BIOAVAILABILITY AND PHARMACOKINETIC STUDIES OF BRANDS OF PARACETAMOL DOSAGE FORMS

BY

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DECLARATION

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

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CERTIFICATION

This thesis entitled BIOAVAILABILITY AND PHARMACOKINETIC STUDIES OF BRANDS OF PARACETAMOL DOSAGE FORMS by MOJIRADE TAIBAT BAKARE meets the regulations governing the award of the degree of MASTER OF SCIENCE of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation

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DEDICATION

Dedicated to my dear mother HAJIA S.O. BAKARE

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ABSTRACT

Total drug content for thirteen different brands of paracetamol tablets and five brands of paracetamol syrup has been evaluated.

Chemical assay methods based on colorimetric and spectrophotometric absorption were employed in the study. The results (except for one brand of paracetamol syrup) complied favourably with the stated amount in the monogram for paracetamol content (95.0 - (5.0)%. The exceptional paracetamol syrup (S2) was found to contain 89.12% of the labelled amount of paracetamol.

Dissolution rate profile of thirteen different lots of paracetamol tablets in 0.1M HC1 solution was investigated by means of U.S.P. XVIII rotating basket method. The drugs showed some similarities as well as some differences in their respective dissolution rate profiles. The differences observed in the dissolution rates of the drugs were probably due to the different manufacturing processes employed by the different manufacturers.

Disintegration time test was carried out on three brands of paracetamol tablets (Al, A2 and A7). There was considerable variation in their disintegration times which could be due to

different types and amount of disintegrant used by the different manufacturers.

In vivo bioavailability study of some brands of paracetamol tablets was carried out in healthy human subjects. A simple, reliable and rapid method of paracetamol estimation, developed by Glynn and Kendal (1975), was adapted for the determination of serum and saliva paracetamol levels. Serum and saliva paracetamol half-lives were calculated by the method of residuals. Area under the concentretion-time curve <AUC> was calculated by the trapezoidal rule and extrapolation to infinity.

Higher but statistically insignificant, levels of paracetamol were detected in saliva compared with the serum for the first 60 minutes following oral ingestion of lg of the drug. There was a good linear correlation between the concentration of paracetamol in serum and saliva. One of the products (A7) was verv slowly absorbed, consistent with its low dissolution rate found *in vitro*. Paracetamol elimination half-lives calculated for serum and saliva were similar.

The *in vivo* data quantitatively correlated with the *in vitro* release of these tablets.

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ABBREVIATIONS

B. P.	=	British Pharmacopoeia
°C	=	Degree centigrade (Celsius)
g	=	Gram
µg	=	Microgramme
mg	=	Milligramme
ml	=	Millilitre
min	=	Minute
hr	=	Hour
HCl	=	Hydrochloric Acid
NaOH	=	Sodium Hydroxide
$NaNO_2$	=	Sodium Nitrite
G.L.C	=	Gas Liquid Chromatography
H.P.L.C	=	High Performance Liquid Chromatography
U.S.P	=	United States Pharmacopoeia
pH	=	Hydrogen-ion concentration
U.V.	=	Ultraviolet

CHAPTER ONE

INTRODUCTION

1.1 *In vitro* Availability Testing

The study of the kinetics of drug release from a solid dosage form is of great importance in the formulation of drugs to ensure that a given dose of an active ingredient is released to the patient, in suitable amounts, after administration.

The dissolution rate test is used for measuring the time required for a given percentage of a drug substance in a solid dosage form to go into solution under specified set of conditions. It is an *in vitro* test and is intended to provide a step towards the evaluation of the physiological availability of the drug substance, but as currently described, it is not designed to measure the safety or the effectiveness of the solid dosage form. Both parameters must be demonstrated initially by means of an appropriate *in vivo* studies and clinical evaluation. Since drug absorption and physiological availability depend on having the drug substance in the

dissolved state, suitable dissolution characteristics are an important property of a satisfactory dosage form.

The physical characteristics and the composition of the drug can have an effect on the rate of disintegration, deaggregation and dissolution of the drug. These can, as such, affect the rate of absorption and resultant blood levels of the drug. When drugs are administered in solid dosage forms, the rate at which they are released into solution may often be slower than other processes involved in absorption, so that dissolution becomes the rate-limiting factor in absorption. In such cases, anything that affects dissolution will also affect absorption. Dissolution can therefore, affect the onset, intensity and duration of a response by controlling the overall bioavailability of the drug from the dosage form.

The steps involved in the disintegration, deaggregation and dissolution of compressed tablets or powders in capsule are shown in Figure 1.

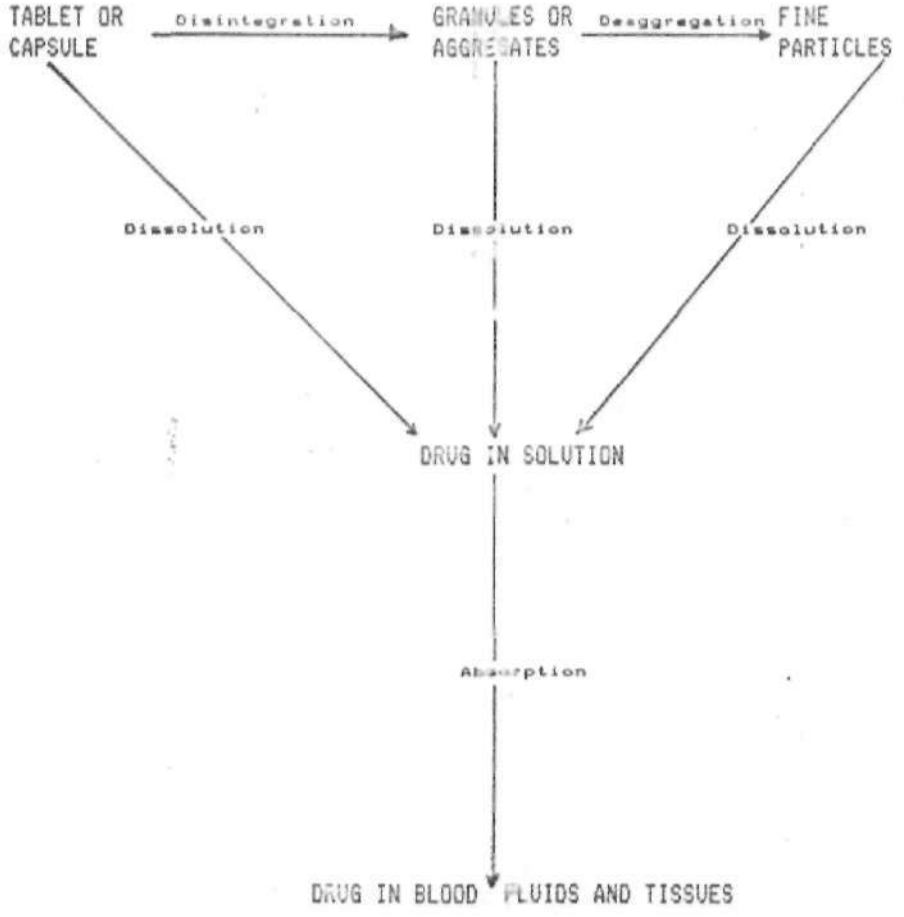


Fig.1: Schematic Representation of Disintegration, Deaggregation and Dissolution of a Drug From Solid Dosage Forms (From Poole, 1979)

Dissolution occurs at all levels from the intact dosage form the granules and the particles. Since the surface area of the drug in the intact dosage form is limited, the rate of dissolution from the intact compact is negligible except for very soluble drugs (Poole, 1979). It has been reported that the rate of absorption of a drug is the function of its dissolution rate (Levy and Hayes, 1960). Wagner (1971b) demonstrated that the processes involved when a solid dosage form is exposed to a fluid is dependent on dissolution.

An *in vitro* dissolution test which correlates quantitatively with dissolution rate-limited drug absorption in man has been developed (Levy et al., 1965). It is important to discover the physical characteristics of a drug which can alter its bioavailability, such as the polymorphic crystal form, choice of the salt form, the particle size, the use of the hydrated or anhydrous form, wettability and solubility, during the chemical testing of the drug prior to sale.

Dissolution rate test is used as a means of quality assurance of a good pharmaceutical dosage form. It is used to ensure that a given product is uniform within the same batch and from batch to batch during an in-process quality control. The characteristics of the pure drug or the effects of

excipients, the processing and manufacturing variables can be assessed using dissolution rate test. Also the stability of a drug after an extended shelf-life can be estimated.

The rate of dissolution depends on the surface area of the solid, which in turn, depends on how finely the drug is comminuted. It also depends on the energy and energy states within crystals of the drug. Noyes and Whitney (1897) incorporated the major factors involved in the rate of dissolution into an equation,

$$ds/dt = ks\ (cs - ct)$$

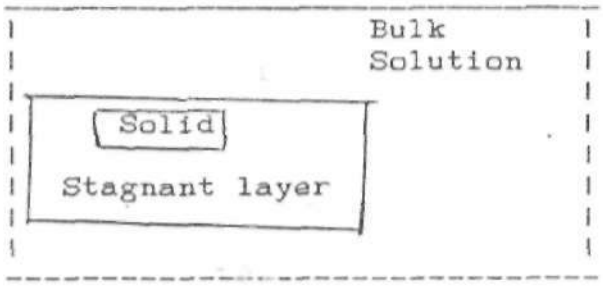
where s is the surface area of the particles,
k is a constant unique to the chemical substance and incorporates energy and entropy factors,
cs is the concentration at saturation, and
ct is the concentration at time t.

K varies widely from drug to drug and some drugs may have slow rate of dissolution despite a fine state of subdivision. Some drugs may exist in more than one crystal form, so that there may be more than one k. For example, some preparations of aluminium hydroxide completely dissolved within thirty minutes, while others showed no appreciable dissolution within one hour. During storage some

drugs change in crystal form. Considerable attention is directed to the dissolution rates of various salts of drugs and to the various crystal forms, in an effort to develop preparations with optimal dissolution rates.

The rate of dissolution of an active ingredient can be altered adversely by improper choice of ingredients and other formulation factors despite the use of solids of appropriate fineness. Because of compaction during tablet manufacture or capsule filling, hydrophobicity imported by tablet lubricants, adsorption of air on the particles often may prove more slowly soluble than larger particles. These properties, therefore, must be recognized and treated more appropriately in the course of a good formulation.

The model belo shows the rate at which a solute goes into solution.



Take a solid particle dispersed in a solvent having a finite thickness, L in centimeter (cm). The layer is an integral part of the solid and is referred to as "stagnant layer". This means that regardless of how fast the solution may be stirred, the stagnant layer remains a part of the surface of the solid moving wherever the particle moves (Ravin, 1980).

Using Fick's law of diffusion, the rate of solution of the solid can be explained as the rate at which a dissolved solute particle diffuses through the stagnant layer to the bulk solution.

The greater the difference in concentration of the solute, C_1 in the stagnant layer, and its concentration, C_2, on the farthest side of the stagnant layer, the fastest the rate of solution.

$$\text{Rate of solution} = \frac{DA(C_1 - C_2)}{L}$$

Acording to Fick's law, the rate of solution is also directly proportional to the area of the solid, A in cm^2, and inversely proportional to the length of the path through which the dissolved solute molecule must diffuse. D is a proportionality constant called the diffusion coefficient in cm^2/sec. The concentration C_1 is usually the saturation solubility as it is

usually larger than C_s. Thus the rate of solution can be expressed as:

$$\text{Rate of Solution} = \frac{DA}{L}(\text{saturation solubility})$$

Factors Influencing Dissolution Rate

The rate of dissolution of a drug can be affected by solubility, particle size, disintegration rate, chemical form and surface area of the solid dosage form (Ravin, 1980).

In the process of an *in vitro* dissolution rate test, it is essential to control some factors which are known to cause variations within and between control laboratories in order to ensure reproducibility of results.

In relation to the dissolution rate apparatus, it is important to use a standardized equipment designed in such a way as to simulate *in vivo* dissolution processes. The British Pharmacopoeia requires the stimer shaft eccentricity to be an imperceptible wobble, the sampling position to be half way between the basket and the wall and half way up the basket; the speed of the drive motor to be constant.

The use of deaerated dissolution medium is specified (B.P., 1980). Aerated medium contains air bubbles which can form on the surface of the basket mesh altering the flow of the medium from the basket. Samples are to be filtered to remove solid particles which will give false spectrophotometric readings.

The temperature of the dissolution medium seems to be the only *in vivo* parameter which can be reproduced with ease in the laboratory. The B. P. and U.S.P. specify a temperature of $37 \pm 0.5^{\circ}C$. This is important since the dissolution of drugs depends on the temperature of the medium.

1.2 *In vivo* Bioavailability Testing

The term bioavailability has been defined as the rate and extent to which the active drug ingredient or therapeutic moiety is absorbed from a drug product and becomes available at the site of action which is normally estimated by its concentration in body fluids, rate of excretion, or acute pharmacological effect (Skelly, 1976). According to this definition, the absorption of an intravenously administered drug is rapid and complete. However, for reasons of convenience and stability, most drugs are administered orally. Therefore, their rate and extent of absorption in an individual are usually not precisely known (Blanchard and Sawchuk, 1979).

Bioavailabilty or bioequivalence studies are usually based on measurement of the active drug moiety and/or its metabolite(s) in biological fluids as a function of time. *In vivo* testing in humans usually dictates that the biological fluid sampled be limited to blood, urine, or saliva.

Drug is administered to subjects who are usually in the fasted state (approximately 12 hours) and who are not permitted food for several hours after receiving drug. Blood samples are then collected after drug administration at a frequency sufficient to permit an estimate of peak plasma or serum concentration of active drug and the total area under the plasma level versus time curve. In most studies where

oral dosage forms are compared, the sampling times after each drug administration will be identical. Typical plasma concentration versus time curve that may be obtained after oral administration of a solid dosage form is shown in Figure 2.

Inspection of Figure 2 permits one to observe the major bioavailability parameters - peak plasma concentration (C_{max}), time to achieve peak plasma concentration (t_{max}), and area under the plasma level versus time curve (AUC). The first two parameters, C_{max} and t_{max}, are related to and used as measures of the rate of availability from the dosage form. The extent of absorption from the dosage form is related to the third parameter, AUC. In a bioequivalence study, the objective is to determine whether the tested dosage form is equivalent to the reference form. Alternatively, one might demonstrate that no statistically significant differences exist between the plasma levels derived from each drug product at each sampling time.

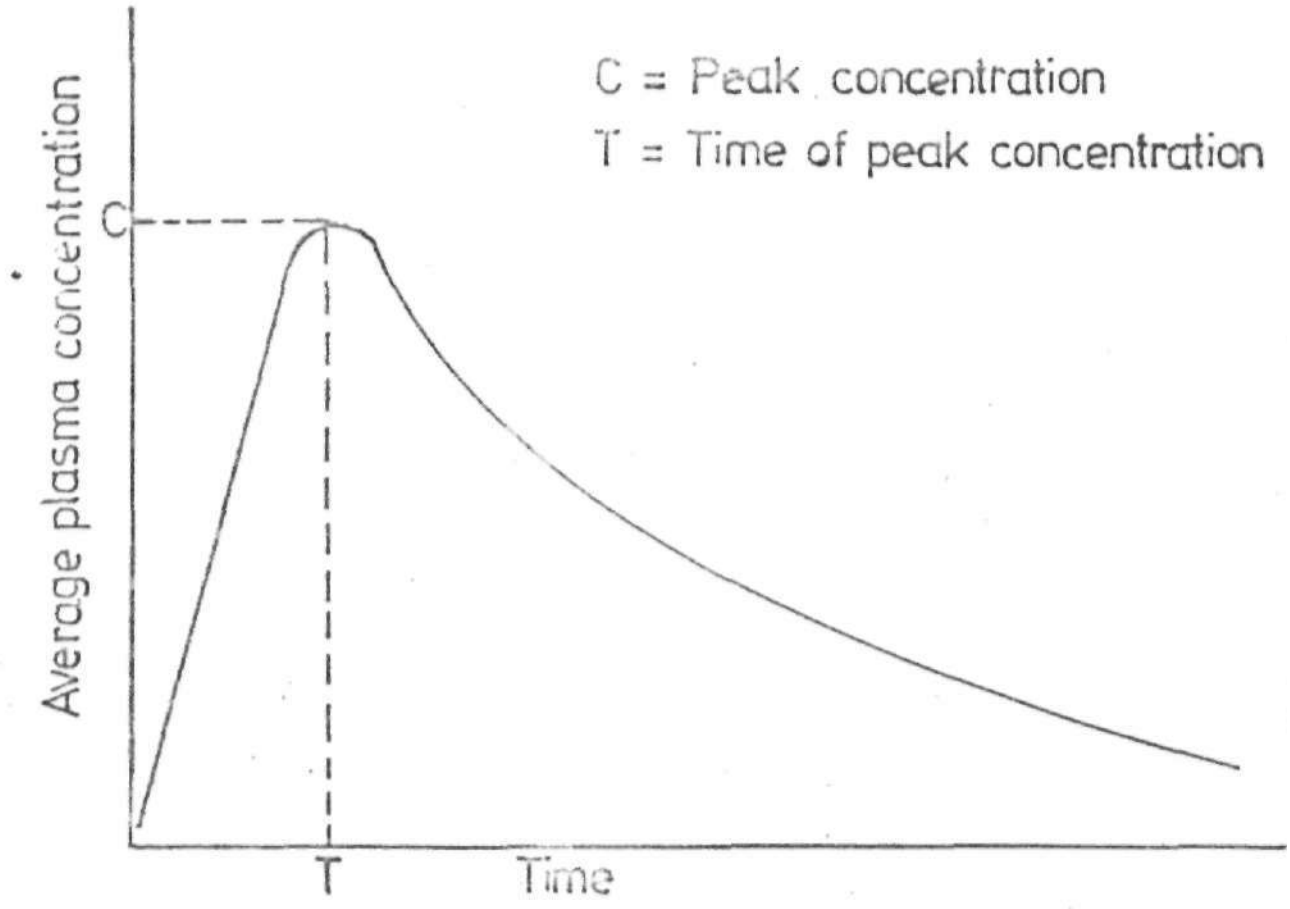


Fig. 2: A typical plasma concentration-time curve.

Determination of the 'absolute' bioavailability of a drug usually involves a comparison of area under the plasma concentration-time curve (AUC) obtained following oral and intravenous administration (for which instantaneous and complete bioavailability is assumed) of the drug. For practical reasons, intravenous administration is often not possible, and in such cases 'relative' bioavailability is determined by comparing the AUC's of the test drug and a secondary reference standard such as an orally administered solution of the drug or a dosage form which has been accepted as a standard (Gibaldi and Perrier, 1975a; Banchard and Sawchuk, 1979).

There are a number of circumstances which arise making it quite difficult to determine the availability of a drug following administration of a single dose. The plasma levels of certain drugs may be very low such that an assay method sensitive enough to measure these levels may not be available. Other drugs have very long half-lives, and the determination of the total area under a plasma concentration-time curve requires plasma samples to be obtained for excessively long periods of time. Thus it is sometimes desirable to determine availability based on steady-state plasma drug levels following multiple dosing (Gibaldi and Perrier, 1975a).

For the estimation of availability, the area under the plasma concentration time curve (AUC) determination is required. The most commonly employed methods for calculating

AUC's are the "cut-and-weigh" method, employment of planimeter and application of trapezoidal rule (Gibaldi and Perrier, 1975c).

1.3 Factors Influencing Drug Bioavailability

The compressed tablet is the most widely used oral dosage form, and is also most commonly involved in drug bioavailability and bioequivalence problems. This is not surprising owing to the reduction in drug surface area in the compressed formulation and the wide variety of drug forms, tableting methodologies, excipients and coating that are used.

Absorption may be affected not only by the different dosage forms, but also by similar dosage forms from different manufacturers and different batches of a dosage form from a single source. The most common conventional oral dosage forms, in decreasing order of relative dissolution and absorption rates are, solutions, suspensions, capsules, tablets, coated tablets and sustained-release preparations.

Dosage form related factors which can produce profound differences in bioavailability include formulation and manufacturing variables such as particle sizes, the chemical form and solubility of the drug; the type and

quantity of excipients used, the compaction pressure and other factors (Blanchard and Sawchuk, 1979).

Dosage form properties often account for some modification in the pharmacological activity of therapeutic agents by altering their intrinsic absorption pattern. The schematic diagram in Figure 3 shows drug concentration in the blood as a function of time after oral administration of a fixed amount of drug in three different doasge forms designated I, II and III. The properties of the dosage form in this hypothetical example produce a change in the overall absorption rate which is reflected in the blood level - time curve.

Activity occurs when the blood concentration reaches the effective drug level (Figure 4). The onset of pharmacological action is significantly earlier with formulation I than with formulation II, and is never attained with formulation III. The duration of the effect is often reflected by the length of time that the blood level of drug is equal to or higher than the minimum effective level.

Again it can be seen from the example in Figure 3 that the duration of effect obtained from formulation I is sginificantly longer than that obtained after equal dose of formulation II.

