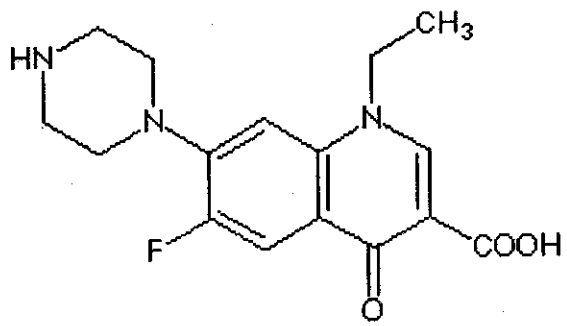
1. Introduction

1.1. Knowledge of the studied drugs

1.1.1.Norfloxacin [66–› 71]



Norfloxacin (Nor.)

Ethyl-6-fluoro-1, 4-dihydro-4-oxo-7-(1-piperazinyl) -3-quinolinecarboxylic acid

Molecular weight : 319.34

Composition: C: 60.18%, H: 5.68%, F: 5.95%, N: 13.16%, O: 15.03%.

Literature reference: *fluorinated quinolone antibacterial.*

Treatment:

Infection caused by susceptible organism, urinary tract infection, antibacterial gastroenteritis.

Dosage	***Treatment***
400 mg b.i.d .(by mouth)	- Urinary tract infections, for7-10 days.
	- Uncomplicated acute cystitis for 3 -10 days.
	- Chronic relapsing urinary tract infection for up to12 weeks.
	- Acute bacterial gastroenteritis for 5 days.
800 mg single dose	- Acute gonococcal urethritis, pharyngitis, proctitis, or cervicitis.
400 mg t.i.d.	– Typhoid fever for 14 days.

Properties:

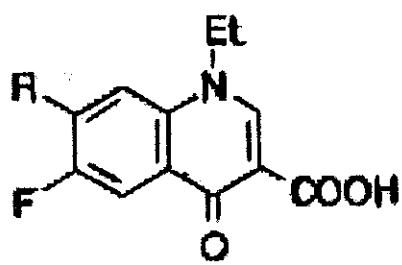
White to light-yellow crystalline powder, hygroscopic, photosensitive, m.p. 220 -221°C. UV max (0.1N NaOH): λ_{max} at 274, 325, 336 nm (molar absorbitivity $\varepsilon = 1.109 \times 10^4$, λ_{max} = 425-437) , pK_{a1} 6.34; pK_{a2} 8.75.

Partition coefficient (octanol/water):

0.46 mg of drug/ml of octanol Soluble at 25 °C, water 0.28; methanol 0.98 ethanol 1.9; acetone 5.1; chloroform 5.5; diethyl ether 0.01; benzene 0.15;ethyl acetate 0.94; octyl alcohol 5.1and in glacial acetic acid 340 mg/ml. Solubility in water is pH dependent, increasing sharply at pH <5 or at pH >10. Hygroscopic in air, forms a hemihydrate.

STORAGE : Store in an airtight container, protected from light.

IMPURITIES



A. R = Cl: 7-chloro-1-ethyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid,

B. R = $NH\text{-}CH_2\text{-}CH_2\text{-}NH_2$: 7-[(2-aminoethyl)amino]-1-ethyl-6- fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid.

The UV - Vis. and IR – spectra of Norfloxacin were recorded as shown in fig. (a_1, a_2).

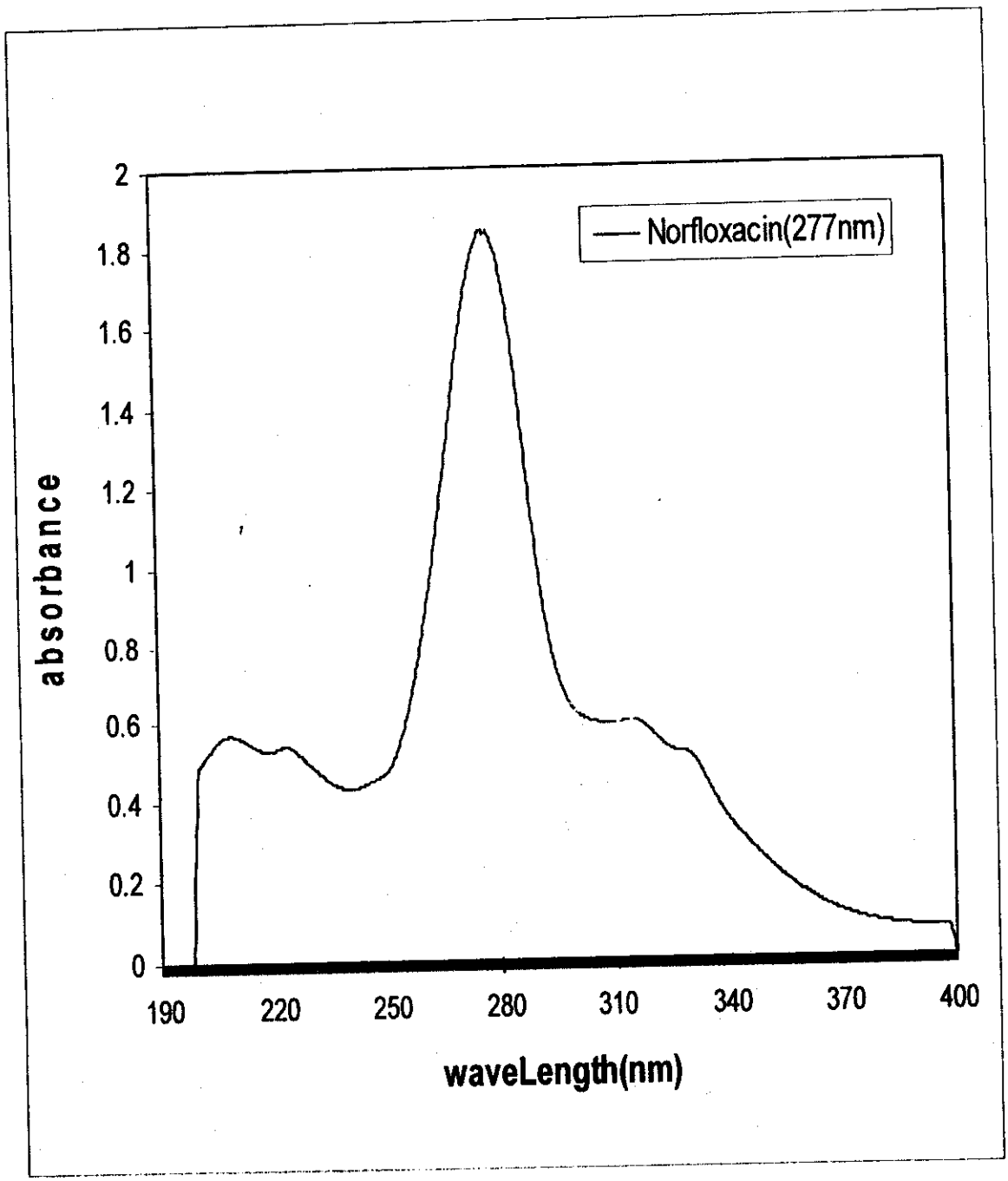


Fig.(a_1): The electronic absorption spectra of ($5x10^{-3}$) M of Norfloxacin in diluted acetic acid solution (2%)

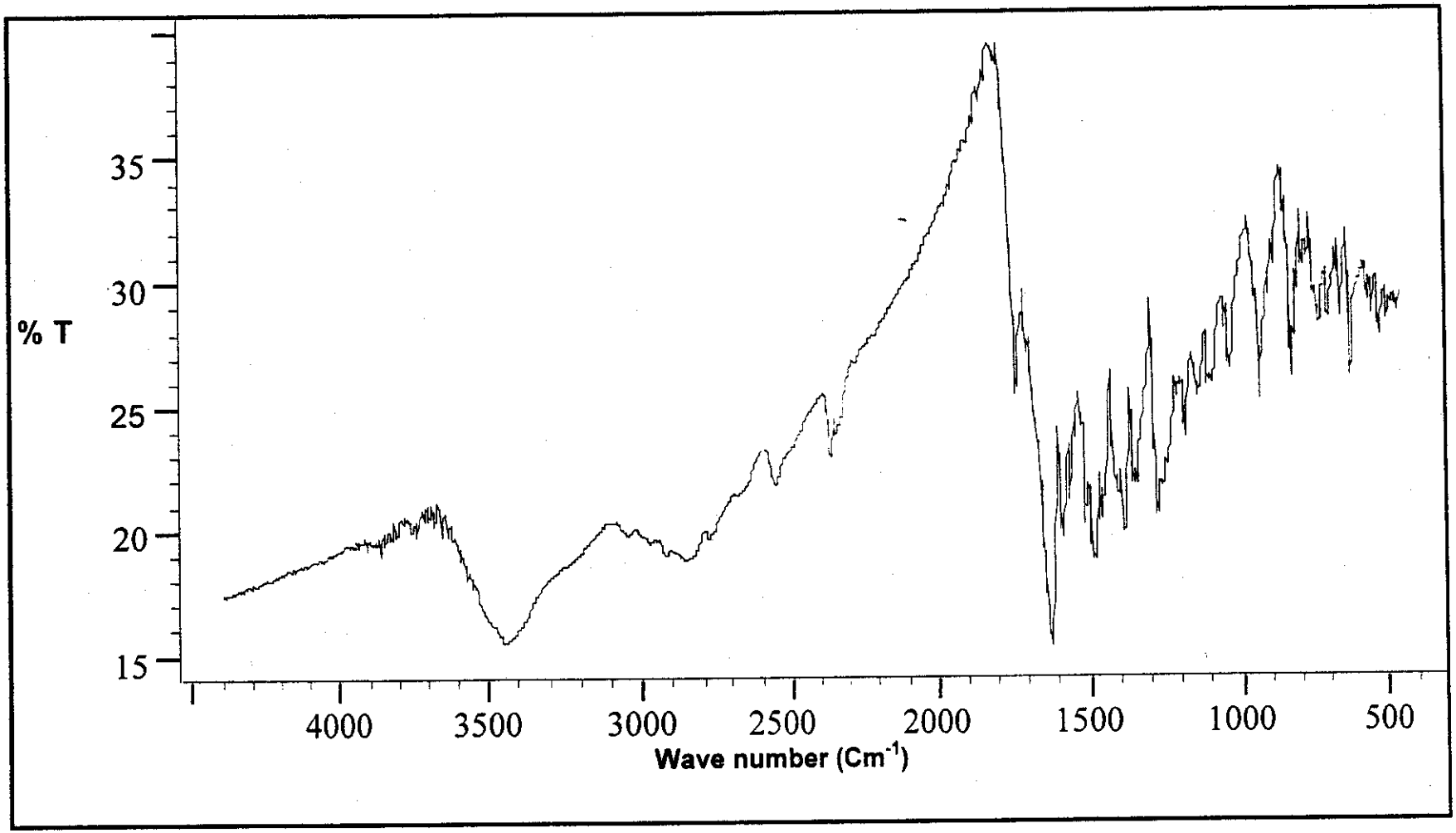
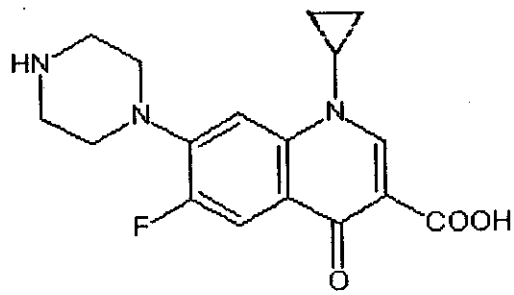


Fig. (a_2): Infrared spectra of Norfloxacin

1.1.2.Ciprofloxacin (66→ 71)



Ciprofloxacin. (Cipro.)

Cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)

-3-quinolinecarboxylic acid

Molecular formula: $C_{17}H_{18}FN_3O_3$

Molecular weight: 331.35

Composition: C: 61.62%, H: 5.48%, F: 5.73%, N: 12.68%, O: 14.49%.

Literature references: Fluorinatedquinolone antibacterial.

spectrum in vitro

Dosage :

Daily dose for adults: 2×1 film –coated tablets (750 mg) for a maximum 2 months in osteomyelitis, and 7-14 days in all other infections .

Properties:

A pale yellow, crystalline powder, practically insoluble in water, very slightly soluble in ethanol and methylene chloride. It is soluble in dilute acetic acid, m.p. 255-257 °C.

Derivative: Monohydrochloride monohydrate

Molecular formula: $C_{17}H_{18}FN_3O_3.HCl.H_2O$

Properties:

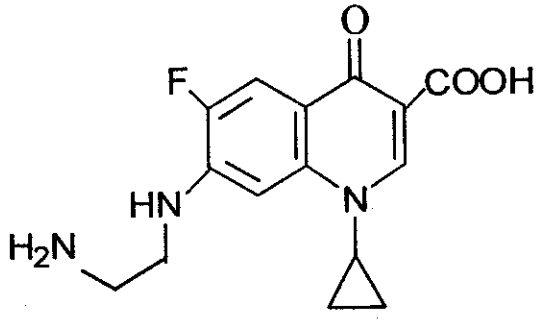
A pale yellow, crystalline powder, soluble in water, slightly soluble in methanol, very slightly soluble in ethanol, practically insoluble in acetone, in ethyl acetate and in methylene chloride, m.p. 318-320 °C.

STORAGE:

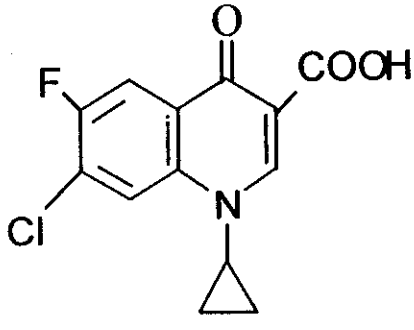
Store in a well-closed container, protected from light.

