Surface-enhanced Raman scattering study of Ag-PVP interactions in the biocompatible Ag@PVP nanoparticles

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Abstract: PVP-capped silver nanoparticles (Ag@PVP) were synthesized through a chemical reduction method using sodium borohydride (NaBH4) as reducing agent in an aqueous medium. The as-prepared nanoparticles were characterized using a Transmission Electron Microscope (TEM), UV-Visible, photoluminescence and Raman spectroscopies. Optical absorption measurements of Ag@PVP nanoparticles showed a strong localized surface plasmon resonance (LSPR). SERS experiments highlighted the regioselective adsorption of PVP on the Ag surface via the Ag-O interactions and the stability of Ag@PVP nanoparticles. The biocompatibility of these nanoparticles was studied using cytotoxicity experiments against HUVEC cells.

Keywords: Silver nanoparticles/ Raman-SERS/ Ag-O interactions/ Biocompatibility/ Cytotoxicity

INTRODUCTION

Nanomaterials are increasingly considered as promising tools with improved diagnostic and therapeutic efficacy. Recent advancements in synthesis and the ability to rationally manipulate silver nanoparticles (AgNPs), such as physical, chemical and biological properties open additional possibilities of application in nanomedicine [1,2,3]. The synthesis of silver nanoparticles can be performed under simple and mild conditions. Typical reducing agents, such as sodium borohydride [4], hydrazine [5], sodium citrate [6], thiols [7], amino acids [8] and polyols [9], are used in the presence of cetyltrimethylammonium bromide (CTAB), polyvinyl alcohol (PVA), cyclodextrin [10], poly(methylhydrosiloxane) [11] or Polyvinylpyrrolidone (PVP) as stabilizing layer. CTAB is classified as a toxic surfactant while PVP is classified as an eco-friendly polymer with hydrophilic properties. The capping of nanoparticles with polymers such as PVP has been identified as a viable method for producing nanoparticles with tunable morphologies and optical properties. Therefore, in order to tune and manipulate colloidal silver nanoparticles, more progress understanding the interfacial interactions between inorganic particles and organic polymers is still required. PVP could bind to silver either through the oxygen or the nitrogen atoms. Several experimental techniques such as NMR and FTIR spectroscopies have been used to study the interaction between PVP and silver [12-15]. Recently, more attention has been given to the theory of molecular aspects to study the regioselective surface adsorption of organic polymers and inorganic nanoparticles such as radial distribution functions (RDF) calculations and density functional theory (DFT) [16,17]. Besides the study of the interaction between PVP molecules and Ag surface, it is as important to study the stability of Ag@PVP nanoparticles which is related to these interactions. In fact, surfactant molecules notably PVP play an important role in controlling the size and the shape of the nanoparticles in solution and the release of Ag+ which is known to be a major factor in the toxicity of AgNPs [18,19].

In the present work, we describe the synthesis of PVP-capped AgNPs (Ag@PVP) by chemical reduction in an aqueous medium. Optical properties

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such as LSPR, SERS, and photoluminescence were studied. SERS experiments were carried out in order to study the adsorption of PVP on the Ag surface and the stability of the Ag@PVP nanoparticles. Their biocompatibility was tested through cytotoxicity experiments against HUVEC cells.

EXPERIMENTAL SECTION

1.Chemicals

Silver nitrate ($AgNO_3$), Polyvinylpyrrolidone (PVP_{K30}) and sodium borohydride ($NaBH_4$) were purchased from Sigma Aldrich.

2. Synthesis of Ag@PVP nanoparticles

PVP-capped silver nanoparticles (Ag@PVP) with a large specific surface area and a narrow size distribution were prepared according to the published protocol described by J.J.Wu et al. with some modifications [20]. A typical synthesis was carried out in a 100 ml, three-neck round-bottomed flask equipped with a condenser : 34 mg of $AgNO_3$ (0.01M) and 90 mg of PVP_{K30} (610^{-5} M) were dissolved in 50 ml of deionized water. This aqueous solution was first heated to 80°C in an oil bath, then 5 ml of a 0.01M freshly prepared $NaBH_4$ solution was quickly added. The reaction mixture was kept under reflux for 20 min with vigorous stirring. A deep yellow colloidal solution was obtained.

