

Monitoring the Interfacial Capacitance at Self-Assembled Phosphate Monolayers on Gold Electrodes upon Interaction with Calcium and Magnesium

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Electrochemical impedance spectroscopy has been used to evaluate the change in interfacial capacitance upon calcium and magnesium coordination to a phosphate-modified electrode. The phosphate electrode was prepared via immobilization of phosphorylated, thiol-containing, serine analogues onto gold. Upon subjection to calcium and magnesium, a substantial drop in capacitance was observed. Magnesium displayed the largest influence on the capacitance: a 27% capacitance drop was observed upon introduction of a 1 mM solution of magnesium ions. The lowered capacitance is a result of a change in the potential and charge distribution at the film/electrolyte interface as the cations coordinate to the phosphate groups. Moreover, the relationship between electrode potential and capacitance has been investigated and reveals a significant difference between monovalent and divalent cations. As complementary information, infrared reflection absorption spectra of the phosphorylated monolayer having different counterions are presented. The results reported in this paper indicate that the phosphorylated amino acid analogue monolayers could be used in investigations of the biochemically important coordination of calcium and magnesium to phosphates and phosphorylated amino acids.

Both calcium and magnesium play very important roles in biological systems. Calcium, for example, is involved in a range of processes occurring at and across cell membranes as well as between cells,^{1–5} while magnesium is important in hormone–

receptor binding⁶ and the classical role in defining the properties of adenosine triphosphate.⁷ These cations are today mostly monitored by using photometry and chromogenic reagents,^{8–10} by ion-selective electrodes, or by atomic absorption spectroscopy.^{6,10} Concerning electrochemistry, ion-selective electrodes^{8–11} are an attractive approach, because they leave the system almost undisturbed and they do not buffer or consume a significant amount of ions. The calcium ion-selective electrodes available today, for example, are constructed from an ordinary glass electrode modified with a PVC gel containing Ca^{2+} ligands.⁸ The disadvantage is that they are difficult to construct reproducibly and have short lifetimes.

Since the first report of self-assembled monolayers (SAM) on gold by Nuzzo and Allara,¹² several examples of SAM-modified gold electrodes have been used in combination with electrochemical techniques for the study of various analytes.¹³ For example, Turyan and Mandler¹⁴ employed voltammetry together with an electrode modified with a 4-(mercaptoethyl)pyridinium monolayer to detect the presence of Cr(VI). The assembly of a molecular film like a SAM at an electrode surface influences the interfacial capacitance, because it appears as a dielectric medium between the electrode and the electrolyte.¹⁵ The capacitance is determined mainly by the thickness and dielectric properties of the molecular film. Functional groups at the ω end of the monolayer, for

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- (1) Takeichi, M. *Annu. Rev. Biochem.* **1990**, *59*, 237–252.
- (2) Geiger, B.; Ayalon, O. *Annu. Rev. Cell Biol.* **1992**, *8*, 307–332.
- (3) Heilbrunn, L. V.; Wiercinski, F. J. *J. Cell. Comput. Physiol.* **1947**, *29*, 15–32.
- (4) Augustine, G. J.; Charlton, M. P.; Smith, S. J. *Annu. Rev. Neurosci.* **1987**, *10*, 633–693.
- (5) Murray, J. M.; Weber, A. *Sci. Am.* **1974**, *230*, 59–71.

- (6) Fawcett, W. J.; Haxby, E. J.; Male, D. A. *Br. J. Anaesth.* **1999**, *83*, 302–320.
- (7) Aikawa, J. K. *Magnesium: Its Biological Significance*; CRC Press: Boca Raton, 1981.
- (8) Petersen, O. H. *Measuring calcium and calmodulin inside and outside cells*; Springer-Verlag: Berlin, 2001.
- (9) Lindfors, T.; Ivaska, A. *Anal. Chim. Acta* **2001**, *437*, 171–182.
- (10) Saris, N. E. L.; Mervaala, E.; Karppanen, E.; Khawaja, J. A.; Lewenstam, A. *Clin. Chim. Acta* **2000**, *294*, 1–26.
- (11) Altura, B. M. *Scand. J. Clin. Lab. Invest.* **1994**, *54*, 5–9.
- (12) Nuzzo, R. G.; Allara, D. L. *J. Am. Chem. Soc.* **1983**, *105*, 4481–4483.
- (13) Flink, S.; van Veggel, F.; Reinhoudt, D. N. *Adv. Mater.* **2000**, *12*, 1315–1328.
- (14) Turyan, I.; Mandler, D. *Anal. Chem.* **1997**, *69*, 894–897.
- (15) Finklea, H. Q. In *Electroanalytical Chemistry*; Bard, A. J., Rubenstein, I., Eds.; Marcel Dekker Inc.: New York, 1996; Vol. 19.

example, receptors, acid/base, or redox functionalities, also contribute to the capacitance, because they, together with the diffuse layer and inner part of the double layer, influence the potential and charge distribution at the electrode/solution interface.^{15–22} The interfacial capacitance is normally evaluated employing electrochemical impedance spectroscopy²³ (EIS). Reports exist in the literature that this technique has been used for the detection of metals, such as copper and nickel,²⁴ and potassium^{25,26} using various SAMs. However, to our knowledge, monolayers modified for the purpose of calcium and magnesium detection by EIS are few, although Terrettaz et al.²⁷ have used the assembly of the Ca²⁺ ligand ETH 1001 on derivatized silicon electrodes as a base for a calcium sensor.

