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ARTICLE TYPE

***In situ* study of the impact of acidic and neutral deposition pH on alkane phosphate film formation and stability on TiO₂**Stephen A. Holt^{*a} Anton P. Le Brun^a Andrew R.J. Nelson^a and Jeremy H. Lakey^{*b}*Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX*

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TiO₂ has been used as a model system for the surface of medical implant devices based upon titanium alloys. Self Assembled Monolayers can be applied via a specific phosphate interaction to modify the surface properties of the TiO₂ substrate. Here, is demonstrated via *in situ* Quartz Crystal Microbalance and Neutron Reflectometry experiments that the deposition of dodecyl phosphate onto TiO₂ proceeds more rapidly at pH 4.5 than at pH 7.0. Conversely, the film stability was enhanced for films deposited at pH 7.0. While the adsorbed amount appeared to be relatively constant after a few minutes incubation time it was found that washing with buffer removed about 50 % of the adsorbed material after these short incubation times. With incubation time of the order of hours the proportion of the film washed off the surface decreased demonstrating that the specific phosphate – TiO₂ interaction was a slow process. slower initial surface interaction at pH 7.0 therefore allowed greater re-arrangement of the dodecyl phosphate resulting in more complete and robust monolayers than at pH 4.5. This was demonstrated by washing the film with buffer of increasing pH of up to 9.5. For the worst performing films, with incubation times of less than 300 min, at least 45 % of the initial monolayer was removed during the pH titration whereas the most robust film (< 5% removed) was deposited at pH 7.0 with an incubation time ca 1000 min.

Introduction

Self-assembled monolayers (SAMs) are widely used to modify the properties of many surfaces. There is a wide interest in and application of surface modification via SAMs for biosensors¹, protein adsorption², cell adhesion³ and other biomaterials applications. A potential use is the coating of medical implant devices, where the generic efficacy of implants can be limited by the presence of the implant causing unwanted side effects; inflammatory responses and fibrous encapsulation being two examples⁴. However, when the surface of an implant is coated with a SAM it may render the implant surface more biocompatible and facilitate incorporation into the host tissue^{5,6}. Many medical implants, such as joint replacements, are made of titanium alloys, thus creating SAMs on TiO₂ (titania), the natural oxide, is of particular interest and relevance.

One of the most commonly used systems to create SAMs on solid supports is thiol chemistry on gold surfaces.⁷⁻¹⁰ The SAM may be introduced to enable some desirable property of the substrate to be used in what may otherwise be a hostile environment. It could also be that the substrate is purely a vehicle to support the SAM and the properties that it presents to the, usually, liquid environment. Clearly, a good understanding of both the deposition and properties of the SAM are required for success in either case. The ability to produce a SAM from a one-step deposition process from aqueous media, under ambient

conditions, is highly desirable, as it is simple with low input energy costs. The Au – thiol SAMs already mentioned typically use ethanolic solutions under ambient conditions. Alkyl phosphates can self-assemble onto metal oxide surfaces in solutions of organic solvent.¹¹ In recent years a procedure has been developed whereby alkane phosphates can be deposited onto metal oxide surfaces from aqueous solutions (by converting the water-insoluble acid to its water-soluble salt) under ambient conditions. This procedure has been carried out on Al₂O₃, Ta₂O₅, Nb₂O₅, ZrO₂, and TiO₂ surfaces^{11,12}.

Typically, alkane phosphates spontaneously assemble on TiO₂ through the phosphate functional group of the molecule. The monolayers formed are ordered and densely packed, similar to those obtained through thioalkanes on gold^{11,13}. SAMs of alkane phosphates are produced through uniform film growth with an initial rapid attachment phase followed by a much slower increase in the grafting density on the surface¹⁴. It has been shown that alkyl phosphates assembled onto TiO₂ show a chain length dependency in their packing density on the surface - the packing density increases with carbon chain length, reaching a maximum density at C₁₆.¹⁵ The stability of alkane phosphate SAMs on TiO₂ have been investigated,¹⁶ with over 80 % of a dodecyl phosphate monolayer lost after one day of immersion in physiological-like Tris buffered saline (TBS) at 37 °C. The rapid degradation of the dodecyl phosphate SAM is demonstrated by the water contact angle of >110° (before immersion) reducing to ~ 63° after one day's immersion in TBS¹⁶. However, the same

