Ultrasonics Sonochemistry xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Ultrasonics Sonochemistry

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

# Formation and distribution of ZnO nanoparticles and its effect on *E. coli* in the presence of sepiolite and silica within the chitosan matrix via sonochemistry

Ezgi Tan [a], Selcan Karakus [a], Gülin Selda Pozan Soylu [b], Özgür Birer [c,d], Yaşar Zengin [b], Ayben Kilislioglu [a,*]

[a] Istanbul University, Faculty of Engineering, Department of Chemistry, 34320 Avcilar, Istanbul, Turkey
[b] Istanbul University, Faculty of Engineering, Department of Chemical Engineering, 34320 Avcilar, Istanbul, Turkey
[c] Koç University, Department of Chemistry, Sarıyer, 34450 Istanbul, Turkey
[d] Koç University, KUYTAM Surface Science and Technology Center, Sarıyer, 34450 Istanbul, Turkey

ARTICLE INFO

*Article history:*
Received 20 June 2016
Received in revised form 24 August 2016
Accepted 24 August 2016
Available online xxxx

*Keywords:*
Sonochemistry
Nanoparticle
Bio-nanocomposite
XPS
SEM
XRD

ABSTRACT

In this study, a new bio-nanocomposite was prepared and characterized with a focus on the formation of hexagonal ZnO and orthorhombic zinc silicate ($Zn_2SiO_3(OH)_2$) phases under ultrasonic irradiation. Chitosan/sepiolite/ZnO and chitosan/silica/ZnO bio-nanocomposites were synthesized using a simple solution method in which extreme physical and chemical conditions created by cavitation within the chitosan solution allowed for the transformation of aqueous $Zn(OH)_2$ to crystallized ZnO and $Zn_2SiO_3(OH)_2$ in room temperature. Both the loading of sepiolite and silica with the zinc precursor significantly influenced the morphology and crystalline structure of the product, however, different zinc compounds were observed. Sepiolite was exfoliated, resulting in a fine, even colloidal solution through ultrasonic dispersion. Exfoliation of sepiolite nanofibers led to the homogeneous dispersion of Zinc in the form of $Zn(OH)_2$ in chitosan matrix. When the same procedure was conducted using the silica component, a formation of ZnO and $Zn_2SiO_3(OH)_2$ was observed, components that were not observed when the procedure was conducted using sepiolite. The average crystalline size of ZnO was calculated as 36 nm for ZnO. In addition, the quantities of crystalline and the ZnO phase volume was determined as 15%. Through zone of inhibition, the silica nanocomposite was discovered to have antibacterial activity. In contrast, the sepiolite compound did not exhibit these properties. We thus hypothesize that $HO^{\cdot}$ radicals, formed during ultrasonic irradiation trigger the formation of a silicate ion ($SiO_3^{2-}$) and formation of ZnO and $Zn_2SiO_3(OH)_2$ species in chitosan/silica/ZnO bio-nanocomposite, which causes to exhibit these antibacterial properties against Gram-negative *E. coli*. Chemical characterization and dispersion of the structure of the ZnO and $Zn_2SiO_3(OH)_2$ phases were done using X-ray diffraction (XRD) and scanning electron microscopy techniques (SEM) with EDAX and X-ray photoelectron spectroscopy (XPS).



## 1. Introduction

Research on the development of nanocomposites with advanced properties such as antibacterial activity has gained significance in the field of nanotechnology. Biopolymers have become an attractive component in the stabilization of host matrices in these materials due to their nontoxic and biodegradable nature. Significant properties can be obtained by the addition and dispersion of small amounts of inorganic additives such as metal or metal oxide nanoparticles into the biopolymer matrix. Homogeneous dispersion, crystalline structure and the chemical properties of nanoparticles are tremendously important in creating a successful bio-nanocomposite with advanced features [1–12]. The final structure depends largely on the extent of inter mixing and compatibility between the organic and the inorganic phases [13–15].

