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Vibrational spectra and normal coordinate analysis on structure of chlorambucil and thioguanine

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Abstract. A normal coordinate analysis on chlorambucil and thioguanine has been carried out with a set of symmetry coordinates following Wilson's F–G matrix method. The potential constants evaluated for these molecules are found to be in good agreement with literature values thereby confirming the vibrational assignments. To check whether the chosen set of vibrational frequencies contribute maximum to the potential energy associated with the normal coordinates of the molecule, the potential energy distribution has been evaluated.

Keywords. Fourier transform infra-red spectrum; Fourier transform Raman spectrum; normal coordinate analysis; potential energy distribution; thioguanine; chlorambucil.

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1. Introduction

Chlorambucil is a chemotherapy drug that has been mainly used in the treatment of chronic lymphocytic leukemia. It is a nitrogen mustard alkylating agent and can be given orally to patients. In the past, it has been used for treating some types of non-Hodgkin lymphoma (Waldenstrm macroglobulinemia, polycythemia vera), trophoblastic neoplasms and ovarian carcinoma. It also has been used as an immunosuppressive drug for various autoimmune and inflammatory conditions, e.g. nephrotic syndrome. Its current use is mainly for CLL as it is well tolerated by most patients, though this has been primarily replaced by fludarabine [1]. Thioguanine is chemically called 2-aminopurine-6-(1H) thione. It is an analogue of naturally occurring purine called guanine, a component of nucleic acid. Pharmaceutically, it is used as antimetabolites [2–4] of folic acid such as amino protein and methoprexate are most effective in the treatment of leukemia [5–10] in children. Thioguanine interferes with the conversion of physiologic purines into nucleic acid,

