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# Halloysite nanotubes as carriers of vancomycin in alginate-based wound dressing

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ABSTRACT

The influence of an inorganic support – halloysite nanotubes – on the release rate and biological activity of the antibiotic encapsulated in alginate-based dressings was studied. The halloysite samples were loaded with approx. 10 wt.% of the antibiotic and then encapsulated in Alginate and Gelatin/Alginate gels. The material functionalized with aliphatic amine significantly extended the release of vancomycin from alginate-based gels as compared to that achieved when silica was used. After 24 h, the released amounts of the antibiotic immobilized at silica reached 70%, while for the drug immobilized at halloysite the released amount of vancomycin reached 44% for Alginate discs. The addition of gelatin resulted in even more prolonged sustained release of the drug. The antibiotic was released from the system with a double barrier with Higuchi kinetic model and Fickian diffusion mechanism. Only the immobilized drug encapsulated in Alginate gel demonstrated very good antimicrobial activity against various bacteria. The inhibition zones were greater than those of the standard discs for the staphylococci and enterococci bacteria tested. The addition of gelatin adversely affected the biological activity of the system. The inhibition zones were smaller than those of the reference samples. A reduction in the drug dose by half had no significant effect on changing the release rate and microbiological activity. The *in vivo* toxicity studies of the material with immobilized drug were carried out with *Acutodesmus acuminatus* and *Daphnia magna*. The material studied had no effect on the living organisms used in the bioassays. The proposed system with a double barrier demonstrated high storage stability.


## 1. Introduction

Halloysite nanotubes (HNTs) are naturally occurring clays composed of aluminosilicate sheets. The wall contains 10–15 bilayers of aluminium and silicon oxide. Unlike kaolinite, HNTs have an additional monolayer of water between adjacent layers. HNTs are characterized by the presence of siloxane groups at the exterior surfaces and aluminol groups at the interior surfaces. As alumina layer is at the inner surface and silica layer at the outer surface, thus the tube surfaces are oppositely charged in water. Different literature sources provide slightly divergent values for the basic dimensions of HNTs, but it can be assumed that the internal diameter (lumen), outer diameter and length of nanotubes are in the range 1–30 nm, 30–100 nm and 0.1–2.0 μm, respectively (Joussein et al., 2005; Pasbakhsh and Churchman, 2015).

HNTs are a low cost, natural mineral nanotubes with a wide range of potential applications in different fields. They have been investigated for thermal resistance, corrosion prevention, in polymerisation reaction, for remediation or as carrier systems (Kamble et al., 2012; Du et al., 2010; Rawtani and Agrawal, 2012; Lvov et al., 2008) and delivery systems (Massaro et al., 2015b, 2016a, 2016b). They have many possible applications in the area of medical science (Lvov et al., 2016a, 2016b; Lvov and Abdullayev, 2013; Massaro et al., 2015a, 2014; Vergaro et al., 2010, 2012), which is not only a consequence of the nanotubes structure (Levis and Deasy, 2002), but also of biocompatibility of the material (Abdullayev and Lvov, 2013). Release of different

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bioactive compounds, like 5-aminosalicylic acid (Aguazzi et al., 2013), tetracycline HCl (Price et al., 2001) or ofloxacin (Wang et al., 2014) from HNTs has been studied. Moreover, surface modification of HNTs can result in improvement in the material properties, *e.g.* modification with dopamine allows enzyme immobilization (Chao et al., 2013), while surface functionalization with 3-Aminopropyltrimethoxysilane increases loading with ibuprofen (Tan et al., 2013). The inorganic HNTs can be also combined with biopolymer materials in order to obtain, *e.g.* scaffolds for tissue engineering (Naumenko et al., 2016) or electrospun fiber membranes for clinical application (Xue et al., 2015).

Halloysite is one of many inorganic carriers of biologically active substances. In our previous work we used modified silica as a carrier of the antibiotic vancomycin (Kurczewska et al., 2015a). Vancomycin is a glycopeptide water-soluble drug, used for the treatment of infection caused by Gram-positive bacteria. Literature provides information on a number of different carriers of this antibiotic. Silica-based mesoporous material SBA-15 has been found to be effective in local delivery of vancomycin (Molina-Manso et al., 2012), while functionalized mesoporous silica participated in the reaction with the antibiotic in order to obtain vancomycin-modified silica nanoparticles for killing pathogenic bacteria (Qi et al., 2013). Also microporous hydroxyapatite fibres have been tested as carriers of the drug (Ravelingien et al., 2010).

