Design of Reverse Micelle with Specific and Controllable pH Value of Polar Core Water: An Idea to Create pH Specific Nanodroplet of Water

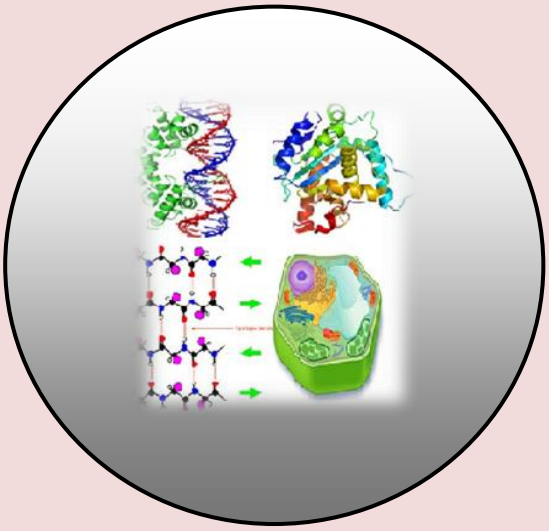
By

Palash Setua

ISSN 2319-3077 Online/Electronic
ISSN 0970-4973 Print

UGC Approved Journal No. 62923
MCI Validated Journal
Index Copernicus International Value
IC Value of Journal 82.43 Poland, Europe (2016)
Journal Impact Factor: 4.275
Global Impact factor of Journal: 0.876
Scientific Journals Impact Factor: 3.285
InfoBase Impact Factor: 3.66

J. Biol. Chem. Research
Volume 36 (1) 2019, Pages No. 225-232



Journal of Biological and Chemical Research

An International Peer Reviewed / Referred Journal of Life Sciences and Chemistry

Indexed, Abstracted and Cited in various International and National Scientific Databases

Published by Society for Advancement of Sciences®

J. Biol. Chem. Research. Vol. 36, No. 1: 225-232, 2019
(An International Peer Reviewed / Refereed Journal of Life Sciences and Chemistry)
Ms 36/01/9009/2019

ISSN 2319-3077 (Online/Electronic)
ISSN 0970-4973 (Print)

Dr. Palash Setua
http:// www.sasjournals.com
http:// www.jbcr.co.in
jbiolchemres@gmail.com

RESEARCH PAPER

Received: 25/03/2019 Revised: 28/04/2019 Accepted: 29/04/2019

Design of Reverse Micelle with Specific and Controllable pH Value of Polar Core Water: An Idea to Create pH Specific Nanodroplet of Water

Palash Setua

Department of Chemistry, Pingla Thana Mahavidyalaya, Maligram- 721140, India

ABSTRACT

In this study I have constructed an aqueous reverse micelle system using a common surfactant molecule AOT [bis(2-ethylhexylsufosuccinate) sodium salt], water and isooctane where pH of the water present inside the core region of water can be controlled using the idea proposed by Henderson Hasselbach to design buffer medium in water solvent. I have used two molecules for construction of the AOT based reverse micelle, one is already reported AOT and the other is its' protonated form AOT-H [bis-2-ethylheylsulfosuccinic acid]. In reverse micelle solution, I controlled the ratio of AOT and AOT-H using the Henderson Hasselbach equation, pH = pK_a + log ([concentration of salt] / [concentration of acid]) where AOT is considered as 'salt' and AOT-H is consider as acid. I observed very good pH control of the reverse micelle core water which satisfies the idea proposed by Henderson Hasselbach.

Key words: AOT, Reverse Micelle, pH control of water core and Henderson Hasselbach idea.

