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Molecular interactions between DNA and an aminated glass substrate

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Abstract

With the development of DNA arrays, the immobilization of DNA strands onto solid substrates remains an essential research topic. DNA arrays have potential applications in DNA sequencing, mutation detection, and pathogen identification. DNA bound to solid substrates must still be accessible and retain the ability to hybridize with its complementary strands. One technology to produce these arrays involves linking DNA molecule probes to a silanized substrate in microspot patterns and exposing them to a solution of fluorescently labeled samples of DNA targets. The behavior of both the target and probe DNA and their interactions with each other at the substrate surface, particularly with respect to molecular interactions, are poorly understood at the present time. The objective of this work is to model simply the interface interactions between DNA and glass slides modified with an aminosilane (γ-aminopropyltriethoxysilane, APTS). In aqueous solutions, DNA behaves as a polyacid over a wide range of pH. A glass substrate treated with APTS is positively or negatively charged, depending on the pH. A model of the surface charge of APTS-treated glass has been developed from results of wetting experiments performed at various pH. It has been demonstrated that the surface charge of APTS-treated glass is well described by a model of constant capacitance of the electrical double layer. A good correlation between experimental data on DNA retention at various pH's and the variation of the surface charge of the APTS-treated glass is obtained. This provides an indication of the role of ionic interactions in the adsorption of DNA molecules onto aminated glass slides.

Keywords: DNA; Glass; Aminosilane; Aminopropyltriethoxysilane; Surface charge; Point of zero charge; Ionic interaction; DNA retention; Wettability; Contact angle; Water; pH

1. Introduction

Over the past years, nucleic acid probes have been extensively used to detect human pathogens in molecular recognition tests. With the development of DNA microarrays, the immobilization of these molecules onto solid substrates remains an essential research topic. When bound to a solid substrate, the DNA molecules must still be accessible and retain the ability to hybridize with their complementary strands. Hybridization of the fluorescently labeled probe to a specific DNA sequence generally indicates that the gene represented by this sequence is expressed. DNA arrays have potential applications in DNA sequencing, mutation detection, and pathogen identification. Preparation of DNA arrays involves binding of DNA molecules to silanized glass substrates in the shape of microspots, or synthesizing DNA on the substrate and exposing the DNA array to a solution of fluorescently labeled samples of DNA targets. This process, which is sometimes called heterogeneous hybridization, allows conducting many different tests in parallel, using very small amounts of expensive DNA probe material. However, the behavior of both the target and probe DNA and their interaction with each other at the liquid/substrate interface are at the present time poorly understood, particularly with respect to specific and nonspecific molecular interactions.

Modified and bare silica or glass substrates are currently used in many fields of technology to immobilize genetic material. Bare silica has been found to be reactive but prone to rapid contamination. Modification of silica or glass surfaces by covalent binding of silanes with surface silanol groups has been extensively studied. Silanization of glass substrates with an aminosilane provides an amine-rich surface which is ideal for attachment of DNA to the solid substrate.

It is generally known that both electrostatic and hydrophobic interactions are major driving forces for protein adsorption at solid/liquid interfaces. However, relatively little is known about how these interactions affect the interfacial behavior of single- or double-stranded DNA molecules.

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It has been demonstrated that DNA oligonucleotides in solution interact with aminopropyl silane treated substrates via ionic interactions [1]. The pH of the medium in which oligonucleotides are present has a strong impact on the amount of material adsorbed and retained on aminated substrates [2].

The objective of this work is to model simply the interaction between DNA molecules and an aminated glass slide as a function of the pH and to use experimental techniques to support the model. The surface charge of the substrate will be studied with the evolution of the wettability of the aminated glass as a function of the pH of the contacting water. The variation of the DNA retention on the substrate as a function of pH will be quantified using fluorescent detection.

2. Theory

2.1. Charge of DNA in solution

DNA is a polymer with an alternating sugar–phosphate sequence. The monomer units of DNA are nucleotides. Each nucleotide consists of a deoxyribose sugar, a nitrogen-containing base attached to the sugar, and a phosphate group. There are four different types of nucleotides found in DNA, differing only in the nitrogenous base: adenine, guanine, cytosine, and thymine.

Native DNA is a double-stranded structure that may be denatured into two single strands in a chaotropic solvent or at high pH values or by heating (melting). The denaturation is a reversible process.

When a single-stranded DNA molecule is in water, ionization of sites on the linkages and bases results in a charge that is pH-dependent. The electrostatic characteristics of a single-stranded DNA molecule are derived from the ionization of the phosphodiester linkages, resulting in one negative charge per nucleotide, and from ionization of bases, resulting in a charge that is base-dependent.

