Acta Ciencia Indica, Vol. XL C, No. 2, 83 (2014)

RARE EARTH β-LACTAM METALLOANTIBIOTICS : (A SYNTHETIC, SPECTRAL & ANTIMICROBIAL APPROACH)

RAJESH KUMAR MISHRA & B.G.THAKUR

Department of Chemistry, C.M. Science College, L.N.M.U., Darbhanga-846004 (Bihar), India

RECEIVED : 8 January, 2014

Three new solid complexes using ligand Cefaclor ("CEF" in brief) and metal ions as La(III), Nd(III) & Sm(III) were synthesized. Cefaclor, a 2nd generation Cephalosporin *i.e.*, β-Lactam antibiotic possess various donor sites for interaction with rare earth metal (III) ions to form complexes of the type "$[ML_2X_2]Cl$" where M = Metal Ions, L = Ligand & X = H_2O with molar ratio of metal : ligand (M : L) of 1 : 2. These complexes were synthesized and characterized by physicochemical and spectroscopic studies. Infrared spectral studies of these complexes suggest that Cefaclor acts as tetradentate ligand and coordinates through endocyclic nitrogen of β-Lactam, Nitrogen of Amide, Nitrogen of 1^0-Amine & Oxygen of Carboxylic acid of dihydrothiazine nucleus to metal ion yielding coordination number ten of the Complex. The complexes have been screened for their antibacterial activity and results were compared with the activity of the uncomplexed antibiotic against *S. Aureus, K. pneumoniae, Salmonella & E. Coli*... etc. The metal complexes were found to be more potent against one or, more bacterial species tested than the uncomplexed Cefaclor.

KEYWORDS : Rare Earth Metal (III) Complexes, β-Lactam antibiotic, UV. IR, ^{1}H NMR, Electronic, Antibacterial & Disc-Diffusion Method.

INTRODUCTION

Metallo-organic Chemistry is becoming an emerging area of research due to the demand for new metal based antibacterial compounds. Most living system contain metal ions for their proper functioning [1-4]. Much research has indicated [5-11] that antibacterial or, antifungal drugs when used as chelates of different metals have enhanced activity. "Cefaclor" is a 2nd generation cephalosporins, a broad spectrum β-Lactam antibiotic with chemical name 3-Chloro-7-D-(2-phenylglycinamido)-3-cephem-4-carboxylic acid monohydrate (Figure-1).

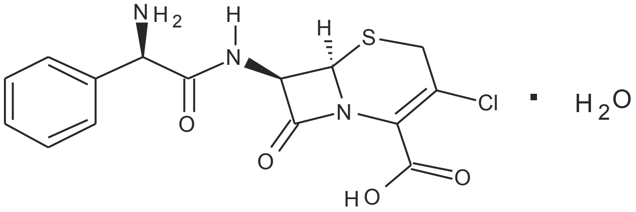

Figure-1: *Cefaclor Monohydrate*

Like all β-Lactams, Cefaclor readily reacts with tissue proteins in-vivo thereby sensitizing susceptible individuals. The allergenic determinants (epitopes) of cephalosporins such as cefaclor are not fully known. This is a consequence of the unstable structures which are formed upon reaction with lysine amino groups in proteins. The primary unstable conjugates are rapidly degraded and transformed into structures where the six membered dihydrothiazine ring is absent [12-13].

Cross reactivity may exist between cephalosporins with similar C-7 side chains and between cephalosporins and penicillins with identical side chains [14]. In practice, this means Cefaclor may cross react with cephalosporins such as Cephalexin, Cephaloglycine, Cefatrizine & semi-synthetic penicillins such as Ampicillin and Amoxicillin.

Clinical pictures typical for immediate reactions to β-Lactams are *Urticaria, with or, without Angioedema & Anaphylaxis.*Anaphylactic reactions to cefaclor have been reported since 80^s [15]. Hama & Mori reported 1988, a high incidence of anaphylactic reactions to Cefaclor [16]. Other kinds of hypersensitivity reactions (non IgE-mediated) to Cefaclor include Serum-Sickness and Erythema multiforma [17].