3. Characterization techniques

The morphology and size distribution of AgNPs were characterized by Transmission Electron Microscopy (TEM) using a JEOL-JEM microscope.

The optical absorbance spectrum of colloidal solution was acquired using a Perkin-Elmer Lambda 11 UV/Vis Spectrophotometer.

The photoluminescence measurements were carried out at room temperature using a Perkin-Elmer spectrophotometer (LS55) with a Xenon lamp source and a 420 nm excitation wavelength.

The Raman experiments were carried out using a Jobin-Yvon XploRa setup. The excitation laser beam (532 nm) was focused onto the sample through the 100X lens of a confocal microscope. The laser power was 15 mw and the Raman spectra were recorded with an accumulation time of 10s.

4. In vitro studies

HUVEC (human umbilical vein endothelial cells) were cultured in Dulbecco's Modified Eage's Medium (DMEM) containing 10% fetal calf serum (FCS) and 1% antibiotics (ATB/ L.Glutamine) at 37°C under a humidified atmosphere in a CO_2 (5%) incubator. One day prior to the experiments, the cells were detached in trypsin–EDTA and grown in a complete medium in 48-well plates at 25 000 cells per well. Silver solutions were injected into the cells at 500µl per well. After 24h of incubation, the MTT assay was performed to determine cell viability [21]. After incubation, the cells were examined under an inverted microscope to ascertain the density of violet spots corresponding to active mitochondria in order to exclude the potential mitochondrial toxicity of the compounds. The supernatant was then removed and the precipitate was dissolved in isopropanol and quantified at 405 nm in a multi-well plate reader (BIOTEK EL800).

RESULTS AND DISCUSSION

1. Characterization of PVP-capped silver nanoparticles (Ag@PVP)

TEM micrographs (figure1) showed spherical AgNPs with an average size of 20 nm coated with PVP molecules. These AgNPs were prepared in an aqueous medium with 0.01 $mol.L^{-1}$ of precursor concentration of and a molar ratio R=0.06 (R = nPVP/ nAg).

The optical absorption of the Ag@PVP solution exhibited a symmetric band at around 400 nm (Figure 2). This band arises from the Localized

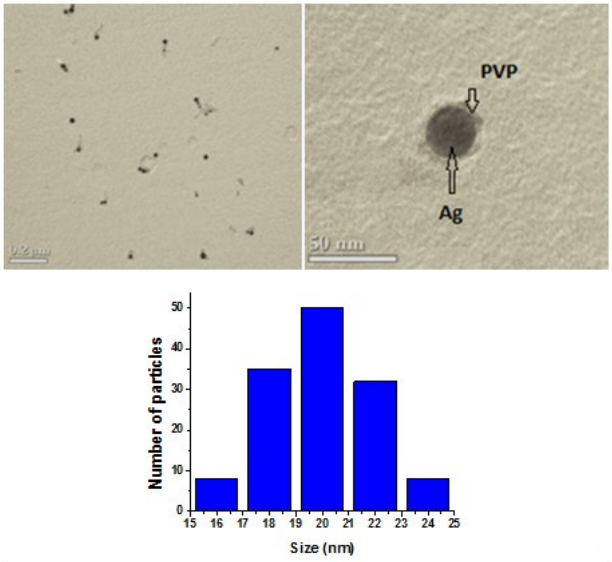

Figure 1: TEM micrographs of Ag@PVP and the corresponding histogram size distribution.

