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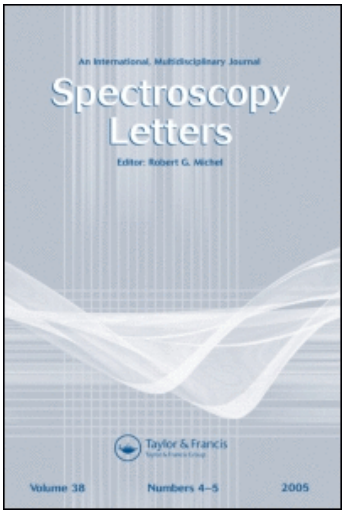

Spectroscopy Letters
Publication details, including instructions for authors and subscription information:
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Electronic and Infrared Spectroscopic Studies of Aggregation of Cholesterol

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To cite this Article Kumar, Sushil , Gupta, Seema and Chandra, Harish(2007) 'Electronic and Infrared Spectroscopic Studies of Aggregation of Cholesterol', Spectroscopy Letters, 40: 4, 583 — 590
To link to this Article: DOI: 10.1080/00387010701301188
URL: http://dx.doi.org/10.1080/00387010701301188

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Spectroscopy Letters, 40: 583–590, 2007
Copyright © Taylor & Francis Group, LLC
ISSN 0038-7010 print/1532-2289 online
DOI: 10.1080/00387010701301188

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Electronic and Infrared Spectroscopic Studies of Aggregation of Cholesterol

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Abstract: A number of reasons and causes has been put forward to understand and explain the process of atherosclerosis, or plaque formation in arteries, and the strategies to combat it. We wish to communicate that the increased level of alkalinity in serum plays an important role in atherosclerosis. On one hand, higher alkalinity helps monomer cholesterol to dimerize, supporting plaque formation. On the other hand, this converts polymer (higher aggregates) into dimer (a lower aggregate). The other finding is that the presence of a certain class of molecules in serum, part of whose bonding structure is $-CH_2-O-CH_2-$, for example, dioxane (maybe as a pollutant), promotes dimerization of cholesterol, which may set in motion the process of plaque formation. At the same time, higher aggregates (insoluble) are converted to dimer (relatively more soluble). This finding could be strategically employed to tackle atherosclerosis.

Keywords: Aggregation of cholesterol, cholesterol, concentration effect, electronic spectroscopy, infrared spectroscopy, pH effect

INTRODUCTION

It is widely believed that the self-association of cholesterol may be involved in the pathogenesis of certain conditions such as atherosclerosis and cholelithiasis.[1] Cholesterol, being an integral part of serum, is basically hydrophobic. So long as its concentration in serum is of the order 10^{-3} M, it is believed to be a monomer[2] and safe, but if its level increases, the aggregation or eventual plaque formation begins in the arteries, leading to the narrowing of

Received 16 September 2006, Accepted 19 January 2007
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the arteries, resulting in the obstructed flow of blood to the heart. The fundamental question that is still being addressed is, "What leads to plaque formation, and what are the factors that promote it?" Once all such factors are identified, then the rates of such processes that lead to the aggregation of cholesterol can be reduced, and the process of atherosclerosis can be slowed down if not prevented altogether. The scientific community in general and the medical fraternity in particular has extensively studied and debated this subject, and debate is still going on. A number of reasons and causes has been put forward.[3,4] These can be divided into two broad categories: (i) genetic and (ii) environmental. Our findings fall in the environmental category. Through this publication, we are trying to draw attention to two factors: (i) higher level of alkalinity in serum, which we think plays an important role in atherosclerosis, and (ii) the presence of a certain class of molecules, for example, dioxane (maybe as pollutants), can also promote atherosclerosis. Staels[5] has postulated, "Inflammatory white blood cells congregate at the damaged sites in the artery walls through their interaction with adhesion molecules. This can then set off a kind of cascading effect on inflammatory processes leading eventually to full-blown atherosclerosis with plaque formation in the artery walls." Staels and Skalen et al. both have essentially commented upon the role of low-density lipoproteins in the process of atherosclerosis. This subject has been studied using different techniques, for example, ^{1}H NMR spectroscopy,[6] vapor pressure osmometry,[7] near infrared (NIR) spectroscopy[8,9] and so forth. However, we provide spectroscopic evidence (UV and IR) in support of other possible causes, which we believe can play important roles in the aggregation of cholesterol.

