Rapid Critical Micelle Concentration (CMC) Determination Using Fluorescence Polarization

Analysis of the Physical-Chemical Properties of Detergents

Paul Held Ph. D., Lab Manager, Applications Department, BioTek Instruments, Inc., Winooski, VT

The determination of the critical micelle concentration (CMC) value of surfactants under different environmental conditions is important for a number of different biological and chemical processes. Because the CMC is not a constant value, shifting with different environmental conditions, it is important that a rapid, reliable and easy methodology be available to facilitate testing. Here we describe the rapid semi-automated determination of CMC values for surfactants in 384-well microplates using fluorescence polarization.

Key Words:

Fluorescence Polarization
Critical Micelle Concentration
CMC
Surfactant
Detergent

Introduction

Surfactants are amphiphilic compounds that have a molecular structure containing both a hydrophilic (water loving) and a hydrophobic (water hating) region. The hydrophobic region is usually a long chain aliphatic hydrocarbon, whereas the hydrophilic portion can be composed of an ionic or non-ionic polar group. The physical nature of these molecules bestows the ability to reduce surface tension of solutions and to self aggregate into colloids known as micelles.

A micelle is an aggregation of surfactant molecules in a colloidal suspension. A typical micelle in aqueous solution forms with the hydrophilic head regions in contact with the water and the hydrophobic aliphatic tail regions buried in the inner portion of the micelle. Useful surfactants are soluble to some degree in aqueous solution and only aggregate into micelles when they reach a sufficient concentration. This concentration is referred to as the critical micelle concentration (CMC) (Figure 1). Below the CMC micelles are not present and the surface tension of the solution decreases and osmotic pressure increases with an increase in surfactant. Above the CMC, the concentration of unaggregated surfactant will stay constant and the number of micelles will increase as the total surfactant concentration increases. This results in increases in solution turbidity and solubilization with increased surfactant concentration. Once the CMC is reached the change in surface tension with surfactant concentration is significantly reduced or eliminated with further increase in surfactant.

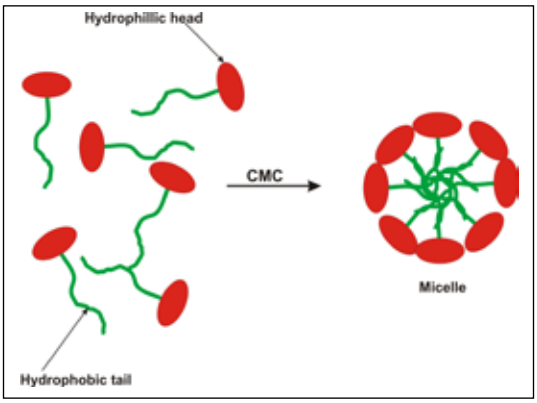


Figure 1. Schematic drawing of Micelle formation.

Amphiphilic surfactants have numerous properties other than lowering of surface tension and are often labeled as to the use (e.g. soap, detergent, wetting agent, dispersant, emulsifier, foaming agent, bactericide, corrosion inhibitor, antistatic agent etc.). While commercially classified by their use, scientifically they are classified based on their dissociation in water. Anionic surfactants dissociate in water into an amphiphilic anion (neg. charge) and a simple cation (e.g. Na^+, K^+). Anionic surfactants are the most commonly used surfactants, accounting for about 50% of the world's production [1].

Nonionic surfactants account for approximately 45% of all surfactants. These agents do not ionize in solution and typically have a hydrophilic group composed of an alcohol, phenol, ether, ester or amide. Many nonionic surfactants contain polyethylene glycol chains. Cationic surfactants form an amphiphilic cation and an anion in aqueous solution. Often this class contains nitrogen compounds such as fatty amine salts of quaternary ammoniums linked to one or more long chain alkyl hydrophobic moieties.

BioTek Instruments, Inc.
P.O. Box 998, Highland Park,
Winooski, Vermont 05404-0998 USA
Phone: 888-451-5171
Outside the USA: 802-655-4740
Email: **customercare@biotek.com**
www.biotek.com

This group is less popular as a result of the cost of their manufacture and is only used when a less expensive substitute cannot be found. Typically they are used as a bactericide, antistatic, and for corrosion inhibition. When a single surfactant molecule contains both anionic and cationic dissociations it is referred to as amphoteric or Zwitterionic. These compounds include synthetic betaines or sulfobetaines and natural substances such as amino acids and phospholipids.

