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Short communication

# Retention of Naproxen enantiomers on the chiral stationary phase Whelk-O1 under reversed-phase conditions. A reconsideration of the adsorption mechanism in the light of new experimental data

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ABSTRACT

A model of adsorption of the Naproxen enantiomers on the (*S*,*S*)-Whelk-O1 chiral stationary phase that we recently proposed was found to be inconsistent with new experimental data. A new model taking into account the variation of the dissociation coefficient of the analyte during elution of the band is discussed.

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In a recent publication [1] we proposed a model of the adsorption of the Naproxen enantiomers on a (*S*,*S*)-Whelk-O1 chiral stationary phase (CSP) from buffered methanol–water solutions. This model assumed the coexistence of two types of adsorption sites, enantioselective (chiral selectors) and nonselective (residual silanol groups) sites. It was assumed that the adsorption of the more strongly retained (*S*)-Naproxen on a chiral selector occured through the formation of a monomolecular adsorption complex whereas that of the less retained (*R*)-Naproxen would involve the formation of monomolecular and bimolecular complexes with bonded ligands. The bimolecular adsorption complex was supposed to result from the interaction of a first adsorbed molecule with a second one from the mobile phase. This model was suggested by the evolution of the band shapes of the less retained enantiomer from bands exhibiting a front shock layer to bands with a rear shock layer when the methanol concentration of the mobile phase increased from 60 to 80 vol.%, as illustrated in Fig. 1. The adsorption isotherms developed based on this set of assumptions allowed an excellent approximation of the experimental elution profiles for both enantiomers and the best-fit isotherm parameters had reasonable values, consistent with their physical meaning. This isotherm model explained the progressive change of shape of the (*R*)-enantiomer band by the influences of the methanol concentration on both adsorption and a dimerization equilibria.

However, new experiments carried out in an extended range of mobile phase pH and ionic strength ($I$) delivered results that question this model. The peak shape of both enantiomers seem to depend on the ionic strength of the mobile phase when its pH is high enough (pH > 6), as illustrated in Fig. 2 where elution profiles are shown for $I = 0.01$ and 0.03 M (pH = 6.35 in both cases) and a constant methanol concentration. These data force a new conclusion, suggesting that the correlation observed in [1] between methanol concentration and peak shape was actually caused by the influence of the methanol–buffer concentration ratio on the mobile phase pH, resulting in an increase of the apparent pH with increasing methanol fraction (see Table 1 in Ref. [1]). The assumption of associative adsorption cannot explain these new results.

An alternative model can be developed, in which we still keep the concept of a two-site surface [2] but assume that the saturation capacity of the nonselective sites (residual silanol groups) is much smaller then that of the enantioselective sites. This assumption is supported by the hydrophobic properties of the Whelk-O1 surface [1]. In contrast to what we initially did, we need to assume that the dissociation degree of Naproxen ($\alpha$) does not remain constant during the elution of its band, which is confirmed by the results of the calculation of $\alpha$ in the buffer systems used, as will be shown below. Furthermore, the neutral (n) and the anionic (an) forms of each Naproxen enantiomer are postulated to adsorb competitively on the same enantioselective sites, but with different equilibrium constants, $K_n$ and $K_{an}$, respectively. The adsorption isotherm of the ionizable compounds on uncharged sites is

$$q = \frac{q_{ns} b_{ns} c}{1 + b_{ns} c} + K_{an} c\alpha + K_n c(1 - \alpha) \quad (1)$$

where $q$ is the overall uptake of Naproxen species and $c$ their overall equilibrium liquid phase concentration. $q_{ns}$ and $b_{ns}$ are the saturation capacity and the adsorption coefficient of the nonselective sites, respectively. Note that the total adsorption of Naproxen (i.e., the sum of the amounts of the neutral and anionic forms

