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Enzyme immobilization on a nanoadsorbent for improved stability against heavy metal poisoning

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ABSTRACT

Magnetic nanoparticles modified with siloxane layers bearing amino and thiol functions have been used for immobilization of urease either by adsorption or via surface grafting. The activity of the immobilized enzyme in the hydrolysis of urea extended to the levels typical of the native enzyme, while its long-term stability in combination with magnetic retraction opened for its repeated use in both analysis and detoxification of bio-fluids. The immobilized urease revealed strongly enhanced stability and 65% activity in the presence of 0.1 mmol/l of Hg^{2+} or 0.3 mmol/l of Cu^{2+} while the native urease did not retain any activity at all. The enzyme grafting was shown to be a potentially perspective tool in alleviation of heavy metal poisoning and to be providing an opportunity for use of the developed adsorbents as both biosensors and bio-reactants for removal of urea from biofluids.

1. Introduction

Express-analysis of the urea content is an important quest for pharmaceutic and food industries and also for environmental monitoring. Its most important applications are, however, in the biomedical and clinical fields. Urea is the most important byproduct of protein metabolism and major organic component of the urine. It is formed in liver and released via kidneys. Certain pathologies such as kidney malfunction, hyperpyrexia, hyperthyroidism, leukemia, burns, dyspeptic diseases, and diabetes have a footprint in the observed levels of urea concentration (2.5–7.5 mmol/l in blood and 10–30 g in total amount of urine released in 24 h) [1,2]. Monitoring of urea in both blood plasma and in urine is thus of prime importance [3]. Urea is commonly detected spectrophotometrically [4], but a few alternative methods have also been elaborated. The urea hydrolysis by urease enzyme has been used for calorimetric [5–7], amperometric [8,9], conductometric [10,11], piezoelectric [12], optical [13] and potentiometric [14,15] detection of urea.

Heavy metals (HM) are broadly spread in nature and exert dual action on the living beings [16–18]. On one hand, even for human beings, they are essential for supporting multiple regular body functions. On the other hand, accumulation of HM in the body is associated with a multitude of health problems. HM represent also destructive and potent environmental hazards, making their detection essential for maintaining of human health [19–21]. An efficient tool for these purposes is exploiting the effects of decrease in the activity of immobilized enzymes in the presence of HM [22]. It is known, that silver and copper ions precipitate proteins and mercury ions interact with -SH groups of proteins deactivating them. In many cases the enzymes inhibited via interaction with HM can be regenerated by treatment with chelating agents, such as, for example, EDTA [23]. The tests based on immobilized enzymes can thus be reused making their application economically feasible. The urease based tests are cost-efficient and highly sensitive, which explains their success in practical application [24]. Monitoring procedures for HM detection, exploiting enzymatic hydrolysis of urea with formation of carbon dioxide and ammonia are well established [25,26]. This work is taking a new step from our earlier research on enzyme immobilization [27,28] by challenging the enzyme activity via poisoning the solution with heavy metal ions.

We aimed here to evaluate the possibility of using urease immobilized on magnetite nanoparticles for quantitative destruction of pathologic urea concentrations in model biological media. We sought also to investigate whether the immobilization was capable to render an enzyme formulation able to resist the heavy metal poisoning and to what extent.

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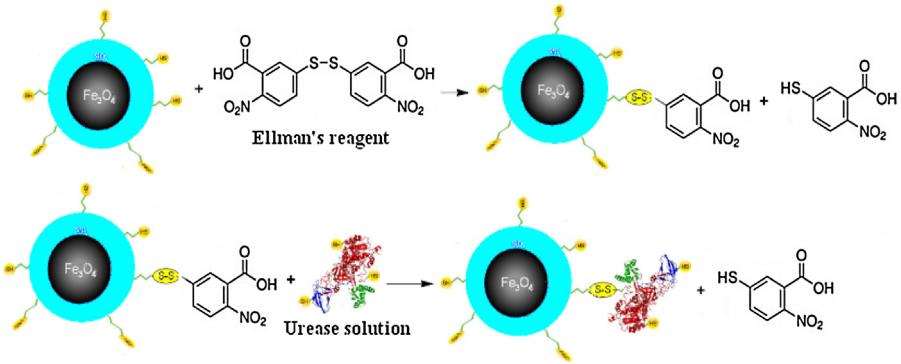

Scheme 1. Grafting of urease on magnetic nanoparticles using Ellman's reagent.

