

Gold electrodes with polyion multilayers and electrostatically bound redox couples

D. A. Lowy and H. O. Finklea*

Department of Chemistry, West Virginia University, Morgantown, WV 26506-6045, U.S.A.

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Abstract—Self-assembled monolayers of alkane thiols with terminal ionized moieties readily adsorb polyions of the opposite charge to form a bilayer structure. A second polyion layer can be adsorbed electrostatically onto the first polyion layer to yield a trilayer. Ellipsometry suggests that the polyion layers have thicknesses comparable to that of the self-assembled monolayer. Each polyion layer can also electrostatically bind a redox couple, although only certain combinations of polyion and redox couple yield a stable redox wave. The reaction of ferroin and other iron complexes onto an anionic surface (monolayer or trilayer) leads to a pH-sensitive surface redox couple believed to be an oligomer of iron hydroxide. A two-terminal pH sensor of the type described by I. Rubinstein [1], and Hickman *et al. Anals Chem* **56**, 1135 (1984). [2] is demonstrated using electrostatically bound redox couples. © 1997 Elsevier Science Ltd. All rights reserved.

Key words: Self-assembled monolayer, polyions, bilayer, trilayer, two-terminal sensor.

INTRODUCTION

In 1984, Rubinstein [1] described a novel voltammetric sensor design in which the reference and sensor redox couples were attached to the same electrode. The formal potential (or peak potential) of the sensing redox centre was measured with respect to the formal potential of the 'built-in' reference redox centre rather than to a conventional SCE or Ag/AgCl reference electrode. Tris(bipyridine)Ru(III/II) adsorbed onto a platinum wire previously dip-coated with Nafion served as the reference (R) coupled. The sensing redox couple (S), chloranil or polyaniline, responded to pH. This system allowed pH measurements to be performed by cyclic voltammetry of a two-terminal system (working and counter electrodes). One advantage of this design is its ease of miniaturization. Similarly, Hickman et al. [2] prepared two-terminal systems in which R was a ferrocenylthiol, HS-(CH₂)_H-Fc, and S a quinone thiol, $HS-(CH_2)_8-Q/QH_2$. The pH of the electrolyte was related to the difference of the anodic peak potentials of the two redox couples. Nernstian response was observed for electrolyte compositions extending from nearly neutral to the very acidic pH

values. The accuracy of pH measurement was compromised by the very broad current peaks of both redox waves and the large peak splittings of the -Q/QH₂ redox couple, which at some pH values resulted in a severe overlap of the two anodic waves. In 1990, Mirkin et al. [3] reported preliminary results on a solid state two-terminal sensor for the detection of carbon monoxide. The sensing site was ferrocenylferrazetine, a complex which showed facile and reversible uptake of CO to form ferrapyrrolinone complexes. Octamethylferrocene, a CO-insensitive compound, was used as the internal reference. Both compounds were imbedded in a solid ionic conducpoly[bis(2-(2-methoxyethoxy)ethoxy)phosphator. zene]. This sensor design was improved by synthesizing a disulfide functionalized ferrocenyl ferraazetine derivative, capable of adsorbing onto Au or Pt by monolayer self-assembly techniques. The chemically inert reference function was ensured by a ferrocene derivative with a mercapto anchor group (11-ferrocenylundecanethiol).

This work was inspired by the manifest advantage of the two-terminal sensor design and by the shortcomings mentioned above. In our approach [4], the sensing and reference redox centres are confined by electrostatic forces in monolayers and multilayers on the gold electrode. The multilayers are also based on ionic binding, in this case, of polyions to a charged substrate. One potential advantage of this design is the flexibility in choosing the redox centres for both the reference and sensing functions, which doesn't require specially synthesized materials.

The construction of complex structures on substrates by electrostatic binding has become popular in recent years [5–39]. These structures are formed by first modifying the entire surface with ions or ionizable compounds, eg thiol-coated metal surfaces, silane-coated or silicon-containing substrates [5, 6]. Multilayers can be attached to this modified surface by consecutively immersing the substrate in solutions of anionic and cationic polyelectrolytes [7–12]. Examples of polyelectrolytes immobilized by these means include protonated poly(allylamine) [13–16], poly(styrene sulfonate) [17], and poly(vinylsulfate) [19].

Many cationic and anionic redox couples are successfully adsorbed into thick (μ m widths) layers of polyelectrolytes. Several examples of this kind of ion-paired systems have been studied by electrochemical techniques [28-39]. Thus, the spontaneous association between the multiply-charged electroactive counterions $[Ru(NH_3)_6^{3+}, Co(NH_3)_6^{3+},$ $Co(2,2'-bipyridine)_{3}^{3+}$, $Os(2,2'-bipyridine)_{3}^{3+}$, Fe(1,10phenanthroline)²⁺ or methyl viologen dications] with poly(acrylate) and/or poly(styrenesulfonate) anions adsorbed onto a pyrolytic graphite electrode surface yielded stable voltammetric behavior [28-33]. Also, multiply charged anions $[Fe(CN)_{b}^{3-4-},$ $Fe(CN)_5(H_{2O})^{2-3-}$, and $Ru(EDTA)H_2O^{-2-}$, where EDTA is ethylenediamine tetraacetate] formed stable complexes with a polyelectrolyte which contained both quaternary and unsubstituted pyridine nitrogen atoms (4,4'-bipyridine molecules attached to the polysiloxane polymer [37] or protonated poly(4vinylpyridine) [38, 39]). In cyclic voltammetry experiments, all the adsorbed redox centres showed a reversible behaviour.