A phosphodiester is a strong acid whose pK is around 1 [3]. Adenine (A) and cytosine (C) bases can be found as neutral or positive forms, whereas thymine (T) is neutral or negative, and guanine (G) can exist in all three states [3]. The ionization of the bases and their corresponding dissociation constants may be described by the equilibria reported in Table 1.

Table 1
Ionization equilibria for the four different bases found in DNA [3]

Base	Equilibrium	Equilibrium constant
Adenine	$AH^+ \leftrightarrow A + H^+$	$K_A = 10^{-3.5}$
Cytosine	$CH^+ \leftrightarrow C + H^+$	$K_C = 10^{-4.2}$
Thymine	$TH \leftrightarrow T^- + H^+$	$K_T = 10^{-9.2}$
Guanine	$GH_2^+ \leftrightarrow GH + H^+$	$K_{G1} = 10^{-2.1}$
	$GH \leftrightarrow G^- + H^+$	$K_{G2} = 10^{-9.2}$

2.2. Ionization of glass and of amine groups of silanized glass

For the description of charge and potential at the oxide/electrolyte interface, different models can be used. A common feature of these models is the notion of charging from dissociation of surface groups and a formulation of mass action laws applied to the solid/liquid interface. In the context of electrokinetic potential measurements, the triple layer model, which comprises a well-confined electric double layer close to the surface and a diffuse layer described by the Gouy–Chapman theory, is considered to be the best tool for a quantitative description [4]. Close to the solid surface, a well-confined electric double layer, also known as the Helmholtz layer, is formed. This is followed by a diffuse layer of counter charges between the outer Helmholtz plane and the neutral bulk of the solution. While the Helmholtz planes are strongly adsorbed, the diffuse layer is considered more mobile.

Several studies show that a simple thermodynamic analysis of the adsorption or desorption of protons allows us to relate the change in the solid/electrolyte interface free energy to the charge density at the oxide surface [5,6]. The well-confined electric double layer close to the solid surface is apparently sufficient to interpret contact angle titration methods, where the point of zero charge of the solid is the unknown parameter. However, simple Helmholtz double-layer description appears to be particularly well adapted to the case of a silanized glass substrate, as shown in this study.

As a first approximation, we assume that a glass surface behaves similarly to an amorphous silica surface. The estimated maximum coverage of silanols on a silica surface is about 4.5 SiOH groups/nm^2 [7]. When silica is submitted to a heat treatment, the number of free surface silanol groups is reduced [7].

SiOH groups are positively or negatively charged, according to the pH. The point of zero charge, pzc, defines the pH at which the surface density of positive charges is equal to the surface density of negative charges [8]. The pzc of silica is around pH 2 to 3 [8,9]. The pK_a of silanol groups is in the range from 5 to 7 [10,11].

We may calculate the ionic species present on a bare glass surface as a function of pH from the average pK_a value of 6 and from the pzc value. These lead to the following equations for the relative density of negative and positive species:

$$\mathrm{SiOH} \leftrightarrow \mathrm{SiO^-} + \mathrm{H^+}, \qquad K_1 = \frac{|\mathrm{SiO^-}||\mathrm{H^+}|}{|\mathrm{SiOH}|} = 10^{-6}, \quad (1)$$

$$\mathrm{SiOH_2^+} \leftrightarrow \mathrm{SiO^-} + 2\mathrm{H^+},$$

$$K = \frac{|\mathrm{SiO^-}||\mathrm{H^+}|^2}{|\mathrm{SiOH_2^+}|} = 10^{-2\mathrm{pzc}}, \quad (2)$$

$$\mathrm{SiOH_2^+} \leftrightarrow \mathrm{SiOH} + \mathrm{H^+}, \qquad K_2 = \frac{|\mathrm{SiOH}||\mathrm{H^+}|}{|\mathrm{SiOH_2^+}|} = \frac{K}{K_1}. \quad (3)$$

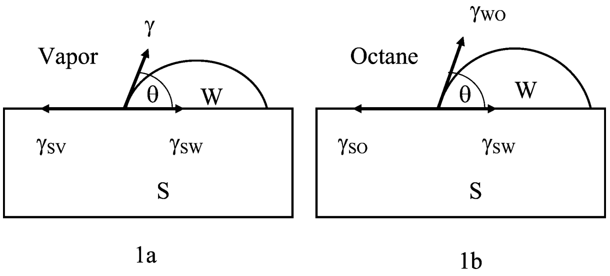

Fig. 1. Young's model for a sessile water drop in the presence of water vapor (a) or octane (b).

