Colloids and Surfaces B: Biointerfaces 183 (2019) 110461

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

# Role of molecular weight and hydrophobicity of amphiphilic tri-block copolymers in temperature-dependent co-micellization process and drug solubility

Chin-Fen Lee[a], Ching-Hsun Yang[b], Tsang-Lang Lin[b], Pratap Bahadur[c], Li-Jen Chen[a,*]

[a] Department of Chemical Engineering, National Taiwan University, Taipei, 10617, Taiwan
[b] Department of Engineering and System Science, National Tsing Hua University, Hsinchu, 30013, Taiwan
[c] Department of Chemistry, Veer Narmad South Gujarat University, Surat, 395 007, India

ARTICLE INFO

*Keywords:*
Polymer micelles
Pluronics
Mixed micelles
Small angle X-ray scattering
Differential scanning calorimetry

ABSTRACT

The binary P123 + F108, + F98, + F88, + F68, + F87 and + P84 systems were used to systematically explore the effect of molecular weight and hydrophobicity of Pluronic on the tendency of cooperative binding between parent copolymers and solubility of drug (ibuprofen) in these mixed Pluronic systems. Temperature-dependent co-micellization process in these systems was carefully investigated by using high sensitivity differential scanning calorimeter (HSDSC), dynamic light scattering (DLS) and small angle X-ray scattering (SAXS). All the HSDSC thermograms for these systems consistently exhibit two endothermic (micellization) peaks apart by at least 13.3 °C. It was evidenced that micelles are mainly formed by P123, the copolymer with a lower critical micelle temperature (CMT), at low temperatures. Raising temperature would dehydrate the other Pluronic with a higher CMT to be integrated into the neat P123 micelles developed at low temperatures. When the temperature is further increased beyond the second endothermic peak, the mixed micelles with a two-shell structure and characteristic corona lengths of their parent copolymers are observed to prove the existence of cooperative binding between parent copolymers. All the binary mixed Pluronic systems used in this study exhibit cooperative binding to form *unimodal* distribution of mixed micelles, except the P123 + F68 system. The SAXS results show that P123 + F68 system at 65 °C exhibits *bimodal* distribution of aggregates with coexisting of neat F68 micelles (65% in number) and P123 + F68 mixed micelles (35% in number). It is interesting to find out that P123 and F68 with distinct polypropylene oxide (PPO) moieties (i.e., a difference of 37 PO units) would exhibit very weak cooperative binding to partially form mixed micelles. Addition of ibuprofen in the P123 + F68 system would substantially enhance the cooperative binding between P123 and F68 to form *bimodal* distribution of aggregates with coexisting of neat F68 micelles (drops down to 30% in number) and P123 + F68 mixed micelles (increases up to 70% in number). For the systems with ibuprofen incorporated, SAXS results demonstrate that the drug is mainly encapsulated in the core of neat micelles developed at low temperatures. The solubility of ibuprofen in the 0.5 wt% P123 + 0.368 wt% P84 system is as high as 2.62 mg/ml, which is 114 times more than that in pure water at 37 °C.

## 1. Introduction

Amphiphilic tri-block copolymers undergo self-assembly in aqueous solutions, in which temperature plays a crucial role in solvent selectivity of certain polymer blocks. Triggered by entropy change of the system while temperature increases, micellization arises where blocks of the molecule with lower solubility becomes dehydrated and shrinks to form core-shell aggregates. It has been proved that micelles formed by amphiphilic block copolymers are able to enhance hydrophobic drug solubility, particle stability, and circulation time of formulations [1]. As a potential carrier for anti-cancer drugs, polymer micelles have been proceeded in the evaluation of several preclinical and clinical trials [2,3]. Among them, Pluronics, one of the amphiphilic tri-block copolymers, become attractive choices for drug carriers due to their intrinsic low- toxicity as well as solubilization capacity of drugs [4–7].

Mixed Pluronic systems have been examined and searched for better combinations between parent copolymers as drug carriers [8]. Gaisford et al. [9] pointed out that binary F77 + F87 system with similar poly

* Corresponding author.
*E-mail address:* ljchen@ntu.edu.tw (L.-J. Chen).

https://doi.org/10.1016/j.colsurfb.2019.110461
Received 25 May 2019; Received in revised form 18 August 2019; Accepted 25 August 2019
Available online 26 August 2019

(propylene oxide) (PPO) block lengths exhibits cooperative binding to form mixed micelles, but not for the F77 + F127 and F87 + F127 systems with distinct chain length of PPO. The cooperative binding between two copolymers with similar hydrophobic moieties has been consistently observed and reported in the literature [9–13]. For example, Liu et al. [10] proved the synergistic self-assembly behavior (cooperative binding) between the two tri-block copolymers $EO_{99}PO_{69}EO_{99}$ (Pluronic F127) and $EO_{45}BO_{14}EO_{45}$ *with distinct hydrophobic chain length*, where EO stands for an ethylene oxide unit [$OCH_2CH_2$], PO for a propylene oxide unit [$OCH_2CH(CH_3)$], and BO for a butylene oxide unit [$OCH_2CH(CH_2CH_3)$]. For the choice of drug carriers for paclitaxel on clinical cancer treatment, polyethylene-polylactic acid and vitamin E-TPGS (an inhibitor of P-glycoprotein) were chosen for the sake of similar chain length [14]. Furthermore, when designating a potential candidate as an injectable or coatable tissue adhesion barrier using Pluronic hydrogel, similar PPO moieties of parent copolymers (e.g. F127 and P123) were considered and chosen to form stable mixed micelles for the residence stability [15]. Recently, a morin hydrated-loaded Pluronic mixed micelle as nanocarriers for the management of Alzheimer's disease was reported, in which F127 and P123 were adopted again based on the same concept of similar PPO moiety [11]. On the other hand, Zhang et al. pointed out that the binary F127 + F68 system would *independently* form micelles simply based on two noticeable micellization (endothermic) peaks observed on the DSC thermogram [16]. They suggested that the first endothermic peak on heating is attributed to F127 micelle formation and the second peak to F68 micelle formation. That is, this binary F127 + F68 system with distinct PPO chain lengths would *not* exhibit cooperative binding.

In contrast, two endothermic peaks were observed on the DSC thermogram and unimodal distribution of the aggregates was determined from the DLS measurements for the binary F127 + L64 system [17]. That is, even the binary system with distinct PPO chain lengths would exhibit cooperative binding to form mixed micelles. Recently, two endothermic peaks were observed on the DSC thermogram and *bimodal* distribution of the aggregates was determined from the DLS measurements for some binary Pluronic systems with distinct PPO chain lengths [12]. Hence, whether a binary Pluronic system with different PPO moieties would exhibit cooperative binding to form mixed micelles still remains an open question and needs to be systematically explored. In addition, the mechanism of mixed micelle formation as well as drug encapsulation in such systems have not been thoroughly examined yet.

In this study, the high-sensitivity differential scanning calorimeter (HSDSC) and dynamic light scattering (DLS) were applied to explore the temperature-dependent micellization process and to investigate correlations between endothermic peaks and size distribution of the mixed systems. Next, differentiation between assemblies in the mixed systems for the more precise probing was conducted through small angle x-ray scattering (SAXS). Upon realization of assembly structure, process of mixing could then be clarified behind co-micellization. Moreover, consistent results are correlated from these techniques applied on the mixed systems with nonsteroidal anti- inflammatory drug - ibuprofen incorporated, in which solubilization capacity characterized by UV-Vis spectroscopy and drug encapsulation process of binary Pluronic systems were simplified.

Factors influencing the critical micellization temperature (CMT) of block copolymers could be traced fundamentally to the relative amount of EO and PO monomers. Connecting factors like CMT, PPO moieties as well as hydrophobicity of the copolymers, discussion of how different core or corona lengths affect molecular association have been demonstrated in this report. We established a series of investigations to study mixing behavior through systematic variations of resemblance between parent copolymers, which could be divided into two perspectives: various hydrophobicities at a fixed PPO moiety (P123 + F88, + F87, and + P84), as well as the different molecular weights at a fixed hydrophilicity (P123 + F108, + F98, + F88, and + F68). We anticipate to clarify the tendencies of mixing behavior between copolymers and the mixing process in binary-Pluronic systems.

