

## ANTIBACTERIAL PROPERTIES OF ZnTiO<sub>3</sub> PREPARED BY SOL-GEL METHOD

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The present study for the ZnTiO<sub>3</sub> synthesis is based on a modified sol-gel method. The Ti(OEt)<sub>4</sub> and Zn-acetate were used as main precursors. The phase formation and structural transformation at every step of the synthesis routes were followed by X-ray phase analysis and IR spectroscopy. The agglomeration tendency and the crystal size were determined by Scanning Electron Microscopy. Submicron powders of cubic ZnTiO<sub>3</sub> were obtained by heating up to 500-550<sup>o</sup>C. It was established that ZnTiO<sub>3</sub> possesses photocatalytical activity against Malachite green organic dye. For the first time it was shown that submicron powders of ZnTiO<sub>3</sub> possess strong antimicrobial activities against high concentration of *Escherichia Coli* bacteria.

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### 1. Introduction

Zinc titanates are attractive materials due to their various applications as paints pigments, fusion cast thermistors, sorbents, microwave dielectrics, dielectric resonators, catalysts, etc. [1-6]. Several compounds are known to exist in the ZnO-TiO<sub>2</sub> system: congruently melting above 1500<sup>o</sup>C Zn<sub>2</sub>TiO<sub>4</sub>; the stable up to 945<sup>o</sup>C ZnTiO<sub>3</sub> and Zn<sub>2</sub>Ti<sub>3</sub>O<sub>8</sub> which was found later [1, 7]. Recently, data concerning the formation of a new ZnTiO<sub>3</sub> (cubic) phase obtained by different methods were reported [8-10] but the investigations on pure ZnTiO<sub>3</sub> are still not exhausted. There are several methods for preparing ZnTiO<sub>3</sub> powders, generally focused on conventional solid state reactions [1, 11], mechanochemical activation [12, 13], precipitation [14, 15] and several variants of the sol-gel technique [1, 11, 17]. During the recent years, the sol-gel method has been regarded as an advantageous one for the synthesis of nanopowders with high purity, ultrahomogeneity, reduction of the sintering temperature, etc. That is why it was selected in order to avoid the ZnTiO<sub>3</sub> decomposition at high temperatures.

It is well known that ZnO and TiO<sub>2</sub> possess antibacterial properties [18-21], but there is no enough information concerning the similar behavior of ZnTiO<sub>3</sub>. This provokes our interest to synthesize that compound and to examine its antibacterial properties.

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## 2. Experimental procedure

### 2.1. Chemicals and preparation of the samples

Zinc acetate (Sigma-Aldrich) and titanium ethoxide (Fluka AG) were used as starting materials. The precursors were dissolved in ethanol at room temperature under intense stirring to achieve complete dissolution and occurrence of hydrolysis processes. The citric acid monohydrate, ethylen glycol and HCl were added. White xerogel was obtained by drying at 110<sup>0</sup>C for 5h. Subsequently, the as-obtained xerogel was subjected to evaporation, drying and calcination, respectively at 400, 500 and 550<sup>0</sup>C for 1h in air.

The phase formation processes were established by Bruker D8 Advance X-ray apparatus. The thermal behaviour of powders was examined by differential thermal analysis (LABSYS<sup>TM</sup> EVO apparatus). The main structural units that determine the short range order in the structure of submicron powders were identified by IR spectroscopy (Nicolet 320 FTIR spectrometer). The particles morphology in the calcinated samples at 500<sup>0</sup>C was observed by Scanning Electron Microscopy (JEOL Superprobe 733). The photocatalytic activity of the powder was studied using Malachite green (MG) (Sigma-Aldrich) dye after subjecting it to UV-radiation. The discoloration of the solutions depending on the time is an indication for the degree of decomposition measured by a Jenway 6400 spectrophotometer.

