3. Results and Discussion

3. 1. Determination of the studied drugs by complex formation with acid dye.

3.1.1.Absorption spectra of the studied drugs with Calconcarboxylic acid (Calc.):-

In order to investigate the optimum conditions for the development of the ion-pair complex formed between the studied drugs and (5.0×10^{-3} M) acid dye Calconcarboxylic acid, some parameters were studied and recorded below.

3.1.1.1. Effect of pH

In order to establish the optimum pH value for each ion-pair formed, Nor., Cipro. and Oflo. was allowed to react with the Calconcarboxylic acid in aqueous buffered solution of in the pH ranges (1.8 -12.0). The absorbance intensity was measured at its λ_{max}.

The highest absorbance values were obtained at pH 4.28 in case of the three drugs, which are selected for ion-pairs formation. These results are shown in Fig. (1). Furthermore, the amount of buffer added was examined and found to be 10 ml for all as shown in Fig. (2). The λ_{max} corresponding to each ion-pair complex of the drugs with Calconcarboxylic acid are 619, 622 and 621 nm in case of Nor., Cipro. and Oflo., respectively, as shown in Fig. (3).

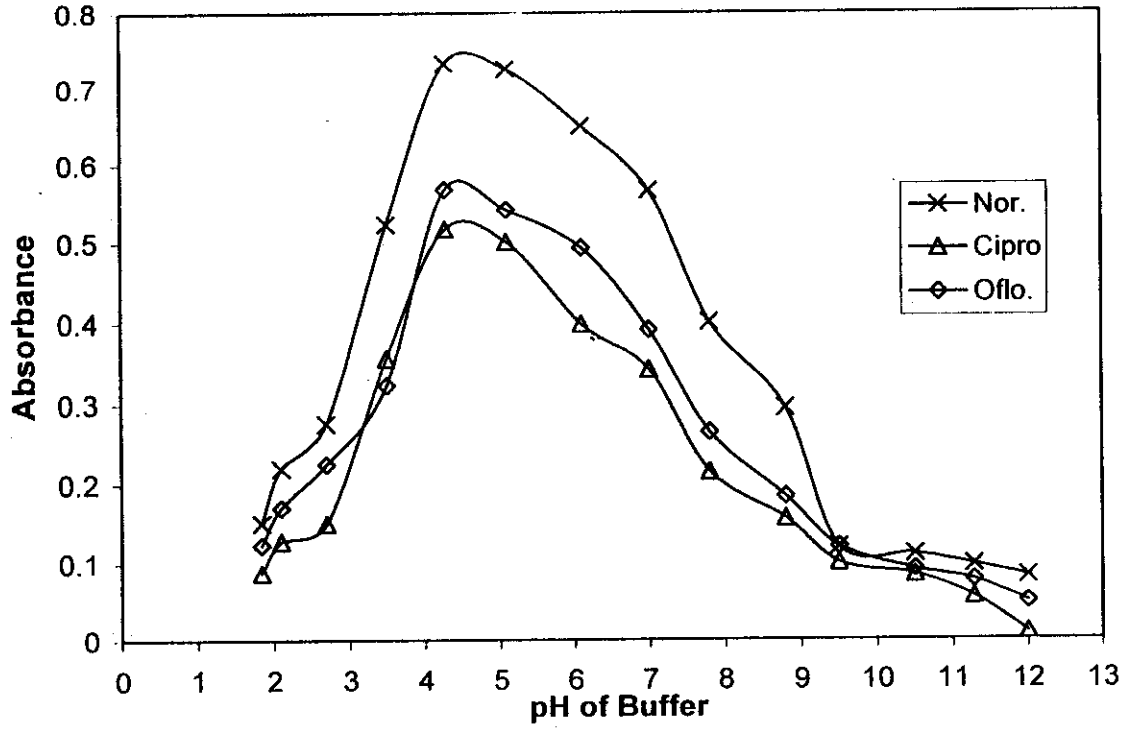

Fig.(1): Effect of pH on the absorption of the studied drugs using Calconcarboxylic acid($5x10^{-3}$M)

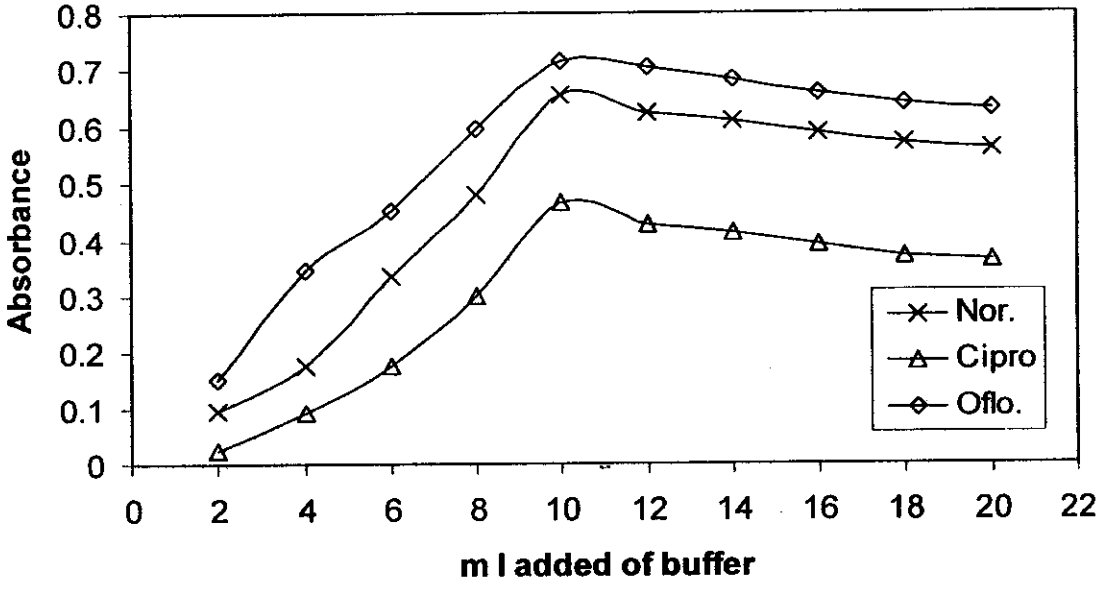

Fig.(2): Effect of ml added of buffer on the absorbance of the studied drugs using Calconcarboxylic acid. (5.0×10^{-3} M)

3. 1. 1. 2. Effect of shaking time

The time required for complete colour development of the ion-pair formed complex between Nor., Cipro. or Oflo. and Calc. was investigated. The shaking time was measured to form precipitate between aqueous and organic layers (new complex) by allowing the reactants to stand for different time intervals. It was observed that the shaking time has an affect on the amount of precipitate, consequently the maximum colour intensity. On allowing the reactants to stand and shaking for different time intervals, it was observed that 5.0 min are quite sufficient to obtain maximum amount of precipitate which was dissolved in acetone to measure the colour intensity. The formed ion-pairs were found to be stable for more than 24 hours after dissolved in acetone and for more time as solids for drugs Nor., Cipro. and Oflo. as shown in Fig. (4).

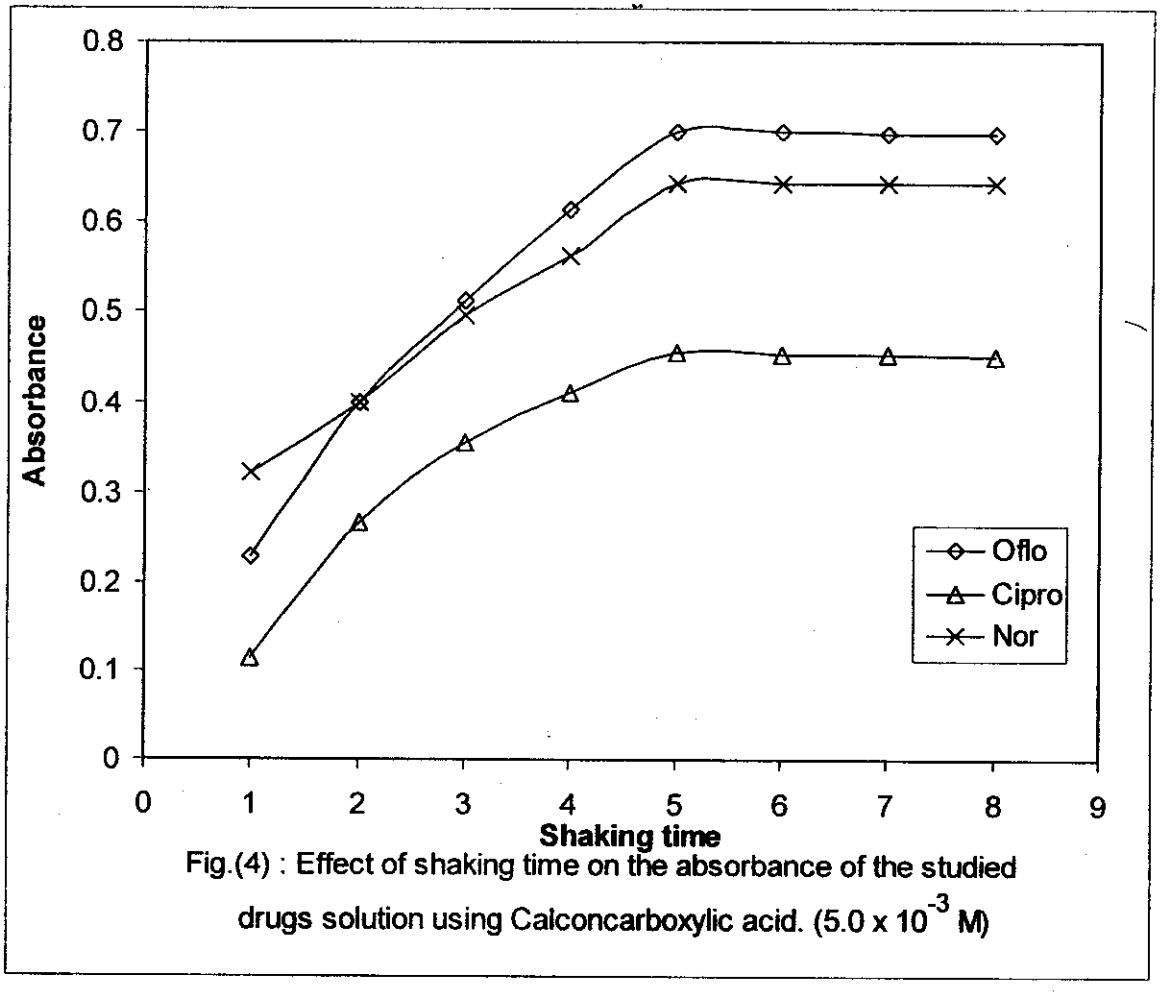

Fig.(4) : Effect of shaking time on the absorbance of the studied drugs solution using Calconcarboxylic acid. (5.0×10^{-3} M)

3. 1. 1. 3. Effect of the polarity of extracting solvent

The polarity of the solvent affects both extraction efficiency and absorbance intensity. The results using different extracting solvents (de - ionized water, benzene, chloroform, carbon tetrachloride, hexane, and dioxan), applying the Calc. reagent on the drugs under consideration indicated that condensate water is the best solvent for the reaction and forming the new blue complex which was added by 3 ml in one batch. Chloroform also is the second best solvent for extraction in case of Nor., Cipro. and Oflo. were selected due to their slightly higher sensitivity and the considerably lower extraction of the reagent itself. Complete extraction was attained by extraction with 20 ml of the solvent in one batch.

3. 1. 1. 4. Effect of reagent concentration

When various concentrations of Calc. were added to a fixed concentrations of Nor., Cipro. and Oflo., 1.0 ml of Calc. (5.0×10^{-3} M) solutions in case of water solvent or 10 ml of Calc. (5.0×10^{-3} M) in case of chloroform solvent as shown in Fig. (5) were found to be sufficient for the production of maximum and reproducible colour intensity. Higher concentration of the reagent decreased the absorbance and colour intensity of the formed ion-pair.

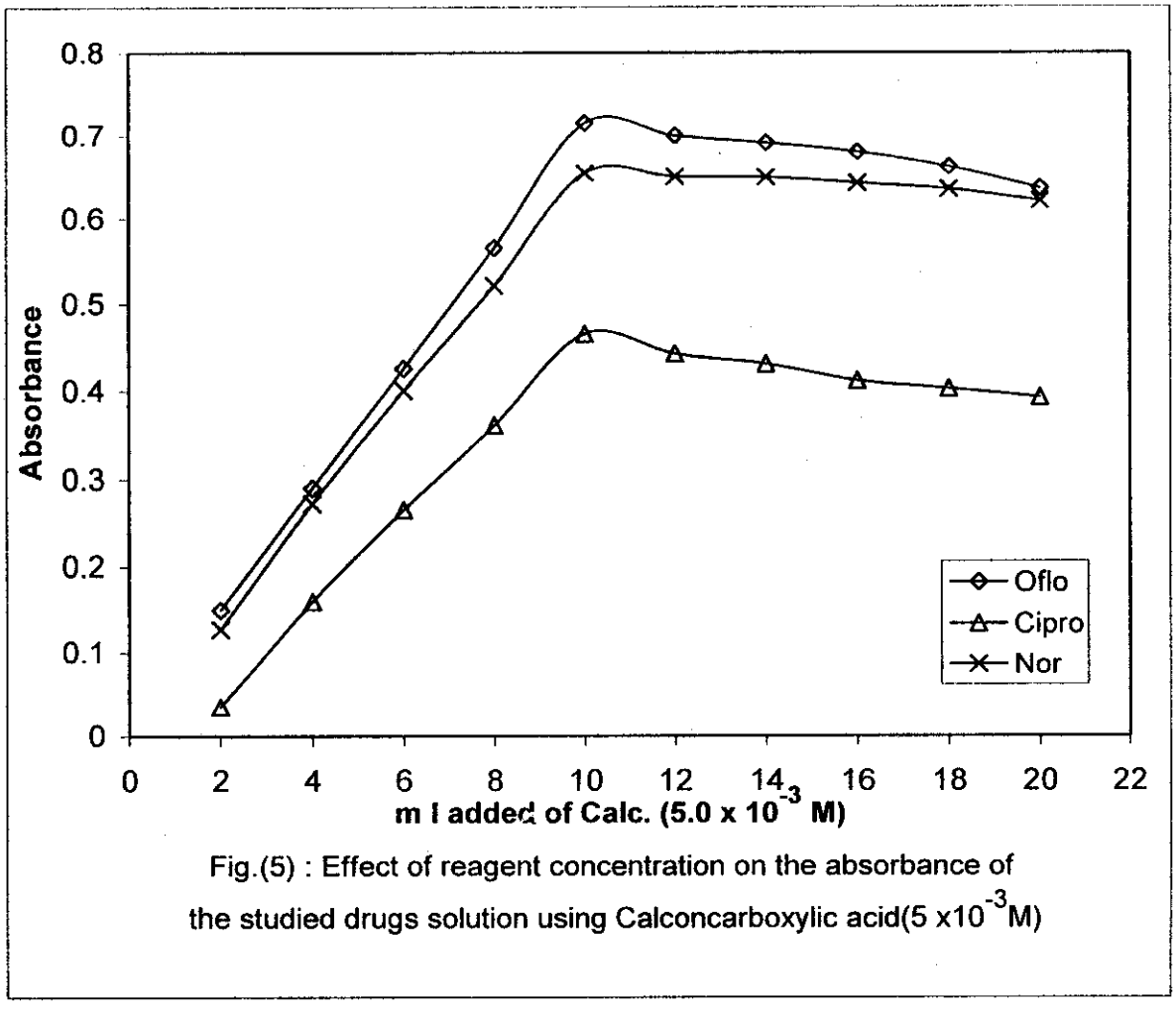

Fig.(5) : Effect of reagent concentration on the absorbance of the studied drugs solution using Calconcarboxylic acid(5 x10^{-3}M)

3. 1. 1.5. Molar ratio of the complexes

In order to investigate the molar ratio of the complexes formed between the drugs under investigation and Calc. at the selected conditions, the molar ratio[72] and continuous variation methods[73-75] were carried out. The results indicated that the molar ratio of the drugs to dye was found to be (1:1) in all ion-pairs formed. The shape of the curves indicated that the complexes were labile, as shown in Fig. (6, 7) The stability constants of the complex were calculated by using the data of the molar ratio[72] and **Job's** continuous variation methods[73] applying **Issa** modification equation[75].The results of the stability constants are recorded in Table(1).

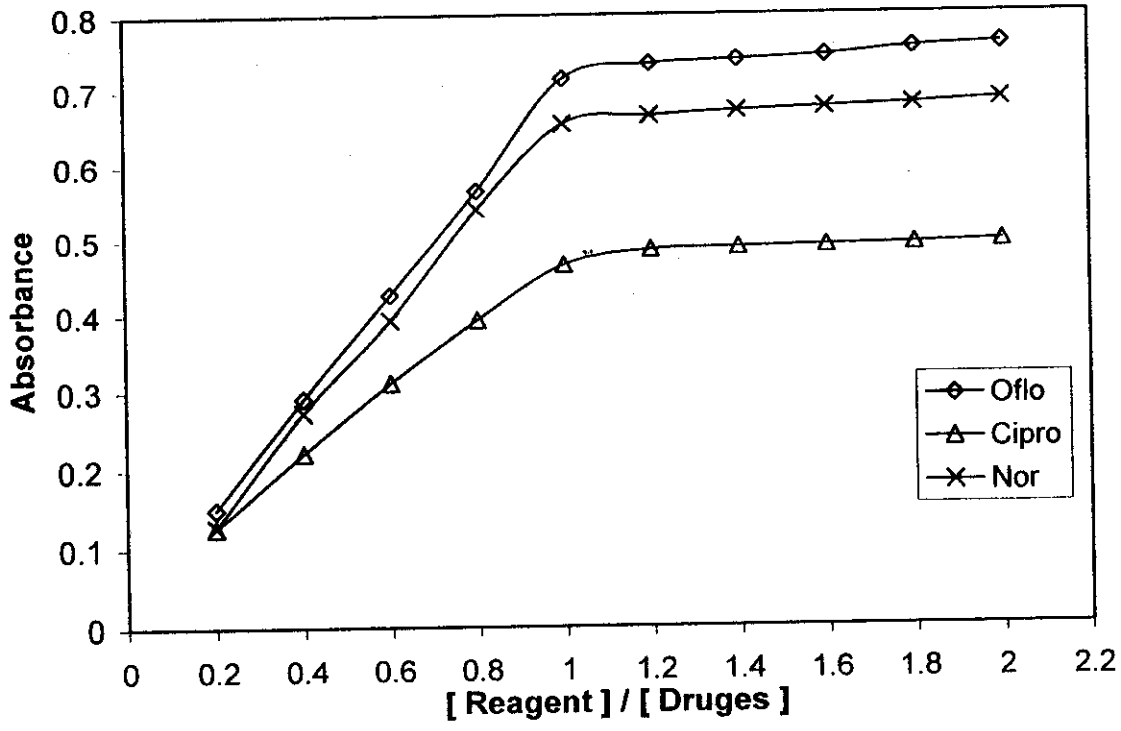

Fig.(6): Molar ratio for Calc.- drugs (5.0×10^{-3} M) u nder consideration

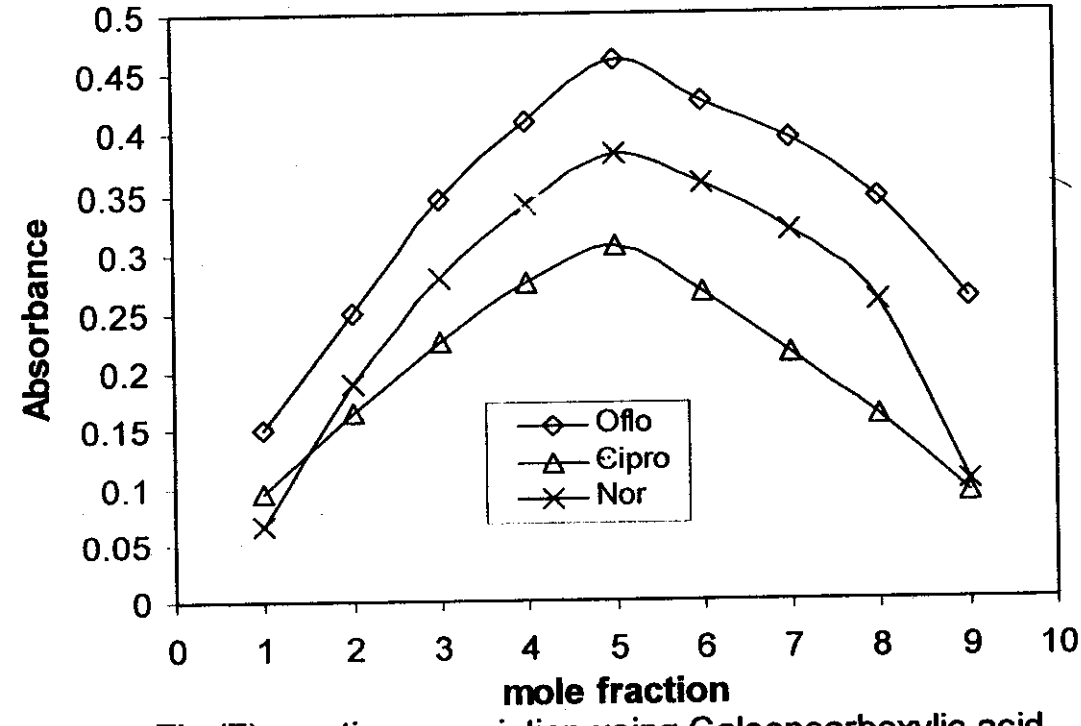

Fig.(7): continous variation using Calconcarboxylic acid (5.0×10^{-3} M) reagent with (5.0×10^{-3} M) of the drugs

3.1.1.6. Interference

No interference (less than ± 3.0% in absorbance is considered non interference) was observed for the determination of Nor., Cipro. and Oflo. with Calc. in the presence of additives and excipients that usually exist in pharmaceutical formula. Also there was no interference from common degradation products which resulted from oxidation of the studied drugs, which are likely to occur under normal storage conditions.

3.1 .1 .7. Evaluation of the stability constants of the ion-pair complexes

Spectrophotometric methods can be applied for the determination of the stability constants of the ion-pair complexes. Generally, the spectrophotometric methods that are usually applied to establish the stoichiometry of the complexes can also be used for the determination of their stability constants in solution. The overall formation constants of the concerned ion-pair complexes were calculated using the spectrophotometric data of the mole ratio and continuous variation methods applying the modification given by **Issa**[75] using the following equation.

$$K_F = (A/A_m) / (1 - A / A_{m.})^{n+1} C_R . n^2$$

where:

A, is the absorbance at reagent concentration C_R.

