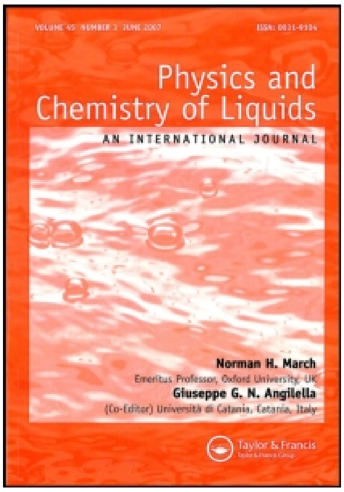

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Studies on the Cholesterol Interactions in Amine-alcohol and Amine-water Mixtures

Paweł Góralski[a]
[a] Department of Physical Chemistry, University of Łódź, Łódź, Poland

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STUDIES ON THE CHOLESTEROL INTERACTIONS IN AMINE-ALCOHOL AND AMINE-WATER MIXTURES

PAWEŁ GÓRALSKI

Department of Physical Chemistry, University of Łódź 91-416 Łódź, Pomorska 18 Poland

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Enthalpies of solution (ΔH_s) of cholesterol and cholesteryl methyl ether in the mixtures of aliphatic amines (triethylamine, di-1-butylamine and tri-1-butylamine) with water, methanol and butan-1-ol have been determined. Using the 'pure base' method, enthalpies of the cholesterol-amine association (ΔH_f) in the amine-water and amine-alcohol mixtures have been calculated. The changes of ΔH_s and ΔH_f values caused by the addition to amine the hydroxyl compounds (R'OH) where discussed. The possibility of formation of amine-R'OH-cholesterol associates involving the hydroxyl group of co-solvent has been suggested in the case of tri-1-butylamine.

KEY WORDS: Enthalpies of solution, cholesterol, amines.

INTRODUCTION

Studies on cholesterol interactions both in cell membranes and in simple isolated systems hold much interest for chemists, physico-chemists as well as for biochemists. Being one of the cell membranes component, cholesterol owing to its specific structure controls the permeability and fluidity of biological membranes. Apart from the specific interactions (hydrogen bonds) of the polar (3-β OH) hydroxyl group, also hydrophobic effects seem to play a considerable part in sterol-liquid interactions. Previous thermochemical studies on the cholesterol interactions have been limited to the systems in which cholesterol interacts either with pure solvents (alcohols[1], amines[2], oxygen proton acceptors[3]) or with proton acceptor compounds in CCl_4 solution[4,5]). The low solubility of cholesterol in water and low solubility of water in CCl_4 make it difficult or impossible to carry out experimental measurements in analogous systems containing water and cholesterol. Thus, there is little information about the direct cholesterol-water interaction. On the other hand, water is one of the principal components of the structure of living organisms in which sterols play a significant part. The role of water in specific interactions between cholesterol and other lipide constituents of cell membranes has been analyzed by Brockerhoff[6], Huang[7,8] and Bush[9,10]. The participation of water molecules in cholesterol–polyene derivatives interactions has been recently investigated by Herve[11]. Either hydration of the hydrogen bonds chol-O–H$\cdots$O$=$C$\langle$ or intermediary role of water molecules in the hydrogen bonds of sterol with amine and carboxyl groups of polyene compounds[11] has been postulated.

The present work was aimed at the determination of the effect of water or alcohols with various chain lengths on the energetics of cholesterol-amine interactions. Our previous studies on cholesterol-aliphatic alcohols systems have shown that in a liquid alcohol system the enthalpy of the cholesterol-alcohol association does not depend on the chain length of aliphatic alcohols and amounts to $-25.7 \pm 0.2\,\mathrm{kJ \cdot mol^{-1}}$. This value is a typical for self-association of liquid aliphatic alcohols[12–14] (-25.0 ± 2.0 $\mathrm{kJ \cdot mol^{-1}}$). The enthalpy of hydrogen bond complex formation (ΔH_f) of cholesterol with aliphatic amines depends on the amine structure[2]. It seems that the energetics of these interaction is considerably affected by steric hindrance of hydrocarbon radicals. The difference of ΔH_f between the complexes 1-butylamine-cholesterol ($-29.6\,\mathrm{kJ \cdot mol^{-1}}$) and tri-1-butylamine-cholesterol ($-15.9\,\mathrm{kJ \cdot mol^{-1}}$) is substantial and contrary to that which could be assumed from the changes in the basicity of amines (eg. Gutman's donor number).

