Synthesis and physicochemical properties of non-ionic and cationic surfactants derived from methionine

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Abstract

Amino acid based surfactants have been widely used as alternative to classical surfactants due to their interesting physicochemical properties, biological activities, biodegradability and low toxicity. The study of the physicochemical properties of surfactants such as critical micelle concentration (CMC) and Krafft temperature is an important aspect in determining their use in detergents or pharmaceutical compositions. In this study, a series of non-ionic and cationic surfactants with varying chain lengths (C_8-C_{12}), namely the *O*-alkyl esters of methionine and their hydrochloride derivatives respectively were synthesized and characterized by spectral data. In view of systematically studying the effect of the head group and chain length on the physicochemical properties, the critical micelle concentrations of both series were evaluated using conductivity measurements and pyrene fluorescence spectroscopy. The Krafft temperatures of selected synthesized methionine-based surfactants were also evaluated. The methionine surfactants were synthesized in good to moderate yield (54 – 81%). For both ester and the hydrochloride series, the CMC of the surfactants were found to decrease with chain length, as commonly observed for all surfactants. The non-ionic *O*-alkyl esters of methionine displayed relatively lower CMC values compared to their ester hydrochloride derivatives for all the chain length studied. The ester hydrochloride derivatives showed an increase in the Krafft temperature with increasing chain length showing that the surfactants with longer chain length has a tendency to micellise at a much higher temperature.

1. Introduction

Surfactants are a class of compounds that have been widely used in the food, pharmaceutical, textile, cosmetics and detergent industries due to their unique properties such as adsorption at interfaces, self-association, solubilisation and interesting biological properties [1, 2]. The strong demand towards the development of more environmentally benign chemicals has led to the use of greener alternatives to replace petroleum-based chemicals for the preparation of surfactants [3]. In this instant, amino acid-based surfactants which constitute a class of compounds with interesting physicochemical properties and a wide variety of biological activities have found numerous applications in the medical and food sector, due to their high level of biocompatibility and biodegradability [4, 5, 6].

For the past few years, different amino acids have been reported to give rise to surfactants with multiple structures, each possessing a unique physicochemical and biological properties depending on the functional groups present on the amino acid head group and the surfactant's tail [7, 8]. Surfactants derived from sulfur-based amino acids such as cysteine have been found to show very interesting and unique physicochemical properties [9-11]. *N*-alkylamine surfactants derived from cystine and cysteine have been reported to form micelles and small lamellar vesicles in aqueous solutions [12]. Amide surfactants derived from cystine have been reported to show interesting surface properties and aggregation behaviour which allowed them to form various structures such as spherical vesicles [13]. However, there are only few reports on the synthesis and physicochemical properties of surfactants derived from methionine.

Our group has been dealing with the synthesis, physicochemical and biological properties of *O*-alkyl esters of aromatic and cyclic amino acids [14-18]. In this study, a series of *O*-alkyl ester of methionine and their hydrochloride derivatives were synthesised and their physicochemical properties were investigated. The effect of chain length and head group structure on the micellisation properties of the methionine esters were studied.

Materials and methods

1.1.Chemicals and instrumentation

L-Methionine and octanol were purchased from BDH Laboratory Supplies (England). Decanol, dodecanol and the fluorescence probe pyrene were purchased from Sigma-Aldrich (St Louis, USA). Silica gel (60-120 Mesh) obtained from Alpha Chemika (India) was used for column chromatography. ^{1}H NMR and ^{13}C NMR spectra were recorded at 250 and 62.9 MHz on a Bruker electro spin NMR spectrometer using $CDCl_3$, D_2O and DMSO-d_6 as solvents. IR spectra were recorded on a Bruker Alpha FT-IR spectrometer. Fluorescence intensities were recorded on a LS 55 Perkin Elmer fluorescence spectrophotometer.

1.2.Synthesis and Characterisation

The methionine esters were synthesized according to our previous procedure reported by our group [14]. L-Methionine (12.1 mmol), *p*-toluene sulfonic acid (12.1 mmol) and the corresponding alcohol (14.5 mmol) were refluxed in toluene (100 ml) for 48 hours using a Dean-Stark apparatus. The crude products were purified by column chromatography on silica gel with EtOAC/Hexane (1:2) to afford the corresponding esters as oils. The free amines were then converted to their corresponding hydrochloride salt by bubbling hydrogen chloride gas.