**Table 1**
Physicochemical characteristics of Pluronic block copolymers used in this study.

| Pluronic | Structure [18–23] | Average Mw[a] | HLB[a] | CP[b] (°C) |
|---|---|---|---|---|
| P123 | $(EO)_{21}(PO)_{67}(EO)_{21}$ | 5750 | 7-12 | 90 |
| F108 | $(EO)_{127}(PO)_{48}(EO)_{127}$ | 14600 | > 24 | > 100 |
| F98 | $(EO)_{118}(PO)_{45}(EO)_{118}$ | 13000 | 28 | > 100 |
| F88 | $(EO)_{97}(PO)_{39}(EO)_{97}$ | 11400 | 28 | > 100 |
| F68 | $(EO)_{80}(PO)_{30}(EO)_{80}$ | 8400 | > 24 | > 100 |
| F87 | $(EO)_{61}(PO)_{40}(EO)_{61}$ | 7700 | > 24 | > 100 |
| P84 | $(EO)_{19}(PO)_{39}(EO)_{19}$ | 4200 | 12-18 | 74 |

[a]Information from BASF ; b Cloud point (CP) of the solution at 1% Pluronic copolymers.

## 2. Materials and method

### 2.1. Materials

Pluronics F98, F88, F87, P84 and F68 were obtained from BASF and Pluronics P123 and F108 were purchased from Sigma-Aldrich. The molecular characteristics and some physical properties of these Pluronics are described in Table 1. The drug ibuprofen, α-methyl-4-(isobutyl) phenyl acetic acid, was bought from Sigma Aldrich. Sodium hydroxide (NaOH) used for calibration of ibuprofen for UV-Vis measurement was bought from SHOWA with purity 96%. All these chemicals were used as received without further purification. Water was purified by double distillation followed by a PURELAB Maxima Series (ELGA Lab Water) purification system with a resistivity better than 18.2 MΩ cm.

### 2.2. High sensitivity differential scanning calorimetry (HSDSC)

HSDSC (VP-DSC, MicroCal) was used to determine critical micellization temperature (CMT) of the solution. The detail experimental procedure can be found in our previous studies [24,25]. All experiments were conducted with a scanning rate of 30 or 60 °C/h from 5 to 120 °C for 7 scans and reproduced at least for 2 times.

### 2.3. Dynamic light scattering (DLS)

Zetasizer Nano system equipped with a He-Ne laser operating at a wavelength 633 nm (Nano-ZS, Malvern) was used to determine particle size distributions of neat and mixed Pluronic solutions. The temperature was controlled in a range of 10–70 °C. The accuracy for aqueous systems using NIST SRM1980 standard reference material is expected to be 0.12 μm cm/V s. Results presented in this study are mainly based on intensity distribution.

### 2.4. UV/Vis Spectroscopy and drug solubility in Pluronic aqueous solution

Pluronic aqueous solutions with an excess drug (ibuprofen) powder was mixed with under the condition of 37 °C for one day at 180 rpm. Solutions were then filtered by a 0.22 μm PTFE filter (Millipore) to remove undissolved drug right before UV/Vis detection. The dissolved ibuprofen concentration was determined by measuring absorbance at 264.8 nm by using UV–vis double beam spectrophotometer (CARY 100nc, Agilent Technologies, Santa Clara, CA, USA). Dilute solutions of ibuprofen dissolved in 0.1 N NaOH were used for the calibration based on Beer-Lambert law.

### 2.5. Small angle X-ray scattering (SAXS)

The SAXS measurements were carried out at the beamline BL23A of the National Synchrotron Radiation Research Center (NSRRC) Hsinchu, Taiwan [26]. The sample-to-detector distance and photon energy were set to be 2700 mm and 14 keV ($\triangle$E/E = 1/7000), respectively, to cover the Q range from 0.008 to 0.35 $Å^{-1}$. Liquid samples were loaded in a cell with a thickness of 5 mm and Kapton-walled windows [27]. Scattered X-ray data were collected by means of a 2D MarCCD detector. The variation of scattering length density (SLD) of $H_2O$, PEO and PPO as a function of temperature was reported in Tables S1 and S2 (supporting information).

## 3. Results and discussion

For the analysis of effect of hydrophobicity of Pluronics on blending, three Pluronics P84, F87 and F88 with a fixed PPO block length (~39 PO units) but different PEO block lengths (see Table 1) were selected. A controlled concentration of PO units of F8x was prescribed. F88 1.0 wt % is equivalent to $8.77 \times 10^{-7}$ m F88 in water. Thus, same concentration $8.77 \times 10^{-7}$ m of F87 and P84 aqueous solutions were prepared and equivalent to 0.675 wt% F87 and 0.368 wt% P84 in water. The HSDSC thermograms of the systems were measured and shown in Fig. **S1A** and could be applied to determine the CMT by using two commonly adopted methods [28,29], i.e., the onset temperature $T_{onset}$ and the peak maximum temperature $T_m$. It is obvious that CMT ($T_{onset}$ and $T_m$) of 0.368 wt % P84 system are lower than those of the other systems due to hydrophobic property of the copolymer (Table S3). The micelle size determined by DLS measurement increases along with a decrease in hydrophobicity (i.e., an increase in PEO chain length) with an order of F88 > F87 > P84.

Consider 1.0 wt % Fx8 aqueous solutions with x = 6, 8, 9, and 10 as an example for different molecular weights of Pluronics with a fixed hydrophobicity. The $T_{onset}$ of F68, F88, F98, F108 and P123 are 47.4, 35.9, 30.7, 29.4, and 17.9 °C, respectively, determined by HSDSC thermograms (Fig. S1B) as listed in Table S3. It is obvious that the CMT increases along with a decrease in molecular weight of the Pluronic Fx8. The micelle size, determined by the DLS and listed in Table S3, increases along with an increase in molecular weight of the Pluronic Fx8.

The Pluronic P123 is chosen as one of the parent copolymers and used throughout the study. The variation of sizes of neat P123 micelles as a function of the temperature ranging from 11 to 65 °C is shown as black dashed line in Fig. 1B. When system temperature T ≦ 17.5 °C, unimers with size of around 4.3 nm exist. When T > 17.5 °C, the particle (micelle) size dramatically increases to around 19.7 nm. It is interesting to consistently find out that the onset temperature ($T_{onset}$) of endothermic peak of the HSDSC thermogram for 0.5 wt% P123 aqueous solution is 17.9 °C (Table S3). When T > $T_{onset}$, the particle (micelle) size remains almost constant around 20 nm up to 49.3 °C.

### 3.1. Effect of hydrophobicity of F8x on the P123 + F8x mixed micelle formation

Three Pluronics P84, F87, and F88 with a fixed PPO block length (~39 PO units, see Table 1) but different PEO block chain lengths (respectively, 19, 61 and 97 EO units) were chosen to explore the effect of hydrophobicity of F8x on the P123 + F8x mixed micelle formation. Note that the molar ratio of F8x/P123 (for x = 4, 7 and 8) is always fixed at 1.01.

It is interesting to point out that all the HSDSC thermograms for the binary P123 + F8x mixed systems exhibit two endothermic peaks as the red solid lines illustrated in Figs. 1A and S5. The first peak appeared on the thermograms of the P123 + F8x systems almost coincides with the endothermic peak of neat P123 system and the second peak is usually located slightly behind the endothermic peak of neat F8x

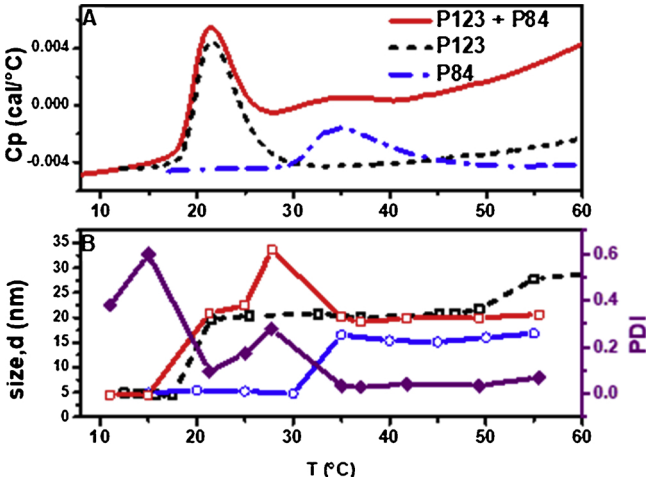


**Fig. 1.** (A) The HSDSC thermograms and (B) variation of particle sizes as a function of temperature measured by the DLS for neat 0.5 wt% P123 (black dashed line and black square),and 0.368 wt% P84 (blue dashed-dotted line and blue circle) systems and the 0.5 wt% P123 + 0.368 wt% P84 mixed system (red solid line and red square). PDI values extracted from DLS (purple solid line and purple diamond). The particle size distributions for 0.5 wt% P123 + 0.368 wt% P84, 0.5 wt% P123 and 0.368 wt% P84 systems measured by the DLS are shown in Figs. S2, S3 and S4, respectively, in the supporting information (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

system. All the CMTs ($T_{onset(m)}$, $T_{m1}$ and $T_{m2}$) of the binary P123 + F8x mixed systems extracted from the HSDSC thermograms are listed in Table 2. For these binary Pluronic mixed systems, three CMTs are defined as $T_{onset(m)}$ stands for the onset temperature of the first endothermic peak; $T_{m1}$ represents the peak maximum temperature of the first endothermic peak; and $T_{m2}$ is the peak maximum temperature of the second endothermic peak.