In this work, the innovative principle of the two-terminal voltammetric sensors comes together with the recently introduced layer-by-layer deposition technique. Various redox couples are attached by ionic forces to multilayer systems built on the surface of a polycrystalline gold electrode. Several types of monolayer, bilayer and trilayer systems are examined:

(1) a self-assembled monolayer of 11-mercaptoundecanoic acid (HS–(CH₂)₁₀–COOH) or 4aminomethylpyridinium-11-mercaptoundecanoamide $(Au/S–(CH₂)_{10}–CO–NHCH₂pyH⁺);$

(2) a bilayer based on an anionic monolayer $(Au/S-(CH_2)_{10}-COO^-)$ to which a cationic poly(4-vinyl-methylpyridinium) (PVPMe⁺) layer is attached; (3) a trilayer in which poly(styrenesulfonate) (PSS⁻) is adsorbed to the preceding bilayer;

(4) a bilayer based on a self-assembled cationic monolayer $(Au/S-(CH_2)_{10}-CO-NHCH_2pyH^+)$ to which an anionic polyelectrolyte (PSS^-) is attached;

(5) a trilayer in which a cationic polyelectrolyte (PVPMe⁺) is adsorbed to the preceding bilayer.

Each of the above systems is potentially capable of adsorbing oppositely charged redox couples to the outermost polyelectrolyte layer, although a stably adsorbed redox species appears to require strong ion-pairing forces between the redox species and the charged sites on the polymer. The efficacy of the various adsorption steps is assessed by ellipsometry and cyclic voltammetry. Adsorption of certain iron complexes onto anionic surfaces results in the formation of a not fully characterized iron redox couple attached to the electrode. One example of a pH sensor is demonstrated using the new iron redox species as the sensing site.

EXPERIMENTAL

Reagents

11-Mercapto-undecanoic acid was synthesized according to the procedures described in the literature [40-42] from technical grade 11-bromoundecanoic acid (Aldrich, Milwaukee, WI, U.S.A.), previously recrystallized from *n*-hexane.

Poly(4-vinyl-1-methyl-pyridinium methylsulfate), PVPMe⁺ MeSO₄⁻. The quaternization procedure of poly(4-vinyl-pyridine), henceforth PVP, was adapted from the method described by Friedman and Schlechter [43–45]. Dimethyl sulfate was employed as the methylating agent.

CAUTION: Dimethyl sulfate is a liquid of b.p. 188.5°C and is practically without odor. Both liquid and vapor dimethyl sulfate are highly poisonous, and the substance should be only used in fume hood with a good draught. Also, goggles and rubber gloves must be worn. Inhalation of vapor may lead to giddiness or even more serious problems. The liquid itself is readily adsorbed through the skin, with toxic results. If the liquid is accidentally splashed upon the hands, wash immediately with copious amounts of concentrated ammonia solution in order to hydrolyze the compound before it can be adsorbed through the skin. Then rub the affected area gently with a wad of cotton wool soaked in ammonia solution [46].

A solution of 1.530 g PVP (MW 200,000, Polysciences, Inc., Warrington, PA, U.S.A.) in 50 mL HPLC-grade methanol (HPLC grade, Fisher Scientific, Malvern, PA, U.S.A., dried over 4A molecular sieves, Malinckrodt, Paris, KY, U.S.A.) was refluxed with a 9-fold molar excess (16.6 g) of dimethyl sulfate (99 + %, Aldrich) for 6 hours, and then magnetically stirred at room temperature overnight. A transparent yellow viscous solution was obtained. In order to remove the unreacted dimethyl sulfate, diethyl ether (Reagent A.C.S., Fisher Scientific) was added to the reaction product. Upon adding the ether, the polymer gathered into a highly viscous white mass. The polymer mass was washed repeatedly with diethyl ether and with acetone (HPLC grade, Fisher Scientific). Then the product was dried to a constant weight in the vacuum oven for 48 hours. Being extremely hygroscopic, the product weight slightly exceeded the maximum theoretical yield for 100% quaternization. The quaternized polymer dissolved readily in deionized water at room temperature to give a clear colorless solution. The product composition was checked by NMR spectroscopy using a JEOL-270 spectrometer. The ¹³C NMR spectrum of PVPMe⁺ (in D₂O) showed a peak at 47.87 ppm, which corresponds to the N-methyl group of the quaternized pyridine ring [47]. The 'H NMR of PVPMe⁺ had a broad peak at 4.06 ppm, which can be assigned to the protons of the N-methyl group [48]. In addition, the presence of the *N*-methyl groups caused significant shifts of the PVP adsorption bands in both the ¹H and the ¹³C NMR spectra. The degree of alkylation could not be assessed from the NMR spectra.

Sodium poly(styrene sulfonate) (completely sulfonated, Polysciences, Inc.), the anionic polyion, was used without further purification.