L-Methionine octyl ester (1a). (Yield: 81%).^{1}H NMR (CDCl3), δ (ppm): 0.86 (t, 3H, *J* 6.3 Hz, $O(CH_2)_7C\underline{H}_3$), 1.25 (m, 10H, $OCH_2CH_2(C\underline{H}_2)_5CH_3$), 1.56 (m, 2H, $OCH_2C\underline{H}_2(CH_2)_5CH_3$), 1.56 (br, 2H, NH_2), 1.73 (m, 1H, $C\underline{H}CHCH_2SCH_3$), 1.98 (m, 1H, $CHC\underline{H}CH_2SCH_3$), 2.08 (s, 3H, $SC\underline{H}_3$), 2.60 (t, 2H, *J* 7.0 Hz, $CH_2C\underline{H}_2SCH_3$), 3.55 (m, 1H, CH), 4.09 (t, 2H, *J* 7.0 Hz, $OC\underline{H}_2CH_2(CH_2)_5CH_3$). ^{13}C NMR (CDCl3), δ (ppm): 14.1 ($O(CH_2)_7\underline{C}H_3$), 15.4 ($S\underline{C}H_3$), 22.6-31.8 ($OCH_2(\underline{C}H_2)_6CH_3$), 30.5 ($CH_2\underline{C}H_2SCH_3$), 34.0 ($\underline{C}H_2CH_2SCH_3$), 53.4 ($\underline{C}H$), 65.2 ($O\underline{C}H_2(CH_2)_6CH_3$), 175.8 (C=O)

L-Methionine octyl ester hydrochloride (2a). (Yield: 70%).^{1}H NMR (CDCl3), δ (ppm): 0.86 (m, 3H, $O(CH_2)_7C\underline{H}_3$), 1.25 (m, 10H, $OCH_2CH_2(C\underline{H}_2)_5CH_3$), 1.65 (m, 2H, $OCH_2C\underline{H}_2(CH_2)_5CH_3$), 2.36 (m, 2H, $C\underline{H}_2CH_2SCH_3$), 2.11 (s, 3H, $SC\underline{H}_3$), 2.80 (m, 2H, $CH_2C\underline{H}_2SCH_3$), 3.65 (m, 1H, CH), 4.19 (m, 2H, $OC\underline{H}_2CH_2(CH_2)_5CH_3$).

L-Methionine decyl ester (1b). (Yield: 66%). ^{1}H NMR (CDCl3), δ (ppm): 0.84 (t, 3H, *J* 6.7 Hz, $O(CH_2)_9C\underline{H}_3$), 1.25 (m, 14H, $OCH_2CH_2(C\underline{H}_2)_7CH_3$), 1.59 (m, 2H, $OCH_2C\underline{H}_2(CH_2)_7CH_3$), 1.59 (br, 2H, NH_2), 1.77 (m, 1H, $C\underline{H}CHCH_2SCH_3$), 2.02 (m, 1H, $CHC\underline{H}CH_2SCH_3$), 2.07 (s, 3H, $SC\underline{H}_3$), 2.59 (t, 2H, *J* 7.3 Hz, $CH_2C\underline{H}_2SCH_3$), 3.55 (m, 1H, CH), 4.08 (t, 2H, *J* 6.7 Hz, $OC\underline{H}_2CH_2(CH_2)_7CH_3$). ^{13}C NMR (CDCl3), δ (ppm): 14.1 ($O(CH_2)_9\underline{C}H_3$), 15.4 ($S\underline{C}H_3$), 22.7-31.9 ($OCH_2(\underline{C}H_2)_8CH_3$), 30.5 ($CH_2\underline{C}H_2SCH_3$), 34.0 ($\underline{C}H_2CH_2SCH_3$), 53.4 ($\underline{C}H$), 65.2 ($O\underline{C}H_2(CH_2)_8CH_3$), 175.9 (C=O).

L-Methionine decyl ester hydrochloride (2b). (Yield: 62%). ^{1}H NMR (CDCl3), δ (ppm): 0.86 (m, 3H, $O(CH_2)_9C\underline{H}_3$), 1.24 (m, 14H, $OCH_2CH_2(C\underline{H}_2)_7CH_3$), 1.64 (m, 2H, $OCH_2C\underline{H}_2(CH_2)_7CH_3$), 2.38 (m, 2H, $C\underline{H}_2CH_2SCH_3$), 2.11 (s, 3H, $SC\underline{H}_3$), 2.76 (m, 2H, $CH_2C\underline{H}_2SCH_3$), 3.55 (m, 1H, CH), 4.21 (m, 2H, $OC\underline{H}_2CH_2(CH_2)_7CH_3$).

