IN VITRO AND INSITU INTERACTIONS BETWEEN PROMETHAZINE HYDROCHLORIDE AND CHLOROQUINE PHOSPHATE

BY

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A

Thesis Submitted to Postgraduate School, Ahmadu Bello University, Zaria in Partial Fulfillment of the requirements for the award of the degree of Master of Science in Pharmacology.

DECLARATION

This is to certify that I carried out the work reported In this Thesis in the Department of Pharmacology and Clinical Pharmacy and Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Under the Joint Supervision of Dr. O. C. Wambebe and Dr. A. Mustapha. 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.

Aminu Abdussalam Abubakar

THESIS APPROVAL

We certify that we have read this study and that in our opinion it conforms to acceptable standard of scholarly presentation and is fully adequate in scope and quality as as thesis for the degree of MASTER OF SCIENCE (Pharmacology).

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DEDICATION

I dedicate this thesis to the memory of my late father Abdussalam Abubakar.

ABSTRACT

1. The Effect of Interaction between promethazine hydrochloride and chloroquine phosphate on their absorption from the rats' gut was studied using the technique of Doluisio et al., (1969).

2. Chemical Interaction between chloroquine phosphate and promethazine hydrochloride was observed In vitro.

3. Promethazine hydrochloride (45 mg) added to a solution of chloroquine phosphate (225 mg) gave rise to a yellowish green solution and later a precipitate (M.P. 206°C) was formed. The latter gave positive result for halogens.

4. Chloroquine phosphate (5 µg/ml) added to promethazine hydrochloride (5 µg/ml) decreased chloroquine (47 %) and promethazine (32 %) U.V. characteristic peaks at 344 nm and 249 nm respectively.

5. Promethazine hydrochloride (5 µg/ml) added to Chloroquine phosphate (25 µg) resulted in a decrease in Chloroquine (94 %) and promethazine (82 %) concentrations.

6. Chloroquine phosphate (5 µg) added to promethazine hydrochloride (25 µg) profoundly decreased the U.V. absorbance of both drugs by 82 %.

7. When chloroquine phosphate (50 mg) was administered with promethazine hydrochloride (50 mg) In situ, the absorption of the former drug was reduced by 9 % at the end of 50 minutes.

8. Concurrent administration of promethazine hydrochloride (2.5 mg) and chloroquine phosphate (45 mg) reduced the extent of Chloroquine absorption by 18 %.

9. Promethazide hydrochloride (1 mg) administered with Chloroquine phosphate (20 mg) led to 20 % reduction in Chloroquine absorption.

10. Chloroquine phosphate (2 mg) administered with promethazine hydrochloride (2 mg) reduced the extent of Chloroquine absorption by 33 %.

11. Promethazine hydrochloride (1 mg) coadministered with chloroquine (2 mg) resulted in 32 % decrease in amount of chloroquine absorbed.

12. Administration of Chloroquine (5 mg) and promethazine (1.25 mg) led to 39 % reduction in Chloroquine absorption.

13. In the doses studies, the most pronounced reduction in Chloroquine absorption (46 %) occurred when chloroquine (2 mg) was coadministered with promethazine (10 mg).

14. The data suggest chemical Interaction between chloroquine phosphate and promethazine hydrochloride. When the two drugs were present in high concentration in solution double decomposition reaction probably occurs via charge transfer complex formation leading to the formation of promethazine phosphate which was precipitated. This is supported by the positive test for phosphorus.

15. The determinants of the interaction both <u>In vitro</u> and <u>In situ</u> seem to be the initial concentration of the two drugs; the ratio in which they were present, and the post-administration time <u>In situ</u>.

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Abbreviation

U. V.	=	Ultravoilet
G.L.C.	=	Gas liquid chromatograph
T.L.C.	=	Thin-layer Chromatography
I.R.	=	Infra-red
M.P.	=	Melting point
%	=	Percentage
g	=	gramme
mg	=	milligram
ug	=	Microgram
B.P.	=	British Pharmacopoeia
^{o}C	=	degrees centigrade (celsius)
cm	=	centimetre
F.I.D	=	Flame ionization detector.
min.	=	minute
ml.	=	millilitre
kg.	=	kilogram
l.	=	Litre.

Chapter 1

PHYSIOLOGY OF THE GASTROINTESTINAL TRACT (GIT)

The three major components of the gastrointestinal tract are the stomach, small intestine and large intestine or colon.

The Stomach:

The stomach acts as a storage organ for food. The oxyntic cells in the stomach produce and secrete hydrochloric acid to a pH of about 1. Secretion of acid is stimulated via the vagus nerve in response to the thought, sight and smell of food. The antral mucosa secretes gastrin which directly stimulates acid secretion in response to mechanical stimulation by food in the antrum or chemically in response to amino acids present in food (Davenport, 1973). Gastric pH may be raised as high as 5 after consumption of normal meal by healthy individuals (James and Pickering, 1949).

The secretion of bicarbonate by the surface epithelium of the gastric mucosa prevents its digestion by acid pepsin. (Gayner and Heylings, 1979).

Peristaltic wave originating from the stomach propels a small part of the stomach's contents at regular intervals through the pyloric sphincter into the duodenum and small intestine.

The Small Intestine

The most important function of the small intestine is absorption of water, salts and food. The physicochemical properties of substances determine their ability to pass the intestinal membrane. Lipid solubility determines the ease with which substances pass through the intestinal membrane. Water soluble substances do not pass through the lipid membrane but rather through water filled pores which exist in the epithelium. These pores are of limited size and therefore, molecular size or diameter is of importance. The bigger the molecule the more difficult its passage through the pores. Since the pores are believed to be electrically charged, the electrical charge on the molecule will also have an influence on the rate of transfer. Thus, highly charged molecules tend to get across with greater difficulty than neutral or uncharged molecules. Since many compounds are partially soluble in both lipid and water, the partition coefficient which is related to their degree of solubility in these two phases will influence their rate of absorption. Some substances are transported across the membrane irrespective of their physiochemical properties due to the presence of specific active transport mechanisms. The intestine possesses enormous capacity for absorption due to its length and the fact that each epithelial cell is itself covered with innumerable microvilli which increases its surface area (about 4500 m^2, Davenport, 1966).

The Large Intestine (colon)

Reabsorption of water predominates in the colon. Secretion of the colon is alkaline due to the secretion of bicarbonate in exchange for chloride. Bicarbonate neutralizes the products of fecal fermentation. The secretion contains no enterokinase, invertase, lipase or trypsin. Lysozyme is present.
No hormonal control of colonic secretion has been identified (Davenport, 1973).

1.2 Drug-Drug Interactions

In the treatment of many diseases the use of combination of drugs is the rule rather than the exception. This polypharmacotherapy provides avenue for drug-drug interactions to occur.
Drug-drug interactions occur with a variety of mechanisms which may be broadly subdivided into:

(1) Pharmacokinetic drug-drug interaction.
(2) Pharmacodynamic drug-drug interaction.
(3) Miscellenous interactions. To this group belong interactions whose mechanisms are not known (Nies, 1974).

1.2.1 Pharmacokinetic Drug Interactions: interactions occur during absorption, metabolism, distribution or excretion and may result in more or less drug/active metabolite at site of action.

Drug Absorption Interactions: the oral route is the commonest, most convenient and preferred route of administration of drugs. Interaction of two or more drugs can occur along the length of the gastrointestinal tract.

Drug absorption interactions may be due to the following mechanisms:

a) Changes in physical properties of gastro intestinal contents.
b) Effect on mucosal permeability and active transport.
c) Alteration in gastro intestinal blood flow.
d) Effect of first pass metabolism.
e) Change in pH of gastro intestinal fluids.
f) Alteration of gastric emptying.
g) Change in gastrointestinal motility.
h) Physicochemical interactions along gastrointestinal tract.
i) Interference with active transport.
j) Unknown mechanisms (Neuvenca, 1979).

Change in physical properties of gastrointestinal Contents.

The volume, composition, viscosity and osmotic pressure of gastrointestinal secretions may be affected by drugs leading to alteration in the deagregation, dissolution and diffusion rates of other drugs (Levy and Rao, 1972). An example is the reduction in absorption of fat soluble vitamins (A,D,K) by use of mineral oil or neomycin (Barrowman et al., 1973).

Mucosal permeability and active transport

Malabsorption syndrome may be produced as a result of toxic effect of neomycin, para-aminosalicylic acid (PAS), and colchicine on intestinal mucosa. This may lead to reduction in the absorption of digoxin, iron, folate and vitamin B_{12}. Malabsorption of vitamin B_{12} has been detected in patients being treated with biguanides or anticonvulsants.

Some drugs such as methyldopa and levodopa are thought to be absorbed by active transport from the small intestine. Chemically similar substances may compete for the same carrier system.

Gastrointestinal blood flow

Changes in blood flow may alter the rate of drug absorption in isolated organs in animals. However, it has not been reported whether drug-induce changes in blood flow modify absorption of other drugs to a clinically significant extent (Neuvonen, 1979).

First pass metabolism: Many Drugs such as propranolol, alprenolol, verapamil, lignocaine and chlormethiazole undergo extensive first pass metabolism and the fraction reaching the systemic circulation unchanged may be very small. Antiepileptic or similar enzyme inducing drugs can reduce the availability of drugs with high first pass metabolism.

pH of the gastrointestinal fluids: For many drugs, pH induced changes in dissolution and gastric emptying may be more important than effect on ionisation. Changes in PH may affect the rate but not the extent of absorption if the drug is not acid labile e.g. aspirin (Neuvonen, 1979).

Gastric Emptying: In some cases, the rate of gastric emptying determines the rate of drug absorption. Drugs with anticholinergic properties, narcotic analgesics, aluminium hydroxide e.t.c. may reduce whereas metoclopramide may accelerate the absorption of other drugs taken concurrently (Gothoni et al., 1972); Nimmo et al., 1975; Hurtwitz, 1977). A decrease in bioavailability of levodopa and penicillins may occur (due to inactivation or metabolism in the stomach) if gastric emptying is delayed (Nimmo, 1976; Prescott, 1974).

Gastrointestinal motility

Beerman and Groschinsky-Grind (1978) reported that oral propantheline decreases the rate but increases the extent of absorption of hydrochlorothiazide. This observation is attributed to the effect of propantheline on gastrointestinal motility.

Physicochemical interaction

Drugs do not compete with one another for passive diffusion since it occurs along the length of the gastrointestinal tract. However, many drugs can physically

interact in the gut to make them non absorbable. For example, cholestyramine binds a number of drugs if they are present simultaneously in the intestine. There was lack of effect of thyroxine when it was administered concurrently with cholestyramine (Northcutt et al, 1969). Kaolin inhibits absorption of lincomycin (Wagner, 1968). Similarly, antacid may reduce the absorption of benzodiazepines through the same mechanism of adsorption (Shader et al; 1977). Adsorption by clay-pectin antidiarrhoeal mixture significantly reduced the rate and extent of absorption of promazine. Similarly, magnesium trisilicate decreases both the rate and extent of nitrofurantoin absorption (Naggar and Khalil, 1979).

Multivalent ions (e.g. Al^{3+}, Ca^{2+}, Mg^{2+}, Zn^{2+} and Fe^{2+}) possess high affinity for tetracyclines. In vitro, they rapidly form tetracyline metal complexes many of which are practically insoluble. Ferrous sulphate tablets ingested simultaneously with doxycycline greatly reduced the absorption of the antibiotic by 50 % (Hussar, 1975). Magnesium trisilicate increases the absorption of bishydroxy coumarin probably via formation of soluble bishydroxy coumarin-magnesium trisilicate chelate (Ambre and Fischer, 1973).

Interactions occuring during distribution.

Some drugs are usually transported in the plasma bound to plasma proteins particularly but not exclusively bound to albumin. The bound fraction is pharmacologically inert and the therapeutically important concentration is that of the free unbound drug which is in dynamic equilibrium with the

bound protein moeity. Disturbance of the equilibrium may lead to clinically important changes in the free active agent. Sulphonamides, salicylates and phenylbutazone by competing for drug binding sites on albumin will displace a number of albumin bound drug and increase the concentration of unbound active agent. The toxicity of methotrexate on the gastrointestinal tract and bone marrow may be enhanced by protein displacement by sulphonamides and salicylates (Dixon, 1965).

Quinacrine is extensively bound in the liver whereas primaquine localizes in the liver, lung and brain. Concommittant adminstration of the two drugs raises the plasma primaquine concentration 5 - 10 fold and its rate of disappearance from the plasma is markedly reduced (Zubrod et al; 1948).

Thus, quinacrine enhances the toxicity of primaquine by altering the metabolism and distribution of primaquine in man. Premature infants possess a defective conjugating system responsible for coupling bilirubin with glucuronic acid. Their blood-brain barrier is also more permeable to bilirubin. Kernicterus results if bilirubin enters the brain (Silverman and Andersen, 1956). Sulphonamides can displace bilirubin from protein binding sites and therefore their use is contraindicated in premature infants.

Drug interaction occuring at site of metabolism

Drug-drug interaction at site of metabolism may be as a result of enzyme induction or inhibition.

Inhibition of metabolising enzymes

One drug may inhibit the metabolism of another drug or of a hormone, neurotransmitter or other endogenous compound. Examples include accumulation of acetaldehyde caused by disulfiram in the presence of ethanol; inhibition of oxidation of monoamine by monoamine oxidase inhibitors. Isoniazid (INH) inhibits the hydroxylation of phenytoin and simultaneous administration of both drugs can cause toxic reactions to phenytoin (Kutt et al.; 1970). However this toxicity manifest itself predominantly in slow acetylators of INH (Kutt et al.; 1966). The metabolism of tolubutamide is reportedly inhibited by bishydroxycoumarin, chloramphenicol and phenylbutazone. Although some of these drugs may displace the hypoglycemic agent from albumin binding sites, the actual interaction is believed to be due to inhibition of drug metabolism (Rowland, 1974). Alcohol either stimulates or inhibits the activity of hepatic enzymes depending on the prevailing circumstances at the time of administration. Chronic alcoholics show decreased sensitivity to sedative drugs. Increased rate of metabolism of tolbutamide, warfarin and phenytoin was observed in alcoholics and may explain the decrease in testosterone levels associated with alcohol consumption (Hussar, 1980). However, acute ingestion of alcohol by nonalcoholic individuals may cause inhibition of hepatic enzymes. This may be responsible for the enhanced sedative effect of sedatives administered together with alcoholic beverage by individuals who are not alcoholics.

Induction of metabolizing enzymes

Induction of drug metabolising enzymes by one drug may result in accelerated inactivation of another drug that is metabolized by the same enzyme system. Phenobarbital is the most widely recognised and one of the most potent of the inducing agents, but chronic use of other barbiturates, phenytoin, griseofulvin, glutethimide and alcohol can also induce microsomal enzymes. The degree of enzyme induction is reported to be under genetic control (Vesell and Page, 1969).

Increased metabolism of steroid hormones by phenytoin or Phenobarbitone has resulted in abnormal dexamethasone suppression test for cushings disease and diminished steroid effect in steroid treated asthmatics (Brooks et al., 1972). Rifampicin has also been shown to induce hepatic mixed function oxygenase enzymes and because of this it interacts with several drugs including oral contraceptives (Mockofink et al., 1973), hypoglycemic agents (Zilly et al., 1975) and oral anticoagulants (O' Reilly, 1974). The effectiveness of prednisolone is considerably reduced in the presence of rifampicin. Since both drugs may occassionally be indicated in treatment of severe tuberculosis, it has been suggested that an alternative to changing to other chemotherapy would be to at least double the dose of prednisolone if rifampicin is to be administered concurrently (McAllister and Thompson, 1983). Rifampicin has also been shown to reduce plasma digitoxin levels due to enzyme induction. As a result of this, acute cardiac failure and pulmonary edema developed. To prevent this, digoxin, which is not metabolized by the liver is prefered (Zilly et al., 1977).

Epileptic patients treated with anticonvulsant drugs showed signs of folate deficiency (Reynolds, 1976), and an increase in the incidence of attack has been observed following folate replacement (Reynolds 1971). Reduction of serum concentration of anticonvulsant drugs as has been demonstrated for phenytoin has been advanced as a cause for the increased fit (Baylis et al., 1971). The effect of some drugs may be decreased in individuals who are heavy smokers. This may be due to increased hepatic enzyme activity resulting from the action of polycyclic hydrocarbons such as 3, 4-benzpyrene which are present in cigarrette smoke. Drugs that are likely to be affected include chlorpropamide, chlordiazepoxide, diazepam, propoxyphene, phenacetin and theophylline. Increased clearance of caffeine by smokers has also been observed. There is enhanced risk of toxicity when oral contraceptives are used by women who smoke since smoking markedly increases the risk of serious cardiovascular effects (e.g. myocardial infarction) especially in women over 35 years of age (Hussar, 1980).

Drug interaction occuring during excretion

One drug may block the renal excretion of another drug by competing for the same tubular transport system. A typical example is the inhibition of penicillin excretion by probenecid thereby increasing penicillin plasma level (Hussar, 1980).

There is enhanced hypoglycemic effect when phenylbutazone is administered with acetohexamide. This is because the active metabolite of acetohexamide, hydroxyhexamide competes with phenylbutazone for tubular secretion. This leads to accumulation of hydroxyhexamide and therefore increased hypoglycemic effect (Field et al., 1967).

The elimination of frusemide involves both renal and non-renal routes (Cuttler et al., 1974). Renal excretion is greater than glomercular filtration rate (GFR) and since the drug is extensively bound to plasma proteins, it is postulated that tubular secretion is the major type of transport (Calesnick et al.; 1966). Frusemide is an organic acid and therefore it is possible that similar compounds may interfere with its tubular transport or its diuretic action or both. Probenecid was observed to reduce renal elimination of frusemide in man with only a mild impairment of its diuretic activity. It was suggested that tubular rather than plasma concentration of frusemide is the main determinant of its diuretic effect (Monnrari et al.; 1977)

Propranolol hasbeen shown to reduce lidocaine elimination in dogs (Branch et al.; 1973) and in human subjects (Ochs, 1980). The fact that lidocaine possesses a narrow therapeutic index prompted Conrad and his colleagues (1983) to study the effect of administration of metoprolol and propranolol on lidocaine elimination in healthy subjedts. They concluded that metoprolol and propranolol reduce lidocaine elimination significantly and urge caution when lidocaine is given to patients who are receiving β-blockers.

They commented that Lidocaine toxicity might result more readily from the combination than when Lignocaine was used alone.

Diphylline is a methylxanthine bronchodilator with a very short biological half life due to elimination by active tubular secretion. The concomittant administration of probeneid (1 g) led to a practical dphylline dosage schedule in aminophylline sensitive patients or those incapacitated by theophylline gastrointestinal side effect (Jarboe, 1983).

Guanethidine-quinidine combination resulted in increased toxicity of the latter drug due to impaired elimination (Schentas et al., 1985).

1.2.2 Pharmacodynamic drug-drug interactions

Alcohol-sedatives: there is increased central nervous system depression in individuals being treated with sedatives or other depressant drugs when they consume relatively large amounts of alcoholic beverages to the extent that such combinations have proven fatal in some individuals (Hussar 1980).

General anaesthetics sensitize the myocardium to the arrythmogenic actions of catecholamines. β-adrenergic blocking agents may mask the signs and symptoms of hypoglycemia and diabetic patients taking propranolol may thus fail to note reactions to insulin or oral hypoglycemic agents in time to prevent dangerous consequences.

In addition, B-adrenergic blocking agents block compensatory mechanisms such as glycogenolysis thus further compounding the problem.

Many patients being treated with an antipyschotic agent such as chorpromazine may also be given antiparkingsonian drug to control extrapyramidal symptoms that may occur. Furthermore a number of psychotic patients may be depressed and a tricyclic antidepresent such as amitryptyline may be added to the therapy. Since each of these agents posses anticholinergic activity, the additive effect may lead to the development of side effects such as dryness of the mouth, blurred vision, urinary retention, constipation and elevation of intraocular pressure. Moreover, it has been observed that excessive anticholinergic effect can cause atropine-like delirium, particularly in geriatric patients. This effect may be misdiagnosed for increase in psychiatric symptoms and may lead to increasing the dosage of the offending therapeutic agent (Hussar, 1975). It was reported that aminophylline antagonized diazepam sedation (Stirt, 1981). This prompted a study of the interaction since it may serve as a possible antidote for diazepam over dosage (Avidson et al., 1982). It was observed that the response to aminophylline was prompt and seemed not to be associated with any unpleasant arousal effect.

It was suggested that the interaction holds out promise that comparably low doses of aminophylline may provide safe antagonism to diazepam sedation.

Cimetidine exaggerates the narcotic effect of methadone. (Sorkin and Opwan, 1983).

The "norepinephrine pump" which physiologically transports norepinephrine into the nerve ending can be blocked by tricyclic antidepressants. Guanethidine also depends on this transport system for its transport to the nerve endings. Therefore, its action can be blocked by tricyclic antidepressants such as amitryptyline, nortryptyline, desipramine, doxepin, et.c. Thus, if guanethidine is added to a regimen which includes tricyclic antidepressants, the former drug will not have any effect. This may result in increase arterial blood pressure to pretreatment levels in hypertensives. The interaction could result in cerebrovascular accident or even death in patients with severe hypertension (Mitchell et al., 1979). It has also been reported that chlorpromazine in doses of 100-400mg/day blocks the norepinephrine pump and can therefore reverse the effects of guanethidine (Janowsky et al., 1972).

1.2.3 Miscellaneous drug-drug interaction.

This include interactions whose mechanisms are unclear or unknown.

Considerably, less warfarin is needed to lower clotting factors in the presence of clofibrate, d and L-thyroxine and anabolic steroids. These drugs enhance the catabolism of clotting factors. In the interaction between warfarin and these drugs there is no alteration in warfarin kinetics, but haemorrhage has been reported (Hussar, 1980).

A diminished tuberculostatic effect may occur if P-aminosalicylic acid (PAS) is administered together with rifampicin because this causes delay and decrease in serum levels of the latter drug. An interval of 8-12 hours is recommended if the two drugs are to be administered concurrently (Domer, 1974).

Bactericidal antibiotic should generally not be administered concurrently with bacteriostatic agents. This is because bactericidal drugs (e.g. penicillin) act on multiplying bacteria that are making new cell walls to exert a lethal effect. If a bacteriostatic agent that stops cell multiplication is administered concurrently, the penicillin derivative cannot exert bactericidal effect because the cells are no longer multiplying.

Lithium is well established in the treatment of mania. Carbamazepine has also been shown to possess antimanic properties.

Chaudry and waters (1983), reported a case of mania in which carbamazepine was used as adjunct to lithium. The patient developed neurotoxicity when carbamazepine was co-added to the treatment, despite plasma concentration of both drugs being within accepted therapeutic levels. In a two-phase study, the authors proved that the neurotoxicity was due to lithium carbamazepine interaction.

Probenecid was reported to improve the antiinflammatory effects of indomethacin without increasing side effects (Brooks et al., 1975).

Aminoglycoside antibiotics, and polymyxins may enhance the action of curare or other neuromuscular junction blockers (Kabins, 1972). This can result in prolonged apnoea in post-operative patients or in difficulty in managing patients with myasthenia gravis.

1.4 Toxicity and side effects of Chloroquine

These include mild and transient headache, visual disturbances, gastrointestinal upset and pruritis. It may cause discoloration of nail beds and mucous membranes. The major untoward effects are pruritis and gastrointestinal discomfort (Rollo, 1980).

Pruritis or itching is a physiologic or sometimes pathologic symptom which occurs in response to a mild, irritative stimuli of the skin. Itching is produced by adequate stimulus of C and probably A delta fibres in the superficial layers of the skin (Winkelman, 1982). A number

of mediators of inflammation have been found to have an effect on pruritis notably histamine which is present in mast cells (Lewis, 1927).

Chloroquine-induced pruritis may have a racial basis. A study of the occurence of the pruritic reaction in 129 negroid, 53 causasoid, 23 Indian, 29 african albino and 8 afro-caucasoid repondents, was reported, (Osifor, 1982). Pruritis during antimalarial therapy was reported by 67.76 % of the negroids, 5.6 % of the caucasoids, 13 % of the indians, 34 % of the african albinos and 12.5 % of the afro-caucasoids. This implied that negroid and others of negroid extraction including albinos are significantly predisposed to have pruritic reaction to chloroquine more then indians and caucasians. The authors suggest that melanization of the skin may not be the sole determinant of the occurence of the reaction and that racial differences in the integument might contribute to the apparent differences in the pruritic reactivity to chloroquine.

In a related study, Olatunde and Obih (1981) observed that the itching reaction did not conform to simple clinical pattern. They suggested a pharmacogenetic basis for the chloroquine induced itching because of its rarity in other environments. Shae and Bernard (1963) tentatively suggested that patients prone to develop chloroquine pruritis may have a slower rate of chloroquine metabolism. Another investigation (Olatunde, 1971) reported that patients who

were susceptible to chloroquine induced itching had significantly higher concentration of unchanged drug and lower concentration of metabolities in their skin compared to skin from other patients indicating that increased liability for this pruritis might be associated with lower rates of chloroquine metabolism.