The equilibrium between protonated amine groups (R–NH_3^+) and amine groups (R–NH_2) is given by

$$\text{R–NH}_3^+ \leftrightarrow \text{R–NH}_2 + \text{H}^+, \qquad K_a = \frac{|\text{R–NH}_2||\text{H}^+|}{|\text{R–NH}_3^+|}. \tag{4}$$

The value of the equilibrium constant K_a may be estimated as being on the order of the quoted K_a value for N-propylamine, i.e., 5.78×10^{-11} (p$K_a = 10.2$). It is known that amine groups of APTS molecules are positively charged up to pH 10, which is equal to the pK_a value [12].

It has been demonstrated that only 30 to 50% of silanol groups may be grafted during silanization reaction [13]. Therefore, the chemical groups present on a silica (glass) surface silanized with APTS are likely to be amine groups and SiOH groups. We will see that our results are in agreement with this description.

2.3. Wettability at various pH

The equilibrium contact angle, θ, of a water sessile drop satisfies Young's equation,

$$\gamma_{SV} = \gamma_{SW} + \gamma \cos\theta, \tag{5}$$

where γ_{SV}, γ_{SW}, and γ are the solid surface free energy of the solid in presence of the water vapor, the interface free energy between water and the solid, and the water surface tension, respectively (Fig. 1a).

When an ionizable solid surface is in contact with an aqueous solution, it acquires a certain charge because of adsorption or desorption of a certain number of protons [5,6]. The water surface tension can be considered as constant when hydrochloric acid or sodium hydroxide is added to modify the pH [14]. Therefore, when the pH of water is changed, the wetting angle, θ, changes accordingly:

$$-d\gamma_{SW} = \gamma\, d(\cos\theta). \tag{6}$$

The surface charge, σ, may be considered as resulting simply from the adsorption of protons (H^+) if the solid surface is positively charged and from the adsorption of OH^- if the solid surface is negatively charged [5]. Using the Gibbs adsorption equation it can be deduced that

$$d\gamma_{SW} = -\Gamma_{H^+} RT\, d\ln|\text{H}^+| = 2.303\Gamma_{H^+} RT\, d(\text{pH}), \tag{7}$$

where Γ_{H^+} is the surface excess concentration of protons. The surface charge density, σ, of the water/solid interface is related to Γ_{H^+} by

$$\sigma = \Gamma_{H^+} F, \tag{8}$$

where F is the Faraday constant. Hence, Eqs. (6)–(8) yield

$$\frac{d(\cos\theta)}{d(\text{pH})} = -\frac{2.303 RT\sigma}{F\gamma}, \tag{9}$$

indicating that the change in θ is controlled by σ. Note that the same Eq. (9) but with the right-hand side of opposite sign, is obtained for a negatively charged surface, due to the adsorption of OH^- groups. At the point of zero charge, pzc, $\sigma = 0$:

$$\frac{d(\cos\theta)}{d(\text{pH})} = 0. \tag{10}$$

Therefore, in the absence of specifically adsorbed ions, a maximum in θ or a minimum in $\cos\theta$ will occur at pzc as the pH of the water drop is scanned [5,6,14].

2.4. Wettability under octane

To measure the wettability as a function of pH for bare glass, the substrate was immersed in liquid octane (Fig. 1b). Under these conditions, the contact angle of water in (nonpolar) octane is only a function of specific, nondispersive, interactions between water and the substrate. This arises from water and octane having the same dispersive or London contribution to their surface tension (21.6 mN m^{-1} for water, 21.3 mN m^{-1} for octane [15,16]).

Another advantage of measuring wettability of water in an octane medium is that finite values of the contact angle can be obtained with a high-surface-energy substrate. For example, clean glass measured in air does not give a finite measurable contact angle with water.

As demonstrated in Section 2.3, a maximum in θ or a minimum in $\cos\theta$ is expected at pzc when the pH of the water drop is changed [6,14].

