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Adsorption of aqueous organic mixtures on a chiral stationary phase with bound antibiotic eremomycin

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ABSTRACT

The adsorption of two typical hydro-organic mobile phases, with methanol and acetonitrile as the organic component, on an antibiotic based chiral stationary phase (CSP) Nautilus-E was studied by the minor perturbation method. In both cases, the excess adsorption of water was positive over a wide range of concentrations from 0 to ~75 or 90 mol% for MeOH or MeCN containing mobile phases, respectively. Such hydrophilic properties of the CSP were attributed to multiple polar functional groups of the chiral ligand and to the residual silanol groups of the silica support. The adsorbed phase was found to be thinner for H_2O–MeOH (~1.1 Å) and thicker for H_2O–MeCN (~9.4 Å). The measurements of the column hold-up volume by different methods allowed us to suggest a model of the adsorbed phase consisting of the volume between bound chiral selectors inaccessible to large size molecules and of the stagnant layer of the mobile phase adsorbed on the external surface of the chiral selectors.

1. Introduction

Adsorption of binary solutions on solids was intensively studied in the 1960s through the 1980s [1–3] in particular to explain properties of HPLC packings in contact with multicomponent mobile phases [4,5]. A solid theoretical basis for these works was provided by Schay and co-workers [1] and Everett [6,7]. This period of “Storm and Stress” had been followed by a peaceful decade of abating interest, not in the last part because the experimental protocol used at that time demanded to keep constant the *mass* of the solution that is not convenient in chromatography where the *volume* of solution is controlled. A revival of attention to this problem was due to a series of publications by Kazakevich et al. [8–10], who not only adopted from earlier works [11,12] a convenient technique to measure excess adsorption isotherms of binary liquid mixtures, the minor perturbation method, but, more importantly, demonstrated the usefulness of the method to study the structure of the adsorbed layer on stationary phases.

This approach has successfully been used by some authors to understand retention mechanisms in reversed phase (RP) liquid chromatography [13,14] and in order to characterize RP stationary phases [15]. It also proved to be informative in chiral chromatography. So, Cavazzini and co-workers studied the adsorption of binary eluents on polar chiral stationary phases (CSPs) to clarify the effect of strong mobile phase additive on enantioseparation [16,17]. The authors of [18] derived information about the composition and thickness of the surface liquid layer on a Whelk-O1 CSP from the excess adsorption isotherms of binary solvents. The purpose of the present work is to apply the method in question to the investigation of surface properties of a silica-based CSP with grafted antibiotic eremomycin (Fig. 1) in contact with typical chromatographic mobile phases. This chiral adsorbent is a recent addition to the family of macrocyclic antibiotic CSPs, demonstrating good separation ability for enantiomers of amino acids [19–21] and 2-arylpropionic acids [22]. This CSP was shown to behave similarly to the representative antibiotic CSP Chirobiotic T (with teicoplanin) in the chromatography of amino acids [20]. Interaction of mixed solvents with the surface of antibiotic-based CSPs including the CSP in question has never been studied before, leaving researchers without proper knowledge of the distribution of the solvent constituents between the stationary and mobile phases.

2. Theoretical

2.1. Excess adsorption

Consider an adsorption equilibrium on a liquid/solid interface, the liquid being a binary mixture. The adsorption measured in both

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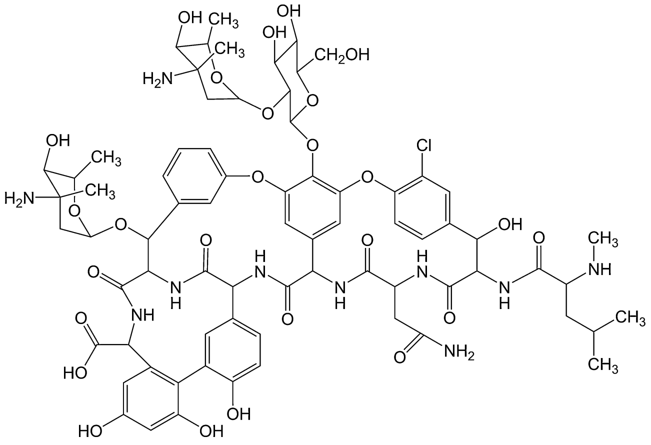

Fig. 1. Eremomycin.

batch and chromatographic experiments is in fact excess adsorption understood in the Gibbsian sense [11,12]. It is defined as the excess amount of a solute present in a system over the amount contained in a hypothetical reference system in which the solute concentration remains uniform throughout the whole liquid phase up to the solid surface and is equal to the bulk solute concentration in the real system. The choice of the reference system is arbitrary but in practice limited by the experimental setup. A natural reference system in the batch method is one containing the same total number of moles n_0 of the liquid phase as in the real adsorption system. Such a selection of the reference system is called the *n* convention [23]. The corresponding adsorption excess of component *i* per unit area of the solid surface is given by

$$\Gamma_i^{(n)} = \frac{n_0(x_{0,i}^l - x_i^l)}{A}, \quad i = 1, 2 \tag{1}$$

where A is the total surface area of an adsorbent, $x_{0,i}^l$ and x_i^l the initial (before contact of a liquid with a solid) and equilibrium molar fraction of component *i*, respectively. The notation $\Gamma_i^{(n)}$ symbolizes the use of the *n* convention. Summing the adsorption excesses for both components of the liquid phase and taking into account that $x_1^l + x_2^l = 1$, we obtain a displacement rule

$$\Gamma_1^{(n)} = -\Gamma_2^{(n)} \tag{2}$$

meaning that positive adsorption (accumulation) of one component of a binary mixture always results in ousting from the surface layer the molecules of the other component and vice versa.