A_m, is the absorbance at full colour development (λ_{max}).

n, is the stoichiometric ratio of the complex.

K_F, is the stability constant.

3. 1. 1. 8. Statistical analysis

The statistical analysis of each variable was made showing the sample mean (χ') and the sample standard error of the mean (S.E.). The mean value and the standard error are calculated according to the following equation:

Mean value $(\chi') = \Sigma_i (\chi_i / n)$

Standard deviation $(S.D.) = [\Sigma_i (\chi_i - \chi')^2 / (n-1)]^{1/2}$

$(S.E.) = (S.D.) / (n)$

Where.

n = Number of observations.

Σ = Summation.

χ_i = Individual observations.

The slope (b) and regression coefficient (r) were calculated using the following equations :

Slope : $(b) = \Sigma_i [(\chi_i - \chi')(Y_i - Y')] / \Sigma_i (\chi_i - \chi')^2$

Regression coefficient:

$$(r) = \Sigma_i [(\chi_i - \chi')(Y_i - Y')] / \{[\Sigma_i (\chi_i - \chi')^2][(Y_i - Y')^2]\}^{1/2}$$

Standard deviation for the slope:

$$(S_b) = [\Sigma_i (Y_i - Y')^2 / n-2]^{1/2} / \Sigma_i [(\chi_i - \chi')^2]^{1/2}$$

Where the fitted Y-values (Y_i') are the points on the calculated regression line corresponding to the individual X-values. Standard deviation of the intercept (SD).

$$S_a = \{\Sigma_i (Y_i - Y_i')^2 / n-2\}^{1/2} \{\Sigma_i \chi_i^2 / n\Sigma_i (\chi_i - \chi')^2\}$$

Relative standard deviation:

$$\% RSD = 100 (SD / X').$$

Relative error

$$RE = 100 (\Delta X' / X').$$

$$\Delta X' = S. t / (n)^{1/2}$$

Where:

t = the tabulated value.

Σ= Summation.

X = independent variable (concentration).

Y = dependent variable (percentage of binding).

n = number of observations.

The regression coefficient (r) of each parameter was calculated and compared with each other. The highest one is the optimum conditions when regression coefficient (r) was calculated and it's result by minus sign denoting that the curve is inverted, if the independent variable X was increased, the dependent Y decreased and vice versa.

3. 1. 1.9. Validity to Beer's law

Under optimum conditions of pH, time, solvent and reagent concentration, some drugs react with anionic dyes to form ion-pair complexes, which are often coloured and can be subsequently measured colorimetrically. This character is applied, for the determination of Nor., Cipro. and Oflo. through measuring the absorbance of the formed coloured ion-pair at corresponding optimum wavelength, using Calc. The various parameters affecting the reaction development were studied. A calibration or, graph was constructed using standard solutions of Nor., Cipro. and Oflo under the optimum conditions, a linear relationship was obtained between the absorbance and concentration of the drugs within the ranges listed in Table (1). The correlation coefficient, slopes and intercepts, standard deviation, relative standard deviation and relative error of the calibration data for Nor., Cipro. and Oflo. are calculated using the equations given above on pages 87-88-89.

The reproducibility of the method was determined by running six replicate samples, each containing 4.0 µg/ml of drug in case of Nor. 5.0 µg / ml in case of Cipro. and 4.0 µg / ml in case of Oflo. At this concentration, the relative standard deviation was found to be ≤ 0.81 % as shown in Table (1). For more accurate results, Ringbom optimum concentration range was determined by plotting log [C] in µg/ml against percent transmittance and the linear portion of the S-shaped curve gave the accurate range of analysis Fig. (8) and the results are recorded in Table (1). The mean molar absorptivity. Sandell sensitivity, detection and quantification limits are calculated from the standard deviation of the absorbance measurements obtained from Beer's law and recorded in Table (1). Representative curves on the validity to Beer's law for Calc., are shown in Fig. (9).

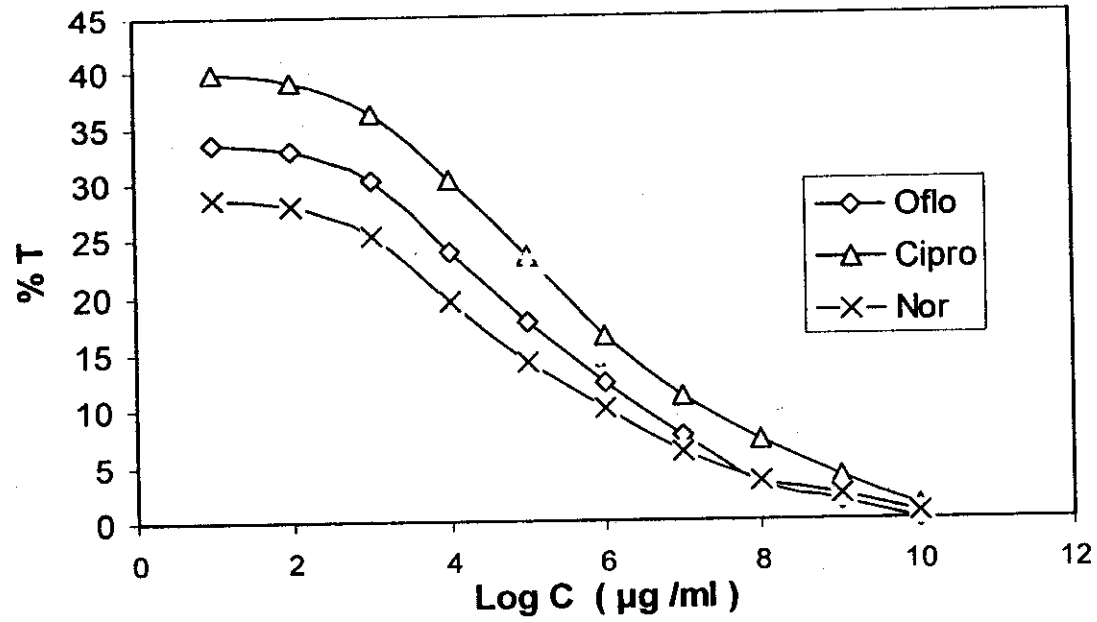

Fig.(8):Ringbom plots for the studied drugs solution using Calconcarboxylic acid (5.0×10^{-3}M)

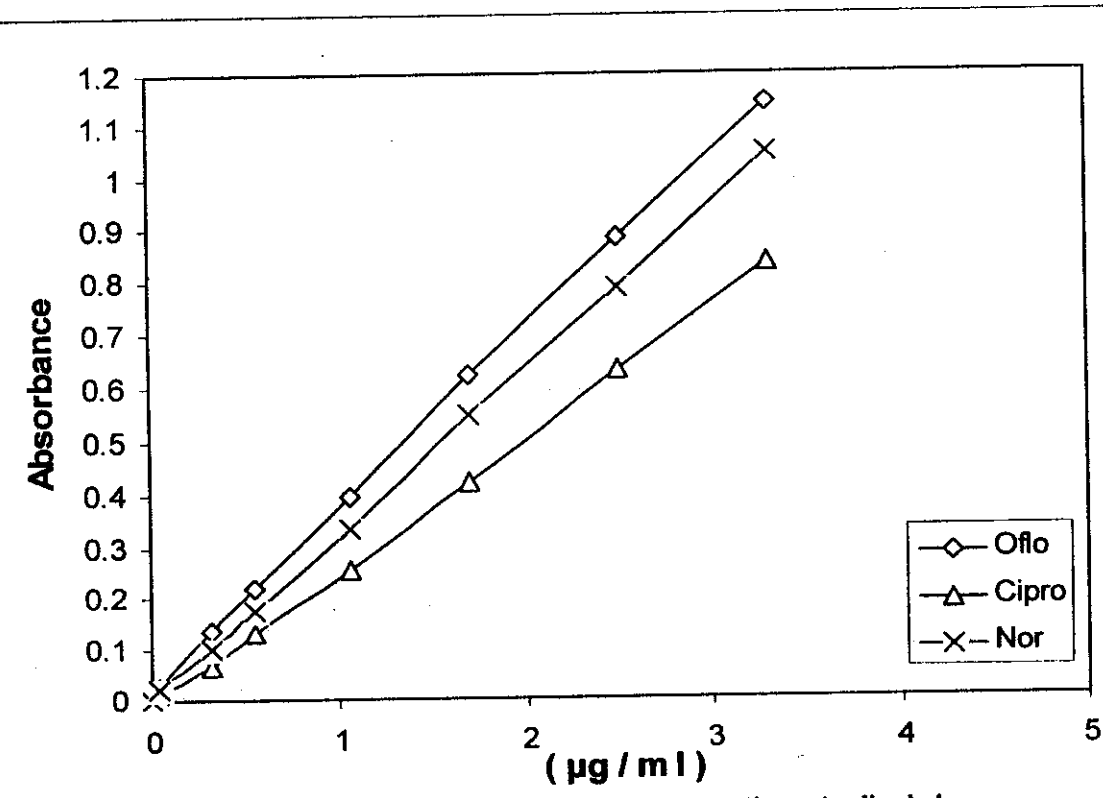

Fig.(9): Application of Beer's law for the studied drugs using the optimum volume of Calconcarboxylic acid (5.0×10^{-3} M)

Table (1) : Analytical data and characteristics of coloured product, precision and accuracy, of the studied drugs using Calc.

Parameters	Calconcarboxylic acid		
	Nor.	Cipro.	Oflo.
pH	4.28	4.28	4.28
Wavelength max.(nm)	619	621.5	621
Stability constant (Log K_F)	8.73	8.11	9.95
Beer's law limits (µg / ml)	0.033 - 3.3	0.033 - 3.3	0.033 - 3.3
Ringbom limits (µg / ml)	0.033-2.525	0.033-2.525	0.033-2.525
Slope (b)	0.316	0.254	0.345
Intercept (a)	0.004	-0.006	0.021
Standard deviation (SD)	0.0042	0.0034	0.0043
Correlation coefficient (r)	0.9998	0.9997	0.9996
Detection limit (µg / ml)	0.013	0.010	0.013
Quantification limit (µg / ml)	0.042	0.034	0.042
Molar absorptivityx10^5 ($mol^{-1}cm^{-1}$)	1:237	0.84	1.246
Sandell sensitivity (µg cm^{-2})	0.0032	0.0039	0.0029
Standard Error* %	0.173	0.138	0.174
RSD %	0.766	0.808	0.687
RE %	0.804	0.847	0.72

*: Average of six determinations.

3. 1. 1. 10. Accuracy and precision

In order to determine the accuracy and precision of the proposed methods, solutions containing three different concentrations of Nor., Cipro. and Oflo. were prepared and analysed in six replicates. The analytical results obtained from this investigation are summarized in Table (2). The percent standard deviations and the percentage range of error at 95% confidence limit were calculated. The results are considered as very satisfactory, at least for the level of concentrations examined.

Table (2): Evaluation of the accuracy and precision of the proposed method using Calc.

Drugs	Taken (µg/ml)	Found (µg/ml)	Recovery (%)	RSD[1] (%)	RE (%)	Confidence[2] Limit
Norfloxacin	1.0	0.985	98.50	0.768	0.807	0.985±0.002
	2.0	1.98	99.00	1.042	1.093	1.98 ± 0.004
	3.0	3.033	101.1	0.753	0.789	3.033± 0.007
Ciprofloxacin	1.0	1.008	100.8	1.766	1.86	1.008± 0.006
	2.0	2.01	100.5	0.719	0.748	2.01± 0.003
	3.0	2.978	99.26	0.443	0.457	2.978± 0.004
Ofloxacin	1.0	0.993	99.3	1.443	1.513	0.993± 0.004
	2.0	1.995	99.75	1.657	1.736	1.995± 0.006
	3.0	3.01	100.3	0.583	0.611	3.01± 0.005

[1]: Relative standard deviation for six determinations.

[2]: 95% confidence limits and five degrees of freedom.

3. 1. 1. 10. Accuracy and precision

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	2.0	1.98	99.00	1.042	1.093	1.98 ± 0.004
	3.0	3.033	101.1	0.753	0.789	3.033± 0.007
Ciprofloxacin	1.0	1.008	100.8	1.766	1.86	1.008± 0.006
	2.0	2.01	100.5	0.719	0.748	2.01± 0.003
	3.0	2.978	99.26	0.443	0.457	2.978± 0.004
Ofloxacin	1.0	0.993	99.3	1.443	1.513	0.993± 0.004
	2.0	1.995	99.75	1.657	1.736	1.995± 0.006
	3.0	3.01	100.3	0.583	0.611	3.01± 0.005

[1]: Relative standard deviation for six determinations.

[2]: 95% confidence limits and five degrees of freedom.

3. 1. 1. 11. Determination of the studied drugs in urine samples by using Calconcarboxylic acid (Calc.).

In this method (extraction by chloroform), 10 ml of the urine aliquot were transferred into 50ml separating funnel and mixed with 10 ml of Calc. (5×10^{-3} M) in case of Nor., Cipro. and Oflo., followed by 10 ml of buffer solution of pH 4.28 . The volume was completed to 50 ml with chloroform to extract the formed complexes with 5 min.shaking time and at room temperature 25 °C, but after shaking, precipitates were formed in the three drugs between aqueous and organic layers and colour changes from violet to blue, then precipitates were filtered, dried and soluble in acetone, time has no affect in this step and the colour become stable for more than 24 hours. The absorbance was measured following the general procedure described above. The relative standard deviation (RSD), recovery and confidence limits of the added drugs are computed and recorded as shown in Table (3).

Table (3): Evaluation of the accuracy and precision of the proposed method for investigated of pharmaceutical forms of Nor., Cipro, Oflo., using Calc.

Dosage forms	Added (µg/ml)	Found (µg/ml)	Recovery (%)	RSD[1] (%)	Confidence[2] limits
Epinor tablets (400 mg per tablet)	-	-	-	-	-
	0.5	0.503	100.6	1.458	0.503±0.003
	1.0	0.99	99.00	1.808	0.99±0.005
	1.5	1.51	100.6	1.017	1.51±0.004
	2.0	1.98	99.00	0.596	1.98±0.005
Noracin tablets (400 mg per tablet)	-	-	-	-	-
	0.5	0.495	99.00	1.91	0.495±0.004
	1.0	1.01	101.0	1.755	1.01±0.006
	1.5	1.498	99.86	1.487	1.498±0.007
	2.0	2.02	101.0	0.663	2.02±0.009
Ciprocine tablets (500 mg per tablet)	-	-	-	-	-
	0.5	0.492	98.4	1.876	0.492±0.003
	1.0	0.993	99.3	0.666	0.993±0.003
	1.5	1.49	99.33	0.617	1.49±0.004
	2.0	1.99	99.5	0.892	1.99±0.008
Ciprobay tablets (750 mg per tablet)	-	-	-	-	-
	0.5	0.502	100.4	0.771	0.502±0.002
	1.0	1.003	100.3	1.042	1.003±0.004
	1.5	1.493	99.53	0.617	1.493±0.004
	2.0	1.985	99.25	0.687	1.985±0.006
Ciprofloxacin tablets (500 mg per tablet)	-	-	-	-	-
	0.5	0.499	99.8	1.262	0.499±0.005
	1.0	1.001	100.1	1.11	1.001±0.007
	1.5	1.506	100.4	0.924	1.506±0.008
	2.0	1.989	99.45	0.55	1.989±0.005
Ciprocine eye drops (500 mg)	-	-	-	-	-
	0.5	0.497	99.4	1.471	0.497±0.004
	1.0	1.002	100.2	2.271	1.002±0.01
	1.5	1.503	100.2	1.061	1.503±0.009
	2.0	1.999	99.95	0.901	1.999±0.011

Table (3) :- continuous

Ciprocine injection vial (200 mg per vial)	-	-	-	-	-
	0.5	0.5001	100.02	1.404	0.5001±0.008
	1.0	0.98	0.98	0.615	0.98±0.005
	1.5	1.501	100.06	0.635	1.501±0.006
	2.0	1.986	99.3	2.189	1.986±0.002
Ofloxacin tablets (200 mg per tablet)	-	-	-	-	-
	0.5	0.491	98.2	1.121	0.491±0.003
	1.0	1.006	100.6	0.809	1.006±0.003
	1.5	1.495	99.66	0.750	1.495±0.004
	2.0	1.995	99.75	0.58	1.995±0.004
Ofloxin tablets (200 mg per tablet)	-	-	-	-	-
	0.5	0.499	99.8	1.355	0.499±0.004
	1.0	0.996	99.6	0.52	0.996±0.002
	1.5	1.499	99.93	0.813	1.499±0.005
	2.0	1.97	98.5	0.333	1.97±0.003
Oflocin tablets (200 mg per tablet)	-	-	-	-	-
	0.5	0.5002	100.04	1.069	0.5002±0.002
	1.0	1.01	101.0	0.615	1.01±0.002
	1.5	1.499	99.93	0.632	1.499±0.003
	2.0	2.003	100.15	0.601	2.003±0.005
Ofloxin eye drops (3 mg per ml)	-	-	-	-	-
	0.5	0.501	100.2	1.464	0.501±0.005
	1.0	1.006	100.6	0.804	1.006±0.004
	1.5	1.5008	100.05	0.698	1.5008±0.005
	2.0	2.01	100.5	1.051	2.01± 0.011

[1]: Relative standard deviation for six determinations.

[2]: 95% confidence limits and five degrees of freedom.

3. 1. 1. 12. Analytical applications

The validity of the proposed procedures is tested for determining Nor., Cipro. and Oflo., in pharmaceutical preparations manufactured in local companies as mentioned before. The concentrations of the studied drugs in dosage forms were calculated from the appropriate calibration graph using standard addition technique. There was no shift in the absorption maximum due to the presence of other constituents in the dosage forms. The results are compared with those obtained by applying the official methods.

The results obtained were compared statistically by the student's t-test and variance ratio F-test with those obtained using the official method on the sample of the same batch. The student's t-test values obtained at 95% confidence level and five degrees of freedom did not exceed the theoretical tabulated value indicating no significant difference between the methods compared. The F-values also showed that there is no significant difference between accuracy of the proposed method and the official ones Tables (4). The accuracy of the proposed method, when applied to pharmaceutical preparations is evaluated by applying standard addition technique. in which variable amounts of the drugs Nor., Cipro. and Oflo., were added to the previously analysed portion of pharmaceutical preparations. The results shown in Tables (5), confirm that the proposed method is not liable to interference by fillers (lactose monohydrate, microcrystallin cellulose, talc powder, explotab, sucrose, lysozyme, sorbitol, povidone, maize starch, sodium acetate, methyl - p-hydroxybenzoate, propyl p-hydroxybenzoate, hydroxy ethyl cellulose, flavours, magnesium stearate) usually formulated with the drugs under consideration. The proposed method is highly sensitive; therefore it could be used easily for routine analysis of both pure forms and pharmaceutical preparations.

Table (4): Evaluation of the accuracy and precision of the proposed and official methods for determination of Nor., Cipro., Oflo., in its pharmaceutical forms using Calc.

Dosage forms	Official method			Proposed method				
	Taken mg	found* mg	Recovery (%)	Taken mg	found* mg	Recovery (%)	t** value	F** test
Epinor tablets (400 mg per tablet)	400	403.5	100.87	400	399	99.75	1.52	1.288
Noracin tablets (400 mg per tablet)	400	398	99.5	400	401.5	100.37	2.505	1.081
Ciprocine tablets (500 mg per tablet)	500	502.3	100.46	500	498.7	99.74	2.185	3.135
Ciprobay tablets (750 mg per tablet)	750	745.8	99.44	750	752	100.26	1.247	3.472
Ciprofloxacin tablets (500 mg per tablet)	500	497	99.4	500	506.1	101.22	0.312	1.070
Ciprocine eye drops(500 mg)	500	498.5	99.7	500	498.9	99.78	0.402	3.599
Ciprocine injection vial (200 mg per vial)	200	201.1	100.55	200	199.3	99.65	0.911	1.164
Ofloxacin tablets (200 mg per tablet)	200	199.2	99.6	200	198.5	99.25	2.449	2.116
Ofloxin tablets (200 mg per tablet)	200	199.6	99.8	200	198.9	99.45	2.12	1.077
Oflocin tablets (200 mg per tablet)	200	201.85	100.92	200	198.1	99.05	1.083	1.087
Ofloxin eye drops (3 mg per ml)	30	29.9	99.66	30	29.6	98.66	1.188	2.77

*: Average of six determinations.
**: Theoretical values for t- and F- values for five degree of freedom and 95% confidence limits are 2.57 and 5.05 , respectively .

Table (5): Determination of the studied drugs Nor., Cipro, Oflo, in its pharmaceutical dosage forms applying the standard addition technique using Calc.

Dosage forms	Taken (µg/ml)	Added (µg/ml)	Found* (µg/ml)	Recovery (%)
Epinor tablets (400 mg per tablet)	1.0	0.0	0.995	99.5
		0.5	1.493	99.53
		1.0	2.02	101.0
		1.5	2.48	99.2
		2.0	3.02	100.66
Noracin tablets (400 mg per tablet)	1.0	0.0	1.002	100.2
		0.5	1.515	101.0
		1.0	1.995	99.75
		1.5	2.49	99.68
		2.0	3.01	100.33
Ciprocine tablets (500 mg per tablet)	1.0	0.0	1.004	100.4
		0.5	1.506	100.4
		1.0	1.98	99.0
		1.5	2.499	99.96
		2.0	2.97	99.0
Ciprobay tablets (750 mg per tablet)	1.0	0.0	0.998	99.8
		0.5	1.497	99.8
		1.0	2.006	100.3
		1.5	2.505	100.2
		2.0	2.992	99.73
Ciprofloxacin tablets (500 mg per tablet)	1.0	0.0	1.001	100.1
		0.5	1.491	99.4
		1.0	2.01	100.5
		1.5	2.49	99.62
		2.0	3.006	100.2
Ciprocine eye drops((500 mg)	1.0	0.0	0.999	99.9
		0.5	1.511	100.73
		1.0	2.007	100.35
		1.5	2.502	100.08
		2.0	2.999	99.96

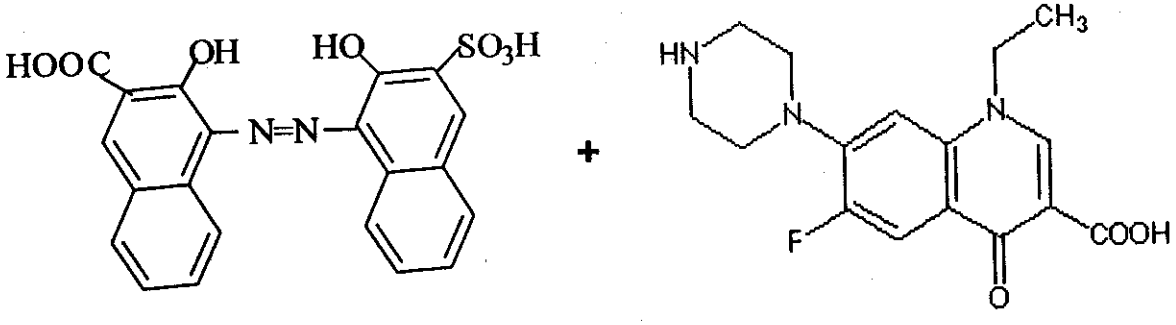
Table (5) :- continuous

Ciprocine injection vial (200 mg per vial)	1.0	0.0	0.997	99.7
		0.5	1.508	100.5
		1.0	1.996	99.8
		1.5	2.498	99.92
		2.0	2.996	99.86
Ofloxacin tablets (200 mg per tablet)	1.0	0.0	1.007	100.7
		0.5	1.499	99.93
		1.0	2.005	100.25
		1.5	2.501	100.04
		2.0	2.986	99.53
Ofloxin tablets (200 mg per tablet)	1.0	0.0	1.001	100.1
		0.5	1.504	100.26
		1.0	1.994	99.7
		1.5	2.489	99.56
		2.0	3.017	100.56
Oflocin tablets (200 mg per tablet)	1.0	0.0	1.01	101.0
		0.5	1.488	99.2
		1.0	2.009	100.45
		1.5	2.5	100.0
		2.0	3.011	100.36
Ofloxin eye drops (3 mg per ml)	1.0	0.0	1.003	100.3
		0.5	1.509	100.6
		1.0	2.01	100.5
		1.5	2.499	99.96
		2.0	3.014	100.47

*: Average of six determinations.