monolayers are much more stable in ambient air with only 20 % of the SAM on TiO₂ lost after two weeks.¹⁶ It has been suggested that the reduced stability in aqueous solutions is due to the relatively small volume of a water molecule, allowing penetration into the film. In contrast, molecules with a larger volume, such as toluene, cannot penetrate into the film and thus the SAM is more stable when immersed in a solution of toluene.¹⁷ Contact angles of methyl terminated phospho-alkane monolayers on TiO₂ exposed to organic solvents with large molecular volumes (>140 Å³), or to air, showed no change in contact angle after exposure. The contact angle remained >100° indicating the presence of a hydrophobic monolayer¹⁷.

The wettability of alkane phosphate SAMs can be modulated by having mixed monolayers of methyl terminated and hydroxyl terminated alkyl chains.^{11, 13} This has paved the way for the creation of more sophisticated SAMs on TiO₂, where the alkane is terminated with oligo(ethylene glycol) for protein adsorption applications¹⁸, small molecule drugs for drug delivery¹⁹ and cell signalling molecules for tissue engineering²⁰. However, the creation of the SAM on the TiO₂ surface is done from a solution of either pure water or organic solvent. Here we investigate the deposition of dodecyl phosphate from aqueous solutions onto TiO₂ at two different pH values (acidic and neutral) and investigate the difference in coverage and packing of the final SAM assembly. To date all measurements of completed alkyl phosphate SAMs on TiO₂ have been carried out in air or under vacuum. This does not reflect the reality of the intended end use of any potential medical implant device, which would be under a liquid environment. In this paper we describe the assembly and structure of SAMs of dodecyl phosphate on TiO₂ at the solid-liquid interface *in situ* and further investigate the stability of the SAMs via pH variation.

Experimental

Materials

All chemicals were purchased from Sigma-Aldrich (Sydney, Australia) unless otherwise stated. Silicon wafers for reflectometry were purchased from EL-CAT (NJ, USA).

Preparation of dodecyl phosphate ammonium salt

We used the method of Hofer *et al.*¹² Briefly, 2.0 g of dodecyl phosphate was dissolved in 200 mL propan-2-ol and heated to 80 °C until fully dissolved. 6 mL of ammonia (25 % aq) was then added and a white precipitate formed. The solution was then cooled on ice and the precipitate filtered under vacuum. The white powder was then washed with ice-cold propan-2-ol. The dodecyl phosphate ammonium salt product was then dried at 60 °C under vacuum overnight.

Buffers and solutions

All buffers were made to a concentration of 10 mM. Acetic acid/sodium acetate was used for pH 4.5 and 5.5, 2-(*N*-morpholino)ethanesulfonic acid (MES) for pH 6.5, Tris-HCl for pH 7.0, 7.5 and 8.5 and glycine for pH 9.5. Buffers made in D₂O were adjusted such that pH = pD – 0.4 to reflect the difference between pH and pD. No binding of the buffers to the titania surfaces was detected by QCM.

Deposition

Alkane phosphate films were assembled both *in* and *ex situ* from aqueous solutions at 0.3 mg/mL with a pH of 4.5 or 7.0. The films studied in the Quartz Crystal Microbalance were deposited *in situ* with short and intermediate incubation times (*ca* 45, 100 and 300 minutes) onto TiO₂ coated chips (QSX-310, Q-sense, Gothenburg, Sweden). For the first 20 minutes of incubation 1 mL of solution flowed over the chips after which the flow was stopped. Samples studied by reflection methods were deposited *in situ* with the same concentration and pH conditions, but there was no solution flow after the initial wash. The substrates were single crystal silicon disks (<111> 100 mm diameter x 10 mm thick) onto which an approximately 270 Å thick TiO₂ film had been deposited (Helia Photonics, Scotland) using ion beam assisted electron beam methods. A further film was assembled *ex situ* at pH = 7.0 with the substrate immersed in solution for four days to assess the impact of incubation time upon film completeness.

