Self-Association of Cholesterol in Carbon Tetrachloride

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Z. Naturforsch. **34a**, 369—374 (1979); received November 21, 1978

Analysis of infrared hydroxyl multimer absorbances of dilute solutions of cholesterol in carbon tetrachloride as a function of the monomer absorbance strongly favours the interpretation of the association of this alcohol with a monomer-dimer-tetramer model up to a molar fraction of 0.012. This model also explains very well the concentration dependence of the apparent dipole moment. Equilibrium constants and dielectric parameters have been determined. Suggestions about the structure of the various associates are made.

Introduction

The resemblance of the infrared spectrum in the hydroxyl stretching range of solutions of cholesterol in non-polar solvents to those of solutions of straight chain alcohols has given raise to an identical interpretation of these spectra, i.e. by the existence of hydrogen-bonded complexes, and dimerization constants have been calculated by a limiting-slope method [1, 2]. The adequacy of the monomer-dimer-tetramer model to explain the behaviour of dilute solutions of aliphatic alcohols in carbon tetrachloride [3, 4] makes a test of this model for dilute solutions of cholesterol in carbon tetrachloride very attractive. This seems relevant because the self-association of this system may be involved in biochemical processes aside from the importance for the general theory of association via hydrogen bonding.

In supplement to the methods used successfully before in the case of alcohols in carbon tetrachloride [4], absorbances due to disturbed hydroxyl groups at several frequencies were determined since these are directly related to the numbers of multimers present and so these absorbances can give a clear picture of the multimers involved. The monomer absorbance is used to compute the equilibrium constants for a given model. Apparent dipole moments are used to test the adequacy of the model derived from the infrared data, and the specific *g*-factors of the multimers involved are calculated.

Experimental Section

Materials

Cholesterol was purchased from Fluka A.G. (puriss. quality) and was used without further purification. Carbon tetrachloride was supplied by Baker (p.A.-quality), dried on $CaSO_4$ and distilled over a glass column.

Experimental Equipment

Dielectric constants and infrared spectra were determined as described previously [4, 5].

Experimental Procedure

The maximum molar fraction used for the infrared measurements was 0.015 in view of the restricted applicability of the monomer-dimer-tetramer model to solutions of straight chain alcohols in carbon tetrachloride [3, 4] and this limiting concentration permits the use of the model of ideal association. The parameters were calculated with molar fractions because this is a less arbitrary concentration unit than molarity or molality. Also calculations with molar concentrations were made and this yielded the same picture within the accuracy of the measurements. The same notation was used as before [4, 5]: i.e. x for the formal (stoichometric) molar fraction cholesterol and C_i $(i = 1, \ldots, n)$ for the fraction of i-mers with respect to the total number of solvent molecules and monomeric units.

Absorbances have been calculated from transmissions relative to the transmission at 3800 cm^{-1}. It has been assumed that the absorption at the frequency of maximum intensity of the monomer peak is due to monomers only, in the low concentration range [4, 6, 7], and that free hydroxyl groups of chain multimers give raise to absorptions at

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somewhat lower frequencies [8]. It follows that the specific absorbance at the frequency of maximum absorption of the monomer peak ($A_{\text{mon}} \equiv (1/l)\ {}^{10}\log I_0/I$) is proportional to the monomer concentration C_1 with a proportionality constant $\varepsilon_{\text{mon}}(1000/\varphi_1)$ (ε_{mon} is the corresponding molar extinction coefficient in $\text{l} \cdot \text{mol}^{-1}\ \text{cm}^{-1}$ and φ_1 is the molar volume of pure carbon tetrachloride in $\text{cm}^3\ \text{mol}^{-1}$).

