing velocity of the displacement train. The rise stops once the temperature becomes so high that the available column length is no longer sufficient for the development of the displacement train. Once the optimum has been reached, these two countervailing effects make the plot plateau. Comparison of Fig. 3 and Fig. 4 shows similar temperature dependence of both the yield and production rate except that the critical temperature range where the production rate falls rapidly is somewhat higher for the throughput than that for the yield. This effect is attributed to a greater decrease in migration time of the displacer front than the resulting reduction in yield in this range. Again at very high temperatures, the kinetics is too fast for any separation to occur.

4.2. Effect of column length and flow velocity

The effect of column length in displacement chromatography without any on-column reaction is illustrated in Fig. 5a at 10°C. It is seen that the separation improves as the column length increases as expected from literature [4,5]. With the 10 cm long column concentration profiles typical for the transient pattern are obtained whereas with the 40 cm long column, isotactic conditions are reached.

With on-column reaction, the effect of column length on displacement with 10, 20 and 40 cm long columns at 10°C is depicted in Fig. 5b. The results illustrate that while the quality of separation in the absence of reaction improves upon increasing the column length before reaching the isotactic pattern, with on-column reaction, however, the advantage of extending the length of the column to attain isotactic condition is thwarted by the interconversion. With increasing column length, the migration time also increases at fixed flow velocity. Fig. 5c shows the concentration profiles for column lengths of 40, 60, 80 and 90 cm. Evidently, the two countervailing effects that were mentioned above continue to take place and the concentration profiles as shown attain a

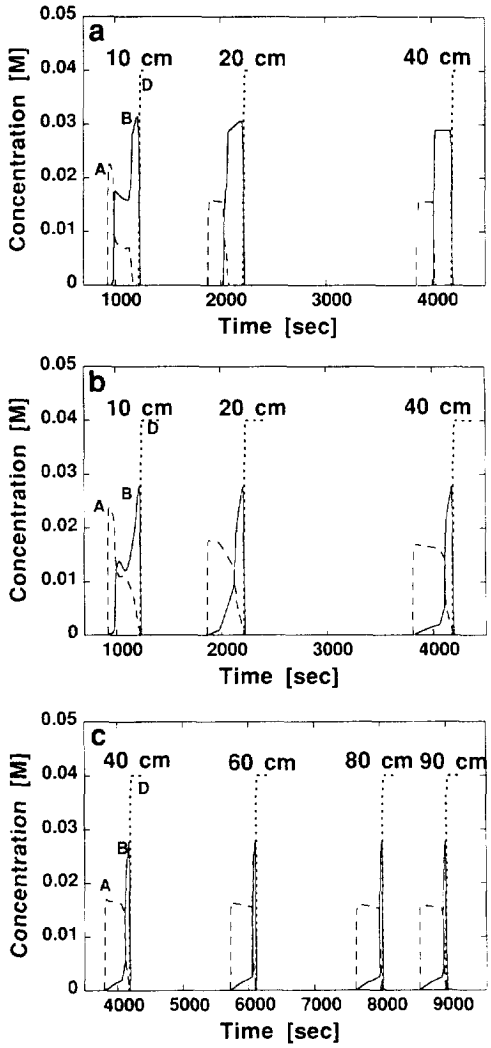


Fig. 5. Simulated concentration profiles in displacement chromatography with various column lengths (a) without reaction and (b) and (c) with isomerization reaction. Conditions same as in Fig. 2 except, column temperature, 10°C.

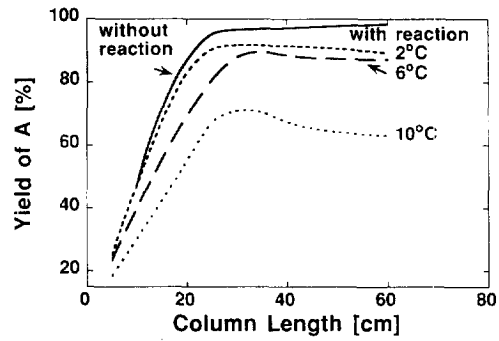


Fig. 6. Graph illustrating the dependence of the yield on the column length for the separation of the interconverting species by displacement chromatography with the column temperature as the parameter. For comparison, the plot for the case of no reaction is also shown. Conditions the same as in Fig. 5.

nearly constant pattern that can be termed as a quasi-isotactic pattern.

The effect of column length on the yield is depicted in Fig. 6 at temperatures of 2, 6 and 10°C. The topmost curve represents the case without on-column reaction at 10°C. For the case of no reaction the yield initially increases sharply till the column length reaches about 20 cm and, thereafter, the yield plateaus at about 95%. In contradistinction, with on-column isomerization the plots show an optimum yield at a column length of approximately 25 cm. With columns shorter than that, the separation process has not developed the quasi-isotactic pattern to yield good separation. Once the optimum length has been reached, however, any further increase in the column length leads to deterioration of the yield due to increasing interconversion that represents greater interference. The optimum becomes more and more pronounced with increasing temperature as the interference by on-column isomerization reaction, which is responsible for the decrease in the yield after the optimum has been reached, increases with the column temperature. Eventually, all plots reach a plateau when the self sharpening effect in displacement chromatography is matched by the countervailing band distortion caused by the reaction.

