OPTOELECTRONICS AND ADVANCED MATERIALS – RAPID COMMUNICATIONS Vol. 4, No. 12, December 2010, p. 2059 – 2063

Nonhydrolytic sol-gel synthesis and antibacterial properties of nanosized TiO_2

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The purpose of this study is to synthesize TiO_2 by a nonhydrolytic sol-gel route and to examine its antibacterial properties. The synthesis of titania was performed by the reaction between titanium tetrachloride and benzyl alcohol at moderate temperature, followed by calcinations at 500 ^oC. The structure and morphology of the resulting particles were characterized by XRD and SEM. The average particles size of synthesized TiO_2 (anatase) was 10-20 nm. The antimicrobial action of the as-prepared TiO_2 was investigated using *Escherichia coli* as test microorganism. It was concluded that the obtained by nonhydrolytic sol-gel method nanosized TiO_2 (anatase) could be successfully used for disinfection of water on illumination with UV light.

(Received October 27, 2010; accepted November 29, 2010)

Keywords: Anatase TiO_2, Sol-gel, Nanoparticles

1. Introduction

The titanium dioxide (TiO_2) is one of the most studied metal oxides during the last 20 years due to its technological and environmental importance. It is known as non-toxic, insoluble in water and stable under UV-radiation compound. TiO_2 has been successfully applied in optics, gas sensing, painting, implants, catalysis, photocatalysis, disinfection, etc [1, 2]. Among the various applications of this oxide, its antimicrobial properties are one of the most attractive and extensively studied topics [3]. In this connection, it is even reported that UV illumination of TiO_2 could slow or halt the development of tumor cells [4].

Due to the broad area of application and the importance of nanosized titania a large number of preparative methods for its synthesis have been reported. Among the most popular synthetic methods are high-temperature processes [5] and liquid-phase (wet chemistry) routes [6].

The use of aqueous sol-gel technology bears the advantages to achieve high purity and superior compositional homogeneity of the products at moderate temperatures with simple laboratory equipment [6, 7]. However, in most cases the reaction rates in aqueous sol-gel chemistry are very fast, especially with transition metal precursors, making it uneasy to control the processes. This can be a major limitation of aqueous sol-gel processes where slight changes in experimental conditions may result in drastic changes in the structure and morphology of the materials [7, 8, 9].

A simple way to circumvent this problem is applying of so called nonaqueous or nonhydrolytic procedures - syntheses performed in organic solvents under exclusion of water [7, 8, 10, 11-16]. The slower reaction rate of nonhydrolytic processes allow for better control over particle size and crystallinity [7]. The organic components in the reaction system not only act as oxygen supplying agents for the metal oxide but also strongly influence particle size, shape and surface properties [7, 8]. Nonhydrolytic sol-gel methods developed for the synthesis of TiO_2 generally involve the reaction of titanium tetrachloride (or alkoxide) [7, 12-14] with either metal alkoxide [15, 16] or an organic oxygen donor such as alcohol [6, 17, 18], ether [15], aldehyde or ketone [13] under exclusion of water. It is known that the photocatalytic activity of TiO_2 strongly depends on the particles size and the type of the precursors. For this reason, many researchers have extensively investigated the relation between the synthesis conditions and the properties of nanosized TiO_2 powders [19]. The anatase nanocrystalline form is of particular interest because it has the highest reactivity in photocatalysis and the best antimicrobial activity [20, 21]. Although a wide variety of approaches for the synthesis of titania have been reported it still remains a particularly active research field.

First Matsunaga et al. [22] reported the photochemical disinfection of some bacteria with platinum doped TiO_2, and since then the photocatalytic bactericidal action of titanium dioxide has obtained increasing scientific attention. Many studies on the antimicrobial effect of UV-illuminated TiO_2 over a wide range of organisms including bacteria [17, 22-39], viruses [29, 40], fungi [41] and cancer cells [18, 42, 43] have been reported. Most of these studies used the conventional powder photocatalyst, commonly the Degussa P25. Unlike them, Jian Zhu et al. (2007) synthesized nanocrystalline anatase by nonhydrolytic sol-gel reaction and studied its

photocatalytic activity toward degradation of phenol [44]. To our knowledge, the bactericidal properties of anatase synthesized by nonhydrolytic route still have not been tested.

