

# Ion-pair adsorption chromatography for process purposes Basic equilibrium studies for the recovery of clavulanic acid by using quaternary ammonium salts

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## Abstract

Ion-pair adsorption chromatography was used to purify clavulanic acid (CA) by employing commercial hydrophobic matrixes in combination with quaternary ammonium salts (QAS). To understand the mechanism governing the interactions between CA, QAS and the matrix, batch experiments were done where the equilibrium relationships for the combinations CA–matrix, QAS–matrix and CA–QAS–matrix were individually investigated. It was shown that the presence of CA can cause a significant concentration-dependent shift in the loading of QAS on the matrix. At high CA concentrations, the total capacity of the system is higher than that expected from a mechanism based purely on ion-pair interactions. These phenomena were analysed qualitatively and semi-quantitatively from the viewpoint of the electrostatic theory and of chemical equilibrium relations between the different species involved in the system. It could be shown that, at the normal conditions used in preparative chromatography, the interactions between CA and the matrix and between CA and QAS in the liquid phase cannot be neglected and have an important contribution to the capacity and affinity of the stationary phase for the adsorbate.

*Keywords:* Ion-pairing reagents; Preparative chromatography; Adsorption isotherms; Thermodynamic parameters; Clavulanic acid; Quaternary ammonium compounds; Charcoal, activated; Amberlite XAD

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## 1. Introduction

The use of ion-pair chromatography as an analytical tool for separating ionic or ionizable substances by HPLC can be traced back to the 1970s [1,2]. In comparison to other possible techniques to analyze such products, it was demonstrated that ion-pair chromatography is potentially more versatile and more prone to optimization due to the many parameters available for fine tuning the method to the

required separation performance [2,3]. In spite of its established use for analytical purposes, this technique has been rarely explored for preparative or process applications until now. The addition of ion-pair forming substances such as trifluoroacetic acid for enhancing the preparative separation of peptides and proteins [4] is one of the main examples for the use of ion-pair chromatography on a larger scale.

Perhaps one of the bottlenecks of its definitive acceptance as a powerful purification tool is the complexity of the mechanisms that rule the interactions between the chemical species involved in such

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a system. Whereas most users follow rules of thumb for the application of this technique, a general controversy about the retention mechanism playing the decisive role in ion-pair chromatography could be noticed in the literature concerning its theoretical aspects [5–7] until the late 1980s. The electrostatic theory developed by Ståhlberg and co-workers during the last eight years [8–11] is by now the most accepted mechanistic explanation for the multiple interaction phenomena occurring in ion-pair chromatography.

According to the electrostatic theory, in an ion-pair system the difference in electrostatic potential between bulk phase and surface that is built up when an electrical double layer of ion-pairing forming molecules and their counterions is formed at the surface generates a supplementary driving force, the so-called surface potential, in addition to the free energy of adsorption. This potential depends on several factors, including the nature of the surface, the ion-pairing substance and the analyte, the interactions between these species, the total ion concentration, the pH and the polarity of the liquid phase and temperature, among others. To convert this very meaningful but extensive set of variables into a manageable and experimentally useful form, a series of simplifying assumptions must be done. This can be accomplished with some ease in the case of analytical applications, due to the fact that the analytical ion-pair chromatography has some well defined characteristics, such as small sample volumes, low analyte concentrations and quasi-steady state run conditions. The assumption that the analyte has negligible interactions with the stationary phase and in this way does not disturb the equilibrium conditions that are established between the ion-pairing substance and the surface is in this case in accordance with the actual situation. Added to a further assumption of a linear isotherm for the analyte, which is reasonable for low analyte concentrations, it allows the simplification of the theory to a ready usable relation between capacity factors and concentration of the ion-pairing substance in the liquid phase.

We have developed a strategy based on commercially available polymeric hydrophobic matrixes (Amberlite XAD) and on activated charcoal (AC) and water-soluble quaternary ammonium salts

(QAS) to separate the  $\beta$ -lactamase inhibitor clavulanic acid (CA) from fermentation broths of *Streptomyces clavuligerus* [12]. The selected stationary phases are the most frequently used hydrophobic materials used in an industrial scale for the adsorption of partially hydrophobic, low-molecular-mass products from fermentation broths. The use of an ion-pairing technique evolved as an attempt to overcome some pitfalls of the classic operations used for the first purification step for this highly unstable product. CA is a  $\beta$ -lactamic acid presenting no group in its structure able to establish strong hydrophobic interactions. Its separation with the help of liquid-liquid extraction or ion-exchange chromatography methods is invariably linked to high losses due to inactivation.

With the purpose of understanding the interactions involved in the selected ion-pair systems, a series of batch experiments and column saturation runs were done with pure solutions of CA. This approach was very useful in disclosing some unique characteristics of the equilibrium behaviour between the ion-pair forming substance, the adsorbate and the matrix. Although the experimental conditions do not fulfil the needed simplifications that allow the use of the quantitative relations derived from Ståhlberg's electrostatic theory, the analysis of the experimental data from this point of view can be done in a qualitative form. To further analyse the results obtained, they were additionally evaluated with the help of chemical equilibrium relations existing among the species present in the systems. The observed phenomena can be useful to complement the present theories that describe the mechanisms involved in ion-pair chromatography.

## 2. Experimental

### 2.1. Chemicals

Resins of the Amberlite XAD series were purchased from Sigma (Deisenhofen, Germany). Activated charcoal (AC) 18–35 mesh ASTM (0.5–1.0 mm) for gas chromatography was bought from E. Merck (Darmstadt, Germany). Benzyltributylammonium chloride (BTBA) and alkylbenzyltrimethylammonium chloride (ABDA) were purchased

from Merck-Schuchardt (Germany). CA was obtained by purification from Augmentan Tabs (the trade mark for the therapeutic formulation of CA, consisting on a combination of potassium clavulanate and amoxicillin in a 1:4 ratio, 625 mg/tab; SmithKline Beecham Pharma, München, Germany), purchased in pharmacies. Other chemicals were obtained from standard sources.

## 2.2. Purification of CA

CA was separated from the amoxicillin present in Augmentan Tabs by successive steps of liquid–liquid extraction with ethyl acetate at pH 2 (volume ratio 2:3) followed by reextraction into 50 mM pH 7 phosphate buffer and adsorption in a column packed with XAD-4 [12]. The reached purity was 96% on the potassium salt basis, equivalent to 80% on the free acid basis.

## 2.3. Preparation of the stationary phases

Amberlite XAD resins were washed several times with methanol and deionized water. AC was washed several times with water. The matrixes were degassed under vacuum to eliminate entrapped air from the pores. XAD and AC were loaded with QAS by shaking known amounts of the matrixes for 48 h with QAS solutions in water. The initial and final concentrations in the liquid phase were measured and used to calculate the total QAS loading by mass balance. All the stationary phases were filtered and blotted out in filter paper to eliminate interparticle fluid. Samples were always weighted on a wet basis, and due to their wet density being nearly 1, the wet mass was considered to be equal to the volume of stationary phase.

## 2.4. Determination of adsorption isotherms

Batch experiments were done in 2-ml microtubes containing 2-ml sample solutions in water at different known initial concentrations of QAS or CA and 100  $\mu\text{g}$  stationary phase. This corresponds to a phase ratio  $\epsilon$ , defined as the liquid phase volume to total (liquid+stationary phase) volume ratio, equal to 0.9524. In some series of isotherms, the initial total ionic concentration was maintained constant by the

addition of a KCl solution in appropriate quantities, complementary to the concentration of CA present, to a total initial concentration of 50 mM  $K^+$ . The tubes were agitated at 20°C in a vortex shaker (Eppendorf Thermomixer 5436, Hamburg, Germany) until equilibrium was attained. In the case of the adsorption isotherms for CA, the matrixes were always pre-loaded with QAS according to a defined equilibrium share. Final concentrations of the product and, when required, of QAS in the liquid phase were determined and the fraction adsorbed on the stationary phase was calculated by mass balance. In all cases, the liquid phase also contained QAS in equilibrium concentrations with the loading of the stationary phase. To ensure optimal conditions for the stability of CA, the pH was always previously controlled at 5.9 by adding diluted KOH or HCl solutions to the samples.

## 2.5. Column runs

Stationary phase was packed in glass columns of 1 cm internal diameter to obtain a bed volume of 5 ml. After being equilibrated with solutions at the desired run conditions (pH, ionic strength, QAS at equilibrium concentrations), aqueous solutions containing 5 mM CA were fed at a constant flow-rate of 9 ml/h controlled by a peristaltic pump (Watson-Marlow 101U, Falmouth, Cornwall, UK). Equilibrium concentrations of the respective QAS were also present in the feeding solution. The outflowing solution was separated by a fraction collector (Pharmacia Frac-300, Uppsala, Sweden) during the whole run, until the column was completely saturated with CA and the product concentration in the outflow equalled the feeding concentration. Breakthrough curves for CA and concentrations of QAS in the outflowing samples were determined.

