V.S. Farafonov [a], **A.V. Lebed** [a], **N.L. Khimenko** [b], **N.O. Mchedlov-Petrossyan** [a]

MOLECULAR DYNAMICS STUDY OF AN ACID-BASE INDICATOR DYE IN TRITON X-100 NON-IONIC MICELLES

[a] V.N. Karazin Kharkiv National University, Kharkiv, Ukraine
[b] V.V. Dokuchaev Kharkiv National Agricultural University, Dokuchaevske, Ukraine

Despite wide laboratory and industrial use, the structure of non-ionic surfactant micelles and the state of molecular probes adsorbed by them are relatively poorly studied by computational methods in comparison to the ionic surfactants. The state of an acid-base indicator dye, namely, 2,6-dinitro-4-n-dodecylphenol, solubilized by micelles of Triton X-100 non-ionic surfactant, was revealed by means of molecular dynamics simulations. Both neutral and ionized protolytic forms were considered. The average positioning of the forms with respect to micelle, composition of their local environment and number of hydrogen bonds with water molecules were obtained and compared with those in ionic micelles. The dye was found to be located on the surface of hydrocarbon core and be significantly less hydrated than in ionic micelles due to covering by polyoxyethylene chains. Both forms are localized similarly, while the anionic form is hydrated stronger than the neutral one, which resembles the situation in ionic micelles. The convergence of simulation results in such micelles is found to be slower than in the ionic micelles, especially for the neutral form.

Keywords: 2,6-dinitro-4-*n*-dodecylphenol, adsorption, localization, hydration, hydrogen bond.

Introduction

Micellar solutions of surfactants are widely used in different fields of science and technology. Besides their versatile applications, micelles serve as (reduced) models of biological membranes, biomolecules, etc. At the same time, acid-base, solvatochromic and fluorescent indicators are unique tools for gaining insight into micellar properties. In this connection, it is of critical importance to know the location of these molecular probes. In our previous publications, we used the molecular dynamics (MD) simulations for solving this problem. The Reichardt's solvatochromic indicators [1,2] and some acid-base indicators [3,4] were studied in micelles of ionic surfactants and conclusions were made about the location and microenvironments of the indicator dyes. The present paper is aimed to extend the research to non-ionic micelles. 2,6-dinitro-4-n-dodecylphenol (DDP) was taken as an indicator dye. This indicator was previously examined both experimentally and computationally in micelles of ionic surfactants [4]. It is a weak acid dissociating by hydroxyl group forming yellow-colored anion. Further in the text, the acidic and anionic forms will be denoted by HA and A$^-$, respectively. Triton X-100 (TX-100 for short, its full chemical name is 4-(1,1,3,3-tetramethylbutyl)phenyl-polyethylene glycol) was chosen because it is one of the most widely used non-ionic amphiphiles. The structures of the compounds are given in Fig. 1.
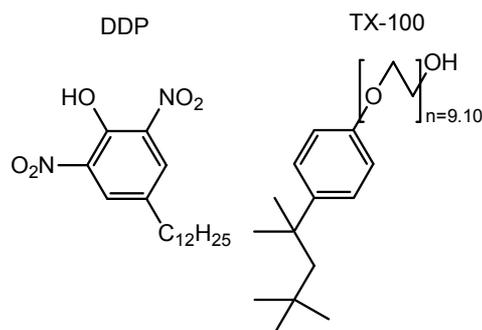
Fig. 1. Molecular structures of DDP and Triton X-100. The industrially available surfactant is a mixture of molecules with n=9 and 10

Despite wide laboratory and industrial application, non-ionic surfactant micelles remain less studied by MD method in comparison to anionic or cationic ones. The gap becomes particularly large for studies of compounds solubilized or adsorbed by such micelles. Considering Triton X family, micellization process of two representatives was examined in large-scale MD simulations by Yordanova et al. [5]. To this end, potential models were thoroughly built in the framework of CGenFF force field. A similar study, but at a hybrid coarse-grained/atomistic resolution, was performed by de Nicola et al. [6]. Interaction of carbon and boron nitride nanotubes with TX-100 micelles was studied by Fatemi and Foroutan [7−9]. Thus, it is timely to include other compounds, including molecular probes, to this list, and perform comparison between the state of a compound in ionic and non-ionic micelles.

