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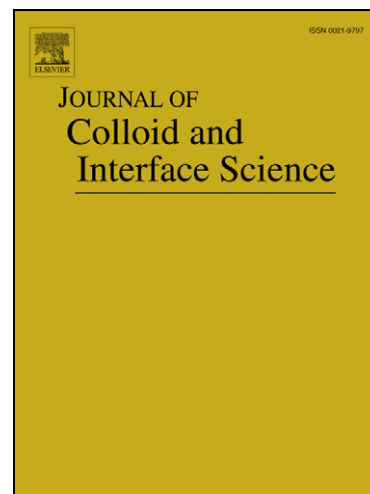
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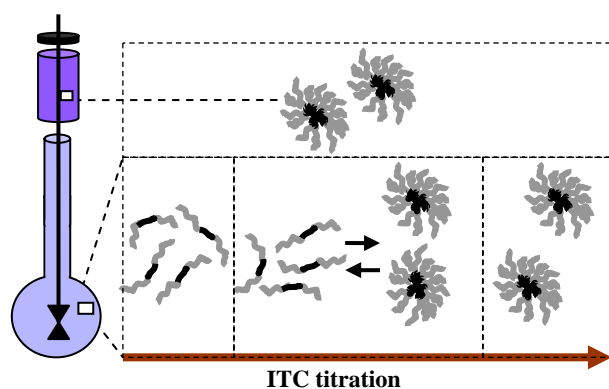
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## Graphical abstract



In the present work ITC was successfully used as a sensitive research tool for examining the effect of temperature and propanediol-1,2 on micellization behavior of Pluronic F127 by determining the CMC and thermodynamic parameters of the micellization ( $\Delta H_{\text{mic}}$ ,  $\Delta G_{\text{mic}}$ ,  $\Delta S_{\text{mic}}$ , and  $\Delta C_p$ ).

**A concise analysis of the effect of temperature and propanediol-1,2 on  
Pluronic F-127 micellization using isothermal titration microcalorimetry**

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**Abstract**

1  
2 This article aims to explore the possibility to use isothermal titration microcalorimetry (ITC) for  
3 the investigation of F127 micellization and to study the effect of temperature and addition of  
4 propanediol-1,2 on F127 micellization behavior. From this work, ITC proved efficient to be  
5 used as a tool for the determination of the critical micellization concentration (CMC) and the  
6 enthalpy of micellization ( $\Delta H_{mic}$ ) of F127, from which the other thermodynamic parameters  
7 were calculated (free energy ( $\Delta G_{mic}$ ), entropy ( $\Delta S_{mic}$ ) and heat capacity of micellization  
8 ( $\Delta C_{p,mic}$ )). The micellization of F127 was confirmed to be a strongly endothermic process with  
9 predominance of hydrophobic interactions ( $T\Delta S_{mic} > \Delta H_{mic}$ ) and the correlation of enthalpy and  
10 entropy of micelle formation exhibits an excellent linearity. The temperature dependence of  
11 F127 micellization was revealed by using ITC, since the CMCs values were respectively 0.197,  
12 0.095, 0.085 and 0.079mM for temperatures 28, 29, 30 and 31°C. Secondly, by the addition of  
13 propanediol-1,2 to the micellization medium containing 1.187mM of F127, the CMC was  
14 shifted to lower values (0.095, 0.081, 0.077, 0.069 and 0.066mM respectively for propanediol-  
15 1,2 concentrations of 0, 1.4, 2.3, 2.8 and 3.7% w/v in the micellization medium). Finally, ITC  
16 was used as diagnostic tool in the aim to check the reproducibility of the experiments  
17 independently on the kinetic and the dynamic aspects related to the micelle formation-breakup.  
18 However, in this work we proved that the use of ITC for the determination of the CMC and  
19 thermodynamic parameters associated to F127 micellization is limited to a range of temperatures  
20 when sigmoidal curves were obtained.  
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38 **Keywords:** Pluronic F127, Poloxamer 407, block copolymer, isothermal titration  
39 microcalorimetry, critical micellization concentration, micellization, thermodynamics,  
40 propanediol-1,2, dynamics  
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## Introduction

