EFFECT OF ULTRA VIOLET RADIATION ON THE STABILITY OF PROGUANIL AND PYRIMETHAMINE

BY

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DECLARATION

This is to certify that the work reported in this thesis was carried out by me under the supervision of Dr. Abdullahi MUSTAPHA, Head of Department of Pharmaceutical Chemistry.

The work of other investigators is acknowledged and referred to accordingly.

I solemnly declare that no part of this thesis has been submitted elsewhere for a degree.

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DEDICATION

This project is dedicated to the

memory of my late friend Mrs. Temitope F. Adeleye.

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ABSTRACT

The influence of UV radiation (366nm) on the stability of proguanil hydrochloride and pyrimethamine sulphate in tablet formulations and in phosphate buffer solutions of different pH values has been evaluated in the present study, UV spectroscopic method for estimating drug content in the various dosage forms was employed. The effect of pH on the UV spectrum of the drugs was investigated» The influence of reaction vessel and pH on the irradiated phosphate buffer solutions was also studied,

In-vitro dissolution rate studies of the drugs were carried out including those tablets that had been subjected to UV irradiation.

The UV spectrum of proguanil hydrochloride was found to be stable at pH values above 2.0 in phosphate buffer solutions. Below pH 2,0 the spectral peak at 232nm disappeared while the peak at 253nm flattened out giving a plateau.

Proguanil hydrochloride was found to enter into photochemical reaction in phosphate buffer solution when subjected to continuous UV irradiation at 366nm. Evidence of photochemical reaction was shown by loss of spectral peaks in phosphate buffer, appearance of a precipitate, colour change of solution, spectral shift and increase in absorbance of the irradiated solution. The photochemical reaction was found to be influenced by the pH of the solution and the type of

glassware in which the reaction was performed.

Assay of irradiated proguanil hydrochloride tablets indicated loss of drug content with time.

Dissolution rate studies of the irradiated tablets showed decrease in dissolution rate: with time

The UV spectrum of pyrimethamine sulphate remained stable at different Values. Pyrimethamine sulphate appeared not to readily enter into photochemical reaction. The formulations used showed signs of degradation by colour change of tablets but there was no loss of drug content on assay.

The dissolution rate of the irradiated pyrimethamine tablets produced by Wellcome Nig. Ltd. increased with time in phosphate buffer solution pH 4.5 while the dissolution rate of irradiated pyrimethamine tablets produced by Quality Control Unit A.B.U. Zaria decreased with time in phosphate buffer solution pH 4.5. This difference observed in the dissolution rates of the formulations has been attributed to the influence of the different excipients used by the different manufacturers.

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ABBREVIATIONS

U.V.	=	Ultraviolet
G.L.C.	=	Gas liquid chromatography
N.M.R.	=	Nuclear magnetic resonance
I.R.	=	Infra red
HPLC	=	High performance liquid chromatography
^{o}C	=	Degree centigrade (Celsius)
MIN	=	Minutes
CONC	=	Concentration
HCl	=	Hydrochloric acid
H_2SO_4	=	Sulphuric acid
NaOH	=	Sodium hydroxide
Ml	=	Millilitre
Cm^3	=	Cubic centimeter
USP	=	United States Pharmacopoeia
NF	=	National Formulary
WHO	=	World Health Organisation
BP	=	British Pharmacopoeia
QCU	=	Quality Control Unit Ahmadu Bello University, Zaria.
ICI	=	Imperial Chemical Industries Limited.

1:1 EFFECT OF ULTRA VIOLET RADIATION ON THE STABILITY OF PROGUANIL AND PYRIMETHAMINE

INTRODUCTION

One of the most important considerations in the formulation of a pharmaceutical dosage form is its stability. The stability of a drug is concerned with the preservation of the characteristics of the drug. A drug is considered adulterated if it does not meet the quality and purity characteristics that it is represented to possess. Schou (1959) defined the stability of a pharmaceutical preparation as the period of time from the completion of the preparation until it no longer fulfils the established specifications. Grimshow (1963) also defined the stability of a drug as the time from the completion of the preparation until the development of an off-odour or off-colour to a point where the article is no longer commercially acceptable.

Stability tests are mandatory as a means of assessing quality assurance of a product. Quality assurance of product does not stop at the release of the product, therefore it is extremely important that the pharmaceutical manufacturer accurately identifies the tendencies of a dosage form to deteriorate with age and environmental conditions by carrying out stability testing. Those aspects of formulation directly concerned with the components used to prepare the pharmaceuticals must be fully understood.

The effect of the container on the product and conversely the effect of product upon the container must be considered. There should be no gross deterioration and any decomposition product must be harmless.

With this knowledge, the manufacturer should be able to predict the shelf-life of his product thus ensuring the safety of the consumer.

Another need for stability testing of pharmaceutical preparations is to maintain sales because a deteriorated product is not the best advertisement for the manufacturer.

Instability of a pharmaceutical preparation can be chemical or physical. Because of the multiplicity of ingredients in most preparations, there exists the possibility of interactions taking place as well as different degradative characteristics. The decomposition of active ingredients can occur through hydrolysis, oxidation-reduction reactions, racemization, photolysis, decarboxylation and ring cleavage. Hydrolysis and oxidation-reduction reactions are most frequently encountered. The factors that affect the rate of these reactions include heat, light, oxygen, moisture and pH.

Hydrolysis

Pharmaceuticals containing ester or amide functional groups are hydrolysed in solution. These include antibiotics, vitamins and anaesthetics.

Esters are hydrolysed into acid and alcohol by fission of the acyl-oxygen linkage the reaction often

being catalyzed by substances like mineral acids and alkalies.

Since a number of drugs degrade through ester hydrolysis, methods to enhance stability of such compounds have been under study. Factors considered include pH, solvent, complexation and modification of chemical structure.

Oxidation - Reduction

The oxidative decomposition of pharmaceuticals is a reaction often mediated by dree radicals or molecular oxygen. It can be catalyzed by heavy metals, hydrogen ion and hydroxyl ion because redox potential is pH dependent for many reactions. Pharmaceuticals that undergo oxidative reactions are generally in the reduced form and minimum decomposition is usually found in the pH range of 3 and 4. In most oxidative reactions the rate is proportional to the concentration of the oxidizing molecule but sometimes it may be independent of the concentration of oxygen in auto-oxidation. The oxidation of unsaturated fats and oils proceed in the presence of atmospheric oxygen according to a free radical chain reaction (Rawlings 1977). Fat soluble vitamins are particularly affected including vitamins A and D so much that most pharmacopoeas permit the addition of antioxidants to such compounds. Prednisolone, morphine, epinephrine are examples of drugs that undergo oxidative decomposition.

The stability of pharmaceuticals undergoing oxidative degradation can be increased by

(i) reduction of oxygen concentration through boiling, filtering, carbon dioxide and nitrogen purging.

(ii) Addition of antioxidants and chelating agents. An antioxidant provides a hydrogen atom or an electron to the free radical, while a chelating agent complexes with trace amounts of heavy metal ions inactivating their catalytic activity.

(iii) It is essential to determine pH of maximum stability and to buffer the solution containing ingredients that are readily oxidized to a pH in the acid range.

Photolysis

Degradative reactions such as reduction - oxidation, ring rearrangement or modification and polymerization can be brought about by exposure to light at particular wavelength. The energy absorbed per mole is greater the shorter the wave-length of light therefore the ultraviolet portion of the light spectrum are more active in initiating chemical reactions than longer wavelength portions of the spectrum.

In systems that are photolyzed, the products are free radicals which undergo subsequent reactions. If the molecules absorbing the radiation take part in the main reaction, it is said to be a photochemical reaction.

The kinetics of photochemical reaction is more complicated than that of thermal reactions because more variables are involved. The intensity and wavelength of light, size and shape of container may greatly affect the rate of reaction. A photochemical reaction may be accompanied by a thermal reaction identical to the photochemical reaction, or opposite to it or entirely different in character. A photochemical reaction may produce a catalyst which then causes a thermal reaction to proceed at a measurable rate. Sometimes an induction period is needed for sufficient quantity of catalyst to accumulate and make the reaction proceed with a measurable velocity. The energy available in a photochemical reaction is much greater than in a thermal reaction and this often changes the character of the reaction.

Due to complexity of photolytic reactions investigations in this area of pharmaceutical stability have been for the most part qualitative in nature. In photodegradative reactions, secondary, primary and zero order reactions are possible.

Felmeister (1964) studied the photodegradation of chlopromazine hydrochloride at 253.5mu and derived the mechanism for the free radical-mediated deterioration. Loss of concentration of chlorpromazine versus photons of light per litre absorbed showed zero order kinetics. Hamlin et-al (1960) studied the

photolytic degradation of alcoholic solutions of prednisolone, hydrocortisone and methylprednisolone exposed to ordinary fluorescent lighting. They found that the two double bonds present in prednisolone and methyl prednisolone make them more susceptible to photodegradation than the one double bond in the A ring of hydrocortisone.

Racemization

An optically active compound may lose its optical activity without changing its chemical composition. This reaction is important to the stability of pharmaceutical formulation since biological effect of one form can be considerably less than that of the other. For example Levo-adrenaline is 15 to 20 times more active than dextro-adrenaline. Solutions of L-adrenaline form a racemic mixture of equal parts of L- and D-adrenaline with pharmacologic activity just over half that of pure L-adrenaline.

By studying the kinetics of racemization and determining rate constant, temperature dependence of reaction and pH dependence, it is possible to establish the optimal conditions for storage of the preparation.

Physical Stability

There is scarcity of reports dealing with physical stability testing. Physical changes in the dosage form upon storage can be as serious as chemical

instability of the active ingredient and sometimes more. Physical changes that can occur include crystal growth, change in crystal form, increase or decrease in dissolution rate and disintegradation time, cracking of emulsions, caking of suspension, colour fading and colour development and sediment development in solution.

There have been studies on the influence of temperature and light on the colour stability of tablets. In one investigation, Everhard and Goodhart (1963) quantitated the relationship of light-catalyzed fading of coloured tablets as a function of time and light intensity. Fading was proportional to the product intensity and time. If the dissolution rate of the active ingredient from the tablet or the tablet disintegradation time were to increase under extended shelf of the tablet formulation, the therapeutic effectiveness of the tablet may be seriously affected.

Effects of storage of phenobarbital and phenacetin tablets on the rates of dissolution have been studied for the three tablet formulations. Tablets prepared with gelatin or polyethylene glycol 6,000 as granulating agents changed little but tablets prepared with carboxymethylcellulose underwent a significant increase in dissolution rate (Solvang and Finholt 1969).

For disperse systems the physical stability of an emulsion or suspension can be influenced by factors affecting the chemical stability of the emulsifier, suspending agent, anti-oxidant microbiological preservative and active substance. It is an established fact that the degree of stability of an emulsion may be measured by the variation of the distribution of sizes of dispersed droplets with time. An emulsion approaching the unstable state is characterized by the appearance of large globules as a result of coalescence and aggregation of small globules. The practical commercial aspect of stability is the observation of creaming and/or separation of water and oil in an emulsion.

Change in crystal form

Some chemically stable drugs may exist in more than one crystal form which differ in melting point, solubility, vapour pressure and density. At a given temperature and pressure only one polymorphic form of a compound is thermodynamically stable while the other form may exist under the same conditions taking from minutes to years to revert to stable state. Metastable forms have a lower melting point and high solubility. Their pharmacological importance depends on the rapidity of the transition to the stable form and the nature of their altered physical properties. The reversion of a well absorbed amorphous novobiocin

to the crystalline poorly absorbed form in suspension illustrates the difficulties that may arise if polymorphism is ignored (Mullins and Macek 1960). Problems that may result from inadequate investigation of polymorphic drug forms are crystal growth in suspension and creams leading to product with poor uniformity, appearance and bioavailability, precipitation of less soluble form in liquid dosage forms and poor chemical stability.

Factors Affecting Drug Stability

Degradative reactions in pharmaceutical formulations take place at definite rates and are chemical in nature. They are dependent on conditions such as concentration of reactants, temperature, pH, catalysts, moisture, solvent, storage and light.

pH

Hydrolytic reactions catalyzed by hydrogen and hydroxyl ions vary considerably with pH. Hydrogen ion catalysis predominates at low pH and hydroxyl ion catalysis at higher pH. At intermediate pH, the rate of reaction can be pH independent or catalyzed by both hydrogen and hydroxyl ions. To determine the influence of pH on degradation reaction the decomposition is measured at different hydrogen ion concentrations. The pH of optimum stability can be determined by plotting log of rate constant versus pH (Lachman 1976).

Temperature

The velocity of degradation of pharmaceutical products is accelerated by heat like many chemical changes. The rule-of-thumb methods indicates that the rate of reaction is doubled for each 10° rise in temperature but this is not generally applicable (Martin 1962). Some deterioration reactions are not measurably influenced over a 10° temperature range while others undergo rapid degradative changes at elevated temperature. Many pharmaceuticals are thermolabile even at room temperature and must be stored in a refrigerator. Low temperature may have deleterious effect too, for example, some antitoxins precipitate and lose their activities when chilled to freezing point (Girgis 1967). To determine the effect of elevated temperature on the stability of formulations, a planned schedule of accelerated tests are carried out. Sams and Carrol (1966) found that heat at 52° has no effect on chloroquine diphosphate in phosphate buffer pH 7.4.

General Acid-Base Catalysis

The chemical instability of a drug is magnified in solution. Buffer solutions are often used in the formulation of pharmaceutical liquids to regulate the pH of the solution. Commonly employed media are acetic acid-acetate, phosphate, phosphate-pthalate borate among other buffers. Although these buffers maintain the pH of solution at a constant level,

they can also catalyze the degradation of dosage forms. It is therefore necessary to evaluate the effect of buffer concentration on the stability of the preparation in addition to the effect of hydrogen and hydroxyl ion. Such effects have been observed, for example, monohydrogen phosphate ion catalyzes the rate of hydrolysis of phenethicillin (Schwartz et-al 1962). In the solvolysis of aspirin anhydride undissociated acetic acid has an inhibitory effect (Garret 1960). General acid-base catalysis has been shown to take place for barbital and phenobarbital (Goyan et-al 1960). Very strong evidence exists to indicate that chloramphenicol degradation is general acid-base catalyzed. Monohydrogen phosphate ions, undissociated acetic acid molecules and citrate-citric acid buffer exert a definite catalytic effect upon the rate of disappearance of chloramphenicol (Higuchi and Marcus 1954).

Concentration of Reactants

The catalysis of a reaction by hydrogen and hydroxyl ions is known as "Specific Acid-Base catalysis". When a drug is positively charged and is undergoing hydrogen ion catalysis, an increase in ionic concentration by the addition of a salt like sodium chloride causes an increase in the rate of degradation (Siegel et-al 1959). A decrease in rate occurs if the positively charged drug is undergoing

hydroxyl catalysis. If the drug is a neutral molecule, changes in ionic strength by the addition of a salt has no effect on the rate of degradation.

The rate of reaction can be influenced by the ionic strength of the solution in accordance with the following equation.

$\log K = \log Ko + 1.02 Z_A Z_B \sqrt{\mu}$.

$Z_A Z_B$ = charges carried by reacting species

μ = the ionic strength

K = Rate constant of degradation.

Ko = Rate constant at infinite dilution.

It is now recognised that not only hydrogen ion and hydroxyl ions but all acidic and basic species as defined in the Bronsted-lowry theory may act as catalysts. Examples include the observations made by Web et-al (1950), Kondritzer (1956) among others.

Solvent Effect

Water is usually the most important constituent in a liquid product. Sometimes it is necessary to eliminate water entirely or in part from the vehicle because of solubility factors or hydrolytic reactions. Frequently used non-aqueous solvents are polyethylene glycol, propylene glycol and fixed oils.

The effects of solvent on the decomposition of drugs are complicated and generalisations cannot ordinarily be made. The reaction of non-electrolytes

appears to be related to solubility parameters of the solvent and solute. For ionic reactions influence of the ionic strength and the dielectric constant of the medium are significant. Solvents other than water may have a catalyzing effect on oxidation-reduction reactions for example aldehyde, ethers and ketones may influence free radical reactions significantly. Partial or full replacement of water with a solvent of lower dielectric constant generally causes a considerable decrease in the velocity of ester hydrolysis. (Bowles 1968).

The solvent therefore affects the stability of drugs to a large degree such that any change in the proportion of a mixture of solvents may change reversibly the stability of the substance. Delgado (1958) found that in multivitamin solution dissolved in a mixture of 20% water and 80% propylene glycol, thiamine mononitrate is more stable than thiamine hydrochloride, while the latter is more stable if propylene glycol is substituted by equal volumes of propylene glycol and glycerine. Gerber et-al (1957) found that ascorbic acid, vitamin B_{12} and ferrous gluconate which are ordinarily incompatible could be combined into an oral preparation by the use of 70% sorbitol solution as the vehicle. The stabilising action of sorbitol was attributed by the authors either to a reduced availability of

water in the system or to complex formation between the polyol and the vitamins.

Moisture

In the preparation of pharmaceutical tablets, it is generally accepted that a small proportion of moisture is present and in some cases this is required to form a coherent tablet. Moisture may also be the major cause of deterioration of pharmaceutical preparations. Traces of moisture due to incomplete dehydration are frequently sufficient to set up slow but progressive solvolytic reactions. Moisture can cause the effervescing ingredients in certain tablets and granules to react slowly with consequent crumbling and decrease or loss of effervescence. An investigation into the effect of moisture in pharmaceutical tabletting was concerned with the compaction of sodium chloride (Rees and Sholton 1971). Wet granulation of powdered materials with hydrophilic adhesives was shown to yield tablets whose mechanical strength is dependent on an optimum moisture content above or below which tablet strength was reduced by various occurrences.

Rees and Hersey (1972) reported that moisture decreases the compact strength by reducing the strength of the interparticulate bonds. Except at low compression force, this effect cancelled out increases in strength due to other mechanisms.

There are some cases where retention rather than elimination of water is desirable. This is true in dermatologic medicaments where humectants like glycerin and sorbitol should be incorporated.

Storage

The storage of drugs on the shelf does lead to deterioration after an extended period of storage under varying environmental conditions of temperature, humidity and light. Container materials mostly used for storage are glass, metal, plastic and rubber. Glass materials can release alkali and insoluble flakes to liquids stored in container. Stability of products packaged in a plastic container is affected in several ways due to container permeability. Penicillin tablets were found to degrade in polystyrene containers due to permeation of water vapour. Product deterioration in plastic containers also occurs due to the fact that air is continuously drawn into the container causing oxidation and drying out of preparation. Plastics also tend to sorb materials from solution causing loss of drug and other materials from solution. Rubber materials also affect product by sorption of active ingredient, antibacterial preservative and other materials.

The stability of some pharmaceuticals is known to be enhanced by storage under relatively low humidity.

Examples of such materials include vitamins, yeast and effervescent products. When the humidity is known at which the product is found to decompose with continued exposure, the type of package can be more wisely chosen.

Ultra-Violet Radiation and Light

Many types of reactions are induced by exposure to light e.g. hydrolysis, autoxidation processes. In some cases substances become coloured e.g. morphine, codeine, quinine become yellowish brown while others fade. When light is absorbed, lines appear in the spectrum of the compound and the groupings responsible, the chromophores are those susceptible to degradation. When degradation occurs, the rate of decomposition is proportional to the light intensity. The rate of change is influenced by temperature, humidity and products of reaction.

There is often a relation between structure of drug and its sensitivity towards light. Phenolic substances develop colours while the presence of a conjugated double bond system in the molecule can induce photolability (Rawlings 1977).

During a photochemical reaction light energy provides activation energy, necessary for a reaction to occur. Activated molecules collide with other molecules raising their kinetic energy and consequently the temperature of the system. The initial photochemical reaction may be followed by a thermal reaction.

1.2 METHODS OF ASSESSING QUALITY ASSURANCE

The quality of a pharmaceutical product is assessed through stability testing. Stability studies demonstrate that the necessary critical characteristics present at the time of production and release can be expected to be present when the dosage form is administered. If safety and efficacy values decline stability studies provide information to determine when and under what conditions the product should be withdrawn from the market.

Effective stability studies must include provisions of (1) Specific chemical and physical characteristics desired at time of administration.

(2) Analytical procedures and tests to determine the degree to which these characteristics are present.

(3) Allowance tolerances and variation for each critical characteristic.

(4) Anticipated shelf life under the most severe anticipated environment before product characteristics are out of specifications.

(5) Recommended physical, chemical and environmental conditions for holding the product during preadministration storage.

Physical and chemical parameters dictate many of the characteristics that will be inherent in the dosage form. Some important considerations are

(i) The crystal form of drug substance when polymorphs have different bioavailability and stability.

(ii) Solubility and stability characteristics in various solvents.

(iii) Acid-base dissociation constants which influence selection of optimum pH levels for the formulations.

(iv) pH values necessary for greatest stability which affects the choice of buffers, chelating agents, and antioxidants used in formulation.

(v) Hardness, friability, dissolution and disintegration times, particle size, solubility, aggregation and wetability of the drug substance in the dosage form.

In-vitro testing methods are available for monitoring these changes and extrapolating stability data. Two types of stability studies may be employed in drug evaluation. The most common is the storage of the drug dosage form in its container under simulated market conditions with assay and control tests made at regular intervals. By varying temperature, humidity and light conditions, approximations to various geographic marketing regions may be made.

The second is accelerated stability study which has demonstrated that by exaggerating environmental conditions in many cases close approximations to actual chemical and physical degradation rates may be extrapolated. This method is used as a predictive tool. Accelerated stability studies which have been well designed and properly performed have permitted accurate prediction of shelf life.

Techniques for evaluating physical and chemical stability include thin-layer chromatography, thermal analysis, diffuse reflectance and spectroscopy. Drugs must be pure before any stability tests are initiated. Most drugs fall into one of four classes; stable at all conditions, stable if handled correctly, e.g. aspirin, moderately unstable even with special handling e.g. vitamins and very unstable.

The objective in the prediction of stability is the determination of those factors that result in alteration of drug entity e.g. heat, light, oxygen, moisture. To determine effect of oxygen, samples of pure drug and drug with water are sealed in ampules some of which are flushed with oxygen and some with nitrogen and stored at different temperatures. Photosensitivity is measured by exposing the surface of compound to light. Moisture sensitivity is determined by placing the drug in open dishes at relative humidities of 30-90%. Samples are monitored regularly for physical changes, water content and chemical degradation.

Methods of studying polymorphs having different bioavailability and stability include:

(a) Dissolution:- Metastable forms are detectable because they have a faster dissolution.

(b) X-ray diffraction and I.R. Spectra:- Differences are observed in X-ray and I.R. spectra.

(c) Differential scanning colorimetry and differential thermal analysis:- Change from one polymorphic form to another involves a change in energy which is thermally detectable with above instruments.

(d) Hot-stage microscope:- On heating to phase transition point, crystal undergoes a change in birefringence and or appearance.

Solubility and stability characteristics in various solvents can be studied by a high temperature kinetic study of drug in solution. Appropriate solvent and buffer systems are selected and reaction rate constants determined at three temperatures in presence and absence of oxygen. An Arrhenius plot is made, heat of activation determined and room temperature stability extrapolated from these data. Generally solubility is determined as equilibrium solubility (Ravin 1969) or pH solubility profile (Dittert 1964).

Dissolution Rate Test

In-vitro dissolution rate tests are intended to measure the time required for a given drug in an

oral solid dosage form to go into solution under a specified set of conditions.

It affords an objective means towards the evaluation of the physiologic availability of the drug in-vitro since availability is largely dependent upon having the drug in the dissolved state.

As a method of assessing quality assurance, suitable dissolution rate is an important property of a good tablet product. Dissolution rate testing is done not only on finished dosage forms but can also be used to assess the characteristics of the pure drug or the effects of excipients, formulation and processing or manufacturing variables. It is also used during in-process quality control to ensure that a given product is uniform within the same batch and from batch to batch.

A dosage form must provide a stable environment during storage so that it can deliver the active ingredient in a form available for absorption. Dissolution rate testing can be used to assess stability after extended shelf-life storage.

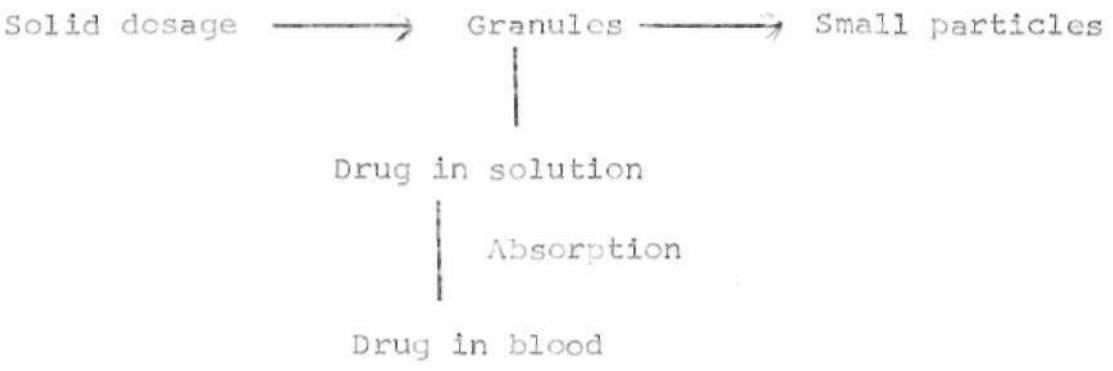
During early stages of product design, dissolution rate tests done in conjunction with in-vivo absorption studies can indicate the extent to which the release characteristics of the dosage form would influence blood levels of the drug. Where evidence for dissolution rate limited absorption exists, monitoring changes in the formulation by

means of dissolution rate tests provides a rational basis for optimizing a formulation.

The factors which affect dissolution rate of drugs include solubility, particle size, disintegration rate, chemical form and surface area.

Levy and Hayes (1960) have shown that absorption rate of drug is a function of its dissolution rate in its particular dosage form.

For a solid dosage form to get into solution and be absorbed it goes through a series of steps which can be represented as shown below.



Initially, the tablet has to be wetted so that the contents can dissolve. The overall process includes disintegration to the fine particles. The granules, aggregates and fine particles are all simultaneously dissolving thus there is an increase of surface area. These processes depend on the stirring, temperature, and contents of the dissolution medium as well as the specific design of the apparatus and its hydrodynamic stability.

The marked difference in blood levels after oral administration of different salts of theophylline

choline, isoprapanolamine and ethylenediamine was attributed to the differences observed in their rates of solution (Remington 1975).

Reduction in particle size is employed in pharmaceutical operations to secure better dispersion of drug in its vehicle. The relationship between particle size and dissolution rate has been investigated. Very fine state of particle division often leads to increase in dissolution rate. Dissolution of a single particle is expressed in terms of particle diameter at any given time by

$$a^2 = a_0^2 - \frac{2D\ C_t}{\rho}$$

a_o = Initial diameter

D = Fick's law constant

C = Concentration of dissolved solid

ρ = Particle density.

Levy and Dare (1964) have shown that rate of dissolution of drug is proportional to surface area of drug and concentration differential.

$$\frac{dc}{dt} = KS(C_s - C)$$

C = Concentration in dissolution medium at time t.

t = time

K = Intrinsic dissolution rate constant

S = Surface area

C_s = Solubility of drug in solvent.

