Available online at www.joac.info

ISSN: 2278-1862

Journal of Applicable Chemistry

2015, 4 (2): 550-556

(International Peer Reviewed Journal)

Synthesis, Characterization and Antitumor activity of Fe_2O_3-Ag_2O-TiO_2 Nanocomposite on MCF-7 Human Breast Cancer Cells

P. Babji[1*], I. Nageswara Rao[1] and S. K. Das[2]

1. Department of Physical, Nuclear Chemistry & Chemical Oceanography, School of Chemistry, Andhra University, Visakhapatnam 530 003, Andhra Pradesh, **INDIA**
2. School of Chemistry, University of Hyderabad, Hyderabad 500 046, Telangana, **INDIA**

Email: babjichemistry007@gmail.com

Accepted on 27th February 2015

ABSTRACT

The study deals with the Magnetic Fe_2O_3-Ag_2O-TiO_2 nanocomposite has been prepared by using sol gel method and characterized by X-Ray Diffraction, Fourier transform-infra red spectroscopy (FT-IR), Field Emission- Scanning Electron Microscopy and Energy dispersed spectroscopy has been applied to investigate the structure and morphology. X-ray diffraction studies of the Fe_2O_3-Ag_2O-TiO_2 show the presence of anatase phase of TiO_2. 1 mmol of the prepared sample from had also shown the presence of anatase phase only. The FE-SEM images of the prepared samples showed the decrease in size and morphological change of the TiO_2 particles when compared to undoped TiO_2. The presence of elements iron, silver, titanium and oxygen were characterized by Energy dispersed spectroscopy (EDS). For comparison, the anticancer activity was carried out by using Temoxifen as a standard with Fe_2O_3-Ag_2O-TiO_2 nanocomposite. The anticancer activity of the synthesized catalysts was investigated by the MCF-7 cell line, and it was found that the Fe_2O_3-Ag_2O-TiO_2 catalysts have better anticancer activity than un-doped TiO_2. The present results showed that this prepared nanocomposite might be a potential alternative agent for human breast cancer therapy.

Keywords: TiO_2, Fe_2O_3-Ag_2O-TiO_2, Anticancer activity, magnetic.

INTRODUCTION

Breast cancer is one of the leading causes of cancer morbidity and mortality among women Worldwide, and the frequency keeps increasing [1-2]. The use of various pharmaceutical nanocarriers has become one of the most important areas of nanomedicine. Ideally, such carriers can be specifically delivered to the pathological areas to provide the maximum therapeutic efficacy. Therefore, it prompted scientists to explore the feasibility of developing nanoparticles as effective drug delivery vehicles to overcome the limited therapeutic efficacy of free drugs and circumvent their toxicity. Metallic nanoparticles like Fe_2O_3, Ag, ZnO and Au are also used for different therapeutics, diagnosis and treatment of cancer cells, but they come with certain drawbacks like during the production of Fe_2O_3 nanoparticles, FeO nanoparticles are also formed as one of the by-product of the other and it is very difficult to completely isolate the two different

nanoparticles and thus their mechanism of action for drug loading and therapeutic activities cannot be assured [3].

Nowadays, some cytotoxic agents are used for its treatment including doxorubicin, daunorubicin, bleomycin, and cisplatin. However, they are costly and known to induce several side effects such as myelosuppression, anemia, and most importantly the generation of cellular resistance. For this, it is important to find alternative therapies or drugs to overcome these drawbacks [4].

A variety of inorganic nanocarriers, such as iron oxide, mesoporous silica, graphene oxide and titanium dioxide nanoparticles, have been studied for successful drug delivery and therapy in cancer treatment [5–10]. Furthermore, metal nanomaterials have also been widely studied as drug carriers, which represent a promising approach to tumors [11, 12]. The titanium dioxide (TiO_2) nanoparticle possesses many attractive features such as excellent biocompatibility, low toxicity, high chemical stability, and unique photocatalytic and sonocatalytic properties. It was found that Fe_3O_4-TiO_2 core–shell nanocomposites with 6–8 nm diameter have been explored as carriers for DOX delivery in drug-resistant ovarian carcinoma cells [10]. In addition, Wenzhi Ren et al. reported an enhanced doxorubicin transport to multidrug resistant breast cancer cells via TiO_2 nanocarriers [13]. Out of the three phases of TiO_2 anatase has higher electron mobility, lower fixed dielectric properties and lower density [14] useful in photocatalytic applications. The present paper describes the antitumor activity of Fe_2O_3-Ag_2O-TiO_2 nanocomposite on MCF-7 cell line.