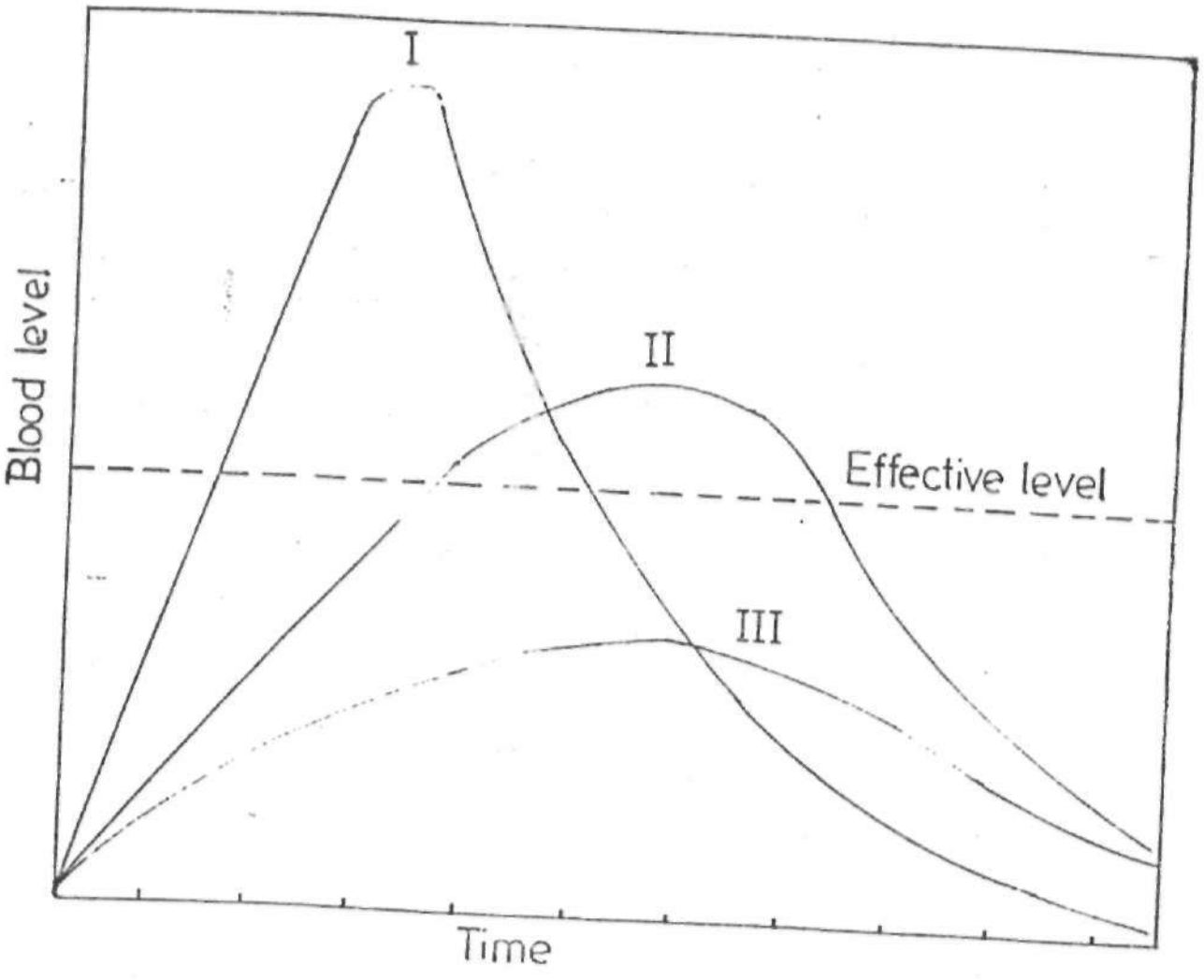


Fig. 7: Blood level-time curves showing possible effects of dosage form on blood levels after oral administration of equal amounts of active agent (from Poole, 1979).

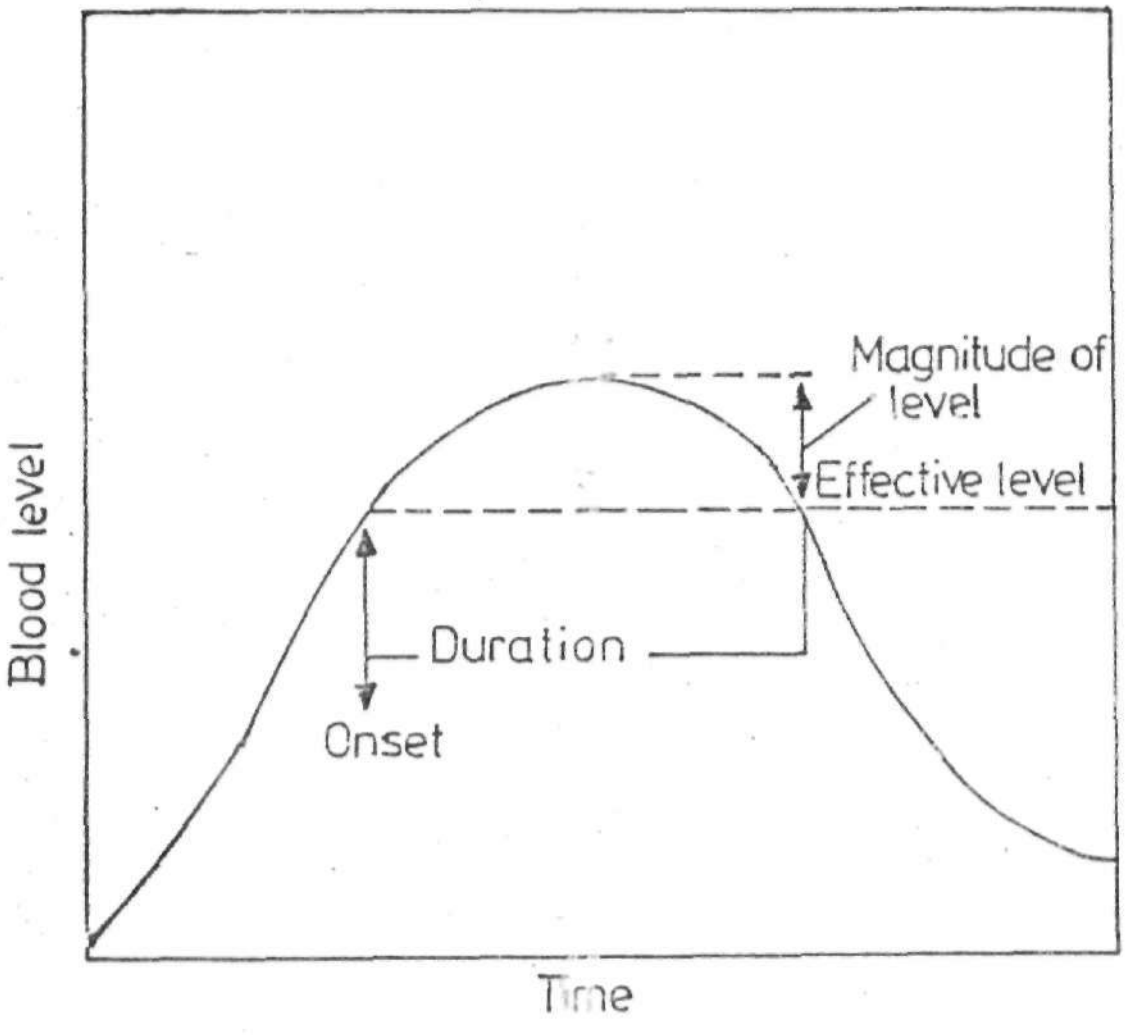


Fig. 4: Relationship of blood level-time curve and pharmacologic effect (from Poole, 1979).

From this general and over-simplified example, it can be seen readily that dosage form factors can have a significant effect on the onset, duration and intensity of pharmacologic action of a particular active component.

Figure 3 also illustrates the effect of formulation on overall biological availability. This availability is frequently expressed as a ratio of the amount of test formulation absorbed to the amount of standard formulation absorbed.

$$\text{Bioavailabilty} = \frac{\text{Area under curve or cumulative amount excreted for test formulation}}{\text{Area under curve or cumulative amount excreted for standard formulation}}$$

A preparation that exhibits 100% availability may be compared with a test dosage form. The ratio obtained for the areas under the two blood level-time curves indicates the availability of the drug from the dosage form. In the example, the areas under the curves for formulations I and II are essentially the same. This means that although the drug is more properly absorbed when administered as formulation I, the total amount absorbed when administered from both forms is approximately the same. However, for formulation III, the area is significantly less (about 50%) than that of the other two. If in the case of the first two formulations the drugs were completely absorbed, the bioavailability of the drug when

administered as dosage form designated III is only about 50%.

The various formulation and dosage form related factors which have been extensively discussed by Poole (1979), Ritschel (1976), Wade (1976) and many writers in this field include: particle size, crystal form, salt, complex, pH and solubility characteristics. Other factors are excipients and adjuvants as well as manufacturing methods. Poole (1979) in his discussion showed that bioavailability of some drugs have been improved by particle size reduction, and how particle size reduction in recent years has been accomplished in several instances by the preparation of microcrystalline molecular dispersions of poorly soluble drugs in solid matrices of water-soluble carriers.

Various adaptations of the technique of solid dispersion have been put forward by different research workers. Fincher (1968) published a review article on the effect of particle size on drug absorption and activity.

The crystal form in which a drug is presented to the patient is an important factor influencing the bioavailability of drugs. The crystal form of a drug substance may change because the substance exhibits polymorphism. Polymorphism has been reported to be relatively common, being exhibited by at least one-third of all organic compounds. Apart from the polymorphic forms in which substances may exist, they can also

occur in non-crystalline or amorphous forms. The energy required for a molecule of drug to escape from the crystal in which it is located is very much greater than that required to escape from an amorphous powder. Therefore, the amorphous form of compound is always more soluble than a corresponding crystal form and may exhibit correspondingly different therapeutic properties (Poole, 1979). Examples of drugs in which this has been observed *in vitro* are novobiocin and chloramphenicol.

The property of many organic medicinal compounds to form solvent addition compounds or solvates may also affect their bioavailability. When water is the solvent associated with the drug moiety, the solvate form is called a hydrate. The solubility and rate of solution of a solvate may significantly differ from that of non-solvated form of the drug. Drugs for which significant differences in dissolution rates have been observed include caffeine, theophylline and glutethimide.

In each of these the anhydrous form dissolved more rapidly. But, for fludrocortisone acetate and succinylsulfathiazole, the solvated forms dissolved more rapidly than the non-solvated forms (Shefter and Higuchi, 1963). Differences in bioavailability between anhydrous and hydrated form of the amphoteric compounds, penicillin and

ampicillin, have been reported by Poole and Bahal, (1968).

Complex formation is a mechanism that can influence the biopharmaceutical properties of a drug. Drugs may interact reversibly to form complexes with substances occurring in the body, with other drugs or with pharmacologically inactive components of the pharmaceutical dosage form. Such a drug complex may differ in its physico-chemical properties from the pure drug. For instance, its ability to permeate biological membranes may become altered. Such complexes are thus pharmacologically inactive since dissociation prior to drug absorption usually fails to occur (Poole, 1979).

The effect of complexation on drug absorption depends on the magnitude of the stability constant of the complex. Frequently there may be little or no effect on the rate of absorption and on the overall bioavailablity. However, some drugs which are intrinsically poorly absorbed can be converted to non-absorbable complexes under certain circumstances and their bioavailability is further decreased. Complex formation according to Poole (1979) can be used in formulation of dosage forms in order to increase solubility or stability of drug substances or drug products. The drug complex in such systems is diluted in the biological fluids after administration which results in the dissociation of the complex with little or no effect on

the overall absorption properties of the active compound.

The components in a dosage form other than the active drug substance may influence the absorption and subsequently the performance of a drug product. These additives or excipients may either facilitate or inhibit the absorptive process. The importance of excipients in the determination of the final drug effect was clearly demonstrated by an outbreak of phenytoin toxicity in Australia. The toxicity occurred when patients previously maintained in a stable dose of 'Dilantin' capsules in which calcium sulphate had been used as inert excipient, were given capsules (containing some quantity of active compound) but with the calcium sulphate substituted for lactose as 'inert' excipient. Presumably, the inorganic ion had complexed with phenytoin reducng the absorption (Wade, 1976).

The role of surface active agents is more complex and not always easy to predict. These substances are frequently used to increase solubility or act as emulsificants, but they may ither increase or decrease absorption depending on the agent, the quantity, the route of administration, the drug in question and the nature of the formulation. It was reported that these surface active agents may change the absorptive surface itself in the small intestine, and that in other cases, the agents promote micelle formation, thus enabling lipid

soluble substances to disperse in an aqueous medium (Wade, 1976).

The factors associated with formulation and processing which may affect dispersion, rate of dissolution and subsequently the bioavailability of drug vary according to the type of preparation. Because tablets are compressed, quick dispersion and dissolution of the drug are more difficult to achieve. It is generally accepted that efficient disintegration is an important requirement for a tablet but it does not necessarily ensure good dispersion and dissolution of the drug because the size and nature of the particles which are produced are also controlling factors. The most important factor is the rate of dissolution of the drug from the disintegration particles (The Pharmaceutical Codex, 1976). Dissolution rate may itself be affected by the granulation, or other mixing procedure; the degree of compression and the presence of fillers, binders or surface active agents.

Interaction between drugs and dosage forms may influence the rate and extent of drug absorption. Such interactions may be direct, as in chelation of tetracycline by polyvalent metal ions, or indirect as with the increased rate of acetaminophen (paracetamol) absorption due to the stimulating effect of metoclopromide on the stomach emptying rate. However, propantheline reduced the rate of

acetaminophen absorption by delaying the stomach emptying rate (Welling, 1980).

The volume of co-administered fluid may have a considerable effect on drug absorption. Although from a purely physical view-point, drug absorption might be expected to occur more readily from concentrated solutions than from dilute solutions, the reverse appears to be the case. The toxicity of various orally dosed organic acids and bases, and also inorganic compounds in rats, was greater from dilute solutions than from concentrated ones containing the same amount of compound (Ferguson, 1962), while the pharmacological activity of sodium phenobarbitone and also the circulating levels of salicylate, were greater from dilute than from concentrated solutions (Borowitz and Moore, 1969). Increased drug bioavailablity from dilute solution may be related to the tendency for a compound to follow net mucosal to serosal fluid flux when hypotonic solutions are introduced into the gastrointestinal tract. However, it may be related also to more rapid stomach emptying of large fluid volumes.

Influence of food and specific dietary components on drug absorption has been extensively reviewed by Welling, (1977) Melander (1978). The reviews reflect the complex and unpredictable nature of drug-food interactions, and the difficulty of establishing patterns which may be used as guidelines for optimum drug therapy. The presence of food has been shown to decrease, delay, increase and often not

to affect drug absorption. Observed effects have been influenced by the type of drug and dosage form, the nature and size of meal, fluid intake, the conditions of the subject and the time sequence between eating and drug administration.

Although drug bioavailability studies are routinely carried out in healthy human volunteers, drugs are administered in practice to patients who may be suffering from a variety of illnesses. Diseases concerning the cardiovascular system, the liver and the gastrointestinal tract may alter circulating drug or metabolite level after oral dosing. Surgical removal of part of the gastrointestinal tract reduces the absorption of most compounds, suggesting that patients who have undergone such procedures may need to be monitored carefully when taking drugs whose absorption effeciency is critical.

1.4 Relationship Between Bioavailability and Dissolution Rate

A dosage form must provide a stable enviroment during storage , so must it deliver the active ingredient in a form available for absorption upon administration. In as much as oral solid dosage forms must dissolve before absorption can occur; dissolution testing provides the means of evaluating this absorption phenomenon. The results of dissolution testing are useful in various ways depending upon the purpose for which the test was performed.

i) In research and development, dissolution testing is performed for assessing factors governing the release of the active ingredient, and more specifically for evaluating the influence of excipeints or other processing variables on dissolution rate and hence on drug bioavailability.

ii) During the early stages of product design, dissolution rate tests done in conjuction with *in vivo* absorption studies can indicate the extent to which the released characteristics of the dosage form would influence blood levels of the drug.

iii) Where evidence for dissolution-rate limited absorption exists, monitoring changes in the formulation, (e.g particle size or salt form) by means of dissolution rate test provides a rational basis for optimizing a formulation.

As previously mentioned, the most widely used dosage form owing to inconvenience and economic factors involved in manufacturing, is the compressed tablet.

However, tablet formulations also pose some of the most difficult pharmaceutical problems which may influence the bioavalability of the active ingredient. one of the major problems encountered with tablets is the decreased effective surface area of the active ingredients as a result of granulation and compression of the drug particles. Unless a compact tablet readily disintegrates and deaggregates when it comes in contact with the body fluids, bioavailability of drugs from such dosage forms usually become reduced. It is now generally recognised that bioavailablity problems may be anticipated from drug products that have slow dissolution rates in water at 37°C. This slow dissolution may be reflective of:-

(1) low solubility of the active drug moiety

(2) the importance of particle size or surface area on dissolution of the active drug

(3) presence of different crystal forms of the drug, and

(4) the presence of inactive excipients in the drug product which may interfere with the dissolution of the active drug.

Good correlation of *in vitro* dissolution data with *in vivo* bioavailability data may permit the establishment of a bioavailability standard for the drug product based on *in vitro* testing.

1.5 The Clinical Significance Of Differences In Drug Bioavailability

A large number of factors have been shown potentially to interefere with drug absorption. The degree of change in clinical response which may accompany altered drug bioavailability is a function both of the drug's mode of action and also the extent of bioavailability variance. It may be difficult to identify a clinical change with altered drug bioavailability due to the many factors contributing to, and the often subjective nature of assessing clinical response.

Nevertheless, several reports have documented actual changes in clinical response which have been associated with bioavailability. Other reports have strongly suggested that changes in the bioavailability of a particular drug would have clinical implications. Most of these compounds have well defined blood or tissue level for therapeutic effect, steep dose-response relationships or narrow therapeutic indices, or have suitable absorption or elimination mechanisms (Azarnoff and Huffman 1976).

When considering the clinical implications of drug bioavailability changes, whether the cause is a pathological condition, drug interaction, product substitution or any other factor, it is appropriate to divide bioavailability changes into three major groups: those which have definitely caused changes in clinical response, those which have not been shown to cause clinical changes but are highly likely to do so, and those which could bring about clinical changes in extreme situations.

1) Bioavailability changes which have caused altered clinical response.

Instances where marked clinical changes have been associated with impaired drug

bioavailability, due either to product substitution or formulation changes have been described for hydrochlorothiazide (Tannenbaum et al., 1968), chlorothiazide and spironolactone (Shaldon et al., 1963), phenytoin (Eadie et al., 1968; Tyrer et al., 1970), indomethacin (Katz et al., 1968), tolbutamide (Carter, 1963), digoxin (Lindenbaum et al., 1961) and dicoumarol (Lozinski, 1960).

2) Bioavailability changes which are likely to have clinical effects.

If a drug meets any of the aforementioned criteria, it is likely that a change in drug bioavailability will have a measurable clinical effect. Included amongst these drugs are the cardiac glycosides, anticoagulants, antihypertensive agents, anticonvulsants, antineoplastic agents and inorganic ions such as lithium. However, in the majority of cases, levels of these compounds in the body greatly exceed the minimum required for activity, and small changes in bioavailability are unlikely to be of clinical significance.

3) Bioavailability changes which may have clinical effects.

A clinical change will occur for all drugs, provided that the change in bioavailability is sufficiently large. Aspirin is considered to have a wide therapeutic index, but zero absorption of a particular formulation of this compound (Morrison and Campbell, 1960) is clearly significant. The clinical effect may be altered by changes in either the rate or extent of drug absorption. Changes in absorption rate are more important for drugs with short biological and pharmacological duration, but the effect tends to be attenuated with repeated dosing.

1.6 Pharmacokinetics

Pharmacokinetics is the study of the rate processes associated with absorption, distribution, metabolism and excretion of a drug, i.e. quantitating drug and/or metabolite(s) levels in body fluids, tissues and excreta at any point in time from the moment of administration until elimination from the body is complete (Kaplan and Jack, 1980). The primary standard of a pharmacokinetic study is based upon the data obtained following intravenous administration.

Since the entire dose administered is placed directly into the blood stream following intravenous administration, the pharmacokinetic parameters obtained are unaffected by all the potential rate-limiting and metabolic factors associated with drug absorption following other routes.

In pharmacokinetics, attempts are made to describe a biological event(s) in mathematical terms, usually by developing mathematical models to represent the body as a system of compartments, even though these compartments often have no apparent physiologic or anatomic reality. The one-compartment model, the simplest model, depicts the body as a single homogenous unit. This model is particularly useful for the pharmacokinetic analysis of blood, plasma or serum concentration, salivary and urinary excretion data for drugs which rapidly distribute between plasma and other body fluids and tissues upon entry into the systemic circulation.

To assume the body behaves as a one-compartment model does not necessarily mean that the drug concentrations in all body tissues at any given time are the same. However, a one-compartment model does assume that any changes that occur in the plasma quantitatively reflects

changes occuring in tissue drug levels. A second criterion for utilizing a one-compartment model is that drug elimination occurs from the body in a first-order fashion (i.e., the rate of elimination of drug from the body at any time is proportional to the amount of drug in the body at that time). The proportionality constant relating the rate and amount is the first-order elimination rate constant which has units of reciprocal time (for example, min^{-1}, hr^{-1}) (Gibaldi and Perrier, 1975b).

Half-life is one of the most useful pharmacokinetic parameters obtained following drug administration. The half-life of a drug is simply the time required to reduce the post-absorption concentration of a drug in the blood stream by fifty percent (Wagner, 1980; Gibaldi and Perrier, 1975b). This parameter is calculated by dividing 0.693 by the calculated slope of each exponential phase of multiphasic blood level curve. Another pharmacokinetic parameter in frequent use is the area under the curve of blood level versus time. The method of calculating area are discussed by various authors (Wagner, 1967; Gibaldi and Perrier, 1975c). Many authors have used area analysis to develop a pharmacokinetic pathway and to obtain the rate constants associated with the

proposed pharmacokinetic model. Total clearance is the sum of individual clearances of the drug by the various organs and tissues. The volume of distribution (Vd) is inversely proportional to blood level, i.e., the larger the volume of distribution the more extensively the drug distributes from the blood stream into the various tissues, organs, and binding sites of the body. There are various ways of calculating Vd depending upon the pharmacokinetic profile of the drug (Gibaldi and Perrier, 1975b).

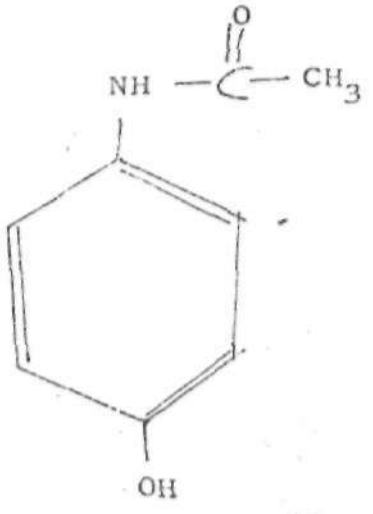
CHAPTER TWO

GENERAL LITERATURE REVIEW

2.1 History and Chemistry

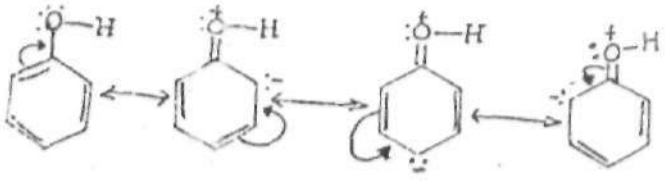
Acetanilide was introduced into medicine as an antipyretic under the name of 'Antifebrin'. It proved to be much more toxic than sodium salicylate and less dangerous compounds were therefore sought. Because it was thought (erroneously, as was later shown) that acetanilide owed its antipyretic action to its conversion in the body to p-aminophenol, derivatives of this last named compound were examined. P-aminophenol itself proved to be as toxic as acetanilide but toxicity was reduced if the hydroxyl or amino group received alkyl or acid substituents respectively. The most useful compounds thus produced were acetophenetidine (phenacetin) and N-acetyl-p-aminophenol paracetamol). But phenacetin is metabolised in the body to paracetamol (Crossland 1980).

Structure



Acetamide, N-(4-hydroxyphenyl-, N-acetyl-P-aminophenol; Paracetamol).

