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pH dependence of tailing in reversed-phase chromatography of a cationic dye: measurement of the strong adsorption site surface density

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Abstract

A question that has interested Dr. J.J. Kirkland is addressed: what is the nature of the silanols that cause tailing to persist at low pH in reversed-phase chromatography? Chromatograms for a cationic dye, 1,1′-didodecyl-3,3,3′3′-tetramethylindocarbocyanine perchlorate (DiI), were studied at varying pH using an Agilent SB-C8 column and 80% ACN/water for six DiI concentrations ranging from 0.9 to 316 μM. The chromatograms showed increased retention and tailing from pH 1 to 5, as expected. Simulations of the chromatograms agreed well with experiment for a bi-Langmuir isotherm with weak (C_8) and strong (silica) adsorption sites. The simulation parameters revealed that the number of strong adsorption sites decreases by 40% from pH 1 to 5, which indicates that the silanols causing tailing are in the SiOH, not the SiO^-, form. This seems paradoxical because tailing increases with increasing pH. The simulation parameters reveal that this increased tailing from pH 1 to 5 owes to doubling of the partition coefficient for DiI to the strong adsorption site, which more than compensates for the decreasing number of sites. We attribute this increased partition coefficient to increased long-range coulombic interactions with the increasingly abundant SiO^- groups at higher pH, which boosts DiI's partition coefficient for both the C_8 and SiOH sites. The picture thus emerges that for DiI, higher pH causes increased tailing because the SiO^- groups exacerbate tailing that actually originates from adsorption to SiOH groups.

Keywords: pH effects; Silanols; Desorption; Peak tailing; Simulation; Tailing; Cationic dye

1. Introduction

The tailing of basic compounds in reversed-phase chromatography is a problem that commonly occurs in the separations of pharmaceuticals, peptides, and proteins [1–4]. Tailing has been linked with residual silanol groups on the surface of the silica stationary phase that remain after surface modification with the reversed-phase layer [5]. These surface sites adsorb basic molecules with a high equilibrium constant and are often referred to as strong adsorption sites in contrast to the hydrophobic interactions between the analyte and reversed-phase layer, which are the weak adsorption sites [6]. The work of Kirkland et al. was the first to reveal that it is not bulk silanols, but rather a sub-population of isolated non-hydrogen bonded silanol groups that are responsible for tailing of basic compounds in reversed-phase chromatography [7,8]. They used a combination of infrared spectroscopy, NMR spectroscopy, and thermogravimetric analysis to quantitatively describe the distribution of hydrogen bonding among the silanol groups on several silica stationary phases. Their work showed that type B silica, which has a higher concentration of surface silanols to give fewer isolated silanols, shows much less tailing than type A silica, which has a lower concentration of silanols to give a higher concentration of isolated silanols. Our group has used chromatographic simulations to measure thermodynamic and kinetic parameters of the interactions between a basic dye and the strong adsorption sites [9], which revealed that the strong adsorption sites occupy on the order of 1 nmol/m^2 of the stationary phase's total surface area. This very low abundance supports Kirkland's notion that rare, isolated silanols underlie the tailing of basic compounds in reversed-phase chromatography.

The ionization state of the silanols that are responsible for tailing is still unknown. The continued decrease in tailing with increasing ionic strength, even at low pH, suggests that SiO^- groups underlie the tailing of basic compounds through ion-exchange [5,10,11]. Since tailing persists at low pH, it has been widely speculated that the silanols that under-

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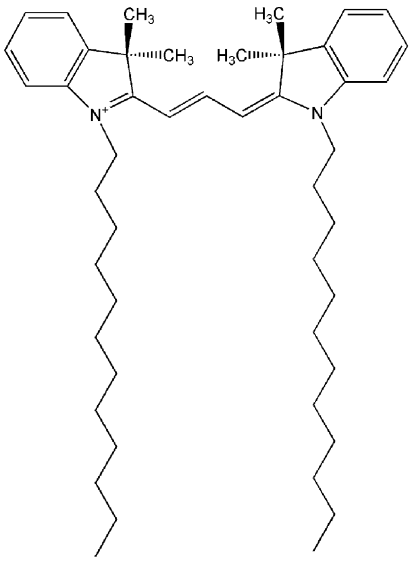
lie tailing are more acidic than the plentiful residual silanols. However, one could argue that an isolated silanol would be less acidic than a hydrogen bonded silanol because the proximity to other silanols would stabilize the anion. Also, some neutral compounds have been found to tail, which suggests a mechanism for tailing other than ion-exchange [12]. A study of the dependence of chromatographic tailing on mobile phase pH can elucidate the charge on the strong adsorption site. Both qualitative and quantitative studies of the effects of mobile phase pH on the retention properties of basic compounds have been reported [13,14]. These studies have measured the ion-exchange properties of various silica stationary phases with Li^+ or bretylium tosylate to determine the acidity, or silanol activity, of these surfaces. However, in light of the findings of Kirkland et al., it is dubious that the number of plentiful silanols would correlate with the number of rare isolated silanols causing tailing. Other studies have used chromatographic simulations to obtain thermodynamic parameters for the interactions of basic compounds with the silica surface [9,15,16], or spectroscopic studies of the interactions of basic compounds with the silica stationary phase [17,18]. These studies have been used to develop improved stationary phases for the reduction of tailing, yet, more physical insight is needed because tailing is still a detrimental problem to the separations field.