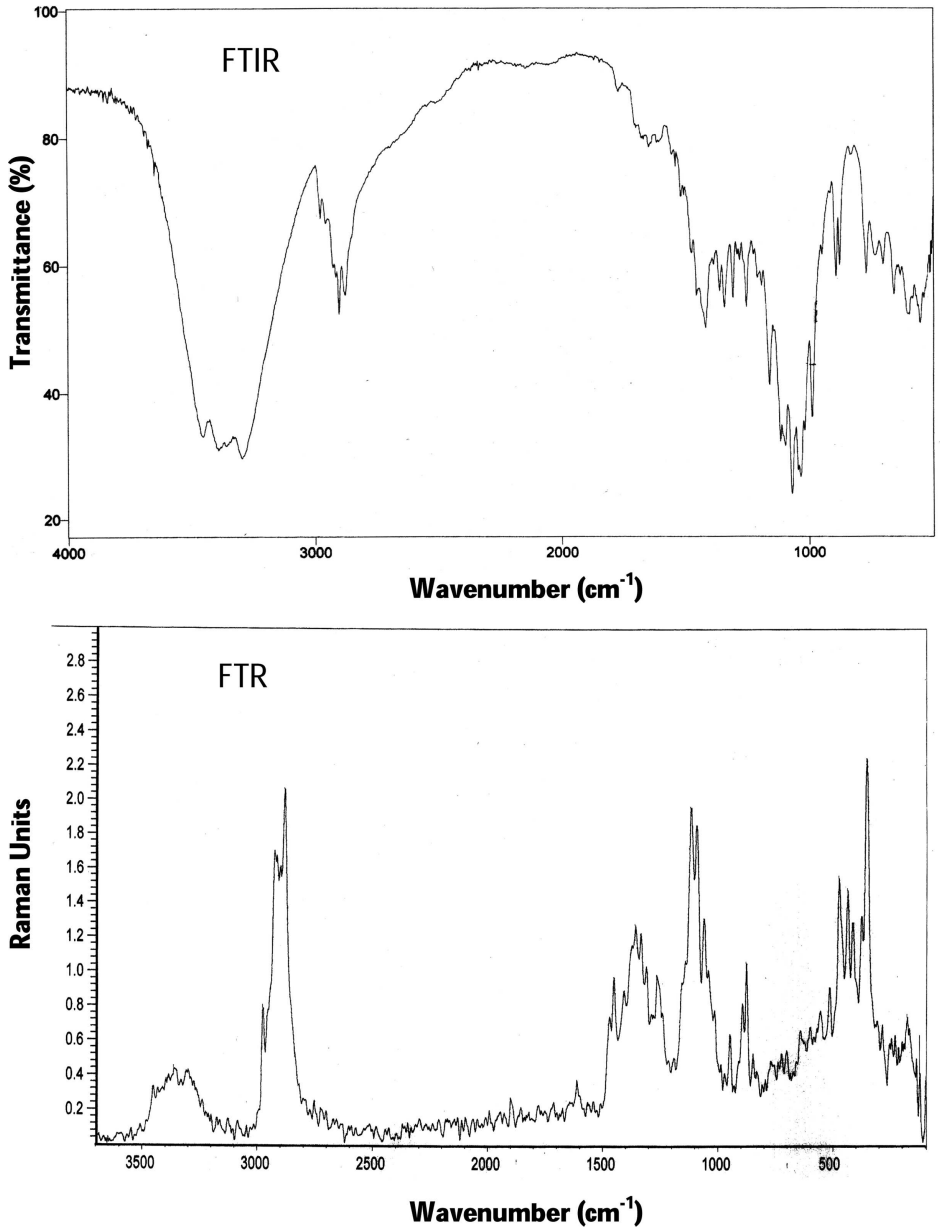

Figure 1. FTIR and FT-Raman spectra of chlorambucil.

i.e. it first converts into deoxyribonucleotide, which blocks DNA synthesis. The increasing interest in the spectroscopic studies of heterocyclic molecules is mainly due to their biological and pharmaceutical importance [11,12]. However, normal coordinate analysis and potential energy distribution associated with each vibrational

mode of chlorambucil and thioguanine have not been carried out so far. Hence, in the present work, the vibrational spectral analyses have been carried out on chlorambucil and thioguanine using normal coordinate analysis. The characteristic vibrational frequencies of these compounds have been identified and assigned based on their relative intensity, characteristic positions and correlation of vibrational bands of related compounds. The present investigation has been undertaken to provide a satisfactory vibrational analysis of chlorambucil and thioguanine through FTIR and FT-Raman spectroscopy and are shown in tables 1 and 2, respectively. To check whether the chosen set of vibrational frequencies contribute maximum to the potential energy associated with the normal coordinates of these molecules, the potential energy distribution (PED) has been evaluated.

2. Experimental

The FTIR spectra of the samples are recorded in the region 4000–400 cm^{-1} in evacuation mode using KBr pellet pressed technique with 4.0 cm^{-1} resolutions and the FT-Raman spectra are recorded in the region 4000–100 cm^{-1} in purge mode using YAG laser of 200 mW. FTIR and FT-Raman spectra of the chosen drugs are presented in figures 1 and 2.

3. Kinetic constants and potential energy distribution

The methods of kinetic constants have been successfully employed by many researchers for the structural elucidation of different types of molecules [13–16]. The elements of inverse kinetic energy matix were formulated using the vectors, which have been evaluated from the expression of the symmetry coordinates in terms of Cartesian displacement coordinates. B' is the transpose of B matrix and is the diagonal matrix of the reciprocal masses of the atoms in the molecule. The frequency assignment is verified by evaluating the potential energy distribution using the relation PED $= F_{ij}L_{ij}^2/\lambda_j$ where PED is the contribution to the potential energy of vibration of the symmetry coordinate whose frequency is j, F_{ij} the force constant and L_{ij}, the L-matrix elements.

4. Normal coordinate analysis of chlorambucil and thioguanine

The structure, orientation and nomenclature of the parameters for chlorambucil and thioguanine molecules are shown in figures 3 and 4. From the structural point of view both molecules belong to C_s point group symmetry. In chlorambucil, CH_2CH_2COOH has been assumed as point mass for the calculation of normal coordinate analysis. The 42 fundamental modes of vibration in thioguanine are distributed as $\Gamma_{vib} = 28A' + 14A''$, and only 37 vibrations have been considered which are $26A'$ and $11A''$ vibrations. The 81 fundamental modes of vibrations in chlorambucil are distributed as $\Gamma_{vib} = 54A' + 27A''$. However, 44 significant vibrations have been considered in the present study, which is distributed as $29A'$ vibrations

