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Experimental and Semi-empirical computations of the vibrational spectra of Methionine, Homocysteine and Cysteine

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Abstract

Homocysteine is an intermediate non-essential amino acid produced during the metabolism of methionine to cysteine. At elevated levels in plasma, homocysteine is a risk factor for Alzheimer's and cardiovascular diseases. The FTIR and FT Raman spectra of Methionine, Homocysteine and Cysteine molecules were recorded in the regions 4000-450 and 4000-100cm^{-1} respectively. The optimized geometry, wave number and other properties were studied using MNDO, AM1 and PM3 semi-empirical methods. A complete vibrational assignment aided by the theoretical harmonic wave number analysis was proposed. The calculated harmonic vibrational frequencies were compared with experimental FTIR and FT Raman spectra. Based on the comparison between calculated and experimental results and the comparison with related molecules, assignments of fundamental vibrational modes were made. The X-ray geometry and experimental frequencies were compared with the results of theoretical calculations.

Key words: FTIR; FT Raman; Semi-empirical method; methionine, homocysteine and cysteine.

Introduction

Homocysteine is a thiol amino acid that lies at a critical branch point in methionine metabolism. It is produced as a result of methylation reactions and represents an intermediate product of the metabolism of the essential amino acid methionine. The importance of methionine in cellular function relies on the fact that together with cysteine, it is a sulfur donor in the cellular metabolism. In addition, methionine is used to synthesize S-adenosyl methionine, an important

methyl donor. Once produced, homocysteine is metabolized via remethylation, resulting in the formation of methionine or via transsulfuration, resulting in the formation of cysteine and finally taurine [1]. The remethylation pathway of homocysteine is strongly dependent on the availability of folic acid in the active form of 5-methyl-tetrahydrofolate and vitamin B12. The latter is essential for optimal activity of the enzyme methionine synthase, which is responsible for methylation of homocysteine to methionine [2]. As the merely two thiol-containing natural amino acids in human body, cysteine and homocysteine are crucial to the physiological balance in living system [3]. Deficiency of cysteine would lead to many diseases, such as hematopoiesis decrease, leukocyte loss, and psoriasis [4], while homocysteine is a risk factor for cardiovascular [5] and Alzheimer's disease [6]. The analysis of homocysteine and cysteine has been carried out with UV-Visible detection and FTIR detection [7].

In order to interpret the spectra of these molecules in an accurate manner, quantum mechanical calculations using semi-empirical methods were carried out on these molecules. MNDO, AM1 and PM 3 semi-empirical computations of the vibrational spectrum and the atomic charges were carried out for methionine, homocysteine and cysteine molecules. The experimental geometric data of the molecules were taken from the crystallographic results of Cambridge crystallographic database [8]. The main aim of the study was to perform the semi-empirical computations to support the wave number assignment of these molecules, as this computational technique is more suitable for the analysis of organic molecules [9].

Materials and Methods

The molecules of methionine, homocysteine and cysteine was purchased from Messrs Sigma–Aldrich Chemical Company, USA with more than 98% purity and was used as such without further purification to record FTIR and FT Raman spectra . The FTIR spectrum of the compound was recorded in the region 4000–400 cm^{-1} in evacuation mode on Bruker IFS 66V spectrophotometer using KBr pellet technique (solid phase) with 400 cm^{-1} resolution. The FT Raman spectrum was recorded using 1064 nm line of Nd : YAG laser as excitation wavelength in the region 3500–100 cm^{-1}on Bruker IFS 66V spectrometer equipped with FRA 106 FT Raman module accessory. The experimental FTIR and FT Raman spectra of the molecules of methionine and homocysteine are presented in figures 1(a), 1(b) and 2(a), 2(b).

