JOURNAL OF MATERIALS SCIENCE: MATERIALS IN MEDICINE 6 (1995) 577–580

In-vitro crystallization of spherulites of monosodium urate monohydrate

S. NARAYANA KALKURA, E. K. GIRIJA, M. KANAKAVEL*, P. RAMASAMY
Crystal Growth Centre, Anna University, Madras, India 600 025
**Analytical Spectroscopy Division, Vikram Sarabhai Space Centre, Trivandrum, India 695 022*

Spherulitic and an aggregate of bow shaped crystals of monosodium urate monohydrate which had a close resemblance to that of those crystals found in gouty synovial fluids and articular cartilage have been grown using tetramethoxysilane and silica gel. Thermogravimetric analysis (TGA), and infrared (IR) and X-ray diffraction were used to analyse the grown samples. Crystals were obtained for a wide range of pH (3–10), without using any biological component at room temperature and were found to be monohydrate form of monosodium urate.

1. Introduction

Several rheumatic diseases are associated with the deposition of crystals in joints, periarticular tissues or elsewhere. Urates are associated with gout, which principally affects the peripheral joints often starting in the foot or hand [1]. The urate is a breakdown product of purine derivatives of nucleic acids, guanine and adenine. Crystals which have been extracted from gouty tophi and synovial fluid are found to be the triclinic form of monosodium urate monohydrate (MSUM). MSUM is the salt of singly ionized state of uric acid. It has also been identified in articular tissues of patients managed with long-term dialysis [2]. The main sites of urate crystallization are articular cartilage, periarticular soft tissues, bursae, epiphyseal bone and kidneys. Tophi can also occur on the ear, on the olecranon and patella bursae and tendon sheaths and less commonly in a number of other sites. The presence of characteristic needle or spherulitic forms of MSUM in synovial fluid and within the synovial leukocytes is recognized as a strong indication of the presence of gouty arthritis. These crystals show negative birefringence using compensated polarizing light microscopy [3]. Recently, Hayes *et al.* made a unique observation of an aggregate of bow-shaped MSUM crystals within the mid-zone of the articular cartilage, this is in contrast to the presence of needle-shaped crystals usually found in synovial fluids [4].

The synthetic crystallization of MSUM usually yielded needle-shaped crystals whereas the crystals grown in the presence of serum, synovial fluid, and components thereof closely resembled the morphology of the MSUM crystals present in the articular cartilage *in vivo* [5]. Synthetic spherulites of MSUM crystals were crystallized from a sodium hydroxide–uric acid solution heated to 60 °C and then cooled to 4 °C [3].

Crystals which have been extracted from gouty tophi and synovial fluid had been analysed by X-ray diffraction. The crystals of MSUM are $[NaC_5H_3N_4O_3 \cdot H_2O]$ and are triclinic with space group P1, $a = 1.0888(5)$ nm, $b = 0.9534(3)$ nm, $c = 0.3567(1)$ nm, $\alpha = 95.06°$, $\beta = 99.47°$, $\Gamma = 97.17°$ and $Z = 2$ [6]. The gel method of crystal growth provides an ideal medium to study crystal deposition diseases owing to their viscous nature providing simulation of cartilage and synovial fluids, which are viscous in nature. Also it provides an inert medium during the process of crystallization [7–9]. Here we report the growth and characterization of synthetic spherulitic and bow-shaped crystals of MSUM without the presence of any biological components, at 27 °C.

2. Experimental methods

For preparing the gel medium, sodium metasilicate $[Na_2SiO_3 \cdot 9H_2O$, AR, JT Baker] and Tetramethoxysilane $[Si(OCH_3)_4$, Riedal-DeHan AG] were used. Uric acid solution was prepared by dissolving 125 mg of uric acid (AR, Loba Chemie) in 50 cm^3 of 0.2 N sodium hydroxide. All reagents used for these experiments were of analar grade. A 1% (v/v) tetramethoxysilane (TMS) solution was prepared by vigorously mixing 7 cm^3 of the above NaOH–uric acid solution (pH adjusted with glacial acetic acid) with 0.07 cm^3 of TMS. After the solution had set, a solution of uric acid in NaOH was allowed to diffuse through the gel medium. The pH of the NaOH–uric acid solution was varied from 3 to 10. The experiments were repeated with different concentrations (0.5–2.0%) of TMS (v/v).

In another single diffusion method, a solution of sodium metasilicate (SMS) of specific gravity 1.03 g/cm^3 was adjusted to a pH of 5 by treating it with glacial acetic acid. This solution was mixed in different ratios with acetone (1 : 1, 1 : 0.05, 1 : 0.025) and

the resulting mixture was allowed to form a gel. A supernatant solution of uric acid in NaOH was poured carefully over the set gel. The experiments were repeated for concentrations ranging from 1.03 to 1.06 g/cm^3 and pH range 6 to 3.