### 2.2. Bactericidal activity measurements

The antimicrobial activity of zinc titanate has been essayed by *Escherichia coli* ATCC 25922 bacterial strain. One colony from fresh 18-h culture of the tested strain on blood agar plate was inoculated in 50 ml nutrient broth and then incubated at 37<sup>0</sup>C for 18 hours. The broth culture was centrifuged at 1000 x g for 10 min, washed and resuspended in sterile phosphate buffer solution (PBS). This solution was suitably diluted to obtain stock solution with cell density of approximately 10<sup>5</sup> cells per ml.

Four flasks were inoculated each with 100 ml of the stock solution of *E. coli* and the samples were treated in the following way: (i) no ZnTiO<sub>3</sub>, no radiation (control sample); (ii) only UV radiation; (iii) 200 mg of ZnTiO<sub>3</sub> were added (kept in the dark); (iv) 200 mg of ZnTiO<sub>3</sub> were added and UV radiation is applied.

For the illumination a UV lamp (Sylvania F6 T5 BLB) with an emission maximum at 365 nm was used. It was situated sidewise at a distance 5 cm to the reaction vessels. ZnTiO<sub>3</sub> was added to cells immediately prior to the reaction and its final concentration in the samples was 2 mg ml<sup>-1</sup>. Aluminum foil was used as a reflective material in order to protect the samples studied in the absence of irradiation. The inoculated flasks were incubated at 25<sup>0</sup>C for 3 hours under continuous stirring with magnetic stirrers.

Dynamics of antimicrobial action of the selected preparations was assessed by killing curves determination. The withdrawn samples (0.1 ml) were inoculated on Mueller-Hinton agar, poured in Petri dishes with thickness of the agar 5 mm for colony counting. The inoculum was performed at the moment of preparation of the suspension (0 min) and at 5<sup>th</sup> min, 15<sup>th</sup> min, 30<sup>th</sup> min, 45<sup>th</sup> min, 60<sup>th</sup> min, 90<sup>th</sup> min, 120<sup>th</sup>, and 180<sup>th</sup> min during the incubation period. The inoculated Petri dishes were incubated at 37<sup>0</sup>C for 24 h. All time kill experiments were performed in duplicate. Time-kill curves were constructed by plotting mean colony counts (CFU/ml) versus time.

## 3. Results and discussion

### 3.1. Results of structural and thermal analyses

The X-ray diffraction patterns of ZnTiO<sub>3</sub> calcinated at different temperatures are shown in Fig. 1. As it is seen the sample at 400<sup>0</sup>C is amorphous, at 500<sup>0</sup>C the strongest peaks of cubic ZnTiO<sub>3</sub> (JCPDS 39-0190) appeared. For comparison Hosono et al. [22] obtained the same phase by sol-gel method at temperatures below 500<sup>0</sup>C using zinc acetylacetonate and titanium

tetraisopropoxide as main precursors. On the other hand using the same precursors Wang et al. [17] prepared the cubic  $\text{ZnTiO}_3$  at  $500^\circ\text{C}$  and their results revealed that at  $800^\circ\text{C}$  several peaks related to the hexagonal  $\text{ZnTiO}_3$  appears, although the cubic crystals are still dominant. In contrast to the above mentioned Golovchansky et al. [23] using titanium tetraisopropoxide and zinc nitrate hexahydrate as precursors successfully prepared single-phase hexagonal  $\text{ZnTiO}_3$  at  $500^\circ\text{C}$  followed by cubic phase and rutile releasing above  $600^\circ\text{C}$ . Our results are closer to those obtained by Aubert et al. [24] and they showed that after the additional heat treatment at  $550^\circ\text{C}$  the peaks intensity increase. The average crystalline size of as obtained at  $500^\circ\text{C}$   $\text{ZnTiO}_3$  particles calculated from the broadening of the diffraction line using Sherrer's equation is about 40-50 nm.

