Chapter 12

CONCLUSION AND FUTURE PLANS

ABSTRACT

According to the findings of the investigations on cholesterol crystal growth, it is advised to get exposed to day light in the morning and evening to control crystallization of cholesterol.

The crude extract of cassia fistula linn. is proved to be an ideal medicine for the control of cholesterol crystallization. Out of the phytocompounds of CF, rhein is found to be more efficient in inhibiting cholesterol.
12.1. CONCLUSIONS

This investigatory study on methods to inhibit cholesterol crystal growth adopts a multipronged approach to achieve its aim. The inhibitory effects of various parameters on cholesterol growth were tested and are presented in this thesis. The crystal growth studies were conducted in silica gel medium by solubility reduction method. In this crystal growth technique crystals were found to grow both inside the gel medium and in the supernatant solution.

For the crystals grown in gel medium, time taken for nucleation and subsequent growth took several days but in the supernatant solution the crystal grew immediately. The crystals grown in both cases had similar morphology and composition. The length and growth rate of the crystals grown in the supernatant solution was much greater. Since the aim of this investigation is the inhibition of the growth of cholesterol crystal and perfection in single crystalline growth is not very relevant, the crystal growth studies were conducted in the supernatant solution. Further, the effect of additive on the crystal growth and the observations on them are more convenient when studies are conducted with crystals grown in the supernatant solution.

Even though the crystals studied were grown over the gel, the pH and density of the gel were found to affect the growth of the crystals. These parameters affected the growth rate, number of crystals grown and length of the cholesterol crystals. The optimum condition for the growth of the crystals for this purpose was determined as the first step of this investigation.
Conclusion and future plans

Chapter 12

The concentration of cholesterol in the top solution had a significant effect on the cholesterol growth. When the concentration of the cholesterol solution was 0.25 % (w/v) the crystals were very thin and needle like. It took about four hours to nucleate and the number of crystals was comparatively less. As the concentration was increased the shape of the crystals changed from needle like to plate like. The number, growth rate and size increased with concentration. As the concentration increased to 1% large sheets of crystals were found to grow across the tube in the supernatant solution. To study the inhibitory effect of additives the optimum value of concentration was fixed at 0.5%. At this concentration the crystals grown had a thin plate like morphology which is similar to the cholesterol crystals grown in human tissues.

Detailed studies were conducted (chapter 7) to attain the optimum condition of investigation on the growth of CH crystals which is compatible with CH crystal growth in biological system. To study the effect of other parameters on cholesterol growth, optimum condition for the growth system was fixed at 0.5% cholesterol solution in a gel of density of 1.0 3g/cc at a pH of 6 and ortho phosphoric acid was used to adjust the pH. Ortho phosphoric acid is a mild acid and is predominantly present in human body.

One of the most significant findings of this research work is the effect of light on the growth of the CH crystals. It was found that cholesterol growth is greatly inhibited by the presence of light. The light of shorter wavelength can inhibit the growth better than light of longer wavelength. The optical effect on cholesterol crystal growth has clinical relevance.
The medicines like Atorvastatin, lovastatin, Simvastatin which are commonly used to control serum cholesterol were first considered as additive material in the top solution for controlling cholesterol crystal growth. Two other materials, potassium and calcium, were also used in this section for investigation. It was found that the number and size/length of the crystals grown in the presence of the statins were much less than that grown in control. In the presence of calcium and potassium the CH crystal growth was found to be enhanced and the number, size/length and the growth rate of cholesterol crystals were greater than those in the control. Further, the increase in the concentration of the additive affected the crystal growth.

The outcome of the experimental studies on the effect of medically used compounds, on growth of cholesterol crystals in gel medium was very encouraging in the sense that in vitro cholesterol growth study can very safely be employed to test the effect of any compound on cholesterol growth.

