International Research Journal of Pharmacy and Pharmacology (ISSN 2251-0176) Vol. 2(5) pp. 97-102, May 2012
Available online http://www.interesjournals.org/IRJPP


*Full Length Research Paper*

# Spectrophotometric determination of norfloxacin in pure and dosage forms by complexation with Fe(III) and Cu(II) ions

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Accepted 02 April, 2012

**Based on the complexation reaction between norfloxacin with Fe(III) and Cu(II), a spectrophotometric procedure for the determination of the drug is proposed. Norfloxacin reacts with Fe(III) and Cu(II) in aqueous buffer solutions whereby a coloured complexes are formed which absorb maximally at 425 and 415 nm, respectively. The different experimental parameters affecting the development and stability of the colour were carefully studied and optimized. The absorbance-concentration plots of Fe(III) and Cu(II) norfloxacin complexes are linear up to 101.16 and 139.10 μg ml$^{-1}$ within molar absorptivities 1.98 x 10$^{-3}$ and 2.17 x 10$^{-3}$ l mol$^{-1}$ cm$^{-1}$, respectively, using borate buffer. The proposed method was further applied to the determination of norfloxacin in pure and dosage forms. The results obtained were in good agreement with those obtained by a reference HPLC method.**

**Keywords:** Norfloxacin determination; Fe(III) and Cu(II) complexation.

## INTRODUCTION

Norfloxacin (1-ethyl-6-fluoro-4-oxo-7-piperazin-1-yl-1H-quinoline-3-carboxylic acid) is a synthetic chemotherapeutic antibacterial agent (Nelson et al., 2007) occasionally used to treat common as well as complicated urinary tract infections (afalsky et al., 2006). It is sold under various brand names with the most common being Noroxin. In form of ophthalmic solutions it is known as Chibroxin (Padeĭskaia, 2003). Norfloxacin (figure 1) interacts with a number of other drugs, as well as a number of herbal and natural supplements. Such interactions increase the risk of anticoagulation and the formation of non-absorbable complexes, as well as increasing the risk of toxicity. Norfloxacin is a broad-spectrum antibiotic that is active against both Gram-positive and Gram-negative bacteria. It functions by inhibiting DNA gyrase, a type II topoisomerase, and topoisomerase IV (Drlica and Zhao, 1997), enzymes necessary to separate bacterial DNA, thereby inhibiting cell division.

Several methods such as fluorometry (Panadero et al., 1995; Rodr´ıguez-D´ıaz et al., 2003), voltammetry (Kapetanović et al., 2000; Belal et al., 1999), titrimetry (Basavaiah et al., 2006), potentiometry (Abulkibash et al., 2003) and high performance liquid chromatography (Argekar et al., 1996; Espinosa-Mansilla et al., 2005/2006) have been used for determination of norfloxacin in different real samples. However, most of these methods are sometimes rather complicated because of need to separate interfering compounds and hazard solvents should be used. In this paper, a new and selective method for determination of norfloxacin using UV-Vis spectrophotometry technique is proposed due to the resulting experimental rapidity, simplicity and the wide range of application (Djurdjević et al., 2000; Fratini and Schapoval, 1996) of this method. The method based on the formation of stable Fe(III) and Cu(II) complexes with norfloxacin. The optimum reaction conditions and other analytical parameters are evaluated. No interferences were observed from excipients and the validity of the method was tested against reference methods. The statistical analysis of the obtained results indicates that the method is precise and accurate.

## Experimental

All reagents and solvents used in the present investigation

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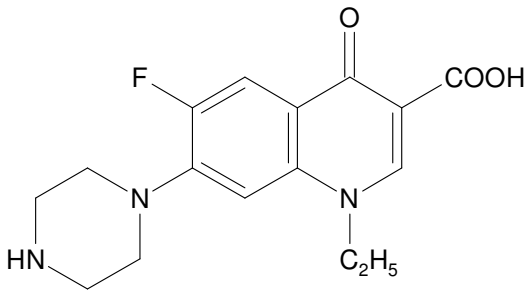


**Figure 1.** Structure of norfloxacin.

were of analytical or pharmacopoeial grade purity from Aldrish, BDH, Riedel de Haen or Fluka.

### Used instruments

A Shimadzu model UV/Vis-1601PC spectrophotometr with 1 cm quartz cells was used to measure the absorbance. The pH measurements were made with **Q** Metrohmdigital model 713 pH-meter with a calomel glass electrode of sensitivity ±0.001 pH units. Hewlett Packard model 1100 high performance liquid chromatography with a variable wavelength detector was used. All measurements were performed at room temperature (28 ±0.01°C).

