American Journal of Chemistry 2012,2(3): 131-136
DOI: 10.5923/j.chemistry.20120203.06

Dynamics of Apolar Guest Solubilized in Bile Salt Micelles: Photochemistry of Acenaphthylene as a Probe to Understand the Supramolecular Characteristics of the Aggregates

Nick Carter, Mahesh Pattabiraman*, Luis Albert Arias

Department of Natural Sciences, Western New Mexico University, Silver City, 88061, USA

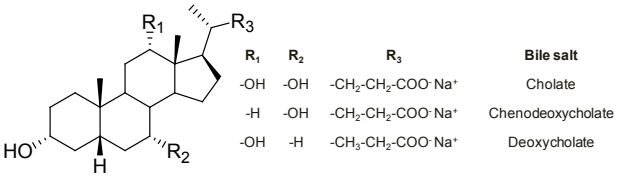
Abstract Product distribution resulting from the excited state reactivity of acenaphthylene is influenced predominantly by multiplicity of the excited state, mobility of the substrate in a given medium, and spatial constraints. The photochemistry of acenaphthylene solubilized by sodium cholate, a member of the atypical micelle forming molecules called bile salts, in water was investigated to understand the interior of the aggregates. Results indicate that the supramolecular characteristics of the aggregates change significantly with concentration which in turn affects the mobility of the encapsulated acenaphthylene guest leading to changes in product distribution from the reaction.

Keywords Bile salts, sodium cholate, micelles, complexation, photochemistry, acenaphthylene, excited state, supramolecular chemistry, green chemistry

1. Introduction

Micelles are supramolecular systems with tremendous applications in biotechnology, catalysis, medicine, nanotechnology, food science, environmental remediation, catalysis, and other areas of practical significance.[1-8] Straight chain amphiphiles such as phospholipids, alkyl sulfonates, tetra alkyl ammonium salts, and alkyl polyglucosides have been well studied for their micelles. But bile salt micelles have remained relatively unexplored,[9,10] especially for application in green chemistry as media to perform chemical transformations.[11-13]

Bile salts are produced in the liver of mammals and are understood to play important roles in digestion of fat.[14-17] Bile salts are family of compounds with steroidal frame work and contain hydroxyl groups (two to three) and an acid group containing side-chain. The steroidal backbone of the molecule assumes a puckered shape in which the hydrophobic methyl groups occupy the convex side and the hydrophilic hydroxyl groups occupy the concave side (Figure 1) rendering the molecules amphiphilic. When dissolved in aqueous solutions at concentrations close to their formal CMC, the bile salts aggregate (Figure 2) to produce primary aggregates composed of 5-10 monomers[18] in which the convex side faces the interior of the aggregate and the concave side faces the aqueous phase. Furthermore, at higher concentrations the primary aggregates are known to aggregate further to yield larger loosely held structure called the secondary aggregates sustained by hydrogen-bonding between the hydroxyl groups of the bile salt and water molecules in the medium.[18-20]



R_1	R_2	R_3	Bile salt
-OH	-OH	$-CH_2-CH_2-COO^-Na^+$	Cholate
-H	-OH	$-CH_2-CH_2-COO^-Na^+$	Chenodeoxycholate
-OH	-H	$-CH_3-CH_2-COO^-Na^+$	Deoxycholate

Figure 1. Chemical structure of bile salt monomers

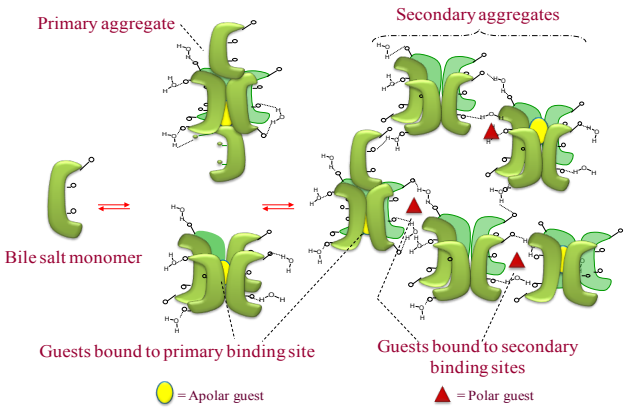


Figure 2. Representation of stepwise aggregation of bile salts in aqueous medium resulting in formation of primary and secondary aggregates