The investigation of solutions containing an addition of water (alcohol) to amines could allow to widen our knowledge about specific cholesterol interaction in systems being more similar to biological systems.

EXPERIMENTAL

Materials

Amines (reagent grade) were distilled from over metallic sodium under reduced pressure. Alcohols were dried by means of molecular sieves and distilled before use. Cholesterol (Sigma, Standard for Chromatography) was dried for over ten hours at a temperature of about 80°C over P_2O_5 in vacuum. Cholesteryl methyl ether (Sigma, Anhydrous) was stored before measurements under vacuum over P_2O_5 for about one week. The weighing of calorimetric ampoules, preparation of non-aqueous solutions and filling of IR cells were carried out in a dry-box. IR measurements were carried out with a Brucker JFS 85 spectrophotometer within the range $4000-3000\,\mathrm{cm^{-1}}$. A resolution of $1\,\mathrm{cm^{-1}}$ was used at the scanning number 128. Calorimetric measurements were carried out with a calorimeter described previously[15]. The accuracy of heat measurement was about $\pm 0.8\%$.

Method

The enthalpy of hydrogen bond complex formation was determined by the 'pure base' method of Arnett *et al.*[16] This method consists of determining the enthalpy of solution of a proton-donor ΔH_S^A and its model ΔH_S^M (a compound in which the proton is substituted by the $-\mathrm{CH_3^-}$ group) in pure base and in a solvent chosen as an inert reference. The enthalpy of complex formation ΔH_f is calculated from the relation:

$$\Delta H_f = (\Delta H_S^A - \Delta H_S^M)_{\mathrm{base}} - (\Delta H_S^A - \Delta H_S^M)_{\mathrm{ref\,solv}}$$

Cholesteryl methyl ether was used as the cholesterol model compound and carbon *tetrachloride as the reference solvent. We have found no concentration dependence of* the enthalpy of solution of cholesterol and cholesteryl methyl ether in the solvent under study. The values of ΔH_s used in calculation are the averages of 8–12 direct measurements of the heat of solution within the concentration range from $5 \cdot 10^{-4}$ mol·dm^{-3} to $3 \cdot 10^{-3}$ mol·dm^{-3}.

RESULTS AND DISCUSSION

Enthalpy of solution of cholesteryl methyl ether

Enthalpies of solution of cholesteryl methyl ether (ChME) and cholesterol in TEA, DBA, TBA and their mixtures with R'OH compounds (where R' = H, Me, Bu) are given in Table 1. In the case of tertiary amines which are not miscible with water within the whole range, aqueous near-saturated amine solutions were studied. The obtained values of the enthalpy of solution of cholesteryl methyl ether show that an addition of water or alcohol, irrespective of its kind and content, always increases the endothermicity of solution. The increase caused by the addition of water seems to be stronger than that due to the addition of alcohol. The low solubility of water in TBA has brought about a relatively small increase in the enthalpy of solution of ChME in the TBA-water (~ 1% mol.) system—lower than that in the TBA-alcohol (10% mol.) system. The increase in the endothermicity of solution of ChME caused by R'OH compounds added to amines suggests the weakness or lack of specific interactions between the oxygen of ChME and protons of water (or alcohol) in solution. Such interactions should make an exothermic contribution to the enthalpy of solution of ChME. The IR spectra of water saturated solutions of TBA and TEA, taken within 3700 – 3100 cm^{-1}, show the presence of band νOH = 3680 cm^{-1}. This band is attributed[18] to the free hydroxyl group in the asymmetric (1 : 1) complex amine-water. The addition of ChME or di-n-butylether brought about no diminuation and/or shift of this band, which can confirm the lack of $>\!N \cdots H{-}O{-}H \cdots O\!<$ (ether) bonds in the amine solution. The

