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Photodegradation studies on chloroquine phosphate by high-performance liquid chromatography

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Abstract: A method based on high-performance liquid chromatography (HPLC) was developed to study degraded chloroquine samples produced after exposure to sunlight in the Sudan. The method was also used to investigate chloroquine photodegradation after irradiation by UV and sunlight at ambient temperature. The study showed that the photodecomposition of chloroquine was pH and solvent dependent. Moreover, the extent of reaction was found to increase in the absence of oxygen. At pH 8, where the reaction rate was high, the photodecomposition was found to follow pseudo-first-order reaction kinetics. The HPLC method developed was also employed to analyse chloroquine and its degradation products in two commercially available brands of chloroquine injections which had been stored under local conditions in the Sudan. A number of degradation products were separated and examined by photodiode array spectroscopy and preparative TLC.

Keywords: *Chloroquine; photodegradation; HPLC; TLC; kinetics.*

Introduction

Chloroquine is extensively used in the Sudan for the prevention and treatment of malaria. The drug is known to be sensitive to sunlight [1–3]. This photosensitivity has been monitored by observing the resultant change of colour, the intensity of which was enhanced by adding reducing agents following irradiation [3]. Paper chromatography of the brown colour formed in a chloroquine solution following irradiation was found to contain a number of uncharacterized irradiation products [4]. Spectrophotometry and spectrofluorimetry have been used to investigate the effect of sunlight and UV irradiation on buffered solutions of chloroquine [5, 6]. The heat stability of chloroquine phosphate in bulk and in tablets has been studied by differential scanning calorimetry (DSC) after exposure to sunlight [7] and by high-performance liquid chromatography (HPLC) after boiling with acid and alkali [8]. Both these methods reported significant loss of chloroquine but did not examine the products of degradation.

In this study the photodecomposition of chloroquine in aqueous solution, after irradiation by UV and sunlight, was investigated using HPLC with both single and multichannel detection. The HPLC method developed to separate the photodecomposition products from the drug was employed for the analysis of chloroquine and its degradation products in two commercially available brands of chloroquine injection which had been stored under local conditions in the Sudan.

Materials and Methods

Chemicals and reagents

Chloroquine phosphate was obtained from Sigma (Poole, UK). Desethyl chloroquine was kindly donated by Dr A.A. Ali (Department of Pharmaceutics, Faculty of Pharmacy, University of Khartoum). Heptane sulphonic acid was obtained from Fisons Scientific (Loughborough, UK). Acetonitrile was HPLC grade obtained from Rathburn Chemicals (Walkerburn, Scotland). All other chemicals were of analytical grade and used as received.

Apparatus

Two HPLC systems were employed. System

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A comprised of a Gilson Model 302 pump and Model 302C manometric module, with a Rheodyne model 7125 loop valve injector (Cotati, CA, USA) supplied with a 20-μl loop; this was connected to a single-channel detector comprising a Pye Unicam PU 4025 variable wavelength UV-detector set at 343 nm (the absorption maximum of chloroquine in water) with a strip-chart recorder. System B comprised an LDC Constametric R300 pump (LDC, Stone, UK) with a Rheodyne Model 7125 Loop-Valve injector provided with a 20-μl loop; this was connected to a multichannel 'rapid scanning' photodiode array detector, the Hewlett–Packard 1040A, interfaced to an HP 9000 series model 300 microcomputer and peripherals for data acquisition, storage, processing and presentation.

Irradiation was carried out using an Immersion-Well Photochemical Reactor (Model Q124, Applied Photophysics, Leatherhead, UK) and a medium-pressure mercury arc lamp (Hanovia, Applied Photophysics) with Pyrex glass sample cell (cut-off at 300 nm) in fixed geometry, all immersed in a water bath (Grant Instruments, Barington, Cambridge, UK) maintained at *ca* 25°.

UV spectra in phosphate buffer (pH 3.5) were run on a Lambda-5 UV–vis spectrophotometer with 1-cm quartz cells (Perkin–Elmer, Beaconsfield, UK).

Fluorescence emission spectra in phosphate buffer (pH 3.5) were obtained using a Perkin–Elmer LS5 spectrofluorimeter and 1-cm quartz cell.

