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A quest for the optimal additive in chiral preparative chromatography

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ABSTRACT

Traditionally, the choice of acid/base additives used in chiral preparative chromatography has not been considered very important. However, it was recently demonstrated that strongly adsorbing additives can result in the most unexpected enantiomer band shapes in modern chiral preparative chromatographic systems. In the present study we demonstrate that, depending on the choice of additive, it is actually possible to obtain the following four binary band-shape compositions when a racemic mixture is injected: (i) anti-Langmuir/anti-Langmuir, (ii) anti-Langmuir/Langmuir, (iii) Langmuir/Langmuir and (iv) Langmuir/anti-Langmuir. Further, we made an advanced numerical investigation, in order to ascertain which one of the four band-shape compositions, is the most favourable one in preparative batch chromatography of a racemic mixture. We found that if the target for purification is either the first eluting enantiomer or both ones, the traditional Langmuir/Langmuir band-shape composition should be chosen. But, if only the second eluting enantiomer is to be purified the optimal situation is the anti-Langmuir/Langmuir band-shape composition. Thus, it was concluded that the best choice of additive depends on which enantiomer is of interest and it is useful to perform a thorough additive screening to find the optimal additive, giving the most advantageous peak shape composition and accordingly the best process performance for a particular separation problem.

1. Introduction

A most peculiar and interesting phenomenon was introduced and investigated both theoretically and experimentally [1–6] around 15 years ago, namely that a solute can have an anti-Langmuirian shape (diffuse front, sharp rear) although its adsorption isotherm is a true Langmuir. The pioneer studies were made on a system comprising of silica as the stationary phase and the mobile phase was dichloromethane containing the additive 2-propanol in different bulk concentrations. The solutes among others were 2-phenylethanol and 3-phenyl-1-propanol [2,4]. Further investigations were made in a reversed-phase ion-pair system containing a binary eluent where the additive was protriptyline, a tricyclic antidepressive with strong UV-properties, and the solutes were substituted benzamides [5]. In this particular system, a diode-array detector allowed the simultaneous recording of the solute- as well as the additive signal. This gave the essential information required to develop a rule of thumb, outlined below, for knowing when anti-Langmuir solute band shapes appear due to an additive, although the solute adsorption isotherm is a true Langmuir [5,6].

For the appearance of anti-Langmuir band shapes two requirements must be fulfilled [5,6]. First, the adsorption strength of the additive must be greater than that of the solute. This condition can be verified if both solute and additive are injected into a chromatographic system comprising an eluent containing only the weak solvent, and not the additive. The first requirement is fulfilled when the retention factor of the additive, k_{add}, is larger than that of the solute, k_{sol}, so that the separation factor, α_0, between the additive and the solute is $\alpha_0 = k_{add}/k_{sol} > 1$. The second requirement is that when a constant concentration of additive is percolated through the column, the additive system peak elutes before the solute at the plateau level of additive, i.e., $\alpha_p \leq 1$. Under all other conditions the solute peak is Langmuir shaped. Exceptions to this rule of thumb can be observed at very large sample loads. Then the large solute and perturbation zones may overlap and interfere, often resulting in loss of separation.

In a more recent paper by Quinones et al. high-concentration system peaks were obtained injecting mixtures of benzyl alcohol and 2-phenylethanol into a reversed-phase system at different relative concentrations of the solutes and 2-methylbenzyl alcohol as the strong additive in the eluent [7]. A most interesting binary band-shape composition was shown where the first band is Langmuir shaped and the second one anti-Langmuir shaped resulting in an improved baseline resolution between the two bands, see Fig. 12 in [7]. It was suggested in the last part of the conclusion that the

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phenomenon may "... provides the knowledgeable separation scientist with another degree of flexibility in the development of new methods to enhance production rate, recovery yield, and/or product purity".

Fast methods for isolation of gram to kilogram amounts of both optical isomers of the candidate drugs are of outmost importance for the pharmaceutical industry today. This has been the case since 1992 when the American Food and Drug Administration (FDA) started to require that both optical isomers of chiral drugs are tested already at an early stage of drug development [8]. Therefore, a possibility to increase both yield and production rate of an existing preparative method should be of outmost importance.

Recently we showed that the above mentioned effects occur in a modern system aimed at chiral, binary preparative separations of enantiomers. More importantly, we demonstrated that we could successfully tune the shapes of the binary profiles by changing the mobile phase composition. The system comprised of a teicoplanin stationary phase (Chirobiotic T) and polar methanol/acetonitrile mobile phases with acetic acid/triethylamine as additives and the solutes were the β-blockers alprenolol, propranolol and atenolol [9]. In this system we could create the interesting binary peak shape composition where the two sharp sides point to each other, i.e., the first peak is anti-Langmuir and the second Langmuir, see Fig. 2b in [9]. Other shapes obtained were anti-Langmuir/anti-Langmuir and the traditional Langmuir/Langmuir. Experiments showed that triethylamine adsorbed strongly to the stationary phase and was the cause of the interesting enantiomer peak shapes. More advanced, truly anti-Langmuirian, models could therefore be rejected. By using the inverse method [10,11] we could determine both the bi-Langmuir adsorption isotherms of the β-blocker enantiomers and the "invisible" additive (triethylamine).