1.5 Aims and objectives of the Project

Clinically, many patients suffer from allergic pruritis following ingestion of chloroquine. It is therefore a normal practice to prescribe an antihistamine to reduce the severity of the allergic pruritis induced by chloroquine. It is not known whether the antihistamine effects the bioavailability of chloroquine to any significant extent which may indicate modification of doses of the drug during malarial chemotherapy.

It is therefore the objective of this project to use the *in situ* technique of Doluisio *et al.*; (1969) in rats to study the effect of promethazine hydrochloride on the absorption of chloroquine phosphate in the small intestine.

MATERIALS AND METHODS

2.1 Materials

Animals: Albino mice (Kinder strain inbred in our Faculty's animal house) weighing 150 - 200 g. and 4 - 5 month old.

Drugs: Chloroquine phosphate (Delagil)(R) containing 100 mg per ampoule of 2 ml (Egyt Pharmacochemical Works, Budapest, Hungary).

Chloroquine phosphate powder supplied by Department of Industrial Pharmacy and Quality Control, Institute of Health, A.B.U., Zaria, Nigeria.

Chloroquine phosphate injection (30 ml) containing equivalent of chloroquine base B.P. 40 mg/ml, chlorbutol 0.5 %, potassium metabisulphite, hydroquinone (Helm Pharmaceuticals GMBH).

Pentobarbitone sodium injection (Sagatal)(R) Manufactured by May and Baker, Great Britain.

Promethazine hydrochloride injection B.P. (Pipolphen)(R) containing 50 mg promethazine hydrochloride in each ampoule of 2 ml (Egyt Pharmacochemical Works, Budapest, Hungary).

Silica gel BDH chemicals products No. 15056, made in England.

All drugs were purchased except chloroquine phosphate powder which was kindly supplied by the Department of Industrial Pharmacy and Quality Control, Institute of Health, Ahmadu Bello University, Zaria, Nigeria. All chemicals used were reagent grade unless otherwise specified. All solutions were prepared using freshly prepared distilled water.

2.2 Physiological Buffer Solution

Phosphate buffer (pH 6.5) was prepared by weighing accurately sodium dihydrogen phosphate (7.3 g), disodium hydrogen phosphate dodecahydrate (7.2 g) and sodium chloride (5 g) and made up to one litre with freshly prepared distilled water. The pH was determined using a pH meter previously calibrated (Pharmaceutical Handbook, 18th edition, 1975).

2.3 Calculations and Statistical analysis (Appendix 2.3)

Assay of Chloroquine Phosphate and Promethazine hydrochloride

Chloroquine phosphate and promethazine hydrochloride used in the study were assayed to ascertain whether they conform to British Pharmacopoeia (B.P.) requirement with respect to content of active ingredient.

Promethazine hydrochloride injection B.P. 1980:

To a volume equivalent to 25 mg promethazine hydrochloride was added sufficient 0.01M hydrochloric acid (HCl) to produce 100 ml. 10 ml of this solution was further diluted to 100 ml with 0.01M HCl; 10 ml of this solution was again diluted to 50 ml with 0.01M HCl and the absorbance of the resulting solution was measured at the maximum at about 249 nm. The content of $C_{17}H_{20}N_2S$. HCl was calculated taking 910 as the value of A (1 %, 1 cm) at the maxima at about 249 nm.

Chloroquine Phosphate:

The procedure followed was as for promethazine hydrochloride except that the absorbance of the solution was measured at 344 nm and the value of A (1 %, 1 cm) was taken to be 371.

2.4 Infra-red Spectroscopy, Gas-liquid Chromatography and Thin-layer Chromatographic Analysis

Infra-red Spectroscopy: The precipitate was washed three times with distilled water, dried in the oven (110°C) for one hour. Its infra-red spectrum was determined using mineral oil dispersion and KBr disks.

Gas-liquid Chromatography

1 ml of chloroquine phosphate solution (50 mg/ml) was placed in a 10 ml extraction tube tapered at one end. Few drops of 20 % sodium hydroxide solution were added and extracted with 50 µl chloroform using single step extraction technique. 3 µl of the chloroform layer was injected into the glc. This procedure was repeated using promethazine hydrochloride solution. The precipitate was dissolved in 5 ml of absolute alcohol with warming and 3 µl was injected into the column under the same operating conditions (Appendix 2.2)

Thin layer Chromatography

Silica gel (30 g) was shaken vigorously with water (60 ml) in a stoppered flask for a period of 90 seconds. A layer (0.25 µm) was prepared by spreading the slurry on glass plates (18 x 20 cm) using Chandon Unoplan(R) Leveller. The plates were placed on the leveller and the adsorbent was applied by means of a movable spreader. The plates were left on the leveller until the surface became dry (after 10 - 20 minutes). They were then transferred to a metal rack and placed in an oven heated to 110°C for one hour.

The solution containing the substance to be analysed together with standards(chloroquine phosphate and promethazine hydrochloride) was applied at a distance 2 cm from the lower edge of the plate by means of micropipettes (10 µl), and allowed to dry before placing in a developing tank.

Solvent Systems: Ammonia; methanol (1.5:100); Acetone: water: Ammonia (90:40:1). Saturation of the chamber (tank) was allowed. This was achieved by introducing the solvent system one hour before developing the chromatogram in order to improve the quality of the separation and enhance reproducibility.

Detection: U.V. light, Dragendorff's reagent. Preparation of Dragendorff's reagent (Appendix 2.1)

2.5.1 Test for Phosphorus: The precipitate (0.05 g) was intimately mixed with sodium peroxide (3 g) and anhydrous sodium carbonate (2 g) in a nickel crucible. The crucible and its content were heated with a small flame, gently at first, afterwards more strongly until the contents were fused and the heating was continued further for 10 minutes. The content of the crucible (after allowing to stand for sometime) were extracted with water (5 ml) and filtered. Excess nitric acid followed by ammonium molybdate solution were added to the filtrate. The presence or absence of yellow precipitate was noted.

2.5.2 Test for Halogens

A solution of the precipitate in ethanol was acidified wity dilute nitric acid. Excess silver nitrate solution was then added. The presence or absence of white precipitate was noted (Vogel, 1972).

2.6 Experimental:

2.6.1 In vitro: Chloroquine phosphate (5 µg/ml) was prepared by diluting 0.1ml chloroquine phosphate injection (50 mg/ml) with distilled water (1000 ml). Promethazine hydrochloride (5 µg/ml) was prepared by diluting 0.1 ml of promethazine hydrochloride injection (25 mg/ml) with distilled water (500 ml). Chloroquine phosphate (225 mg/4.5 ml) was mixed with promethazine hydrochloride (45 mg/1.8 ml) in a sample bottle. 0.1 ml of the mixture was diluted to 100 ml with distilled water, and the absorbance taken. The concentration of each drug was calculated. Any change in colour and or precipitation was noted. The change in the intensity of the colour with time was also noted.

2.6.2 Insitu rat gut technique

The technique of Doluisio et al., (1969) was adopted. Perfusion solution: The perfusion solution used for irrigating the gastrointestinal tract contained 1.45×10^{-1}M Nacl, 4.56×10^{-3}M KCl; 1.25×10^{-3}M $CaCl_2$; and 5.00×10^{-3}M NaH_2PO_4.

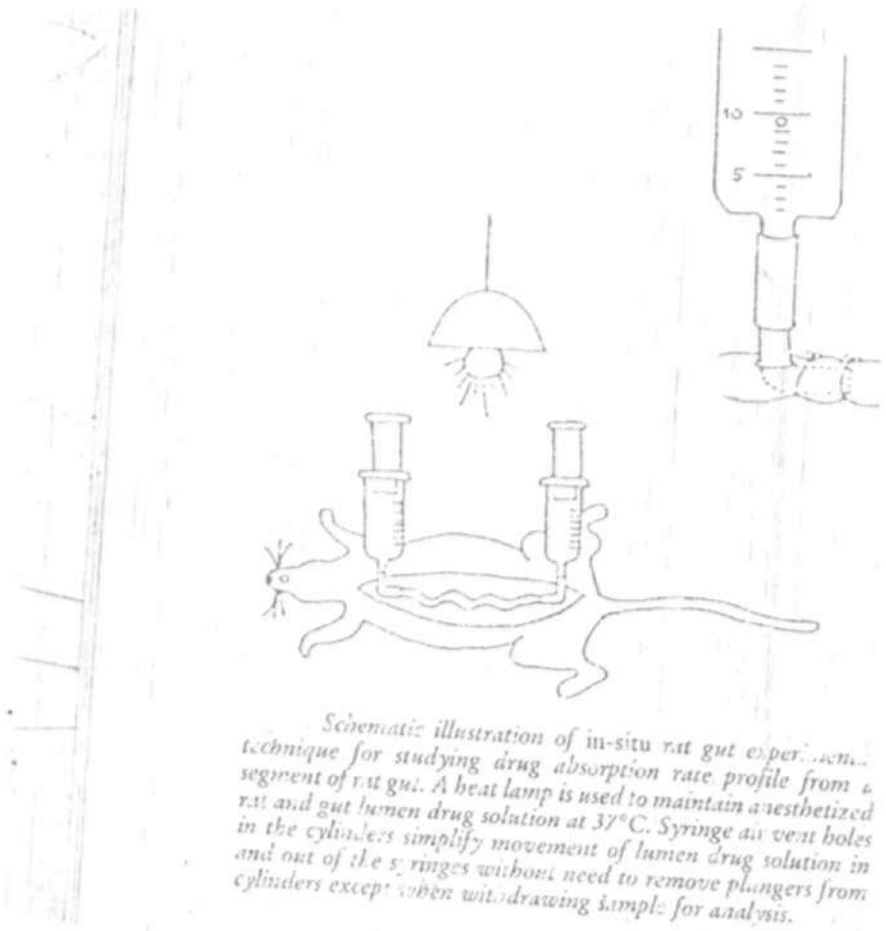
Drug solutions were made isotonic and buffered at pH 6.5 with Sorensen's Phosphate buffer. All solutions were maintained at 37°C ± 0.5°C during administration.

Test Animals. Male albino rats (Wistar strain) weighing 150 - 200 g were fasted 16 - 24 hour prior to surgery; however drinking water was readily accessible. The rats were kept in cages having wide floors to prevent coprophagy. The rats were anaesthetised with pentobarbitone sodium (sagatal)(R) using an intraperitoneal injection of 40 mg/kg body weight.

Intestinal absorption procedure:

The small intestine was exposed by a mid-line abdominal incision, and two L-shaped glass cannulae were inserted through small slits at the duodenal and illeal ends as illustrated (fig. 2.1). Care was taken to handle the small intestine gently and to reduce surgery to a minimum in order to maintain an intact blood supply. The cannulae were secured by ligation with silk suture, and the intestine returned to the abdominal cavity to aid in maintaining its integrity. Four centimetre segments of tygon tubing were attached to the exposed ends of both cannulae and a 10 ml hypodermic syringe containing perfusion fluid warmed to 37°C was attached to the duodenal cannula. As a means of clearing the gut contents, perfusion fluid was then passed slowly and discarded through the the illeal cannula until the effluent solution was clear.

The remaining perfusion solution was carefully expelled from the intestine by pumping air with the aid of the



Schematic illustration of in-situ rat gut experiment technique for studying drug absorption rate profile from a segment of rat gut. A heat lamp is used to maintain anesthetized rat and gut lumen drug solution at 37°C. Syringe air vent holes in the cylinders simplify movement of lumen drug solution in and out of the syringes without need to remove plungers from cylinders except when withdrawing sample for analysis.

Fig.2.1(Adopted from Swintosky, J. V. and Pogonowska-wala, E: Pharmacy International (May) 1982, P. 164.)

syringe and drug solution (9 ml) was immediately introduced into the intestine using the syrirge. The time was noted (by starting a stopwatch) and the illeal cannula was simultaneously connected to another 10 ml syringe. This arrangement enables one to move the lumen solution into either the illeal or the duodenal syringe and to remove 0.1 ml aliquot and return the

remaining solution to the intestine within 10 - 15 seconds. To ensure uniform drug solution concentration throughout the gut segment, aliquots were removed from the two syringes alternately.

Chloroquine phosphate solution or promethazine hydrochloride solution was administered alone and 0.1 ml samples were withdrawn for analysis at five minutes interval for fifty minutes. The two drugs were then mixed (at various dosage ratios) and the procedure repeated as for single drug solution. Each sample (0.1 ml) was diluted with distilled water (20 ml) and the absorbance measured at 344 nm and 249 nm for chloroquine phosphate and promethazine hydrochloride determination respectively, using U.V. spectrophotometer (SP8-100 Pye Unicam, Ltd., England).

Chapter 3

RESULTS

3.1 Assay of Chloroquine phosphate and Promethazine hydrochloride

Both chloroquine phosphate (98.65 %) and promethazine hydrochloride (95.6 %) conformed to the British Pharmacopoeia requirement (95 - 105 %) with respect to content of active ingredient (Appendix 3.1).

3.2 In Vitro Chloroquine Phosphate - Promethazine hydrochloride Interaction

Promethazine hydrochloride when added to a solution of chloroquine phosphate decreased the latter's absorbance measured at 344 nm. Conversely, chloroquine phosphate when added to a solution of promethazine hydrochloride decreased its absorbance when the latter was determined at 249 nm. For example, when chloroquine phosphate solution (225 mg/4.5 ml) was added to promethazine hydrochloride solution (45 mg/1.8 ml) and the absorbance of the mixture was determined at 344 nm and 249 nm, there was a decrease in both chloroquine concentration (14 %) and promethazine hydrochloride concentration (56.15 %). (Appendix 3.2). Similar but more pronounced effect was observed in dilute solution (5 - 50 µg). When chloroquine phosphate (5 µg/ml. fig. 3.2.1), was added to promethazine hydrochloride (5 µg/ml, fig. 3.2.2), there was 47 % decrease in chloroquine concentration and 32 %

decrease in promethazine concentration (fig. 3.2.3). When one part of chloroquine phosphate (5 µg) and five parts of promethazine hydrochloride (25 µg, fig. 3.2.4) were mixed, chloroquine phosphate concentration was decreased by 82 %, while that of promethazine was decreased by 32.79 % (fig. 3.2.5). One part of promethazine hydrochloride (5 µg) added to five parts of chloroquine phosphate (25 ug, fig. 3.2.6) resulted in 94.31 % decrease in chloroquine phosphate conentration and 82.79 % decrease in promethazine hydrochloride concentration (fig. 3.2.7).

Both chloroquine phosphate and promethazine hydrochloride solutions are normally colourless. However, when the two solutions were mixed, there was an immediate development of a yellowish green colour and later a precipitate was formed. The same phenomenon was observed with chloroquine phosphate injection containing an antioxidant (potassium metabisulphite) and an antibacterial agent (chlorbutol). Similar observation was made with a solution of chloroquine phosphate powder after promethazine hydrochloride was added. In fig. 3.2.8 is shown the U.V. spectrum of the coloured mixture (chloroquine phosphate was used as the reference).

The mixture of chloroquine phosphate and promethazine was acidified and extracted in chloroform (fig. 3.2.26). When promethazine hydrochloride was used as reference in the U.V. analysis of the chloroform

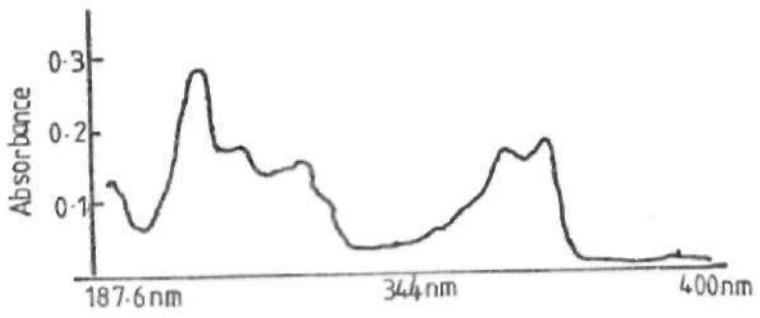


Fig.3.2.1 U.V Spectrum of chloroquine phosphate (5µg/ml)

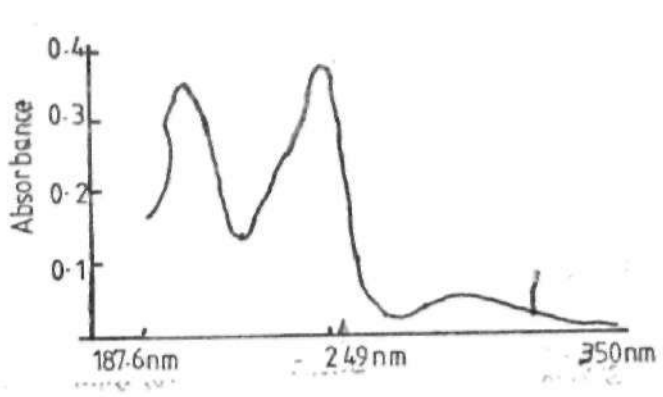


Fig.3.2.2 U·V· Spectrum of promethazine hydrochloride (5µg/ml)

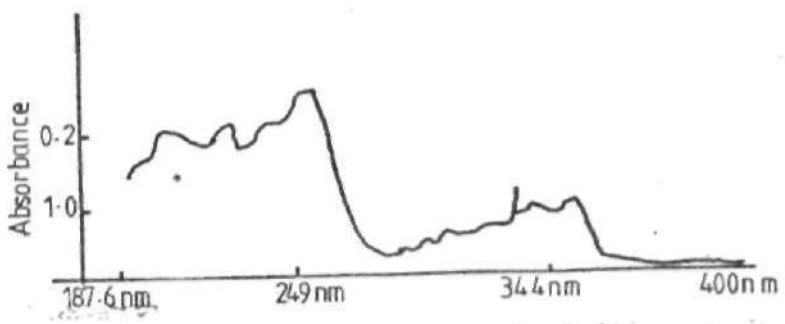


Fig.3.2.3 U·V· Spectrum of chloroquine phosphate (5µg/ml) and promethazine hydrochloride (5µg/ml)

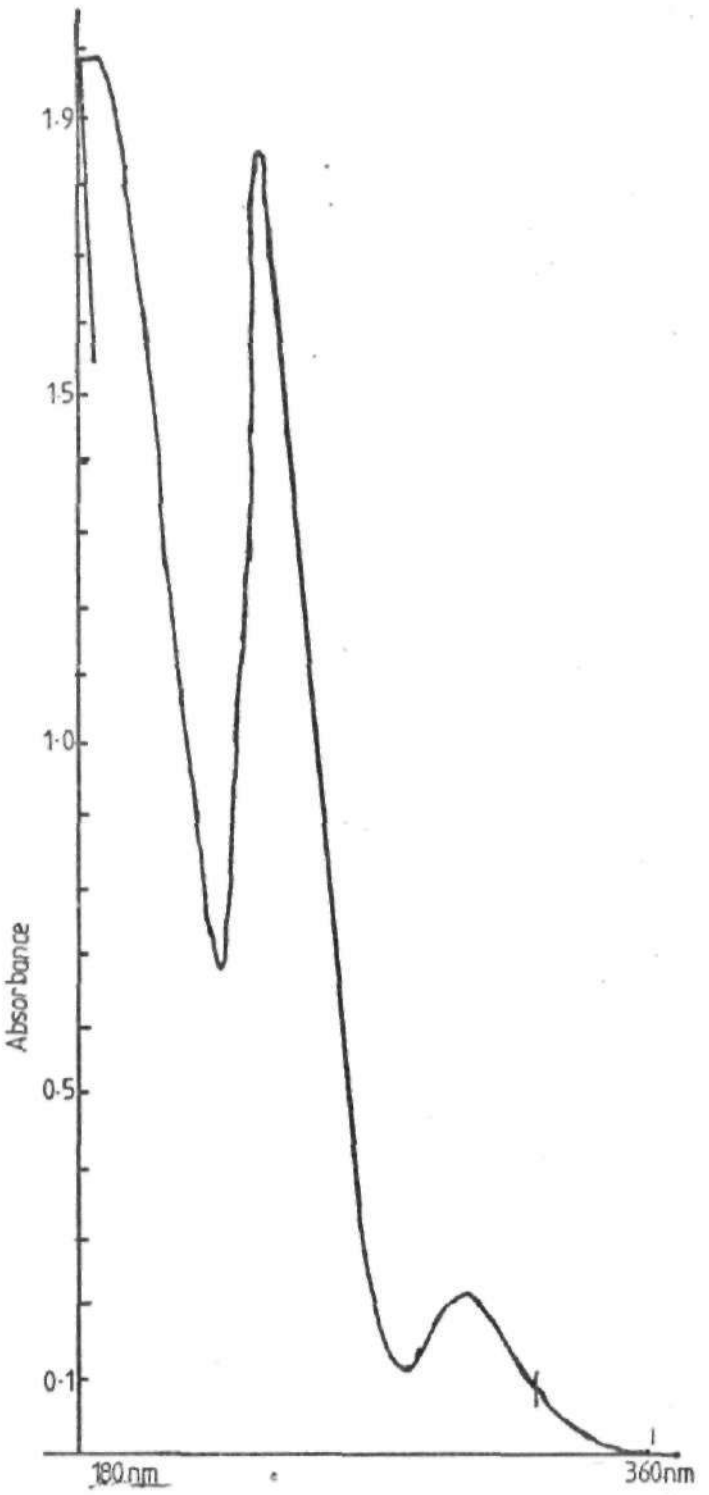


Fig 3.2.4 U. V. Spectrum of promethazine hydrochloride (25 µg/ml

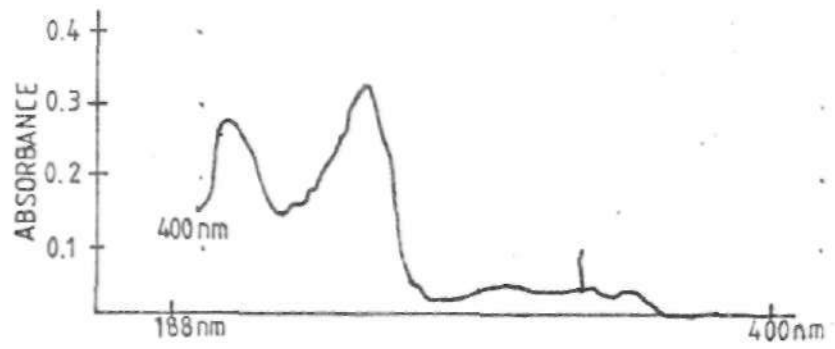


Fig. 3.2.5 U.V. Spectrum of chloroquine phosphate (5µg) and promethazine hydrochloride (25µg)

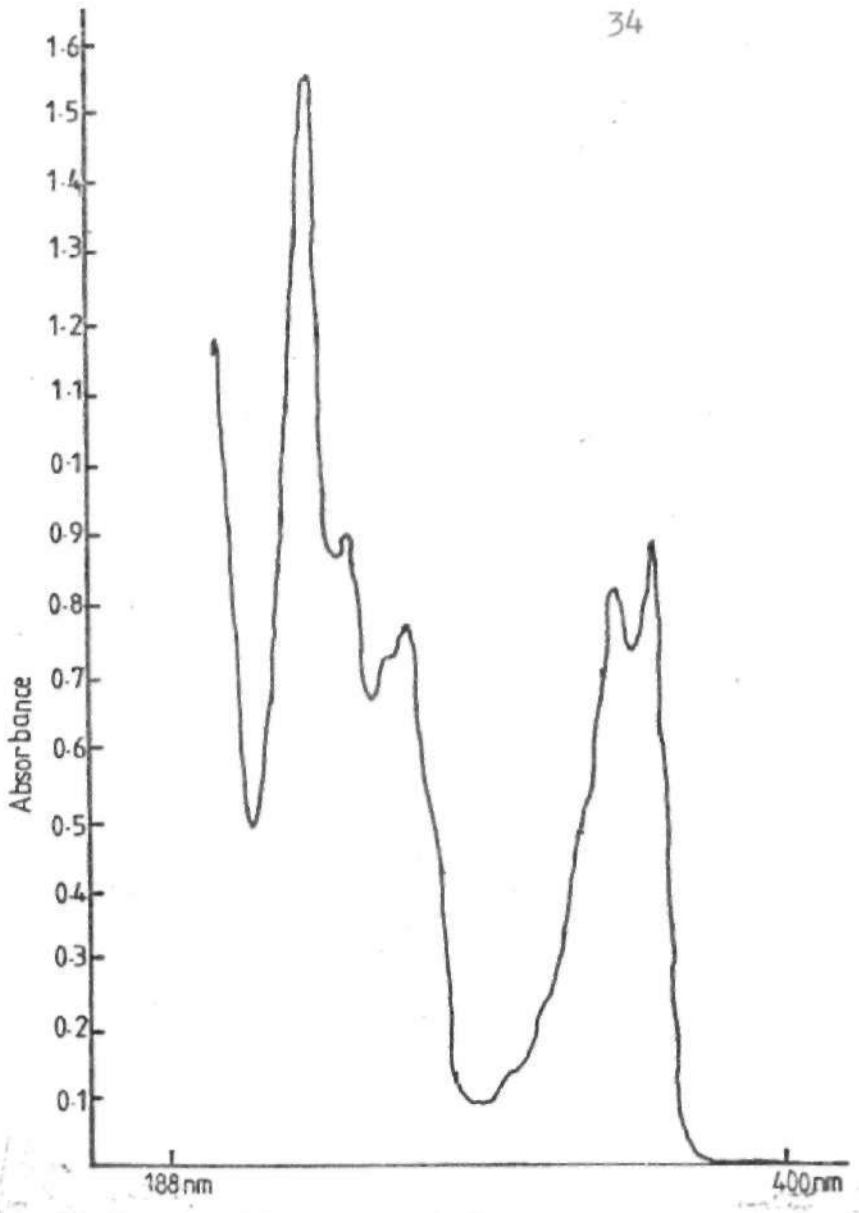


Fig. 3.2.6 U·V Spectrum of chloroquine phosphate (25 µg/ml)

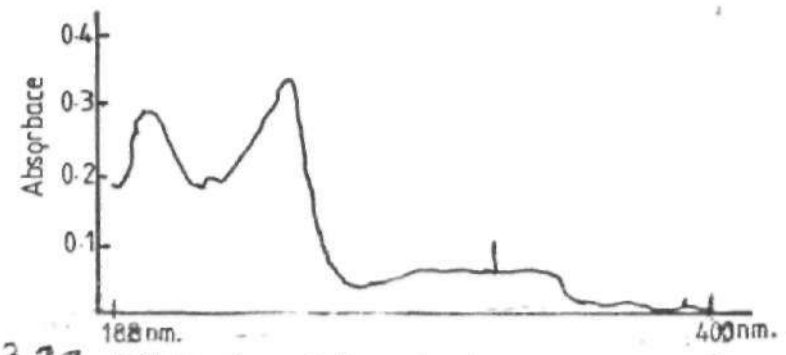


Fig 3.2.7 U.V. Spectrum of chloroquine phosphate (25µg) and promethazine thydrochloride (5µg)

extract two peaks were obtained at 375 nm and 418 nm (fig. 3.2.9). Only the peak at 375 nm was observed when chloroquine phosphate was used as reference.

