

Investigation of the Electronic Structure of Catecholamine Neurotransmitters. III. Quantitative Analysis of the FT-IR Spectra

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HIGH resolution FT-IR spectra of some catecholamine neurotransmitters namely, dopamine, norepinephrine and epinephrine are reported and compared to that of the parent compound, catechol. The spectra are presented and analyzed in a comparative study aiming at the understanding of intramolecular interactions that exist in this class of compounds. Substituent effects are analyzed, and discussed. Quantitative assignments and interpretation of the type and extent of interaction are facilitated via *ab initio* MO vibrational computation.

Catecholamines⁽¹⁾ are neurotransmitters⁽²⁾ that have been found in many human tissues⁽³⁾. Their action is controlled by the central nervous system in a manner that seems critical to mankind^(4,5). Several reviews exist that summarize the physiological⁽⁶⁾ and the biochemical⁽⁷⁾ characterization of catecholamine neurotransmitters⁽⁸⁻¹³⁾. Although the IR- spectra of catecholamines were reported⁽¹⁴⁾ long time ago yet these spectra are of low resolution and are in general ill-interpreted⁽¹⁴⁾. In most cases, the reported IR-spectra are qualitatively presented and discussed.

In the present work the high resolution IR spectra of the studied catecholamines are presented and analyzed in a comparative study aiming at the understanding of intramolecular interactions that exist in this class of compounds. Analyzing substituent effects on apparent skeletal structure, which has been taken to be catechol, can trace structural similarities and differences. The strategy that has been followed in the present analysis is to compare the

band positions and intensities of some selected functional groups, in the studied catecholamines, to those of catechol. Quantitative assignments and interpretation of the type and extent of interaction are facilitated via *ab initio* MO vibrational computation.

Experimental

All compounds studied in the present work are of analytical grade and were used without further purification.

The high resolution FT-IR spectra were all measured for a 0.5 % KBr discs using a high resolution Shimadzu FT-IR 8201 PC instrument.

Method of calculation

All quantum mechanical vibrational analysis carried out in the present work were performed at the *ab initio* LCAO-MO level of theory⁽¹⁵⁾ using a minimal STO-3G basis set⁽¹⁶⁾. The G94w program was used through out the present work⁽¹⁷⁾. Equilibrium geometries and other ground state properties at this level of theory were extracted from the work of Hilal *et al*⁽¹⁸⁾.

Results and Discussion

The electronic interactions that we are concerned with may be considered as substituent electronic effect on the system concerned. In particular, we will focus our attention on the type and extent of intramolecular interactions that may exist between the two polar groups that characterize catecholamines, namely the -OH and -NH₂ groups. Such substituent effect can be transmitted by several distinct mechanisms⁽¹⁹⁾, the most important of which are:

- 1) I_{σ} effect: the induction effect of polar group transmitted by diminishing relay along a system of σ -bond
- 2) The F effect: the field effect of a polar group transmitted through space to a site where it affects a measurement.
- 3) The resonance effect R: which results from the interaction of substituent

orbital of suitable symmetry with the π orbitals of the unsaturated nucleus and can lead to charge transfer either to or from the substituent.

- 4) The π orbital effect: the repulsion of the filled π orbitals of the nucleus caused by substituent induced π^* mixing; no CT is induced.
- 5) π_F and π_σ effects: the disturbance of the π system of the nucleus by the 1_σ and F effect of the substituent.

1- FT-IR spectrum of catechol

Figure 1 presents a high resolution FT-IR spectrum of catechol measured for a KBr pellet of 0.5 % catechol. The spectrum shows 35 peaks all are well resolved. This spectrum has been well characterized and all bands have been assigned. In the present discussion we will restrict ourselves to some finger print absorptions that will be traced in all catecholamines. These vibrations and their assignments are given in Table 1.

