CHEMICAL ASSAY AND PHARMACOKINETIC BEHAVIOUR OF FOUR BRANDS OF CHLOROQUINE TABLETS

BY

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A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL AHMADU BELLO UNIVERSITY, ZARIA, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN PHARMACOLOGY

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DECLARATION

I hereby declare that the work reported in this thesis was carried out by me under the joint supervision of Dr. Ibrahim Abdu-Aguye and Dr. Iliyasu Mohammed. It has not been presented in any previous application for higher degree. The work of other investigators are acknowledged and referred to accordingly.

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CERTIFICATION

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Dedicated to My Dear Parents and Loved One

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ABSTRACT

The amount of active ingredient in four brands, of chloroquine tablet were determined by the chemical assay method as described in B, P, (1980) for both chloroqulne phosphate and sulphate tablets. The results (except for one brand) complied favourably with stated amount:; in the monogram for chloroquine content (92.5-107.5%). The exceptional brand was found to contain 58.31 per cent of the labelled amount of chloroquine.

Pharmacokinetic behaviour of the four brands of chloroquine tablets was studied in 25 healthy adult volunteers. The H.P.L.C. assay as described by Qgunbona *et* al. (1986) was adapted for the estimation of plasma choloroquine concentration. Area under the plasma concentration-time curve for the firet 6 hours AUC^{o-sbr} was calculated by the trapezoidal rule. Pharmacokinetics of chloroquine in individual volunteers was calculated from parameters generated by $^{H}KINBMP^{M}$, a modified version of SIKP as described Johnston (1985). The mean parameters determined from these data wore compared by Student t test.

The absorption of all the brands was rapid with a lag time ranging from 0 to 7.3min and t_{max} occurring between 2,1 to 4h and o_{mftM} ranging from 111.3 to 583.1 ng/ml. There was no significant difference in the mean lag times but **the** c_{max} and $AUC_{(0-8h)}$ of the substandard brand differed significantly from the others. Furthermore, analysis of the AUCs revealed that none of the four brands was bioequivalent to the other during the first 8h of the study and only two could be considered to be bioequivalent at the end of the study.

The data suggest that a few of the drugs in use in Bigeria may be Mfaked" and could contribute to therapeutic failures and other problems such as the emergence of resistance and dictate the **need** for more stringent control of the drug scene. However, the purity of the active ingredients in the brands of chloroquine used in this study is assured by the basic similarity in most pharmacokinetic parameters.

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ABBREVIATIONS

B. P.	=	British Pharmacopoeia
CQ	=	Chloroquine
g	=	Gramme
µg	=	Micogramme
mg	=	Milligramme
ml	=	Millilitre
min	=	Minute
h	=	Hour
HCl	=	Hydrochloric acid
kg	=	Kilogramme
L	=	Litre
NaOH	=	Sodium Hydroxide
HPLC	=	High Performance Liquid Chromatography
µL	=	Microlitre
uv	=	Ultra-violet

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 HISTORY AND CHEMISTRY

Malaria is one of the most prevalent of human diseases in the Tropics; in the past this was so in parts of the Temperate Regions. In the early writings in Egypt, India and China it is one of the oldest infections mentioned. Its clinical symptoms were fully described by Hippocrates 400 years before the christian era (Bruce-Chwatt *et al.*, 1986).

Several unsuccessful attempts at treating this disease were made using flowers, leaves and roots of many plants. In China for at least 2000 years Qing haosu (*Artemisia annua*) was used. Although this had some undoubted medicinal effects, the first potent remedy against malaria was discovered only in the 17th century following the contact of Europe with the New World. This remedy was found in the bark of "fever tree" that grew on the mountainous slopes of the Peruvian

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Andes. This tree was later to be known as Cinchona in 1749 (Bruce-Chwatt *et al.*, 1986).

In 1820 two French chemists Pierre Pelletier and Joseph Caventou isolated two basic alkaloids of Cinchona - Quinine and Cinchonine. Subsequently various salts of quinine were manufactured on a wide scale in many parts of the world for the treatment of malaria. The demand for the drug became in excess of the supply especially during the civil war in the United States of America, resulting in the search for synthetic antimalarials.

In 1920 the first synthetic antimalarial was produced as an 8-aminoquinoline (Pamaquine). This was found to be highly active against avian *Plasmodia* but not human malaria. It had a limited action on the asexual forms of *Plasmodium falciparum* and toxicity that was by no means negligible. So the search for better quinine substitutes continued.

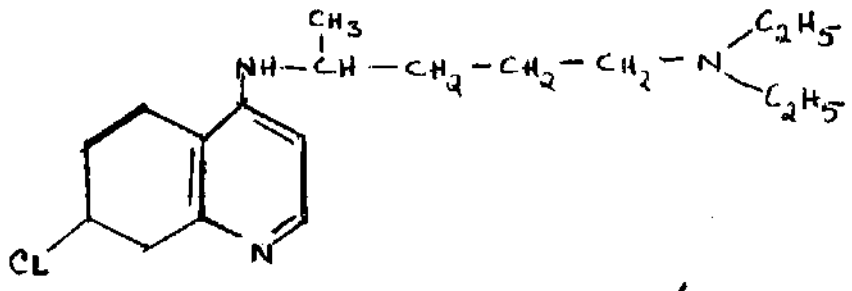
In 1932, Kikuth announced the discovery by Mauss and Mietzsch of a series of compounds synthesized by attaching the basic side chain evolved for pamaquine to other heterocyclic compounds. The quinoline ring was replaced by acridine - a yellow dye - and one of the compounds

of the series so synthesized was mepacrine. This drug was considered to have rather serious side effects. And this made it unpopular.

Then came 1934 and chloroquine was synthesized as Resochin by Andersag. By changes in the basic side chain attached in position 4 of the quinoline nucleus (6-methoxyquinoline) of the cinchona alkaloid. It was then used as an antimalarial just before the Second World War (Bruce-Chwatt *et al.*, 1986).

Subsequently, collective and co-ordinated extensive clinical studies done in the United State (U.S.A.), Germany and by the French in 1944, pointed to chloroquine as an outstanding antimalarial drug faster in therapeutic action than any other antimalarial drug then known. It was found to be highly effective against the asexual blood stages of all species of malarial parasites and an active gametocytocidal agent against *Plasmodium vivax, ovale, malariae* and immature forms of *falciparum* (Bruce-Chwatt *et al.*, 1986).

STRUCTURE



7-Chloro-4-(4'-diethylamino-1'-methylbutylamino) quinoline.

1.2 PHARMACOLOGY

1.2.1 Clinical Uses

Following the publication of a statement approved by the board for coordination of malaria studies affirming its therapeutic potentials in malaria (Loeb *et al.*, 1946) chloroquine was introduced into therapeutics shortly after the second world war.

Since its introduction, chloroquine has been the drug of choice for therapy of this disease. This is largely because it is cheap, safe widely available and until recently highly efficacious in the treatment of the three most important species of malaria parasites.

Apart from its antimalarial activity, chloroquine has been used extensively in the treatment of hepatic amoebiasis and rheumatoid arthitis (Freedman, 1960). Other uses include treatment of discoid lupus erythematosus, photoallergic reactions and as an anti-arrhythmic alternative to quinine. Giardiasis and Clonorchiasis have also been treated with the drug (Rollo, 1980a and 1980b).

1.2.2 MODE OF ACTION

Both the antimalarial and antibacterial actions of chloroquine is said to be by inhibition of DNA replication and RNA biosynthesis (Bowman and Rand, 1984). This drug inhibits DNA polymerase enzyme markedly; this is brought about by interference with the parasites ability to digest haemoglobin which deprives it of essential amino acids and eventually leads to interference with DNA replication (Krogstad and Schlesinger, 1987).

1.2.3 SIDE EFFECTS AND TOXICITY

This is minimal at doses employed for therapy or suppression. It is not unusual to have abdominal discomfort nausea, vomiting, headache., dizziness, blurring of vision, mental and physical weakness, fatigue and pruritus after a single therapeutic dose. Severe adverse reactions are rare. These include cardiovascular collapse, dyskinesia, neuromuscular disorders and hearing loss. These however disappear if the drug is stopped except dyskinesia and hearing loss that persist (Salako, 1984).

Children are more susceptible to the cardiovascular effects of this drug especially after parenteral administration.

1.2.4 PARASITE RESISTANCE

Barely a decade after its introduction in malaria chemotherapy, chloroquine resistant strains of *Plasmodium falciparum* started developing in different parts of the world. It was first suspected in Thailand in 1957 and found in patients in Colombia in 1959 (W.H.O., 1967). Since then it has been spreading. Strains of the parasite resistant to chloroquine have been reported from more than forty countries in South America, East and Central Africa, India subcontinent, Southeast Asia, the Malay archipelago and Oceania (Centre for Disease Control, 1985)

In Africa, strains of *Plasmodium falciparum* in Ethopia, the Sudan and possibly Nigeria are somewhat less sensitive to chloroquine although they still respond to the normal therapeutic doses (Salako, 1987). Chloroquine resistant *Plasmodium falciparum* was first reported from East Africa

(Fogh *et al.*, 1976).This was confirmed in 1979 in a traveller returning from Tanzania to the United States who had blood isolates of the parasite showing a high degree of resistance *in vitro* (Campbell *et al.*, 1979). Since that time resistant strains have been reported in nations of East and Central Africa, Cameroon in 1985 (Centre for Disease Control, 1987), Gambia (Menon *et al.*, 1987) and Benin (LeBras *et al.*, 1986) and South Eastern Nigeria (Ezedinachi, 1987) all in West Africa. Occasional resistance of *Plasmodium falciparum* to chloroquine has also been reported from Madagascar and Zambia, but confirmation is pending (Centre for Disease Control, 1986). Quite recently *in vitro* sensitivity studies of *Plasmodium falciparum* to some antimalarials were carried out in Kinshasha. Results showed high prevalence of resistance to both chloroquine and pyrimethamine (Nguyen-Dinh *et al.*, 1987).

In West Africa, *Plasmodium falciparum* is still largely sensitive to chloroquine and despite increasing evidence of resistance in the continent, this drug remains the mainstay of antimalarial treatment for most of the continent. In Zaria *in vivo* sensitivity studies of *Plasmodium falciparum* to chloroquine showed no evidence of

resistance to the drug (Midala *et al.*, 1988). Malawi in 1984, after assessing the efficacy of chloroquine in 224 children adopted chloroquine as the primary therapy for malaria (Khoromana *et al.*, 1984). Outside the continent, the drug is still largely used in places like Haiti, Central and South America, where in most cases it is administered with or without primaquine once or twice monthly to a large segment of the population (Mass drug administration) in malaria control programmes (Centre for Disease Control, 1986). On a global basis, this drug is still largely recommended for malaria control. The World Health Organisation (W.H.O., 1979) in recognition of the differences in malaria control programme objectives, proposed four general objectives known as Tactical Variants for Malaria Control Programmes. As part of Tactical variant I, it recommended that in a partially immune population, a single dose of a 4-aminoquinoline drug may be sufficient but its effectiveness should be confirmed by *in-vivo* studies in different areas of the country (Centre for Disease Control, 1986).

