International Journal of Pharmaceutics 463 (2014) 161–169

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Synthesis and characterization of a novel controlled release zinc oxide/gentamicin–chitosan composite with potential applications in wounds care

Bogdan Stefan Vasile[a], Ovidiu Oprea[a,*], Georgeta Voicu[a], Anton Ficai[a], Ecaterina Andronescu[a], Andrei Teodorescu[a], Alina Holban[b]

[a] University Politehnica of Bucharest, Faculty of Applied Chemistry and Materials Science, Gh. Polizu 1, Bucharest, Romania
[b] University of Bucharest, Faculty of Biology, Department of Microbiology and Immunology, Intr. Portocalelor 1-3, Bucharest, Romania

ARTICLE INFO

Article history:
Received 15 August 2013
Received in revised form 3 November 2013
Accepted 18 November 2013
Available online 26 November 2013

Keywords:
Zinc oxide
Chitosan
Gentamicin
Controlled release
Improved wound dressing
Antimicrobial effect

ABSTRACT

Freshly prepared ZnO nanoparticles were incorporated into a chitosan solution in weight ratios ranging from 1:1 to 12:1. Starting from the ratio of 3:1 the chitosan solution was transformed into a gel with a high consistency, which incorporates 15 mL water for only 0.1 g solid substance. The powders obtained after drying the gel were characterized by X-ray diffraction (XRD), transmission electron microscopy (TEM) and thermal analysis (TG-DSC). The electronic (UV–vis), infrared (FTIR) and photoluminescence (PL) spectra were also recorded. ZnO particles were coated with gentamicin and incorporated into the chitosan matrix, to yield a ZnO/gentamicin–chitosan gel. The release rate of gentamicin was monitored photometrically. This ZnO/gentamicin–chitosan gel proved great antimicrobial properties, inhibiting *Staphylococcus aureus* and *Pseudomonas aeruginosa* growth in both planktonic and surface-attached conditions. The results indicate that the obtained composite can be used in cutaneous healing for developing improved wound dressings, which combine the antibacterial activity of all three components with the controlled release of the antibiotic. This wound dressing maintains a moist environment at the wound interface, providing a cooling sensation and soothing effect, while slowly releasing the antibiotic. The system is fully scalable to any other soluble drug, as the entire solution remains trapped in the ZnO–chitosan gel.

1. Introduction

Beside its various technical applications (Vaja et al., 2012, 2013), zinc oxide may be also used in a series of cosmetic products, sun protection cream, acne treatment or for wound dressings. Application of zinc oxide has been shown to accelerate the healing of both chronic and acute wounds and it also exhibits antibacterial, antifungal and anti-inflammatory properties (Selvam et al., 2012; Subhasree et al., 2012; Vlad et al., 2012). ZnO effect on wounds epithelialization as well as its bacteriostatic properties promotes it as an effective ingredient for topical wound dressings. It can be used in the treatment of various dermatitis, diaper rashes, diaper wipes, blisters, and open skin sores (Kumar et al., 2012a; Shalumon et al., 2011).

Chitosan, an abundant natural polysaccharide well known for its biodegradable and non-toxic properties, is popular in the field of biomedicine due to its interesting physicochemical properties and potential for a wide range of applications (Casettari et al., 2012; Grumezescu et al., 2012a,b; Sogias et al., 2012). One of the applications that received much attention in the last years is related to the potential uses of chitosan in the process of wound healing (Archana et al., 2013; Ribeiro et al., 2013; Wijekoon et al., 2013).

Incorporation of zinc oxide nanoparticles into chitosan comes as one of natural solutions to develop new bandages for wound dressing (Kumar et al., 2012a,b), as the antibacterial activity of the composite ZnO–chitosan is also well documented in the literature (El Shafei and Abou-Okeil, 2011; Liu and Kim, 2012; Perelshtein et al., 2013).

Gentamicin belongs to the class of aminoglycoside antibiotics, widely used to treat many types of serious infections, particularly those caused by Gram-negative bacteria. It is usually used to treat skin infections, soft tissues and bone infection, and also extreme burns-associated infections (Builders et al., 2013; Manjunatha et al., 2010). Rapid debridement of burn wounds and the application of effective topical and systemic antimicrobial agents can improve the efficacy of burn therapy (Sun et al., 2012).

Many types of nosocomial pathogens are drug-resistant and patients infected with these drug-resistant pathogens are at a high risk. As different bacterial strains become increasingly resistant

* Corresponding author. Tel.: +40 724697047.
E-mail address: ovidiu73@yahoo.com (O. Oprea).

0378-5173/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ijpharm.2013.11.035