INTRODUCTION

Reverse micelle is an assembly of surfactant molecules when they are dissolved in nonpolar hydrocarbon medium. Surfactant are amphiphilic molecules with one polar (head group) and one nonpolar hydrocarbon (tail) part. So when I dissolve surfactant molecule in the nonpolar solvent it tries to shade its polar head group from the unfavourable nonpolar interaction of the hydrocarbon medium and form a self assembled structure where all the tail parts remain directed towards the hydrocarbon medium and polar head groups towards the core of the aggregate. The polar core created inside the reverse micelle is the most interesting part of these systems from both fundamental and applied consideration (Lusi et al, 1984). It has been applied as the encapsulation medium in different field of research from nanomaterial synthesis, medicinal chemistry, material science and biology, especially as a mimicking system of intracellular space for protein or biorelevant molecules (Lusi et al, 1988, Nandi et al, 2000, Zhou et al, 2010, Yeung et al, 2012 and Murakami et al, 2011). It has already been proved that properties of the water present in the nanometer size water droplet present inside reverse micelle are different from the properties of the bulk water (Nandi et al, 2000). pH of the water medium plays a significant role in controlling the proton dependent kinetics of the simple chemical reaction as well as it has the ability to change the kinetics and structure of much larger complex biomolecules (Levinger et al, 2011).

In case of biomolecule like protein it plays crucial role because structure of biomolecules are sensitive towards the pH change of the medium (Stigter et al, 1991). To avoid this problem, biomolecules and protein are studied in buffer medium with specific pH value. Buffer action and the pH of the buffer media is best explained by the well known Henderson-Hasselbach equation,

$$pH=pK_a+\log\left(\frac{\text{congugate base}}{\text{acid}}\right) \quad \text{.....(1)}$$

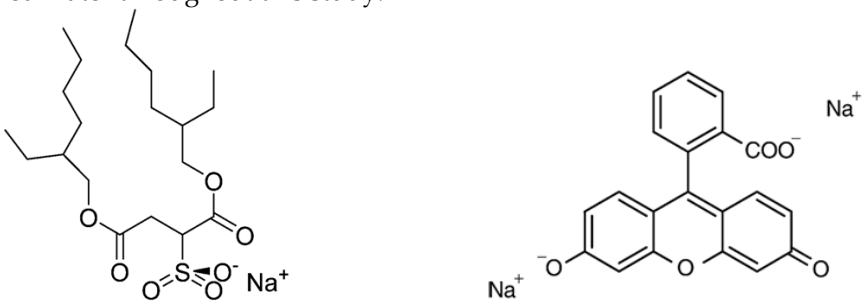
Where, [acid] is the molar concentration of the undissociated weak acid, [conjugate base] is the molar concentration of the dissociated form and pK_a represent the [- log(K_a)] where K_a is the acid dissociation constant of that particular acid (de Levie, 2010). We can control the pH of the buffer media within the approximate ± 1 range of the pK_a value. Some studies had already been done in reverse micelle media to address this problem. Hasegawa et al. Showed a buffer like action and constant pH value fixed at pH ~ 5 in AOT reverse micelle medium (Hasegawa, 2001). They assumed suppression of the electrolytic dissociation strength of $-SO_3Na$ group present in the AOT molecule when they form reverse micelle. This property change from strong to weak electrolyte and with assumption of plausible $-SO_3H$ group formation they explained their results. Baruah et al. (Baruah et al, 2006) observed near neutral pH of the AOT reverse micelle core with the assumption that proton concentration remains lower in the core region compare to the interfacial region where $-SO_3^-$ group of AOT molecules remain present. They proposed a concept of ion exchange between the interfacial sodium ions with the H^+ ion. But later this concept of proton exchange with sodium counter ion was proven wrong with neutral surfactant forming reverse micelle. Another aspect of this problem is the limitation in the IUPAC definition of pH which is not valid in the very small nonometer size reverse micelle. According to modern IUPAC concept, $pH=-\log[a_{H^+}]$ where a_{H^+} is the activity of the proton in the solution, and is valid only within the pH range from 2 to 12 and at ionic strength below 0.1 mol/lit. In reverse micelle media, ionic strength is much higher considering the proton activity constant. So considering these limitation people have described reverse micelle pH a apparent or relative pH. This apparent pH is considered equivalent to the bulk water domain pH sensed by a probe molecule which is also used to sense the pH environment of the reverse micelle medium. Considering the complicacy and delicacy of the reverse micelle media (where an average one proton per reverse micelle can produce a dramatic change in the environment) (Levinger et al, 2011) I did not use any buffer solution or any acidic or basic solution for the construction of reverse micelle. I approach in a direct manner to address this problem of constant pH or buffer like action inside reverse micelle and also to check the feasibility of designing a specific pH reverse micelle based nanoreactor or chamber. In this study I incorporated the acid form of the AOT [bis (2-ethylhexyl) sulfosuccinate sodium salt] molecule (containing $-SO_3H$ functional group instead of $-SO_3Na$) into the reverse micelle assembly formed by the normal AOT molecule (with $-SO_3Na$) with particular ratio and use pure double distil water as polar solvent. The impacts are analysed directly from the view of fundamental and applied consideration.

