
Pankaj Singla,†‡ Onkar Singh,‡ Shagun Sharma,‡ Kai Betlem,§ Vinod K. Aswal,∥ Marloes Peeters,⊥ and Rakesh Kumar Mahajan†∥

†Department of Chemistry, UGC-Centre for Advanced Studies-I, Guru Nanak Dev University, Amritsar 143005, India
‡Faculty of Science and Engineering, Division of Chemistry and Environmental Sciences, Manchester Metropolitan University, John Dalton Building, Chester Street, Manchester M15 6BH, U.K.
§Département de Physique, ULB, CP 238, av. F. D. Roosevelt, Bruxelles B-1050, France
∥Solid State Physics Division, Bhabha Atomic Research Centre, Mumbai 400085, India
⊥School of Engineering, Newcastle University, Merz Court, Newcastle upon Tyne NE17RU, U.K.

Supporting Information

ABSTRACT: Pluronics (tri-block copolymers) have a significant role in the pharmaceutical industry and are being used to enhance the solubility and delivery of hydrophobic drugs in different marketed formulations. However, instability and unsatisfactory drug-loading capacity are the major weak spots of these pluronic micelles. The present research work is designed to solve the existing issues by the solubilization study of hydrophobic drugs in different pluronic micelles at variable temperatures. The solubilization of the hydrophobic antiepileptic drug lamotrigine (LAM) in five different pluronic micelles viz. P84, P85, F127, F108, and F68 was studied at different temperatures, 37, 47, and 57 °C, using UV−visible spectroscopy. The solubilization of LAM in pluronic micelles increased with the increase in temperature. Small-angle neutron scattering (SANS) measurements were used to observe the morphological and structural changes taking place in pluronics by increasing the temperature. The SANS results showed the morphological changes of spherical P84 micelles to prolate ellipsoidal micelles at 57 °C due to remarkable increase in the aggregation number. This morphological conversion was further confirmed by the heat transfer method (HTM) and dynamic light scattering (DLS) measurements. DLS measurements confirmed that LAM-loaded micelles showed a greater hydrodynamic diameter \( D_h \) compared to unloaded micelles, assuring LAM solubilization in the pluronic micelles. The rate of controlled release of LAM from five different pluronic micelles was accessed by using different kinetic models to evaluate the in vitro release profile. This is the first report in which HTM measurements are established for the analysis of morphological changes in the thermoresponsive pluronic micelles in real time. The present work corroborates how we can control the drug-loading capacity, morphological structure of the drug carrier, as well as drug release by simply changing the temperature of pluronic micellar media.

1. INTRODUCTION

Poor water solubility of drugs is the most important hurdle in the pharmaceutical industry and research. The development of better formulations of approved drugs is one of the most successful strategies of scientific and industrial importance.1−3 In this context, delivery of hydrophobic drug via pluronic micelles is an interesting alternative. Pluronics, triblock copolymers, are US-FDA approved excipients for many marketed formulations and are currently used in parenteral injections. Pluronics consist of hydrophilic block poly-(ethylene oxide) (PEO) and hydrophobic block poly-(propylene oxide) (PPO) in a triblock structure of PEO−PPO−PEO. The hydrophilic PEO block of the pluronic micelle is effective against serum protein adsorption, which improves the stability of the nanomicellar system and increases drug delivery. Pluronics play a significant role in pharmaceutical performance to increase the solubility, bioavailability, release, and stability of hydrophobic drug molecules.4−6 Pluronics modulate the drug efflux transporter P-glycoprotein on the blood brain barrier (BBB) results in the enhancement the BBB penetration of pluronic-loaded hydrophobic drugs. According to recent findings, in the drug

Received: April 3, 2019
Accepted: June 18, 2019
Published: June 28, 2019

DOI: 10.1021/acsomega.9b00939
ACS Omega 2019, 4, 11251−11262

ACS Publications © 2019 American Chemical Society

11251
resistance, P-glycoproteins are over-expressed and pluronic can act as modulators of P-glycoproteins; hence, as a drug carrier for the treatment of multidrug-resistant diseases, pluronic micellar formulations can be useful.9–13

Lamotrigine (LAM), 3,5-diamino-6-(2,3-dichlorophenyl)-1,2,4-triazine, is a novel antiepileptic drug, and it is also used in the treatment of primary and secondary tonic–clonic seizures and seizures associated with Lennox–Gastaut syndrome. The antiepileptic effect of LAM is due to the stabilizing presynaptic neuronal membranes; moreover, LAM inhibits sodium currents by selectively binding with the inactivated state of the sodium channel and results in repressing the release of the excitatory amino acid (such as, glutamate and aspartate).14,15 LAM belongs to the biopharmaceutical classification system class II drug with a pH of 5.70. It is practically insoluble in ethanol, and the solubility of LAM in water is only 0.17 mg/mL at 25 °C.16 Therefore, the dissolution of LAM is the rate-limiting step for its efficient absorption and bioavailability in the body. Different research scientists have attempted to increase the water solubility and dissolution of LAM to improve its therapeutic effects. The following strategies have been employed: use of β-cyclodextrin inclusion complexes, LAM salts, and solid dispersions of LAM.17–20 To enhance the therapeutic efficacy of LAM, its aqueous solubility needs to be improved. In order to achieve this goal, we have prepared the five different pluronic micellar formulations at three different temperatures to optimize the solubility of LAM.

