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QUALITATIVE ANALYSIS ON THE INFRARED BANDS OF TETRACYCLINE AND AMPICILLIN

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This work presents Fourier Transform Infrared spectra of important antibiotics, Tetracycline and Ampicillin. Tetracycline and Ampicillin are the major drugs used for the treatment of fatal bacillary and coccal infections. The vibrational spectra of these two antibiotics have been examined through Infrared spectroscopy.

Key Words: Fourier Transform Spectroscopy; Tetracycline and Ampicillin; Vibrational Assignments; Qualitative Analysis

Introduction

A great many drugs fall into one of the two broad categories—chemotherapeutic and compound acting on the central nervous system. Chemotherapeutic agent is a substance that inhibits or destroys an infectious organism such as pathogenic bacteria or parasites, which has invaded a host. Antibiotics are chemotherapeutic agents that are produced by microorganisms and are toxic to other organisms, particularly bacteria and viruses. Tetracycline and Ampicillin are the major drugs and since they became available in 1945, it has saved thousands of human lives from fatal bacillary infections like plague. Recently, during the epidemic of pneumonic plague at Surat, tetracycline was prescribed for prevention and cure. It was internationally accepted that tetracycline was the best choice of treatment.

Experimental

Tetracycline and Ampicillin were prepared using standard methods in a pharmaceutical laboratory at Madras and the samples were of high purity. The infrared spectra of the samples were recorded using Bruker IFS66V *at RSIC*, IIT, Madras over the region 4000–400 cm^{-1}.

The frequencies observed in the spectra of Tetracycline and Ampicillin were summarized with their band assignments in Tables I and II respectively and the spectra were reploted and are shown in Figs 1 and 2.

Qualitative Analysis on the Vibrational Bands

The molecular formula of Tetracycline $C_{22}H_{24}N_2O_8$ and Ampicillin $C_{16}H_{19}N_3O_4S$ and they all belong to C_5 symmetry.

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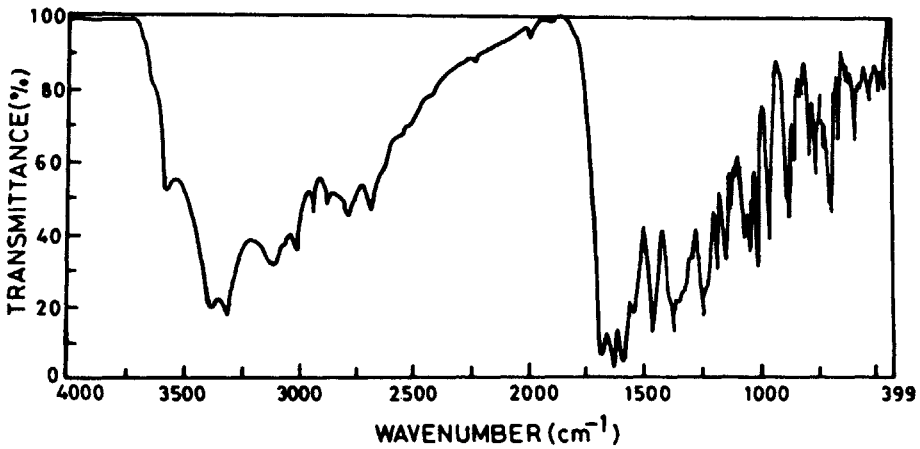