This motivated our study to synthesize anatase TiO_2 by a nonhydrolytic sol-gel route and to examine its antibacterial properties. In the present work we report a nonhydrolytic synthesis of pure anatase nanoparticles by the reaction between titanium tetrachloride and benzyl alcohol. The bactericidal effect of thus obtained titania was examined on *Escherichia coli* bacteria at dark conditions and in the presence of UV radiation.

2. Experimental

Materials

Titanium(IV) chloride (purity ≥99.0%) was purchased from Fluka and benzyl alcohol (≥99.5%), absolute ethanol and diethyl ether were supplied by Merck. All the chemicals were used without further purification.

Preparation of titanium dioxide

The synthesis of titanium oxide nanoparticles was carried out following the procedure described by Niederberger et al. [4] which is based on the nonhydrolytic sol-gel reaction of benzyl alcohol and titanium tetrachloride. Benzyl alchohol has a low toxicity and is approved for use in food and cosmetics [21].

The reaction between titanium chloride and benzyl alcohol was performed in a regular glass beaker at a controlled heating. In a typical preparation 4 ml $TiCl_4$ was slowly added to a beaker containing 80 ml benzyl alcohol under vigorous stirring at room temperature. In the mixing, special attention was taken because of the violent reaction. Initially, the reaction mixture was colored in dark red and contained some white fluffy precipitates that are completely dissolved on mixing. The container was covered with a Petri dish and the reaction mixture was stirred continuously. Following this procedure two samples were obtained: *Sample 1*: The reaction mixture was kept at 60^{o}C under continuous stirring for 30 min. After that heating was stopped, the mixture was stirred for 8 hours and left for aging at room temperature. A white thick suspension appeared in 20 days; *Sample 2*: The reaction mixture was kept at 100 ^{o}C under continuous stirring for 8 hours and then left for aging at room temperature for two weeks. The resulting white thick suspensions were centrifuged at 3500 rpm for 15 min and the supernatant was discarded by decantation. The white precipitate was then washed two times with absolute ethanol and three times with diethyl ether (1×20 ml). After every washing step, the solvent was separated by centrifugation. The collected materials were dried in air overnight and then ground into a fine powder. The obtained powders were calcinated at 500^{o}C for 4 hours.

The samples obtained were characterized by X-ray diffraction (XRD-Bruker D8 Advance, Cu Ka radiation). The microheterogeneity, and the size of the crystals were determined by Scanning Electron Microscopy (JEOL Superprobe 733). The photocatalytic activities of the synthesized powders were evaluated by degradation of a model aqueous solution of Malachite Green (MG) after subjecting it to UV-radiation. The characteristic absorption of MG at 618 nm can be chosen to monitor the photocatalytic degradation process. The decomposition degree was determined by a Jenway 400 spectrophotometer.

The antimicrobial action of the prepared TiO_2 *(Sample 2)* was investigated using *Escherichia coli* as test microorganism. The bacteria growth was examined by the effect of UV light alone, in the presence of TiO_2 at dark conditions and in the presence of both – TiO_2 and UV radiation. The experiments were done in suspension containing initial cell concentration of 185000 colony forming units (CFU)ml^{-1} and TiO_2 concentration of 1 gL^{-1}. The experiments were continued up to 180 min at temperature of 25^{o}C.

Bactericidal activity measurements

Bacterial strain

Escherichia coli was selected as a representative microorganism of waterborne pathogens**.** *E. coli* ATCC 25922 was used in all experiments performed because this strain is susceptible to antimicrobials and serves as a control in susceptibility testing of enterobacteria according to the Clinical and laboratory Standards Institute (CLSI) guidelines.

Bacterial culture

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°C for 18 hours. The broth culture was centrifuged at 1000 x g for 10 min and then washed twice with 50 ml of sterile phosphate buffered saline (PBS) – pH 7.2. The final pellets were resuspended with PBS and the turbidity was adjusted to be optically comparable to that of the 0.5 McFarland corresponding to the density of 1.5 x 10^8 cells per ml. This solution was diluted 1000 times to obtain stock solution with cell density of approximately 10^5 cells per ml.

