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Photoreactivity of biologically active compounds. XV. Photochemical behaviour of mefloquine in aqueous solution

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The photochemical degradation of mefloquine in aqueous solution was studied as a function of pH, oxygen concentration, buffer concentration and ionic strength. The influence of various scavengers on the degradation process was examined. Formation of superoxide was studied and a reaction pathway was proposed. Seven of the main degradation products were isolated and identified. Reaction mechanisms for the formation of the various products were postulated. Photochemical degradation of mefloquine in aqueous solution seems to take place from the neutral form of the molecule by formation of the cation radical from the excited triplet.

1. Introduction

Mefloquine hydrochloride (MQ) is a blood schizonticide belonging to the quinolinemethanol class of antimalarials. Mefloquine is still restricted in use for the treatment of malaria, and will be the drug of choice in areas with high prevalence of multiple drug resistance. Side effects which may involve phototoxic reactions like visual disturbances, rash and urticaria and leucopenia or leucocytosis are reported.

Mefloquine absorbs radiation in the UV part of the spectrum. The substance acts as a photosensitizer *in vitro*, leading to the formation of singlet oxygen and superoxide and induces photopolymerization of lens proteins [1–3]. The phototoxic potential is demonstrated to be dependent on the ionized form of the molecule [1]. At physiological pH mefloquine consists of a mixture of the monocation (94%) and the neutral drug molecule (6%). Previous studies have shown that the drug forms several photodecomposition products in solution [4]. However, the photoreactivity of MQ in aqueous solution is not characterized in detail. The aim of this study was to obtain information on the photodegradation of mefloquine in various dissociation forms and to evaluate the mechanisms involved in the photodegradation process. Knowledge about the photoreactivity of this compound is important in order to evaluate both therapeutic aspects and adverse effects of this drug.

2. Investigations, results and discussion

2.1. Degradation of mefloquine as a function of pH and oxygen concentration

The pK_a value of 8.4–8.6 is given for the piperidine nitrogen in mefloquine [5, 6]. The pK_a value of the quinoline nitrogen is postulated to be in the range pH 4–5.5 [1]. The degradation process was studied at pH 3.4, 7.4 and 9.4, representing the dication, monocation and neutral form of MQ, respectively. The oxygen concentration of the samples was varied by flushing with helium or oxygen during irradiation. The results are presented in Fig. 1–2. There is an apparent increase in degradation rate with an increase in pH indicating that the photodegradation process is strongly dependent on the state of ionization of MQ (Fig. 1). The dicationic form of MQ is almost not photolyzed (~1%) under the given experimental conditions while the monocationic- and the neutral forms of the