IMPURITIES:

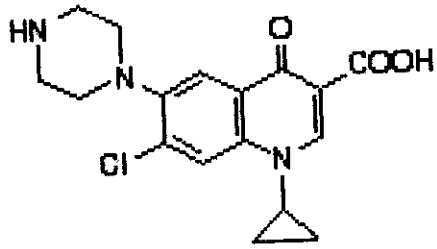
A. 7-[(2-aminoethyl) amino]-1-cyclopropyl-6-fluoro-1,4-dihydro - 4 - oxo-quinoline-3 -carboxylic acid (ethylenediamine compound),



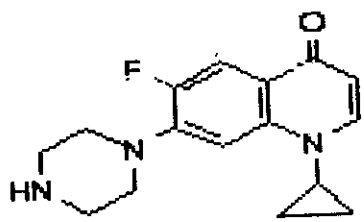
B. 7-chloro-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-3-quinoline -3-carboxylic acid (fluoroquinolonic acid),



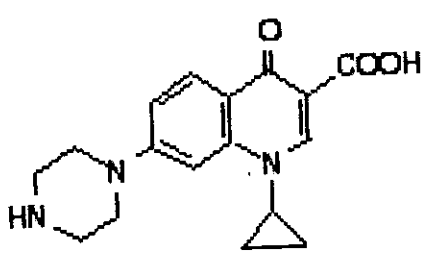
C. 7-chloro-1-cyclopropyl-1,4-dihydro-4-oxo-6-(piperazine-1-yl)-quinoline-3 -carboxylic acid (by-compound A),



D. 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(piperazin-1-yl)-quinoline (decarboxylated compound).



E.1-cyclopropyl-1,4-dihydro-4-oxo-7-(piperazin-1-yl)quinoline - 3- carboxylic acid (desfluoro compound).



The UV - Vis. and IR – spectra of Ciprofloxacin were recorded as shown in fig. (b_1, b_2).

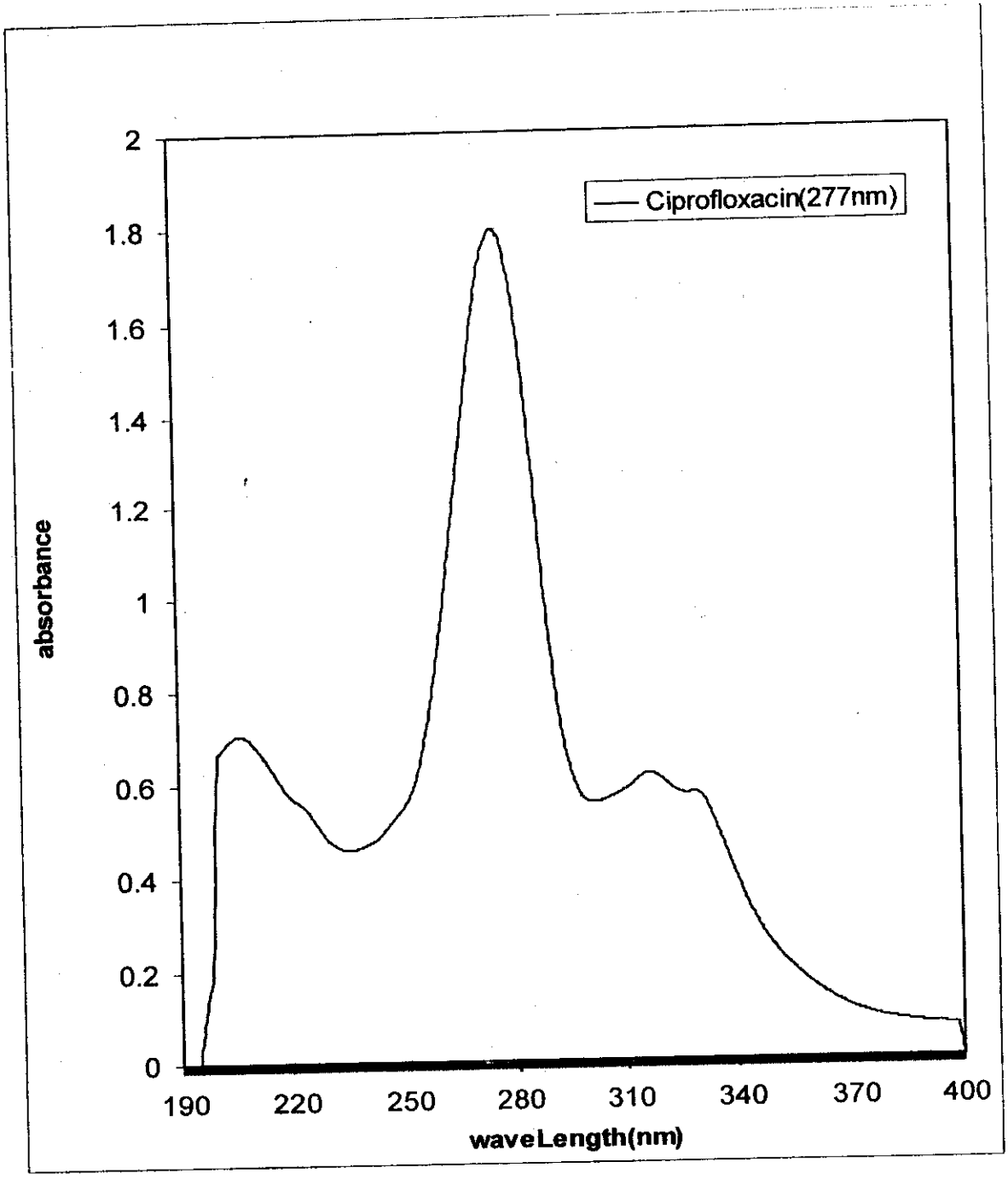


Fig.(b_1): The electronic absorption spectra of ($5x10^{-3}$) M of Norfloxacin in diluted acetic acid solution (2%)

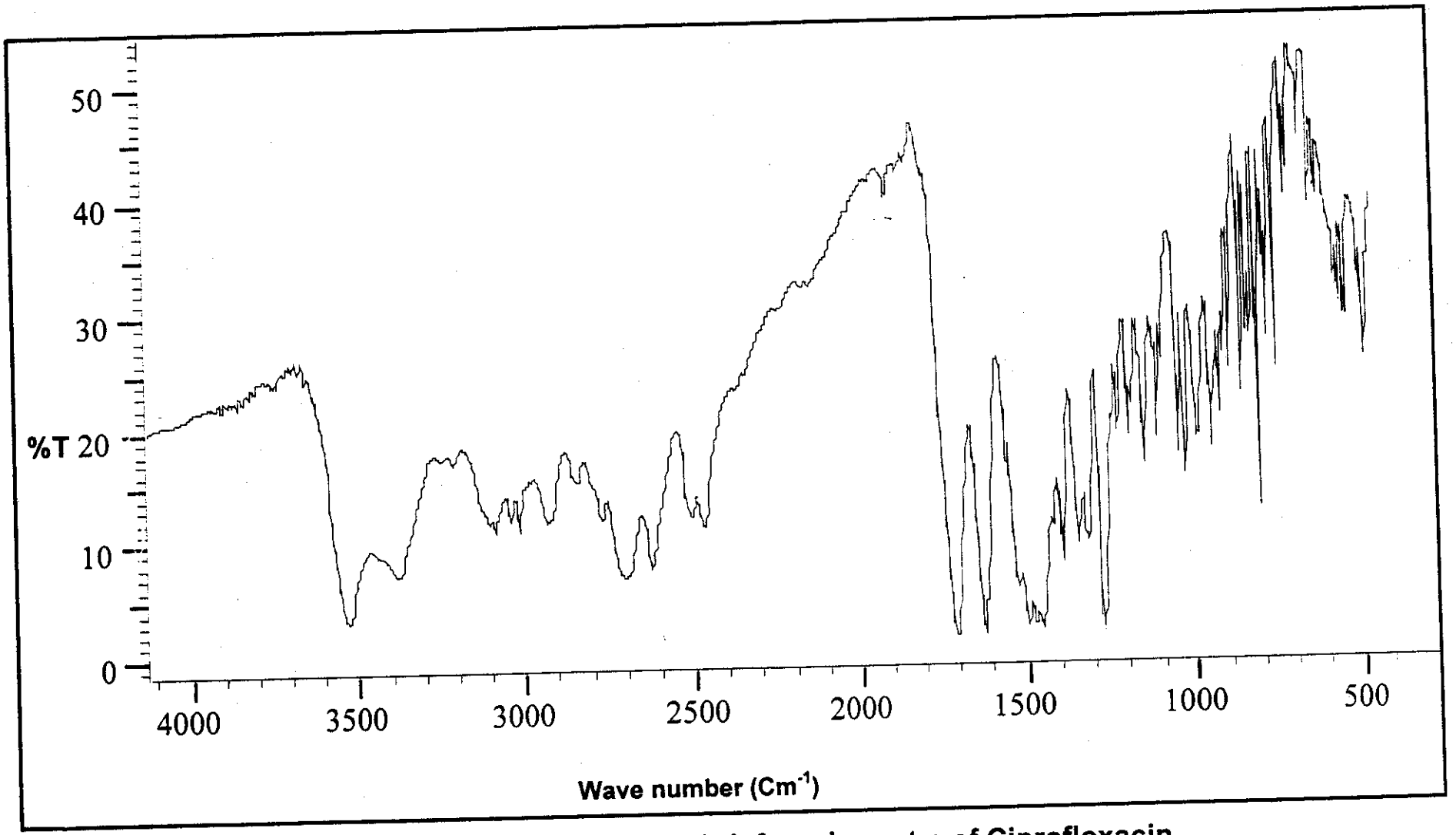
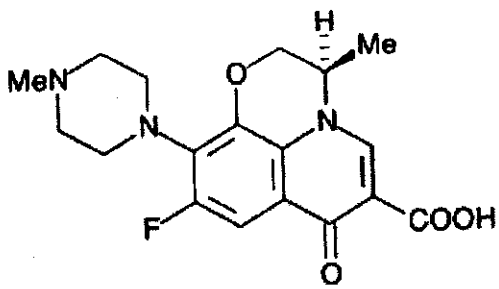


Fig. (b_2): Infrared spectra of Ciprofloxacin

1.1.3. Ofloxacin (66-67-69-70-71)



Ofloxacin (Oflo.)

9-fluoro-3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-2,3-dihydro-7H-pyrido-[1,2,3-de][1,4]benzoxazine-6-carboxylic acid,

Molecular formula: $C_{18}H_{20}FN_3O_4$

Molecular weight: 361.37

Composition: C:59.83%, H :5.58% F: 5.26%, N: 11.63%, O: 17.7%.

Literature references: antibacterial fluorinated quinolone

Dosage	***Treatment***
2×1(200mg) tablet daily	- infections of the respiratory tract - Infections of skin, soft tissue - Infections of upper urinary tract - Infections of the abdominal cavity
2×1/2 (200mg) tablet daily	- infections of lower urinary tract

in most cases a course of treatment lasting 7-10 days is sufficient .

Properties: Colorless needles from ethanol m.p. 250-257 °C

Broad spectrum: λ_{max} at 208, 233, 273,

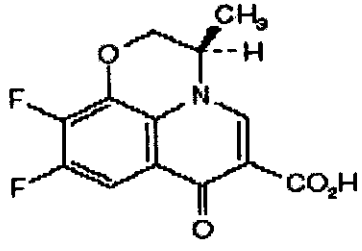
CHARACTERS

A pale yellow or bright yellow, crystalline powder, slightly soluble in water, soluble in glacial acetic acid slightly soluble to soluble in methylene chloride, slightly soluble in methanol.

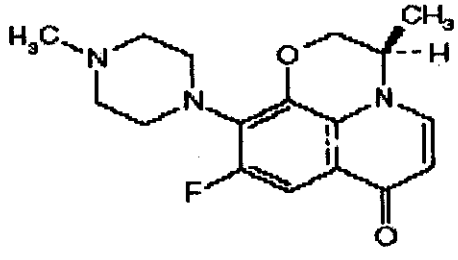
STORAGE

Store in an airtight container, protected from light.

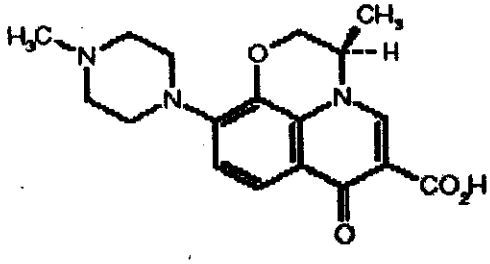
IMPURITIES



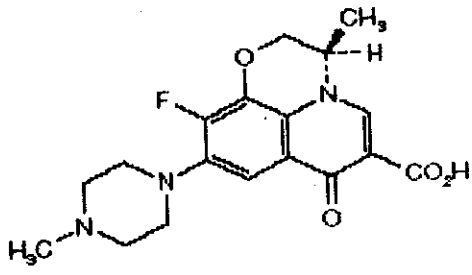
A- 9,10-difluoro-3-methyl-7-oxo-2,3-dihydro-7H-pyrido-[1,2,3de]- [1,4] - benzoxazine-6-carboxylic acid



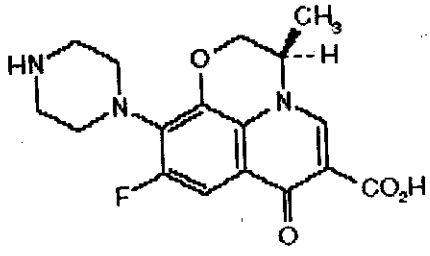
B- 9-fluoro-3-methyl-10-(4-methylpiperazin-1-yl)-2,3-dihydro-7H- pyrido - [1,2,3-de][1,4]benzoxazin-7-one,



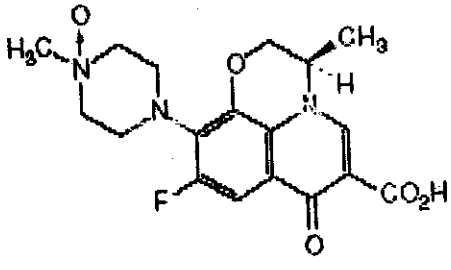
C- 3-methyl-10-(4-methylpiperazin-1-yl)-7-oxo-2,3-dihydro-7H-pyrido[1,2,3-de][1,4]benzoxazine-6-carboxylic acid,



D- 10-fluoro-3-methyl-9-(4-methylpiperazin-1-yl)-7-oxo-2,3-dihydro-7H-pyrido[1,2,3de][1,4] benzoxazine-6-carboxylic acid,



E- 9-fluoro-3-methyl-10-(piperazin-1-yl)-7-oxo-2,3-dihydro-7H-pyrido[1,2,3-de][1,4]benzoxazine-6-carboxylic acid,



F- 9-fluoro-3-methyl-10-(4-methyl-4-oxopiperazin-1-yl)-7-oxo-2,3-dihydro -7H-pyrido[1,2,3-de][1,4]benzoxazine-6-carboxylic acid.

The UV - Vis. and IR – spectra of Ofloxacin were recorded as shown in fig. (c_1, c_2).

