Electronic Supporting Information for

Simultaneous preconcentration and fluorescence detection of ATP by a hybrid nanocomposite of magnetic nanoparticles incorporated mixed metal hydroxide

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1. Chemicals

ATP was purchased from Sigma-Aldrich (USA). Aluminum chloride (AlCl₃), magnesium chloride (MgCl₂), ammonium hydroxide (28% NH₄OH) and hydrochloric acid (37% HCl) were received from QRec (New Zealand). Ferrous sulfate (FeSO₄), sodium hydroxide (NaOH), hydrogen peroxide (30% H₂O₂) and tris(hydroxymethyl)aminomethane were purchased from Carlo Erba Reagents. (Italy). Ferric chloride (FeCl₃) was obtained from Riedel-de Haën (Germany). OPD was received from Sigma (USA). Acetic acid (CH₃COOH) was purchased from RCI Labscan. All aqueous solutions were prepared from ultrapure water with a specific resistivity of 18.2 M Ω cm from a RiOsTM Type I Simplicity 185 system (Millipore water). All reagents were used as received without further purification.

2. Instrumentation

Fluorescence spectra were recorded using an RF-6000PC spectrofluorometer (Shimadzu). The absorbance was recorded with an Agilent HP spectrophotometer. The morphology of the MNPs and MNPs@MMH was observed on a dual beam scanning electron microscope/focused ion beam (SEM/FIB) (FEI Helios NanoLab G3 CX Dual Beam, FEI, USA). Fourier transform infrared (FT-IR) spectra were recorded on a TENSOR27 infrared scanner (Bruker, Germany) with a spectral range of 4000 to 500 cm⁻¹. The magnetic properties were investigated by measuring magnetization (M) versus magnetic field (H) using the vibrating sample magnetometer (VSM) option in a VersaLab instrument (Quantum Design, USA) with a maximum applied field of 10 kOe. X-ray diffraction (XRD) patterns were recorded using an XRD instrument (Model D8 Advance, Bruker AXS, Germany) with Cu K α radiation ($\lambda = 0.1514$ nm) in the 2 θ range of 10° to 80°. The pH values of the solutions were measured with a UB-10 Ultra Basic pH meter (Denver Instrument).

3. Synthesis of Fe₃O₄ magnetic nanoparticles (MNPs)

Fe₃O₄ magnetic nanoparticles were prepared by coprecipitation of ferric (Fe³⁺) and ferrous (Fe²⁺) ions in alkaline solution. FeCl₃ (1.6200 g) and FeSO₄ (1.3960 g) were dissolved in deoxygenated water, and the mixture was heated to 90°C under vigorous stirring. Then, the NH₄OH solution was added to the mixture solution, and the reaction was maintained for 1 h. Finally, the as-prepared MNPs were washed with water and dried in an oven. The working MNP suspension was prepared by using 0.1500 g of dry MNPs in 10.0 mL of DI water.



Fig. S1 FT-IR spectrum of (a) ATP, (b) MNPs and (c) MNPs+ATP



Fig. S2 Magnetic field dependence of the magnetization of MNPs in the presence and absence of ATP



Fig. S3 (a) Fluorescence spectra of DAP in 0.8 M Tris-HCl buffer pH 8.0 generated from the mixture of 200 μ M OPD and 200 mM H₂O₂ using *in situ* and *ex situ* prepared MNPs@MMH in the absence (----) and presence (.....) of 10 μ M ATP (b) The corresponding degree of fluorescence decreasing (F₀-F) after adding 10 μ M ATP. Fluorescence measurements were performed with a fluorescence spectrometer (Edinburgh Analytical Instruments, FS5).