K varies widely from drug to drug and some drugs may have a slow rate of dissolution despite a fine state of subdivision. Polymorphic drugs have more than one K (Remington 1975).

Assessing Quality Assurance During Manufacture And Finished Product

Total quality assurance certifies that each manufactured batch of product meets the established quality standards. It provides for the authorization of the release of manufactured product to the market based on laboratory testing which can be physical, chemical or biological.

Chemical and physical tests include tests for appearance, colour, odour, identity, optical rotation, specific gravity, solubility, viscosity, disintegration time, hardness, friability, average weight or volume per unit weight, content uniformity, moisture content and assay for active ingredient, impurities, and degradation products.

Macro and microbiological assays and tests for safety, toxicity, pyrogenicity, sterility, phenol coefficient, antiseptic activity and preservative action are also carried out.

Through purity and identity tests, the quality of drug is established. The assay measures the concentration of the drug in the dosage form. Most drugs are assayed by quantitative physico-chemical means. Where this is not possible macro/microbiological procedure is used. Biological testings

utilize intact animals, animal preparation, isolated living tissues or micro-organisms. To prevent objectionable micro-organisms in drug products not required to be sterile, the USP under microbial limit tests gives tests for total counts of viable micro-organisms.

An important test performed on the finished dosage form to guard against adulteration is the safety or toxicity test. Reference standards are used. Reference standards are prepared and distributed by USP or NF. WHO provides International Standards concerned mainly with standards for sera, vaccines, toxins, vitamins and endocrine extracts.

Pharmaceutical analysis include gravimetric, and volumetric assay methods, techniques like partition and adsorption chromatography, GLC, HPLC, UV and IR spectrophotometry, complexometry, chelatometry, nonaqueous titrimetry, fluorometry, polarography, differential scanning, calorimetry, X-ray spectroscopy, NMR, autoradiography, thermogravimetry and mass spectroscopy.

Analytical work involved may range from elemental analysis of compound to chemical and functional group determination as in complex medicinals like reserpine.

Automated instrumentation of analysis is also used. It has great potential for routine testing. Compendial methods for testing drugs are largely based on UV absorption measurements. An automated continuous

system for repetitive spectral scanning is the autoanalyzer which is capable of accepting tablet, capsule, powder, solution and suspension samples for analysis as required. Other automated systems include the titralyzer, Robot Chemist and Assayomat. The pharmaceutical industry employs detectors, scanners, readers, sensors, indicators, comparators and feed back mechanisms at various stages of manufacture. These mechanical and electronic inspection devices are employed to supplement and extend the capabilities of human beings.

Because of the vast amount of records maintained in a quality assurance programme the storage, and retrieval of data can be achieved using computer. Data is converted to digitized form and stored on computer tapes, cards, discs or in case storage.

The batch or control number is very important as it is the direct key by which the entire quality assurance programme in production, control, storage and distribution can be unlocked.

LITERATURE REVIEW

1.3 PROGUANIL

Proguanil is employed as an antimalarial agent and folic acid antagonist. It is effective as a causal prophylactic, a potent schizonticide and is lethal to actively developing pre-erythrocytic tissue forms of some plasmodium. It exerts a sterilizing effect on gametocytes.

History

Proguanil was discovered in Britain during the second world war. It was synthesized by Curd and Associates (1945). Early members of its congeners were synthesized as pyrimidine derivatives because of the known importance of pyrimidine-containing compounds in cell metabolism. Later members of the series including proguanil were simplified by opening the pyrimidine ring to give a biguanide.

Proguanil was first employed as an antimalarial (Adams and co-workers 1945) at the Liverpool School of Tropical Medicine after which it was given extensive clinical trial. Unfortunately, the value of the drug is now compromised by the development of resistant strains.

The fact that only single doses of antimalarial drugs are effective for only brief periods stimulated Thompson and co-workers (1963) to synthesize compounds that might act for much longer periods of time. Their optimism stemmed from the remarkably high potency of

the triazine metabolite of proguanil called cycloguanil pamoate (4,6-diamino-1-(p-chlorophenyl)-1,2-dihydro-2, 2-dimethyl-s-triazine). This metabolite was prepared and found to possess the desired properties. It was marketed under the proprietory name CAMOLAR. Camolar has been withdrawn from the market.

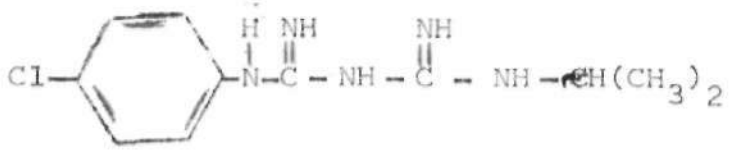
The prolonged period of protection afforded by this preparation was found to be due to the release of the active moiety from a depot formed at the site of injection and not to a systemic reservoir formed after administration.

Studies in man showed that a single intramuscular injection of 5mg/kg protects against P.falciparum and vivax for several months. Up to 80% remains at injection site two weeks after administration and small amounts can be found for as long as fifty-six weeks.

Due to incidence of resistant strains, clinical trials have involved the use of two depot preparations in combination. This is the triazine plus acedapsone (the diacetyl-4'4'-diaminodiphenylsulfone DADDS). It is marketed under the proprietory name Dapolar.

Chemistry

Proguanil is N¹-p-chlorophenyl-N^5-isoprophyl biguanide with the molecular structure:-



The molecular formular is $C_{11}H_{16}ClN_5$- and its molecular weight is 253.2. It occurs as colourless crystals which melt between 130-131°C and at 129°C when crystallized from toluene. It is used as proguanil hydrochloride. Its molecular formular is $C_{11}H_{16}ClN_5$. HCl. Molecular weight 290.2 and melting point 245°C. It is a white odourless powder having a bitter taste. It is soluble 1 in 110 parts of water and is more soluble in hot water, soluble 1 in 40 parts of ethanol (96%) and is practically insoluble in chloroform and ether. It is extracted by organic solvents from aqueous alkaline solutions.

The B.P. gives various methods of spectrophotometric identification. The methods used for analysis include ultraviolet radiation, paper and thin layer chromatography, infra-red radiation and high-performance liquid chromatography (Selkirk et-al 1980).

Pharmacological Effects

Malaria is a serious disease which affects about 15-20 million people in the world while about 40 million have the risk of infection. Endemic regions include Africa, Asia, South and Central America and the islands of S.W. Pacific. The most common pathogenic species of plasmodium inducing malaria are: Plasmodium vivax, Plasmodium falciparum, Plasmodium malariae and Plasmodium ovale. The efficacy of drugs in the treatment of malaria is related to the species of infecting parasite and its stage of development.

Many investigations have been carried out to determine the value of proguanil as a suppressive agent and causal prophylactic, as an effective schizonticide, gametocide, its mode of action with reference to effects on exoerythrocytic forms and its toxic effects.

Proguanil in falciparum malaria is a complete causal prophylactic with definite lethal action on pre-erythrocytic forms. A single dose of 50 to 100mg given 39-131 hours after exposure to infective mosquito bites affords complete protection. Lethal action is not exerted on the sporozoites which appear to be insusceptible. In vivax malaria it is a partial causal prophylactic in all instances and sexual parasites fail to gain access to blood while drug is being taken. During this period sub-innoculation tests are negative. This suggests an inhibitory action on schizogony of pre-erythrocytic forms.

Mackerras (1947) also showed that in vivax malaria trophozoites may grow to full size in presence of Proguanil. Its lethal action is only exerted upon the dividing nucleus which is rapidly destroyed. Plasmodium malariae was shown to be less susceptible to proguanil than vivax. Schizogony may occur up to the 20th hour after starting therapy and merozoites may grow to full size before being destroyed. Thompson et al (1963) have demonstrated that a single dose of a repository preparation of the triazine metabolite protected mice from infection with plasmodium berghei for up to two months and rhesus monkeys from infection with P. cynomolgi for up to eleven months. Schmidt and co-workers (1963) showed the same preparation protected monkeys for up to eight months.

Coatney et al (1963) evaluated effectiveness in man and found that the repository preparation of cycloguanil pamoate (Cl-501) when given as a single intramuscular injection at a dosage of 5mg/kg body weight can protect man from vivax malaria for a minimum of six months and a maximum of fourteen months.

Among the antimalarials reported to possess anti-arrhythmic activity are 4-aminoquinoline derivatives, 8-aminoquinolines, acridine and cinchona alkaloids. Arora and Madan (1956) investigated the anti-arrhythmic action of proguanil and its metabolite and compared them with quinidine for their action in

auricular fibrillation induced by acetylcholine, auricular flutter produced by injury stimulation procedure, and ventricular arrhythmias induced by petroleum ether and adrenaline. They were found to possess a weak quinidine-like activity.

Abdel-Aziz et-al (1971) reported that in isolated spontaneously contracting rat uterus pretreated with estradiol dipropionate proguanil was inhibitory. Progesterone treatment decreased inhibitory action. Inhibition occurs in early and late pregnancy. Action was blocked by atropine sulfate methysergide or a histamine antagonist.

Cotton and Sutorius (1971) also reported that proguanil inhibits action of glycose-6-phosphate dehydrogenase (G6PDH) from purified yeast or whole tissue extracts of erythrocytes and skin. Inhibition was competitive for NADP. This can have far reaching effects since G6PDH is the rate limiting point of entry into the pentose phosphate shunt which is the sole producer of extra-mitochondrial NADPH and produces ribose for nucleic acid synthesis.

Burn and Vane (1949) demonstrated that the action of acetylcholine on isolated rabbit auricles is gradually changed by exposure to proguanil from an inhibitory action to a stimulant action with a diminution in the size of the spontaneous contractions.

Absorption and Excretion

Proguanil is slowly but adequately absorbed from the gastrointestinal tract. Black (1946) performed

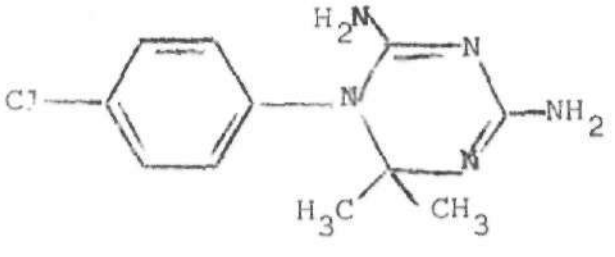
experiments to obtain some idea of the absorption rate of proguanil. He found that specimen collected half an hour after administration went through schizogony but after one, two and four hours, morphological changes occurred that is vacuolation, disappearance of and failure of chromatin division. Peak plasma levels were attained between two to four hours. Plasma concentration declined rapidly and was practically zero at twenty four hours. During prolonged administration, the drug accumulates only to a slight extent in the tissues. In man 40-60% of the absorbed drug is excreted in the urine and approximately 10% is excreted directly into the intestinal tract and eliminated in the feaces. In man 60% of the drug excreted in the urine is the parent compound and 30% is the triazine metabolite. Due to rapid elimination from the body, the triazine metabolite cannot be used directly.

Olunbona and Olowu (1985) investigated the effect of age on the cumulative amount of unchanged drug excreted in man. Their result showed that with increase in age up to 40 years there seems to be an increase in the percentage of an oral dose of proguanil excreted unchanged in the urine. In the young adults aged 30-40 years the cumulative amount of unchanged drug excreted in urine in 48 hours was 33.6% of the oral dose. This was significantly different from the value for children and teenagers (8-18 years) who

excreted 22.5%. In the age groups 19 - 29 years and 41-51 years, the values were significantly different from that of children and teenagers but not significantly different from that of the young adults (P 0.05).

Metabolism

Hawking (1947) showed evidence that metabolites of proguanil were responsible for activity. The two metabolites were isolated and identified as N'-p-chlorophenyl biguanide (PBG) and the triazine, 1-p-chlorophenyl-2-4-diamino-, 6-dihydro-1,3,5 triazine (CGT) (Carrington et al. 1951, Crounse 1951).



CGT

The active isomer is readily converted to an inactive isomer which is 4-amino-2-p-chloroaniline-1:6-dihydro-6:6-dimethyl-1:3:5 triazine. It is also readily hydrolysed to N'-p-chlorophenyl biguanide. Metabolism takes place in the liver. Armstrong (1973) showed that the formation of CGT involves binding to cytochrome P-448. N-Dealkylation is also mediated by a mixed function oxidase N-dealkylase which involves

binding to cytochrome P-450. He also observed specie difference in metabolism. PBG is the only metabolite isolated from rats and mice.

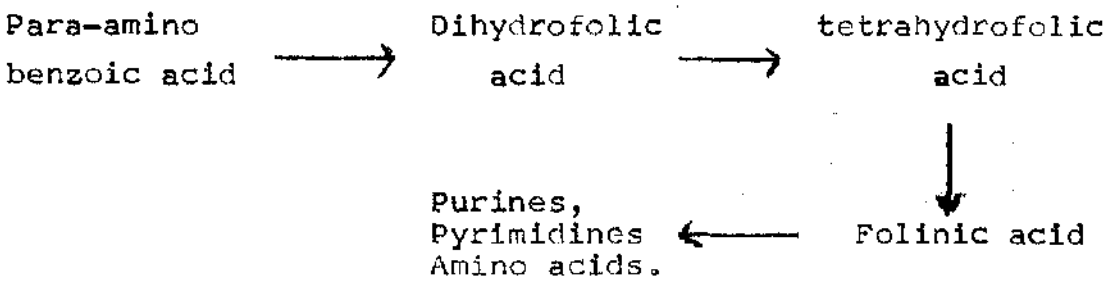
Further work suggested that the triazine metabolite is responsible for antimalarial activity (Crowther 1953). PBG has no measurable anti-malarial activity.

The metabolism of CG and CGT was also investigated by Smith and Co-workers (1961) in monkeys and man. When tested for antimalarial activity via same route of administration, CG was found to be 2-4 times more active than CGT. Their work with CG and its ethyl and n-propyl analogs suggested that it is very difficult to account for all the antimalarial activity of CG solely on the basis of the formation of an active triazine derivative from an inactive parent compound.

This leads to the conclusion that a very significant part of the antimalarial activity resides in the parent compound CG.

Mechanism of action

Proguanil and its metabolite cycloguanil inhibit conversion of dihydrofolate to tetrahydrofolate in parasite. The biochemical pathway involved is the synthesis of folinic acid from para-aminobenzoic acid. Folic acid whether preformed or synthesized is then used for the synthesis of nucleic acid, purines and pyrimidines.



The enzyme dihydrofolate reductase converts dihydrofolate to tetrahydrofolate with NADPH. It is present in both parasite and host. The drug binds to and inhibits dihydrofolate reductase. Its selective toxicity against parasite is due to the fact that they are more strongly bound to the enzyme of parasite than host enzyme.

Laser and co-workers (1975) have demonstrated that proguanil may exert chemotherapeutic effect by inducing premature lysis of parasitized cells.

Side Effects and Toxicity

In its use as an antimalarial, proguanil is remarkably free from toxic implications. The difference between effective therapeutic dose and toxic dose is very considerable. Significant toxic symptoms are not likely to follow its use in any dosage regimen adopted for purposes of therapy, suppression and causal prophylaxis. Large doses like 1g daily may cause vomiting, abdominal pain and diarrhoea. Excessive amounts may cause hematuria and transient appearance of casts in urine. Accidental overdosage as much as 14.5g has been followed by complete recovery. Margin of safety is quite satisfactory.

Gon and Reid (1975) reported a case of hemolytic anaemia and recurrent subacute disseminated intravascular coagulation associated with malarial prophylaxis in which the breakdown product of pyrimethamine and especially proguanil were incriminated as causative agents.

Black water fever apparently precipitated by proguanil has been reported (Singh 1953). Sen (1950) also reported a case of accidental poisoning.

Resistance

As an antimalarial agent, a high degree of acquired resistance to proguanil has been demonstrated conclusively in all types of plasmodia where it was sought. The characteristic appears to be stable Goodman and Gilman (1975). Proguanil resistance of a high degree has occurred in Plasmodium falciparum in South East Asia, West Africa and India. This trend has been attributed to unduly low or too widely spaced doses as an important causal factor. West African Plasmodium falciparum are naturally relatively insensitive to schizonticidal action of proguanil.

Bishop (1962) and McConnachiew (1948) reported that treatment of P. gallinaceum with representative sulphonamides such as sulphadiazine and sulphanilamide rapidly potentiated the development of proguanil resistance.

The works of Williamson (1972), Ferone and co-workers (1970) have shown that resistance to the antifols appears to be due to the development by

parasite of a mutant enzyme with decreased affinity for drug and is produced in larger quantity than normal enzyme thus compensating for the presence of the inhibitory drug.

Uses

Proguanil is employed as an antimalarial agent and folic acid antagonist. It is effective as a causal prophylactic, a potent schizonticide and is lethal to actively developing pre-erythrocytic tissue forms of some plasmodium. It exerts a sterilizing effect on gametocytes.

In vivax malaria, it controls acute clinical attack. It is an effective suppressive but does not result in radical cure. It has been effective in treatment of chloroquine resistant malaria when given with dapsone. Proguanil has been recommended for use during pregnancy.

Proguanil has been used for many years in tropical splenomegaly (Sagoe 1970). It is safe and mode of administration is simple-one 100mg tablet daily. Reports from endemic areas show that idiopathic tropical splenomegaly has been successfully treated with proguanil. It is preferred to splenectomy which has been the preferred treatment. Relapse usually occurred on discontinuance of proguanil but symptoms were controlled again when treatment recommenced.

Two groups of investigators from Costa Rica (Pena 1965, Beltran and co-workers 1966) have reported good results with cycloguanil pamoate in the treatment of leishmaniasis in their countries with particular advantage of only one injection being necessary. Their work was confirmed by Johnson (1968) who reported complete healing in twenty six cases studied but seven failed to respond. Pena (1965) in his report on treatment of American leishmaniasis observed improvement in all cases and complete clinical cure was obtained in 80% of patients.

1:4 PYRIMETHAMINE

History and Chemistry of Pyrimethamine

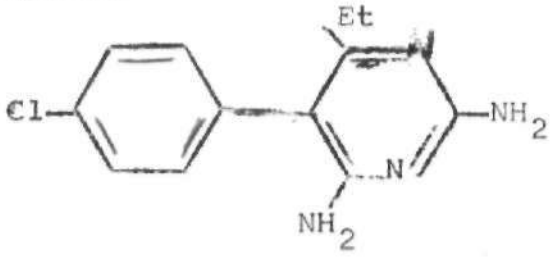
Several 2,4-diaminopyrimidines were found to antagonize competitively folic acid and folinic acids in the growth of Lactobacillus casei.

Prediction was made that L.casei would not be unique in its sensitivity to these substances.

Experiments with the diaminopyrimidines in the treatment of malaria in experimental animals bore out prediction; a high degree of antimalarial activity was found in several of them.

The most active, pyrimethamine, was later found to be highly effective against the plasmodia infecting man and has since been widely used for prophylaxis and suppression.

Pyrimethamine is 2,4-diamino-5-(P-Chlorophenyl)-6-ethyl pyrimidine



The molecular formular is $C_{12}H_{13}ClN_4$ and its molecular weight is 248.7. It is a white crystalline powder insoluble in water, soluble 1 in 200 parts of ethanol and 1 in 125 parts of chloroform. It melts between 239-242^{o}C. It is extracted by organic solvents from aqueous alkaline solutions.

Methods of identification include chromatography infra red absorption, UV absorption and HPLC.

Pharmacology

Pyrimethamine is an antimalarial drug. Its antimalarial effects are identical to those of proguanil but with higher potency. The major use is in prophylaxis and suppression and in concurrent therapy.

Pyrimethamine is well absorbed after oral administration and peak plasma concentrations are reached about two hours after a dose is taken. It is mainly concentrated in kidneys, lungs, liver and spleen. It is eliminated slowly and half-life is about four days. Suppressive concentration remain in the blood for two weeks. Metabolites appear in urine but there are few data on their structure or antimalarial activity. It is excreted in the milk of nursing mothers.

Mechanism of Action

Like proguanil, pyrimethamine inhibits the enzyme dihydrofolate reductase which catalyzes the reduction of dihydrofolate to tetrahydrofolate with NADPH.

Toxic Effects/Contraindications

With the recommended dosage of 25mg once weekly no significant toxic symptoms have been reported for pyrimethamine but 25mg given daily over a prolonged

period may cause depression of haemopoiesis due to the interference of the drug in metabolism of folic acid. Skin rashes may also occur. Excessive doses may produce megaloblastic anaemia, leucopenia, thrombocytopenia. Vomiting, convulsions and respiratory failure and even deaths have occurred in children.

It has been found to cause disruption of embryogenesis in rats (Dyban et-al 1965, Pozhidaev 1967).

Pyrimethamine is contraindicated in leukemia patients with pneumocystis carinii pneumonia. It should not be used after methotrexate in meningeal leukemia and should not be given to patients between courses of antineoplastic agents.

Resistance to Pyrimethamine

Resistant strains have arisen when small doses of pyrimethamine alone were used for long periods of time. They may also be cross-resistant to other dihydrofolate reductase inhibitors.

Therapeutic Uses

As an antimalarial drug, the main value of pyrimethamine is as a suppressant. It also affects gametocytes, rendering them incapable of complete development in the mosquito. A dosage of 25mg once weekly is usually adequate for the prophylaxis

of all forms of malaria. It has little effect in the treatment of actual primary attack of malaria and is slow in clearing parasitemia.

In combination with short-acting sulfonamide it is useful for the treatment of acute attacks of uncomplicated chloroquine-resistant P. falciparum malaria. Its combination with sulfadoxine is particularly appropriate for prophylaxis when given at intervals of 2 to 4 weeks.

Pyrimethamine given concurrently with triple sulfonamides has been found useful in the treatment of toxoplasmosis (Giracid 1960). Some success has followed its use in the treatment of polycythemia vera and in the prophylaxis of meningeal leukemia (Armata 1979). In combination with sulphadiazine it has been used in prophylaxis and treatment of pneumonia due to pneumocystis carinii (Dutz and co-workers 1976).

Aims and Objectives of the present study

Proguanil hydrochloride and pyrimethamine sulphate are among the drugs that are used in the treatment of malaria. They are commonly used in Nigeria for prophylaxis. Many pharmaceutical dosage form products tend to deteriorate with age and environmental conditions. It is therefore important to assess this tendency through stability studies thus ensuring the shelf-life stability of the products. There are few publications which deal with the stability of proguanil hydrochloride. Koskinen et-al (1972) determined the stability constant for protonation of proguanil hydrochloride. The values were given as $\log K^{\circ}_{1} = 11.3$, $\log K^{\circ}_{12} = 2.02$. They also determined the stability constant of Cu(II) complexes of proguanil at zero ionic strength and 25°. The values they got are $\log K_{1} = 8.7$, $\log K_{2} = 7.8$. Elmarakby (1982) investigated the effect of ultraviolet radiation, sunlight, heat and storage on proguanil hydrochloride. She concluded that proguanil hydrochloride solution did not show signs of photodegradation when irradiated at 254nm and 366nm. In addition proguanil hydrochloride tablets were found to be unstable to heat but stable on the shelf for up to eight years. British Pharmacopoeia indicates that proguanil hydrochloride tablets should be stored away from light therefore it will be of interest to see the effect of light on the

tablet especially on the dissolution rate. Dissolution rate tests have satisfactory predictive value in assessing biological equivalence of drug preparations. It was decided to carry out a more detailed investigation of the effect of UV radiation on the stability of proguanil hydrochloride in buffered solutions of pH values 4.5, 6.9 and 8.0 and in the tablet formulations.

There is no literature on the stability of pyrimethamine sulphate to light and as an important prophylactic antimalarial used in the tropics it was decided to extend this study to include pyrimethamine.

It is also envisaged that the results of this investigation would stimulate interest in research from other people on the stability of the two drugs since there is very little work carried out in this area.

CHAPTER 2

2:1 MATERIALS

Physiological buffer solutions

See Appendix 1 for method of preparation.

Drugs

Pyrimethamine sulphate powder supplied by Quality Control Unit Ahmadu Bello University Zaria.

Pyrimethamine sulphate tablets (Daraprim) supplied by Quality Control Unit Ahmadu Bello University Zaria. (QCU).

Batch number 5 = B5

Batch number 7 = B7.

Pyrimethamine sulphate tablets bought from local chemist (Dangi) in Zaria made by Wellcome Nigeria Limited.

Batch number 191 11U = B191

Proguanil hydrochloride tablets supplied by Quality Control Unit, Ahmadu Bello University, Zaria.

Batch number 19 = B19

Batch number 20 = B20

Proguanil hydrochloride tablets (Paludrine) bought from local chemist in Zaria produced by Imperial Chemical Industries Limited (ICI).

Batch number PL 29/5079 = ICI B29

Proguanil hydrochloride powder supplied by Quality Control Unit Ahmadu Bello University Zaria.

2.2 ASSAY

Pyrimethamine Tablets

20 tablets of pyrimethamine were accurately weighed and average weight of one tablet determined. They were powdered and quantity of the powder equivalent to 25mg of pyrimethamine was accurately weighed and dissolved in a litre of 0.005N HCl solution. The solution was filtered. Appropriate dilutions were made and UV absorbance taken at 273nM.

25mg of pyrimethamine powder was accurately weighed, dissolved in one litre of 0.005N HCl solution, equivalent dilutions were made and UV absorbance taken at 273nM.

Drug content of tablet was calculated from the ratio:-

$$\frac{\text{Absorbance of tablet}}{\text{Absorbance of powder}} \times \frac{100}{1}\%$$

Proguanil tablets

20 tablets of proguanil hydrochloride were weighed and the average weight of one tablet determined. The tablets were powdered and a quantity of the tablet equivalent to 100mg of proguanil hydrochloride was dissolved in sufficient distilled water to make one liter of solution. The solution was filtered, appropriate dilutions made and the UV absorbance taken at 253nm.

100mg of proguanil hydrochloride powder was also dissolved in one liter of solution, appropriate dilutions made and the UV absorbance taken. Content of drug was calculated as for pyrimethamine.

2.3 EFFECT OF pH ON UV SPECTRA

Proguanil hydrochloride

The effect of pH on the UV spectrum of proguanil hydrochloride was investigated by preparing solutions of 10 µg/ml and 4 µg/ml in 0.1N H_2SO_4, 0.1N HCl, buffered solutions of pH 1.5, 2.5, 3.5, 4.5, 6.9, 7.5 and running the UV spectra of the solutions.

Pyrimethamine Sulphate

The effect of pH on the UV spectrum of the drug was investigated by preparing solutions of 10 µg/ml in 0.1N HCl and in buffer solution pH 4.5 and running the UV spectra of the solutions. The drug dissolves in pH 4.5 after warming and leaving on the bench for sometime. The drug was insoluble in buffer solutions of pH 6.9 and 8.0.

Caliberation Curve

Standard solutions were prepared as follows:

100 µg of proguanil hydrochloride was accurately weighed and dissolved in 100ml of the appropriate solvent.to give a solution of 1mg/ml. 10ml of the solution was diluted to 100ml to give a solution 100 µg/ml. The solution of 100 µg/ml was further diluted as shown below and the UV absorbance of the solutions taken at the appropriate wavelength of maximum absorption.