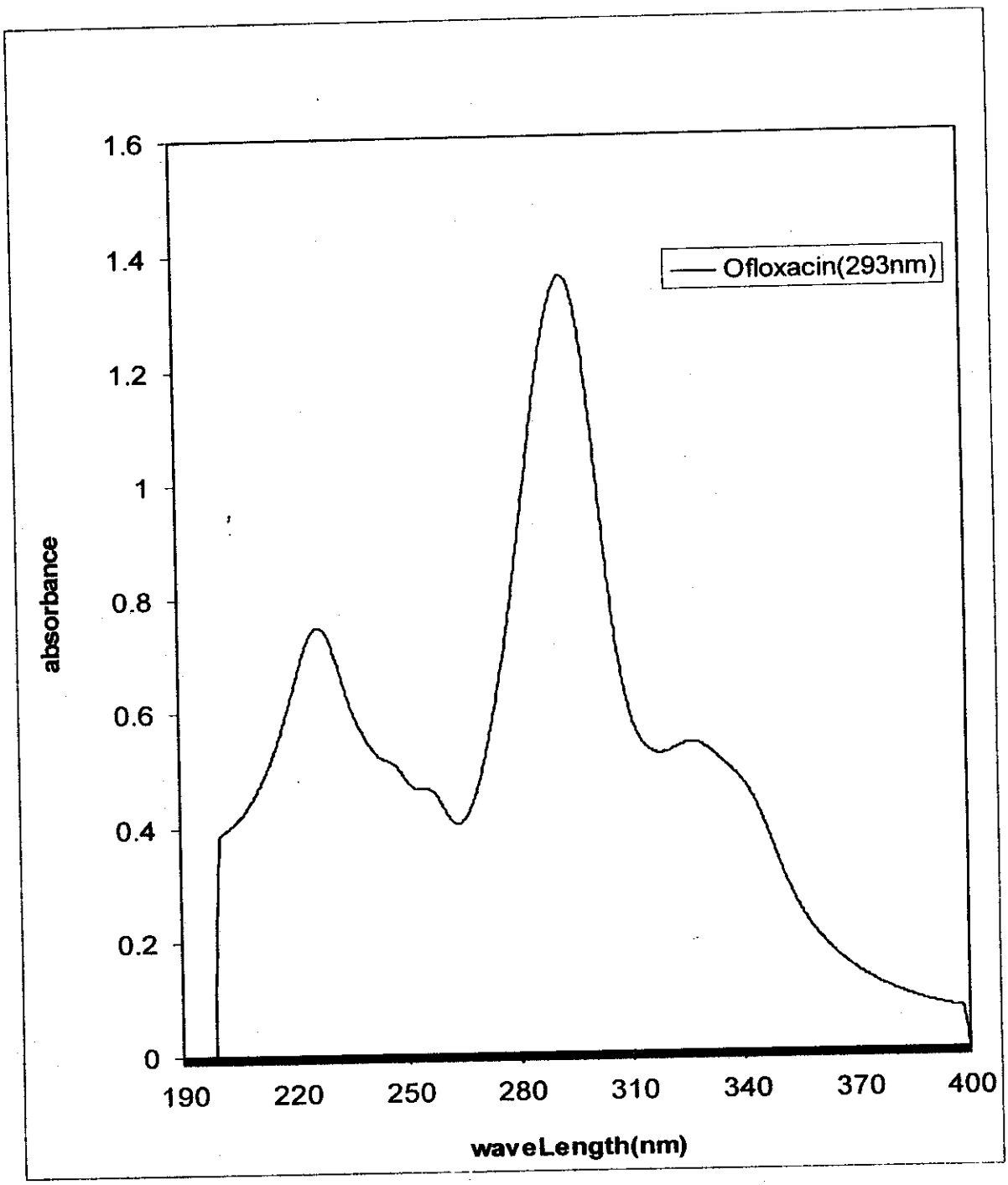


Fig.(c_1): The electronic absorption spectra of ($5x10^{-3}$) M of Ofloxacin in diluted acetic acid solution (2%)

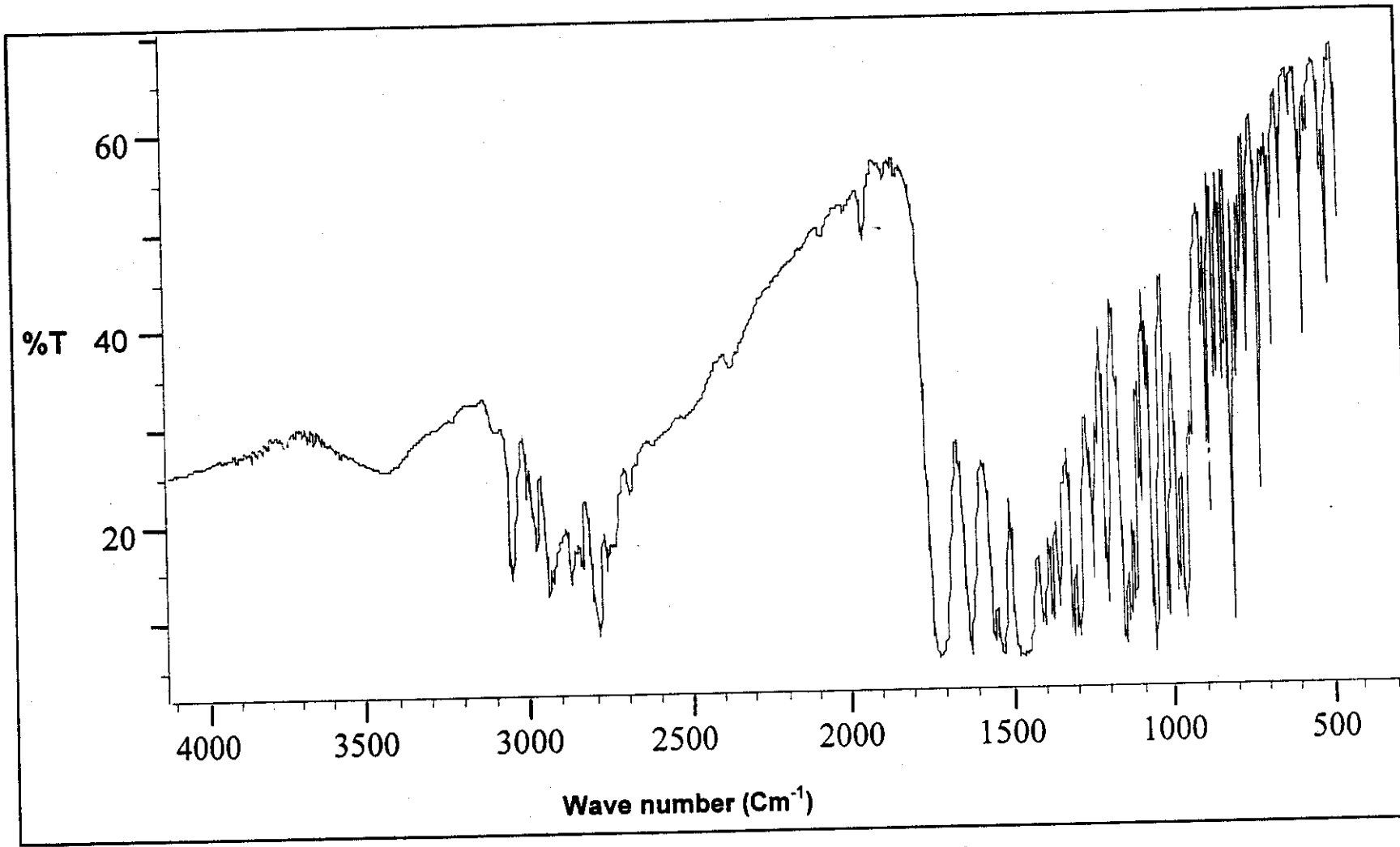


Fig. (c_2): Infrared spectra of Ofloxacin

1.2 Literature suvey for the determination of drugs

1.2.1 Literature suvey for the determination of norfloxacin

1.2.1.1 Spectrophotometric methods

El Khateeb et al. [1]; studied that the two spectrophotometric procedures for the selective determination of norfloxacin (NF) in the presence of its decarboxylated degradant were described. The first depends upon measurement of the pH-induced absorbance difference (Δ A) of the drug solution between 0.1 N HCl and 0.1 N NaOH at 280 nm. The second involves chelation of the intact drug with Fe^{2+} in acetate buffer pH 5.7 ± 0.1 to form a yellow colored chelate which absorbs at 358 nm. The two procedures were applied for the determination of the drug both in pure form and in tablet form. The methods retained their accuracy in the presence of up to 62 and 76% degradants, respectively.

Wang, et al. [2]; found that norfloxacin (NFX) was proposed as reagent for the derivative spectrophotometric determination of Nd^{3+}, Ho^{3+}, and Er^{3+} in mixed rare earths. The absorption spectra of 4f electron transitions of the systems of Nd^{3+}, Ho^{3+}, and Er^{3+} complexes with norfloxacin in presence of cetylpyridinium chloride were studied by normal and derivative spectra. The absorption bands found normally at 575 nm for Nd^{3+}, 450 nm for Ho^{3+} and 523 nm for Er^{3+} were enhanced markedly. Using the second derivative spectrum, Beer's Law was obeyed from 5.0×10^{-5} to 2.5×10^{-4} mol/l for Nd^{3+}, Ho^{3+}, and Er^{3+}, respectively. The RSDs (relative standard deviation) are 1.0, 1.4, and 1.1% for 6.9×10^{-5} mol/l of Nd^{3+}, 6.1×10^{-5} mol/l of Ho^{3+}, and 6.0×10^{-5} mol/l of Er^{3+}, respectively. A method for the direct determination of Nd^{3+}, Ho^{3+}, and Er^{3+} in mixts. of rare earth elements with good accuracy and selectivity was described.

Singhvi et al. [3]; indicated a simple and accurate method for the simultaneous determination of metronidazole and norfloxacin in combined dosage form was based on the use of the fourth derivative UV spectroscopy. Two wavelengths were chosen for this method were 294.6 nm and 313.2 nm. Beer's Lambert law was obeyed in the concentration range 0-40 for metronidazole and for norfloxacin 0-14 µg/ml. Results of analysis were validated statistically.

Mohan, et al. [4]; examined glenn's method of orthogonal polynominal function as applied to the absorption curve of Norfloxacin for its determination in eye ointments and tablets. The quadratic polynominal coefficient had a linear relation in the concentration range of 5-50 µg/ml of the drug. The absorbance was measured at 6 points equally spaced at 2 nm intervals over the wavelength range of 278-288 nm.

Wang, et al. [5]; investigated a portion of lanthanid solution was mixed with 3 ml 0.05M-norfloxacin and 3 ml of pH 8.8 buffer (prepared from 1M-ammonia solution and ammonium chloride) and the volume was made up to 10 ml with H_20. The solution was allowed to stand for 10 min after which the second derivative spectrum was recorded against a reagent blank as reference using $\Delta\lambda$ = 1.0 nm, bandpass = 1.0 nm and scan rate = 20 nm/min. The amplitudes were measured at 480.5(+) and 482.5(-) nm for Pr^{3+}. A Shimadzu UV-240 spectrophotometer with 4 cm cells was used. Derivative spectra were obtained with a Shimadzu derivative spectrum attachment optional program/interface model OP1-2 (1st to 4th derivative, $\Delta\lambda$ = 1, 2 and 4 nm). The pH was measured with a pHs-2 meter. The absorption bands of 4f-electron transitions of Pr^{3+} complex with norfloxacin were enhanced markedly. Using the second

derivative spectra, the calibration graph was linear from 5-35 µg/ml of Pr^{3+}. The method was successfully used for the determination of Pr^{3+} in mixed rare earths.

Babu, et al. [6]; designed a simple, sensitive and accurate spectrophotometric method for the determination of norfloxacin in pharmaceutical formulations was based on the formation of a pink chromogen with Rose Bengal in H_2SO_4 medium. This compound had absorption maxima at 570 nm and obeyed Beer's law in the concentration range 0-8 µg/ml. The color was stable for more than 1 h.

Takacs-Novak et al. [7]; determined the acid-base equilibrium of several diprotic amphoteric drugs, namely, niflumic acid, norfloxacin, piroxicam, pyridoxine and 2-methyl-4-oxo-3H-quinazoline-3-acetic acid have been characterized in terms of microconstants and tautomeric ratios. A multiwavelength spectrophotometric (pH metric) titration method for determination of acid dissociation constants (pK_a values) of ionizable compound. developed previously was applied for this purpose. Microspeciation was investigated by three approaches: (1) selective monitoring of ionizable group by spectrophotometry, (2) deductive method and (3) k_Z (stability constant) method for determination of tautomeric ratio from co-solvent mixtures. The formulation for (3) has been derived and found to invoke fewer assumptions than a reported procedure (ref.Takacs-Novak [7]). It has been shown that the (pH metric) technique, for such types of ampholytes, is able to deduce the microconstants and tautomeric ratios which are in good agreement with literature data.

Xuan et al. [8]**;-** examined a sample equivalent to 0. 1-12 µg pipemidic acid (I), 0.3-16 µg norfloxacin (II) and 0. 1 -18 µg ciprofloxacin lactate (III) was treated with 2.5 ml p-nitrophenol and made up to 10 ml with H_20 of pH 7. The mixture was heated at 40°C for 40 min. After cooling, the absorbance was measured at 404, 407 and 403 nm for I, II and III, respectively. Beer's law was obeyed in the ranges stated for each of the drugs (molar absorbitivity $\varepsilon = 3.5 \times 10^4$, 1.1×10^4 and 3.9×10^4 for I, II and III, respectively). The complexes had a 1:1 stoichiometry and were formed by a charge-transfer mechanism .The method was applied to tablets and the results were in good agreement with the official methcds.

More et al. [9]**;** investigated simultaneous estimation of the concentrations of norfloxacin (NIF) and tinidazole (TZ) in pharmaceuticals containing just one or both drugs. In 0.01 N-acetic acid, the absorption maxima was 277.4 nm for NF and 317.4 nm for TZ; both drugs showed isoabsorptivity at 304.8 nm. The absorptivity values obtained were employed in simultaneous equations using Cramer' rules with or without matrices; details were given. Recoveries were >99% and RSD were 0.045-0.89%.

Song et al. [10]**;** prepared sample containing aqueous pipemidic acid (I) was treated with 1.5 ml 6 mM-2,4 dinitrophenol and then diluted with H_2O to 10 ml. After heating at 50 °C for 50 min and cooling to room temperature, the absorbance of the solution was measured at 404 nm ($\varepsilon = 2.4 \times 10^4$) vs. a reagent blank. Similarly, norfloxacin (II) and ciproffoxacin lactate (III) were determined with measurement at 397.4 nm ($\varepsilon = 1.34 \times 10^4$) and 398.4 nm ($\varepsilon = 4.2 \times 10^4$), respectively. The calibration graphs for I, II and III were linear from 0. 15 -16, 0.25-15 and 0. 12-14 µg/ml respectively. The recoveries were >99% and RSD were

1.43-3.65%. The method was appliede to I, II and III tablets. The results were compared with those in the literature The reaction mechanism is discussed.