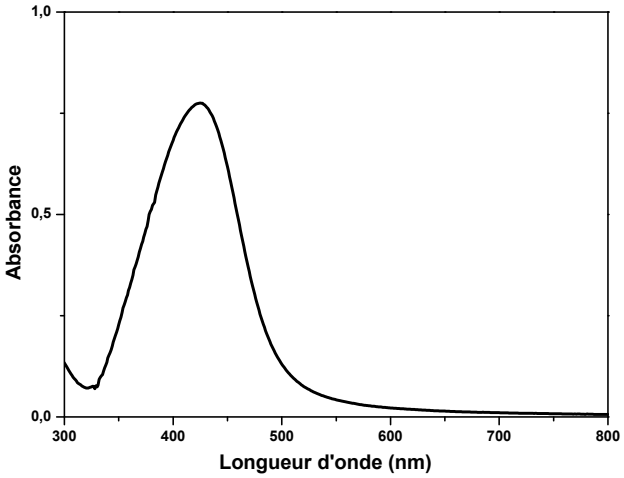

Figure 2: UV-Vis absorption spectrum of Ag@PVP colloid

Surface Plasmon Resonance (LSPR) attributed to the collective oscillation of conduction electrons induced by an electromagnetic field [22] and confirmed the formation of spherical silver nanoparticles with a good distribution as illustrated in Figure 1.

The photoluminescence spectrum of PVP-capped silver nanoparticles (Figure 3) showed an intense emission peak in the visible region at 435 nm when the synthesized Ag@PVP was excited with 420 nm radiation. Ratan Das et al. results showed that the PVP molecules excited with 380 nm to 460 nm wavelength provide any band in the emission visible region of Ag@PVP [23]. Therefore, the origin of the fluorescence of Ag@PVP can be attributed to the interband transition by the absorption of the incident radiation and the promotion of the d-band electrons of the silver nanoparticles to higher electronic states in the sp-band [24,25].

2. Adsorption of PVP on the Ag surface: SERS measurements

Several studies have been reported to clarify the interactions between silver nanoparticles and PVP molecules where two potential absorbing groups are available, a carboxyl and the pyrrolidyl nitrogen group. H.Wang et al based on FTIR spectroscopy suggested that these interactions depend on the silver nanoparticles size [26]. Using the SERS correlated through the DFT quantum chemical calculations, Phumlane.S.Mduli et al proved that the pyrrolidone are selectively adsorbed on silver surface via the non bonding electrons of the carbonyl group [27].

Thereby, Ag-O account for the interaction between PVP molecules and Ag surface and the formation of PVP capped silver nanoparticles.

2.1 SERS spectrum of Ag@PVP

The SERS spectrum of Ag@PVP (Figure 4) showed an intense peak located at 235 cm^{-1} attributed to the Ag-O vibration .This could be compared to the observed results by P. S. Mdluli et al. [27] and Gao et al [28] who showed the Ag–O vibration respectively at 223 cm^{-1} and 233 cm^{-1}. Furthermore, there is no band stretching in the 1500 cm^{-1} and 1700 cm^{-1} range which are the typical features of the C-N and C=O vibrations of PVP molecules.

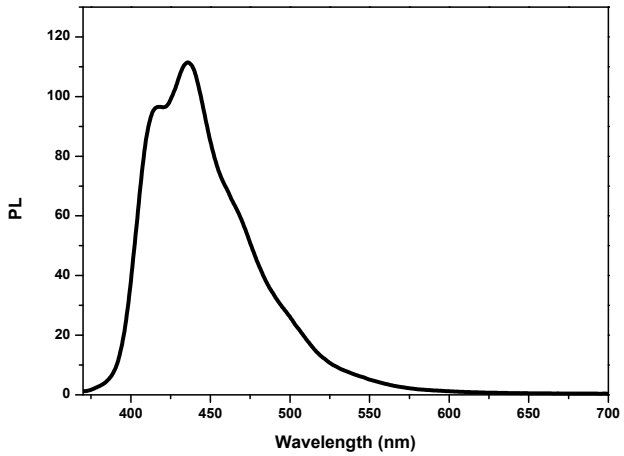

Figure 3: Photoluminescence spectrum of Ag@PVP colloid excited with 420 nm radiation).