Considerable effort has been made on predicting CMC values for surfactants. Accurate prediction of CMC prior to synthesis of new compounds would enable the customization of surfactants to meet specific needs [14]. For some surfactant types the predicted CMC agrees quite well with the observed values under defined conditions, primarily in simple aqueous solution. Empirical relationships have been utilized to generate mathematical relationships between surfactant structure and CMC. Most notable is the linear relationship between the log of CMC and the number of alkane carbon atoms in linear alkyl hexaethoxylates [14]. Thermodynamic models have also been used to predict CMC for various surfactants [17]. Most recently a systematic quantitative structure-property relationship (QSPR) approach has allowed for predictive modeling equations to be generated for more classes of surfactants [18]. Despite the effort made in predicting surfactant behavior under many conditions, the only way to determine the CMC is to do so empirically.

Critical micelle concentrations have been experimentally determined using a number of different methodologies. UV-spectroscopy of benzoylacetone (BZA) has been used to determine CMC [13]. BZA exists in an equilibrium mixture of keto and enol tautomers when dissolved in water. The amount of enol tautomer increases dramatically in the presence of surfactant above the CMC, as the enol form partitions to the inner portion of the micelle [13]. Lipid soluble dyes such as Hoechst 33342 [6] or Nile red [7] demonstrate enhanced fluorescence in a hydrophobic environment such as when micelles begin to form and the dye partitions in its hydrophobic core. Nile red's spectral shift in different solvents has also been exploited to monitor micelle formation [8]. The fluorescent compound pyrene exhibits five major vibrational fluorescent peaks, which vary depending on the solvent. The ratio of the fluorescence intensity of peak 1 to that of peak 3 is indicative of the local environment. The pyrene 1:3 ratio plots of surfactant titrations generate decreasing sigmoidal shaped curves. Surfactant concentrations below the micelle concentration result in a polar environment indicative of a high peak 1 to peak 3 ratio. As surfactant concentrations increase and approach the CMC, the pyrene 1:3 ratio begins to decrease rapidly to reaching a new lower constant value that reflect the 1:3 ratio at surfactant concentrations above the CMC [9].

In addition to fluorescent methods, changes in conductivity [10], increase in light scattering [11] and even solid state electrodes [12] have been used to determine critical micelle concentrations of surfactant molecules in solution.

The fluorescence polarization of fluorescent molecules that have been modified to interact with micelles can be exploited to determine CMC of surfactants [3]. 5-dodecanoylaminofluorescein (DAF), is essentially a fluorescent probe connected to an aliphatic tail, which can be inserted into the micellar inner region, but not become completely immersed in the interior of the micelle. By doing so the effective molecular volume of the fluorescent compound DAF is that of the micelle, which is significantly larger than the lipophilic probe alone. As such the rotational speed differential can be exploited through fluorescence polarization measurements.

Fluorescence polarization (FP) is a fluorescence detection technique first described in 1926 by Perrin [2]. It is based on the observation that fluorescent molecules, when excited by polarized light, will emit polarized light. In solution, the polarization of the emitted light is inversely proportional to the molecule's rotational speed, which is influenced by molecular volume or by approximation, molecular weight. Fluorescence polarization is measured using the ratio of the fluorescence emission returned through two polarizing filters, one parallel ($\parallel$) to and one perpendicular ($\perp$) to the plane of polarized excitatory light. Fluorescence polarization (P) is calculated using the following formula, where G is an instrument and assay dependant correction factor.

$$\text{Eq 1.} \quad P = \frac{(\parallel exp - \parallel blank) - G * (\perp exp - \perp blank)}{(\parallel exp - \parallel blank) - G * (\perp exp - \perp blank)}$$

Data is often multiplied by 1000 and expressed as millipolarization (mP).

Calculation of CMC:
The fluorescence polarization data generated in these experiments produces a sigmoidal shaped curve that can be described using a 4-parameter logistic curve fit [5] which is given by

$$\text{Eq 2.} \quad \gamma = \frac{A - D}{1 + (x/C)^B} + 1$$

Where variable y corresponds to the polarization value of a given surfactant concentration at a concentration x and A is the theoretical response at the lower concentration, B is the relative slope of the curve at its inflection point, C is the concentration value at the inflection point, and D is the response at the highest concentration.

While there are a number of different methods to calculate the CMC value from experimental plots. One method that was originally described for use with the pyrene 1:3 method [4], utilizes the interception of the rapidly changing portion of the curve and the nearly horizontal lower concentration portion of the curve [3]. This method can be modified for use with an increasing sigmoidal curve that is observed with fluorescence polarization (Figure 2).

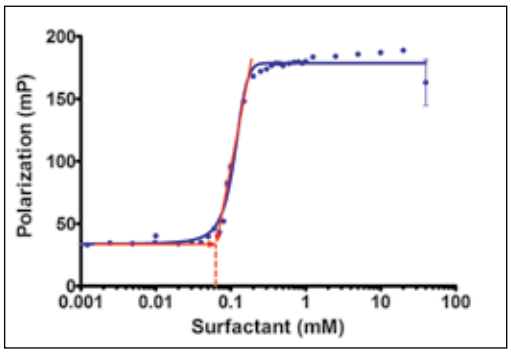


Figure 2. Schematic of CMC determination from Sigmoidal shaped plot.