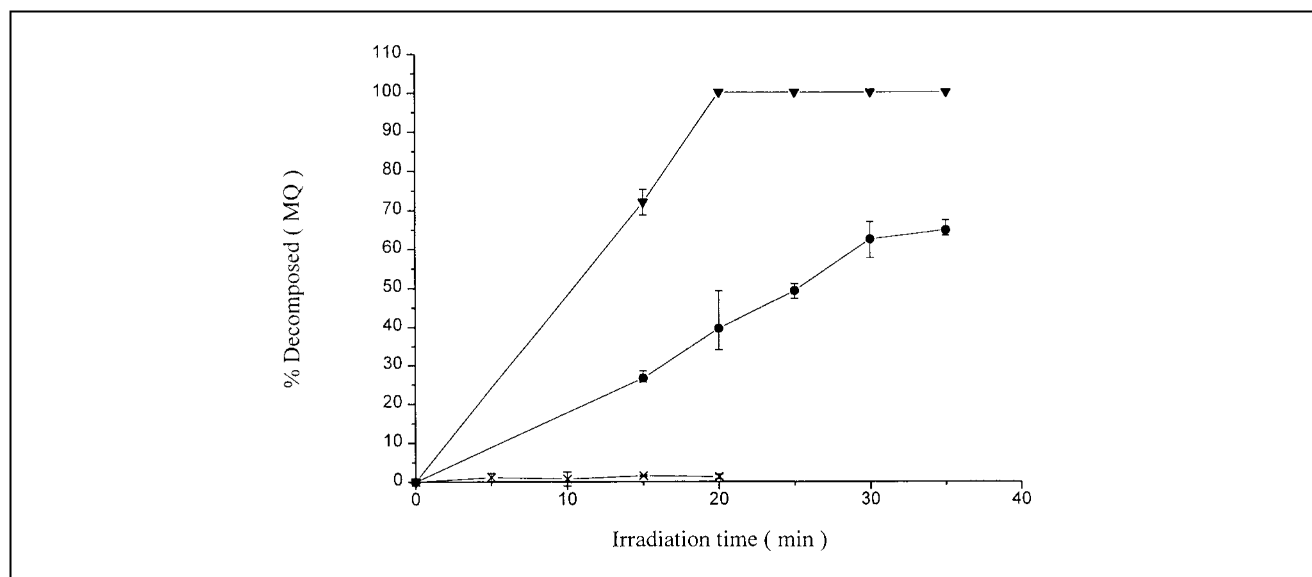


Fig. 1: Degradation of mefloquine in air saturated samples at various pH as a function of irradiation time (n = 3). Each result is the mean of three measurements. × = pH 3.4 ● = pH 7.4 ▼ = pH 9.4

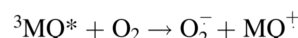
molecule undergo photodecomposition. The degradation rate is further increased by an increase in pH above 9.4 (data not shown). This indicates that the degradation occurs on the neutrale molecule. The presence of the monocation is, however, important to keep the molecule in solution. Flushing the samples with helium during irradiation leads to a substantial increase in degradation rate at pH 7.4 and 9.4, while an increase in oxygen level has a retarding effect on the degradation process. This is demonstrated in Fig. 2. The oxygen level did apparently not influence the degradation of MQ at low pH. The monoprotonated form of MQ is previously shown to be phosphorescent [1]. The ratio between phosphorescence and fluorescence calculated as the ratio between the areas under the uncorrected phosphorescence emission spectrum (460–600 nm) and the uncorrected fluorescence emission spectrum (370–450 nm) is found to be 115 (excitation 310 nm). This indicates that the triplet excited state of MQ is favoured compared to the singlet excited state which is further emphasized by previous results showing that MQ is an efficient producer of singlet oxygen [1, 2]. As oxygen is an efficient quencher of triplet states, the results indicate that the triplet state is essential for the degradation of MQ. This is further emphasized by laser flash photolysis studies (to be published).

2.2. *Influence of scavengers on the degradation process*

Mannitol (hydroxyl radical scavenger) or sodium azide (singlet oxygen quencher) was added to the samples to study the influence of reactive oxygen species (ROS) on the photodegradation of MQ at pH 7.4. Exposure at 310 nm (30 min, monochromator) resulted in a loss of 67% of the active compound in plain buffer. Addition of mannitol had no effect on the degradation process (65% loss after 30 min) while sodium azide had a strongly retarding effect on the reaction (9% decomposed after 30 min). Addition of EDTA did not influence the results. The inhibitory effect caused by sodium azide can be due to quenching of the excited triplet of MQ or to quenching of singlet oxygen, the last indicating that singlet oxygen is taking part in the degradation process. A 60% reduction in phosphorescence signal was, however, observed when sodium azide was added to the MQ sample.
Quenching of the mefloquine triplet makes therefore one likely explanation for the observed inhibitory effect caused by sodium azide.

2.3. *Photoionization and formation of superoxide*

To detect the formation of superoxide during irradiation of MQ the reduction of ferricytochrome C (Cyt C) was followed by measuring the absorption at 550 nm. The data is presented in the Table. MQ induces reduction of Cyt C indicating the formation of superoxide. This is consistent with previous results [1]. The process is clearly inhibited be superoxide dismutase (SOD) and also by sodium azide. The latter indicates that the formation of superoxide takes place from the excited triplet of MQ by a red-ox process:



$$^3MQ^* + O_2 \rightarrow O_2^{-} + MQ^{+}$$

The cation radical of MQ should then result. The cation radical of MQ can further decompose leading to the formation of degradation products. Photoionization should be more supported in water than in a less polar solvent like methanol. When MQ is irraditated in methanol, the degradation process is indeed much slower than in buffer pH 7.4 (29% decomposed in methanol compared to 67% in buffer after 30 min exposure). Formation of the cation radical of MQ was confirmed by laser flash photolysis and pulse radiolysis (to be published). Mannitol has no inhibitory effect on the reduction of Cyt C indicating that the hydroxyl radical is of little importance in the process (Table). The photodecomposition products of MQ are less potent as inducers of superoxide than the parent compound illustrated by the small effect on Cyt C caused by the pre-irrdiated MQ sample.