The main part of this research work was conducted on the study of the growth of cholesterol crystals and the effect of the phyto compounds present in the flower petals of the plant cassia fistula Linn. In pharmacognosy and ayurvedic medicine, all the parts of this plant are attributed with a large number of medicinal properties. Major cardiac problems are caused by the crystallization of cholesterol in the lesions which subsequently caused atherosclerosis. Cardiac protection is one of the important properties of this plant material. Hence the extract of the plant material was considered as the additive in the top solution for testing its effectiveness on the cholesterol crystal growth.
Growth experiments conducted using the flower extract added to the superannuated solution of cholesterol showed that it has got an inhibitory effect on the crystallization. Crystals formed in the control tube had plate-like morphology. In the treatments the addition of the plant extract showed not only a delay in nucleation but also a change in morphology. The crystal turned from plate-like to needle-like and also with a wool-like appearance. The change in morphology is a clear indication of inhibition of cholesterol crystal growth which may be attributed to the phytoactive compounds in the flower extract. This fact is supported by IR studies in which the hydroxyl bonds observed in CH crystals grown in control were absent in the crystals grown after treatment with flower extract. In oxidation of secondary alcohol to ketone, one learns to expect the disappearance of hydroxyl (O-H) stretch and appearance of carbonyl (C=O). X-ray studies proved that the CH crystals are triclinic with the cell parameters as follows:

For hydrous CH crystal (C_{27}H_{46}O·H_2O):
\[ a = 12.39 (3); \quad b = 12.41 (3); \quad c = 34.36 (6) \text{ Å} \]

For anhydrous form of the crystal (C_{27}H_{46}O):
\[ a = 14.172 (7); \quad b = 34.209 (18); \quad c = 10.481 (5) \text{ Å} \]
\[ \alpha = 94.64 (4)^\circ, \quad \beta = 90.67 (4)^\circ, \quad \gamma = 90.32 (4)^\circ. \]

Cassia fistula Linn. is found to bloom in the peak of summer season. The ultra violet spectrum of the flower extract has an emission peak at 285.5 nm. The ultra violet spectrum of cholesterol has an absorption peak at 295.5 nm. This particular correlation might have set an unfavorable condition for cholesterol
crystal growth in the presence of CF extract, i.e. it can inhibit the growth of CH crystals. The studies on the cholesterol growth in the presence of the CF flower extract had proved that this assumption is true. The experimental studies on cholesterol growth in the presence of the flower extract (chapter 10) and its lipid components (chapter 11) showed that the size, number and growth rate of cholesterol crystals decrease with the increase in the concentration of the plant extract. The studies with the different components in the extract of CF showed that the phytocompound rhein had maximum inhibitory effect on the growth of CH crystals.

Thus we can conclude that the addition of Cassia fistula flower extract in the growth stage can reduce the growth of cholesterol crystal i.e. additional crystallization of cholesterol can be avoided. Therefore Cassia fistula has suitable medicinal values without side effect for the control of cholesterol.

Another important outcome of the study of the effect of additives on cholesterol growth in gel medium is that invitro studies can be effectively adopted for testing the nature of materials which can be used as medicines for the prevention of CH crystallization. A number of diseases like atherosclerosis, gout, gall stones, kidney stones etc are caused by crystallization of materials. Thus invitro studies can be employed to check the effectiveness of new medicines. Such an effort can help as to save the life of some guinea pigs, rats and other animals used for the purpose.
12.2. Future plans

The most important outcome of this research work is that phytocompounds are effective in reducing/controlling cholesterol crystal growth and invitro studies may be employed successfully. The research work conducted by the author was able to bring out only a quality level exploration in this aspect. This work opens a new pathway to approach health related matters in the framework of physical concepts. We plan to conduct further studies on the effect of all the components in various parts of the plant cassia on the CH crystal growth. This understanding can further be extended to study the effect of phytocompounds present in other medicinal plants on CH crystal growth. These studies can help us to evolve a better strategy to combat with atherosclerosis, the most fatal disease in this world. A comprehensive survey on the occurrence of cholesterol related diseases and its correlation with socio-economic-region-wise distribution is also on the plan. An investigation in a theoretical and quantitative manner to describe the control of cholesterol crystal formation and the liquid crystalline behavior of cholesterol related compounds is also on cards.