Arch. Pharm. Res. Vol. 17, No. 6, pp. 415-419, 1994

Studies of Complex Formation between the Bromophenol Blue and some Important Aminoquinoline Antimalarials

Saadia Mohamed El-Ashry[1], Fatma Ahmed Aly[2] and Amina Mohamed El-Brashy[2]

[1]*Medicinal Chemistry and* [1]*Analytical Chemistry Departments, Faculty of Pharmacy, University of Mansoura, Mansoura 35516, Egypt.*

(Received July 14, 1994)

A simple and rapid colorimetric method for the assay of amodiaquine hydrochloride, chloroquine phosphate and primaquine phosphate is described. The method is based on the interaction of the drug base with bromophenol blue to give a stable ion-pair complex. The spectra of the complex shows a maxima at 415-420 nm with high apparent molar absorptivities. Beer's law was obeyed in the concentration range 1-8, 2-10 and 2-12 $\mu g \cdot ml^{-1}$ for amodiaquine hydrochloride, primaquine phosphate and chloroquine phosphate respectively. The proposed method was applied to the determination of these drugs in certain formulations and the results were favourably comparable to the official methods.

Key words: Bromophenol blue, Amodiaquine, Chloroquine, Primaquine, Colorimetry, Dosage forms

INTRODUCTION

Aminoquinolines are widely prescribed therapeutic agents used as antimalarials. Due to their medicinal importance, several methods have been reported for their determination, either perse or in dosage forms. These drugs have been determined by titrimetry (Walash *et al.*, 1983); colorimetry; through formation of chargetransfer complexes using tetracyano-ethylene (Ibrahim *et al.*, 1989a), iodine (Abdel-Salam *et al.*, 1986) and chloranilic acid (Mahrous *et al.*, 1986); by acid dye technique (Sastry *et al.*, 1986); by the formation of ternary complexes with cobalt thiocyanate (Abou-Ouf *et al.*, 1980) or with tannic acid and 4-methylaminophenol sulphate (Sastry *et al.*, 1989).

Amodiaquine has been determined by spectrophotometry using 2,2-diphenyl-1-picrylhydrazyl (Emara, 19 88), potasium periodate (Sanghi *et al.*, 1990) or using 1-benzothiazolinone (Rao *et al.*, 1989).

Other reported methods for the determination of these antimalrials include fluorimetry (Adelusi and Salako, 1980; Ibrahim *et al.*, 1989b) and high perfomance liquid chromatography (Sanghi *et al.*, 1990: Williams *et al.*, 1990).

Bromophenol blue has been reported to be used for the determination of pharmaceutical basic compounds (Sadana *et al.*, 1986; Buyuktimkin and Buyuktimkin, 1986; Zarapker *et al.*, 1987; Shingbal and Kara, 1988; Martinez *et al.*, 1990). The aim of the present work is to extend the application of bromophenol blue to include some aminoquinoline antimalarials through ion-pair complex formation between bromophenol blue and aminoquinoline derivatives in dry chloroform.

Correspondence to: Fatma Ahmed Aly, Faculty of Pharmacy, University of Mansoura, Mansoura 35516, Egypt.

MATERIALS AND METHODS

Apparaturs

All colorimetric determinations were performed with a Perkin Elmer Spectrophotometer with 1 cm quartz cells.

Reagents and Materials

Solutions of 0.1% (w/v) and 1×10^{-4} M bromophenol blue (Darmstadt, FRG) in chloroform (Merck) were prepared.

The studied aminoquinoline antimalarials were amodiaquine hydrochloride, chloroquine phosphate and primaquine phosphate, all were of pharmaceutical grade. Representative dosage forms (Table III) were obtained from commerical sources. The purity of these drugs was checked using the official methods (BP,

Table I. Collective data of the aminoquinoline salts with bromophenol blue

Compound	Concentration range ($\mu g \cdot ml^{-1}$)	λmax nm.	ε $L \cdot mol^{-1} cm^{-1}$	Regression equation	Correlation coefficient
Amodiaquine hydrochloride	1.0- 8.0	420	5.5×10^4	C=8.4902A-0.0281	0.9998
Chloroquine phosphate	2.0-12.0	415	4.3×10^{-4}	C=12.1735A-0.0038	0.9998
Primaquine phosphate	2.0-10.0	415	4.4×10^{-4}	C=10.3669A+0.0624	0.9997

C=concentration in $\mu g \cdot ml^{-1}$, A=absorbance at λmax.