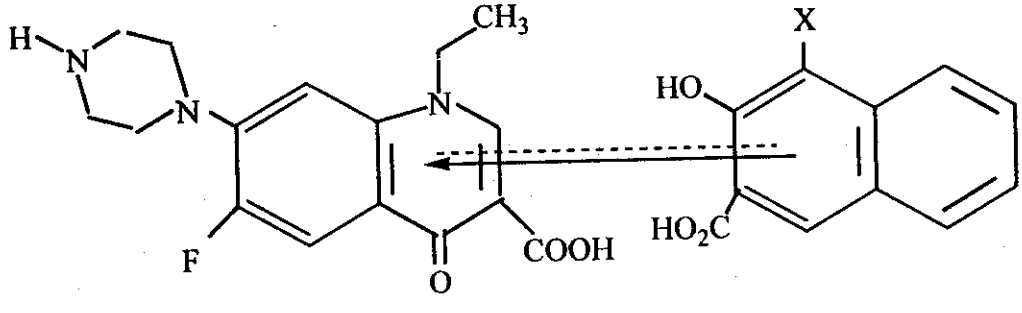
3. 1. 1.13. Suggested mechanism according to visible spectra measure :-

Drug – reagent reaction can be stated that the addition compound is formed through a charge transfer from the reagent (Calcon, Erioch or Aliz.) as electron donor to the drug (Nor., Cipro. or Oflo.) as electron acceptor. CT complex formed (Calcon with drugs) exhibits maximum absorbance at λ_{max} 619, 621.5 and 621 nm in case of Nor., Cipro,and Oflo., respectively as shown by the mechanism in **Fig (10).**

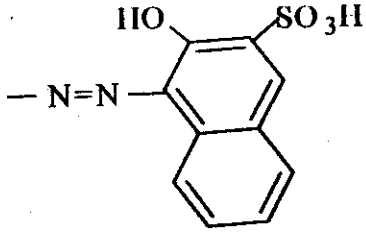


Calconcarboxylic acid **Norfloxacin**

pH = 4. 28



Charge transfer complex

Where x = 

3. 1. 2. Absorption spectra of the studied drugs with Eriochromeblack T (EBT)

In order to investigate the optimum conditions for the development of the ion-pair complex formed between the studied drugs and (5.0×10^{-4}M) EBT, some parameters were studied and recorded below.

3.1.2.1. Effect of pH

In order to establish the optimum pH value for each ion-pair formed, Nor., Cipro. or Oflo. was allowed to react with the EBT in aqueous buffered solution in the pH ranges (1.8 -12.0). The absorbance intensity was measured at its λ_{max} .The highest absorbance values were obtained at pH 4.28 in case of the three drugs, which are selected for ion-pairs formation. These results are showed in Fig. (11). Furthermore, the amount of buffer added was examined and found to be 10 ml for all complexes as shown in Fig. (12).The λ_{max} corresponding to each ion-pair complex of the drugs with EBT at 505 nm in case of Nor., and Cipro., 504 nm in case of Oflo as shown in fig. (13).

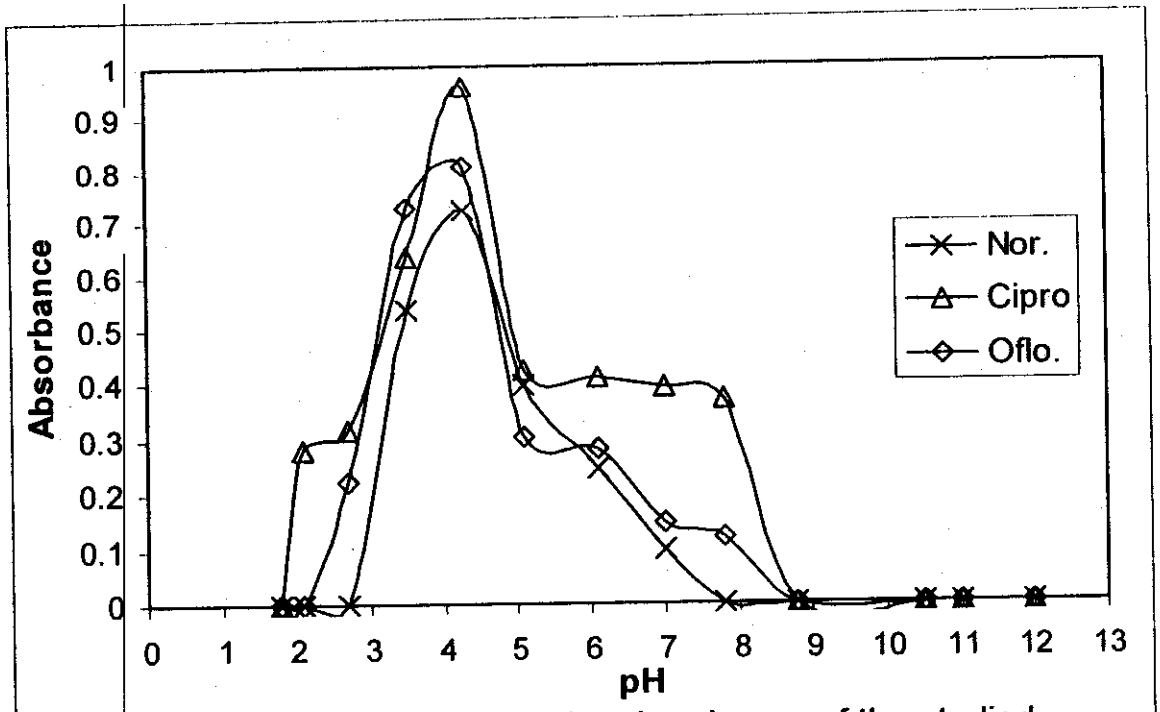


Fig.(11): Effect of pH on the absorbance of the studied drugs using EBT (5.0×10^{-3} M)

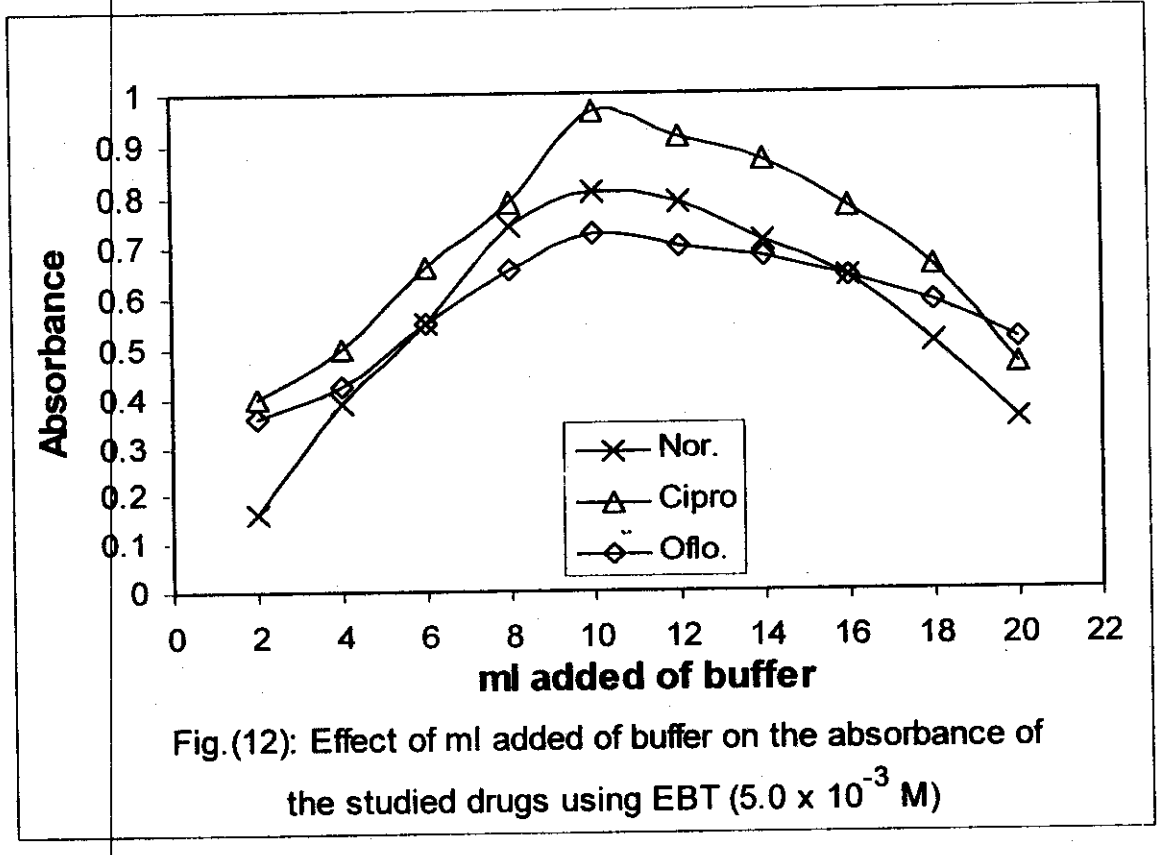


Fig.(12): Effect of ml added of buffer on the absorbance of the studied drugs using EBT (5.0×10^{-3} M)

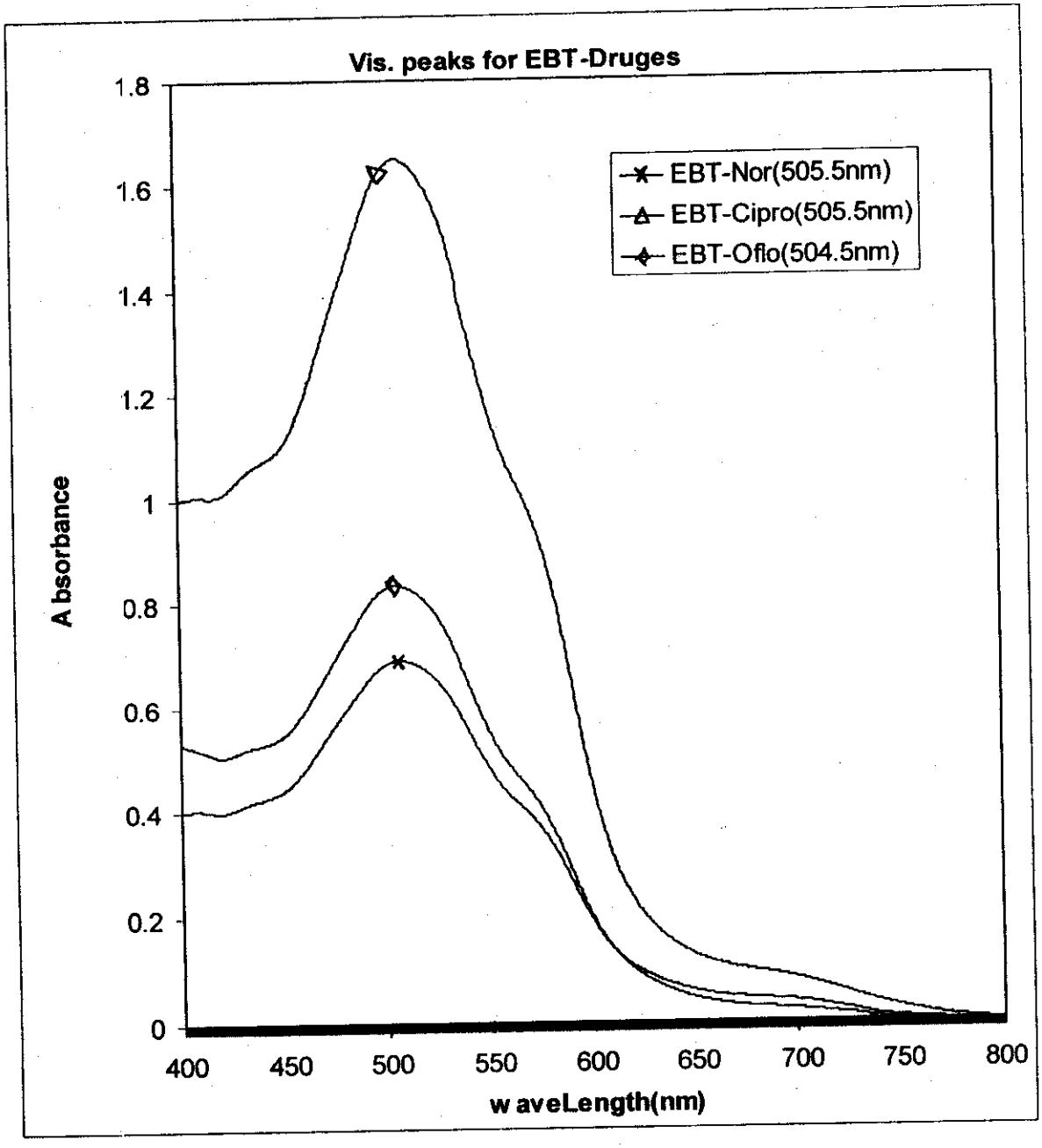


Fig.(13): Absorption of new complexes of the studied drugs with acid dye EBT (5.0×10^{-3} M)

3. 1. 2. 2. Effect of shaking time

The time required for complete colour development of the ion-pair formed complex between Nor., Cipro. or Oflo. and EBT was investigated. Allowing the reactants to stand and shaking for different time intervals it was observed that, shaking time needed to form precipitate between aqueous and organic layers (new complex), has an affect on the amount of precipitate, consequently the maximum colour intensity. So that, shaking time required for complete colour development of ion-pair formed between the drugs and EBT was investigated, it was observed that 3.0 min are quite sufficient to obtain maximum amount of precipitate (fig. 14) which is dissolved in acetone, the absorbance was the measured to test the maximum colour intensity. The formed ion-pairs were found to be stable for more than 24 hours after dissolution in acetone and for more time as solid in drugs Nor., Cipro. and Oflo.

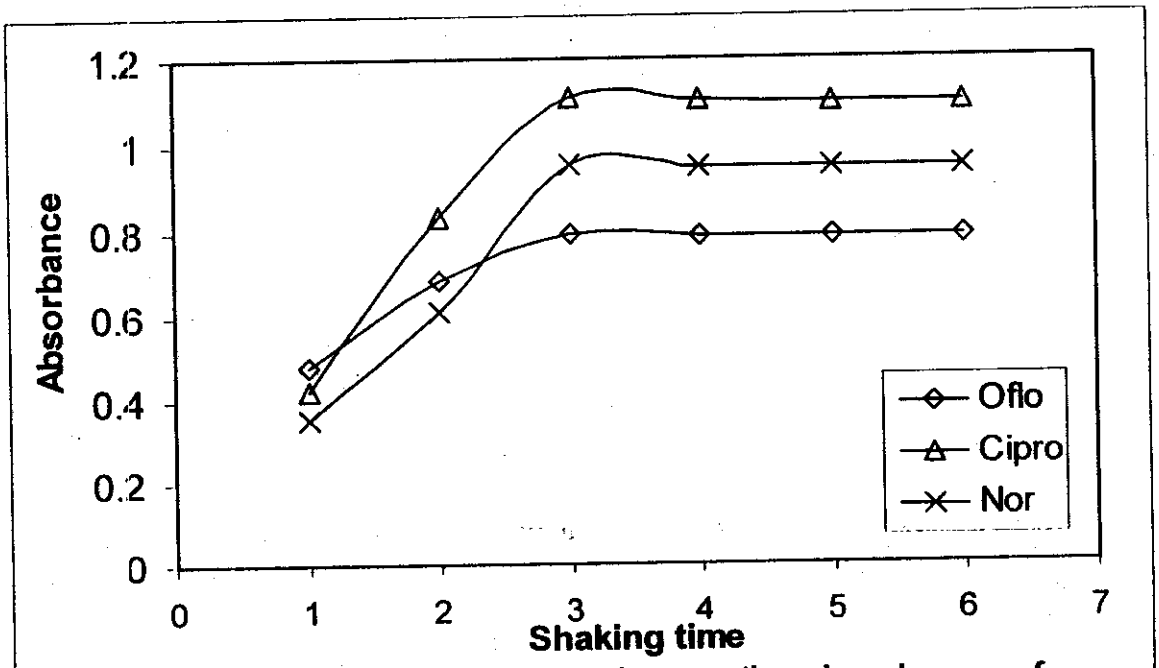


Fig.(14) : Effect of shaking time on the absorbance of the studied drugs solution using EBT(5.0×10^{-3}M)

3. 1. 2. 3. Effect of the polarity of extracting solvent

The polarity of the solvent affects both extraction efficiency and absorbance values. The results using different extracting solvents (condensate water, benzene, chloroform, carbontetrachloride, hexane, dioxan), applying the EBT reagent on the drugs under consideration indicated that ,chloroform is the best solvent for extraction in case of Nor., Cipro. and Oflo. This solvent was selected due to its slightly higher sensitivity and the considerably lower extraction of the reagent itself. Complete extraction was attained by extraction with 20 ml of the solvent in one batch.

3. 1. 2. 4. Effect of reagent concentration

When various concentrations of EBT were added to a fixed concentrations of Nor, Cipro. or Oflo., 10 ml of EBT (5.0×10^{-3} M) solutions with chloroform solvent, as shown in Fig. (15) were found to be sufficient for the production of maximum and reproducible colour intensity. Higher concentration of the reagent decreased the absorbance and colour intensity of the formed ion-pair.

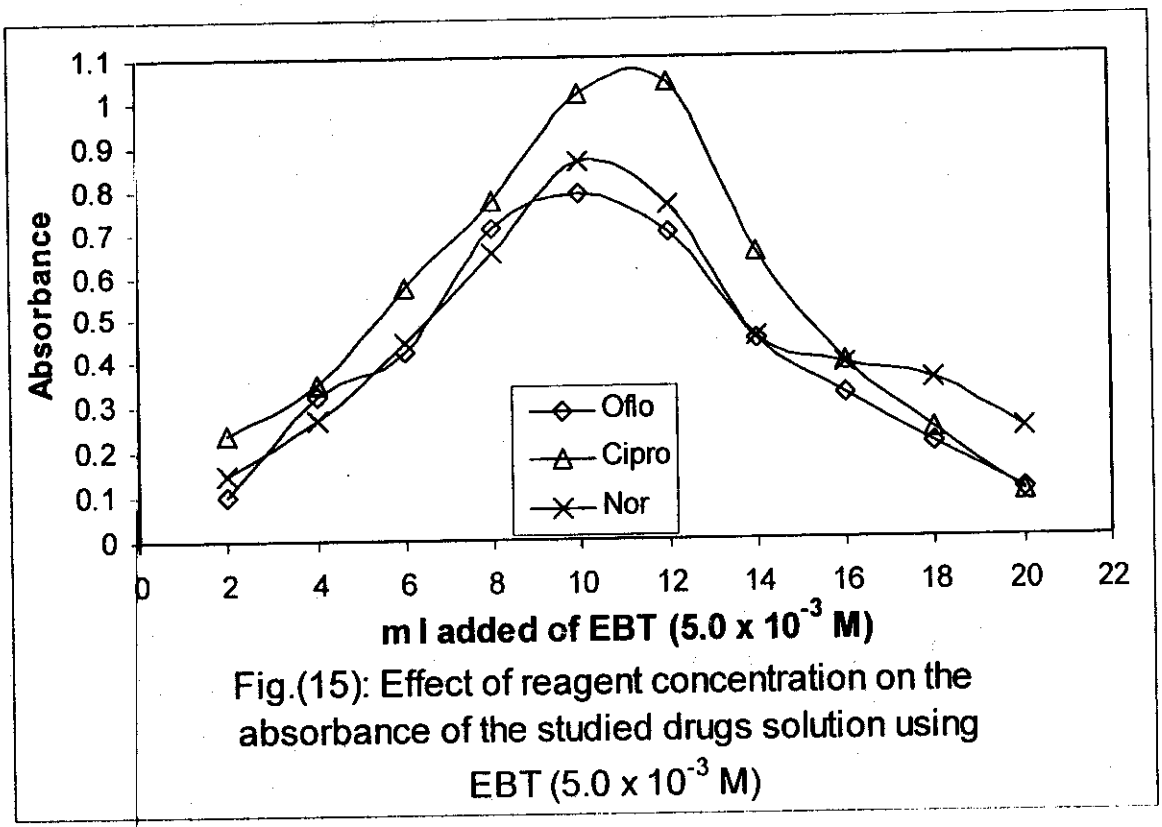


Fig.(15): Effect of reagent concentration on the absorbance of the studied drugs solution using EBT (5.0×10^{-3} M)

3. 1. 2. 5. MoLar ratio of the complexes

In order to investigate the molar ratio of the complexes formed between the drugs under investigation and EBT under selected conditions, the molar ratio[72] and continuous variation methods[73 -75] were carried out. The results indicated that the molar ratio of the drugs to dye was found to be (1:1) in all ion-pairs formed. The shape of the curves indicated that the complexes were labile, as shown in Fig's. (16,17). The stability constant of the complex was calculated by using the data of the molar ratio[72] and **Job's** continuous variation methods [73] applying **Issa** modification equation[75] .The results of the stability constants are recorded in Table (6).