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MATERIALS AND METHODS

Cholesterol (99.5%) purchased from E. Merck was used after crystallization. Spectrograde quality (E. Merck, India), dioxane, carbon tetrachloride, tetrahydrofuran, and acetonitrile were used and dried by usual procedure. Water purified by reverse osmosis technique (pH 6.85) was preferred. UV spectra were recorded on Cary 100 Bio (Australia) UV-Vis spectrophotometer. Spectra of concentrated solutions were recorded in 2-mm pathlength quartz cells while spectra of very dilute solutions were recorded in 50-mm pathlength quartz cells. Spectral bands were identified through derivative plots. In experiment where dioxane was added to the solutions, blank experiment was done to ensure that the transmission through the UV cells was not less than 10% at 215 nm, the cutoff region for dioxane. In actual experiment, dioxane was added in both the cells, and baseline linearity was checked after each addition of 20 μL. Infrared spectra were recorded in solution phase on a Perkin Elmer (UK) 2000 Fourier transform infrared spectrophotometer.

RESULTS AND DISCUSSION

UV spectra of very dilute solution of cholesterol (ca. 10^{-5} M) in water showed a band at 199 nm (pi $\rightarrow$ pi*) (marked through derivative plots only), Fig. 1A. However, when a spectrum of concentrated solution (absorbance ca. 2.0) was recorded, a broad band at 205.5 nm (second derivative) was observed, Fig. 1B. This indicates that the occurrence of the band at 205.5 nm is associated with higher concentration. The solubility of cholesterol in water is very low, which means a state of saturation is reached at much lower concentration than in solvents where the solubility is higher (e.g., carbon tetrachloride). The implication of this is that the processes or phenomenon (here we mean by

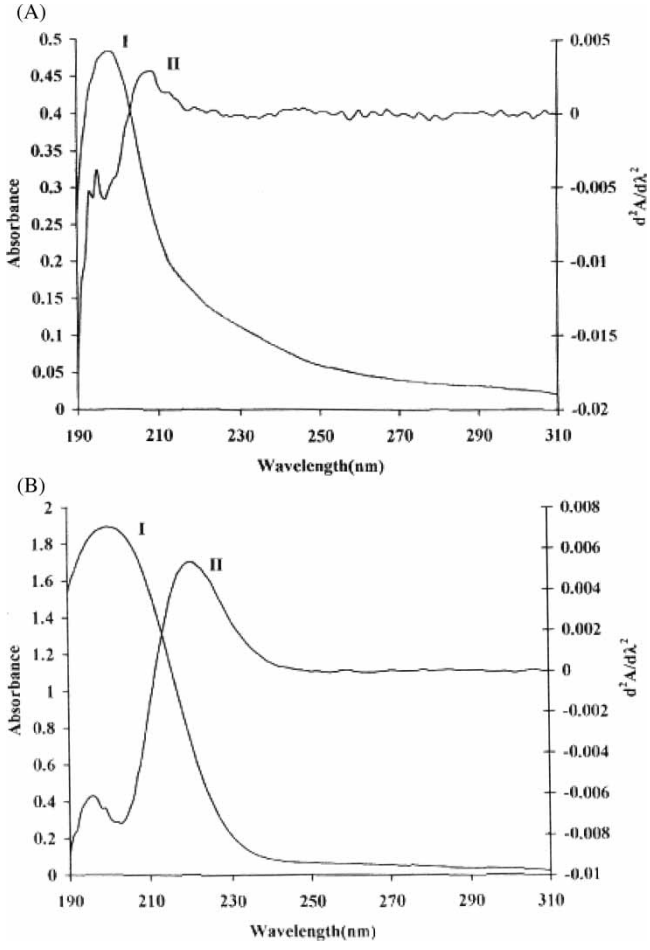

Figure 1. (A) UV spectra of cholesterol in water. I (ca. 10^{-5} M); II, second derivative plot (B) UV spectra I (ca. 10^{-3} M); II, second derivative plot.

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aggregation) come into operation in solvents of higher solubility at much higher concentration, whereas it may come into operation in water at much lower concentration. It is reported[2] that at ca. 10^{-3} M in carbon tetrachloride, cholesterol exists mainly as a monomer, but at ca. 10^{-2} M or at higher concentration, a higher order of self-associates (e.g., dimer, trimer, tetramer, etc.; open chain or cyclic) are formed. Hence, the band at 205.5 nm (in water) could be partly due to dimer, trimer, tetramer, or even hexamer[10] (maybe open chain and/or cyclic or even in the stacked form).

Each time 40 μL of dioxane was added to both the cells, a total of 120 μL was added. The band at 205.5 nm showed a gradual red shift and decrease in intensity (strongly in the beginning), finally stabilizing at 211.0 nm with a significant decrease in intensity, Fig. 2 (λ_{max} in dioxane is observed at 215.0 nm). The band turned relatively sharper, which may be an indication that cholesterol existing in different forms has preferentially been transformed to a single species.

Because there is a significant decrease in the intensity of the band, it can be interpreted that monomer is being preferentially transformed into a single higher order species (e.g., into dimer). Infrared studies described in the later part of this paper have helped us reach this conclusion.

