Mapana J Sci, 15, 1 (2016), 1-16
ISSN 0975-3303 | doi:10.12723/mjs.36.1

Study of Role of Silver Nanoparticles on Spectroscopic Properties of a KetocyanineDye

J Thipperudrappa[*], U P Raghavendra[†], H R Deepa[‡] and M Basanagouda[§]

Abstract

The role of silver nanoparticles on spectroscopic properties of the ketocyaninedye 2,5-di[(E)-1-(4-dimethylaminophenyl) methylidine]-1-cyclopentanone (2,5-DMAPMC) has been investigated using absorption and fluorescence spectroscopy. Silver nanoparticles are synthesized by chemical reduction method and estimated size from SEM measurements is 22 nm. The changes in absorption spectrum of dye in the presence of silver nanoparticles suggest the possible interaction with silver nanoparticles. The Stern-Volmer plot of fluorescence quenching is found to be nonlinear showing positive deviation. The magnitude of quenching rate parameter and fluorescence lifetime measurements indicates the presence of both collisional and static quenching mechanisms. The binding constant and number of binding sites for static part of the quenching have been

---

[*] Department of Physics, BNM Institute of Technology, Bengaluru, India; jtrphy2007@gmail.com (Corresponding author)

[†] Department of Physics, Bangalore Institute of Technology, Bengaluru, India.;r.puttaswamy@gmail.com

[‡] Department of Physics, BNM Institute of Technology, Bengaluru , India; deepa_rshankar@yahoo.co.in

[§] P.G. Department of Studies in Chemistry, K.L.E. Society's P.C. Jabin Science College, Hubballi; India.mahanteshachem@gmail.com

estimated from the fluorescence data. The role of diffusion and electron transfer processes in fluorescence quenching mechanism has been discussed.

Keywords: Ketocyanine dye, Silver nanoparticles, Quenching, Binding site, Electron transfer

1. Introduction

Ketocyanine dyes are one of the important class of dyes which exhibit excellent solvatochromic behavior. These dyes are reported to be promising probes for monitoring micro-polarity, metal ion sensing, hydrogen-bond donating interaction, investigation of the cell membrane structures, evaluating the micro-environmental characteristics of biochemical and biological systems and many more [1-6]. A ketocyanine dye 2,5-di[(E)-1-(4-dimethylaminophenyl) methylidine] -1- cyclopentanone(2, 5-DMAPMC) is also reported to be exhibiting such interesting properties. Even though thorough investigations have been carried out with respect tophotophysics and photochemistry of 2,5-DMAPMC, there is a dearth of information on the interaction of this dye with nanoparticles in general and silver nanoparticles in particular.

The investigations on interaction of dyes with nanoparticles has attracted significant attention due to the fact that integration of nanotechnology with biology and medicine is expected to produce major advances in molecular diagnostics, therapeutics and bioengineering [7,8]. Among the metal nanomaterials, silver nanoparticles have received considerable attention due to their good antibacterial, antifungal and antiseptic properties. It has been reported that silver nanoparticles in the size regime 1-10 nm bind to HIV-1 virus and prevent it from bonding to host biological cells [9]. Excellent probe nature of 2,5-DMAPMC, and potential applications of dye-nanoparticle systems, motivated us to carry out the present work.

In continuation of our investigation on spectroscopic properties of fluorescent organic molecules and because of the importance of study of interaction of fluorescent molecules with silver nanoparticles, the present investigation is carried out. The aim of the present work is to systemically study the role of silver nanoparticles on absorption and fluorescence properties of 2,5-DMAPMC. Experimental data is analysed by using Benesi-Hildebrand model, Stern-Volmer (S-V) equation andRehm-Weller equation for electron transfer.

2. Material and Methods

2.1. Materials

Ketocyanine dye 2,5-DMAPMCwas synthesized in our lab and its structure is confirmed by NMR. The molecular structure of dye is shown in Figure 1. Methanol used in the present study is of spectroscopic grade and is obtained from S.D. Fine Chemicals Ltd., India. Analytical grade silver nitrate ($AgNO_3$) and sodium borohydride ($NaBH_4$) were also obtained from S.D.Fine Chemicals, India. Double distilled water was used in the preparation of aqueous solutions.