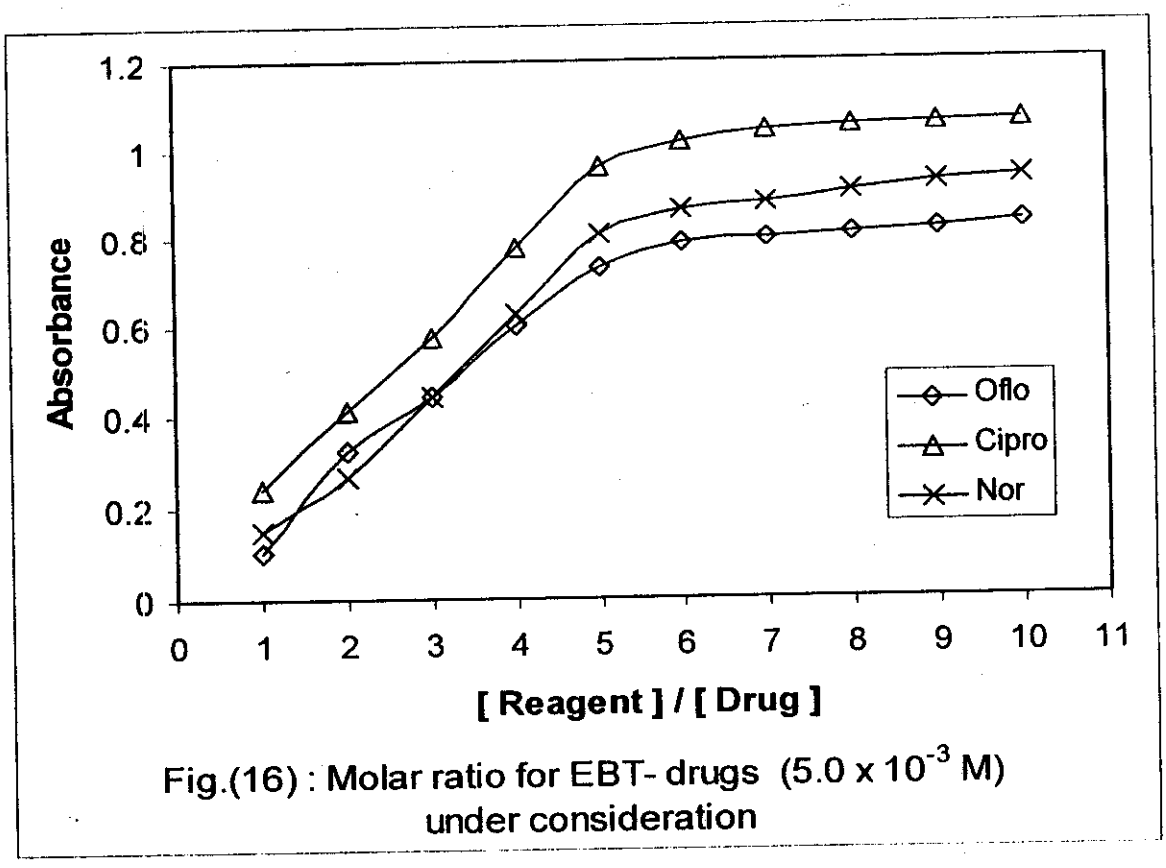


Fig.(16) : Molar ratio for EBT- drugs (5.0×10^{-3} M) under consideration

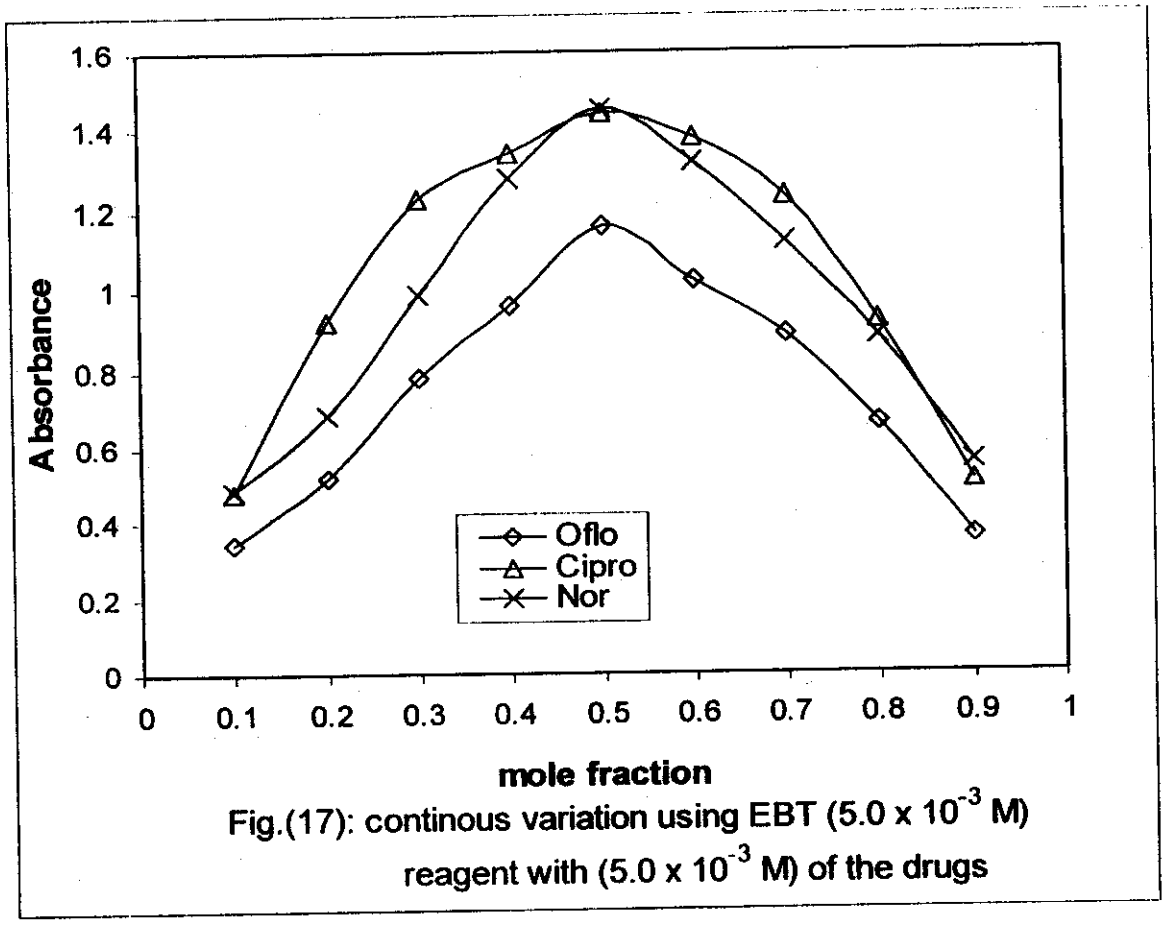


Fig.(17): continous variation using EBT (5.0×10^{-3} M) reagent with (5.0×10^{-3} M) of the drugs

Table (6): Analytical data and characteristics of coloured product, precision and accuracy, of the studied drugs using EBT

Parameters	Eriochromeblack T		
	Nor.	Cipro.	Oflo.
pH	4.28	4.28	4.28
Wavelength max.(nm)	505.5	505.5	504.5
Stability constant (Log K_F)	8.67	8.16	8.17
Beer's law limits (µg / ml)	0.11-2.65	0.11-2.65	0.11-2.65
Ringbom limits (µg / ml)	0.17-2.25	0.17-2.25	0.17-2.25
Slope (b)	0.447	0.52	0.36
Intercept (a)	-0.002	-0.016	0.0019
Standard deviation (SD)	0.0051	0.005	0.0044
Correlation coefficient (r)	0.9999	0.9996	0.9998
Detection limit (µg / ml)	0.016	0.015	0.013
Quantification limit (µg / ml)	0.052	0.05	0.044
Molar absorptivityx10^5 ($mol^{-1}cm^{-1}$)	1.749	1.72	1.288
Sandell sensitivity (µg cm^{-2})	0.0022	0.0019	0.0028
Error* %	0.211	0.208	0.182
RSD %	1.004	0.88	1.067
RE %	1.05	0.92	1.12

* : Average of six determinations.

3. 1. 2. 6. Validity to Beer's law

Under optimum conditions of pH, time, solvent and reagent concentration, some drugs react with anionic dyes to form ion-pair complexes, which are often coloured and can be subsequently measured colorimetrically. This character is applied, for the determination of Nor., Cipro. and Oflo. through measuring the absorbance of the formed coloured ion-pair at the corresponding optimum wavelength, using EBT Various parameters affecting the color development were studied. A calibration graph was constructed using standard solutions of Nor., Cipro. and Oflo under the optimum conditions, a linear relationship was obtained between the absorbance and concentration of the drugs (fig.18) within the range listed in Table (6). The correlation coefficient, slopes and intercepts, standard deviation, relative standard deviation and relative error of the calibration data for Nor., Cipro. and Oflo. are calculated using the equations given above on pages 87-88-89 .

The reproducibility of the method was investigated by running six replicate samples, each containing 4.0 µg/ml of drug in case of Nor., 5.0 µg/ml in case of Cipro. and 4.0 µg/ml in case of Oflo. at this concentration, the relative standard deviation was found to be ≤ 1.067 % as shown in Table (6). For more accurate results, Ringbom optimum concentration range was determined by plotting log [C] in µg/ml against percent transmittance and the linear portion of the S-shaped curve gave the accurate range of analysis Fig. (19) and the results are recorded in Table (6). The mean molar absorptivity. Sandell sensitivity, detection and quantification limits are calculated from the standard deviation of the absorbance measurements obtained from Beer's law and recorded in Table (6).

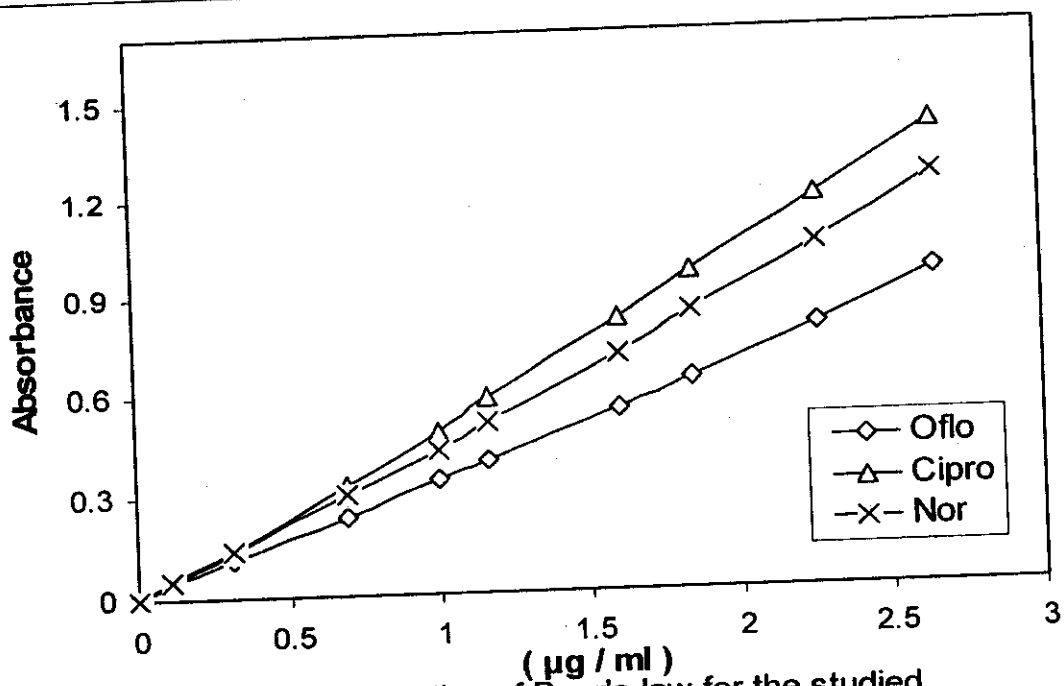


Fig.(18) : Application of Beer's law for the studied drugs using the optimum volume of EBT(5.0×10^{-3} M)

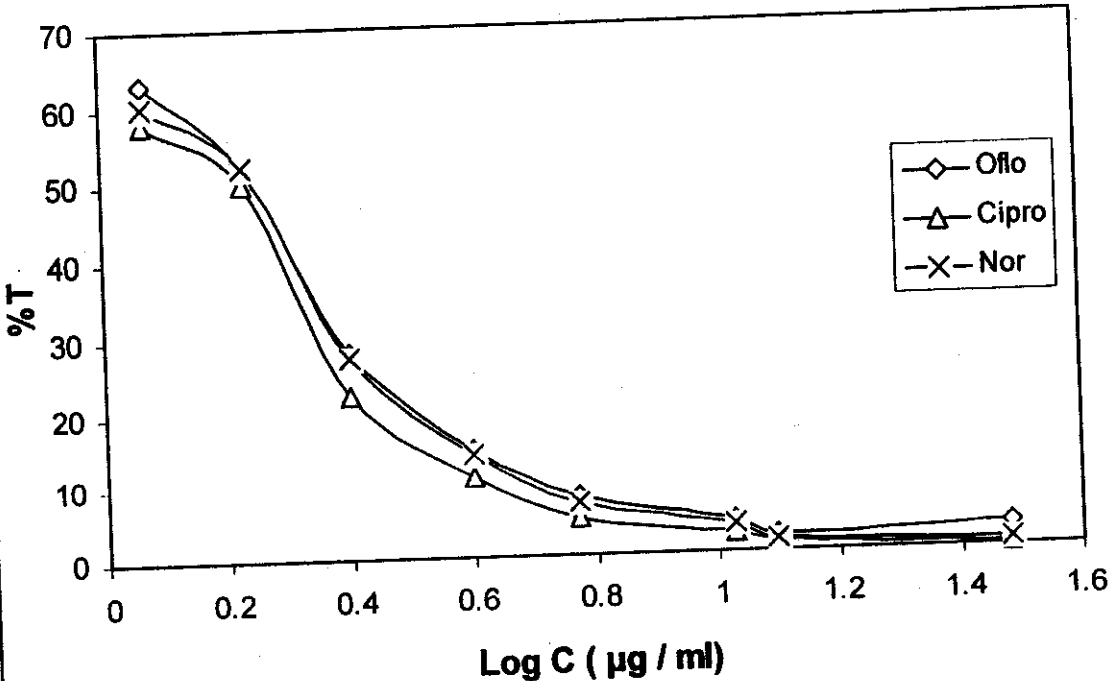


Fig.(19):Ringbom plots for the studied drugs solution using EBT(5.0×10^{-3}M)

3. 1. 2. 7. Accuracy and Precision

In order to determine the accuracy and precision of the proposed methods, solutions containing three different concentrations of Nor., Cipro. or Oflo. were prepared and analysed in six replicates. The analytical results obtained from this investigation are summarized in Table (7). The percent standard deviations and the percentage range of error at 95% confidence level were calculated. The results are considered as very satisfactory, at least for the level of concentrations examined.

Table (7): Evaluation of the accuracy and precision of the proposed method using EBT

Drugs	Taken (µg/ml)	Found (µg/ml)	Recovery (%)	RSD[1] (%)	RE (%)	Confidence[2]
Norfloxacin	1.0	1.004	100.1	1.054	1.104	1.004±0.004
	2.0	2.01	100.5	0.8	0.839	2.01±0.007
	3.0	3.02	100.66	1.307	1.371	3.02±0.008
Ciprofloxacin	1.0	0.996	99.6	1.224	1.284	0.996±0.006
	2.0	2.015	100.75	0.267	0.279	2.015±0.003
	3.0	2.987	99.56	0.657	0.688	2.987±0.009
Ofloxacin	1.0	0.998	99.8	0.235	0.247	0.998±0.002
	2.0	2.02	101.0	0.494	0.518	2.02±0.005
	3.0	3.025	100.83	0.540	0.566	3.025±0.004

[1]: Relative standard deviation for six determinations.

[2]: 95% confidence limits and five degrees of freedom.

3. 1. 2. 8. Determination of the studied drugs in urine samples by using Eriochromeblack T (EBT)

10 ml of the urine aliquot were transferred into a 50 ml separating funnel and mixed with 10 ml of EBT (5×10^{-3} M) in case of Nor., Cipro. or Oflo., followed by 10 ml of buffer solution of pH 4.28. The volume was completed to 50 ml with chloroform to extract the formed complexes with 3 min.shaking time and at room temperature (25 °C), but after shaking, precipitates were formed in the three drugs between aqueous and organic layers and the colour changed from violet to red. The precipitates were filtered, dried and dissolved in acetone, time has no affect in this step and the colour was stable for more than 24 hours. The absorbance was measured following the general procedure described above. The relative standard deviation (RSD), recovery and confidence limits of the added drugs are computed and recorded as shown in Table (8).

Table (8): Evaluation of the accuracy and precision of the proposed method for investigated of pharmaceutical forms of Nor., Cipro, Oflo., using EBT

Dosage forms	Added (µg/ml)	Found (µg/ml)	Recovery (%)	RSD[1] (%)	Confidence[2] limits
Epinor tablets (400 mg per tablet)	-	-	-	-	-
	0.5	0.499	99.92	1.818	0.499±0.004
	1.0	1.005	100.56	1.776	1.005±0.005
	1.5	1.51	100.66	1.017	1.51±0.004
	2.0	2.001	100.05	0.888	2.001±0.008
Noracin tablets (400 mg per tablet)	-	-	-	-	-
	0.5	0.505	101.0	1.774	0.505±0.005
	1.0	1.003	100.3	1.755	1.003±0.006
	1.5	1.508	100.53	1.488	1.508±0.00
	2.0	2.005	100.25	1.036	2.005±0.009
Ciprocine tablets (500 mg per tablet)	-	-	-	-	-
	0.5	0.501	100.24	2.422	0.501±0.007
	1.0	0.998	99.85	0.666	0.998±0.003
	1.5	1.502	100.11	0.617	1.502±0.004
	2.0	2.011	100.55	0.892	2.011±0.007
Ciprobay tablets (750 mg per tablet)	-	-	-	-	-
	0.5	0.504	100.9	1.52	0.504±0.008
	1.0	1.001	100.1	1.0418	1.001±0.004
	1.5	1.494	99.65	1.598	1.494±0.011
	2.0	1.996	99.8	0.9059	1.996±0.009
Ciprofloxacin tablets (500 mg per tablet)	-	-	-	-	-
	0.5	0.499	99.9	1.262	0.499±0.005
	1.0	1.003	100.3	1.144	1.003±0.007
	1.5	1.499	99.95	1.045	1.499±0.008
	2.0	2.014	100.7	1.125	2.014±0.018
Ciprocine eye drops (500 mg)	-	-	-	-	-
	0.5	0.499	99.88	1.468	0.499±0.006
	1.0	1.008	100.8	1.494	1.008±0.007
	1.5	1.490	99.33	0.891	1.49±0.009
	2.0	1.99	99.5	0.686	1.99±0.009

Table (8) :- continuous

Ciprocine injection vial (200 mg per vial)	-	-	-	-	-
	0.5	0.500	100.04	1.076	0.5000.007
	1.0	1.001	100.1	0.568	1.001±0.005
	1.5	1.499	99.99	0.571	1.499±0.006
	2.0	1.998	99.9	2.12	1.998±0.005
Ofloxacin tablets (200 mg per tablet)	-	-	-	-	-
	0.5	0.499	99.98	0.736	0.499±0.003
	1.0	0.992	99.25	1.0993	0.992±0.006
	1.5	1.495	99.68	0.545	1.495±0.004
	2.0	1.993	99.65	0.936	1.993±0.008
Ofloxin tablets (200 mg per tablet)	-	-	-	-	-
	0.5	0.500	100.02	1.088	0.500±0.004
	1.0	0.991	99.1	0.997	0.991±0.004
	1.5	1.507	100.46	0.764	1.507±0.005
	2.0	1.993	99.68	0.616	1.993±0.006
Oflocin tablets (200 mg per tablet)	-	-	-	-	-
	0.5	0.496	99.2	0.696	0.496±0.002
	1.0	1.003	100.34	1.128	1.003±0.004
	1.5	1.507	100.49	0.528	1.507±0.003
	2.0	2.02	101.0	0.656	2.02±0.007
Ofloxin eye drops (3 mg per ml)	-	-	-	-	-
	0.5	0.502	100.4	1.704	0.502±0.006
	1.0	0.998	99.8	0.745	0.998±0.004
	1.5	1.500	100.02	0.46	1.500±0.004
	2.0	2.01	100.5	1.0149	2.01±0.010

[1]: Relative standard deviation for six determinations.

[2]: 95% confidence limits and five degrees of freedom.

3. 1. 2. 9. Analytical applications

The validity of the proposed procedures is tested for determining Nor., Cipro. and Oflo., in pharmaceutical preparations manufactured in local companies as mentioned before.

The concentrations of the studied drugs in dosage forms were calculated from the appropriate calibration graph using the standard addition technique. There was no shift in the absorption maximum due to the presence of other constituents in the dosage forms. The results are compared with those obtained by applying the official methods. The results obtained were compared statistically by the student's t-test and variance ratio F-test with those obtained using the official method on the sample of the same batch. The student's t-test values obtained at 95% confidence level and five degrees of freedom did not exceed the theoretical tabulated value indicating no significant difference between the methods compared. The F-values also showed that there is no significant difference between accuracy of the proposed and the official method Table (9). The accuracy of the proposed method when applied to pharmaceutical preparations was evaluated by applying standard addition technique. in which variable amounts of the drugs Nor., Cipro. or Oflo., were added to the previously analysed portion of pharmaceutical preparations. The results shown in Table (10), confirm that the proposed method is not liable to interference by fillers (lactose monohydrate, microcrystallin cellulose, talc powder, explotab, sucrose, lysozyme, sorbitol, povidone, maize starch, sodium acetate, methyl - p-hydroxybenzoate, propyl p-hydroxybenzoate, hydroxy ethyl cellulose, flavours, magnesium stearate) usually formulated with the drugs under consideration. The proposed method is highly sensitive; therefore it could be used easily for routine analysis of both pure forms and pharmaceutical preparations.

Table (9): Evaluation of the accuracy and precision of the proposed and official methods for determination of Nor., Cipro., Oflo., in its pharmaceutical forms using EBT

Dosage forms	Official method			Proposed method				
	Taken mg	found* mg	Recovery (%)	Taken mg	found* mg	Recovery (%)	t** value	F** test
Epinor tablets (400 mg per tablet)	400	399	99.75	400	398.7	99.67	0.988	1.938
Noracin tablets (400 mg per tablet)	400	402	100.5	400	401.1	100.28	0.336	2.26
Ciprocine tablets (500 mg per tablet)	500	497.2	99.45	500	498.9	99.78	0.791	1.62
Ciprobay tablets (750 mg per tablet)	750	743	99.07	750	745.3	99.37	0.91	1.893
Ciprofloxacin tablets (500 mg per tablet)	500	498.5	99.7	500	499	99.8	1.59	3.23
Ciprocine eye drops(500 mg)	500	501.3	100.26	500	502.1	100.42	2.32	2.46
Ciprocine injection vial (200 mg per vial)	200	201.0	100.5	200	200.46	100.23	1.604	1.329
Ofloxacin tablets (200 mg per tablet)	200	198.0	99.0	200	199.2	99.6	1.946	1.39
Ofloxin tablets (200 mg per tablet)	200	198.7	99.35	200	199.74	99.87	1.28	1.057
Oflocin tablets (200 mg per tablet)	200	198.9	99.45	200	200.87	100.43	0.307	1.746
Ofloxin eye drops (3 mg per ml)	30	29.77	99.23	30	30.05	100.16	1.85	1.307

* : Average of six determinations.