1.3 PHARMACOKINETICS

1.3.1 Absorption.

Chloroquine is rapidly and almost completely absorbed from the intestinal tract. The drug is detectable in plasma within 30 minutes of oral ingestion. The plasma concentration reached in the first 30 minutes after oral administration of 10mg/kg dose of the drug is usually greater than the therapeutic level for sensitive *Plasmodium falciparum* (30µg/l) (Adelusi *et al.*, 1982; Walker et *al.*, 1983a; Gustafsson et *al.*, 1983a).

Adelusi *et al* (1982) after administering chloroquine, 10mg/kg body weight, to healthy adult volunteers and children with malaria found the drug to have an absorption half life of 0.56 hour and achieved a peak plasma concentration of 250µg/l in 2 hours. Similar studies done using 300mg of chloroquine in both solution and tablet forms in healthy volunteers showed a peak plasma conentration of 55-94ng/ml (mean 73ng/ml) in 1.5 to 6 hours and 56-102ng/ml (mean 76ng/ml) in 1 to 6 hours respectively (Gustafsson *et al.*, 1983a). In another study involving children being treated

for malaria with chloroquine (10mg/kg body weight) peak plasma concentrations between 62 and 263ng/ml were obtained in 1 to 8 hours. Mean plasma concentration after ½h was 67.7 ±46.8ng/ml (Walker *et al.*, 1983a). Studying single dose kinetics of the drug and its major metabolite desethylchloroquine (DECQ) in healthy subjects, chloroquine was found to have plasma peak concentration occuring in 6 hours Frisk-Holmberg *et al.*, 1984). On using whole blood after administering 620mg of chloroquine base, peak concentrations of 1058-1313ng/ml were obtained in 1 to 2 hours and an initial rapid decline in plasma concentration was observed between 5 and 24 hours (Adjapon-Yamoah *et al.*, 1986).

Administered intramuscularly in rats using 10g/kg body weight, peak plasma concentrations were obtained in 30 minutes (Salako and Adelusi, 1983). In healthy volunteers however, after administering 200mg of the drug intramuscularly, there was rapid absorption with an average peak plasma concentration of 539.26± 134.9ng/ml occuring at a mean time (t_{max}) of 0.13± 2.13 hours. On co-administration with 4mg chlorpheniramine intramuscularly, these values

were 513.36 ± 159.2ng/ml and 0.16 ± 3.2 hours respectively (Ogbuokiri, 1988).

Peak plasma concentration of 478 to 1200ng/ml chloroquine were obtained in 5 to 15 minutes (mean 12min) after intravenous administraion of 300mg of the drug (Gustafsson *et al.*, 1983a).

1.3.2 DISTRIBUTION

Chloroquine is extensively bound in tissues being concentrated in lysozomes and selectively in melanin containing organs in the body like the skin and retina. It is also concentrated in malaria infested erythrocytes. Ratios of 21:1 erythrocyte to plasma concentrations was obtained in children with acute malaria at the height of parasitaemia. This however fell to a steady value of about 5.3:1 four days after parasitaemia had completely disappeared (Adelusi *et al.*, 1982). Other cellular elements of blood like platelets and granulocytes also concentrate chloroquine more than erythrocytes. (Bergquist and Domeij-Nyberg, 1983). In healthy volunteers, erythrocytes concentrations 2 to 5 times higher than that of

plasma have been found (Frisk-Holmberg *et al.*, 1984). The highest concentrations of the drug was found in the lungs and the lowest concentrations in the heart of rats after administration of the drug (Funmilayo and Ajayi, 1986). Chloroquine also concentrates in tissues with a developed reticuloendothelial system. Whole blood concentrations are usually 8 to 10 times greater than the plasma concentrations (Frisk-Holmberg *et al.*, 1984). By virtue of its extensive affinity for tissues, redistribution from the periphery to plasma is the rate limiting step for the elimination of the drug from the body thus implying a multicompartment model for chloroquine kinetics.

Chloroquine is 46 to 70% (mean 61± 9%) bound to protein and this is mainly to the albumin and α_1- acid glycoprotein fractions. This binding is thought to be an important determinant of its pharmacokinetics or response (Walker *et al.*, 1983). *In vitro* studies have shown that chloroquine binds to glass significantly decreasing its availability in samples collected in such containers in concentrations up to forty per cent (40%). This however is not the case with plastics where the binding is very little (Geary *et al.*, 1983).

Chloroquine has an oral bioavailability of over 70%. Gustafsson *et al* (1983a) after administration of 300mg of chloroquine orally in both solution (Malarex® solution, A/S Dumex, Copenhagen, Denmark) and tablet forms (Klorokin tablets, Aco Lakaedel AB, Stockholm, Sweden) obtained mean bioavailability values of 78% and 89% respectively.

As a result of its extensive binding to tissues such as spleen, kidney and liver with concentrations of up to 300 to 500 times that of plasma (Berliner *et al*, 1948; McChesney and McAuliff, 1961 and McChesney *et al*, 1966), distribution and binding, it has a large volume of distribution about 200 times total body fluid volume, and values ranging from 111 to 285L/kg were similarly calculated after single doses of the drug intravenously (Gustafsson *et al.*, 1983a). A mean value of 59± 24L/kg was found using whole blood (Adjepon-Yamoah *et al.*, 1986).

1.3.3 ELIMINATION

Metabolism is by side chain de-ethylation leading to formation of the main metabolite desethylchloroquine (Gustafsson *et al.*, 1983a;

McChesney *et al.*, 1966; Fletcher *et al.*, 1975). This metabolite usually has a peak concentration about the same time as the parent drug (Walker *et al.*, 1983a; Gustafsson *et al.*, 1983a), but the blood concentrations are generally higher than those of the parent drug after 48 hours (Adjepon-Yamoah *et al.*, 1986). Apart from exhibiting the same profile of distribution and tissue binding (Fletchner *et al*, 1975) this metabolite also has similar antimalarial activity against chloroquine sensitive *Plasmodia* as the parent drug (Verdier *et al.*, 1984). Individual variations do exist in the pattern of distribution of chloroquine and its main metabolite desethylchloroquine. Subjects prone to chloroquine induced pruritus usually have a higher concentration of the drug and a lower concentration of the metabolite in their skin than other subjects (Olatunde, 1971).

Elimination of chloroquine from the body is a very slow process. Tissue elimination is much slower than that of plasma thus simulating separate compartments (Adelusi and Salako, 1982a, 1982b). Plasma half-life (t½) varies between 3 days (Adelusi *et al.*, 1982, McChesney *et al.*, 1962, Walker *et al.*, 1983) and 205 days (Alving *et al.*, 1948). This obviously reflects a

multicompartment distribution. This drug and its main metabolite are detectable in plasma for up to 56 days after a 300mg dose (Gustafsson *et al.*, 1983a).

The main route of elimination is via the kidney. Chloroquine is excreted predominantly in the unchanged form (about 70 percent) (McChesney *et al.*, 1966; Gustafsson*et al.*, 1983a) while the excreted metabolite, desethylchloroquine constitutes about twenty-five percent (25%) of the excreted parent drug. Chloroquine is detectable in urine over a period of 120 days after a dose of 300mg. Its maximum excretion is within the first 24 hours where about 10 percent of administered dose excreted, subsequently urinary excretion decreases exponentially with total 4-aminoquinolines urinary recovery of between 50 percent and 60 percent of administered dose (Gustafsson *et al.*, 1983a).

The total plasma clearance (Clp) is between 750 and 1050ml/min. Renal clearance accounts for more than 50 percent of the total clearance of chloroquine. Gustafsson *et al.*(1983), after administering 300mg of chloroquine base to healthy volunteers obtained a renal clearance of between 400 and 450ml/min. This suggested an active

tubular secretion of the drug into urine. In another study after administering 620mg of chloroquine base orally to healthy volunteers, using whole blood, a mean renal clearance of 83± 20ml/min was obtained. This accounted for more than 60 percent of a mean apparent total clearance of 128± 25ml/min (Adjepon-Yamoah *et al.*, 1986)

As far as disease states are concerned, chloroquine has a longer elimination half-life in patients with renal failure (Salako *et al*, 1984) and it has been shown to have a reduced rate of metabolism in kwashiorkor states (McChesney and Wharton, 1970).

1.4 OBJECTIVE AND SCOPE OF STUDY

It has been stated that over sixty percent of the therapeutic drugs available in Nigeria are fake, adulterated or expired and this in some hospitals is said to be causing increasing number of deaths. This new generation of fake and adulterated drugs includes complately inert or even potentially harmful substances marketed as antibiotics, analgesics, steroids and antimalarial (Lancet, 1988).

A drug is considered as fake where it significantly departs either in name, content, packaging or labelling from the claims being put forward. A variance of this would include "Bad Copies"; such drugs fail to meet established products requirement either in quality or quantity of active ingredients or in either pharmacokinetic profiles (Igboechi, 1988).

There exist variations in the manufacturing processes of different manufacturers and this could only imply that brands of the same drug could contain diferent amounts of the active ingredient even when they meet the official requirement. In a situation where the "principle of *caveat emptor*" (let the buyer beware) cannot operate normally when the layman acquires prescription drugs, this may lead to undesirable effects.

It is the primary responsibility of the manufacturer to ensure that drug quality is reproducible not only within a single batch but also from one batch to another. It was in recognition of this fact that in 1967, the Draft Requirements for Good Manufacturing Practice a.k.a G.M.P guidelines was introduced by the World Health Organisation (W.H.O.). This was an attempt

to increase drug safety and ensure world wide standards of drug quality in areas of purity, content of active ingredients, content uniformity, bioavailability and lastly stability.

Quite recently, the W.H.O. adopted the concept of "Essential Drugs Programme", this refers to drugs which satisfy the health care needs of majority of the population. These drugs should be available at all times, in adequate amounts and in appropriate dosage forms at all levels of the health care delivery system (Akala, 1988). The operative words in the policy are: *safe* and *effective* which imply that these drugs should be of high quality and that this quality should be assured at various levels of the drug delivery system (W.H.O., 1982).

Chloroquine is one of the "Essential Drugs" in Nigeria. It is widely used and is available without prescription in this environment. It comes under diverse names either as single preparation or in combination with other drugs such as paracetamol and vitamins in "fever medicines". This means that a patient could commonly be ingesting a drug containing a large or very inadequate amounts of chloroquine without knowing.

The emergence of chloroquine resistant *Plasmodium falciparium* is increasing in the West African sub-region. Among the many reasons advanced for this is the so-called "drug pressure" whereby the drug is commonly wrongly used (Peters, 1982 and Onori, 1984). This could also be related to quality of chloroquine itself.