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3 The thermosensitive properties of pharmaceutical formulations based on (ethylene  
4 oxide)<sub>97</sub>(propylene oxide)<sub>69</sub>(ethylene oxide)<sub>97</sub> block copolymers known under the generic name  
5 of poloxamer 407 and the trade name of Pluronic F127, are of utmost importance in  
6 pharmaceutical formulation. Indeed, under appropriate concentration conditions in aqueous  
7 medium these systems are fluid at room temperature (25°C) facilitating their administration (for  
8 instance, via syringes), and in the form of gel above sol-gel transition temperature promoting  
9 prolonged release of active drugs at body temperature (37°C). Thermosensitive and  
10 mucoadhesive gels based on Pluronic F127, hydroxypropylmethylcellulose and propanediol-1,2  
11 were recently developed by Koffi and co-workers [1,2] and appeared to be interesting systems  
12 for the rectal administration of quinine. Despite the fact that extensive works have been done  
13 concerning the rheological characterization and the evaluation of biological effects of these  
14 ternary systems, physico-chemical evaluation of diluted formulations by the investigation of  
15 micellization is still lacking. Since micellization represents the very first step in the gelation  
16 process, the characterization of the micellization under variable parameters such as temperature  
17 and addition of propanediol-1,2 in the micellization medium would represent an original  
18 strategy to understand the parameters which govern the thermoreversible property of this  
19 system.  
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36 The process of micellization of Pluronics can be induced by increasing the block copolymer  
37 concentration to be above the critical micellization concentration (CMC) (i.e. the concentration  
38 at which the micelle formation starts in solution) and/or adjusting the temperature to exceed the  
39 critical micellization temperature (CMT) (i.e. the copolymer solution temperature at which the  
40 micelle formation starts). The CMC of Pluronics is a widely studied phenomenon: it has been  
41 determined using various techniques such as surface tension measurements [3], Fourier  
42 transformed infrared spectroscopy [4,5], <sup>1</sup>H-NMR relaxation studies [6,7], dynamic light  
43 scattering, fluorescence spectroscopy [8] and differential scanning microcalorimetry which  
44 allows to determine the enthalpy change related to the micellization process from integration of  
45 the heat capacity versus the temperature [9,10]. These experiments also provide the onset  
46 temperature for micellisation *i.e.* the CMT. Nowadays, isothermal titration microcalorimetry  
47 (ITC) is the only technique capable of measuring the critical micellization concentration and the  
48 enthalpy of micellization ( $\Delta H_{mic}$ ) of a surfactant from a single experiment without the necessity  
49 of any probe [11-21]. Other thermodynamic parameters related to the micellization namely the  
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1 free energy ( $\Delta G_{\text{mic}}$ ), the entropy ( $\Delta S_{\text{mic}}$ ) and the heat capacity ( $\Delta C_{\text{p,mic}}$ ) of micellization can be  
2 calculated from the experimentally determined CMC and  $\Delta H_{\text{mic}}$  [11-21].  
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5 Considering the wide use of ITC for the characterization of micellar-based systems [22], there  
6 are unfortunately only very sparse data for the use of this technique to investigate Pluronic F127  
7 micellization. Indeed, the only study performed on Pluronic F127 deals with its association with  
8 sodium dodecyl sulphate [23]. In this context, the first purpose of the present work was to  
9 explore the possibility to use ITC for the characterization of micellization behavior of Pluronic  
10 F127 (CMC,  $\Delta H_{\text{mic}}$ ,  $\Delta G_{\text{mic}}$ ,  $\Delta S_{\text{mic}}$  and  $\Delta C_{\text{p,mic}}$ ) and to extract advantages of this technique for the  
11 studied system. Secondly, micellization of F127 was investigated under variable parameters  
12 such as temperature and addition of propanediol-1,2 in the micellization medium. Finally, the  
13 reproducibility of ITC experiments was checked by studying successively (i) the effect of the  
14 method of preparation of F127 micellar solution (ii) the duration between the preparation of the  
15 micellar solution and ITC experiments, and (iii) the effect of the time interval between two  
16 injections during ITC experiment on the CMC and thermodynamic parameters of the  
17 micellization.  
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## 31 2. Experimental section

### 32 2.1. Materials

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38 The Poloxamer 407 (European Pharmacopea 5<sup>th</sup> Edition 5.6) available with the trade name of  
39 Lutrol F 127 and Pluronic F127 (pharmaceutical grade), was a gift from BASF and was used  
40 without further purification. According to the supplier, the weight average molecular weight  $M_w$   
41 is about 13,500 g.mol<sup>-1</sup> and the number average molecular weight  $M_n$  is about 9,300g.mol<sup>-1</sup>  
42 (from gel permeation chromatography). Previous studies on poloxamers have shown that these  
43 copolymers which are synthesized via anionic polymerization are in fact heterogeneous in  
44 composition and may contain for instance some diblock components [24,25]. This variability in  
45 composition depends on the supplier. As many other authors working on poloxamers we chose  
46 to study a pharmaceutical grade sample without further purification in order to remain close to  
47 actual use of this copolymer in the pharmaceutical industry. Propanediol-1,2 was purchased  
48 from Prolabo, Paris, France.  
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### 60 2.2. Preparation of the F127 solutions

1 In order to achieve ITC experiments, diluted solutions are required. The Pluronic F127 aqueous  
2 solutions were prepared according to two protocols:  
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6 In the first one (denoted protocol 1), F127 solutions were prepared from the volumic dilution in  
7 MilliQ<sup>®</sup> water of F127 gels at a concentration of 16% w/w. These gels were prepared by weight  
8 according to the so-called “cold method” by using a mixer equipped with a turbine adapted to  
9 the mixing of viscous preparations (Rayneri-turbotest, Rayneri, France). For the preparation of  
10 the gels, the F127 powder was gradually added under agitation (1000 rpm) at 4°C to a liquid  
11 phase which consisted in propanediol-1,2 aqueous solutions at different concentrations (0, 15,  
12 25, 30, 40% w/w). After complete dissolution of F127, each Pluronic gel was equilibrated  
13 overnight at 4°C [1,2]. The obtained gels were then diluted with water and the final proportions  
14 of propanediol-1,2 in the resulting solutions were (0, 1.4, 2.3, 2.8 and 3.7% w/v). In this case,  
15 the final concentration of F127 solution was 1.5% w/v (1.187mM).  
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26 In the second protocol (denoted protocol 2), solutions of F127 (1.187mM) were directly  
27 prepared by the dissolution of the corresponding weight of F127 powder in propanediol-1,2  
28 aqueous solutions at different concentrations (0, 1.4, 2.3, 2.8 and 3.7% w/v). The dissolution of  
29 F127 powder was achieved by using a mixer equipped with a turbine (Rayneri-turbotest,  
30 Rayneri, France).  
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### 37 **2.3. Isothermal Titration Microcalorimetry**

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40 An isothermal calorimeter (ITC) (MicroCal Inc., USA) was used for determining from a single  
41 titration curve simultaneously the CMC and  $\Delta H_{mic}$ . The ITC instrument was periodically  
42 calibrated either electrically using an internal electric heater, or chemically by measuring the  
43 dilution enthalpy of methanol in water. This standard reaction was in excellent agreement (1-  
44 2%) with MicroCal constructor data.  
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52 Typically, aliquots of 5 $\mu$ l of F127 solution were delivered over 20s and the corresponding heat  
53 flow was recorded as a function of time. The time interval between two consecutive injections  
54 was 400s and agitation speed was 394 rpm along all the experiment. Using the interactive  
55 software, an injection schedule was automatically carried out by setting the number of  
56 injections, volume of each injection, and time between each injection. Each titration was  
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performed three times to ensure reproducibility of the results. The reported values are the mean  $\pm$  standard deviation of the three experiments. Data analysis was carried out using Microcal ORIGIN 7 software.

