

Comparative Study on Antimicrobial Efficiency of AgSiO₂, ZnAg and Ag-Zeolite for the Application of Fishery Plastic Container

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Abstract

AgSiO₂, ZnAg and Ag-Zeolite were investigated for antimicrobial activity using Gram-negative *Pseudomonas aeruginosa*, *Shewanella putrefaciens* and Gram-positive *Clostridium*, *Listeria monocytogenes* as target microorganisms by disk diffusion and Agar plate MIC method. These bacteria are responsible for microbiological and chemical spoilage of sea food mainly and from the result we found that Silver zeolite, AgSiO₂ has good antimicrobial activity for all target microorganisms except for *clostridium perfringem*. ZnAg has also shown good antimicrobial properties against all target microorganisms except *Listeria monocytogenes*. Only ZnAg is capable to reduce *clostridium perfringem* as compare to Silver zeolite and AgSiO₂.

Keywords: AgSiO₂; ZnAg; Ag-Zeolite; Antimicrobial agents; *Pseudomonas aeruginosa*

Introduction

Antibacterial agents are of relevance to a number of industrial sectors including environmental, food, synthetic textiles, packaging, healthcare, medical care, as well as construction and decoration. They can be broadly classified into two types, organic and inorganic. Organic antibacterial materials are often less stable particularly at high temperatures and/or pressures compared to inorganic antibacterial agents [1]. This presents a potential obstacle for the product formulation. As a consequence, inorganic materials such as metal and metal oxides have attracted lots of attention over the past decade due to their ability to withstand harsh process conditions.

Antimicrobial packaging is a promising form of active food packaging, in particular for fish products. Since microbial contamination of these foods occurs primarily on the surface, due to post-processing handling, attempts have been made to improve safety and to delay spoilage by use of antibacterial sprays or dips. However, direct surface application of antibacterial substances onto foods have limited benefits because the active substances are neutralized on contact or diffuse rapidly from the surface into the food mass. On the other hand, the incorporation of SZ inhibited the growth of the bacteria tested under anaerobic conditions. Kawahara examined and found that the MIC of Silver zeolite ranged between 256 and 2048 µg/ml, which corresponded to a range of 4.8-38.4 µg/ml of Ag⁺ [2]. This result suggested that Bactericidal or bacteriostatic agents into fish formulations may result in partial inactivation of the active substances by product constituents and is therefore expected to have only limited effect on the surface microflora. At the first stage of spoilage fish have very bad odor, which was undesirable by most of the consumers, so antimicrobial agents which have the capacity to reduce the odor is very helpful to minimize this problem while trying to enhance shelf life of fish. Traditionally, antimicrobial agents are added directly to the foods; however, their activity may be inhibited by many substances in the food itself, diminishing their effectiveness. In such cases, the use of antimicrobial container for transporting fish can be more efficient than adding antimicrobial agents directly to the food since these may selectively and gradually migrate from the container onto the surface of the food.

Pseudomonas aeruginosa, *Shewanella putrefaciens*, *Clostridium*,

Listeria monocytogenes is one of the most important bacteria for responsible of fish spoilage during the transportation of fresh harvested fish [3]. Seafood is a highly perishable product. *Listeria monocytogenes*, *Salmonella* and *Staphylococcus aureus* are common Pathogens to seafood; however, others such as *Clostridium botulinum* Type E, *Vibrio* species and *pseudomonas* are more commonly associated with or exclusive to marine food products. Packaging of seafood products has historically been passive, or used to protect from oxygen, desiccation and microbial contamination. Tamper-evident packaging became the norm 30 years ago [4] (Table 1). Silver nanoparticles (NPs) or silver ions have long been known to have strong inhibitory and bactericidal effect in solutions and composites with silica films and particles (Ag/SiO₂) with developed surface area. The antibacterial effects of AgSiO₂ hollow composite powders against *Escherichia coli*, *Staphylococcus aureus* and *bacillus* were reported. Hollow silica nano-spheres and nanotubes were synthesized as hosts for the immobilization of silver. It was observed that both composites had excellent antibacterial performance. The combination of silver and silica for the Synthesis of nanoparticles has been widely studied.

ZnO particles are effective for inhibiting both Gram-positive and Gram-negative bacteria. They even have antibacterial activity against spores that are high-temperature and high-pressure resistant [1,5].