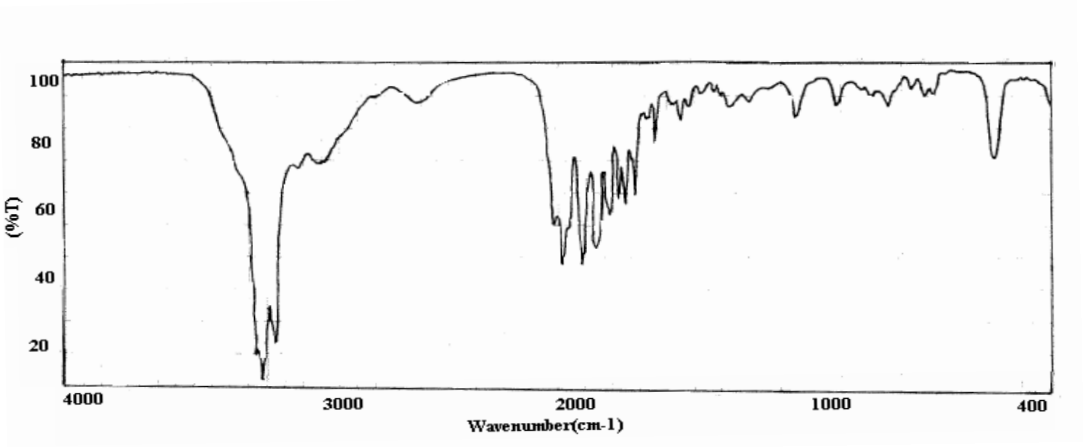


Fig 1(a) FTIR spectrum of methionine

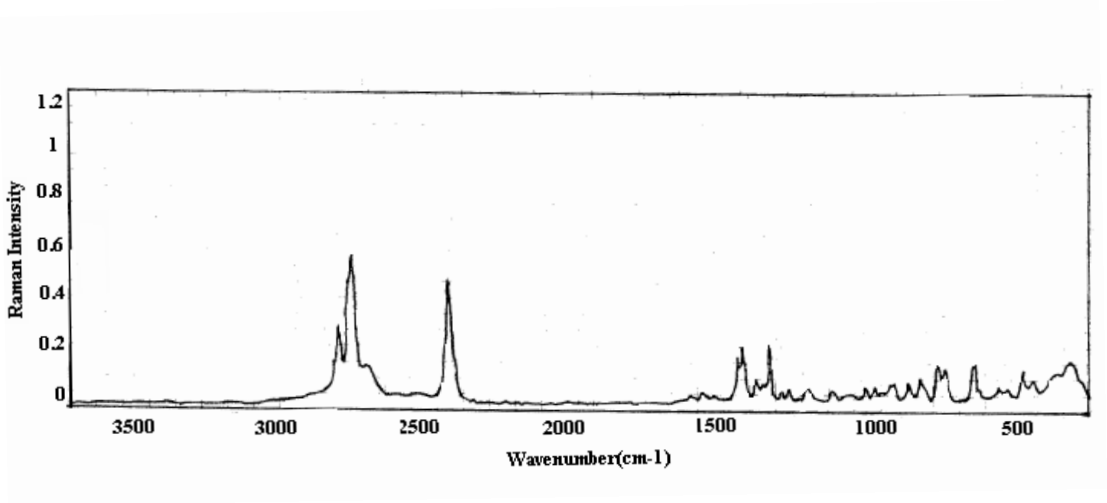


Fig 1(b) FT-Raman spectrum of methionine

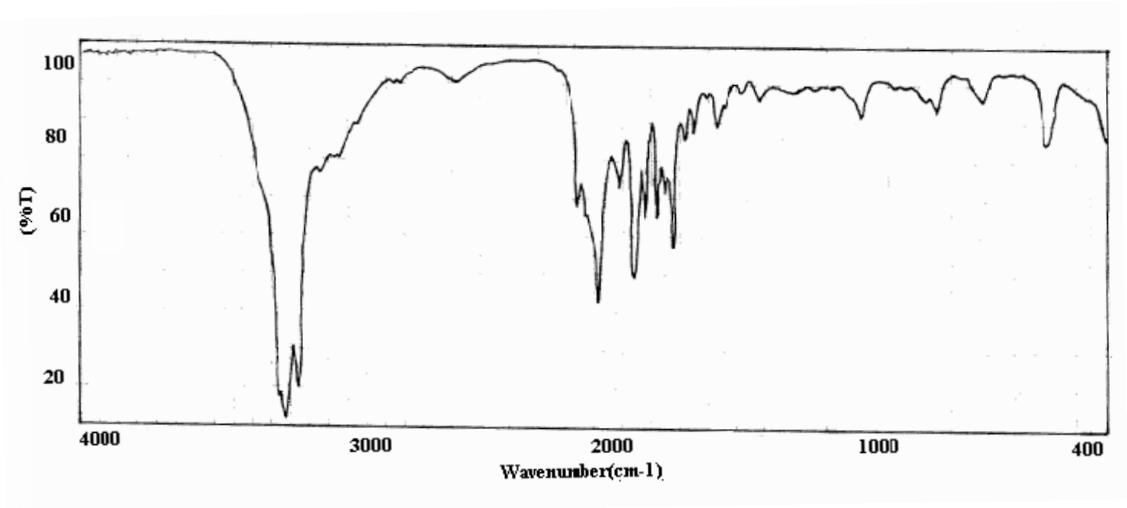


Fig 2(a) FTIR spectrum of homocysteine

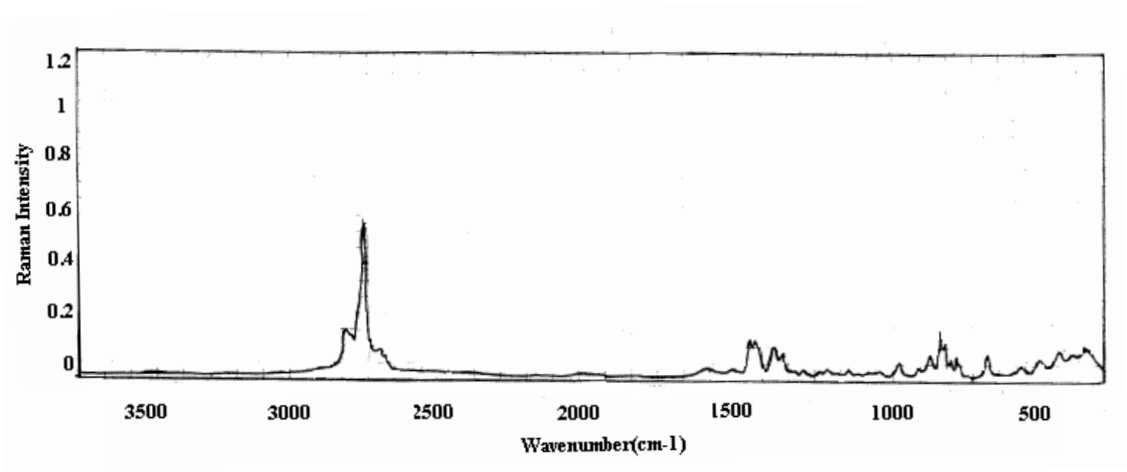


Fig 2(b) FT-Raman spectrum of homocysteine

The entire calculations performed at MNDO, AM1 and PM3 levels were on an AMD 4000+/3.2 GHz personal computer using Gaussian03 program package [10]. The basis sets used in semi-empirical calculations are specially optimized minimal basis sets composed of Slater-type orbitals. The optimized structural parameters were used in the vibrational wave number calculations at the MNDO, AM1and PM3 levels to characterize all stationary points as minima. Then, vibrationally averaged nuclear positions of methionine, homocysteine and cysteine were used for harmonic vibrational wave number calculations resulting in IR and Raman wave numbers together with intensities. Vibrational wave numbers computed by PM3 method have been adjudicated to be more reliable than those obtained by the MNDO and AM1 methods. The assignment of the calculated wave numbers was supported by the animation option of chemcraft, a graphical interface for Gaussian programs, which gives a visual presentation of the shape of the vibrational modes [11].

Results and Discussion

3.1 Geometric parameters

In this work, we performed full geometry optimization of the three molecules. The optimized structure parameters of the molecules calculated by semi-empirical MNDO, AM1 and PM3 methods listed in table 1 and 2 are in accordance with atom numbering scheme given in figure 3, 4 and 5 respectively. For methionine, the PM3 method leads to geometry parameters, which are close to experimental data [8]. A statistical treatment of these data (figure 6, 7 and 8) shown at the bottom of the tables reveal that for the bond lengths PM3 is much better than the MNDO geometry. The correlation coefficient for bond length in the case of methionine was 0.9971 using the PM3 method. For homocysteine and cysteine molecules, again the PM3 method leads to geometry parameters which are close to experimental data [8] and the correlation coefficient for the bond length were 0.9956 and 0.9951 respectively. The agreement for bond angles is not as good as that for the bond distances. The slight variation with the experimental value is due to the fact that the optimization was performed in an isolated condition, whereas the crystal environment affected the experimental X-ray structure.