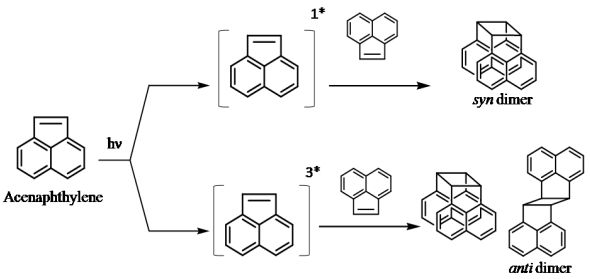
* Corresponding author:
pattabiramanm@wnmu.edu (Mahesh Pattabiraman)
Published online at http://journal.sapub.org/chemistry

The primary and secondary aggregates contain small pockets of empty space which are capable of accommodating guests of appropriate size and polarity. The pockets of space in the primary aggregates (primary binding sites) tend to be highly non-polar, and rigid, while the same in the secondary aggregates (secondary binding sites) tend to be more polar, less rigid, and relatively voluminous. The primary binding sites of bile salt micelles are excellent binding sites for nonpolar guest molecules such as polyaromatic hydrocarbons while the secondary aggregates accommodate large, polar guest molecules. Unlike the well-studied long chain micelles, bile salt micelles exhibits a much more complex aggregation pattern and possess unique supramolecular potentials, resulting from their distinct binding sites, such as tunability, compartmentalization of binding sites, and guest induced aggregation.[21-25]

In spite of their dynamic nature and great supramolecular potential bile salts still remain under-studied in several aspects – especially in terms of application as a green medium for chemical transformations. Therefore, we attempted to study the photocycloaddition of acenaphthylene in the medium for two primary reasons: (a) photochemistry of acenaphthylene has been used as a probe to gain insight into the mobility and immediate spatial environment of the encapsulating medium, (b) the reaction would also allow us to explore the bile salts aggregates as a green medium to substitute for organic solvents to perform photodimerization reactions. Herein, we report the outcome of our detailed studies exploring the interior of the bile salt aggregates using photochemistry of acenaphthylene as a probe.

2. Results and Discussion

2.1. Photochemistry of Acenaphthylene



Scheme 1. Photochemistry of acenaphthylene

Acenaphthylene (ACN) yields two dimers upon irradiation that are stereo isomers (Scheme 1) - the *syn* and the *anti*.[26,27] The proportion of *syn* and the *anti* dimers in the product mixture is dependent on three main factors, viz. multiplicity of excited state, concentration, and polarity of medium. Investigations conducted by Cowan et. al.[26] led them to ascertain that acenaphthylene in its singlet (S_1) excited state yields the *syn* dimer while the triplet (T_1) state yields both *anti* dimer (~90%) and *syn* dimer (~10%). With respect to concentration: higher concentration of acenaphthylene favors the formation of *syn* dimer over *anti*, and dilution results in the increase of *anti* dimer formation, which is in fact a consequence of the singlet-triplet dependent photochemistry; at high concentrations acenaphthylene has greater probability of reacting from the short lived singlet state to yield the *syn* dimer. At lower concentrations due to its shorter lifetime, the probability of S_1 surviving long enough to encounter another acenaphthylene in the ground state is reduced. Hence the proportion of the *syn* dimer in the product mixture decreases resulting in the reduction of *syn* dimer proportion.

Due to the medium influenced photochemistry, ACN has been often utilized to understand the dynamics of encapsulated guest in unexplored medium.[28-32] Under the same consideration, we have performed the photochemistry of ACN in NaCh to understand the nature of the medium.

In order to understand the nature of the bile salt micelles as a homogeneous medium and the variation in supramolecular characteristics of the aggregates with changes in concentration, the photochemistry of ACN was explored by observing product distribution with changes in concentration of sodium cholate.

2.2. Inclusion of Acenaphthylene within Sodium Cholate Aggregates

The procedure for complexation of guest involves initial sonication of mixture of sodium cholate and acenaphthylene in water, followed by even stirring of solution for six hours. Solubilization of guest within the aggregates is easily noted by observing increasing solution homogeneity over time. Evidence of guest included within aggregates may be obtained by recording ^{1}H NMR of an identical solution prepared in D_2O (Figure 3) where aromatic guest signals could be seen alongside those of host's aliphatic signals. Acenaphthylene does not dissolve in water to any detectable extent in NMR even after sonication followed by stirring for extended durations.

