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Ag-doped TiO_2 Nanocomposite Prepared by Sol Gel Method: Photocatalytic Bactericidal Under Visible Light and Characterization

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Abstract

In this reaserch, photocatalyst titanium dioxide was doped with silver and modified by polyethylene glycol by sol gel method and the samples were characterized by X-ray diffraction (XRD) and scanning electron microscopy (SEM). The purpose of the present study was to evaluate the photocatalytic bactericidal effects of prepared nanocomposite on human pathogenic bacteria under visible light irradiation whereas; many studies have been published on the use of titanium dioxide as a photocatalyst, which decomposes various organic compounds. We observed that TiO_2 reveals the bactericidal property against the Staphylococcus aureus, Shigella dysanteriae, Salmonella enterica subsp. enterica serovar Paratyphi bacteria and pathogenic fungi Candidia albicans which is increased by the essence of silver and visible light.

1. Introduction

Nano particle are of great interest due to their high surface-to-volume ratios and size dependent properties. Among those nanoparticles, titanium dioxide (TiO_2) is the most commonly used semiconductor photocatalyst, because of its physical and chemical stability, high catalytic activity, high oxidative power, low cost and ease of production [1].

UV light illumination over TiO_2 produces electrons and holes. The valence band holes are powerful oxidants, while the conduction band electrons are good reductants [2]. TiO_2 photocatalysts have been found to kill cancer cells,

bacteria, viruses, and algae under UV illumination [3]. However, most applications involve near-UV light irradiation (Eg > 3.2 eV), practically limiting the use of solar spectrum. In addition, the high rate of photo-generated electron–hole recombination in TiO_2 particles results in a low efficiency of photo-catalysis [4]. To solve these problems, numerous strategies have been proposed. The chemical modification of TiO_2, by doping the lattice with a transition-metal (TM) ion, has proven to be effective in the extension of the absorption threshold toward the visible region [5]. Among TM ions, Ag has drawn considerable attention because Ag particles act as better electron traps, preventing photo-generated charge carrier recombination and facilitating electron excitation by creating a local electrical field [4]. In addition, Silver-doped titanium dioxide nanoparticles became of current interests because of both their effects on the improvement of photocatalytic activity of TiO_2 and their effects on antibacterial activity [6].

Ag has been used as bactericidal agent in hygiene and medicinal applications for thousands of years. Both ions Ag^+ and nanoparticles of silver were shown to have antibacterial activity.

Silver ions could affect the bacterial membrane respiratory electron transport chains and DNA replication components. However, the bacterial-killing enhancement of photocatalysis might not apply to all forms of silver coating. These results indicated that Ag/TiO_2 composted materials may contain the advantages of both materials: silver has a higher antimicrobial activity, and TiO_2 can last longer, and able to be controlled by illumination [7].

In this paper, the Ag/TiO_2 catalyst was prepared by the sol–gel method and The surface structure of the powder was modified by adding polyethylene glycol (PEG) into the TiO_2 sol. The samples were characterized by XRD and SEM. Also its antibacterial effect was investigated in dark and visible light irradiations.

2. Experimental

2.1 Materials and Characterization

All the materials including tetrabutyl ortho titanate (TBOT), ethanol, acetylacetone (acac), hydrochloric acid, silver nitrate and polyethylene glycoland (PEG) were provided by Merck Company and without any further purification. Deionized (DI) water that was prepared by an ultra pure water system type smart-2-pure, TKA, Germany, was used throughout.

The products obtained were characterized by a Powder X-ray diffraction (Philips X'pert Pro MPD) and Scanning Electron Microscopy (Philips XL-30ESM).

2.2. Synthesis of pure TiO_2, Ag/TiO_2 and $Ag/PEG\text{-}TiO_2$ nanocomposite

Undoped and Ag-doped TiO_2 nanocomposites were synthesized by sol gel method. In this method, TBOT (2.5 ml) was added dropwise to a solution of 10 ml ethanol and 2.5 ml acac at room temperature, and stirred for 30 min. Then, 2.0 ml DI water was added to the above solution and pH was adjusted to 1.8 with HCl. 0.0014 g $AgNO_3$ (as a source of silver) was added into prepared sol. Afterwards, 1.0 g PEG were added to the above solution. A stable sol was finally obtained after stirring for 2 h. The sol was heated in a steam bath and then the concentrated solution was placed at 60 °C for 48 h. The dried solution was annealed at 500 °C for 1 h. By this method, 3 types of dried bulk powders, Pure TiO_2, Ag/TiO_2 and $Ag/PEG\text{-}TiO_2$, were prepared.