Chromatography

High-performance liquid chromatography. Separations were carried out on a (100 × 4.6 mm) chromatographic column packed with 5-μm Hypersil (Shandon-Southern, Runcorn, Cheshire, UK). The mobile phase comprised acetonitrile–KH_2PO_4, to which heptane sulphonic acid and triethylamine were added. Mobile phase composition and flow rate were optimized by systematic investigation of a degraded sample of chloroquine, as discussed below.

Thin-layer chromatography (TLC). A solution of chloroquine phosphate (5 mg ml^{-1}) in water was exposed to sunlight during March–April for 21 days in the Sudan. A small amount of the yellow solution produced was applied to a silica gel TLC plate (SI60G, Merck, Darmstadt, Germany) and developed using acetylacetate–absolute ethanol–ammonia (s.g. 0.880) (25:1:1, v/v/v) as mobile phase, optimized on the basis of earlier work [9]. The spots separated were visualized under UV light. The decomposition products were separated using preparative TLC and extracted from the silica with methanol. The UV and fluorescence emission spectra (λ_{ex} = 340 nm; slit-widths, 5 nm) of the extracts were recorded and compared.

Effect of pH on photodegradation

Chloroquine phosphate solutions (20 μg ml^{-1}) were prepared in 0.1 M phosphate buffer solutions at pH 5.0, 7.0 and 8.0. The solutions were placed in Pyrex cells and irradiated for 4 h. At the end of the irradiation period the pH was adjusted to 3.5 with orthophosphoric acid and the UV spectrum and HPLC chromatogram of each were obtained.

Effect of solvent on photodegradation

Three solutions of chloroquine phosphate (50 μg ml^{-1}) were prepared in: (a) chloroform; (b) 20% v/v acetonitrile–phosphate buffer (0.1 M; pH 9); and (c) phosphate buffer alone (0.1 M; pH 9). The solution of chloroquine in chloroform was prepared by basification with addition of 1 M NaOH (1 ml) to aqueous chloroquine solution (2 mg ml^{-1}); the mixture was then extracted four times with 10-ml portions of chloroform. The combined chloroform extracts were washed four times with 10 ml water.

Each of the three solutions was transferred to a Pyrex cell and irradiated for 6 h. At the end of irradiation 1 ml of chloroform solution was evaporated to dryness with a stream of nitrogen and the residue was reconstituted in 1 ml of phosphate buffer (pH 3.5). The pH of the two aqueous solutions was adjusted to 3.5 with orthophosphoric acid and the solutions were examined by HPLC. The chromatograms obtained were compared with those of non-irradiated solutions.

Effect of oxygen on photodegradation

Chloroquine solution (50 μg ml) in phosphate buffer (0.1 M; pH 9) was placed in two Pyrex cells and irradiated for 4 h; the solution in one of the cells was flushed with nitrogen for 15 min prior to irradiation and nitrogen bubbling was continued throughout the irradi-

ation. At the end of the irradiation period the pH of the two solutions was adjusted to between 3 and 5 with orthophosphoric acid then each was subjected to HPLC. A similar study was conducted at pH 8.0.

Kinetic studies on chloroquine photodegradation

A solution of chlorine phosphate (20 μg ml^{-1}) was prepared in 0.1 M phosphate buffer (pH 9.0), placed in a Pyrex cell and irradiated. Aliquots (4 ml) were taken at 0, 30, 60, 90 and 120 minutes after the start of irradiation. The pH of each aliquot was adjusted to 3.5 and duplicate 20-μl injections of each were made and chromatographed. The mean height of the chloroquine peak in each chromatogram obtained was measured and the logarithm plotted against time.

Results and Discussion

Development of HPLC separation

The HPLC method was developed using system A on the basis of the degraded chloroquine sample produced by exposure to sunlight in the Sudan. An ODS-Hypersil packing was employed because of its high selectivity and specificity in earlier work [10]. The acetonitrile concentration was varied from 15 to 40% v/v using a nominal concentration of ion-pair reagent initially (5 mM heptane sulphonic acid). A pH of 3.5 was used in order to ensure that chloroquine was in the cationic form (pK_a = 8.4). On the basis of preliminary experiments, it was found that adding triethylamine (5 mM) yielded improved peak shape, probably due to its effect on residual uncapped silanols on the packing material [11]. These observations correspond with those of many workers in the authors' laboratories, concerned with the separation of basic compounds [12]. As can be seen from Fig. 1 at least four degradation products were detected at this stage. The fourth degradation product (peak e) was found to be unstable and could not be further examined. Their relative peak capacity ratios (k') are described in Fig. 2. From this it can be seen that excellent resolution of all four peaks was achieved at 18% v/v acetonitrile, when the overall retention time was about 20 min.