Experimental setup

For all the experiments sterile glass flasks were used. Four flasks were inoculated each with 100 ml of the stock solution of *E. coli* ATCC 25922. First flask served as a control of bacteria growth – no TiO_2 or radiation is applied. It is kept in the dark. To the second flask 100 mg of TiO_2 powder were added, also kept in the dark. The mixture in the third flask was contacted with UV radiation only, and to the fourth flask both 100 mg of TiO_2 were added and UV radiation is applied. The experimental setup is shown in Fig.1. For the illumination a UV lamp with an emission maximum at about 365 nm was used, since it matches well the band gap of anatase [23-25]. It was

situated sidewise at a distance 5 cm to the reaction vessels. TiO_2 was added to cells immediately prior to the reaction and its final concentration in the samples was 1 mg ml^{-1}. Aluminum foil was used as a reflective material in order to protect the samples studied in the absence of irradiation. All experiments were conducted by continuous stirring with magnetic stirrers to ensure best mixing and to prevent settling of the TiO_2 particles. The inoculated flasks were incubated at 25°C for 3 hours.

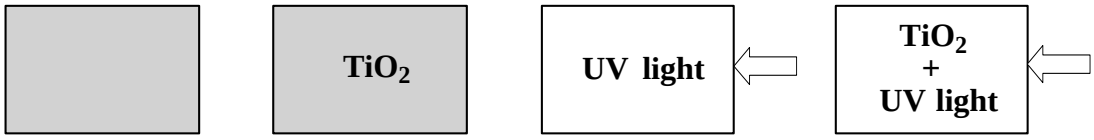

Fig. 1. Schematic diagram of the experimental setup.

Time-kill experiments

Dynamics of antimicrobial action of the selected preparations was assessed by killing curves determination. 0.1 ml of the undiluted suspension, and 10^{-1} and 10^{-2} dilutions were inoculated on Mueller-Hinton agar, Becton Dickinson Microbiology Systems (Cockeysville, Md.), 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^{th} min, 15^{th}min, 30^{th} min, 45^{th} min, 60^{th} min, 90^{th} min, 120^{th}, and 180^{th} min during the incubation period. The inoculated Petri dishes were incubated at 37° C for 24 h. The number of colony forming units (CFU) per 1 ml was determined according to the formula:

CFU/ml = the number of colonies × 10 × reciprocal value of the dilution.

All time kill experiments were performed in duplicate. Time-kill curves were constructed by plotting mean colony counts (CFU/ml) versus time.

2. Results and discussion

Characterization of titanium dioxide

Fig. 2 presents the XRD patterns of commercial (a) and synthesized TiO_2 (b). The three strongest interplanar distances of anatase (TiO_2) appear at 3.51; 1.89 and 1.66 (JCPDS 78-2486). The XRD analysis was made in order to check and compare the particle size in all samples. The average crystalline size of as obtained TiO_2 calculated from the broadening of the diffraction line using Sherrer's equation is from 4 to 30 nm. The Rietveld analysis of sample 1 was performed by PowderCell program and it proved the X-ray diffraction results (Fig . 3).

Fig. 4 (a, b) shows the SEM images of sample 1, while 4 (c, d) present the SEM images of sample 2. Comparing both samples it can be seen that the grain size of sample 1 is a little smaller than that of sample 2 (c and d). XRD results have already confirmed this fact. Obviously, sample 2 shows aggregation of grains.

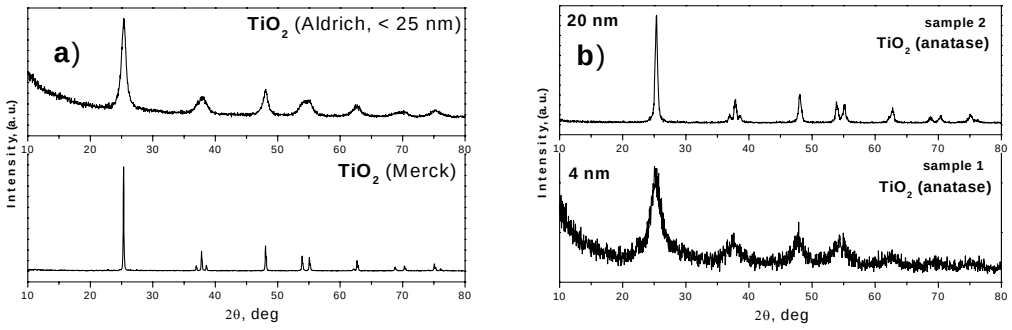

Fig. 2. XRD patterns of pure TiO_2 – anatase (Merck and Aldrich) and samples 1 and 2.