In a 4-parameter logistic fit the slope of the tangent line can be described using the equation

Eq 3. $$y = LS * Log(x) + LSb$$

Where LS is the LogSlope and LSb is the y intercept of the line. The LogSlope (LS) can be calculated from the individual parameters of the 4-parameter logistic equation used to describe the original data using the equation

Eq 4. $$LS = B * (D - A) * Ln(10)/4$$

LSb is calculated using the information provided by the inflection point according to the equation.

Eq 5. $$LSb = (A + D)/2 - LS * Log(C)$$

As previously stated the CMC value is the intersection (Ix) between the lower horizontal portion of the curve and the tangent line. Thus

Eq. 6. $$A = LS * Log(Ix) + LSb$$

or

$$Log(Ix) = A - LSb/LS$$

The antilog of the resultant value is the calculated CMC value.

Materials and Methods

The surfactants domiphen bromide (P/N 247480), sodium dodecyl sulfate (P/N L4509), N-Benzyl-N,N-dimethyl-1-dodecanaminium chloride (P/N 13380), sodium tetradecyl sulfate (P/N293938), sodium decyl sulfate (P/N 71443), Niaproof® 4 (P/N N1404), sodium octyl sulfate (P/N O4003), Ipegal 630 (P/N 18896), Triton X-100 (T8787), polysorbate 20 (P/N P1379), polysorbate 40 (P/N P1504), polysorbate 60 (P/N 95754), polysorbate 80 (P/N 59924), zwittergent (P/N T7763) were purchased from Sigma-Aldrich (St. Louis, MO). Stock solutions of these surfactant compounds were made in Milli-Q water. Solid black 384-well microplates (3573) were obtained from Corning (Corning, NY) and 5-dodecanoylamino-fluorescein (P/N D109) was purchased from Life Technologies. A 5x stock solution of Hepes buffer (125 mM pH 8.0) and 2.5x solutions of sodium chloride (0.25%, 2.5%, and 25% w/v) were prepared, filter sterilized and stored at room temperature.

Assays were run in 384-well plates such that different compound dilutions, fluorescent stains, buffer constituents and salt concentrations could be used interchangeably. Reagents were added as 5x or 2.5x solutions to achieve the intended final concentrations. Compound dilutions were made fresh daily from 200 mM stock solutions and pipetted (15 µL) into microplates manually. After compound titration 5x Hepes reaction buffer (pH 8.0) solution (15 µL) was added using a syringe pump on a MultiFlo™ automated dispenser (BioTek Instruments, Winooski VT). Sodium chloride solutions (30 µL) were then immediately added using the MultiFlo peripump dispenser. For experiments where multiple salt concentrations were required, different tubes from the 8-tube peristaltic pump pulled from different reagent reservoirs. After 3-minute incubation, 15 µL of DAF fluorescent dye (5 µM) was added using a MultiFlo syringe pump dispenser. The fluorescent polarization was measured after 20 minute incubation using a Synergy™ Neo (BioTek Instruments, Winooski, VT). Parallel and perpendicular readings were made simultaneously using filter cubes 4 and 61 with 485/20 excitation and 528/25 emission filters. The assay workflow is presented in Figure 3.

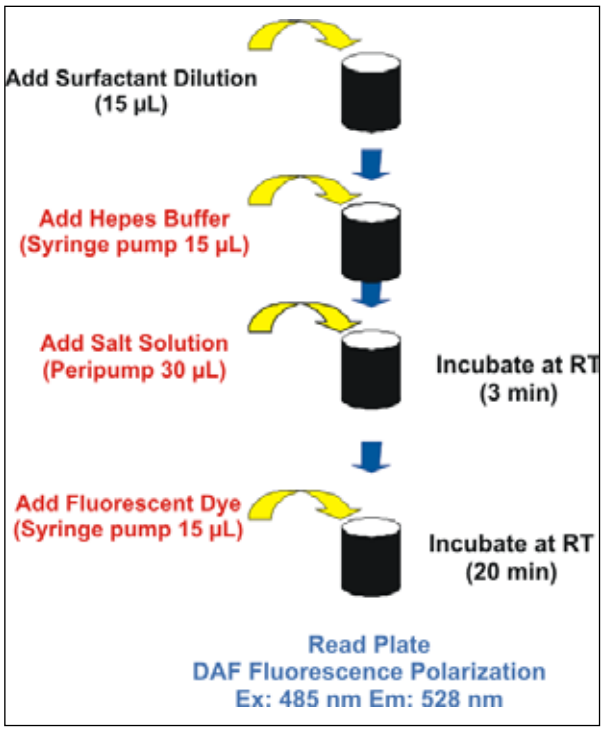


Figure 3. CMC determination assay process.

