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Controlled release of preservatives using dealuminated zeolite Y

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Abstract

This study demonstrates that dealuminated zeolite Y can act as a depot after adsorption of phenol derived preservatives. Upon suspension of zeolite loaded with the preservative m-cresol, equilibrium was quickly reached between free and adsorbed m-cresol. The equilibrium concentration of m-cresol was below 1 mM; however, it was enough to kill bacteria such as *Escherichia coli* and *Staphylococcus aureus* under metabolically active conditions. Killing of bacteria was not obtained under non-proliferating conditions and m-cresol was only released from the zeolite upon bacterial activity. Together, these results demonstrate an interesting potential use of dealuminated zeolite Y containing adsorbed preservatives for preventing microbial growth in numerous applications in industry and clinical setting.

Keywords: Anti microbial activity; Benzalkonium chloride; Dealuminated zeolite; m-cresol

1. Introduction

Zeolites are tectosilicates consisting of corner-sharing AlO_4 and SiO_4 tetrahedrals that form well-defined channels and cavities, with apertures that range from 2.5 to 7.5 Å in diameter [1]. The properties of zeolites are determined by their chemical composition and the size and dimensionality of the channel system. The ratio of Si/Al is a chemical feature of zeolites that can be varied either during synthesis or post-synthetically [2]. The zeolites commonly used have a low Si/Al ratio (usually 1 to 4) and thus a high capacity for ion-exchange. The capacity decreases, however, if the Al content is reduced and zeolites with a high Si/Al ratio (usually between 100 to 400), termed dealuminated or ultra-stable zeolites due to their high thermostability [3], are more hydrophobic in their adsorption properties. Molecules that pass through the zeolite pores can be adsorbed by the zeolite and up to 30% by weight of the zeolite can be bound [4].

Various molecules such as tensides [4], phenol and its derivates [5] have been reported to be adsorbed by dealuminated zeolites. Proteins and other macromolecules are too large to enter the zeolite pores and thus protein adsorption can only occur on the external surface of the zeolite [6]. Small dealuminated zeolite particles have been used as carriers to deliver proteins as well as low molecular weight biomolecules into phagocytosing cells [7].

Silicates have been described as reservoirs for controlled release in various systems [8,9]. Mesoporous silica has been addressed as a potential controlled release system [10] and several methods for the preparation of porous silica have been reported [11–13].

Commonly used zeolites with a low Si/Al ratio have an ion-exchange capacity and mainly been used as a reservoir and release system of ions. Zeolites have especially been used as a release system in soil fertilizing applications [14,15]. Dealuminated zeolites are more hydrophobic in their adsorption properties and adsorb mainly un-charged molecules. This paper describes the use of dealuminated zeolite Y as a reservoir and controlled release system of phenol based preservatives.

2. Materials and methods

Crystalline particles of dealuminated zeolite Y, USY (Si/Al ratio: 240), with an average particle size of 4 μm were obtained from Tosoh Corporation, Japan. Sintered particles of dealuminated zeolite Y having a particle size of 63 to 150 μm, were manufactured by Tosoh Corporation, Japan from USY (Si/Al ratio: 260) and 15% alumina as binder.

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Bacterial strains used were *Escherichia coli* CU1867 (ATCC 47092) and *Staphylococcus aureus* (ATCC 6538).

Phenol, m-cresol, benzoic acid, thymol and benzalkonium chloride were obtained from Sigma, St. Louis, MO, USA.

2.1. Adsorption of m-cresol and benzalkonium chloride to dealuminated zeolite Y

Benzalkonium chloride or m-cresol were solubilized in water or phosphate buffer saline (PBS) and various amounts of the preservatives were added to crystalline particles of dealuminated zeolite Y, 25 mg/ml final concentration. The samples were Vortex mixed for 30 s before the zeolite particles were removed by centrifugation for 5 min at 13,000×*g*. The concentration of m-cresol or benzakonium chloride remaining in the supernatants was determined by absorbance at 280 nm.

2.2. Adsorption of m-cresol by sintered particles of dealuminated zeolite Y and the release of adsorbed m-cresol

Sintered particles of dealuminated zeolite Y, 40 mg/ml, were incubated with m-cresol (23.1 mM, 46.2 mM or 92.5 mM solubilized in water) on a rocking table for 2 h at room temperature. The zeolite particles were harvested on a glass funnel and dried over night at 60 °C. The amount of m-cresol in the filtrate after glass funnel filtration was determined by absorbance at 276 nm and used to calculate the amount of m-cresol adsorbed by the zeolite. After incubation with 23.1 mM, 46.2 mM and 92.5 mM m-cresol, respectively, 0.6 μmol, 1.1 μmol and 1.8 μmol m-cresol were adsorbed per mg zeolite.