Zhao et al. [11]; found that the charge transfer (CT) reaction between 7,7,8,8-tetracyanoquino -di- methane (TCNQ) as a pi -electron acceptor and cinnarizine,,analgin (dipyrone), norfloxacin as electron donors have been studied by spectrophotornetric method. The charge transfer complexes between TCNQ and these drugs are stable blue color, therefore a simple, rapid, accurate and sensitive method for determination of these drugs has been developed. The optimization of the experimental conditions was described. Beer's - Lambert law is obeyed in the ranges 2-18, 2-18 and 4-32 µg/ml for cinnarizine, dipyrone and norfloxacin, respectively. The apparent molar absorptivity (ε) of the CT complex, at 7-13 nm is 1, 58 x 10^4; 1, 71 x 10^4 and 8.91 x 10^3 mol^{-1} cm^{-1}, respectively. The composition of all these CT complexes are found to be 1: 1 by different methods. The RSD (n = 10) are <3%. The proposed method has been applied to the determination of these drugs in each of their pharmaceutical dosage forms with satisfactory results.

El Walily et al. [12]; made two experimental conditions for the determination of the fluoroquinolone antibiotic, norfloxacin (1) by spectrophotometry with 2,4-dir:itrofluorobenzene (DNFB) [Sanger's reagent] were investigated. For method (i), portions (0.2-0.8 ml) of a standard 500 µ g/ml aqueous solution of I were diluted to 1 ml with H_20 and 0.6 ml 1.3% DNFB in acetone was added. The mixture was heated for 20 min at 60 °C on a water bath and after cooling , 2 ml 0.5M saturated HCl gas in dioxan (decolorizing agent) was added. The mixture was diluted to 10ml with dioxan and the absorbance was measured at 365 nm (ε = 7.19 x10^4) vs. a DNFB blank. For (H), portiong (0.2-0.8 ml)

of a standard 100 μ g/ml solution of 1 in DMSO were mixed with 0.5ml 0.2% IDNFIB in DMSO. The mixture was diluted to 10 ml with solvent and the absorbance was measured at 410 nm (ε = 3.64 x10^4) vs. a DIVISO blank. Calibration graphs were linear (r = 0.9999 and 0.9996, respectively) from 10-40 and 2-8 μ g/ml , respectively, for I and II, with corresponding detection limits of 0.47 and 0.27 μg/ml .The methods were applied to the analysis of 1 in five pharmaceutical preparations. Results (tabulated) gave recoveries of 97.28-100.34 and 97.54-99.85 %, respectively, of labelled values, for I and II, with corresponding RSD of 0.66-1.37 and 0.61-1.49%, respectively. Well with the USP XXIII method (United States Pharmacopoeia XXIII, United States)

Amin [13]; found a simple, rapid. accurate and sensitive spectro-photometric method for the determination of norfloxacin (I), ofloxacin (II) and ciprofloxacin (III) was described. This method is based on the formation of an ion pair with Sudan III in aqueous acetone medium [40% (v/v) acetone]. The coloured products were measured at 567, 565 and 566 nm for I, II and III, respectively. The optimization of various experimental conditions was described. Beer's law is obeyed in the range 0.4 -12.0, 0.4-8.8 and 0.4-10.4 μg/ml of I, II and III, respectively. For more accurate results, Ringborn optimum concentration ranges were 0.8-11.2, 0.6-8.5 and 0.8-10.0 μ g/ml, respectively. The results obtained showed good recoveries of 100 ± 1.12, 100 ± 1.15 and 100 ± 1.17 % with relative standard deviations of 0.67, 0.83 and 1.08 % for I, II , and III, respectively. The molar absorptivity and sensitivity were also calculated. Applications of the proposed method to representative pharmaceutical formulations were successiully presented.

Gupta et al. [14]; studied samples of norfioxacin (NF) and tinidazole (TD) dissolved in DMF and Subjected to spectrophotometric analysis.

The drugs had zero absorbances at 272 nm for NF and 381 nm for TD. It was found that at the zero crossing point on the first order spectrum of one drug, the other drug showed substantial absorbance. Recoveries were close to 100%, and curves showed good linearity in the ranges of 0-24 µg/ml NF and 0-32 µg/ml TD.

Rizk et al. [15]**:,** investigated a derivative UV-spectrophotometric analytical procedure developed for the determination of three 4-quinoloe antibacterials :norfloxacin(NFX) ciprofloxacin (CFX), and sparfloxacin (SFX). The method depends on the complexation of Cu (II) with the studied compounds in aqueous medium. A third order, measurement was applied for their quantification, a linear correlation was established between the amplitude of the peak and concentration for all the studied drugs in the range of 15-80, 35-120, and 200-700 µg/ml with minimum detect ability of 1.0, 1.3, and 5.1 µg/ml for NFX, CFX, and SFX respectively. The method was successfully applied for accurate, sensitive, and selective determination of the studied drugs in bulk and tablets formulation with average percentage recoveries(R%) of 99.22±1.55 to 100.33±1.16. The results obtained were favourably compared with those of the reference method. The method was also used to determine sparfloxacin in spike human plasma and urine. The results obtained were satisfactory, accurate, and precise.

Amin et al. [16]**;** studied the complexation between norfloxacin, ciprofloxacin and oftoxacin with tungstate, molybdate and vanadate was studied by conductimetric titrations, potentiometric titrations and spectrophotornetry to calculate the stoichiometric ratio and stability constants. Drug solutions were mixed with 2 ml tungstate, molybdate or vanadate solution, 6 ml of buffer to the optimum pH for each complex and absorbance read after 3 min against the recommended λ_{max}. Results

were presented and showed a recovery of 99.7% with RSD <1.4% and agreed well with the results obtained by official methods.

Ragab and Amin [17]; determined three accurate, rapid and simple atomic absorption spectrometric, conductometric and colorimetric methods for the estimation of norfloxacin (NRF), ciprofloxacin (CIP), ofloxacin (OFL) and enrofloxacin (ENF). The proposed methods depend upon the reaction of ammonium reineckate (NH_4 ReO_4) with the studied drugs to form stable precipitate of ion-pair complexes, which was dissolved in acetone. The pink coloured complexes were determined either by AAS or colorimetrically at .max 525 nm directly using the dissolved complex. Using conductimetric titration, the studied drugs could be evaluated in 50 % (v/v) acetone in the range 5.0–65, 4.0–48, 5.0–56 and 6.0–72 µg/ml of NRF, CPF, OFL and ENF, respectively. The optimizations of various experimental conditions were described. The results obtained showed good recoveries(R%) of 99.15 ± 1.15, 99.30 ± 1.40, 99.60 ± 1.50, and 99.00 ± 1.25% with relative standard deviations of 0.81, 1.06, 0.97, and 0.69% for NRF, CIP, OFL, and ENF, respectively. Applications of the proposed methods to representative pharmaceutical formulations are successfully presented.

Suslu and Tamer [18]; spectrophotometric three simple methods were developed, accurate and sensitive for determination of enoxacin.The methods based on extraction of this drug into chloroform as ion pairs with sulphonphthalein dyes as bromophenol blue and bromocresol purple. The optimum conditions of the reactions were studied and optimized. The absorbance of yellow products was measured at 412 nm for enoxacin–bromophenol blue and 410 nm for enoxacin–bromocresol purple. Linearity ranges were found to be 2.0–20.0 µg /ml for enoxacin–bromophenol blue and 0.77–17.62 µg /ml for

enoxacin–bromocresol purple. The detection limits(DL) were found to be 0.084 µg /ml and 0.193 µg /ml for enoxacin–bromophenol blue and enoxacin–bromocresol purple, respectively. The composition of the ion pairs was found 1:1 by continuous variation method. The developed methods were applied successfully for the determination of this drug in pharmaceutical preparation. The data obtained by developed methods were compared with the spectrophotometric method in literature. No differences were found statistically.

Mohamed et al. [19]; investigated two simple, rapid and sensitive spectrophotometric methods for the determination of levofloxacin, norfloxacin and ciprofloxacin have been performed in pure form, pharmaceutical tablets and spiked human urine. Both methods are based on the formation of a binary complex between the drugs and one of the two xanthenes dyes, eosin Y or merbromin in aqueous buffered medium. Under the optimum conditions, the binary complexes showed absorption maxima at 547 nm for eosin Y and 545 nm for merbromin. Using eosin Y, the calibration graph was linear over the range 2–8 µg/ ml for the three drugs with mean percentage recoveries 99.94 ± 0.65, 99.97 ± 0.68 and 100.01 ± 0.61 for levofloxacin, norfloxacin and ciprofloxacin, respectively, while in case of merbromin, the concentration range was 2–15 µg/ ml with mean percentage recoveries 99.96 ± 0.49, 100.02 ± 0.51 and 99.98 ± 0.51 for the three drugs, respectively. The proposed methods were successfully applied to determine these drugs in their tablet formulations and spiked human urine and the results compared favorably to that of reference methods. The suggested methods have the advantage of being applicable for the determination of the three drugs without prior extraction. They are recommended for quality control and routine analysis where time, cost effectiveness and high specificity of analytical techniques are of great importance.

1.2.1.2 High performance liquid chromatography(HPLC) methods

Li,[20] ; investigated a review of the advances in methods for the analysis of fluoroquinolone antibacterials, including HPLC, UV, visible and fluorescence spectrophotometry, TLC and titrimetry was presented Examples are tabulated. (50 references).

Hong et al. [21]; studied sample, equivalent to one capsule obtained from the contents of several capsules, was dissolved in 0.1 % H_3P0_4 and complete to 500 ml and filtered. Portions (2 ml) of filtrate were diluted with the mobile phase to 20 ml. portion (10 µl) of the diluted solution were analysed for norfloxacin (1) by HPLC on a 5 µ m Shim-pack ODS column(dimension not given), operated at 35°C, with 0.1% H_3P0_4 /methanol (11:5) of pH 2.7 containing 1% triethyl;amine as mobile phase (1 ml/min) and detection at 278 nm; quantitation was by the external-standard method. The calibration graph was linear from 5-60 mg/l of 1. The solution was stable for 8 h. Degradation products and excipients did not interfere. The average recovery was 99.8% with RSD (n = 7) of 0.4%. Results were compared with those obtained by non aqueous titrimetry and UV- spectrophotometry,

Niu et al. [22]; investigated a mixture of pipemidic acid, norfloxacin, ciprofloxacin hydrochloride, fleroxacin and ofloxacin (I) together with p-aminophenol (internal standard) pefloxacin mesylate in 0.1M-HCl was analysed by TLC on a silica gel GF254 plate with $CHCl_3$ /methanol /concentrated ammonia H_2O (15:10:3) as mobile phase, and the chromatogram was examined under a UV lamp at 254 nm, indicating that all spots were cleanly separated. The above six 4-quinolone drugs

and p-aminobenzoic acid (internal standard) were separated by HPLC on a 5 μ m (Waters C18 column 15 cm x 4.6 mm), with phosphate buffer containing 0.98 mg/ml sodium heptanesulfonate at pH 2.4/methanol (13:7) as mobile phase at 1 ml/min and detection at 277 nm. Enantiomers of 1 were similarly separated by HPLC with 6 mM-L-phenylalanine / 3 mM-$CuSO_4$ (1:1), adjusted to pH 3.5 with NaOH/methanol (3:1) as mobile phase and detection at 293 nm. All components were separated by HPLC in these two HPLC systems. Studies on the drugs by UV spectrophotometry and chemical identification through colour reaction were also carried out.

1.2.1.3 Capillary electrophoresis methods

Barbosa et al. [23]; examined the advantages of using capillary electrophoresis over other methodologies for pK_a values of drugs in hydroorganic media were discussed. The focus of the discussion based upon the pK_a values of a series of quinolones determined in acetonitrile/H_20 mixtures by capillary electrophoresis, LC, potentiometric, and spectrophotometric methods.

Fierens et al. [24]; applied capillary electrophoresis (CE) to the study of 10 quinolones of first and second generation — nalidixic acid, oxolinic acid, pipemidic acid, cinoxacin, norfloxacin, ciprofloxacin, ofloxacin, pefloxacin, fleroxacin, and flumequine. Separation was performed on a fused silica capillary (75 mm–60 cm) using a phosphate buffer (pH 7.0,125 mM). Detection was at 214 nm. Only norfloxacin and ciprofloxacin cannot be separated in this way. Because of the specificity

of the method, the identification of the individual quinolones by their migration time was possible. The same system has been applied for the quantitative determination of quinolones in tablets and capsules. Some parameters (linearity, precision, accuracy) were validated. Especially the possibility of simultaneous quantification and identification of the active ingredient in the finished product is very attractive.

1.2.1.4 Flourimetric methods:-

Diaz,-M et al. [25]; studied the modification of the fluorescent properties of norfloxacin in acid solution after prolonged exposure to fluorescent light and the possibility of using a fluorimetric method.to check the photo degradation of norfloxacin samples were studied. Spectrophotometry was performed at 278 nm and fluorimetry at 445 nm (excitation at 330 nm). No statistically significant modifications of the UV signals were apparent, but an increase of the fluorescent signal after light exposure appeared, which led to an increase of the average recovery up to 100.27% over 15 months. Using a validated HPLC method for photo stability studies of the drug (ref. Cordoba-Borrego et al., Ibid., in press), a loss of 5% with respect to the initial drug amount was observed. Results indicated that fluorescence analysis was the method of choice for the study of photo degradation of norfloxacin.

Gao et al. [26]; examined two samples were obtained from 10 pulverized tablets, on of which was dissolved in and diluted with anhydrous ethanol while the other in H_20 containing trace HCl to 250 ml. To determine trimethoprim (I), a portion of the ethanolic solution was treated with a defined amount of ethanolic I standard and more ethanol to 10 ml by an H-point standard-addition method and fluorescence absorbances were measured at 380 and 410 nm. The amount of I in the

sample was then calculated (equation given). Norfloxacin II in the aqueous solution was directly determined after dilution by measuring fluorescence intensity at 451 nm (excitation at 320 nm). Calibration graphs for I and II were linear up to 3 and 4 mg/l, respectively. Average recoveries for I and II were 100.4 and 100% with RSD of 1.76 and 1.62 %, respectively. Results were compared with those obtained by HPLC.