Quartz Crystal Microbalance with Dissipation (QCM-D)

A QsenseE4 instrument (Q-sense, Gothenburg, Sweden) was used with flow controlled by a peristaltic pump (Ismatec SA, Glattbrugg, Switzerland). A flow rate of 50 µL/min was used for all solutions. The sensor chambers were thermostatted at 25 °C. Sensor crystals coated with TiO₂ were used for all experiments. The sensor crystals were cleaned by UV-ozone for 10 minutes, rinsed with 2 % (v/v) Hellmanex (a detergent based cleaning solution from Hellma) followed by copious rinsing with >18 MΩ cm water and drying under nitrogen. The sensors had a final clean by UV-ozone for 10 minutes immediately before use. The piezoelectric quartz crystal was excited at its fundamental frequency (5 MHz) and any change in frequency (Δ*f*) for the third, fifth, seventh, ninth and eleventh overtones was noted. A decrease in frequency corresponds to an increased mass on the surface of the sensor. For each overtone the change in dissipation (Δ*D*) was also measured. The dissipation is the proportion of energy dissipated during one cycle of the frequency oscillation and provides information on the viscoelastic properties of the materials deposited on the sensor surface. When a complete monolayer is formed on the surface, and the change in dissipation is negligible, the monolayer can be considered rigid and the Sauerbrey equation, below, is valid and can be used to calculate the mass absorbed to the surface per unit area (Δ*m*):

$$\Delta m = -(\Delta f \rho_q v_q) / (2 n \sqrt{F}) \quad [1]$$

where ρ_q is the density of quartz (2648 kg m⁻³), v_q is the speed of sound through quartz (3340 m s⁻¹), F is the fundamental frequency (5 MHz) and n is the overtone number. From this the area per molecule (APM) can be calculated:

$$APM = N_A \frac{M_r}{\Delta m} \quad [2]$$

where M_r is the relative molecular mass (264 g mol⁻¹) and N_A is Avogadro's number.

Neutron Reflection

Specular neutron reflection probes the scattering length density profile normal to an interface. Scattering length density is a measure of how strongly a neutron is scattered by a given

Fig. 1. The QCM-D trace of dodecyl phosphate ammonium salt at a bulk concentration of 0.3 mg mL^{-1} deposited onto titania surfaces at pH 4.5 (squares) and pH 7.0 (circles). The arrows indicate the points at which the post-deposition buffer wash occurred. The displayed traces are of the 5th overtone at (A) short incubation time, and (B) intermediate incubation time.

material. The neutron reflection (NR) experiments were carried out on the *Platypus* time-of-flight neutron reflectometer at the OPAL 20 MW research reactor (Sydney, Australia)^{21,22}. Here we used a frame rate of 20 Hz, with a fractional wavelength resolution of 4%. The specular reflectivity was collected at three incident angles, $\theta = 0.45^\circ$, 1.6° and 4.5° using a wavelength (Å) range of 3.5–16 Å. The resulting Q range is 0.01–0.25 Å⁻¹:

$$Q = \frac{4\pi \sin \theta}{\lambda} \quad [3]$$

Data collection times were 15 minutes for 0.45° and 1.6° and 90 minutes for 4.5° . Direct beam measurements through the silicon substrate were collected for each incident angle under the same collimation conditions. Data was reduced using the SLIM reduction package.²³ The reflectivity profiles from each incident angle were re-binned to instrument resolution, corrected for background and detector efficiency and stitched together at the appropriate overlap regions. Additionally, the *Platypus* data collection system is configured so that time slicing of the data can be performed post acquisition. Therefore, data was collected during film assembly over many hours and then sliced into whatever time profile was required to follow the assembly kinetics of the SAM.

Neutron Reflection Data Analysis

Data analysis was carried out using the MOTOFIT reflectometry analysis package²⁴. The NR data was initially fitted via a series of slabs representing the real space scattering length density profile perpendicular to the surface. Each layer is described by three parameters: a thickness, a scattering length density (SLD) and a Gaussian interfacial roughness. Subsequently the fit was reparameterised so that the slab or layer corresponding to the alkane phosphate was described using the APM and a thickness. The APM calculation by definition includes the scattering due to both the molecule and any water that may have penetrated the film. The APM of a layer used in the NR data fitting is defined as follows:

