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Crystallization of cystine

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Cystine is an amino acid, which is a constituent of the urinary stones. It also is deposited in eyes, thyroid glands, bone marrow and white blood corpuscles. Crystals of cystine have been grown by both solution and gel methods. Small bunched hexagonal crystals, along with many other morphological crystals of size 0.3 mm across, were obtained. The crystals were analysed by X-ray diffraction and IR analyses.

1. Introduction

Crystal deposition diseases such as urinary stones, kidney stones, gallstones, gout, etc. are found to be the leading causes of hospitalization and surgery [1]. It is estimated that from 4 to 15% of the adult male population in various countries and about half that many females will develop kidney stones [2]. This makes it a social and economic problem of considerable magnitude. Among the constituents of kidney and urinary stones, cystine (a dibasic sulfur containing amino acid) $[-S-CH_2-CH-(NH_2)-COOH]_2$ forms a non-salt type organic compound [3]. It accounts for about 2.8% of the total composition of urinary stones [4]. The size of cystine stones varies from small to coral size (sizes vary from pinhead size to about 1 cm in diameter [5, 6]). Although cystinosis is a rare inherited disease, the pathologies behind it vary from mild to severe nephropathic cystinosis. Various renal functions are impaired and affected patients die at an early age with all manifestations of acute renal failure. Fanconi syndrome (a hereditary disorder of renal tubular function in which there is a defective reabsorption of glucose, amino acids, uric acid, phosphate, calcium and potassium) is believed to exist only in association with cystinosis. Apart from this, cystinosis may occur in many tissues and organs, particularly in the reticuloendothelial system (lymph nodes, liver, spleen and bone marrow) throughout the body [7]. Struvite ($MgNH_4PO_4 \cdot 6H_2O$) forms about 15.4% of the composition of renal stones. These stones have a high degree of recurrence (about 39% [8]). Cystine can act as a seed and favours struvite crystallization by epitaxial overgrowth [9]. Cystine is found to crystallize in a hexagonal system with space group $p6_122$ and unit cell dimensions a = 0.5422 nm and c = 5.6275 nm [10].

In vivo, it is difficult to see cystine crystals in fixed specimens because of their high solubility during fixation [11]. The *in vivo* process leading to the formation and growth of crystals in biological fluids, which is influenced by various factors, is a very complex one and is not yet fully understood. As *in vivo* experiments are possible only to a limited extent, *in vitro* experiments have to be attempted to provide a better understanding of cystine-crystal-related diseases. The growth of crystals *in vivo* will be dependent on the same factors of solubility, nucleation and growth rates as *in vitro* [4]. The study of nucleation and growth of cystine crystals *in vitro* will be an aid for medical and pharmaceuticals research. The investigations of either solution or solid phase phenomena will yield useful information regarding the etiologies of calculi. The activities of various inhibitors covering a wide range of molecular weight, which are present in urine, can be studied by knowing the growth and dissolution of stones and stone mineral phases [12]. A crystal deposition process is essentially crystallization from solutions. The gel method of crystal growth is a suitable method to study crystal deposition diseases because their viscous nature provides simulation of synovial fluid, cartilage and other biological fluids which are viscous in nature [13]. Crystallization of cystine is very difficult owing to its very low solubility in water and also its insolubility in alcohols. So far there have been no reports in the literature on the crystallization and growth kinetics of cystine. Here we report the crystallization of cystine by solution and gel methods. The experiments were conducted at 27 °C.

2. Experiments and observations

L-cystine was procured from SIGMA (USA) and all other reagents used for these experiments were of analar (AR) grade. A solution was prepared by dissolving a small amount of L-cystine in a warm dilute (1 N) ammonia solution. Warm dilute 5% (v/v) acetic acid was gradually added just to bring the solution to the point of precipitation. The warm solution was then allowed to cool down slowly. The following chemical reactions were expected, leading to the crystallization of cystine.

When dissolved in ammonia, cystine forms its amide, which when treated with acetic acid forms cystine. Cystine crystals of spherical bunched hexagonal and rectangular morphologies were found to grow in a day. The hexagonal crystals were about 0.3 mm

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$$[-S-CH_2-\underset{\displaystyle NH_2}{\underset{|}{CH}}-COOH]_2 + 2NH_3 \rightarrow [-S-CH_2-\underset{\displaystyle NH_2}{\underset{|}{CH}}-CONH_2]_2 + 2H_2O$$

$$[-S-CH_2-\underset{\displaystyle NH_2}{\underset{|}{CH}}-CONH_2]_2 + 2CH_3COOH \rightarrow [-S-CH_2-\underset{\displaystyle NH_2}{\underset{|}{CH}}-COOH]_2 + 2CH_3CONH_2$$

across and 0.1 mm in thickness.

