Utilization of Nanotechnology for Development of Antimicrobial Zeolities Surfaces

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The current study aims to develop stable antimicrobial materials containing silver and zinc ion–exchanged zeolites. Faujasite X and Linde type A zeolites were synthesized, and following ion exchange with Ag+ and Zn++ ions they were found to exhibit antimicrobial effects against *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*, *Candida albicans*, and *Aspergillus niger*. Zeolites X and A; containing silver and zinc ions were then mixed with various coating materials, including paints and polypropylene, to develop antimicrobial composites. The long-term antimicrobial characteristics of zeolite-containing composite materials were investigated by inoculating selected microorganisms onto the surface of the materials. The results indicated that the higher the zeolite concentration present in the composite, the more long-term antimicrobial activity was achieved. Silver ion–exchanged zeolites were more effective against bacterial and candidal species, while zinc zeolites exhibited noticeable antifungal properties. Materials manufactured with metal-ion–exchanged zeolites would prevent microbial growth on surfaces, reducing cross-contamination and infection risk as well as the microbial degradation of products.

Key words : Silver, zinc, Zeolite, Antimicrobial, Coating, Polypropylene, Paint.

Microbial species including fungi, yeast, and bacteria can live almost anywhere on the earth, and some may be primary and opportunistic pathogens causing clinically important diseases in human beings, animals, and plants. In the early 1900s, infectious diseases were the most common cause of death worldwide[1]. Current technology is available to control pathogenic microbial flora under *in vivo* and *in vitro* conditions with use of antimicrobial agents such as antibiotics, antiseptics, disinfectants, and synthetic drugs. Over the last century the number of deaths originating from microbial infections has decreased considerably with the development of antimicrobial agents. On the other hand, microorganisms have developed resistance to certain antibiotics due to misuse and overuse[2]. The use of high dose antibiotics resulted in microorganisms with acquired resistance such that the effectiveness of some of the available antibiotics has been invalidated[3, 4]. Both Gram-positive and Gram-negative bacterial pathogens that develop drug resistance in hospitals compromise our ability to treat serious infections[5, 6, 7]. This challenging and dynamic pattern of infectious diseases and the emergence of antibiotic resistance demands longer-term solutions[8, 9]. Toxicity, adverse drug reactions, and drug resistance have led scientists to develop novel and safer antimicrobial agents that are effective against most microorganisms.

Natural and manufactured surfaces provide a shelter for microorganisms where they can survive and proliferate. As they increase in number, they secrete extracellular matrix proteins

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which act as a barrier to external threats and make them 1000 times less sensitive to biocides and antimicrobials[10]. Microbial contamination of surfaces, especially in the hospital environment, is the major cause of the spread of infection between patients[11, 12, 13]. Microbial species including human pathogens can easily adapt to the surface of various materials and survive more than 90 days[14]. A possible solution for preventing surface contamination is the frequent use of disinfectants. However, they have adverse effects on the environment and may not be an economical approach[15]. Therefore, developing novel, safe, and cost-effective antimicrobial surfaces that inhibit microbial growth is of considerable interest to scientists.

Zeolites are inorganic, nanoporous crystalline solids[16, 17]. The negatively charged aluminosilicate structure is balanced with exchangeable alkaline or alkaline earth metal cations. The cation exchange capacity of zeolites can be altered by the SiO_2:Al_2O_3 ratio of the framework. Zeolites are used in petrochemical industries[18]; detergent production[19]; aquaculture, agriculture, and horticulture[20]; medical applications[21]; and water treatment[22]. Recent studies have reported several types of zeolites with various ion exchange capacity and antimicrobial activity. Silver, zinc, and copper-exchanged natural zeolites have been investigated for their antibacterial activity[23]. Acrylic resin containing silver and zinc zeolites have an anticandidal effect against *Candida albicans*[24]. In addition, insulated ducts containing silver zeolite installed in healthcare settings display a remarkable antifungal effect against *Aspergillus niger*[25].

