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Chemoinhibition of Mineralization of Urinary Stone Forming Minerals by Magnesium and Zinc Ions in Aqueous and Urinary Milieu

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Chemoinhibition of mineralization of urinary stone forming minerals *viz.*, calcium oxalate and calcium phosphate by magnesium and zinc ions, under different concentrations, have been studied in aqueous as well as urinary milieu. Inhibition efficiency has been studied in an experimental model. Crystals were isolated from the centrifugates of inhibited solutions in aqueous medium and their infrared spectra were studied. Results revealed that magnesium ions at different concentrations show uniformly good inhibition of mineralization of calcium phosphate by 22.59-30.63 % above blank in aqueous and 18.01-29.67 % above blank in urinary media. Zinc ions, however, showed a good inhibition of calcium oxalate mineralization in the range of 6.50-17.07 % above blank in aqueous and 0-35.32 % above blank in urinary media. Infrared spectra of the crystals, isolated from the centrifugates of inhibited solutions, evidenced a bridging mode of C=O of oxalate and a coordinated mode of phosphate. This suggests soluble complexation to be the probable mechanism behind inhibition of oxalate and phosphate mineralization by magnesium and zinc ions.

Key Words: Urolithiasis, Urinary stones, Urolithogenesis, Chemoinhibition of urolithogenesis, Magnesium, Zinc.

INTRODUCTION

The urinary stone formation is related to the level of inhibitors of calculogenesis in urine[1]. Human urine is known to contain some low as well as high molecular weight inhibitors. These are citrate, pyrophosphate, nephrocalcin, glycosaminoglycans, magnesium and zinc. However, the mechanism of action of inhibitors has not yet been clearly established. The anions like citrate and pyrophosphate have been speculated to act by soluble-chelation of calcium ions. So far as the mechanism of action of inhibitor cations like magnesium and zinc ions are concerned, it is not yet well unraveled. Effect of magnesium and zinc on urolithiasis risk factors have been studied[2-4]. Attempts to correlate urinary zinc levels to urolithiasis have been made[3,4]. However, magnesium and zinc's inhibitory capacities towards lithogenesis in the urinary tract have not yet been quantified and the corresponding chemical mechanisms have not been elucidated. A quest in this direction would be of applied value. Magnesium and zinc are nutritionally essential for the body. Magnesium is a macronutrient

while zinc is a micronutrient. Magnesium is seen both in intracellular and extracellular fluids. Its requirement is about 300 mg/day. Normal serum level of Mg^{2+} is 2-3 mg/dl (1-1.5 mmol/L). Mg^{2+} is the activator of many enzymes requiring ATP[5]. Normal serum level of zinc is 100 µg/dl (15 µmol/L). Requirement of zinc for adults is 1-1.5 mg/day; in pregnancy and lactation, 1.5-2 mg/day. More than 300 enzymes are zinc dependent[6].

Thus in view of biological importance of magnesium and zinc and also with a view to understand their applicability as inhibitors in urolithiasis, we have presently studied the inhibition efficiency of magnesium and zinc ions towards the mineralization of urinary stone forming minerals *viz.*, calcium oxalate and phosphate, in aqueous as well as urinary media. An attempt has been made to unfold, tentatively, the mechanism of inhibition by these inhibitors.

EXPERIMENTAL

All chemicals used were of analytical reagent grade. Crystalloid forming solutions, *viz.*, solution of calcium chloride, trisodium phosphate and disodium oxalate of 0.01 M concentrations were prepared in distilled water. Inhibitor solutions *viz.*, 0.02, 0.01, 0.002, 0.001 M solutions of magnesium sulphate and zinc sulphate were prepared separately in distilled water as well as in urine. The urine sample for this purpose was collected from a healthy 35 years old male in a sterilized plastic container. A 24 h urine output was collected and a bit of camphor was added as a preservative. It was used out in minimum possible time after collection.

Inhibition experiments: An experimental model was designed in which the two salt forming solutions *e.g.*, sodium phosphate and calcium chloride (for calcium phosphate) were taken in two separate burettes (50 mL) and were allowed to fall simultaneously and slowly (drop wise) with equal speed into a beaker containing 50 mL of inhibitor ($MgSO_4$ or $ZnSO_4$ solution). The whole operation took about 40 min. At the end the contents of beaker were digested in a hot water bath for 10 min, cooled to room temperature and centrifuged in small volumes. The total centrifugate was collected. Next, the calcium content of the centrifugates in case of calcium phosphate mineralization experiments and oxalate content of the centrifugate in case of calcium oxalate mineralization experiments were determined.