It was decided to examine the effect of ion-pair concentration on retention with 18% v/v acetonitrile to see if further resolution could be achieved. It was also of interest to establish which of the degradation peaks was able to form an ion-pair in the eluent. As can be seen from Fig. 3, the k' of all the detectable peaks increased with increasing ion-pair concentration, confirming that all the degradation products were cationic and able to form ion pairs at this pH.

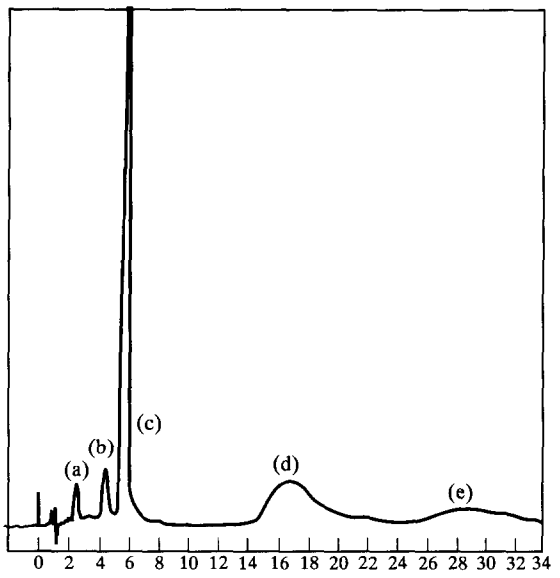

Figure 1
HPLC separation of degraded sample of chloroquine phosphate after exposure to sun in the Sudan. For experimental details, see text. Key: a, b, d, e: degradation products; c, chloroquine.

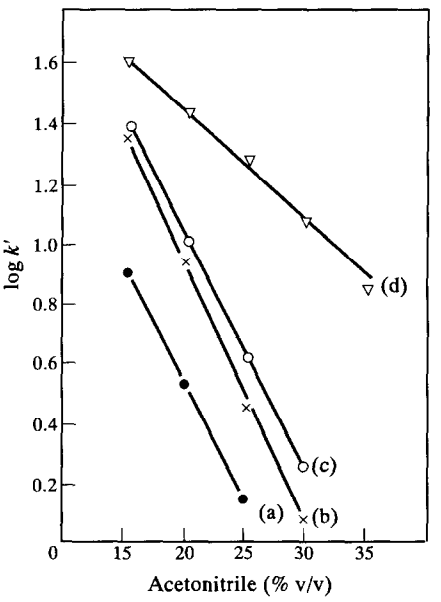

Figure 2
Relationship of log k' for three degradation products (cf. Fig. 1) with acetonitrile concentration (heptane sulphonic acid, 5 mM; TEA, 5 mM; pH 3.5) (for details see text).

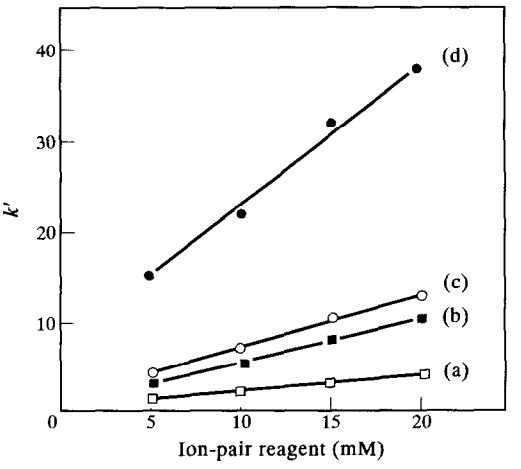

Figure 3
Relationship of k' for each of the three degradation products (cf. Fig. 1) with ion-pair concentration (acetonitrile concentration, 18% v/v; phosphate buffer pH 3.5; TEA, 5 mM).

At this stage it was concluded that the optimum eluent composition giving adequate resolution of all the detectable decomposition products was: acetonitrile–KH_2PO_4 (0.1 M; pH 3.5) (18:82,% v/v) containing 5 mM heptane sulphonic acid and 5 mM triethylamine, at 1 ml in^{-1}.

