Physical Chemistry

Antibacterial Activity of Metal-Containing Heulandite

Vladimer Tsitsishvili*, Nanuli Dolaberidze, Nato Mirdzveli**, Manana Nijaradze**, Zurab Amiridze**, Bela Khutsishvili****

* *Academy Member, Petre Melikishvili Institute of Physical and Organic Chemistry, Ivane Javakhishvili Tbilisi State University, Tbilisi, Georgia*
** *Petre Melikishvili Institute of Physical and Organic Chemistry, Ivane Javakhishvili Tbilisi State University, Tbilisi, Georgia*

Zeolite-containing silver, copper or zinc are recognized as a promising material for the development of new antibacterial substances. The purpose of the work was to obtain materials containing the indicated bioactive metals based on pre-heat- and acid-treated samples of heulandite-bearing tuff from the Rkoni plot of the Tedzami-Dzegvi deposit, Georgia, to establish their composition and properties, as well as to study their activity against bacteria and fungi. It has been established that heating of natural heulandite leads to stepwise dehydration proceeding up to ≈800°C, amorphization starting at ≈250°C, and structural changes at temperatures above 500°C, while treatment in acidic solutions leads to significant dealumination and decationization, an increase in the specific volume of micropores and the area of the adsorbing surface, as well as a decrease in the average diameter of mesopores. The enrichment of zeolite with metals was carried out by ion exchange in solutions of the corresponding salts; samples containing 244 mg/g silver, 43.3 mg/g copper and 59.8 mg/g zinc were obtained from untreated heulandite; in pre-treated samples, the metal content is usually lower, with the exception of pre-calcined samples, in which the copper content is 65-80 mg/g. According to the results of tests carried out by the disk diffusion method, pre-treatment does not increase the bacteriostatic activity of samples enriched with silver and copper, but acid treatment initiates the inhibition of the growth of *Escherichia coli*, *Staphylococcus aureus* and fungal microorganisms *Candida albicans* and *Aspergillus niger*, and also slightly increases the activity against *Bacillus subtilis* and practically does not change the activity against *Salmonella enteritidis* of zinc-containing samples.

heulandite, silver, copper, zinc, bacteriostatic activity

Among the materials advanced for the development of new antibacterial substances, crystalline microporous aluminosilicates $M_x[Al_xSi_yO_{2(x+y)}]\cdot mH_2O$, known as zeolites [1], in which usual alkali or alkaline-earth metal ions M^+ are partially replaced by such bioactive metal ions as Ag^+, Cu^{2+}, Zn^{2+}, etc., are recognized as promising [2,3]. It is found that biocidal formulations with such metal-containing zeolites (MZs) do not cause allergic reactions; they are nontoxic, odorless, and considered to be safe for the environment [4]. MZs were obtained back in the mid-1980s, but their intensive research began in the 21st century, the results obtained to date are reflected in numerous publications and summarized in reviews [5,6]. According to known results, synthetic and natural zeolites enriched with silver, copper and zinc exhibit antimicrobial activity against a wide range of pathogens, and it is believed, that the Ag-Zs are the most active, although there are disadvantages to using silver ions, including high cost, instability of Ag^+ in aqueous solutions, tendency to be reduced to Ag^0, and reaction with sulfate and other anions to form insoluble salts [7]. It was also found that the activity of MZs against pathogens is determined not only by the silver, copper, etc. ions released into the liquid medium, but also by the type of a carrier zeolite [8,9]. A comparison of the activity against bacteria and fungi of synthetic type A zeolite, natural phillipsite, analcime and heulandite enriched with silver, copper and zinc [10,11] and showed a fairly high activity of heulandite, despite the relatively low ion exchange capacity [11]. It should be taken into account that heulandite-clinoptilolite is the only natural zeolite whose safety is recognized by EU legislation [12], so it is advisable to carry out the following studies with this type of natural zeolite having the crystal chemical data $|Ca_4(H_2O)_{24}|[Al_8Si_{28}O_{72}]$-**HEU** [13].