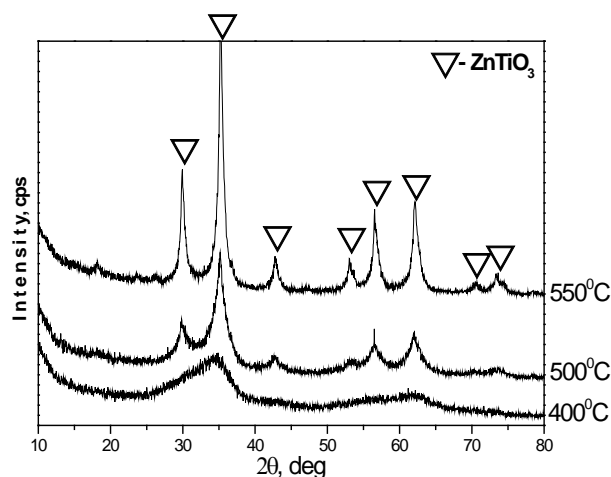


Fig. 1. XRD patterns of  $\text{ZnTiO}_3$  after calcination at different temperatures

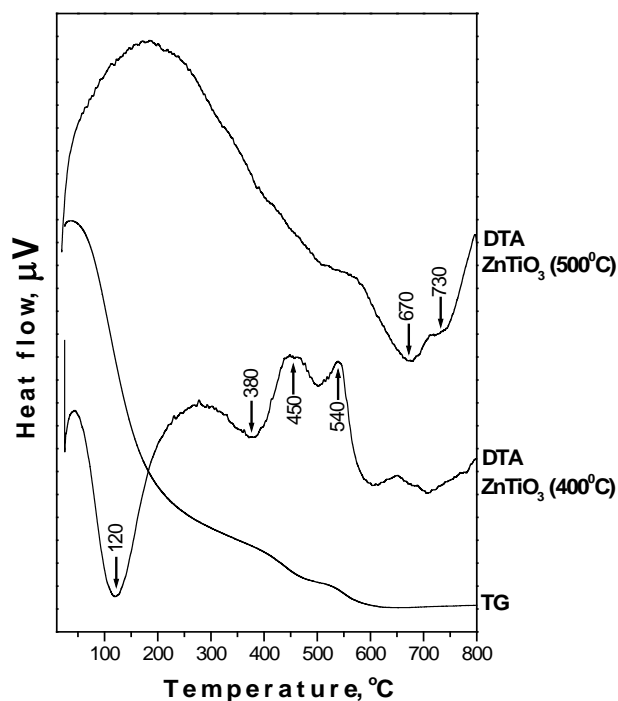


Fig. 2. DTA curves of the samples calcinated at  $400$  and  $500^\circ\text{C}$

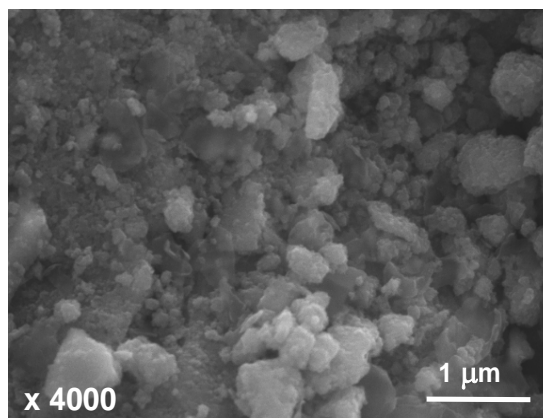


Fig. 3. SEM image of  $\text{ZnTiO}_3$  obtained at  $500^\circ\text{C}$

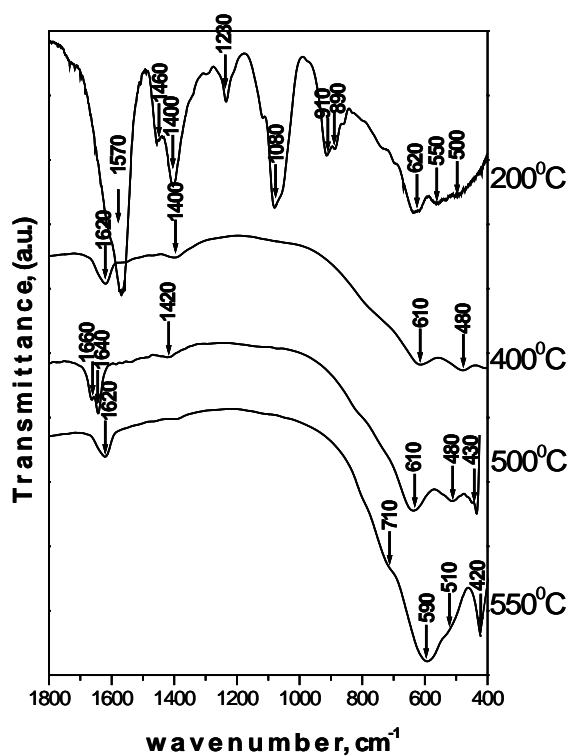


Fig. 4. IR spectra of  $\text{ZnTiO}_3$  at different temperatures

The DTA curve of the previously heat treated at  $400^\circ\text{C}$  sample (Fig. 2) reveals two endothermic ( $120$  and  $380^\circ\text{C}$ ) and two exothermic peaks at  $450$  and  $540^\circ\text{C}$ . The endothermic effects can be attributed to the dehydration of the sample, while the exothermic ones could be related to the combustion of the organic residues and crystallization of the amorphous sample with formation of  $\text{ZnTiO}_3$  phase ( $540^\circ\text{C}$ ). It is interesting to note that both exothermic peaks (at  $450$  and  $540^\circ\text{C}$ ) are not observed in the DTA curve of the previously heat treated at  $500^\circ\text{C}$  sample. This proves that the last exothermic effect is connected to the crystallization process which is in good accordance with the X-ray diffraction results (Fig. 1). Our results are compatible to those obtained by Wang et al. [17]. The SEM result showed that the  $\text{ZnTiO}_3$  sample calcinated at  $500^\circ\text{C}$  consisted of agglomerates (Fig. 3) with average particle size below  $100$  nm.

The IR spectra of  $\text{ZnTiO}_3$  calcinated at different temperatures ( $200$ ,  $400$ ,  $500$  and  $550^\circ\text{C}$ ) are shown in Fig. 4. There is significant difference in the IR spectra of samples heat treated at  $200$  and  $400^\circ\text{C}$  because the absorption peaks for organic species disappeared at  $400^\circ\text{C}$ . This is an