The aim of this study was to synthesize silver- and zinc-ion–loaded antimicrobial zeolites (antibacterial, anticandidal, and antifungal) for the manufacture of durable antimicrobial composite surfaces using paints and polymers.

MATERIALS AND METHODS

Materials and reagents

The sodium aluminate, sodium hydroxide, and sodium metasilicate pentahydrate (Na_2O:SiO_2:$5H_2O$) and colloidal silica (Ludox:SiO_2:$5H_2O$) used for zeolite synthesis were obtained from Sigma–Aldrich. Steel plates, surface coating materials, and polypropylene (PP) surfaces were supplied by a leading appliance company (VESTEL; powder coating material, acrylic paint, and polyester paint). The silver nitrate ($AgNO_3$) and zinc chloride ($ZnCl_2$) used for the ion-exchange process were obtained from Sigma–Aldrich. The potato dextrose agar (PDA), Sabouraud dextrose agar (SDA), tryptic soy agar (TSA), Sabouraud dextrose broth (SDB), and tryptic soy broth (TSB) used in antimicrobial activity tests were purchased from Merck (Darmstadt, Germany). A 6-branch manifold filtration system and incubator shaking cabinet (CERTOMAT BS-T; Sartorius, Germany) were used during the zeolite synthesis and ion exchange processes.

Zeolite synthesis

During the study, 2 types of zeolites with different Si:Al ratios were synthesized as described previously by our group[26]. Synthesis gel formulas of the zeolites are listed in Table (1). Sodium metasilicate pentahydrate (SMS) and Ludox were used as the silica source, sodium aluminate was used as the aluminum source, and sodium hydroxide was the source of the balancing cation. Chemicals were weighed and placed into polyethylene Erlenmeyer flasks. The required amounts of SMS or Ludox were put into Erlenmeyer flasks along with the required amount of water. Hydrothermal synthesis of zeolites took place in an oven at 90°C for 3 days. At the end of the crystallization period zeolites were filtered using vacuum filtration and placed into an oven for 24 h at 90°C[27]. Dried zeolite samples were ground using a mortar and pestle.

Ion-exchange processes of zeolites

Zeolite (80 g/L) samples were mixed with 1 M silver nitrate ($AgNO_3$) and 1 M zinc chloride ($ZnCl_2$) solutions individually. Mixtures were shaken at 200 rpm for 3 days in dark medium at room temperature. At the end of the incubation period, zeolites were filtered by vacuum filtration and put into an oven at 90°C for 24 h. Dried zeolites were ground using a mortar and pestle.

Modified disk diffusion assay

The standard NCCLS disk diffusion assay[28] was modified and used to assess antimicrobial activity against each microorganism tested. Briefly 100 ìL of suspensions containing 108 CFU/mL bacteria, 106 CFU/mL yeast, and 104 spore/mL fungus were prepared from freshly grown cultures and spread on TSA, SDA, and PDA, respectively. The blank disks (6 mm in diameter) were wetted with 20 ìL of sterile distilled water and impregnated with approximately 40 mg of metal-ion–loaded zeolite samples. Disks carrying zeolites were placed on inoculated plates. Sterile, distilled water impregnated blank disks were used as negative controls. Ofloxacin (5 ìg/disk) and nystatin (100 U/disk) were used as positive controls for bacteria and fungi, respectively. The inoculated plates were incubated for 24 h at 36 ± 1°C for bacterial strains and 48 h for yeast strains and 72 h at 27 ± 1°C for fungal species. Antimicrobial activity in the modified disk diffusion assay was evaluated by measuring the zone of inhibition against test microorganisms[29]. Each test was repeated at least twice.

