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Magnetic $CoFe_2O_4$@ melamine based hyper-crosslinked polymer: A multivalent dendronized nanostructure for fast bacteria capturing from real samples

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Polymeric compounds are main types of advanced materials to prepare antibacterial coating as well as water treatment system hence this work was aimed to prepare a polymeric nanostructure with excellent bacteria capture efficiency. Dendronized melamine – resorcinol was synthesized by a condensation reaction. To simplify polymer collection from aqueous solutions, a magnetic nanocomposite of the polymer was also prepared. For this purpose, $CoFe_2O_4$ nanoparticles were synthesized by solid-state combustion route using cellulose as fuel. Bacteria removal efficiency was studied by uptake of Gram-negative (*Escherichia coli*) and Gram-positive (*Bacillus subtilis*) bacteria from water, milk and fruit juice samples. Effective parameter on the capturing efficiency including; solution pH, contact time and nanocomposite dosage were optimized. Results confirmed the positive role of presented nanostructure for fast capturing of bacterial pathogens with high efficiency (more than 99%).
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1. Introduction

Pathogenic microorganisms cause prevalence of infections hence they are in the spotlight of the healthcare field. In other words, infections by harmful fungi, bacteria, protozoa, and viruses are a great concern in the modern lifestyle especially in food packaging, dental and surgery equipment and water purification systems (Muñoz-Bonilla et al., 2012). Relative to direct exposure to infection, bacterial resistant to disinfection is a more and more important threat to public health. Since microbe growth on a surface generates a biofilm which acts as a shelter for the embedded microbes and facilitates gene exchange between them which increases bacterial resistance to antibiotics (Littunen et al., 2016). In other words, bacteria biofilm release toxic chemicals which accelerate degradation of underlying cells. Therefore there are growing demands for antimicrobial materials to eliminate the need for disinfection as well as controlling bacteria growth (Izquierdo-Barba et al., 2011; Karam et al., 2015).

Rapid advances in nanotechnology offer some unique opportunities for expanding of antimicrobial materials (Meziani et al., 2016). In other words, nanotechnology fulfilled the escalating request of artificial material to cope with microorganism infections (Prabhu et al., 2015). In fact, this technology has responded to this challenge by various bioactive nanomaterials such as silver (Ibrahim and Hassan, 2016), carbon-based nano-compounds (Liu et al., 2011), alumina (Mu et al., 2015), metal oxides (Wei et al., 2011) and polymer nanocomposites (Orsuwan et al., 2016; Zhou et al., 2016). Among various types of antimicrobial agents, polymeric materials have attracted great interest owing to superior efficacy and reduced environmental toxicity (Huang et al., 2016). Up to now, two major strategies were developed for antimicrobial modification of polymers. The first approach is physically incorporation of antibiotics, halogens, heavy metals and quaternary ammonium compounds on polymer structure. The second approach includes covalently bonding antimicrobial agents on polymer matrix (Mohy Eldin et al., 2016). The first strategy is effective but it is limited due to "leaching" nature of the biocidal

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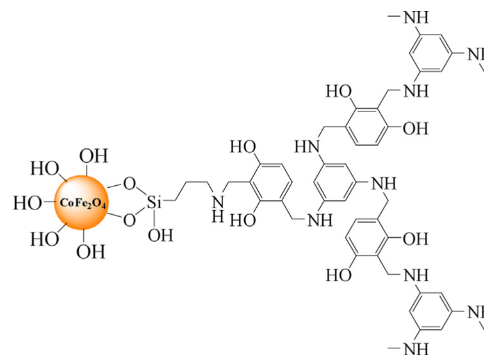
action which releases secondary contaminant. The second strategy is more desired as a durable antimicrobial system (Goy et al., 2016). Covalently attached antibacterial functional groups on the polymer structure include amine, carboxylic acid, quaternary amino groups and phenolic compounds (Makvandi et al., 2016; Qi et al., 2004). Among various polymeric functional groups, phenolic compounds are unique natural distributed materials in bark and fruits of many plants, which serves as defense against plant pathogens and abiotic stress conditions. Moreover, the antimicrobial and antioxidant activity of vegetable and medicinal plants due to the presence of phenolic compounds are well known in recent decades (Sahiner et al., 2016). According to the above-mentioned merits, this work focuses on preparing an efficient antimicrobial system based on polymer nanocomposite. The mentioned polymer is a combination of phenolic and amine containing functional groups. In fact, antibacterial characteristic of amino and phenolic groups are served in synergism way onto one dendronized structure. 1,3,5-triamino triazine (melamine) and resorcinol were reacted with condensation Michael-reaction using p-formaldehyde as a linker. The prepared dendronized polymer is a candidate for water purification, therefore, to facilitate handling of the antimicrobial compound from reaction vessel a covalently bonded magnetic polymer nanocomposite over amino functionalized $CoFe_2O_4$ was also prepared through in situ polymerization. The $CoFe_2O_4$ nanoparticles were synthesized by a simple solid-state combustion route. Cellulose was applied as fuel. In fact, hydroxyl groups in cellulose structure trap Fe and Co ions through complexation moreover CH bonds of cellulose act as electron acceptor agent (Alves et al., 2013; Varma et al., 2016). *Escherichia coli* (*E. coli*) and *Bacillus subtilis* (*B. subtilis*) ATCC 6633 was selected as sample pathogens. *E. coli* is gram-negative and *B. subtilis* is a gram-positive bacterium which is widely distributed in nature. Expose to *E. coli* cause a variety of diseases such as gastroenteritis, dysentery, urinary tract infection, and septicemia. Besides, *B. subtilis* with endospore-forming abilities may proliferate in water and foods hence efficient elimination of these bacteria are vital in human health and environmental safety (Chang et al., 2016). Effective parameter on the capturing efficiency including; solution pH, contact time and nanocomposite dosage were studied and results are discussed in detail.