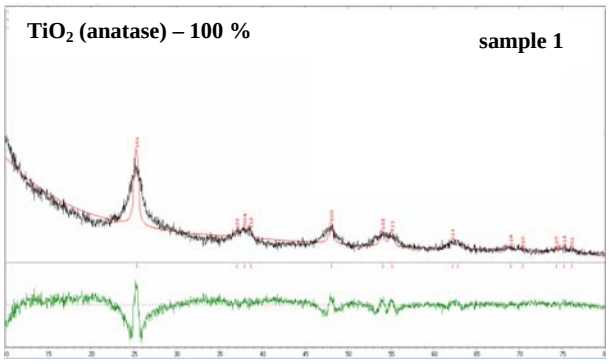

Fig. 3. Rietvield refinement of sample 1 performed by PowderCell program.

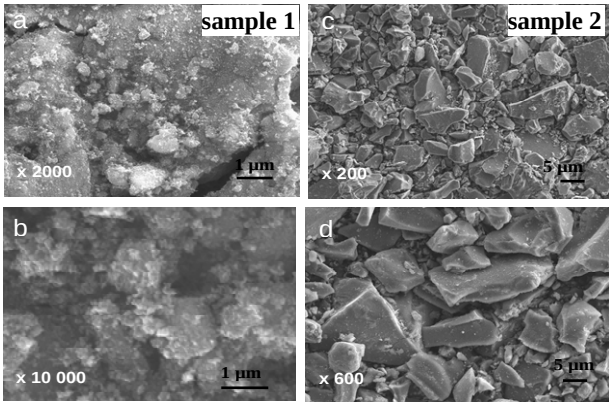

Fig. 4. SEM micrographs of samples 1 (a, b) and 2 (c, d) at different magnifications.

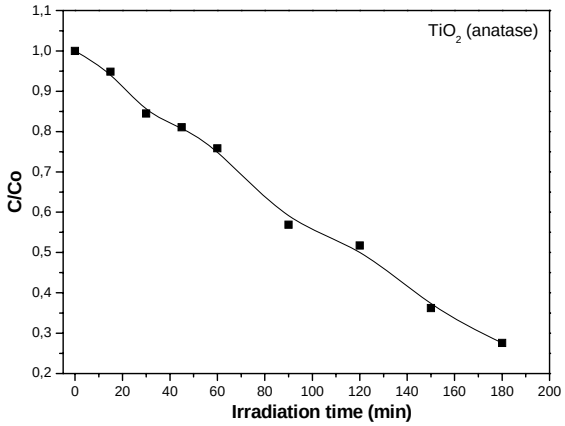

Fig. 5. Photocatalytic activities of sample 2.

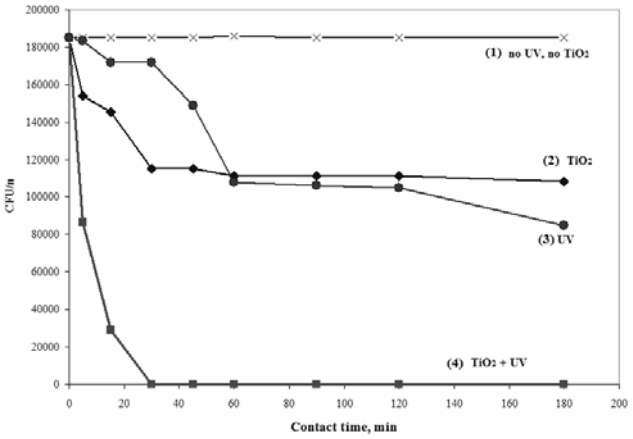

Fig. 6. Time-kill curves of E. coli in sample 2 at different exposures: (1) no TiO_2 and no UV radiation; (2) in the presence of TiO_2 at dark conditions; (3) UV light alone; (4) in the presence of TiO_2 and UV radiation.

The Fig. 5 presents the UV–Vis absorption spectra of an aqueous solution of MG (initial concentration: 5 ppm 150 mL) by TiO_2. It can be seen that the photodegradation of MG was completed in 180 min.