1ml	in 25ml	=	4 µg/ml
2ml	in 25ml	=	8 µg/ml
2.5ml	in 25ml	=	10 µg/ml
3ml	in 25ml	=	12 µg/ml
4ml	in 25ml	=	16 µg/ml
5ml	in 25ml	=	20 µg/ml
6ml	in 25ml	=	24 µg/ml
6.5ml	in 25ml	=	26 µg/ml

Graphs of absorbance versus concentration were plotted. Slopes, intercepts and correlation coefficients were calculated using a T1 programmable 58 Texas instrument.

Dissolution Test

For the dissolution test, the USP XIX basket method is described here. The basic parts of the apparatus consists of a 1000ml-vessel made of glass, a variable speed drive, and cylindrical basket. The vessel is cylindrical with a flat bottom having and internal diameter of 100mm. It has a flanged upper rim. The vessel is fitted with a lid which has a number of openings one of which is central.

The variable speed motor causes a basket to rotate in the vessel. The speed of the motor is capable of being varied and being maintained within 5 percent of the required speed. The speed used in the present work is 100 revolutions per minute. The motor drives the basket by means of a shaft which is

approximately 6mm in diameter and about 300mm long in such a way that the basket revolves smoothly and without perceptible wobble. The shaft passes through the central opening in the lid of the vessel and is positioned so that its axis is not more than 0.2cm from the vertical axis of the vessel. It carries at its lower end a stainless steel disc of 25mm diameter in which there is a 2mm vent.

The cylindrical stainless steel basket assembly is made of woven wire cloth with a nominal aperture size of 425 um. It is joined down one side with a narrow welded seam and with a flanged rim at each end. The top of the basket is attached to the disc on the driving shaft by three clips.

The vessel is securely clamped in a water bath in such a way that displacement vibration from other equipment including the water circulating device is minimized. The water bath is set to maintain the temperature of the dissolution medium in the glass vessels at $37^{\circ} \pm 0.5^{\circ}C$ and keep the bath fluid in constant smooth motion.

The dosage unit is placed in a dry basket at the beginning of each test, the basket is lowered into position before the rotation is started.

This method was used in carrying out the dissolution tests in this work.

Procedure

One litre of the dissolution medium was placed in the flask of the dissolution apparatus and immersed in a constant temperature water bath maintained at $37^{\circ} \pm 0.5^{\circ}C$. This was allowed to equilibrate. A tablet was placed in the basket which was then immersed into the flask and rotated at 100 revolutions per minute starting from a noted time. Samples were withdrawn at ten minute intervals filtered and diluted. An equivalent volume of the dissolution medium previously maintained at $37^{\circ} \pm 0.5^{\circ}C$ was replaced into the dissolution medium. The dissolution runs were performed at least in triplicate. Samples of the dissolved drug withdrawn at given time intervals were assayed by reference to a standard caliberation curve. Cumulative concentration at a given time was calculated using the formula (Wurster and Taylor 1965)

$$Cn = Cs + \frac{X}{1000}$$

Cn denotes the cumulative concentration of drug released at time t if the previous samples had not been removed.

Cs denotes the spectrophotometrically measured concentration

X denotes the volume of sample removed at each time withdrawal was made.

For proguanil 5ml samples were withdrawn and diluted to 50ml. For pyrimethamine 10ml. Samples were withdrawn at each time interval and no dilution was made. 1000 is the volume of the dissolution medium in millilitres.

The formula makes cumulative correction for the previously removed samples not returned to the medium. Where a calibration curve had a significant intercept, concentrations Cs were calculated using

$$X = \frac{Y - c}{m.}$$

2.5 ULTRA-VIOLET IRRADIATION

Procedure

Proguanil hydrochloride solution

Proguanil hydrochloride powder 100mg was dissolved in 100ml of buffer solution pH 4.5 to give a solution of 1mg/ml. 1ml of the solution was diluted to 100ml to give a solution of 10 µg/ml. 10ml of the solution was put in two sample bottles and irradiated at 366nm with UV lamp. Camag UV lamp type 2900 with fixed wavelength at 366nm was used for irradiation. The time of irradiation extended from 8-426 hours. The UV spectra of irradiated samples and the corresponding non-irradiated samples (control) at room temperature were run on a Bye Unicam spectrophotometer SP8-100. Solutions of proguanil hydrochloride powder in pH 6.9 and pH 8.0 buffers were

similarly treated. The experiment was repeated and the time of irradiation limited to 8-168 hours. The influence of reaction vessel was also investigated. This was done by repeating the procedure using five sample bottles and one silica cuvette.

Pyrimethamine Sulphate Solution

Pyrimethamine sulphate powder 10mg was accurately weighed and dissolved in 100ml of phosphate buffer solution pH 4.5 to give a solution of 10 ug/ml. This solution was treated as described for proguanil hydrochloride. Pyrimethamine sulphate was insoluble in pH 6.9 and pH 8.0 buffer solutions, therefore, the experiment could not be carried out at these pH values.

Proguanil hydrochloride tablets

Proguanil hydrochloride tablets 100mg were placed in a clean watch glass and irradiated with a 366nm UV lamp. The time of irradiation extended from one hour to two months. Samples were removed at intervals of 12, 48, 96 hours, two weeks, one month and two months, respectively. Dissolution rate tests of the samples were carried out. Non-irradiated tablets at room temperature were used as control.

Pyrimethamine sulphate tablets

Pyrimethamine sulphate tablets 25mg were similarly treated as described for proguanil hydrochloride tablets.

2.6 ASSAY OF IRRADIATED TABLETS

Proguanil hydrochloride tablets 100mg were placed in a clean watch glass and irradiated with a 366nm UV lamp. The time of irradiation extended for three weeks. Samples were removed at intervals of 4,7, 10, 14 and 21 days and assayed as described for non-irradiated tablets. Non-irradiated tablets at room temperature were used as control.

Pyrimethamine sulphate tablets

Pyrimethamine sulphate tablets 25mg were similarly treated as described for proguanil hydrochloride tablets.

CHAPTER 3

RESULTS AND DISCUSSION

The effect of UV radiation on the stability of proguanil hydrochloride and pyrimethamine sulphate tablets and solutions has been investigated. The influence of reaction vessel and pH on the irradiated solutions were studied. Also stated in this chapter are the results of the effects of UV radiation on the dissolution rate profiles of proguanil hydrochloride and pyrimethamine sulphate tablets in phosphate buffered solution pH 4.5. The effect of pH on the UV spectra of the drugs was studied.

3.1 EFFECT OF pH ON THE UV SPECTRA OF:-

(a) Proguanil hydrochloride solution

It was observed that the UV-spectra of proguanil hydrochloride solution in 0.1N H_2SO_4, 0.1N HCl and in phosphate buffer solutions pH 1.5, 2.5, 3.5, 4.5, 6.9, and 7.5 were markedly different. The spectrum in 0.1N acid and pH 1.5 were similar showing a flattened peak (fig. 1). The spectra of the drug in buffer solutions of pH 2.5, 3.5, 4.5, 6.9, 7.5 were similar showing the same criteria for the two peaks at 253nm and 232nm (fig. 1). The disappearance of peak at 232nm at low pH could be assigned to proton uptake by the amine groups of proguanil. The effect of the amine substituents would be nullified giving different wavelength of maximum absorption in acid

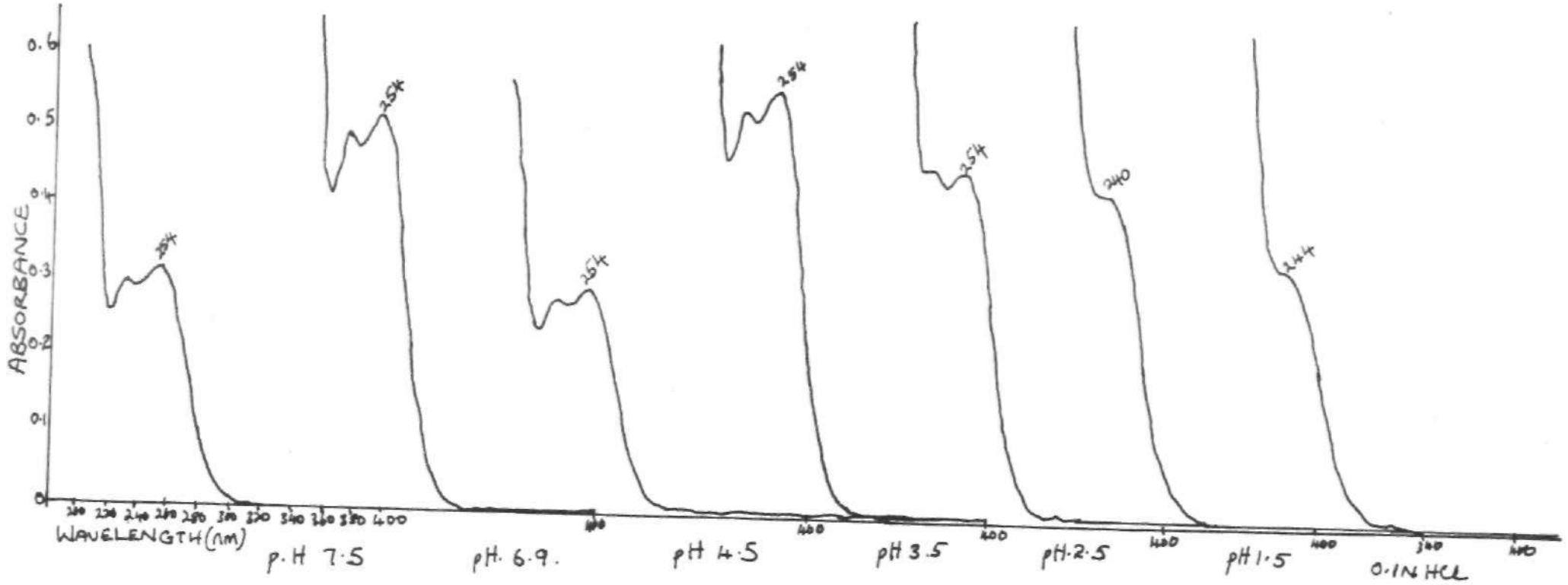
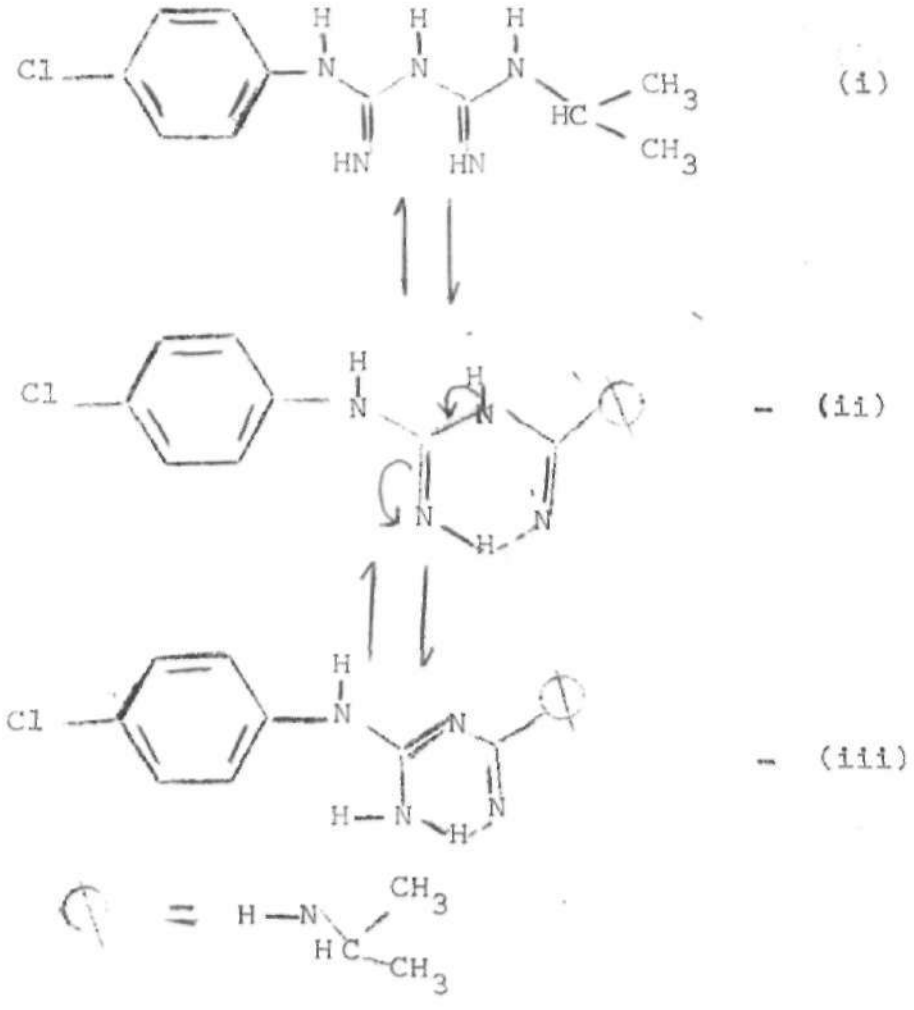


Fig 1. Effect of pH on the uv spectrum of proguanil hydrochloride.

and alkaline medium. No bathochromic shift observed between pH 2.5 and pH 7.5 but rather a flattening of the peak at 253nm with a point of inflexion at 248nm was quite evident. This seems to indicate that protonation resulted in another reaction within the medium that led to loss of peak. Although this work was not aimed at establishing the mechanism of this reaction, a proposed possible reaction of proguanil hydrochloride in strong acid medium which could lead to the loss of the spectral peak at 232nm is shown in Scheme I.

SCHEME 1



1. The possibility of the 6-membered ring formation plus the electronegativity of nitrogen allows an intramolecular H-bonding by N_6 and N_8 to form (ii).

2. Tautomeric shift of the proton on N_3 to N_6 allows the biguanide structure to rearrange to a conjugated system as in (iii). The peak at max 232nm is probably due to this chromophore.

3. In strong acid media (pH 2) all the nitrogen are almost certainly protonated. The reduced electronegativity of nitrogen with consequent disruption of the H-bond the loss of the conjugated system and the extension of the double bonds would explain the disappearance of the peak at max 232nm. The peak at max 253nm is probably due to the p-aminochlorobenzene. In strong acid medium, the nitrogen is protonated and the spectrum of this moiety reduces essentially to that of benzene whose absorptions at max 203.5 (7400) and 254 (204) would be in agreement with the observed spectra.

Pyrimethamine sulphate solution

The UV spectra of pyrimethamine sulphate solutions in 0.005N HCl, 0.1N HCl and in buffer solution pH 4.5 were similar. The drug was not soluble in pH 6.9 and pH 7.5 (See fig. 2).

3.2 ASSAY

Assay of proguanil hydrochloride tablets used (ICI B19 and B20) shows that there was no considerable difference in their content of proguanil hydrochloride (Table 1). BP stated drug content is 95-105%.

Assay of pyrimethamine sulphate tablets (B5, B7 and B191) shows that there was no considerable difference in their content of pyrimethamine sulphate (Table 2). BP stated drug content is 92.5-107.5%. From the results it can be concluded that the batches of the drugs used (proguanil hydrochloride B19, B20 and ICI, pyrimethamine sulphate B5, B7 and B191) complied with the official B.P (1980) specification for drug content.

3.3 DISSOLUTION TEST

Linear calibration curves with good correlation coefficients of proguanil hydrochloride are shown in figures 3a, 3b and 3c. The dissolution rates of the tablets containing proguanil hydrochloride (designated as ICI, B19 and B20) in phosphate buffer solutions pH 2.0, 4.5, and 7.2 calculated as the percent proguanil dissolved at various time periods are shown in Tables 3-5). Figures 4-6 graphically represent the time dissolution rate profiles.

Figure 4 shows the comparison between in-vitro dissolution rate profile of proguanil tablets in phosphate buffer solution pH 2.0. Each point is average of triplicate determinations. The dissolution

TABLE 1: QUANTITATIVE DETERMINATION OF PROGUANIL HYDROCHLORIDE TABLETS

Compound	Drug Content (in mg)	Percentage	S.D
QCU B19	99.39	99.89	± 0.62
QCU B20	101.93	101.93	± 0.08
ICI	97.61	97.61	± 0.31

BP 1980 specified drug content = (95-105)%

S.D. = Standard deviation.

TABLE 2: QUANTITATIVE DETERMINATION OF PYRIMETHAMINE SULPHATE TABLETS

Compound		Drug Content (in mg)	Percentage(%)	S.D.
QCU	B5	25.08	100.31	± 2.15
QCU	B7	25.22	100.88	± 0.89
	B191	25.29	101.16	± 0.73

BP 1980 specified drug content = (92.5-107.5)%

S.D. = Standard deviation.

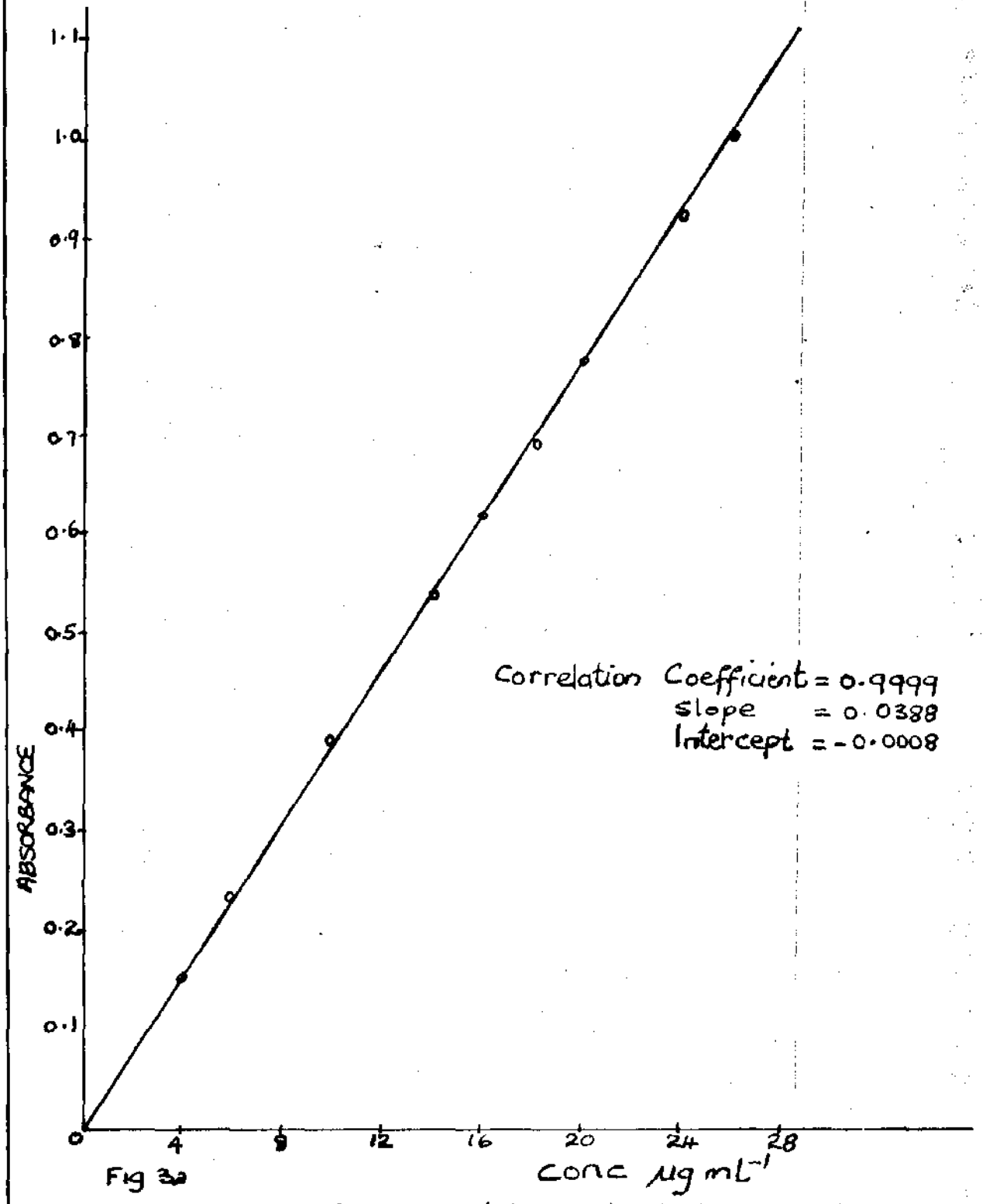


Fig 3a

Calibration curve for proguanil hydrochloride in phosphate buffer solution pH 2.0 using uv spectraphotometer.

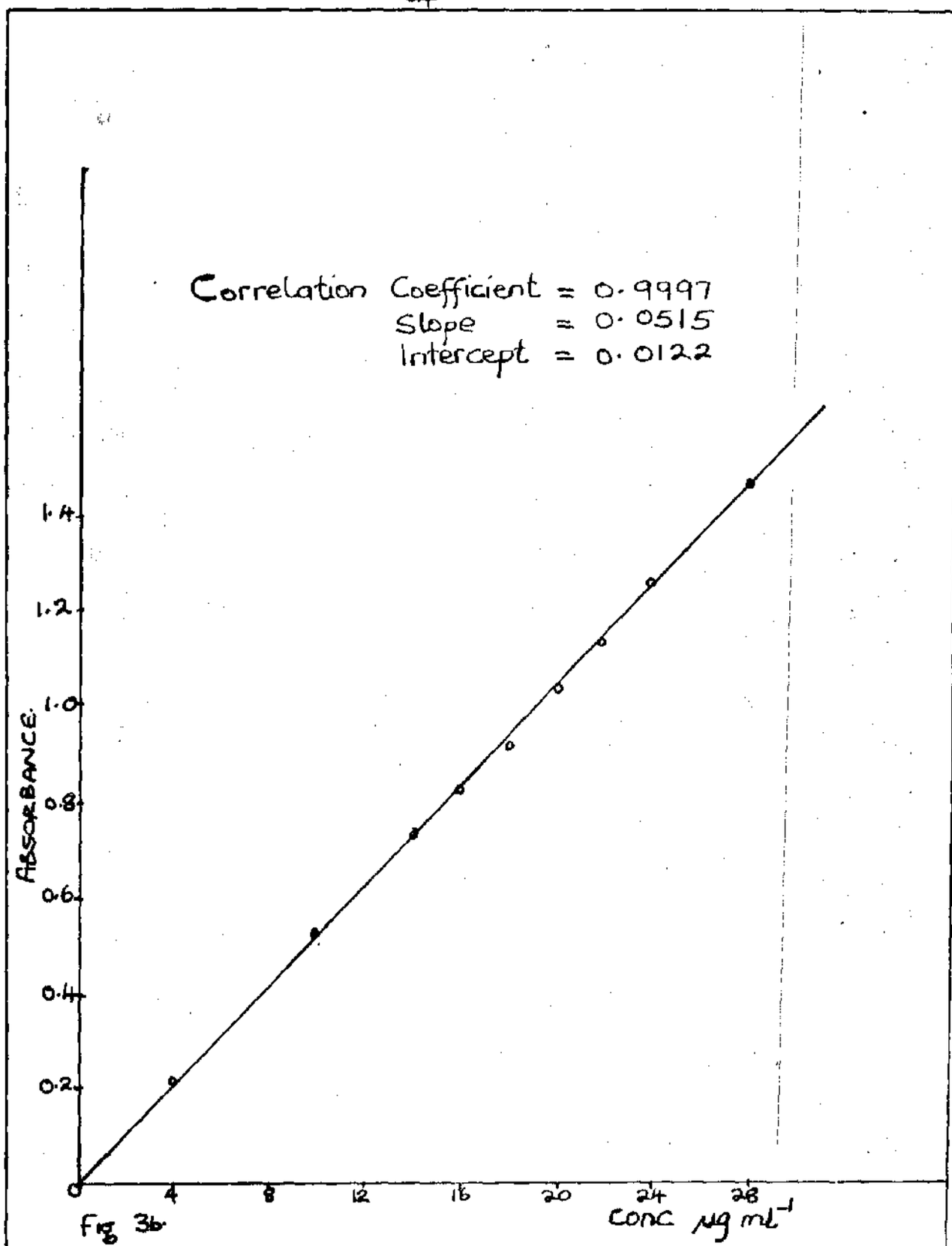


Fig 36

Calibration Curve for proguanil hydrochloride in phosphate buffer solution pH 4.5 using UV spectrophotometer.

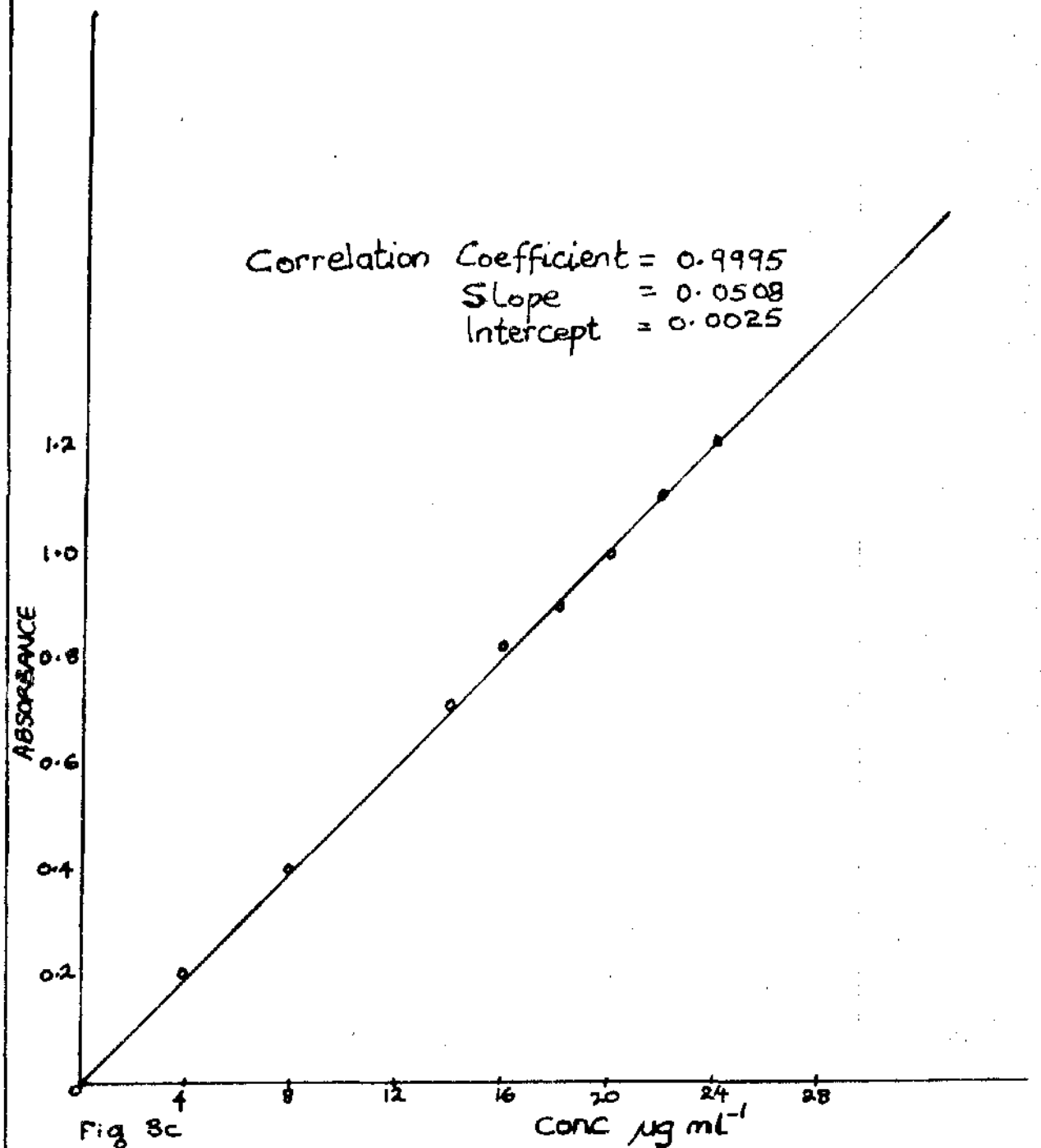


Fig 8c

Calibration Curve for proguanil hydrochloride in phosphate buffer solution pH 7.2 using UV spectrophotometer.