The micellization of F127 was studied by varying two parameters namely the temperature and the proportion of propanediol-1,2 in the micellization medium. A couple of experiments was hence achieved:

- To study the effect of temperature on micellization behavior, the experiments were performed by using a solution of F127 (1.187mM) in MilliQ<sup>®</sup> water at constant temperature throughout titration. A series of experiments were conducted by varying the temperature for each titration from 5 to 60°C.
- To evaluate the effect of propanediol-1,2 on F127 micellization, the injecting syringe was filled by solutions of F127 at a fixed concentration of 1.187mM containing propanediol-1,2 at different concentrations (0, 1.4, 2.3, 2.8 and 3.7% w/v). The measurement cell contains a solution of propanediol-1,2 in the same proportion as the F127 solution in the syringe. The temperature was fixed at 29°C for all these experiments.

Finally, to check the reproducibility of ITC results a series of experiments were conducted:

- Comparison between micellization experiments of F127 solutions (1.187mM) obtained according to protocols 1 and 2 detailed in section 2.2.
- Comparison between the micellization experiments of F127 solutions (1.187mM) immediately diluted from gels according to protocol 2 and the same solutions after 12 months of storage.
- By varying the time interval between two injections of F127 solution at 1.187mM prepared according to protocol 1 (300, 400, 500 and 600s).

### 3. Results and discussion

On injection of a small amount of F127 micellar solution into water contained in the sample cell, there was a characteristic exothermic heat flow effect as shown in Figure 1.A. After every new injection of F127 solution the heat flow effect was smaller since the concentration difference between the two solutions, in the syringe and in the sample cell, was smaller. Integration of each peak versus time allowed to obtain the curve of the heat as a function of F127 concentration in

the sample cell (Figure 1.B), from which the enthalpy of micellization and the CMC were determined. In many works, results were expressed in terms of the enthalpy of demicellization ( $\Delta H_{\text{demic}}$ ) [11-13], or in terms of enthalpy of micellization ( $\Delta H_{\text{mic}}$ ) [14-16]. Since results obtained from these two considerations are similar ( $\Delta H_{\text{mic}} = -\Delta H_{\text{demic}}$ ), we suggest that it is thermodynamically more significant to comment results expressed by the enthalpy of micellization which is, here, the effective studied phenomena.

### 3.1. Analysis of the enthalpograms

After the dilution of the Pluronic F127 micellar solution in water and according to the temperature of the interaction, two types of enthalpograms were obtained: sigmoidal curves which were observed for temperatures ranging between 28 and 31°C (301-304 K) (Figure 1) and non sigmoidal curves which were obtained for temperatures above 304 K (31°C) (Figure 2. A and B). According to the type of curve obtained, two methods were used for the analysis of the enthalpograms:

*Method 1.* When sigmoidal curves were obtained, the CMC corresponded to the concentration where the first derivative of the curve in Figure 1.B displayed a maximum as presented in Figure 1.C. The heat of micellization  $\Delta H_{\text{mic}}$ , was equal to the enthalpy difference between the two extrapolated lines in Figure 1.B [3,16,21,23,26-28].

*Method 2.* Curves that did not present clear sigmoidal features were analyzed by a data treatment analogous to the one suggested by many authors [12,21,23,29]. Linear fits of the data sets in the lower and upper concentration domains were performed. Figure 2.B shows how to determine the ST (start of transition) and ET (end of transition) corresponding respectively to the start and the end of micellization process. The intercepts of the two straight lines were determined and the difference between the two intercepts yielded the enthalpy of the micellization.

### 3.2. Calculation of the free energy ( $\Delta G_{\text{mic}}$ ), entropy ( $\Delta S_{\text{mic}}$ ) and heat capacity of micellization ( $\Delta C_{\text{p mic}}$ ) of F127

The free energy of micellization,  $\Delta G_{mic}$ , corresponding to the standard free energy change for the transfer of one amphiphilic molecule from solution to the micellar phase, in the absence of electrostatic interactions (the PEO-PPO-PEO copolymers are non-ionic), is given by the expression:

$$\Delta G_{mic} = RT \ln(CMC') \quad (\text{Eq.1})$$

where R is the gas constant ( $8.314 \text{ J.K}^{-1}.\text{mol}^{-1}$ ) and T is the absolute temperature of the experiment. In this equation, CMC' is the critical micellization concentration expressed in mole fraction units. The change in entropy  $\Delta S_{mic}$  for the F127 micellization was obtained based on the second law of thermodynamics by using the Gibbs-Helmholtz equation:

$$\Delta S_{mic} = \frac{\Delta H_{mic} - \Delta G_{mic}}{T} \quad (\text{Eq.2})$$

The thermal heat capacity of micellization  $\Delta C_{p,mic}$  could be evaluated from the following relationship:

$$\Delta C_{p,mic} = \left( \frac{\partial \Delta H_m}{\partial T} \right)_p \quad (\text{Eq.3})$$

### 3.3. Effect of temperature on the micellization of F127

- CMC,  $\Delta H_{mic}$ ,  $\Delta G_{mic}$

This study was performed on F127 solution prepared according to protocol 1. Upon temperature increase three different types of dilution enthalpograms were obtained.

