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Research paper

Kinetics of release and antibacterial activity of salicylic acid loaded into halloysite nanotubes

L. Ghezzi[a], A. Spepi[a], M. Agnolucci[b], C. Cristani[b], M. Giovannetti[b], M.R. Tiné[a], C. Duce[a,*]

[a] Department of Chemistry and Industrial Chemistry, University of Pisa, Via Moruzzi 3, I-56124 Pisa, Italy
[b] Department of Agriculture, Food and Environment, University of Pisa, Via del Borghetto 80, I-56124 Pisa, Italy

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ABSTRACT

In this work halloysite (Hal) nanotubes were used as nanocontainers for salycilic acid (SA) in a perspective of its use in active packaging for food industry. The system Hal/SA was investigated for its ability to stabilize Hal suspensions by turbidimetry, its release kinetics in water by UV spectroscopy and its antibacterial activity against *Pseudomonas fluorescens* IMA 19/5 by Isothermal Micro Calorimetry (IMC). IMC is a sensitive and non-destructive technique and allows the study of a wide range of relatively slow processes (hours and days) in solutions. The system Hal/SA resulted to stabilize Hal suspension in water and to release SA in a controlled way over 50 h. Moreover the SA released by Hal/SA showed an antibacterial activity at lower concentrations than free SA, likely due to the close contact of bacteria and Hal in the reaction vessel.

1. Introduction

Halloysite (Hal) nanotubes are naturally occurring aluminosilicate that have been massively exploited as nanocontainers in recent years due to their biocompatibility and absent cytotoxicity at concentrations up to 0.2 mg/ml (Vergaro et al., 2010; Lai et al., 2013; Lvov et al., 2014; Massaro et al., 2017). In particular, Hal has a hollow tubular structure of 10–15 bilayers of aluminosilicate layers with SiOH and AlOH groups on the external and internal surfaces, respectively (Abdullayev et al. 2012; Yuan et al., 2015; Massaro et al., 2017). The Hal dimensions depend on the Hal deposit, with a length of 0.2–1.5 µm and inner and outer diameters of 10–30 nm and 40–70 nm, respectively (Abdullayev et al. 2012; Yuan et al., 2015; Massaro et al., 2017). Several works investigated the properties of pristine and modified Hal (Duce et al., 2015; Bretti et al., 2016; Presti et al., 2016; Massaro et al., 2017), its loading and sustained release of active chemical and biochemical molecules (Viseras et al., 2008; Yuan et al., 2012; Aguzzi et al., 2013; Lvov and Abdullayev, 2013; Lun et al., 2014; Tan et al., 2014; Abdullayev and Lvov, 2016; Della Porta et al., 2016; Li et al., 2016; Duce et al., 2017) and its possible use in composite and materials with tailored properties (Lvov and Abdullayev, 2013; Zhang et al., 2016; Massaro et al., 2017).

Salicylic acid (SA) is a natural compound used in a wide range of pharmaceutical formulations and as an additive for food (Baxter et al., 2001) and cosmetics (Coleman and Brody, 1997) due to its bactericidal and antiseptic properties. Moreover it is used to model natural organic matter (NOM) in both experimental and theoretical studies of NOM adsorption on different kinds of mineral surfaces, including clays (Yost et al., 1990; Biber and Stumm, 1994; Kubicki et al., 1997; Biddeci et al., 2016; Makaremi et al., 2017).

The system Hal/SA was previously obtained and characterized (Spepi et al., 2016). Different experimental conditions were tried in order to obtain the maximum loading of SA. The better results were obtained using Hal etched with H_2SO_4 2 M at 25 °C for 48 h and a solution of sodium salicylate (NaSA) at pH 8. As reported previously (Abdullayev et al. 2012; Yuan et al., 2015), mild acid or alkaline treatments slightly enlarged the Hal lumen, but substantially maintained Hal structure. Indeed, SEM X-ray elemental analysis showed that Hal pre-treated with H_2SO_4 2 M at 25 °C for 48 h etching slightly reduced the aluminum/silicon ratio from 1, for pristine Hal, to 0.8 for etched Hal, while SEM images revealed that the nanotubes structure was maintained. (Spepi et al., 2016) Nitrogen Adsorption/Desorption Isotherms showed also a similar pore distribution for Hal etched at 25 °C and pristine Hal, with a slightly higher pore volume and BET surface area (BET area (m^2/g): 70.9 pristine Hal; 113.6 etched Hal; Pore volume (ml/g): 0.1637 pristine Hal; 0.2488 etched Hal).