The purpose of this work is to use a combination of experimental chromatography with varying mobile phase pH in conjunction with chromatographic simulations to determine whether the strong adsorption site is the SiO^- or SiOH group that is contributing to tailing. A quantitative description of chromatographic zones entails the determination of the thermodynamic and kinetic parameters that describe the interaction between the analyte and the strong adsorption site, as well as the strong adsorption site surface coverage. Chromatograms of a cationic dye are obtained for pH 1 to 6.3 mobile phases, and chromatographic simulations are used to obtain a value for the equilibrium adsorption constant for the strong adsorption site (k_{strong}), and the contribution of the strong and weak adsorption sites to the total retention of DiI (k'_{strong} and k'_{weak}). These simulation parameters are used to calculate the free energy (ΔG_{strong}), surface density ($\Gamma_{sat,strong}$), and DiI desorption time (τ_{strong}) for the strong adsorption sites as a function of mobile phase pH. The pH dependence of the number of strong adsorption sites reveals the ionization state of the silanol groups that contribute to tailing.

2. Experimental section

The cationic dye, 1,1′-didodecyl-3,3,3′3′-tetramethylindocarbocyanine perchlorate (DiI, 1), was obtained from Molecular Probes and was used as the analyte. Acetonitrile obtained from Aldrich as HPLC grade, and water purified to a resistance of 18 MΩ cm using a Barnstead E-pure system were used in the mobile phase. Other standard chemicals and reagents were purchased from commercial suppliers.



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The chromatograph used in these experiments was an Agilent 1100 with an isocratic pump, vacuum degasser, and diode-array absorbance detector. The column temperature was set to 20 °C for all experiments using a thermostatted column compartment. The column was purchased from Agilent and was 8 cm by 4.6 mm. The stationary phase was non-endcapped Zorbax SB-C8, which is high-purity type B silica modified with sterically protected diisopropyl n-octyl silane, reported by the manufacturer to be stable from pH 1 to 6. This column was chosen because it is known to cause tailing of basic analytes with acidic mobile phases. The dry weight of silica was measured using a commercial bench-top balance, and falls within the manufacturers reported range. The mobile phase was 80/20%, acetonitrile/water (v/v). The pH of the aqueous portion of the mobile phase was measured with a Beckman pH electrode, and was adjusted by adding HCl until the desired value was obtained. The appropriate amount of KCl was added to keep a constant ionic strength of 100 mM for all mobile phases. For all chromatograms, the injected volume was 10 μL and the flow rate was 1.0 mL/min. The injected concentrations were 0.9, 6, 16, 87, 245, and 316 μM. Three replicate runs were performed to establish the reproducibility of the chromatograms.

Details of the simulation software, kindly donated by Prof. Georges Guiochon of the University of Tennessee, are provided elsewhere [9]. The simulations use the bi-Langmuir model to describe adsorption of analyte onto a stationary phase containing two sites. The equilibrium-dispersive model was used to account for all the sources of zone broadening, which combines all sources of non-equilibrium broadening into one axial dispersion term and assumes fast exchange of the analyte between stationary and mobile phases [19]. There are four independent parameters in the chromatographic simulations: the partition coefficient for

Table 1
Results obtained from the simulations of DiI chromatograms showing the best-fit equilibrium binding constant (k_{strong}) and corresponding free energy (ΔG_{strong}), the partition coefficient of the strong (k'_{strong}) and weak sites (k'_{weak}), and the desorption time ($\tau_{desorption}$) for DiI and the strong adsorption site for the indicated mobile phase pH