## 2.6. Analytical methods

CA in pure water solutions was determined by measuring the rate of its reaction with imidazole during the linear phase and correlating it with the concentration present in the sample: a 1-ml sample containing 0–0.2 mM CA (0–40 mg/l) was mixed with 50  $\mu\text{l}$  imidazole reagent (10.31 g imidazole p.A. is dissolved to 50 ml with deionized water and 5

M HCl to a final pH value of 6.8) and the formation of the reaction product was immediately followed at 311 nm for 1 min in a LKB Ultrospec Plus UV-Vis spectrophotometer (Cambridge, UK). The reaction slope is linearly correlated to the CA present in the samples. A standard curve was obtained using a solution of Augmentan Tabs as standard for the antibiotic. The amoxicillin present in the samples does not react with imidazole at the test conditions and thus does not interfere with the results.

QAS (BTBA and ABDA) concentrations in aqueous solutions were determined spectrophotometrically at 260 nm (LKB Ultrospec Plus UV-Vis spectrophotometer).

### 3. Theoretical aspects

#### 3.1. Chemical equilibrium relations

In an ion-pair adsorption system, the interdependent equilibrium relations that describe the possible interactions between the stationary phase M and the existing relevant species in the liquid phase, an ion-pair forming substance B (for the sake of simplicity the ionic charges will not be represented) and an adsorbate A, can be represented by the following equations:



In most practical cases, there is no difference between the complexes  $A \cdot BM$ ,  $BA \cdot M$  and  $AB \cdot M$  or  $B \cdot AM$  and  $BA \cdot M$ , so that Eqs. (4–7) overlap themselves in describing the existing adsorbed species. Although these considerations lead to sim-

plifications of the equation system, the usefulness of those relations for describing a real system is limited due to the existence of too many variables that usually cannot be independently measured, as is the case for the distinction between species A and AB or B and AB in the liquid phase, and consequently species  $A \cdot M$  and  $AB \cdot M$  or  $B \cdot M$  and  $AB \cdot M$  in the stationary phase. Thus, the measurable concentrations in the system will always be composed by two variables, which cannot be independently quantified:

$$q_{A_{\text{total}}} = q_{AM} + q_{ABM} \quad (8)$$

$$q_{B_{\text{total}}} = q_{BM} + q_{ABM} \quad (9)$$

$$c_{A_{\text{total}}} = c_A + c_{AB} \quad (10)$$

$$c_{B_{\text{total}}} = c_B + c_{AB} \quad (11)$$

The relation between the equilibrium concentration of a species in the stationary phase and in the liquid phase can be described by the partition coefficient  $\lambda$ :

$$\lambda = \frac{q}{c} \quad (12)$$

The equilibrium conditions of the adsorption of an adsorbate on a matrix can be represented by a general Langmuir equation for real surfaces [13]:

$$q = \sum_{i=1}^{i=\infty} q_{\text{max}_i} \frac{c}{c + K_{d_i}} \quad (13)$$

Eq. (13) states that the adsorption in a real surface can be considered as an infinite sum of ideal Langmuir surfaces having each a characteristic maximal capacity  $q_{\text{max}}$  and dissociation constant  $K_d$ . The representation for one ideal surface is the special case for  $i=1$ , where all active sites are energetically equal:

$$q = q_{\text{max}} \frac{c}{c + K_d} \quad (14)$$

For very low adsorbate concentrations or in systems where the stationary phase shows low affinity for the adsorbate ( $K_d \gg c$ ), Eq. (14) simplifies to a linear isotherm:

$$q = ac \quad (15)$$

so that

$$\lambda = \alpha \quad (16)$$

The symbols A and B used here refer to CA and QAS, respectively.

### 3.2. Electrostatic relations

According to the electrostatic theory, the adsorption isotherm for a species on a surface can be represented by the following relation [8]:

$$\frac{q}{q_{\max}} = \frac{ce^{(-\Delta G^\circ - F\psi_0)/RT}}{1 + ce^{(-\Delta G^\circ - F\psi_0)/RT}} \quad (17)$$

which is the extended form of a Langmuir isotherm ( $1/K_d = e^{-\Delta G^\circ/RT}$ ) with a term for the effect of the surface potential added to the free energy of adsorption  $\Delta G^\circ$ . The surface potential  $\psi_0$  is a complex function of the sum of ionic species adsorbed at the surface including their electric charge, so that if, for example, ions with equivalent opposite charges are adsorbed in equal amounts at the surface, its total electric potential will be annulled [8]. In the case of ion-pair adsorption chromatography, the assumption that the only species that interacts with the surface is the ion-pairing substance simplifies the function  $\psi_0$  and allows its evaluation as an empirical function of the concentration of adsorbed ion-pairing molecules only. This last value can be directly correlated to the concentration of this substance in the liquid phase through its isotherm. As a further simplification, at low sorbate concentrations (when  $q \ll q_{\max}$ ) its isotherm can be considered as a linear relation and can be expressed by [8]:

$$\lambda_a = \frac{q_a}{c_a} = q_{\max} e^{(-\Delta G_a^\circ - z_a F \psi_0)/RT} \quad (18)$$

where the subscript a refers to the analyte in contrast to the ion-pairing substance. In Eq. (18), the electric potential will be positive when the charges of both sorbate and ion-pairing substance are equal and negative when they are opposite. The reference condition where the value of  $\psi_0$  is stated as zero is conveniently related to the system in the absence of ion-pairing substance, so that, considering  $q_{\max}$  to be equal in the presence or in the absence of ion-pairing substance, the following relation is obtained [10]:

$$\lambda_a = \lambda_{a0} e^{(-z_a F \psi_0/RT)} \quad (19)$$

or

$$\psi_0 = -\frac{RT}{z_a F} \ln \frac{\lambda_a}{\lambda_{a0}} \quad (20)$$

Eqs. (19,20) represent the most useful derivation of the electrostatic theory for practical purposes, as it enables the quantitative evaluation of the electrical surface potential from the measurement of the partition coefficient (or capacity factor) of a sorbate in the presence and in the absence of ion-pairing substance. These two variables are easily measurable and are mainly a function of the quantity of ion-pairing substance in the system, when all mentioned simplifications are considered.

## 4. Results and discussion

### 4.1. Isotherms for QAS in the absence of CA

Preliminary experiments were done using different hydrophobic matrixes (AC, XAD-2, XAD-4, XAD-7 and XAD-16) and QAS with different apolar group: tetraethylammonium chloride, TEA, tetrabutylammonium hydrogensulphate, TBA, benzyltributylammonium chloride, BTBA and alkylbenzyltrimethylammonium chloride, ABDA (in this case, alkyl refers to a saturated chain containing 11 to 16 carbon units). Based on the capacity and affinity of the matrixes for the ammonium salts and further on the capacity and affinity of the ion-pair systems for CA, two configurations were chosen as the most adequate for continuing the experiments: XAD-4–ABDA and AC–BTBA. The isotherms for the equilibrium between QAS and stationary phase in the case of these two systems at 21°C are given in Fig. 1 and the parameters for the Langmuir isotherm description calculated by fitting the experimental data to Eq. (14) are shown in Table 1.

To evaluate the influence of the total ionic concentration on the equilibrium between QAS and stationary phase, the equilibrium conditions for a series of batch experiments for the systems XAD-4–ABDA and AC–BTBA at equal initial QAS concentration in the liquid phase (20 mM) but different concentrations of KCl were measured. The results can be seen in Fig. 2 and demonstrate that the

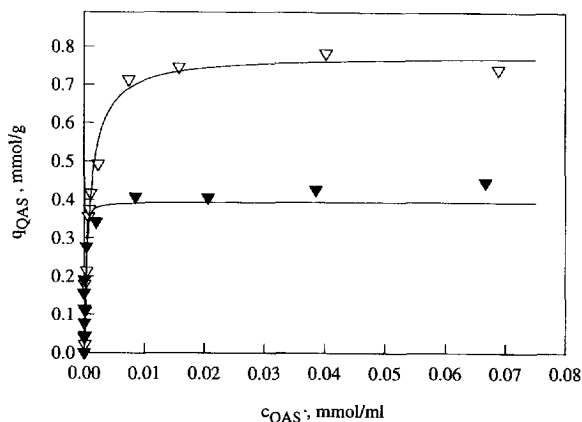


Fig. 1. Isotherms for the adsorption of ABDA on XAD-4 ( $\nabla$ ) and BTBA on AC ( $\blacktriangledown$ ) at variable ionic concentration, 21°C and  $\epsilon=0.9524$ . The curves represent the simulated data with a Langmuir model (Eq. (14)) and the parameters shown in Table 1.