The pH for promethazine hydrochloride and chloroquine phosphate injections were 5.9 and 4.4 respectively (they satisfied B.P. requirement). The mixture of the two drugs had a pH of 5.1. When the pH of chloroquine phosphate injection was raised to 5.2 (by the addition of ammonium hydroxide), there was no precipitation observed. Upon acidification of the coloured solution (with 5 % HCl), the colour disappeared.

Thin-layer chromatography: No additional spots were observed when mixture of chloroquine phosphate and promethazine hydrochloride were spotted. Similarly no additional spots were observed with chloroquine phosphate containing antibacterial and antioxidant. However, the chloroform and water extracts showed faint spots with R_f values of 0.74. (Chloroquine phosphate and promethazine hydrochloride had R_f values of 0.41 and 0.58 respectively, figs. 3.2.10; 3.2.11).

The precipitate formed was not soluble in moderate amount (10 ml) of chloroform, benzene, heptane and diethylether. However, it was soluble in absolute alcohol on warming. Prolonged warming of the solution of the precipitate resulted in the development of red colouration. The visible and

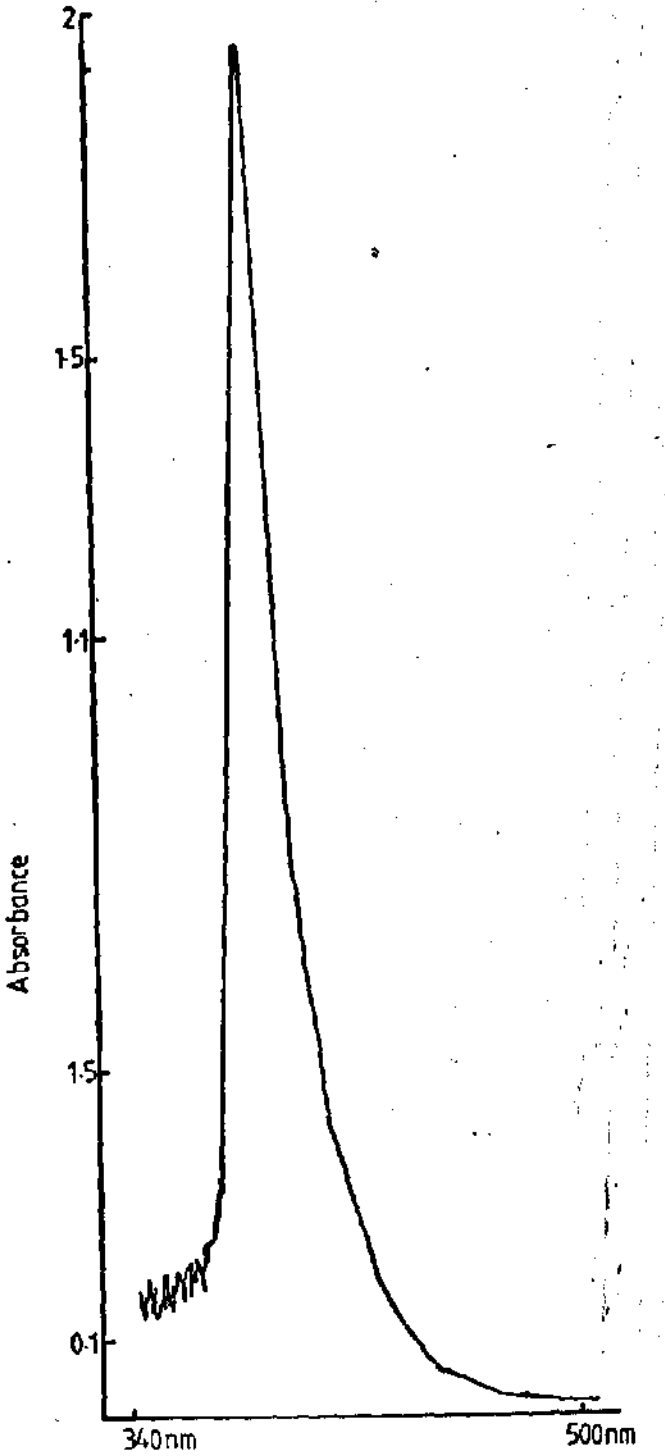
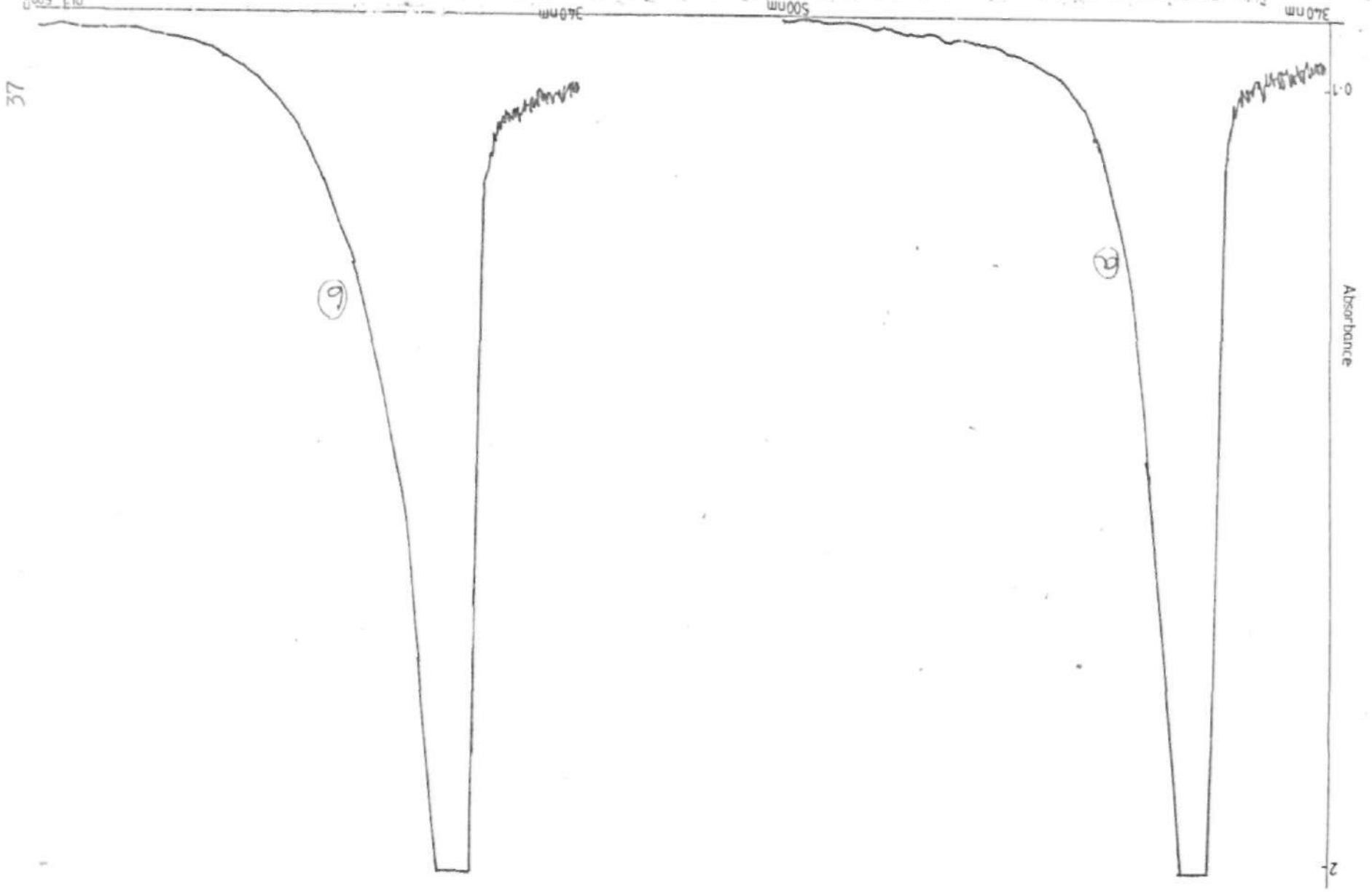


Fig.3.2.8 U.V. Spectrum of coloured mixture of chloroquine phosphate and promethazine hydrochloride



U.V. Spectrum of coloured mixture of promethazine hydrochloride and chloroquine phosphate (a) and chloroform extract of the mixture (b) (chloroquine phosphate was used as reference)

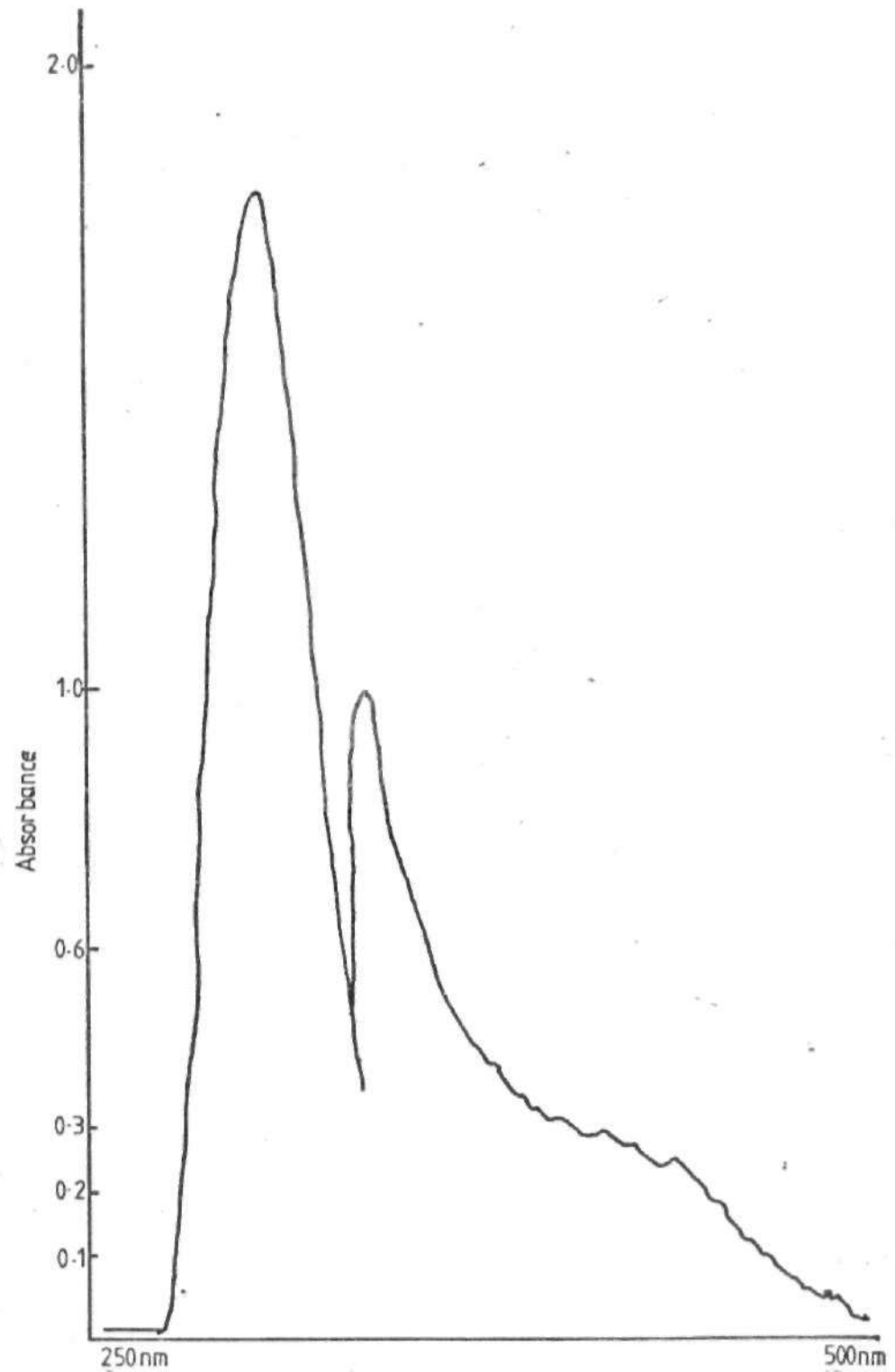


Fig. 3.2.9 UV. Spectra of chloroform extract of chloroquine phosphate-promethazine hydrochloride mixture (promethazine hydrochloride as reference)

ultraviolet (500 nm - 310 nm) of the red solution was determined using chloroquine phosphate as blank (fig. 3.2.12). No spectrum was obtained when promethazine hydrochloride was used as blank (fig. 3.2.13). The spectrum obtained when distilled water was used as blank is shown in fig. 3.2.14. The ultraviolet spectrum of the precipitate, promethazine hydrochloride and chloroquine phosphate were determined (fig. 3.2.15; 3.2.16; 3.2.17) respectively.

Infra-red spectroscopy (I.R.): In fig. 3.2.18, 3.2.19, 3.2.20 and 3.2.21 are shown the infrared spectrum of the precipitate and chloroquine phosphate in KBr disks and liquid paraffin respectively. The spectrum of liquid paraffin (neat) is also included (fig. 3.2.22). The reported principal peaks for promethazine hydrochloride (KBr disks) are 757 or 1459 cm^{-1}, 1222 cm^{-1} (Clarke, 1971) and also 1336 cm^{-1}, 1129 cm^{-1}, 758 and 733 cm^{-1} (The Pharmaceutical Codex). The I.R. spectra of the precipitate did not correspond with that of chloroquine phosphate. However, the I.R. spectrum of the precipitate corresponded with most of the reported values of the principal peaks for promethazine hydrochloride.

Gas-liquid Chromatography (GLC): The following g.l.c. retention times were observed for Chloroquine, promethazine and precipitate (figs. 3.2.23, 3.2.24, 3.2.25).

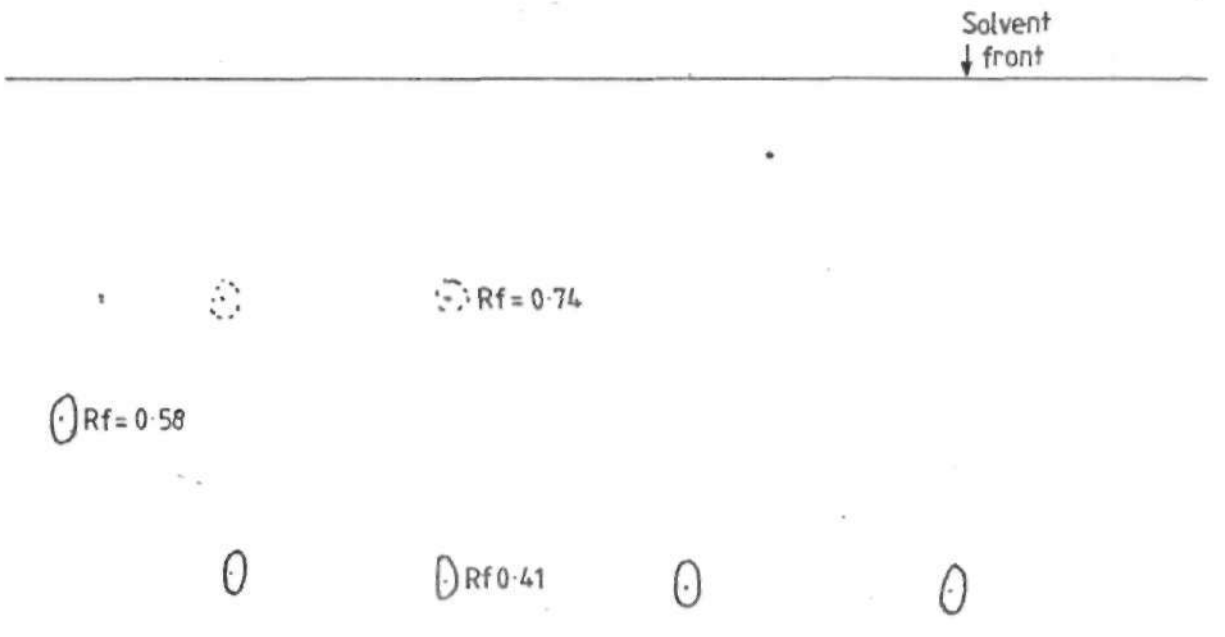


Fig. 3.2.10 TLC of chloroquine phosphate, promethazine hydrochloride, chloroform and water extracts of mixture of chloroquine phosphate and promethazine hydrochloride

Key:

Prometh : promethazine hydrochloride

Chlorof extr Chloroform extract

Water extr : water extract

ChlAA : Chloroquine phosphate containing antibacterial and antioxidant.

Chl: Chloroquine phosphate

Mobile phase: Ammonia · methanol (1.5 : 100)

Visualizing reagent : Dragendorff reagent.

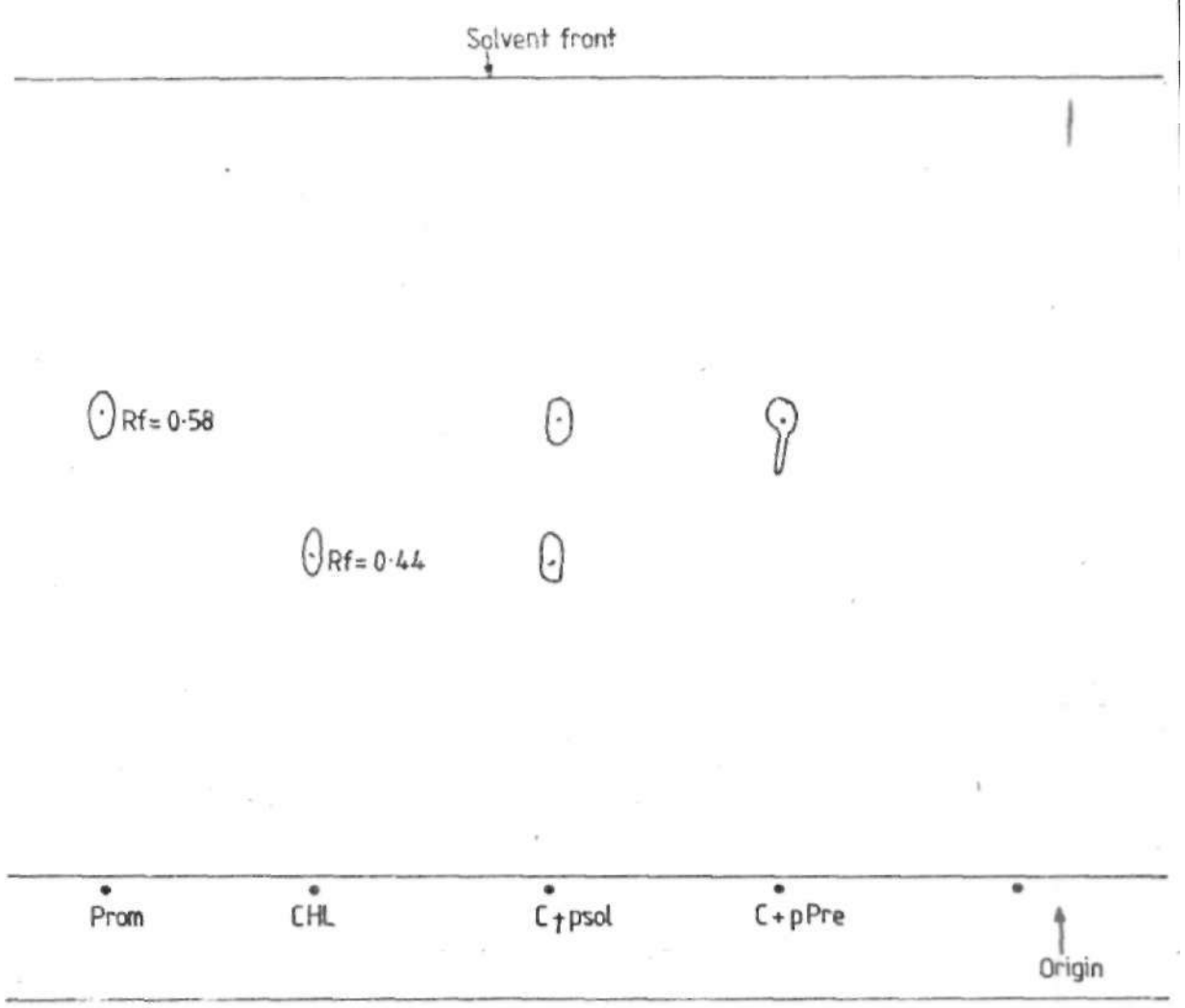


Fig. 3.2.11 TLC of precipitate, supernatant solution, promethazine hydrochloride and chloroquine phosphate

Key :

Prom = Promethazine Hydrochloride
CHL = Chloroquine phosphate
C+psol = Chloroquine phosphate and promethazine hydrochloride solution
C+pPre = Precipitate

Mobile phase : Ammonia : methanol
(1·5 : 100)

Visvalizing agent : U.V. light

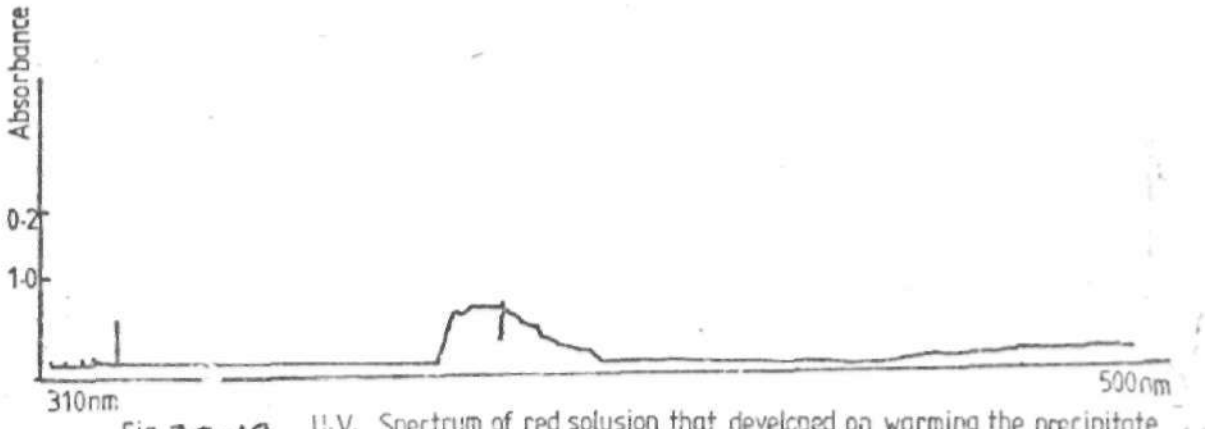


Fig. 3.2.12 U·V. Spectrum of red solusion that develcped on warming the precipitate absolute alcohol



Fig. 3.2.13 No spectrum obtained when promethazine hydrochloride was used as blank.