pH	k_{strong}	ΔG_{strong} (kJ/mole)	k'_{strong}	k'_{weak}	k'_{strong}/k'_{weak}	$\tau_{desorption}$ (ms)
1	214,000 ± 13,000 (6%)	−27.9 ± 0.1	41.8 ± 2.0	40.5 ± 2.0	1.03	78.5 ±4.0
2	243,000 ± 25,000 (10%)	−28.1 ± 0.3	43.5 ± 3.7	43.0 ± 3.7	1.01	79.9 ± 6.7
3	254,000 ± 30,000 (12%)	−28.2 ± 0.3	46.2 ± 4.6	44.6 ± 4.6	1.01	76.7 ± 4.7
4	345,000 ± 50,000 (15%)	−28.9 ± 0.3	53.9 ± 4.6	51.8 ± 4.6	1.01	78.5 ± 5.5
5	430,000 ± 65,000 (15%)	−29.4 ± 0.4	54.2 ± 6.9	57.3 ± 6.9	0.86	82.5 ± 6.3

the weak site (k'_1), the equilibrium binding constant for the strong site (k_2), the saturated surface coverage of the strong site in relation to the total saturated surface coverage of all sites ($\Gamma_{sat,strong}/\Gamma_{sat}$), and the number of theoretical plates (N). N was measured experimentally using a 0.9 μM solution of DiI, and 0.1–5 mL/min flow rates ($N = 6000$), and was used as a simulation parameter. This left only three parameters to be determined from the simulations: k'_1, k_2, and $\Gamma_{sat,strong}/\Gamma_{sat}$. The saturated surface coverage of DiI on the stationary phase was assumed to be on the order of 1 μmol/m^2 based on the steric constraints for packing a full layer of DiI on the stationary phase. To determine the best-fit parameters for the average of three sets of experimental chromatograms, k_{strong} was systematically increased, starting at 100,000, while optimizing k'_{strong} and k'_{weak} until the best fit was achieved. All reported ranges for k_{strong}, k'_{strong}, and k'_{weak} in Table 1 were obtained from the simulation results of three replicate sets of experimental chromatograms.

The fluorescence spectra of DiI adsorbed to chromatographic particles (Zorbax SB-C8, Agilent) were obtained using a Zeiss Axiovert 200 microscope with a 100× oil immersion objective (N.A. 1.4) to both direct the excitation light onto a single silica particle and to collect the emitted light. The 514 nm laser line from an Ar^+ laser was used as the excitation source, which was filtered from the spectrum using optical filters. The fluorescence emission was focused onto the slit of a Holospec monochrometer (Kaiser Optical Systems) and the spectrum was then captured using a Pentamax ICCD camera (Princeton Instruments). The software program Winview 32 v2.5 was used to capture images, and the software package Scion Image v4.0 was used to analyze the resulting spectrum.

3. Results and discussion

Chromatograms corresponding to six concentrations (0.9, 6, 16, 87, 245, and 316 μM) of the cationic dye, DiI, are shown in Fig. 1A. These concentrations are typical of the range used in analytical-scale separations. The chromatograms were obtained with an 80/20%, acetonitrile/water, pH 1 mobile phase using a non-endcapped, reversed-phase, type B silica stationary phase. The chromatograms of DiI show tailing that increases with concentration, which is referred to as nonlinear tailing. Nonlinear tailing results from saturation of the strong adsorption sites at high analyte concentrations [20,21]. We previously showed that the adsorption of DiI onto this type of stationary phase for a 90/10 %, acetonitrile/water, pH 2 mobile phase follows a bi-Langmuir adsorption isotherm with strong and