The O-H region of the spectrum shows two strong absorptions at 3450 and 3325 cm^{-1} . The former is sharp and more intense. These absorptions are assigned to O-H stretching vibrations which has been shifted by about 300 cm^{-1} from their standard values. This shift implies hydrogen bonding. Theoretically, ab initio STO-3G vibrational analysis has predicted two bands at 3736 and 3723 cm^{-1} . It is apparent that the theoretical model predicts the strong coupling between the O-H stretching vibrations satisfactorily yet, it is not able to detect H-bonding in catechol. However, the intensity ratio of the two peaks is predicted correctly (~ 1.3). The aromatic C-H vibrations appear as a complex structure in the 3051- 2723 cm^{-1} region with moderate intensity. Theoretically, one single absorption at 3700 cm^{-1} accounts for the ring C-H stretching in poor agreement with the experimental observation. Nevertheless, the O-H bending and the C=O stretching modes are strongly coupled and responsible for the absorption in the 1400 - 1280 cm^{-1} range in much better agreement with the experimentally observed absorption.

In general, two strong bands can be directly associated with the phenolic group. These are the $\nu_{\text{C-O}}$ and a deformation of the COH, viz., $\nu_{(\text{OH})}$. It was suggested²⁰ that these modes interact and result in a strong absorption profile in the 1350 - 1200 cm^{-1} range. However, analysis of the spectra of anisols led to

the conclusion that it is better to assign the 1350 cm^{-1} band to the ν_{OH} while the one at 1200 cm^{-1} may be safely assigned to the $\nu_{\text{C-O}}$. This last assignment has been adopted throughout our analysis of the spectra of catecholamines.

2- FT-IR spectra of catecholamine hormones

Figure 2 presents the high-resolution FT-IR spectrum of dopamine measured for KBr pellet of 0.5 % dopamine. The broad strong absorption profile in $3350 - 3100\text{ cm}^{-1}$ region is due to N-H and O-H stretching vibrations. The $\nu_{\text{N-H}}$ stretching is assigned to the 3230 cm^{-1} absorption whereas, the 3344 cm^{-1} one is due to O-H stretching. Comparing ν_{OH} in dopamine with that reported in Table 1 for catechol, indicates that ν_{OH} is shifted to lower wavenumbers by at least 100 cm^{-1} . Furthermore, the frequency of the N-H stretching (3230 cm^{-1}) is considerably low as compared to that of free N-H stretching (3500 cm^{-1}). These observations lead to the conclusion that there exists appreciable intramolecular interaction between the OH and NH_2 groups in dopamine. Here electronic and asymmetry effects play a major role in determining the extent of interaction. To investigate this point a little bit further, and exploring the nature of interaction, let us examine what happens to aromatic C-H stretching. In the case of catechol, a complex profile in the $3000 - 2800\text{ cm}^{-1}$ range account for the aromatic C-H stretch, the more intense of which is at 3051 cm^{-1} . The corresponding profile in dopamine covers almost the same range with the most intense absorption at 3041 cm^{-1} , almost at the same position as that in catechol. This might suggest that the interaction between the OH groups, in one hand, and the ethylamino group, in the other hand is a through-space interaction. That is, in dopamine the predominant interaction is an F-effect the field effect of the polar groups transmitted through space and affect the frequency of the stretching vibrations of the OH and amino groups. Table 2 presents the theoretical IR parameters computed for dopamine at the ab initio STO-3G level of theory. As expected, the STO-3G frequencies are all high as compared to those observed experimentally. This is a general trend for minimal basis sets. Nevertheless, the present calculations predicted the observed IR pattern satisfactorily. The coupled IR intensities are in much better agreement with the experimentally observed ones.

TABLE 1. Comparison between the theoretically computed and experimentally observed FTIR spectral characteristics of catechol.

Experimental		Theoretical		Assignment and Description
ν , cm^{-1}	I %T	ν , cm^{-1}	I, KM/mol	
3450	21.87	3736	11.06	O-H stretching
3325	28.62	3723	14.91	O-H stretching
3051	47.96			Aromatic C-H stretching
2979	53.34	3708	29.48	
2854	56.87			
2723	58.40			
1363	28.89	1360	57.57	$\delta(\text{OH})$
1166	32.10	1191	28.16	$\nu(\text{C=O})$
1469	34.50	1492	50.51	Aromatic $\nu(\text{C=C})$
1602	55.61	1587	84.78	

TABLE 2. Theoretically computed IR characteristics of Dopamine.

