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EFFECTS OF CRITICAL MICELLE CONCENTRATION OF ANIONIC SURFACTANTS AND THEIR TOXICITY TO AQUATIC ORGANISMS

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ABSTRACT. *The critical micelle concentration (CMC) is the concentration of surfactants above which micelles are formed. The effects of CMC of methyl ester sulfonates (MES) on ecotoxicological behaviour of freshwater organisms in predicting the risk levels contributed from the surfactant used were determined. The surface tension of palm-based MES with various carbon chain lengths (C_{12}, C_{14} and C_{16}) was measured to determine the CMC. Ecotoxicity tests were conducted on three different aquatic organisms: green algae (Raphidocelis subcapitata), freshwater crustacean (Daphnia magna) and freshwater fish (Tilapia nilotica). The effective concentration of MES that caused 50% fish mortality (LC_{50}), crustacean immobilization (EC_{50}) and algae inhibition (EC_{50}) was determined. Through surface tension analyses, the CMC obtained for MES C_{12}, C_{14} and C_{16} was 1000 mg/L, 900 mg/L and 12 mg/L, respectively. The LC_{50} of MES C_{12}, C_{14} and C_{16} were 391 mg/L, 22.6 mg/L and 12.6 mg/L, respectively, in fish. The crustacean EC_{50} of MES C_{12}, C_{14} and C_{16} were >100 mg/L, 77.6 mg/L, and 1.15 mg/L. Meanwhile, algae EC_{50} of MES C_{12}, C_{14} and C_{16} was 541 mg/L, 399 mg/L and >10 mg/L, respectively. Relative comparison showed that D. magna was observed to be more sensitive compared to R. subcapitata and T. nilotica towards MES of the same chain length. A linear relationship was observed between CMC and ecotoxicity values. The lower the CMC value, the lower is the LC_{50} or EC_{50} value and the surfactant becomes more toxic. It is suggested that the CMC value can be used as a toxicity indicator for anionic surfactant by considering that the EC_{50} value of a surfactant will be reached before its CMC value.*

KEYWORDS: CMC, Ecotoxicity, Aquatic organisms, Environment

INTRODUCTION

The anionic surfactants are best known for their wide use which contributed about 60% of the world surfactant production. Excessive use of any type of surfactant and their disposal in the environment, especially in an aquatic environment, could seriously affect the ecosystems, hence should be monitored and regulated (Ivanković and Hrenović, 2010). Many aquatic toxicity studies toward surfactants have been conducted due to the public concerns (Fernández-Serrano *et al.*, 2014; Jurado *et al.*, 2012b; Ríos *et al.*, 2017; Jurado *et al.*, 2012a). The ecotoxicity of different commercial surfactants (six anionics, two amphoteric and one nonionic) towards planktonic freshwater green algae and marine diatoms have also been studied by Pavlic *et al.*, (2005) whereby all of these commercial surfactants caused toxic effects on freshwater green algae and marine diatoms.

The toxicity of surfactants in an aquatic environment is affected by their chemical properties. The study by Calamari and Marchetti (1973) reported the increase in toxicity of surfactants with increasing cellular permeability of aquatic species in response to the surface tension reduction. The toxicity value of a surfactant has also been correlated with its critical micelle concentration (CMC). The CMC is a concentration of surfactant at which it forms micelles. The study conducted by Hisano and Oya (2010) revealed the reduction of ecotoxicity of sodium linear alkylbenzene sulfonate (LAS) sample as the CMC of this surfactant increased.

Methyl ester sulphonate (MES) is an anionic surfactant derived from sulphonation of palm-based fatty acid methyl esters. It is used as the active ingredient in laundry detergent due to its performance such as excellent detergency and less sensitivity to water hardness. The global market of fatty methyl ester sulfonates (FMES) is expected to reach USD 2.49 billion by 2025 according to Grand View Research due to consumers' awareness on cleanliness and environmental-friendly issues (Market Research Store, 2015b). The major manufactures of MES are Stepan Company (United States of America), Lion Corporation (Japan), Jiangsu Haiqing Biotechnology (China), Huish Detergents (United States of America), Guangzhou Lonkey Industrial Co Ltd (China), and KL-Kepong Oleomas (Malaysia) (Market Research Store, 2015a).