### Solutions and reagents

Stock solution (2 x $10^{-3}$ M) of norfloxacin (Asta) was prepared by dissolving the accurately weighed amount in glacial acetic acid and the volume was completed to the mark with bidistilled water. Metal stock solution (0.1 M) was prepared by dissolving the appropriate amount of ferric chloride anhydrous (Fluka) and copper(II) acetate monohydrate (Merck) in double distilled water and was standardized by the recommended method (Vogel, 1986). Working solutions were prepared by suitable dilutions with deionized water. The solutions used for investigation of the effect of diverse ions were prepared from sulfate or nitrate of the tested cations and sodium or potassium salts of the tested anions. The universal, acetate, borate and phosphate buffer solutions of varying pH values were prepared as described by Britton (1952).

### Recommended procedures

A series of solutions containing up to 4.0 ml of buffer solution, 1 ml (0.1 M) of the metal ions and 0.2-2.8 ml (1 x $10^{-2}$ M) of norfloxacin was mixed in 10 ml measuring flask and then diluted up to the mark with water. The mixture was allowed to stand for 10 min. The absorbance was measured at the maximum wavelength ($\lambda_{max}$) against a blank solution prepared in the same manner but not contains metal ions. The calibration graphs were prepared by using the same procedure (at least seven concentration points) and were linear passing through the origin.

### General procedure for the determination of norfloxacin in tablets

To minimize tablet composition variation, ten tablets were finely ground. A portion of powder was placed in 100 ml glass stopper flask and dissolved in glacial acetic acid and the volume was completed to the mark with bidistilled water. The assay was completed as described previously. To check the applicability of the developed procedure for the analysis of norfloxacin in tablets, the results were compared with those of a reference method (HPLC) (Through personal communication of Egyptian International Pharmaceutical Industries Co, 16294/1989).

## RESULTS AND DISCUSSION

### Study of reaction between norfloxacin with Fe(III) and Cu(II) ions

Investigations were carried out to establish the most favorable conditions for the complexation reaction of norfloxacin with Fe(III) and Cu(II) ions, to achieve the optimum conditions for maximum colour development in the determination of norfloxacin.

### Electronic spectra and selection of the suitable wavelength

The absorption spectra of the norfloxacin complexes with Fe(III) and Cu(II) were shifted to longer wavelength compared with that of norfloxacin itself (Figure 2). This shift of the absorption maxima can be attributed to the decrease in the energy gap between the excited and ground states upon complex formation [Babko and Philipenko, 1971). T he absorption spectra of the

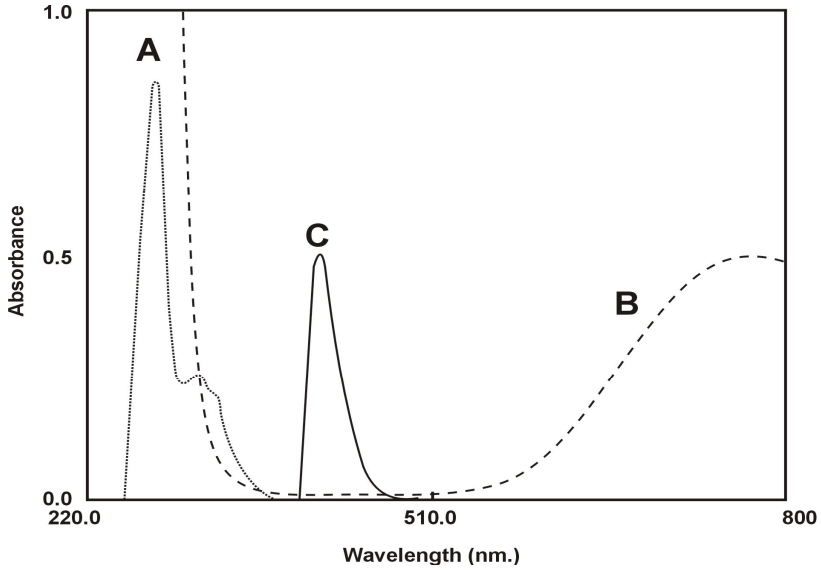


**Figure 2.** The electronic absorption spectra of norfloxacin, Cu(II)-norfloxacin complex and $Cu(CH_3COO)_2$.
A- Norfloxacin against buffer as a reference.
B- $Cu(CH_3COO)_2$ against buffer as a reference.
C- Cu(II)-norfloxacin complex against $Cu(CH_3COO)_2$ and buffer as a reference.

norfloxacin complexes in the UV–Vis region exhibits maximum absorption at 425 and 415 nm for Fe(III) and Cu(II) complexes, respectively, using the same amount of the metal ion as a blank. These longer wavelength peaks have been used in all subsequent measurements of the absorbance. At these wavelengths the absorption of both norfloxacin and metal solution were negligible (figure 2).

