PAPER

A procedure for estimating the surface dipole potential of monolayers adsorbed on electrodes

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The extrathermodynamic potential difference $\Delta\phi$ across an electrified interface enclosed between a bulk metal M and a bulk aqueous phase does not depend on the content of the interface, at constant applied potential E. By equating at constant E the expression of $\Delta\phi$ for an electrode coated by a self-assembled monolayer (SAM) to that for the corresponding bare electrode immersed in the aqueous solution of a nonspecifically adsorbed electrolyte, it is possible to estimate the surface dipole potential χ_{SAM} of the SAM. If the molecules of the SAM form a bond M–X with the metal M, this procedure requires an independent knowledge of the surface dipole potential χ_{SAM}^{M-X} due to such a bond. The other way round, if the χ_{SAM} value is known by independent means, the procedure allows an estimate of χ_{SAM}^{M-X}. The self-consistency of this procedure was tested with SAMs of ten different thiolated peptides covalently bound to a mercury electrode, where χ_{SAM} can be determined independently by expanding a mercury drop. The procedure was then applied to the estimate of the χ_{SAM} value of a peptide SAM on a polycrystalline gold electrode.

1. Introduction

The absolute potential difference $\Delta\phi$ across an electrified interface interposed between a bulk metal and a bulk aqueous phase at a given applied potential is the same independent of the content of the electrified interface. Thus, one can equate the extrathermodynamic expression of $\Delta\phi$ for an electrode coated by a self-assembled monolayer (SAM) to that for the corresponding bare electrode immersed in the aqueous solution of a nonspecifically adsorbed electrolyte at the same applied potential. To obtain useful information from such an equation, all the contributions to $\Delta\phi$ must be adequately estimated.[1–3] In the case of a SAM-coated electrode, $\Delta\phi$ can be described as the sum of the following contributions: (i) the surface dipole potential, χ_{ml}^M, due to electron spillover; (ii) the surface dipole potential, χ_{ml}^{M-X}, due to any covalent or polar bond M–X between the metal and the molecules forming the SAM; (iii) the potential difference, $\Delta\phi_{ml}$, across the monolayer; and (iv) the potential difference, $\Delta\phi_d$, across the diffuse layer. In the case of a bare electrode in an aqueous solution of a nonspecifically adsorbed electrolyte, $\Delta\phi$ lacks contribution (ii). Moreover, contribution (iii) consists of the potential difference, $\Delta\phi_{M-OHP}$, across the "inner" layer enclosed between the metal surface plane and the locus of the center of charge of hydrated ions in their position of closest approach to the metal, *i.e.* the outer Helmholtz plane (OHP). There is strong

experimental evidence that, to a good approximation, $\Delta\phi_{M-OHP}$ depends only on the charge density σ_M on the metal surface and not on the electrolyte concentration.[4,5] The same is true for the inner layer capacitance $C_i = d\sigma_M/d(\Delta\phi_{M-OHP})$.

In what follows, a dipole-moment vector is conventionally regarded as directed from the negative to the positive pole and its magnitude is taken as positive when the dipole points its positive pole toward the bulk metal. For both a SAM-coated and a bare electrode, the surface dipole potential due to electron spillover can be written in the form $\chi_{ml}^M = \chi^M + \delta\chi_{ml}^M$, where χ^M is the positive surface dipole potential at the interface between the metal M and vacuum, whereas $\delta\chi_{ml}^M$ is its change when vacuum is replaced by the monolayer of molecules in direct contact with the metal surface (water molecules in the case of the bare metal). $\delta\chi_{ml}^M$ is due to the perturbation of the metal electron density tail induced by the presence of this monolayer, independent of its being physisorbed (as in the case of water) or chemisorbed.[1,3]

The potential difference, $\Delta\phi_{ml}$, across a monolayer adsorbed on the metal surface without bond formation is commonly estimated on the basis of the following assumptions.[3,6,7] The normal component, $\mu_\perp$, of the permanent dipole moment of the adsorbed molecules is assumed to remain constant over the potential range of stability of the SAM, where its differential capacitance is practically independent of the applied potential and does not show pseudocapacitance peaks due to any drastic dipole reorientations. The contribution from these permanent dipoles to $\Delta\phi_{ml}$ is, therefore, given by $\chi_{ml} = N\mu_\perp/(\varepsilon_0\varepsilon_{ml})$, where N is the number of moles of the adsorbed species per unit surface, ε_0 is the permittivity of free space and ε_{ml} is the static dielectric constant of the SAM. The contribution to $\Delta\phi_{ml}$ from the distortional