MATERIAL AND METHODS

Materials Used

Bis (2-ethylhexyl) sulfosuccinate sodium salt (AOT) (figure 1) was purchased from sigma-Aldrich and purified according to the previously reported literature procedure (Eicke et al, 1974). Fluorescein sodium salt (figure 1) was purchased from sigma Aldrich and use without further purification. Isooctane of highest purity grade was purchased from Sigma-Aldrich and was used as received. Amberlyst 15 as purchased from Sigma-Aldrich to convert the sodium salt form of sulfonate group ($-SO_3Na$) of AOT surfactant to its acid form ($-SO_3H$). For simplicity, from here and onwards I shall represent the $-SO_3H$ functional group containing AOT as AOT-H. I constructed the pH response profile of flourescein in different pH range with the following standard buffers:

(a) KCl/HCl 0.2 M (pH range 1.5 to 2.2) (b) potassium hydrogen phthalate / HCl 0.1 M (pH range 2.2 to 4.0) (c) potassium hydrogen phthalate / NaOH 0.1 M (pH range 4.1 to 5.9) (d) potassium dihydrogen phosphate / NaOH 0.1 M (pH range 5.8 to 8.0) (e) sodium tetraborate / HCl 0.025 M (pH range 8.1 to 9.2) (f) sodium tetraborate / NaOH 0.025 M (pH range 9.2 to 10). All the chemicals used for the buffer preparation were purchased from sigma Aldrich and were used as received. I used double distilled water throughout this study.



AOT

Figure 1. Chemical structure of probe molecule (fluorescein sodium salt) and surfactant (AOT) molecule.

Instruments Used

UV-Vis absorption spectra were measured with the Shimadzu UV-Vis spectrophotometer UV-1800. Spex Fluorolog-3 was used to measure the excitation spectra. Dynamic light scattering measurements were performed with Malvern zs particle size analyser instrument. Final pH of the buffer solution was measured with Sistronic pH meter which was previously calibrated with three standard buffer solutions with pH value 10.01, 7.0 and 4.01.