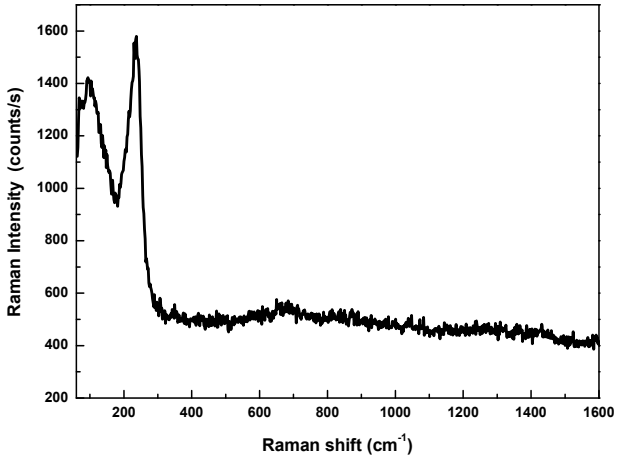

Figure 4: Raman spectrum of Ag@PVP colloid with 532 nm excitation wavelength, 1% of the initial power (15 mW) and 10s of accumulation time

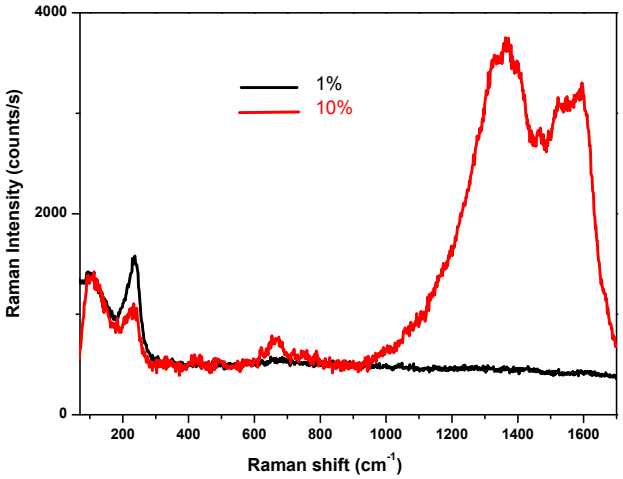

Figure 5: SERS spectra of Ag@PVP recorded with 1% and 10% of the incident power (15 mW) excited with 532 nm wavelength

2.2 Stability study of the Ag-O band

In order to study the stability of Ag@PVP we made remedies to the stability of Ag-O band by Surface Enhanced Raman Scattering. The incident laser power, incident laser wavelength and temperature were varied.

2.2.1 Incident power effect

SERS spectra of Ag@PVP were reccorded with 1% and 10% of the initial power (15 mW) excited with 532 nm to study the effect of incident power on the Ag-O band located at 235 cm^{-1} (Figure5).

When the Raman spectrum was recorded at 1% of the initial power, a strong band centred at 235 cm^{-1} was observed, whereas for the one recorded at 10%, the Ag-O intensity decreased and two vibration modes were located at 1369 cm^{-1} and 1590 cm^{-1} respectively. These vibration modes are assigned to the G and D bond characteristic of amorphous carbon [29]. The G peak is attributed to the E2g mode while the D band can be attributed to in-plane A1g (LA) zone-edge mode [30,31]. The increase of the incident power from 1% to 10% of initial power promoted the degradation of the PVP molecules adsorbed at the AgNP surface and the breaking of the Ag-O bond.

2.2.2 Incident laser wavelength effect

Two laser excitations, 532 nm and 638 nm, were used and the SERS spectra of Ag@PVP were shown in Figure 6.

The spectra were normalized to the same accumulation time and laser power in order to compare the relative intensities of the Raman scattering excited with different excitation lines. The increase of incident laser wavelength from 532 nm to 638 nm causes a decrease in the intensity of the Ag-O band from 50000 to 30000 (counts/s). As expected, the overall Raman intensity is at a maximum for excitation at 532 nm because this is the nearest in length to that of the silver nanoparticle resonance (LSPR). The incident power was limited to 1% of initial power to avoid the degradation of the organic molecules surrounded AgNPs by laser heating.