Computations

MD simulations were performed at the standard conditions (temperature of 298 K and pressure of 1 bar), Berendsen thermostat with time constant of 1 ps and Berendsen barostat with time constant of 1.5 ps were employed for this. Time step was equal to 1.6 fs, the 3D periodic boundary conditions were imposed. Electrostatic interactions were computed by means of the PME method, while van der Waals interactions were cut off at 1 nm. All covalent bonds were constrained. GROMACS 5 software package was used.

The potential model for DDP was taken from our previous works [4]. It was derived in the basis of the common well-validated OPLS-AA force field. For TX-100, it was straightforward to build the model because for all parameters, the standard values present in the force field were suitable. We used value $n=10$

(Fig. 1) for simulations.

The initial configuration was prepared in several steps. First, an empty micelle was made by equilibration of a preassembled bilayer of 110 molecules put in cubic water cell with edge length of 9.7 nm. The number of monomers corresponds to the experimental aggregation number [10]. After 50 ns of simulation at the stated conditions, a rod-like micelle was formed (Fig. 2). Then, a cavity was created near the surface of micelle following the protocol, described in detail elsewhere [1−4]. In the cavity, a dye molecule was placed, and the cavity was collapsed providing a configuration where dye is solubilized in micelle. For each dye and each protolytic form, three configurations were prepared in such a way.

For each system, simulations were performed starting from these 3 configurations, and corresponding 3 sets of computed characteristics were averaged. Simulation length was 50 ns for the anionic form and 130 ns for the neutral one. The analysis was performed based on the last 40 ns of runs, while the time before were discarded as equilibration.

Results and discussion

First, we focused on the properties of the micelle itself. The final equilibrated shape is a curved rod with highly irregular surface, with diameter of ~5 nm and length in unbend state of ~12 nm, as was determined by visual inspection in VMD program (including van der Waals radii of atoms). The micelle consists of hydrocarbon core composed by atoms belonging to benzene ring and aliphatic radical attached to it and hydrophilic shell formed by polyoxyethylene chains. The core has diameter of ~3 nm and length of ~10 nm, while the chains extend as far as 1 nm out of it. To obtain quantitative understanding of the density of the hydrophilic shell,
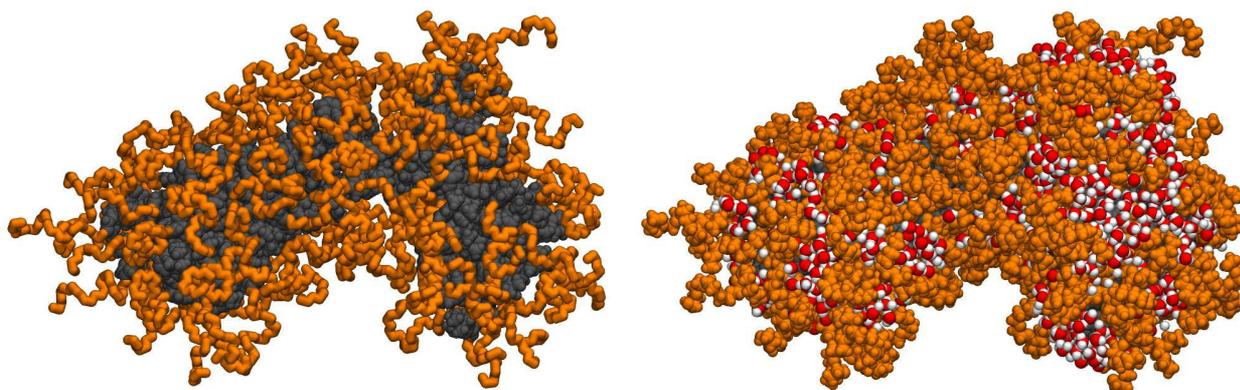
Fig. 2. Equilibrated Triton X-100 micelle. Hydrocarbon core is colored gray, hydrophilic shell is colored orange. On the left image, polyoxyethylene chains are depicted as simplified; on the right image, water molecules within 0.6 nm of hydrocarbon core are shown as well