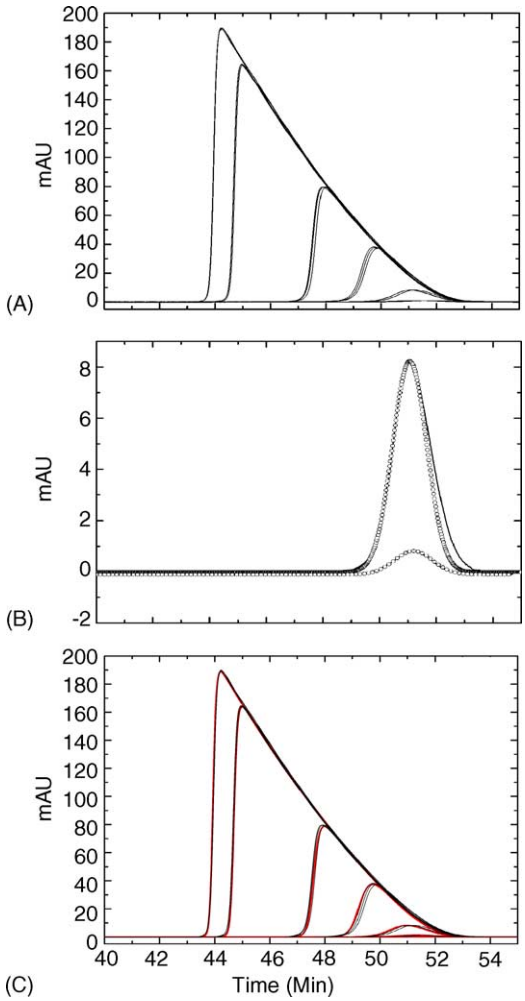

Fig. 1. (A) Chromatograms of the cationic dye DiI obtained at concentrations of 0.9, 6, 16, 87, 245, and 316 μM. Shown are the results from three replicate runs at each concentration. The separations were performed on a Zorbax RX-C8 reversed-phase column with the following conditions: 80/20% acetonitrile/water, pH 1; 20 °C; 1 mL/min flow rate. (B) Chromatograms of 0.9 and 6 μM DiI (solid line) plotted with corresponding Gaussian curves (circles) showing the onset of tailing. (C) The DiI chromatograms shown in A (black lines) plotted with the best-fit simulation results (red lines).

weak adsorption sites:

$$\Gamma = \Gamma_{\text{sat,strong}} \frac{k_{\text{strong}} c}{1 + k_{\text{strong}} c} + \Gamma_{\text{sat,weak}} \frac{k_{\text{weak}} c}{1 + k_{\text{weak}} c} \quad (1)$$

where Γ is the total surface coverage of DiI on the stationary phase with a solution concentration of c, and a saturated surface coverage of $\Gamma_{\text{sat,weak}}$ and $\Gamma_{\text{sat,strong}}$ for weak and strong sites that have equilibrium adsorption constants of k_{weak} and k_{strong}, respectively [6,9]. The abundant, weak sites allowed for fast elution and the rare strong sites caused the nonlinear tailing. This same model presumably applies to the chromatograms in Fig. 1, obtained with an 80/20% acetonitrile/water mobile phase at pH 1, which still show significant tailing despite the lower pH.

The analyte concentration where the onset of nonlinear tailing is seen in the chromatograms of Fig. 1 can be used to estimate the equilibrium adsorption constant for the strong adsorption site. Fig. 1B shows an expanded view of the DiI chromatograms for the two lowest concentrations that were studied. Also shown in the figure are the Gaussian fits of these chromatograms. While the lowest DiI concentration fits to a Gaussian curve, the chromatogram corresponding to 6 μM DiI shows significant tailing. The onset of tailing between 0.9 and 6 μM points to a value of k_{strong} between 170,000 and 1,110,000, since k is the inverse of the concentration at which half the strong sites are occupied.

Pioneering work by Guiochon and others makes the quantitative description of the tailing of basic compounds in reversed-phase chromatography possible [6,16,22]. This work has enabled the development of simulation software that uses the bi-Langmuir isotherm to describe adsorption of analyte onto the stationary phase and the equilibrium dispersive model to describe the sources of zone broadening. Shown in Fig. 1C are simulation results with the best-fit simulation parameters overlapped with the experimental chromatograms of Fig. 1A. The best-fit parameters were determined by systematically increasing the value of k_{strong} starting at 100,000 and concomitantly varying the parameters that describe the contribution of the strong and weak sites to the partition coefficient. The best-fit k_{strong} value for the chromatograms shown in Fig. 1 was 214,000 ± 13,000, corresponding to a ΔG value of −27.9 ± 0.1 kJ/mole. It is not possible to measure k_{weak} from the simulations because the weak adsorption sites do not saturate under these conditions. The contribution of the strong adsorption sites to the retention of DiI under these conditions is about equal to the weak adsorption sites ($k'_{\text{strong}}/k'_{\text{weak}}$=1.03). This agrees with previous results for DiI on this type of stationary phase when the mobile phase was 90/10% acetonitrile/water ($k'_{\text{strong}}/k'_{\text{weak}} = 0.98$) [9].

We sought to study the chromatograms of DiI under conditions of increasing pH of the mobile phase to determine how the number of strong adsorption sites varies with pH. Shown in Fig. 2 are the chromatograms of DiI obtained with mobile phases ranging from pH 2 to 5. The reported pH was measured with a pH electrode for the aqueous portion of the mobile phase prior to mixing with acetonitrile, which is a consistent measurement. The actual pH of the mobile phase after mixing with acetonitrile is higher than that reported here [23]. The same concentrations of DiI were used to obtain the chromatograms shown in Figs. 1 and 2. The chromatograms of DiI show both an increase in retention

