Observation of cholesterol nucleation in a magnetic field

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The *in vitro* studies on the nucleation, growth and other fundamental aspects of cholesterol crystallization have received considerable attention, as it plays a vital role in the formation of atheroclerotic plaques and gallbladder stones. The cholesterol was crystallized in methanol, ethanol, acetone and isopropanol at the physiological temperature of 37°C in the presence and absence of a low static magnetic field at 0.1 T. The presence of magnetic field was found to have a significant effect on the metastable zone width and induction period of cholesterol.

Keywords: cholesterol; metastable zone width; induction period; interfacial energy; magnetic field

1. Introduction

In recent years there has been an increased interest in the biological effects of magnetic fields (Chiriac & Simionescu, 2000). Human beings are normally exposed to static and time varying magnetic fields varying from 0.3 μT in houses and offices to 2.0 T found in medical applications by using magnetic resonance imaging and spectroscopy (Miller, 1974; Stuchly, 1986). Recently, artificial hearts have been designed with magnetic and electromagnetic (0.04 to 0.4 T) devices (Maslen *et al.*, 1998). The presence of magnetic field (0.08 to 0.3 T) influenced the crystallization of diamagnetic biominerals like calcium phosphate (Sorensen & Madsen, 2000; Madsen, 1995). At 0.11 T, the conversion process of methacrylic polyester (a diamagnetic polymer) synthesis is affected (Chiriac & Simionescu, 2000). The magnetic field effect on the crystallization of proteins was first reported concurrently by Ataka *et al.* and Sazaki *et al.* in 1997. Many subsequent crystallization studies on protein have reported crystal orientation in the direction of magnetic field, when a reasonably high intensity field was used (Astier *et al*., 1998; Wakayama, 1998; Yanagiya *et al*., 1999). The lyzozyme crystals were found to orient even in a low magnetic field of intensity 0.64 T as reported by Tanimoto *et al.* (2002). The growth rate of lysozyme crystals was reduced to 60% at 11 T (Yanagiya *et al*., 2000). But, the increased growth rate and decrease in the metastable zone width under 0.3 T of diamagnetic material was reported by Freitas *et al.* (1999). The experiments were performed under presence of 0.1 T intensity magnetic field, known to have perceptible effect on diamagnetic materials. Cholesterol is found in cell membranes, and is supposed to be an etiological agent in artheroclerosis and cholelithiasis (Admirand & Small, 1968; Small & Shipley, 1974). The crystallization and effect of solvents on the crystal habit of cholesterol have been reported (Toor *et al.*, 1978; Garti *et al.*, 1981; Kalkura *et al.*, 1991).

The *in vivo* processes leading to crystal deposition of cholesterol are very complex. It is therefore difficult to gain insight into the nucleation and growth aspects of the pathogenesis from *in vivo* experiments. Through *in vitro* studies, however, it is possible to gather data on conditions of nucleation, factors controlling the precipitation and the velocity of crystal growth, such information is vital for developing an understanding of why crystals grow in some patients but not in others. The fundamental nucleation parameters such as metastable zone width, induction period of cholesterol crystallization has not been reported so far. Here we report the various nucleation parameters of cholesterol nucleation in the presence and absence of a magnetic field. The metastable zone width was calculated for various solvents at different temperatures. The induction period was measured for various supersaturation levels and the interfacial energy at the crystal–solution interface were evaluated for different solvent systems.

2. Experiment

Reference standard grade anhydrous cholesterol was purchased from Sisco Research Laboratories, Pvt. Ltd. (Mumbai, India) were further recrystallized from acetone and kept in dark in a vacuum desiccator. The organic solvents that were either spectroscopic or analytical grade were used as such.

2.1. Solubility

The solubility of cholesterol was determined for four different temperatures 27, 32, 37 and 42°C. The solubility was determined by dissolving the excess amount of crystalline solute in the solvent in an air tight teflon capped flat-bottom container maintained at a constant temperature bath of accuracy ±0.05°C and the solution was continuously stirred with the help of an immersible magnetic stirrer for about 12 hours. After attaining the saturation, the equilibrium concentration of the solute was analyzed gravimetrically.