Studies on degradation products

Using TLC, the number of detectable degradation products was confirmed to be four. Preparative LC was used to isolate sufficient quantities of each degradation product in order both to obtain a UV-spectrum, and to further examine each by HPLC in order to correlate the results of the two methods (Table 1). As is evident from Fig. 4, the UV-spectra are closely similar (λ_{max} = 343 nm) at pH 3.5, indicating that the fundamental chromophore had remained intact.

To further examine the nature of the decomposition products, fluorescence spectra were

Table 1
Comparison of HPLC and TLC data for photodegradation products in sample of irradiated sample of chloroquine phosphate. The k' values, K'_R values (i.e. capacity ratio expressed relative to that of chloroquine) and R_f values were obtained using conditions described in the text

Product no.	k'	K'_R	R_f
a	1.5	0.34	0.12
b	3.2	0.727	0.28
c	4.4	1.000	0.4
d	15.1	3.43	0.8
e	27.6	6.27	0.97

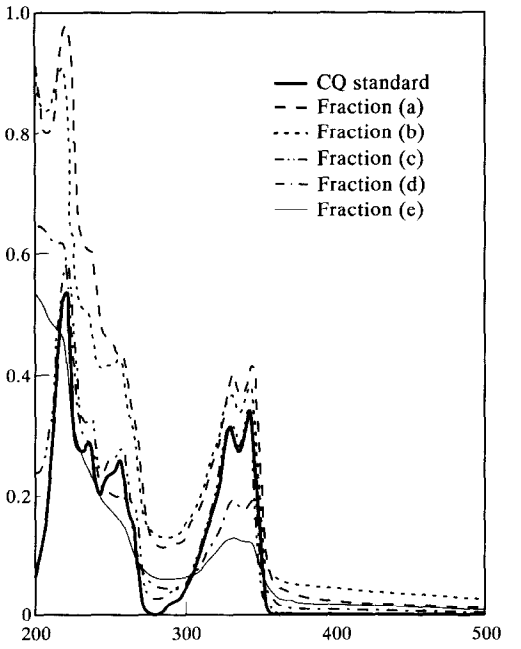

Figure 4
UV spectra in phosphate buffer (pH 3.5), of chloroquine reference and of each degradation product obtained by preparative TLC. Key: ———, chloroquine phosphate reference; — — —, fraction (a); - - - fraction (b); — · — · — · —, fraction (c) (chloroquine); - · - fraction (d) ——— fraction (e).

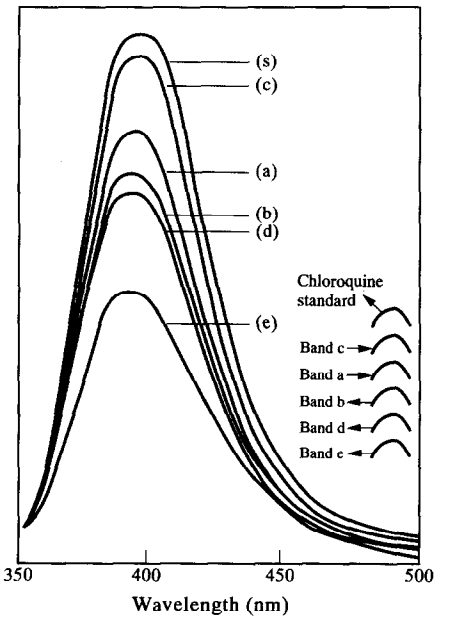

Figure 5
Fluorescence spectra in phosphate buffer (pH 3.5) of chloroquine reference standard and peaks extracted by prep. TLC. Key: a, b, d, e correspond to degradation products in Fig. 1; c is residual chloroquine after photolysis; s represents the chloroquine reference standard.