$$APM = \frac{\sum b - V \rho_{soln}}{t(p_L - \rho_{soln})} \quad [4]$$

Where $\sum b$ is the sum of the scattering lengths of the molecule (Å); t , the thickness of the layer (Å); ρ_L , the SLD of the layer (Å⁻²), V , the molecular volume of the molecule (Å³); and ρ_{soln} the SLD of the solution. The molecular volumes were calculated from Molinspiration Property Calculation Service²⁵. The solution consisted of buffer made with D₂O (SLD = $6.35 \times 10^{-6} \text{ Å}^{-2}$) or mixtures of D₂O and H₂O (SLD = $-0.55 \times 10^{-6} \text{ Å}^{-2}$). The solution SLD could therefore be matched to that of the TiO₂ film, referred to as TiO₂M. This effectively makes the TiO₂ film invisible to neutrons and highlights the scattering from the monolayer. See Table S1 for a full listing of calculated SLD values. The APM can readily be converted into a mass of material per unit area.

The alkane phosphate monolayer was partitioned into two layers for the reflectometry modelling. A superior fit was obtained when the phosphate headgroup and the alkane tail were delineated in the model. The APM was constrained to be the same for each layer. Equation [4] enables different levels of hydration in each layer to account for any packing variation between the phosphate headgroup and the alkane tail. Parameterising the model in this way has two main benefits. The first is that the number of modelled parameters is reduced. Secondly, it enforces the prior knowledge that there is one headgroup per tail. This is more effective than trying to back calculate an APM from SLD values derived independently from each layer.

Parameter uncertainties were obtained using a Monte Carlo^{26,27} resampling procedure. The real dataset is fitted via a genetic algorithm minimisation in MOTOFIT²⁴ to attain the best fit. The parameter ranges for this fit are then used in the resampling procedure where 1000 separate datasets are ‘synthesized’ from the original dataset by adding Gaussian noise to each data point based on the counting statistics of the real data. These synthesised datasets are fitted individually. The fits to the synthetic data were analysed by histogramming the fitted values. The distribution of each parameter was statistically analysed with the parameter value taken as the midpoint of the 95 % confidence interval and its uncertainty from the standard deviation of the distribution.

Results:

Deposition of dodecyl phosphate ammonium salt

The dodecyl phosphate ammonium salt was deposited from solutions at pH values of 4.5 and 7.0, with Fig. 1 showing the impact of incubation time. The larger the change in frequency,

Figure 2. The third (○), fifth (□), seventh (△), ninth (▽) and eleventh (◇) overtones for the deposition of 0.3 mg mL⁻¹ dodecyl phosphate ammonium salt on titania as measured by QCM-D. The change in frequency (black lines, left axis) and change in dissipation (grey lines, right axis) are shown for a) deposition at pH 4.5 and b) deposition at pH 7.0.

Fig. 3. Kinetic NR datasets, over a restricted Q range, showing the TiO₂ surface, black trace, and then two minute data slices at the indicated times. All datasets are for surface in contact with the incubating solution made in D₂O buffer. (a) pH 4.5 and (b) pH 7.0. In this and all subsequent NR datasets error bars are only shown where they are larger than the symbol used.

left hand axis, the greater the mass deposited onto the surface. The rate of deposition is faster at pH 4.5 than at pH 7.0 as evidenced by the more rapid decrease in the Δf signal. The amount assembled onto the TiO₂ surface at each pH reaches a similar saturation point (Fig. 1b) after about 360 minutes. The overtones all overlap, indicating that a complete layer has formed which is rigid with little water associated (Fig. 2). The Sauerbrey equation is therefore valid. The absorbed masses are 2.88 and 2.40 mg m⁻² at pH 4.5 and 7.0 (Fig. 1b) respectively before a buffer wash. The APM, at this stage, is calculated to be 15.2 and 18.3 Å² for pH 4.5 and 7.0 respectively. These values are consistent with a complete monolayer. When a buffer wash is carried out, non-chemically bonded material is removed from the TiO₂ surface, with a greater loss observed in the pH 4.5 buffer solution at both the short (Fig. 1a) and intermediate (Fig. 1b) incubation times. The loss of material leads to a corresponding increase in the area per molecule. Table 1 gives a full listing of the QCM-D deposition results.