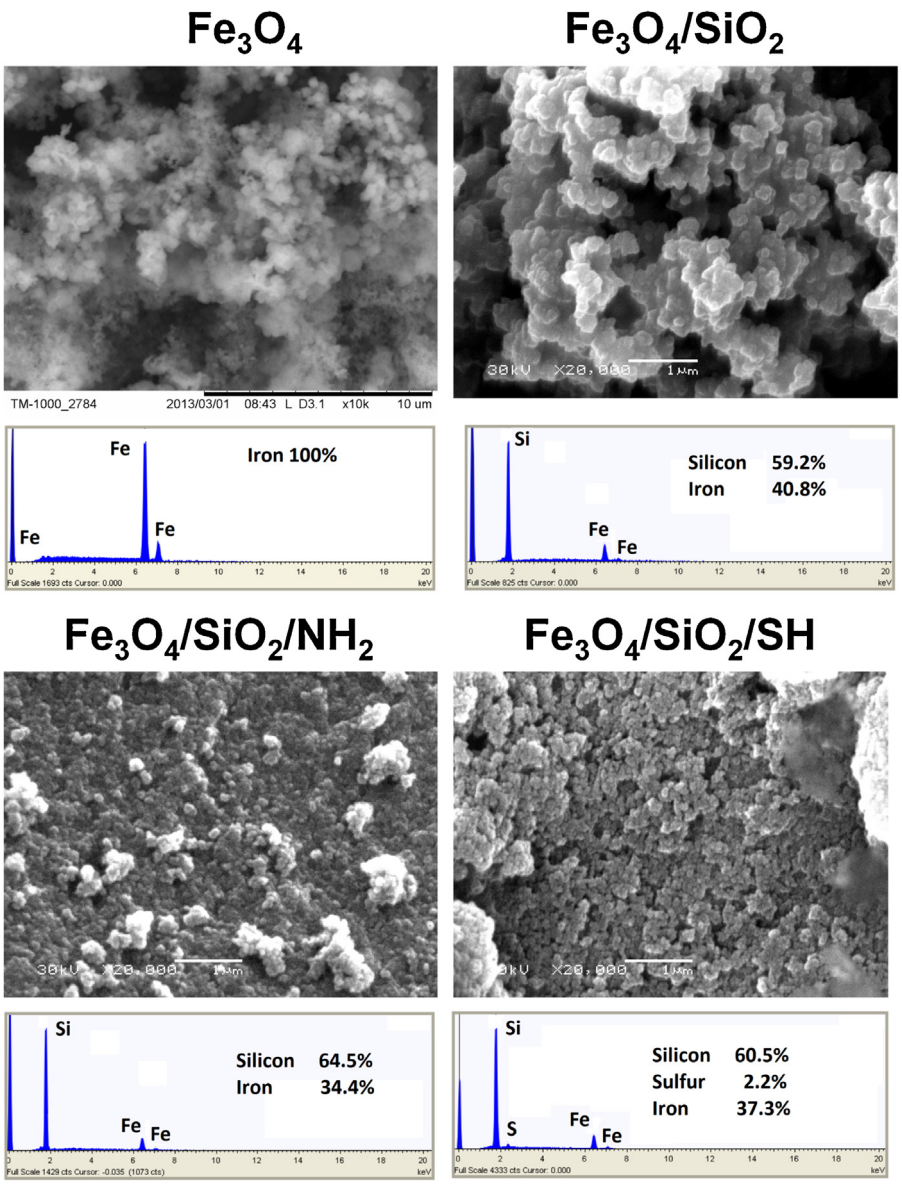

Fig. 1. SEM micrographs and EDS analysis of initial and functionalized magnetite particles.

2. Experimental

The following reagents were used in the present study: tetraethoxysilane, $Si(OC_2H_5)_4$ (TEOS, Aldrich, 98%); 3-aminopropyltriethoxysilane, $(C_2H_5O)_3Si(CH_2)_3NH_2$ (APTES, Aldrich, 99%); 3-mercaptopropyltrimethoxy-silane, $(CH_3O)_3Si(CH_2)_3SH$ (MPTMS, Aldrich, 95%); iron(II) chloride tetrahydrate, $FeCl_2{\cdot}4H_2O$ (Aldrich, 97%), iron(III) chloride