The apparent dipole moments have been calculated from the experimental values of ε with the following formula [9]

$$\langle\mu^2\rangle_{\text{app}} = g\,\mu_1{}^2 = \frac{9\,kT\,(2\,\varepsilon + \varepsilon_\infty)^2}{4\pi\,N_{\text{A}}\,x\,(\varepsilon_\infty + 2)^2\,(2\,\varepsilon + 1)} \cdot \left[\frac{\varphi\,(\varepsilon - 1)}{\varepsilon} - \frac{3\,(1 - x)\,(\varepsilon_0 - 1)\,\varphi_1}{2\,\varepsilon + \varepsilon_0} - \frac{3\,x\,(\varepsilon_\infty - 1)\,\varphi_2}{2\,\varepsilon + \varepsilon_\infty}\right]. \qquad (1)$$

Here ε is the dielectric constant of the solution, ε_∞ is the dielectric constant of induced polarization for the pure solute, ε_0 is the dielectric constant of the pure solvent and φ and φ_2 are the molar volumes of respectively the solution and the pure solute in $\text{cm}^3\ \text{mol}^{-1}$.

φ_2 was determined from φ and φ_1 assuming additivity of the molar volumina. ε_∞ was calculated from the molar refraction of cholesterol obtained from extrapolation of these properties of the solutions to the pure compound, yielding reasonable values of about 2.46, where the atomic polarization was accounted for in an approximative way by taking $\varepsilon_\infty = 1.05\,n^2$.

Infrared Results

The monomer peak is situated at 3622 cm^{-1}; this wavenumber slightly increases with increasing temperature, while within the accuracy of the measurements it is independent of the concentration. At somewhat higher concentration a subsidiary peak (low multimer peak) appears at about 3500 cm^{-1}; this wavenumber increases with increasing temperature and also slightly with decreasing concentration. At yet higher concentration a peak (high multimer peak) appears at about 3350 cm^{-1}.

The absorbance at the frequency of maximum absorption of the low multimer peak ($\equiv A_{\text{dim}}$) can be analyzed as a function of A_{mon} and of x. Both A_{dim}/x against x and $A_{\text{dim}}/A_{\text{mon}}$ against A_{mon} yield at all temperatures (25 °C—45 °C) a finite limiting slope, pointing to the presence of an appreciable amount of dimers in the concentration range considered. If it is assumed that only two multimers are involved (dimer and n-mer), the following formula is valid for every absorbance A, determined at some fixed frequency, in the region of the multimer peaks:

$$\frac{A}{A_{\text{mon}}} = \frac{\varphi_1}{1000}\,\frac{\varepsilon_2\,K_2}{\varepsilon^2_{\text{mon}}}\,A_{\text{mon}} + \left[\frac{\varphi_1}{1000}\right]^{n-1}\frac{\varepsilon_n\,K_n}{\varepsilon^n_{\text{mon}}}\,A^{n-1}_{\text{mon}} \qquad (2)$$

where ε_2 and ε_n are the molar extinction coefficients in $\text{l} \cdot \text{mol}^{-1}\ \text{cm}^{-1}$ at that frequency for dimers and n-mers respectively.

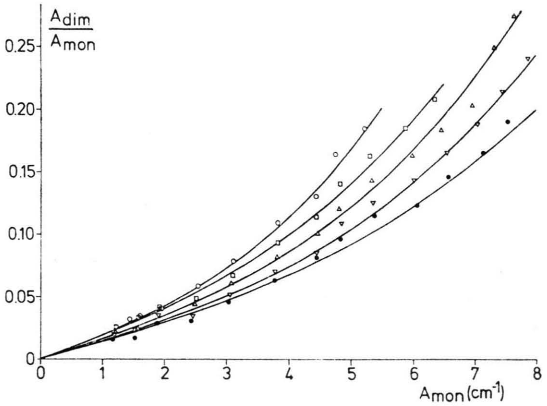

Fig. 1. $A_{\text{dim}}/A_{\text{mon}}$ as a function of A_{mon}, ○ 25°, □ 30°, △ 35°, ▽ 40°, ● 45°. The lines have been drawn with the parameters of the best monomer-dimer-tetramer fit.