The reader was controlled and the data captured using Gen5™ Data Analysis Software (BioTek Instruments). Experimental differences in fluorescence polarization data was adjusted using the G-factor to return a value of 29 mP for free unbound DAF tracer. The data was automatically plotted as a 4-parameter logistic fit and the CMC calculated by the Gen5 software.

Results

When different surfactant molecules are compared, markedly different curve shapes and CMC values are evident. Of the molecules tested, the non-ionic polysorbate 20 (Tween 20) had the lowest CMC, while sodium dodecyl sulfate (SDS) formed micelles at much higher molar concentrations.

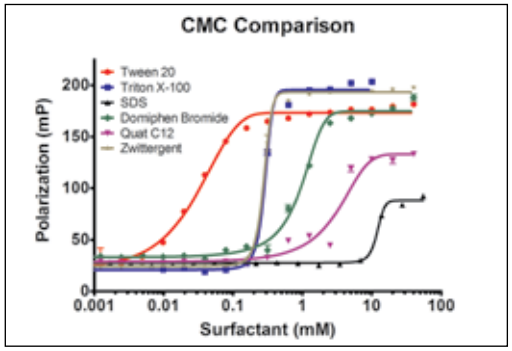


Figure 4. **Comparison of surfactant CMC determination using fluorescence polarization.** The fluorescence polarization of DAF was plotted as a function of detergent concentration.

In addition to the differential concentration at which micelles begin to form, it is important to note that the polarization value in the presence of micelles of each compound is quite different, despite correcting the 100% unbound tracer polarization wells to the same value. This suggests that each surfactant forms micelles of different sizes.

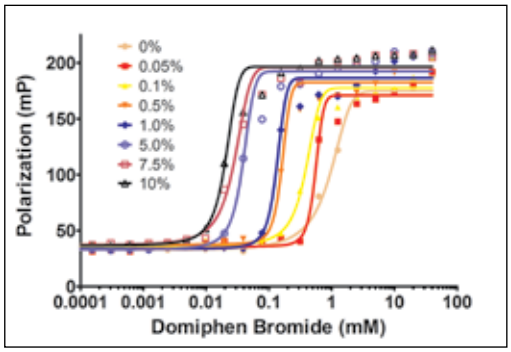


Figure 5. **Effect of sodium chloride concentration on Domiphen bromide CMC.** The fluorescence polarization of DAF was measured in the presence of domiphen bromide compound titrations in various concentrations of sodium chloride.

The concentration at which many surfactants form micelles is effected by sodium chloride concentration. As demonstrated in Figure 5, the fluorescence polarization curves and by inference the CMC for domiphen bromide is affected by the amount of sodium chloride present. The marked increase in polarization, which denotes the formation of micelles occurs at lower concentrations in the presence of sodium and chloride ions. The CMC for each concentration can be determined mathematically (Table 1) and the fold change from the no salt value plotted as a function of sodium chloride concentration (Figure 6). With increasing sodium chloride concentration, the CMC of domiphen bromide decreases nearly 50-fold. However, this large effect is not uniform with all surfactants as polysorbate 20 is minimally affected by changes in sodium chloride. This is no doubt due to it being a non-ionic detergent and so the salt will not affect micelle formation to the same degree.

NaCl Conc (% w/v)	CMC (mM)
0.0	0.351
0.05	0.389
0.1	0.192
0.5	0.0817
1.0	0.0657
5.0	0.0153
7.5	0.0104
10.	0.00791

Table 1. Determined CMC values for domiphen bromide at different sodium chloride concentrations.

Hydrogen ion concentration can play a significant role in micelle formation also. As demonstrated in Figure 7. domiphen form micelles at an approximately 50-fold lower concentrations in an acidic environment, as compared to a neutral pH. SDS and Ipegal 630 are much less affected by hydrogen ion concentration, showing little to no change from pH 5.5 to 9.0.

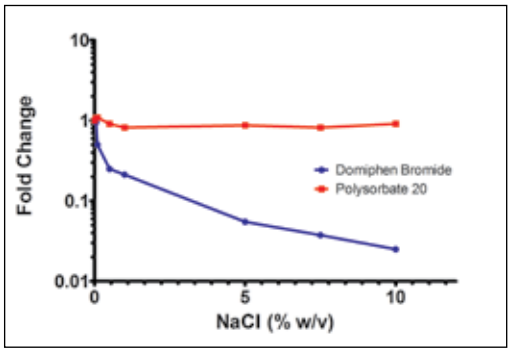


Figure 6. **Comparison of salt effect with various surfactants.** The fluorescence polarization of DAF was determined in the presence of different detergent and sodium chloride concentrations. The fold change in CMC values relative to the non salt control was then plotted as a function of sodium chloride concentration.

