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Dedicated to the memory of
Professor Victor-Emanuel Sahini (1927–2017)

PHYSICOCHEMICAL STUDIES ON KETOPROFEN ENCAPSULATED IN PLURONIC F127 NANOMICELLES FOR DRUG APPLICATIONS

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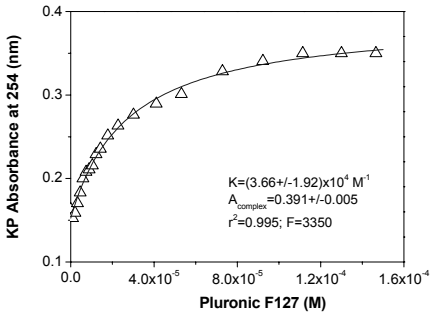
An important pharmacological property of micelles is the ability to increase the solubility of poorly water soluble drugs, which enhances their bioavailability. The goal of this study is to provide means to understanding the mechanisms by which Pluronic F127 micelles solubilize and interact with Ketoprofen (KP), a poorly soluble anti-inflammatory drug. Physicochemical methods including surface tension, dynamic light scattering and UV-visible spectroscopy have been used in this respect. The surface tension results prove that drug binds to the copolymer and promotes its micellization. Addition of KP entails increasing of the hydrodynamic radius of the Pluronic F127 micelles and is an additional proof of drug uptake. The absorption spectroscopy allows quantifying the drug-Pluronic F127 micelle interaction in terms of binding constant and Gibbs free energy of binding.



INTRODUCTION

Delivery systems such as liposomes, micro particles, nanoassociates, nanoparticle, polymer-conjugates and micelles contribute to improved transport of low therapeutic index drugs.[1] Among this various drug delivery systems, those based on synthetic polymers are considered particularly promising.[2] When water is present, amphiphilic polymers tend to self-associate creating micelles, which can be used as drug encapsulating carrier systems, ensuring the transport to specific sites of action, the minimization of drug degradation and loss, the prevention of harmful side effects, and improving the treatment efficacy.[3]

One interesting amphiphilic polymer is Pluronic F 127 (Figure 1. A). It is a triblock copolymer with a central hydrophobic block of propylene oxide flanked by two hydrophilic blocks of ethylene oxide.

Pluronic F127 has low molecular weight, is water-soluble and presents self-association ability due to relatively high critical micelle concentration (cmc). Others characteristics such as critical micelle temperature, association number, micelle size and thermodynamic properties in the dilute regime have been widely investigated.[4, 5]

Drugs solubilisation by copolymeric micelles depends on the interaction between the drug and the micellar core formed by the hydrophobic propylene oxide blocks.[6] Drugs may be solubilized in the hydrophobic core and/or in the interface of micelles. Thus, predominant location of the drug depends on its hydrophobicity and interactions with the copolymer.[7] The interactions between the

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drug molecules and the non-ionic micellar copolymeric systems can be described by the hydrophobic effect and the hydrogen bonds effect (determined by the association of drug molecules with the polyethylene glycol head groups of the copolymer).

Ketoprofen (KP) (Figure 1 B), is a poorly water-soluble (water solubility ≈ 0.13 mg·mL^{-1} at 25°C), non-steroid anti-inflammatory drugs. As most anti-inflammatory drugs, KP produces adverse effects to the human body. By incorporating the drug in a delivery system its bioavailability may be enhanced and the adverse effects reduced. Various polymers such as sodium alginate, poly lactic acid, chitosan, poly amino acids are used for the development of drug delivery systems containing nanoparticles, micelles, polymer conjugates.[8]

In this context, it is important for us to understand the behaviour of non-ionic micelles in presence of drugs. Although several general rules have been developed regarding the solubilisation of poorly-soluble drugs by micelles, the complete mechanism is not fully understood and despite the large number of publications devoted to the interaction between drugs and copolymer micelles, the results are still hazy.[9]

The goal of this study is to provide a molecular-level understanding of the interaction and solubilisation mechanism of KP molecules in Pluronic F127 aqueous micelles. The study was done by surface tension, dynamic light scattering (DLS) and UV-visible spectroscopy. The surface tension brought information about the adsorption ability at water/air interface of Pluronic F127 in the presence of KP. The micelle size of surfactant in the absence and presence of KP was evaluated by DLS. The interaction of fixed concentration of KP with Pluronic F127 was investigated by UV-visible spectroscopy and the results allowed determining the binding constant of KP and the Gibbs free energy of binding.

