An ultrasensitive sandwich enzyme immunoassay with Glucometer

Readout for Portable and Quantitative Detection of Cronobacter

Sakazakii

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1. Synthesis of silica-coated magnetic nanoparticles (MNP)

The Fe₃O₄ were synthesis by the precipitation method. The details of the procedure are described in the following: 1.35 mM FeCl₂·4H₂O were dissolved in 70 mL distilled water, and stirred vigorously in a nitrogen atmosphere. 2.7 mM FeCl₃·4H₂O and 5 mL ammonium hydroxide were added quickly into the Erlenmeyer flask. The as-received solution was heated to 80 °C and stirred continuously for 60 min. After cooled down, the obtained black powder was washed with distilled water several times and dispersed in 30 mL distilled water.

The method to synthesis MNP is a modified stöber process. Typically, 1 mL MNP water dispersion, 60 mL ethanol, 9 mL ammonium hydroxide were added into a 150 mL Erlenmeyer flask sequentially. Then magnetic stirring was applied to obtain a homogeneous mixture. Next, 1 mL of TEOS was injected into the reaction system. After stirred at room temperature for 24 h, the MNP microspheres were separated and washed repeatedly with ethanol and deionized water to remove nonmagnetic by-products. Finally, MNP were obtained.

2. Synthesis of Silica nanoparticles (SiNP)

The details of the procedure are described in the following: 7.5 mL cyclohexane, 1.77 g Trition X-100, 1.8 mL 1-hexanol and 480 μ L H₂O were added into 50 mL Erlenmeyer flask and stirred for 20 min to ensure water completely dispersed into cyclohexane. Afterwards, 100 μ L TEOS were added to this reverse microemulsion system followed by 100 μ L ammonium hydroxide (25-28 wt %) to catalyze the hydrolization of TEOS. After stirring 24 h, the microemulsion was broken by adding 10 mL acetone. The SiNps were separated from the supernatant by centrifugation at 10000 rpm for 3 min and washed with water and ethanol three times each.