** : Theoretical values for t- and F- values for five degree of freedom and 95% confidence limits are 2.57 and 5.05 , respectively .

Table (10): Determination of the studied drugs Nor., Cipro, Oflo, in its pharmaceutical dosage forms applying the standard addition technique using EBT

Dosage forms	Taken (µg/ml)	Added (µg/ml)	Found* (µg/ml)	Recovery (%)
Epinor tablets (400 mg per tablet)	1.0	0.0	0.998	99.8
		0.5	1.498	99.86
		1.0	2.009	100.46
		1.5	2.501	100.04
		2.0	2.974	99.13
Noracin tablets (400 mg per tablet)	1.0	0.0	0.999	99.9
		0.5	1.499	99.93
		1.0	2.012	100.6
		1.5	2.507	100.28
		2.0	3.004	100.13
Ciprocine tablets (500 mg per tablet)	1.0	0.0	0.997	99.7
		0.5	1.485	99.0
		1.0	1.982	99.1
		1.5	2.483	99.34
		2.0	2.967	98.9
Ciprobay tablets (750 mg per tablet)	1.0	0.0	1.008	100.8
		0.5	1.488	99.22
		1.0	2.004	100.2
		1.5	2.518	100.73
		2.0	2.983	99.44
Ciprofloxacin tablets (500 mg per tablet)	1.0	0.0	1.001	100.1
		0.5	1.515	101.0
		1.0	2.022	101.1
		1.5	2.497	99.88
		2.0	2.498	99.93
Ciprocine eye drops(500 mg)	1.0	0.0	1.002	100.2
		0.5	1.501	100.07
		1.0	1.987	99.35
		1.5	2.497	99.88
		2.0	3.003	100.1

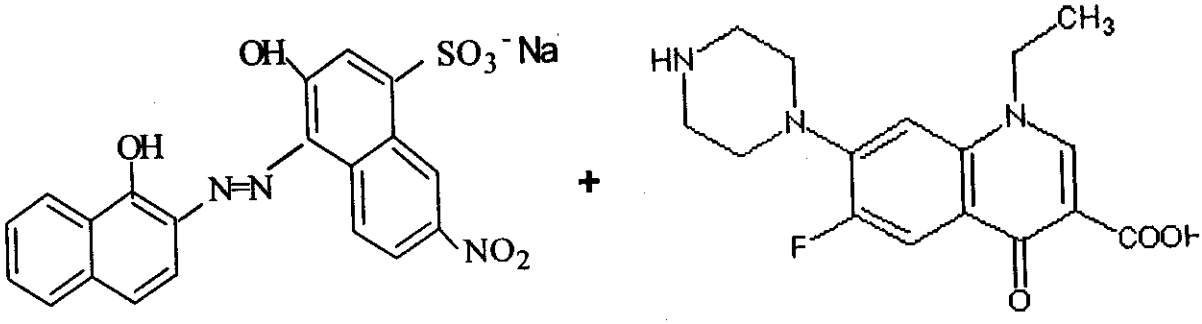
Table (10):- continuous

Ciprocine injection vial (200 mg per vial)	1.0	0.0	1.004	100.4
		0.5	1.499	99.93
		1.0	2.02	101.0
		1.5	2.522	100.88
		2.0	2.999	99.96
Ofloxacin tablets (200 mg per tablet)	1.0	0.0	0.991	99.1
		0.5	1.496	99.73
		1.0	2.006	100.3
		1.5	2.494	99.76
		2.0	2.975	99.16
Ofloxin tablets (200 mg per tablet)	1.0	0.0	1.005	100.5
		0.5	1.488	99.2
		1.0	2.001	100.05
		1.5	2.487	99.48
		2.0	2.998	99.93
Oflocin tablets (200 mg per tablet)	1.0	0.0	1.002	100.26
		0.5	1.504	100.2
		1.0	1.98	99.0
		1.5	2.498	99.92
		2.0	2.993	99.76
Ofloxin eye drops (3 mg per ml)	1.0	0.0	0.992	99.2
		0.5	1.494	99.63
		1.0	2.004	100.02
		1.5	2.505	100.03
		2.0	2.998	99.93

*: Average of six determinations.

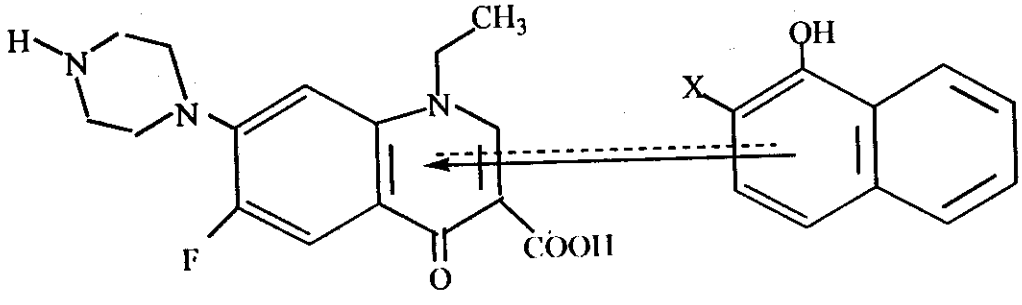
3. 1. 2. 10. Suggested mechanism

Drug – reagent reaction can be stated that the addition compound is formed through a charge transfer from the reagent (Calcon, Erioch or Aliz.) as electron donor to the drug (Nor., Cipro. or Oflo.) as electron acceptor. Charge transfer (CT) complex formed (Erioch. with drugs) exhibits maximum absorbance at λ_{max} 505.5 nm in case of Nor., 505.5 nm in case of Cipro., and 504.5 nm in case of Oflo., as shown by the mechanism in **Fig (20)**.



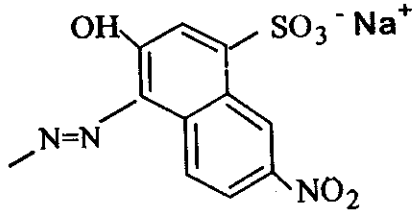
Eriochromeblack T **Norfloxacin**

pH = 4. 28



Charge transfer complex

Where x =



3.1.3. Absorption spectra of the studied drugs with Alizarin red S (Aliz.)

In order to investigate the optimum conditions for the development of the ion-pair complex formed between the studied drugs and Alizarin red S (5.0×10^{-3}M), some parameters were studied and recorded below.

3.1.3.1. Effect of pH

In order to establish the optimum pH value for each ion-pair formed, Nor., Cipro. and Oflo. was allowed to react with the Alizarin red S in aqueous buffered solution of the pH ranges (1.8 -12.0).The absorbance was measured at its λ_{max}.

The highest absorbance values were obtained at pH 2.7 in case of the three drugs, which are selected for ion-pairs formation. These results are shown in Fig. (21). Furthermore, the amount of buffer added was examined and found to be 5 ml for all complexes as shown in Fig. (22). The optimum wavelength corresponding to each ion-pair complex of the drugs with Alizarin red S at 515, 525and 554 nm in case of Nor., Cipro, and Oflo., respectively, as shown in Fig. (23),

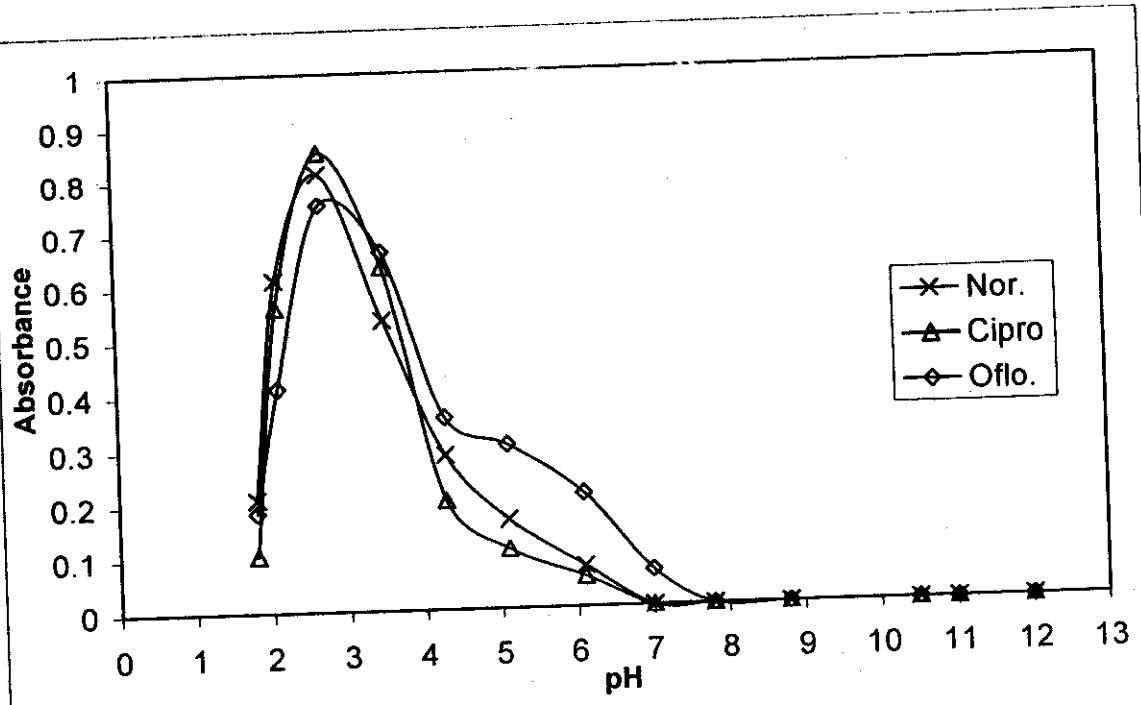


Fig.(21): Effect of pH on the absorbance of the studied drugs using Aliz. (5.0×10^{-3} M)

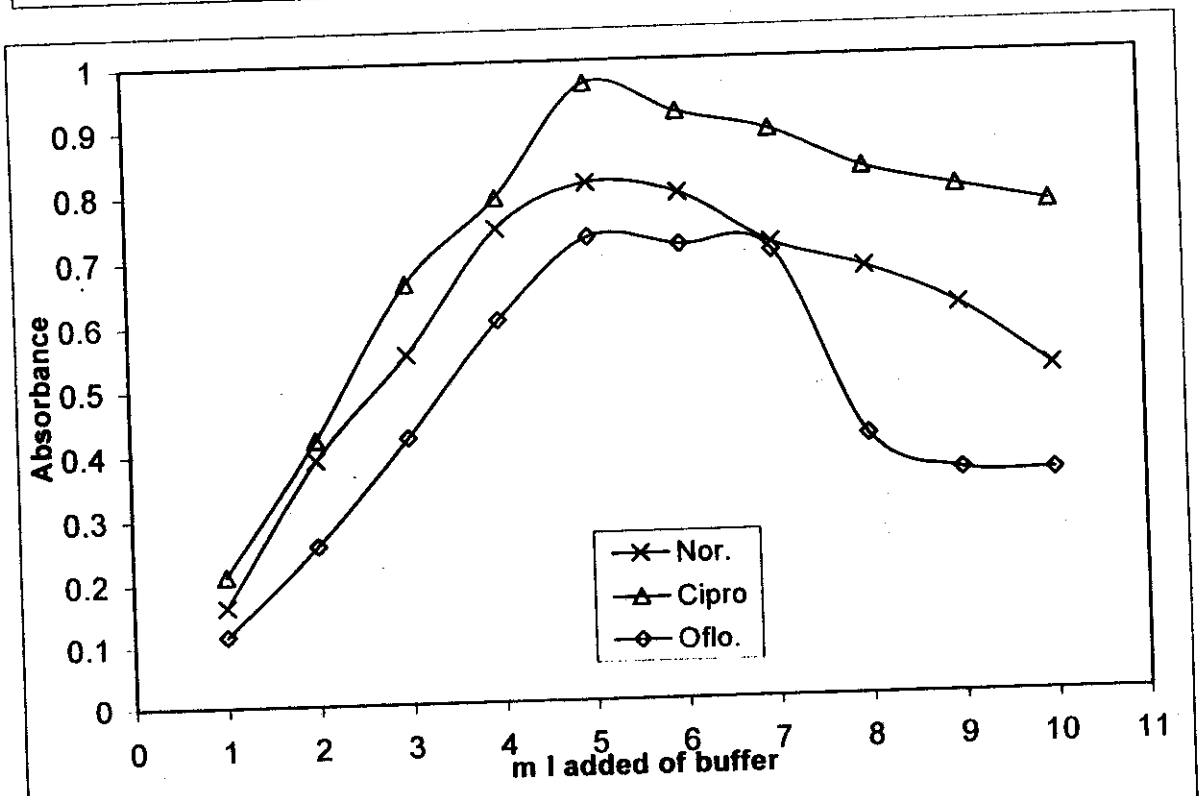


Fig.(22): Effect of ml added of buffer on the absorbance of the studied drugs using Aliz. (5.0×10^{-3} M)

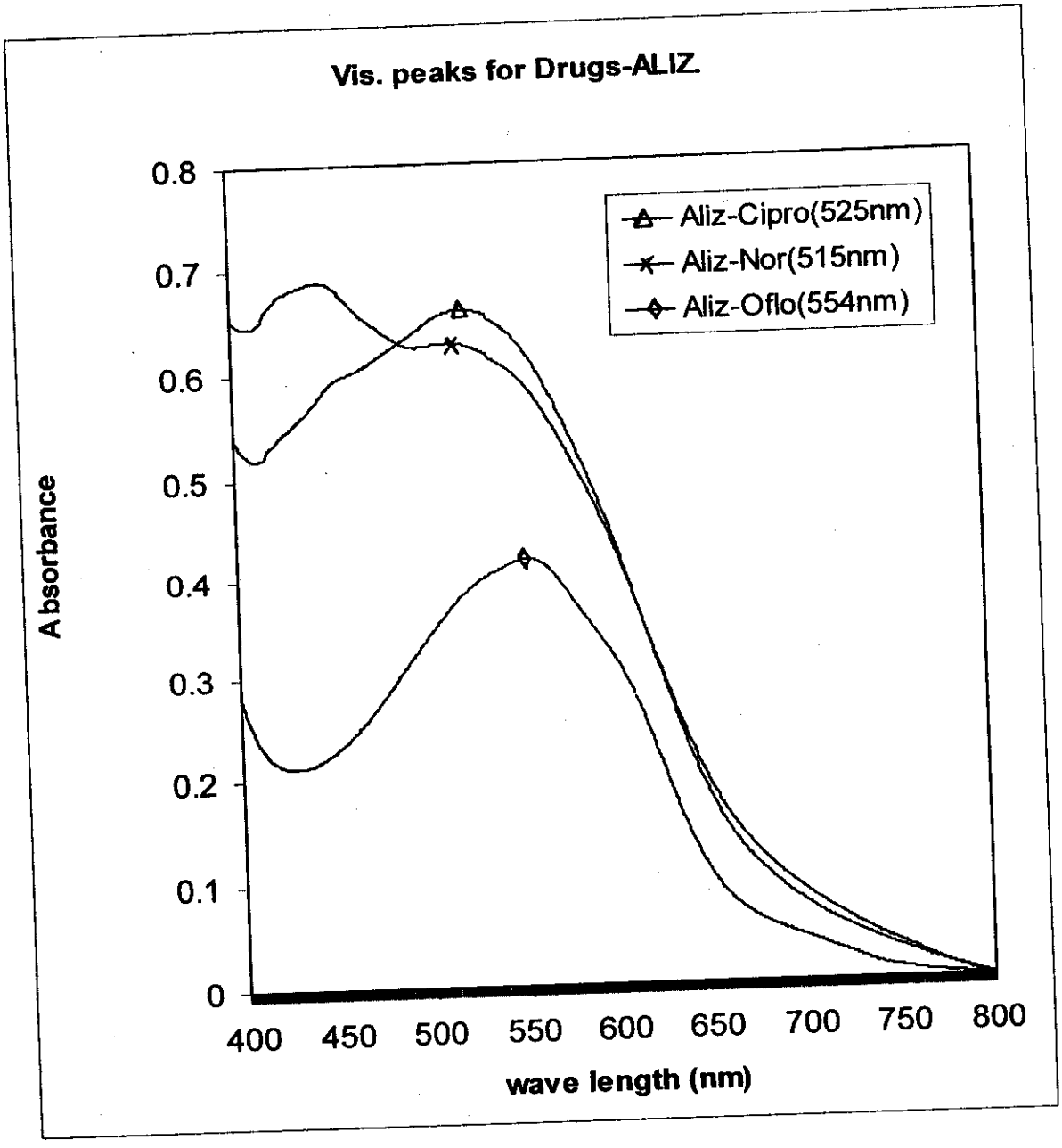


Fig. (23): Absorption of new complexes of the studied drugs with acid dye Aliz. (5.0×10^{-3} M)

3. 1. 3. 2. Effect of shaking time

The time required for complete colour development of the ion-pair complex formed between Nor., Cipro. or Oflo. and Alizarin red S was investigated. Allowing the reactants to stand and shaking for different time intervals it was observed that, shacking time needed to form precipitates between the aqueous and organic layers (new complex), has an affect on the amount of precipitate, consequently the maximum colour intensity. The shaking time required for complete colour development of ion-pair formed between the drugs and aliz. was investigated., it was observed that 6.0 min are quite sufficient to obtain maximum amount of precipitate which is dissolved in acetone to give measure maximum colour intensity. The formed ion-pairs were found to be stable for more than 24 hours after soluble in acetone and for more time as solid in drugs Nor., Cipro. or Oflo., as shown in fig. (24).

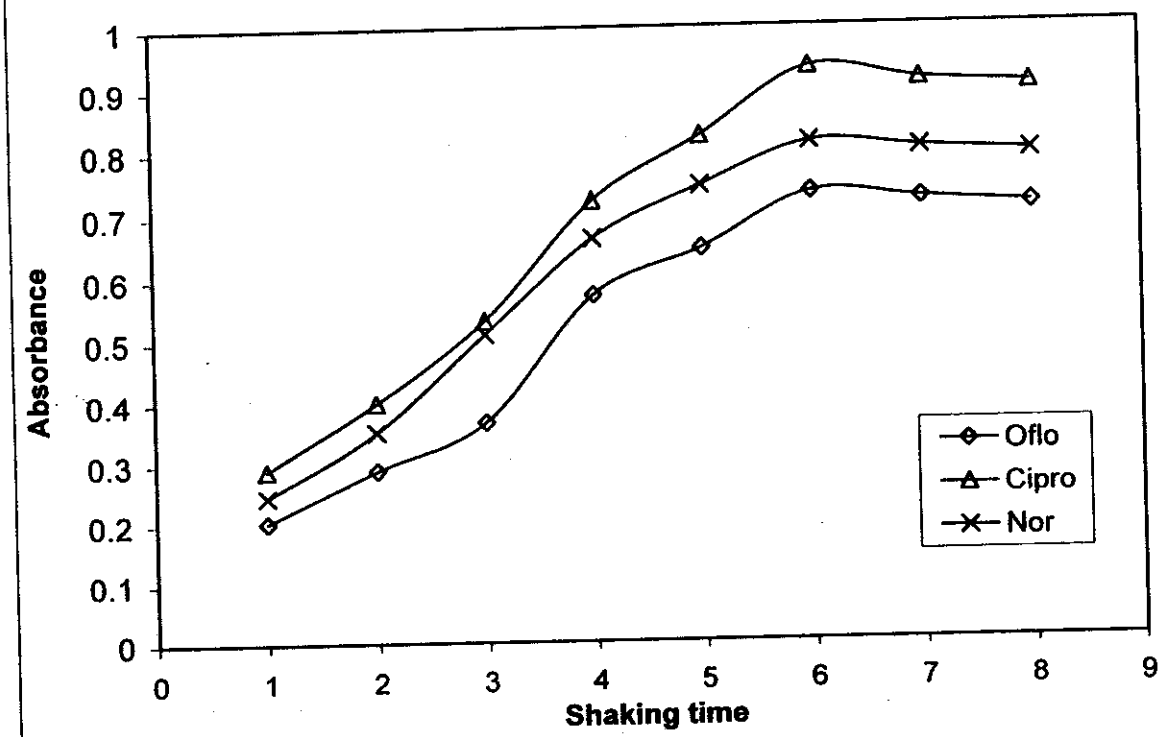


Fig.(24) :Effect of shaking time on the absorbance of the studied drugs solution using Aliz. (5.0×10^{-3} M)

3. 1. 3. 3. Effect of the polarity of extracting solvent

The polarity of the solvent affects both extraction efficiency and absorbance intensity. The results using different extracting solvents (condensate water, benzene, chloroform, carbontetrachloride, hexane, dioxan), applying the Alizarin red S reagent on the drugs under consideration indicated that, chloroform is the best solvent for extraction in case of Nor., Cipro. or Oflo. were selected due to their slightly higher sensitivity and the considerably lower extraction of the reagent itself. Complete extraction was attained by extraction with 25 ml of the solvent in one batch.

3. 1. 3. 4. Effect of reagent concentration

When various concentrations of Aliz. were added to a fixed concentrations of Nor., Cipro. and Oflo., 10 ml of Alizarin red S (5.0 x 10^{-3} M) solutions with chloroform solvent as shown in Fig. (25) were found to be sufficient for the production of maximum and reproducible colour intensity. Higher concentration of the reagent decreased the absorbance and colour intensity of the formed ion-pair.