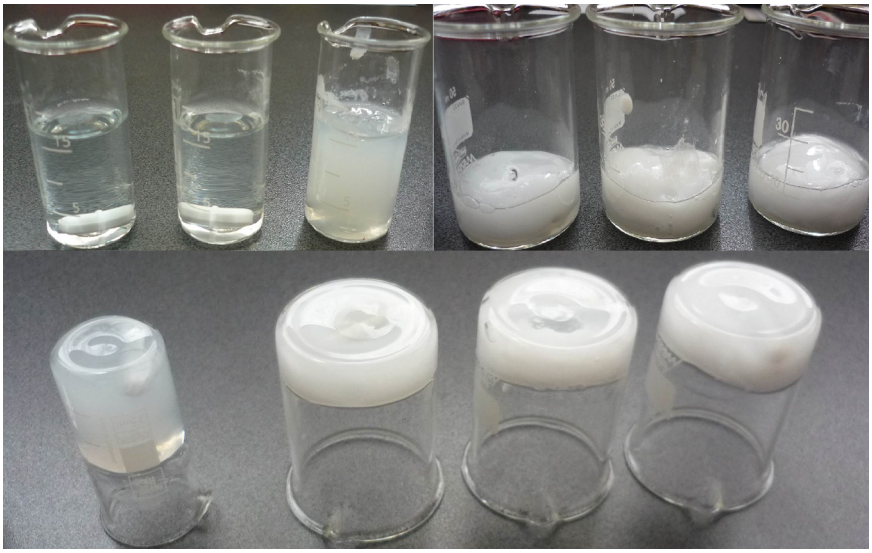

Fig. 1. The ZnO–chitosan gels. On top row (from left to right) synthesis with ZnO:chitosan ratio of 1:1; 2:1; 3:1; 4:1; 5:1 and 6:1. On bottom row, the ZnO–chitosan gels have high consistency starting with ZnO:chitosan ratio 3:1.

to antibiotics, the search for new strategies to deal with resistant bacteria is emerging (Grumezescu et al., 2012c). Researchers need to identify and develop next generation drugs or agents to control bacterial infections (Grumezescu et al., 2012d). The main strategy is to combine different antibacterial agents to overcome the resistance of the microorganisms and to obtain synergic antibacterial activity (Andronescu et al., 2012; Saviuc et al., 2011; Song et al., 2013; Zabielinski et al., 2013). Controlled release and targeting of drugs is another modern approach that is trying to improve the efficiency of various substances (Eidi et al., 2012; Grumezescu et al., 2012e; Mihaiescu et al., 2011, 2012). In a previous study (Voicu et al., 2013) we proved that gentamicin coated ZnO nanoparticles can be successfully used to design a large spectrum-active antibacterial wound dressing material composed by synergistically acting components.

The aim of this study was to develop a newly ZnO–chitosan based controlled release nanosystem, with great absorptive properties, for improving the efficiency of antibiotics in external infections. This material may be successfully used in wound care, the potential applications being derived from its enhanced antibacterial activity, along with the high water content that helps in maintaining a moist environment at the wound interface, providing a cooling sensation and soothing effect, while slowly releasing the antibiotic, on the other side.

2. Materials and methods

2.1. Experimental procedure

Zinc acetate dihydrate, $Zn(Ac)_2{\cdot}2H_2O$, with 99.9% purity was obtained from Merck. Absolute ethanol was used as received from Sigma without further purification. Gentamicin solution with a concentration of 10 mg/mL was supplied by Sigma. Chitosan (CAS 9012-76-4) was used as received from Sigma.

ZnO synthesis was done as described before (Oprea et al., 2011). ZnO–gentamicin hybrid material synthesis was performed as described in Voicu et al. (2013).

ZnO–chitosan and ZnO/gentamicin–chitosan composites were obtained starting from the relevant ZnO or ZnO/gentamicin nanopowders and chitosan solution in acetic acid 1%, as follows. 0.0250 g chitosan were dissolved in 10 mL acetic acid solution (1%). Freshly prepared ZnO nanoparticles (from 0.0250 g up to 0.3000 g) were suspended in 5 mL bi-distilled water and ultrasonicated for 5 min. The milky ZnO suspension was quickly added under magnetic stirring over the chitosan solution. For the formulation with weight ratio ZnO/chitosan of 1:1 and 1:2 a viscous, clear solution is obtained. For the formulation with weight ratio ZnO/chitosan $\geq$3:1, the ZnO–chitosan gel (opalescent-white) is formed in few seconds, and the magnetic bar is immobilized inside the gel (Fig. 1). The ZnO–chitosan gel can be extracted from the baker, and cut in any shape, just like rubber. It is stable up to three days in laboratory atmosphere, but can be kept over three months in water, with no further swelling (Fig. 2).

For the synthesis of ZnO/gentamicin–chitosan gel, ZnO/gentamicin hybrid was obtained starting from 0.3000 g ZnO and 5 mL gentamicin solution (0.0500 g gentamicin). The 5 mL aqueous solution containing the ZnO/gentamicin nanopowder was added over 10 mL CH_3COOH solution 1% of 0.0250 g chitosan.

The ZnO–chitosan and ZnO/gentamicin–chitosan nanopowders were obtained by drying the appropriate gel and fine grinding the films that result.

2.2. Experimental techniques

(a) *Electron microscope images*. The transmission electron images were obtained on dried, finely powdered samples using a Tecnai^{TM} G^2 F30 S-TWIN high resolution transmission electron microscope from FEI, operated at an acceleration voltage of 300 kV obtained from a Schottky field emitter with a TEM point resolution of 2 Å and line resolution of 1.02 Å.

(b) *X-ray diffraction*. X-ray powder diffraction patterns were obtained with a Shimadzu XRD6000 diffractometer, using Cu Kα (1.5406 Å) radiation operating with 30 mA and 40 kV in the 2θ range 10–70°. A scan rate of 1° min^{-1} was employed.

(c) *Thermal analysis*. Thermal behaviour of the ZnO–chitosan gel, ZnO–chitosan and ZnO/gentamicin–chitosan nanopowders were followed by TG-DSC with a Netzsch TG 449C STA Jupiter. Samples were placed in alumina crucible and heated with 10 K min^{-1} from room temperature to 900 °C, under the flow of 20 mL min^{-1} dried air.