V.S. Farafonov, A.V. Lebed, N.L. Khimenko, N.O. Mchedlov-Petrossyan

we computed the fraction of O atoms of the chains (out of $11 \times 110 = 1210$ in total) within a range of distances from the surface of hydrocarbon core. The graph is presented in Fig. 3,a (an interval of 40−50 ns of trajectory was used for computation). The distance from an O atom to the core surface was calculated as the center-to-center distance from it to the closest atom belonging to the core. Estimated in such a way, shell thickness equals 0.8 nm (that is consistent with the results of visual examination), while the half of O atoms are collected within 0.4 nm from the surface (i.e. lay immediately on it without water molecules located in between).

Now we turn to describing DDP−micelle complex. In comparison with moderately ellipsoidal micelles of ionic surfactants, characterization of localization of dye molecules with respect to highly non-spherical rod-like micelles is much more difficult task. Usually used distribution functions of distance or angle between micelle center of mass (COM) and some atoms of the dye molecule are not insightful here. Instead, we computed distribution functions of distance between the surface of hydrocarbon core and chosen the following dye atoms: hydroxyl O atom (denoted by O_H) and the first C atom of dodecyl radical (denoted by C_α) (Fig. 3,b). Again, the distance to its surface was approximated by the distance to its closest atom. This approximation does not allow distinguishing between location immediately on the surface of the core and inside it: in both cases, the distance will correspond to the closest contact distance between the atoms that is determined by the sum of their van der Waals radii. For H and C or O atoms, it equals to ~0.28 nm [11]. However, larger distances are unambiguous.

As is seen from computed distributions, most of the time, the distance between dye atoms and hydrocarbon core is <0.37 nm with the peak at 0.32 nm (C_α) or 0.28 nm (O_H), which indicates the phenyl ring stays inside the core or immediately on its surface. For O_H atom, the values longer than 0.45 nm seldom occur indicating the hydroxyl group is able to advance towards water for short time, but not far (<0.6 nm); this tendency is two times stronger for the anionic form. Thus, there is no evidence for the phenyl moiety as a whole to be located inside the hydrophilic polyoxyethylene shell or on the interface between the shell and bulk water.

For clarity, several configurations generated by MD are shown in Fig. 4 allowing visual perception of the dye locus.

The composition of microenvironment of the dye molecule was inspected via the following approach. The atoms, surrounding the molecule, were classified in three categories: atoms of water molecules, atoms of hydrocarbon core and atoms of hydrophilic shell; and the average populations of each category were computed. The results are shown in Fig. 5. Fraction of water in ME computed in such a way represents an average measure of the hydration of the molecule.

