

# Assessment of the Antiviral Properties of Zeolites Containing Metal Ions

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**Abstract** The antiviral properties of zeolite (sodium aluminosilicate) powders amended with metal ions were assessed using human coronavirus 229E, feline infectious peritonitis virus (FIPV), and feline calicivirus F-9. Zeolites containing silver and silver/copper caused significant reductions of coronavirus 229E after 1 h in suspension. The silver/copper combination yielded a  $>5.13\text{-log}_{10}$  reduction within 24 h. It was also the most effective ( $>3.18\text{-log}_{10}$ ) against FIPV after 4 h. Other formulations were ineffective against FIPV. On plastic coupons with incorporated silver/copper-zeolites,  $>1.7\text{-log}_{10}$  and  $>3.8\text{-log}_{10}$  reductions were achieved for coronavirus 229E and feline calicivirus within 24 h, respectively. Silver/copper zeolite reduced titers of all viruses tested, suggesting that it may be effective against related pathogens of interest [i.e., SARS coronavirus, other coronaviruses, human norovirus (calicivirus)]. Of note, it was effective against both enveloped and nonenveloped viruses. Metal-zeolites could therefore possibly be used in applications to reduce virus contamination of fomites and thus the spread of viral diseases.

**Keywords** Coronavirus · Calicivirus · Fomites · Antiviral · Copper · Silver

## Introduction

By July of 2003, 8,098 probable cases of severe acute respiratory syndrome (SARS) resulting in 774 deaths had

been reported to the World Health Organization (WHO) from 29 countries on five continents (Centers for Disease Control and Prevention 2003; World Health Organization 2004). A novel coronavirus, SARS coronavirus (SCoV) was isolated from patients (Ksiazek et al. 2003; Navas-Martín and Weiss 2004). Before the identification of SCoV, two coronaviruses were known to infect humans, strains 229E and OC43 (Navas-Martín and Weiss 2004). These cause mild, self-limiting, upper respiratory tract infections (Myint 1994) and belong to the Group I and Group II coronaviruses, respectively. SCoV possesses characteristics specific to all three coronavirus groups (Navas-Martín and Weiss 2004), but is not closely related to any (Poutanen et al. 2003). It is apparently an animal virus that recently adapted to cross the species barrier, allowing for human-to-human transmission (Antia et al. 2003).

Human norovirus (NoV) causes illness in an estimated 23 million people in the United States each year, resulting in 50,000 hospitalizations and 310 deaths (Mead et al. 1999). It has been suggested that NoV may be the leading cause of foodborne illness in the United States (Widdowson et al. 2005), responsible for approximately 66% of all cases with known etiologies (Mead et al. 1999) and at least 50% of all foodborne outbreaks of gastroenteritis (Centers for Disease Control and Prevention 2006). NoV was identified in 93% of nonbacterial gastroenteritis outbreaks by the Centers for Disease Control and Prevention (CDC) between 1997 and 2000 in the United States (Fankhauser et al. 2002). Similarly, surveillance by the Foodborne Viruses in Europe network found that NoV was responsible for greater than 85% of all nonbacterial gastroenteritis outbreaks from 1995 to 2000 (Lopman et al. 2003).

Nonenveloped viruses are typically more resistant to environmental conditions and the action of antimicrobials

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than enveloped viruses (Watanabe et al. 1989; Barker et al. 2001). Feline calicivirus has been found to persist for up to 28 days in a dry environment at room temperature (Doultree et al. 1999). Also, in a study by Smid et al. (1991), rabbit hemorrhagic disease virus (also a calicivirus) survived for at least 105 days in a dried state at room temperature. Viruses that cause symptoms such as vomiting or diarrhea are likely to contaminate the environment. In one study, 607 of 680 (89%) norovirus outbreaks were linked to person-to-person transmission (Evans et al. 1998) that included poor hand hygiene as well as surface-to-surface transmission (Barker et al. 2001). Also, successive outbreaks of norovirus infections in passengers on cruise ships on separate trips have strongly implicated environmental contamination (Barker et al. 2001). Enveloped viruses are typically less stable in the environment, yet the SCoV is able to survive on fomites for up to 96 h (Duan et al. 2003). The transmission of SCoV is believed to be multifactorial, with evidence from previous outbreaks suggestive of at least some role for contaminated fomites in the transmission of the virus (Dowell et al. 2004; Chu et al. 2005).