Method of Preparation

I prepared AOT-H molecule from the purified AOT following the ion exchange method using the highly acidic Amberlyst 15 regin. This simple and single step conversion process with ~100% yield has already been used to convert AOT to its acid form (Wang et al, 2012). I dissolved 500 mg purified and dried AOT in 50 ml diisopropyl ether and stirred overnight with 5 g Amberlyst 15 resin. Then the mixture was filtered and the resin was washed with 10 ml diisopropyl ether. Extra diisopropyl ether was removed by evaporator and the product was vacuum dried for 72 hours at room temperature. I followed the standard method of reverse micelle preparation to prepare 0.2 M AOT reverse micellar solution in isooctane (Hazra et al, 2003). I followed the direct weighing and addition process for AOT addition, in brief I added calculated amount of AOT to a specific volume of isooctane to produce the required volume of reverse micelle solution where specific volume equals to required volume. For mixed AOT and AOT-H reverse micelle preparation, I kept the overall surfactant concentration fixed at 0.2 M i.e. in all sample [AOT] + [AOT-H] = 0.2 M. I changed the molar ratio of AOT to AOT-H by changing the relative proportion of each component keeping the total surfactant concentration at 0.2 M. I generated reverse micellar solution with different water loading by injecting calculated volume of double distilled water from a calibrated microlitre syringe into the dry reverse micelle medium. Water loading or water content of a particular reverse micelle system is generally described by a term w_0 value. It represents the molar concentration of water to surfactant. In case of pure AOT reverse micelle system, w_0 = [water]/ [AOT]. In case of AOT reverse micelle mixed with AOT-H, w_0 = [water] / ([AOT] + [AOT-H]). Solutions are homogenised by vortex. For excitation spectra measurement I kept the fluorescence concentration ~10^{-6} M for all samples.

RESULTS AND DISCUSSION

In this study, I have used the optical spectroscopy method based on organic probe molecule to sense the pH of the AOT reverse micellar medium. This method solely depends on the structural change of the probe molecules with the variation of the proton concentration in the medium.

This structural change is quantitatively reflected in their optical spectrum which can be easily monitor by optical spectroscopy instrument (Hasegawa, 2001, El Seoud et al, 1982, 1997, Miguel et al, 2001 and Vodolazkaya et al, 2010). In most of the cases this structural change is triggered by at least one labile proton which remain present in the optical probe molecule and whose attachment or detachment is directly controlled by the pH of the surrounding environment. Results obtained using these invasive technique shows some dependency on the probe location inside the studied media (Baruah et al, 2006). Reverse micelle is micro heterogeneous media. So when I incorporate probe molecule inside water reverse micelle it shows some deviation from its normal behaviour which I generally observe in bulk water environment (Smith et al, 1980, Fujii et al, 1979). Moreover in reverse micelle medium probe molecules may remain present in different location like interfacial reverse micellar region or core of the reverse micelle. It was assumed that different local environment inside reverse micelle may affect the optical response of the probe molecules (Crans et al, 2012). Realizing and accepting these difficulties still researchers are using this invasive technique with difference responsive probe (e.g. optical or NMR) to explore the pH property of those complicated system. This is mainly due to the long list of applicability of the reverse micelle system in various field of research and unavailability of any better method. Previous studies on the pure AOT reverse micelle system reported that this reverse micelle system has slightly acidic (Hasegawa, 2001) or near neutral (Baruah et al, 2006) environment in its water core. So based on these observation and with the initial assumption that mixing of AOT-H with AOT will gradually make the aqueous domain of the mixed reverse micelle more acidic, I started our work. I used sodium salt of fluorescein as optical probe because it shows large pH response range from slightly alkaline to highly acidic value. Moreover being sodium salt it shows good solubility in aqueous environment.

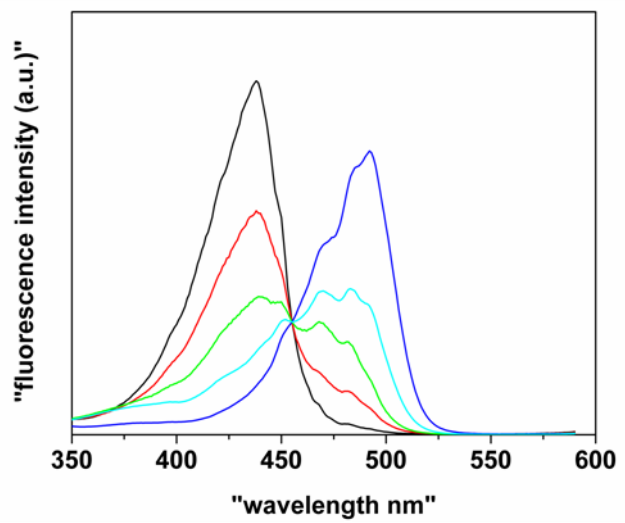


Figure 2. Variation of excitation spectra of fluorescein λ_{emi}=600 nm with the variation of pH in aqueous medium. Spectra are normalized at 455 nm wavelength (iso-emissive point). Black at pH = 1.84, red pH = 2.84, green pH = 3.87, cyan pH = 5.86 and blue pH = 8.07.