4-Aminomethyl-1-methylpyridinium-11mercapto-undecanoamide, methyl sulfate (HS-UDA-AMPMe⁺MeSO₄). 4-(Aminomethyl)pyridine-11mercaptoundecanoamide (HS-UDA-AMP) was synthesized from 4-(aminomethyl)pyridine (98%, Aldrich) and 11-mercapto-undecanoic acid (prepared in our laboratory, 99%) in acetone (previously degassed with argon) in the presence of dicyclohexylcarbodiimide (99%, Aldrich). The mixture was stirred overnight in anhydrous methylene chloride (HPLC grade, Fisher Scientific) at room temperature. After filtering off the silver-white dicyclohexylurea, the reaction product was purified by liquid chromatography in a column filled with silica gel and an eluant of CHCl₃ and CCl₄ (in the ratio of 2:1, v/v; both solvents were HPLC grade. Fisher Scientific). An orange-yellow, almost odorless reaction product was obtained (yield: 96.1%). FT-IR showed only one N-H stretching band at 3302 ± 2 cm⁻¹ (corresponding to secondary amides) and strong absorptions at 1658 cm⁻¹(C=O stretching, amide I band), and 1538 cm⁻¹(N–H bending, amide II band). The ¹H NMR spectrum, taken in CDCl₃, contained a multiplet at 6.48 ppm (1H) which corresponds to the N-H proton [48]. Next, HS-UDA-AMP was quaternized to HS-UDA-AMPMe⁺MeSO₄⁻⁻ bv refluxing HS-UDA-AMP with dimethyl sulfate in anhydrous methanol for 8 hours. The unreacted Me₂SO₄ was removed by dissolving the reaction product in methanol and evaporating the liquid phase. The pale yellow quaternized reaction product had a m.p. of 32.5-34 C. and was soluble in both methanol and water.

Tris(1,10 - phenanthroline)Co(II) perchlorate, Co(phen) $_{3}^{2+}$, was obtained by the precipitation of CoCl₂.6H₂O (99.8%, Fisher Scientific) with 1,10phenanthroline, 1-hydrate (Reagent ACS, 97%, Eastman Organic Chemicals, Rochester, NY, U.S.A.) in the presence of LiClO₄ (A.C.S. reagent, Aldrich). Ferrocenylmethyltrimethylammonium iodide, Fc- NMe_3^+ , was prepared by the quaternization of N,N-dimethylaminomethylferrocene (98 + %, Strem Chemicals, Newburyport, MA, U.S.A.) with methyl iodide (99.5%, Aldrich). Potassium ferrocyanide (certified A.C.S., Fisher Scientific); ferrocenecarboxylic acid, Fc-COOH (97%, Aldrich); hexammineruthenium (III) chloride, [Ru(NH₃)₆]Cl₃(99%, Strem); 1,10-phenanthroline ferrous perchlorate, $Fc(phen)_{3}^{2+}$ (G. Frederick Smith Chemical Co., Columbus, OH, U.S.A.); 5-chloro-1,10-phenanthroline (98%, Aldrich) and 5-nitro-1,10-phenanthroline (98%, Aldrich) were used as received. $Cu(EDTA)^{2-}$ and Cr(EDTA)⁻ were obtained in aqueous solution by mixing together stoichiometric amounts of Na₂EDTA (certified A.C.S., Fisher Scientific) with the corresponding metal chloride. Osmium(II)tris(2,2'-bipyridine) perchlorate, $Os(bpy)_{3}^{2+}$, was prepared from K₂OsCl₆ (Johnson Matthey Catalog Co., Ward Hill, MA, U.S.A.) and 2,2'-bipyridine (99 + %, Aldrich) according to the recommendations of Dwyer and co-workers [49-51]. Tris(5-chloro-phenanthroline)Fe(II) perchlorate, Fe(Clphen) $_{3}^{2+}$ and tris(5-nitrophenanthroline)Fe(II) perchlorate. Fe(NO₂phen) $^{2+}_{3+}$, were prepared by adding a 3-fold of molar excess of the respective ligand to the aqueous solution of FeSO₄.7H₂O (Certified A.C.S., Fisher Scientific). The mixtures were magnetically stirred for 30 min at room temperature, and then the reaction product was precipitated with an excess of LiClO₄.3H₂O (98%, Aldrich). The brick red products were collected by filtration and washed with de-ionized water (yield: 85-87%). Tables 1 and 2 list the redox couples and their formal potentials: Table I is organized according to the charge of the redox centre, and their strength of binding. Table 2 contains the cases where the identity of the bound redox centre is clearly different from the generating redox system.

Alkaline Clark and Lubs buffers [52] were used as the supporting electrolyte. They were prepared by dissolving the necessary amounts of boric acid (analytical reagent, Mallinckrodt) and sodium hydroxide (98.6%, Mallinckrodt) in de-ionized water (17.8 M Ω cm, DF Corning Mega-Pure System).

PROCEDURES

Monolayer and multilayer deposition

Gold mirrors (1000 Å Au on top of 50 Å Ti; Evaporated Metal Films, Inc.) on 3.0 cm × 1.0 cm float glass were used for ellipsometric measurements, and polycrystalline bulk gold flags (99.95%, Johnson Matthey Electronics, Ward Hill, MA, U.S.A.) of approximately 1 cm² surface area were employed for cyclic voltammetry. The gold mirrors were cleaned with hot $H_2SO_4 + H_2O_2$ (3:1, v/v). Warning: this reaction mixture, sometimes known as 'piranha' solution, reacts extremely violently with organic materials. It should only be used in a fume hood with a good draught. Rubber gloves and eye protection must be worn. After each brief immersion in 'piranha' solution, the mirrors were thoroughly rinsed with abundant amounts of de-ionized water. Polycrystalline bulk gold flags were cleaned by repeated heatings to incandescence in a Bunsen burner flame and quenching in deionized water.

A self-assembled monolayer (SAM) of each thiol was deposited on the gold substrate by multiple immersions of the gold flag or gold mirror in an acetonitrile (HPLC grade, Fisher Scientific) solution of the thiol $(5 \times 10^{-4} \text{ mol dm}^{-3})$ for a total immersion time of about 30 minutes. After emersion, the gold was rinsed several times with pure acetonitrile, and dried with a stream of warm air.