Table 1 Enthalpies of solution of cholesterol and cholesteryl methyl ether (ChME) in amines and its mixtures with hydroxyl compounds.

Solvent	ΔH_s/kJ·mol^{-1} *ChME*	ΔH_s/kJ·mol^{-1} *Cholesterol*
TEA[a]	23.3 ± 0.4	8.4 ± 0.2
TEA – H_2O (~3% mol.)	26.0 ± 0.4	8.2 ± 0.2
TEA – MeOH (10% mol.)	25.6 ± 0.3	9.6 ± 0.2
TEA – BuOH (10% mol.)	24.5 ± 0.2	9.1 ± 0.2
DBA[a]	20.6 ± 0.3	5.5 ± 0.1
DBA – H_2O (10% mol.)	23.1 ± 0.3	5.5 ± 0.1
DBA – H_2O (20% mol.)	23.7 ± 0.4	5.7 ± 0.1
DBA – MeOH (10% mol.)	22.8 ± 0.3	6.2 ± 0.1
TBA[a]	22.2 ± 0.4	14.0 ± 0.2
TBA – H_2O (~1% mol.)	23.1 ± 0.5	12.9 ± 0.2
TBA – MeOH (10% mol.)	25.3 ± 0.4	12.5 ± 0.2
TBA – BuOH (10% mol.)	23.3 ± 0.4	11.6 ± 0.2
CCl_4[b,c]	23.5 ± 0.5	31.2 ± 0.3

[a] – ref. 2 [b] – ref. 3 [c] – ref. 17

increase in the enthalpy of solution of ChME in amine-water (amine-alcohol) mixtures can neither be a result of dipol interactions. Such interaction would rather make an exothermic contribution to the heat of solution. The observed changes in ΔH_S can be rather ascribed to the 'structure-making' effect of addition of water (or alcohol) to the liquid amine. The increase in number of hydrogen bonds per volume unit of solution can bring about an increase in the cavitation effect. The endothermic effect of creation of a cavity able to contain the dissolved cholesterol molecule should increase. This conclusion seems to be confirmed by the enthalpies of solution of cyclopentane in DBA, TBA and in their mixtures with MeOH (10% mol)[19]. The heat of solution of cyclopentane is more endothermic in amine-MeOH (10% mol.) mixture by about 0.6–0.8 kJ · mol^{-1} than that in pure amines.

Enthalpy of solution of cholesterol

An addition of water (alcohol) to amines brings about increase in the enthalpy of solution of cholesterol in the case of TEA and DBA, and decrease in this value for TBA. The observed phenomenon probably results from the overlapping of various effects. One of them can be the cavitation effect appearing also in the case of ChME (endothermic contribution), while the other one can consist of possible additional specific interactions of cholesterol or its amine complexes. The observed changes in ΔH_s can suggest that the addition of wather (alcohol) to amines with a average steric hindrance (TEA, DBA) inconsiderably changes the energetics of the cholesterol-solvent interaction.

Solutions of tri-1-butylamine show different behaviour. An addition of 1% of water to TBA causes cholesterol to dissolve in this medium more exothermically by over 1 kJ · mol^{-1}. An addition of 10% of alcohol brings about a decrease in the cholesterol enthalpy of solution (increase in exothermicity) by 2.0 ± 0.5 kJ · mol^{-1}. The appearance of this additional exothermic effects in TBA-R'OH mixtures can be accounted for by the intensification of the interactions or appearance of new ones in the TBA-water-cholesterol (TBA-alcohol-cholesterol) system. Such interactions can be better described by the values of enthalpy of cholesterol association (ΔH_f) in the system under investigation.