There are very limited reports in the literature on the effect of temperature on the solubilization of hydrophobic drugs in the pluronic micelles. Basak and Bandopadhyay described the encapsulation of the three drug molecules, erythromycin, ibuprofen, and aspirin in pluronic F127 micelles using dynamic light scattering (DLS) and fluorescence measurements. They observed that the average hydrodynamic radii (Dh) of the pluronic F127 micelles were increased upon drug incorporation.21 Kadam et al. reported the solubilization of the hydrophobic drug carbamazepine in pluronic micelles (P103, P123, P84, and F127) at two different temperatures by employing UV spectroscopy and DLS measurements. They observed that the solubilization capacity of carbamazepine in the pluronic micelles increased with the increase in temperature and concentration of pluronics.22 Raval et al. studied the micellar behavior of the aqueous solutions of six pluronics viz. P84, F88, P103, P105, P123, and F127 in water and studied the solubility of three poorly water soluble anticancer drugs, genistein, paclitaxel, and quercetin, using UV–visible spectroscopy, high-performance liquid chromatography, and DLS. All these investigated drugs showed improved solubility in pluronic P123 and P103 micelles with the increase in temperature and salt concentration. In vitro drug-release profiles of these formulations demonstrated slower release of the drugs in pluronic micelles. The in vitro cytotoxicity of the drug-loaded pluronic micelles was studied on a breast cancer MCF-7 cell line, which showed higher anticancer activity as compared to the bare drug. These results show that pluronics are promising candidates for anticancer drug delivery.23 In contrast to these studies, the main objective of our work is to find the relation between temperature, the structure of the pluronics, and its drug solubilization capacity. For this purpose, we have studied the solubilization of the hydrophobic drug LAM in five different pluronic micelles viz. P84 (PEO 19PPO 41PEO 19), P85 (PEO 26PPO 40PEO 26), F127 (PEO 100PPO 46PEO 100), F108 (PEO 135PPO 50PEO 135), and F68 (PEO 27PPO 27PEO 72) with 40, 50, 70, 80, and 80% PEO content, respectively, at different temperatures (37, 47, and 57 °C) using UV–visible measurements. The molecular structures of LAM and different pluronics are presented in Scheme 1.

Scheme 1. Molecular Structure of (a) Pluronics and (b) LAM

![Scheme 1](image)

The solubility pattern of the drug in the different pluronics follow the order P84 > P85 > F127 > F108 > F68 at all % w/v. These results can be explained by taking into consideration the PEO effect and the PPO effect of the different pluronics. Pluronic P84 shows higher solubilization than pluronic P85, irrespective of the presence of almost similar PO units (43 and 40 PPO units in P84 and P85, respectively) and differing in the presence of hydrophilic PEO units (2 × 19 and 2 × 26 PEO units in P84 and P85, respectively). On the other hand, pluronics F127 and F108 with small difference in PO units (65 and 50 PPO units in F127 and F108, respectively) and unloaded micelles has been studied employing DLS measurements. Furthermore, the effect of the temperature and LAM solubilization in the investigated different pluronic micelles has been adjudged employing indigenously built small-angle neutron scattering (SANS) and the heat transfer method (HTM).24–26 In vitro drug release studies were performed to evaluate the relation of LAM release from these micelles with respect to increase in the temperature.

2. RESULTS AND DISCUSSION

2.1. Solubilization of LAM. 2.1.1. Effect of Concentration. The solubilization of LAM in the different pluronic F68, F108, F127, P85, and P84 micellar solutions was determined by UV–visible spectroscopy. The UV absorption spectra of LAM solubilized in the F108, F127, P85, and P84 micelles were measured in the concentration range of 1–5% w/v at 37 °C (Figure S1, Supporting Information). The UV absorption of LAM increased significantly in all studied pluronic solutions except for pluronic F68, implying that the pluronic micelles are able to solubilize LAM. The difference in behavior of pluronic F68 has been ascribed to its inability to self-assemble at the studied concentration and temperature, thereby, making it difficult to solubilize the drug.27,28 The results for the solubilization capacities of the different pluronics for LAM are summarized in Figure 1a.

The solubility pattern of the drug in the different pluronics follow the order P84 > P85 > F127 > F108 > F68 at all % w/v. These results can be explained by taking into consideration the PEO effect and the PPO effect of the different pluronics. Pluronic P84 shows higher solubilization than pluronic P85, irrespective of the presence of almost similar PO units (43 and 40 PPO units in P84 and P85, respectively) and differing in the presence of hydrophilic PEO units (2 × 19 and 2 × 26 PEO units in P84 and P85, respectively). On the other hand, pluronics F127 and F108 with small difference in PO units (65 and 50 PPO units in F127 and F108, respectively) and unloaded micelles has been studied employing DLS measurements. Furthermore, the effect of the temperature and LAM solubilization in the investigated different pluronic micelles has been adjudged employing indigenously built small-angle neutron scattering (SANS) and the heat transfer method (HTM).24–26 In vitro drug release studies were performed to evaluate the relation of LAM release from these micelles with respect to increase in the temperature.
The solubility in water also increases with increase in temperature due to thermal agitation, in addition to the fact that LAM in the area for solubilization of LAM in the pluronic micelles (Supporting Information). This was attributed to the increase of pluronics as the length of the corona increases. Our results are in agreement with the work of Alexandridis et al., which states that the micellization capacity of pluronics decreases as the PEO units forming the hydrophilic corona or PPO, forming the hydrophobic core region of the pluronic micelles decreases as the temperature is the driving force for the solubilization of drugs. Upon increasing the temperature, there was dehydration of the PEO region and this leads to a decrease in the solubilizing area available for the drug. The micellar growth has been confirmed by the SANS measurements (Section 2.3). It is also important to mention that the CMT (critical micelle temperature) value of the pluronic P84, P85, F127, F108, and F68 (at 5% w/v) is 23, 25, 19.5, 24.5, and 40 °C, respectively. Pluronic F127 has the lowest CMT value, and hence, the drug solubilization capacity of this pluronic is lower than pluronic P85 and P84 that have the high CMT values. From these results, it was concluded that the solubility of drugs is not only dependent on the CMTs of the pluronics. Other important molecular characteristic such as PEO content also plays an important role in the solubilization of the drugs. The compilation of the hydrophobic drug LAM solubilization data for a wide range of pluronic block copolymers at different temperatures presented in this article is, to the best of our knowledge, the most complete in literature.