Preparation of antimicrobial composites

Stainless steel plates (5 × 5 cm) were coated with a powder coating material used in the household appliance industry and polyester paint. The PP plates were painted with acrylic paint. Briefly, commercially-pure powder coating material was mixed with different concentrations of zeolite samples (7%, 10%, and 12% w/w). Then 100 mg of mixture was poured onto a metal plate and oven-dried at 120°C for 1 h. In addition, polyester paints were mixed with zeolite samples (7%, 10%, and 12% w/w). Finally, different concentrations of zeolite samples (10%, 12%, and 15% w/w) were mixed with acrylic paint. Type X silver zeolites were used for the manufacture of antimicrobial PP surfaces. Zeolite samples [7% and 10% (w/w) ratio] were mixed with melted PP bulk until homogeneity was achieved in an extruder (200–220°C).

Antimicrobial activity tests of prepared antimicrobial surfaces

The antimicrobial activity of the surface-modified samples was investigated for selected microorganisms (Table 2). Antimicrobial composite specimens painted with mixtures containing metal-ion–loaded zeolite samples were placed into sterile Petri dishes. The modified surface of the sample was placed facing upward, and 1 mL of TSB, PDA, or SDB was poured onto the surface for bacteria, yeast, and fungus respectively. Then 100 ìL of suspensions containing 106 CFU/mL bacteria, 104 CFU/mL yeast, and 103 spore/mL fungus were added to the medium on the surface. The Petri dishes were capped to prevent medium evaporation and incubated for 24 h at 36 ± 1°C for bacterial strains and 48 h for *Candida albicans* and for 7 days at 27 ± 1°C for *Aspergillus niger*. Stainless steel and PP plates painted with commercial paints were used as negative controls. After incubation, a 100 ìL sample was transferred into TSB, SDB, and PDB and serially diluted. From each dilution a 100 ìL sample was plated on TSA, SDA, and PDA and cultured at the appropriate temperature to detect bacterial, yeast, and fungal growth, respectively. Surface modified specimens were re-inoculated with selected microorganisms (bacteria, yeast, and fungus) at 15 day intervals for 1 year.

RESULTS AND DISCUSSION

Biocidal activities of the zeolite samples tested based on disk diffusion assay revealed that pure zeolites X and A did not have any antimicrobial activities, whereas both silver and zinc ion–exchanged zeolites exhibited remarkable inhibition zones around the samples for all tested microorganisms. The diameters of the inhibition zones are given in Table (2). The metal-ion–exchanged zeolites display variable antimicrobial activity against all microorganisms tested in the current study. Similar results have been reported in previous studies showing that Ag^{+} and Zn^{+2} ion–exchanged zeolites had inhibitory effects on several microbial species including bacteria, yeasts, and fungi [23, 30, 31].

In the current study, composite materials containing various concentrations of silver and zinc zeolites are examined for their antimicrobial activity and stability against the microorganisms tested. Others added nanostructured silver vanadate to water-based paints and found it effective against methicillin-resistant *Staphylococcus aureus* (MRSA), *Enterococcus faecalis*, *Escherichia coli*, and *Salmonella enterica*[32]. Another study indicated that stainless steel surfaces coated with paints containing a silver and zinc zeolite showed profound antimicrobial activity against *Bacillus* spp[33]. The addition of copper and silver nanoparticles to architectural paint provided antimicrobial properties against fungi including *Aspergillus niger*, *Paecilomyces variotii*, *Penicillium funiculosum*, *Chaetomium globosum* mixture, and the bacterial strain

Pseudomonas aeruginosa[34]. However, little is known about the stability and durability of antimicrobial materials or their antimicrobial efficacy against a wide range of microorganisms including bacteria, yeast, and fungi.

In the present work, different surfaces coated with various paints containing silver and zinc ion–exchanged zeolites were evaluated for their antimicrobial activity. Zinc ion–exchanged zeolite compositions were used for white powder coating materials and polyester paints; there were no noticeable changes in the color of composite materials. In addition, silver colored powder coating material, polyester paint, and acrylic paints were mixed with silver ion–exchanged zeolites, and there were no noticeable change in these surfaces either. On the other hand, for the PP surfaces there was a remarkable change in surfaces containing silver zeolite. The color of PP surfaces changed to a brownish color after 7% and 10% silver zeolite additions.