This paper aims to determine the effects of CMC of MES on aquatic toxicity in predicting the risk levels contributed by the surfactant used.

MATERIALS AND METHODS

Test substances

Palm-based MES with various carbon chain lengths (C_{12}, C_{14} and C_{16}) were produced from palm stearin methyl esters at the Malaysian Palm Oil Board (MPOB). Potassium dichromate, $K_2Cr_2O_7$ 99.9% AR Grade, from Friendemann Schmidt, Germany, was used as the reference compound.

Surface tension measurement

The CMC values were determined by measuring the surface tension of different concentrations of surfactant solutions at test temperature (25 ºC) using a tensiometer model Tensiometer K100 (Kruss GmbH, Germany) equipped with a 2 cm platinum plate. The stock solution of MES (500 mg/L) was prepared by diluting the surfactant in deionized water. From the stock solution, a series of MES solutions (at different concentrations) were prepared. The platinum plate was cleaned and heated to a reddish orange colour with a Bunsen burner before use. A graph of surface tension value against MES concentration was plotted. The concentration at which discontinuous change in slope occurs is defined as the CMC.

Aquatic toxicity of surfactant

The aquatic toxicity of MES was tested using three different test organisms namely green algae (*Raphidocelis subcapitata*), freshwater crustacean (*Daphnia magna*) and freshwater fish (*Tilapia nilotica*). The method used for ecotoxicity test using fish and freshwater crustacean were according to the test guideline OECD 203, Fish acute toxicity test and OECD 202, *Daphnia* sp., acute immobilisation test, respectively. Both methods were well described by Razmah *et al.* (2015).

The procedures for ecotoxicity test using freshwater algae were briefly described according to test guideline OECD 201, Algae growth inhibition test (Siti Afida *et al.*, 2017). The green algae, *R. subcapitata* (ATCC® 22662™) obtained from the American Type Culture Collection (Maryland, USA) was used as the test species. Five concentrations were prepared for each MES sample with a separation factor not exceeding 3.2, *i.e.* 0 mg/L, 62 mg/L, 197 mg/L, 627 mg/L and 2000 mg/L. These test solutions were exposed to exponentially-growing cultures of *R. subcapitata* and incubated in an incubator (EYELA FLI-2000, Japan,) at 25ºC, 14 hours light cycle (4000 Lux) and 10 hours dark cycle, and shook at 100 rpm. After 72 hours of exposure, the number of algae cells was measured using a particle counter (Beckman Counter Z2, USA). The average growth rate of algae was calculated using the following formula:

$$\mu_{i-j} = \frac{\ln X_j - \ln X_i}{t_j - t_i} \ (\text{day}^{-1}) \cdot$$

where:
μ_{i-j} is the average specific growth rate from time i to j;
X_i is the biomass at time *i*;
X_j is the biomass at time *j*
t is the period of test

Meanwhile, the percent inhibition of algae growth rate was calculated using the following formula:

$$\%I_r = \frac{\mu_C - \mu_T}{\mu_C} \times 100$$

where:
$\%I_r$ is the percent inhibition in average specific growth rate;
μ_C is the mean value for average specific growth rate (μ) in the control group;
μ_T is the average specific growth rate for the treatment replicate.

Calculation of effective concentration (EC_{50})

The EC_{50} (effective concentration at 50% algae growth inhibition) values of the samples were determined from the plot of percentage of growth rate inhibition against concentration. All calculations were tabulated using Microsoft Excel.

The respective LC_{50} (lethal concentration which kills 50% of the *T. nilotica*) and EC_{50} (concentration which immobilizes 50% of the *D. magna* after exposure) values for toxicity tests of fish and freshwater crustaceans were calculated via probit analysis with 95% confidence limits using Statistical Package for the Social Sciences (SPSS) software.

