Published in final edited form as:

Chem Commun (Camb). 2011 May 21; 47(19): 5527–5529. doi:10.1039/c1cc10605h.

A facile surfactant critical micelle concentration determination

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Abstract

Liquid surface curvature variations in microplate wells due to different liquid surface tension cause significant signal change in spectroscopic measurement using a plate reader with a vertical detecting light beam. The signals have been quantitated and used to develop a method for facile surfactant critical micelle concentration determination.

Surfactants are an important class of substance; they prefer to stay on the liquid surface when dissolved in aqueous solution and they decrease the liquid surface tension.¹ Above a certain point where the surface concentration is saturated, surfactants begin forming self-assembled aggregates, usually starting as micelles, leaving the solution surface property unchanged. The corresponding concentration is surfactant critical micelle concentration (CMC), a key parameter of surfactants. Surface tension measurement provides a universal method for CMC determination, but it is complicated and time consuming.^{2, 3} Methods have been developed for determining surfactant CMC values more easily and rapidly (for extensive references see supplementary materials 1). These methods made use of changes in other properties associated with micelle formation, in order to bypass the laborious surface tension measurement, including light scattering,^{4, 5} electrophoresis and conductivity,^{6–8} or the use of dyes that specifically interact with micelles as probes.^{9–11} These fast methods, although applicable in certain circumstances, suffered from compatibility of the specific measurement methods with myriad kinds of surfactants with diverse properties. A simple and fast CMC determination that can be universally applied to most surfactants is still the main challenge in this field.^{9, 12}

Here we report a facile surfactant CMC determination by probe-free quantitative monitoring of the variation of liquid surface properties of surfactant solutions via the resulting liquid surface curvature changes in microplate wells. The method requires microliters of surfactant solutions and standard microplate absorbance measurements.

Surfactant solutions have an effect on liquid surface curvature in thin wells. An example is shown in Figure 1. A constant volume of solution with varying concentrations of surfactant was distributed in the wells of a 384-well microplate (Greiner Bio-One). The variation in liquid surface curvature was distinguishable by eye (Figure 1A). The curved liquid surface formed in the microplate wells is a capillary like effect caused by different surface interactions. The curvature is determined by liquid surface tension, solid surface tension of

the microplate coating material, solid-liquid interface tension, gravity, and the size and shape of the wells. In a given type of microplate at the same experimental conditions such as temperature, air pressure and moisture, all other factors are fixed and the surface curvature is solely dependent on properties of the liquid surface.

The liquid surface curvature variations caused different signal responses in plate reader measurements using a vertical detecting light beam. Other studies have utilized this phenomenon to measure surface effects.^{13, 14} In this study, the signal variations were quantitatively measured using a plate reader (Molecular Devices SpectraMax M5) with the vertical detecting light beam offset from the center of the well, as was carried out by Cottingham.¹⁴ A 1 mm offset in a 384-well microplate gave an absorbance value of ~1 unit for the most curved liquid surface (using surfactant concentration above its CMC, such as 1 mM Triton X-100), and was selected to give maximum sensitivity. The detecting light wavelength was set at 900nm to minimize interference from sample chromophores. A significant dynamic range was obtained as a result of the surface curvature variations, despite the absence of absorbing chromophores in the solution. Reproducible signals were obtained with the proper internal reference (Figure 1B, details see supplementary materials 2).

The absorbance-like signal due to a curved liquid surface is the result of light refractions that change the detecting light path so that it partly escapes detection by the photon detector. The light path is shown in Figure 1C and the mechanism can be reduced to a trigonometric problem from which a quantitative relationship between absorbance-like signal value and liquid surface curvature can be deduced based on the law of refraction (Snell's Law), although it is dependent on plate reader, microplate type, and instrumental settings (details see supplementary materials 3). In an instrument with a detecting light beam width comparable to the dimensions of the plate well, as is the case in the M5 plate reader, continuous liquid surface curvature change causes a gradual loss of light reaching the detector, resulting in a gradual absorbance change, which can be quantitatively calibrated to the liquid surface curvature.