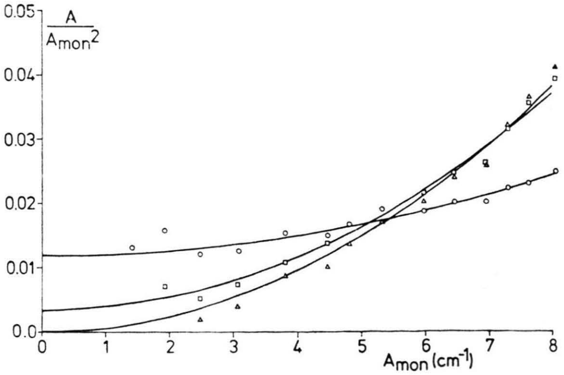

Fig. 2. The absorbance at 3520 cm^{-1} ○, at 3440 cm^{-1} □ and at 3410 cm^{-1} △ over the second power of the corresponding monomer absorbance as a function of this monomer absorbance at 35 °C. The lines have been drawn with the parameters of the best monomer-dimer-tetramer fit.

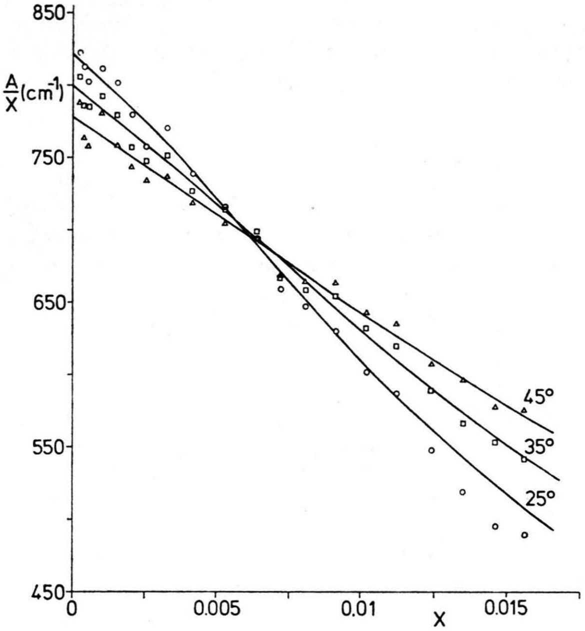

Fig. 3. A_{mon}/x at ○ 25 °C, □ 35 °C, △ 45 °C. The lines have been drawn with the parameters of the best monomer-dimer-tetramer fit.

The analysis of A_{dim} in this way (A_{dim} for A in formula (2)) yielded a good fit for $n=4$ (Fig. 1), pointing to a monomer-dimer-tetramer model and so the absence of an appreciable quantity of trimers.

Absorbances at eight frequencies between 3330 cm^{-1} and 3520 cm^{-1} have been analyzed with formula (2) and good fits have been found in all cases for $n=4$, also pointing to a monomer-dimer-tetramer model and the absence of an appreciable amount of trimers. An example of the curves obtained is shown in Figure 2. At 25 °C and 30 °C deviations occur above a molar fraction of 0.012, pointing to a multimer larger than a tetramer at higher concentrations.

It should be pointed out that this analysis is based on the a priori plausible assumption that there is no absorption due to monomers at the low-multimer peak, so that the curves in Fig. 1 should go through the origin. Assuming a finite monomer contribution to the absorption in this frequency range, Tucker and Becker [6] were able to interpret the concentration dependence of the absorption of t-butyl alcohol in perdeutero-hexadecane at 3510 cm^{-1} with the help of a monomer-trimer equilibrium. We investigated the possibility of this by plotting A/A_{mon} as a function of A^2_{mon}. Although for the wave numbers around 3500 cm^{-1} the data can be fitted in this way by a straight line, the intercept has a maximum as a function of frequency, instead of decreasing monotonously with decreasing frequency as should be expected. At the lower wavenumbers a higher multimer than the trimer is required to fit the data, and so no reduction of the number of parameters is obtained.

Concluding from the above to the preponderance of dimers and tetramers, we used the dependence of A_{mon} on x to calculate the dimerization and tetramerization constant and the monomer molar extinction coefficient ε_{mon}, with