Most recently, a theoretical study was carried out based on the available thermodynamic information for the enantiomers of these three β-blockers on the teicoplanin chiral stationary phase mentioned [12]. The optimization of the process performance when performing repetitive batch injections was investigated using the equilibrium dispersive model. It was concluded that for a proper model-based optimization it is absolutely necessary to consider the invisible additive perturbation peak when defining the cycle time. Furthermore, it was indicated that both productivity and yield could be improved for the two unusual shape combinations in comparison to the traditional Langmuir/Langmuir case. Unfortunately, since the three β-blockers had very different binding capacities, solubility and retention times it was not possible to draw any general conclusions. Moreover, the fourth band-shape composition was lacking (Langmuir/anti-Langmuir).

For proper numerical optimization of a preparative chromatographic system it is important to consider (i) what should be optimized, (ii) the experimental parameters that are going to be tuned and (iii) the constraints imposed on the system [13]. Normally, when the costs cannot be properly estimated, one investigates the maximum production rate using a yield constraint.

The aim of the present study is twofold. The first aim is to perform a systematic numerical investigation of the advantages and disadvantages of the four different band-shape compositions possible when using a racemic mixture with respect to preparative process performance, i.e., yield and productivity. Here, we also investigate the impact of the column efficiency on the preparative performance. The four different band-shape compositions are: (1) both bands being anti-Langmuir, (2) the first band being anti-Langmuir and the second Langmuir, (3) both bands being Langmuir and finally (4) first band Langmuir and second anti-Langmuir. In case 2, the two sharp ends of respective enantiomer band are pointing towards each other resulting in increased baseline resolution, see Fig. 12 in [7] and Fig. 2b in [9], and in case 4 the two sharp sides are instead pointing in opposite directions. Visually, these two situations look advantageous as compared to the traditional Langmuir/Langmuir case, but we cannot know the truth without a careful numerical investigation under otherwise more or less identical conditions. In order to generate the different band shapes above we fix the adsorption isotherm parameters of the enantiomers and carefully select four sets of additive adsorption isotherm parameters. The second aim is to investigate if there actually exists a "perfect" additive for a particular purification problem. Here we will use an advanced optimization routine to find the additive adsorption isotherm parameters that facilitates the most productive separation process. This corresponds to a thorough additive screening in the method development laboratory.

2. Theory

In this study the equilibrium dispersion model was used to describe the chromatographic process [13]. This model assumes instantaneous solute equilibrium between the mobile and stationary phases and that all dispersive contributions are lumped together into a single apparent dispersion constant. We have one partial differential mass balance equation, with initial and boundary conditions, per component,

$$\begin{cases} \dfrac{\partial C_i(x,t)}{\partial t} + F\dfrac{\partial q_i(x,t)}{\partial t} + u\dfrac{\partial C_i(x,t)}{\partial x} = \dfrac{L\,u}{2\,N_{\mathrm{ap}}}\dfrac{\partial^2 C_i(x,t)}{\partial x^2}, \\ 0 \le x \le L, \quad t \ge 0, \quad i = 1, \ldots n, \\ C_i(x,0) = C_{0,i}, \\ C_i(0,t) = \varphi_i(t). \end{cases} \tag{1}$$

Here n is the number of components, $C_i(x,t)$ and $q_i(x,t)$ are mobile and stationary phase concentrations of each component i at time and space coordinates t and x, L is the column length, F is the column phase ratio, u is the linear flow velocity and N_{ap} is the apparent number of theoretical plates, here assumed to be equal for all components. In this study, there were three components; two enantiomers denoted by sub-indices 1 and 2, and an additive denoted by sub-index "add". The boundary conditions, or injection profiles, φ, can be measured experimentally, but here they are assumed to be square pulses. The initial condition, C_0, describes the column state prior to injection, i.e., the initial concentration of the component in the mobile phase. In this study, we assumed that the column was equilibrated with an eluent containing the additive with concentration C_{add}, and used the same additive concentration in the injected sample. The elution profiles at the outlet $C_i(L,t)$ was calculated by solving the mass balance equations using a modified Rouchon method as described elsewhere [13]. The Rouchon method assumes identical N_{ap} for all components.

Using a proper adsorption isotherm function, $q_i(x,t)$, it is possible to calculate quite realistic estimates of the solute and the additive elution profiles. In this study we will use the competitive Langmuir adsorption isotherm model,

$$q_i(C_1, C_2, C_{add}) = \frac{a_i C_i}{1 + b_1 C_1 + b_2 C_2 + b_{\mathrm{add}} C_{\mathrm{add}}}, \quad i = 1, 2, \text{ add}, \tag{2}$$

where a and b are the adsorption isotherm parameters. A similar model (the competitive bi-Langmuir model [13]) has successfully been used previously to account for the adsorption of the additive triethylamine and β-blocker enantiomers [9]. In that experimental study Langmuirian additive perturbation peaks and Langmuirian/anti-Langmuirian enantiomer peaks were observed and properly described by the chosen model. In the present study we choose a single-site model, for the sake of simplicity.

One of the most frequently considered performance criteria is the *production rate*, Pr_i, which is defined to be,

$$Pr_i = \frac{n_{\mathrm{coll},i}}{t_{\mathrm{c}}}, \quad i = 1, 2, \tag{3}$$