Characterization of polymeric-mixed micelles applied as pseudo-stationary phase in MEKC

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ABSTRACT

The characterization of a polymeric mixed-micelle system applied as pseudostationary phase in micellar electrokinetic chromatography (MEKC) is presented. This system consists of sodium dodecyl sulfate (SDS), cholic acid and a polymeric tensioactive, Tetronic 1107®. This combination of the three tensioactive agents has been previously applied in a MEKC system to resolve nine steroid hormones in urine samples. In a physicochemical study of a set of test molecules and nine steroid hormones, different parameters have been calculated not only for the polymeric mixed-micelles system but also for SDS and cholic acid as single tensioactive agents and in combination. The parameters presented were micellar phase residence times, \( t_{mic} \), micellar proportion, \( t_{prop,mic} \), CLogP\(_{50}\) and methylene selectivity. A comparison of each parameter in the different micellar systems allowed to demonstrate that the retention and selectivity of each micellar system applied as PSP is related to the hydrophobicity of the test molecules. An exception was found in the steroid groups, where only the polymeric mixed-micelle system showed different \( t_{prop,mic} \) values specially for intermediate and high hydrophobic steroids. The order of capability for interaction with the analytes of the studied pseudostationary phases was demonstrated by the CLogP\(_{50}\) values and the comparison of methylene selectivity. Moreover, cloud point and the CMC values for Tetronic were presented to complete the characterization of the polymeric mixed-micelle system. In addition, dynamic light scattering analysis and transmission electron microscopy demonstrated that the combination of SDS, cholic acid and Tetronic constitutes a polymeric mixed-micelle system. 

KEYWORDS: hydrophobicity, mixed-micelles, polymers, pseudostationary phase, retention

ABBREVIATIONS

Andros, androstenedione; CA, cholic acid; Cort, cortisol; Dh, hydrodynamic diameter; DHEA, dehydroepiandrosterone; DLS, dynamic light scattering; E2, estradiol; E3, estriol; E1, estrone; HSDB, Hazardous Substances Data Bank; PAN, 1-(2-pyridylazo)-2-naphthol; PEO-PPO, poly (ethylene oxide)-poly (propylene oxide); Pg, progesterone; PSP, pseudostationaryphase; SDHEA, dehydroepiandrosterone sulfate; SDS, sodium dodecyl sulfate; TEM, transmission electron microscopy; To, testosterone

1. INTRODUCTION

Micellar electrokinetic chromatography (MEKC) was introduced by Terabe in 1984 as a unique mode of capillary electrophoresis (CE) that allows resolution of both neutral and ionic compounds [1]. The mechanism of separation of the analytes

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In a previous work, we have developed a polymeric-mixed micelles MEKC system using two conventional tensioactive agents such as cholic acid (CA), a trihydroxy bile acid, SDS, and also a polymeric micelle, poloxamine (Tetronic 1107®) for the simultaneous determination of nine steroids in human urine [8]. Although, the application of mixed-micelles like SDS and CA in MEKC had previously been described [9-10], the addition of poloxamine as part of the PSP was presented for the first time.

Poloxamines like Tetronic 1107® are amphiphilic poly(ethylene oxide)-poly(propylene oxide) (PEO-PPO) block copolymers containing two tertiary amine groups in the center of the molecule enabling temperature and pH sensitiveness (Figure 1). At alkaline pH values, the micelles are larger and the size distribution more homogeneous. Poloxamines as polymeric micelles are frequently used in the pharmaceutical technology field as drug delivery agents [11-12].

In MEKC system, the characterization of the PSP helps to understand the behavior of the different micellar systems with respect to the analytes, with particular emphasis in the study of the relationship between retention and hydrophobicity. The retention in MEKC can be characterized using parameters such as micellar phase residence times, micellar proportion, CLogP50 values and methylene selectivity. These parameters have been previously evaluated in MEKC systems with one micellar agent [13] and with the combination of two tensioactive agents [14] using a set of test molecules.