Simultaneous blank experiments with water/urine in place of inhibitor solution were also carried out for evaluating the inhibition efficiency of inhibitors compared to water/urine. All experiments were conducted at room temperature (20-25 ºC).

Estimations and calculations: Calcium was estimated by complexometric method using standard disodium EDTA solution[7]. Oxalate was estimated by permaganatometry using standard $KMnO_4$ solution[8].

While calculating the calcium contents of centrifugate, an EDTA titre value, equivalent to the total inhibitor solution (Mg^{2+} or Zn^{2+} solution) was deducted from the total titre value (equivalent to the centrifugate). This was done because Mg^{2+} or Zn^{2+} that is present in the centrifugate, would also consume some EDTA.

While calculating the oxalate content of the centrifugate in case of experiments with inhibitor solution in urinary media, a permanganate titre value equivalent to 50 mL urine was deducted from the total titre value (equivalent to the centrifugate). This was done because urine itself would consume some permanganate due to its own oxalate and probably other reducing substances.

Inhibition efficiency of the inhibitor solutions (including that of water/urine) was calculated using the formula:

$$\text{Inhibition efficiency (\% inhibition)} = \frac{\text{Ca}^{2+} \text{ or oxalate in centrifugate}}{\text{Total Ca}^{2+} \text{ or oxalate in experiment}} \times 100$$

Total Ca^{2+} in experiment means the Ca^{2+} content of 50 mL 0.01 M $CaCl_2$ solution (used in the experiment), which was determined separately. Similarly, the total oxalate in experiment means the oxalate content of 50 mL 0.01 M sodium oxalate solution (used in the experiment), which was determined separately.

From the percentage inhibition values, the increase of inhibition efficiency of inhibitors over blank (water/urine) were calculated out. Percentage increase of inhibition efficiency of inhibitors, relative to blank (water/urine), were also calculated out using the formula:

$$\text{Percentage increase of inhibition efficiency relative to blank} = \frac{\text{Increase of \% inhibition over blank}}{\text{\% Inhibition by blank}} \times 100$$

Isolation of crystals from the centrifugates of inhibition experiments in aqueous media: Separate inhibition experiments were conducted in the above experimental model using 50 mL each of 0.02 M concentration of salt forming solutions (calcium chloride and sodium phosphate/oxalate solutions) and 50 mL of 0.02 M inhibitor solution (magnesium sulphate/zinc sulphate solution) in aqueous medium.

The centrifugate from these inhibition experiments (after removing precipitates) were subjected to crystallization by evaporating off to a small volume. The crystals, so obtained, were filtered off from the mother liquor and dried at 110 ºC in an air oven and preserved over fused calcium chloride. Infrared spectra (FTIR) of these crystals were recorded in the range 4000-500 cm^{-1} in KBr phase, on a Shimadzu 8201 PC infrared spectrophotometer.

RESULTS AND DISCUSSION

Inhibition data of magnesium sulphate and zinc sulphate towards calcium oxalate mineralization in aqueous and urinary media are mentioned in Tables 1 and 2, respectively. Inhibition efficiency of magnesium sulphate and zinc sulphate towards calcium phosphate mineralization in aqueous and urinary media are mentioned in Tables 3 and 4, respectively.

TABLE-1
INHIBITION OF MINERALIZATION OF CALCIUM OXALATE BY MAGNESIUM AND ZINC IONS IN AQUEOUS MEDIA

Inhibitor	Strength of inhibitor solution (M)	Oxalate in solution (mg)	Oxalate precipitated (mg)	Inhibition (%)	Increase of inhibition over blank (%)	% Increase of inhibition relative to blank (%)
Water (Blank)	–	1.34	42.66	3.04	–	–
$MgSO_4$	0.020	3.54	40.46	8.04	5.00	164.47
$MgSO_4$	0.010	2.22	41.78	5.05	2.01	66.12
$MgSO_4$	0.002	1.66	42.34	3.77	0.73	24.01
$MgSO_4$	0.001	1.54	42.46	3.49	0.45	14.80
$ZnSO_4$	0.020	8.85	35.15	20.11	17.07	561.51
$ZnSO_4$	0.010	5.53	38.47	12.56	9.52	313.15
$ZnSO_4$	0.002	4.65	39.35	10.57	7.53	247.69
$ZnSO_4$	0.001	4.20	39.80	9.54	6.50	213.81

