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Antibacterial activity of *Carum copticum* extract loaded $MnFe_2O_4$ nanoparticles coated with PEGylated chitosan

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ABSTRACT

To deal with antibiotic resistance, utilization of natural antibacterial agents such as essential oils and extracts of medicinal plants seems necessary. *Carum copticum* is a traditional famous spice and food preservative in Indian medical system. *C. copticum* seeds extract have wealthy antibacterial compounds, hence to enhance stability and antibacterial activity, we loaded CE on PEGylated chitosan-$MnFe_2O_4$ NPs, which has significant antibacterial activity especially against gram negative bacteria. The nanoparticle size and crystal structure, respectively demonstrate by XRD and SEM image. CE loading analysis was investigated by FT-IR. VSM was developed to evaluate synthesized NPs magnetic properties. Releasing profile and actual CE loading were determined by UV–vis spectroscopy at (λ = 275 nm). Nanoparticle size was measured approximately 21 nm with spherical and uniform shape. MIC of CE-PEG-chitosan-$MnFe_2O_4$ NPs were determined by liquid broth dilution method. Chitosan as biodegradable and compatible natural polymer not only has antibacterial effect, but also improves nanoparticles characteristics. We stabilized NPs with PEG against RES which increases NPs circulation in-vivo.

1. Introduction

Microorganisms-resistance against many existing synthetic antibiotics is the serious leading to gain medical plants benefits. *Carum copticum* belongs to Apiaceae family. The seeds of *C. copticum* are used as spice in some regions of the world, including India, Pakistan and Egypt (Zahin et al., 2010). Therapeutic applications of *C. copticum*, involve antimicrobial, anticeptic, antiviral, amoebiasis expectorant, antiparasitic, antilithiasis, anti-cholinergic, antiplatelet-aggregatory, antihypertensive, antispasmodic, anticonvulsant, antitussive, anti-inflammatory, anxiolytic, antioxidant, tracheal relaxant, carminative, bronchodilator, analgesic antimutagenic effects (Balaji et al., 2012; Dashti-Rahmatabadi et al., 2007). These pharmalogical effects are regarding to phytochemical compounds such as tannins and dietary fiber, starch, aminoacids like lysine and threonine, steroptin, thymine, iron, calcium (Gilani et al., 2005). Our previous study of antibacterial effect of PEG-chitosan-$MnFe_2O_4$ NPs was revealed significant effect against both gram negative and positive bacteria, because of multifunctional bactericidal activity of these nanoparticles (Esmaeili and Ghobadianpour, 2016). Targeting drug delivery is the second purpose of loading *C. copticum* seeds extract on $MnFe_2O_4$ NPs, due to their, small size, low toxicity, magnetic properties, biocompatibility, and biodegradability and high stability (Esmaeili and Hadad, 2015). To modify sufface of NPs, we coated them with biodegradable, biocompatible, nonimmunogenic and nontoxic polymer Chitosan (Liu et al., 2006). We functionalized NPs with hydrophilic polymer PEG, to reduce agglomeration tendency of nanoparticles due to their high surface energy, and protected them from RES, so their circulation time in blood were increased (Qu et al., 2013). In this study we evaluated the possible antibacterial effect of ethanolic extract of *C. copticum* seeds and CE-PEG-chitosan-$MnFe_2O_4$ NPs on gram negative and gram positive bacteria. We employed XRD and VSM analysis, SEM image, to investigate nanoparticles characteristics, FT-IR to confirm loading polymers and CE extract, UV–vis spectroscopy to evaluate actual CE loaded and releasing from nanoparticles. Scheme 1 shows

Abbreviations: PEG, poly ethylene glycol; RES, reticuloendothelial system; FT-IR, fourier transform infrared spectroscopy; XRD, x-ray diffraction; SEM, scanning electron microscopy; SAR, specific absorption rate; TEM, transmission electron microscopy; VSM, vibrating sample magnetometery; UV-vis, ultraviolet-visible spectrophotometery; VRSA, vancomycin resistance staph aureus; MRSA, methycilin resistance staph aureus; vanco-PEG-ch-$MnFe_2O_4$, vancomycin-PEG-chitosan-$MnFe_2O_4$; PBS, phosphate buffer saline; MHA, muller hinton agar; CFU, colony formation unit; MHB, muller hinton broth; MIC, minimum inhibitory concentration; FCC, face centered cubic; FWHM, full width at half maximum.

