International Journal of Current Research in Chemistry and Pharmaceutical Sciences
www.ijcrcps.com Volume 1 Issue: 4 2014 Pages:01-07

(p-ISSN: 2348-5213; e-ISSN: 2348-5221)

RESEARCH ARTICLE

A STUDY ON SYNTHESIS AND CHARACTERIZATION OF BENZOTRIAZOLINIUM PICRATE CRYSTALS

T. DHANABAL[1*] AND G. AMIRTHAGANESAN[2]

[1]Department of Chemistry, Muthayammal College of Engineering, Rasipuram, Namakkal-637 408, Tamil Nadu, India.
[2]Department of Chemistry, Sri Ramakrishna Mission Vidyalaya College of Arts and Science, Coimbatore-641 020, Tamil Nadu, India

*Corresponding Author: dhanabal27@gmail.com

Abstract

Single crystals of benzotriazolinium picrate were synthesized and crystallized using slow evaporation solution growth method at room temperature. The synthesized crystals were characterized through powder X-ray diffraction method, mechanical, dielectric and antimicrobial studies. The sharp and well defined Bragg peaks observed in the powder X-ray diffraction pattern confirm the crystalline nature of the compound. The mechanical properties of the crystal was measured by Vicker's microhardness tester and found that the synthesized crystal belongs to soft material. The dielectric constant and dielectric loss of the crystal decrease with increase in frequency. The antibacterial and antifungal activities of the crystal were studied by disc diffusion method and found that the compound shows g.

Keywords: Characterization; Growth from solution; Powder X-ray diffraction; dielectric study; Inhibition efficiency.

Introduction

Charge transfer crystals play a central role in bioelectrical and biological systems such as bactericides, fungicides, insecticides and various light-driven physical and chemical processes (Roy *et al.,* 2005; Brown and S.F. Mason, 1962; Sondhi *et al.,* 2001; Zhao *et al.,* 1999). The charge transfer interaction of organic crystals were used in different pharmaceuticals (Amin and El-Beshbeshy, 2001; Andrade *et al.,* 2000), micro-emulsion (Eychmuller and Rogach, 2000) and also as organic semiconductors (Petrosyan *et al.,* 2005). The proton-transfer from organic acid to amines may take place readily with very low activation energy in contrast with the attack of the amine to the carbonyl carbon leading to amide formation (Prasad and Williams, 1991). Highly nitrated small ring heterocycles are good candidates of energetic materials because of the increased performance from the additional energy released upon opening of the strained ring system during decomposition (Petrosyan *et al.,* 2005). The presence of three electron withdrawing nitro groups makes it as a good π acceptor for neutral carrier donor molecule (Xue and Ratajczak, 2005; Farrell *et al.,* 1985; Franchini *et al.,* 1988). In addition, picric acid is used at human theraphy like treatment of burns, aniseptic and astringent agent (Elmosallamy, 2004; Maurya *et al.,* 2003).

In the present work, we reported synthesis, mechanical, dielectric and biological activities of (hereafter abbreviated as BTP). The compound was

characterized by powder X-ray diffraction method, mechanical, dielectric and antimicrobial studies.

Experimental details

Synthesis of BTP crystals

Single crystals of BTP were grown by slow evaporation solution growth method at room temperature. One mole benzotriazole and one mole of picric acid reacted to form benzotriazolinium picrate crystal. Methanolic solution containing analytical grades of one mole of each of the substances were prepared separately. The two solutions were mixed together and stirred well for about 6 h to get a homogeneous solution using mechanical stirrer and the resulting solution was filtered in a 100 ml clean dry beaker through a Whatman 40 filter paper. After filtration, the filtrate was kept in dust free environment for crystallization. The beaker was covered by an ordinary filter paper. Care was taken to minimize the temperature gradient and mechanical shack.

The compound, benzotriazolinium picrate is formed as per the following scheme.1.

Under the experimental conditions the bright, transparent and yellow coloured BTP crystals were obtained within 20 to 25 days. The grown crystals were collected from the mother liquid by using well cleaned forceps.

