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Amino acid separation by preparative temperature-swing chromatography with flow reversal[1]

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Abstract

A special type of adsorptive parametric pumping called temperature-swing chromatography is experimentally investigated for a new application: the separation of amino acids. Experiments have been done with a mixture of arginine, histidine and lysine in acidic solution (HCl). A multicomponent equilibrium model has been used to simulate the experimental results and to investigate some possible ways to improve the separation. It is shown that it is possible to separate these amino acids, and the composition of the products strongly depends on the experimental conditions. Arginine with purity greater than 95% can be obtained by applying these methods.

Keywords: Preparative chromatography; Temperature-swing chromatography; Parametric pumping; Adsorption isotherms; Amino acids

1. Introduction

Due to both the more and more rigorous requirement of environmental protection and the raw-material need of fine chemical industry, increasing attention is paid to the utilization of biomass origin wastes. After acidic, basic or enzymatic hydrolysis of protein containing materials, a mixture of amino acids is obtainable, which can be used as natural source for producing pure amino acids.

Generally the individual amino acids are isolated by multistep adsorption, ion-exchange methods. These processes have in common the necessity of using several chemical reagents even to buffer the mixtures during the saturation or to elute the fixed amino acids and regenerate the bed. It can therefore be said that these chemicals provide the driving force of the separation. Due to the use of chemical regenerants, regeneration effluents are produced which must be reprocessed in order to eliminate polluting products and/or to recover the reagents.

A class of processes called "thermofractionation" [1] allows substituting low-temperature energy for chemicals. This is analogous to distillation [2,3] in which the mixture is separated into two or more fractions without the intervention of other chemical species. In thermofractionation heat provides the driving force of the separation and it uses the displacement of a separation equilibrium between the

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phases under the influence of a temperature variation. The advantages of thermofractionation are the following: (i) it does not consume chemical products, (ii) it does not dilute the products, (iii) it uses heat at a low level: 10 to 90°C, and (iv) it is possible to reach very high-grade separation. Among these processes parametric pumping is a technique which has been studied extensively.

In this article a new type of adsorptive parametric pumping, called temperature-swing chromatography (described later), is experimentally investigated from the point of view of separating basic amino acids (arginine, histidine, lysine), and a multicomponent, nonlinear equilibrium model is used to predict the separation and to search the optimum conditions of the operation. Usually the amino acid mixtures to be separated are acidic. We consider here HCl as acidic medium.

2. Parametric pumping

Parametric pumping is a separation process invented by Wilhelm et al. in 1966 [4] which consists of a mobile phase percolated through a fixed phase alternating in one direction and then in the other, and the changes in temperature are applied to the phases in contact simultaneously with the changes in flow direction. It makes possible the enrichment of a given component at one end of the column and its depletion at the other end.

The principle of parametric pumping is illustrated in Fig. 1. In this figure the steps of the cyclic operation are illustrated with the help of a McCabe–Thiele-type diagram.

Among other things, the two isotherms (hot and cold) can be seen in the figure and it is clear that the uptake is higher at low temperature than at high temperature.

The operation starts with a first step to obtain the initial conditions:

Step 1: the column is equilibrated at the cold temperature with the solution to be separated and the bottom reservoir is filled with the same solution (point A on the isotherms).

After this a cycle consists of the following steps:

Step 2: the column is heated to the hot temperature (point B on the isotherms).

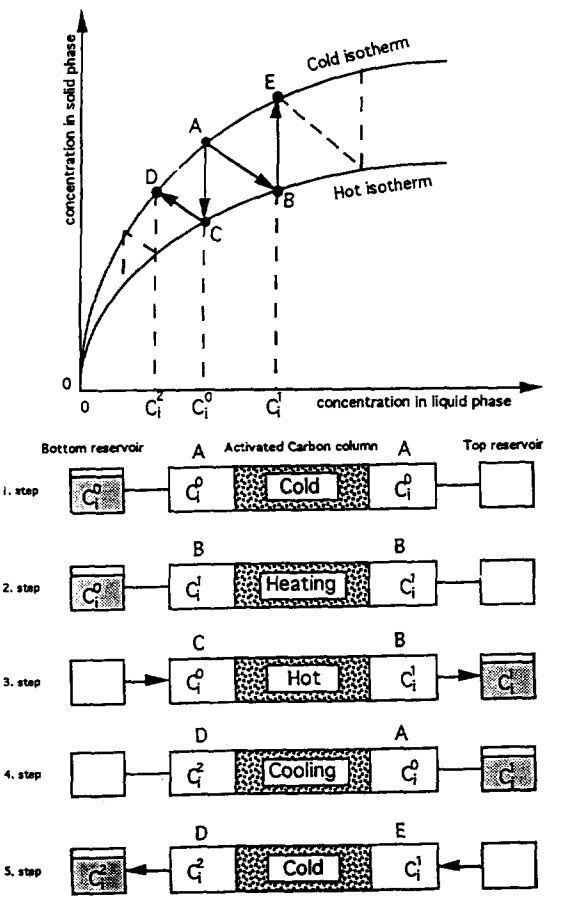

Fig. 1. Principle of parametric pumping.

Step 3: the solution from the bottom reservoir is pumped to the top reservoir through the column at the hot temperature (points C and B on the isotherms).

Step 4: the column is cooled to the cold temperature (points D and A on the isotherms).

Step 5: the solution from the top reservoir is pumped to the bottom reservoir through the column at the cold temperature (points E and D on the isotherms).

Steps 2 to 5 are repeated until the desired cycle number is reached.

It can be also seen in the figure that the concentration of component i increases in the top reservoir and decreases in the bottom reservoir. This model gives a good qualitative understanding of how the separation develops, but it is usually insufficient to represent results quantitatively.

The analogy with distillation has been described

by Grévillot and Tondeur [2,3]. In parametric pumping, the upward flow during the hot half-cycle is analogous to the vapour in distillation and the downward flow during the cold half-cycle is analogous to the liquid in distillation. The adsorption isotherms at the two temperatures play the role of the liquid–vapour equilibrium curve. Parametric pumping can be operated either at total reflux (batch) or at partial reflux in several ways (continuous, semi-continuous, intermediate feed position, ...). The temperature change can be applied to the column itself (direct mode) or by the fluid itself (recuperative mode).

Since 1966 many experimental and theoretical extensions have been made to the basic technique.