The drug loading efficiency (DLE) is the percentage of solubilized LAM from the LAM fed in pluronic micelles during the solubilization process and can be calculated from the following eq 1.

\[
DLE = \frac{\text{total weight of drug solubilized} - \text{weight of drug in water}}{\text{weight of drug fed initially}} \times 100
\]

The values of DLE of 1–5% w/v pluronics at 37 °C and 5% w/v pluronics at 37, 47, and 57 °C are depicted in Figure S3a,b respectively (Supporting Information).

The DLE of P84 is higher as compared to different pluronics under study at all concentrations and temperatures by virtue of its hydrophobicity. It is well established that hydrophobicity of pluronics can be improved by increasing the PPO content. Accordingly, P84, having a 60% PPO content which is relatively higher than other pluronics under study, displayed the highest DLE. The partition coefficient is a crucial parameter used for the assessment of the micellar systems as potential drug carriers. The micellar/water partition coefficient can be calculated from the photo-spectrometric results and is defined as the ratio of the amount of drug solubilized in the micelle and the drug...
present in water at a particular amphiphilic concentration (eq 2).

\[
P = \frac{S_{\text{tot}} - S_{w}}{S_{w}}
\]

(2)

where \(S_{\text{tot}}\) and \(S_{w}\) is the total solubility of LAM and solubility of LAM in water, respectively. The partition coefficients have been computed, depicted in Figure S4a for pluronic F108, F127, P85, and P84 micelles in the concentration range of 1−5% w/v at 37 °C and shown in Figure 2a for 5% F68, F108, F127, P85, and P84 at different temperature viz. 37, 47, and 57 °C. It was observed that the value of the partition coefficient increased by increasing the temperature among all pluronic. The highest value of the partition coefficient was noted in P84 micelles at all temperatures, confirming the better partitioning of solubilized LAM in these micelles compared to other investigated pluronic micelles. Higher partitioning of LAM in P84 micelles at all temperatures revealed the higher drug solubilization in the core of these micelles by inducing a favorable hydrophobic environment compared to other investigated pluronic micelles.

The standard free energy of solubilization (\(\Delta G^\circ\)) can be calculated from the partition coefficient and is represented by the following expression

\[
\Delta G^\circ = -RT \ln P
\]

(3)

where \(R\) denotes the gas constant (J/mol), \(T\) is the temperature (in Kelvin), and \(P\) is the partition coefficient. The \(\Delta G^\circ\) value depicts the Gibbs free energy of transfer of 1 mol of the drug in the micellar phase. The \(\Delta G^\circ\) values are depicted in the Figure S4b for pluronic F108, F127, P85, and P84 micelles in the concentration range of 1−5% w/v at 37 °C and shown in Figure 2b for 5% F68, F108, F127, P85, and P84 at different temperature viz. 37, 47, and 57 °C. Figure 2b reveals that the values of \(\Delta G^\circ\) are negative except pluronic F68 at 37 and 47 °C, showing that the process of LAM solubilization in pluronic micelles is spontaneous and transfer of water molecules from the micellar core induces a more hydrophobic environment. The positive \(\Delta G^\circ\) values of pluronic F68 might be due to the inability of F68 to form the micelles at the concentration and temperature (37 and 47 °C) being studied.

2.2. Dynamic Light Scattering. DLS experiments were performed to find the hydrodynamic diameter (\(D_h\)) of pluronic micelles as a function of temperature increase as depicted in Figure 3a,b for the \(D_h\) of aqueous solutions of 5% w/v P84 and F127 at increasing temperatures 37, 47, and 57 °C. \(D_h\) of aqueous solutions of 5% w/v P85 and 5% w/v F108 and F68 at increasing temperatures 37, 47, and 57 °C are presented in Figure S5a−c (Supporting Information), respectively. In the case of hydrophobic pluronics, viz. P84 and P85, the micellar size was observed to increase with increasing the temperature, which revealed that aggregation is stimulated by the increase in temperature. At 37 °C, the \(D_h\) of P84 micelles is 13.54 nm, but at the temperature 47 and 57 °C, \(D_h\) of P84 micelles increased from...
13.54 to 18.16 nm and 37.54 nm, respectively; hydrophobic pluronic P85 exhibits similar increase in \(D_h\) of micelles. Pluronics F127 and F108 formed loosely packed micelles involving few monomers, so an increase in temperature results in the removal of water and formation of more closely packed micelles with altogether decrease in the \(D_h\) of the micelles. In the case of very hydrophilic pluronic F68, different peaks correlating to micelles, clusters, and unimers are present at 37 °C. Increase in the temperature, decreases the polydispersity and makes the unimers and clusters disappear, which results in the single micellar peak. \(D_h\) of 5% w/v P84 and F127, LAM loaded and unloaded micelles in aqueous solution at 37, 47, and 57 °C were measured and are depicted in Figure 4a,b, respectively.

