

# Preparation and Antibacterial Properties of $\text{Ag}^+$ -Exchanged Tobermorite-Chitosan Films

Andrew P. Hurt, George J. Vine, Samantha E. Booth, and Nichola J. Coleman

**Abstract**—Silver-exchanged zeolites and clays are used in polymer composites to confer broad-spectrum antimicrobial properties on a range of functional materials. Tobermorite is a layer lattice mineral whose potential as a carrier for  $\text{Ag}^+$  ions in antibacterial composites has not yet been investigated. Accordingly, in this study, synthetic tobermorite was ion-exchanged with 10 wt% silver ions and the resulting material was incorporated into a composite film with chitosan. Chitosan is a biocompatible, biodegradable derivative of chitin, a polysaccharide obtained from the shells of crustaceans. The solvent-cast  $\text{Ag}^+$ -exchanged tobermorite-chitosan films were found to exhibit antimicrobial action against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*.

**Keywords**—Antimicrobial, chitosan, silver, tobermorite.

## I. INTRODUCTION

**T**OBERMORITE is a rare naturally occurring layer lattice mineral of ideal formula  $\text{Ca}_5\text{Si}_6\text{O}_{16}(\text{OH})_2 \cdot 4\text{H}_2\text{O}$  whose structure comprises infinite layers of Ca-O polyhedra bound on both sides by silicate chains running along the *b*-axis (as shown in Fig. 1) [1], [2]. These layers are stacked in the *c*-axis direction creating channels, similar to those found in zeolites, which house water molecules and labile ion-exchangeable  $\text{Ca}^{2+}$  and  $\text{Na}^+$  cations.

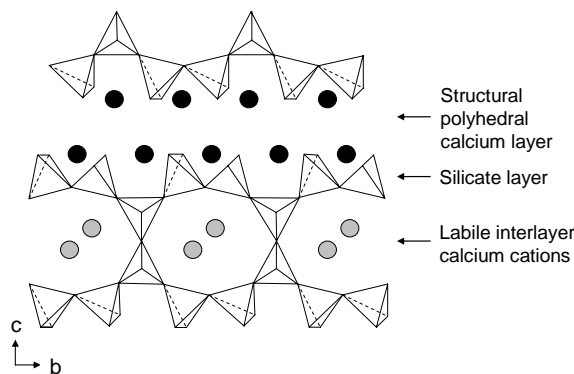


Fig. 1 Structure of tobermorite in the *bc* plane

Previous research has indicated that the exchange of interlayer  $\text{Na}^+$  and  $\text{Ca}^{2+}$  for  $\text{Ag}^+$  cations within the tobermorite structure confers antimicrobial activity against gram positive *Staphylococcus aureus* and gram negative *Pseudomonas aeruginosa* [2]. Silver-exchanged zeolites and clays have been used in polymer composites to confer broad-spectrum antimicrobial properties on a range of technical and biomedical materials [2], [3]; however, to date, the incorporation of  $\text{Ag}^+$ -bearing tobermorite in antibacterial composites has not yet been investigated.

Chitosan (shown in Fig. 2) is the partially N-deacetylated derivative of chitin, a structural mucopolysaccharide obtained on an industrial scale from the shells of crustaceans [3]-[5]. Chitosan affords many advantages over synthetic petroleum-based polymers as it is a readily abundant renewable resource which is biodegradable, non-toxic, edible and biocompatible. Current and prospective applications of chitosan include: medical textiles, sutures and implants; wound dressings; tissue engineering scaffolds; pharmaceuticals; cosmetics; food packaging materials; and water filtration media [3]-[5].

