International Journal of Research in Engineering and Science (IJRES)
ISSN (Online): 2320-9364, ISSN (Print): 2320-9356
www.ijres.org Volume 10 Issue 12 ǁ December 2022 ǁ PP. 432-438

New 2-amino-5-butyl-4-(pyren-1-yl)isophthalonitrile aqueous nanoparticles: Synthesis, photophysical nature and cell imaging potential

Ananada S. Kudale[a,e]*, Anna D. Gophane[b], Chetan B. Aware[c], Santosh B. Kamble[d], Vasant B. Helavi[a]

a. Department of Chemistry, Rajaram College, Kolhapur 416004, MS, India.
b. Department of Zoology, Shivaji University, Kolhapur 416004, MS, India
c. Department of Biotechnology, Shivaji University, Kolhapur 416004, MS, India
d. Yashavantrao Chavan Institute of Science (Autonomous), Karmveer Bhaurao Patil University, Satara, MS, India
e. Regional Forensic Science Laboratory, Tararani Chowk, Kolhapur 416001, MS, India

**Corresponding Author: Email: as.kudale@gmail.com, as.kudale@gov.in*

Abstract

In this paper, we report the preparation of bright fluorescent 2-amino-5-butyl-4-(pyren-1-yl)isophthalonitrile (2A5B4PIPN) nanoparticles (NPs) in aqueous medium by reprecipitation method without use of stabilising agent and used in fluorescence cell imaging. The average particle size of fluorescent NPs in water was found to be 90 nm which was confirmed by Field Emission Scanning Electron Microscopy (FESEM) as well as dynamic light scattering (DLS) shows average particle size in water is 101 nm. It was observed that the NPs of 2A5B4PIPN showed blue shift (278 nm) in UV-vis spectra as compared to 2A5B4PIPN in acetone (345 nm) solution. Interestingly, NPs of 2A5B4PIPN had comparatively shorter fluorescence lifetime (5.40×10^{-11} s) than that in dilute acetone solution (9.86×10^{-11}s). Furthermore, we successfully screened the potential of newly prepared NPs of 2A5B4PIPN for the fluorescence imaging of human oral mucous cells.

Keywords: *Fluorescence; Organic nanoparticles; Human oral mucous cell imaging; Pyrene; Dicyanoaniline.*

Date of Submission: 09-12-2022 Date of acceptance: 23-12-2022

I. Rationale

Recent years, synthesis of the organic nanoparticles and their uses in bio-imaging probe attracted the attention of several researchers, which is due to their simple protocol, easy to handle and can be prepared in large quantity with low cost. [1-6] thus, due to size-dependent optical and electronic properties, fluorescent organic nanoparticles (FONPs) have been employed in different scientific areas [7-14]. ONPs shown to be much safer as compared with inorganic NPs (INPs), as INPs are toxic and non-biodegradable, thus, limit their use in the biomedical applications [15]. Consequently, several FONPs were prepared, studied and screened in various bio-imaging applications [16-19].

Very recently, the use of structurally diverse mono/di/tri-heterocyclyl-2,6-dicyanoanilines for the imaging of MCF-7 and THP-1 cell lines [20] and utilization of these entities for the DNA and RNA binders as efficient non-toxic ds-RNA selective fluorescent probes were reported [21]. Therefore, expands the scope of 2,6-dicyanoanilines and related compounds for consideration in biological applications. Various methodology of synthesis of 2,6-dicyanoanilines and associated compounds in the several fields were described and evaluated in number of research articles [22-31]. However, use of 2,6-dicyanoanilines and associated compounds have never been explored for preparation of FONPs and use them for bio-imaging or any other biological application. On the other hand, pyrene based systems are popular in the literature for the fluorescence and imaging applications [32-34].

Taking account into uses of dicyanoanilines and pyrene derivatives, we design to combine these two moieties, thus, prepared fluorescent 2-amino-5-butyl-4-(pyren-1-yl) isophthalonitrile (2A5B4PIPN) (Fig. 1). Furthermore, 2A5B4PIPN used to prepare FONPs in aqueous solution by reprecipitation method without use of any stabilizers (sodium dodecyl sulfate (SDS), cetyltrimethylammonium bromide (CTAB) *etc*. As the biological systems are more compatible with aqueous medium, therefore, we further explored the potential of 2A5B4PIPN NPs for fluorescence imaging of human oral mucous cells. The formation of 2A5B4PIPN NPs were determined

by UV-vis spectroscopy, fluorescence spectroscopy and controlled size was confirmed by dynamic Light Scattering (DLS) and Field Emission Scanning Electron Microscopy (FESEM) techniques.