Paracetamol, like salicylamide, is weakly acidic due to the hydroxyl group directly attached to the nucleus. Because of the resonance, the oxygen atom of the OH group acquires a positive charge and so proton release is facilitated:



Considering the -$NHCOCH_3$ side chain, the lone pair of the NH_2 group enters into resonance with the carbonyl group, and consequently resonance with the ring is strongly diminished. This gives the drug a very weak basic character.



The antipyretic acitivity of the compound resides in the aminobenzene structure. Introduction of other radicals into the hydroxyl group of para-aminophenol and into the free amino group of aniline reduces toxicity without loss of antipyretic action.

Best results are obtained with phenolic alkyl ethers (ethyl in phenacetin) and with the amide (acetyl in phenacetin and acetaminophen).

2.2 Absorption and Metabolism

Paracetomol is generally readily absorbed from the gastrointestinal tract after oral administration. The concentration in plasma reaches a peak in about thirty minutes to one hour and the therapeutic half-life is approximately three hours (Swinyard, 1980). Paracetamol is generally absorbed from the stomach and the intestine (Jaffe et al, 1971). Weikel and Lish (1969) found that in rats paracetamol is absorbed from the stomach and all portions of the small intestine and that it is relatively insensitive to changes in gastric emptying rates.

Factors Influencing Absorption Of Paracetamol From the Gastrointestinal Tract

It is generally recognised that administration of drugs by the oral route a short time before or after meal may alter absorption of the drug from the gastrointestinal tract. Generally, meals have been reported to retard absorption (Jaffe et al, 1971). Certain specific dietary components can significantly alter the absorption pattern of orally administered paracetamol. A preceding meal of high fat or protein content or a balanced test-meal did not

delay significantly absorption of paracetamol whereas a high carbohydrate meal, especially one with large amounts of pectin, reduced absorption by 60 - 76% in the first 1.5 hours compared with the fasting state (Jaffe et al, 1971).

McGilveray and Mattok (1972) studied the effect of food and sleep on the absorption and excretion of paracetamol. Concomitant administration of food produced little effect on the extent of its absorption. Less paracetamol is absorbed and excreted when the drug was taken before sleep than after morning ingestion. They are, however, not sure of the cause of the reduced absorption.

Chiou (1975) reported that food could significantly reduce the absorption rate and peak blood levels of paracetamol in humans; it had no significant effect on total bioavailability, as indicated by similar areas under blood concentration curves of paracetamol. He suggested that differences in the contribution of the first-pass effect for rectal and orally administered paracetamol would not be of clinical significance.

If paracetamol is metabolised appreciably during absorption, its systemic availability should be relatively increased by administering

its precursor, acetophenetidine, which has its phenolic group blocked and therefore protected from conjugation in the gut wall (Levy, 1971).

Paracetamol is relatively uniformly distributed throughout most body fluid (Flower et al., 1985). Gwilt et al (1963) demonstrated that paracetamol is distributed evenly in all tissues with the exception of fat in dogs. The main metabolic pathway of paracetamol disposition is by conjugation to the glucuronide and sulphate (Cummings et al., 1967; Levy, 1971; Levy et al., 1975; Ramachander et al., 1973; Levy and Regardh, 1971; Levy and Yamada, 1971; Mitchell et al., 1974 and Mrochek et al., 1974.).

In normal subjects, less than 50% of paracetamol is usually excreted in the urine while the rest is eliminated largely as the glucuronide and sulpahte conjugates (Cummings et al., 1967; Levy and Regardh, 1971; Levy and Yamada, 1971; Mitchell et al., 1974 and Mrochek et al, 1974.).

Studies have been conducted on the elimination of paracetamol and its metabolites. Results indicated that after oral administration of 1g dose of paracetamol, the average recovery of drug in all forms was 92%, mostly as acetaminophen glucuronide and acetaminophen sulphate which are

non-toxic (Figure 5). Only 3.4% of the dose was recovered as free paracetamol (Levy and Yamada, 1971; Miller and Fischer, 1974 and Prescott, 1971).

The normal plasma elimination half-life of paracetamol is about 2hr, but can be considerably longer after massive dose (Prescott et al, 1971). Paracetamol is the major metabolite of phenacetin and acetanilide (Batty and Robinson, 1977). Miller and Fischer (1974) concluded that rat is not an appropriate animal model for paracetamol metabolism in humans. Paracetamol appears to be excreted in the urine primarily as acetaminophen sulphate when administered to rats in doses comparable to human doses. In contrast, the glucuronide conjugate constitutes the largest fraction of the dose of paracetamol excreted in the urine by humans. However, children have less capacity for glucuronidation of the drug than do adults.

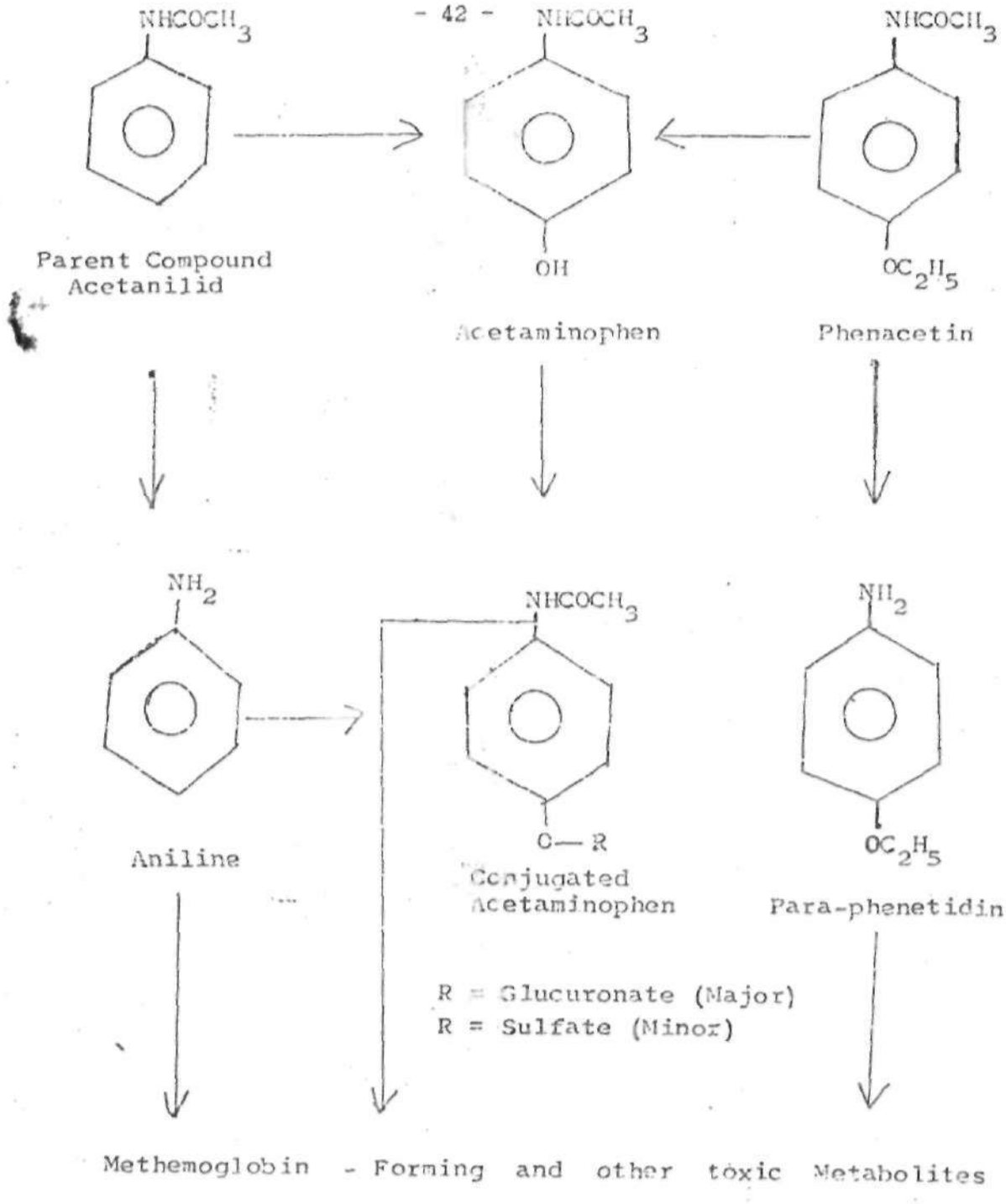


Fig. 5: Structural formulas of major para-aminophenol derivatives, and their interrelations (from Flower et al. 1985).

The ratio of glucuronide and sulphate metabolites varies widely between the different species; mice excrete more glucuronide than sulphate (Dybing, 1975). Administration of paracetamol with single and multiple doses of aspirin produce no effect on paracetamol metabolism in man (Amsel and Davison, 1972).

Excretion rate of the glucuronide and sulphate metabolites of paracetamol was reduced after administration of salicylamide, and paracetamol reduced the formation of the sulphate metabolite of salicylamide (Levy and Yamada, 1971). Ascorbic acid caused a significant decrease in the fraction of paracetamol excreted as the sulphate and a significant increase in the fraction excreted as paracetamol and paracetamol glucuronide, but it had no effect on the recovery of total paracetamol i.e the sum of paracetamol, its glucuronide and its sulphate (Houston and Levy, 1976).

Since paracetamol is eliminated largely by conjugation with glucuronic acid and sulphate, Lowenthal et al (1976) concluded that that the kidneys do not contribute significantly to the formation of these metabolites. They mentioned that anephric patients, unlike normal subjects,

showed pronounced accumulation of acetaminophen glucuronide and sulphate in plasma.

2.3 Therapeutic Significance of Paracetamol

Paracetamol is one of the most frequently used mild analgesic and antipyretics (Repta and Hack, 1973; Plankogiannis and Saad, 1978; Chiou, 1975; Levy, 1971 and Swinyard, 1980). The antipyretic analgesics are widely used to relieve low intensity pain and reduce fever. They are effective in treating pain arising from intergumental structures and malaise associated with viral infections, especially those components associated with headache and muscle aching (Bonica and Halpern, 1972). Paracetamol is particularly useful as an analgesic-antipyretic in patients sensitive to aspirin. It rarely induces untoward effects and is usually well tolerated by aspirin-sensitive patients.

Paracetamol may reduce the post traumatic inflammatory response and may be preferable to acetylsalicylic acid in bilateral oral sugery (Lokken and Skjelbred, 1980). However, paracetamol is therapeutically inferior to both indomethacin and aspirin in the treatment of rheumatoid

arthritis (Watson et al, 1975). Paracetamol does not produce any abnormality indicative of an adverse reaction in therapeutic doses and in the presence of chronic liver disease (Benson, 1983). The effective serum concentration of paracetamol for analgesia and antipyresis is 10 - 20 microgram per ml and is usually attained after single doses of less than 2g is taken orally.

Mechanism of Action

Fever, such as those produced by bacterial endotoxins or viruses, are probably caused by endogenous pyrogens derived from polymorphonuclear leucocytes. Endogenous pyrogens act directly upon the thermoregulatory neurones in the hypothalamus to increase the 'set-point' temperature (Parkhouse, 1979). Paracetamol inhibits the enzyme prostaglandin synthetase in the brain. By preventing the biosynthesis of prostaglandin, the antipyretic analgesics could inhibit the generation of prostaglandin E type substances by pyrogen, a process necessary for an increase in the 'set-point' temperature (Flower and Vane, 1972). There is evidence to suggest that paracetamol may be more effective against enzyme in the CNS than those in the periphery. This fact may partly account for its well-documented ability

to reduce fever and to induce analgesia, effects that involve actions on neural tissue (Flower et al., 1985).

2.4 Toxicity

In recommended therapeutic dose, paracetamol is usually well tolerated. Skin rash and other allergic reactions occur occasionally. The rash is usually erythematous or urticarial, but sometimes it is more serious and may be accompanied by drug fever and mucosal lesions (Flower et al., 1985). When high doses are ingested, paracetamol undergoes N-hydroxylation to form N-acetylbenzoquinoneimine, a highly reactive intermediate. This metabolite reacts with sulfohydryl groups in proteins and glutathione. (See Figure 6).

When hepatic glutathione is depleted, after massive single doses, the excess reactive arylating intermediate covalently binds to vital hepatocellular macromolecules, leading to necrosis (Flower et al, 1985). The continuing problem of acute hepatic necrosis following paracetamol overdose has been reviewed (Barwick et al, 1976; Fernandez and Brito, 1977; Boyer and Rouff, 1971;

Byer and Traylor, 1982 and Rosenberg and Neelon, 1978). Children have hitherto been considered to be relatively resistant to the hepatotoxic effect of paracetamol and severe liver damage was thought to be very uncommon. One possible explanation is that its metabolism in children is different, with a greater proportion being converted to the sulphate conjugate (Prescott, 1980).

Severe hepatic damage was likely if the patient had vomited or had a stomach washout within six hours of paracetamol overdose (Gazzard et al, 1977). A toxic reaction of paracetamol on a patient's bone marrow has been reported by Mackinnoon and Menon (1974).

Intravenous N-acetylcysteine is now considered the treatment of choice for paracetamol poisoning. It is more effective than intravenous cysteamine or methionine in preventing liverr damage. It also prevents renal failure and death when given within ten hours of ingestion (Maurer and Zeisler, 1978; Jacobsen and Wiik-Larsen, 1981). Prescott and Mathew (1974) reported that cysteamine is the treatment of choice for paracetamol poisoning, but the safety of cysteamine in man is yet to be established.

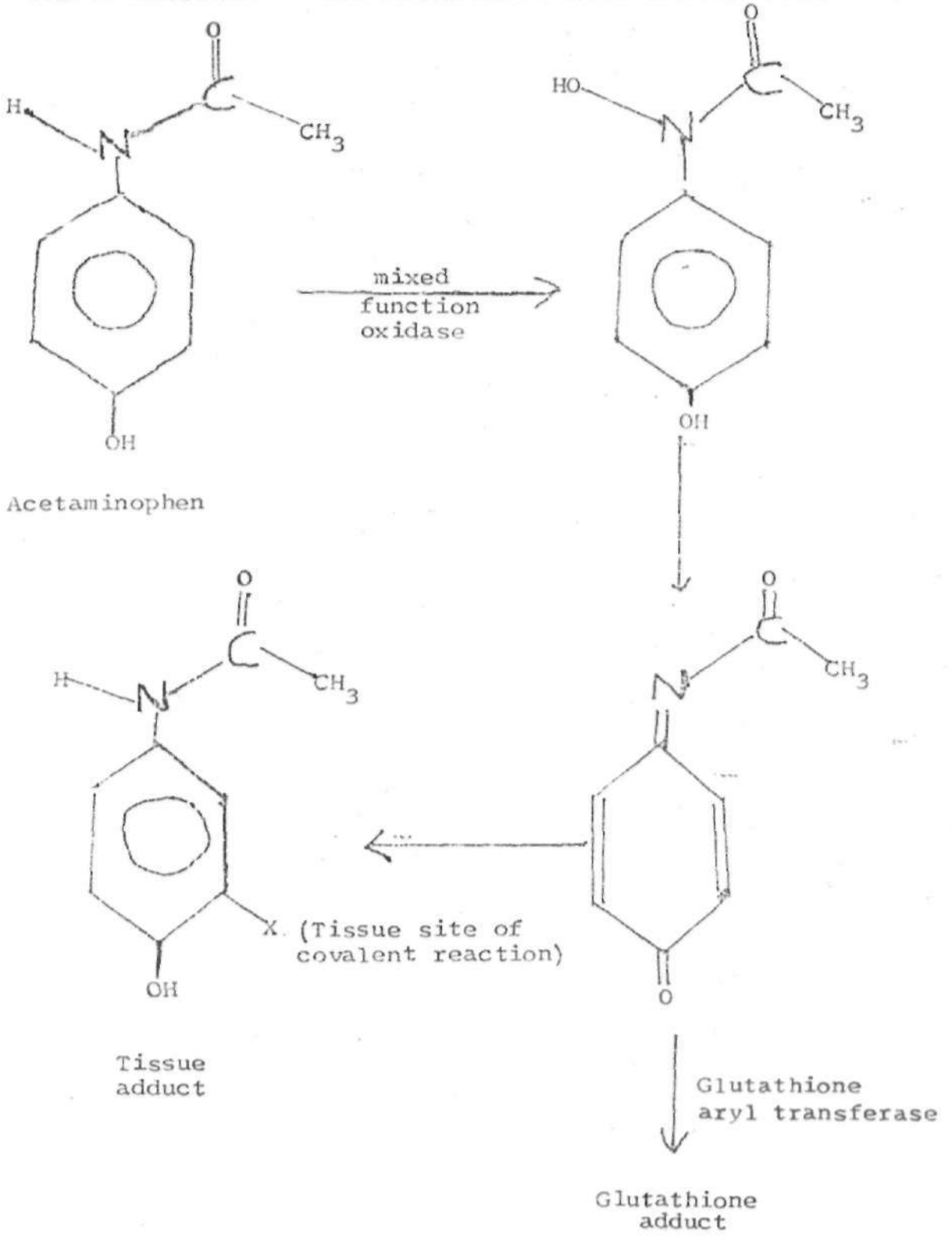


Fig. 6: Formation of Reactive Intermediates During Metabolism of Environmental Substances and Drugs (From Benet and Sheiner, 1985).

From studies in patients with paracetamol overdose it was found that severe hepatic lesions occurred when the plasma paracetamol concentrations reached more than 300 µg per ml after four hours; none occurred in patients with concentrations of less than 120 µg per ml. There was no evidence of liver damage in 11 or 12 patients with plasma concentrations of less than 50 µg per ml at 12 hours, but hepatic necrosis developed in 16 of 18 with concentrations greater than 50 µg (Prescott et al, 1971).

Symptoms of overdose include vomiting, gastrointestinal haemorrhage, liver damage, cerebral oedema and renal tubular necrosis. Hyperglycaemia and hypoglycaemia have been reported after overdose with paracetamol and hypoglycaemia has followed a therapeutic dose in a child. Liver necrosis may be fatal several days after overdose (Martindale, 1982).

2.5 Assay Methods

While the need to determine potency may almost always be ultimately related to health services, the immediate purposes of particular assays may vary greatly. The type and design of assay procedure employed vary according to the concentration and the medium of analysis.

Several methods for the quantitative determination of paracetamol as such and in pharmaceutical preparations are available. Most of them are colrimetric and require the hydrolysis of paracetamol to p-aminophenol. The earliest colorimetric assay of paracetamol in plasma was proposed by Brodie and Axelrod (1949). After acid hydrolysis, the formed p-aminophenol is diazotized and coupled to alpha-naphthol, and the azo dye is measured by spectrophotometer. The method is non-specific and time consuming (Wallace et al., 1973). Other procedures utilise the reaction of p-aminophenol with $NaNO_2$-HCl to form the coloured 2-nitro-p-aminophenol (Chambers and Jones, 1976; Glynn and Kendal, 1975; Wiener, 1977) and with Vanillin to a stable imine with an absorbance maximum at 395 nm (Plankogiannis and Saad, 1978). These procedures resulted in good selectivity for paracetamol. They found no

interference from the sulphate and glucuronide conjugates of paracetamol.

P-aminophenol can also be coupled with phenol in the presence of hypobromite to form an indophenol dye (Welch and Conney, 1965) which may be interfered with by ascorbic acid (Swale, 1977).

Chafetz et al (1971) described a simple and selective colorimetric assay for paracetamol and its tablet preparations in which the drug is measured as 2-nitro-4-acetamidophenol after reaction with nitrous acid. Daly et al. (1972) adapted the method of Chafetz et al. (1971), using an automated assay procedure. The method gave an excellent recovery data with commercial paracetamol formulations. Inamdar and Kaji (1969), working separately, dissolved paracetamol in methanol and treated it with hydrochloric acid and sodium nitrite to obtain a stable yellowish derivative. The method gave satisfactory results and it works with all usual dosage forms of paracetamol, in usual combinations.

Prescott (1971) reported a sensitive and specific method for the estimation of phenacetin and paracetamol in plasma and urine by a G.L.C. technique using trimethylsilyl derivatives. The disadvantages of this method are that it has a

significant time requisite and absolute dryness is required to achieve an effective silanizing reaction (Hosoya and Oka, 1969; Wilkson and Way, 1969).

Gotelli et al. (1977) reported a reverse phase H.P.L.C. assay of paracetamol with U.V. detection at 254 nm; it requires only 0.1ml plasma with a sensitivity of 0.5 µg paracetamol per ml and analysis time of 5 minutes. The extraction technique is rapid and includes acetoacetanilde as the internal standard. Of 36 other drugs tested, only theophylline interfered with the determination of paracetamol. Horwitz and Jatlow (1977) also employed an octadecyl silica reverse phase column. Horwitz and Jatlow (1977) included an ether extraction step and reported no assay interference by theophylline. This may be due to the ether extraction step rather than the ethyl acetate used by Gotelli et al. (1977).

The recovery of paracetamol was 65% with ether and above 80% with ethyl acetate. Addition of silylation of the octadecyl silica phase in order to mask free Si-OH functions improves the separation of paracetamol and its metabolites from endogenous substances in urine (Knox and Jurand, 1977).

Blair and Rumack (1977) described a microscale H.P.L.C. assay for paracetamol on a cation exchange column requiring only 1.5 µl plasma. The retention times of paracetamol and its internal standard, N-butyryl- p- aminophenol, were 30 and 50 minutes respectively, a circumstance that limits the applicability of this assay (Sadee and Beelen, 1980).

2.6 Objectives and Scope of the Study

The influx of imitation drugs into our country has been reported (Pharmanews, 1983). Analysis by some pharmaceutical companies showed that most of these drugs contained lower and in a few cases, higher percentage of the active ingredients. Some were found to contain no active ingredients at all.

In some cases, there is virtually nothing to distinguish an imitation drug from the original product as both brand names and packs are found to be similar in all respects. An example is the imitation of ICI's 'Fetmosol' antiseptic soap which did not contain the active ingredient, monosulfiram. Furthermore, some products found in the market, have names like "Telamycin",

"Terrimycin" and "Teramycin", all of which resemble Pfizer's "Terramycin". The company's VIBRAMYCIN has also been copied as "Neora-vibramycin". Imitation drugs such as "Ketras", "kentax" and "Tetras" have been designed to simulate ICI's broad spectrum antihelmintic "Ketrax".

The manufacturer's have been unable to take any effective action against such widespread drug imitations because of their inability to trace the sources. It is believed that most of them are imported into the country from Asian and European countries in collaboration with some local businessmen.

Due to variations in the manufacturing processes from different manufacturers, different brands of the same drug may not contain the same amount of active ingredient, although they may all meet other official requirement. The overall effect is that a patient changing from one brand to another may not obtain the desired effect at the required time.