El- Kommos,et al.[27]; were developed simple, rapid, reliable, and sensitive spectrofluorometric methods for the determination of eight quinolone antibacterials namely ciprofloxacin, norfloxacin, lomefloxacin, difloxacin, amifloxacin, pefloxacin, ofloxacin, and nalidixic acid. The methods depend on the chelation of each of the studied drugs with zirconium, molybdenum, vanadium or tungsten to produce fluorescent chelates. Different factors affecting the relative fluorescence intensity of the resulting chelate were studied and optimized. At the optimum reaction conditions, the drug - metal chelate showed λ_{max} from 274 to 295 nm and emission maxima ranging from 409 to 495 nm. The chelates were found to be stable at room temperature for 2 days and show good stability upon increasing temperature to 50 °C for about 1 h. Rectilinear calibration graphs were obtained in the range of 10 - 60 µg/ ml for each of the investigated drugs and the limits of detection and quantitation ranged from 1.21 to 2.05 and from 4.05 to 6.82 µg/ml, respectively. The molar ratios of the formed chelates were determined by Job's method and their association constants were also calculated. The developed methods were applied successfully for the determination of the studied drugs in their pharmaceutical dosage forms with a good precision and accuracy compared to official and reported methods as revealed by t - and F-tests. They were also applied for the determination of studied drugs in spiked urine and plasma samples.

drugs (LOM, FLX, CPFX and NOR) in tablets with mean percentage recovery(R%) 99.81±1.12, 99.94 ±0.92, 99.24± 1.36 and 99.88± 0.81, respectively.

1.2.2 Literature suvey for the determination of ciprofloxacin

1.2.2.1. Spectrophotometric methods

Abdel-Gawad et al. [30]; studied the determination of ciprofloxacin in pure form and in tablets through charge-transfer complexation reactions. Three methods are based on the reaction of this drug as an n-electron donor with 2,3-dichloro-5,6-dicyano-p-benzoquinone (DDQ), 7,7,8,8,-tetracyanoquinodimethane (TCNQ), and p-chloranil (CL) as acceptors to give highly colored complex species.The colored products were quantitated spectrophotometrically at 460, 843, and 550 nm for DDQ, TCNQ, and CL, respectively. Optimization of the different experimental conditions was described. Beer's law is obeyed in the concentration ranges 5-50, 1.5 -15, and 20-200 µg/ ml ciprofloxacin, but the concentration ranges for best accuracy are 10-48, 2.5-15, and 35-195 µg/ml of drug for DDQ, TCNQ, and CL, respectively. The relative standard deviations were <1.5%. Applications of the suggested methods to ciprofloxacin tablets were presented and compared with the USP method. The stability constant of the 1:1 DDQ and CL complexes were1.09 x 10^4 and 2.58 x 10^4respectively, whereas for the1:2 TCNQ complex it was 3.62 x 10^8.

Bungalowala et al. [31]; were developed simple and accurate procedure for the simultaneous determination of ciprofloxacin in tablet pharmaceutical formulation. The methods use derivative spectrophotometry and Q-test . Ciprofloxacin have absorption maxima at 273.2 nm in 10% N,N-dimethylformamide. This obeys the Beer's law in the concentration ranges used for this method (5-25 µg /ml). The

method were validated statistically and by recovery studies.

Xuan, Chun Sheng et al. [32]; were described a simple, quick and sensitive spectrophotometric method for the determination of pipemidic acid, norfloxacin, and ciprofloxacin lactate. The assay is based on a reaction with p-nitrophenol in aqueous medium, apparently with a charge-transfer mechanism, yielding 1:1 complexes with the analytes. The complexes of the 3 respectively analytes had max. absorption at 404, 407, and 403 nm. Optimum conditions for the determination linear calibration range, and apparent molar absorptivities were reported. The method is highly accurate and was applied to the determination of the 3 compounds in tablets. The analysis results were in good agreement with data obtained by official methods.

Bombale et al. [33]; developed two simple, precise and economical procedures for simultaneous determination of ciprofloxacin and tinidazole in 2 component tablets . The methods employ a program in the multicomponent mode of analysis of the instrument used and absorbance ratios for the simultaneous determination of the two drugs. In 0.01N acetic acid, ciprofloxacin had an absorbance max. at 276 nm, while tinidazole had an absorbance max. at 317 nm and isosbestic point at 297 nm. Both the drugs obeyed the Beer's Law in the concentration ranges employed for these methods. The results of analysis were validated statistically and by recovery studies. No interference from excipients was observed

Gursoy et al. [34]; characterized ciprofloxacin liposomes using derivative ultraviolet spectrophotometric determinations where neutral, (-) and (+) charged encapsulated liposome formulations prepared with egg yellow phosphatidycholine (EPC):Chol and dipalmitoyl phosphatidylcholine (DPPC):Chol lipids were investigated for their

loading capacity, encapsulation % and release properties. Ciprofloxacin (CF) in the liposomes were estimated using derivative UV spectroscopy. Among the liposome formulations DPPC:Chol and EPC:Chol neutral formulations had a significantly higher loading capacity and slowest release rate compared with (-) and (+) charged liposomes. The derivative UV spectroscopy was found to be an easy and sensitive method for direct estimation of CF in liposomes.

Meyyanathan et al. [35]; developed a spectrophotometric method for the determination of a fluoroquinolone antibiotic in its pharmaceutical dosage forms using 0.2% ceric ammonium sulfate (CAS) in 2 N sulfuric acid. The reddish brown chromogen reaction product had an absorption max. at 484 nm and was stable for 40 min. Beer's law was obeyed in the concentration range of 10.0-80.0 µg/ml. The reproducibility of the method was 99.1-99.9%.

Avadhanulu et al. [36]; examined a simple spectrophotometric method for the determination of certain fluoroquinolone drugs such as ciprofloxacin, norfloxacin, pefloxacin, lomefloxacin and enrofloxacin in their pharmaceutical dosage forms using ammonium reineckate reagent was based on the formation of precipitate between the drugs and ammonium reineckate, which are soluble in aqouse 50% acetone. This reagent is specific for precipitate of primary and secondary amines. The precipitate drug-reagent complex was separated by filtration and dissolved in aqueous 50% acetone and the absorbance of the resulting pink solution was measured at 524 nm against reagent blank.

Li, et al. [37]; developed a sensitive centrifugation spectrophotometric method for the determination of ciprofloxacin

hydrochloride (CPFX). CPFX can react with phloxine to form a precipitate of charge-transfer complex with a compound of 1:2 by mole ratio and Job's methods. The precipitate. was centrifuged and dissolved in aqueous alcohol, It showed an absorption peak at 546 nm with a molar absorptivity of 2.71 x 10^4 l /$mol^{-1}cm^{-1}$. Beer's law was obeyed in the range of 0 - 22.1 mg/l of CPFX. The presented method has been applied to the determination of CPFX in tablets.

Hopkala, et al. [38]; studied the application of the first-, second-, third- and fourth-order derivative UV spectrophotometric methods for the determination of ciprofloxacin, norfloxacin and ofloxacin in tablets , by using the "peak-zero" (P-0) and "peak-peak" (P-P) techniques of measurement were developed for the determination of ciprofloxacin, norfloxacin and ofloxacin in tablets. The calibration curves are linear within the concentration range of 2.0-12.0 µg/ml for ciprofloxacin, 1.0-10.0 µg/ ml for norfloxacin and 2.5-15.0 µg/ ml for ofloxacin. The procedure is simple, rapid and the results are reliable.

Djurdjevic, et al. [39]; estimated an analytical procedure for the determination of ciprofloxacin in serum without previous extraction has been developed. The determination was carried out using iron (III) nitrate as chromogenic agent, with the addition of sodium dodecylsulfate, at pH = 3.0. Absorbance was measured at 430 nm. The range of linearity was between 0.5 - 20.0 µg/ml with a detection limit 0.2 µg/ml.

Rizk et al. [40]; developed an accurate simple and selective kinetic procedure for the determination of certain 4-quinolones namely, norfloxacin (I), ofloxacin (II), enrofloxacin (III), fleroxacin (IV), ciprofloxacin (V) and pefloxacin (VI) was described. The procedure is

based on reacting the studied compound in acidic media (0.1M HCl) with 3-methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH) in presence of cerium (IV) ammonium sulfate as an oxidant at room temperature for a fixed time of 20 min. for (I), (III), 12 min for (II) and 30 min for IV, V and VI, then the absorbance of the reaction product was measured at 630 nm. The concentration of the studied compounds was computed using the corresponding calibration curve equation for the fixed-time method. The absorbance-concentration plot was rectilinear over the range 20-100 µg/ml for (I), 2 - 20 µg/ml for (II), 10 - 74µg/ml for (III), 10 - 60 µg/ml for IV, 10 - 50 µg/ml for IV and 8 - 40 µg/ml for VI.The determination of the studied compound by the fixed-concentration and rate constant methods was feasible with the calibration equation obtained, but the fixed-time method proved to be more applicable. The procedure was applied successfully to estimat tablets and ampoules and the results obtained were compared statistically with the reference methods.

El-Adl et al. [41]; described a simple and sensitive spectrophotometric procedure for the quantitative determination of certain fluoroquinolones (ciprofloxacin, enoxacin, and norfloxacin) either in authentic samples or in their pharmaceutical formulations by using dichloromaleimide derivative as a chromogenic reagent .The cited drugs were interacted with a synthetic reagent, [N-2,6-dimethylphenyl-2,3-dichloromaleimide] forming an intense colour which can be measured at λ_{max} 514 nm. The optimum interaction conditions, molar ratio of the reactants, and calibration graphs have been studied. A comparative study with the official pharmacopoeia methods of assay of the cited drugs showed no significant difference between them. Furthermore, the proposed procedure was more sensitive and selective. Moreover, the reaction product was isolated and subjected to the structural study using

IR and NMR spectroscopy.

Dominguez et al. [42]; studied the post-antibiotic effects of gentamicin and ciprofloxacin at 1 x, 2 x and 4 x MIC on Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus were studied using a spectrophotometric method and the classic method of viable counts on agar as a reference of the growth kinetics was carried out by viability counting on the plate every hour and by means of the optical dextro of the cultures measured by spectrophotometry at a wavelength of 450 nm. No statistically significant differences were found between the results obtained with the spectrophotometric method and the reference method. The former method was much quicker, much easier to use and to replicate.

Bungalowala et al. [43]; developed derivative spectrophotometric and Q-analysis methods for the simultaneous determination of ciprofloxacin and tinidazole in tablet formulations, Crushed tablets equivalent to 10 mg ciprofloxacin were dissolved in 10 ml DMF and the solution was diluted to 100 ml with H_2O. After filtration and dilution, a solution of concentration equivalent to 10 µg/ml ciprofloxacin was obtained. The solution was derivatized and then the absorbance measured at 253 and 299.6 nm to determine, for ciprofloxacin and tinidazole, respectively. In the second method, absorbances were measured at the isobestic point (297 nm) and at the wavelength of maximum absorption of tinidazole (318.4 nm). Substitution of these values in mathematical equations allowed the concent rations of each drug to be calculated. Recovery studies for both methods gave 99-101 % the proposed methods were accurate, simple and rapid for routine simultaneous determinations of the two drugs. The derivative method of

analysis overcomes the interference due to spectral overlap by selecting a suitable order of derivatization and corresponding derivative interval.

Li et al. [44]; centrifuged portions of a standard ciprofloxacin hydrochloride (I) solution with 0.5 ml aqueous 2 mM-phloxine and H_20 to 5 ml after storing 3 min, at 2500 rpm for 10 min. The liquid was decanted the precipitate was washed with 5 ml H_20 and centrifuged for 10 min. The solid was dissolved in 75 % ethanol to 10 ml and the absorbance was measured at 546 nm ($\varepsilon = 2.71 \times 10^4$) vs. a reagent blank. Beer's law was obeyed from 5-60 μ M / l, with a detection limit of 2μ M. The method was applied to the analysis of (I) in tablets. Reaction mechanism was discussed.

Hu et al. [45]; examined sample solution (5 ml) was diluted with 20 ml borax buffer of pH 9 before adding 5 ml 3 mM-chloranil and H_20 to 50 ml and heating at 40°C for 40 min. After cooling, absorption was scanned between 300 and 450 nm. Fluoroquinolone antibiotics studied were enoxacin, ciprofloxacin (I), sparfloxacin, pefloxacin, fleroxacin and levofloxacin. Effect of structures of the compounds on their charge-transfer reaction for use in their determination was investigated, particularly with more detailed studies on (I) and its photodegradation mixtures. The piperazine group as well as substituents at positions 3, 4 and 5 interfered.

Vega et al. [46]; analysed metronidazoie in parenteral admixture with ciprofloxacin by first-derivative spectrophotometry using the zero-crossing point of measurement. The procedure did not require prior separation steps. The method was found to be linear ($r > 0.999$) from 2.5-10 μ g/ml for metronidazole in absence or presence (at constant concentration) of ciprofloxacin. The first derivative method was applied

for the analysis of intravenous admixture of metronidazole and ciprofloxacin and proved to be rapid, accurate and reproducible .

Mostafa et al. [47]; described a spectrophotometric method for the determination of the antibacterial quinolone derivatives, ciprofloxacin, enrofloxacin and pefloxacin, through their charge-transfer complex formation with three different acceptors. Chloranilic acid was utilized for their determination, forming charge-transfer complexes with λ_{max} =520 nm . The proposed method was applied for analysis of ciprocin tablets, enroxil oral solution, peflacin ampoules and peflacin tablets with accurate mean recovery % 99.58 ± 1.25, 99.94 ± 0.96, 100.91 ± 1.59 and 99.86 ± 1.003. Also, tetracyanoethylene (TCNE) was utilized in the determination of the compounds concerned forming charge transfer complexes with maximum absorbances at 335 nm for ciproftoxacin and at 290 nm for both enrofloxacin and pefloxacin. The procedure was applied for analysis of ciprocin tablets, enroxil 10% oral solution, peflacine tablets and peflacine ampoules with mean recovery %of 99.4 ± 1,27, 99.95 ± 0.9, 98.98 ± 1.565 and 99.88± 0.998, respectively. Also, 2,3 dichloro-5,6-daicyano-p-benzoquinone(DBQ) was utilized for the determination of pefloxacin forming a charge-transfer complex with maximum absorbance at 460 nm. The procedure was applied for the analysis of peflacine tablets and peflacine ampoules with mean % accuracies of 100.4 ± 0.76 and 99.91 ± 0.623, respectively, statistical analysis of the results obtained showed no significant differences between the proposed methods and other official and reported methods as evident from the t-test and variance ratios.