ν cm^{-1}	Intensity ^a	Force constant ^b	Assignments
1158	7.67	1.71	ν (C-O)
1261	14.61	2.08	
1291	2.58	1.86	
1365	28.47	1.71	$\delta(\text{OH})$
1374	12.11	1.51	ν (C-O)
3567	10.17	8.00	ν (N-H)
3612	3.16	8.19	ν (O-H)
3696	3.9	8.93	

^a in KM/mol ^b in m dyne/Å

Now let us turn our attention to a slightly complex case, namely, that of norepinephrine. Figure 3 presents the high-resolution FT-IR spectrum of norepinephrine measured for KBr pellets of 0.5 % norepinephrine. The fingerprint regions show the same general features as those observed in the spectrum of dopamine. Two sharp strong intensity peaks at 3265 and 3232 cm^{-1} are observed and may be attributed to the NH and OH stretching modes. A rather complex profile is observed to cover the 3000 - 2470 cm^{-1} range. This profile may solely be assigned to the aromatic and the aliphatic C-H stretching vibrations. Careful examination of this spectrum reveals the following:

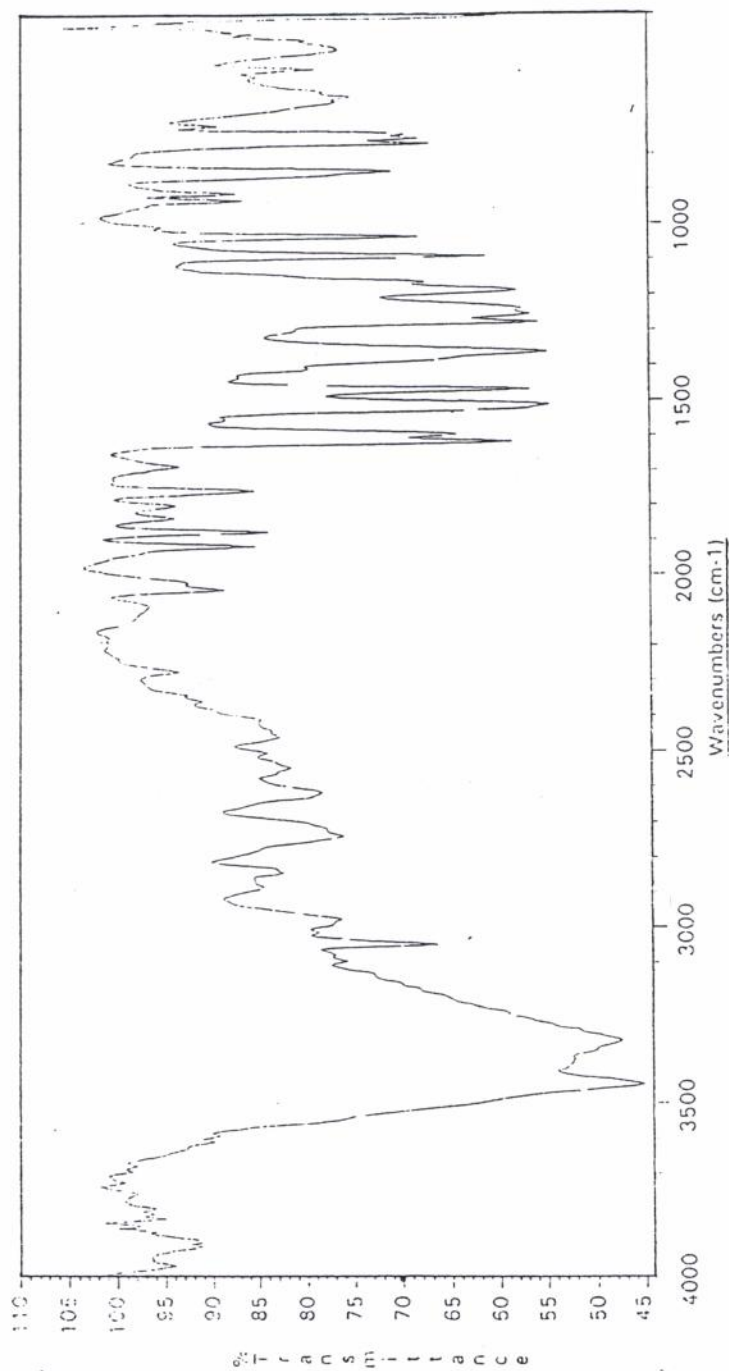


Fig. 1. FT-IR spectrum of Catechol (0.5% KBr pellet)