Figs. 1B and S5 also illustrate the variation of size of aggregates (determined by the DLS) as a function of temperature for the neat Pluronic and binary P123 + F8x mixed systems. For example, as the system temperature increases, the particle size of neat 0.368 wt% P84 system increases from 5.2 nm to 15.9 nm and remains almost constant at T > $T_m$ = 34.7 °C, as illustrated in Fig. 1B. When T < $T_{onset(m)}$(17.6 °C), the binary 0.5 wt% P123 + 0.368 wt% P84 mixed system exists in the form of unimers with particle (unimer) size around 4.5 nm. Further increase in temperature up to 21.3 °C would dramatically increase the particle (micelle) size to around 20.9 nm and even 33.6 nm at 27.8 °C, as the red line shown in Fig. 1B. Note that the first peak for the binary P123 + P84 mixed system ($T_{m1}$ = 21.5 °C) almost coincides with the $T_m$ of neat P123 system (21.7 °C).

When the system temperature increases, the polydisperse index (PDI), an index of degree of uniformity of aggregate size, of the P123 + P84 mixed system decreases from 0.60 (at 15.0 °C) to 0.10 (at 21.3 °C) (Fig. 1B). Keep rising temperature promotes assembling process until the $T_m$ of neat P84. The appearance of the second peak may suggest that P84 actively participates in the micellization process at $T_{m2}$ = 34.8 °C by integrating the P84 into mixed P123 + P84 micelles, as the sudden drop in micelle size around 35.0 °C illustrated in Fig. 1. The size of mixed micelles remains around 20.2 nm within the temperature range from 35.0 to 56.0 °C. The PDI of the mixture decreases from 0.10 (at 21.3 °C) to 0.04 (at 35 °C) and remains always smaller than 0.1 up to 56 °C, as the purple line illustrated in Fig. 1B. Raising temperature continues promoting assembling process and unifying the aggregations in which monodisperse system was performed.

The HSDSC thermograms and variation of particle size as a function of temperature for the other binary P123 + F87 and P123 + F88 mixed systems, as illustrated in Fig. S5, demonstrate a similar tendency to that of the P123 + P84 mixed system. The $T_{m1}$ and $T_{m2}$ are located rather

**Table 2**
The CMT ($T_{onset(m)}$, $T_{m1}$ and $T_{m2}$) determined from the HSDSC thermograms, mixed micelle size, PDI and FWHM at temperature > $T_{m2}$ determined from the DLS for the binary mixed Pluronic systems.

| Binary system | 0.5 wt% P123 + 1 wt% F108 | 0.5 wt% P123 + 1 wt% F98 | 0.5 wt% P123 + 1 wt% F88 | 0.5 wt% P123 + 1 wt% F68 | 0.5 wt% P123 + 0.675 wt% F87 | 0.5 wt% P123 + 0.368 wt% P84 |
|---|---|---|---|---|---|---|
| $T_{onset(m)}$[1] (°C) | 17.3 | 17.9 | 17.8 | 18.0 | 17.6 | 17.6 |
| $T_{m1}$[2] (°C) | 21.3 | 21.3 | 21.2 | 21.4 | 21.3 | 21.5 |
| $T_{m2}$[3] (°C) | 35.3 | 36.6 | 42.9 | 59.6 | 43.7 | 34.8 |
| $D_h$* (nm) | 30.6 (36-65 °C) | 28.1 (42-68 °C) | 29.3 (49-65 °C) | 32.3 (60-67 °C) | 24.2 (54-58 °C) | 20.2 (35-56 °C) |
| PDI | 0.05 (45.2 °C) | 0.05 (42.0 °C) | 0.07 (43.0 °C) | 0.23 (66.7 °C) | 0.06 (43.6 °C) | 0.04 (49.3 °C) |
| FWHM | 19.5 | 18.5 | 20.1 | 40.3 | 15.3 | 6.73 |

[1]Temperature at the onset point of the first peak. 2Temperature at the peak maxima of the first peak. 3Temperature at the peak maxima of the second peak. *The mixed micelle diameter $D_h$ is determined by averaging over the mixed micelle size measured by the DLS in this temperature range ($T > T_{m2}$) indicated in the parentheses.

close to $T_m$ of neat P123 system (21.7 °C) and the $T_m$ of the other parent copolymer (F87 or P84) respectively. When the system temperature increases, particle size dramatically develops from unimers' scale to around the size of P123 micelles at $T_{m1}$. When $T > T_{m2}$, the aggregation sizes drop and remain stable up to high temperature. The mixed micelle size of the P123 + P84 system is consistently smaller than that of mixtures P123 + F88 and P123 + F87. It is attributed to the shortest PEO chain length of P84 among all the copolymers that leads to the smallest hydrodynamic diameter (see Table 2). Combining P84 with P123 develops a stable, unimodal system, in which particles are made to be monodisperse as illustrated in Fig. 1B. In other words, enhancing hydrophobicity of the system does not necessarily decrease the uniformity of assemblies.

Furthermore, the binary P123 + P84 system was chosen to further investigate the conformation of mixed micelle by the SAXS. The SAXS profiles of all the neat Pluronic systems were fitted to the Polycore model [30], a simple core-shell model with two density levels, to determine the conformation of micelle. The core radius and shell thickness of P123 micelles at 25 °C are, respectively, 5.3 and 3.2 nm (Table 3), consistent with literature data from both SANS and SAXS [31–33]. The core SLD is not a fitting parameter but an assigned value in the data regression analysis. For example, the core SLDs for the neat P123 system at 25, 37 and 45 °C are $9.40 \times 10^{-6}$, $9.33 \times 10^{-6}$ and $9.28 \times 10^{-6}$ Å$^{-2}$, respectively. The core radius slightly increases to 5.8 nm as the temperature is raised up to 45 °C due to an increase in the aggregation number from 82 (at 25 °C) to 101 (at 45 °C). The SAXS profile for neat 0.368 wt% P84 system at 25 °C (below $T_{onset}$ = 29.8 °C), as illustrated in Fig. 2, is fitted with the Debye function [34,35] to find out that the P84 molecules in the aqueous solution exist in the form of unimers with a radius of gyration of 2.0 nm. When the system temperature increases, more P84 unimers are assembled to form micelles, consistent with that of Liu et al. [36] and Jain et al. [37].