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Micellar phase residence time, $t_{mic}$, is the time spent by the analyte in the micellar phase and the micellar proportion, $t_{prop,mic}$ characterizes the

![Figure 1. Chemical structure of poloxamine, Tetronic 1107®.](image-url)
interaction between the analytes and the PSP. Moreover, using a set of test molecules with known hydrophobicity (LogP), it is possible to calculate the CLogP_{50} value for each one in a MEKC system. This parameter expresses the value of hydrophobicity of a virtual analyte spending exactly 50% of its migration time in the pseudostationary phase. Low values of CLogP_{50} indicate that micellar system weakly interacts with the analyte, and high values of this parameter suggest a very strong interaction between the hydrophobic analyte and the PSP.

Methylene selectivity is used as a parameter for characterization of the interaction between the PSP and structurally related compounds, showing the ability of the PSP to distinguish between analytes differing in their structures in a methylene group. Therefore, this parameter is an indicator of hydrophobicity of the PSP [15]. Methylene selectivity of the PSP can be determined from the micellar proportion values of different test molecules.

The aim of this work was to characterize and to compare in terms of retention and hydrophobicity the developed polymeric-mixed micelle system applied in the resolution of steroids with different structures. In addition, dynamic light scattering analysis (DLS) was used to determine the diameter of the micelles, and their morphology was characterized by transmission electron microscopy (TEM). The cloud point and the CMC value of Tetronic are also presented for completing the study of characterization.

To our knowledge, the characterization of a polymeric-mixed micelle system consisting of three tensioactive agents is reported for the first time in this study.

2. MATERIALS AND METHODS

2.1. Chemicals and reagents

Acetophenone, propiophenone, butyrophenone, hexanophenone, octanophenone, dodecaphenone, cortisol (4-pregnene-11\beta,17\alpha,21-triol-3,20-dione) (Cort), androstenedione (4-androstene-3,17-dione) (Andros), estradiol (1,3,5(10)-estratien-3,17-\beta-diol) (E_2), dehydroepiandrosterone (5-androsten-3\beta-ol-17-one) (DHEA), testosterone (4-androsten-17\beta-hydroxy-ol-3-one) (T_0), dehydroepiandro-sterone sulfate (5-androsten-3\beta-ol-17-one sulfate) (SDHEA), estrone (1,3,5(10)-estratien-3-ol-17-one) (E_1), progesterone (4-pregnene-3,20-dione) (Pg), sodium dodecyl sulfate (SDS), sodium cholate hydrate (CA) and 1-(2-pyridylazo)-2-naphthol (PAN) were purchased from Sigma (St. Louis, MO, USA). Tetronic 1107® was a gift from BASF Corporation (Florham Park, NJ, USA). Sodium monohydrogen phosphate, sodium borate 10-hydrate, tetrahydrofuran, ethanol and methanol were HPLC grade and supplied by E. Merck (Darmstadt, Germany). Ultrapure water was obtained from EASY pure™ RF equipment (Barnstead, Dubuque, IA, USA). All solutions were filtered through a 0.45 µm nylon membrane (Micron Separations Inc., Westboro, MA, USA) and degassed before use.

2.2. Instrumentation and electroophoretic conditions

Analysis was carried out with a P/ACE™ MDQ Capillary electrophoresis system (Beckman, Fullerton, CA, USA). Uncoated fused silica capillaries (Microsolv technology, Eatontown, NJ, USA) of 50 cm (40 cm length to detector) x 75 µm i.d. were used. The capillary temperature was maintained at 25 °C, and UV detection was set at two different wavelengths, 210 nm and 254 nm. Samples were injected under 0.5 psi pressure for 3 s and electrophoretic system was operated under positive polarity and a constant voltage of 18 kV.

The five characterized PSPs in this study were based on SDS, CA (single and in combination) and polymeric mixed-micelles system. The MEKC systems were prepared in 5 mM borate-5 mM phosphate buffer (pH = 8.0) as described in Table 1.

2.3. Stock and standard solutions

Stock solutions of nine steroids containing E1, E2 and E3 at 1 mg/ml, T0 and Pg at 2 mg/ml, DHEA and SDHEA at 9 mg/ml, and cortisol at 6 mg/ml were prepared by dissolution in methanol. A set of solutions of test molecules (1 mg/ml) were also prepared in methanol. The selected test molecules were acetophenone, propiophenone, butyrophenone, hexanophenone and octanophenone. Dodecaphenone and methanol were used as micelle and EOF marker, respectively.
Table 1. Composition of MEKC systems.