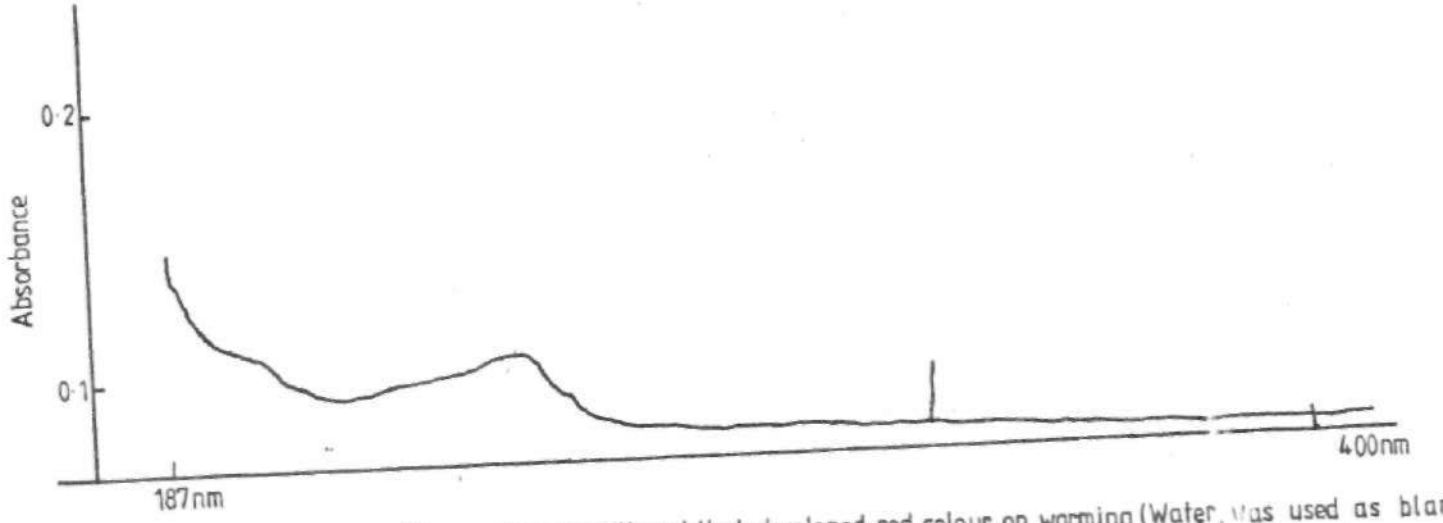


Fig. 3.2.14 The ultraviolet spectrum of the precipitate methanol that developed red colour on warming (Water was used as blank).

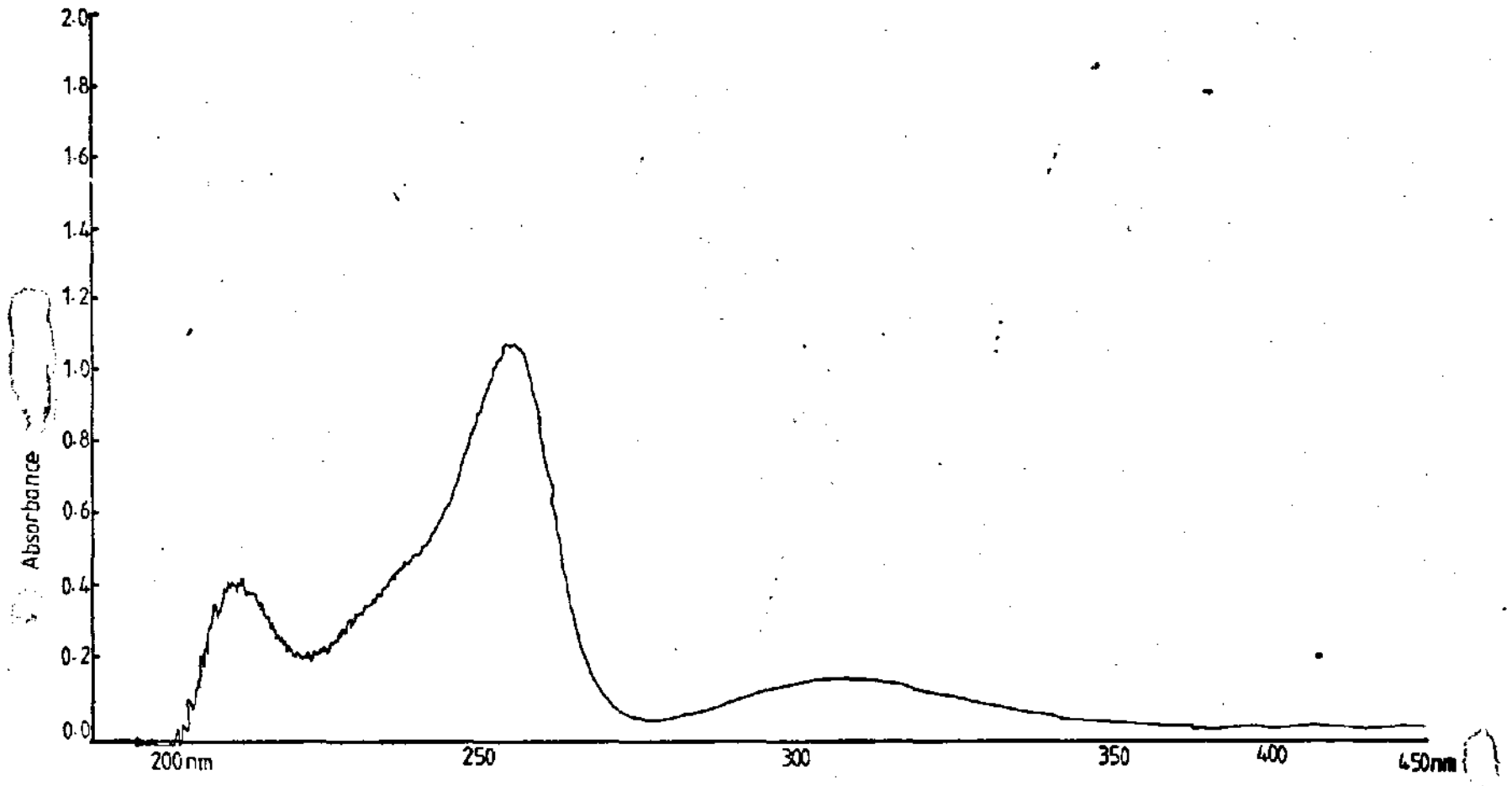


Fig. 3.2.15 U.V. Spectrum of precipitate in ethanol

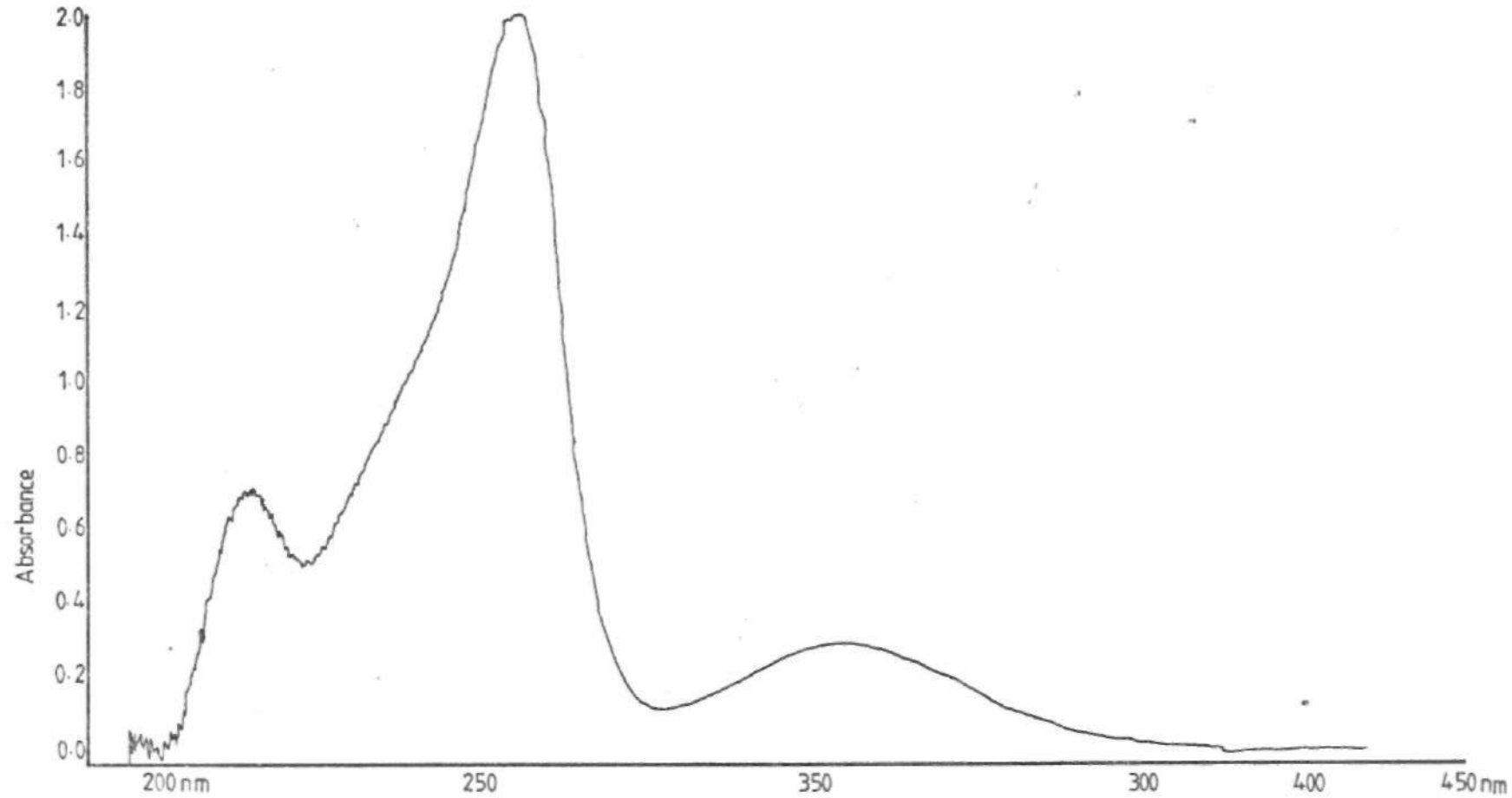


Fig. 3.2.16 U.V. Spectrum of promethazine hydrochloride (in water)

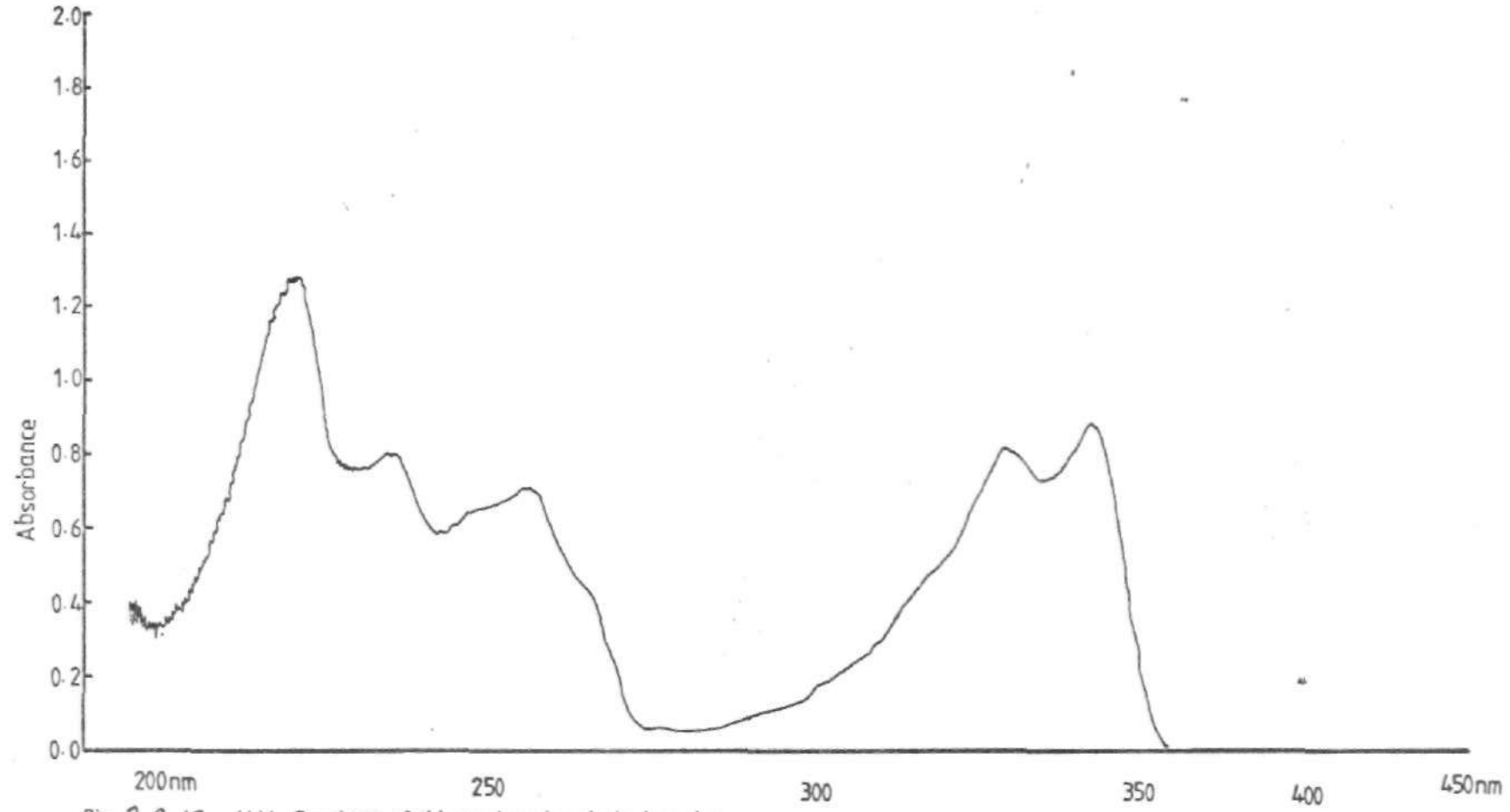


Fig. 3.2.17 U.V. Spectrum of chloroquine phosphate in water

Substance	Retention time (minutes)
Chloroquine	8
Precipitate	3.9
Promethazine	3.9
?Impurity	2.8

Melting point: The precipitate had a melting point range of 205 - 206°C.

Test for presence of phosphorus in the precipitate:

A yellow precipitate was formed indicating the presence of phosphorus.

Test for presence of halogens in the precipitate:

No white precipitate was formed indicating the absence of halogens.

3.3 In situ Rat Gut Technique

When chloroquine phosphate (50 mg) was administered with promethazine hydrochloride (50 mg), the amount of chloroquine phosphate absorbed was reduced by 9 % (table 3.3, fig. 3.3.1). Similarly, when chloroquine phosphate (45 mg) was administered concurrently with promethazine hydrochloride (2.5 mg) the absorption of chloroquine was decreased by 18 % (tables 3.3.2, and fig. 3.3.2). When administered in the ration

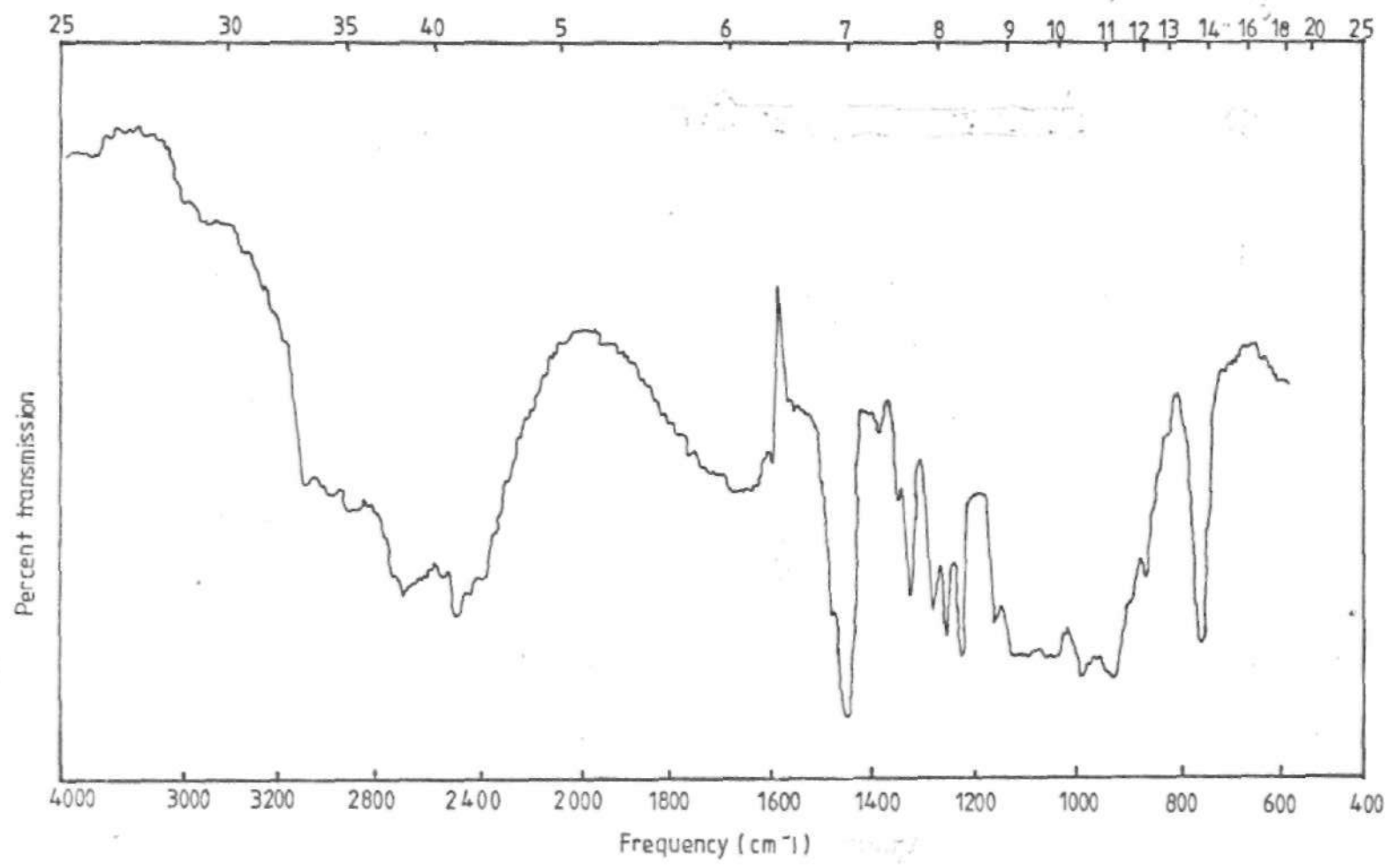


Fig. 3.2.18 I.R. Spectrum of "precipitate" (K Br)

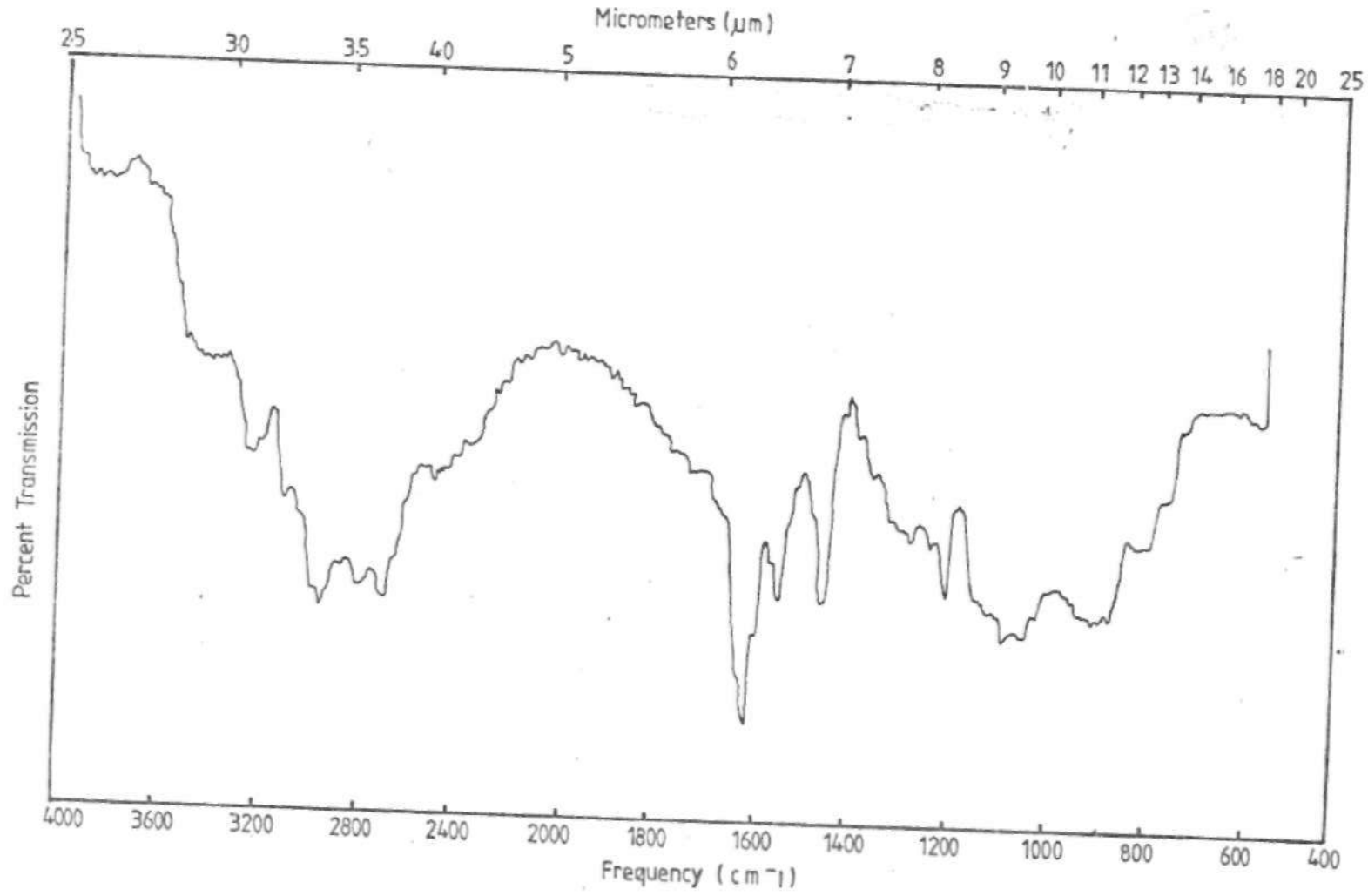


Fig. 3·2·19 I·R· Spectrum of chloroquine phosphate (K Br)