(a) At low temperatures, from 5 to 27°C, very weak enthalpy was observed as in Figure 2.C and D. This type of curve was obtained in either of the two following situations: (i) if the temperature was below the CMT of the poloxamer solution in the syringe; then the weak enthalpy effect was solely due to the dilution of the unimers in the measurement cell. According to the literature, the CMT values of F127 solution at concentrations of 1 and 2% were 21.9 and 23.1°C respectively [24] indicating that the F127 at a concentration of 1.5% (1.187mM) was in the form of unimers below this range of temperatures. (ii) if the temperature of the experiment was above the CMT of the F127 solution in the syringe (around 23°C) the observed enthalpy corresponded to the breakup of the micelles to unimers (demicellization) and to the dilution of

1 the resultant unimers, the final concentration of the Pluronic F127 molecules in the measurement  
2 cell remaining below their CMC throughout the experiment. Indeed, the concentration of F127  
3 in the measurement cell is varied from 0 to 0.207 mM (abscissa axis in figure 2.D) during the  
4 ITC experiment and we suggest that the CMC in the measurement cell was not reached for  
5 temperatures below 27°C. This suggestion is in accordance with the literature since the CMT  
6 values of F127 at a concentration of 0.198 mM was 28°C [30].  
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11 (b) For temperatures ranging between 28 and 31°C, the injected Pluronic solution was then  
12 clearly above its CMT. As a consequence, the CMC was reached in the measurement cell during  
13 titration. In this case, sigmoidal enthalpograms were obtained as shown in Figure 1.B. Each  
14 enthalpogram could be divided into three concentration ranges denoted (1), (2) and (3) reflecting  
15 different molecular organizations in the measurement cell (Figure 1.B). Step (1) corresponded to  
16 the first few injections: the final concentration of Pluronic F127 molecules in the measurement  
17 cell remained below their CMC and the strong enthalpic effects (in absolute values) was due to  
18 the breakup of the micelles to unimers and to the dilution of the resultant unimers. In step (2) if  
19 more micellar solution of F127 was added to the sample cell, a clear decrease of the absolute  
20 values of the heat effects was observed indicating that the micelles of the added micellar  
21 solution did not all breakup and the critical micellar concentration was reached. In step (3) when  
22 more micellar F127 solution was added above the CMC, the micelles did no longer dissociate in  
23 the measurement cell and the injected micellar solution was diluted in the measurement cell  
24 leading to an observed heat of micelle dilution. The CMC of F127 was determined according to  
25 method 1 of the analysis of the enthalpograms (CMC: 0.197, 0.095, 0.085, 0.079 mM for  
26 temperatures 28, 29, 30 and 31°C respectively) (Table 1). These values obtained from ITC  
27 experiments are perfectly in agreement with data reported in other works using other techniques.  
28 For example, the CMT of F127 solutions at concentrations of 0.079 mM and 0.198 mM were  
29  $31\pm 1^\circ\text{C}$  and  $28\pm 1^\circ\text{C}$  respectively determined by probe spectroscopy [30].  
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49 (c) The same experiments achieved at higher temperatures (from 32 to 60°C) led to curves  
50 which did not exhibit the previous sigmoidal shape. The non-sigmoidal shapes obtained showed  
51 an abrupt change in the heat of micellization indicating that the association of unimers into  
52 micelles occurred at very low concentrations of F127 in the measurement cell attained from the  
53 very first injections of the copolymer solution. If more micellar F127 solution was added beyond  
54 the aggregation concentration, the micelles no longer dissociate and the observed heat was due  
55 to the dilution of micelles. In this temperature range only the previously described steps 2 and 3  
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1 can be observed during the experiment. For the range of temperatures from 32 to 40°C,  $\Delta H_{mic}$   
2 was obtained according to the second method described for the analysis of the enthalpograms.  
3 This method was applied for the determination of ST and ET for many amphiphiles, when non-  
4 sigmoidal curves were obtained [12,21,23,29]. Unfortunately, this method did not give a precise  
5 estimation of these two parameters for the studied system. Indeed, for higher temperatures (45-  
6 60°C) the determination of  $\Delta H_{mic}$  became inaccurate or even impossible. Furthermore, when  
7 non-sigmoidal curves are obtained, the CMC can not be calculated. Only ST and ET providing a  
8 rough estimation of the start and the end of the micellization can be determined in these cases  
9 (Table 2).

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18 In the present work, ITC was proved to be efficient for the determination of the CMC and the  
19 thermodynamic parameters associated to the micellization of F127 only when sigmoidal curves  
20 are obtained. The calorimetric traces obtained upon addition of F127 at a concentration of  
21 1.187mM in water allowed the determination of the  $\Delta H_{mic}$  which was found positive  
22 independently on the temperature of the experiment (Table 2), indicating that the transfer of  
23 unimers from solution to the micelles is an endothermic process. This is in agreement with  
24 previously reported results on the micellization of Pluronics [4,5,21,24,30,31]. From the  
25 sigmoidal curves obtained in the temperature range from 28 to 31°C, it was possible to follow  
26 the temperature dependence of F127 micellization process. From the results in Table 1, evidence  
27 that the increase of the temperature generates a decrease of the CMC of F127 as has been found  
28 previously by other techniques than ITC [32]. The increment of temperature resulted in a slight  
29 decrease of the  $\Delta G_{mic}$  (-20.90 to -23.38 kJ.mol<sup>-1</sup> for temperatures ranging from 28°C to 31°C  
30 respectively) indicating that the micellization is more spontaneous when the temperature of the  
31 experiment is increased.  
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#### 45 • **Molecular mechanism of the effect of the temperature on F127 micellization**