In another method cystine was crystallized by the solubility reduction technique using gels. A small amount of cystine was dissolved in sodium meta silicate solution of specific gravity 1.04 g/cm^3 using a magnetic stirrer, the pH was adjusted to 6 by treating it with glacial acetic acid and the solution was allowed to set. The solution gelled in approximately 48 h. Crystals started growing during the gelling process. Crystals of cystine having different morphologies, namely single, twinned and bunched hexagonals, cubic, rectangular and needles were obtained (Figs 1–5).

Experiments were repeated by varying the pH from 5 to 10 and the gel density from 1.03 to 1.06 g/cm^3. Crystals were obtained for gel density from 1.03 to 1.05 g/cm^3 and for pH from 5 to 7. A gel density of 1.03 g/cm^3 and a pH of 6 was found to be the best condition for growth of crystals. When the gel density is greater than 1.05 g/cm^3 and for pH greater than 7, there was no crystallization.

In another modification of the above method, crystallization was achieved by pouring a supernatant solution of cystine dissolved in dilute ammonia solution over the gel. Here the gel was prepared by treating a solution of 1.04 g/cm^3 sodium meta silicate with glacial acetic acid. Crystals of bunched hexagonal morphologies were obtained in a day at the gel–solution interface.

In another modification of the single diffusion technique, a solution of sodium meta silicate of specific gravity 1.04 g/cm^3 was adjusted to pH = 6 by treating it with glacial acetic acid. Then the solution was mixed with acetone in 2:1 ratio and was allowed to set. It took approximately 70 h for the solution to

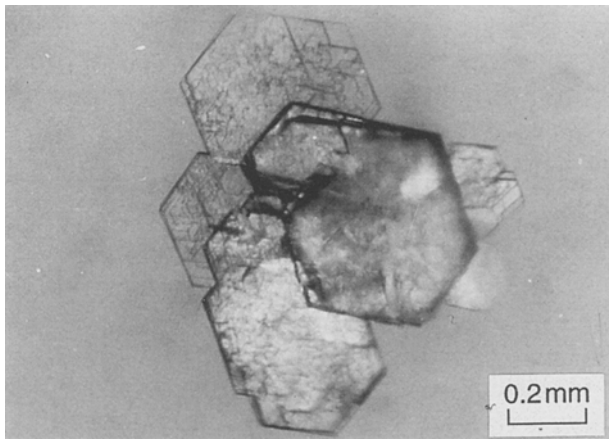

Figure 2 Bunched hexagonal crystal of cystine.

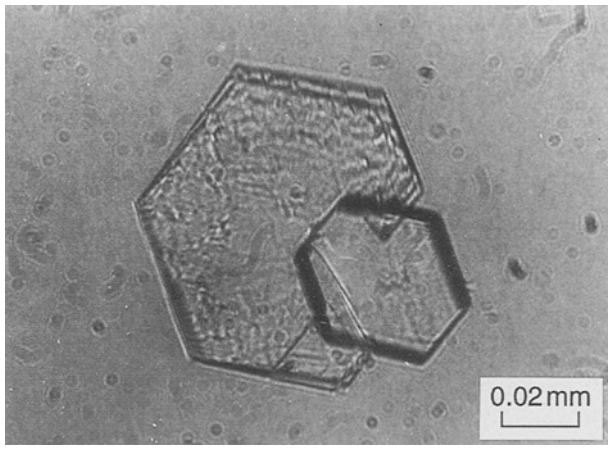

Figure 3 Twinned hexagonal crystal of cystine.

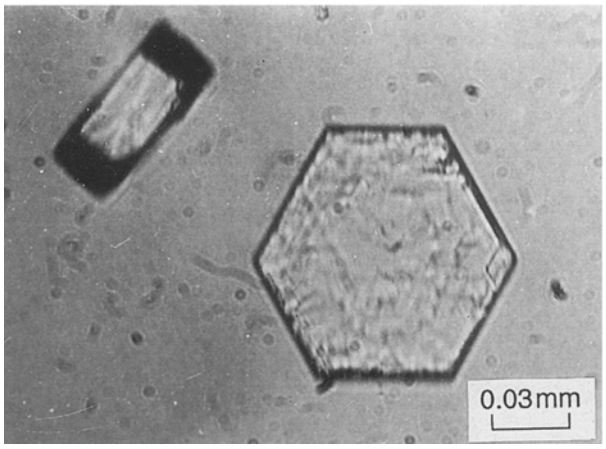

Figure 1 Single crystals of cystine.

