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RESEARCH ARTICLE

CRYSTALIZATION OF CHOLESTEROL CRYSTAL BY GEL METHOD

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ABSTRACT

Cholesterol ($C_{27}H_{46}O$) is the most abundant and best known steroid in the animal kingdom. In the present study the crystallization of pure cholesterol monohydrate crystals in gel medium. In human body, precipitation of cholesterol gallstones occurs due to a defect of crystallization inhibiting or an abundance of crystallization promoting factors. The physical/ chemical events useful method for the identification of factors delaying or preventing precipitation of cholesterol crystals and therefore, gallstone formation in humans .The Gel grown cholesterol crystals are characterized by using FT-IR,FT-Raman and XRD methods.

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INTRODUCTION

Cholesterol ($C_{27}H_{46}O$) is the most abundant and best known steroid in animal kingdom. It is found in brain, nerve tissue and cell membranes[1]. It is a major constituent of crystalline material in gallstones[2]. Cholesterol has low solubility in water but it is soluble in organic solvents such as ethanol, acetone, methanol and benzene. Cholesterol was crystallized from methanol as a solvent and the effect of solvent on the crystal structure was studied. Cholesterol crystal has needle like morphology in silica gel in methanol as a solvent[3-4]. Gel is an ideal medium to grow biological crystals since its structure is similar to the mucus in the living organisms[5-9]. The internal surface of the organs in animals is invariably covered with mucus membrane. This material has open structure containing pores of different sizes. These pores can act as nucleation centers for the growth of crystals. Even at low super saturation, specific molecules can segregate creating critical nuclei to enhance the growth of crystalline materials. In the present work cholesterol is grown in silica gel medium using methanol as a solvent. The effect of temperature and concentration on the growth is also studied.

MATERIALS AND METHODS

The single test-tube diffusion method (Henisch 1988) was employed for growing cholesterol crystals in the gel medium. To prepare the silica hydro- gel, aqueous sodium meta silicate (SMS) solutions of 1.03 specific gravity was prepared and Methanol were mixed in appropriate amount and was acidified by acetic acid so that the PH of the mixture could be set with in 6.0. This mixture was allowed to set in to the gel form for 4 days.

For growing pure cholesterol crystals the supernatant solution was prepared by dissolving 2%(w/v) concentration of cholesterol (purity 99.99%) in methanol solvent and the solution was poured carefully over the top of the silica gel with out disturbing the latter. Within 24 hours needle like crystals were found to grow in the supernatant solution . The length of the needle goes up to 2 to 4 cm. This lengthening of the needle depends on the length of the liquid column over the gel. The grown crystals were harvested within 21 days [10-12].

RESULTS AND DISCUSSION

FT-IR spectroscopic studies on Cholesterol Crystals

Fig.2 shows the IR spectrum of cholesterol crystal. FT-IR spectrum cholesterol crystal was recorded SHIMAZDU 400-4000cm^{-1} range in chemistry department at Annamalai university. The respective assignments of the vibrations are given in table1. The bands observed at 3420 cm^{-1} indicate the presence of water group along with the hydroxyl group which is attached to the cholesterol ring. The bands observed at 2193-2931cm^{-1} region in the spectrum stem from the C-H stretching modes of aromatic compounds. C=O stretching observed at 1651 and 1507cm^{-1}. C-H deformation band observed at 1457cm^{-1}.Both CH_2 and CH_3 groups give rise to bands at 1384cm^{-1}, owing to hydrogen bending vibrations. C-H-in-plane bend at frequency 1274cm^{-1}. The bands C-H-in-plane bend observed at frequency 1149cm^{-1}. C-C-C-in-plane bend at frequency 1113cm^{-1}. The ring deformation of cholesterol can be assigned at 1050cm^{-1}.Ring breathing observed at frequency953cm^{-1}. C-C-C Streching observed at frequency 853cm^{-1}. Skeletal distortion observed at frequency791cm^{-1}. Ring deformation observed at frequency 701cm^{-1}. C-OH-in-plane bend observed at frequency 568 cm^{-1}.