<table>
<thead>
<tr>
<th></th>
<th>SDS (mM)</th>
<th>CA (mM)</th>
<th>Tetrahydrofuran % v/v</th>
<th>Methanol % v/v</th>
<th>Tetronic 1107® % w/v</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDS 10</td>
<td>10</td>
<td>-</td>
<td>2.5</td>
<td>2.5</td>
<td>-</td>
</tr>
<tr>
<td>SDS 50</td>
<td>50</td>
<td>-</td>
<td>2.5</td>
<td>2.5</td>
<td>-</td>
</tr>
<tr>
<td>CA</td>
<td>-</td>
<td>50</td>
<td>2.5</td>
<td>2.5</td>
<td>-</td>
</tr>
<tr>
<td>SDS/CA</td>
<td>10</td>
<td>50</td>
<td>2.5</td>
<td>2.5</td>
<td>-</td>
</tr>
<tr>
<td>SDS/CA/Tetronic</td>
<td>10</td>
<td>50</td>
<td>2.5</td>
<td>2.5</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Standard solutions were obtained by appropriate dilution with 0.5 mM borate-0.5 mM phosphate buffer (pH = 8.0) at final concentrations of 2 µg/mL (E1, E2 and E3), 4 µg/mL (To and Pg), 18 µg/mL (DHEA and SDHEA), 12 µg/mL (Cort) and 10 µg/ml (test molecules and dodecaphenone).

2.4. Calculation method

Micellar phase residence times, micellar proportion, CLogP50 value and methylene selectivity have been calculated according to reported works [13-14].

Briefly, micellar phase residence time, $t_{mic}$ is calculated as it follows:

$$t_{mic} = t_{mc}k'$$

(1)

where $t_{mc}$ is the migration time of the micelle and $k'$ is the normalized retention factor.

The normalized retention factor $k'$ is calculated by the equation 2:

$$k' = \frac{(t_m-t_0)}{(t_{mc}-t_0)}$$

(2)

where $t_m$ is the migration time of the analyte and $t_0$ is the migration time of EOF.

The relationship between $t_{mic}$ and CLogP (calculated LogP) is expressed as

$$\text{CLogP} = A \log t_{mic} + B$$

(3)

where A and B are the slope and the intercept, respectively.

Micellar proportion, $t_{prop,mic}$ is given by the equation:

$$t_{prop,mic} = \frac{t_{mic}}{t_m}$$

(4)

The relationship between $t_{prop,mic}$ and CLogP is expressed as

$$\text{CLogP} = A \log t_{prop,mic} + B$$

(5)

where A and B are the slope and the intercept, respectively. The values of CLogP of the test molecules as well as the steroids were taken from United States National Library of Medicine (HSDB) [16].

Methylene selectivity (A) is given by

$$\log t_{prop,mic} = A \log Z + B$$

(6)

where Z is the number of carbons of the alkyl chain of the test molecules and A and B are the slope and the intercept, respectively.

2.5. CMC method

The CMC value for Tetronic 1107® was determined by the dye solubilization method [17]. Solutions of the polymeric surfactant at eight different concentrations were prepared in the range from 0.00 to 3.00% w/v. The selected dye used in this test was 1-(2-pyridylazo)-2-naphthol (PAN) dissolved in pentane at 1.6 mM concentration. An aliquot of 0.1 mL of PAN solution was added to 5 mL of each polymeric surfactant solution, and incubated at room temperature for 60 minutes. At the end of the incubation time, the absorbance of each solution was measured by spectrophotometry at 470 nm.

2.6. Dynamic light scattering (DLS)

The average hydrodynamic diameter ($D_h$) and size distribution of each system was measured on a Malvern Zetasizer Nano ZS (Malvern Instruments, UK) equipped with a back-scattering detector (173 degrees). $D_h$ was expressed as size distribution...
by number. Data are expressed as the average of at least six measurements. Samples were passed through a 0.45 µm filter prior to each assay.