1993; USP XXII, 1990).

Preparation of Sample Solutions

A 100 mg portion of the drug salt was transferred into a 60 ml separator with a small volume of distilled water and made alkaline with 6 M ammonia solution. The liberated base was then extracted with five portions (15 ml) of chloroform. The combined chloroform extracts were dehydrated by shaking with anhydrous sodium sulphate for 5 min. The extract was filtered, into a 100 ml volumetric flask. The sodium sulphate and the filter were rinsed with chloroform and the washings were added to the filtrate. The solution was diluted to the mark with chloroform. This solution was further diluted with chloroform to give a 50 $\mu g \cdot ml^{-1}$ drug working solution. A 1×10^{-4} M solution was prepared as described above.

General Procedure

Accurately measured volumes of the drug in chloroform in the concentration range cited in Table I were transferred into a series of 10 ml volumetric flasks. Bromophenol blue in chloroform (0.1% 2 ml) was added to each flask and the solutions were mixed and diluted to volume with chlroform. The absorbance was measured immediately at 415-420 nm against a reagent blank prepared simultaneously.

Procedure for Dosage Forms

An accurately weighed amount of the powdered tablets or measured volume of the mixed ampoules equivalent to 25 mg of the drug was transferred into a 60 ml separator with a small volume of distilled water and made alkaline with 6 M ammonia solution. An assay solution was prepared in a similar manner as the sample solution utilizing chloroform as an extracting solvent. An aliquot of the chloroform extract in the concentration range cited in Table I was transferred into a 10 ml volring flask and diluted as described under general procedure. The contents of the tablets or ampoules were calculated from the calibration graphs previously prepared with the authentic drugs or from the linear regression equations.

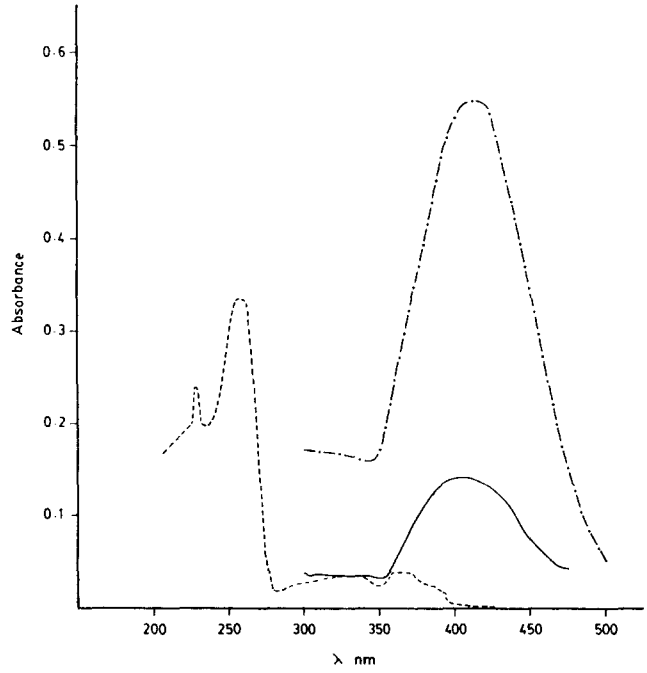

Fig. 1. Absorption spectra of: ---, primaquine bromophenol blue ion-pair complex (6 $\mu g \cdot ml^{-1}$). —, bromophenol blue (0.02%) and ---, primaquine (6 $\mu g \cdot ml^{-1}$).