MATERIALS AND METHODS

All compounds were used as received. Pluronic F127 (formula EO_{100}-PO_{65}-EO_{100}, nominal molecular weight 12600 g·mol^{-1}) was provided by BASF. The molecular weight of the PO segment is 3780 and 70 %wt. of the chain is made up of EO. KP was purchased from Sigma-Aldrich. Aqueous solutions were prepared with distilled water obtained with a Simplicity UV Millipore apparatus.

Sample preparation

Pluronic F127 aqueous solutions were prepared by dissolving the copolymer in water under gentle stirring using the "cold method".[10] Solution series were prepared by dilution of a 8 ·10^{-3}M stock solution. An aqueous solution of KP was added to the Pluronic F127 solutions and the samples were sonicated for 8 h to allow incorporation of KP into the micelles. Temperature was kept at 25°C throughout all experiments. All the samples were equilibrated for at least 24 h before the measurements.

Surface tension of aqueous solutions of Pluronic F127 in the absence and presence of fixed concentration of KP were measured at 25°C by computer controlled ring method. The data were collected from a K11, Krüss tensiometer. The error of measurements was of ± 0.08%. The standard deviation of the mean never deviated ± 1.2% of the mean. The precision of the surface tension apparatus was 0.1 mN·m^{-1}.

The diameters of the Pluronic F127 micelles in absence and presence of KP were estimated by measuring their DLS with a Zeta seizer, Nano ZS (Malvern) Instruments. Analyses were performed with at 633 nm "red" laser at an angle of 173°. The hydrodynamic radii (R_h) of the micelles were obtained from the Stokes-Einstein equation (eq. 1), assuming that the aggregates were spherical and non-interacting[11]:

$$R_h = k_B T/3\pi\eta D \quad (1)$$

where k_B is the Boltzmann constant, T is the absolute temperature, η is the viscosity of the solvent, $D = \Gamma/k^2$ is the diffusion coefficient obtained from the average characteristic line width (Γ) and k^2 is the magnitude of the scattering vector.

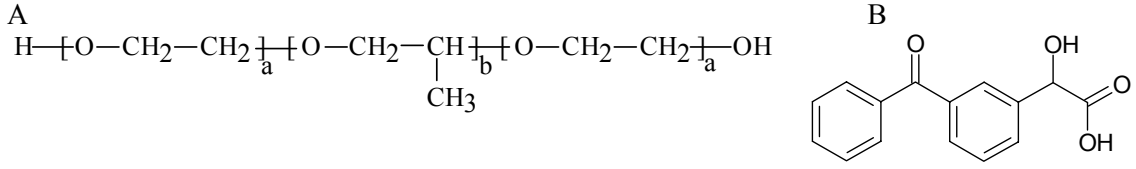

Fig. 1 – Chemical structures of (A) Pluronic F127 where a = 100 (ethylene oxide units) and b = 65 (propylene oxide units) and (B) Ketoprofen.

UV-vis spectra of aqueous KP ($1.15 \cdot 10^{-5}$ M) and KP encapsulated in Pluronic F127 micelles were recorded with a Varian-Cary 100 Bio spectrophotometer using 1 cm quartz cells, in the range 200–800 nm.

RESULTS AND DISCUSSION

1. Surface tension measurements

Surface tension measurements have been used to determine the cmc of Pluronic F127, the critical aggregation concentration (cac) and the complete uptake concentration (cuc) of KP by the surfactant micelles. The cac represents the onset of KP incorporation into the Pluronic F127 micelles, and cuc is the concentration where almost all drug molecules are solubilised in the copolymer aggregates (Table 1).