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49 A great deal of efforts has been devoted to explain how Pluronic block copolymers micelles are  
50 formed and to describe the molecular mechanism by which the temperature affects the  
51 micellization. Whatever the temperature, when the concentration of Pluronics is above the  
52 CMC, the poly(propylene oxide) (PPO) segments are preferentially located in the core of the  
53 micelles, whereas the strongly hydrated poly(ethylene oxide) (PEO) segments are primarily  
54 located in the outer region of the micelles [9,24,30,33,34].  
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1 Small-angle neutron scattering (SANS) has been used to study the temperature dependence of  
2 the structure of PEO-PPO-PEO block copolymer micelles [35-38]. It was shown that the  
3 micellar core cannot be composed of PPO segments only but must contain significant quantities  
4 of water.  
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8 It is well understood that there exists hydrogen bonding between water and surfactant molecules.  
9 While the temperature increases, some of the existing hydrogen bonds would rupture due to  
10 thermal fluctuation, which makes the surfactant molecules more hydrophobic [39]. On  
11 increasing the temperature, hydrophilicity of the surfactant molecules decreases owing to the  
12 smaller probability of hydrogen bond formation at higher temperatures. Water content in the  
13 micellar core decreases and the micellization is favored resulting in a decrease of the CMC.  
14 Furthermore, the increase in temperature causes the decrease in hydration of the hydrophilic  
15 POE and POP groups which favours micellization [39]. The endothermic nature of the  
16 micellization for Pluronic surfactants arises from the desolvation of the PPO groups. Indeed,  
17 water of solvation, which adopts an ice-like structure around the PPO groups, is lost as the  
18 temperature is increased [40].  
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30 For the range of temperatures varying from 28 to 31°C, the increase of the temperature led to an  
31 increase of the enthalpy of micellization (Table 1). It should be noted that the heat of  
32 micellization for pluronic triblock copolymers also increases, as expected along with an increase  
33 of temperature in literature [21,24,41]. The determination of the temperature dependence of  
34  $\Delta H_{mic}$  actually helps to decide whether more hydrophobic surface is exposed to water when the  
35 aggregates associate into micelles. In the case of surfactants, the heat capacity of micellization  
36 has been found to be proportional to the hydrophobic surface area of amphiphiles that become  
37 excluded from water through micellization. In other words, the increase of temperature results  
38 on a decrease of the exposed hydrophobic surface area of the F127 and an increase of  $\Delta C_{p,mic}$   
39 observed in Table 1.  
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50 The entropy of micellization was found positive and it increased significantly with temperature.  
51 Our findings obtained using ITC are in complete agreement with the results of studies on the  
52 micellization of Pluronics determined by using probe spectroscopy [30], surface tension,  
53 differential scanning calorimetry [24] and high-sensitivity differential scanning calorimetry  
54 experiments [42].  
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From the comparison of entropy and enthalpy of micellization, we can conclude that the major driving forces for F127 micelle formation are the hydrophobic interactions because  $T\Delta S_{mic} > \Delta H_{mic}$ : the micellization process of F127 turns to be entropy-governed. The hydrophobicity of both PPO and PEO segments increases with temperature, which leads to the packing of more PPO segments in the core, expulsion of water from the micellar core and shrinking of PEO [35-37]. According to Liu et al [43], the positive  $\Delta S_{mic}$  values are due to the destruction of the ordered hydrogen bounded water structure in the vicinity of the polymer chain. A large gain in entropy occurs when water molecules in hydration shells around the hydrophobic parts of the unimeric amphiphiles are released during the micellization process resulting in an increase of the degree of freedom.

- **Enthalpy-entropy compensation**

The micellization process of F127 has been shown to exhibit enthalpy-entropy compensation phenomenon (figure 3). The linearity of the plot ( $r^2=0.9997$ ) indicates that for the aggregation process, changes in  $\Delta H_{mic}$  are compensated by changes in  $\Delta S_{mic}$ . The slope of the correlation line corresponds to the correlation temperature ( $T_c$ ) which characterizes solvent-solute interaction [44]. The compensation temperature  $T_c$  is 298.08 K. This value is very close to that obtained for pluronics F88 and 68 (294.9 K) [45] but slightly larger than the one obtained by other research work [42] (281.9 K) for 12 Pluronics. Noteworthy that the linear correlation between enthalpy and entropy change have often been observed in studies of isothermal process where, typically, the temperature, or the composition of the solvent is varied in a systematic way [42,43,45-47].

### **3.4. Effect of propanediol-1,2 on the micellization of F127**

From the results in Table 3, it can be clearly seen that the presence of propanediol-1,2 at concentrations from 1.4 and 3.7% w/v in the micellization medium led to driving the micellization boundary toward lower concentrations as exemplified by a decrease of the CMC and more negative values of  $\Delta G_{mic}$  in presence of propanediol-1,2. Concerning the other thermodynamic parameters, both  $\Delta H_{mic}$  and  $\Delta S_{mic}$  appear to decrease monotonically with an increase of propanediol-1,2 concentration.

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These variations in the CMC and the thermodynamic parameters are expected because it was demonstrated by previous works that the presence of an organic co-solvent alters dramatically the manner in which the surfactant self-assembles to form micelles [48-52]. Many research works reported that the solvent tends to interact with surfactant and may alter the structure of the micelle. Several mechanisms explaining the effect of the glycol- and polyol-type solvent into poloxamer micellization have been reported into the literature. Kabalnov et al [53] suggested that the polyol, which is depleted from a surfactant layer, dehydrates the layer by an osmotic effect. Studies on the effect of polyols (e.g., glycerol, propanediol-1,2, butanediol-1,3) on the phase behavior and microstructure of alkyl-oligo(ethylene oxide) ether surfactants using small-angle X-ray scattering have been reported [54,55]. The observed modification of micelles structure induced by polyols has been explained by dehydration of the POE chain of the surfactants and corresponding decrease of the surfactant hydrophilicity upon the addition of polyols. It was reported that the propanediol-1,2 is considered as PPO-resembling glycol [56]. Indeed, propanediol-1,2 has a preference to locate in the relatively apolar PPO-rich domains and participate together with the block copolymer in the formation of the interface between the PEO-rich and the PPO-rich domain. It was also demonstrated that propanediol-1,2 is responsible on the swelling of both PEO and PPO blocks [57].

As the CMC of F127 is smaller when the concentration of propanediol-1,2 is increased, one would expect  $\Delta H_{mic}$  to be more positive. As exactly the opposite variation of the enthalpy is observed, one explanation of this result is that the inter-molecular hydrogen bonds between water and propanediol-1,2 present an increasing contribution when the concentration of propanediol-1,2 is increased. The hydrogen bonds could also be responsible of a lower degree of freedom and in turn a lower value of  $\Delta S_{mic}$ .