NR analysis of the deposition process revealed the same trends and similar adsorbed amounts derived from the QCM-D data. The TiO₂ films were first characterised against D₂O and H₂O for each substrate revealing a completely dense TiO₂ film (98 - 101%

theoretical density) with thicknesses ranging from 270 – 300 Å and a Gaussian surface roughness of less than 5 Å over the beam footprint (30 x 50 mm²).

When deposition was undertaken at pH 4.5 the layer appeared to be complete in the time required to exit the instrument enclosure and open the neutron shutter to enable data collection. Figure 3a shows the pre-deposition data, the first two minutes of recorded data, then two minutes slices after 10, 50 and 350 minutes. It is clear that the signal, while very different from the initial TiO₂ film, changes very little during the first 50 minutes of incubation. After 350 minutes there is a minor variation in the intensity (but not position) of the fringes. At pH 7.0, in agreement with the QCM-D data, we observe more variation in the data over the first four hours of incubation (Fig. 3b). Once again the film appears almost completely formed in the time taken to start the experiment with no variation in signal over the first ten minutes. However, after 60 minutes there is a more noticeable increase in fringe intensity but no further variation over the next 180 minutes.

Figure 4 shows data and real space profiles for monolayers produced at pH 4.5 and 7.0. Figures 4 a&b show data collected at an intermediate time (360 minutes) against a D₂O subphase and

again after 1140

Fig. 4. (a) Full NR datasets (symbols) and fits (lines) of a dodecyl phosphate film deposited at pH 4.5 after a buffer wash in a D₂O (□) and TiO₂M (■) subphases. (b) Corresponding real space density profiles corresponding to the fits in panel (A). (c) Full NR data sets (symbols) and fits (lines) of a dodecyl phosphate film deposited in D₂O at pH 7.0 for 425 minutes before buffer wash (□) and after buffer wash in D₂O (■) and 12 % D₂O (△) subphases. (d) The corresponding real space density profile of the fits in panel (C). The symbols in (A) and (C) correspond to those in (B) and (D) respectively.

minutes and washing against a TiO₂M subphase. The density profile has been set so that the 'zero' position (Fig. 4b) corresponds to the TiO₂ film / solution interface. As the two datasets were collected on the same substrate the silicon oxide and TiO₂ parameters (such as SLD, thickness and roughness) were constrained to be equal between contrasts. This principle was followed wherever possible. Figures 4 c&d show similar results and fits for a preparation at pH 7.0. In this case there is an intermediate incubation (425 minutes) with an unwashed D₂O subphase, and 1020 minutes incubated washed datasets against D₂O and a 12% D₂O / 88% H₂O mixture. The density profile (Fig. 4d) reveals that the lowest SLD for the tail region was observed for the 425 unwashed sample, indicating the highest adsorbed amount. It can also be seen that there is a difference in the subphase SLD for the 425 minutes profile and the D₂O washed profile. The cell had previously been filled with H₂O and then washed with a minimal volume of dodecyl phosphate in D₂O buffer, clearly not enough to completely exchange the solution. The washed dataset was collected after the cell volume had been

exchanged more than ten times resulting in complete solution exchange.

Table 2 lists the APM, tail thickness and surface excess of alkane phosphate monolayer from all NR experiments after incubation at the indicated times.

Stability of the dodecyl phosphate films with increasing pH

Figure 5 shows the impact of pH titration on three films deposited in the QCM. After 330 minutes incubation for pH 4.5, Fig. 5a, the usual loss of mass on washing (~ 50 %) is observed. The pH 4 washes after that had very little impact on the monolayer. After 107 minutes incubation at pH 7.0 (Fig. 5b) there was an initial loss of material (~ 20 %) when the surface was washed with buffer. Further material was removed upon washing with pH 4.5 buffer (~ 10%). The monolayer was then relatively stable until the pH 8.5 and 9.5 washes where finally there is only 50 % of the monolayer remaining. After 310 minutes of incubation at pH 7.0 in the QCM-D (Fig 5c) a more stable film was produced. Upon washing about 40% of material was lost from the surface. The

SAM was then very stable until the pH 9.5 wash where a further 20 % of the SAM was removed.