2.3. Measurements of bioactivity (antibacterial and antifungal)

2.3.1. Microbial strains:

For antimicrobial test of the Pure TiO_2, Ag/TiO_2 and $Ag/PEG\text{-}TiO_2$, powders, 4 microorganisms were individually tested. Following microbial strains were provided by Iranian research organization for science and technology (IROST) and used in the research. Staphylococcus aureus (ATTC 29737), Shigella dysanteriae (PTCC 1188), Salmonella enterica subsp. enterica serovar Paratyphi A (PTCC 1230) and Candidia albicans (ATTC 10231). Bacterial strains were cultured overnight at 37º C in nutrient agar (NA) and fungi were cultured overnight at 30º C in Saburaud dextrose agar (SDA).

2.3.2. MIC (minimal inhibitory concentration)

The inocula of the microbial strains were prepared from 12 h broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity. Pure TiO_2, Ag/TiO_2 and $Ag/PEG\text{-}TiO_2$ nanocomposites dissolved in 10% dimethylsulfoxide (DMSO) was first diluted to the highest concentration (5 mg/ml) to be tested, and then serial two-fold dilutions were made in a concentration range from 0.078 to 5 mg/ml in 10 ml sterile test tubes containing brain heart infusion (BHI) broth for bacterial strains and sabouraud dextrose (SD) broth for yeast. The 96-well plates were prepared by dispensing 95 µl of the cultures media and 5 µl of the inoculum into each well. A 100 µl aliquot from the stock solutions of the plant extracts initially prepared at the concentration of 5 mg/ml was added into the first wells. Then, 100 µl from their serial dilutions was transferred into six consecutive wells. The last well containing 195 µl of the cultures media without the test materials and 5 µl of the inoculum on each strip was used as the negative control. The final volume in each well was 200 µl. Contents of each well were mixed on plate shaker at 300 rpm for 20 s and then incubated at appropriate temperatures for 24 h. Microbial growth was determined by the presence of a white pellet on the well bottom and confirmed by plating 5 µl samples from clear wells on NA medium. The MIC value was defined as the lowest concentration of the plant extracts required for inhibiting the growth of microorganisms. All tests were repeated two times.

2.3.3. Zones of inhibition (Disc diffiusion assay):

Determination of antimicrobial activity of Pure TiO_2, Ag/TiO_2 and $Ag/PEG\text{-}TiO_2$ nanocomposites were accomplished by agar disc diffiusion method. (NCCLS, 1997). At first 4 mg of Pure TiO_2, Ag/TiO_2 and $Ag/PEG\text{-}TiO_2$ powder was dissolved in strilled 1 ml DMSO 10%. Antimicrobial tests were carried out by the disc diffiusion method reported by Murray et al.(1999). Using 100µl of suspension containing 10^8 CFU/ml of bacteria, 10^6 CFU/ml of yeast (Candidia albicanse) in this study.The discks (6 mm in diameter) impregnanted with 50µl of each suspensions of Pure TiO_2, Ag/TiO_2 and $Ag/PEG\text{-}TiO_2$ powder films and DMSO (as negative control) were placed on the incubated agar. The inoculated plates were incubated for 24 h at 37 ºC for bacterial strains and 48-72 h at 30 ºC for yeast isolate in both light and dark conditions. The diameters of inhibition zones were used as a measure of antimicrobial activity and each assay was repeated twoice.

3. Results and discussion

3.1. Antimicrobial activity

Determining the MIC values of antibacterial agents is a valuable means for comparing the

antibacterial effectiveness of the agents. The MIC values were the lowest concentration of nanocomposites in aqueous solution that inhibited visual growth after 24 h of incubation. Thus, lower MIC value means higher bioactivity. The minimal inhibitory concentration of microorganism growth for as-prepared Pure TiO_2, Ag/TiO_2 and $Ag/PEG\text{-}TiO_2$ nanocomposites was estimated using bacteria S. aureus, Salmonella paratyphi, Shigella dysanteriae and pathogenic fungi Candida albicans. The effect of silver loading on MIC value is presented in Table 1. For pure TiO_2, the inhibition in microorganism's growth was not observed even for photocatalyst concentration below 500 μ g/ml. The essence of silver and visible light lead to decreasing of the MIC value related to bioactivity enhancement.