The nondispersive interaction between water and the substrate, I_{SW}^{nd}, may be deduced from the Young and Dupré equations,

$$\gamma_{SO} = \gamma_{SW} + \gamma_{WO} \cos\theta, \tag{11}$$

$$\gamma_{Si} = \gamma_S + \gamma_i - I_{Si}^{d} - I_{Si}^{nd}, \tag{12}$$

where subscript S represents the solid, and subscript i the liquid phase (water, W or octane, O), and θ is the water contact angle under octane. I_{Si}^{d} is the solid/liquid dispersive interaction (the sum $I_{Si}^{d} + I_{SW}^{nd}$ in Eq. (12) corresponds to the solid/liquid work of adhesion as expressed in the Dupré equation). Since $I_{SW}^{d} \approx I_{SO}^{d}$ and $I_{SO}^{nd} = 0$, I_{SW}^{nd} satisfies

$$I_{SW}^{nd} \approx \gamma_W - \gamma_O + \gamma_{WO} \cos\theta, \tag{13}$$

where γ_W (72.8 mN m^{-1}), γ_O (21.3 mN m^{-1}), and γ_{WO} (51 mN m^{-1}) are respectively the water and octane surface tensions and the water/octane interface tension. The last is not affected by the water pH [14].

From the nondispersive (hydrogen bond) energy of interactions, at pzc, expressed per unit of interface area, $I_{\mathrm{SW}}^{\mathrm{nd}}$, and from the molar energy of hydrogen bonds, E, on the order of 24 kJ/mol [17], we can estimate the number, n, of hydrogen bonds per unit interface area ($n \approx (I_{\mathrm{SW}}^{\mathrm{nd}}/E) \times 6.02 \times 10^{23}$), which corresponds to the surface density of hydroxyl groups on the solid surface.

3. Experimental methods

3.1. Sample preparation

The glass slides have a thickness of 1.1 mm and the format of microscope slides ($1 \times 3''$). The bare glass slides were cleaned by pyrolysis in an oven to burn organic contamination. The pyrolyzed samples were held in a glass rack and placed in a glass container covered with aluminum foil and closed with a glass lid. Following removal from the oven, the glass samples remained clean for as long as 1 week, provided the lid was not opened. Following opening of the container, the clean glass samples were used within 5 min. The low level of organic contamination was checked by controlling that water spread fully on glass slides. The glass substrate was treated with APTS molecules by using a proprietary process leading to a uniform coating with thickness on the order of one monolayer. After coating, the glass slides were placed in sealed packaging filled with nitrogen to prevent carbonatation of amine functions from carbon dioxide in air. Colloidal gold staining (Colloidal Gold Total Protein Stain, Bio-Rad Laboratories) verified uniformity of the surface charge on the coating.

3.2. Determination of the pzc of substrates by wettability measurements

The surface charge of bare glass and APTS-treated glass has been probed by measuring the water contact angle as a function of pH.

Contact angle measurements were made using a Ramé–Hart contact angle goniometer. The average water contact angle as a function of pH was obtained from measurements on five different glass slides of both APTS-coated and uncoated glass. Two measurements were made on one sessile drop for each pH value and on each slide (10 contact angle measurements per pH value). The water drops had a volume of 2 µl. Water was purified by ionic exchange (Elgastat, UHP), leading to a resistivity of 18 MΩ cm, followed by organic removal.

To measure the wettability as a function of pH for bare glass, the substrate was first immersed in octane (octane > 99%, Aldrich). Water wetting measurements were made also on APTS-coated glass in air as a function of pH. Adding HCl or NaOH to water varied the pH of water drops. The pH values were controlled with a pH meter (Basic 20, Crison).

3.3. Measurement of DNA retention

DNA retention was studied on APTS glass slides with double-stranded DNA obtained from polymerase chain reaction (PCR). The DNA used is the Bacillus subtilis 2 (BS2) gene of 1600 base pairs, 10% of molecules being labeled with Cy3 dye to measure the retention by fluorescence.

The DNA molecules, dissolved in a water/ethylene glycol mixture (1 : 4 by volume), were printed on APTS glass slides with a pin spotter in the form of small spots (Cartesian Technologies, dot diameter ≈ 250 µm). The pH of the water part in the solvent mixture was varied from 1 to 12.

The amount of DNA bound to the substrate was evaluated from the fluorescent intensities of spots measured with a scanner (Genepix Scanner (Cy3-wavelength, $\lambda = 532$ nm)). Fluorescent intensities were measured before and after the printed slides were washed with a prehybridization solution (50 ml of water, 25 ml of formamide, 25 ml of SSC 20X, and 0.5 ml of SDS 10X). The printed slides were dried for 4 h at 80 °C, before and after rinsing. The DNA retention is measured as the ratio of the fluorescent intensities before and after washing.

