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# Synthesis, Characterization, Spectrofluorometric and Antibacterial Activity Studies of Moxifloxacin-Zirconium Complex

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SPECTROSCOPIC methods such as mass, FT-IR and nuclear magnetic resonance in combination with thermal analysis measurements were used to verify and describe the physicochemical properties of the synthesized moxifloxacin (MOX) Zirconium (IV) metal complex. The spectroscopic and elemental analysis data support the formation of the complex with the formula $C_{21}H_{23}FN_3O_4Zr(H_2O)_2.0.5H_2O$. Results revealed that complexation between Zirconium (IV) and moxifloxacin exhibited significant increase in antibacterial activity especially against Gram negative organisms.

In addition, a simple, rapid, reliable, and sensitive spectrofluorometric method is developed for the determination of MOX. The method depends on the chelation of MOX with zirconium (IV) to produce fluorescent chelate (MOX/ Zr). Different factors affecting the relative fluorescence intensity of the resulting chelate were studied and optimized. The relationship between the concentration and relative fluorescence intensity was rectilinear in the range of 0.1–4 µg/ml. The limits of detection and quantitation are 0.06 and 0.11 µg/ ml, respectively.

At the optimum reaction conditions, the drug–metal chelate showed excitation maximum at 333 nm and emission maxima at 485nm. The developed method was applied successfully for the determination of the studied drug in its pharmaceutical dosage forms with a good precision and accuracy.

**Keywords:** Moxifloxacin, Metal complexation, Spectrofluorometric, Thermal analysis and Antibacterial.

Moxifloxacin (1-cyclopropyl-6-fluoro -1,4-dihydro-8- methoxy-7- [(4*aS*,7*aS*)-octahydro -6H-pyrrolo [3,4-b]pyridin-6-yl]4-oxo-3 quinoline carboxylic acid) (Fig.1) is a fourth-generation synthetic 8-methoxyquinolone derivative of fluoroquinolone antibacterial agents. It was discovered in 1999 by addition of an

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azabicyclo-substitution at C-7, which is associated with activity against a broad spectrum of pathogens, encompassing Gram-negative and Gram-positive bacteria[1]. In addition, the presence of a methoxy group at the C-8 position was associated with a decreased propensity for development of resistance phototoxicity and enhanced activity against tuberculosis[2-4]; it also includes antibiotic resistant *Streptococcus pneumonia*. It is available for oral and parenteral administration. IV formulation of MOX will now allow treatment of very ill hospitalized patients with respiratory or skin infections [3,5,6] .

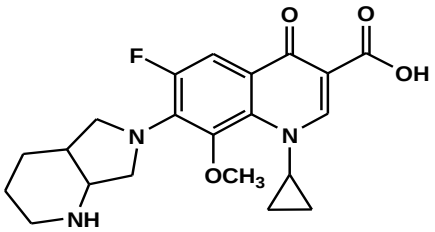



**Fig.1. Structure of moxifloxacin.**

Interaction studies between drugs and transition metals are an important research area in bioinorganic chemistry [7-9]. The action of many drugs is dependent on coordination with metal ions[7] and/or the inhibition[8] of formation of metalloenzyme [10]. Transition metals are integral part of an organic structure performing a vital function in the organism during the biological process of drug utilization in the body. The reduction of metals below certain limit results consistently in a reduction of physiologically important function [11]. Although, the absorption of quinolone drugs is lowered when they are administered simultaneously with multivitamins, magnesium or aluminium containing antacids and others cations [12,13], the proposed mechanism of the interaction is chelation between the 4-oxo and adjacent carboxyl group of quinolone and metal cations [10,14-16]. Since these functional groups are required for antibacterial activity, it could be anticipated that all of the quinolones could be interacting with metal ions[15]. Literature survey assembled a number of different complexation of quinolones [17-19] .

The methods employed in the literature include high-Performance Liquid Chromatography [20, 21]; square-wave voltammetry by interaction of MOX with Cu(II)[6]; potentiometric and UV spectrophotometric measurements through complex formation with gadolinium(III) ion[22]. Recently, MOX-copper complexes were synthesized, characterized and screened for anti-proliferative and apoptosis-inducing activity against multiple human breast cancer cell lines[23].

Based on few reports in the literature about Zr (IV) fluoroquinolones complexation we aimed to: (i) synthesis and characterization of (MOX)/Zr(IV) chelate using different techniques including FT-IR spectroscopy, NMR spectroscopy, elemental analysis, mass spectrometry, thermogravimetric and differential thermal analysis (ii) Screening of the antibacterial activity of the prepared complex (iii) Studying the spectrofluorometric properties and stoichiometry of chelation between

moxifloxacin and zirconium(IV) in solution, also, application of the developed procedures for determination of moxifloxacin in pure and pharmaceutical dosage forms.