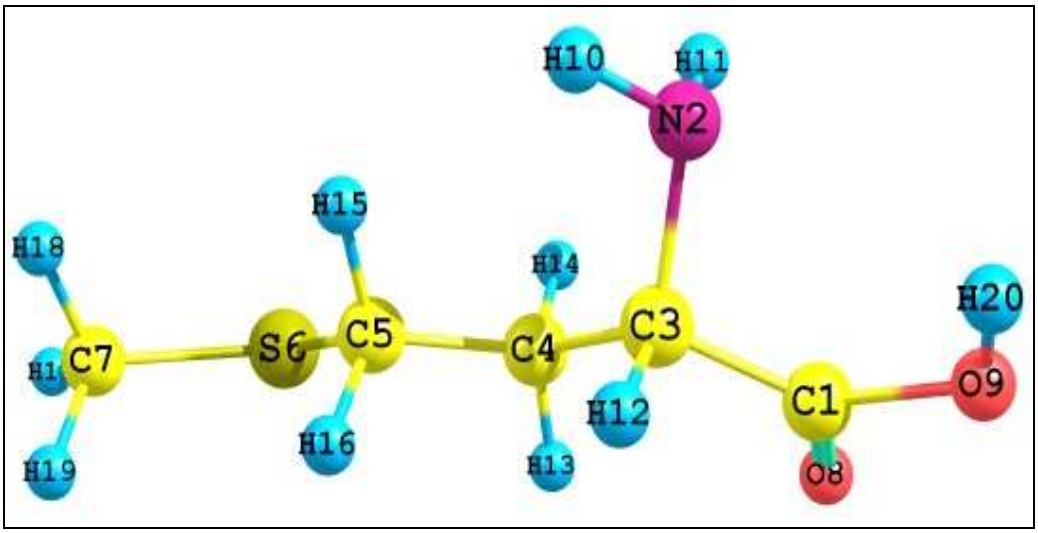


Fig 3. Geometry of Methionine optimized by PM3

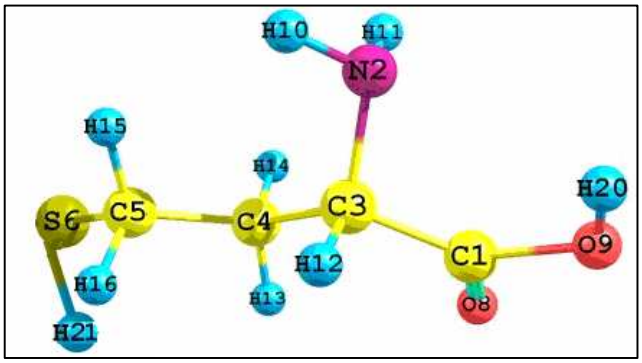


Fig 4. Geometry of Homocysteine optimized by PM3

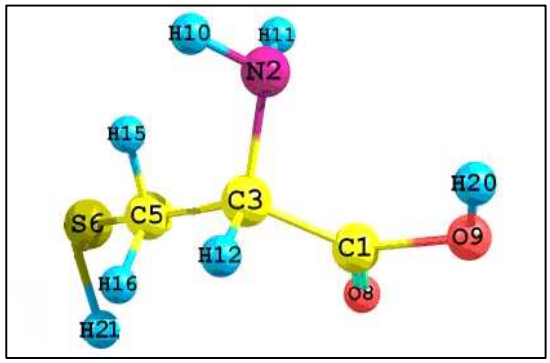


Fig 5. Geometry of Cysteine optimized by PM3

Table 1. Selected bond lengths for methionine, homocysteine and cysteine

Atoms	Bond length (A°)											
	Methionine				Homocysteine				Cysteine			
	X-ray	MNDO	AM1	PM3	X-ray	MNDO	AM1	PM3	X-ray	MNDO	AM1	PM3
C1–O8	1.208	1.227	1.230	1.213	1.208	1.231	1.234	1.218	1.208	1.231	1.232	1.218
C1–O9	1.338	1.359	1.361	1.350	1.338	1.356	1.363	1.352	1.338	1.354	1.361	1.350
C1–C3	1.509	1.556	1.527	1.533	1.509	1.554	1.527	1.527	1.509	1.547	1.523	1.527
O9–H20	0.972	0.947	0.968	0.950	0.972	0.950	0.972	0.953	0.972	0.950	0.972	0.952
C3–C4	1.523	1.559	1.537	1.534	1.523	1.559	1.541	1.536				
C3–C5									1.523	1.556	1.536	1.536
C3–H12	1.113	1.124	1.135	1.120	1.113	1.123	1.135	1.120	1.113	1.123	1.135	1.122
C3–N2	1.468	1.470	1.449	1.486	1.468	1.465	1.441	1.481	1.468	1.470	1.446	1.483
N2–H11	1.035	1.008	1.001	0.999	1.035	1.008	1.001	0.999	1.035	1.008	1.001	0.998
N2–H10	1.035	1.008	1.001	0.998	1.035	1.008	1.002	0.997	1.035	1.008	1.000	0.998
C4–H13	1.113	1.115	1.124	1.111	1.113	1.114	1.122	1.110				
C4–H14	1.113	1.113	1.122	1.111	1.113	1.115	1.124	1.110				
C4–C5	1.523	1.539	1.505	1.517	1.523	1.539	1.505	1.517				
C5–H15	1.113	1.112	1.117	1.106	1.113	1.112	1.117	1.106	1.113	1.114	1.120	1.110
C5–H16	1.113	1.112	1.118	1.107	1.113	1.112	1.121	1.109	1.113	1.111	1.117	1.107
C5–S6	1.815	1.736	1.768	1.825	1.815	1.730	1.766	1.817	1.815	1.728	1.762	1.815
S6–C7	1.815	1.723	1.750	1.800								
S6–H21					1.345	1.302	1.321	1.308	1.345	1.302	1.327	1.307
C7–H17	1.113	1.107	1.115	1.097								
C7–H18	1.113	1.107	1.113	1.096								
C7–H19	1.113	1.107	1.113	1.096								
CC		0.9834	0.9920	0.9971		0.9822	0.9899	0.9956		0.9809	0.9897	0.9951
MAD		0.0209	0.0161	0.0098		0.0225	0.0204	0.0144		0.0255	0.0207	0.0168
RMS		0.0337	0.0248	0.0173		0.0319	0.0237	0.0187		0.0337	0.0248	0.0208

Fig 6. Calculated (PM3) bond lengths and bond angles in comparison with experimental data for methionine

