Development of an Ecological-friendly Method for Ciprofloxacin Determination and Cloud Point Extraction in Pharmaceuticals using Fe(II) (FeSO₄.7H₂O)

Abbas S. Fahad¹, Mohammed Z. Thani², Asmaa M. Abdullah³, Saadiyah A Dhahir⁴
¹,²,³Department of Chemistry, College of Science, Al-Mustansiriyah University, Baghdad-Iraq.
⁴Department of chemistry, College of science for women, University of Baghdad-Iraq.

²Corresponding Author: Mohammed.chem@uomustansiriyah.edu.iq.

Abstract. In this study, new spectrophotometric methods development for evaluation of ciprofloxacin are described. The first method including conversion of ciprofloxacin to colored complex with Fe (II) in the alkaline medium. The colored product has a yellow color with absorbance at λmax 450 nm. Between the concentration range (2-90 µg.mL⁻¹), the Beer’s law is obeyed with correlation coefficient (R²) as 0.9996, limit of detection as 0.365 µg.mL⁻¹, limit of quantification as 1.189 µg.mL⁻¹. Secondly, cloud point extraction was used to determine of a trace amount of the colored product in the first method followed by measuring with a UV-Vis spectrophotometer. The linearity of calibration curve was above the range of (1-50µg.mL⁻¹), the correlation coefficient (R²) was 0.9995. The Limit of detection (LOD) and Limit of quantification (LOQ) were found to be 0.129 and 0.387 µg.mL⁻¹ respectively. This method was successfully employed for ciprofloxacin detection in the several pharmaceutical samples by Rec. Was rang between (98-102.8).

Key word: Ciprofloxacin, Metal Fe(II), Cloud point Extraction, Ecological-friendly

1. Introduction
Ciprofloxacin [1, cyclopropyl-6-fluoro- 1,4 – dihydro – 4- oxo -7- ( 1- piperazinyl ) -3- quinolone carboxylic acid ] (Figure 1), is used to for treat the of bacterial infections. It is believed that the mode of action of the quinolone family is through binding DNA gyrase enzyme [1,2]. It proves to be effective in the treatment of infections caused by microorganisms such as, Escherichia coli, Haemophilus ducreyi, Neisseria gonorrhoeae, Mycobacterium intracelluar, Brucella campylobacter, Pseudomonas aeroginosa, Enterobacteriaceae, Vibrio, Haemophilus influenza, Neisseria meningitides, Moraxella catarrhalis and Bacillus anthracis [3]. Ciprofloxacin can be estimated using different analytical methods like HPLC [4-9], capillary electrophoresis [10,11], fluorimetry [12], chemiluminometry [13], voltammetry [14, 15], high performance capillary electrophoresis [16], spectrofluorimetry [17] and ISE-based potentiometry [18]. The reproducible, sensitive, accurate and precise analytical methods are required to an evaluation of ciprofloxacin in the pharmaceutical samples. Therefore, we existed in this work new methods for determination of ciprofloxacin using complication reaction with iron metal and pre-concentration, extraction it by cloud point extraction (CPE). CPE technique has become increasing popular compared with classical extraction methods due to the advantage of low consumption of organic solvents, rapid
phase separation, high enrichment factor, low cost and high recovery. This technique is attractive that decreases the consumption and exposures to the solvent is CPE and also, it decreases extraction time and disposal costs that have been used for pre-concentration of ciprofloxacin after the formation of a complex which poorly water-soluble \(^{19-29}\). The aim of the present work, is to combine and develop of cloud point extraction (CPE) with a spectrophotometric method to determine the ciprofloxacin, as high sensitive method.

![Ciprofloxacin structure](image)

### 2. Experimental

The spectra and absorbance measurement were achieved in single beam UV-Vis spectrophotometer 160 equip with (1cm ) quartz cell. The pH values were measured by metlar pH meter. All chemicals and reagents used without further purification and freshly prepared. However, ciprofloxacin was purchased from the general company for the manufacture of medicines and medical supplies the state company for drugs industry and medical appliances samarra –Iraq and (FeSO\(_4\).7H\(_2\)O) from Merck company (MSD). A stock iron solution (250 µg/ mL) was prepared by dissolving (0.025 g )of FeSO\(_4\). 7H\(_2\)O in D.W and diluting to (100 mL). Stock ciprofloxacin solution ( 250 µg/mL) was prepared by dissolving ( 0.025 g ) in D.W and dilution to the mark in ( 100 mL ) volumetric flask. 0.01M of hydrochloric acid, 0.01 M sodium hydroxide, 10 % of TritonX-100.