2.7. Transmission electron microscopy (TEM)

The morphology of the polymeric mixed-micelle system and Tetronic 1107® (0.05% w/v in the same condition of the MEKC system without SDS and CA, Table 1) was studied by means of transmission electron microscopy (Philips CM-12 TEM instrument, FEI Company, Eindhoven, The Netherlands). The sample was prepared according to Moretton et al. [18]. Briefly, samples of 5 µL of each MEKC system were placed on a grid covered with Fornvar film. After 30 s, the excess was carefully removed with filter paper and 5 µL of a 2% w/v uracetyl acetate solution was added. After 30 s, the excess was removed and 5 µL of distilled water was added, maintained for 30 s, and removed. Finally, samples were dried in a closed container filled with silicagel and analyzed.

2.8. Cloud point temperature

Cloud point of Tetronic 1107® (0.05% w/v) was measured with and without SDS (10 mM). Cloud point was determined by heating two sealed vials containing each one of the solution in a well-stirred heating bath. The heating rate was increased about 1 °C per minute up to 170 °C.

3. RESULTS AND DISCUSSION

In a previous work we had developed a polymeric mixed-micelle MEKC system for the simultaneous analysis of nine steroids with different hydrophobicity in urine samples [8]. The polymeric mixed-micelles used as PSP consisted of SDS/CA/ Tetronic 1107® (10 mM/50 mM/0.05% w/v) mixed with 2.5% v/v methanol and 2.5% v/v tetrahydrofuran in 5 mM borate-5 mM phosphate buffer, pH 8.0.

The application of a polymeric mixed-micelle system involves different aspects. Firstly, the separation of high hydrophobic molecules using SDS as PSP is often unsuccessful [9, 19-20]. Moreover, the employment of mixed micelles like SDS and CA in MEKC has previously been described for the resolution of different compounds from intermediate to high hydrophobicity, including some steroid groups, given the increase of micelle polarity with respect to the single SDS micelles [9-10]. However, the use of SDS mixed with CA was unsuccessful to separate the more hydrophobic steroid hormone pair (E2 and Pg), making it necessary to add another surfactant to the electrolyte. Polymeric micelles used as PSP improve solubilization capacity of hydrophobic compounds with respect to regular micelles. In this case, a polymeric tensioactive like poloxamine (Tetronic® 1107) allowed the complete resolution of the steroids (8). Esaka et al. have reported the use of mixed-micelle SDS and Tween 20 (polyoxyethylenesorbitan monooleate) or Brij 35 (polyoxyethylene lauryl ether) systems to improve selectivity and efficiency in the analysis of hydrophobic, ionic and non ionic compounds [21-22]. In these systems the incorporation of a non ionic surfactant (Tween 20 or Brij 35) possessing polyether chains, into the SDS system was found to cause hydrogen-bonding interactions between the mixed-micelle system and the high hydrophobic analytes. With this in mind, the addition of a polymeric micelle with groups, poly oxide ethylene and poly oxide propylene (Tetronic) to a mixed micelle with SDS and CA could be the explanation of the resolution of the steroid group analyzed.

Regarding the addition of organic solvents like methanol and tetrahydrofuran to mixed tensioactives, it is known that incorporation of organic solvents modulates the separation of steroids and helps in their resolution due to the fact that the organic solvents enlarge the elution window allowing better separation [23]. Therefore, in order to compare the results reported in the previous paper, we studied the MEKC systems in the presence of organic solvents (Table 1).

Micellar phase residence time, micellar proportion, CLogP₅₀ value and methylene selectivity were determined not only for the polymeric-mixed micelle system (SDS/CA/Tetronic) but also for SDS, CA used as single tensioactive agent or combined, in order to explain the behavior of the steroids studied in the previous work.

3.1. Micellar phase residence times and micellar proportions for different pseudostationary phases

One of the parameters studied to characterize the properties of micellar system is micellar phase
Table 2. Micellar phase resident times ($t_{\text{mic}}$) and micellar proportions ($t_{\text{prop,mic}}$) calculated on different pseudostationary phases.