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polarization of the SAM, due to induced dipoles, is equated to the ratio of the charge density σ_M on the metal to the differential capacitance C_{ml} of the monolayer. This amounts to assuming that the small AC signal superimposed on the bias potential E in electrochemical impedance measurements of C_{ml} affects the induced dipole moment of the adsorbed molecules, but not their permanent dipole moment. Thus, we have:

$$\Delta\phi_{ml} = \chi_{ml} + \sigma_M/C_{ml} \quad (1)$$

The estimate of $\Delta\phi_{ml}$ is more complicated if the SAM adsorbed on the metal surface forms a bond with the metal surface atoms. A typical example is offered by thiol monolayers tethered to a metal, which are chemisorbed in the form of thiolates. In this case, the charge density $\sigma_M(E_i)$ at a potential E_i at which the SAM is chemisorbed is obtained by measuring the charge density $Q(E_i \rightarrow E_f)$ involved in a potential step from E_i to a final potential E_f negative enough to allow the complete desorption of the SAM, if such a potential is experimentally accessible; $\sigma_M(E_i)$ is then given by $[\sigma_M(E_f) - Q(E_i \rightarrow E_f)]$, where $\sigma_M(E_f)$ is the charge density independently measured at E_f on the uncoated electrode. The charge density $\sigma_M(E_i)$ is thermodynamically significant, because it is measured without having recourse to models, but it should by no means be regarded as the actual charge density located on the metal surface; it is just the positive charge density required to compensate the negative charge density due to the chemisorbed thiolate molecules, in order to maintain the electroneutrality of the whole electrified interface at E_i. Whether electron transfer from the sulfur atom to the metal is total, partial or absent cannot be established on the basis of thermodynamic arguments and can only be hypothesized on the basis of extrathermodynamic assumptions. In this case, σ_M in eqn (1) must be replaced by a charge density q, equal to the sum of σ_M and the charge density on the thiolate groups.[7] This charge density was identified with that experienced by the diffuse layer ions. This amounts to disregarding discreteness-of-charge effects within the monolayer. With this approximation, the charges of the negative and positive poles of the permanent dipoles of the SAM can be regarded as smeared out on the two planes where they are located. Since the electric field created by a uniform planar charge distribution of infinite extent does not depend on the distance from the plane, the electric fields created by these two charge distributions, which have equal magnitude and opposite sign, cancel out and thus have no effect on the diffuse layer ions. These ions are only sensitive to q. The q value can be estimated by measuring the diffuse layer capacitance, C_d, at different concentrations c of a 1,1-valent electrolyte and by inserting pairs of the corresponding C_d and c values in the following expression of the Gouy–Chapman theory:

$$q = -\sqrt{\frac{4R^2T^2}{F^2}C_d^2 - \frac{2RT\varepsilon_w c}{\pi}} \quad (2)$$

Here ε_w is the dielectric constant of water and R, T, F retain their usual significance.

A SAM-coated hanging mercury drop electrode exhibits a unique advantageous feature with respect to the case of SAMs supported by solid metals such as gold, in that it allows an independent estimate of χ_{ml}. This is because the mercury drop can be expanded gradually, determining a progressive tilt of the

permanent dipoles of the SAM without resulting in the incorporation of water molecules in the SAM. In fact, if water molecules entered the SAM during drop expansion, they would cause an abrupt increase in the SAM capacitance, which has never been observed.[7–10] It was shown elsewhere[7] that χ_{ml} is directly provided by the opposite of the slope of the quantity:

$$\xi(\theta) \equiv q(\theta)\cos\theta/C_{ml} + \Delta\phi_d[c,q(\theta)] \quad (3)$$

against $\cos^2\theta$ at constant applied potential, where θ is the tilt angle of the self-assembled dipolar molecules with respect to the monolayer normal. Here, $q(\theta)$ is the charge density experienced by the diffuse layer ions during the drop expansion, and $\Delta\phi_d[c,q(\theta)]$ is the potential difference across the diffuse layer, regarded as a function of the electrolyte concentration c and charge density $q(\theta)$. The latter is obtained by adding to the q value estimated at the unexpanded drop the charge increments accompanying the gradual expansion of the drop. It should be noted that the slope of the $\xi(\theta)$ vs. $\cos^2\theta$ plot measures the deviation of the electrified interface from the behavior of a parallel plate capacitor. Thus, if the SAM molecules point the positive pole of their dipoles toward the electrode (i.e., if $\mu_\perp$ is positive), a drop expansion decreases the positive potential difference created by the dipole moments as a consequence of their tilt. Consequently, during the drop expansion, a positive charge must flow to the electrode surface along the external circuit, in addition to that due to the charging of the double layer, in order to maintain the potential difference across the whole electrified interface constant. This causes the $\xi(\theta)$ function to move in the positive direction with increasing tilt (i.e., with decreasing $\cos^2\theta$), imparting a negative slope to the $\xi(\theta)$ vs. $\cos^2\theta$ plot. The linear increase of ξ with a decrease of $\cos^2\theta$ excludes the possibility of a breakdown of the SAM during the drop expansion.

