Monatsh Chem (2009) 140:9–14
DOI 10.1007/s00706-008-0022-5

ORIGINAL PAPER

Kinetic spectrophotometric determination of amodiaquine and chloroquine

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Received: 26 February 2008 / Accepted: 2 June 2008 / Published online: 14 August 2008

Abstract Two simple, sensitive, and selective spectrophotometric methods were developed for determining amodiaquine (AQ) and chloroquine (CQ) based on their oxidation with potassium iodate and potassium bromate, respectively. The initial rates of oxidation of AQ and CQ were monitored at 342 and 343 nm, the wavelengths of maximum absorptions of the two drugs. The various experimental parameters affecting oxidation reactions were thoroughly studied and optimized. Beer's law was obeyed for 0.2–4.0 and 0.5–5.0 μg cm^{-3}, with correlation coefficients of 0.9999 and 0.9998 ($n = 6$) and a detection limit (based on the $3S_b$-criterion) of 0.04 and 0.06 μg cm^{-3} for AQ and CQ. The proposed methods were conveniently applied to determining AQ and CQ in pure and dosage forms.

Keywords Kinetic analysis · Oxidation of amodiaquine and chloroquine · Bromate and iodate · Pure and dosage forms

Introduction

Amodiaquine, 4-[(7-chloro-4-quinolinyl)amino]-2-[(diethylamino)methyl]phenol (AQ), and chloroquine, N^4-(7-chloro-4-quinolinyl)-N^1,N^1-diethyl-1,4-pentanediamine (CQ), (Scheme 1) are aminoquinoline derivatives commonly used to prevent and treat malaria, the widely spreading protozoan disease causing the death of about 1.1–2.7 million people per year and of 25% of child mortality in Africa [1]. Because of their medicinal importance, several methods have been reported for determining AQ and CQ, either in pure or dosage forms.

The US pharmacopoeia [2] has adopted ultraviolet (UV)-absorption spectrophotometry at 342 and 343 nm for determining AQ and CQ in pharmaceutical dosage forms, respectively, whereas the British pharmacopoeia [3] has adopted a titrimetric method for determining CQ. Other analytical methods for the assay AQ and CQ include liquid chromatographic [4–6], potentiometric [7–9], and spectrophotometric methods [10–20]. Although these techniques provide the required sensitivity, they suffer from disadvantages such as limited selectivity, the need for extensive sample extraction, and/or the long time for assaying. Therefore, the development of rapid, simple, sensitive, and selective spectrophotometric methods for determining AQ and CQ in pharmaceutical formulations is desirable.

On the other hand, kinetic spectrophotometric methods of analysis are becoming increasingly popular because of their simplicity, speed, and precision compared with

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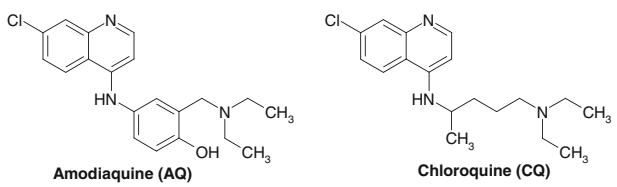

Scheme 1

equilibrium methods [21]. Moreover, these methods offer enhanced selectivity arising from the fact that measurements are carried out at the beginning of the reaction when the perturbation of other species present in the system is less likely to occur. Furthermore, the initial rate is derived from measuring the absorbance change with time instead of measuring a fixed absorbance value and therefore eliminating the possibility of any interference from any absorbing species in the complex sample matrix.

The aim of this work was to develop rapid, simple, sensitive, and selective kinetic methods for determining AQ and CQ in pharmaceutical dosage forms. The kinetic methods, reported here for the first time, are based on the reaction of the drug with iodate and bromate in the presence of sulfuric acid, where the initial rates of drug consumption were spectrophotometrically monitored.

Results and discussion

Hemolytic and antimalarial effects of aminoquinoline antimalarials depend on the formation of reversible redox intermediates formed during biotransformation of the drug in the host [22]. Thus, AQ was determined by extraction spectrophotometry based on its oxidation with periodate [14], persulphate [18], or chloramine-T [19] to give semiquinone intermediate [23] that is further oxidized to the pale orange–red colored product 2-diethylaminomethyl-*N*-(7-chloro-4-quinolyl)-1,4-benzoquinoneimine, which is finally extracted in ether [18] or chloroform [14, 19] and exhibited absorption maximum at 560 nm [18] or 442 nm [14, 19].