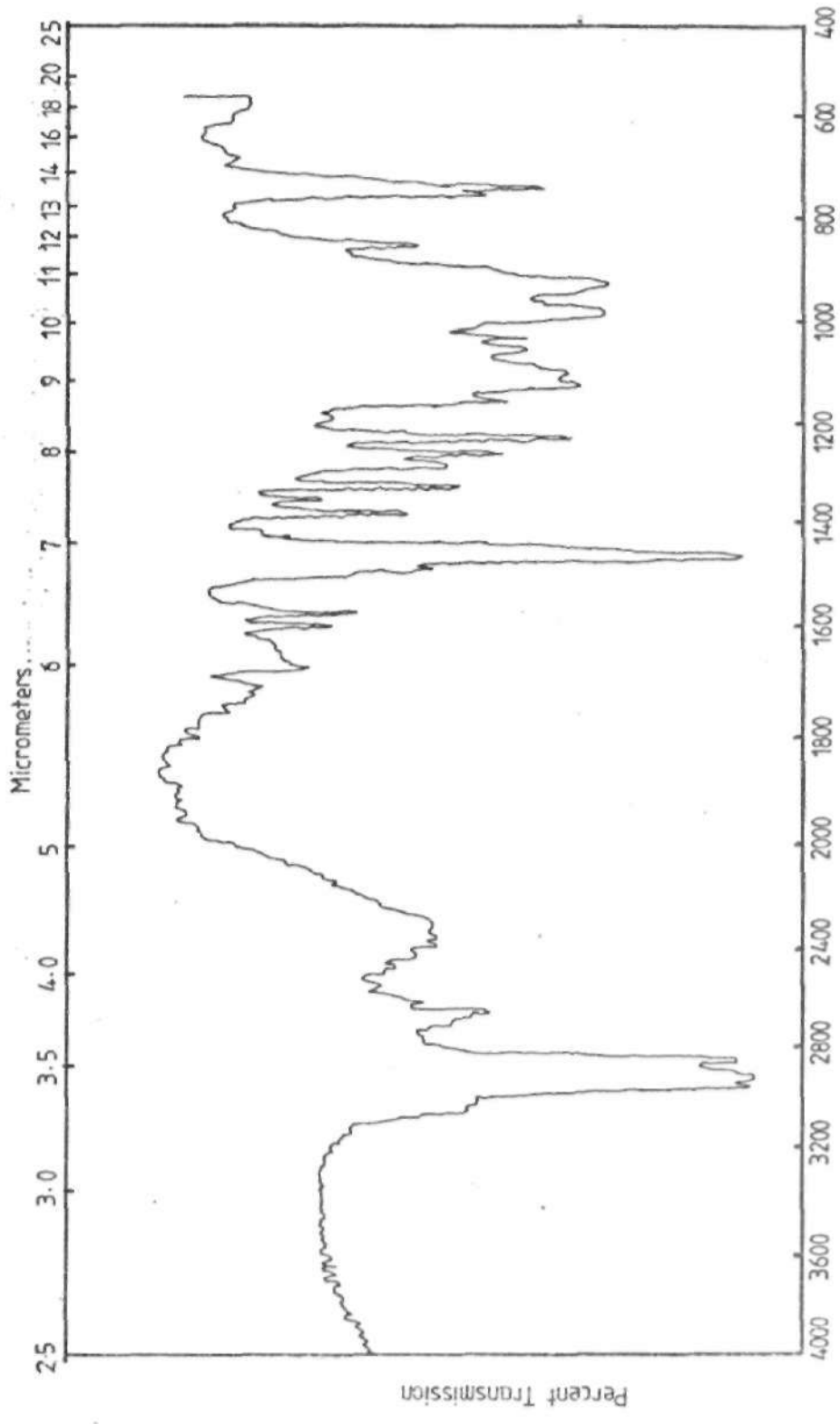


Fig **3.2.20** I.R spectrum of precipitate (liquid paraffin)

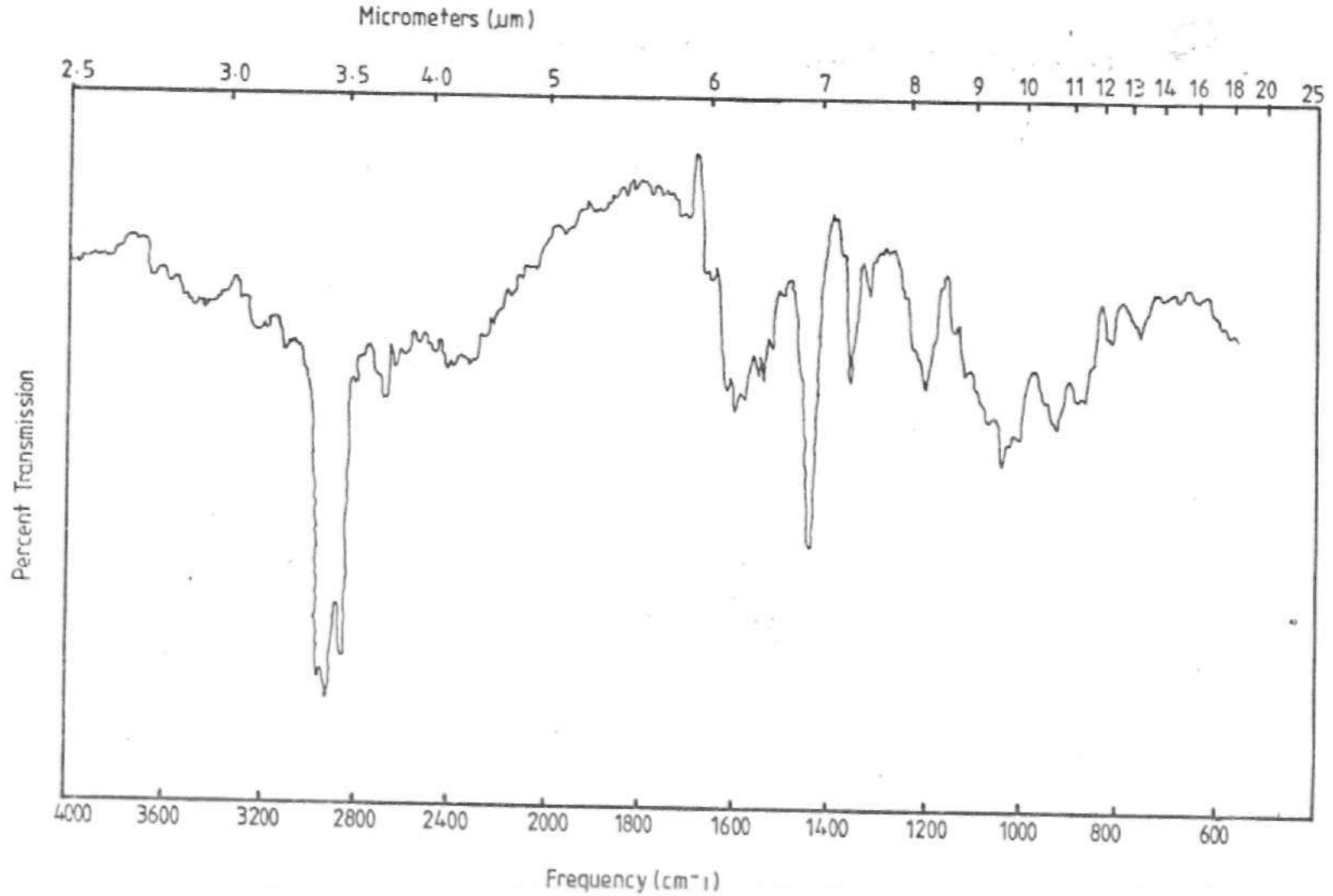


Fig. 3.2.21 I·R spectrum of chloroquine phosphate (liquid paraffin)

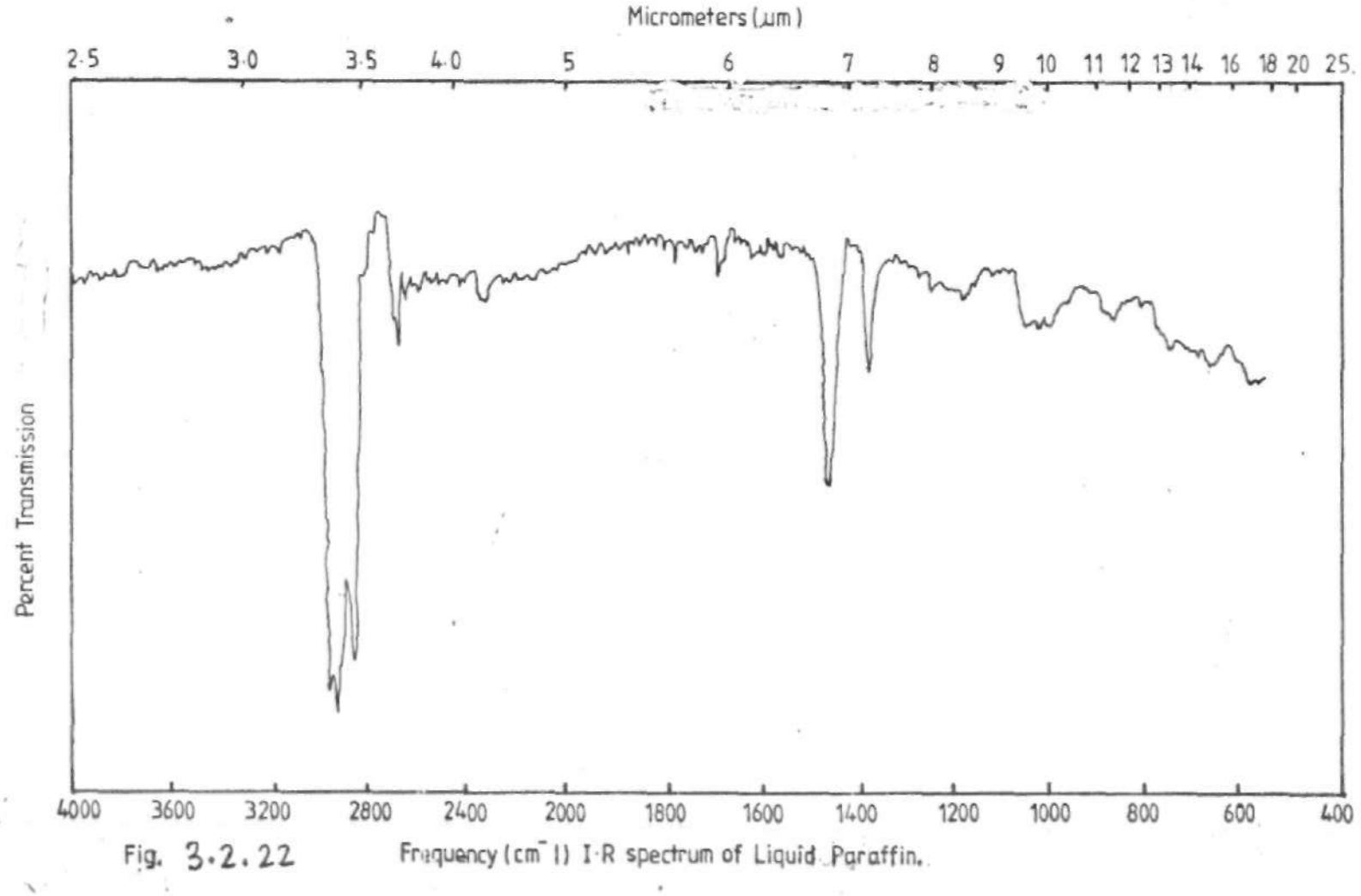

Micrometers (μm)
2·5
3·0
3·5
4·0
5
6
7
8
9
10
11
12
13
14
16
18
20
25
Percent Transmission
4000
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
600
400
Frequency (cm⁻¹) I·R spectrum of Liquid Paraffin.

Fig. 3.2.22

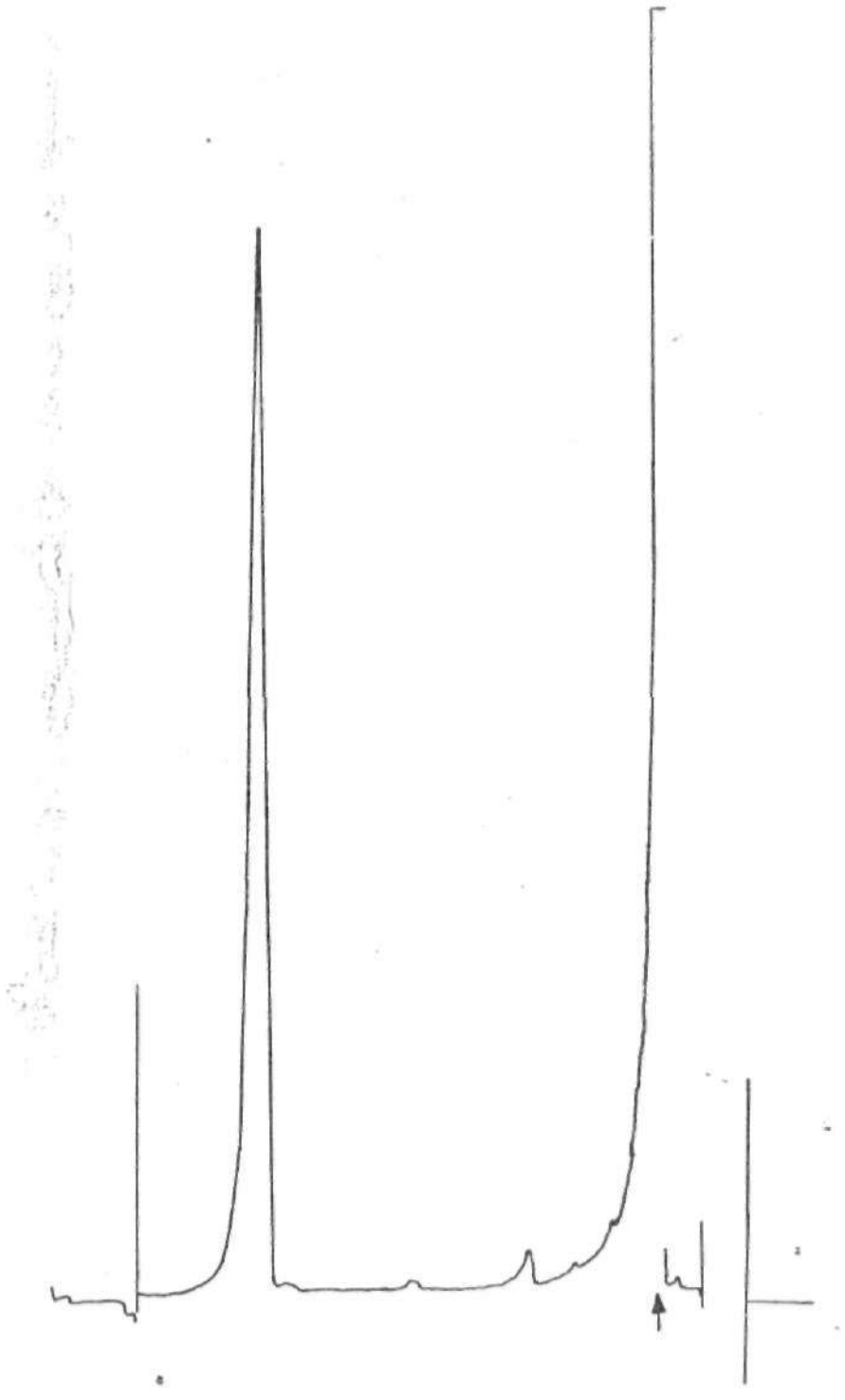


Fig. 3.2.23 G.L.C. of chloroquine

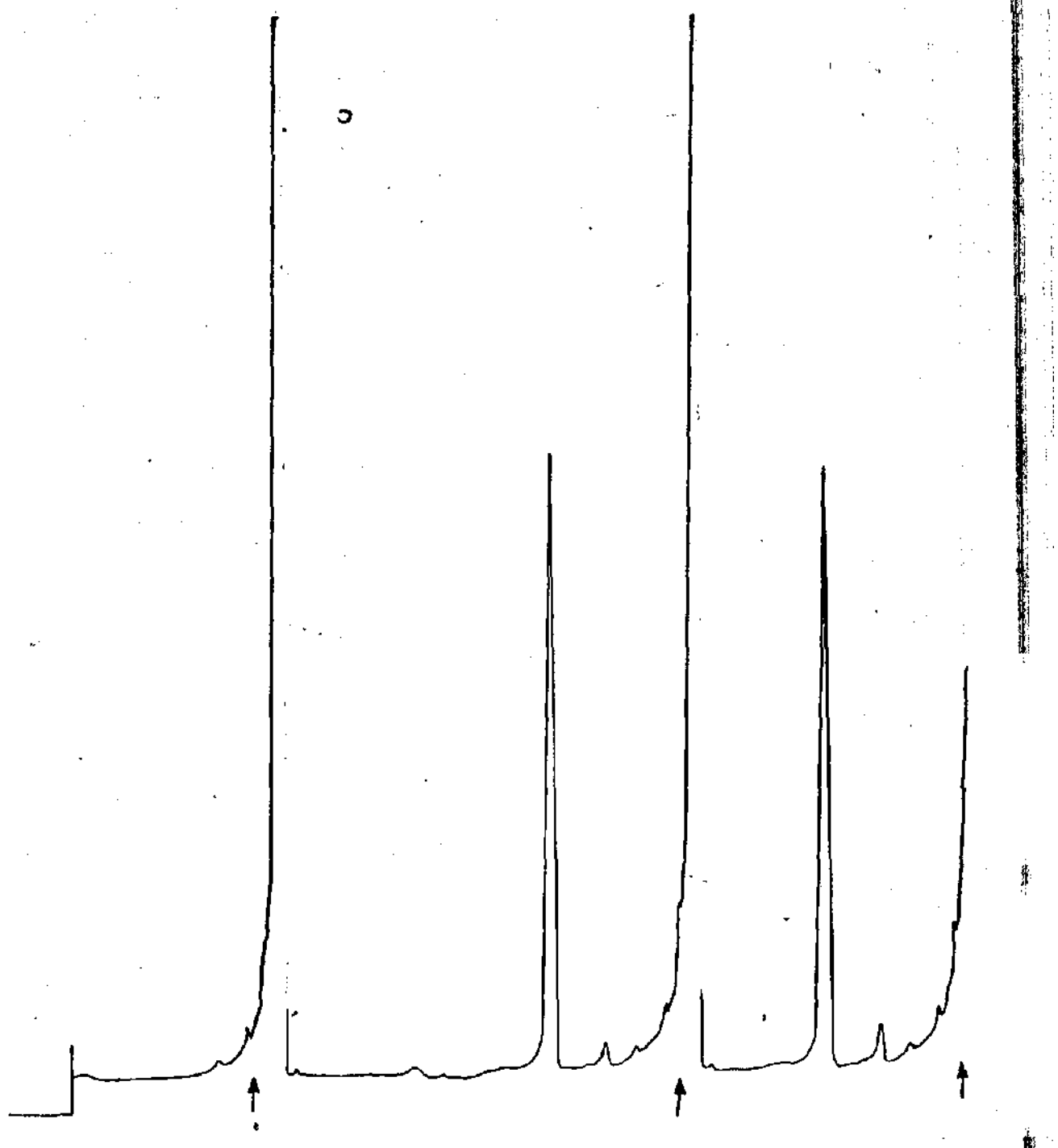


Fig. 3.2.24 GLC of promethazine in chloroform showing also the chloroform solvent peak

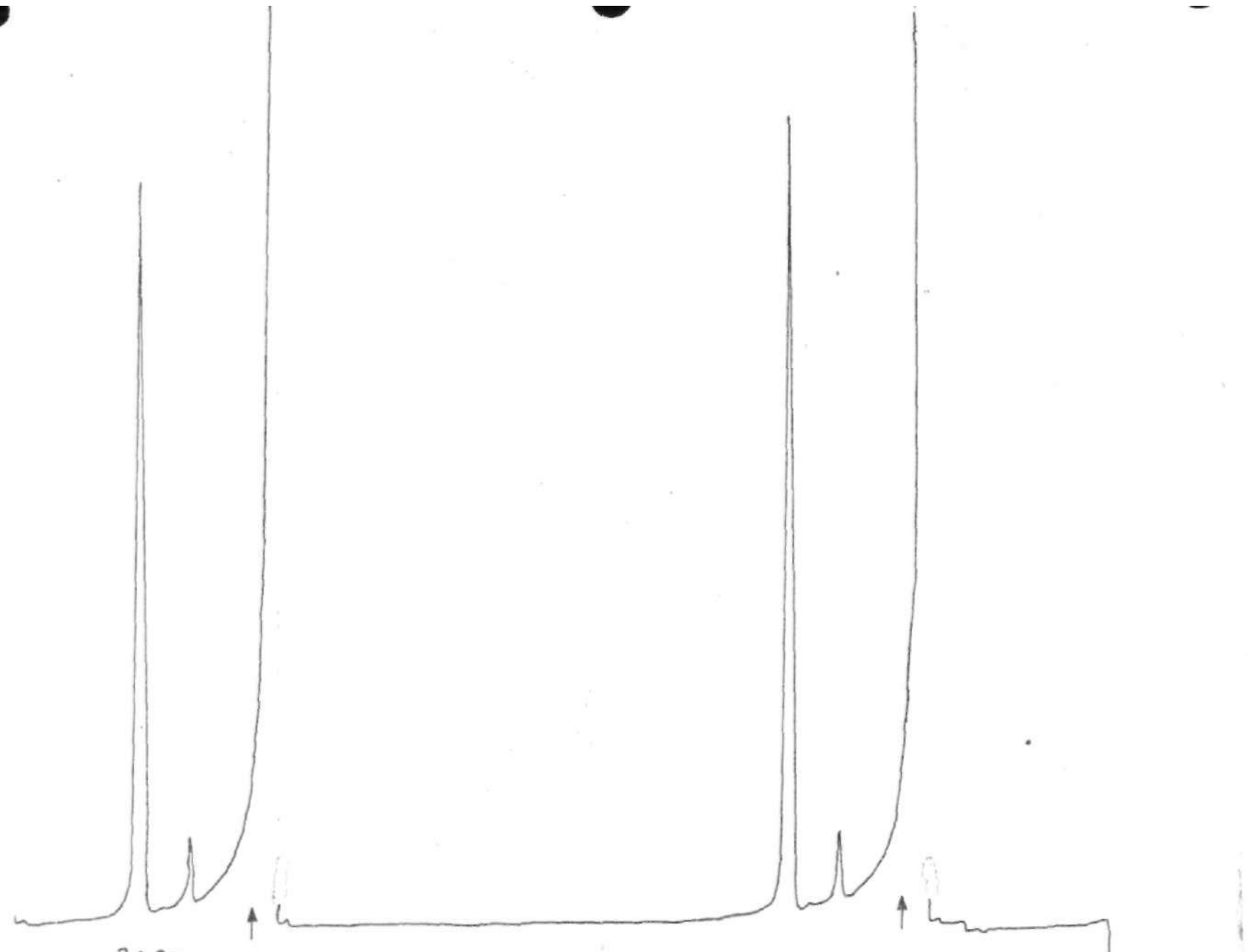


Fig. 3.2.25 G.L.C. of precipitate in ethanol

1:20 (promethazine hydrochloride to chloroquine phosphate), there was 20 % reduction in the amount of chloroquine phosphate absorbed (tables 3.3.3, 3.3.4 and fig. 3.3.3; 3.3.4).

In low doses, when chloroquine phosphate (2 mg) was administered together with promethazine hydrochloride (2 mg) i.e. in the ratio 1:1, there was reduction in amount of chloroquine phosphate absorbed (33 %, table 3.3.5, 3.3.6 and, fig. 3.3.6). Similarly, there was a reduction (32 %) in the amount of chloroquine absorbed when chloroquine phosphate (2 mg) was coadministered with promethazine hydrochloride (1 mg) in the ratio 2:1 (table 3.3.8 and fig. 3.3.8). In the doses studied, the highest reduction in chloroquine phosphate absorption (46 %) occured when chloroquine phosphate (2 mg) was administered with promethazine hydrochloride (10 mg) in the ratio 1:5 (tables 3.3.5, and fig. 3.3.5). Administration of chloroquine phosphate (5 mg) and promethazine hydrochloride (1.25 mg) resulted in a significant reduction (39.6 %) in the amount of chloroquine phosphate absorbed (table 3.3.7; fig. 3.3.7). In both high and low doses, the combination of chloroquine phosphate and promethazine hydrochloride in the ratio 20:1 respectively, resulted in approximately equal reduction in chloroquine absorption (20 %, table 3.3.9 and fig. 3.3.9).