This work aims to show that equating the extra-thermodynamic expression of $\Delta\phi$ for a SAM-coated electrode to that for the corresponding bare electrode, immersed in the aqueous solution of a nonspecifically adsorbed electrolyte at the same applied potential, allows an approximate estimate of the surface dipole potential χ_{ml} of the SAM, without recourse to the expansion procedure. The resulting method can, therefore, be extended to solid metal supports. In the present work, it will be applied to the estimate of the surface dipole potential of a series of peptides based on the α-aminoisobutyric acid (Aib) unit and thiolated either at the C- or N-terminus. As opposed to peptide systems based on coded α-amino acids, which start to form helices only for rather long oligomers,[11] Aib homopeptides have the peculiarity of forming stiff 3_{10}-helices even with a low number of monomeric units.[12] This is due to the marked steric hindrance of the α-carbon, which results in a restricted torsional freedom. Rigidity is granted by the presence of strong intramolecular C=O···H–N hydrogen bonds which, in turn, generate a strong molecular dipole moment that has its positive pole on the nitrogen terminus of the peptide.[13] The increase of Aib units is accompanied by an increase of the number of intramolecular hydrogen bonds and a concomitant increase of the peptide stiffness.[12c] The peptides examined in the present work are labeled as 3+, 3−, 5+, 5−, 6+, 6−, 7+ and 8+, where the digit denotes the number of intramolecular hydrogen bonds and the sign denotes whether the N-terminus (+) or the C-terminus (−) is

thiolated. The couples of peptides 3+/3−, 5+/5− and 6+/6− were devised in such a way as to keep constant both their lengths and the nature of the hydrophobic group (*tert*-butyl) facing the solution. Fig. 1 shows the primary structure and the ball-and-stick model of the thiolated peptides 3+ and 3−. The hydrogen bonds, denoted by dashed segments, form rings of ten atoms and involve three Aib residues. Each residue contributes 0.20 nm to the length of the peptide along the helical axis, such that the helix pitch amounts to 0.60 nm. The 3+ peptide consists of five Aib residues that form three hydrogen bonds. Each additional residue increases the number of hydrogen bonds by one unit.

2. Experimental

Water, obtained from an inverted osmosis unit, was distilled and then further distilled from alkaline permanganate. Merck suprapur® KCl was baked at 500 °C before use to remove any organic impurities. Tetramethylammonium chloride (TMACl) was purchased from Merck and used as such. The procedure followed for the synthesis of the thiolated Aib homopeptides 6+, 6−, 7+ and 8+ was the same as that adopted in ref. 12*g* and 14 for the synthesis of 3+, 3−, 5+ and 5−. Solvents, salts, and reagents used for these syntheses are described in ref. 14.

All measurements were carried out in aqueous solutions of KCl. A homemade hanging mercury drop electrode (HMDE) was used, which is described elsewhere.[15] A homemade glass capillary with a finely tapered tip, about 1 mm in outer diameter, was employed. The capillary and mercury reservoir were thermostatted at 25 ± 0.1 °C in a water-jacketed box to avoid any changes in drop area due to a change in temperature. The HMDE has a stainless steel cylindrical piston that compresses the mercury contained in a reservoir at the top of the capillary, causing its extrusion from the capillary tip. The piston is driven
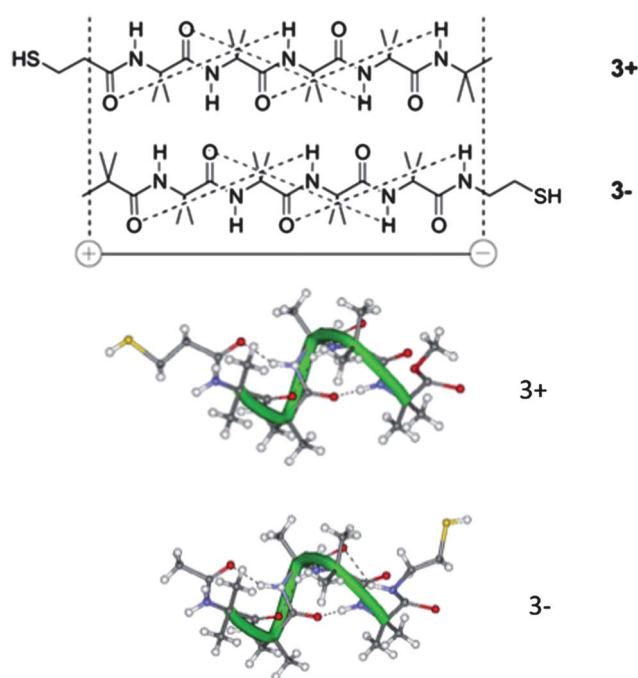
Fig. 1 Primary structure and ball-and-stick model of the cAib-peptides 3+ and 3−. Hydrogen bonds are denoted by dashed segments.