L-Methionine dodecyl ester (1c). (Yield: 54%). ^{1}H NMR (CDCl3), δ (ppm): 0.85 (m, 3H, $O(CH_2)_{11}C\underline{H}_3$), 1.24 (m, 18H, $OCH_2CH_2(C\underline{H}_2)_9CH_3$), 1.60 (m, 2H, $OCH_2C\underline{H}_2(CH_2)_9CH_3$), 1.60 (br, 2H, NH_2), 1.74 (m, 1H, $C\underline{H}CHCH_2SCH_3$), 2.00 (m, 1H, $CHC\underline{H}CH_2SCH_3$), 2.08 (s, 3H, $SC\underline{H}_3$), 2.60 (m, 2H, $CH_2C\underline{H}_2SCH_3$), 3.62 (m, 1H, CH), 4.11 (m, 2H, $OC\underline{H}_2CH_2(CH_2)_5CH_3$). ^{13}C NMR (CDCl3), δ (ppm): 14.1 ($O(CH_2)_{11}\underline{C}H_3$), 15.4 ($S\underline{C}H_3$), 22.7-31.9 ($OCH_2(\underline{C}H_2)_{10}CH_3$), 30.5 ($CH_2\underline{C}H_2SCH_3$), 32.8 ($\underline{C}H_2CH_2SCH_3$), 53.3 ($\underline{C}H$), 65.3 ($O\underline{C}H_2(CH_2)_{10}CH_3$), 175.7 (C=O).

L-Methionine dodecyl ester hydrochloride (2c). (Yield: 50%). ^{1}H NMR (CDCl3), δ (ppm): 0.85 (m, 3H, $O(CH_2)_{11}C\underline{H}_3$), 1.24 (m, 18H, $OCH_2CH_2(C\underline{H}_2)_9CH_3$), 1.60 (m, 2H, $OCH_2C\underline{H}_2(CH_2)_9CH_3$), 2.35 (m, 2H, $C\underline{H}_2CH_2SCH_3$), 2.10 (s, 3H, $SC\underline{H}_3$), 2.70 (m, 2H, $CH_2C\underline{H}_2SCH_3$), 3.62 (m, 1H, CH), 4.11 (m, 2H, $OC\underline{H}_2CH_2(CH_2)_5CH_3$).

1.3.Critical micelle concentration (CMC) determination

The CMC of the cationic methionine ester hydrochlorides were determined by measuring conductivity values as a function of ester concentration in deionized water at 25°C. For the non-ionic methionine esters, the CMC were determined using the pyrene fluorescence measurement at varying temperature (25ºC, 35ºC and 40ºC). Stock solution of pyrene solution in methanol (10 μL, 0.1 mM) was transferred into vials. After evaporating the methanol, surfactant solutions with varying concentrations were added to the vials. The solutions were shaken on a mechanical shaker for 24 hours and the fluorescence spectra of the solutions were recorded over the spectral range 350 to 450 nm at an excitation wavelength of 334 nm. The emission was recorded at 373 (I1) and 384 (I_3) nm. The CMC of the methionine surfactants were obtained from the plot of the ratio of the intensities of the first and the third vibronic peaks in the fluorescence spectrum (I_1/I_3) as a function of the surfactant concentrations.

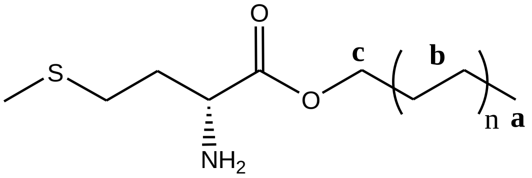
1.4. Krafft temperature determination

To measure the Krafft temperature of the synthesized esters, 0.01M solutions of the methionine esters (C_8, C_{10} and C_{12}) were prepared in deionized water and placed in the refrigerator for 24 hours at 4 ºC. The conductivity was noted for every one-degree increase in the temperature of the solution under gentle stirring.