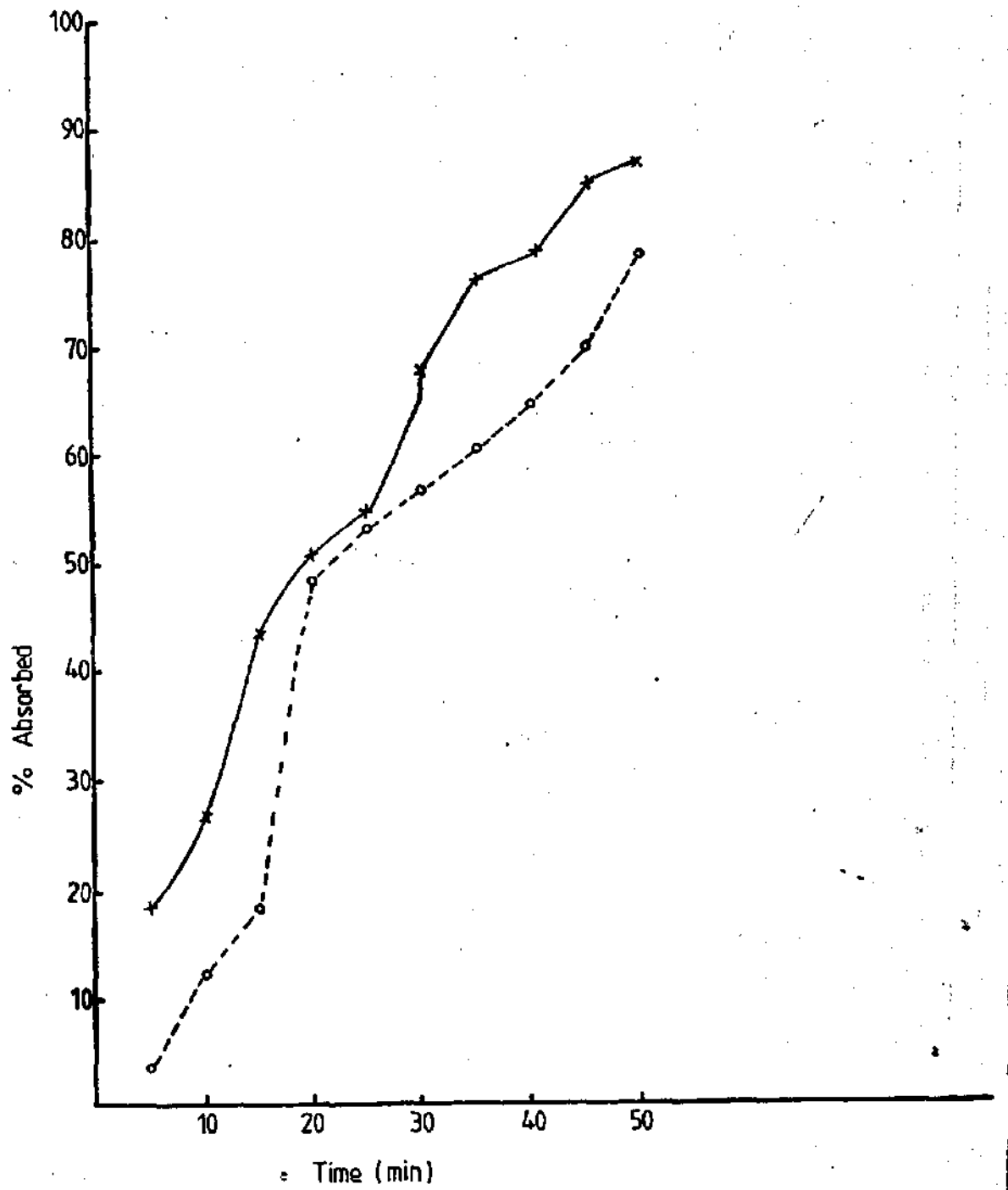


Fig. 3.3.1 Effect of promethazine hydrochloride (50 mg) on absorption of chloroquine phosphate (50 mg)

X—X Chloroquine phosphate

o---o Chloroquine phosphate and Promethazine hydrochloride

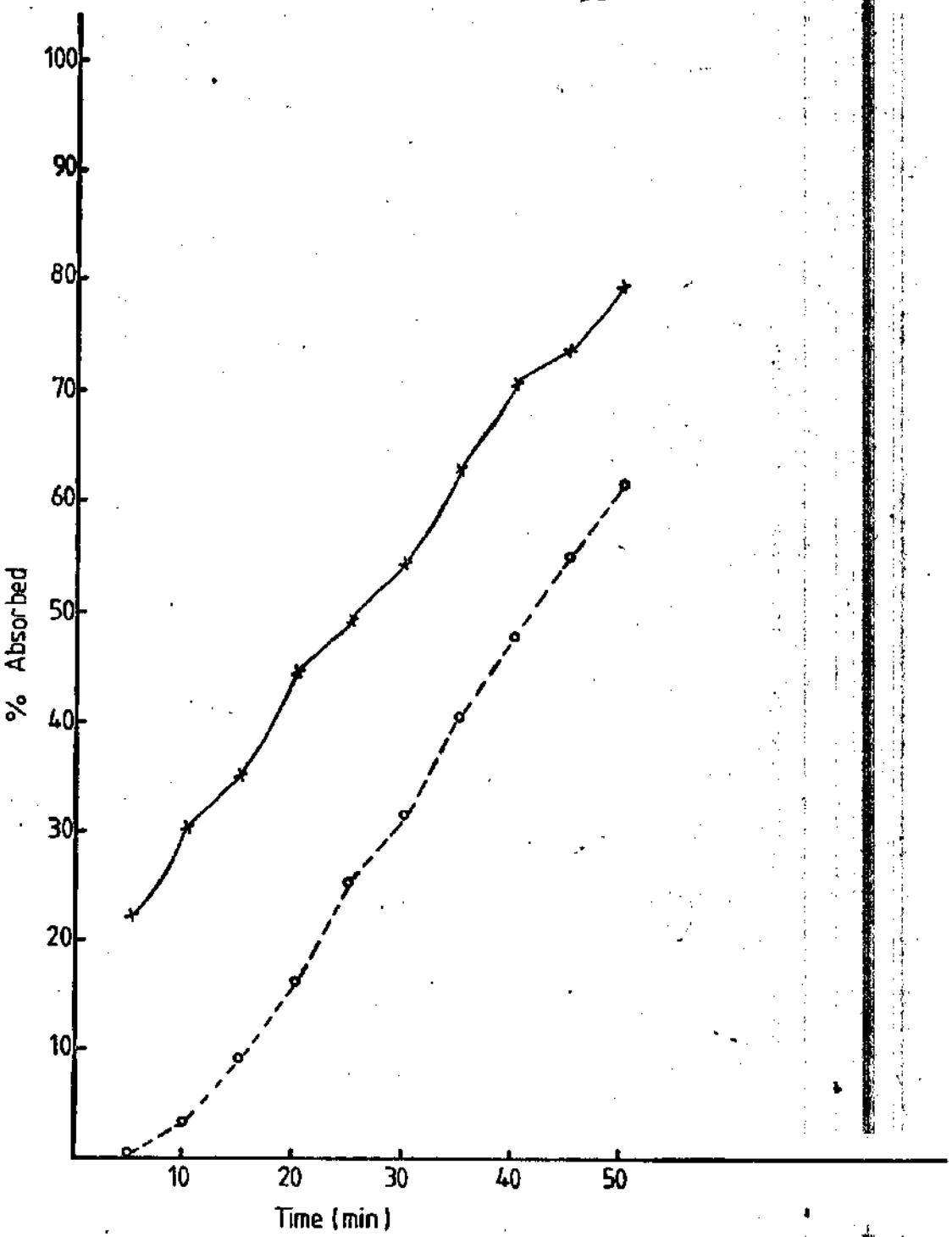


Fig. 3.3.2 Effect of promethazine hydrochloride (2.5mg) on absorption of chloroquine phosphate (45 mg)

x - x Chloroquine Phosphate only.

o----o Chloroquine phosphate and Promethazine hydrochloride

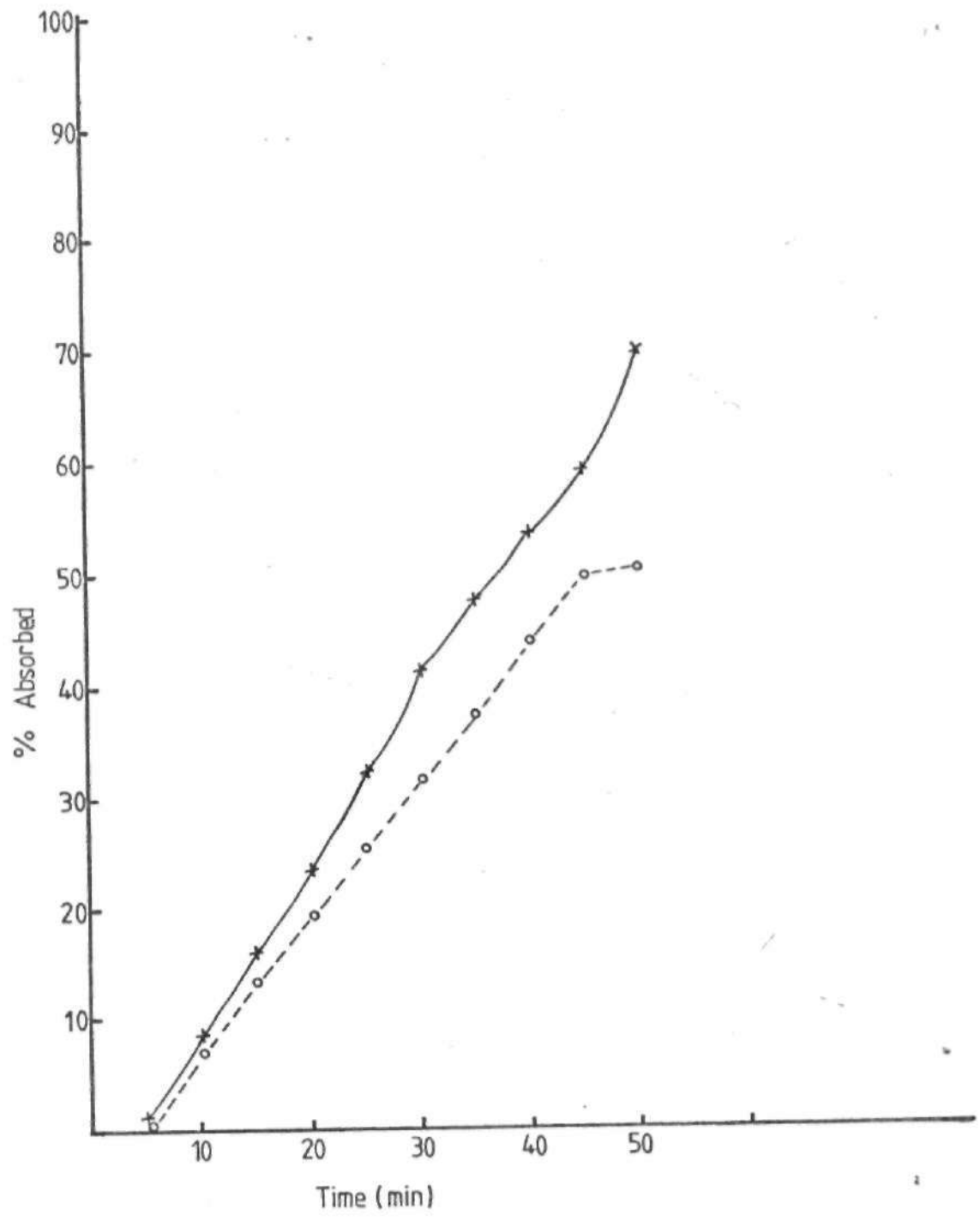


Fig.3.3.3 Effect of promethaze hydrochloride (1 mg) on absorption of chloroquine phosphate (20 m

X—X Chloroquine phosphate only

o--o Chloroquine phosphate and Promethaze hydrochlride

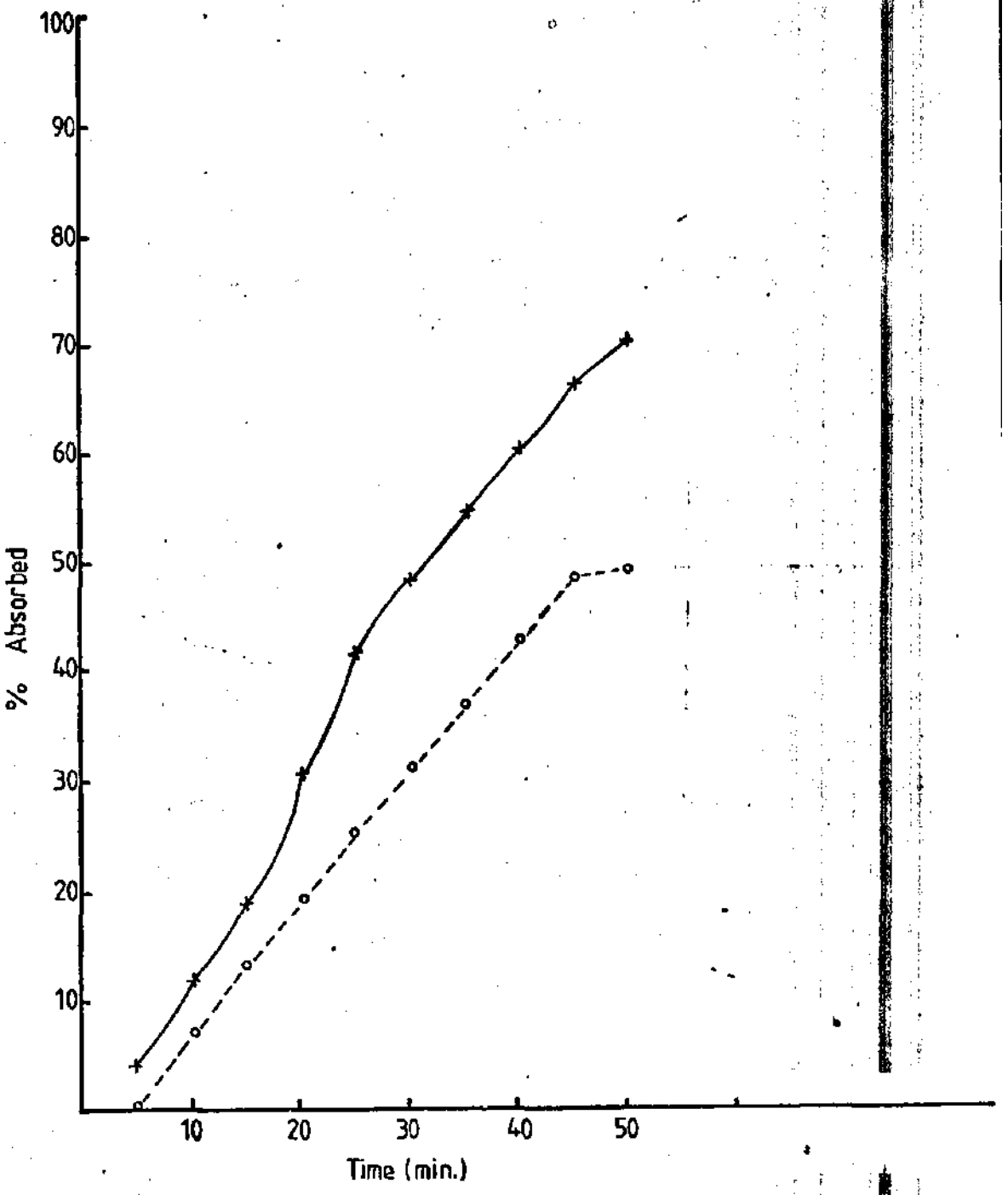


Fig. 33.4.

Effect of promethazine hydrochloride (1·25mg) on absorption of chloroquine phosphate (25mg.)

X—X Chloroquine phosphate only

o--o Chloroquine phosphate and promethazine hydrochloride

When promethazine hydrochloride (20 mg) was administered with chloroquine phosphate (20 mg), there was a 42.4 % reduction in the amount of promethazine hydrochloride absorbed (tables 3.3.10 and fig. 3.3.10). However, only 19 % decrease in promethazine hydrochloride absorption was observed when it was combined with chloroquine in the ratio 1:20 (promethazine hydrochloride (1 mg), chloroquine phosphate (20 mg, See tables 3.3.11 and fig. 3.3.11).

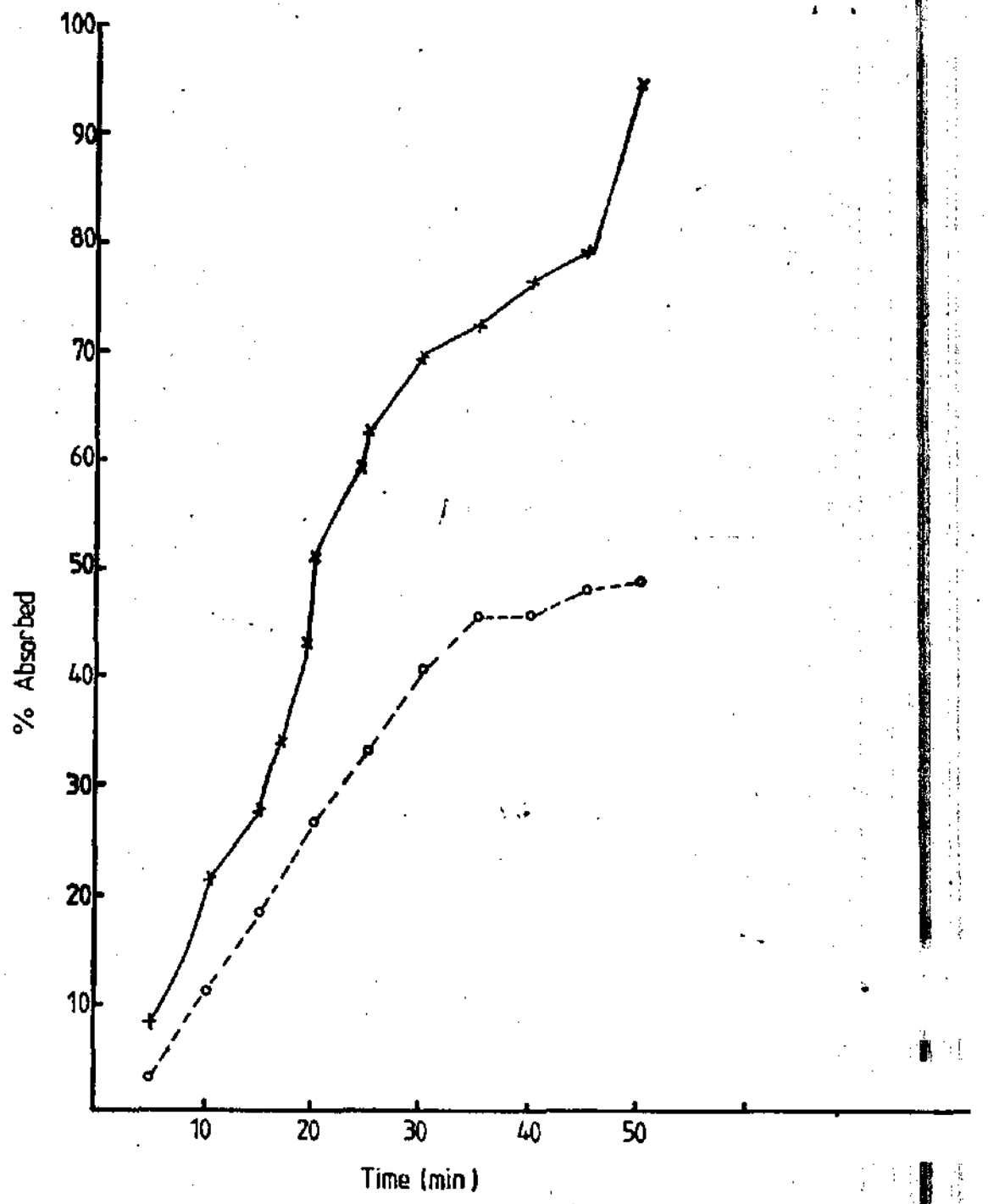


Fig. 3.3.5

Effect of promethazine hydrochloride (10 mg) on absorption of chloroquine phosphate (2 mg)

X⟷X Chloroquine phosphate only

o---o Chloroquine phosphate and promethazine hydrochloride

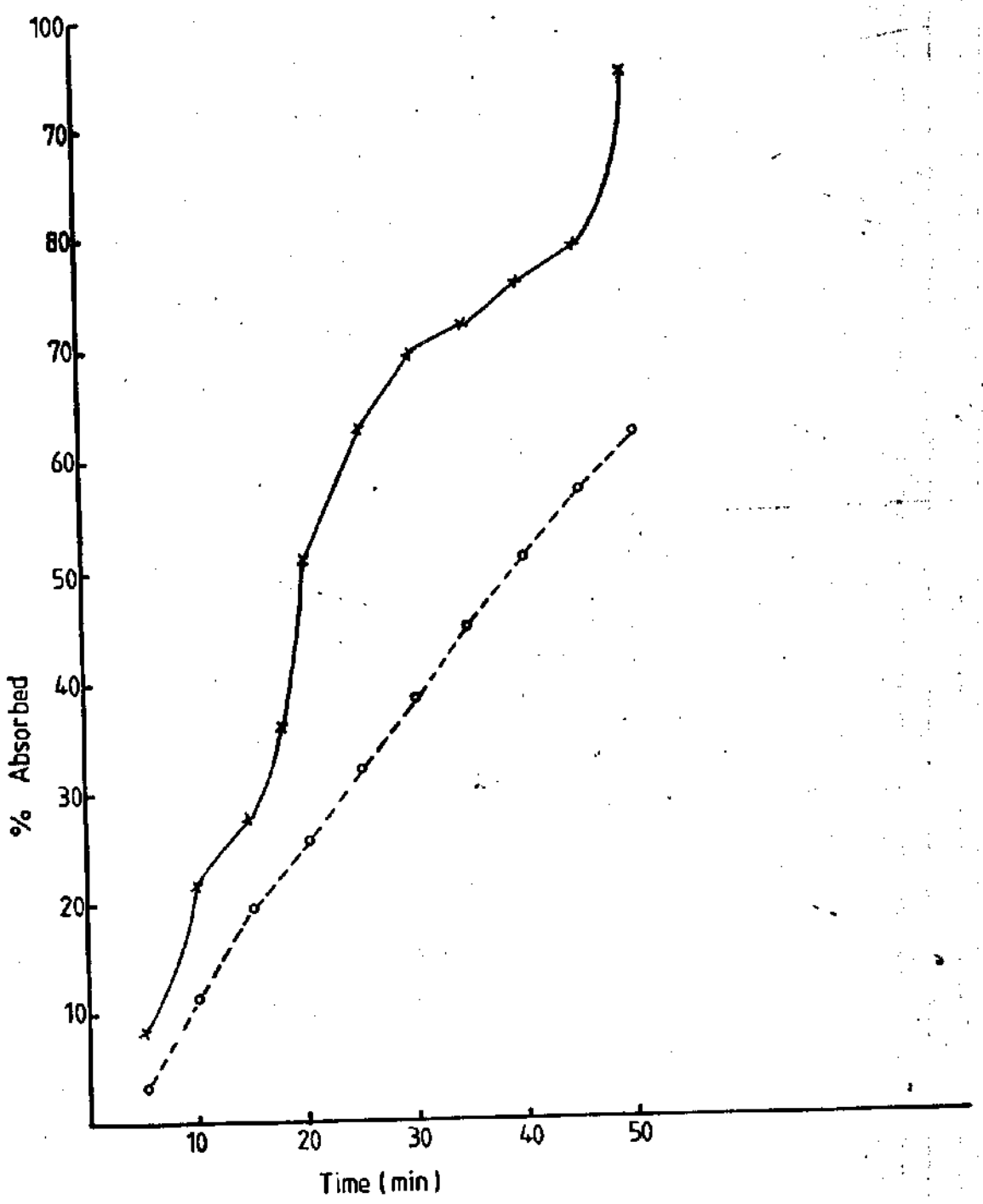


Fig 3.3.6 Effect of promethazine hydrochloride (2 mg) on absorption of chloroquine phsophate (2 m

X—X Chloroquine phosphate only

o--o Chloroquine phsophate and promethazine hydrochloride

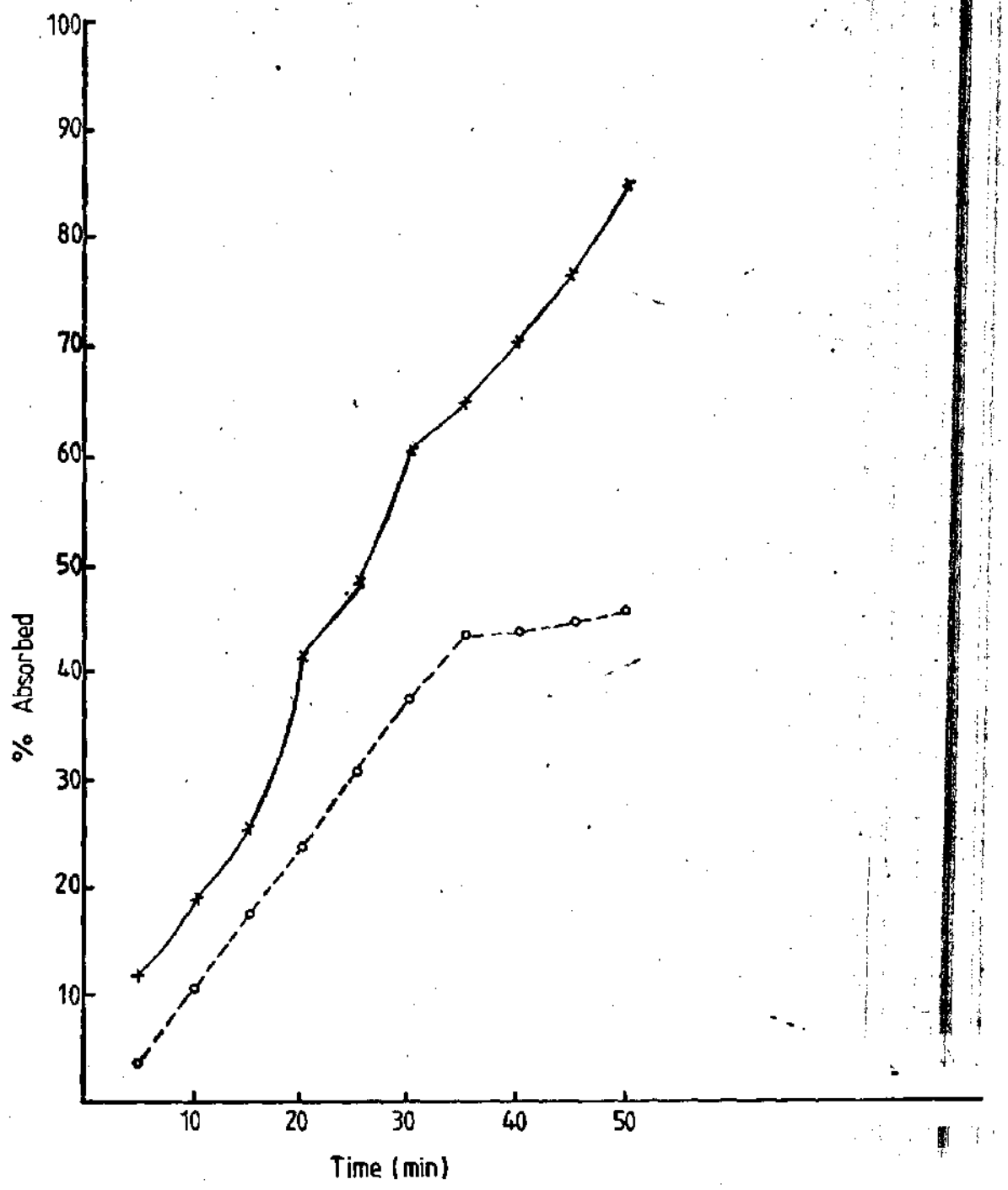


Fig 3.37 Effect of promethazine hydrochloride (1·25mg) on absorption of chloroquine phosphate (5mg)