This study is designed to investigate the amount, if any, of the active ingredients in four commonly available brands of chloroquine in Zaria. The work will also attempt to determine the differences, if any, in early and late pharmacokinetics of these products.

For this study, the High Perfomance Liquid Chromatograph assay method would be used to determine chloroquine concentrations in plasma and a chemical assay method for determination of chloroquine base as described in British Pharmacopoea (1980) would be adopted.

CHAPTER TWO

MATERIALS AND METHOD

2.1 Drugs

Tablets of four commonly available brands of chloroquine were purchased across the counter. Nivaquine (M & B) was one of these. The batch numbers, dates of manufacture and expiration were recorded. For identification the various brands were labelled Brand I-IV respectively. Table I shows the details of each brand.

2.2 EQUIPMENT

(a) A Varian Model 5000 Liquid Chromatograph fitted with a fixed wavelength (254nm) uv detector. It is fitted with a 10µl loop through a Varian manual loop valve injector. The dectector output was connected to a Varian Model 9176 recorder. A MicroPak MCH-10µm reverse-phase column (300x4nmI.D) was used.

(b) A 100µl syringe

(c) A Whirlmixer (C. P. Ltd., U.K.)

(d) Gallenkamp centrifuge.

Table 2.1

DETAILS OF PROPRIETARY PREPARATIONS OF CHLOROQUINE USED IN THE STUDY

COMPOUND	MANUFACTURER	BATCH Nº	DATE OF MAN.	EXP.DATE	BRAND Nº
Chloroquine-phosphate B.P	EMZOR Chemist Lagos	2228	Feb 1988	Jan 1991	I
"	EMCEEA Ltd., Lagos	EC04	Jan 1987	Nov 1991	II
EVANS chloro-quine phosphate B.P.	GLAXO Nig. Ltd., Lagos	ZA122004	Jan 1988	Jan 1991	III
NIVAQUINE	MAY AND BAYER Lagos	Lot IU 469	-	July 1991	IV

(e) Micropipette of 5,10,20, and 100µl volume capacities.

(f) Plastic 10ml pipette.

(g)Analyticol balance, Type H35 AR Mettler.

2.3 REAGENTS

Soduium Hydroxide pellets - (BDH chemical, Poole, England)

Methanol - (G.P.R, Hopkin and William, Chadwell Health, Essex England)

Hydrochloric acid - Analar grade (BDH Chemicals, Poole, England)

n - Hexane (B.D.H. Chemicals, Poole, England)

Papaverine (Sigma Chemical Co., U. S. A.)

Perchloric acid (" " ")

Sodium dihydrogen phosphate pellets (B. D. H. Chemicals Poole, England)

Blank plasma. - (Ife University Teaching Hospital, Ile-Ife)

Bromocresol green solution (B. D. H. Chemicals, Poole, England

Anaesthetic ether (B. D. H. Chemicals, Poole, England

2.4 SUBJECTS

Twenty-five Normal healthy adult volunteers (2 females, 23 males), aged 21-34 years (mean 23.4 years) old and weighing between 48.7 and 72.3kg (mean 58.52kg) participated in the study.

2.5 EXPERIMENTAL PROTOCOL:

2.5.1 CHEMICAL ASSAY.

For the phosphate tablets, 0.65g of each brand was accurately weighed and dissolved in 30ml of water separately in a conical flask. 3ml of sodium hydroxide solution was added and extraction with successive quantities each of 20ml of anaesthetic ether effected until extraction was complete. The combined ether extracts was washed with successive quantities each of 10mls of water until washing were no longer alkaline to titan Yellow paper. The combined washings were shaken with 25ml of anaesthetic ether, the ether was then added to the combined ether extracts. Ether was evaporated and heating continued until the volume was reduced to about 2.5ml.

50ml of 0.1N Hyrochloric acid was added, warmed to dissolve the base, cooled and the excess acid titrated with 0.1N sodium hydroxide using bromocresol green solution as indicator.

The whole proceedure was repeated without the chloroquine phosphate. The difference between the titrations was taken as the amount of 0.1N Hydrochloric acid (HCl) required by the chloroquine phosphate. Each millilitre of 0.1N HCl difference was taken to be equivalent to 0.02579g of the chloroquine base.

For the sulphate tablet, 0.5g of the tablet was used instead of 0.65g and here each millilitre of 0.1N HCL is equivalent to 0.02090g of the base.

2.5.2 PHARMACOKINETIC STUDIES

Each of the volunteers who claimed not to have taken chloroquine since two months prior to entry gave their informed consent to participate in the study which was approved by the Ethical Committee of Ahmadu Bello University Teaching Hospital, Zaria. The study took place in the Clinical Pharmacology Unit of that hospital.

The subjects were randomly divided into four groups and the groups were further randomly assigned to one of each brand of chloroquine

All subjects fasted overnight and remained so for the first three hours of the study after which a cup of orange squash and 4 pieces of biscuits (TUC) were served. They were allowed any meals of their choice after the fourth hour following the ingestion of chloroquine.

The study was a randomised double blind one. On arrival at the Trial Room, a 21G cannula was inserted in the antecubital vein and 5ml of blood taken (0min). 600mg of the appopriate brand of chloroquine base was then administered orally to each volunteer with about 100ml of water. Further blood sampling via the indwelling cannula was taken at 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8 and 24 hours and then on days 3 and 7 after drug administration. Blood samples were collected into heparinized plastic tubes and immediately centrifuged at 2000g for 10 minutes to obtain plasma samples which were then stored at -20°C in tightly covered plastic containers until analysed.

2.6 HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC ASSAY

2.6.1 CALIBRATION CURVE

A stock solution of chloroquine (100µg/ml) was prepared using distilled water. Out of this a solution was made to obtain 10µg/ml. In perparing the calibration curve, solutions of chloroquine ranging frome 50 to 1000ng/ml were used. To 1ml of blank plasma in extraction tube was spiked the desired chloroquine concentration and 20µl of 5µg/ml of internal standard (papaverine), 1ml of 2N sodium hydroxide 3ml of n-hexane added. Whirlmixing was done for 30 seconds and then centrifuging for 10min at 2000g to separate the two layers. The hexane layer was transfered into a tapered end tube and to this was added 100µl of the mobile phase followed by mixing for 1min and centrifuging at 2000g for 5min. The hexane layer was then rejected and 10µl aliquot of the aqeous phase was then injected into the HPLC. The peak height ratios of chloroquine to internal standard were calculated and this was used for the calibration curve. This HPLC assay is a modified method of that described by Ogunbona *et al*, (1986).

2.6.2 ANALYSIS OF PLASMA SAMPLES

The High Performance Liquid Chromatograph (HPLC) assay method of Ogunbona *et al* (1986) which is specific for chloroquine and its metabolites was adopted. Here in place of 1ml of blank plasma was substituted 1ml of the desired plasma sample and the whole process as for the calibration curve carried out. The peak height ratios of the chloroquine to internal standard were calculated and these used to derive the effective concentrations of the chloroquine base by reference to the calibration curve.

2.7 DATA HANDLING

The results of the chemical assay were carefully recorded. Mean percentage chloroqine content were calculated and compared with official B. P. C. requirements.

Individual plasma chloroquine concentrations for the various brands calculated from the volunteers studies were tabulated and the mean levels for the various brands determined and compared. Appropriate graphs were drawn.

Estimates of C_o, k_e, k_a and t_{lag} were made from a log concentration-time curve and a curve of residuals and submitted to a computer program "KINEMP" (a modified version of SIMP as described by Johnston, 1985) which was used to determine the correct values. The pharmacokinetic parameters of chloroquine in plasma for the first 8hr of the study were then calculated by using these values in the appropriate equations. The program assumed a one-compartment open model with a lag period, i.e

$$C = C_o e^{-\beta(t-t_{lag})} - e^{-\alpha(t-t_{lag})}$$

where

C = concentration at time t,

β = elimination rate constant, and

α = absorption rate constant.

The $AUC_{(0-\infty)}$ was calculated using the trapezoid rule. Mean pharmacokinetic parameters for the various brands were determined and compared for statistically significant differences.

Assuming the mean 8h level to be C_o, the terminal elimination rate constant, ke_r, was determined from the equation:

$$C = C_o e^{-k_e t}$$

from which the mean terminal half-life and $AUC_{(0-\infty)}$ were determined.

The relative bioavailability of chloroquine in the various brands under study was determined by estimating the ratio:-

$$\frac{\text{mean AUC of a particular brand}}{\text{mean AUC for brand IV}} \times 100$$

during the periods (0-8h) and (0-∞). The two relevant brands were considered bioequivalent if the value obtained was between 80 and 120%.

2.8 STATISTICAL ANALYSIS

Student 't' test for paired data and F test was used for analysis and $P > 0.05$ was considered not significant.

CHAPTER THREE

RESULTS

3.1 *IN VITRO* STUDIES

3.1.1 Chemical Assay of Chloroquine Tablets

All the preparations with the exception of Brand II contained between 92.43 and 95.20 per cent of the active ingredient. Brand II contained only 58.31 per cent of the active ingredient. Thus the different brands with the exception of Brand II complied with the B. P. (1980) specification of drug content (92.5 - 107.5 per cent). The detailed values for the drug content and percentage composition are given in Table 3.1.

Table 3.1

QUANTITATIVE DETERMINATION OF CHLOROQUINE BASE IN THE VARIOUS BRANDS OF TABLETS USED IN THIS STUDY

BRAND	COMPOSITION	PERCENTAGE CONTENT OF CHLOROQUINE BASE			
		1	2	3	Mean±S.D.
I	CQ Phosphate	92,20	95,10	92,40	93,23±1,6
II	CQ Phosphate	57,98	58,50	58,45	58,31±0,3
III	CQ Phosphate	95,10	95,23	95,27	95,20±0,001
IV	CQ Sulphate	92,37	92,50	92,42	92,43±0,001

3.2 *IN VIVO* STUDIES

3.2.1 Compliance

Seven of the volunteers did not complete the study, having failed to turn up for all the scheduled blood sampling. Three did not return after the first eight hours of the study. None of the subjects reacted to the drugs used in this investigation.

3.2.2 Calibration Curve

The calibration curve of chloroquine in plasma is shown in Figure 1. It is linear with a correlation coefficient (r) of 0.987. The coefficient of variability at all concentrations used in the curve was less than 5%.