On the other hand, preliminary experiments in this study showed that AQ and CQ react with oxidizing agents to give pale yellow oxidized products, where AQ was more susceptible to oxidation because of the presence of the labile *p*-aminophenol moiety. However, the instability of the initially formed semiquinone intermediates and the long time required to attain the extractable quinoneimine end products pose considerable restrictions to conventional kinetic measurements based on monitoring the final products. Therefore, in this work and for the sake of simplicity and saving time, the initial rates of reactions were followed spectrophotometrically for the disappearance of AQ and CQ at the wavelengths of maximum absorptions of the two drugs, 342 and 343 nm (Fig. 1a, b). Therefore, the effects of various experimental parameters affecting oxidation of AQ and CQ were carefully studied and optimized. Such factors were changed individually, whereas the others were kept constant. The studied factors included type and concentration of the oxidizing agent and acid medium, reaction temperature, and the presence of excipients.

Effects of oxidizing agents

In the presence of sulfuric acid, reactions of AQ and CQ with $K_2S_2O_8$, KIO_4, $KClO_3$, and $(NH_4)VO_3$ were very slow and/or gave nonlinear *A*–*t* graphs; however, reactions with KIO_3 and $KBrO_3$ gave measurable-rate values with linear *A*–*t* graphs. Therefore, the effects of KIO_3 and $KBrO_3$ concentrations were thoroughly investigated (Fig. 2a, b).

The effect of iodate concentration was studied in AQ oxidation over the range of 0.2–5.0 mmol dm^{-3} KIO_3. The

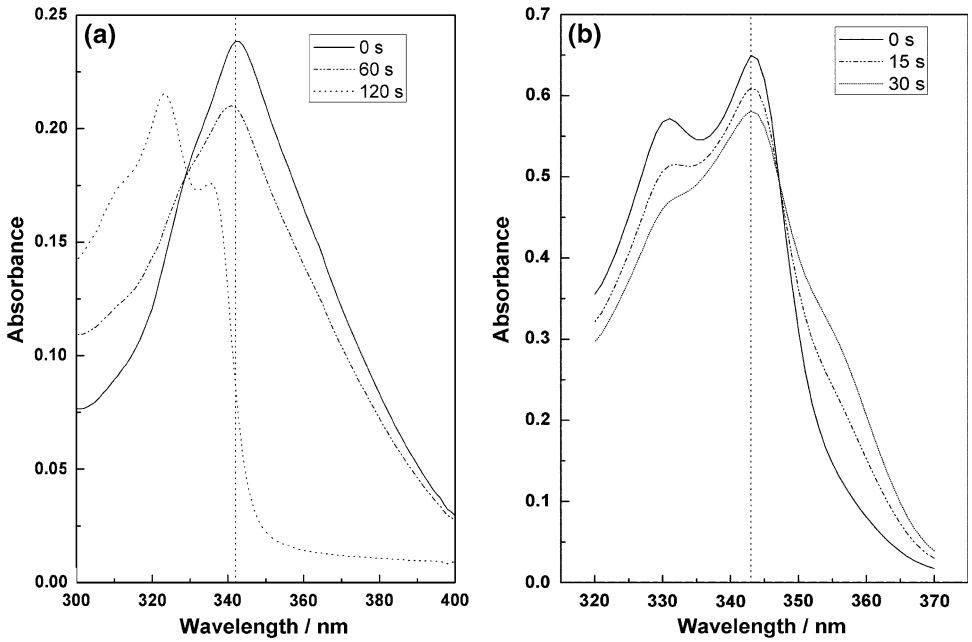

Fig. 1 Absorption spectra of the oxidation of **a** 4.0 μg cm^{-3} AQ with 3.0 mmol dm^{-3} iodate in the presence of 2.0 mol dm^{-3} H_2SO_4 at 50 °C. Spectra were recorded 0, 60, and 120 s after mixing; and **b** 5.0 μg cm^{-3} CQ with 10.0 mmol dm^{-3} bromate in the presence of 4.0 mol dm^{-3} H_2SO_4 at 50 °C. Spectra were recorded 0, 15, and 30 s after mixing