TABLE 3.1: DISSOLUTION RATE DATA OF THE THREE LOTS OF PROGUANIL HYDROCHLORIDE TABLETS IN PHOSPHATE BUFFER SOLUTION pH 2.0.

TIME MINUTES	Percentage Released (%)		
	B19	B20	ICI
10	84.81	63.32	52.13
20	87.63	89.07	76.90
30	88.12	92.42	82.58
40	88.61	93.37	84.88
50	88.65	93.44	86.26
60	88.69	93.52	86.27
70	88.73	93.59	86.29
80	88.76	94.10	86.30
90	89.24	94.17	86.31

TABLE 3.2: DISSOLUTION RATE HALF-LIVES ($T_{50\%}$) OF PROGUANIL HYDROCHLORIDE TABLETS IN PHOSPHATE BUFFER SOLUTION pH 2.0.

TABLET	$T_{50\%}$ (MINUTES)
B19	6.0
B20	8.0
ICI	9.5

KEY

B19, B20 = Quality Control Products.

ICI = Imperial Chemical Industries Product.

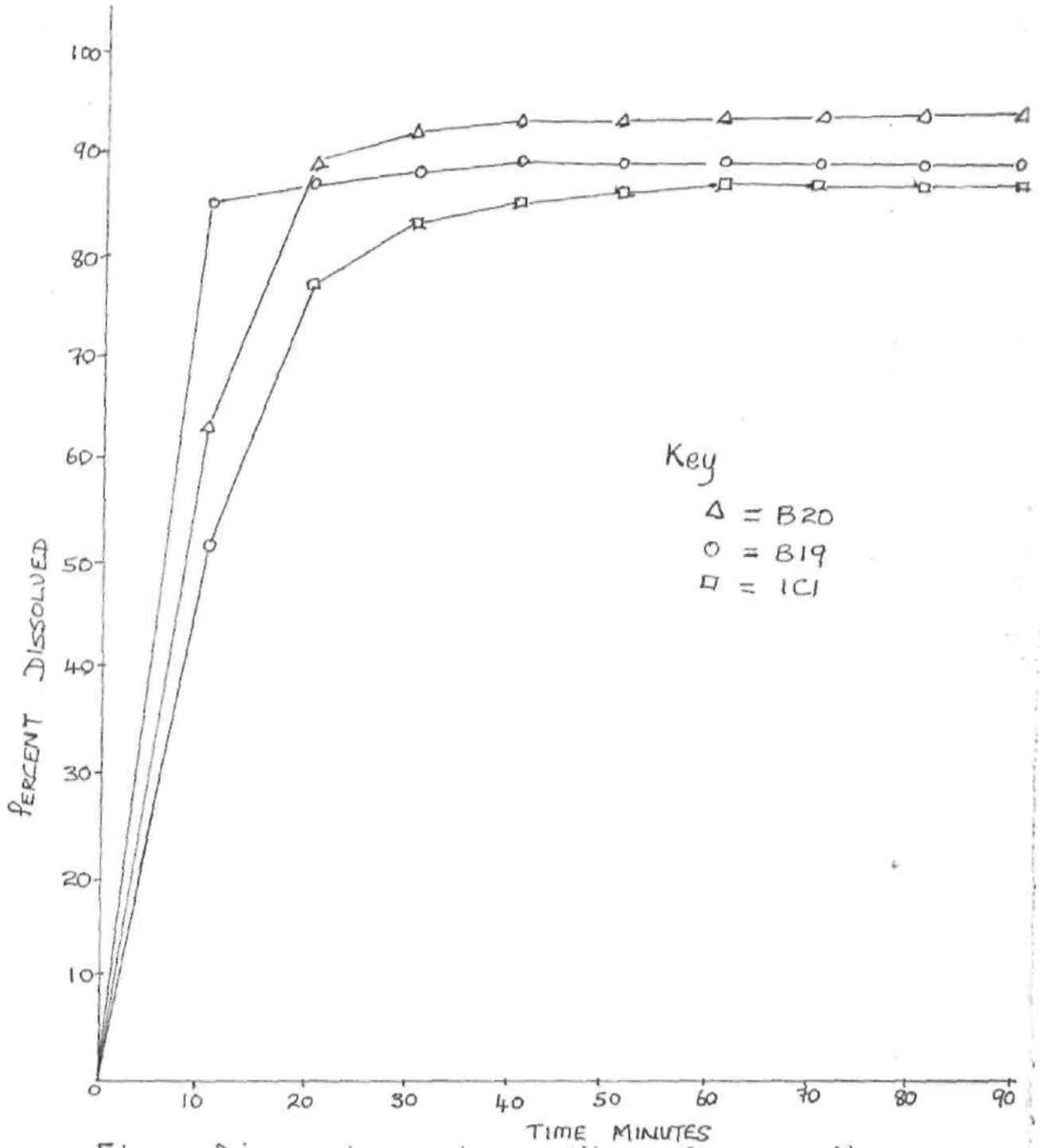


Fig 4: Dissolution rate profiles of proguanil hydrochloride tablets in pH 2.0

rate increased for the first thirty minutes and finally became constant. The dissolution half-lives T50% expressed as the time required for 50% of the drug to be released in the dissolution medium were found to be 6.0, 8.0, and 9.5 minutes for B19, B20 and ICI respectively. T50% was less than ten minutes for all the batches. Although 100% release was not achieved in any of the batches from the 30th minute the cumulative percentage release became constant. It appears that the dissolution medium was probably saturated so that no more drug went into solution.

Figure 5 shows the comparison between in-vitro dissolution rate profile of proguanil hydrochloride tablets in phosphate buffer solution pH 4.5. At pH 4.5 there was an initial rapid release such that in the first 10 minutes more than 70% of drug was released into the dissolution medium. T50% were found to be 3.5, 4.8, and 6.5 minutes for B19, B20 and ICI respectively.

Saturation of medium was not observed at pH 4.5 and cumulative release was not significantly different for the three batches (See table 4). Cumulative percentage release was significantly different from values at pH 2.0 (Paired t-test p 0.001).

The best dissolution occurred at pH 4.5 probably indicative of the fact that this drug dissolves best in a weakly acidic medium. Extrapolating the in-vitro results to in-vivo situation maximum

TABLE 4.1 DISSOLUTION RATE DATA OF THE THREE LOTS OF PROGUANIL HYDROCHLORIDE TABLETS IN PHOSPHATE BUFFER SOLUTION pH 4.5

TIME MINUTES	Percentage Released (%)		
	B19	B20	ICI
10	75.95	72.85	72.27
20	84.34	78.12	80.78
30	88.26	83.42	87.71
40	91.70	85.30	89.81
50	94.15	87.68	91.79
60	96.62	89.38	93.26
70	98.09	90.51	95.26
80	98.56	91.93	96.49
90	99.04	92.67	98.75

TABLE 4.2: DISSOLUTION RATE HALF-LIVES ($T_{50\%}$) OF PROGUANIL HYDROCHLORIDE TABLETS IN PHOSPHATE BUFFER SOLUTION pH 4.5

TABLET	$T_{50\%}$(MINUTES)
B19	3.5
B20	4.8
ICI	6.5

KEY

B19, B20 = Quality Control Products.

ICI = Imperial Chemical Industries Product.

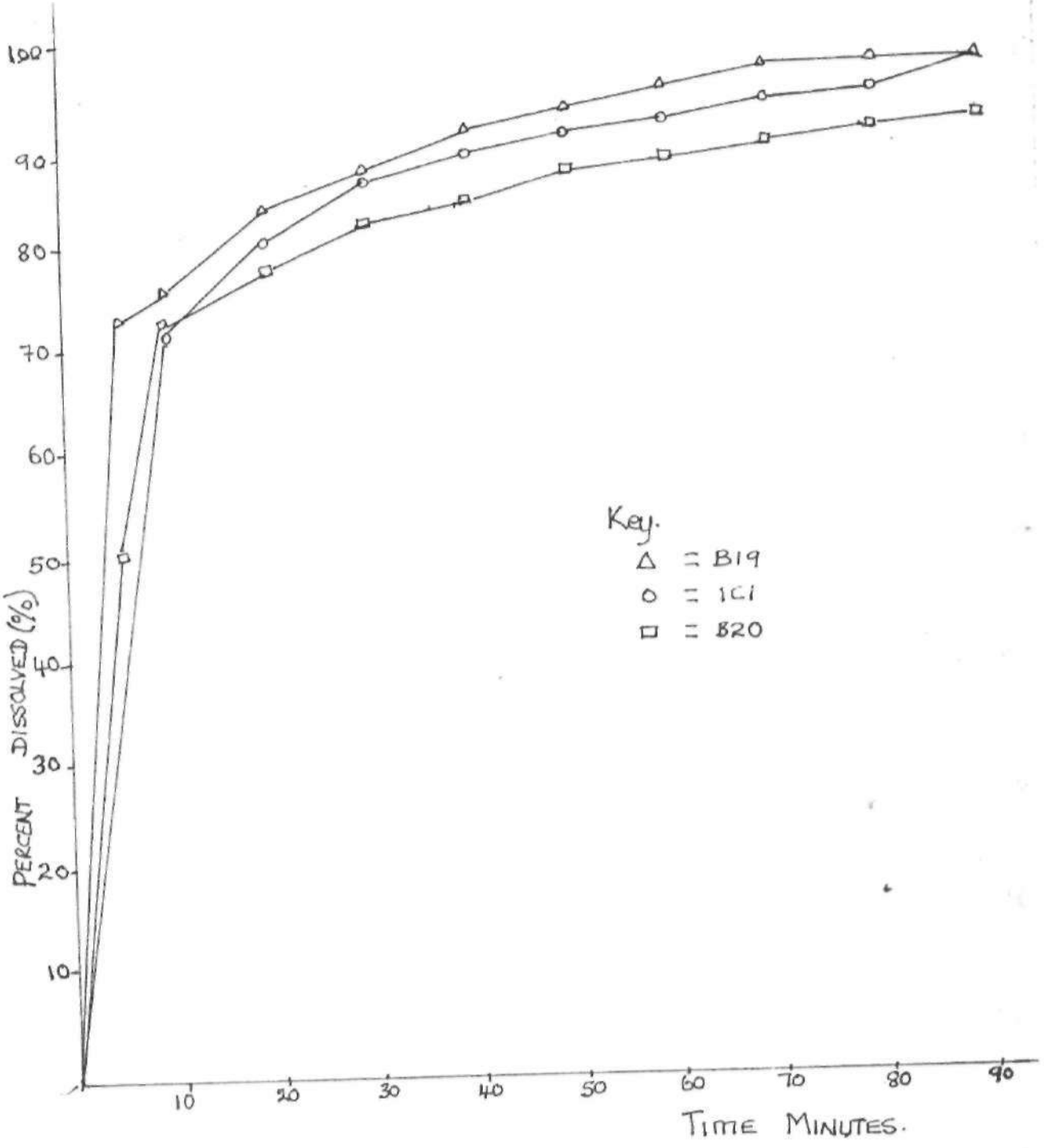


Fig 5 Dissolution rate profiles of proguanil hydrochloride tablets in buffer solution pH 4.5.

absorption of proguanil could be expected to occur in the proximal part of the small intestine. This is very likely because the drug is basic and at pH 2.0 it will be almost wholly cationic in the stomach and the cation being hydrophilic is not appreciably absorbed. In a less acidic medium the unionised lipid soluble form of the drug will predominate to ensure good absorption.

Figure 6 shows the comparison between the dissolution rate profile of proguanil hydrochloride tablets in phosphate buffer solution pH 7.2. At pH 7.2 there is very high initial drug release, above 80% of the drug is in solution by the 10th minute. At about 30 minutes a regular pattern had been initiated for all the batches, the dissolution rate increased and finally became constant. There is no significant difference in the rate and extent of the drug released. If this were to simulate in-vivo condition, at this pH, the three batches could be said to be bioequivalent. T50% were found to be 6.0, 6.0 and 6.5 minutes for B19, ICI and B20 respectively.

The official BP 1980 requirement for dissolution rate test states that for each dosage form tested, the amount of active ingredient in solution is not less than 70% of the stated amount after 45 minutes unless otherwise specified in the monograph. At all pHs these results complied with this specification.

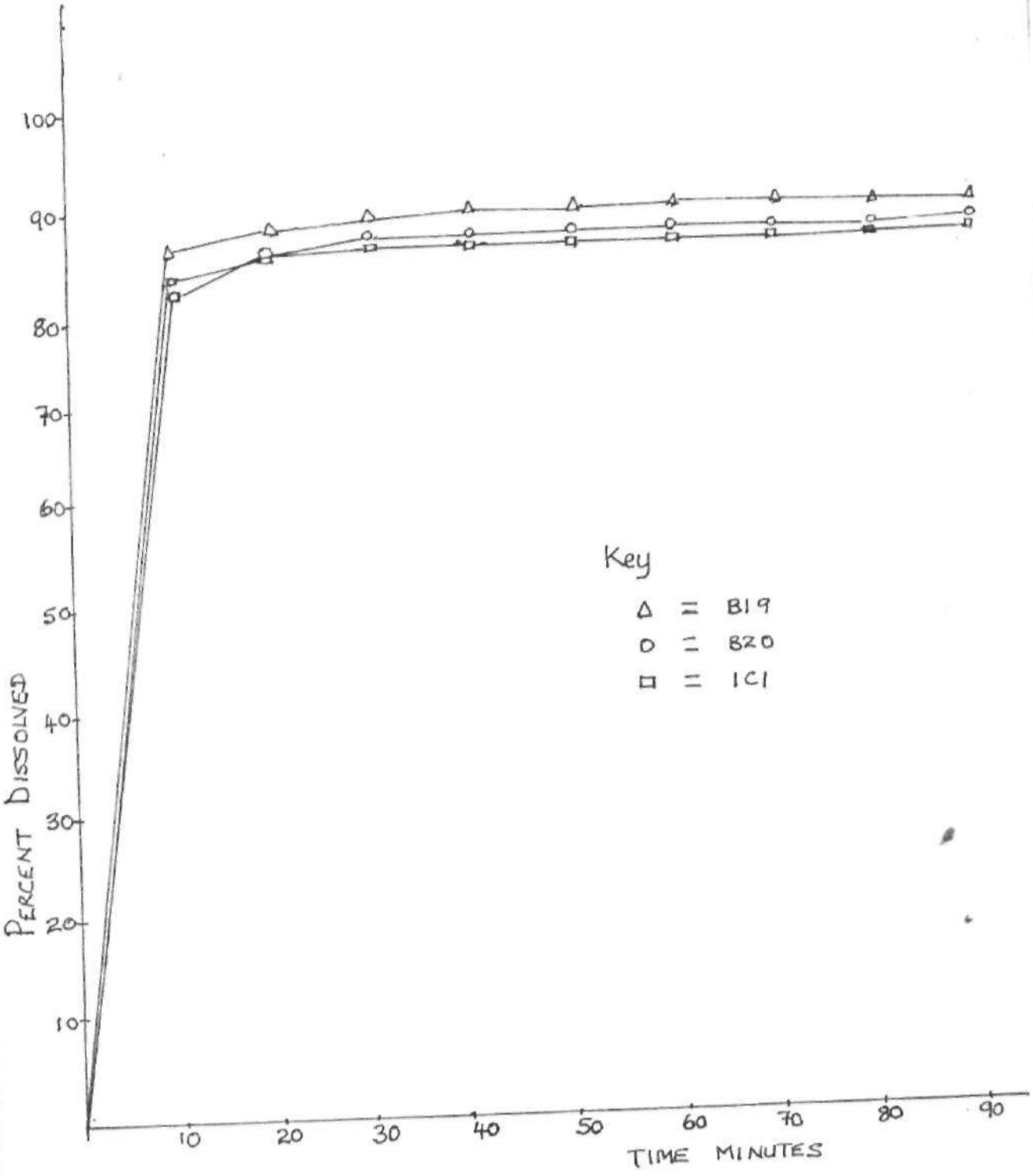


Fig 6. Dissolution rate profiles of proguanil hydrochloride tablets in buffer solution pH 7.2

TABLE 5.1: DISSOLUTION RATE DATA OF THE THREE LOTS OF PROGUANIL HYDROCHLORIDE TABLETS IN PHOSPHATE BUFFER SOLUTION pH 7.2.

TIME MINUTES	Percentage Released (%)		
	B19	B20	ICI
10	86.71	82.34	84.15
20	88.09	85.43	85.99
30	89.47	86.78	86.47
40	89.52	87.26	86.49
50	89.56	87.30	86.51
60	89.61	87.34	86.53
70	89.65	87.38	86.09
80	89.69	87.42	86.55
90	89.72	87.45	86.56

TABLE 5.2: DISSOLUTION RATE HALF-LIVES ($T_{50\%}$) OF PROGUANIL HYDROCHLORIDE TABLETS IN PHOSPHATE BUFFER SOLUTION pH 7.2.

TABLET	$T_{50\%}$ (MINUTES)
B19	6.0
B20	6.5
ICI	6.0

KEY

B19, B20 = Quality Control Product.

ICI = Imperial Chemical Industries Product.

At the given pH, temperature, agitation and other unidentified variables the results indicate the solubilities of the drug in the preparations.

3.4 IRRADIATION OF PROGUANIL HYDROCHLORIDE TABLETS

The results of the effect of UV irradiation on the dissolution rate profile of proguanil hydrochloride tablets in phosphate buffer solution pH 4.5 are shown in Figures 7-8 and Tables 6-7.

It can be seen there was variation in the c cumulative percentage release of the non-irradiated and irradiated tablets in the buffer solution at pH 4.5 although neither the degree of agitation (100 r.p.m) nor the volume (1000ml) of the dissolution fluid was varied.

An average decrease of 21% of the cumulative percent release of the non-irradiated drug was observed. The results obtained were subjected to statistical (paired t-test) analysis which indicated a significant difference between the results of the non-irradiated and irradiated tablets at the 5% confidence level. See tables 6 and 7.

UV irradiation affects the dissolution rate profile of proguanil hydrochloride tablets in phosphate buffer solution pH 4.5.

3.5 IRRADIATION OF PROGUANIL HYDROCHLORIDE SOLUTION IN BUFFER SOLUTIONS pH 4.5, pH 6.9 and pH 8.0

When this experiment was first performed in buffer solution pH 4.5 using silica cuvettes, the

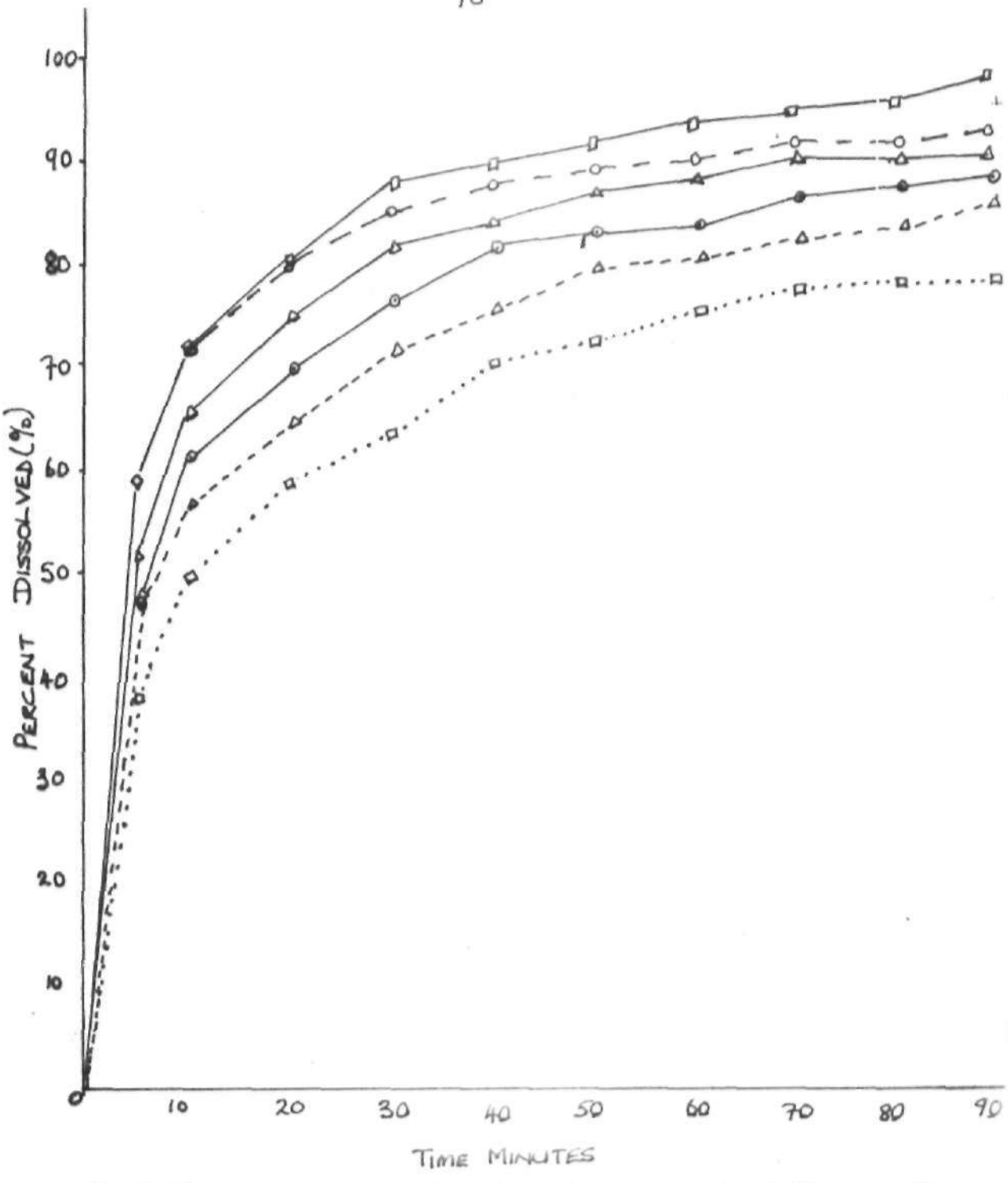


Fig 7. Effect of uv irradiation(316nm) on the dissolution profile of proguanil hydrochloride tablets(IC1) in buffer pH 4.5.

Key:

—□—□—□ = Non irradiated tablets
– o – – o – – o = Tablets irradiated for 12hours.
—△—△—△ = Tablets irradiated for 96hours
—⊙—⊙—⊙ = Tablets irradiated for 2weeks
– – △ – – △ – – △ = Tablets irradiated for 1 month
. . □ . . . . □ . . . . □ = Tablets irradiated for 2 months.

TABLE 6: EFFECT OF UV RADIATION ON THE DISSOLUTION RATE PROFILE OF PROGUANIL HYDROCHLORIDE TABLETS (ICI) IN PHOSPHATES BUFFER SOLUTION pH 4.5

TIME		Percentage Released (%)						
		HOURS OF IRRADIATION						
MINUTES	NI	12	24	48	96	2 weeks	1 month	2 months
5		60.24	64.02	59.90	52.25	47.41	46.50	37.91
10	72.27	**72.52**	**74.59**	**72.50**	**66.34**	**61.82**	57.25	50.38
20	80.78	80.57	80.59	81.59	75.38	70.35	64.67	58.84
30	87.71	85.06	85.09	86.09	81.89	76.68	72.06	63.73
40	89.81	87.53	87.56	88.56	84.35	81.66	76.09	70.55
50	91.79	89.01	89.45	90.02	86.81	82.56	80.16	73.00
60	93.26	90.45	90.92	92.52	88.26	84.84	80.61	75.87
70	95.26	91.91	92.39	92.96	89.72	87.11	83.32	78.31
80	96.49	92.36	92.84	93.41	90.15	91.36	83.77	78.69
90	98.75	92.81	93.28	93.86	90.59	91.87	86.50	79.10
	P	<0.001	<0.02	<0.05	<0.001	<0.001	<0.001	<0.001

NI = Non Irradiated tablet.

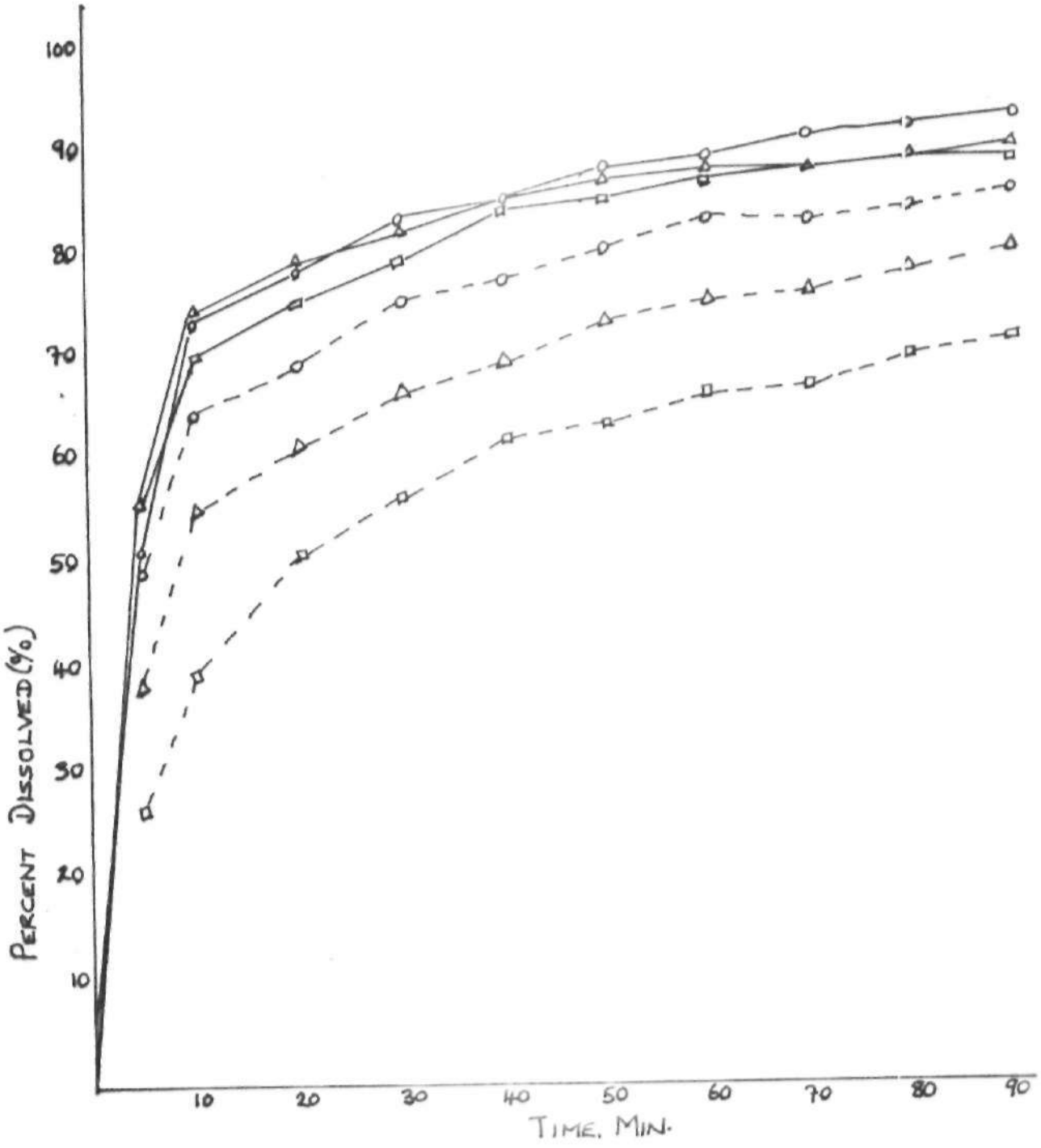


Fig 8 Effect of uv irradiation on the dissolution rate profile of proguanil hydrochloride tablets (acu BZO) in buffer solution pH 4.