Enthalpy of formation of cholesterol-amine associates

The values of the enthalpy of formation of cholesterol-amine associates obtained by the pure base method are given in Table 2. An addition of a compound containing hydroxyl group (R'OH), irrespective of the size of R', brings about increase in the absolute value of the enthalpy of formation of cholesterol-amine associates (ΔH_f). In the case of di-1-butylamine, good miscibility with water makes measurements possible at the water content in amine being 10% mol. and 20% mol. As is seen, the increase in water content from 10% to 20% slightly changes the ΔH_f value of cholesterol-amine associates. The present data point to the following direction of changes in the enthalpy of association of cholesterol in the amine-R'OH systems:

$$\text{amine} < \text{amine-BuOH} < \text{amine-MeOH} < \text{amine-water}$$

Table 2 Enthalpies of cholesterol-amine association in pure amines and in amine-R′OH mixtures.

Solvent	ΔH_f/kJ·mol^{-1}	*Solvent*	ΔH_f/kJ·mol^{-1}	*Solvent*	ΔH_f/kJ·mol^{-1}
TEA[a]	−22.6	DBA[a]	−22.8	TBA[a]	−15.9
TEA − H_2O (~3% mol.)	−24.9	DBA − H_2O (10% mol.)	−25.3	TBA − H_2O (~1% mol.)	−17.9
TEA − MeOH (10% mol.)	−23.7	DBA − H_2O (20% mol.)	−25.7	TBA − MeOH (10% mol.)	−20.5
TEA − BuOH (10% mol.)	−23.1	DBA − MeOH (10% mol.)	−24.3	TBA − BuOH (10% mol.)	−19.4

[a] − ref. 2.

Table 3 Enthalpies of transfer of cholesterol (*A*), amine (*B*) and cholesterol-amine complexes (*AB*) from pure amine to amine-R′OH (10% mol.) mixtures.

Amine-R′OH mixture	$\Delta H_{tr}^{(A)}$/kJ·mol^{-1}	$\Delta H_{tr}^{(B)}$/kJ·mol^{-1}	$\Delta H_{tr}^{(AB)}$/kJ·mol^{-1}
DBA − H_2O (10% mol.)	0.0	0.01	−2.5
DBA − MeOH (10% mol.)	0.7	−0.01	−0.8
TEA − MeOH (10% mol.)	1.2	−0.02	0.1
TEA − BuOH (10% mol.)	0.7	−0.04	0.2
TBA − MeOH (10% mol.)	−1.5	0.03	−6.1
TBA − BuOH (10% mol.)	−2.4	0.06	−5.8

The addition of R'OH to amine affects the enthalpy of formation of cholesterol-amine associates the more the smaller is the R' radical. An exception is the TBA-water mixture (~ 1% mol.) whose enthalpy of association is lower than that in the TBA-alcohol systems (10% mol.). A reason for this comes probably from the high difference in concentration of the co-solvent R'OH added to amine.

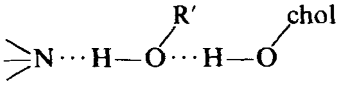
It is observed in the IR spectra of the TEA-water-cholesterol system that the band $\nu OH = 3680\ cm^{-1}$ of the assymmetric complex TEA-water does not change its position and intensity under the influence of the cholesterol added. It follows from this that the free proton of hydroxyl group in the TEA-water associates is not engaged in an additional hydrogen bond. A gradual addition of cholesterol causes only an increase in absorption within $3200 - 3300\ cm^{-1}$ i.e. the formation of the cholesterol complexes. The relatively small steric hindrance of ethyl groups and the possibility of formation of direct hydrogen bonds with TEA would suggest that the increase in ΔH_f values (Table 2) can be rather associated with changes in solvation of the interacting components or TEA-cholesterol complexes.

