Synthesis and Antibacterial Properties of ZnO/Clinoptilolite and $TiO_2/ZnTiO_3$/Clinoptilolite Powders

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The purpose of this study was to estimate the antimicrobial activity of ZnO/clinoptilolite and $ZnTiO_3$/ clinoptilolite samples in solid media (agar plates) against Gram-negative Escherichia coli ATCC 25922 and Gram-positive Staphylococcus aureus ATCC 25923. Samples of clinoptilolite rich tuff (from Beli Plast deposit in Eastern Rhodopes, Bulgaria) treated with oxalic acid were obtained. The ZnO and $TiO_2/ZnTiO_3$ were additionally incorporated into the clinoptilolite samples. The structure, morphology and elemental composition of the pre-treated clinoptilolite samples were characterized by XRD, SEM and EDX analysis. The antibacterial activity was investigated by exposing Escherichia coli (E. coli) and Staphylococcus aureus (S. aureus) in nutritive media to the ZnO, $TiO_2/ZnTiO_3$, ZnO/clinoptilolite and $TiO_2/ZnTiO_3$/clinoptilolite samples. A strong bactericidal action of both samples against E. coli and S. aureus was observed. Moreover, the $TiO_2/ZnTiO_3$/ clinoptilolite sample exhibited a strong antibacterial effect in the presence of E. coli (11 mm), while another sample (ZnO/clinoptilolite) showed strong antibacterial effect against S. aureus (15 mm).

Keywords: ZnO nanoparticles, clinoptilolite, $ZnTiO_3$, antibacterial properties, Escherichia coli, Staphylococcus aureus

Zeolites belong to a series compounds of hydrated aluminosilicates with open porous structure capable of absorbing large quantities of water molecules in the vicinity of room temperature even at low pressures. Among all natural materials, they have been intensively studied because their active sites where cationic exchange could occur, are stable in aqueous solutions at different *p*H. The ion exchange properties of natural zeolites are very promising in applications as sorption of harmful compounds from various systems or for their utilization as carriers of useful elements. Pretreatment of natural zeolites by acids, bases, surfactants, etc. is an important method to improve their ion-exchange capacity [1-4]. Zeolites acquire microbicidal properties after being ion exchanged with one or more "antibiotic cations" (Ag^+, Zn^{2+}, Cu^{2+}) often in combination with ammonium, NH_4^+ [5]. Natural clinoptilolite is a mineral of volcanic activity origin forming large industrial deposits. Clinoptilolite is one of the most important natural zeolites because it is found in large deposits worldwide. The clinoptilolite rocks contain hardly separable impurity minerals, first of all quartz, micas and others [5]. Clinoptilolite is crystalline, hydrated aluminosilicate (Si/Al > 4) of alkali and alkaline earth metals having an infinite, open, three-dimensional structure, three types of non-planar channels limited by a system of 8 and 10 tetrahedral elliptic-shaped rings with free diameters of 0.41x 0.47 nm, 0.40 x 0.55 nm, and 0.44 x 0.72 nm, respectively, a porosity of about 34% and a cationic exchange capacity (CEC) up to 2.3 meq/g [6, 7]. The exchangeable cations are located in the specific sites of clinoptilolite, coordinated with a different number of water molecules and oxygen atoms. Some advantages of the clinoptilolite as one of the most researched natural zeolites have been summarized recently [8].

It is well known that ZnO and TiO_2 possess antibacterial properties, and they were extensively studied as antimicrobial agents mainly under UV light irradiation [9-15]. It was found that TiO_2 is very effective in killing *Escherichia coli* (*E. coli*) [9], while ZnO is most effective in disinfection of *Staphylococcus aureus* (*S. aureus*) [13]. There is no detailed information concerning the similar behaviour of $ZnTiO_3$. Recently, it was found that nanosized $ZnTiO_3$ powders obtained by hydrolytic and nonhydrolitic sol-gel method exhibited good antibacterial activity against *E. coli* [16].

The purpose of this study is to prepare and evaluate the antibacterial activity of natural zeolite (clinoptilolite) modified with previously synthesized by combustion gel method ZnO and $TiO_2/ZnTiO_3$ powders. The antibacterial tests were performed in solid media (agar nutritive) against the cells of *E. coli* and *S. aureus*.

Experimental part

Materials and methods

The clinoptilolite – rich tuff employed in the current study is a natural zeolite from Kardjali deposit, Bulgaria. The zeolitic mineral was powdered and sieved, and then the powder with average particle size of 0.1 mm was selected in order to perform the experiments.