The pH of the NaSA loading solution was set to 8 in order to maximize the negative charge of salicylate, while remaining within the pH range of 4–8.5 where the inner surface of HNTs was positively charged. (Abdullayev et al., 2012; Yuan et al., 2015; Spepi et al., 2016) Under such conditions, TG and TG-FTIR data showed that the amount of salicylate retained was 10.5% (w/w) and that the NaSA thermal

* Corresponding author.
E-mail address: celia.duce@unipi.it (C. Duce).

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degradation was drastically modified, when NaSA was located inside Hal, suggesting a strong interaction of the salicylate moiety chemisorbed on the aluminum hydroxide surface. Scanning transmission electron microscopy (STEM) revealed that after NaSA loading, the empty lumen of the HNT was no longer visible (Spepi et al., 2016). NaSA inside Hal lumen produced a partial pore blockage and a reduction in the BET area, micropore area, micropore volume parameters, and mesoporosity of the material. ATR–FTIR interfacial spectra revealed that the Ph–OH group seemed to be involved in the interaction between salicylate and aluminum, with a weakening of the hydrogen bond. The splitting of the ν_{as} and ν_s of $-COO^-$ into two components suggested the presence of different complexes Hal/SA. The experimental spectra seemed to refer to a NaSA molecule in solution and adsorbed in Hal with a large number different configurations and DFT calculations indicated that the salicylate preferred to adsorb in a monodentate and bridging mode rather than a bidentate mode. In fact, the vibrational spectra of the monodentate and bridging adsorbate models also compared better with the experimental one (Spepi et al., 2016).

To the best of our knowledge, data on the behaviour of Hal/SA system in suspension in aqueous medium are lacking, although they could be exploited in a perspective of Hal/SA use in active packaging for food industry. The concept of active food packaging was developed in the last decade and entails not only food protection, but also positive effects on its safety. One of the most interesting example was the incorporation of antimicrobial compounds into food packaging materials (Galotto et al., 2015). When antimicrobial substances are incorporated into polymers, such antimicrobial films release active compounds and display continuous antimicrobial effects on the food surface during the exposure time, thus increasing consumer safety as the antimicrobial compounds are included in the packaging structure (instead of being directly added to food) and are released in small amounts on the food surface. According to literature, the packaging industry already focused its attention on polymer-clay nanocomposites (Azeredo, 2009) and, even if montmorillonite was the most studied clay filler, there is increasing research interest in potential applications of Hal as filler for polymer nanocomposites (Pasbakhsh et al., 2016). To this aim, Hal nanotubes were tested very recently as active food-packaging material based on starch-halloysite nanocomposites incorporating antimicrobial peptides (Meira et al., 2017) and on poly-lactic acid(PLA)-halloysite nanocomposites using Hal as nanocontainers to carry antimimicrobial ZnO agent within the PLA matrix (De Silva et al., 2015).

Concerning future applications, the use of SA loaded Hal in polymer nanocomposites appears very promising. Besides reinforcement of the food packaging materials, the new formed nanostructure could provide an additional antimicrobial activity thanks to the loaded SA (as already observed by De Silva et al., 2015).

Most of nano-additives (including Hal) were incorporated at 0.1–5% w/w in the packaging material, particularly films. They may be incorporated into polymers by melt- or solvent-compounding. Thermal polymer processing methods, such as extrusion and injection molding, may be used with thermally stable antimicrobials. SA doped Hal can withstand temperatures up to 200–300 °C and therefore could be incorporated as a thin co-extruded layer with processable polymers at such temperature range. The polymer could be selected from the group consisting of polyethylene (PE), poly(vinyl alcohol) (PVA) and bio-based plastics and their blends, that are commonly used in food packaging (Galotto et al. 2015). As organic polymers are not soluble in water or polar solvents, the melt compounding is preferred. Conversely, for polymers like PVA or its blends, the nanocomposite can be also prepared in solution, i.e. by ultrasonicating SA-doped HNTs in the polymer solution.