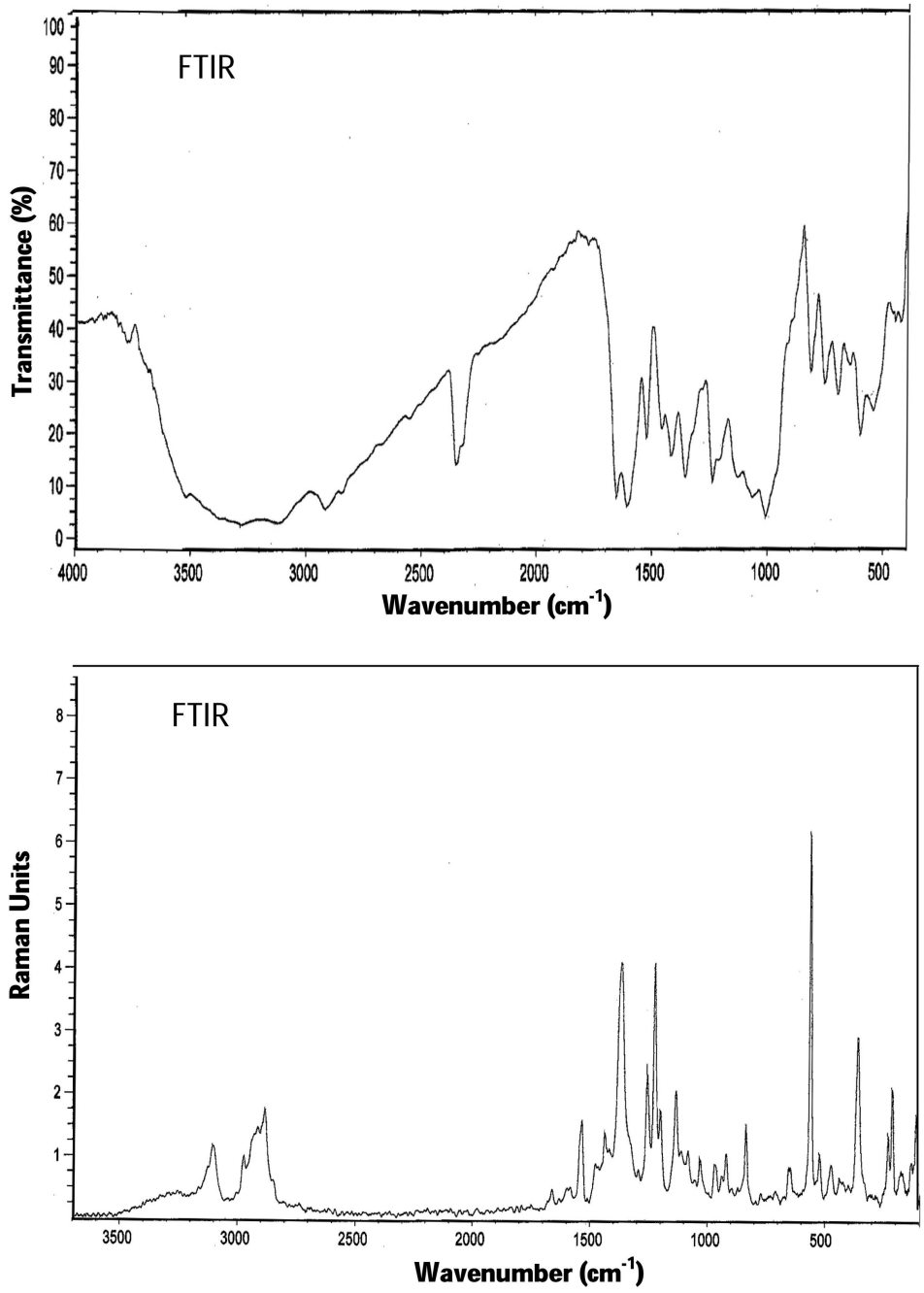

Figure 2. FTIR and FT-Raman spectra of thioguanine.

