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Biocidal efficacy, biofilm-controlling function, and controlled release effect of chloromelamine-based bioresponsive fibrous materials

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Abstract

In this study, 2-amino-4-chloro-6-hydroxy-*s*-triazine (ACHT) was synthesized through controlled hydrolysis of 2-amino-4,6-dichloro-*s*-triazine (ADCT). A simple pad-dry-cure approach was employed to immobilize ACHT onto cellulosic fibrous materials. After treatment with diluted chlorine bleach, the covalently bound ACHT moieties were transformed into chloromelamines. The structures of the samples were fully characterized with NMR, UV/VIS, DSC, TG, iodometric titration and elemental analyses. The chloromelamine-based fibrous materials provided potent, durable, and rechargeable biocidal functions against bacteria (including multi-drug resistant species), yeasts, viruses, and bacterial spores. SEM studies demonstrated that the new fibrous materials could effectively prevent the formation of biofilms, and controlled release investigations in vitro suggested that the biocidal activities were bioresponsive. Biocidal mechanisms of the chloromelamine-based fibrous materials were further discussed.

Keywords: Biocidal polymers; Cotton cellulose; Biofilm; Bioresponsive; Controlled release

1. Introduction

Fibrous materials, such as gowns, uniforms, face masks, bedding materials, drapes, pillows, mattresses, dishcloths, aprons, etc., are widely used in healthcare-, institutional- and household-settings. Unfortunately, like other polymeric materials, fibrous materials are susceptible to contamination of various microorganisms including pathogenic bacteria, viruses, yeasts and spores, with some species surviving for longer than 90 days [1–4]. The deposited microorganisms could be liberated and re-dispersed into the air [5], or transferred to the surrounding environments through direct or indirect contact [6–10]. Therefore, in real applications, contaminated fibrous materials can be important sources of cross-infections, which have already caused serious outbreaks of nosocomial infections in healthcare facilities [11–16]. As "one ounce of prevention equals a pound of cure" in dealing with the global concern of emerging and re-emerging infectious diseases [17,18], there is a clear need to control microbial contaminations on fibrous materials in order to reduce the incidence of infections.

One of the most effective approaches in controlling microbial contamination is to introduce biocidal functions into the target materials [19]. Following the pioneering work of Gagliardi [20], which described the principles and strategies for imparting antimicrobial activities into fibrous materials, various biocidal agents, including antibiotics [21–23], metal ions [24], quaternary ammonium salts [25–27], phosphonium compounds [27,28], *N*-halamines [29,30], etc., have been incorporated into fibrous materials. The antimicrobial activities and mechanisms of these agents differ considerably. Among them, organic *N*-halamines have been demonstrated to be a class of effective, durable and reachargeable antimicrobial agents with low toxicity and little environmental concern [31–38].

An *N*-halamine can be defined as a compound containing one or more nitrogen–halogen covalent bonds, in which the positive halogen provides antimicrobial properties [31]. Based on their chemical structures, organic *N*-halamines can be classified into three types: imide *N*-halamines, amide

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N-halamines, and amine *N*-halamines. Due to the differences in the localized chemical environments, their stabilities follow the order of imide *N*-halamines < amide *N*-halamines ≪ amine *N*-halamine, and their antimicrobial activities have a trend of imide *N*-halamines > amide *N*-halamines ≫ amine *N*-halamine [31]. In other words, the stability and antimicrobial activity of *N*-halamines are determined by opposite factors.

One of the research interests of this group is the development of *N*-halamine-based polymeric materials to achieve biocidal and biofilms-controlling functions [34,39–41]. Our most recent efforts have been focused on a class of unique *N*-halamines, chloromelamines, which have already been safely used as water and food disinfectants [42]. Structurally, chloromelamines belong to amine *N*-halamines. However, because of the strong electron withdrawing effect of the triazine rings, their chemical environments are similar to those of amide *N*-halamines. Therefore, it is expected that the biocidal activity and stability of chloromelamines may be between "normal" amine and amide *N*-halamine. This can be an attractive characteristic for biocidal treatments of polymeric materials in applications that require both strong biocidal activity and good stability.

To provide detailed information about the performance of chloromelamine-based polymeric materials, in this study, 2-amino-4-chloro-6-hydroxy-*s*-triazine (ACHT) was synthesized through the hydrolysis of 2-amino-4,6-dichloro-*s*-triazine (ADCT), and then immobilized onto cotton cellulose using a pad-dry-cure approach. After chlorine bleach treatment, the immobilized ACHT moieties were transferred into chloromelamines. The resultant fibrous materials were challenged with bacteria, yeasts, viruses, and spores to determine the biocidal activities. Moreover, the biofilm-controlling functions and controlled release effects of the covalently bound chlorines were evaluated, and the biocidal mechanisms were discussed.