indication for the complete removal of the organics. In the other two spectra (at 500 and 550<sup>0</sup>C) the bands in the absorption range 700-400 cm<sup>-1</sup> are dominant. They are related to the vibrations of TiO<sub>6</sub> units in ZnTiO<sub>3</sub> [1, 25]. By the way it is well known that the bands corresponding to ZnO<sub>n</sub> polyhedra are in the same absorption range and it is not possible to be distinguished in this case [26, 27]. There is a difference in the band positions in both IR spectra irrespective that according to X-ray diffraction, presence of cubic ZnTiO<sub>3</sub>, only at these temperatures was established. The observed distinction in the IR spectra at 500 and 550<sup>0</sup>C probably is due to some transformations in the short range order of the building units. Additional experiments are needed in order to elucidate this task.

Figure 5 presents UV-Vis absorption spectra of an aqueous solution of MG (initial concentration: 5 ppm 150 mL) by ZnTiO<sub>3</sub>. It can be seen that the photodegradation of MG was completed in 180 min and it is compatible to the photocatalytic activity of ZnO [28].

### 3.2. Bactericidal action of zinc titanate

As can be seen from Fig. 6, the bactericidal action of UV radiation alone was lower comparing to that in the presence of ZnTiO<sub>3</sub>. After 60 min irradiation only 18% of bacteria were killed in the sample to which only UV radiation was applied; about 86% of bacteria were killed in the sample treated with ZnTiO<sub>3</sub> in the dark; and in the sample treated with combination of ZnTiO<sub>3</sub> and UV radiation almost all of bacteria were killed. In the last case the bactericidal process was virtually completed after 90 min, while the complete killing of bacteria treated only with ZnTiO<sub>3</sub> was achieved after 120 min. Obviously the removal efficiency due to the joint action of UV irradiation and ZnTiO<sub>3</sub> was increased in comparison to UV irradiation or ZnTiO<sub>3</sub> alone. For comparison the antibacterial effect of ZnTiO<sub>3</sub> is similar to those of pure ZnO and TiO<sub>2</sub> [29-31].