The potentiality of the technique was demonstrated by Wilhelm and Sweed [5] when they obtained a separation factor of 10^5 in a toluene–*n*-heptane–silica gel system under total reflux conditions.

Pigford et al. [6] originated an important and simple equilibrium theory (two primary assumptions of the theory were: instantaneous local equilibrium throughout the adsorption column with linear equilibria and absence of axial dispersion), and derived mathematical expressions for the performance of the batch parametric pump.

By extending the equilibrium theory, Chen et al. [7–9] have derived mathematical expressions for the performance of batch, continuous, and semi-continuous parametric pumps.

For getting more realistic results than by using the equilibrium theory, Gupta and Sweed [10] worked out a model which takes into account the non-equilibrium effects.

Sabadell and Sweed [11] carried out separation by a recuperative mode pH pump in which pH control was maintained by acid addition at one of the end reservoirs. Shaffer and Hamrin [12] combined affinity chromatography and parametric pumping to reduce enzyme concentration in an aqueous solution. Also, pH pumping was used by Chen et al. [13–17] for the separation of proteins like hemoglobin and albumin.

Oren and Soffer [18–21] extended parametric pumping to electrochemical systems for water desalting and separating isotopes.

Several other reviews [22–25] also show the interest in using parametric pumping for different kinds of separations.

3. Adsorption isotherms

To obtain a separation by parametric pumping it is necessary that the adsorption isotherms vary with temperature. We measured these isotherms for arginine, histidine and lysine on Reanal activated carbon at two temperatures, 288 and 363 K, by frontal method in a column [26]. Physical characteristics of the adsorbent are collected in Table 1.

Before use, the activated carbon was washed with HCl and rinsed with demineralyzed water. For each isotherm point the feed was a pure amino acid at the desired concentration in 0.1 *M* HCl solution. For a given amino acid, a first point of the isotherm was obtained by measuring the breakthrough curve using a feed with a low amino acid concentration. After this, the next point was obtained with a feed of greater amino acid concentration. Several points for each isotherm were determined in this way.

For each point the volume of breakthrough (defined as the inflexion point of the front) allows to calculate the amino acid uptake, which is:

$$q_{A_i} = \frac{(V_B - V_D)(C^2_{A_i} - C^1_{A_i})}{V_c} \quad (1)$$

where V_B is the volume of breakthrough, V_D is the bed dead volume, V_c is the volume of adsorbent in the column, $C^1_{A_i}$ and $C^2_{A_i}$ are the concentrations of amino acid in the liquid phase before and after the frontal chromatographic run, and q_{A_i} is the amount of adsorbed amino acid expressed on the basis of a volume unit of adsorbent.

The results are shown in Fig. 2–4. The following facts are clearly seen in these figures: (i) the amino acid uptake at cold temperature is always greater than at hot temperature, (ii) at cold temperature the

Table 1
Physical characteristics of the adsorbent

Specific surface area S_{BET} (m^2/g)	330.33
Pore volume (cm^3/g)	0.16
Average pore diameter (nm)	1.95
Particle size (μm)	<60

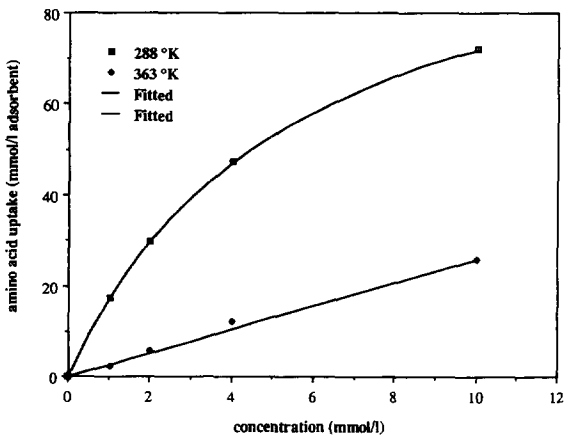

Fig. 2. Hot and cold isotherms of arginine.

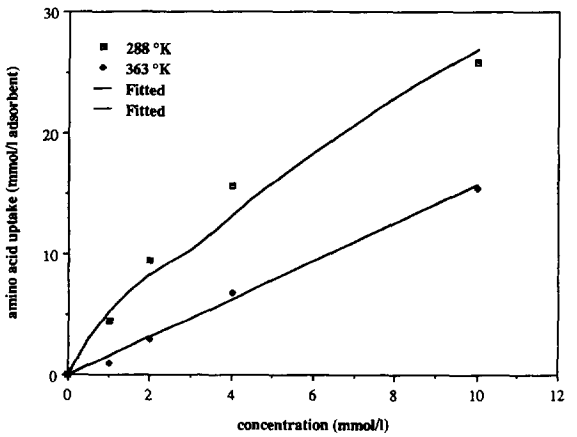

Fig. 3. Hot and cold isotherms of histidine.

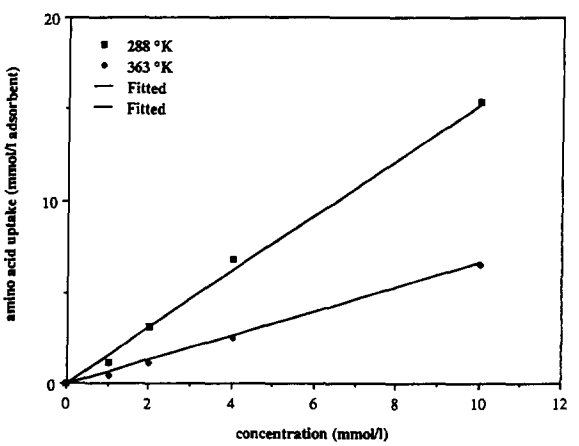

Fig. 4. Hot and cold isotherms of lysine.

decreasing order of amino acid uptake is arginine, histidine, lysine, (iii) decreasing order of the temperature dependence of the isotherm is also arginine, histidine, lysine, and (iv) the histidine uptake seems to exhibit a sigmoid-type isotherm with a slope which first increases and then decreases.

At cold temperature Langmuir-type isotherms were fitted to the measured values:

$$q_{A_i} = Q_{A_i} \frac{k_{A_i} C_{A_i}}{1 + k_{A_i} C_{A_i}} \tag{2}$$

where Q_{A_i} and k_{A_i} are the parameters of a Langmuir-type isotherm, C_{A_i} is the concentration of amino acid i in liquid-phase and q_{A_i} is the amino acid concentration in adsorbent phase.