## Effect of pH and buffer solution

In order to determine the optimum pH value for complex formation, the drug was allowed to react with the metal ions in borate buffer of pH 8.0-10.0, phosphate buffer of pH 5.8-8.0 and universal buffer of pH 2.0-12.0. The most suitable pH values for the formation of the complexes were determined by scanning the absorption spectra of the metal complexes in solutions of different pH values using the same amount of the metal ion and the buffer as a blank within the wavelength range 200-800 nm. It was found that the borate buffer of pH 9.0-9.4 is the most suitable for developing Fe(III) and Cu(II) complexes. In case of Fe(III) and Cu(II) norfloxacin complexes, the universal and phosphate buffers cause fading of the colour of complexes due to interference from the buffer constituents.

## Influence of time and sequence of addition

The effect of time on complex formation was studied by measuring the absorbance of norfloxacin-metal complexes at different time intervals. The results show that the complexes are formed instantaneously and remain constant for more than 24 h. The obtained results for the effect of different sequences of addition (ligand-metal-buffer, metal-buffer-ligand and ligand-buffer-metal) to select the most suitable one for developing the colored complexes showed that the sequence ligand–metal–buffer was the best one for the formation of Fe(III) and Cu(II) complexes with norfloxacin.

## Effect of metal ion concentration

The effect of metal ion concentration on the absorbance of norfloxacin complexes was investigated by varying the metal ion concentration while other variables were held constant. A constant and maximal absorbance was obtained when the metal ion concentration exceeded three times the reagent concentration. So, the metal ion concentration should be used in large excess during norfloxacin determination.

## Effect of foreign ions

No interference was observed (relative error < 2.5% is considered no-interference) in the determination of norfloxacin with Cu(II) or Fe(III) from the presence of additives and excipients that are usually present in pharmaceutical formulations (noroxin tablets) such as magnesium citrate, microcrystalline cellulose,

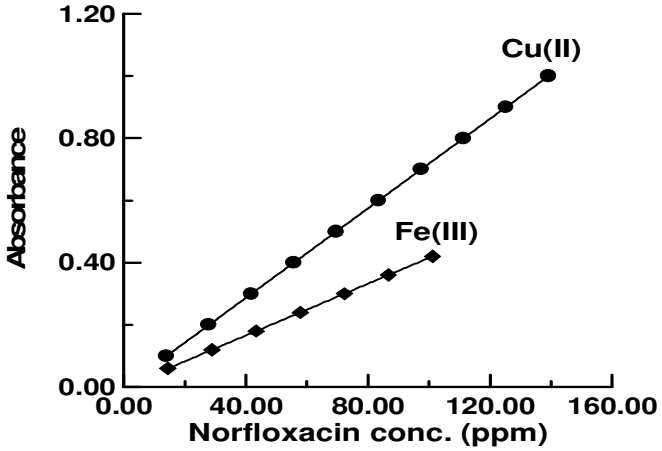


**Figure 3.** Absorbance vs concentration plots for Fe(III)-norfloxacin (λ = 425 nm) and Cu(II)-norfloxacin (λ = 415 nm) complexes.

croscarmellose sodium, hydroxypropylmethyl cellulose and titanium dioxide.

## Composition, stability constants and free energy changes

Stoichiometry of norfloxacin complexes formed in the solution was determined spectrophotometrically applying the continuous variation (Issa et al., 1975) and mole ratio (Zayan et al., 1973) methods. The obtained results revealed the formation of 1:1 (M:L) norfloxacin complexes with Fe(III) and Cu(II) ions. The logarithmic stability constants (log $\beta_n$) and the free energy changes ($\Delta G^*$) of the formed complexes were calculated from the data of continuous variation and mole ratio methods applying equations **1** and **2** (Harvey and Manning, 1950).

$$\beta_n = \frac{\frac{A}{A_m}}{[1-\frac{A}{A_m}]^{n+1} C_l^n n^2} \rightarrow (\mathbf{1})$$

$\Delta G^* = -2.303\ RT \log \beta_n \rightarrow (\mathbf{2})$

where $\beta_n$ is the stability constant of the metal chelate, A is the absorbance at ligand concentration $C_L$, $A_m$ is the absorbance at full color developed, n is the order of the complex formed, T is the absolute temperature and R is the gas constant.

## Calibration graphs and statistical treatments of results

Under the optimum reaction conditions for the spectrophotometric determination of norfloxacin by chealation with Fe(III) and Cu(II) ions, obeyance to Beer's law was tested. On plotting the absorbance as a function of norfloxacin concentration, straight lines were obtained up 101.16 and 139.10 µg $ml^{-1}$ using Fe(III) and Cu(II), respectively, in presence of borate buffer (figure 3). The optimum ranges for the determination of norfloxacin were determined from Ringbom plots and the results are summarized with the other analytical parameters in Table 1. The apparent molar absorptivities were 1.98 x $10^{-3}$ and 2.17 x $10^{-3}$ l $mol^{-1}$ $cm^{-1}$ whereas the Sandell's sensitivities (Sandell, 1959) were 0.147 and 0.161 µg $ml^{-1}$. The high values of correlation coefficients and small values of standard deviations indicate the good linearity of all calibration graphs and the confirmatory of Beer's law to absorbance measurements.