IgE antibodies to Cefaclor have been detected in vitro in some recent studies by *Korean & Spanish* teams. Thus Kim and co-workers described 4 cases of anaphylaxis caused by cefaclor in which a specific IgE response to cefaclor was demonstrated using an ELISA [18]. In *Spain,* Torres and her team evaluated the in-vitro IgE response to Cefaclor in 7 patients who had experienced anaphylaxis or, urticaria [19].

Cefaclor is active against many bacteria including both gram (-)ive & gram (+) ive. It has also been reported to cause a serum-sickness like reactions in children [20-21].The frequency and severity of serum-sickness like reactions in children has led researchers to question its role in pediatric illness [22]. Cefaclor is passed into the breast milk in small quantities, but is generally accepted to be safe to take during breastfeeding [23]. Cefaclor is not known to be harmful in pregnancy [24].

The ligand *i.e.*, Cefaclor is a white to off white crystalline powder, slightly soluble in water and insoluble in alcohol & chloroform.

A large number of ligands containing N, O & S donor atoms coordinate with rare earth metals forming stable complexes have been reported [25-29]. In view of this, the synthesis, characterization, spectral and antimicrobial screening of a few complexes of Cefaclor with La(III), Nd(III) & Sm(III) metal ions have been studied. For this very purpose different analytical tools have been used. The prepared metal chelates have been found to be more active against *Salmonella, E.coli, K.pneumoniae & S.Aureus...etc* than the uncomplexed Cefaclor. We are in process of synthesizing and study of complexes with other β-Lactam antibiotics also.

EXPERIMENTAL

Materials & Methods :

Solvents used were of analytical grade and all metal (III) were used as chloride salts. Metal salts *i.e.*, $LaCl_3$, $NdCl_3$, $SmCl_3$ (of 99.99% purity) were purchased from Indian Rare Earth Udyog Mandal, Kerala, India and ligand *i.e.*, Cefaclor were purchased from CDH fine chemicals. Molar conductance of the complexes was measured by Systronics digital conductivity meter model-304 in 1×10^{-3} M DMF Solution. Magnetic moment of the complexes were measured by *Gouys* method in Bohr-Magneton unit at room temperature using Hg[Co(NCS)$_4$] as the calibrant in *Inorganic Research Laboratory, P.G. Dept. of Chemistry, L.N.M.U Darbhanga*.

Elemental analysis of the complexes were carried out at *CDRI Lucknow*. Nitrogen content was estimated using elemental analyser *Heraeus Carlo Erba-1108* at CDRI. IR Spectra of the ligand and complexes were recorded on a *Perkin-Elmer FTIR Spectrophotometer* using *KBr pellet method.* The *^{1}H NMR* Spectra of the complexes was recorded in DMSO-d$_6$. All chemical shift values are expressed in δ-scale downfield. Molecular wt. of the complexes were determined by *Camphor-Rast Method.* Electronic spectra of the complexes were recorded by the courtesy of CDRI & Department of Chemistry, IIT Delhi. For determining anrtibacterial action of the complexes experiment was carried out against two non-pathogenic bacteria *E.Coli*, S. aureus and two K. pneumoniae & Salmonella using Agar-Plate diffusion technique [30].

The meat peptone agar was used as culture growth medium. All the solutions of alloy films were prepared by dissolving 1mg/ml of the substance in propylene glycol and stabilized by adopting the usual method in autoclave. The disc-diffusion method uses whatman filter paper discs of diameter 5mm charged with appropriate concentration of test drug. The discs were stored dry in cold. A suitable dilution of broth-culture was flooded on the surface of a nutrient agar on a plate.