RESULTS AND DISCUSSION

Critical micelle concentration (CMC) of methyl ester sulfonates (MES) homologues

The surface tensions of MES were determined and plotted against concentrations to obtain CMC values. The surface tension plots for MES C_{12}, MES C_{14} and MES C_{16} are presented in Fig. 1, 2 and 3, respectively. The CMC value refers to the concentration at which discontinuous change in surface tension slope occurs. The surfactant‘s monomers assemble to form a closed aggregate (micelle) in which the hydrophobic tails are shielded from water while the hydrophilic heads face the water at CMC.

The MES exhibited an approximately linear decrease in surface tension followed by a plateau. The CMC values of MES C_{12}, C_{14}, and C_{16} obtained were 1000 mg/L, 900 mg/L, and 12 mg/L, respectively. The CMC is correlated with the number of hydrophobic tails of MES. The MES becomes less polar and less soluble in water with a higher number of hydrophobic tails. MES C_{12} had the highest CMC value and was more soluble in water compared to MES C_{14} and MES C_{16}. It can be concluded that as the chain length of MES increases, the CMC value of MES decreases.

Sanchez leal *et al.* (1991) also reported an excellent linear relationship between the CMC value and molecular weight of anionic surfactant. Becher *et al.* (1984) established an equation to relate CMC with the number of carbons and ethoxy groups. The CMC increases with a decrease in the partial charge of the head groups, indicating an increase in solubility of the surfactant molecule as the charge is more widely distributed throughout the molecule. As the carbon chain length of MES increases, the micelles are formed at lower concentrations and are less soluble in water.

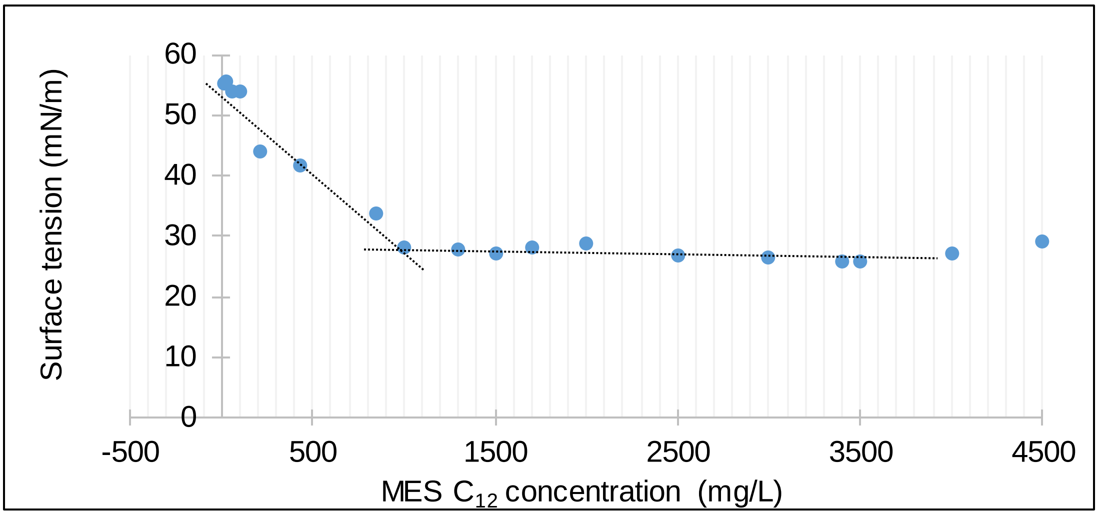

Figure 1: Critical micelle concentration (CMC) for methyl ester sulfonates (MES)