In the case of histidine, the sigmoid shape of the isotherm was fitted by two Langmuir-type isotherms. The first one is valid when the histidine concentration is less than 3 mmol/l and the second one is used when the concentration is greater than 3 mmol/l.

At hot temperature linear isotherms were assumed, which is close to the reality as Figs. 2–4 show:

$$q_{A_i} = K^h_{A_i} C_{A_i} \tag{3}$$

where $K^h_{A_i}$ is the slope of the hot, linear, adsorption equilibrium isotherm. The equilibrium data are shown in Table 2.

Considering a thermal direct-mode parametric pumping device with a hot upward half-cycle and a cold downward half-cycle, all of the amino acids will mainly concentrate in the top reservoir and deplete in the bottom reservoir if there is not too much competition between amino acids (that is the case if the initial amino acid concentrations are low, as in

Table 2
Parameters of the isotherms at 288 and 363 K

Amino acid	288 K		363 K
	Q_{A_i}	k_{A_i}	$K^h_{A_i}$
Lysine	396	0.004	0.664
Histidine	19.4	0.376	1.57
	88.8	0.0436	
Arginine	111	0.184	2.59

our experiments). In this way, concentration of amino acids in different degrees is available, but the method is not suitable for separating them. For this purpose it is necessary to use a special type of parametric pumping called temperature-swing chromatography.

4. Temperature-swing chromatography

Temperature-swing chromatography (TSC) is a kind of batch parametric pumping. In contrast with traditional direct-mode batch parametric pumping, in this case the column is not equilibrated with the solution to be separated before starting the experiment (first step in Fig. 1 is missing), so the column is filled with fresh adsorbent. Before starting the experiment the whole solution to be separated is poured into the top reservoir. In TSC, a cycle consists of the following steps: (i) cold flow (V_{cold}) from the top reservoir to the bottom reservoir, (ii) heating, (iii) hot flow (V_{hot}) from the bottom reservoir to the top reservoir ($V_{hot} < V_{cold}$), and (iv) cooling. As a result of each cycle $V_{cold} - V_{hot}$ net volume leaves the top reservoir. The cycles are repeated until the top reservoir becomes empty, then the column is regenerated at the hot temperature.

In Grévillot's terms, this is analogous to batch rectification. The cold flow of TSC is analogous to the upward flow of vapour in batch rectification and the hot flow of TSC is analogous to the downward flow of liquid in batch rectification. In batch rectification there is no feed and a part of the top product is withdrawn and this happens also in temperature-swing chromatography, since the volume of hot flow is less than the volume of cold flow. The adsorption isotherms at the two temperatures play the role of the liquid–vapour equilibrium curve. In batch rectification only the 'light' components can reach the top of the column and the 'heavy' components remain at the bottom of the column. In TSC the components with less affinity to the adsorbent break through the column and the components with higher affinity remain at the top reservoir end of the column. In batch rectification the reflux makes the separation sharper and in TSC the hot flow, which regenerates the column, also sharpens the separation. Batch rectifications may be carried out in two ways: (i) constant overhead product composition, variable reflux ratio; (ii) variable overhead product composition, constant reflux ratio. The same operation types exist in TSC.

It follows from the foregoing that TSC is capable to use the differences between the individual cold isotherms and the degree of temperature dependence of adsorption isotherms to separate arginine, histidine and lysine as shown later in the Experimental section.

4.1. Theory of temperature-swing chromatography

Let us consider temperature-swing chromatography with a cold downward half-cycle and a hot upward half-cycle. We assume instantaneous local equilibrium, plug flow, instantaneous heating and cooling and flow reversal in phase with temperature change.

In our case the system contains three amino acids: arginine, histidine and lysine, in 0.1 *M* HCl. The material balance over a differential volume element of an adsorption column is:

$$v\frac{\partial C_{A_i}}{\partial z} + \frac{\partial C_{A_i}}{\partial t} + \frac{1-\epsilon}{\epsilon}\frac{\partial q_{A_i}}{\partial t} = 0 \tag{4}$$

where C_{A_i} is the concentration of a given amino acid in the fluid-phase, q_{A_i} is the amino acid concentration in the solid-phase, v is the interstitial velocity and $\epsilon = 0.35$ is the bed void fraction.

To solve these equations it is necessary to have a description of the uptake equilibrium on the adsorbent. The multicomponent uptake of amino acids at the cold temperature can be expressed as:

$$q_{A_i} = Q_{A_i}\frac{k_{A_i}C_{A_i}}{1+\sum_{i=1}^{3}k_{A_i}C_{A_i}} \tag{5}$$

The amino acid uptake at the hot temperature is expressed by Eq. 3. The concentrations before and after the temperature change are related by:

$$C^{c}_{A_i} + \nu\frac{Q_{A_i}k_{A_i}C^{c}_{A_i}}{1+\sum_{k=1}^{3}k_{A_i}C^{c}_{A_i}} = C^{h}_{A_i} + \nu K^{h}_{A_i}C^{h}_{A_i} \tag{6}$$

where ν is the mass capacity ratio in the bed, and $C^{c}_{A_i}$ and $C^{h}_{A_i}$ are the liquid-phase concentrations at cold and hot temperatures, respectively.

The model was solved with a finite-difference method. To carry out the numerical computation, the boundary conditions and the initial conditions are assigned. These conditions always change when a new half-cycle is started, so there is no reason to go into details. The model was used to predict the amino acid separation and to investigate possible ways to improve the separation.

5. Experimental

5.1. Materials and methods of analysis

For experiments the activated carbon and the amino acids were obtained from Reanal Factory of Laboratory Chemicals (Budapest, Hungary). The purity of the amino acids were greater then 99%, and other chemicals were of analytical reagent grade.

The amino acid concentrations were measured by an amino acid analyzer (Aminochrom II, OE-914, Labor-MIM, Budapest, Hungary). The analyzer operates on the base of the colour reaction of amino acids with ninhydrin solution.

5.2. Experiments

The schematic diagram of the experimental apparatus is shown in Fig. 5. A jacketed glass column (25 cm×1.0 cm I.D.) was filled with activated carbon and reservoirs were connected to both ends (the extra-column void volume was 1 cm^3). Constant temperature of the column was maintained by use of thermostatted baths, which supplied water at 288 and 363 K. The movement of the solution through the column was accomplished by use of a peristaltic pump. The flow-rate was 2 cm^3/min.