Polyion layers and redox centres were adsorbed onto 11-mercaptoundecanoic acid SAMs using the following steps:

(i) The carboxylic groups of the SAM were ionized by rinsing the surface with $5 \times 1 \text{ cm}^{-3}$ aliquots of borate buffer (pH 9), and then washed with an abundant volume of de-ionized water.

(ii) The SAM-coated electrode was immersed for 10-30 minutes in a saturated aqueous solution containing a cationic redox couple, such as ferroin or a ferroin derivative.

(ii') Alternatively, PVPMe⁺, a polycation, was adsorbed onto the monolayer (with its negatively charged surface) from an aqueous solution of pH 8–10. The deposition solution contained 2.4 mg cm⁻³ of the polymer (corresponding to the concentration of 2×10^{-2} mol dm⁻³ relative to the monomer). After 3–5 minutes, the substrate was removed and thoroughly washed with deionized water.

(iii) The bilayer system formed in (ii'), with an outermost cationic layer, was soaked for 3-5 minutes in an alkaline solution of an anionic redox couple, such as $[Fe(CN)_6]^{-4}$, $Cu(EDTA)^{2-}$, $Cr(EDTA)^{-}$ or Fc-COO⁻ (typical concentrations of 10^{-3} mol dm⁻³), and subsequently rinsed with water.

(iii') Alternatively, poly(styrene sulfonate) (PSS⁻) was adsorbed onto the cationic bilayer system (ii') from a 0.21 mg cm⁻³ solution in water (corresponding to the concentration of 10^{-3} mol dm⁻³ relative to the monomer) to form a trilayer system.

(iv) Cationic redox couples, such as $Co(phen)_{3}^{2+}$, Fc-NMe₃⁺ or methyl viologen, were adsorbed onto the trilayer from their respective millimolar solutions in 5–8 minutes. In each case, a water rinse removed any weakly adsorbed material.

Similar steps were used to build monolayer and

Table 1.

| Organo matallio | and | inorgania | redov | counles | with | reday | notantials i | n the | notential | window | of | nold |
|-----------------|-----|-----------|-------|---------|------|-------|--------------|-------|-----------|--------|----|------|
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| Bound redox couple | System | Supporting electrolyte for bound couples | E ^{O'} (bound) [V/SCE] | $\Delta E_{\rm p}$ [mV] | Strength of binding ^e | E ^{o,} (bulk) [V/SCE] |
|---|--|---|---------------------------------------|-------------------------|--|--------------------------------------|
| Ru(NH ₃) ³⁺ | UDA ⁻ - AMPMe ⁺ /PSS ⁻ UDA ⁻ /PVPMe ⁺ /PSS ⁻ | Borate ^a , pH 9.0 0.1 <i>M</i> KCl, pH 3.1 Borate ^a , pH 9.0 | -0.17 -0.26 -0.30 | 110 225 130 | weak | —0.19 pH 10 |
| $Co(phen)_{3}^{2+}$ | UDA ⁻ UDA ⁺ /PVPMe ⁺ /PSS ⁻ | Borate ^b pH 9.6 | +0.07 +0.09 | 320 310 | medium weak | +0.13 pH 9.4 |
| Co(bpy) ₃ ²⁺ | UDA- | Borate ^b pH 9.6 | +0.22 | 218 | medium | 0.21 pH 9.6 |
| Os(bpy) ²⁺ | UDA- UDA-/PVPMe+/PSS- | Borate ^a pH 9.0 | +0.59 +0.57 | 40 102 | weak | +0.57 pH 0.0 |
| Fc-CH ₂ -NMe ₃ ⁺ | UDA ⁻ UDA ⁻ /PVPMe ⁺ /PSS ⁻ | 0.1 <i>M</i> Na ₂ SO ₄ / /0.01 <i>M</i> Na ₂ HPO ₄ pH 9.4 | +0.41 +0.40 | 40 63 | medium | +0.37 pH 0.0 |
| Fe(CN) ⁴⁻ | -AMPMe ⁺ UDA ⁻ /PVPMe ⁺ | 0.1 MKCl, pH 3.1 Borate ^a , pH 9.0 | +0.23 +0.21 | 21 60 | strong | +0.19 pH 7.0 |
| AQ-1,8-disulfonic acid | UDA ⁻ /PVPMe ⁺ | Borate ^a pH 9.0 | -0.51 | 33 | weak | —0.57 pH 9.0 |
| FcCOO- | - AMPMe+ | 0.5 MKCl, pH 8.0 | +0.28 | 60 | medium | +0.28 pH 10.0 |

AQ—anthraquinone; bpy—2,2'-bipyridine; Fc—ferrocene; phen—1,10-phenanthroline; UDA—anionic monolayer (ionized 11-mercapto-undecanoic acid); AMPMe⁺—cationic monolayer (4-aminomethyl-1-methylpyridinium-11-mercapto-undecanoamide); PVPMe⁺—poly(4-vinyl-1-methyl-pyridinium; PSS⁻—poly(styrene sulfonate); E^{or}—formal potential vs. SCE; ΔE_p —peak separation at a 0.1 Vs⁻¹ scan rate; borate: borate buffer or Clark and Lubs buffer solutions, "50 mL (0.1 mol dm⁻³H₃BO₃ + 0.1 mol dm⁻³KCl) + 20.8 mL NaOH diluted to 100 mL with de-ionized water; ^b50 mL H₃BO₃ + 36.9 mL NaOH diluted to 100 mL with de-ionized water; ^cstrength of binding—see text.

