Separation of steroid compounds by overloaded preparative chromatography with precipitation in the fluid phase

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Abstract

In order to gain a commercially acceptable yield compared to the adsorption capacity of the column, a rather large amount of sample is separated with preparative chromatography. During the competitive adsorption of the sample-components, the species adsorbing better can force the rest out of the adsorbent phase. As a consequence, the concentrations of the later components may increase in the fluid phase to a level that those species start to precipitate. A mathematical model which takes the precipitation and dissolution into consideration is presented in this paper. Data calculated by this model are compared to that we obtained by using a previous mathematical model as well as the experimental results gained by a laboratory scale separation of steroid compounds on an organic polymer adsorbent. The equilibrium adsorption–desorption and equilibrium precipitation–dissolution is the first approximation of the complicated process mentioned above. In our next publication we are to extend the model with kinetic terms belonging to adsorption–desorption and precipitation–dissolution process respectively. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

In the preparative chromatography, compared to the adsorption capacity of the equipment, a large amount of sample is prepared, in order to be economical. The concentrations of the components bound to the solid phase depend on their concentrations in the fluid phase and on the equilibrium relationships. In case of rapid adsorption kinetics, the fluid and solid phase are in equilibrium with each other. In our previous articles [1,2] we presented a model which describes the adsorption process taking place in a chromatographic column where the multi-

component equilibrium can be described with the competitive Langmuir adsorption isotherm equation. According to this model, if the fluid phase to be separated contains more strongly adsorbing components, then they will displace the less adsorbable species, which had been adsorbed on the solid phase during the earlier adsorption process. Thus the concentration of the less strongly adsorbing components will increase in the fluid phase. According to the equilibrium model, this increase can exceed even the solubility of the component. High concentration levels can be reached not only in the period of sample feeding but also during elution or during displacement chromatography as it can be realized from Guiochon's fundamental work [3]. In these cases, reaching the limit of solubility the components will start to crystallize.

When separation of aqueous solutions of hydroxy-lated steroid compounds takes place, the common algorithm does not give back the concentration profile that has been measured on an organic polymer adsorbent. To gain more adequate data we built a glass preparative chromatographic equipment in which crystallization was detected. So that we can follow the precipitation, our model [1] should be modified.

2. Experimental

Our experiments were carried out at 22°C in a glass column with length of 103 cm and internal diameter of 1.14 cm, which was packed by an organic polymer adsorbent (DIAION–SP–207). We were to keep the volumetric rate of drum-filtered

fermentation liquid at a level of about 8.0 cm^3/min and the liquid was flowing in direction from top to the bottom in the column. During 480 min 3840 cm^3 liquid entered the column. The inlet aqueous drum-filtered fermentation liquid was saturated with two steroid compounds, DH AL and an apolar component AP. The inlet concentrations were 0.84 mgDH/cm^3 liquid, 1.26 mgAL/cm^3 liquid and 0.2 mgAP/cm^3 liquid respectively.

Liquid samples were taken at 9 points, that were about equally distributed along the column. Including the inlet and outlet, we had a total of 11 measuring points to follow the liquid concentration profile in the column. We collected liquid samples in every 2 h and measured DH, AL concentrations with an analytical HPLC as shown in Fig. 1.