Fig 1

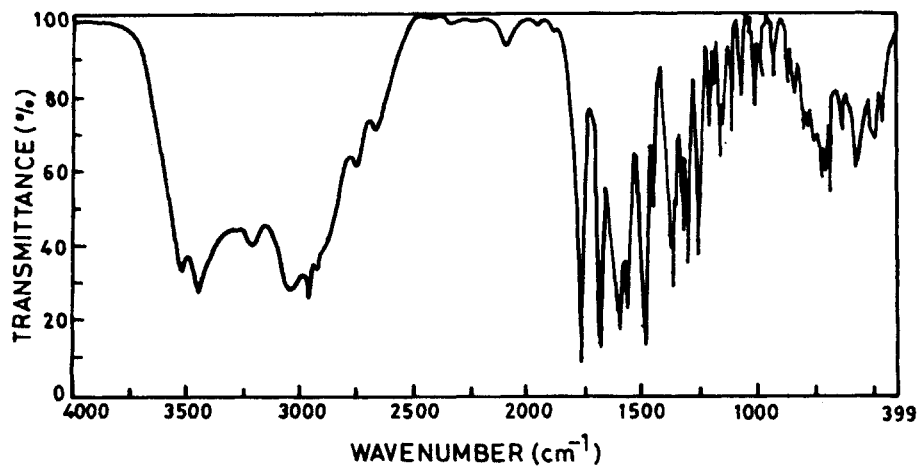

Fig 2

(*A*) *TETRACYCLINE*

Aromatic Ring Vibrations

Most mononuclear and polynuclear aromatic compounds have three or four peaks in the region 3080-3010 cm^{-1}. This being due to stretching vibrations of the ring C–H bonds and these have strong-medium intensity. A strong band observed at 3049 cm^{-1} is assigned to C–H vibrations of the phenyl ring.

A number of C–H in-plane deformation bands occur in the region 1290-1000 cm^{-1}, the bands are usually being sharp, but of weak-to-medium intensity. However, these bands are not normally of importance for interpretation purposes although they can be used. In fact, a number of interactions are possible, thus necessitating great care in the interpretation of bands in the region-polar ring substituents may result in an increase in the intensity of these bands. Addi-

tional difficulties may also arise due to the presence of the other bands in the region. The frequencies of C – H out of plane deformation vibrations are mainly determined by the number of adjacent hydrogen atoms on the ring and not very much affected by the nature of substituent[1-2]. These bands give important means for determining the type of aromatic substitution. These C – H in plane bending vibrations and out of-plane bending vibration are presented in the Table I.

The ring carbon-carbon stretching vibrations occur in the region 1625-1530 cm^{-1}. For six-membered ring there are two or three bands in this region due to skeletal vibrations, the strong usually being at about 1500 cm^{-1}[3-7]. In general, the bands are of variable intensity and are observed at 1625-1590 cm^{-1}, 1590-1575 cm^{-1}, 1525-1470 cm^{-1} and 1465-1430 cm^{-1} for substituted benzenes. In the spectrum of Tetracycline several aromatic phenyl ring vibrations are observed. In line with the above statement the band observed at 1452 cm^{-1} and 1527, 1552, 1584, 1618 and 1666 cm^{-1} are assigned to C=C ring stretching. The bands in the aromatic ring deformation region are quite sensitive to the change in the nature and position of substituents[8-9] although other bands depend mainly on the distribution and number of substituents rather than on their chemical nature or mass so that these latter vibrations together with the out-of-plane vibrations of the ring hydrogen atoms are extremely useful in determining the positions of substituents. For monosubstituted aromatics, the bands due to the out-of-plane ring deformation vibration occur in the region 410-550 cm^{-1}. Hence, the bands observed at 501 cm^{-1} and 567 cm^{-1} are due to out-of-plane ring deformation of aromatic phenyl ring.

Methyl Group Vibrations

Methyl group has two types of stretching vibrations, one is symmetrical contraction of the C – H bonds (or) expansion of C – H bonds and another is anti-symmetrical contraction or the expansion of C – H bonds. These vibrations always occur below 3000 cm^{-1}. Hence, the bands of medium intensity are observed at a frequency of 2866 cm^{-1} and 2277cm^{-1} are assigned to anti-symmetrical and symmetrical stretching vibrations respectively. The bands observed at 1452 cm^{-1} and 1358 cm^{-1} are attributed to unsymmetrical and symmetrical bending vibrations of C – H group respectively. It is possible for us to assign tentatively a band at 1358 cm^{-} may due to Terminal dimethyl bending vibration, since terminal dimethyl group of steroids absorb at around 1374-1360 cm^{-1}[10].