## Experimental

*Apparatus*

Ex-situ FTIR spectra were taken of lightly loaded (<1%) thin discs of KBr-supported test materials at 4000 to 400 $cm^{-1}$ with the resolution of 4 $cm^{-1}$, using a model 410 Jasco FT-IR spectrophotometer (Japan). The $^{1}$H-NMR spectra were recorded on a JEOL 500 MHz spectrometer using DMSO-$d_6$ as a solvent and TMS as internal standard. Mass spectra (MS) were taken on an AEIMS 30 Mass spectrometer at 70eV. Thermal analysis was performed by 30H Shimadzu analyzer (JAPAN). Thermogravimetric and Differential thermal analysis curves were recorded while heating a small portion (10-15 mg) of the drug compound up to 800 °C at 10 °C/min in a $N_2$ atmosphere (20 $cm^3$/min) of the test gas. A highly sintered α-$Al_2O_3$ (Shimadzu Corp.) was the thermally inert reference material for the DTA. The abbreviation ML stands for mass loss. Elemental analysis was performed at the Unit of Microanalysis, Assiut University (Assiut, Egypt).

The pH values of solutions were measured using an Orion Research Model 601A digital pH-meter. All calculations were carried out on IBM computer using Microsoft excel 2002 for windows ME. SMAC program [24] was used for all statistical methods.

*Materials and reagents*

Moxifloxacin hydrochloride was obtained from the (Medical Union utical; MUP, Ismalia, Egypt). Zirconium nitrate was 99% and purchased from (BDH laboratory reagents, England). All solvents and reagents used for the preparation of complex were of analytical grade. Double distilled water was used. Samples used in this study were supplied by their respective manufactures and were used without further purification.

*Pharmaceutical formulations*

Moxacin tablets (Medical Union pharmaceutical; MUP, Ismalia, Egypt) labeled to contain 400 mg anhydrous MOX per tablet, batch number 102883. A Moflox tablet (Global Napi /Wockhardt, Cairo, Egypt) labeled to contain 400 mg MOX, batch number 101806 and a Moxiflox tablet (Eva-pharma, Cairo, Egypt) labeled to contain 400 mg MOX, batch number 103335.

*Preparation of moxifloxacin-zirconium complex in the solid state*

Appropriate quantity (0.5 mmol, 0.218 g) of MOX hydrochloride was dissolved in distilled water (5 ml) which was then added to the solution of $Zr(NO_3)_4$ (0.5 mmol, 0.169 g) in distilled water (5 ml). Triethylamine solution (1 mol/l) was added wise drop, maintaining the pH between 7.5 and 8.0. The reaction mixture was stirred at room temperature for two days. The solid precipitate obtained was filtered off

under vacuum, washed with water and methanol, and dried under vacuum at room temperature. The complex prepared is indicated throughout the text by the abbreviation (MOX/Zr). mp: > 300 ºC. % Yield = 65 %. IR data ($cm^{-1}$): 1650, 3400 (br). $^1$H NMR (DMSO-$d_6$) δ ppm: 0.85-2 (m, 5 H); 2.55- 4.3(m, 12H); 3.68(s, 3H); 7.75 (d, 1H); 8.78 (s, 1H). ES-MS: $C_{21}H_{23}FN_3O_4Zr$ $(H_2O)_2.0.5H_2O$ (Scheme 1); $M^+$.-$2H_2O$ = 499(3%); 402 (100 %); 358 (6 %). Anal. Calc.: C, 47.00; H, 5.26; N, 7.83. Found: C, 47.6; H, 6.00; N, 7.80.

*Spectrofluorometric study*

*Preparation of standard solution*

Stock solution containing 1.0 µg/ml of MOX hydrochloride was prepared in doubled distilled water. Working standard solutions containing 0.1-0.7 µg/ml were prepared by suitable dilution of the stock solution with doubled distilled water.

*Effect of pH*

Accurately measured 1.0 ml of standard solution containing 0.5 µg/ml of the drug was transferred into 10 ml calibrated flask. One milliliter of the metal solution (0.85 mg/ml) was added and then pH was adjusted by using acetate-HCl buffer solution of the respective pH 2.0-6.0. The volume was completed with methanol and the relative fluorescence intensities were measured against reagent blank treated similarly at the excitation (336 nm) and emission (486 nm) maxima specific for the drug.

*Effect of different diluting solvents*

Different diluting solvents were tested to choose the most suitable one for the chelate formation. The investigated solvents include; water, methanol, 2-propnanol, DMF, acetone and ethanol.

*Effect of metal ion concentration*

The effect of zirconium concentration on the relative fluorescence intensity was studied by using different metal concentration in the range of 0.1-2.0 µg/ml, keeping all the other variables constant.

*Effect of reaction time*

Keeping all the other variables constant, the relative fluorescence intensities of the resulting solution was measured at time intervals ranging from 1 min. to 24 hr at ambient temperature against reagent blanks treated similarly at the excitation and emission maxima specified for the drug.

*General analytical procedure*

Accurately measured one milliliter aliquot volume of the standard 0.5 µg/ml or sample solutions were transferred into 10 ml calibrated flask. One milliliter of the metal solution containing (0.85 mg/ml) was added and pH was adjusted to 4.0 using one milliliter of acetate buffer solution. The volume was completed with methanol. The relative fluorescence intensity of the resulting solution was measured against reagent blank treated similarly at the excitation and emission maxima specific for the drug.

