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Crystal growth of some urinary stone constituents: II. In-vitro crystallization of hippuric acid.

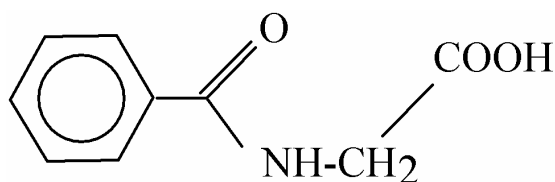
Hippuric acid [C₆H₅CONHCH₂COOH], one of the organic chemical constituents of urinary stone is crystallized in silica gel under suitable pH conditions by double diffusion method. The grown crystals were characterized by density measurement, Fourier transform infrared spectroscopy, X-ray powder diffraction and thermogravimetric analysis.

Keywords: hippuric acid, urinary crystal, gel growth, XRD, FTIR, TGA/DTA

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Introduction

Hippuric acid, also called as N-Benzoylglycine, is a colourless crystal obtained from the urine of domestic animals and humans. The most frequent renal calculi found in humans are products of the different modifications of calcium oxalate, and hippuric acid is one of the least frequent.



Chemical Structure of hippuric acid.

Hippuric acid, when present in urine in high concentration, may increase the solubility of calcium oxalate. The differences in the excretion of hippuric acid between normal adults and oxalate stone formers is very important because hippuric acid appears to be a natural regulator of urinary saturation with regard to calcium oxalate crystallization.

Moreover, toluene, also known as methylbenzene, an aromatic hydrocarbon solvent which is widely used in industries, is metabolized into hippuric acid and can be recovered in the urine of humans exposed to toluene. Low-level, chronic exposure as well as acute exposure to toluene may result in central nervous system depression and decreased memory (AXELSON, 1977). Symptoms include headache, dizziness, fatigue, muscular weakness, drowsiness, and incoordination with staggering gait, skin paresthesia, collapse, and coma (USEPA, 1980).

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Urinary stones grow in a gel-like medium. The crystal growth by gel method provides simulation of synovial, cartilage and other biological fluids (ARCHILLES et al. 1995). Gel growth (*in vitro*) of some urinary stone constituents, viz., calcium oxalate monohydrate and dihydrate (COM and COD), calcium hydrogen phosphate (CHP) and ammonium magnesium phosphate (AMP) and the inhibitory role played by extracts or juices of natural products in crystal growth were studied earlier (NATARAJAN et al. 1997).

Crystallization of the hippuric acid in gel medium is not reported in literature. Crystal growth of hippuric acid by gel technique and its characterization are reported in this present work. Studies of urinary hippuric acid concentration and its link in the growth of urinary stones among toluene exposed persons may be helpful for monitoring urinary stone disease.

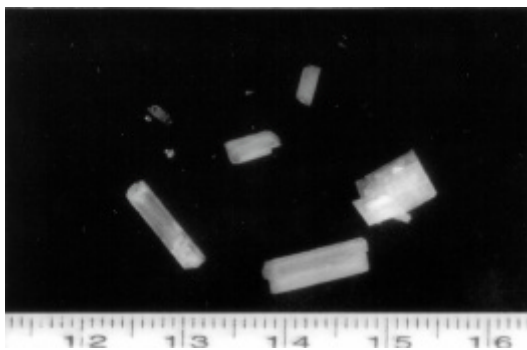


Fig. 1: Rectangular plates of hippuric acid crystals.

2. Crystal growth by gel method

Silica gel prepared from an aqueous solution of sodium metasilicate ($\text{Na}_2\text{SiO}_3 \cdot 5\text{H}_2\text{O}$), (S. D. Fine chem. Ltd.) was used as crystal growth medium. U-tube (of size $18.0 \times 1.75 \text{ cm}^2$) was used as crystal growth vessel. Crystal growth by double diffusion method was employed for the crystallization. The pH of the gel solution of specific gravity 1.06 gm/cm^3 was adjusted by acetic acid (1:1). The concentration of the reactants, viz., hippuric acid dissolved in sodium hydroxide and diluted hydrochloric acid, placed over the set gel in the two limbs of the U-tube, was varied. Different pH and concentration of the gel solution were tried, to get best conditions for crystal growth. The best growth condition was determined based on the number of crystals obtained, their transparency, sizes and morphology.

For the best crystal growth, the concentration of the reactants were: 0.5 M hippuric acid dissolved in 0.2 M sodium hydroxide placed over the set gel in one limb and diluted hydrochloric acid (1:5) in the other and the gel pH was 6.0 Rectangular plates of hippuric acid of size $10.0 \times 4.0 \times 1.0 \text{ mm}^3$ were formed at the gel-solution interface in the limb having diluted hydrochloric acid. The crystals were carefully removed after three weeks and were photographed (Fig. 1).