$$A_{\text{mon}} = (1000/\varphi_1)\, \varepsilon_{\text{mon}}\, C_1 \tag{3}$$

and

$$x = C_1 + 2K_2\, C_1{}^2 + 4K_4\, C_1{}^4\,. \tag{4}$$

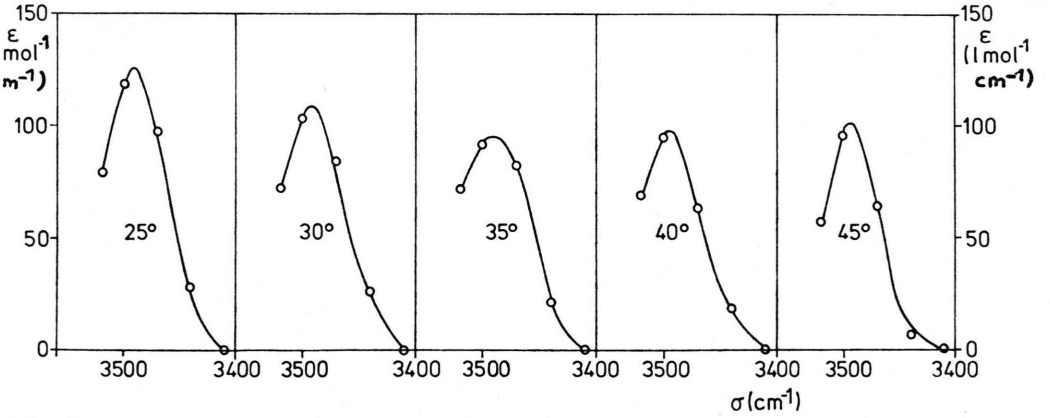

Fig. 4. Dimer absorption obtained by plotting the dimer extinction coefficient calculated at some wavenumber against this wavenumber at 25 °C, 30 °C, 35 °C, 40 °C and 45 °C. The lines have been drawn as an aid to the eye.

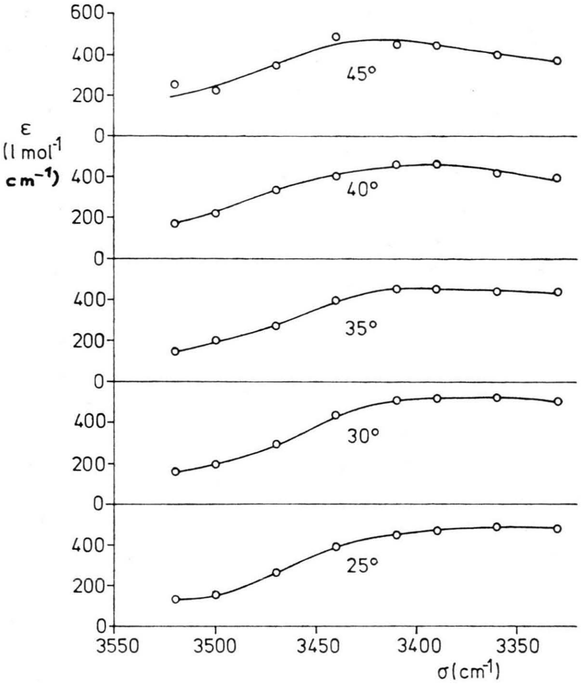

Fig. 5. Tetramer absorption obtained by plotting the tetramer extinction coefficient at some wavenumber against this wavenumber at 25 °C, 30 °C, 35 °C, 40 °C and 45 °C. The lines have been drawn as an aid to the eye.

Table 1. Association parameters of cholesterol in carbon tetrachloride.

$t°$ (°C)	ε_{mon} l · mol.$^{-1}$ cm^{-1}	K_2 m.f.u.	$10^3 K_4$ m.f.u.	μ_1^2 (D^2)	g_2	g_4
25	79.8	11.3	112	2.770	1.33	0.48
30	79.4	11.7	65.7			
35	78.7	10.4	53.4	2.700	1.32	0.58
40	78.1	9.9	37.1			
45	77.5	9.3	25.6	2.680	1.24	0.70

$\Delta H_2 = -8$ kJ/Mol $\quad \Delta H_4 = -\ 59$ kJ/Mol
$\Delta S_2 = -7$ J/deg Mol $\quad \Delta S_4 = -101$ J/deg Mol

The graphical method and the computer method outlined in Ref. [4] yielded a good fit of the experimental data, with again at 25 °C and 30 °C deviations above a molar fraction of 0.012 pointing to a larger multimer than the tetramer (Fig. 3 shows the results at 25 °C, 35 °C and 45 °C).