Characterization techniques

The powder XRD patterns of the complexes were obtained using Phillips PW 3710 diffractometer with Cu Kα radiation (λ = 1.54060 Å) at room temperature and the samples were scanned over the range of 0-80º at a scan rate of 1º/min. The mechanical properties of the compounds were measured using Vicker's microhardness test. The dielectric properties of the crystals were studied at room temperature using a TH 2816 A DIGITAL LCRZ METER in the frequency region from 50 Hz to 5 MHz.

Antimicrobial study

Preparation of chemical extract

100 µg of the chemical compound was weighed. Then it was mixed with 1 ml of the DMSO solvent. The filtrate was obtained by filtration with Whatman No.1 filter paper and the filtrate disc was kept in test tube containing chemical compound. The disc was allowed to dry in laminar airflow chamber. Control was maintained by adding solvent on the disc.

Antibacterial activity

The antibacterial activity of newly synthesized crystal was tested in vitro against four Gram-positive bacteria *Staphylococcus epidermidis*, *Staphylococcus aureus* (Lab isolate), *Enterococcus faecalis* and *Staphylococcus aureus* (Clinical isolate) and five Gram-negative bacteria *Proteus* sp., *Escherichia coli, Pseudomonas aeruginosa*, *Pseudomonas* sp. and *Klebsiella pneumoniae* (Cruickshank *et al.,* 1995; Collins, 1976). Media with DMSO solvent was set up as control. The discs measuring 5 mm in diameter were prepared from Whatman No.1 filter paper sterilized by dry heat at 140ºC for 1 h. The sterile discs previously soaked in a concentration of the test compounds were placed in a nutrient agar medium. The petri plates were invested and kept in an incubator for 24 h at 37ºC and growth was monitored both visually. The screening was performed at 100 µg/ml concentration of test crystals and antibiotic disc. Tetracycline (30mg/disc, Hi-Media) was used as control. Logarithmic serially two fold diluted amount of test crystals and controls was inoculated within the range 10^{-4}-10^{-5} cfu/ml. To obtain the diameter of zone, 0.1 ml volume was taken each and spread on agar plates. The number of colony forming units (cfu) was counted after 24 h of incubation at 35ºC. After incubation the zone of inhibition was measured and expressed as mm in diameter.

Antifungal activity

The newly synthesized crystal was also screened for its antifungal property against *Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus* and *Penicillium* sp. in DMSO solvent by using standard agar disc diffusion method. The synthesized compounds were dissolved in DMSO solvent and media with DMSO was set up as control. All cultures were routinely maintained on Sabouraud Dextrose Agar (SDA) and incubated at 28°C. Spore formation of filamentous fungi was formed from seven day old culture on sterile normal solution, which was diluted to approximately 105 cfu/ml. The culture was centrifuged at 1000 rpm, pellets was resuspended and diluted in sterile Normal saline solution (NSS) to obtain a viable count 105 cfu/ml. With the help of spreader, 0.1 ml volume of

approximately diluted fungal culture suspension was taken and spread on agar plates. The fungal activities of compounds were compared with Nystatin (30g/disc Hi-Media) as standard drug. The cultures were incubated for 48 h at 37ºC and the growth was monitored. Antifungal activity was determined by measuring the diameters of the zone (mm) in triplicate sets.

Results and discussion

Powder X-ray diffraction pattern method

The powder X-ray diffraction pattern of BTP crystal is shown in Figure 1. The sharp and well defined Bragg peaks observed in the powder X-ray diffraction pattern confirm its crystallinity. In the powder XRD pattern most of the peaks were indexed. From the observed 2θ values the lattice parameters and the unit cell volume have been calculated using CRYSFIRE software package. The unit cell parameters obtained are a = 9.0349Å, b = 11.6272Å, c = 12.5848Å, α = γ = 90°, β ≠ 90° and unit cell volume is 1420.45Å^3.The unit cell parameters indicates that BTP belongs to monoclinic system. The peaks corresponding to 410 plane has a maximum count of 3300. This is the strongest diffraction peak in the powder X-ray diffraction pattern.