by a micrometric head (no. 350-541 Mitutoyo, Japan) equipped with a digital millesimal position sensor with a precision of 2 μm piston shift; this allowed us to estimate changes in drop area as little as of 0.04 mm² and to form highly reproducible drops by the piston movement, with a reproducibility of 1%.[15] The self-assembly of the thiolated-peptide monolayers on the HMDE was carried out by keeping the mercury drop, 22 × 10⁻³ cm² in surface area, immersed in 1 mg mL⁻¹ ethanol solutions of the thiolated peptides for 90 min. The SAM-coated mercury drop was then extracted from the ethanol solution and, after allowing the ethanol to evaporate in a nitrogen atmosphere, it was immersed in the working aqueous solution of 0.1 M KCl. The peptide-coated mercury electrode was stabilized by scanning the applied potential E several times over the potential range where the SAM was found to be more stable and reproducible; this range was determined by first recording the curve of the differential capacitance C against E over the whole accessible potential range, from −0.20 to −1.80 V *vs.* the normal calomel electrode (NCE). The mercury-supported SAMs so obtained were stable for hours in aqueous 0.1 M KCl. This denotes the practical insolubility of the peptides in aqueous solution and excludes the possibility of inorganic ions penetrating across the SAM and reaching the mercury surface.

Drop expansion measurements were carried out by expanding the SAM-coated mercury electrode by consecutive steps, manually operating the micrometric head that moves the piston, and by recording the charge following each expansion step as a function of time. The resulting charge *vs.* time curves have the shape of sloping steps, whose duration, of about 0.25 s, measures the time actually spent to expand the drop manually. The charge Q involved in the overall drop expansion was obtained by summing the heights of all the steps recorded during the gradual drop expansion. An analogous procedure was followed in drop contraction measurements. Drops were expanded up to about 90% of their initial area, in 10 to 15 steps, whereas they were contracted down to no more than 10%, in one or two steps.

The capacitance C_{ml} of the monolayer for the different SAMs was measured at a fixed applied potential chosen within the range of higher stability of the given SAM by electrochemical impedance spectroscopy (EIS), using a three electrode system. The impedance spectrum was fitted by an equivalent circuit consisting of a series of three RC parallel combinations (RC meshes), which simulated the chemisorbed monolayer, the diffuse layer and the aqueous solution adjacent to the SAM. The diffuse layer capacitance, C_d, was found to be much higher than the monolayer capacitance, C_{ml}, and could not be determined with sufficient accuracy. The accuracy with which the RC mesh of the aqueous solution could be determined was also low; in fact, the C and R values of a 0.1 M KCl aqueous solution are of the order of a few nF cm⁻² and a few Ω cm², respectively. Hence, the RC mesh of the solution is centered at a frequency $f = (2\pi RC)^{-1}$ of the order of 10⁷ Hz, and its effect is entirely negligible at frequencies <10⁴ Hz. The curves of the differential capacitance C against the applied potential E were determined by AC voltammetry, upon measuring the quadrature component of the current at 75 Hz as a function of E and converting the current into the capacitance on the basis of a calibration curve determined with a high-precision capacitor. At 75 Hz, the phase angle determined independently by EIS is ≥85°, denoting the negligible contribution of the

in-phase component of the current. Moreover, at this frequency the effect of the solution resistance is entirely negligible. Considering that C_d is $\gg C_{ml}$, the differential capacitance C determined by AC voltammetry at 75 Hz is almost coincident with the monolayer capacitance C_{ml} determined by EIS.

A gold bead electrode was fabricated from a polycrystalline gold wire (0.5 mm in diameter) of superior purity (99.99%) by melting one end of a clean wire into a small spherical bead in gas–air flame. The geometrical area, obtained by measuring the diameter of the electrode with a microscope, was about 0.13 cm². Before functionalization, the electrode was treated with hot 0.8% (w/w) chromic acid in concentrated sulfuric acid for 10 min, thoroughly rinsed with distilled water, dried with a flux of hot air and cleaned in the reductive flame of a Bunsen burner. Immediately afterwards, the electrode was immersed in aqueous 0.05 M HClO$_4$ and subjected to repeated potential scans between −0.45 and +1.50 V vs. a Ag|AgCl|1 M KCl reference electrode. It was then immersed in an ethanol solution of the 3+ thiolated peptide for about 24 h, then rinsed with ethanol and dried.

