CHAPTER 9

Abstract

Heart failure due to occluded coronary arteries is a major cause of death in our society. When the amount of cholesterol in the blood exceeds that required for the synthesis of membranes, bile salts and hormones pathological accumulation of cholesterol can develop in blood vessels resulting in atherosclerotic plaques. The main constituent of atherosclerotic plaques is cholesterol monohydrate crystals. The statins, medicinal products derived from fungi, are commonly used to treat patients with hypercholesterolemia. These compounds resemble mevalonate and are competitive inhibitors of HMG-CoA reductase. It is reported that the statin treatment lowers serum cholesterol by as much as 30%. In this section it is attempted to study the effect of statins on invitro cholesterol crystallization by gel method. Together with these statins, the effect of minerals calcium and potassium is also studied and presented in this chapter.
9.1. Introduction

Cholesterol is the abundant and best known steroid in the animal kingdom. It is found in brain, nerve tissue and cell membranes. It is a major constituent of crystalline material in gallstones and also acts as the precursor of bile acid and steroid hormones. Cholesterol has low solubility in water but it is soluble in organic solvents such as ethanol, methanol and benzene. The solubility of cholesterol in various solvents has been widely studied and reported. Cholesterol was crystallized from different solvents under various conditions and the effect of solvent on the crystal structure was studied. Gel is an ideal medium to grow biological crystals since its structure is similar to the mucus in the living organisms. The internal surface of the organs in animals is invariably covered with mucus membrane. Gel material has open structure containing pores of different sizes. These pores can act as nucleation centers for the growth of crystals. Even at low supersaturation, specific molecules can segregate creating critical nuclei to enhance the growth of crystalline materials. The studies have shown that cholesterol monohydrate crystals can be grown in gel medium and that these crystals grow in fibrous and platelet forms. In the present work cholesterol is grown in sodium metasilicate gel medium using 99.9% pure AR grade ethanol as solvent. The effectiveness of different medicines/chemicals in controlling the growth of cholesterol crystal in gel medium of different pH values was studied.

Cholesterol is formed from acetyl-CoA in a complex series of reactions through the intermediates β-hydroxy-β-methylglutaryl-CoA (HMG-CoA), mevoalonate and two activated isoprenes. The medicines used to reduce serum cholesterol include the statins like lovastatin, simvastatin, atorvastatin, compactin,
pravastatin etc. These compounds resemble mevalonate in structure and are competitive inhibitors of HMG-CoA reductase and thus inhibit cholesterol synthesis. Hence the study on the inhibitory nature of statins on the cholesterol growth can be very helpful in understanding the invitro inhibitory nature of other less known compounds.

The advanced atherosclerotic lesion is characterized by the formation of microscopic cholesterol crystals that contribute to mechanisms of inflammation and apoptotic cell death. These crystals develop from membrane cholesterol domains, a process that is accelerated under conditions of hyperlipidemia and oxidative stress. In this study, the comparative effects of hydroxymethylglutaryl-CoA (HMG-CoA) reductase inhibitors (statins) on oxidative stress-induced cholesterol domain formation were tested in model membranes containing physiologic levels of cholesterol using small angle x-ray diffraction approaches. In the absence of HMG-CoA reductase, only the atorvastatin active \( \sigma \)-hydroxy metabolite (ATM) blocked membrane cholesterol domain formation as a function of oxidative stress. This effect of ATM is attributed to electron donation and proton stabilization mechanisms associated with its phenoxy group located in the membrane hydrocarbon core. ATM inhibited lipid peroxidation in human low density lipoprotein and phospholipids in a dose-dependent manner, unlike its parent and other statins (pravastatin, rosuvastatin, simvastatin). These findings indicate an atheroprotective effect of ATM on membrane lipid organization through a potent antioxidant mechanism.

Cholesterol is grown in sodium meta silicate gel medium using 99.9% pure AR grade ethanol as solvent. The effect of pH variation and effectiveness of different chemicals in controlling the growth of cholesterol crystal in gel medium...
was studied in detail. The medicinal materials used here are lovastatin, simvastatin and artrovastatin. The effect of two other materials, potassium and calcium, on invitro crystal growth was also studied and is reported in this chapter.