***Wang* et al.** [48]; performed performed determination of the entrapment efficiency of ciprofloxacin liposomes by cation exchange resin-first order derivative spectrophotometry where two portions (0.2 ml)

of the sample solution were separately diluted to 5 ml with de-ionized H_20 to obtain solutions A and B. Solution A was applied to a column packed with type 731 strongly cationic exchange resin, the column-was washed with de-ionized H_20 and the eluate was collected and diluted to 5 ml with H_20. Portions (1 ml) of the solution and 1 ml solution B were separately diluted to 10 ml with 95% ethanol. Their absolute amplitudes (D1 and D2) of the first-derivative spectral peak of ciprofloxacin (I) at 264 nm were measured for calculation of entrapment efficiency (E) using a formula of E = D1/D2 x 100% for determination of I by the method. The calibration graph was linear 14-42 µM.Recoveries were 98.12-100.58% with RSD (n = 5) of less than or equal to 1.81 %.

Nagaralli et al. [49]; proposed two simple, sensitive and accurate spectrophotometric methods for the determination of amoxycillin (AMX), ciprofloxacin (CPF) and piroxicam (PIR) in pure and pharmaceutical preparations. The methods *are* based on the measurement of absorbances of tris (o-phenanthroline) iron (II) [method A] and tris (bipyridyl) [Method B] complexes at λ_{max} 510 and at 522nm, respectively. Reaction conditions have been optimize- to obtain coloured complexes of higher sensitivity and longer stability. The absorbances were found to increase linearly with increase in concentrations of AMX, CPF and PIR which were corroborated by correlation coefficient values. The complexes obeyed Beer's law over the concentration ranges of 0.06-5.2, 0.04-7.2 and 0.2-6.5 µg/ml for AMX, CPF and PIR, respectively, in method A, and of 0.05-8.5, 0.05-9.0 and 0.05-6.5 µg/ml for AMX, CPF and PIR, respectively, in method B. The developed methods have been successfully applied for the determination of AMX, CPF and PIR in bulk drugs and pharmaceutical formulations. The common excipients and additives did not interfere in their determinations. The results obtained by

the proposed methods have been statistically compared by means of Student t-test and by the variance ratio F-test.

Wei et al. [50]; proposed ciprofloxacin (CPFX) as a reagent for the derivative spectrometric determination of praseodymium in mixed rare earths. The absorption spectra of 4f electron transitions of the praseodymium complex with CPFX were studied by normal and derivative spectrophotometry. The stoichiometry of the praseodymium-CPFX complex was calculated by the mole ratio and continuous variations methods. A ratio of Pr to CPFIX of 1:3 was found. The absorption bands of 'the 4f electron transitions of the complex were enhanced markedly. Using the third derivative spectrum. Beer's law was obeyed up to 35 µg/ml of praseodymium. The RSD is 0.62% for 14 µg/ml ol praseodymium. The detection and quantification limits were 0.17 and 0.56 µg/ml of praseodymium, respectively. A method for the direct determination of praseodymium in mixtures of rare earths with good accuracy and selectivity is described .

Pascual - Reguera, et al. [51]; developed a simple and inexpensive method for the determination of ciprofloxacin using solid-phase spectrophotometry. The intrinsic absorbance of ciprofloxacin fixed on a dextran-type cation-exchange resin, Sephadex SP C-25, was measured directly at 277 and 380 nm after packing the gel beads in a 1-mm cell. Using a sample volume of 10 ml, the calibration graph was linear over the range 0.05–0.3 mg/ ml with a RSD of 1.11%. The sensitivity obtained is 40 times higher than that of the corresponding solution method. The method was applied to the determination of ciprofloxacin in pharmaceutical preparations and was validated by standard addition.

Rodryguez - Dyaz [52]; used terbium(III) and europium(III) as dry reagents for the simultaneous determination of ciprofloxacin and tetracycline in serum and urine samples. These lanthanide ions were immobilised by adsorption in paper strips, which were previously treated with sucrose. The terbium(III)-ciprofloxacin chelate presents an intense luminescence at λ_{max} = 284 nm and λ_{max} = 545 nm and the europium(III)-tetracycline chelate does it at λ_{max} = 395 nm and λ_{max} = 615 nm. Luminescence measurements have been performed using the time-resolved mode. The linear ranges of the calibration graphs using standard solutions are 0.03–1.5 μg/ml for ciprofloxacin and 0.03–2.5 μg/ml for tetracycline and the calculated 3 σ (σ =standard deviation) detection limits are 9 and 11 μg/ml, for ciprofloxacin and tetracycline, respectively. Quantification limits (10σ) in the original human urineand serum samples were 125 and 1.3 μg/ml, respectively, for ciprofloxacin, and 75 and 0.8 μg/ml, respectively, for tetracycline. The features of the dry reagent method have been compared to those provided by the measurements carried out in solution. Mixtures of ciprofloxacin and tetracycline ranged in weight ratios between 5:1 and 1:10 were satisfactorily resolved with errors < 6% using the dry reagent method, which was applied to the analysis of several serum and urine samples with recoveries ranging from 88.7 to 109.3%.

1.2.2.2 High performance liquid chromatography(HPLC) methods :-

Tipre et al. [53]; presented the analysis of ciprofloxacin (I) and tinidazole(II) simultaneous in table by HPLC and by simultaneous equation (SE) and absorption factor (AF) spectrophotometry. Twenty tablets were weighed accurately, powdered and an amount of powder, equivalent to 50 mg was dissolved in 25 ml methanol by warming for 15 min on a water bath. The solution was diluted to 50 ml with methanol and

filtered. The filtrate was diluted with methanol to give concentrations of 5 and 6 μ g/ml of I and II and 20 μl portions were analysed by HPLC on a micro Bondapak-NH_2 column (30 cm x 3.9 mm) operated at 50°C with methanollethyl acetate/H_20 acetic acid as mobile phase (0.7 ml/min) and detection at 285 nm. The calibration graph was linear from 2-10 μg/ml of I and II for (SE) spectrophotometric analysis , 20 tablets were dissolved in methanol (as above) and diluted to give concentrations of 5 and 6 μ g/ml of I and II in DMF. The absorbance of the solution (as well as those of standards) was measured at 278 nm and 318 nm, respectively, for I and II. The calibration graphs were linear frorn 2-10 μ g/ml of each analyte and the simultaneous equations (given) were used to calculate the concentrations of each in mixtures. For AF spectrophotometry, the absorbances of the diluted tablet solutions were measured at 272 and 400 nm, respectively, for I and II. The ratio of absorbance of II at 400 and 272 nm was found to be constant over the Beer's range from 2-10 μg/ml of I and II. Results (tabulated) from validation studies showed that the recoveries of both I and II from tablets were quantitative

1.2.2.3 Fluorimetric methods

Zhou et al. [54]***;*** heated sample (250 mg), equivalent to 0.75 mg ciprofloxacin hydrochloride (I), to boiling with 10 ml 1.0 M-NaOH and 5 ml anhydrous ethanol with stirring. On cooling to room temperature in an ice-bath for 20 min, the mixture was filtered and the filtrate was neutralized with HCl and diluted with H_20 to 25 ml. A portion of the solution was treated with 1 ml HCl sodium citrate buffer of pH 5 and 1ml 10mM-$AlCl_3$, and H_20 was added to 10 ml. After 15 ml storage at room temperature, the fluorescence intensity was measured at 420 nm (excitation at 360 nm). The calibration graph was linear from 0.62-3.1 μg/ml of 1. Average recovery was 102.2% with RSD of 1.9%. There was

no interference. Results were compared with those obtained by UV-spectrophotometry.

1.2.3 Literature suvey for the determination of ofloxacin

1.2.3.1 Spectrophotometric methods

Zhao et al. [55]; studied the charge-transfer (CT) complex formed between ofloxacin as the donor and 7,7,8,8-tetra-cyanoquinodimethane (TCNQ) as the acceptor in methanol-acetone medium by a spectrophotometric method. Beer's law is obeyed in the range of 0-15 mg/l of ofloxacin. The apparent molar absorptivity of the CT complex at 743 nm was 3.58×10^4 l mol^{-1} cm^{-1}. The composition of CT complex was found to be 1:1 by Bent-French and curved intersection methods. The relative standard deviation was less than 3% (n=8). The method was applied to the determination of ofloxacin in tablets with satisfactory results.

Mashru et al. [56]; examined a simple and sensitive spectrophotometric method for the determination of pefloxacin (PFL) and ofloxacin (OFL) involved the reaction of these drugs with ferric nitrate in nitric acid medium and have absorption maxima at 462 and 445 nm, for PFL and OFL.respectively. The method is precise and accurate.

Wang et al. [57]; investigated the determination of ofloxacin in tablets by spectrophotometry was based on the formation of an orange complex with ferric chloride in DMSO-MeOH, which exhibited λ_{max} at 420 nm. The linear concentration range was 25-150 µg/ml.The average recovery was 99.78% with RSD of 0.19%.

Nie, et al. [58]; presented the spectrophotometric method for determination of chlorhexidine acetate and ofloxacin in Jieyinling solution. The chlorhexidine content was determined by wavelength spectrophotometry at 255 and 269.4 nm. The content of ofloxacin was determined by spectrophotometry at 326 nm. The average recovery for chlorhexidine acetate was 99.6%, with RSD of 0.8%, and that for ofloxacin was 99.8%, with RSD of 0.3%. The method was simple, fast, and accurate.

Djurdjevic et al. [59]; studied the complex formation equilibria between Al^{3+} ion and ofloxacin in 0. 1 M-LiCl at 298 K by potentiometry using a pH meter and by spectrophotometry. Structures of the various complexes formed were presented and the mechanism of the formation of the complexes and their possible implications on Al toxicity were discussed.

: ***Feng et al.*** [60]; investigated portions of a standard ofloxacin (I) solution were treated with 2 ml of 0.04% tetracyanoethylene in acetone and the mixture was diluted with acetone to 5 ml. The solution was heated at 50°C for 30 min and the absorbance was measured at 409 nm ($\varepsilon = 2.8 \times 10^4$) vs. a reagent blank Beer´s law was obeyed for to 12 µg/ml of I. The method was applied to the analysis of (I) in a commercial tablet. The recovery was 99.5% (based on listed value) with RSD (n =10) of 0.72 %.Results were compared with those obtained by UV- spectrophotometry.

Wang et al. [61]; determined Pr, Nd, Ho and Er in rare earth mixtures, as complexes with ofloxacin,by spectrophotometry using second-derivative spectra. A known volume of lanthanide solution was

mixed with 0.03 M-ofloxacin (5 ml) and buffer (pH 8.6, 3 ml). The solution was diluted to 10 ml with distilled water and allowed to stand for 30 min. Second-derivative spectra were recorded against a reagent blank using 4 cm cells, $\Delta\lambda$= 1 nm, band pass = 1 nm and scan rate 20 nm/min. Details were given of the range of wavelengths over which amplitudes were measured. Synthetic samples were prepared and analysed. the results were tabulated and showed good agreement with the expected values. Two reference materials were analysed for Pd and Nd and results were in goodagreement with certified values. In this method Beer's law was obeyed for 7-50 µg/ml for Pr, Nd, Ho and Er with RSD of the absorption signals of 3.8, 2.2, 2.6 and 3.2% respectivel

Panzade et al. [62]; applied a simultaneous equation method to the determination of ofloxacin (OFX) and tinidazole (TZ) in tablets. $\Delta\lambda$ for solutions of 10 µ g/ml OFX and 30 µ g/ml TZ were determined as 294 nm and 317 nm, respectively. Beer's law was obeyed up to 20 and 60 µg/ml of OFX and TZ, respectively. (ε)values were calculated at 294 nm and 317 nm by solving simultaneous equations. The method was applied to tablets (equivalent to 20 mg OFX) which were dissolved in 0.1 N-NaOH solution, filtered and diluted to 10 µ g/ml. The analysis was carried out on two different tablet brands, and the results are tabulated. Method validation was carried out by adding the known amount of drugs in final dilution and determining any loss from the total content. Recoveries were 99.98-100.21 and 100.02-100.14% for OFX in the two brands; the corresponding values for TZ were 101.10 and 99.96-100.23%.

Quan et al. [63]; formed a new charge-transfer complex by the reaction of ofloxacin with p-nitrophenol(4-niltrophenol). The complex had

strong absorbance of 302 nm while ofloxacin had no absorbance at the same wavelength .The absorbance coefficient, the molar ratio, and the dissociation constant of the complex were studied. The linear calibration range was 2 μ g/ml to 80 μ g/ml for ofloxacin.

Li Ming Du et al. [64]; developed a spectrofluorimetric method for the determination of three fluoroquinolones antibacterials, namely, ofloxacin (OFL), levofloxacin (LEV), lomefloxacin (LOM), and pipemidic acid (PIP) through charge transfer (CT) complex formation with 7,7,8,8-tetracyanoquinodimethane (TCNQ). TCNQ was found to react with these drugs to produce stable complexes and the fluorescence intensity of the complexes was enhanced in 15–90 times higher than that of the studied drugs itself. The formation of such complexes was also confirmed by both infrared and UV–visible spectra measurements. The different experimental parameters that affect the fluorescence intensity were carefully studied. At the optimum reaction conditions, the rectilinear calibration graphs were obtained in the concentration range 0.02–2.2 μg/ml for the investigated drugs and the limits of detection ranged from 0.006 to 0.016 μg/ml .The proposed procedures could be applied successfully for the determination of the investigated drugs in their pharmaceutical dosage forms with a good precision and accuracy compared to official and reported methods as revealed by *t*- and *F*-tests. Also they were applied to determine spiked urine and plasma samples.

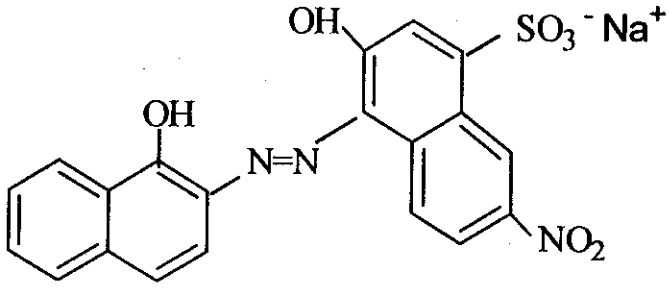
1.2.3.2 Oscillopolarographic titration methods

Zhang et al. [65]; dissolved pulverized tablets or injection solution, equivalent to 10~20 mg ofloxacin (I), in 10 ml acetic acid

sodium acetate of pH 4.5 and ≤ 3.2 molar ratio of sodium tetraphenyl borate was added .After diluted with H_2O to 50 ml standing for 10 min and filtration a 25 ml portion of filtrate was mixed with 0.5 g sodium acetat oscillopolarographic titration at alternating-current voltage of 1.1 V and direct-current voltage of -0.45 V, with several drops of 10 M NaOH to give two incision points appeared while stirring and with 5 mM-thallium(I) sulfate as titrant until the incision points disappeared (end-point). An equation for calculation of the amount of (I) is provided. Excipients did not interfere. By standard addition method, the average recovery was100.2%, with RSD of 0.56%. Results were comparable with those obtained by spectrophotometry.