In the case of aqueous Pluronic F127 solution, a substantial decrease in surface tension even at very low copolymer concentrations is seen, reflecting the surface activity of the copolymer (Figure 2). Two breaks in the curve of surface tension against log concentration are observed. These two break points in surface tension–log concentration plots extending over a wide range of concentrations have been observed for EO-PO block copolymers, particularly at ambient temperatures and for polymers with high percentage of EO.[12] The first break is attributed to the rearrangements of the copolymer molecules at the air/water interface and the second is identified as the cmc. The first break is observed at a characteristic copolymer concentration ($2.4 \cdot 10^{-6}$M), after which the surface tension values continue to decrease until a second break appears. The further increase of copolymer concentration determines invariable surface tension. The higher concentration break in the surface tension curve corresponds to the cmc ($6.35 \cdot 10^{-4}$M) a value in agreement with the literature,[13] and signifies the formation of polymolecular micelles. The cmc is most simply defined as the concentration of copolymer at which micelles are formed. When the copolymer concentration is below the cmc, the copolymers exist in the form of unimers. However, as the copolymer reaches a concentration just above its cmc, a dynamic equilibrium is established between unimers and micelles. Then, as the concentration of Pluronic F127 continues to increase above the cmc, the number of micelles raises, while the concentration of unimers remains constant (equal to cmc).

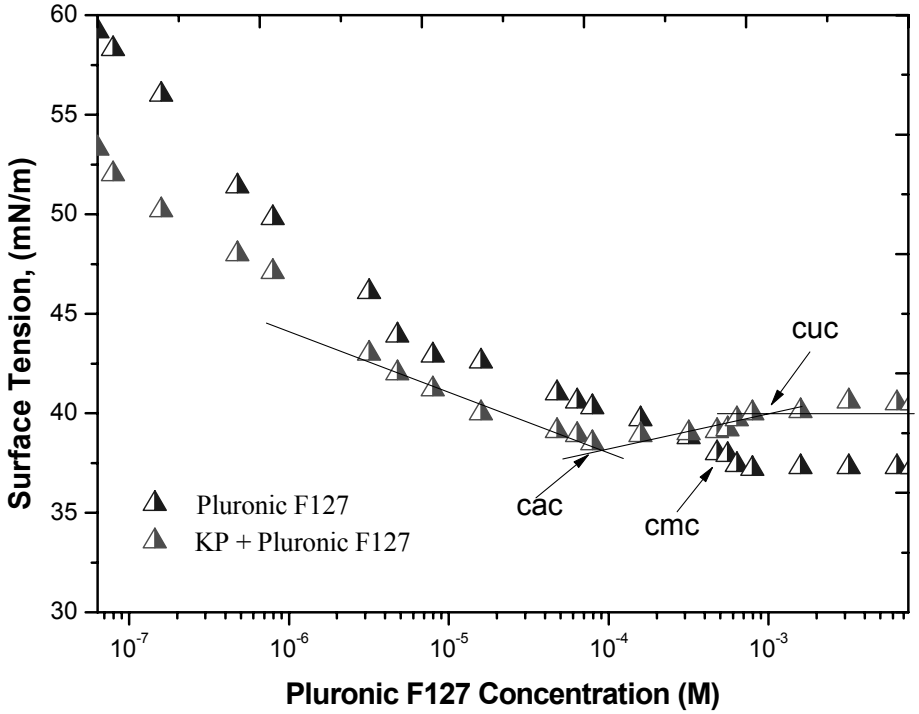

Fig. 2 – Surface tension data for aqueous solutions of (blue triangles) Pluronic F127 and (red triangles) Pluronic F127 + KP system, plotted as a function of copolymer concentration.

