

The combined effect of silanols and the reversed-phase ligand on the retention of positively charged analytes

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Abstract

The nature of the interaction of positively charged analytes with the surface of reversed-phase bonded phases has been investigated as a function of both pH and volume fraction of organic modifier. Studies of the combined effect of both the parameters have been previously reported by us, and the data presented here further demonstrate a multiplicative interaction between pH and the concentration of organic modifier in the mobile phase. Fitting of the data as functions of pH and eluent composition clearly shows that the hydrophobically assisted ion-exchange process dominates over a purely reversed-phase or a pure ion-exchange retention mechanism. The underlying theory is developed in detail, and the mechanism is elucidated using several reversed-phase packings of substantially different character.

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1. Introduction

It has been understood since the beginnings of HPLC that the interaction of an analyte with the reversed-phase sites of a packing is not the only factor responsible for retention. In addition, silanols interact with analytes containing basic functional groups. This can create excessive retention [1] or tailing [2,3]. As early as the late 1970s and early 1980s, attempts were made to understand the combined effect of reversed-phase and “silanophilic” interaction [1,4–9].

Recently, there has been renewed interest in the interaction of charged analytes with reversed-phase packings, specifically with surface silanols on silica or hybrid-based bonded phases [10–17]. Some of the previous work reflects a continued concern about the effects of silanols on the peak shape obtained with different packings ([13], and references therein), the selectivity of the stationary phase [18–23] or the repro-

ducibility of packings towards positively charged analytes [17]. Other studies are driven by the desire to understand the loadability of basic compounds in preparative chromatography [14,15,24].

In the classical model of the interaction of a charged analyte with the surface of a silica-based reversed-phase packing, it was assumed that two separate mechanisms take place [8,9]. One of these is the reversed-phase mechanism encountered otherwise with purely hydrophobic molecules. In addition, an interaction of suitable analytes with the surface silanols is occurring as well. If the second mechanism is ion-exchange, we can summarize the classical dual mechanism as an additive mechanism:

$$k = k_{\text{rp}} + k_{\text{ex}} \quad (1)$$

where k is the retention factor, k_{rp} is the contribution of the reversed-phase process and k_{ex} the contribution of the ion-exchange with surface silanols. This formalism is equivalent to the two types of sites being on separate particles or even in tandem columns.

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Neue et al. [10] formulated a model in which the total free energy of transfer of the solute is taken as the sum of independent contributions from hydrophobic and Coulombic effects which is equivalent to a multiplicative formulation of Eq. (1). Essentially, the purely hydrophobic retention process combines with the ion-exchange process as follows:

$$k = k_{\text{rp}}k_{\text{ex}} \quad (2)$$

where k_{rp} is the contribution of the reversed-phase process and k_{ex} the contribution of the ion-exchange with surface silanols. This ad-hoc model explains the increase in ionic interaction with increased hydrophobic interaction, but it fails in two important points. Firstly, it cannot account for the change in retention with a change in the hydrophobicity of the packing. Secondly, the retention will disappear as *either* the ionic interaction or the hydrophobic interaction vanishes. Neither can be the case (e.g. it has been shown that retention remains when all ionic interactions are eliminated), and a more thorough model is required.

Such an improved model was proposed by Yang et al. [11]. The model combines our model of multiplicative interaction (called a one-site hydrophobically assisted ion-exchange in [11]) with a purely hydrophobic retention. Thus, there are two mechanisms that act in parallel: pure hydrophobic interaction and the multiplicative mechanism of combined hydrophobic and ion-exchange interaction. The following equation shows this model:

$$k = k_{\text{rp}}(1 + k_{\text{ex}}) = k_{\text{rp}} + k_{\text{rp}}k_{\text{ex}} \quad (3)$$

As the ionic interaction increases, the multiplicative interaction between reversed-phase and ion-exchange dominates. As the ion-exchange interaction vanishes, the purely hydrophobic interaction dominates. One may call the second term either an ion-exchange-assisted hydrophobic interaction or a hydrophobically assisted ion-exchange process.

From the form of Eq. (3), it is clear that as the hydrophobic interaction decreases, the retention disappears. Yet this cannot be the case either, since now the ion-exchange process should take over. We therefore need to formulate a third case, where the interaction with silanols is the dominant step, and the combined hydrophobic and ion-exchange interaction is a possible step that accompanies the ionic interaction. Although Yang et al. [11] did not find such a mechanism on the silica-based reversed-phase packings that they studied, they did encounter it on a polybutadiene-coated zirconia when used in phosphate media. Mixed-mode interactions of this nature also emerge on the surface of a mixed-mode divinylbenzene-based ion-exchanger, where the longer-range ion-exchange interaction combines with the hydrophobic interaction with the DVB backbone [25]. Both phenomena, the augmentation of hydrophobic retention with ion-exchange and the augmentation of ion-exchange with hydrophobic interaction have been reported for ion-exchange materials by Lee and O'Gara [25]. The mixed-mode interaction of trialkylammonium ions on a styrene-divinylbenzene-based ion-

exchanger has also been reported by Rahman and Hoffman, but no quantitative description was given [26].

On the surface of a silica-based reversed-phase packing, we find hydrophobic bonded-phase ligands and silanols. In principle for the interaction between a hydrophobic and positively charged analyte with such packings, we may find pure hydrophobic interactions, pure silanophilic interactions or combinations of both with the multiplicative interaction, as shown in Eq. (4):

$$k = k_{\text{rp}} + k_{\text{rp}}^*k_{\text{ex}}^* + k_{\text{ex}} \quad (4)$$

It is possible that the retention factors underlying the combined effect, which are marked here with an asterisk, and the retention factors for the individual interactions are different. Such events could be caused, if the sites responsible for the multiplicative interaction were substantially different than for example the sites responsible for the purely hydrophobic interaction. Due to the substantially different nature of the packings used in this study, it is unlikely that a special site distinct from those sites responsible for the individual interactions is causing the multiplicative interaction. For the sake of simplicity, we will therefore assume in this paper that the single retention factors and the retention factors in the multiplicative term are identical. On a series of packings with variable ratios of reversed-phase ligands and silanols, the mixture of the three mechanisms varies with the packing. The particular mixture of properties is a function of the details of the design of the packing. In this paper, we will study several packings of substantially different character to examine the nature of the interaction between a hydrophobic quaternary amine and these packings.

As will become clear in the discussion of the underlying theory and the results in this paper, the complex combined mechanism cannot be uncovered with measurements in a single mobile phase composition, in addition not even under conditions where only the pH is altered without varying the concentration of organic solvent. This has been the drawback of previous investigations. It also answers the question why such a fundamental effect, i.e. the multiplicative combination of hydrophobic and silanophilic interaction, has remained hidden in 30 years of HPLC, and was unearthed only recently [10,11].

An interesting aspect of the inorganic-organic hybrid packing underlying the XTerra packings is the fact that the $\text{p}K_{\text{a}}$ -values of the surface silanols shift into the alkaline pH compared to a silica-based packing [10]. This is the expected shift based on the differential effect of an oxygen versus an alkyl substituent on the acidity of the SiOH group. This phenomenon has been confirmed by Méndez et al. [12]. The consequence of this is a much lower silanol activity compared to silica-based packings. This needs to be taken into account in the interpretation of the chromatographic retention data of ionic and ionizable analytes. Méndez et al. [12] have also corroborated the difference in the ionization between a classical silica and a high-purity silica, as well as for the respective

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