interaction intensity between QAS and matrix increases with the increase of the total ionic strength of the liquid phase. This phenomenon is predictable from the nature of the interactions that predominate in these systems, which are partially or totally hydrophobic and thus depend on their available hydration water content and, consequently, on the ionic strength. From the electrostatic point of view, at higher ionic strengths the repulsive electrostatic effect generated by already adsorbed ions on their own adsorption is reduced due to the shielding of the charges on the surface by the higher concentration of counterions in the liquid phase. Beyond a KCl concentration of 50 mM, the influence of the ionic strength on the interactions between QAS and matrix levels off. Therefore, this value was chosen for

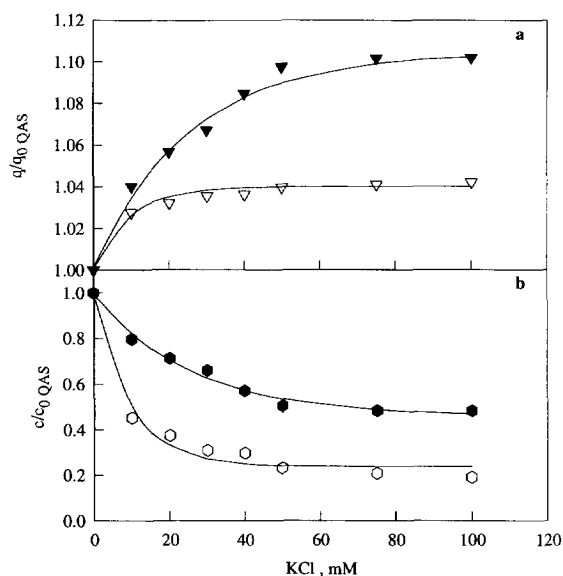


Fig. 2. Influence of the ionic concentration on the relative concentration of QAS (a) in the stationary phase and (b) in the liquid phase for the systems XAD-4-ABDA ( $\nabla$ ,  $\circ$ ) and AC-BTBA ( $\blacktriangledown$ ,  $\bullet$ ) at 21°C and  $\epsilon=0.9524$ . The reference values  $c_0$  and  $q_0$  for no addition of KCl (variable ionic concentration) are described in Table 2.

subsequent experiments at constant ionic concentrations.

#### 4.2. Isotherms for CA in the absence of QAS

Fig. 3 shows the isotherms for the adsorption of CA on AC and XAD-4 in the absence of QAS. In one of the experimental series, the total initial ionic concentration in the liquid phase was maintained

Table 1

Isotherm parameters calculated by fitting the experimental equilibrium data for the systems XAD-4-ABDA, AC-BTBA (Fig. 1) and XAD-4-CA, AC-CA (Fig. 3) to the Langmuir model [Eq. (14)]

Adsorption system	$q_{max}$ , mmol/g	$K_d$ , mmol/ml	$\alpha$
XAD-4-ABDA, variable i.c.	0.780	$9.7 \times 10^{-4}$	
AC-BTBA, variable i.c.	0.395	$8.9 \times 10^{-5}$	
XAD-4-CA, variable i.c.	0.211	0.045	3.77
constant i.c.	0.189	0.044	3.39
AC-CA, variable i.c.	0.201	$8.2 \times 10^{-4}$	
constant i.c.	0.302	$1.8 \times 10^{-3}$	

i.c. = ionic concentration.

For the system XAD-4-CA, the parameter  $\alpha$  for the linear isotherm [Eq. (15)] is also shown.

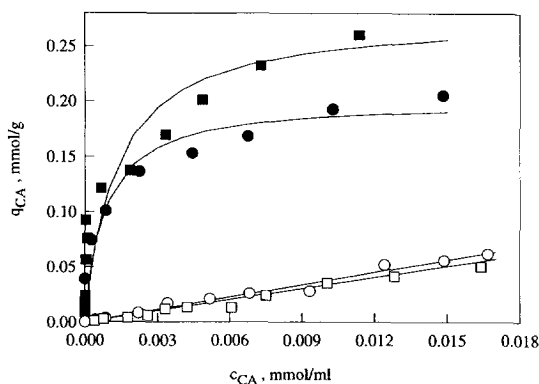


Fig. 3. Isotherms for the adsorption of CA on XAD-4 at variable ( $\circ$ ) and constant ( $\square$ ) ionic concentration and on AC at variable ( $\bullet$ ) and constant ( $\blacksquare$ ) ionic concentration at 21°C and  $\epsilon = 0.9524$ . In the samples with constant ionic concentrations, the total  $K^+$  concentration is 50 mM. The curves represent the simulated data with a Langmuir model [Eq. (14)] and the parameters shown in Table 1.

constant by adding different amounts of KCl complementary to the amount of CA, so that the total concentration of the potassium cation was 50 mM in all samples. Comparing the isotherms at constant and at variable ionic concentrations, it can be seen that this parameter does not have any meaningful influence on the affinity between XAD-4 and CA, whereas the interactions between AC and CA are enhanced at higher ionic strength in the whole concentration range (see experimental data). The absence of strong hydrophobic groups in the structure of CA and the supposedly homogeneous hydrophobic surface of XAD-4 explain the low affinity between these two materials and the relative insensitivity of their weak interactions towards changes in the ionic strength in the investigated range. In this case, as the repulsive electrostatic potential generated by already adsorbed molecules is very low due to the low concentration of adsorbed CA, increasing the ionic strength does not proportionate a significant neutralisation effect on the surface potential. On the other hand, the heterogeneous surface of AC offers a much higher affinity and capacity for CA in the investigated concentration range, and therefore the ionic strength has a measurable improving effect on the interactions between AC and CA. For HPLC systems, Ståhlberg and Bartha could rationalize the effects of the ionic strength on the equilibrium

between sorbate and matrix by using the modified Langmuir relation that includes a term for the surface potential [Eq. (17)], which can be evaluated from data for capacity factors for two sorbates having charges of different sign in the absence and in the presence of ion-pairing reagent [11]. The same procedure to estimate the surface potential in the present study is difficult due to the very weak interactions between CA and XAD-4 and the heterogeneous nature of AC, so that the original Langmuir relation [Eq. (14)] was used instead. The parameters for a Langmuir description of the equilibrium conditions calculated by fitting the experimental data to Eq. (14) are shown in Table 1. Once again, the low affinity of XAD-4 for CA can be seen from the high value obtained for the dissociation constant  $K_d$ . In this case, the Langmuir relation can be simplified to the linear relation [Eq. (15)], whose parameter  $\alpha$ , calculated from a linear regression fit to the experimental data, is also listed in Table 1. For the system AC–CA, it is not reasonable to fit the data to Eq. (15), as even in the very low concentration range no linear relation between  $q$  and  $c$  could be approximated.

#### 4.3. Isotherms for CA in the presence of QAS

It is known that the capacity and affinity of ion-pair systems for a species A depend on the loading of the matrix with the ion-pair forming species B, which, according to the electrostatic theory, can be directly related to the surface potential created by the double layer formed by the ion-pairing substance. In a column configuration, this relationship is reflected by the dependence of the retention times or capacity factors of A on the concentration of B measured in the liquid phase at the column outlet at equilibrium conditions. In a batch configuration, this effect can be observed by measuring the isotherms of the adsorption of A on the matrix pre-loaded with B at different equilibrium conditions. Preliminary batch experiments were done where the capacity of the ion-pair systems for CA was measured at different equilibrium loadings of the matrix with QAS (data not shown), which correspond to different total amounts of QAS distributed between liquid and stationary phases or to different equilibrium points in Fig. 1. An increase in the capacity of the systems for

Table 2

Equilibrium concentrations for the systems XAD-4-ABDA and AC-BTBA used as initial conditions for the batch and column experiments

System	XAD-4-ABDA	AC-BTBA
$c_{\text{QAS}}$ , mmol/ml (variable i.c.)	0.001	0.003
$q_{\text{QAS}}$ , mmol/g (variable i.c.)	0.382	0.340
$c_{\text{QAS}}$ , mmol/ml (constant i.c.)	$2 \times 10^{-4}$	0.002
$q_{\text{QAS}}$ , mmol/g (constant i.c.)	0.398	0.360

i.c. = ionic concentration.

CA followed by a decrease for increasing QAS loading could be observed, especially in the case of the system XAD-4-ABDA. For the tested concentration range, the capacity of the system AC-BTBA for CA was less dependent on the loading of the matrix with QAS than that of the system XAD-4-ABDA.