RESULTS AND DISCUSSION

Bromophenol blue reacts as an acidic ion-pairing reagent. It has been used for the determination of pharmaceutical basic compounds through either dye salt formation technique and extractive spectrophotometric measurment of the complex formed in non polar solvent or through ion-pair complex formation (Sadana *et al.*, 1986; Buyuktimkin and Buyuktimkin, 1986; Zarapker *et al.*, 1987; Shingbal and Kara, 1988; martinez *et al.*, 1990) followed by extractive spectrophotometric (Shingbal and Kara, 1988) or turbidimetric (Martinez *et al.*, 1990) measurement.

The studied drugs, amodiaquine, chloroquine and primaquine have high electron density sites, so they may act as powerful electron donors. In chloroform these drugs exhibit absorption maxima in the ultraviolet region at 260-270 nm. Upon addition of bromo-

phenol blue, a pronounced bathochrmic shift to 415-420 nm is observed (Fig. 1). This change in the spectra may be attributed to the formation of neutral ion-pair complexes.

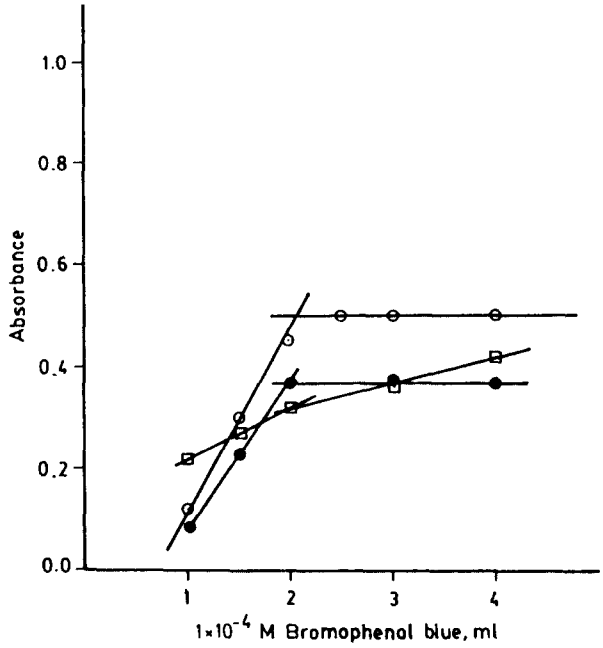

Fig. 2. Mole ratio plot of aminoquinolines-bromophenol blue complexes 1×10^{-4} M; ○—○ amodiaquine (1 ml); ●—●, chloroquine (1 ml); □—□ Primaquine (1 ml).

The different experimental parameters affecting the formation of the complex were extensively studied to determine the optimal conditions for the assay procedure. The reaction was studied as a function of the volume of the reagent, solvent, reaction time and stability. Maximum absorbance was attained using 2 ml of a 0.1% bromophenol blue solution. Different solvents such as chloroform, acetonitrile, dichloromethane and dichloroethane were tested. Chloroform was found to be the solvent of choice for the formation of the complex because of the greater molar absorptivity of the complex. The reaction product was formed immediately at room temperature and it was stable for more than one hour.

Under the described experimental conditions, standard calibration graphs were constructed by plotting absorbance versus concentration. Conformity with Beer's law was evident in the concentration ranges cited in Table I. Linear regression analysis of the results showed that a rectilinear relationship exists between the absorbance at λmax and the concentration over the ranges cited in Table I.

The stoichiometry of bromophenol blue and the studied drugs was achieved by the mole ratio method (Jop, 1971) and it was found that the complexes are formed in the ratio (1 : 2) drug : reagent (Fig. 2).

The appreciable values of molar absorptivities and the considerable stability of the complexes formed permit a successful application of the proposed me-

Table II. Assay of some aminoquinoline salts by bromophenol blue in chloroform

Compound	Proposed method		Official method (BP, 1993; USP, 1990)	
	Concentration [μg·ml^{-1}]	Recovery* [%]	Concentration [mg]	Recovery [%]
Amodiaquine hydrochloride	1.0	99.07	200.0	100.69
	2.0	100.48	350.0	100.83
	4.0	99.06	350.0	98.63
	8.0	99.41	400.0	101.39
	6.0	101.41	300.0	100.00
Mean± S.D.		99.89± 0.92		100.31± 1.06
Chloroquinone phosphate	2.0	101.46	500.0	100.97
	4.0	100.34	600.0	100.97
	6.0	101.38	700.0	101.94
	8.0	100.38	800.0	99.03
	10.0	100.63		
	12.0	99.39		
Mean± S.D.		100.60± 0.70		100.73± 1.22
Primaquine phosphate	2.0	101.61	500.0	100.17
	4.0	100.05	550.0	101.53
	5.0	100.77	600.0	101.30
	6.0	99.52	700.0	100.39
	8.0	100.56		
	10.0	100.15		
Mean± S.D.		100.44± 0.66		100.85± 0.67

*Mean of three determinations.

