

Sonocatalytic Damage of Composite TiO_2/ZnO Powder to Bovine Serum Albumin under Ultrasonic Irradiation¹

Jun Wang^{a,b}, Shixian Wang^a, Bin Liu^b, Ying Guo^b, Liquan Zhang^b,
Xia Chen^a, Rui Xu^a, and Xiangdong Zhang^a

^a Department of Chemistry, Liaoning University, Shenyang 110036, P. R. China

^b Department of Pharmacy, Liaoning University, Shenyang 110036, P. R. China

e-mail: wangjun890@126.com

Received December 11, 2008

Abstract—The composite TiO_2/ZnO was prepared by mixing nano-sized TiO_2 and ZnO powders directly, in the molar proportion of 4:6, followed by heat-treatment at 500°C for 40 min. The products were characterized by powder X-ray diffraction and transmission electron microscopy. The test of sonocatalytic activities of the composite TiO_2/ZnO powders was carried out through the damage of bovine serum albumin (BSA). Otherwise, the effects of several factors on the damage of BSA molecules were evaluated by means of UV-vis and fluorescence spectra. It was found that the damage degree was aggravated with the increase of ultrasonic irradiation time and composite TiO_2/ZnO addition amount. These research results were of great significance for driving sonocatalytic method to treat tumor in clinic application.

DOI: 10.1134/S0020168510060154

INTRODUCTION

In recent years, semiconductor TiO_2 , as an important photocatalyst, has widely been used for degradation of environmental contaminants [1, 2]. It is based on the generation of hydroxyl radicals ($\cdot\text{OH}$) which can cause various chemical reactions due to the strong oxidation ability [3]. Moreover, the TiO_2 particles can be engulfed by macrophage cells around blood vessels in normal tissue and then eliminated from human body [4]. Accordingly, the TiO_2 particles used in design system for photocatalysis, as a new anticancer modality, were also considered. However, the photocatalytic method needs complicated equipments. Meanwhile, it can only cure shallow tumor because of the short penetration distance of light. For deep tumor, it must need the aid of fiber to reach interior tumor tissue. Fortunately, the sonocatalytic method can overcome these disadvantages because of the strong ability to penetrate and low energy consumption, which is basically no harm to human body. Hence, adopting sonocatalytic method to restrain and destroy tumor cells, especially for treating the tumor cells of deep tissue has attracted more and more researchers' attention [5, 6].

However, due to strong photocatalytic activity and poor selectivity, TiO_2 particles can produce overmany $\cdot\text{OH}$ radicals and then kill normal cells. Otherwise, the isoelectric point of TiO_2 particles is about pH 6.0, and that of most biomolecules is close to pH 5.0. Hence, both of them are negatively charged when they are at physiologi-

cal acidity (pH 7.4). Since they are unapproachable under well-balanced condition, the biomolecules are hard to be destroyed. Semiconductor ZnO is also a good photocatalyst [7]. Moreover, its isoelectric point is about pH 9.3, so it is easy to interact with biomolecules due to ZnO particle with positive charge at pH 7.4. Thus, adding adequate ZnO to TiO_2 not only could raise the isoelectric point and increase the scope of interaction with biomolecules, but also reduce the side effects caused by only using TiO_2 [8]. That is, it might destroy some biomolecules selectively and effectively through cavitation oxidation.

Serum albumin is the most abundant protein in the blood plasma and acts as transportation and deposition of endogenous and exogenous substances in body [9]. Due to the physiological importance and easy separation and purification, bovine serum albumin (BSA) is often selected as a globular protein model [10]. For the reason given above, in this paper, composite TiO_2/ZnO powder is prepared and used as a sonocatalyst to damage BSA for the first time. Maybe it is a valuable reference to treat tumor on the base of further investigation.

EXPERIMENTAL

Materials Nano-sized anatase titanium dioxide (TiO_2) and zinc oxide (ZnO) powders were bought from Haerbin Chemistry Reagent Company (Haerbin, China) and Hangzhou Wanjiang Xincailiao Limited Company (Hangzhou, China), respectively. BSA was purchased from Aoboxing Biotechnological Company (Beijing,

¹ The article is published in the original.