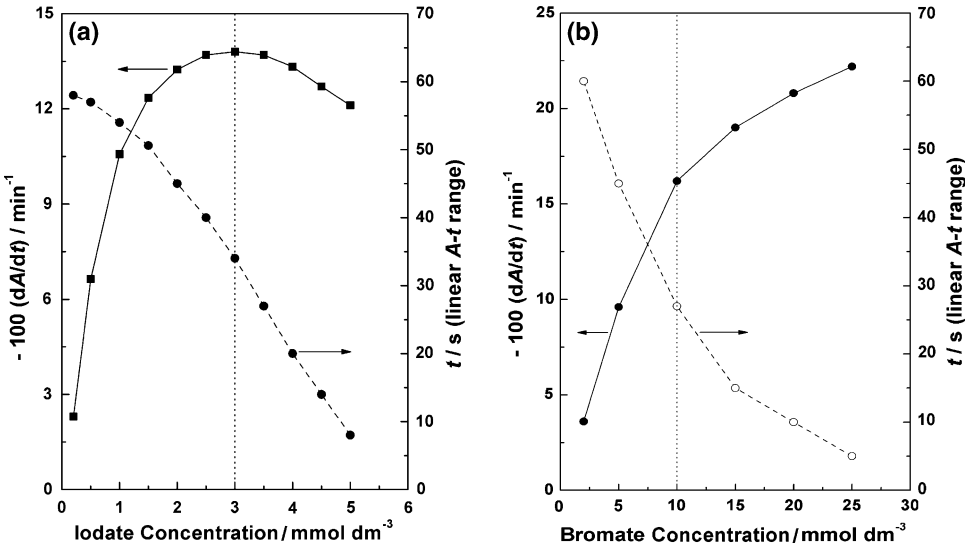

Fig. 2 **a** Effect of potassium iodate concentration on the rate of consumption 4.0 μg cm^{-3} AQ in the presence of 2.0 mol dm^{-3} H_2SO_4 at 50 °C, against water as a reference; and **b** effect of potassium bromate concentration on the rate of consumption 5.0 μg cm^{-3} CQ in the presence of 4.0 mol dm^{-3} H_2SO_4 at 50 °C, against water as reference

initial rate of AQ consumption [−100(d*A*/d*t*)] increased with iodate concentrations up to 2.5 mmol dm^{-3} and then remained almost constant up to 3.5 mmol dm^{-3} IO_3^-. Meanwhile, the linear range of the *A*–*t* graphs decreased with increasing the iodate concentration (Fig. 2a). Therefore, an iodate concentration of 3.0 mmol dm^{-3} was adopted in the recommended procedure to give high sensitivity and moderate linear *A*–*t* ranges. The facile reaction of AQ with KIO_3 may be attributed to the presence of the labile *p*-aminophenol moiety [18, 21].

On the other hand, the reaction of CQ with KIO_3 was too slow for convenient measurements; therefore, the stronger oxidant $KBrO_3$ was studied and gave convenient rate values with good linear *A*–*t* ranges.

The effect of bromate concentration was studied in CQ oxidation over the range of 2.0–25.0 mmol dm^{-3} $KBrO_3$. The initial rate of CQ consumption [−100(d*A*/d*t*)] gradually increased with bromate concentrations, whereas the linear ranges of the *A*–*t* graphs gradually decreased (Fig. 2b). Therefore, a bromate concentration of 10.0 mmol dm^{-3} was adopted in the recommended procedure to give moderate sensitivity and linear *A*–*t* ranges.

Effect of sulfuric acid concentration

Preliminary experiments showed that a strong acid medium was required for AQ and CQ oxidation with KIO_3 and $KBrO_3$, respectively. Nitric and perchloric acids were excluded due to their oxidizing properties. Hydrochloric acid was also excluded to avoid the complications arising from reactions of Cl^- ions with IO_3^- and BrO_3^- ions. Therefore, the effects of various concentrations of sulfuric acid were studied in AQ and CQ oxidation with 3.0 mmol dm^{-3} KIO_3 and 10.0 mmol dm^{-3} $KBrO_3$ (Fig. 3a, b).

The initial rate of AQ disappearance increased gradually with sulfuric acid concentration up to 3.5 mol dm^{-3}, whereas the linear ranges of the *A*–*t* graphs decreased gradually. Therefore, a sulfuric acid concentration of 2.0 mol dm^{-3} was adopted in the recommended procedure to give moderate rate values and a linear *A*–*t* range (Fig. 3a).

Moreover, the initial rate of disappearance of CQ increased gradually with sulfuric acid concentration up to 5.0 mol dm^{-3}, whereas the linear ranges of the *A*–*t* graphs decreased gradually. Therefore, a sulfuric acid concentration of 4.0 mol dm^{-3} was adopted in the recommended procedure to give moderate rate values and a linear *A*–*t* range (Fig. 3b).