Before starting the experiment all the solution to be separated was poured into the top reservoir, in all cases it was 1000 cm^3, and the column was thermostatted at the cold temperature. The experiment was started by displacing V_{cold} volume of the solution at

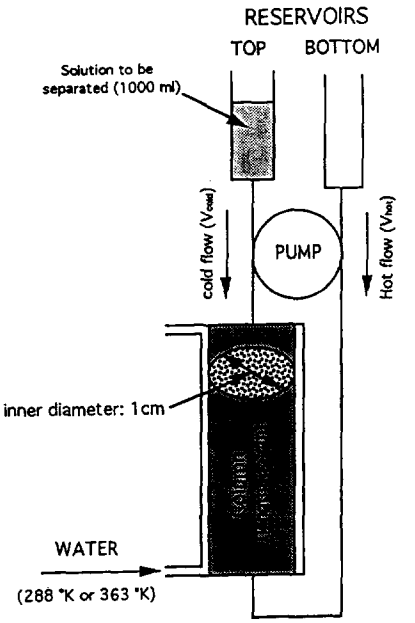

Fig. 5. Schematic diagram of the experimental apparatus.

the cold temperature from the top reservoir to the bottom reservoir. Then the column was heated and V_{hot} ($V_{hot} < V_{cold}$) volume of the bottom reservoir solution was displaced back from the bottom reservoir to the top reservoir. The first cycle was finished by cooling the column. These operations were repeated until the whole solution was displaced from the top reservoir to the bottom. Then the column was regenerated at the hot temperature with 100 cm^3 of 0.1 *M* HCl solution. In this way two products were obtained: one was the solution in the bottom reservoir (P1) and the other (P2) was the solution obtained after the hot regeneration with HCl. During the experiments, after every half-cycle samples were taken from the mixed reservoirs for analytical purposes.

Five chromatographic runs were carried out under the conditions shown in Table 3. In runs 1–3 the effect of the cold displaced volume on the separation was investigated, while the 'reflux ratio' (defined as V_{hot}/V_{cold}) was kept constant: 0.625. In runs 3–5 the effect of the 'reflux ratio' was investigated while the cold displaced volume was constant: 120 cm^3.

Table 3
Experimental conditions

Run	V_{cold} (cm^3)	V_{hot} (cm^3)	Amino acid	Initial amino acid conc. (mmol/l)	HCl conc. (mol/l)	Volume of the bed (cm^3)
1	40	25	arginine	1	0.1	24.5
			histidine	1		
			lysine	1		
2	80	50	arginine	1	0.1	23
			histidine	1		
			lysine	1		
3	120	75	arginine	1	0.1	21
			histidine	1		
			lysine	1		
4	120	60	arginine	1	0.1	21
			histidine	1		
			lysine	1		
5	120	40	arginine	1	0.1	23.5
			histidine	1		
			lysine	1		

6. Discussion

6.1. Advantage of temperature-swing chromatography in amino acid separation

It was mentioned before that traditional thermal batch parametric pumping is not suitable to separate the amino acids, but only to concentrate them in different degrees in one reservoir.

The advantage of TSC compared with simple frontal chromatography (FC) is well visible in Fig. 6. This figure shows the concentration of arginine in the bottom reservoir as a function of the effluent volume. In the frontal chromatographic experiment 1000 cm^3 solution was displaced from the top reservoir to the bottom without changing the temperature and the flow direction. All the effluent was collected and mixed in the bottom reservoir. In the case of TSC also 1000 cm^3 solution was displaced from the top reservoir to the bottom, but several temperature and flow direction changes were applied (run 4). One can see that, in the case of FC, the concentration in the bottom reservoir increases after some delay. At the end, the reservoir contains 1000 cm^3 of a solution at about 0.73 mmol/l and about 0.27 mmol are on the adsorbent. In the case of TSC, the concentration of arginine in the bottom reservoir remains zero until 900 ml and thereafter starts to increase. Thus, the appearance of arginine in the bottom reservoir is delayed relatively to FC. In fact, FC can be considered as a limiting case of TSC where only one half-cycle (cold) is performed. Therefore, by adjusting the operating parameters in TSC (cold and hot displacement volumes), the arginine appearance in the bottom reservoir can be chosen.

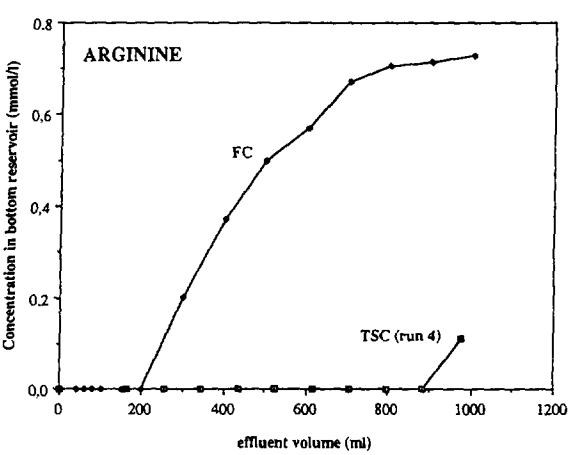

Fig. 6. Advantage of temperature-swing chromatography.

6.2. Effect of cold flow

The results of the experiments and calculations are shown in Fig. 7–11 and in Table 4. Every figure consist of a part 'a', which shows the concentrations in the bottom reservoir (the volume of this solution increases from cycle to cycle), and a part 'b' which shows the concentrations in the top reservoir. The amino acid concentrations are lower than the initial concentrations in the bottom reservoir and greater than the initial concentrations in the top reservoir.

In Figs. 7–9 the results of runs 1–3 are shown. It is clear in the parts 'a', that according to the cold isotherms the lysine concentration is the greatest, the histidine concentration is less and there is only little

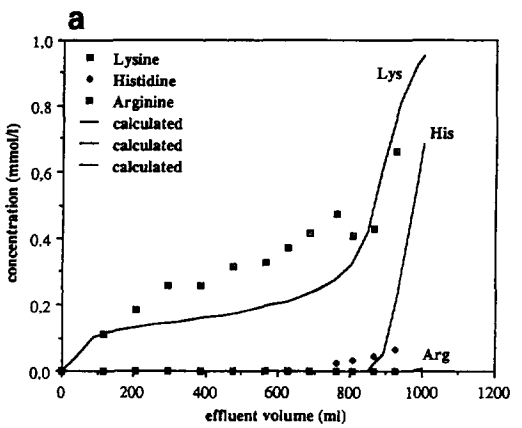

Fig. 7. (a) Bottom reservoir concentration in run 1. (b) Top reservoir concentration in run 1.