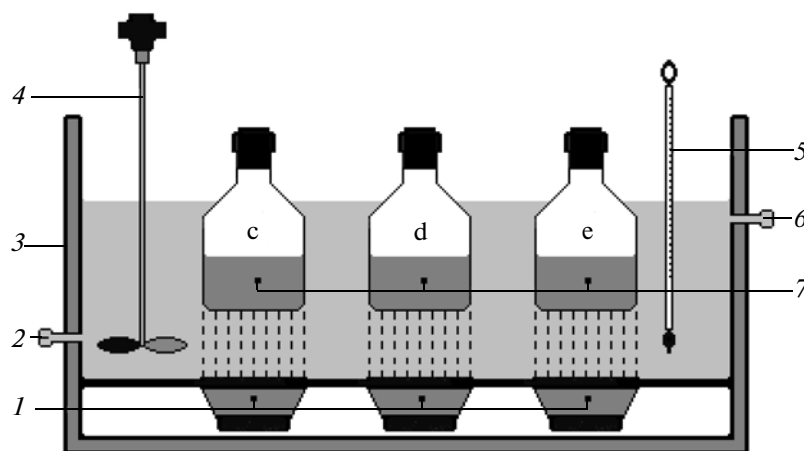


Fig. 1. Schematic illustration of experimental setup: 1—ultrasonic transducer, 2—intake, 3—flume, 4—stirrer, 5—thermometer, 6—outlet, 7—reactor; c—BSA + ultrasound + ZnO, d—BSA + ultrasound + ZnO/TiO₂, e—BSA + ultrasound + TiO₂.

China) and was used without further purification. All other reagents were of analytical grade. Doubly distilled water was used as a solvent throughout the experiment.

Apparatus. The UV-vis and fluorescence spectra were recorded on a UV-vis spectrometer (Cary-50, Varian Company, USA) and a fluorescence spectrometer (Cary-300, Varian Company, USA), respectively. The XRD pattern was determined by X-ray diffraction measurement (RINT 2500, XRD-Rigaku Corporation, Japan). The TEM observation was carried out using a transmission electron microscope (JEM-3010, JEOL, Japan). The Controllable Serial-Ultrasonics apparatus (KQ-100, Kunshan Ultrasound Apparatus Company, China) shown in Fig. 1 was adopted as irradiation source.

Preparation of composite TiO_2/ZnO powders. The preparation of composite TiO_2/ZnO powders was as follows: firstly, TiO_2 and ZnO powders were put into doubly distilled water with a series of molar proportion of TiO_2 and ZnO. Then they were ultrasonically dispersed in water and collected by centrifugation. After being dried using an oven, the mixtures were ground thoroughly in an agate mortar. Then the mixture with a TiO_2/ZnO molar proportion of 4:6 was divided into several parts in crucibles, followed by heat-treatment for different temperatures in the range of 200–600°C. At last, all the heat-treated powders were cooled to room temperature naturally for sonocatalytic measurements.

Experiment process. Five BSA solutions (1.0×10^{-5} mol/L) marked as a–e were prepared. Nano-sized TiO_2 , composite TiO_2/ZnO and nano-sized ZnO powders, whose addition amount were all 1.0 g/L, were placed into c, d and e, respectively. Afterwards, b, c, d and e were placed in an ultrasonic irradiation apparatus. After 3.0 h, samples (c, d and e) were centrifuged to remove the catalysts and their transparent liquids were analyzed. The UV-vis and fluorescence spectra of each sample solution

were determined, in order to evaluate the damage to BSA molecules. After this, the effects of molar proportion of TiO_2 and ZnO, heat-treated temperature and heat-treated time on sonocatalytic activity of composite TiO_2/ZnO powder were reviewed by the numbers. In addition, the effects of ultrasonic irradiation time and composite TiO_2/ZnO adding amount on sonocatalytic damage of BSA molecules were also studied.

RESULTS AND DISCUSSION

XRD and TEM of heat-treated composite TiO_2/ZnO powder. The X-ray diffraction patterns of TiO_2 , composite TiO_2/ZnO with molar proportion of 4:6 and ZnO powders were shown in Fig. 2. The pronounced characteristic diffraction peaks of anatase TiO_2 at $2\theta = 25.00$ and 48.00° and three sharp peaks from 30 to 40° of ZnO with very high intensity are found. Moreover, these diffraction peaks are also found in the XRD pattern of composite TiO_2/ZnO powder. It indicates that the composite powder really contains the two crystalline phases belonging to anatase TiO_2 and blende ZnO.

Figure 3 gives the TEM images of the three samples mentioned above. The individual TiO_2 and ZnO particles have a size range of 25–30 and 30–45 nm, respectively. Whereas, the particle size of the composite TiO_2/ZnO powder is about 35–55 nm and becomes larger than those of pure TiO_2 and ZnO particles after heat-treatment. The reason for this result is that TiO_2 and ZnO powders have different isoelectric points which are 6.0 for TiO_2 and 9.3 for ZnO. When they are in distilled water (about pH 7.2), TiO_2 is negatively charged while ZnO is positively charged. They probably combine with each other. Thereby, the particle size is larger than either of them.