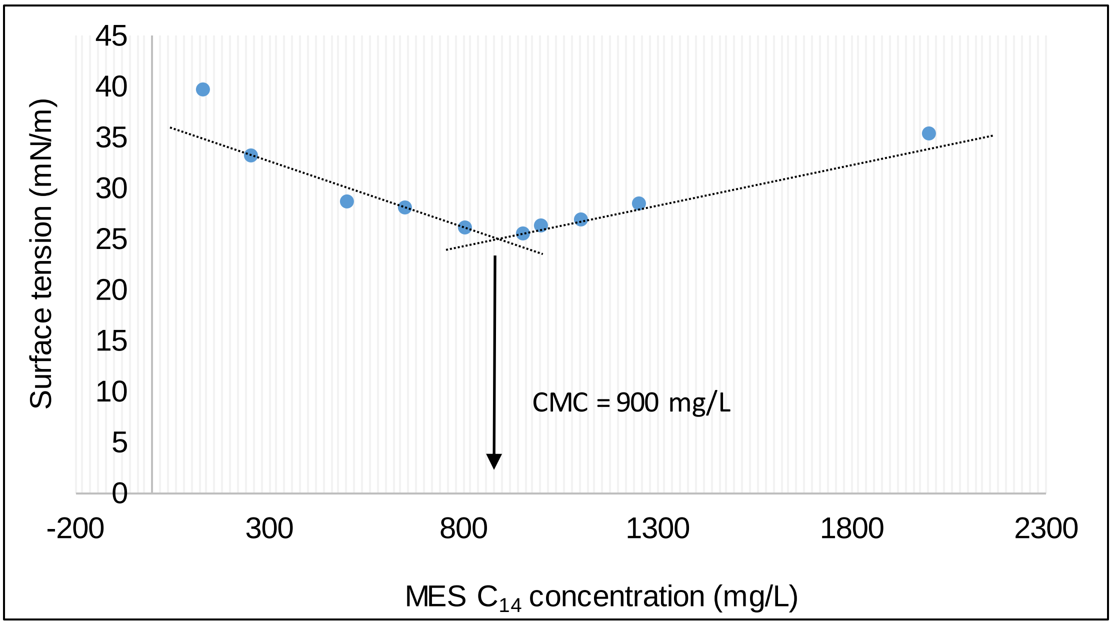

Figure 2: Critical micelle concentration (CMC) for methyl ester sulfonates (MES) C_{14}

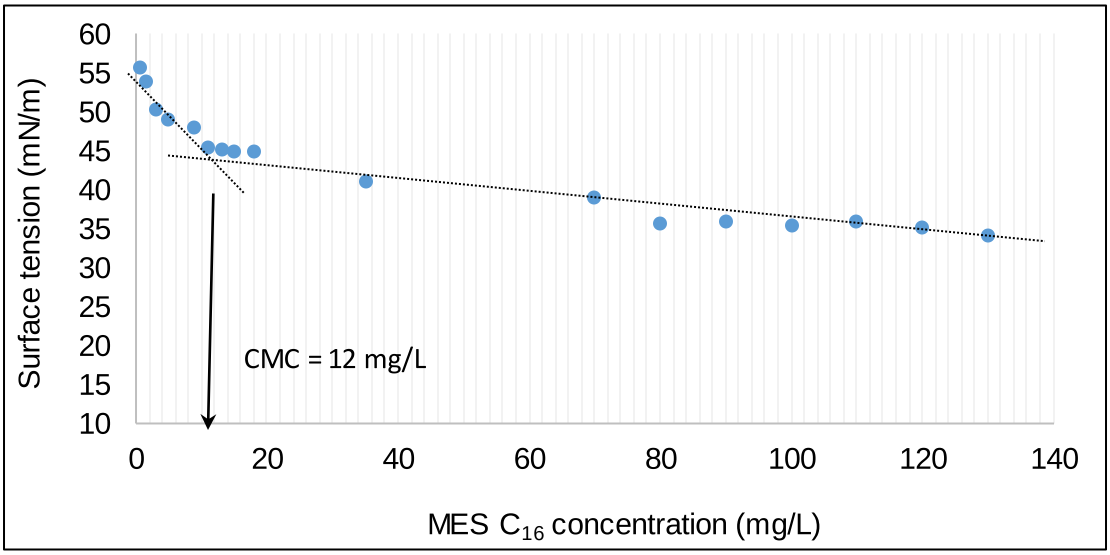

Figure 3: Critical micelle concentration (CMC) for methyl ester sulfonates (MES) C_{16}