Various amounts of dried zeolite particles containing adsorbed m-cresol were re-suspended in one ml PBS. After 1 h at a rocking table the samples were centrifuged 13,000×*g* for 5 min and the supernatants were removed. The zeolite particles were carefully re-suspended in new portions of 1 ml PBS, incubated for another hour at a rocking table, centrifuged and after removal of the supernatants, re-suspended in PBS. The amount of released m-cresol into the PBS supernatants after each 1-h incubation with zeolite particles was determined by absorbance at 276 nm.

2.3. Adsorption of benzalkonium chloride by sintered particles of dealuminated zeolite Y and the release of adsorbed benzalkonium chloride

Sintered particles of dealuminated zeolite Y, 40 mg/ml, were incubated with 15 mM benzalkonium chloride in water on a rocking table for 2 h at room temperature. The zeolite particles were harvested on a glass funnel and dried over night at 60 °C. The amount of benzalkonium chloride in the filtrate after glass funnel filtration was determined by absorbance at 263 nm and used to calculate the amount of benzalkonium chloride adsorbed by the zeolite. After incubation, 0.3 μmol benzalkonium chloride was adsorbed per mg zeolite.

Twenty milligrams of dried zeolite particles containing adsorbed benzalkonium chloride was re-suspended in 1 ml PBS. After 30 min at a rocking table the samples were centrifuged, 13,000×*g* for 5 min, and the supernatant was removed. The zeolite particles were carefully re-suspended in a new portion of 1 ml PBS, incubated for another 30 min at a rocking table, centrifuged and after removal of the supernatant, re-suspended in PBS. Totally five re-suspensions were made in PBS and the concentration of benzalkonium chloride in the PBS supernatants was determined by absorbance at 263 nm. Finally, the zeolite particles were re-suspended in three portions, each of 1 ml ethanol and the concentration of benzalkonium chloride in the ethanol supernatants were determined by absorbance at 263 nm.

2.4. Inhibition of bacterial growth by release of m-cresol

2.4.1. E. coli

Sintered particles of dealuminated zeolite Y, 40 mg/ml, were incubated with 92.5 mM m-cresol in water on a rocking table for 2 h at room temperature. As a control, zeolite particles were incubated with water. The zeolite particles were harvested on a glass funnel and dried over night at 60 °C. The amount of m-cresol in the filtrate after glass funnel filtration was determined by absorbance at 276 nm and used to calculate the amount of m-cresol adsorbed by the zeolite. After incubation, 1.6 μmol m-cresol was adsorbed per mg zeolite (0.17 mg m-cresol/mg zeolite).

Five milligrams of dried zeolite particles containing adsorbed m-cresol or zeolite particles without any adsorbed m-cresol was suspended in 200 μl LB-Medium (LB-Broth) containing 8×10^5 cfu/ml of *E. coli* strain CU1867. Duplicates were made of each zeolite preparation. After 5 min the zeolite particles had sedimented and 100 μl of the LB-medium above the sedimented zeolite particles was carefully removed. The sedimented zeolite particles containing entrapped *E. coli* (8×10^4 cfu) were incubated at room temperature or at 8 °C. After 18 h at room temperature or 2.5 days at 8 °C the zeolite particles were each re-suspended in 1 ml LB-Medium and the number of colony forming units in the suspensions was determined by a serial dilution plated on LB-agar plates and incubated overnight at 37 °C.

2.4.2. S. aureus

Sintered particles of dealuminated zeolite Y, 40 mg/ml, were incubated with 92.5 mM or 46.2 mM m-cresol in water on a rocking table for 2 h at room temperature. As a control zeolite particles were incubated with water. The zeolite particles were harvested on a glass funnel and dried over night at 60 °C. Adsorbed m-cresol (mg m-cresol/mg zeolite) was determined by measuring the amount of released m-cresol after dilution of the zeolite particles to 0.1 mg zeolite/ml. The amount of m-cresol was determined by absorbance at 276 nm and was found to 0.12 mg m-cresol respectively 0.1 mg m-cresol per mg zeolite.

After loading with 92.5 mM and 46.2 mM m-cresol the zeolite preparations contained 0.12 respectively 0.1 mg m-cresol adsorbed per mg zeolite. In order to get a maximal total release capacity corresponding to 3 mg m-cresol/ml after re-suspension of the zeolite particles, 25 mg respectively 30 mg of loaded zeolite particles was re-suspended per ml and incubated with *S. aureus*. An accredited laboratory measured the number of colony forming units after incubation with *S. aureus*