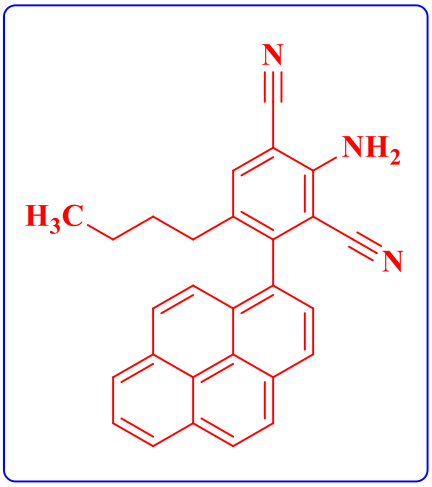

Fig. 1. Structure of 2A5B4PIPN.

Specifications Table

Subject area	*Organic chemistry, fluorescence spectroscopy, physical organic chemistry*
Compound	*2-amino-5-butyl-4-(pyren-1-yl)isophthalonitrile aqueous nanoparticles*
Data category	*Spectral, physiochemical*
Data acquisition format	*UV-vis, fluorescence, DLS and FESEM, fluorescence microscopy*
Data type	*Analyzed*
Procedure	*New 2-amino-5-butyl-4-(pyren-1-yl)isophthalonitrile aqueous nanoparticles prepared by reprecipitation method.*
Data accessibility	*Manuscript*

II. Experimental

2.1 Materials and methods

The 2A5B4PIPN was used as it is synthesized by using synthetic method reported in our earlier report [35]. UV-vis absorption spectra were recorded on UV-vis double beam spectrophotometer (Analytik Jena Specord Plus) and fluorescence spectra were recorded on spectrofluorometer (FP-8300, JASCO, Japan). The required aqueous solution was prepared with doubly distilled water. The particle size distribution of NPs in aqueous suspension was measured by Dynamic Light Scattering (DLS) analysis on Malvern Instruments Ltd., UK. The size and morphology of NPs were observed by using FESEM on FEI Nova Nano SEM 450 instrument. The fluorescence lifetimes were recorded on Horiba Sci. NL (Japan) in the time scan 500 ps to 1µsec by Time Correlated Single Photon Counting (TCSPC) method at respective emission and excitation wavelength. Cell imaging experiments were carried out by using Zeiss Axio-scope A1 trinocular phase contrast microscope with fluorescent attachment.

2.2 Preparation of 2A5B4PIPN NPs

3 mL of a 2A5B4PIPN solution in acetone (0.0159 M) was injected by a micro syringe into 70 mL distilled water with vigorous stirring for 2 h and further resulting mixture was sonicated for 25 min at 27 ^{o}C to form stable nanoparticles of square shaped morphology.

2.3 Cell imaging experiments

In the present work, we screened imaging potential of 2A5B4PIPN NPs in aqueous suspension against human oral mucous epithelial cells. Ethical approval for fluorescence imaging study on human oral mucous cells was obtained from the D. Y. Patil Medical College, Kasba Bawada, Kolhapur, MS, India (Ref. No. DYPMCK/166/2018; Dated 21/08/2018). The experiments were performed on oral mucous cells of two healthy male donors in the age group of 30-35 by informed consents.

2.3.1Preparation of slides for imaging study

Clean steel spoon was used to collect the oral epithelial cells by scratching spoon against oral epithelium of the mouth and thin smear was prepared on clean glass slides to obtain uniform layer of collected

cells. After 5 min, a few drops of NPs (200 µL) and diluted solution of 4′,6-diamidino-2-phenylindole (DAPI) were added over the separate slides on which smear of mucous cell were prepared and covered with cover glass. The slides were kept for incubation for 15 min and then the cells were observed using bright field and FITC spectrum blue (Chroma 3100) comprising 25 mm diameter filters, excitation filter (D350/50), beam splitter (400 DCLP) and emission filter (D460/50) fluorescence microscope at 400× magnification and images were captured.