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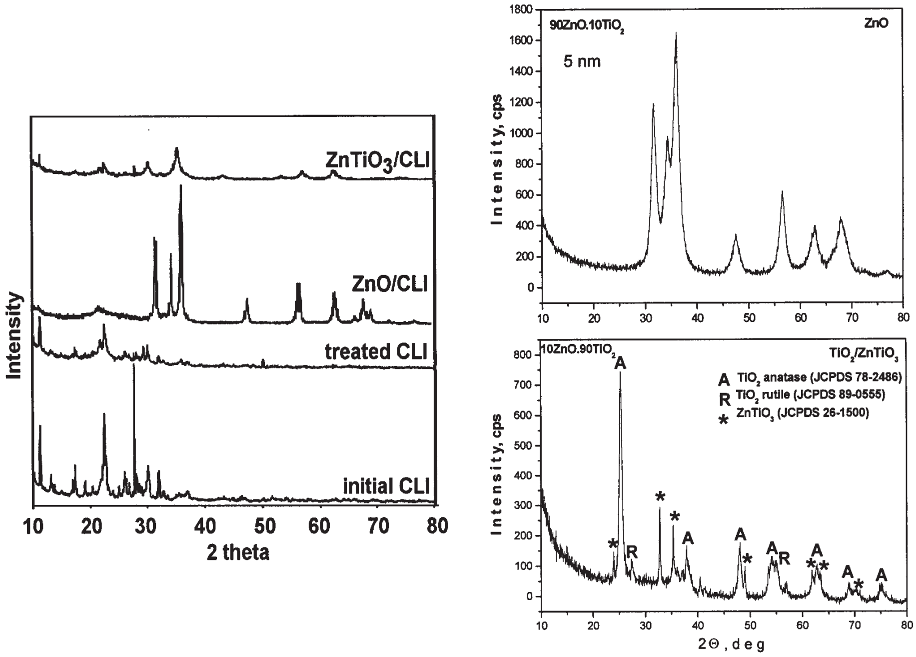


Fig. 1. XRD patterns of: (a) natural clinoptilolite – rich tuff (initial CLI), and oxalic acid pre-treated (treated CLI), ZnO/clinoptilolite and TiO_2/$ZnTiO_3$/ clinoptilolite and (b) ZnO and TiO_2/ $ZnTiO_3$ powders

The starting materials for obtaining the ZnO and TiO_2/ $ZnTiO_3$ powders were: zinc nitrate – $Zn(NO_3)_2.6H_2O$ (Merck), Zn acetate - $Zn(CH_3COO)_2.2H_2O$ (Merck), titanium (IV) ethoxide (Fluka AG) - $Ti(OC_2H_5)_4$, titanium (IV) butoxide (Sigma-Aldrich) - $Ti(OC_4H_9)_4$ and ethylene glycol ($C_2H_6O_2$). Both powders were ynthesized applying combustion gel synthesis and the main scheme for their synthesis is previuosly discussed elsewhere [17].

For the antibacterial tests *Escherichia coli* (*E. coli*) ATCC 25922 and *Staphylococcus aureus* (*S. aureus*) ATCC 25923 used were both obtained from the American Type Culture Collection (ATCC).

Preparation of TiO_2/$ZnTiO_3$/clinoptilolite

The chemical composition of the tested natural zeolite sample is (wt%): SiO_2 – 66.47; Al_2O_3 –10.75; Fe_2O_3 – 0.77; K_2O – 2.94; Na_2O – 0.44; CaO – 4.78; MgO– 1.50; TiO_2 – 0.11; MnO – 0.12; loss of ignition – 11.61; total 99.49%, the major component (70%) is clinoptilolite in multicationic form together with some impurities such as clays dolomite, 15%, and opal-cristobalite, 10%, feldspar, ~3%, and mica, about 2%. [18]. In order to improve the clinoptilolite content and the cationic exchange capacity, the natural zeolite mineral was acid treated with 1M oxalic acid ($H_2C_2O_4$. $2H_2O$) at 100°C for 5h with a liquid/solid ratio of 5:1. The solids were vacuum filtered, several times washed with deionized water and dried at 105ÚC [19].

For obtaining TiO_2/$ZnTiO_3$/clinoptilolit sample, previously synthesized by combustion gel method TiO_2/$ZnTiO_3$ powder (fig. 1a) was used as it was mentioned above. The TiO_2/$ZnTiO_3$/clinoptilolit sample was prepared by solid state dispersion (SSD) of $ZnTiO_3$. Initially involves mixing of TiO_2/ $ZnTiO_3$ and zeolite using ethanol in agate mortar; then the solvent was removed by evaporation with mixing. As prepared by this method samples were dried at 110°C and calcined in air at 550°C for 5h to obtain TiO_2/$ZnTiO_3$/ clinoptilolite.