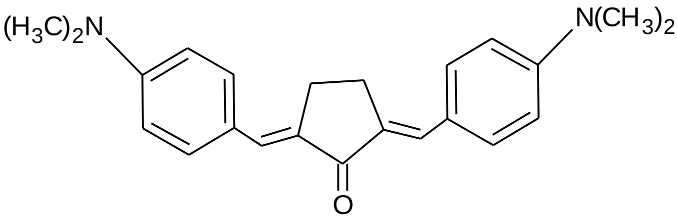

Fig 1. Molecular structure of 2,5-DMAPMC

2.2. Absorption, fluorescence and lifetime measurements

The absorption and fluorescence spectra were recorded in methanol in the presence of silver nanoparticles by using UV-VIS spectrophotometer (Model: Shimadzu UV-1800) and fluorescence spectrophotometer (Model: Hitachi F-2700) respectively at room temperature. In order to reduce the self-absorption effects, measurements were carried out at low concentration of dye ($2x10^{-5}$ M).

Fluorescence lifetime measurements were carried out using a picosecond time correlated single photon counting (TCSPC-Model:

chronosBH, Make: ISSINCUSA) spectrometer. This set up has lifetime measurement range of 10^{-11}s to 10^{-2}s. The excitation source used is a light emitting diode with excitation wavelength of 480 nm. The detector system comprises of photomultiplier tube (PMT) which operates in the wavelength range 185nm – 850nm. The emission wavelength is at 607 nm. The minimum time channel width of this setup is 820 fs and it has total useful count rate up to 4 MHz. The observed fluorescence decay curves were analyzed using a reconvolution program, which is an iterative nonlinear least squares fit method. The goodness of fit was evaluated by $\chi 2$ criterion and visual inspection of residuals of the fitted function to the data. In the present case the decay profile was bi-exponential.

2.3. Synthesis and estimation of size of silver nanoparticles

Silver nanoparticles were synthesized by chemical reduction method which involves the reduction of silver nitrate solution by sodium borohydride solution. The details of synthesis were reported in our previous work [10]. In this method, sodium borohydride and silver nitrate were used in 6:1 ratio for better stability of silver nanoparticles as suggested by Solomon [11].

The size of silver nanoparticles was estimated by employing Scanning Electron Microscope (SEM) using CARL ZEISS ULTRA-55 FESEM under high vacuum mode. For the preparation of substrate for SEM, the sample holder was covered with double sided carbon tape and a small quantity of silver nanoparticles was added to the carbon tape. This was degassed for 24 hours in vacuum desicator. The SEM image of silver nanoparticles is shown in Figure. 2. The SEM image indicates the presence of spherical nanoparticles with few other shapes as well. The average size of silver nanoparticles is found to be 22 nm.

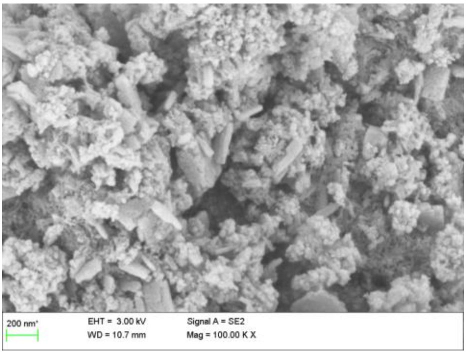

Fig 2.SEM image of silver nanoparticles.

2.4. Cyclic voltametric measurements

The oxidation potential of 2,5-DMAPMCin methanol was measured by cyclic voltametry experiment on the AUTOLAB electrochemical device using a cell equipped with three electrodes, the working electrode (Pt disc), the counter electrode (Pt disc) and the reference electrode (Saturated Calomel Electrode (SCE)). The cyclic voltammograms were recorded at a scan rate of 100 mV S^{-1}.

