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# Nanoconjugates of ferrocene and carbon-encapsulated iron nanoparticles as sensing platforms for voltammetric determination of ceruloplasmin in blood

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ABSTRACT

The nanoparticles comprising of iron core and carbon shell were decorated with ferrocene derivatives: ferrocenecarboxaldehyde (Fc-1) and ferrocenecarboxaldehyde oxime (Fc-2). A microdrop of suspension of the nanoconjugate was placed on a glassy-carbon electrode to prepare the recognition/sensing layer. Drying and purification of the sensing layer resulted in a well-defined and stable square-wave voltammogram of the ferrocene moiety. The height of the voltammetric peak increased in the presence of ceruloplasmin. That increase was linearly dependent on the logarithmic concentration of ceruloplasmin in blood. The applied external magnetic field was a factor which yielded better sensitivity and repeatability of the sensor response. The linearity of sensor response was found to be between 0.001 and 10 μg $dL^{-1}$ and 0.05–10 μg $dL^{-1}$ for both nanoconjugates: Fe@C-Fc-1 and Fe@C-Fc-2, in the presence and absence of the magnet, respectively. The obtained detection limit (LOD) for Fe@C-Fc-1 was found to be 0.60 and 0.10 μg $dL^{-1}$ in the absence and presence of magnetic field, respectively, whilst for Fe@C-Fc-2 was 0.4 and 0.07 μg $dL^{-1}$ in the absence and presence of a magnet, respectively. The proposed method is selective because the presence of common antioxidants in blood did not interfere significantly with the determination of the concentration of ceruloplasmin.

## 1. Introduction

In the last 25 years the transport of electrons in biological molecular systems, e.g. proteins and metaloproteins became one of the most frequently examined interdisciplinary (biology and electrochemistry) phenomenon (Wang, 2001). The full knowledge about electron transport in such systems and the ability of controlling that process is of the highest importance, because biologically active macromolecules are widely applied. They take part in various metabolic processes and are the key factors in the photosynthesis and breathing. They are also employed in miscellaneous scientific and commercial activities: construction of fuel biocells, electronic devices and biosensors (Freire et al., 2003). However, there is still a big challenge for electrochemists to obtain well defined and reproducible current signals of metalloproteins. Behind that problem is the fact that the electroactive centers of metalloproteins are deeply embedded in the protein shell and this fact significantly hampers the process of exchange electrons between the electrode and the protein (Xu et al., 2007).

The simplest and most wanted way of the electron transfer between metalloproteins and the electrode is the direct exchange of electrons (DET) (Ghindilis et al., 1997). To actuate the DET, the protein must be immobilized on the electrode surface. After immobilization the distance between the electroactive centre and the electrode surface should be as short as possible (Zhao et al., 2015). Therefore, the shortest distance between the prostatic group of the metalloprotein and the conducting material is only guaranteed by such orientation of the molecule (Freire et al., 2003; Kowalczyk et al., 2016; Matysiak et al., 2015a, 2015b, 2015c). As it was mentioned above, after routine immobilization of a metalloprotein on a non-modified electrode, particularly on the bare metal, it is very difficult to get the direct electron transfer. This is not only because of the improper protein orientation but also due to the protein denaturation and/or deactivation (Cracknell et al., 2008). The latter process very efficiently blocks the exchange of electrons with the electrode surface (Rusling, 1998). To avoid the deactivation of proteins the electrode surface can be covered with a variety of modifiers (Scouten et al., 1995). They have some impact on the protein orientation but also protect the electrode metal against the possible loss of the electroactivity (Stellwagen, 1978). Thiols with suitable functional

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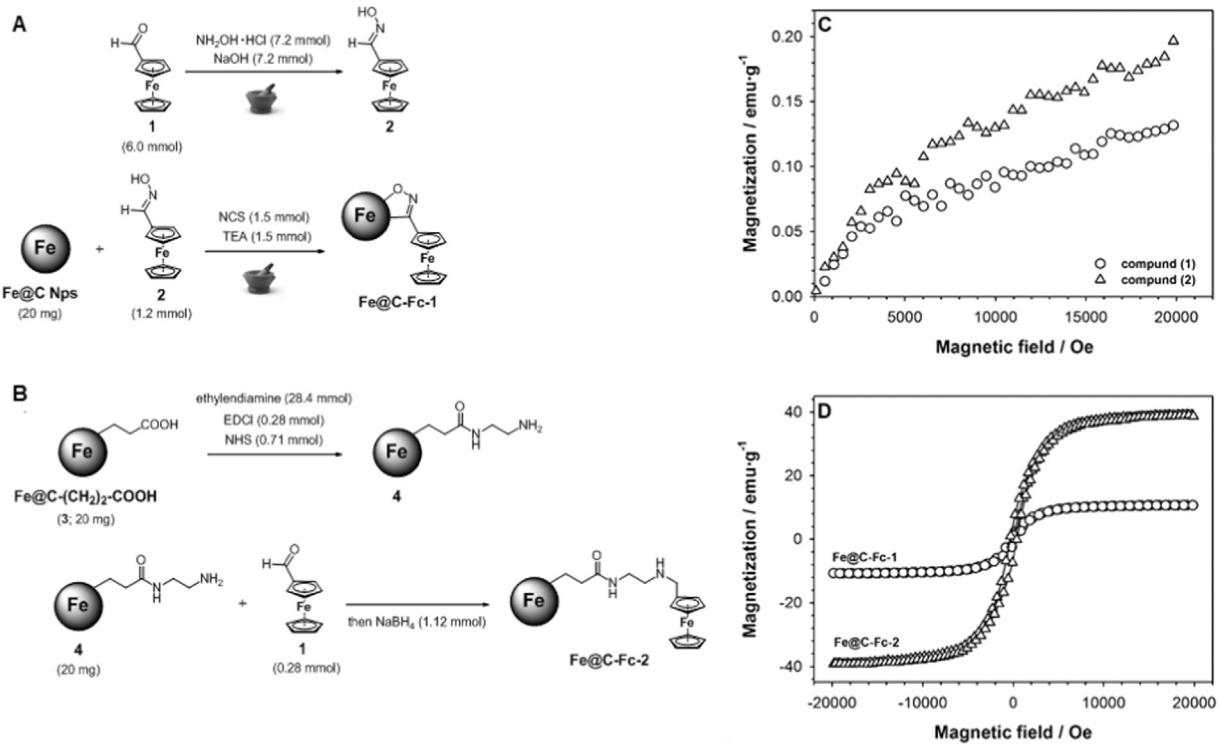