We have previously described impedance measurements on SAMs of hydroxyl- and phosphate-terminated amino acid analogue thiols assembled on gold electrodes.²⁸ The finding was that an increased capacitance was observed as phosphates was introduced. This fact can be explained by the introduction of more charged and polar groups in the ω end of the monolayer. On the basis of these results and the well-known fact that calcium and magnesium can coordinate to organic phosphates, the aim of the present paper is to employ EIS for evaluation of the interfacial capacitance as calcium and magnesium coordinate to a phosphate modified monolayer.

EXPERIMENTAL SECTION

Assembly. Substrates used for assembly were gold-coated (2000 Å) glass plates. Prior to assembly, the gold substrates were cleaned in TL1, a mixture of water, 30% hydrogen peroxide, and 25% ammonia (Merck) (5:1:1), at 80 °C for 10 min. The monolayers were prepared from ethanol (Kemetyl, Haninge, Sweden) solutions, having a thiol concentration of 0.5 mM, by placing the cleaned gold surface in the solution for at least 15 h. The substrates were removed from the solution, gently rinsed in ethanol followed by a 5-min ultrasonication in ethanol. Prior to use in experiments, the surfaces were dried under a stream of nitrogen.

Infrared Spectroscopy. Reflection–absorption (RA) spectra were recorded on a Bruker IFS66 Fourier transform spectrometer equipped with a grazing angle of incidence reflection accessory aligned at 85°. A liquid-nitrogen-cooled MCT detector was used. Interferograms were apodized using a three-term Blackmann–Harris function before transformation. The spectra were recorded by averaging 3000 interferograms (10 min) at 2 cm^{−1} resolution.

Ellipsometry. Single-wavelength ellipsometry was performed on a Rudolph AutoEL ellipsometer. The light source was a He–Ne laser with wavelength 632.8 nm at an angle of incidence of 70°. The fresh gold plates were measured prior to incubation with thiol to obtain reference values. As a model, ambient/organic film/gold, assuming an isotropic transparent organic layer having $n = 1.5$, was used. The thickness was calculated by the Rudolph AutoEL ellipsometer software as an average value of three individual spots on each surface.

Electrochemistry. The impedance data and cyclic voltammograms were recorded using an Autolab PGSTAT 20 (EcoChemie, Utrecht, The Netherlands), equipped with a FRA2 frequency response analyzer module. All experiments were performed in the three-electrode mode using a Ag/AgCl reference electrode and a platinum wire as counter electrode. The SAM-coated gold surfaces (working electrodes) were brought into contact with the solution via pressfitting to an O-ring in the side of the Kel-F-based electrochemical cell.²⁹ The exposed area of the electrode was 0.2 cm². The impedance data were recorded in a solution of 100 mM NaCl (Merck) or 100 mM KNO₃ (Merck) at 21 logarithmically distributed frequencies in the range 200 Hz to 2 kHz. The amplitude was 5 mV, and the DC electrode potential was 0.0 V (vs Ag/AgCl). The interfacial capacitances were obtained by fitting the impedance data to an equivalent RC circuit, where R represents the solution resistance in series with C, the total capacitance. The capacitance was evaluated with the constant-phase element (CPE) technique,²³ using the FRA 2.3 software (EcoChemie). Cyclic voltammograms were recorded by scanning the potential from −0.30 (vs Ag/AgCl) to +0.60 V and back, employing a scan rate of 10 mV/s. Prior to the scan, the electrode was kept at −0.30 V for 10 s. To provide redox species, a 1.0 mM K₃Fe(III)(CN)₆ (Merck) solution also containing 100 mM KNO₃ as supporting electrolyte was used.

Calcium and Magnesium Additions. Prior to all measurements, the electrodes were subjected to 1 mM EDTA (Merck) in 100 mM sodium chloride in order to remove any coordinated calcium and magnesium (impurities). Additions of calcium or magnesium chloride (Merck and Sigma, respectively) were made to an electrolyte solution of 30 mL of 100 mM sodium chloride in the cell, followed by a 5-s manual stirring. Appropriate volumes of 30 mM calcium or magnesium chloride dissolved in 100 mM sodium chloride were added.