In order to exert a required pharmacological effect, a drug must be absorbed and at a rate and to an extent that will produce adequate drug concentrations at the site of action.

It has now become apparent however, that the assurance of the pharmaceutical equivalence does not always ensure equivalent biological performance of drug products. Thus identical dosage forms containing equal amounts of active ingredients and conforming to official standards may not provide active drug to the body in an identical fashion. Similarly drug products that are pharmaceutical equivalents may not be "bioequivalent drug products". The major cause of differences in absorption of drug from various formulations is in the dissolution rate profile.

Considerable efforts have been expended on the development of *in vitro* method capable of predicting *in vivo* bioavailability of drug products. To date, no one has developed a single system or model that can universally permit such predictions. Good correlations of *in vitro* dissolution data with *in vivo* bioavailability data may permit the establishment of bioavailalbility standard for the drug product based on *in vitro* testing.

The abuse of the analgesic-antipyretic drug, paracetamol may lead to hepatic necrosis. This danger coupled with the drugs widespread use and increasing importance due to the recent dangers attributed to the use of acetylsalicylic

acid, necessitates specific and rapid detection of the compound in biological specimens.

This study is designed to investigate the presence of and the percentage of the active ingredients in thirteen brands of paracetamol tablets and five brands of paracetamol syrup. The release rates of the thirteen lots of paracetamol tablets will be examined by means of USPXVIII Rotating basket method. The work also intends to carry out *in vivo* bioavailability and pharmacokinetic studies of some brands of the paracetamol tablets in serum and saliva using healthy human subjects. The *in vivo* data will be correlated with the *in vitro* release rate of these tablets.

The study will employ a quantitative U.V. spectrophotometric technique for determining paracetamol in tablets, U.S.P. (1980) method for determining paracetamol in syrup and an adaptation of a simple, rapid and selective colorimetric estimation of paracetamol in tablets, serum and saliva.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Drugs

Paracetamol B.P. 500mg per tablet.

Compound	Manufacturer	Batch No.	Designation
Calpol	Wellcome Nigeria Limited, Oba Akran Avenue, Ikeja, Lagos	UN/783/A	A1
Painax	CIBA-GEIGY, S.A, Barcelona, Spain, for Swiss Nigeria Chemical Company, Lagos, Nigeria	X005	A2
Panadol	Winpharm [P] Sterling-Winthrop House, Onslow Street, Guildford, Surrey.	PL0071/5074	A3
Mycetamol	Lek Yugoslavia for C.O.A (Pharmaceutical Division), Lagos, Nigeria	076872/4	A4

Compound	Manufacturer	Batch No.	Designation
Paramol	Biode Pharmaceutical Industries Limited, Lagos, Nigeria	L28B	A5
Acamol	Ikapharm, Dizengoff, W.A., Nigeria Limited,	011862	A6
Remidol	Ranbaxy (India) MF, Ranbaxy Montari Nigeria Limited	45485	A7
Anagol	Barewa Pharmaceutical Limited Olugbo Close, Shasha Road, Akowonjo, Dopemu, Lagos Nigeria	00368	A8
Paracetamol B.P.	Southshourne Ltd., 155 Praed Street, London W2 1RL	0401/506	A9
Paracetamol B.P.	Harris Pharmaceutical Ltd., London	555	A10
Paracetamol B.P.	Helm Pharmaceuticals, G.M.B.H Hamburg, W. Germany	5521084	A11

Compound	Manufacturer	Batch No.	Designation
Paracetamol B.P.	H. N. Morton and Co., Ltd, London	41241	A12
Paracetamol B.P.	The Wallis Laboratory, 11 Camford Way, Luton LU3 3AN	50668	A13
	Paracetamol Syrup		
Paradol	Willinger Pharmacy and Gen. Enterprises (Nig) Ltd. 46 Yoruba Road, Sabon Gari, Kano	0137	S1
Nycetamol	Houry International Commodities Nigeria	859113	S2
Pancemol	Embechem Limited, Ikeja Lagos, Nigeria	11270	S3
Remidol'	Ranbaxy Montari Nig. Ltd. Sango-Otta, Nigeria	3585	S4
Calpol	Wellcome Nig. Ltd., Oba Akran, Avenue, Ikeja, Lagos	UN/285/B	S5

' 5 ml of Remidol syrup = 31.25mg/ml all others = 24 mg/ml

3.2 Equipment

SP8 - 100 Ultraviolet spectrophotometer (PYE-UNICAM LTD, York Street, Cambridge, England CB1 2PX)

ERWEKA dissolution apparatus and accessories, Erweka Appatebau G.M.B.H. 6056. Heusenstamm Kr. Offenbuch - Main

Analytical balance, Type H35 AR Mettler. Gallenkamp

Silica Cuvettes B.S. 3875.3 10 mm F.O.

Thermometer - 31/3 Quickfit

Reagents

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

Sodium Hydroxide pellets - (BDH Chemicals, Poole, England).

10% Sodium Nitrite - (May and Baker Ltd., Dagenham, England)

15% Sulphamic acid - (BDH Chemicals Ltd., Poole, England

10% Trichloroacetic acid - (BDH Chemicals Ltd., Poole England)

Chloroform - Analar grade (BDH Chemicals Ltd. Poole, England)

Diethylether - Analar grade (May and Baker Ltd., Dagenham, England)

Sodium Hydrogen carbonate - (G.P.R., Hopkin & Williams, Chadwell Heath, Essex, England).

Sodium Carbonate - (G.P.R., Hopkin & William, Chadwell Heath, Essex, England)

Methanol - (G.P.R., Hopkin & William, Chadwell Heath, Essex, England).

3.3 a.1 Chemical Assay (Spectrophotometric method)

Twenty paracetamol tablets were accurately weighed and the average weight determined . They were powdered and a quantity of the powder equivalent to 0.15g of paracetamol was weighed and added to 50ml of 0.1M NaOH in a 200ml flat bottom flask, diluted with 100ml of water, and shaken for 15 minutes. Sufficient water was added to produce 200ml. The solution was filtered, 10ml of the filtrate was diluted to 100ml with water. 10ml of the resulting solution was added to 10ml of 0.1M NaOH, diluted to 100ml with water and the absorbance of the resulting solution measured at

the maximum at about 257 nm. The percent content of paracetamol was calculated taking 715 as the value of A(1%, 1cm) at the maximum at about 257 nm (B.P., 1980). Formula:

$$\frac{\text{Absorbance}}{\text{Weight of the drug in g X A}} \text{ X Dilution Factor}$$

A (1 per cent, 1 cm) = A/cd

where A (1 per cent, 1 cm) is the absorbance of a 1 cm layer of a 1% w/v solution (1 g in 100 ml) of the absorbing solute. It is also called the specific absorbance; c is the concentration of the absorbing solute expressed as %w/v and d is the thickness of the absorbing layer in centimeter.

3.3a.2 Chemical assay (Colorimetric method)

Colorimetric determination of paracetamol was carried out using the method of Glynn and Kendal (1975) except that the 10% trichloroacetic acid was not used. 0.1g equivalent of the powdered drug was dissolved in 50ml of water in a 200ml flat-bottom flask; sufficient distilled water was added to produce 200ml. 1ml of the resulting solution was added to 1ml 6N hydrochloric acid and 2ml of 10% sodium nitrite in a 25-ml test tube.

After allowing the contents to stand for 2 minutes, 2ml of 15% sulfamic acid was added carefully, followed by 5ml 10% NaOH. The ensuing yellow colour was measured at 430 nm against a reagent blank of water. The percent content of paracetamol was calculated by the following formula:

$$\% \text{ paracetamol} = \frac{\text{Conc. of sample x Dilution factor}}{\text{weight of the sample}} \times \frac{100}{1}$$

Calibration Curves

100mg of paracetamol powder was weighed and dissolved in water, transferred into a 100-ml volumetric flask, and diluted to volume with distilled water to obtain standard paracetamol solution of 100 µg/ml. 1.0, 2.0, 4.0, 5.0, 6.0 and 7.0 ml of the standard solution were transferred to series of 10-ml volumetric flasks, enough distilled water was added to volume. Solutions of various concentrations (100 - 700 µg) were obtained, and treated as mentioned in the assay. The ensuing yellow colour was measured at 430 nm against a reagent blank of water. The absorbances obtained were plotted against the respective concentrations.

3.3.a.3 Syrup Assay

An accurately measured volume of paracetamol syrup, equivalent to 250mg of paracetamol was transferred into a 250-ml volumetric flask. 2ml of 1N NaOH was added, diluted with water to volume and mixed. 2ml of this solution was transfered into a 100-ml beaker, 1 drop of concentrated HCl was added, swirled to mix, and 3.0g of purified siliceous earth was then added mixed and transferred to chromatographic column. The beaker was scrubbed with 1g of purified siliceous earth, mixed with 2 drops of water; the washings were transferred to the column, and tapped gently. A pledget of fine glass wool was placed on top of the column. The column was washed with 100ml of water-saturated chloroform, and the eluate with 150 ml of water-saturated ether, and collected in 400-ml beaker. The ether was evaporated on a steam bath with the aid of a current of air just to dryness. The residue was dissolved in a solvent mixture consisting of 1ml of dilute hydrochloric acid per 100ml of methanol, and transferred to a 50-ml volumetric flask. The beaker was rinsed with the solvent mixture, the rinsings were added to the flask, diluted with the sovent mixture to volume, and mixed. 10.0ml of the resulting solution was transferred into another 50-ml volumetric flask and diluted with the solvent mixture to volume. The absorbance was determined in 1-cm cell at the wavelength

of maximum absorbance at about 249 nm, using a solvent mixture consisting of 1ml of 0.1N hydrochloric acid per 100ml of methanol as blank. The percent content of paracetamol was calculated by the formula:

$$\frac{\text{Concentration of sample (mg) X Dilution Factor}}{250\text{mg}} \times \frac{100}{1}$$

Calibration Curve

50 mg of paracetamol powder was accurately weighed, and dissolved in 100ml of distilled water in 100ml volumetric flask. Thus, the standard solution contains 500 µg/ml. 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 ml of the standard solution were transferred to series of 100-ml volumetric flasks, sufficient solvent mixture (1ml of dilute HCl per 100ml of methanol) was added to volume. Solutions of various concentrations (5 to 30 µg) were obtained. The absorbance was determined in 1cm cell at the wavelength of maximum absorbance at about 249 nm using a solvent mixture consisting of 1ml of 0.1M hydrochloric acid per 100ml of methanol as blank. The graph of absorbance versus concentration was plotted.

3.4 *In Vitro* Experimental

The USP XIX basket method is described for the dissolution rate tests. The apparatus consists of a cylindrical, flat bottom glass vessel with an internal diameter of 100mm, a flanged upper rim, and a capacity of 1000ml; a variable speed motor; a cylindrical stainless steel basket assembly made of woven wire cloth, with a nominal aperture size of 425 micrometer; joined down one side with a narrow welded seam and with a flanged rim at each end. The variable speed motor causes the basket to rotate in the vessel. A speed of 100 revolutions per minute is used. The motor drives the basket by means of a shaft which is approximately 6 mm in dimeter and about 300 mm long in such a way that the basket revolves smoothly and without perceptible wobble.

The shaft passes through the vessel and opening in the lid of the vessel and is positioned so that its axis is not more than 2mm from the vertical axis of the vessel. It carries at its lower end a stainless steel disc of 25mm in diameter in which there is a 2mm vent.

The vessel is securely clamped in a water bath in such a way that displacement vibration from other equipment including water circulating device is minimised. The water bath is set to

maintain the temperature of the dissolution medium in the glass vessel at 37±0.5°C. The assembled apparatus is shown in Figure 7.

Procedure

One litre of 0.1M hydrochloric acid, as the dissolution medium, was introduced into the vessel and immersed in a constant temperature water bath, maintained at 37±0.5°C. For each test, one tablet was placed in the basket. When the required tempearture was attained, the apparatus was assembled and the distance between the bottom of the basket and the interval surface of the vessel adjusted to between 18 and 22 mm. The motor was started at a rotational speed of 100 revolutions per minute. A sample of 5ml of the dissolution medium was withdrawn at 1, 5, 10, 20, 40 and 60 minutes. Samples were withdrawn from a point half way between the basket wall and the wall of the vessel, level with the mid-point of the side of the basket, to ensure that the normal pattern of flow of the dissolution medium was not disturbed. An equivalent volume (5ml) of the dissolution medium previously maintained at 37±0.5oC was replaced into the dissolution medium. The dissolution run was performed five times. Samples of the dissolved drug were filtered,

diluted to 50ml and assayed by reference to a standard calibration curve. The absorbance of each diluted sample was measured at a maximum of 257 nm.

Cumulative concentration at a given time was calculated using the formula of Wurster and Taylor (1965).

$$C_n = C_{measured} + \frac{x}{1000} \cdot \sum_{s=1}^{n=1} (C_{measured})$$

$C_{measured}$ denotes the spectrophotometrically measured concentration.

Cn denotes the concentration of nth sampling expected in the medium if previous samples had not been removed.

x is the volume of the sample removed at each time withdrawal was made.

1000 is the volume of the dissolution medium in millilitres.

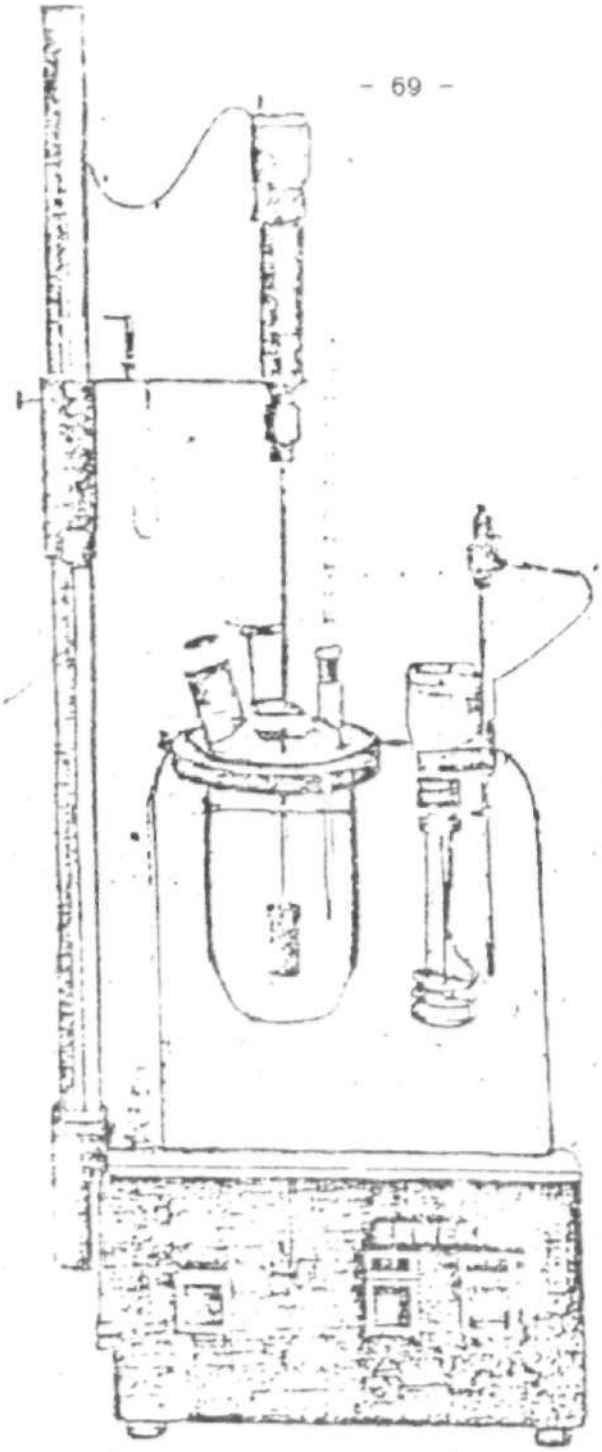


Fig. 9. Assembled apparatus for determination of dissolution rate.
(From USP XVIII)

Calibration Curve

50mg of paracetamol powder was accurately weighed, and dissolved in 100ml of 0.1M hydrochloric acid in a 100-ml volumetric flask. Thus, the standard solution contains 500 µg/ml. 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 ml of the standard solution were transferred into series of 100-ml volumetric flasks; enough hydrochloric acid was added to volume. Solutions of various concentrations (5 - 30 µg) were obtained. Absorbances were measured at 257 nm in 1-cm cell using 0.1M hydrochloric acid as blank. Graph of absorbance versus concentration was plotted.

3.5 Disintegration Tests

Apparatus: As described in B.P.(1980). See Appendix 11.

Procedure: One tablet each was placed in the tubes of the basket; a disc was added to each tube. The assembly was suspended in the beaker containing distilled water maintained at 37±0.5°C and the apparatus was operated for fifteen minutes.

3.6 *In Vivo* Absorption Studies of Paracetamol In Human Volunteers

Subjects:

Normal healthy adult volunteers, 24 - 30 years old participated in the study.

Drugs:

Calpol (Wellcome Nigeria Ltd.) Batch No. UN/783A

Remidol (Ranbaxy Acutari Nigeria Ltd.) Batch No. 45485

Painax (CIBA-GEIGY S.A.; Barcelona, Spain for Swiss Nigeria Chemical Company Batch No. X005

Protocol

See Tables 1 and 2

Eight normal healthy adult volunteers (7 males and1 female) took part in the study. The protocol of the study was explained to each of them and an informed consent was obtained. No drugs including alcohol, was allowed a few days before the study and each subject fasted overnight before taking part on the following day. None of the volunteers smoked cigarettes.

The study was a randomised cross-over double blind one with a wash-out period of at least one week between attendances by any individual. Four of the subjects received oral solutions of the tablets and the other four the solid dosage forms of the same tablets. On the day of each test, prior to oral ingestion of the 2X500mg tablets each volunteer attended the trial room and was allowed a 5-minutes relaxation on a couch. A cannula was placed in an antecubital vein and 5 ml of blood taken (0 minute). A 2-ml blank saliva sample was also collected after stimulation with a rubber band which was chewed for a few minutes.

Further blood sampling via the indwelling cannula was carried out at 5, 10, 15, 20, 30, 60, 90, 120, 180 and 240 minutes after ingestion of the tablets. Stimulated saliva samples were also collected at the specified time. All volunteers remained on the couch and no food or other drink was allowed during the four hours of the study. Blood was collected into plain glass tubes and allowed to clot. Serum was obtained by centrifuging for 15 minutes. Using Pasteur pippetes, the serum was transferred into bijoux bottles, stoppered and stored alongside the saliva samples which were collected in plain plastic

containers in a deep freezer at -20°C until analysed.

Analysis

The method of Glynn and Kendal (1975) which is specific for paracetamol without interference from the sulphate and glucuronide conjugates of paracetamol was adopted. 1ml of the sample was added to a 25 ml stoppered centrifuge tube containing 2 ml 10 % trichloroacetic acid. After thorough mixing, the tube was centrifuged briefly and the clear supernatant decanted into a 25 ml test tube containing 1 ml 6N hydrochloric acid and 2 ml 10% sodium nitrite. After allowing the contents to stand for 2 minutes, 2 ml of 15% sulphamic acid was added carefully, followed by 5 ml 10% NaOH. The ensuing yellow colour was measured at 430 nm against a reagent blank of water. The absorbances of the control serum and saliva (obtained prior to administration of the tablets) were substracted from the absorbances of the serum and saliva specimens obtained subsequently. The samples were assayed by reference to standard calibration curves.

Calibration Curves

Serum

5mg of paracetamol powder was weighed and dissolved in distilled water, transferred into a 100-ml volumetric flask and made to volume with distilled water. A standard paracetamol solution of 50 μg per ml was obtained. 1.0, 2.0, 3.0, 5.0 and 6.0 ml of the standard solution were transferred into a series of 10-ml volumetric flasks. 1ml blank serum was added to each flask and diluted to volume with distilled water. Solutions of various concentrations (5 - 30 μg) were thus obtained.

Saliva

5mg of paracetamol powder were weighed and dissolved in distilled water, transferred into a 100-ml volumetric flask and made to volume with distilled water. A standard solution of 50 μg per ml was obtained. 1.0, 2.0, 3.0, 4.0 and 6.0 ml of the standard paracetamol solution were transferred into a series of 10-ml volumetric flasks. 1ml blank saliva was added to each flask, and made to volume with distilled water. Solutions of various concentrations (5 - 30 μg) were thus obtained.

All samples were treated as mentioned in the colorimetric method assay procedure. The absorbances were measured at 430 nm in 1-cm cells against a reagent blank of water. Graphs of absorbance versus concentration were plotted.

3.7 Results

The results of the various studies were carefully recorded. Tables were drawn where appropriate as were graphical representations. The data were statistically analysed where appropriate. Student's paired t test was used for all the data except for those from dissolution studies where one-way analysis of variance was found to be more suitable. P values greater than 0.05 was considered to be statistically significant.

Table 1

Randomized Scheme For Oral Administration of Paracetamol Solution (1g)

	WEEK		
Subject	1	2	3
I	Calpol	Painax	Remidol
II	Painax	Remidol	Calpol
III	Remidol	Calpol	Painax
IV	Painax	Remidol	Calpol

Table 2

Randomized Schem For Oral

Administration of Paracetamol

Tablet (1g)

	WEEK		
Subject	1	2	3
I	Painax	Calpol	Remidol
II	Calpol	Remidol	Painax
III	Remidol	Painax	Calpol
IV	Painax	Remidol	Calpol

CHAPTER FOUR

RESUTS

4.1a Chemical Assay Of Paracetamol Tablets

Linear calibration curve with good correlation coefficient ($r = 0.999$) of paracetamol is shown in Figure 8. All the preparations investigated contained between 99.15 and 101 per cent of the active ingredient. Thus, the different brands of paracetamol tablets used in this study have complied with the B.P. (1980) specification of drug content (95.0 - 105.0)%. It can also be seen that the results obtained with the two methods of analysis adapted for the purpose of comparison (Spectrophotometric and Colorimetric methods of assay) are very similar. Values for the drug content and percentage composition are given in table 3.