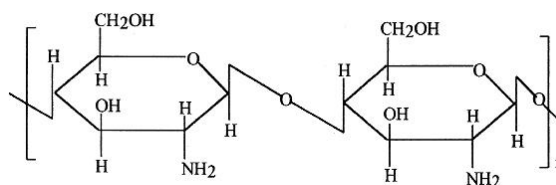


Fig. 2 Structure of chitosan

In this study, synthetic tobermorite was prepared hydrothermally and characterised by X-ray diffraction (XRD) and X-ray fluorescence spectroscopy (XRF). The tobermorite was then ion-exchanged with silver ions. Tobermorite-chitosan films were prepared by solvent casting, characterised by Fourier Transform infrared spectroscopy (FTIR) and their antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* was evaluated by the inhibition zone method.

## II. EXPERIMENTAL

### A. Preparation

Tobermorite samples were prepared in triplicate by heating mixtures of 6.8 g of sodium metasilicate pentahydrate, 1.47 g of calcium oxide and 60 cm<sup>3</sup> of 1.0 M sodium hydroxide solution at 100 °C in hermetically sealed PTFE reaction vessels for 19 days. The products were washed with deionised

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water to pH ~7 and dried in air at 40 °C to constant mass. The products were characterised by powder XRD analysis which was performed on a Philips D8 diffractometer (with Cu K $\alpha$  = 1.5406 Å, a step size of 0.019 ° and a measuring time of 141.8 s/step) and X-ray fluorescence spectroscopy [2].

Labile interlayer sodium and calcium ions of the tobermorite lattice were exchanged for silver ions by contacting 0.5 g of tobermorite with 200 cm<sup>3</sup> of 3.0 M silver nitrate solution for 48 hours at 25 °C. After contact, the supernatant liquor and ion exchanged solids were separated by gravitational filtration and the recovered solutions were analysed for silver by atomic absorption spectroscopy using a Pye Unicam SP 9 spectrophotometer. The Ag<sup>+</sup>-exchanged tobermorites were dried in air at 40 °C and stored in the absence of light in air-tight polypropylene containers.

Tobermorite-chitosan composite films were prepared by solvent casting. Low molecular weight chitosan (*ex. Sigma*) was dissolved in a 2% ethanoic acid solution and tobermorite was added at a chitosan:tobermorite ratio of 10:7 by mass. The mixtures were stirred for 2 hours, cast onto polycarbonate plates and dried in air at 60 °C for 2 days to produce films approximately 7 µm thick. Composite films prepared with 'as synthesised' and Ag<sup>+</sup>-exchanged tobermorite were labelled CT and CT-Ag, respectively, and were characterised by transmission FTIR using a Perkin Elmer Paragon spectrometer.

#### B. Antibacterial Properties of CT and CT-Ag Films

The antimicrobial activities of the CT and CT-Ag composite films were evaluated using a semi-quantitative inhibition zone method against gram positive *Staphylococcus aureus* NCIMB 9518, gram negative *Escherichia coli* NCIMB 9132 and gram negative *Pseudomonas aeruginosa* NCIMB 8628. In each case, 100 cm<sup>3</sup> of Nutrient Broth (Oxoid) was inoculated with 0.1 cm<sup>3</sup> of an overnight culture of the bacterium. The cultures were then incubated for 24 hours, with shaking, at 37 °C. 0.2 cm<sup>3</sup> of the resulting cultures were spread in quadruplicate on nutrient agar plates. 8 mm discs were punched from the CT and CT-Ag composite films and one disc was placed in the centre of each spread plate. The plates were examined for clear zones after incubation at 37 °C for 24 hours. Plate counts indicated that the final population densities of the plates spread with *S. aureus*, *E. coli* and *P. aeruginosa* were approximately 1.0 x 10<sup>9</sup>, 5.9 x 10<sup>8</sup>, and 5.9 x 10<sup>9</sup> colony forming units per plate.

### III. RESULTS AND DISCUSSION

#### A. Characterisation

The powder XRD pattern of the synthetic tobermorite prepared for this study is shown in Fig. 3 and closely resembles that of other phase-pure tobermorites reported in the literature [1], [6]. The actual formula of the synthetic tobermorite, Ca<sub>4.55</sub>Na<sub>0.44</sub>Si<sub>6.00</sub>O<sub>16.77</sub>·6.4H<sub>2</sub>O, was calculated from the XRF oxide analysis which is listed in Table I.