Finally, a meaningful and important characteristic of the medium is the number of hydrogen bonds formed by the species of interest with accessible water molecules (n_{HB}). The following geometric criteria were employed for identification of the presence of a hydrogen bond: (i) distance between donor and acceptor no longer than 0.35 nm; (ii) angle ∠(donor, H atom, acceptor) not less than 150^0. These criteria are standard for GROMACS
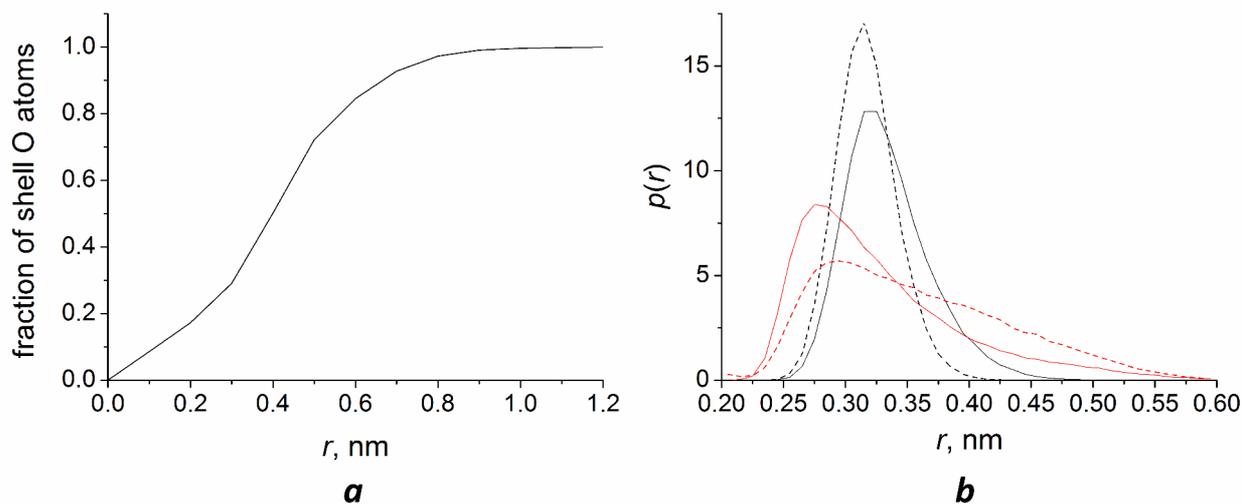
Fig. 3. a − fraction of O atoms of polyoxyethylene chains within some distance from the hydrocarbon core; b − distribution functions of distances between the surface of TX-100 micelle hydrocarbon core and hydroxyl O_H, C_α atoms of DDP. Red curves are for O_H atom, black curves are for C_α atom, solid curves are for neutral form, and dashed curves are for anionic form
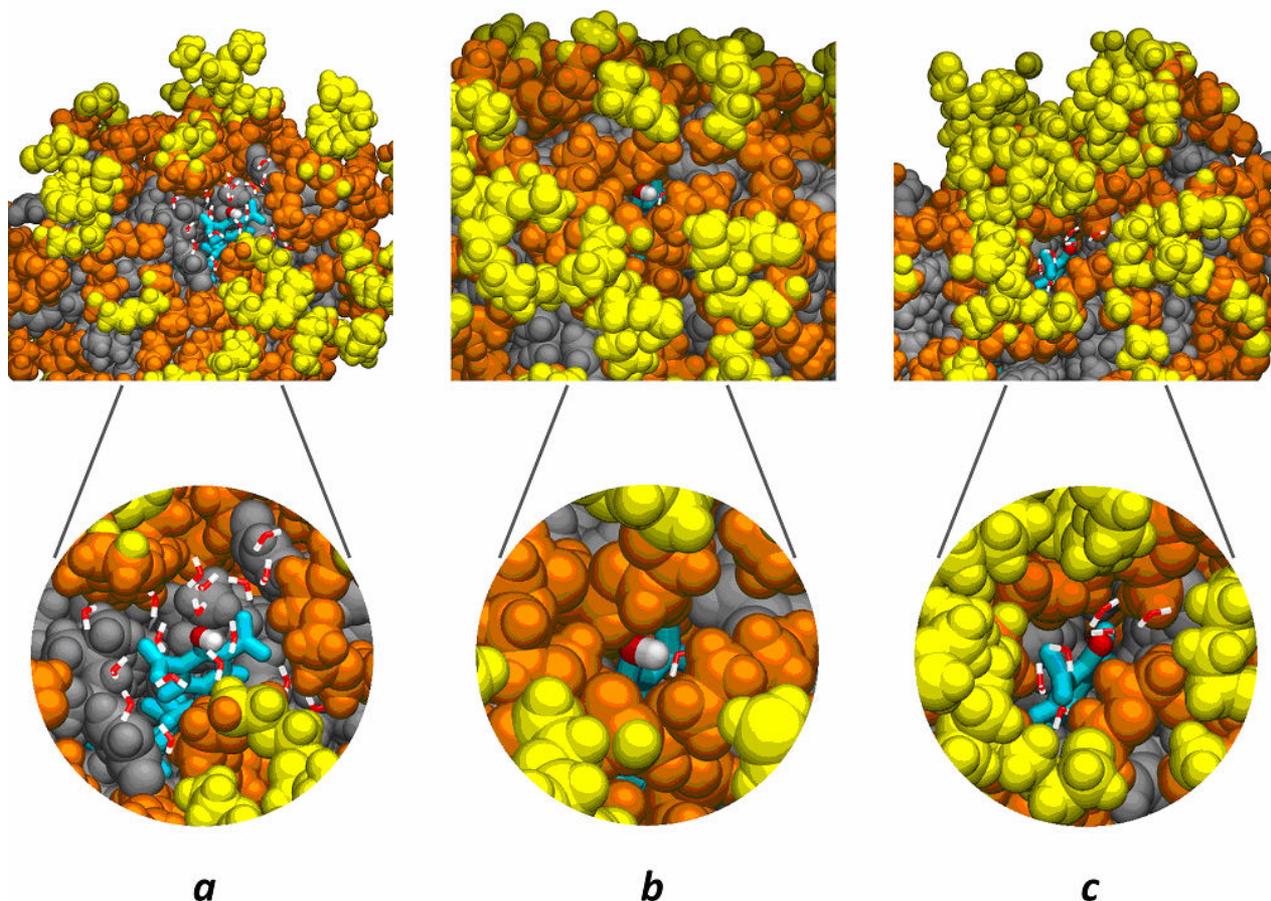
Fig. 4. Instantaneous configurations of DDP in TX-100 micelle: a − highly hydrated neutral form, b − neutral form almost screened from water, c − anion at intermediate hydration. Hydrocarbon core is colored gray, atoms of hydrophilic shell within 0.6 nm from surface are colored orange and other its atoms are colored yellow. Water molecules within 0.5 nm of the dye are shown as lines. Local environment of the dye is shown in insets
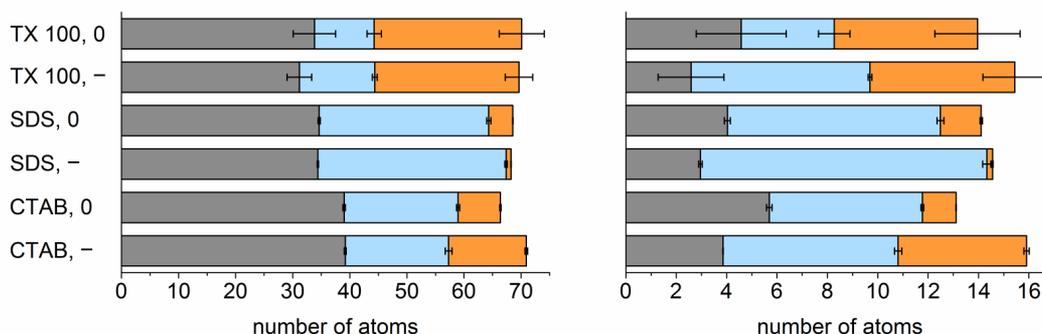
Fig. 5. Compositions of microenvironment of DDP molecule (left) and its hydroxyl O atom (right) in TX-100 micelles. The first section (gray) shows the number of hydrocarbon core atoms, the second one (light blue) shows the number of water atoms, and the one (orange) shows the number of hydrophilic shell or headgroups atoms