Bactericidal action of titanium dioxide

It was found that the antibacterial activities of TiO_2 only and under UV light alone were roughly the same – the number of *E. coli* cells was reduced approximately 50% for 60 min (Fig. 6). Ultraviolet radiation deleterious effects on bacterial cell have been long recognized. The decrease in the amount of *E. coli* bacteria in the presence of TiO_2 without radiation may be explained with the absorption of some microorganisms by the TiO_2 particles. The combination of TiO_2 and UV radiation led to the complete killing of bacteria in 30 min (Fig. 6). Obviously, the joint action of UV irradiation and TiO_2 was markedly increased in comparison to UV irradiation or TiO_2 alone.

3. Conclusions

Nanosized TiO_2 has been obtained by nonhydrolitic sol-gel synthesis. The obtained TiO_2 showed good antimicrobial properties, nevertheless its low photocatalytic activity. The near-UV illumination of TiO_2 was very effective in removing *E. coli* bacteria. The action of as obtained TiO_2 is better than those obtained by other methods. The removal efficiency due to the joint action of UV irradiation and TiO_2 was markedly increased in comparison to UV irradiation or TiO_2 alone. The synthesized TiO_2 (anatase) by nonhydrolitic method could be successfully used for disinfection on illumination with UV light.

Acknowledgements

The study was performed with financial support of The Ministry of Education and Science of Bulgaria, The

National Science Fund of Bulgaria, Contract No TK-X-1702/07. Thanks are also due to the Financial Support of National Centre for New Materials UNION, Contract No DO-02-82/2008.

