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# Spectrophotometric Determination of Quinolones by Charge Transfer Complexation with Chloranilic Acid: Synthesis and Characterization

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## Abstract

A simple and sensitive spectrophotometric method has been described for the assay of quinolones in bulk drug and in pharmaceutical formulations. The developed method is based on the formation of colored charge transfer complexes of quinolones with chloranilic acid in acetonitrile solvent. The formed complexes absorbed at 417, 436, 419 and 436 nm for sparfloxacin, enoxacin, norfloxacin and levofloxacin respectively. Beer's law is obeyed in the concentration range of 0.5-7, 1-10, 1-10 and 1-5 µg $mL^{-1}$ with LLOD values 0.036, 0.0041, 0.0344 and 0.0063 ng $mL^{-1}$ respectively. The data are discussed in terms of molar absorptivity, association constant and Gibb's free energy. Spectral characteristics including oscillator's strength, dipole moment, ionization potential, energy of complexes and resonance energy have been determined. Benesi-Hildebrand plots for each complex have been constructed. Structural characteristics of synthesized charge transfer complexes were determined by IR spectroscopy. The applicability of the method was demonstrated by determination of studied drugs in commercial tablets with satisfactory results. No interference from excipients was observed in the formulation.

**Keywords:** Charge transfer complexes; Quinolones; Chloranilic acid; Benesi-Hildebrand plot

## Introduction

Quinolones are broad spectrum antibiotic drugs [1,2] frequently prescribed for the treatment of wide range of bacterial infections usually caused by Gram-negative and Gram positive bacteria by inhibition of their DNA gyrase [3,4]. Structurally, all the quinolones contains carboxylic group at position 3 and carbonyl group at position 4 that is why they are usually called 4-quinolones.

A number of analytical methods have been reported in literature for the determination of quinolones in bulk drug, pharmaceutical formulation and body fluids including capillary electrophoresis [5], potential gradient detection method [6], HPLC [7], MLC method with fluorescence detection [8], fluorimetric [9] LC–MS/MS [10] and spectrophotometric methods [11]. In the past era, a number of spectrophotometric methods for the determination of verapamil [12], gabapentin [13], quinolone antibiotics [14], metformin [15], ascorbic acid [16] and montelukast [17] have been developed by our research fellows. Methods for the determination of quinolones have been developed by our research fellows earlier [12,14,18].

In the present study we aimed to describe the rapid and accurate spectrophotometric methods based on charge transfer complexes of sparfloxacin (SPAR), enoxacin (ENO), norfloxacin (NOR) and levofloxacin (LEVO) (Figure 1) with chloranilic acid (ChA). The optimum reaction conditions of the developed methods have been established, besides, the oscillator strength (f), dipole moment (µ), ionization potential ($I_p$), energy of CT complex ($E_{CT}$) and resonance energy ($R_N$) were evaluated. The association constant ($K_c$) and standard free energy changes (ΔG°) have also been determined. The solid complexes were synthesized and then characterized by IR spectroscopy.

## Experimental

### Materials

Analytical grade acetonitrile was used throughout the research. Reference standards of sparfloxacin and enoxacin were kindly gifted by Abbott laboratories (Pakistan) Ltd, norfloxacin by Hilton Pharma and levofloxacin by Aventis Pharmaceutical Laboratories Ltd. Pharmaceutical formulations Sparaxin® 100 mg, Enoxabid® 400 mg, Floxin® 400 mg and Levoxin® 250 mg were purchased from the local pharmacy (Karachi, Pakistan). Chloranilac acid was purchased from Merck Schuchardt OHG, Darmstadt, Germany. Double distilled deionized water was used throughout the work.

### Instruments

Electronic spectra of quinolones and its complexes were recorded in the region 200-800 nm using Shimadzu 1800 double beam UV–visible spectrophotometer version 2.32 software with quartz cells of 1.0 cm path length. The FT-IR spectra were obtained from KBr discs using Shimadzu Prestige-21200VEC version 1.2 software.

### Standard stock solutions

Stock solution of 100 µg $mL^{-1}$ was prepared by dissolving 10 mg pure drug in 100 mL acetonitrile. Working standard solutions were prepared by suitable dilutions of stock solution with same solvent. 0.1% ChA was prepared fresh daily in acetonitrile.