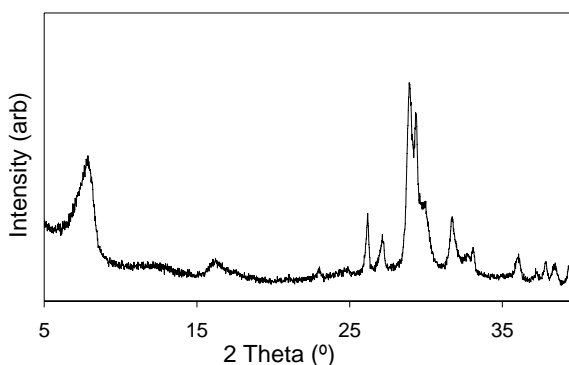


Fig. 3 Powder XRD data for tobermorite

TABLE I  
OXIDE COMPOSITION OF TOBERMORITE SAMPLES

Oxide	Tobermorite (mass %)	Ag-Exchanged Tobermorite (mass %)
SiO <sub>2</sub>	48.40	39.58
CaO	34.25	24.99
Na <sub>2</sub> O	1.83	0.14
Ag <sub>2</sub> O	-	11.71
H <sub>2</sub> O	15.46	23.55

The uptake profile of silver ions by tobermorite is plotted in Fig. 4 and demonstrates that equilibrium uptake of 0.96 mmol g<sup>-1</sup> is achieved within the first 24 hours of contact. This loading corresponds to approximately 10 wt% of silver.

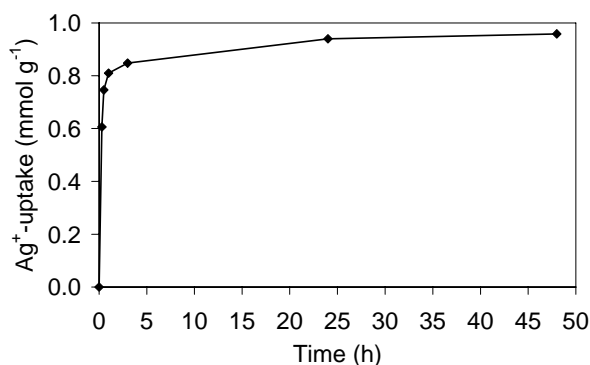


Fig. 4 Uptake of Ag<sup>+</sup> ions by tobermorite

The XRF oxide analysis of the Ag<sup>+</sup>-exchanged tobermorite is listed in Table I and indicates that 91% and 11% of the original Na<sup>+</sup> and Ca<sup>2+</sup> ions are, respectively, readily exchanged for Ag<sup>+</sup> ions from aqueous solution. These data demonstrate that essentially all of the interlayer cations are exchanged for Ag<sup>+</sup> ions and that the remaining four Ca<sup>2+</sup> ions per unit formula which constitute the structural polyhedral Ca-O layer do not undergo exchange under the selected experimental conditions. The equilibrium silver uptake capacity observed for tobermorite exceeds those reported for palygorskite (0.6 wt%) and montmorillonite (8.37 wt%) clays and falls within the range reported for various zeolites (3–18.4 wt%) [7]–[11]. The formula of the Ag<sup>+</sup>-exchanged tobermorite is Ca<sub>4.06</sub>Na<sub>0.04</sub>Ag<sub>0.92</sub>Si<sub>6.00</sub>O<sub>16.54</sub>·11.9H<sub>2</sub>O (based on the XRF

oxide analysis listed in Table I).