Because the hydrophilic polar region of the molecule is dependent on the ionization state of the molecule, positively charged molecules such as domiphen bromide would certainly be expected to be influenced by hydrogen ion concentration and their effectiveness at forming micelles at low pH is not surprising. Non-ionic detergents (e.g. Ipegal 630) are more resistant to changes in hydrogen ion concentration. Somewhat surprising is the resistance of the CMC for the negatively charged SDS to change with the pH levels tested. Although the slight decrease in the fold change at a pH of 9.0 for SDS suggests that micelles may form at significantly lower surfactant concentrations at higher pH.

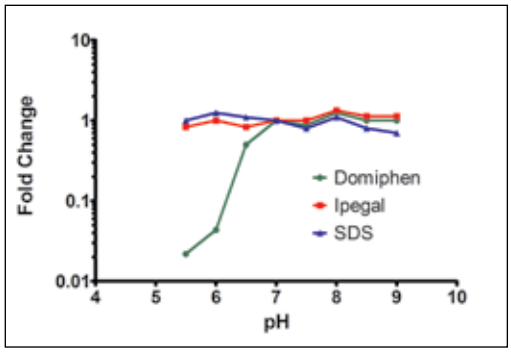


Figure 7. **Effect of pH on CMC value for select amphiphilic surfactants.** The fluorescence polarization of DAF was determined using several detergents and phosphate buffers at differing pH levels. The CMC for each compound at each pH was calculated and the fold change from pH 7.0 plotted.

In addition to the hydrophilic head portion of the surfactant molecule, the hydrophobic aliphatic "tail" can also influence the CMC for different surfactants. When three alkyl sulfate compounds differing in only the length of the aliphatic tail are compared, significant differences in the CMC are observed. In these examples an approximate 10 fold decrease in the CMC is seen with each 2-carbon addition to the aliphatic tail (Figure 8). Sodium decyl sulfate (C10) forms micelles at a concentration of 40.085 mM, while sodium tetradecyl sulfate (C14) has a CMC of 0.904 mM (Table 2).

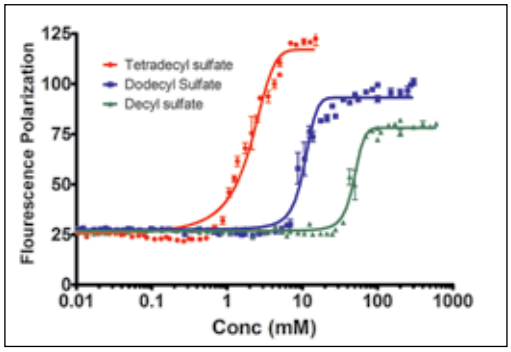


Figure 8. **Effect of Aliphatic side chain with quaternary amine surfactant.** The fluorescence polarization of DAF in the presence of three quaternary compound titrations was determined and plotted.

This 10-fold decrease in CMC with increasing tail length is not an absolute phenomenon. As demonstrated in Figure 9, where three different polysorbate surfactants are compared, only small differences in the CMC is observed with molecules having different length aliphatic tails. Polysorbate 20 and 60, which have tail lengths of 12 and 18 respectively has a decrease in CMC of approximately 2-fold, despite an increase in tail length of 6 carbons. In addition to length, the structure of the aliphatic tail can have a significant influence on the CMC. Branching of the aliphatic hydrocarbon tail results in a substantial increase in the concentration required for micelle formation. As demonstrated in Figure 10, two alkyl sulfate compounds, NiaProof® 4 and sodium tetradecyl sulfate have markedly different CMC values despite having the same formula weight and the same number of hydrocarbons in their aliphatic tails. NiaProof® 4 has several side chains in the aliphatic tail while tetradecyl sulfate does not (See Appendix A). Disruption of close fitting molecules with branching in the micelle is most likely the cause for higher CMC values.