### General procedure

Into four different series of 10 mL volumetric flasks, aliquots of SPAR, ENO, NOR and LEVO were transferred to get final concentration ranges 0.5-7, 1-10, 1-10 and 1-5 µg $mL^{-1}$ respectively. To each flask 1 mL ChA was added, pink colored charge transfer complexes were immediately formed for SPAR, NOR and LEVO, whereas purple color was obtained for ENO complex at room temperature (25°C), volumes of flasks were brought to mark to get the above concentrations by acetonitrile, absorbance were measured against reagent blank

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treated similarly. Standard calibration graph was prepared by plotting absorbance of complexes against concentration of quinolones.

### Pharmaceuticals formulations

Ten tablets of each formulation were separately weighed and finely powdered, accurately weighed portion of powder equivalent to 10 mg was dissolved in acetonitrile and shaken well for proper mixing. These solutions were allowed to stand for 30 min and then sonicated for complete solubilization of drugs. Then the contents were filtered to separate the insoluble excipients and volume was completed with the same solvent to get the final concentration of 100 µg $mL^{-1}$. The procedure was continued as described under general procedure.

### Synthesis of Solid CT Complexes

Equimolar quantities of drug and ChA were dissolved in 10 ml acetonitrile and refluxed on a water bath for 1.5 hrs, reaction was continuously monitored by TLC using solvent system methanol and chloroform (9:1). When all the reactants changed into product, then collected by filtration, excess solvent was evaporated to dryness. The resultant solid material was thoroughly washed to remove the remaining traces of reactant. These materials were then dissolved and re-crystallized in acetonitrile and then characterized by UV-visible and FT-IR spectroscopy.

## Results and Discussion

### Strategy to develop and design the proposed method

The proposed method was designed to develop charge transfer complexation reaction between quinolones as donor and ChA as pi-acceptors. The absorbance of formed charge transfer complexes was measured on a UV/visible spectrophotometer. Selection of drug was based on its frequent use against serious infections caused by bacteria. The mechanism of reaction is based on the transfer of electron from electron rich donor having lone pair of electron to electron deficient pi-acceptors, which further dissociates due to high ionizing power of the polar solvent, and leads to the formation of radical ions. The proposed reaction mechanism is illustrated in Scheme 1.

### Reaction and spectral characteristics

Pink colored charge transfer complexes were immediately formed for SPAR, NOR and LEVO, whereas purple color was obtained for ENO at room temperature (25°C) in acetonitrile medium. The coloration of complexes was not associated with any of the reactants. The newly formed complexes exhibit absorption band at 417, 436, 419 and 436 nm for SPAR, ENO, NOR and LEVO respectively. The electronic absorption spectra are shown in Figure 2.

### Optimization of reaction conditions

Optimum conditions necessary for quick charge transfer complex formation were established by investigating a number of parameters and observing their effect on the absorbance of the colored product. Solvents like acetonitrile, methanol and water were tested, among them, acetonitrile was found to be the suitable solvent giving higher molar absorptivity values. Optimum reaction time was determined by monitoring the absorbance of the developed colored complex at different time intervals at ambient temperature (25 ± 5°C) for all the reagents. Complete color development was attained instantaneously for all the studied quinolones; these complexes were found to be stable for 24 hrs at -20°C.

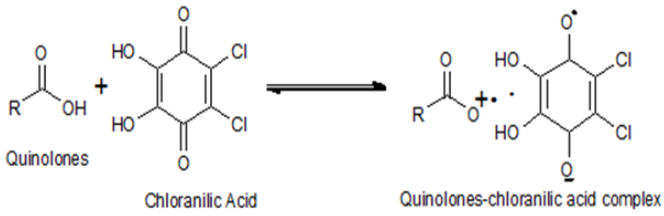
### Stoichiometric relationship

The composition of charge transfer complexes of studied quinolones with ChA were determined spectrophotometrically by applying Job's method [19] using equimolar solution which indicated that interaction of all the quinolones with ChA occurs on equimolar basis (Figure 3).

### Linearity, accuracy and precision

From the above described analytical conditions, linear calibration graph was obtained between absorbance verses concentration of quinolones. Beer's law was obeyed in the concentration range of 0.5-7, 1-10, 1-10 and 1-5 µg $mL^{-1}$ for SPAR, ENO, NOR and LEVO respectively with correlation coefficient greater than 0.998 in each case. Regression characteristics including slope, intercept, correlation coefficient and also the molar absorptivity values for each drug are given in Table 1. Lower limit of detection and quantitation were determined to establish the sensitivity of the method, LLOD values were calculated to be 0.036, 0.0041, 0.0344 and 0.0063 ng $mL^{-1}$ respectively.