Ecotoxicity of methyl ester sulfonates (MES) homologues towards *Raphidocelis subcapitata, Daphnia magna* and *Tilapia nilotica*

Individual toxicity values for different chain lengths of MES are shown in Table 1. The LC_{50} of MES C_{12}, C_{14} and C_{16} was 391 mg/L, 22.6 mg/L and 12.6 mg/L, respectively, in fish. The crustacean EC_{50} for MES C_{12}, C_{14} and C_{16} was >100 mg/L, 77.6 mg/L and 1.15 mg/L, respectively. Meanwhile, the algae EC_{50} of MES C_{12}, C_{14} and C_{16} was 541 mg/L, 399 mg/L and >10 mg/L, respectively.

The toxic effects of MES with the same chain length were higher towards *D. magna* compared to *T. nilotica* and *R. subcapitata*. The *R. subcapitata* was less sensitive towards the MES. According to Razmah *et al.* (2015), *D. magna* was more sensitive to the ecotoxicity effects of MES compared to *T. nilotica*.

MES C12, the shortest carbon chain length, was least toxic among other MES chain lengths with a toxicity range from 100 mg/L to 541 mg/L. The toxicity values of MES increased as the number of carbon chain lengths increases. MES C12 can be classified as practically non-toxic according to GESAMP (2014) since the EC_{50} value is higher than 100mg/L.

Table 1: Acute ecotoxicity of MES towards *Raphidocelis subcapitata, Daphnia magna and Tilapia nilotica*

Test Organisms	**MES C_{12}**	**MES C_{14}**	**MES C_{16}**
Raphidocelis subcapitata (EC_{50}, mg/L)	541	399	>10
Daphnia magna * (EC_{50}, mg/L)	>100	77.6	1.15
Tilapia nilotica * (LC_{50}, mg/L)	391	22.6	12.6

* Data published by Razmah *et al.* (2015)

The aquatic toxicity for anionic surfactants such as MES depends mainly on the length of the carbon chain of the molecule. The toxicity level of a substance correlates with the chain length of the alkyl group (Toshiharu *et al.*, 2006). This correlation has also been observed in the homologues of alcohol sulphates and alkylbenzene sulphonates in which the longer the carbon chain, the more toxic the anionic surfactant (Fendinger, 1994; Protokor, 1992). The possible reason for toxicity increase with homologue chain length might be due to greater interaction of the heavier homologues with cell membranes (Ivankovic and Hrenovic, 2010). However, a systematic dependence of the toxicity on the chain length is only recognizable in fully water-soluble compounds (Garcia *et al.*, 2001). The ecotoxicity trend of MES was also reported by Razmah *et al.* (2016) by which MES of shorter carbon chains were less toxic than MES with longer carbon chains. Nevertheless, this palm-based MES are not expected to cause any environmental concerns on aquatic organisms due to their rapid biodegradation properties in the environment and only 10%–30% of MES are used in detergent products (Razmah *et al.*, 2016).

The effects of CMC and ecotoxicity towards several test organisms are shown in Fig. 4. It can be seen that CMC is clearly related to toxicity as the substances are more toxic with lower CMC values, depending on the organisms assayed.