Table 1

Cmc, cac and cuc values for Pluronic F127 in absence and presence of KP

Compounds	**cmc (M)**	**cac (M)**	**cuc (M)**
Pluronic F127	$6.35 \cdot 10^{-3}$	-	-
Pluronic F127 + KP	-	$7.14 \cdot 10^{-5}$	$7.94 \cdot 10^{-3}$

A very interesting adsorption phenomenon observed commonly during micellization studies is the reduction of cmc upon drug solubilisation. The surface tension data in Figure 1 shows that the KP molecules alter the adsorption isotherm of the copolymer at the water/air interface. Similar adsorption behaviour was reported in the literature for copolymer Eudragit copolymer/Flurbiprofen mixture[14] and polymer/surfactant complexes.[15] Several distinct regions in the surface tension graphs of the Pluronic F127/KP mixture are delimited between the break points. The drug lowers the surface tension (below that of the polymer alone) until the first break point (cac) is reached on the adsorption isotherm. In this particular case, cac is below cmc. It represents the concentration at which the drug starts to be solubilized into the surfactant micelles. Below the cac, the drug molecules are hydrophobic and form complexes with amphiphilic Pluronic F127 unimers at the water/air interface. However, above the cac, a slight increase in surface tension isotherm occurs. The increase in surface tension is caused by desorbing some of the drug molecules and/or polymer/drug complexes from the water/air interface to bulk. This increase takes place until the second break point (cuc) of the Pluronic F127/KP adsorption isotherm. The third region corresponds to the concentrations at which almost all drug molecules are loaded in the micelles. A similar behaviour was observed for the Pluronic /Flurbiprofen system.[16]

2. Dynamic light scattering measurements

The encapsulation of KP into Pluronic F127 micelles was verified by DLS measurements, by determining the micelle hydrodynamic radii (R_h) in the absence and presence of KP at two different polymer concentrations. The variation of the R_h of the Pluronic F127 micelles and of the Pluronic F127/KP is presented in Table 2. For the Pluronic F127 of $1.59 \cdot 10^{-3}$M, the R_h was of 1.51 nm. When KP was introduced in the micellar system, the R_h increased to 2.02 nm. This proves that the drug has been encapsulated inside the micelle. By increasing the copolymer concentration, the empty micelle dimension increases to 2.13 nm. KP addition does not significantly affect the micelle dimension (R_h = 2.05 nm). This is because at high copolymer concentration the drug is solubilised in a larger number of micelles.

Table 2

The Pluronic F127 micelle hydrodynamic radii (R_h) in the absence and presence of KP (*Pdi – polydispersion index)

Samples	R_h(nm)	Pdi*
Pluronic F127 ($1.59 \cdot 10^{-3}$M)	1.51	0.25
Pluronic F127($1.59 \cdot 10^{-3}$M) + KP	2.02	0.34
Pluronic F127 ($3.9\ 10^{-3}$M)	2.13	0.28
Pluronic F127 ($3.9\ 10^{-3}$M)+ KP	2.05	0.32

3. UV-vis spectroscopy

The absorption measurements were used to quantify the binding constant, K_b, of the drug-Pluronic F127 complex. The variation of absorbance at the characteristic wavelength of 254 nm for KP as a function of Pluronic F127 concentration is presented in Figure 3. The symbols in Figure 3 represent the experimental data and the full line is the result of nonlinear fitting using Eq. (2):

$$A \quad \frac{A_0 + A_b K_b [\text{Pluronic F127}]}{1 + K_b [\text{Pluronic F127}]} \qquad (2)$$

where, A is the measured absorbance, A_0 is the absorbance of the drug in the absence of surfactant, A_b is the absorbance of the drug bound to copolymer micelles, and [Pluronic F127] is the working molar concentration of polymer. This equation allowed the best fitting in obtaining K_b when the concentration of the bound drug is smaller than its initial concentration. [17]

The absorbance increases with surfactant concentration up to a concentration of approximately $1.2 \cdot 10^{-5}$M, marking the encapsulation of drug in Pluronic F127 micelles. Above $1.2 \cdot 10^{-5}$M, the absorbance seems to reach a limiting value and becomes almost constant showing that the micelles were saturated with KP.

