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A novel, rapid and automated conductometric method to evaluate surfactant–cells interactions by means of critical micellar concentration analysis

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ABSTRACT

Conductometry is widely used to determine critical micellar concentration and micellar aggregates surface properties of amphiphiles. Current conductivity experiments of surfactant solutions are typically carried out by manual pipetting, yielding some tens reading points within a couple of hours. In order to study the properties of surfactant–cells interactions, each amphiphile must be tested in different conditions against several types of cells. This calls for complex experimental designs making the application of current methods seriously time consuming, especially because long experiments risk to determine alterations of cells, independently of the surfactant action. In this paper we present a novel, accurate and rapid automated procedure to obtain conductometric curves with several hundreds reading points within tens of minutes. The method was validated with surfactant solutions alone and in combination with *Saccharomyces cerevisiae* cells. An easy-to use R script, calculates conductometric parameters and their statistical significance with a graphic interface to visualize data and results. The validations showed that indeed the procedure works in the same manner with surfactant alone or in combination with cells, yielding around 1000 reading points within 20 min and with high accuracy, as determined by the regression analysis.

1. Introduction

The chemistry of cationic, anionic or non-ionic, surfactants and their aggregates in water, or in organic solvents, has a significant importance in various areas spanning from basic research to industrial and environmental applications [1–3]. Aqueous micellar solutions catalyze chemical reactions [4], substitute organic solvents [5], allow oil recovery [6], act as dispersing and templating agents in nanomaterial chemistry [7], represent reaction media with water pools reactions [8]. The large number of potential applications induced many researchers to synthesize surfactants tailored for specific roles [9–13]. The surfactant specificity is well demonstrated when interacting with bio-molecules, in fact each amphiphile can exert different actions with diverse target macromolecules such as proteins, DNA etc. [14–17]. The use of surfactants as biocides against bacteria, virus and fungi is one of their most appreciated features [18–26].

Conductometric measurements are commonly used for the determination of critical micellar concentration (hereinafter referred as *c.m.c.*) and counterion fraction (α) [27], yielding curves whose slopes are determined by charged molecules mobility in water. Typical curves are characterized by two branches with the first steeper than the second. This change is due to the ion mobility caused by the micellar formation and therefore the flex between the two branches corresponds to the *c.m.c.* Since a surfactant interacting with a macro-molecule has less mobility, this phenomenon can be observed by a reduction of the conductometric curve slope [28]. *Vice versa* surfactants with biocidal effect, likely disaggregate the cell membrane causing the release of conducting materials in aqueous solution with an increase of the curve slope after the cell damage [15].

For this reason, conductometry is a promising technique in surfactant–cells interactions studies [15,25] and can be conveniently applied to these analyses instead of other procedures [29–36].

This methodology applied to surfactants is inexpensive, accurate and gives important information on the interactions that can occur between surfactant monomers or micelles and cells.

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Conductivity experiments of surfactants–cells mixtures can be carried out by manual pipetting specific aliquots of surfactant solution and of the target cells, to increase the surfactant concentration while keeping constant that of the cells (hereinafter referred to as "point by point procedure"). The accuracy of the experiment depends on the number of the reads acquired, and therefore on the time spent on the experiment. On the other hand, extending the experiment time, changes in the cell physiology can be often produced, independently of the surfactant action. This fact can produce an experimental noise, difficult to subtract from the signal, referring to the actual action exerted by the amphiphile against the cells. The combination of these experimental requirements calls for a rapid conductometric method able to obtain several reading points in a short time with at least the same accuracy obtainable by conventional procedures with sole surfactants.

In this paper we present a novel, rapid and automated conductometric method able to obtain accurate curves, with ten to hundred times more points than the "point by point" procedure, in short time using surfactants alone or in combination with cells. Statistical analysis can be carried out with a simple dedicated R script, producing *c.m.c.* evaluation with a graphic display. This method is accomplished with common tools present in every laboratory and therefore does not require additional dedicated equipment. It was validated using the well-known cethyltrimethylammonium bromide (CTAB) and the novel amphiphile *N*-tetradecyltropinium bromide as surfactants, and the yeast *Saccharomyces cerevisiae* as model cells. The procedure yielded ten to hundred times more reads than the current methods with five to ten times fold reduction; the increased number of reading points allowed a more careful *c.m.c.* evaluation and an easy to read result display. Finally, the conductometric experiments of surfactants and yeast cells mix showed the presence of otherwise not detectable inflection points, whose meaning is still under investigation.