Zeolite (sodium aluminosilicate) powders (AgION Technologies, Wakefield, MA, USA) form porous crystals. Metal ions may reside within these pores and zeolites can act as ion exchangers, exchanging metal ions for other cations in the environment. Although the effect of metal-zeolites has been documented in numerous studies with bacteria (Bright et al. 2002; Takai et al. 2002; Cowan et al. 2003; Rusin et al. 2003; Kwakye-Awuah et al. 2008), the use of zeolite powders containing heavy metal ions to reduce coronaviruses and caliciviruses has not been previously reported. This paper describes the antiviral effect of suspensions of zeolite powders amended with silver (Ag), copper (Cu), and zinc (Zn) ions in phosphate-buffered saline against human coronavirus 229E and feline infectious peritonitis virus (FIPV; feline coronavirus). This report also includes tests of the survival of human coronavirus 229E, FIPV, and feline calicivirus on the surfaces of plastics with zeolite containing Ag and Cu ions incorporated into the plastic.

Human coronavirus 229E and FIPV were employed in this study as surrogates for other coronaviruses. Feline calicivirus was also included as a surrogate for NoV. There is currently no practical method for propagating human NoV in cell culture monolayers. Feline calicivirus, on the other hand, grows readily in cell culture. It is in the same family as human NoV and is commonly used as a NoV surrogate in experiments (Slomka and Appleton 1998; Clay et al. 2006) because of its biochemical and genetic similarities to NoV (Jiang et al. 1993).

## Materials and Methods

### Virus Preparation

Human coronavirus strain 229E (ATCC #VR-740) was obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). It was maintained on MRC-5 (fetal human lung fibroblast, ATCC #CCL-171) cell line monolayers with minimal essential medium (MEM, modified with Earle's salts, Irvine Scientific, Santa Ana, CA, USA) containing 2% fetal bovine serum (FBS, Hyclone, Logan, UT, USA) at an incubation temperature of 35°C with 5% CO<sub>2</sub>. Coronavirus 229E replicates better at this temperature than at 37°C. Feline infectious peritonitis virus (FIPV; ATCC #VR-990) and feline calicivirus strain F-9 (ATCC #VR-782) were maintained in the same manner on CRFK (Crandell Reese feline kidney, ATCC #CCL-94) cell line monolayers.

Viruses were purified by centrifugation (750×g) to remove cell debris followed by polyethylene glycol (9% PEG, 0.5 mol/l NaCl) precipitation. Viral titrations were performed using the Reed-Muench method (Payment and Trudel 1993) to determine the tissue culture infectious dose that affected 50% of the cultures (TCID<sub>50</sub>).

### Metal-Zeolite Powders in Suspension

Coronavirus strains 229E and FIPV were added to Erlenmeyer flasks containing 30.0 ml of phosphate buffered saline (PBS, pH 7.4; Sigma-Aldrich, St. Louis, MO, USA) with 10.0 mg of suspended zeolite test powder [either unamended powder, 20.0% Ag (w/w), 3.5% Ag/6.5% Cu, or 0.6% Ag/14% Zn/80% ZnO] (AgION Technologies, Wakefield, MA, USA). Positive control flasks without zeolite powder were also included. All experiments were performed in duplicate.

The positive control flasks (without zeolite powders) were sampled immediately ( $t = 0$  h) by removing 1.0 ml from each flask and placing it into 1.0 ml of D/E neutralizing broth (Remel, Lenexa, KS, USA). The 2.0 ml volumes were mixed thoroughly and placed into 4.0 ml of PBS (pH 7.4). All test flasks were then placed on an orbital shaker (200 rpm) at room temperature (23°C) and were sampled at 1, 4, and 24 h in the manner described previously. All samples were frozen in 1.0 ml aliquots at -80°C. Frozen aliquots were subsequently assayed using the Reed-Muench TCID<sub>50</sub> method as before (Payment and Trudel 1993).