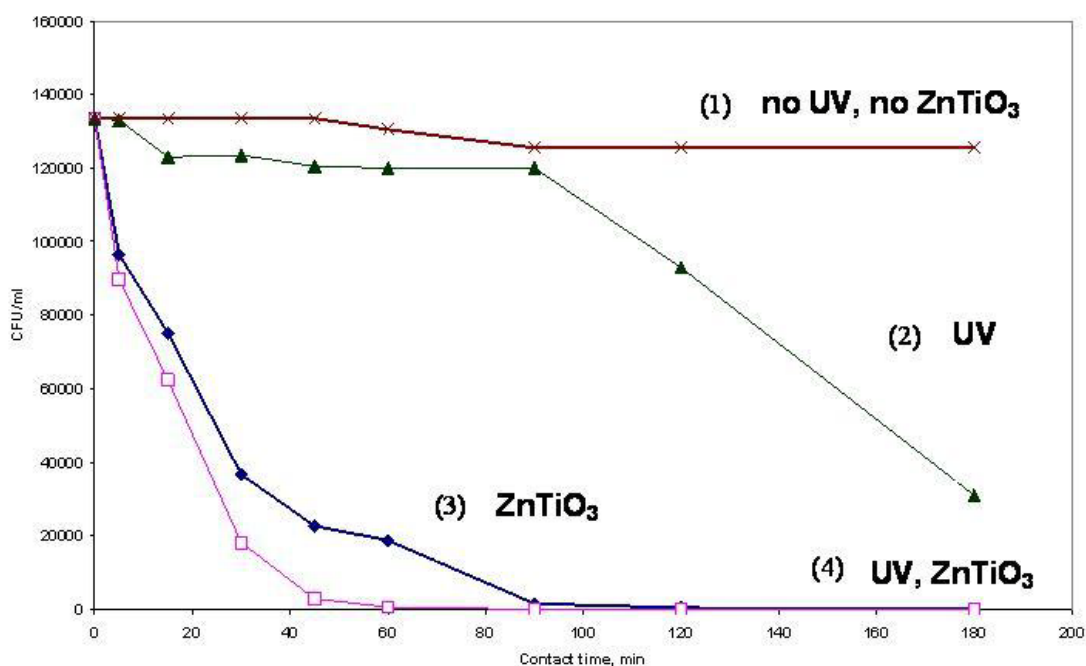


Fig. 5. Time-kill curves of *E. coli* in samples at different exposures: (1) no ZnTiO<sub>3</sub> and no UV radiation; (2) UV light alone; (3) in the presence of ZnTiO<sub>3</sub> at dark conditions; (4) in the presence of ZnTiO<sub>3</sub> and UV radiation.

#### 4. Conclusion

Submicron powders of cubic ZnTiO<sub>3</sub> were obtained by heating up to 500<sup>0</sup>C using titanium ethoxide and zinc acetate as main precursors. It was established that ZnTiO<sub>3</sub> possesses photocatalytical activity against Malachite green organic dye. For the first time it was shown that ZnTiO<sub>3</sub> possesses strong antimicrobial activities against high concentration of *E. coli* bacteria. The removal efficiency due to the joint action of UV irradiation and ZnTiO<sub>3</sub> was increased in comparison to UV irradiation or ZnTiO<sub>3</sub> alone.