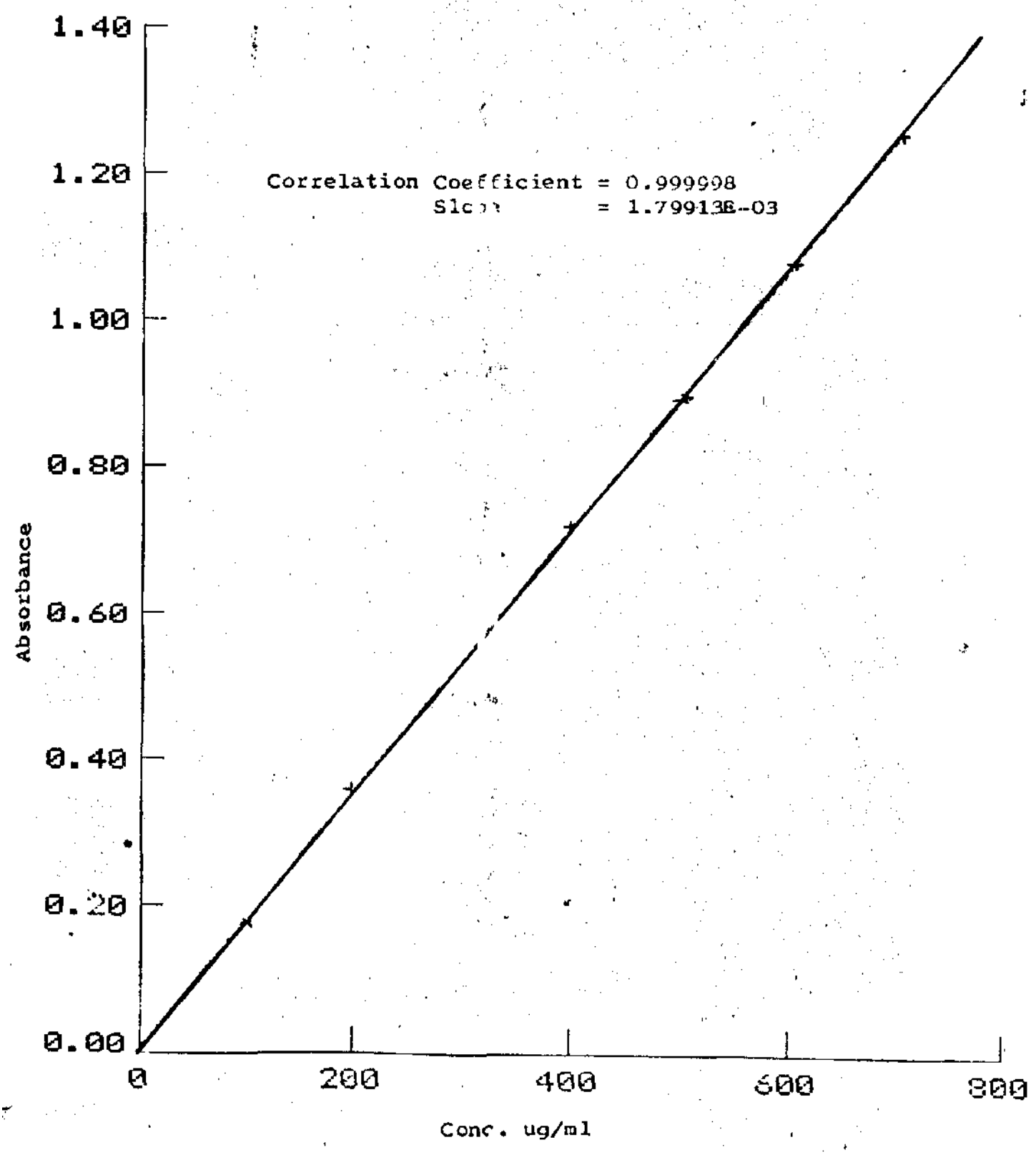


Fig. 7: Calibration curve for paracetamol in 10% sodium hydroxide solution using UV Spectrophotometer.

Table 3

QUANTITATIVE DETERMINATION OF PARACETAMOL TABLETS

(SPECTROPHOTOMETRIC AND COLORIMETRIC METHODS OF ANALYSIS)

COMPOUND	DRUG CONTENT		PERCENTAGE ± S. D.	
	Spectrophotometric method	Colorimetric method	Spectrophotometric method	Colorimetric method
A1	505,90	502,50	101,18 ± 0,24	100,50 ± 1,06
A2	502,40	501,35	100,48 ± 0,62	100,27 ± 0,47
A3	502,80	508,25	100,56 ± 0,47	101,65 ± 1,24
A4	509,15	499,95	101,83 ± 0,35	99,99 ± 0,58
A5	502,85	503,50	100,57 ± 0,81	100,70 ± 0,40
A6	504,00	501,20	100,80 ± 0,57	100,24 ± 0,11
A7	498,50	497,15	99,70 ± 0,14	99,43 ± 0,18
A8	498,15	497,20	99,63 ± 0,52	99,44 ± 0,54
A9	501,15	501,95	100,23 ± 0,52	100,59 ± 0,35
A10	508,90	503,85	101,78 ± 1,41	100,77 ± 0,47
A11	501,95	498,05	100,39 ± 0,55	99,61 ± 0,16
A12	499,45	496,75	99,89 ± 0,51	99,15 ± 0,58
A13	499,80	499,65	99,96 ± 0,24	99,93 ± 0,61

S.D. = Standard deviation

4.1b Chemical Assay Of Paracetamol Syrup

Linear calibration curve with good correlation coefficient (r = 0.999) of paracetamol is shown in Figure 9. Four brands (Paradol, Pancemol, Remidol and Calpol) out of the five different brands of paracetamol syrups used complied with the official USP (1980) specification for paracetamol elixir (95.0 - 105.0)%. Nycetamol syrup (S2) contains less than 95% of the labelled amount of paracetamol. See Table 4.

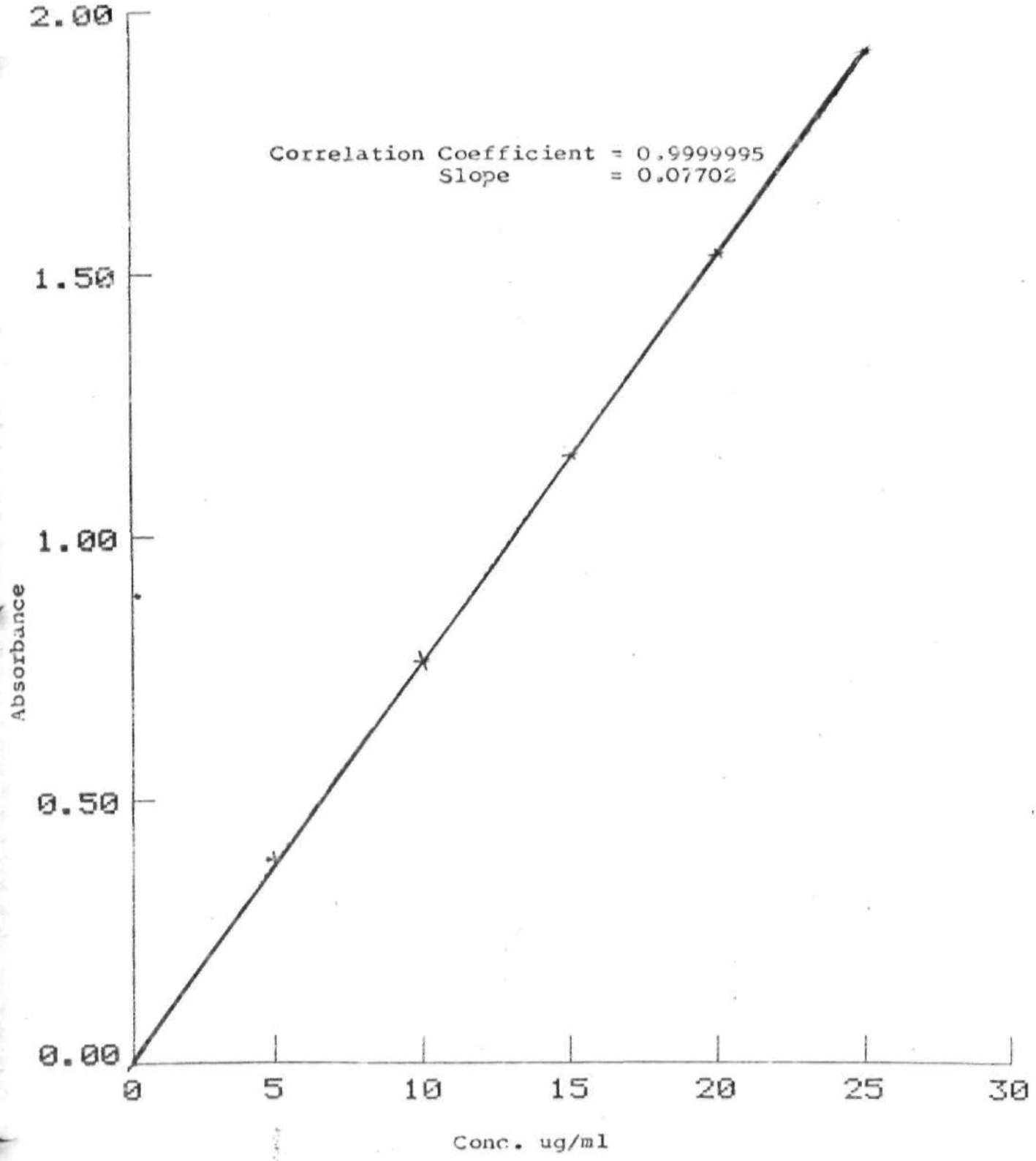


Fig. 8: Calibration curve for paracetamol in a solvent mixture (1ml of dilute HCl per 100ml of methanol) using UV Spectrophotometer.

TABLE 4

QUANTITATIVE DETERMINATION OF PARACETAMOL SYRUP

USING U.S.P. METHOD

Compound	pH Range	Drug Content (mg)	Percentage ± S.D.
S1	5,0	249,60	99,84 ± 0,37
S2	6,0	222,80	89,12 ± 0,56
S3	5,0	253,65	101,46 ± 0,30
S4	5,0	245,53	98,21 ± 0,35
S5	5,0	252,85	101,14 ± 0,15

S.D. = Standard deviation

4.2 Dissolution Rate Tests

Linear calibration curve of paracetamol is shown in Figure 10 (r = 0.999). The dissolution rate profiles of the different brands of paracetamol tablets in 0.1M HCl solution are given as the cumulative percentage of the active ingredients released from the drugs at various time intervals. (See Table 5). The tabulated values are the mean of five dissolution runs for each brand of paracetamol tablet. Figure 11 is the graphical representation of the dissolution rate profiles for the thirteen brands of paracetamol tablets in 0.1M HCl solution.

The dissolution half-life ($T_{50\%}$) expressed as the time required for 50% of the drug to be released into the dissolution medium was found to be less than three minutes for all the brands with the exception of Remidol (A7). The corresponding time required for this preparation was 21 minutes a period almost seven times longer than for the rest of the brands (See Table 6). More than 75% dissolution was achieved for all the brands in 40 minutes.

It can also be seen that for all the brands, the dissolution rate increased rapidly in the first 10 minutes becoming constant soon afterwards. Indeed, complete release of the active

ingredient was attained for all the brands in 10 minutes except A7 which released 28%. However the variations in the dissolution rates for all brands at the various times differed significantly ($P < 0.0001$). Complete dissolution was achieved for A7 only at 60 minutes.

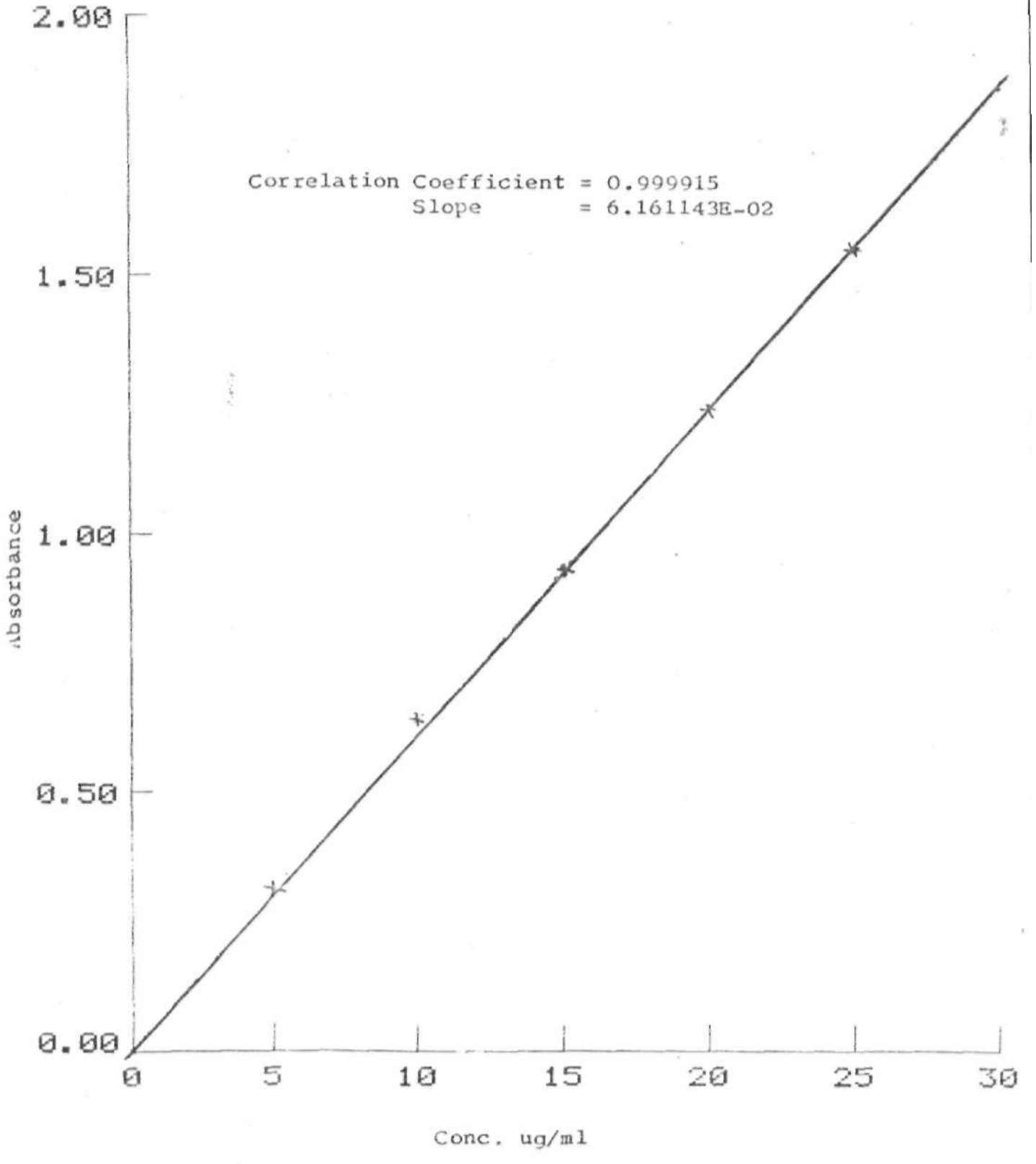


Fig. 10: Calibration curve for paracetamol in 0.1N HCl solution using UV Spectrophotometer.

TABLE 5

COMPARISON BETWEEN *IN VITRO* DISSOLUTION RELEASE RATES OF DIFFERENT BRANDS OF PARACETAMOL TABLETS IN 0,1M HCl MAINTAINED AT 37 ± 0,5°C USING USP XVIII ROTATING BASKET METHOD WITH A STIRRING RATE OF 100 r. p. m.

	Cumulative Percentage Released ± S. D.					
Compound	1 minute	5minutes	10 minutes	20 minutes	40 minutes	60 minutes
A1	46,00±2,73	100,28±2,83	100,38±0,37	99,29±0,25	98,78±0,50	98,26±2,59
A2	30,00±3,17	90,15±7,24	100,60±1,79	99,10±1,83	99,59±1,29	99,08±0,76
A3	45,00±1,95	93,22±2,58	100,89±2,82	100,19±0,42	99,69±0,25	99,48±0,27
A4	26,00±0,87	88,13±2,50	100,57±0,64	99,07±1,32	99,56±2,57	97,04±0,97
A5	42,00±1,03	86,21±0,38	96,64±2,05	97,52±3,23	97,90±3,39	98,18±3,96
A6	45,00±1,95	88,23±0,62	100,67±0,32	97,17±0,25	96,65±0,54	95,12±0,62
A7	5,00±0,70	10,03±0,87	28,00±4,49	48,52±0,73	82,06±0,72	100,87±0,32
A8	60,00±2,93	96,90±0,57	98,78±0,30	98,57±0,34	98,76±0,62	98,24±2,62
A9	30,00±1,67	76,75±6,48	99,53±3,50	101,03±2,85	99,59±1,64	98,02±1,53
A10	31,60±3,15	90,16±0,66	97,61±0,61	99,09±0,29	97,58±1,56	97,05±1,36
A11	56,00±3,41	97,28±0,39	100,77±1,35	100,27±3,77	99,76±1,32	99,55±1,36
A12	28,30±4,54	85,14±3,31	99,17±0,85	100,06±4,54	99,56±1,01	99,35±0,24
A13	36,00±3,54	90,20±5,40	99,60±2,10	100,10±0,29	99,60±2,10	99,10±0,47

S.D = Standard deviation

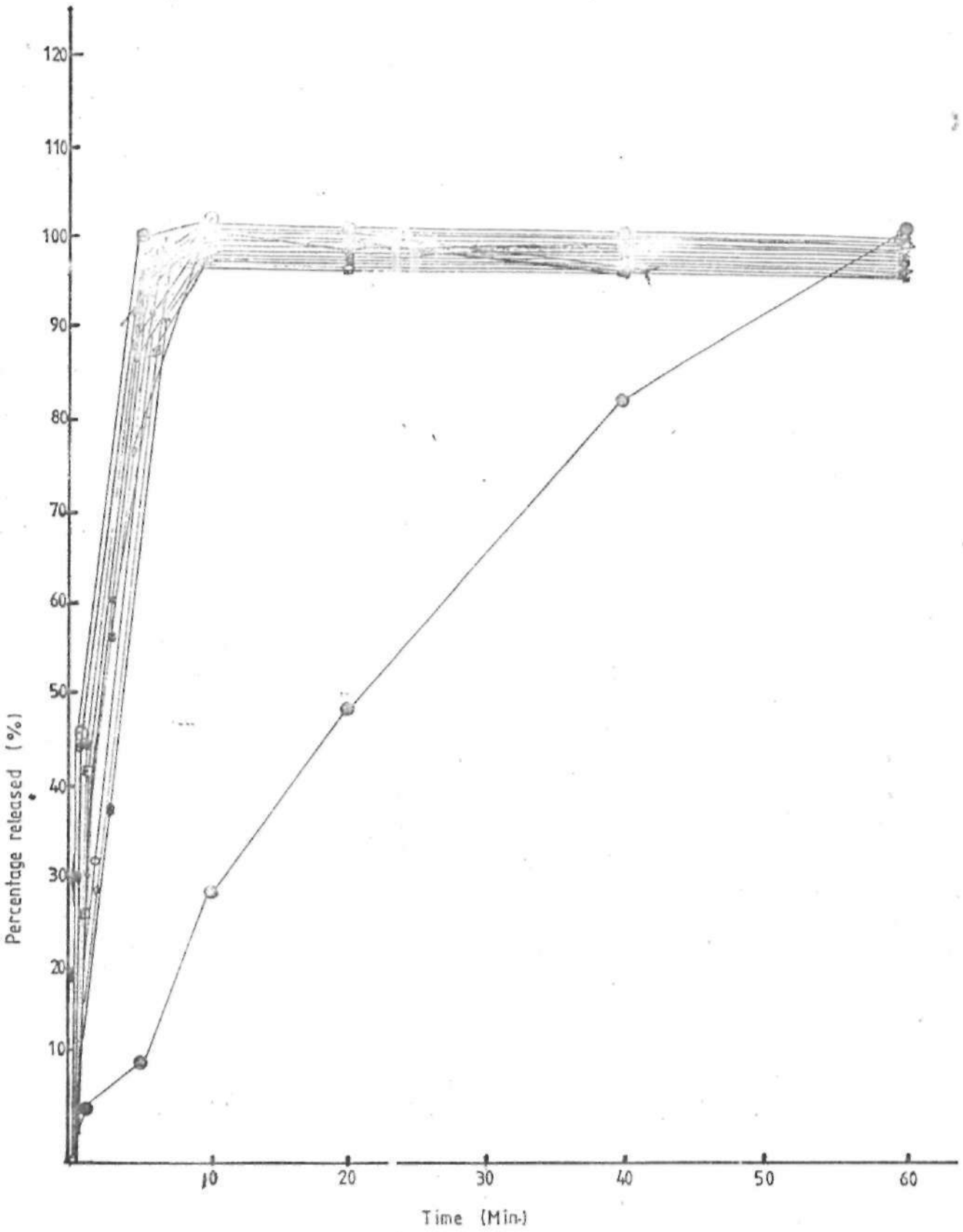


Fig. 11: Comparison between in-vitro dissolution rate profiles of paracetamol tables A_1 (⊘), A_2 (●), A_3 (⊖), A_4 (□), A_5 (⊟), A_6 (▣), A_7 (■), A_8 (×), A_9 (▷), A_{10} (⊕), A_{11} (⊗), A_{12} (◇), A_{13} (◆) in O.1M HCl using USP XVIII rotating basket method.

TABLE 6

DISSOLUTION HALF-LIFE ($T_{50\%}$) OF PARACETAMOL TABLETS IN 0.1M HCl

Compound	$T_{50\%}$ (Minutes)
A1	1,2
A2	2,5
A3	1,5
A4	2,8
A5	1,5
A6	2,0
A7	21,0
A8	1,0
A9	2,7
A10	2,2
A11	2,9
A12	1,0
A13	2,8

4.3 Disintegration Times

The times taken for samples of tablets of Calpol, Painax and Remidol to disintegrate in water maintained at 37±0.5°C are shown in table 7. It can be seen that it takes Calpol about as twice as long as Painax to disintegrate. Remidol, however, requires about twelve times the time required for Painax. Compared to the latter, both times are statistically significant ($P < 0.05$).

TABLE 7

DISINTEGRATION TIMES FOR PARACETAMOL TABLETS

Tablet No,	Disintegration Time (min)		
	A1	A2	A7
1	6,0	2,5	11,0
2	7,0	3,0	27,0
3	7,0	3,0	35,0
4	7,5	3,5	50,0
5	-	-	75,0
6	-	-	90,0
Mean ± S,D	6,90±0,63	3,0±0,41	48,0±29,9

S,D, = standard deviation

4.4 *In vivo* Absorption Studies

Linear calibration curves with good correlation coefficient (r = 0.999) of paracetamol are shown in Figures 12 and 13 for serum and saliva samples respectively. Following administration of 1g paracetamol tablets, mean peak serum concentrations (C_{max}) ranged from 20.0 to 21.96 µg/ml. The mean peak salivary concentrations (C_{max}) ranged from 20.92 to 24.13 µg/ml. Time to reach peak concentrations (t_{max}) ranged from 0.33 to 1.5h.

The mean recovery of paracetamol after oral administration of 1g dose is summarised in Tables 8 and 9 for serum and saliva samples respectively. The serum and saliva concentrations - time curves are shown in Figures 14 and 15.