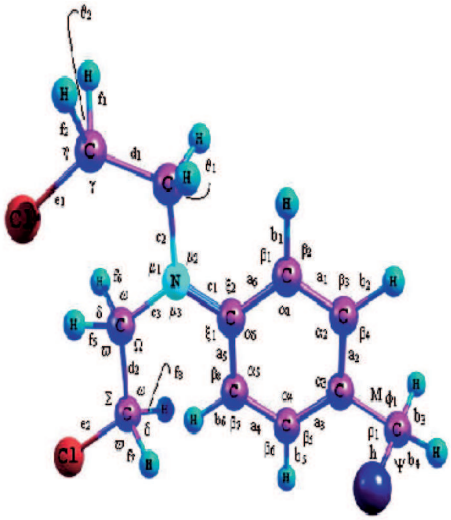

Figure 3. Structure, nomenclature and parameters of chlorambucil.

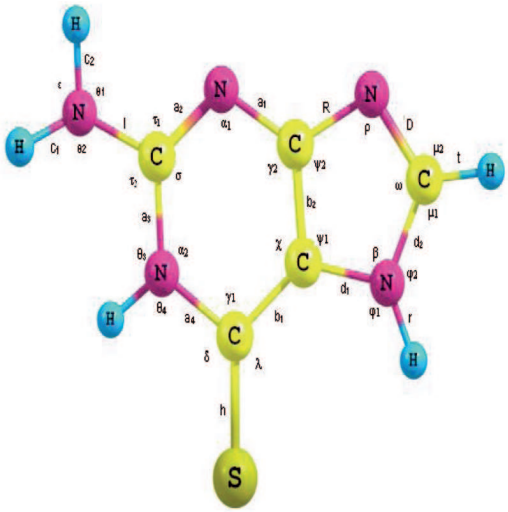

Figure 4. Structure, nomenclature and parameters of thioguanine.

and $15A''$ vibrations. The geometrical parameters were taken from literature [17]. The symmetric coordinates for both these molecules are constructed and used.

4.1 *Vibrational analysis of chlorambucil and thioguanine*

4.1.1 *Aromatic CC vibrations*: The ring carbon–carbon stretching vibrations [18] occur in the region 1625–1430 cm^{-1}. Neville and Shurvell [19] have identified

Table 1. Vibrational assignments and potential energy distribution of chlorambucil.

Symmetry coordinate	Frequency (cm^{-1})		Assignments	PED (%)
	FTIR	FT-Raman		
A' species				
S_1	1475(w)	1473(w)	Ring C–C symmetric stretching	93
S_2	2926(w)	2932(w)	C–H symmetric stretching	89
S_3	1308(s)	–	C–N symmetric stretching	90
S_4	2877(m)	2884(s)	C–H symmetric stretching	91
S_5	552(s)	560(w)	C–Cl symmetric stretching	90
S_6	1455(w)	1453(m)	C–C symmetric stretching	92
S_7	1253(s)	1265(m)	C–C(X) stretching	85
S_8	1034(s)	–	C–C stretching	86
S_9	539(m)	540(m)	C–C–C symmetric bending	60
S_{10}	1096(w)	1094(s)	C–C–H symmetric bending	38
S_{11}	948(w)	949(m)	C–C–N symmetric bending	56
S_{12}	565(m)	578(w)	C–C–C symmetric bending	61
S_{13}	632(vw)	645(vw)	C–N–C symmetric bending	50
S_{14}	1387(w)	1380(w)	H–C–H symmetric bending	84
S_{15}	1069(s)	1061(m)	C–C–H symmetric bending	38
S_{16}	1044(s)	1040(w)	C–C–H symmetric bending	37
S_{17}	1361(m)	1373(w)	H–C–H symmetric bending	84
S_{18}	1418(m)	–	H–C–N bending	46
S_{19}	658(m)	–	C–C–C bending	65
S_{20}	948(w)	949(m)	C–C–H bending	44
S_{21}	815(w)	–	H–C–H bending	67
S_{22}	906(w)	910(m)	C–C–H bending	43
S_{23}	891(s)	899(m)	C–C–N bending	47
S_{24}	470(w)	475(s)	C–C–Cl bending	47
S_{25}	–	404(m)	H–C–Cl bending	44
S_{26}	1281(w)	–	H–C–N bending	49
S_{27}	876(s)	877(s)	C–C–N bending	38
S_{28}	435(w)	438(s)	C–C–Cl bending	50
S_{29}	460(w)	465(m)	H–C–Cl bending	52
A'' species				
S_{30}	1647(w)	1640(w)	Ring C–C asymmetric stretching	90
S_{31}	2977(m)	2977(m)	C–H asymmetric stretching	86
S_{32}	1341(s)	1349(m)	C–N asymmetric stretching	87
S_{33}	2915(w)	2910(w)	C–H asymmetric stretching	89
S_{34}	640(w)	642(w)	C–Cl asymmetric stretching	86
S_{35}	1555(w)	1550(w)	C–C asymmetric stretching	90
S_{36}	596(w)	600(w)	C–C–C asymmetric bending	57
S_{37}	1225(w)	1230(w)	C–C–H asymmetric bending	36
S_{38}	988(s)	990(w)	C–C–N asymmetric bending	53
S_{39}	632(w)	630(w)	C–C–C asymmetric bending	58
S_{40}	701(s)	700(w)	C–N–C asymmetric bending	48
S_{41}	1506(w)	1520(w)	H–C–H asymmetric bending	81
S_{42}	1209(w)	1210(w)	C–C–H asymmetric bending	36
S_{43}	1192(w)	–	C–C–H asymmetric bending	34
S_{44}	1518(w)	1529(w)	H–C–H asymmetric bending	81

