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THE EFFECT OF TEMPERATURE TREATMENT TiO_2 NANOPARTICLES ON ANTIBACTERIAL PROPERTIES

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Abstract

The synthesis of TiO_2 nanoparticles (NPs) under various temperature treatments was described and TiO_2 NPs was subsequently tested as an antibacterial agent. Scanning electron microscopy (SEM), Raman spectroscopy, Dynamic light scattering (DLS) were used to confirm structure of TiO_2 NPs and detect differences between individual batches treated with different temperature. Antibacterial properties were tested on *Escherichia coli* (E. coli). TiO_2 NPs as photocatalyst was incubated with bacterial cells under ambient light. Changes in temperature treatment can affect diameter size and crystal structure of TiO_2 NPs as well as its antibacterial properties.

Keywords: Titanium dioxide, pathogenic bacteria, nanoparticles, antibacterial properties, *E.coli*, nanomaterial

1. INTRODUCTION

Bacterial infections are source of serious diseases and can be risk for human health. Antibiotics are relatively cheap, high-yield, widely used solution of bacterial infections. But there have been increasing number of resistant bacteria and even super bacteria, which are resistant to almost all spectrum of antibiotics [1]. Antibiotics have been widely used in livestock production and veterinary medicine to increase production [2-6]. But antibiotics are water-soluble and 30-90 % antibiotics can be excluded in environment [7]. Nanomaterials are perspective solution of this global issue, because the way of interaction with bacteria is different in compare with antibiotics. Antibiotics can diffuse and need penetrate bacterial cell wall in contrast with nanomaterials which act with direct contact with bacterial cells [7-9]. Titanium dioxide (TiO_2) nanoparticles (NPs) is widely known antibacterial agent which was even approved by the American Food and Drug Administration (FDA) for use in human food and materials for food-contact [10]. TiO_2 shows photocatalytic activity and after illumination with light can kill bacteria with contact. After illumination hydroxyl radicals (OH) and reactive oxygen species (ROS) are generated onto TiO_2 and react with bacteria by oxidizing the polyunsaturated phospholipid component of the cell membrane [11-13]. OH is more effective for E.coli inactivation than common disinfectants (e.g. ozone, chlorine or chlorine dioxide) [14]. There are many factors which can affect antibacterial properties of TiO_2 e.g. crystal phase or size [15]. It is general opinion that anatase TiO_2 NPs are more toxic than rutile NPs by inducing stronger oxidative stress [15]. It is also widely known that smaller particles are more toxic. In this present work effect of applied temperature in synthesis of TiO_2 on size, crystal phase and antibacterial properties was evaluated.

2. MATERIAL AND METHODS

2.1. Preparation of TiO_2

Titanium isopropoxide (IV) (Sigma-Aldrich, 97 %) was diluted in isopropyl alcohol (Lachner, 99.7 %). TiO_2 NPs were precipitated on dropwise addition of alkaline Milli-Q water (pH 8). The as-prepared precipitate was washed with Milli-Q water triplicate and heated at 200 °C, 300 °C, and 500 °C [16].

2.2. Scanning Electron Microscopy

The structures of the TiO_2 NPs were characterized by scanning electron microscopy (SEM). For documentation of the nanoparticles structure, a MIRA3 LMU (Tescan, Brno, Czech Republic) was used. This model is equipped with a high brightness Schottky field emitter for low noise imaging at fast scanning rates. The SEM was fitted with In-Beam SE detector. For automated acquisition of selected areas a TESCAN proprietary software tool called Image Snapper (Tescan, Brno, Czech Republic) was used. The software enabled automatic acquisition of selected areas with defined resolution. An accelerating voltage of 15 kV gave satisfactory results regarding maximum throughput.

2.3. Dynamic light scattering

Particle size was measured using the Zetasizer Nano ZS instrument (Malvern Instrument Ltd, UK). The parameters of particle size measurements were follows: refraction index of the dispersive phase of 3.00 and 1.333 for the dispersive environment, adsorption coefficient 10−3, temperature 25 °C, equilibration time 120 s, measurement angle of 173° backscatter. For measurement, disposable cuvettes type ZEN 0040, were used, containing 50 µL of sample.

2.4. Raman spectroscopy

The samples of TiO_2 obtained at three different temperatures were characterized by Raman spectroscopy. Measurements were performed on an InVia Reflex Raman microspectrometer (Renishaw, Wotton-under-Edge, UK) equipped by the 514.5 nm line of an argon laser for excitation. A Leica microscope equipped with a standard 50x objective were used. Scans of 10 s were accumulated 5 times and the laser power was typically set to 0.2 mW at source. Resulting spectra were baseline-corrected in WiRE 3.4 oftware (Renishaw, Wotton-under-Edge, UK).