3. Results and Discussion

3.1. Role of silver nanoparticles on absorption characteristics of 2,5-DMAPMC

The optical absorption spectrum of synthesized silver nanoparticles in water shows a surface plasmon resonance peak at 388 nm(Fig.3).

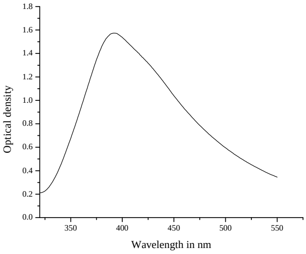

Fig3.Absorption spectrum of silver nanoparticles.

The optical absorption spectrum of dye 2,5-DMAPMC in methanol was recorded in the absence and presence of silver nanoparticles. The concentration of silver nanoparticles was varied from 0 to 31.72µM with respect to the analytical concentration of silver. The absorption spectrum of 2,5-DMAPMC in the absence and presence of silver nanoparticles is shown in Figure 4.

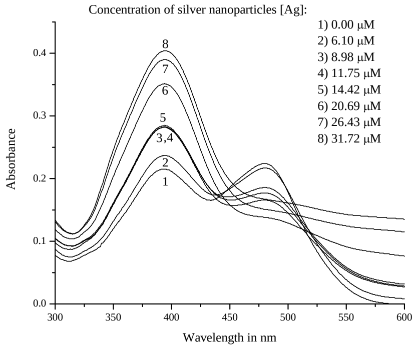

Fig 4. Absorption spectra of 2,5-DMAPMCin the absence and presence of silver nanoparticles.

The increase in optical density at the absorption maximum of dye and broadening of spectra is observed in the presence of silver nanoparticles. Also, the absorption maximum of dye is slightly blue shifted with the addition of silver nanoparticles. These changes in absorption spectrum indicate the possible interaction between 2,5-DMAPMC and silver nanoparticles. The changes in intensity of absorption peak as a result of formation of surface complex can be used to obtain association constant (k_a) of dye with silver nanoparticles in the ground state according to Benesi-Hildebrand equation as given below [12].

$$\frac{C}{\Delta A} = \frac{1}{\Delta\varepsilon_a} + \frac{1}{\Delta\varepsilon_a k_a [Ag]} \tag{1}$$

Where C is the concentration of dye, ΔA is the change in absorbance of dye with and without silver nanoparticles at its $\lambda_{\max}$, $\Delta\varepsilon_a$ is the change in absorption coefficient and $[Ag]$ is the analytical concentration of silver. The plot of $\frac{C}{\Delta A}$ versus $\frac{1}{[Ag]}$ according to equation (1) is found to be linear and is shown in Figure5.

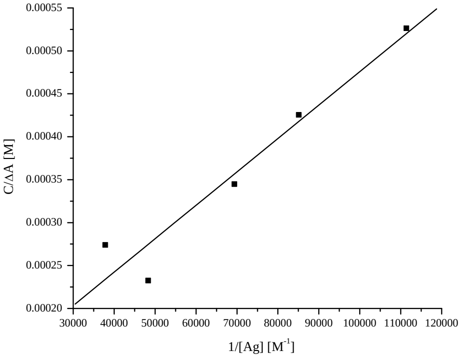

Fig 5.Plot of C/ΔA versus 1/[Ag] for 2,5-DMAPMC.

From the slope and intercept of this plot, $k_a$value is found to be 2.22 x 10^4 M^{-1}. The value of $k_a$is within the range of those previously reported for other dyes [10, 13-15]. This indicates the strong association between silver nanoparticles and the dye investigated.

3.2. Influence of silver nanoparticles on fluorescence characteristics of 2,5-DMAPMC

The steady state fluorescence spectrum of 2,5-DMAPMCin methanol is recorded in the absence and presence of silver nanoparticles. The fluorescence spectrum of 2,5-DMAPMCin the absence and presence of silver nanoparticles is shown in Figure 6.