MATERIALS AND METHODS

Ferric Nitrate, silver nitrate and tetrabutyl titanate (TBOT) were purchased from Merck., Ltd., Chaina. Anhydrous ethanol was obtained from Hangzhou Changzheng Chemical Reagent Co., Ltd., Chaina.

Preparation of Fe_2O_3-Ag_2O-TiO_2 nanocomposite: 0.02 mol of tetrabutyl orthotitanate were added drop wise into 50 mL absolute ethanol to give a solution which was then vigorously stirred for 20 min at room temperature and add 3 drops of Conc. HNO_3. The desired amounts, 1.0 mmol of ferric nitrate and 1.0 mmol of silver nitrate (the molar ratio of Cu to Zn was 1:1) were then added in the reaction mixture while stirring continuously for 60 min until the Fe and Ag were dissolved. An amount of 2.0 mL of deionized water was then added to the above solution. Afterwards, the resultant solution mixture was maintained at room temperature with continuous stirring for 2 h to form a gel which was then aged for 12 h at room temperature. After being dried at 80^0 C for 24 h were obtained and then annealed at 400 ^{0}C for 3 h. An un-doped TiO_2 (anatase) sample was also prepared by adopting the above procedure without adding the ferric nitrate and silver nitrate which is pure TiO_2. The doping concentrations are expressed as mmol of titanium atom.

Catalyst characterization: Crystalline structure and crystallinity of the Fe_2O_3-Ag_2O-TiO_2 nanocomposite were examined using an X-ray Diffractometer (Shimadzu, XRD-6000) equipped with Cu Kα radiation source using Ni as filter at a setting of 45 kV/40 mA. A 2θ scan range from 10 to 90 ^{0}C, a scanning step size of 0.01 ^{0}C and a scintillation counter detector was used. Curve fitting and integration was carried out using proprietary software from Philips X'Pert high score plus. Fourier transform infrared (FT-IR) spectroscopy was carried for a Fe_2O_3-Ag_2O-TiO_2 nanocomposite was obtained in the range 4,000 to 500 cm^{-1} with an IR-Prestige-21 Shimadzu FT-IR spectrophotometer, by KBr pellet method. The FE-SEM micrographs were obtained using a JEOL 6335F FE-SEM microscope equipped with a Thermo Noran energy dispersive spectroscopy (EDS) detector. The presence of elemental iron, silver, titanium and oxygen was confirmed through EDAX. The EDAX observations were carried out in STIC, CUSAT, (JOEL Model JED-2300).

Cell culture: Human cancer cell lines used in this study were procured from National Centre for Cell Science, Pune. All cells were grown in Minimal essential medium (MEM, GIBCO) supplemented with 4.5

g L^{-1} glucose, 2 mM L-glutamine and 5% fetal bovine serum (FBS) (growth medium) at 37°C in 5% CO_2 incubator.

RESULTS AND DISCUSSION

XRD patterns:

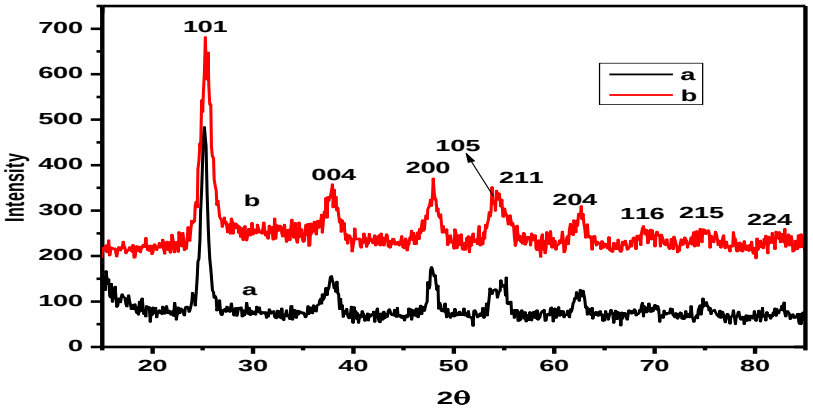


Fig. 1. XRD patterns of (a). un-doped TiO_2 and (b). 1mmol of Fe_2O_3-Ag_2O-TiO_2 nanocomposite

Fig. 1 shows the XRD patterns of un-doped TiO_2 (curve a), and 1mmol of Ferric nitrate and 1mmol of Silver nitrate TiO_2 (curve b) powders. It is found that all of the crystal phase is anatase for all of the samples [15]. No diffraction peak corresponding to the Fe and Ag was detected. The reason could be due to the fact that the content of Fe and Ag might be too small to detect. The shape of the diffraction peaks of all the photo catalysts was consistent with that of un-doped TiO_2. The well-defined diffraction peaks with 2θ are at about 25°, 38°, 48°, 54°, 54°, 62°, 68°, 70°, 74° and 82° which are assigned to the (101), (004), (200), (105), (211), (204) (116), (220), (215) and (224) crystal planes, respectively. This XRD characteristic pattern is consistent with the standard JCPDS values of anatase TiO_2 (JCPDS Card No. 21-1272) [16, 17] with tetragonal structure and did not appear in rutile and brookite form.

Fourier Transform Infrared Spectroscopy:

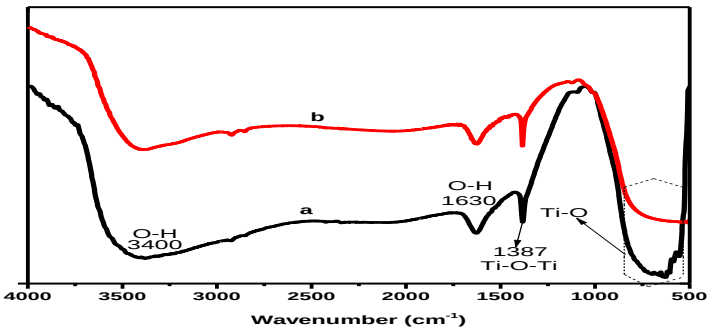


Fig. 2. FT-IR spectra of (a) Un-doped TiO_2 and (b) 1mmol of Fe_2O_3-Ag_2O-TiO_2 nanocomposite.

The FTIR spectra of the all samples in the frequency range of 400–4000 cm^{-1} are shown in Fig. 2. All the samples show peaks corresponding to the stretching vibration of O–H and bending vibrations of adsorbed water molecules around 3200–3400 cm^{-1} and 1600 cm^{-1} respectively [15, 18, 19]. Furthermore, the broadening of ~3400cm^{-1} O-H stretching vibration the formation of a different -OH group, and most probably as Ti-OH surface group. The broad intense band in the range of 450–700 cm^{-1} is due to the Ti–O stretching and Ti–O–Ti bridging stretching modes [16, 17]. Iron and silver co-doped TiO_2 intensity has reduced compared to un-doped TiO_2. No additional peaks are present upon Fe and Ag deposition, supporting the efficient dispersion of iron and silver, and it indicates the absence of clusters of iron and silver, which is in good agreement with the XRD analysis result.

Field Emission-Scanning Electron Microscopic Study:

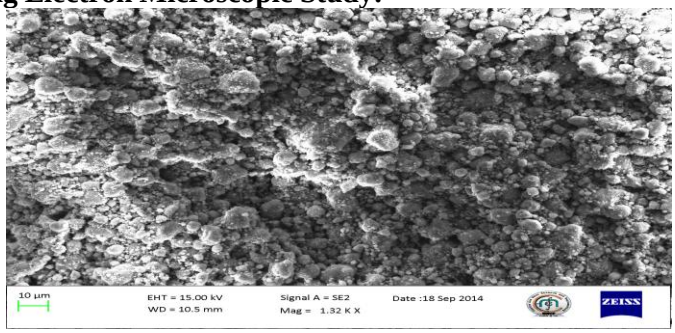


Fig. 3. FE-SEM images of 1mmol Fe_2O_3@Ag_2O@TiO_2 nanocomposite.

