IJFANS INTERNATIONAL JOURNAL OF FOOD AND NUTRITIONAL SCIENCES
ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 11, Iss 11, Dec 2022

AIEE BEHAVIOUR OF CTAB STABILISED PNs FOR DETERMINATION OF ATP

D. K. Dalavi
Associate Professor, Department of Chemistry,
Bhogawati Mahavidyalaya, Kurukali, Affiliated to Shivaji University, Kolhapur.
Email: dkdalavi@gmail.com

ABSTRACT

Cetyl trimethyl ammonium bromide stabilized perylene nanoparticles (CTAB-PNs) 20 to 31 nm in diameter were prepared by modified reprecipitation method in aqueous solution under ultrasonic treatment. A spectrofluorimetric method for the quantitative determination of ATP based on the fluorescence quenching of CTAB-PNs by ATP in aqueous solution was proposed. Under the most favourable conditions, the fluorescence intensity of PNs monitored at excitation wavelength λ_{ex} = 380 nm was quenched by gradual addition of concentration of ATP. The fluorescence quenching results found to fit into Stern-Volmer relation in the range of 0.5 to 15 µM with a correlation coefficient of 0.9970. The limit of detection was 3.92 µM. The method based on fluorescence quenching was successfully applied for quantitative determination of ATP.

KEYWORDS: Adenosine-5-triphosphate (ATP), Perylene nanoparticles (PNs), Fluorescence quenching.

INTRODUCTION

In recent years, Aggregation Induced Enhanced Emission (AIEE)[1-8] organic nanoparticles have attracted considerable research interests owing to their selective binding with analyte molecules, more variability and flexibility in materials synthesis and nanoparticle preparation, high water solubility, peculiar size-dependent optical and electronic properties.[1-4] Fluorescent inorganic semiconductor or metal nanoparticles have been extensively investigated due to their quantum confinement effects. Since such effects were not expected for organic nanoparticles, least attempts have been made to synthesis and characterize to them. The methods of preparation of inorganic nanoparticles are lengthy, complicated and expensive. In contrast, preparation of fluorescent organic nanoparticles is easy, rapid and cost effective method by means of 'reprecipitation method'.[2-6] Reprecipitation is carried out by rapidly injecting microamounts of a solution of an organic compound in a good solvent to a poor solvent. The good solvent disperses the compound and a sudden change in environment for organic molecules induce precipitation in the form of nano or microcrystal dispersion.

In this report, fluorescent, water-soluble perylene nanoparticles were prepared by modified reprecipitation method. Aqueous suspension of perylene nanoparticles exhibits large Stoke's shifted enhanced emission owing to specific nanoclusters formed by π-π stacking and fluorescence quenching studies of perylene nanoparticles by the gradually addition of Adenosine-5-triphosphate (ATP) which linear fit into the conventional linear Stern-Volmer relationship.

Adenosine-5-triphosphate (ATP) is the prime energy source in living organisms. Adenosine-5-monophosphate AMP and ATP plays a significant role in the regulation of cellular metabolism and biochemical pathways in cell physiology and extensively used as an indicator for cell viability and cell injury in living organisms. Many diseases demonstrate an abnormal content of AMP, such as cardiovascular disease,[9] parkinsonism,[10] and Alzheimer's.[11] Therefore, sensitive and selective determination of ATP is crucial for clinical diagnosis as well as biochemical study.

To date, several approaches have been developed for the recognition of ATP, such as chromatography, fluorescent biosensor, fluorescent chemosensors, bioluminescence, chemiluminescence, electrochemical.[12-15] colorimetric detection.[16] ATP aptamer based methods. However, the chromatography based methods have tedious separation of sample and lower precision of detection. The aptamer-based ATP detection is generally labelled aptamer methods. The new fluorescent biosensor based on fluorescence quenching method with high sensitivity and selectivity has been proposed for the determination of ATP.