Chemically, sepiolite is a hydrous magnesium silicate whose individual particles have a needle-like morphology. Because of its high surface area and porosity, sepiolite can be used as a nano filler for biopolymers. It exhibits microfibrous morphology with particle sizes in a wide range and shows alternation of blocks and tunnels that grow in the fiber direction [16–18].

Sonochemical method is reliable to get homogeneously dispersed inorganic and organic phases. The sonochemical method has been used extensively to generate novel materials with unusual properties [19,20]. The growth and collapse of these micro

* Corresponding author.
*E-mail address:* ayben@istanbul.edu.tr (A. Kilislioglu).

http://dx.doi.org/10.1016/j.ultsonch.2016.08.027
1350-4177/

bubbles cause an energy transfer from an acoustic wave to vapor inside the bubbles [21–23]. This extraordinary energy creating process creates reactive free species such as HO· radicals that are observed to significantly change chemical processing [24,25]. In the experimental conditions, the HO· radicals are spontaneously reacted with –OH groups on the surface of $SiO_2$. For $SiO_2$, the relative rates of aqueous $H_2O_2$ decomposition are in the same order as the HO· radical reaction probability, which suggested a silicate ion followed by the formation of ZnO and $Zn_2SiO_3(OH)_2$ [26–28].

The chemical reactions which are expected to occur in the solution are shown as following [29];

$$Zn(NO_3)_2 \longrightarrow Zn^{2+} + 2NO_3^- \quad (1)$$

$$Zn^{2+} + 2OH^- \xrightarrow[\text{Sound waves}]{} Zn(OH)_2 \quad (2)$$

$$Zn(OH)_2 \longrightarrow ZnO + H_2O \quad (3)$$

Different species of $O^{2-}$·, $H_2O_2$ and hydroxyl radicals are also produced. In this study we studied the radical species formed as a silicate mineral – a component of the calamine mineral with a formula of $Zn_2SiO_3(OH)_2$. This silicate mineral has been historically mined from the upper parts of zinc and lead ores, associated with smithsonite, $ZnCO_3$. They are assumed to be the same mineral and ware labelled under the same name of calamine. In the second half of the 18th century it was discovered that these two different minerals were both present in calamine. The silicate is the rarer of the two [30]. This important result is due in part by the surfaces of the silica particles.

In this current study, we tested the antibacterial effect of a nontoxic and biodegradable bio-nanocomposite by zone of inhibition method [31]. We used a natural aminopolysaccharide biopolymer chitosan as the organic matrix and $SiO_2$ or sepiolite components in order to get a stronger structure. Finally we focused on formation of hexagonal ZnO and orthorhombic zinc silicate ($Zn_2SiO_3(OH)_2$) phases under ultrasonic irradiation. X-ray diffraction (XRD) was used to characterize the crystallographic structure of the bio-nanocomposites. Scanning electron microscopy (SEM) was used to evaluate the size and the homogenous nano particle settlement in biopolymer matrix. The absence of Zinc oxide nanoparticles on 10 nm thickness of the surface was evaluated by X-ray photoelectron spectroscopy (XPS).

## 2. Experimental

### 2.1. Materials

Chitosan (medium molecular weight) (Aldrich), Oxalic acid (Merck), Zinc nitrate hexa hydrate ($Zn(NO_3)_2{\cdot}6H_2O$) (Merck), Sodium hydroxide (Merck), silica gel (Merck), sepiolite (Hekimhan, Turkey).

### 2.2. Preparation of Nano-biocomposites

Chitosan/sepiolite/ZnO and chitosan/silica/ZnO bio- nanocomposites were synthesized by simple solution method under ultrasonic irradiation. A solution of chitosan (2 g/135 ml) in 0.2 M oxalic acid was prepared at 40 °C in ultrasonic bath for 60 min. Due to its low solubility the solution was kept overnight at room temperature.