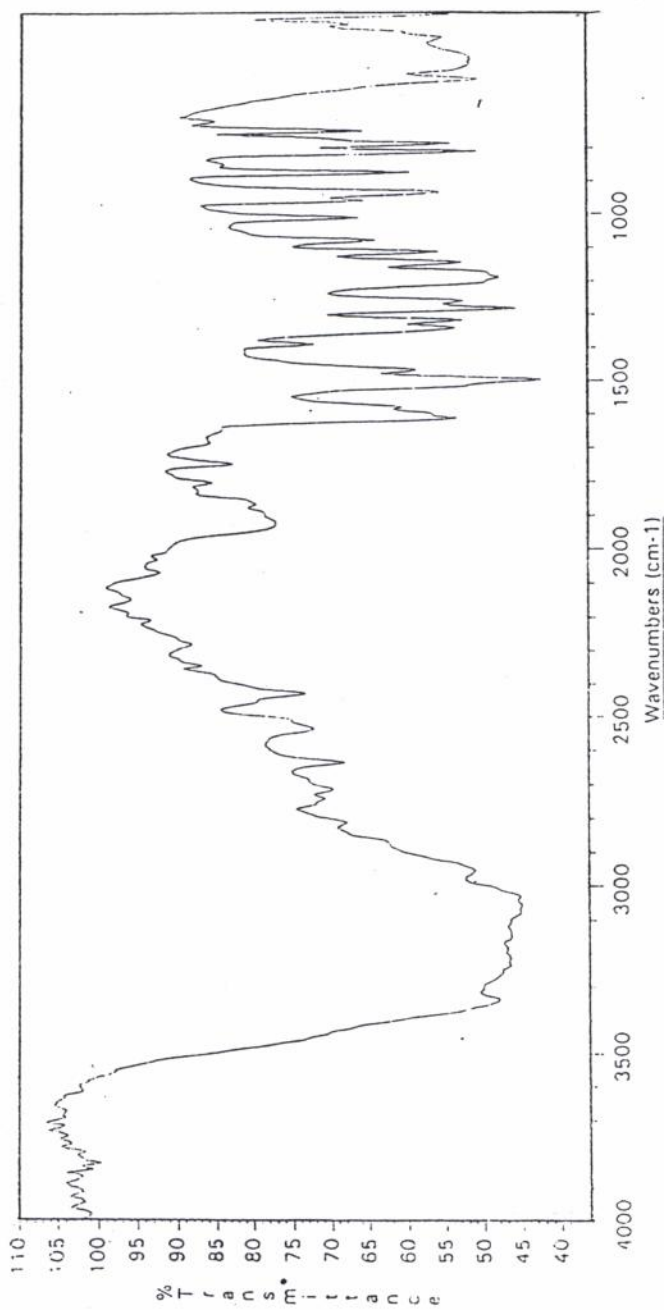


Fig. 2. FT-IR spectrum of Dopamine (0.5% KBr pellet)

