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An electrogenerated poly(pyrrole-benzophenone) film for the photografting of proteins

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A photoreactive organic polymer was prepared by oxidative electrochemical polymerization of a pyrrole-benzophenone derivative on conductive surfaces; the resulting polypyrrolic film allowed, upon irradiation, the reagentless covalent grafting of proteins.

Deposition of biological macromolecules with controlled spatial resolution is the subject of increasing research efforts owing to its potential application in the field of biochips and miniaturized biosensors. Among the conventional immobilization methods, photopatterning has been widely used for the fabrication of bioanalytical devices.[1] Actually, immobilization of biomolecules by light is topically addressable and compatible with biological functions. In addition, this reagentless approach based on a light-induced reaction between a photoreactive group and C–H bonds, is easily applicable to a wide variety of biomolecules.[2–6] However, photoimmobilization previously required the immobilization of a highly uniform and fully active layer of photoreactive groups on transducer surfaces.

Among the conventional procedures of surface functionalization with molecular reagents, only the electrochemical deposition of organic polymers allows the reproducible functionalization of conductive microsurfaces of complex geometry with a precise spatial resolution.[7] In addition, the electrogeneration of polymer films that is compatible with bulk manufacturing procedures, provides densely packed multilayers of active species. The quality of the latter (absence of defects and chemical stability) constitutes an attractive advantage for the regularity at the molecular level of additional functionalization of the electrode *via* the polymer film. This communication describes a new photo-electrochemical method for the immobilization of biological macromolecules which combines the advantages of photolithography with those of the electrochemical addressing of polymer films.

We report herein the first successful synthesis and electropolymerization of a benzophenone derivative and the use of the resulting polypyrrole film for the photoimmobilization of biomolecules (Scheme 1).

In this study, the pyrrole monomer (**1**) functionalized with a photoreactive benzophenone group was prepared by the esterification of the 3-benzoylbenzoic acid with the 1-(3-hydroxypropyl)pyrrole using the carbodiimide method.[8] The pyrrolic benzophenone was purified by silica gel chromatography and characterized by H^1 NMR and IR spectra as well as by EI mass spectrum.[9] The electrochemical behavior of the pyrrole benzophenone **1** (2.0×10^{-3} M) was investigated by cyclic voltammetry in CH_3CN containing 0.1 M n-Bu_4NClO_4. Upon reductive scanning with a sweep rate of 0.1 V s^{-1}, the cyclic voltammogram displays a reversible peak system (−1.96 V *vs* Ag/Ag^+ 1.0×10^{-2} M in CH_3CN) due to the one-electron reduction of the carbonyl group (Fig. 1).

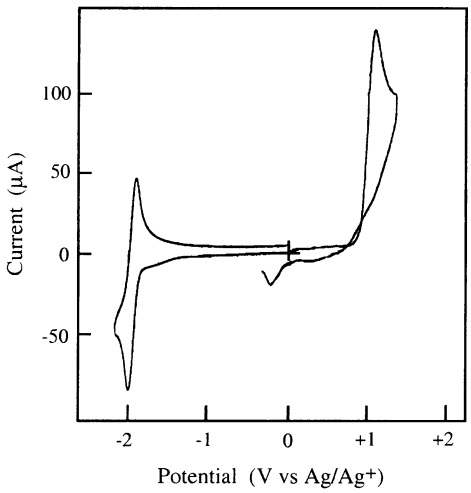


Fig. 1 Cyclic voltammogram recorded at a platinum electrode (diameter 5 mm) of **1** in CH_3CN + 0.1 M nBu_4 $NClO_4$. Scan rate 0.1 V s^{-1}.

Upon oxidative scanning, an irreversible peak is observed at 1.06 V corresponding to the oxidation of the pyrrole group. Electropolymerisation of **1** (3.0×10^{-3}M) was accomplished by repeated potential cycling over the range 0–0.86 V (Fig. 2).