2.4. *Influence of buffer concentration and ionic strength on the degradation*

Solutions of 0.045 M phosphate buffer pH 7.4 containing MQ (2.8×10^{-5} M) were prepared. While maintaining the pH, solutions with various ionic strength ($\mu = 0.09$–0.30)

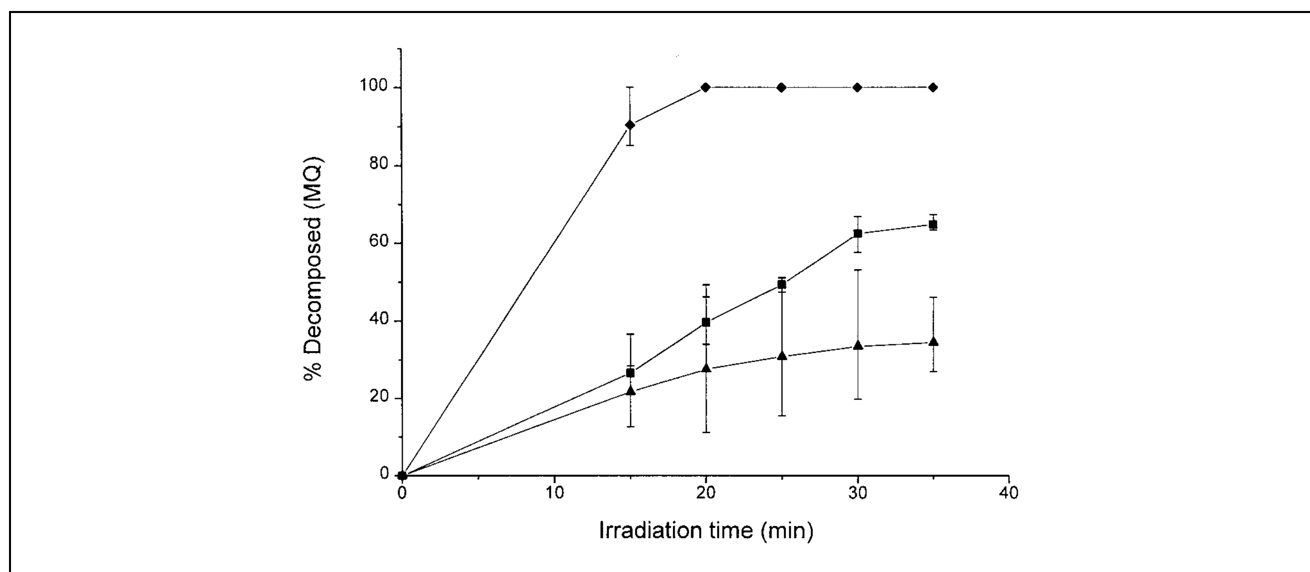


Fig. 2: Degradation of mefloquine at pH 7.4 at various oxygen concentrations as a function of irraditation time (n = 3). Each result is the mean of three measurements. ■ = air ▲ = oxygen flushed samples ◆ = helium flushed samples

Table: Reduction of ferricytochrome C measured as a change in absorbance at 550 nm in the presence of MQ as a function of irradiation time (n = 3)

Sample	Irradiation time (min) $\Delta A_{550\,nm}$ 0	20	40	60
MQ	0.008 (0.003–0.013)	0.019 (0.01–0.032)	0.025 (0.015–0.035)	0.027 (0.021–0.032)
MQ + SOD	0.001 (< 0–0.003)	0.003 (0.001–0.006)	0.004 (0.002–0.007)	0.005 (0.003–0.008)
Pre-irr. MQ	0.005 (0.003–0.009)	< 0	< 0	0.0011 (0.001–0.021)
MQ + NaN_3	0.001 (< 0–0.006)	0.005 (0.003–0.006)	0.005 (< 0–0.006)	0.005 (< 0–0.007)
MQ + MAN	0.001 (< 0–0.003)	0.014 (0.010–0.018)	0.022 (0.016–0.029)	0.024 (0.020–0.028)

