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Engineered mixed oxide-based polymeric composites for enhanced antimicrobial activity and sustained release of antiretroviral drug

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ABSTRACT

Here, pH-responsive engineered polymeric composites were fabricated from sodium alginate and mixed Cu/Zn oxides. The resulting alginate–$Cu_xZn_{1-x}O$ composites were characterized by FTIR, SEM and XRD, then used as an efficient carrier for the antiretroviral drug (zidovudine, AZT) and exhibited remarkable antibacterial properties. The resulting polymeric composites had specific surface areas of 185.2–198.6 m^2/g as confirmed by the Brunauer–Emmett–Teller analysis. The metal oxide distribution within the alginate matrix was confirmed from the X-ray diffraction and scanning electron microscopy analyses. The zidovudine, an antiretroviral drug was encapsulated in 30 mg of alginate–$Cu_{0.7}Zn_{0.3}O$ with 68% encapsulation efficiency. The release of AZT in simulated intestinal fluid (pH 7.4) was studied, a slow and sustained release of AZT (~96.2%) was observed. The AZT release kinetics is sufficiently described by the Korsmeyer–Peppas model and follows the Fickian transport profile. Results herein demonstrated that A–$Cu_{0.7}Zn_{0.3}O$, A–$Cu_{0.3}Zn_{0.7}O$ and $Cu_{0.5}Zn_{0.5}O$ exhibited excellent bacterial devastation property. A dose of 8 μg/mL A–$Cu_{0.7}Zn_{0.3}O$ and 13 μg/mL A–$Cu_{0.3}Zn_{0.7}O$ are sufficient to completely killed *E. coli* DH5a and *S. aureus* NSUHS-151 within 24 h.

1. Introduction

In the past few decades, there have been great advances and increasing research efforts in the pharmaceutical and biomedical industries due to the growing number of diseases. Concerted attention has been directed towards the development of antibacterial materials and effective carrier-based drug delivery system as it protects the drug molecules and improves its bioavailability after oral administration [1,2].

Unlike the conventional oral and intravenous drug delivery methods where drugs are distributed to both infected and healthy sites, controlled drug delivery system ensures drugs are released at the diseased site within a desired therapeutic range. The carrier plays a significant role in controlled drug delivery system, practically, the drug release from any carrier depends upon the degradation of the carrier, microstructure of carrier, the solubility of the drugs, and the bond between the carrier and drug [3,4]. Biopolymers are often used in the design of drug delivery system owing to their excellent properties such as biocompatibility, environmental sensitivity, biodegradability and non-toxicity, etc. [1,5–11]. In particular, electrospun gliadin fibres and Janus nanofibers have been reported to demonstrate acceptable drug sustained release profile compared with the conventional drug delivery systems [7,8]. However, due to poor mechanical properties and high swelling tendency in an aqueous environment, the direct use of some of these biopolymers is practically limited.

Lately, the development of biopolymer-inorganic composites as drug delivery vessels has attracted substantial attention due to their unique properties [12,13]. The synergistic effect of inorganic materials and biopolymers could enhance the drug entrapment, mechanical properties and sustained drug release behaviour [13,14]. Also, the properties of the resultant composite could be further modified by altering the content and type of inorganic materials [12].

Among the biopolymers that are sensitive to external stimuli such as pH, ionic strength etc., a naturally occurring non-toxic polysaccharide, alginate is highly attractive and well-studied pH-sensitive biopolymer. The sodium salt of the alginic acid (SA) is water-soluble, rapidly undergoes sol-gel transformation in response to multivalent metal cations and thus frequently investigated for encapsulation of various therapeutic agents [6,15,16]. Unfortunately, the fast disintegration of the ionotropically-gelled alginate in the intestinal fluid which leads to rapid drug release and low entrapment efficiency of water-soluble bioactive agents in the gelled alginate remain a serious challenge for developing sustained drug delivery systems [13,17].

The introduction of inorganic materials into the alginate matrices provides a convenient route to circumvent drawbacks of alginate and

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produce biopolymer–inorganic hybrids that contain properties of both the polymer and inorganic guest in a single material [18–21].

