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In situ synthesis of stable mixed ligand Fe^{2+} complexes on bipyridinyl functionalized electrodes and nanotube supports†

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A simple method is presented to synthesize asymmetric mixed ligand iron(II) diimine complexes using bipyridinyl functionalized carbon nanotubes. The synthesis of these complexes was realized using subsequent dip coating processes. The *in situ* formed mixed ligand complexes were used in aqueous media to act as building blocks in biosensor devices.

Tris-bipyridyl metal complexes showed, among others, their usefulness in electrochemical or electrochemiluminescent setups due to the easy accessibility to a wide range of derivatives.[1] The introduction of appropriate functionalities through the chemical modification of the ligand can "tune" the redox or photochemical behaviour of the metal centre.[2] Such functional ligands can also be used as building blocks for (bio)-molecular scaffolds.[3,4] Hetero ligand complexes constitute an attractive platform for the combination of different functionalities. Therefore, such diimine complexes, on one part, decorated with functional groups for attachment on the electrode matrix, and, on the other part, functionalized with an affinity partner, can act as a bridge for the specific immobilization of bioreceptor units. Furthermore, the electroactivity of the metal center may be used as a redox probe for impedimetric measurements. However, even though homo ligand diimine iron complexes of the general formula $Fe(N–N)_3$ have been comprehensively investigated,[5] there are only a few examples of asymmetric diimine Fe complexes[6–8] due to their high ligand exchange rate.[5]

In this context, we present an innovative approach to synthesize stable mixed ligand iron diimine complexes directly on bipyridinyl functionalized carbon nanotubes (Fig. 1) and their use as building blocks in biosensor devices. These iron(II) complexes were constituted of bipyridinyl ligands bearing pyrene or biotin groups. The pyrene residues allow the iron attachment onto carbon nanotubes (CNT) *via* π-stacking interactions while biotin groups can be used for the subsequent anchoring of biotinylated proteins *via* the formation of an avidin bridge by avidin–biotin interactions.

In order to evaluate the possibility to form stable asymmetric diimine iron complexes, we studied the electrochemical behaviour

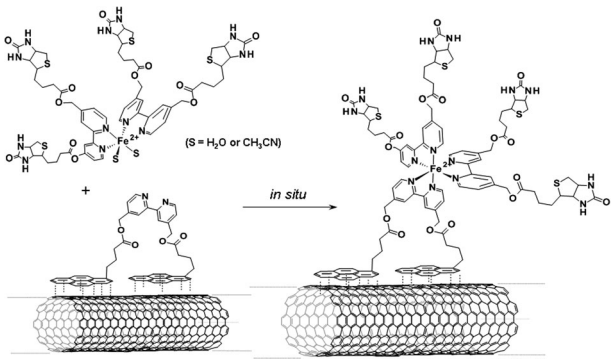

Fig. 1 Synthesis scheme of the mixed ligand iron complex.

of the bipy-pyrene (L_2) ligand and its appropriateness to form a monolayer on the nanotube sidewalls. In this context, CNT modified electrodes were incubated in a DMF solution of L_2 (2.3 mM) and then transferred into pure CH_3CN containing 0.1 M TBAP. The π-stacked ligand molecules were then electropolymerized by controlled potential electrolysis at 0.9 V *vs.* Ag/Ag^+ 10 mM in CH_3CN. Fig. 2A shows the cyclic voltammogram of the L_2 functionalized nanotube electrode. Despite the high capacitive current due to the nanotube deposits and the formation of an ultrathin polymer film, one reversible peak system may be observed at −1.0 V (ΔE = 0.14 V) followed by two reduction peaks at −1.68 and −2.13 V. These systems are attributed to the polymerized pyrene groups[9] and the two successive one-electron reductions of the 4,4′ substituted 2,2′-bipyridine esters,[10] respectively. In the positive range of the electrogenerated L_2 film, the reversible oxidation of the polypyrene film around 0.6 V remains almost invisible due to the thin film thickness.