10 ml 0.002 M NaOH solution dropped in 10 ml 0.001 M Zn $(NO_3)_2{\cdot}6H_2O$ under continuous magnetic stirring (labelled as Z1). In this study 0.005 M $Zn(NO_3)_2{\cdot}6H_2O$ was also used and it's assigned as Z5. 0.1 g $SiO_2$ (labelled as Si1) was added into the solution and then it was immersed into an ultrasonic bath for 5 min. The samples included 0.5 g $SiO_2$ were labelled as S5. Then the mixture was added into 20 ml chitosan solution and homogenized for 5 min using sonoplus homogenizer (frequency 35 kHz, 320 W, Sonoplus, Bandelin, Germany). The nano-biocomposites were dried at 50 °C to a constant weight.

### 2.3. Material characterization

X-ray diffraction (XRD, PAN analytical Xpert-Pro, Cu Kα: 1.5406 Å, 40 kV, 40 mA, Bruker D8 Advance X-ray Diffractometer), scanning electron microscopy (SEM, Quanta FEG 450). The specimen was coated with a thin conducting (tens of nanometers) layer of gold X-ray photoelectron spectroscopy (XPS, Thermo Scientific K-alpha).

### 2.4. Antibacterial tests

A zone inhibition test was performed on an agar plate to test the antibacterial properties of the newly synthesized material. In this experiment, the bacteria strain used was the *Escherichia coli*. The dishes were incubated at 37 Müller Hinton medium was used on two separate petri dishes.

## 3. Results and discussion

### 3.1. X-ray diffraction analysis

The XRD patterns of chitosan/silica/ZnO (labelled as chitosan/Si1 /Zn1) and chitosan-sepiolite-Zn bio-nanocomposites are presented in Figs. 1 and 2. The complexation between oxalic acid and chitosan can be formed chitosan–oxalic acid salt or chitosan oxalate.

The loading of sepiolite and silica with zinc precursor significantly influences the morphology and crystal structure of the obtained products. For the samples labelled as chitosan Si1 Zn1 (see Table 1), the obtained diffraction data (Fig. 1) was similar to those mixed with hexagonal ZnO (JCPDS 36-1451) and orthorhombic zinc silicate ($Zn_2SiO_3(OH)_2$) phases. In addition, the sample prepared with sepiolite shows characteristic peaks associated with the $Zn(OH)_2$. However, hexagonal ZnO phases were not detected for chitosan/Si5/Zn1 and chitosan/Si1/Zn5 samples (Fig. 2). Although it is not possible to claim a critical role of mass with limited experimental data, we speculate that there might be a role for masses of silica and zinc in the formation of nanoparticles since these two phases were not observed when the procedure was conducted using various amounts of silica and zinc.

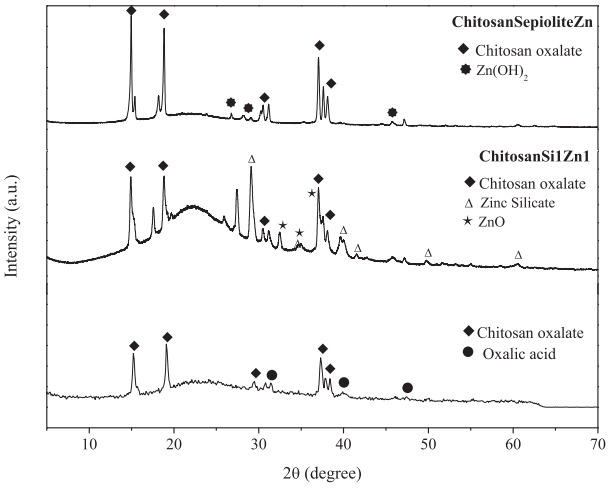


**Fig. 1.** XRD pattern of chitosan/sepiolite/Zn, chitosan/Si1/Zn1 and chitosan film.