In recent years, silver has either been incorporated into polymers or in some cases coated onto polymers to reduce this microbial contamination by inhibiting biofilm formation. Silver-loaded zeolites can act as an inorganic reservoir and release silver ions in a controlled way. Zeolites are crystalline-hydrated aluminosilicates with a framework structure consisting of cavities or pores that are occupied by cations or water molecules. Silver zeolite is made by complexing alkaline earth metal with crystal aluminosilicate, which is partially

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Product	Specific spoilage bacteria	Reference
Iced marine fish	<i>Shewanella putrefaciens</i>	Lone Gram and Paw Dalgaard
Iced freshwater fish	<i>Pseudomonas spp.</i>	
CO ₂ -packed chilled fish	<i>Photobacterium phosphoreum</i>	
Fresh harvested Fish	<i>Shewanella putrefaciens</i> , <i>Photobacterium phosphoreum</i> , <i>Pseudomonas spp.</i> , <i>Aerobic spoilers</i> , <i>Vibrionaceae</i>	Owen P. Fraser and Sam Sumar
Iced storage Fish	<i>Pseudomonas sp. and S. putrefaciens</i>	L.Gram and H.H.Huss
Ambient Storage Fish	<i>mesophilic Vibriouweae</i>	
Iced store tropical fresh water	<i>Pseudomonas spp.</i>	

Table 1: Bacterial spoilage compound for sea food.

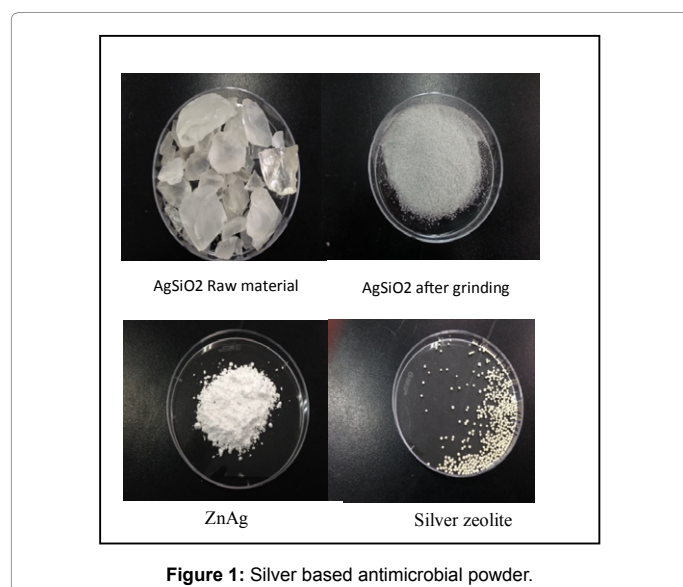


Figure 1: Silver based antimicrobial powder.

replaced by silver ions using ion exchange method. In Japan, ceramics are manufactured coated with silver zeolite to apply antimicrobial property to their products. These ceramics are used for food preservation, disinfection of medical products, and decontamination of materials [2,6]. In recent years, there has been renewed interest by researchers, drug companies, food production, packaging and powder coating industries to investigate novel types of safe and cost-effective antimicrobial materials. However, the longevity of silver and the extent to which it persists in its antimicrobial activity has not been established and neither is the mechanism of antimicrobial action of silver ions fully understood [2]. Zeolites are one of such additives and have high surface area and cationic exchange properties. The physicochemical properties that make zeolite so attractive for Trimethylamine are also expected to enhance adsorption of VOCs and odor emitted from Fish.

The objectives of current study is to investigate the antibacterial activity of AgSiO₂, ZnAg, AgZeolite, particles against, *Pseudomonas aeruginosa*, *Shewanella putrefaciens*, (Gram Negative) *Clostridium*, *Listeria monocytogens* (Gram positive).