The parameters determined are given in the Table and were used to calculate ε_2 and ε_4 at each analyzed frequency from the coefficients of the best fits of A in A_{mon} with formula (2) for $n = 4$. These molar extinction coefficients were plotted as a function of the corresponding wavenumber, yielding a kind of decomposition of the multimer absorption in a dimer absorption (Fig. 4) and a tetramer absorption (Figure 5).

Dielectric Results

The apparent dipole moment at 25 °C, 35 °C and 45 °C first increases and afterwards decreases as a function of the concentration (Fig. 6) as it has been found for straight chain alcohols in carbon tetrachloride [10, 11, 12]. The finite limiting slope in Fig. 6 gives another independent indication that an appreciable amount of dimers is present.

If we adopt the description of $\langle\mu^2\rangle_{\text{app}}$ in specific g-factors as developed before [4, 5, 9], we get

$$x\,\langle\mu^2\rangle = \sum_i i\,K_i\,(C_1)^i\,g_i\,\mu_1{}^2\,. \tag{5}$$

If only two kinds of multimers are present, i.e. dimers and n-mers, in the concentration range considered, it can be concluded that the dimers have $g_2 > 1$ and the n-mers $g_n < 1$. With the

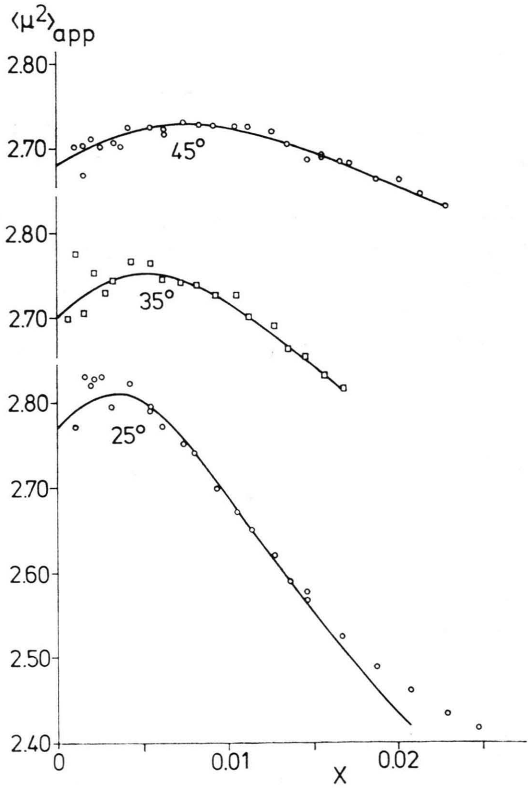

Fig. 6. Values of $\langle\mu^2\rangle_{\text{app}}(D^2)$ as a function of the formal molar fraction at 25 °C, 35 °C and 45 °C. Curves have been drawn with the parameters of the best monomer-dimer-tetramer fit.

equilibrium constants computed from A_{mon} and x, C_1 can be calculated for every formal molar fraction x and the adequacy of the monomer-dimer-tetramer model can be tested by a computer fit of $x\langle\mu^2\rangle_{\text{app}}$ or $x\langle\mu^2\rangle_{\text{app}}/C_1$ in C_1 and formula (5) for $i=1$, 2 and 4, or with a somewhat transformed formula:

$$\frac{x\langle\mu^2\rangle_{\text{app}}}{C_1^2\,\mu_1^2}-\frac{1}{C_1}=2K_2\,g_2+4K_4\,g_4\,C_1^2 \qquad (6)$$

and the l.h.s. can be plotted against C_1^2.

This yields a good fit of the experimental data as can be seen in Fig. 6, only at 25 °C deviations occur at molar fractions larger than 0.012, pointing to multimers larger than tetramers with a relatively larger dipole moment. The best fit parameters of formula (6) (μ_1^2, g_2 and g_4) are given in the Table.

Discussion

From the experimental data given above it can be concluded that the monomer-dimer-tetramer model gives a good description of the association of cholesterol in carbon tetrachloride. The equilibrium constants given in the Table point to a stronger association of cholesterol than of straight chain alcohols in carbon tetrachloride [3, 4]. The validity of this model for cholesterol is limited to a smaller concentration range than for straight chain alcohols.