III. Results and Discussion

3.1 Comparison of photophysical properties of 2A5B4PIPN and its aqueous NPs

The UV-vis spectra of dilute solution of 2A5B4PIPN in acetone and aqueous NPs were recorded (Fig. 2. a and b). The UV-vis spectra of aqueous NPs showed blue shift of about 67 nm as compared to dilute solution of 2A5B4PIPN in acetone (Table 1) which indicates that the formation of the H-aggregates in the aqueous medium [36].

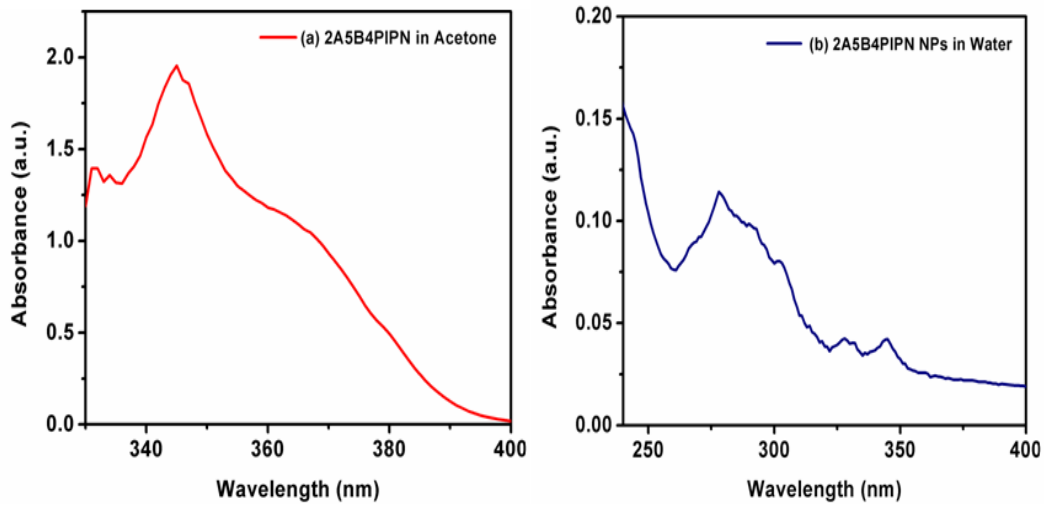

Fig. 2. UV-vis absorption spectra of a) 2A5B4PIPN in acetone**;** b)2A5B4PIPN NPs in water

Table 1 UV-vis observations of 2A5B4PIPN.

UV-vis spectra of 2A5B4PIPN	Concentration	Wavelength (λ_{max}) nm
in acetone	1.06×10^{-3} M	345
aqueous NPs	0.68×10^{-3} M	278

The fluorescence excitation and emission spectra of the dilute solution of 2A5B4PIPN in acetone and corresponding aqueous NPs is depicted in Fig. 3 and Fig. 4 which reflects the significant change in the photophysical behavior of the compound in dilute solution and in NPs form (Table 2). This variation in excitation and emission spectra of 2A5B4PIPN in acetone and aqueous suspension may be a consequence of presence of NPs of varying sizes.