| Source redox couple | System | Supporting electrolyte for bound couples | E ^{O'} (bound) [V/SCE] | $\Delta E_{\rm p}$ [mV] | Strength of binding ^c | E ^{O'} (bulk) [V/SCE] |
|--|--------------------------|--|---------------------------------------|----------------------------|--|--------------------------------------|
| Fe ^{II} /Fe(phen) ²⁺ | UDA- UDA-/PVPMe+/PSS- | Borate ^b pH 9.6 | -0.32 - 0.33 | 25 20 | strong strong | + 0.83 ^d pH 0.0 |
| Fe ^{II} /Fe(Clphen) ²⁺ | UDA- | Borate ^b pH 9.6 | -0.39 | 60 | strong | +0.88 ^d pH 0.0 |
| $Fe^{II}/Fe(NO_2phen)_3^{2*}$ | UDA- | Borate ^b pH 9.6 | -0.41 | 70 | strong | + 1.02 ^d pH 0.0 |
| Fe(CN) ⁴⁻ | UDA- | Borate ^a pH 9.0 | -0.35 | 40 | weak | +0.19 pH 7.0 |
| Fc-CH ₂ -NMe ₃ | UDA- | 0.1 <i>M</i> Na ₂ SO ₄ pH 9.4 | -0.41 | 70 | medium | +0.37 pH 0.0 |
| Cu(EDTA) ^{2–} | UDA-/PVPMe+ | Borate ^a pH 9.0 | -0.29 | 230 | medium | +0.06 pH 9.4 |
| Cr(EDTA) ²⁻ | UDA- | Borate ^b pH 3.5 9.4 | +0.28 | 80 | strong | — 1.23° рН 5.1 |

Table 2. Organo-metallic and inorganic complexes that generate a new redox centre

Clphen—5-chloro-1,10-phenanthroline; NO₂phen—5-nitro-1,10-phenanthroline; $*50 \text{ cm}^3$ (0.1 mol dm⁻³ H₃BO₃ + 0.1 mol dm⁻³ KCl) + 20.8 cm³ NaOH diluted to 100 cm³ with de-ionized water; $^{6}50 \text{ cm}^{3} \text{ H}_{3}\text{ BO}_{3} + 36.9 \text{ cm}^{3} \text{ NaOH diluted}$ to 100 cm³ with de-ionized water; $^{6}51 \text{ cm}^{3} \text{ H}_{3}\text{ BO}_{3} + 36.9 \text{ cm}^{3} \text{ NaOH diluted}$ to 100 cm³ with de-ionized water; $^{6}50 \text{ cm}^{3} \text{ H}_{3}\text{ BO}_{3} + 36.9 \text{ cm}^{3} \text{ NaOH diluted}$ to 100 cm³ with de-ionized water; $^{6}50 \text{ cm}^{3} \text{ H}_{3}\text{ BO}_{3} + 36.9 \text{ cm}^{3} \text{ NaOH diluted}$ to 100 cm³ with de-ionized water; $^{6}50 \text{ cm}^{3} \text{ H}_{3}\text{ BO}_{3} + 36.9 \text{ cm}^{3} \text{ NaOH diluted}$

multilayer systems with attached redox couples in which the SAM has a cationic charge.

Post-deposition characterization

Following deposition of each layer and/or attachment of a redox couple, the layers confined to the gold mirror support were dried with a stream of warm air and analyzed by a manual nulling ellipsometer with a HeNe laser source. Calculated thicknesses of the films assumed that the films were transparent with an index of refraction of 1.5 [53]. Also, after each adsorption step, the system confined to the gold flag was analyzed by cyclic voltammetry in a supporting electrolyte free of any redox couple. Cyclic voltammetry (cv) experiments were performed with a Voltammograph CV-27 (Bioanalytical Systems, Inc., West Lafayette, IN, U.S.A.), an Omnigraphic 100 Recorder (Houston Instruments, Austin, TX, U.S.A.), and a computerized data collection system, based on Asystant (Macmillan Software Co., U.S.A.) software. A three-compartment glass cell divided by porous glass frits permitted isolation of the gold flag working electrode from an SCE reference and a Pt counter electrode. The electrolyte varied with the experiment (Tables 1 and 2) but was typically adjusted to an alkaline pH for multilayers containing 11-mercaptoundecanoate and to an acidic pH for multilayers containing HS-UDA-AMP.

RESULTS AND DISCUSSION

Ellipsometry of polyion multilayers

The layer thickness was monitored by the change of the ellipsometric parameters Δ and Ψ for one gold mirror during the sequential deposition of a HS-(CH₂)₁₀-COOH monolayer, a HS-(CH₂)₁₀-COO⁻/PVPMe⁺ bilayer, and the adsorption of $Fe(CN)_{6}^{4-}$ to the bilayer. We found that the layer thickness for both the mercaptocarboxylic acid and the cationic polyion corresponded to about one monolayer (ca. 1 nm). In this case, no more than a monolayer of the polyion is adsorbed even after multiple immersions in the polyion solution (the second immersion caused a thickness increase of only 0.1 nm). An alkaline wash is a necessary step in the adsorption of PVPMe⁺ onto a 11-mercaptoundecanoic acid SAM; un-ionized SAMs do not adsorb significant amounts of the polyion (in the latter case the increase of the layer thickness was of only 0.3 nm).