2.2. Supersolubility and induction period measurement

A constant volume of 5 ml of solution was used in all the experiments with the help of a micropipette. The solution was preheated to 5–10°C above the saturation temperature for homogenization and left at the superheated temperature for 6 hours before cooling. It was continuously stirred using an immersible magnetic stirrer and paddle arrangement to ensure homogeneous concentration and temperature throughout the entire volume of the solution. Metastable zone width of cholesterol in the four organic solvents was measured using the conventional polythermal method reported elsewhere (Zaitseva *et al.*, 1995). The average cooling rate was kept at 0.1ºC per hour, slow enough compared to the expected induction time for nucleation. The equilibrium-saturated solution was cooled from the overheated temperature until the first visible crystal with ~0.1mm length in the longest diameter was observed. Since the time taken for the formation of the first visible nucleus after the attainment of the critical nucleus is very small, the first crystal observed was taken as the critical nucleus. Induction period of cholesterol in four organic solvents were measured using the isothermal method. The saturated solution was cooled to the desired temperature and was maintained at that temperature and the time taken for the formation of the first crystal was measured. The solubility, supersolubility, induction period curves for different temperatures were as shown in Fig. 2–6. The experimental supersolubility and induction period measurements have < 5% standard deviation errors - checked 8 times by repeating the experiments. The average values observed from duplicate experiments were taken for analysis.

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2.3. Magnetic field set up

A pair of permanent bar magnets of 0.1T strength which was insulated by thin polythene cover to avoid water contamination were kept inside the constant temperature water bath (±0.1°C) and the crystallization solution was kept in between the magnets. The metastable zone and induction period for cholesterol crystallization in the presence of magnetic field were determined as described above. The magnetic field profile was calibrated with the Gaussmeter. The schematic diagram of the magnetic field as shown in Fig.1.

2.4. Interfacial energy

An understanding of the interfacial free energies that control the nucleation and growth of crystals is important to elucidate the mechanisms of pathological mineralization. The interfacial energy is calculated from the induction period measurements. At small thermodynamic driving forces for crystallization or dissolution, the low interfacial tensions increase the tendency of precipitation or dissolvation. Here, the metastable solutions were held at constant supersaturation. The interfacial tension in the present investigation has been calculated on the basis of the classical theory of homogeneous nucleation of spherical nuclei. The kinetic nature of metastable supersaturation leads to an equation for the nucleation rate (Zettlemayor *et al*., 1969) as given by,

$$J = A \exp \frac{-16\,\pi\,(\sigma)^3\,V^2}{3\,(kT)^3\,\ln(S)^2} \qquad (1)$$

where J is the rate of nucleation, A a constant, σ the interfacial energy, k the Boltzmann constant, T the absolute temperature, V the molecular volume and S the relative supersaturation with respect to the equlibrium saturation at the absolute temperature.

Interfacial energy (or) surface tension is given by

$$\sigma = RT[3m/(16\pi V^2 N)]^{1/3}\ \ mJ/m^2 \qquad (2)$$

where R is the gas constant, T the absolute temperature, m slope value of the graph drawn between ln x_n and inverse of the induction period, x_n mole fraction of the solute, V molar volume of the crystal, N Avogadro´s number (6.022 x 10^{23} /gram).