9.2. Experimental set up

The single test tube diffusion method \(^9\) was employed for growing cholesterol crystals in the gel medium. The stock solution of specific gravity 1.03 was prepared by dissolving sodium meta silicate powder in double distilled water at room temperature. The solution was filtered and kept in a clean flask. This solution was mixed with ortho-phosphoric acid to adjust the pH of the solution. By varying the pH the density of the gel could be controlled. In the present case gel of pH 5 to 6 were employed to study the effectiveness of different medicines on the growth of cholesterol crystals.

Gel is an ideal medium to grow biological crystals since its structure is similar to the mucus in the living organisms. The internal surface of the organs in animals is invariably covered with mucus membrane. This material has an 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 nuclear to enhance the growth of crystalline materials.

The supernatant solution was prepared by dissolving required amount of cholesterol (CDH, AR) in 99.9% pure AR grade ethanol. Here 0.5% (w/v) solution of cholesterol in ethanol is used as stock solution. From this 0.5% (w/v) solution of cholesterol in ethanol, 2ml is pipetted out slowly to the gel surface in the test tube.
The cholesterol crystals formed after one hour. 0.5% (w/v) solution of cholesterol in ethanol, added as supernatant solution was treated as the control in the growth system in all the experiments throughout this study for the cholesterol crystal growth.

9.3. **Effect of cholesterol crystal growth at different pH values of the gel in the presence of pharmaceutical medicines.**

A comprehensive study was conducted since the findings could develop new strategies to control cholesterol growth. In this section control tubes were set with gel of pH values 5, 5.5 and 6. Atorvastatin (A), Lovastatin (L) & Simvastatin(S) are the medicines and potassium (K) & calcium (Ca) are biomaterials available in market and were taken as additives in the cholesterol growth system. The studies were carried out, with additives of known concentrations, at varying pH values.

Experiments were conducted in a series of test tubes at pH values ranging from 5 to 6 and known concentration of the additives were added, keeping a control tube (C) of 2ml of 0.5% (w/v) of cholesterol in ethanol. Various combinations of additives and pH values were conducted, as a function of time. Three types of treatments given to the sets of experiments are described as follows.

A 0.5 % solution of the three pharmaceutical compounds and biominerals were prepared in absolute alcohol and were used in different concentration for each set of studies. For these experiments concentrations of 1, 2 and 3 ml of 0.5 % solution of pharmaceutical medicines were used in T1, T2 and T3 respectively. T1, T2 and T3 are the treatment sequence in progression.
The three medicines, lovastatin, Simvastatin, atorvastatin and the two biomaterials, potassium & calcium taken for the investigations and its effect on crystal growth, are explained in the following sections (9.3.1, 9.3.2, 9.3.4 and 9.3.5). In the case of atorvastatin a second method of application was tested to clarify the former method. A second sequence of studies and experiment was set up where the sequence of addition, atorvastatin, potassium and calcium, were modified.

9.3.1. Effect of lovastatin on cholesterol growth

To study the effect of medicines on cholesterol growth in gel medium, lovastatin was first considered. Control and treatment tubes were set up as described in the experimental set up given above. The growth of CH crystals was observed after one hour in the control tubes. The crystals were clearly visible after 24 hours and were thin and plate like. The dimensions of the crystals were greater at pH 6 and the maximum number of crystals was at pH 5.

The experiment in T 1 set, crystal growth started within 2 hours and visible after 24 hours. The number and length of CH crystals grown were less than that in the control. The crystals formed were narrow and plate like.

In the treatment 2 and treatment 3, the similar growth patterns were observed. It is also observed that from control to experimental set up 3, the number and length of CH crystals formed decreased proportionately. The photograph (Fig.9.1) shows a comparison of the growth of cholesterol crystals in a control and treatmented set of experiments.
Figure 9.1. Cholesterol growth in control experiment (left) and in presence of additives (right).

The observations for the control and other sets of experiments are incorporated in tables 9.1, 9.2 and 9.3 and depicted in figures 9.2, 9.3 and 9.4.

Table 9.1. Effect of the concentration of lovastatin on crystal growth with respect to pH.