EXPERIMENTAL

1 Reagents

All chemicals used were of analytical reagent grade and used as received without further purification. All aqueous solutions were prepared with doubly distilled water. Perylene was purchased from Sigma Aldrich. Cetyl trimethyl ammonium bromide (CTAB) procured from Spectrochem Pvt. Ltd. Mumbai. ATP was purchased from s d fine-chem Ltd. (Mumbai, India).

2 Instrumentation

The absorption spectrum was acquired at room temperature on UV-3600 Shimadzu UV−VIS−NIR spectrophotometer with the use of 1.0 cm quartz cell. Fluorescence measurement of solutions was made with PC based spectrofluorophotometer (JASCO Model FP-8300, Japan). Both excitation and emission slits were fixed at 10 nm. An excitation wavelength of 380 nm was obtained from the excitation spectrum and the emission spectrum was monitored at this excitation wavelength. The particle size distribution and zeta potential of PNs in aqueous suspension was measured by dynamic light scattering (DLS) with a Zeta Sizer Nano ZS (Malvern Instruments Ltd., U. K.). A scanning electron microscope, SEM (JEON-6360 Japan) was used to examine the morphology and size of the nanoparticles. The pH of solutions was measured with digital pH meter model LI-120 (ELICO Hyderabad, India) with a combined glass electrode.

3 Preparation of Perylene nanoparticles

Aqueous suspension of Perylene nanoparticles were prepared by the modified reprecipitation method in presence of CTAB surfactant.[17-19] 0.1 ml of a perylene solution in acetone (1 mM) was injected by a microsyringe into 100 ml CTAB aqueous solution (0.5 mM) with vigorously stirring at ambient temperature to assemble a pale-yellow colloid. Then the content was sonicated for 30 min at room temperature to disperse the nanoparticles into aqueous solution. Hence, stable dispersions of perylene nanoparticles were formed due to CTAB surfactant.

RESULTS AND DISCUSSION

1 Particles size distribution PNs

In the present work, cationic surfactant CTAB used as an additive was able to control the size and shape of organic nanoparticles obtained by the reprecipitation method. The particle size distribution of nanoparticles in aqueous suspension was examined by Dynamic Light Scattering (DLS). The average particle size of perylene nanoparticle is about 24 nm and a range of particle size distribution from 20 nm to 31 nm.

2 Photophysical properties of nanoparticles

The photophysical properties of perylene nanoparticles have provided information about the formation of nanoparticles and have also facilitated to investigate the suitability of nanoparticles for sensing relevance. In recent years, UV-vis absorption spectroscopy was the most commonly used to obtain qualitative and/or quantitative knowledge concerning the aggregation of nanoparticles.[20] UV-vis absorption spectra of perylene nanoparticles in aqueous suspension and dilute solution of perylene in acetone displays in Fig. 1 reveals the absorption maximum attributed at 470 nm of perylene nanoparticle was red-shifted the structured spectrum of dilute solution in acetone with maximum at 434 nm. In general, the J-structure those molecules arrange into a slanted stack, and the transition to the lower couple excited state of molecules is allowed. Accordingly, the absorption is red-shifted and the emission is stronger than that of the monomer. However, in case of perylene nanoparticle emission is broad and quiet weak. These results recommend that the molecules in nanoparticles may be oriented in a less optimal way of J-aggregation.[20-21] The bathochromic shift (red shift) indicates that the molecules undergo aggregation.

3 Stoke's shift

The Stoke's shift estimated as a difference between excitation and fluorescence energy for perylene nanoparticles suspension is 8832 cm^{-1}. This value is significantly larger than the Stoke's shift of dilute solution is 356 cm^{-1}. The observed large Stoke's shift of nanoparticles is recognized to the aggregation of molecules by л-л interaction between closely stacked adjacent molecules due to this a gradual increase in the excitonic coupling effect by which the exciton relaxes to an energetically lower lying excited state and

hence, the emission of perylene nanoparticles originates from a lower lying excited state as compared to the isolated perylene molecules.[17,22] As a result, perylene nanoparticles with a large Stoke's shift which leads to enhanced emission of aggregated molecules.

