Interaction Between Cholesterol and Non-ionic Surfactants Studied by Thin-Layer Chromatography

E. Forgács,¹,* T. Cserháti,¹ O. Farkas,¹ A. Eckhardt,² I. Miksik,² and Z. Deyl²

¹Research Laboratory of Materials and Environmental Chemistry, Chemical Research Center, Hungarian Academy of Sciences, Budapest, Hungary
²Institute of Physiology, Czech Academy of Sciences, Prague, Czech Republic

ABSTRACT

The interaction between non-ionic surfactants and cholesterol has been studied by reversed-phase thin-layer chromatography (RP-TLC) using cholesterol impregnated TLC plates and methanol–water mixtures as mobile phases. The \( R_M \) values obtained were in linear correlation with the methanol concentration of the mobile phase. The intercept obtained from linear regression analysis (\( R_{M0} \)), being characteristic for the strength of interaction, and the slope (\( b \)), being related to the surface area of

*Correspondence: E. Forgács, Research Laboratory of Materials and Environmental Chemistry, Chemical Research Center, Hungarian Academy of Sciences, P.O. Box 17, H-1525 Budapest, Hungary; E-mail: forgacs@chemres.hu.

1981
surfactants in contact with cholesterol, have been determined. Stepwise regression analysis (SRA) was performed to find relationship between the structural parameters of surfactants and strength of interaction. The results show that stacking interaction exists between cholesterol and the aromatic ring of the surfactants. The number of ethylene oxide units and length of the carbon chain in the surfactant molecules have significant effect on the strength of the interaction between the compounds studied.

**Key Words:** Interaction; Cholesterol; Non-ionic surfactants; Thin-layer chromatography.

**INTRODUCTION**

Non-ionic surfactants are amphipathic molecules consisting of a hydrophobic (alkylated phenol derivatives, fatty acids, long chain linear alcohols, etc.) and a hydrophilic part (generally an ethylene oxide chain). Because of their favorable physicochemical characteristics, they are extensively used in agrochemical,[11] industrial,[2,3] and household products[4] as detergents, emulsifiers, and dispersing agents. They have also been successfully used in various pharmaceutical formulations.[5] Non-ionic surfactants show manifold biological activities and also exert toxic side effects. It has been reported that they significantly inhibit the mineralization of phenantrene in soil, probably by the interaction with the membrane of soil microflora.[6] Non-ionic surfactants with an average ethoxylate chain length of 9–12 monomer units were toxic to a polyaromatic-hydrocarbon-degrading *Mycobacter* species.[7] The toxicity of decaethoxylated nonylphenol non-ionic surfactant to *Campylobacter gracilis* has also been studied.[8] It has been further established that non-ionic surfactants readily bind to proteins,[3,9] thereby modifying, structure,[10,11] physicochemical properties, and enzymatic activity.[12,13] The inhibition of esterase,[13] glucose oxidase,[14] and adenosine-triphosphatase[15] activity by surfactants was also reported. It was demonstrated that metal ions are significantly more toxic to *Caenorhabditis elegans* when combined with non-ionic surfactants.[16] It has been established, many times, that the toxicity of non-ionic surfactants depends on both the length of the polar ethylene oxide chain[17] and on the character of the hydrophobic moiety.[18]

Cholesterol is an important component of biological membranes. It constitutes the third group of membrane lipid sterols beside phospholipids and glycolipids.[19] Cholesterol molecules decrease the permeability of the phospholipid bilayer and increase its stability.[20] Surfactants might bind to cholesterol or to other lipids in the membrane, influencing structure and causing malfunction. This effect may depend on the strength of interaction between phospholipids and surfactant compounds.[21]
Various chromatographic methods have been extensively used for the study of molecular interactions.[22] Thin-layer chromatography (TLC) techniques applied for the determination of molecular interactions allow the simultaneous measurement of several compounds, so its application is recommended when large numbers of retention data are needed.

Multivariate mathematical–statistical methods, such as stepwise regression analysis (SRA), have been recently used to extract maximum information coming from complex data structures.[23] By application of SRA, it is possible to find relationships between physicochemical parameters and biological and biochemical properties of the molecules, and this method is capable of eliminating the insignificant independent variables from the selected equation.

The aim of the work was to study the interaction between cholesterol and non-ionic surfactants as a function of the structure of the latter. Our experiments have been focused on revealing the potential existence of interaction between cholesterol and non-ionic surfactants, with concomitant selection of structural parameters of surfactants exerting significant effect on the binding process.