UV-vis and Fluorescence spectra of BSA solution under ultrasonic irradiation in the presence of composite

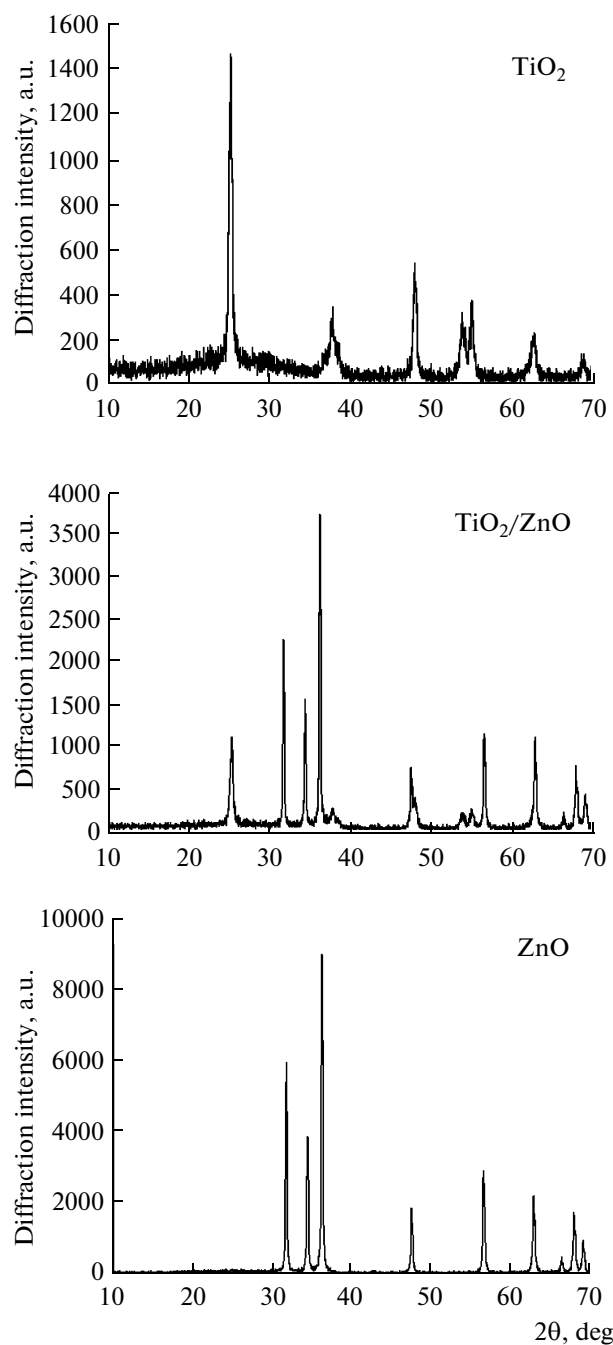


Fig. 2. XRD of TiO_2 , composite TiO_2/ZnO and ZnO powders after heat-treatment.

TiO_2/ZnO powder. It can be seen in Fig. 4a that the only ultrasonic irradiation (curve 2) has very little effect on the UV-vis spectra of BSA solution compared with the original BSA solution (curve 1). However, the UV-vis spectra of BSA solutions in the presence of catalysts (curve 3–5) show obvious hyperchromic effects under ultrasonic irradiation. Moreover, the sequence of sonocatalytic activity is $\text{ZnO} < \text{composite } \text{TiO}_2/\text{ZnO} < \text{TiO}_2$. It indicates one-