3. Characterization

3. 1 X-ray powder diffractogram

The crystals of hippuric acid were carefully removed, dried and powdered. X-ray powder diffractogram (XRD) was recorded using a Siemens X-ray powder diffractometer (Model

D5000). CuK α radiation (of wave length 1.5418 Å) was used and the XRD is shown in Fig. 2. The XRD of the grown hippuric acid was matched with the organic database using computer and the result matched well with that of hippuric acid.

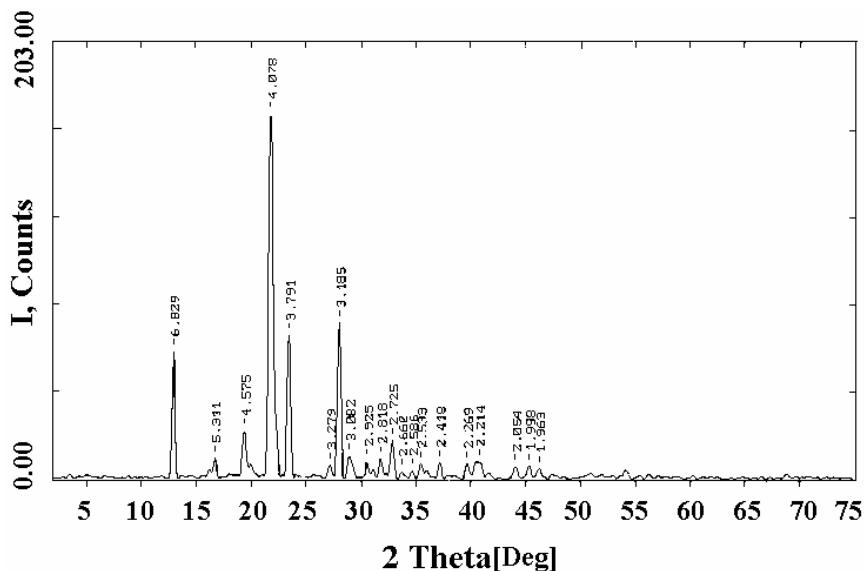


Fig. 2: X-ray powder diffractogram of grown hippuric acid.

3.2 Measurement of the density of the crystal

The density of the crystal was determined using the floatation method. Xylene (density: 0.89g/cm³) and carbon tetrachloride (density: 1.59 g/cm³) were used for the experiment. The density of hippuric acid was determined as: 1.38 ± 0.02 g/cm³. The expected value of density is 1.385 g/cm³ (WEAST, 1973). There is good agreement between the measured and standard value, confirming the identity of the substance.

3.3 Fourier transform infrared (FTIR) spectra

IR spectra is used in organic chemistry for qualitative and quantitative analysis. The FTIR spectra of the grown hippuric acid was recorded in the KBr phase in the frequency region 400 – 4000 cm⁻¹ using Jasco Spectrometer FTIR, model 410. The recorded FTIR spectra (Fig. 3) was compared with the standard spectra of the functional groups (SOCRATES, 1980).

The characteristic peaks at 3344 cm⁻¹, 1487 cm⁻¹ and 436 cm⁻¹ due to asymmetric stretching, deformation and bending respectively, of N-H group confirmed the existence of aromatic amine group. Broad band due to C-H stretching (3085 cm⁻¹) and band due to ring stretching (1560 cm⁻¹) established the existence of benzene ring structure. In-plane deformation vibrations (1257-999 cm⁻¹) and out of plane deformation vibrations (849-660 cm⁻¹) of the C-H group occurred, confirming the ring structure is monosubstituted benzene. Intense absorption peak (1751 cm⁻¹) due to asymmetric stretching vibrations of C=O group indicated the presence of carboxylic acid. Aromatic ketones have strong absorption between

600 cm^{-1} and 580 cm^{-1} (GIANTURCO, PITCHER, 1963) due to the in-plane deformation vibration of the C-CO group and a band occurred at 544 cm^{-1} confirming this. $\text{CH}_2\text{-CO}$ deformation (1410 cm^{-1}) supported the presence of methylene group. Thus, the presence of all the functional groups occurring in hippuric acid, were confirmed. All the observed vibrational frequencies and their tentative assignments are listed in Table 1.

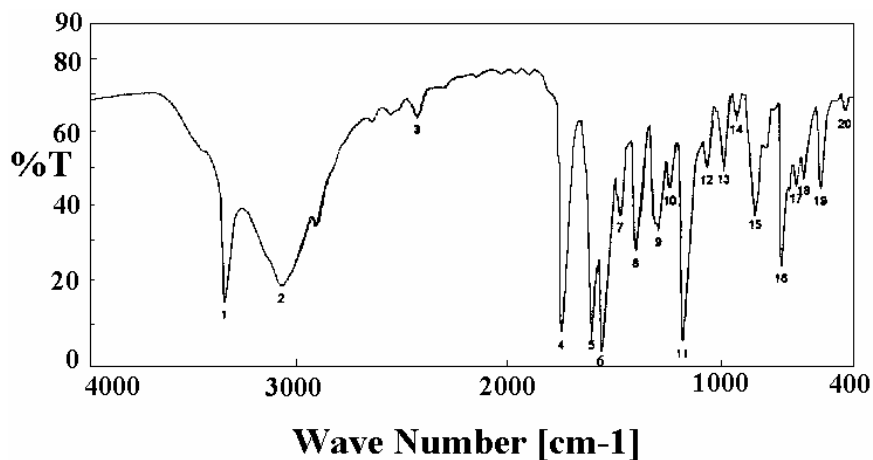


Fig. 3: FTIR spectra of hippuric acid.