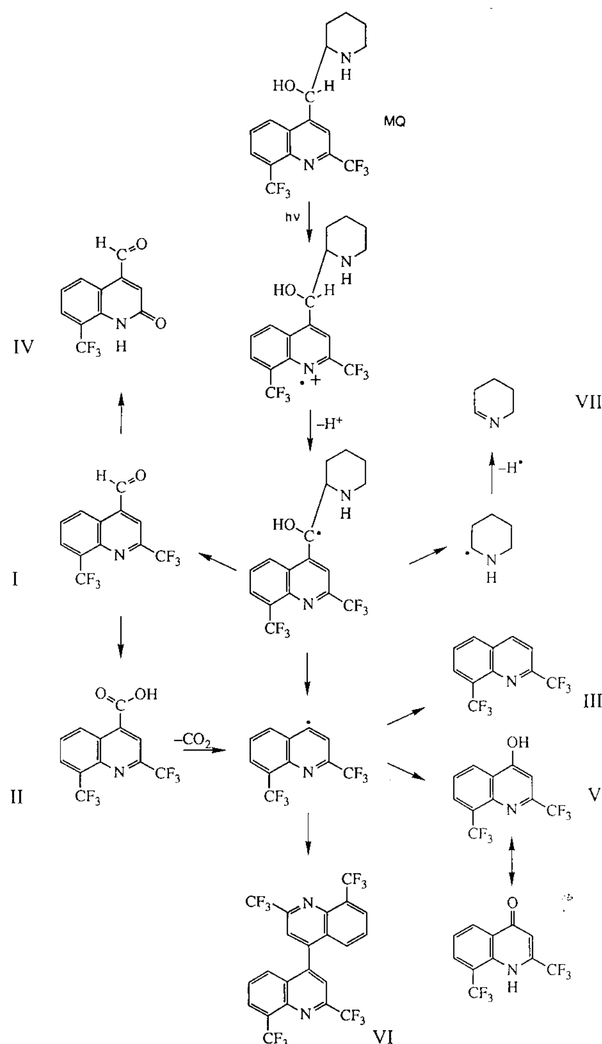
$A_{550\,nm}$ = change in absorbance units at 550 nm
MQ = meflloquine + Cyt C
MQ + SOD = mefloquine + Cyt C + superoxide dismutase
Pre-irr. MQ = pre-irradiated mefloquine + Cyt C
MQ + NaN_3 = mefloquine + Cyt C + sodium azide
MQ + MAN = mefloquine + Cyt C + mannitol

were investigated for the ionic strength effect on the photostability of MQ. The ionic strength was adjusted by addition of sodium chloride. There was a slight decrease (from 0.04 to 0.02 min^{-1}) in the observed first order rate constant for the degradation of MQ by an increase in μ from 0.09 to 0.3. It has previously been reported that an increase in ionic strength could have a photostabilizing effect by providing a protective film of solvated ions around the reacting molecule [7]. The buffer systems used in the studies of MQ degradation all contain phosphate ions. This ion is known to influence the photochemical properties of compounds (e.g. tyrosine) [8]. An increase in degradation of MQ was observed by an increase in concentration of phosphate ions at constant pH and μ. No shift in the absorption spectrum was detected with an increase in phosphate concentration, e.g. ground state complex formation is unlikely. Proton transfer from the excited state can however, be facilitated in the presence of nearby proton acceptors like phosphate ions from the bulk solution [9]. An increase in phosphate ion concentration can therefore favour the formation of the neutral form of MQ at pH values below the pK_a value. Assumed that the degradation takes place from the neutral molecule this will lead to an increase in degradation rate.

2.5. Formation of degradation products

The quantum yield for the degradation of mefloquine in phosphate buffer at pH 7.4 was measured to be 0.06 (λ = 315 nm). Three of the previously described degradation products of mefloquine hydrochloride [4] and four new degradation products were isolated and identified. The identification was based on MS (EI, CI, high resolution) as it turned out to be difficult to isolate enough of each compound to obtain an NMR spectrum due to the low solubility of MQ at pH 7.4. Isolation and identification by MS was performed in triplicate. The results obtained from the three series of experiments were consistent. The spectral and analytical data of compound V were compared to a reference sample. Postulated mechanisms for the photolysis of MQ and formation of the main degradation products are presented in the Scheme. Formation of compound IV involves the loss of fluorine. An increase in concentration of fluoride ions in the samples as a function of irradiation time (monochromator, pH 7.4) could be observed by use of a fluoride electrode. The ob-

Scheme 1



Postulated mechanisms for the photolysis of mefloquire and the formation of the main degradation products in aqueous solution (pH = 7.4)

served signals were outside the linear range of the electrode and quantitation of the fluoride content was therefore difficult. The electrode signal did, however, increase with a factor 460 after 150 min exposure; the size of the final signal corresponding to a fluoride concentration of approximately 10^{-5} M.