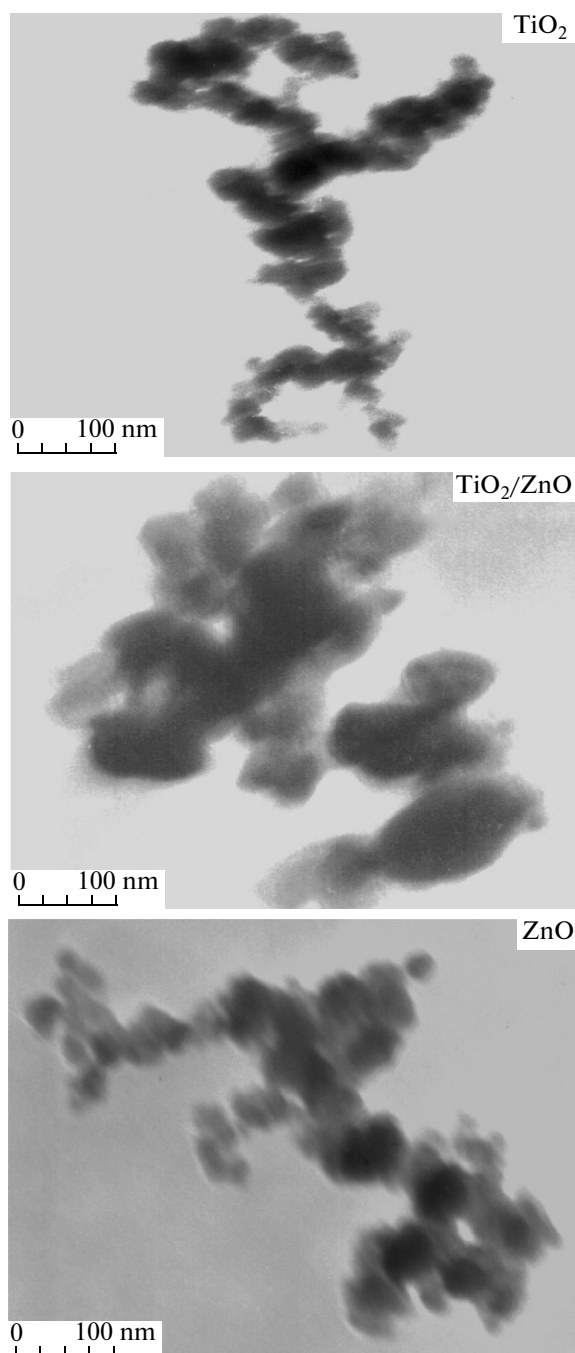


Fig. 3. TEM of TiO_2 , composite TiO_2/ZnO and ZnO powders after heat-treatment.

fold ultrasonic irradiation can damage the BSA molecules slightly, but ZnO , composite TiO_2/ZnO and TiO_2 can aggravate the damage of BSA molecules. The reason may be explained as follows: firstly, the associated actions of ultrasonic irradiation and various catalysts (ZnO , composite TiO_2/ZnO and TiO_2) produce $\cdot\text{OH}$ radicals. They attack the bisulfur bonds between two peptide chains and hydrogen bonds between amino acid residual groups,

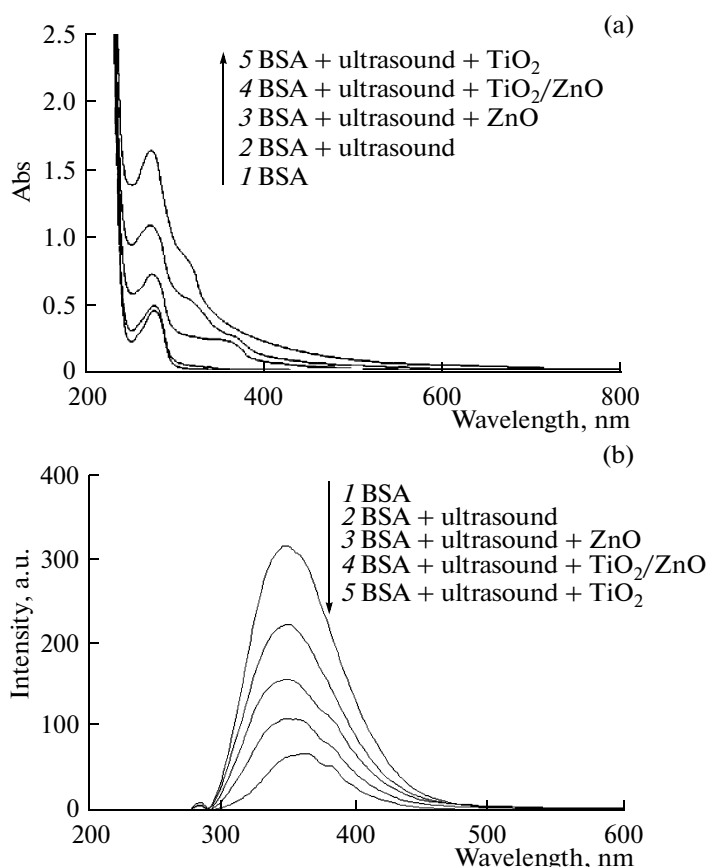


Fig. 4. UV-vis (a) and Fluorescence (b) spectra of BSA solution (1.0×10^{-5} mol/L) under different conditions after 3.0 h.

resulting in the extension of peptide chain and the exposure of tryptophan (Trp) and tyrosine (Tyr) residues. Thereby the absorbencies of BSA solution enhance obviously. Secondly, $\cdot\text{OH}$ powder produces much more $\cdot\text{OH}$ radicals than composite TiO_2/ZnO or ZnO powder. So the damage of BSA in the presence of TiO_2 is more serious.

The corresponding results can be further validated via fluorescence spectra shown in Fig. 4b. The sequence of fluorescence intensity is the original BSA solution (curve 1), onefold ultrasound (curve 2), ultrasound + ZnO (curve 3), ultrasound + TiO_2/ZnO (curve 4) and ultrasound + TiO_2 (curve 5). It may be attributed to the fact that all the combinations of ultrasound and catalysts destroy the Trp and Tyr residues which mainly contribute to fluorescence spectra of BSA molecules, so that the fluorescence intensity reduces. The catalytic activity of TiO_2 is too high. Perhaps, the composite TiO_2/ZnO is more suitable to be adopted for the damage of biomolecules in intending sonocatalytic treatment of tumors.

The effect of molar proportion of TiO_2 and ZnO on sonocatalytic activity of composite TiO_2/ZnO powder. In

order to evaluate the effect of molar proportion on the catalytic activity of composite TiO_2/ZnO powder, the molar proportion of TiO_2 and ZnO was varied from 10:0 to 0:10.