The appearance and the continuous growth of a reversible oxidation wave at 0.32 V clearly indicate the formation of a

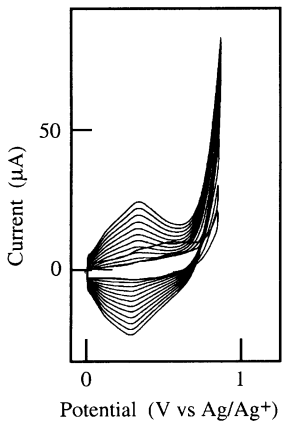
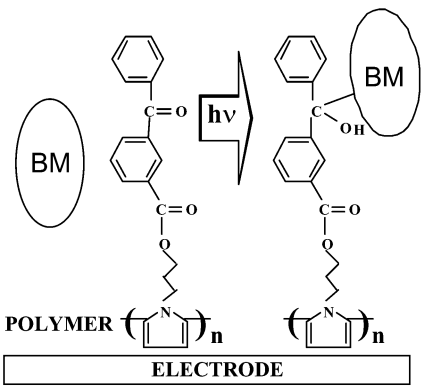


Fig. 2 First 12 scans in the oxidative electropolymerization of **1** (3.0×10^{-3} M) in CH_3CN + 0.1 M nBu_4 $NClO_4$; scan rate 0.1 V s^{-1}.



Scheme 1 Schematic representation of the photoactivation of an electrogenerated poly(pyrrole-benzophenone) film for the grafting of biomolecules (BM).

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polymeric coating on the electrode surface. The resulting electrode was transferred after thorough rinsing to a CH_3CN + 0.1 M nBu_4NClO_4 solution free of monomer **1**.

As expected the cyclic voltammogram of this electrode exhibits two reversible peak systems at $E_{1/2}$ = 0.32 V and $E_{1/2}$ = −2.00 V assigned to the oxidation of the polypyrrolic matrix and to the one-electron reduction of the polymerized benzophenone groups respectively (Fig. 3).

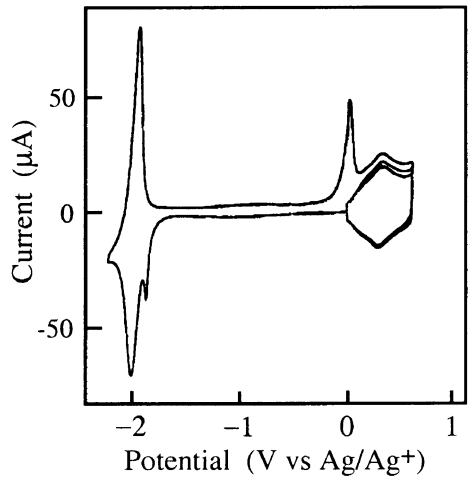

Fig. 3 Cyclic voltammogram of the polymerized **1** (Γ_1 = 1.8 10^{-8} mol cm^{-2}) in CH_3CN + 0.1M nBu_4NClO_4 scan rate 0.1 V s^{-1}.

Among the photoreactive groups applicable for the photoimmobilisation, benzophenone has been widely used due to its chemical stability and the low energy required for its activation.[5,6,10] In particular, the excited triplet state of benzophenone was generated by irradiation at wavelengths = 350 nm that are compatible with most biological macromolecules. Since the photoactivation of benzophenone leads to a radical-mediated bond formation with a variety of substrates, the possibility of generating covalent linkages between the poly **1** film and target biomolecules upon irradiation was investigated (Scheme1).