FT-Raman spectral analysis of cholesterol cryst

FT-Raman spectra of the cholesterol crystal methanol solvent as shown in fig3. Table.2 shows vibrational assignment of cholesterol crystal. The C-H stretching vibration gives rise to bands in the 2800-3100cm^{-1} region (L.J.Bellamy,1966 and M.Rangacharyulu, D.Premas warup, 1978).The raman bands at 2932cm^{-1}, 2867$cm^{-1}$are assigned to these modes. Bands involving the in-plane bending vibration of C-H are observed in the region 1000-1300cm^{-1}. Thus the raman bands at 1272cm^{-1}, 1175cm^{-1} assigned to the C-H in-plane bending modes.The C-H out-of-plane bending vibration depends on the substituents and absorbs in the region 800-1090cm^{-1}(L.J.Bellamy,1966 and M.Rangacharyulu, D.Premas warup, 1978). The Raman frequencies being 1086, 1056, 1006, 984cm^{-1}.

The skeletal stretching of C-C bonds (L.J.Bellamy,1966 and M.Rangacharyulu, D.Premas warup, 1978). Absorbs in the

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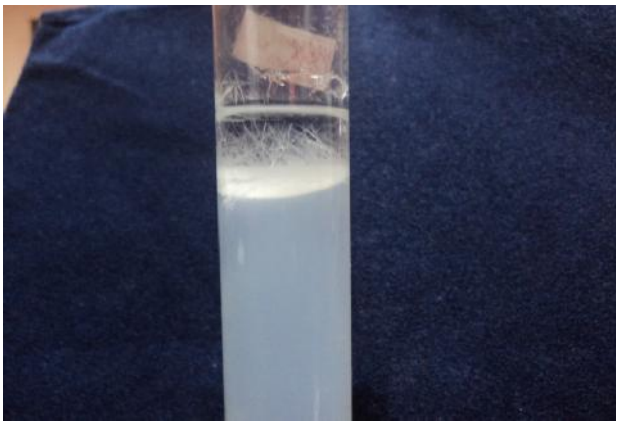

Fig.1 Cholesterol Crystals grown in gel medium

region 1300-1600cm^{-1} and around 1000cm^{-1}. All the above modes, except the ring breathing mode, are practically insensitive to the substitution. The ring breathing mode may shift to low values in the case of bulky substituents. The Raman bands in these region are assigned to this mode at 958cm^{-1}. These ring deformation modes are due to C-C-C in-plane bending and C-C-C out-ofplane bending.

Table.1 FT-IR Spectrum of cholesterol crystal

IR Frequency (cm^{-1})	Assignments
3420	O-H strech
2931	C-H stretching
2855	C-H stretching
2193	C-H stretching
1651	C=O stretching
1507	C=O stretching
1457	C-H deformation band
1409	C-H deformation band
1384	Combination band
1274	C-H in- plane bend
1149	C-H in- plane bend
1113	C-C-C in-plane bend
1050	Ring deformation
953	Ring breathing
853	C-C-C Stretching
791	Skeletal distortion
701	Ring deformation
568	C-OH in-plane bend

The in-plane bending modes generally occur around 700-900cm^{-1} region, where as out-of-plane bending modes occur around 500cm^{-1}. These bands observed in these region740cm^{-1}. The C-OH in- plane bending is observed at 606 cm^{-1}. In hydroxy sustituted hetero cyclic rings, keto forms usually found to exist.

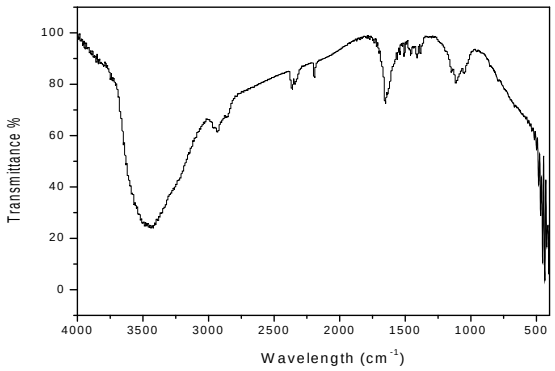

Fig.2 FT-IR Spectrum of Cholesterol Crystal

Table.2 FT-Raman frequencies (in cm^{-1}) with tentative assignment for Cholesterol crystal