Preparation of ZnO/clinoptilolite

Submission of ZnO nanoparticles (5nm) (fig. 1b) on the clinoptilolite support was achieved by the base hydrolysis. First, clear ethanolic solution containing zinc acetate, (1.0975g $Zn(ac)_2.2H_2O$/50 mL) was obtained. In this solution the support – clinoptilolite tuff treated with oxalic acid was added and mixed. The addition of lithium hydroxide solution and $Zn(OH)_2$ at *p*H 8 led to precipitation. The obtained precipitate was subsequent shaked for 2 h at 20°C. Finally, the sample was filtered, washed with ethylic alcohol and dried at 110°C. The ZnO nanoparticles were obtained by calcination at 550°C in air for 5h at a heating rate of 1°C/min.

Characterization of the samples

The phase formation of the obtained powders were established by X-ray diffraction (Bruker D8 Advance X-ray apparatus) between 10° and 70° 2θ. The morphology of the powders was observed from a VEGA 11 LSH Scanning Electron Microscope (TESCAN, Czech Republic). Samples were deposited on a sample holder with an adhesive carbon foil. The energy dispersive X-ray spectra – EDX patterns – were carried out using a Quantax QX2 system (Bruker/ Roentec, Germany).

Method for qualitatively determination of inhibition zone

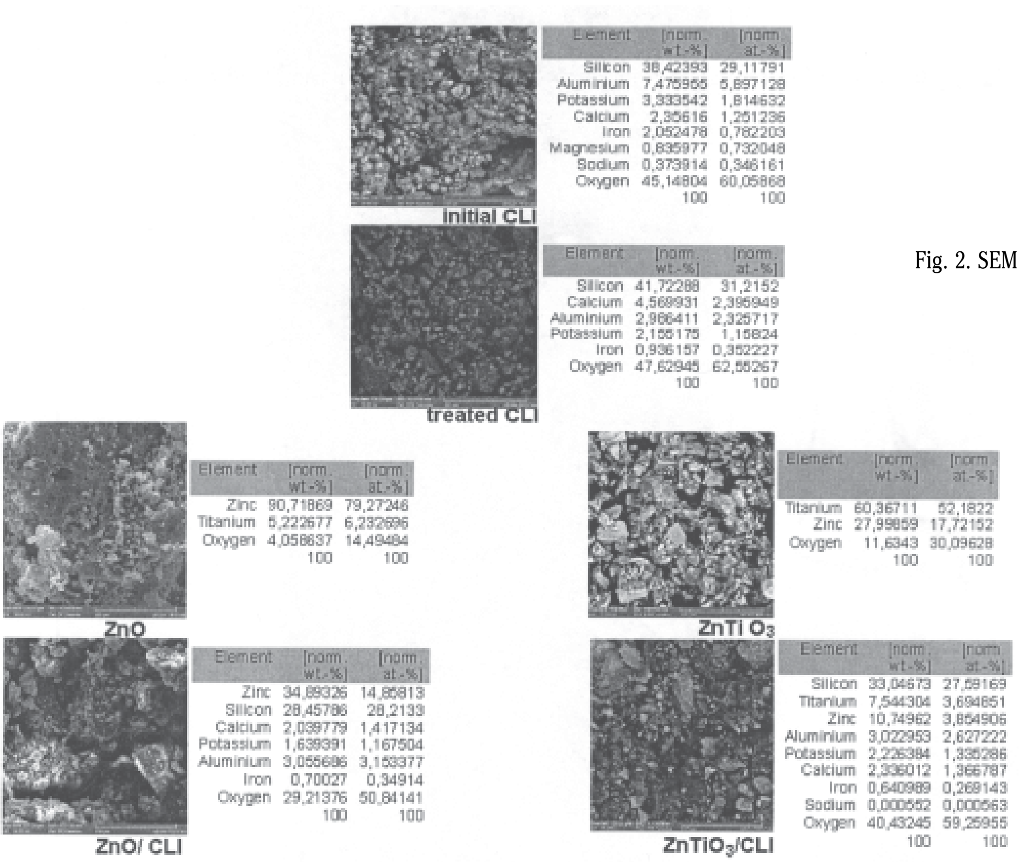
The strains of microorganisms were cultured aerobically at 37°C for 18h in 10mL nutrient broth. 20 ± 2mL of sterilized nutrient agar (solid media), dispersed into each standard flat bottom Petri dish to obtain firmly solid agar before inoculating. 2.0mL of inoculum from the ten times diluted inoculum cultured was transferred on the surface of the sterile agar area of a Petri dish. After that the tested samples were gently pressed to contact intimately the agar surface and the inoculum. The obtained mixtures were incubated at 37°C for 24 h . The antibacterial activity is observed when a clear zone of inhibition of the bacterial growth around the tested samples becomes visible.