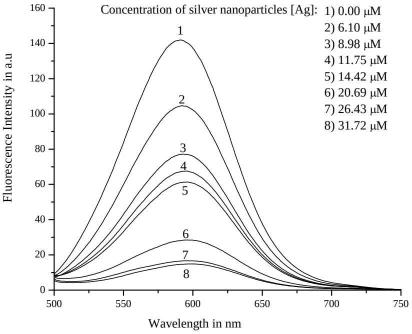

Fig 6. Fluorescence spectra of 2,5-DMAPMCin the absence and presence of silver nanoparticles.

From, Figure. 6, it is noticed that as the concentration of silver nanoparticles increases, the shape of fluorescence band does not change but there is a red shift of 8 nm as the concentration of silver nanoparticles is increased from 0 to 31.72 μM. Also, there is an appreciable quenching of fluorescence intensity of the dye with increase in the concentration of silver nanoparticles. To understand the quenching of fluorescence intensity, Stern-Volmer (SV) plot is drawn as shown in Figure 7according to the following equation [16]:

$$\frac{I_0}{I} = 1 + K_{SV}[Ag] \tag{2}$$

whereI_0 and I are fluorescence intensities of dye respectively in the absence and presence of silver nanoparticles and K_{SV} is Stern-Volmer quenching constant. From Figure 7, it is clear that SV plot of fluorescence quenching is non-linear showing positive deviation. The value of K_{SV} was determined from the lower portion of SV plot and is found to be 3.37 x 10^4 M^{-1}. The quenching rate parameterk_q (= K_{SV}/τ_0) is found to be 1.07 x 10^{15} $M^{-1}s^{-1}$ where τ_0 is the fluorescence lifetime of 2,5-DMAPMCin the absence of silver nanoparticles (0.0315 ns). It is well known that the value of maximum collisional k_q for various quenchers to bio molecules is of the order of 10^{10} $M^{-1}s^{-1}$[16]. But, in the present case, higher quenching rate parameter of the order of $10^{15}$$M^{-1}s^{-1}$was obtained. This shows that quenching of 2,5-DMAPMCby silver nanoparticles

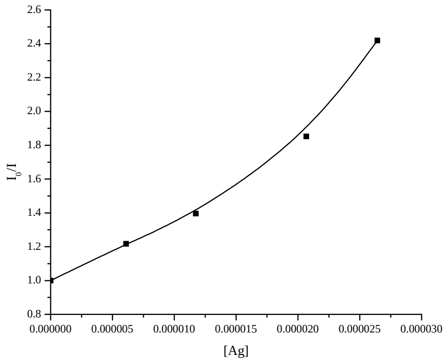

Fig 7. SV plot for 2,5-DMAPMC.

could be due to static quenching, which arises because of the formation of complex between the dye and silver nanoparticles. In order to find out whether quenching is purely static, fluorescence lifetime (τ) measurements were carried out. The typical fluorescence decay profile in the absence and presence of silver nanoparticles is shown in Figure 8. The fluorescence decay curves of 2,5-DMAPMC with and without silver nanoparticles are double exponential according to

$$I\ (t) = a_1 exp\ (-t/\tau_1) + a_2 exp\ (-t/\tau_2)$$

where the subscripts 1 and 2 refer to the shorter and longer pre-exponential components respectively of the decay and, $a_1$and $a_2$are the corresponding relative amplitudes. The lifetime values were obtained by fitting experimental decay profiles to double exponential function (using reconvolution procedure) to get χ^2 values very close to unity. From Figure 8, it is clear that fluorescence lifetime of 2,5-DMAPMCdid not change with increase in the concentration of silver nanoparticles (overlapping of decay profiles). Therefore, positive deviation in SV plot is mainly due to static quenching process [16].

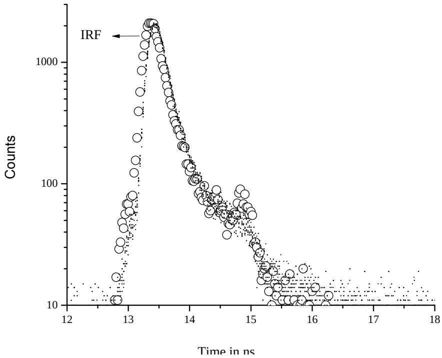

Fig 8. Fluorescence decay profile of 2,5-DMAPMCin the absence and presence of silver nanoparticles.