Material and Methods

Silver based material

AgSiO₂, ZnAg and AgZeolite used in the present study contained silver ions. All test materials were supplied by the South Korea Company shown in Figure 1. Silver based antimicrobial powder and made into suspension in sterile distilled water at concentrations ranging from 5.0-20.0 mg/ml (Table 1) before testing. Preset amount

Independent variables	Levels of independent Variables (mg/ml)				Dependent variable
	5	10	15	20	
AgSiO ₂	5	10	15	20	<i>Pseudomonas aeruginosa</i>
ZnAg	5	10	15	20	<i>Shewanella putrefaciens</i>
AgZeolite	5	10	15	20	<i>clostridium</i>
					<i>Listeria monocytogens</i>

Table 2: Experimental Design.

of dry Antimicrobial nanoparticles was mixed with distilled water in a glass beaker with the aid of a magnetic stirrer. Once particles were dispersed in water, the beaker was placed in an ultra-sonicator (The reason for the use of sonication was to break down the agglomerates as seen) After 30 min of sonication, the master Antimicrobial nano-fluid was produced To enhance the stability of the suspension, two dispersants, PVP and PEG400, were used to produce suspensions of Antimicrobial The amount of the dispersant was 10% of the amount of antimicrobial suspension prepared were autoclaved at 121°C for 15 min.

Microorganisms

Pseudomonas aeruginosa KCCM 11266, *Shewanella putrefaciens* KCCM 41648, *Clostridium perfringens* KCCM 40947, *Listeria monocytogens* were obtained from the Korea Culture Center of Microorganisms. The strains of bacteria were stored in 20% glycerol solution at -8°C. They were thawed and incubated in brain heart infusion broth (Food packaging lab Korea) at 37°C for 20 h. The cells were in stationary phase and washed once in sterile saline (0.85 w/v%), and then resuspended in saline and adjust the turbidity using spectrophotometer at approximately 10⁹ CFU/ml. The tube containing the bacterial suspension was immersed in ice water before use in the experiments in Table 2.

Antimicrobial activity

To evaluate if antimicrobial packaging have an effect on microorganisms present in the sea foods, agar plate methods, and minimally inhibitory concentrations (MIC), have been used using similar methods to those used to evaluate antimicrobials alone. In Japan, a method referred to as 'Film Contact Method' is used as a standard to assess the ability of products containing antimicrobials to impart antimicrobial properties to products. The method was developed for inorganic antimicrobials such as silver substituted zeolites. It is appropriate for films and sheets and consists of inoculating bacteria on the test specimen and incubating and counting the bacteria under specified conditions. The intent is to determine the resistance of the plastic to microbial growth, but it may also serve to determine if polymers are 'self-sterilizing'. In Table 3 standard method has been given for testing antimicrobial efficiency. MIC can indicate the antimicrobial strength of the polymer and allows the comparison of

Bacteria	MIC(ZnAg) Concentration of antibacterial agents			
	5 mg/ml	10 mg/ml	15 mg/ml	20 mg/ml
<i>Shewanella gaetbuli</i>	+	-	-	-
<i>Pseudomonas aeruginosa</i>	+	+	+	-
<i>Clostridium perfringem</i>	+	-	-	-
<i>Listeria monocytogens</i>	+	+	+	+
(ZnAg) Disk Diffusion (zone of inhibition mm)				
<i>Shewanella gaetbuli</i>	19	21	24	25
<i>Pseudomonas aeruginosa</i>	9	10	14	17
<i>Clostridium perfringem</i>	-	-	11	12
<i>Listeria monocytogens</i>	-	-	-	-

Table 3: (ZnAg) Disk Diffusion and agar plate MIC for *Pseudomonas aeruginosa*, *Shewanella gaetbuli*, *Clostridium*, *Listeria monocytogen*.

the polymer's antimicrobial activity to that of the antimicrobial alone. The method consists in seeding a series of tubes containing growth medium with the target microorganism and with polymers containing different concentrations of antimicrobial. The tubes are incubated for a pre-determined period of time and visually inspected for microbial growth (turbidity). MIC is the lowest concentration of an antimicrobial in a polymer resulting in the complete inhibition of growth of a test microorganism.

Test of antibacterial properties: Agar cup diffusion method was employed to obtain the susceptibility pattern of microorganisms against each silver-based material. Briefly, 20 mL of Nutrient agar was poured into an 80 mm-petri dish. The medium was allowed to cool and plates were then inoculated with 10⁸ CFU/mL of bacteria. All disks and materials were sterilized in an autoclave before experiments. The antibacterial activities of AgSiO₂, ZnAg and AgZeolite nano composites were measured by two methods: paper disk diffusion assay and minimal inhibitory concentration (MIC). The disk diffusion assay was performed by placing an 8 mm disk saturated with 5-20 mg/ml AgSiO₂, ZnAg and AgZeolite nano composite aqueous slurry onto an agar plate seeded with various microorganisms. After 24 h of incubation, the diameters of the inhibition zones were measured. MIC values were determined as the lowest concentration of AgSiO₂, ZnAg and AgZeolite nano composite where the absence of growth was recorded. At the end of the incubation period, the plates were evaluated for the presence or absence of growth.