Table 1 Area per molecule and deposited amount (Surface Excess) from QCM-D experiments for dodecyl phosphate SAM deposition at the pH and incubation times indicated. Errors are the standard deviation of the results from four chips exposed under identical conditions in parallel.

	pH 4.5		pH 7	
	Prewash	Washed	Prewash	Washed
Deposition Time	45 Minutes		48 Minutes	
APM ($\text{\AA}^2$)	16.0 (0.2)	22.0 (0.5)	28.5 (1.0)	46.6 (1.1)
Surface Excess (mg/m^2)	2.74 (0.04)	1.99 (0.06)	1.54 (0.05)	0.94 (0.02)
Deposition Time	105 Minutes		105 Minutes	
APM ($\text{\AA}^2$)	17.7 (1.1)	41 (17)	10.4 (0.1)	13.0 (2.0)
Surface Excess (mg/m^2)	2.48 (0.15)	1.07 (0.46)	4.23 (0.37)	3.37 (0.52)
Deposition Time	240 Minutes		300 Minutes	
APM ($\text{\AA}^2$)	15.2 (0.1)	33.5 (9.2)	17.5 (0.6)	29.2 (3.9)
Surface Excess (mg/m^2)	2.88 (0.02)	1.31 (0.36)	2.50 (0.09)	1.50 (0.2)
Deposition Time	320 Minutes		350 Minutes	
APM ($\text{\AA}^2$)	14.8 (0.5)	30 (3)	18.3 (0.6)	27.6 (3.5)
Surface Excess (mg/m^2)	2.97 (0.09)	1.46 (0.15)	2.40 (0.04)	1.59 (0.16)

Table 2 Alkane tail length, area per molecule and deposited amount (Surface Excess) from NR experiments for dodecyl phosphate SAM deposition at the pH and incubation times indicated. Errors are calculated via the Monte Carlo resampling process described in the text and propagated through to derived parameters.

	pH 4.5	pH 7	
	Washed	Prewash	Washed
Deposition Time	320 Minutes		
APM ($\text{\AA}^2$)		13.2 (0.3)	13.3 (0.3)
Tail thickness ($\text{\AA}$)		12.76 (0.76)	12.9 (0.82)
Surface Excess (mg/m^2)		3.32 (0.07)	3.29 (0.07)
Deposition Time	1140 Minutes	1020 Minutes	
APM ($\text{\AA}^2$)	15.21 (0.24)	14.7 (0.2)	18.0 (0.3)
Tail thickness ($\text{\AA}$)	17.24 (0.53)	14.3 (0.4)	12.4 (0.5)
Surface Excess (mg/m^2)	2.393 (0.038)	2.48 (0.03)	2.02 (0.03)
Deposition Time	4320 minutes		
APM ($\text{\AA}^2$)		13.28 (0.12)	
Tail thickness ($\text{\AA}$)		18.9 (3.5)	
Surface Excess (mg/m^2)		2.74 (0.03)	

The subphase pH was stepped from 4.5 to 9.5 for samples produced on *Platypus* at pH 4.5 and 7.0. Figure 6, where longer tail length and smaller APM indicate a more complete SAM, presents a summary of the data fitting as a function of pH exposure. See Fig. S1 for the data and fits for pH 4.5 (1140 minutes) and pH 7.0 (1020 minutes) preparations from which the APM was calculated. At pH 4.5 there is an initial loss of material when the surface is washed with pH 5.5 buffer, the APM and tail

length is then relatively constant as the pH is increased to 9.5 (Fig. 6). This is consistent with what is observed with QCM-D (Fig. 5). The 1020 minute incubation pH 7.0 surface was exposed

Fig. 5. pH titrations of a monolayer of dodecyl phosphate on titania deposited at (a) pH 4.5 with 330 minutes incubation, (b) pH 7.0 with 105 minutes incubation and (c) pH 7.0 with 310 minutes incubation. The figure shows the change in frequency (circles, left axis) and change in dissipation (squares, right axis) on the fifth overtone. The arrows indicate the times at which initial buffer wash and subsequent pH washes occurred.

to pH 4.5 buffer with the APM and tail length unchanged from the washed values at pH 7.0 (Table 2).