*Analysis of dosage form*

An accurately weighed amount, equivalent to 10 mg of the drug from composite of 20 powdered tablets was transferred into 100ml calibrated flask and diluted to the mark with doubled distilled water, sonicated for 20 min and filtered off to obtain solution of 100 µg/ml. Further dilutions are carried out to obtain sample solution containing (0.3 µg/ml) then the general procedure was followed.

*Determination of molar ratio*

Equimolar concentrations of the drug and metal ($1.0 \times 10^{-4}$ M) were prepared. Aliquots of each solution were added in different ratios to a series of 10ml calibrated flask. The total volume of both the drug and metal was adjusted to 5ml. The pH was adjusted to 4.0 using 1ml of acetate buffer solution and then the volume was completed to 10 ml with methanol. The relative fluorescence intensity was measured at its respective maxima [25] .

*Antibacterial investigation*

(MOX/Zr) complex in comparison with the free ligand MOX as a reference was tested against four bacterial strains, *Staphylococcus aureus* (*s.aureus)*, *Bacillus subtillus (B. subtilis*), *Escherichia coli (E.coil)* and *pseudomonas aeruginosa* (*p. aeruginosa)*. Bacterial strains were supplied by Department of Microbiology, Faculty of Pharmacy, Minia University. Suspension of each microorganism was prepared to $10^6$ colony forming units (CFU/ml). The tested compounds were dissolved in DMSO to an initial concentration of 10 mg/ml and dilutions of the test compounds were prepared at concentrations 2, 4, 8, 16, 32, µg/ml. Moxifloxacin was used as a reference antibiotic, it was dissolved in DMSO at a concentration of 10 mg/ml and the five dilutions of it were prepared as the tested compounds. All strains were cultured on Muller-Hinton agar medium which was supplied from Oxoid Chemical Co. UK. and prepared according to the instructions of the manufacturers. The media were molten on a water bath, inoculated with 0.5 ml of the culture of the specific microorganism and poured into sterile Petri dishes to form a layer of about 3-4 mm thickness. The layer was allowed to cool and harden. With the aid of cork-porer, cups of about 9 mm diameter were done. Antibacterial activity was investigated using Agar cup diffusion technique[26], Different concentrations of the tested compound in DMSO was placed separately in cups in the agar medium. All plates were incubated at 37ºC overnight. The inhibition zone was measured after 24 hr. The minimum inhibitory concentration (MIC) is the intercept of the grave of logarithm concentrations versus diameter of the inhibition zones.

## Results and Discussion

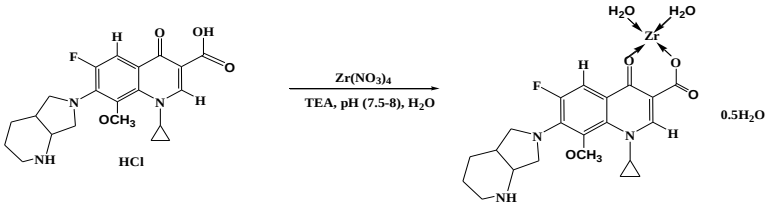
*Characterization of moxifloxacin-zirconium complex*

The physical properties of MOX ligand and the complex are listed in Table 1. The melting point of the complex is higher than that of the ligand revealing that the complex is much more stable than ligand. The complex is stable in air and insoluble in water, methanol, acetone and dimethyl formamide.

**TABLE 1. Physical properties of moxifloxacin and its zirconium complex.**

| Molecular formula | Molecular weight | Yield (%) | Color | mp (°C) |
|---|---|---|---|---|
| $C_{21}H_{24}FN_3O_4.HCl$ | 437.15 | - | Pale yellow | 238-242 |
| $C_{21}H_{23}FN_3O_4Zr\ (H_2O)_2.0.5H_2O$ | 535.1 | 65 | orange | > 300 |

The proposed structure of moxifloxacin and its complex with Zirconium (IV) (MOX/ Zr) is shown in Scheme 1.




**Scheme 1. Synthesis of (MOX/ Zr) complex $C_{21}H_{23}FN_3O_4Zr(H_2O)_2.0.5\ H_2O$.**

*IR spectrum*

Comparing the main FT-IR frequencies of metal complexes with that of moxifloxacin (Fig. 2), the following was found (i) in the spectrum of the ligand (MOX), the two strong absorption peaks at 1727 and 1620 $cm^{-1}$ are characteristic to (υCOOH) and (υCO), respectively [27]. (ii) Different from the spectrum of the ligand (MOX), the band at 1727 $cm^{-1}$ for the complex completely vanished due to deprotonation of –COOH group and formation of Zr- O bond Scheme 1. The peak at 1620 $cm^{-1}$ was retained in the IR spectrum of (MOX/ Zr) but shifted to 1636. (iii) For the complex, the bands positioned at the ranges of 1580-1500 $cm^{-1}$ and 1480-1420 $cm^{-1}$ may be attributed to the asymmetric and symmetric vibrations of the –COO group. (iv) New vibrating absorptions were observed in the range of 650- 550 $cm^{-1}$, which were characterized as the absorption of M-O bonds [28]. (v) The broad band at > 3000 $cm^{-1}$ is characteristic for the secondary amino group and coordinated water (Fig. 2). So we excepected as reported that the complexation occurred between the ring C-4 carbonyl and the carboxylate at position 3 [29].