Microhardness measurement

The microhardness of BTP was studied using Vickers microhardness tester fitted with a Vickers diamond pyramidal indentor. Hardness of the crystal was studied with the applied loads of 25, 50 and 100 g. For microhardness measurement, the crystal surface was carefully lapped and polished to avoid surface effects which influence the hardness value strongly. The selected surface of the crystal was polished using a velvet cloth to get a smooth surface. The indentation time was fixed as 10 s for each trial. Vickers microhardness value was calculated using the following equation

$H_v = 1.8544\ P/d^2$ kg/mm^2, where P is the applied load, d is the diagonal length of the indentor impression and H_v is the hardness number.

A plot of between hardness and applied load is shown in Figure 2. From the plot, it is observed that the hardness increases up to 50 g and above 50 g crack appears as evident from the upward trend in the line. The plot of log d against log P is shown in Figure 3. According to Mayer's law, $P = ad^n$ where 'n' is the work hardening co-efficient and 'a' is a constant for a given material. Using this law, the work hardening co-efficient, n is calculated from the slope of the straight line obtained by plotting log d and log P (Figure 3). According to Onitsch (1950) if n is less than 1.6 then the material is a hard material and if n greater than 1.6 it is a soft material. The value of 'n' for the crystal is found to be 1.5 and hence it is a soft material

Dielectric studies

Study of the frequency dependence of dielectric properties unveil useful information about structural changes, defect behavior and transport phenomena (Lin *et al.,* 2001). The sample was polished on soft tissue paper. The opposite parallel faces of the crystals were coated with high grade silver paste placed between two copper electrodes and thus parallel plate capacitor was formed. In organic crystals, the dielectric response is good in the lower frequency region. Hence, the experiments were carried out in the lower frequency region only. The capacitance and dielectric loss were measured for different frequencies from 50 Hz to 5 MHz. The dielectric constant (ε_r) was calculated using the following relation,

$$\varepsilon_r = C_t / \varepsilon_o A$$

where C is the capacitance, d is the thickness of the crystal, ε_o is the vacuum dielectric constant and A is the area of the crystal.

The variation of dielectric constant with log υ is shown in Figure 4. From the graph, it is observed that the dielectric constant decreases with increase in frequency. The values of dielectric constants are found to be high at lower frequencies and they are low at higher frequency region. The variation of dielectric constant value of the material may be due to the contribution of all the four polarizations, namely, space charge, dipolar, electronic and ionic polarization.

The variation of dielectric loss with log υ is shown in Figure 5. The dielectric losses decrease with increase in frequencies. At higher frequency region the values of dielectric loss are low and at lower frequency region the values of dielectric loss are high. The low values of dielectric loss suggest that the quality of the grown crystal is moderately good.