### Plastics with Incorporated Metal-Zeolites

Plastic coupons (5 cm by 5 cm) with either 5 or 10% (w/w) zeolite (containing 3.5% Ag and 6.5% Cu ions) incorporated

into the plastic during manufacture prior to molding were used in this set of experiments. To further clarify, the test coupons all contained zeolites amended with 3.5% Ag and 6.5% Cu (w/w), but with differing amounts [5 or 10% w/w] of this Ag/Cu zeolite incorporated into the plastic. The plastic coupons were sanitized with 70% ethanol, allowed to air dry, and then evenly inoculated using a sterile glass rod with 0.1 ml of diluted virus (human coronavirus 229E or feline calicivirus). Three control coupons (without zeolite) were sampled immediately using a sterile polyester swab dipped in 1.0 ml of D/E neutralizing broth (Remel, Lenexa, KS, USA) to determine the original virus titer recovered. The remaining coupons were then placed in humidity chambers at a relative humidity of approximately 95% and incubated at room temperature (23°C). At 1, 4, and 24 h, the coupons were swabbed as before. Because the experiment was conducted in a nonsterile environment, samples were filtered using a 0.22- $\mu\text{m}$  pore size Acrodisc® syringe filter (Pall, Ann Arbor, MI, USA) pre-wetted with 3% beef (pH 7.0) extract to remove any contaminating bacteria/fungi and then frozen in 1.0 ml aliquots at  $-80^{\circ}\text{C}$ . All experiments were performed in triplicate. Frozen aliquots were subsequently enumerated in duplicate using a plaque-forming assay (for feline calicivirus) described by Bidawid et al. (2003) or the Reed-Muench TCID<sub>50</sub> method (for coronavirus 229E) as described previously (Payment and Trudel 1993).

#### Statistical Analysis

A Student's *t* test was used to compare the viral counts recovered from the flasks containing test powder suspensions and test plastic coupons to those recovered from the positive controls.

## Results

### Metal-Zeolite Powders in Suspension

Amended zeolite powder suspensions were compared to determine which heavy metal combinations demonstrated the greatest activity against human coronavirus 229E. Unamended powder was used as a control to evaluate the effect of adsorption. The effect of Cu alone was undetermined. The results of the suspension tests are presented in Table 1. The results from the flasks containing zeolite control powder indicate that removal of virus was not due to adsorption by zeolite particles. Of the powder suspensions tested, the 3.5% Ag/6.5% Cu ion combination was the most efficacious, yielding a 1.08- $\log_{10}$  reduction of 229E after 1 h, a 2.06- $\log_{10}$  reduction after 4 h, and a  $>5.13$ - $\log_{10}$  reduction after 24 h of exposure. The greatest reductions observed for the other amended powders were following 24 h of exposure; nevertheless, the reductions at 24 h were not significantly greater ( $P = 0.274$ ) than those after 4 h of exposure.

The 3.5% Ag/6.5% Cu combination was also effective ( $>3.18$ - $\log_{10}$  reduction) against FIPV within 4 h; however, neither of the other formulations was effective against FIPV, even after 24 h of exposure.

### Plastics with Incorporated Metal-Zeolites

The results for the virus survival on the plastics with incorporated Ag/Cu-zeolite are shown in Table 2. Significant reductions were observed for coronavirus 229E on the Ag/Cu-zeolite plastic coupons after 24 h of exposure with a 1.84- $\log_{10}$  and a 1.77- $\log_{10}$  reduction achieved on the 5% and 10% (wt/wt) zeolite coupons, respectively. The

**Table 1** Log<sub>10</sub> reduction of coronaviruses after exposure to zeolite test powders amended with heavy metals

Virus	Time (h)	Positive control <sup>a</sup>	Zeolite control <sup>b</sup>	Amended zeolite powder (w/w)		
				3.5% Ag 6.5% Cu	20% Ag	0.6% Ag 14% Zn 80% ZnO
229E (human)	1	0.00 ± 0.00	0.00 ± 0.24	1.08* ± 0.07	0.43* ± 0.09	0.50 ± 0.24
	4	0.70 ± 0.00	0.26 ± 0.28	2.06* ± 0.18	1.28* ± 0.12	1.30 ± 0.00
	24	0.59 ± 0.14	0.16 ± 0.05	$>5.13^* \pm 0.00^c$	1.92* ± 0.47	1.45 ± 0.66
FIPV (feline)	1	0.16 ± 0.12	0.08 ± 0.13	1.91* ± 0.31	0.14 ± 0.61	0.50 ± 0.66
	4	0.01 ± 0.20	0.08 ± 0.20	$>3.18^* \pm 0.00^c$	0.40 ± 0.69	0.42 ± 0.48
	24	0.10 ± 0.36	0.35 ± 0.43	$>3.18^* \pm 0.00^c$	0.30 ± 1.52	0.53 ± 1.06