Results and Discussion

Evaluation of antibiotic properties

ZnAg: Using the agar disk diffusion method as a screening test, all of silver materials showed antimicrobial effects against all of test organisms with a zone of inhibition ranging from 9 to 25 mm (Table 2). Percival, Song, Lara, Chaloupka have reported that silver has a direct or indirect effect on microbial cells [7-10]. No growth inhibition was observed when exposed to the carriers without ZnAg. The MICs and MLCs of silver materials are shown in Table 1. All the test microorganisms were inhibited and killed at >10.0 mg/ml of silver materials except for that of ZnAg against *Listeria monocytogens*. Antibacterial effects in the form of inhibition zones, evaluated by the disk diffusion assay of the ZnAg are shown in Figure 2. The clear zones of ZnAg against *Pseudomonas aeruginosa*, *Sh. putrefaciens*, and *Clostridium perfringem*, are determined but for *L. monocytogens* no growth inhibition observed Zhang Lingling investigated and found that the ZnO nano fluids have bacteriostatic activity against *E. coli* [11]. The antibacterial activity increases with increasing nanoparticle

concentration and increases with decreasing particle size (Table 3).

Silver zeolite (AgZ): AgZ materials showed antimicrobial effects against all of test organisms with a zone of inhibition ranging from 11 to 23 mm. The MLCs of AgZ materials are shown in Table 2. All the test microorganisms were inhibited and killed at >10.0 mg/ml of AgSiO₂ materials except against *Clostridium*. In Figure 3 Antibacterial effects in the form of inhibition zones and MIC agar plates, are shown. The clear zones of AgZ against *Pseudomonas aeruginosa*, *Sh. putrefaciens*, and *L. monocytogens*, are determined but for *Clostridium perfringem* no growth inhibition observe as shown in Figure 3. Saengmee-Anupharb also found that The MIC of AgZ, AgZrPSi and AgZrP was 10.0 g/L whereas MLC ranged between 10.0-60.0 g/L [12]. This result explained by the Krishnani in his investigation the diameter of the inhibition zones for *E. coli*, *V. harveyi*, *V. cholerae* and *V. parahaemolyticus*, respectively, increased from 0.5 to 2.3 cm, 0.6 to 2.4 cm, 0.3 to 1.65 cm and 0.3 to 1.7 cm with increasing concentrations of silver ion-exchanged zeolite from 10 to 400 μg (Table 4 and Figure 4) [13].

AgSiO₂: AgSiO₂ materials showed antimicrobial effects against all of test organisms with a zone of inhibition ranging from 7 to 26 mm (Table 4). The MLCs of AgSiO₂ materials are shown in Table 4. All the test microorganisms were inhibited and killed at >10.0 mg/ml of AgSiO₂ materials except against *Listeria monocytogens* and *Clostridium*. Figure

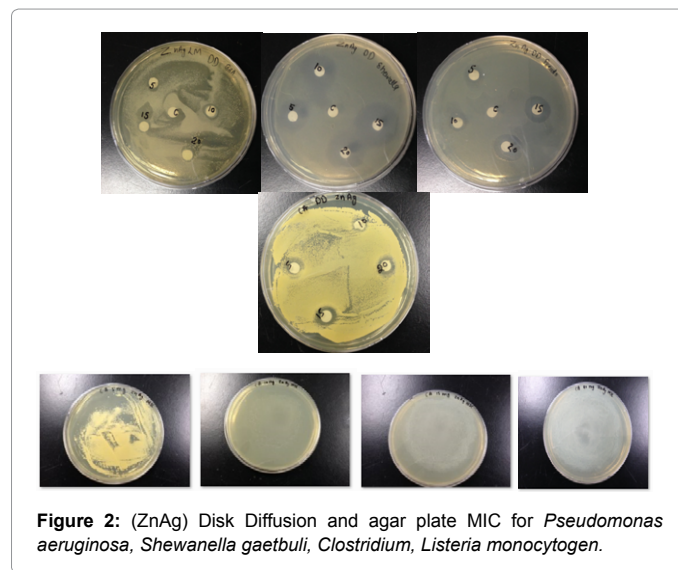


Figure 2: (ZnAg) Disk Diffusion and agar plate MIC for *Pseudomonas aeruginosa*, *Shewanella gaetbuli*, *Clostridium*, *Listeria monocytogen*.