### 3.5. Reproducibility of the experiments under variation of experimental parameters related to the dynamics of F127 micelles

It is now accepted that when an equilibrated, concentrated polymer micelle solution is added to water, a new equilibrium might be established sufficiently rapidly on the time scale of the experiment. Nevertheless, in some cases, the process of micelle breakup is extremely slow or “frozen”, with the systems taking days, months or even longer times to reach equilibrium, after being submitted to a perturbation that affects micelles [58-60]. In these situations the micellar systems stay in a non-equilibrium state because the kinetic of any equilibration process is frozen

1 at the time scale of the experiment. In this part of study, ITC was used as diagnostic tool in the  
2 aim to check the reproducibility of the experiments independently on the kinetic and the  
3 dynamic aspects related to the micelle formation-breakup.  
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6 In the case of kinetically frozen polymeric micelles, the enthalpogram would represent only the  
7 heat of dilution of the micelles whatever the temperature of the ITC experiment. In the present  
8 study, the sigmoidal curves obtained upon the dilution of the F127 solution (Figure 1)  
9 demonstrated that the micelles are in equilibrium and that both the demicellization upon dilution  
10 and re-equilibration processes of F127 micelles are sufficiently fast compared to the time  
11 between two injections. The time interval between 2 injections (300, 400, 500 and 600s) has no  
12 significant influence on the results of the CMC and the thermodynamic parameters related to the  
13 micellization of the F127 (Table 4).  
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23 Finally, in this part of the work, special care was taken to investigate the effect of the solution  
24 preparation method on the micellization behavior of F127. In general, the dynamic structure of  
25 micelles depends on the method of preparation. When a solid polymer is directly dissolved in  
26 water, micellization equilibrium can be established reasonably rapidly. However, in some  
27 micellar solutions, a system will reach equilibrium within experimentally reasonable time scales  
28 (a few hours). In this case, micellar solutions might be annealed by simple standing at room  
29 temperature over long periods of time (several days) [12]. For this reason, two protocols for the  
30 preparation of F127 solution were assessed in the present work. The first one consisted on the  
31 dilution of the gels and the second one consisted on the direct preparation of the solution by  
32 dissolving F127 solid powder into water. The results demonstrated that the method of  
33 preparation of the micellar solution has no significant effect on the CMC and  $\Delta H_{mic}$  values  
34 (results not shown). Finally, the results obtained immediately after the preparation of the F127  
35 micellar solution are not significantly different from those obtained after 12 months of storage  
36 indicating that thermodynamic equilibrium was reached rapidly after the preparation of the  
37 micellar solution.  
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#### 51 **4. Summary and Conclusions**

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53 This work proved that Isothermal titration microcalorimetry can be used as a sensitive research  
54 tool for examining the micellization behavior of Pluronic F127 by determining the CMC and  
55 thermodynamic parameters. It was demonstrated that the formation of block copolymer micelles  
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1 is dependent on the temperature and the composition of the medium resulting in a decrease of  
2 the CMC upon a small increase in temperature and by the addition of propanediol-1,2. The  
3 micellization behavior of F127 was independent on the method of preparation of the F127  
4 micellar solution and on the interval time between 2 injections. Whatever the experimental  
5 conditions and medium composition, micellization process of F127 is spontaneous and turns to  
6 be entropically driven. Nonetheless, the use of ITC for the determination of the CMC and the  
7 thermodynamic parameters associated to the micellization of F127 solution (1.187mM) is  
8 limited to a range of temperatures (28-31°C) when sigmoidal curves were obtained.  
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## Figures

### Figure captions

**Figure 1.** Typical data obtained from ITC experiments performed with a pluronic solution of F127 at a concentration of 1.187mM in water when the temperature of the experiment is fixed at 30°C (i.e. above the CMT of the 1.187mM F127 solution). Panel (A) shows exothermic heats released upon injection of the F127. Panel (B) shows integrated heat data, leading to a sigmoidal micellization curve. The CMC is defined as the concentration where the first derivative of the curve B reaches a maximum (C).

**Figure 2.** Typical data obtained from ITC experiments performed with a pluronic solution of F127 at a concentration of 1.187mM in water when the temperature of the experiment is fixed at 34°C. Panel (A) shows exothermic heats released upon injection of the F127. Panel (B) shows integrated heat data, giving a sigmoidal micellization curve. ST and ET represent respectively the start and the end of the micellization determined according to method 2 described in the section “analysis of enthalpograms”. Lower panels represent a typical ITC data for the dilution of F127 solution at a concentration 1.187mM in water when the temperature of the experiment is fixed at 10°C. Panel (C) shows exothermic heats released upon the injection of the F127. Control consisted on the injection of water into water contained in the measurement cell. Panel (D) shows the integrated heat data.

**Figure 3.** Enthalpy-entropy compensation plot corresponding to the micellization of F127 (1.187mM)

(i) in water at various temperatures (○). Data available in table 1.

(ii) in presence of propanediol-1,2 at 29°C (■). Data available in table 3.