*$^1$H NMR spectrum*

$^1$H NMR gives information about the environment in which the nuclei of atoms are found in molecules. The $^1$H-NMR spectrums showed the absence of the COOH proton at δ 15.12 ppm confirming that the complex is formed between the carboxylate oxygen and ring C-4 carbonyl. The spectrum of the complex of MOX zirconium metals showed broad signals that were attributed to what is known as paramagnetic characters[30]. Generally, minor changes had been demonstrated in the chemical shifts of the prepared complex on comparison with their corresponding signals in the parent MOX, this was explained previously by the change in the counteranion [30]. The characteristic doublet signal for H-5 appeared at about δ 7.75 ppm for the complex while appeared at δ 7.80 ppm in case of the parent drug. The singlet signal characteristic for H-2 appeared at

δ8.78 ppm that is slightly downfield shifted by δ 0.1 ppm than the corresponding H in case of MOX (δ 8.68 ppm).

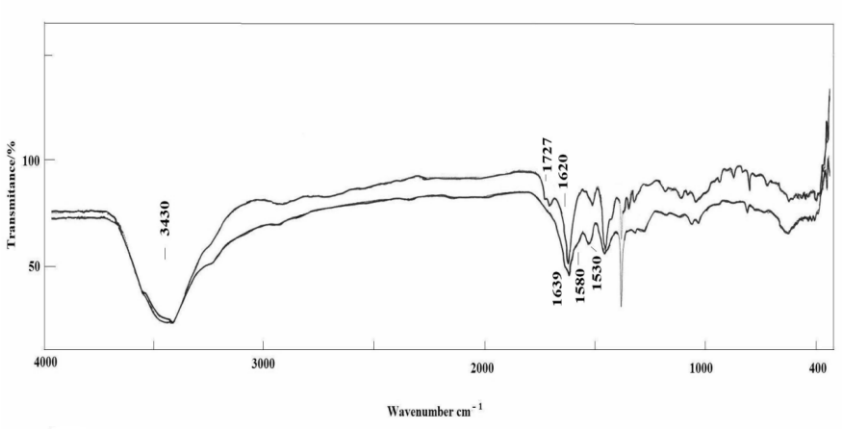



**Fig. 2. FT/ IR spectra of moxifloxacin(A) and its zirconium complex(B).**

*Mass Spectrum*

In the mass spectrometer (Fig.3), the sample is ionized to produce a charged molecule and a series of ionic fragments. The assortment of charged particles is then separated according to their mass to charge ratios (m/z) and displayed as a mass spectrum. In general, it is believed that the most prominent fragmentation pathway of the complex occurs through stepwise removal of water followed by the metal giving the most intense peak (base peak) which corresponds to the ligand. Mass spectroscopy in combination with elemental analysis is used in determination of the formula of (MOX/Zr) complex. In mass spectrum, the actual molecular weight is expected to be 535.1, the coordination water molecules seem to be absent giving the fragment $M^+$-$2H_2O$ at 499. This is an indication of the weak bonding of water molecules with the main fragments. After removal of all water molecules, the sequence of fragmentation is proposed to be through decarboxylation giving the fragment at 446.4. Another fragmentation pathway is proposed to occur through removal of water molecules and Zr giving the most stable fragment at 402.3 corresponding to the parent drug, moxifloxacin.

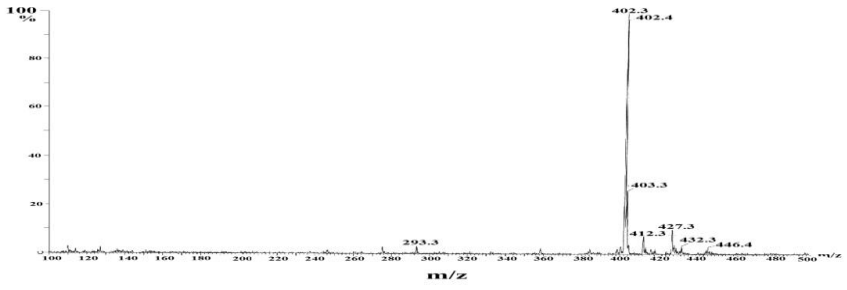



**Fig. 3. Mass spectrum of MOX/ Zr complex.**

*TG and DTA analysis*

Thermal analysis results (Fig.4) reveal that the decomposition course of (MOX/Zr) commences near 50°C and terminates at ≥ 580°C, encompassing endothermic and exothermic events.