In another modification, SMS gel was prepared without the addition of acetone, and a dilute solution of NaOH–uric acid was allowed to diffuse into the gel for 4 weeks. Then the supernatant solution was replaced by dilute HCl.

The grown crystals were analysed using thermogravimetric analysis (TGA), infrared transmission spectroscopy (IR) and X-ray diffraction (XRD). The TGA was carried out on a Dupont Modular Thermal Analysis System (heating rate: 10 °C/min). IR spectra of the samples grown were recorded using a Perkin-Elmer IR spectrophotometer, model 283. X-ray diffractograms of the grown crystals were recorded in a Philips PW/1729 system using CuK_α radiation.

3. Results and discussion

In the case of the experiments with TMS, spherulites of MSUM were found to grow inside the gel medium (Fig. 1). The spherulitic crystals were spherically symmetrical, radiating crystal aggregates with needle-like projections at the periphery. They showed a close resemblance to the "beach ball" structures identified in gouty synovial fluid by Fiechtner and Simkin [3]. Along with the growth of these spherulites, bow-shaped crystals of MSUM were also found (Fig. 2). The spherulites were in the order of 1 mm in diameter. The experiments with SMS also yielded spherulites of MSUM similar to that obtained in the above experiments. We were able to crystallize MSUM in a wide range of pH (10 to 3) with TMS gels. Needle-shaped crystals of MSUM were obtained for values of pH below 6 (Fig. 3).

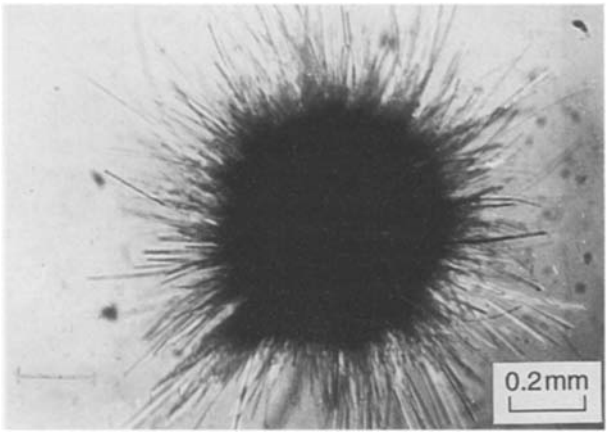

Figure 1 Spherulites of monosodium urate monohydrate (MSUM).

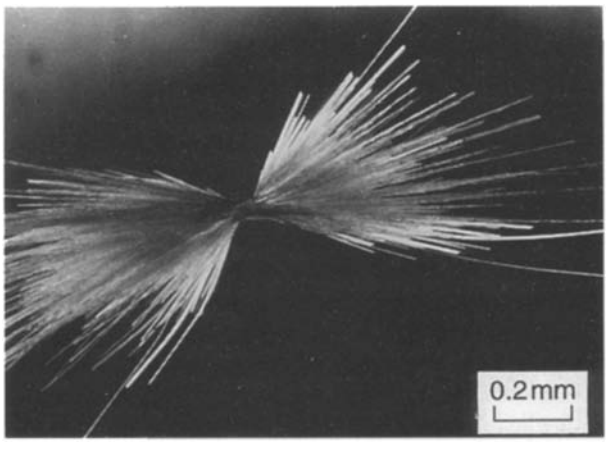

Figure 2 Aggregate of bow shaped crystals of MSUM.

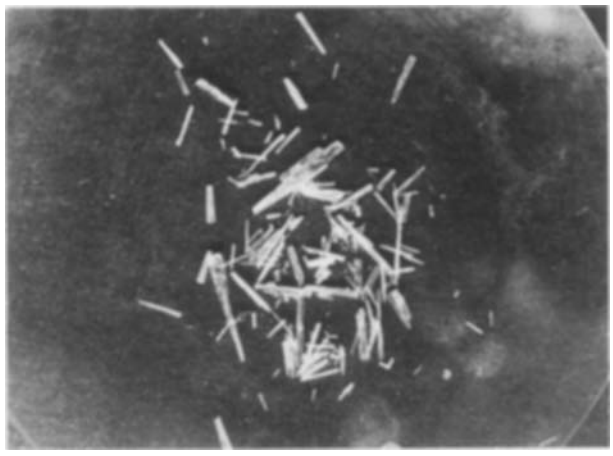

Figure 3 Needle-shaped crystals of MSUM (× 2).