To cover large pH scale it undergoes more number of structural change compare to other optical probe (Crans, 2012) but still it has been successfully used to sense the pH of different reverse micelle system (Hasegawa, 2001 and Vodolazkaya et al, 2010). pH dependent optical response of fluorescein can be monitored by both the UV-Vis absorption and florescence excitation spectroscopy. I used fluorescence excitation spectra of fluorescein due to its superior sensitivity and accuracy in comparison to the UV-Vis absorption spectra (Hasegawa, 2001). Figure 2 shows the changes in the excitation spectra of fluorescein with the variation of pH in water medium where pH was controlled by different buffer medium mentioned in the experimental section. Previous study on fluorescein showed that it remain present in four form namely cationic, neutral, anionic and di-anionic with prolytic constants pK_1 = 2.08, pK_2 = 4.31 and pK_3 = 6.43 respectively (Sjoback et al, 1995).

In strongly acidic condition, an emission band centred ~435 nm is observed; this is originated from the cationic form of the species. With slight increase in pH value a shoulder like emission band in the excitation spectrum centred ~470 nm is observed which is originated from the neutral form of the molecule. In alkaline line pH range, the peak position was shifted to ~490 nm which was originated from the di-anionic form of fluorescein molecule (Sjoback et al, 1995).

I follow the intensity ratio method and plotted the ratio of 435 nm intensity to 490 nm intensity (I_{435}/I_{490}) with the variation of pH to construct the pH reference calibration curve (figure 3)

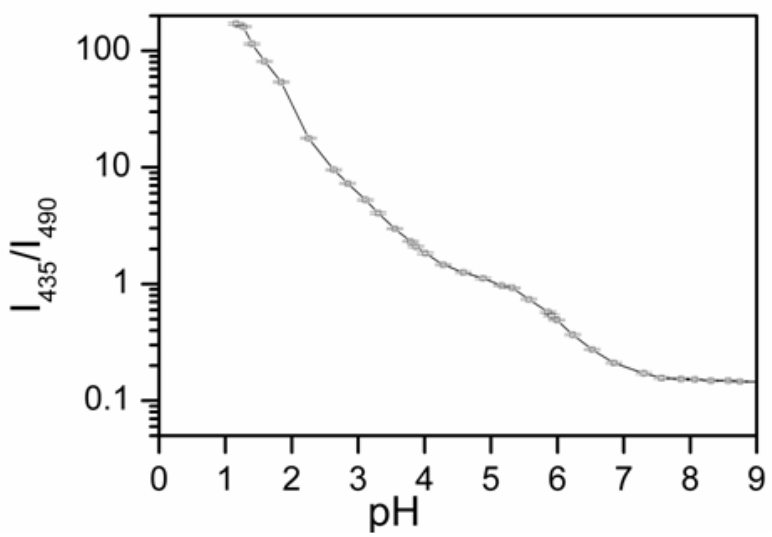


Figure 3. Variation in excitation spectra intensity ratio at 435 nm to 490 nm (I_{435}/I_{490}) of fluorescein as a function of pH controlled by different buffer.