the IR bands at 1470, 1484, 1561 and 1575 cm^{-1} in diazepam and closely related compound of benzodiazepines due to aromatic CC stretching vibrations. Based on these factors, in the present study the FTIR bands present at 1481 cm^{-1} and

Table 2. Vibrational assignments and potential energy distribution of thioguanine.

Symmetry coordinate	Frequency (cm^{-1})		Assignments	PED (%)
	FTIR	FT-Raman		
A' species				
S_1	1373(s)	1369(s)	C–N symmetric stretching	82
S_2	1481(w)	1480(m)	Ring C–C symmetric stretching	89
S_3	1230(m)	1228(s)	C–N symmetric stretching	95
S_4	3287(w)	3280(w)	N–H symmetric stretching	94
S_5	1339(m)	–	C–NH2 stretching	88
S_6	2901(w)	2906(w)	N–H stretching	96
S_7	679(m)	676(w)	C=S stretching	55
S_8	2921(w)	2920(w)	N–H stretching	97
S_9	2850(w)	–	C–H stretching	89
S_{10}	1507(w)	1510(w)	C=N stretching	74
S_{11}	1114(w)	1120(w)	C–N stretching	96
S_{12}	430(w)	429(w)	C–N–C symmetric bending	25
S_{13}	444(m)	433(w)	C–C–N symmetric bending	37
S_{14}	1820(w)	–	N–C=N symmetric bending	64
S_{15}	776(s)	780(w)	C–N–H symmetric bending	13
S_{16}	1850(w)	–	C–C=S bending	37
S_{17}	1946(w)	–	N–C=S bending	40
S_{18}	1584(w)	–	C=C–N symmetric bending	54
S_{19}	1072(w)	1070(w)	N–C–H symmetric bending	60
S_{20}	790(m)	797(s)	C–N–H symmetric bending	14
S_{21}	1582(s)	1586(s)	H–N–H bending	44
S_{22}	1664(m)	1668(w)	N=C–N bending	28
S_{23}	599(w)	–	C–C=C bending	57
S_{24}	1636(m)	1625(m)	N–C=N bending	28
S_{25}	558(m)	560(w)	C–N=C bending	57
S_{26}	420(w)	420(w)	C–N–C bending	25
A'' species				
S_{27}	1433(w)	1439(w)	C–N asymmetric stretching	80
S_{28}	1541(s)	1550(w)	Ring C–C asymmetric stretching	89
S_{29}	1250(s)	1259(m)	C–N asymmetric stretching	91
S_{30}	3335(w)	–	N–H asymmetric stretching	92
S_{31}	469(w)	460(w)	C–N–C asymmetric bending	23
S_{32}	476(w)	475(w)	C–C–N asymmetric bending	35
S_{33}	1900(vs)	–	N–C=N asymmetric bending	59
S_{34}	837(m)	846(w)	C–N–H asymmetric bending	12
S_{35}	1674(w)	–	C=C–N asymmetric bending	51
S_{36}	1166(w)	–	N–C–H asymmetric bending	57
S_{37}	822(w)	810(w)	C–N–H asymmetric bending	11

1541 cm^{-1} in thioguanine are assigned to aromatic CC symmetric and asymmetric stretching vibrations and the bands at 1475, 1455, 1647 and 1555 cm^{-1} in chlorambucil have been assigned to CC symmetric and asymmetric stretching vibrations, respectively.