3. Experimental

3.1. Materials

Mefloquine hydrochloride was generously provided by Mepha AG, Aesch, Switzerland. The sample was used as received. The purity was tested by HPLC and was found to be higher than 99%. Sodium azide (NaN_3), superoxide dismutase (SOD, from bovine erythrocytes), etylenediaminetetraacetic acid (EDTA), Cytochrome C (from bovine heart) and D-mannitol were all obtained from Sigma Chemical Co., St. Louis, USA. 2,8-bis-(trifluoromethyl)-4-quinolinol was obtained from Aldrich-Chemie, Steinheim, Germany. All other chemicals used were of p.a. grade.

3.2. Equipment

3.2.1. HPLC

The concentration of MQ was measured by reversed phase HPLC. The separation was performed on a 15 cm × 3.9 mm Nova Pak^R C_{18} column (Waters, Milford, MA, USA). The mobile phase was composed of 0.045 M KH_2PO_4/acetonitrile (69:31) and adjusted to pH 3.4 with orthophosphoric acid. A flow rate of $1.2\ ml \cdot min^{-1}$ was used (HPLC pump: Spectra Physics SP8700). MQ was detected at 315 nm (Shimadzu UV-Vis Detector SPD 10A). The samples were injected by a Shimadzu auto injector SIL-6A. Data acquisition was completed using a Shimadzu C-R3A integrator. The retention time of MQ was 14 min.

3.2.2. Fluoride electrode

The formation of fluoride ions was measured by a Orion Model 720A meter equipped with a Orion combination fluoride electrode (Orion Research Inc., Cambridge, MA, USA).

3.2.3. Absorbance measurements

Ultraviolet-visible absorption measurements were made using a Shimadzu UV-260 UV-Visible recording spectrophotometer.

3.2.4. Fluorescence and phosphorescence measurements

Fluorescence and phosphorescence emission spectra were recorded on a Perkin Elmer LS 50B luminescence spectrometer. Phosphorescence was measured at 77 K in EPA or ethanol (EPA is a mixture of diethylether, isopentane and ethanol in the ratio 5:5:2). Excitation wavelengths: 285 nm and 310 nm. Emission 370–450 nm (fluorescence), 460–600 nm (phosphorescence). To study the effect of sodium azide on the formation of MQ triplet 5 mg of MQ were dissolved in 200 ml ethanol. This solution, (4 ml) was mixed with 20 ml of a saturated solution of sodium azide in ethanol.

3.2.5. Mass spectrometry

Electron impact (EI) MS, chemical ionization (CI) MS (ionization gas: methane) and high resolution MS (HRMS) were obtained with a VG Pro-Spec (Fisons Instruments) spectrometer via direct inlet. The probe temperature and the ion potential were 220 °C/70 eV, 250 °C/47 eV and 220 °C/70 eV for the EI, CI and HRMA analysis, respectively.

3.3. Buffer systems

Buffers (0.045 M) were prepared in distilled water using analytical grade reagents as follows: pH 3.4, KH_2PO_4 -orthophosphoric acid, pH 7.4 and 9.4, KH_2PO_4–NaOH.

3.4. Irradiation conditions

3.4.1. Isolation of degradation products

Heraeus immersion lamp system (120 W) with emission 240–600 nm. A glass filter was placed between the sample and the light source to obtain light above 300 nm.

3.4.2. Studies of degradation rate, formation of fluoride ions, degradation quantum yield

Monochromator f 3.4, 900 W xenon arc lamp, Applied Photophysics Ltd., Surrey, England, operated with a bandwidth of 20 nm at the irradiation wavelength (310 nm). The intensity of the irradiation was adjusted to 60 mW/cm^2 at the surface of the cuvette by a Thermopile voltmeter (Applied Photophysics Ltd.) calibrated against a black body irradiator. Stirring was maintained during exposure.

3.4.3. Formation of superoxide, effect of EDTA

Suntest CPS (1.8 kW xenon burner), Heraeus GmbH, Hanau, Germany, equipped with a quartz-glass dish with IR reflective coating and a glass filter (emission 310–800 nm). The intensity of the irradiation was adjusted to 83 mW/cm^2 at the surface of the cuvette by a Thermopile voltmeter (Applied Photophysics Ltd.) calibrated against a black body irradiator. Stirring was maintained during exposure.

