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Newly designed silver coated-magnetic, monodisperse polymeric microbeads as SERS substrate for low-level detection of amoxicillin

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ABSTRACT

We report the preparation of silver-coated magnetic polymethacrylate core–shell nanoparticles for use in surface-enhanced Raman scattering based drug detection. Monodisperse porous poly (mono-2-(methacryloyloxy)ethyl succinate-*co*-glycerol dimethacrylate), poly (MMES-*co*-GDMA) microbeads of *ca.* 5 μm diameter were first synthesized through a multistage microsuspension polymerization technique to serve as a carboxyl-bearing core region. Microspheres were subsequently magnetized by the co-precipitation of ferric ions, aminated through the surface hydroxyl groups and decorated with Au nanoparticles via electrostatic attraction. An Ag shell was then formed on top of the Au layer through a seed-mediated growth process, resulting in micron-sized monodisperse microbeads that exhibit Raman enhancement effects due to the roughness of the Ag surface layer. The core–shell microspheres were used as a new substrate for the detection of amoxicillin at trace concentrations up to 10^{-8} M by SERS. The proposed SERS platform can be evaluated as a useful tool for the follow-up amoxicillin pollution and low-level detection of amoxicillin in aqueous media.

1. Introduction

Surface-enhanced Raman scattering (SERS) has been used as an efficient tool for the sensing of molecules in fields such as materials science, biochemistry, biosensing, catalysis chemistry and electrochemistry [1,2]. Gold, silver, copper and similar metals are typically utilized for Raman enhancement, and the surface architectures of metallic surfaces heavily influence the intensity and distribution of the SERS signal [3]. In recent years, various SERS substrates have designed in the form of nanoparticles, nanostars, nanopyramids, nanorods, and hybrid materials for identifying chemical structures of species [4–10]. Working with nanostructures is highly difficult because of coagulation and toxicological problem and not easy to follow up in the physical systems. In the case of nanostructures, the reproducibility of SERS signal is also poor because of non-uniform aggregation of these materials [11]. As such, an ideal SERS substrate should eliminate the problems associated with nanoparticle use and storage in addition to exhibiting an ideal SERS profile.

In this study, monodisperse-porous microspheres were chosen over nanoparticles for the preparation of a novel SERS substrate, primarily due to their advantage of handling [12–14]. Nanoscale roughnesses on the surface of porous microspheres are sufficient to create the local confinement in electromagnetic fields required for the SERS effect. The ends of nanorods, tips of nano-triangles and edges of nanocubes have previously been reported to exhibit such localized field enhancements, serving as “hot spots” for the enhancement of the SERS signal and potentially producing enhancement factors sufficient for the detection of single molecules [15,16]. Magnetic materials and imprinting technologies were also used in tandem with SERS-active nanoparticles for the design of optimal SERS detection systems [17–19]. Core-shell systems are especially popular for the fabrication of multi-functional magnetic SERS substrates of this type [20–23].

SERS substrates are generally tested using SERS-active dye molecules such as Rhodamine 6G and methylene blue [16,24]. Their practical use also extends to the characterization of cells, bacteria, proteins, drugs, DNA, organic pollutants and various chemical species in the present day [22,25–28]. The detection of various drugs was also performed using SERS in the past decade, and is of substantial importance due to the health and ecological risks

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associated with antibiotic run-off [29].

The detection and the quantitative analysis of amoxicillin, a moderate spectrum β-lactam antibiotic, were performed at concentrations down to 1 μg/ml by SERS [30–33]. In this study, we report the fabrication of a new kind of silver-coated magnetic, porous polymer microspheres as a SERS substrate. A model SERS-active dye, methylene blue, was used as test material for the evaluation of substrate performance, while the aforementioned drug, amoxicillin and the commercial product of amoxicillin, were analyzed to sub-molar concentrations as proof-of-concept to evaluate the efficiency of the material for chemical characterization.

2. Experimental

2.1. Materials

Glycidyl methacrylate (GMA), glycerol dimethacrylate (GDMA), mono-2-(methacryloyloxy) ethyl succinate (MMES), (3-aminopropyl)triethoxysilane (APTES), ethylbenzene (EB), polyvinyl pyrrolidone K-30 (PVPK-30), tetrahydrofuran (THF), sodium dodecyl sulphate (SDS), Iron (II) chloride tetrahydrate ($FeCl_2 \cdot 4H_2O$), Iron (III) chloride hexahydrate ($FeCl_2 \cdot 6H_2O$), triethylenamine (TEA), ammonium hydroxide (NH_4OH), hydrochloric acid (HCl), formaldehyde (HCHO), silver nitrate ($AgNO_3$), chloroauric acid ($HAuCl_4$), polyvinyl alcohol (PVA, 87–89% hydrolyzed, average molecular weight 85,000–146,000 Da), sodium citrate tribasic dihydrate, toluene, methylene blue were purchased from Sigma–Aldrich. All chemicals used as received. Except benzoyl peroxide (BPO) dried in vacuum at 30 °C, was obtained from Aldrich. The initiator, 2,2′-azobisizobutyronitrile (AIBN) was crystallized from methanol, and absolute ethanol was obtained from Merck A.G., Darmstadt, Germany. Distilled deionized (DDI) water was supplied from Millipore/Direct Q-3UV water purification system. Amoxicillin was obtained from Faculty of Pharmacy at Hacettepe University. Largopen was supplied from Bilim İlaç San. ve Tic. AŞ as a commercial amoxicillin.

