Hydrophobic magnetic nanoparticles induce selective bioelectrocatalysis

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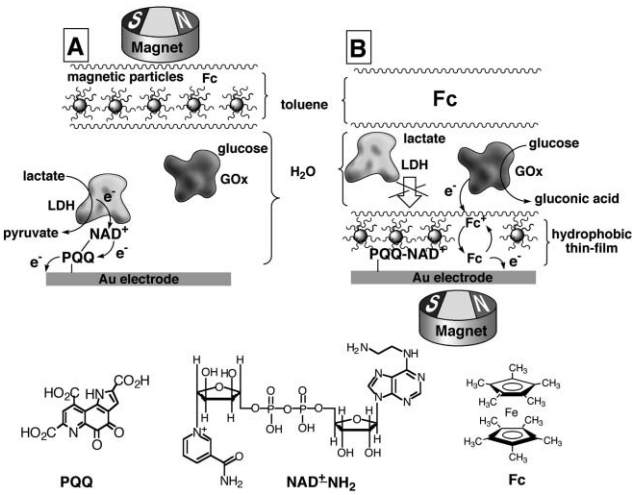
Hydrophobic magnetic nanoparticles were used to sense selectively lactate and glucose at the same applied potential.

Magnetic particles are widely used for separation of bioaffinity complexes,[1] and for the construction of magnetic nanostructures.[2] Recently, functionalized magnetic particles were employed for the ON–OFF switching of bioelectrocatalytic processes.[3b] Also, the rotation of functionalized magnetic particles on electrode supports was used to amplify bioelectrocatalytic reactions and biosensing processes such as DNA or antigen–antibody interactions.[3] Magnetic nanoparticles (NPs) modified with a hydrophobic capping layer were used to control the hydrophilic–hydrophobic properties of electrode surfaces.[4] By the magnetic attraction or retraction of the magnetic NPs to or from the electrode surface, electrochemical and electrocatalytic processes at the electrode could be regulated, and electroactive species confined to the electrode surface could change reversibly their redox properties from "aqueous-type" to organic phase behavior.[5] The biosensing of different analytes by enzyme-electrodes is often perturbed by the non-specific electrochemical oxidation (or reduction) of interfering compounds. For example, the electrochemical detection of glucose is interfered by ascorbate, uric acid or paracetamol.[6] Also, the parallel detection of different analytes in a mixture is a challenging topic in bioelectronics. Redox-functionalized magnetic particles were used to analyze different substrates by tuning the electrochemical potential regions applied on the system.[7] Here we report on the application of magnetic NPs capped with a hydrophobic layer, and a two-phase toluene–aqueous electrolyte assembly, as a system that enables the parallel sensing of two analytes, *e.g.*, lactate or glucose, and the specific analysis of a substrate in the presence of an interfering compound, *e.g.*, the analysis of glucose in the presence of ascorbate.

The system for the parallel analysis of lactate and glucose is depicted in Scheme 1. A Au electrode (0.3 cm^2 area exposed to the solution) is modified with a pyrroloquinoline quinone (PQQ) monolayer to which N^6-(2-aminoethyl)-β-nicotinamide adenine dinucleotide (NAD^+-NH_2) is covalently linked (surface coverage *ca.* 1.1×10^{-10} mol cm^{-2}).[8] The system consists of two phases. The enzymes lactate dehydrogenase (LDH, EC 1.1.1.27 from rabbit muscle, type II) and glucose oxidase (GOx, EC 1.1.3.4 from *Aspergillus niger*) and the respective substrates, lactate and glucose, are solubilized in the lower aqueous electrolyte phase (0.1 M phosphate buffer pH = 8.0). The upper phase consists of toluene in which hydrophobic undecanoate-capped magnetite NPs (average diameter *ca.* 4.5 nm, saturated magnetization 38.5 emu g^{-1}) are solubilized, 1 mg mL^{-1}.[4] Also, the hydrophobic decamethylferrocene (Fc), 2×10^{-3} M, is solubilized in the toluene phase. Fig. 1(A) shows the cyclic voltammograms of the PQQ–NAD^+-modified electrode in the absence of lactate in the aqueous phase, curve (a), and in the presence of two different concentrations of lactate, curves (b) and (c), respectively. In the absence of lactate only the cyclic voltammogram of PQQ is observed, $E^\circ = -0.16$ V *vs.* SCE (pH = 8.0). Addition of lactate results in the formation of electrocatalytic anodic currents, due to the oxidation of lactate biocatalyzed by LDH and mediated by the NAD^+–PQQ-dyad.[8] As the concentration of lactate is elevated, the electrocatalytic anodic current is enhanced. That is, the LDH biocatalyzed oxidation of lactate to pyruvate yields NADH that is re-oxidized by PQQ. The electrochemical oxidation of $PQQH_2$ leads to the continuous oxidation of the biocatalytically generated NADH, and to the observed bioelectrocatalytic anodic current, Scheme 1(A). Fig. 1(C), curve (a) shows the derived calibration curve corresponding to the amperometric responses of the system at different concentrations of lactate. It should be noted that the system in this configuration does not lead to any oxidation of glucose due to the lack of an electron mediator for activating GOx. Upon exclusion of lactate from the aqueous phase, and adding different concentrations of glucose to the aqueous phase, no amperometric responses are observed, Fig. 1(C), curve (b). In fact, the amperometric responses of the system in the presence of lactate