utility gmx hbond that was used for analysis. The obtained n_{HB} values are listed in Table. For completeness, the values for water and surfactants studied before are computed and listed as well.

In general, the dye appeared much less hydrated in these non-ionic micelles than in ionic ones. The cause is the bulky hydrophilic shell in the former, which expels a plenty of water from the dye vicinity, as can be seen in Fig. 5. This stays in contrast to the situation in ionic micelles, where hydrophilic

headgroups occupy much less space and screen the molecule from water to lesser extent. This may appear contradictory to the common opinion that non-ionic micelles are well-hydrated, but as we emphasized in the paper devoted to the Reichardt's dye, the microenvironment of the adsorbed species may strongly differ from average composition of the region it resides in.

Average numbers of hydrogen bonds formed by DDP with water in various solutions. Uncertainty is no more than 0.1, except A⁻ in CTAB, where it equals ±0.16

Surfactant	$n_{HB}(HA)$	$n_{HB}(A^-)$
pure water	3.3	8.1
TX-100	1.2	3.5
SDS	2.5	6.4
CTAB	1.7	4.4

It can be seen that the microenvironments of both forms are almost similar despite the difference in charge. This stays in line with the situation in micelles of SDS and CTAB. Oppositely, the amount of water around the hydroxyl group and, consequently, the number of hydrogen bonds formed by it are highly different that is also typical of SDS, but not of CTAB.