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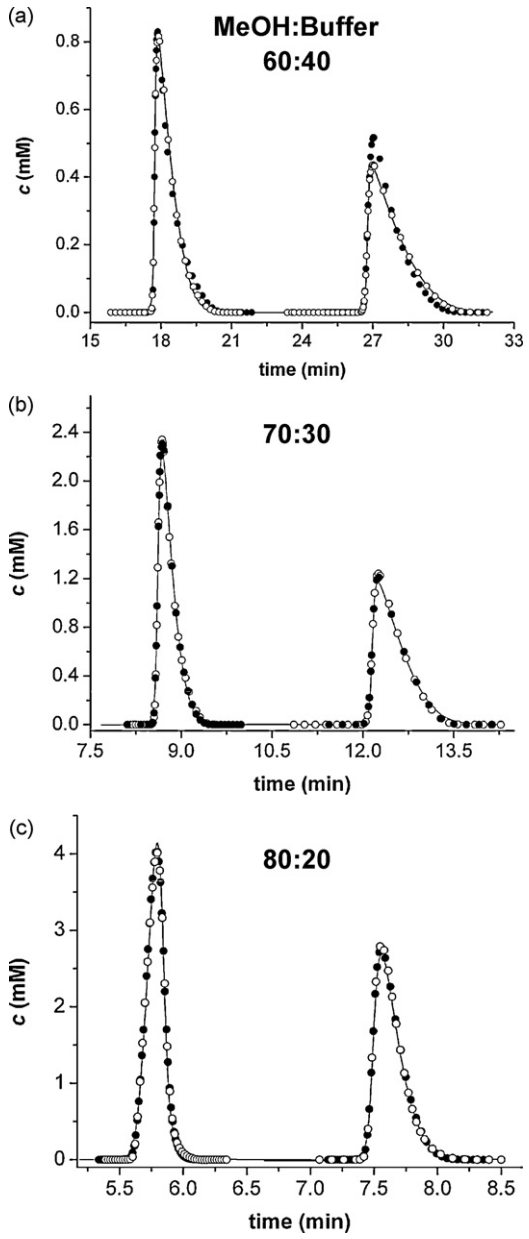


**Fig. 1.** Experimental (lines) and simulated (symbols) elution profiles of (*R*)-Naproxen (the first peak) and (*S*)-Naproxen (the second peak) for different methanol–acetic buffer (0.09 M $CH_3COOH$ + 0.03 M $CH_3COONa$) ratios. The open circles show results of calculations made according to the old model described in [1], the filled circles show the profiles calulated using Eq. (1) of this work. Conditions: (250 mm × 4.6 mm) (*S,S*)-Whelk-O1 column, temperature 34.7 °C, flow rate 1 ml/min, sample concentration 34.8 mM, and sample size 20 μl.

adsorbed) on the nonselective sites is approximated by a Langmuir local isotherm and the adsorption of either form of Naproxen on the chiral selectors is approximated by a linear local isotherm with corresponding adsorption constants. This latter simplification is supported by the fact that, in the investigated range of concentrations, the Naproxen adsorption isotherms were only slightly nonlinear [1]. Taking into account that the adsorbed amounts measured were much lower than the chiral selector surface density, the nonlinear behavior of the overall isotherm should be ascribed to the nonlinear contribution of adsorption on the sparse nonselective sites.

The dissociation degree of Naproxen is calculated by solving the set of dissociation equilibrium equations for mixtures of Naproxen and buffer (acetic) acid in the presence of a buffer salt according

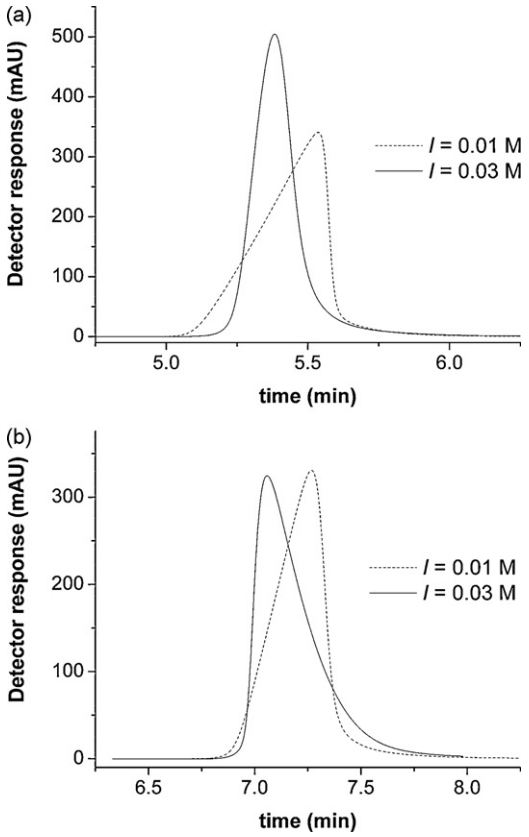


**Fig. 2.** Chromatograms of (*R*)-Naproxen (a) and (*S*)-Naproxen (b) for methanol–water (80:20, v/v) mobile phases with different ionic strengths. The ionic strength was set at the required value by dissolution of proper amounts of $CH_3COOH$ and $CH_3COONa$ in the methanol–water mixture. Temperature was 27 °C, other conditions the same as in Fig. 1.

to a procedure given in [3]. Numerical values of the dissociation constants for methanol–water solvents were found in [4,5]. Fig. 3 shows plots of $\alpha$ as a function of the total Naproxen concentration, *c*, for the 3 mobile phase compositions examined in [1]. For the purpose of numerical calculation of the elution profiles those curves were approximated with second order polynomials.