RESULTS AND DISCUSSION

The phosphate-containing thiols used for assembly of the SAMs are depicted in Figure 1. The molecules (1a and 1b) are based on 3-mercaptopropionic acid. The characterization of such SAMs by IR-spectroscopy, X-ray photoelectron spectroscopy, contact angle, and ellipsometry will be described elsewhere.³⁰ We have previously reported characterization and electrochemical evaluation²⁸ of assemblies on the basis of similar analogues synthesized from 16-mercaptohexadecanoic acid (Figure 1c). From the latter work, it was concluded that changing the polarity or the charge distribution at the terminus of the monolayers by introducing phosphate groups influenced the interfacial capaci-

(16) Andreu, R.; Fawcett, W. R. *J. Phys. Chem.* **1994**, *98*, 12753–12758.

(17) Andreu, R.; Calvente, J. J.; Fawcett, W. R.; Molero, M. *Langmuir* **1997**, *13*, 5189–5196.

(18) Andreu, R.; Calvente, J. J.; Fawcett, W. R.; Molero, M. *J. Phys. Chem. B* **1997**, *101*, 2884–2894.

(19) Fawcett, W. R.; Fedurco, M.; Kovacova, Z. *Langmuir* **1994**, *10*, 2403–2408.

(20) Gao, X.; White, H. S. *J. Electroanal. Chem.* **1995**, *389*, 13–19.

(21) Smith, C. P.; White, H. S. *Langmuir* **1993**, *9*, 1–3.

(22) White, H. S.; Peterson, J. D.; Cui, Q.; Stevenson, K. J. *J. Phys. Chem.* **1998**, *102*, 2930–2934.

(23) Macdonald, J. R. *Impedance Spectroscopy*; Wiley: New York, 1987.

(24) Stora, T.; Hovius, R.; Dienes, Z.; Pachoud, M.; Vogel, H. *Langmuir* **1997**, *13*, 5211–5214.

(25) Bandyopadhyay, K.; Shu, L. H.; Liu, H. Y.; Echegoyen, L. *Langmuir* **2000**, *16*, 2706–2714.

(26) Terrettaz, S.; Vogel, H.; Grätzel, M. *Langmuir* **1998**, *14*, 2573–2576.

(27) Terrettaz, S.; Vogel, H.; Grätzel, M. *J. Electroanal. Chem.* **1992**, *326*, 161–176.

(28) Ekeröth, J.; Björefors, F.; Borgh, A.; Lundström, I.; Liedberg, B.; Konradsson, P. *Anal. Chem.* **2001**, *73*, 4463–4468.

(29) Yang, Z. P.; Engquist, I.; Liedberg, B.; Kauffmann, J. M. *J. Electroanal. Chem.* **1997**, *430*, 189–195.

(30) Ekeröth, J.; Borgh, A.; Uvdal, K.; Liedberg, B.; Konradsson, P. *J. Colloid Interface Sci.* Submitted.

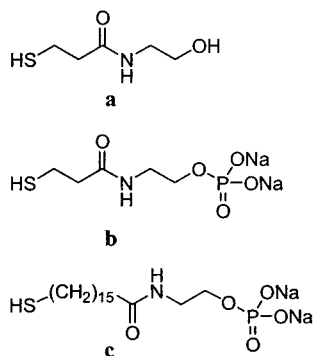


Figure 1. Functionalized thiols used to form the monolayers: (a) alcohol-terminated thiol, (b) phosphate-terminated thiol, and (c) phosphate-terminated thiol synthesized from 16-mercaptohexadecanoic acid.

tance. The aim of this work was to use EIS to demonstrate the influence on the capacitance from calcium and magnesium ion coordination to phosphate modified surfaces. Several factors influence the potential and charge distribution and, hence, the interfacial capacitance at monolayer modified electrodes.^{15–22} The polarity and charge of functional groups together with the length and insulating properties of the alkane chains are most important. Penetration of electrolyte ions and solvent molecules into the monolayer increases the capacitance, and it is expected to occur for monolayers based on short alkane thiols. About 10 methylene units are necessary to form a dense assembly and to prevent ion and solvent penetration. In addition, defects in the gold substrate or adsorbed impurities can cause pinholes and defects in the monolayer that have negative effects on the insulating properties. The structuring of a thin layer of water molecules close to the monolayer also has an effect on the interfacial capacitance.^{16,18,19} Because of the monolayer proximity, this water layer has a lower dielectric constant, as compared to bulk water. Moreover, ions in the diffuse part of the double layer influence the total capacitance, but this contribution is normally negligible at high supporting electrolyte concentrations. Evaluation of the contribution from each of these factors, for example, by using a more complex equivalent circuit to fit the impedance data, is difficult, since they more or less influence each other. It is also not obvious if the factors should be treated in series or in parallel to each other. The simple series RC circuit was, therefore, used because the Bode and impedance plots for the monolayers of the analogues in Figure 1 did not indicate that a more advanced equivalent circuit was necessary, regardless if calcium or magnesium was present in the solution or not.