It attracts attention that uncertainties for microenvironment in TX-100 are unusually big compared to those in ionic micelles. They stem from discrepancies between the values computed based on three runs. Actually, the convergence of simulation results appeared to be unexpectedly slow, especially for the neutral form. To struggle it, we had to extend simulations of HA to 130 ns, and even this duration appeared insufficient to reach complete coincidence. We think that the cause is that the local environment of the dye molecule in TX-100 is more viscous because of the presence of bulky hydrophilic shell immediately around the hydrocarbon core that moves much slower than the ionic headgroups. Also, it allows for higher variations of dye hydration (from complete covering from water molecules to giving them free access), while small headgroups of ionic surfactants does not allow complete covering. The ionized hydroxyl group has stronger affinity to water than the neutral, thus, complete covering is less typical of it in non-ionic micelles, too. So that, the application of advanced sampling techniques like replica exchange MD [12] may be strongly needed for these systems despite their relative simplicity.

Actually, these observations about localization and hydration of DDP molecule, as well as computational difficulties may be relevant for other acid-base dyes adsorbed by TX-100 micelles, too, because all of them seem to be caused by the nature of hydrophilic shell.

Conclusions

Molecular dynamics simulation of acid-base bye 2,6-dinitro-4-n-dodecylphenol in micelles of non-ionic surfactant Triton X-100 was performed. The results show that the dye molecule (its phenyl ring) in both forms (neutral and anionic) is immersed into hydrocarbon core having hydroxyl group located on the surface of the core mostly keeping contact with water molecules. The dye is much less hydrated than in ionic micelles of sodium dodecyl sulfate or cetyltrimethylammonium bromide; the number of hydrogen bonds formed with water molecules is smaller, as well. Unexpectedly long simulation times were needed to reach sufficiently converged results of different runs, which is likely caused by the bulky nature of hydrophilic shell. These observations may be relevant for other acid-base dyes adsorbed by these micelles. The use of advanced techniques like replica exchange MD may be important for such systems.

REFERENCES

1. *Farafonov V.S., Lebed A.V., Mchedlov-Petrossyan N.O.* Character of localization and microenvironment of solvatochromic Reichardt's betaine dye in sodium n-dodecyl sulfate and cetyltrimethylammonium bromide micelles: molecular dynamics simulation study // Langmuir. − 2017. − Vol.33. − No. 33. − P.8342-8352.

2. *Farafonov V.S., Lebed A.V., Mchedlov-Petrossyan N.O.* An MD simulation study of Reichardt's betaines in surfactant micelles: unlike orientation and solvation of cationic, zwitterionic, and anionic dye species within the pseudophase // Kharkov Univ. Bull., Chem. Ser. − 2018. − Vol.30. − P.27-35.

3. *Mchedlov-Petrossyan N.O., Farafonov V.S., Lebed A.V.* Examining surfactant micelles via acid-base indicators: Revisiting the pioneering Hartley-Roe 1940 study by molecular dynamics modeling // J. Mol. Liq. − 2018. − Vol.264. − P.683-690.

4. *In search* of an optimal acid-base indicator for examining surfactant micelles: Spectrophotometric studies and molecular dynamics simulations / Mchedlov-Petrossyan N.O., Farafonov V.S., Cheipesh T.A., Shekhovtsov S.V., Nerukh D.A., Lebed A.V. //

Colloids Surf., A. − 2019. − Vol.565. − P.97-107.

5. *Yordanova D., Smirnova I., Jakobtorweihen S.* Molecular modeling of Triton X micelles: force field parameters, self-assembly, and partition equilibria // J. Chem. Theory Comput. − 2015. − Vol.11. − P.2329-2340.

6. *Self-assembly* of Triton X-100 in water solutions: a multiscale simulation study linking mesoscale to atomistic models / De Nicola A., Kawakatsu T., Rosano C., Celino M., Rocco M., Milano G. // J. Chem. Theory Comput. − 2015. − Vol.11. − No. 10. − P.4959-4971.