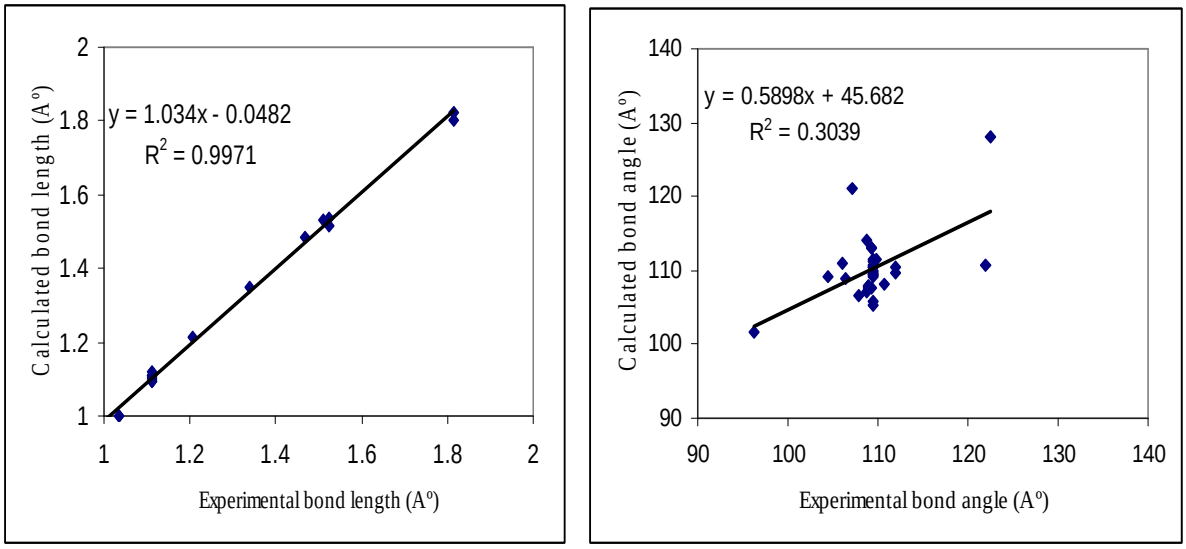


Fig 7. Calculated (PM3) bond lengths and bond angles in comparison with experimental data for homocysteine

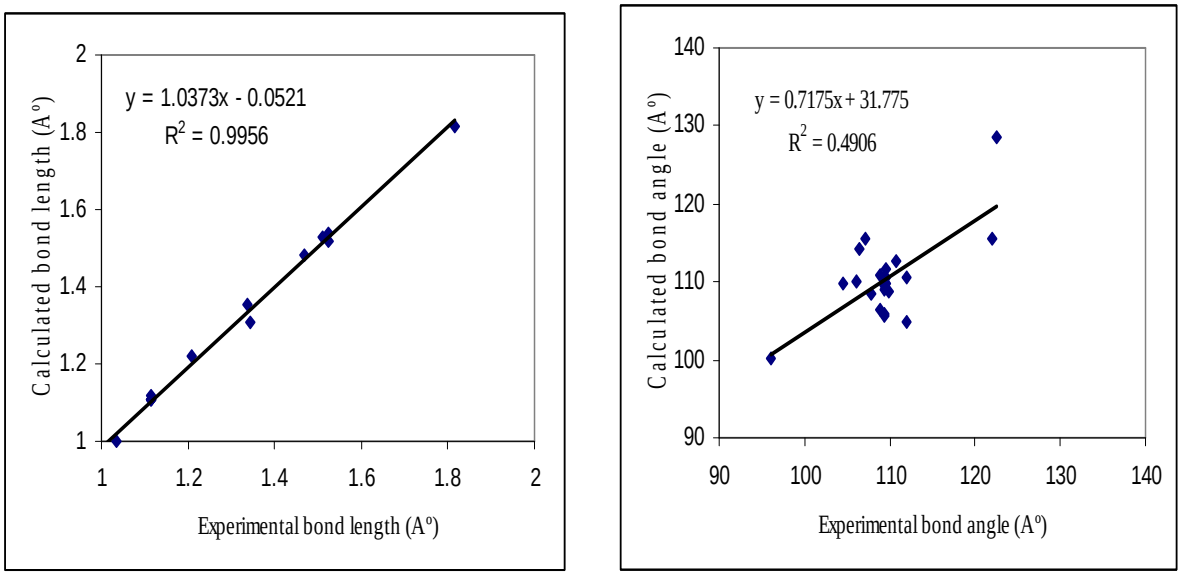


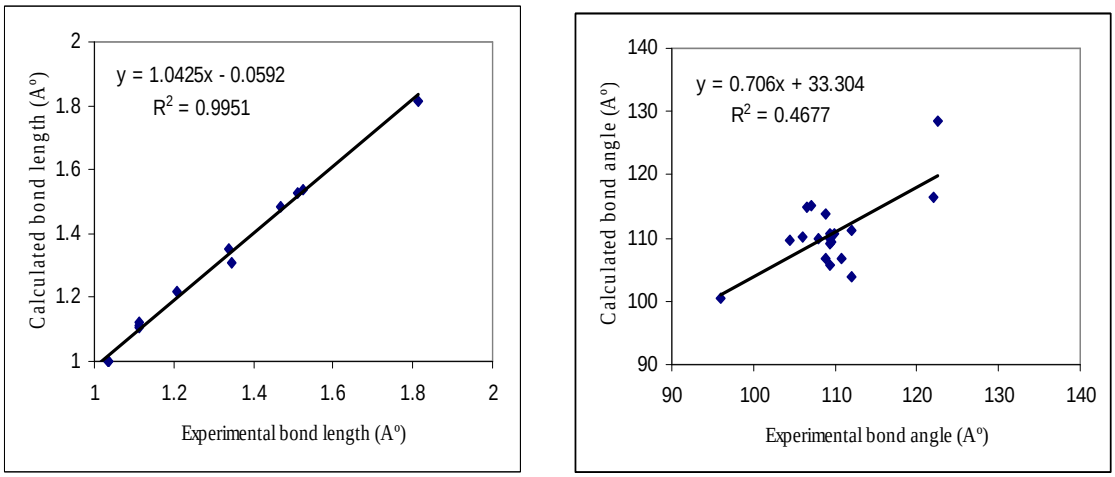
Table 2. Selected inter-axial angles for methionine, homocysteine and cysteine