From these preliminary results, an optimal equilibrium loading of the matrix with QAS was selected for each stationary phase, corresponding in both cases to an initial QAS concentration in the liquid phase of 20 mM in a batch system with a phase ratio  $\epsilon = 0.9524$ . At this condition, the equilibrium conditions for the systems XAD-4-ABDA and AC-BTBA at variable and constant ionic strength are those shown in Table 2. Fig. 4 represents the

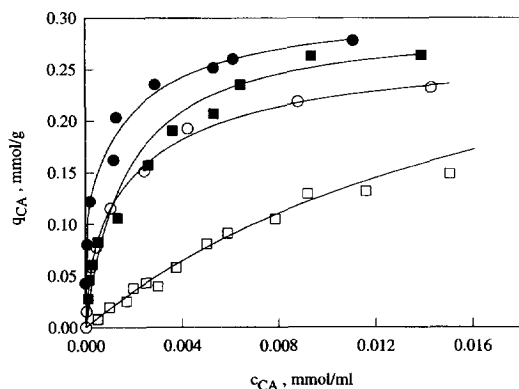


Fig. 4. Isotherms for the adsorption of CA on XAD-4-ABDA at variable ( $\circ$ ) and constant ( $\square$ ) ionic concentration and on AC-BTBA at variable ( $\bullet$ ) and constant ( $\blacksquare$ ) ionic concentration at 21°C,  $\epsilon = 0.9524$ . In the samples with constant ionic concentrations, the total  $K^+$  concentration is 50 mM. The initial equilibrium conditions between matrix and QAS are described in Table 2. The curves represent the simulated data with a Langmuir model [Eq. (14)] and the parameters shown in Table 3.

isotherms for CA on XAD-4-ABDA and AC-BTBA at these initial conditions. These isotherms were also fitted to a non-modified Langmuir model [Eq. (14)] and the calculated parameters are shown in Table 3. Especially for the low ionic strength experiment in the low concentration range, the presence of ABDA greatly increases the total capacity of XAD-4 for CA. The same increase is not so evident in the system based on AC, as AC alone has a good affinity for CA. It seems that the presence of QAS causes no enhancement in the adsorption of CA on AC. However, it has been shown through desorption experiments that the recovery of CA from the system AC-BTBA is much more effective than that from AC alone and can be done by using aqueous salt solutions [12]. In the case of AC, the desorption of CA occurs only by employing hydrophilic organic solvents and reaches at best half the recovery obtained when the system also contains BTBA. Therefore, it can be supposed that there is a significant shift in the nature of the interactions when an ion-pairing substance is introduced in the system AC-CA, although this phenomenon cannot be deduced directly from the observation of the adsorption isotherms.

The influence of the total ionic concentration in the liquid phase on the isotherms for CA can be seen in Fig. 4. Comparing the curves obtained without and with addition of KCl, one can observe that the affinity of the system XAD-4-ABDA for CA decreases significantly at a higher total ionic concentration. In the case of CA-BTBA this effect is less important. The difference in the capacity and affinity for CA cannot be explained by any decrease in strength of the interactions between QAS and stationary phase, as it was already shown (Fig. 2) that an increase in the ionic strength actually in-

Table 3

Isotherm parameters calculated by fitting the experimental equilibrium data for the systems XAD-4-ABDA-CA and AC-BTBA-CA (Fig. 4) to the Langmuir model [Eq. (14)]

Adsorption system	$q_{\text{max}}$ , mmol/g	$K_d$ , mmol/ml
XAD-4-ABDA-CA, variable i.c.	0.243	$1.1 \times 10^{-3}$
constant i.c.	0.390	0.0203
AC-BTBA-CA, variable i.c.	0.257	$2.6 \times 10^{-4}$
constant i.c.	0.297	$1.7 \times 10^{-3}$

i.c. ionic concentration.



increases the loading of the matrix. Also the capacity and affinity of the matrix XAD-4 for CA are not influenced by the ionic strength (Fig. 3). Therefore, it is clear that the deterioration of the surface potential and therefore of the capacity and affinity of XAD-4–ABDA for CA at higher ionic strength is due to weaker ionic forces between the layer of adsorbed QAS and CA. In the case of AC–BTBA–CA, it could be that this negative effect at the level QAS–CA is compensated by enhanced hydrophobic interactions between CA and AC at higher ionic strength (see Fig. 3).

To further investigate these effects, the isotherms for CA on XAD-4–ABDA and on AC–BTBA at the same initial loading condition were measured again and the final concentration of QAS in the liquid phase attained in each equilibrated sample was simultaneously measured. The initial conditions are those listed in Table 2. Fig. 5a and b show the

equilibrium concentrations measured for CA and for QAS in each system at varying ionic concentration, Fig. 5c and d show the same experiments at constant initial total ionic concentration. For these experiments, a wider concentration range of CA was investigated to evaluate also the equilibrium shift effects that occur at higher concentrations. The results shown in Fig. 5a to d reveal some important aspects of the multiple interactions that take place in ion-pair systems.

With the introduction of CA in the liquid phase of a batch adsorption system pre-equilibrated with QAS, two dynamic phenomena occur simultaneously. On the one side, CA diffuses in the direction of the matrix surface loaded with QAS. On the other side, QAS diffuses from the stationary phase back to the liquid phase. It seems that, because CA and QAS can build complexes AB in both phases, the presence of CA in the liquid phase causes the diffusion of

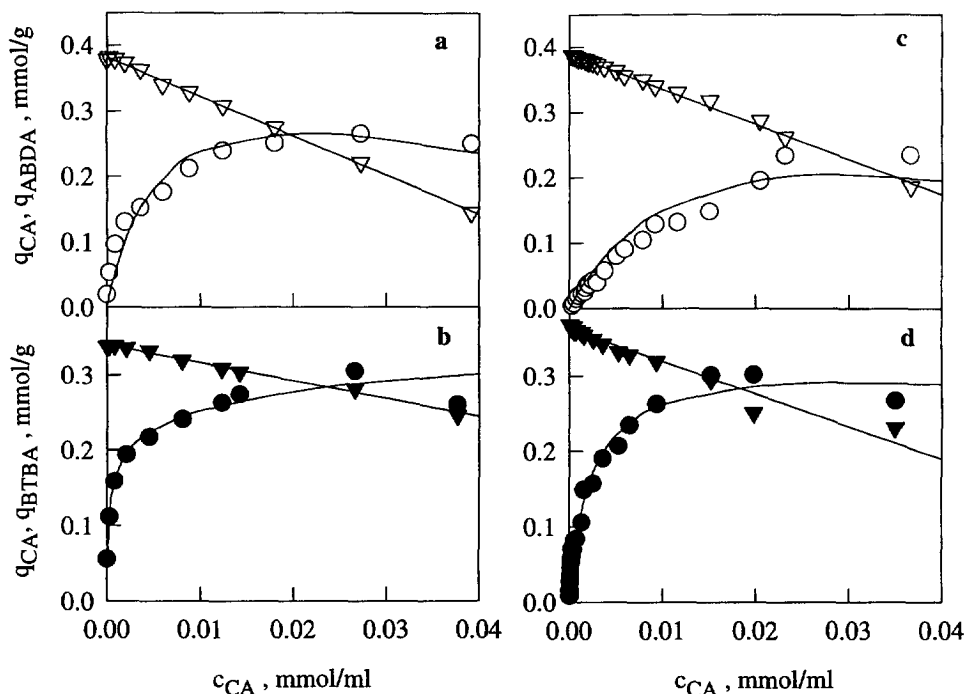


Fig. 5. Equilibrium conditions for the adsorption of CA and QAS at 21°C and  $\epsilon=0.9524$  as a function of different equilibrium concentrations of CA in the liquid phase for the systems XAD-4–ABDA (a) and AC–BTBA (b) at variable ionic concentration; and XAD-4–ABDA (c) and AC–BTBA (d) at constant ionic concentration. (a) and (c)  $q_{CA}$  (○),  $q_{ABDA}$  (▽); (b) and (d)  $q_{CA}$  (●),  $q_{BTBA}$  (▼). In the samples with constant ionic concentrations, the total  $K^+$  concentration is 50 mM. The initial equilibrium conditions between matrix and QAS are described in Table 2. The curves represent the simulated data with the modified double Langmuir model represented by Eq. (24) and the parameters shown in Table 6.

QAS molecules from the stationary phase in direction of the liquid phase, causing a decrease in the loading. As can be seen from Fig. 5a–d, the desorption of QAS is apparently dependent on the concentration of CA in the liquid phase in a direct form. QAS and CA diffuse in contrary directions until equilibrium conditions are once again established. In the new equilibrium condition, CA and QAS are distributed between stationary and liquid phase in such a way that the loading of the matrix with QAS molecules, and consequently the surface potential, is different from that observed in the absence of CA. This dislocation effect can be observed in the whole concentration range, although for very low adsorbate concentrations it can be neglected.

A second very important observation from the results shown in Fig. 5a–d is that the total capacity of the ion-pair systems for CA can be higher than the loading of the surface with QAS molecules for very high CA concentrations. As the ratio between the adsorbed molecules CA and QAS could be at best equimolar (for the total saturation of the QAS layer with CA molecules, as expected from a purely stoichiometric reasoning), it means that a second type of interaction in the investigated systems must be taken into account, which is based on the direct interaction between CA and the unloaded matrix surface. At these conditions, an analysis of the interaction phenomena just from the viewpoint of the electrostatic theory can no more be done, as the experimental conditions deviate too much from those that support the case of an ideal surface loaded homogeneously with a double ionic layer that is not disturbed by the presence of the adsorbate in the liquid phase.