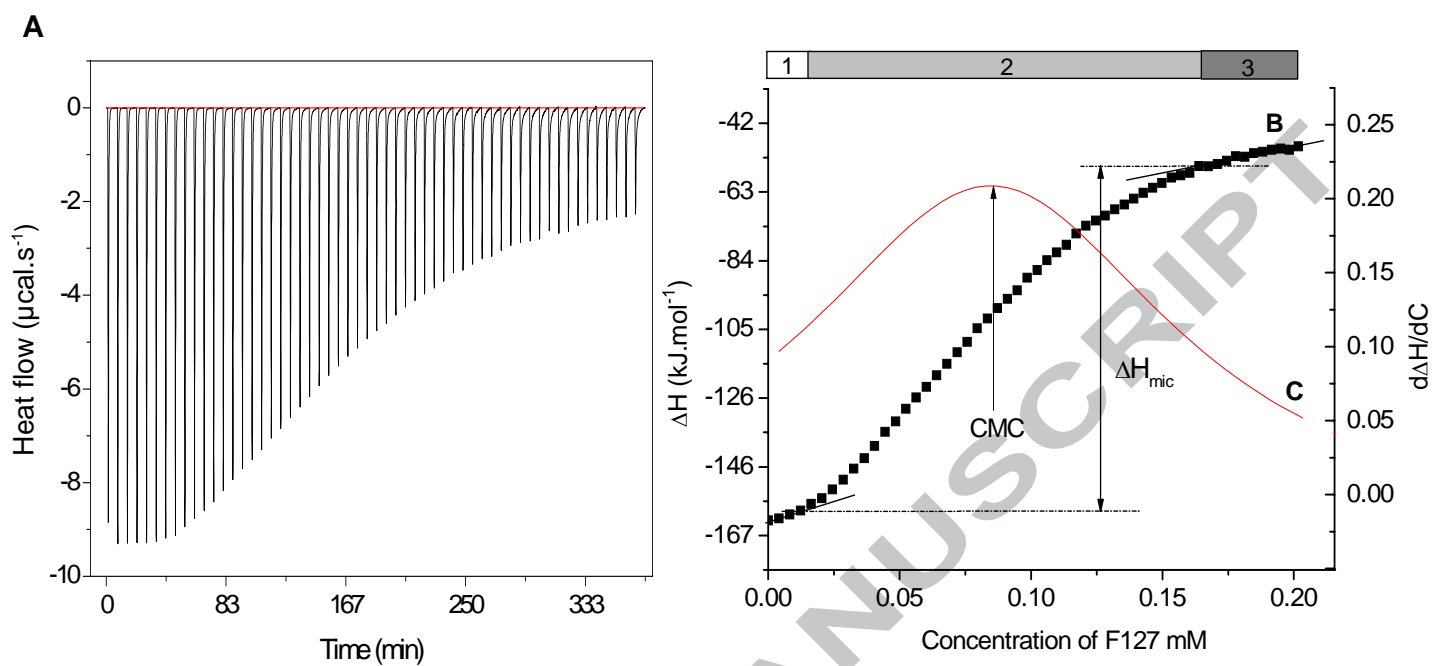


Figure 1

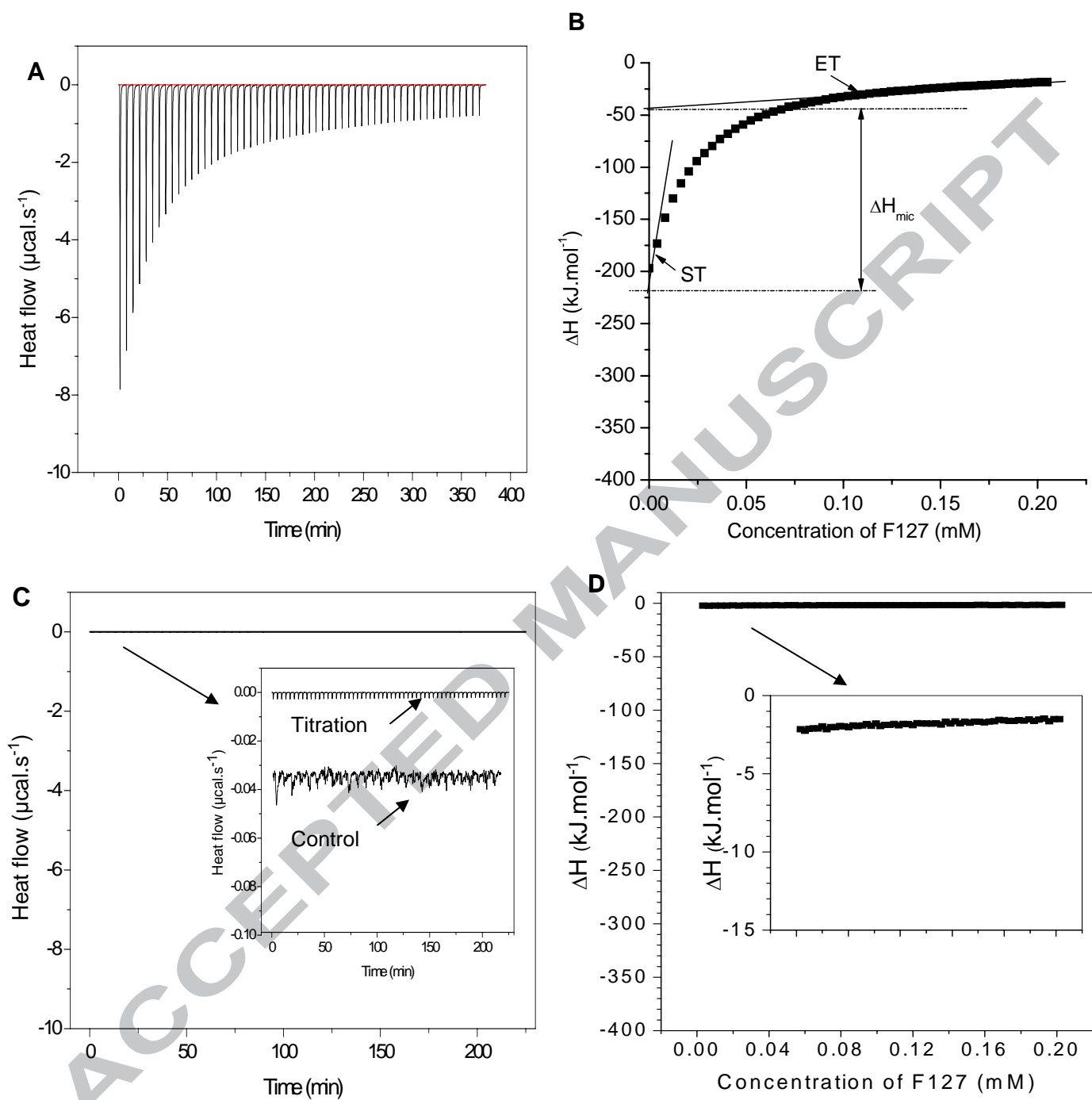


Figure 2

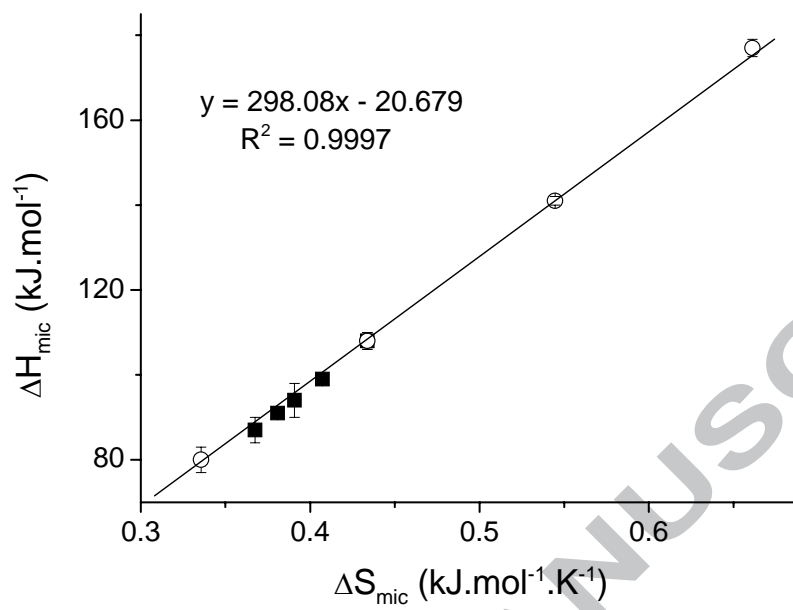


Figure 3

## Tables

**Table 1.** Micellization thermodynamic data determined from ITC measurements performed with F127 solution at a concentration of 1.187 mM in water for temperatures ranging from 28 to 31°C. Data extracted from the sigmoidal micellization curve according to method 1 (materials and methods section, analysis of the enthalpograms).

T	CMC mM of F127	$\Delta H_{mic}$ kJ.mol <sup>-1</sup> of F127	$\Delta G_{mic}$ kJ.mol <sup>-1</sup> of F127	$T\Delta S_{mic}$ kJ.mol <sup>-1</sup>	$\Delta C_{p,mic}$ kJ.mol <sup>-1</sup> .K <sup>-1</sup>
301K (28°C)	0.197 ± 0.001	80 ± 3	-20.90 ± 0.01	101 ± 3	28
302 K (29°C)	0.095 ± 0.005	108 ± 2	-23.25 ± 0.12	131 ± 2	33
303 K (30°C)	0.085 ± 0.001	141 ± 1	-23.13 ± 0.02	165 ± 1	36
304 K (31°C)	0.079 ± 0.002	177 ± 2	-23.38 ± 0.06	201 ± 2	-

(-) the last value of  $\Delta C_{p,mic}$  can not be calculated.

**Table 2.** Micellization thermodynamic data determined from ITC measurements performed with a F127 solution at a concentration of 1.187 mM for temperatures ranging from 5 to 60°C.

T	CMC <sup>a</sup> mM of F127	ST <sup>b</sup> mM of F127	ET <sup>b</sup> mM of F127
278-300 K (5-27°C)	-	-	-
301K (28°C)	0.197 ± 0.001	-	-
302 K (29°C)	0.095 ± 0.005	-	-
303 K (30°C)	0.085 ± 0.001	-	-
304 K (31°C)	0.079 ± 0.002	-	-
305 K (32°C)	-	0.024	0.139
306 K (33°C)	-	0.024	0.153
307 K (34°C)	-	0.016	0.142
308 K (35°C)	-	0.016	0.109
308.5 K (35.5°C)	-	0.016	0.138
309 K (36°C)	-	0.008	0.147
310 K (37°C)	-	0.008	0.146
311 K (38°C)	-	0.006	0.163
312 K (39°C)	-	0.003	0.160
313 K (40°C)	-	0.003	0.139
315 K (42°C)	-	0.003	0.142