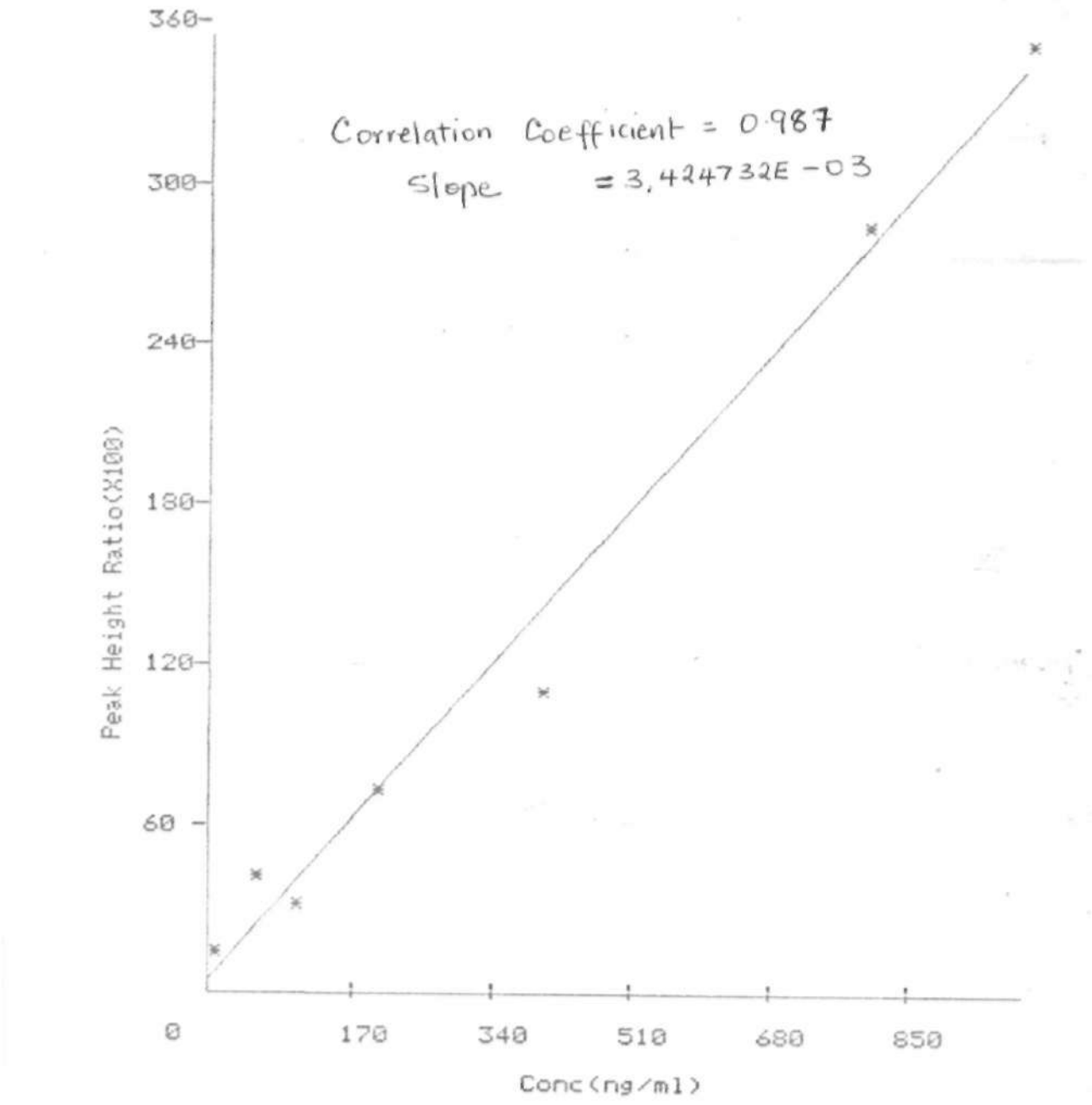


Fig. 1 Calibration curve of Chloroquine in plasma.

3.2.3 Plasma Concentration of Chloroquine

Following the oral administration of 600mg of chloroquine base, mean plasma chloroquine concentrations obtained ranged from 8.4 to 655.9ng/ml. The levels obtained for Brand II were outstandingly lower than those for other brands (Table 3.2). The individual levels obtained are shown in Appendix I.

The log mean plasma concentration-time curve for the entire 168h period of study is shown in Figure 2. It can be seen that chloroquine appears to undergo a biexponential decay within this period. The first phase (β-phase), occurring before 8h seems to fit a one-compartment open model (Figures 3 and 4). The second (z-phase) occurred between 8h and the end of the study (Figure 5).

Table 3.2

The mean plasma concentration of chloroquine resulting from the oral administration of a single 600mg dose of various brands to several volunteers after an overnight fast

Time (hr)	BRANDS			
	I	II	III	IV
0	0,0	0,0	0,0	0,0
0,5	200,1 ± 142,4	13,1 ± 7,8	42,8 ± 13,5	40,7 ± 44,5
1,0	367,2 ± 214,9	61,7 ± 41,4	111,4 ± 58,3	184,5 ± 124,3
1,5	368,9 ± 167,1	72,1 ± 46,2	226,0 ± 11,2	264,4 ± 191,9
2,0	299,8 ± 168,4	99,6 ± 32,9	196,4 ± 83,4	665,9 ± 200,0
2,5	302,9 ± 169,4	65,3 ± 39,2	293,9 ± 84,5	436,3 ± 146,9
3,0	351,9 ± 220,7	59,8 ± 42,3	280,3 ± 104,2	246,8 ± 60,5
4,0	449,2 ± 168,3	75,1 ± 37,3	226,2 ± 31,9	170,8 ± 40,1
6,0	294,8 ± 103,8	67,8 ± 11,8	169,2 ± 42,7	416,8 ± 455,8
8,0	239,8 ± 135,1	147,8 ± 124,6	97,2 ± 31,4	155,1 ± 69,3
24,0	276,9 ± 105,6	33,9 ± 23,4	78,4 ± 43,5	100,7 ± 88,9
72,0	148,3 ± 82,9	41,9 ± 1,4	47,5 ± 11,1	40,1 ± 16,6
168,0	126,0 ± 202,2	8,4 ± 5,8	42,9 ± 19,1	98,2 ± 99,6

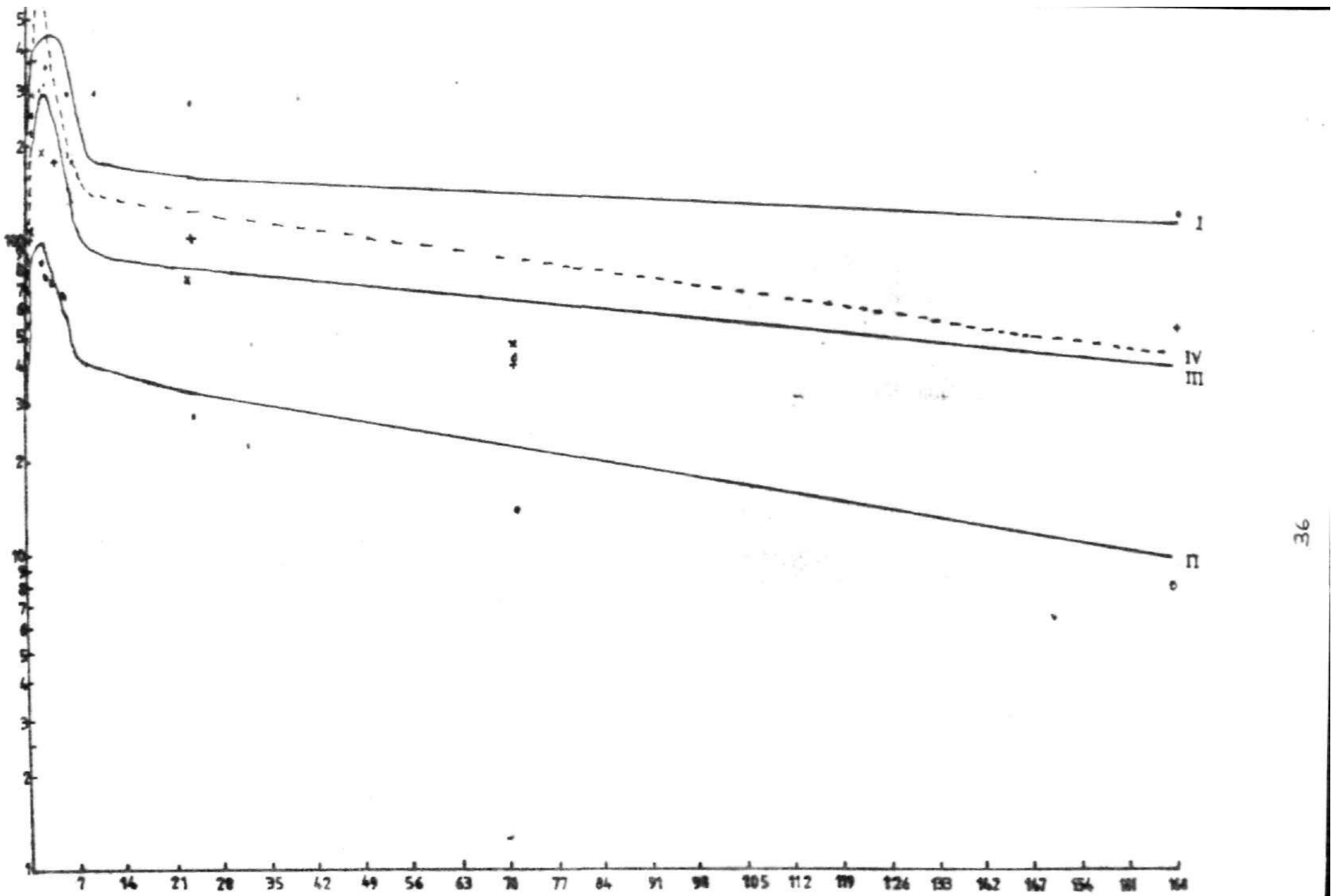


Fig. 2. Log Mean Plasma Chloroquine [redacted]centration-time curve during the period of study.