This surface was then remarkably stable up to pH 9.5. A different trend is observed for the 320 minute pH 7.0 incubated surface. This sample initially seemed to have a more complete SAM than the long incubation sample but proved to be the least stable

Fig. 6. Summary of fits to the pH titration on NR *in situ* films, 1140 minutes incubation at pH 4.5 (□), 310 minutes incubation at pH 7.0 (■) and 1020 minutes incubation at pH 7.0 (△). (a) Dodecyl tail lengths and (b) APM as a function of subphase pH. Lines are a guide to the eye, error bars are determined from the Monte Carlo resampling procedure as described in the text.

monolayer against increasing pH. By mass, 44 % of the initial SAM remained on the surface at pH 9.5. This is consistent with observations of a dodecyl phosphate monolayer that had 100 minutes of incubation time (Figure 5b). There is an initial loss of material when the buffer is switched from pH 7.0 to pH 4.5 with an 18 % loss of mass from the surface. The monolayer remains relatively stable from pH 4.5 to pH 7.5 with little mass variation. However at pH 8.5 and above a large mass loss is observed with only 46 % of the original mass remaining on the TiO₂ surface at pH 9.5 (Figure 5b).

Discussion:

Deposition of dodecyl phosphate ammonium salt

It is clear that a consistent picture, if exposure times are taken into account, is attained for all experiments. Also there is good agreement between the results obtained by QCM-D and NR, sample areas ~ 1 cm² and ~ 200 cm² respectively. The deposition

at pH 4.5 proceeds more rapidly and usually results in a greater surface loading before buffer washing than at pH 7.0. In both NR and QCM-D experiments the pre-wash state always displays more material than the washed state. This differential decreases with incubation time. The pH 4.5 depositions also tend to lose proportionally more material at the shorter incubation times. There is a range of isoelectric points for TiO₂ in the literature,²⁸ from 4.7 to 6.2. At pH 4.5 then one would expect the TiO₂ to have no net charge or be weakly negative while at pH 7 the surface will have a net negative charge. Phosphoric acid is protonated in a weakly acidic solution only becoming deprotonated, with a net charge of -2, in strongly basic solutions.²⁹ The measured pK values for dodecyl phosphate are 2.8 and 7.2 below the critical micelle concentration (CMC) of 0.77 mg mL⁻¹.

Therefore at pH 4.5 the surface is likely to be slightly negatively charged with the concentration of dodecyl phosphate used, the dominate species is likely to be negative with a singly deprotonated phosphate (i.e. a net charge of -1).²⁹ At pH 7.0 the surface will be negative with the dodecyl phosphate distributed between singly and doubly negatively charged species. Nakayama *et al*²⁹ found it difficult to determine the pK values precisely, therefore at pH 7.2 it is reasonable to assume singly and doubly deprotonated species with a bias to the latter. At pH 9.5 one would anticipate a negative charge on the TiO₂ surface with the dodecyl phosphate possessing a double negative charge. While there is a difference in the charge based interaction at pH 4.5 and pH 7.0 which influences the deposition rate, the completeness of the monolayer quality is not affected. The final SAM is therefore determined by the specific chemistry of the phosphate / TiO₂ interaction. It has previously been observed that considering the variation of the IEP of a range of metallic oxides did not predict SAM formation with dodecyl phosphate.¹² It has been shown that when forming SAMs of alkane phosphates on TiO₂ there is a two-step kinetic process where the adsorbate – substrate interactions dominate the formation of the SAM. Our results confirm this as the variation in pH predominantly affects the kinetics of formation rather than the final SAM.

Stability of the dodecyl phosphate films with increasing pH

Figure 7 shows data that summarises the SAM stability when exposed to buffers with increasing pH. This data is calculated by comparing the mass of monolayer on the surface with the mass after washing. It is quite clear from this figure that the best performance was obtained from a film deposited at pH 7.0 (Fig. 7b) for 1020 minutes (NR) where only about 5% of the monolayer was removed as sequential exposure to a series of pH values. This confirms that electrostatic attraction was not the determinant of film formation as both the surface and phosphate group would be negatively charged at this pH. The film deposited in the QCM-D for 105 minutes at pH 4.5 (Fig. 7a) appears to show anomalous results, when the error bars are considered it is apparent that the variability in the data is within the errors of the measurement. This data is the average over four channels on the QCM-D with the error shown as the standard deviation, clearly there are significant differences in the adsorbed amount after washing for these times. Table 1 shows the initial pre-washed and washed amounts for this deposition where it can be seen that the washed APM is 41 Å², equal to about 30 % surface coverage.