Chronocoulometric, AC voltammetric and EIS measurements were carried out with an Autolab instrument PGSTAT 12 (Echo Chemie, Utrecht, The Netherlands) supplied with a FRA2 module for impedance measurements, SCAN-GEN scan generator and GPES 4.9007 software. Potentials were measured vs. a Ag|AgCl electrode immersed in the KCl working solution, and are referred to the normal calomel electrode (NCE).

3. Results and discussion

To verify the possible reductive desorption of the thiolated Aib-peptide SAMs at sufficiently negative potentials, their differential capacitance C was measured by AC voltammetry over the potential range from −0.20 to −2.15 V in aqueous TMACl. In fact, the double-layer region on bare mercury in this electrolyte extends beyond −2.15 V before the onset of hydrogen evolution. All SAMs employed in this work were found to be completely desorbed between −2.00 V and −2.15 V, as demonstrated by the coincidence of the C vs. E curves on bare and SAM-coated mercury at these far negative potentials. Fig. 2 shows plots of the differential capacitance curves of Hg-supported SAMs of 6−, 6+, 7+ and 8+ peptides in aqueous 0.1 M KCl, over the potential ranges in which the SAMs were stabilized by means of repeated potential scans.
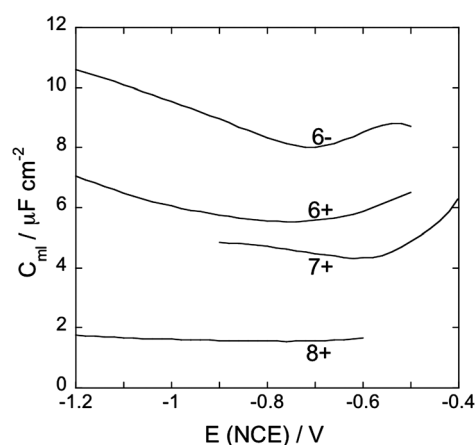
Fig. 2 Curves of the differential capacitance C against the applied potential E for Hg-supported 6−, 6+, 7+ and 8+ SAMs in aqueous 0.1 M KCl, over the potential ranges in which they were stabilized.

In a previous work[14] we estimated the charge density q experienced by the diffuse-layer ions at Hg-supported 3+, 3−, 5+, and 5− SAMs on the basis of the Gouy–Chapman theory, upon measuring the diffuse-layer capacitance C_d at different electrolyte concentrations by EIS. However, the monolayer capacitance C_{ml} of the 6+, 6−, 7+ and 8+ SAMs is lower than those of the 3+, 3−, 5+, and 5− SAMs, and tends to obliterate the appreciably higher diffuse-layer capacitance C_d, in series with it. Therefore, we adopted a different strategy, which consists in contracting slightly the SAM-coated mercury drop immersed in aqueous 0.1 M KCl, measuring the charge Δq accompanying this contraction, and dividing Δq by the decrease ΔA in drop area. In view of the very low solubility of Aib peptides, in no case will the adsorbed molecules in excess be desorbed from the mercury drop. Two possible scenarios can be envisaged. If the SAM is not tightly packed, the contraction will increase the number density N of the adsorbed molecules, causing an unwanted increase in q. Conversely, if the SAM is tightly packed, the excess molecules will accumulate at the neck of the mercury drop, leaving the N and q values unaltered. To verify if this was the case, the drop contraction procedure was also applied to the 3+ and 3− SAMs, for which q values had already been estimated using the Gouy–Chapman theory.[14] Differences were found to be within the limits of experimental error. Table 1 summarizes the C_{ml} and q values

Table 1 Parameters for the adsorption of ten thiolated molecules on mercury from aqueous 0.1 M KCl, double-layer parameters on bare mercury in aqueous solutions of a nonspecifically adsorbed electrolyte at the same applied potential, and χ_{ml} values estimated by the drop-expansion method (column a) and by the present procedure (column b). *The last row reports corresponding values on polycrystalline gold*