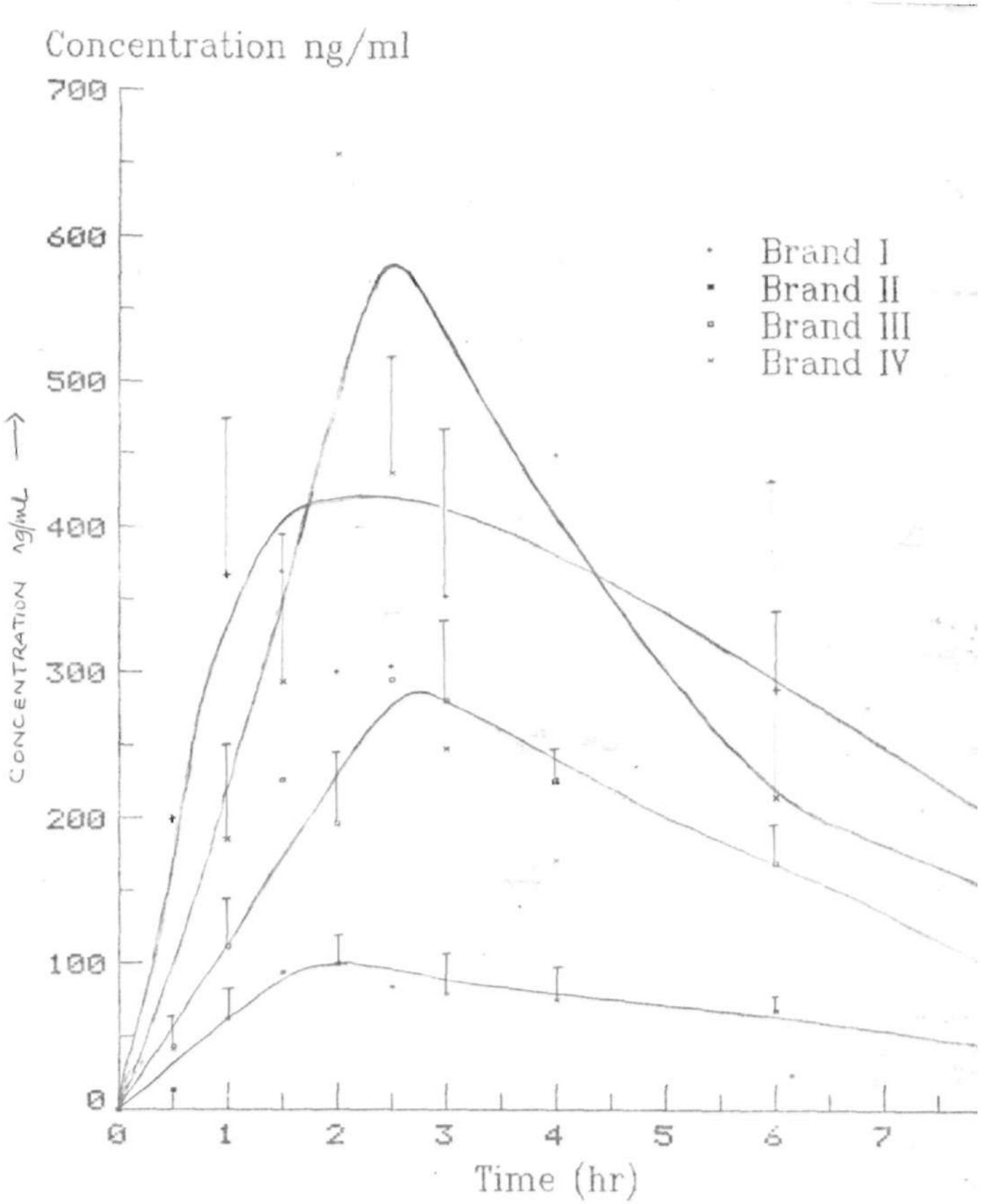


Figure 3 PLASMA CHLOROQUINE LEVELS (Mean ± S.E.M) in healthy volunteers following 600mg Chloroquine base orally (THE FIRST EIGHT HOURS)

Concentration ng/ml

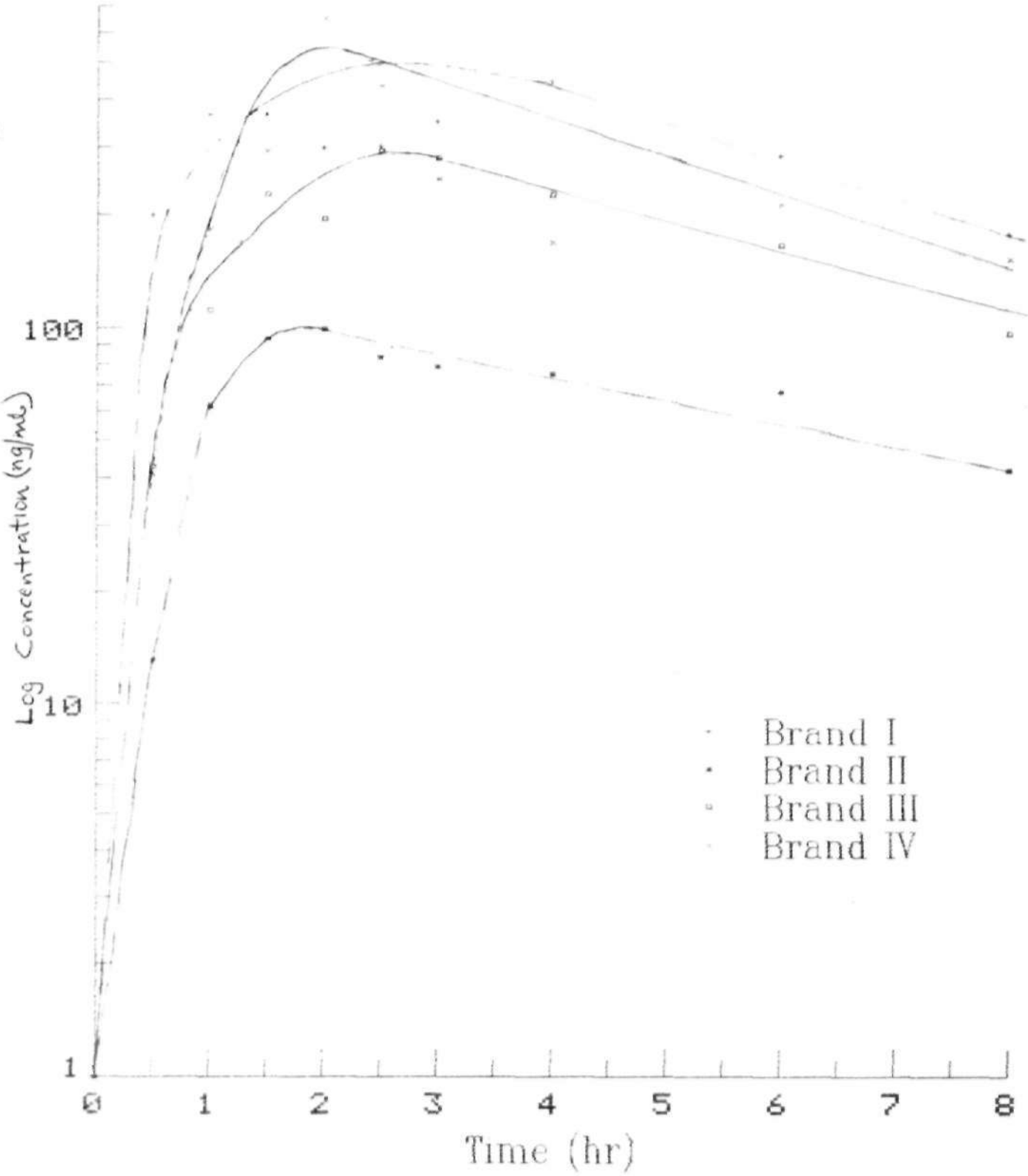


Fig. 4. Log mean plasma chloroquine concentration - time curve for the first 8hr of the study.

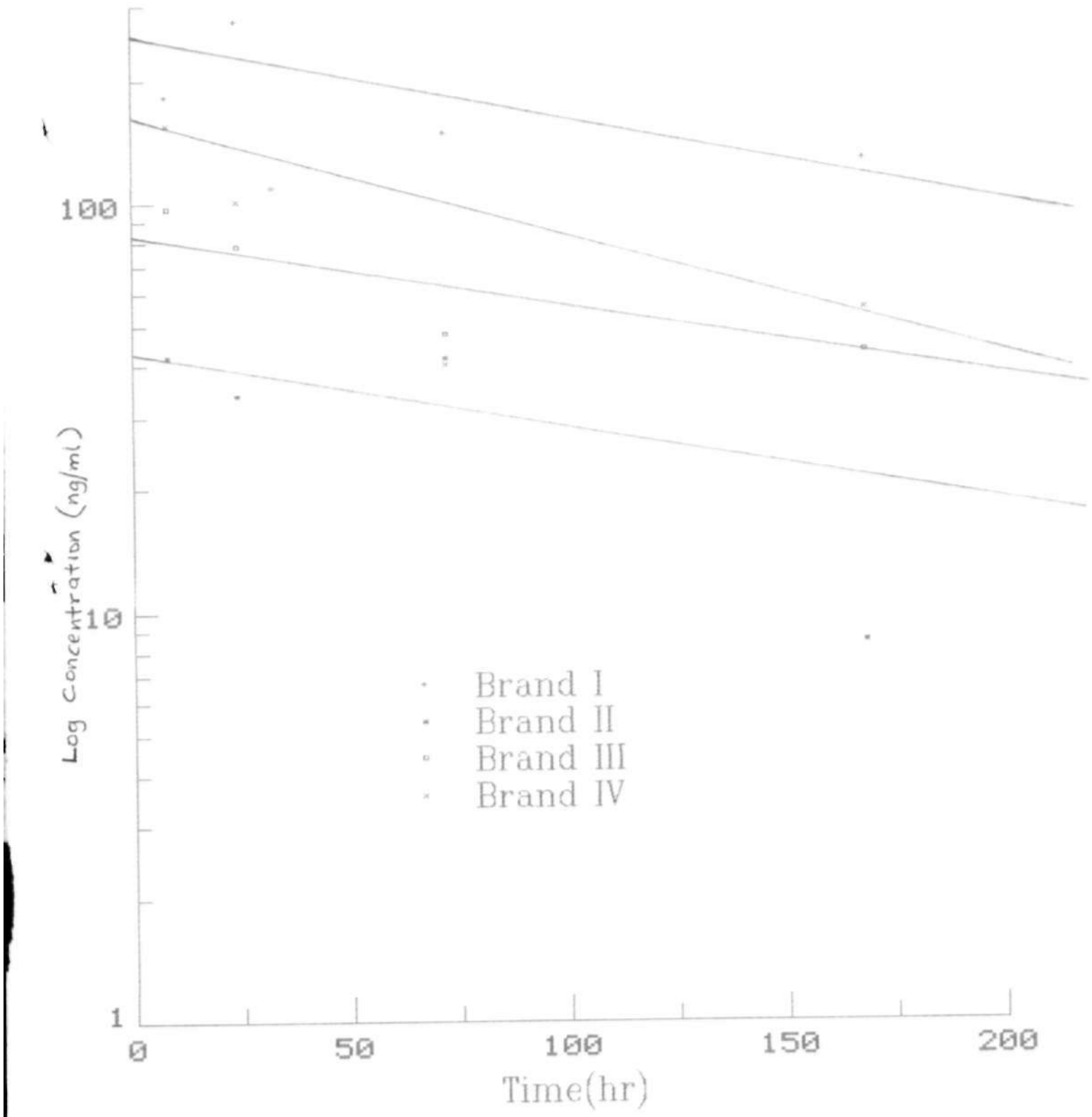


Fig 5 Log mean plasma chloroquine concentration-time curve for the terminal phase of the study.

3.2.4 Pharmacokinetics

3.2.4.1 *Acute or β-phase*

The individual and mean pharmacokinetic parameters calculated during the acute decay phase are shown in Tables 3.3 - 3.7.