2.3. SANS Measurements. Micellar structure of the 5% w/v pluronics, viz. F68, F108, F127, P85, and P84 were obtained from SANS measurements at different temperatures: 37, 47, and 57 °C. Scattering curves of pluronic P84 and F68 are presented in Figure 5a,b respectively, and pluronic P85, F127, and F108 are presented in Figure S7a–c (Supporting Information), respectively. At 37 °C, the SANS distribution curves of all pluronics show the existence of correlation peaks which arise because of the hard sphere repulsion between the micelles except pluronic F68.41 The presence of unimers was only observed in the pluronic F68 at 37 °C due to the high CMT value of F68 (CMT of 5% w/v 68 = 40 °C).42 The value of aggregation number \(N_{agg}\) is higher in the pluronic P84 than that in P85, F127, and F108. The higher \(N_{agg}\) value of hydrophobic pluronic P84 is attributed to the minimal stability of this pluronic in water as compared to other investigated pluronics.43 The higher aggregation number of P84 is responsible for its larger hard sphere radii \(R_{HS}\) than P85 \(R_{HS} = 71.9\) and P85 \(R_{HS} = 71.9\). Conversely, the hard sphere radius values are higher in hydrophilic pluronics, F108 \(R_{HS} = 102.5\) and F127 \(R_{HS} = 102.0\) than in pluronic P84, which signifies the more intermicellar interaction of F108 and micelles than that of P84 due to the longer corona region. Volume fraction \(\phi\) values of hydrophilic pluronics, F127 \(\phi = 0.158\) and F108 \(\phi = 0.142\) are higher as compared to the hydrophobic pluronics P84 \(\phi = 0.098\) signifies the greater \(D_2O\) penetration in the hydrophilic corona and core region of these hydrophilic pluronics (F108 and F127).44 Various key parameters from the model for the SANS measurements viz. core radius \(R_c\), polydispersity, hard sphere radius \(R_{HS}\), volume fraction \(\phi\), and aggregation number \(N_{agg}\) are tabulated in Table 1. The increase in temperature from 37 to 47 °C and 57 °C persuades the increase of the core radius \(R_c\) and the aggregation number \(N_{agg}\). There is evidence to suggest a decrease in the hard sphere radius of hydrophilic pluronics (F108 and F127) when temperatures increased from 37 to 57 °C, conferring the removal of water from the corona region. Increase in the hard sphere radius of the hydrophobic pluronics (P84 and P85) as increase in the temperature (37–57 °C) is due to the increase in the aggregation numbers of these hydrophobic pluronics. In the case of pluronic P84, volume fraction \(\phi\) decreases as the

![Figure 4](image_url) Intensity weighed size-distribution profiles for empty and LAM-loaded pluronic micelles (a) P84 and (b) F127 measured by DLS.

![Figure 5](image_url) SANS data pattern (with error bars in experimental data) for (a) 5% w/v pluronic P84 (b) 5% w/v pluronic F68 at 37, 47, and 57 °C.
temperature is increased. At 37 °C, the volume fraction ($\phi$) was 0.089 and this falls to ($\phi$) = 0.081 at 47 °C and ($\phi$) = 0.060 at 57 °C. A similar decrease was also observed in the other investigated pluronic except F68. The unimers of F68 converted into the micelle with volume fraction ($\phi$) = 0.065 at 47 °C, and at 57 °C, aggregation of these micelles was increased but volume fraction was also increased from ($\phi$) = 0.065–0.140. The volume fraction of F68 micelles increases, and the dehydrating effects of the rise of temperature can be ascribed to the transformation of remaining F68 unimers to contribute to form more micelles.45 At 57 °C, pluronic P84 with 40% PEO content converted from spherical micelles to prolate ellipsoidal micelles. The correlation peak tends to be weak in the scattering curve at 57 °C and cannot be explained employing a spherical shape micelles-based model.46,47 At higher temperature, the aggregation number ($N_{agg}$) increases, 

Table 1. Various Key Model Parameters: Core Radius ($R_c$), Polydispersity, Hard Sphere Radius ($R_{HS}$), Volume Fraction ($\phi$), and Aggregation Number ($N_{agg}$) for 5% w/v Pluronic (F68, F108, F127, P85, and P84) at 37, 47, and 57 °C before and after Solubilization of LAM