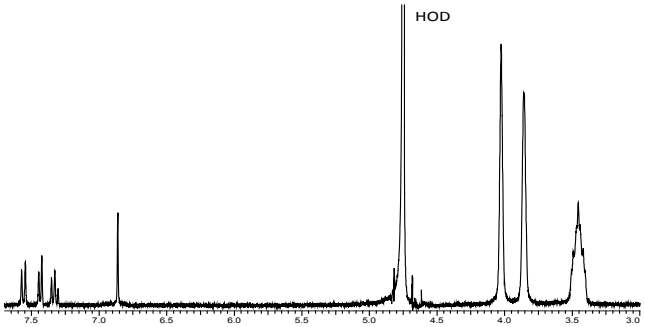


Figure 3. Partial ^{1}H NMR spectrum of acenaphthylene (0.66 mM) solubilized in D_2O by sodium cholate aggregates (45 mM) shows the aggregate mediated dissolution of guest in aqueous medium

2.3. Photoreactivity of Acenaphthylene in Sodium Cholate

Table 1 presents the product distribution resulting from the irradiation of solutions in which concentration of NaCh was varied incrementally (5 mM to 250 mM) while that of the guest ACN was maintained at 0.66 mM. The concentra-

tion range for NaCh in the experiments were chosen to entail equilibrium situations in which the predominant equilibrium species shift from NaCh monomer (at lower concentrations) to primary aggregates to secondary aggregate (at higher concentrations).

Table 1. Product selectivity observed in photochemistry of acenaphthylene (ACN) incorporated in presence of varying amounts of sodium cholate in aqueous sodium chloride solution

[NaCh] mM	Predominant aggregate type[15]	% *syn*	% *anti*	*syn*:*anti*
5	Monomer-Primary	66	34	1.94
20	Primary	71	29	2.45
40	Primary-Secondary	78	22	3.55
90	Secondary	84	16	5.25
140	Secondary	87	13	6.69
190	Secondary	86	14	6.14
250	Secondary	88	12	7.33

Note: Concentration of acenaphthylene was fixed at 0.66 mM. 25 mM sodium chloride aqueous solution was used to prepare sodium cholate solution. Conversion in all cases was between 30-40%. Entries are average of three independent experiments.

Though the formal CMC of NaCh is commonly accepted to be between 15 mM and 21 mM,[33-36] unlike straight chain surfactants, it has been established in literature that bile salts form micelles at concentrations well below the CMC. The same fact was corroborated in our studies where we observed that ACN (0.66 mM) was in fact solubilized by NaCh at concentration as low as 5 mM. The solution, upon irradiation resulted in the formation of 66 % *syn* dimer and 34 % *anti* dimer. Whereas, irradiation of the same amount of ACN solubilised in 20 mM NaCh, the concentration in which the equilibrium predominantly lies towards primary aggregates, the medium favored the formation of *syn* dimer by 71 % over 29% for *anti*. The trend of increase in *syn* dimer proportion with increase in concentration of bile salt persisted as the concentration of NaCh was increased further to 40 mM, 90 mM, and 140 mM. At 140 mM of NaCh, 87 % of *syn* and 13 % of *anti* dimer were observed, and at concentrations higher than 90 mM there were no significant changes in the *syn*/*anti* proportions.

Table 2. Product distribution obtained from the irradiation of varying amounts acenaphthylene in presence of a constant concentration of sodium cholate[a] in water

[ACN] mM	% *syn*	% *anti*	*syn*:*anti*
0.66	83	17	4.26
1.5	80	20	4.88
2.5	81	19	5.25
3.5	83	17	3.34
4.5	82	18	4.26

Note: Concentration of sodium cholate was maintained at 45 mM. 25 mM sodium chloride aqueous solution was used to prepare sodium cholate solution. The conversion in all cases was between 30-40%.

Table 2 presents the results of photochemistry of ACN performed in which the concentration of the host was maintained constant at 45 mM while that of the guest was varied between 0.66 mM and 4.5 mM. Considering an average aggregation number of 6 for the primary aggregates, the mean occupancy numbers for the aforementioned concentrations vary between 0.09 and 0.6. There were no significant changes in product distribution for the seven fold increase in guest concentration. The correlation between dimer proportion and NaCh concentration is represented as a plot in Figure 4.

Control studies were also performed to serve as reference points to help understand the effect of spin multiplicities in the product distribution within the medium (Table 3). Irradiations were performed in O_2 purged solution, a triplet quencher, resulted in 94 % *syn* dimer – 11 % higher formation of *syn* dimer compared to the same in N_2 purged solution.