Table 3 demonstrates the reproducibility of the thicknesses for each part of a trilayer and the effect of adsorbing a redox couple. In general, the thickness of the self-assembled monolayer and each polyion layer is reproducible to within a fraction of a monolayer. The third layer, the polyanion, also has a thickness of about 1 nm. These results are consistent with the thickness measurements by Lvov

Table 3. Mean values of the layer thickness for the charged layers in multilayer systems

| Examined layer | Individual layer thick- ness ^a [nm] | | | | |
|---|---|--|--|--|--|
| HS-(CH ₂) ₁₀ -COOH | 1.2 ± 0.3 | | | | |
| PVPMe ⁺ | 1.4 ± 0.4 | | | | |
| PSS ⁻ | 1.4 ± 0.2 | | | | |
| $[Fe(CN)_{6}]^{4-}$ | 0.3 ± 0.2 | | | | |
| (attached to bilayer) | | | | | |
| Fe(II) redox couple (attached to trilayer) | 0.3 ± 0.2 | | | | |

"The figures in this table are mean values for six experiments, \pm refers to 90% confidence interval. Individual layer width refers to changes in thickness after the deposition of the indicated layer.

et al. [8, 26], who used a small angle X-ray scattering method to probe the thickness of polyion multilayers. The ferri/ferrocyanide redox couple adsorbed to the bilayer does not affect the apparent thickness within experimental uncertainty. Neither does the generation of the iron redox centre modify the thickness of the trilayer. Because of the very small thicknesses in the bilayer and trilayer structures, the redox couples are close to both the electrode and the solution to which they are responding; both chemical communication with the solution and electron transfer with the electrode should be rapid in these multilayers. The multilayers share this advantage with electroactive monolayers but without paying a synthetic price in the preparation of new molecules.

Electroactive multilayers

Ferrocyanide ions are tightly adsorbed to a self-assembled cationic monolayer of protonated HS-UDA-AMP (Fig. 1) or a monolayer of the corresponding quaternized derivative (HS-UDA-AMPMe⁺). Less than 35% of the adsorbed $[Fe(CN)_6]^{3-/4-}$ redox couple is lost from the electrode surface over 110 scans. Also, the redox couple exhibits nearly reversible behaviour (fast electron transfer kinetics) as evidenced by a small peak separation ($\Delta E_p = 24 \text{ mV}$). This observation is consistent with the hypothesis that the ferri/ferrocyanide ions reside near the external surface of the alkanethiol monolayer. The peak half-widths (ca. 160 mV) are much larger than the ideal 90 mV. This observation can be interpreted as a spread in formal potentials of the redox centres caused by varying degrees of ion-pairing between the redox couples and the surface cationic sites. A similar limiting coverage is obtained with ferrocyanide attached to a bilayer system with an outermost cationic surface (see the discussion concerning the two-terminal bilayer assembly). The main difference for ferrocyanides electrostatically trapped in the cationic polyion of a bilayer coating is an increase in the peak splitting $(\Delta E_p$ is typically in the range from 85 to 120 mV), indicating that the redox centres are now trapped further away on average from the electrode surface.

In all bilayer and bilayer coatings examined, the moles of bound ferrocyanide ions are estimated to be much less than the moles of available cationic sites in the adsorbed polyion. Assuming that the pyridine rings in PVP are completely quaternized, and that



Fig. 1. Ferrocyanide attached to a cationic monolayer (HS-UDA-4-AMPH⁺) on gold flag (0.1 V s⁻¹, Na₂SO₄, pH 3.0); A-3rd scan (E^{o'} = 0.109 V vs SCE, $\Delta E_p = 24 \text{ mV}$; mean peak area: 1.4 μ C), B-110th scan.



Fig. 2. Two new redox couples attached by electrostatic forces: A—redox couple obtained by the immersion of an anionic trilayer system $(Au/S-(CH_2)_{10}-COO^{-}/PVPMe^{+}/PSS^{-})$ in alkaline Fe(phen)³⁺ solution; B—redox couple generated by the immersion of a monolayer $(Au/S-(CH_2)_{10}-COO^{-})$ in alkaline Cr(EDTA)²⁻ solution.

about half of these cationic sites are ion-paired with the carboxylate moieties of the SAM, then the theoretical density of positive sites that can bind ferrocyanide ions is approximately 2×10^{-10} mol cm⁻². This estimate is based on the volume of a 10^{-7} cm thick PVPMe⁺MeSO₄ layer with a density of 0.8-1.0 g cm⁻³, and a monomer unit formula weight of 231. However, the largest ferrocyanide coverage achieved for a bilayer system was 5.7×10^{-11} mol cm⁻². Consequently, even with the strong ion-pairing tendencies of ferrocyanide, electrostatic binding does not create a densely packed electroactive layer. For comparison, Bowden et al. [54], found that a nearly close-packed monolayer of cytochrome $c(2 \times 10^{-11} \text{ mol cm}^{-2})$ could be electrostatically bound to the surface of a SAM with ionized terminal carboxyl groups, but only in dilute electrolyte.

Competitive adsorption of ferrocyanide and ferricyanide onto thicker films of quarternized PVP (with thickness typically in the range of 500 ± 50 nm), reveals a much greater affinity of the polycation for the more hydrophobic ferricyanide [55, 56]. However, in those experiments, the ferricyanide was reversibly bound, and could be ejected from the polycation upon reduction of the redox centres.