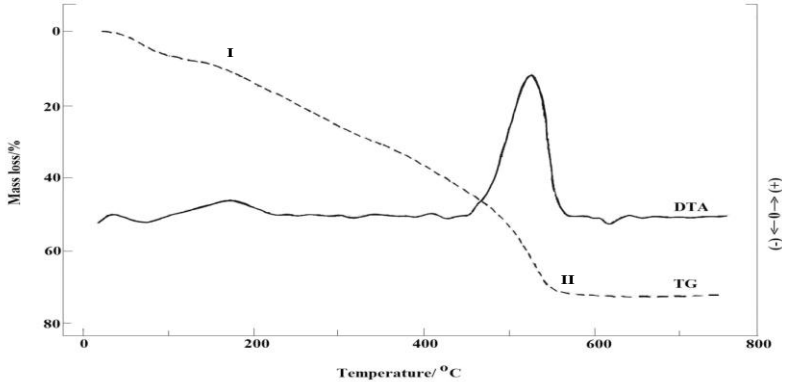



**Fig. 4. TG (---) and DTA(—) curves obtained at (10 °C/min) of MOX/Zr complex.**

*Dehydration reactions*

TG and DTA results of (MOX/Zr) shows broad endothermic event in the temperature range 50-110°C, characteristic of the removal of hydrating water.

*Decomposition reactions*

(MOX/Zr) complex begins to decompose at ~ (120-130 °C) through overlapped exothermic events that bring the total ML for (MOX/Zr) to (73-73.5%). These values are close to those calculated for the formation of the final decomposition product; the white colored $ZrO_2$ (73.5 %). The DTA curve resolve two exothermic events in the temperature ranges at (100-220 °C) and at (440-580°C). The slow mass loss detected over the temperature range (220-440 °C) monitored by the slopping plateau (Fig. 4) shown to lead to the significant mass loss pertaining to event II may be explained by the occurrence of different and consecutive pyrolytic activities during heating of the sample.

*Spectrofluorometric study results*

The solution of the studied drug has weak native fluorescence (Fig.5) however in the presence of zirconium the fluorescence intensity increases substantially while the excitation and the emission maxima remain practically in the same position. The sensitivity is enhanced by 10 folds due to the formation of a chelate in which the metal ion is bonded to the carbonyl and carboxylate oxygen of the quinolone. The drug-metal chelate showed excitation maximum at 333 nm and emission maxima at 485 nm.

The effect of the time allowed for the reaction to take place on the relative fluorescence intensities of the formed chelate was studied. It was found to have no

significant effect indicating that the chelation reaction between the studied drug and the metal is spontaneous under the specified conditions. Thus, measurements were carried out immediately after completion with solvent in the subsequent experiments.

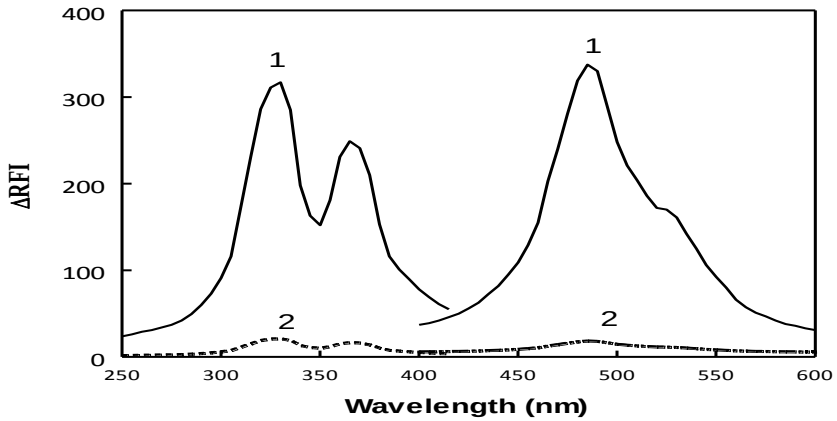



**Fig. 5. Excitation (left) and emission (right) spectra of (1) moxifloxacin (0.5 μg ml-1) with zirconium (0.85 mg/ml) and (2) moxifloxacin (0.5 μg/ ml) alone.**

*Optimization of the reaction variables*

The influence of pH on the relative fluorescence intensity of the formed metal MOX -chelate was studied at its respective maxima using acetate buffer solutions of pH range (2.0-6.0). The relative fluorescence intensity was maximum at pH range of (3.0-5.0), therefore buffer solution pH 4 was used in all subsequent experiments (Fig.6).

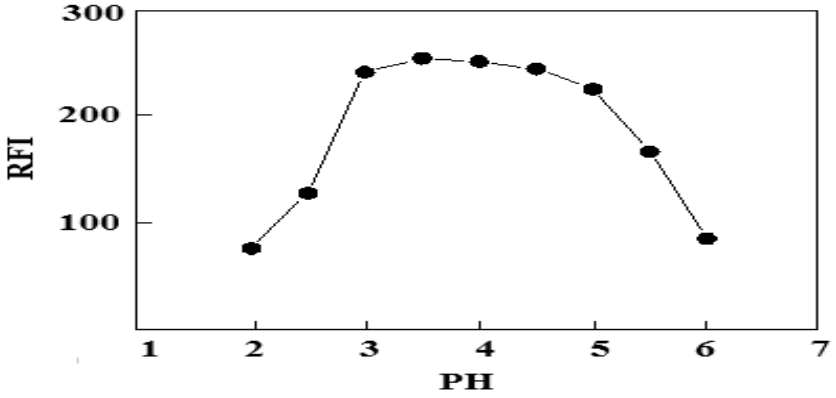



**Fig. 6. Effect of pH on the relative fluorescence intensity of moxifloxacin (0.5 μg/ml) with zirconium (0.85 mg/ml).**

The influence of the metal ion concentration was studied in the range (0.1–2) μg/ml. The relative fluorescence intensity increased with increasing metal concentration up to 0.8 mg/ml but leveled off at higher concentration (Fig.7). Thus, the final metal ion concentration of 0.85 mg/ml was used in all subsequent experiments for the studied drug.