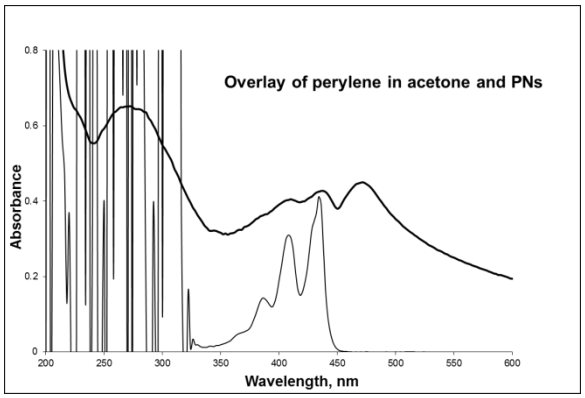

Fig. 2: UV-vis absorption spectra of dilute solution of perylene in acetone (dark line) and perylene nanoparticles in aqueous solution (soft line).

4 Effect of coexisting species on fluorescence quenching of PNs by ATP

To explore the selectivity of the method using perylene nanoparticles[5] as a probe for ATP in aqueous solution, the changes in fluorescence intensity of the probe were measured in the presence of co-existing ions such as CH_3COO^-, Mg^{++}, Na+, IO_3^-, SO_4^{2-}, CO_3^{2-}, NO_3^-, BrO_3^-, Zn^{++}, Cl^-, Br^-, Ag^+, ClO_3^-, SO_3^-, F^- and mixture of co-existing anions solution of 10 µM solution concentrations each. The fluorescence quenching response only for ATP solution and no other anions caused observable quenching in these experiments. This is because of ATP specifically adsorbed on the positively charged surface of PNs than other anion resulting in fluorescence quenching.

5 Selective fluorescence quenching of PNs by

The development of analytical method of high sensitivity and selectivity has been always a challenge in analytical chemistry. In conventional photophysical quenching experiments, Stern-Volmer relationship is used to describe quenching processes whereby the fluorescence intensity and lifetime data of nanoparticles are fitted to eqn (1).

$$\frac{F_0}{F} or \frac{\tau_0}{\tau} = K_{SV}[Q]+1 = k_q\tau_0[Q] \quad (1)$$

Where F_0 and F are the fluorescence intensities of the fluorophore, perylene nanoparticles in the absence and presence of quencher, respectively. τ_0 and τ are the respective equivalent excited state lifetimes in the absence and presence of quencher, K_{sv} is the Stern-Volmer quenching constant, [Q] is the concentration of the quenching species and k_q is the quenching rate constant.

The fluorescence emission spectra using CTAB stabilized Perylene nanoparticles as a probe with different amounts of ATP in an aqueous solution were recorded in Fig. 2. The figure reveals the fluorescence intensity of the probe as PNs was significantly quenched regularly in the gradual addition of an ATP in the concentration range of 0.5 - 15 µM. The quenching results fit into the conventional linear Stern-Volmer relationship.

$$\frac{F_0}{F} = K_{SV}[Q]+1 \quad (2)$$

The plot of changes in fluorescence intensity (F_0/F) *versus* the increasing concentration of ATP is shown in Fig 3. The obtained experimental data for ATP fitted well to the following empirical equation.

$$F_0 / F = 0.206x + 1 \quad (3)$$

The linear relationship in the range of 0.5-15.0 µM has a correlation coefficient of R^2= 0.9970 (n=3). The limit of detection based on the definition by equation,[17]

$$LOD = 3\sigma / k, \tag{4}$$

where σ is the standard deviation of the y- intercepts of the regression lines and k is the slope of calibration graph. Here, the limit of detection (LOD) was 3.92 µM. The method has the advantages of lower detection limit (LOD), hence, this probe is selectively and sensitively recognized for ATP.