Consistently higher concentrations of paracetamol in saliva relative to those in serum were observed during the first 60 minutes following oral ingestion. There was a good correlation between serum and saliva paracetamol levels (r = 0.82, 0.98; 0.94 , 0.95 and 0.76, 0.99 for the solution and solid dosage form of Calpol, Painax and Remidol respectively). However, the difference between the serum and salivary concentration did not reach a level of statistical significance except for painax at 5 minutes (P <

0.00023) and 15 minutes ($P < 0.00002$) and for Remidol at 5 minutes ($P < 0.0302$), 30 minutes ($P < 0.0344$ and at 90 minutes ($P < 0.0244$).

The pharmacokinetic parameters obtained from the mean concentration data for the three brands of paracetamol tablets in serum and saliva of healthy human subjects are shown in Tables 10 and 11 respectively. The similarities between the pharmacokinetic parameters for Calpol and Painax in both serum and saliva are obvious. However some of the parameters calculated for Remidol differ from those of the other two. Based on the parameters calculated using the serum concentration data, compared to Calpol, the AUC_{0-4hrs} for Remidol is statistically higher ($P < 0.000015$); the absorption half life is about four times longer ($P < 0.00005$) and time to peak is also about four times longer ($P < 0.05$).

A correlation between *in vitro* and *in vivo* data is demonstrated for two of the three brands of paracetamol (Painax and Calpol) by plotting the percent of the dose absorbed at 5, 10, and 15 minutes after drug administration versus the percent of paracetamol dissolved *in vitro* in one-third of the respective time period. ($r = 0.9793$ and 0.9999 respectively, see Figure 16a). Correlation of *in vivo* absorption and *in*

vitro dissolution data was demonstrated for paracetamol (Remidol) by plotting the percent of the dose absorbed at 5, 10, 15 and 20 minutes after drug administration versus the percent of paracetamol (Remidol) dissolved *in vitro* at the same time ($r = 0.9904$). Figure 16b is a graphical representation.

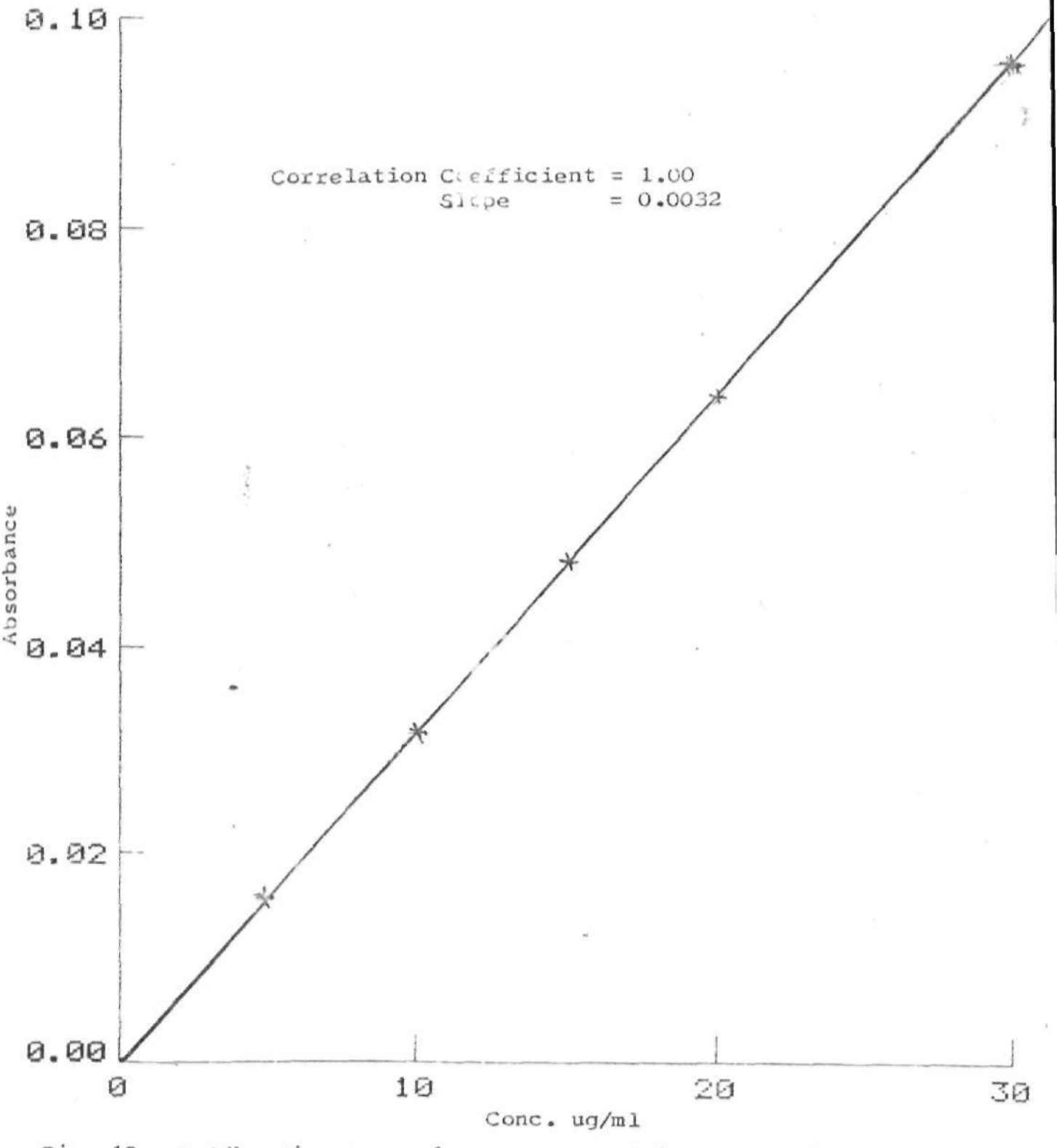


Fig. 12: Calibration curve for paracetamol in serum using UV Spectrophotometer.

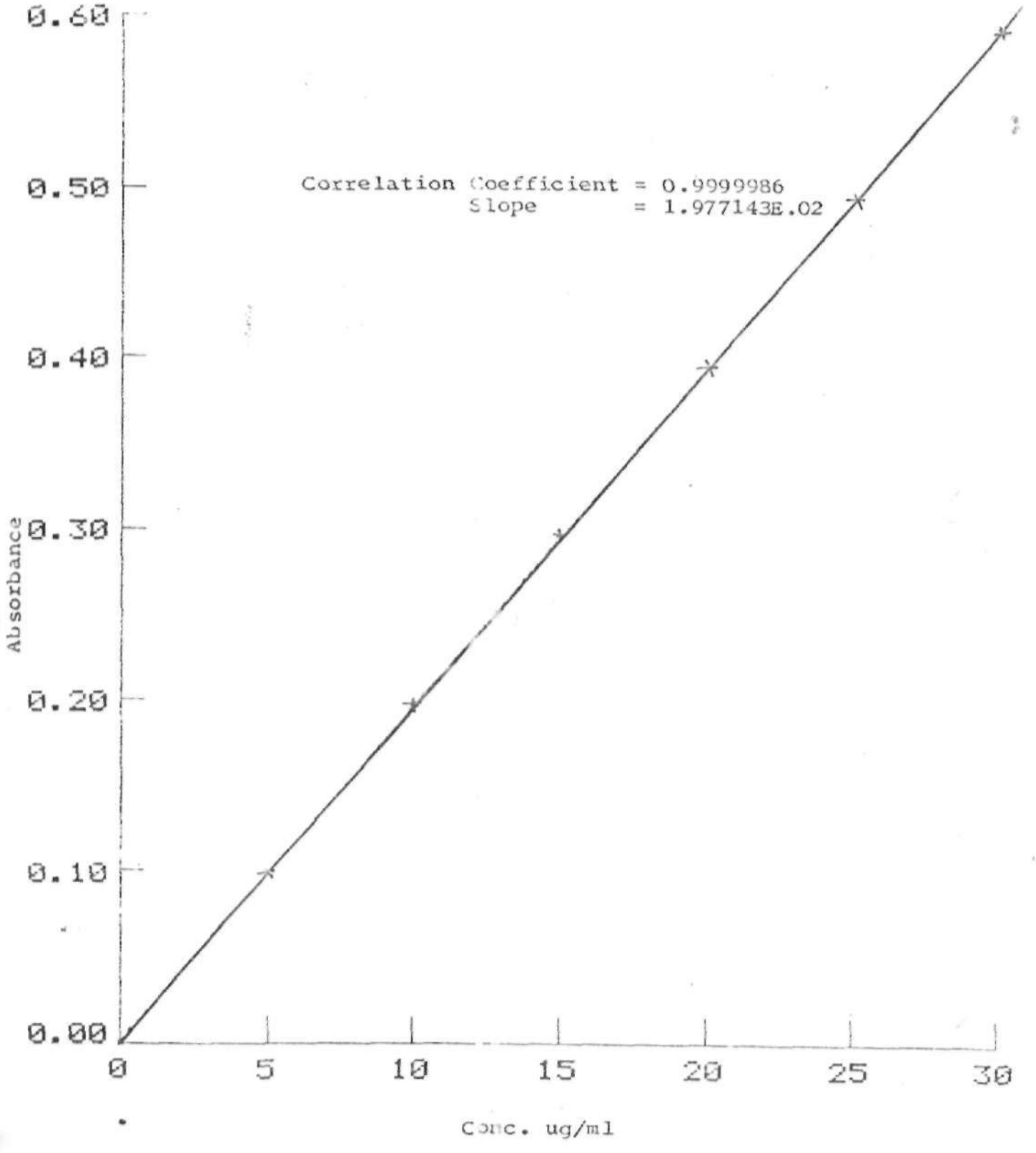


Fig. 13: Calibration curve for paracetamol in saliva using UV Spectrophotometer.

TABLE 8

MEAN SERUM PARACETAMOL CONCENTRATIONS IN HEALTHY SUBJECTS FOLLOWING ORAL ADMINISTRATION OF PROPRIETARY BRANDS (1g) IN SOLUTION AND TABLET FORMS

Time (Min)	CONCENTRATION (µg/ml) ± S.D.					
	Calpol		Painax		Remidol	
	Solution	Tablets	Solution	Tablets	Solution	Tablets
5	8,91±1,32	6,10±0,7[illegible]	7,97±0,41	6,10±0,91	2,43±0,53	2,10±0,57
10	12,50±1,51	11,30±1,3[illegible]	18,28±4,55	11,20±1,03	3,75±0,43	4,60±1,02
15	15,33±1,78	16,60±2,1[illegible]	21,17±2,17	15,20±1,07	7,03±0,74	7,30±1,03
20	21,96±1,43	20,80±1,38	18,67±1,21	21,00±1,04	9,14±0,60	9,50±1,04
30	18,83±1,28	18,80±1,37	16,72±1,16	18,90±1,03	12,50±2,10	11,20±1,07
60	16,88±1,22	14,60±1,36	14,39±0,68	14,80±1,03	18,13±2,08	19,00±1,07
90	13,83±2,94	13,30±1,16	11,17±0,69	13,20±1,03	21,25±2,58	20,90±0,78
120	11,25±3,09	11,60±1,37	10,86±0,69	11,70±1,03	15,32±3,58	17,70±0,99
180	10,63±2,86	9,10±0,9[illegible]	10,32±0,76	9,90±0,87	10,63±2,35	14,80±1,26
240	9,61±2,66	6,00±0,9[illegible]	10,16±0,69	6,50±1,38	10,39±2,19	10,70±1,26

S.D. = Standard deviation

TABLE 9

MEAN SALIVARY PARACETAMOL CONCENTRATIONS IN HEALTHY SUBJECTS FOLLOWING ORAL ADMINISTRATION OF PROPRIETARY BRANDS (1g) IN SOLUTION AND TABLET FORMS

Time (Min)	CONCENTRATION (µg/ml) ± S.D.					
	Calpol		Painax		Remidol	
	Solution	Tablets	Solution	Tablets	Solution	Tablets
5	14,23±0,78	6,13±0,09	11,46±2,60	9,60±0,97	4,39±3,33	3,31±3,31
10	18,11±0,14	10,29±1,23	18,91±2,98	11,75±1,21	10,27±	5,83±1,69
15	21,63±1,23	15,98±1,79	21,13±2,16	21,87±1,21	12,55±2,61	8,73±1,01
20	23,35±1,57	22,66±1,84	18,85±1,31	23,14±1,21	13,59±5,98	10,93±1,06
30	18,82±1,01	20,58±2,57	15,52±0,92	20,91±1,24	13,72±0,53	13,06±6,57
60	15,90±0,60	17,36±3,96	13,13±1,42	16,84±1,15	16,24±0,79	20,34±1,23
90	14,79±0,62	15,18±2,63	12,87±1,69	15,07±1,31	19,80±3,86	23,84±3,86
120	13,29±0,72	13,36±2,85	12,51±1,99	12,94±1,05	20,92±3,57	21,35±3,58
180	12,38±0,29	10,56±3,36	11,89±1,67	10,78±1,14	20,60±3,51	16,28±2,49
240	11,48±0,48	7,01±2,50	11,18±1,32	7,76±0,69	18,60±1,42	13,01±1,45

S.D. = Standard deviation

TABLE 10

PHARMACOKINETIC PARAMETERS OF PARACETAMOL DERIVED FROM THE MEAN SERUM CONCENTRATION DATA AFTER A SINGLE 1g ORAL DOSE.

Parameter	Compound		
	Calpol	Painax	Remidol
Lag Time (min)	3,00	2,10	4,00
Absorption half-life			
$t_{\frac{1}{2}\alpha}$ (hr)	0,09	0,11	0,36
Absorption rate constant			
K_a (hr^{-1})	7,70	6,30	1,93
Maximum Concentration			
C_{max} µg/ml	20,80	21,00	20,90
Time to Peak t_{max} (h)	0,33	0,33	1,50
Elimination Half-life			
$t_{\frac{1}{2}\beta}$ (h)	2,36	2,65	2,70
Elimination Rate Constant			
K_e (h^{-1})	0,292	0,26	0,26
Area Under the Curve			
AUC_{0-4h} (µg/ml/hr	46,20	47,30	59,38
$AUC_{0-\infty}$	66,60	72,30	101,08

TABLE II

PHARMACOKINETIC PARAMETERS OF PARACETAMOL DERIVED FROM THE MEAN SALIVARY CONCENTRATION DATA AFTER A SINGLE 1g ORAL DOSE

Parameter	Compound		
	Calpol	Painax	Remidol
Lag Time (min)	2,6[illegible]	3,70	5,10
Absorption Half-life			
$t_{1/2\alpha}$ (h)	0,0[illegible]7	0,065	0,34
Absorption Rate Constant			
K_a (h^{-1})	7,1[illegible]	10,66	2,04
Maximum Concentration			
C_{max} (µg/ml)	22,20	22,70	23,84
Time to Peak t_{max} (h)	0,3[illegible]	0,33	1,50
Elimination Half-life			
$t_{1/2\beta}$ (h)	2,35	2,72	2,56
Elimination Rate Constant			
K_e (h^{-1})	0,2[illegible]5	0,255	0,271
Area Under the Curve			
AUC_{0-4h} (µg/ml/hr	49,4[illegible]	52,50	64,60
$AUC_{0-\infty}$	69,8[illegible]	82,56	106,50

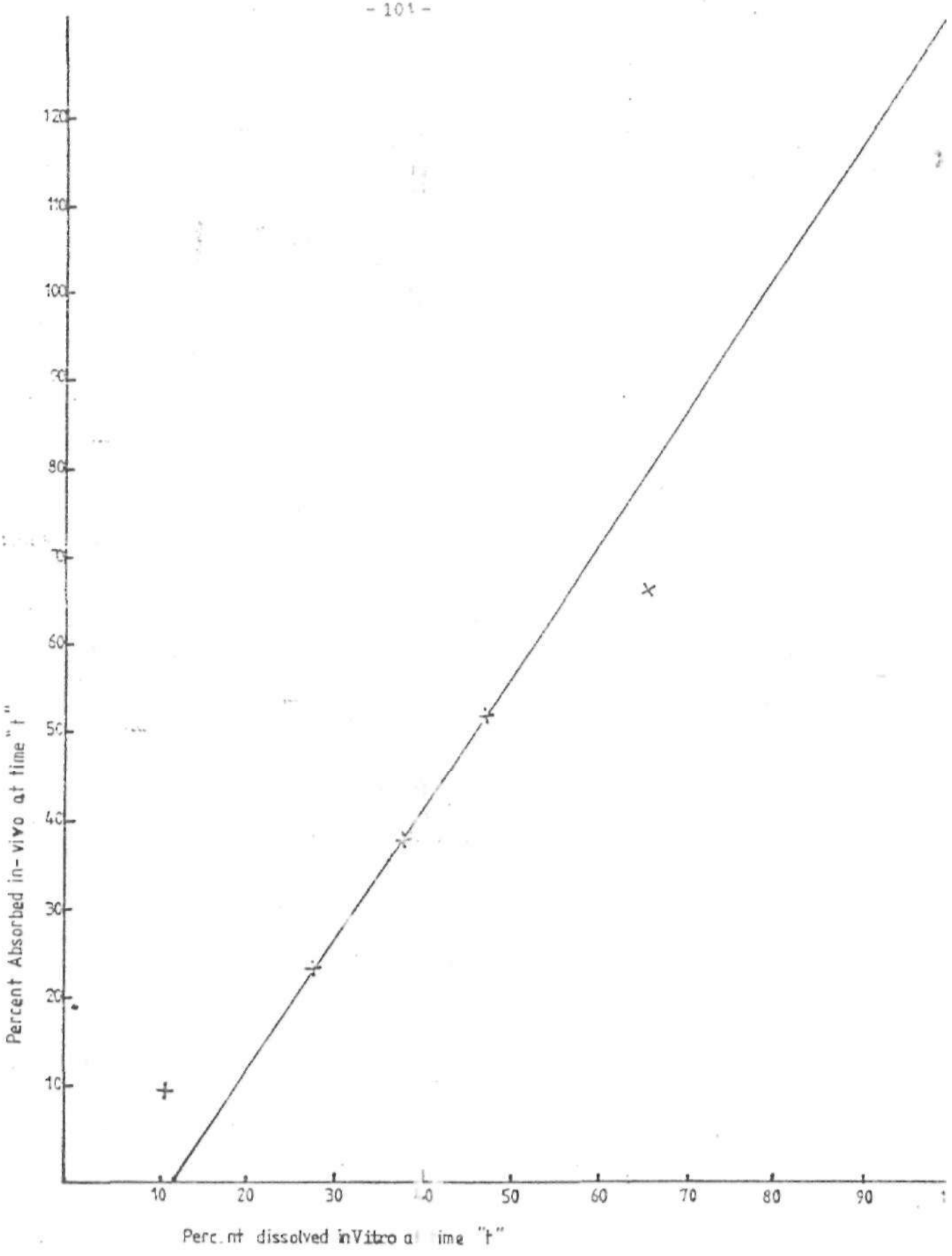
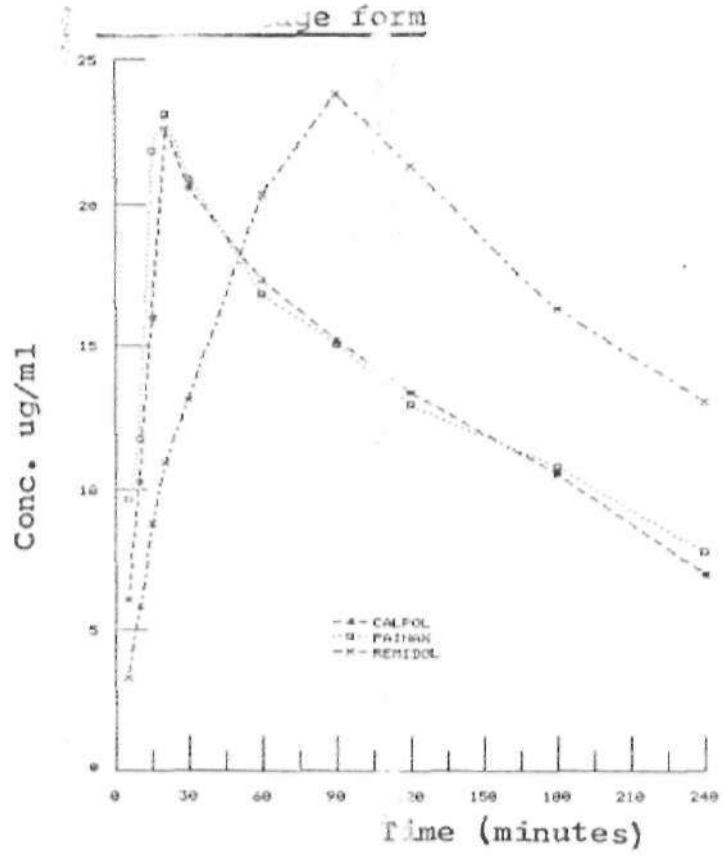


Fig. 16b: Plot of percent of paracetamol (Remidol) absorbed at various times after oral administration versus percent dissolved in-vitro at the respective times.



Solution form

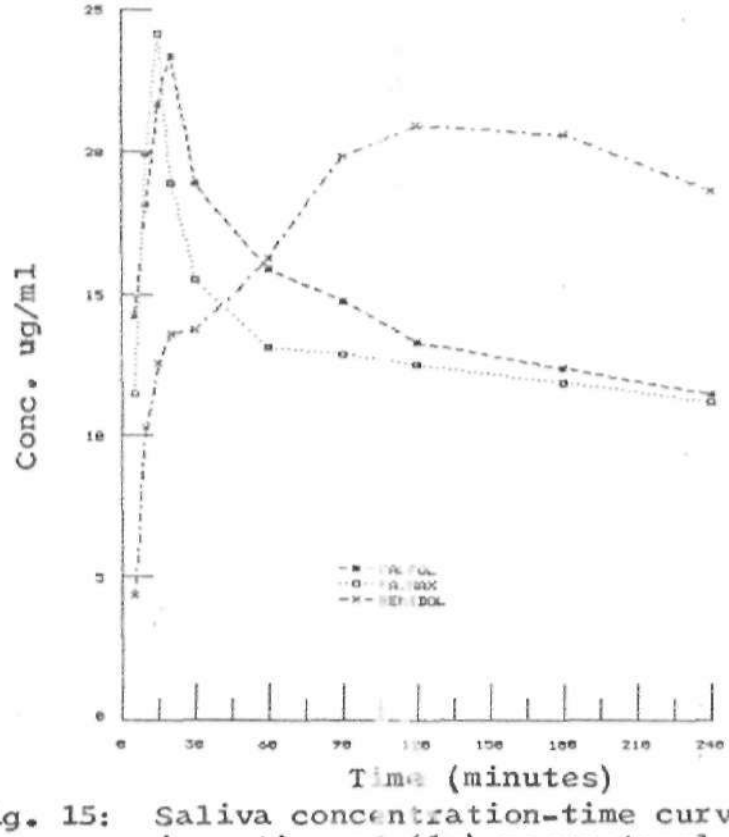


Fig. 15: Saliva concentration-time curves after oral ingestion of (1g) paracetamol administered as solid dosage form and solution form respectively.