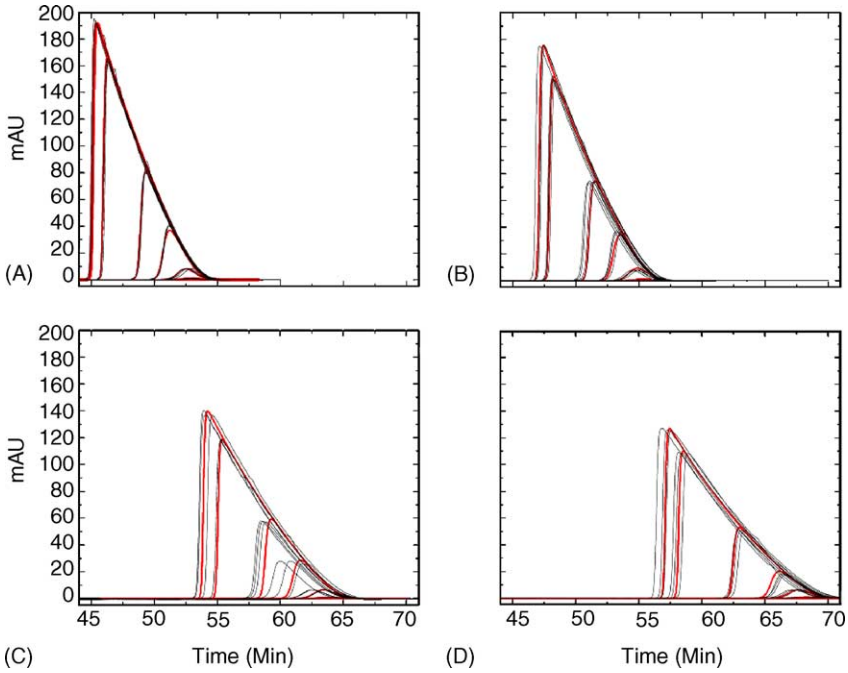

Fig. 2. DiI chromatograms (black lines) and best-fit simulation results (red lines). All conditions are the same as those listed in Fig. 1, except: 80/20% acetonitrile/water, (A) pH 2; (B) pH 3; (C) pH 4; (D) pH 5.

time with increasing mobile phase pH and an increase in the amount of tailing at high DiI concentrations, which is expected for organic cations in reversed-phase chromatography [13,23–25]. DiI is an ideal probe to study the pH dependence of the silica stationary phase because it is a quaternary amine, and positively charged for all pH values. Therefore, changes in the DiI chromatograms with increasing pH can be attributed to the acidity of the silica stationary phase. The pK_a of the silanol group on type B silica has been measured by others. The literature reports a wide range of values that typically center around 6 in water [26]. This would be higher for acetonitrile/water mixtures. Since the operating pH is below the pK_a, the average surface charge on the silica stationary phase progressively increases by a factor of 10 for each unit increase in the pH from 1 to 5. The increase in retention and tailing from pH 1 to 5 for the chromatograms of Figs. 1 and 2 qualitatively reinforces the widely held notion that the strong adsorption sites are SiO^- groups.

Plotted with the experimental DiI chromatograms in Fig. 2 are the simulation results for each set of chromatograms with the best-fit parameters, which are listed in Table 1. There is less reproducibility in the experimental chromatograms with increasing pH of the mobile phase. This is probably the result of the mobile phase being composed of a mixture of KCl and HCl to obtain the desired pH (i.e., a buffered system was not used). The spread in the experimental chromatograms was used to express the uncertainty in the simulation parameters.

The saturated coverage of the analyte on the strong adsorption sites, $\Gamma_{sat,strong}$, was determined through the phase ratio (ϕ_s), [9] which is related to the total surface area of the stationary phase (A) obtained from the manufacturer's reported surface area and the measured dry weight of silica, and also the measured volume of mobile phase in the column (V_m) as shown in Eq. (2). The concentration term in the denominator accounts for the standard state and provides a unitless phase ratio. The phase ratio is related to the partition coefficient, k_{strong}, and the capacity factor in the limit of low analyte concentrations, $k'_{strong,\lim c\to 0}$, for the strong adsorption:

$$\phi_s = \Gamma_{sat,strong}\frac{A}{V_m 1M} \quad (2)$$

$$k'_{strong,\lim c\to 0} = k_{strong}\phi_s \quad (3)$$

sites (Eq. (3)). For the material used in this work, $\phi_s = \Gamma_{sat,strong} \times 2.3 \times 10^5\ m^2/L$. At pH 1, the saturated surface coverage of strong adsorption sites was found by the best-fit simulation parameters and Eqs. (2) and (3) to be $8.5 \pm 0.4 \times 10^{-10}$ mol/m^2. The saturated coverage of strong adsorption sites can be interpreted as the number density of strong adsorption sites that are accessible to DiI. For these results in 80/20% acetonitrile/water, the value of $\Gamma_{sat,strong}$ is 40% lower than it is for the same stationary phase in 90/10% acetonitrile/water (1×10^{-9} mol/m^2). This is consistent with