Preparation of Metal(III)-Complex :

For the preparation of metal (III) complexes with cefaclor different molar ratio of metal: cefaclor as 1 : 2 were used. The complexes having a molar ratio of metal:cefaclor as 1:2 were prepared by adding a solution of the corresponding metal (III) chloride (0.01 mol) in ethanol (25 ml) to a solution of cefaclor (0.02 mol) in a mixture of water-ethanol (25 ml, 1 : 1 v/v). The mixture was refluxed for 1h on a water bath and concentrated to half volume. Then on cooling to room temperature, the colored solid complexes got precipitated, which was filtered, washed repeatedly with distilled water & ethanol. Now, the complexes were dried over anhydrous calcium chloride in dessicator.

RESULTS & DISCUSSION

All the Ln(III) complexes were obtained in powder form with characteristic color. Analytical data, magnetic moment, molar conductance and color of all the three complexes are represented in Table-1. These data of all the complexes confirms to 1 : 2 stoichiometry. All these complexes are non-hygroscopic and they are soluble in DMF & DMSO & insoluble in common organic solvents. Magnetic moments of the complexes are in good agreement with the theoretical values calculated by Van-Vleck [31]. All the metal complexes decompose above than 300°C.

Table-1 : Analytical, Magnetic Susceptibilty, Molar Conductance data of “Ln(III)-CEF” Complex.

				% (Obs./Cal.)						
S. No	**Complex**	**M. Formulae**	**M. Wt (Obs./Cal.)**	**C**	**H**	**N**	**O**	**S**	**Cl**	**Ln**
1.	[La(CEF)$_2$(H$_2$O)$_2$]Cl	$C_{30}H_{26}LaCl_3N_6O_{10}S_2$	938.64/ 938	38.39/ 38.37	2.81/2.77	8.99/ 8.95	17.09/17.05	6.86/6.82	11.25/ 11.19	14.85/ 14.81
2.	[Nd(CEF)$_2$(H$_2$O)$_2$]Cl	$C_{30}H_{26}NdCl_3N_6O_{10}S_2$	943.75/ 943	38.22/ 38.17	2.80/2.75	8.97/ 8.90	17.02/16.96	6.83/6.78	11.19/ 11.13	15.34/ 15.27
3.	[Sm(CEF)$_2$(H$_2$O)$_2$]Cl	$C_{30}H_{26}SmCl_3N_6O_{10}S_2$	949.23/ 949	38.01/ 37.93	2.77/2.73	8.94/ 8.85	16.94/16.85	6.79/6.74	11.09/ 11.06	15.89/ 15.80

S.No	**Complex**	**Colour**	**Decomposition Temp(^{0}C)**	**Λ_m (Ohm^{-1}cm^{2}mol^{-1})**	**$\mu_{eff.}$(in B.M.)**
1.	$[La(CEF)_2 (H_2O)_2]Cl$	Light Yellow.	337	9.2	Dia.
2.	$[Nd(CEF)_2(H_2O)_2]Cl$	Pale Yellow.	331	12.5	3.61
3.	$[Sm(CEF)_2(H_2O)_2]Cl$	Yellowish White.	318	10.3	1.68

Electronic Spectral Studies :

Electronic spectral data for the solutions of some lanthanide (III) complexes were given in table-2. Lanthanum (III) has no significant absorption in the UV-Visible region. The absorption bands of the Nd^{+3} & Sm^{+3} in the visible and near infrared region appears due to the transition from ground state $^4I_{9/2}$ & $^6H_{5/2}$ respectively to the excited states *i.e,* J-levels of $4f^n$-configuration [32]. The Nehlauxetic ratio (β) has been determined by the method of JØrgenson [33] using the relation :

$$(1-\beta)=\frac{\nu_{\text{Aquo}}-\nu_{\text{Complex}}}{\nu_{\text{Aquo}}}$$

The covalence factor ($b^{1/2}$), metal-ligand covalency % *i.e.*, Sinhas parameter (δ%) and covalency angular overlap parameter (η) have been calculated by using following three expressions [34] :

$$b^{1/2}=\frac{1}{2}[(1-\beta)^{1/2}] \quad \vdots \quad \delta\%=\left[\frac{(1-\beta)}{\beta}\right]\times 100 \quad \vdots \quad \eta=\left[\frac{1-\beta^{1/2}}{\beta^{1/2}}\right]$$

The +ve values of (1-β) and δ% supports the evidence of strong covalent bonding in all the synthesized chelated Lanthanide (III)-Complexes [35].