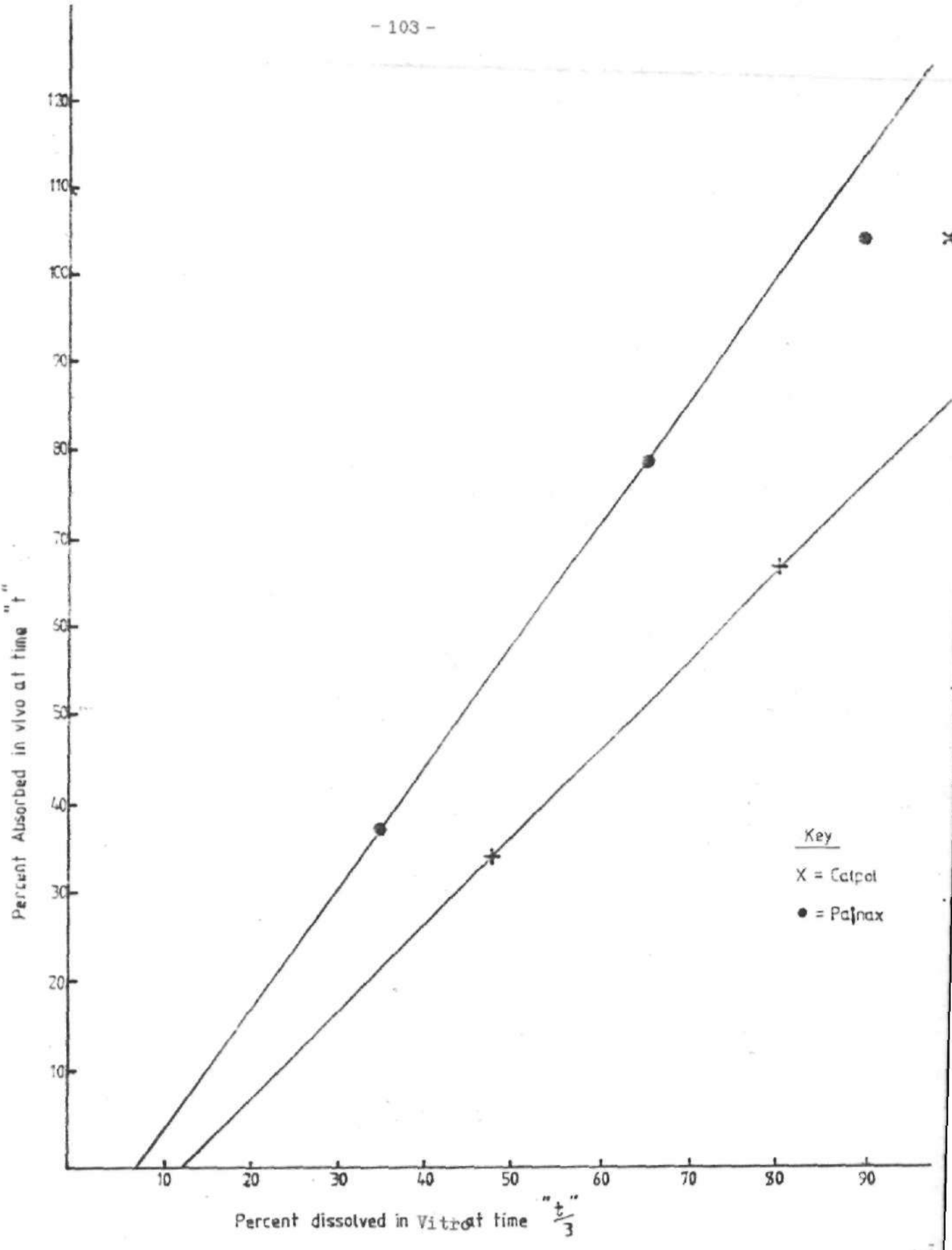


Fig. 16a: Plot of percent of paracetamol absorbed at various times after oral administration versus percent dissolved *in-vitro* at one-third the respective times.

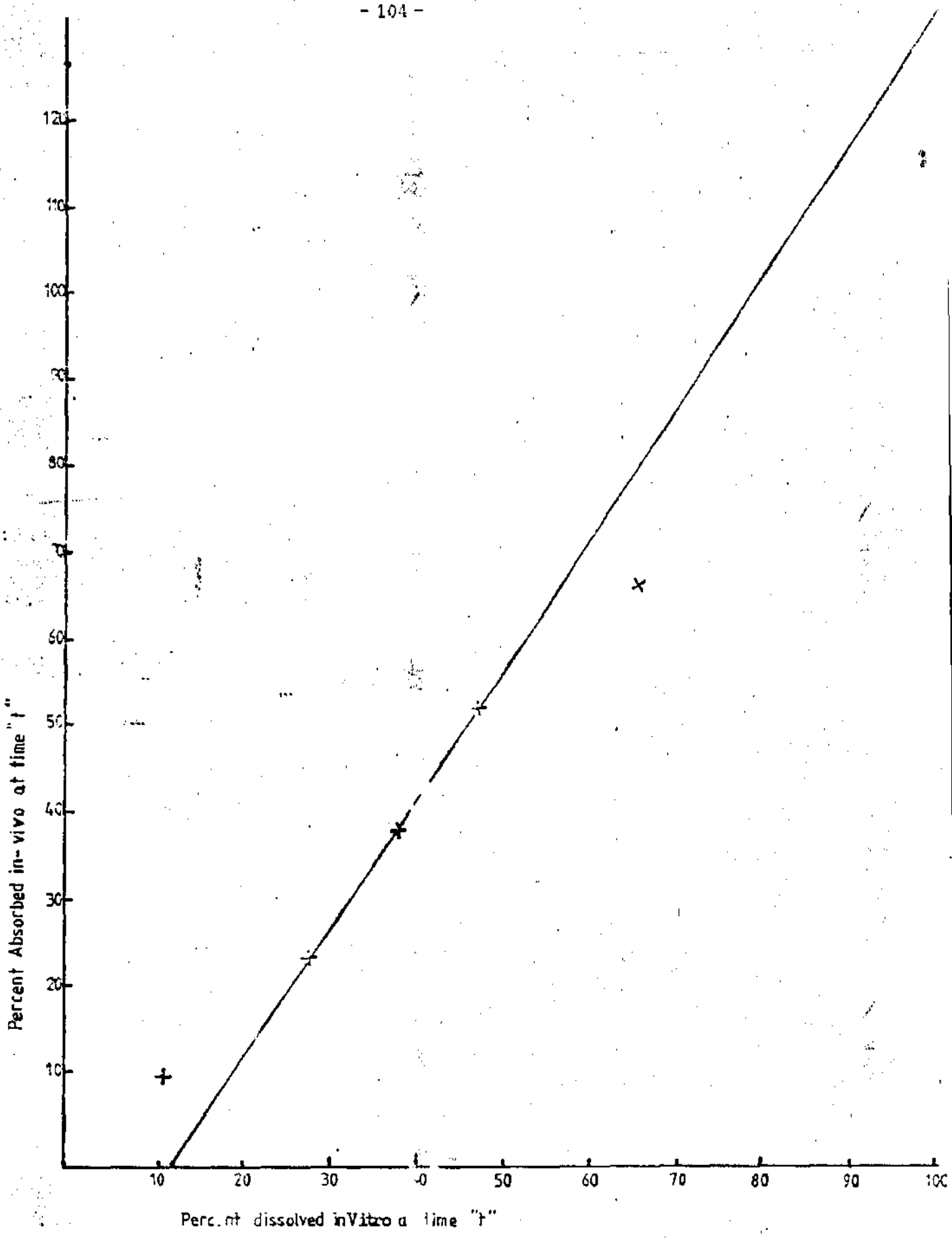
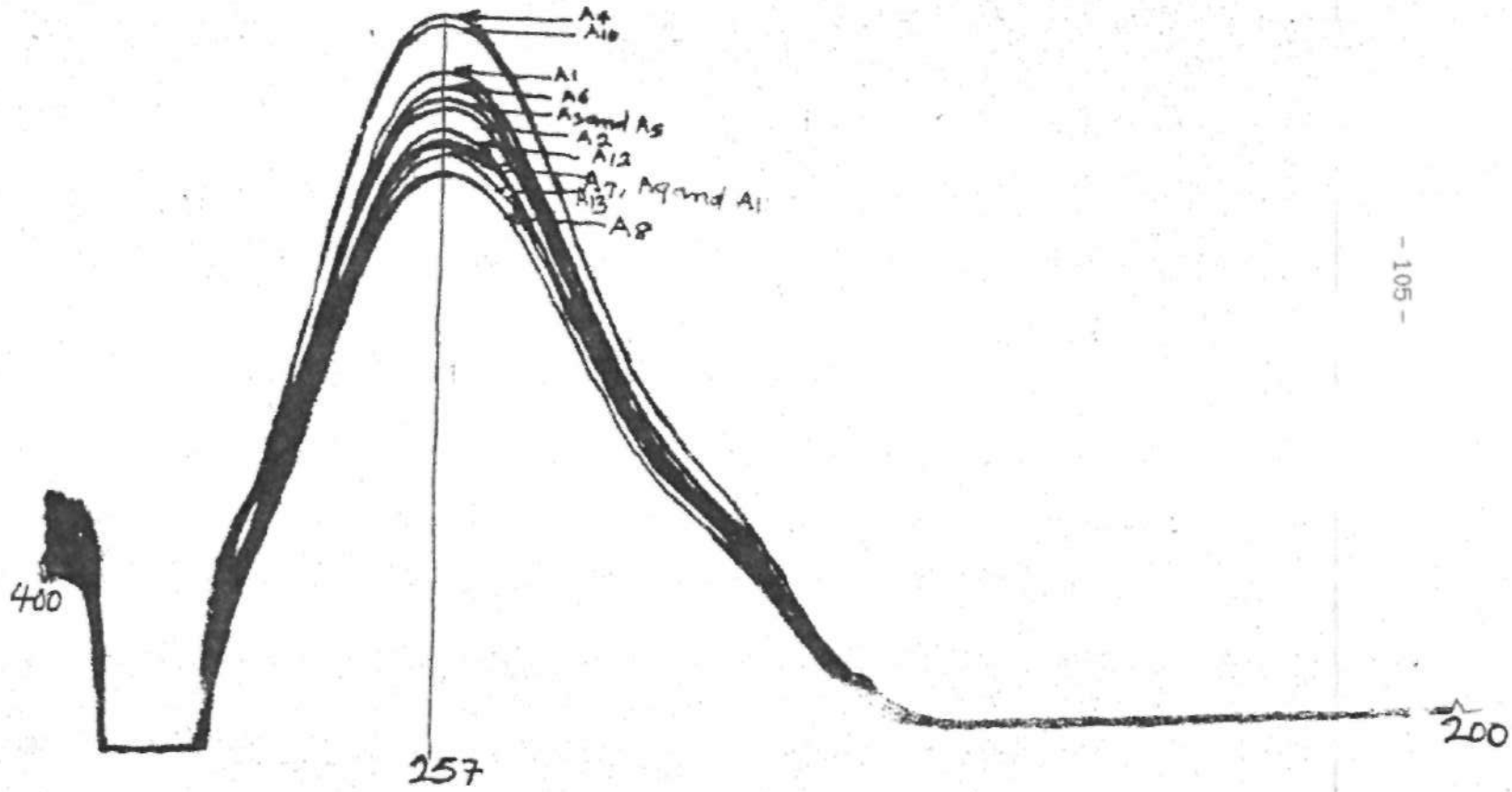


Fig. 16b: Plot of percent of paracetamol (Remidol) absorbed at various times after oral administration versus percent dissolved in-vitro at the respective times.



Wavelength (nm).

Fig. 17: Typical Absorption Spectra for Thirteen Brands of Paracetamol Tablets in 0.1M Sodium Hydroxide Solution (200 - 400 nm).

CHAPTER FIVE

DISCUSSION

5.1 Content of Active Ingredient

The presence and the percentage of active ingredient in paracetamol tablets and syrups were quantitatively determined. The results obtained from this study showed that all the brands of paracetamol tablets and syrups (with the exception of brand S2 of the syrup) tested complied with the official requirement (95.0 - 105.0%) of paracetamol content.

The result further showed that chemical equivalence between the different brands of paracetamol tablets analysed have been established indicating conformity with the general manufacturing practice by the different manufacturers. Although chemical equivalence may not necessarily infer physiological equivalence, however, taking into consideration other factors, it can be said that the different generic products of paracetamol employed in this study may infer similar bioavailability data following administration.

Paracetamol syrup (brand S2) was found to contain 89.12% of the labelled amount of paracetamol and so, did not comply with the official requirement for paracetamol content. Wagner (1971a) reported controlled studies which have largely shown inequivalence rather than equivalence of marketed drug products containing the same drug in the same type of dosage form made by two or more manufacturers. The active ingredient used to formulate the product is probably not of suitable purity or the mixing operation was inefficient (Abdel-Monem, 1978; Wagner, 1971a).

5.2 Dissolution Rate Studies

The results from this study have demonstrated the differences as well as the similarities in the dissolution rates of different brands of paracetamol tablets. For example, brands A2, A3, A8, A10, A11 and A13 gave a dissolution-(T_5) ranging from 90 to 97%. Dissolution value-(T_5) for brands A4, A5, A6 and A12 ranged from 85 to 88%. Furthermore, the results of the *in vitro* dissolution tests established the superiority of A8 and A12 over the

rest of the brands in attaining faster availability (50% dissolution in 1 minute).

The variations found in the dissolution rate of the paracetamol tablets could be explained by various formulation factors. Some of these factors could be the amount and type of diluent (Marlowe and Shangraw, 1967), binder and disintegrant and other adjuvants (Jacob and Plein, 1968; Suren, 1970). Method of incorporation of the ingredients, the compressional force and speed of compression have also been among other factors leading to large variations in the dissolution rates of tablets (Vanodtshoorn et al., 1971, Khan and Rhodes, 1972).

Levy and Procial (1962) observed an unusual behaviour in the dissolution rate profile of alluminium acetyl salicylic acid in 0.1M HCl, due to formation of film coating on the drug. Higuchi and Hamlin (1963) reported that the rate of release of an amine drug from a pellet of a weak acid salt of the amine, in acid solution was controlled by a coat which was formed by precipitation of a weak acid into the pellet surface.

Morozowich et al. (1962) noted that the release of benzphetamine pamoate in 0.12M HCl was controlled by a layer of pamoic acid which was

deposited on the surface. Levy and Gumtow (1963) observed that active lubricant sodium lauryl sulfate enhanced markedly the dissolution rate of salicylic acid contained in compressed tablets. The effect could be attributed to the interfacial tension lowering of the surfactant. Their results 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. The observation can also be attributed to greater local solubilization, increased drug wettability and particle size reduction. It has been established that a drug will dissolve more rapidly when its specific particle size is decreased (Goldberg et al., 1966). Increased bioavailabilty following particle size reduction has been observed with griseofulvin (Ravin, 1980). Also, since the dissolution rate is directly proportional to surface area, a reduction in particle size will result in increased surface area and consequently in rapid dissolution.

Brand A7 (Reridol) however, showed a relatively lower dissolution rate. Dissolution half-life (T_{50}) for this product was 21 minutes. Dissolution of a drug from a tablet or other dosage form involves a disintegration or deaggregation step during which the solid drug

particles are released to the dissoluion medium. Once released, the individual drug particles undergo dissolution. Tablet dissolution is thus a composite of the sequential time-dependent processes of tablet disintegration and particle dissolution. The extent of drug release from a tablet with time can be related to the sequential processes of tablet disintegration and particle dissolution (Nelson and Miller, 1979). They described the dissolution of the drug particles by an exponential equation:

$$F = 1 - e^{-kt},$$

where F is the fraction of the mass of particles that dissolve after exposure of the dissolution medium for a time t, and k is an experimentally determined rate constant. Then the relationship between tablet disintegration and dissolution is given by:

$$W = \frac{1}{k}\frac{dm}{dt} + M$$

The fraction of the tablet that has disintegrated after exposure to the medium for time t is W, and M is the fraction of the drug in the tablet that has dissolved at time t. The present finding could be explained by the Nelson and Miller equation. Disintegration test conducted

for Remidol is a reflection of what was found with its *in vitro* dissolution rate; dissolution half-life was 21 minutes and its mean disintegration time was 48 minutes. The corresponding findings for Calpol were 1.2 and 3 and for Painax 2.5 and 7 minutes respectively. The official B.P. (1980) time-limit for tablet disintegration is 15 minutes. Remidol (A7) failed the official requirement for tablet disintegration.

Kitazawa et al (1977) reported that increase in hardness of tablets will result in an increase in the disintegration time and a decrease in the dissolution rate. This could be another factor contributing to the slow release rate of brand A7. Unlike other manufacturers, this manufacturer might have used excess quantity of binder and compressional force. Due to the insufficient number of tablets, it was not possible to carry out hardness and friability tests.

Type and amount of disintegrant employed may also affect the disintegratin time. Wagner (1971b) reported that in a comparative study of seven disintegrants, compressed at three concentrations (5, 10 and 15% in a base of spray-dried lactose using magnesium stearate as

lubricant with direct compression), most rapid disintegration was achieved with corn starch at 10 and 15% regardless of storage temperature.

Generally, many drug manufacturers use a wide range of formulation and manufacturing variables in the production process. Most likely, a number of these variables as discussed above could have contributed to the difference found in the pharmaceutical availability of Remidol as compared with other brands studied.

5.3 *In vivo* Bioavailability Studies

Higher levels of paracetamol were detected in saliva compared with serum for the first 60 minutes following oral ingestion of 1g of the drug, the level thereafter being comparable. The finding is in agreement with the data of Glynn and Bastain (1973); Adithan and Thangam (1982) and Kamali et al. (1987). Similarly, identical saliva and serum paracetamol elimination half-lives, established in our study were also in agreement with the data of Adithan and Thangam (1982), Kamali et al. (1987). Ogunbona et al. (1986) noted that, in the study of salivary excretion of chloroquine in man, the peak concentration (C_{max})

occurred at the same time in serum and saliva and suggested that equilibrium is reached rapidly between them, in line with Posti's hypothesis that saliva should be regarded as an integral part of the central compartment (Posti, 1982) rather than a 'deep' pharmacokinetic compartment as suggested by Galeazzi et al. (1976).

There were differences as well as similarities in the calculated individual pharmacokinetic parameter. (See Appendices 7 - 10). For example, the time to reach peak concentrations (t_{max}) and elimination half-lives calculated for each person were similar for Calpol and Painax but, slightly different for Remidol. Other parameters are also slightly different within subjects, in agreement with the findings of Levy et al. (1965) and Albert et al. (1974). The differences observed in the individual parameters are due to individual variability or varying degree of the hepatic first-pass effect. An orally administered drug may be destroyed or metabolised within the gut lumen or by enzymes in the mucosa. Because of the anatomical arrangement of the hepatic portal circulation, drugs absorbed into the portal vein are also exposed to the liver before reaching the site of action in the systemic

circulation. Drug metabolism prior to the drug entering into the systemic circuit at any of these sites after oral administration is referred to as presystemic or first-pass elimination. Pharmacokinetic theory permits some reasonable predictions of the magnitude of the first-pass effect on drugs that are eliminated entirely by apparent first-order hepatic biotransformation (Perrier and Gibaldi, 1972; Perrier et al., 1973).

Although many factors influence the rate of drug absorption, pharmaceutical formulation and the characteristics of the subjects are major sources of individual variations. Subject characteristics can produce striking differences in the efficacy of a given drug product. One characteristic that influences drug absorption is the rate of gastric emptying. It may affect both the rate and extent of absorption of the drug (Abdel-Monem and Henkel, 1978; Rawlins, 1979). Gastric emptying is an important rate-limiting step in the absorption of paracetamol (Clements et al., 1978). Intrasubject variability in drug disposition is well documentated (Koup and Gibaldi, 1980). The degree of intrasubject variability changes from drug to drug and may differ from one panel of subjects to another

depending on age and other factors (Koup and Gibaldi, 1980).

Matin et al. (1974) have suggested that for a lipid soluble weakly acidic compound such as paracetamol (Pka = 9.51) the saliva to serum concentration ratio (R) could be predicted on the basis of the degree of ionization in the two fluids by the following equation:

$$R = \frac{1 + 10^{(PHs - Pka)}}{1 + 10^{(PHp - Pka)}}$$

where PHs = PH of saliva and PHp = PH of serum. If total drug concentration (i.e., bound + unbound) is measured, then the ratio would be predicted by a derived equation:

$$R = \frac{1 + 10^{(PHs - Pka)}}{1 + 10^{(PHp - Pka)}} \times \frac{Fp}{Fs}$$

where Fp = fraction of unbound drug in serum, and Fs = fraction of unbound drug in saliva.

Paracetamol is not bound to saliva protein and only minimally bound to plasma protein (Kamali et al., 1987). Also, under conditions of stimulated salivary flow, as was used in our experiments, the PH of saliva approaches that of serum (Dawes and Jenkins, 1964). Because of this, it would be expected from the equations of Matin et al. (1974) that the saliva to serum concentration ratio for paracetamol would approach unity as found in this study which is thus in agreement with those of Glynn and Bastain (1973) and Lowenthal et al. (1976).

Recently, Poeti (1982) developed an anatomical-physiological model to explain the finding of higher salivary drug levels relative to serum drug levels during the early stages following oral ingestion of a drug. In this model it is hypothesized that (1) during absorption the concentration of drug in arterial blood is higher than that in venous blood. This concentration difference being at any moment directly proportional to the rate of absorption, and (2) the concentration of drug in saliva is in equilibrium with that in arterial blood. The duration of the elevated saliva to serum concentration ratio for paracetamol is consistent with the suggestion that the elevated ratio will

occur during absorption, and the patterns of saliva/serum paracetamol concentration-time curves are in agreement with the general patterns predicted by Posti (1982).

One conclusion derived by Posti (1982) is that the difference in AUC between the saliva and serum drug concentration-time curves within appropriate time limits during absorption is proportional to the amount of drug present in the system at time t. Kamali et al. (1987) tested the appropriateness of the Posti model to the present findings with paracetamol. They suggested that the elevated saliva to serum concentration ratio for paracetamol observed during the first one hour following oral ingestion is related to ongoing absorption of paracetamol into the arterial system.