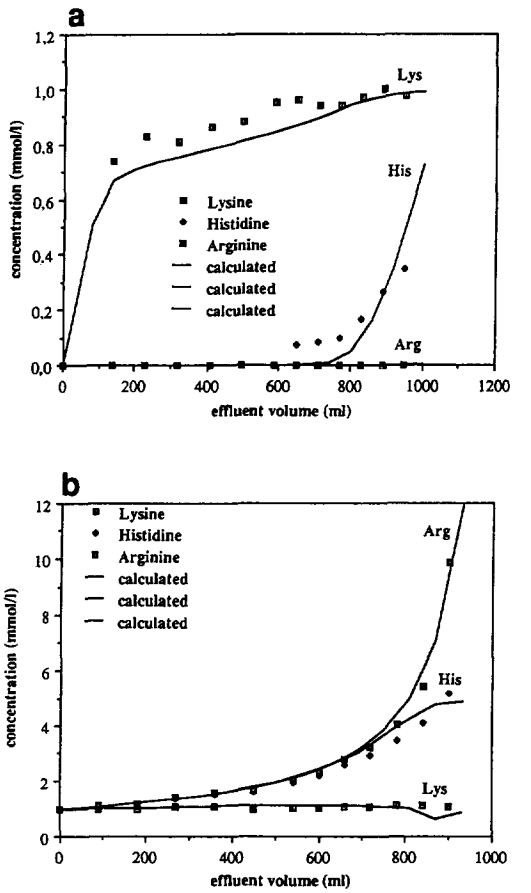

Fig. 8. (a) Bottom reservoir concentration in run 2. (b) Top reservoir concentration in run 2.

or no arginine in the bottom reservoir. It can also be seen that in run 1 (Fig. 7, $V_{cold} = 40$ cm^3) the lysine and histidine concentrations in the bottom reservoir are less than in run 2 (Fig. 8, $V_{cold} = 80$ cm^3) or in run 3 (Fig. 9, $V_{cold} = 120$ cm^3). Simultaneously, in the top reservoir the concentration of lysine is the least, that of histidine is greater and the arginine concentration is the greatest, as can be observed in the parts 'b'.

Since the lysine uptake at the cold temperature and the temperature dependence of its isotherm are the smallest, lysine breaks through the column, and the greater the displaced volume is, the more lysine is transferred to the bottom reservoir. Consequently, increasing the volume of cold flow, the lysine

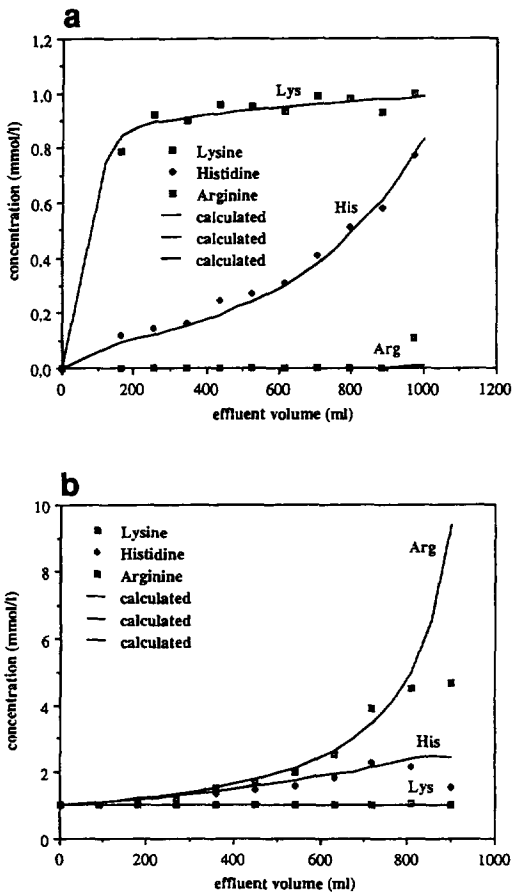

Fig. 9. (a) Bottom reservoir concentration in run 3. (b) Top reservoir concentration in run 3.

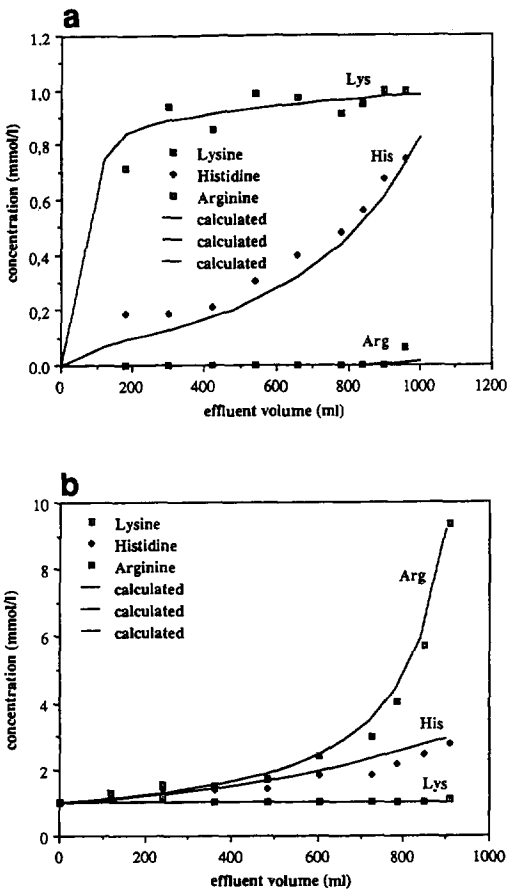

Fig. 10. (a) Bottom reservoir concentration in run 4. (b) Top reservoir concentration in run 4.

concentration increases in the bottom reservoir, and in runs 2 and 3 (Fig. 8 and Fig. 9) it practically reaches the initial concentration (the total amount of lysine is pushed to the bottom reservoir). In the meantime the lysine concentration in the top reservoir is the least due to its greatest concentration in the bottom reservoir. In terms of distillation analogy lysine is the 'light' component which is mainly in the top product (or in the P1 product, that is the bottom reservoir solution in our case).