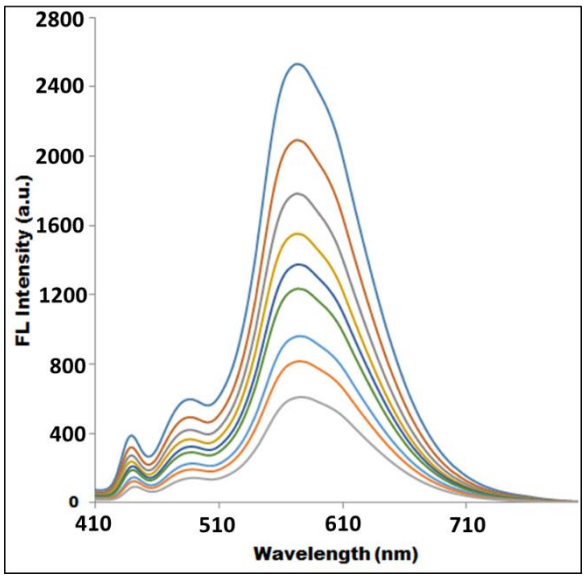

Fig. 2: Fluorescence emission spectra of CTAB-PNs in presence of different amounts of ATP (0.5-15.0 µM).

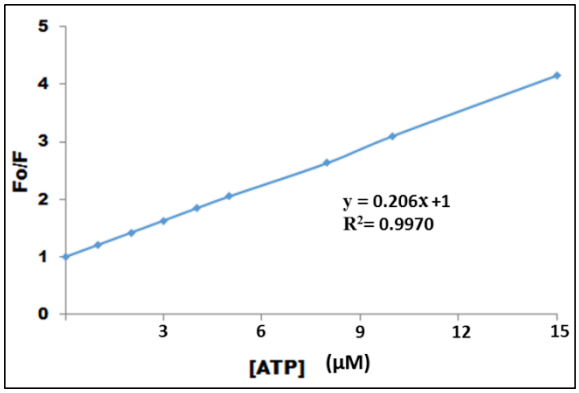

Fig. 3: Stern-Volmer plot of F_0/F versus addition of different amounts of OHCbl solution.

6. Fluorescence quenching mechanism

The quenching of fluorescence intensity of a fluorophore by a competing deactivating process resulting from the specific interaction between fluorophore and quencher substance is known as fluorescence quenching. Several mechanisms have been proposed for the fluorescence quenching emission from nanoparticles, including non-radiative recombination pathways, energy transferring, charge diverting, electron transfer process, collisional quenching and ground state complex formation, surface adsorption and others. Quenching of a fluorophore arises by the formation of a non-fluorescent complex between a fluorophore and another fluorophore or non-fluorescent molecule. This mechanism is known as static quenching. In static quenching, two molecules interact by proton-coupled electron transfer through the formation of hydrogen bonds. In aqueous solutions, electrostatic, steric and hydrophobic forces control the formation of hydrogen bonds. When this complex absorbs energy from light, the excited state immediately returns to the ground state without emission of a photon and the molecules do not emit fluorescent light. The Stern-Volmer model decisively predicts, if quenching is dynamic in nature, the plots of relative intensities

and relative lifetimes should be identical and if quenching is static in nature, the lifetime of the probe will remain unchanged.

Careful observation of Fig. 2 reveals that the emission of nanoparticles is quenched significantly and regularly on the addition of ATP solution in the concentration range of 0.5-15.0 µM. As per the binding mode, CTAB stabilized the surface of the monodispersed PNs and is bound to ATP by electrostatic interaction to form stable non-fluorescent ground state complex, which contributes fluorescence quenching of nanoparticles.

The probable fluorescence quenching mechanism of CTAB-PNs by solution of ATP and formation of non-fluorescent ground state complex.

7 Determination of ATP from pharmaceutical samples

Under the most favourable conditions mentioned above, the present method was fruitfully applied to determine ATP from synthetic and different environmental water samples by a standard addition method. Recovery values obtained are in the range 95.10% to 99.15%, which demonstrated the method based on the 'off-fluorescence' of CTAB-PNs that can effectively recognize ATP.

