



Charge binding of rhodamine derivative to OH⁻ stabilized nanomaghemite: Universal nanocarrier for construction of magnetofluorescent biosensors

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ARTICLE INFO

Article history:

Received 11 October 2011

Received in revised form 3 February 2012

Accepted 6 February 2012

Available online 13 February 2012

Keywords:

Maghemite nanoparticles

Rhodamine isothiocyanate

Glucose oxidase nanocatalyst

Biosensor

OH⁻ groups

ABSTRACT

Superparamagnetic nanoparticles (20–40 nm) of maghemite, $\gamma\text{-Fe}_2\text{O}_3$, with well-defined stoichiometric structure, are synthesized by the borohydride reduction of ferric chloride at an elevated temperature (100 °C) followed by thermal treatment of the reaction product. Prepared maghemite nanoparticles reveal excellent colloidal stability for a long time without the necessity for any additional surface modification. These colloidal features are due to surface stabilizing OH⁻ groups, which act as charge barriers preventing a particle aggregation and enabling a reversible binding of various oppositely charged organic substances. Such binding with rhodamine B isothiocyanate results in the fluorescent magnetic nanocarrier providing, at the same time, a spacer arm for covalent immobilization of other biosubstances including enzymes. In this work, we exploit this general applicability of the developed nanocarrier for covalent immobilization of glucose oxidase. This is the first reported example of magnetically drivable fluorescent nanocatalyst. The immobilized enzyme creates a 3–5 nm thick layer on the nanoparticle surface as proved by high-resolution transmission electron microscopy. This layer corresponds to 10 enzyme molecules, which are bound to the nanoparticle surface as found by the fluorimetric determination of flavin adenine dinucleotide. The developed magnetic fluorescent nanocatalyst, showing a rate constant of 32.7 s^{-1} toward glucose oxidation, can be used as a biosensor in various biochemical, biotechnological, and food chemistry applications. The presence of the nanocatalyst can be simply monitored by its fluorescence; moreover, it can be easily separated from the solution by an external magnetic field and repeatedly used without a loss of catalytic efficiency.

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1. Introduction

Magnetic nanoparticles of iron oxides, namely maghemite (i.e. $\gamma\text{-Fe}_2\text{O}_3$) and magnetite (i.e. Fe_3O_4), play a key role in modern biomedical and biotechnological applications. Among various magnetic species, nanosized iron-oxide-based magnetic particles possess many superior properties including magnetic (e.g. superparamagnetism, high values of saturation magnetization, easy control by small magnetic fields) and biochemical (e.g. non-toxicity, biodegradability, biocompatibility) characteristics [1–8] that

empower their prominent application position in diverse fields of medicine.

Particles such as cross-linked iron oxide (CLIO) [9,10], ultra-small superparamagnetic iron oxide (USPIO) [11–13], and monocrySTALLINE iron oxide nanoparticles (MIONS) [14,15] have all been developed as imaging agents in magnetic resonance imaging (MRI). Some of the reported particles are likely to be taken up by macrophages and immune cells and can be used to image lymph nodes and inflammatory tissues.

Cell labeling is another biomedical field where iron oxide nanoparticles have been found very effective. Commonly, surfaces of magnetic nanoparticles are coated with compounds of mostly organic nature favoring either non-specific or specific cell labeling. Thus, a magnetic nanoparticle is functionalized with specific ligands for targeting cell membrane receptors. For example, Lunov

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