After frontal adsorption, the column was washed with 2000 cm^3 of distilled water. Then the adsorbent
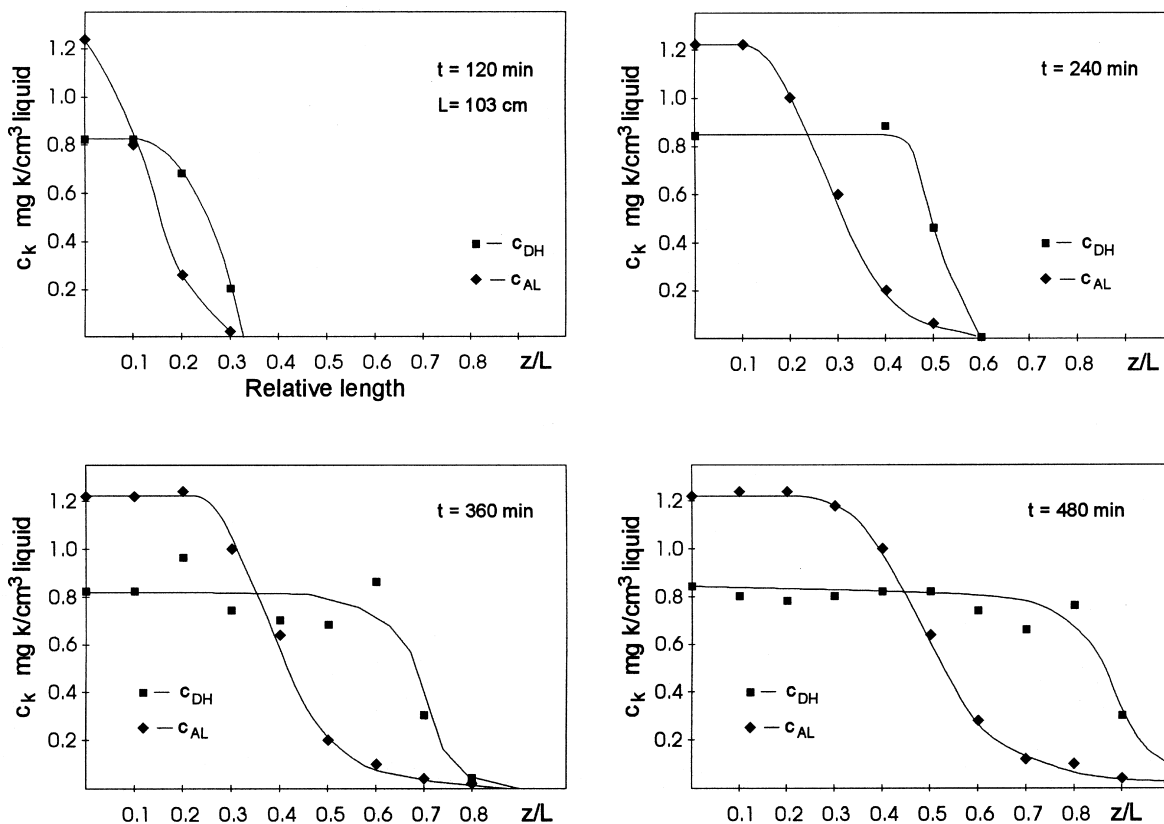

Fig. 1. Axial concentration distributions in the liquid phase after frontal adsorption. 120 min, 960 cm^3 liquid; 240 min, 2000 cm^3 liquid; 360 min, 3000 cm^3 liquid; 480 min, 3900 cm^3 liquid.
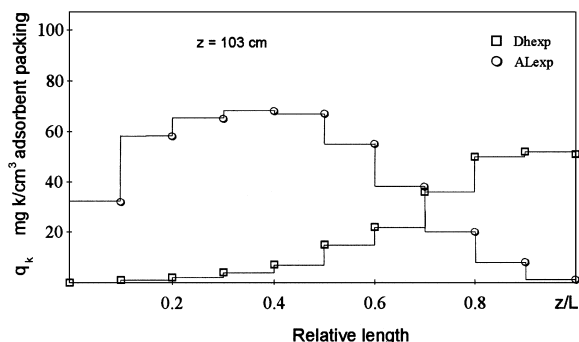
Fig. 2. Adsorbent phase axial concentration distribution after 3900 cm^3 fermentation liquid and 2000 cm^3 distilled water flowing at 22°C.

Fig. 3. Adsorption isotherms of DH steroid and AL steroid on DIAION–SP–207 adsorbent in aqueous solution at 22°C.

packing was cut into about 10 cm slices from top to the bottom. By means of an analytical HPLC, we determined the distributions of the components by elution those from the slices of adsorbent column with pure methanol (300 cm^3), see Fig. 2.

Table 1
Single Langmuir isotherm constants

Component	a_k cm^3 liquid/g ads	b_k cm^3 liquid/mg
1 DH, c_1	3000.0	30.3
2 AL, c_2	15870	42
3 AP, c_3	84435.0	97.00

2.1. The mathematical model

We described the adsorption of the components by competitive Langmuir adsorption isotherm equation:

$$q_k = \frac{a_k c_k}{1 + \sum_0^K b_k c_k} \tag{1}$$

where q_k is the concentration of component k in the adsorbent phase, mg k/g dry adsorbent; a_k is the Langmuir isotherm constant, cm^3 liquid/g dry adsorbent; and b_k is the Langmuir isotherm constant, cm^3 liquid/mg k.