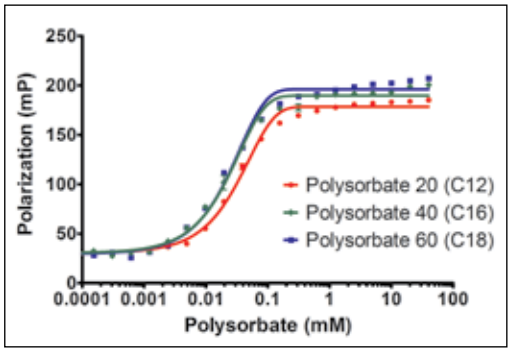


Figure 9. **Effect of Aliphatic side chain length with polysorbate compounds.**

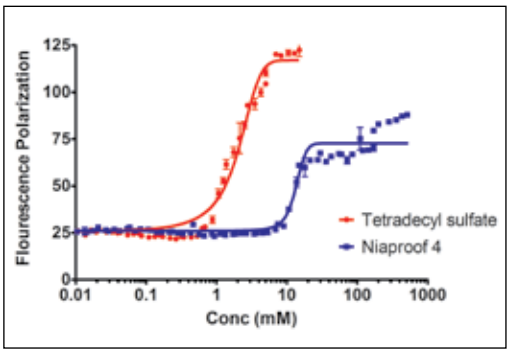


Figure 10. **Effect of branching in aliphatic side chain with akyl sulfate surfactants.** The fluorescence polarization of DAF in the presence of one branched and one unbranched alkyl sulfate compound titrations was determined and plotted.

In addition the presence of a double bond does not significantly influence the formation of micelles with polysorbate compounds (Figure 11) Polysorbate 60 and polysorbate 80 differ by the presence of a double bond linkage present in the aliphatic tail (see appendix). When the CMC value is calculated only a very small difference is observed (Table 2).

Compound	CMC (mM)
Quat C12	3.375
Quat C14	0.194
Quat C16	0.0421
Polysorbate 20	0.00694
Polysorbate 40	0.00381
Polysorbate 60	0.00367
Polysorbate 80	0.00300

Table 2. **Determined CMC values for Quat and polysorbate surfactants with different aliphatic tails.**

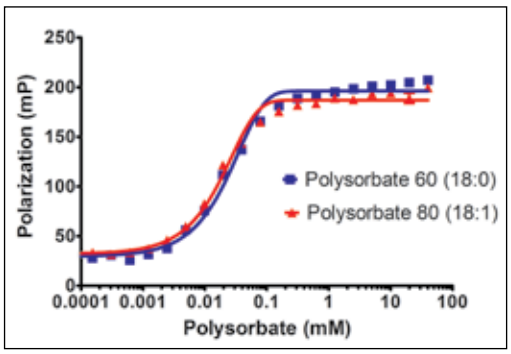


Figure 11. **Affect of double bond structure in aliphatic side chain with polysorbitans surfactants.**

Discussion

The critical micelle concentration (CMC) of a surfactant is an important physical parameter. When surfactants are present at concentration above the CMC they can act as emulsifiers, allowing normally immiscible compounds to dissolve in the solvent. The normally insoluble compound is sequestered in the micelle core, while the head group interacts with the solvent. The most significant example of this would be the use of detergents, where poorly water soluble "dirt", namely oils and waxes, are removed by surfactants in water, when water alone would not normally remove them.

The size and type of structure of the polar head region of the surfactant molecule plays a significant role in surfactant's CMC under different conditions. Small ionic heads typically form micelles at higher molar concentrations than surfactants with large non-ionic compounds. While increasing aliphatic tail length reduces the CMC value for surfactants, compounds with small polar heads are influenced by the length of the aliphatic tail to a much greater extent than surfactants with large non-ionic polar-regions. The ionic alkyl sulfate compounds tested show an approximately 10 fold decrease in CMC with each additional increase of a 2 carbon length. Non ionic detergents such as simple polyoxyethylenes (e.g. Triton X-100) or the polysorbitans, such as polysorbate 20, are polyoxyethylene derivatives which show more modest decreases.

Preparations of many non-ionic surfactants are often mixtures of slightly different compounds with the number of oxyethylene moieties represented as an average. All of the polysorbitans have a total of 20 oxyethylene moieties linked to a sorbitol sugar (see Appendix), with differences being in the fatty acid tail, which are designated by the number present in the name. Ipegal 630 and Triton X-100, while having different names and described uses in the literature, are essentially the same molecule and have approximately 9 oxyethylene groups (see Appendix). A number of studies have shown that head size of alcohol ethoxylates directly influences the CMC. Head size is proportional to the number of ethylene oxides present [16]. In either molecular class the polar head structure is relatively large as compared to the ionic polar regions of the positively charged Quat compounds or the negatively charged SDS. The CMC for these compounds appear to be much less influenced by length of the aliphatic tail.

The effect of sodium chloride on micelle formation is two-fold. Sodium chloride significantly decreases the CMC of ionic surfactants such as domiphen bromide. In addition polarization values of surfactants above the CMC in the presence of salt are higher than those without, which suggest that the size of the micelle aggregates is larger. A slight increase in micelle size is also observed with non-ionic surfactants. This increase in size could be manifested by either more molecules on average in each micelle or a swelling of the micelles as a result of ionic forces.

