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DETERMINATION OF CRITICAL MICELLE CONCENTRATION (CMC) OF DIFFERENT PLURONICS WITH TWO DIFFERENT METHODS

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البلورونك هى بوليمرات مخلقه تحتوى على عديد الأوكسى اثيلين و عديد الأوكسى بروبيلين وتستخدم على نطاق واسع فى الصياغات الصيدليه كمادة تساعد على تكوين المستحلبات وكمادة مذيبه. وفى هذه الدراسة تم تعيين الدرجه الحرجه لتكوين الميسل لمجموعه من البلورونك فى محاليلها وهى ل ٦١ ، ل٦٢ ، ل٦٣ ، ل٦٤ ، ل٩٢ ، ف٣٨ وف٦٨ بطريقتين مختلفتين: الطريقه الأولى هى تعيين التوتر السطحى للمحاليل والطريقه الثانيه هى طريقه فلوريمتريه باستعمال ثنائى فنيل الهكسترايين. وقد وجد أنه بزيادة الصفه المحبه للماء فى البلورونك يقل تركيز الدرجه الحرجه لتكوين الميسل و أيضا وجد أن الطريقه الفلوريمتريه أكثر حساسيه و دقه من الطريقه المعتمده على تعيين التوتر السطحى للمحاليل.

Pluronics are series of closely related block copolymers of ethylene oxide and propylene oxide and are used primarily in pharmaceutical formulation as emulsifying or solubilizing agents. In this study, the critical micelle concentration (CMC) of different pluronics such as L61, L62, L63, L64, L92, F38, and F68 was measured by two different methods. The first method is the surface tension method (ring method); the second one is fluorimetrical method by using DPH (1,6 diphenyl hexatriene). It was found that, by increasing the lipophilicity of the block copolymer (increase the propylene oxide moiety) the CMC value increases. Also, the fluorimetric method is very sensitive, accurate and more reproducible than surface tension method.

INTRODUCTION

Surfactants are amphiphilic molecules that contain a nonpolar segment, commonly called the tail, and a polar segment called the head. This characteristic, being an amphiphilic molecule, leads to aggregation. When a surfactant is dissolved at low concentrations, the molecules exist as individual entities. However, as the concentration of the surfactant increases the molecules tend to associate to form aggregates. The aggregation number indicates how many molecules are present in an aggregate, and often there will be a rather narrow size range. In aqueous solution, the hydrophobic tails of the surfactant associate, leaving the head group (hydrophilic) exposed to the solvent. The simplest of such aggregates, with an approximately spherical shape, are called micelles. The transition from a monomeric solution to an aggregate form can be seen as a change in the slope of plots against surfactant concentration of many physical properties (eg. Viscosity, conductance, and surface tension).[1-3]

The concentration at which this change takes place is called the critical micelle concentration (CMC). Once the micelles are formed, further increase of the surfactant concentration does not significantly change the concentration of the free monomer. The surfactant added is incorporated completely into the micelles, i.e., the concentration of the free surfactant molecules remains constant after the micelles are formed. In nonpolar solvents, the hydrophilic segment can be poorly solvated, so the head will form the interior of the aggregates. The hydrophobic segments surround the polar core and are responsible for the solubility of the aggregates.[3] The structures formed are the so-

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called reveres micelles. The aggregates can be formed at low surfactant concentrations, but the aggregation numbers for reveres micelles are usually relatively small. In fact the CMC is a transition region over a small composition range, so exact determination is difficult.[4] However, experimental data show an apparent break between two lines.

Surfactants are extremely important in studies of biological membranes due to their ability to solubilize the membrane.[5] It is known that above a particular concentration, called the critical micelle concentration (CMC), surfactant molecules self-associate to form thermodynamically stable aggregates called micelles.[6] The determination of CMC of surfactants has been studied by many physicochemical techniques. These include measurement of light scattering,[7] surface tension,[8] hydrodynamic properties[9] and changes in absorbance or fluorescence upon dye solubilization.[10]

Poloxamers, i.e., triblock copolymers of the type poly ethylene oxide/poly propylene oxide/poly ethylene oxide, are available commercially under the trade name Pluronics, have been widely used as nonionic surfactants in pharmaceutical and cosmetic industries, since these copolymers have low toxicity and have the ability to form a clear solution in aqueous media.[11]

In this study, the CMC of different pluronics was measured by surface tension method (ring method) and fluorimetrical method by using DPH.