**EXPERIMENTAL**

**Materials**

DC-Aluminium oxide F$_{254}$ plates 20 × 20 cm and DC-silica gel 60 plates, 20 × 20 cm, were obtained from Merck (Darmstadt, Germany). Solvents were purchased from Carlo Erba S.p.a. (Milano, Italy), Koch-Light Ltd (Haverhill, Suffolk, England), and Reanal (Budapest, Hungary). These included chloroform, acetone, and methanol. All of the solvents were spectroscopic grade. Water was purified with a Milli-Q system (Millipore, Milford, MA). Cholesterol used in the method complied with the requirements of European Pharmacopeia (EPC 2155000). The names and the chemical structures of the surfactant molecules are shown in Table 1 and Fig. 1, respectively.

**TLC**

Cholesterol (5 g) was dissolved in chloroform–acetone (1:1). Aluminum oxide plates were impregnated by overnight predevelopement in this solution of cholesterol without any pretreatment. Silica gel plates were also impregnated using the same procedure. Surfactants were separately dissolved in acetone at a concentration of 10 mg mL$^{-1}$, and 5 µL of the solutions were spotted on the plates. In order to elucidate the influence of structural parameters on the cholesterol–surfactant interaction, the surfactants were chosen systematically.
The number of ethylene oxide units changed from 2 to 50, while the hydrophobic part included aromatic rings, esthers, unsaturated, and saturated aliphatic chains. Methanol–water mixtures were used as mobile phases in the concentration range of 0–50% of methanol in steps of 5%. Developments were carried out in sandwich chambers (22 × 22 × 3 cm$^3$) at ambient temperature, the distance of development being about 15 cm. After development, the plates were dried at room temperature, and the spots were detected with modified Burger reagent.$^{[24]}$ The orange spots were clearly observable on a yellow background.

The $R_M$ value characterizing the molecular hydrophobicity in reversed-phase thin-layer chromatography (RP-TLC) was calculated for each solute in each mobile phase as follows:

$$R_M = \log \left( \frac{1}{R_f} - 1 \right)$$  \hspace{1cm} (1)

where $R_f$ is the retention factor value of the analyte.

**Table 1.** Chemical structure of non-ionic surfactants; $n_e$ is the average number of ethylene oxide groups in the molecule.

<table>
<thead>
<tr>
<th>No.</th>
<th>General structure</th>
<th>$n_e$</th>
<th>Commercial name</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>a</td>
<td>10</td>
<td>Arcopal N 100</td>
<td>Hoechst AG$^a$</td>
</tr>
<tr>
<td>2</td>
<td>a</td>
<td>11</td>
<td>Arcopal N 110</td>
<td>Hoechst AG</td>
</tr>
<tr>
<td>3</td>
<td>a</td>
<td>13</td>
<td>Arcopal N 130</td>
<td>Hoechst AG</td>
</tr>
<tr>
<td>4</td>
<td>a</td>
<td>15</td>
<td>Arcopal N 150</td>
<td>Hoechst AG</td>
</tr>
<tr>
<td>5</td>
<td>a</td>
<td>23</td>
<td>Arcopal N 230</td>
<td>Hoechst AG</td>
</tr>
<tr>
<td>6</td>
<td>a</td>
<td>30</td>
<td>Arcopal N 300</td>
<td>Hoechst AG</td>
</tr>
<tr>
<td>7</td>
<td>b</td>
<td>10</td>
<td>Sapogenat T 100</td>
<td>Hoechst AG</td>
</tr>
<tr>
<td>8</td>
<td>b</td>
<td>11</td>
<td>Sapogenat T 110</td>
<td>Hoechst AG</td>
</tr>
<tr>
<td>9</td>
<td>b</td>
<td>13</td>
<td>Sapogenat T 130</td>
<td>Hoechst AG</td>
</tr>
<tr>
<td>10</td>
<td>b</td>
<td>18</td>
<td>Sapogenat T 180</td>
<td>Hoechst AG</td>
</tr>
<tr>
<td>11</td>
<td>b</td>
<td>50</td>
<td>Sapogenat T 500</td>
<td>Hoechst AG</td>
</tr>
<tr>
<td>12</td>
<td>c</td>
<td>20</td>
<td>Tween 40</td>
<td>Atlas GmbH$^b$</td>
</tr>
<tr>
<td>13</td>
<td>c</td>
<td>20</td>
<td>Tween 80</td>
<td>Atlas GmbH</td>
</tr>
<tr>
<td>14</td>
<td>d</td>
<td>2</td>
<td>Genapol O 20</td>
<td>Hoechst AG</td>
</tr>
<tr>
<td>15</td>
<td>d</td>
<td>12</td>
<td>Genapol O 120</td>
<td>Hoechst AG</td>
</tr>
<tr>
<td>16</td>
<td>e</td>
<td>20</td>
<td>Myrj 48</td>
<td>Atlas GmbH</td>
</tr>
<tr>
<td>17</td>
<td>e</td>
<td>30</td>
<td>Myrj 51</td>
<td>Atlas GmbH</td>
</tr>
<tr>
<td>18</td>
<td>e</td>
<td>40</td>
<td>Myrj 52</td>
<td>Atlas GmbH</td>
</tr>
<tr>
<td>19</td>
<td>e</td>
<td>50</td>
<td>Myrj 53</td>
<td>Atlas GmbH</td>
</tr>
<tr>
<td>20</td>
<td>e</td>
<td>23</td>
<td>Brij 35</td>
<td>Atlas GmbH</td>
</tr>
</tbody>
</table>