However, the composition, structure and properties of zeolite can be changed by heat or chemical treatment. Heating the zeolite leads to dehydration and amorphization of the crystal structure, acid treatment increases effective surface area and “opens” the micropores, since M^+ cations sometimes block the “entrance windows” [14]. Our research of heulandite-containing tuff from the Dzegvi-Tedzami deposit [15,16] has shown that an acidic environment leads to significant dealumination (Si/Al molar ratio increases from 3.6 to 9.5 after processing with 2.0 mol/L HCl solution) and decationization (total charge of M^+ decreases from 1 to 0.68) of the sample; solutions of hydrochloric acid gradually dissolve the zeolite microporous crystal structure without amorphization and lead to a sharp increase in the volume of micropores (from ≈7 to 80-90 mm^3/g) and surface area (from ≈13 to 120-175 m^2/g), as well as to changes in the mesoporous system, causing to the prevalence of pores with a diameter up to 4 nm; heulandite heating leads to stepwise dehydration proceeding up to ≈800°C, amorphization starting at ≈250°C, and structural changes at temperatures above 500°C.

The main purpose of this paper is to examine bacteriostatic activity of preliminary heat- and acid-treated samples of recently characterized [17] heulandite of Georgian origin selected as raw material to create new bactericidal zeolite filter materials for purification and desinfection of water from various sources.

Materials and Methods

Materials. Heulandite-chabazite-containing rock from the Rkoni plot of Tedzami deposit (Eastern Georgia) was crushed in a standard crusher, fractionated to a particle size of 1-1.4 mm (14-16 mesh), washed with distilled water to remove clay impurities, and dried at a temperature of 95-100°C. Zeolite phase content of the material was 90%, empirical formula $(Na_{0.25}K_{0.06}Ca_{0.19}Mg_{0.15})[AlSi_{3.6}O_{9.2}]\cdot 3H_2O$, weight ratio of heulandite and chabazite phases 8:1, framework impurity $Ti_{0.005}$, mineral impurities $Fe_{0.2}$ and $Ca_{0.14}$ per Al atom. Calcination of prepared samples in the temperature range up to 800°C was carried out in muffle furnace B400/410 (Naberthem, Carl Stuart Group), details are described in [15]. Acid treatment was carried out by processing 10 g of sieved, washed and dried zeolite sample with 100 ml of 0.5, 1.0, and 2.0 mol/L solutions of hydrochloric acid in a shaking water bath (OLS26 Aqua Pro, Grant Instruments, US) operating in linear mode at 75°C; the total processing time was 6 hours, details are described in [16]. The enrichment was carried out by ion exchange reactions in 1 mol/L solutions of silver(I) nitrate $AgNO_3$, copper(II) chloride dihydrate $CuCl_2{\cdot}2H_2O$, and zinc(II) chloride $ZnCl_2$ (p.a., obtained from Merck KGaA, Darmstadt, Germany, and used without any additional purification) at a solid:liquid ratio of 1:10 at room temperature, with stirring for 6 h; the prepared samples were dried at 100-110°C.

Characterization. Chemical composition of samples was calculated from the X-ray energy dispersive spectra (XR-EDS) obtained from high performance scanning electron microscope JSM-6490LV (JEOL, Japan) equipped with INCA Energy 350 XRED analyzer (Oxford Instruments, UK).

Bacteriostatic properties of prepared MZs were determined by the disk diffusion (Kirby-Bauer) method in standard conditions using the cultures of Gram-negative bacteria *Escherichia coli* and *Salmonella enteritidis*, Gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis*, fungal pathogenic yeast *Candida albicans* and a fungus *Aspergillus niger* placed (10^9 CFU/cm^3) on Mueller–Hinton agar (3 mm deep) poured into 100 mm Petri dishes. Before testing the antibacterial activity, all MZ samples were sterilized at 70°C for 2 hours in a dry sterilizer. For testing, 0.2 g of powdered MZs in the form of a tablet pellets with a diameter of 8 mm was placed into the plates. The plates contaminated with bacteria were incubated at 37°C over 5% CO_2 medium, the plates contaminated with fungal microorganisms were incubated at 25°C during 3-4 days and, finally, the width of inhibition zone of each sample in the plates was measured at the end of the first day; all experiments were done in triplicate**.**

Results and Discussion

Chemical composition. According to the XR-EDS data, the degree of enrichment for silver ranges from 43 to 51%, for copper within 34-44%, and for zinc 34-38%, while the degree of decationization does not exceed 14% for enrichment with silver and 6% for enrichment with copper and zinc. The specific content of silver, copper and zinc in the treated samples calculated from the XR-EDS data in assumption that each aluminum atom in the zeolite framework "holds" three water molecules, and the conditional atomic mass of compensating cations other than the introduced metal is 30 a. m. u., results are presented in Table.