The established method provided a facile method to measure CMC. The samples for CMC measurements were prepared by serial dilution of surfactant using a 96-well plate, and then direct transfer into a 384-well plate for measurement. This protocol avoided the air bubbles introduced by direct dilution of the samples in the 384-well plate without compromising the simplicity and throughput. On-site dilution in 384-well plate was feasible, although it caused additional signal noise may due to the bubbles introduced (supplementary materials 4). Representative surfactants of different classes were tested including anionic surfactants, cationic surfactants, zwitterionic surfactants and nonionic surfactants. Their absorbance vs. concentration curves are shown in Figure 2A. Based on the mechanism of micelle formation, there is an inflection point in the surface tension vs. concentration curve at the surfactant CMC, which is observed in all surfactants tested (see supplementary materials 4 for determination of the actual inflection point). Three distinguishing features of the measurement are worth noting: 1) All tested surfactants showed a sharp inflection point in the curves that enabled sensitive determination of their CMC values; 2) All displayed similar magnitude of absorbance changes and shared similar curves around their CMC independent of the charge, class of surfactant, and order of magnitude of the CMC, indicating the universality of the method. 3) The method is straightforward and probe-free, requiring only μ l of samples and a direct absorbance measurement, so that it is suitable for high-throughput measurement using an automated liquid delivery system. Without probe interference and incompatibility, the main restriction for most spectroscopic CMC determinations is lifted,^{9, 10} providing a solution to the long lasting problem of an accurate, fast, simple and universal CMC determination.

The CMC values determined from the new method are listed in Table 1, both in 50 mM phosphate buffer pH 7.0 and in water. For comparison, CMC values were also determined using a well established although low throughput method involving pyrene as a fluorescent probe¹¹ (for details see supplementary materials 5). The CMC values showed relative standard deviations of less than 5% for parallel measurements using the high throughput absorbance method described here. For all surfactants tested, no statistically significant differences were observed between the absorbance method and the pyrene fluorescence method (Figure 2B). In general, measured CMC's fall into a wide range in the literature, because of the differences in experimental conditions. In Table 1 we demonstrate the variations caused by different buffer and salt concentration, especially for charged surfactants.^{4, 8, 17} Different sources of surfactants may also contribute to these differences (see Table 1 and references in supplementary material 6).

Liquid surface curvature change on which the current measurement is based is a cooperative effect involving liquid surface tension, liquid-solid interface tension and solid surface tension of microplate coating materials. Different type of microplates with different coating materials may give a different signal response. We observed that some types of microplates were not suitable for curvature based determination because the water solution without surfactant already had a highly curved liquid surface, thus significantly reducing the sensitivity of the measurement (data not shown), as reported by other groups.¹⁴ Fortunately, the widely used microplate with an untreated plastic surface is suitable for the liquid surface curvature based measurement, with a nearly flat liquid surface observed in the absence of surfactant.

Liquid volume also had an effect on signal response by affecting the detecting light path. The liquid surface meniscus was not affected over a wide range of liquid volumes (as long as there was enough liquid and enough free space in the well for forming a full liquid meniscus) so that CMC value was independent of the volume selected for the serially diluted samples (supplementary materials 7). Signals reached equilibrium in ~30 minutes, and remained unchanged for a couple of hours (data not shown). The 30 minute incubation of sample in the microplate before measurement was required to get reproducible results.

In conclusion, we have presented a facile surfactant CMC determination by probe-free measurement. The surface tension-caused liquid surface curvature change in microplate wells was detectable by absorbance measurements. The measurement requires μ l of sample, is tunable and potentially high-throughput. The ubiquitous role of surface tension and the simplicity and versatility of the method suggest new application opportunities.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by National Institutes of Health (NIH) grants AI060361 and NS059403 to M. G. and intramural funding from Beijing Institute of Pharmacology & Toxicology to L. C. The authors thank Dr Lei Guo for providing the Fluorescence Spectrometer, and Juan Chen, Baohua Zheng, and Kun Wang in Beijing Inst. Pharmacol. Toxicol. for assistance in sample preparation for CMC determination using the pyrene fluorescence method.

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Figure 1.