7. *Fatemi S.M., Foroutan M.* Study of dispersion of carbon nanotubes by Triton X-100 surfactant using molecular dynamics simulation // J. Iran Chem. Soc. − 2015. − Vol.12. − P.1905-1913.

8. *Fatemi Sh. M., Foroutan M.* Molecular dynamics simulations studies of Triton surfactant-wrapped single-walled carbon nanotubes surface // J. Adv. Phys. − 2016. − Vol.5. − No. 2. − P.129-133.

9. *Fatemi Sh.M., Foroutan M.* Study of the dynamic behavior of boron nitride nanotube (BNNT) and Triton surfactant complexes using molecular dynamics simulations // Adv. Sci. Eng. Med. − 2014. − Vol.6. − No. 5. − P.583-590.

10. *Grieser F., Drummond C.J.* The physicochemical properties of self-assembled surfactant aggregates as determined by some molecular spectroscopic probe techniques // J. Phys. Chem. − 1988. − Vol.92. − P.5580-5593.

11. *Zefirov Yu.V., Zorkii P.M.* Van der Waals radii and their application in chemistry // Russ. Chem. Rev. − 1989. − Vol.58. − No. 5. − P.421-440.

12. *Replica* exchange molecular dynamics: a practical application protocol with Solutions to common problems and a peptide aggregation and self-assembly example / Qi R., Wei G., Ma B., Nussinov R. // Peptide Self-Assembly. Methods in Molecular Biology. − 2018. − Vol.1777. − P.101-119.

ДОСЛІДЖЕННЯ МЕТОДОМ МОЛЕКУЛЯРНОЇ ДИНАМІКИ КИСЛОТНО-ОСНОВНОГО ІНДИКАТОРНОГО БАРВНИКА В НЕІОНОГЕННИХ МІЦЕЛАХ ТРИТОН Х-100

В.С. Фарафонов, О.В. Лебідь, Н.Л. Хименко, М.О. Мчедлов-Петросян

Незважаючи на широке лабораторне та промислове використання, структура міцел неіоногенних поверхнево-активних речовин (ПАР) та стан адсорбованих ними молекулярних зондів є відносно мало дослідженими розрахунковими методами, порівняно з іоногенними ПАР. Стан кислотно-основного індикаторного барвника 2,6-динітро-4-н-додецилфенолу, солюбілізованого міцелами неіоногенної ПАР Тритон Х-100 визначені за допомогою молекулярно-динамічного моделювання. Розглянуті і нейтральна, і іонізована протолітичні форми. Середнє положення форм відносно міцели, склад їх локального середовища та кількість водневих зв'язків з молекулами води розраховані та порівняні з такими в міцелах іоногенних ПАР. Виявлено, що барвник розташований на поверхні вуглеводневого ядра та є значно менш гідратованим, ніж у іонних міцелах, за рахунок укриття поліоксіетиленовими ланцюгами. Обидві форми локалізовані подібно, проте аніонна форма гідратована сильніше за нейтральну, що подібно до становища в іонних міцелах. Збіжність результатів моделювання виявлена повільнішою, ніж в іонних міцелах, особливо для нейтральної форми.

Ключові слова: 2,6-динітро-4-*н*-додецилфенол, адсорбція, локалізація, гідратація, водневий зв'язок.

MOLECULAR DYNAMICS STUDY OF AN ACID-BASE INDICATOR DYE IN TRITON X-100 NON-IONIC MICELLES

V.S. Farafonov [a, *], A.V. Lebed [a], N.L. Khimenko [b], N.O. Mchedlov-Petrossyan [a]

[a] **V.N. Karazin Kharkiv National University, Kharkiv, Ukraine**
[b] **V.V. Dokuchaev Kharkiv National Agricultural University, Dokuchaevske, Kharkiv region, Ukraine**

* **e-mail: farafonov@karazin.ua**

Despite wide laboratory and industrial use, the structure of non-ionic surfactant micelles and the state of molecular probes adsorbed by them are relatively poorly studied by computational methods in comparison to the ionic surfactants. The state of an acid-base indicator dye, namely, 2,6-dinitro-4-n-dodecylphenol, solubilized by micelles of Triton X-100 non-ionic surfactant, was revealed by means of molecular dynamics simulations. Both neutral and ionized protolytic forms were considered. The average positioning of the forms with respect to micelle, composition of their local environment and number of hydrogen bonds with water molecules were obtained and compared with those in ionic micelles. The dye was found to be located on the surface of hydrocarbon core and be significantly less hydrated than in ionic micelles due to covering by polyoxyethylene chains. Both forms are localized similarly, while the anionic form is hydrated stronger than the neutral one, which resembles the situation in ionic micelles. The convergence of simulation results in such micelles is found to be slower than in the ionic micelles, especially for the neutral form.