Thiol	E (NCE) mV	q μC cm^{-2}	C_{ml} μF cm^{-2}	σ_M μC cm^{-2}	C_i μF cm^{-2}	χ^{Hg-S} mV	χ_w mV	χ_{ml} (a) mV	χ_{ml} (b) mV
3+	−750	−4.0	11	−5.47	20	−60	+45	+220	+182.3
3−	−950	−6.0	11	−8.94	17.5	−60	+60	+220	+136.0
5+	−750	−3.0	8.5	−5.47	20	−60	+45	+220	+161.3
5−	−750	−3.0	8.5	−5.47	20	−60	+45	+220	+161.3
6+	−700	−2.7	5.6	−4.51	21.5	−60	+40	+333	+354.0
6−	−800	−2.8	8.3	−6.36	19.5	−60	+60	+105	+99.2
7+	−650	−2.3	4.4	−3.55	23	−60	+20	+530	+434.6
8+	−900	−3.5	1.6	−8.10	18	−60	+60	+1550	+1822
EO3	−750	−3.5	11	−5.47	20	+300	+45	(−214)	−228.0
TP	−1000	−3.0	11	−9.75	17	+300	+60	(−515)	−590.8
On Au 3+	E(NCE) +350	q +1.5	C_{ml} 11	σ_M +11	C_i 48	χ^{Au-S} −60	χ_w −60		χ_{ml} +258

for the different Aib-peptide SAMs, as well as the applied potentials E at which they were determined. The table also reports these quantities for peptides 3+, 3−, 5+ and 5−, as estimated in ref. 14.

3.1 Estimate of χ_{ml} by the drop-expansion method

After determining the q and C_{ml} quantities, the surface dipole potential χ_{ml} of the adsorbed Aib-peptides was estimated from the slope of the $\xi(\theta)$ function in eqn (3) against $\cos^2\theta$. The charge density $q(\theta)$ during the progressive expansion of the SAM-coated mercury drop was obtained by extruding mercury gradually from the capillary by manual advancement of the piston of the mercury reservoir and by recording the charge increment, ΔQ, flowing as a consequence of each piston advancement.[7] Summing the charge increment involved in each piston advancement to the sum of all preceding charge increments yields the charge $Q(\theta)$; $q(\theta)$ is then given by $[Q(\theta) + Aq]/A(\theta)$, where A and q are the drop area and the charge density of the initial unexpanded drop and $A(\theta)$ is the drop area during the gradual drop expansion. The cosine of the tilt angle θ is just given by the $A/A(\theta)$ ratio. The diffuse-layer potential $\Delta\phi_d[c,q(\theta)]$ in the expression of $\xi(\theta)$ was calculated from the Gouy–Chapman theory and makes only a very small contribution to $\xi(\theta)$. Fig. 3 shows $\xi(\theta)$ vs. $\cos^2\theta$ plots for the Hg-supported 6−, 6−, 7+ and 8+ SAMs in 0.1 M KCl. The opposites of the slopes resulting from their linear fits yield the χ_{ml} values summarized in Table 1. Upon considering the limits of experimental errors in the estimate of q and C_{ml} and their propagation in the estimate of the $\xi(\theta)$ function, the surface dipole potentials χ_{ml} for all SAMs are affected by a probable error of 20%.

The monolayer capacitance C_{ml} of the Aib-peptides thiolated at the N-terminus decreases gradually with an increase in chain length while the surface dipole potential χ_{ml} increases, as expected. However, in the 6− < 7+ < 8+ series, χ_{ml} increases with chain length more than linearly. This behavior points to a progressive decrease of the peptide tilt with respect to the normal to the electrode surface, up to the attainment of a particularly high value for 8+. The 3− and 5− peptides are characterized by
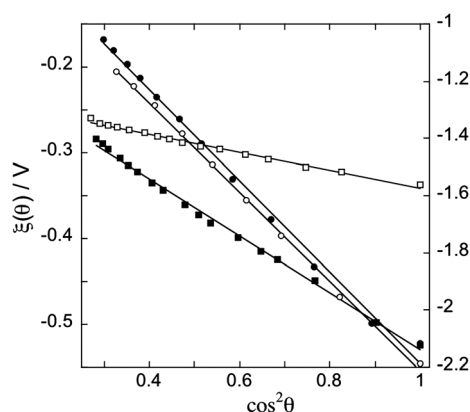
Fig. 3 Plots of $\xi(\theta)$ against $\cos^2\theta$ for Hg-supported SAMs of 6− at −800 mV (open squares), 6+ at −700 mV (solid squares), 7+ at −650 mV (solid circles) and 8+ at −900 mV (open circles) in aqueous 0.1 M KCl. The plots of 6−, 6+ and 7+ refer to the left vertical axis, the plot of 8+ refers to the right one.

