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Antibacterial sustained-release coatings from halloysite nanotubes/waterborne polyurethanes

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ABSTRACT

Natural and safe antibacterial nanoparticles based on carvacrol loaded halloysite nanotubes and their waterborne polyurethane nanocomposite coatings with antibacterial and antibiofilm properties are presented. Halloysite nanotubes are natural clay nanoparticles with a hollow tubular structure that allows loading and sustained release of active agents. In this study, halloysite nanotubes were efficiently loaded with carvacrol, the active agent of essential thyme oil. Encapsulated carvacrol molecules were demonstrated to be released from halloysite nanotubes in a sustained manner over one week and effectively inhibit the growth of a pool of pathogenic microorganisms. Carvacrol loaded halloysite nanotubes were further investigated as antibacterial nanofillers in polymeric nanocomposites by incorporating them into waterborne polyurethane coatings. Polyurethane nanocomposite films containing 5 wt.% carvacrol loaded halloysite nanotubes showed uncompromised thermal and mechanical stability as compared to neat polyurethane films. Carvacrol/halloysite nanotubes/polyurethane films demonstrated sustained release of carvacrol and antibacterial activity on representative pathogens, *Aeromonas hydrophila*, as evidenced by growth inhibition in agar diffusion assays and reduction in bacterial count upon exposure to nanocomposite films. Furthermore, these nanocomposite films inhibited bacterial colonization on their surfaces at least for two days demonstrating their applicability as anti-biofilm surface coatings. Composed of safe and natural components, the sustained-release antibacterial coatings presented here have strong potential for being widely utilized to prevent and mitigate bacterial infections on materials surfaces without raising toxicity concerns.

1. Introduction

Pathogenic microorganisms and infections caused by them are of great concern not only for the medical field but also for materials science. Adhesion of bacterial cells onto surfaces and interfaces initiates bacterial colonization and formation of resilient bacterial communities called biofilms, which turn these surfaces and interfaces susceptible to bacterial infections [1–3]. Materials that have the ability to kill pathogenic bacteria and prevent bacterial colonization are desired for utilization in several application areas such as food-contact materials, textiles, water purification systems, prosthetic devices and hospital equipment surfaces. While antibacterial materials benefit community health by controlling bacterial infections, they also greatly contribute to the prevention of industrial economic losses caused by biofouling.

Several approaches have been proposed to obtain antibacterial activity by the modification of surface pattern of materials to prevent bacterial adhesion or by the use of active agents to provide contact killing effect. These approaches include the manipulation of surfaces with steric barriers like polymer brushes [4–6], modification of the surface pattern [7–9], manipulation of hydrophobicity [10,11], and conjugation of materials' surface with polycations [12–15], or antimicrobial peptides [16]. Another approach that is more versatile and effective is the utilization of nanoparticles and polymeric nanocomposites as antibacterial coatings or materials. Metal and metal oxide nanoparticles including silver, copper, gold, titanium and zinc along with carbon based nanoparticles have been demonstrated to have antibacterial properties which are reflected on their polymeric nanocomposites [17–22]. Among these, silver nanocomposites which demonstrated significant antibacterial effect based on the release of silver ions have attracted great

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attention. Several nanocomposites containing silver nanoparticles alone and in combination with other nanofillers have been prepared with natural and biodegradable polymeric matrices [23–28]. While metal nanoparticles are effective antibacterial agents, concerns related to their safety and environmental effects limit their widespread use, commercialization and public acceptance especially in biomedical and food-contact applications. Therefore, there exists a significant need for natural antibacterial nanoparticles that are effective against pathogenic bacteria without raising any toxicity concerns and can be incorporated into nanocomposites with antibacterial effects. Essential oils that are volatile components of herbs and spices are widely studied due to their antibacterial properties [29,30] and attempts to incorporate them into structural materials and surfaces to act against pathogenic bacteria has been reported. One approach that has been studied widely is the direct incorporation of essential oils or their active components into polymeric materials [31–34]. While this method results in polymeric materials with some antibacterial activity, the fact that antibacterial agents are immediately released in an uncontrolled manner from the polymers diminishes the long term antibacterial activity of these materials. As another approach, active components of essential oils have been adsorbed on montmorillonite clay platelets which were then incorporated into polymeric materials as nanofillers. However, poor compatibility of montmorillonite clay with polymer matrices requires the use of compatibilizers or leads to poor mechanical properties in resulting nanocomposites [35,36]. While these examples demonstrate the utilization of essential oils in bulk polymeric materials such as thermoplastics, incorporation of essential oils into surface coating formulations with sustained release behavior has not been reported previously.

