Permeability improvements of electropolymerized polypyrrole films using dissolvable nano-CaCO₃ particle templates

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To overcome this issue, nanoporous structures as bioelectrochemical interfaces were proposed using carbon nanotubes, template-assisted or self-organized formation of nanostructure polymers. In this context, we report here the original combination of functionalized polypyrrole films with CaCO₃ nanoparticles as temporary templates. The strategy consists in the initial deposition of CaCO₃, followed by the electropolymerisation of the pyrrole monomer and, finally, the dissolution of the carbonate particles leading to a highly porous functional polypyrrole film.

Experimental part

Methods and instrumentation

The calcium carbonate nanoparticles (nano-CaCO₃) were prepared and characterized by ultrasonic precipitation using organic additives, 11-Pyrrol-1-yl-undecanoic acid, (11-pyrrolyl-1-undecyl) triethyl-ammonium tetrafluoroborate (poly(pyrrole-ammonium)) were synthesized as described. Histidine-tagged glucose oxidase was synthesised as described previously in the literature.

All other reagents were of analytical grade and used as received without further purification. Na₂HPO₄, NaH₂PO₄ and glucose were purchased from Sigma whereas hydroquinone and CuCl₂ were obtained from Prolabo. All aqueous solutions were prepared in deionized distilled water. Solutions of glucose were prepared daily and stored overnight to reach mutarotational equilibrium before use. LiClO₄ was purchased from Fluka and acetonitrile (HPLC grade) from Rathburn.

Electrochemical investigations were performed using an Autolab potentiostat 100 (EcoChemie, Utrecht, The Netherlands). All electrochemical studies were performed using a conventional three-electrode system. A saturated calomel electrode (SCE) was used as a reference in water solution. An Ag/Ag⁺ electrode (AgNO₃ 10⁻² mol L⁻¹ in CH₃CN + 0.1 mol L⁻¹ LiClO₄) was used as reference in the acetonitrile electrolyte. The working electrodes were of platinum and glassy carbon (diameters 3 and 5 mm). These electrodes were carefully polished with 1 μm diamond paste and
then were rinsed with acetone and ethanol before use. The electrochemical experiments were carried out on an Autolab PGstat 100 potentiostat and the amperometric experiments on a Tacussel PRG-DL potentiostat.

Permeability measurements were carried out using the hydroquinone redox probe (1 × 10⁻³ mol L⁻¹) in phosphate buffer solution (0.1 mol L⁻¹, pH 7.0). The rotating disk voltammograms were recorded at 20 mV s⁻¹, at rotation rates varying from 250 to 1000 rpm.

Topographic images were recorded using a Keyence 3D laser scanning microscope.

Elaboration of modified electrodes

A nano-CaCO₃ colloidal suspension (4 mg mL⁻¹) was prepared by stirring nano-CaCO₃ in deionized water overnight. After sedimentation of undispersed particles, the supernatant was sonicated for 5 minutes in order to destroy aggregates leading to a homogenized size distribution of the nanocrystals, 13 The average CaCO₃ particle sizes obtained in this way are shown in Table 1. The sizes are in submicron ranges, and about 10% have sizes below 200 nm, which allowed, according to our technique presented above, the separation of nano-sized CaCO₃ particles to be used in preparing the modified electrodes. 20 µL of the obtained aqueous nano-CaCO₃ dispersion was cast on the electrode surface and dried at 60 °C in an oven for 5 minutes. This procedure led to an adherent nano-CaCO₃ film and was repeated 2 times.

This step is followed by electropolymerisation of the pyrrolic monomers. In the case of amphiphile pyrrole-ammonium electro-polymerisation, a drop of the mixture of pyrrole-ammonium (3 × 10⁻³ mol L⁻¹ in H₂O) and CaCO₃ (4 mg mL⁻¹) in a ratio of 1/5 was deposited on the surface of the layers of CaCO₃, dried at room temperature and then electropolymersised in H₂O + 0.1 mol L⁻¹ LiClO₄ at +0.72 V vs. SCE. For pyrrole-NTA electropolymerisation, a drop of pyrrole-NTA (4 × 10⁻³ mol L⁻¹ in CH₃CN) was spread onto the electrode surface, dried at room temperature and then electropolymersised at +0.9 V vs. Ag/Ag⁺ in CH₃CN + TBAP (0.1 mol L⁻¹). In order to evaluate the permeability of the films, the electroreactivity of both polypyrrole films was destroyed by electrochemical overoxidation of the polymers.

To dissolve CaCO₃ particles, the electrodes were stirred in HCl solution at pH 2 (10⁻² mol L⁻¹) for 1 h and then washed under stirring in H₂O + 0.1 mol L⁻¹ LiClO₄ for 30 min.