The SAXS profiles for the P123 + P84 mixed system, as illustrated in Fig. 2, were fitted to the Debye and Polycore model. The core radius and shell thickness of the P123 + P84 mixed system at 25 °C are similar to that of neat P123 micelles, implying the micelles are formed mainly by P123 molecules. As temperature is increased up to 37 °C, volume fraction of unimers drops from 0.38 to 0.11%, indicating most of copolymers already assembled into aggregates. The core-shell thickness of the micelles in the mixed system remains similar to the characteristic length of neat P123 micelles. However, volume fraction of the micelle increases from 1.5 to 4.1% at the expense of the decrease in volume fraction of unimers simultaneously (from 0.38 to 0.11%) when the temperature is increased from 25 to 37 °C. It is plausible to conjecture that the amount of the decrease in volume fraction of P84 unimers was integrated into the P123 micelles to form the P123 + P84 mixed micelles. At 45 °C, stable mixed micelle structure were obtained with aggregation number $N_{agg}$ = 63, in-between those of neat systems

**Table 3**
SAXS results for neat P123 and P84 systems and binary P123 + P84 system.

| SAXS parameters | 0.5 wt% P123 | | | 0.368 wt% P84 | | | 0.5 wt% P123 +0.368 wt% P84 | | |
|---|---|---|---|---|---|---|---|---|---|
| | 25 °C | 37 °C | 45 °C | 25 °C | 37 °C | 45 °C | 25 °C | 37 °C | 45 °C |
| $\phi_{unimer}$[a] (%) | – | – | – | 0.5 ± 0.1 | 0.38 ± 0.1 | – | 0.38 ± 0.03 | 0.11 ± 0.05 | – |
| $R_g$[b] (nm) | – | – | – | 2.0 | 2.5 | – | 1.5 | 0.6 | – |
| $\phi_{micelle}$[c] (%) | 1.8 ± 1.0 | 1.7 ± 1.0 | 1.7 ± 0.2 | – | 1.1 ± 0.2 | 1.8 ± 0.3 | 1.5 ± 0.2 | 4.1 ± 0.5 | 5.0 ± 0.4 |
| $R_{core}$[d] (nm) | 5.3 | 5.5 | 5.8 | – | 4.1 | 4.4 | 5.0 | 5.5 | 5.1 |
| $R_{shell}$[e] (nm) | 3.2 | 3.3 | 3.2 | – | 2.6 | 2.0 | 3.3 | 3.2 | 3.7 |
| D[f] (nm) | 17.0 | 17.6 | 18.0 | – | 13.4 | 12.8 | 16.7 | 17.4 | 17.6 |
| σ[g](%) | 0.17 ± 0.02 | 0.15 ± 0.02 | 0.18 ± 0.02 | – | 0.18 ± 0.03 | 0.18 ± 0.03 | 0.15 ± 0.01 | 0.13 ± 0.02 | 0.11 ± 0.01 |
| Shell SLD ($10^{-6}$ Å$^{-2}$) | 9.62 | 9.58 | 9.58 | – | 9.57 | 9.60 | 9.63 | 9.55 | 9.48 |
| n[h] ($10^{15}$ cm$^{-3}$) | 6.4 | 5.5 | 5.1 | – | 11.2 | 14.6 | 5.8 | 14.4 | 16.6 |
| $N_{agg}$[i] | 82 | 95 | 101 | – | 25 | 36 | – | 63 | 63 |
| Hydro $D_h$[j] (nm) | 19.5 | 20.0 | 20.7 | – | 16.5 | 15.1 | 20.9 | 20.1 | 19.9 |

[a]$\varphi_{unimer}$: volume fraction of unimers. [b]$R_g$: radius of gyration of unimers. [c]$\varphi_{micelle}$: volume fraction of micelles. [d]$R_{core}$: average core radius. [e]$R_{shell}$: shell thickness. [f]D: diameter of micelle. [g]σ: core polydispersity. [h]n: Micelle number density. [i]$N_{agg}$: aggregation number of the micelle. [j]$D_h$: hydrodynamic diameter of micelle measured by the DLS.

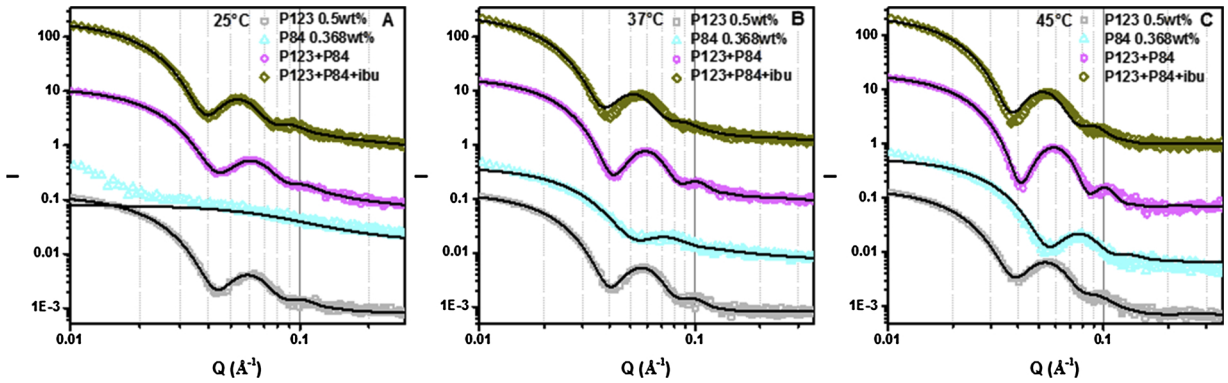


**Fig. 2.** SAXS profiles of 0.5 wt% P123, 0.368 wt% P84, 0.5 wt% P123 + 0.368 wt% P84 with/without ibuprofen incorporated at (A) 25 °C, (B) 37 °C, and (C) 45 °C. (□) neat P123; (△) neat P84; (○) P123 + P84; (◇) P123 + P84 with ibuprofen. The black solid lines are the fitting results.

($N_{agg}$ = 36 for neat P84 system and $N_{agg}$ = 101 for neat P123 system). Owing to relatively short PPO block length of P84, it may be easy for P84 molecules being integrated into the P123 micelles formed at lower temperatures with very similar corona lengths (shell thickness) due to the PEO chain length of P84 similar to that of P123. That implies that the binary P123 + P84 system exhibits cooperative binding to form mixed micelles.

The hydrodynamic diameter $D_h$ is determined by the DLS based on the diffusion of the particles (micelles). The particle (micelle) moves through a liquid medium along with a solvation layer on the surface of particle. Thus the hydrodynamic diameter $D_h$ gives the micelle size including the hydration layer. Consequently, the hydrodynamic diameter $D_h$ is slightly larger than the diameter D determined by the SAXS without considering the hydration layer, as shown in Table 3.

### *3.2. Effect of molecular weight of Fx8 on the P123 + F × 8 mixed micelle formation*

Pluronics Fx8 (x = 10, 9, 8 and 6) with a fixed PEO/PPO ratio (80/20) were used to blend with Pluronic P123 exploring the effect of molecular weight on the (0.5 wt%) P123 + (1.0 wt%) Fx8 mixed micelle formation. Molar ratios of Fx8/P123 for x = 10, 9, 8 and 6 are 0.79, 0.88, 1.01, and 1.37, respectively.

The HSDSC thermograms and temperature-dependent size of aggregates for the binary P123 + F108, + F98, and + F68 mixed systems, as illustrated in Fig. S6, exhibit similar tendency to that of the P123 + P84 mixed system. In general, both P123 and Fx8 exist as unimers in aqueous solution when the temperature is below the CMT ($T_m$) of P123. The particle size dramatically increases when the temperature is raised to the $T_m$ of P123 due to the micellization of P123. Furthermore, the size gradually increases along with temperature to a maximum value near the $T_{onset}$ of neat Fx8 system, then drops and remains almost constant when the temperature is above the $T_{m2}$ of the HSDSC thermogram for the P123 + F × 8 mixed system. Note that the unimodal distribution of particle size (temperature above the $T_{m2}$ of the second endothermic peak of the HSDSC thermogram) was consistently observed for all the binary P123 + F × 8 mixed systems.

The PDI could also provide practical information of cooperative binding between the parent copolymers. For binary P123 + F × 8 mixed systems, PDIs were always larger than 0.4 below 21 °C indicating various sizes of particles coexisting in the solution including unimers and some large aggregates which is often believed as impurities accompanied with copolymers [29]. When the temperature increases up to near the $T_m$ of neat P123 system, PDI value decreases to around 0.17. Further increase in temperature to the $T_{m2}$ of the second peak of the HSDSC thermograms results in the drop of PDI to below 0.1 and remains almost constant, except the P123 + F68 mixed system (Fig. S7). The PDI value of the P123 + F68 mixed system undergoes an abrupt rise when the temperature is near $T_{onset}$ of neat F68 and drops down to 0.20 with particle size around 27 nm. Note that the PDI of the binary Pluronic system gradually increases as difference of PPO moieties between parent copolymers increases.

In addition to the PDI and size of aggregates (Figs. S8 and S7), two binary P123 + F108 and P123 + F68 systems were selected to further investigate chain length effect by using the SAXS (Figs. S10 and S9). The neat F108 and F68 micelles are formed with small core radius and relatively large shell thickness, as reported in Tables S4 and 4, consistent with that of Fan et al. [35]. Mixing behavior of these two binary systems shows similar tendencies to that of the P123 + P84 system, in which P123-micelle dominates the system at low temperatures and coexists with unimers of the other copolymer. However, it becomes distinctive between the P123 + F108 and P123 + F68 systems at temperature higher than their $T_{m2}$.