Exfoliated nanocomposites were reported to exhibit the best properties due to the optimal interaction clay/polymer (De Azeredo, 2009). Exfoliation could be attained via previous chemical modification of the Hal, for example by using organic ammonium ions which help improve the compatibility with organic polymers.

Data on the behaviour of Hal/SA system in suspension are useful to understand the mechanism of Hal aggregation in water and to investigate different ways to stabilize Hal dispersions. In fact, the absorption of negatively charged molecules inside Hal lumen enhanced the Hal dispersion stability by increasing the net negative charge and electrostatic repulsion of Hal (Cavallaro et al., 2012).

The in vitro release studies of encapsulated molecules (drugs, proteins, DNA, corrosion inhibitors etc.) showed a controlled release in aqueous medium that can be further extended by exploiting tube-stoppers, shells and the creation of Hal-polymer nanocomposites (Lvov et al., 2016, 2014). The profile of guest molecules release from Hal lumen was widely described and discussed in terms of Peppas model (Lvov and Abdullayev, 2013). Differently, the group of Viseras proposed a model based on a combination of first order desorption kinetics that successfully fitted the 5-aminosalicylic acid release from Hal (Viseras et al., 2008; Aguzzi et al., 2013).

In vitro antibacterial, antimicrobial and anticancer tests of new Hal formulations are generally performed by monitoring selected bacterial or cellular/microbial activity on Petri plates added with Hal complexes and comparing it with their standard growth (Abdullayev et al., 2009; Lvov and Abdullayev, 2013; Riela et al., 2014; Biddeci et al., 2016; Makaremi et al., 2017). In this work, the antibacterial activity of pure SA and Hal/SA system was tested against *P. fluorescens* by using Isothermal Micro Calorimetry (IMC) in order to have direct information of Hal/SA antimicrobial activity in aqueous suspension. IMC measures the heat involved in biological processes in vitro and it is successfully employed for microorganism detection and discrimination, studies of microbial processes and tests of antimicrobial and antibacterial activities of various chemicals (Von et al., 2009; Braissant et al., 2010a, 2010b, 2013; Vazquez et al., 2014). IMC is a sensitive and non-destructive technique and allows the study of a wide range of relatively slow processes (hours and days) in solutions. Microbial cultures in liquid media are placed in the measurement vessel and the heat flow signal is registered. The inoculum of the active agent on the microbial cultures modifies or inhibits the heat evolved. IMC is a promising tool for medical, environmental and food microbiology. (Rong et al., 2007; Von Ah et al., 2009; Braissant et al., 2010a, 2010b; Gardikis et al., 2017).

The aim of this work was to characterize the behaviour of Hal/SA in aqueous medium. In particular Hal/SA was here investigated for its capability of stabilizing Hal suspensions by turbidimetry, for its release kinetics in water by UV spectroscopy and for its antibacterial activity against *Pseudomonas fluorescens* IMA 19/5, a bacterial species active in food spoilage (Gram et al., 2002), by Isothermal Micro Calorimetry (IMC).

2. Experimental

2.1. Materials

Pristine halloysite nanotubes, Sodium salycilate (NaSA) (99.5%), NaOH (≥ 97.0, pellets) were purchased from Sigma Aldrich and used without further purification. SA loaded Hal (Hal/SA) was prepared according to the literature (Spepi et al., 2016; Duce et al., 2017). The suspension of Hal in water was prepared by adding 5 g of etched Hal to a concentrated solution of NaSA in water (1 g/ml) and the pH was adjusted at 8 with NaOH 0.1 M. The suspension was evacuated in a vacuum jar, kept under vacuum for 3 h, and then cycled back to atmospheric pressure. This process was repeated three times. Finally, Hal was separated from the solution by centrifugation, washed with water, dried in an oven at 70 °C. The amount of salicylate retained was determined by thermogravimetry resulting in a 10.5% (w/w). The characterization of the empty and loaded systems by means of SEM, SEM X-ray Elemental Analysis, STEM, TG, TG-FTIR, Nitrogen Adsorption/Desorption Isotherms was reported in Spepi et al., 2016. *Pseudomonas*