essentially the same positive χ_{ml} values as the corresponding peptides 3+ and 5+. This implies that the 3− and 5− peptides have the dipole moment directed toward the metal, namely from the N- to the C-terminus, even though in the free state it is directed in the opposite direction. In a previous work[14] this apparently anomalous behavior was explained by the strong interfacial electric field created by the negative charge density q causing an inversion of the dipole moment of the peptide molecules thiolated at the C-terminus. This reorientation requires the cleavage of the intramolecular H bonds responsible for the 3_{10}-helix and the formation of intermolecular H-bonds with a favorable orientation of the C=O bond dipole moments. The 6− peptide has also a positive dipole potential χ_{ml}, although it is appreciably smaller than that of the corresponding 6+ peptide. It is possible that the increased length makes intermolecular H-bond formation more difficult, leading to a more disordered SAM; this might explain the relatively high C_{ml} value of the 6− SAM (8.3 μF cm^{-2}), as compared with that (5.6 μF cm^{-2}) of the 6+ SAM.

3.2 Estimate of χ_{ml} by equating $\Delta\phi$ for a SAM-coated electrode to that for a bare electrode

Let us now estimate the above χ_{ml} values without having recourse to the drop expansion method, by equating the extra-thermodynamic expression of $\Delta\phi$ for a SAM-coated mercury electrode to that for a bare mercury electrode in a nonspecifically adsorbed electrolyte, at the same applied potential:[1]

$$\Delta\phi = \chi^{Hg} + \delta\chi^{Hg}_{ml} + \chi^{Hg-S} + \chi_{ml} + q/C_{ml} + \Delta\phi_d = \chi^{Hg} + \delta\chi^{Hg}_w + \chi_w + \sigma_M/C_i + \Delta\phi'_d \quad (4)$$

Here, χ^{Hg} is the surface dipole potential at the interface between mercury and vacuum, whereas $\delta\chi^{Hg}_{ml}$ and $\delta\chi^{Hg}_w$ express its change when vacuum is replaced by the peptide SAM and by the water molecules in direct contact with bare mercury, respectively. In the third member of this equation, which refers to bare mercury, χ_w is the surface dipole potential of the water molecules adjacent to the mercury surface, σ_M is the thermodynamic charge density on mercury, and C_i is the capacitance of the inner layer. $\Delta\phi'_d$ is the potential difference across the diffuse layer, which is calculated by the Gouy–Chapman theory at the charge density σ_M and for a 0.1 M concentration of a nonspecifically adsorbed 1,1-valent electrolyte. The two quantities $\delta\chi^{Hg}_{ml}$ and $\delta\chi^{Hg}_w$, which express the perturbation of the metal electron density tail induced by the presence of the adjacent monolayer, independent of its being physi- or chemisorbed, can be regarded as approximately equal to a good approximation.[1,3] Eqn (4) can, therefore, be written in the form:

$$\chi_{ml} = \chi_w + \sigma_M/C_i + \Delta\phi'_d - \chi^{Hg-S} - q/C_{ml} - \Delta\phi_d \quad (5)$$

Values of σ_M and C_i at all applied potentials of interest can be extracted from Grahame's accurate differential capacitance measurements at the interface between mercury and aqueous solutions of the nonspecifically adsorbed salt NaF.[5] Tabulated values of σ_M at different NaF concentrations, obtained by interpolation and extrapolation from Grahame's data, were reported by Russell.[16] The σ_M values reported in Table 1 were

obtained at the appropriate applied potentials by interpolation from Russell's tabulated values for 0.1 M NaF. The C_i values were obtained from the C_i *vs. E* curve for the Hg–aqueous NaF interface in ref. 5, Fig. 1. The χ_w values reported in Table 1 were obtained from Fig. 4 in ref. 17, upon ascribing to the extra-thermodynamic value of χ_w at the potential of zero charge the commonly accepted value of -70 mV.[3]

The only parameter on the right-hand side of eqn (5) that is critical to estimate is the surface dipole potential χ^{Hg-S} stemming from the electron density flow through the atoms involved in the formation of the Hg–S bond. In a recent work[1] we estimated the surface dipole potential, χ^{M-S}, of the metal–sulfur bond of SAMs of different thiolated and sulfidated molecules supported by Hg, Au and Ag, on the basis of extrathermodynamic considerations. The χ^{M-S} values were found to depend much more on the direction of the dipole moment normal component of the adsorbed molecules than on their nature or on that of the metal support. Thus, χ^{M-S} varies from $+300$ to $+450$ mV when passing from a Hg- to a Au-supported SAM of the same sulfidated polyethyleneoxy chain with the dipole moment pointing toward the aqueous solution. Moreover, the χ^{M-S} values for Hg-supported SAMs of a sulfidated polyethyleneoxy chain and of the four Aib-peptides 3+, 3−, 5+, and 5−, all having the dipole moment normal component directed toward the bulk metal, range from -40 to -60 mV, in spite of their different chemical nature. Therefore, as a rough approximation, we may set $\chi^{M-S} \approx -60$ mV for adsorbates pointing their dipole moment toward the bulk metal and $\approx +400 \pm 50$ mV for adsorbates pointing their dipole moment toward the solution, provided a reasonable guess can be made about the direction of the adsorbate dipole.