Table. Specific content (mg/g) of silver, copper and zinc in the acid- and heat-treated samples

Introduced metal		Ag	Cu	Zn
Untreated sample		244	43.3	59.8
Concentration of HCl solution, mol/L	0.5	153.2	41.0	38.7
	1.0	137.0	26.8	30.9
	2.0	123.4	24.8	31.4
Calcination temperature,°C	400	132	79.4	48.5
	500	54.5	65.4	34.3
	600	40.8	67.0	24.3
	700	49.4	78.6	31.9
	800	23.5	80.3	24.4

Compared to untreated samples, preliminary acid treatment causes a decrease in the silver content by approximately two times, copper by 1.75 times, zinc by 1.9 times, while the specific aluminum content as a result of acid treatment decreases by approximately 2.8 times. Heat treatment leads to a decrease in the silver content by an order of magnitude, and the zinc content by approximately two times, but the copper content even increases.

Bacteriostatic activity. The results of the Kirby-Bauer test versus bioactive metal content in zeolite are summarized in Figs. 1 and 2, and it is found that as a result of acid treatment, the activity of silver and copper forms against *E. coli* is reduced by approximately 55% and 15%, respectively, and completely disappears against *Salmonella*, despite the preservation of a fairly high content of bioactive metals in the samples. However, the zinc-enriched form, which is practically inactive against *E. coli*, acquires quite high activity as a result of acid treatment; preliminary acid treatment has little effect on the activity of the zinc form against *Salmonella*.

The silver and copper forms inhibit the growth of both gram-positive bacteria with the exception of the silver form of the sample preliminary treated in a concentrated HCl solution (2.0 mol/L), which does not show activity against *Bacillus subtilis*. The initial zinc form does not inhibit the growth of *Staphylococcus*, but is very active against *Bacillus*

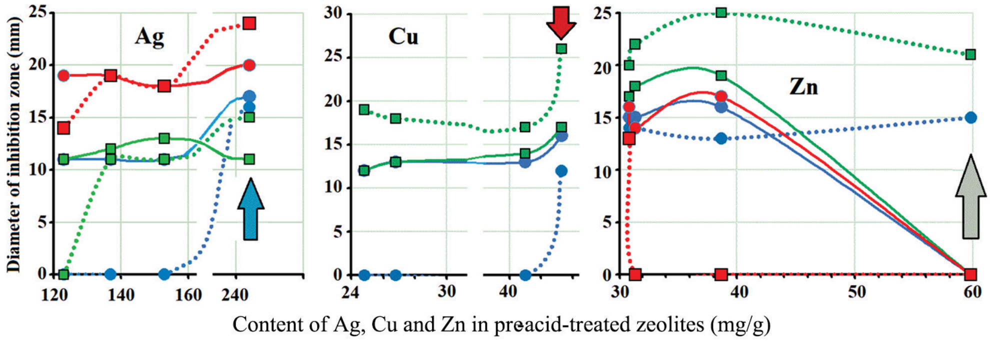

Fig. 1. Dependence of the diameter of the inhibition zones of the growth of microorganisms (blue circles and a solid line – *E. coli*, blue circles and a dotted line – *Salmonella*, green squares and a solid line – *Staphylococcus*, green squares and a dotted line – *Bacillus*, red circles and a solid line – *Candida*, red squares and dotted line – *Aspergillus*) on the content of metals in pre-acid-treated samples; arrows show the metal content in the untreated samples.