## Determination of norfloxacin in pure and dosage forms

In order to determine the accuracy and precision of the proposed method, a solution containing 27.82 µg/ml of norfloxacin was prepared and analyzed using proposed method at the recommended pH value and the suitable wavelengths for each metal ion (six determinations each). The values of relative errors are 1.05% and 0.98% whereas the coefficient of variations are 0.54% and 0.43% using Fe(III) and Cu(II), respectively. The proposed method was extended for the determination of norfloxacin in tablets manufactured in the local company, In order to validate the methodology. The concentration of norfloxacin in the dosage form was calculated from the appropriate calibration graphs (figure 3). The performance of the developed procedure was assessed by calculation of t-test and F- ratio compared with the reference procedure (HPLC) (Through personal communication of Egyptian

**Table 1.** Spectrophotometric analytical characteristics of norfloxacin complexes with Fe(III) and Cu(II) ions

| Metal ion ($\lambda_{max}$) | M/L ratio | log $\beta_n$ (- $\Delta$G) | $\varepsilon$ x $10^{-3}$ (S.S.) | U.L. Beer (R.R.) | C.C. (S.D.) | R.S.D.* (% E) | t-test* (F-ratio) |
|---|---|---|---|---|---|---|---|
| $Fe^{3+}$ (425) | 1:1 | 4.57 (3.45) | 1.98 (0.147) | 101.16 (31.62-101.16) | 0.9997 (0.0197) | 0.347 (±1.35) | 1.08 (1.13) |
| $Cu^{2+}$ (415) | 1:1 | 2.89 (2.08) | 2.17 (0.161) | 139.10 (37.93-126.47) | 0.9973 (0.0194) | 0.353 (±1.27) | 1.10 (1.25) |

$\lambda_{max}$ – a suitable wavelength in nm; log $\beta_n$ – logarithm of stability constant; ($\Delta$G*) – free energy change (KJ $mol^{-1}$); $\varepsilon$ – molar extinction coefficient (L $mol^{-1}$ $cm^{-1}$). C.C. – correlation coefficient; (S.S.) – Sandell's sensitivity; U.L. Beer – upper limit in Beer's law plot (µg $mL^{-1}$); (R.R.) – Ringbom range (µg $mL^{-1}$); (S.D.) – standard deviation; *Average of six determinations; R.S.D. – relative standard deviation; (%E) – relative error; t- and F- values for 5 degree of freedom and 95% confidence level.

**Table 2.** Spectrophotometric determination of norfloxacin in tablets by the proposed procedure and the official methods.

| Proposed procedure | | | | | | Official procedure | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Metal used | Certified conc. (mg) | Found conc. (mg) | % Recovery | t-value | F-ratio | Taken conc. (mg) | Found conc. (mg) | % Recovery | R.S.D. | Ref. |
| $Fe^{3+}$ | 50 | 50.055 | 100.11 | 1.130 | 1.220 | 400 | 399.82 | 99.955 | 0.69 | [18] |
| $Cu^{2+}$ | 50 | 49.984 | 99.968 | 1.033 | 1.634 | 400 | 399.82 | 99.955 | 0.80 | [5] |

Number of replicates is 6. Theoretical F-values at 95% confidence level = 5.05.
Theoretical t-values at 95% confidence level = 2.57.

International Pharmaceutical Industries Co, 16294/1989). From Table 2, it is found that for the investigated method F-values = 1.13 and 1.25 where t-values = 1.08 and 1.10 for five degree of freedom (P=0.05) and six replicates (n=6) at 95 % confidence level. The tabulated values did not exceed the theoretical F-(5.05) and t-values (2.57) (Miller, 1986) which means that all samples are not subject to systematic error (accurate), i.e. no significant difference between accuracy of the proposed and the official procedures. Also, the values of standard deviations (S.D.), relative standard deviations (R.S.D.) and percentage error are very small indicating high precision (Hinchen, 1969) of the investigated method.

## CONCLUSIONS

The present research work has demonstrated the feasibility of the use of UV-Vis spectroscopy and complexation reaction for determination of norfloxacin. The determination process based on the ability of norfloxacin to form stable 1:1 (M:L) complexes with Fe(III) and Cu(II) ions. The proposed method is applied for determination of norfloxacin in pure and dosage forms with high precession and good accuracy.

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