A Bode plot, i.e., the logarithm of the total impedance and phase angle as a function of the logarithm of the frequency (1 Hz to 10 kHz), for the phosphate-modified monolayer (Figure 1b) is presented in Figure 2a. As reported before,²⁸ the best correlation of the impedance data to the equivalent circuit was obtained when using frequencies from 200 Hz to 2 kHz. At frequencies above 5 kHz, a nonrandom deviation was observed (probably involving stray capacitances from the system), and large residuals were present at frequencies below 50 Hz. Figure 2b represents the impedance plot, that is, the imaginary impedance as a function of the real impedance for the phosphate-modified monolayer, using frequencies from 200 Hz to 2 kHz. A fit to the impedance data in

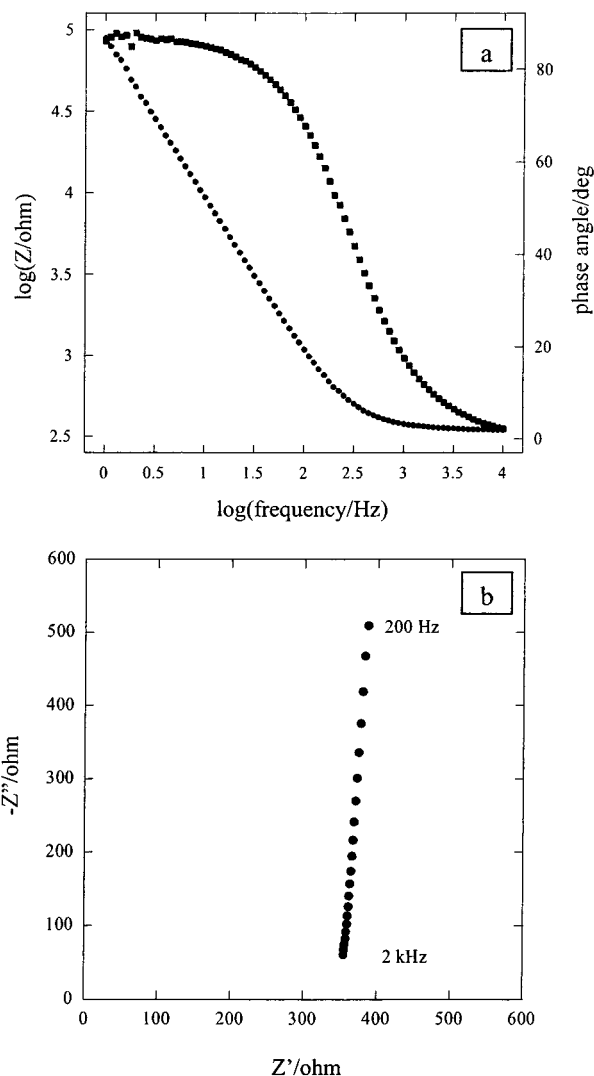


Figure 2. (a) Bode plot and (b) impedance plot for the phosphate-terminated monolayer in Figure 1b in 100 mM NaCl. In the Bode plot, dots represent the total impedance, and squares represent the phase angle.

Figure 2b did not result in a vertical line, which is expected for a series RC circuit when the capacitor is ideal. As a consequence, a constant-phase element (CPE) was employed during the data evaluation. The CPE is based on the term $(\omega C)^\alpha$, which is believed to account for nonideal behavior, for example, surface roughness.²³ The value of the exponent α in the present experiments was from 0.93 to 0.95. Omitting the CPE resulted in a substantially worse correlation of the fit.

Phosphate-Terminated Monolayers. Table 1 summarizes the measured interfacial capacitance for the monolayers of the substances in Figure 1 when 1.0 mM Ca^{2+} or Mg^{2+} is added to a solution containing 100 mM NaCl. The ellipsometric thicknesses of the three different monolayers are also presented. As can be seen, the thickness of the propionic acid based phosphate is somewhat higher than expected, most likely due to a hydration layer surrounding the phosphates. To estimate the uncertainty in the capacitance for each of the three monolayers, the 95% confidence interval was calculated on the basis of the data obtained in the 100 mM NaCl solution (and for the thinner phosphate monolayer also in the presence of 1 mM Ca^{2+} or Mg^{2+}). As will

Table 1. Ellipsometric Thickness and Capacitance Values for the Monolayers in Figure 1 in the Absence and Presence of Calcium and Magnesium^a

monolayer	ellipsometric thickness (Å) ^c	100 mM KNO ₃ (μF cm ⁻²)	100 mM NaCl (μF cm ⁻²)	1.0 mM CaCl ₂ ^d (μF cm ⁻²)	1.0 mM MgCl ₂ ^d (μF cm ⁻²)
-OPO ₃ ²⁻	17.7	6.52	6.58 ± 0.09 (4)	5.59 ± 0.05 (7)	4.90 ± 0.09 (5)
-OH	9.0	7.93	7.82 ± 0.07 (4)	7.88	7.86
-OPO ₃ ²⁻ ^b	23.7	2.22	2.19 ± 0.09 (5)	2.17	2.10