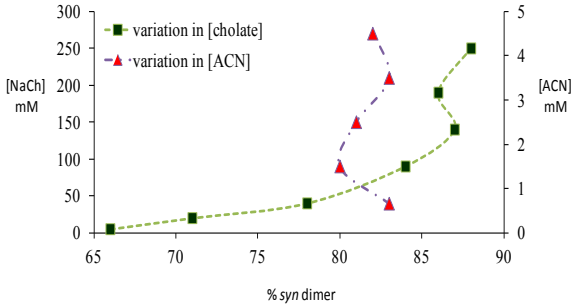


Figure 4. Photoproduct distribution from reaction of ACN and variation with concentration changes

When Eosin-Y, a triplet sensitizer, was added to the solution of ACN solubilised in NaCh, 56% *syn* dimer resulted with *anti* dimer still being the minor product. When a solution of identical concentration of ACN in hexane was irradiated in presence of Eosin-Y, the *anti* dimer was the major product.

Table 3. Product distribution obtained from the irradiation of varying amounts acenaphthylene in presence of a constant concentration of sodium cholate[a] in water

Medium	Condition	Role	% *syn*	% *anti*
NaCh	N_2-purged		83	17
NaCh	O_2-purged	Triplet quencher	94	6
Hexane	Eosin-Y	Triplet sensitizer	45	55
NaCh	Eosin-Y	Triplet sensitizer	56	44

a) Concentration of acenaphthylene 1.3 mM and that of NaCh 45 mM. b) Concentration of Eosin-Y was approximately 10^{-5} M. The solutions were irradiated with a 410 nm cut-off filter.

2.4. Discussion of Results

Selectivity in product distribution of acenaphthylene in constrained medium is shown to be primarily controlled by two factors: the spin states of ACN in the excited state,[21,22] and the availability of space, where spatial limitations favor the more compact *syn* dimer over *anti*.[28] The possibility of a ground state complex of acenaphthylene preoriented to form the *syn* dimer could be ruled out based on the reports by Bohne et. al.[27] as this would require multiple occupancy for ACN within the primary aggregates of sodium cholate – primary binding sites are too small to accommodate two guest molecules simultaneously.

To gauge the influence of the spatial factor alone on the

product distribution, the contribution from the spin factor was eliminated by using triplet quenchers and sensitisers (Table 3). In the absence of spin state manipulating species, ACN reacts to yield 83% *syn* and 17% *anti* dimer. Whereas, the same for oxygen purged solution (oxygen being a triplet quencher) yielded almost exclusively *syn* dimer. The formation of as much as 94% of *syn* dimer in the medium is indicative of the fact that the medium does not thwart the near quantitative formation of *syn* dimer.

On the other hand, when the reaction was performed with Eosin-Y in the medium, a triplet sensitizer, yielded 56 % *syn* dimer; comparing this with the Eosin-Y sensitized reaction in hexane where no spatial constraints exist, 11 % more *syn* dimer (Table 3) was obtained within the aggregates. Considering the results from control studies presented in table 3, it is evident that the formation of *syn* dimer is favored within the primary aggregates most likely due to its compactness in shape, as the space available for binding within the aggregates is both limited and very rigid.

The notable feature in the photodimerization of acenaphthylene within in sodium cholate micelles is the observed trend of increase in *syn* dimer formation within increase in micelle concentration until 90 mM and no significant changes thereafter (Figure 4). By correlating the bile salt equilibrium dynamics with the trend in *syn*:*anti* proportion in reaction outcome with changes in NaCh concentration, it is apparent that as the concentration of bile salt increases, the equilibrium shifts towards the secondary aggregates formation the photochemistry of acenaphthylene is steered towards the formation of *syn* dimer in higher proportion.

In order to explain the observed trend in product distribution, we propose the following: acenaphthylene, being highly nonpolar, is expected to be solubilised in primary aggregates only. At low concentrations of NaCh secondary aggregates is expected to be absent and, therefore, ACN will be solubilised mostly by primary aggregates. In this situation, the probability of dimerization of ACN in its S_1 and T_1 states are limited by the diffusion of the primary aggregates – the mobility of the guest is only as fast as the mobility of the primary aggregate itself. Since the S_1 state is short-lived than the T_1 state (by three orders of magnitude) the low NaCh concentration situation favors reactivity from the T_1 state more than the S_1 as the latter would be the first to "die out" due to relaxation.