X—X Chloroquine phosphate only

o--o Chloroquine phosphate and promethazine hydrochloride

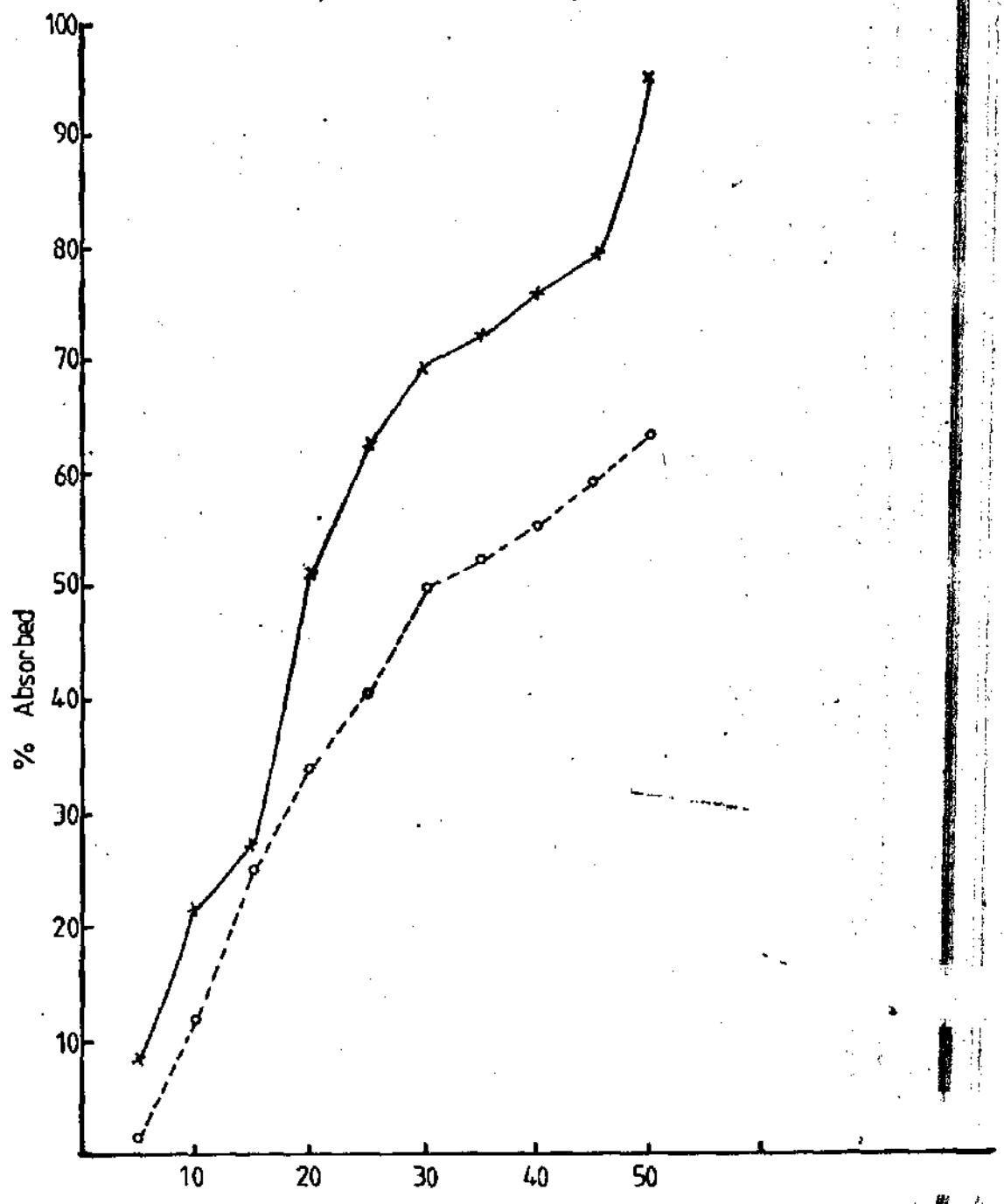


Fig. 3.3.8

Effect of promethazine hydrochloride (1 mg) on absorption of chloroquine phospha

X—X Chloroquine phosphate only

o---o Chloroquine phosphate and promethazine hydrochloride

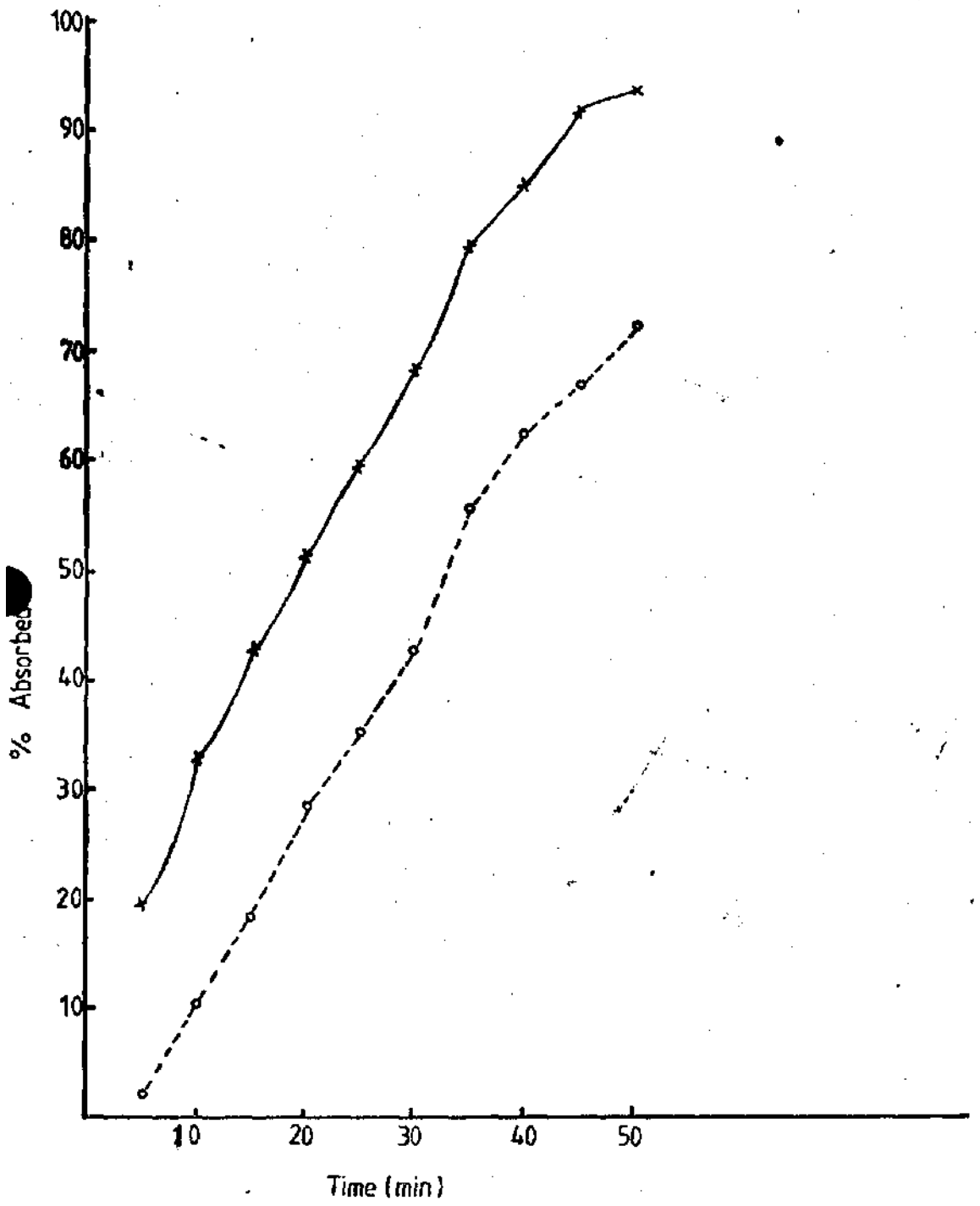


Fig 3.3.9 Effect of promethazine hydrochloride(0·125mg) on absorption of Chloroquine phospate (2·5mg)

x— x Chloroquine Phosphate only.

o--o Chloroquine Phosphate
and
Promethazine Hydrochloride.

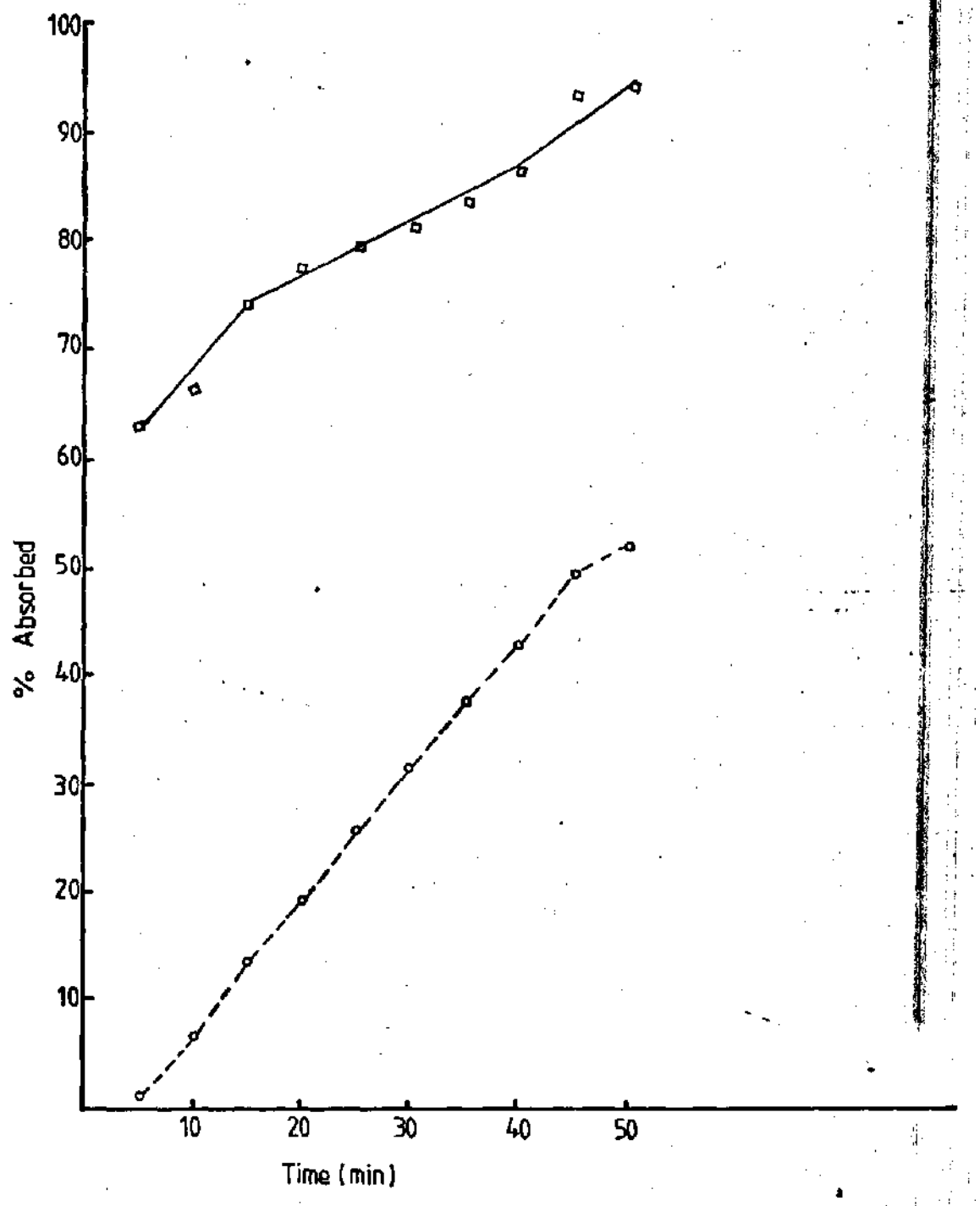


Fig.3.4o Effect of chloroquine phosphate (20 m)
on absorption of promethazine hydrochloride (20 mg)
□—□ Promethazine hydrochloride only
o--o Promethazine hydrochloride and chloroquine phosphate

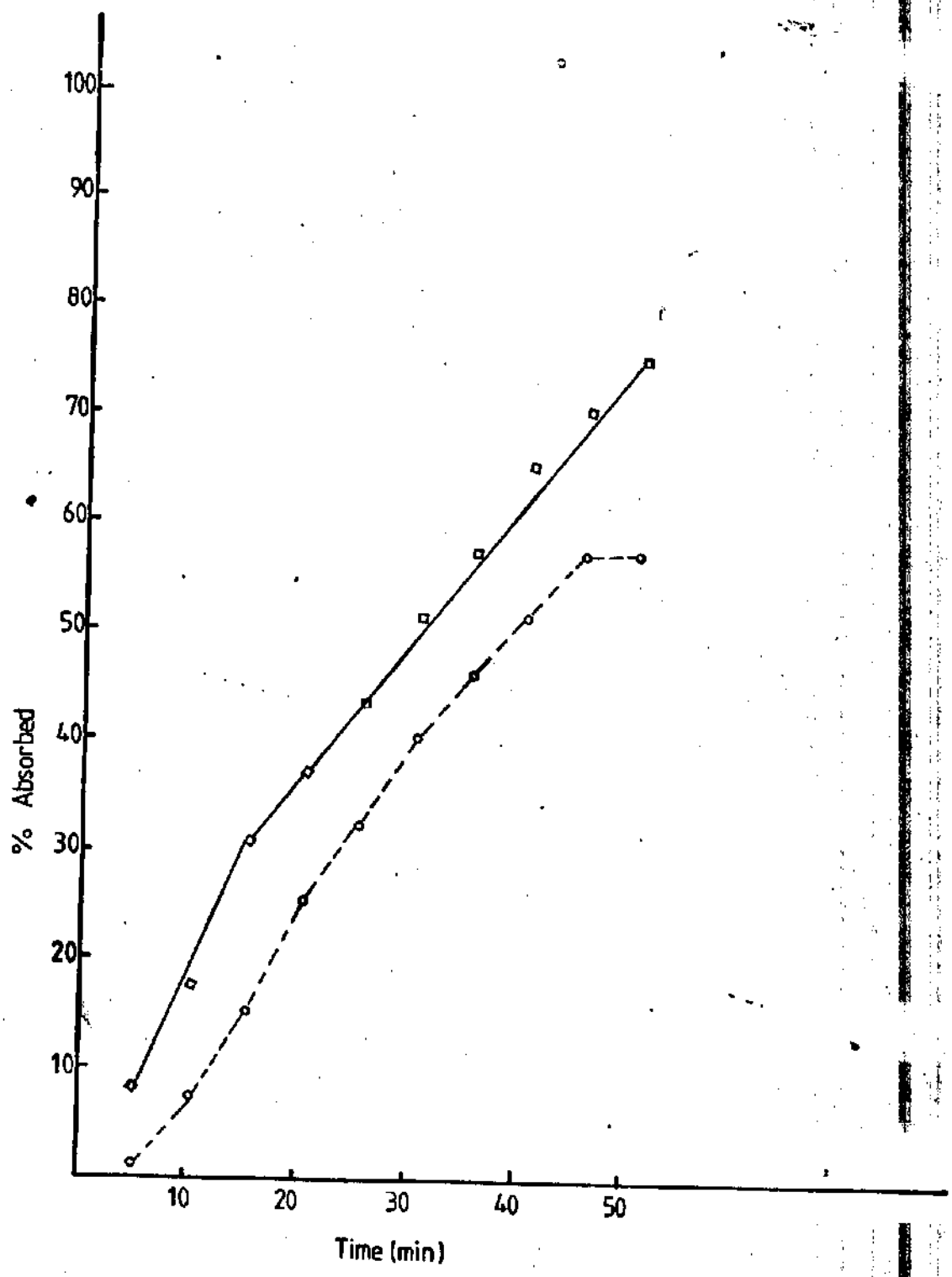


Fig. 3.3.11

Effect of chloroquine phosphate (20 mg) on absorption of promethazine hydrochloride (1mg)

□—□ Promethazine hydrochloride

o--o Promethazine hydrochloride and chloroquine phosphate

TABLE 3.3.1 EFFECT OF PROMETHAZINE HYDROCHLORIDE (50 mg) ON ABSORPTION OF CHLOROQUINE PHOSPHATE (50 mg).

N = 3

TIME (Min)	AMOUNT CHL (mg) MEAN ± S.E.M	ABSORBED CHL + PROM MEAN ± S.E.M	% ABSORBED CHL	CHL + PROM
5	8.77 ± 0.50	1.99* ± 0.02	17.54	3.98
10	13.35 ± 1.2	6.02* ± 1.02	26.70	12.04
15	21.70 ± 0.44	9.17* ± 0.27	43.40	18.34
20	25.48 ± 0.42	24.34 ± 0.86	50.96	48.68
25	27.23 ± 1.41	26.53 ± 0.9	54.46	53.06
30	33.83 ± 2.40	28.33 ± 1.3	67.66	56.66
35	38.15 ± 4.09	30.14 ± 0.88	76.30	60.28
40	39.22 ± 0.54	32.22* ± 1.07	78.44	64.44
45	42.73 ± 4.42	34.96* ± 2.24	85.46	69.92
50	43.54 ± 0.42	39.0 ± 0.93	87.08	78.0

N = 3

*represents P < 0.0025, student t-test compared to chloroquine phosphate (50 mg). Chl. and Prom represent chloroquine phosphate and promethazine hydrochloride respectively.

N = 3 for all values in tables 3.3.1 - 3.3.11

TABLE 3.3.2 EFFECT OF PROMETHAZINE HYDROCHLORIDE (2.5 mg) ON ABSORPTION OF CHLOROQUINE PHOSPHATE (45 mg)

N = 3

TIME (Min)	AMOUNT CHL (mg) MEAN ± S.E.M	ABSORBED CHL + PROM (mg) MEAN ± S.E.M.	% ABSORBED CHL	CHL + PROM
5	10.04 ± 1.29	0.10* ± 0.02	22.31	0.22
10	13.63 ± 0.65	1.45* ± 0.016	30.28	3.22
15	15.84 ± 1.12	4.17* ± 1.09	35.20	9.26
20	20.10 ± 0.85	7.27* ± 0.38	44.66	16.15
25	22.32 ± 0.45	11.37* ± 0.39	49.6	25.26
30	24.43 ± 1.57	14.28* ± 0.50	54.28	31.73
35	28.33 ± 1.01	18.02* ± 0.43	62.95	40.04
40	31.83 ± 0.65	21.55* ± 0.27	70.7	47.88
45	33.15 ± 1.79	24.87* ± 0.56	73.65	55.2
50	35.73 ± 0.58	27.75* ± 1.85	79.40	61.66

N = 3

*represents P < 0.0025, student t-test compared to Chloroquine phosphate (45 mg). Chl and Prom represent Chloroquine phosphate and Promethazine hydrochloride respectively.

TABLE 3.3.3 EFFECT OF PROMETHAZINE HYDROCHLORIDE (1.25 mg) ON ABSORPTION OF CHLOROQUINE PHOSPHATE (25 mg)

N = 3

TIME (min)	AMOUNT ABSORBED CHL (mg) + MEAN ± S.E.M.	CHL+PROM (mg) MEAN ± S.E.M	% ABSORBED CHL	CHL+PROM
5	1.18 ± 0.09	0.12* ± 0.05	4.72	0.48
10	3.01 ± 0.26	1.76* ± 0.04	12.04	7.04
15	4.79 ± 0.44	3.29 ± 0.45	19.16	13.16
20	7.65 ± 0.34	4.82* ± 0.09	30.60	19.28
25	10.29 ± 0.71	6.29 ± 1.88	41.16	25.16
30	12.07 ± 0.59	7.77* ± 1.34	48.28	31.08
35	13.74 ± 1.05	9.23 ± 3.60	54.96	36.92
40	15.16 ± 0.57	10.67 ± 0.41	60.64	42.68
45	16.51 ± 0.20	12.12 ± 0.58	66.04	43.48
50	17.67 ± 0.28	12.28* ± 1.52	70.68	49.12

N = 3

*represent P < 0.005, student t-test compared to Chloroquine phosphate (25 mg). Chl. and Prom represent Chloroquine phosphate and promethazine hydrochloride respectively.

TABLE 3.3.4 EFFECT OF PROMETHAZINE HYDROCHLORIDE (1 mg) ON ABSORBATION OF CHLOROQUINE PHOSPHATE (20 mg).

N = 3

TIME (Min)	AMOUNT CHL (mg) MEAN ± S.E.M	ABSORBED CHL + (PROM) (mg) MEAN ± S.E.M	% ABSORBED CHL	CHL+ PROM
5	0.30 ± 0.05	0.07* ± 0.009	1.50	0.35
10	1.68 ± 0.14	1.46 ± 0.14	8.40	7.30
15	3.23 ± 0.36	2.66 ± 0.23	16.15	13.30
20	4.72 ± 0.22	3.89 ± 0.45	23.60	19.45
25	6.51 ± 0.43	5.08 ± 0.02	32.55	25.40
30	8.36 ± 0.60	6.32 ± 0.30	41.80	31.60
35	9.54 ± 0.23	7.55 ± 0.46	47.70	37.75
40	10.71 ± 0.35	8.74 ± 0.20	53.55	43.70
45	11.83 ± 0.77	9.91 ± 0.77	59.15	49.55
50	14.18 ± 0.48	10.09* ± 0.72	70.90	50.45

N= 3

*rrepresents P < 0.05, student t-test compared to Chloroquine phosphate (20 mg). Chl and Prom represent Chloroquine phosphate and Promethazine hydrochloride respectively.

TABLE 3.3.5 EFFECT OF PROMETHAZINE HYDROCHLORIDE (10mg) ON ABSORPTION OF CHLOROQUINE PHOSPHATE (2mg).

TIME (Min)	AMOUNT CHL (mg) MEAN ±S.E.M	ABSORBED PROM+CHL(mg) MEAN±S.E.M	% ABSORBED CHL	CHL+PROM
5	0.17±0.01	0.06*±0.01	3.5	3.0
10	0.43±0.03	0.22*±0.01	21.5	11.0
15	0.55±0.02	0.37±0.01	27.5	18.5
20	1.02±0.5	0.53*±0.03	51.0	26.5
25	1.25±0.01	0.66*±0.005	62.5	33.0
30	1.39±0.01	0.81*±0.09	69.5	40.5
35	1.44±0.02	0.92*±0.02	72.0	45.5
40	1.52±0.02	0.92*±0.03	76.0	45.5
45	1.58±0.01	0.96*±0.03	79.0	48.0
50	1.90±0.05	0.97*±0.08	95.0	48.5

*represents P ≤ 0.0025, Student t-test compared to Chloroquine phosphate (2mg). Chl and Prom represent chloroquine phosphate and Promethazine hydrochloride respectively.

TABLE. 3.3.6 EFFECT OF PROMETHAZINE HYDROCHLORIDE (2mg) ON ABSORPTION OF CHLOROQUINE PHOSPHATE (2mg).