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#### References

- [1] O. Yamaguchi, M. Morimi, H. Kawabata, et al., J. Amer. Ceram. Soc. **70**, c97-8 (1987)
- [2] A. Baumgrate, R. Blachnik, J. Alloys Compd **210**, 75 (1994)
- [3] Z. Chen, A. Derking, W. Kot, M. Dijk, J. Catal. **161**(2), 730-41 (1996)
- [4] H. Kim, S. Nahm, J. Byun, J. Am. Ceram. Soc. **82**(12), 3476-80 (1999)
- [5] H. Kim, J. Byun, Y. Kim, Mater. Res. Bull., **33**(6), 963-73 (1998)
- [6] S.F. Wang, M. Lu, F. Gu, C. Song, D. Xu et al. Inorg. Chem. Commun. **6**(2), 185-88 (2003)
- [7] F.H. Dulin, D.E. Rase, J. Amer. Ceram. Soc. **43**, 12-5 (1960)
- [8] C. Li, Y. Bando, M. Nakamura, N. Kimizuka et al., Mater. Res. Bull., **35**, 351-358 (2000)
- [9] Y.L. Chai, Y.S. Chang, G.J. Chen, Y.J. Hsiao, Mater. Res. Bull., **43**, 1066-1073 (2008)
- [10] X. Xing, J. Luo, G. Liu, L. Qiao, J. Meng, J. Amer. Ceram. Soc., **89**(3), 1125-1128 (2006)
- [11] Y. Chang, Y.H. Chang, I.G. Chen et al. Ceram. Intern., **30**, 2183-2189 (2004)
- [12] P. Botta, E. F. Aglietti, J. Porto Lopez, J. Mater. Sci., **39**, 5195-5199 (2004)
- [13] N. Labuss, N. Obradovic, T. Sreckovic, Sci. and Sintering, **37**, 115-122 (2005)
- [14] H. W. Yao, R.B. Feng, Mining Safety Environ Protect, **28**, 20 (2001)
- [15] R. P. Gupta, S.K. Gangwal, S. Jain, US Patent N 5,714,431 (1998)
- [16] Y. S. Chang, Y.H. Chang, I.G. Chen et al., J. Cryst. Growth., **243**, 319(2002)
- [17] S.F. Wang, F. Gu, M.K. Lu et al., Mater, Res, Bull, **38**, 1283-1288 (2003)
- [18] L.K. Adams, D. Lyon, P.J. Alvarez, Water Res ., **40**, 3527-3523 (2006)
- [19] A. Stoyanova, M. Sredkova, A. Bachvarova-Nedelcheva, R. Iordanova, Y. Dimitriev, H. Hitkova, Tz. Iliev, Opt. Adv. Mater. - RC, **4** (12), 2059-2063 (2010)
- [20] L. Zhang, Y. Jiang, Y. Ding, M. Povey, D. York, J. Nanoparticle Res., **9**, 479-489 (2007)
- [21] N. Jones, B. Ray, K. Ranjit, A. Manna, FEMS Microbiol. Lett., **279**, 71-76 (2008)
- [22] E. Hosono, S. Fujihara, M. Onuki, T. Kimura, J. Amer. Cer. Soc., **87**(9), 1785-1788 (2004)
- [23] A. Golovchansky, H. Kim, Y. Kim, J. Kor. Phys. Soc., **32**, S1167-S1169 (1998)
- [24] T. Aubert, F. Grasset, M. Potel, V. Nazabal, T. Cardinal, St. Pechev, N. Saito et al., Sci. Technol. Adv. Mater., **11**, 044401-9(2010)
- [25] B. G. Shabalin, Mineral. Zh., **4**, 54-61 (1982)
- [26] M. Mancheva, R. Iordanova, Y. Dimitriev, J. Alloys Compd, **509**(1), 15-20 (2011)
- [27] M. Andres-Verges, M. Martinez-Gailego, J. Mater. Sci., **27**, 3756-62 (1992)
- [28] Y. Dimitriev, Y. Ivanova, A. Staneva, L. Alexandrov, M. Mancheva, R. Yordanova, C. Dushkin, N. Kaneva, C. Iliev, J. Univ. Chem. Tech. Metall., **44**(3), 235-242 (2009)
- [29] C. Srinivasan, N. Somasundaram, Current Science, **85**, 1431-38 (2003)
- [30] K. Hirota, M. Sugimoto, M. Kato, K. Tsukagoshi, T. Tanigawa, H. Sugimoto, Ceram Intern **36**, 497-506 (2010)
- [31] C. Karunakaran, V. Rajeswari, P. Gomathisankar J Alloys Comp **508**, 587-591 (2010)