Scheme 1 Magneto-switchable selective sensing of glucose or lactate using the hydrophobic magnetic NPs for gating the bioelectrocatalytic processes: (A) The magnetic NPs are retracted from the electrode. (B) The magnetic NPs are attracted to the electrode.

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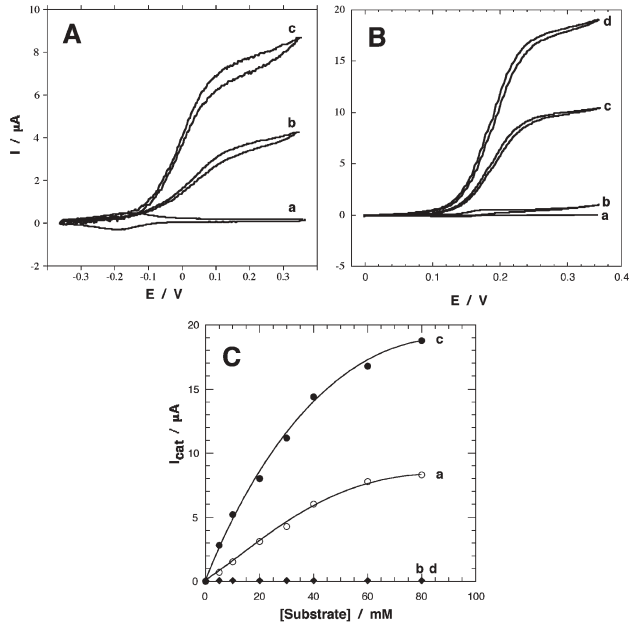
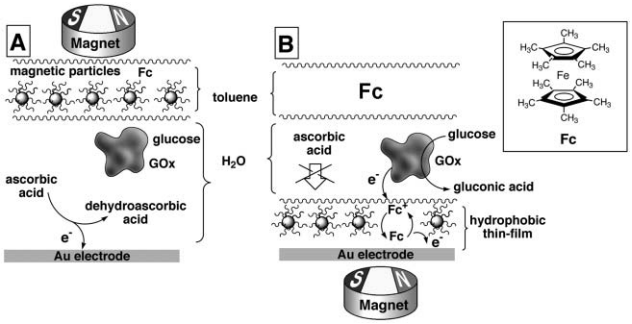


Fig. 1 Cyclic voltammograms recorded at the NAD^+–PQQ-functionalized Au electrode in the presence of GOx and LDH in the aqueous solution and hydrophobic magnetic NPs in the toluene phase: (A) Upon retraction of the magnetic NPs from the electrode surface and in the presence of different concentrations of lactate in the aqueous phase: (a) 0 mM, (b) 20 mM, (c) 80 mM. (B) Upon attraction of the magnetic NPs to the electrode surface: (a) in the absence of Fc in the toluene phase and in the presence of glucose, 50 mM, and lactate, 50 mM, in the aqueous phase. In the presence of Fc in the toluene phase and in the presence of different concentrations of glucose in the aqueous phase: (b) 0 mM, (c) 20 mM, (d) 80 mM. (C) Calibration plots derived from the respective cyclic voltammograms (at E = 0.35 V *vs.* SCE) in the presence of variable concentrations of: (a) lactate upon retraction of the magnetic NPs from the electrode, (b) glucose upon retraction of the magnetic NPs from the electrode, (c) glucose upon attraction of the magnetic NPs to the electrode, (d) lactate upon attraction of the magnetic NPs to the electrode. The data were recorded in the presence of GOx (1 mg mL^{-1}) and LDH (1 mg mL^{-1}) in the aqueous solution (0.1 M phosphate buffer, pH = 8.0) and magnetic NPs (1 mg mL^{-1}) and Fc (2×10^{-3} M) in the toluene phase. Potential scan rate, 5 mV s^{-1}.