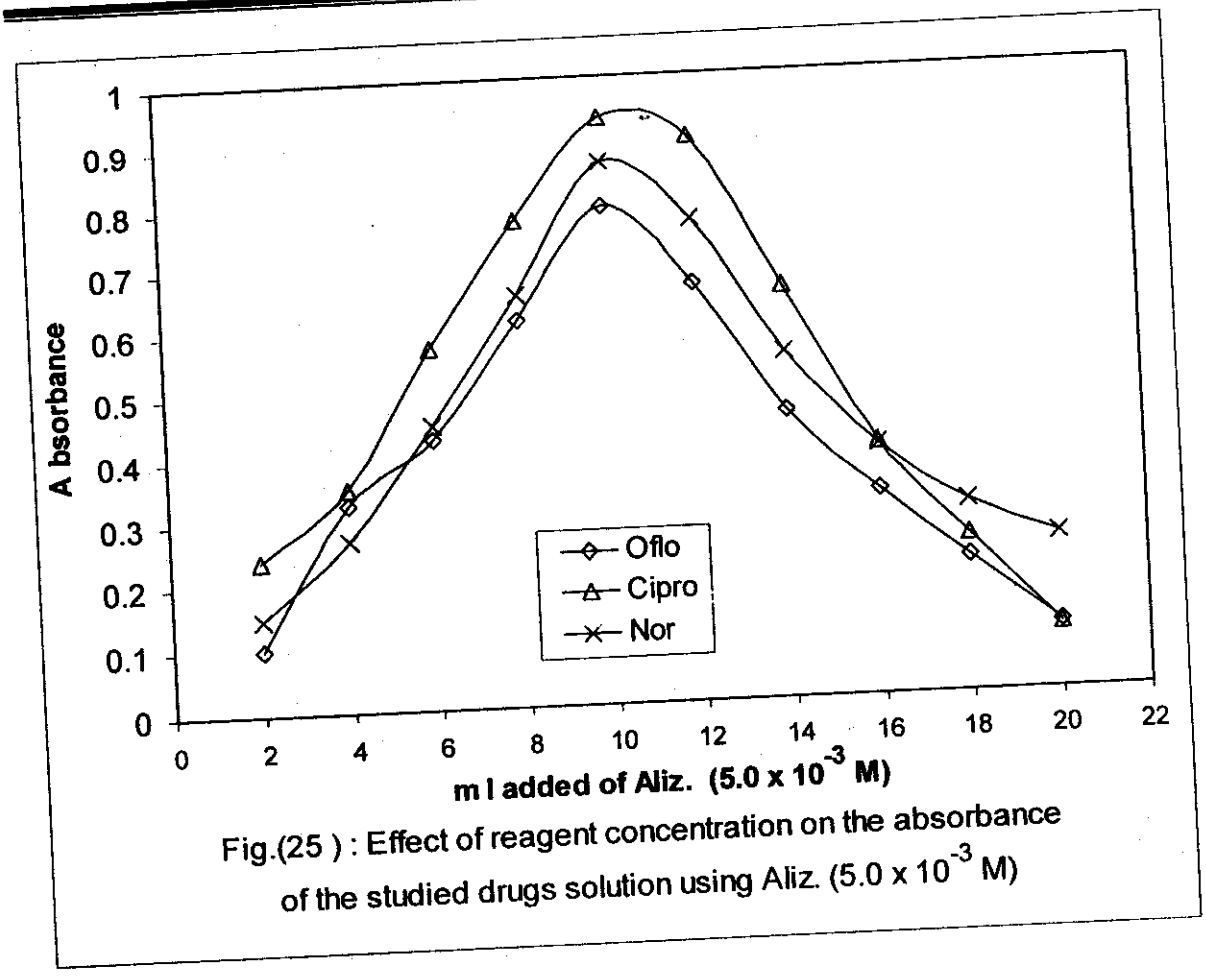


Fig.(25) : Effect of reagent concentration on the absorbance of the studied drugs solution using Aliz. (5.0×10^{-3} M)

3. 1. 3. 5. MoLar ratio of the complexes

In order to investigate the molar ratio of the complexes formed between the drugs under investigation and Aliz. at the selected conditions, the molar ratio[72] and continuous variation methods[73 - 75] were utilised. The results indicated that the molar ratio of the drugs to dye was (1:1) in all ion-pairs formed. The shape of the curves indicated that the complexes were labile, as shown in Fig's. (26,27) .The stability constants of the complex were calculated by using the data of the molar ratio[72] and **Job's** continuous variation methods[73] applying **Issa** modification equation[75].The results of the stability constants are recorded in Table(11).

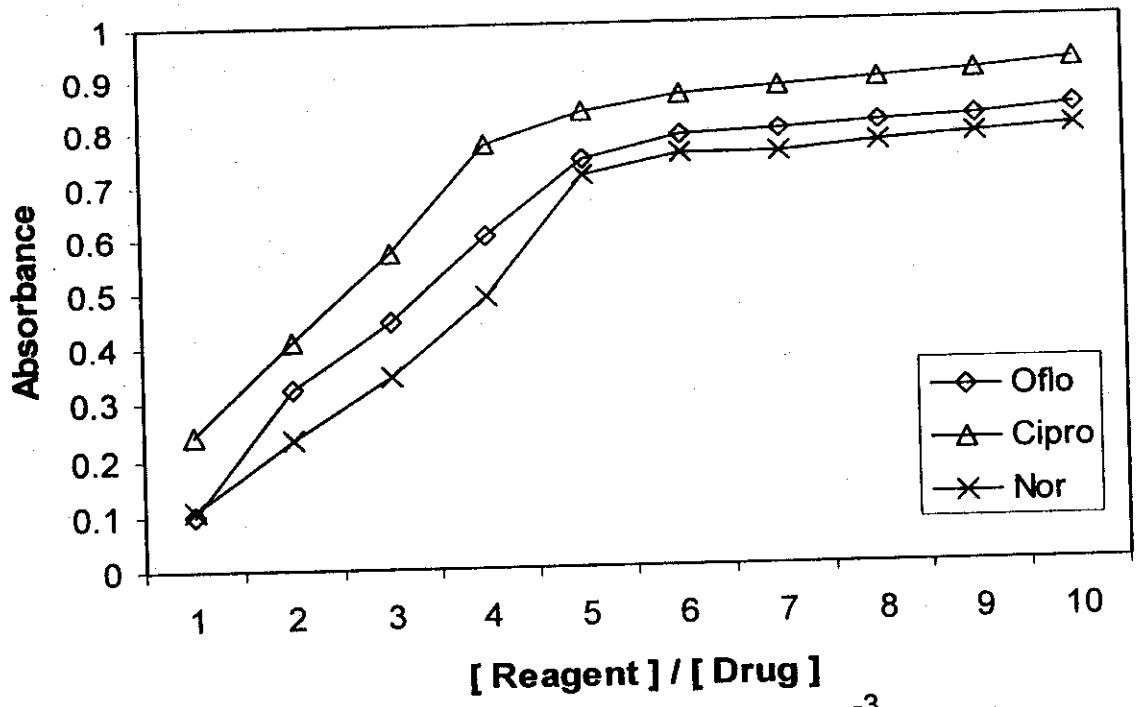


Fig.(26): Molar ratio for Aliz- drugs (5.0×10^{-3} M) under consideration

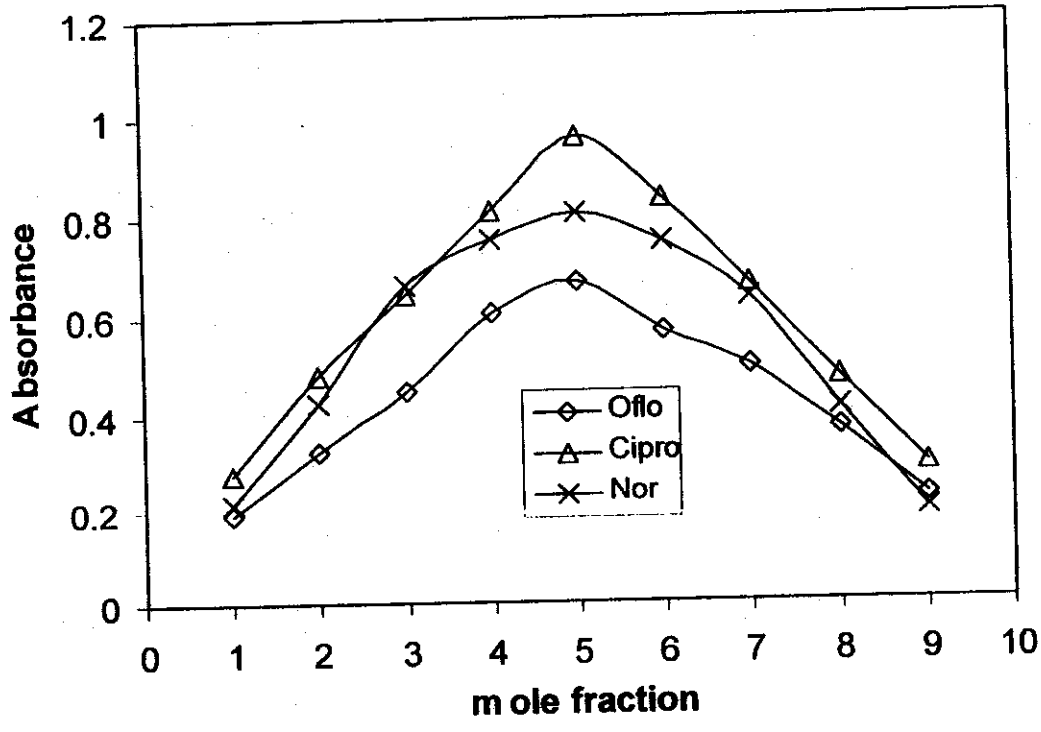


Fig.(27): continuous variation using Aliz. (5.0×10^{-3} M) reagent with (5.0×10^{-3} M) of the drugs

Table (11): Analytical data and characteristics of colored product, precision and accuracy, of the studied drugs using Alizarin red S

Parameters	Alizarin red S		
	Nor.	Cipro.	Oflo.
pH	2.73	2.73	2.73
Wavelength max.(nm)	515	525	554
Stability constant (Log K_F)	8.27	8.8	9.2
Beer's law limits (µg / ml)	0.17-3.3	0.17-3.3	0.17-3.3
Ringbom limits (µg / ml)	0.25-3.3	0.25-3.3	0.25-3.3
Slope (b)	0.224	0.252	0.306
Intercept (a)	-0.006	0.007	0.019
Standard deviation (SD)	0.0053	0.004	0.0039
Correlation coefficient (r)	0.9998	0.9997	0.9997
Detection limit (µg / ml)	0.016	0.012	0.0117
Quantification limit (µg / ml)	0.0527	0.041	0.039
Molar absorptivityx10^5 ($mol^{-1}cm^{-1}$)	0.878	0.834	1.105
Sandell sensitivity (µg cm^{-2})	0.0045	0.004	0.0033
Error* %	0.215	0.164	0.159
RSD %	1.428	0.932	0.724
RE %	1.498	0.979	0.759

*: Average of six determinations.

3. 1. 3. 6. Validity to Beer's law

Under optimum conditions of pH, time, solvent and reagent concentration, some drugs react with anionic dyes to form ion-pair complexes, which are often coloured and can subsequently measured colorimetrically. This character is applied, for the determination of Nor., Cipro. and Oflo. through measuring the absorbance of the formed coloured ion-pair at corresponding optimum wavelength, using Alizarin red S. Various parameters affecting the reaction development were studied. A calibration graph was constructed using standard solutions of Nor., Cipro. or Oflo under the optimum conditions, a linear relationship was obtained between the absorbance and concentration of the drugs within the range listed in Table (11). The correlation coefficient, slopes and intercepts, standard deviation, relative standard deviation and relative error of the calibration data for Nor., Cipro. and Oflo. are calculated using the equations given above on pages 87-88-89.

The reproducibility of the method was determined by running six replicate samples, each containing 4.0 µg/ml of drug in case of Nor., 5.0 µg/ml in case of Cipro. and 4.0 µg/ml in case of Oflo. At this concentration, the relative standard deviation was found to be ≤ 1.428 % as shown in Table (11). For more accurate results, Ringbom optimum concentration range was determined by plotting log [C] in µg/ml against percent transmittance and the linear portion of the S-shaped curve gave the accurate range of analysis Fig. (28) and the results are recorded in Table (11). The mean molar absorptivity. Sandell sensitivity, detection and quantification limits are calculated from the standard deviation of the absorbance measurements obtained from Beer's law and recorded in Table (11). Representative curves on the validity to Beer's law for Aliz. , are shown in Fig's. (28-29).

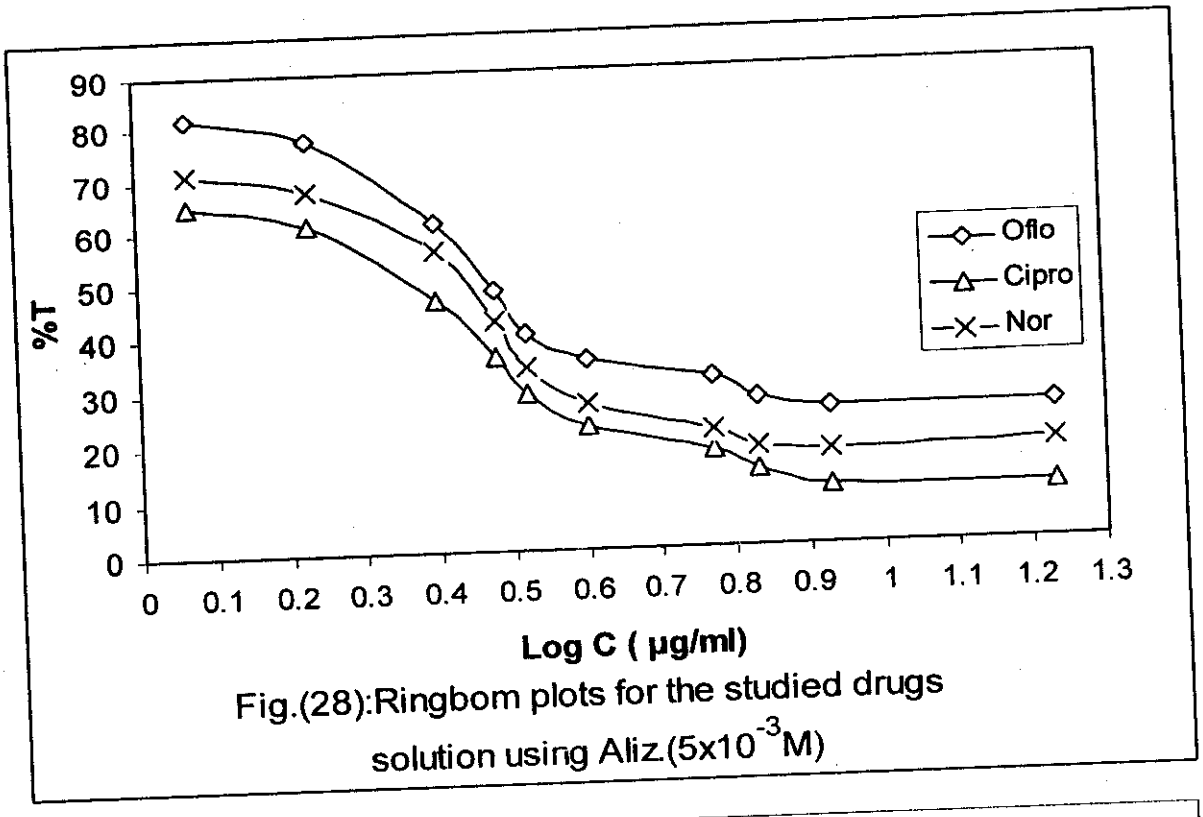


Fig.(28):Ringbom plots for the studied drugs solution using Aliz.($5x10^{-3}$M)

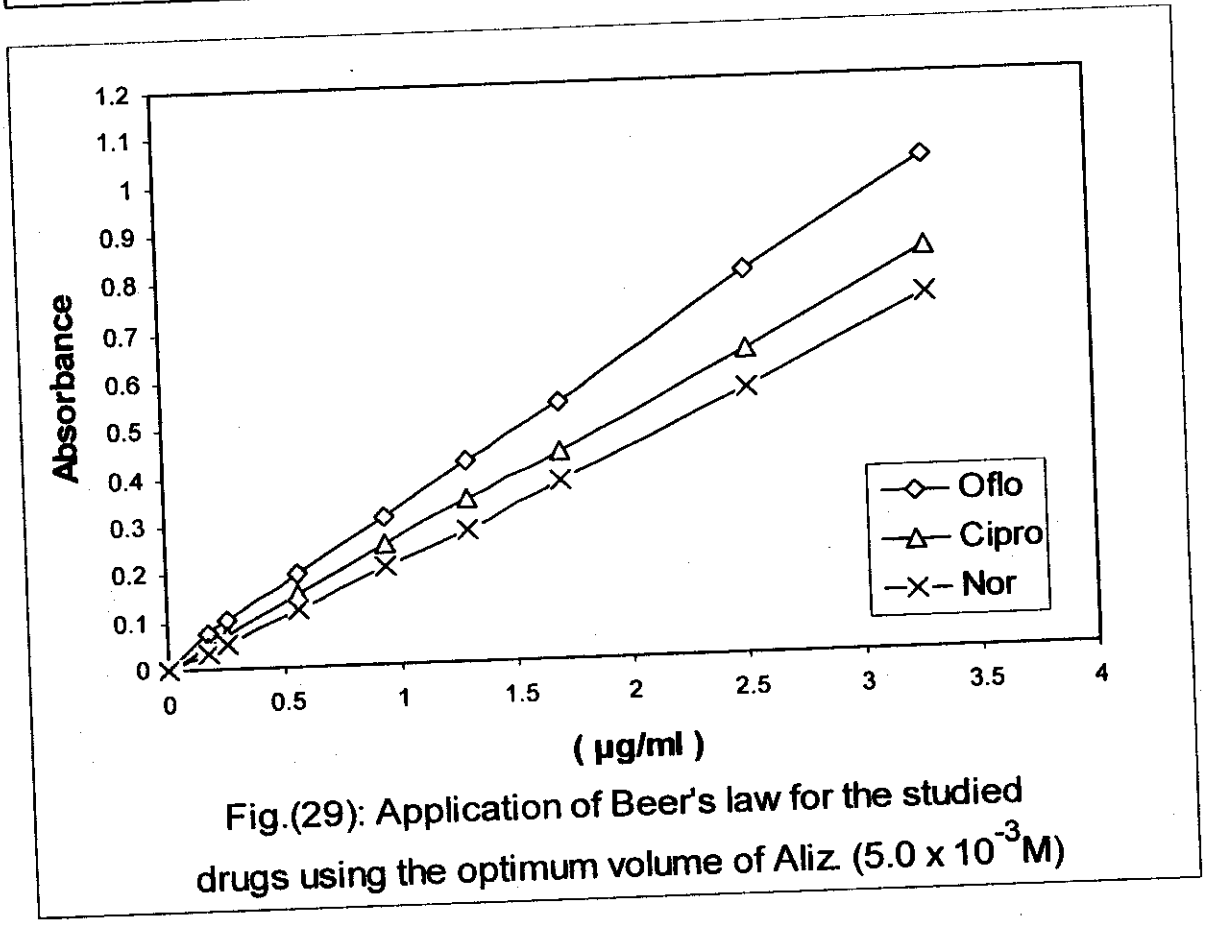


Fig.(29): Application of Beer's law for the studied drugs using the optimum volume of Aliz. (5.0×10^{-3}M)

3. 1. 3. 7. Accuracy and Precision

In order to determine the accuracy and precision of the proposed methods, solutions containing three different concentrations of Nor., Cipro. or Oflo. were prepared and analysed in six replicates. The analytical results obtained from this investigation are summarized in Table (12). The percent standard deviations and the percentage range of error at 95% confidence level were calculated. The results are considered as very satisfactory, at least for the level of concentrations examined.

Table (12): Evaluation of the accuracy and precision of the proposed method using Aliz.

Drugs	Taken (µg/ml)	Found (µg/ml)	Recovery (%)	RSD[1] (%)	RE (%)	Confidence[2] limit
Norfloxacin	1.0	1.005	100.5	1.191	1.249	1.005±0.007
	2.0	1.996	99.9	0.725	0.76	1.996±0.006
	3.0	3.001	100.03	1.367	1.434	3.001±0.008
Ciprofloxacin	1.0	0.99	99.0	1.724	1.809	0.99±0.009
	2.0	1.987	99.35	0.473	0.496	1.987±0.005
	3.0	2.97	99.0	0.513	0.538	2.97±0.007
Ofloxacin	1.0	0.986	98.6	0.461	0.484	0.986±0.004
	2.0	2.012	100.6	0.473	0.497	2.012±0.004
	3.0	2.983	99.43	0.826	0.867	2.983±0.008

[1]: Relative standard deviation for six determinations.

[2]: 95% confidence limits and five degrees of freedom.

3. 1. 3. 8. Determination of the studied drugs in urine samples by using Alizarin red S.

10 ml of the urine aliquot were transferred into 50 mL separating funnel and mixed with 10 ml of Alizarin red S (5×10^{-3} M) in case of Nor., Cipro. and Oflo., followed by 5 ml of buffer solution of pH 2.7 . The volume was completed to 50 ml with chloroform to extract the formed complexes with 6 min,shaking time at room temperature (25 °C). After shaking, precipitates were formed in the three drugs between aqueous and organic layers and colour changes from violet to red. The precipitates were filtered, dried and turned soluble in acetone; time has no affect in this step and the colour become stable for more than 24 hours. The absorbance was measured following the general procedure described above. The relative standard deviation (RSD), recovery and confidence limits of the studied drugs are computed and recorded as shown in Table (13).

Table (13): Evaluation of the accuracy and precision of the proposed method for investigated of pharmaceutical forms of Nor., Cipro, Oflo., using Alizarin red S.