3.3. Estimation of binding constant and number of binding sites

For the static quenching, if it is assumed that there are independent binding sites to a set of equivalent sites on a macromolecule, the apparent binding constant and the number of binding sites can be determined according to the following equation [17].

$$\log\left[\frac{I_0-I}{I}\right]= \log k_b + n \log [Ag] \tag{3}$$

where k_b is the binding constant of silver nanoparticles with 2,5-DMAPMC, which can be determined from the intercept of the plot of $\log\left[\frac{I_0-I}{I}\right]$ versus $\log[Ag]$ as shown in Figure 9.

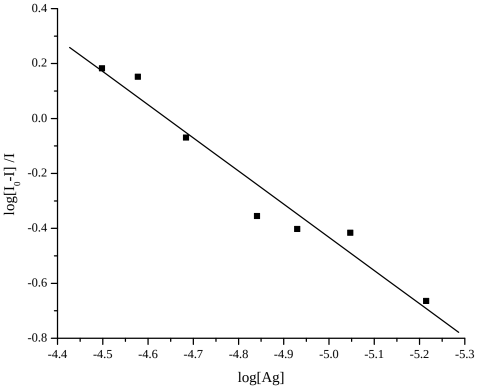

Fig 9. Plot of $\log\left[\frac{I_0-I}{I}\right]$ versus log [Ag] for 2,5-DMAPMC.

The calculated value of binding constant (k_b) is 4 x $10^5 M^{-1}$and number of binding sites (n) is 1.2. The value of 'n' implies that there exists only one binding site in 2,5-DMAPMCfor silver nanoparticles.

3.4. Role of diffusion in fluorescence quenching

To investigate the role of diffusion in fluorescence quenching, the diffusion rate parameter $k_d$is estimated using Umberger-Lamer equation [18] as given below.

$$k_d = 4\pi N' DPR \tag{4}$$

where N' is Avogadro's number per millimole, D is the sum of diffusion coefficients of dye and silver nanoparticles, and is calculated using Stokes-Einstein relation [19], P is the probability of quenching per encounter and is taken to be unity, and R is the sum of molecular radii of dye and silver nanoparticles. The radius of dye molecule was estimated using the method suggested by J.T.

Edward [20]. The value of k_d calculated using equation (4) is found to be 14.85 x 10^{10} $M^{-1}s^{-1}$, it is clear that value of k_d is smaller than k_q. This indicates that the fluorescence quenching of 2,5-DMAPMCby silver nanoparticles is not just controlled by diffusion. This is the expected result for static quenching process.

3.5. Role of energy transfer and electron transfer in fluorescence quenching

Since there is no overlap of absorption spectrum of silver nanoparticles with the emission spectrum of 2,5-DMAPMC, the possibility of energy transfer process between silver nanoparticles and 2,5-DMAPMCis ruled out. Hence the study of electron transfer process is taken up in the present investigation. The role of electron transfer in fluorescence quenching of 2,5-DMAPMCby silver nanoparticles is explained by the well-known Rehm-Weller formula [21]. Rehm-Weller formula given by equation (5) is used to understand the thermodynamic feasibility of the excited singlet state electron transfer reaction.

$$\Delta G_{et} = E_{1/2}^{(ox)} - E_{1/2}^{(red)} - E^{*} + C \quad (5)$$

whereΔG_{et} is the free energy change for the electron transfer reaction, $E_{1/2}^{(ox)}$ is the oxidation potential of 2,5-DMAPMC(0.0029 V as obtained from cyclic voltametry measurements) and $E_{1/2}^{(red)}$ is the reduction potential of silver nanoparticles. The reduction potential of silver nanoparticles (- 0.09 V) with silver as reference electrode is taken from literature [22]. The reported reduction potential of silver nanoparticles is converted against saturated calomel electrode (SCE) by subtracting 0.3 V from the measured potential [23]. $E^{*}$is the excited singlet state energy of 2,5-DMAPMC(2.59eV) which is calculated using the crossing point of emission and excitation spectra and C is the coulombic term (0.00245eV). The ΔG_{et} value has been calculated and is found to be -2.20 eV. The higher negative value of ΔG_{et} indicates the presence of electron transfer process which is thermodynamically favorable [24, 25]. In order to understand the extent of contribution of electron transfer process in overall quenching mechanism, the rate constant for electron transfer $k_{et}$is calculated according toequation (6) in accordance with Marcus theory [26].