Histidine isotherms and the temperature dependence of its isotherm occupy an intermediate position between lysine and arginine. Due to this fact its concentrations in the two reservoirs are also between the concentrations of lysine and arginine. In run 1 (Fig. 7) – when the volume of cold flow V_{cold} is the least – histidine does not break through the column, so its concentration in the bottom reservoir is practically zero and in the top reservoir it is practically equal to the concentration of arginine. In runs 2 and 3 (Fig. 8 and Fig. 9), while increasing V_{cold} more and more histidine breaks through the column, and therefore the histidine concentration increases in the bottom reservoir. In the distillation analogy histidine is a 'medium' component, which can be either in the top product or in the bottom (or either in the P1 product or in the P2 in our case), depending on the experimental conditions.

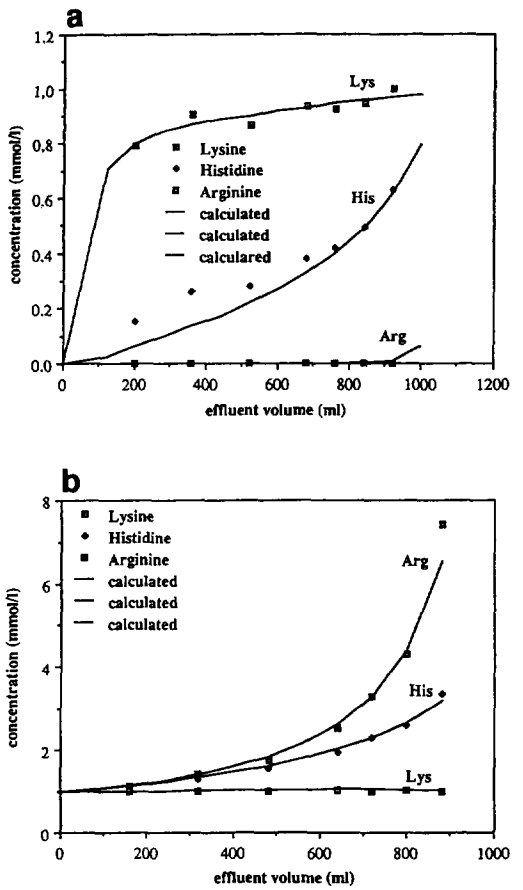

Fig. 11. (a) Bottom reservoir concentration in run 5. (b) Top reservoir concentration in run 5.

Table 4
Experimental final amino acid concentrations

Experiment	Amino acid	P1 (1000 cm^3) (mmol/l)	P2 (100 cm^3) (mmol/l)
Run 1	arginine	0.0	6.4
	histidine	0.075	6.5
	lysine	0.65	1.5
Run 2	arginine	0.13	6.24
	histidine	0.71	1.355
	lysine	0.984	0.21
Run 3	arginine	0.22	6.22
	histidine	0.83	0.93
	lysine	1.0	0.22
Run 4	arginine	0.11	8.15
	histidine	0.81	1.17
	lysine	1.0	0.23
Run 5	arginine	0.21	3.57
	histidine	0.81	0.79
	lysine	1.0	0.12

Since arginine has the greatest affinity for the adsorbent and its isotherm has the greatest temperature dependence in runs 1–3 arginine never breaks through the column. That is the reason why its concentration in the bottom reservoir is zero, and the greatest in the top reservoir. In terms of distillation analogy arginine is the 'heavy' component, which is mainly in the bottom product (or in the P2 product in our case).

Comparing the products P1 (1000 cm^3 solution in the bottom reservoir) and P2 (100 cm^3 solution, obtained after the hot regeneration) in runs 1–3 (see Table 4), we can conclude that the compositions of the products strongly depend on the volume of cold flow. If it is small, like in run 1, then P1 is almost pure lysine and P2 is a mixture of mainly arginine and histidine. If it is large, like in run 3, then P1 is a mixture of lysine and histidine and a little arginine, but P2 is mainly arginine. Another thing which can be pointed out is that P1 product concentrations depend only on the operating parameters of TSC while P2 product concentrations depend in addition on the elution parameters. There are thus several degrees of freedom to optimize any separation by this method.

6.3. *Effect of 'reflux ratio'*

In runs 3–5 the effect of 'reflux ratio' (V_{hot}/V_{cold}) on the separation was investigated. The volume of cold flow was 120 cm^3 in these experiments and the volume of hot flow was the varied parameter. In fact in this way the "reflux ratio" was changed from 0.625 to 0.5 and 0.33 in runs 3–5, respectively. The results are shown in Figs. 9–11.

Since there are no significant differences between these figures, we can conclude that in this range the effect of 'reflux ratio' is not considerable. This is no longer true out of this range, as we will show later by simulations with the model.

6.4. Discussion of the calculated results and simulations

The results of the calculations are also shown in Figs. 7–11. We might as well say at first sight that the calculated results are in good agreement with the measured ones; however, it is worth to arrange the results into groups and to investigate them according to the volume of cold flow.

6.4.1. Low volume of cold flow ($V_{cold} = 40\ cm^3$, Fig. 7)

In this case the cold flow is so small that neither arginine nor histidine break through to the bottom of the column (except a little histidine at high effluent volume). Accordingly, the descriptions of arginine and histidine concentrations both in the top and in the bottom reservoir are good; histidine is a little bit underestimated in the bottom reservoir and overestimated in the top reservoir, but this can be explained by the neglect of dispersive effects (mass transfer kinetics and flow hydrodynamics).

The most critical component in this group is the lysine, because this V_{cold} value is very close to the breakthrough volume one can calculate according to its cold isotherm; therefore, the effect of dispersion and the accuracy of the description of the isotherms might be very important. It can be seen in Fig. 7 that the prediction of lysine concentration is qualitatively good. Some discrepancy can probably be attributed to the inaccurate description of the isotherms and the dispersion.

6.4.2. Medium volume of cold flow ($V_{cold} = 80\ cm^3$, Fig. 8)

V_{cold} being greater than the breakthrough volume of lysine, it breaks through completely. It can be seen in the figure that the descriptions of lysine concentration profiles, both in the bottom and in the top reservoir, are better than in the previous case. Since its breakthrough is complete, the dispersion effects are not so important, therefore the slight differences may be caused by the inaccurate isotherm description.