CONCLUSIONS

CTAB-PNs by reprecipitation method was applied successfully as a fluorescent probe for the determination of ATP from from pharmaceutical formulations using fluorescence quenching method. The calibration curve was linear over the concentration range 0.5-15 µM with a correlation coefficient of 0.9970. The developed method is simple, rapid, reproducible, selective and free from interference of excipients. The content of ATP in synthetic and pharmaceutical samples determined by the present method agreed with the reference method with satisfactory recovery.

ACKNOWLEDGEMENTS

We gratefully acknowledge Department of Chemistry, Shivaji University, Kolhapur and Department of Chemistry, Bhogawati Mahavidyalaya, Kurukali for providing research facilities, equipments, etc.

REFERENCES

1. H. Yan, H. Li, *Sens. Actuators, B*, 148, **2010**, 81.
2. An, B.K.; Kwon, S.K.; Jung, S.D.; Park, S. Y., *J. Am. Chem. Soc.* **2002,** *124* (48), 14410-14415.
3. Dalavi, D. K.; Kamble, A.; Bhopate, D. P.; Mahajan, P. G.; Kolekar, G. B.; Patil, S. R., **2015,** *5* (85), 69371-69377.
4. H.-B. Fu, J.-N. Yao, *J. Am. Chem. Soc.*, 123, **2001**, 1434.
5. Dalavi, D. K.; Bhopate, D.; Bagawan, A.; Gore, A.; Desai, N.; Kamble, A.; Mahajan, P.; Kolekar, G.; Patil, S. R., *Anal. Methods*, **2014,** *6* (17), 6948-6955.
6. Dalavi, D. K.; Suryawanshi, S. B.; Kolekar, G. B.; Patil, S. R., *Anal. Methods*, **2018**.
7. D. Horn, J. Rieger, *Angew. Chem. Int. Ed.*, 40, **2001**, 4330.
8. AiFang Zheng, JinLong Chen, Genhua Wu, Ganlin Wu, Yuan Guang Zhang, HePing Wei, *Spectrochimica Acta Part A*, **2009**, 74, 265-270.
9. Ronald Eisler, *Biological Report*, **1986**, 85(1.6).
10. A. K. Singh, V. K. Gupta, Barkha Gupta, *Analytica Chimica Acta*, **2007**, 585, 171-178.
11. Dinesh Mohan, Charles U. Pittman Jr., *Journal of Hazardous materials*, **2006**, 137, 762-811.
12. Takashi Sumida, Tamami Ikenoue, Kazuhide Hamada, Akhmad Sabarudin, Mitsuko Oshima, Shoji Motomizu, *Talanta*, **2005**, 68, 388-393.
13. Takashi Sumida, Akhmad Sabarudin, Mitsuko Oshima, Shoji Motomizu, *Analytical Sciences*, **2006**, 22, 161-164.
14. Magdalena Stanislawska, Beata Janasik,Wojciech Wasowicz, *Talanta*, **2013**, 117, 14-19.
15. Marcio F. Bergamini, Daniela P. dos Santos, Maria Valnice Boldrin Zanoni, *Sensors and Actuators B*, **2007**, 123, 902–908.
16. M.A. Elbagermi, A.I Alajtal, H.G.M. Edwards, *APCBEE Procedia*, **2013**, **5**, 378-382.

17. Asli Baysal, Suleyman Akman, Seda Demir, Mehmet Kahraman, *Microchemical Journal*, **2011**, 99, 421-424.
18. Takashi Sumida, Akhmad Sabarudin, Mitsuko Oshima, Shoji Motomizu, *Analytical Sciences*, **2006**, 22, 161-164.
19. Magdalena Stanislawska, Beata Janasik,Wojciech Wasowicz, *Talanta*, **2013**, 117, 14-19.
20. Bente Gammelgaard, Yi-ping Liao, Ole Jons, *Analytica Chimica Acta*, **1997**, 354, 107-113.
21. Hong-Bing Fu, Jian-Nian Yao, *J. Am. Chem. Soc.*, **2001**, 123, 1434-1439.
22. Roohollah Torabi Kachoosangi, Richard G. Compton, *Sensors and Actuators B*, **2013**, 178, 555–562.