Table III. Determination of aminoquinoline salts in pharmaceutical preparations

Preparation	Proposed method		Official method	
	Concentration [$\mu g \cdot ml^{-1}$]	Recovery [%]	Concentration [mg]	Recovery [%]
Camoquin tablets[a]	3.0	100.95	300.0	98.63
(200 mg Amodiaquine hydrochloride/tab.)	4.0	101.18	300.0	100.72
	5.0	99.62	300.0	100.54
	6.0	100.01		
Mean± S.D.		100.44± 0.65		99.96± 1.16
Primaquine phosphate tablets	3.0	100.57	500.0	102.44
(200 mg/tab.)	4.0	101.34	500.0	101.99
	5.0	102.01	500.0	102.67
	7.0	100.12		
Mean± S.D.		101.01± 0.72		102.37± 0.35
Dagrinol tablets[b]	3.0	98.79	500.0	97.83
(250 mg chloroquine phosphate/tab.)	5.0	99.25	500.0	98.84
	9.0	98.59	500.0	98.44
	7.0	97.96		
Mean± S.D.		98.65± 0.46		98.37± 0.51
Dagrinol ampoules[b]	4.0	101.75	500.0	101.16
(250 mg chloroquine phosphate/ampoule)	6.0	100.45	500.0	102.38
	8.0	101.29	500.0	101.25
	10.0	100.91		
Mean± S.D.		101.10± 0.48		101.60± 0.68

[a] Product of Nile Co.
[b] Product at Alex. Co.

Table IV. Statistical analysis of aminoquinolines and official methods

Compound	Amodiaquine hydrochloride		Chloroquine phosphate		Primaquine phosphate	
Function	Proposed	Official	Proposed	Official	Proposed	Official
No. of Exp.	5	5	6	4	6	4
Mean± S.D.	99.89± 0.92	100.31± 1.06	100.60± 0.70	100.73± 1.22	100.44± 0.66	100.85± 0.67
Variance	0.846	1.124	0.490	1.488	0.436	0.449
Student's-t-test	0.67 (2.31)	0.22 (2.31)	0.96 (2.31)			
Variance ratio (F test)	1.33 (6.39)	3.04 (5.41)	1.03 (5.41)			

The figures in brackets are the tabulated values at P=0.05 (Sanders *et al.*, 1976).

thod for the determination of these compounds either in pure form (Table II) or in their representative dosage forms (Table III).

Statistical analysis (Sanders *et al.*, 1978) (Table IV) was performed and showed that the proposed and the official methods are equally accurate. The proposed method is more sensitive than the official procedures, as a small amount (1 $\mu g \cdot ml^{-1}$) can be determined with good accuracy. These advantages suggest its application in the analysis and quality control of these antimalarials in their pharmaceutical preparations. Substances having no basic centers are not expected to interfer, since extraction of the antimalarial bases precedes the colour reaction.

The most striking feature of the proposed method over the reported methods is the higher sensitivity achieved. Moreover, the formation of the colour probe is instantaneous, thus, the method can be readily applied to automated analysis or coupled with high performance liquid chromatography or flow injection analysis techniques. Comparing the proposed method with the USP spectrophotometric method described for amodiaquine hydrochloride and chloroquine phosphate, the USP method seems to be more simple but the proposed method is more sensitive, more selective and it has a lower detection limit.

REFERENCES CITED

Abdel-Salam, M., Issa, A. S. and Lymona, H., Utility of charge-transfer complex formation for spectrophotometric detrmination of some antimalarials. *J. Pharm.*

Belg., 41, 314-318 (1986).