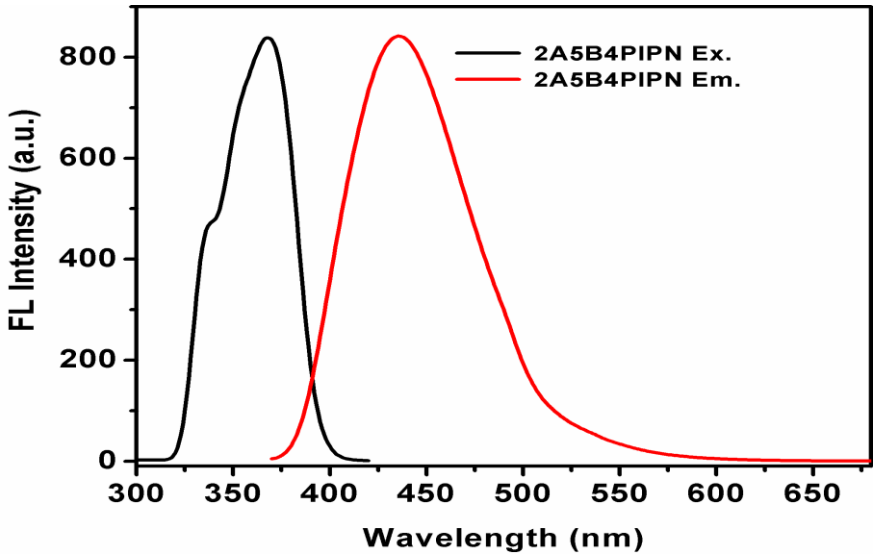

Fig. 3. Fluorescence excitation and emission spectra of 2A5B4PIPN in acetone.

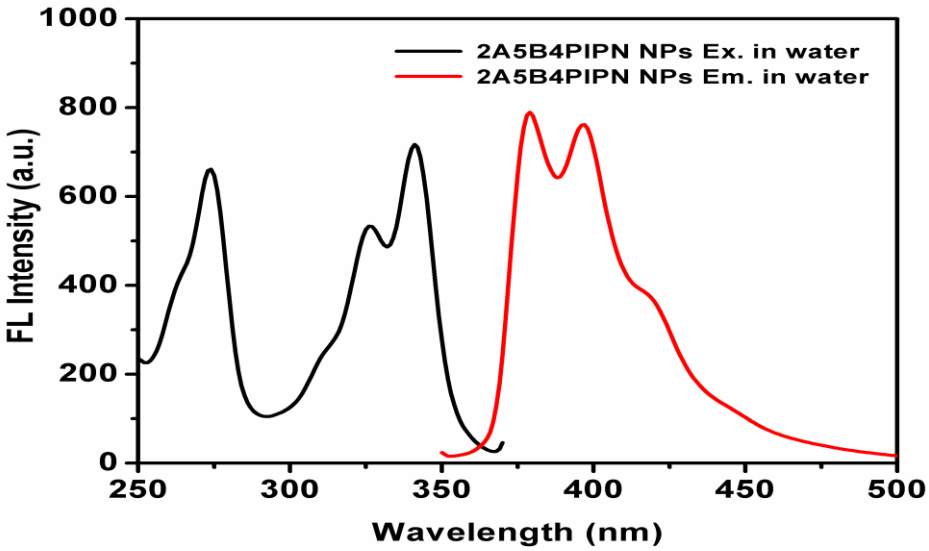

Fig. 4. Fluorescence excitation and emission spectra of 2A5B4PIPN NPs in water

Table 2 Fluorescence observations of 2A5B4PIPN in acetone and in aqueous NPs

Fluorescence spectra	λ Ex (nm)	λ Em (nm)	Intensity	Conc^{n}	FL parameters	
					Slit width	Sensitivity
2A5B4PIPN in acetone	367	435	841	1.06×10^{-3} M	5-5′	Very low
2A5B4PIPN aqueous NPs	341	379	789	0.68×10^{-3} M	5-5′	Low

3.2 Fluorescence lifetime of 2A5B4PIPN NPs

Figure 5 shows the fluorescence decay profile of aqueous NPs (a) and 2A5B4PIPN (b) in dilute acetonitrile solution. It was observed that aqueous NPs of 2A5B4PIPN have shorter lifetime (5.40×10^{-11} s) than that in acetonitrile solution (9.86×10^{-11} s). The shorter lifetime of 2A5B4PIPN NPs may be a result of fast re-combination of excited state electron to ground state as compared to its dilute solution in acetone.

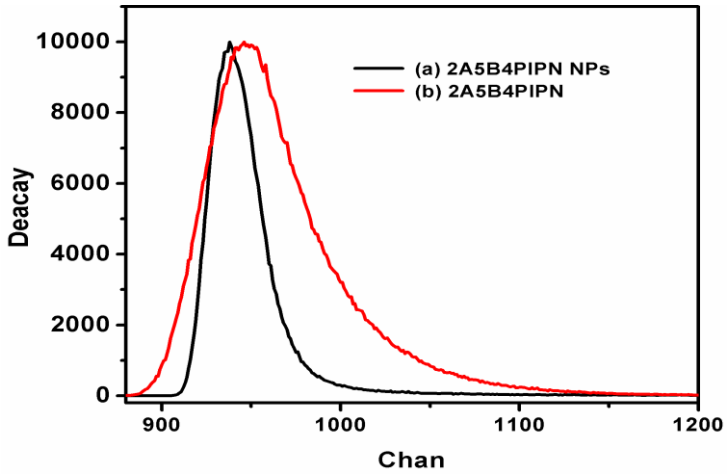

Fig.5. Fluorescence lifetime of a) 2A5B4PIPN NPs in water; b) 2A5B4PIPN in acetone.