2.2.3 Temperature effect

We systematically studied the effect of increase of temperature on Raman band intensity. The

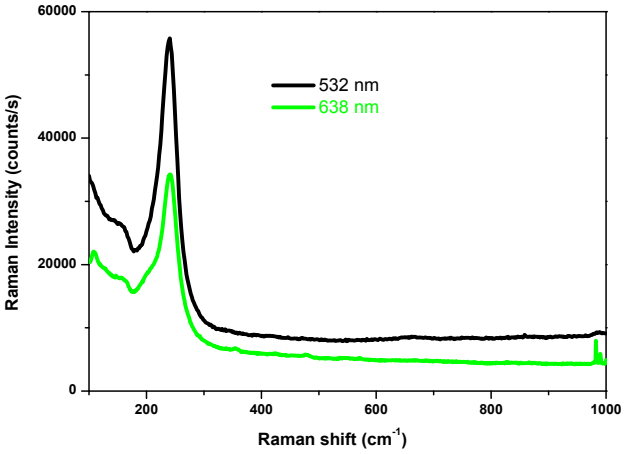

Figure 6: SERS spectra of Ag@PVP excited with two laser excitations: 532 nm and 638 nm and 1% of the initial power (15mW)

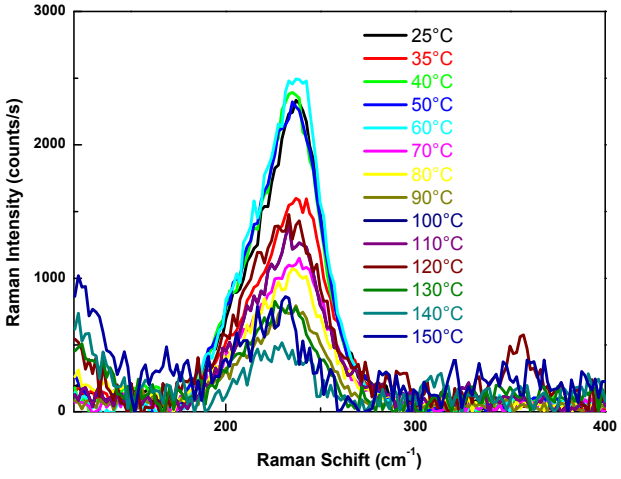

Figure 7: SERS spectra of Ag@PVP recorded in the 100 - 350 nm range excited with 532 nm at 1% of initial power (15 mW) at different temperatures

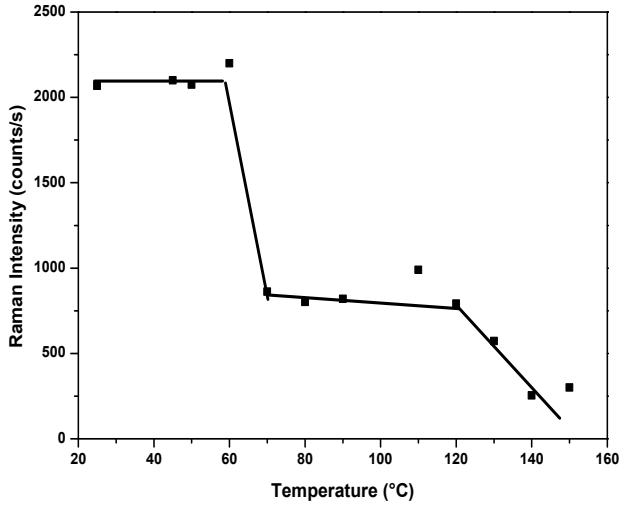

Figure 8: Variation of the Raman Intensity as a function of temperature (25°C - 150°C)

Raman-SERS spectra are presented in Figure 7.