For purposes of comparison, the pharmacokinetic parameters derived for Brand IV (Nivaquine®, May and Bayer) were used as standard. The mean peak plasma concentration (C_{max}) ranged from 111.3 to 583.1ng/ml. The value obtained for Brand II was statistically different from that obtained for Brand IV ($P < 0.05$). In this respect Brands I and III appear to be similar to Brand IV. The mean time to reach peak plasma concentration (t_{max}) ranged from 2.1 to 4.0h. These times for Brands I and III differed significantly from that of Brand IV ($P < 0.05$). The calculated mean lag times (t_{lag}) were 0.7 to 7.3 and did not differ significantly from each other ($P > 0.05$).

Compared to the other brands, The area under the plasma concentration-time curve up to 8h (AUC_{0-8h}) for brand II was only about one-half of the others and was statistically different from

that for Brand IV (P = 0.0003). Although the calculated mean clearance (Cl) for Brand II was about twice that of Brand IV, none differed significantly from the latter with respect to this parameter.

The mean elimination half-lives for the acute decay phase ($t_{\alpha\beta}$) were similar for Brands II, III and IV. The value obtained for Brand I differed significantly from that of Brand IV and III (P = 0.0002).

The results of the determination of the relative bioavailabilties can be seen in Table 3.9. None of the brands I-III was bioequivalent to brand IV during the period 0-8 hours.

3.2.4.2 *Terminal or z-phase*

During the z-phase the calculated half-lives ($t_{\infty z}$) were 5.3, 6.2, 4.0 and 5.5 days for Brands I to IV respectively. The corresponding terminal elimination rate constant, $k_{\infty z}$, as well as the $AUC_{(0-\infty)}$ are also shown in Table 3.8.

Table 3.9 shows that brands III and IV can be considered to be bioequivalent during the period 0-∞.

Table 3.3

PHARMACOKINETIC PARAMETERS OF CHLOROQUINE IN PLASMA OF INDIVIDUAL VOLUNTEERS FOLLOWING THE INGESTION OF 600mg BASE IN TABLET FORM (THE FIRST EIGHT HOURS)

BRAND I

PARAMETER	VOLUNTEER NUMBER				
	1	2	3	4	5
t_{lag} (min)	0,1	3,0	0,0	0,1	0,2
k_a (h^{-1})	0,1	0,2	0,1	0,1	0,1
t_{max} (h)	7,0	3,3	6,4	7,3	9,0
k_e (h^{-1})	2,0	0,4	0,4	1,6	2,1
$t_{1/2}$ (h)	0,3	1,9	1,9	0,4	0,3
C_{max} (ng/ml)	238,4	478,6	501,4	551,2	715,4
t_{max} (h)	6,0	4,0	4,0	4,0	3,0
$AUC_{(0-\infty)}$ (ng/ml,h)	1811,0	1961,0	1191,8	3926,0	3212,0
Cl (L/h)	139,4	225,0	270,4	84,3	105,9

Table 3.4

PHARMACOKINETIC PARAMETERS OF CHLOROQUINE IN PLASMA OF INDIVIDUAL VOLUNTEERS FOLLOWING THE INGESTION OF 600mg BASE IN TABLET FORM (THE FIRST EIGHT HOURS)

BRAND II

PARAMETER	VOLUNTEER NUMBER			
	1	2	3	4
t_{lag} (min)	0,0	6,0	3,4	3,6
k_a (h^{-1})	0,3	0,1	0,1	0,3
$t_{1/2}$ (h)	2,5	4,7	5,3	2,2
k_e (h^{-1})	0,5	0,7	1,5	0,4
t_{max} (h)	1,5	1,0	0,5	1,6
C_{max} (ng/ml)	104,1	129,9	120,5	90,8
t_{max} (h)	3,0	2,0	2,0	2,5
$AUC_{(0-\infty)}$ (ng/ml,h)	516,0	588,0	664,0	372,0
Cl (L/h)	1015,7	588,9	350,6	1111,7

Table 3,5

PHARMACOKINETIC PARAMETERS OF CHLOROQUINE IN PLASMA OF INDIVIDUAL VOLUNTEERS FOLLOWING THE INGESTION OF 600mg BASE IN TABLET FORM (THE FIRST EIGHT HOURS)

BRAND III

PARAMETER	VOLUNTEER NUMBER			
	1	2	3	4
t_{lag} (min)	6,0	11,0	7,0	5,0
k_e (h^{-1})	0,4	0,3	0,2	0,2
t_{up} (h)	1,8	2,5	3,1	3,5
k_a (h^{-1})	0,5	0,4	0,4	0,3
t_{up} (h)	1,4	1,7	1,9	2,0
C_{max} (ng/ml)	172,2	343,2	358,3	358,0
t_{max} (h)	2,5	2,5	2,5	3,0
$AUC_{(0-8h)}$ (ng/ml,h)	1398,0	1692,0	1147,0	1016,0
Cl (L/h)	387,4	277,0	295,9	399,0

Table 3.6

PHARMACOKINETIC PARAMETERS OF CHLOROQUINE IN PLASMA OF INDIVIDUAL VOLUNTEERS FOLLOWING THE INGESTION OF 600mg BASE IN TABLET FORM (THE FIRST EIGHT HOURS)

BRAND IV

PARAMETER	VOLUNTEER NUMBER				
	1	2	3	4	5
t_{lag} (min)	12,0	6,0	6,0	12,0	0,0
k_a (h^{-1})	0,3	0,3	0,4	0,3	0,2
t_{ma} (h)	2,2	2,4	1,8	2,2	2,9
k_e (h^{-1})	0,5	0,6	0,8	0,5	0,4
t_{me} (h)	1,4	1,1	0,9	1,4	1,6
C_{max} (ng/ml)	291,9	403,0	653,2	674,1	899,1
t_{max} (h)	2,5	2,0	2,0	2,0	2,0
$AUC_{(0-8h)}$ (ng/ml,h)	990,0	2063,0	2550,0	1732,4	1806,0
Cl (L/h)	274,3	228,7	186,5	1111,7	259,0

Table 3,7

MEAN (± S.D) PHARMACOKINETIC PARAMETERS OF CHLOROQUINE IN PLASMA OF SEVERAL VOLUNTEERS FOLLOWING THE INGESTION OF 600mg BASE IN TABLET FORM IN 4 BRANDS (THE FIRST EIGHT HOURS)

PARAMETER	BRAND NUMBER I	II	III	IV
t_{lag} (min)	0,7± 1,3	1,8± 5,3	7,3± 2,6	6,4± 6,3
k_a (h^{-1})	0,1± 0,4	0,2± 0,1	0,3± 0,1	0,3± 0,1
t_{ab} (h)	6,6± 2,1	4,7± 3,3	2,7± 0,7	2,3± 0,4
k_e (h^{-1})	1,3± 0,8	0,8± 0,5	0,4± 0,1	0,6± 0,2
t_{max} (h)	1,0± 0,9	1,2± 0,5	1,8± 0,3	1,3± 0,3
C_{max} (ng/ml)	497,0± 171,7	111,3± 17,6	307,9± 91,0	583,1±237,8
t_{max} (h)	4,0± 1,2	2,4± 0,5	2,6± 0,3	2,1± 0,2
$AUC_{(0-\infty)}$#	2400,4±1083,0	535,0± 124,3	1313,3±298,1	1828,3±567,5
Cl (L/h)	165,0± 79,6	766,7± 358,7	339,8± 62,3	412,0±392,6

= (ng/ml.h)

Table 3.8

PHARMACOKINETIC PARAMETERS OF CHLOROQUINE IN PLASMA OF SEVERAL VOLUNTEERS FOLLOWING THE INGESTION OF 600mg BASE IN TABLET FORM IN 4 BRANDS (DURING THE TERMINAL PHASE [8-168h])

PARAMETER	BRAND NUMBER			
	I	II	III	IV
k_{el} (h^{-1})	0,005	0,004	0,007	0,005
$t_{1/2}$ (days)	5,3	6,2	4,0	5,5
$AUC_{(0-\infty)}$≠	51401,1	9055,5	19073,4	22673,7

≠ = (ng/ml.h)

Table 3.9

THE RELATIVE BIOAVAILABILTY OF CHLOROQUINE IN THE VARIOUS BRANDS USING BRAND IV AS STANDARD

PERIOD	RELATIVE BIOAVAILABILTY (%)			
	I	II	III	IV
0 - 8h	131	29	72	100
0 - ∞	227	40	84	100

CHAPTER FOUR

DISCUSSION

4.1 IN VITRO STUDIES.

4.1.1 Chemical Assay of Chloroquine Tablets.

The results obtained from the chemical assay showed that three of the brands (1, III and IV) of chloroquine tablets tested complied with the official requirement 92.5 to 107.5 percent of chloroquine content. Brand II was found to contain 58.31 percent of the labelled amount and so, did not comply with the official requirement for chloroquine content (B.P.C, 1980).

Wagner (1971a) reported controlled studies which have largely shown inequivalence of marketed drug products containing the same drug in the same type of dosage form made by two or more manufacturers. The active ingredient used to formulate the product is probably not of suitable purity or the mixing operation was inefficient (Abdel-Monem 1978; Wagner, 1971a).

The result shows a non comformity with the general manufacturing practice by the manufacturers of brand II. this brand could be termed "fake" since it is adequately labelled and branded with respect to generic name but fail to comply with stated quantities of active ingredients (Igboechi, 1988)

4.2 *IN - VIVO* STUDIES

4.2.1 Pharmacokinetics

The four brands all showed a rapid absorption phase and were detectable in plasma within 30 minutes of oral administration. With the exception of brand II, the other preparations achieved a therapeutic plasma level for plasmodium falciparum (30μg/l) within 30 minutes of oral administration. The findings are in agreement with those of Adelusi *et al*, (1982) and Walker *et al* (1983). these authors also observed that malaria had no effect on the pharmacokinetics of chloroquine except for an increased concentration of the drug in red blood cells.

Walker *et al* (1983), found mean half hour plasma chloroquine concentrations of 67.7 ± 46.8ng/ml at the commencement of the administration of the drug in a dose of 25mg/kg body weight in 3 divided doses for three days to children with malaria. The mean half hour plasma chloroquine concentrations found in all brands in this study which range from 13.1 to 200.2ng/ml are comparable with his results.

The mean peak plasma concentrations for the four brands used ranged from 111.3 to 583.1ng/ml. Similar values were found by Walker *et al.*, (1983) in children with malaria. As expected, higher values were obtained after parenteral administration of the drug (Ogbuokiri, 1988 and Gustafsson *et al.*, 1983).