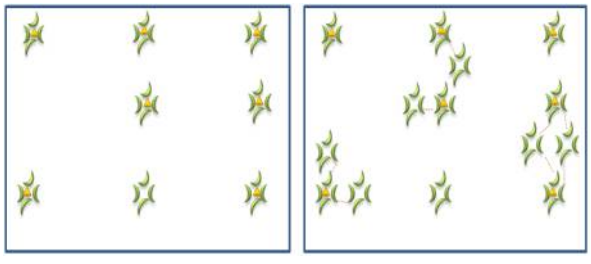


Figure 5. Pictorial representation of two different scenarios – low concentration (left) and high concentration (right) of NaCh – to explain the variation in product distribution obtained from the photochemistry of ACN

On the other hand, at higher concentrations of NaCh the equilibrium shifts towards the formation of secondary aggregates which are conglomerates of primary aggregates. The secondary aggregates could be envisioned as a 'micro-environment of micellar network' (depicted in Figure 5) of primary aggregates loosely held together by forces of hydrogen-bonding interaction between the hydroxyl groups of bile salt and water molecules. Within this 'micellar network' the local mobility of solubilised guest molecule such as ACN would be significantly enhanced as multiple primary aggregates are held close to each other with the NaCh monomers engaged in the dissociation-association equilibrium. Due to enhanced mobility the probability of apolar guest solubilised within the primary aggregates, the probability of ACN reacting from its singlet excited state is greatly increased as the mobility of ACN is no longer limited to the diffusion of the primary aggregates only. This in turn would reflect in the increased formation of *syn* dimer - as is the case.

This model explains the observed increase in *syn* dimer formation with increasing bile salt concentration until the concentration reaches 140 mM. For concentration increase beyond 140 mM there appears to be no concomitant change in supramolecular characteristics of the aggregates. Based on the fact that there is no significant increase in the formation of *syn* dimer beyond 140 mM, we believe that 140 mM is the concentration at which the equilibrium attributes in favour of secondary aggregates are at their highest. We also infer that increasing concentration of NaCh beyond 140 mM does not enrich the medium with secondary aggregates at the same rate observed between the 20 mM – 140 mM bracket. In order to confirm that it is the concentration of secondary aggregates and the proximity of the primary aggregates within the secondary aggregates that affects the photoproduct distribution of acenaphthylene photodimers, we performed experiments in which concentration of ACN was varied while that of Nach was maintained at 45 mM (Table 2). In this case, as expected, not much variation in product distribution was observed which substantiates the hypothesis that at lower concentrations of NaCh, the reactivity is limited by the diffusion of primary aggregates. The clear influence of sodium cholate assembly equilibrium on the photochemical outcome of acenaphthylene is evident from the trend presented in figure 4, where *syn*:*anti* ratio changes with NaCh concentration. Our studies have lead us to understand the dynamic nature of the bile salt aggregates and the mobility of guest molecule within the assembly.

3. Conclusions

Sodium cholate aggregates were employed to perform the photochemical dimerization of acenaphthylene. Concentration variation experiments and control experiments were performed to understand the interior of the primary aggregates and the shift in supramolecular characteristics of the bile salt micro-heterogeneous medium. Results indicate that

the tight spaces within primary aggregates favor the formation of *syn* dimer over the spatially demanding *anti* dimer. In addition the concentration dependent photochemistry suggests that at lower concentrations of NaCh the mobility of the guest is limited by the diffusion characteristics of the primary aggregates. Whereas, at higher concentration of NaCh the predominance of secondary aggregates act as a micro-network of primary aggregates increasing the mobility of the guest molecules. The reaction provides insight into the mobility and spatial constraint of apolar guest molecules encapsulated within the bile salt micellar system.

4. Experimental

Materials

Sodium cholate (NaCh), sodium chloride, anhydrous sodium sulfate, and eosin-Y, were procured from Sigma Aldrich Co. and used without further purification. Acenaphthylene procured from Sigma Aldrich Co. was 95% acenaphthylene and 5% acenaphthene as indicated in the manufacturer's label and analyzed by us in the GC. The compound was purified by vacuum sublimation technique during which acenaphthylene sublimed on the upper walls of the sublimator while acenaphthene remains at the bottom. This procedure was repeated to obtain acenaphthylene more than 98% pure.