To explore further, we studied the phenomenon in acetonitrile where the solubility of cholesterol is higher. Qualitatively, results were similar, but a smaller amount of dioxane was needed. This is due to the fact that water being hydroxylic forms a much stronger hydrogen bond with the cholesterol molecule, while acetonitrile associated with the cholesterol molecules.

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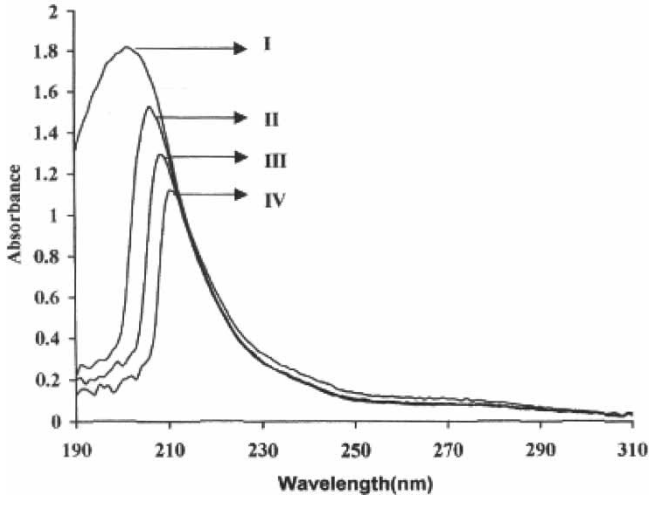

Figure 2. Incremental addition of dioxane to the cholesterol in water. I, cholesterol in water; II, 2% (v/v) dioxane added; III, 4% (v/v) dioxane added; IV, 6% (v/v) dioxane added.

Therefore, the process of dimerization has to compete strongly with the process of formation/breaking of the hydrogen bond of cholesterol with water.

The addition of tetrahydrofuran or crown ether (much stronger effect) to cholesterol in acetonitrile had a similar effect. The common nature in all the three substances—dioxane, tetrahydrofuran, and crown ether—is that these have bonding -CH_2-O-CH_2- as part of the molecular framework. This observation suggests that this kind of bonding plays an important role in dimerization.

Infrared spectral studies were carried out for better understanding the problem and confirmation of results. The infrared spectra of cholesterol (0.2 M) in CCl_4, Fig. 3A(1), were qualitatively identical with the reported one[2] except for some degree of shift. Three bands in the region 3300–4000 cm^{-1}, at 3346 cm^{-1}(III), 3492 cm^{-1}(II), and 3621 cm^{-1}(I), were identified. A band at 3346 cm^{-1} (III) (lit. value 3330 cm^{-1}) due to higher aggregate, a band at 3492 cm^{-1} (II) (lit. value 3470 cm^{-1}) as shoulder due

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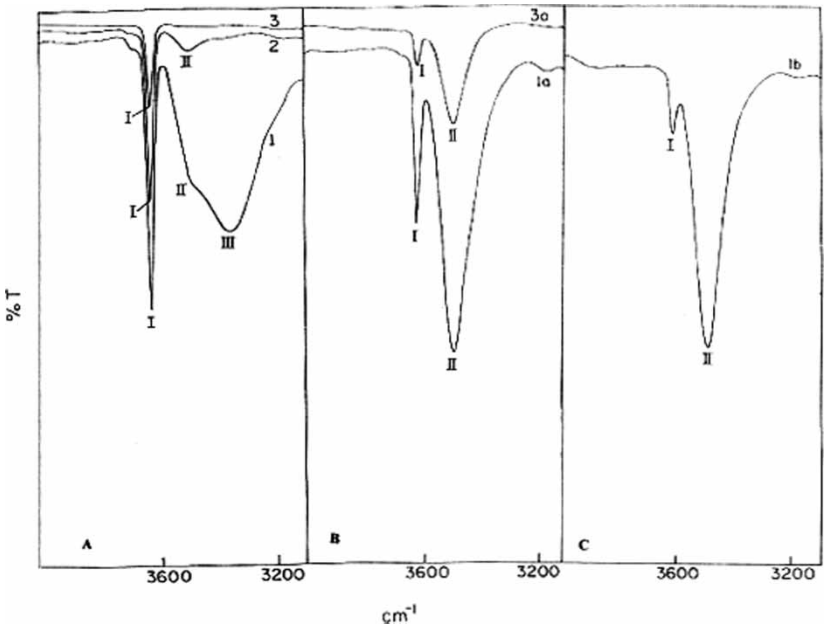