Table 2. Electronic spectral data along with band-assignment (in cm^{-1}) and related bonding parameters of "Ln (III)- CEF " Complex.

Complex	**Band Assignments**	**Bands(in cm^{-1})**	**Calculated Bonding Parameter**				
			(1-β)	β	**$b^{1/2}$**	**δ(%)**	η
$[Nd(CEF)_2(H_2O)_2]Cl$	$^4I_{9/2}\rightarrow{}^4G_{9/2}$	19270	0.0102	0.9898	0.0504	1.0305	0.0052
	$\rightarrow{}^4G_{5/2},{}^2G_{7/2}$	17385	0.0113	0.9887	0.0531	1.1429	0.0057
	$\rightarrow{}^4F_{9/2}$	14640	0.0041	0.9959	0.0320	0.4116	0.0021
	$\rightarrow{}^2S_{3/2},{}^4F_{7/2}$	13230	0.0046	0.9954	0.0339	0.4621	0.0024
	$\rightarrow{}^4F_{5/2},{}^4H_{9/2}$	12560	0.0064	0.9936	0.0400	0.6441	0.0033
$[Sm(CEF)_2(H_2O)_2]Cl$	$^6H_{5/2}\rightarrow{}^7F_{5/2}$	33467	0.0016	0.9984	0.0200	0.1602	0.0090
	$\rightarrow{}^4H_{7/2}$	28240	0.0045	0.9955	0.0335	0.4520	0.0023
	$\rightarrow{}^4I_{7/2}$	26170	0.0061	0.9939	0.0390	0.6137	0.0031
	$\rightarrow{}^4I_{15/2}$	22570	0.0040	0.9960	0.0316	0.4016	0.0021

IR-Spectral Studies:

IR-Spectra of Cefaclor and its Ln(III) metal complexes were reported in Table-3. Cefaclor has various potential donor sites. A comparison between the IR-Spectra of cefaclor and its Ln (III) complexes provides evidence in support of bonding sites in the Cefaclor-Ln(III) complexes. The IR-Spectra of Cefaclor and its metal(III) complexes were recorded in the range of 4000-400cm^{-1}. In the IR-Spectra of Cefaclor $\nu_{C\text{-}N(\beta\text{-Lactam})}$, $\nu_{\text{-}NH}$ of Amide & $\nu(\text{-}NH_2)$ of 1^0-Amine stretching frequency was observed at 1185 cm^{-1}, 2983cm^{-1}, & 3375 cm^{-1} respectively which was shifted in the range of (1350-1070) cm^{-1}, (2950-2910) cm^{-1} & (3280-3250) cm^{-1} in the the spectra of all Ln(III)-complexes respectively. The band at 1742 cm^{-1} assigned due to $\nu_{c\text{–}o}$ of carboxylic acid of dihydrothiazine nucleus of Cefaclor which was shifted to lower frequencies in the range of 1620-1600cm^{-1} in the spectra of all the rare earth metal (III)-cefaclor complexes. The broad absorption band at 3428-3400 cm^{-1} in the spectra of all the metal complexes provide evidence in support of coordinated water molecule to central metal ion [36]. Shifting of these bands in all the complexes indicate that there is a coordinate covalent bonding through endocyclic N of β-Lactam, Amide, 1^0-Amine & O of carboxylic acid of dihydrothiazine nucleus of cefaclor and O of water molecule with Ln (III)- Central metal ion [37-38]. All of the above mentioned IR-Spectral data confirms coordination number ten of the metal complexes.