Large interindividual variations in chloroquine pharmacokinetics have been observed in various subjects. The rate constants of absorption (k_a) and elimination ($k_{\alpha\beta}$) have been found to decrease with increasing dose. However, this was not the case with the terminal elimination rate constant (ke_α) (Frisk-Holmberg *et al.*, 1984). This variation is somewhat shown in this study where the mean t_{max} for the brands varied from 2.1 to 4.0 hours whereas individuals were observed to

have a t_{max} range from 2 to 6 hours. The t_{max} for healthy volunteers are usually shorter than those of patients with malaria. This is thought to be due to the gastrointestinal disturbances associated with this disease (Walker *et al.*, 1983). The mean t_{max} values in this study are similar to those observed by Walker *et al.*, 1983 and as expected are longer than those obtained after parenteral administration of the drug (Gustatsson *et al.*, 1983; Ogbuokiri, 1988). However the t_{max} from individual data compare favourably well with those obtained by Frisk-Holmberg *et al.* (1984) and Aderounmu *et al.* (1986).

The differences observed in individual parameters are due to individual variability or varying degree of the presystemic elimination. An orally administered drug may be destroyed or metabolised within the gut lumen or by enzymes in the mucosa. pharmacokinetic theory permits some reasonable predictions of the magnitude of the first pass effect on drugs that are eliminated entirely by apparent first order hepatic biotransformation (Perrier and Gibaldi, 1972, Perrier*et al.*, 1973).

Despite other factors influencing rate of drug absorption pharmaceutical formulation and the charateristics of the subjects are major sources of individual variations. Subject characteristics can produce striking differences in the efficacy of a given drug product. One characteristic that influences drug absorption is the rate of gastric emptying. It may affect both the rate and extent of absorption of the drug (Abdel-Monem and Henkel, 1978; Rawlins, 1979). Gastric emptying is not known to be an important rate limiting step in the absorption of chloroquine; however, chloroquine does decrease the oral-caecal transit time by about two hours (4.3 ± 0.2h to 2.6 ± 0.1h) (Adjepon-Yamoah *et al.*, 1985).

Intrasubject variability in drug disposition is well documented (Koup and Gibaldi, 1980). The degree of intrasubject variability changes from drug to drug and may differ from one panel of subjects to another depending on age and other factors (Koup and Gibaldi, 1980).

This study clearly showed that all four brands of chloroquine undergo a multiexponential decay phase. This multiexponential model is due to tissues behaving as a separate compartment with a slower elimination rate constant than plasma

(Adelusi and Salako, 1982a, 1982b). Adjepon-Yamoah *et al.* (1986), observed a multiexponential decay with three discernable linear phases for chloroquine; the first phase which is the acute accuring before 24 hours, the second phase between 24 hours and 7 days and a third phase thereafter. Long terminal half-lifes ($t_{1/2\beta}$) as calculated for all the brands used in this study, are similar to those observed in adults by McChesney *et al.* (1962) and in children by Walker *et al* (1983)m and Adelusi *et al.* (1982). These long half-lives are due to extensive tissue binding of chloroquine. This implies that a tissue/plasma equilibrium is not achieved for many weeks after single dose of chloroquine (Adelusi and Salako, 1983).

Measured area under the plasma concentration curve to 8 hours (AUC_{0-8h}) using the trapezoid rule did not differ significantly for brands I, III and IV but was smaller for brand II. This decreased AUC can be explained by considering the fact that this brand contained a significantly smaller amount of the active ingredient. This would suggest a lower bioavailability of the drug since the absorption rate constant is similar to that of the other brands.

CONCLUSIONS & RECOMMENDATIONS.

The brands of chloroquine employed in this study except for brand II which contained 58.31 percent of the active ingredient complied with the official B.P (1980) requirement for chloroquine content (92.5-107.5%).

Similar studies done in Zaria using paracetamol tablets and syrups showed a non conformity with G.M.P guidelines by one of the manufacturers of a syrup brand (Bakare, 1987). These findings appear to suggest that if wide scale Quality Control studies are carried out regularly, quite a sizeable number of the drugs in the market may be discovered to be "faked". These substandard drugs would ultimately lead to treatment failure in most cases to the detriment of the patient and invariably loss of credibility on the part of the professionals.

This study has clearly demonstrated that chemical equivalence as shown by brands I, III and IV does not necessarily confer bioequivalence. This obviously could be attributed to formulation problems. Active ingredient in brand I was released faster than any of the brands and this

could easily lead to toxicity with this brand. On the contrary, brand III which was bioequivalent to brand IV after the first 8 hours of the study, clearly demonstrated a case of slower release than the 'standard formulation'.

The cost of health care is currently exorbitant in our environment and those involved in the payments (Government and public) should get value for their money. This makes it mandatory for there to be routine analysis and stringent Quality Control of pharmaceuticals (both imported and locally manufactured) at source, at level of the Government (the various Food and Drug Administrations) and at other levels (Teaching Hospitals and other research bodies). This routine analysis should in all instances include both chemical assay and pharmacokinetic studies. The pharmacokinetic studies are imperative because of the differences in drug handling by individuals and races.

There is the need for similar studies involving brands of other drugs other than those mentioned here and possibly drug interaction studies to be done routinely in this environment if the patients are to derive greater benefit than harm from their prescribed drugs.

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APPENDIX 1A

PLASMA CONCENTRATION OF CHLOROQUINE (ng/ml) IN FIVE HEALTHY VOLUNTEERS FOLLOWING ORAL ADMINISTRATION OF 600mg OF THE BASE AFTER OVERNIGHT FAST.

BRAND I

Time (hr)	VOLUNTEERS 1	2	3	4	5	Mean (±SD)
0	0,0	0,0	0,0	0,0	0,0	
0,5	52,3	68,8	211,7	278,9	388,9	200,1 ± 142,4
1,0	104,4	179,3	439,8	513,3	599,1	367,2 ± 214,9
1,5	135,3	291,0	360,7	531,4	525,9	368,9 ± 167,1
2,0	148,2	200,2	235,4	375,4	539,7	299,8 ± 158,4
2,5	78,2	170,8	402,1	388,3	475,3	302,9 ± 169,4
3,0	150,2	204,3	352,8	336,9	715,4	351,9 ± 220,7
4,0	154,9	478,6	501,4	551,2	560,3	449,2 ± 168,3
6,0	238,4	244,3	246,0	450,3	-	294,8 ± 103,8
8,0	111,7	146,7	440,1	188,4	311,9	239,8 ± 135,1
24,0	154,9	252,9	254,3	260,2	452,1	276,9 ± 105,6
72,0	55,9	113,2	124,6	171,4	276,9	248,3 ± 82,9
168,0	17,9	29,4	56,7	39,4	486,8	126 ± 202,2

APPENDIX 1B

PLASMA CONCENTRATION OF CHLOROQUINE (ng/ml) IN FOUR HEALTHY VOLUNTEERS FOLLOWING ORAL ADMINISTRATION OF 600mg OF THE BASE AFTER OVERNIGHT FAST.

BRAND II

Time (hr)	VOLUNTEERS 1	2	3	4	Mean (±SD)
0,0	0,0	0,0	0,0	0,0	
0,5	18,8	4,1	16,4	-	13,1 ± 7,8
1,0	30,6	60,6	120,5	34,9	61,7 ± 41,4
1,5	5,2	93,8	111,9	76,4	72,1 ± 46,2
2,0	57,0	129,9	120,6	90,8	99,6 ± 32,9
2,5	89,7	68,8	93,8	8,8	65,3 ± 39,2
3,0	104,1	71,1	61,4	2,6	59,8 ± 42,3
4,0	67,3	106,4	101,1	25,6	75,1 ± 37,3
6,0	74,7	66,4	78,3	51,7	67,8 ± 11,8
8,0	20,6	63,2	274,3	233,1	147,8 ± 124,6
24,0	27,3	45,6	58,2	4,4	33,9 ± 23,4
72,0	-	40,9	42,9	-	41,9 ± 1,4
168,0	5,2	4,4	5,2	17,0	8,4 ± 5,8

APPENDIX IC

PLASMA CONCENTRATION OF CHLOROQUINE (ng/ml) IN FOUR HEALTHY VOLUNTEERS FOLLOWING ORAL ADMINISTRATION OF 600mg OF THE BASE AFTER OVERNIGHT FAST.

BRAND III

Time (hr)	VOLUNTEERS				Mean (±SD)
	1	2	3	4	
0,0	0,0	0,0	0,0	0,0	0,0
0,5	-	-	30,2	52,3	42,8 ± 13,5
1,0	72,9	77,3	98,2	197,2	111,4 ± 58,3
1,5	-	-	218,1	233,9	226,0 ± 11,2
2,0	91,7	169,9	279,6	244,3	196,4 ± 83,4
2,5	172,3	343,3	358,3	301,9	293,9 ± 84,5
3,0	134,0	277,4	351,9	358,0	280,3 ± 104,2
4,0	230,8	244,6	249,5	179,9	226,2 ± 31,9
6,0	122,6	195,8	213,7	144,6	169,2 ± 42,7
8,0	96,7	103,2	132,6	56,4	97,2 ± 31,4
24,0	28,2	-	100,5	106,4	78,4 ± 43,5
72,0	-	47,0	58,9	36,7	47,5 ± 11,1
168,0	34,9	42,6	69,4	24,7	42,9 ± 19,1

APPENDIX 10

PLASMA CONCENTRATION OF CHLOROQUINE (ng/ml) IN FIVE HEALTHY VOLUNTEERS FOLLOWING ORAL ADMINISTRATION OF 600mg OF THE BASE AFTER OVERNIGHT FAST.