Dosage forms	Added (µg/ml)	Found (µg/ml)	Recovery (%)	RSD[1] (%)	Confidence[2] limits
Epinor tablets (400 mg per tablet)	-	-	-	-	-
	0.5	0.502	100.4	1.204	0.502± 0.004
	1.0	1.01	101.0	1.047	1.01± 0.005
	1.5	1.51	100.66	1.847	1.51± 0.013
	2.0	1.991	99.55	0.41	1.991± 0.004
Noracin tablets (400 mg per tablet)	-	-	-	-	-
	0.5	0.5005	100.1	1.774	0.5005± 0.005
	1.0	1.001	100.1	1.755	1.001± 0.006
	1.5	1.4983	99.89	1.4883	1.4983 ± 0.007
	2.0	1.9826	99.13	1.299	1.9826 ± 0.008
Ciprocine tablets (500 mg per tablet)	-	-	-	-	-
	0.5	0.5003	100.06	1.602	0.5003± 0.005
	1.0	0.9895	98.95	1.527	0.9895± 0.008
	1.5	1.4874	99.16	0.741	1.4874± 0.005
	2.0	1.9962	99.81	0.964	1.9962± 0.008
Ciprobay tablets (750 mg per tablet)	-	-	-	-	-
	0.5	0.4996	99.92	1.461	0.4996 ± 0.008
	1.0	1.0067	100.67	1.76	1.0067± 0.007
	1.5	1.5024	100.16	0.522	1.5024 ± 0.004
	2.0	1.9966	99.83	0.917	1.9966 ± 0.009
Ciprofloxacin tablets (500 mg per tablet)	-	-	-	-	-
	0.5	0.4987	99.74	0.79	0.4987± 0.003
	1.0	0.995	99.5	1.346	0.995 ± 0.007
	1.5	1.5135	100.9	0.876	1.5135 ± 0.008
	2.0	2.02	101.0	0.839	2.02 ± 0.011
Ciprocine eye drops (500 mg)	-	-	-	-	-
	0.5	0.5028	100.56	0.936	0.5028 ± 0.004
	1.0	0.9923	99.23	0.577	0.9923 ± 0.003
	1.5	1.4944	99.63	1.025	1.4944± 0.01
	2.0	2.010	100.51	0.686	2.010 ± 0.010

Table (13):- continuous

Ciprocine injection vial (200 mg per vial)	-	-	-	-	-
	0.5	0.501	100.2	0.97	0.501 ± 0.007
	1.0	0.988	98.8	0.682	0.988 ± 0.006
	1.5	1.5066	100.44	0.671	1.5066 ± 0.007
	2.0	2.0086	100.43	0.924	2.0086 ± 0.005
Ofloxacin tablets (200 mg per tablet)	-	-	-	-	-
	0.5	0.4999	99.99	0.892	0.4999 ± 0.003
	1.0	0.9994	99.94	1.51	0.9994 ± 0.008
	1.5	1.4827	98.85	0.852	1.4827 ± 0.006
	2.0	1.98	99.0	0.765	1.98 ± 0.008
Ofloxin tablets (200 mg per tablet)	-	-	-	-	-
	0.5	0.495	99.0	1.006	0.495 ± 0.004
	1.0	0.997	99.7	1.1	0.997 ± 0.006
	1.5	1.5084	100.56	0.639	1.5084 ± 0.005
	2.0	2.001	100.05	0.76	2.001 ± 0.008
Oflocin tablets (200 mg per tablet)	-	-	-	-	-
	0.5	0.5037	100.74	1.6	0.5037 ± 0.006
	1.0	0.9926	99.26	0.88	0.9926 ± 0.004
	1.5	1.4923	99.49	0.567	1.4923 ± 0.005
	2.0	1.9978	99.89	0.578	1.9978 ± 0.009
Ofloxin eye drops (3 mg per ml)	-	-	-	-	-
	0.5	0.499	99.8	1.44	0.499± 0.007
	1.0	0.9968	99.68	1.022	0.9968 ± 0.006
	1.5	1.515	101.0	0.68	1.515 ± 0.005
	2.0	2.002	100.1	0.92	2.002 ± 0.010

[1]: Relative standard deviation for six determinations.

[2]: 95% confidence limits and five degrees of freedom.

3. 1. 2. 9. Analytical applications

The validity of the proposed procedures is tested for determining Nor., Cipro. and Oflo., in pharmaceutical preparations manufactured in local companies as mentioned before. The concentrations of the studied drugs in dosage forms were calculated from the appropriate calibration graph using the standard addition technique. There was no shift in the absorption maximum due to the presence of other constituents in the dosage forms. The results are compared with those obtained by applying the official methods. The results obtained were compared statistically by the student's t- test and variance ratio F-test with those obtained using the official method on the sample of the same batch. The student's t - test values obtained at 95% confidence level and five degrees of freedom did not exceed the theoretical tabulated value indicating no significant difference between the methods compared. The F-values also showed that there is no significant difference between the accuracy of the proposed and the official method Table (14). The accuracy of the proposed method when applied to pharmaceutical preparations is evaluated by applying the standard addition technique. in which variable amounts of the drugs Nor., Cipro. or Oflo., were added to the previously analysed portion of pharmaceutical preparations. The results shown in Tables (14,15) , confirm that the proposed method is not liable to interference by fillers (lactose monohydrate, microcrystallin cellulose, talc powder, explotab, sucrose, lysozyme, sorbitol, povidone, maize starch, sodium acetate, methyl-p-hydroxybenzoate, propyl -p-hydroxybenzoate, hydroxy ethyl cellulose, flavours, magnesium stearate) usually formulated with the drugs under consideration. The proposed method is highly sensitive, therefore it could be used easily for routine analysis of both pure forms and pharmaceutical preparations of the drugs considered.

Table (14) : Evaluation of the accuracy and precision of the proposed and official methods for determination of Nor., Cipro., Oflo., in its pharmaceutical forms using Aliz.

Dosage forms	Official method			Proposed method				
	Taken mg	found* mg	Recovery (%)	Taken mg	found* mg	Recovery (%)	t** value	F** test
Epinor tablets (400 mg per tablet)	400	395.16	98.79	400	397.56	99.39	1.12	1.727
Noracin tablets (400 mg per tablet)	400	397.84	99.46	400	401.56	100.39	2.08	2.392
Ciprocine tablets (500 mg per tablet)	500	501.7	100.34	500	503.4	100.68	1.81	2.55
Ciprobay tablets (750 mg per tablet)	750	744.975	99.33	750	746.85	99.58	1.94	1.67
Ciprofloxacin tablets (500 mg per tablet)	500	499.4	99.88	500	498.1	99.62	2.24	1.97
Ciprocine eye drops(500 mg)	500	498.15	99.63	500	500.5	100.1	2.13	1.685
Ciprocine injection vial (200 mg per vial)	200	198	99.0	200	199.46	99.73	1.74	1.76
Ofloxacin tablets (200 mg per tablet)	200	197.86	98.93	200	200.38	100.19	2.16	1.91
Ofloxin tablets (200 mg per tablet)	200	201.02	100.51	200	200.46	100.23	0.92	1.5
Oflocin tablets (200 mg per tablet)	200	200.32	100.16	200	199.3	99.65	1.107	1.22
Ofloxin eye drops (3 mg per ml)	30	29.97	99.9	30	29.955	99.85	0.75	2.18

* : Average of six determinations.

** : Theoretical values for t- and F- values for five degree of freedom and 95% confidence limits are 2.57 and 5.05 , respectively .

Table (15): Determination of the studied drugs Nor., Cipro, Oflo, in its pharmaceutical dosage forms applying the standard addition technique using Alizarin red S.

Dosage forms	Taken (µg/ml)	Added (µg/ml)	Found* (µg/ml)	Recovery (%)
Epinor tablets (400 mg per tablet)	1.0	0.0	1.001	100.1
		0.5	1.499	99.93
		1.0	1.997	99.84
		1.5	2.501	100.1
		2.0	2.967	98.89
Noracin tablets (400 mg per tablet)	1.0	0.0	0.992	99.23
		0.5	1.498	99.88
		1.0	1.996	99.79
		1.5	2.501	100
		2.0	3.03	101
Ciprocine tablets (500 mg per tablet)	1.0	0.0	1.001	100.1
		0.5	1.495	99.66
		1.0	1.976	98.81
		1.5	2.478	99.1
		2.0	2.976	99.19
Ciprobay tablets (750 mg per tablet)	1.0	0.0	1.001	100.1
		0.5	1.513	100.9
		1.0	1.98	98.99
		1.5	2.477	99.09
		2.0	2.983	99.42
Ciprofloxacin tablets (500 mg per tablet)	1.0	0.0	1.004	100.4
		0.5	1.495	99.69
		1.0	1.986	99.3
		1.5	2.505	100.2
		2.0	3.025	100.8
Ciprocine eye drops(500 mg)	1.0	0.0	0.995	99.49
		0.5	1.485	99
		1.0	1.995	99.75
		1.5	2.484	99.35
		2.0	2.988	99.61

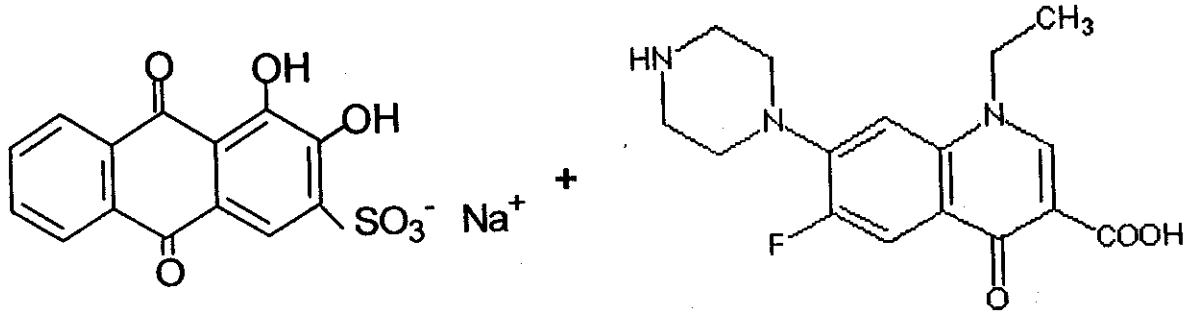
Table (15):- continuous

Ciprocine injection vial (200 mg per vial)	1.0	0.0	1.009	100.9
		0.5	1.495	99.66
		1.0	1.995	99.77
		1.5	2.506	100.2
		2.0	3.015	100.5
Ofloxacin tablets (200 mg per tablet)	1.0	0.0	0.995	99.54
		0.5	1.492	99.44
		1.0	1.998	99.88
		1.5	2.48	99.21
		2.0	2.964	98.8
Ofloxin tablets (200 mg per tablet)	1.0	0.0	1.003	100.3
		0.5	1.499	99.95
		1.0	2.007	100.3
		1.5	2.475	99.0
		2.0	2.992	99.72
Oflocin tablets (200 mg per tablet)	1.0	0.0	1.007	100.7
		0.5	1.506	100.4
		1.0	1.998	99.91
		1.5	2.495	99.8
		2.0	3.005	100.2
Ofloxin eye drops (3 mg per ml)	1.0	0.0	0.99	99.0
		0.5	1.499	99.9
		1.0	2.002	100.1
		1.5	2.505	100.2
		2.0	2.979	99.3

*: Average of six determinations.

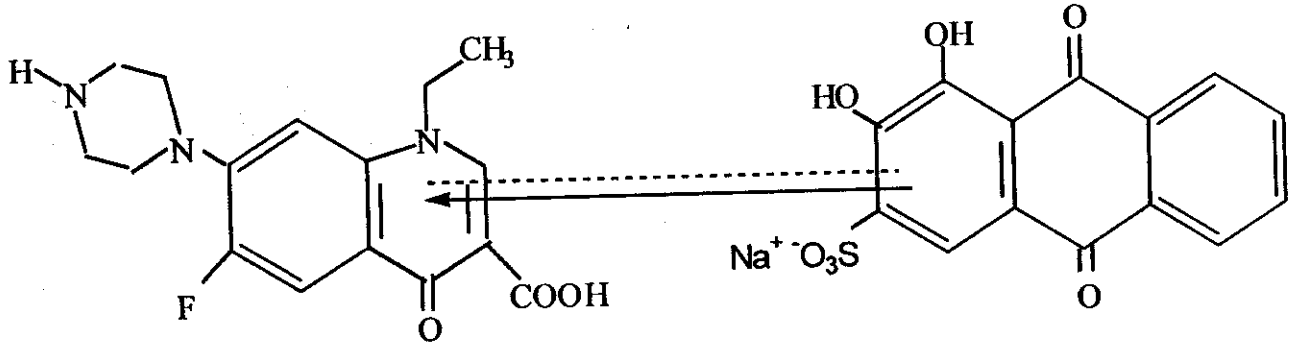
3. 1. 3. 10. Suggested mechanism

Drug – reagent reaction can be stated that the addition compound is formed through a charge transfer from the reagent (Calcon, Erioch or Aliz.) as electron donor to the drug (Nor., Cipro. or Oflo.) as electron acceptor. CT complex formed (Aliz. with drugs) exhibits maximum absorbance at λ_{max} 515, 525 and 554 nm in case of Nor., Cipro.and Oflo., respectively as shown by the mechanism in **Fig (30)**.



Alizarin red S **Norfloxacin**

pH = 2.73



Charge transfer complex

Support for the type of formed complex

The reaction of the drugs under study with the reagents used leads to some obvious colour changes. The colour change was considered by many authors to be due to the formation of some sort of ion pairs between the anion of one component and the cation of the other. As shown above the ion pair formation was represented for the reactions concerned.

As a matter of fact, an electrostatic attraction between the ions to form the ion pair, although can cause some electronic polarizations in both ions forming the ion pair, yet such type of interaction can not lead to obvious coloration and hence drastic changes of absorption spectra (figs.3, 13,23 and 31). The figure denotes the appearance of some new bands, which are not present in the original spectra of the drugs or reagents.

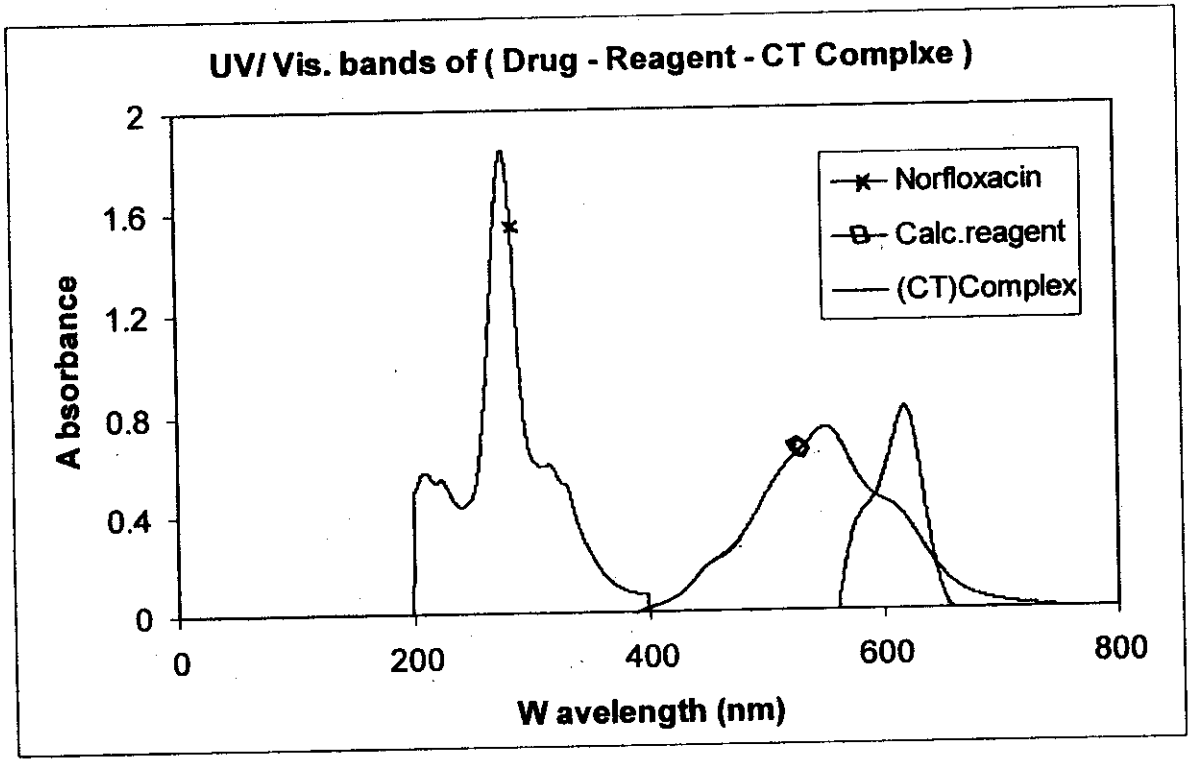


Fig. (31): The electronic absorption spectra of Norfloxacin – Calconcarboxylic acid reagent – new CT complex

The appearance of the new bands can be attributed to the occurrence of a charge transfer between the two components of the ion pair

$$\underset{\text{Cation}}{C^+} + \underset{\text{anion}}{A^-} \rightarrow \underset{\text{ion pair}}{C^+ \ldots\ldots A^-} \rightarrow \underset{\text{ion pair CT complex}}{C^+ \ldots\ldots \leftarrow \ldots\ldots A^-}$$

To obviate the origin of the CT interaction, the ionization potentials of the two components of the ion pair (CT complex) were determined from the electronic absorption spectra of the respective anions and cations using the relation:-

$$I_p = a + b\,E$$

where a and b are constants having the values (4.39 and 0.587)[76] , (5.158 and 0.778)[77], or (5.011and 0.7011)[78] and E is the energy of the $S_0 \rightarrow S_1$ electronic transition in .e.v.

The drugs exhibit the same wavelength, so they have similar ionization potentials. The reagents on the contrary have different spectra and accordingly different ionization potentials. The mean values thus obtained for the ionization potentials of the reagents and the drugs are given in the following table.

table (16):- Ionization potentials of the drugs and reagents

	CALCON	EBT	Aliz.	Drugs (Nor., Cipro. Oflo.)
I_p (e.v)	6.642	6.718	6.692	7.768

The data reveal that the I_p values for the reagents are lower than those of the drugs. Accordingly, it is expected that the charge transfer originates from the reagent anion to the cation of the drug within the ion pair structure.

To confirm this point of view the energy of the charge transfer was determined from the λ_{max} values of the new bands appearing in the spectra of the ion pair CT complexes using the relation:-

$$E_{CT} = 1241.6 / \lambda_{max}^{(CT)} \quad e.v$$

The values of E_{CT} obtained are collected in the following table.

table (17):- Charge transfer energies of the different CT complexes

E_{CT} (e.v)	Calcon.	EBT	Aliz.
Nor.	2.006	2.456	2.411
Cipro	1.998	2.456	2.365
Oflo.	1.999	2.461	2.241

To judge this point the electron affinity of the drugs was determined using the relation given by Briegleb[79] in the form

$$E_{CT} = I_p - E_A + C$$

where:

E_{CT}: energy of charge transfer

I_p : ionization potential of the donor

E_A: electron affinity of the donor

C : coulomic force between the electrons transferred and the positive hole left behind it. The values of C was given by many authors are (-3.7, -4.2, -4.7, -5.2 or -5.6) e.v.

Based on this, the values of E_A for each C value were determined; the data of E_A obtained are collected in the following table.

Table(18):- Electron affinity Values for the drugs obtained for different coulomic force (C).

C / E_A(e.v)	3.7	4.2	4.7	5.2	5.6
Calcon	0.693	0.193	-0.307	-0.807	-1.207
E.B.T.	0.671	0.171	-0.329	-0.807	-1.229
Aliz.	0.676	0.176	-0.324	-0.824	-1.224
Drugs	0.373	-0.127	-0.627	-1.127	-1.527
Calcon-Nor.	0.936	0.436	-0.064	-0.564	-0.964
E.B.T.-Nor.	0.562	0.062	-0.438	-0.938	-1.338
Aliz.-Nor.	0.581	0.081	-0.419	-0.919	-1.319
Calcon -Cipro.	0.944	0.444	-0.056	-0.556	-0.956
E.B.T.-Cipro.	0.562	0.062	-0.438	-0.938	-1.338
Aliz.-Cipro.	0.627	0.127	-0.373	-0.873	-1.273
Calcon -Oflo.	0.943	0.443	-0.057	-0.557	-0.957
E.B.T.-Oflo.	0.557	0.057	-0.443	-0.943	-1.343
Aliz.-Oflo.	0.751	0.251	-0.249	-0.749	-1.149

These data reveal that the most probable value for C would be (-5.2 or -4.7) ev. Though the value -4.7 ev. gave more acceptable results.

A further support for the occurrence of (CT) can be gained by examining the IR spectra of the solid complexes in comparison to those of their components. The data are collected in tables (19-20-21-22).

Table (19):- γ_{CH} bands in the IR spectra of EBT, Ciprofloxacin and their CT complex (ν' = wave number cm^{-1}, I= intensity of peaks)

EBT		Ciprofloxacin		EBT - Cipro. complex	
$\nu' cm^{-1}$	I	$\nu' cm^{-1}$	I	$\nu' cm^{-1}$	I
-	-	944	46.7	942	63
882	65.7	890	37.6	892	52.7
-	-	838	34.3	832	45.5
804	63.4	804	56.3	806	44.9
790	67.1	-	-	790	45.3
-	-	786	28.7	768	43.2
738	70.7	750	37.7	740	46.5
-	-	716	24.9	-	-
-	-	704	23.3	-	-
650	81.5	666	21.6	652	59.9

Table (20):- γ_{CH} bands in the IR spectra of Calconcarboxylic acid, Ciprofloxacin and their CT complex

Calcon		Ciprofloxacin		Calcon.- Cipro. complex	
$\nu' cm^{-1}$	I	$\nu' cm^{-1}$	I	$\nu' cm^{-1}$	I
958	58.7	-	-	-	-
-	-	944	46.7	-	-
-	-	890	37.6	890	20.1
888	63.8	-	-	-	-
844	48.8	-	-	-	-
-	-	838	34.3	830	12.9
808	49.6	-	-	-	-
-	-	804	56.3	804	13.3
788	54.3		-	-	-
-	-	786	28.7	760	19.2
748	69.3	750	37.7	-	-
730	73.7	-	-	-	-
-	-	716	24.9	-	-
712	55.4	-	-	-	-
-	-	704	23.3	668	16.8
650	84	666	21.6	650	27.5

Table (21):- γ_{CH} bands in the IR spectra of Alizarin red S, Ciprofloxacin and their CT complex

Alizarin red S		Ciprofloxacin		Aliz.-Cipro. complex	
ν cm^{-1}	I	ν cm^{-1}	I	ν cm^{-1}	I
-	-	944	46.7	942	74.5
-	-	890	37.6	890	68.4
868	82.7	-	-	-	-
-	-	838	34.3	830	61.5
824	66.8	-	-	-	-
804	75.1	804	56.3	806	68.4
-	-	786	28.7	-	-
778	77	-	-	-	-
-	-	750	37.7	748	59.3
728	78.9	-	-	-	-
-	-	716	24.9	-	-
712	111.5	-	-	720	68.9
-	-	704	23.3	-	-
680	79.1	-	-	-	-
668	71.3	-	-	668	57.8
-	-	666	21.6	636	77.3

Table (22):- γ_{CH} bands in the IR spectra of for Alizarin red S, Norfloxacin and their CT complex

Alizarin red S		Norfloxacin		Aliz.-Nor. complex	
ν cm^{-1}	I	ν cm^{-1}	I	ν cm^{-1}	I
-	-	932	144.9	934	80.2
-	-	902	128.7	-	-
868	82.7	886	124.6	-	-
-	-	848	118.2	-	-
824	66.8	828	141.2	824	68.1
804	75.1	806	128	796	69.6
-	-	786	124.6	-	-
778	77	770	123.5	-	-
-	-	-	-	744	68.2
728	78.9	736	132.9	-	-
712	111.5	-	-	720	72.9
-	-	696	132.1	-	-
680	79.1	-	-	-	-
668	71.3	668	125.5	638	78.1