Table-3: IR Spectral data (in cm^{-1}) of ligand and complexes :

Functional Group	**Ligand CEFACLOR**	**Complexes**		
		La(III)	**Nd(III)**	**Sm(III)**
$>N-$	1185	1182	1075	1348
$-\underset{\underset{O}{\|}}{C}-OH$	1742	1603	1618	1608
$-NH_{(Amide)}$	2983	2918	2943	2938
$-NH_{2(1^0\text{-}Amine)}$	3375	3252	3278	3267

NMR Spectral studies :

^{1}H NMR Spectra of ligand and its Ln (III)- Chelates were taken in DMSO-d_6. All the protons were found to be in their expected region [39]. A multiplet signals in the region of δ 7.60-7.98 ppm appeared due to aromatic ring protons [40]. In the spectra of "Ln(III)-CEF" Complexes, the signals due to –COOH group protons show downfield shift appearing in the region of δ 2.0-2.2 ppm, COOH Proton signal disappears in the complex indicating that the proton of –COOH is replaced during complex formation. The conclusions drawn from these studies provide evidence in support of the mode of bonding as discussed above from their IR-Spectra. It was also observed that DMSO did not have any coordinating effect either on the spectra of the ligand and their metal complexes.

Magnetic Susceptibility Studies :

Unlike the d-electrons of the transition metal ions, the f-electrons of the lanthanide ions are almost unaffected by the chemical environment and the energy levels are the same as in the free ion due to very effective shielding by the overlying $5s^2$ and $5p^6$ shells. The magnetic moment values of the complexes showed that only Ln(III) complex is diamagnetic while the rest are paramagnetic, showing close agreement with the calculated values except for the Sm(III), indicating an insignificant participation of the 4f-electrons in the bonding. The relatively high value obtained in the case of Samarium(III) complex may be due to small J-J separation, which leads to the thermal population of the higher energy levels and show susceptibilities due to first order Zeemann effect [41].

Antimicrobial Studies :

Antimicrobial studies of Cefaclor alone & their all the Ln(III) complexes indicates that all the complexes are highly effective than the uncomplexed Cefaclor as is evident from Table-4.

Table-4 : Minimum Inhibitory Concentration (in μg/ml) values of Cefaclor and their Ln(III) complexes :

Complexes	*Salmonella*		*E. Coli*		*K. pneumoniae*		*S.Aureus*	
$[La(CEF)_2(H_2O)_2]Cl$	51	59	115	123	117	119	120	123
$[Nd(CEF)_2(H_2O)_2]Cl$	57	61	123	127	112	126	124	127
$[Sm(CEF)_2(H_2O)_2]Cl$	55	64	118	125	119	128	121	128

CONCLUSION

Electronic. IR, & NMR spectral data suggest a coordination number ten for these complexes. The proposed structure (Figure-2) of the complexes may be :

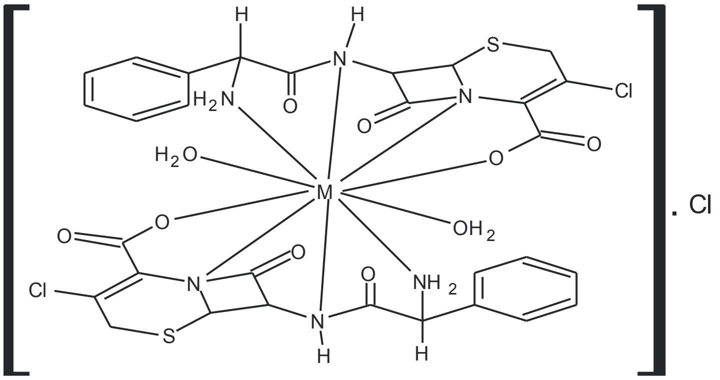

Figure-2: Proposed structure of "M(III)-CEF" Complex.