Keywords: 2,6-dinitro-4-*n*-dodecylphenol; adsorption; localization; hydration; hydrogen bond.

REFERENCES

1. Farafonov V.S., Lebed A.V., Mchedlov-Petrossyan N.O. Character of localization and microenvironment of solvatochromic Reichardt's betaine dye in sodium n-dodecyl sulfate and cetyltrimethylammonium bromide micelles: molecular dynamics simulation study. *Langmuir*, 2017, vol. 33, pp. 8342-8352.

2. Farafonov V.S., Lebed A.V., Mchedlov-Petrossyan N.O. An MD simulation study of Reichardt's betaines in surfactant micelles: unlike orientation and solvation of cationic, zwitterionic, and anionic dye species within the pseudophase. *Kharkov University Bulletin Chemical Series*, 2018, vol. 30, pp. 27-35.

3. Mchedlov-Petrossyan N.O., Farafonov V.S., Lebed A.V. Examining surfactant micelles via acid-base indicators: revisiting the pioneering Hartley-Roe 1940 study by molecular dynamics modeling. *Journal of Molecular Liquids*, 2018, vol. 264, pp. 683-690.

4. Mchedlov-Petrossyan N.O., Farafonov V.S., Cheipesh T.A., Shekhovtsov S.V., Nerukh D.A., Lebed A.V. In search of an optimal acid-base indicator for examining surfactant micelles: spectrophotometric studies and molecular dynamics simulations. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 2019, vol. 565, pp. 97-107.

5. Yordanova D., Smirnova I., Jakobtorweihen S. Molecular modeling of Triton X micelles: force field parameters, self-assembly, and partition equilibria. *Journal of Chemical Theory and Computation*, 2015, vol. 11, pp. 2329-2340.

6. De Nicola A., Kawakatsu T., Rosano C., Celino M., Rocco M., Milano G. Self-assembly of Triton X-100 in water solutions: a multiscale simulation study linking mesoscale to atomistic models. *Journal of Chemical Theory and Computation*, 2015, vol. 11, pp. 4959-4971.

7. Fatemi S.M., Foroutan M. Study of dispersion of carbon nanotubes by Triton X-100 surfactant using molecular dynamics simulation. *Journal of the Iranian Chemical Society*, 2015, vol. 12, pp. 1905-1913.

8. Fatemi S.S.M., Foroutan M. Molecular dynamics simulations studies of Triton surfactant-wrapped single-walled carbon nanotubes surface. *Journal of Advanced Physics*, 2016, vol. 5, no. 2, pp. 129-133.

9. Fatemi S.M., Foroutan M. Study of the dynamic behavior of boron nitride nanotube (BNNT) and Triton surfactant complexes using molecular dynamics simulations. *Advanced Science, Engineering and Medicine*, 2014, vol. 6, no. 5, pp. 583-590.

10. Grieser F., Drummond C.J. The physicochemical properties of self-assembled surfactant aggregates as determined by some molecular spectroscopic probe techniques. *Journal of Physical Chemistry*, 1988, vol. 92, pp. 5580-5593.

11. Zefirov Yu.V., Zorkii P.M. Van der Waals radii and their application in chemistry. *Russian Chemical Reviews*, 1989, vol. 58, no. 5, pp. 421-440.

12. Qi R., Wei G., Ma B., Nussinov R. Replica exchange molecular dynamics: a practical application protocol with solutions to common problems and a peptide aggregation and self-assembly example. In: Nilsson B., Doran T. (eds.) *Peptide Self-Assembly. Methods in Molecular Biology*, 2018, vol. 1777, pp. 101-119.