The potential use of copper oxide (CuO), zinc oxide (ZnO) and other metal oxide nanomaterials in biomedical applications is increasingly gaining attention in the medical and scientific communities [4,22–24]. Palanikumar et al. [4] synthesized nano ZnO for controlled delivery of amoxicillin and reported that the drug loading efficiency of as-prepared ZnO nanoparticles is dependent on the concentration of drug and size of nanoparticles. Finally, they concluded that the amoxicillin-loaded ZnO nanoparticles exhibited good antibacterial activity against infectious Gram-negative and Gram-positive pathogenic bacteria. Azam et al. [25] prepared CuO nanoparticles via citric precursors and reported that the monodispersed CuO demonstrated enhanced antibacterial activities against pathogenic bacterial strains.

To this end, we prepared different Cu/Zn mixed oxides ($Cu_xZn_{1-x}O$) nanoparticles and introduced into alginate moiety, the resultant alginate–$Cu_xZn_{1-x}O$ composite is designated as A–$Cu_xZn_{1-x}O$ (A–$Cu_{0.7}Zn_{0.3}O$, A–$Cu_{0.3}Zn_{0.7}O$ and A–$Cu_{0.5}Zn_{0.5}O$). The nanoengineered Cu/Zn mixed oxide-based polymeric composites were utilized for controlled release of an antiretroviral drug, zidovudine, chemically known as azidothymidine (AZT). AZT belongs to the group of poorly water-soluble drugs [7,8] and its therapeutic use of AZT is seriously limited by its poor oral bioavailability (<65%), haematological toxicity and significant short biological half-life (<3 h) [16,26]. To reduce side effects of the conventional release of AZT in patients, the as-fabricated alginate–$Cu_xZn_{1-x}O$ composites were utilized for the controlled release formulations to provide desirable therapeutic AZT release profiles.

Herein, we present experimental investigations of AZT release mechanisms and kinetics of A–$Cu_xZn_{1-x}O$ in simulated fluids of pH 1.2–7.4 at temperatures below and very close to the physiological body temperature (i.e. 25 °C and 37 °C, respectively). The structure, morphology and properties of the as-prepared materials were characterized with Fourier transform infrared (FTIR), X-ray diffraction analyses (XRD) and Scanning electron microscopy (SEM). Finally, the antibacterial activities of A-$Cu_xZn_{1-x}O$ and $Cu_xZn_{1-x}O$ against Gram-positive bacteria (*S. aureus*) and Gram-negative bacteria (*E. coli*) were systematically investigated.

2. Experimental

2.1. Materials

All reagents were used as received from suppliers without further purification. Sodium alginate (SA) of pharmaceutical grade (viscosity average molecular weight: 53,000 and M/G ratio:1.6) was purchased from Merck (Germany). Sodium hydroxide, calcium chloride, Zn $(NO_3)_2 \cdot 6H_2O$ and Cu $(NO_3)_2 \cdot 6H_2O$ were purchased from Sigma-Aldrich (USA). The model organisms acquired from ATCC Bacteriology Collection (USA) were Gram-positive bacteria (*S. aureus* NSUHS-151) and Gram-negative bacteria (*E. coli* DH5a). The strains were grown aerobically in nutrient broth for 24 h at 37 °C before usage as target organisms. Double-distilled water was used throughout to prepare the solutions in this study.

2.2. Synthesis of $Cu_xZn_{1-x}O$ and alginate-metal mixed oxides (A–$Cu_xZn_{1-x}O$)

Firstly, the single/mixed oxides were prepared via co-precipitation of hydroxycarbonates from the corresponding metal nitrates [23]. Specifically, 6.42 g of $M(NO_3)_2$ was reacted with an aqueous solution of $NaHCO_3$ (0.12 mol) in a closed flask under vigorous stirring at 90 °C for 180 min. The reaction pH ~8.5 was maintained by 7% hydroxycarbonate and 0.0254 g of surfactant, cetyltrimethylammonium bromide according to the following reaction; $2M(NO_3)_2 + 4NaHCO_3 \rightarrow M_2CO_3(OH)_2 + 4NaNO_3 + 3CO_2$, M is either Cu, Zn or a mixture of Zn and Cu in the atomic ratio 30:70 or 50:50. After the reaction is complete, the resultant product was filtered and repeatedly washed with distilled water, dried at 80 °C and then calcinated at 450 °C for 120 min to obtain the corresponding oxides ($Cu_xZn_{1-x}O$).