Finally, it can be noted from the last experimental points that apparently a decrease of the capacity of the system for CA occurs in the high CA concentration range. A more precise investigation of this aspect would require the use of very concentrated solutions of CA, which was not feasible due to practical limitations.

#### 4.4. Column runs

To evaluate the effect of the equilibrium shift verified in batch experiments on the dynamic be-

haviour of the ion-pair system in a column configuration, column runs were made with stationary phase which was pre-loaded with QAS to equilibrium conditions. The feeding solution contained 0.005 mmol/ml CA and the equilibrium concentration of QAS (0.001 mmol/ml ABDA or 0.003 mmol/ml BTBA). The breakthrough curves for CA were determined and the concentrations of QAS in the outlet were simultaneously measured. Fig. 6 shows the obtained concentration profiles for CA and QAS. It can be seen that the gradual saturation of the stationary phase with CA causes the desorption of QAS during the run. For these runs, the constantly changing conditions in the column do not enable the maintenance of a constant ionic concentration throughout the run.

As for the batch experiments at variable ion concentrations, the increase in the capacity of the stationary phase for QAS molecules ( $c/c_{\text{QAS f}} < 1$ ) for low CA concentrations was also observed here. This effect was most evident for the system XAD-4–ABDA, which, as already shown from the batch data,

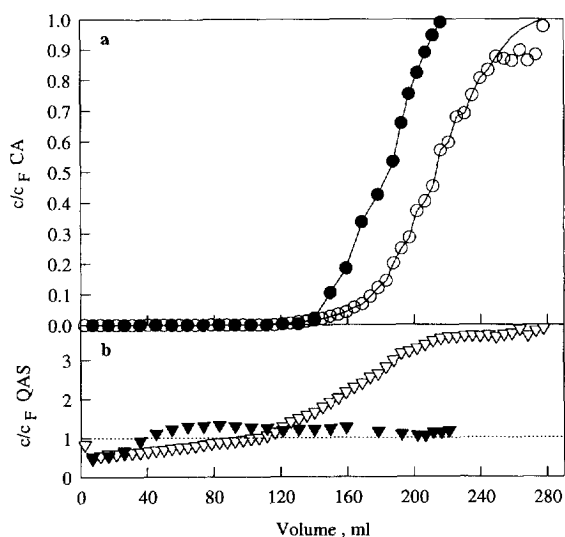


Fig. 6. (a) Breakthrough curves of CA on a 5-ml column packed with XAD-4–ABDA (O) and AC–BTBA (●) at 21°C, feeding rate 9 ml/h and feeding CA concentration  $c_{\text{f CA}} = 0.005$  mmol/ml. (b) Concentration profiles of ABDA (▽) and BTBA (▼) at the outlet, with initial equilibrium conditions between matrix and QAS are described in Table 2.  $c_{\text{f ABDA}} = 0.001$  mmol/ml,  $c_{\text{f BTBA}} = 0.003$  mmol/ml.

is more sensitive to variations in CA concentrations in the liquid phase than the system AC–BTBA.

An interesting consequence of these phenomena is the increase in the overall capacity of the stationary phase for CA in a column configuration in comparison with the expected capacity from the batch runs. Table 4 contains the theoretical capacity of the columns for CA and for QAS predicted from the equilibrium conditions shown in Fig. 5 for  $c_{CA} = 0.005$  mmol/ml and their real capacity calculated from the numerical integration of the experimental breakthrough curves. For both systems, there is an important difference between the real and the theoretical capacities. This difference is most significant for the system XAD-4–ABDA, where the real capacity for CA in the column was two times higher than that predicted from the batch isotherms. Table 4 also contains the average experimental value for the matrix loading with QAS obtained by an integral mass balance in the whole system (initial QAS loading plus total QAS fed minus total QAS eluted) if the stationary phase is considered to be homogeneously loaded (no concentration gradients of QAS along the bed). In the case of XAD-4–ABDA, the initial equilibrium loading of QAS (0.382 mmol/g) at first increased and then decreased to a final value of 0.294 mmol/g (calculated by mass balance from the data shown in Fig. 6) when the column was saturated with CA, giving a global volume-weighted average for the whole run of  $q_{QAS} = 0.365$  mmol/g ( $q_{QAS}/q_{QAS 0} = 0.96$ ). Along the column, the concentration of QAS in the liquid phase varied from 0.001 mmol/ml in the feeding point to the measured concentrations shown in Fig. 6b in the outlet. The value for  $q_{QAS}$  at equilibrium with a solution containing CA at 0.005 mmol/ml (the concentration of

CA in the liquid phase at a condition of saturated column) in a comparable batch configuration is about 0.35 mmol/g ( $q_{QAS}/q_{QAS 0} = 0.92$ ) (from Fig. 5a), whereas the respective simultaneous concentration of QAS in the liquid phase is 0.0025 mmol/g. The same analysis for the system AC–BTBA shows a decrease in the loading of BTBA from the equilibrium value of 0.340 mmol/g at the beginning of the run to 0.315 mmol/g at the saturated condition (calculated from the experimental data shown in Fig. 6b), giving a global volume-weighted average for the whole run of 0.338 mmol/g ( $q_{QAS}/q_{QAS 0} = 0.994$ ). In a batch configuration, the expected equilibrium condition for QAS in presence of CA at a concentration  $c_{CA} = 0.005$  mmol/ml would be  $q_{QAS} = 0.328$  mmol/g ( $q_{QAS}/q_{QAS 0} = 0.965$ ) and  $c_{QAS} = 0.0036$  mmol/ml. The possible meaning of these experimental values will be dealt with in the next sections.

#### 4.5. Phenomenological analysis from the electrostatic viewpoint

According to the electrostatic theory, the surface potential generated by the formation of a double layer on an adsorption surface is responsible for the ionic interactions between the loaded surface and an adsorbate, the intensity of which is proportional to the magnitude of the surface potential. As the analyte concentrations in the system are generally very low, it is assumed that its interaction with the matrix is negligible and therefore does not interfere with the equilibrium between the ion-pairing substance and the matrix. In the case of the systems investigated, it could be shown that the interactions between the ion-pairing substance QAS and the adsorbate CA in

Table 4

Experimental data from the column runs for the systems XAD-4–ABDA–CA and AC–BTBA–CA (Fig. 6),  $c_{CA f} = 0.005$  mmol/ml;  $c_{QAS f} = 0.001$  mmol ABDA/ml for XAD-4–ABDA,  $c_{QAS f} = 0.003$  mmol BTBA/ml for AC–BTBA

Adsorption system	XAD-4–ABDA–CA	AC–BTBA–CA
Theoretical capacity for CA, mmol/g	0.197	0.254
Real column capacity for CA, mmol/g	0.402	0.297
Theoretical capacity for QAS, mmol/g	0.382	0.340
Real overall average column capacity for QAS during the saturation run (mmol/g)	0.365	0.338

The initial equilibrium conditions for the columns are the same as Table 2.

the liquid phase as well as those between CA and the matrix cannot be neglected throughout the whole concentration range dealt with. In fact, it seems that the interactions QAS–CA in the liquid phase in such batch systems cause a desorption of QAS and consequently a negative effect on the surface potential, especially at high CA concentrations. Moreover, the direct interactions between CA and the matrix have an additional negative effect on the surface potential, as CA and QAS have opposite charges. The quantitative analysis of the experimental data with the help of Eq. (19) or Eq. (20) is therefore not possible in the whole experimental range, and the evaluation of the real capacity of the batch system should include the interactions of all species involved, including those related to Eqs. (1,2,5,7), which represent the different possible interactions between CA and matrix or CA and QAS in the liquid phase.

In the case of the system XAD-4–ABDA and especially for low CA concentrations, however, these interactions can be neglected and it can be supposed that CA does not influence the interaction between QAS and matrix. For the concentration range 0–0.005 mmol/ml CA, where the adsorption behaviour can be linearized, the equilibrium data shown in Fig. 5a and c was analysed according to Eq. (20) and the values for the surface potential  $\psi_0$  were calculated. Together with the average values for  $\lambda_{a0}$  and  $\lambda_a$  from the experimental data in this concentration range, they are listed in Table 5. The same analysis could not be done for the system AC–BTBA, where no concentration range could be defined within which  $\lambda_{a0}$  and  $\lambda_a$  can be considered constant without introducing too much error in the calculations.

From the results shown in Table 5, it can be seen that the ionic strength has a significant overall

influence on the surface potential of the system. It has already been shown that the interactions ABDA–XAD-4 are positively affected by the ionic strength and the values for  $\lambda_{a0}$  for variable and constant ionic strength in Table 5 are practically similar (there is even a small positive effect of the ionic strength on this parameter). Therefore, it can be shown that, in the case of the system XAD-4–ABDA, the effect of the ionic strength on the surface potential is reflected only at the level of the interactions CA–ABDA. Through an extension of this reasoning for the case of AC–BTBA–CA, it can be speculated that the negative effect of the higher ionic strength on the interactions QAS–CA is counterbalanced by its positive effect on the interactions CA–AC, which cannot be neglected.