With the exception of three samples all SAMs tested had about 50 – 60 % of the washed monolayer remaining after treatment with increasing pH solutions. When alkane thiol films on gold are exposed to varying pH a complete monolayer is stable from pH 1

Fig. 7 A summary of all data showing the stability of the SAM to washing with solutions of increasing pH where 100 % represents the amount of material on the surface after washing at the deposition pH. (A) pH 4.5, incubation times 105 minutes (□), 320 minutes (■) and 1140 minutes (△) and (B) pH 7.0 incubations times 105 minutes (□), 300 minutes (■), 310 minutes (△) and 1020 minutes (○), where the lines are a guide to the eye.

to 12 with no or insignificant amounts of material lost from the gold surface³⁰. Above pH 12 (sodium hydroxide solutions) material loss from the gold surface is observed and it was speculated that the loss of material was due to direct attack from the hydroxide ions or degradation of the gold surface rather than a change in the protonation state of the thiol³⁰. Our results also suggest that once a complete SAM is formed it is resistant to pH variation, demonstrating that the phosphate groups are covalently bound to the TiO₂ surface and that the loss of material from SAMs with short incubation times (i.e. where there is an incomplete monolayer) is likely due to direct attack from anions rather than from a change in the protonation state of the phosphate group. The indirect study of the stability of alkane phosphates in physiological-like buffers at 37 °C revealed a rapid loss of material from TiO₂ surfaces¹⁶. This rapid loss of material was attributed to the hydrolytic instability of phosphate group on Ti and the enhanced degradation in the presence of buffer over

plain water due to the presence of sodium and chloride ions¹⁶. Mani and co-workers¹⁶ also showed a reduced degradation of dodecyltrichlorosilane SAMs on TiO₂ in buffer compared to dodecyl phosphate and this was attributed to a stronger Si-O-Ti bond. We can therefore conclude from our results that the loss of SAM material from incomplete monolayers at high pH is from enhanced hydrolysis of material at the surface rather than a change in the protonation state of the phosphate head group.

Conclusions

We observed an initially faster association of the dodecylphosphate with the TiO₂ surface at pH 4.5 than at pH 7.0 which was attributed to differences in surface charge. Our results show, both in the QCM-D and NR that pH stability is enhanced for monolayers prepared at pH 7.0. According to a study³¹ of the deposition of octadecyl phosphonic acid onto zirconated silicon, ZrO₂ and TiO₂ powders the key parameter is the strength of the phosphate – metal cation bond. It was observed by ³¹P solid-state NMR that the chemical shift of the phosphorous peak was least for the TiO₂ surface, attributed to incomplete de-protonation of the phosphonic acid headgroup. Under the deposition conditions used in this work we would expect that the phosphate headgroup would be more protonated at pH 4.5 which may explain the reduced stability of pH 4.5 deposited films. The slower deposition rate observed at pH 7.0 may have allowed rearrangement of the dodecylphosphate into a more densely packed SAM. Furthermore it also seemed that shorter incubation times were required in the QCM-D than in the NR cell. This could be related to both the much active area of the QCM-D chip as well as some difference in the solution flow conditions. There was no solution flow after the initial wash over samples produced for NR, whereas for QCM-D the surfaces had solution flowing across them for 20 minutes followed by static incubation. The QCM-D does though apply a small perturbation to the chip, which may have impacted upon the mass transfer to the surface. It would be interesting to repeat the NR depositions under flow conditions.

Notes and references

- ^a Bragg Institute, Australian Nuclear Science and Technology Organisation, Locked Bag 2001, Kirrawee DC 2234, Australia. Tel: +61 29717 3173; E-mail: sph@ansto.gov.au
- ^b Institute for Cell and Molecular Biosciences, Newcastle University, Newcastle upon Tyne NE2 4HH, United Kingdom. Fax: +44 191 2227242; Tel: +44 191 2228865; E-mail: jeremy.lake@ncl.ac.uk
- † Electronic Supplementary Information (ESI) available: [ESI includes a table of the relevant calculated SLD values and a figure showing the pH series data and fits]. See DOI: 10.1039/b000000x/
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