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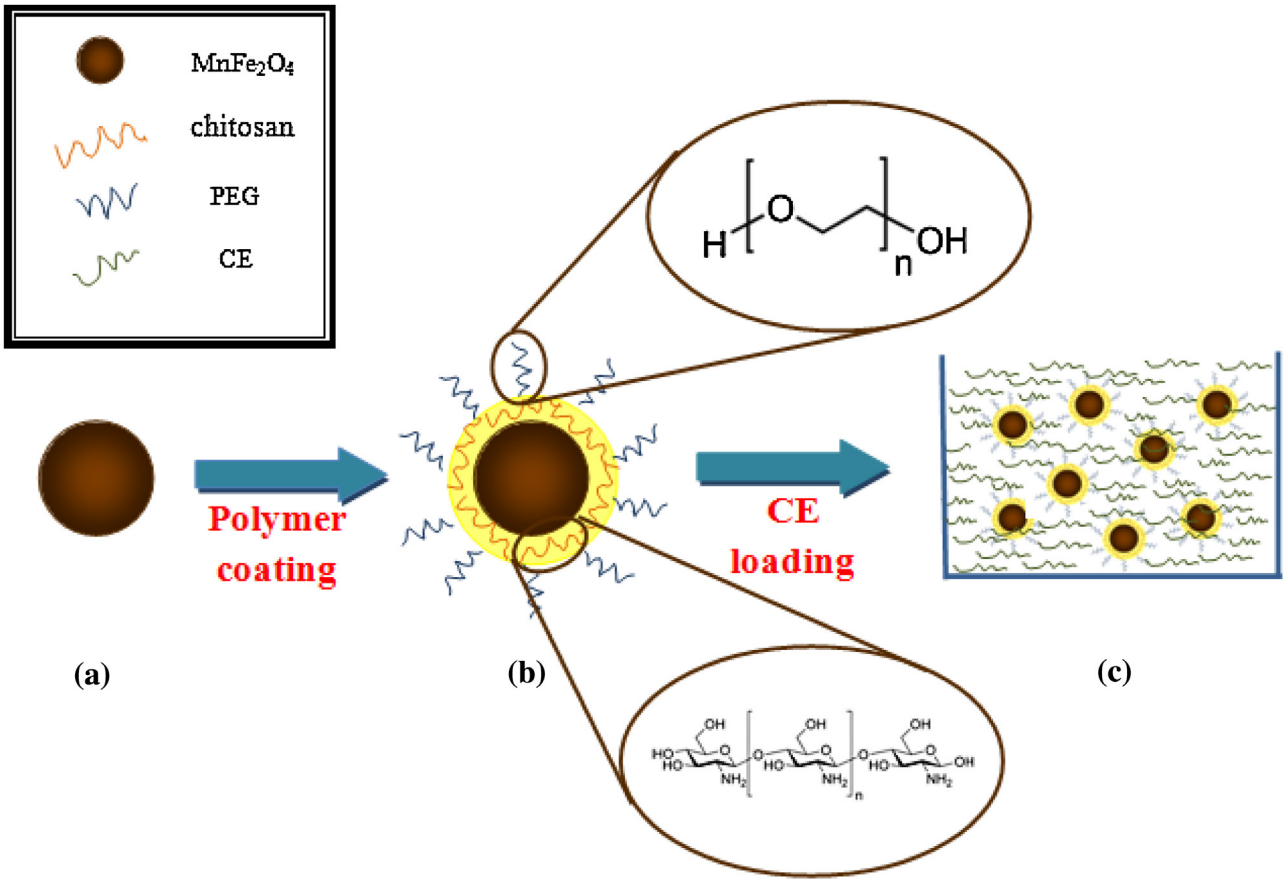

Scheme 1. Preparation of CE-PEG-chitosan-$MnFe_2O_4$ NPs: $MnFe_2O_4$ NPs (a), PEG-chitosan-$MnFe_2O_4$ NPs (b), CE-PEG-chitosan-$MnFe_2O_4$ NPs.

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2. Experimental

2.1. Chemicals and culture media

Medium molecular weight Chitosan (75–85% degree of deacetylation), Tween 80 (26 kDa), PEG (400), from Sigma Chemical Co. $FeCl_3{\cdot}6H_2O$ (98%), $MnCl_2{\cdot}4H_2O$ (98%), glutaraldehyde, NaOH (99%), from Merck Co. Seeds of *C. copticom* was purchased from local market. MHA and MHB were procured from Merck Co.

2.2. Bacterial strains

Staphylococcus epidermitis (ATCC 12228), *Staphylococcus aureus* (ATCC 25923), *Bassilus subtilis* (ATCC 1720), *Klebsiella pneumoniae* (ATCC 1290) *Pseudomonas aeroginos* (ATCC 1430), *Escherchi coli* (ATCC 1399) were donated from Islamic Azad University of Pharmaceutical Sciane.

2.3. C. copticum *extraction and isolation*

50 g finely, dried powder of *C. copticum* seeds, were dissolved in ethanol 70%, mixture shaked at room temperature at 90 rpm. After homogenizing of solvent and powder, the solution was filtered by Watman paper. To evaporate the solvent we were used rotary. Afterward to examine antibacterial activity of pure extract, we keep it cool at 8 °C in sterile tubes.

2.4. Superparamagnetic $MnFe_2O_4$ nanoparticles synthesis

Superparamagnetic $MnFe_2O_4$ nanoparticles were prepared by co-percipitation method. 7 g $FeCl_3{\cdot}6H_2O$ (98%) and 3.2 g $MnCl_2.4\,h_2O$ (98%) were dissolved in 230 ml distilled water. 8 g NaOH (99%) was added to solution and stirred vigorusley for 20 min under nitrogen atmosphere at 80 °C. Resulting nanoparticles were separated by magnetic decantation and quickly washed 3 times with ethanol (100%).

2.5. $MnFe_2O_4$ nanoparticles coating by PEGylated chitosan

0.5 g chitosan was added to 100 ml solution of 2% (V/V) glacial acetic acid and stirred for 30 min at 60 °C to get transparent. 0.5 g $MnFe_2O_4$ nanoparticles were added to solution and stirred 30 min 20 min sonicated at room temperature. 1 ml of 2% glutaraldehyde and 0.5 g PEG were added to mixture and stirred 15 min at 65 °C. 0.002 g Tween 80 was added to mixture and incessantly mixed for 1 h. PEGylated chitosan NPs decantated magnetically and washed several times with distilled water and dried (Esmaeili and Ghobadianpour, 2016). Tween 80 was surfactant for the system.

2.6. Preparation of CE-PEG-chitosan-$MnFe_2O_4$ NPs

To prepare CE-PEG-chitosan-NPs 0.5 g PEG-chitosan-NPs were dispersed in 100 ml phosphate buffer saline pH = 7.4, different amount of CE with polymer: drug ratio (w/w) 1:1, 2:1, 3:1 and 4:1 seperately dissolved in solution and shaked gentley for 24 h at room temperature.