Effect of reaction temperature

AQ and CQ oxidation with iodate and bromate were studied at different temperatures (30–65 °C). The initial AQ and CQ disappearance rates gradually increased with temperature (Fig. 4). However, a working temperature of 50 °C was adopted in the recommended procedure.

Effects of excipients

A systematic study of the effects of excipients was performed by adding a known amount of excipient to 5.0 cm^3 of 10.0 μg cm^{-3} AQ or CQ, filtering off the insoluble excipient when necessary, washing the residue, diluting the combined filtrates and washings in a 25-cm^3 volumetric flask, and analyzing an aliquot of 1,500 mm^3 following the recommended procedure. The results revealed that 200-fold excess of excipients—such as lactose, glucose, mannitol, starch, talc, hydroxypropyl methylcellulose, polyethylene glycol, polysorbate 80, calcium phosphate dibasic, stearic

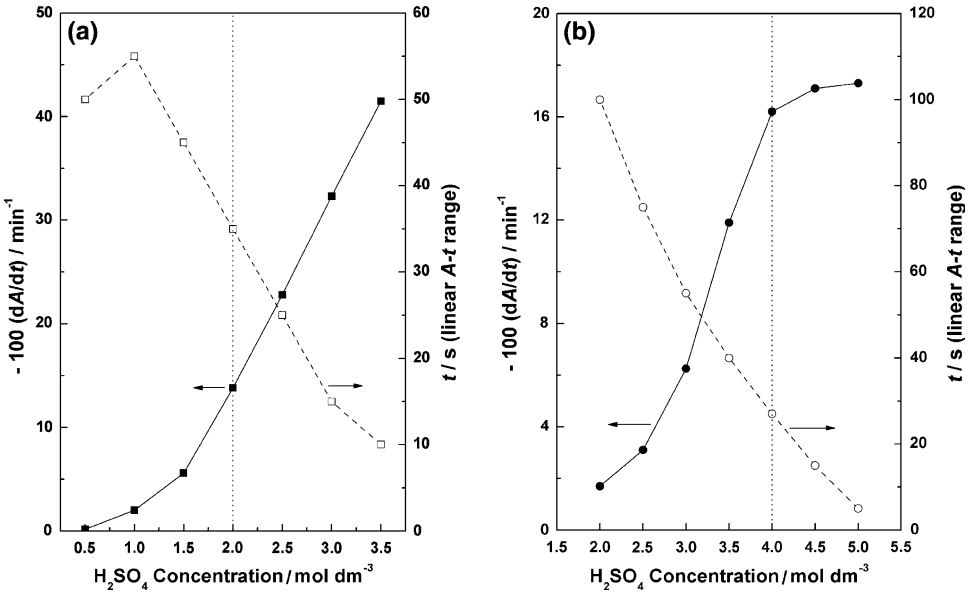

Fig. 3 Effect of sulfuric acid concentration on the rate of consumption of **a** 4.0 μg cm^{-3} AQ in the presence of 3.0 mmol dm^{-3} iodate and **b** 5.0 μg cm^{-3} CQ in the presence of 10.0 mmol dm^{-3} bromate. Except for the abscissa variable, other conditions were as given in Fig. 2

acid, magnesium stearate, and titanium dioxide that are commonly found in pharmaceutical formulations of AQ and CQ and can represent a potential source of interference in other methods—do not interfere in the proposed method.

Calibration graphs, limits of detection and precision

Calibration graphs [$-100(\mathrm{d}A/\mathrm{d}t)$ vs. conc.] obtained, following the recommended procedures, were found to be linear for 0.2–4.0 and 0.5–5.0 μg cm^{-3}, with correlation coefficients of 0.9999 and 0.9998 ($n = 6$) and a detection limit—based on the $3S_b$-criterion, three times the standard deviation (SD) of the intercept divided by the slope of the respective calibration graph [24]—of 0.04 and 0.06 μg cm^{-3} for AQ and CQ, respectively. Linear regression data analysis gave the following equations at 50 °C:

$$-100\mathrm{d}A/\mathrm{d}t = ((3.505 \pm 0.023)[\mathrm{AQ}]) - (0.316 \pm 0.051), \quad r = 0.9999$$

$$-100\mathrm{d}A/\mathrm{d}t = ((3.306 \pm 0.021)[\mathrm{CQ}]) - (0.283 \pm 0.070), \quad r = 0.9998,$$

where [AQ] and [CQ] are the concentration of AQ and CQ in μg cm^{-3}.