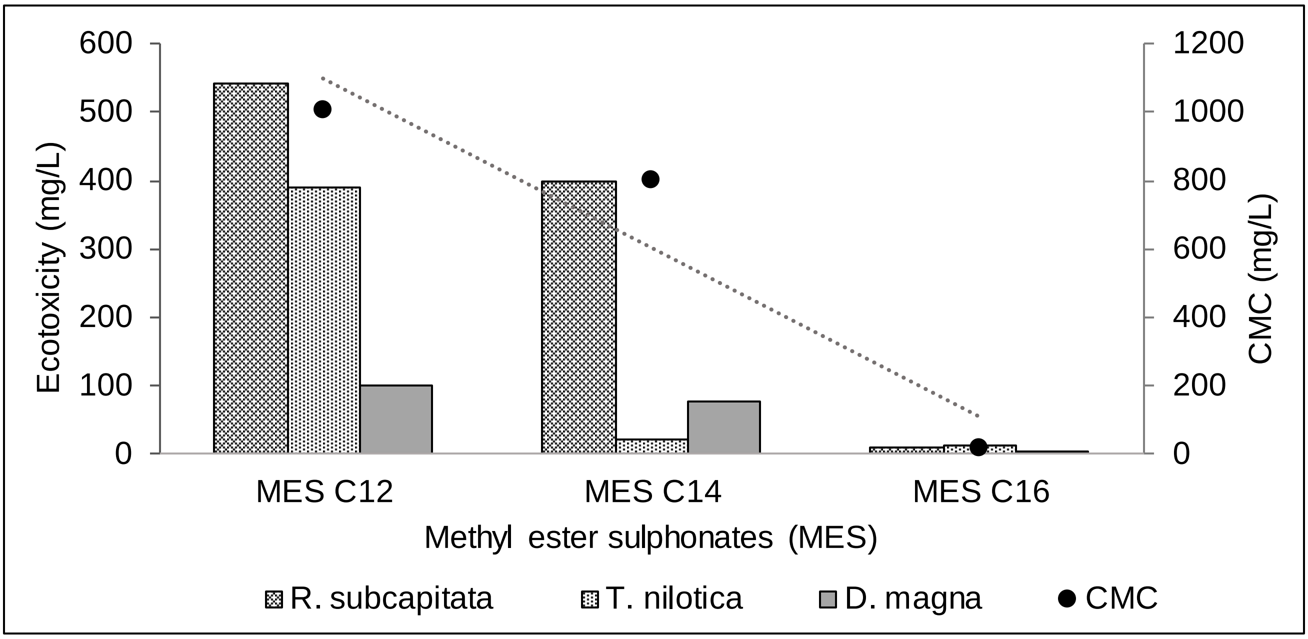

Figure 4: The effects of CMC and ecotoxicity towards *Raphidocelis subcapitata, Daphnia magna,* and *Tilapia nilotica.*

Fernández-Serrano *et al.* (2014) reported that when the CMC of anionic surfactant or mixtures increased, the toxicity of anionic surfactant towards three different organisms (*Vibrio fischeri, Daphnia magna,* and microalgae) decreased. Meanwhile, Inacio *et al.*, (2011) reported that the toxic effects of surfactant depended on its hydrophilic head groups, whereby the toxicity level was significantly lower in polar surfactants than the non-polar surfactants. This observation is related to the penetration of surfactant into phospholipids of the organism's cellular membrane. In terms of surfactant, the longer the length of the alkyl chain, the higher the hydrophobicity, which then allows the surfactant to penetrate and interact with the membrane phospholipids. The interaction between surfactant and cell membranes can destabilise and/or destruct the organism's cell membranes and increase the toxicity level of a surfactant (Fernández-Serrano' *et al.*, 2014; Inácio *et al.*, 2011).

CONCLUSION

In conclusion, the CMC of MES decreased with the increase in chain length of MES. Similarly, the ecotoxicity of MES increased with the increase in chain length of MES. The toxic effects of MES with the same chain length were higher towards *D. magna* compared to *T. nilotica* and *R. subcapitata*. There is a strong relationship between the CMC and ecotoxicity values of MES surfactant, whereby the CMC value decreases as the ecotoxicity of MES increases. The CMC value can be used as a toxicity indicator for anionic surfactant by considering that the EC_{50} value of a surfactant will be reached before its CMC value.

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