2.5. Cultivation of bacteria strains

Bacterial culture Escherichia coli NCTC 13216 was cultivated in Muller-Hinton broth (MHB; Oxoid, Hampshire, UK) overnight at 37 °C and 150 rpm.

2.6. Minimal inhibitory concentration

The bacterial culture was diluted with physiological solution to a concentration ~1×10^6 CFU/mL. The bacterial concentration was measured by optical density at 600 nm (OD_{600}). Then, the bacterial culture was diluted 100 times. The 75 µL of the bacterial suspension was added into a 96-well microplate and mixed with TiO_2 nanoparticles in ratio 1:1, with total volume 150 µL. The TiO_2 nanoparticles concentration range was from 0.5 mg/mL to 0.063 mg/mL. In case of control, bacteria were diluted only with MHB. The microplate was irradiated with visible light for 30 minutes and placed in Plate Shaker-Thermostat (Biosan, Riga, Latvia) for 16 hours at 37 °C and 800 rpm.

3. RESULTS

The SEM micrographs (**Figure 1**) confirmed nanostructure of TiO_2 and showed structural differences between samples treated at various temperature conditions. It can be easily seen that NPs prepared at 200 °C (A) are

smaller than NPs prepared at 500 °C (C). Average size of TiO_2 NPs at different temperature treatment was confirmed by DLS and results are displayed in **Table 1.** There can be observed trend between increasing temperature during synthesis and increasing size of NPs.

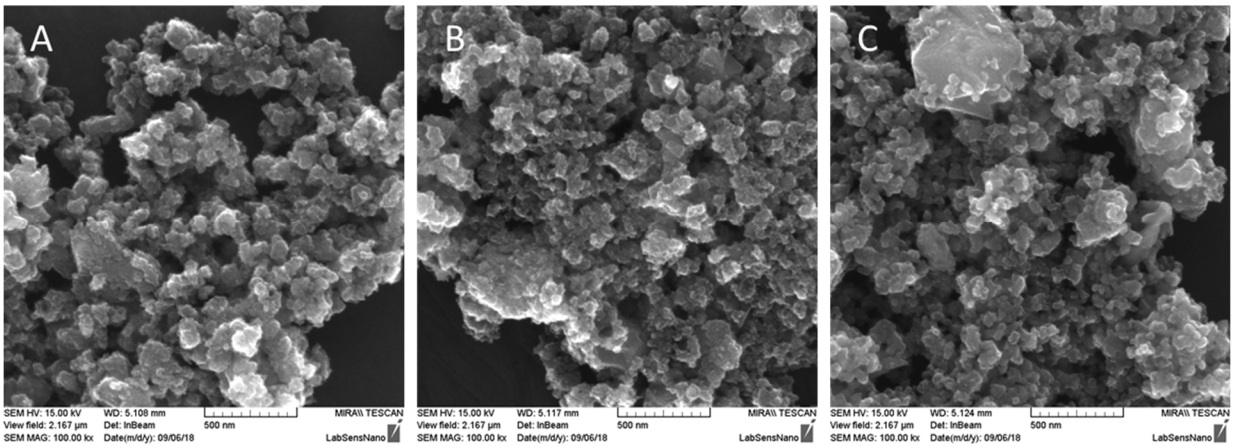

Figure 1 Images of TiO_2 NPs under different heating conditions (A) 200 °C, (B) 300°C, (C) 500°C by using SEM

Table 1 Size parameters of TiO_2 NPs under different temperature treatment by using DLS

Treating temperature of TiO_2 (°C)	Average particle size (nm)
200	92
300	129
500	846

Raman spectroscopy of the three samples synthesized at 200, 300 and 500 °C, respectively, revealed the anatase type of TiO_2 structure. Temperature-dependent spectral changes were observed. The shift of wavenumber position of the band corresponding to Eg(1) phonon mode towards lower value was observed with increasing temperature (**Figure 2**). Namely, at 200°C it was detected at 148.7 cm^{-1}, whereas at 300°C and 500°C the detected position was 147 and 143.7 cm^{-1}, respectively. According to Choi et al. [17], the shift in wavenumber position of the Raman band likely reflects the differences in particle sizes. Simultaneously, the band widths decreased with an increased temperature; for FWHM values corresponding to the band of Eg(1) phonon mode see the **Table 2**. It is interpreted to be a result of increasing crystallinity of anatase with higher temperature.