**Fig. 1.** Synthesis of Fe@C-Fc-1 (A) and Fe@C-Fc-2 (B). C: Magnetization of ferrocenecarboxaldehyde (1) and ferrocenecarboxaldehyde oxime (2). D: Magnetic hysteresis loop for nanoconjugates Fe@C-Fc-1 and Fe@C-Fc-2.

groups such as $-COOH$ and $-NH_2$ are the most frequently used modifiers (Lötzbeyer et al., 1994). Thiols are well self-organizing molecules on gold surfaces and form compact monolayers which can be further conjugated with conducting polymers (Wang et al., 2009) and nanomaterials (Ivnitski et al., 2008), including gold nanoparticles (Zhang et al., 2005) and carbon nanotubes Cai and Chen (2004).

The mediators are applied in the case of inappropriate immobilization of a protein on the electrode surface. The mediators are compounds or ions which participate in the electron transfer process (MET) (Chaubey and Malhotra, 2002). Such compounds should be stable and their electrode processes should be fast (Chaubey and Malhotra, 2002). In addition, the mediators should quickly and reversibly react with the proteins and become resistant to the changes of pH. Ideally, it is expected that the overpotential for the regeneration of the oxidized form of the mediator should be very low and the reduced form should not react with oxygen (Chaubey and Malhotra, 2002). The mediator molecules should also quickly diffuse throughout the protein channels and this feature is required for the effective transport of electrons from the protein electroactive centre to the electrode.

The electron transport with the use of a modifier can be either of homogeneous or heterogeneous in nature. The first type takes place when both: mediator and protein is in one phase and can freely diffuse (Dominguez-Benetton et al., 2013). In the heterogeneous transport, one of the participating agents, either mediator or metalloprotein, is immobilized on the electrode surface, whist the second one is present in the solution (Chaubey and Malhotra, 2002). If the mediator is anchored on the electrode surface, the stability of that component is very important. A stable binding/connection can be obtained by employing such vectors as carbon paste, colloidal compounds, composite materials, hydrogels and conducting polymers (Dominguez-Benetton et al., 2013). The presence of the mediator not only increases the electron transfer rate. Another advantage is related to the fact that the protein does not have to be in a direct contact with the electrode and therefore the risk of denaturation is substantially limited.

Ferrocene and its derivatives are ideal candidates for mediators in the electron exchange between the protein and the electrode surface. They can be easily functionalized and therefore they can possess the needed specific properties. In our research we have used novel nanoconjugates of ferrocene and carbon-encapsulated iron nanoparticles (Fe@C Nps). The voltammetric and spectroscopic characterization of the nanoconjugates have been performed. Their analytical functionality against the selected metaloproteins in the real blood samples has been demonstrated. Additionally, the magnetic properties of the nanoconjugates have been examined.

## 2. Experimental section

### 2.1. Materials and reagents

All used chemicals were of the highest purity available and are listed in Supporting information, Section 1. Carbon-encapsulated iron nanoparticles were synthesized using a carbon arc discharge route according to the procedure described elsewhere (Bystrzejewski et al., 2013). The graphite electrodes doped with Fe (45 wt%) were used as anodes. The raw product was purified in boiling 3 M HCl (8 h) with subsequent washing in water and ethanol. The purification was necessary to remove all the non-encapsulated or partially encapsulated iron nanoparticles.

Human ceruloplasmin (Cp), human transferrin (Tf), $KH_2PO_4$, $K_2HPO_4$, $K_2SO_4$, were obtained from Sigma-Aldrich. The initial Cp solution of 10 μg $dL^{-1}$ (determined as suggested in (Ehresmann et al., 1973) was prepared in 0.02 M phosphate buffer (PB) with addition of 0.150 M $K_2SO_4$ of pH 7.0 and was stored at + 4 °C until use. All solutions were prepared using Milli-Q water of a conductivity of 0.056 μS $cm^{-1}$.

#### 2.1.1. Synthesis of material Fe@C-Fc-1 (Fig. 1A)

The synthesis was based on the grinding-induced 1,3-cycloaddition reaction described elsewhere (Kasprzak et al., 2017). In brief, first step of the protocol covered the synthesis of ferrocenecarboxaldehyde oxime