#### 2.1. General procedure of ciprofloxacin complication

An aliquot of (10 mL) solution containing (1 mL) of ciprofloxacin (250 µg/mL), was mixed with (0.2mL) of pH 8.3(Ammonia, ammonium chloride), (0.6mL) of FeSO\(_4\).7H\(_2\)O (250 µg / mL) and then mixture was completed to 10 mL with D.W. The yellow solution formed that gave absorbance at \(\lambda_{\text{max}}\) 450 nm.

#### 2.2. General procedure of CPE for Ciprofloxacin

Various concentrations (1-50µg / mL) of ciprofloxacin complex put in the (10 mL) centrifuge tubes, then 1.8 mL of Triton-X-100 and D.W was added to make total volume of solution to 10 mL. The mixture solution kept in the thermostatic bath (80\(^{\circ}\)C) for 40 min. Separation of two phases was carried out by
centrifugation (Hermle Labor Technik GmbH-Z200 A Universal Compact Centrifuge) for 1 min at 1000 rpm. The mixture was cooled to increase the viscosity of the surfactant-rich phase and aqueous phase was easily disposed by decantation. The rich-surfactant phase from this procedure was diluted with 1 mL of methanol and transferred into quartz cell to measure its absorbance at 450 nm.

2.3. Pharmaceutical preparations procedure

Ciprofloxacin tablets provided from Dar Al-Dawa or the state company for drugs industry and medical appliances samarra –Iraq and Pharma International company containing (500 mg) were carefully weight, then average tablets weight was extracted. The valet weight (0.737gm for one table) was dissolved in D.W to ensure the complete solubility, then made up to volumetric flask (100 mL) and the solution was filtrated.

3. Results and Discussion

Absorption spectra of ciprofloxacin and its Fe(II) complex in pH 8.3 were measured against reagent blank as the reference (Figure.2). The complex formation was accompanied with red shift of $\lambda_{\text{max}}$ of ciprofloxacin(450 nm) by125nm. Investigations were achieved to establish the most favorable conditions. The effect of each of the following variable on the reaction was studied.

3.1 Study of optimization reaction of complication

Different parameter affected absorbance of complication solution, such as pH, type of buffer, volume of buffer, concentration of metal, temperature and time of reaction. The influence of pH on the absorbance at a fixed concentration of complex was investigated in the range pH (1-13.5) using NaOH (0.1M) and HCl(0.1M). Various types of buffer solutions such as (Ammonia/ammonium chloride, Sn( OH)3/SnCl2 and Li(OH).H2O/Li1 ) were examined. The best one is ammonium buffer and optimum value was pH8.3 (Figure.3). Thus, (0.2 mL) of buffer solution (Ammonia/ammonium) pH 8.3 was chosen in the subsequent experiments.

Figure 2. Absorption spectra of ciprofloxacin and its complex Drug, Metal Fe$^{2+}$ against Water and Complex CIP-Fe$^{2+}$ against a blank prepared under the same conditions without drug.

Figure 3. Effect of PH on complication of 25 $\mu$g.mL$^{-1}$ Ciprofloxacin with Fe(II)
The influence of the amount of ion metal (Fe$^{+2}$) was studied by varying concentrations of (Fe$^{+2}$) used from 5-62.5 μg/mL in the complication process and it was found that 15 μg/mL gave the optimum absorption intensity as shown in (Figure 4). The temperature and reaction time was investigated. It was observed that the temperature 25 °C and reaction time 10 min were the optimum conditions to obtain the heights absorbance for ciprofloxacin, as shown in (Figure 5) and (Figure 6). The continuous variation and mole ratio methods were used to estimate the stoichiometry of ciprofloxacin: Fe(II) ratio. The results showed that the ratio of 1:1 (drug: Fe$^{+2}$) (Figure 10 and Figure 11).
3.2 Analytical Data: Under the enhanced conditions, the absorption intensity of ciprofloxacin drug increases linearly as the concentration of ciprofloxacin drug increases.

3.3 Precision and Accuracy: The accuracy was evaluated by determination of the percentage, relative error and recovery and the precision was estimated by the percentage relative error (RSD%) (Table. 2). Table. 1 shows the comparison between the spectrophotometric method and cloud point extraction and we observed that the second method gives the best results.