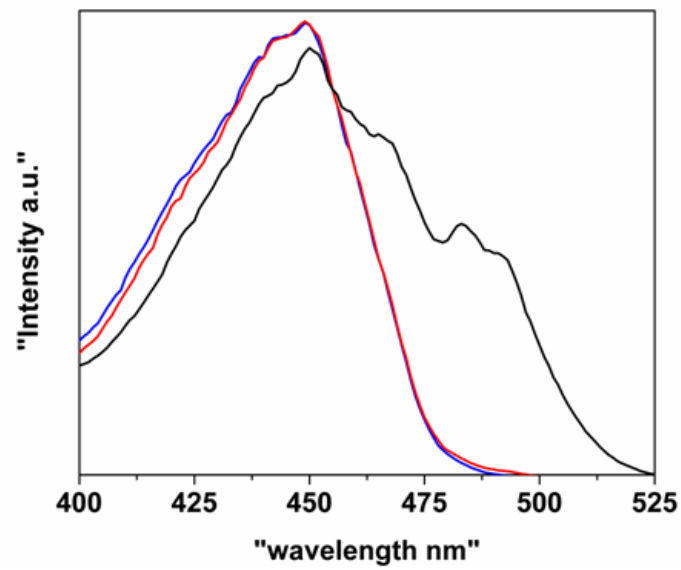


Figure 4. Excitation spectra of fluorescein observed in mixed AOT reverse micelle condition with the molar ratio of AOT: AOT-H = 99: 01 (black), 98: 02 (red) and 80: 20 (blue).

This calibration curve was used for measuring the relative pH of the reverse micelle water pool. I followed the standard method of AOT reverse micelle preparation. Mixed AOT reverse micelle solution were prepared by mixing necessary amount of AOT-H with AOT and kept the total surfactant concentration ([AOT]+[AOT-H]) fixed at 0.2 M. Water stock solution of sodium fluorescein was used to incorporate the probe molecule inside reverse micelle. Water volume of probe solution used was considered during the w_0 value calculation of the reverse micellar solution. Figure 4 shows the excitation spectra of fluorescein probe molecule observed in mixed AOT reverse micelle with different AOT: AOT-H molar ratio.

I carried out the details study in two w_0 condition at $w_0 = 12$ and $w_0 = 20$. I selected these two higher w_0 values because in these conditions fraction of bulk like water molecules become higher than the fraction of interfacial water which would be in favour of this pH measurement method. It has already been reported that absorption and fluorescence spectra of probe molecules are different bellow $w_0 = 5$ and become consistent with bulk water when w_0 value crosses the $w_0 = 10$ limit (Hasegawa, 2001 and Hasegawa et al, 1996, 1994). So I chose $w_0 = 12$ and 20 condition for our study. Figure 5 shows the relative pH of the reverse micellar water nanopool sensed by fluorescein molecule with the increase in percentage of AOT-H from 1% to 20%.

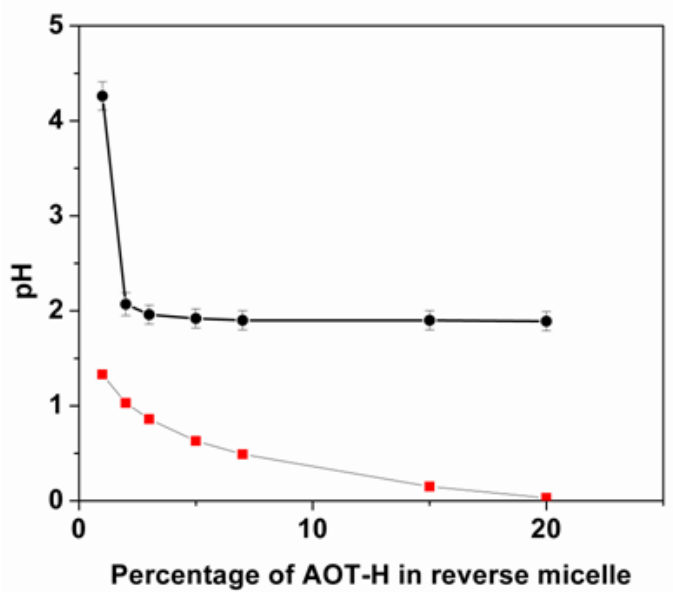


Figure 5. Black points show the variation of pH in the AOT reverse micellar system with AOT-H percentage. Red points are theoretically calculated considering 100% dissociation of the AOT-H molecules in the reverse micellar assembly.