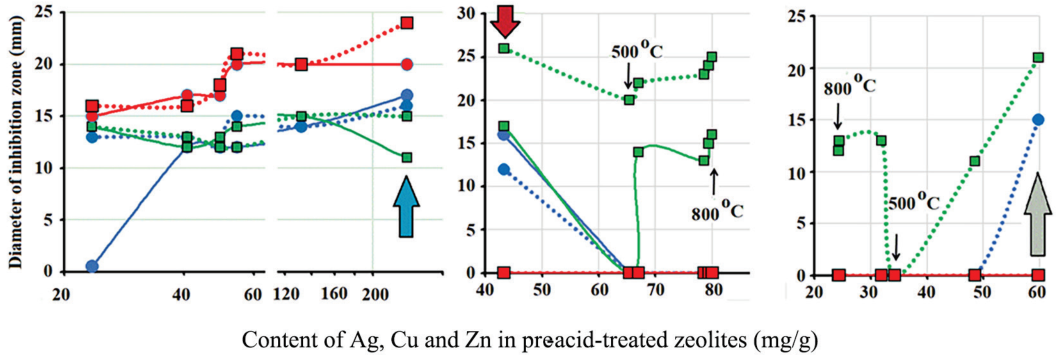

Fig. 2. Dependence of the diameter of the inhibition zone of the growth of microorganisms on the content of metals in pre-heat-treated samples; arrows show the metal content in the untreated samples.

subtilis; preliminary acid treatment gives the zinc form quite high activity against *Staphylococcus* and slightly increases activity against *Bacillus subtilis*.

Of the not pre-treated metal-enriched heulandites, only the silver form inhibits the growth of fungal microorganisms, the copper and zinc forms are inactive. Preliminary acid treatment somewhat reduces the activity of the silver form; the copper form remains inactive; the zinc form acquires sustained activity against *Candida* and "unexpected" activity against *Aspergillus* in a sample pretreated with a 1 mol/L HCl solution. Pre-heat-treated zinc-containing samples lose activity against *Salmonella*, but retain activity against *Bacillus subtilis*, although calcination at 500°C also leads to loss of activity.

Pre-heated copper and zinc forms, despite the content of bioactive metals, do not inhibit the growth of gram-negative bacteria. The silver form loses activity against *Escherichia coli* only after preheating the sample at 800°C, that is, when it is completely amorphized, but retains activity against *Salmonella*.

The silver and copper forms inhibit the growth of both gram-positive bacteria, with the exception of the copper form of the sample preheated at 500°C, which is inactive against staphylococcus. The zinc form does not inhibit the growth of staphylococcus at all, but is very active against *Bacillus subtilis*, with the exception of the sample preheated at 500°C and partially amorphized [15]. At higher temperatures, new microporous phases are formed that can "accommodate" ions of bioactive metals, which explains the activity of samples heated to 600-800°C. The high activity of the copper form against *Escherichia coli* and, in particular, *Bacillus subtilis* is noteworthy, when the diameter of the inhibition zone is almost twice that of the silver form. Of the pre-calcined samples, only the silver form inhibits the growth of fungal microorganisms, copper and zinc forms are inactive.

The dependence of the diameter of the inhibition zone of the growth of microorganisms on the specific content of silver in the zeolite indicates a weak correlation between activity against fungal microorganisms and the content of biologically active metal. The activity of samples pre-calcined and treated with acid and then enriched with silver differs significantly. Thus, the silver content in samples heated at 400°C and treated with a 1.0 mol/L HCl solution is approximately the same, 132 and 137 mg/g, respectively, but the sample partially amorphized by preheating suppresses the growth of salmonella, while a sample dealuminated by acid treatment is absolutely inactive.

Although the preheated samples had higher copper content compared to the acid-treated samples (60-80 and 25-40 mg/g, respectively), they were only active against Gram-positive bacteria. The activity is not related to the degree of amorphization, so a sample heated at 800°C, when the only microporous phase in the zeolite remains an admixture of chabazite, shows activity comparable to the activity of the original sample. It is likely that some of the copper atoms are contained occluded copper chloride particles, do not enter an ionic state, and do not participate in the process of inhibiting bacterial growth.

Conclusions

As a rule, pre-acid and heat treatments do not increase the bacteriostatic activity of samples obtained by ion exchange in a solution of silver nitrate, the only exception being the slightly increased activity of samples pre-treated with acid against staphylococcus. In relation to fungal microorganisms, the treated samples retain high bacteriostatic activity, which decreases by no more than 35% when the silver content in zeolites decreases tenfold. A significant difference in the activity of samples partially amorphized by preheating and samples dealuminated by acid treatment, containing approximately the same amount of silver, indicates the significant role of the zeolite matrix in the process of suppressing the growth of microorganisms.

Preliminary acid and heat treatments do not increase the bacteriostatic activity of samples obtained by ion exchange in a copper chloride solution. In the first case, this may be due to the lower copper content in acid-treated zeolites, and in the second case, due to the retention of copper ions in a bound state by amorphous samples.