TABLE-2
INHIBITION OF MINERALIZATION OF CALCIUM OXALATE BY MAGNESIUM AND ZINC IONS IN URINARY MEDIA

Inhibitor	Strength of inhibitor solution (M)	Oxalate in solution (mg)	Oxalate precipitated (mg)	Inhibition (%)	Increase of inhibition over blank (%)	% Increase of inhibition relative to blank (%)
Urine (Blank)	–	6.10	37.90	13.86	–	–
$MgSO_4$	0.020	28.26	15.74	64.22	50.36	363.34
$MgSO_4$	0.010	22.72	21.28	51.63	37.77	272.51
$MgSO_4$	0.002	12.75	31.25	28.97	15.11	109.02
$MgSO_4$	0.001	6.10	37.90	13.86	0.00	0.00
$ZnSO_4$	0.020	21.64	22.36	49.18	35.32	254.83
$ZnSO_4$	0.010	14.96	29.04	34.00	20.14	145.31
$ZnSO_4$	0.002	10.53	33.47	23.93	10.07	72.65
$ZnSO_4$	0.001	6.10	37.90	13.86	0.00	0.00

TABLE-3
INHIBITION OF MINERALIZATION OF CALCIUM PHOSPHATE BY MAGNESIUM AND ZINC IONS IN AQUEOUS MEDIA

Inhibitor	Strength of inhibitor solution (M)	Ca^{2+} in solution (mg)	Ca^{2+} precipitated (mg)	Inhibition (%)	Increase of inhibition over blank (%)	% Increase of inhibition relative to blank (%)
Water (Blank)	–	5.88	19.00	23.63	–	–
$MgSO_4$	0.020	13.50	11.38	54.26	30.63	129.62
$MgSO_4$	0.010	14.73	10.15	52.20	28.57	120.90
$MgSO_4$	0.002	14.01	10.87	51.52	27.89	118.02
$MgSO_4$	0.001	11.50	13.38	46.22	22.59	95.60
$ZnSO_4$	0.020	19.74	5.14	79.34	55.71	235.76
$ZnSO_4$	0.010	16.62	8.26	66.79	43.16	182.65
$ZnSO_4$	0.002	14.91	9.97	59.91	36.28	153.53
$ZnSO_4$	0.001	12.26	12.62	49.26	25.63	108.46

TABLE-4
INHIBITION OF MINERALIZATION OF CALCIUM PHOSPHATE BY MAGNESIUM AND ZINC IONS IN URINARY MEDIA

Inhibitor	Strength of inhibitor solution (M)	Ca^{2+} in solution (mg)	Ca^{2+} precipitated (mg)	Inhibition (%)	Increase of inhibition over blank (%)	% Increase of inhibition relative to blank (%)
Urine (Blank)	–	10.80	14.08	43.40	–	–
$MgSO_4$	0.020	18.18	6.70	73.07	29.67	68.36
$MgSO_4$	0.010	17.49	7.39	70.29	26.89	61.96
$MgSO_4$	0.002	15.28	9.60	61.41	18.01	41.49
$MgSO_4$	0.001	12.95	11.93	52.04	8.64	19.90
$ZnSO_4$	0.020	14.16	10.72	56.90	13.50	31.10
$ZnSO_4$	0.010	12.56	12.32	50.47	7.07	16.29
$ZnSO_4$	0.002	11.69	13.19	46.97	3.57	8.22
$ZnSO_4$	0.001	11.67	13.21	46.89	3.49	8.04