$^a$Hoechst Aktiengesellschaft, 8230 Frankfurt am Main 80, Germany.

$^b$Atlas Chemie, Essen, 45141 Goldschmidtstrasse 100, Germany.
Figure 1. General structure of surfactant molecules; $n$ is the average number of ethylene oxide groups in the molecule, $x + y + 
+ y + z + w = n$. 
In order to assess the relative strength of surfactant–cholesterol inter-
action and the surface area of surfactants in contact with cholesterol, linear 
correlations were calculated between \( R_M \) and the methanol concentration 
\((c, \text{vol.} \%)\) in the mobile phase separately for each of the surfactants:

\[
R_M = R_{M0} + bc
\]

(2)

where \( R_M \) is the value for a surfactant determined at \( c \, \text{vol.} \% \) methanol 
concentration, \( R_{M0} \) (intercept) is considered as related to the strength of the 
molecular interaction between the surfactants and cholesterol, and \( b \) (slope) 
is related to the surface area of surfactants in contact with cholesterol.\(^{[25]}\)

**Mathematical–Statistical Methods**

To determine the effect of the individual structural parameters of the 
surfactants on their binding capacity to cholesterol, SRA was applied. The 
following parameters were included in the SRA: the length of the carbon 
chain \((c_{\text{length}})\), the presence or absence of ring structure indicated with 1 or 
0, respectively \((n_{\text{rings}})\), the average number of ethylene oxide groups \((n_e)\), 
and the presence or absence of ether bonds indicated with 1 or 0, respectively 
\((b_{\text{ether}})\) in the surfactants as independent variables. The dependent variables 
were the \( b \) and \( R_{M0} \) values of Eq. (2) (see Table 2).

Although the relationship between the \( R_M \) values of Myrj 51, 52, 53, and 
the methanol concentration was not linear, all the results obtained were also 
included in the SRA calculation; the \( b \) values of these compounds were set 
to zero, and the \( R_{M0} \) values were considered as a mean of the \( R_M \) values of 
these surfactants. The \( R_{M0} \) value of Tween 80 was not included in the calcu-
lation because its structure cannot described by simple parameters. The accep-
tance level for the individual independent variables was 95%. Calculations 
were carried out with the STATISTICA 5.5 software package (StatSoft Inc., 
Tulsa, OK).

**RESULTS AND DISCUSSION**

Generally, no migration was observed in the case of the silica plates. 
Basic aluminum oxide is assumed to be more liable for adsorption of choles-
terol compared to an acidic silica support. In the case of alumina plates, two of 
the surfactants [Genapol O 20 (14) and Myrj 48 (16)] did not migrate at all in 
any concentration of the mobile phase investigated. Comparing the structure 
of the surfactants, it is worthy to note that non-migrating molecules do not
contain aromatic rings. Due to the large polarity difference between water and cholesterol, pure water does not migrate on cholesterol impregnated plates, therefore, no migration was observed for the surfactant with this mobile phase. Tween 40 (12) migrated with 30% methanol concentration in the mobile phase and above, and Genapol O 120 (15) migrated with 50% methanol concentration. Thus, having an insufficient number of points, we did not investigate the correlation in this case. In the case of three surfactants [Myrj 51 (17), Myrj 52 (18), Myrj 53 (19)], the methanol concentration did not influence the $R_M$ values significantly. These surfactants have an ester function and apparently their interaction with methanol and water is similar. Thus, in 13 cases, strong correlation was established between the methanol concentration and $R_M$ values, as is shown in Table 2. The $R_M$ values decrease with increase in methanol concentration, i.e., these compounds do not show any anomalous retention behavior in this concentration range that would invalidate Eq. (2). The value of the regression coefficients ($r$) was over 0.89 in each case, indicating that the ratios of variance explained by the independent variable are high.