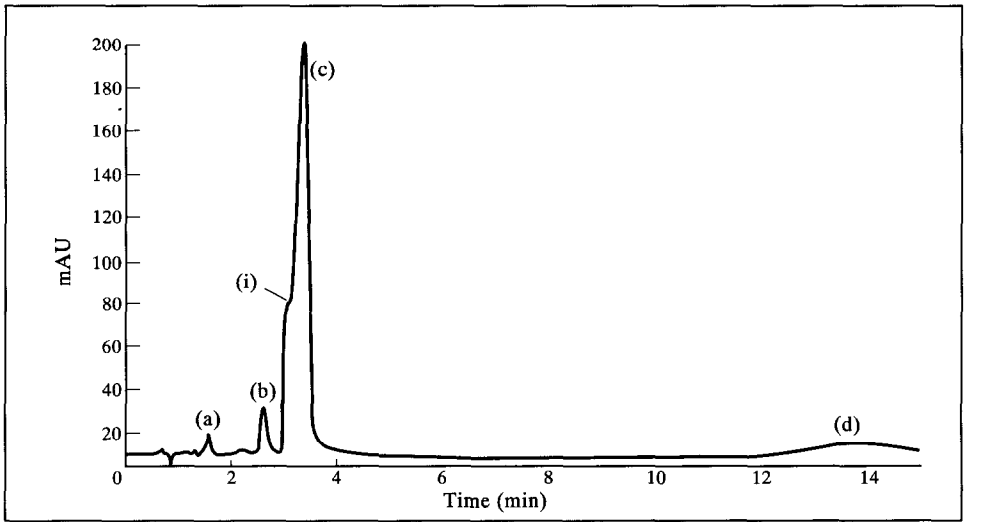

Figure 6
Analysis of chloroquine sample (cf. Fig. 1) after photodegradation, analysed by chromatographic system B. Key: a, b, d, degradation products; i, inflection on peak; c, chloroquine. Peak e in Fig. 1 was detected but is not displayed.

obtained in phosphate buffer (pH 3.5) for each (Fig. 5). In this experiment also, the emission spectra were closely similar (λ_f = 400 nm), confirming the structural similarities of the fluorophores concerned.

In order to further examine the nature of the degradant peaks, HPLC system B was employed to generate spectrochromatograms of the sunlight-degraded sample from Sudan (Fig. 6). However, interpretation of these data indicated that a fifth degradation product was present, as is evident from the three-dimensional plot (Fig. 7a) and the contour plot (Fig. 7b). Comparison of the chromatograms (Fig. 1 vs Fig. 6) indicates the presence of a shoulder on the leading edge of the chloroquine peak. This was confirmed by transforming this chromatogram to the second derivative in the time domain, when the shoulder on the leading edge of the chloroquine peak (c) could be clearly seen. That this was not detectable by system A is attributable to the difference in performance of system A (N = 18,450 plates m^{-1}) and B (N = 22,260 plates m^{-1}) for chloroquine due to the smaller flow cell (4.5 μl) in B than in A (8 μl).

Once again it was evident that the spectra for all detectable peaks were closely similar. However, spectral normalization (Fig. 8) indicated that the spectrum of the leading edge component (i) on peak C was slightly different from the spectra respectively at the apex and on the trailing edge.

Photodegradation studies

Chloroquine is observed to gradually change in colour on exposure to light. This photolability [13, 14] has been observed for other related antimalarials. Quinine is known to undergo a rapid photoreduction when irradiated in concentrated acid. However, in dilute acid quinine mainly gives a polymeric product [14]. A quinoline dimer (2,2-biquinolyl) has been isolated when pure quinoline was irradiated [15]. Irradiation of a number of N-heterocyclic compounds in neutral ethers, alcohols and organic acids has been found to lead to a general substitution reaction on C_2 and C_4 [16, 17]. These reactions are thought to occur [16, 17] through an intermediate radical.

Chloroquine in aqueous solutions is expected to undergo similar reactions on irradiation to give a dimer. This could either be through association of two quinoline nuclei at C_2 or through the substitution of the alkyl chain of one molecule on C_2 of the other. A dimer through association of two amino alkyl chains would also be anticipated, since irradiation of amines is observed to give diamino compounds through dehydrogenation of the carbon atom linked to the amine [15].

These products are expected to be less polar than chloroquine and in fact compounds that move faster than chloroquine on TLC (Table 1) were obtained for chloroquine solutions exposed to sun. Products of shorter R_f values were also obtained. HPLC has also shown the