$$k_{et} = \nu \exp\left(\frac{-\Delta G^*}{kT}\right) \tag{6}$$

where ΔG^* is free energy of activation and is given by

$$\Delta G^* = \frac{(\Delta G_{et} + \lambda)^2}{4 \times \lambda} \tag{7}$$

and v is the frequency factor whose value ranges from 10^{12} to 10^{14}s^{-1} depending on the systems [27, 28]. In the present study, maximum value of v (=10^{14} s^{-1}) is considered for k_{et}calculation. In equation (8) λ is the total reorganization energy, given by the sum of the internal reorganization energy λ_{in} and solvent reorganization energy λ_s:

$$\lambda = \lambda_{in} + \lambda_s \tag{8}$$

λ_{in}has an insignificant contribution, which is an expectable fact in diffusion conditions [29]. In case of electron transfer between organic molecules diffusing freely in solution, the solvent reorganization energy $\lambda_s$is considered to be the most important term [30].The value of $\lambda_s$is calculated according to equation (9)

$$\lambda_S = e^2\left(\frac{1}{2r_D} + \frac{1}{2r_A} - \frac{1}{r_{DA}}\right) \times \left(\frac{1}{\mu^2} - \frac{1}{\varepsilon}\right) \tag{9}$$

where μ and ε are refractive index and dielectric constant of solvent (methanol), $r_A$is the radius of electron acceptor (4.34Å for 2,5-DMAPMC), r_D is radius of electron donor (silver nanoparticles, 220 Å) and $r_{DA}$is considered to be equal to the sum of r_D and $r_A$values. The value of k_{et} calculated according to equation (6) is 3.08 x 10^5 s^{-1}. The magnitude of k_{et} indicates the substantial contribution of electron transfer in overall quenching mechanism.

4. Conclusions

The role of silver nanoparticles on 2,5-DMAPMChas been studied. The absorption spectral changes indicate the possible interaction between the dye and silver nanoparticles. The observed fluorescence quenching of 2,5-DMAPMCby silver nanoparticles is due to static quenching mechanism. For static quenching the binding constant and the number of binding sites were estimated from fluorescence data. It has been observed that fluorescence quenching is not solely controlled by diffusion. Electron transfer between the dye and silver nanoparticles also playsa role in overall quenching mechanism. To the present day, this is the first study on roleof silver nanoparticles on spectral properties of 2,5-DEAPMC.

Acknowledgements

Authors thank Visvesvaraya Technological University, Belgaum, India for providing financial assistance to carry out this work through Research Grant Scheme (Grant No.VTU/Aca./2011-12/A-9/763 dated 5th May 2012).

References

[1] A.Lobnik and O.S. Wolfbeis, “Polarity studies on ormosils using a solvatochromicfluorescent probe”, *Analyst*, vol. 123, pp.2247-2250, 1998. doi:10.1039/a804583f.

[2] C. Reichardt,“Solvatochromic Dyes as Solvent Polarity Indicators”, *Chem. Rev.,*vol. 94, pp. 2319-2358, 1994.

[3] A.O. Doroshenko, A.V. Grigorovich, E.A.Posokhov, A.G.Pivovarenko and A.P.Demchenko, “Bis-Azacrown Derivative of Di-Benzylidene-Cyclopentanone as Alkali EarthIon Chelating Probe Spectroscopic Properties, Proton Accepting ability and Complex Formation with Mg2+ andBa2+ Ions”, *Mol. Eng.,* vol. 8, pp.199-215, 1998.doi:10.1023/A:1008393201193.