Scheme 1. Synthetic procedure of BTP crystal

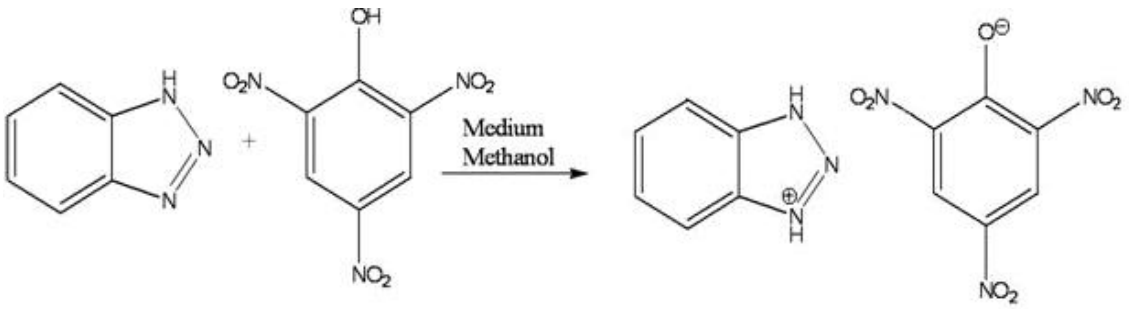

Figure 1. Powder X-ray diffraction pattern of BTP crystal

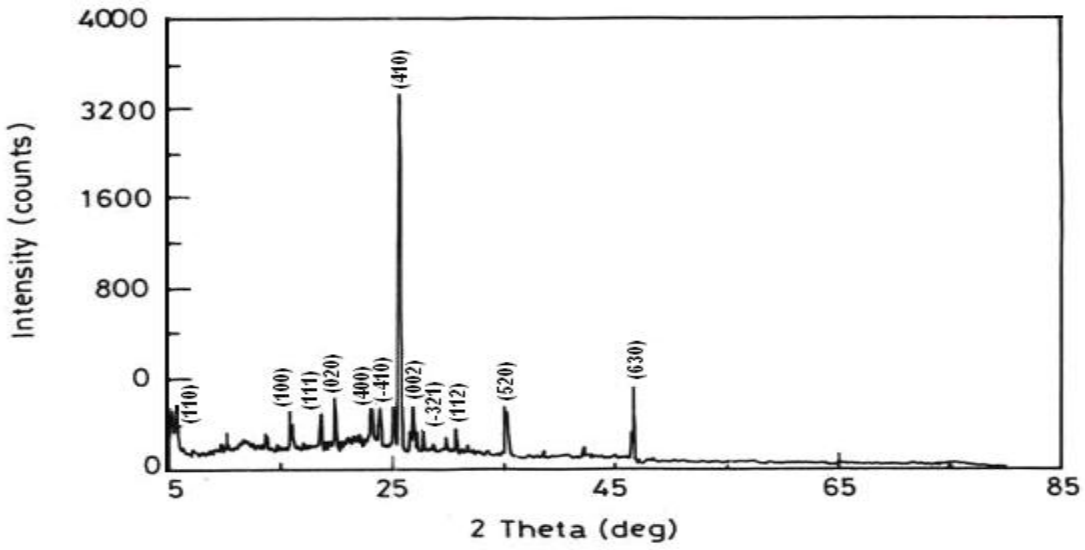

Figure 2. Variation of hardness with applied load of BTP crystal

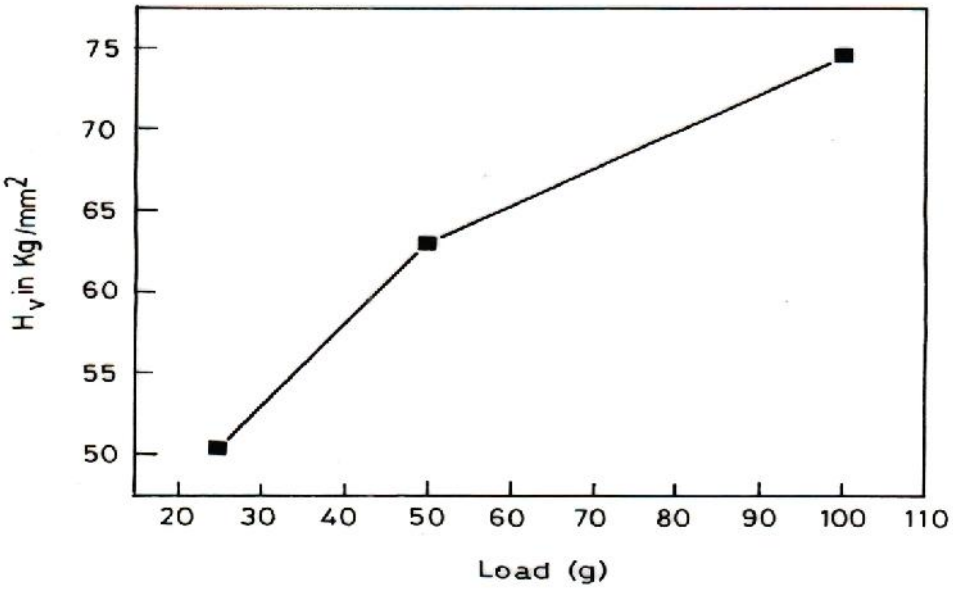

Figure 3. Plot of log d versus log P of BTP crystal

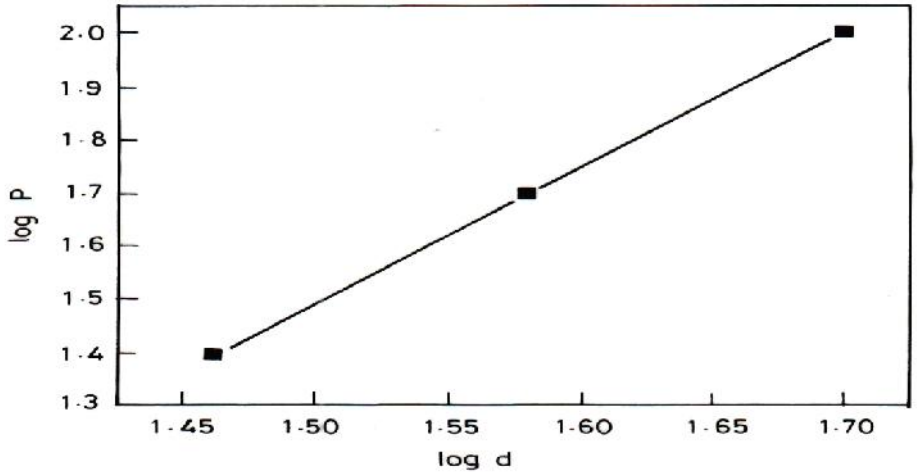

Figure 4. Variation of dielectric constant with log frequency

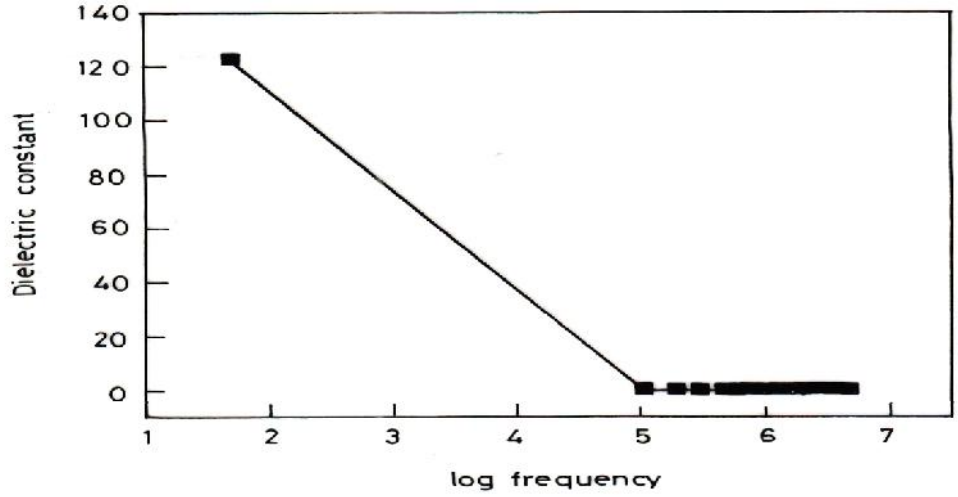

Figure 5. Variation of dielectric loss with log frequency

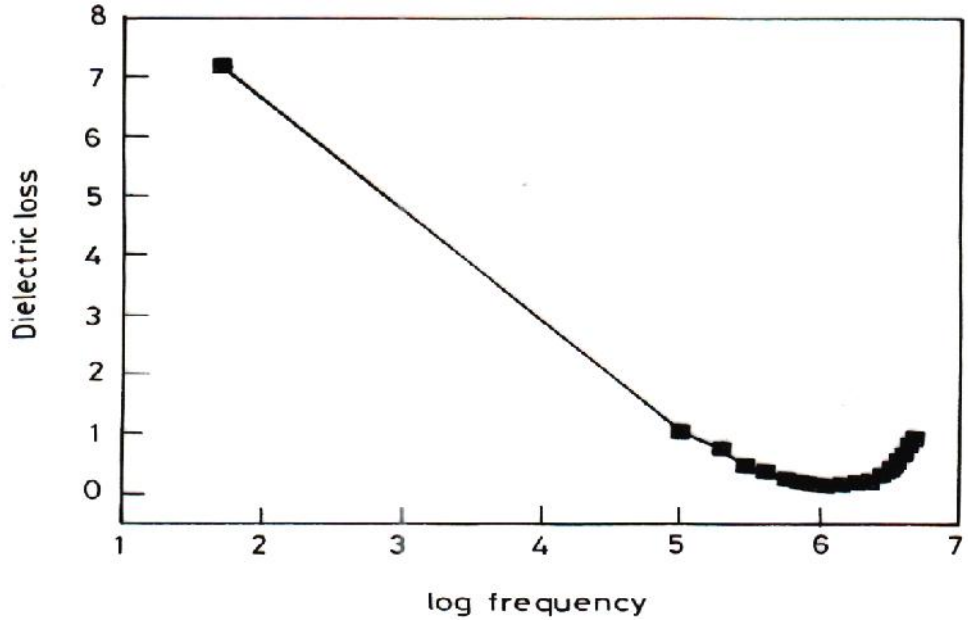

Table. 1 Antibacterial activity of BTP crystal at a concentration of 100 µg/ml.

Bacteria	BTP crystal	Tetracycline
Proteus sp.	16	19
Staphylococcus aureus (Lab isolate)	26	20
Esherichia coli	29	24
Pseudomonas aeruginosa	15	23
Klebsiella pneumoniae	20	18
Staphylococcus epidemidis	20	25