The mixed micelle formation was detected in the mixture P123 + F108 with a two-shell structure. The PolyTwoShell model was applied to analyze the SAXS data of the binary Pluronic systems. With the PolyTwoShell model, it is possible to distinguish two different SLD values of PEO regions inside the corona due to the different PEO block lengths between P123 and F108. At 45 °C, the thickness of the first shell is 3.1 nm and the second is 5.8 nm for the P123 + F108 system. The thickness of the first shell is very close to the corona length of P123 micelles (3.2 nm) and the sum of two shell thickness is 8.9 nm, which is very close to the corona length of neat F108 system, 7.6 nm. It is interesting that the SLD of the first shell ($9.65 \times 10^{-6}$ Å$^{-2}$) is higher than that of the second shell ($9.38 \times 10^{-6}$ Å$^{-2}$) and both of them are within the SLD values of pure water ($9.33 \times 10^{-6}$ Å$^{-2}$) and PEO ($1.09 \times 10^{-5}$ Å$^{-2}$) (Table S4), revealing that the PEO concentration in the first shell is higher than that in the second shell. The region consisting of both the PEO chains of P123 and F108 is thus identified as the first shell, while the region consisting of PEO chain of only F108 stretching toward solution is recognized as the second shell. Consequently, cooperative binding between P123 and F108 with comparable PPO block chain lengths was confirmed. Fig. 3 shows the schematic diagram (upper row) of evolution of temperature-dependent micellization process and micelle conformation for the binary P123 + F108 system.

It is interesting to point out that the conformation of mixed micelles of the P123 + F68 system is different from that of the other systems. The SAXS profiles and fitting results for the binary P123 + F68 system are illustrated in Fig. S9 and listed in Table 4, respectively. Table 4 shows that the binary P123 + F68 system at 65 °C (above the $T_{m2}$) exhibits bimodal distribution of aggregates, i.e., coexisting of neat F68 micelles and mixed micelles of P123 + F68. Mixed micelles with core radius of 7.1 nm and total shell thickness of 4.8 nm (composed of two shell thicknesses, 1.4 and 3.4 nm) were obtained. On the other hand, there exists another size of micelles with core radius of 1.9 nm and shell thickness of 4.2 nm coexisting with the mixed micelles in the solution taking up 4.6% in volume fraction. Note that this micelle conformation

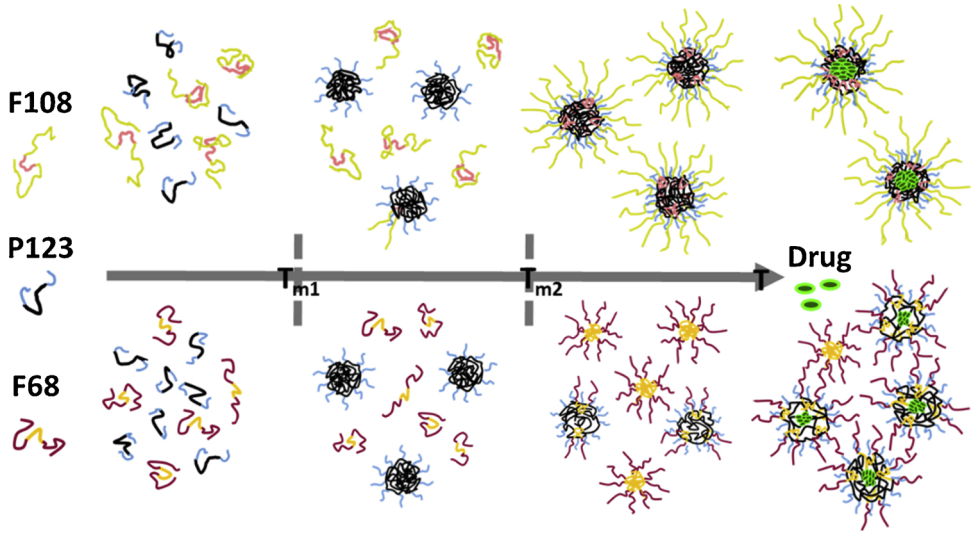


**Fig. 3.** Schematic diagram of micellization process as a function of temperature and addition of drug (ibuprofen) for binary P123 + F108 (upper row) and P123 + F68 (lower row) systems.

is very similar to that of neat F68 micelles ($R_{core}$ = 1.9 nm and $R_{shell}$ = 4.3 nm). Thus these relatively small micelles (D = 12.2 nm) are classified as neat F68 micelles. The two-sphere model provides better regression to the scattering intensities with much smaller $\chi^2 = 781$ compared to that ($\chi^2 = 1742$) from only one sphere model. Furthermore, according to the number densities of micelles reported in Table 4, the number ratio of neat F68 micelles and mixed micelles coexisting in the binary P123 + F68 system at 65 °C is 65/35. That is, the majority of F68 would form neat F68 micelles, instead of the P123 + F68 mixed micelles, implying only partially cooperative binding between P123 and F68. The schematic diagram (lower row) of evolution of temperature-dependent micellization process and micelle conformation for this binary P123 + F68 system is illustrated in Fig. 3.

The SAXS data demonstrate that the P123 + F68 system exhibits bimodal distribution of aggregates at 65 °C, however, the DLS data show the unimodal distribution of aggregates at temperatures higher than $T_{m2}$, as shown in Fig. S6. It seems that there is a discrepancy between the SAXS and DLS results. The DLS results were double-checked to further report the PDI and full-width at half maxima (FWHM) of size distribution peak for all the mixtures at temperature above $T_{m2}$ in Table 2. The FWHM for the system P123 + F108, + F98 and + F88 are all around 19.0 nm and PDI consistently as small as 0.06 ± 0.01. However, the FWHM and PDIs for the P123 + F68 system at 66.7 °C are 40.3 nm and 0.23, respectively, implying relatively broad size distribution of aggregates. Note that the SAXS results demonstrate the bimodal distribution with P123 + F68 mixed micelles of D = 23.8 nm and neat F68 micelles of D = 12.2 nm coexisting. The size difference between these two micelles is only 11.6 nm in diameter that is too small to be discernible by the DLS. It is plausible to conjecture that the DLS results merge these two populations of micelles (bimodal distribution) into a broad unimodal distribution with FWHM = 40.3 nm.

### 3.3. Solubility and encapsulation process of ibuprofen in binary Pluronic systems

The CMTs of binary Pluronic systems in the presence of nonsteroidal anti-inflammatory drug, ibuprofen (( ± ) 2-(4-isobutylphenyl) propionic acid), were carefully examined by the HSDSC. Neat P84 and F108 systems (Fig. S11) as well as binary P123 + P84 and + F108 systems (Fig. S12) were chosen to demonstrate the effect of drug loaded