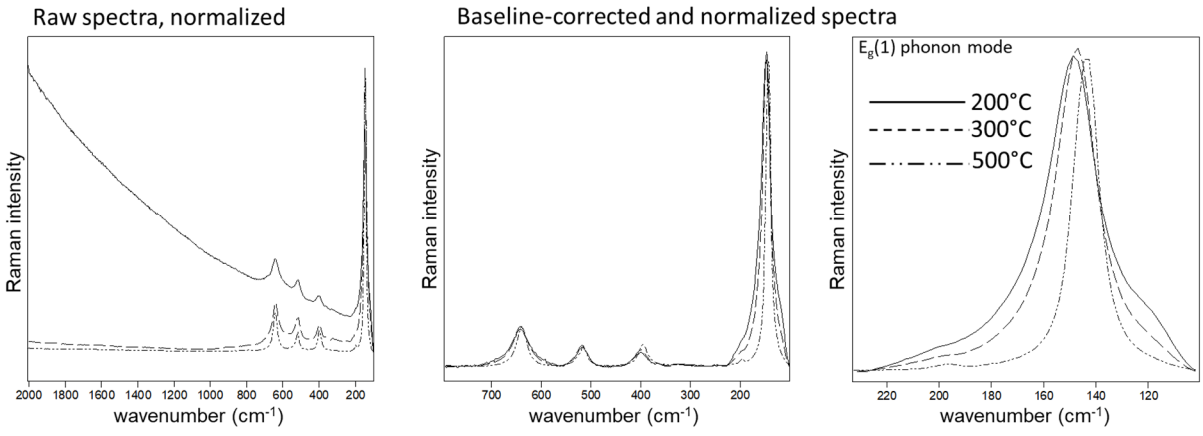

Figure 2 Raman spectra of TiO2 NPs under different heating conditions

Table 2 The position and FWHM of the Eg mode in the TiO_2 NPs

Treating temperature of TiO_2 (°C)	Position of the Eg(1)	FWHM Eg(1)
200	148.7	25.1
300	147.0	19.3
500	143.7	11.6

FWHM, full width at half maximum.

Antibacterial properties of three samples of TiO_2 were tested on *E.coli* as a model organism. The best results exhibited sample prepared at 300 °C in the highest concentration (**Figure 3**). The reason of the best efficiency of TiO_2 NPs treated at 300 °C is still small size and possible presence of large amount organic residues in sample treated at 200 °C, which can difficult the adsorbate- adsorbent interaction [16]. Differences between other samples and concentrations were not so significant although all samples exhibited antibacterial activity. Each experiment was repeated three times. The student t-test was used to evaluate statistically significant differences ($p < 0.05$) between experimental group and control group. The null hypothesis that the frequency was not different between different samples was rejected at a value less than or equal to 0.05. Error bars such as standard error were used.

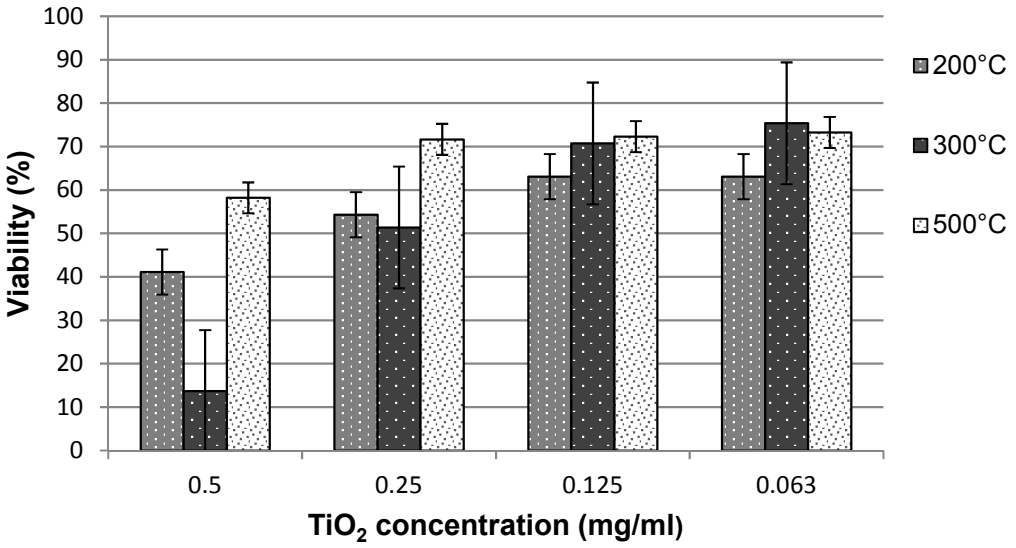

Figure 3 Minimal inhibitory concentration results of TiO_2 NPs after 30 minutes ambient light irradiation

4. CONCLUSION

In this study TiO_2 NPs were synthesized under different temperature treatment. The size of NPs increasedwith increase of heating temperature. The anatase type of TiO_2 structure was detected in all samples. The best antibacterial properties exhibited sample prepared at 300 °C in concentration 0.5 mg/ml against *E.coli* as a model organism.

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