1.3.Reagents

1.3.1. Eriochromeblack T (E B T) [66, 67, 70,71]



Eriochromeblack T (E B T)

Hydroxy-4-[(1-hydroxy-2-naphthalenyl)azo]-7-nitro-1-naphthalenesulfonic acid monosodium salt; C.I. Mordantblack T

Molecular formula: $C_{20}H_{12}N_3NaO_7S$

Molecular wieght: 461.39

Composition:

C: 52.06%, H: 2.62%, N: 9.11%, Na: 4.98%, O: 24.27%, S: 6.95%

Properties:

Brownish-black powder with a faint metallic sheen . Soluble in hot water giving a reddish-brown solution when cold, Violet brown precipitate with excess HCl. Deep blue, then red in aqueous solution of NaOH. Soluble in concentrated H_2SO_4 giving a blackish-blue solution which yields a brown precipitate on dilution.

USE:

To dye wool from an acid bath reddish-black, which can be converted to blue-black after chroming.

As indicator in the determination of the total calcium and magnesium content of water produces a red colour with calcium, magnesium, zinc and certain other metals in alkaline solutions. When metal ions are absent, for example in the presence of an excess of disodium edetate, the solution is blue.

Store *:* in an airtight container protected from light

The UV- Vis. and IR – spectra of EBT were recorded as shown in fig. (d_1,d_2).

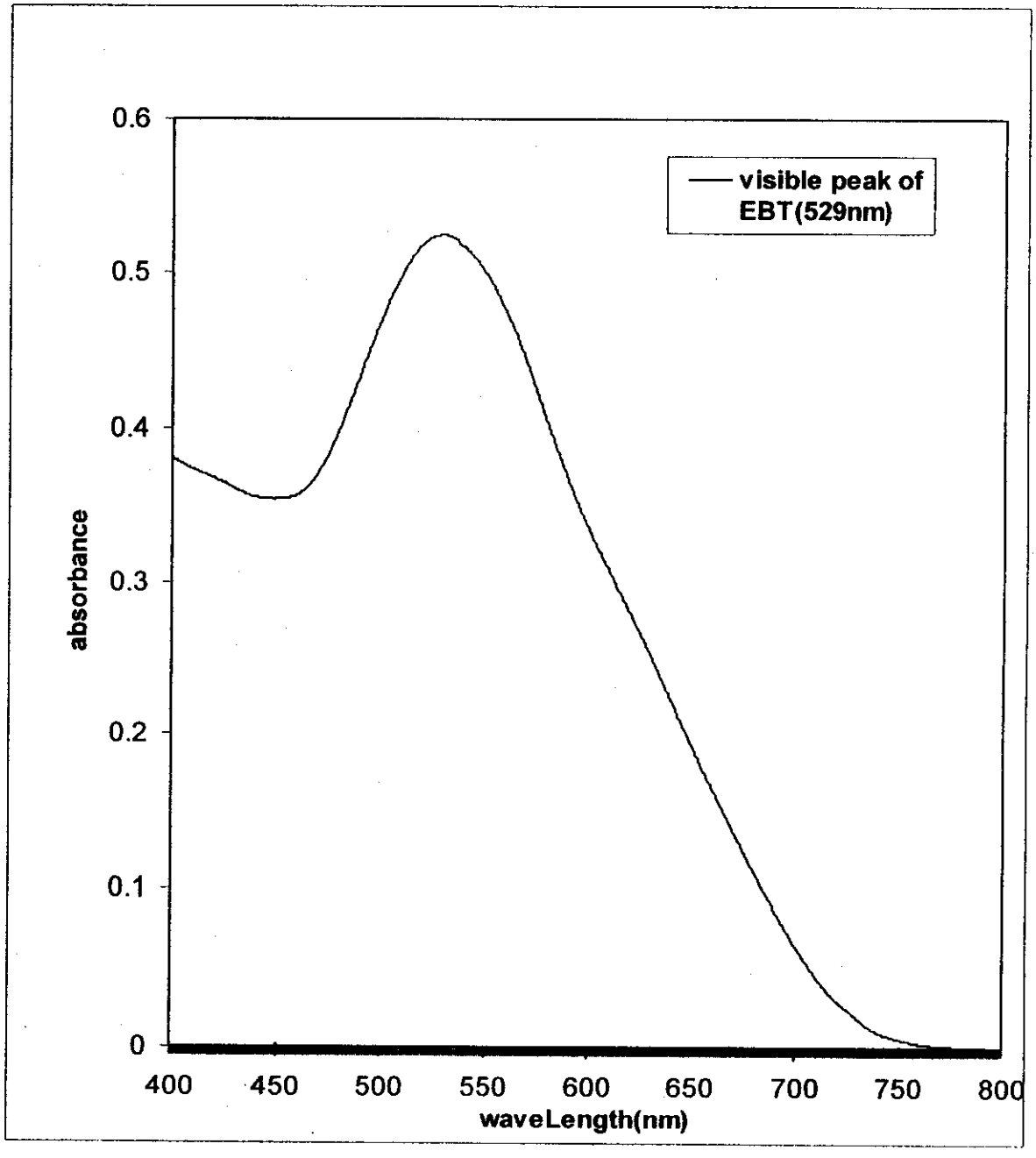


Fig.(d_1): The electronic absorption spectra of ($5x10^{-3}$) M of Eriochromeblack T in diluted NaOH solution (2%)

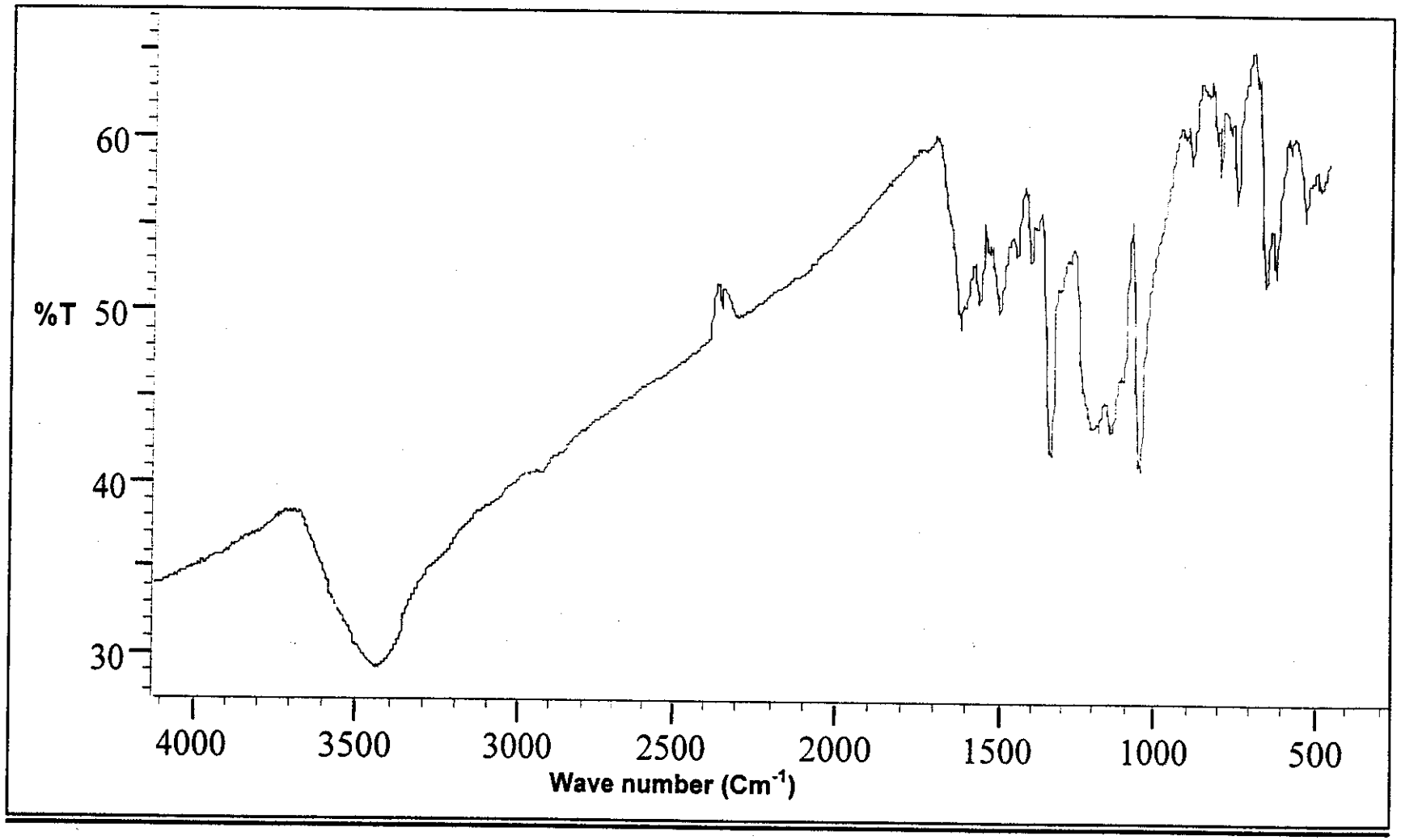
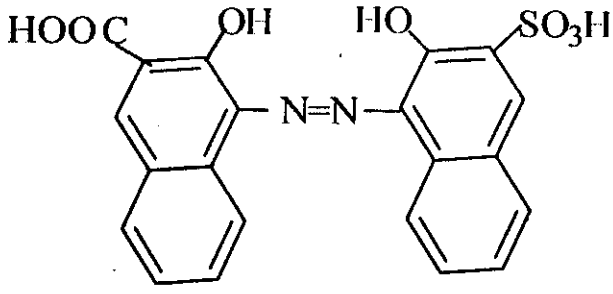


Fig. (d_2): Infrared spectra of Eriochromeblack T

1.3.2. Calconcarboxylic acid(Calc. [66,67,70,71]



2-Hydroxy-1-(2-hydroxy-4-sulpho-1-naphthylazo)naphthalene-3-carboxylic acid.

Molecular formula:. $C_{21}H_{14}N_2O_7S\ 3H_2O$

Molecular wieght: 492.5

Compositio : C: 51.7%, H: 4.0%, N: 5.7%, O: 32.5%, S: 6.5%

Properties:

A brownish-black powder, slightly soluble in water, very slightly soluble in acetone and in alcohol, sparingly soluble in dilute solutions of sodium hydroxide.

USE

Mix 1 part of calconcarboxylic acid R with 99 parts of NaCl R.

Test for sensitivity. Dissolve 50 mg of calcon carboxylic acid triturate in a mixture of 2 ml of strong sodium hydroxide solution R and 100 ml of water . The solution is blue but becomes violet on addition of 1 ml of a 10 g/l solution of magnesium sulphate R and 0.1 ml of a 1.5 g/l. Solution of calcium chloride R and turns pure blue on addition of 0.15 ml of 0.01 M sodium

Store : in an airtight container protected from light

The UV- Vis. and IR – spectra of Calconcarboxylic acid were recorded as shown in fig. (e_1 ,e_2).

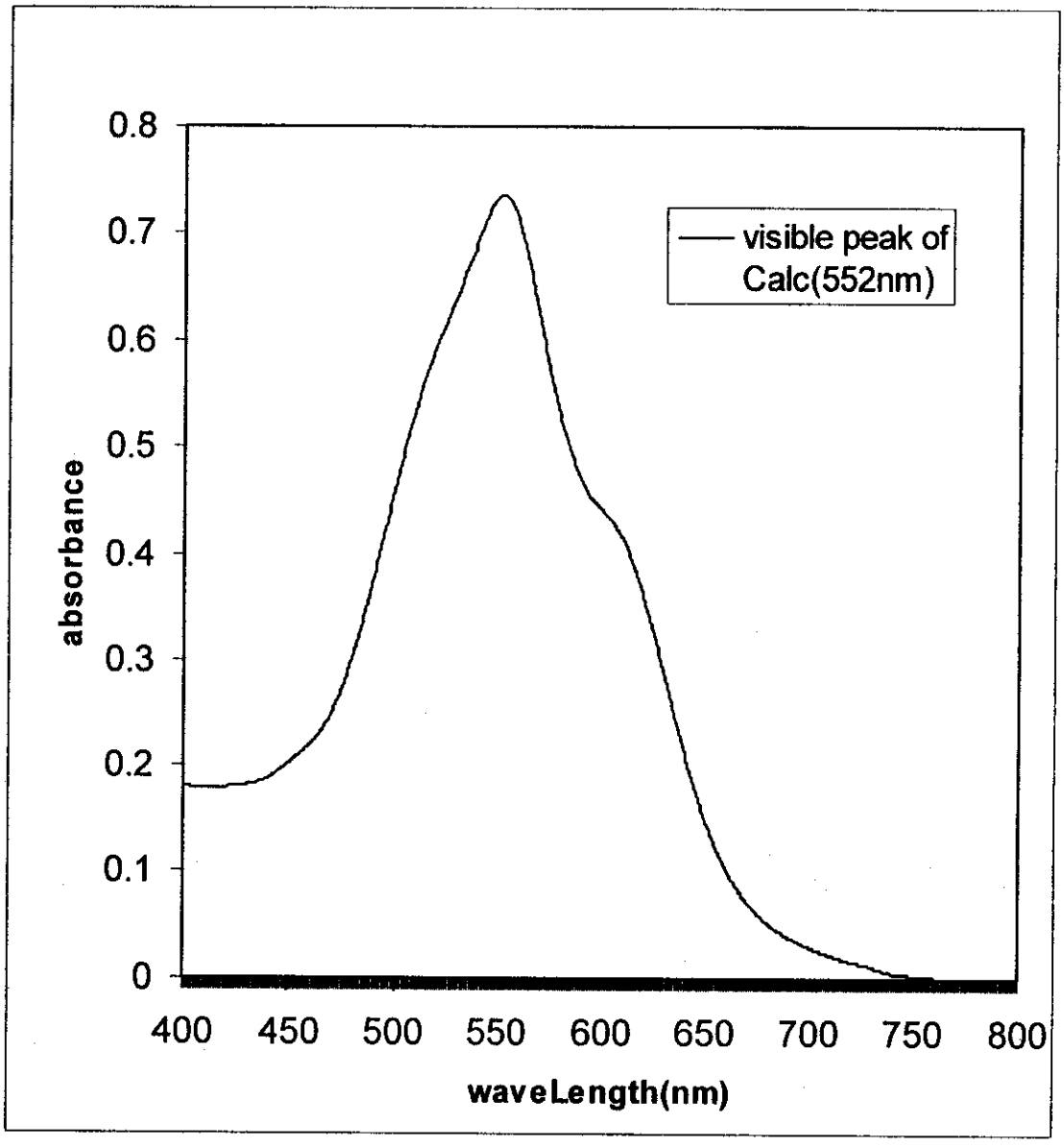


Fig.(e_1): The electronic absorption spectra of ($5x10^{-3}$) M of Calconcarboxylic acid in diluted NaOH solution (2%)