Tables 1 and 2 provide a survey of cationic and anionic redox couples which have been bound to monolayer (ie, just the SAM), bilayer and trilayer systems. Not all combinations of redox couple and charged surface layers result in useful electroactive layers in relatively high ionic strength electrolytes. The rating used for the strength of binding (column

5 in Tables 1 and 2) is based on the following observations obtained from cv's: strong-no significant decrease in cv peak areas for over 50 potential scans; medium-no significant decrease in the peak areas for only 5 to 10 potential scans; weak-rapid loss of peak areas during the first 3-5 potential scans. It may be possible to increase the stability of some of the weak or medium combinations by decreasing the ionic strength of the electrolyte, but that would hinder the ultimate utility of the sensor. The most stable electroactive multilayers contain the $[Fe(CN)_6]^{3-4-}$ redox couple bound to a cationic surface, or an iron(III/II) wave generated during exposure of an anionic surface to an alkaline solution of an iron complex (Table 2). The successful adsorption of ferrocyanide is probably caused by the pronounced tendency of this anion to form strong ion-pairs [37, 38], especially with multiply charged cations like PVPMe⁺ or PVPH⁺ (mixing aqueous solutions of PVPMe+ and ferrocyanide results in immediate precipitation).

The new redox couple produced during the immersion of an anionic coating in alkaline (pH 9.6) saturated $Fe(phen)_{3}^{2+}$ perchlorate solution for 10-30 minutes has formal а potential of $E^{0'} = -0.34 \pm 0.01$ V vs SCE (Fig. 2, curve A), well negative of the +0.83 V vs SCE formal potential of ferroin [57, 58]. Once formed, the redox wave is stable over hundreds of potential scans; only 10% of the attached redox couple are lost over the first 50-200 scans. The coverage is dependent primarily on the length of time for which the anionic surface is exposed to the alkaline ferroin solution. The coverage is not limited by two-dimensional packing, since in

some cases, apparent coverages far exceed 10^{-10} mol cm⁻². Peak splittings and peak half-widths are variable. In all cases, the redox couple responsible for this wave is generated only when both the deposition step and the potential scans are performed in alkaline buffer supporting electrolyte (eg borate buffer, at pH 9.0 and 9.6, or in carbonate/bicarbonate buffer, at pH 10.0), The iron-based redox couple is formed on both a Au/S-(CH₂)₁₀-COO⁻ SAM and a Au/S-(CH₂)₁₀-COO⁻/PVPMe⁺/PSS⁻ trilayer system. On the other hand, no redox wave appears when a bilayer system with an external cationic layer, Au/S-(CH₂)₁₀-COO⁻/PVPMe⁺, or a nonionic octadecylthiol monolayer is immersed in alkaline ferroin solution. The necessity of having an anionic surface implies that the redox couple and/or its precursors are cationic. The redox wave at -0.3 to -0.4 V vs SCE is also obtained during the immersion of an anionic surface (monolayer or trilayer) in alkaline solutions containing other iron complexes leg, Fe(II) tris(phenanthroline) with a nitro- or chloro-substituent on the 5 position of the phenanthroline ring, the water-soluble ferrocene quaternary amine, and ferrocyanide (Table 2)]. Thus, the redox wave is not related to the parent complex, but is likely to be caused by traces of Fe²⁺ or Fe³⁺ ions generated by decomposition of the iron complex in alkaline solution. Nevertheless, this phenomenon could not be reproduced by using a common ferrous salt, such as FeSO₄, at micromolar concentrations (higher concentrations, of course, form precipitates of iron hydroxides). The redox wave is pH sensitive, with a Nernstian response in the pH range from 7.2 to 12.8 (see the section on the two-terminal bilayer sensor). In more acidic solutions, the redox couple is lost rapidly, while at pH values greater than 12.8, the redox process becomes very irreversible, with peak separations up to 200 mV.

By considering the cationic nature and the Nernstian behaviour of the iron-related redox species, we hypothesize that it is probably a form of Fe(II) hydroxide. The ferrous hydroxide is generated in situ by traces of Fe^{2+} or Fe^{3+} ions from the slow decomposition of the iron complex present in an alkaline solution. A large number of ferrous hydroxide/carboxylate complexes are known for both mono- and polycarboxylic acids [59-63]. Although these complexes are usually not stoichiometric compounds, they can be described with approximate molecular formulas. Furthermore, their physical and chemical properties are reproducible when prepared under similar conditions [59-63]. A general scheme of the interaction of the ferrous ions with the anionic monolayer and the trilayer system terminating in PSS⁻, respectively, is shown in equations (1) and (2):

$$xAu/S-(CH_2)_{10}-COO^- + yFe^{2+} + zOH^- \rightarrow$$

$$\rightarrow [(Au-S-(CH_2)_{10}-COO)_x Fe_y (OH)_z] \qquad (1)$$

$$x[CH_2-CH(C_6H_4-SO_3^{-})] + yFe^{2+} + zOH^{-} \rightarrow$$

$$\rightarrow [CH_2-CH(C_6H_4-SO_3)], Fe_y(OH)_2].$$
(2)

Based on known complexes, the stoichiometric coefficients can vary over the ranges x = 1-7, y = 1-3, and z = 1-4. These species are not expected to be stable at very low and very high pH values, in agreement with the known instability of the new iron redox species.

Given the structural similarity of several iron and chromium complexes [59], it should be possible to attach a chromium redox couple, which should parallel the behaviour of the iron couple, to the anionic electrode surface. Thus, a self-assembled monolayer (Au/S-(CH₂)₁₀-COO⁻) soaked in alkaline Cr(EDTA)⁻ solution for 30 minutes leads to a relatively broad but stable redox wave at +0.28 V vs SCE which is believed to be caused by analogous chromium hydroxy species (Fig. 2, curve B). This redox potential is about 1.5 V more positive than the formal potential of Cr(EDTA)^{-,2-} [64, 65].

Despite the promising simplicity, the attachment of redox couples to the electrode surface by ionic forces has several drawbacks. Firstly, only a few redox couples bind strongly. Secondly, the few that do bind well show non-ideal electrochemical behaviour (broad cv waves). Hence, we focus on one combination of two redox centres that do bind strongly to illustrate a sensor application.