Enterococcus faecalis	20	26
Staphylococcus aureus (Clinical isolate)	27	23
Pseudomonas sp.	-	22

Table. 2 Antifungal activity of BTP crystal at a concentration of 100 µg/ml.

Fungi	BTP crystal	Nystatin
Aspergillus niger	28	23
Penicillinium sp.	19	21
Aspergillus flavus	19	22
Candidia albicans	-	23
Aspergillus fumigatus	-	21

In lower frequency region the dielectric loss shows larger values due to the ionic mobility associated with the material (Shinichi *et al.,* 1990).

Antimicrobial activity studies

A small amount of (about 100 µg) of the synthesized crystal, BTP was weighed and dissolved in 1 ml of DMSO solvent to obtain stock solutions. The diameter zones were measured which exhibit the growth of tested microorganism. The microbial activities of BTP crystal have been studied for its antibacterial and antifungal activities using disc diffusion method.

Antibacterial activity study

Antibacterial *in vitro* study, the bacterial species *Proteus* sp, *Staphylococcus aureus* (Lab isolate), *Escherichia coli, Pseudomonas aeruginosa, Pseudomonas sp, Klebsiella pneumoniae, Staphylococcus epidemidis, Enterococcus faecolis* and *Staphylococcus aureus* (Clinical isolate) were prepared at a concentration of 100 µg/ml. Tetraglycine was used as a standard drug for the comparison bacterial results and the results obtained are given in Table 1.

From the data, it is observed that the BTP crystal has good inhibition activities against *Escherichia coli, Staphylococcus aureus* (Lab isolate), Klebsiella pneumoniae and *Staphylococcus aureus* (Clinical isolate). The BTP crystal has remarkable inhibitory action against *Pseudomonas* sp., *Proteus* sp. and *Pseudomonas aeruginosa* bacterial species. The crystal has no inhibition activity against *Pseudomonas* sp. bacteria.