The experiments were conducted in duplicate at room temperature. The original titer was  $5.0 \times 10^5$  TCID<sub>50</sub>/ml for human coronavirus and  $5.6 \times 10^3$  TCID<sub>50</sub>/ml for feline coronavirus. The  $\pm$  indicates the standard deviation for the duplicate samples

\* Reduction was statistically significant ( $P \leq 0.05$ ) in comparison to the positive control

<sup>a</sup> Virus, phosphate buffered saline (PBS) and D/E neutralizer

<sup>b</sup> Virus, phosphate buffered saline (PBS), unamended zeolite powder, and D/E neutralizer

<sup>c</sup> Below the detection limit

**Table 2** Log<sub>10</sub> reduction of viruses on plastic coupons impregnated (5% or 10%) with zeolite powder (containing 6.5% copper, 3.5% silver ions)

Virus	Time (h)	Positive control <sup>a</sup>	5% Zeolite (w/w)	10% Zeolite (w/w)
Coronavirus 229E	1	0.22 ± 0.51	0.93 ± 0.05	0.80 ± 0.00
	4	0.50 ± 0.61	0.52 ± 0.47	0.44 ± 0.24
	24	0.67 ± 0.61	1.84* ± 0.20	1.77* ± 0.24
Feline calicivirus	1	0.04 ± 0.03	0.25* ± 0.06	0.67* ± 0.14
	4	0.17 ± 0.08	0.64* ± 0.19	0.96 ± 1.45
	24	0.40 ± 0.32	3.84 ± 1.02	5.05* ± 0.21

The experiment was conducted in triplicate at room temperature. The original titer was  $4.0 \times 10^5$  TCID<sub>50</sub>/ml for human coronavirus and  $5.0 \times 10^6$  PFU/ml for feline calicivirus. The ± indicates the standard deviation for the triplicate samples

\* Reduction was statistically significant ( $P \leq 0.05$ ) in comparison to the positive control

<sup>a</sup> Plastic coupons without zeolite

reductions for feline calicivirus were greater, including a 3.84-log<sub>10</sub> reduction on the 5% Ag/Cu-zeolite coupons and a 5.05-log<sub>10</sub> reduction on the 10% Ag/Cu-zeolite coupons after 24 h.

## Discussion

To date, there have been no detailed studies of the interaction between heavy metals and viruses. Viruses that contain sulfhydryl termini may bind silver, interfering with viral replication (Davies and Etris 1997). Silver may also modify the adsorption of viruses to host cells (Tzagoloff and Pratt 1964). Thurman and Gerba (1989) suggested that viral inactivation might not require a metabolic process. For instance, the virus may be immobilized to a surface, the host-cell receptors may be blocked, or the nucleic acid within the viral capsid may be inactivated.

Copper is toxic to most microorganisms at higher concentrations, possibly due to the blocking of functional groups on proteins and the inactivation of enzymes (Faundez et al. 2004). Zinc oxide produces an active oxygen species at its surface that has a similar oxidative effect to hydrogen peroxide when it dissociates. This may damage the viral capsid and allow more metal ions inside the virus.

Unlike the respiratory disease caused by coronavirus 229E, FIPV causes gastrointestinal symptoms. The fact that Ag/Cu zeolite is effective against two substantially different coronaviruses suggests that it may also be effective in reducing the SCoV which causes severe respiratory disease, but which may also have a gastrointestinal component and is shed in the feces for greater than 10 weeks (Leung et al. 2003). The Ag/Cu zeolite was also effective against the nonenveloped feline calicivirus whose physical properties differ greatly from the enveloped coronaviruses.

Zeolite powders containing antiviral heavy metals have many potential applications. They may be added to materials such as plastics, paints, and synthetic fabrics

(Quintavalla and Vicini 2002; Takai et al. 2002), and may be bonded to surfaces such as stainless steel (Bright et al. 2002; Cowan et al. 2003; Rusin et al. 2003). The effectiveness of the Ag/Cu zeolite against substantially different viruses appears promising for its potential use in applications to reduce environmental contamination of fomites by viral pathogens and thus the spread of diseases. Additional tests utilizing zeolites containing copper ions alone or in combination with various metals against other disparate viruses are needed.

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