**Table 4**
SAXS results for neat F68, P123 + F68 and P123 + F68 + ibuprofen systems.

| SAXS Parameters | 1 wt% F68 | | | 0.5 wt% P123 + 1 wt% F68 | | | | P123 + F68 + ibuprofen | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 25 °C | 37 °C | 65 °C | 25 °C | 37 °C | 65 °C two-sphere model | | 25 °C | 37 °C | 65 °C two-sphere model | |
| $\phi_{unimer}$[a] (%) | 0.5 ± 0.1 | 0.7 ± 0.1 | 0.2 ± 0.05 | 0.5 ± 0.04 | 0.5 ± 0.05 | – | – | 0.5 ± 0.05 | 0.5 ± 0.03 | – | – |
| $R_g$[b] (nm) | 3.3 | 4.1 | 1.2 | 3.2 | 2.7 | – | – | 3.3 | 3.2 | – | – |
| $\phi_{micelle}$[c] (%) | – | – | 4.9 ± 0.4 | 1.4 ± 0.2 | 2.0 ± 0.2 | 10.7 ± 0.7 | 4.6 ± 0.7 | 2.4 ± 0.3 | 2.8 ± 0.2 | 14 | 0.7 |
| $R_{core}$[d] (nm) | – | – | 1.9 | 5.2 | 5.5 | 7.1 | 1.9 | 5.9 | 6.8 | 6.4 | 1.9 |
| $R_{shell}$[e] (nm) | – | – | 4.3 | 3.1 | 3.6 | 1.4/3.4 | 4.2 | 3.6 | 3.0 | 1.3/3.2 | 3.9 |
| D[f] (nm) | – | – | 12.4 | 16.6 | 18.2 | 23.8 | 12.2 | 19.0 | 19.6 | 21.8 | 11.6 |
| σ[g] (%) | – | – | 0.56 ± 0.04 | 0.14 ± 0.03 | 0.14 ± 0.02 | 0.21 ± 0.02 | 0.55 ± 0.04 | 0.15 ± 0.02 | 0.17 ± 0.02 | 0.35 | 0.02 |
| Shell SLD ($10^{-6}$ Å$^{-2}$) | – | – | 9.47 | 9.63 | 9.55 | 9.70/9.27 | 9.33 | 9.57 | 9.57 | 9.66/9.30 | 9.44 |
| n[h] ($10^{15}$ cm$^{-3}$) | – | – | 22.8 | 5.9 | 6.0 | 13.4 | 22.6 | 6.42 | 6.77 | 18.3 | 8.1 |
| $N_{agg}$[i] | – | – | 21 | – | – | 56 | 21 | 82 | 77 | 63 | 9 |
| Hydro $D_h$[j] (nm) | 6.3 | 6.2 | 18.7 | 26.7 | 21.1 | 30.9 | | 30 | 33 | 35 | |
| PDI[k] | 0.22 | 0.23 | 0.16 | 0.22 | 0.13 | 0.23 | | 0.22 | 0.19 | 0.09 | |

[a]$\varphi_{unimer}$: volume fraction of unimers. [b]$R_g$: radius of gyration of unimers. [c]$\varphi_{micelle}$: volume fraction of micelles. [d]$R_{core}$: average core radius. [e]$R_{shell}$: shell thickness. [f]D: diameter of micelle. [g]σ: core polydispersity. [h]n: micelle number density. [i]$N_{agg}$: aggregation number of the micelle. *j*$D_h$: hydrodynamic diameter of micelle measured by the DLS. [k]PDI: Polydisperse Index calculated by DLS measurement.

on micellization process. The ibuprofen concentration used in this study is one fifth of the saturated concentration of each system at 37 °C. According to the HSDSC thermograms, addition of ibuprofen into the systems substantially decreases the CMT of copolymers either in neat or binary systems. For instance, $T_m$ of neat F108 1.5 wt% system without ibuprofen is 35.7 °C and 33.0 °C for the system with ibuprofen. Furthermore, the area of micellization peak for the system with ibuprofen added is obviously much smaller than that of the system without drug revealing smaller endothermic heat needed for micellization process.

The ibuprofen solubility in the mixed-Pluronic systems was then evaluated by UV/Vis spectrometry. Effect of molecular weight and hydrophilicity of copolymers on drug incorporation were then carefully examined. It has been found [12] that Pluronics with larger molecular weight (F108 in the Fx8 series) and more hydrophobic characteristics (P84 in the F8x series) exhibit better solubilization capacity for ibuprofen. In this study, the hydrophobic Pluronic P123 with relatively large molecular weight was introduced into the system to enhance the ibuprofen solubility in the binary Pluronic system.

The solubility of ibuprofen in the binary P123 + F88 and P123 + F87 systems, as illustrated in Fig. 4, are indeed 1.95 and 1.56 times that of neat F88 and F87 systems [12], respectively. However, the solubility of ibuprofen in the binary 0.5 wt% P123 + 0.368 wt% F84 system almost remains the same as that in the neat 0.868 wt% P84 system. Furthermore, ibuprofen solubility in pure water is 0.0206 mg/ml at 35 °C and 0.0264 mg/ml at 40 °C [38], that could be linearly interpolated to estimate the solubility of ibuprofen at 37 °C around 0.0229 mg/ml. The solubility of ibuprofen in the P123 + P84 system is 114 times higher than that in pure water.

Although those three binary P123 + F88, P123 + F87 and P123 + F84 systems have the same total amount of PO units, that does not lead to identical solubilization capacity of ibuprofen. Effect of PEO chain length of F8x on the solubility of ibuprofen in the mixed Pluronic P123 + F8x systems at a fixed PPO chain length is in the order of F84 > F87 ≈ F88. It should be pointed out that in this study all the solubility data were measured at 37 °C, which is lower than $T_{m2}$ of the P123 + F88 and P123 + F87 systems. Slightly lower solubility of ibuprofen in the P123 + F88 and P123 + F87 systems at 37 °C may be due to not all the F88 (or F87) molecules being integrated into mixed micelles. Note that the P123 + P84 system exhibits the most outstanding solubilization capacity of ibuprofen among all the binary Pluronic systems at 37 °C in this study. Hydrophobicity of Pluronic copolymer again plays an important role in drug loading. It may be attributed to the longer PEO chain inducing more steric hindrance for the drug entrapment for F88 and F87.

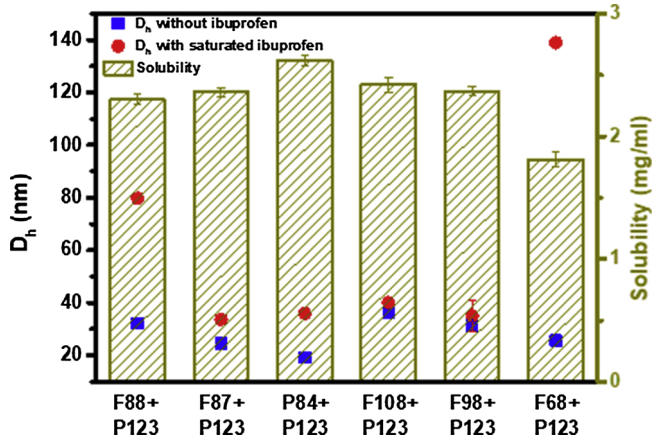


**Fig. 4.** Hydrodynamic diameter of aggregates ($D_h$, nm) and solubility of ibuprofen in binary mixed Pluronic P123 + F8x (x = 8, 7 and 4) and P123 + F × 8 (x = 10, 9 and 6) system at 37 °C.

There is an obvious increase in size of aggregates after the addition of ibuprofen, as the hydrodynamic diameter of aggregates shown in Fig. 4, implying that most of ibuprofen are entrapped inside the micelles to enlarge the size of micelles. The size of aggregates for the P123 + F88 system is obviously larger than the other two P123 + F87 and P123 + P84 systems in the presence/absence of ibuprofen. Also, PDI for the P123 + F88 system with ibuprofen loaded is 0.12, and drops to only 0.08 and 0.04 for the, respectively, P123 + F87 and P123 + P84 system with ibuprofen loaded. Hence, unimodal distribution of aggregates are still maintained after drug loading in P123 + F87 or + P84 with exceptional drug solubilization capacity.

For system P123 + F × 8 (x = 10, 9, 8 and 6), solubility of ibuprofen (Fig. 4) increases by 1.7 times higher than that of the neat F108 or F98 system [12]. The ibuprofen solubility in the P123 + F68 system even shows 4 times higher than that of neat F68 system due to intrinsically low solubility of ibuprofen in the neat F68 system [12]. It is interesting to find out that the solubility of ibuprofen in the mixed Pluronic P123 + F × 8 systems with different molecular weights at the fixed PEO/PPO mass ratio (80/20) is in the order of F108 ≈ F98 ≈ F88 > F68. With the addition of P123, the effect of block chain length of Fx8 on ibuprofen solubility is smeared. On the other hand, it is obvious to observe that the longer the block chain length, the smaller the micelle size. It is evidenced that PEO chains on each side of the Pluronic molecules function as a stabilizer dispersing particles in aqueous solution. The longer the corona chains, the stronger the ability of micelles dispersing with small volume in solution. PDIs for the three mixtures P123 + F108, + F98 and + F68 after loading drug are 0.25, 0.28 and 0.06, respectively, at 37 °C.