TIME (Min)	AMOUNT ABSORBED CHL(mg) MEAN ±S.E.M	CHL+PROM MEAN ± S.E.M	% ABSORBED CHL.	CHL+PROM.
5	0.17±0.01	0.06*±0.01	8.5	3.0
10	0.43±0.03	0.22*±0.01	21.5	11.0
15	0.55±0.02	0.39*±0.03	27.5	19.5
20	1.02±0.05	0.51*±0.01	51.0	25.5
25	1.25±0.01	0.64*±0.12	62.5	32.0
30	1.39±0.01	0.76*±0.06	69.5	38.0
35	1.44±0.02	0.89*±0.005	72.0	44.5
40	1.52±0.02	1.02*±0.005	76.0	51.0
45	1.58±0.01	1.14*±0.03	79.0	57.0
50	1.90±0.05	1.24*±0.10	95.0	62.0

*represents $P < 0.01$ Student t-test compared to Chloroquine phosphate (2mg). Chl. and Prom represent Chloroquine Phosphate and Promethazine hydrochloride respectively.

TABLE. 3.3.7 EFFECT OF PROMETHAZINE HYDROCHLORIDE (1.2mg) ON ABSORPTION OF CHLOROQUINE PHOSPHATE (5mg).

TIME (Min)	AMOUNT ABSORED CHL(mg) MEAN±S.E.M	CHL+ PROM(mg) MEAN ± S.E.M	% ABSORBED CHL	CHL+PROM
5	0.61±0.005	0.19*±0.005	12.2	3.8
10	0.95±0.02	0.52*±0.026	19.0	10.4
15	1.29±0.45	0.88±0.07	25.8	17.6
20	2.06±0.12	1.19*±0.04	41.2	23.8
25	2.38±0.21	1.54±0.03	47.6	30.8
30	3.03±0.10	1.87*±0.04	60.6	37.4
35	3.26±0.14	2.16±0.42	65.2	43.2
40	3.52±0.18	2.17±0.19	70.4	43.4
45	3.84±0.13	2.22*±0.18	76.8	44.4
50	4.25±0.25	2.27*±0.17	85.0	45.4

* represents P < 0.0025, student t-test compared to Chloroquine phosphate (5mg). Chl. and Prom represent Chloroquine phosphate and Promethazine hydrochloride respectively.

TABLE. 3.3.8 EFFECT OF PROMETHAZINE HYDROCHLORIDE (1mg) ON ABSORPTION OF CHLOROQUINE PHOSPHATE (2mg)

TIME (Min)	AMOUNT ABSORBED CHL(mg) MEAN ± S.E.M	CHL+PROM (mg) MEAN ± S.E.M	% ABSORBED CHL	CHL+PROM
5	0.17±0.01	0.03*±0.005	8.5	1.5
10	0.43±0.03	0.24*±0.02	21.5	12.0
15	0.55±0.02	0.51±0.007	27.5	25.5
20	1.02±0.05	0.68*±0.19	51.0	34.0
25	1.25±0.01	0.81*±0.06	62.5	40.5
30	1.39±0.01	0.99*±0.01	69.5	49.5
35	1.44±0.02	1.04*±0.005	72.0	52.0
40	1.52±0.02	1.10*±0.07	76.0	55.0
45	1.58±0.01	1.18±0.02	79.0	59.0
50	1.90±0.05	1.26*±0.10*	95.0	63.0

*represents P< 0.005 Student t-test compared to Chloroquine phosphate (2mg). CHL. and Prom. represent Chloroquine Phosphate and Promethazine hydrochloride respectively.

TABLE. 3.3.9 EFFECT OF PROMETHAZINE HYDROCHLORIDE (0.125mg) ON ABSORPTION OF CHLROQUINE PHOSPHATE(2.5mg)

TIME (Min)	AMOUNT CHL(mg) MEAN ± S.E.M	ABSORBED CHL+PROM (mg) MEAN ± S.E.M	% ABSORBED CHL.	CHL.+PROM
5	0.49±0.01	0.05*±0.01	19.6	2.0
10	0.82±0.05	0.26*±0.02	32.8	10.4
15	1.07±0.02	0.46*±0.03	42.8	18.4
20	1.28±0.03	0.71*±0.05	51.2	28.4
25	1.49±0.03	0.88*±0.02	59.6	35.2
30	1.71±0.09	1.07*±0.03	68.4	42.8
35	1.99±0.06	1.39*±0.05	79.6	55.6
40	2.13±0.03	1.55*±0.11	85.2	62.0
45	2.29±0.07	1.67*±0.02	91.6	66.8
50	2.34±0.05	1.80*±0.025	93.6	72.0

*represents P < 0.0025, student t-test compared to Chloroquine phosphate (2.5mg). Chl and Prom. represent Chloroquine phosphate and Promethazine hydrochloride respectively.

TABLE 3.3.10 EFFECT OF CHLOROQUINE PHOSPHATE (20 mg) ON ABSORPTION OF PROMETHAZINE HYDROCHLORIDE (20 mg).

N = 3

TIME (Min)	AMOUNT ABSORBED PROM (mg) MEAN ± S.E.M	CHL. + PROM (mg) MEAN ± S.E.M.	% ABSORBED PROM	CHL + PROM
5	12.64 ± 0.65	0.2* ± 0.05	63.2	1
10	13.31 ± 0.62	1.37* ± 0.03	66.55	6.85
15	14.86 ± 0.19	2.7* ± 0.2	74.3	13.5
20	15.47 ± 0.30	3.85* ± 0.81	77.35	19.25
25	15.83 ± 0.47	5.15* ± 0.35	79.15	25.75
30	16.30 ± 0.70	6.29* ± 0.42	81.5	31.45
35	16.73 ± 0.79	7.55* ± 0.28	83.65	37.45
40	17.31 ± 0.58	8.69* ± 0.28	86.60	43.45
45	18.67 ± 0.74	9.92* ± 0.02	93.35	49.6
50	18.83 ± 0.50	10.4* ± 0.35	94.4	52.0

N = 3

*represents $P < 0.0025$ student t-test compared to Promethazine hydrochloride (20 mg). Chl. and Prom. represent Chloroquine phosphate and Promethazine hydrochloride respectively.

TABLE 3.3.11 EFFECT OF CHLOROQUINE PHOSPHATE (20 mg) ON ABSORPTION OF PROMETHAZINE HYDROCHLORIDE (1 mg).

N = 3

TIME (min)	AMOUNT PROM (mg) MEAN ± S.E.M	ABSORBED CHL+PROM (mg) MEAN ± S.E.M	% ABSORBED PROM	CHL+ PROM
5	0.08 ± 0.01	0.01* ± 0	8	1
10	0.12 ± 0.01	0.07* ± 0.005	12	7
15	0.31 ± 0.05	0.15 ± 0.02	31	15
20	0.37 ± 0.01	0.25 ± 0.02	37	25
25	0.44 ± 0.01	0.32* ± 0.01	44	32
30	0.51 ± 0.04	0.40* ± 0.01	51	40
35	0.58 ± 0.04	0.46 ± 0.01	58	46
40	0.65 ± 0.02	0.51* ± 0.01	65	51
45	0.71 ± 0.05	0.57 ± 0.005	71	57
50	0.76 ± 0.02	0.57* ± 0.01	76	57

N

*represent P < 0.01 Student t-test compared to Promethazine hydrochloride (1 mg). Prom, and Chl. represent Promethazine hydrochloride and chloroquine respectively.

Chapter 4

DISCUSSION

It is evident from the results that there is a chemical interaction between chloroquine phosphate and promethazine hydrochloride. It appears that the determinants of the extent of the interaction include the initial concentration of the two drugs, the ratio in which they were present and the post administration time.

At high concentration *in vitro* (chloroquine phosphate (225 mg) and promethazine hydrochloride (45 mg; i.e. ratio 5:1), the reduction in promethazine hydrochloride concentration (56 %) was profoundly greater than that of chloroquine phosphate (14 %). However, using the same ratio but at a lower concentration(chloroquine phosphate (25 µg) and promethazine hydrochloride (5 µg) the reduction in the concentration of chloroquine (94.31 %) was more than that of promethazine hydrochloride (82.79 %). The difference between the *in vitro* observations made at various concentrations may be due to the fact that at high concentration, chemical reaction probably occurs.

The colour change and later precipitation observed were not likely to be as a result of oxidation of promethazine by atmospheric oxygen since the same phenomenon was observed in this study with chloroquine phosphate containing an antioxidant. Moreover, the product

of promethazine oxidation by atmospheric oxygen is blue in in colour (USP - 19th edition, 1975) while the colour observed was yellowish green. In addition, oxidation of promethazine by atmospheric oxygen takes relatively longer time to manifest itself whereas the colour change observed occured immediately chloroquine phosphate and promethazine hydrochloride were mixed. Other causes of precipitation include pH change, addition of co-solvent and double decomposition reactions.

In this study, pH changes may not be a major contributory factor because the pH of the mixture of chloroquine phosphate and promethazine hydrochloride (pH 5.1) was within the British Pharmacopoeia requirement for promethazine hydrochloride injection (pH 5-6). Moreover, when the pH of chloroquine phosphate injection was raised to pH 5.2, there was no precipitation.

Both chloroquine phosphate and promethazine hydrochloride were formulated in water, thus making the addition of cosolvent unlikely as the cause of precipitation.

The ultraviolet spectrum of the precipitate (fig. 3.2.15), is identical to that of promethazine hydrochloride (Fig. 3.2.16,) and no U.V. spectrum of the solution of the precipitate in ethanol was obtained when promethazine hydrochloride was used as the reference, thus indicating that the two may be identical (Fig. 3.2.13). Further

support for this claim is provided by the fact that the infrared spectrum of the precipitate corresponds with the reported principal peaks for promethazine (figs. 3.2.18 - 3.2.22). In addition, the g.l.c. retention time (3.9 min) of the precipitate was identical to that of promethazine (Fig. 3.2.23, 3.2.24).

Although the melting point of phosphates may be higher than that of hydrochlorides, the precipitate had a melting point (206°C) lower than that of the hydrochloride probably due to impurities. U. V. and g.l.c. results were not identical with that of promethazine hydrochloride. This suggests the formation of a salt of promethazine with a different melting point from promethazine hydrochloride. The new salt formed was probably promethazine phosphate since test for phosphorus was positive while test for chloride ions was negative

It is likely that not all of the promethazine hydrochloride was converted to Promethazine phosphate in the presence of chloroquine phosphate since it was possible to detect promethazine in the supernatant solution of the mixture after the precipitate was formed.

The reduction in absorption observed *in situ* due to the interaction between chloroquine phosphate and promethazine hydrochloride reflected the *invitro* observation. For example, the reduction in chloroquine absorption (9 %) at high dose *in situ* compares favourably with the reduction in chloroquine concentration (14 %) observed *in vitro*.

Similarly, there was correlation between the reduction of promethazine absorption observed *in situ* (42 %) and finding (56 %) at high concentration.

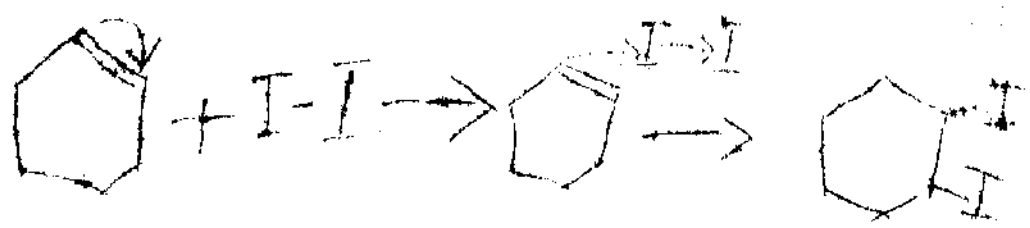
When chloroquine phosphate (2 mg) was administered with promethazine phosphate (10 mg), the reduction in chloroquine absorption (46.5 %) was less pronounced than the corresponding reduction observed *in vitro* (82 %) when chloroquine phosphate (5 µg) was mixed with promethazine hydrochloride (25 µg). The data generated from the study cannot explain this observation. Approximately, the same reduction in chloroquine absorption occured at both high and low doses (20 %) when chloroquine phosphate and promethazine hydrochloride were administered in the ratio 20:1 respectively. When chloroquine phosphate (45 mg) was administered with promethazine hydrochloride (2.5 mg), there was 18 % decrease in absorption of the former drug. These results indicate that chloroquine-promethazine interaction occured at all dose-concentration combinations studied. The extent of the interaction cannot be predicted and did not seem to follow any defined pattern. However, the extent of reduction in chloroquine absorption observed *in situ* correlates more with the effect observed *in vitro* at low concentration. At high concentration, there was probably a chemical reaction between chloroquine phosphate and promethazine hydrochloride involving the formation of a coloured intermediary product.

The colour that developed immediately after mixing chloroquine phosphate and promethazine hydrochloride was proably due to the formation of charge transfer complex. This phenomenon is a short-range intermolecular interaction involving the transfer of an unpaired electron from a donor (D) to an acceptor (A) molecule yielding a complex which may later dissociate into two free radicals:



The donor sources are either π- electrons or non bonded electrons. Typical donor molecules are either unsaturated compounds (ethylenic or acetylenic), aromatics with electron donating substituents, or groups with unshared electron pairs such as alcohols, ethers, thiols, sulphides, halides and amines. π-Electron donors usually give rise to weaker complexes than those derived from nonbonded electron donors. Typical acceptors are π dificient systems, particularly benzenoid aromatics with electron with drawing substituents or heteroaromatics.

The interaction between cyclohexene and iodine is well established as a simple example of charge transfer interaction (Stenlake, 1979):



Cyclohexene--iodine complex (Brown) 1,2-di-iodocyclohexane (Colourless)

An intense new band characteristic of the complex but not of neither component alone can be detected by spectral differences or sometimes by the formation of coloured complexes (Epstein et al.; 1964). A new broad absorption band appearing at longer wavelength than the spectra of either reactant suggests that a charge transfer complex is formed (Brook and Gruthuman, 1968).

The above proposal is supported by the observation of a new band (λ max C.a 300 nm, fig. 3,2.8) which is different from that of chloroquine phosphate (λ max = 344 nm) or promethazine hydrochloride (λ max = 249 nm). Further evidence is provided by the report that chloroquine is considered to act as a result of intercalation between a proportion of the base pairs of the DNA double helix, stabilized by charge transfer interactions (Stenlake, 1979). Proton magnetic resonance studies have established charge-transfer interactions between chloroquine phosphate and both Cyclic adenosine monophosphate (CAMP) and

guarosine monophosphate (GMP) which support the view that DNA complexes may be similarly stabilized (Sternglanz, Yielding and pruit, 1969).

Like many antihistamines, promethazine forms aggregates or miscelles (Attwood et al., 1974). Miscellization is a form of self complexation and both miscelle formation and interactions with extraneous materials decrease the proportion of free drug available for passage through membranes.

Charge transfer complexes of bromine and iodine with promethazine which form in acetonitrile have been detected by the use of conductrimetric titrations (Guttman and Keyzer, 1968).

There is thus evidence to suggest that both chloroquine phosphate and promethazine hydrochloride are capable of undergoing charge transfer interactions.

It is most likely that double decomposition reaction followed the formation of charge transfer complex. This is because precipitation did not occur immediately after mixing chloroquine phosphate and promethazine hydrochloride. If the precipitate formed was due to acid-base interaction, it would be expected to occur immediately. Furthermore, the yellowish green colour became faint when the precipitate was formed. This implied that it may be transitory.

A scheme of the probable reaction is as follows:-

Chloroquine phosphate + Promethazine hydrochloride → Chloroquine hydrochloride (soluble) + Promethazine phosphate (insoluble)

A feature of double decomposition reaction is the formation of unionized molecule of relatively low solubility in water (The Pharmaceutical Handbook, 18th edition, 1975). This explains why the promethazine phosphate formed was precipitated (because it was insoluble). Shearer and Miller (1975) reported that in the preparation of infrared spectrum of promethazine hydrochloride, it is preferable to use mineral oil dispersion rather than potassium bromide disks because the possibility exists for salt interchange between bromide and chloride. This is a further proof that an exchange between chloride and phosphate could occur.

Chloroquine hydrochloride solution exhibits the same maxima and minima at the same wavelength as that of similar solution of USP chloroquine phosphate (USP XVIII) therefore no difference was observed in in UV spectrum of the supernatant of the mixture and that of chloroquine phosphate alone (compare fig. 3.2.17, with fig. 3.2.27).

The relevance of the observed interaction lies in its potential clinical significance. Promethazine hydrochloride is recommended to be taken one hour before administration of chloroquine phosphate, however, patient compliance cannot be guaranteed. Moreover, promethazine hydrochloride tablets are usually sugar coated (Martindale, The Extra Pharmacopoeia 16th edition, 1973). This means that one hour

is the upper limit for their disintegration (Burlinson, 1968). And since dissolution must precede absorption, the possibility of both chloroquine phosphate and promethazine hydrochloride interacting along the gastrointestinal tract exists. Furthermore, phenothiazines and other antihistamines have been reported to delay gastric emptying (Nimmo, 1979). Some drugs are absorbed much more rapidly from the upper small intestine than from the stomach because of the greater surface area of the intestine (Levine, 1970). The rate of gastric emptying may therefore limit the rate of drug absorption and is particularly important in the context of interadtions Thus, it is likely that the two drugs may even interact in the stomach due to delayed gastric emptying caused by promethazine hydrochloride.

In the treatment of acute malaria, it is usual to give chloroquine phosphate injection especially in those patients who cannot swallow the tablets (e.g. babies or adults who vomit the drug). If such patients complain about itching (from past experience with chloroquine) promethazine hydrochloride could be included in the prescription. It is a common practice in hospitals to combine two injectables apart for the same patient in one syringe prior toinjection. This is supposedely more convenient for the patient and the nurses as well. However, this provides a perfect opportunity for chemical interaction to occur.

Promethazine hydrochloride is subject to extensive first pass metabolism in humans. The oral bioavailability was reported to be only 25 %, although absorption exceeded 80 % (Taylor et al.,1983). Interaction with chloroquine prior to absorption can further reduce the amount absorbed and hence the fraction that is systemically available. Similarly, decreased amount of chloroquine reaching the systemic circulation as a result of interaction with promethazine could lead to subtherapeutic blood levels of chloroquine which may result in therapeutic failure or emergence of chloroquine insensitive strains of plasmodium.

SUMMARY AND CONCLUSION

From the study, it has been established that there is a chemical interaction between chloroquine phosphate and promethazine hydrochloride. The Interaction was studied both *in vitro* and *in situ*.

In vitro: It was observed that the pattern of the interaction depends on the concentration of the two drugs and the ratio in which they were present. When both drugs were present in high concentration (10 - 250 mg), relatively less chloroquine (and more promethazine) was involved in the interaction. Conversely, at low concentration (5 - 50 µg), more chloroquine and less promethazine was engaged in the interaction. It can be suggested that at high concentration, a charge-transfer complex was formed which was responsible for the colour development. Double decomposition reaction probably follows, leading to the formation of insoluble precipitate of promethazine phosphate. The presence of the latter was confirmed by U.V.; g.l.c. and I.R. studies. The positive result for phosphorus and negative result for halogens confirm that the precipitate was promethazine phosphate and *not* hydrochloride.

In situ: Promethazine hydrochloride reduced the extent of absorption of chloroquine phosphate from rat small intestine. The result obtained from *in situ* studies correlates with the observation made *in vitro* at low concentration. However,

the post administration time *in situ* also influences the pattern of the interaction. The conclusion arrived at is that promethazine interacts with chloroquine and may be of clinical significance in humans.

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APPENDIX 2.1

Preparation: of Dragendorff's reagent.

Solution I: Bisthmus Oxynitrate (0.85 g)
Glacial acetic acid (10 mls)
Distilled water (40 mls).

Solution II: Potassium Iodide (8g)
Distilled water 20 ml.

Solutions I and II were mixed to prepare the stock solution; 10 mls of stock solution was diluted with 20 mls glacialacetic acid and the volume adjusted to 10 ml with distilled water (Iriah, 1984).

APPENDIX 2.2

Gas-Liquid Chromatograph: Pye Unicam series 204 fitted with FID, quarter-Inch Injector system (Pye Unicam); Recorder No. PM 8251 (Phillips, Holland); OV-17 3 % on gas Chrome Q (100/120 mesh) packed in 1.2 M glass column, i.d. 2 mm; conditioned at 300°C and Silinised with 3 x 5 µl hexamethyldisilazane. The following conditions were employed:

Nitrogen: 1.5 kgf/cm^2

Hydrogen: 1.8 kgf/cm^2

Air: 1 kgf/cm^2

Column temp: 300°C

Injection port temp: 300°C

Detector temp: 260°C

Attenuation: 512 x 1

Detector: FID

APPENDIX 2.3

Calculations and Statistical analysis.

The formula used in calculating chloroquine phosphate and promethazine hydrochloride concentrations from their respectively absorbances was $A(1\ \%, 1\ cm) = \frac{A}{c.d}$

Where

C = concentration (% w/v)

d = pathlength (cm)

A = Absorbance

A (1 %, 1 cm) = specific absorption coefficient

A (1 %, 1 cm) for chloroquine phosphate at 344-nm = 371

A (1 %, 1 cm) for chloroquine phosphate at 249 nm = 307

A (1 %, 1 cm) for promethazine hydrochloride at 249 nm = 910

Chloroquine phosphate absorb at 249 nm (the mamax for promethazine hydrochloride). Therefore, in the determination of promethazine hydrochloride concentration from a mixture containing chloroquine phosphate, the absorbance due to the latter must be substracted i.e.

$$\left(\text{Absorbance Reading Of Mixture}\right) - \frac{\left(\text{Absorbance due to Chloroquine at 344 nm}\right)}{\left(A\ (1\ \%, 1\ cm) \text{ for chloroquine at 344}\right)} \times A\ (1\ \%, 1\ cm) \text{ for chloroquine at 249 nm.}$$

Statistical analysis

The student t-test was used to determine whether the results were statistically significant or insignificant. Results obtained at five minutes

interval for chloroquine phosphate alone were compared with the values obtained when promethazine was added to chloroquine at corresponding time interval. The formula used was:-

$$\frac{X-Y}{\sqrt{\frac{}{n_1+n_2}} \times \sqrt{\frac{(S_1)^2(n_1-1)+(S_2)^2(n_2-1)}{n_1+n_2}}}$$

Where:

X = Values obtained with Chloroquine phosphate alone.

Y = Values obtained with chloroquine phosphate and promethazine hydrochloride.

S_1 = Standard deviation for values obtained with chloroquine and promethazine.

n_1 = number of samples of chloroquine.

n_2 = number of samples of chloroquine and promethazine.

A probability level of 5 % or less is regarded as significant.

Assay of Chloroquine Phosphate and Promethazine hydrochloride

	Absorbance 344 nm	249 nm
Chloroquine phosphate	0.183	
Promethazine hydrochloride		0.435

The dilute solution (50 ml) should contain 0.2500 mg if its active ingredient content is 100 %.

For Promethazine hydrochloride:

$$A(1\%, 1\ cm) = \frac{A}{c.d}$$

$$916 = \frac{0.435}{c \times 1}$$

$$c = 0.000\ 47802\ g/100\ ml = 0.2390\ mg/50\ ml.$$

$$\frac{0.2390}{0.2500} \times 100 = 95.6\ \%$$

For Chloroquine Phosphate.

$$371 = \frac{0.183}{c \times 1}$$

$$C = 0.00049326\ g/100\ ml = 0.2466\ mg/50\ ml.$$

$$\frac{0.2466}{0.2500} \times 100 = 98.65\ \%$$

APPENDIX 3.2

Chloroquine phosphate 225 mg/4.5 ml

+

Promethazine hydrochloride 45 mg/1.8 ml.

The absorbance of 0.1 ml of the mixture made up to 100 ml with 0.1N Hcl was found to be 1.259 at 344 nm and 1.326 at 249 nm.

$$A(1\%, 1\ cm) = \frac{A}{c.d}$$

$$371 = \frac{1.259}{c \times 1} \qquad C = \frac{1.259}{371} = 0.00339\ g/100\ ml = 3.393\ mg/100\ ml$$

0.1 ml of the mixture contained 3.3935 mg of chloroquine phosphate and 6.3 ml will contain 213.7924 mg.

Therefore % loss for Chloroquine = $\frac{(250 - 213.7924)}{250} \times 100 = 14.48\ \%$

For promethazine hydrochloride: there has to be correction for chloroquine phosphate absorption at 249 nm.

$$\frac{\text{(Absorbance of Chloroquine at 344 nm)}}{\text{A (1\%, 1cm) for chloroquine at 344 nm)}} \times \begin{pmatrix}\text{A 1\%, km/ for}\\ \text{chloroquine at}\\ \text{(249 nm}\end{pmatrix}$$

$\frac{1.259}{371} \times 307 = 1.041$. This value must be substracted from the absorbance of the mixture at 249 nm, in order to get absorbance due to promethazine hydrochloride.

1.326 - 1.041 = 0.285.

$$910 = \frac{0.285}{c \times 1} \quad = C = 0.000313\ g/100\ ml = 0.313\ mg/100\ ml$$

0.1 ml of the mixture contained 0.3131 mg of promethazine hydrochloride and 6.3 ml will contain 19.730 mg % Loss for Promethazine $\frac{(45 - 19.730)}{45} \times 100 = 56.15\ \%$