3.3 Size distribution and morphology

The formation of NPs in aqueous medium was characterized by DLS (Fig. 6) and FESEM (Fig. 7). The DLS analysis shows the average particle size of aqueous 2A5B4PIPN NPs fall around 101 nm. The FESEM analysis shows the formation of square shaped morphology with average particle size of 90 nm. The larger size of 2A5B4PIPN NPs in aqueous solution exhibited in DLS analysis may be attributed to hydrodynamic layer present on the surface of NPs which certainly allows its stability in aqueous medium.

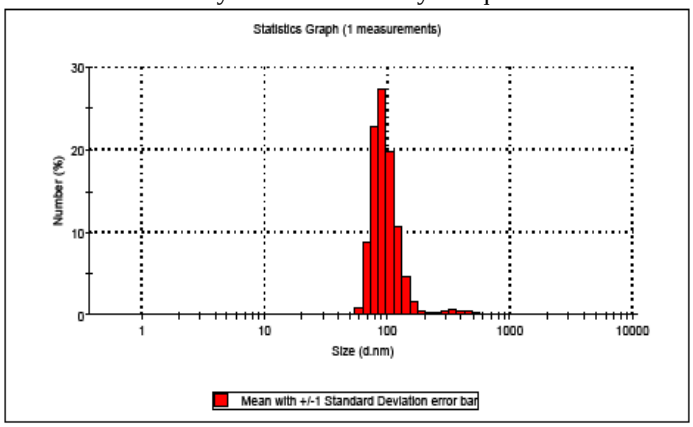

Fig. 6. Size distribution histograms of 2A5B4PIPN aqueous NPs.

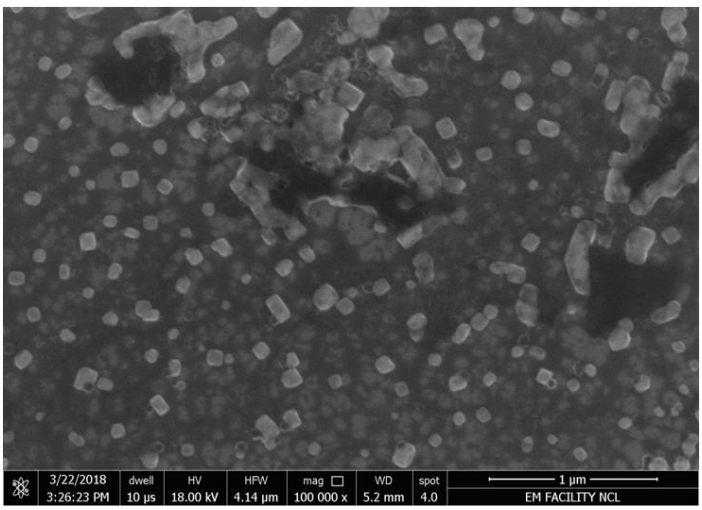

Fig. 7. FESEM Image of 2A5B4PIPN NPs.

3.4 Cell imaging

We screened the fluorescence imaging proficiency of 2A5B4PIPN NPs in reference with DAPI which is a well known nuclear counter stain and images were captured under bright field and fluorescence microscope as presented in Fig. 8 (A, B, C, a, b, c). It was noted that the cells incubated with the DAPI, the nuclei are stained blue while in the case of cells incubated using 2A5B4PIPN NPs showed affinity mainly to the membranes of nuclei as well as cell membrane and membrane part of cell organelles. Thus cells incubated with 2A5B4PIPN NPs showed bright blue emission and explores the picture of cell with cell organelles.