2. Materials and methods

2.1. Materials

2.1.1. Surfactants employed

CTAB surfactant was purchased from Sigma–Aldrich and recrystallized from methanol–acetone mixture before use. *N*-tetradecyltropinium bromide was synthesized and purified using methods reported elsewhere [15].

2.1.2. Culture and growth conditions

Yeast strain *S. cerevisiae* LCF 520 was obtained from the internal collection of the Microbial Genetics and Phylogenetics Laboratory of the Department of Pharmaceutical Sciences (University of Perugia), its classification was previously carried out by 26S rDNA sequencing (data not shown). Pre-culture and subsequent culture were inoculated at OD_{600} = 0.2 in 100 mL of YEPD + chloramphenicol medium (Yeast Extract 1%, Peptone 1%, Dextrose 2% – chloramphenicol 0.5 g/l – Difco Laboratories, Detroit, MI, USA) and grown 24 h at 25 °C, with 150 rpm shaking.

2.2. Methods

2.2.1. c.m.c. experiments with surfactant alone

Conductivity was measured on a CRISON GLP-31 conductivity meter at 25.0 ± 0.1 °C (Pharmacia Biotech Multitemp III thermostat). The conductivity meter was connected to a computer via RS-232. A Watson-Marlow 323 peristaltic pump (with Watson-Marlow tubes) was used for the surfactant solutions additions. Surfactant solution was added at 0.0143 mL/s rate to a measuring chamber maintained at 25.0 °C in a water bath and stirred at 100 rpm. The rate of the pump was determined by gravimetric determination of the flow. The conductivity values were acquired by the conductivity meter with the frequency of one per second and transferred to the computer via RS-232 port, using the SerialNGAdvDemo free-software for data acquisition (www.domis.de). The recorded data were analyzed in the free R statistical software (cran.r-project.org).

2.2.2. Conductivity experiments of surfactants in the presence of S. cerevisiae cells

The yeast cell suspension was centrifuged (1500*g*, 5 min), washed twice with HCl 5×10^{-3} M and then three times with bidistilled water to standardize the supernatant conductivity to a value below 10 μS/cm. A JASCO V-530 spectrophotometer was used to determine cell suspension concentration. In order to have linearly reproducible figures of cell density, all spectrophotometric measures were carried out by diluting the suspensions to densities ranging from OD_{600} = 0.1 to OD_{600} = 0.5. The final optical density was obtained by multiplying the dilution factor and the actual spectrophotometric measure.

Each cell suspension was standardized at an optical density OD_{600} = 5.00 which resulted optimal for conductivity measures. In order to keep the cell density constant, the peristaltic pump added the same volume of surfactant and of double density cell suspension (OD_{600} = 10.0). The procedure of data acquiring was identical to the one used for *c.m.c.* experiment of a single surfactant.

2.2.3. The CMC R-script

Conductometric curves obtained by increasing surfactant concentration show almost two linear trends of which the former steeper than the second. The *c.m.c.* is normally calculated as the concentration (*x*-axis) value corresponding to the intercept point between the two linear regressions.

The CMC (for *c.m.c.* determination) script was developed in the free statistical software R (www.cran.org) and works equally well in Vista-Win7-Win8 and iOS environments. The software implemented two algorithms for the *c.m.c.* determination. One (Method A) is based on the rationale that *c.m.c.* can be calculated as the concentration corresponding to the point of the conductometric curve where the steepness changes between the first and the second branch, while the other approach (Method B) is the usual procedure based on the intercept of the two regression curves.

Method A was carried out with a reiterative sliding window algorithm (Eq. (1)) to calculate the R values (Pearson correlation) of the regression, calculated for each *i*th point of the curve, in which the window width (*w*) is predetermined by the operator, *k* is the conductivity and *C* is the compound concentration.

$$der = cor(k_{(i \div i+w)}, C_{(i \div i+w)}) \quad (1)$$

The minimum *der* value found throughout the curve corresponds to the CMC*min* (*c.m.c.* with the minimum derivative point algorithm) and is a first estimate of the *c.m.c.* In order to avoid problems in determining the CMC*min* of conductometric curves presenting a low steepness initial part, a correction algorithm has been implemented that does not consider the extremes of the curve, with the rationale that in any case the CMC*min* is found next to the center.

Method B uses the CMC*min* point to divide the conductometric curve into two branches and finds the part of each branch with the optimal linear fitting, by applying Eq. (2), where *tr* is the correlation (*R*) threshold (i.e. the minimum *R* value of the regression curve) chosen by the operator. Typically, *tr* values above 0.98 are used.

$$der_i > tr \quad (2)$$

Once the best fitting parts of the two branches have been found, the algorithm calculates the intercept (CMC value) and the R^2 of the two regression curves.