Reproducibility of the method was measured for a series of six




**Scheme 1:** Schematic diagram of reaction of quinolones with ChA.

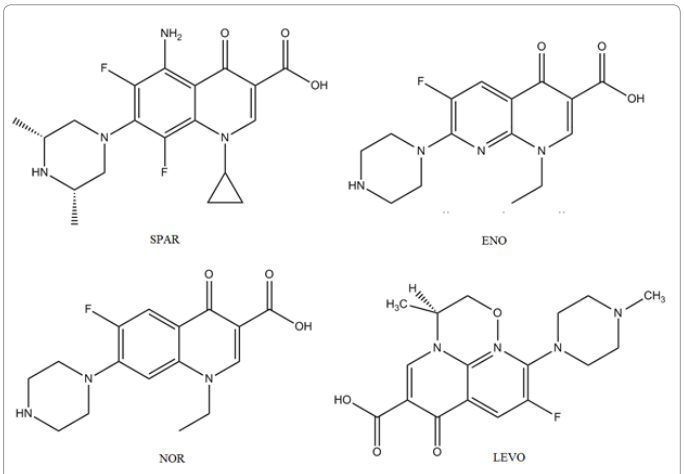



**Figure 1:** Chemical structure.

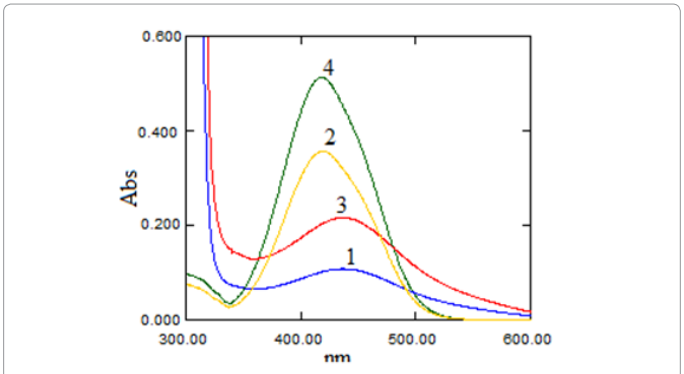



**Figure 2:** UV spectra of SPAR[1], ENO[2], NOR[3] and LEVO[4].

determinations at five concentration levels, data of percent relative standard deviation obtained for each drug are reported in Table 2. The %RSD values in the range of 0.04-0.85, 0.23-1.80, 0.27-0.71 and 0.19-0.39 confirm the sensitivity of method. Accuracy of method was ascertained by analyzing three replicates of studied quinolones at different concentration levels in its pharmaceutical formulation. Satisfactory recovery data was obtained in the range of 99.2-100.0, 99.4-100.4, 99.2-99.6 and 99.7-100.1%, respectively. The results of % recovery values and % error are given in Table 3.

## Application of the proposed method

The proposed method was applied successfully on commercial tablets of SPAR, ENO, NOR and LEVO, the results obtained are reported in Table 3 which showed good percent recovery within the limit indicating the accuracy and precision of the method. Molar absorptivity, correlation coefficient, detection limit and variance speak of good sensitivity of the proposed method. Therefore, it is concluded that the proposed method is free from constant error independent of the quinolones concentration.

## Interference from excipients

A systemic study was performed to determine the effect of inactive ingredients commonly used in pharmaceutical formulations by scanning blank solution containing 10 mg of pure quinolones and also the placebo solutions prepared by separately mixing 10 mg of quinolones with pyrrolidone (10 mg), lactose (10 mg), talc (20 mg), magnesium stearate (15 mg) and starch (10 mg) in 100 mL volumetric flask. The percent recovery values given in Table 4 indicate that there is no effect of common excipients present in pharmaceutical formulations.

## Determination of oscillator strength (f) and transition dipole moment (μ)

Experimental oscillator strength (*f*) and transition dipole moment (μ) is calculated from CT spectra making use of equation (1) and (2) [20,21].

$$f = (4.319\times10\text{-}9)\ \varepsilon_{max}.\nu_{1/2} \quad (1)$$

$$\mu = 0.0958\ (\varepsilon_{max}.\nu_{1/2}/\nu_{max})^{1/2} \quad (2)$$

where $\varepsilon_{max}$ is the molar extinction coefficient at maximum absorbance, $\nu_{1/2}$ is the band-width at half absorbance in $cm^{-1}$ and $\nu_{max}$ is wave number in $cm^{-1}$. The calculated values are reported in Table 5.