Mass Balance Experiments:

Mass balance experiments were carried out to confirm that product selectivities observed were not due to selective decomposition, loss, inclusion, or exclusion of reaction components. A standard solution with equal amounts of the acenaphthylene and an external standard (2mg each in 5mL of hexane-ethyl acetate) for GC (naphthalene) was prepared and injected in the GC. The ratio of the peak intensities was taken as a standard for 1:1 mixture by mass. To determine the mass balance after photochemistry, the irradiated samples were extracted and mixed with an equal mass (as that of the reactive guest taken) of internal standard and analyzed in the GC. The ratio of all peaks in the GC run with respect to the internal standard was considered to calculate the mass balance. In all cases the mass balance was estimated to be at least 80%.

Complexation, irradiation and extraction procedure:

A standard solution of acenaphthylene (5mg/mL) in dichloromethane was prepared. Volume corresponding to 0.5 mg was pipetted out and solvent evaporated to obtain a thin film of the compound. 20 mL of 25 mM sodium chloride solution, 45 mg of sodium cholate were added to the acenaphthylene thin film. Sonication for 15 minutes and allowing the solution to stir for 2 hours resulted in formation of clear yellow colored solutions. The solutions were filtered with a Whatmann filter paper of fine porosity and slow flow rate. The filtrate was purged with nitrogen for 15 minutes, sealed and irradiated with a 410 nm cut-off filter. The cut-off filter was used to prevent dimers from absorbing any radiation from the photochemical lamp source.

After irradiation, the solution was diluted by adding 30 mL of deionized water. Extraction of the starting material and reactants was performed in a separatory funnel with 30 mL of ethyl acetate and 15 mL of acetonitrile mixture. The organic layer was dried over anhydrous sodium sulfate and analyzed in a Shimadzu model 4020 GC-MS fitted with an Agilent SE-30 column. The identities of the dimers were also established by ^{1}H NMR spectroscopy.

Reported values

All values reported in tables are averages of three independently performed experiments. The averages are rounded off to the closest unit and the error limit for reported values is ± 3%.

Analysis of photodimers of acenaphthylene:

The identities of the photoproducts were confirmed by NMR spectroscopy recorded on a Bruker Avance 300 MHz spectrometer and referenced using the residual solvent signal as the internal standard. The chemical shifts were matched with those reported in literature.[22]

Syn dimer: ^{1}H NMR ($CDCl_3$) δ 7.18 (d, 4H, J = 8.0 Hz), 7.15 (d, 4H, J = 7.5 Hz), 7.03 (d, 4H, J = 7.3 Hz), 4.85 (s, 4H).

Anti dimer: ^{1}H NMR ($CDCl_3$) δ 7.74 (d, 4H, J=8 Hz), 7.60 (d, 4H, J = 7.9 Hz), 7.01 (d, 4H, J = 7.9 Hz), 4.11 (s, 4H).

For routine analysis and quantitative determination of products and reactant composition in the reaction mixture, samples were analyzed as such after extraction in a GC-MS.

GC-MS program: Initial temp 100° C, Initial time 1 minute, Initial rate 10° C/minute, final temperature 300° C and final time 5 minutes. Retention times: ACN at 7.5 min, *syn* dimer at 13.3 min and anti dimer at 16.1 min.

ACKNOWLEDGEMENTS

The work presented in the manuscript was performed with the financial support from VP's office at WNMU. MP thanks Mr. Ross Fischer in the Dept. Of Natural Sciences, WNMU for his support, and Dr. Gopalan (New Mexico State University, Las Cruces) for helping us in acquiring NMR spectra for characterization. MP is very thankful to Dr. V. Ramamurthy for providing insight and advice in this project.

REFERENCES

[1] A. Singh, J.D. VanHamme, and O.P. Ward, *Biotechnol. Adv.*, 2007, 25 (1), 99.

[2] A.S. Yazdi, *Trends Anal. Chem.*, 2011, 30 (6), 918.

[3] A.N. Lukyanov, and V.P. Torchilin, *Adv. Drug Delivery Rev.*, 2004, 56 (9), 1237.

[4] E. Semo, E. Kesselman, D. Danino, Y. D. Livney, *Food Hydrocolloids*, 2007, 21, 5-6, 936.

[5] S. Paria, *Adv. Colloid Interface Sci.*, 2008, 138 (1), 24.

[6] S. Kim, Y. Shi, J. Kim, K. Park, and J. Cheng, *Expert Opin. Drug Discovery*, 2010, 7 (1), 49.