To extrapolate the quantitative treatment of the experimental data to higher CA concentration ranges, the surface potential  $\psi_0$  for the systems XAD-4–ABDA and AC–BTBA was calculated considering that  $\lambda_{a0}$  and  $\lambda_a$  are not constant but can be represented by a Langmuir relation, with the parameters shown in Tables 1 and 3. Fig. 7a and b show the values found for  $\psi_0$  as a function of the CA concentration and of the adsorbed QAS from the data of Fig. 5a–d. From the curves for the system XAD-4–ABDA, it can be shown that the strong decrease in the potential verified for very low ionic concentrations is mainly a consequence of the variation of

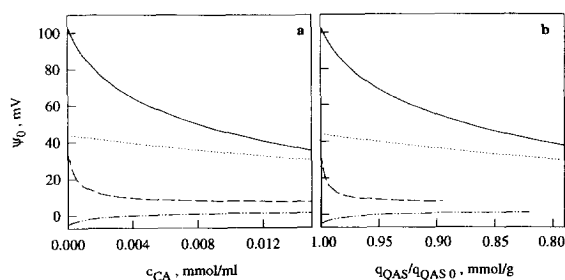


Fig. 7. Values for the surface potential  $\psi_0$  for the systems XAD-4–ABDA at variable (—) and constant (·····) ionic concentration, and AC–BTBA at variable (– –) and constant (– · – ·) ionic concentration calculated from the data in Fig. 5 with help of Eq. (20), considering  $\lambda_{a0}$  and  $\lambda_a$  to be described by Langmuir relations [Eq. (14)] with the parameters listed in Tables 1 and 3. (a)  $\psi_0$  as a function of the CA concentration in the liquid phase, reference condition for  $\psi_0=0$  in the absence of CA; (b)  $\psi_0$  as a function of the relative concentration of QAS in the stationary phase, reference condition for  $\psi_0=0$  in the absence of CA.

Table 5

Calculated values for the average partition coefficients  $\lambda_{a0}$  and  $\lambda_a$  and for the surface potential  $\psi_0$  for the system XAD-4–ABDA–CA at variable and at constant ionic strength

	Variable ionic strength	Constant ionic strength
$\lambda_{a0}$	3.8	4.3
$\lambda_a$	228	16.7
$\psi_0$ , mV	103.8	34.3

The considered concentration interval for CA is 0–0.005 mmol/ml,  $R=1.987$  cal/mol K,  $T=294$  K,  $F=23.06$  cal/mV mol.

the ionic strength. The steady, linear decrease observed for the curve of  $\psi_0$  at constant ionic strength can then be directly correlated to the desorption of QAS from the surface due to the increase in the concentration of CA in the liquid phase. In the case of the system AC–BTBA, especially in the higher concentration range, there is no variation in the surface potential. The low values calculated for  $\psi_0$  reflect the small difference verified in the capacity of AC for CA in the presence and in the absence of BTBA. However, as already commented, it has been shown through desorption experiments that the nature of the interactions in the system AC–BTBA–CA is very different from that in the system AC–CA [12]. This means that there is a shift from the direct adsorption of CA on AC to that intermediated by QAS molecules. The low values for  $\psi_0$  can therefore also qualitatively reflect the fact that CA and QAS both adsorb directly at the surface, generating surface potentials of opposite charges. At constant ionic strength, the surface potential is nearly zero, pointing to the hypothesis that the available surface of AC is equivalently shared for the direct adsorption of both species. The adequacy of this analysis could be questioned, as the experimental conditions are far away from those idealized for the use of the simplified equations of the electrostatic theory to calculate the surface potential  $\psi_0$ . Nevertheless, the observed phenomena can be well understood from the electrostatic point of view when the different variables affecting the surface potential (simultaneous adsorption and desorption of ion-pairing substance and of opposite charged adsorbate) are qualitatively considered. To provide an additional point of view on these phenomena taking the other variables into account, an analysis based on the chemical equilibrium relations represented by Eqs. (1–7) was done and is presented in the next item.

As for the batch experiments, the analysis of the deviation of the expected capacity for CA observed in the column runs is made more difficult by the fact that the concentrations of CA in the system are relatively high. Moreover, the total amount of CA in the system increases during the run and there is a real elimination of QAS from the system. From the average values for  $q_{\text{QAS}}$  evaluated before for column and batch configurations, a higher column capacity should be actually expected for the system XAD-4–

ABDA, due to a higher average surface potential in the column configuration (see Fig. 7b and values for  $q_{\text{QAS}}/q_{\text{QAS}0}$  calculated above). However, this effect alone cannot explain a column capacity for CA that is twofold higher than the batch capacity. Therefore, the column experiments were also further analysed with the help of a reasoning based purely on chemical equilibrium considerations [Eqs. (1–7)].

#### 4.6. Phenomenological analysis from the chemical equilibrium viewpoint

In view of the difficulties that hinder the analysis of batch ion-pair systems from the electrostatic point of view, especially at high adsorbate concentrations, some considerations can be done on the interactions occurring in ion-pair systems from the viewpoint of a mechanistic model based on equilibrium relations. Fig. 8 is a schematic representation of a model adsorption surface which has two relevant types of active sites, composing the surfaces  $M_1$  and  $M_2$ . These surfaces have different capacities and affinities for the species CA and QAS. In the absence of QAS, CA adsorbs on the surface  $M_1$ , which has the maximal capacity  $q_{\text{max}1}$  for this species. In the presence of QAS, a second active surface is formed as a consequence of the adsorption of QAS molecules on the active surface  $M_2$ . Due to steric hindrance or to the active sites being actually shared between both model surfaces (competition effects), these surfaces may not be entirely accessible at the same time for the two kinds of interactions, i.e. they may be partially overlapped or sterically inaccessible. The total capacity for CA is then composed by the sum of the capacities due to the surfaces  $M_1$  and  $\text{QAS}\cdot M_2$ , each one weighted by an “interference” parameter, which is dependent on the amount of CA present in the system in an unknown manner. CA is present in both the liquid and stationary phase as such or as the complex  $\text{CA}\cdot\text{QAS}$ . As the total concentration of CA in the system increases, the molecules of QAS distribute themselves between the stationary and the liquid phase so that the concentration of QAS in the liquid phase – as QAS or as  $\text{CA}\cdot\text{QAS}$  – increases too. The amount of active sites  $\text{QAS}\cdot M_2$  decreases and in consequence, a certain number of active sites  $M_1$  is once again available. For this reason, the total capacity of the system may

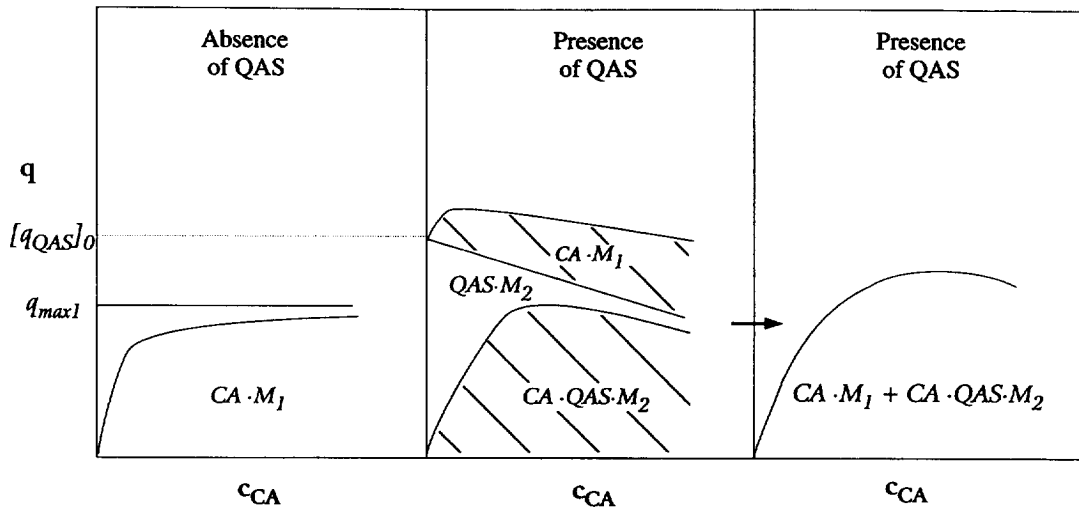


Fig. 8. Scheme for the explanation of the equilibrium phenomena occurring in a batch ion-pair system:  $q_{\max 1}$  = maximal capacity for CA in the absence of QAS;  $[q_{\text{QAS}}]_0$  = initial loading of QAS;  $M = M_1 + M_2$  - matrix;  $CA \cdot M_1$  = complex CA-matrix;  $QAS \cdot M_2$  = complex QAS-matrix;  $CA \cdot QAS \cdot M_2$  = complex CA-QAS-matrix.

show a maximum and afterwards decrease, or eventually increase steadily or show a saturation pattern, as a normal adsorption isotherm. From this point of view, the phenomenon ruling the capacity of the system for CA is the difference between the increase in active sites  $M_1$  and the decrease in active sites  $QAS \cdot M_2$  with increasing CA concentration.