4. Results and discussion

4.1. Charge of single-stranded DNA in solution

Considering that in each nucleotide of DNA, the phosphate group may lead to one negative charge and the bases lead to positive, negative, or neutral charges, a simple calculation of the net average charge per nucleotide as a function of pH is possible. Assuming a probability of presence of 25% for each base per nucleotide, the average net charge per nucleotide, C/N, may be estimated as

$$\begin{aligned} \mathrm{C/N} \approx & -\frac{1}{1+|\mathrm{H}^+|/K_{\mathrm{ph}}} + 0.25\frac{1}{1+K_{\mathrm{A}}/|\mathrm{H}^+|} \\ & + 0.25\frac{1}{1+K_{\mathrm{C}}/|\mathrm{H}^+|} - 0.25\frac{1}{1+|\mathrm{H}^+|/K_{\mathrm{T}}} \\ & + 0.25\frac{|\mathrm{H}^+|^2 - K_{\mathrm{G1}}K_{\mathrm{G2}}}{K_{\mathrm{G1}}K_{\mathrm{G2}} - K_{\mathrm{G1}}|\mathrm{H}^+| + |\mathrm{H}^+|^2}, \end{aligned} \tag{14}$$

where K_{ph} $(= 10^{-1})$ corresponds to the ionization of phosphodiester groups and K_{A}, K_{C}, K_{T}, K_{G1}, and K_{G2}, are the dissociation constants given in Table 1. The resulting charge as a function of pH is plotted in Fig. 2. Equation (14) predicts that a single strand of DNA becomes negatively charged between pH 1 and pH 2. The solid line of Fig. 2 represents the contribution of phosphate groups, which are negatively charged from pH 0. This line may be considered as representing the charge of a double-stranded DNA polymer.

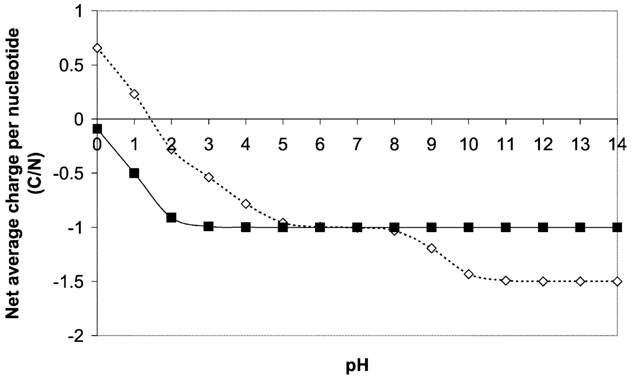

Fig. 2. Calculated net average charge per nucleotide of a double- or single-stranded DNA molecule in solution. The solid line represents the charge of a double-stranded DNA (phosphate groups). The dotted line represents the charge of a single-stranded DNA.

In fact, the presence of phosphates increases all the dissociation constants of the bases by a few tenths of a pH unit. This shift is due to the electrostatic attraction between the phosphate groups and any positively charged species, combined with the repulsion between the phosphate groups and any negatively charged species [3].

Above pH 2, a single strand of DNA may be considered as negatively charged. This is probably also true for double-stranded DNA, due to the dominant contribution of phosphate groups. The molecules may be seen as acidic polyelectrolytes.

4.2. Bare glass pzc

Figure 3 shows the variation of the water contact angle in octane as a function of the pH for bare glass. We observe that the wetting angle is clearly higher at pH 3, indicating a point of zero charge, pzc, at approximately pH 3. This pzc for a bare glass surface is close to that for a silica surface [8–12,15]. Therefore, we deduce that the equilibrium constant of silanol protonation (Eq. (3)) is on the order of 1.

We may deduce the ionic species present on bare glass as a function of pH from the equilibrium constants of dissociation or protonation of SiOH groups. It may be deduced that 50% of silanol groups are positively charged at pH 0. At pH 6, 50% of the silanol groups are negatively charged. Between these two values, the ionization of the silanol groups is weak. The fraction of positively and negatively charged silanol groups is on the order of 0.1% at pH 3.

The water contact angle in octane may be also used to estimate the density of silanol groups at the glass surface. The number of charged SiOH groups is very limited at the pzc (0.2%) and the contact angle in octane is 19.5°. We may consider that nondispersive interaction between water and the noncharged glass surface, $I_{\mathrm{sw}}^{\mathrm{nd}}$, at pH 3 is primarily generated by hydrogen bonding. From the nondispersive (hydrogen bonds) energy of interactions expressed per unit of interface area, $I_{\mathrm{sw}}^{\mathrm{nd}}$, and from the molar energy of hydrogen bonds, E, of 24 kJ/mol, the number, n, of hydrogen bonds per unit interface area ($n \approx (I_{\mathrm{sw}}^{\mathrm{nd}}/E) \times 6.02 \times 10^{23}$) is obtained. This gives about 2.5 silanol groups per square nanometer on the glass surface. This value is comparable to the density of silanol groups measured on silica that has been heat-treated at 500 °C [7].