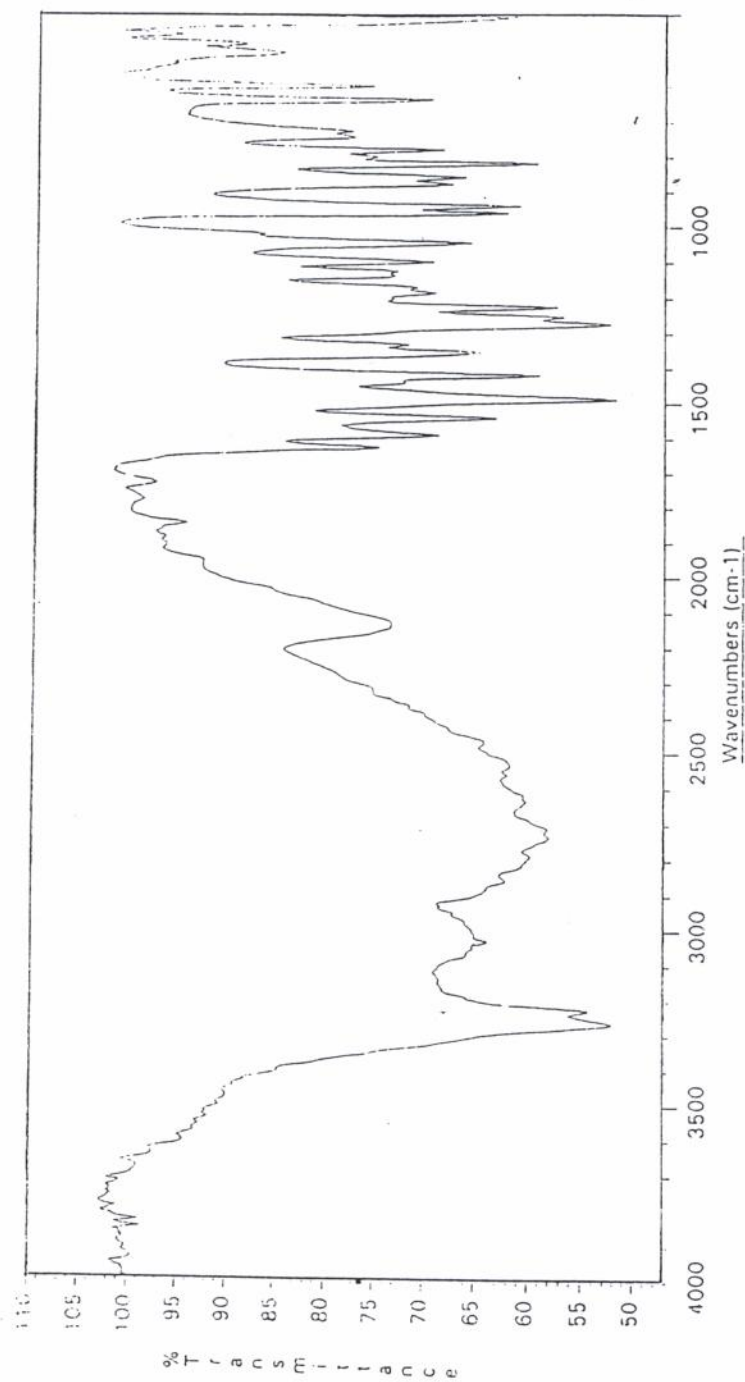


Fig. 3. FT-IR spectrum of Norepinephrine (0.5% KBr pellet)

- 1) The $\nu_{\text{N-H}}$ is little affected by the presence of the OH group in the β carbon of the ethylamino group. Thus, in dopamine this vibration appears at 3230 cm^{-1} at almost the same position as that observed in the case of norepinephrine. Furthermore, its intensity is reduced but slightly as compared to that of dopamine; the intensity ratio is 0.88.
- 2) The OH stretching vibration is shifted by 80 cm^{-1} to the short wavenumber side, and retains its intensity.
- 3) The aromatic C-H stretching profile although covers the same frequency range yet its intensity pattern is quite different as compared to that observed in the case of dopamine. Thus, the first band in this profile appears in the spectrum of dopamine at 3041 cm^{-1} with 12 % transmittance in comparison with a 3037 cm^{-1} (44 % transmittance). This reduction in the intensity is a direct consequence of the β -OH substitutions.

The above observation lead to the conclusion that F-effect is still operative between the two polar OH and NH_2 groups. The predominant interaction is a through-space transmittance of polar effect which has but negligible effect on the aromatic ring. The presence of the OH group in the ethylamino side chain affects, on the other hand, the ring vibrations. This effect is reflected in a considerable reduction in band intensity. This is through bond interaction an I_{σ} effect which is inductive in nature. The polar OH group transmits this inductive effect by diminishing relay along a framework of the aromatic ring. The presence of a C-H group prevents any conjugative interaction between the OH group and the aromatic ring and thus rules out the possibility of a resonance interaction (R). The theoretically computed IR characteristics of norepinephrine is given in Table 3 at the ab initio STO-3G level of theory. Figure 4 presents the high resolution FT-IR spectrum of epinephrine measured for a KBr pellets of 0.5% epinephrine. In the finger print region, the spectrum shows one very sharp absorption at 3329 cm^{-1} that overlap strongly with another less intense absorption which appears as a shoulder in its short wavenumber wing. These can safely be assigned to $\nu_{(\text{NH})}$ and $\nu_{(\text{OH})}$. The aromatic $\nu_{(\text{C-H})}$ profile appears as usual at 3000 cm^{-1} . The features of aliphatic C-H stretch seem different from those observed for dopamine and for norepinephrine. Thus, although $\nu_{(\text{C-H})}$ aliphatic covers almost the same range in all three catechol amines, yet the intensity pattern seems quite different; in epinephrine, this aliphatic $\nu_{(\text{C-H})}$ appears more intense and broad. This is a consequence of the I_{σ} inductive effect of the methyl group which is transmitted through the amino into ethyl σ -frame. The presence of the N-methyl group does