<table>
<thead>
<tr>
<th>system</th>
<th>temperature (°C)</th>
<th>core radius $R_c$ (nm)</th>
<th>polydispersity $\Sigma$</th>
<th>hard sphere radius $R_{HS}$ (nm)</th>
<th>volume fraction $\phi$</th>
<th>aggregation number $N_{agg}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>5% w/v P84</td>
<td>37</td>
<td>5.21</td>
<td>0.19</td>
<td>8.20</td>
<td>0.089</td>
<td>159</td>
</tr>
<tr>
<td></td>
<td>47</td>
<td>5.65</td>
<td>0.19</td>
<td>8.82</td>
<td>0.081</td>
<td>203</td>
</tr>
<tr>
<td></td>
<td>57</td>
<td>$a = 13.5, b = 5.15$</td>
<td>0.19</td>
<td>8.90</td>
<td>0.060</td>
<td>403</td>
</tr>
<tr>
<td>5% w/v P84 + LAM drug</td>
<td>37</td>
<td>5.18</td>
<td>0.19</td>
<td>8.30</td>
<td>0.095</td>
<td>156</td>
</tr>
<tr>
<td></td>
<td>47</td>
<td>5.54</td>
<td>0.19</td>
<td>8.85</td>
<td>0.093</td>
<td>191</td>
</tr>
<tr>
<td></td>
<td>57</td>
<td>$a = 12.8, b = 5.13$</td>
<td>0.19</td>
<td>8.95</td>
<td>0.065</td>
<td>360</td>
</tr>
<tr>
<td>5% w/v P85</td>
<td>37</td>
<td>4.47</td>
<td>0.18</td>
<td>7.19</td>
<td>0.098</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td>47</td>
<td>4.82</td>
<td>0.18</td>
<td>7.52</td>
<td>0.097</td>
<td>122</td>
</tr>
<tr>
<td></td>
<td>57</td>
<td>5.10</td>
<td>0.18</td>
<td>7.90</td>
<td>0.094</td>
<td>145</td>
</tr>
<tr>
<td>5% w/v P85 + LAM drug</td>
<td>37</td>
<td>4.45</td>
<td>0.18</td>
<td>7.23</td>
<td>0.098</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>47</td>
<td>4.79</td>
<td>0.18</td>
<td>7.63</td>
<td>0.098</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td>57</td>
<td>5.04</td>
<td>0.18</td>
<td>7.95</td>
<td>0.094</td>
<td>140</td>
</tr>
<tr>
<td>5% w/v F127</td>
<td>37</td>
<td>5.44</td>
<td>0.38</td>
<td>10.20</td>
<td>0.158</td>
<td>108</td>
</tr>
<tr>
<td></td>
<td>47</td>
<td>5.78</td>
<td>0.34</td>
<td>10.10</td>
<td>0.151</td>
<td>130</td>
</tr>
<tr>
<td></td>
<td>57</td>
<td>5.94</td>
<td>0.31</td>
<td>10.10</td>
<td>0.138</td>
<td>141</td>
</tr>
<tr>
<td>5% w/v F127 + LAM drug</td>
<td>37</td>
<td>5.42</td>
<td>0.38</td>
<td>10.25</td>
<td>0.158</td>
<td>107</td>
</tr>
<tr>
<td></td>
<td>47</td>
<td>5.76</td>
<td>0.34</td>
<td>10.20</td>
<td>0.151</td>
<td>129</td>
</tr>
<tr>
<td></td>
<td>57</td>
<td>5.90</td>
<td>0.30</td>
<td>10.15</td>
<td>0.138</td>
<td>138</td>
</tr>
<tr>
<td>5% w/v F108</td>
<td>37</td>
<td>4.79</td>
<td>0.49</td>
<td>10.25</td>
<td>0.142</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>47</td>
<td>5.33</td>
<td>0.46</td>
<td>10.20</td>
<td>0.140</td>
<td>132</td>
</tr>
<tr>
<td></td>
<td>57</td>
<td>5.82</td>
<td>0.40</td>
<td>10.10</td>
<td>0.130</td>
<td>173</td>
</tr>
<tr>
<td>5% w/v F108 + LAM drug</td>
<td>37</td>
<td>4.67</td>
<td>0.48</td>
<td>10.30</td>
<td>0.145</td>
<td>89</td>
</tr>
<tr>
<td></td>
<td>47</td>
<td>5.24</td>
<td>0.44</td>
<td>10.30</td>
<td>0.143</td>
<td>126</td>
</tr>
<tr>
<td></td>
<td>57</td>
<td>5.66</td>
<td>0.39</td>
<td>10.20</td>
<td>0.140</td>
<td>159</td>
</tr>
<tr>
<td>5% w/v F68</td>
<td>37</td>
<td>$R_g = 2.11$ nm (unimer)</td>
<td>1.98</td>
<td>7.35</td>
<td>0.065</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>47</td>
<td>3.88</td>
<td>0.55</td>
<td>7.10</td>
<td>0.130</td>
<td>88</td>
</tr>
<tr>
<td>5% w/v F68 + LAM drug</td>
<td>37</td>
<td>$R_g = 2.08$ nm (unimer)</td>
<td>2.13</td>
<td>7.40</td>
<td>0.082</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>47</td>
<td>3.37</td>
<td>0.56</td>
<td>7.25</td>
<td>0.140</td>
<td>57</td>
</tr>
</tbody>
</table>

Figure 6. SANS data pattern (with error bars in experimental data) for (a) P84 and (b) F68, 5% w/v in the absence and presence of LAM drug 37, 47, and 57 °C.
$N_{agg} \approx 159$ at $37 \, ^\circ C$ to $N_{agg} \approx 203$ at $47 \, ^\circ C$ and $N_{agg} \approx 403$ at $57 \, ^\circ C$, leading to an increase in the micelle core radius. When the length of the core region exceeds the stretched length of the PEO corona region of the pluronic micelles, it tends to shape conversion from spherical to prolate ellipsoidal micelles. It is reported in the literature that when the temperature increases, the aspect ratio also increases, which results in anisotropic growth and change in morphology of micelles to worm-like and cylindrical structures.48,49

Another important reason behind this kind of behavior is change in the PEO region into a less polar form with increase in temperature; moreover, PEO region of the P84 micelle interacts less favorably with water molecules. A weak interaction between the PEO region and water molecules at $57 \, ^\circ C$ results in the higher packing density at the PEP−PPO−PEO interface, which favors the morphological conversion of spherical pluronic micelles to prolate ellipsoidal micelles.47

The results showed no structural transformations of other pluronics due to insignificant increase in the aggregation number ($N_{agg}$). In case of pluronic P85 with 50% PEO content, $N_{agg} \approx 98$ at $37 \, ^\circ C$ to $N_{agg} \approx 122$ at $47 \, ^\circ C$ and $N_{agg} \approx 145$ at $57 \, ^\circ C$, in pluronic F127 with 70% PEO content, $N_{agg} \approx 108$ at $37 \, ^\circ C$ to $N_{agg} \approx 130$ at $47 \, ^\circ C$ and $N_{agg} \approx 141$ at $57 \, ^\circ C$, in pluronic F108 with 80% PEO content $N_{agg} \approx 96$ at $37 \, ^\circ C$ to $N_{agg} \approx 132$ at $47 \, ^\circ C$ and $N_{agg} \approx 173$ at $57 \, ^\circ C$, in pluronic F68 with 80% PEO content $N_{agg} \approx 11$ at $47 \, ^\circ C$ to $N_{agg} \approx 88$ at $57 \, ^\circ C$. A decrease in the interactions between water and pluronic P84 on an increase in temperature was the main reason for the increase in the aggregation number. Instead of the longer corona region of the other investigated micelles enable to retain the interactions of pluronics with water.