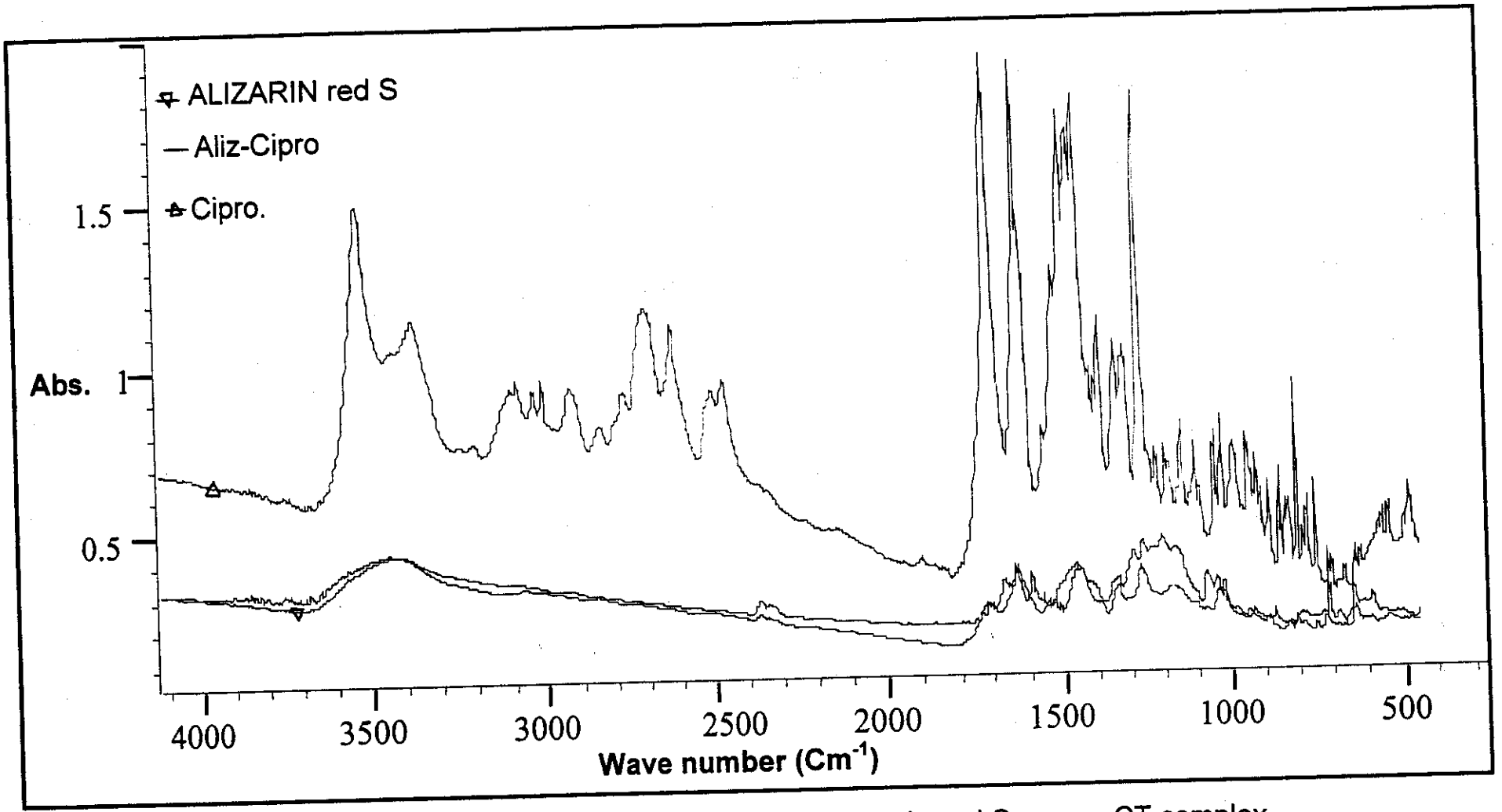


Fig.(32): IR spectra of Ciprofloxacin – Alizarin red S – new CT complex

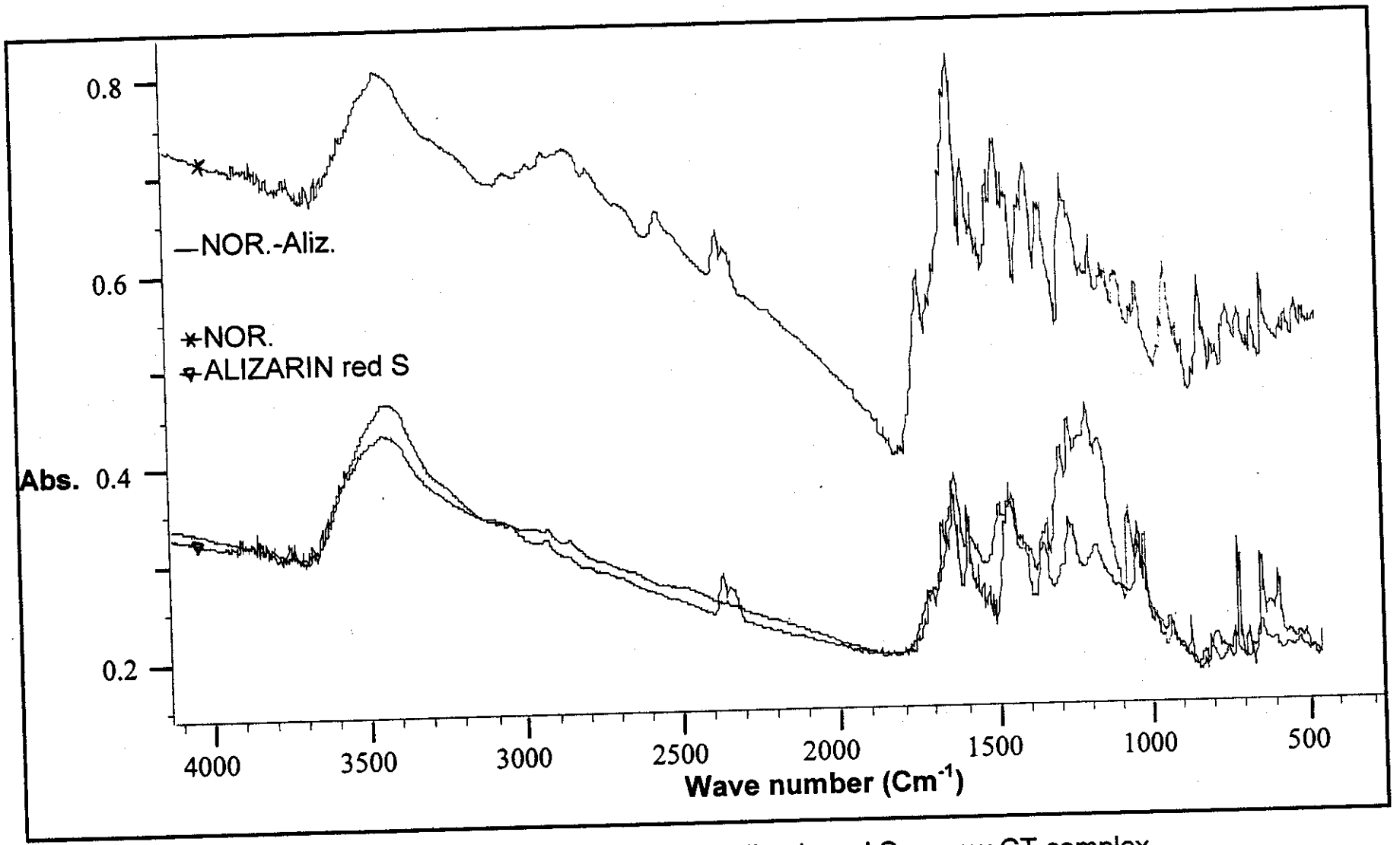


Fig.(33): IR spectra of Norfloxacin – Alizarin red S – new CT complex

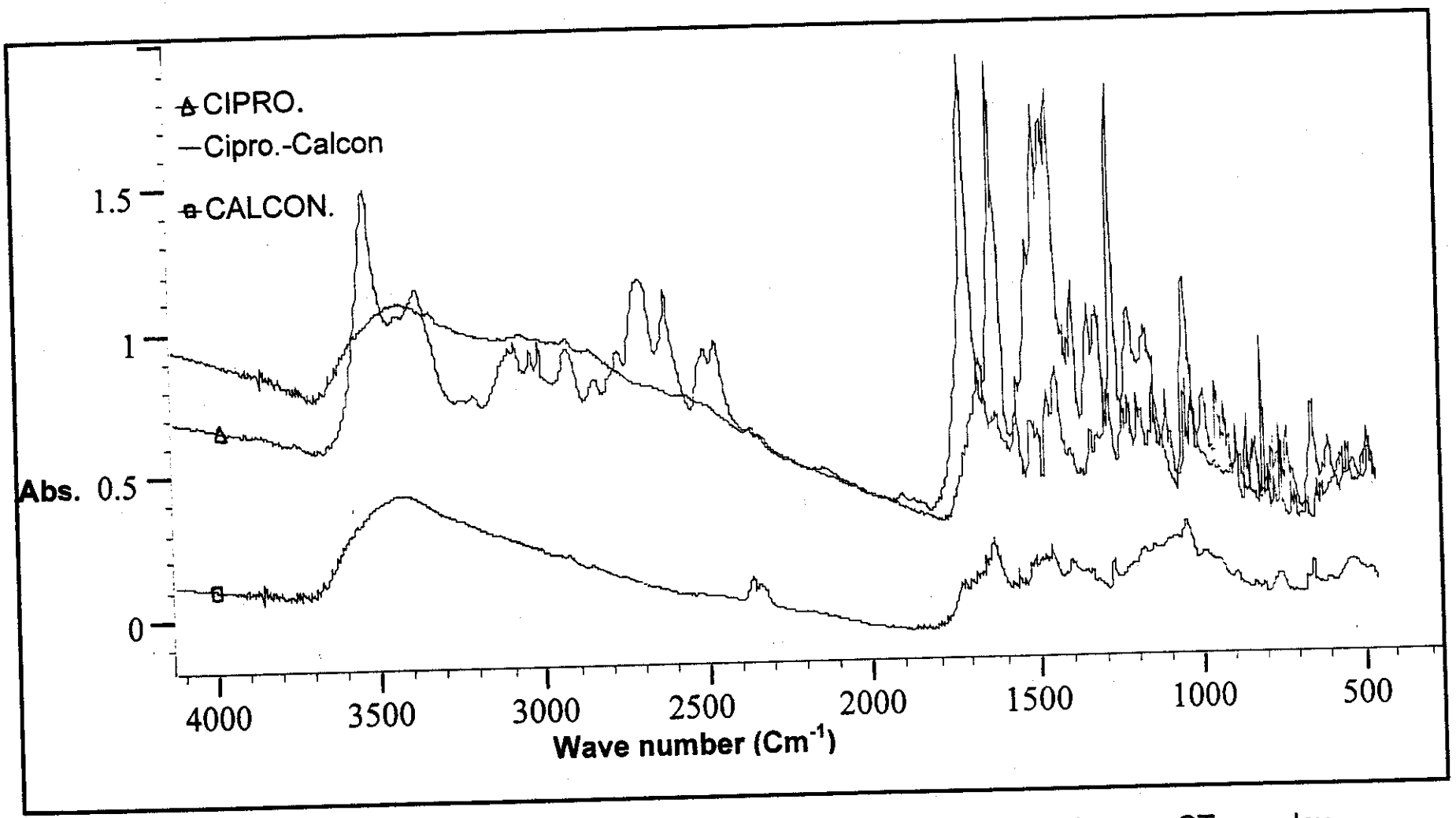


Fig.(34): IR spectra of Ciprofloxacin – Calconcarboxylic acid – new CT complex

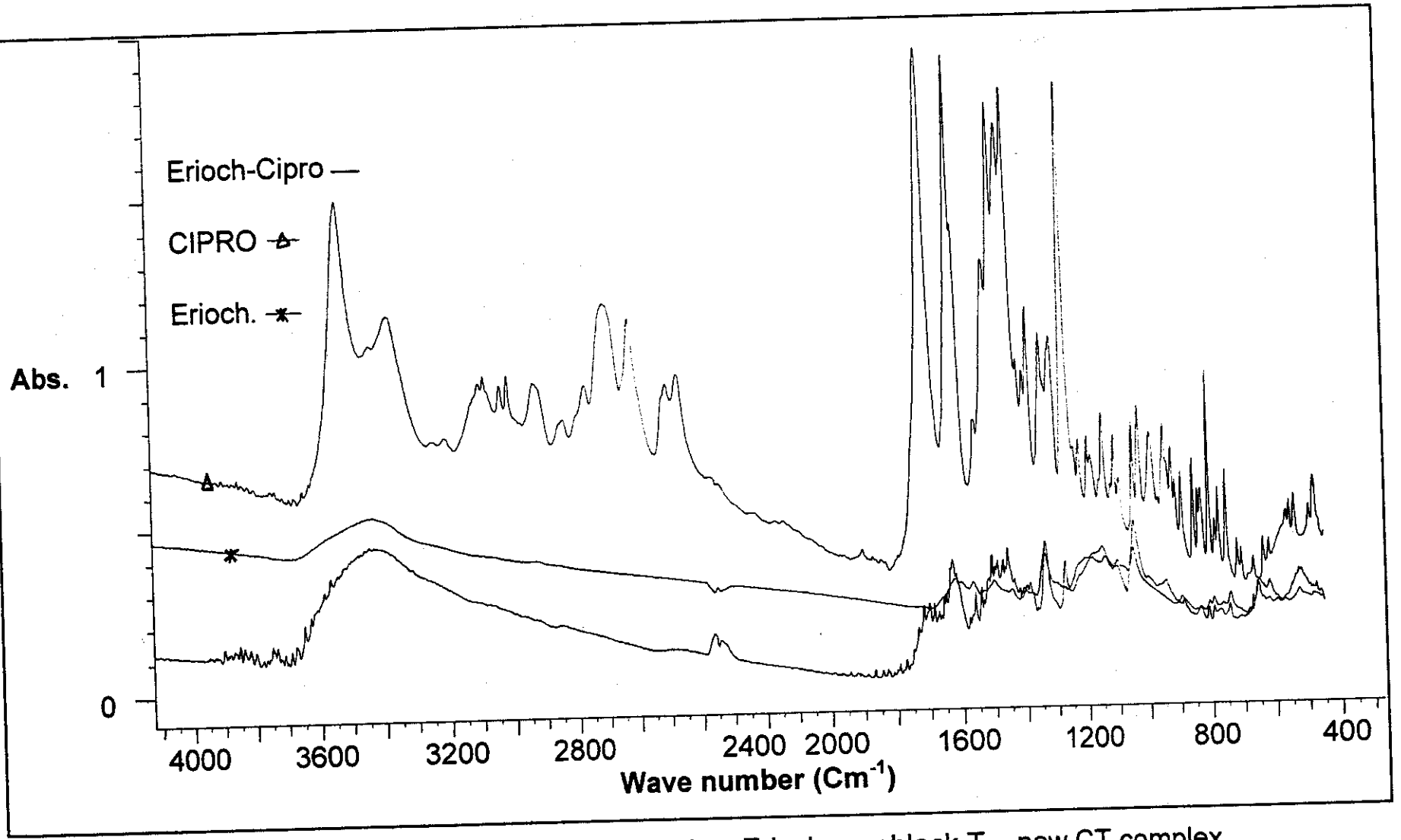


Fig.(35): IR spectra of Ciprofloxacin – Eriochromeblack T – new CT complex

As a matter of fact, previous studies on IR spectra of charge transfer complexes revealed that the intermolecular electron transfer from the donor to the acceptor showed that the bands of the donor component shifted to higher wave numbers while that of the acceptor was shifted to lower values [80-81]. Most obvious were the shifts of the γ_{CH} bands of both components

It is obvious that the bands due to the γ_{CH} of the donor reagent are shifted to higher wave numbers while those for the drugs display a shifted to smaller values.

The same type of shift was observed for the C=O band of the quinon ring as shown in table (23).

Table (23):-Values for the C=O bands of drugs, reagents and their CT complexes.

ν C=O band cm^{-1}				
Drugs \ Reagents		Erioch.-Drugs bands value	Aliz. .-Drugs bands value	Calcon.-Drugs bands value
Cipro.	1708	1664	1628	1634
Oflo.	1716	1700	1644	1650
Nor.	1734	1700	1629	1634

The behavior reflects that the charge transfer interaction takes place from the reagents used to the drug molecules.

A further support for the occurrence of the charge transfer interaction in the compounds under study can be gained from the fact that the ion pair complexes gave ESR spectra (fig.36) while the components are ESR silent

The ESR spectra of the ion pair complexes gave obvious signals with g_{eff} at (2.149) and (1.9616). The signal has a rather broad appearance with doubled peaks, a behavior which is very identical to the

the ESR spectra of charge transfer complexes involving $\pi \rightarrow \pi^*$charge transfer interaction[82].

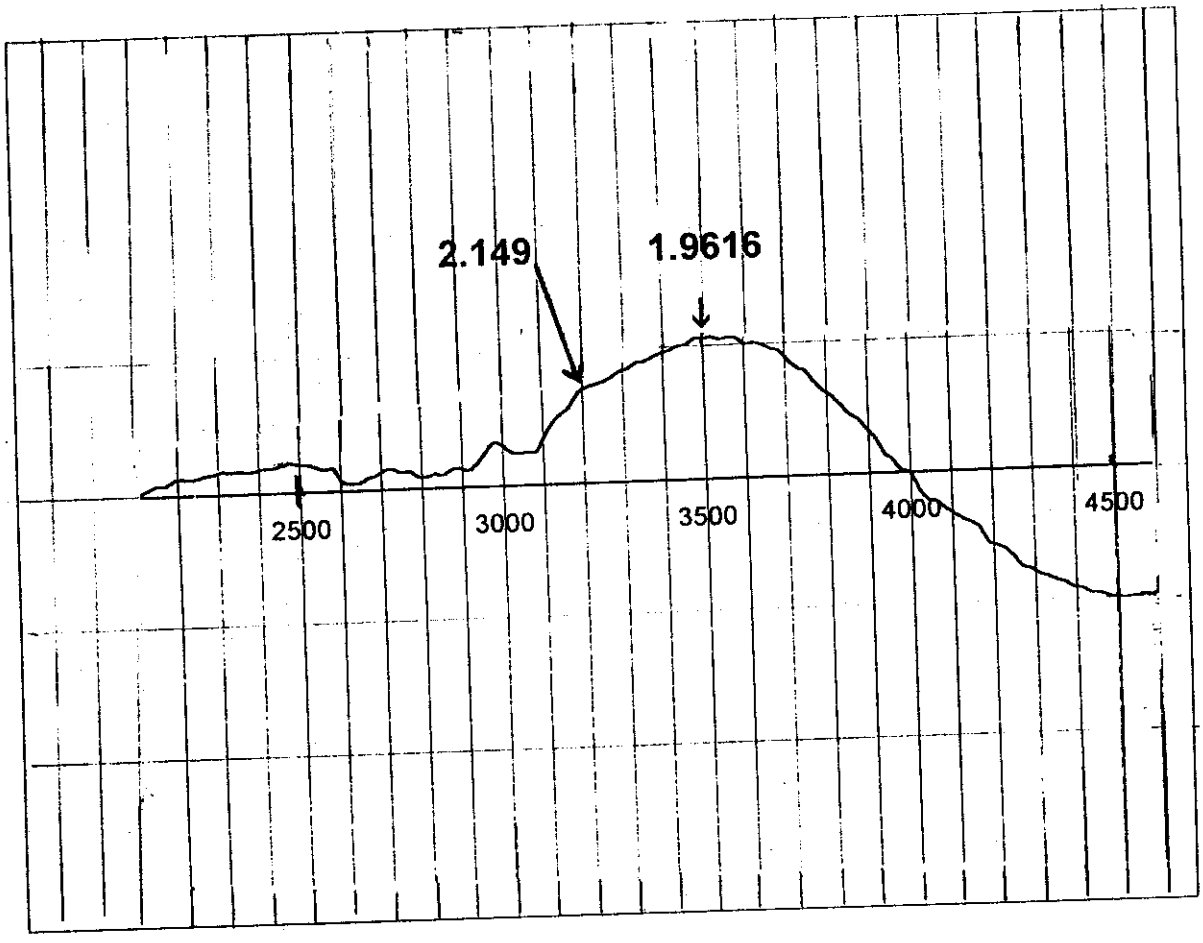


Fig. (36) :- ESR chart spectra of Eriochromeblack T – Norfloxacin charge transfer complex

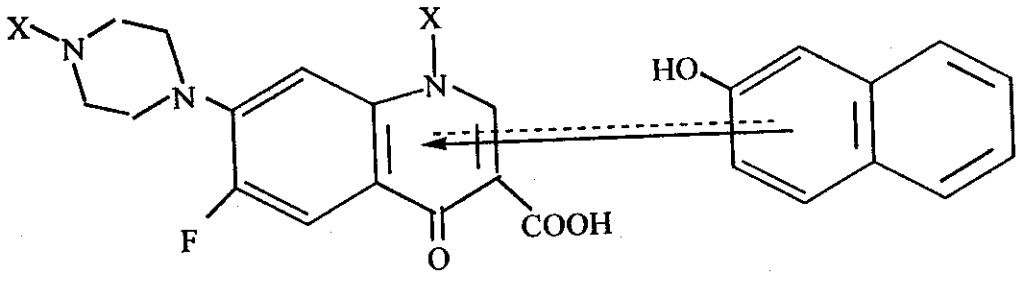
The bonding of the reagents with the drugs

As gained from the data obtained above for the study of the addition compounds formed between the three reagents used Eriochrome black T, Alizarin red S or Calconcarboxylic acid and the three drugs under study namely Ciprofloxacin, Ofloxacin or Norfloxacin using UV / Vis., IR, ESR spectra, it can be stated that the addition compound is formed through a charge transfer from the reagent as electron donor to the drug as electron acceptor.

As a matter of fact the charge transfer takes place from the HOMO of the part possessing the highest electron density on the donor to the LUMO of the part having the lowest electron density on the acceptor[83].

Considering the three reagents used, it is obvious that the electron donor centers of the reagents is the hydroxynaphthyl moiety of Eriochromeblack T, the benzene ring carrying the two hydroxyl groups of Alizarin red S and the hydroxycarboxynaphthyl moiety of Calcon-carboxylic acid, will be the donating parts. On the other hand, the acceptor center on the drugs will be the benzquinazolone moiety. This fact is gained from the obvious shift of the carbonyl band of this moiety at 1708, 1716 and 1734 cm^{-1} for the three drugs respectively to smaller wavenumbers in the IR spectra of the charge transfer complexes table (23).

Based on these conclusions, the CT interaction in the different cases can be formulated as follows:-



Previous studies of the charge transfer complexes of a variety of components indicated that the two interacting molecules are situated parallel to each other with the charge transfer direction perpendicular to the planes of the two moieties involved in the charge transfer [79-84-85]. In addition, X - ray studies of oriented single crystals of picric acid charge transfer complexes with aromatic amines revealed that the distance between the two molecules forming the CT complex amounted to 2.7 – 3.4 Å [86, 87].