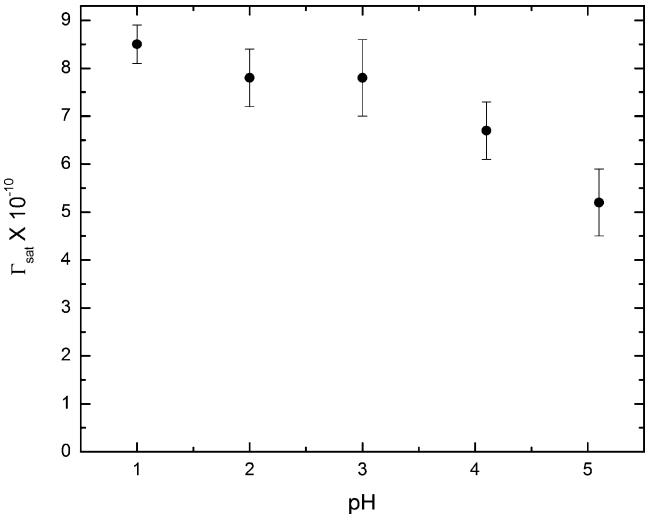

Fig. 3. Plot of the saturated surface coverage of the strong adsorption sites obtained from the best-fit simulation parameters of the data shown in Figs. 1 and 2 versus pH of the mobile phase.

the observation made using single molecule spectroscopy that higher acetonitrile compositions enhance the abundance of strong adsorption sites [27]. The number density of accessible strong adsorption sites is on the scale of nmoles/m^2, which is more than 1000-fold lower than the population of residual silanols. This rarity supports Kirkland's idea that it is the unusual, isolated with respect to hydrogen bonding silanols, not the plentiful residual silanols, that cause tailing in reversed-phase chromatography.

Plotted in Fig. 3 are the calculated $\Gamma_{sat,strong}$ values calculated from the best-fit simulation parameters of the chromatograms as a function of the mobile phase pH. The $\Gamma_{sat,strong}$ value drops from $8.5 \pm 0.4 \times 10^{-10}$ at pH 1 to $5.5 \pm 0.7 \times 10^{-10}$ mol/m^2 at pH 5, indicating that there are fewer strong adsorption sites at pH 5 than at pH 1. This points to SiOH as the strong adsorption site for DiI between pH 1 to 5, and it indicates that DiI does not specifically adsorb to SiO^- groups. This result indicates that DiI is a hydrogen bond acceptor. As the pH of the mobile phase increases, a portion of the isolated SiOH groups are converted to SiO^-, and $\Gamma_{sat,strong}$ decreases. This is the first reported quantitative result obtained from chromatographic data pointing to neutral SiOH as the strong adsorption site, to our knowledge. Hydrogen bonding donors, such as protonated amines, lysine and histidine, could hydrogen bond to the isolated SiO^- groups, which would be excellent hydrogen bond acceptors. The results described here apply to analytes that are hydrogen bond acceptors.

The value of k_{strong} increases with pH while the concentration of strong adsorption sites decrease slightly with pH. This reveals that the increased tailing with pH is due entirely to an increase in k_{strong}. We suggest that a long-range coulombic contribution from SiO^- sites would give an additive contribution to the free energy of

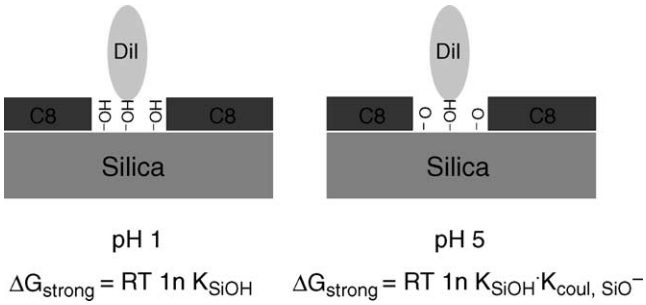

Fig. 4. Schematic diagram showing the adsorption of DiI onto a C8 modified silica surface at pH 1 (left) and pH 5 (right). Defects in the alkyl layer leave residual silanols on the silica surface. At pH values between 1 and 5, the DiI adsorbs to SiOH sites on the stationary phase through hydrogen bonding. As the pH of the mobile phase increases, there is an increase in the ionization of the residual silanols. The increase in chromatographic tailing observed with increasing pH is due to the contribution of long-range electrostatic interactions between DiI adsorbed to SiOH sites and the increasing concentration of SiO^- groups.

adsorption.