are not affected by any concentration of glucose in the aqueous phase. Attraction of the hydrophobic magnetic NPs to the electrode support, by means of an external magnet (NdFeB/Zn-coated magnet with the remanent magnetization of 10.8 kG), alters the bioelectrocatalytic functions of the system, Scheme 1(B). Fig. 1(B), curve (b) shows the cyclic voltammogram of the system in the absence of glucose and lactate in the aqueous phase. Only the weak response (due to the slow scan rate) of the ferrocene derivative (Fc) is observed, E° = 0.16 V *vs.* SCE. Exclusion of Fc from the toluene phase, but in the presence of lactate, 50 mM, and glucose, 50 mM, in the aqueous phase, results in the electrical response shown in Fig. 1(B), curve (a). That is, the magnetic attraction of the magnetic NPs to the electrode support blocks the bioelectrocatalytic oxidation of lactate. Also, the results imply that upon the attraction of the hydrophobic magnetic NPs to the electrode, the ferrocene (Fc) solubilized in the toluene layer and co-adsorbed to the magnetic NPs is electrically contacted with the electrode (*vide infra*). Fig. 1(B), curves (c) and (d) show the cyclic voltammograms of the system recorded in the presence of different concentrations of glucose, when the hydrophobic magnetic NPs carrying Fc are attracted to the electrode. Bioelectrocatalytic anodic currents, as a result of the oxidation of glucose, are observed, and as the concentration of glucose is elevated the anodic currents are higher. The oxidation of glucose biocatalyzed by GOx is mediated by decamethylferrocene (Fc) as schematically depicted in Scheme 1(B). The hydrophobic magnetic NPs transported by means of the external magnet, from the toluene phase to the electrode surface, carry with them a thin co-adsorbed layer of toluene.[5] This organic thin film includes the hydrophobic ferrocene derivative (Fc), that activates the bioelectrocatalyzed oxidation of glucose. That is, the electrogenerated ferrocenyl cation mediates the oxidation of the flavoenzyme GOx, and this catalyzes the oxidation of glucose to gluconic acid. Fig. 1(C) curve (c) shows the derived calibration curve, corresponding to the bioelectrocatalytic anodic currents in the presence of variable concentrations of glucose. It should be noted that the bioelectrocatalytic oxidation of lactate is totally blocked by the attracted magnetic NPs with the co-adsorbed toluene, Fig. 1(C), curve (d). Also, the amperometric analysis of glucose is not interfered with or affected by any lactate concentration in the system. Retraction of the magnetic NPs from the electrode support by means of the external magnet regenerates the bioelectrocatalytic activity of the system towards the oxidation of lactate, while the biocatalyzed oxidation of glucose is blocked. Thus, the hydrophobic magnetic NPs allow the selective analysis of the substrates by two different enzymes.

The use of hydrophobic magnetic NPs to eliminate the perturbation of the amperometric biocatalytic analysis of a substrate by an electroactive interferant is exemplified in Scheme 2, using ascorbate as a typical interferant for the bioelectrocatalytic analysis of glucose. The system consists of a two-phase aqueous–toluene system. The aqueous phase includes glucose, GOx and the interfering compound, ascorbate. In the upper toluene phase the hydrophobic magnetic NPs, 1 mg mL^{-1}, and decamethylferrocene (Fc), 2×10^{-3} M, are solubilized. Fig. 2(A) shows the cyclic voltammograms of the system upon dissolution of different concentrations of ascorbic acid in the aqueous phase. As the concentration of ascorbate increases, the amperometric responses of the system as a result of the electrochemical oxidation of ascorbate are enhanced. Fig. 2(C), curve (c), shows the derived calibration curve corresponding to the



Scheme 2 Magneto-switchable selective sensing of glucose or ascorbate using the hydrophobic magnetic NPs for gating the bioelectrocatalytic processes: (A) The magnetic NPs are retracted from the electrode. (B) The magnetic NPs are attracted to the electrode.