Frequencies(cm^{-1})	Tentative Assignments
2932	C-H streching vibration
2867	C-H streching vibration
1671	C=O stretch
1439	Skeletal ring streching
1329	Skeletal ring streching
1272	C-H in-Plane bending
1175	C-H in-plane bending
1130	C-C-C in-plane bending
984	C-H out-of-plane bending
958	Ring breathing
881	C-C-C stretching
740	Ring deformation
700	Water twisting
606	C-OH in-plane bending
547	C=O in plane bending
426	C-O-C in plane bending

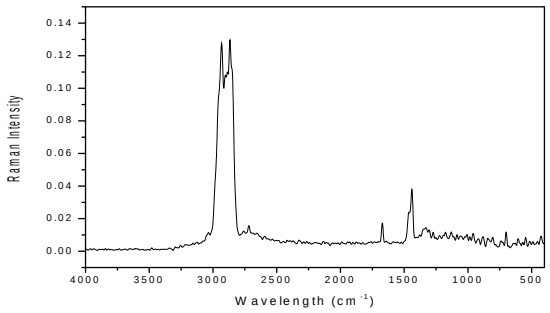

Fig.3 FT-Raman Spectrum of Cholesterol crystal

This pionts out that the proton of the OH group is not firmly attatched.the strong bandat 1671cm $^{-1}$ and medium one at 547cm^{-1} in Raman spectrum are indicative of the C=O stretching and bending vibrations and also explain the existence of tautomerism in this crystal.

Table. 3 powder XRD data of Cholesterol crystal

Pos.[º2Th.]	Height[cts]	FWHM Left[º2Th.]	d-spacing[Å]	Rel.Int.[%]
12.90(2)	23(6)	0.18(5)	6.85487	1.70
15.499(2)	305(13)	0.153(6)	5.71259	22.07
18.122(1)	1381(27)	0.139(3)	4.89133	100.00
23.407(3)	325(29)	0.12(2)	3.79743	23.51
26.087(6)	99(17)	0.10(3)	3.41310	7.17
28.74(1)	66(14)	0.13(5)	3.10424	4.81
31.438(8)	74(15)	0.08(4)	2.84324	5.36
34.16(3)	24(12)	0.11(8)	2.62292	1.75
39.606(9)	64(10)	0.12(3)	2.27367	4.66
42.380(4)	240(17)	0.11(1)	2.13107	17.36
45.178(7)	51(8)	0.08(2)	2.00536	3.72
48.005(8)	46(7)	0.09(2)	1.89367	3.35
50.85(3)	15(4)	0.3(1)	1.79410	1.08

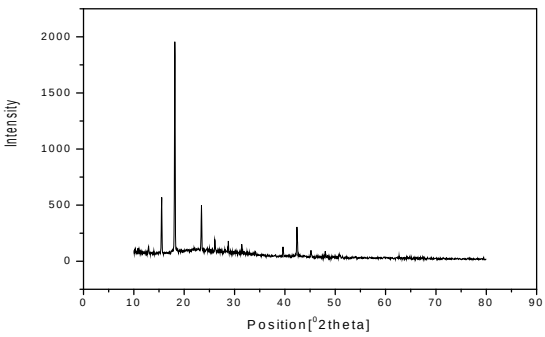

Fig.4 Powder XRD analysis of Cholesterol crystal

Powder X-ray diffraction of Cholesterol Crystal (XRD)

XRD patterns of cholesterol crystal were shown in fig.4 and table.3 shows XRDdata of Cholesterol crystals..XRDanalysis was recorded using XPERT-PRO Diffractometer with cu kα-Radiation.The cholesterol monohydrate is known to crystallize in triclinic structure with the following parameters; a=12.39Å, b=34.46Å,c=12.41Å, α=91.90º,β=98.1º, γ = 100.80º. In the present work, Fig.4 corresponds to cholesterol and all observed peaks have been indexed by using JCPDS data (No.48-2178, 07-0742).

CONCLUSION

Long needle like cholesterol crystals have been grown by gel method using Methanol solvent. FT-IR and FT-Raman results confirmed the presence of functional groups of cholesterol. Powder XRD confirms the crystal nature of the cholesterol crystal

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