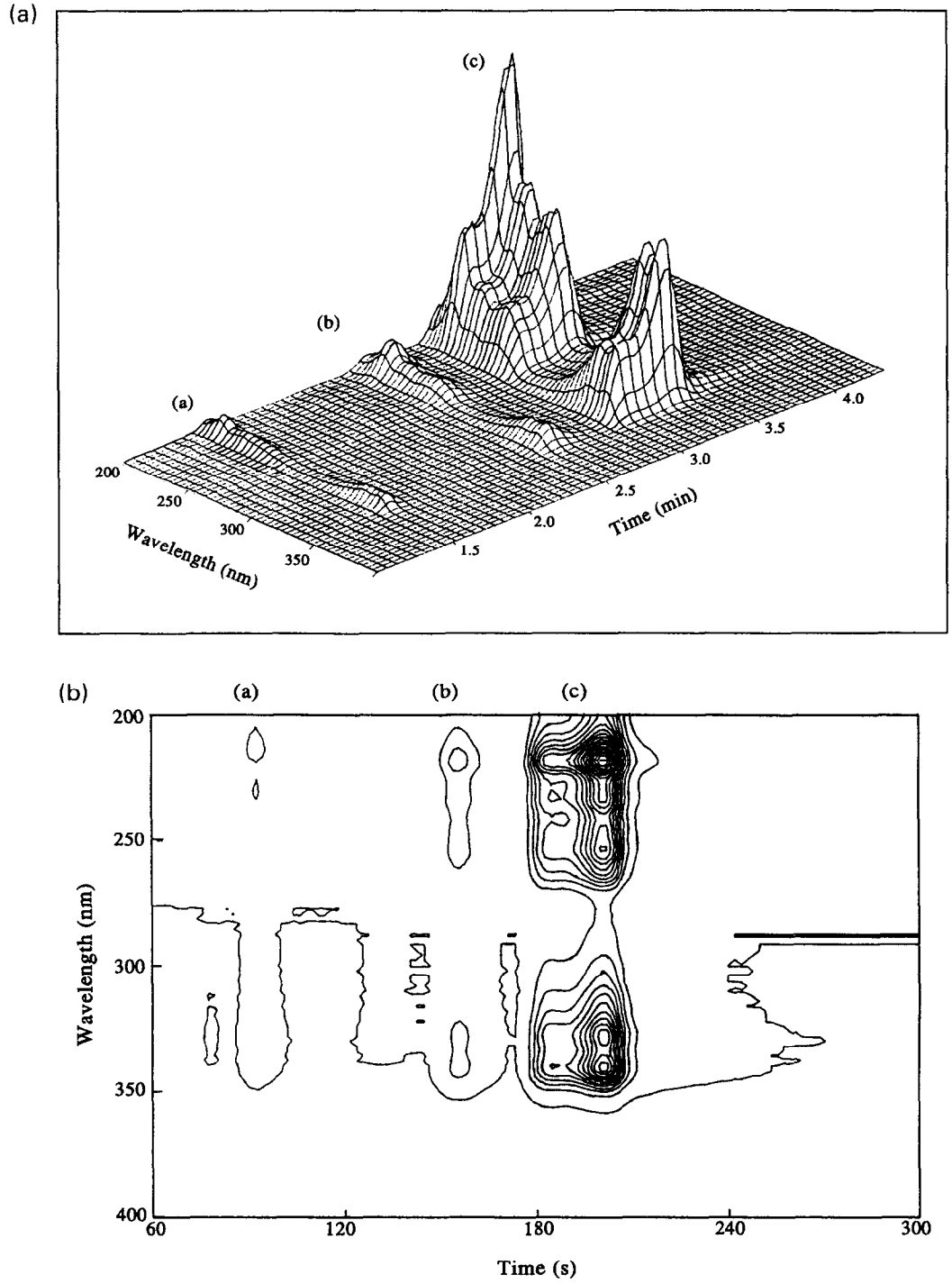

Figure 7
(a) Spectrochromatogram of degraded chloroquine sample obtained by photodiode array detection, showing degradation products a, b, and residual chloroquine, c. (b) Contour plot showing distorted contours associated with peak c, indicating lack of homogeneity.

presence of decomposition products which are eluted at longer retention times than chloroquine (Fig. 1). Peaks d and e are very broad and they constitute the major degradation products, judging by their peak areas. Peak (b) was shown to co-elute when injected with added desethyl-chloroquine. This suggests that chloroquine is de-ethylated to a small extent on irradiation.