Key.

o—o—o = Non Irradiated tablets.
Δ—Δ—Δ = Tablets irradiated for 12 hours
□—□—□ = Tablets irradiated for 48 hours
o---o---o = Tablets irradiated for 2 weeks
Δ---Δ---Δ = Tablets irradiated for 1 month
□---□---□ = Tablets irradiated for 2 months

TABLE 7: EFFECT OF UV RADIATION ON THE DISSOLUTION RATE PROFILE OF PROGUANIL HYDROCHLORIDE TABLET QCU B20 IN PHOSPHATE BUFFER SOLUTION pH 4.5.

TIME		Percentage Released (%)					
		HOURS OF IRRADIATION					
MINUTES	NI	12	48	96	2 weeks	1 month	2 months
5	51.02	55.90	54.94	56.41	49.05	38.27	26.49
10	72.85	73.86	71.21	70.23	64.01	55.14	39.38
20	78.12	79.13	75.49	75.49	69.24	60.81	51.34
30	83.42	81.98	79.49	79.49	74.49	66.03	56.50
40	85.30	84.84	82.33	84.30	76.82	69.30	61.69
50	87.68	86.92	86.17	85.20	79.65	73.24	63.47
60	89.38	87.64	87.58	87.29	82.98	74.90	66.23
70	90.51	88.07	88.00	87.71	83.39	76.25	67.37
80	91.93	88.98	89.41	88.92	83.79	77.60	69.67
90	92.67	90.39	89.84	89.35	85.67	79.94	70.99
	P	< 0.5	< 0.5	< 0.001	< 0.001	< 0.001	< 0.001

NI = Non-Irradiated tablets.

solutions were subject to evaporation although the cuvettes were covered. This made it difficult to follow the reaction and laboratory specimen bottles were employed in place of the cuvettes.

Significant differences were recorded both in the absorbances and spectral picture of duplicate experiments at the same pH. At pH 6.9 in one experiment the peak at 232nm disappeared after sixteen hours while in the duplicate it disappeared after twenty four hours. At pH 8.0 the peak at 232nm became the major peak after 16 hours while in the duplicate, there was no visible change even after 426 hours. At pH 4.5 no visible change was observed in the spectra even after 426 hours (See figures 9 (1a-1b), 10(1a-1b), 11 and table 8).

These results were indication that the reaction vessel could have influenced the reaction to an appreciable extent and so the above observations cannot be attributed to pH changes. To investigate the influence of the glass vessel used for the reaction, a set of experiments were performed at each pH using the same sample bottles. It was observed that at pH 4.5, evidence of photochemical reaction appeared after 8 hours of irradiation. Black precipitate was observed. The peak at 232nm became the major peak after 16 hours and disappeared after 24 hours. This observation was made in one experiment out of six. In two other experiments,

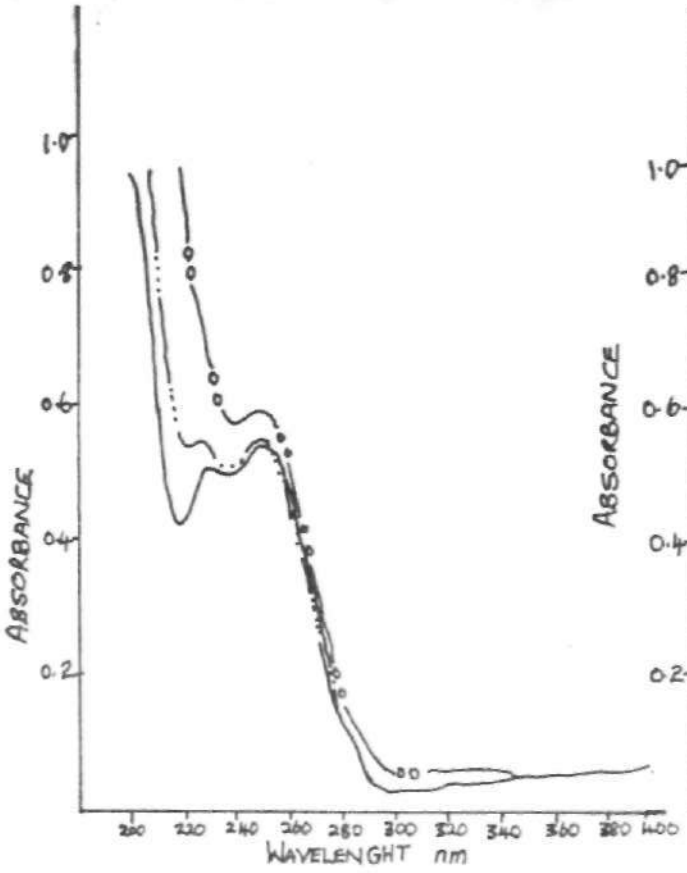


Fig 9a. Uv spectrum of proguanil hydrochloride after 16 hours exposure to uv irradiation in phosphate buffer solution pH 6.9.

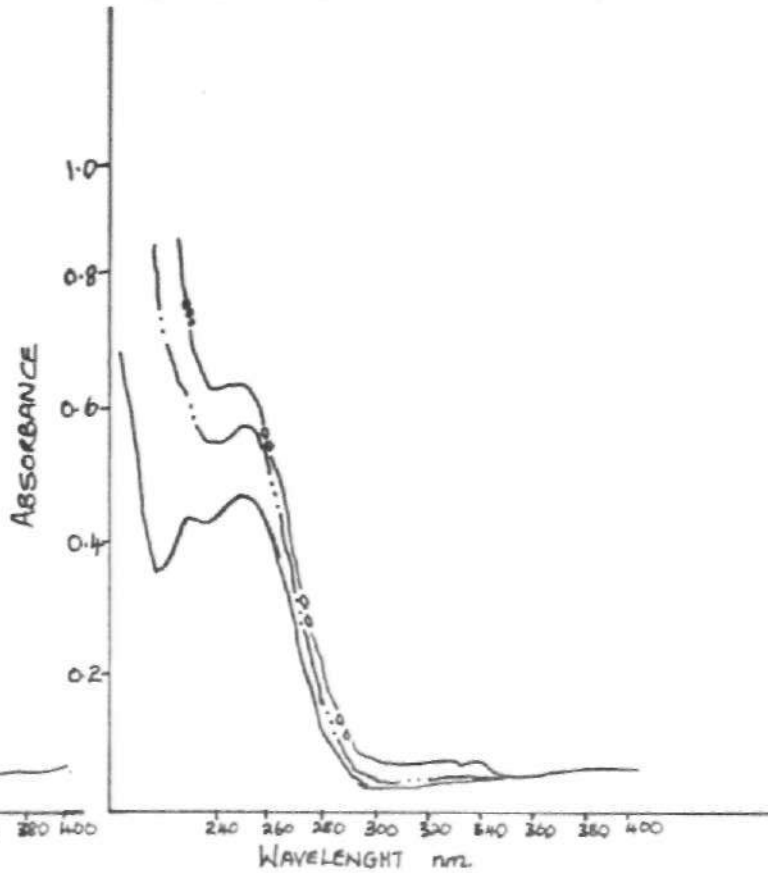


Fig 9b. Uv spectrum of proguanil hydrochloride after 24 hours exposure to uv irradiation (366nm) in phosphate buffer solution pH 6.9.

Key.

———— = Non irradiated solution.

—·—·— } = Irradiated solutions.

—•o—•• —

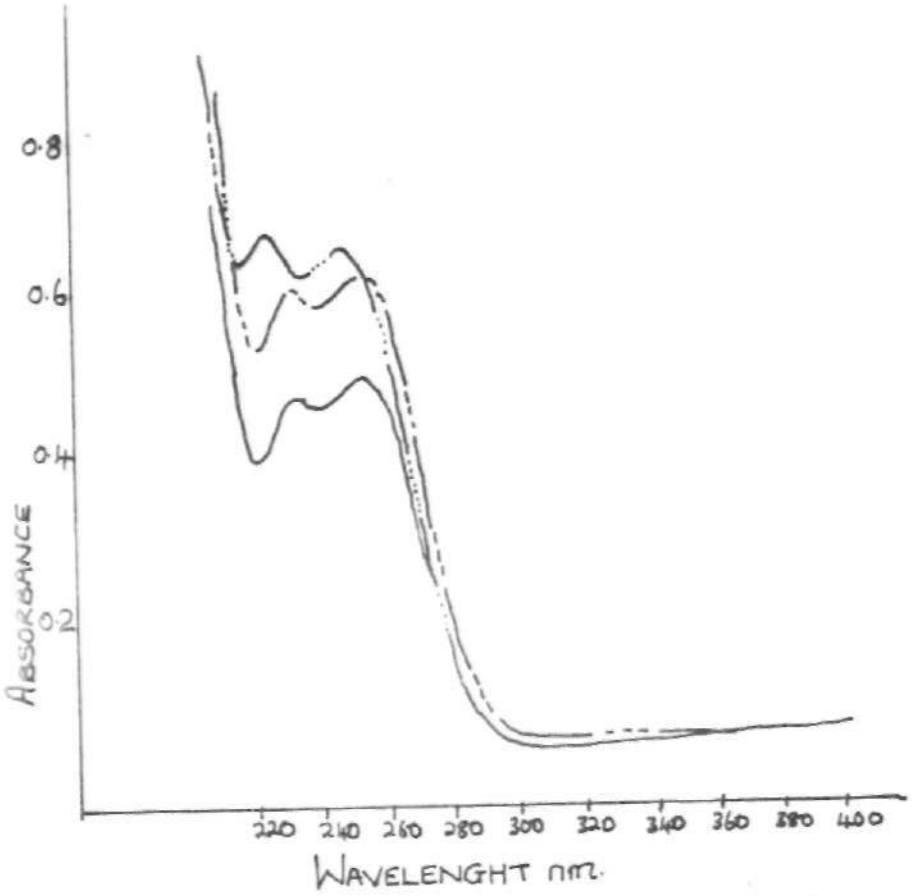


Fig 10a. Uv spectrum of proguanil hydrochloride after 16hours exposure to uv irradiation(366nm) in buffer solution pH 8.0 (1).

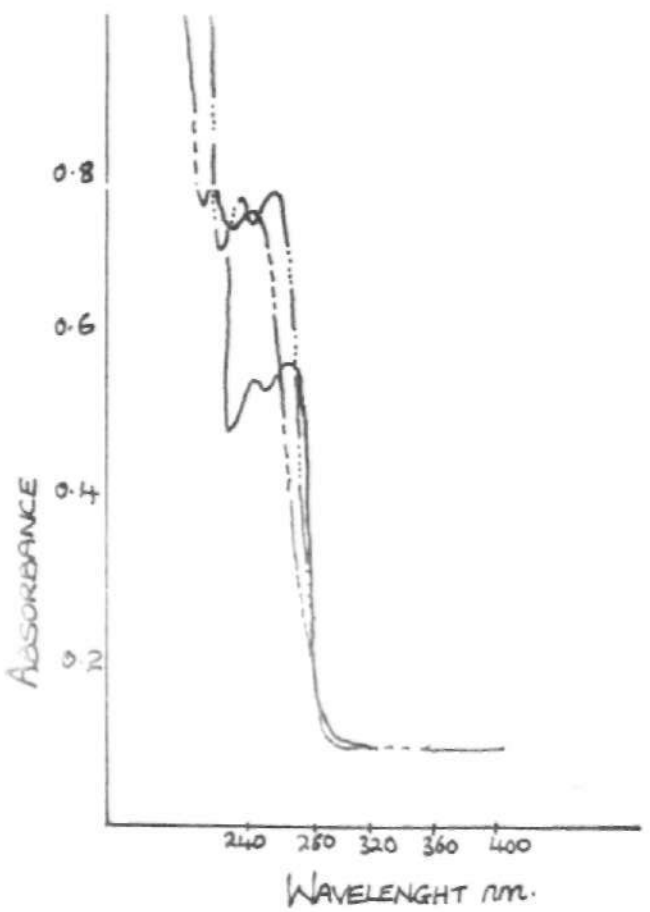


Fig 10b. Uv spectrum of proguanil hydrochloride after 426hours exposure to uv irradiation in buffer solution pH 8.0.

Key. _____ Non irradiated solution

_ _ _ _ _ } Irradiated solutions

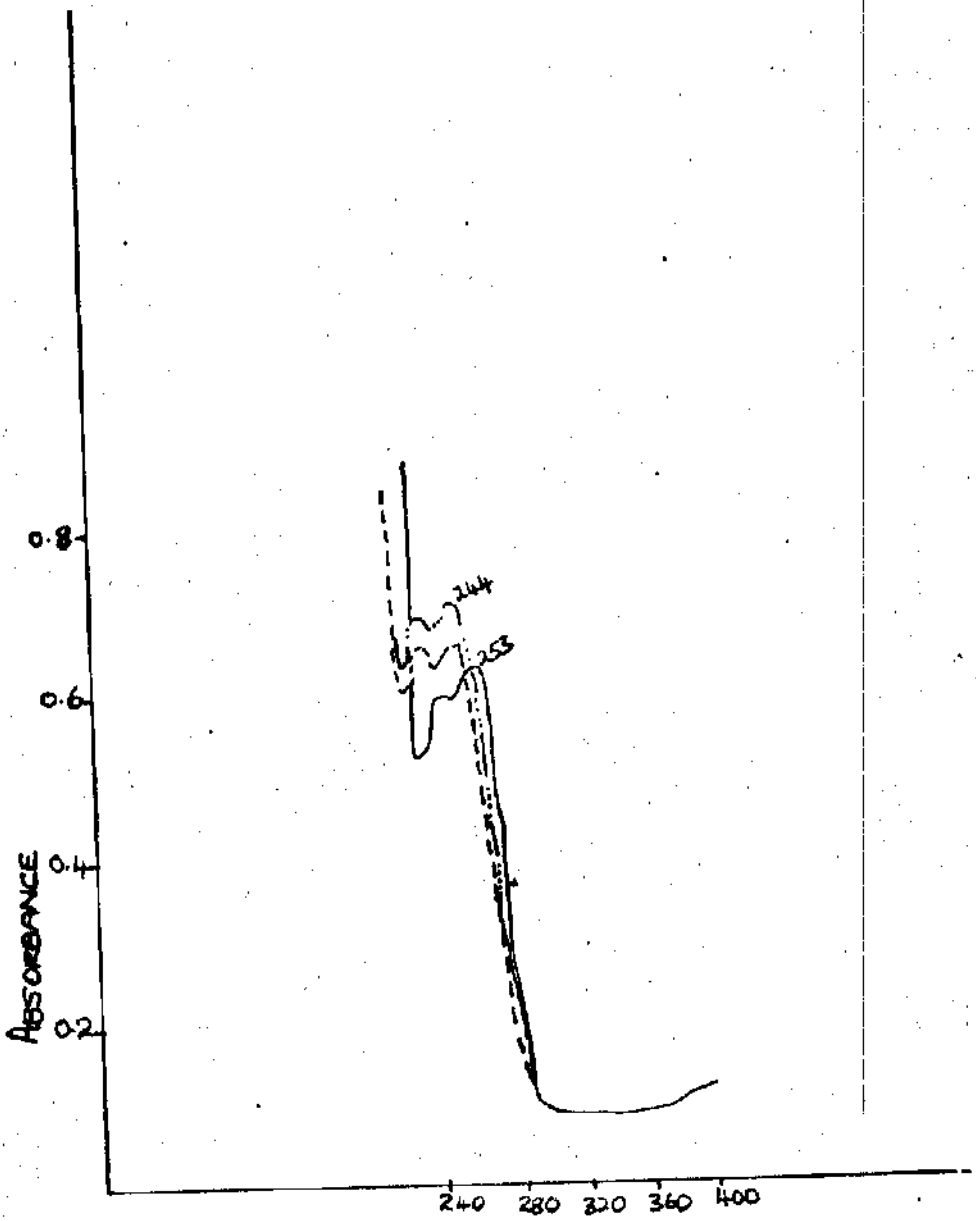


Fig 11 UV spectrum of proguanil hydrochloride after 426 hours irradiation (366nm) in phosphate buffer solution pH 4.5.

Key. ____ = Non irradiated solution.

---} = Irradiated solutions.

TABLE 8: EFFECT OF UV RADIATION ON PROGUANIL HYDROCHLORIDE IN PHOSPHATE BUFFER SOLUTIONS (pH 4.5, 6.9 AND 8.0) AT 366nm.

TIME	ABSORBANCE								
	pH 4.5			pH 6.9			pH 8.0		
HOURS	1	2	NI	1	2	NI	1	2	NI
8	0.486	0.477	0.457	0.463	0.460	0.465	0.535	0.539	0.450
16	0.488	0.477	0.457	0.539	0.470	0.465	0.536	0.591	0.450
24	0.500	0.489	0.457	0.594	0.503	0.430	0.530	0.586	0.450
32	0.519	0.503	0.465	0.598	0.503	0.430	0.530	0.586	0.450
40	0.519	0.503	0.465	0.614	0.505	0.365	0.538	0.590	0.450
48	0.523	0.506	0.465	0.648	0.537	0.355	0.538	0.608	0.450
60	0.523	0.506	0.467	0.678	0.537	0.345	0.538	0.608	0.450
96	0.528	0.525	0.464	0.703	0.556	0.340	0.568	0.643	0.450
124	0.535	0.525	0.465	0.725	0.557	0.340	0.569	0.648	0.460
220	0.558	0.544	0.468	0.725	0.557	0.340	0.598	0.651	0.462
330	0.608	0.588	0.470	0.725	0.557	0.343	0.648	0.662	0.462
426	0.610	0.588	0.470	0.725	0.557	0.345	0.662	0.665	0.460

the peak at 232nm flattened out and remained the major peak (Fig. 12c). In the other three experiments, there was no change in the spectral peaks (Fig. 12d). In all experiments there was increase in absorbance at 253 and 232nm.

At pH 6.9, only two experiments were compared. After 8 hours the difference in the absorbance between the two peaks decreased from 0.04 to 0.01. In one experiment, the peak at 232nm disappearance after 16 hours, the peak at 253nm flattened after 40 hours and there was colour change in solution from colourless to yellow between 96-124 hours. The peak at 253nm disappeared completely after 426 hours. Similar observations were not made in the duplicate experiment. The peak at 232nm disappeared after 32 hours, there was no colour change in reaction medium even after 426 hours. The peak at 253nm remained stable even after 124 hours and flattened after 426 hours. (Fig. 13a - 13c).

In buffer solution pH 8.0 in one experiment the peak at 232nm disappeared within 8 hours. In all experiments except the silica cuvette experiment, black precipitate was observed by the 96th hour. In all experiments, there was increase in absorbance of peaks at 232nm and 253nm respectively (See Fig. 14a - 14b and Table 11).

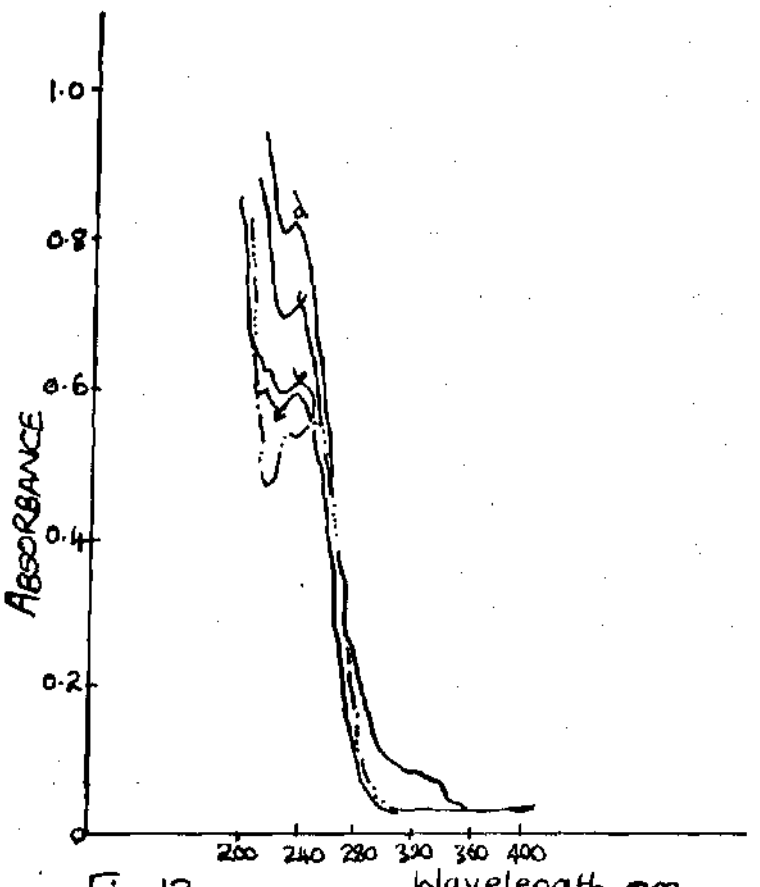


Fig 12

UV spectrum of proguanil hydrochloride in phosphate buffer pH 4·5 after UV irradiation

Key:- _ . _ . _ Non irradiated solution

a = 8 hours irradiation
b = 16 hours irradiation
c = 48 hours irradiation
d = 96 hours irradiation

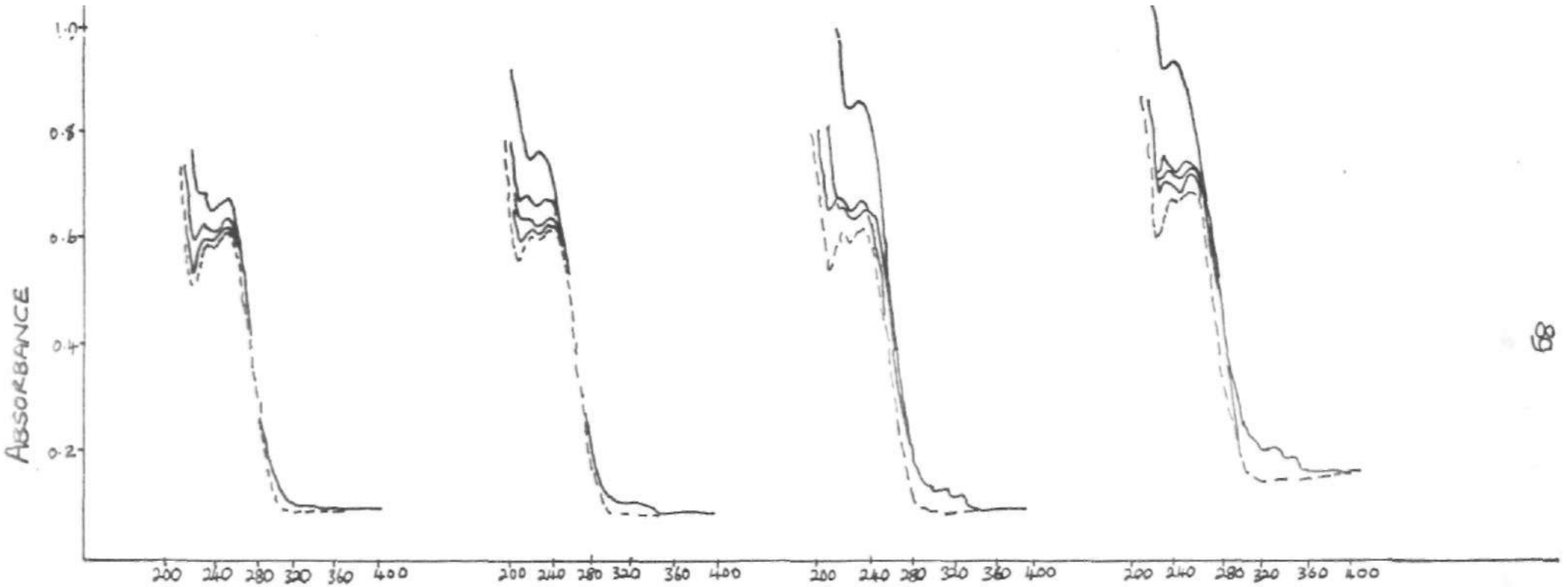


Fig 12a Fig 12b Fig 12c Fig 12d.

Influence of reaction vessel on uv spectrum of proguanil hydrochloride in buffer solution pH 4.5 on irradiation at 366nm.

Key.

Fig 12a = 16 hours irradiation
Fig 12b = 48 hours irradiation
Fig 12c = 72 hours irradiation
Fig 12d = 144 hours irradiation
- - - - - = Non irradiated solution.

TABLE 9: EFFECT ON UV RADIATION ON PROGUANIL HYDROCHLORIDE IN BUFFER SOLUTION pH 4.5 AT 366nm.

TIME	Absorbance						
HOURS	SILICA CELL	EXPT. 1	EXPT. 2	EXPT. 3	EXPT. 4	EXPT. 5	NI
8	0.550	0.546	0.586 +	0.539	0.541	0.548	0.535
16	0.554	0.546	0.610	0.541	0.541	0.562	0.535
24	0.558	0.550	0.621 *	0.543	0.553	0.583	0.530
48	0.558	0.550	0.732	0.562	0.582	0.591	0.535
72	0.558	0.552	0.732	0.562	0.583	0.595	0.538
96	0.566	0.557	0.807	0.563	0.591	0.601	0.538
144	0.572	0.568	0.807	0.567	0.595	0.612	0.538

\+ = Appearance of black precipitate.

* = Loss of spectra peak at 232nm

NI = Non-Irradiated Solution.