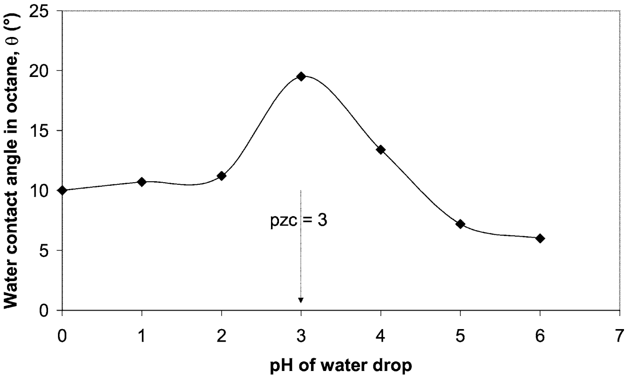

Fig. 3. Determination of the point of zero charge, pzc, of bare glass.

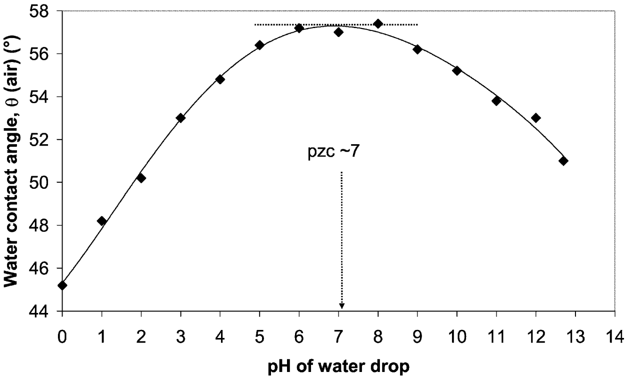

Fig. 4. Determination of the point of zero charge, pzc, of APTS slides by wettability measurements. The maximum of the wetting angle corresponds to the pzc (at pH ≈ 7) of the surface.

4.3. Pzc of silanized glass

The variation of the water contact angle in air as a function of the pH of the water drop is presented in Fig. 4. The maximum of the contact angle on APTS slides is obtained at pH ≈ 7, implying that the pzc of the APTS treated glass is around pH 7. This result is in perfect agreement with the study of Golub et al. [18] on silica gels modified with APTS.

We cannot consider that the APTS surface is just a cover of amine functions, since amine functions are positively charged up to pH ≈ 11. Neutrality of the APTS surface at pH 7 implies the presence of negatively charged species. The SiO^- species from SiOH are a probable candidate.

We model the surface charge of an APTS slide by considering that it is composed of amine and silanol groups. The number of ionizable surface sites, c, is considered constant. We define α as the fraction of amine groups and $(1-\alpha)$ as the fraction of silanol groups. At the pH corresponding to the

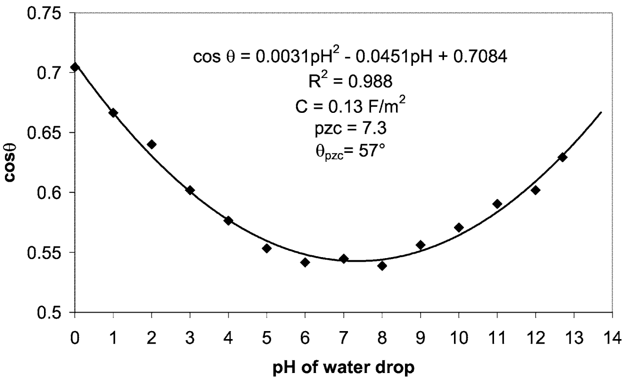

Fig. 5. Parabolic variation of the cosine of the water contact angle on the APTS-treated glass as a function of the pH of the water drop. The solid line represents a parabolic fit of experimental data.

pzc, the densities of positive and negative charges are equal:

$$|\mathrm{SiO}^-| = |\mathrm{SiOH}_2^+| + |\mathrm{R{-}NH}_3^+|. \tag{15}$$

At pH 7, the fraction of positively charged silanol groups is negligible. Therefore, Eq. (15), when combined with Eqs. (1) and (4), becomes

$$|\mathrm{SiO}^-| = \frac{(1-\alpha)c}{1+10^{\mathrm{p}K_1-7}} = |\mathrm{R{-}NH}_3^+| = \frac{\alpha c}{1+10^{7-\mathrm{p}K_a}}. \tag{16}$$

Solving this equation yields $\alpha = 0.48$. This is in good agreement with reported studies of APTS on silica [13]. This value of α implies that the surface densities of amine and silanol groups are about equivalent on APTS-treated glass. The amine density on the APTS substrate is therefore of the order of $0.48 \times 2.5 = 1.2$ groups per nm^2.