2. Results and discussions

2.1. Synthesis and characterisation

In order to systematically study the effect of head group and alkyl chain length on the physicochemical properties of sulfur-based surfactants, *O*-alkyl esters of methionine and their ester hydrochloride derivatives having chain length of C_8 to C_{12} were synthesized. The esters were synthesized by the acid-catalyzed condensation of methionine with varying long chain alcohols. The esters were then converted to their respective ester hydrochloride derivatives by protonation of the amino group. An overall decrease in the yield of the methionine esters were observed upon increasing the alkyl chain length of the surfactants. The IR, ^1H and ^{13}C NMR spectra were concordant with the expected structures. Presence of the peaks at 1736 - 1738 cm^{-1} in the IR spectrum confirmed the formation of the ester linkage. The peaks corresponding to the protons (position **a** and **b**) in the alkyl chain appeared at δ 0.84 – 1.60 ppm in the ^{1}H NMR spectrum. The protons at position c appeared much more downfield (δ 4.08– 4.11 ppm).



n= 3, 4, 5

Figure 1: General structure of the methionine alkyl ester

The singlet at δ 2.07 - 2.08 ppm corresponded to the S-CH_3 moiety. The carbonyl carbon appeared at δ 175.7 - 175.9 ppm in the ^{13}C NMR spectra. Further details of the spectral data are given in the experimental section.

2.2. Physicochemical properties

2.2.1. Critical micelle concentration

The critical micelle concentration of the methionine esters were determined by pyrene fluorescence spectroscopy while the CMC of the methionine ester hydrochlorides were determined by conductivity measurements. Figure 2 shows the plot between conductivity as a function of methionine dodecyl ester hydrochloride (**2c**).

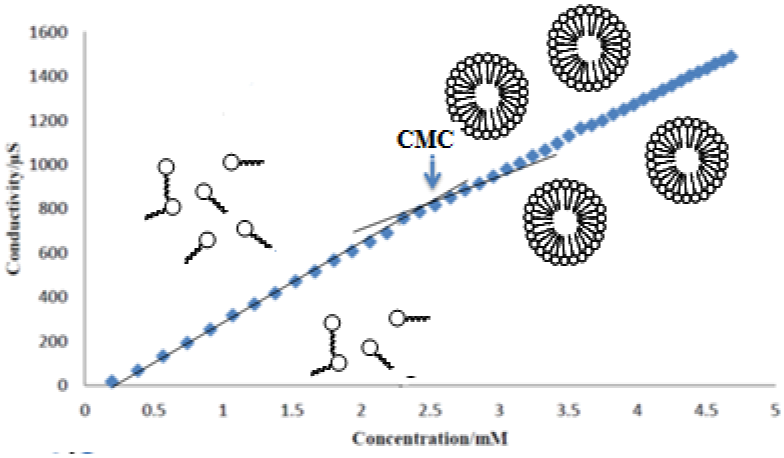


Figure 2: Variation of conductivity with concentration of methionine dodecyl ester hydrochloride

For the determination of the CMCs of the non-ionic methionine esters, pyrene fluorescence measurements were used since the surfactants are uncharged and therefore have no conductivity values. Pyrene molecules are highly hydrophobic and they are preferentially solubilised within the hydrophobic interior of micellar aggregates and are strongly distributed into the micelle as soon as they form [19]. This causes an abrupt decrease in the I_1/I_3 ratio at the CMC. Figure 3 represents the fluorescence spectrum of pyrene in the presence of methionine octyl ester (**1a**).

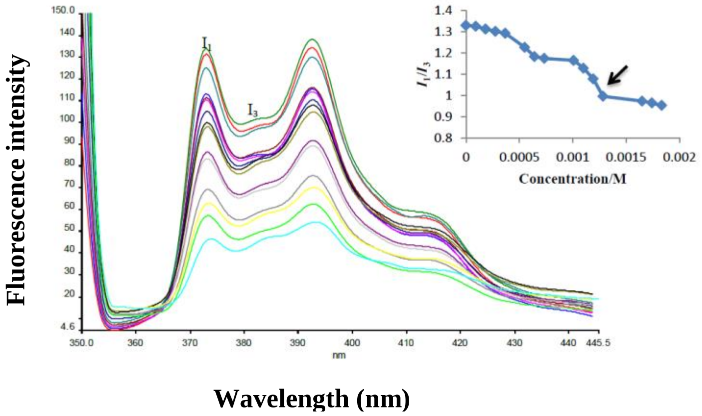


Figure 3: Fluorescence spectra of pyrene in the presence of methionine octyl ester (1a)

The CMCs of the methionine esters and their respective hydrochloride derivatives are shown in Table 1.