These data demonstrate that the use of DAF fluorescence polarization as a means to determine the CMC values for surfactants is not only easy and accurate, but that the method can also be easily scaled for large sample numbers. Unlike fluorescence intensity, fluorescence polarization uses a ratio of two measurements on each well, correcting for differences in intensity brought about by experimental conditions, such as pH, temperature, and surfactant concentration. The Synergy Neo reader is a high throughput reader specifically designed for the measurement of large numbers of samples. The reader uses modular optic cubes to measure numerous read modalities, which include UV-Vis absorbance, luminescence, fluorescence intensity, time resolved fluorescence, HTRF®, AlphaScreen®, and fluorescence polarization. In regards to fluorescence polarization, the reader is capable of simultaneously determining parallel and perpendicular measurements. Gen5™ software (BioTek instruments) not only controls reader function, but also is capable of automatically performing the 4-parameter logistic fit and calculating CMC values.

References

1. Salager, J-L, (2002) Surfactants Types and Uses FIRP Booklet # E300-A

2. Perrin, F. (1926) Polarization de la Lumiere de Fluorescence. Vie Moyenne de Molecules dans L'etat Excite. J. Phys Radium 7:390.

3. Thorsteinsson, M.V., J. Richter, A.L. Lee, and P.DePhillips (2005) 5-Dodecanoylamino-fluorescein as a probe for the determination of critical micelle concentration of detergents using fluorescein anisotropy. *Analytical Biochemistry* **340:**220-225.

4. Aguiar,J., P. Carpena, J.A. Molina-Bolivar, and C.Carnero Ruiz (2003) On the Determination of the Critical Micelle Concentration by the Pyrene 1:3 Ratio Method, J. Colloid and Interface Science, 258:116-122.

5. Maciel, R. (1985) Standard Curve Fitting in Immunodiagnostics: A Primer J. Clinical Immunassay 8:98-106.

6. Jumpertz, T., B. Tschapek, N. Infed, S.H.J. Smitz, R. Ernst, and L. Schmitt (2011) High-throughput evaluation of the Critical Micelle Concentration of Detergents, *Analytical BioChemistry* **408:**64-70.

7. Greenspan, P. and S.D. Fowler (1985) Spectrofluorometric Studies of the Lipid Probe Nile Red. *J. Lipid Research*, **26:**781-789.

8. Stuart, M.C.A., J.C. van de Pas, J.B.F.N. Engberts (2005) The Use of Nile Red to Monitor the Aggregation Behavior in Ternary Surfactant-Water-Organic solvent systems. *J. Phys. Organic Chemistry*, **18:**929-934.

9. Wolszczak, M. and J. Miller (2001) Chracaterization of non-ionic surfactant aggregates by fluorometric techniques, *J. Photochemistry and Photobiology* **147:**45-54.

10. Rodriguez, J.R. and J. Czapkiewicz (1994) Conductivity and dynamic light scattering studies on homologous alkbenzyldimethylammonium chlorides in aqueous solutions, *Colloids and Surfaces* **101:**107-111.

11. Khougas, K., Z. Gao, and A. Eisenberg (1994) Determination of the Critical Micelle Concentration of Block Copolymer Micelles by Static Light Scattering, *Macromolecules* **27:**6341-6346.

12. Mihali, C., G. Oprea, and E. Cical (2008) Determination of Critical Micelar Concentreation of anionic Surfactants using Surfactants-Sensible Electrodes, Chem Bull. POLITEHNICA Univ **53:**159-162.

13. Dmoinguez, A., A. Fernández, N. González, W. Iglesias, and L. Montenegro (1997) Determination of Critical Micelle Concentration of Some Surfactants by Three Techniques, *J. Chemical Education*, **74:**1227-1231.

14. Huibers, P.D.T., V.S. Lobanov, A.R. Katritzky, D.O. Shah, and M. Karelson (1996) Prediction of Critical Micelle Concentration Using a Quantitative Structure-Property Relationship Approach. 1. Nonionic Surfactants, *Langmuir*, **12:**1462-1470.

15. Kubota, Y. M. Kodama, and M.Miura (1979) Bull

16. Rulison C. (1995) Critical Micelle Concentration as a Function of Head Group size for alkyl alcohol Ethoxylates. Application Note #201 Kruss USA.

17. Nagarajan, R. and E. Ruckenstein (1991) Theory of Surfactant Self-Assembly: A Predictive Molecular Thermodynamic Approach, Langmuir 7:2934-2969.

18. Mozrzymas, A. and B. Rozycka-Roszak (2010) Prediction of Critical Micelle Concentration of nonionic surfactants by quantitative structure property Relationship. *Combinatorial. Chemistry and High Throughput Screening* **13(1):**39-44.