To immobilise the histidine-tagged glucose oxidase (GOX), the modified electrodes (before or after treatment with acid) were immersed in CuCl₂ (10⁻² mol L⁻¹) and in acetate buffer solution (0.1 mol L⁻¹, pH 4.8) for 20 minutes. The excess of CuCl₂ was removed by successively stirring the modified electrodes in 0.5 mol L⁻¹ sodium chloride solution for 5 minutes and then in phosphate buffer solution (0.1 mol L⁻¹, pH 7) for 5 minutes. The resulting electrodes were finally dried at room temperature. Then 20 µL of phosphate buffer (0.1 mol L⁻¹, pH 7) containing histidine-tagged GOX (2 mg mL⁻¹) was deposited on the electrode surface for 15 min. The electrodes were rinsed for 5 minutes by stirring in phosphate buffer solution (0.1 mol L⁻¹, pH 7) to remove not firmly immobilized enzymes.

Results and discussion

Permeability studies

To evaluate the permeability improvements of this template-assisted nanostructuring of functional polymer films, two N-substituted pyrrole derivatives were electropolymerized on the nano-CaCO₃ deposits by controlled potential electrolysis (CPE) (2.58 mC cm⁻²). Nano-CaCO₃ particles with different sizes and structures were studied to determine the most appropriate template material (see characteristics in Table 1).

The permeability of the modified electrodes was evaluated firstly via the electrochemical behaviour of hydroquinone as the redox probe. Fig. 1A shows the cyclic voltammograms of hydroquinone (10⁻² mol L⁻¹) within the potential range −0.4 V to 0.6 V in 0.1 mol L⁻¹ phosphate buffer (pH 7) (a) at a bare glassy carbon electrode, (b) after electropolymerization of pyrrole-ammonium on the nano-CaCO₃ (P5) deposit, and (c) after dissolution of the nano-CaCO₃ templates in acidic aqueous solution. As expected, a decrease in peak system intensity of the redox probe at Em,pa = 0.40 V and Ep,pc = −0.18 V was observed. For instance, the cathodic peak current intensity

![Fig. 1](image-url)
for hydroquinone decreases from $-80$ to $-45$ $\mu$A (Fig. 1A, curves a and b). This phenomenon is simply due to the formation of the template-polymer layer, which constitutes a barrier towards the diffusion of hydroquinone. After dissolution of the nano-CaCO$_3$ templates in acidic aqueous solution, the anodic and cathodic current intensities of the redox couple increase. This clearly indicates a better diffusion of hydroquinone due to an improved permeability of the polymer film. To confirm these results, electrochemical permeability measurements were performed using rotating disk electrodes. The Koutecky–Levich curves were obtained by plotting the current intensities of the redox probe as a function of the inverse square root of the rotation speed, 250–1000 rpm (Fig. 1B). The steady-state limiting currents were obtained for the matrix CaCO$_3$-poly(pyrrole-ammonium) before and after incubation in acidic aqueous solution.

The plots of the reciprocal of the limiting current versus the reciprocal of the square root of the rotation rate for both template-poly(pyrrole-ammonium) modified electrodes, before and after dissolution of nano-CaCO$_3$, show a linear behaviour with the same slope as for a bare glassy carbon electrode (data not shown). As previously reported$^{14}$ these straight lines give a positive intercept whose values depend on the permeability of the films. The permeability value (Pm) increases from $3.1 \times 10^{-2}$ cm s$^{-1}$ to $6.0 \times 10^{-2}$ cm s$^{-1}$ after dissolution of the P5 nano-CaCO$_3$ template sample by soaking in acidic aqueous solution (pH 2) for one hour. After the removal of the nano-CaCO$_3$ template, cavities are formed within the polymer film leading to a better diffusion of hydroquinone through the polymeric layer. It is noteworthy that this nanostructure poly(pyrrole-ammonium) film shows increased permeability compared to those reported for similar polypyrrole films.$^{15}$

To evaluate the influence of size and morphology of the nano-CaCO$_3$ particles on the permeability after dissolution, identical experiments were performed using different particle sizes and structures of CaCO$_3$ (aragonite, calcite, and vaterite). The results are summarized in Table 1.

It appears that the highest permeabilities for electrogrogenated and nanostructured poly(pyrrole-ammonium) films were obtained using the P4 and P5 CaCO$_3$ samples. These carbonates have a big amount of calcite and a high number of spherical particles (with the vaterite structure). It is also known that vaterite is better soluble than aragonite in aqueous solution.$^{16}$

As expected, after acid treatment the permeability increases for all composites. In detail, using the P3 carbonate sample, the permeability of the polymer film greatly increased (3.31 times) after template dissolution. The obtained value ($5.3 \times 10^{-2}$ cm s$^{-1}$) is close to the best value obtained when using the P5 carbonate sample ($6.0 \times 10^{-2}$ cm s$^{-1}$). This could be explained by the increased average particle sizes of the P3 carbonate sample that appears to be advantageous for the film permeability.