Preliminary acid treatment significantly affects the bacteriostatic activity of samples obtained by ion exchange in a solution of zinc chloride, initiating inhibition of the growth of *Escherichia coli*, staphylococcus and fungal microorganisms, as well as slightly increasing activity against *Bacillus subtilis*; activity against salmonella remains virtually unchanged. Preliminary thermal treatment does not improve the disinfecting properties of zinc-containing samples.

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ფიზიკური ქიმია

ლითონშემცველი ჰეილანდიტის ანტიბაქტერიული აქტივობა

ვ. ციციშვილი*, ნ. დოლაბერიძე**, ნ. მირძველი**, მ. ნიჟარაძე**, ზ. ამირიძე**, ბ. ხუციშვილი**

** აკადემიის წევრი, ივანე ჯავახიშვილის სახ. თბილისის სახელმწიფო უნივერსიტეტი, პეტრე მელიქიშვილის სახ. ფიზიკური და ორგანული ქიმიის ინსტიტუტი, თბილისი, საქართველო*
*** ივანე ჯავახიშვილის სახ. თბილისის სახელმწიფო უნივერსიტეტი, პეტრე მელიქიშვილის სახ. ფიზიკური და ორგანული ქიმიის ინსტიტუტი, თბილისი, საქართველო*

ვერცხლის, სპილენძის ან თუთიის შემცველი ცეოლითები აღიარებულია, როგორც საკმაოდ პერსპექტიული მასალები ახალი ანტიბაქტერიული საშუალებების შესაქმნელად. სამუშაოს მიზანი იყო აღმოსავლეთ საქართველოს თეძამი-ძეგვის საბადოს რკონის უბნიდან მოპოვებული ჰეილანდიტშემცველი ქანის წინასწარ თერმულად და მჟავათი დამუშავებული ნიმუშების საფუძველზე მითითებული ლითონების შემცველი მასალების მიღება, მათი შემადგენლობისა და თვისებების დადგენა, აგრეთვე ბაქტერიებისა და სოკოების მიმართ მოქმედების შესწავლა. დადგენილია, რომ ჰეილანდიტის გათბობა იწვევს ≈800°C-მდე მიმდინარე ეტაპობრივ გაუწყლოებას, ≈250°C-ზე დაწყებულ ამორფიზაციასა და სტრუქტურულ ცვლილებებს 500°C-დან, ხოლო მჟავა ხსნარებში დამუშავება იწვევს მნიშვნელოვან დეალუმინირებას და დეკატიონირებას, მიკროფორების მოცულობისა და ადსორბციული ზედაპირის ფართობის ზრდას, აგრეთვე მეზოპორების საშუალო დიამეტრის შემცირებას. ცეოლითის ლითონებით გამდიდრება განხორციელდა შესაბამისი მარილების ხსნარებში იონმიმოცვლის მეთოდით; 244 მგ/გ ვერცხლის, 43,3 მგ/გ სპილენძის და 59,8 მგ/გ თუთიის შემცველი ნიმუშები მიღებული იყო დაუმუშავებელი ჰეილანდიტიდან; წინასწარ დამუშავებულ ნიმუშებში, როგორც წესი, არის ლითონების უფრო დაბალი შემცველობა, გარდა წინასწარ კალცინირებული ნიმუშებისა, სადაც სპილენძის შემცველობა არის 65-80 მგ/გ. დისკ-დიფუზიური მეთოდით ჩატარებული ტესტების შედეგების მიხედვით, წინასწარი დამუშავება არ ზრდის ვერცხლითა და სპილენძით გამდიდრებული ნიმუშების ბაქტერიოსტატიკურ აქტივობას, მაგრამ მჟავათი დამუშავება იწვევს თუთიის შემცველი ნიმუშების მიერ ბაქტერიების *Escherichia coli*-სა და *Staphylococcus aureus*-ის, სოკოვანი მიკროორგანიზმების *Candida albicans*-ისა და *Aspergillus niger*-ის ზრდის ჩახშობას, ასევე ოდნავ ზრდის აქტივობას *Bacillus subtilis*-ის მიმართ და პრაქტიკულად არ ცვლის აქტივობას *Salmonella enteritidis*-ის მიმართ.

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