The isotherms of the pure components (AL, DH > 99% w/w purity standards Richter Gedeon Ltd.) measured in aqueous phase on DIAION–SP–207 adsorbent at 22°C are shown in Fig. 3. The constants of the single isotherms (Table 1) give the constants of Eq. (1). The Langmuir constants of apolaric component were only estimated (the Langmuir isotherm shape was controlled in the usual $1/q$ versus $1/c$ diagram).

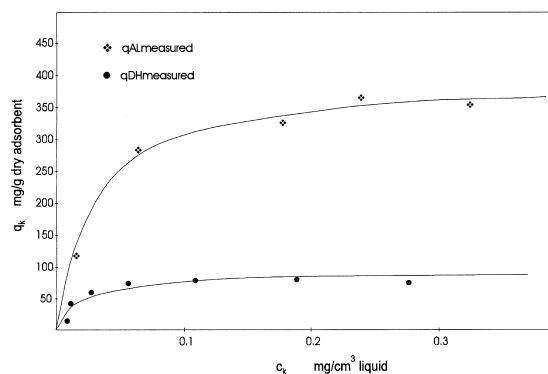The component balance equation for $k = 1 = $ DH, $k = 2 = $ AL and $k = 3 = $ AP components:

$$v_0 \frac{\partial c_k}{\partial z} + \rho_b \frac{\partial q_k}{\partial t} + \left(\epsilon - \sum_{k=1}^{3} s_k \right) \frac{\partial c_k}{\partial t} + \rho_k \frac{\partial s_k}{\partial t} = 0 \tag{2}$$

where v_0 is velocity at the free cross section of the column, cm/min; z is axial distance of the column, cm; ϵ is free volume coefficient of the column, cm^3 free volume/cm^3 column; ρ_b is bulk density of the adsorbent phase, g dry adsorbent/cm^3 column packing; s_k is free volume ratio of kth solid component, cm^3 solid k in free volume/cm^3 column; and ρ_k is solid-phase density of component k, mg k/cm^3 solid.

If:

$$c_k < c_{k,s} \quad \text{then } s_k = 0 \text{ and } \frac{\partial s_k}{\partial t} = 0 \tag{3}$$

where $c_{k,s}$ is the solubility of component k in fermentation liquid (mg k/cm^3 liquid). In this case, Eq. (2) is reducing to Eq. (4):

$$v_0 \frac{\partial c_k}{\partial z} + \rho_b \frac{\partial q_k}{\partial t} + \left(\epsilon - \sum_{k=1}^{3} s_k \right) \frac{\partial c_k}{\partial t} = 0 \tag{4}$$

If:

$$c_k \geq c_{k,s} \quad \text{then } c_k = c_{k,s} \text{ and } \frac{\partial c_k}{\partial t} = 0 \tag{5}$$

and Eq. (2) becomes:

$$v_0 \frac{\partial c_k}{\partial z} + \rho_b \frac{\partial q_k}{\partial t} + \rho_k \frac{\partial s_k}{\partial t} = 0 \tag{6}$$

The free volume coefficient $\epsilon - \Sigma_{k=1} s_k$ always is to be corrected after the equilibrium precipitation or dissolution.

With the help of Eq. (7), we can calculate $q_{k,c}$ the excess adsorbent concentration of component k in crystal (precipitate) form. $q_k + q_{k,c}$ is the total concentration of component k in the adsorbent and in crystal phase in the free volume of the packing:

$$q_{k,c} = \frac{1}{\rho_b} s_k \rho_k \tag{7}$$

where $q_{k,c}$ is the adsorbent concentration of component k in crystal form, mg k/g dry adsorbent.

Initial conditions:

$$0 \leq z \leq L \quad c_k(z,0) = 0 \quad s_k(z,0) = 0 \quad q_k(z,0)$$
$$= 0 \quad k = 1, 2, 3 \tag{8}$$

Boundary conditions:

$$c_k(0,t) = c_k^0 \quad k = 1, 2, 3 \quad t < t_m \tag{9}$$

$$c_k(0,t) = 0 \quad k = 1, 2, 3 \quad t > t_m \tag{10}$$

t_m denotes the time of the end of frontal adsorption.