Converting liquid surface curvature variations in microplate wells into quantitative absorbance signals. (A) Various liquid surface curvatures are formed in microplate wells for solutions with different surface tensions by the capillary effect. Photograph of a Greiner 384-well black/clear microplate, two rows of wells at top, middle and bottom contain 30 μ l Triton X-100 solution in serial dilution, the last two wells in each row contained buffer only; the remaining rows are empty. The liquid surface curvature variations are distinguishable by eye from their different reflection of light. (B) Absorbance signal vs. Triton X-100 concentration in the microplate wells measured using a Molecular Device SpectraMax M5 plate reader by offset the detecting light beam 1 mm from the well center and detected at 900 nm wavelength. (C) Light paths in the instrument for absorbance measurement. The light beam was offset from the well center to enable refraction from a different normal at different liquid surface curvature. The vertical detecting light beam (red line) passes through the liquid (azure shadow)-air interfaces with a two-step refraction and reaches the detector with a shift from the original vertical location. The shift value can be calculated by solving the triangles with known h_1 , h_2 , offset, and the refractive index of liquid and air, n_2 , n_1 , at a given liquid surface curvature radius R .^[14]

Figure 2.

(A) Absorbance vs. concentration curves of surfactants of different classes. Surfactants were dissolved in 50 mM phosphate buffer pH 7.0. The samples were prepared as a group of 12 by serial dilution in a 96-well plate, and then direct transfer in odd or even wells of a 384-well plate (30 μ l/well), the last two rows contained an internal reference of 1 mM Triton X-100. The absorbance was measured with the plate reader setting as in Figure 1 and normalized as described in supplementary material 2. Error bars are standard deviations from quadruplicate repeats. Anionic surfactants: Sodium octyl sulfate (C₈SO₄), sodium decyl sulfate (C₁₀SO₄), sodium dodecyl sulfate (C₁₂SO₄), 1-decanesulfonic acid sodium (C₁₀SO₃); Cationic surfactants: decyltrimethylammonium bromide (C₁₀NBr), dodecyltrimethylammonium bromide (C₁₂NBr), tetradecyl trimethylammonium bromide (C₁₄NBr), hexadecyltrimethylammonium bromide (C₁₆NBr), Octadecyltrimethylammonium bromide (C₁₈NBr); Zwitterionic surfactants: 3-(N, N-dimethylpalmityl-ammonium) propanesulfonate (SB3-16), nonionic surfactants: Tween20, Triton X-100 (Triton). (B): comparison between CMC determined by current method and pyrene method (detail see supplementary material 5).

Table 1

CMC values of surfactant measured

Surfactants	CMC (mM) ^a			Literature results
	Absorbance (Buffer)	Absorbance (H ₂ O)	Pyrene (H ₂ O)	
SB3-16 [*]	0.023 (0.004)	0.040	0.034	0.028–0.029 ^{13, 15}
C ₁₈ NBr [*]	0.019 (0.000) ^b	0.164	0.133	
Tween20 [*]	0.037 (0.006)	0.026	<0.048 ^c	0.028–0.054 ^{13, 16}
Triton X-100 [*]	0.125	0.19	0.27	0.12–0.29 ^{8, 9, 11–13}
Triton X-100 ^{**}		0.076	0.083	
C ₁₆ NBr [*]	0.124	0.78	0.80	0.2–1 ^{8, 11}
SDS [*]	1.81 (0.27)	8.2 (0.1) ^b	6.4	0.52–35 ^{4, 8, 11–13, 17}
SDS ^{***}		3.9	2.9	
C ₁₄ NBr [*]	1.18 (0.06)	3.0	2.4	3.4–3.94 ^{4, 18}
C ₁₀ SO ₄ [*]	11.83 (4.49)	32	24	8–9.1 ¹⁹
C ₁₂ NBr [*]	8.29 (0.32)			14.6–16 ^{18, 20, 21}
C ₁₀ NBr [*]	73.7 (1.36) ^b	58	61	60–69.2 ^{11, 18}
C ₁₀ SO ₃ [*]	30.9 (8.27)	29	32	~30 ²²
C ₈ SO ₄ [*]	27.8 (2.02)	121	104	199 ²¹

^{*} Sigma reagents;^{**} SERVA reagent;^{***} Amresco reagent;^a Precision of the measurement (in brackets) from different batches of Sigma surfactants measured at different times;^b Precision of parallel measurements, conducted in the same plate;^c no inflection observed at low concentration.