The FTIR spectra of the tobermorite-chitosan composite films CT and CT-Ag are shown in Fig. 5. The broad signals at  $3400\text{ cm}^{-1}$  and  $1600\text{ cm}^{-1}$  arise from interlayer water molecules and silanol groups (Si-OH) of the tobermorite and also the hydroxyl groups of the chitosan. The combination band at  $1060\text{ cm}^{-1}$  is assigned to various Si-O stretching modes and the sharp signals at  $\sim 680\text{ cm}^{-1}$  arise from Si-O bending modes. Carbonate ion stretching vibrations give rise to the signal at  $1440\text{ cm}^{-1}$  and indicate that calcium carbonate is present in the tobermorite sample following exposure to atmospheric carbon dioxide. The bands at  $1540\text{ cm}^{-1}$  and  $1410\text{ cm}^{-1}$  are attributed to N-H bending modes and C-H stretching modes of the chitosan, respectively, and the weak signal at  $2900\text{ cm}^{-1}$  arises from C-C stretching vibrations.

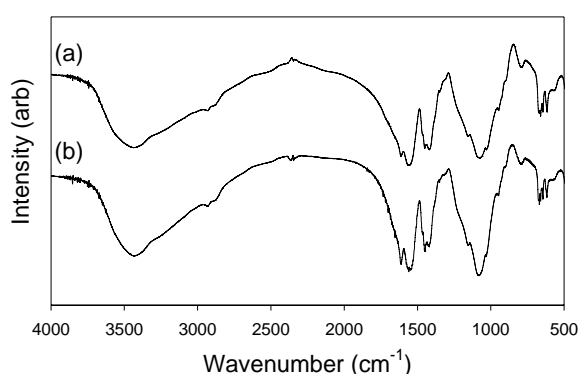


Fig. 5 FTIR spectra of (a) CT and (b) CT-Ag films

The incorporation of silver ions within the tobermorite matrix has no significant impact on the FTIR spectrum of this mineral phase.

#### B. Antibacterial Properties of CT and CT-Ag Films

The results of the *in vitro* antibacterial zone inhibition assays are listed in Table II. The composite films incorporating Ag<sup>+</sup>-exchanged tobermorite, CT-Ag, exhibited distinct clear antimicrobial zones against all three microorganisms. Conversely, films prepared from the 'as synthesised' tobermorite, CT, failed to exhibit a discrete inhibition zone against any of the bacteria using this disc method.

TABLE II  
ZONE INHIBITION DATA FOR TC AND TC-AG

Bacterium	Zone for TC (mm)	Zone for TC-Ag (mm)
<i>S. aureus</i>	0	$0.81 \pm 0.13$
<i>E. coli</i>	0	$1.07 \pm 0.13$
<i>P. aeruginosa</i>	0	$1.44 \pm 0.13$

#### C. Potential Applications of Ag<sup>+</sup>-Exchanged Tobermorite-Chitosan Films

Silver-bearing zeolites and clays have been incorporated into a range of polymer composites to confer antimicrobial

properties to wound dressings, bioactive implants, and food packaging materials [2]. This research has demonstrated that Ag<sup>+</sup>-exchanged tobermorite-chitosan composite films exhibit antimicrobial action against both gram positive and gram negative microorganisms. It should be noted that calcium silicate substrates (such as minerals, ceramics, glasses, and gels) and their breakdown products are generally acknowledged to be biocompatible and do not elicit a toxic response from the body. Hence, in composites for biomedical and food packaging applications, the tobermorite lattice as a carrier for silver ions has a distinct advantage over those of zeolites and many types of clay whose degradation products contain cytotoxic aluminium species. As previously mentioned, chitosan is also a more desirable alternative to petroleum-based polymers for biomedical and food packaging applications as it is biodegradable, non-toxic and biocompatible.

#### IV. CONCLUSION

Solvent casting from weakly acidic solutions of chitosan mixed with Ag<sup>+</sup>-exchanged tobermorite can be used to prepare composite films. These films exhibit inhibitory action against the common pathogenic microorganisms, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*.

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