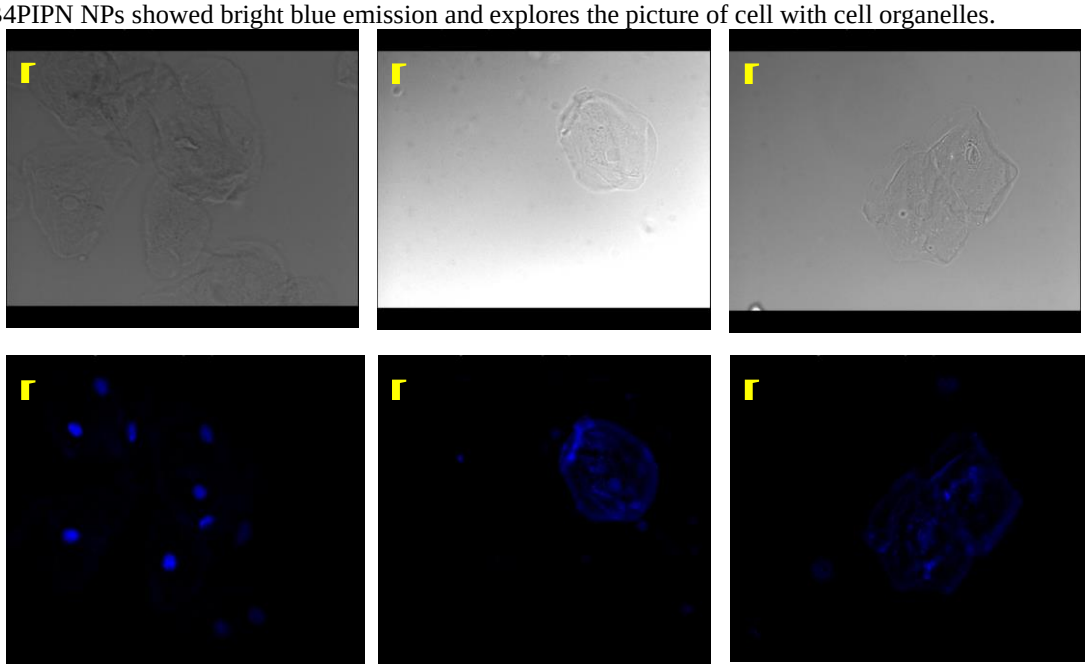

Fig. 8.i)Upper part- bright field microscopic images of human oral mucous cells incubated with [A]: DAPI, [B] and [C]: 2A5B4PIPN NPs; **ii)Lower part**- images under fluorescence microscope incubated with [a]: DAPI, [b] and [c]: 2A5B4PIPN NPs.

IV. Conclusion

We successfully synthesized 2-amino-5-butyl-4-(pyren-1-yl)isophthalonitrile NPs in aqueous medium and further demonstrated their fluorescence imaging application on human mucous cells. This is first example of dicyanoaniline class of compound where fluorescent organic NPs of 2A5B4PIPN prepared in water without any stabilizer like SDS or CTAB *etc*. This study provides the advantage of low concentration aqueous NPs of 2A5B4PIPN for cell imaging application. We are looking forward to modify the structure of present molecule in order to get the specificity in the fluorescence imaging. We believe that the current study will lead the way in development of new and smart dicyanoaniline based nano-probes for imaging research in biology, biomedical and sensing applications.

Acknowledgements

Dr. Ananada S. Kudale thanks Government of Maharashtra, Home department for granting permission for Ph. D. research work and Director, Forensic science laboratories, Mumbai for the necessary support. Dr. Anil H. Gore (R. C. Shahu College, Kolhapur) gratefully acknowledges Science and Engineering Research Board (SERB), New Delhi (Government of India) for the partial financial support (File no. YSS/2015/001806). Dr. Chetan B. Aware acknowledges to Department of Biotechnology Government of India for Interdisciplinary Programme of Life Sciences for Advanced Research and Education (IPLS) (Referece no. BT/PR4572/INF/22/147/2012). We greatly acknowledge the help of Dr. Rakesh K. Sharma, M. D., Dean, D. Y. Patil Medical College, Kolhapur for help in presenting proposal for institutional ethical approval and Dr. Amrut Bhosale, Department of Zoology, Shivaji University, Kolhapur for help in cell imaging experiments.

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Graphical abstract