not only affect the aliphatic C-H stretching vibrations but also shifts $\nu_{\text{(NH)}}$ to frequencies that overlap with $\nu_{\text{(OH)}}$. The spectrum of epinephrine is therefore similar to those presented before with minor differences, which can be accounted for on the basis of the local action of the methyl group. The STO-3G theoretically computed IR characteristics of epinephrine are given in Table 4.

It is interesting to analyze the behavior of the angle bending mode $\nu_{\text{(OH)}}$ and the ring stretching $\nu_{\text{(C-O)}}$ which are highly characteristic for the phenolic structures. Table 5 presents the band positions and intensities of $\nu_{\text{(OH)}}$ and $\nu_{\text{(C-O)}}$ in all compounds studied. It is remarkable to notice that in all the recorded spectra $\nu_{\text{(OH)}}$ has a value of $1350 \pm 10 \text{ cm}^{-1}$ whereas, $\nu_{\text{(C-O)}}$ has a value of $1200 \pm 10 \text{ cm}^{-1}$. These values are typical and elaborate upon our previous conclusions concerning the type and extent of intramolecular interactions in the studied catecholamines.

In phenol a highly characteristic band near $1350 \pm 20 \text{ cm}^{-1}$ is observed and assigned to $\nu_{\text{(C=C)}}$ of the aromatic ring⁽²¹⁾. This vibration may couple to the OH deformation and therefore intensifies and shifts to higher frequencies. In substituted phenols this band may shift up to 1660 cm^{-1} . Inspection of the IR spectrum of catechol (Fig. 1) show several combination bands in the $1600\text{-}1300 \text{ cm}^{-1}$ range, two of which has been safely assigned⁽²²⁾ to $\nu_{\text{(C=C)}}$ these are the 1467 cm^{-1} (34 % T) and the 1602 cm^{-1} (55 % T) absorptions.

These assignments have been confirmed by the ab initio STO-3G calculations carried out in the present work. The relative intensity ratio of these two peaks has also been predicted nicely by the MO results (c.f. Table 1). It is surprising to notice that in all of the studied catecholamines the $\nu_{\text{(C=C)}}$ absorption appears in almost the same position, that is $1590 \pm 10 \text{ cm}^{-1}$ (35 \pm 10 % T). Furthermore, the band structure in the $1600 - 1400 \text{ cm}^{-1}$ region is almost the same in the studied compounds. Table 5 lists the band structure in this range, in the studied catecholamines together with the assignments of the characteristics of the $\nu_{\text{(C-C)}}$ in each case. The theoretically computed characteristics of the $\nu_{\text{(C-C)}}$ band structure in the studied catecholamines are gathered in Table 6. The agreement between the theoretical results and the experimental observations is rather good. In fact, this region of the spectrum has been predicted nicely by the STO-3G basis. This prediction, in fact, enabled the safe assignments for the $\nu_{\text{(C-C)}}$ in this region which is highly crowded by combinations and perhaps overtones.

TABLE 3. Theoretically computed characteristics of Norepinephrine as calculated using the STO-3G basis set*.

ν cm^{-1}	Intensity	Force constant	Assignments
1183	28.69	1.21	ν (C-O)
1273	3.65	2.49	
1291	16.25	1.62	δ (OH)
1353	11.18	2.03	
1374	9.8	1.48	
3561	12.31	8.13	ν (N-H)
3580	4.79	8.05	ν (OH)

*See footnote in table (1).

TABLE 4. Theoretically computed IR characteristics of epinephrine as calculated using the STO-3G basis set*.

ν cm^{-1}	Intensity	Force constant	Assignments
1206	10.99	1.65	ν (C-O)
1291	18.84	1.62	
1342	14.2	2.2	δ (OH)
1357	6.79	1.82	
1378	6.54	2.31	
3540	7.40	7.67	ν (N-H)
3561	12.01	8.10	ν (OH)

*See footnote in table (2).