From Fig. 5a it is observed that the sonocatalytic activity for the damage of BSA decreases with the increase of ZnO amount in the composite TiO_2/ZnO powders. When the molar proportion of TiO_2 and ZnO is set at 4:6, the high catalytic activity appears. The reason for this phenomenon is $\cdot\text{OH}$ radicals produced by TiO_2 is more than those produced by ZnO , owing to the high catalytic activity of TiO_2 . So the addition of ZnO leads to the decline of catalytic activity of TiO_2 . When the molar proportion of TiO_2 and ZnO is appropriate (e.g. 4:6), selectivity of composite TiO_2/ZnO is promoted, so the absorbency of BSA enhances. The same results can also be verified by fluorescence spectra in Fig. 5b. Therefore, the molar proportion of 4:6 was adopted in the following experiments.

The effect of heat-treated temperature on sonocatalytic activity of composite TiO_2/ZnO powder. In order to investigate the effect of heat-treated temperature on the catalytic activity of composite TiO_2/ZnO powder, the heat-treated temperature ranges from 200 to 600°C.

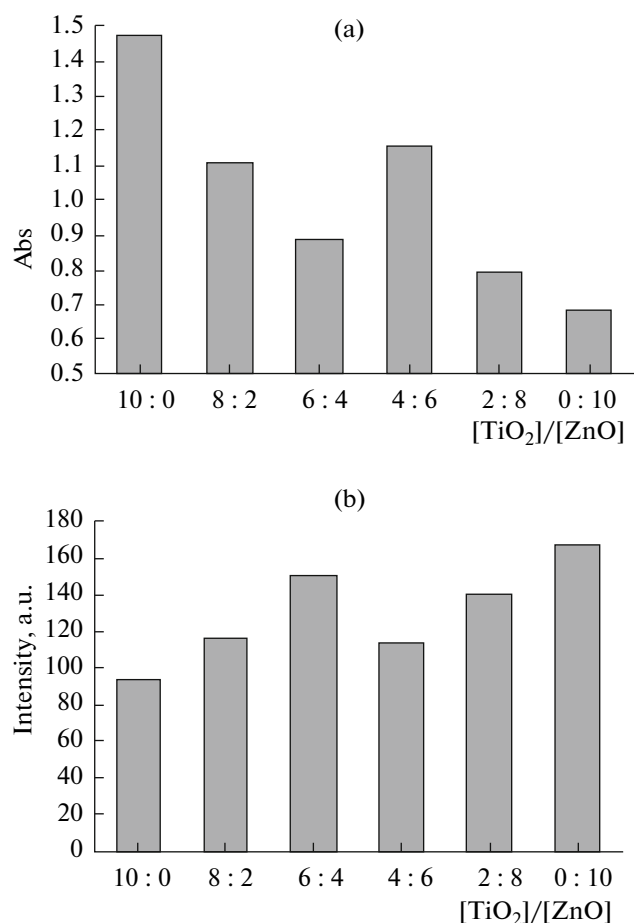


Fig. 5. Changes of absorbency (a) and intensity (b) of BSA solution (1.0×10^{-5} mol/L) with molar proportion of composite TiO_2/ZnO powder.

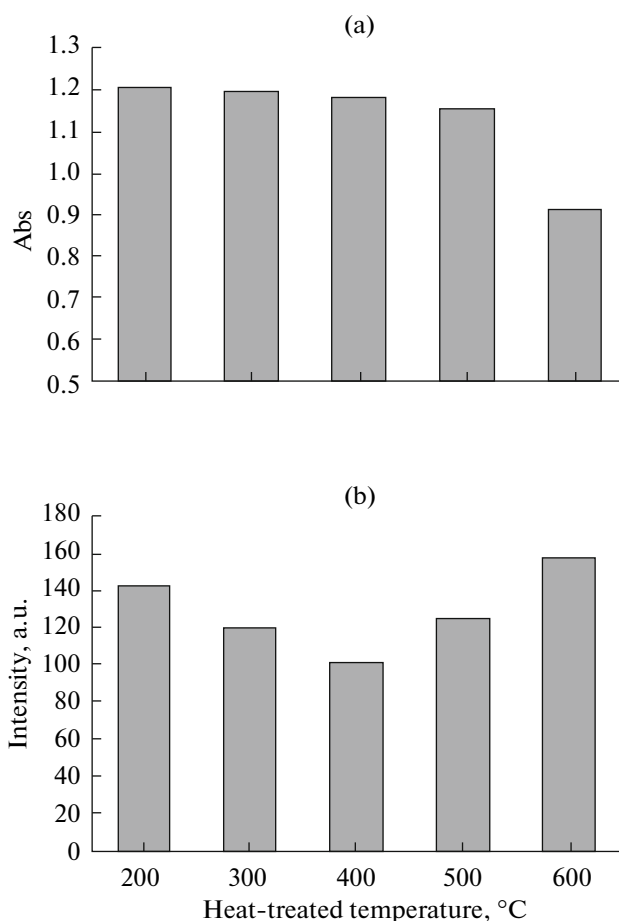


Fig. 6. Changes of absorbency (a) and intensity (b) of BSA solution (1.0×10^{-5} mol/L) with heat-treated temperature of composite TiO_2/ZnO powder.