## Determination of ionization potential (Ip) of free donor

The ionization potential (Ip) of free donor was calculated by applying the relationship given in equation (3) [22]

$$I_P = 5.76 + 1.53 \times 10\text{-}4\ \nu_{CT} \quad (3)$$

where $\nu_{CT}$ is the wave number in $cm^{-1}$ corresponding to the charge transfer band of complex formed between donor and acceptor. The values thus determined are given in Table 5.

## Determination of resonance energy ($R_N$) and energy of charge transfer complex ($E_{CT}$)

The resonance energy of charge transfer complex in the ground state is determined by Brieglab and Czekalla given below [23]:

$$\varepsilon_{max} = 7.7 \times 10^{-4}/\ [h\nu_{CT}/\ R_N\text{-}3.5] \quad (4)$$

The energy of charge transfer complexes was calculated using the following equation (4) [21]:

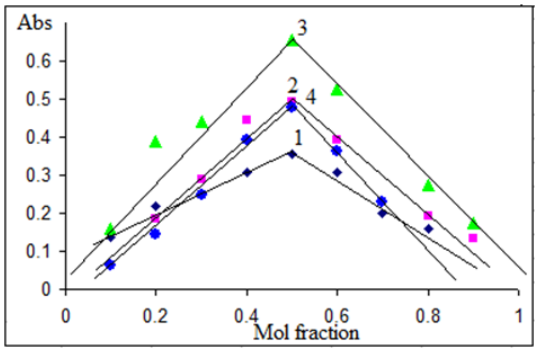



**Figure 3:** Job plot for charge transfer complexes of SPAR[1], ENO[2], NOR[3] and LEVO[4] with ChA.

| Parameters | SPAR | ENO | NOR | LEVO |
|---|---|---|---|---|
| $\lambda_{max}$ (nm) | 417 | 436 | 419 | 436 |
| Linearity range μgmL$^{-1}$ | 0.5-7 | 1-10 | 1-10 | 1-5 |
| Molar absorptivity | 5.51 x10$^6$ | 3.33 x 10$^6$ | 4.18 x 10$^6$ | 5.18 x 10$^6$ |
| Slope | 0.148 | 0.114 | 0.142 | 0.149 |
| Intercept | -0.0385 | -0.0392 | -0.0418 | -0.0153 |
| Correlation coefficient | 0.9985 | 0.9986 | 0.9989 | 0.9994 |
| LLOD ngmL$^{-1}$ | 0.036 | 0.0041 | 0.0344 | 0.0063 |
| LLOQ μgmL$^{-1}$ | 0.11 | 0.0124 | 0.1043 | 0.019 |

**Table 1:** Optimum conditions and analytical parameters.

| SPAR | | ENO | | NOR | | LEVO | |
|---|---|---|---|---|---|---|---|
| Conc | %RSD | Conc | %RSD | Conc | %RSD | Conc | %RSD |
| 2 | 0.04 | 1 | 1.80 | 1.50 | 0.27 | 1.0 | 0.26 |
| 3 | 0.26 | 2 | 0.83 | 2.25 | 0.71 | 1.5 | 0.39 |
| 4 | 0.05 | 4 | 0.53 | 3.00 | 0.39 | 2.0 | 0.22 |
| 5 | 0.65 | 6 | 0.23 | 3.75 | 0.54 | 2.5 | 0.26 |
| 6 | 0.85 | 7 | 0.23 | 5.00 | 0.57 | 3.0 | 0.19 |

**Table 2:** Precision of method.

| Sparaxin | | Enoxabid | | Floxin | | Levoxin | |
|---|---|---|---|---|---|---|---|
| % Rec | % Err | % Rec | % Err | % Rec | % Err | % Rec | % Err |
| 100.0 | 0.041 | 100.4 | 0.157 | 99.2 | 0.893 | 100.0 | 0.041 |
| 99.9 | -0.025 | 99.7 | -0.034 | 99.6 | 0.312 | 100.0 | 0.144 |
| 99.9 | 0.052 | 99.4 | 0.681 | 99.4 | 0.811 | 99.7 | 0.361 |
| 99.3 | 0.893 | 99.8 | 0.365 | 99.4 | 0.638 | 99.9 | 0.047 |
| 99.2 | 0.642 | 100.0 | 0.280 | 99.5 | 0.502 | 100.1 | -0.282 |