<table>
<thead>
<tr>
<th>Sl. no.</th>
<th>Experiment</th>
<th>Rate of variation of crystal length (cm.) as a function of pH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>24 hr</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>1</td>
<td>C</td>
<td>1.08</td>
</tr>
<tr>
<td>2</td>
<td>T 1</td>
<td>0.78</td>
</tr>
<tr>
<td>3</td>
<td>T 2</td>
<td>0.57</td>
</tr>
<tr>
<td>4</td>
<td>T 3</td>
<td>0.37</td>
</tr>
</tbody>
</table>
Figure 9.2 (a). Effect of crystal growth in presence of lovastatin as a function of pH, concentration and time.

Figure 9.2 (b). Average crystal growth profile.

From the observations depicted in fig 9.2 (a) & 9 (b) we can see that the growth rate and length of crystals decrease with the increase in concentration of the additive medicine in the top solution. This aspect is also evident in the fig 9.3 where
it was observed that with the increase in the amount of lovastatin added, the number of CH crystals decreased. Further, as pH of the gel changed from 5 to 6 with medicines the number of CH crystals grown decreased.

<table>
<thead>
<tr>
<th>Sl. no.</th>
<th>Experiment</th>
<th>Number of crystals formed with respect to pH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>5.0</td>
</tr>
<tr>
<td>1</td>
<td>Control (C )</td>
<td>18</td>
</tr>
<tr>
<td>2</td>
<td>Treatment 1</td>
<td>12</td>
</tr>
<tr>
<td>3</td>
<td>Treatment 2</td>
<td>9</td>
</tr>
<tr>
<td>4</td>
<td>Treatment 3</td>
<td>8</td>
</tr>
</tbody>
</table>

Table 9.2. Crystal formation as a function pH in the presence of Lovastatin

![Graph showing crystal formation](image)

Figure 9.3. Number of crystals grown in the control and treatments on addition of Lovastatin for various pH values of gel medium.
9.3.2. Effect of the medicine simvastatin on cholesterol growth.

This study is similar in all respects as already mentioned in 9.3.1. except a new additive simvastatin was used instead of lovastatin. The growth of crystal, its dimension and number were determined in control as well as the three sets of experiments. Comparable results were obtained in all the sets as in the previous case. This conforms that the addition of a statin definitely inhibits the growth of cholesterol crystal under the controlled experimental conditions.

The number and length of CH crystals grown were taken as indicators of growth rate and a photograph of a sample growth system is given in figure 9.1. Observations on the growth rate in terms of length and number of CH crystals on addition of simvastatin are given in table 9.5 & 9.6 and the details are plotted in figures 9.4 (a), (b) & 9.5.

**Table 9.5. Effect of the concentration of simvastatin on Crystal growth with respect to pH.**

<table>
<thead>
<tr>
<th>Sl.no.</th>
<th>experiment</th>
<th>Rate of variation of crystal length (cm.) as a function of pH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>24 hr</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>1</td>
<td>C</td>
<td>1.09</td>
</tr>
<tr>
<td>2</td>
<td>T 1</td>
<td>0.77</td>
</tr>
<tr>
<td>3</td>
<td>T 2</td>
<td>0.56</td>
</tr>
<tr>
<td>4</td>
<td>T 3</td>
<td>0.37</td>
</tr>
</tbody>
</table>
Figure 9.4 (a). Effect of crystal growth in presence of simvastatin as a function of pH, concentration and time.

Figure 9.4 (b). Average crystal growth profile.
Table 9.6. Crystal formation as a function pH in the presence of simvastatin

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Experiment</th>
<th>Number of CH crystals grown at different pH values of the gel</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>1.</td>
<td>control</td>
<td>15</td>
</tr>
<tr>
<td>2.</td>
<td>Treatment 1</td>
<td>13</td>
</tr>
<tr>
<td>3.</td>
<td>Treatment 2</td>
<td>12</td>
</tr>
<tr>
<td>4.</td>
<td>Treatment 3</td>
<td>10</td>
</tr>
</tbody>
</table>

Figure 9.5. Number of crystals grown in gel medium of various pH values on addition of simvastatin
9.3. 3. Spectral analysis of CH crystals grown in the presence of Medicines Lovastatin and Simvastatin (L&S)

Absorption methods based upon ultra violet (UV), Visible and infra red (IR) radiations find wide spread application in the quantitative and qualitative analysis of molecular species. Radiation in this region is of sufficient energy to cause transitions of valence electrons. For any particular peak to occur, the magnitude depends upon the capture cross section of the species and the probability of an energy absorbing transition. The information which electronic spectroscopy yields may be classified as follows.