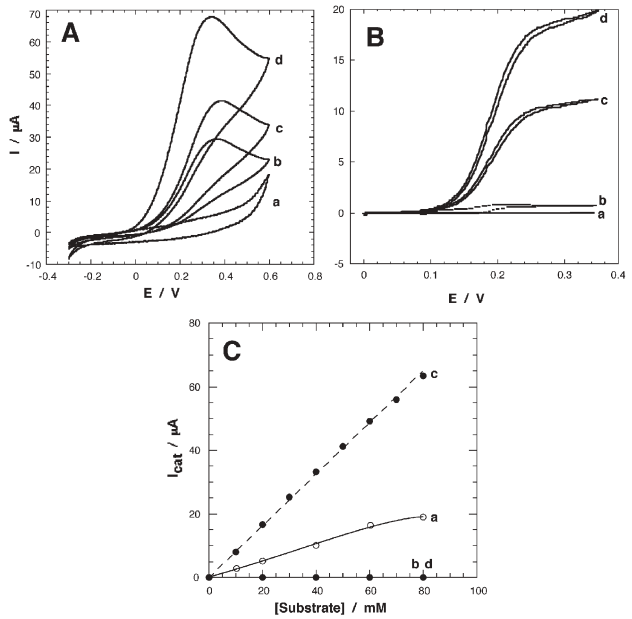


Fig. 2 Cyclic voltammograms recorded on a Au electrode in the presence of GOx in the aqueous solution and hydrophobic magnetic NPs in the toluene phase: (A) Upon the retraction of the magnetic NPs from the electrode surface and in the presence of different concentrations of ascorbate in the aqueous phase: (a) 0 mM, (b) 30 mM, (c) 50 mM, (d) 80 mM. (B) Upon the attraction of the magnetic NPs to the electrode surface: (a) in the absence of Fc in the toluene phase and in the presence of glucose, 50 mM, and ascorbate, 50 mM, in the aqueous phase, (b)–(d) in the presence of Fc in the toluene phase and in the presence of different concentrations of glucose in the aqueous phase: (b) 0 mM, (c) 40 mM, (d) 80 mM. (C) Calibration plots derived from the respective cyclic voltammograms (at E = 0.35 V *vs.* SCE) in the presence of variable concentrations of: (a) glucose upon attraction of the magnetic NPs to the electrode, (b) glucose upon retraction of the magnetic NPs from the electrode, (c) ascorbate upon retraction of the magnetic NPs from the electrode, (d) ascorbate upon attraction of the magnetic NPs to the electrode. The data were recorded in the presence of GOx (1 mg mL^{-1}) in the aqueous solution (0.1 M phosphate buffer, pH = 8.0) and magnetic NPs (1 mg mL^{-1}) and Fc (2×10^{-3} M) in the toluene phase. Potential scan rate, 5 mV s^{-1}.

currents generated by the system upon oxidation of ascorbate. Control experiments indicate that within the entire range of glucose concentrations employed no bioelectrocatalyzed oxidation of glucose is observed because of the lack of an electron transfer mediator that activates GOx, Fig. 2(C), curve (d).

Magnetic attraction of the hydrophobic magnetic NPs with the co-adsorbed toluene layer that includes Fc to the electrode surface, results in the cyclic voltammograms shown in Fig. 2(B). In the absence of Fc in the toluene phase and in the presence of glucose/GOx and ascorbate in the aqueous phase no amperometric response is observed, curve (a). The low capacitance of the background current is consistent with the formation of a hydrophobic layer on the electrode.[4] The lack of the amperometric response implies that the oxidation of ascorbate is totally blocked, and that in the absence of the electron mediator Fc no bioelectrocatalyzed oxidation of glucose takes place. The cyclic voltammogram of the system that includes Fc in the toluene phase, but in the absence of glucose in the aqueous phase, is shown in Fig. 2(B), curve (b). Only the redox response of Fc is visible. Fig. 2(B) curves (c) and (d) show the cyclic voltammograms of the system in the presence of Fc and at different concentrations of glucose. The bioelectrocatalytic anodic currents originate from the ferrocene-mediated oxidation of glucose by GOx, Scheme 2(B). Fig. 2(C), curve (a), shows the derived calibration curve that corresponds to the amperometric responses of the system in the presence of different concentrations of glucose. The system does not react to a broad range of ascorbate concentrations, Fig. 2(C), curve (b), and the electrochemical analysis of glucose is not interfered with ascorbate. That is, the magnetic attraction of the hydrophobic magnetic NPs with the co-adsorbed toluene and co-carried Fc to the electrode surface blocks the ascorbate oxidation. Although ferrocenyl cation is known to catalyze the oxidation of ascorbate,[9] this process is presumed inefficient in the system where the electron mediator is confined to the toluene film. The electrical oxidation of Fc in the toluene thin film yields, however, the ferrocenyl derivative that activates GOx towards the biocatalyzed oxidation of glucose.

In conclusion, the present study has demonstrated the use of hydrophobic magnetic NPs in controlling bioelectrocatalytic processes. This allows the simultaneous analysis of two different substrates. The study has also revealed the use of the hydrophobic magnetic NPs in the elimination of the perturbing effects of interferants on the bioelectrocatalytic sensing of substrates.

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