2.2. The numerical solution of the mathematical model

The finite difference method we have used is written more widely in publication [1].

$$\frac{\partial c_k}{\partial t} = \frac{v_0 \frac{N^2}{\rho_b} \frac{\partial c_k}{\partial z} - a_k c_k \sum_0^K b_i \frac{\partial c_i}{\partial t} + a_k c_k b_k \frac{\partial c_k}{\partial t}}{a_k c_k b_k - N a_k - \frac{(\epsilon - \sum s_k)}{\rho_b} N^2} \tag{11}$$

where N is the denominator of competitive Langmuir adsorption equilibrium equation:

$$N = 1 + \sum_{k=1}^{3} b_k c_k \tag{12}$$

$$\frac{\partial q_k}{\partial t} = \sum_{i=1}^{3} \frac{\partial q_k}{\partial c_i} \frac{\partial c_i}{\partial t}$$

$$= -\frac{a_k c_k}{\left(1 + \sum_{i=1}^{3} b_i c_i\right)^2} \sum_{i=1}^{3} b_i \frac{\partial c_i}{\partial t} + \frac{a_k}{1 + \sum_{i=1}^{3} b_i c_i} \frac{\partial c_k}{\partial t} \tag{13}$$

After this, in Eq. (6), the velocity of change of the free volume ratio of solid component s_k can be determined.

In the partial-differential component balance equation, we did not take the axial diffusion into consideration but during the numerical solution we divided the time and the length into intervals to bring some numerical dispersion into the model. That is why the axial distance step is a parameter to be fitted. (Measured data: HETP[cm] = $9.48 \times v_o$[cm/s] + 0.79)

A computer program based on the above mentioned mathematical model was written for IBM PC in Pascal language. The program is available at the Department of Chemical Engineering, University of Veszprem

Variables of the mathematical model and the input data of the computer program can be given as follows.

3. Results

Variables of the mathematical model and the input data of Krom_N.pas and Krom_S.pas computer programs can be seen in Tables 2 and 3.

In Figs. 4–6 we represented the result we have got without precipitation–dissolution. It can be seen that DH and AL liquid concentrations versus axial distance have maximum values and the DH component is "running out" from the column (Fig. 4).

The agreement between the measured and calculated adsorbent phase concentrations after the distilled water washing period are not well enough (Fig. 5).

In Fig. 6, we had represented the calculated q_{DH}, q_{AL}, q_{AP} adsorbent phase concentrations versus axial distance after frontal adsorption ($Q = q_{DH} + q_{AL} + q_{AP}$).

Table 2
Variables of the mathematical model

v_0	Velocity at free cross section	cm/s
z	Axial distance	cm
ϵ	Free volume coefficient	cm^3 liquid/cm^3 column
ρ_b	Packing density of adsorbent	g/cm^3 column packing
s_k	Free volume ratio of solid copmonent k	cm^3 solid k/cm^3 column
ρ_k	Solid phase density of component k	mg k/cm^3 solid
c_k	Liquid concentration	mg k/cm^3 liquid
$c_{k,s}$	Solubility of component k in fermentation liquid	mg k/cm^3 liquid
q_k	Adsorbent concentration	mg k/g dry adsorbent
$q_{k,c}$	Adsorbent concentration k in crystal form	mg k/g dry adsorbent

Table 3
Input data[a]

k	a_k cm^3 liquid g dry ads.	b_k cm^3 liquid mg k	c_k mg k cm^3 liq.	ρ_k mg k cm^3 solid
DH	3000	30.3	0.84	1275
AL	15870	42.0	1.26	1178
AP	84435	97	0.20	1070

[a] $k=3$; I.D.$=1.14$ cm; $L=103$ cm; $\epsilon=0.679$ cm^3 liquid/cm^3 column; $B=8$ cm^3/min; $\rho_b=0.369$ g/cm^3 column; NTP$=10$.

In case of Figs. 7–9, we had used the mathematical model, where we took the precipitation–dissolution into consideration.