<table>
<thead>
<tr>
<th>LogP</th>
<th>SDS 10</th>
<th>SDS 50</th>
<th>CA</th>
<th>SDS/CA</th>
<th>SDS/CA/Tetronic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$t_{\text{mic}}$</td>
<td>$t_{\text{prop,mic}}$</td>
<td>$t_{\text{mic}}$</td>
<td>$t_{\text{prop,mic}}$</td>
<td>$t_{\text{mic}}$</td>
</tr>
<tr>
<td>Acetophenone</td>
<td>1.6</td>
<td>0.57</td>
<td>0.14</td>
<td>2.10</td>
<td>0.41</td>
</tr>
<tr>
<td>Propiophenone</td>
<td>2.2</td>
<td>1.13</td>
<td>0.26</td>
<td>3.67</td>
<td>0.58</td>
</tr>
<tr>
<td>Butirophenone</td>
<td>2.5</td>
<td>2.08</td>
<td>0.41</td>
<td>6.05</td>
<td>0.75</td>
</tr>
<tr>
<td>Hexanophenone</td>
<td>3.5</td>
<td>6.97</td>
<td>0.82</td>
<td>11.87</td>
<td>0.94</td>
</tr>
<tr>
<td>Octanophenone</td>
<td>4.6</td>
<td>10.60</td>
<td>0.96</td>
<td>14.47</td>
<td>0.99</td>
</tr>
<tr>
<td>Cort</td>
<td>1.6</td>
<td>4.94</td>
<td>0.63</td>
<td>11.50</td>
<td>0.88</td>
</tr>
<tr>
<td>E3</td>
<td>2.5</td>
<td>4.38</td>
<td>0.59</td>
<td>15.17</td>
<td>0.95</td>
</tr>
<tr>
<td>Andros</td>
<td>2.7</td>
<td>10.31</td>
<td>0.86</td>
<td>13.15</td>
<td>0.92</td>
</tr>
<tr>
<td>SDHEA</td>
<td>2.8</td>
<td>3.41</td>
<td>0.52</td>
<td>6.45</td>
<td>0.72</td>
</tr>
<tr>
<td>E1</td>
<td>3.1</td>
<td>11.88</td>
<td>0.90</td>
<td>14.66</td>
<td>0.95</td>
</tr>
<tr>
<td>DHEA</td>
<td>3.2</td>
<td>10.36</td>
<td>0.87</td>
<td>14.66</td>
<td>0.95</td>
</tr>
<tr>
<td>To</td>
<td>3.3</td>
<td>10.30</td>
<td>0.86</td>
<td>15.17</td>
<td>0.95</td>
</tr>
<tr>
<td>Pg</td>
<td>3.9</td>
<td>16.17</td>
<td>0.98</td>
<td>15.17</td>
<td>0.95</td>
</tr>
<tr>
<td>E2</td>
<td>4.0</td>
<td>13.72</td>
<td>0.93</td>
<td>14.03</td>
<td>0.94</td>
</tr>
</tbody>
</table>

$t_{\text{mic}}$ values were calculated by equation 1.  
$t_{\text{prop,mic}}$ values were calculated by equation 4.  
RSD values of the $k'$ data were less 2% ($n = 6$).
residence time ($t_{mic}$) and it was calculated according to equation 1.

The relationship between the $t_{mic}$ and the hydrophobicity of the test molecules and steroids has been evaluated for the following MEKC systems: SDS (50 mM and 10 mM), CA (50 mM), a combination of SDS/CA (10 mM/50 mM) and a mixed-polymeric MEKC system SDS/CA/Tetronic (10 mM/50 mM/0.05% w/v) (Table 1).

The results of $t_{mic}$ are presented in Table 2 for comparison.

PSPs using only SDS as tensioactive agent (10 mM and 50 mM) presented high values of $t_{mic}$ for test molecules with respect to CA as single tensioactive agent. In addition, a remarkable increment was observed in the $t_{mic}$ for octanophenone (a test molecule with high hydrophobicity) in the polymeric mixed-micelle system (Table 2). Moreover a good correlation between the log $t_{mic}$ and CLogP values of the test molecules was found for all studied PSPs (Table 3; Figure 2).