In Fig. 7 the production rate is plotted as a function of column length. We can see that unlike the yield in Fig. 6, the rate of production has an optimum when displacement takes place without interference by the reaction. This is because once the isotactic pattern is formed further increase in column length increases only the migration time but not the yield. It is seen that interference by on-column isomerization results in a shift in the optimum column length range from 25–30 cm in Fig. 6 to 15–20 cm in Fig. 7. This is so because the lower yield with a shorter column is more than compensated for by a reduction in the migration time. An increase in the column temperature is accompanied by a further decrease in the optimum column length

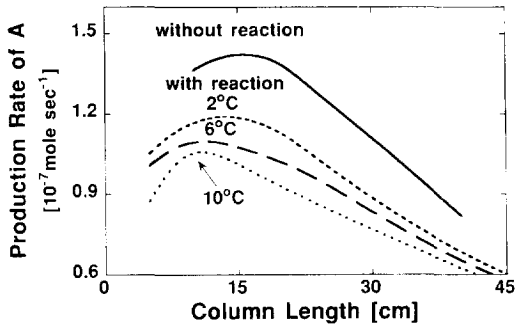


Fig. 7. Graph illustrating the dependence of the rate of production on the column length for the separation of the interconverting species by displacement chromatography with the column temperature as the parameter. For comparison, the plot for the case of no reaction is also shown. Conditions the same as in Fig. 5.

which decreases from 15 cm at 2°C to 10 cm at 10°C. This illustrates that short migration times obtained with short columns at elevated temperatures are particularly advantageous because they engender less interconversion and hence relatively high production rates.

Simulation results on the effect of the flow velocity showed that in the system under consideration, there is a reciprocal relationship between the flow velocity and the column length, i.e. the effect of increasing flow velocity is identical to that of decreasing the column length, provided the migration time is kept constant. Thus, the data presented in this section can be *mutatis mutandis* applied to describe the effect of flow velocity as well.

4.3. Effect of displacer concentration

Fig. 8a shows displacement chromatograms obtained by simulations using displacer concentrations of 0.02, 0.1 and 0.2 *M* for the case when no reaction occurs. According to the literature [4,5], an increase in the displacer concentration is usually beneficial to the separation since the displacement train travels faster so that the time of separation decreases and the concentrations of the zones increase. As shown in Fig. 8a, at the lowest displacer concentration, component A elutes with a long tail because the displacer concentration is lower than the critical value required for the displacement of the less adsorbed component. Upon increasing the displacement concentration to

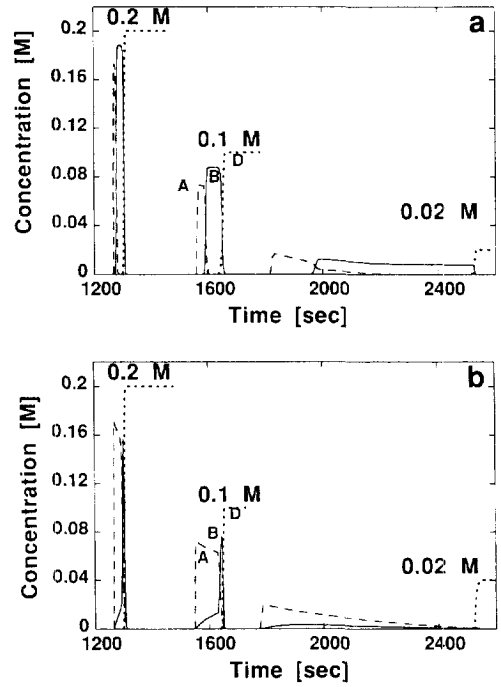


Fig. 8. Simulated concentration profiles in displacement chromatography at various displacer concentrations (a) without reaction and (b) with isomerization reaction. Conditions the same as in Fig. 2 except, column temperature, 22°C and column length, 25 cm.

0.1 *M*, nearly isotactic conditions are reached and the separation improves. However, when the displacer concentration is further increased to 0.2 *M*, the bands become so narrow that the recovery of the pure components is impeded [43].