A quantification of the total capacity based on these considerations should then be possible with the help of the following relation:

$$q_{CA} = x_1 q(c_{CA})_{M_1} + x_2 q(c_{CA})_{M_2} \quad (21)$$

where the parameters  $x_1$  and  $x_2$  account for the mutual "interference" of both surfaces on each other and the functions  $q(c_{CA})_{M_1}$  and  $q(c_{CA})_{M_2}$  are the isotherms for the adsorption of CA on  $M_1$  and on  $QAS \cdot M_2$ , respectively. If these two functions can be represented in a Langmuir form, a modified double Langmuir relation [Eq. (13) with  $i=2$ ] results:

$$q_{CA} = x_1 \cdot q_{\max 1} \frac{c_{CA}}{c_{CA} + K_{d1}} + x_2 \cdot q_{\max 2} \frac{c_{CA}}{c_{CA} + K_{d2}} \quad (22)$$

In the analysed ion-pair systems, the practical use

of such a relation is hindered by the fact that actually none of the parameters  $x_1$ ,  $x_2$ ,  $q_{\max 1}$ ,  $q_{\max 2}$ ,  $K_{d1}$  or  $K_{d2}$  is actually constant, because of the complex pattern of interactions between the involved species. Qualitatively, however, each one of these parameters can be analysed as follows:

1.  $x_1$  and  $x_2$ : depend on the amounts of QAS and of CA present in the system. For the sake of simplicity, these parameters can be considered constant throughout the concentration range, so that each surface  $M_1$  or  $QAS \cdot M_2$  is available for CA in a proportion varying between 0 for no availability (maximal interference) and 1 for maximal availability (no interference at all).
2.  $q_{\max 1}$  and  $K_{d1}$ : the capacity and dissociation constant of the surface  $M_1$  for CA could vary due to interactions between CA and QAS. However, it can be anticipated without too much error that these parameters can be calculated from the isotherms for CA in the absence of QAS (Fig. 3, Table 1), if the other conditions (pH, ionic strength) are equal in both systems.
3.  $K_{d2}$ : the dissociation constant of the surface  $QAS \cdot M_2$  is a measure of the affinity of CA for the stationary phase, which should be a function of

the ratio between the affinity of CA for the adsorbed form of QAS and that of CA for the QAS in the liquid phase, i.e. a function of the ratio  $K_{ABM}/K_{AB}$ , equilibrium constants for Eqs. (4,1), respectively. For same initial conditions in the batch system (total amount of QAS, pH, ionic strength, etc.),  $K_{d2}$  can be considered to be constant.

4.  $q_{\max 2}$ : the maximal capacity of the surface QAS- $M_2$  for CA should be, from a stoichiometric viewpoint, equivalent to the adsorbed quantity of QAS on the stationary phase. It has been shown that this quantity can be represented as a linear function of the concentration of CA in the liquid phase (Fig. 5):

$$q_{\text{QAS}} = q_{\text{QAS}}|_{c_{\text{CA}}=0} - m \cdot c_{\text{CA}} \quad (23)$$

5. For a single batch configuration with the same initial conditions for  $\epsilon$ , total amount of QAS, pH and ionic strength, the parameters  $m$  and  $q_{\text{QAS}}|_{c_{\text{CA}}=0}$  can be directly calculated from experimental data as the curve  $q_{\text{QAS}}$  versus  $c_{\text{CA}}$  shown in Fig. 5. The decreasing linear function between  $q_{\text{QAS}}$  and  $c_{\text{CA}}$  reflects the dislocation of QAS from the surface to the liquid phase caused by the presence of CA in the liquid phase. Therefore, the parameter  $m$  should be proportional to the ratio of the affinity of CA for QAS in the liquid phase to that of CA for the adsorbed form of QAS, i.e. a function of the ratio  $K_{AB}/K_{ABM}$ , equilibrium constants for Eqs. (1,4), respectively. Further, it should be also a function of the initial loading conditions of the matrix, of the ionic strength, pH, etc.

Considering the points above, if the parameters are handled as constants, Eqs. (21–23) can be combined and a relation follows that can be used to fit the experimental data:

$$q_{\text{CA}} = x_1 \cdot q_{\max 1} \frac{c_{\text{CA}}}{c_{\text{CA}} + K_{d1}} + x_2 \cdot (q_{\text{QAS}}|_{c_{\text{CA}}=0} - m \cdot c_{\text{CA}}) \cdot \frac{c_{\text{CA}}}{c_{\text{CA}} + K_{d2}} \quad (24)$$

After calculating the parameters  $m$  and  $q_{\text{QAS}}|_{c_{\text{CA}}=0}$  with the help of Eq. (23) from the data shown in Fig. 5, and knowing the values for  $q_{\max 1}$  and  $K_{d1}$  from Table 1, the curves  $q_{\text{CA}}$  versus  $c_{\text{CA}}$  of Fig. 5 were fitted to Eq. (24) to obtain  $x_1$ ,  $x_2$  and  $K_{d2}$ . Table 6 shows the values calculated for the parameters of Eqs. (23,24) from the best fitting curves. These curves are also the ones which are represented by the lines in Fig. 5a–d. In general, there is good agreement between experimental and simulated data. The model is able to describe the capacity decrease verified at high CA concentrations, although in this range the deviations between experimental data and simulation predictions are also larger. The values found for  $x_1$  and  $x_2$  can be interpreted as an indication that in the case of the system XAD-4-ABDA the two adsorption mechanisms are practically independent and do not interfere with each other, i.e. there are two types of active sites, each showing a selective affinity for each CA or QAS. On the other hand, the two active surfaces in the system AC-BTBA seem to be only partially available, which means that either there is a steric hindrance effect from one type of active sites on the other or that there are active sites which are common to the two

Table 6

Isotherm parameters calculated by fitting the experimental equilibrium data for the systems XAD-4-ABDA-CA and AC-BTBA-CA (Fig. 5a–d) to the proposed ion-pair equilibrium model [Eqs. (23,24)]

Parameters	XAD-4-ABDA-CA		AC-BTBA-CA	
	Variable i.c.	Constant i.c.	Variable i.c.	Constant i.c.
$q_{\text{QAS}} _{c_{\text{CA}}=0}$ , mmol/g	0.382	0.389	0.340	0.364
$m$	6.00	5.34	2.38	4.35
$x_1$	0.95	1.00	0.85	0.88
$x_2$	1.00	0.82	0.29	0.24
$K_{d2}$ , mmol/ml	$5.9 \times 10^{-3}$	0.014	$2.9 \times 10^{-4}$	0.0105

i.c. = ionic concentration.

surfaces  $M_1$  and  $M_2$ . This interpretation is reinforced by the conclusions that could be taken from the electrostatic analysis of this system, which gave very low values for the surface potential. As already commented, results from desorption experiments show the partial shift of the interactions mechanism from direct adsorption of CA on AC to its indirect adsorption by mediation of a layer of adsorbed QAS, when in presence of this substance [12]. From these two interpretations, one can suppose that both mechanisms are equivalently present in the system AC–BTBA–CA and CA and QAS compete for the same sites. These two species of opposite charges annul the surface potential generated on the surface of AC. In this case, the use of a model based on multi-component adsorption would be perhaps more convenient for the description of the isotherms instead of a model based on the addition of two kinds of active surfaces.

The values obtained for the parameter  $m$  indicate that the ratio  $K_{AB}/K_{ABM}$  is higher for the system XAD-4–ABDA than for the system AC–BTBA. The higher affinity between BTBA and AC can be also seen from the value for the dissociation constant  $K_d$  in Table 1, which is ten times lower than the same parameter for the system XAD-4–ABDA. This could explain why more ABDA diffuses from the XAD-4 active surface back to the liquid phase in the presence of CA than BTBA does from the surface of AC. The fact that less ABDA diffuses at higher ionic strengths (see values for  $m$  in Table 6) is consistent with the data shown in Fig. 2. In the case of AC–BTBA, the back diffusion of BTBA is stronger at higher ionic strengths, an indication that other interactions of ionic nature play a role in the adsorption of BTBA on the surface of AC. These interactions could be most probably of the kind represented by Eq. (5).