Scattering curves of pluronics P84 and F68 in the presence of LAM are shown in Figure 6a, b, respectively; pluronic P85, F127, and F108 in the presence of LAM are presented in Figure S8a–c (Supporting Information), respectively. The scattering data from solutions of all pluronic micelles show features that are observed in the absence of the LAM drug. The SANS results evidenced no change in the scattering features in the presence of LAM. However, in the absence of LAM, the scattering intensity decreased at low values of $q$ region, when compared to data from pluronic micelles, in the presence of LAM. However in the absence of LAM, the scattering intensity decreased at low values of $q$ region, when compared to data from pluronic micelles. These observations indicate that the drug affects either the aggregation number or size but does not affect the shape of pluronic micelles. SANS experiments on micellar solutions in the presence of LAM suggest that the number density of micelles increases as the pluronic micelle aggregation number decreases. This may be attributed to the rise in the micellar volume fraction due to the presence of the hydrophobic drug LAM.50 Upon the addition of LAM, the size of cores and the coronas of the micelles increased; the values are tabulated in Table 1. A

Figure 7. Resistance vs temperature profiles of pluronic micelles at variable temperature (a) P84 and (b) F127 (c) P84 in the presence of the LAM drug (d) F127 in the presence of the LAM drug.
significant decrease in the aggregation number of the pluronic micelles indicates that in the presence of the LAM drug, micelle formation occurred even at lesser pluronic monomers. The concentration of pluronic F108, F127, P85, and P84 are above the cmc values; in fact, the remaining monomers of pluronics participate in the formation of new micelles. Prescott et al. also explored the effect of addition of flurbiprofen, drug, cosolvent, and temperature on the pluronic micellization using SANS measurements. The scattering pattern obtained from SANS measurements revealed the attractive interactions in the micelles and/or the morphological change in the micelles at the higher flurbiprofen concentrations (1% w/v). These results demonstrate that these important factors must be considered carefully for clinical or industrial applications, such as effective and stable drug delivery systems.

2.4. HTM Measurements. The working temperature range was chosen on the basis of the morphological transitions observed in pluronic micelles with SANS measurements. The heat transfer resistance, \( R_{th} \), was calculated using eq 4 given below

\[
R_{th} = \frac{T_1 - T_2}{P}
\]

(4)

Here, \( T_1 \) and \( T_2 \) is the temperature of the copper block and temperature measured by the thermocouple that is placed in the liquid at 1.7 mm above the copper black, and \( P \) is the power applied. \( R_{th} \) measurements of pluronic P84 and pluronic F127 are displayed in Figure 7a,b, respectively.

The noise in the \( R_{th} \) signal is due to small fluctuations of the power input related to the PID controller. It is noteworthy that irreversible changes on the electrode surface are observed as a decrease in the \( R_{th} \) upon heating. Moreover, the temperatures at which the \( R_{th} \) drops are detected correspond well to the main phase-transition temperature observed by the SANS measurement: the pretransitional behavior starts at 55.0 °C, and the transition is finished at 58 °C (Figure 7a), intimating that the modification in \( R_{th} \) correlates to a phase change between spherical micelles and the prolate ellipsoidal micellar states upon heating (\( R_{th} \) value). At the temperature where morphological changes occur (spherical micelles to prolate ellipsoidal micelles), there is decrease in the surface area of the micelles due to the elongated structure that increase the movement of the current resulting in the decrease in the thermal resistance value. There are no additional morphological changes observed in the next temperature cycle. In the case of pluronic F127, there is a continuous decrease in the \( R_{th} \) upon heating, whereas the \( R_{th} \) increased to the same value upon cooling. In this case, there is no drop in the \( R_{th} \) value, confirming that there are no morphological changes occurring on the surface. The LAM-loaded pluronic P84 micelles shown in Figure 7d demonstrate a drop in the \( R_{th} \) starting at 52 °C in the first heating cycle that ends at 58 °C, which is not observed in any later heating and cooling cycles. When comparing the temperature at which transition starts between the LAM-loaded pluronic P84 micelles and unloaded pluronic P84, there is a difference of 3 °C, favoring the morphological changes to occur at a lower transition temperature in the presence of LAM. LAM-loaded F127 micelles (Figure 7d) also does not show any significant difference in the \( R_{th} \) value, which corresponds to a consistent morphology of the micelles.