Figure 3. FTIR spectra of cholesterol in carbon tetrachloride and addition of dioxane to it. (A) 1. Conc. 0.20 M [I = 3621 cm^{-1} (monomer)]; [II = 3492 cm^{-1} (dimer); III = 3346 cm^{-1} (higher aggregates). 2. Conc. 0.06 M (only band I and II were observed). 3. Conc. 0.014 M (only band I was observed). (B) 5% (v/v) dioxane added to solutions no. 1 and 3 (Fig. 3A). 1a. Band I = 3621 cm^{-1} (monomer); band II = 3492 cm^{-1} (dimer). 3a. Band I = 3621 cm^{-1} (monomer); band II = 3496 cm^{-1} (dimer). (C) 2.5% (v/v) dioxane added to solution 1a (Fig. 3B). 1b. Band I = 3621 cm^{-1} (monomer). Band II = 3492 cm^{-1} (dimer).

to dimer, and a clear band at 3621 cm^{-1} (I) (lit. value 3620 cm^{-1}) to monomer have been assigned. This assignment is of the same order as reported.[2] Then, the spectrum was recorded at lower concentration (0.06 M) Fig. 3A(2). Only two bands at 3621 cm^{-1} (I) and at 3492 cm^{-1} (II) were observed [band at 3346 cm^{-1} (III) was missing] indicating that at this concentration, higher order aggregates are not formed. This observation is in agreement with literature. Another spectrum was recorded at 0.014 M concentration, Fig. 3A(3). Only one weak band at 3622 cm^{-1} (I) (assigned due to monomer) was observed.

This experimental observation clearly points out the following: (i) with the increase in concentration, higher aggregates are formed, and (ii) the aggregation is intermolecular in nature. Dioxane was added to both reference and sample cell, Fig. 3B(1a). Band III at 3346 cm^{-1} disappeared. Band II at 3492 cm^{-1} became stronger while band I (due to monomer) got weaker but sharper. This suggests that higher aggregates and monomer have been converted to dimer. This observation fortifies our conclusion drawn through UV studies. The equilibrium now exists between monomer and dimer. When more of dioxane was added, Fig. 3C(1b), the peak at 3492 cm^{-1} (II) assigned to dimer became stronger while that due to monomer got weaker.

At the lower concentration (0.014 M) study [where only one band at 3622 cm^{-1} (I) was observed], when dioxane was added, a new strong band was observed at 3496 cm^{-1} (II) while the band at 3621 cm^{-1} (I) became much weaker, Fig. 3B(3a).

Similar kinds of studies were performed in CH_3CN, and observations were fairly identical.

The observed effect can be explained as follows. Dioxane, a weak base, provides basic environment that facilitates dimerization. This hypothesis can be easily verified by adding ions, which are negatively charged and are part of serum (e.g., bicarbonate ions, hydroxyl ions, chloride ions, etc.). When sodium hydroxide, sodium bicarbonate, and sodium chloride solutions were added to the cholesterol solution in water, a similar kind of effect was observed. All these ions have one thing in common; when added to the neutral water solution, the level of alkalinity is raised, and this favors dimerization. If this is true, then with the addition of trace amount of acid to the solution to which sodium hydroxide had been added, the original spectrum should be restored. Indeed this was observed. This suggests that alkaline environment favors dimerization whereas acidic favors monomerization. The physiologic implication of this is that persons whose serum pH is relatively higher or people suffering from higher blood pressure are more prone to the aggregation of cholesterol, or the process of atherosclerosis is facilitated. It is no surprise that patients suffering from heart disease are advised to keep blood pressure low. This result has an important implication; if the pH level of the serum is slightly reduced under medical supervision, then the process of plaque formation can be slowed down if not reversed completely.

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The other aspect of this observation on which we would like to comment is that when a certain class of molecules part of whose bonding structure consists of $-CH_2-O-CH_2$ (e.g., dioxane, etc.) gets into serum, perhaps inadvertently or as pollutants, it helps monomeric cholesterol to dimerize eventually leading to higher aggregates or atherosclerosis. But at the same time, higher aggregates (insoluble) are converted to dimer (relatively more soluble), the thus stage is set for the gradual removal of plaque, if suitable nontoxic molecules consisting of $-CH_2-O-CH_2-$ bonding are used.

CONCLUSIONS

Through this communication, we are trying to draw attention to the fact that higher level of alkalinity favors aggregation of cholesterol in serum plasma. We have also made an attempt to link higher blood pressure with the aggregation of cholesterol. The bonding arrangement $-CH_2-O-CH_2-$ of molecules, like dioxane, facilitates dimerization of monomers on one hand and converts higher aggregates to dimers on the other hand.

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ACKNOWLEDGMENTS

Seema Gupta acknowledges the financial support from DST, Government of India. We are grateful to the reviewers for their helpful suggestions and constructive comments. This work was part of the Ph.D. thesis of S.K. submitted to Delhi University in 2005.

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