As shown in Fig. 6a, the absorbency of BSA solution decreases slightly before 500°C , and then fleetly decreases. It indicates that the sonocatalytic activity of composite TiO_2/ZnO powder hardly changes in the range of low heat-treated temperatures. Only when the heat-treated temperature exceeds 500°C , the sonocatalytic activity begins to decrease obviously. Nevertheless, Fig. 6b gives the lowest fluorescence intensity at heat-treated temperature of 400°C . It means that the composite TiO_2/ZnO powder heat-treated at 400°C is more conducive to the sonocatalytic damage of BSA molecules.

The effect of heat-treated time on sonocatalytic activity of composite TiO_2/ZnO powder. In order to determine the effect of heat-treated time on the catalytic activity of composite TiO_2/ZnO powder, the heat-treated time was changed from 20 to 60 min.

From Fig. 7a it can be seen that the absorbency of BSA solution increases gradually before 40 min, and then it begins to decrease obviously. It indicates that the highest catalytic activity of composite TiO_2/ZnO powder appears

at heat-treated time of 40 min. Correspondingly, the fluorescence spectra give the lowest fluorescence intensity in Fig. 7b at the same heat-treated time. So the composite TiO_2/ZnO powder heat-treated for 40 min was adopted in our experiments.

The effect of ultrasonic irradiation time on damage of BSA. The changes of UV-vis and fluorescence spectra of BSA solutions were reviewed by varying ultrasonic irradiation time within 5.0 h at intervals of 1.0 h.

From Fig. 8a it can be seen that the absorbency of BSA solution under onefold ultrasonic irradiation increases only slightly. Comparatively, the absorbencies of BSA solutions in the presence of catalysts all increase gradually with the increase of ultrasonic irradiation time. Furthermore, the composite TiO_2/ZnO powder shows higher sonocatalytic activity than that of pure nano-sized ZnO powder but lower than that of pure nano-sized TiO_2 powder. It is because that the three catalysts all produce more and more $\cdot\text{OH}$ radicals along with the increase of ultrasonic irradiation time, especially TiO_2 . The correspond-

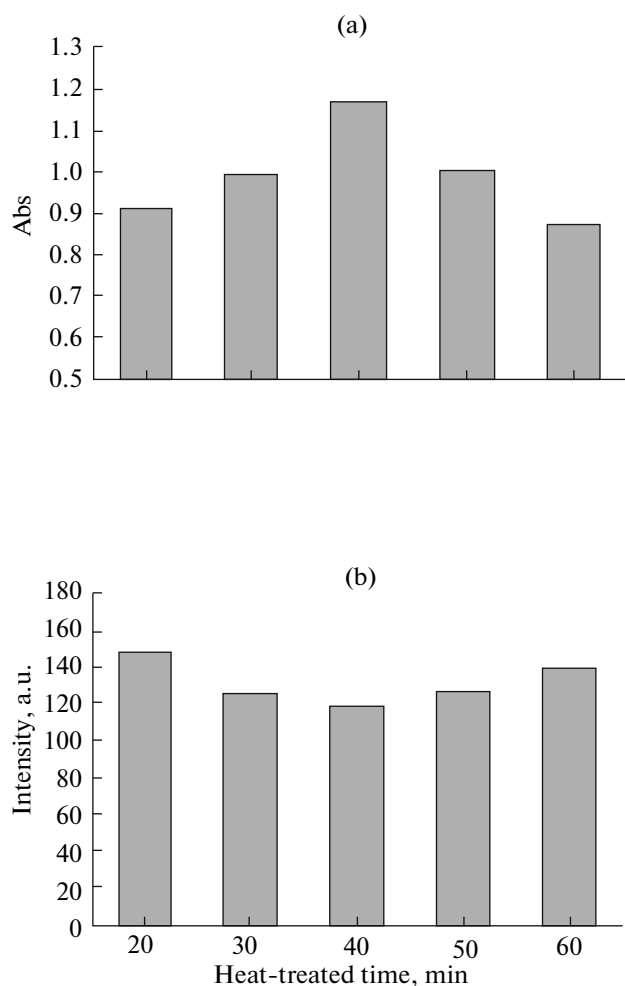


Fig. 7. Changes of absorbency (a) and intensity (b) of BSA solution (1.0×10^{-5} mol/L) with heat-treated time of composite TiO_2/ZnO powder.

ing-results can be further proved via fluorescence spectra as depicted in Fig. 8b. With increase in ultrasonic irradiation time, the typical fluorescence quenching is observed. And the quenching sequence is $\text{ZnO} < \text{composite } \text{TiO}_2/\text{ZnO} < \text{TiO}_2$. These results suggest that the microenvironment around chromophore of BSA molecules is changed after the addition of the three catalysts, respectively. The Trp and Tyr residues are destroyed, so the fluorescence intensities of BSA reduce.