After functionalization and characterization of the nanotube deposits with bipyridinyl ligands, the asymmetric $[Fe^{II}(L_1)_2L_2]^{2+}$ was formed by incubation of the modified electrode in a dry CH_3CN solution of $[Fe^{II}(L_1)_2S_2]^{2+}$ (5 mmol L^{-1}) at low temperature for 25 minutes. After rinsing the electrode with acetonitrile, the *in situ* formed asymmetric complex was examined by cyclic voltammetry (Fig. 2B). As expected, the cyclic voltammogram of the resulting electrode clearly shows changed features compared to the preceding one related to the uncoordinated polymerized ligand. In the negative region, three cathodic peaks appear at −1.75 V, −1.97 V and −2.24 V. The potential of these signals are in good accordance

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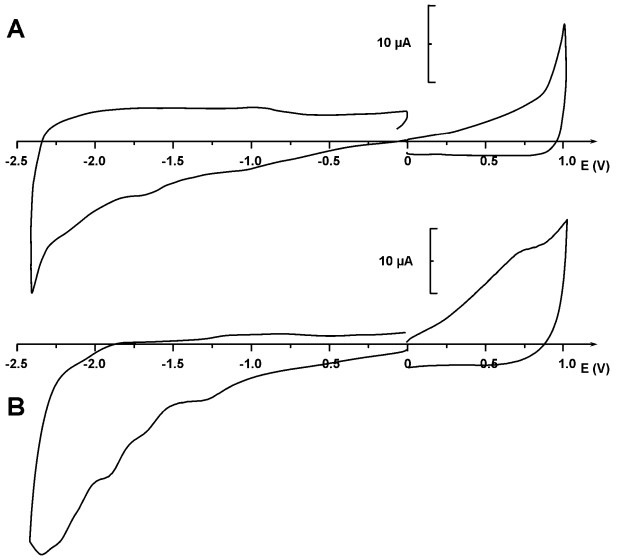

Fig. 2 (A) Cyclic voltammogram of the ultrathin polypyrene-bipyridinyl film on carbon nanotube modified Pt-electrodes. (B) Cyclic voltammogram of the mixed ligand complex $[Fe^{II}(L_1)_2L_2]^{2+}$ formed *in situ* on the functionalized carbon nanotubes. Cyclic voltammograms were recorded in the positive part from 0.0 to 1.0 V and in the negative part from 0.00 to −2.4 V in CH_3CN + 0.1 M TBAP at room temperature. Scan rate: 100 mV s^{-1}.

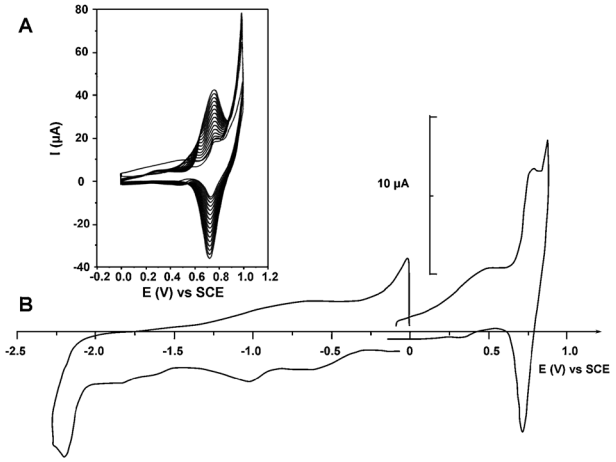

Fig. 3 (A) Oxidative polymerization of the reference complex $[Fe^{II}(L_2)_3]^{2+}$ (0.1 mM in CH_3CN) on a Pt-electrode (5 mm in diameter) by repeated potential scanning between 0 V and 1 V. (B) Cyclic voltammograms recorded at the Pt-electrode of the electropolymerized reference complex. The cyclic voltammograms were recorded in CH_3CN + 0.1 M TBAP at room temperature in a glove box. Scan rate: 100 mV s^{-1}.