The lower MIC value (the highest bioactivity) was observed for Shigella dysanteriae in the presence of Ag/TiO_2 and $Ag/PEG\text{-}TiO_2$ sample. It was noticed that silver nanoparticles revealed higher antimicrobial activity against Gram-negative bacteria Shigella dysanteriae than for Gram-positive bacteria S. aureus.

This study showed that Gram-positive bacteria were more resistant to photocatalytic disinfection than Gram-negative bacteria. The difference is usually ascribed to the difference in cell wall structure between Gram-positive and Gram-negative bacteria. Gram-negative bacteria have a triple-layer cell wall with an inner membrane (IM), a thin peptidoglycan layer (PG) and an outer membrane (OM), where as Gram-positive bacteria have a thicker PG and no OM [8].

Based on the zone of inhibition analysis, shown in Table 2, it was observed that in the light situation, when of media was subjected to modified surface and silver, inhibition zone of Shigella dysanteriae, Salmonella paratyphi and S. aureus was increased dramatically, followed whith the conditional growth of Salmonella paratyphi only in visible light condition. Conversely, in the same conditions, Candida albicans didn't illustrate any inhibition zone.

The images of the zone of growth inhibition for S. aureus, Shigella dysanteriae and pathogenic fungi Candida albicans are shown in Fig. 1.

Growth inhibition zones appearing around the spots were lined for easier detection (see Fig. 1).

3.1.1. Mechanism of antimicrobial activity

The killing mechanism involves degradation of the cell wall and cytoplasmic membrane due to the production of reactive oxygen species (ROS) such as hydroxyl radicals and hydrogen peroxide. This initially leads to leakage of cellular contents then cell lysis and may be followed by complete mineralisation of the organism.

TiO_2 is a semiconductor. The adsorption of a photon with sufficient energy by TiO_2 promotes electrons from the valence band (e_{vb}^-) to the conduct ion band (e_{cb}^-), leaving a positively charged hole in the valence band (h_{vb}^+; Eq. 1). The band gap energy of anatas is approx. 3.2 eV, which effectively means that photocatalysis can be activated by photons with a wave length of below approximately 385 nm. The electrons are then free to migrate within the conduction band. The holes may be filled by migration of an electron from an adjacent molecule, leaving that with a hole, and the process may be repeated. The electrons are then free to migrate within the conduct ion band and the holes may be filled by an electron from an adjacent molecule. This process can be repeated.

Thus, holes are also mobile. Electrons and holes may recombine (bulk recombination) a non-productive react ion, or, when they reach the surface, react to give reactive oxygen species

(ROS) such as $O_2^{-\bullet}$ (Eq. 2) and $OH^{\bullet}$ (Eq. 3). These in solution can react to give H_2O_2 (Eq. 4), further hydroxyl (Eq. 5) and hydroperoxyl (Eq. 6) radicals. Reaction of the radicals with organic compounds results in mineralisation (Eq. 7). Bulk recombination reduces the efficiency of the process, and indeed some workers have applied an electric field to enhance charge separation, properly termed photoelectrocatalysis [9].

Table 1. Minimum inhibitory concentration (MIC) of Pure TiO_2, Ag/ TiO_2 and Ag/PEG-TiO_2 for microbial growth (bacteria and fungi).

Microbial strains	MIC (μg/ml)					
	Pure TiO_2		Ag/TiO_2		Ag/PEG-TiO_2	
	Dark	Visible light	Dark	Visible light	Dark	Visible light
Staphylococcus aureus (ATTC 29737)	≥500	≥500	500	500	500	500
Shigella dysanteriae (PTCC 1188)	≥500	125	500	62.5	500	62.5
Salmonella Paratyphi A (PTCC 1230)	≥500	125	≥500	125	500	125
Candidia albicans (ATTC 10231)	500	500	250	500	250	500

Table 2. Zone of microorganism's growth inhibition.