The effect of addition amount of composite TiO_2/ZnO powder on damage of BSA. Figure 9 shows the effects of different composite TiO_2/ZnO addition amounts (0.0–2.5 g/L) on the sonocatalytic damage of BSA molecules.

It can be seen in Fig. 9a that the hyperchromic effects of BSA solutions become more and more obvious with the increase of catalyst addition amount. But the catalytic activity of TiO_2 is the highest. And those of composite TiO_2/ZnO and ZnO are the second highest and lowest,

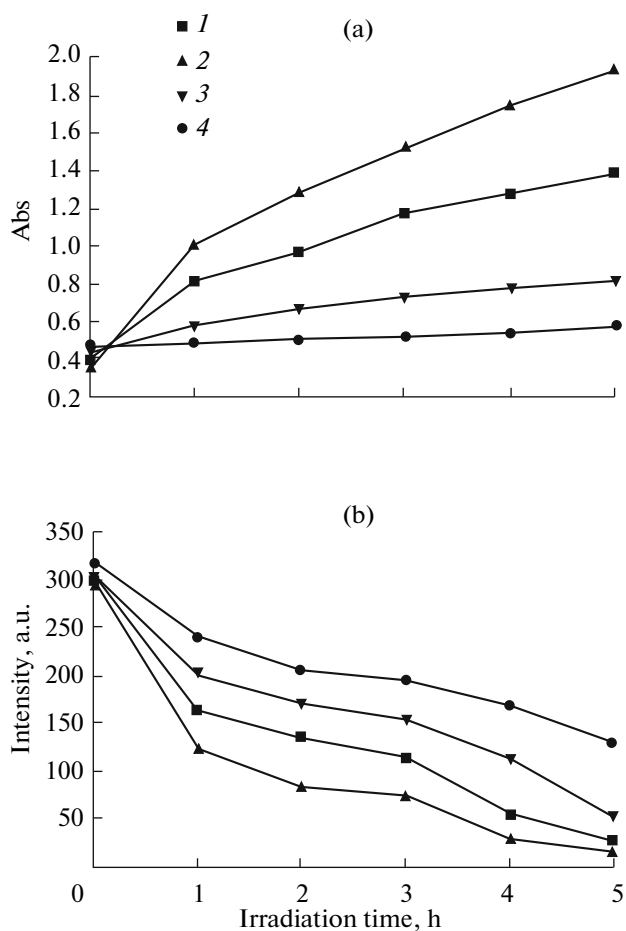


Fig. 8. Changes of absorbency (a) and intensity (b) of BSA solution (1.0×10^{-5} mol/L) with ultrasonic irradiation time: 1—BSA solution + ultrasound + TiO_2/ZnO , 2—BSA solution + ultrasound + TiO_2 , 3—BSA solution + ultrasound + ZnO , 4—BSA solution + ultrasound.

respectively. It is thought to be owing to the increase of the number of the $\cdot\text{OH}$ radicals produced by these catalysts. By comparison, no matter which kind of catalysts, the absorbency of BSA has slight hypochromic effect without ultrasonic irradiation. Perhaps, it attributes to the decrease of BSA molecules which are caused by the increase of catalyst particles. On the other hand, the redundant catalyst particles in BSA solutions mutually defilade one another, resulting in weakening their own sonocatalytic abilities.

The corresponding phenomena can be further validated by means of fluorescence spectra. As depicted in Fig. 9b, with the increase of the amount of catalyst, the fluorescence intensities of BSA solutions are reduced under ultrasonic irradiation for the three cases, but those without ultrasonic irradiation are almost constant. Therefore, it is concluded under ultrasonic irradiation these catalysts can actively damage BSA.