Furthermore, in order to investigate structures of drug loaded micelles as well as process of drug encapsulation, we conducted a series of experiments of DLS and SAXS at a fixed ibuprofen concentration of 0.66 mg/ml incorporated in the P123 + P84, + F108 and + F68 systems. For the P123 + P84 + ibuprofen system, according to the DLS, particle size remains stable at temperature higher than 25 °C (Table S5). PDI dropped to 0.06 from 0.10 after the addition of drug, which indicates promotion of micellization and unification of aggregations in the presence of drug. The SAXS results demonstrate that the addition of ibuprofen would decrease the volume fraction of P84 unimers from 0.38% to 0.22% for the P123 + P84 system at 25 °C (Table S5), implying more P84 unimers integrating into the aggregations for drug encapsulation. Mixed micelles in the presence of ibuprofen with core radius of 6.2 nm and shell thickness of 3.1 nm formed at 25 °C that is similar to that of the P123 + P84 mixed micelles (5.1 and 3.7 nm for core radius and shell thickness, respectively, at 45 °C). It is interesting to note that core radius enlarges from 5.1 to 6.2 nm after drug incorporation, which could be also evidenced by the form factor shift to lower q region on the scattering pattern (Fig. 2). Therefore, it is believed that the P123 + P84 mixed micelles are formed and simultaneously incorporates the ibuprofen inside the core region at 25 °C. It is consistent with the results from HSDSC (Fig. S12) in which area of the first endothermic peak diminishes prominently for the drug-loading system compared to that without drug indicating less energy required for the micellization in the presence of drug. When the temperature is increased even higher than 37 °C, more copolymers develop into assemblies since the micelle number density dramatically increases from $9.38 \times 10^{15}$ (25 °C) to $11.8 \times 10^{15}$ $cm^{-3}$ (37 °C) along with decreasing volume fraction of unimers. Micelles with stable structures were observed until 45 °C. According to the observations on the P123 + P84 micellization process with drug incorporated, some mixed micelles formed by P123 and P84 copolymers with drug enclosed into its core region at 25 °C. Increasing temperature continues promoting mixed micelle formation till 45 °C.

Variation of the micellization process as a function of temperature for the P123 + F108 + ibuprofen system as reported in Table S5 is similar to that of the P123 + P84 + ibuprofen system. Owing to the higher CMT of neat F108 system compared to that of neat P84 system,

micelles are formed mainly by P123 with some F108 copolymers at 25 °C since similar core-shell structure of P123 was observed. The dramatic increase in core radius from 4.9 to 6.5 nm indicates that ibuprofen is also incorporated in the micelle core. Mixed micelle formation with drug stably encapsulated was thus observed when temperature higher than 37 °C.

The P123 + F68 + ibuprofen system also demonstrates similar tendencies. Interestingly, bimodal distribution of aggregates with neat F68 micelles and mixed micelles coexisting was still observed in the P123 + F68 + ibuprofen system at 65 °C, as the SAXS results reported in Table 4. However, the addition of ibuprofen indeed enhances cooperative binding between copolymers P123 and F68, as one can see that the number ratio of neat F68 micelles and mixed micelles becomes 30/70. The number density of mixed micelles (and neat F68 micelles) in the P123 + F68 + ibuprofen system is substantially larger (and smaller) than that in the P123 + F68 system without ibuprofen. That is, much more F68 molecules are integrated into P123 micelles to form mixed micelles for encapsulating ibuprofen, compared to the system without ibuprofen, as schematically illustrated in Fig. 3.

## 4. Conclusions

In this study, HSDSC, DLS and SAXS were applied to explore cooperative binding between parent copolymers and solubility of drug (ibuprofen) in binary mixed Pluronic systems. The binary P123 + F108, + F98, + F88 and + F68 systems were chosen to systematically explore the effect of molecular weight of Fx8 and the binary P123 + F88, + F87 and + P84 systems were used to systematically explore the effect of hydrophobicity of Pluronic on the temperature-dependent micellization process in the binary Pluronic systems.

All the HSDSC thermograms for these binary Pluronic systems consistently exhibit two endothermic (micellization) peaks. The block copolymer (P123) with a lower CMT would undergo dehydration at $T_{m1}$ to form neat P123 micelles. This was further verified by the SAXS for P123 dominated micelles coexisting with unimers of the other copolymer at low temperatures. Since the volume fraction of unimers of the other copolymer decreases along with increasing temperature, the other parent copolymer is gradually integrated into neat P123 micelles. When the temperature is further increased beyond its $T_{m2}$, the Pluronic with a higher CMT would then dehydrate to form the mixed micelles. Also, it was found that corona length of the micelles increases to the characteristic length of the more hydrophilic one indicating the integration of the other copolymer to form mixed micelles. Consider the binary P123 + F108 system as an example. All the binary mixed Pluronic systems, except the P123 + F68 system, examined in this study exhibit cooperative binding to form unimodal distribution of mixed micelles. The SAXS result shows that the binary P123 + F68 system at 65 °C exhibits bimodal distribution of aggregates with coexisting of neat F68 micelles and P123 + F68 mixed micelles. That is, the P123 and F68 would exhibit rather weak cooperative binding to form mixed micelles and majority of F68 would self-assemble into neat F68 micelles.

The parent copolymers, such as, P123 + F88, P123 + F87, and P123 + F84, with dissimilar PPO chain lengths apart by 28 PO units still exhibit cooperative binding to form unimodal distribution of mixed micelles. While the parent copolymers P123 + F68 have different PPO chain lengths apart by 37 PO units, that weakens cooperative binding between P123 and F68 to form neat F68 micelles instead. The evolution of temperature-dependent micellization process and micelle conformation for the binary P123 + F108 and P123 + F68 systems are concluded and schematically illustrated in Fig. 3.

Addition of ibuprofen into the binary Pluronic system significantly decreases the CMT and endothermic heat of micellization. On the study of hydrophobic effect, solubilization capacity of P123 + F8x systems is in the order of P84 > F87 ≈ F88. Moreover, the participation of P123 smeared effect of molecular weight of Pluronic Fx8 on solubilization capacity, which is in the order of F108 ≈ F98 ≈ F88 > F68. The SAXS results demonstrate that the drug (ibuprofen) is mainly encapsulated in the core of neat P123 micelles at low temperatures. Increasing temperature continues triggering the micellization process forming mixed micelles with stable two shells structure. Moreover, addition of ibuprofen promotes cooperative binding between P123 and F68 to enhance mixed micelle formation leading to an increase (or a decrease) in number density of mixed micelles (or neat F68 micelles) compared to the system without ibuprofen.

## Declaration of Competing Interest

The authors declare no competing financial interest.

## Acknowledgment

This work was supported by the Ministry of Science and Technology of Taiwan via grant numbers: NSC102-2923-E-002-005-MY3 and MOST105-2221-E-002-209-MY3.

## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.colsurfb.2019.110461.