Concentrations above 1% of TMS and 1.05 g/cm^3 of SMS, with all other conditions remaining the same, did not yield any crystals. This is probably due to the decrease in the pore size of the gel medium as the concentration is increased. At low concentrations (0.1% TMS or 1.02 g/cm^3 of SMS) and low pH values (< 3), they tended to be unstable and there was a considerable delay in proper setting of the gel.

The sodium hydroxide–uric acid solution which was used as supernatant solution in the experiments is understood to have not reacted in any way with the gel medium. During the diffusion of the supernatant solution into the gel medium, solubility of MSUM is reduced and this leads to supersaturation inside the gel, thereby causing nucleation and subsequent growth of crystals. In the case of SMS the addition of acetone seemed to aid the supersaturation of MSUM during diffusion through the gel medium.

In the third modification, where dilute HCl was allowed to diffuse into the acetone-free gel; platy, transparent single crystals of uric acid were found to grow throughout the gel medium (Fig. 4). The dilute HCl which diffused through the gel had a displacement reaction with sodium hydroxide–uric acid solution present in the gel medium producing uric acid dihydrate crystals [7].

The TGA of the grown samples showed a weight loss corresponding to the loss of one mole of water of hydration. The IR spectrum of the crystals was recorded in a KBr pellet (Fig. 5). The O–H stretching vibrations are expected to occur at around 3600 cm^{-1} in the IR spectra. The band observed in the IR spectra at 3602 cm^{-1} is attributed to O–H stretching vibrations. The bands in the region 2700–3100 cm^{-1} are due to the C–H stretching mode [10]. The strong

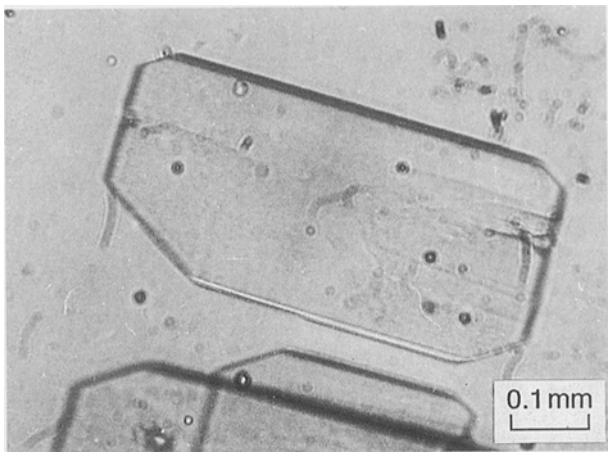

Figure 4 Platy uric acid dihydrate crystals.

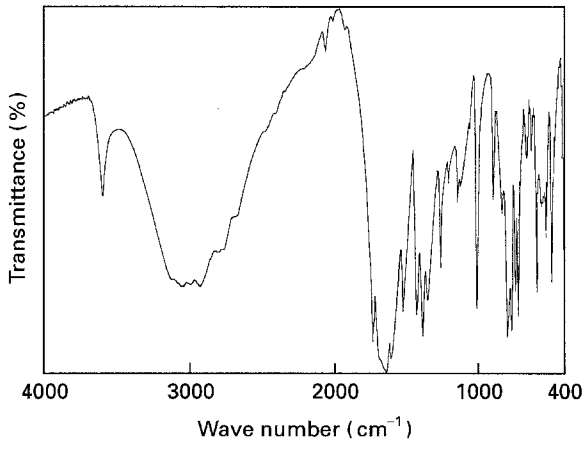

Figure 5 IR spectrum of MSUM.

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

Wave number	Assignments
3602	Water and O–H stretch
3052	C–H stretch
1738	C=O stretch
1653	
1529	C–C and C–N stretch
1427	
1385	
1352	O–H deform
1259	Ring vibration
1136	
1003	
885	N–H out-of-plane and in-plane bending
799	
768	
742	
721	
598	Skeletal ring deformations
530	
491	

bands observed at 1738 and 1653 cm^{-1} have been assigned to carbonyl stretching vibrations. The bands at 491, 536, 598, 721, 742, 767, 798, 885 and 1003 cm^{-1} are due to ring vibrations. The observed vibrational frequencies and their assignments are listed in Table I

