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Synthesis of PVA-CAP-based biomaterial in situ dispersed with Cu nanoparticles and carbon micro-nanofibers for antibiotic drug delivery applications

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ABSTRACT

A novel pH-sensitive and water-soluble polyvinyl alcohol (PVA)-cellulose acetate phthalate (CAP) composite-based biomaterial was prepared, in which the multi-scale web of copper (Cu)-grown carbon micro-nanofibers (Cu-ACF/CNFs) was in situ dispersed during a synthesis stage. PVA-CAP and Cu-nanoparticles (NPs) were used as an encapsulating agent and nano-antibiotics, respectively. The web of Cu-ACF/CNF was prepared by growing CNFs on the activated carbon microfiber (ACF) substrate by chemical vapor deposition using Cu NPs as the catalyst. The novel step of the synthesis included esterification of polyvinyl acetate (PVAc) to produce a PVA gel to which the ball-milled Cu-ACF/CNF was blended at the incipience of the gel formation to produce the PVA-CAP-Cu-ACF/CNF metal-carbon-polymeric composite film. The in vitro dissolution tests revealed that the encapsulating polymeric composite was dispersible in water and its rate of dissolution was high at pH > 6.5. The antibacterial tests performed on the material demonstrated its effectiveness against both gram negative *Escherichia coli* and gram positive *Staphylococcus aureus* bacterial strains. The Hixson-Crowell kinetic model described the dissolution profiles of the material. The method of preparation is novel, simple, and environmentally friendly. The prepared biomaterial may be used in several biomedical applications, including wound healing and the controlled release of drugs in the antibiotic delivery system.

1. Introduction

At present, considerable reductions in morbidity and mortality rates due to infectious diseases have been achieved with the advent of antibiotics. However, the pathogens gradually develop resistance against antibiotics, reaching a critical level that invalidates majority of the commercially available antimicrobial drugs. The problem of bacterial resistance to antimicrobial agents is being resolved by developing new materials and/or modifying existing antibiotic agents. In this context, the development of drug delivery systems has attracted considerable attention [1–4]. Enhanced benefits of such systems may be realized by developing nanomaterials that are instantly responsive to therapeutic requirements and may be released to the site of infections at controlled rate with maximum effectiveness and without side-effects [5,6].

Activated carbon microfiber (ACF) has been extensively used as a substrate for several metal nanoparticles (NPs), including Cu, Ag, Fe, Zn, and Ni to prepare a multi-scale web of carbon micro-nanofibers (ACF/CNFs) [7–10]. Such webs prepared by growing CNFs on ACFs by chemical vapor deposition (CVD) using metal NPs as the catalyst have been applied to different environmental remediation and biological applications.

Cu NPs are well known antimicrobial agents. These NPs adhere to bacteria and inhibit protein synthesis, thereby preventing bacteria multiplication. Cu NPs are effective against *Escherichia coli* and *Staphylococcus aureus* bacterial strains, which are inherently present in burns and surgical wounds as well as in diabetic foot-ulcer infections [11,12]. The previous study has shown that Cu-ACF/CNF has insignificant cytotoxicity compared with the other carbon-based materials such as carbon nanotubes (CNTs) and activated carbon [10]. Therefore, the hierarchical web of ACF/CNF dispersed with Cu NPs can be potentially used as nano-antibiotics.

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With regard to polymeric materials used as a carrier, several types of polymers such as poly vinyl alcohol (PVA), polylactic acid, poly lactide-co-glycolide (PLGA), and chitosan have been used as encapsulating agents for the controlled release of drugs [13–15]. However, major drawbacks exist in these materials, including weak interaction between the biopolymers and drugs and the rapid collapse of the polymeric encapsulating agents during drug release process. Different methods including blending, crosslinking of co-polymers have been shown to be useful for improving the performance of polymeric carriers [16–18].

The present study describes the synthesis of a PVA and a cellulose acetate phthalate (CAP)-based material as an encapsulating agent for the Cu NPs-grown ACF/CNFs for biomedical applications. PVA and CAP are non-toxic, biocompatible, environmentally benign and biodegradable materials. PVA has been used in the synthesis of hydogels, opthalimic films, and transdermal drug delivery systems [19,20]. CAP is extensively used in the coating of many drugs because of its resistance against acidic environment. CAP exhibits both antibacterial and antiviral properties and is, therefore, used in the synthesis of anti-hepatitis-, anti-HIV- and anti-bacterial vaginosis agents. It also enhances the permeability of the drug molecules through cellular membranes [21]. Because of these unique properties, the PVA-CAP composite polymeric film is used in this study as the encapsulating agent for the Cu NPs-grown ACFs/CNFs in situ dispersed in the polymeric matrix.

The novelty of the present study is as follows. (1) Cu-ACF/CNF was in situ added to PVA at the incipience of the gel-formation. No surfactant, which is generally skin-irritant, was used to disperse Cu-ACF/CNF in the CAP solution; instead, CAP itself acted as a dispersing agent. (2) The dissolution rate of PVA-CAP composite film was controlled by adjusting the relative amount of CAP in the composite. (3) Cu NPs used as the catalyst to grow CNF on ACF acted as an antibacterial agent as well, and (4) CNFs had the dual roles of holding Cu NPs to their surface, thereby controlling the release of metal ions to the specific site of application, as well as enhancing the mechanical strength of the produced polymeric film.