The relationship between the $R_{M0}$ values of three characteristic surfactants, Arcopal N 130 (3), Sapogenat T 130 (9), Sapogenat T 500 (11), and the methanol concentration in the mobile phase is shown in Fig. 2. It can be seen that the two lines of Arcopal N 130 and Sapogenat T 130 (both having

Table 2. Parameters of the linear relationship between the $R_M$ values of surfactants and the methanol concentration (vol.% in the mobile phase.

<table>
<thead>
<tr>
<th>No. of surfactant</th>
<th>$R_{M0}$</th>
<th>$s_{RMO}$</th>
<th>$b$</th>
<th>$s_b$</th>
<th>$r$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.74</td>
<td>$1.10 \times 10^{-1}$</td>
<td>$3.3 \times 10^{-2}$</td>
<td>$3.7 \times 10^{-3}$</td>
<td>0.9636</td>
</tr>
<tr>
<td>2</td>
<td>1.80</td>
<td>$1.19 \times 10^{-1}$</td>
<td>$3.1 \times 10^{-2}$</td>
<td>$4.0 \times 10^{-3}$</td>
<td>0.9650</td>
</tr>
<tr>
<td>3</td>
<td>1.63</td>
<td>$1.40 \times 10^{-1}$</td>
<td>$3.9 \times 10^{-2}$</td>
<td>$4.7 \times 10^{-3}$</td>
<td>0.9388</td>
</tr>
<tr>
<td>4</td>
<td>1.89</td>
<td>$8.30 \times 10^{-2}$</td>
<td>$3.6 \times 10^{-2}$</td>
<td>$2.8 \times 10^{-3}$</td>
<td>0.9879</td>
</tr>
<tr>
<td>5</td>
<td>1.72</td>
<td>$1.35 \times 10^{-1}$</td>
<td>$4.4 \times 10^{-2}$</td>
<td>$4.5 \times 10^{-3}$</td>
<td>0.9625</td>
</tr>
<tr>
<td>6</td>
<td>1.27</td>
<td>$2.02 \times 10^{-1}$</td>
<td>$4.0 \times 10^{-2}$</td>
<td>$6.8 \times 10^{-3}$</td>
<td>0.9226</td>
</tr>
<tr>
<td>7</td>
<td>1.58</td>
<td>$1.34 \times 10^{-1}$</td>
<td>$2.9 \times 10^{-2}$</td>
<td>$4.5 \times 10^{-3}$</td>
<td>0.9364</td>
</tr>
<tr>
<td>8</td>
<td>1.62</td>
<td>$1.56 \times 10^{-1}$</td>
<td>$3.5 \times 10^{-2}$</td>
<td>$5.2 \times 10^{-3}$</td>
<td>0.9206</td>
</tr>
<tr>
<td>9</td>
<td>1.57</td>
<td>$1.25 \times 10^{-1}$</td>
<td>$3.8 \times 10^{-2}$</td>
<td>$4.2 \times 10^{-3}$</td>
<td>0.9595</td>
</tr>
<tr>
<td>10</td>
<td>1.25</td>
<td>$1.69 \times 10^{-1}$</td>
<td>$3.0 \times 10^{-2}$</td>
<td>$5.7 \times 10^{-3}$</td>
<td>0.9178</td>
</tr>
<tr>
<td>11</td>
<td>0.95</td>
<td>$1.55 \times 10^{-1}$</td>
<td>$3.2 \times 10^{-2}$</td>
<td>$5.2 \times 10^{-3}$</td>
<td>0.9476</td>
</tr>
<tr>
<td>13</td>
<td>1.13</td>
<td>$5.70 \times 10^{-2}$</td>
<td>$1.0 \times 10^{-2}$</td>
<td>$1.7 \times 10^{-3}$</td>
<td>0.9000</td>
</tr>
<tr>
<td>13</td>
<td>1.60</td>
<td>$1.19 \times 10^{-1}$</td>
<td>$2.5 \times 10^{-2}$</td>
<td>$3.6 \times 10^{-3}$</td>
<td>0.9585</td>
</tr>
</tbody>
</table>

Note: $R_{M0}$, related to the strength of surfactant and cholesterol interaction; $s_{RMO}$, standard deviation of $R_{M0}$; $b$, related to specific surface area of surfactants in contact with the cholesterol surface; $s_b$, standard deviation of $b$; $r$, regression coefficient.
the same number of ethylene oxide units; \( n = 13 \) are quite close to each other. For Sapogenate T 500, which has a much larger ethylene oxide chain \( (n = 50) \), the \( R_{M0} \) value is much smaller while the value of the slope is similar to the other two surfactants. This behavior of the surfactants refers to the key role of the number of the ethylene oxide units in the molecules in determining the strength of interaction between cholesterol and non-ionic surfactants.