The stability of all surfaces coated with different paints, including various amounts of silver and zinc zeolites (7% – 15%), is given in Table (3). Silver and zinc ion–embedded zeolite X (AgX and ZnX) exhibited longer antimicrobial activity in comparison to silver or zinc ion–embedded zeolite A (AgA or ZnA). This result could be a consequence of the high and sudden release rate of ions in the case of zeolite A[35]. Zeolite A releases loosely bound metal ions faster than zeolite X so that the remaining metal content may not be sufficient to inhibit microbial growth.

The inhibitory effect of steel plates treated with commercial and antimicrobial powder coating containing silver and zinc ion–exchanged zeolites is shown in Table (3). According to the results, the addition of 7% silver zeolite (w/w) to the powder coating was sufficient to provide antimicrobial activity against the tested strains for 45 days. Moreover, AgX (10%) and ZnA (10%) were found to be free of *Aspergillus niger* contamination up until day 120 of inoculation.

Polypropylene surfaces including 7% and 10% AgX, prepared by extrusion and thermoforming, displayed the shortest duration of antimicrobial activity among surfaces, as expected. As showed in table 3 *Aspergillus niger* growth on a pure PP surface and the inhibitory effect of AgX-PP composite against the fungal isolates. Although silver zeolite–enhanced PP surfaces exhibited antimicrobial efficiency for at least 30 days, their maximum efficiency period was 60 days. This may be due to the rigid structure of the PP material. Metal ions bound to zeolite structures could not be released fast enough to inhibit microbial growth for longer periods on PP surfaces. Therefore, antimicrobial activity on the PP surfaces was relatively short-term in comparison to coated materials. Our data supports the findings of previous studies that reported that silver, copper, and zinc ion–exchanged zeolite/polyurethane

Table 1. Zeolite gel formulations

Sample name	Synthesis gel formula	Silica source	Type of zeolite
Zeolite X	4.64 Na_2O:Al_2O_3:3.2 SiO_2:400 H_2O	SMS	Faujasite X (FAU X)
Zeolite A	2 Na_2O:Al_2O_3:1.6 SiO_2:200 H_2O	Ludox	Linde type A (LTA)

Table 2. Antimicrobial effect of silver (Ag^+) and zinc (Zn^{2+}) loaded zeolites for microorganisms based on inhibition zones in disk diffusion assay

Microorganism	AgX	ZnX	AgA	ZnA	PC	NC
Escherichia coli	11 ± 2	13 ± 2	12 ± 2	11 ± 2	28	0
Pseudomonas aeruginosa	12 ± 2	7 ± 1	12 ± 3	7 ± 1	24	0
Staphylococcus aureus	13 ± 1	14 ± 2	13 ± 2	14 ± 1	20	0
Candida albicans	33 ± 5	19 ± 2	29 ± 4	16 ± 1	20	0
Aspergillus niger	7 ± 2	36 ± 2	8 ± 1	36 ± 4	20	0

AgX: silver zeolite type X; ZnX: zinc zeolite type X; AgA: silver zeolite type A; ZnA: zinc zeolite type A; PC: ofloxacin (5 ìg/disk) and nystatin (100 U/disk) for bacteria, candida, and fungus, respectively; NC: pure zeolite type A and X samples.

composites have antimicrobial effects against *Escherichia coli*, methicillin-resistant *Staphylococcus aureus*, and *Pseudomonas aeruginosa*[36, 37].

In the case of PP surfaces coated with acrylic paint containing ion-exchanged zeolites, microbial growth inhibition was observed for more than 200 days (Table 3). Durability of antimicrobial efficiency was found to be directly proportional to the concentration of zeolite samples in material surfaces. These results are consistent with those reported previously[38]. As the concentration of Ag zeolites increased, the stability of antimicrobial activity progressed in parallel.