2. Experimental

2.1. Materials and instruments

$Fe(NO_3)_3{\cdot}9H_2O$, $Co(NO_3)_2{\cdot}6H_2O$ and cellulose microcrystalline were used to prepare $CoFe_2O_4$ nanoparticles. Aminopropyltriethoxysilane (APTES), resorcinol, melamine, p-formaldehyde (FA) and dimethyl sulfoxide (DMSO) were applied for nanocomposite preparation. Bacteria were supplied from Institute Pastor, Tehran, Iran.

The prepared composite were characterized by powder X-ray diffraction analysis (XRD), Field emission scanning electron microscopy (FE-SEM) and transmission electron microscopy (TEM), Fourier transforms infrared spectra (FT-IR), elemental mapping, Raman spectroscopy, thermal gravimetric analysis (TGA), vibrating sample magnetometry (VSM) and zeta potential measurements. XRD was recorded with Philips powder diffractometer, X' Pert MPD, using Cu-Kα radiation at $\lambda = 1.540589$ Å. FESEM, elemental mapping, and TEM carried out using SIGMA VP ZEISS and JEM-2010, Japan. FT-IR was measured with Equinox 55 Bruker at the wavenumber of 400–4000 cm^{-1}. VSM was recorded with MDKFD instrument, Iran. Zeta potential property was analyzed by Zeta Potential Analyzer (Malvern, United Kingdom). Raman spectra were recorded with Nd: YAG laser, Takram P50C0R10 – Teksan. A digital pH-meter (model 692, metrohm, Herisau, Switzerland), was used for the pH adjustment.



Scheme 1. Schematic illustration of magnetic dendronized melamine – resorcinol nanocomposite.

2.2. Synthesis of nanoparticles, polymer, and nanocomposite

$CoFe_2O_4$ nanoparticles were prepared by solid-state combustion rout. In a typical run; 0.62 g of $Co(NO_3)_2{\cdot}6H_2O$ and 1.7 g of $Fe(NO_3)_3{\cdot}9H_2O$ was mixed in a mortar for 2 min. Then, 1.0 g of crystalline cellulose was added to them and mixed for 5 min. The mixture was combusted at 150 °C for 5 min then further heated at 400 °C for 1 h to remove residual nitrate and carbon from nanoparticle structure. To synthesis polymer, 0.5 g of $CoFe_2O_4$ was added to 50 mL DMSO containing 0.5 g of melamine and 1.0 g of resorcinol. After 30 min stirring, 0.5 mL of APTES and 0.3 g of FA was added to the mixture. To assist hydrolysis of APTES 0.5 mL of distilled water was also added to the reaction vessel. The mixture was refluxed for 24 h at 100 °C along with magnetic stirring using a glassy magnet bar and stirrer. At the end of the reaction the product was separated by external magnetic field, washed with ethanol and distilled water then dried at 80 °C for 6 h. The polymer was also synthesized same to nanocomposite in absence of $CoFe_2O_4$ nanoparticles. For this purpose, 0.5 g of melamine, 1.0 g of resorcinol and 0.3 g of FA was dissolved in 50 mL of DMSO. After refluxing at 100 °C for 24 h the mixture was concentrated in one third and 50 mL distilled water was added to the reaction vessel. The products were collected by centrifugation at 4000 rpm for 10 min then washed once with 5 mL of DMSO, ethanol and distilled water. The product was dried at 80 °C for 6 h. Prepared composite is schematically illustrated at Scheme 1.

2.3. Bacteria capture experiment

To evaluate the antibacterial activity of as-synthesized composite, the Gram-negative strain of *E. coli* and gram-positive strain of *B. subtilis* was used as model pathogens. The growth cultivates medium includes 100 mL of Luria Broth (LB) which contain 5 gL^{-1} bacto-yeast extract, 15 gL^{-1} tryptone and 5 gL^{-1} NaCl. After shacking the strains in an incubator at 30 °C for 32 h, cells were separated by centrifugation (4000 × g for 10 min at 4 °C). The growth pellets were washed with 0.9% of NaCl at pH 7.0 and re-suspended in the physiological saline to obtain a cell density of 1.5×10^8 Colony Forming Unit per milliliter (CFU mL^{-1}). To perform bacteria capture experiments, 20 mg of the nanocomposite was added in the 20 mL bacteria solutions with a concentration of 1.5×10^8 CFU mL^{-1}. The mixture was incubated for 20 min then the particles were separated from the suspension. Bacteria capture efficiency was determined after culturing 1.0 mL of diluted stock sample solution or supernatant (10^{-6} time) in LB agar plates and removal percentages (%R)

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