Thermodynamically, KP interaction with polymer molecules is characterized by the binding constant and the free energy of dye-polymer complex formation, ΔG_b^0. In this context, the interaction has been evaluated at constant KP concentration and increasing polymer concentration. The values of absorbance have been used to calculate the binding constant (K_b) by non-linear regression (full line in Figure 3) assuming a 1:1 interaction between KP and the copolymer micelle, and using equation (2).[17] The results are presented in Table 3.

The Gibbs free energy of binding of KP to copolymer micelles, ΔG_b^0, can be obtained using equation (3):

$$G_b^0 \quad -RT\ln K_b \qquad (3)$$

where R is the gas constant and T the absolute temperature. The value obtained for K_b is of 2.92×10^4 M^{-1} and for ΔG_b^0 of -22.47 kJ·mol^{-1}. The value of binding constant is in the range of the solubilisation constants of other drugs in copolymer micelles.[17] The value obtained for ΔG_b^0 confirms the formation of a stable complex of dye-polymer which consumes a low amount of energy.

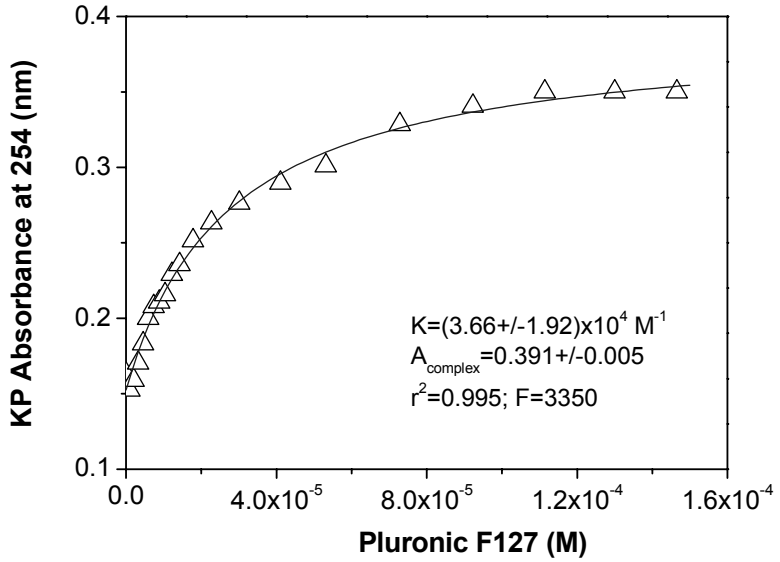

Fig. 3 – The variation of KP absorbance at 254 nm *vs*. Pluronic F127 concentration. [KP] = 1.15×10^{-5} M. The symbols are experimental points. The curve is the nonlinear fitting using Eq. (2).

Table 3

Binding constant (K_b), the Gibbs free energy of binding (ΔG_b^0) and the absorbance of KP bound to Pluronic F127 (A_b)

Sample	K_b(M^{-1})	ΔG_b^0 (kJ·mol^{-1})	A_b(complex)
Pluronic F127/KP complex	$(2.92 \pm 0.26) \cdot 10^4$	-22,47	0.391±0.005

CONCLUSIONS

This study provides valuable insights regarding Ketoprofen encapsulation in Pluronic F127 nanomicelles by surface tension, DLS and UV-visible spectroscopy. The surface tension results unveil that Pluronic F127 forms hydrophobic associates which are able to solubilise the KP. DLS data confirms the encapsulation of KP in copolymer micelles. The UV-visible data and the applied physical model allowed computing the binding constant of drug to the Pluronic F127 micelles. Furthermore, the quantification of the drug-Pluronic F127 micelle interaction in terms of binding constant and Gibbs free energy of binding proved that a stable drug-polymer complex is formed.

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