For the preparation of A–$Cu_xZn_{1-x}O$, initially, 1.5% of aqueous sodium alginate (SA) solutions were prepared under constant stirring by dissolving 1.5 g SA in distilled water at room temperature for 120 min. To these solutions, 100 mg mixed metal oxides ($Cu_{0.7}Zn_{0.3}O$, $Cu_{0.5}Zn_{0.5}O$ or $Cu_{0.7}Zn_{0.3}O$) were introduced and stirred constantly for 60 min at 300 rpm, then sonicated for 45 min to obtain homogeneous solutions. To the prepared solutions, 1% $CaCl_2$ solution was added drop by drop and subjected to microwave (MW) irradiation (450 W) for 5 min. The obtained alginate–$Cu_xZn_{1-x}O$ composites were washed several times with deionized water and dried in a vacuum oven at 60 °C. The amount of mixed metal oxides in the composites was determined by atomic absorption spectroscopy and found to be ~35 wt%.

2.3. Characterization and instrumentation

The morphology and elemental composition of the prepared samples were characterized by scanning electron microscope (SEM) integrated with energy dispersive spectroscopy (EDS) instrument (JSM-7500F field SEM). Fourier-transform infrared (FT-IR) spectra were collected via a Perkin Elmer-Spectrum Spectrometer in the range of 400–4000 cm^{-1} using pressed KBr pellets. To identify the crystal structure of the metal oxides and metal mixed-oxides polymeric composites, X-ray diffraction (XRD, Bruker AXN) measurements were carried out using Cu-Kα radiation (l = 1.542 Å) at 40 kV and 30 mA over the 2θ range 10–80°. Thermo-gravimetric analysis (TGA) was performed on a TA instruments (Model: STA, Q600, USA). 1.0 g samples were heated from 20 to 950 °C at a heating rate of 10 °C/min in the N_2 gas atmosphere (flow rate 50 mL/min).

2.4. Swelling ratio measurements

The swelling ratios of A–$Cu_{0.7}Zn_{0.3}O$ and A–$Cu_{0.3}Zn_{0.7}O$ were examined by immersing known weights of dried samples in a simulated intestinal fluid, SIF (pH = 7.4, 0.68% (w/v) KH_2PO_4 and 0.77% (v/v) NaOH without pancreatin), simulated gastric fluid, SGF (pH = 1.2, 0.7% (v/v) HCl and 0.2% (w/v) NaCl without pepsin) and simulated body fluid, SBF (pH = 6.5) at 37 ± 0.5 °C until equilibrium was established. The samples weights were measured periodically after the droplets of test media adhered on the surface of the swollen samples were carefully removed by blotting with soft tissue paper. The difference in weight was noted before (W_o) and after (W_1) swelling using electronic balance (Sartorius, Model: BSA224S-CW, China). The swelling studies were conducted in triplicate and the percentage swelling ratio, SR (%) was computed from the average results using the following mathematical relation:

$$SR(\%) = \left(\frac{W_1 - W_o}{W_o}\right) 100 \quad (1)$$

2.5. Drug loading and encapsulation efficiency

The AZT belongs to the group of poorly water-soluble drug. Hence, known amounts of AZT were added to distilled water spiked to pH 7.5 and subjected to agitation of 300 rpm for 6 h at 37 °C. The known weights of dried mixed metal oxide-based polymeric composites were placed into 100 mL of freshly prepared 50–200 mg/L AZT solution for 24 h at 37 ± 0.5 °C. The AZT molecules were physically absorbed into the MPC matrix via equilibrium swelling method. Thereafter, the drug-loaded MPC were filtered from the solution and the remaining drug solution was collected to estimate the quantity of unentrapped AZT during the process.

The AZT loading and encapsulation efficiencies were estimated as per the procedure reported elsewhere [16] with a slight modification.