In this case, when the experiment starts, the breakthrough volumes of arginine and histidine are greater than V_{cold}. After 600 cm^3 solution has been displaced to the bottom reservoir, histidine begins to appear in the solution pumped through the column due to the following: the concentrations in the top reservoir increase, the isotherm is not linear so the increase of the adsorbent capacity is not directly proportional to the concentration increase and since the volume of cold flow is constant the breakthrough volume of histidine becomes less than V_{cold} (and in the meantime arginine also pushes histidine from the top end of the column to the bottom end, due to the increasing competition with increasing concentration). At first the appearance of histidine in the bottom reservoir is underestimated (and the concentration in top reservoir is overestimated) due to the neglect of dispersion and maybe due to the inaccurate description of the isotherms. After 900 cm^3 solution has been displaced to the bottom reservoir the histidine concentration in this reservoir becomes overestimated (and underestimated in the top reservoir), and this is undoubtedly due to the inexact isotherm description in the higher histidine concentration range.

Arginine does not break through the column, so the predictions of arginine concentrations in the reservoirs are as good as in the previous case, that is for low volume of cold flow.

6.4.3. High volume of cold flow ($V_{cold} = 120\ cm^3$, Figs. 9–11)

The calculated results of histidine and lysine and the measured ones are in good agreement, since both of them break through the column completely.

Since arginine does not break through the column (except a little at high effluent volume), the predictions of its concentrations in the reservoirs are very good. It can be also observed that decreasing the reflux ratio, the calculated arginine concentration in the bottom reservoir increases, and this is also because of the less effective hot regeneration.

The effect of reflux ratio has been studied by specific simulations. For these simulations, the bed volume is 20 cm^3, $V_{cold} = 120\ cm^3$ and V_{hot} is raised from 20 to 100 cm^3. Initial composition and volume of the solution are the same as in the experiments. Results of calculations are shown in Fig. 12 for arginine. One can see in this figure that the 'reflux

ratio' has no significant effect on the separation in a wide range (from 0.33 to 0.83), but at extremely small value (0.166) the separation goes to ruin. This behaviour is analogous to distillation, where a decrease in reflux ratio leads to less separation. In the present system, there are two joined reasons: first, at constant volume of cold flow a decrease of reflux ratio means a decrease of the volume of hot flow, that is an insufficient regeneration of the column; thus the concentration fronts are closer to the bottom reservoir end of the column than in case of a larger hot flow. Second, the number of cycles required to pump 1000 cm^3 from the top reservoir to the bottom reservoir decreases with the reflux ratio. Thus the column is submitted to less cycles when the reflux ratio decreases and, consequently, the separation is worse.

7. Possible ways to improve the separation of basic amino acids

This part of the paper is clearly theoretical work and the aim is only to flash some ideas how to improve the separation, therefore the discussions are not so detailed. We stressed the separation of arginine from lysine and histidine, so the expression 'improve the separation' means how to produce purer arginine. Note that the results presented here for the different methods are examples only and probably not the optima of the given method.

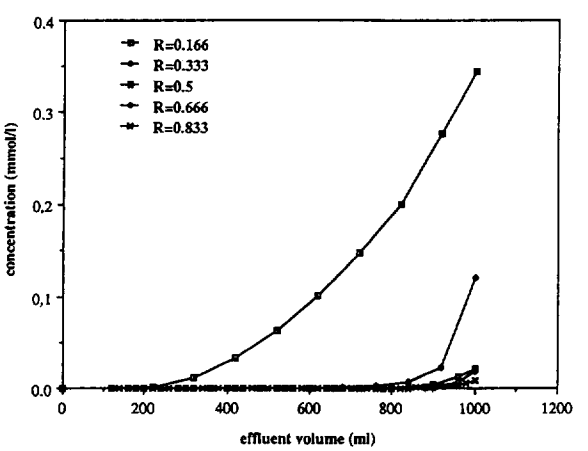

Fig. 12. Effect of 'reflux ratio' on the arginine concentration in the bottom reservoir.

7.1. First possibility to improve the separation: varied volumes of flow

In the earlier experiments and calculations we used constant volumes for cold and hot flow in a given experiment, and we could produce P1 and P2 products with a certain composition. If the goal of the work is to separate arginine from lysine and histidine, then it is necessary to move the most possible of histidine and lysine to the bottom reservoir, because the purity of arginine in P2 becomes better in this way.

For achieving greater purity of arginine, a method was developed where the volumes of cold and hot flow were not constant but depended on the arginine concentration at the outlet of the column. The solution is kept flowing at the cold temperature until the arginine concentration reaches a certain value at the outlet of the column, and then half of this V_{cold} is made to flow at the hot temperature in the opposite direction to regenerate the column. In this way the most histidine and lysine is moved to the bottom reservoir without arginine breaking through.

The results, compared with the results of constant streaming TSC (with $V_{cold} = 120$ cm^3 and $V_{hot} = 60$ cm^3), are shown in Table 5. Slight improvement in quality of P2 can be seen.

7.2. Second possibility to improve the separation: columns at different temperatures

In the previous cases the solution of hot flow which regenerated the column was a histidine and lysine containing solution and in this way we moved these back to the top reservoir. We tried to avoid this return of the two components to the top reservoir by simulating a system of two columns at different temperatures. The schematic diagram of the system is shown in Fig. 13. Column 1 is thermostatted at the cold temperature and column 2 at the hot temperature. Varied volumes of flow are used (like in the previous case) and the concentration of arginine is controlled at the output of column 1. Since column 2 is thermostatted at the hot temperature, its amino acid uptake is not significant. After the arginine

Table 5
Results of the improved amino acid separations

Method	Arginine in P2 (mmol/l; mol%)	Histidine in P2 (mmol/l; mol%)	Lysine in P2 (mmol/l; mol%)
TSC (with constant flow)	9.41	1.55	0.15
	84.6%	13.9%	1.41%
TSC (with varied flow)	9.33	1.27	0.16
	86.7%	11.8%	1.5%
TSC (columns at different temperatures)	9.25	0.98	0.15
	89.13%	9.43%	1.42%
Parametric pumping (PP)	36.89	0.19	0.012
	99.45%	0.51%	0.03%

For PP, values are for the top reservoir after 25 cycles.

concentration has reached a certain value the hot column 2 is cooled, the cold column 1 is heated and the direction of the flow is changed. In such a way, column 1 is regenerated by a solution which has just left the cold column 2, so its histidine and lysine content is lower. P2 product of the method is obtained by hot regeneration of column 1.