It can be seen, that the liquid concentration versus axial distance have not maximum and the DH is not "running out" from the column (Fig. 7).

The calculated and measured adsorbent concentrations (after washing with water period) agreed
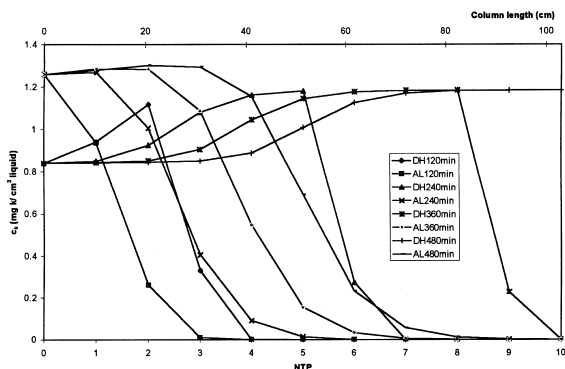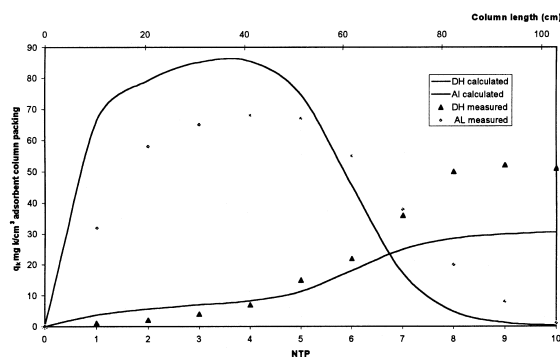

Fig. 5. Calculated and measured adsorbent phase concentrations without precipitation after washing with water.

well enough in case of AL but not in case of DH (Fig. 8).

In Fig. 9 we had represented the adsorbent phase concentrations after frontal adsorption. It is interesting that — because of equilibrium precipitation–

Fig. 4. Calculated liquid phase concentrations without precipitation at different times of frontal adsorption.
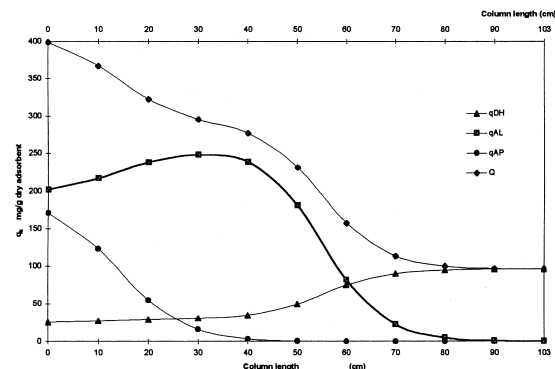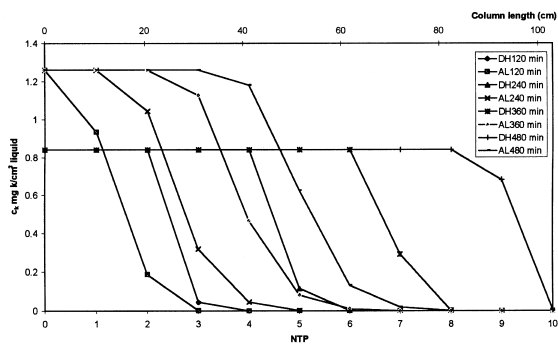

Fig. 6. Calculated adsorbent phase concentrations without precipitation after frontal adsorption.

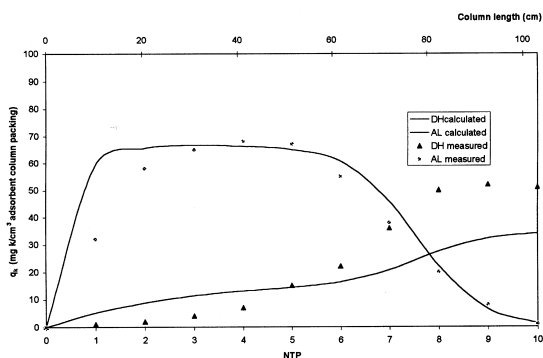Fig. 7. Calculated liquid phase concentrations with precipitation at different times of frontal adsorption.