A study of Table-1 suggests that magnesium sulphate has a moderate inhibition efficiency towards calcium oxalate mineralization. At 0.02 M concentration, $MgSO_4$ has a net inhibition of 8.04 % which is 5 % more than that by water (blank). Compared to water the percentage inhibition increased by 164.47 %. With decreasing concentrations of $MgSO_4$ the inhibition efficiency decreases. At very low concentration (0.001 M) its inhibition is only slightly higher than that of water. In urinary medium (Table-2) $MgSO_4$ seems to function as a better inhibitor of oxalate mineralization. At 0.02 M concentration the net inhibition is as high as 64.22 %, which comes to 61.18 % higher than that of water and 50.36 % higher than that of urine. Urine itself has inhibited up to 13.86 % which is 10.82 % higher than that of water. This inhibition efficiency of pure urine might be due to its natural inhibitors like citrate, pyrophosphate, *etc*. With decreasing concentration of Mg^{2+}, the inhibition has been found to gradually decrease and becomes equal to that of pure urine at 0.001 M strength. Thus only up to 0.002 M strength Mg^{2+} retains it own inhibition power. Calcium oxalate is the most frequently occurring constituent of urinary calculi[9-12]. It is also the most stubborn constituent. A moderate inhibition of oxalate by Mg^{2+} up to as low as 0.002 M concentration, particularly in urinary medium, suggests that this metal ion would be a useful inhibitor of stone formation in the urinary tract.

So far as calcium phosphate inhibition is concerned, the Mg^{2+} shows a good inhibition in aqueous media (Table-3). The net inhibition at 0.02 M being 54.26 %. However, since phosphate has relatively better solubility than oxalate, water itself has shown 23.63 % inhibition. As such, compared to water it is only 30 % increase of inhibition by 0.02 M $MgSO_4$. With decrease of concentrations of Mg^{2+}, the phosphate inhibition decreases only slightly. Even at 0.001 M, the inhibition is 22.59 % higher than water, which comes to a 95 % increase over blank. This shows that Mg^{2+} is a good sequesterant of calcium phosphate even at very low concentration. In urinary media (Table-4) too, Mg^{2+} shows more or less similar trend of inhibition. Urine

itself has exhibited as high as 43.4 % inhibition of phosphate. This high value might be due to urinary citrate, which is a potent inhibitor of phosphate.

A study of Table-1 shows that zinc sulphate is also a moderately good inhibitor of calcium oxalate precipitation in aqueous medium, The inhibition is slightly more than that of $MgSO_4$ under similar experimental conditions. At 0.02 M concentration, Zn^{2+} inhibits up to 20.11 % of oxalate which is 17.07 % more than that by water. With decrease of concentration of Zn^{2+} the inhibition efficiency gradually decreases. Even at a very low concentration of 0.001 M, the inhibition is almost 3 times (9.54 %) of water. In urinary media $ZnSO_4$, once again, showed a good inhibition of oxalate (Table-2). The net inhibition values in urinary media have been found to be quite higher compared to that in corresponding aqueous media. This is because urine itself has an inhibition efficiency of 13.86 %. At a very low concentration of 0.001 M, Zn^{2+} in urine shows inhibition just equal to that of pure urine, showing, thereby, that at very low concentration Zn^{2+} fails to inhibit appreciably.

So far as calcium phosphate mineralization is concerned, $ZnSO_4$ exhibits a good inhibition in aqueous media (Table-3). Compared to water (blank), 0.02 M Zn^{2+} shows an increase of 55.71 % inhibition. With the decrease in concentration of inhibitor (Zn^{2+}), the inhibition efficiency has been found to decrease only slightly. In urinary media, $ZnSO_4$ does not show up as a good inhibitor for calcium phosphate (Table-4). The net inhibition by Zn^{2+} at 0.02 M concentration is only 56.9 % which is just 13.5 % above that of the urine (blank). With a decrease of concentration the net inhibition decreases slightly and at very low concentration (0.001 M), Zn^{2+} has an inhibition almost equal to pure urine *i.e.*, with almost no additional increase over blank.

On the whole, it looks that Mg^{2+} is a uniformly good inhibitor of calcium phosphate mineralization, whereas, Zn^{2+} is a better inhibitor of calcium oxalate mineralization. The underlying mechanism behind inhibition might be the soluble complexation of phosphate/oxalate by Mg^{2+}/ Zn^{2+} (inhibitor). It looks Zn^{2+} forms a soluble stable complex with oxalate ions and, in turn, screens the later (oxalate) from Ca^{2+}. Oxalate precipitation is, thus, inhibited. Mg^{2+}, on the other hand, seem to have comparatively (compared to Zn^{2+}) less affinity for oxalate, hence unable to sequesterate much oxalate in solution. Calcium oxalate precipitation is thus only slightly inhibited, However, at least some inhibition by Mg^{2+} points to the fact that a relatively more soluble (compared to calcium oxalate) magnesium oxalate complex of some stoichiometry participates in the entire solution equilibria in the experiment.