The graph in Fig. 5 gives the variation of the cosine of the water contact angle as a function of pH. It appears qualitatively that the curve has a parabolic shape and is symmetrical about the pzc. This curve is compatible with the constant capacitance model formed by an electrical double layer.

If the double layer forms a capacitor of capacitance C (per unit area), the surface charge satisfies

$$\sigma = CV, \tag{17}$$

where V is the surface potential. The energy of the capacitor, E, contributes to the water/solid interface free energy, so that the variation of the interface free energy may be also written as

$$dE = -d\gamma_{\mathrm{SW}} = d\left(\frac{1}{2}CV^2\right) = \sigma\, dV. \tag{18}$$

This is equivalent to the Lippman equation in electrowetting. Considering that $V = 0$ at pzc, combining Eqs. (6), (7), and (18) leads to the variation of the cosine of the wetting angle with the pH of water:

$$\begin{aligned}\cos\theta &= \cos\theta_{\mathrm{pzc}} + \frac{CV^2}{2\gamma}\\ &= \cos\theta_{\mathrm{pzc}} + \frac{C}{2\gamma}\left(2.303\frac{RT}{F}\right)^2(\mathrm{pzc}-\mathrm{pH})^2.\end{aligned} \tag{19}$$

Equation (19) justifies the parabolic variation with pH of the cosine of the wetting angle of water on the APTS-treated glass. The solid line of Fig. 5 represents the parabolic fit of the experimental points. R^2, the correlation coefficient, is very close to 1 (0.988). From the constant term of the parabolic fit, it is possible to determine precisely the pzc of the solid surface as pH 7.3 and the value of the wetting angle at pzc of 57°. The similarity with electrowetting becomes evident if θ_{pzc} is considered as equivalent to $\theta_{V=0}$ in electrowetting.

Hence, the surface charge of the APTS-treated glass leads to the formation of an electrical double-layer, comparable to the Helmholtz double-layer description, when placed in contact with water. The simple model of a constant capacitor for this double layer is in good agreement with the experimental results. The value of the constant capacitance is found to be equal to 0.13 F/m^2. Considering a value of 80 for the water dielectric constant leads to a distance d between the inner and outer plane of the double layer of 5.5 nm ($d = \varepsilon_0\varepsilon_r/C$). The pzc at pH 7.3, instead of pH 7, does not significantly alter the fraction of amine functions of the total ionizable surface sites (0.49 versus 0.48).

4.4. DNA retention as a function of the proton concentration

The DNA retention, expressed as a fraction of the amount of DNA retained by the substrate after washing, appears to be strongly dependent on the proton concentration of the printing solution, as shown in Fig. 6. An increased DNA retention is observed at the highest proton concentrations. The four curves correspond to four different APTS slides with four printed DNA dots per proton concentration value.

We can deduce that a positively charged substrate is favorable to having a high retention of printed DNA. This effect occurs despite the fact that the dry DNA spots are then washed by the same buffer solution.

At high proton concentrations, between 10^{-1} and 10^{-2} mol/l, the phosphate groups of DNA are negatively charged

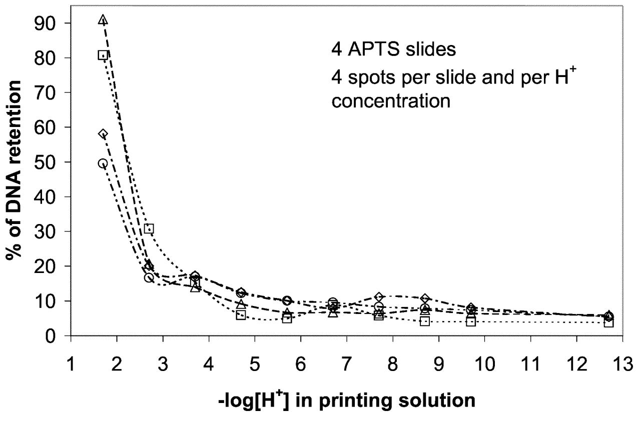

Fig. 6. DNA retention as a function of the proton concentration in the DNA printing ink.