EXPERIMENTAL

Materials

Different grades of pluronics, PF-68, PF-38 from BASF AG, Ludwigshafen, Germany, PL 61, PL 62, PL 63, PL 64 and PL 92 from C. H. Erbsloech. Desseldorf, Germany. DPH (1,6 diphenyl-1, 3,5-hexatriene) from EGA-Chemie, Steinheim, Germany. Hepes buffer (N-2-Hydroxyethylpiprazine-N-2-ethanesulfonic acid) from Sigma Co., St Louis, USA. All other chemicals were of analytical grades.

Methods

Determination of CMC of Pluronics by DPH (1,6 diphenyl hexatriene)

a) Preparation of pluronic solution
Serial dilutions of pluronics with Hepes buffer pH 7.4 were prepared, with a volume of 10 ml for each concentration. Five ml of this solution were used as sample and the other 5.0 ml as a blank.

b) Preparation of DPH solution
0.232 μg DPH was used as probes in 5.0 ml solution equivalent to (0.2 μM/L). For this purpose, 1.16 mg DPH was weighed accurately and dissolved in 1.0 ml of tetrahydrofuran. 200 μl from this solution were pipetted and diluted to 50 ml with methanol.

c) Samples preparation and measurements
50.0 μl DPH-methanol solution was pipetted accurately in brown vials and introduced in a dissicator. Methanol was evaporated using rotation evaporator. 5.0 ml pluronic solutions were added to each vial. The vials were shaken strongly to dissolve the dry DPH film and incubated in a dark place for 16 hr.
The fluorescence intensity was measured against blank solution treated in the same manner as the samples without DPH solution. The excitation wavelength was 366 nm. and the emission wavelength was 430 nm. The width of band was 10 nm. at a constant temperature of 25°.[10] All experiments were done in duplicate and the average fluorescence was calculated.

Determination of CMC of pluronics by surface tension method

This method was carried out using Kruesse K10 digital tensiometer (Wilhelmy Co., Frankfurt, Germany) with platinum ring. In this method a serial dilutions of different pluronics were prepared in Hepes buffer pH 7.4. Each sample was about 10.0 ml Samples were incubated for 24 hr and then the surface tension (γ) of these solutions was measured at room temperature. Surface tension data, as a function of concentration (C) were determined employing the ring-detachment method.[12]

The following standardized procedures were adopted:

- All measurements were made in triplicate at room temperature and all reported values were averaged.
- All glass apparatus used, as well as the platinum ring, were cleaned with freshly prepared chromic acid and then thoroughly rinsed with distilled water.
- Each solution contained in the dish was set aside for 10 min prior to the actual measurement of surface tension.

RESULTS AND DISCUSSION

The objective of this investigation was to establish a relatively fast and accurate method for the CMC determination. So that, we choose two different methods namely, surface tension method, which is the most, and popular one and fluorimetric method. The principle of the DPH assay of CMC is that the DPH fluorescence will be greatly enhanced above the CMC due to its incorporation into the hydrophobic interior of the micelle.[10] In order to determine the best condition for this assay we examined the dependence of the fluorescence intensity upon pluronic concentration. By increase the concentration of pluronic, the fluorescence was weak at the lowest concentration of copolymer, and then rises rapidly and finally level off. This is most easily interpreted as follows: the rapid rise in fluorescence occurs at and above the CMC of the pluronic. As the amount of copolymer is increased, the number of micelles increased and the amount of bound DPH, and therefore, fluorescence increased. At very high pluronic concentration, all DPH is bound and so fluorescence levels off. The CMC is given by the intersection of the straight line through the fluorescence at low copolymer concentrations with a straight line through the fluorescence values in the region of rapid intensity increase.