**Table 3:** Accuracy of method.

| Excipients | % Recovery | | | |
|---|---|---|---|---|
| | SPAR | ENO | NOR | LEVO |
| Pyrrolidone | 99.72 | 100.24 | 99.75 | 99.96 |
| Lactose | 99.38 | 99.40 | 100.91 | 100.02 |
| Talc | 100.81 | 99.32 | 99.42 | 100.77 |
| Magnesium stearate | 99.57 | 101.06 | 100.37 | 99.11 |
| Starch | 98.26 | 99.64 | 99.31 | 98.17 |

**Table 4:** Recovery of quinolones in presence of different excipients.

| Complex | f x 10$^2$ | μ | Ip | $E_{CT}$ | $R_N$ | Kc x10$^2$ (lit/mol) | ΔG° (KCal) |
|---|---|---|---|---|---|---|---|
| SPAR | 30.13 | 516.65 | 9.43 | 2.98 | 0.85 | 2.09 | 4.52 |
| ENO | 16.72 | 393.62 | 9.27 | 2.85 | 0.81 | 2.15 | 4.54 |
| NOR | 13.37 | 345.06 | 9.41 | 2.97 | 0.85 | 2.07 | 4.52 |
| LEVO | 16.21 | 387.56 | 9.27 | 2.85 | 0.81 | 2.06 | 4.51 |

**Table 5:** Spectrophotometric results.

$$E_{CT} = 1243.667/\lambda_{CT} \quad (5)$$

where $\lambda_{CT}$ is the wavelength of CT band.

### Determination of association constants and standard free energy changes

More detailed examination was made for newly formed complexes, by applying Benesi-Hildebrand plot [24], absorbance was measured on cells with optimum 1 cm path length. Values of formation constants (Table 5) are calculated by using equation 1. The concentration of donor $[D_o]$ was varied and concentration of acceptor $[A_o]$ was kept constant.

$$[A_o]/A = 1/K\,[D_o].\,\varepsilon + 1/\varepsilon \quad (6)$$

where, K is the association constant, A is absorbance, ε is molar extinction coefficient and $[A_o]$ and $[D_o]$ are the initial concentrations of the acceptor and donor respectively ($[A_o] >> [D_o]$). In both cases, sharp straight lines were obtained on plotting the values of $1/D_o$ versus $A_o/A$, as shown in Figure 4. The data obtained throughout this calculation is given in Table 6.

The standard free energy changes (ΔG°) associated with quinolones charge transfer complexation reactions were calculated from the association constants by applying equation (2) [25], values of ΔG° for each complex are given in Table 5.

$$\Delta G° = -2.303RT \log K_C \quad (7)$$

where, ΔG° is the free energy change of the complex in KJ $mol^{-1}$, R is the gas constant (1.987 cal $mol^{-1}$ $deg^{-1}$), T is temperature in Kelvin and $K_c$ is the association constant of drug-acceptor complexes.

### Spectral studies

IR spectra of free donor and formed complexes were recorded using KBr discs to determine the structure of complexes which showed disappearance of broad hydroxyl band in the formed charge transfer complexes of quinolones supporting the conclusion that the interaction has occurred at –carboxylic acid group. Figure 5 represents the IR spectra all the studied quinolones.

## Conclusion

Aim of present study was to develop simple and economic method for the determination of quinolones in bulk drug and pharmaceutical formulations. Methodology involved charge transfer complex formation of drugs with ChA at room temperature in acetonitrile solvent. Linear calibration curves were obtained with correlation coefficient greater than 0.998. Spectral characteristics including oscillator's strength, dipole moment, ionization potential, energy of complexes, resonance energy, association constant and Gibb's free energy changes have been determined. Benesi-Hildebrand plots for each complex have been constructed. Further, solid charge transfer complexes of quinolones were synthesized and characterized by IR spectroscopy.