Table 1 summarizes the q, C_{ml}, σ_M, C_i, and χ_w values at the appropriate applied potentials E for all Aib-peptides, the χ_{ml} values estimated by the expanding procedure (column *a*) as well as the χ_{ml} values calculated from eqn (5) by setting $\chi^{Hg-S} = -60$ mV (column *b*), in consideration of the fact that their dipole moments point toward the metal. The latter χ_{ml} values are in fairly good agreement with those estimated by the drop expansion method. These results prompted us to apply this procedure to two Hg-supported SAMs for which q and C_{ml} values had been estimated in previous works. These adsorbates, whose q and C_{ml} values are reported in Table 1 together with the corresponding applied potentials, are triethyleneoxythiol (EO3)[18] and HS(CH$_2$)$_2$[Aib-Glu(OTeg)]$_2$-Aib-Ala-OH (TP),[19] where Teg stands for triethylene glycol monomethyl ether. The resulting χ_{ml} values, reported in Table 1, are in fairly good agreement with those obtained by an approximate expression of the potential difference across the electrified interface that ignores the Hg–S bond and is given by (see eqn (6) in ref. 1 and eqn (4)):

$$\Delta\phi'' = \Delta\phi - \chi^{Hg} - \delta\chi^{Hg}_{ml} - \chi^{Hg-S} \approx E(\text{V/SCE}) + 0.210 \text{ V} = \chi_{ml} + q/C_{ml} + \Delta\phi_d \quad (6)$$

The χ_{ml} values between round brackets in column (*a*) were calculated by this equation.

3.3 Application to a SAM on a gold electrode

The use of eqn (5) for the estimate of χ_{ml} can also be extended to solid-supported SAMs, provided a reliable value can be ascribed

to the χ^{M-S} surface dipole potential. This, in turn, requires a reasonable guess about the direction of the dipole moment of the adsorbed molecules of the SAM. In the case of a gold bead coated with a 3+ SAM, it is quite reasonable to assume that the normal component of the peptide dipole, $\mu_\perp$, is directed toward the metal. The dipole potential χ^{Au-S} was, therefore, set equal to that, -60 mV, used for χ^{Hg-S}. The charge q experienced by the diffuse layer ions on the Au-supported 3+ SAM at $+0.350$ V was determined by measuring the diffuse layer capacitance C_d by EIS at KCl concentrations, c, increasing from 0.002 to 0.032 M by 0.2 logarithmic steps. To avoid considering the surface roughness of the gold bead, its effective surface area was estimated by making the rough assumption that the monolayer capacitance C_{ml} of the 3+ SAM on Au is equal to that, 11 μF cm^{-2}, on Hg. The q value, obtained by inserting pairs of corresponding values of C_d and c into eqn (2), amounts to $+1.5$ μC cm^{-2}. The C_i and σ_M values on bare gold in aqueous NaF were obtained from Fig. 2 and 4 of ref. 20 and amount to about 48 μF cm^{-2} and $+11$ μC cm^{-2}, respectively. Finally, the surface dipole potential, χ_w, of the water molecules on bare gold at the high charge density of $+11$ μC cm^{-2} was assumed, as a rough approximation, to be equal in magnitude and opposite in sign to the limiting positive χ_w value on bare mercury, which is approximately equal to $+60$ mV.[3] All the above parameters are summarized in the last row of Table 1. With these parameters and the $\Delta\phi_d$ and $\Delta\phi'_d$ values calculated by the Gouy–Chapman theory for $c = 0.1$ M and for the q and σ_M values, respectively, a dipole potential $\chi_{ml} = +258$ mV for the 3+ monolayer on gold was estimated from eqn (5). This value is relatively close to that estimated on a Hg support.

In spite of the rough assumptions made in the latter estimate, this example shows that the present procedure can be applied to solid-supported SAMs if one can make a reasonable guess about the orientation of the dipole moment of the adsorbed molecules and, hence, about the value of the χ^{M-S} dipole potential. The other way around, if one can assume that the surface dipole potential χ_{ml} of a given SAM, as estimated on Hg by the drop expansion method, is approximately equal to that on a metal M of interest, such as Au or Ag, eqn (5) can be used to estimate the corresponding χ^{M-S} value.

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