[4] A.O. Doroshenko, L.B. Sychevskaya, A.V. Grygorovych and V.G.Pivovarenko, “Fluorescence Probing of Cell Membranes with Azacrown Substituted Ketocyanine Dyes”, *J.Fluoresc.,* vol. 12, pp. 455-464, 2002.doi:10.1023/A:1021374212498.

[5] K. Rurack, M.L.Dekhtyar, J.L. Bricks, U.Resch-Genger and W.Rettig, “Quantum Yield Switching of Fluorescence by Selectively Bridging Single and Double Bonds in Chalcones: Involvement of Two Different Types of Conical

Intersections”, *J. Phys.Chem A*, vol. 103, pp. 9626-9635, 1999. doi:10.1021/jp992878m.

[6] M.V. Barnabas, A.D. Liu, A.D.Trifunac, V.V.Krongauz and C.T. Chang,“Solvent effects on the photochemistry of a ketocyanine dye and its functional analog. Michler'sketone”, *J.Phys Chem.*, vol. 96, pp. 212-217, 1992. doi:10.1021/j100180a041.

[7] N.L. Rosi and C.A.Mirkin,“Nanostructures in biodiagnostics”,*Chem. Rev.*, vol. 105, pp. 1547-1562, 2005. doi:10.1021/cr030067f.

[8] P.Alivisatos, “The use of nanocrystals in biological detection”, *Nat.Biotechnol.*,vol. 22, pp. 47-52, 2004. doi:10.1038/nbt927.

[9] J.L. Elechiguerra, J.L. Burt, J.R.Morones, A. Camacho-Bragado, X. Gao, H.H. Lara and M.J. Yacaman, “Interaction of silver nanoparticles with HIV-1”, *J.Nanobiotechnol.*, vol. 3, pp. 6-16, 2005. doi:10.1186/1477-3155-3-6.

[10] H.R.Deepa, H.M. Suresh Kumar, M. Basanagouda and J.Thipperudrappa,“Influence of silver nanoparticles on absorption and fluorescence properties of laser dyes”,*Can. J. Phys.,* vol. 92, pp. 163-167, 2014. doi:10.1139/cjp-2013-0133.

[11] S.D.Solomon, M.Bahadory, A.V.Jeyarajasingam, S.A.Rutkowsky, C.Boritzand L.Mulfinger, “Synthesis and study of Silver Nanoparticles”, *J. Chem. Educ.*, vol. 84, pp. 322-325, 2007.doi:10.1021/ed084p322.

[12] H.A.Benesi and J.H. Hildebrand,“A Spectrophotometric Investigation of the Interaction of Iodine with Aromatic Hydrocarbons”, *J. Am. Chem. Soc.,*vol. 71, pp. 2703-2707, 1949.doi:10.1021/ja01176a030.

[13] M. Umadevi, N.A.Sridevi, A.S.Sharmila, B.J.M. Rajkumar, M.B. Mary, P.Vanelle, T. Terme and O. Khoumeri,“Influence of Silver Nanoparticles on 2,3-Bis(Chloromethyl)Anthracene-1,4,9,10-Tetraone”, *J.Fluoresc.*, vol. 20, pp.153-161, 2010, doi:10.1007/s10895-009-0533-4.

[14] P. Manikandan and V.Ramakrishnan,"Spectral Investigations on N-(2-Methylthiophenyl)-2-Hydroxy-1-Naphthaldimine by Silver Nanoparticles: Quenching", *J.Fluoresc.*, vol. 21, pp. 693-699, 2011. doi:10.1007/s10895-010-0757-3.

[15] S.R.Kavitha, M.Umadevi, P.Vanelle, T.Terme, O.Khoumeri and B. Sridhar, "Spectroscopic studiesof 1,4-dimethoxy-2,3-dimethylanthracene-9,10-dione on plasmonicsilver nanoparticles", *Spectrochim.Acta. Part A* ,vol. 133, pp. 472-479, 2014. doi:10.1016/j.saa.2014.06.007.

[16] J.R.Lakowicz,: Principles of fluorescence spectroscopy", 3rd edn., New York; Springer, 2006.