^a Where the confidence interval is reported, the number in parentheses represents the number of separate substrates; the other data in the table represent the average from at least two substrates. ^b Synthesized from 16-mercaptohexadecanoic acid. ^c Estimated uncertainty, ±1 Å. ^d 100 mM NaCl as supporting electrolyte.

be discussed below, the capacitance decreases significantly as Ca²⁺ or Mg²⁺ coordinate to the phosphate, derived from mercaptopropionic acid (Figure 1b). Furthermore, the choice of cation (Na⁺ or K⁺) or anion (Cl⁻ or NO₃⁻) in the electrolyte does not significantly influence the results. As a control for the changes in capacitance of the phosphate-modified surface, the corresponding alcohol was subjected to the same changes in calcium and magnesium concentration. No significant change in the capacitance due to calcium or magnesium additions was found in this case. Neither was it possible to detect any significant response toward the addition of calcium or magnesium for the serine analogue based on 16-mercaptohexadecanoic acid (Figure 1c). The absence of the phosphate functionality or extension of the carbon chain clearly removes the cation coordination effect on the capacitance.

Calcium and magnesium have the correct charge and size to effectively be coordinated to the phosphate. Thus, the rules of correct size and charge are fulfilled.³¹ This is reflected by the very low solubility products for calcium (2×10^{-33}) and magnesium (1×10^{-24}) phosphate. The parallel between inorganic phosphates and organic phosphomonoesters is not obvious, but for phosphomonoesters, at least part of the coordination ability to calcium and magnesium is retained. In this case, however, magnesium is the stronger phosphate coordinator, as indicated by the results. For monovalent ions, the situation is different. The ions cannot fit into an appropriate lattice, which makes the ionic compound less stable. Generally, common monovalent ions, such as sodium and potassium, have few insoluble salts. The monovalent cations maintain the charge neutrality in the form of loosely coordinated ion pairs. The most reasonable explanation for the observed capacitance drop of the phosphate molecular films upon introduction of calcium or magnesium is a stronger neutralization of the phosphate charges. This is a consequence of the coordination ability of these ions with the phosphate. Several publications^{18,32-36} in the literature report on the influence of electrolyte counterions on the electric double layer with respect to SAMs modified with redox-active groups. A second probable effect of more strongly coordinated counterions is a decrease in the number of ions penetrating into the monolayer. In comparison to the less strongly coordinated monovalent ions, which can penetrate further into

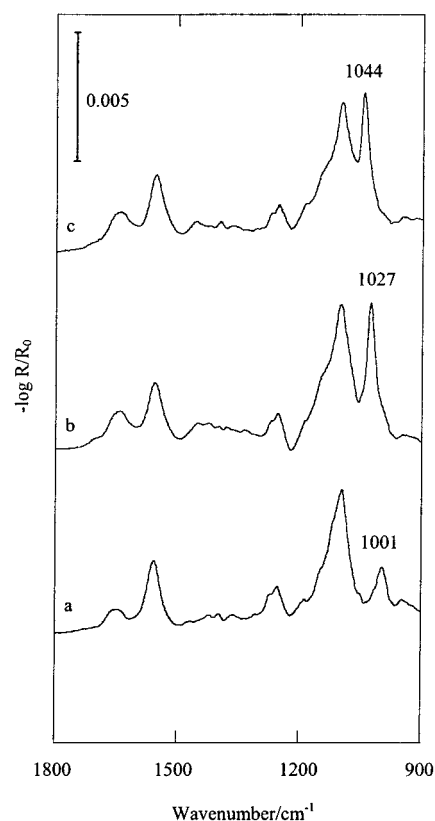


Figure 3. IR spectra for the phosphate-terminated monolayer in Figure 1b with (a) sodium, (b) calcium, and (c) magnesium as counterion.

the monolayer while still maintaining the charge neutrality, this will also contribute to a lowered interfacial capacitance.

The effect of counterion exchange can also be seen by infrared spectroscopy (Figure 3). A significant and sharp absorption is seen in the 1000–1050 cm⁻¹ region as calcium or magnesium is introduced. This stretch is ascribed to the P–O stretch as the phosphate coordinates different counterions. The weak peak at 1001 cm⁻¹ for sodium is enhanced and shifted to 1027 cm⁻¹ for calcium and to 1044 cm⁻¹ for magnesium. Magnesium, in comparison to calcium, has a stronger coordination to organic phosphates, which causes the bond order between phosphorus and oxygen to increase, and the vibration of the bond shifts toward higher energy. It is also likely that the structure of the monolayer changes upon stronger coordination of counterions. The molecules could tilt relative to the surface in order to accommodate calcium and magnesium. The change in intensity

(31) Wulfsberg, G. *Principles of Descriptive Inorganic Chemistry*; University Science Books: Mill Valley, 1991.