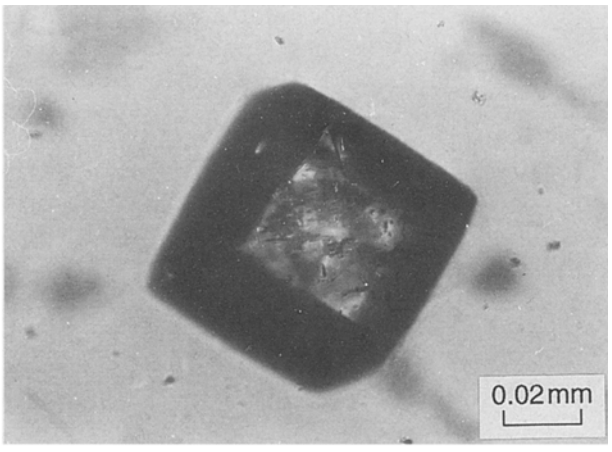

Figure 4 Cubic crystal of cystine.

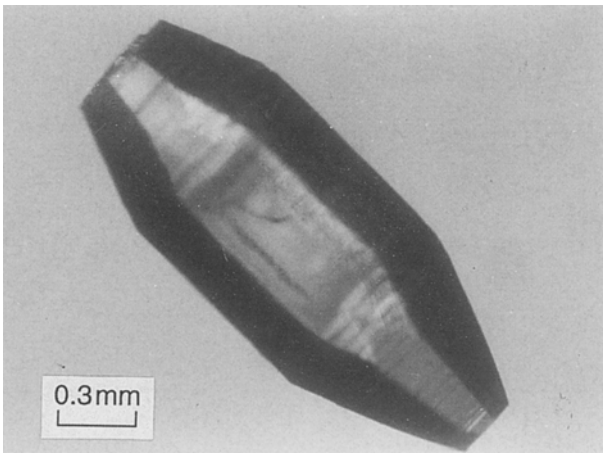

Figure 5 Bipyramidal crystal of cystine.

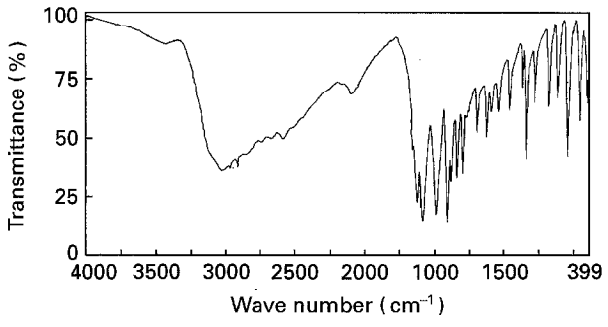

Figure 6 IR spectrum of cystine crystals.

gel. A solution of cystine in 1 N ammonia was poured over the gel medium. Crystals started growing at the gel–solution interface and also inside the gel medium. Crystals of spherical bunched hexagonals of size varying from 0.5 to 3 mm were obtained at the gel–solution interface. Crystals of dendritic morphology were obtained inside the gel medium. The same experiments were repeated with methanol and ethanol, and also for the ratios 1:1 and 1:2. These variations did not have any visible effect on the type of crystals grown. The organic solvents added to the gel medium reduce the amount of water present in the gel so that during the diffusion of cystine into the gel its solubility is gradually reduced because of the change in water concentration. This facilitates nucleation and subsequent growth of crystals in the gel medium [14].

Crystals were analysed by infrared transmission spectroscopy and X-ray powder diffraction. The IR spectrum was recorded in a KBr pellet and is reproduced in Fig. 6. The absorption band at 3026 cm^{-1} has been assigned to CH stretching vibrations. The two bands at 1622 and 1584 cm^{-1} are assigned to NH_2 deformation. The band observed at 1408 cm^{-1} is related to the mixed vibrational modes of C–H bending and COO^- stretching modes. The bands at 1382 cm^{-1} and 1337 cm^{-1} have been assigned to C–H bending and C–C stretching, respectively. C–S stretching vibration was seen at 675 and 615 cm^{-1}. The sharp band at 540 cm^{-1} is attributed to the S–S stretching mode. The recorded infrared spectrum of the grown samples were in good agreement with the reported literature values [15]. The observed vibrational frequencies and their assignments are listed in Table I. X-Ray powder diffraction analysis of the grown sample also agree well with the reported literature values [12]. Further characterization and kinetic studies of the grown crystals are in progress.

TABLE I Spectral data and assignments (cm^{-1})

Wave number	Assignments
3026	CH stretch
1622 1584	NH_2 deformation
1408	CH bending and COO^- stretching
1382	CH bending
1337	C–C stretching
847 846 777	NH out-of-plane and in-plane bending
675 615	C–S stretching
540	S–S stretching

3. Conclusions

Cystine, which has a low aqueous and organic solubility, can be crystallized in solution and in aqueous gel media. The knowledge of the kinetics of crystallization and dissolution of crystals is essential for the development of suitable methods for stone removal and also to find the factors that can prevent their further growth in our body system. These studies could be applied to determine the mechanism of crystallization of cystine stones, which are responsible for the diseases related to cystine stone formation.

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