

Research Article

FLUORESCENCE SPECTRAL STUDY OF THE SOLUBILIZATION OF BENZO [a] PYRENE IN MICELLAR SURFACTANT SOLUTION

***Sunil Kumar Jangir and Seema Acharya**

Department of Chemistry, Jai Narain Vyas University, Jodhpur (Raj.) India

**Author for Correspondence*

ABSTRACT

Benzo[a]pyrene is one of a group of chemicals called polycyclic aromatic hydrocarbon, PAHs for short, and found in cigarette smoke, in the exhaust from automobiles and coal tar. Polycyclic aromatic hydrocarbons (PAHs) are toxic environmental pollutants that are known or suspected carcinogens or mutagens. Bioremediation has been used as a general way to eliminate them from the contaminated sites or aquifers, but their biodegradation is rather limited due to their low bioavailability because of their sparingly soluble nature. The presence of surfactants can increase the solubility of PAHs and hence potentially increase their bioavailability. Micellar solubilization of benzo[a]pyrene in nonionic and ionic surfactants heteromicroenvironment is monitored by fluorescence and absorption spectral techniques has been reported by the authors. The influence of surfactant, concentration and working experimental conditions on the fluorescence spectra of benzo[a]pyrene is thoroughly evaluated and discussed. The increase in fluorescence intensity in micellar media can be attributed to the increase in quantum efficiency suggests that the suspended hydrophobic benzo[a]pyrene molecules have been solubilized. The solubilizing action has been supplemented and confirmed by few theoretically calculated spectral parameters like, empirical fluorescence coefficient (k_f), quantum yield (ϕ_f), molar extinction coefficient (ϵ) and Stokes' shift values.

Keywords: *Surfactants, Benzo[a]pyrene, Fluorescence, Solubilization*

INTRODUCTION

Fluorescent technique, due to its high sensitivity and great selectivity is being used widely in biochemical, biomedical, pharmaceutical, agriculture, forensic and environmental pollution control analysis (Lakowicz, 1999). Its advantage is extremely high sensitivity and selectivity even single molecule can be detected and it achieves a high spatial resolution and time resolution in combination with microscopic techniques as laser techniques, respectively (Sharma and Schulman, 1999). In material sciences, this is used to study structure and dynamics of surfaces, particularly in this area of biochemistry and molecular genetics, fluorescence spectroscopy has become a dominating technique. Together with the latest imaging techniques, fluorescence spectroscopy allows a real time observation of the dynamics of intact biological system with an unprecedented resolution (Andreef and Pinkel, 1999).

Polycyclic aromatic hydrocarbons (PAHs) are organic contaminants of concern for human health and natural ecosystems due to their known carcinogenic and mutagenic properties (Enzmingher and Ahlert, 1987). Their low water solubility limits their availability to microorganisms, which is a potential problem for bioremediation processes. Surfactant-mediated biodegradation is a promising alternative because the presence of surfactants can increase the solubility of PAHs and hence potentially increase their bioavailability (Li and Chen, 2009). The surfactants assemble into micelles at the critical micelle concentration (CMC) and the interior of the micelles provides a hydrophobic environment to solubilise nonpolar compounds such as hydrocarbons (Elworthy *et al.*, 1968, Guerin and Jones, 1988). Therefore, only concentrations above CMC enhance the mobility and apparent solubility of PAHs (Edwards *et al.*, 1991). Surfactants because of their ability to solubilize the membrane proteins are extremely important in simulating the complex environmental condition present in larger bioaggregates such as biological membranes (Tanford 1973). However, high surfactant concentrations may be not only toxic for microorganisms (Laha and Luthy, 1992), but they can also reduce the adhesion of bacteria to hydrophobic surfaces (Rosenberg and Rosenberg, 1995).

Research Article

The enhanced solubility in the micellar solution can be attributable to the increased biodegradation and bioavailability of substrate to microorganism. Both positive and negative effects have been reported on surfactants on microbial utilization of PAHs. The positive effects are generally attributable to the increased solubility/dissolution these compounds by surfactants which enhances their bioavailability. The negative effects are contributed by a variety of factors, which include toxicity of surfactants to microorganism, preferential degradation of surfactants and limited bioavailability of substrate solubilized in surfactant micelles

In this paper we report here the investigation carried out on solubilization of benzo[a]pyrene occurring in the presence of surfactant micelles employing fluorescence and absorption measurements. The result have been interpreted from the determination of quantum yield of benzo[a]pyrene fluorescence in micellar medium.

MATERIALS AND METHODS

All the fluorimetric and absorption experiments were carried out with Perkin- Elmer fluorescence spectrophotometer model no. 204 A with a synchronized model no. 056 strip chart recorder and Hewlet Packard (HP) 8452 A diode array spectrophotometer, respectively. The stock solution of analytically pure benzo[a]pyrene (Sigma Chemicals) was prepared in distilled methanol. All the experiments were made at room temperature (23⁰-25⁰C) and 1% methanolic medium keeping the final concentration of benzo[a]pyrene at 1×10^{-5} M. All the surfactants used were either of sigma (USA) or BDH product. The following surfactants were employed.