2.2. Synthesis of monodisperse-porous poly(MMES-co-GDMA) microbeads

Monodisperse poly (GMA) seed latex 2 μm in size was prepared by dispersion polymerization [34]. Plain poly (MMES-*co*-GDMA) microbeads were produced by a multistage shape-template polymerization technique. In the first step, the diluent EB (2.5 ml) was emulsified in DDI water (50 ml) including SDS (0.125 g) by sonication for 6 min. Poly (GMA) seed latex (1 mL, solid content 0.3 g) was added into this emulsion and sonicated for 6 min. The emulsion was magnetically stirred at room temperature for 24 h. Next, the functional monomer (MMES, 1.0 g), the crosslinking agent (GDMA 5.0 mL) and the initiator (BPO, 0.25 g) was mixed together and added into DDI water (50 mL) including SDS (0.125 g). The monomer phase was emulsified by sonication for 5 min. The second emulsion was then poured into the first emulsion containing swollen seed particles. The final emulsion was stirred at magnetically at room temperature for 24 h. Aqueous PVA solution (10 mL, 8% wt/wt) was added into the final emulsion and the resulting dispersion was left at 80° C in a shaking water-bath at 120 cpm for 24 h. Monodisperse-porous poly (MMES-*co*-GDMA) microbeads were isolated by successive centrifugation and decantation steps and washed respectively by EtOH, THF, EtOH several times to remove the unreacted monomers and poly (GMA) template.

2.3. Magnetization of monodisperse-porous poly(MMES-co-GDMA) microbeads

The magnetization of microbeads was performed according to the literature [35]. Typically, 1 g of poly (MMES-*co*-GDMA) microbeads were dispersed in 50 mL of DDI water. The dispersion was placed in an ice-bath under nitrogen atmosphere. 0.268 g of $FeCl_2 \cdot 4H_2O$ and 0.4 g $FeCl_2 \cdot 6H_2O$ was dissolved in 10 ml DDI water in N_2 atmosphere. The mixed salt solution was added into the dispersion containing polymer microbeads and the ice-bath was removed. The medium was evacuated till no air bubble was observed. Then a light brown mixture was formed and immediately immersed in a water-bath at 85° C. 12.5 ml of NH_4OH (25% wt/wt) was added into this mixture and the color was changed into black. The resulting dispersion was mechanically stirred at 85° C for 1 h and then cooled to room temperature. The magnetic poly (MMES-*co*-GDMA) microbeads were separated from the liquid part with a magnet and washed by DDI water and 0.1 M HCl.

2.4. Amine functionalization of magnetic-poly(MMES-co-GDMA) microbeads

Primary amine groups were attached onto magnetic poly (MMES-*co*-GDMA) microbeads via the reaction between the hydroxyl groups of magnetic microbeads and triethoxysilane groups of APTES. For this purpose, 0.5 g of dry, magnetic poly (MMES-*co*-GDMA) microbeads were redispersed in toluene (20 ml). Then 4 ml of APTES and 0.3 ml TEA was added into this mixture. The resulting dispersion was refluxed for 6 h. Amine functionalized magnetic microbeads were separated from reaction medium by a magnet and washed with ethanol several times.

2.5. Gold decoration of primary amine functionalized magnetic-poly(MMES-co-GDMA) microbeads

Frens's method was applied for gold decoration of primary amine functionalized magnetic-poly (MMES-*co*-GDMA) microbeads [36]. For this purpose, 0.02 g $HAuCl_4$ was dissolved in 24 ml DDI water and heated up to boiling under magnetic stirring. 0.07 g sodium citrate tribasic dihydrate in 1 ml water was added into boiling gold solution. The solution was kept for 10 min at boiling point until the color changed from light yellow to dark red. Then the solution was left for cooling at room temperature. After cooling down, 25 mg of primary amine functionalized magnetic-poly (MMES-*co*-GDMA) microbeads were added into gold solution under mechanical stirring. The solution was mechanically stirred at room temperature for 6 h for the attachment of gold nanoparticles onto the primary amine functionalized magnetic polymer microbeads. The gold nanoparticle decorated microbeads were separated by a magnet and washed with DDI water several times.

2.6. Growth of silver shell on gold decorated-magnetic polymer microbeads

Silver shell growth protocol in Wang's study was modified as follows [14]. 0.3 g $AgNO_3$ and 0.025 g sodium citrate tribasic dihydrate were dissolved in 25 ml DDI water by ultrasonication for 5 min. After adding 25 mg of gold decorated magnetic microbeads, pH was set to 10 by using 1 M ammonia solution. Under mechanical stirring, HCHO solution (10% wt/wt) was added into the basic dispersion and the resulting dispersion was left for 6 h for completion of the shell growth reaction. Finally, Ag shell coated magnetic beads were collected by a magnet and washed by water several times.