By relying on figure 8 which showed the variation of Raman Intensity as a function of temperature, three temperature intervals can be seen: the signal strength was almost the same between 25 °C and 60 °C (5% change); a sharp decline in the intensity was observed at 70 °C (over 50%) and remained almost stable up to 110 °C; then it decreased to almost extinction at 140 °C. High temperatures could damage the chemical environment of the AgNPs and thus lead to a collapse of the Ag-PVP link.

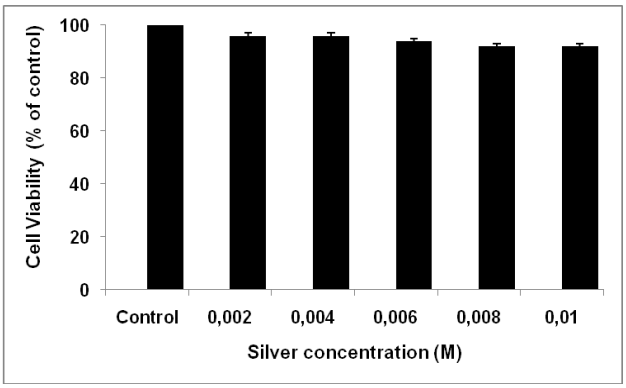

Figure 9: Histogram of the cytotoxicity of PVP-capped silver nanoparticles (Ag@PVP), cell viability is expressed as a percentage of untreated control culture

3. Cytotoxicity of Ag@PVP

The cytotoxicity of various concentrations of Ag@PVP solutions (0.002M to 0.01M) was tested and the resulting histogram (Figure 9) showed a great viability rate of the cells for all concentrations up to 90%.

The cell viability study result at different concentrations clearly showed the biocompatibility of Ag@PVP. This has been attributed to the presence of PVP capping which serves as a protective layer and reduces the release of deleterious silver ions. It has already been reported that the surface modified nanoparticles have good biocompatibility when compared to those of pristine nanoparticles [32]. Therefore, capping with a polymer protects the mitochondrial activity thereby improving the viability of cells exposed to nanoparticles. These properties can be advantageous and make the as-prepared Ag@PVP good candidates for biological and biomedical applications.

CONCLUSION

In this work PVP-capped spherical silver nanoparticles (Ag@PVP) were prepared by chemical reduction. UV-Visible and photoluminescence (PL) studies showed absorption and emission spectra in the visible range which are related to the LSPR properties.

Surface enhanced Raman spectroscopy (SERS) measurements of Ag@PVP showed a strong band located at 235 cm^{-1} attributed to the Ag-O interactions from the carbonyl group of the PVP molecules and silver surface. SERS measurements highlighted the stability of Ag-O band in a temperature range less than 70°C.

The cytotoxicity of the Ag@PVP solution was tested using the MTT test and showed a great viability rate of HUVEC cells which makes it a good candidate for biomedical applications.

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REFERENCES

[1] A. Meddahi-pellé; A. Legrand; L.Louedec; D.Letourneur; L.Leibler; *Angew.Chem.Int.Ed* **2014**, 53, 6369

[2] V.K. Vidhu; Daizy Philip, *Spectrochim. Acta A, Spectroscopic,* **2014**, 117, 102

[3] H.S. Desarkar; P. Kumbhakar; A.K. Mitra, *J.Lumin,* **2013**, 134, 1

[4] M.P. Mallin; C.J. Murphy; *Nano Lett,* **2002**, 2, 1235

[5] T. Pal; D.S. Maity; A. Ganguly, *Analyst,* **1986**, 111, 1413

[6] S. Link; Z.L. Wang; M.A. El-Sayed, *J. Phys. Chem. B,* **1999**, 103, 3529.

[7] E.M.S. Azzam; A.F.M. El-Frarrge; D.A. Ismail; A.A. Abd-Elaal, *J. Dispers. Sci. Technol,* **2011**, 32, 816

[8] Z. Zoya; A.M. Maqsood; F.M. Al-Nowaiserb, K. Zaheer, *Colloids Surf. B,* **2010**, 81, 587

[9] Y.G. Sun; Y.N. Xia, *Science,* **2002**, 298, 2176

[10] C. H. Bernard Ng; J.Yang; W. Yip Fan, *J. Phys. Chem. C,* **2008**, 112, 4141

[11] B. P. S Chauhan; R. Sardar, *Macromol*, **2004**, 37, 5136.