4.1.2 *N–H vibrations*: Hetero-aromatics containing an N–H group show its stretching absorption in the region 3500–3220 cm^{-1}. The position of absorption in the region depends upon the degree of hydrogen bonding, and hence on the physical

state of the sample or the polarity of the solvent [20]. Primary amines examined in dilute solution display two weak absorption bands, one near 3500 cm^{-1} and the other near 3400 cm^{-1}. These bands represent, respectively the asymmetrical and symmetrical N–H stretching modes [21]. In the present work, the IR bands observed at 3287 and 3335 cm^{-1} and the bands at 3280 cm^{-1} in Raman spectrum were assigned to N–H symmetric and asymmetric vibrations of thioguanine.

4.1.3 *C–N and C=N vibrations*: The ring C=N and C–N stretching vibrations [19] occur in the region 1615–1575 cm^{-1} and 1520–1465 cm^{-1}. The medium to weak absorption bands for the C–N linkages in amines appear in the region 1200–1020 cm^{-1}. Mohan *et al* [22] have identified the stretching frequency of C=N bond in benzimidazole at 1617 cm^{-1}. Gunasekaran and Leela Abraham [23] have observed the C–N stretching band at 1312 cm^{-1} in benzocaine. Referring to the above assignments the FTIR bands at 1373 and 1433 cm^{-1} in thioguanine are assigned to C–N symmetric and asymmetric stretching vibrations and the bands present at 1308 and 1341 cm^{-1} in chlorambucil have been assigned to C–N symmetric and asymmetric stretching vibrations, respectively. Also the bands observed at 1507 cm^{-1} in FTIR and 1510 cm^{-1} in FT-Raman could be attributed to C=N stretching vibrations of thioguanine which is in good agreement with the literature value [24,25].

4.1.4 *C–Cl vibrations*: The C–Cl stretching vibrations give generally strong bands in the region 710–505 cm^{-1}. Compounds with more than one chlorine atom exhibit very strong bands due to the asymmetric and symmetric stretching modes. Vibrational coupling with other groups may result in a shift in the absorption to as high as 840 cm^{-1}. For simple organic chlorine compounds, C–Cl absorptions are in the region 750–700 cm^{-1} whereas for the trans- and gauche-forms they are near 650 cm^{-1} [25,26]. Gunasekaran *et al* [21] have observed the FTIR band at 583 cm^{-1} and Raman band at 582 cm^{-1} in diazepam. Considering these factors, the bands observed at 552 and 640 cm^{-1} in FTIR and 560 and 642 cm^{-1} in FT-Raman have been assigned to C–Cl symmetric and asymmetric stretching vibrations in chlorambucil. The above result is in close agreement with the literature values [27,28].

4.1.5 *Deformation vibrations*: In benzene, six ring deformation frequencies are observed. Of these, three arise from in-plane bending vibrations corresponding to 606 and 1010 cm^{-1} mode and the remaining three are derived from out-of-plane bending vibrations corresponding to 404 and 711 cm^{-1} mode of vibrations. The in-plane bending mode of benzene splits into two components in substituted benzenes while both these components are reducing heavily in metal isomers of disubstituted benzenes [14,15]. In the present investigation, the bands observed at 599 cm^{-1} in thioguanine have been assigned to C–C–C bending and 539, 565, 596 and 632 cm^{-1} in the FTIR spectra have been assigned to C–C–C symmetric and asymmetric bending vibrations, respectively. The FTIR bands observed at 948 and 988 cm^{-1} in chlorambucil could be assigned to C–C–N symmetric and asymmetric bending vibrations, respectively. Apart from these vibrations, other bending vibrations of the compound are assigned in the proper region in analogy with the related molecules.

5. Conclusion

A complete vibrational band assignment has been made for thioguanine and chlorambucil using FTIR and FT-Raman spectra. The vibrational band assignments have been made in analogy of the related compounds. Further, to check whether the chosen set of vibrational frequencies contributes to the maximum value of potential energy associated with normal coordinates of the molecules, the PED has been calculated. It has been observed that the potential energy distribution of all the fundamental vibrations is satisfactory and it confirms the present assignments.

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