At 1% AOT-H condition, I observed a pH value ~4.2. When the AOT-H percentage was increased to 2% the pH value decreased to 2.2 with a sharp fall in the pH value. At 3% mixing I observed a pH value ~1.95, pH value decreased but the decrease was very less compare to the initial decrease of the pH value. Further increase in the AOT-H percentage, I observed very less pH decrease in the reverse micelle medium. Practically the pH value was fixed ~1.85 at 15% or 20% mixing of AOT-H. Above results clearly showed that there are buffer type control mechanisms which control the pH of the water core inside the mixed AOT reverse micelle medium. As SO_3H is a strong acid group so its pK_a value is low and the buffering action is occurring in the lower pH range. Again if I see the theoretically calculated pH value of the solution considering 100% dissociation of $-SO_3H$ groups then it is clear that dissociation of the $-SO_3H$ group is suppressed in the reverse micelle environment in comparison to bulk water which is in accordance of the previous observation that report the deviated behaviour inside reverse micelle. This may arises from the change in the physical properties of core water molecules or may be from the changed behaviour of the the probe molecule.

CONCLUSION

This work shows some deviation from bulk properties but undoubtedly established that Idea of Henderson-Hasselbach can be successfully used to create pH specific nano droplet using the process of reverse micelle formation.

ACKNOWLEDGEMENTS

I am very thankful to Dr. Nilmoni Sarkar for his guidance throughout his research life. I gratefully acknowledge the financial support from UGC minor research project [F.No.PSW-230/15-16 (ERO)], India.

REFERENCES

Baruah, B., Roden, J.M., Sedgwick, M., Correa, N.M., Crans, D.C. and Levinger, N.E. (2006). When is water not water? Exploring water confined in large reverse micelles using a highly charged inorganic molecular probe. *J. Am. Chem. Soc.* 128: 12758-12765.

Crans, D.C. and Levinger, N.E. (2012). The conundrum of pH in the water nanodroplets: sensing pH in reverse micelle water pools. *Acc. Chem. Res.* 45: 1637-1645 and references therein.

de Levie, R. (2010). Potentiometric pH measurements of acidity are approximations, some more useful than others. *J. Chem. Educ.* 87: 1188-1194 and references therein.

Eicke, H.F. and Christen, H. (1974). Nucleation process of micelle formation in apolar solvents. *J. Colloid Interface Sci.* 48: 281-290.

El Seoud, O.A. (1997). Use of NMR to probe the structure of water at interface of organized assemblies. *J Mol. Liq.* 72: 85-103.

El Seoud, O.A., Chinelatto, A.M. and Shimizu, M.R. (1982). Acid-base indicator equilibrium in the presence of aerosol-OT aggregates in n-heptane-ion exchange in the reverse micelles. *J. Colloid Interface Sci.* 88: 420-427.

Fujii, H., Kawai, T. and Nishikawa, H. (1979). Determination of pH in reversed micelles. *Bull. Chem. Soc. Jpn.* 52: 2051-2055.

Hasegawa, M. (2001). Buffer likes action in water pool of Aerosol OT reverse micelle, *Langmuir* 17: 1426-1431.

Hasegawa, M., Sugimura, T., Suzaki, Y., Shindo, Y. and Kitahara, A. (1994). Microviscosity in water pool of aerosol OT reversed micelle determined with viscosity-sensitive fluorescence probe, auramine O, and fluorescence depolarization of xanthene dyes. *J. Phys. Chem.* 98: 2120-2124.