A large group of systems, used as drug carriers are hydrogels (Peppas et al., 2000; Hamidi et al., 2008). Vancomycin can be encapsulated within hydrogel beads (Lin et al., 2010) or it can be covalently bonded in order to form antibacterial hydrogel (Lakes et al., 2014). Hydrogels with vancomycin can be applied as wound healing dressings (Zhang et al., 2008; Zhao et al., 2014). Modern dressings should not only increase patients' comfort, but mostly they are designed to create appropriate environment around a wound that facilitates and participates in its healing (Boateng et al., 2008; Ovington, 2007). Alginate is relatively common drug carrier in modern dressings. It is a natural polysaccharide polymer composed of alternating residues of 1–4 α-L-guluronic (G-blocks) and β-D-mannuronic acid (M-blocks). Gel formation of alginate is carried out under mild environment, and it is possible thanks to the reaction with divalent cations (Ca, Ba, Sr). Calcium alginate wound dressing with impregnated vancomycin has been investigated as a system for treatment of surgical infections (Lin et al., 1999). However, more sophisticated systems,- ensuring a slow-release of an active substance, are continuously searched for. One of the proposed solutions is the use of bilayer films, in which one layer contains the active drug and the other provides an additional barrier that must be overcome by this drug to reach the treatment site (Thu et al., 2012; Thu and Ng, 2013). In our previous studies we investigated the properties of Vancomycin immobilized at the functionalized silica surfaces, encapsulated in alginate-based gel (Kurczewska et al., 2015b). The system proposed was investigated as a potential modern wound dressing with a slowly-released antibiotic.

There are also some other materials that are composed of halloysite and alginate. Bionanocomposite beads of halloysite/alginate have been characterized and studied as a new drug carriers (Chiew et al., 2014; Karnik et al., 2015). A hybrid nanocomposite containing halloysite nanotubes, chitosan and sodium alginate has been used for a sustained release of an analgesic (Li et al., 2016).

In this work, encouraged by a high binding capacity of different drugs by halloysite, we modified the system with a double barrier. The main aim of the present study was to investigate halloysite nanotubes as carriers of vancomycin, to demonstrate the influence of surface functionalization with (3-Aminopropyl)-trimethoxysilane and finally to investigate the effect of inorganic carrier on release rate and biological activity of vancomycin in potential wound dressings, in which halloysite with ionically-bonded drug is encapsulated in alginate-based matrix.

## 2. Materials and methods

### 2.1. Materials

Halloysite nanotubes (HNTs), (3-Aminopropyl)-trimethoxysilane (APTS), alginic acid sodium salt, gelatin powder and glycerol were commercial products of Aldrich and were used as received. Vancomycin hydrochloride was used as a commercial product of Xelia Pharmaceuticals ApS (Denmark). Hydrochloric acid, ethanol and toluene were purchased from POCH (Poland). All the chemicals were of analytical grade. Demineralized water was used for aqueous solutions preparation.

### 2.2. Synthesis of APTS-modified halloysite

HNTs were first purified with 10% (v/v) hydrochloric acid solution for 24 h at room temperature. Then purified nanotubes were suspended in dry toluene with (3-Aminopropyl)-trimethoxysilane. The mixture was refluxed under constant stirring for 12 h. A calcium chloride drying tube was used to maintain a dry environment. Then the product was filtered off and washed several times with toluene and ethanol to remove the excess of organosilane. Finally APTS-modified halloysite (HNTs_APTS) was dried overnight at 120 °C.

### 2.3. Characterization of unmodified and modified halloysite

Transmission electron microscope (TEM) images were recorded on a Hitachi HT7700 microscope, operating at accelerating voltage of 100 kV. The infrared spectra were taken on an IFS 66v/s Fourier transform infrared (FTIR) spectrophotometer from Bruker, equipped with an MCT detector (125 scans, resolution 2 $cm^{-1}$). The spectra were recorded in the 400–4000 $cm^{-1}$ range for KBr pellets. The thermogravimetric studies were carried out in a Setsys 1200 apparatus (Setaram) at a heating rate of 10 °C/min under helium atmosphere. Elemental analysis was carried out on a Vario ELIII (Elementar, USA) analyzer.

### 2.4. Drug loading and fabrication of alginate-based dressings

Three different HNT samples were used for vancomycin immobilization: commercial product (HNTs), halloysite activated with hydrochloric acid (HNTs_HCl) and halloysite modified with (3-Aminopropyl)-trimethoxysilane (HNTs_APTS). The procedure of drug loading was analogous to that presented for the silicas (Kurczewska et al., 2015b). A schematic presentation of the preparation of vancomycin immobilized at HNTs_APTS is shown in Fig 1.

Alginate-based dressings were prepared only for the samples with vancomycin immobilized at the HNTs_APTS according to the procedure previously described (Kurczewska et al., 2015b). In brief, for Alginate dressing 1.50 ± 0.02 g of sodium alginate (6%, w/v) was dissolved in water. Then 1.5 mL of a plasticizer (glycerol) was added and finally 0.25 or 0.50 ± 0.05 g of the HNTs_APTS was added under rapid stirring of the sample. For making Gelatin/Alginate dressing, at first 1.50 ± 0.04 g of gelatin powder was dissolved in hot water. The following steps were the same as for Alginate gel (alginate was dissolved in the gelatin solution). Alginate or Gelatin/Alginate gels were poured into petri dishes (diameter 7.5 cm) and