Pluronic block copolymer can form (mostly) spherical micelles in aqueous solutions above a certain copolymer concentration. CMC of this copolymer depends on the HLB and molecular weight. Figures 1-3 show the dependency of the fluorimetric intensity on the pluronic concentrations and also the CMC of Pluronics L61, L62 and L92 which determined by this method, while Figures 4-6 show the plot of the surface tension against pluronic concentration and the CMC of the same surfactants which determined by the surface tension method. All plots show that, the surface tension varies linearly with concentration of copolymer up to a certain concentration characteristic of each copolymer, where surface tension remains essentially constant with further increase in concentration. The CMC of each pluronic is determined graphically from the intersection of the linear portion of each plot. Table (1) shows the CMC of different pluronics and their standard deviation values. It was found that, by increasing the hydrophilicity from L61 to F38 the CMC decreases with the exception of L92, that depends on HLB of pluronic. From another side, F38 and F68 have the same HLB but F38 has CMC smaller than F68 that may be due to the difference in the molecular weight.

These results show that, the fluorimetric method is sensitive and reproducible because the standard deviation values for the mean of two experiments is lower than the standard deviation values of three experiments for the surface tension method.

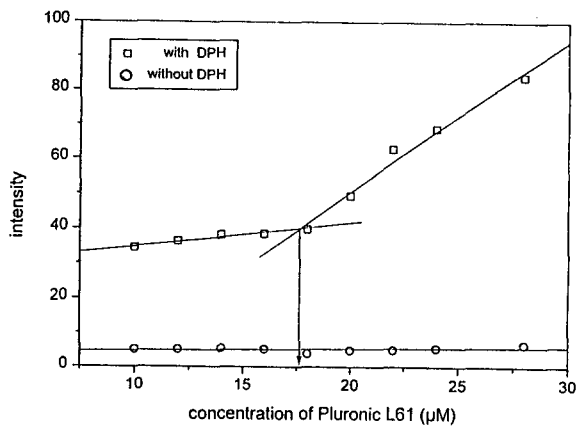

Fig. 1: Determination of critical micelle concentration of Pluronic L61 in Hepes buffer pH 7.4 by DPH method.

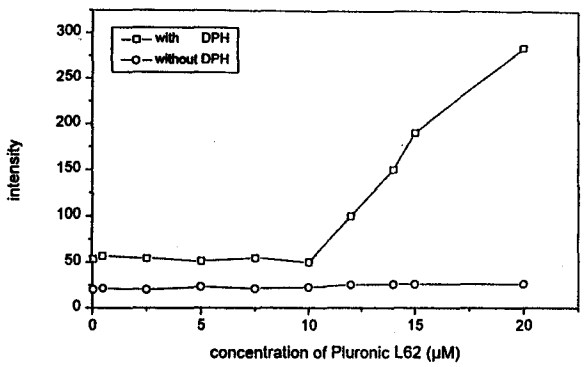

Fig. 2: Determination of critical micelle concentration of Pluronic L62 in Hepes buffer pH 7.4 by DPH method.

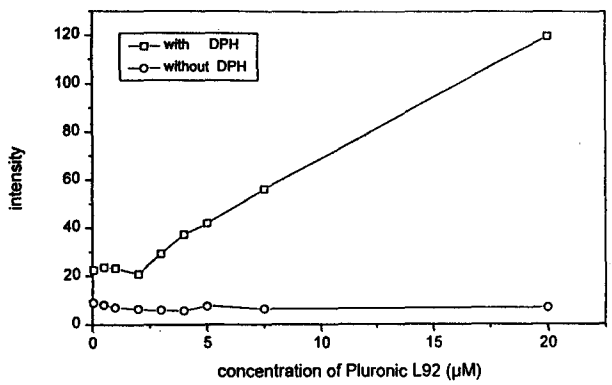

Fig. 3: Determination of critical micelle concentration of Pluronic L92 in Hepes buffer pH 7.4 by DPH method.

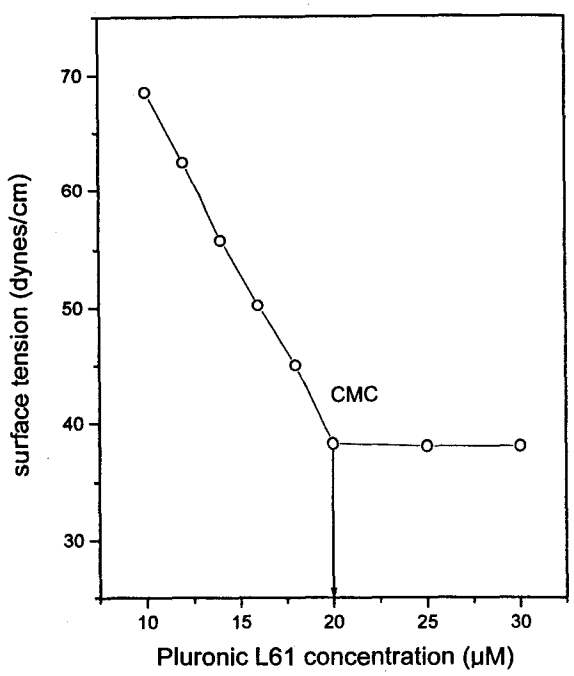

Fig. 4: Determination of critical micelle concentration of Pluronic L61 in Hepes buffer pH 7.4 by surface tension method.