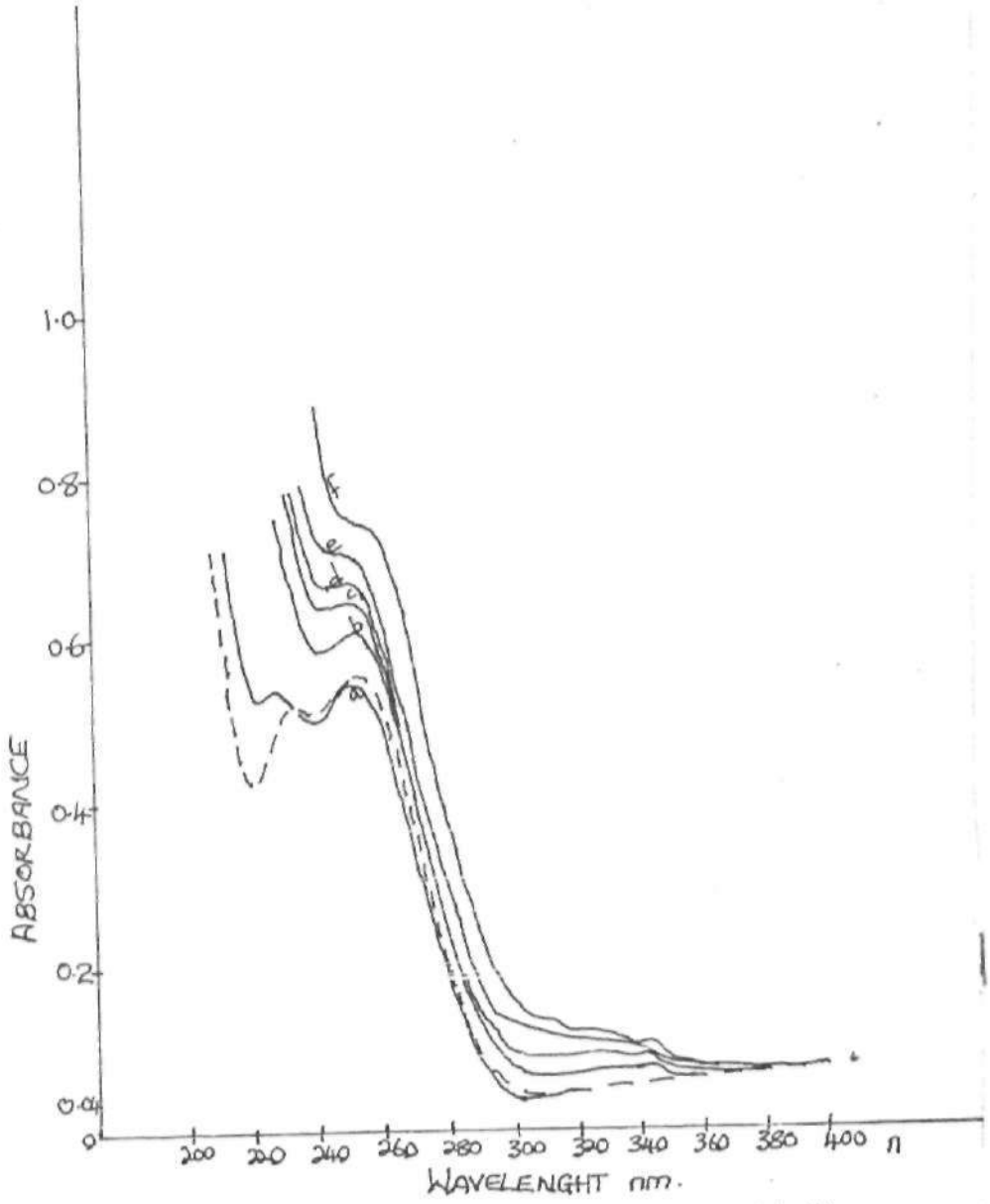


Fig 13 Uv spectrum of proguanil hydrochloride in phosphate buffer pH 6.9 after UV irradiation (366nm).

Key: - - - - = Non irradiated solution
a = 8 hours irradiation
b = 16 hours irradiation
c = 24 hours irradiation
d = 48 hours irradiation
e = 96 hours irradiation
f = 124 hours irradiation.

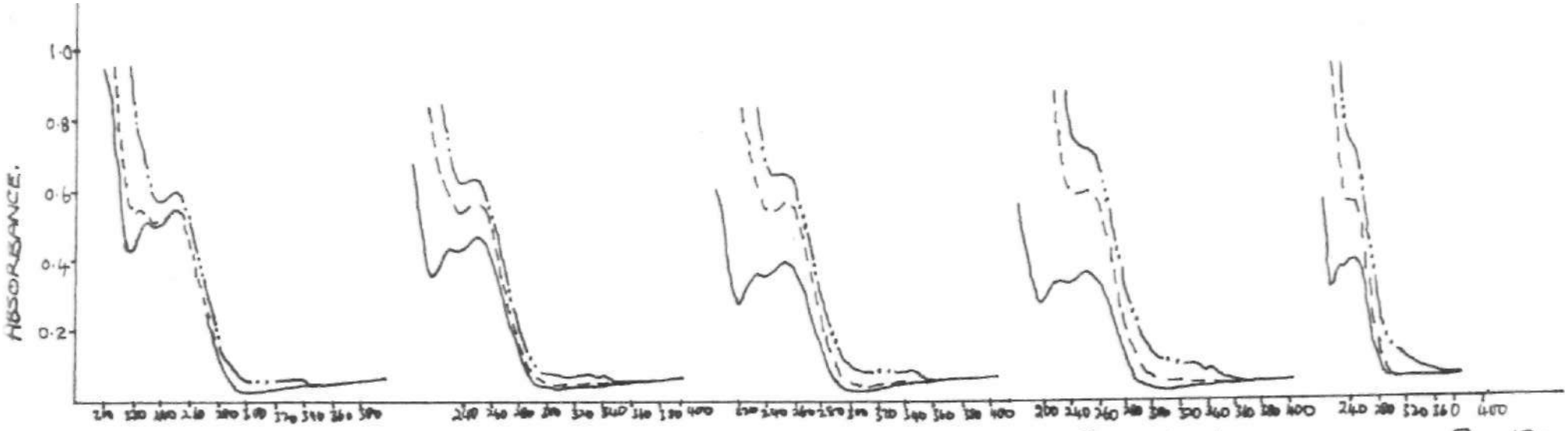


Fig 13a Fig 13b Fig 13c Fig 13d Fig 13e

Effect of reaction vessel on UV spectrum of proguanil hydrochloride solution in buffer solution pH 6·9 (366nm)

Key: Fig 13a = 16hours irradiation, Fig 13b = 24hours irradiation, Fig 13c = 40hours irradiation
Fig 13d = 124hours irradiation Fig 13e = 426 hours irradiation.

——— = Non irradiated solution.

—·····— } Irradiated solutions.
- - - - - -

TABLE 10: EFFECT OF UV RADIATION ON PROGUANIL HYDROCHLORIDE IN PHOSPHATE BUFFER SOLUTION pH 6.9 AT 366nm.

TIME	ABSORBANCE		
HOURS	EXPT 1	EXPT 2	NI
8	0.483	0.460	0.470
16	0.539	0.470	0.470
24	0.594	0.503	0.430
32	0.598	0.503	0.430
40	0.614	0.505	0.375
48	0.648	0.537	0.355
60	0.678	0.537	0.345
96	0.703*	0.556	0.340
124	0.725	0.557	0.340
426	0.727+	0.557	0.340

Key NI = Non Irradiated Solution

= Disappearance of peak at 232nm

\+ = Disappearance of peak at 253nm

= Flattening of peak at 253nm

* = Solution changed colour to yellow.

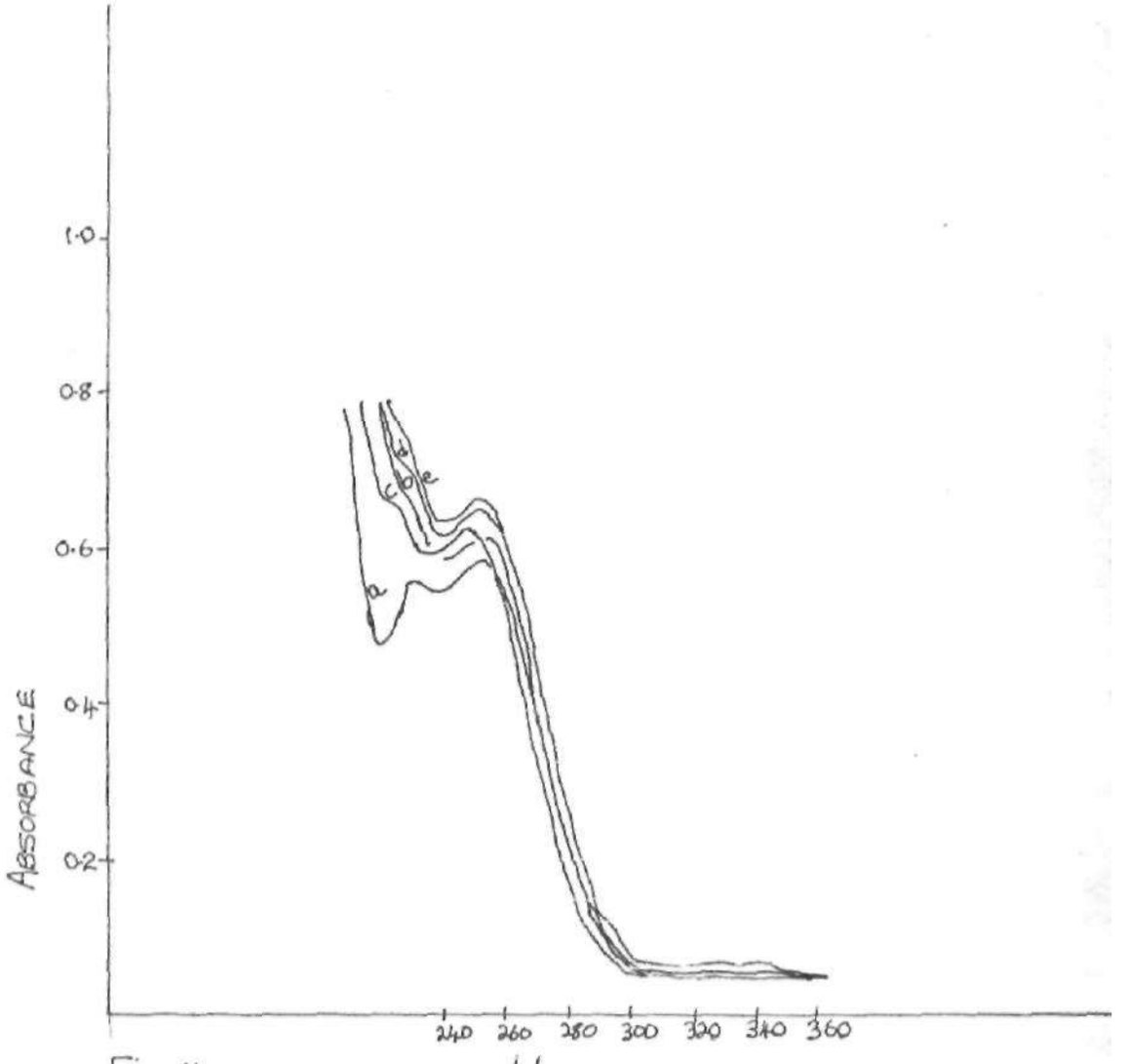


Fig 14

UV spectrum of proguanil hydrochloride after irradiation (366nm) in phosphate buffer pH 8·0

Key: a = Non irradiated solution.
b = 8 hours irradiation
c = 16hours irradiation
d = 48 hours irradiation
e = 72 hours irradiation

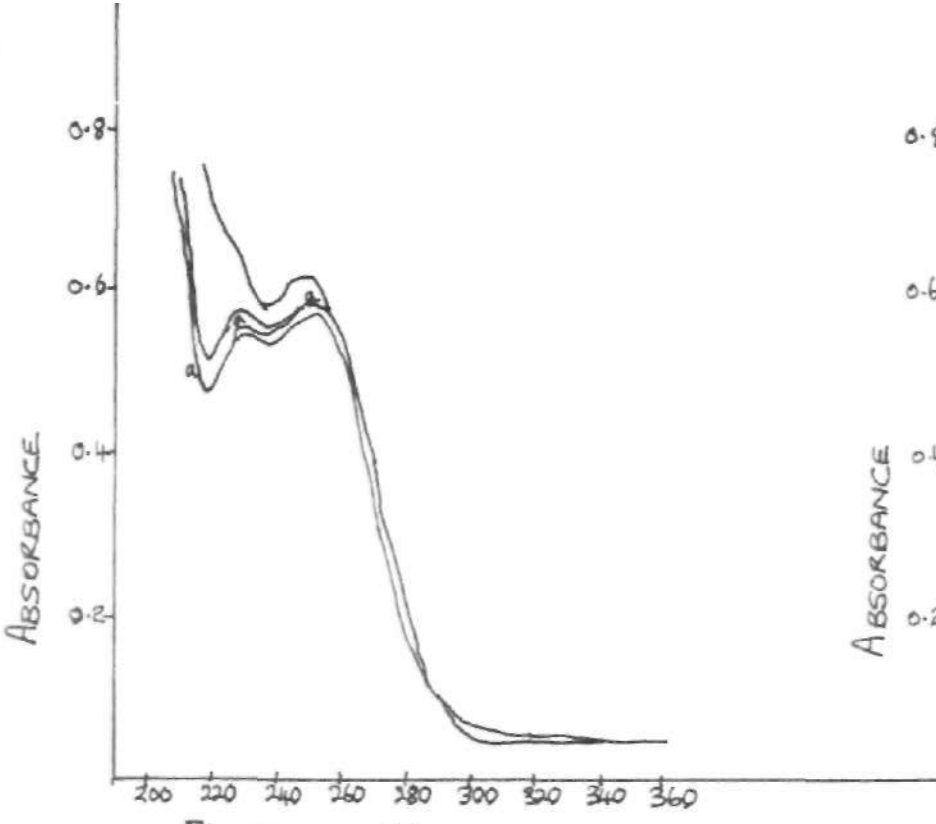


Fig 14a, WAVELENGHT nm.

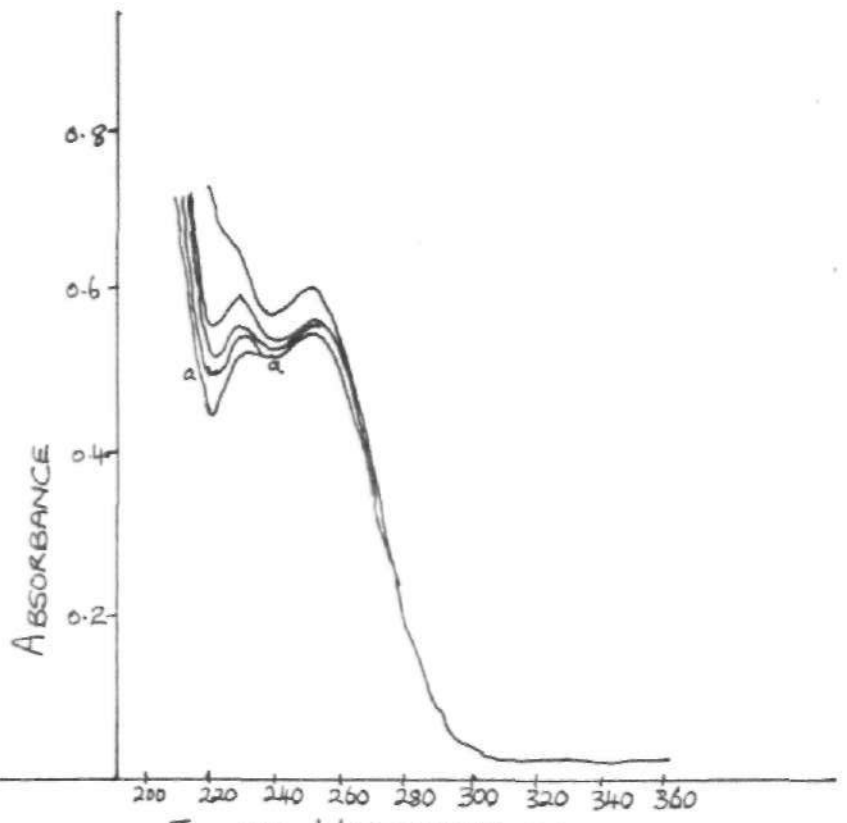


Fig 14b. WAVELENGHT nm.

Influence of reaction vessel on uv spectrum of proguanil hydrochloride after irradiation in phosphate buffer solution pH 8·0.

Key.

Fig 14a = 8 hours irradiation
Fig 14b = 48 hours irradiation
a = Non irradiated solution.

TABLE 11: EFFECT OF UV RADIATION ON PROGUANIL HYDROCHLORIDE IN PHOSPHATE BUFFER SOLUTION pH 8.0 AT 366nm.

TIME	ABSORBANCE					
HOURS	EXPT. 1	EXPT. 2	EXPT. 3	EXPT. 4	EXPT IN SILICA CELL	NI
8	0.499	0.548 +*	0.503	0.498	0.496	0.514
16	0.499	0.551	0.503	0.498	0.502	0.510
24	0.509	0.563	0.510 +	0.503	0.507	0.515
48	0.533	0.590	0.519	0.515 +	0.519	0.517
72	0.542 +	0.613	0.522	0.519	0.542	0.517
96	0.540	0.613	0.521	0.519	0.555	0.517

Key: + = Appearance of black precipitate

* = Disappearance of peak at 232nm

NI = Non-Irradiated Solution.

Silica cuvettes were again employed in these experiments. Evaporation was minimized by covering the cuvette first with a plastic cover and then aluminium foil. At pH 4.5 there was increase in absorbance of both peaks at 232nm and 253nm respectively. At pH 8.0, the peak at 232nm became the major peak after 96 hours. No precipitate was however observed using the silica cuvettes.

From these observations, the type of glass in which this reaction occurs is very important because a photochemical reaction depends on the number of photons of light absorbed. If the glass is such that it absorbs a large percentage of the incident light ther photochemical reaction may not occur.

This study further supports the previous findings of Sams and Carrol (1966) who studied the UV irradiation of chloroquine diphosphate in 0.1M phosphate buffer pH 7.4. They observed that UV irradiation resulted in marked depression of the characteristic peaks at 328nm and 342nm and a significant increase of absorption in 267-300nm range. The shift did not occur when irradiation was done at pH 5.0.

This work also establishes the fact that proguanil hydrochloride in phosphate buffer solution pH 4.5 - pH 8.0 readily enters into photochemical reaction.

Influence of pH on the photochemical reaction was determined by comparing the results obtained from the same sample bottle. This was done on the assumption that the radiation absorbed by a given sample bottle remained unchanged at the different pH 4.5, pH 6.9, and pH 8.0.

The result is shown in table 12 and fig. 15a-15c. There is increased absorbance in the three media with the greatest increase at pH 4.5. The peak at 232nm disappeared within 8 hours in pH 8.0 and within 24 hours in pH 4.5 and pH 6.9. Black precipitate was observed within 8 hours in pH 4.5 and pH 8.0. No precipitate was observed in pH 6.9. The photochemical reaction of proguanil hydrochloride in phosphate buffer solutions appears to be dependent on pH. The rate of reaction under the same conditions appears to be in the order pH 8.0 > pH 4.5 > pH 6.9.

Fig 16 shows the combined influence of pH and reaction vessel. Evidence of a photochemical reaction was observed at pH 4.5, 6.9, and 8.0 within the first eight hours of irradiation either by the appearance of a black precipitate or change in spectral peak. The peak at 232nm disappeared within 8 hours in 8.0, 16 hours in pH 6.9 and 24 hours in pH 4.5. No precipitate was observed in pH 6.9 and the colour change observed in pH 6.9 was not observed in the other buffer solutions pH 4.5 and pH 8.0.

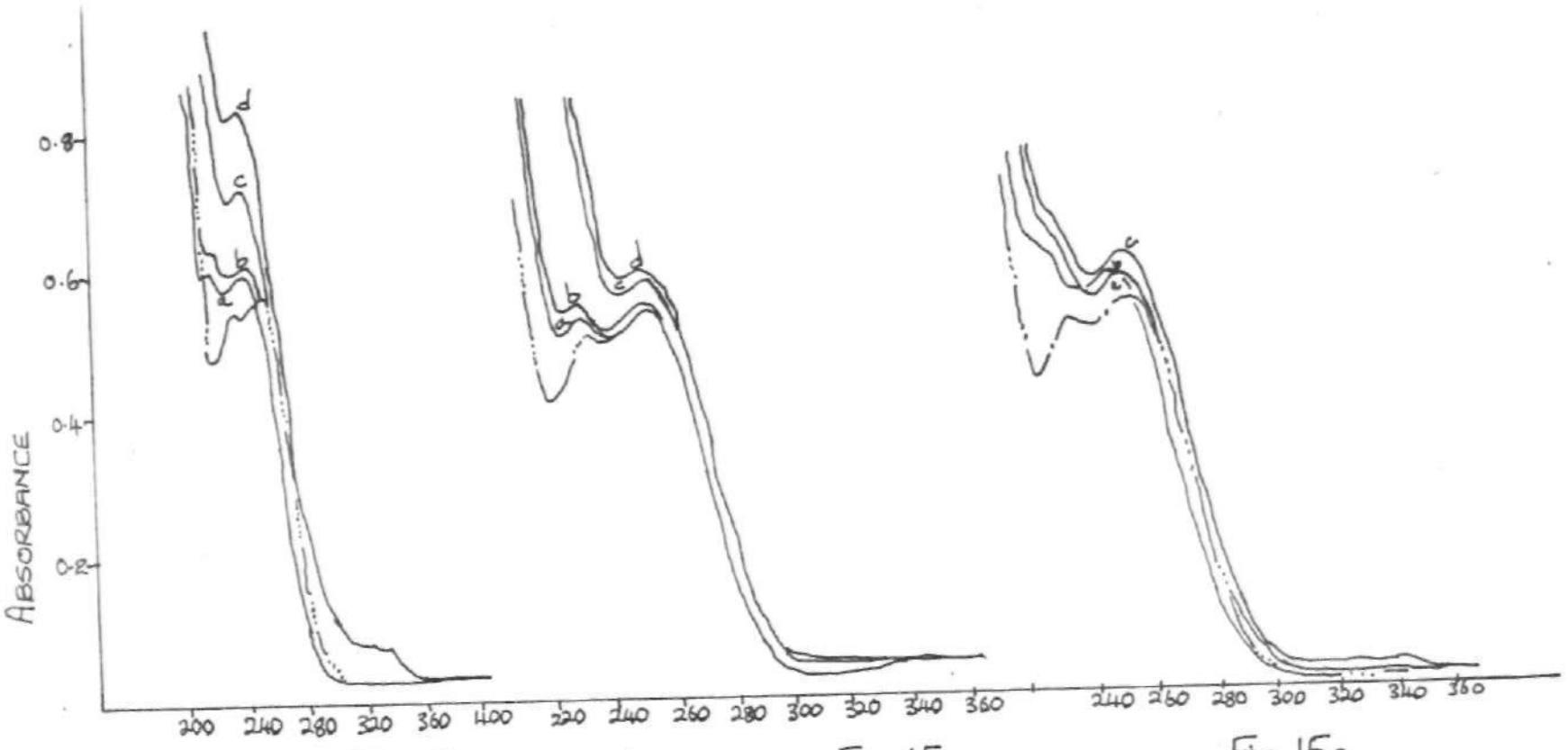


Fig 15a. Fig 15b Fig 15c

Influence of pH on the UV spectrum of proguanil hydrochloride after UV irradiation (366nm) in phosphate buffer solution (pH 4.5, 6.9, and 8.0).

Key.

Fig 15a = Irradiation in buffer pH 4.5.
Fig 15b = Irradiation in buffer pH 6.9.
Fig 15c = Irradiation in buffer pH 8.0.

——·——·—— Non irradiated solution.
a = 8 hours irradiation
b = 16 hours irradiation
c = 48 hours irradiation
d = 96 hours irradiation

TABLE 12: INFLUENCE OF pH ON THE IRRADIATION OF PROGUANIL HYDROCHLORIDE IN PHOSPHATE BUFFER SOLUTIONS (pH 4.5, 6.9, 8.0) AT 366nm.

TIME	ABSORBANCE		
HOURS	pH 4.5	pH 6.9	pH 8.0
8	0.586^{+}	0.460	0.548^{*+}
16	0.610	0.470	0.551
24	0.621*	0.503*	0.563
48	0.732	0.537	0.590
72	0.732	-	0.613

Key: * = Disappearance of peak at 232nm

\+ = Appearance of black precipitate.

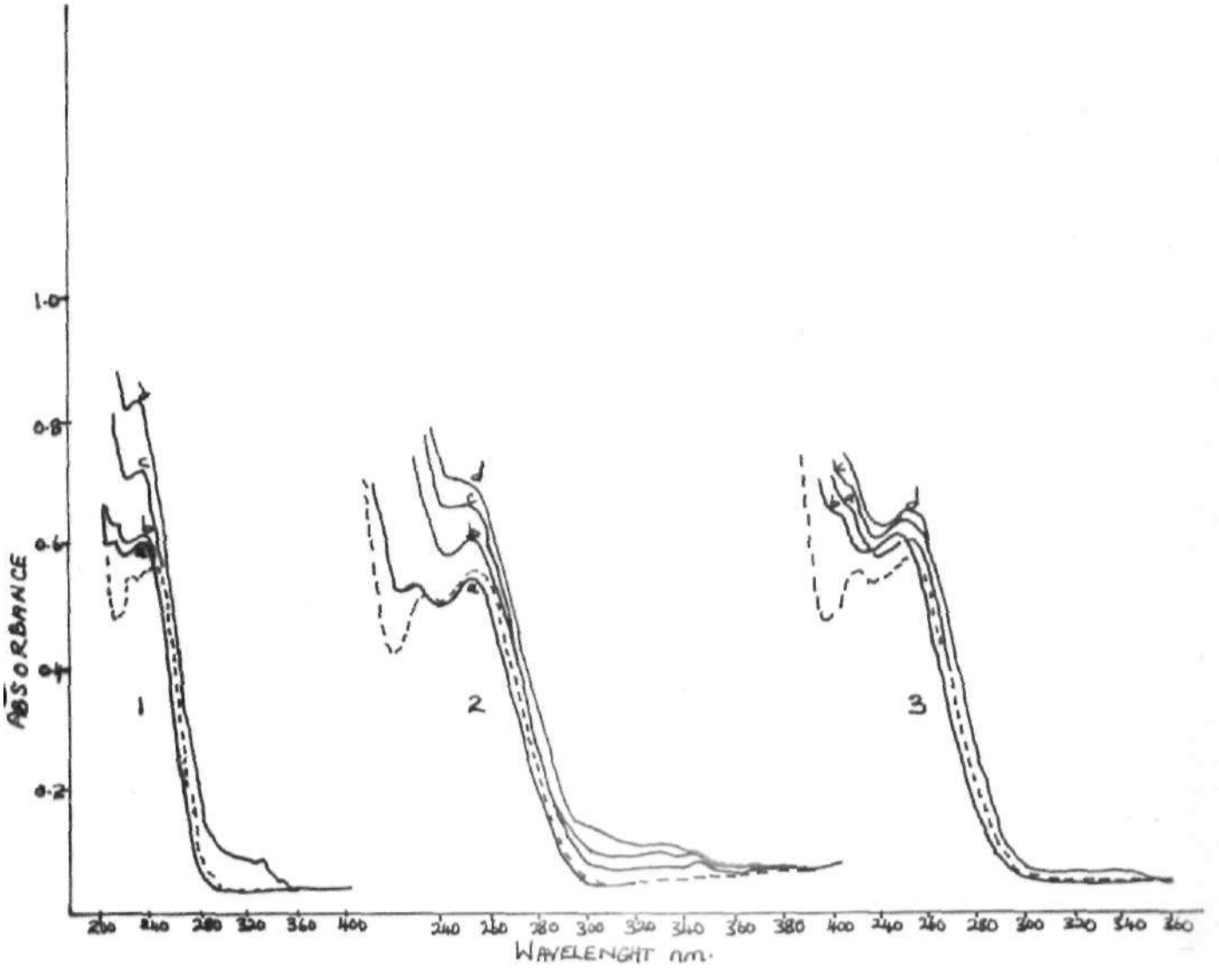


Fig 16 Combined influence of pH and reaction vessel on the UV spectrum of proguanil hydrochloride on irradiation(366nm) in phosphate buffer solution pH 4.5, 6.9, and 8.0.