Microbial Community	Zone of inhibition, diameter of zone (mm)					
	Pure TiO_2		Ag/ TiO_2		Ag/PEG-TiO_2	
	Dark	Visible light	Dark	Visible light	Dark	Visible light
Staphylococcus aureus (ATTC 29737)	10	10	10	12	10	11
Shigella dysanteriae (PTCC 1188)	11	15	11	15	11	16
Salmonella Paratyphi A (PTCC 1230)	-	-	-	12	-	15
Candidia albicans (ATTC 10231)	11	-	12	-	12	-

Contact between the cells and TiO_2 may affects membrane permeability, but is reversible. This is followed by increased damage to all cell wall layers, allowing leakage of small molecules such as ions. Damage at this stage may be irreversible, and this accompanies cell death. Furthermore, membrane damage allows leakage of higher molecular weight components such as proteins, which may be followed by protrusion of the cytoplasmic membrane into the surrounding

medium through degraded areas of the peptidoglycan and lysis of the cell. Degradation of the internal components of the cell then occurs, followed by complete mineralisation. The degradation process may occur progressively from the side of the cell in contact with the catalyst. Fig. 2 showes Scheme for photocatalytic killing and destruction of bacteria on TiO_2. Direct oxidation of cell components can occur when cells are in direct contact with the catalyst. Hydroxyl radicals and H_2O_2 are involved close to and distant from the catalyst, respectively. Furthermore, $OH^{\bullet}$ can be generated from reduction of metal ions [10]. Ag enhances photocatalys is by enhancing charge separation at the surface of the TiO_2 [11]. Ag^+ is antimicrobial and can also enhance generation of ROS (Eqs. 8, 9 and 10).

$$TiO_2 + h\upsilon \rightarrow e_{cb}^- + h_{vb}^+ \quad \text{(Eq. 1)}$$
$$O_2 + e_{cb}^- \rightarrow O_2^- \quad \text{(Eq. 2)}$$
$$h_{vb}^+ + H_2O \rightarrow OH^{\bullet} + H^+_{aq} \quad \text{(Eq. 3)}$$
$$OH^{\bullet} + OH^{\bullet} \rightarrow H_2O_2 \quad \text{(Eq. 4)}$$
$$O_2^- + H_2O_2 \rightarrow OH^{\bullet} + OH^- + O_2 \quad \text{(Eq. 5)}$$
$$O_2^- + H^+ \rightarrow OOH^{\bullet} \quad \text{(Eq. 6)}$$
$$OH^{\bullet} + \text{Organic} + O_2 \rightarrow H_2O\ , CO_2 \quad \text{(Eq. 7)}$$
$$Ag^+ + O_2^{-\bullet} \rightarrow Ag + O_2 \quad \text{(Eq. 8)}$$
$$Ag + O_2^{-\bullet} \rightarrow Ag^+ + O_2^{2-} \quad \text{(Eq. 9)}$$
$$H_2O_2 + Ag \rightarrow OH^- + OH^{\bullet} + Ag^+ \quad \text{(Eq. 10)}$$

3.2. X-ray diffraction (XRD) analysis

There are three main polymorphs of TiO_2: anatase, rutile and brookite. The majority of studies show that anatase was the most effective photocatalyst and that rutile was less active; the differences are probably due to differences in the extent of recombination of electron and hole between the two forms [12].

Fig 3 (a–c) shows the XRD patterns of Pure TiO_2, Ag/TiO_2 and $Ag/PEG\text{-}TiO_2$ nanocomposites