2.5. In Vitro Drug Release. The release of LAM from pluronic formulations at 37 and 57 °C is presented in Figure 8a,b, respectively. The figure depicts that the drug release starts with an initial burst followed by a slow release of LAM from the formulations prepared at 37 °C was 2, 1, 0.5, and 0.5 h faster than formulations prepared at 57 °C in pluronic P84, P85, F127, and F108, respectively. LAM-loaded hydrophilic pluronics of F108 and F127 that are prepared at 37 °C showed 100% drug release in 2 and 2.5 h, respectively. However, a slower release rate was seen in the case of loaded hydrophobic pluronics P84 and P85 as they showed 90% LAM release in 4.5 and 5 h, respectively, because most of the LAM was solubilized in the core of these micelles. Five different models, viz. zero-order kinetics, first-order kinetics, Higuchi kinetics, the Hixson–Crowell model, and the KP model, were used to evaluate the data of drug release. The most fitted model was selected based on comparing the coefficient of determination. To find the release phenomenon of LAM from the pluronic micelles, correlation coefficient and rate constant were computed for all five models. From the R² values, it was ascertained that for the LAM release profiles of formulations solubilized at 37 °C, the best fit model was the Hixon model for all pluronic micelles, except the F108 micelle for which the best fitted model was KP model; the values are tabulated in Table 2. According to the KP equation,
Di transport. The Hixson model values, which are tabulated in Table 3. The value of n observed in the best solubilized at 57 °C in case of F108 suggested that the release follows the zero order kinetics. In vitro drug release revealed that the release behavior of LAM can be modulated by simply changing the temperature. In summary, we have presented LAM solubilization in different Pluronic Formulations. Di II transport, and Hixson model. The Hixson model parameters such as the drug loading capacity, partition coefficient, and standard free energy of solubilization (ΔG°) were determined. Among all investigated pluronics, P84 has the best solubilization capacity in the temperature range, 37−57 °C. SANS experiments revealed that pluronic P84 micelles converted in prolate ellipsoidal micelles from spherical micelles at 57 °C. This was confirmed by DLS and HTM and is due to higher hydrophobicity of P84 compared to other investigated pluronics. The SANS results showed that the aggregation number increases in hydrophobic pluronic P84 as the temperature is increased and that is the driving force for the morphological changes in the micelles. HTM results revealed that the conversion of the spherical micelles to prolate ellipsoidal micelles starts at 55 °C and ends at 58 °C. However, the hydrophilic pluronic F127 does not show any significant change in the Rsh value, confirming that no morphological changes occur as a function of temperature. In vitro drug release revealed that the release behavior of LAM can be modulated by simply changing the temperature for preparation of pluronic micelle formulation. Therefore, this work opens many new areas to modulate the drug-loading capacity of the hydrophobic drug in the different pluronic micelles by varying the temperature and drug release behavior. For the first time, we have reported the use of HTM to determine morphological changes of thermoresponsive micelles.

4. MATERIALS AND METHODS

4.1. Materials. Pluronics P84, P85, F127, F108, F68, and LAM, with purities of ≥98% were procured from Sigma-Aldrich. All other chemicals used were of analytical grade and used without any further purification. For the preparation of solutions, double distilled water was used.

4.2. Methods. 4.2.1. Solubilization Study of LAM Drug. To investigate the solubilization capacity, preweighed amounts of LAM were added to the vials containing 5 mL of different pluronic P84, P85, F127, F108, and F68 micellar media. The sample vials were stirred at a temperature of 37, 47, and 57 ± 0.1 °C for 12 h after which they were filtered using a millipore filter (0.20 μm) to remove the LAM that did not dissolve in the micelles. The concentration of solubilized LAM in the micelles was determined using a Shimadzu (UV-1800) UV−vis double beam spectrophotometer. The λmax of LAM was found to be 303 nm. Molar absorption coefficient of LAM was 5.9832 × 10^3 L mol⁻¹ cm⁻¹ as calculated from the calibration curve. The pluronic concentration was kept the same in both the reference and the measurement cell to eliminate the effect of the pluronics on the UV absorbance. Molar absorption coefficient has been used to determine the amount of LAM solubilized in the pluronic micelles.

4.2.2. DLS Measurements. The hydrodynamic diameter (Dh) of pluronic micelles in the presence and absence of LAM was measured using a Malvern Zetasizer Nanoseries Nano-ZS instrument equipped with He−Ne laser (λ = 632.8 nm) at 37, 47, and 57 ± 0.1 °C at a scattering angle of 173°. All samples were filtered with millipore filter (0.20 μm) to avoid the interference of contaminants.

4.2.3. SANS Measurements. The SANS measurements were carried out on an indigenously built SANS instrument operating at the Dhruva reactor, Bhabha Atomic Research Centre (BARC), Mumbai, India. The mean wavelength of the monochromatized beam was 5.2 Å⁻¹ [wavelength resolution (Δλ/λ) of approximately 15%] and was equipped with a linear position sensitive detector (angular range 0.5−

---

**Table 2. Rate Constants and Correlation Coefficients Using Different Kinetic Models for the Release Behavior of LAM from Different Pluronic Formulations Solubilized at Temperature of 37 °C**

<table>
<thead>
<tr>
<th>system</th>
<th>zero order kinetics</th>
<th>first order kinetics</th>
<th>Higuchi kinetics</th>
<th>Hixson–Crowell model</th>
<th>KP model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R²</td>
<td>K₀ (h⁻¹)</td>
<td>R²</td>
<td>K (h⁻¹)</td>
<td>R²</td>
</tr>
<tr>
<td>P84</td>
<td>0.871</td>
<td>30.72</td>
<td>0.795</td>
<td>2.099</td>
<td>0.958</td>
</tr>
<tr>
<td>P85</td>
<td>0.858</td>
<td>33.05</td>
<td>0.771</td>
<td>2.168</td>
<td>0.955</td>
</tr>
<tr>
<td>F127</td>
<td>0.911</td>
<td>23.88</td>
<td>0.783</td>
<td>2.239</td>
<td>0.950</td>
</tr>
<tr>
<td>F108</td>
<td>0.967</td>
<td>39.51</td>
<td>0.743</td>
<td>2.122</td>
<td>0.951</td>
</tr>
</tbody>
</table>

**Table 3. Rate Constants and Correlation Coefficients Using Different Kinetic Models for the Release Behavior of LAM from Different Pluronic Formulations Solubilized at the Lowest Temperature (57 °C)**