[12] C. E. Hoppe ; M.Lazzari ; I. Pardin˜as-Blanco ; M. Arturo Lo´pez-Quintela, *Langmuir,* **2006**, 22, 7027

[13] N. Giri1; R. K. Natarajan; S. Gunasekaran; S. Shreemathi, *Arch. Appl. Sci. Res,* **2011**, 3, 624

[14] S. Li ; P. Liu ; Q. Wang, *Appl. Surf. Sci,* **2012**, 263, 613

[15] M. Gao ; L.Sun ; Z.Wang ; Y. Zha, *Mat. Sci. Eng C,* **2013**, 33 , 397

[16] A. Kyrychenko ; O. M. Korsun ; Iurii I. Gubin ; Sergiy M. Kovalenko ; Oleg N. Kalugin, *J. Phys. Chem. C,* **2015**, 119, 7888

[17] A.M. Abdelghany ; M.Sh. Mekhail ; E.M. Abdelrazek ; M.M. Aboud, *J.Alloys Compd,* **2015**, 646, 326

[18] E. J. Kim ; J. H.Yeum ; J. H. Choi, *J. Mater. Sci. Technol, 2014*, 30 , 107

[19] S. Yu ; Y. Yin ; J. Chao ; M. Shen ; J. Liu, *Environ. Sci. Technol,* **2014**, 48, 403

[20] J.J. Wu ; G.J. Lee ; Y.S. Chen ; T.L. Hu, *Curr.Appl Phys,* **2012**, 12, 1

[21] G.Taju ; S. Abdul Majeed ; K.S.N Nambi ; A.S. Sahul, *Comp.Biochem.Physiol.C:Toxicol,* **2014**, 161, 41

[22] T. Endo; A. Shibata; Y. Yanagida; Y.Higo; T.Hatsuzawa. *Mater. Lett*, **2010**, 64, 2105

[23] R. Das; S. Sarkar, *Opt. Mater,* **2015**, 48, 203

[24] N. Jayaprakash; J. Judith Vijaya L; John Kennedy; K. Priadharsini; P. Palani, *Mater.Sci.Eng.C,* **2015**, 49, 316

[25] M.Fayaz; C.S. Tiwary; P.T. Kalaichelvan; R. Venkatesan, *Colloids.Surf.B,* **2010**, 75, 175

[26] H. Wang; X.Wang; S.Ding, *Mater. Chem. Phys*, **2005**, 94, 449

[27] P. S. Mdluli; N.M. Sosibo; N. Revaprasadu; P. Karamanis; J. Leszczynski, *J. Mol. Struct*, **2009**, 935, 32

[28] Y. Gao; P. Jiang; D.F. Liu; H.J. Yuan; X.Q. Yan; Z.P. Zhou; J.X. Wang, L. Song, L.F. Liu, W.Y. Zhou, G. Wang, C.Y. Wang, S.S. Xie, *J. Phys. Chem. B,* **2004**, 108, 12877.

[29] Y. Borodko; S. E. Habas; M. Koebel; P.Yang; H. Frei; Gabor A. Somorjai. *J.Phys.Chem.B* **2006**, 110, 23052

[30] R.N. Gayen; A.K. Pal, *Appl.Surf.Sci*, **2010**, 256, 6172

[31] D. Kang, M ; Hakamatsuka, K.Kojima; M. Tachibana, *Diamond.Relat.Mater,* **2010**, 19, 578

[32] S.R. Kumar; L.F. Marianna; S. Gianni; A.J. Nathanael; S.I. Hong; T.H. Oh; D. Mangalaraj, C. Viswanathan; N. Ponpandian, *Mater. Res. Exp*, **2014**, 1, 1.