Fig. 1 compares the experimental chromatograms of the Naproxen enantiomers taken from [1] with those calculated using: (1) the isotherm model described in that earlier publication and (2) the isotherm in Eq. (1), with details of the numerical proce-

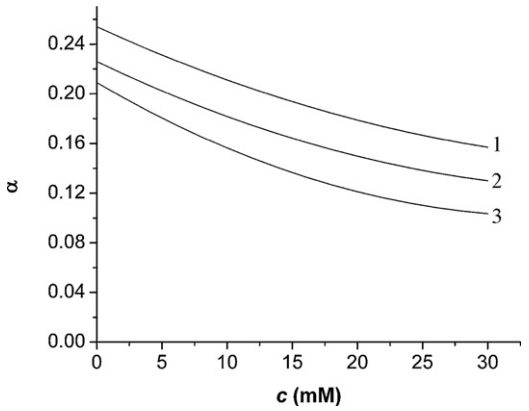


**Fig. 3.** Dissociation degree of Naproxen as a function of its concentration for different compositions of the methanol–acetic buffer mixture (v/v): 60:40 (1), 70:30 (2), and 80:20 (3). The acetic buffer was 0.09 M $CH_3COOH$ + 0.03 M $CH_3COONa$.

**Table 1**
Best-fit parameters of the adsorption isotherm Eq. (1).

| Coefficient | Methanol–buffer [a] ratio (v/v) | | |
|---|---|---|---|
| | 60:40 | 70:30 | 80:20 |
| $q_{ns}$ (mM) | 1.02 | 0.69 | 0.07 |
| $b_{ns}$ ($mM^{-1}$) | 1.92 | 0.60 | 1.49 |
| $K_{an}(R)$ | 7.43 | 4.67 | 0.92 |
| $K_n(R)$ | 11.56 | 4.10 | 2.46 |
| $K_{an}(S)$ | 66.85 | 29.49 | 6.93 |
| $K_n(S)$ | 0.52 | 0.58 | 2.74 |
| RSD [b] (%) | 2.5 | 0.7 | 1.0 |

[a] 0.09 M $CH_3COOH$ + 0.03 M $CH_3COONa$.
[b] Relative standard deviation, the ratio of the cumulative standard deviation computed by the elution profiles of the two enantiomers to the average apex concentration.

dure given in [1]. The best fit parameters of the model in Eq. (1) are listed in Table 1. The figure shows a good agreement between the experimental results and the profiles calculated with the new model, except for the mobile phase containing 60 vol.% of methanol, in which case extending the model by using a Langmuir rather then a linear term for the enantioselective adsorption allows a significant improvement of the agreement (not reported data). The shape of a peak profile is determined by the relative importance of the contributions of adsorption of the charged and the neutral species in the total retention of Naproxen. When prevails the adsorption of the anions the band exhibits a front shock layer. In contrast, when retention is substantially accounted for by the adsorption of the neutral species, the band exhibits a rear shock layer.

Thus, new experimental data demands that the model presented earlier and postulating the associative adsorption of the less retained enantiomer be abandoned. Instead, we must consider that the retention mechanisms of the dissociated and neutral Naproxen species are different, a feature neglected earlier [1]. Since the dissociation degree of Naproxen acid depends on its concentration in the mobile phase, a difference in their adsorption mechanism can explain the evolution of the peak shape as a function of the mobile phase composition. Even if the model expressed by Eq. (1) does not give the same high quality of approximation of band profiles as the one demonstrated in an earlier work [1], it explains the new experimental findings and can be extended to improve the accuracy of the band profile prediction.

## References

[1] L. Asnin, F. Gritti, K. Kaczmarski, G. Guiochon, J. Chromatogr. A 1217 (2010) 264–275.
[2] T. Fornstedt, P. Sajonz, G. Guiochon, Chirality 10 (1998) 375–381.
[3] F. Gritti, G. Guiochon, J. Chromatogr. A 1216 (2009) 1776–1788.
[4] F.Z. Oumada, C. Ràfols, M. Rosés, E. Bosch, J. Pharm. Sci. 91 (2002) 991–999.
[5] E. Bosch, P. Bou, H. Allemann, M. Rosés, Anal. Chem. 68 (1996) 3651–3657.