For calcium phosphate inhibition, on the other hand, slightly reverse is the trend. Mg^{2+} proved to be a better inhibitor than Zn^{2+}. Relative affinities between the cation (Mg^{2+} on Zn^{2+}) and anion (PO_4^{3-}) seem to play role, once again. Due to poor chelating ability of phosphate ion, Zn^{2+} fails to stabilize the former in solution. Magnesium ion, however, might be forming a relatively more covalent (Mg^{2+} has a higher ionic potential) and less aggregating salt.

Infrared studies of crystals isolated from the centrifugates of inhibition experiments in aqueous media: In the study of the IR spectra of crystals isolated from the centrifugates, we are mainly interested on the nature of oxalate/phosphate bands. It is known fact that these crystals are not likely to be pure compounds and rather contain more than one ingredients left out in the centrifugates. However, they definitely contain the sequestered (inhibited) portion of oxalate/phosphate.

Metal oxalates are known to exhibit C=O stretching vibration in wide variation[13]; the frequencies ranging from above 1700 cm^{-1} to as low as 1650-1600 cm^{-1}. The C=O group might be terminal or bridged ones. Presently, the crystals, isolated from the centrifugate of the reaction mixture of calcium chloride, sodium oxalate and magnesium sulphate, showed C=O stretching vibration at 1630 cm^{-1}. The crystals from the centrifugate of the reaction mixture of calcium chloride, sodium oxalate and zinc sulphate exhibited C=O stretching vibration at 1624 cm^{-1}. A quite low position of ν(C=O) band in these crystals suggest that the C=O groups are not free but are rather bridged ones[13-15]. Most probably the C=O groups might be bridging the Ca^{2+} and Mg^{2+}/Zn^{2+} (inhibitor) ions. The low position of C=O vibration might also be due to a chelation of oxalate with the Mg^{2+}/Zn^{2+} (inhibitor ion) resulting ion in coordinated C=O groups and consequent polymeric association through C=O bridging. Thus, in any case, soluble chelation of oxalate by the inhibitor cation seems to be the mechanism behind inhibition of calcium oxalate mineralization by the inhibitors (Mg^{2+}/Zn^{2+}).

The phosphate ion (PO_4^{3-}) has a tetrahedral (T_d) symmetry and shows 4 infrared absorption modes[16]. These are symmetric P-O stretching (ν_1) asymmetric P-O stretching (ν_3) and the two O-P-O bending modes (ν_2 and ν_4). In a non-equivalent force field around the phosphate ion, however, there occurs distortion from the tetrahedral symmetry[16,17]. In case of ionic phosphate, the totally symmetric stretching mode (ν_1) is Raman active, but in coordinated phosphates this band becomes IR active[18]. Presently the infrared spectra of the crystals, obtained from the centrifugate of reaction mixture of calcium chloride, sodium phosphate and magnesium sulphate, showed a band of medium intensity at 1094 cm^{-1}. This band may be assigned to asymmetric P-O stretch (ν_3). The symmetric P-O stretch (ν_1) showed rather low at 915 cm^{-1}. Weak bands at 680 and 604 may be assigned to the two split components of O-P-O bending mode (ν_4). Relatively low position of ν_3 band coupled with split of ν_4 band suggests a coordinated nature of phosphate in the crystals[18,19]. In the infrared spectra of the crystals from the centrifugate of calcium chloride, sodium phosphate and zinc sulphate, the ν_3 band showed at 1155 cm^{-1} as a double headed peak. The ν_1 band has been observed just as a shoulder at *ca.* 950 cm^{-1}. The ν_4 band, however, has been found to split into two, showing at 671 and 602 cm^{-1}. Split of ν_3 and ν_4 bands suggests that the phosphate in the crystals is not ionic but is rather in some coordinated state. Thus, it seems, Mg^{2+} or Zn^{2+} inhibits calcium phosphate mineralization through sequestering-complexation of phosphate.