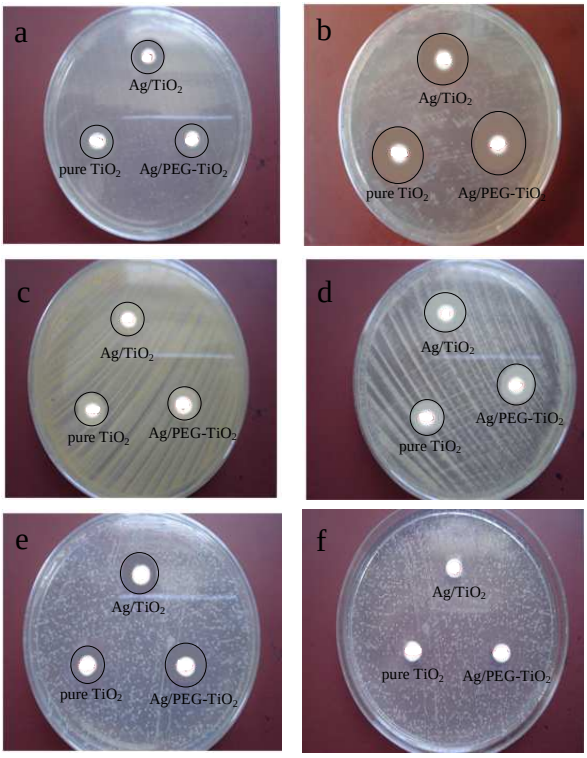

Fig. 1. inhibition zone of a) Shigella dysanteriae, b) S. aureus c) Candida albicans, when of media was subjected to pure TiO_2, Ag/TiO_2 and $Ag/PEG\text{-}TiO_2$.

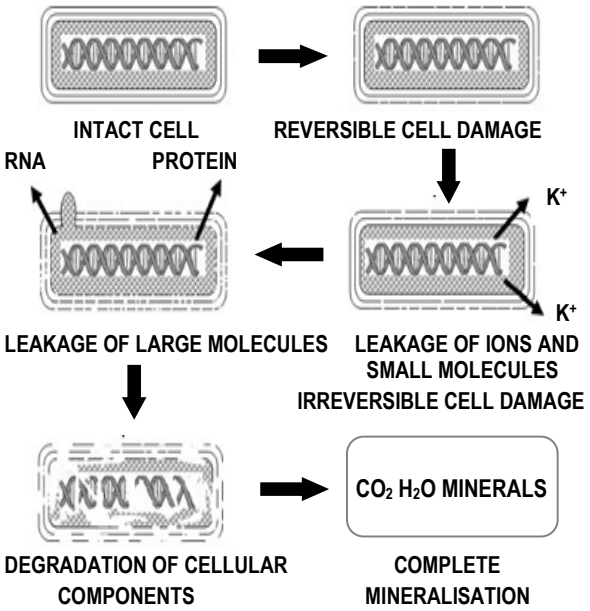

Fig. 2. Scheme for photocatalytic killing and destruction of bacteriaon TiO_2.

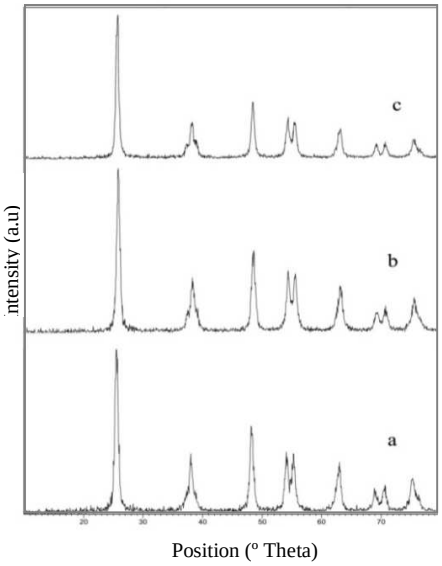

Fig. 3. XRD patterns of (a) Pure TiO_2, (b) Ag/TiO_2 and (c) Ag/PEG-TiO_2.

respectively. For all samples (1 0 1), (0 0 4) and (2 0 0), diffraction peaksappearing at 2β values of 25.5°, 38.0° and 48.3° respectively, matches well with the standard TiO_2 diffraction pattern and All the relatively sharp peaks could be indexed as anatase TiO_2 corresponding to the reported values Joint Committee on Powder Diffraction Standards (JCPDS) card No. 04-0477 [13].It can be seen that all pure and doped samples are well crystallized and anatase phase is the only constituent of the nanocrystals. The average particle size was estimated from the Scherrer equation on the anatase diffraction peaks:

$$D = \frac{K\lambda}{\beta\, Cos\theta}$$

Where D is the crystal size of the catalyst, λ the X-ray wavelength, β the full width at half maximum (FWHM) of the diffraction peak (radian), K= 0.89 and θ is the diffraction angle at the peak maximum.

Average crystal size of pure TiO_2, Ag/TiO_2 and Ag/PEG-TiO_2 were calculated to be 22.10, 18.50 and 17.23 nm respectively. It has been found that the particle size reduces as a result of Ag addition.