Results and discussions

Sample characterization

XRD patterns of the starting natural clinoptilolite – rich tuff (initial CLI), oxalic acid pre-treated (treated CLI), ZnO/ clinoptilolite, TiO_2/$ZnTiO_3$/clinoptilolite as well as of pure ZnO and TiO_2/$ZnTiO_3$ powders are shown in figure 1a and b.

The main crystalline phase in initial volcanic tuff is clinoptilolite, the position and intensities of the reflections correspond to the literature data [4, 18-22]. The peak positions of the reflections in the pre-treated samples are similar, with minor changes in the position and intensity caused by removal of additionally species, as well as by dealumination of zeolite [23]. Besides, during the treatment with oxalic acid, the removal of Ca^{2+}, K^+, Mg^{2+} and Fe^{3+}



initial CLI

Element	[norm. wt.-%]	[norm. at.-%]
Silicon	38,42393	29,11791
Aluminium	7,475965	5,897128
Potassium	3,333542	1,814632
Calcium	2,35616	1,251236
Iron	2,052478	0,782203
Magnesium	0,835977	0,732048
Sodium	0,373914	0,346161
Oxygen	45,14804	60,05868
	100	100

treated CLI

Element	[norm. wt.-%]	[norm. at.-%]
Silicon	41,72288	31,2152
Calcium	4,569931	2,395949
Aluminium	2,986411	2,325717
Potassium	2,155175	1,15824
Iron	0,936157	0,352227
Oxygen	47,62945	62,55267
	100	100

ZnO

Element	[norm. wt.-%]	[norm. at.-%]
Zinc	90,71869	79,27246
Titanium	5,222677	6,232696
Oxygen	4,058637	14,49484
	100	100

ZnTi O$_3$

Element	[norm. wt.-%]	[norm. at.-%]
Titanium	60,36711	52,1822
Zinc	27,99859	17,72152
Oxygen	11,6343	30,09628
	100	100

ZnO/ CLI

Element	[norm. wt.-%]	[norm. at.-%]
Zinc	34,89326	14,85813
Silicon	28,45786	28,2133
Calcium	2,039779	1,417134
Potassium	1,639391	1,167504
Aluminium	3,055686	3,153377
Iron	0,70027	0,34914
Oxygen	29,21376	50,84141
	100	100

ZnTiO$_3$/CLI

Element	[norm. wt.-%]	[norm. at.-%]
Silicon	33,04673	27,59169
Titanium	7,544304	3,694851
Zinc	10,74962	3,854906
Aluminium	3,022953	2,627222
Potassium	2,226384	1,335286
Calcium	2,336012	1,366787
Iron	0,640989	0,269143
Sodium	0,000552	0,000563
Oxygen	40,43245	59,25955
	100	100

Fig. 2. SEM micrographs and composition of the samples

Table 1
ANTIBACTERIAL ACTIVITY OF INVESTIGATED SAMPLES

Samples		**Antibacterial tests – results**			
Pure powders	**Modified CLI powders**	**Inhibition diameter *(E. coli)***		**Inhibition diameter *(S. aureus)***	
ZnO	ZnO/CLI	9 mm	6mm	10 mm	**15**mm
Anatase, ZnTiO$_3$ and Rutile	TiO$_2$/ZnTiO$_3$/CLI	8 mm	**11**mm	11 mm	8mm

ions took place, associated with the increase of the amorphous state and modification of the pore size corresponding to the H-form of clinoptilolite [24]. The tuff rich in clinoptilolite has some impurities such as plagioclase, sanidine, muscovite (illite), quartz, calcite, dolomite, and less potassium feldspar. In the sample pre-treated with oxalic acid (CLI treated) is also present calcium oxalate monohydrate ($CaC_2O_4.H_2O$).

Figure 2 shows the SEM micrographs and the corresponding samples composition. The SEM images reveal that crystal morphology of clinoptilolite is composed of flat (blade) and small particles. The particles size is very similar in appearance with the natural and treated clinoptilolite but there are difference between ZnO/CLI and $ZnTiO_3$/CLI composites. From the compositions it is evident the absence of iron and sodium and the reduction of magnesium and aluminium content in the natural clinoptilolite pre-treated with oxalic acid.