References

[1] U. Diebold, Surface Science Reports, **48**, 53-229 (2003).
[2] P. A. Christensen, T.P. Curtis, T.A. Egerton, S.A.M. Kosa, J.R. Tinlin, Applied Catalysis B: Environmental **41**, 371–386 (2003).
[3] C. Srinivasan and N. Somasundaram, Current Science, **85** (10), 1431 (2003).
[4] R. Cai, Y. Kubota, T. Shuin, H. Sakai, K. Hashimoto, A. Fujishima, Cancer Research, **52**, 2346 (1992).
[5] P. W. Morrison, Jr., R. Raghavan, A. J. Timpone, C. P. Artelt, S. E. Pratsinis, Chem. Mater., **9**, 2702 (1997).
[6] M. Niederberger, M. H. Bartl, G. D. Stucky, Chem. Mater., **14** (10), 4364–4370 (2002).
[7] M. Niederberger, G. Garnweitner, Chemistry - A European Journal, **12** (28), 7282-7302 (2006).
[8] M. Niederberger, Accounts of Chemical Research, **40**(9), 793 (2007).
[9] P. H. Mutin and A. Vioux, Chem. Mater., **21(**4), 582–596 (2009).
[10] A. Vioux, Chem. Mater., **9**, 2292-2299 (1997).
[11] I. Djerdj, D. Arcon, Z. Jaglicic, M. Niederberger, Journal of Solid State Chemistry, **181**, 1571 (2008).
[12] G. Garnweitner and M. Niederberger, J. Am. Ceram. Soc., **89** (6), 1801 (2006).
[13] G. Garnweitner, M.Antonietti, M.Niederberger, Chemical Communications, **3**, 397-399 (2005)
[14] T. Kotsokechagia, F. Cellesi, A. Thomas, M. Niederberger, N. Tirelli, Langmuir, **24** (13), 6988 (2008).
[15] P. Arnal, R. J. P. Corriu, D. Leclercq, P. H. Mutin, A. Vioux, Chem. Mater., **9** (3), 694 (1997).
[16] T. J. Trentler, T. E Denler, J. F. Bertone, A. Agrawal, V. L. Colvin, Journal of the American Chemical Society, , **121** (7), 1613 (1999).
[17] M. Niederberger, M. H. Bartl, G. D. Stucky, J. Am. Chem. Soc., **124**, 13642 (2002).
[18] C. Wang, Z.-X. Deng, G. Zhang, S. Fan, Y. Li, Powder Technology, **125** (1), 39 (2002).
[19] M. Ivanda, S. Music, S. Popovic, M. Gotic, Journal of Molecular Structure, **480-481**, 645 (1999).
[20] M. Machida, K, Norimoto, T. Kimura, Journal of the American Ceramic Society, **88** (1), 95 (2005).
[21] B. Nair, International Journal of Toxicology, **20** (suppl 3) 23 (2001).
[22] T. Matsunaga, R. Tomoda, T. Nakajima, H. Wake, Microbiology Letters, **29** (1-2), 211 (1985).
[23] Y. S. Chai, J. C. Lee, B. W. Kim, Korean J Chem Eng., **17** (6), 633 (2000).
[24] A. G. Rincón, C. Pulgarin, N. Adler, P. Peringer, Journal of Photochemistry and Photobiology A: Chemistry, **139**, 233 (2001).
[25] M. Cho, H. Chung, W. Choi, J. Yoon, Water Res., **38** (4), 1069 (2004).
[26] T. Matsunaga, R. Tomoda, T. Nakajima, N. Nakamura, T. Komine, Applied and Environmental Microbiology, **54** (6), 1330 (1988).
[27] J. C. Ireland, P. Klostermann, E. W. Rice, R. M. Clark, Applied and Environmental Microbiology, **59** (5), 1668 (1993).
[28] C. Wei, W. Y. Lin, Z. Zainal, N. E. Williams, K. Zhu, A. P. Kruzic, R. L. Smith, K. Rajeshwar, Environ. Sci. Technol., **28** (5), 934 (1994).
[29] R. J. Watts, L. S. Kong, M. P. Orr, G. C. Miller, B. E. Henry, Water Res., **29** (1), 95-100 (1995)
[30] M. Bekbolet, C. V. Araz, Chemosphere, **32**(5), 959 (1996).
[31] P.-C. Maness, S. Smolinski, D. M. Blake, Z. Huang, E. J. Wolfrum, W. A. Jacoby, Applied and Environmental Microbiology, **65** (9), 4094–4098 (1999)
[32] J. A. Herrera Melian, J.M. Dona Rodriguez, A. Viera Suarez, E. Tello Rendon, C. Valdes do Campo, J. Arana, J. Perez Pena, Chemosphere, **41**, 323-327 (2000)
[33] I.-H. Cho, I.-Y. Moon, M.-H. Chung, H.-K. Lee, K.-D. Zoh, Water Science&Technology: Water Supply, **2** (1) 181 (2002).
[34] J. C. Yu, H. Y. Tang, J. Yu, H.C. Chan, L. Zhang, Y. Xie, H. Wang, S.P. Wong, Journal of Photochemistry and Photobiology A: Chemistry, **153**, 211–219 (2002)
[35] P. A. Christensen, T. P. Curtis, T. A. Egerton, S.A.M. Kosa, J.R. Tinlin, Applied Catalysis B: Environmental, **41**, 371 (2003).
[36] J. A. Ibánez, M. I. Litter, R. A. Pizarro, Journal of Photochemistry and Photobiology A: Chemistry, **157**, 81 (2003).
[37] D. Gumy, C. Morais, P. Bowen, C. Pulgarin, S. Giraldo, R. Hajdu, J. Kiwi, Applied Catalysis B: Environmental, **63**, 76–84 (2006)
[38] A. R. Rahmani, M. R. Samarghandi, M. T. Samadi, F. Nazemi, J. Res. Health Sci., 9 (1), 1-6 (2009)
[39] A. G. Rincón, C. Pulgarin, Catalysis Today, **101**, 331 (2005).
[40] S. Lee, M. Nakamura, S. Ohgaki, Journal of Environmental Science and Health, Part A, **33** (8), 1643 (1998).
[41] C. Maneerat, Y. Hayata, International Journal of Food Microbiology, **107**, 99 (2006).
[42] H. Sakai, E. Ito, R.-X. Cai, T. Yoshioka, Y. Kubota, K. Hashimoto, A. Fujishima, Biochimica et Biophysica Acta (BBA) - General Subjects, **1201** (2), 259 (1994).
[43] Y. Kubota, T. Shuin, C. Kawasaki, M. Hosaka, H. Kitamura, R. Cai, H. Sakai, K. Hashimoto, A. Fujishima, Br. J. Cancer, **70**, 1107 (1994).
[44] J. Zhu, J. Yang, Z.-F. Bian, J. Ren, Y.-M. Liu, Y. Cao, H.-X. Li, H.-Y. He, K.-N. Fan, Applied Catalysis B: Environmental, **76**, 82 (2007).

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