<table>
<thead>
<tr>
<th>system</th>
<th>zero order kinetics</th>
<th>first order kinetics</th>
<th>Higuchi kinetics</th>
<th>Hixson–Crowell model</th>
<th>KP model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R²</td>
<td>K₀ (h⁻¹)</td>
<td>R²</td>
<td>K (h⁻¹)</td>
<td>R²</td>
</tr>
<tr>
<td>P84</td>
<td>0.950</td>
<td>16.40</td>
<td>0.838</td>
<td>2.176</td>
<td>0.969</td>
</tr>
<tr>
<td>P85</td>
<td>0.949</td>
<td>18.60</td>
<td>0.898</td>
<td>2.225</td>
<td>0.953</td>
</tr>
<tr>
<td>F127</td>
<td>0.916</td>
<td>16.43</td>
<td>0.933</td>
<td>2.291</td>
<td>0.968</td>
</tr>
<tr>
<td>F108</td>
<td>0.980</td>
<td>16.87</td>
<td>0.823</td>
<td>2.263</td>
<td>0.989</td>
</tr>
<tr>
<td>F68</td>
<td>0.961</td>
<td>30.53</td>
<td>0.802</td>
<td>2.239</td>
<td>0.990</td>
</tr>
</tbody>
</table>

0.5 ≤ n (diffusional coefficient) is described with a Fickian diffusion mechanism, 0.5 < n < 1.0 anomalous (non-Fickian) diffusion, 1.0 = n case II transport, and n > 1.0 with super case II transport. The diffusional coefficient (n) value less than 0.5 in case of F108 suggested that the release follows the Fickian diffusion. Whereas in case of the formulations solubilized at 57 °C, the best-fitted model was KP for all pluronic micelles except F127 micelles, which followed the Hixson model values, which are tabulated in Table 3. The value of n observed in the best fitted model for F68, F108, P85, and P84 pluronic micelles was observed to be more than 0.5, indicating anomalous (non-Fickian) diffusion.

3. CONCLUSIONS

In summary, we have presented LAM solubilization in five different pluronics, viz. P84, P85, F127, F108, and F68, using UV−visible spectroscopy. The solubilization in all pluronics was enhanced by increasing the temperature, which changes the polarity of the micelles leading to dehydration and an increase in the aggregation number. Different solubilization parameters such as the drug loading capacity, partition coefficient, and standard free energy of solubilization (ΔG°) were determined. Among all investigated pluronics, P84 has the best solubilization capacity in the temperature range, 37−57 °C. SANS experiments revealed that pluronic P84 micelles converted in prolate ellipsoidal micelles from spherical micelles at 57 °C. This was confirmed by DLS and HTM and is due to higher hydrophobicity of P84 compared to other investigated pluronics. The SANS results showed that the aggregation number increases in hydrophobic pluronic P84 as the temperature is increased and that is the driving force for the morphological changes in the micelles. HTM results revealed that the conversion of the spherical micelles to prolate ellipsoidal micelles starts at 55 °C and ends at 58 °C. However, the hydrophilic pluronic F127 does not show any significant change in the Rsh value, confirming that no morphological changes occur as a function of temperature. In vitro drug release revealed that the release behavior of LAM can be modulated by simply changing the temperature for preparation of pluronic micelle formulation. Therefore, this work opens many new areas to modulate the drug-loading capacity of the hydrophobic drug in the different pluronic micelles by varying the temperature and drug release behavior. For the first time, we have reported the use of HTM to determine morphological changes of thermoresponsive micelles.

11259

DOI: 10.1021/acsomega.9b00939
ACS Omega 2019, 6, 11251−11262
of these temperature cycles were performed and after the using a constant heating or cooling rate of 11.67 °C/h. Subsequently, these electrodes were dried at 50.0 ± 0.1 °C for 30 min and mounted on the flow cell shown in Scheme 2, as per the flow cell design described in ref 26.

**Scheme 2. Schematic Presentation of Morphological Changes of Spherical Micelles (Left Side, before Heating) into Prolate Ellipsoidal Micelles after Reaching the Temperature 55 °C (Right Side) Represented by Heat Flow**

After stabilizing for 30 min at 35 °C in a phosphate-buffered saline (PBS) solution, the temperature was increased to 70 °C and subsequently cooled down to the starting temperature using a constant heating or cooling rate of 11.67 °C/h. Three of these temperature cycles were performed and after the final run, the solution was kept at 35 °C for 30 min.

**4.2.5. In Vitro LAM Release from Pluronic Micelles.** In vitro release profiles of LAM from different micelles were evaluated using the dialysis release method. A solution of LAM-loaded pluronic micelles (2 mL) was introduced into a dialysis membrane bag (HiMedia, molecular weight cut-off between 12,000 and 14,000) and submerged into 100 mL of a PBS solution (pH = 7.4), which acts as release medium to create a concentration gradient. The dialysis release system was maintained at 37 °C with constant stirring at 250 rpm. Samples were taken from the release medium at regular specified time intervals, and the media was replenished with the same volume of fresh release media. The collected samples were filtered with 0.20 μm filters before determining the amount of LAM release using a spectrophotometer.

**ASSOCIATED CONTENT**

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.9b00939.

Theoretical details of SANS measurements; UV spectra of different pluronic at different temperatures and corresponding data; plots of DLE and partition coefficients of pluronic; intensity weight plots for pluronic P85, F108, and F68; and SANS scattering curves for pluronic P85, F127, and F108 (PDF)

**AUTHOR INFORMATION**

**Corresponding Author**
*E-mail: rakesh_chem@yahoo.com. Fax: +91 183 2258820*

**ORCID**
Vinod K. Aswal: 0000-0002-2020-9026

**Notes**
The authors declare no competing financial interest.

**ACKNOWLEDGMENTS**

P.S. is thankful to DST New Delhi (Department of Science and Technology) for INSPIRE Fellowship and Newton Bhabha PhD Placement Programme funded by British Council and DST. Financial Support by DST (P. no. SR/S1/PC-02/2011) and UGC New Delhi [F. no. 42-278/2013 (SR)] is also strongly acknowledged. M.P. thanks the Engineering and Physical Research Council for funding, grant number EP/R029269/1.

**REFERENCES**