$$\Delta G_{strong} = RT\ln(k_{strong}) = \Delta G_{SiOH} + \Delta G_{coul} = RT\ln(K_{SiOH}K_{coul}) \quad (4)$$

$$\Delta G_{weak} = RT\ln(k_{weak}) = \Delta G_{C_8} + \Delta G_{coul} = RT\ln(k_{C_8}k_{coul}) \quad (5)$$

By these relations, the ratio k_{strong}/k_{weak} would be independent of pH. While k_{weak} is not known, it is proportional to k'_{weak}, assuming that the number of weak sites is independent of pH. Indeed, the ratio of capacity factors, k'_{strong}/k'_{weak}, is constant from pH 1 to 4, where the number of strong adsorption sites changes negligibly. This constant ratio of capacity factors supports the interpretation that k_{coul} is causing both k'_{strong} and k'_{weak} to increase together, as in Eqs. (4) and (5). Our interpretation is that, k_{coul} increases with pH due to the increasing number of SiO^- sites, and this is shown schematically in Fig. 4. The variation of k_{strong} with pH is thus attributed to long-range coulombic interactions with SiO^- sites, rather than specific adsorption to SiO^- sites. These results explain that SiO^- sites do play a role in exacerbating tailing, which is consistent with increased ionic strength and decreased pH both reducing tailing, but the problematic sites that originate the tailing for DiI are the rare, isolated SiOH groups.

The desorption time of DiI from the strong adsorption sites ($\tau_{desorption}$) can be used to establish that the strong adsorption sites do not change between pH 1 and 5. It is possible to calculate $\tau_{desorption}$ using the simulation parameters, and the following expression:

$$C = 2R(1-R)(\tau_{sum} + f\tau_{strong}) \quad (6)$$

where the C term is from the van Deemter equation, R is the retention ratio, τ_{sum} is the contribution to zone broadening from all sources except desorption from the strong

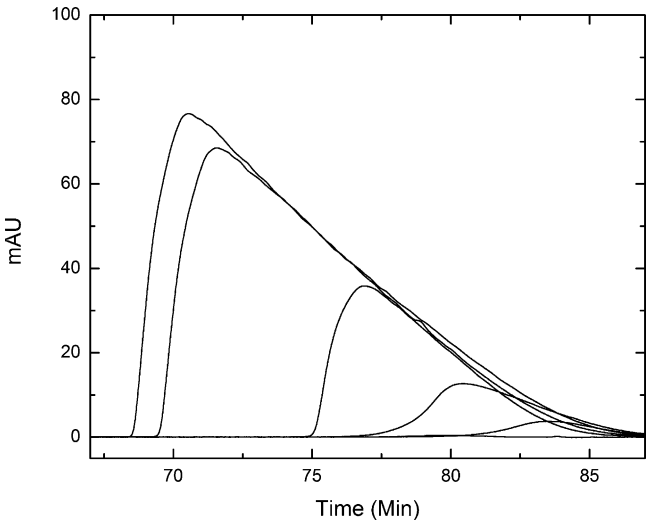

Fig. 5. DiI chromatograms over the same concentration range listed in Fig. 1, obtained with a mobile phase containing 80/20% acetonitrile/water, pH 6.3. Both fronting and tailing are present in the chromatograms.

adsorption site, which is denoted as τ_{strong}, and f is the contribution of the strong sites to the total partition coefficient. τ_{sum} can be measured from a compound that negligibly interacts with the strong adsorption sites, such as tetracene. This has been demonstrated for a 90/10% acetonitrile/water, pH 2 mobile phase with DiI [9]. Shown in Table 1 are the calculated desorption times for DiI on the strong adsorption sites for pH 1–5 mobile phases. Within experimental error, the desorption time remains constant as a function of pH, which supports the conclusion that the same strong adsorption sites are contributing to the chromatograms at each pH.

The chromatographic behavior of DiI changes above pH 5. Shown in Fig. 5 are the chromatograms of DiI obtained with a pH 6.3 mobile phase for 0.9, 6, 16, 87, 245, and 316 μM DiI. In addition to the tailing behavior that was observed in the chromatograms below pH 5, there is also fronting behavior. The abrupt appearance of fronting in the DiI chromatograms at pH 6.3 suggests a dramatic transition in the nature of the surface groups. Fronting behavior in the chromatograms indicates a deviation from the bi-Langmuir adsorption isotherm, which can be due to interactions of adsorbing DiI with neighboring adsorbed DiI, if more than a monolayer of adsorbate forms, or if the surface is not homogenous. Carbocyanine dyes are known to form aggregates at solid/solution interfaces [28]. Preliminary evidence points to the formation of molecular aggregates of DiI on the silica surface above pH 5. Shown in Fig. 6 are the fluorescence spectra of DiI adsorbed to 5 μm Zorbax SB-C8 particles at pH 1 and pH 6.3. At pH 6.3 the DiI fluorescence spectrum is shifted to higher wavelengths (red-shifted) compared to the spectrum of DiI adsorbed to the silica particles at pH 1. The observed red-shift indicates aggregation of the dye molecules on the chromatographic surface at high pH despite their extremely low surface concentration [28,29]. This suggests the close proximity of the silanol sites, or small patches of silanol sites, as originally suggested by Lochmüller [30].