A) Nonionic: Polyoxyethylene tertoctyl phenol (TX-100), Polyoxyethylene sorbitan monolaurate (Tween-80) and Polyoxyethylene sorbitan monopalmitate (Tween-40)

B) Cationic : Cetyltrimethyl ammonium Bromide (CTAB), Cetylpyridinium chloride (CPC) and Cetylpyridinium bromide (CPB)

C) Anionic : Dodecylbenzene sodium sulphonate (DBSS), Dioctylsodium sulposuccinate (DSSS) and Sodiumlauryl sulphate (SLS)

The purity of surfactant was checked by determining their CMC values with the help of surface tension measurement, employing drop weight method. The absolute fluorescence quantum yield (Φ_f) of the compound was calculated relative to anthracene solution as standard. Each time the total intensity of fluorescence emission was measured for the standard and the sample from the area of the fluorescence spectrum recorded over the whole range of emission under identical conditions, Molar extinction coefficient (ϵ) data have been reported in term of its logarithm $\log \epsilon$, the Stokes' shift data been calculated in different micellar media and are expressed in term of nanometers

RESULTS AND DISCUSSION

The Maximum excitation and emission wavelength of benzo[a]pyrene was observed at 296 nm and 414 nm respectively. Fluorescence intensity increased monotonically with increasing concentration of the nonionic surfactants with 5 nm red shift. Among nonionic surfactants TX-100 showed maximum effect. On addition of anionic surfactant the emission intensity, initially decreased and a further increase in surfactant concentration in solution caused substantial enhancement in emission peak height. On addition of cationic surfactant for solution of benzo[a]pyrene the emission intensity decreased. The minimum and maximum fluorescence intensity in absence and presence of nonionic, anionic and cationic surfactants are given in Table 1. The fluorescence spectral changes on addition of TX-100 are as given in Figure 1.

The absorbance of benzo[a]pyrene was found to be maximum at 285 nm. The effect of all the three classes of surfactants on absorption spectra showed a similar trend to that of fluorescence spectra. The fluorescence quantum yield values obtained showed parallel trends to fluorescence intensity. Molar extinction coefficient values showed maximum effect in nonionic surfactants. Stokes' shift for benzo[a]pyrene at room temperature was increased with its rising concentration. The quantum yield values increased with increasing concentration of the nonionic surfactants and were found to be highest when TX-100 was added to benzo[a]pyrene solution. Enhancement in the fluorescence intensity of the

Research Article

Table 1: Relative fluorescence emission intensity (F.I.) of the benzo[a]pyrene (1×10^{-5} M) with different surfactants

($\lambda_{\text{ex}} = 296 \text{ nm}$, $\lambda_{\text{em}} = 414 \text{ nm}$, P.M. Gain = 2 Sensitivity Range = 0.3)

S. No.	% of TX-100	F.I.	% of DBSS	F.I.	% of CPB	F.I.
1.	0.000	21	0.000	21	0.000	21
2.	0.01	23	0.01	22	0.01	19
3.	0.07	29	0.07	24	0.07	16
4.	0.5	33	0.5	26	0.5	15

compound on adding surfactant can be attributed to the increase in the quantum efficiency of fluorescence. Furthermore the quantum yield of fluorescence was higher in nonpolar medium, because of the lesser effect of other deactivation processes which compete with fluorescence (Shizuka *et al.*, 1985).