The systems containing tri-1-butylamine seem to behave differently. The increase in ΔH_f caused by the addition of R'OH is higher that for amines with smaller steric hindrance (TEA, DBA). In the spectra of the systems: TBA-cholesterol and TBA-water-cholesterol, one can observe the band $\nu OH = 3620\ cm^{-1}$ which can be attributed to the free hydroxyl group of cholesterol, unbounded in a hydrogen bond complex. Analogous band in the spectra containing TEA was not observed even at higher cholesterol contents. The presence of dissolved and unbounded by H-bonds molecules of cholesterol in TBA results probably from:

a) the steric hindrance impeding the formation of hydrogen bonds with the free electron pair of nitrogen $\geqslant N \cdots H{-}O{-}chol$, and

b) dispersion interactions between alkyl radicals of cholesterol and TBA, facilitating cholesterol solubility. Surely, it is owing to these interactions that the ability of cholesterol to extraction from blood is the highest for tertiary amines and rapidly increases with the alkyl chain length[20].

The distinct increase in the enthalpy of association in the system TBA-cholesterol, caused by the addition of R'OH, could be accounted for by the formation of analogous interactions as in Scheme 1.



$$\geqslant N\cdots H{-}\overset{R'}{O}\cdots H{-}\overset{chol}{O} \qquad (1)$$

The formation of the TBA-cholesterol associates through hydroxyl group seems to be probable for TBA due to the steric effect impedding the formation of direct $\geqslant N \cdots H{-}O{-}chol$ bonds. Such associates would be an analogy of linear self-associates appearing in alcohols. The interactions between cholesterol and water molecules in the TBA-cholesterol-water system are suggested also by the increase in the solubility of cholesterol in TBA brought about by an addition of about 1% mol of water.

The changes in the enthalpy of formation of the cholesterol-amine associates caused by the addition of alcohol are better seen when analysing the values of the enthalpy of the complex transfer ($\Delta H_{tr}^{(AB)}$) from pure amine to the mixed solvent amine-alcohol.

Assuming that the $\Delta H_f(S)$ values are the enthalpies of association in solvent (S) we can calculate $\Delta H_{tr}^{(AB)}$ from the following cycle:

$$\begin{tikzcd}
A(S1) \arrow[d, "\Delta H_{tr}^{(A)}"'] & + & B(S1) \arrow[d, "\Delta H_{tr}^{(B)}"'] \arrow[r, "\Delta H_f(S1)"] & AB(S1) \arrow[d, "\Delta H_{tr}^{(AB)}"'] \\
A(S2) & + & B(S2) \arrow[r, "\Delta H_f(S2)"] & AB(S2)
\end{tikzcd}$$

$$\Delta H_{tr}^{AB} = \Delta H_{tr}^{A} + \Delta H_{tr}^{B} - \Delta H_f^{(S1)} + \Delta H_f^{(S2)}$$

For comparison we selected the systems in which the R'OH content was the same (10% mol.). The values of the enthalpy of transfer of components and complexes from pure amine to amine-R'OH mixtures are given in Table 3. Enthalpies of complex transfer can be interpreted as changes in the complex solvation due to exchange of solvent. In the discussed case the $\Delta H_{tr}^{(AB)}$ values can be also caused by the formation of new type of associate from the complexes amine-cholesterol and amine-R'OH (eg. as that given in scheme 1). The interpretation of results of $\Delta H_{tr}^{(AB)}$ concerning DBA is difficult due to the intricate character of the water-secondary amine interactions. Analysing the tertiary amines, it is easy to observe that the effect of the added R'OH is considerably higher for TBA (about $6\,\mathrm{kJ \cdot mol^{-1}}$), while for TEA it is within the error limits, zero. This can additionaly suggest a different mechanism of interactions in TBR-R'OH-cholesterol and TEA-R'OH-cholesterol systems.

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