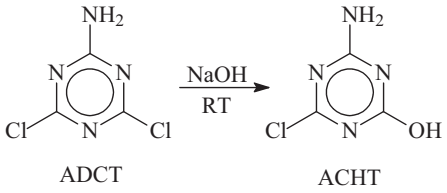
2. Materials and methods

2.1. Materials

Bleached cotton knit fabrics (catalog number: 489; weight: 175 g/m^2) were purchased from Testfabrics, Inc. (West Pittston, PA). Before immobilization reactions, the fabrics were treated with boiling acetone for 30 min to remove possible impurities. 2-amino-4,6-dichlorotriazine (ADCT) was provided by Monomer-Polymer & Dajac Labs, Inc. (Feasterville, PA), which was purified by two recrystallizations from ether and one from benzene. Other chemicals were obtained from Fisher Scientific (Fair Lawn, NJ) and used as received.

2.2. Synthesis of ACHT

ACHT was synthesized through controlled hydrolysis of ADCT using a modified method reported previously [43], as shown in Scheme 1. In the current study, ADCT (16.5 g, 0.1 mol) was suspended in 250 ml of distilled water containing 4.4 g (0.11 mol) of sodium hydroxide. The mixture was stirred at room temperature for 15 h. After filtration, 7.32 g of un-reacted ADCT were removed. The clear, colorless filtrate was cooled to 0–5 °C and neutralized (pH 6.8–7.0) with glacial acetic acid. The white solid was collected by filtration, washed with cold water, dried in air, and recrystallized from hot water to yield 4.59 g of ACHT (Yield: 56.3%, based on the amount of reacted ADCT).



Scheme 1. Synthesis of ACHT through controlled hydrolysis of ADCT.

2.3. Immobilization of ACHT onto cotton cellulose

A known amount of ACHT was dissolved in distilled water containing 2 wt% of NaOH and 0.05 wt% of a nonionic wetting agent (TX-100) to form an aqueous solution containing 4 wt% of ACHT. A pad-dry-cure approach was used to immobilize ACHT onto cellulose. Our previous studies have demonstrated that this method could provide high reaction efficiency in a relatively short period of time [44]. In this treatment, cotton fabrics were dipped into the ACHT solution, padded through a laboratory wringer (Atlas Electric Devices Co., Chicago, IL) to 100% wet pickup (three repeats), wrapped in aluminum foil, and cured in an oven at 120 °C for 20 min. The fabrics were then washed thoroughly with a large amount of distilled water, dried at room temperature and stored in a desiccator to reach a constant weight.

2.4. Chlorination of ACHT-immobilized cotton fabrics

The ACHT-immobilized cotton fabrics were immersed in diluted chlorine bleach (Clorox Company, Oakland, CA) solutions containing 3000 ppm of active chlorine and 0.05 wt% TX-100 at room temperature for 30 min under constant shaking. The bath ratio was kept at 30:1. After chlorination, the fabrics were washed with a large amount of distilled water (the washing water was tested with KI/starch to ensure that most of the free chlorines were removed), air-dried, and stored in a dessicator to reach constant weights.

The chlorine content of the treated fabrics was determined by iodimetric titration [44]. Briefly, about 0.5 g of the chlorinated ACHT-immobilized fabrics was cut into small pieces and then added into 40 ml of absolute ethanol containing 2 g KI. The mixture was vigorously stirred at room temperature for 60 min under N_2 atmosphere. The iodine released during the oxidation reaction of KI by the covalently bound chloromelamine structures was titrated with 0.01 mol/l of sodium thiosulfate aqueous solution. The same amount of unchlorinated ACHT-immobilized fabrics was also titrated using the same method as the control. The chlorine content of the sample was calculated according to the following equation:

$$[\mathrm{Cl}] = \frac{35.5}{2} \times \frac{(V_s - V_c) \times 10^{-3} \times 0.01}{W_s} \times 10^6, \quad (1)$$

where [Cl] was the chlorine content of the sample (ppm); V_s and V_c were the volumes (ml) of the sodium thiosulfate aqueous solutions consumed in the titration of the sample and control, respectively; and W_s was the weight of the chlorinated ACHT-immobilized fabrics (g).

2.5. Characterization

Elemental analysis was conducted by Quantitative Technologies Inc. (Whitehouse, NJ). Thermal properties of the samples were tested on Shimadzu DSC-60 and Shimadzu TGA-50 (Shimadzu, Kyoto, Japan) at a heating rate of 10 °C/min under N_2 atmosphere. UV/VIS spectra were recorded on a Beckman DU 520 General Purpose UV/VIS