The morphology and structure of the as prepared samples were investigated by field emission scanning electron microscopy (FE-SEM). According to the FE-SEM, the morphology of the Fe_2O_3-Ag_2O-TiO_2 nanocomposite was observed and approximately spherical, in which the Fe and Ag deposited with titanium dioxide nanoparticles were in aggregated form. This reveals that the powder particles are slightly agglomerated and the closed view of spherical nanoparticles has showed (Fig. 3).

Energy Dispersive X-ray (EDX) spectra:

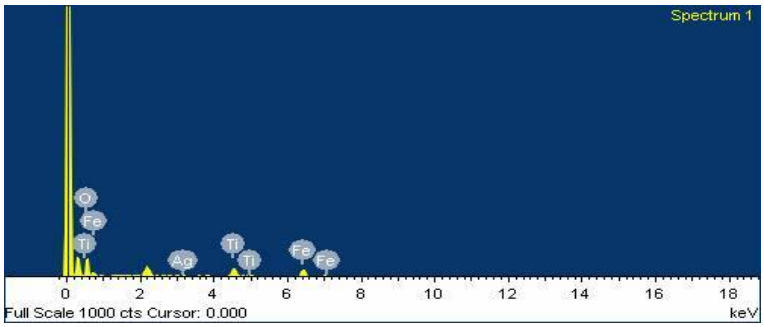


Fig. 4. EDS spectra of 1mmol of Fe_2O_3-Ag_2O-TiO_2 nanocomposite.

The energy dispersive X-ray (EDX) spectra of the Fe_2O_3-Ag_2O-TiO_2 nanocomposite was shown in Fig.4 respectively. The peaks corresponding to titanium, oxygen and the respective deposited metals of iron and silver can be confirmed by fig 4. The results of an elemental analysis confirmed the homogenous distribution of metal nanoparticles in the TiO_2 lattice.

In vitro assay for cytotoxicity activity (MTT assay): The MTT assay developed by Mosmann [20] was modified and used to determine the inhibitory effects of test compounds on cell growth *in vitro*. In brief, the trypsinized cells from T-25 flask were seeded in each well of 96-well flat-bottomed tissue culture plate at a density of $5x10^3$ cells/well in growth medium and cultured at 37°C in 5% CO_2 to adhere. After 48hr incubation, the supernatant was discarded and the cells were pretreated with growth medium and were subsequently mixed with different concentrations of test compounds (12.5,25,50,100,200 µg mL^{-1}) in triplicates to achieve a final volume of 100 µL and then cultured for 48 h. The compound was prepared as 1.0 mg m L^{-1} concentration stock solutions in DMSO. Culture medium and solvent are used as controls. Each well then received 5 µL of fresh MTT (0.5mg m L^{-1} in PBS) followed by incubation for 2h at 37°C. The supernatant growth medium was removed from the wells and replaced with 100 µL of DMSO to solubilize the colored formazan product. After 30 min incubation, the absorbance (OD) of the culture plate was read at a wavelength of 492 nm and 620nm reference filter on an ELISA reader, Anthos 2020 spectrophotometer.