In the case of the group of steroids with different hydrophobicity, the $t_{mic}$ values presented in the MEKC systems with SDS, 10 mM and 50 mM, were higher than the values of $t_{mic}$ calculated using solely CA as tensioactive agent (Table 2). However, the $t_{mic}$ values of steroids from intermediate to high hydrophobicity with values of LogP from 3 to 4, respectively, were similar for each MEKC system with SDS or CA alone or combined. In this sense, the mixed-polymeric PSP presented different $t_{mic}$ values for all tested steroids and especially the differentiation of $t_{mic}$ values for the two highest hydrophobic steroids studied (Pg and E2). In addition, a poor correlation between log $t_{mic}$ and CLogP values of steroid groups is presented in Table 3 using SDS 50 mM as the PSP. However, a better correlation is obtained if SDHEA is excluded from the other studied steroids (Table 3). SDHEA was the only steroid hormone of the group with an ionic form. Therefore, although the hydrophobic interaction between the analytes and the PSP is the main driving force in MEKC [24], the elution of steroids in the polymeric mixed-micelle system does not seem to be the only mechanism of interaction, for example hydrogen bond donor or acceptor. The best correlation of $t_{mic}$ values and hydrophobicity was found for CA/SDS and polymeric mixed-micelle system in both cases with or without SDHEA (Table 3).

Micellar proportion, $t_{prop,mic}$ expresses the fraction of time spent by the analyte in the PSP. The $t_{prop,mic}$ values have been determined in the five studied MEKC systems for test molecules as well as for steroids and they have been calculated by equation 4. The results are shown in Table 2.

Micellar proportion values for test molecules showed a good correlation between hydrophobicity of the molecules and retention in the PSP.

In the case of steroids groups, different aspects can be pointed out. Steroids in the range from

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| Table 3. Relationship between hydrophobicity (CLogP) and micellar phase residence time ($t_{mic}$). |
|-----------------------------------------------|----------------|-------|----------------|----------------|----------------|
|                                               | SDS 10         | SDS 50 | CA             | SDS/CA         | SDS/CA/Tetronic |
| Alkyl-phenones                                |                |       |                |                |                |
| A                                             | 2.162          | 3.220 | 1.910          | 2.587          | 2.789          |
| B                                             | 2.017          | 0.365 | 2.450          | 1.406          | 0.922          |
| $R^2$                                         | 0.944          | 0.903 | 0.945          | 0.959          | 0.916          |
| With SDHEA                                    |                |       |                |                |                |
| A                                             | 2.272          | 1.986 | 4.376          | 4.513          | 3.236          |
| B                                             | 0.908          | 0.801 | -0.848         | -0.712         | -0.648         |
| $R^2$                                         | 0.553          | 0.106 | 0.633          | 0.911          | 0.894          |
| Without SDHEA                                 |                |       |                |                |                |
| A                                             | 3.325          | 12.78 | 4.762          | 4.509          | 3.235          |
| B                                             | -0.203         | -11.67| -1.114         | -0.706         | -0.646         |
| $R^2$                                         | 0.746          | 0.476 | 0.718          | 0.910          | 0.893          |

Fitting parameters and correlation coefficients were calculated by equation 3.
3.2. CLogP50 values

CLogP50 value calculated by the equation 5 represents the hydrophobicity of a virtual molecule which spends exactly 50% of its migration time in the PSP. A low CLogP50 value represents that micellar system weakly interacts with the analyte, and a high value indicates a strong interaction between the analyte and the PSP [13-14].

CLogP50 values of test compounds as well as steroids are summarized in Table 4. The lowest CLogP50 values were for SDS 50 mM system and the highest values were for CA 50 mM. This finding was in agreement with the results reported by Yang and Khaledi [27]. The best capability of the PSPs to interact with test molecules was in the

intermediate to high hydrophobicity (LogP 2.5 - 4) spend the same time in the PSP based on SDS 50 mM. This observation is in agreement with reports of different authors indicating that MEKC systems using only SDS as PSP for the separation of some hydrophobic compounds may be unsuccessful [25-26]. MEKC system using SDS and CA in combination as PSP presented different \( t_{prop, mic} \) values for steroid groups. This finding was in agreement with the results previously reported by Wiedmer et al. indicating that the mixed micellar system composed by SDS and CA can effectively be used for the separation of hydrophobic corticosteroids by MEKC [10]. However, using the polymeric mixed-micelle system we found that the most hydrophobic steroids spend different retention time in the PSP.

![Figure 2](image-url)

**Figure 2.** Relationship between hydrophobicity (LogP) and micellar phase residence times (\( t_{mic} \)) for test molecules on the different pseudostationary phases calculated in Table 2. Fitting parameters and correlation coefficients taken from Table 3.
follows order, SDS 50 mM > SDS/CA/Tetronic > CA/SDS > SDS 10 mM > CA 50 mM.