Atoms	Bond angle (A°)											
	Methionine				Homocysteine				Cysteine			
	X-ray	MNDO	AM1	PM3	X-ray	MNDO	AM1	PM3	X-ray	MNDO	AM1	PM3
C3–C1–O9	107.1	119.35	119.85	121.21	107.1	114.71	115.84	115.40	107.1	114.42	113.53	115.01
C3–C1–O8	122.5	125.54	126.07	128.02	122.5	126.56	127.68	128.56	122.5	126.43	129.00	128.61
O8–C1–O9	122.0	115.11	114.01	110.74	122.0	114.71	115.84	115.40	122.0	119.15	117.45	116.38
C1–O9–H20	106.1	114.09	110.56	110.98	106.1	115.65	109.78	110.11	106.1	115.49	109.72	110.26
C1–C3–C4	109.9	112.80	108.59	111.52	109.9	110.27	106.78	108.63				
C4–C3–N2	108.8	116.90	115.88	114.20	108.8	111.26	113.09	110.96				
C1–C3–N2	110.7	105.26	111.12	108.06	110.7	113.08	116.71	112.73	110.7	105.38	109.74	106.74
N2–C3–H12	108.8	107.28	107.10	106.99	108.8	106.24	106.90	106.33	108.8	107.22	106.87	106.84
C4–C3–H12	109.4	106.70	108.00	109.10	109.4	108.24	108.24	109.50				
C1–C3–H12	107.9	107.46	105.61	106.61	107.9	107.49	104.54	108.56	107.9	108.94	108.08	109.86
C1–C3–C5									109.9	113.23	107.53	110.60
C5–C3–N2									108.8	115.08	116.18	113.72
C5–C3–H12									109.4	106.74	108.19	108.98
C3–N2–H11	109.5	111.32	110.98	109.90	109.5	112.15	111.26	110.46	109.5	111.57	111.54	110.41
C3–N2–H10	109.5	110.38	110.13	109.42	109.5	110.39	110.64	109.80	109.5	109.85	110.39	109.46
H10–N2–H11	104.5	105.82	109.26	109.25	104.5	106.16	109.22	109.67	104.5	105.83	110.18	109.70
C3–C4–H13	109.4	107.82	108.24	109.57	109.4	110.07	110.04	110.62				
C3–C4–C5	109.5	114.95	111.37	111.60	109.5	115.01	111.20	111.56				
C3–C4–H14	109.4	110.17	109.75	110.55	109.4	107.75	107.73	109.05				
C5–C4–H14	109.4	108.95	110.38	110.37	109.4	108.36	110.08	109.60				
H13–C4–H14	109.4	106.48	106.90	105.19	109.4	106.20	107.64	105.70				
C5–C4–H13	109.4	108.11	110.07	109.34	109.4	109.06	110.06	110.14				
S6–C5–C4	106.5	110.23	107.17	108.97	106.5	114.15	112.72	114.09				
C4–C5–H15	109.4	110.77	111.28	111.36	109.4	110.22	110.22	110.19				
S6–C5–H15	112.0	109.19	109.90	109.73	112.0	109.43	110.56	110.70	112.0	105.54	104.02	103.77
S6–C5–H16	112.0	109.59	110.23	110.38	112.0	105.08	104.51	104.86	112.0	110.10	110.93	111.21
H15–C5–H16	109.4	106.60	107.53	105.73	109.4	106.87	107.77	105.95	109.4	106.36	107.68	105.66
C4–C5–H16	109.4	110.40	110.75	110.65	109.4	110.74	110.83	110.63				
S6–C5–C3									106.5	114.58	113.55	114.95
C3–C5–H15									109.4	108.46	110.30	109.95
C3–C5–H16									109.4	111.30	110.07	110.68
C5–S6–H21					96.0	102.84	99.77	100.19	96.0	102.97	99.56	100.49
C5–S6–C7	96.3	108.01	102.09	101.76								
H17–C7–S6	109.3	106.94	106.11	107.55								
H17–C7–H18	109.0	108.48	108.96	107.78								
H17–C7–H19	109.0	108.48	108.95	107.80								
H18–C7–H19	109.0	108.22	108.57	107.55								
H18–C7–S6	109.3	112.30	112.07	112.95								
H19–C7–S6	109.3	112.30	112.09	112.98								
CC		0.2277	0.4027	0.3039		0.3725	0.4883	0.4906		0.4356	0.5400	0.4876
MAD		3.2425	2.5647	2.9491		3.2358	3.1204	2.8481		3.8370	3.3345	3.5700
RMS		4.4484	3.6879	4.2154		4.2193	3.8651	3.7823		4.6580	4.1968	4.4763

Geometrical parameters determined with X-ray diffraction method from ref [8]

CC, Correlation coefficient; MAD, Mean arithmetic deviation; RMS, Root mean square error

Fig 8. Calculated (PM3) bond lengths and bond angles in comparison with experimental data for cysteine



3.2 Vibrational assignments

The FT-IR and FT-Raman spectra of methionine, homocysteine and cysteine molecules were recorded. None of the predicted vibrational spectra have any imaginary frequency, implying that the optimized geometry is located at the local lowest point on the potential energy surface. We know that MNDO, AM1 and PM3 potentials systematically overestimate the vibrational wave numbers. These discrepancies were corrected either by computing anharmonic corrections explicitly or by introducing a scaled field [12] or directly scaling the calculated wave numbers with the proper factor [13]. Theoretical and experimental results of the title molecules are shown in table 4, 5 and 6. The PM3 method is superior to MNDO and AM1 methods in terms of realistic reproduction of both band intensity distribution and general spectral features.

3.2a C=O/C-O Vibrations: The structural C=O has an excellent group frequency, which is described as a stretching vibration. Since the C=O group is a terminal group, only the carbon is involved in a second chemical bond. This reduces the number of force constants determining the spectral position of the vibration. The C=O stretching vibration usually appears in a frequency range that is relatively free of other vibrations. For example in many carbonyl compounds the double bond of the C=O has a force constant different from those of such structural units such as C=C, C-C, C-H, etc.; only structural units such as C=C have force constants of magnitudes similar to that of the C=O group. The C=C vibration could interact with the C=O if it was of the same species, but generally it is not. Almost all carbonyl compounds have a very intense and narrow peak in the range of 1800-1600cm^{-1}. In this study the C=O stretching vibration was observed at 2110 cm^{-1} for methionine, 2100cm-1 for homocysteine and 2082 cm^{-1} for cysteine. This is in agreement with the theoretically predicted frequency obtained by the AM1 method. The C-O stretching is observed at 1585cm^{-1} and 1587cm-1 in the FTIR spectrum of methionine and homocysteine respectively [14,15]. The FT-Raman wave numbers also agree with the reported values.

3.2b O-H Vibrations: The OH group vibrations are likely to be most sensitive to the environment. So they show pronounced shifts in the spectra of the hydrogen bonded species. The

non-hydrogen bonded or a free hydroxyl groups absorbs strongly in the 3700cm^{-1} and 3550cm^{-1}. It is reduced in five or six member ring system [16,17]. The out-of-plane bending vibrations of C-OH were observed at 1310 cm^{-1} of methionine and 1308 cm^{-1} for cysteine. At lower frequencies that absorption at 552cm^{-1}, 470cm^{-1} and 615cm^{-1} corresponds to C-OH vibrations for methionine, homocysteine and cysteine respectively.

3.2c C-H Vibrations: In aromatic compounds [18,19], C-H stretching frequencies appear in the range of 3100-3000cm^{-1}. C-H in-plane bending in the range of 1300-1000cm^{-1} and C-H out of plane bending vibration in the range 1000-750 cm^{-1}. For the C-H stretching vibration observed for methionine, homocysteine and cysteine are 2852 cm^{-1}, 2850 cm^{-1} and 2725cm^{-1} respectively. The intensity is weak and there is excellent correlation with the calculated value using PM3 model.