2. Materials and method

2.1. Materials

Polyvinyl acetate (PVAc) was purchased from Sigma Aldrich, India. Methanol, methyl acetate, sodium hydroxide, $Cu(NO_3)_2 \cdot 3H_2O$, tryptone, yeast extract, sodium dodecyl sulfate (SDS), Triton-x-100 and ethylene diamine tetra acetate (EDTA) were purchased from Merk, India. CAP was purchased from Merck (Germany). Sodium chloride (NaCl) and agar were purchased from Hi-media, India. Hydrogen, nitrogen, and C_2H_2 (AAS grade) were purchased from Sigma Gases, India. All reagents used were of high purity grade. The phenolic resin precursor-based ACFs were procured from Kynol, Japan.

2.2. Preparation of Cu-ACF/CNF

The as-received ACF samples were treated with 5 ml of 0.03 M nitric acid in 1 l of DI water at 80 °C for 2 h to remove any impurities from their surfaces. Next, ACFs were washed several times with DI water until the surface became neutral. The solution of 0.4 M $Cu(NO_3)_2 \cdot 3H_2O$ in DI water was prepared to impregnate the ACFs using the wet incipient method. SDS (0.3%, w/w) was used as a surfactant to increase mono-dispersion and avoid agglomeration of the metal ions in the solution. The metal salts impregnated ACFs were dried at room temperature (~30 °C) for 3 h, followed by drying at 120 °C for 12 h in a vacuum oven. Cu NPs were produced in situ on the ACFs by calcination at 200 °C for 4 h in the presence of nitrogen, followed by H_2 reduction at 350 °C for 2 h. CNF was grown on the Cu NPs-dispersed ACFs by CVD at 650 °C for 30 min, using acetylene (C_2H_2) as the carbon source. The method of preparation is described in detail in a previous study [9].

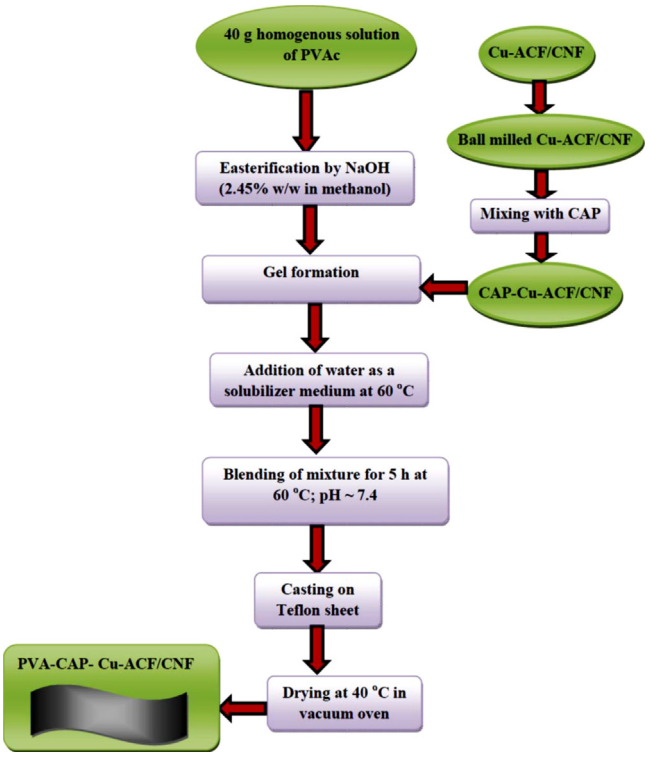

Fig. 1. Flow-sheet for the synthesis of PVA-CAP-based polymeric film dispersed with Cu-ACF/CNF.

The prepared Cu-ACF/CNF was ball milled in CAP to produce the slurry of micron/nano size-fibers (100–500 nm). To prepare the dispersion of Cu-ACF/CNF in CAP, approximately 0.1–0.5 g of Cu-ACF/CNF was cut into small pieces. The samples were transferred to a nano ball-mill (Retsch, Germany) and milled at 40 Hz-speed for 35 min. The ball milled Cu-ACF/CNF along with different amounts (2.5–30%, w/w) of powder CAP were added to 50 ml of DI water. The mixture was continuously stirred at 120 ppm for 2 h. The solution containing CAP and dispersed Cu-ACF/CNF were subsequently added to the reactant mixture for esterification, as described in the following section.

2.3. Synthesis of pH sensitive biomaterial

Fig. 1 describes the preparation steps for the biomaterial. The primary step consisted of the formulation of PVA from PVAc by esterification. A mixture of 124 g-PVAc in 200 cc-volume of methanol was stirred in a beaker to prepare a homogenous solution. Approximately 40 g of the prepared solution was transferred to a 2-1-3 round bottom flask. The temperature of the reaction mixture was kept constant at 60 °C. 25 ml-methanol, 22 ml-methyl acetate and 0.1 ml-DI water were added to the mixture. The suspension mixture was continuously stirred at 120 rpm until the solution was clear. Next, 10 ml of methanolic solution hydroxide (2.5% NaOH in methanol) was added to the clear solution. After approximately 15 min, the PVA-gel was formed. At the incipience of the gel formation, a mixture of the ball-milled micron sized web of Cu-ACF/CNF dispersed in CAP was added to the reactant mixture.

After approximately 60 min of adding the mixture of ball milled Cu-ACF/CNF and CAP, a black gel of PVA-CAP-Cu-ACF/CNF blend was produced. Next, 100 ml of milli-Q water was added to the