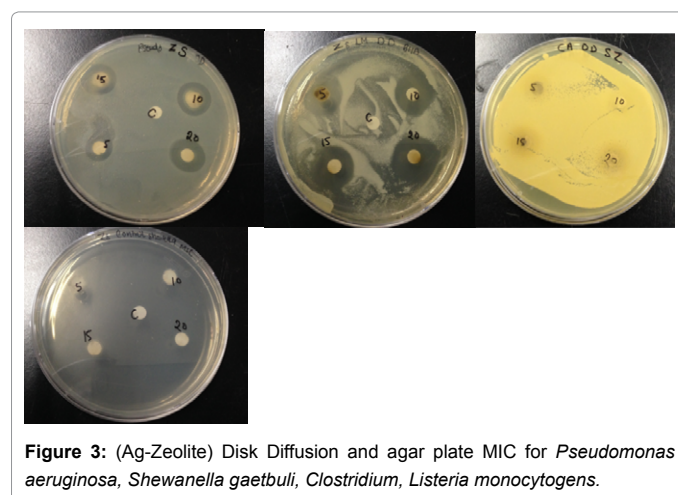


Figure 3: (Ag-Zeolite) Disk Diffusion and agar plate MIC for *Pseudomonas aeruginosa*, *Shewanella gaetbuli*, *Clostridium*, *Listeria monocytogen*.

Bacteria	MIC(ZnAg) Concentration of antibacterial agents			
	MIC (silver zeolite)			
	5 mg/ml	10 mg/ml	15 mg/ml	20 mg/ml
<i>Shewanella gaetbuli</i>	+	+	-	-
<i>Pseudomonas aeruginosa</i>	-	-	-	-
<i>Clostridium perfringem</i>	+	+	+	+
<i>Listeria monocytogens</i>	+	-	-	-
(silver zeolite)Disk Diffusion (zone of inhibition mm)				
<i>Shewanella gaetbuli</i>	No growth	No growth	No growth	No growth
<i>Pseudomonas aeruginosa</i>	11	16	16	17
<i>Clostridium perfringem</i>	-	-	-	-
<i>Listeria monocytogens</i>	19	20	20	23

Table 4: (Ag-Zeolite) Disk Diffusion and agar plate MIC for *Pseudomonas aeruginosa*, *Shewanella gaetbuli*, *Clostridium*, *Listeria monocytogens*.

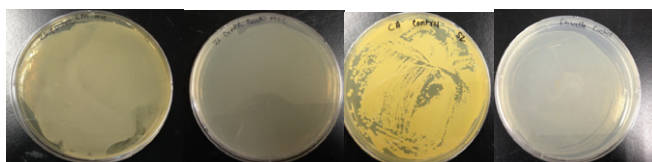


Figure 4: Control for *Pseudomonas aeruginosa*, *Shewanella gaetbuli*, *Clostridium*, *Listeria monocytogens*.

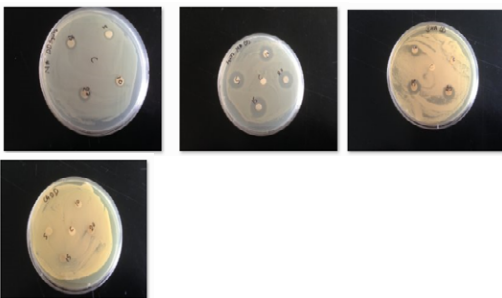
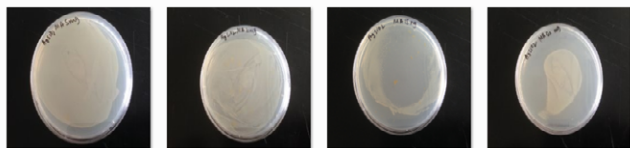


Figure 5: (AgSiO₂) Disk Diffusion and agar plate MIC for *Pseudomonas aeruginosa*, *Shewanella gaetbuli*, *Clostridium*, *Listeria monocytogens*.

