



NOTE

Effect of Cucurbitacin L on the Crystal Defects of Cholesterol Monohydrate

N. SAMPATH KUMAR^{1,*} and G. MADHURAMBAL²

¹Department of Chemistry, Chettinad College of Engineering & Technology, Karur-639 114, India

²Department of Chemistry, ADM College for Women, Nagapattinam-611 001, India

*Corresponding author: Tel: +91 4324 250930; E-mail: arunaiguru@gmail.com

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Cholesterol crystals were crystallized from acetone with and without cucurbitacin L by gel method. The morphology of the cholesterol crystal changed from plate shape to needle shape crystals. The change in the crystal defects of pure cholesterol crystal like inter planar distance, dislocation density, grain size and micro strain due to the presence of cucurbitacin L as a dopant is presented by using XRD data.

Key Words: Gel method, Morphology, Inter planar distance, Dislocation density, Grain size, Micro strain.

Cholesterol ($C_{27}H_{46}O$) is the most important steroid which is found in most of the parts of the body. The deposition of cholesterol crystals may produce cardiovascular diseases. Phytoactive steroids like cucurbitacins are used to control cholesterol deposition by Ayurvedic medicines. The morphology of cholesterol crystals in blood vessels is reported to be plate like shape¹. The *in vitro* cholesterol crystals also have the same morphology as like in blood vessels². Cholesterol was crystallized from different solvents under various conditions and the effect of the solvents on cholesterol crystals were reported³.

Gel is an ideal medium to grow cholesterol crystal because its structure is similar to the mucus in the living organisms and the effect of cucurbitacin L on the growth of cholesterol crystals in sodium metasilicate (SMS) medium is discussed. Cucurbitacin L, a triterpenoid extracted and isolated from the fruits of *Citrullus colocynthis* by solvent extraction⁴. Plant sterols which have similar structure to cholesterol and they can influence the nucleation of cholesterol. The influence of sterols on cholesterol is characterized by X-ray analysis.

The single test-tube diffusion method was employed for growing cholesterol crystals in the gel medium⁵. The stock solution of specific gravity 1.03 g/cc was prepared by dissolving sodium metasilicate powder in double distilled water at room temperature. The solution was filtered and kept in a flask.

This solution was mixed with acetic acid to adjust the pH of the solution to 5.6. The gels were set in 4 days and the acetone solution of cholesterol and the acetone solutions of cucurbitacin were prepared as supernatant solutions.

The control was prepared by mixing 5 mL of acetone with 1 % (W/V) cholesterol in acetone solution and kept with out

any disturbance. Test sample for the treatment of effective fraction of herbal constituent was kept by adding the acetone solutions of cucurbitacin L in varying concentrations *viz.*, 10, 20, 30, 40 and 50 % to the top solution. All tubes were observed and noted that there is change in the nucleation of cholesterol crystal in the presence of cucurbitacin L. The optimum conditions set for the growth of crystals were tabulated in the following Table-1.

TABLE-1
OPTIMUM CONDITIONS SET FOR THE
GROWTH OF CHOLESTEROL CRYSTAL

Density of sodium meta silicate solution	1.03
Acid used to adjust pH of gel	Acetic acid
pH value of set gel	5.6
Temperature	32 °C
Concentration of Cholesterol solution	1 % (W/V)
Concentration of cucurbitacin solutions	0.001 g/L
Solvent used	Acetone
Gel setting period	4 days
Observed period of growth	70 days

XRD studies were conducted to characterize the crystals grown in gel media in the presence of cucurbitacin L. X-Ray powder diffraction method is one of the most widely used analytical methods where the intensity of the radiation diffracted by the crystalline material is measured by an electronic counting system. The X-ray diffractogram of the grown crystals in the presence of cucurbitacin L were recorded in a computer controlled rigaku, ultima III diffractometer using CuK_{α} radiation. The X-ray powder diffraction analysis was done and the

cell parameters of cholesterol grown under inhibition are found⁶ to be $a = 12.00$; $b = 32.2$; $c = 8.5 \text{ \AA}$. There are 8 independent molecules of cholesterol per unit cell $z = 8$ and it has the triclinic symmetry. The effect of cucurbitacin L on the growth specimen has obviously an inhibitory nature which not only reduced the growth rate and also changed the nature of the material (Table-2).

TABLE-2
CRYSTAL DEFECTS OF CHOLESTEROL
MONOHYDRATE PURE AND DOPED CRYSTAL

Nature of the crystal	I/I ₀	Dislocation density	Inter planar distance (Å)	Grain size (Å)	Micro strain
ChM1	100	0.00386	5.22	16.11	0.0225
ChM2	100	0.00386	5.22	16.09	0.0225

ChM1 = ChM crystal; ChM2 = Crystal grown with cucurbitacin I

Both the pure cholesterol monohydrate (ChM) crystal and ChM crystal grown in the presence of cucurbitacin L have the identical value for dislocation density (0.00386) at the peak having maximum I/I₀ value. With respect to the second highest peak, the dislocation density of the pure and doped crystals is 0.00467 and 0.00466. Cucurbitacin L decreased the dislocation density by 0.00001 units.

The interplanar distance of the pure and doped crystal of ChM is 5.22 Å. The introduction of the cucurbitacin L during crystallization of ChM did not affect the inter planar distance of the pure crystal. With reference to the second highest peak, there is no variation in the interplanar distance of the pure and doped crystal.

The inhibitor cucurbitacin L affected the average grain size of ChM crystal. The variation in the grain size among the ChM crystals grown with and without the inhibitor shown in the graph. From the above graph, it is evident that the inhibitor

which has a significant role in the reduction of average crystalline size from 16.11-16.07 Å. With reference to the second highest peak, the grain size of the doped crystals was found to be lesser than the pure crystal. This may be due to the dehydration of the ChM material in the presence of inhibitor which is further supported by the change in the morphology of the crystals⁷.

There is no variation in the micro strain values of the pure cholesterol crystals and doped cholesterol crystals. During the course of the crystallization no strain put up on the cholesterol crystal by the inhibitor.

Conclusion

The introduction of cucurbitacin L during the crystallization of cholesterol monohydrate by gel method, which did not change the crystal defects like dislocation density, inter planar distance and the micro strain. But there is a noticeable reduction in the grain size of the pure cholesterol monohydrate crystal.

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