Abou-Ouf, A. A., Hassan, S. M. and Metwally, M. E., Spctrophotometric determination of primaquine phosphate in pharmaceutical preparation. *Analyst,* 105, 113-118 (1980).

Adelusi, S. A. and Salako, L. A., Improved fluorimetric assay of chloroquine in biological samples. *J. Pharm. Pharmacol.,* 32, 711-714 (1980).

British Pharmacopoeia, London, Her Majesty's Stationary Office (1993).

Buyuktimkin, N. and Buyuktimkin, S., Ion-pair spectrophotometric assay of oxolamine. *Pharmazie,* 41, 287-288 (1986).

Emara, K. M., Spectrophotometric determination of amodiaquine hydrochloride using 2,2-diphenyl-1-picrylhydrazyl. *Alexandria J. Pharm. Sci.,* 2, 179-181 (1988).

Ibrahim, F. A., El-Brashy, A. M. and Belal, F., Spectrophotometric determination of some pharmaceutically important aminoquinoline antimalarials. *Mikrochim. Acta.,* 1, 321-327 (1989a).

Ibrahim, F. A., Belal, F. and El-Brashy, A., Fluorometric determination of som aminoquinoline antimalarials using Eosin. *Microchem. J.,* 39, 65-70 (1989b).

Job, P., Spectrochemical Methods of Analysis, Wiley Interscience, New York, 9, 346 (1971).

Mahrous, M. S., Abdel-Salam, M., Issa, A. S. and Abdel-Hamid, M., Colorimetric determination of some antimalarials. *Talanta,* 33, 185-186 (1986).

Martinez, C. J., Sanchez, S. A. and Navasquillo, S. S., Determination of diphenylhydramine hydrochloride by flow injection with bromophenol blue and turbidimetric measurment. *Analyst,* 115, 855-858 (1990).

Rao, G. R., Murty, S. S. N. and Raju, I. R. K., Spectrophotometric determination of amodiaquine hydrochloride in dosage forms. *Indian Drugs,* 27, 74-75 (1989).

Sadana, G. S., Sane, R. T., Ozarkar, S. G., Spare, D. S. and Nayak, N. G., Extractive spectrophotometric method for determination of ranitidine hydrochloride from pharmaceutical preparations. *Indian Drugs,* 23, 573-574 (1986).

Sanders, D. H., Murph, A. F. and Eng, R. J., Statistics, McGraw Hill, New York (1976).

Sanghi, S. K., Verma, A. and Verma, K. K., Determination of amodiaquine in pharmaceuticals by reaction with periodate and spectrophotometry or by high performance liquid chromatography. *Analyst,* 115, 333-335 (1990).

Sastry, B. S., Rao, E. V., Tummura, M. K. and Sastry, C. S. P., Extractive spectrophotometric determination of some antimalarials using fast green. *Indian Drugs,* 24, 105-108 (1986).

Sastry, B. S., Rao, J. V., Prasad, T. N. V. and Sastry, C. S. P., Estimation of some aminoquinoline antimalarials using tannic acid. *Indian J. Pharm. Sci.,* 51, 109-110 (1989).

Shingbal, D. M. and Kara, R., Extractive spectrophotometric method for determination of flufenamine hydrochloride from its tablets. *Indian Drugs,* 25, 341-343 (1988).

The United States Pharmacopoeia XXII, National Formularly XVII, Rockville, USP Convention, Inc. (1990).

Walash, M. I., Rizk, M., Abou-Ouf, A. A. and Belal, F., Determination of some quinoline derivatives with organic brominating agents. *Anal. Lett.,* 16, 129-148 (1983).

Williams, H. L., Johnson, D. J., Collins, H. H., Martin, S. K. and Ramuz, H., Simultaneous determination of chloroquine and selected calcium channel blockers by high performance liquid chromatography. *Anal. Chim. Acta,* 228, 155-157 (1990).

Zarapker, S. S., Rele, R. V. and Shah, V. M., Simple extractive colorimetric determination of pipazethate hydrochloride from pharmaceutical preparations. *Indian Drugs,* 24, 445-449 (1987).