(32) Redepenning, J.; Flood, J. M. *Langmuir* **1996**, *12*, 508–512.

(33) Gobi, K. V.; Ohsaka, T. *J. Electroanal. Chem.* **2000**, *485*, 61–70.

(34) Creager, S. E.; Rowe, G. K. *J. Electroanal. Chem.* **1997**, *420*, 291–299.

(35) Rowe, G. K.; Creager, S. E. *Langmuir* **1991**, *7*, 2307–2312.

(36) Smith, C. P.; White, H. S. *Anal. Chem.* **1992**, *64*, 2398–2405.

in the stretch at $\sim 1650\text{ cm}^{-1}$, for example, could be interpreted as a change in tilt angle of the molecules, according to the surface selection rule.³⁷

Alcohol-Terminated Monolayer. The penetration of ions into the monolayer is most likely the explanation for the relatively high capacitance value obtained for the alcohol-terminated monolayer (Table 1). Although this monolayer is expected to have a higher capacitance as compared to the phosphate monolayer because it is thinner, a direct comparison is difficult, because the larger polarity and charges of the phosphate groups will increase the capacitance for the phosphate monolayer. Penetration of electrolyte ions into the thinner alcohol monolayer is, on the other hand, more likely, because it is uncharged and less insulating. However, no significant change in capacitance was observed when calcium or magnesium was added, because these cations do not change the charge or polarity of the alcohol groups. This result indicates that the decrease in capacitance observed for the phosphate monolayer in Figure 1b is mainly due to the calcium/magnesium phosphate coordination.

Phosphate-Terminated Monolayer with Extended Carbon Chain. The change in capacitance upon coordination of calcium or magnesium decreases when the interaction takes place further away from the gold electrode, that is, by using a longer carbon chain in the thiol forming the monolayer. The influence on the capacitance from penetration of ions or solvent molecules into the monolayer is also much less pronounced because of the long and hydrophobic carbon chains. When a phosphate-modified monolayer based on a serine analogue thiol containing 15 methylene units (Figure 1c) was used, no significant change in capacitance was observed when calcium or magnesium was added to the solution. This is probably explained by the fact that the phosphate part of the monolayer acts as a relatively large capacitance in series with a capacitance corresponding to the long and hydrophobic carbon chains.³⁸ The influence from the phosphates is then negligible, because the total capacitance is dominated by the smaller capacitance represented by the carbon chains (capacitances in series are added reciprocally). This is also in line with previous results²⁸ where removal of protective groups, generating two charges at each phosphate group, was shown to have little effect on the total capacitance for a phosphate-terminated monolayer based on the thiol in Figure 1c.

Electrode Potential and Insulating Properties. The electrode potential influences the capacitance for monolayers with acid/base or redox functionalities.^{15–17,19,21,22} The electrode potential also affects the presence and penetration of electrolyte ions and solvent molecules in monolayers with poor insulating properties. From Figure 4, it is concluded that the capacitance for the monolayer of the shorter phosphate (Figure 1b) in only 100 mM NaCl is dependent on the electrode potential in the range -0.30 to 0.60 V. These potential limits were chosen in order to avoid faradaic reactions from dissolved oxygen and oxidation/reduction of the thiols. Because the phosphates have no acid/base- or redox-properties, and because the contribution from the diffuse layer can be neglected, this result is probably due to the potential-driven penetration of electrolyte ions into the monolayer. Increasing the potential up to 0.60 V favors the presence of anions in the

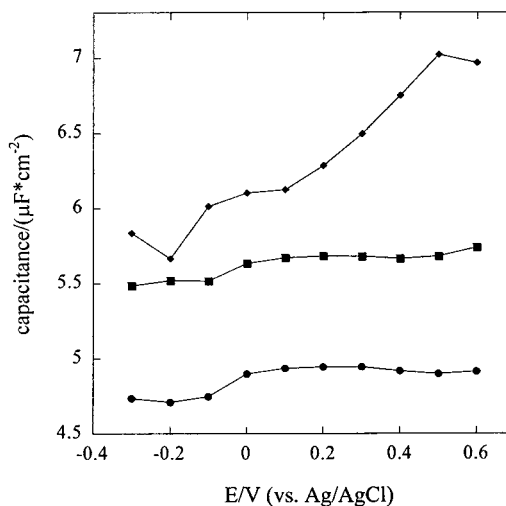


Figure 4. Capacitance as a function of the electrode potential (vs Ag/AgCl) for the phosphate-terminated monolayer in Figure 1b in 100 mM NaCl (◆), 1.0 mM Ca^{2+} and 100 mM NaCl (■), and 1.0 mM Mg^{2+} and 100 mM NaCl (●).

monolayer, and at more cathodic potentials, cations can penetrate into the monolayer and cause an increase in the capacitance. A minimum is present at ca -0.2 V. Because this monolayer was not treated with EDTA to remove impurities before use, the capacitance values are lower than expected from Table 1. The capacitance for the less insulating alcohol-terminated monolayer was even more influenced by the electrode potential (data not shown). A minimum in the capacitance was in this case detected at $+0.2$ V. The electrode potential may also influence the weak coordination of sodium ions to the phosphates and the water layer outside the monolayer, but these factors are probably less important when compared to the penetration of ions.