- Information about functional groups.
- General information about the electronic structure of organo-metallic compounds.
- Information regarding electronic effects in specific bands.
- Nature of charge transfers complexes.
- Energies of the commonly occurring transitions of the type UV-Visible-Near IR Spectrum of the crystal was taken using a spectrophotometer.

9.3.3.1. Analysis – Infrared Spectroscopy.

Absorption of IR radiation is confined largely to molecular species for which a small energy difference exists between various vibrational and rotational states. In order to absorb IR radiation, a molecule must undergo a net change in dipole moment as a consequence of its vibrational motion. Only under these circumstances can the alternating field of the radiation interact with the molecule. Vibrational energy levels are quantized and the energy difference between quantized states
corresponds to the readily accessible regions of IR from about 4000 to 200 cm\(^{-1}\) for each vibrational state.

The Infrared absorption of cholesterol was recorded in a Perkin Elmer spectrometer 983 in KBr matrix. IR of pure cholesterol and that on addition of two sample medicines, Lovastatin and Simvastatin (L&S) are included in this section in figures 9.6, 9.7 & 9.8. Fig.9.7 shows that the crystal grown in the presence of lovastatin has water of crystallization, as is evident from the peak at 3448.63 cm\(^{-1}\) (relating to symmetric OH stretching) and at 1647.87 cm\(^{-1}\) (relating to H-OH bending). These bands in the IR spectra are usually ascribed to the molecules being involved in hydrogen bonding of different extents. The band at 2936.89 cm\(^{-1}\) indicates the O-H stretch. The peak at 1367.27 cm\(^{-1}\) represents the C-O stretch.

Figure 9.6. IR spectrum of pure cholesterol crystal
Fig. 9.7. Infrared Absorption spectrum of cholesterol with Lovastatin added

A relatively intense and diagnostic absorption owing to a C-O stretching is present in the region of 1100.58 cm\(^{-1}\). The revelation of well pronounced peaks at 798.75 cm\(^{-1}\), 471.72 cm\(^{-1}\) (C-O scissor motion). These peaks confirm the presence of cholesterol, with the presence of water of crystallization, agreeing quite well with the reported values in the literature and with respect to pure cholesterol crystals.

Fig. 9.8. Infrared Absorption spectrum of cholesterol with Simvastatin
grown in control Fig.9.6 shows that the cholesterol crystal grown in the presence of the medicine simvastatin has water of crystallization as evident from the peak at \(3533.16\text{cm}^{-1}\) (relating to symmetric OH stretching) and at \(1635.84\text{cm}^{-1}\) (relating to H-OH bending). These bands in the IR spectra are usually ascribed to the molecules being involved in hydrogen bonding of different extents. The band at \(3056.07\text{cm}^{-1}\) indicates the O-H stretch.

The peak at \(1338.61\text{cm}^{-1}\) represents the C-O stretch. A relatively intense and diagnostic absorption owing to a C-O stretching is present in the region of \(1098.75\text{cm}^{-1}\). The revelation of well pronounced peaks at \(775.43\text{cm}^{-1}, 409.04\text{cm}^{-1}\) (C-O scissor motion), \(520.53\text{cm}^{-1}\) [ring formation + \(\delta\) (C-H-O)]. These peaks confirms the presence of cholesterol, with the presence of water of crystallization and also agrees quite well with the reported values in the literature.

### 9.3.3.2. Analysis – UV spectroscopy

The position and intensity of an ultraviolet absorption band are usually reported by the wavelength of maximal absorption. For any particular peak to occur, the magnitude depends upon the capture cross section of the species and the probability of an energy absorbing transition. UV spectrum of cholesterol crystals grown in control and those grown in treatments with the medicine lovastatin are considered here for a comparison.