Construction of a two-terminal sensor system

A two-terminal sensor system requires two redox groups bound to the same electrode surface. Given the results in the preceding section, we designed a procedure designed to create a sensor for pH measurement using five steps; cv's obtained at the end of each step reveal the formation of the relevant redox centres:

(i) a self-assembled monolayer of 11-mercaptoundecanoic acid is deposited on the gold flag electrode (Fig. 3, curve A);

(ii) the iron(II) redox couple is attached from ferroin solution onto the ionized carboxylate groups of the monolayer (Fig. 3, curve B);

(iii) PVPMe⁺ is attached to the monolayer system with the already confined Fe(II) redox couple. Pronounced loss of the iron redox wave is observed (Fig. 3, curve C);

(iv) Ferrocyanide is adsorbed to the outermost cationic layer (Fig. 3, curve D);

(v) the Fe(II) redox couple is regenerated by additional soaking of the system in ferroin solution (Fig. 3, curve E).

A proposed schematic picture of the bilayer assembly is shown in Fig. 4. The confinement of the ferrocyanide redox centers to the outer polycation layer is supported by the noticeable increase in peak splitting compared to that observed when ferrocyanide is adsorbed to just a cationic SAM (Fig. 1).



Fig. 3. Construction of an electrode with two attached redox centres. A—Self-assembled 11-mercaptoundecanoic acid monolayer on Au flag (0.1 Vs⁻¹, pH 9.0 borate buffer); B—After adsorption of the Fe redox couple from alkaline ferroin solution; C—After PVPMe⁺ deposition; D—After adsorption of Fe(CN)¹₆⁻; E—After second adsorption of Fe redox couple from alkaline ferroin solution.

It is necessary to generate first the Fe(II) redox couple (step (ii)) and then to replenish its coverage after the deposition of ferrocyanide (step (v)). If step (ii) is omitted, then the new Fe(II) redox couple does not form on the PVPMe⁺ cationic surface (step (v)). The renewal of the redox wave suggests that the proposed Fe hydroxy oligomers are forming at specific sites not covered by PVPMe⁺.

The bilayer assembly can be used for pH measurement. The formal potential of the new iron couple shifts in a Nernstian fashion with the proton concentration while the $[Fe(CN)_6]^{3-4-}$ couple



Fig. 4. Schematic picture of a bilayer system with adsorbed cationic and anionic redox couples.



Fig. 5. Cyclic voltammograms of the two-terminal bilayer system for decreasing pH values: A-pH 9.0; B-pH 8.00; C-pH 6.42 (0.1 Vs⁻¹, borate buffer). The pH values were adjusted with 0.1 mol dm⁻³ HCl solution.

maintains a stable potential value ($E^{0'}$ = +0.22 ± 0.04 V vs SCE) over neutral and alkaline pHs. The positions of the two redox waves are shown relative to the SCE reference electrode as the pH of the supporting electrolyte is decreased from pH 9.0 to 6.42 (Fig. 5, curves A–C), and then increased from



Fig. 6. Cyclic voltammograms of the two-terminal bilayer system for increasing pH values: A—pH 7.70; B—pH 8.02; C—pH 8.60; D—pH 9.50; E—pH 10.24. The pH values were adjusted with 0.1 mol dm⁻³ NaOH solution.



Fig. 7. pH dependence of the iron formal potential relative to the internal reference (ferrocyanide-wave). The line is a linear regression fit of the data between pHs 6.4 and 9.5, with a correlation coefficient of 0.975. The slope of the calibration curve is 59 mV (pH unit)⁻¹.

7.70 to 10.24 (Fig. 6, curves A-E). The formal potential of the sensing redox centre relative to that of the reference redox centre displays a Nernstian shift with respect to pH $[-59 \text{ mV} \text{ (pH unit)}^{-1}$, see Fig. 7]. One immediate problem noticeable in Figs 5 and 6 is in the large variation of peak shape, area and peak half-width for the same wave over the working pH range. In contrast, the same features for ferro/ferricyanide redox centres are relatively stable, although the precision in measurement of its formal potential is compromised by the somewhat broad waves and large peak splitting. Another inconvenience is the permanent loss of the sensing redox site at high and low pH values. An advantage of the described system, is, however, that pH measurements are based on the relative peak positions of the sensing and the reference site, rather than on the peak currents or areas. Therefore these measurements can be performed successfully as long as the two waves can be detected.

CONCLUSIONS

A new approach in designing two-terminal voltammetric sensors relies on the adsorption of redox couples to monolayer, bilayer and trilayer systems based on ionized self-assembled thiol monolayers and adsorbed polyions. A redox wave, believed to be due to an iron hydroxy species, appears on anionic electrode surfaces after exposure to iron complexes in alkaline solutions. This redox wave in combination with electrostatically bound ferro/ferricyanide forms the basis of a two-terminal pH sensor. One advantage of this approach is the ease of assembly of the multilayer systems using readily available redox couples. Disadvantages include the lack of kinetically fast and stable redox couples with formal potentials within the potential window of gold and with strong ion-pairing tendencies on immobilized charged sites. The large half-width of the redox waves and the significant peak splitting of the kinetically slower couples (*eg* ferri/ferrocyanide) degrade the analytical signal based on the difference in formal potentials. The carboxylate-terminated SAM limits the current design to neutral and alkaline electrolytes, while alkylthiol monolayers with 4aminomethylpyridinum moieties should be effective in acidic supporting electrolytes.

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