The influence from the electrode potential when calcium or magnesium ions are present in the solution was less pronounced (see Figure 4). This indicates that fewer ions are present in the monolayer, as discussed above, and that the coordination of calcium or magnesium is not to a large extent influenced by the electrode potential between -0.30 – 0.60 V.

The poor insulating properties were also demonstrated with cyclic voltammetry using $1.0\text{ mM Fe(III)(CN)}_6^{3-}$ as the redox species (Figure 5). For the alcohol-terminated monolayer, the reduction peak potential in the voltammogram was, for example, shifted only 5 mV more negative, as compared to the reduction peak potential at a naked gold electrode. The small influence of the alcohol monolayer on the redox reactions was also obvious, because the peak current for the reduction was reduced by only 14% . For the thinner phosphate-modified monolayer, the reduction peak potential was shifted 300 mV more negative as compared to an unmodified gold electrode. This large shift is mainly due to the influence from the negatively charged phosphate groups and only to a small extent to the better insulating properties. The large shift in peak potential makes a direct comparison of the peak currents unfair. However, the magnitude of the faradaic current for the phosphate-modified monolayer reveals that the blocking properties are poor. With these poor insulating properties, it is difficult to obtain information on pinholes and defects in the monolayers from CV experiments. The more pronounced penetration of ions for the alcohol monolayer was also obvious when

(37) Valiokas, R.; Svedhem, S.; Svensson, S. C. T.; Liedberg, B. *Langmuir* **1999**, *15*, 3390–3394.

(38) Swietlow, A.; Skoog, M.; Johansson, G. *Electroanalysis* **1992**, *4*, 921–928.

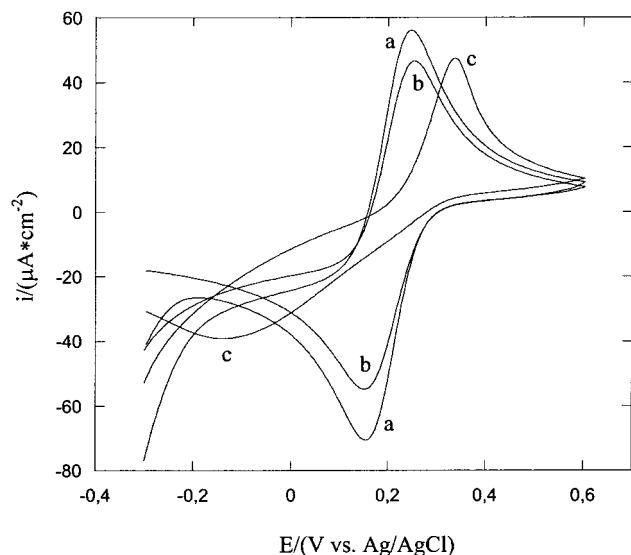


Figure 5. Cyclic voltammograms for (a) a naked gold electrode, (b) the alcohol-terminated monolayer in Figure 1a, and (c) the phosphate-terminated monolayer in Figure 1b, in 1.0 mM $K_3Fe(III)-(CN)_6$ and 100 mM KNO_3 . The scan rate was 10 mV/s.

comparing the charging currents in the voltammograms in the absence of the redox species (data not shown). The poor insulating properties can cause interferences in the evaluation of the interfacial capacitance due to faradaic currents. In real life samples, however, this can be accounted for by introducing a charge-transfer resistance and Warburg impedance into the equivalent circuit used for evaluation of the impedance data. Faradaic reactions can also be accounted for by choosing a detection potential at which such reactions are minimized.

Sensor Aspects. To summarize, electrochemical evaluation of the interfacial capacitance when calcium or magnesium interacts with a phosphate modified monolayer is an interesting possibility. The capacitance is influenced by the change in polarity and the charge of the phosphate groups, and the presence of ions and solvent molecules in the monolayer cannot be neglected. The reproducibility between substrates and the response to changes in the concentration of the analyte are, however, important properties when evaluating a possible sensor application. The reproducibility between individual substrates is very good for the monolayers in Table 1. For example, the 95% confidence interval for the phosphate-modified monolayer in the presence of 1.0 mM Ca^{2+} is $5.59 \pm 0.05 \mu F \cdot cm^{-2}$ ($n = 7$). The main contribution to the uncertainty is probably from variations between the gold substrates. In an application for which the real time change in capacitance on a single substrate is monitored, the uncertainty in the capacitance evaluation is expected to be better.