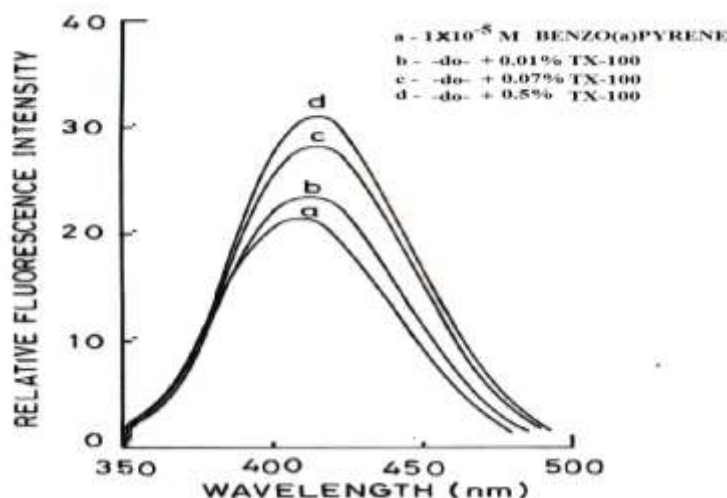


Figure 1: Influence of TX-100 on fluorescence intensity of benzo[a]pyrene

Thus, increase in quantum yield suggest that the surfactants have solubilized the suspended molecule of benzo[a]pyrene in solution. The result show that TX-100 micelles have solubilized benzo[a]pyrene very efficiently even at its low concentration. To explain its action, an oblate ellipsoid model has been postulated for TX-100. Although a spherical model requires mixing of the hydrophobic part and the hydrophilic part, while the octyl phenyl groups and the polyoxyethylene groups of TX-100 can separate each other and each layer packs well in the oblate ellipsoid model. This model, therefore predicts the hydrophobic and less fluid interior of TX-100 micelles. This fact has also been supported by some authors (Kano *et. al* 1981). The non polar environment of the TX-100 micelles interior be preferable to incorporate hydrophobic benzo[a]pyrene molecule. The highest solubilizing effect of TX-100 may also be due to the preference of ether linkage in it, while the other nonionic surfactants employed were esters. The higher polarity of the ionic micelles may be ascribed to the loose fluctuating and disorder in structure of these micelles. Benzo[a]pyrene must leave its aggregate and exclude water molecules inside the ionic micelle. These processes should cause slow solubilization. It is assumed that ionic micelles are too hydrophilic to solubilize the hydrophobic benzo[a]pyrene molecule to larger extent. However, in the case of cationic surfactants fluorescence intensity was quenched. This indicates electrostatic preferential interaction between the π electrons of the solubilize molecule and cationic head group of the surfactant which may result in change in geometry of the solubilize molecule where it loses coplanarity leading to decrease in emission intensity.

Absorption spectra of benzo[a]pyrene are very less affected in micellar media as compared to the fluorescence spectra. This may be because absorption is less sensitive to its environment as compared to fluorescence. No major change in the nature of absorption spectrum indicates no structural change due to complex formation or dissociation between benzo[a]pyrene in the ground state and the surfactant. Blue

Research Article

shift obtained in the absorption maxima may be because of the difference in solvation energy of the solubilize molecule in the ground state and the excited state. The sufficiently large values of $\log \epsilon$ are assigned to the $\pi - \pi^*$ transitions and also confirms the increasing trend of Stokes' shift values.

Conclusion

The present analysis and interpretation suggest that experimental results obtained and the theoretically calculated spectral data are found to be in good agreement. This proves the validity of the investigation made. This work gives a review of surfactant effects on the biodegradation of solubilization of benzo[a]pyrene, a member of common and toxic pollutants (PAHs) that have raised significant environmental concerns. Surfactants have been proven to be important vehicles for the recovery of these compounds from contaminated soil or aquifers due to the solubilization process.

ACKNOWLEDGEMENT

The authors are thankful to Head, Department of Chemistry, J.N. Vyas University, Jodhpur for providing necessary research facilities.

REFERENCES

- Andreeff M and Pinkel D (1999).** *Introduction to Fluorescence In Situ Hybridization : Principles and Clinical Applications* (Wiley Intersciences, New York).
- Edwards DA, Luthy RG and Liu Z (1991).** Solubilization of polycyclic aromatic hydrocarbons in micellar non-ionic surfactant solutions. *Environmental Science and Technology* **25** 127-133.
- Elworthy PH, Florence AT and Macfalan CB (1968).** *Solubilization by Surface Active Agents and its Application in Chemistry and Biological Sciences* (Chapman & Hall, London).
- Enzmingher JD and Ahlert RC (1987).** Environmental fate of polynuclear aromatic hydrocarbons in coal tar. *Environmental Technology Letters* **8** 269-278.
- Guerin WF and Jones GE (1988).** Mineralization of phenanthrene by a Mycobacterium sp. *Applied and Environmental Microbiology* **54** 937-944.
- Kano K, Goto H and Ogawa T (1981).** Solvent effect on fluorescence of intramolecular heteroexcimer system and its use for investigation polarity of microscopic environment. *Chemistry Letters* 653-656.
- Laha S and Luthy RG (1992).** Effects of non-ionic surfactants on the solubilization and mineralization of phenanthrene in soilwater systems. *Biotechnology and Bioengineering* **40** 1367-1380.
- Lakowicz JR (1999).** *Principles of Fluorescence Spectroscopy* (Plenum Press, New York).
- Li JL, and Chen BH (2009).** Surfactant-mediated biodegradation of polycyclic aromatic hydrocarbons. *Materials* **2** 76-94.
- Rosenberg M and Rosenberg E (1995).** Bacterial adherence at the hydrocarbon-water interface. *Oil and Petrochemical Pollution* **2** 155-162.
- Sharma A and Schulman SG (1999).** *Introduction to Fluorescence Spectroscopy* (Wiley Intersciences New York).
- Shizuka H, Ekushima M, Fuzu K, Kobayashi T, Ohtani H and Hohino M (1985).** Proton-induced quenching of Methoxynaphthalenes studied by laser flash photolysis and inclusion effect of beta-cyclodextrin on the quenching. *Bulletin of the Chemical Society of Japan* **58** 2107.
- Tanford C (1973).** *The Hydrophobic Effect : Formation of Micelles and Biological Membranes* (Wiley, New York).