The dimers seem to be mainly open as indicated by the values of g_2, and within the accuracy of the measurements no unambiguous conclusions about the temperature dependence of this property can be drawn. The dimer spectrum (Fig. 3) shows only a slight increase of the wavenumber and a decrease of the extinction coefficient at the frequency of maximum absorption with increasing temperature.

The tetramers must have some specific features to explain their preponderance, and the dielectric data suggest that the tetramers have for the greater part some kind of cyclic structure. This makes the existence of an appreciable amount of cyclic dimers unlikely since the simultaneous existence of cyclic dimers and cyclic tetramers without the occurrence of an appreciable amount of trimers seems improbable. As for all alcohols investigated in the same way g_4 has a value of about 0.5 [3, 4, 13]; it is unlikely that this value refers to an equilibrium between open and cyclic tetramers (the latter with $g_4^c=0$). In our view (see discussion in Ref. [13]) the g_4 and K_4 calculated refer to a cyclic tetramer with many different conformations and so giving raise to absorption over a relatively large frequency range (Fig. 5), and the temperature dependence of g_4 may point to an increase of the contribution due to symmetric conformations of the tetramer with decreasing temperature. The shift to lower frequency of the maximum absorption (Fig. 5) with decreasing temperature may be related to this.

In view of the deviations from the monomer-dimer-tetramer model shown by straight chain alcohols and cholesterol in carbon tetrachloride, it can be tried to make qualitative suggestions about the associates occurring at higher concentration. From the monomer absorbance at 25 °C and at a molar fraction of 0.015 it can be inferred that about 4% of the cholesterol molecules is present in the form of an associate larger than a tetramer. The absence of an appreciable amount of trimer makes a possible cooperative effect in the formation of open chain multimers rather small, and the equilibrium constant for a linear pentamer can be estimated by the fourth power of the dimerization constant. It is clear that deviations due to such a kind of pentamers and higher linear multimers occur at much higher molar fraction than 0.015.

Comparison of the deviations of the dielectric and infrared behaviour from the monomer-dimer-tetramer model, in the case of cholesterol somewhat tentative since the maximum concentration measured is rather low, shows that the apparent dipole moment deviates less from the monomer-dimer-tetramer model at higher concentration than can be expected from open chain multimers. The most obvious indication is that straight chain alcohols at 25 °C reach a minimum g-factor of about 0.7 at a molar fraction of about 0.1 [8, 10, 14], while the deviation of the apparent dipole moment is already detectable at a molar fraction of about 0.02. This makes a chain structure rather improbable, and it seems more plausible that these larger multimers are formed by addition of monomers to cyclic tetramers, implicating that two hydrogen bonds can be made to one oxygen atom, as proposed before by Meeussen and Huyskens [15] and by Smith [16].

It must be remarked that a comparison of monomer concentrations obtained from infrared absorbances and those obtained from activity determinations as it is done by these authors, offers rather ambiguous evidence for the existence of such a kind of multimers, since a hydroxyl group acting only as a hydrogen bond acceptor gives probably raise to a presumably sharp absorption maximum at a slightly lower frequency than the monomer absorption [8]. So the quantitative influence of this kind of hydroxyl groups on the monomer absorbance can be neglected at least at low concentrations.

The above arguments suggesting that this particular structure for associates prevails at higher concentration, are equally valid for alcohols in alkanes [3, 10, 14]. This points to the existence of associates with the same structure in these system, as also tetramers form the preponderant species at low concentrations in this case. Smith [16] showed that in this way the experimental data can be explained more or less quantitatively over a large concentration range if simplifications are made.

It seems very attractive to study association between cholesterol and various hydrogen bond acceptors, like ethers and esters, because this interaction can be involved in biochemical processes, e.g. in relation with the permeability of cell membranes [17]. Preliminary experiments with tetrahydrofuran as hydrogen bond acceptor point to 1:1 complexation with cholesterol at low concentration with an equilibrium constant about equal to the dimerization constants. This suggests that cholesterol-lecithin interaction can occur via hydrogen bond formation as has been stated before [18], and study of this system by infrared measurements may yield information about this point.

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