N – H Stretching Vibrations and its Bending Vibrations

The frequency of the N – H stretching is reduced by hydrogen bonding. Overlapping occurs in the observed position of N – H and O – H stretching frequency so that an unequivocal differentiations in structures is sometimes impossible. Dilute solutions in non polar solvents, amides show two moderately intense N – H stretching frequencies, corresponding to anti-symmetrical and symmetrical N – H stretching vibrations. These bands occur near 3520 cm^{-1} and 3400 cm^{-1} respectively. But in the spectra of solid samples, these bands are observed near 3450 cm^{-1} and 3180 cm^{-1} because of hydrogen bonding[11]. In

Table I

Fourier transform spectrum and vibrational frequencies of tetracycline

Infrared Frequency (cm^{-1})	Intensity*	Description
501	VVW	C – C out-of-plane bending
567	M-W	C – C out-of-plane being
641	M-W	C – C in-plane bending/aromatic C – H out-of-plane bending
678	M	Aromatic C – H out-of-plane bending
744	M	Aromatic C – H out-of-plane bending/C – C stretching/C-C stretching of breath type
771	M-W	C – C stretching/aromatic C – H out-of-plane bending/C-C stretching/C – C stretching of breath type
839	M-W	C – N stretching
950	M	C – N stretching
1002	M-S	C-O stretching/C – H in plane bending
1037	S	C – O stretching/C-N stretching/C – H in plane bending
1112	S	C – C stretching/C – N stretching/C – H in plane bending
1137	M	C – C stretching
1178	S	C – N stretching/C – C stretching
1234	S	C-N stretching/C – C stretching
1358	VS	C – O stretching/symmetric CH_3 bending/Terminal gem dimethyl bending
1452	VS	Unsymmetrical CH_3 bending/ring C=C stretching
1527	VS	NH in plane bending (Sci) ring C=C stretching
1552	VS	ring C=C stretching
1584	VS	Ring C=C stretching/C = O stretching
1618	VS	Ring C=C stretching/C = O stretching
1666	VS	Ring C=C stretching/C = O stretching
1900	VVW	2 × 950
2533	W	3550-1002
2674	M	C – H stretching of Methyl group
2776	M	C – H stretching of Methyl group
2866	M	Symmetrical stretching of Methyl group
2916	M	Anti-symmetrical stretching of Methyl group
3000	M	Aromatic C – H stretching
3049	S	Symmetric N – H stretching/aromatic C – H stretching
3108	VS	Anti-symmetric N – H stretching
3312	VS	Associated hydroxyl group absorption
3383	VS	Associated hydroxyl absorption
3550	M	Associated hydroxyl absorption

VS:- Very Stong; S:- Strong; M:-Medium; W:- Weak; VVW:- Very Very Weak

the FTIR spectrum of Tetracycline, O – H stretching vibrations occur at the regions of N – H stretching vibration, makes interpretation very difficult. In line with the reference[11] there might be a possibility that N – H stretching vibrations are shifted, being a solid sample. Hence bands at 3049 cm^{-1} and 3108 cm^{-1} are assigned to symmetrical N – H stretching vibrations and in the spectrum of Tetracycline the N – H band has been augmented by O – H bands in the region of 3550 cm^{-1} to 3313 cm^{-1}. Two bands of equal intensity and a band energy having medium intensity have been assigned to OH stretching vibrations.

(B) AMPICILLIN

Methyl Group Vibrations

Methyl group has three stretching vibrations. However, only two absorption bands are normally observed in spectra. In practice, there are two asymmetric vibrations but they cannot be distinguished and so the methyl group is commonly observed to have two C – H stretching absorption bands at 2962 cm^{-1} and 2872 cm^{-1}. In the spectrum of Ampicillin two anti-symmetric stretching vibrations of C – H are observed at 2969 cm^{-1} and 2933 cm^{-1}. The band observed at 2733 cm^{-1} is due symmetric stretching of methyl group.