318-333 K (45-60°C)	-	-	-

a. Midpoint of the transition from sigmoidal curve, data treatment according to method 1 (see analysis of the enthalpogram section).

b. ST and ET values calculated according to method 2 (analysis of enthalpogram section).

-. The parameters could not be determined from the experiment.

**Table 3.** Micellization thermodynamic data determined from ITC measurements performed with a F127 solution at a concentration of 1.187mM in water/propanediol-1,2 mixtures at various concentrations of propanediol-1,2 (0, 1.4, 2.3, 2.8 and 3.7% w/v) when the temperature is fixed at 29°C.

Propanediol in water % w/v	CMC mM of F127	$\Delta H_{mic}$ kJ.mol <sup>-1</sup> of F127	$\Delta G_{mic}$ kJ.mol <sup>-1</sup> of F127	$T\Delta S_{mic}$ kJ.mol <sup>-1</sup>
0	0.095 ± 0.005	108 ± 2	-23.25 ± 0.12	131 ± 2
1.4	0.081 ± 0.001	99 ± 1	-23.65 ± 0.02	123 ± 1
2.3	0.077 ± 0.007	94 ± 4	-23.78 ± 0.20	118 ± 4
2.8	0.069 ± 0.008	91 ± 1	-24.06 ± 0.21	115 ± 1
3.7	0.066 ± 0.004	87 ± 3	-24.17 ± 0.17	111 ± 3

**Table 4.** Micellization thermodynamic data determined from ITC measurements performed with a F127 solution at a concentration of 1.187mM in water. The time interval between 2 injections was varied from 300 to 600 s and the temperature of the experiments was fixed at 29°C. Solutions were prepared according to protocol 1.

Time interval between 2 injections (s)	CMC mM of F127	$\Delta H_{mic}$ kJ.mol <sup>-1</sup> of F127	$\Delta G_{mic}$ kJ.mol <sup>-1</sup> of F127	$T\Delta S_{mic}$ kJ.mol <sup>-1</sup>
300	0.095 ± 0.004	109 ± 1	-23.25 ± 0.10	132 ± 1
400	0.095 ± 0.005	108 ± 2	-23.25 ± 0.12	131 ± 2
500	0.097 ± 0.003	109 ± 1	-23.20 ± 0.07	132 ± 1
600	0.096 ± 0.005	108 ± 2	-23.23 ± 0.12	131 ± 2