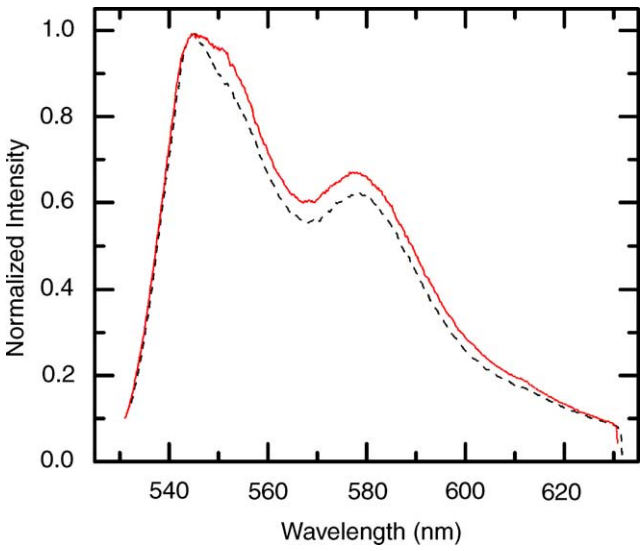

Fig. 6. Fluorescence spectra of DiI adsorbed to Zorbax SB-C8 particles obtained with 514 nm excitation. The solution concentration of DiI was 100 μM with 80/20% acetonitrile/water, pH 1 (dotted black line) or pH 6.3 (solid red line). The filter used to remove the excitation light contributes to the response observed at low wavelengths. The spectrum at pH 6.3 is red shifted compared to that at pH 1, indicating aggregation of the DiI molecules on the silica surface at high pH.

The results presented here demand some explanation of why hydrogen bonding with rare SiOH sites gives tailing but coulombic interactions with the abundant SiO^- groups does not. The short answer to this question is that it is the size of the partition coefficient that affects tailing. This implies that hydrogen bonding gives a much higher partition coefficient than coulombic interactions. One can use first principles of thermodynamics to confirm that this makes sense. The strength of a typical hydrogen bonding interaction from two species coming together in aqueous solution can be estimated from studies of protein folding. For example, when a protein folds in water to allow one carbonyl group and one amino group to hydrogen bond, the free energy change is on the order of 6 kJ/mol [31–33]. The strength of a coulombic interaction is determined by the surface potential, ψ, which is related to the adsorption energy through $\Delta E = zF\Psi$. It is possible to calculate the surface potential of the stationary phase by estimating the surface density of ionizable silanol groups. The surface density of alkylsilane modified silanols is about 3.4 μmol/m^2 for this stationary phase, the surface density of silanols on the unmodified silica is 8 μmol/m^2 [5], and it was assumed that 10% of the residual silanols can be ionized at the pK_a value, which is about 6 for this stationary phase [8,24]. At pH 5 in an 80% ACN mobile phase, the surface potential of the C_8 modified silica stationary phase is estimated to be 4 mV, which gives an estimated coulombic interaction energy of 0.4 kJ/mol. The ratio of the partition coefficients for hydrogen bonding and coulombic interaction would thus be $\exp(6\,\text{kJ}/0.4\,\text{kJ}) = 10^6$. The much higher partition coefficient for hydrogen bonding explains why coulombic interactions with the SiO^- groups do not cause tailing at the pH values used here. Working at a pH greater than the pK_a of the silanols, e.g., neutral pH, would generate surface potentials that would allow the coulombic interactions alone to cause tailing, without requiring the presence of rare, isolated silanols.

4. Conclusions

The results reported herein support the notion that the tailing of an organic cation, DiI, in reversed-phase chromatography at low pH is the result of a small and neutral subpopulation of the total residual silanols, and they refute the notion that these isolated silanols must be highly acidic compared to the majority of the residual silanol groups. Increasing mobile phase pH causes increased surface charge, and the resulting long-range coulombic interactions between the adsorbed DiI on the strong adsorption sites and the SiO^- groups exacerbates the tailing that originates from the neutral SiOH groups, but the coulombic interactions are not enough to cause tailing by themselves at these pH values for DiI.

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