3.2d CH_2 vibrations: The assignments of CH_2 group frequency involves six fundamentals namely CH_2 symmetric stretch; CH_2 asymmetric stretch; CH_2 scissoring and CH_2 rocking which belongs to in-plane vibrations and two out-of –plane vibrations viz., CH_2 wagging and CH_2 twisting modes, which are expected to be depolarized. The asymmetric CH_2 stretching vibrations are generally observed in the region 3100-3000cm^{-1}, while the symmetric stretch will appear between 3000 and 2900 cm^{-1} respectively [20,21,22]. The symmetric stretching of CH_2 was observed at 2970 cm^{-1} for methionine. The asymmetric stretching was observed at 2925cm^{-1}, 2930 cm^{-1} and 2945 cm^{-1} for methionine, homocysteine and cysteine respectively. The theoretically computed values were slightly higher than the experimental values. The CH_2 wagging mode at 1324cm^{-1} for methionine and its derivatives exactly coincided with the computed values. The other vibrations namely CH_2 rocking, CH_2 symmetric bending, had their theoretically predicted values in good agreement with the experimentally obtained results. The weak bands at 750 - 770 cm^{-1} corresponding to rocking mode of CH_2 for all the three molecules agree with the PM3 model.

3.2e CH_3 vibrations: If a CH_3 group is present in a compound it provokes to exist two asymmetric and one symmetric stretching vibration. Two methyl groups which are in conjugation with the adjacent carbonyl group may alter the electronic structure of the compound and influence the vibrational frequencies and intensities [23,24,25]. The IR band observed at 1385 cm^{-1} is assigned to the scissoring vibration of CH_3 for methionine and that observed at 879cm^{-1} is due to torsion. For homocysteine and cysteine, in the same frequency range the same CH_2 vibrations are observed.

3.2f NH_2 Vibrations: For CH-NH_2 the vibrational modes are the C-H stretching modes, the NH_2 scissors, N-H stretching modes, NH_2 wag, C-N stretching and the torsion about the C-N bond [26]. The band at 78-cm^{-1} corresponds to the NH_2 wag and the band at 1616cm^{-1} corresponds to the C- NH bending motions [27,28]. The in-plane bending of NH_2 for methionine was observed at 1615 cm-1 and for homocysteine it was at 1633cm^{-1}. For the same vibration a strong absorbance was noted at 1583 cm^{-1} for cysteine. The wagging mode of methionine was assigned to the frequency at 1259cm^{-1}. The band in the region 1210cm^{-1} – 1179 cm^{-1} exhibits the torsion of NH_2 and those at 423cm^{-1}-362cm^{-1} exhibits the rocking motion of NH_2 for homocysteine. These agree with the computed values using the PM3 model. For cysteine the out-of-plane bending vibrations of the NH_2 is prominent in the frequency 1341cm^{-1} and the NH_2 rocking is observed in the frequency 934cm^{-1}.

Table 3. Observed FTIR, FT-Raman and calculated wave numbers for methionine using MNDO, AM1 and PM3 methods.

Vibrational assignment	Calculated wave number (cm^{-1})							
	Observed Wave number (cm^{-1})		MNDO		AM1		PM3	
	FTIR	FT-Raman	Wave number	Rel Int.	Wave number	Rel Int.	Wave number	Rel Int.
$\gamma_s CH_2$	2970(w)	2985(w)	3269	5	3069	1	3024	3
$\gamma_{as} CH_2$	2925(s)	2917(s)	3235	2	3035	4	2973	2
$\gamma_{as} CH_2$	2889(w)	2849(m)	3217	2	3009	4	2941	4
γCH	2852(w)	2890(w)	3193	1	2966	2	2837	2
$\gamma_s C{=}H + \alpha COH$	2110(s)	2100(s)	2112	86	2073	78	1983	100
δNH_2	1615(w)	1620(w)	1811	1	1721	2	1658	5
$\gamma_s CCOH + \delta NH_2 + \alpha COH$	1585(s)	-	1545	100	1537	100	1431	96
δCH_2	1523(w)	1520(w)	1459	4	1451	10	1405	0
δCH_3	1420(m)	1425(w)	1451	4	1432	2	1393	2
δCH_2	1415(w)	1411(w)	1448	9	1407	0	1384	5
δCH_3	1385(w)	1380(w)	1443	1	1397	2	1381	3
$\delta CH_2 + \omega CH_2 + \alpha CCH$	1345(m)	1340(w)	1437	8	1383	6	1351	20
ωCH_3	1324(w)	1325(w)	1429	1	1381	0	1329	2
αCOH	1310(w)	1300(w)	1423	0	1364	4	1315	20
$\beta CCH + \tau NH_2 + \gamma CC$	1290(w)	1284(w)	1415	27	1352	1	1288	1
$\omega CH_2 + \beta CCH + \beta NH$	1276(w)	-	1376	1	1338	1	1242	5
$\omega NH_2 + \omega CH_2 + \alpha CCH$	1259(w)	1251(w)	1371	2	1270	2	1202	8
$\tau CH_2 + \beta CNH + \alpha CCH$	1190(w)	1190(w)	1345	17	1206	1	1179	5
$\omega CH_2 + \rho NH_2 + \alpha CCH$	1150(m)	1162(w)	1249	0	1175	2	1141	7
$\tau CH_2 + \beta CNH + \alpha CCH$	1115(w)	1116(w)	1246	0	1081	0	1134	0
$\omega CH_2 + \beta CNH + \alpha CCH$	1088(w)	1080(w)	1233	2	1013	30	1100	1
$\omega NH_2 + \alpha CCH\text{-}CH$	1032(m)	1030(w)	1205	5	986	5	1077	7
$\tau CH_2 + \beta CNH + \alpha CH$	1000(w)	-	1157	1	963	4	1034	0
$\beta NH_2 + \beta CCH$	992(w)	990(w)	1071	21	941	2	998	5
τCH_3	957(w)	964(w)	1040	1	919	0	944	1
$\tau CH_2 + \rho CH_2 + \beta NH_2 + \beta CH$	915(w)	-	1016	1	801	2	930	1
τCH_3	879(w)	880(s)	1001	2	792	0	920	0
$\omega CH_2 + \beta CNH + \alpha CCH$	810(w)	810(w)	977	0	762	6	890	0
ρCH_2	772(w)	765(w)	963	3	729	2	784	1
ρCH_2	750(w)	743(w)	914	1	599	5	775	1
$\gamma CH_3\text{-}SH_2$	727(w)	705(w)	811	1	544	1	753	3
$\beta O{=}C\text{-}COH + \rho CCH$	684(m)	685(w)	753	4	460	29	695	2
$\delta OH\ {-}C{=}O + \alpha CCH + \alpha CNH$	652(w)	662(w)	642	10	422	3	554	7
$\omega NH_2 + \rho CH_2 + \alpha COH$	627(w)	-	576	2	380	6	516	1
βCOH	552(s)	550(s)	396	0	323	4	448	36
$\rho CH_2 + \alpha COH + \beta CH\text{-}NH_2$		412(w)	381	18	359	3	403	9
Lattice vibration		357(w)	357	24	230	9	369	3
$\beta COH + \omega CH_3 + \rho CH_2$		277(w)	321	7	195	2	295	2
$\beta C\text{-}NH_2 + \omega CH_2 + \alpha COH$		225(w)	269	5	109	0	251	3
$\rho CH_2 + \alpha COH$		180(w)	251	11	99	1	238	11

Table 4. Observed FTIR, FT-Raman and calculated wave numbers for homocysteine using MNDO, AM1 and PM3 methods.