The UV spectrum of pure cholesterol is given in fig 9.9. The UV-Visible absorption spectrum of cholesterol crystal grown in the presence of Lovastatin in top solution is depicted in figure 9.10. The peak observed in the spectrum is assigned to
the modes by comparing them with standard values of the elements available in literature.

![UV spectrum of cholesterol](image)

**Figure 9.9. UV visible spectrum of cholesterol**

![UV spectrum with Lovastatin added](image)

**Fig.9.10. UV visible spectrum of cholesterol with Lovastatin added.**
The UV-Visible absorption spectrum of cholesterol crystal grown in the presence of Simvastatin is depicted in figure 9.11. The peak observed in the spectrum is assigned to the modes by comparing them with standard values of the elements available in literature. The slight peak obtained around 250 nm is assigned to the cholesterol group. Since the sample size taken was small, the spectral details are rather vague. Still, the presence of cholesterol was evident from the spectrum.

The UV and IR spectra of the crystals thus showed that the composition of cholesterol crystals are not much affected by the presence of medicine even though they can inhibit the nucleation and growth of the crystals.

Fig.9.11. UV visible spectrum of cholesterol with Simvastatin added.
9.3.4. Effect of the medicine atorvastatin on cholesterol growth.

In this experimental set up a modified sequence of application of addition of the inhibitor, atorvastatin, was employed. It is anticipated that it may produce a more refined output.

9.3.4.1. Experimental set up

Experiments were conducted at pH 5 and varying the time of addition and quantity of additives. In the experimental set up T1, a mixture of 2 ml of cholesterol solution 0.5% (w/v) and 2ml of Atorvastatin solution (0.5%) in ethanol was added as top solution. Whereas, in the second set (T2) the same quantity of atorvastatin was added after a time lag of 24 hours. The growth rate, crystal size and number of crystals were studied in parallel. These two types of treatments were kept as growth systems along with a control system and the observations are presented in the following section.

9.3.4.2. OBSERVATIONS

In this experimental set up control and T 2, within 24 hours crystals were grown to the size of 1.1 cm whereas in T 1, because of the addition of atorvastatin the growth was found to be only 0.2 cm. After 24 hours the same quantity of the additive was added in T1 and the subsequent changes in all the three experiments were evaluated. The results obtained are shown in table no. 9.7 and figure 9.13.

In T1 the crystal growth was almost uniform and reached to an ultimate value of 0.6cm in 120 hours. On the contrary in T 2, the growth rate was reversed from a value of 1.1 cm to 0.7 in 120 hours, almost coinciding with that of a corresponding value of T1, because of the addition of atorvastatin within a time lag of 24 hours.
This provides very important information that the introduction of an additive like atorvastatin has a profound effect on crystal growth at any stage in the progress of crystal growth. This may find application in the medical field and elsewhere. The number of crystals formed in C, T1 and T2 are 14, 6 & 6 respectively, which also is in conformation with the above statement. An illustration of the pattern of growth in control and treatment is given in Fig.9.14.

Table 9.7. Effect of the medicine atorvastatin on the length of cholesterol crystals grown at constant pH

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Experiment</th>
<th>Length (cm) of CH crystals grown as a function of time (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>24</td>
</tr>
<tr>
<td>1</td>
<td>C</td>
<td>1.10</td>
</tr>
<tr>
<td>2</td>
<td>T1</td>
<td>0.20</td>
</tr>
<tr>
<td>3</td>
<td>T2</td>
<td>1.10</td>
</tr>
</tbody>
</table>

Figure 9.13. Effect of artrovastatin on the growth rate / length of cholesterol crystals.
Figure 9.14. Photograph of the pure cholesterol growth (left) and very few number of growth (right) when atorvastatin was added

9.3.5. Effect of calcium and potassium on cholesterol growth

Calcium and potassium are two important minerals found in blood vessel along with several substances. These have a great influence in metabolism and body functions. Clinically, both play advantageous and disadvantageous roles. Here, we added these materials in the top solution of the cholesterol growth system in gel media of different pH values and different concentration of additive solution.