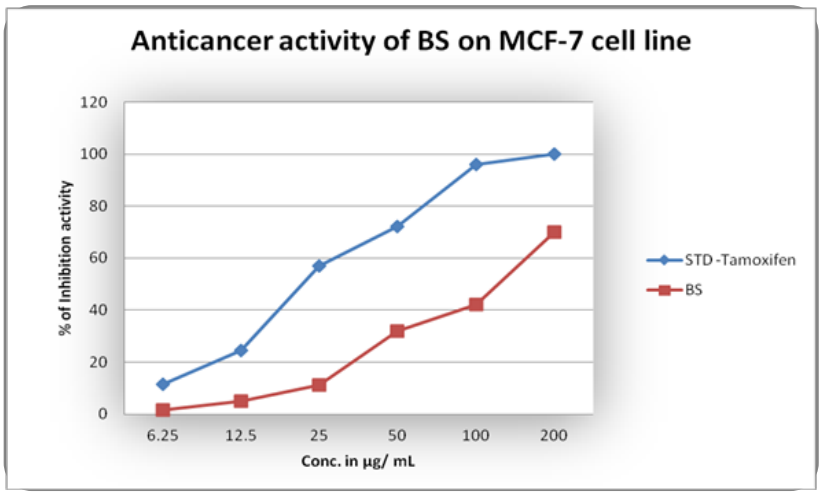
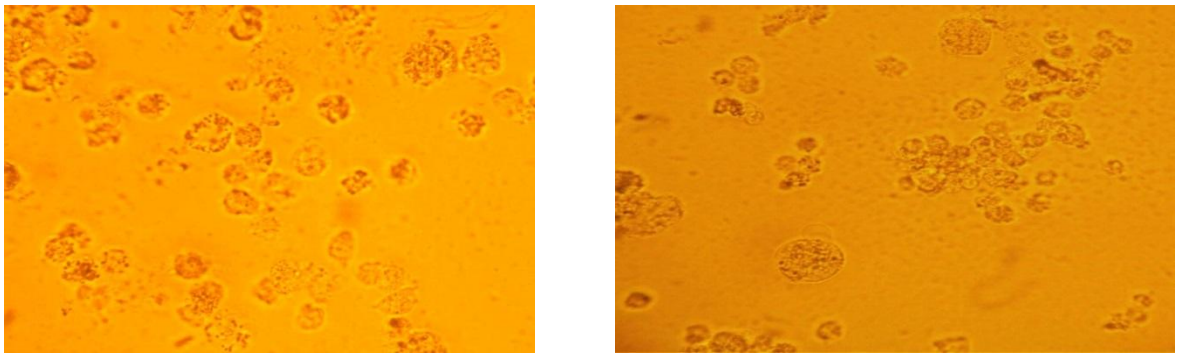


Fig. 5. STD is Temoxifen and BS is Fe_2O_3-Ag_2O-TiO_2 nanocomposite.

Anticancer activity of Fe_2O_3-Ag_2O-TiO_2 nanocomposite was evaluated on MCF-7 cells in comparison to Tamoxifen as a standard. *In vitro* cytotoxic activity against MCF7 cell line at different concentrations was evaluated and compared with the standard drug Temoxifen. The *in vitro* screening of the present nanocomposite showed potential cytotoxic activity against the human breast cancer (MCF 7) cell line. The plates were observed under an inverted microscope to detect morphological changes showed in fig. 6. The result showed that MTT cells proliferation were significantly inhibited by Fe_2O_3-Ag_2O-TiO_2 nanocomposite. Thus the synthesized nanocomposite was found to be potently cytotoxic agent against MTT cell line. These results indicate that the sensitivity of human cancer cell line for cytotoxic drugs is higher than that of Vero cell line for the same cytotoxic agents.

Growth inhibition was calculated by the following formula:

% Growth inhibition = $100-[OD_{test\ sample}-OD_{blank}/OD_{test\ sample}-OD_{control}]x100$



a) b)

Fig.6. a) Temoxifen, b) Fe_2O_3-Ag_2O-TiO_2 nanocomposite.

APPLICATIONS

The prepared Fe_2O_3-Ag_2O-TiO_2 nanocomposite showed anticancer activity for the MCF-7 cell line. These results suggested that the simple and cost effective method and shows excellent adsorption removal properties on dyes for industrial applications.

CONCLUSIONS

The Fe_2O_3-Ag_2O-TiO_2 nanocomposite was characterized using XRD, FT-IR, FE-SEM and EDS. The results showed that, the product was in the form of anatase phase. The nanocomposite prepared by sol gel method showed excellent anticancer activity than TiO_2. These results suggested that the simple and cost effective method and shows excellent adsorption removal properties on dyes for industrial applications. The overall results indicated that the colloidal Fe_2O_3-Ag_2O-TiO_2 nanocomposite has antitumor activity through induction of apoptosis in MCF-7 breast cancer cell line, suggesting that present nanocomposite might be a potential alternative agent for human breast cancer therapy.