Conclusion

Presently, it is observed that magnesium sulphate and zinc sulphate solution, under different concentrations, exhibit moderate to good efficiency of inhibition towards mineralization of urinary stone forming minerals *viz.*, calcium oxalate and calcium phosphate, in aqueous as well as urinary milieu. Mg^{2+} has proved to be a comparatively better inhibitor for calcium phosphate mineralization, while Zn^{2+} proved out to be a comparatively better inhibitor of calcium oxalate mineralization. Infrared studies suggested that the magnesium or zinc ions inhibit calcium phosphate or calcium oxalate mineralization by sequestering complexation (soluble chelation) of phosphate or oxalate. Calcium oxalate forms a stubborn constituent of urinary calculi. Its inhibition by Zn^{2+}, particularly in urinary medium, would be of applied value in the prevention and control of urolithiasis.

Both magnesium and zinc are essential metals for life process. Both these metal ions are found in a number of foods. Chief source of magnesium are cereals, beans, leafy vegetables and fish. Dietary sources of zinc are grains, beans, nuts, cheese, meat and shellfish. Homoeostasis of magnesium is maintained by excretion through urine. Normal 24 h urinary level of magnesium is 100-200 mg. Zinc is mainly excreted through urine. Thus both Mg^{2+} and Zn^{2+} form part of the urinary system and any excess of them can be renally handled under normal renal function. In the process of excretion through urinary tract these metal ions would inhibit stone meneralization in the tract.

Thus the calcium urolithiasis patients should be benefited by magnesium and zinc supplementation, particularly, if they are deficient in these metals.

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REFERENCES

1. Y.M.F. Maricker, Essentials of Nephrology and Urology, in ed.: R. Kasivishweswaran, Assoc. eds.: V.N. Acharya, M.S. Rao, V. Sakhuja and S. Bhat, Churchil Livingstone Pvt. Ltd., New Delhi, India, p. 329 (1994).
2. P.C. Hallson and G.A. Rose, *Br. J. Urol.*, **61**, 382 (1988).
3. K. Komleh, P. Hada, A.K. Pendse and P.P. Singh, *Int. J. Urol. Nephrol.*, **22**, 113 (1990).
4. Taner Ozurtas, Gurer Yaket, Mahir Gulec, Muhittin Serdar and Turker Kutluay, *Urol. Internat.*, **72**, 233 (2004).
5. D.M. Vasudevan and S. Sreekumari, Text Book of Biochemistry, Jaypee Brothers Medical Publishers (P) Ltd., New Delhi, India, edn. 2, p. 433 (1999).
6. D.M. Vasudevan and S. Sreekumari, Text Book of Biochemistry, Jaypee Brothers Medical Publishers (P) Ltd. New Delhi, edn. 2, p. 440 (1999).
7. A.I. Vogel, Text Book of Quantitative Inorganic Analysis, E.L.B.S. and Longman, London, edn. 4, p. 320 (1978).
8. A.I. Vogel, Text Book of Quantitative Inorganic Analysis, E.L.B.S. and Longman, London, edn. 4, p. 352 (1978).

9. R. Ahlawat, M.C. Goel and A. Elhence, *Natl. Med. J. India*, **9**, 10 (1996).
10. S.K. Ratan, V. Bhatnagar, D.K. Mitra, N. Basu and L.K. Malhotra, *Indian Pediatr.*, **39**, 819 (2002).
11. R. Kumar, R. Kapoor, B. Mittal, A. Kumar and R.D. Mittal, *Indian J. Clin. Biochem.*, **18**, 209 (2003).
12. M.S. Ansari, N.P. Gupta, A.K. Hemal, P.N. Dogra, A. Seth, M. Aron and T.P. Singh, *Int. J. Urol.*, **12**, 12 (2005).
13. A.K. Banerjee, S.S. Prakash and S.K. Roy, *J. Indian Chem. Soc.*, **62**, 818 (1985).
14. J. Fujita, K. Nakamoto and M. Kobayashi, *J. Phys. Chem.*, **61**, 1014 (1957).
15. S. De, S. Barros and S.A. Friedberg, *Phys. Rev.*, **141**, 637 (1966).
16. K. Nakamoto, Infrared Spectra of Inorganic and Coordination Compounds, John Wiley & Sons, Inc. New York, p. 103 (1966).
17. T.R.N. Kutty, *Indian J. Chem.*, **8**, 655 (1970).
18. R. Coomber and W.P. Griffith, *J. Chem. Soc. A*, 1128 (1968).
19. T.V.R.K. Rao and S. Bano, *J. Indian Coun. Chem.*, **17**, 35 (2000).

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