In the case of the steroids group, the migration behavior of the analytes does not follow an order of hydrophobicity, and not even correlates with each other. To evaluate the capability of the different PSPs to interact with the analytes, we analyzed the $t_{prop,mic}$. The evaluation between SDS 10 and 50 shows that with a higher concentration of SDS, $t_{prop,mic}$ of the analytes is nearly 0.9 which implies that the analytes are strongly retained in the micelle, and they cannot be resolved. Meanwhile, CA shows a high capability to resolve analytes with a CLogP below 3, but more hydrophobic analytes are greatly retained, with a $t_{prop,mic}$ above 0.9 (Table 2). Moreover, a poor correlation between CLogP and $t_{prop,mic}$ was observed when SDS was the PSP (10 and 50 mM) (Table 4). Analyzing both surfactants, SDS and CA, we found that the correlation between CLogP and $t_{prop,mic}$ improves, approaching one as for polymeric mixed-micelle system (Table 4).

### 3.3. Methylene selectivity

Methylene selectivity is a parameter that indicates the relative retention of the members of homologous series differing only in one methylene group.

Methylene selectivity was calculated for alkylphenone test molecules according to equation 6 and the results are presented in Table 5 for the studied five MEKC systems, showing the highest methylene selectivity for the system prepared with SDS 50 mM as PSP and the lowest value for the polymeric mixed-micelle system. These results confirm that using SDS as PSP, the elution order is in agreement with hydrophobicity of the molecules.

### 3.4. CMC values

Tetronic 1107® possesses two pKa values, 5.6 and 7.9 [12]. However, although the pH value of the buffer employed in all studied MEKC systems was 8.0, in practice, if Tetronic is used as the only tensioactive, test molecules did not differ from the EOF; therefore Tetronic under experimental conditions was considered to be an uncharged tensioactive.

For determination of CMC of conventional surfactants, different methods have been reported such as surface tension, electric conductivity, dye micellization and CE [28]. However there are few reports showing the micelle formation of polymeric tensioactives with PPO groups with the help of surface tension measurement, but most of them are generally very weakly surface active and, hence, do not give the clear break in the surface tension versus concentration plots. This is probably due to the weaker hydrophobic properties of PPO groups in comparison with the completely saturated hydrocarbon tails of conventional surfactants [29]. Based on these facts, dye solubilization method was chosen for CMC determination due to its simplicity and applicability to uncharged surfactants like Tetronic.

<table>
<thead>
<tr>
<th></th>
<th>SDS 10</th>
<th>SDS 50</th>
<th>CA</th>
<th>SDS/CA</th>
<th>SDS/CA/Tetronic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkyl-phenones</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>3.572</td>
<td>3.228</td>
<td>3.690</td>
<td>3.931</td>
<td>3.756</td>
</tr>
<tr>
<td>R²</td>
<td>0.493</td>
<td>0.083</td>
<td>0.663</td>
<td>0.902</td>
<td>0.878</td>
</tr>
<tr>
<td>CLogP&lt;sub&gt;50&lt;/sub&gt;</td>
<td>2.031</td>
<td>1.648</td>
<td>0.760</td>
<td>1.190</td>
<td>1.341</td>
</tr>
<tr>
<td>Steroids</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>6.844</td>
<td>2.405</td>
<td>4.171</td>
<td>5.217</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>4.123</td>
<td>3.938</td>
<td>4.308</td>
<td>4.203</td>
<td>4.385</td>
</tr>
<tr>
<td>R²</td>
<td>0.892</td>
<td>0.828</td>
<td>0.917</td>
<td>0.915</td>
<td>0.881</td>
</tr>
<tr>
<td>CLogP&lt;sub&gt;50&lt;/sub&gt;</td>
<td>3.126</td>
<td>1.878</td>
<td>6.412</td>
<td>2.947</td>
<td>2.815</td>
</tr>
</tbody>
</table>

Fitting parameters and correlation coefficients were calculated by equation 5.
This method is based on the principle that the solubilization of a hydrophobic dye in a surfactant solution only takes place if micelles are present. The measured intensity of each solution prepared as described in section 2.5 was plotted as a function of surfactant concentration. In agreement with previously published works [17] three regions of the diagram can be distinguished, and the points can be fitted on two lines with different slopes. The intersection point between these two lines corresponds to the CMC value under this experimental condition tested.