Key.

1 = Irradiation in pH 4.5
2 = Irradiation in pH 6.9
3 = Irradiation in pH 8.0.
a = 8hours irradiation
b = 16hours irradiation
c = 48hours irradiation
d = 96hours irradiation.

In buffer solution pH 6.9 there is evidence of absorption at 344nm but the concentration of the product was probably too small for a well defined peak (see figure 13).

The observation of precipitates may be indicative of catalysis in these reactions. The catalyst could be produced from the glass vessel which is able to release alkali ions in addition to various cations. Its alkaline surface may raise the pH of pharmaceutical and induce chemical reaction. These results indicating the photochemical reaction of proguanil hydrochloride are consistent with the observed complexity of photolytic reactions. The kinetics of photochemical reactions is more complicated than thermal reactions because more variables are involved. The intensity and wavelength of light, the size and shape of the container may greatly affect the reaction. A photochemical reaction may be accompanied by a thermal reaction identical to the photochemical reaction or opposite to it. A photochemical reaction may produce a catalyst which then causes a thermal reaction to proceed at a measurable rate. Sometimes an induction period is necessary while a sufficient quantity of catalyst is being accumulated to make reaction proceed at a measurable rate (Lachman 1976).

3.6 ASSAY OF IRRADIATED TABLETS

The results are shown in table 13.

Proguanil hydrochloride tablets

Proguanil hydrochloride tablets showed significant decrease in drug content ($P < 0.05$) after UV irradiation (366nm). Approximately 9% of the drug was lost after three weeks of irradiation. British Pharmacopoeia 1980 specified drug content for proguanil hydrochloride tablets is 95-105%. Proguanil hydrochloride tablets failed to comply with this specification after three weeks of UV radiation (366nm). Drug content after three weeks irradiation was determined to be 88.15%.

Pyrimethamine sulphate

Pyrimethamine sulphate tablets showed no decrease in drug content after three weeks of UV irradiation (366nm), British Pharmacopoea 1980 specified drug content for pyrimethamine sulphate is 92.5 - 107.5%. Irradiated pyrimethamine sulphate tablets comply with this specification. Drug content after three weeks of irradiation was determined to be 100.3%. This is an indication that pyrimethamine sulphate is probably stable to UV radiation (366nm).

TABLE 13: QUANTITATIVE DETERMINATION OF PROGUANIL HYDROCHLORIDE TABLETS AND PYRIMETHAMINE SULPHATE TABLETS AFTER UV IRRADIATION (366nm) FOR PERIOD OF THREE WEEKS.

TIME	PERCENTAGE (%)		
DAYS	PROGUANIL HYDROCHLORIDE	PYRIMETHAMINE QCU B7	SULPHATE Wellcome B191
0	97.61	100.88	101.16
4	95.52	100.57	101.49
7	92.15	100.11	100.62
10	90.62	100.15	101.37
14	88.63	98.45	100.82
21	88.15	100.30	-

P 0.05

3.7 DISSOLUTION RATE TEST OF PYRIMETHAMINE SULPHATE TABLETS

Linear calibration curves with good correlation coefficients of pyrimethamine sulphate are shown in Figures 17a and 17b.

In figure 18 and Table 14 are shown the in-vitro dissolution rates of pyrimethamine sulphate tablets in 0.1N HCl from two different manufacturers, Quality Control Unit, Ahmadu Bello University, Zaria (QCU B5, QCU B7) and Wellcome Nigeria Limited (B191). From the results, it is apparent that there was a gradual release of drug for the first 60 minutes after which the cumulative release of pyrimethamine into the dissolution medium became constant. Cumulative percent release at the 60th minute decreased in the order B7 > B191 > B5.

The dissolution half-lives T50% were found to be 8.5, 15 and 17.5 minutes for B7, B191 and B5 respectively (Table 14.2).

Cumulative percent release became constant although 100% release was not achieved. This reflects the solubility of the drug in the medium.

Fig. 19 and Table 15 show the in-vitro dissolution rates of pyrimethamine sulphate in phosphate buffer solution pH 4.5. The dissolution rate profile showed a consistent gradual release of the drug into the dissolution medium. Large variation in dissolution rate is apparent from the T50% values which were

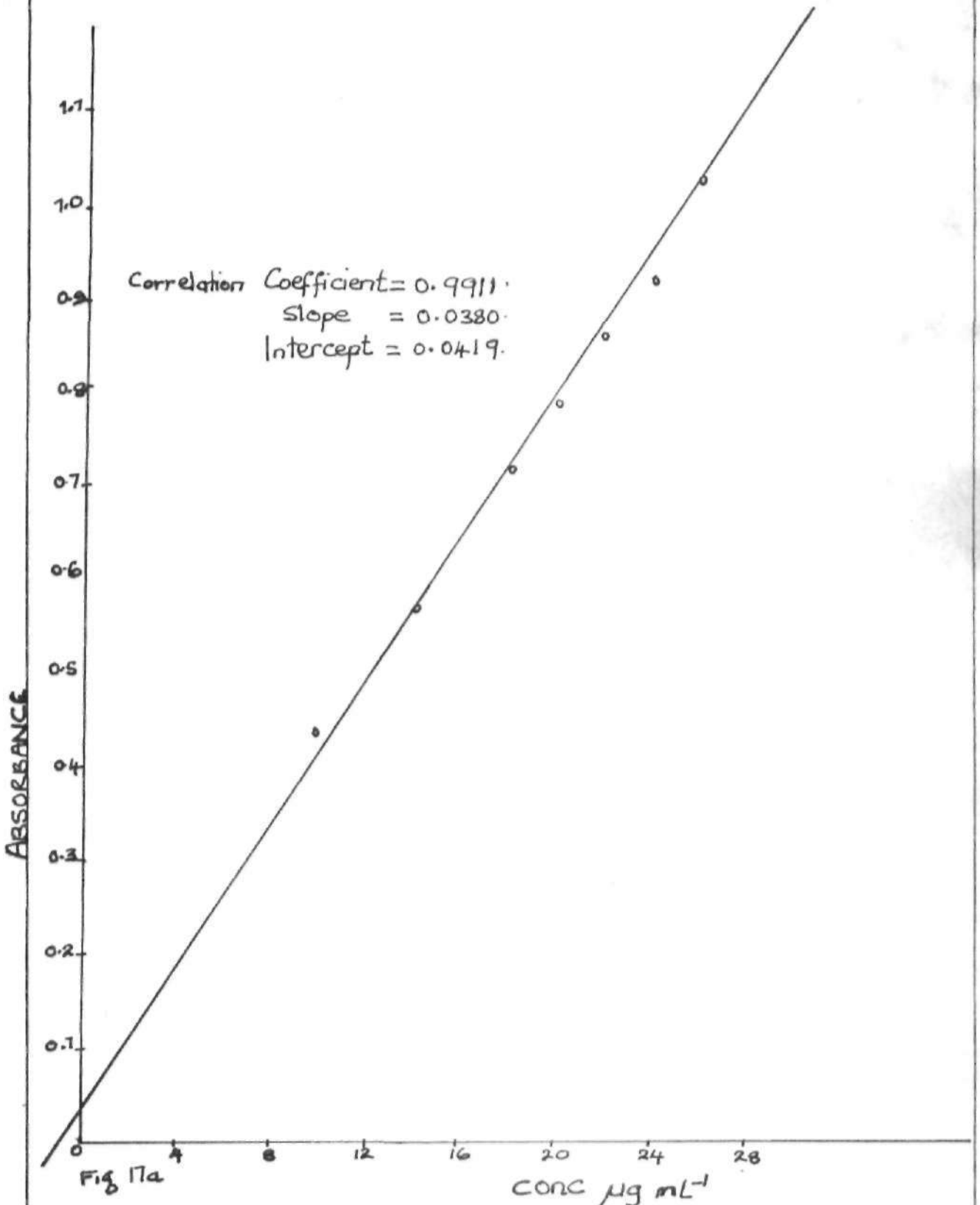


Fig 17a

Calibration Curve for pyrimethamine sulphate in 0.1 N. HCl using U.V spectrophotometer.

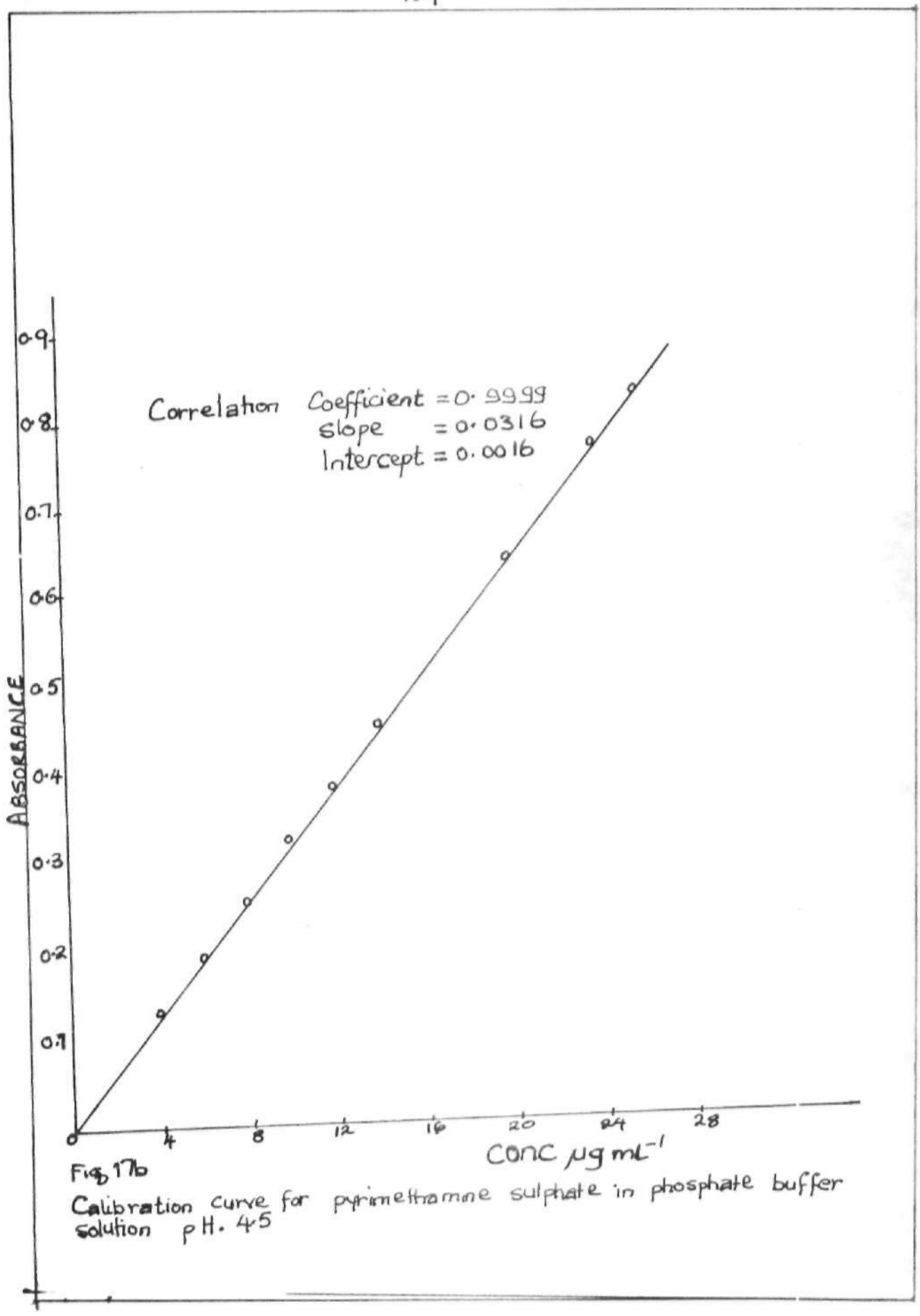


Fig 17b

Calibration curve for pyrimethamine sulphate in phosphate buffer solution pH. 4.5

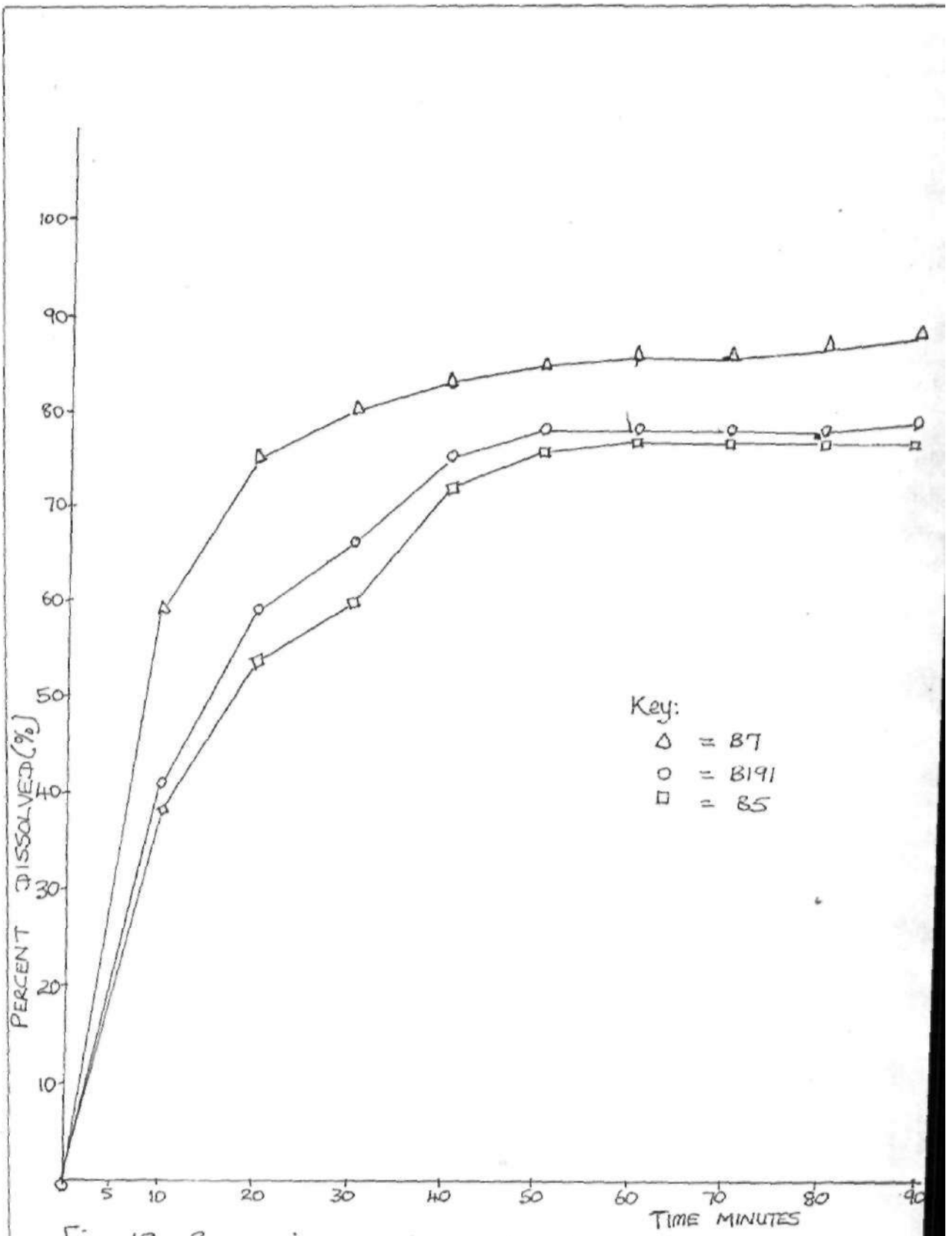


Fig 18: Comparison between in-vitro dissolution rate profiles of pyrimethamine sulphate B7, B5, Wellcome B191 in 0·1N HCL.

TABLE 14.1: DISSOLUTION RATE PEOFILES OF PYRIMETHAMINE SULPHATE TABLETS IN 0.1N HCl.

TIME	Percentage Released (%)		
MINUTES	QCU B5	QCU B7	B191
10	37.76	58.64	41.16
20	53.69	74.97	58.53
30	64.91	80.20	66.35
40	71.77	82.69	75.13
50	75.58	85.24	77.94
60	76.76	86.18	78.18
70	76.86	86.38	78.32
80	76.96	87.11	78.43
90	77.27	87.23	78.93

TABLE 14.2: DISSOLUTION RATE HALF-LIVES ($T_{50\%}$) OF PYRIMETHAMINE SULPHATE TABLETS IN 0.1N HCl.

TABLET	$T_{50\%}$ (MINUTES)
B7	8.5
B191	15.0
B5	17.5

KEY

B191 = Wellcome Product

B5, B7 = Quality Control Products.

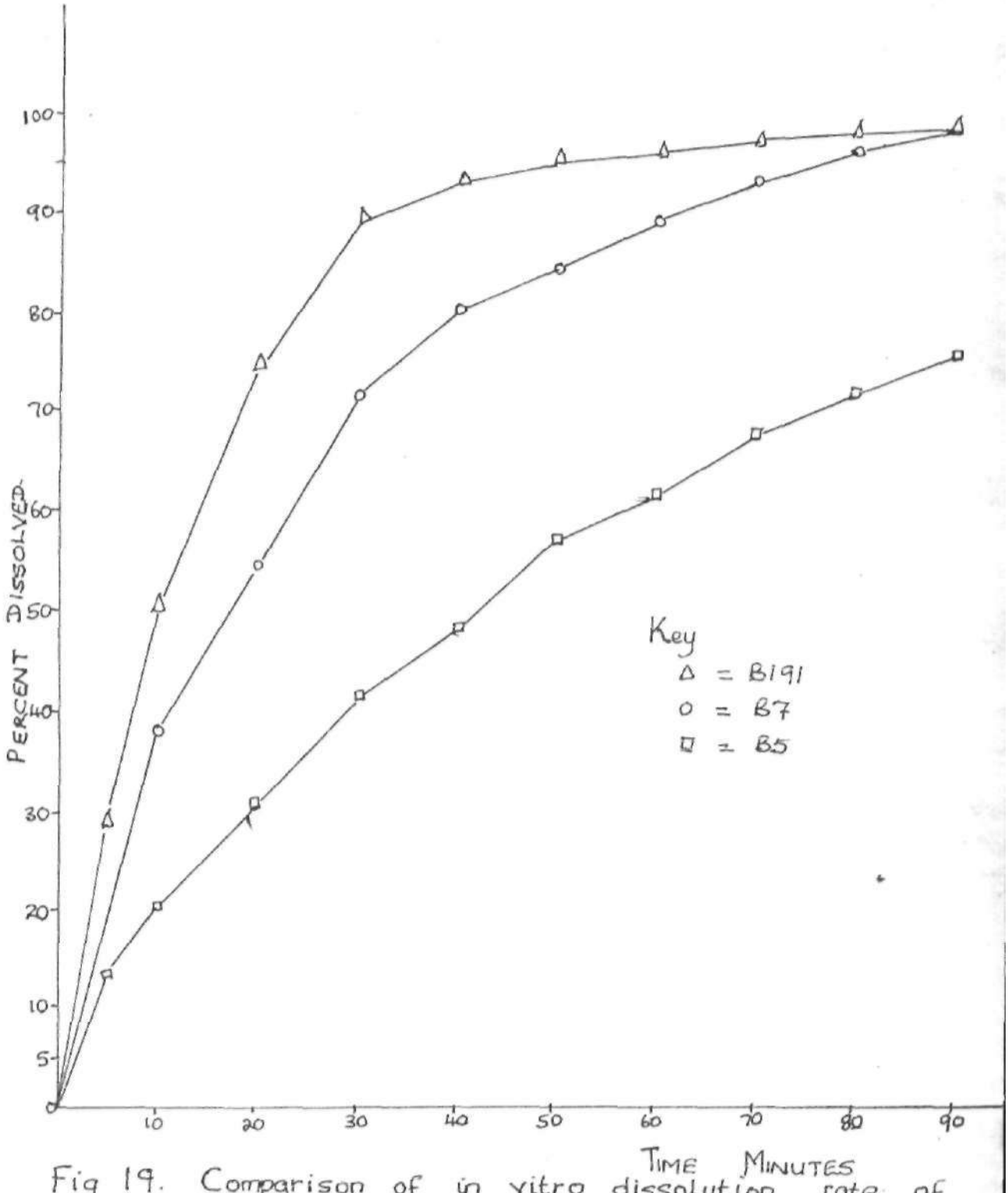


Fig 19. Comparison of in vitro dissolution rate of pyrimethamine sulphate B5, B7, Wellcome B191 in phosphate buffer solution pH 4.5

TABLE 15.1: DISSOLUTION RATE PROFILES OF PYRIMETHAMINE SULPHATE TABLETS IN PHOSPHATE BUFFER pH 4.5.

TIME	Percentage Released (%)		
MINUTES	QCU B5	QCU B7	B191
10	19.67	37.57	50.11
20	29.64	54.20	73.74
30	40.50	70.99	89.30
40	48.07	80.41	93.93
50	56.72	84.17	95.44
60	60.86	89.15	96.36
70	67.23	92.75	97.28
80	71.47	96.64	97.61
90	74.94	98.35	98.33

TABLE 15.2: DISSOLUTION RATE HALF-LIVES ($T_{50\%}$) OF PYRIMETHAMINE SULPHATE TABLETS IN PHOSPHATE BUFFER SOLUTION pH 4.5.

TABLET	$T_{50\%}$ (MINUTES)
B191	9.5
B7	17.5
B5	42.0

KEY

B191 = Wellcome Product.

B5, B7 = Quality Control Product.

found to be 9.5, 17.5, and 42 minutes for B191, B7 and B5 respectively. The cumulative percent release after 90 minutes is similar for Wellcome and B7 being 98.33% and 98.35% respectively. B5 released 75% of the drug into the dissolution medium after 90 minutes.

The variation in T50% between the products of the two manufacturers could probably reflect the influence of aging or nature of the other ingredients employed in the manufacture of the tablet on the dissolution rate. This may have therapeutic implications. For a patient changing from either B191 or QCU B7 to QCU B5 there may be chances of undermedication.

Saturation of the dissolution medium which was observed in 0.1N HCl solution after 60 minutes was not observed in buffer solution pH 4.5. Cumulative percentage of drug released increased consistently in buffer solution pH 4.5. This difference probably reflects the solubility of the drug in the different media. Pyrimethamine sulphate was insoluble in buffer solution pH 6.9 and above. Similar to proguanil hydrochloride, pyrimethamine sulphate probably dissolves best in a weakly acidic medium. BP 1980 specification for dissolution rate test states that for each dosage form tested the amount of active ingredient in solution is not less than 70% of the stated amount in 45 minutes unless otherwise specified in the monograph.

In 0.1N HCl solution the dissolution test of the three batches from the different manufacturers c complied with the BP 1980 specification. In phosphate buffer solution pH 4.5, batch 5 of QCU failed to comply with the specification.

3.8 IRRADIATION OF PYRIMETHAMINE SULPHATE TABLETS AT 366nm

The results are shown in Figures 20-21 and Tables 16-17.

Pyrimethamine sulphate tablets from Wellcome Nigeria Limited batch 119 showed an increase in dissolution rate after 15 hours of irradiation. Cumulative percentage of drug released in the first twenty minutes increased from 73.74% to 94.4%. On irradiation the tablets changed colour from white to yellow. Only the surface directly exposed to the UV lamp changed colour, the underside of the tablets retained the original white colour.

$T_{50\%}$ was calculated to be 10 minutes and 4.5 minutes for the non-irradiated and irradiated tablets respectively. This represents more than 50% increase in the dissolution rate. This may have significant influence on the bioavailability.

Levy and Procknal (1962) observed an unusual behaviour due to film formation in the dissolution rate profile of aluminium acetyl salicylic acid in

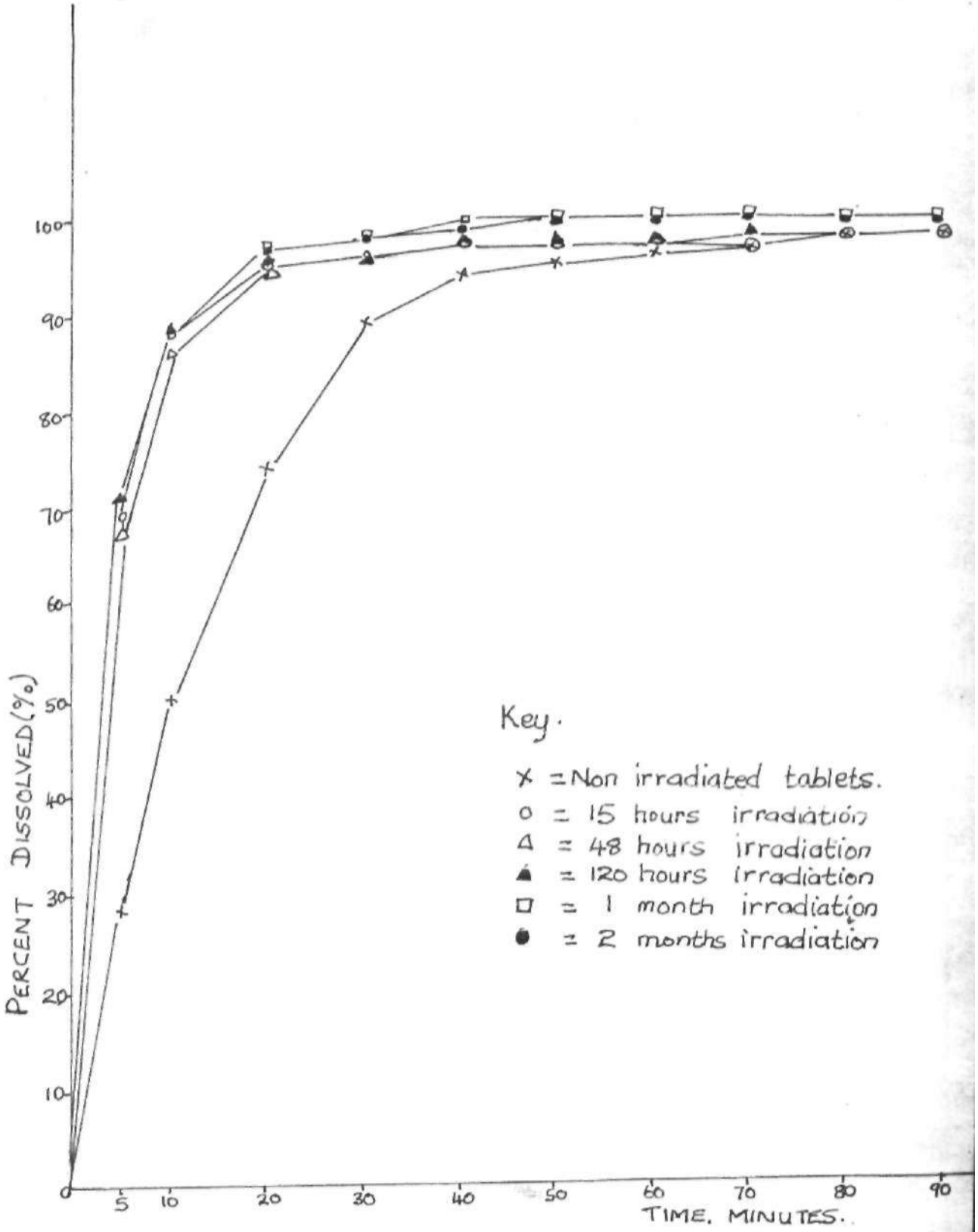


Fig 20 Comparison between dissolution rate profiles of pyrimethamine sulphate tablets B191 in buffer solution pH 4.5 after UV irradiation at 366nm.