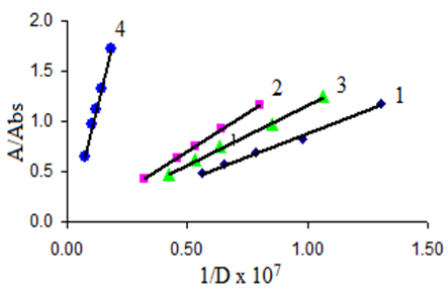



**Figure 4:** BH plot for ChA complexes with SPAR[1], ENO[2], NOR[3] and LEVO[4].

| Complex | D (M) x $10^{-7}$ | A (M) x $10^{4}$ | Abs | 1/D x $10^{7}$ | A/Abs x $10^{-3}$ |
|---|---|---|---|---|---|
| SPAR | 0.13 | 4.78 | 0.0706 | 7.84 | 6.78 |
| | 0.77 | 4.78 | 0.4081 | 1.31 | 1.17 |
| | 1.02 | 4.78 | 0.5762 | 0.98 | 0.83 |
| | 1.28 | 4.78 | 0.6943 | 0.78 | 0.69 |
| | 1.53 | 4.78 | 0.8410 | 0.65 | 0.57 |
| ENO | 1.25 | 4.78 | 0.4113 | 0.80 | 1.16 |
| | 1.56 | 4.78 | 0.5203 | 0.64 | 0.92 |
| | 1.88 | 4.78 | 0.6434 | 0.53 | 0.74 |
| | 2.19 | 4.78 | 0.7550 | 0.46 | 0.63 |
| | 3.13 | 4.78 | 1.1199 | 0.32 | 0.43 |
| NOR | 0.47 | 4.78 | 0.1834 | 2.13 | 2.61 |
| | 0.94 | 4.78 | 0.3848 | 1.06 | 1.24 |
| | 1.18 | 4.78 | 0.4931 | 0.85 | 0.97 |
| | 1.57 | 4.78 | 0.6428 | 0.64 | 0.74 |
| | 1.88 | 4.78 | 0.7764 | 0.53 | 0.62 |
| LEVO | 5.54 | 4.78 | 0.2779 | 0.18 | 1.72 |
| | 6.93 | 4.78 | 0.3605 | 0.14 | 1.33 |
| | 8.31 | 4.78 | 0.4301 | 0.12 | 1.11 |
| | 9.70 | 4.78 | 0.4956 | 0.10 | 0.97 |
| | 13.85 | 4.78 | 0.7321 | 0.07 | 0.65 |

**Table 6:** The values of $[A_o]$/Abs and 1/ $[D_o]$ for ibuprofen complexes.

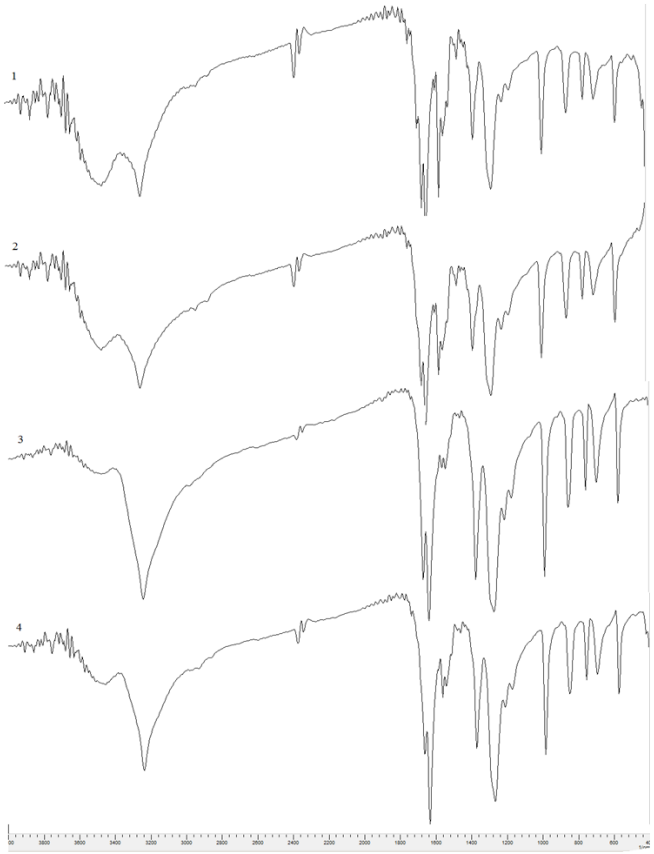



**Figure 5:** IR spectra of ChA complexes with SPAR[1], ENO[2], NOR[3] and LEVO[4].

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