The small values of SD of the slopes and intercepts indicate the high accuracy and precision of the proposed methods. Moreover, the precision of the method was further assessed according to the International Union of Pure and Applied Chemistry (IUPAC) recommendations [24] by analyzing 0.5, 1.0, 2.0, 3.0, and 4.0 μg cm^{-3} AQ and CQ in aqueous solutions following the recommended procedures and gave quantitative recoveries of ≥99.5%, where the within-day relative SDs (RSDs) were ≤1.8%, the between-day RSDs were ≤2.1% ($n = 6$), and the Student's t test values were ≤1.9, showing that the t test could not detect any systematic error and revealing the

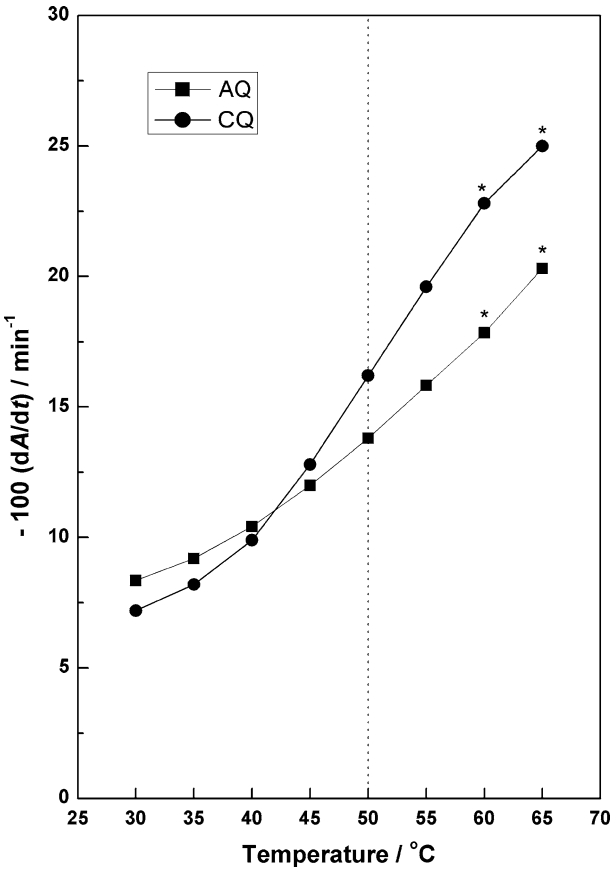

Fig. 4 Effects of temperature on AQ and CQ oxidation rates. Except for the abscissa variable, conditions were as given in Fig. 2. *Asterisks* denote data points with poor linearity of the *A–t* graphs

high accuracy and precision of the proposed method. (The tabulated t value for the 95% confidence level and $n = 6$ is 2.45 [24].)

Applications

The proposed methods were directly applied to the quantification of AQ and CQ in dosage forms obtained from the local market (Table 1). The data were compared with those obtained following the standard pharmacopoeial methods (USP 2007 [2]) based on UV spectrophotometry at 342 and 343 nm for AQ and CQ. Statistical analysis of the results did not detect any significant difference between the performances of the proposed methods and the standard pharmacopoeial methods regarding accuracy and precision as revealed by the Student's t test and the variance ratio F test [24].

Conclusion

It can be concluded that the proposed methods have the advantages of high sensitivity (small values of the limit of detection), simplicity, no need for extraction or separation steps before the analysis, and the absence of any interference from tablet excipients. In addition, statistical treatment of analytical results showed that the proposed methods are accurate and precise, as indicated by the good recoveries of the drugs, the low RSD values (both within day and between day), and the good agreement with standard US pharmacopoeial methods [2]. The above findings substantiate the usefulness of the proposed methods for the assay and quality control of AQ and CQ in their dosage forms.

Experimental

Apparatus

Kinetic measurements were made on a UV–Vis 1601 Shimadzu double-beam spectrophotometer (Kyoto, Japan) equipped with a thermostated cell holder with 10-mm matched cells. The cell compartment of the spectrophotometer was thermostatically controlled by circulating water from a PolyScience (IL, USA) thermostated water bath with a temperature stability of ±0.1 °C. Eppendorf vary pipettes (Westbury, NY, USA), 10–100 and 100–1,000 mm^3, were used to deliver accurate volumes.