5 shows Antibacterial effects in the form of inhibition zones and MIC agar plates, are shown. The clear zones of AgSiO₂ against *Pseudomonas aeruginosa*, *Sh. putrefaciens*, and *L. monocytogens*, are determined but for *Clostridium perfringem* no growth inhibition observed as shown in Figure 3. Kim Hwan Young also observed similar results of disk diffusion and MIC for AgSiO₂ against *S. aureus*, *P. aeruginosa*, *C. albicans*, *P. citrinum*, and *A. niger*. In Figure 5 control sample shows positive growth for *Pseudomonas aeruginosa*, *Shewanella gaetbuli*, *Clostridium*, *Listeria monocytogens* with zero concentration of selected antimicrobial. In Figure 6 graphical representations of disk diffusion has been shown (Table 5).

Applications

It is beneficial to know the potential application of AgSiO₂, ZnAg

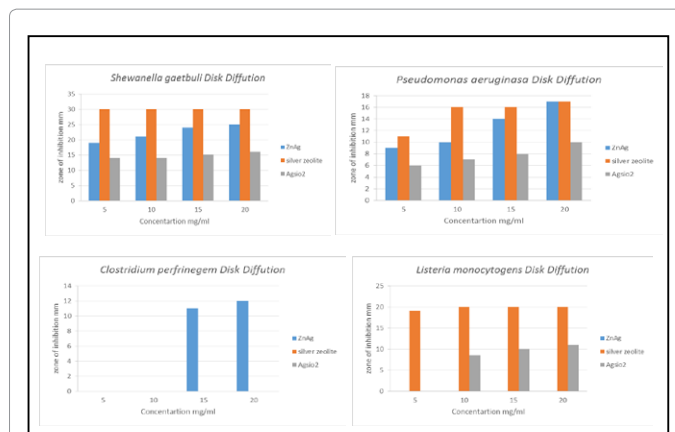


Figure 6: Disk diffusion diameter of Zone of inhibition for *Shewanella gaetbuli*, *Pseudomonas aeruginosa*, *Clostridium*, *Listeria monocytogens* (ZnAg, Silver zeolite, AgSiO₂).

Bacteria	Concentration of antibacterial agents				
	MIC(AgSiO ₂)				
	5 mg/ml	10 mg/ml	15 mg/ml	20 mg/ml	25 mg/ml
<i>Shewanella gaetbuli</i>	+	+	-	-	-
<i>Pseudomonas aeruginosa</i>	+	+	-	-	-
<i>Clostridium perfringem</i>	+	+	+	+	+
<i>Listeria monocytogens</i>	+	+	+	+	+
AgSiO ₂ Disk Diffusion (zone of inhibition mm)					
<i>Shewanella gaetbuli</i>	14	14	15	16	
<i>Pseudomonas aeruginosa</i>	6	7	8	10	
<i>Clostridium perfringemii</i>	-	-	-	-	
<i>Listeria monocytogens</i>	-	7	10	11	

Table 5: (AgSiO₂) Disk Diffusion and agar plate MIC for *Pseudomonas aeruginosa*, *Shewanella gaetbuli*, *Clostridium*, *Listeria monocytogens*.

and AgZeolite in manufacturing of fish boxes with incorporation of tested antimicrobial agents. The result of the study has shown that tested antimicrobial agents are effective against sea food spoilage bacteria at low concentrations. Krishnani also reported in his application suggestion for use of silver-zeolite that Silver-zeolite can be proposed as a low cost material for bactericidal and ammonia removal activity for aquaculture use [13]. However future studies on the sustainability of these antimicrobial agents, changes in the physical properties of the incorporated material and the toxicity related to the food products.

Conclusion

This study concluded that the Silver zeolite and AgSiO₂ is capable to reduce the number of bacterial load for all target microorganisms except *clostridium perfringem*. ZnAg is also capable to reduce the number of bacterial load for all target microorganisms except *Listeria monocytogens*. Only ZnAg is capable to reduce *clostridium perfringem* as compare to Silver zeolite and AgSiO₂.

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