## References

[1] G. Abdelbary, A. Makhlouf, Adoption of polymeric micelles to enhance the oral bioavailability of dexibuprofen: formulation, in-vitro evaluation and in-vivo pharmacokinetic study in healthy human volunteers, Pharm. Dev. Technol. 19 (2014) 717–727.
[2] J.W. Valle, A. Armstrong, C. Newman, V. Alakhov, G. Pietrzynski, J. Brewer, S. Campbell, P. Corrie, E.K. Rowinsky, M. Ranson, A phase 2 study of SP1049C, doxorubicin in P-glycoprotein-targeting pluronics, in patients with advanced adenocarcinoma of the esophagus and gastroesophageal junction, Invest. New Drugs 29 (2011) 1029–1037.
[3] J. Shen, Q. Yin, L. Chen, Z. Zhang, Y. Li, Co-delivery of paclitaxel and survivin shRNA by pluronic P85-PEI/TPGS complex nanoparticles to overcome drug resistance in lung cancer, Biomaterials 33 (2012) 8613–8624.
[4] C.F. de Freitas, I.R. Calori, A.C.P. da Silva, L.V. de Castro, F. Sato, D.S. Pellosi, A.L. Tessaro, W. Caetano, N. Hioka, PEG-coated vesicles from Pluronic/lipid mixtures for the carrying of photoactive erythrosine derivatives, Colloids Surf. B. Biointerfaces 175 (2019) 530–544.
[5] M. Younus, A. Hawley, B.J. Boyd, S.B. Rizwan, Bulk and dispersed aqueous behaviour of an endogenous lipid, selachyl alcohol: effect of Tween 80 and Pluronic F127 on nanostructure, Colloids Surf. B Biointerfaces 169 (2018) 135–142.
[6] V. Patel, D. Ray, A. Bahadur, J. Ma, V. Aswal, P. Bahadur, Pluronic®-bile salt mixed micelles, Colloids Surf. B Biointerfaces 166 (2018) 119–126.
[7] H.-L. Sung, Y.-L. Fan, Kimberly Yeh, Y.-F. Chen, L.-J. Chen, A new hydrate form of diflunisal precipitated from a microemulsion system, Colloids Surf. B Biointerfaces 109 (2013) 86-73.
[8] S. Jafarzadeh-Holagh, S. Hashemi-Najafabadi, H. Shaki, E. Vasheghani-Farahani, Self-assembled and pH-sensitive mixed micelles as an intracellular doxorubicin delivery system, J. Colloid Interface Sci. 523 (2018) 179–190.
[9] S. Gaisford, A.E. Beezer, J.C. Mitchell, Diode-array UV spectrometric evidence for cooperative interactions in binary mixtures of pluronics F77, F87, and F127, Langmuir 13 (1997) 2606–2607.
[10] T. Liu, V.M. Nace, B. Chu, Self-assembly of mixed amphiphilic triblock copolymers in aqueous solution, Langmuir 15 (1999) 3109–3117.
[11] M. Singh, V. Thakur, R. Deshmukh, A. Sharma, M. Rathore, A. Kumar, N. Mishra, Development and characterization of morin hydrate-loaded micellar nanocarriers for the effective management of Alzheimer's disease, J. Microencapsulation 35 (2018) 137–148.
[12] C.-F. Lee, H.-W. Tseng, P. Bahadur, L.-J. Chen, Synergistic effect of binary mixed-Pluronic systems on temperature dependent self-assembly process and drug solubility, Polymers 10 (2018) 105.
[13] G.E. Newby, I.W. Hamley, S.M. King, C.M. Martin, N.J. Terrill, Structure, rheology and shear alignment of Pluronic block copolymer mixtures, J. Colloid Interface Sci. 329 (2009) 54–61.
[14] Z. Fan, C. Chen, X. Pang, Z. Yu, Y. Qi, X. Chen, H. Liang, X. Fang, X. Sha, Adding vitamin E-TPGS to the formulation of Genexol-PM: specially mixed micelles improve drug-loading ability and cytotoxicity against multidrug-resistant tumors significantly, PLoS One 10 (2015) e0120129.
[15] S.H. Oh, J.G. Kang, J.H. Lee, Co-micellized Pluronic mixture with thermo-sensitivity and residence stability as an injectable tissue adhesion barrier hydrogel, J. Biomed. Mater. Res. B Appl. Biomater. 106 (2018) 172–182.
[16] M. Zhang, M. Djabourov, C. Bourgaux, K. Bouchemal, Nanostructured fluids from pluronic® mixtures, Int. J. Pharm. 454 (2013) 599–610.
[17] A.M. Pragatheeswaran, S.B. Chen, C.-F. Chen, B.-H. Chen, Micellization and

gelation of PEO-PPO-PEO binary mixture with non-identical PPO block lengths in aqueous solution, Polymer 55 (2014) 5284–5291.
[18] S. Gaisford, A.E. Beezer, J.C. Mitchell, Diode-array UV spectrometric evidence for cooperative interactions in binary mixtures of pluronics F77, F87, and F127, Langmuir 13 (1997) 2606–2607.
[19] C. Chaibundit, N.M. Ricardo, Fd.M. Costa, S.G. Yeates, C. Booth, Micellization and gelation of mixed copolymers P123 and F127 in aqueous solution, Langmuir 23 (2007) 9229–9236.
[20] S.R. Croy, G.S. Kwon, The effects of Pluronic block copolymers on the aggregation state of nystatin, J. Control. Release 95 (2004) 161–171.
[21] A.V. Kabanov, E.V. Batrakova, N.S. Melik-Nubarov, N.A. Fedoseev, T.Y. Dorodnich, V.Y. Alakhov, V.P. Chekhonin, I.R. Nazarova, V.A. Kabanov, A new class of drug carriers: micelles of poly (oxyethylene)-poly (oxypropylene) block copolymers as microcontainers for drug targeting from blood in brain, J. Control. Release 22 (1992) 141–157.
[22] T. Sakai, P. Alexandridis, Single-step synthesis and stabilization of metal nanoparticles in aqueous Pluronic block copolymer solutions at ambient temperature, Langmuir 20 (2004) 8426–8430.
[23] S.A. Tanner, S. Amin, C.J. Kloxin, J.H. van Zanten, Microviscoelasticity of soft repulsive sphere dispersions: tracer particle microrheology of triblock copolymer micellar liquids and soft crystals, J. Chem. Phys. 134 (2011) 174903.
[24] H.-W. Tsui, J.-H. Wang, Y.-H. Hsu, L.-J. Chen, Study of heat of micellization and phase separation for Pluronic aqueous solutions by using a high sensitivity differential scanning calorimetry, Colloid Polym. Sci. 288 (2010) 1687–1696.
[25] Y.-H. Hsu, H.-W. Tsui, S.-Y. Lin, L.-J. Chen, The origin of anomalous positive heat capacity change upon micellization of Pluronic triblock copolymer F108 in aqueous solutions: effect of PEO-PPO diblock impurities, Colloids Surf. A Physicochem. Eng. Asp. 509 (2016) 109–115.
[26] U.-S. Jeng, C.H. Su, C.-J. Su, K.-F. Liao, W.-T. Chuang, Y.-H. Lai, J.-W. Chang, Y.-J. Chen, Y.-S. Huang, M.-T. Lee, A small/wide-angle X-ray scattering instrument for structural characterization of air–liquid interfaces, thin films and bulk specimens, J. Appl. Crystallogr. 43 (2010) 110–121.
[27] P.-W. Yang, T.-L. Lin, Y. Hu, U.-S. Jeng, A time-resolved study on the interaction of oppositely charged bicelles–implications on the charged lipid exchange kinetics, Soft Matter 11 (2015) 2237–2242.
[28] H.-W. Tsui, Y.-H. Hsu, J.-H. Wang, L.-J. Chen, Novel behavior of heat of micellization of Pluronics F68 and F88 in aqueous solutions, Langmuir 24 (2008) 13858–13862.
[29] Y.-H. Hsu, H.-W. Tsui, C.-F. Lee, S.-H. Chen, L.-J. Chen, Effect of alcohols on the heat of micellization of Pluronic F88 aqueous solutions, Colloid Polym. Sci. 293 (2015) 3403–3415.
[30] P. Bartlett, R. Ottewill, A neutron scattering study of the structure of a bimodal colloidal crystal, J. Chem. Phys. 96 (1992) 3306–3318.
[31] S. Manet, A. Lecchi, M. Impéror-Clerc, V. Zholobenko, D. Durand, C.L. Oliveira, J.S. Pedersen, I. Grillo, F. Meneau, C. Rochas, Structure of micelles of a nonionic block copolymer determined by SANS and SAXS, J. Phys. Chem. B 115 (2011) 11318–11329.
[32] J. Bhattacharjee, G. Verma, V. Aswal, P. Hassan, Small angle neutron scattering study of doxorubicin-surfactant complexes encapsulated in block copolymer micelles, Pramana 71 (2008) 991–995.
[33] S. Alexander, T. Cosgrove, T.C. Castle, I. Grillo, S.W. Prescott, Effect of temperature, cosolvent, and added drug on pluronic–flurbiprofen micellization, J. Phys. Chem. B 116 (2012) 11545–11551.
[34] P. Debye, The intrinsic viscosity of polymer solutions, J. Chem. Phys. 14 (1946) 636–639.
[35] L. Fan, M. Degen, N. Grupido, S. Bendle, P. Pennartz, Effects of molecular weight, temperature and salt on the self assembly of triblock copolymer solutions, Mater. Sci. Eng. A 528 (2010) 127–136.
[36] Y. Liu, S.-H. Chen, J.S. Huang, Small-angle neutron scattering analysis of the structure and interaction of triblock copolymer micelles in aqueous solution, Macromolecules 31 (1998) 2236–2244.
[37] N. Jain, V. Aswal, P. Goyal, P. Bahadur, Salt induced micellization and micelle structures of PEO/PPO/PEO block copolymers in aqueous solution, Colloids Surf. A Physicochem. Eng. Asp. 173 (2000) 85–94.
[38] L.C. Garzón, F. Martínez, Temperature dependence of solubility for ibuprofen in some organic and aqueous solvents, J. Solution Chem. 33 (2004) 1379–1395.