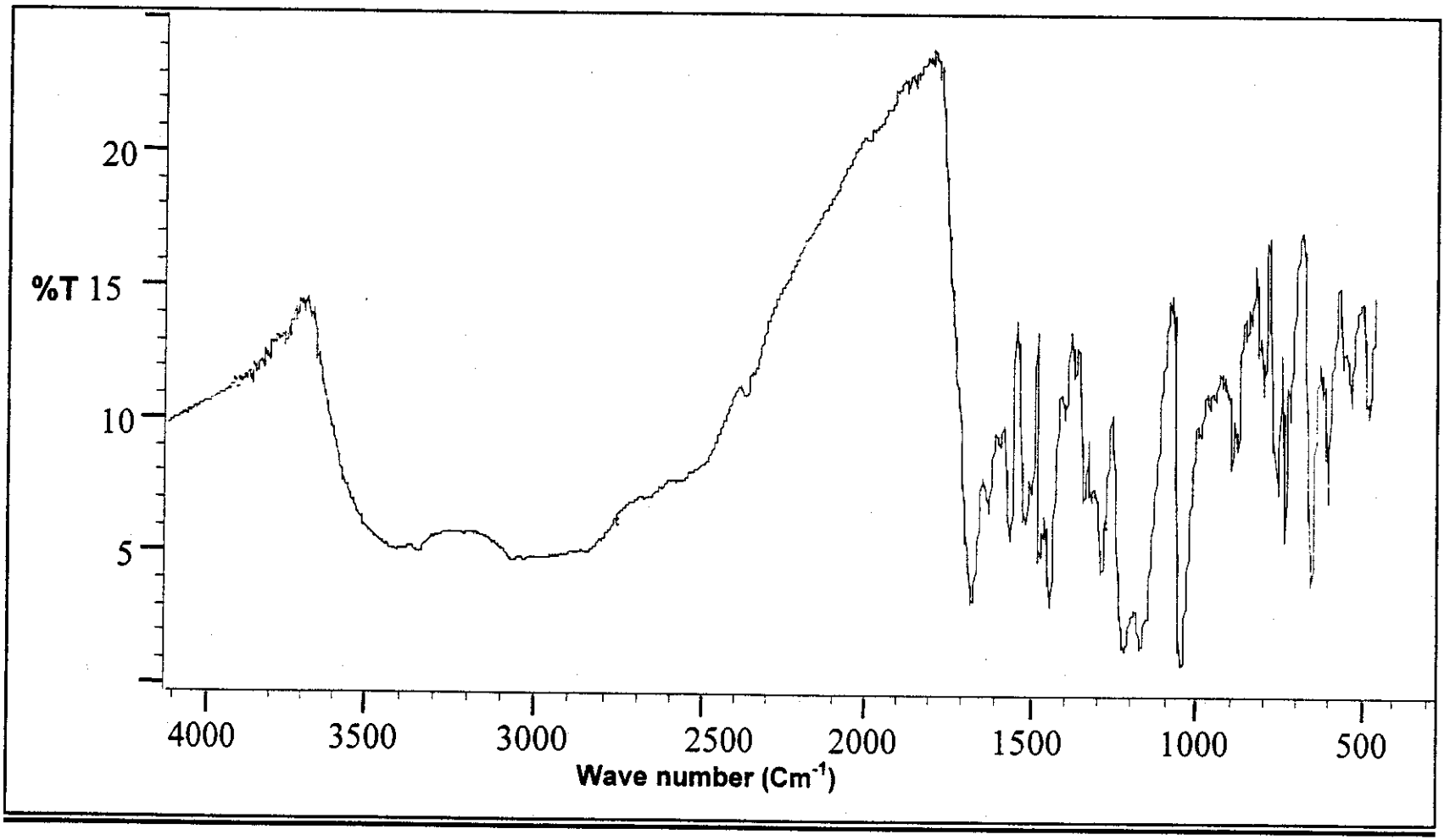
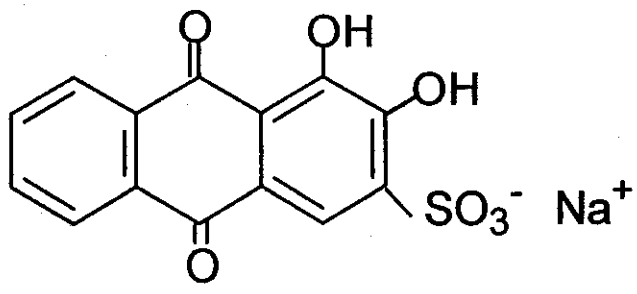


Fig. (e_2): Infrared spectra of Calconcarboxylic acid

1.3.3. Alizarin red S *(Aliz.)* [66,67,70 ,71]



***Alizarin* red S**

9, 10 - Dihydro-3, 4-dihydroxy-9, 10-dioxo-2-anthracenesulfonic acid monosodium salt (Sodium Alizarinsulfonate)

Molecular formula: $C_{14}H_7NaO_7S$

Molecular weight: 342.26

Composition: C: 49.13%, H: 2.06%, Na: 6.72%, O: 32.72%, S: 9.37% .

Literature references:

Occurs in the root of the madder plant (Rubia tinctorum L., Rubiaceae; Krappwurzel) in combination with 2 mols glucose, called ruberythric acid. Was known and used in ancient Egypt, Persia, and India. Synthesized from 2-anthraquinonesulfonic acid sodium salt

Properties:

Orthorhombic, orange needles by sublimation or from absolute alcohol . Solvated scales from diluted alcohol or by evaporation from ether. Sublimes at 110 °C (2 mm Hg). mp 290 °C bp 430 °C. Soluble in water at 18 °C, 2.1×10^{-6} mols/l; at 25 °C 2.5×10^{-6}mols/l. Soluble in 300 parts boiling water; moderately soluble in alcohol, freely in hot methanol

and in ether at 25 °C, also soluble in benzene, toluene, xylene, pyridine, carbon disulfide, glacial acetic acid. Soluble in water solution of alkalies with blue color, but without fluorescence. Fluorescent solution indicate unchanged 2-anthraquinone sodium sulfonate.

USE:

In the manufacture of acid and chrome dyes for wool; acid-base indicator and in the determination of fluorine, usually used as a 1% aqueous solution. pH: 3.7 yellow, 5.2 purple; as a reagent for aluminum and stain in microscopy; in spot tests

The UV - Vis. and IR – spectra of Alizarin red S were recorded as shown in fig. (f_1 ,f_2).

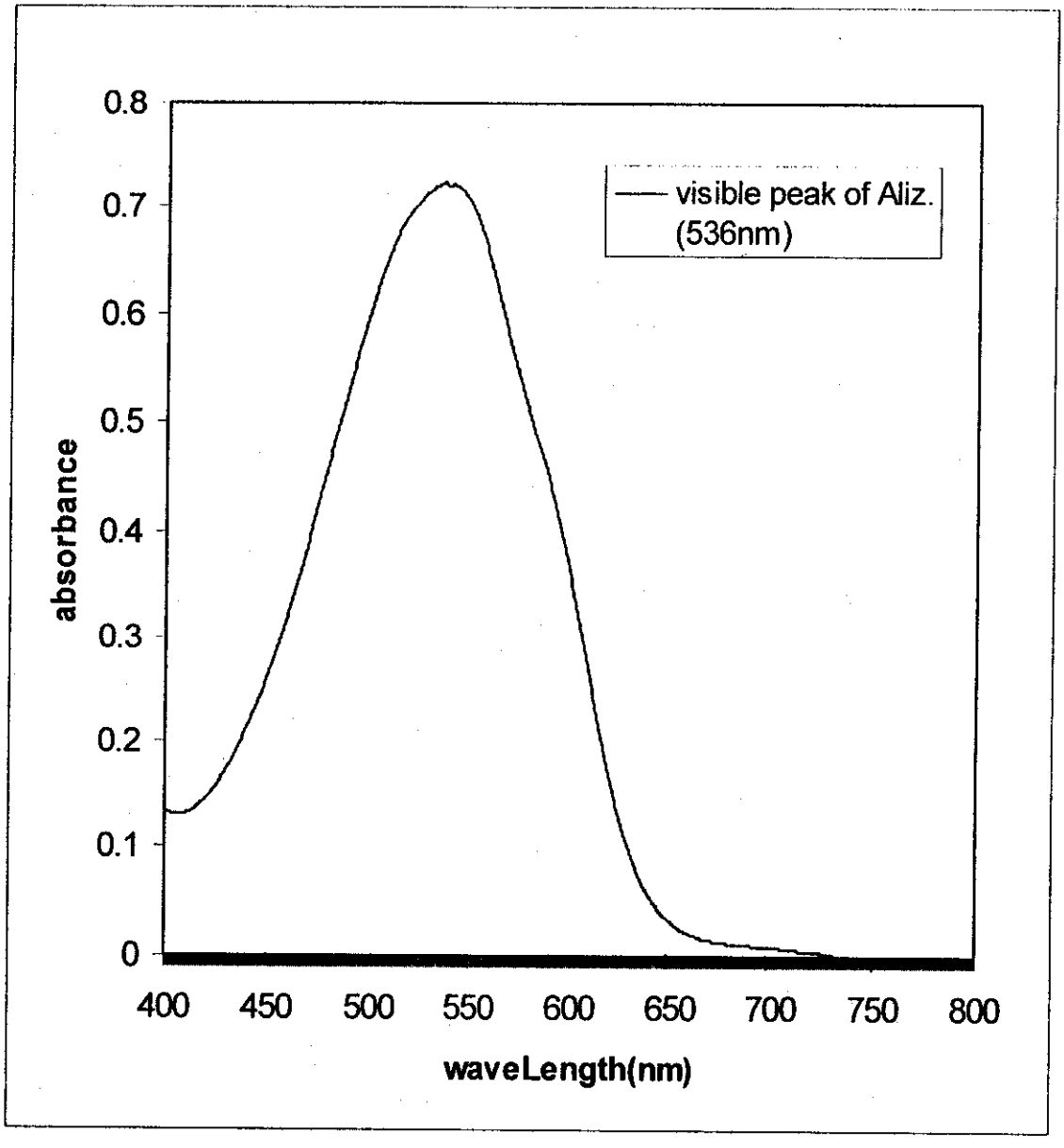


Fig.(f_1): The electronic absorption spectra of ($5x10^{-3}$) M of Alizarin red S in diluted NaOH solution (2%)

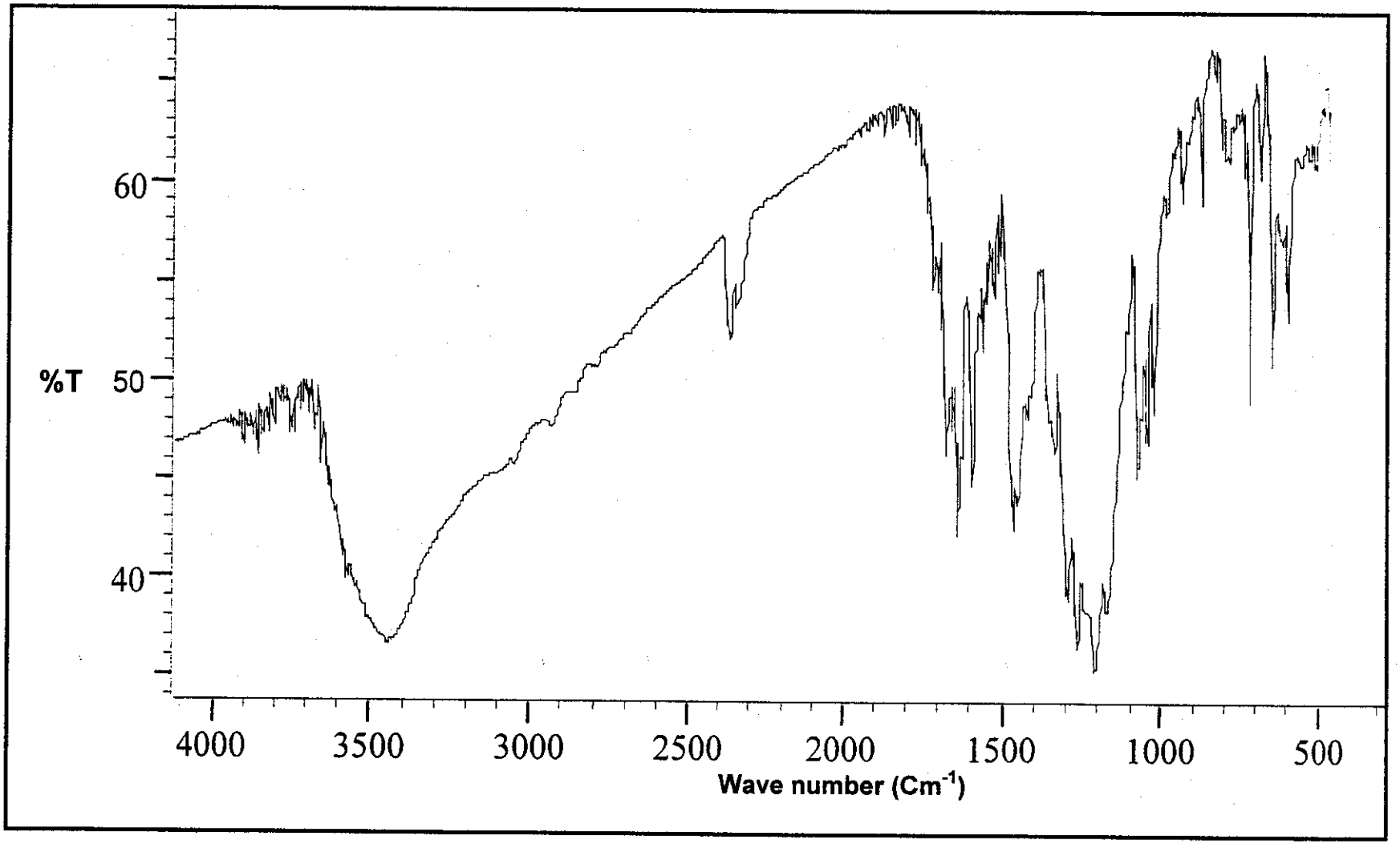


Fig. (f_2): Infrared spectra of Alizarin red S