Antifungal activity study

The BTP crystal was also studied for its antifungal activities at a concentration of 100 µg/ml using Nystatin as a standard drug for the comparison of antifungal activities. The antifungal inhibition activity results of BTP crystal are given in Table 2. From the table, it found that the BTP crystal has good inhibition results *against Aspergillus niger, Aspergillus flavus, Penicillium* sp. and the crystal shows no inhibitory activity against *Candida albicans* and *Aspergillus fumigatus* fungal species

Conclusions

Single crystals of BTP crystal were grown by slow evaporation solution growth method at ambient temperature from its aqueous solutions. The powder X-ray diffraction pattern was used to confirm its crystallinity and most of the predominant Bragg peaks were indexed. The Vicker's microhardness tester was studied to find out the mechanical strength of the crystal and shows that the crystal belongs to soft material category. The dielectric constant and dielectric loss of the crystal decrease with increase in frequency. The synthesized compound shows good inhibition efficiency against various bacteria and fungi species.

Acknowledgements

The authors gratefully acknowledge the School of Chemistry, University of Hyderabad, Hyderabad for their instrumental facilities. I would like to thank The Management and Principal of Sri Ramakrishna Mission Vidyalaya College of Arts and Science, Coimbatore - 641 020, Tamil Nadu, India and the Management of Muthayammal College of Engineering, Rasipuram, Namakkal-637 408, Tamil Nadu, India.

References

Amin, A.S., and El-Beshbeshy, A.M. 2001. Microchim. Acta 137 : 57.

Andrade, S.M., S.M.B. Costa and Pansu, R. 2000. J. Colloids Interface Sci. 226: 260.

Brown, D.J. and Mason, S.F. 1962. The Pyrimidines, Interscience Publishers, John Wiley & Sons, New York.

Collins, A.H., 1976. Microbiology Method, 2nd ed., Butterworth, London, 1976.

Cruickshank, R., J.P. Duguid, B.P. Marmion and Awain, R.H.A. 1995. Medicinal Microbiology, 12th ed., 11, Churchill Livingstone, London. pp. 196.

Elmosallamy, M.A.F., 2004. Anal. Sci. 20: 285-290.

Eychmuller, A., and Rogach, A.L. 2000. Pure Appl. Chem. 72: 179.

Farrell, P.G., F. Terrier and Schaal, R. 1985. Tetrahedron Lett. 26: 2433-2435.

Franchini, G.C., A. Marchetti, L. Tassi and Tosi, G. T. 1988. J. Chem. Soc. Faraday Trans. 184:4427-4438.

Lin, H.M., Y.F. Chen, J.L. Shen and Chou, W.C. 2001. J. Appl. Crystallogr. 89: 4476 .

Maurya, R.C., P. Sharma and Roy, S. 2003. Synth. React. Inorg. Metal. Org. Chem. 33: 683-698.

Onitsch, E.M., 1950. Microskope, 95:12.

Petrosyan, H.A., H.A. Karapetyan, M. Yu Antipin and Petrosyan, A.M. 2005. J. Cryst. Growth. 275:1919-1925.

Prasad, P.N., and Williams, D.J. 1991. Introduction to nonlinear optical effects in organic molecules and polymer, Wiley, New York.

Roy, D.K., A. Saha and Mukherjee, A.K. 2005. Spectrochim. Acta Part A 61. 2017.

Shinichi, H., C.K. Pan, O. Hirishi, U. Hirishi and Yoshithiro,I. 1990. J. Mater. Sci. 25: 493.

Sondhi, S.M., M. Johar, S. Rajvanshi, S.G. Dastodar, R. Shukla, R. Raghubir and Lown, J.W. 2001. Aust. J. Chem. 54 : 157.

Xue, D., and Ratajczak, H. 2005. J. Mol. Struct. Theochem. 716:207-210.

Zhao, F.L. B.Z. Xu, Z.Q. Zhang and Tong, S.Y. 1999. J. Pharm. Biomed. Anal. 21: 355.