3.4 Optimization Study of cloud point extraction for ciprofloxacin

A series of different types of surfactant (Triton-X100, TritonX-114, Tween-80, Tween-20, SDS and CTAB) and volumes in the range (0.2-2.6 mL) to improve CPE was investigated. The results illustrate that the absorption intensity will increasing with surfactant conc., consequently, 1.8 mL of (10%, TX-100) was chosen as optimum (Figure.7). The influence of temperature and extraction time has also been investigated by various temperature (60-90 °C) and extraction time (20-70 min). The results showed that 80 °C and 40 min were the best and selected as optimum (Figure.8) and (Figure.9). The centrifugation speed and time were studied to gain excellent extraction, the centrifugation speed and time were investigated in the range (1000-6000 rpm) and (1-6 min) respectively. It found that 1000 rpm and 1min were chosen as optimum. Methanol was added to the surfactant-rich phase after cloud point extraction as the surfactant-rich phase was highly viscous to ease its transfer into sample cell. The amount of (1 mL) methanol was chosen to have amount of sample for transferring and measuring the absorption intensity of the sample.

Analytical data of CPE for ciprofloxacin drug: Under the optimum conditions founded by CPE method for the determination of ciprofloxacin and linear calibration curve was established by plotting concentration of ciprofloxacin versus absorbance (1-50 µg.mL⁻¹).

Table 1. Comparison the analytical parameter of determination CIP drug between two method

| Parameters  | Before CPE | After CPE |
|-------------|------------|-----------|
| λ<sub>max</sub> nm | 450 | 450 |
### Table 2. Application of the proposed CPE for the evaluation of ciprofloxine

| Drug       | Before cloud point extraction | Relative Error (%) | Recovery (%) | Average Recovery (%) | RSD% (n=5) |
|------------|-------------------------------|--------------------|--------------|-----------------------|------------|
|            | Conc. of drug µg/mL⁻¹ |                         |              |                       |            |
|            | Taken   | Found                           |              |                       |            |
| Ciprodar   | 10      | 9.79                       | -2.1         | 96.4                  | 98         | 5.0   |
|            | 20      | 20.25                      | 1.25         | 101.2                 | 2.8        |       |
|            | 30      | 30.3                       | 1.00         | 101.0                 | 3.2        |       |
| Cipopharm  | 10      | 9.44                       | -5.6         | 98.0                  | 98.9       | 5.1   |
|            | 20      | 19.4                       | -3           | 99.5                  | 4.4        |       |
|            | 30      | 29.6                       | -1.33        | 99.3                  | 2.5        |       |
|            | After cloud point extraction |                      |              |                       |            |
|            | 10      | 10.1                       | 1.0          | 101                   | 102.2      | 4.2   |
|            | 20      | 21.4                       | 7.0          | 107                   | 1.9        |       |
|            | 30      | 29.6                       | -1.3         | 98.6                  | 3.9        |       |
| Ciprodar   | 10      | 10                         | 0            | 100                   | 102.8      | 4.0   |
|            | 20      | 22.2                       | 11           | 111                   | 1.1        |       |
|            | 30      | 29.3                       | -2.3         | 97.6                  | 1.7        |       |
| Cipopharm  | 10      | 10                         | 0            | 100                   | 102.8      | 4.0   |
|            | 20      | 22.2                       | 11           | 111                   | 1.1        |       |
|            | 30      | 29.3                       | -2.3         | 97.6                  | 1.7        |       |

### Table 3. Comparison the values of LOD and LOQ of the CPE method with various methods reported in literature

| Method                                | LOD µg/mL | LOQ µg/mL | Ref.   |
|---------------------------------------|-----------|-----------|--------|
| Titrimetric                           | 0.05      | 0.09      | [30]   |
| A first-derivative spectrophotometric | 0.18      | 0.60      | [31]   |
| Cloud point extraction                | 0.77      | 2.57      | [32]   |
| HPLC                                  | 0.11      | 0.35      | [33]   |
| HPLC                                  | 0.0587    | 0.1779    | [34]   |
| Liquid-Liquid                         | 0.19      | -         | [35]   |
| Self-Assembled Gold Nanofilm          | 0.25µM    | -         | [36]   |
| HPLC                                  | 0.16      | 0.28      | [37]   |
| matrix is potential synchronous spectrometry | 0.0135   | -         | [38]   |
| Reverse flow injection spectrophotometric | 0.20      | 0.69      | [39]   |
| Cloud point extraction                | 0.075     | 0.129     | Present work |

### 4. Conclusion

A simple, precise, accurate, ecological-friendly and sensitive spectrophotometric method for the estimation of trace amount of ciprofloxacin. The first method containing convention ciprofloxacin to the colored product was measured using UV-Vis spectrophotometry the second method is cloud point extraction that
was to determination and pre-concentration of ciprofloxacin complex. The method is cheap and less time consuming compared with the other methods like, SPE, liquid-liquid extraction and HPLC.

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6. References

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