Most reliable absorption bands below 1500 cm^{-1} are deformations. Methyl group has two deformations, the symmetrical (~ 1380) and the anti-symmetrical (~ 1450). The bands observed at 1496 cm^{-1} and 1457 cm^{-1} are assigned to anti-symmetrical bending of CH group and the bands observed at 1373 cm^{-1}, 1389 cm^{-1} (doublet) and 1334 cm^{-1} are assigned to symmetrical bending of C – H group. The doublet at around 1373 cm^{-1} may be due to gem-dimethyl group, since these gem-dimethyl group show characteristic doublet near 1375 cm^{-1} [12-15].

Carbonyl Vibrations

The carbonyl group is important in organic chemistry and its characteristic frequency has been extensively studied in a wide range of compounds. It is necessary to exercise some caution since, we have assumed a very simple picture of the carbonyl vibration. The carbonyl vibration is not located entirely within the carbonyl bond but involves some of the other atoms in the molecule. Changes in the composition and structures on the rest of the molecule will therefore exert a mechanical influence on the carbonyl frequency, which will vary from one molecule to another molecule. In the case of cyclic molecule on the other hand, it is this mechanical effect which is primarily responsible for the observed increase in the carbonyl frequency as the ring size decreases below six atoms. The carbonyl group in the four membered ring will have a strong absorption at around 1775 cm^{-1}. Hence, a band observed 1774 cm^{-1} is assigned to carbonyl vibrations[14]. Two strong bands at 1688 cm^{-1} and 1607 cm^{-1} are assigned to carbonyl vibrations of carboxylic group (COO^-) and peptite respectively.

Vibrations of Carboxylic Acids of Heterocyclic

The most diagnostic feature of a carboxylic acid spectrum is a very broad absorption frequency extending from 2500 cm^{-1} to 3300 cm^{-1}. The factor

Table II

Fourier transform infrared spectrum and vibrational frequencies of Ampicillin

Infrared Frequency (cm^{-1})	Intensity*	Description
450	VVW	C-C out-of-plane bending
500	VVW	C-C out-of-plane bending
589	W	C-C out-of-plane bending
646	W	S-C stretching/N – H wagging
697	M-W	Symmetric S – C stretching/N – H wagging
711	M-W	Anti-symmetric S – C stretching/C – C stretching/N – H wagging/C – C stretching breath type
736	M-W	Anti-symmetric S – C stretching/C – C stretching/N-H wagging/C – C stretching breath type/C – H out of plane bending
800	W	C – C stretching/N-H wagging/C – H out plane bending
850	VVW	C-C stretching C – N stretching
883	W	C-C stretching/C – OH bending
929	W	C-C stretching/C-OH bending
983	VVW	C-C stretching
1020	VVW	C – C stretching/C-H in plane bending
1078	VVW	C – C stretching/C – H in plane bending
1118	VVW	C-C stretching
1169	VVW	C – N stretching/C – H in plane bending
1217	W	C – H in plane bending/C – N stretching/C – O stretching
1263	W	C – O stretching/Interaction band C – N – H
1307	VVW	C – O stretching
1334	M	C – O stretching
1389	M	Symmetric bending of gem dimethyl group/C – OH in plane bending/C – N stretching of peptide
1373	M-S	Symmetric bending of gem dimethyl group/C – OH in plane bending/C – N stretching of peptide
1457	S	Unsymmetrical bending of CH_3 group/C=C ring stretching/C – N stretching of pepide
1496	S	Unsymmetrical bending of CH_3 group/C=C ring stretching
1574	VS	N – H in plane bending/Interaction band C – N – H/C – C ring stretching
1607	S	Carbonyl absorption of peptide
1688	VS	Carbonyl absorption of COO^- group
1774	VS	Carbonyl absorption of Four mem. ring
1900	VS	1217 + 697
1966	VVW	1217 + 736
2083	VVW	1169 + 929
2250	VVW	2 × 1118
2333	VVW	2 × 1169