9.3.5.1. Experimental set up

In this section we adopted the method tested in section 9.3.3. That is, the additive solution was added in the top solution of cholesterol in the second day of growth. Such a procedure had changed the growth rate of crystals with respect to that of the control system. In this part, growth study was conducted on gel of pH
values 5, 5.5 and 6. Experimental tubes were prepared by adding 2 ml of cholesterol solution (0.5%) over the gel and observations were started. After 24 hours 1% ethanol solution of calcium and potassium were added in different amounts in the different treatments. The tubes in which crystals had grown for a period of 24 hours were taken and the different treatments were set with 1ml and 2ml of the solution of calcium or potassium over the top solution. The different treatments considered are listed in the table 9.8.

Table 9.8. Designation of treatments set with potassium and calcium.

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>K 1-</td>
<td>2ml of 0.5% cholesterol +1ml of 1% K solution.</td>
</tr>
<tr>
<td>K 2-</td>
<td>2ml of 0.5% cholesterol +2ml of 1% K solution</td>
</tr>
<tr>
<td>Ca 1-</td>
<td>2ml of 0.5% cholesterol +1ml of 1% Ca solution</td>
</tr>
<tr>
<td>Ca 2-</td>
<td>2ml of 0.5% cholesterol +2ml of 1% Ca solution</td>
</tr>
</tbody>
</table>

The treatment tubes 2 had more of additive in the top solution. Experimental tubes were set up with such treatments on gel of pH values 5, 5.5 and 6 and observations were taken on all the growth systems. The observations showed that the number of crystals grown in the treatments was greater than that in control. Also as the amount of additive increased, the number of crystals increased. The observations are depicted in figure.9.15.
Figure 9.15. Number of crystals in control and treatments as a function of pH

The variation in the growth rate, in terms of length of the crystals, on addition of different minerals of varied concentrations as the top solution over the gel of different pH values is given in figure 9.16. It can be concluded that in all the cases the growth rate in control was the least. The length / growth rate increased in the presence of the additive and further increased with the concentration of the additive in the top solution. These observations are tabulated and depicted in the figure 9.16. Figures 9.15 and 9.16 show that with the addition of calcium and potassium in the top solution, the number and length / growth rate of cholesterol crystals increased in the gel medium. It can be concluded that calcium and potassium are favorable to the growth of cholesterol crystals in gel medium.
Figure 9.16. Effect of potassium and Calcium on cholesterol growth.
9.4. CONCLUSION

In this chapter the effect of the medicines Lovastatin, Simvastatin, Atorvastatin, potassium and calcium on the cholesterol growth is discussed. In the control, the growth started after one hour of the addition of top solution. The growth of the crystal (length) was high in pH 6. The number of crystal was high in pH5. In treatments where medicines Lovastatin, Simvastatin and Atorvastatin were added, the growth rate of CH crystals was less because these materials lower the rate of nucleation. But when calcium and potassium were added, the growth rate of CH crystals increased. This may be attributed to the fact that presence of cations enhances the nucleation process of crystals. The IR and UV spectral analysis showed that the presence of the additive materials does not change the structure of cholesterol. The growth observations showed that the presence of the additives modify the rate of nucleation and growth of cholesterol crystals.

The cholesterol crystals have a thin plate like structure. When the growth medium becomes acidic the growth rate of cholesterol crystal is high. When it becomes basic, the growth rate is decreased and the number of nucleation of cholesterol is decreased. It is necessary to control cholesterol crystal formation in our body because it is harmful to health and can cause many diseases. This study showed that the medicines which are commonly administered to reduce serum cholesterol are also effective in reducing the nucleation and growth of cholesterol crystals in gel medium. The cholesterol crystals have assume a thin plate like structure in control and in all treatments. Calcium and potassium are found to be favourable to CH crystal growth and cannot reduce the growth of cholesterol.
crystals. The amount of potassium and calcium should not exceed the optimum value required for the functioning of the body; otherwise it can also cause cholesterol crystallization. This part of the investigation gave us more encouragement in employing gel method for testing of the effectiveness of phytocompounds to control the growth of cholesterol crystals.

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    Chicago, Chicago 1.1, 2.1, 2.2


    A.1, A.2

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