Fig. 8. Calculated adsorbent phase concentrations with precipitation after washing with water.

Fig. 9. Calculated adsorbent phase concentration with crystallisation after frontal adsorption.

dissolution — about the 50–75% of the total fixed DH ($q_{DH} + q_{DH,c}$) is in solid form ($q_{DH,c}$) at the top of the column (Fig. 9A). The solid part of AL ($q_{AL,c}$) is only about 10% compared to the total fixed AL ($q_{AL} + q_{AL,c}$) at the top of the column. The total capacity ($Q = q_{DH} + q_{DH,c} + q_{AL} + q_{AL,c} + q_{AP}$) is also represented in Fig. 9A.

In Fig. 9B we can see that the $q_{DH,c}$ is about 10–15% and $q_{AL,c}$ about 5% of the Q value (Q is the summarized adsorbent and solid concentration for DH, AL and AP, see above).

In Fig. 9C we had given the s_k (cm³ solid/cm³ column) values, which have maximum 0.015 for DH and 0.003 for AL. The s_k values are only some percents (0.4–2.2%) of the $\epsilon = 0.679$ cm³ liquid/cm³ column volume. That is why the precipitate do not block the column free cross section.

In Fig. 10A and B we had represented the measured and calculated liquid concentration (with
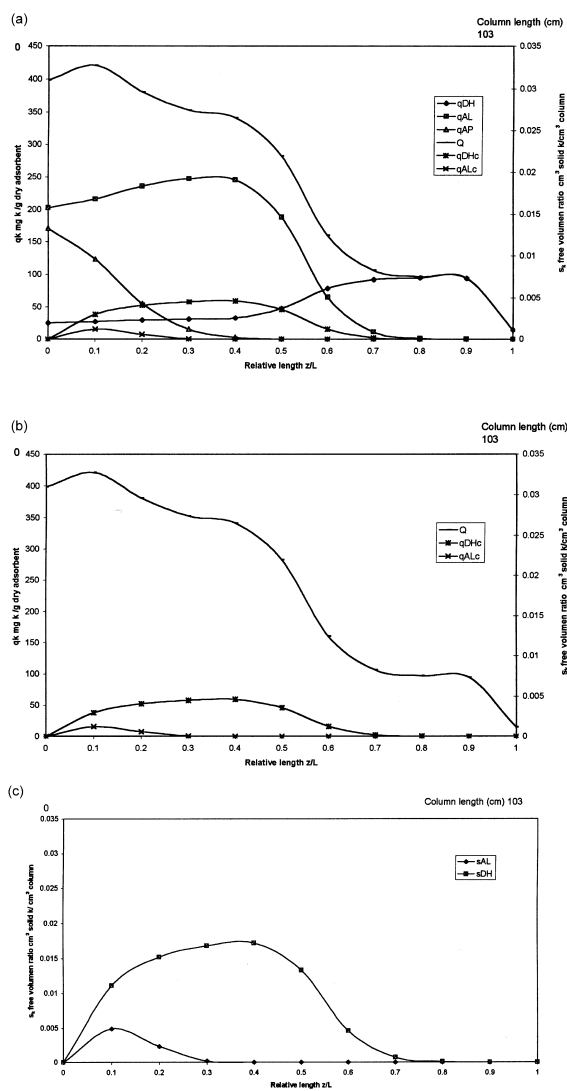
and without precipitation) versus axial distance curves after frontal adsorption for DH and AL compounds. It can be seen that the mathematical model, where we had used equilibrium precipitation and dissolution, results good agreements between experimental and calculated values. The model without precipitation–dissolution in case of DH had given practically unacceptable differences between measured and calculated values (Fig. 10A and B).
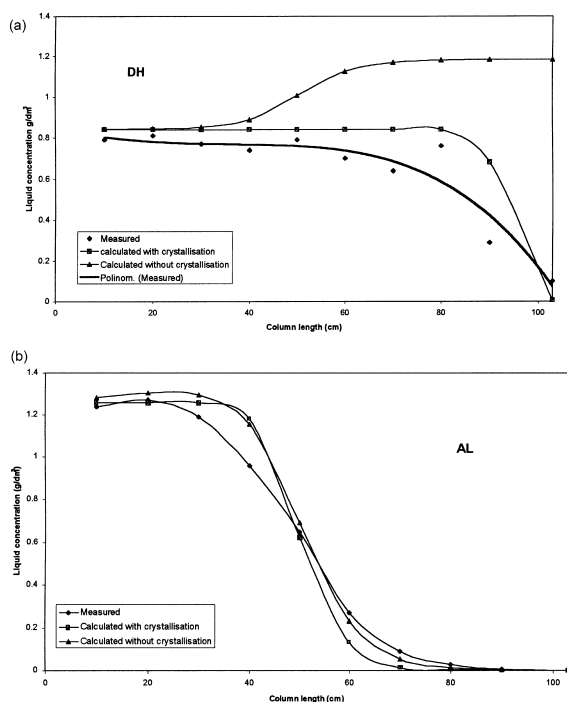
Fig. 10. **(A)** Calculated and measured liquid phase DH concentration at 480 min (at the end of the frontal adsorption). **(B)** Calculated and measured liquid phase AL concentration at 480 min (at the end of the frontal adsorption).