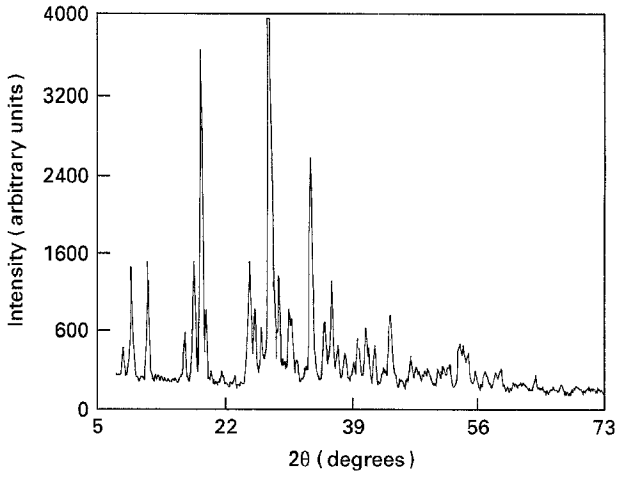

Figure 6 X-ray diffractogram of MSUM.

TABLE II X-ray diffraction data of MSUM (CuK_α radiation, θ is the glancing angle)

Sin^2 θ (observed)	Sin^2 θ (calculated)	(h k l)
0.0070	0.0090	(1 0 0)
0.0101	0.0100	(0 1 0)
0.0207	0.0180	(1 1 0)
0.0247	0.0250	(2 0 0)
0.0267	0.0310	(0 2 0)
0.0470	0.0515	(3 0 0)
0.0485	0.0528	(0 0 1)
0.0544	0.0580	(1 0 1)
0.0590	0.0589	(2 2 0)
0.0637	0.0647	(0 3 0)
0.0698	0.0676	(1 1 1)
0.0713	0.0751	(1 3 0)
0.0833	0.0812	(0 2 1)
0.0857	0.0854	(2 1 1)
0.0916	0.0902	(1 2 1)
0.0946	0.0959	(2 3 0)
0.1012	0.1011	(3 0 1)
0.1126	0.1117	(0 4 0)
0.1292	0.1287	(4 2 0)
0.1349	0.1312	(5 0 0)
0.1645	0.1633	(0 4 1)

and they are in good agreement with the reported literature values [11]. The X-ray powder diffraction patterns of the grown samples are reproduced in Fig. 6. Both the calculated and observed values of sin^2 θ were seen to be in good agreement with those reported in the literature for MSUM [6]. The indices of various reflections thus obtained are listed in Table II. The results of the above analyses clearly identify the grown crystals to be monosodium urate monohydrate.

4. Conclusions

Spherulitic and bow-shaped crystals of MSUM, which have been identified in articular cartilage and synovial fluids, were successfully grown *in vitro*, in gels without using any biological components. The existing standard method to grow spherulites of MSUM is to slow cool a solution of NaOH–uric acid solution kept at

pH 8.9 from 60 °C to 4 °C [3]. Here we have succeeded in growing spherulites of MSUM in a wide range of pH (3–10) at room temperature, without resorting to any heating of the solutions. The characterization by XRD and IR has shown the crystals to be structurally identical to those found in the gouty synovial fluid and within the mid-zone of articular cartilage. TGA has further confirmed the monohydrate nature of the crystals grown. These experiments could be used to identify growth inhibitors that can suppress the formation of crystals in joints, and also to find a suitable solvent to dissolve them *in vivo*, so that they could be treated without the surgical removal of crystalline deposits.

References

1. P. A. DIEPPE and P. CALVERT, "Crystals and joint disease", (Chapman & Hall, London, 1983.)
2. J. E. Z. CANER and J. L. DECKER, *Amer. J. Med.* **36** (1964) 571.
3. J. F. FIECHTNER and P. A. SIMKIN, *JAMA* 245 (1981) 1533.
4. A. HAYES, I. G. TURNER, K. A. POWELL and P. A. DIEPPE, *J. Mater. Sci. Mater. Med.* **3** (1992) 75.
5. N. W. MCGILL and P. A. DIEPPE, *Brit. J. Rheum.* **30** (1991) 107
6. N. S. MANDEL and G. S. MANDEL, *J. Amer. Chem. Soc.* **98** (1976) 2319.
7. S. N. KALKURA, V. K. VAIDYAN, M. KANAKAVEL and P. RAMASAMY, *J. Cryst. Growth* **132** (1993) 617.
8. S. N. KALKURA and S. DEVANARAYANAN, *ibid.* **110** (1991) 265.
9. S. DEVANARAYANAN and S. N. KALKURA, *J. Mater. Sci. Lett.* **10** (1991) 497.
10. L. J. BELLAMY, "Infrared spectra of complex molecules" (Chapman & Hall, London, 1975).
11. V. R. KODATI, A. T. TU and J. L. TURUMIN, *Appl. Spectrosc.* **44** (1990) 1134.

Received 14 June 1994
and accepted 27 March 1995