with the $E_{\frac{1}{2}}$ values previously reported for the reversible one-electron reduction of the 4,4′-bis(biotin)-2,2′bipyridine ligand of a tris(bipyridyl) iron(II) complex.[3] As a consequence, these peak systems were attributed to the one-electron reductions of the different bipyridinyl esters of the mixed complex.[11] Concerning the electroactivity of the polypyrene skeleton, the reduction wave of the polypyrene groups, which is expected around −1.0 V, seems to be negatively shifted to −1.25 V. In the positive region, an anodic peak appears at 0.63 V that may be ascribed to the $Fe^{II/III}$ redox couple corroborating thus the formation of the asymmetric iron complex onto the ligand film. In order to attribute, unambiguously, the different redox signals ascribed to the asymmetric complex, the electrochemistry of the redox behaviour of the symmetric reference complex $[Fe^{II}(L_2)_3]^{2+}$ has been investigated.

The $[Fe^{II}(L_2)_3]^{2+}$ complex (1 mM in CH_3CN) was thus electropolymerized on Pt. Upon oxidative scanning between 0 and 1.0 V, the complex exhibits for the first scan the reversible $Fe^{II/III}$ transition followed by an irreversible peak at 0.95 V. This peak can be attributed to the oxidation of the pyrene group to its cationic radical.[12] The electropolymerization of $[Fe^{II}(L_2)_3]^{2+}$ was continued by repeated potential cycling over the range 0.0–1.0 V leading to a continuous decrease in the intensity of the oxidation peak and the growth of the reversible $Fe^{II/III}$ peak system at 0.74 V (Fig. 3A). Furthermore, a less pronounced redox system appears and grows with each further cycle around 0.6 V indicating the formation of a polymeric coating on the electrode surface. Upon transfer into a complex free CH_3CN + 0.1 M $LiClO_4$ solution, the voltammogram of the resulting electrode between 0.0 V and 0.9 V clearly shows an anodic prepeak at 0.45 V followed by a reversible peak system at $E_{1/2}$ = 0.72 V (Fig. 3B). These peak systems are assigned to the electroactivity of the polypyrene film and the $Fe^{II/III}$ redox couple, respectively.[13,14] In the negative region, an ill-defined reversible peak system around $E_{1/2}$ = −0.77 V ascribed to the polypyrene film reduction was followed by three distinct reduction waves situated at −1.64 V, −1.75 V and −2.10 V. The latter were attributed to the successive one-electron reduction of the three bipyridinyl ester ligands of the symmetric iron complex.[10] Although their potential values are slightly positively shifted, the similarity of the electrochemical behaviour of the electropolymerized iron complex with that of the modified electrode incubated with $[Fe^{II}(L_1)_2S_2]^{2+}$ corroborates the *in situ* formation of the asymmetric $[Fe^{II}(L_1)_2L_2]^{2+}$ complex.

In order to further prove the concept of the *in situ* synthesis of $[Fe^{II}(L_1)_2L_2]^{2+}$ and its stability, we evaluated its properties for the anchoring of proteins onto the nanotube surface. Biotinylated glucose oxidase (B-GOx) was chosen as a protein model. The activity of this enzyme was used to determine the effective attachment of a protein onto the nanotube coating. Nanotube modified Pt-electrodes were thus functionalized with poly-L_2 onto which the mixed ligand Fe^{II} complex was formed as described above. The biotinylated bipyridine ligands L_1 allow the anchoring of biotinylated bioreceptor units *via* avidin bridges thanks to the strong affinity interactions between the protein avidin and four biotins.[15] In this context, the prepared electrodes were successively incubated for 20 minutes in avidin, and then in biotin tagged glucose oxidase solutions. Glucose oxidase converts glucose into gluconolactone by reducing oxygen to hydrogen peroxide. The presence of immobilized B-GOx can therefore be detected *via* the electro-oxidation of the enzymatically produced H_2O_2 at the underlying Pt electrode biased at 0.6 V *vs.* SCE.[16] Amperometric measurements of glucose were performed at 0.6 V in 0.1 M phosphate buffer solution at pH 7.0 at 25 °C. Fig. 4a shows the amperometric current response as a function of glucose concentration. The calibration curve was linear in the range between 2×10^{-7} M and 3.5×10^{-3} M and curved gradually for higher concentrations, a pseudoplateau was reached for glucose