With the aim to demonstrate the benefits of such template-assisted nanostructuring of polymer films for biosensing applications, the influence of such a procedure on the efficiency of protein immobilization was examined with GOX as the enzyme model. Taking into account that poly(pyrrole-ammonium) films were mainly used for the entrapment of enzymes during their electrogrogenation process, the dissolution of the nano-CaCO$_3$ template inside the polypyrrole-GOX matrix should induce a drastic loss of GOX activity. For this reason and to confirm the appropriateness of this setup for other polypyrrole films, this procedure was applied to the elaboration of biosensors with polypyrrole-NTA films. With such polymers, the anchoring of proteins occurs after the polymer formation via the coordinative affinity system NTA/Cu$^{2+}$/histidine-tagged protein and hence can be carried out after acid treatment. Moreover, the amount of attached proteins should be proportional to the presence of nanopores within the polypyrrole backbone. As previously described for poly(pyrrole-ammonium) films, the same permeability investigations were performed for polypyrrole-NTA films under identical conditions revealing an increase of permeability after acid treatment by using the P5 sample. As a control experiment, a polypyrrole-NTA film was electrogrogenated without calcium carbonate nanoparticles. This film shows permeabilities of $4.45 \times 10^{-3}$ cm s$^{-1}$ before and of $3.57 \times 10^{-3}$ cm s$^{-1}$ after acid treatment. Such acid treatment reduces, therefore, the permeability of the polypyrrole film, contrary to the polypyrrole-NTA film after dissolution of the nano-CaCO$_3$ particles.

The cavities formed in the polymer matrix after the dissolution of nano-CaCO$_3$ particles were analyzed using a 3D laser scanning microscope. Fig. 2A shows the 3D topography of a poly(pyrrole-NTA) film electropolymerized onto a drop-cast nano-CaCO$_3$ deposit. The surface of the electrogrogenated polymer is relatively uniform and shows an average roughness of 400 nm (Fig. 2A). After dissoluion of the nano-CaCO$_3$ template, the topography of the electrode surface became cragged again showing cavities within the size distribution of the nano-CaCO$_3$ templates (Fig. 2B). The average roughness increased to 800 nm.

These nano-structured polypyrrole-NTA films were formed on Pt-electrodes and tested towards the immobilization of histidine-tagged GOX to determine the influence of the change in the polymer topography. For this purpose, the resulting biocomposites were applied in the electro-enzymatic detection of glucose as a model for the enzymatic reaction. GOX catalyses the oxidation of glucose to gluconolactone and H$_2$O$_2$ in the presence of dioxygen. The bioelectrodes were, therefore, polarized at 0.70 V in 0.1 mol L$^{-1}$ phosphate buffer (pH 7, 25 °C) in order to detect, by electro-oxidation, the enzymatically generated H$_2$O$_2$. Fig. 3 shows the anodic current response of the enzyme electrodes as a function of the glucose concentration for the nano-CaCO$_3$-polypyrrole-NTA-Pt-electrode (a) before and (b) after acid treatment.

![Fig. 2](https://via.placeholder.com/150)

**Fig. 2** Confocal 3D microscope images of glassy carbon electrodes modified with nano-CaCO$_3$-polypyrrole-NTA (A) before and (B) after acid treatment.
The glucose sensitivity, determined by the slope of the linear part of the calibration curves, is 0.28 and 1.00 mA mol⁻¹ L cm⁻² for the configuration before and after acid treatment, respectively (Fig. 3). The drastic improvement in glucose sensitivity by a factor of 3.57 reflects a better permeation of substrates and products of the enzyme reaction through the composite polymer due to the nanocavities and channels chemically generated by the CaCO₃ dissolution.

The obtained calibration curves also reveal a twofold increase in the maximum current density (Jₘₐₓ) from 5 to 10 μA cm⁻² after template dissolution. Since Jₘₐₓ is directly proportional to the amount of immobilized biocatalytic units, this corroborates the increase in the immobilized amount of histidine-tagged GOX. The template-assisted nanostructuring of poly(pyrrole-NTA) films leads to a higher specific surface of the polymer with a larger accessibility for biomolecules of high molecular mass like GOX. Even when these performances are still slightly below those of other glucose sensor setups using nanostructured electrodes,¹⁷,¹⁸ this principle can still be optimized and even combined with other structuring strategies.

Conclusion

In this article, we present an elegant alternative for template-assisted nanostructuring of electrogenerated polymer films using CaCO₃ nanoparticles. The polymer was formed on the nanostructured templates, which led, after dissolution of the template, to a highly porous polymer film. Two N-substituted polypyrrole films, electrogenerated by potentiostatic methods were tested, clearly demonstrating a permeability increase of these polymeric materials. Moreover, this template procedure creates a nanoporous polymeric structure that allows the permeation and the anchoring of macro-molecular biomolecules opening attractive perspectives for the development of immunosensors and protein sensors.

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Notes and references