In relation to the experiments undertaken in column configuration, a qualitative analysis with help of chemical equilibrium relations could explain both the profile for QAS at the outflow and the higher column capacity for CA when compared to the capacity expected from batch tests. The initial increase in the capacity of XAD-4 for ABDA could be due to the simultaneous increase of the ionic concentration when CA is fed to the column. Later,

the displacement of the adsorbed ABDA to the liquid phase caused by the rising CA concentrations will overlap this positive effect, leading to a decreasing overall capacity of the matrix for ABDA. In the case of AC–BTBA, the same phenomena occur, but in addition the displacement of BTBA by CA can be compensated by the interaction of these molecules with the newly adsorbed molecules of CA on the surface [Eq. (5)].

In relation to the capacity of the column for CA, some comments must be done on the dynamic conditions of this system when compared to a batch configuration. For very low feeding rates, it can be assumed that equilibrium is attained throughout the column (a condition that was fulfilled in the experiments described). However, the fact that the system is constantly being fed with a solution containing QAS with the original equilibrium concentration and that the desorbed quantity of QAS is constantly being withdrawn from the column means that there is a lower concentration of this species in the liquid phase as should be expected in a batch system, where all desorbed QAS accumulates in the liquid phase. The average ionic strength in the liquid phase is therefore lower than in the equivalent batch system. Additionally, it means that in the equilibrium model formulated as Eq. (24) the surface  $QAS \cdot M_2$  is still being reduced along the experiment, but its affinity for CA (represented by  $K_{d2}$ ) is higher, because the average distribution of QAS between the phases,  $\lambda_{QAS}$ , is higher. From the global mass balance done for the column runs, the average value of  $\lambda_{QAS}$  for the system XAD-4–ABDA during the saturation step is 297, whereas the same value for a batch system containing 0.005 mmol/ml CA at equilibrium would be 141. In the case of the system AC–BTBA, the same parameter for the column run is 104 and for the batch run would be 91. Those values are qualitatively in accordance with the capacity enhancement found in the column configuration for the systems XAD-4–ABDA and AC–BTBA. However, to take into account the simultaneous effect of the reduced ionic strength and of the increased affinity of CA for the stationary phase on the capacity of the system for CA on a more quantitative basis would require a much more extensive set of experimental data than that shown here.



## 5. Conclusions

The analysis of the behaviour of an ion-pair adsorption system with the help of batch experiments could elucidate some features of the interactions that take place among the many different chemical species during the contact time between matrix, ion-pair forming substance and adsorbate. Some phenomena occurring in these systems differ from the behaviour of the quasi-stationary, continuous column configurations well known from analytical applications of the ion-pair chromatography. Therefore, the electrostatic theory in its simplified form could only be used to give a qualitative insight on these phenomena, as the surface potential of batch ion-pair systems has a very dynamic behaviour and depends on multiple interactions between the species present in such systems. Nevertheless, with the help of its simplified form, this theory pointed out to the different characters of the two systems investigated. To further analyse their behaviour, a semi-quantitative description was done with the help of chemical equilibrium relationships alone. However, a very extensive set of experimental data would be needed to describe all possible interferences from variables as pH and ionic strength in the many equilibrium relations coexisting in such systems. Some qualitative conclusions about the observed phenomena withdrawn from these two insights will be commented here.

In face of the total capacity of the ion-pairing systems for the adsorbate measured at high adsorbate concentrations being higher than the adsorbed quantities of ion-pairing substance, there is evidence that the direct interaction between matrix and adsorbate, without the intermediation of the ion-pairing substance, is an important factor defining the overall capacity of the system. This is especially true when the unloaded matrix already has a specific affinity for the adsorbate. At these conditions, from the point of view of chemical equilibrium relationships coexisting in these systems, one must suppose the existence of at least two active surfaces that can be either mutually independent or not, depending on the type of matrix and of ion-pair forming substance used. One surface possesses greater affinity for the ion-pair forming substance. This surface is responsible for the

adsorption based on ion-pair phenomena. The second surface has greater affinity for the adsorbate itself, and is responsible for an additional adsorption effect based on other interactions. As a consequence of the existence of two distinct adsorption mechanisms, the use of a mathematical model based on a modified double Langmuir relation has proven to be adequate for the description of the isotherms for the ion-pair system. Other authors [15,16] had already shown that models based on two active site types can explain some characteristic features for the ion-pair chromatography. The developed model can also predict the decrease in capacity for CA at high CA concentrations as a consequence of the decrease in the concentration of adsorbed QAS. From the viewpoint of the electrostatic theory, this decrease in the capacity of a batch ion-pair system can be qualitatively predicted in face of the phenomena observed here, as the surface potential will decrease due to the simultaneous decrease in the concentration of adsorbed QAS and increase in the concentration of adsorbed CA. The coexistence of these two adsorption mechanisms can explain why the use of ion-pair chromatography leads sometimes to peak splitting under specific pH and ionic strength conditions [14]. If these conditions permit the simultaneous occurrence of both kinds of adsorption mechanisms at a similar degree of relevance, the adsorbate molecules can be retarded by two very different ways. If, on the other hand, one of two mechanisms prevails through selected eluent conditions, the adsorbate peak will be dislocated to one or the other extreme of the possible retention time range.

The mutual equilibrium shift verified for CA and for QAS under different conditions for batch and for column configurations is an indication that the formation of a complex CA·QAS in the liquid phase is an important phenomenon in the ion-pair chromatography and should be taken into account to describe the mechanism involved in this technique, when the system under study contains significant amounts of adsorbate in the liquid phase. In the investigated batch system, the increase of the concentration of CA led to dramatic shifts of the partition coefficients of QAS, which can have as a consequence the partial shift of the relevant adsorption mechanisms from ion-pairing to simple

adsorption effects. In an analytical column where the sample dislocates along the equilibrated stationary phase, the high local concentration of adsorbate in the peak zone could also lead to significant temporary shifting effects of the ion-pair forming substance, that tend to reequilibrate themselves after the peak has passed. These effects are dependent on the sample concentration, and can therefore cause the dependence of the retention time of a species or of the peak shape on the sample concentration, a generally known phenomenon for ion-pair chromatography that has been also sometimes reported [17–19]. Usually, however, the sample concentrations in the analytical chromatography are very low and the interference of the adsorbate on the equilibrium between ion-pairing substance and matrix can be neglected.

Finally, the well known fact that the retention times or capacity factors show a maximum for a particular concentration of the ion-pair forming substance and then decreases for higher concentrations can be understood in the light of the equilibrium considerations given in this paper. First of all, there is an increase in the ionic strength of the system, which can have a significant negative effect on the affinity of the loaded stationary phase for the adsorbate or on the surface potential. Furthermore, for the majority of the usually employed ion-pairing substances, increasing their total amount in the system leads to a decrease in their partition coefficient between stationary and liquid phase. Therefore, the equilibrium conditions for CA are also shifted towards the liquid phase due to its interaction with the QAS molecules. A maximum should only be absent in the cases where the adsorption of the ion-pairing substance on the matrix show a linear or unfavourable (concave upward) isotherm, i.e. where the partition coefficient is constant or increases with the increase of the concentration in the liquid phase.

In conclusion, to consider the effects relevant for the ion-pair chromatography as a preparative or purification tool, it is important not only to have previous knowledge of the equilibrium behaviour between the chosen combination of ion-pairing substance and matrix, but also the interactions between adsorbate and matrix without mediation of ion-pairs and the mutual dependence of the partition behaviour of the ion-pair forming substance and the adsorbate

in the presence of each other are to be considered, as these additional effects can be significant for the behaviour of the ion-pairing system under non-stationary, dynamic operation conditions, in batch as well as in column configurations.

## 6. Symbols

$c$	Concentration in the liquid phase, mmol/ml
$F$	Faraday number, cal/mV mol
$\Delta G^\circ$	Free energy of adsorption, cal/mol
$K$	Equilibrium constant
$K_d$	Dissociation constant, mmol/ml
$m$	Parameter in Eq. (24)
$q$	Concentration in the stationary phase, mmol/g
$q_{\max}$	Maximal concentration in the stationary phase, mmol/g
$R$	Universal gas constant, cal/mol K
$T$	Absolute temperature, K
$x$	Fractional availability of active surface
$z$	Ionic charge
$\alpha$	Constant for the linear isotherm
$\epsilon$	Phase volume ratio, liquid to total volume
$\lambda$	Partition coefficient
$\lambda_0$	Partition coefficient for CA in the absence of QAS
$\psi_0$	Surface potential, mV

### 6.1. Abbreviations and subscripts

0	Initial condition
1	Related to the adsorption active surface $M_1$
2	Related to the ion-pair adsorption active surface $\text{QAS} \cdot M_2$
a	Related to the adsorbate
A	Generic adsorbate species
ABDA	Alkylbenzyltrimethylammonium chloride
AC	Activated charcoal
B	Generic ion-pair forming substance
BTBA	Benzyltributylammonium chloride
CA	Clavulanic acid
f	Feeding
M	Matrix
QAS	Quaternary ammonium salt

TBA Tetrabutylammonium hydrogensulphate  
TEA Tetraethylammonium chloride

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