Study of antibacterial activity

As it was mentioned above the *E. coli* and *S. aureus* were used in order to evaluate the antibacterial capability of the ZnO, TiO_2/$ZnTiO_3$, ZnO/clinoptilolite and TiO_2/$ZnTiO_3$/ clinoptilolite samples. Table 1 showed the obtained results from the antibacterial tests.

During the experiments each of the circular specimens with 5 mm diameter (filter paper with specific pressed sample) was gently pressed on the *E. coli* and *S. aureus* inoculated agar surface before incubation. After incubation at 37°C for 24 h bacteria inhibition take place and a zone of inhibition appeared around the samples. No clear zone of inhibition was seen around the blank experiments. The bactericidal action of all samples was observed for both bacteria (figs. 3 and 4).

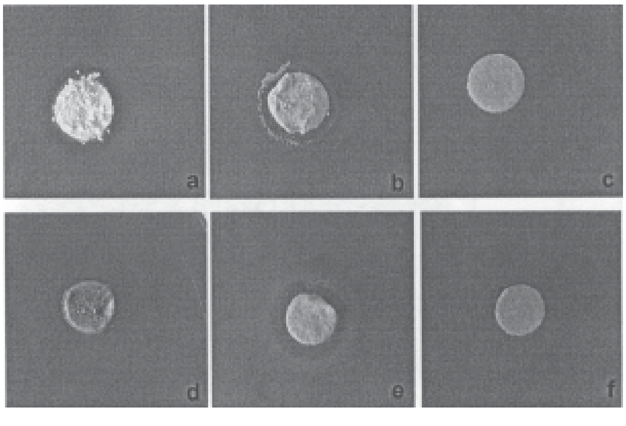


Fig. 3. Growth inhibition of the samples for *E. coli*: a - ZnO, b – TiO_2/$ZnTiO_3$, c - blank, d - ZnO/CLI, e - TiO_2/$ZnTiO_3$/CLI, f - blank

It was established that the introducing of zeolite to the pure ZnO samples significantly improved the antimicrobial effect against *S. aureus* (15 mm), while the other sample (TiO_2/$ZnTiO_3$/ zeolite) exhibited better antimicrobial effect towards *E. coli* (11 mm).

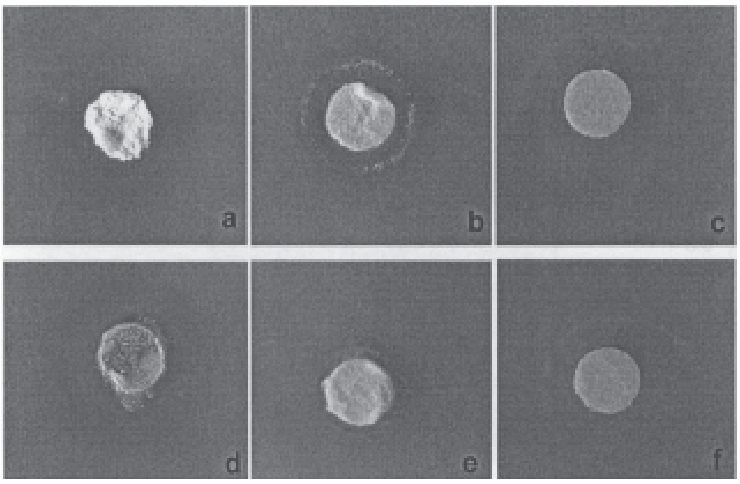


Fig. 4. Growth inhibition of ZnO/CLI and ZnO/SBA-15
S. aureus: a - ZnO, b - $TiO_2/ZnTiO_3$, c - blank,
d - ZnO/CLI, e - $TiO_2/ZnTiO_3$/CLI, f - blank

Conclusions

The acid pre-treated natural clinoptilolite from Kardjali deposit, Bulgaria has been chosen as matrices for the encapsulation of ZnO nanoparticles taking into account that its channels limit the particle size of ZnO during the growth and $ZnTiO_3$.

The ZnO NPs (5 nm) were loaded into the clinoptilolite matrix in solution phase, from zinc acetate dihydrate dissolved in ethanol followed by precipitation with lithium hydroxide at *p*H 8 and calcinations at 550^0C.

The as prepared ZnO, $TiO_2/ZnTiO_3$, ZnO/CLI and $TiO_2/ZnTiO_3$/CLI were tested as antibacterial agents against *Escherichia coli ATCC 25922 and Gram-positive Staphylococcus aureus ATCC 25923.* It was established that introducing of zeolite to the ZnO sample led to improving of its antimicrobial effect against *S. aureus*, while $TiO_2/ZnTiO_3$/zeolite sample exhibited better antibacterial properties toward *E. coli*.

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