Chain Length	Methionine Esters	CMC (mM)	Methionine Ester Hydrochloride	CMC (mM)
8	1a	1.28	2a	4.74
10	1b	0.63	2b	3.42
12	1c	0.25	2c	2.31

In both methionine esters and the ester hydrochloride series, the CMC was found to decrease with increasing chain length of the surfactants as observed in previously reported surfactant series. A linear relationship exists between chain length and CMC for a homologous series according to Kleven's equation (Equation 1).

$$\text{Log CMC} = A - Bn \qquad \textbf{Equation 1}$$

where n is the number of carbon atoms in the alkylchain and A & B are constants [20]. A linear relationship was determine for the methionine ester and their hydrochloride derivatives yielding Equations 2 and 3 respectively.

$$\text{Log CMC} = -1.6952 - 0.078n$$ **Equation 2**

$$\text{Log CMC} = -1.4598 - 0.1773n$$ **Equation 3**

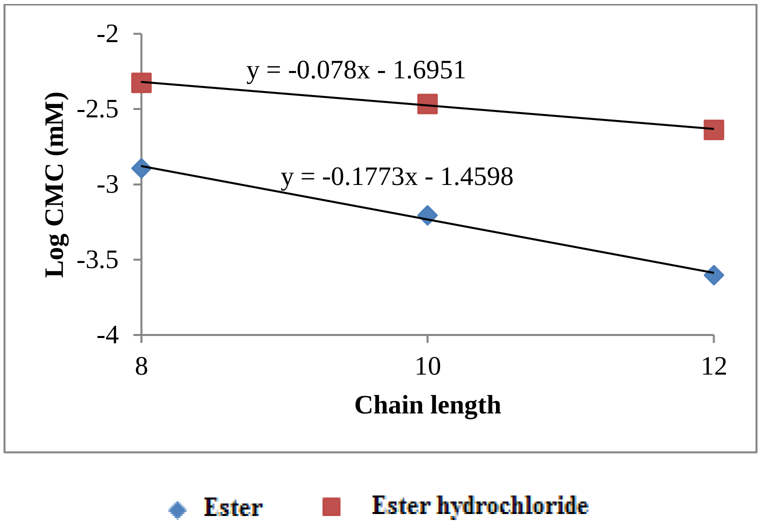


Figure 4: Variation of CMC with chain length of methionine esters and their ester hydrochlorides

The CMC of the methionine ester hydrochloride was found to be higher than that of the non-ionic methionine esters. This was due to the presence of the cationic head group which caused repulsion between the surfactants, hence decreasing their ease of micellisation.

2.2.2. Krafft point

Surfactants exhibit their micellar properties only above a certain temperature called the Krafft temperature (T_K), which is defined as the minimum temperature above which the surfactants are able to form micelles in aqueous solution. The Krafft temperature of the methionine ester hydrochlorides were determined. The T_K of the methionine ester hydrochloride was taken by the abrupt change in conductivity versus temperature plot (Figure 5).

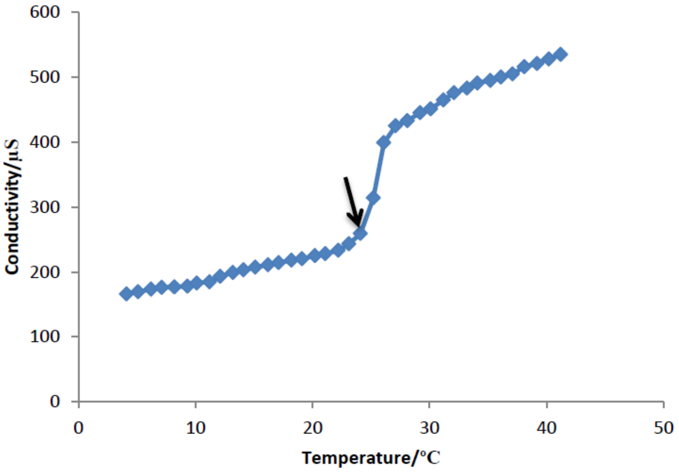


Figure 5: Variation of conductivity with temperature of methionine d solution

The krafft temperature of cetyl trimethylammonium bromide (CTAB) was found to be 22C which is comparable to that reported previously. The Krafft temperatures of methionine ester hydrochlorides was found to increase with increasing chain length showing that these surfactant derivatives have a tendency to micellise at a much higher temperature with increasing chain length.