SRA has found linear correlations between the binding characteristics \( (R_{M0}, b) \) and the selected structural parameters of the surfactants, as shown in Figure 2.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure2}
\caption{The relationship between the \( R_{M} \) values of Arcopal N 130, Sapogenat T 130, Sapogenat T 500, and the methanol concentration in the mobile phase \( (c, \text{vol.} \%) \).}
\end{figure}
in Table 3. The significance level was over 99.9% (see in Table 3, calculated $F$ values) and the ratios of variance explained were about 90% (see in Table 3, $r^2$ values) in each instance. The good statistical parameters suggest that the dependent variables included in the calculation can be employed for the prediction of the strength of interaction between cholesterol and surfactants.

The presence of an aromatic ring in the surfactants led to elevation of the interacting surface area of the molecules. This fact can be explained by the occurrence of a stacking interaction between the aromatic ring of the surfactant and the basic unit of the cholesterol. The interacting area between the cholesterol and the surfactant molecule seems to decrease by the increasing length of the carbon chain of the surfactants. This phenomenon can be explained by putative steric hindrance of stacking interaction by a longer carbon chain.

The strength of the interaction between cholesterol and surfactant molecules decreases with the increasing carbon chain length of the surfactants. The number of ethylene oxide units is also in inverse relationship with the strength of the interaction. This is probably due to the fact that the hydrophilic ethylene oxide units increase their solubility in the mobile phase, pulling the surfactants towards the liquid phase.

Table 3. Relationship between the structural parameters of non-ionic surfactants and their binding characteristics to cholesterol.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>$b$</th>
<th>$R_{M0}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$a$</td>
<td>$5.37 \times 10^{-2}$</td>
<td>2.93</td>
</tr>
<tr>
<td>$b_1$ (%)</td>
<td>$-6.53 \times 10^{-1}$</td>
<td>$-5.50 \times 10^{-1}$</td>
</tr>
<tr>
<td>$x_1$</td>
<td>$c_{\text{length}}$</td>
<td>$n_{\text{etox}}$</td>
</tr>
<tr>
<td>$b_2$ (%)</td>
<td>65.44</td>
<td>43.56</td>
</tr>
<tr>
<td>$b_2$ (%)</td>
<td>$3.45 \times 10^{-1}$</td>
<td>$-7.14 \times 10^{-1}$</td>
</tr>
<tr>
<td>$x_2$</td>
<td>$n_{\text{rings}}$</td>
<td>$c_{\text{length}}$</td>
</tr>
<tr>
<td>$r^2$</td>
<td>0.9160</td>
<td>0.9002</td>
</tr>
<tr>
<td>$F_{\text{calc}}$</td>
<td>65.45</td>
<td>33.08</td>
</tr>
</tbody>
</table>

Note: Results of stepwise regression analysis ($n = 15$, $F_{99.9} = 2.81$). $a$, intercept; $b_1$ and $b_2$, regression coefficients; $b_1$ (%) and $b_2$ (%), path coefficients (dimensionless numbers indicating the relative impact of the individual independent variables on the dependent variable); $r^2$, coefficient of determination (indicates the ratio of variance explained by the independent variables); $F$, calculated value of Fisher significance test.
CONCLUSION

An RP-TLC method using cholesterol impregnation seemed to be appropriate for examination of strength of interaction between cholesterol and non-ionic surfactants. SRA was successfully applied to evaluate the retention data of TLC. It can be concluded that stacking interaction exists between the basic unit of the cholesterol and the aromatic ring of the surfactants. The strength of interaction was mainly influenced both by the length of the carbon chain and the number of ethylene oxide units, in the surfactant compounds.

ACKNOWLEDGMENT

This work was supported by the Czech-Hungarian Cooperation Program.

REFERENCES


Received December 2, 2003
Accepted January 20, 2004
Manuscript 6346G