4. Summary

The authors worked out a new mathematical model for fixed bed chromatographic column taking into the consideration the precipitation–dissolution phenomena. Having modeled the phenomena, using numerical mathematical method (finite differences), a computer program was written for IBM–PC in Turbo–Pascal language. For the experiments drum filtered fermentation liquid (DH, AL steroids and AP apolar compound in water) and DIAION–SP–207 adsorbent was used.

The measured liquid phase concentration–time–axial distance and adsorbent phase concentration–time–axial distance curves were compared with calculated values (with and without precipitation dissolving processes). After calculation the DH compound in 50–75% and the AL in 10% exist in crystal form at the top of the column, which caused only a little change in free volume coefficient ($s_{DH} =$

0.015, $s_{AL} = 0.03$, $\epsilon = 0.679$). This little ϵ change cannot cause the plugging of the column free section.

The calculated and measured values practically have a good agreement, if we use the equilibrium precipitation–dissolution in the mathematical model (Fig. 10A and B).

In our next publication we are going to extend our mathematical model with kinetic terms belonging to adsorption–desorption and precipitation–dissolution respectively.

5. Nomenclature

c_k	liquid concentration of component k, mg k/cm^3
q_k	concentration of component k at the adsorbent, mgk/g dry adsorbent
a_k	Langmuir isotherm constant, cm^{3}liquid/ g dry adsorbent
b_k	Langmuir isotherm constant, cm^{3}liquid/ mg k
N	denominator of competitive Langmuir equation, see Eq. (12)
NTP	number of theoretical plates
v_0	velocity for the column free cross section, cm/min
z	axial distance, cm
t	time, min
t_m	time of the end of frontal adsorption, min
ϵ	free volume coefficient, cm^3 liquid/cm^3 column
s_k	free volume ratio of kth solid component, cm^{3}solid cryst/cm^3 column
ρ_b	bulk density of the adsorbent, g dry adsorbent/cm^3 packing
ρ_k	solid-phase density of component k, mg k/cm^3 solid
DH c_1	liquid concentration of DH steroid, mg/cm^3 liquid
AL c_2	liquid concentration of AL steroid, mg/cm^3 liquid
AP c_3	liquid concentration of apolar compound, mg/cm^3 liquid
$c_{k,s}$	solubility of component k in fermentation liquid, mg k/cm^3 liquid

q_{DH}　q_1　DH concentration on adsorbent, mg/g dry adsorbent

q_{AL}　q_2　AL concentration on adsorbent, mg/g dry adsorbent

q_{AP}　q_3　apolar compound concentration on adsorbent, mg/g dry adsorbent

$q_{k,c}$　　kth adsorbent concentration in crystal form in free volume of column, mg k/g dry adsorbent

Q　　summa ($q_k + q_{k,c}$), mg/g dry adsorbent

s_{DH}　　DH free volume ratio, $cm^3 cryst/cm^3$ column volume

s_{AL}　　AL free volume ratio, $cm^3 cryst/cm^3$ column volume

k　　number of component

exp　　experimental

est　　estimated

calc　　calculated

meas　　measured

Indexes

k　　component

b　　bulk

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