BRAND IV

Time (hr)	VOLUNTEERS					Mean (±SD)
	1	2	3	4	5	
0,0	0,0	0,0	0,0	0,0	0,0	
0,5	0,0	-	2,9	52,3	98,8	40,7 ± 44,5
1,0	36,4	138,7	140,8	241,6	365,1	184,5 ± 124,3
1,5	143,7	148,7	171,7	261,9	595,9	264,4 ± 191,3
2,0	-	403,0	663,2	674,1	893,1	655,9 ± 200,0
2,5	291,9	330,7	373,9	563,8	621,4	436,3 ± 146,9
3,0	167,6	-	233,9	280,7	305,1	246,8 ± 60,5
4,0	116,4	-	177,9	175,8	213,1	170,8 ± 40,1
6,0	122,3	234,6	236,6	264,3	1226,4	416,8 ± 455,8
8,0	95,8	119,6	145,8	163,4	250,7	155,1 ± 59,3
24,0	31,2	50,6	58,2	113,4	250,1	100,7 ± 88,9
72,0	28,5	21,4	42,6	43,2	64,7	40,1 ± 16,6
168,0	36,4	49,7	45,9	86,1	273,1	98,2 ± 99,6

```
10 REM KINEMP IS A NON LINEAR CURVE FITTING PROGRAM
20  REM THE EQUATION WHICH IS TO BE USED TO FIT
30  REM THE DATA IS IN THE SUB ROUTINE AT LINE
40  REM 9000
50  REM
60  REM LAST MODIFIED 3rd JANUARY 1986
70 INPUT "DO YOU WISH TO READ DATA FROM A FILE (Y/N)";an$
80 IF an$="N" OR an$="n" THEN GOTO 220
90 IF an$="Y" OR an$="y" THEN GOTO 110
100 GOTO 70
110 INPUT"PLEASE TYPE RELEVANT FILE NAME TO READ FROM";filename$
120 OPEN "i",1,filename$
130 INPUT £1,n1
140 DIM C(N1),T(N1),R(N1)
150 REM*************
160 FOR L = 1 TO N1: INPUT £1, Tentry, Centry
170 REM************************************************************
180 T(L) =Tentry:C(L) = Centry
190 REM
200 NEXT L
210 CLOSE
220 INPUT "Please supply the weight measurement of the concentration,
e.g. ng"; W$
230 INPUT "Now supply the volume measurement of the concentration, e.g
. ml"; Y$
240 INPUT "Finally, is TIME units in hr (Y/N)?"; Z$
250 IF z$="Y" OR z$="y" THEN GOTO 280
260 IF z$="N" OR z$="n" THEN GOTO 270
270  fact=1:GOTO 290
280 fact=60
290 INPUT "NUMBER OF POINTS",N1
300 DIM C(N1), T(N1),R(N1)
310 FOR L = 1 TO N1
320 INPUT T(L),C(L)
330 NEXT L
340 INPUT "NUMBER OF PARAMETERS",N
350 DIM E(N+2,N+1),F(N),P(N),S(N),V(N)
360 FOR L= 1 TO N
370 INPUT "PARAMETER ESTIMATE",E(L,1)
380 S(L) = E(L,1)
390 NEXT L
400 REM
410 REM INITIAL MATRIX
420 FOR L = 1 TO N
430 FOR L1 =2 TO N+1
440 LET E(L,L+1)=E(L,1) + S(L)*(SQR(N+1)-1)/(N*SQR(2))
450 NEXT L1
460 LET E(L,L+1)=E(L,1) + S(L)*(SQR(N+1)+(N-1))/(N*SQR(2))
470 NEXT L
480 REM INITIAL SS AND VERTEX COUNT
490 FOR L = 1 TO N+ 1
500 FOR L1 = 1 TO N
510 P(L1) = E(L1,L)
520 NEXT L1
530 GOSUB 1280
540 E(N+1,L) = S1
550 E(N+2,L) =1
560 NEXT L
570 GOTO 1150
```

```
580 REM DISPLAY interim results
590 IF Y THEN PRINT "ITERATION NO ";Y ELSE PRINT "INITIAL MATRIX"
600 FOR L = 1 TO N+2
610 PRINT E(L,1),E(L,N+1)
620 NEXT L
630 X = E(N+1,N+1)/E(N+1,1)-1
640 IF X < 0.0002 THEN GOTO 1350 ELSE GOTO 650:REM CHECK FOR CONVERGEN
CE.
650 REM VERTEX SUM AND AVERAGE
660 FOR L = 1 TO N
670 S = 0
680 FOR L1 = 1 TO N
690 S = S+E(L,L1)
700 NEXT L1
710 F(L) = S/N
720 NEXT L
730 REM NEW VERTEX
740 REM REFLECTION
750 FOR L = 1 TO N
760 V(L) = F(L) + (F(L)-E(L,N+1))
770 P(L) =V(L)
780 NEXT L
790 GOSUB 1280
800 X = S1
810 IF S1<E(N+1,N) THEN 950
820 IF S1>E(N+1,N+1) THEN 890
830 REM CONTRACTION
840 FOR L =1 TO N
850 P(L) =F(L)+ 0.5*(F(L)-E(L,N+1))
860 NEXT L
870 GOSUB 1280
880 GOTO 1000
890 REM BACK CONTRACTION
900 FOR L=1 TO N
910 P(L) =F(L) - 0.5*(F(L)-E(L,N+1))
920 NEXT L
930 GOSUB 1280
940 GOTO 1000
950 REM EXPANSION
960 FOR L=1 TO N
970 P(L) =F(L)+2*(F(L)-E(L,N+1))
980 NEXT L
990 GOSUB 1280
1000 IF S1 <X THEN GOTO 1040 ELSE S1 = X
1010 FOR L = 1 TO N
1020 P(L) = V(L)
1030 NEXT L
1040 REM EXCHANGE
1050 IF S1>E(N+1,N+1) THEN X = N ELSE X = N+ 1
1060 FOR L = 1 TO N
1070 LET E(L,X) = P(L)
1080 NEXT L
1090 E(N+1,X) = S1
1100 E(N+2,X) = 0
1110 REM INCREMENT VERTEX COUNT
1120 FOR L = 1 TO N
1130 E(N+2,L) = E(N+2,L)+1
1140 NEXT L
1150 REM VERTEX SORT ROUTINE
1160 FOR L = 1 TO N
1170 FOR L1 = L TO N+1
```

```
1180 IF E(N+1,L) <E(N+1,L1) THEN GOTO 1240
1190 FOR L2=1 TO N+2
1200 X = E(L2,L)
1210 E(L2,L) = E(L2,L1)
1220 E(L2,L1) = X
1230 NEXT L2
1240 NEXT L1
1250 NEXT L
1260 LET Y = Y+1
1270 GOTO 580
1280 REM SUM OF SQUARES ROUTINE AND EQUATION TO BE FITTED.
1290 S1 = 0
1300 FOR L2 = 1 TO N1
1310 R(L2)=P(1)*(EXP(-P(2)*(T(L2)-P(4)))-EXP(-P(3)*(T(L2)-P(4))))
1320 S1 = S1 + (R(L2) - C(L2))*(R(L2)-C(L2)): REM Weights can be inser
ted here,e.g., multiple (R(L2) - C(L2))*(R(L2) - C(L2)) by 1/(
C(L2)*C(L2)) to give each point equal weight.
1330 NEXT L2
1340 RETURN
1350 REM ******** SUBROUTINE FOR CALCULATING AREA
1360 AUC = 0
1370 FOR L3 = 2 TO N1: AUC = AUC + (C(L3)+C(L3-1))* 0.5 * (T(L3)-T(L3-
1)):NEXT
1380 TOTALAUC = AUC + C(N1)/E(2,1)
1390 Cl = D/TOTALAUC : REM *** Clearance is calculated ***
1400 LPRINT: REM TURNS THE PRINTER ON
1410 LPRINT "SIMPLEX TERMINATED AFTER ";Y; "ITERATIONS ":LPRINT:LPRINT
1420 INPUT "DOSE, ...Please supply the dose in the same unit as per y-
axis,e.g if concentration was in microgram per ml, a dose of 1
0mg will be given simply as 10000"; D:PRINT:PRINT:PRINT
1430 LPRINT "************** RESULTS OF CALCULATIONS ******************
**":LPRINT
1440 Cl = D/TOTALAUC : REM *** Clearance is calculated ***
1450 INPUT "BIOAVAILABILITY,... a bioavailability of 90% will be suppl
ied as 0.9; 80% as 0.8 etc; However when the bioavailability i
s unknown simply supply 0, in which case the program will assume compl
ete bioavailability"; B
1460 IF B <> 0 THEN GOTO 1500
1470 VD = Cl/E(2,1):REM **Formula to calculate volume of distribution*
*
1480 LPRINT "VOLUME OF DISTRIBUTION (";Y$;")";:LPRINT TAB(48) " = "; V
D
1490 GOTO 1520
1500 VD = (D/E(2,1)) * B: REM VOLUME OF DISTRIBUTION WHERE BIOAVAILABI
LITY IS KNOWN
1510 LPRINT "VOLUME OF DISTRIBUTION (";Y$;")";:LPRINT TAB(48) " = "; V
D
1520 TB = 0.693/E(2,1)
1530 LPRINT "CLEARANCE (";Y$;"/";Z$;")";:LPRINT TAB(48) " = "; Cl
1540 LPRINT "INTERCEPT ON Y-AXIS (C";:LPRINT CHR$(27)"S";CHR$(1); "o";
:LPRINT CHR$(27)"T";:LPRINT ")";:LPRINT TAB(50) " = "; E(1,1)
1550 LPRINT "ELIMINATION RATE CONSTANT (K";:LPRINT CHR$(27)"S";CHR$(1)
;"e";:LPRINT CHR$(27)"T";:LPRINT ")";:LPRINT " (";W$;"/";Y$;"/
";Z$;")";:LPRINT TAB(50) " = "; E(2,1)
1560 LPRINT "ABSORPTION RATE CONSTANT (K";:LPRINT CHR$(27)"S";CHR$(1);
"a";:LPRINT CHR$(27)"T";:LPRINT ")";:LPRINT " (";W$;"/";Y$;"/"
;Z$;")";:LPRINT TAB(50) " = "; E(3,1)
1570 LPRINT "LAG TIME (";Z$;")";:LPRINT TAB(48) " = "; E(4,1)
1580 LPRINT "ELIMINATION HALF-LIFE";:LPRINT CHR$(27)"S";CHR$(1);"(t-ha
lf beta)";:LPRINT CHR$(27)"T";:LPRINT "(";Z$;")";:LPRINT TAB(5
0) " = "; TB
```

```
1590 LPRINT "ABSORPTION HALF-LIFE";:LPRINT CHR$(27)"S";CHR$(1);"(t-hal
f alpha)";: LPRINT CHR$(27)"T";:LPRINT "(";Z$;")";:LPRINT TAB(
50) " = "; 0.693/E(3,1)
1600 LPRINT "AUC";:LPRINT CHR$(27)"S";CHR$(1);"(0 -";T(N1);")";:LPRINT
 CHR$(27)"T";:LPRINT "(";W$;"/";Y$;".";Z$;")";:LPRINT TAB(50)
" = "; AUC
1610 LPRINT "AUC";:LPRINT CHR$(27)"S";CHR$(1);"(0 - INFINITY)";:LPRINT
 CHR$(27)"T";:LPRINT"(";W$;"/";Y$;".";Z$;")";:LPRINT TAB(50) "
 = "; TOTALAUC:LPRINT:LPRINT
1620 LPRINT "&&&&&&&&&&&&&& SOME STATISTICAL JARGON **********
**":LPRINT
1630 LPRINT "SUM OF SQUARES = "; E(5,1):LPRINT
1640 GOSUB 1280
1650 FOR L = 1 TO N1
1660 IF L = 1 THEN LPRINT "X - VALUES", "Y - VALUES", "CAL Y - VALUES"
1670 LPRINT T(L),C(L),R(L)
1680 NEXT L
1690 REM WE ARE ABOUT TO FINISH
1700 INPUT"Do you wish to plot curve (y/n)?";an$
1710 IF an$="Y" OR an$="y" THEN GOTO 1730
1720 IF an$="N" OR an$="n" THEN  END
1730 INPUT "Filename ";Name$
1740 OPEN "o",1,Name$
1750 WRITE £1,n1
1760 FOR 1=1 TO n1
1770 t(1)=t(1)*fact
1780 WRITE £1,CINT(t(1)),CINT(c(1)),CINT(r(1))
1790 NEXT 1
1800 CLOSE
1810 RUN "kingraph"
```