Illumination experiments were performed in the presence of bovine serum albumin (BSA) as the model protein.[11] After irradiation, the resulting electrode was thoroughly washed and transferred into CH_3CN + 0.1 M nBu_4NClO_4 to examine the electrochemical behavior of the irradiated poly **1** film. It appears that the reversible system at $E_{1/2}$ = − 1.96 V corresponding to the formation of the benzophenone radical anion presents only *ca*. 56% of its initial current intensity. In order to determine the presence of photochemically grafted BSA molecules on the poly **1** film, the mass-transfer process through the polymer was examined in acetonitrile solution by rotating disk electrode voltammetry with decamethyl ferrocene (DmFc) as a bulky electroactive probe. As expected, the permeability of the polypyrrole film for DmFc decreases from 2.1×10^{-4} to 6.2×10^{-5} cm s^{-1} illustrating the steric constraints generated by the light-induced attachment of BSA molecules to the polymerized benzophenone groups. It should be noted that the same experiment carried out with a nonirradiated poly **1** electrode does not lead to a decrease in DmFc permeation through the polymeric film. The IR spectrum of the poly **1** film showed before the irradiation step, identical main peaks than the monomer **1** due to pyrrole, ketone and ester stretching. In contrast, the irradiated electrode presents three main broad IR bands centred on 3350, 1670 and 1550 cm^{-1}. The latter are similar to those observed for the IR spectrum of BSA, namely 3500, 1690 and 1550 cm^{-1} (amide absorption bands) and hence indicate the successful photografting of the protein.

In order to corroborate the photoimmobilization of protein on the functionalized polypyrrole film, two poly **1** platinum electrodes were soaked in an aqueous solution of glucose oxidase (GOX), one being irradiated and the other being in the dark. In the presence of oxygen this enzyme catalyzes the oxidation of glucose and the production of H_2O_2. Consequently, the presence of immobilized GOX was detected through the electrochemical oxidation of H_2O_2 at the underlying electrode surface.[12] The maximum current density (J_{max}) determined at glucose saturating conditions was markedly higher for the irradiated poly **1** electrode (5.5 μA cm^{-2}) than for the electrode unexposed to light (1.4 μA cm^{-2}). Since J_{max} is directly proportional to the enzyme loading, this difference clearly indicates that the photografting process leads to the immobilization of a higher amount of GOX than a simple non-specific adsorption. In addition, the comparison between this value and the J_{max} value (3.7 μA cm^{-2}) corresponding to a monomolecular layer of biotinylated GOX immobilized *via* avidin–biotin bridges,[13] illustrates the efficiency and the non-denaturating character of the GOX attachment by irradiation.

The results described herein demonstrate the easy and electrochemically controlled elaboration of a polypyrrolic film exhibiting photografting abilities for biomolecule immobilization. This grafting technique can be useful for supporting bioactive material but could affect its 3D structure. The usefulness of the method, consequently, may be hampered by a loss of biological activity that must be controlled in each case.

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8 3-Benzoylbenzoic acid (2 mmoles) and dicyclohexylcarbodiimine (2 mmoles) were dissolved in CH_2Cl_2 (20 cm^3). The resulting solution was reacted with 1-(3-hydroxypropyl)pyrrole (1.66 mmoles) and dimethylaminopyridine (0.66 mmoles) for 48 h in a dry box. After addition of water, the product was extracted with CH_2Cl_2. The extract was dried over Na_2SO_4 and filtered and CH_2Cl_2 was removed on a rotary evaporator. The product was dissolved in Et_2O and purified by chromatography on a silica column eluted with CH_2Cl_2 to give a white product. The yield was 61%.
9 H^1 NMR (200 MHz, $CDCl_3$) data for **1** : δ(ppm) 2.20(m,2H) 4.10(t,2H), 4.29(t,2H), 6.10(t,2H), 6.60(t,2H), 7.52(m,4H), 7.80(d,2H), 7.98(d,1H), 8.22(d,1H), 8.40(s,1H). FTIR(CsI) data for **1**: 3099, 3064, 2960, 1721, 1661, 1600 cm^{-1}. Mass data for **1**: 333, 209, 152, 124.
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11 Poly **1** electrodes were placed in deoxygenated aqueous solutions containing bovine serum albumin or glucose oxidase (5 mg ml^{-1}) and were irradiated at room temperature for 1 h with a high pressure mercury lamp (250 W) through IR and UV cutoff filters with a surface light intensity of 200 mW cm^{-2}.
12 After incubation with GOX, the poly **1** electrodes were thoroughly rinsed with 0.1 M phosphate buffer. The amperometric measurements of glucose were carried out in stirred air-saturated 0.1 M phosphate buffer (pH 7) by potentiostating the modified electrodes at 0.6 V *versus* an aqueous saturated calomel electrode.
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