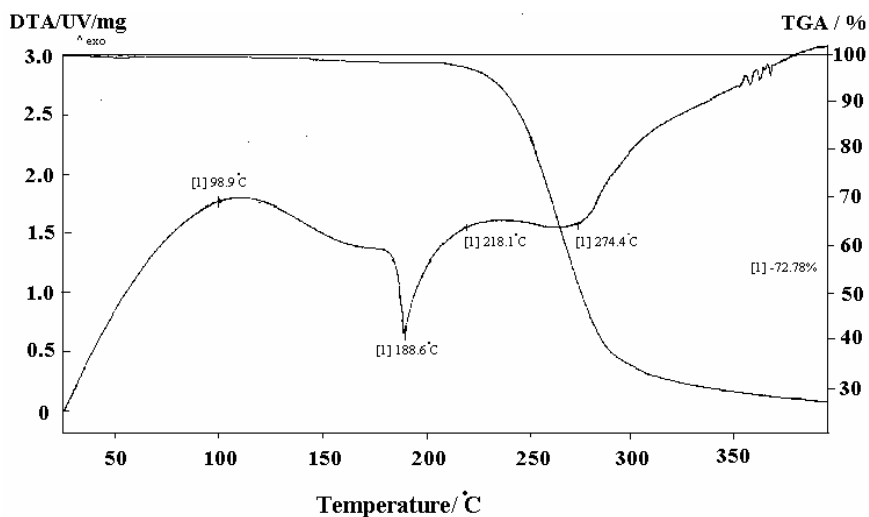


Fig. 4: TGA/DTA of hippuric acid.

3.4 Thermogravimetric analysis (TGA) and Differential Thermal Analysis (DTA)

TGA and DTA studies for hippuric acid were made using NETZSCH-Gerätebau STA 409PC Thermal Analyser and are depicted in Fig. 4. The thermal analysis was carried out in the

temperature range between 30°C and 400°C. An endothermic peak was obtained at 188.6°C, the melting point of hippuric acid. Slow decomposition took place between 218.1°C and 274.4°C with a weight loss of 68.8% due to the expulsion of a H₂O molecule and atoms of carbon, hydrogen and oxygen from the chain.

Table 1: FTIR Spectral Data

Wave number cm ⁻¹	Tentative assignments
3344	NH asymmetric stretching
3085	CH stretching
2800	CH (in CH ₂) stretching
2474	
1751	C=O stretching
1608	NH asymmetric stretching
1560	Ring stretching
1487	NH deformation
1410	CH ₂ -CO deformation
1308	C=O stretching
1257	CH in plane deformation
1178	CH in plane deformation
1080	CH in plane deformation
999	CH in plane deformation
943	
849	CH out of plane deformation
723	CH out of plane deformation
660	CH out of plane deformation
625	
544	C-CO deformation
436	NH bending

4. Conclusions

Rectangular plates of hippuric acid, a urinary stone constituent, were crystallized in gel by double diffusion method in sodium metasilicate for the first time. The XRD studies confirmed the structural identity of the grown hippuric acid crystals. FTIR of the hippuric acid crystals revealed the presence of functional groups. The endothermic peak obtained at the melting point of hippuric acid in the TGA/DTA characteristics substantiated the grown substance as hippuric acid. The density of the grown hippuric acid crystals matched the standard density value (WEAST, 1973).

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References

- ARCHILLES, W., FREITAG, R., KISS, B., RIEDMILLER, H.: *J. Urol.* **154** (1995) 1552
- AXELSON, O.: Current Aspects of Solvent Related Disorders. In *Developments in Occupational Medicine*, Yearbook Medical Publishers Inc., Chicago 1977.
- GIANTURCO, M. A., PITCHER, R. G.: *App. Spectrosc.* **19** (1963) 109.
- NATARAJAN, S., RAMACHANDRAN, E., BLISIN SUJA, D.: *Cryst. Res. Technol.* **32** (1997) 551.
- SOCRATES, G.: *Infrared Characteristic Group Frequencies*, Wiley-Interscience, Chichester, 1980.
- U. S. ENVIRONMENTAL PROTECTION AGENCY,: *Toluene, Health and Environmental Effects*, Office of Solid Waste, Washington, D.C., 1980.
- WEAST, R. C.: *CRC Handbook of Chemistry and Physics*, 54th edition, CRC Press, Ohio, 1973.