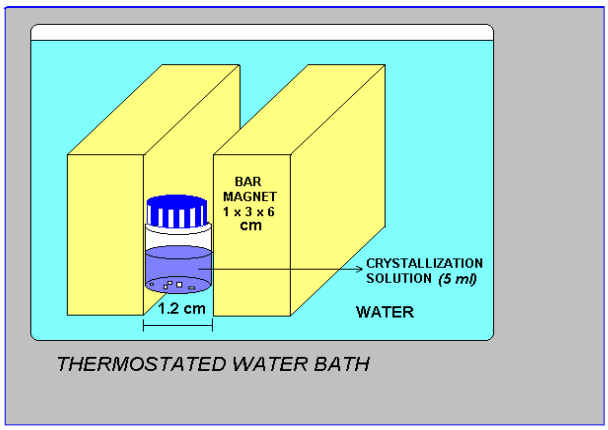

Figure 1 Schematic diagram of magnetic field setup

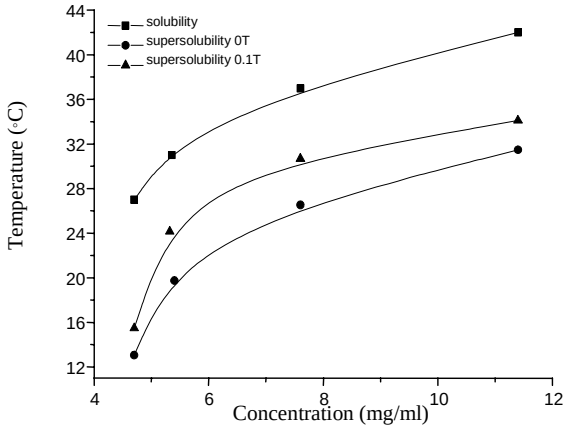

Figure 2 Cholesterol solubility in methanol

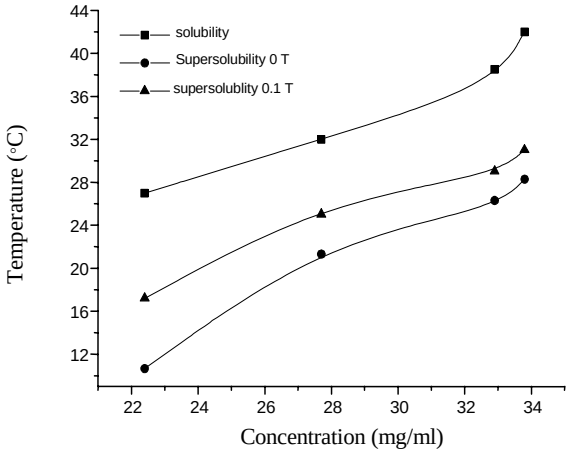

Figure 3 Cholesterol solubility in acetone

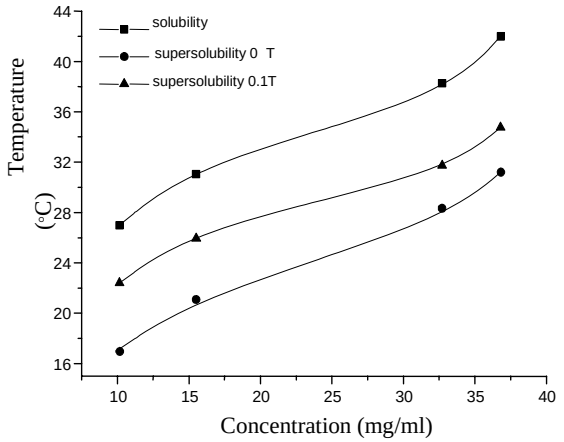

Figure 4 Cholesterol solubility in ethanol

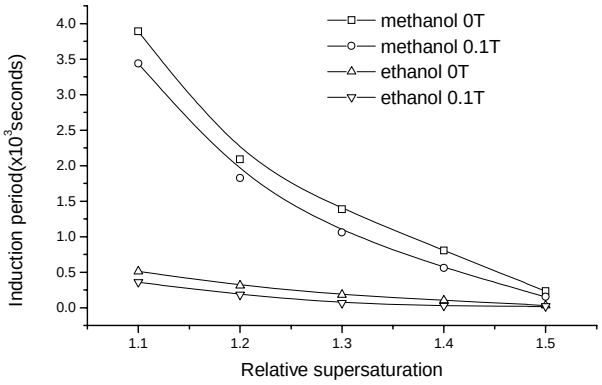

Figure 5 Cholesterol induction period in methanol and ethanol

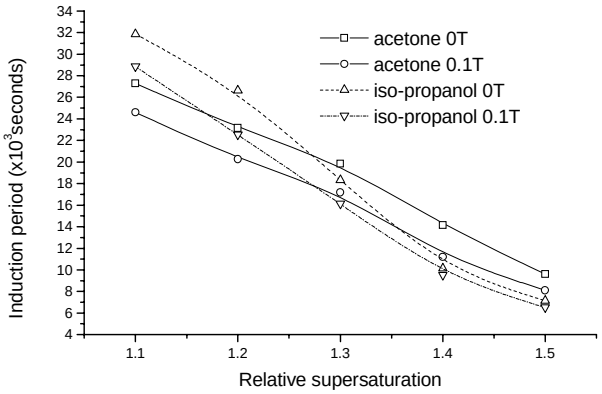

Figure 6 Cholesterol induction period in acetone and isopropanol

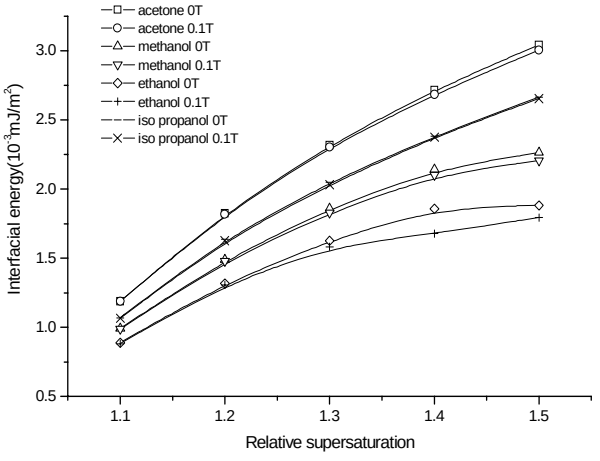

Figure 7 Cholesterol interfacial energy in organic solvents

3. Results and discussion

Solubility of anhydrous cholesterol in four organic solvents such as acetone, methanol, ethanol and isopropanol were determined at temperatures varying from 27 to 42°C in steps of 5°C. The solubility has a positive temperature coefficient for all the solvents. The solubility increases in the order of methanol < acetone < ethanol < isopropanol. The solubility increased with the decrease in polarity of the solvent. The solubility of cholesterol in acetone and ethanol are almost identical at 37°C. The metastable zone width of cholesterol in the above four organic solvents were determined experimentally. The solubility and supersolubility curve are drawn for each solvent (Fig. 2–4). In the presence of magnetic field, the metastable zone width decreases irrespective of the solvent used. The induction time of cholesterol crystallization decreases in the magnetic field for all the four solvents (Fig. 5 and 6).

From the induction time, the interfacial energy was calculated with respect to relative supersaturation (Fig. 7). Cholesterol induction period at the lower supersaturation are in the order of isopropanol > acetone > methanol > ethanol. There was a decrease in the induction period in the presence of magnetic field irrespective of the solvents used. The interfacial energy