Vibrational assignment	Calculated wave number (cm^{-1})							
	Observed Wave number (cm^{-1})		MNDO		AM1		PM3	
	FTIR	FT-Raman	Wave number	Rel Int.	Wave number	Rel Int.	Wave number	Rel Int.
$\gamma_{as}CH_2$	2970(w)	2976(w)	3241	1	3038	16	2976	6
$\gamma_{as}CH_2$	2930(s)	2934(s)	3219	2	3012	0	2946	1
γCH	2850(w)	2874(w)	3200	0	3960	5	2846	3
γC=O + αCOH	2100(s)	-	3197	2	2078	100	1971	100
γSH	1677(s)	1640(w)	2104	100	2042	25	1710	10
δNH_2	1633(w)	-	1811	0	1725	2	1660	2
γCO + αCOH	1587(s)	1585(w)	1578	39	1536	50	1436	37
δCH_2	1462(m)	1460(w)	1464	3	1469	16	1404	1
δCH_2	1388(w)	1382(w)	1457	0	1415	28	1376	1
δCH_2 + αCCH	1344(w)	1350(w)	1445	0	1407	5	1347	2
βCH + αCNH + αCOH	1327(w)	1320(m)	1431	0	1396	1	1290	4
ωCH_2 + τNH_2 + βCCH	1286(w)	1283(w)	1413	34	1354	0	1242	6
ωCH_2 + βCCH + αCOH	1255(s)	1251(w)	1366	6	1277	1	1232	24
τNH_2 + ωCH_2 + αCOH	1210(w)	1202(w)	1337	17	1254	2	1203	2
τNH_2 + βCCH	1179(w)	1180(w)	1261	1	1248	2	1173	6
ωNH_2 + τCH_2 + βCCH	1162(w)		1244	0	1197	0	1141	5
τCH_2 + τNH_2	1100(w)	1112(w)	1216	0	1113	1	1128	1
ωNH_2 + αCCH	1090(w)	1090(w)	1174	5	1096	0	1103	4
τCH_2 + ρNH_2 + αCSH	1014(w)	1010(w)	1135	10	1044	18	1066	1
τCH_2 + βCH + αCSH	1004(m)	1005(w)	1094	21	1033	22	1052	7
ρNH_2 + ρCH_2 + βCCH + αCSH	995(w)	990(w)	1060	3	972	34	1007	2
τCH_2 + βCH + αSH + βNH	966(s)	962(w)	995	11	891	3	994	0
αSH + αOH + βNH + βCH	914(w)	920(w)	945	2	796	4	927	5
τCH_2 + αSH	860(s)	850(w)	907	2	741	0	870	0
ρCH_2	769(w)	788(w)	780	2	703	10	784	2
ρCH_2 + αSH	736(w)	745(w)	724	5	596	23	735	1
δCH_2 + βCOH + βCH + αSH	648(w)	650(s)	667	12	565	45	684	6
βCOH + βO=C-COH	544(w)	548(s)	557	8	535	14	567	4
βCOH	470(m)	456(w)	493	51	443	3	523	41
ρNH_2 + ρCH_2 + αCOH		423(w)	393	3	346	2	479	6
ρNH_2 + ωCH_2 + αCOH		362(w)	333	2	286	1	368	5
β CH_2 + αC-C=O		328(w)	312	0	282	1	313	3
Lattice vibration		227(w)	290	14	234	22	292	0
αC- NH_2 + βCOH		185(w)	262	6	177	16	244	1

3.2g S-H Vibrations: The thiol group present in the molecules of homocysteine and cysteine exhibits characteristic frequencies which agree well with the theoretically computed values using the PM3 model. The stretching vibrations of methyl bonded sulphur are observed at 727 cm^{-1} for methionine. The S-H stretching vibration in homocysteine and cysteine are in the range 1677cm^{-1}- 1650cm^{-1} respectively. The in- plane bending of C-SH is observed in the frequency range of 1014cm^{-1} to 995$cm^{-1}$for homocysteine and the in-plane bending of C-SH, prominent in cysteine is found in the range of 1075cm^{-1} to 1000cm^{-1}. These in-plane and out-of-plane bending vibrations of C-SH are in good agreement with the PM3 model of the semi-empirical technique.

Table 5. Observed FTIR, FT-Raman and calculated wave numbers for cysteine using MNDO, AM1 and PM3 methods.

Vibrational assignment	Calculated wave number (cm^{-1})							
	Observed Wave number (cm^{-1})		MNDO		AM1		PM3	
	FTIR	FT-Raman	Wave number	Rel Int.	Wave number	Rel. Int.	Wave number	Rel Int.
$\gamma_{as}CH_2$	2945(s)	2942(s)	3022	0	3029	9	2966	7
γCH	2725(w)	2843(w)	2976	6	2965	8	2837	7
γC=O + δC-OH + δC-O	2082(s)	-	2946	0	2086	100	1671	100
γSH	1650(m)	1644(w)	2846	3	2012	34	1709	12
δNH_2	1583(s)	1580(w)	1871	100	1715	1	1654	5
τC-OH + γC-CH + αCCH	1405(s)	1408(w)	1710	9	1545	37	1451	47
δCH_2	1373(w)	1370(w)	1660	2	1449	2	1376	5
ρNH + ωCH_2 + αCCH	1341(s)	1345(w)	1436	37	1412	30	1281	0
ωCH_2 + βCCH + βCOH	1308(m)	1310(s)	1404	1	1383	4	1266	2
τNH_2 + βCOH	1237(w)	1243(w)	1376	1	1362	0	1244	27
ωCH_2 + ρNH_2 + βCCH	1211(w)	1210(w)	1347	2	1275	1	1202	9
τNH_2 + αC-CH	1163(w)	1174(w)	1290	4	1262	1	1145	1
ωNH_2 + αCCH	1131(s)	1147(w)	1242	6	1193	1	1116	7
ρHC-CH_2 + τNH_2 + αCSH	1075(s)	1070(s)	1232	24	1166	3	1103	1
ωNH_2 + ρHC-CH_2 + αCSH	1014(w)	1015(w)	1203	2	1117	0	1048	5
ρCH_2 + ωNH_2 + αCCH + αCSH	1005(s)	1000(s)	1173	6	1010	5	1025	13
τNH_2 + ρCH_2 + αCSH	934(s)	954(w)	1141	5	963	20	988	6
τNH_2 + τCH_2 +βCCH + αCOH	882(w)	880(w)	1128	1	948	38	877	1
τCH_2 + αCSH	823(w)	824(s)	1103	3	838	1	814	0
ρCH_2 + αCSH	691(w)	695(s)	1066	1	757	4	734	2
ρCH_2 + βC-COOH	678(w)	-	1052	7	712	9	700	7
ρCH_2 + αCCOH + βCCH	637(w)	-	1007	2	644	7	558	7
βOH	615(s)	615(w)	994	0	550	43	517	39
ωNH_2 + ωCH_2 + αCOH	542(s)	545(w)	927	5	515	12	435	12
βC- NH_2 + αCOH		434(w)	870	0	363	0	352	1
βC- NH_2 + ρCH_2 + αCCO		337(m)	784	1	311	0	299	0
τNH_2 + ρCH_2 + αC=OH		289(s)	735	1	282	1	267	1
τNH_2		210(w)	684	6	214	21	227	16
αC- NH_2 + ρCH_2 + αCSH		183(w)	567	4	207	11	188	2