In the experiment in Figure 6, the capacitance of the phosphate monolayer was measured every 2 min as consecutive additions of calcium (Figure 6a) and magnesium (Figure 6b) were performed in the concentration range 1.0 μM to 2.0 mM. This concentration range more than well covers the interesting biological concentrations. Each data point represents the capacitance evaluated by fitting the impedance data to a series RC circuit using frequencies from 200 Hz to 2 kHz. The stronger coordination of magnesium to phosphates is very pronounced, as indicated by, for example, the larger shift in capacitance at an addition

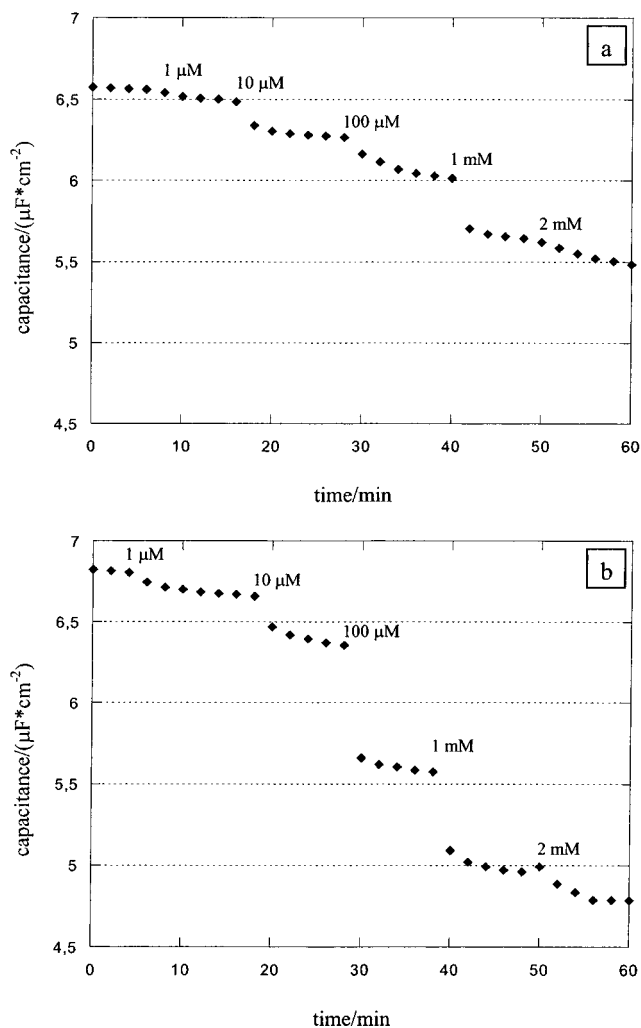


Figure 6. The capacitance of the phosphate-terminated monolayer in Figure 1b at different concentrations of (a) calcium and (b) magnesium in 100 mM NaCl.

corresponding to 100 μM . The decrease in capacitance is almost doubled for magnesium, as compared to calcium, at 2 mM.

A few other aspects also have to be faced. Increased selectivity between calcium and magnesium would be beneficial. In this case, EGTA is an interesting substance, because it predominantly binds calcium. In competition with the phosphates, this predominance could increase the selectivity. Furthermore, the reusability of the surfaces is important. In the experiments in this paper, we have reproducibly used EDTA to remove any coordinated calcium and magnesium. The results indicate a good reusability for the surfaces, and in our tests, we reused them five times with no sign of sensitivity loss. The third main concern is the nonspecific interaction and adsorption to the surface that is encountered in real life samples. One approach to this problem is to use ion-permeable membranes in order to prevent biomolecules from reaching the surface. In this way, real-life samples may be evaluated without the need for preparation.

CONCLUSIONS

We have presented a new approach using phosphorylated molecular film electrodes to detect the presence of and changes in calcium and magnesium concentrations. A substantial drop in

capacitance was experienced by EIS as these ions interact with the molecular film. It was also shown that the interaction must take place close to the electrode surface, for example, by using short alkane thiols. The modified electrodes have good reproducibility and are reusable via EDTA treatment. In addition, the possibility for miniaturization exists, which could provide a means to measure changes in biological systems, perhaps even inside single cells. A problem yet to be solved is the selectivity problem. There are a number of solutions to this, and the most straightforward one is a combination with the presence of EGTA to prevent calcium from interacting with the phosphate surface. We see a great potential in using these surfaces for calcium and magnesium ion concentration measurements. Moreover, the strong interaction between phosphates and counterions in itself

raises many interesting fundamental questions. These layers could also very well be used for following and studying biomineralization processes, such as the formation of soft minerals (e.g., apatite).

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