was found to decrease in the presence of magnetic field. Based on our experimental observation, it is not directly possible to infer on the growth rate from the critical nuclei to visible nucleus. However, magnetic field perceptibly attenuates the growth rate, it is likely that the nucleation rate has been significantly enhanced. We postulate, at a particular critical supersaturation in the presence of magnetic field, the interaction between the growth surface and the molecules in the mother solution are greater in the magnetic field. Subsequent reduction of the interfacial energy would promote the nucleation of cholesterol. The morphology of the cholesterol crystals obtained from the induction period experiments reveals the characteristic needle crystals from acetone, thin plates in methanol, thick platelets from ethanol and plates from isopropanol. The habit changes are mainly due to the interactions between the cholesterol solute and the solvent. The detailed studies involving habit, structure, and magnetic field are in progress and will be reported elsewhere.

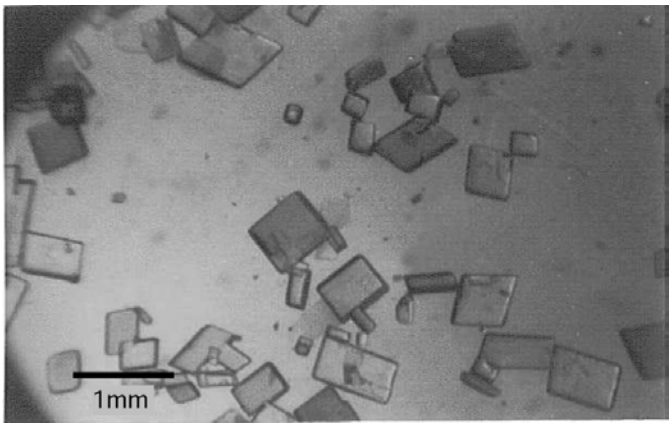

Figure 8 Cholesterol platelets at 1.2 relative supersaturation in ethanol.

4. Conclusions

The ascending order of solubility in methanol, acetone, ethanol and isopropanol are due to the decrease in polarity of the solvents. The induction time for crystallization of cholesterol is found to decrease even in the presence of a very low magnetic field of 0.1 T. From the induction time measurements the interfacial energy of the solutions were calculated. The morphological changes are due to the interfacial energy relation between the each solvent and the cholesterol. There was no change in morphology due to the presence of this magnetic field. This study on cholesterol crystallization under magnetic field is significant considering the fact that the artificial heart pumps have magnetic devices in them.

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