In order to benefit from the antibacterial effects of essential oils to the best in the form of safe antibacterial nanocomposite coatings, they need to be efficiently encapsulated within natural and nontoxic nanocontainers that allow their sustained release and can be incorporated into a suitable polymeric coating system. Halloysite nanotubes (HNTs) are hollow tubular aluminum silicate nanoparticles with a high aspect ratio that can be utilized as nanocarriers and can be effectively dispersed in polymeric matrices resulting in active agent releasing nanocomposites [37–39]. Their nontoxic nature [40,41], effective encapsulation capacity and suitability for incorporation into polymers to prepare nanocomposite materials render HNTs ideal nanocontainers for antibacterial agents. An ideal polymer coating system for the effective utilization of such essential oil loaded halloysite nanotubes as sustained-release antibacterial nanofillers would be environmentally friendly waterborne polyurethanes which are widely employed in industrial applications due to their versatile chemistry to yield coatings with tunable thermo-mechanical properties [42].

Herein we studied the encapsulation of carvacrol, the active component of essential thyme oil, within HNTs to prepare natural and safe antibacterial nanoparticles, and their incorporation into waterborne polyurethane dispersions, to obtain sustained-release antibacterial and antibiofilm surface coatings.

2. Experimental methods

2.1. Materials

HNTs were provided by Eczacibasi Esan (Turkey). Carvacrol was supplied by Tokyo Chemical Industry Co. (Japan). The antibacterial activity of carvacrol loaded HNTs was determined by using different bacterial strains including *Aeromonas hydrophila* (*A. hydrophila*, ATCC 35654), *Pseudomonas putida* (*P. putida*, ATCC 49128), *Listeria monocytogenes* (*L. monocytogenes*, ATCC 7644) and *Staphylococcus aureus* (*S. aureus*, ATCC 25923). All bacterial strains were purchased from Medimark (France). Tryptic soy broth (TSB), Nutrient broth (NB), Brain-heart infusion (BHI) and Agar powder were purchased from Biolife (Italy). Anionic, aqueous polyurethane (PU) dispersion based on a polyester-polyol was kindly supplied by Punova R&D and Chemicals Inc. (Turkey) with a 35 wt.% solid content.

2.2. Loading of HNTs with carvacrol

In order to load HNTs they were mixed with liquid carvacrol with a ratio of 0.1 g HNT per 1 mL carvacrol. Three different protocols were followed for the loading; (i) Ultrasonication: HNT-carvacrol mixture was subjected to ultrasonication with a microprobe (Qsonica, Q700) for 30 min with 2 s pulse on and 5 s pulse off time in an ice bath. (ii) Vacuum application: HNT-carvacrol mixture was transferred into a vacuum jar connected to a vacuum pump and 1 mbar pressure was applied for 30 min to remove air inside HNTs followed by application of atmospheric pressure for 10 min to allow carvacrol molecules enter evacuated HNTs. The cycle was repeated twice to increase loading efficiency. (iii) HNT-carvacrol mixture was subjected to ultrasonication and then treated with vacuum application as described in the first and second protocols above, respectively. For all three protocols, solid phase in the resulting suspension comprising carvacrol loaded HNTs was separated by centrifugation at 5000 rpm for 5 min, and the excess carvacrol was removed. Carvacrol loaded HNTs were washed with ethanol once or twice by centrifugation to remove surface adsorbed carvacrol molecules and were dried overnight at room temperature in an open container. Dry loaded HNTs were kept in a closed container at room temperature.

2.3. Determination of carvacrol loading efficiency

Carvacrol loading efficiency was determined by thermogravimetric analysis (TGA) on a DTG-60H (Shimadzu, USA) instrument. Samples of carvacrol loaded HNTs and unloaded HNTs were heated in an alumina pan from 30 to 1000 °C at a rate of 10 °C/min under nitrogen flow. The resulting temperature dependent weight loss percentages were analyzed using TA-60WS Collection software. Loading efficiency was calculated as the difference in total weight loss of carvacrol loaded HNT sample and unloaded HNT sample.

2.4. Imaging of HNTs with transmission electron microscopy

Transmission electron microscopic (TEM) analysis of loaded and unloaded HNTs was performed using JSM-2000FX (JEOL, Japan) at an operating voltage of 160 kV, using a 200 mesh Copper grid (Formvar film).

2.5. Measurement of carvacrol release from HNTs

The release rate of carvacrol from HNTs was measured by TGA under isothermal conditions on DTG-60H (Shimadzu, USA). Samples of carvacrol loaded HNTs and unloaded HNTs in an alumina pan were kept at 30 °C for one week under air flow and the percent weight loss was monitored as a function of time.

2.6. Antimicrobial activity of carvacrol loaded HNTs

The agar diffusion method was used to evaluate the antimicrobial activity of carvacrol loaded HNTs against *A. hydrophila*, *P. putida*, *L. monocytogenes* and *S. aureus*. 3 mL overnight cultures of bacteria were grown in appropriate growth media. *A. hydrophila* were grown in TSB at 30 °C, *P. putida* were grown in NB at 30 °C, *L. monocytogenes* were grown in BHI at 37 °C and S. *aureus* were grown in TSB at 37 °C on a shaker incubator (200 rpm). 0.1 mL of overnight