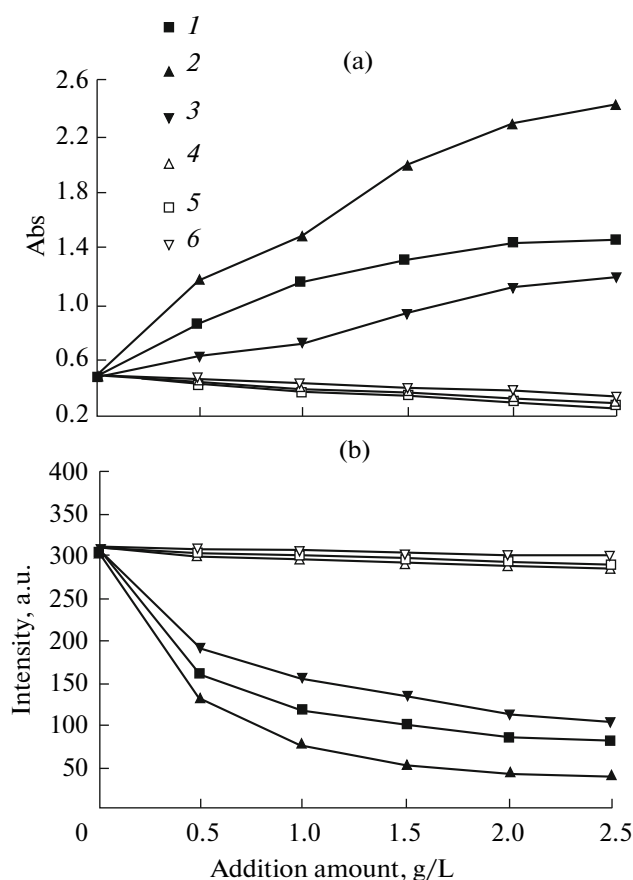


Fig. 9. Changes of absorbency (a) and intensity (b) of BSA solution (1.0×10^{-5} mol/L) with addition amount of composite TiO_2/ZnO powder: 1 — BSA solution + ultrasound + TiO_2/ZnO , 2 — BSA solution + ultrasound + TiO_2 , 3 — BSA solution + ultrasound + ZnO , 4 — BSA solution + TiO_2/ZnO , 5 — BSA solution + TiO_2 , 6 — BSA solution + ZnO .

CONCLUSIONS

In this paper, the composite TiO_2/ZnO powder was prepared by mixing nano-sized TiO_2 and ZnO powders directly. The damage of BSA molecules under ultrasonic irradiation in the presence of composite TiO_2/ZnO powder was detected by means of UV-vis and fluorescence

spectra. The results showed the composite TiO_2/ZnO powder with the molar proportion of 4:6 heat-treated at 500°C for 40 min displayed comparatively high sonocatalytic activity. Furthermore, the damage degree of BSA molecules was aggravated with the increase of ultrasonic irradiation time and composite TiO_2/ZnO addition amount. Hence, it can be inferred from the experimental results that the combination action of ultrasonic irradiation and composite TiO_2/ZnO powder can easily damage the BSA molecules.

REFERENCES

1. Linsebigler, A.L., Lu, G.Q., and Yates, J.T., Photocatalysis on TiO_2 Surfaces: Principles, Mechanisms, and Selected Results, *Chem. Rev.*, 1995, vol. 95, no. 3, pp. 735–758.
2. Dimitrijevic, N.M., Saponjic, Z.V., Rabatic, B.M., and Rajh, T., Assembly and Charge Transfer in Hybrid TiO_2 Architectures Using Biotin-Avidin as a Connector, *J. Am. Chem. Soc.*, 2005, vol. 127, no. 5, pp. 1344–1345.
3. Cai, R.X., Kubota, Y.N., Shuin, T.R., et al., Induction of Cytotoxicity by Photoexcited TiO_2 Particles, *Cancer Res.*, 1992, vol. 52, pp. 2346–2348.
4. Kubota, Y., Shuin, T., Kawasaki, C., et al., Photokilling of T-24 Human Bladder Cancer Cells with Titanium Dioxide, *Br. J. Cancer.*, 1994, vol. 70, pp. 1107–1111.
5. Chen, L., Rivens, I., Haar, G.T., et al., Histological Changes in Rat Liver Tumors Treated with High-Intensity Focused Ultrasound, *Ultrasound Med. Biol.*, 1993, vol. 19, no. 1, pp. 67–74.
6. Umemura, S., Kawabata, K., Sasaki, K., et al., Recent Advances in Sonodynamic Approach to Cancer Therapy, *Ultrason. Sonochem.*, 1996, vol. 3, no. 3, pp. S187–S191.
7. Hariharan, C., Photocatalytic Degradation of Organic Contaminants in Water by ZnO Nanoparticles: Revisited, *Appl. Catal., Ser. A*, 2006, vol. 304, pp. 55–61.
8. Richard, C., Boule, P., and Aubry, J.M., Oxidizing Species Involved in Photocatalytic Transformations on Zinc Oxide, *J. Photochem. Photobiol., Ser. A*, 1991, vol. 60, no. 2, pp. 235–243.
9. Carter, D.C. and Ho, J.X., Structure of Serum Albumin, *Adv. Protein Chem.*, 1994, vol. 45, pp. 153–203.
10. Wang, J., Wu, J., Zhang, Z.H., et al., Sonocatalytic Damage of Bovine Serum Albumin (BSA) in the Presence of Nanometer Anatase Titanium Dioxide (TiO_2), *Ultrasound Med. Biol.*, 2006, vol. 32, no. 1, pp. 147–152.