Another useful choice of a reference system, the *V* convention [23], assumes that (i) the volume of the liquid phase in the reference system is equal to that in the real system and (ii) that the volumes of the liquid phase before and after contact with the adsorbent are the same. The corresponding specific adsorption excess of component *i* is expressed through the volume of the liquid phase V_0 and the molar concentrations of *i* before ($c_{0,i}$) and after (c_i) equilibrium is established:

$$\Gamma_i^{(v)} = \frac{V_0(c_{0,i} - c_i)}{A}, \quad i = 1, 2 \tag{3}$$

The *V* convention is natural to chromatographic experiment, in which the volume of the liquid phase remains constant, limited by the column walls. Guggenheim and Adam [23] have shown that the specific excess adsorption values based on different conventions relate one to another as

$$\Gamma_i^{(n)} = \Gamma_i^{(v)} - x_i^l\left(\Gamma_1^{(v)} + \Gamma_2^{(v)}\right) \tag{4}$$

This transformation is necessary in order to make use of the elaborate theory of adsorption equilibrium of the batch experiment [6,7,24] for the analysis of experimental data obtained by means of chromatography. There have been disputes, still unresolved, about thermodynamic consistency of Eq. (4) [24–26], which is undoubtful only for ideal systems. A current approach consists in using this expression and neglecting a probable inconsistency in results, which is not larger than a few percent, as estimated from the volume contraction effect for the mobile phases studied.

2.2. Excess adsorption, total adsorbed amount, and thickness of the adsorbed layer

The concept of surface excess, although directly relating to experimentally measured quantities, has an inherent drawback that impedes its use in studying the adsorption equilibrium. Consideration of this phenomenon in terms of the mass action law (see Eq. (11) below) requires the use of the total surface concentrations of the liquid phase components rather than the excess quantities. A definition of the surface concentration must rely on a certain model of the surface layer as it requires assigning a specific volume to the surface layer. The simplest model was developed by Guggenheim and Adam [23] who suggested the division of the liquid phase by two parts. One part, adjoining to the solid surface and extending normally to the surface at a distance τ, is assumed to contain all excess amounts of the liquid phase components. This is the adsorbed phase. Above this layer of thickness τ, there is the bulk phase where the component concentrations are supposed to be equal to the equilibrium ones. The specific total content of component *i* in the adsorbed phase is

$$q_i = \Gamma_i^{(v)} + \tau c_i \tag{5}$$

or

$$q_i = \Gamma_i^{(n)} + x_i^l q^\infty \tag{6}$$

In Eq. (6), the thickness of the adsorbed phase is implicitly included in the saturation capacity of the adsorbed layer, $q^\infty = q_1 + q_2$. Indeed, general logics suggests that the number t of monomolecular layers constituting the adsorbed phase is given by [27]

$$t = a_1^* q_1 + a_2^* q_2 \tag{7}$$

The molar cross-sectional areas a_i^* of the compounds are, in general, functions of the surface concentration. Neglecting this circumstance and also assuming that t is constant, a useful expression for the molar fraction x_1^a of component 1 in the adsorbed layer is derived from Eqs. (6) and (7) [24,27]:

$$x_1^a = \frac{t x_1^l + a_2^* \Gamma_1^{(n)}}{t + (a_2^* - a_1^*)\Gamma_1^{(n)}} \tag{8}$$

The application of Eqs. (7) and (8) requires knowledge of a_i^*. In this study, we use the values recommended by Gritti and Guiochon [27]: 78,000; 130,000; 160,000 m^2/mol for water, methanol, and acetonitrile, respectively.

Depending on the convention chosen, both Eqs. (5) and (8) may be used to evaluate the thickness of the adsorbed layer, although in somewhat different terms, provided that additional assumptions are made. The first of them is that this characteristic does not depend on the composition of the liquid adsorbed. To determine τ from Eq. (5), one should also assume, following Schay et al. [1], that the derivative ($\mathrm{d}q_i/\mathrm{d}c_i$) is equal to zero within the concentration range corresponding to the linear part of the excess adsorption isotherm. Then, in that concentration interval, $\tau = -(d\Gamma_i^{(v)}/dc_i)$.

Eq. (8) in combination with the thermodynamic condition for two-phase coexistence $(\partial x_1^a/\partial x_1^l)_T > 0$ [28] allows evaluation of