Where, M(III) = La(III), Nd(III) & Sm(III)

ACKNOWLEDGEMENTS

We express our sincere gratitude to Professor L.K. Mishra, Department of Chemistry, Science College, Patna University for providing valuable suggestions to carry out this work. We are also glad to acknowledge the CDRI & Deptt. of Chemistry, IIT Delhi, for carrying out the elemental and spectral analysis. One of the author (B.G.Thakur) expresses their sincere thanks to UGC New Delhi for providing Major Research Project under XIth plan.

REFERENCES

1. Norden, B., Lincoln, P., Akerman, B., Tuitec, In E., Sigel, A., Sigel, S., Editors : *Metal ions in biological systems*, New York: Marcel Dekker, Vol. **33** (1996).
2. Dr. Williams, *The metals of life*, London : Van Nostrand (1971).
3. Sigel, H., Mc Cormick, D.B., On the discriminating behavior of metal ions and ligands with regard to their biological significance, *Acct Chem Res*., **3(6)**, 201-207 (1970).
4. Dr. Williams, *Metals, ligands & Cancer: Chem Rev*., **72(3)**, 203-213 (1972).
5. Albert, A., The variety of effects of chelating agents on organisms, *Aust J Sci.*, **30(1)**, 1-7 (1967).
6. Kirschner, S., Wei, Y. K., Francis, D., Bergman, J.G., Anticancer and potential antiviral activity of complex Inorganic Compounds, *J. Med. Chem*., **9**, 369-372 (1966).
7. Sekhon, Bhupinder Singh, Metalloantibiotics and antibiotic mimics- an overview. *J. Pharm. Educ. Res.*, Vol. **1**, Issue No. **1** (June 2010).
8. Ming, Li-June, Structure and function of Metalloantibiotics, *Med. Res. Rev.*, Vol. **23**, No. **6**, 697-762 (2003).
9. Barton, J.K., Metal Nucleic Acid Interactions, *Bio-Inorganic Chemistry*, London University Science Books (1994).
10. Hinton, J., Koeppe, R.E., Complexing properties of Gramicidins, *Metal Ion Biol Syst*, **173**, 19 (1985).
11. Claussen, C.A., Long, E.C., Nucleic acid recognition by metal complexes of bleomycin, *Chem Rev*, **2797[32]**, 99 (1999).
12. Venemalm, L., Pyrazinone Conjugates as Potential Cephalosporin Allergens, *Bioorg Med Chem Lett*., **11**, 1869-70 (2001).
13. Perez-Inestrosa, E., Suau, R., Montanez, M.I., Rodriguez, R., Mayorga, C., Torres, M.J., Blanca, M., Cephalosporin chemical reactivity and its immunological implications, *Curr Opin Allergy Clin : Immunol*, **5**, 323-30 (2005).
14. Antunez, C., Blanca-Lopez, N., Torres, M.J., Mayorga, C., Perez-Inestrosa, E., Montanez, M.I., Fernandez, T., Blanca, M., Immediate allergic reactions to cephalosporins: Evaluation of crossreactivity with a panel of penicillins and cephalosporins, *J. Allergy Clin Immunol*, **117**, 404-10 (2006).
15. Nishioka, K., Katayama, I., Kobayashi, Y., Takijiri, C., Anaphylaxis due to cefaclor hypersensitivity, *J. Dermatol*, **13 (3)**, 226-27 (1986).
16. Hama, R., Mori, K., High incidence of anaphylactic reactions to cefaclor, *Lancet*, **1**, 1331 (1988).
17. Joubert, G.I., Hadad, K., Matsui, D., Gloor, J., Rieder, M.J., Selection of treatment of cefaclor associated urticarial, serum sickness-like reactions and erythema multiforme by emergency pediatricians: lack of a uniform standard of care, *Can J. Clin Pharmacol*, **6(4)**, 197-01 (1999).
18. Kim, S.H., Choi, J.H., Park, H.S., Heterogeneity of the IgE response to allergenic determinants of cefaclor in serum samples from patients with cefaclor-induced anaphylaxis,: *Ann. Allergy Asthma Immunol*, **94(6)**, 700-04 (2005).
19. Antunez, C., Blanca-Lopez, N., Torres, M.J., Mayorga, C., Perez-Inestrosa, E., Montanez, M.I., Fernandez, T., Blanca, M., Immediate allergic reactions to cephalosporins : Evaluation of crossreactivity with a panel of penicillins and cephalosporins, *J. Allergy Clin. Immunol*, **117(2)**, 404-10 (2006).
20. Herbert, A., Sigman, E., Levy, M., Serum sickness like reactions from cefaclor in children, *J. Am. Acad Dermatol*, **25**, 805-8 (1991).
21. Parra, F., Igea, J., Martin, J., Alonso, M., Lezaun, A., Sainz, T., *"Serum sickness like syndrome associated with cefaclor therapy", Allergy*, **47(4 pt. 2)**, 439-40 (1992).
22. King, B.A., Geelhoed, G.C., Adverse skin and joint reactions associated with oral antibiotics in children : the role of cefaclor in serum-sickness like reactions, *J. Pediatr Child Health*, **39(9)**, 671-81 (December 2003).
23. Lact MED. "Summary of Cefaclor's use during Lactation". *National library of medicine* (May 2011).
24. Ito, S., Blajchman, A., Stephenson, M. *et al*, "Prospective follow-up of adverse reactions in breast-fed infants exposed to maternal medication". *Am J. Obstet Gynecol*, **168(5)**, 1393-1399 (1993).
25. Keshavan, B. and Radhika, R.T.. *Indian J. Chem.*, **39A**, 425 (2000).
26. Joshi, C.P., Jagtap, C.S. and Chalgfrie, S.V., *Indian J. Pharm. Sci*., **49**, 818 (1987).
27. Talani, J.G. and Modi, R.M., *J. Ind. Chem. Soc*., **60**, 944 (1983).
28. Murthy, P. Radhakrishna and Rao, V. Ramachandra, *Curr. Sci .(India)*, **36**, 233 (1967).