*VS = Very Strong; S = Strong; M = Medium; W = Weak; VVW = Very Very Weak

(*Table II Contd.*)

(*Contn. of Table II*)

Infrared Frequency (cm^{-1})	Intensity*	Description
2350	VVW	1118 + 1217
2650	M-W	Characteristic absorption of Carboxylic acids
2733	M	Symmetric stretching of CH_3 group
2933	S	Anti-symmetric stretching of CH_3 groups
2969	S	Anti-symmetric stretching of CH_3 groups
3033	S	Aromatic C – H stretching
3216	S	N – H symmetric stretching
3447	S	N-H anti-symmetric stretching
3500	S	O – H stretching

*VS:- Very Strong; S:- Strong; M:- Medium; W:- Weak; VVW:- Very Very Weak

which is responsible for the diffuse shape and relatively low frequency is hydrogen bonding[14]. This important characteristic features are observed over a region of 3250-2750cm^{-1}. The important recognizable absorption of carboxylic acids are (*i*) C – O stretch (*ii*) C – O – H in-plane-bend (*iii*) C – O – H out-of-plane bend (*iv*) C = O stretching.

Two bands arising from C – O stretching and C – O – H in-plane-bending appear in the spectra of carboxylic acids near 1320-1210 cm^{-1} and near 1440-1395 cm^{-1} respectively. Both of these bands involve some interaction between C – O stretching and in-plane C – O – H bending[11]. The bands observed at 1217 cm^{-1}, 1263 cm^{-1}, 1307 cm^{-1} and 1334 cm^{-1} are assigned to C – O stretching vibration of carboxylic group. The bands observed at 1373 cm^{-1} and 1389 cm^{-1} are assigned to C – OH in plane bending vibration. A strong band at 1688 cm is due to carbonyl absorption of carboxylic group in the heterocyclic (five member) nucleus.

Heterocyclic Vibrations

Usually heterocyclic compounds may be five-membered ring or six-membered ring, containing hetero atoms like oxygen, nitrogen and sulphur. In the synthetic heterocyclic compound, Ampicillin, S – C stretching vibrations are observed in the region of 650-750 cm^{-1}[15]. Hence, the bands of medium to weak intensity at 646, 697, 711 and 736 cm^{-1} are assigned to heterocyclic S – C stretching vibration[15]. The other heterocyclic vibrations are C – C stretching vibrations. These vibrations are presented in the Table II.

Aromatic Ring Vibrations

The C-H stretching vibration of most of the aromatic compounds are found in the region of 3080-3010 cm^{-1}[1-2]. A strong band observed at 3033 cm^{-1} is assigned to C – H stretching vibrations of the phenyl ring. The bands in the region 1625-1530 cm^{-1} are may be due ring carbon-carbon stretching vibration[1-2]. Generally, the bands due to ring carbon-carbon stretching vibration having strong to medium intensity are observed at 1625-1590 cm^{-1}, 1590-1575

cm^{-1}, 1525-1170 cm^{-1} and 1465-1430 cm^{-1}. Hence the bands observed at 1574 cm^{-1}, 1496 cm^{-1} and 1457 cm^{-1} are attributed to C – C ring stretching. Remaining bending vibrations of the aromatic ring and O – H stretching are presented in the Table II.

Conclusion

Thus a complete vibrational band assignment has been made available for the compounds of pharmaceutical importance viz., Tetracycline and Ampicillin through infrared spectroscopy. The results are briefly discussed with respect to their relative magnitudes and intensity of the specific modes of vibrations. The vibrational investigations available in the present study can be used for the qualitative analysis of these compounds.

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