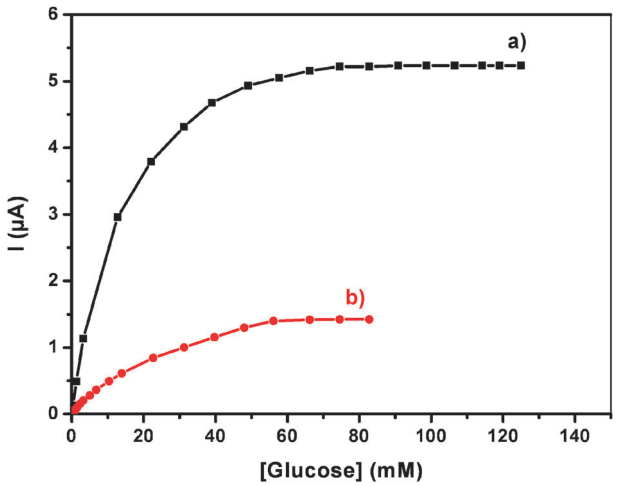

Fig. 4 Calibration curves for glucose (a) at a mixed ligand complex $[Fe^{II}(L1)_2L_2]^{2+}$–CNT electrode and (b) at a mixed ligand complex $[Fe^{II}(L1)_2L_2]^{2+}$ film formed onto a Pt electrode modified by an electrogenerated poly L_2 polymer. Both electrodes were subsequently incubated in avidin and B-GOx (both: 0.5 mg mL^{-1} in 0.1 M phosphate buffer solution, pH 7.0) to form the bioelectrodes. The amperometric measurements were performed in 0.1 M phosphate buffer solution (pH 7.0) at 25 °C and at E_{app} = 0.6 V *vs.* SCE.

concentrations higher than 70 mM. The enzyme electrode shows a sensitivity (determined as the slope of the linear range) and a maximum current density of 3.61 ± 0.05 mA M^{-1} cm^{-2} and 26.5 ± 2.2 μA cm^{-2}, respectively. These obtained values confirm the anchoring of the biotinylated enzyme and hence the formation of the mixed tris-bipyridinyl ligand iron complex at the CNT modified electrodes. The reproducibility of the enzyme electrode performance and the stability of the maximum current response with time demonstrate the iron complex stability in physiological solutions, at room temperature and in the presence of air.

In order to evaluate the benefit of functionalizing nanotube deposits by such kinds of mixed iron complexes, a blank experiment was performed under identical conditions, just without nanotubes. Fig. 4b shows the resulting calibration curve for glucose. The obtained sensitivity and maximum current density of 0.33 mA M^{-1} cm^{-2} and 6.0 μA cm^{-2}, respectively, are clearly below the values of the mixed ligand complex formed on nanotubes. The maximum current density commonly corresponds to the maximum rate achieved when the total amount of enzyme is saturated by glucose. This current density is thus proportional to the amount of active enzyme immobilized. As a consequence, it appears that the immobilized amount of B-GOx is 4 times higher on the CNT electrode, highlighting the surface amplification brought by the nanotubes. Assuming that the B-GOx electrode without CNT deposit leads to a compact enzyme monolayer and taking into account that the maximum coverage of glucose oxidase for a close-packed monolayer corresponds to 2.96×10^{-12} mol cm^{-2},[17] the immobilized amount of B-GOx onto the CNT electrode was estimated roughly to be 11.2×10^{-12} mol cm^{-2}.

An easy approach is presented to form asymmetric mixed ligand iron complexes which are stable under physiological conditions that allows their use in bioanalytical devices. This approach consists in precedent functionalization of carbon nanotubes with a bipyridinyl ligand onto which the stable mixed ligand iron complex of the general formula $[Fe^{II}(L_1)_2L_2]^{2+}$ can be formed just by incubation of the nanotubes in a solution of the precursor complex $[Fe^{II}(L_1)_2S_2]^{2+}$. This approach represents an original and simple way to obtain metal–organic nanotube derivatives opening a wide range of possibilities beyond bioanalytics by combining the specific properties of the component like the metal center or the functionalities on the remaining bipyridinyl groups.

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