Reagents

All reagents were of analytical grade and were used as received. Fresh distilled, deionized water was used throughout. Amodiaquine dihydrochloride dihydrate and chloroquine diphosphate were purchased from Sigma (St. Louis, MO, USA). Sulfuric acid, potassium iodate, and potassium bromate were purchased from Merck (Darmstadt, Germany). Tablets containing the drug were obtained from the local market. Stock standard solutions of 100 μg cm^{-3} AQ or CQ were prepared in 0.01 mol dm^{-3} H_2SO_4 and were further diluted when required. Aqueous working solutions of 12.0 mol dm^{-3} H_2SO_4, 90 mmol dm^{-3} KIO_3, and 100 mmol dm^{-3} $KBrO_3$ were also prepared.

Table 1 Determination of amodiaquine (AQ) and chloroquine (CQ) in pharmaceutical preparations

Analyte/preparation	Nominal concentration	Found ± SD[a]		t[c]	$F_{6,6}$[c]
		Pharmacopoeial method[b]	Proposed method		
Amodiaquine hydrochloride					
Flavoquine[d]	200 mg/tab	199.2 ± 1.0	198.6 ± 1.2	0.86	1.4
Chloroquine phosphate					
Chloroquine[e]	80 mg/5 cm^3 susp.	80.4 ± 1.1	80.0 ± 0.9	0.63	1.5
Chloroquine[e]	250 mg/tab	248.0 ± 2.1	249.1 ± 1.9	0.87	1.2
Alexoquine[f]	250 mg/tab	249.1 ± 2.4	249.6 ± 2.1	0.35	1.3
Cidoquine[g]	250 mg/tab	245.3 ± 2.4	244.7 ± 2.2	0.41	1.2

[a] Data are averages of six replicate determinations ($n = 6$). Reaction conditions were those given in the recommended procedures

[b] Following the assay conditions given in the USP 2007

[c] The tabulated Student's t test ($t_{(n = 6)}$) values and the Variance ratio ($F_{6,6}$) values at the 95% confidence level are 2.45 and 4.28

[d] Product of Roussel, Paris, France

[e] Product of Pharco Pharmaceutical Company, Egypt

[f] Product of Alexandria Company for Pharmaceuticals, Egypt

[g] Product of CID Company for Pharmaceuticals, Egypt

Treatment of tablets

The contents of ten tablets under investigation were weighed, ground into fine powder, and mixed well. An accurately weighed portion of the powder equivalent to one tablet of amodiaquine dihydrochloride or chloroquine diphosphate was transferred to a 50-cm^3 beaker containing about 25 cm^3 of water and 100 mm^3 of the working sulfuric acid solution, placed in the ultrasonic bath for 10 min, and filtered into a 100-cm^3 volumetric flask. The residue and filter paper were thoroughly washed with several portions of water. The washings and extracts were combined in the same measuring flask, completed to the mark with water, and further diluted if required.

Recommended procedure for determining AQ

Transfer aliquot volumes containing $\leq$12 μg of the unknown AQ solution or the working AQ standard solution into a dry thermostated spectrophotometric cell, add 500 mm^3 of the working sulfuric acid solution and dilute with water to 2,900 mm^3. Add 100 mm^3 of the working KIO_3 solution, shake well, return the cell to its holder, and start recording the absorbance change with time at 342 nm for 90 s, against water as a reference, allowing a lag time of 30 s. Calculate the initial rate of AQ consumption from the slope of the *A*–*t* graph, the rate of decrease of absorbance $[-100(\mathrm{d}A/\mathrm{d}t)\ \mathrm{min}^{-1}]$. Determine the content of the tablet either from the similarly constructed calibration graph or its linear regression equation.

Recommended procedure for determining CQ

Transfer aliquot volumes containing $\leq$15 μg of the unknown CQ solution or the working CQ standard solution into a clean, dry, and thermostated spectrophotometric cell. Add 1,000 mm^3 of the working sulfuric acid solution, dilute with water to 2,700 mm^3, and add 300 mm^3 of the working $KBrO_3$ solution. Shake well, return the cell to its holder, and start recording the absorbance change with time at 343 nm, against water as a reference. Calculate the rate of CQ consumption—the rate of decrease of absorbance $[-100(\mathrm{d}A/\mathrm{d}t)\ \mathrm{min}^{-1}]$—from the initial linear part of the *A*–*t* graph within 30 s of pressing the start button. Determine the content of the tablet either from the similarly constructed calibration graph or its linear regression equation.

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