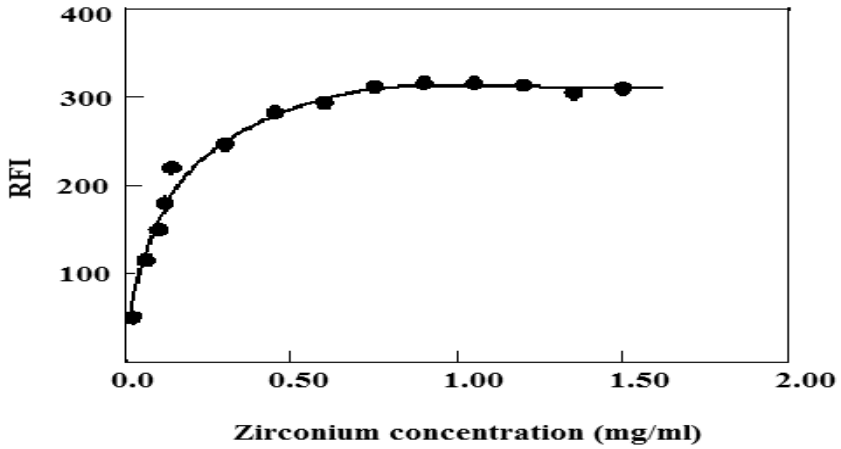



**Fig. 7. Effect of metal ion concentration on the relative fluorescence intensity of moxifloxacin (05 µg/ ml) and zirconium.**

The effect of various solvents of different polarities and hydrogen bonding capacities on the relative fluorescence intensities of the formed chelate was studied (Table 2). It was observed that the position of the excitation and emission maxima was changed by using solvents of different polarities. The stock shifts ($\lambda_{em}$-$\lambda_{ex}$) were also affected by changing the solvent**.** The relative fluorescence intensities of the formed chelate are greatly affected by solvent used and it was much higher in methanol. Based on the results obtained, methanol was chosen as the most suitable solvent throughout the reactions.

**TABLE 2. Effect of solvents on the fluorescence intensity (RFI) of the formed chelate of moxifloxacin (0.5 µg/ml) with zirconium.**

| Solvent | parameter | | | |
|---|---|---|---|---|
| | $\lambda_{ex}$(nm) | $\lambda_{em}$(nm) | $\Delta\lambda$ | RFI |
| Water | 328 | 479 | 151 | 306 |
| Methanol | 333 | 485 | 152 | 332 |
| Propan-2-ol | 336 | 484 | 148 | 310 |
| DMF | 330 | 489 | 159 | 308 |
| Acetone | 381 | 487 | 106 | 329 |
| Ethanol | 332 | 483 | 151 | 330 |

The molar ratio between MOX and Zr(IV) in the buffered aqueous solution was studied using Job's method [31] . Equimolar solution of both the drug and the metal were prepared. The results revealed that Zr(IV) : drug ratio was 1:2 (Fig.8 & 9). The suggested mechanism of the reaction could be based on the formation of a chelate in which the metal ion is bonded to the carbonyl and carboxylate oxygen of the quinolone.