Hasegawa, M., Yamasaki, Y., Sonta, N., Shindo, Y., Sugimura, T. and Kitahara, A. (1996). Clustering of aerosol OT reverse micelles as studied by nonradiative energy transfer of solubilised probes. *J. Phys. Chem.* 100: 15575-15580.

Hazra, P., Chakraborty, D. and Sarkar, N. (2003). Solvation dynamics of coumarin 153 in aqueous and non-aqueous reverse micelle. *Chem. Phys. Lett.* 371: 553-562.

Levinger, N.E., Rubenstrunk, L.C., Baruah, B. and Crans, D.C. (2011). Acidification of reverse micellar nanodroplets by atmospheric pressure CO_2. *J. Am. Chem. Soc.* 133: 7205-7214 and reference therein.

Luisi, P.L., Giomini, M., Pileni, M.P. and Robinson, B.H. (1988). Reverse micelle as a host for proteins and small molecules. *Biochim. Biophys. Acta.* 947: 209-246.

Lusi, P.L. and Straube, B. E. (1984). Reverse micelle, Eds. Plenum Press: New York.

Miguel, M.D., Burrows, H.D., Pereira, M.A.E. and Varela, A.P. (2001). Probing solute distribution and acid base behaviour in water in oil microemulsion by fluorescence techniques. *Colloids Surf. A* 176: 85-99.

Murakami, H., Nishi, T. and Toyata, Y. (2011). Determination of structural parameters of protein containing reverse micellar solution by near-infrared absorption spectroscopy. *J. Phys. Chem. B* 115: 5877-5885.

Nandi, N., Bhattacharyya, K. and Bagchi, B. (2000). Dielectric relaxation and solvation dynamics of water in complex chemical and biological systems. *Chem. Rev.* 100: 2013-2045.

Pileni, M.P. (2006). Reverse micelles used as a template: a new understanding in nano crystal growth. *J. Exp. Nanosci.* 1: 13-27.

Sjoback, R., Nygren, J. and Kubista, M. (1995). Absorption and fluorescence properties of fluorescein. *Spectrochimi Acta A* 51: L7-L21.

Smith, R.E. and Luisi, P.L. (1980). Micellar solubilisation of bio-polymers in hydrocarbon solvent. 3. Empirical definition of an acidity scale in reverse micelles. *Helv. Chim. Acta.* 63: 2302-2311.

Stigter, D., Alonso, D.O.V. and Dill, K.A. (1991). Protein stability: electrostatic and compact denatured state. *Proc. Natl. Acad. Sci.* 88: 4176-4180.

Vodolazkaya, N.A., McHedlov-Petrossyan, N.O., Salamanova, N.V., Surov, Y.N. and Doroshenko, A. O. (2010). Molecular spectroscopy studies of solvent properties of dispersed 'water pools' fluorescein and 2, 7-dichlorofluorescein in reversed AOT-based microemulsions. *J. Mol. Liq.* 157: 105-112.

Wang, J., de Jeu, W.H., Muller, P., Moller, M. and Mourran, A. (2012). Thin film structure of block copolymer-surfactant complexes: strongly ionic bonding polymer systems. Macromolecules 45: 974-985 and reference therein.

Yeung, P.S.-W. and Axelsen, P.H. (2012). The crowded environment of a reverse micelle induces the formation of β- strand seed structures for nucleating amyloid fibril formation. *J. Am. Chem. Soc.* 134: 6061-6063.

Yu, Z.J., Zhou, N.F. and Neuman, R.D. (1992). The role of water in the formation of reversed micelles: an antimicellization agent. *Langmuir* 8: 1885-1888.

Zhou, J., Wei, C., Jia, G., Wang, X., Feng, Z. and Li, C. (2010). Formation and stabilisation of G-quadruplex in nanosized water pools. *Chem. Commun*. 46: 1700-1702.

Corresponding author: Dr. Palash Setua, Department of Chemistry, Pingla Thana Mahavidyalaya, Maligram- 721140, India
Email: palashsetua@gmail.com