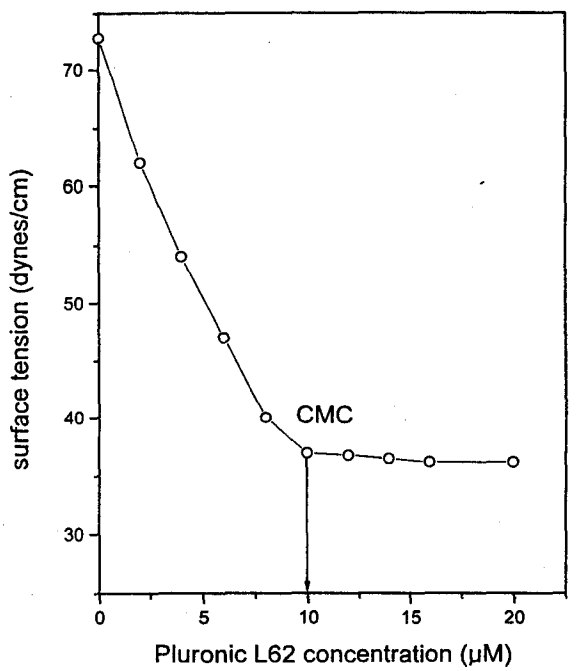

Fig. 5: Determination of critical micelle concentration of Pluronic L62 in Hepes buffer pH 7.4 by surface tension method.

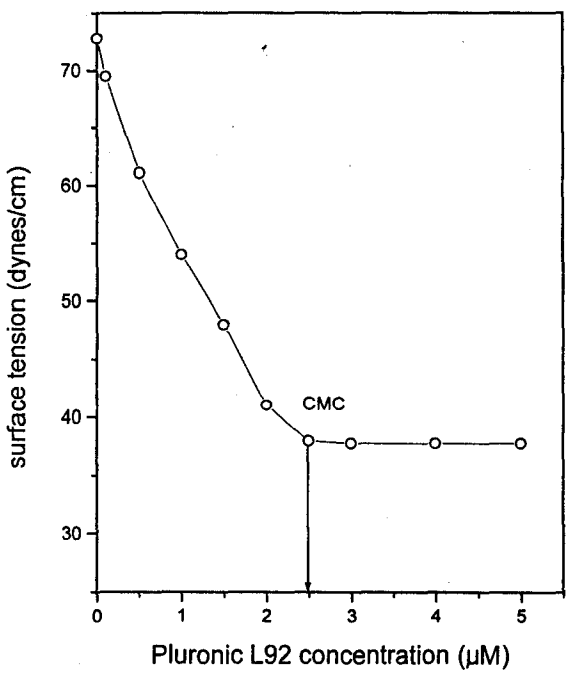

Fig. 6: Determination of critical micelle concentration of Pluronic L92 in Hepes buffer pH 7.4 by surface tension method.

Table 1: CMC values of Pluronics block copolymers.

Pluronics grade	Molecular weight	HLB	CMC (μM) determined by surface tension method	CMC (μM) determined by fluorimetric method
PL 61	2000	2.0	20.0 ± 2.1	17.6 ± 0.5
PL 62	2500	4.1	10.0 ± 2.0	10.0 ± 0.6
PL 63	2700	6.0	8.0 ± 1.5	7.3 ± 0.4
PL 64	2900	8.0	6.5 ± 1.3	5.0 ± 0.35
PL 92	3650	4.0	2.5 ± 1.5	2.0 ± 0.22
PF 68	8400	16.0	8.2 ± 1.8	6.9 ± 0.32
PF 38	4750	16.0	7.5 ± 1.6	5.2 ± 0.41

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