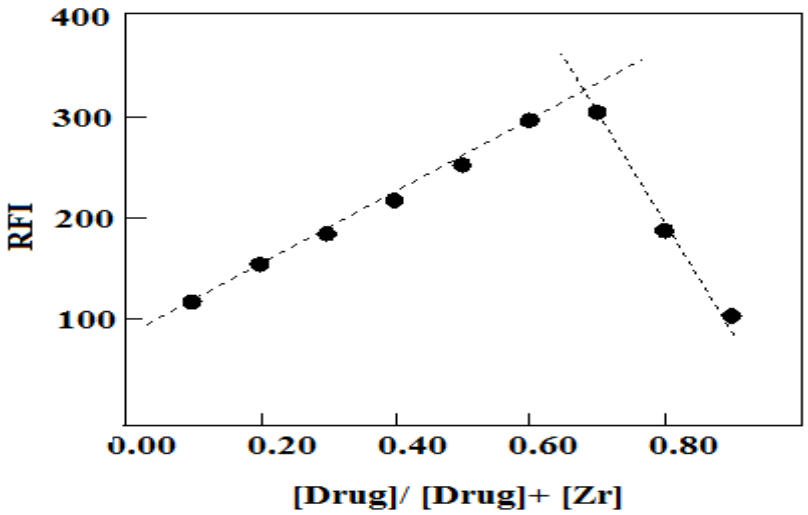



**Fig. 8. Job's plots for the reaction of MOX with Zr(IV) in aqueous buffered solution.**

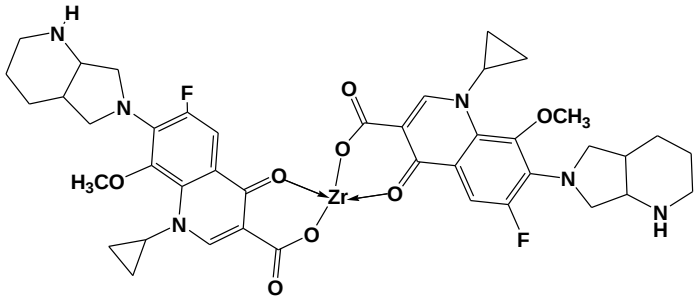



**Fig. 9. Proposed structure for the spectrofluorometric studied chelate.**

*Validation of the proposed method linearity, detection and quantitation limits*

Under the above-mentioned optimum conditions, the calibration graphs correlating the relative fluorescence intensity (RFI) with the corresponding concentration of the drug were constructed. Regression analysis for the results was carried out using least-square method. The correlation coefficients were 0.9969 and the general concentration range was 0.1-4 μg/ml (Table 3). The limit of detection (LOD) and limit of quantitation (LOQ) were calculated using the formula: LOD or LOQ = $\kappa SD_a/b$, where $\kappa$ = 3.3 for LOD and 10 for LOQ, $SD_a$ is the standard deviation of the intercept, and b is the slope. The LOD and LOQ values were from 0.06 and 0.11 μg/ml, respectively.

**TABLE 3. Analytical parameters of spectrofluorimetric method of moxifloxacin with zirconium.**

| Parameters | Value |
|---|---|
| Linear range ($\mu gml^{-1}$) | 0.10-4.0 |
| Limit of detection ($\mu gml^{-1}$) | 0.06 |
| Limit of quantitation ($\mu gml^{-1}$) | 0.11 |
| Slope (b) | 250.6 |
| S.D. of slope $(S)_b$ | 6.385 |
| Intercept on the ordinate (a) | -9.286 |
| S.D. of the intercept on the ordinate $(S)_a$ | 6.448 |
| Number of points (n) | 8 |
| Correlation coefficients (r) | 0.9969 |

*Effect of interfering substances:* The assay result was unaffected by the presence of excipients as shown (Table 4) by the excellent recoveries (98.62±1.1 to 100.4±0.44) obtained when analyzing the studied drugs in presence of commonly encountered excipients. As samples containing a fixed amount of the drug (0.1 μg/ml) and excipients (50 μg/ml) were measured, no interference was observed from commonly used excipients such as starch, lactose, glucose, fructose, sucrose and magnesium stearate. This fact indicates good selectivity of the method to determine the studied drugs both in raw material and in their dosage forms.

**TABLE 4. Effect of excipients on the determination of moxifloxacin (100 ng/ml) using the proposed specroflourometric method.**

| Excipient (50 μg/ml) | Recovery (%) ± SD[a] |
|---|---|
| Starch | 98.62±0.59 |
| Talc | 99.9±0.41 |
| Lactose | 100.4±0.44 |
| Glucose | 99.62±0.63 |
| Sucrose | 98.85±0.65 |
| Magnesium stearate | 100.15±0.65 |

*Analysis of pharmaceutical formulations*

The proposed method was applied to the determination of MOX in commercial tablets. Five replicate determinations were made for each pharmaceutical dosage form. The proposed and reported methods[(32)] were applied to the determination of studied drugs in tablets containing the studied drug. The recovery of the drug was calculated by comparing the concentration obtained from the dosage form solution with those of the pure drugs. The results on analyzing the commercial tablets are obtained by the proposed method and the reported method (Table 5). In the t- and F-tests, no significant differences were found between the calculated and theoretical values (95% confidence) of both the proposed and reference methods and this indicated similar precision and accuracy between them.

**TABLE 5. Determination of moxifloxcin in its pharmaceutical dosage forms.**

| Pharmacetutical product | Proposed method (%) ± SD (n= 5) | Official or reported method (%) ± SD (n= 5) | t-test | F-test |
|---|---|---|---|---|
| Moxacin tablets | 100. 61 ± 0.78 | 99. 38 ± 1.20 | 1.91 | 2.37 |
| Moflox tablets | 99. 67 ± 1.41 | 98. 95 ± 1.63 | 0.75 | 1.30 |
| Moxiflox tablets | 99. 34 ± 0.84 | 99. 29 ± 1.01 | 0.09 | 1.45 |

* Tabulated value at 95 % confidence limit; F = 6.34 and t = 2.306

*Antibacterial activity*

From the MIC results (Table 6), it is clear that both moxifloxacin and its corresponding zirconium complex are more active against Gram negative strains (*E.coli* and *P.aeruginosa*) than the Gram positive strains (*S. aureus* and *B. subtilis*). Furthermore, it is obvious that the MOX/Zr complex is superior in its antibacterial activity against the Gram negative strains than the parent moxifloxacin. The increased antibacterial activity of (MOX/Zr) may be explained by change in cell permeability through lipid membrane that enhances the penetration of complexes into the lipid membranes [33]. Inversely, the literature results revealed that lipophilicity of the molecule and hence penetration into bacterial cell is not the only factor which can affect its activity [34] . Indeed, other factors like the affinity of the compounds for their target DNA Gyrase and Topoisomerase IV can affect their MIC [35] .