At pH 5 chloroquine is little affected by

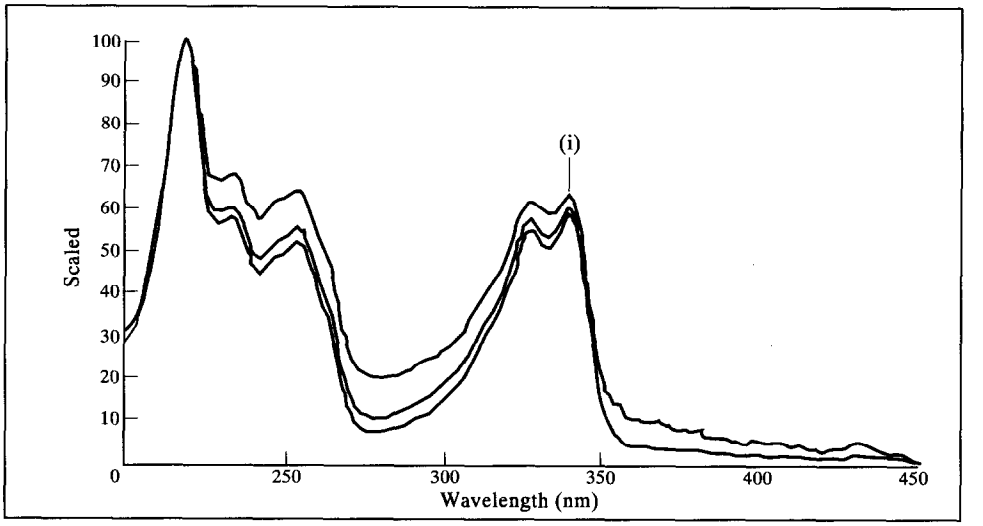

Figure 8
Normalized spectra for peak (c) taken at apex, mid-point of trailing edge, and at inflection point, i.

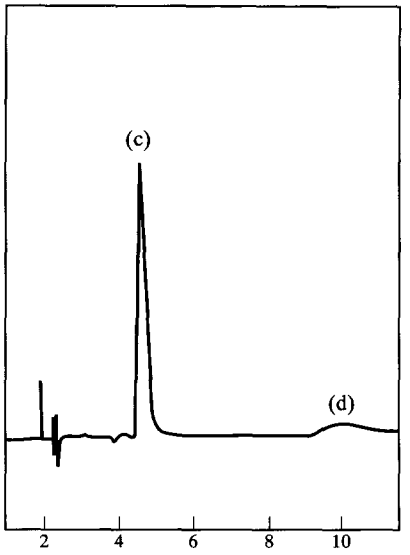

Figure 9
HPLC analysis of chloroquine sample irradiated at pH 5 for 4 h. Key: c, chloroquine; d, degradation product.

irradiation. The chromatogram of the solution showed a small amount of component d (Fig. 9). A considerable decomposition (~50%) was observed for a solution of chloroquine at pH 9 when irradiated for 2 h. At pH 9 the chromatogram of the irradiated solution revealed a number of products, eluting at very short retention times. However, no peaks were obtained at retention times longer than chloroquine.

The pattern of chloroquine degradation was found to be different depending on the solvents used. The rate of photodegradation of chloroquine in phosphate buffer at pH 9 yielded 60% degradation on irradiation for 4 h. A similar rate was found in the chloroform extracts. This has serious implications for accuracy in the context of analysing chloroquine in biological samples, where multi-extraction processes involving high pH and organic solvents are generally used.

In the presence of nitrogen the rate of photodegradation of chloroquine was found to increase by 30% relative to that in the presence of oxygen. The lower photodegradation of chloroquine in the presence of oxygen has been commented upon previously [17] in studies on the mechanism of photoreaction of quinoline derivatives.

Assuming that first-order or pseudo first-order kinetics might apply to the photodegradative loss of chloroquine, a plot of log A_t vs t was found to be linear, where A_t is the absorbance (i.e. peak height) at any time t, representing the amount of chloroquine remaining, A_0 is the initial concentration of chloroquine in relative units and k is the first-order rate constant (s^{-1}). Linear regression yielded the equation:

$$\log A_t = 0.702 - 8.12 \cdot 10^{-5} t$$

from which the first-order rate constant is $k = 1.88\ 10^{-4}\ s^{-1}$, under these particular experimental conditions at pH 9.0.