In Fig. 8b the effect of displacer concentration is illustrated in the case when the interconversion interferes with the displacement process. At the lowest displacer concentration, the displacement profile is similar to that in overloaded elution but when the displacer concentration is increased to 0.1 *M*, the quality of separation improves significantly. At displacer concentration of 0.2 *M* the profile of A is not as narrow as shown in Fig. 8a and hence the separation is better. The relatively large zone of A with respect to that shown for the absence of reaction in Fig. 8a is due to the equilibrium constant of the reaction which favors the formation of A as discussed above in conjunction with the effect of temperature. The large zone of A allows the use of

higher displacer concentrations than the case of without reaction shown in Fig. 8a and so the separation improves when the displacer concentration increases from 0.1 M to 0.2 *M* as shown in Fig. 8b.

In Fig. 9 the dependence of the yield in the absence of reaction on the displacer concentration illustrates the optimum that is expected from the literature [4] and from the results depicted in Fig. 8a. The results of simulation when the on-column isomerization interferes with the separation, are also presented in Fig. 9 at temperatures of 2 and 22°C, and they illustrate mainly the effect of kinetics which is a strong function of temperature. As shown in Fig. 9, the isomerization reaction reduces the yield at 22°C due to formation of the reaction zone in a similar way as illustrated in Fig. 8b. Also, the increase in band size of component A due to favorable kinetics, results in a shift in the optimum displacer concentration to a slightly higher value than that in the case with no reaction. At 2°C with reaction, we see that the optimum lies at 0.07 *M* since at low temperatures the critical displacer concentration is smaller so that the curve shifts towards lower displacer concentrations.

The effect of displacer concentration on the rate of production is illustrated in Fig. 10 for the cases of no reaction and reaction at column temperatures of 2 and 22°C. The plots are similar to those shown for

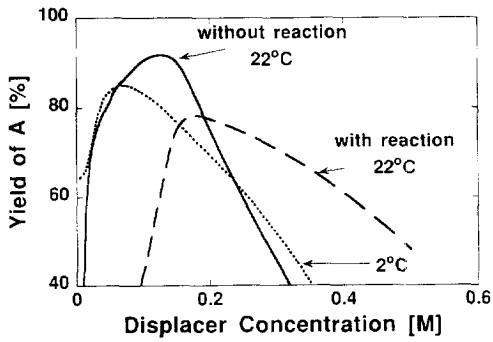


Fig. 9. Graph illustrating the dependence of the yield on the displacer concentration in the separation of the interconverting species by displacement chromatography with the column temperature as the parameter. For comparison, the plot for the case of no reaction is also shown. Conditions the same as in Fig. 8.

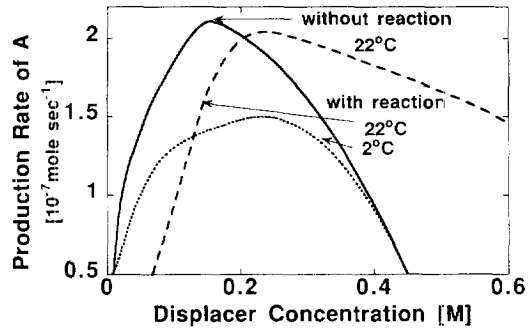


Fig. 10. Graph illustrating the dependence of the rate of production on the displacer concentration in the separation of the interconverting species by displacement chromatography with the column temperature as the parameter. For comparison, the plot for the case of no reaction is also shown. Conditions the same as in Fig. 8.

the yield in Fig. 9 and both show optima. The production rate is highest when there is no reaction and the displacer concentration is 0.15 *M*. With on-column isomerization at 22°C, the results are similar to those obtained in the non-reactive system at the same temperature and the optimal displacer concentration is 0.2 *M*, similar to the respective values in Fig. 9. The optimal displacer concentration, however, shifts from its original value of 0.07 *M* in Fig. 9 to 0.2 *M* upon lowering the temperature to 2°C. The explanation of this shift rests with the faster migration velocity of the displacer front at the higher concentration so that the decrease in yield at higher displacer concentration is more than compensated by the decrease in migration time. In contrast to the previous cases where production rates improve as we lower the temperature, here we find that the production rate is higher with reaction at 22°C than at 2°C. This is because the higher migration velocities at elevated temperatures result in shorter migration times and consequently higher production rates, the lower yield notwithstanding.

5. Conclusions

A mathematical model has been developed and the resulting non-linear equations solved in order to simulate the effect of on-column isomerization re-

action on the separation of the two interconverting conformers by displacement chromatography. The parameters used in the present study correspond to those associated with the separation of *cis–trans* isomers of peptidyl-proline dipeptides as most of the data used correspond to chromatographic and electrophoretic measurements that were performed in our laboratory for separation of the two conformers of the phenylalanine–proline dipeptide. The results presented here show that high yields and production rates for a complicated case as the present one can only be obtained under optimized conditions of pH, temperature and displacer concentration when the reaction kinetics as well as the chromatographic conditions favor the formation of the component of interest. Further extension of this work to longer peptides and proteins would require use of a more sophisticated model for adsorption isotherms. Nevertheless, the study shows that computer simulations can be a useful adjunct for optimization of the operating conditions.

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