(Fig. 2). The silanol groups become positively charged, increasing the net positive charge of the substrate. Thus, it is likely that the increase of DNA retention at very high proton concentrations is due to ionic interactions between negatively charged phosphate groups of DNA and the positively charged amine and silanol groups of the substrate.

Therefore, the DNA printing conditions, and in particular the proton concentration, have a strong impact on DNA retention, even after washing.

5. Conclusion

Wetting experiments performed at various pH reveal that a glass surface treated with APTS is positively charged below pH 7 and negatively charged above pH 7, although the amine groups alone are positively charged up to pH ≈ 11.

A simple model allows an estimate of the net surface charge of silica or glass treated with APTS. The APTS substrate is positively charged below pH 7 and negatively charged above pH 7, due to the contribution of amine and free silanol groups. The model indicates that the surface is composed of about 50% amine groups and 50% silanol functions. This result is in good agreement with the conclusions of former studies of the adsorption of APTS on silica [13,18]. The origin of these free silanol groups may be the glass surface or hydrolyzed alkoxy groups of the APTS silane.

A good correlation between the DNA retention data and the variation of the surface charge of APTS silanized glass has been obtained. The retention of DNA is improved when DNA spots are printed in acidic conditions, probably because phosphate groups of DNA are negatively charged from pH 0 and both the amine functions of APTS and the silanol groups are positively charged at this pH.

This study may be considered as an attempt to describe the ionic interactions between DNA molecules and functionalized substrates and their consequences on DNA retention. The subject is quite complex. It is evident that DNA may interact with the surface via other interactions, as hydrophobic interactions. However, our results show evidence of the important role played by ionic interactions in the retention of DNA molecules on aminated substrates, such as APTS-treated slides.

We have demonstrated that the electrical double layer formed at the interface between water and the APTS-treated glass is perfectly described by a model of constant capacitance of the double layer. The electrical behavior of the APTS-treated glass in contact with water is remarkable and it may be used to better control the retention of DNA and the properties of the functionalized substrate.

References

[1] V. Chan, S.E. McKenzie, S. Surrey, P. Fortina, D.J. Graves, J. Colloid Interface Sci. 203 (1998) 197.

[2] V. Balladur, A. Theretz, B. Mandrand, J. Colloid Interface Sci. 194 (1997) 408.

[3] C.R. Cantor, P.R. Schimmel, Biophysical Chemistry I. The Conformation of Biological Macromolecules, Freeman, New York, 1980.

[4] S. Koch, P. Woias, L.K. Meixner, S. Drost, H. Wolf, Biosensors Bioelectron. 14 (1999) 417.

[5] L.-K. Chau, M.D. Porter, J. Colloid Interface Sci. 145 (1991) 283.

[6] E. McCafferty, J.P. Wightman, J. Colloid Interface Sci. 194 (1997) 344.

[7] L.T. Zhuravlev, Colloids Surf. A Physicochem. Eng. Asp. 74 (1993) 71.

[8] G.A. Parks, Chem. Rev. 65 (1965) 177.

[9] A. Carré, F. Roger, C. Varinot, J. Colloid Interface Sci. 154 (1992) 174.

[10] M.L. Hair, W. Hertl, J. Phys. Chem. 74 (1970) 92.

[11] R.A. Van Wagenen, J.D. Andrade, J.B. Hibbs, J. Electrochem. Soc. 123 (1976) 1438.

[12] M. Bezanilla, S. Manne, D.E. Laney, Y. Lyubchenko, H.G. Hansma, Langmuir 11 (1995) 655.

[13] E.F. Vansant, P. Van der Voort, K.C. Vranken, Characterization and Chemical Modification of the Silica, in: Studies in Surface Science and Catalysis, Vol. 93, Elsevier, Amsterdam, 1995.

[14] C. Vittoz, M. Mantel, J.C. Joud, J. Adhesion 67 (1998) 347.

[15] W. Birch, S. Carré, A. Carré, in: Proceedings EURADH '2000, 5th European Adhesion Conference, 2000, p. 242.

[16] W. Birch, S. Mechken, A. Carré, in: K.L. Mittal (Ed.), Proceedings of the International Symposium on Surface Contamination and Cleaning, MST Conferences, Newark, NJ, 2001, to be published.

[17] J.N. Murrell, A.D. Jenkins, Properties of Liquids and Solutions, Wiley, New York, 1994.

[18] A.A. Golub, A.I. Zubenko, B.V. Zhmud, J. Colloid Interface Sci. 179 (1996) 482.