[7] K. Trickett, and J. Eastoe, *Adv. Colloid Interface Sci.*, 2008, 144 (1-2), 66.

[8] L. Kumar, T. Mahajan, and D. D. Agarwal, *Ind. Eng. Chem. Res.*2012, 52 (5), 2227.

[9] M. Pattabiraman, L.S. Kaanumalle, and V. Ramamurthy, *Langmuir*, 2006, 22 (5), 2185.

[10] S. Bhat, and U. Maitra, *Molecules*, 2007, 12(9), 2181.

[11] F. Jiang, J. Du, X. Yu, J. Bao, and X. Zeng, *J. Colloid Interface Sci.*, 2004, 210 (2), 497.

[12] U. Tonellato, *Pure Appl. Chem.*, 1998, 70(10), 1961.

[13] F. Trentin, A. Scarso, and G. Strukul, *Tetrahedron Lett*. 2011, 52 (51), 6978.

[14] A. Schreiber, F. R. Simon, *J, Pediatr. Gastroenterol. Nutr.* 1983, 2(2), 337.

[15] P. Mukerjee, Y. Moroi, M. Murata, and A. Y. S. Yang, *Hepatology*, 1984, 4 (5 suppl), 61s.

[16] P. Mukerjee, and J. R. Cardinal, *J. Pharm. Sci.* 1976, 65 (6), 882.

[17] P. Ekwall, *J. Colloid Sci.* 1954, (Suppl. 1), 66.

[18] L. Hao, R. Lu, D.G. Leaist, and P. R. Poulin, *J. Soln. Chem.* 1997, 26 (2), 113.

[19] D.M. Small, In *The Bile Salts*; P.P. Nair, D. Kritchevsky, Eds.; Plenum Press: New York, 1971, Vol. 1, pp 249-256.

[20] D.M Small, S.A. Penkett, and D. Chapman, *Biochim. Biophys. Acta*, 1969, 176 (1), 178.

[21] R. Li, E. Carpentier, E. D. Newell, L. M. Olague, E. Heafey, C. Yihwa, and C. Bohne, *Langmuir*. *2009*, 25 (24), 13800.

[22] G. Conte, R. Di Blasi, E. Giglio, A. Parretta and N. V. Pavel, *J. Phys. Chem.*, 1984, 88 (23), 5720.

[23] A. Jover, F. Meijide, E. Rodríguez Núñez and J. Vásquez Tato, M. Mosquera. *Langmuir*, 1997, 13 (2), 161.

[24] L. L. Amundson, R. Li, and C. Bohne, *Langmuir*, 2008, 24 (16), 8491.

[25] C. Ju, and C. Bohne, *Photochem. Photobiol.* 1996, 63 (1), 60.

[26] D. O. Cowan, and R. L. E. Drisko, *J. Am. Chem. Soc*. 1970, 92 (21), 6286.

[27] N. Haga, H. Takayanagi, and K. Tokumaru, *J. Org. Chem.*1997, 62 (11), 3734.

[28] M. Yoshizawa, Y. Takeyama, T. Okano, and M. Fujita. *J. Am. Chem. Soc.* 2003, 125 (11), 3243.

[29] V. Ramesh, and V. Ramamurthy, *J. Photochem.* 1984, 24 (4), 35.

[30] L. S. Kaanumalle, and V. Ramamurthy, *Chem. Commun.* 2007, (10), 1062.

[31] D. Madhavan, K. Pitchumani. *Photochem. Photobiol. Sci.* 2003, 2(2), 95.

[32] Y. Matsuo, T. Fukunaga, N. Tokura, T. Fukutsuka, and Y. Sugie. *Trans. Mat. Res. Soc. Jpn*. 2003, 28(3), 589.

[33] L. B. Pa´rtay, M. Sega, and P. Jedlovszky, *Langmuir*, 2007, 23 (24), 12322.

[34] P. Perez-Tejeda, R. Risquez, A. R. Perez, A. Terriza, and M. P. Leon. *All Res. J. Chem.* 2010, 1 (1), 13.

[35] A. Coello, F. Meijide, E. R. Nunez, and J. V. Tato, *J. Pharm. Sci.* 1996, 85(1), 9.

[36] S. Reis, C. G. Moutinho, C. Matos, B. de Castro, P. Gameiro, J. L. F. Lima, *Anal. Biochem.* 2004, 334, 117–126.