29. Dong, Wenkui, Yang, Rudong and Ma, Ling, *Indian J. Chem*., **37A**, 332 (1998).
30. Adams, H., Bailoy, N.A., Fenton, D.E., Mood, R. and Latour, J.M., *Inorg. Chim. Acta*., **135**, L1 (1987).
31. Van Vleck, J.H. and Frank, N., *Phys. Rev*., **34**, 1494 (1929).
32. Agarwal, R.K. and Agarwal, Himanshu, *Synth. React. Inorg. Met.-Org. Chem*., **31**, 263 (2001).
33. Jorgenson, C.K. and Judd, B.R., *Mol. Phys*., **8**, 281 (1964).
34. Tandon, S.P., Mehta, P.C., *J. Chem. Phys*., **52**, 4313 (1970).
35. Singh, B., Sahai, Preeeti and Singh, Praveen K., *Indian J. Chem.*, **35A**, 494 (1996).
36. Nakamoto, K., *Infrared Spectra of Inorganic and Coordination Compounds*, 2nd Edn., New York, Wiley Interscience (1970).
37. Ferraro, J.R., *Low frequency vibrations of Inorganic and Coordination Compounds*. Plenum Press, New York (1970).
38. Bellamy, L.J., *The Infrared spectra of complex molecules. Methuen*, London (1964).
39. Simmons, W.W., *The Sadtler Handbook of Proton NMR Spectra. Sadtler Research Laboratories*, Inc: (1978).
40. Singh, N.K. and Agrawal, Namita Rani, *Indian J. Chem.*, **13**, 1549 (2001).
41. Phillips, J.P. and Merritt, L.L., *J. Am. Chem. Soc.*, **71**, 3984 (1949).

❑