γ-stretching; δ-scissoring; ω-wagging; ρ-rocking; τ-twisting; α-in-plane bending; β-out-of plane bending

3.3 Other molecular properties

3.3a. Mulliken charges

The calculation of effective atomic charges plays an important role in the application of quantum mechanical calculations to molecular systems. Our interest here is the comparison of different methods to describe the electron distribution in methionine, homocysteine and cysteine, as broadly as possible and assess the sensitivity of the calculated charges to changes in the choice of the semi-empirical method. The Mulliken charges calculated using different semi-empirical methods are listed in table 6 for methionine, homocysteine and cysteine. From these results it will be possible to attribute the change in charge distribution to the choice of the method which depends on the change due to polarization.

Table 6 Mulliken atomic charges of methionine, homocysteine and cysteine for different semi-empirical methods.

Atom with numbering	Methionine			Homocysteine			Cysteine		
	MNDO	AM1	PM3	MNDO	AM1	PM3	MNDO	AM1	PM3
C1	0.3468	0.3326	0.3916	0.2858	0.2595	0.3330	0.3265	0.3060	0.3694
N2	-0.2568	-0.3076	-0.0298	-0.2500	-0.2953	-0.0206	-0.2520	-0.2866	-0.0099
C3	-0.0040	-0.1109	-0.1587	0.0498	-0.0492	-0.1018	0.0493	-0.0469	-0.0988
C4	-0.0418	-0.1923	-0.0307	-0.0198	-0.1627	-0.1174			
C5	-0.0914	-0.2636	-0.2067	-0.0796	-0.2345	-0.1817	-0.1272	-0.2902	-0.2189
S6	0.0278	0.0445	-0.0070	0.0413	-0.0460	-0.0218	0.1035	0.0247	0.0245
C7	-0.0420	-0.3145	-0.2034						
O8	-0.2815	-0.2704	-0.3108	-0.3314	-0.3272	-0.3554	0.3288	-0.3227	-0.3633
O9	-0.2784	-0.2985	-0.2873	-0.2732	-0.3013	-0.2868	-0.2510	-0.2631	-0.2426
H10	0.1015	0.1422	0.0363	0.1119	0.1522	0.0429	0.0995	0.1404	0.0270
H11	0.1056	0.1458	0.0383	0.1036	0.1446	0.0368	0.1032	0.1436	0.0341
H12	0.0426	0.1144	0.0844	0.0522	0.1251	0.0917	0.0699	0.1488	0.1144
H13	0.0407	0.1166	0.0909	0.0402	0.1203	0.0961			
H14	0.0396	0.1166	0.0922	0.0153	0.0907	0.0640			
H15	0.0131	0.0913	0.0760	0.0211	0.1009	0.0765	0.0270	0.1063	0.0818
H16	0.0220	0.0998	0.0780	0.0615	0.1468	0.1184	0.0254	0.1055	0.0820
H17	0.0289	0.1164	0.0875						
H18	0.0096	0.0949	0.0679						
H19	0.0101	0.0956	0.0684						
H20	0.2079	0.2469	0.2228	0.2051	0.2374	0.2205	0.2040	0.2322	0.2079
H21				-0.0338	0.0387	0.0055	-0.0494	0.0123	-0.0078

Table 7. Theoretically computed thermal energies (kcalmol^{-1}), zero-point vibrational energies (kcalmol^{-1}), rotational constants (GHz), and entropies (cal mol-1 K-1) for methionine, homocysteine and cysteine

Parameters	Methionine			Homocysteine			Cysteine		
	MNDO	AM1	PM3	MNDO	AM1	PM3	MNDO	AM1	PM3
Thermal energy	116.888	111.373	109.481	96.736	91.388	88.736	77.131	72.437	70.420
Zero-point energy	109.749	104.078	102.040	90.679	85.248	82.394	71.856	67.169	64.928
Rotational constants									
	3.524	3.557	3.557	3.348	3.630	3.400	2.814	2.941	2.905
	0.488	0.525	0.498	0.691	0.721	0.705	1.498	1.545	1.383
	0.457	0.472	0.458	0.633	0.633	0.634	1.202	1.170	1.141
Entropy									
Total	110.258	112.416	113.531	99.284	100.498	100.866	91.722	91.410	93.829
Translational	40.908	40.908	40.908	40.613	40.613	40.613	40.287	40.287	40.287
Rotational	30.393	30.279	30.621	29.776	29.653	29.737	28.541	28.493	28.640
Vibrational	38.957	41.229	42.262	28.896	30.232	30.515	22.894	22.630	24.902

3.3b. Thermodynamic properties

The calculated thermodynamic parameters are presented in table 7. Scale factors have been recommended [29] for an accurate predication in determining the zero-point vibrational energies and the entropy S. The variation in the zero point vibrational energies seems to be insignificant. The changes in the total entropy of methionine, homocysteine and cysteine at room temperature found using different semi-empirical methods are only marginal.

Conclusion

The results of the study lead to the following conclusions: (i) The frequency assignments using the FTIR and FT-Raman spectra was performed for the first time for methionine, homocystine and cysteine molecules. The theoretical semi-empirical calculations of the vibrational spectra of the molecule presented in this paper were compared with the FTIR and Raman spectra. (ii) Geometries were reported within the limits of accuracy of available experimental data. The molecular geometry of methionine, homocystine and cysteine molecules were best at the PM3 level of the various semi-empirical methods employed. (iii) Mulliken charges of methionine, homocystine and cysteine molecules at different levels were calculated and the results discussed.

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