**TABLE 6. Minimum inhibitory concentration (MIC) of Moxifloxacin and its Zirconium complex.**

| Compound | Minimum inhibitory concentration (MIC) (µg/ml) | | | |
|---|---|---|---|---|
| | Gram negative | | Gram positive | |
| | ***P.aeruginosa*** | ***E.coli*** | ***S. aureus*** | ***B. subtilis*** |
| **Mox** | 22 | 18 | 36 | 25 |
| **Mox/ Zr** | 2 | 3 | 35 | 21 |

## Conclusions

MOX/Zr(IV) complex has been synthesized and identified by spectroscopic, thermal and elemental analysis techniques. The complex is identified to be with the molecular formula $C_{21}H_{23}FN_3O_4Zr(H_2O)_2.0.5H_2O$. The spectroscopic and elemental analysis results revealed that the complex formed involves direct coordination of the metal ion to quinolone ring carbonyl and carboxylic oxygen in the ratio of 1:1. The thermal decomposition of the complex proceeds in two steps; dehydration followed by decomposition. The results showed significant increase in antibacterial activity of MOX/Zr complex as compared with uncomplexed one (MOX) especially against the tested Gram negative strains. The chelation between the studied drug and zirconium metal was employed in the

development of a simple, rapid, reliable and sensitive spectrofluorometric method for assay of MOX. The developed methods were applied successfully for the determination of the studied drugs in their pharmaceutical dosage forms with a good precision and accuracy. The proposed method is suitable for the routine quality control of the drug alone and in tablets or capsules without fear of interference caused by excipients. The results of studying the molar ratio between MOX and Zr(IV) using Job's method revealed a 1:2 ratio for zirconium to moxifloxacin.

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# تحضير و توصيف و دراسة التألق الطيفى والفاعلية كمضاد للبكتريا لمتراكب الموكسيفلوكساسين مع الزركونيوم

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تم فى هذا البحث دراسة الخواص الفيزيائية و الكيميائية للمتراكب المحضر من احد مشتقات الكينولون المضاده للبكتريا و هو موكسيفلوكساسين مع الزركونيوم (IV) نيترات. وقد تم أستخدام الطرق الطيفيه مثل الاشعة تحت الحمراء ، الرنين النووى المغناطيسى ، الاشعة السينية ، مطياف الكتلة والتحليل الدقى للعناصر بالاضافة الى استخدام التحليل الحرارى لتحديد الخواص الفزيوكيميائيه للمتراكب الناتج. و قد اثبتت النتائج تكوين متراكب له الصيغه الجزيئية التالية $[MOX/\ Zr\ (H_2O)_20.5H_2O]$. وقد تم استنتاج ان الموكسيفلوكساسين احادى الارتباط عبر مجموعة الكربونيل و اكسجين من مجموعة الكربوكسيل مع الزركونيوم. وقد اثبتت الدراسات الحراريه ان المتراكب المتكون يفقد جزيئات الماء عند درجات حرارة منخفضة من (50 -110°م) ويتبعه تفكك عند درجات حرارة اعلى من (110-580°م) ، و ان هذا المتراكب له درجة ثبات عاليه. وقد وجد أن للمتراكب المحضر تأثير مضادا للبكتريا الموجبة والسالبة المستخدمة يوازى أو فى معظم الاحيان اكثر من الكينولون المقابل بافضلية كمضاد للبكتريا السالبة عنه ضد البكتيريا الموجبة . و قد تم تفسير هذه النتائج بناء على التغيير فى الخواص الفزيوكيميائيه للمتراكب الناتج.

ومن ناحية اخرى تم عمل دراسة باستخدام مقياس التالق الطيفى لتعيين شدة طيف الموكسيفلوكساسين و تعتمد هذه الطريقة على تكوين متراكب ذا شدة طيف عالى نتيجة تفاعل الموكسيفلوكساسين مع محاليل ملح الزركونيوم فى وسط حمضى عند اس هيدروجينى 4. وقد تم التوصل الى ان هذا التفاعل يؤدى الى تكوين متراكب ثنائى له شدة طيف عالية يسهل قياسها لتعيين تركيز المركب المستخدم و قد اعتمد التفاعل على عدة عوامل منها تركيز محلول الملح للفلز المستخدم و تركيز ايون الهيدروجين و كذلك على نوعية المذيب المستخدم. و قد تم دراسة هذه النتائج و اختيار انسب الظروف للحصول على افضل نتائج ممكنه للتفاعل و قد تم كذلك تعيين نسبة التفاعل الجزيئى و ثوابت الارتباط للمتراكب و تم تطبيق الطريقة المقترحة لاستنباط طريقة سريعة و بسيطة و حساسة لتقدير مركب الموكسيفلوكساسين فى شكله الصيدلى.