In this study different concentrations of zeolite samples were selected for various materials. These concentrations can be altered according to the desired durability of the antimicrobial effect. However, using a concentration of silver ion–exchanged zeolite below 7% is not appropriate as there is no significant antimicrobial effect below that point. Silver and zinc ions are released at a controlled rate and ensure long-term antimicrobial protection on surfaces. However, silver ion–exchanged zeolites exhibited greater inhibitory effect against bacteria than zinc ion–exchanged zeolites on all surfaces. These results may be explained by the low antibacterial effect of Zn^{2+} zeolites as reported previously[39]. On the other hand, Zn^{2+}-loaded zeolites displayed better antifungal effects than Ag^{+} loaded zeolites in general. Similar findings have been reported in previous studies[40, 26]. As zinc, but not silver, is an essential element for fungal species and necessary for fungal metabolism, transportation of zinc into the cytoplasm is easier than transport of the silver

Table 3. Durability of antimicrobial effect of surface containing metal-ion-loaded zeolites

Microorganism	Powder coating											
	AgX (7%)	AgX (10%)	AgX (12%)	ZnX (7%)	ZnX (10%)	ZnX (12%)	AgA (7%)	AgA (10%)	AgA (12%)	ZnA (7%)	ZnA (10%)	ZnA (12%)
Escherichia coli	75>	105>	105>	45>	75>	105>	45>	75>	90>	45>	60>	90>
Pseudomonas aeruginosa	90>	105>	135>	60>	90>	105>	60>	90>	105>	60>	75>	105>
Staphylococcus aureus	45>	75>	105>	30>	60>	90>	45>	75>	90>	30>	60>	75>
Candida albicans	90>	105>	150>	75>	90>	120>	75>	90>	120>	75>	75>	105>
Aspergillus niger	90>	135>	180>	105>	150>	255>	75>	105>	135>	90>	135>	180>

	Polyester paint											
	AgX (7%)	AgX (10%)	AgX (12%)	ZnX (7%)	ZnX (10%)	ZnX (12%)	AgA (7%)	AgA (10%)	AgA (12%)	ZnA (7%)	ZnA (10%)	ZnA (12%)
Escherichia coli	45>	75>	90>	30>	60>	75>	45>	60>	90>	30>	45>	75>
Pseudomonas aeruginosa	60>	90>	105>	45>	60>	90>	45>	75>	105>	30>	60>	75>
Staphylococcus aureus	60>	75>	105>	60>	60>	90>	60>	75>	90>	45>	60>	90>
Candida albicans	75>	90>	120>	60>	90>	90>	60>	90>	105>	60>	75>	90>
Aspergillus niger	45>	75>	105>	60>	75>	120>	45>	75>	90>	60>	75>	105>

	Acrylic paint						Polypropylene surface	
	AgX (10%)	AgX (12%)	AgX (15%)	AgA (10%)	AgA (12%)	AgA (15%)	AgX (7%)	AgX (10%)
Escherichia coli	60>	90>	225>	45>	75>	225>	30>	30>
Pseudomonas aeruginosa	105>	120>	210>	75>	75>	210>	45>	45>
Staphylococcus aureus	105>	105>	225>	45>	90>	195>	30>	45>
Candida albicans	150>	165>	285>	60>	75>	240>	45>	60>
Aspergillus niger	135>	240>	360<	45>	60>	285>	30>	45>

AgX: silver zeolite type X; ZnX: zinc zeolite type X; AgA: silver zeolite type A; ZnA: zinc zeolite type A; >: the day of microbial growth detection; <: Existence of antimicrobial effect more than 360 days.

ions[41]. Although zinc is necessary for the fungal system, it can display biocidal activity at high concentrations. Therefore, zinc ions released from zeolite samples may have accumulated in fungal cells, resulting in greater fungicidal activity, with respect to silver ions.

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