TABLE 16: COMPARISON BETWEEN IN-VITRO DISSOLUTION RATES OF PYRIMETHAMINE SULPHATE TABLETS WELLCOME B191 IN PHOSPHATE BUFFER SOLUTIONS pH 4.5 AFTER UV IRRADIATION OF TABLETS AT 366nm FOR A PERIOD OF 15 HOURS TO 2 MONTHS

TIME	Percentage Released				
	HOURS OF IRRADIATION				
MINUTES NI	15	48	120	1 month	2 months
5 28.47	68.80	66.62	71.17	70.35	71.35
10 50.11	87.68	85.68	88.48	87.33	88.40
20 73.74	94.48	95.03	94.72	97.29	97.06
30 89.30	95.61	95.77	95.85	98.43	98.41
40 93.93	95.95	96.50	96.39	99.61	99.18
50 95.44	96.27	96.84	96.92	99.78	99.64
60 96.36	96.60	97.37	97.25	99.94	99.70
70 97.28	96.72	97.70	97.58	100.09	99.86
80 97.61	97.04	98.02	97.70	100.25	100.00
90 98.33	97.94	98.34	98.21	100.38	100.14

NI = Non-Irradiated tablets.

0.1N HCl. Levy and Gumtow (1963) observed that a water soluble surface active lubricant sodium lauryl sulfate enhanced markedly the dissolution rate of salicylic acid contained in compressed tablets. This effect appeared to be due to interfacial tension lowering of the surfactant. Their result showed that a surfactant can enhance the dissolution rate of drugs contained in compressed tablets and cause them to be more rapidly and more completely absorbed. Higuchi and Hamlin (1963) examined the problem in which rate of release in acid solution of an amine drug from a pellet of a weak acid salt of the amine was controlled by a coat which was formed by precipitation of a weak acid onto the pellet surface. Morozowich et-al (1962) noted that the release of benzphetamine from pellets of benzphetamine pamoate in 0.12N HCl was controlled by a layer of pamoic acid which was deposited on the pellet surface.

The unexpected increase in dissolution rate of irradiated pyrimethamine sulphate tablets by Wellcome (B191) could be attributed to complex formation induced by UV irradiation which enhanced the dissolution rate. Light could have affected one of the inactive ingredients used in the formulation. The product of reaction probably complexed with pyrimethamine in which pyrimethamine remained stable but more soluble. The complex may have formed on the tablet periphery as a film, on dissolution after the complex had gone,

the core was left with the unaffected part and rate of dissolution dropped to that of the non-irradiated tablet. Change of colour of pyrimethamine tablet may be as a result of the presence of the complex. A change of colour of pharmaceutical product is usually a visual indication that some form of chemical or photochemical decomposition is occurring (Rawling 1977).

Grimshow (1963) defined the stability of a pharmaceutical preparation as the period of time from the completion of the preparation until the development of an off-odour or off-colour to a point where the article is no longer commercially acceptable. This preparation having developed an off-colour on irradiation to a point where the article is no longer commercially acceptable is an indication of photo-degradation by one of the other ingredients employed in its manufacture.

Pyrimethamine sulphate tablets produced by Quality Control Unit A.B.U., Zaria showed a decrease in dissolution rate after UV irradiation (see Table 17)

$T_{50\%}$ was calculated to be 17.5, and 21.5 minutes respectively for the non-irradiated and irradiated tablets (Fig. 21).

From the assay of irradiated tablets, decrease in drug content was not observed even after three weeks of irradiation. Significant decrease in dissolution rate was observed from the 15th hour of irradiation

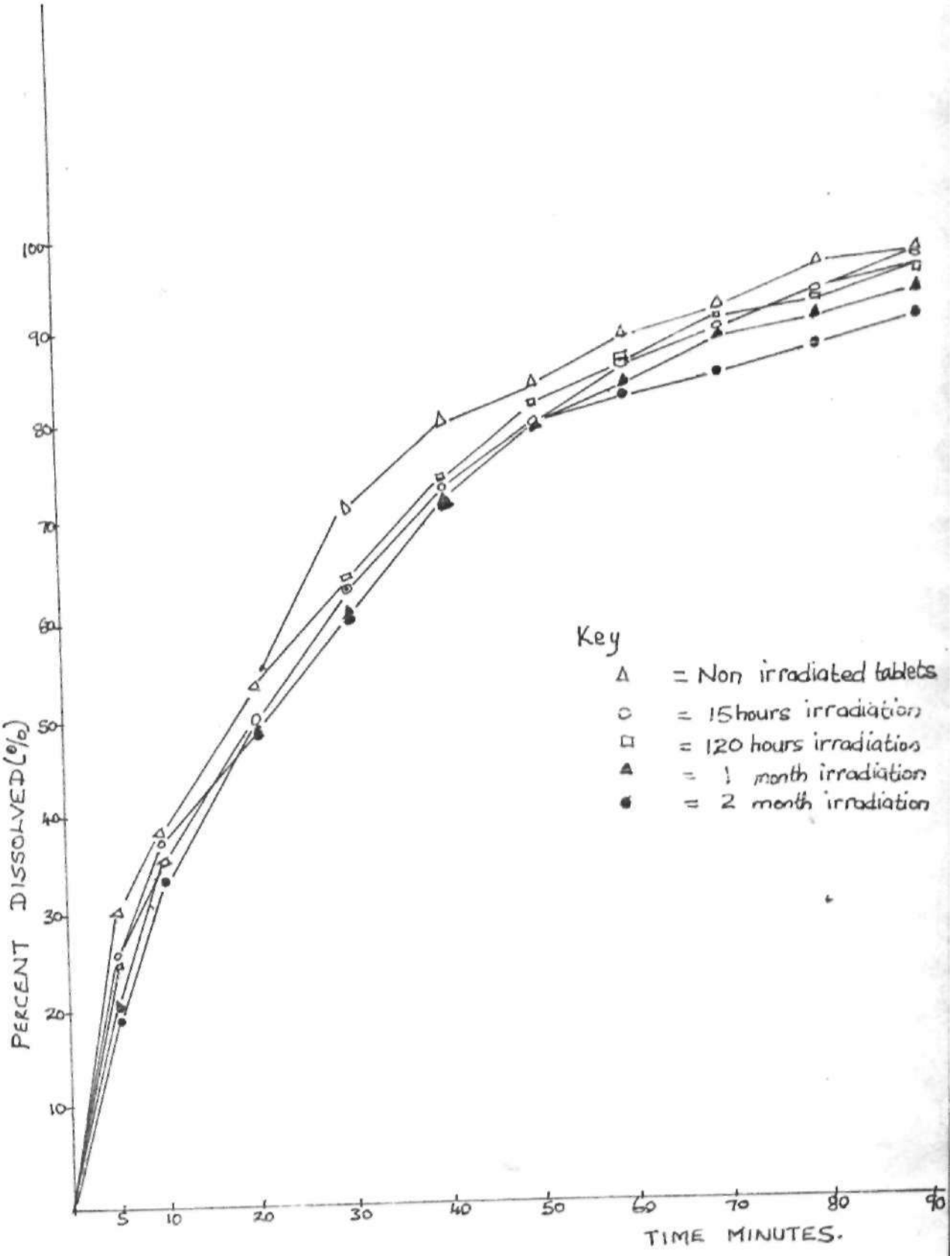


Fig 21. Comparison between dissolution rate profiles of pyrimethamine sulphate tablets BCU 87 in buffer solution pH 4.5 after UV irradiation at 366nm.

TABLE 17: COMPARISON BETWEEN IN-VITRO DISSOLUTION RATES OF PYRIMETHAMINE SULPHATE TABLETS QCU B7 IN PHOSPHATE BUFFER SOLUTION pH 4.5 AFTER UV IRRADIATION OF TABLETS AT 366nm. FOR THE PERIOD OF TIME 15 HOURS TO 2 MONTHS.

TIME	Percentage Released (%)					
		HOURS OF IRRADIATION				
MINUTES	NI	15	48	120	1 month	2 months
5	29.74	24.58	18.64	24.19	23.79	20.42
10	37.57	35.14	33.10	37.51	37.31	36.29
20	54.20	49.76	49.68	52.56	48.59	49.13
30	70.09	62.55	63.06	63.98	60.77	59.73
40	80.41	72.08	75.18	74.50	72.07	72.22
50	84.17	80.13	81.27	81.99	79.71	80.05
60	89.15	85.86	85.82	85.36	84.05	83.02
70	92.75	90.46	91.02	90.94	89.22	85.90
80	96.64	93.50	94.06	92.79	91.66	88.14
90	98.35	97.56	96.93	96.97	93.72	91.22
	P	< 0.001	< 0.01	< 0.01	< 0.001	< 0.001

NI = Non-Irradiated tablets.

therefore decrease in dissolution rate is not due to decrease in drug content. The work of Cooper and Brecht (1957), show that surfactants in tablet formulation could effectively reduce disintegration time. Levy and Gumtow (1963) also observed that the lubricant magnesium stearate retarded the dissolution rate of salicylic acid contained in compressed tablets. This retarding effect was partially attributed to delayed granule disintegration and reduced contact between drug and solvent. Decrease in dissolution rate observed here for pyrimethamine sulphate in QCU B7 could be due to the effect of UV radiation on a lubricant which resulted in modification of tablet disintegration time thereby affecting the dissolution rate. The similarity between the observations made here and the work of Levy and Gumtow (1963) on the effect of lubricant on the dissolution rate of salicylic acid contained in compressed tablets is shown in figures 22a - 23b.

3.9 IRRADIATION OF PYRIMETHAMINE SULPHATE IN PHOSPHATE BUFFER SOLUTION pH 4.5.

The results are shown in Table 10 and Figure 24.

On irradiating pyrimethamine sulphate in phosphate buffer solution pH 4.5 at 366nm an increase in absorbance was observed. This remained consistent until the irradiation was stopped after 96 hours. Neither depression of peak nor spectral shift was observed. In two out of the six experiments carried out,

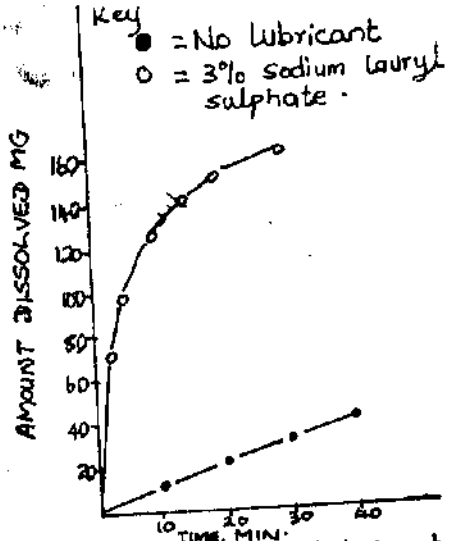


Fig 22a. Effect of lubricant on dissolution rate of salicylic acid contained in compressed tablets. (formula B).

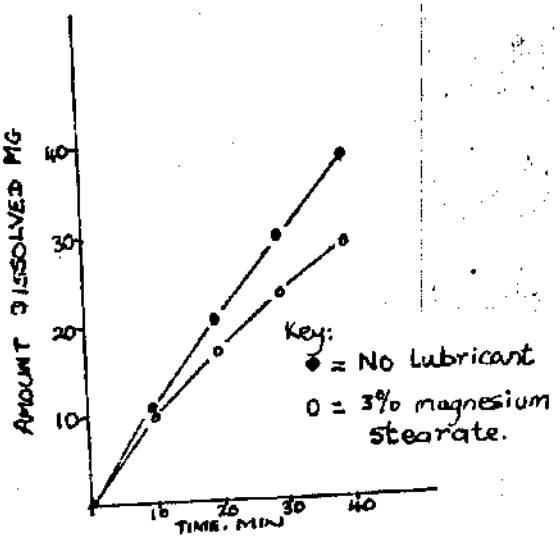


Fig 22b. Effect of lubricant on dissolution rate of salicylic acid contained in compressed tablets (formula B)

Adapted from Levy/Gumtow (1963). J. Pharm. Sc. 52: 114

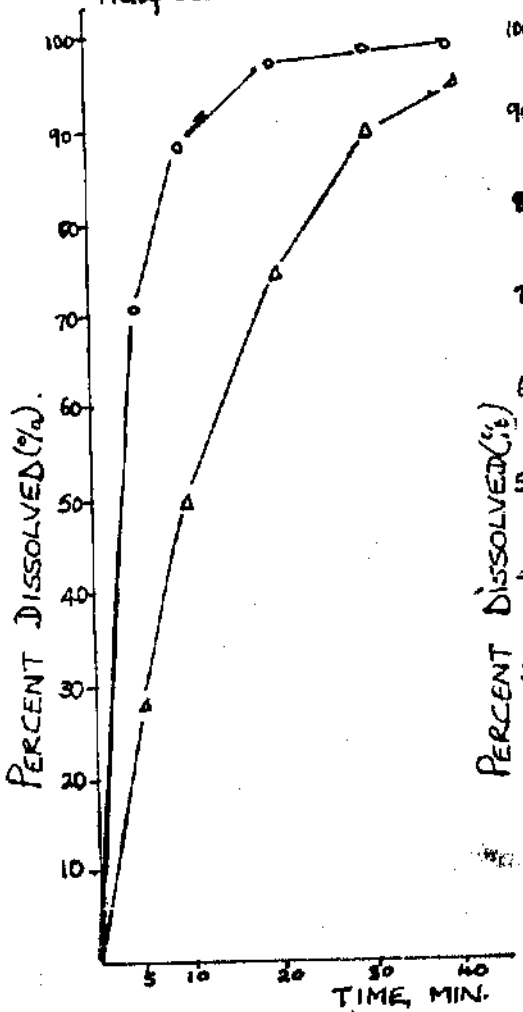


Fig 23a: Effect of uv radiation on dissolution rate of pyrimethamine tablets B191 after 2 months irradiation (366nm).

Key: ○ = Irradiated tablets
△ = Non irradiated tablets

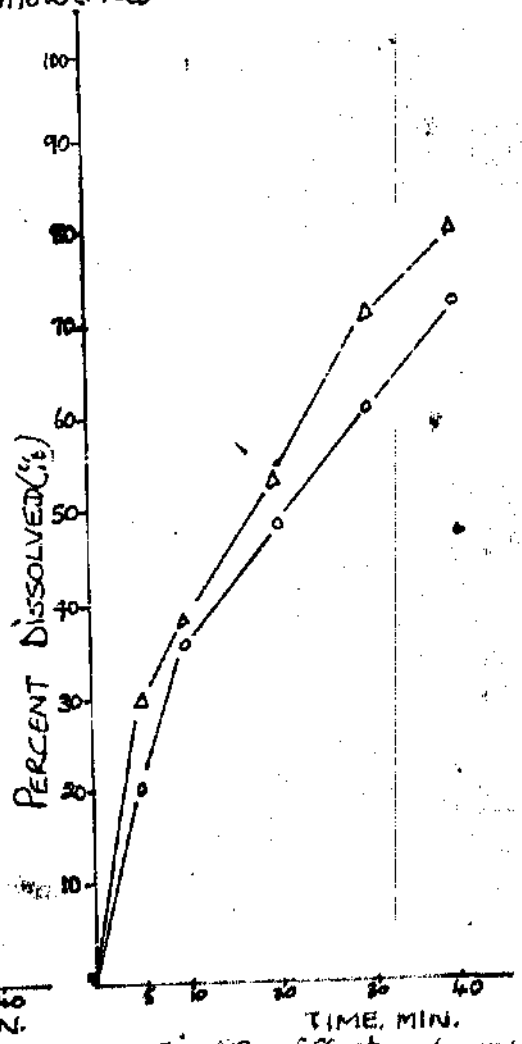


Fig 23b: Effect of uv radiation on dissolution rate of pyrimethamine tablets QCU B7 after 2 months irradiation (366nm).

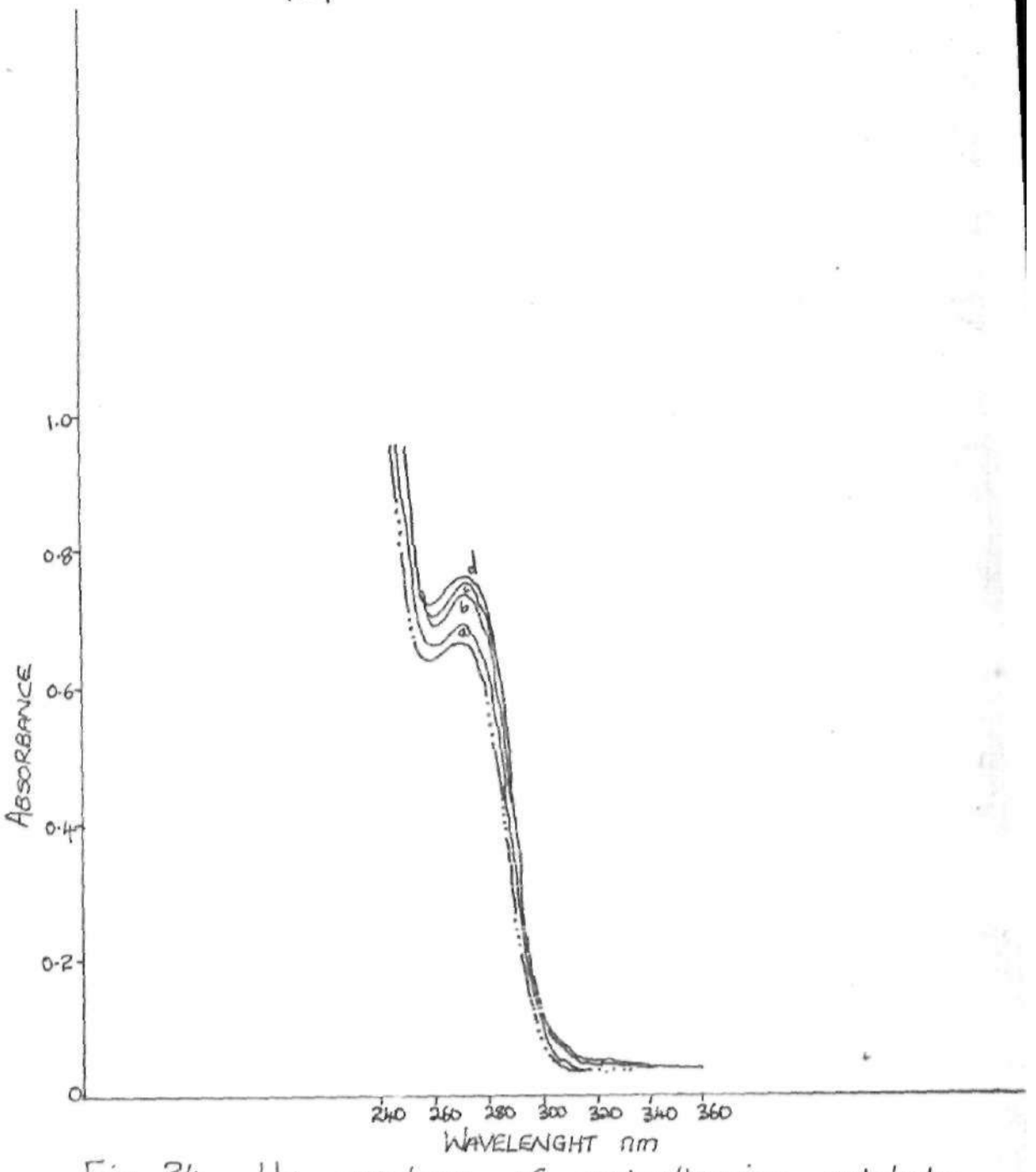


Fig 24 Uv spectrum of pyrimethamine sulphate after uv irradiation (366nm) in phosphate buffer solution pH 4.5.

Key: ——·····—— = Non irradiated solution
a = 8 hours irradiation
b = 20 hours irradiation
c = 44 hours irradiation
d = 96 hours irradiation

TABLE 18: EFFECT OF UV RADIATION ON PYRIMETHAMINE SULPHATE IN PHOSPHATE BUFFER SOLUTION pH 4.5 AT 366nm.

TIME	ABSORBANCE					
HOURS	EXPT. 1	EXPT. 2	EXPT. 3	EXPT. 4	EXPT. 5	N
8	0.608	0.620	0.634	0.611	0.606	0.603
20	0.625	0.673*	0.655	0.616	0.645	0.603
44	0.626	0.726	0.650*	0.617	0.654	0.600
96	0.639	0.731	0.667	0.627	0.668	0.606

Key: * = Appearance of black precipitate.

NI = Non Irradiated Solution.

black precipitate was observed.

It is known that when certain compounds which undergo photochemical reactions are exposed to UV light they usually showed an increase in absorbance of their UV spectra followed by destruction of characteristic peaks (Sams and Carrol 1966, Storck 1965).

The observations made on irradiation of pyrimethamine sulphate are not totally consistent with the above findings. It seems that this drug probably does not readily undergo photochemical reaction when exposed to UV radiation of long wavelength (366nm) especially in phosphate buffer pH 4.5.

CONCLUSION

From the discussion above on the effect of UV radiation on the stability of proguanil hydrochloride and pyrimethamine sulphate (tablets and phosphate buffer solutions), it can be concluded that proguanil hydrochloride is probably unstable to light especially UV radiation while pyrimethamine sulphate appear to be stable to UV radiation (366nm).

The dissolution rate profiles indicate that both drugs seem to have optimum dissolution in weakly acidic medium using phosphate buffer solutions. The rate and extent of drug release were different for the different batches used especially pyrimethamine sulphate tablets. This result confirms the need for batch to batch uniformity in the quality control of pharmaceutical products. The dissolution rate test of irradiated pyrimethamine sulphate tablets emphasize the changes that could take place in a pharmaceutical preparation due to improper exposure during transportation or storage thus affecting the efficacy of the drug.

This work has shown that in the analysis of proguanil hydrochloride especially in quantitative work, strong acid medium should be avoided. In analysis involving phosphate buffers, it is recommended that work should not be carried out below pH 2.0. for the stability of the spectral peaks at 253 and 232nm to be retained.

Irradiation of the drugs in phosphate buffer solutions indicated that proguanil hydrochloride readily enters into photochemical reactions. Accordingly it is recommended that in the formulation of suspensions, elixirs, and syrups containing proguanil hydrochloride the drug should be protected from light to avoid degradation. The pH of such formulations should be adjusted to pH 4.5 to enhance solubility.

Although pyrimethamine sulphate appeared not to enter readily into photochemical reaction black precipitate was observed in some of the irradiated solutions. It is therefore recommended that in the storage of liquid formulations containing pyrimethamine sulphate, the type of glassware employed should be carefully evaluated and be protected from light.

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APPENDIX I

Preparation of physiological buffer solutions

pH 1.5

2.0gm. of sodium chloride was accurately weighed and dissolved in sufficient distilled water to make one litre. It was warmed to 37°C and adjusted to pH 1.5 with U.S.P. diluted hydrochloric acid.

pH 2.0

2.0gm of sodium chloride was accurately weighed and dissolved in sufficient distilled water to make one litre. It was warmed to 37°C and adjusted to pH 2.0 with U.S.P. diluted hydrochloric acid.

pH 4.5

6.8gm of potassium di-hydrogen orthophosphate was accurately weighed and dissolved in sufficient distilled water to make one litre. It was warmed to 37°C and adjusted to pH 4.5 with 1N HCl.

pH 6.9

3.40gm of potassium di-hydrogen orthophosphate and 3.55gm of di-sodium hydrogen orthophosphate (anhydrous) were accurately weighed and dissolved in distilled water to make one litre. It was warmed to 37°C and adjusted to pH 6.9 with 1N HCl or 1N NaOH.

pH 7.2

6.8gm of potassium di-hydrogen orthophosphate was dissolved in sufficient distilled water to make one litre. It was warmed to 37°C and to pH 7.2 with 40% NaOH.

pH 8.0

6.8gm of potassium di-hydrogen orthophosphate was dissolved in sifficient distilled water to make one litre. It was warmed to 37°C and adjusted to pH 8.0 with 40% NaOH.

APPENDIX II

Paired t-test - Effect of one month UV irradiation on pyrimethamine sulphate tablets QCU B7 at 366nm

TIME MINUTES	A	B	A - B	d^2
5	29.74	23.79	5.95	35.40
10	37.57	37.31	0.26	0.68
20	54.20	48.59	5.61	31.47
30	70.99	60.77	10.22	104.45
40	80.41	72.07	8.34	64.56
50	84.17	79.71	4.46	19.89
60	89.15	84.05	5.10	26.01
70	92.75	89.22	3.53	12.46
80	96.64	91.66	4.98	24.80
90	98.35	93.72	4.72	21.44
		SUM	53.08	345.506
		MEAN	5.308	

$n = 10$

$\bar{d} = 5.308$

$d = 53.08$

$(d)^2 = 2817.49$

$d^2 = 345.51$

$\frac{d^2}{n} = 281.75$

$$SD^2 = \frac{d^2 - \frac{(d)^2}{n}}{n-1}$$

$SD^2 = 7.084$

$\frac{SD^2}{n} = 0.7084$

$\sqrt{\frac{SD^2}{n}} = 0.8417$

$$t = \frac{\bar{d}}{\sqrt{\frac{SD^2}{n}}} = 6.31$$

$n-1 = 9$

$P < 0.001$

A = Cummulative percentage drug release of non-irradiated tablets

B = Cummulative percentage drug release of irradiated tablets