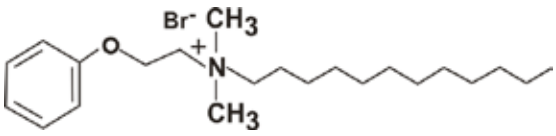
Acknowledgements

I would like to acknowledge Eddie Delpierre (BioTek Instruments) and Sara Held (Community College of Vermont) for their assistance with the mathematical determination of CMC values from the 4-parameter logistic fit equitation.

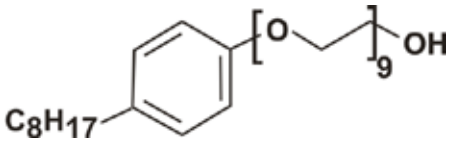
APPENDIX

Structures of Surfactant Compounds

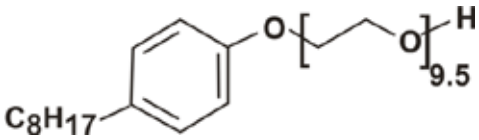
Domiphen Bromide



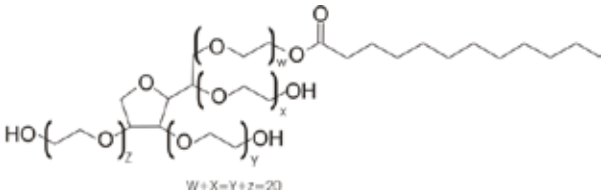
Ipegal 630



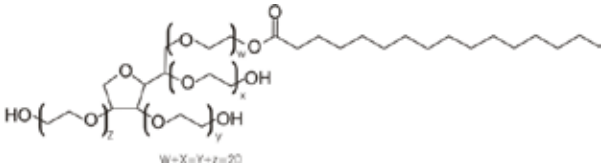
Triton X 100



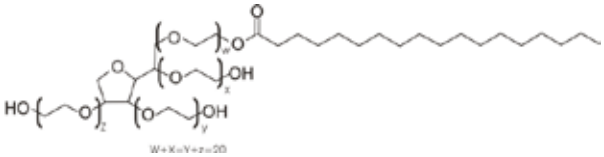
Polysorbate 20



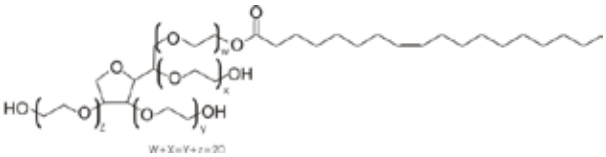
Polysorbate 40



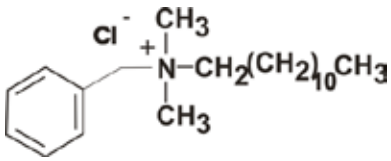
Polysorbate 60



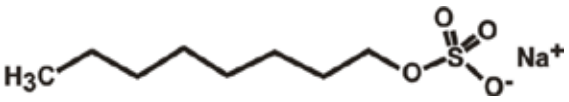
Polysorbate 80



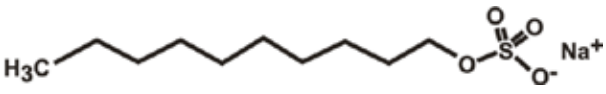
Quat C12



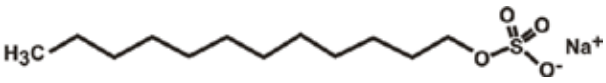
Sodium Octyl Sulfate



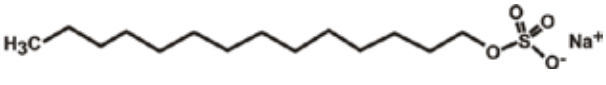
Sodium Decyl Sulfate



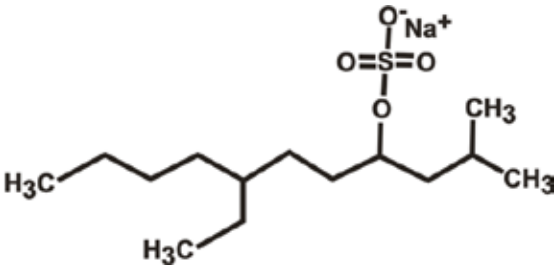
Sodium Dodecyl Sulfate



Sodium Tetradecyl Sulfate



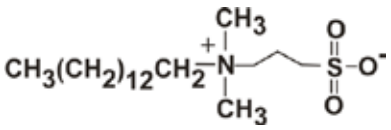
NiaProof® 4



APPENDIX

Structures of Surfactant Compounds

Zwittergent



DAF