TABLE 5. Comparison between δ (OH) and ν (C-O) in the IR spectra of the studied compounds.

Compound	δ (OH)	Intensity, % T	ν (C-O)	Intensity, % T
Catechol	1363	29	1186	32
Dopamine	1344	37	1207	26
Norepinephrine	1357	41	1207	50
Epinephrine	1350	35	1203	38

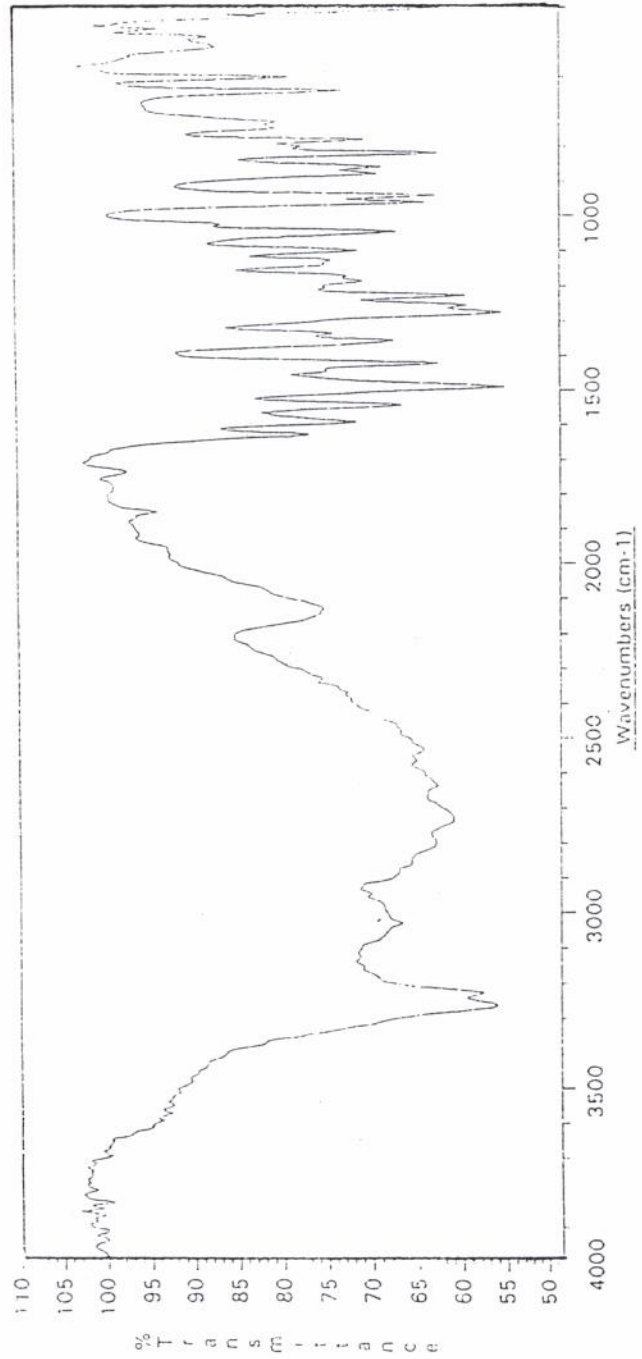


Fig. 4. FT-IR spectrum of Epinephrine (0.5% KBr pellet)

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دراسة التركيب الجزيئي للموصلات العصبية من مشتقات الكتيكول أمين ٣. تحليل كمي لطيف

FT-IR

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المملكة العربية السعودية

لقد تم قياس طيف الأشعة تحت الحمراء فى ظروف فصل عالية للموصلات العصبية من مشتقات الكتيكول أمين وبالتحديد الدوبامين والنورأبينفرين والابينفرين . وقد أجريت دراسة مقارنة مع طيف المركب الاب لهذه المجموعة وهو مركب الكتيكول. ولقد تم تحليل الطيف لهذه المركبات بهدف فهم التأثيرات المتبادلة داخل هذه المجموعة من الموصلات العصبية وكذلك دراسة تأثير مجموعات الاحلال على الطيف الاب ، وقد تم شرح وتحليل النتائج كميًا باستخدام حسابات المدارات الجزيئية للحركات الاهتزازية لهذه الجزيئات .