3.3. Scanning electron microscopy (SEM)

Fig. 4a and b shows the SEM photographs of pure TiO_2 and Ag/PEG-TiO_2 nanocomposite powders. In (a) shows that the product mainly contained undulatory texture. On the contrary, Ag/PEG-TiO_2 (b) nanocomposite powders very rough surface morphologies that seems to indicate the presence of very large pores in the matrix. This indicated that the specific surface areas of Ag/PEG-TiO_2 nanocomposite powders were larger than that of pure TiO_2 powders and there would be more reactive sites participating in the photoreaction.

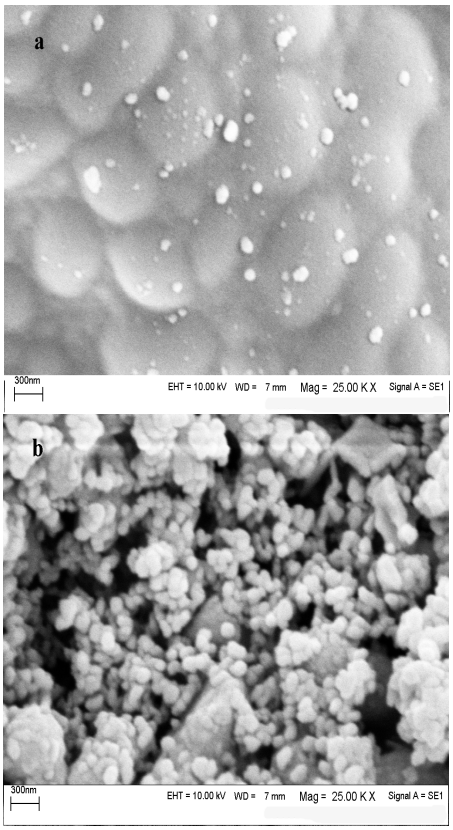

Fig. 4. SEM photographs of (a) Pure TiO_2, (b) Ag/PEG-TiO_2

4. Conclusion

The Ag doped TiO2 nanoparticles, modified by PEG, were successfully prepared by a simple sol gel dip coating method as antimicrobial materials. The antimicrobial susceptibility was tested using bacteria S. aureus, Salmonella paratyphi, Shigella dysanteriae and pathogenic fungi Candida albicans. The obtained results showed that bioactivity of differed depending on microbial strain, Ag content and presence of visible light and silver/ TiO_2 composted materials may contain the advantages of both materials: silver has a higher antimicrobial activity, and TiO_2 can last longer, and able to be controlled by illumination.

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References

[1] M. K. Seery, R. George, P. Flori, S. C. Pillai. J Photochem. Photobiol. A-Chem. 189 (2007) 258–263.

[2] D. P. Macwan. Pragnesh. N. Dave. Sh. Chaturvedi, J. Mater. Sci. 46 (2011) 3669–3686.

[3] A. Fujishima, X. Zhang, D. A. Tryk, Surf. Sci. Rep. 63 (2008) 515–582.

[4] N. Bahadur, K. Jai, A.K. Srivastava, Govind, R. Gakhar, D. Haranath, M.S. Dulat, Mater. Chem. Phys. 124 (2010) 600–608.

[5] M. Logar, B. Jancar, Sa. Sturm, D.Suvorov. Langmuir, 26 (2010) 12215–12224.

[6] A. Zielinska, E. Kowalska, J. W. Sobczak, I. Łacka b, M. Gazda, B. Ohtani, J. Hupka, A. Zaleska, Separ. Purif. Tech. 72 (2010) 309–318.

[7] M. Wong, D. Sun, H. Chang. Plos one 5 (2010) 10394.

[8] H. A. Foster, I. B. Ditta, S. Varghese, A. Steele, Appl. Microbiol. Biotechnol. 90 (2011) 1847 –1868.

[9] J. Harper, P. Christensen, T. Egerton, J. Appl. Electrochem. 31(2000) 623–628.

[10] T. Sato, M. Taya, Biochem. Eng. J. 30 (2006) 199–204.

[11] J. Musil, M. Louda,R. Cerstvy,P. Baroch, I. Ditta,A. Steele,H. Foster, Nanoscale Res. Lett. 4 (2009) 313–320.

[12] T. Miyagi, M. Kamei, T. Mitsuhashi, T. Ishigaki, A. Yamazaki, Chem. Phys. Lett. 390 (2004) 399–402.

[13] D. Wang, B. Yu, F. Zhou, C. Wang, W. Liu, Mater. Chem. Phys. 113 (2009) 602–606.