A first inflexion point was observed at concentration of Tetronic of 0.055% w/v but a second point was presented at 0.63% w/v (RSD = 1.8). Therefore, the value of CMC obtained for Tetronic 1107® in this experiment was the latter and it was in good agreement with the values reported in literature [12]. This result indicates that the concentration of Tetronic applied in the mixed polymeric system was close to the first point of inflexion. A possible explanation for this fact is discussed by different authors who mention that the determination of CMC of this type of tensioactives like Tetronic, presents two breaks in the diagram representing measured intensity vs. surfactant concentration. The second break at high concentration corresponds to the real CMC value. The appearance of two breaks was previously described for a broad molecular weight distribution of polyethers [30] or the formation of unimolecular micelles or oligomers before the CMC is reached [31].

### 3.5. DLS results

The effect of the interaction of ionic and non ionic surfactants on the micellization behavior in aqueous solutions has been described by many authors. Desai et al. described the addition of an anionic surfactant like SDS to a solution of a non ionic surfactant such as polyoxyethylene-sorbitanmonoooleate (Tween 80), leading to the formation of a non ionic surfactant-SDS complex (or a mixed micelle) [32]. Ganguly et al. reported that the values of the concentration of each surfactant and the working temperature can lead to the formation of either mixed micelles or different types of mixed aggregates [33].

Nambam and Phillips [34] determined the size and zeta potential of polymer solutions with increasing concentrations of SDS. Their results suggest that, at low concentrations of SDS, its negative molecules are associated by charge, with hydrophobic central PPO core of the pure polymer micelles. This imparts negative charges to these mixed aggregates, and the intra-aggregate repulsion increase and leads to the breakdown of the large copolymer-rich micelles into smaller copolymer-rich micelles. At a certain concentration of SDS, the micelles of copolymer-rich micelles are destroyed, and the freed polymer unimers form aggregates with SDS micelles. This system is dominated by a mixture of SDS-rich micelles and pure SDS micelles. These reported results are in agreement with those obtained in our work. In our case the hydrodynamic diameter of the micelles of Tetronic 1107® (18.8 nm) is substantially greater than the diameter of the micelles on the polymeric mixed-micelle (1.5 nm), similar to the hydrodynamic diameter of SDS 10 mM micelles (2.3 nm).

### 3.6. TEM

The morphology of Tetronic and polymeric mixed-micelle investigated by TEM is displayed in Figure 3. Micelles showed the characteristic spherical morphology and the co-existence of aggregates of different sizes. Solvent evaporation and shrinkage of the structures during sample preparation usually affect the size and size.
The characterization of a polymeric mixed-micelle MEKC system is reported here for the first time. Using a set of test molecules, different parameters have been calculated not only for characterization of the polymeric mixed micelle system but also for the most commonly used surfactants alone and combined.

A good correlation between $t_{\text{mic}}$, $t_{\text{prop, mic}}$, and hydrophobicity of test molecules has been demonstrated. An exception is the steroid groups, where only the polymeric mixed micelle system showed different $t_{\text{prop, mic}}$ values especially for intermediate and high hydrophobic steroids. The CLogP50 results demonstrated the capability of each PSP to interact with the test molecules is in the order SDS 50 mM > SDS/CA/Tetronic > CA/SDS > SDS 10 mM > CA 50 mM. Moreover, $t_{\text{prop, mic}}$ values of the test molecules were applied to compare methylene selectivity of the PSP as well.

In addition, the diameter of the particles measured by DLS as well as the morphology presented by TEM could demonstrate that the combination of SDS/CA/Tetronic is a mixed-micelle system. The cloud point value obtained for Tetronic in presence of SDS allow us to confirm this statement.

CONCLUSION
The characterization of a polymeric mixed-micelle MEKC system is reported here for the first time. Using a set of test molecules, different parameters have been calculated not only for characterization of the polymeric mixed micelle system but also for the most commonly used surfactants alone and combined.

ACKNOWLEDGEMENT
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**Figure 3.** TEM micrograph of a) SDS/CA/Tetronic(20000X), (b) Tetronic 1107 0.05% w/v (20000X).
REFERENCES