3.5. Preparative TLC

The stationary phase was silica gel, precoated 0.25 mm (Merck), the mobile phase was isopropanol/ 25% ammonia (9:1).

3.6. Preparation of samples

For all experiments except 3.6.1 a stock solution of MQ in methanol was used (1.4×10^{-3} M) and diluted to the final concentration in buffer.

3.6.1. Isolation of degradation products

MQ (9 mg) was dissolved directly in 500 ml phosphate buffer pH 7.4 and irradiated (immersion lamp) for 40 min. After irradiation the sample was extracted with 2 × 100 ml dichloromethane. The combined extracts were evaporated to dryness under vacuum. The residue was stored in the refrigerator overnight. The residue was dissolved in acetonitrile (400 μl). After separation by TLC the degradation products were extracted from the silica gel with acetonitrile. The fractions were centrifuged for 10 min at 2000 ×g. The supernatant was evaporated to dryness under vacuum and stored in the freezer until further analyzed. Irradiation and isolation of degradation products from 20 samples was needed to obtain sufficient material for the MS analysis. The experiment was carried out in triplicate.

3.6.2. Degradation rate

MQ (2.4×10^{-5} M) was dissolved in buffer or methanol in the presence or absence of quenchers ($10^{-2} - 10^{-3}$ M) and irradiated (monochromator) for 30 min under continous stirring. The concentration of MQ was measured by HPLC at intervals of 5 min exposure. To obtain variations in the oxygen level the samples were flushed with helium or oxygen 15 min prior to-and during exposure.

3.6.3. Reduction of ferricytochrome C (Cyt C)

To study the reducing effect of MQ on Cyt C during irradiation (Suntest) samples containing Cyt C (0.2 mg/ml) were prepared in buffer pH = 7.4. Test samples with MQ (2.8×10^{-5} M) and reference samples (no MQ) were irradiated from 0–60 min under constant stirring. To some samples sodium azide (10^{-3} M), mannitol (10^{-3} M) or SOD (300 units/ml) was added. The absorbance at 550 nm was measured after irradiation and compared to a corresponding sample stored in the dark. The reducing effect of the combined degradation products was tested using a pre-irradiated MQ sample (irradiated for 2.5 h in the monochromator, i.e. MQ almost completely decomposed) instead of MQ.

3.6.4. Effect of EDTA

MQ (2.8×10^{-5} M) in buffer pH 7.4 was irradiated under constant stirring (Suntest) in the presence of EDTA (2×10^{-7} M, 1×10^{-6} M).

3.6.5. Formation of fluoride ions

MQ (2.8×10^{-5} M) in buffer pH 7.4 was irradiated (monochromator) for 2.5 h. The degradation process was followed by means of HPLC. The concentration of F^- was measured by use of a fluoride electrode.

3.6.6. Influence of ionic strength and concentration of buffer salt

The ionic strength of samples of MQ ($2.8 \cdot 10^{-5}$ M) in buffer pH 7.4 was adjusted ($\mu = 0.09$–0.3) by addition of NaCl. The concentration of phosphate was varied by a factor 6 keeping the ionic strength constant ($\mu = 0.3$) by addition of NaCl. The samples were irradiated (monochromator) and analyzed by HPLC.

3.7. Quantum yield measurements

The reaction quantum yield measurements were carried out using a ferrioxalate chemical actinometer [10]. The samples were irradiated in the monochromator at 315 nm. The number of MQ molecules reacted per unit time per unit volume as a function of exposure time was quantified by means of HPLC. Dark controls showed no detectable degradation of MQ. The number of photons absorbed by the drug molecules per unit time (N_{drug}) was

calculated by using the following expression [11]:

$$N_{drug} = (\text{slope (set 1)} - \text{slope (set 2)})\, N_A R / \Phi_{Fe}\, \varepsilon_{510}\, l$$

N_A is Avogadro's number, ε_{510} is the molar absorbtivity of the Fe(II)-phenanthroline complex at 510 nm (12590 l/mol cm), R is a correction factor that takes into account the dilutions involved in the colorimetric assay and l is the cell pathlength used for measuring the absorbance of the Fe(II)-phenanthroline complex at 510 nm.

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