The results, compared with the results of the previous calculations, are shown in Table 5. Further slight improvement of arginine purity was available in this way.

It is interesting to observe that while the histidine mole fraction – comparing with the results of TSC – decreases in both methods, the lysine mole fraction increases a little bit. This can be explained by the competition for the adsorbent active sites. When the histidine concentration is greater in the top reservoir (consequently the histidine mole fraction in P2 is also greater), then the lysine uptake decreases because of the competition. If histidine in the top reservoir is less, then the adsorbent can adsorb more lysine, thus its concentration (or mole fraction) in P2 becomes greater.

7.3. Third possibility to improve the separation: traditional batch parametric pumping after TSC

We have mentioned before that traditional batch parametric pumping is not good for separating the amino acids, but only to concentrate them to different degrees. This is true when the initial concentrations are so low (each of them was 1 mmol/l in the previous cases) that the isotherms are linear. In that case the components behave independently. If the concentrations are higher, then due to the effect of the competition for the adsorbent active sites a separation can be achieved. The product P2 of the previous case, in Section 7.2, has such high concentrations. Thus traditional batch parametric pumping on this solution can be applied in order to separate its components. Simulations were performed with the solution P2 of Section 7.2 using a 20 cm^3 bed volume and 10 cm^3 displaced volume. The

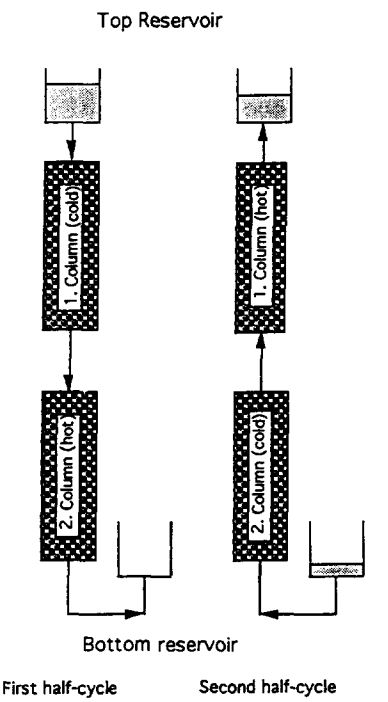

Fig. 13. Temperature-swing chromatography with two columns at different temperatures.

results of the batch parametric pumping calculation are shown in Table 5 and in Fig. 14.

It can be seen in Fig. 14 that the concentrations (log scale) of histidine and lysine in the top reservoir fall to very low values. Since the concentrations of histidine and lysine are very small compared with the concentration of arginine (the histidine initial concentration is 0.1 times the arginine initial concentration, and the lysine initial concentration is 0.016 times the arginine one), they are pushed from the top reservoir end of the column to the bottom reservoir end of the column by arginine. This is very advantageous for further improving the purity of arginine, and it can be seen in Table 5 that almost pure arginine can be obtained in this way.

8. Conclusions

Amino acid separation by a special type of parametric pumping called temperature-swing chromatography was experimentally and theoretically studied.

The effects of two parameters on the separation of basic amino acids (arginine, histidine and lysine) – namely the effect of the volume of cold flow and the effect of 'reflux ratio' (V_{hot}/V_{cold}) – were investigated.

It was shown that the compositions of the products strongly depend on the volume of cold displaced solution. Either a solution of pure lysine (P1) and a mixture of arginine and histidine (P2) can be obtained, if V_{cold} is small, or a mixture of lysine and histidine (P1) and an arginine-rich solution (P2), if V_{cold} is large.

The effect of 'reflux ratio' is not significant in a wide range, but if it is too small then the separation is lost.

Three possibilities to improve the separation were also theoretically investigated. In these cases we stressed the separation of arginine from histidine and lysine, and we were able to produce arginine with a purity of 95–99%. Experimental investigation and optimization of these methods will be the object of future work.

As conclusions, we have shown that TSC is potentially promising for the separation of amino acids, and there are several methods to operate such a system using one or more columns. This work can also be extended to the separation of other molecules.

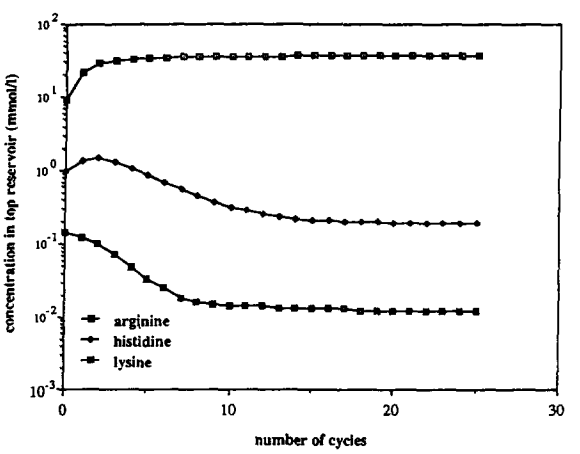

Fig. 14. Batch parametric pumping on P2 product.

9. Notation

C_{A_i}	liquid-phase amino acid concentration (mmol/l)
$C^c_{A_i}$	liquid-phase amino acid concentration at cold temperature (mmol/l)
$C^h_{A_i}$	liquid-phase amino acid concentration at hot temperature (mmol/l)
$K^h_{A_i}$	slope of the hot isotherm
k_{A_i}	parameter of a Langmuir-type isotherm (l/mmol)
Q_{A_i}	maximum of a Langmuir-type isotherm (mmol/l adsorbent)
q_{A_i}	adsorbent amino acid concentration (mmol/l adsorbent)
t	time (s)
V	volume of the solution to be separated (l)
V_B	volume of breakthrough (l)
V_c	volume of adsorbent (l)
V_D	bed dead volume (l)
V_{cold}	volume of cold flow (l)
V_{hot}	volume of hot flow (l)
v	interstitial velocity (cm/s)
z	bed axial position (cm)
ϵ	bed void fraction
ν	mass capacity ratio in the bed

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