Analysis of chloroquine at different wave-

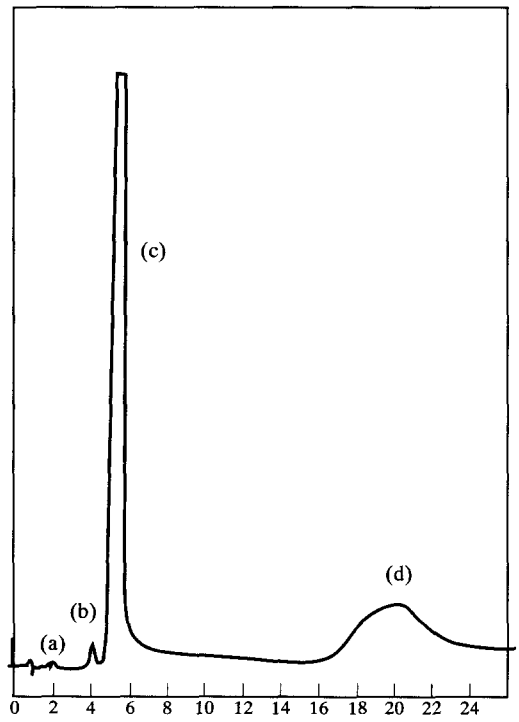

Figure 10
Chromatogram of chloroquine in ampoule after storage under shelf conditions at *ca* 30°C for 1 year.

lengths (200, 254, 272 and 343 nm) using the photodiode array detector system B did not reveal any new peaks, except for the leading edge inflection. The UV spectra of the detectable decomposition peaks were very similar to that of chloroquine, indicating structural similarities. This highlights another source of error when relatively non-specific methods (e.g. UV, fluorescence) are used for the analysis of chloroquine in the presence of its degradation products in field studies in tropical countries. Moreover, routine HPLC methods for analysing chloroquine may also suffer from these problems, unless special measures are taken [9].

By way of illustrating the utility of the HPLC method developed, ampoules of two brands of chloroquine were analysed. This showed that one of the brands studied contained a considerable amount of the major photodecomposition product detected (peak d, Fig. 10). These ampoules had been stored for *ca* 12 months in a pharmacy in Khartoum under shelf conditions at ambient temperature (*ca* 30°C). This confirmed the necessity to store these injections at +4°C in the dark.

These studies illustrate the need for careful control of storage conditions for chloroquine intended for local use in tropical climates. The methods developed permit satisfactory control to be exercised, whilst at the same time allowing the degradation products to be subjected to further study. Further work in progress is directed towards the characterization of these degradation products, and the assessment of the antimalarial activities of chloroquine preparations stored under various tropical conditions.

References

[1] *British Pharmacopoeia* pp. 103 and 746. HMSO, London (1980).
[2] D.D. Hong, *Chloroquine Phosphate*, in *Analytical Profiles of Drug Substances* (K. Florey, Ed.), p. 62. Academic Press, New York (1976).
[3] G. Urban and W.A. Behrendt, *Arch. Exptl. Pathol. Pharmacol.* **238**, 140–142 (1960).
[4] G. Urban and W.A. Behrendt, *Proc. Intern. Congr. Photobiol.*, Copenhagen, 591–593 (1960).
[5] W.M. Sams and N.V. Carroll, *Arch. Dermatol.* **93**, 123–128 (1966).
[6] J.A. Owoyale and Z. Elmarakby, *Int. J. Pharm.* **12**, 265–269 (1982).
[7] G. Mulakozi, I. Edafiogho, A.M. Mulakozi and A. Diete-Spiff, *J. Therm. Anal.* **32**, 1139–1143 (1987).
[8] V. Das Gupta, *Anal. Lett.* **19**, 1523–1532 (1986).
[9] A.N. Abdelrahman, E.I.A. Karim and K.E.E. Ibrahim, *J. Pharm. Biomed. Anal.*, in press.
[10] Y. Bergqvist and M. Frisk-Holmberg, *J. Chromatogr.* **22**, 119–127 (1980).
[11] B.J. Clark, A.F. Fell, H.P. Scott and D. Westerlund, *J. Chromatogr.* **286**, 261–273 (1984).
[12] J.S. Kiel, S.L. Morgan and R.K. Abramson, *J. Chromatogr.* **320**, 313–323 (1985).
[13] G.C. Kyker, M.M. McEwen and W.E. Cornatzer, *J. Biol. Chem.* **162**, 353 (1945).
[14] V.I. Stenberg, E.F. Travecedo and W.E. Musa, *Tetrahed. Lett.* **25**, 2031–2033 (1969).
[15] K. Pfordte and G.- Leuschner, *Ann.* **646**, 25–30 (1961).
[16] A. Castellano and A. Lablache-Combier, *Tetrahedron* **27**, 2303–2315 (1971).
[17] F.R. Stermitz, C.C. Wei and C.M. O'Donnell, *J. Amer. Chem. Soc.* **92**, 2745–2752 (1970).