[17] Y.J.Hu, Y. Liu, J. Wang, X.H. Xiao and S.S.Qu,"Study of the interaction between monoammoniumglycyrrhizinate and bovine serum albumin", *J. Pharm. Biomed. Anal.*, vol. 36, pp. 915-919, 2004. doi:10.1016/j.jpba.2004.08.021.

[18] J.Q.Umberger and V.K.LaMer, "The Kinetics of Diffusion Controlled Molecular andIonic Reactions in Solution as Determined by Measurements of the Quenching of Fluorescence", *J.Am. Chem. Soc.*, vol. 67, pp. 1099-1109, 1945.doi:10.1021/ja01223a023.

[19] G.H. Malimath and G.C.Chikkur,"Role of energy migration in an organic liquid scintillator system in the 20–70°C temperature range", *Appl.Radiat.Isot.*, vol. 45, pp. 143-147, 1994. doi:10.1016/0969-8043(94)90002-7.

[20] J.T.Edward, "Molecular volumes and the Stokes–Einstein equation", *J. Chem. Educ.*vol. 47, pp. 261-270, 1970.doi:10.1021/ed047p261.

[21] D. Rehm and A. Weller,"Kinetics of Fluorescence Quenching by Electron and H-Atom Transfer", *Isr. J. Chem.*, vol. 8, pp. 259-271, 1970. doi:10.1002/ijch.197000029.

[22] Z.J. Jiang, C.Y. Liu andY.J. Li,"Electrochemical Studies of Silver Nanoparticles Tethered onSilica Sphere", *Chem.Lett.*, vol. 33, pp. 498-499, 2004. doi:10.1246/cl.2004.498.

[23] V.Anbazhagan and R.Renganathan,"Investigation of the fluorescence quenching of 2,3- diazabicyclo[2.2.2]oct-2-ene

(DBO) by certain substituted uracils", *J.Lumin.*, vol. 129, pp. 382-388, 2009. doi:10.1016/j.jlumin.2008.11.006.

[24] K. Kikuchi, T.Niwa, Y. Takahashi, H. Ikeda and T.Miyashi,"Quenching mechanism in a highly exothermic region of the Rehm-Weller relationship for electron-transfer fluorescence quenching", *J. Phys. Chem.*, vol. 97, pp. 5070-5073, 1993. doi:10.1021/j100121a037.

[25] S.Nath, H. Pal, D.K.Palit, A.V.Sapre and J.P. Mittal,"Steady-State and Time-Resolved Studies onPhotoinduced Interaction of Phenothiazine and 10-Methylphenothiazine with Chloroalkanes", *J. Phys. Chem.A,*vol 102, pp. 5822-5830, 1998.doi:10.1021/jp9726145.

[26] R.A. Marcus and N.Sutin,"Electron Transfer in Chemistry and Biology", *Biochim.Biophys.Acta,* vol. 811, pp. 265-322, 1985. doi:10.1016/0304-4173(85)90014-X

[27] J.M.Chen, T.I. Ho and C.Y.Mou,"Experimental investigation of excited-state electron-transfer reaction: effects of free energy and solvent on rates", *J. Phys. Chem.*, vol. 94, pp. 2889-2896, 1990. doi:10.1021/j100370a030.

[28] N.Sutin,"Nuclear, electronic, and frequency factors in electron transfer reactions"*Acc.Chem. Res.*, vol 15, pp. 275-282, 1982.doi:10.1021/ar00081a002.

[29] C.Tablet and M.Hillebrand, "Quenching of the fluorescence of 3-carboxy-5,6-benzocoumarin by aromatic amines",*J.Photochem.Photobiol. A*:*Chem.*, vol. 189, pp.73-79,2007. doi:10.1016/j.jphotochem.2007.01.009.

[30] H.N. Gosh, H. Pal, D.K.Palit, T. Mukherjee and J.P. Mittal,"Interaction of the excitedsinglet state of disubstitutedanthraquinones with aliphatic and aromatic amines: a fluorescence quenching study", *J.Photochem.Photobiol.A Chem.*, vol. 73, pp. 17-22, 1993.doi:10.1016/1010-6030(93)80028-8.