3. Conclusion

The methionine esters with varying chain lengths (C_8 - C_{12}) and their ester hydrochloride derivatives were successfully synthesized in good to moderate yields. The CMC of the methionine esters and their hydrochloride derivatives were found to decrease with chain length, as observed for previously reported surfactants. The methionine ester hydrochloride derivatives have a less tendency to micellise compared to their non-ionic ester derivatives due to the repulsion caused by their cationic head groups. An increase in chain length of the methionine ester hydrochloride caused an increase in the Krafft temperature of the surfactants, suggesting that the methionine surfactants with longer chain length micellise at higher temperatures.

References

[1] Attwood, D., Florence, A.T. Surfactant Systems. Their chemistry, pharmacy and Biology, Chapman and Hall, London, 1983.

[2] Fendler, J.H. Membrane mimetic chemistry. Characterisation and applications of micelles, microemulsions monolayers, bilayers, vesicles, host-guest systems and polyions. John Wiley and Sons, New York, 1982.

[3] Nnanna, I. A., Xia, J. Protein Based Surfactants: Synthesis, Physicochemical Properties and Applications, Surfactant Science Series, 101, Marcel Dekker, New York, USA 1998.

[4] Chandra, N., Tyagi, V. K., 2013. Synthesis, properties and applications of amino acid based surfactants. J. Dispersion Sci. Technol, 34, 800.

[5] Infante, M. R., Perez, L., Pinazo, A., Clapes, P., Moran, M. C., Angelet, M, . Garcia, M.T., Vinardell, M. P., 2004. Amino acid based surfactants. C. R. Chim, 7, 583.

[6] Holmberg, K., 2004. Preparation, application and biodegradability, 2nd edition, surfactant science series. Marcel Dekker, NewYork

[7] Pinazo, A., Manresa, M.A., Marques, A.M., Bustelo, M., Espuny, M.J., Perez, L., 2016. Amino acid based surfactants-New antimicrobial agents. Adv. Colloid. Int. Sci, 228, 17-39.

[8] R. Bordes, K. Holmberg, Adv. Colloid Interface Sci. 2015, 222, 79.

[9] Faustino, C.M.C., Calado, A.R.T., Garcia-Rio, L., 2010. Dimeric and monomeric surfactants derived from sulfur-containing amino acids. Journal of colloid and interface science, 351, 472-477.

[10] Faustino, C.M.C., Calado, A.R.T., Garcia-Rio, L., 2010. New urea-based surfactants derived from α-ω-amino acids. Journal of Physical chemistry, 113, 977-982.

[11] Branco, M.A., Pinheiro, L., Faustino, L., 2015. *Colloids and Surfaces A: Physicochemical and Engineering Aspects,* 480, 105-112.

[12] Yoshimura, T., Sakato, A., Tsuchiya, K., Ohkubo, T., Sakai, H., Abe, M., Esumi, K., 2007. Journal of Colloid and Interface Science, 308, 466-473

[13] Fan, H., Han, F., Liu, Z., Qin, L., Li, Z., Liang, D., Ke, F., Huang, J., Fu, H., 2008. Journal of Colloid and Interface Science, 321, 227-234.

[14] Joondan, N., Jhaumeer-Laulloo, S., Caumul, P., 2014. Microbiol. Res. 169, 675-685

[15] Joondan, N., Caumul, P., Akerman, M., Jhaumeer-Laulloo, S., 2015 Bioorg. Chem. 58, 117-129

[16] Joondan, N., Jhaumeer-Laulloo, S., Caumul, P., 2015. J. Surfactants Deterg. 18, 1095-1104

[17] Joondan, N., Jhaumeer-Laulloo, S., Caumul, P., Marie, D.E.P., Roy, P., Hosten, E., 2016. Colloids. Surf. A. Physicochem. Eng. Asp. 511, 120-134

[18] Joondan, N., Caumul, P., Jhaumeer-Laulloo, S., 2017. J. Surfactants Deterg. 20, 103-115

[19] Pineiro, C., Novo, M., Al-Soufi, W., 2015. Fluorescence emission of pyrene in surfactant solutions. Adv Colloid Int Sci. 215, 1-12.

[20] Klevens, H.B.,1953. Structure and aggregation in dilute solutions of surface active agents. J. Am Oil Chem Soc, 30, 74–80.