ACKNOWLEDGMENTS

The author is thankful to UGC-BSR, New Delhi for financial support. The auther is also thankful Department of Inorganic & Analytical Chemistry, Andhra University, VSP for providing laboratory facilities. The auther express gratitude to University of Hyderabad for providing spectral data.

REFERENCES

[1] C. DeSantis, R. Siegel, P. Bandi, A. Jemal, *CA-Cancer J. Clin,* **2011**, 61, 408–418.
[2] D. R. Youlden, S. M. Cramb, N. A. Dunn, J. M. Muller, C. M. Pyke, P. D. Baade, *Cancer Epidemiol,* **2012**, 36, 237–248.
[3] Wahajuddin, Sumit Arora, *International journal of nanomedicine,* **2012,** *7*, 3445-3471.
[4] D. W. Kim, G. H Hong, H. H Lee, S. H Choi, B. G Chun, C. K Won, I. K Hwang, M. H Won, *Neuroscience,* **2007,** 117(3), 387-400.
[5] E. S. Lee, Z. Gao, Y. H. Bae, *J. Controlled Release*, **2008,** 132, 164–170.
[6] S. Bhattacharyya, R. A. Kudgus, R. Bhattacharya, P. Mukherjee, *Pharm. Res*, **2011,** 28, 237–259.
[7] F. M. Kievit, F. Y. Wang, C. Fang, H. Mok, K. Wang, J. R. Silber, R. G. Ellenbogen, M. Zhang, *J. Controlled Release,* **2011,** 152, 76–83.

[8] H. Meng, M. Liong, T. Xia, Z. Li, Z. Ji, J. I. Zink, A. E. Nel, *ACS Nano*, **2010,** 4, 4539–4550.
[9] J. Wu, Y. Wang, X. Yang, Y. Liu, J. Yang, R. Yang, N. Zhang, *Nanotechnology*, **2012,** 23, 355101–355109
[10] H. C. Arora, M. P. Jensen, Y. Yuan, A. Wu, S. Vogt, T. Paunesku, G. E. Woloschak, *Cancer Res*, **2012,** 72, 769–778.
[11] P. K. Jain, X. Huang, I. H. El-Sayed, M. A. El-Sayed, *Acc. Chem. Res*, **2008,** 41, 1578–1586.
[12] V. Biju, T. Itoh, A. Anas, A. Sujith, M. Ishikawa, *Anal. Bioanal. Chem*, **2008,** 391, 2469–2495.
[13] W. Ren, L. Zeng, Z. Shen, L. Xiang, A. Gong, J. Zhang, C. Mao, A. Li, T. Paunesku, G. E. Woloschak, N. S. Hosmaned, A. Wu, *RSC Adv*, **2013,** 3, 20855–20861.
[14] H. M. Moghaddam, S. Nasirian, *South African Journal of Science*, **2011,** *107* (3-4), 01-05.
[15] N. Venkatachalam, M. Palanichamy, V. Murugesan, *J. Mol. Catal. A: Chem*, **2007,** 273, 177–185.
[16] J. Tao, Y. Shen, F. Gu, J. Zhu, J. Zhang, *J. Mater. Sci. Technol*, **2007,** 23, 513–516.
[17] T. C. Jagadale, S. P. Takale, R. S. Sonawane, H. M. Joshi, S. I. Patil, B. B. Kale, S. B. Ogale, *J. Phys. Chem. C*, **2008,** 112, 14595–14602.
[18] N. Venkatachalam, M. Palanichamy, B. Arabindoo, V. Murugesan, *J. Mol. Catal. A: Chem*, **2007,** 266, 158–165.
[19] X. Chen, X. Wang, X. Fu, *Energy Environ. Sci*, **2009,** 2, 872–877.
[20] T. Mosmann, *J. Immunol. Methods*, **1983**, 65, 55-63.