Comparing the bioavailability of the three brands of paracetamol tablets used in this study, it is obvious from the results obtained that, the major cause of differences in absorption of paracetamol in these preparations is their differing dissolution rates. This is reflected in the longer absorption half-life found *in vivo* for brand A7. However, measured area under the serum concentration curve to 4h (AUC_{0-4h}) using

the trapezoid rule did not differ significantly for brands A1 and A2 but was greater for brand A7.

This increased AUC can be explained by considering the fact that, the time required to reach peak concentration is representative of the rate of drug absorption while AUC is proportional to the amount or extent of drug absorption. The greater the total amount of drug absorbed the greater the AUC will be and the greater is the bioavailability (Abdel-Monem and Henkel, 1978). Absorption may be rapid (large Ka) with a low bioavailability (brands A1 and A2) or, conversely, the absorption may be slow (small Ka) with a bioavailability near unity (brand A7). There is, of course, a limit to how slow absorption may be without reducing the bioavailability. This is determined by how long the drug remains in the gastrointestinal tract. Following oral administration, drug that has not been absorbed within 18 - 36h is excreted in the faeces. Consequently absorption half-lives greater than 4 - 6h are associated with decreased bioavailablity, and the absorption process probably does not strictly obey first-order kinetics (Tozer, 1979). Due to difficulty in getting suitable subjects, it was not possible to carry out in vivo study using paracetamol syrup.

The linearity of the plots shown as Figures 16a and 16b indicate a successful correlation of *in vivo* with *in vitro* data. It illustrates that dissolution is the rate-determining step in the absorption of paracetamol taken orally in solid form. The deviation of the last experimental points from the line is due apparently to concentration build-up of drug in the *in vitro* dissolution medium.

Dissolution rate is proportional to the difference between the concentration of a saturated solution C_s and the instantaneous concentration C : Dissolution Rate = $k(C_s - C)$ and the increased degree of saturation during the last stages of the *in vitro* test will slow down the dissolution process (Noyes and Whitney, 1897). This does not occur *in vivo* where build-up of concentration is prevented by continous absorption of dissolved drug (Levy, 1964). The positive intercept at the abscissa of the experimental lines shown in Figures 16a and 16b indicates a lag-time which probably reflects the time necessary for the initial quantity of dissolved drug to diffuse through gastric fluid to the mucosa and from there to the blood stream. This takes apparently less than three minutes as indicated in Table 10. Similar results were

obtained in correlation studies of *in vivo* with *in vitro* data for aspirin (Levy, 1964) and frusemide (Kingsford et al., 1984).

CONCLUSION

The brands of paracetamol tablets (A1 - A13) used satisfied the official B.P. (1980) requirement. Similarly, the paracetamol syrups employed in this study except for one brand (S2 which contained 82.12%) complied with the official specification (U.S.P., 1980) for paracetamol content (95.0 - 105.0)%.

It has been demonstrated in the present study that the rate and extent of drug release for various brands of paracetamol tablets were different, although some brands showed similarities in their dissolution rates. The variation could be due to the different manufacturing processes employed by the different manufacturers.

The result of the disintegration test conducted for three brands of paracetamol showed that the differences in the disintegration time are statistically significant. ($P < 0.05$). The variation could be due to the amount of the binder, type and amount of disintegrant and the compressional force used by the different manufacturers.

Higher concentrations of paracetamol were detected in saliva compared with serum for the first 60 minutes following oral ingestion, the levels thereafter being comparable. Serum/saliva paracetamol elimination half-lives calculated were similar. Measured area under the concentration-time curve (AUC) differed in the serum and saliva, the difference being proportional, during absorption, to the amount of drug present in the system at time t (Kamali et al., 1987). There were marked differences in the rate of absorption by each subject. This could be due to a varying degree of the hepatic first pass effect or individual variability.

Most important drugs which are administered orally in tablet form are sometimes poorly and incompletely absorbed due to improper formulation. This has resulted, on some occasions, in consequencies to the patient and misjudgement of appropriate dosage regimens. Adequate official standards for compressed tablets are needed urgently to prevent similar occurrences in the future.

Saliva concentrations of paracetamol could have two uses: its specific identification and estimation in the saliva of patients suffering from paracetamol overdose may assist the clinician

to decide further treatment; and the measurement of the biological or systemic availability of paracetamol formulations could be achieved without the discomfort, possible hazard and necessary attendance of medical staff required for repeated venepunctures. Saliva level determination proved as useful as the serum levels in the determination of some pharmacokinetic parameters of the drug.

There is need for further studies involving the interactions of paracetamol with other commonly used drugs, with pharmacologically inactive components of the pharmaceutical dosage forms and physico-chemical interactions between the component(s) of a meal and paracetamol.

The results of this study dictate the need for more stringent quality control studies of drugs which are meant for use in our environment. This will ensure that only drugs of proven quality are available to our people.

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APPENDICES

APPENDIX 1

Chemical Assay

Of Paracetamol Tablets

Compound	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12	A13
Absorbance:													
at 257nm	,695	,632	,638	,765	,639	,659	,572	,567	,611	,760	,625	,586	,591
at 430nm	1,160	1,058	1,066	1,283	1,025	1,100	0,958	0,950	1,033	1,257	1,050	0,973	0,992

APPENDIX 2A

Dissolution Rate Studies of Paracetamol Tablets In 0.1M HCl Maintained at 37±0.5°C Using USP XVIII Rotating Basket Method.

Absorbance measured at 257 nm

Time(Min,)	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12	A13
1	,348	,185	,283	,166	,258	,283	,033	,366	,185	,200	,348	,175	,225
5	,620	,565	,575	,537	,533	,537	,066	,606	,475	,565	,608	,525	,565
10	,633	,620	,620	,620	,599	,620	,175	,616	,619	,608	,620	,619	,619
20	,616	,616	,619	,616	,604	,600	,298	,612	,620	,616	,619	,619	,619
40	,608	,616	,616	,600	,602	,583	,508	,608	,616	,600	,616	,616	,616
60	,600	,608	,612	,598	,601	,575	,620	,600	,600	,584	,612	,612	,612

APPENDIX 2B

Dissolution Rate Studies of Paracetamol Tablets In 0.1M HCl Solution

Absorbance at 257 nm, Spectrophotometrically measured concentration ($C_{measd.}$)

Time (Min.)	Concentration ($C_{measd.}$) mg/1000ml.												
	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12	A13
1	280	150	225	130	208	225	25	300	150	158	280	141	180
5	495	448	462	438	427	437	49	479	373	448	480	423	451
10	496	491	490	491	470	489	140	478	487	476	489	486	498
20	470	474	477	474	466	469	239	468	484	474	478	479	501
40	458	466	465	457	457	450	401	459	468	456	464	467	498
60	447	453	454	444	448	434	487	447	450	444	453	456	496

APPENDIX 2C

Dissolution Rate Studies of Paracetamol Tablets In 0.1M HCl Solution

Cumulative Concentration (C_n)

Time (Min.)	Concentration (C_n) mg/1000ml												
	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12	A13
1	280	150	225	130	208	225	25	300	150	158	280	141	180
5	501	451	466	440	431	441	50	485	384	451	486	425	447
10	512	503	504	502	483	503	142	494	498	488	504	498	486
20	496	496	501	495	488	486	243	493	505	496	501	500	478
40	494	498	498	487	489	483	410	494	498	488	499	498	466
60	492	495	497	485	490	476	504	492	490	485	498	497	453

APPENDIX 3

Serum Concentration of Unchanged Paracetamol (µg/ml) In 4 Healthy Subjects Following Oral Administration of A Solution of the Tablets (1g) After Overnight Fast

	Concentration (µg/ml)											
Subjects	I			II			III			IV		
Time (Min)	Calpol A1	Painax A2	Remidol A7	Calpol A1	Painax A2	Remidol A7	Calpol A1	Painax A2	Remidol A7	Calpol A1	Painax A2	Remidol A7
5	7,50	7,50	2,50	10,63	8,13	1 88	9,06	7,81	3,13	8,44	8,44	2,19
10	12,19	15,00	3,50	14,69	16,25	3,75	11,88	25,00	4,00	11,25	16,88	3,75
15	14,38	18,75	7,50	17,81	20,00	5,25	15,38	23,44	7,81	13,75	22,50	6,56
20	20,00	17,50	10,00	23,44	18,13	8 44	22,19	20,31	9,38	22,19	18,75	8,75
30	17,19	15,63	15,00	20,31	15,94	10 00	19,06	17,19	11,88	18,75	18,13	13,13
60	15,63	14,69	19,69	18,44	15,00	17,81	17,19	14,44	15,31	16,25	13,44	19,69
90	9,69	11,56	23,44	16,56	11,88	19,69	15,00	10,94	18,44	14,06	10,31	23,44
120	6,88	11,25	15,00	14,06	11,56	19,69	12,50	10,63	10,94	11,56	10,00	15,63
180	6,56	10,94	11,25	13,13	10,94	13,44	11,88	10,00	7,81	10,94	9,38	10,00
240	5,63	10,63	10,63	10,64	10,63	13,13	10,94	10,00	7,81	10,94	9,38	10,00

APPENDIX 4

Serum Concentration of Unchanged Paracetamol (ug/ml) In 4 Healthy Subjects Following Oral Administration of The Tablets (1g) After Overnight Fast

	Concentration (µg/ml)											
Subjects	I			II			III			IV		
Time (Min)	Calpol	Painax	Remidol	Calpol	Painar	Remidol	Calpol	Painax	Remidol	Calpol	Painax	Remidol
	A1	A2	A7	A1	A2	A7	A1	A2	A7	A1	A2	A7
5	6,30	5,90	1,60	6,30	5,0	2,80	5,00	6,30	1,60	6,60	7,20	2,20
10	10,60	11,30	3,40	11,30	10,0	5	10,00	10,90	4,50	13,10	12,80	5,30
15	15,60	15,30	5,90	15,90	14,1	7,50	15,00	14,70	8,40	19,70	15,60	7,20
20	19,10	20,90	8,10	21,60	19,70	9,70	20,30	21,30	10,60	22,20	22,20	9,70
30	17,20	18,80	9,70	19,40	17,50	11,30	18,10	19,10	12,20	20,30	20,00	11,60
60	13,10	14,70	17,50	15,10	13,40	19,10	14,10	15,00	20,00	16,30	15,90	19,40
90	11,90	13,10	20,00	14,10	11,90	20,90	12,80	13,40	21,90	14,40	14,40	20,90
120	10,00	11,60	16,30	12,20	10,30	17,80	10,90	11,90	18,40	13,10	12,80	18,40
180	8,10	9,70	13,10	9,70	8,50	14,70	8,40	10,00	15,60	10,00	10,90	15,90
240	5,00	5,60	10,00	5,90	5,00	11,30	5,90	7,80	12,20	7,20	7,50	9,40

APPENDIX 5

Saliva Paracetamol Concentration (ug/ml) In 4 Healthy Subjects

Following Oral Administration of a Solution of Tablets (1g)

After Overnight fast

	Concentration (µg/ml)											
Subjects	I			II			III			IV		
Time (Min)	Calpol	Painax	Remidol	Calpol	Painax	Remidol	Calpol	Painax	Remidol	Calpol	Painax	Remidol
	A1	A2	A7	A1	A2	A7	A1	A2	A7	A1	A2	A7
5	12,85	9,92	5,85	14,25	9,92	0,45	14,58	15,08	6,06	14,24	10,12	4,96
10	17,61	17,85	8,73	17,85	22,91	7,79	17,85	17,85	8,93	17,77	19,5	7,89
15	19,84	21,82	11,71	21,92	23,06	10,76	21,92	29,73	11,91	21,33	23,11	10,57
20	21,08	16,86	12,60	24,84	18,10	11,66	22,81	19,69	12,80	22,91	19,34	12,35
30	17,85	14,88	13,69	19,69	14,14	12,95	17,50	16,07	13,89	18,83	15,87	12,85
60	16,12	11,38	16,27	16,07	12,15	14,93	15,38	14,14	16,76	14,88	13,99	15,82
90	14,88	10,81	17,85	15,18	11,66	16,51	13,89	14,09	18,35	14,14	13,99	24,99
120	12,15	10,12	23,31	13,79	11,16	17,21	12,90	13,99	23,80	13,39	13,89	17,80
180	11,90	9,92	21,72	12,40	10,67	20,38	11,91	13,14	22,22	12,40	13,00	16,81
240	10,91	9,82	19,14	11,41	9,92	17,30	10,91	12,2	19,64	11,90	12,00	16,47

APPENDIX 6

Saliva Paracetamol Concentration (µg/ml) In 4 Healthy Subjects

Following Oral Administration of The Tablets (1g)

After Overnight fast

	Concentration (µg/ml)											
Subjects	I			II			III			IV		
Time (Min)	Calpol	Painax	Remidol	Calpol	Painax	Remidol	Calpol	Painax	Remidol	Calpol	Painax	Remidol
	A1	A2	A7	A1	A2	A7	A1	A2	A7	A1	A2	A7
5	6,00	9,50	2,70	6,00	8,10	3,90	6,20	10,30	3,20	6,10	9,80	4,20
10	9,90	11,30	3,90	8,50	9,90	5,20	11,10	12,20	7,10	10,90	12,60	5,73
15	17,90	21,40	7,20	13,70	19,80	8,60	15,10	22,10	8,80	16,10	22,60	9,60
20	20,80	22,60	9,30	22,30	21,10	10,80	21,00	22,30	11,00	24,70	23,80	11,80
30	17,90	20,90	12,70	20,30	19,30	12 40	18,90	19,80	12,60	22,70	22,10	13,60
60	14,40	16,70	18,30	16,20	15,20	20,10	14,80	16,30	20,30	18,60	17,50	21,10
90	12,90	15,20	21,30	14,70	13,70	23,80	13,40	13,80	23,80	17,20	16,40	24,70
120	10,90	13,30	17,60	12,80	11,80	20,60	11,60	11,90	24,70	15,20	14,50	20,80
180	9,30	10,70	14,50	7,90	9,20	17,60	9,90	10,40	16,00	12,70	12,00	15,90
240	4,50	8,00	11,30	7,4	6,70	14,10	6,00	7,90	12,40	7,1	7,90	9,70

APPENDIX 7

PHARMACOKINETIC PARAMETERS OF PARACETAMOL IN SERUM OF INDIVIDUAL VOLUNTEERS FOLLOWING THE INGESTION OF 1G IN SOLID DOSAGE FORM

(a) Calpol

	VOLUNTEER NUMBER				
PARAMETER	I	II	III	IV	MEAN(±S.D)
AUC_{0-4hr} (µg/hr)	41.17	48.10	43.84	51.33	46.1(4.5)
C_{max} (µg/ml)	19.1	21.6	20.3	22.2	20.9(1.4)
t_{max}	0.33	0.33	0.33	0.33	0.33
Ke (hr^{-1})	0.36	0.34	0.35	0.32	0.34(0.02)
$t_{\frac{1}{2}\beta}$ (hr)	1.90	2.06	1.96	2.18	2.03(0.12)
Ka (hr^{-1})	8.24	7.47	7.60	9.42	8.18(0.89)
$t_{\frac{1}{2}\alpha}$ (hr)	0.084	0.093	0.091	0.074	0.086(.01)
t_{lag} (min)	2.52	2.65	3.15	3.04	2.84(0.34)

(b) Painax

	VOLUNTEER NUMBER				
PARAMETER	I	II	III	IV	MEAN(±S.D)
AUC_{0-4hr} (µg/hr)	46.45	41.89	48.46	51.60	47.1(4.07)
C_{max} (µg/ml)	20.9	19.7	21.3	22.2	21.03(1.04)
t_{max} (hr)	0.33	0.33	0.33	0.33	0.33
Ke (hr^{-1})	0.34	0.36	0.29	0.29	0.32(0.04)
$t_{\frac{1}{2}\beta}$ (hr)	2.06	1.90	2.38	2.40	2.19(0.25)
Ka (hr^{-1})	7.76	7.63	7.64	8.25	7.82(0.29)
$t_{\frac{1}{2}\alpha}$ (hr)	0.089	0.091	0.091	0.084	0.09(0.003
t_{lag} (min)	2.78	3.05	2.59	2.38	2.71(0.3)

(c) Remidol

	VOLUNTEER NUMBER				
PARAMETER	I	II	III	IV	MEAN(±S.D)
AUC_{0-4hr} (µg/hr)	57.17	59.84	62.99	60.77	60.2 (2.41)
C_{max} (µg/ml)	20.00	20.9	21.90	20.90	20.9 (0.78)
t_{max} (hr)	1.5	1.5	1.5	1.5	1.5
Ke (hr^{-1})	0.51	0.40	0.30	0.57	0.45(0.12)
$t_{\frac{1}{2}\beta}$ (hr)	1.37	1.72	2.28	1.21	1.65(0.65)
Ka (hr^{-1})	0.77	1.03	1.41	0.79	1.0 (0.3)
$t_{\frac{1}{2}\alpha}$ (hr)	0.90	0.67	0.49	0.87	0.73(0.19)
t_{lag} (min)	2.75	0.82	2.70	1.37	1.91(0.97)

APPENDIX 8

PHARMACOKINETIC PARAMETERS OF PARACETAMOL IN SALIVA OF INDIVIDUAL VOLUNTEERS FOLLOWING INGESTION OF 1g IN SOLID DOSAGE FORM

(a) Calpol

	VOLUNTEER NUMBER				
PARAMETER	I	II	III	IV	MEAN(±S.D)
AUC_{0-4hr} (µg/hr)	44.5	48.3	47.1	58.7	49.7(6.2)
C_{max} (µg/ml)	20.8	22.3	21.0	24.7	22.2(1.8)
t_{max} (hr)	0.33	0.33	0.33	0.33	0.33
Ke (hr^{-1})	0.38	0.37	0.33	0.32	0.35(0.03)
$t_{1/2}$ (hr)	1.84	1.87	2.12	2.19	2.01(0.18)
Ka (hr^{-1})	8.07	5.55	7.51	6.10	6.81(1.2)
$t_{1/2a}$ (hr)	0.09	0.13	0.09	0.11	0.10(0.02)
t_{lag} (min)	2.98	2.73	2.58	2.96	2.81(0.19)

(b) Painax

	VOLUNTEER NUMBER				
PARAMETER	I	II	III	IV	MEAN(±S.D)
AUC_{0-4hr} (µg/hr)	53.5	47.7	51.0	57.5	52.4(4.1)
C_{max} (µg/ml)	22.6	21.1	22.3	23.8	22.5(1.1)
t_{max} (hr)	0.33	0.33	0.33	0.33	0.33
Ke (hr^{-1})	0.31	0.34	0.31	0.30	0.31(0.02)
$t_{1/2}$ (hr)	2.27	2.03	2.21	2.32	2.21(0.13)
Ka (hr^{-1})	7.94	7.80	9.14	8.27	8.26(0.60)
$t_{1/2a}$ (hr)	0.09	0.10	0.08	0.08	0.08(0.005
t_{lag} (min)	1.69	2.13	1.56	1.90	1.82(0.25)

(c) Remidol

	VOLUNTEER NUMBER				
PARAMETER	I	II	III	IV	MEAN(±S.D)
AUC_{0-4hr} (µg/hr)	59.6	68.9	69.8	66.7	66.3(4.6)
C_{max} (µg/ml)	21.3	23.8	23.3	24.7	23.3(1.8)
t_{max} (hr)	1.5	1.5	1.5	1.5	1.5
Ke (hr^{-1})	0.37	0.37	0.44	0.59	0.44(0.13)
$t_{1/2}$ (hr)	1.89	1.86	1.6	1.18	1.60(0.4)
Ka (hr^{-1})	1.18	0.93	0.98	0.85	0.98(0.17)
$t_{1/2a}$ (hr)	0.59	0.75	0.72	0.82	0.72(0.12)
t_{lag} (min)	1.95	0.0	1.09	0.23	1.09(1.22)

APPENDIX 9

PHARMACOKINETIC PARAMETERS OF PARACETAMOL IN SERUM OF INDIVIDUAL VOLUNTEERS FOLLOWING THE INGESTION OF 1g IN SOLUTION

(a)Calpol

	VOLUNTEER NUMBER				
PARAMETER	I	II	III	IV	MEAN(±S.D)
AUC_{0-4hr} (µg/hr)	37,95	59,35	54,60	51,70	50,9 (9,2)
C_{max} (µg/ml)	20,00	23,44	22,19	22,19	21,96(1,4)
t_{max} (hr)	0,33	0,33	0,33	0,33	0,33
Ke (hr^{-1})	0,48	0,21	0,21	0,21	0,28(0,14)
$t_{1/2}$ (hr)	1,45	3,27	3,34	3,24	2,83(0,92)
Ka (hr^{-1})	6,89	8,08	7,45	7,40	7,46(0,49)
$t_{1/2a}$ (hr)	0,10	0,09	0,09	0,09	0,09(0,01)
t_{lag} (min)	1,50	0,57	0,97	1,17	1,05(0,39)

(b) Painax

	VOLUNTEER NUMBER				
PARAMETER	I	II	III	IV	MEAN(±S.D)
AUC_{0-4hr} (µg/hr)	48,00	49,26	48,29	45,46	47,8(1,62)
C_{max} (µg/ml)	18,75	20,00	23,44	22,50	21,2(2,17)
t_{max} (hr)	0,25	0,25	0,25	0,25	0,25
Ke (hr^{-1})	0,17	0,19	0,33	0,30	0,25(0,08)
$t_{1/2}$ (hr)	4,07	3,71	2,09	2,32	3,05(0,99)
Ka (hr^{-1})	22,70	26,33	96,22	22,95	42,05(36,2)
$t_{1/2a}$ (hr)	0,003	0,026	0,007	0,003	0,01(0,01)
t_{lag} (min)	3,48	3,63	4,71	3,55	3,84(0,58)

(c) Remidol

	VOLUNTEER NUMBER				
PARAMETER	I	II	III	IV	MEAN(±S.D)
AUC_{0-4hr} (µg/hr)	56,66	58,83	43,05	54,72	53,3(7,05)
C_{max} (µg/ml)	23,44	19,69	18,44	23,44	21,25(2,58)
t_{max} (hr)	1,5	1,5	1,5	1,5	1,5
Ke (hr^{-1})	0,40	0,34	0,43	0,54	0,43(0,08)
$t_{1/2}$ (hr)	1,75	2,01	1,63	1,28	1,67(0,30)
Ka (hr^{-1})	1,61	1,03	1,68	1,18	1,38(0,32)
$t_{1/2a}$ (hr)	0,43	0,68	0,41	0,59	0,53(0,13)
t_{lag} (min)	3,90	2,45	1,60	4,03	3,00(1,17)

APPENDIX 10

PHARMACOKINETIC PARAMETERS OF PARACETAMOL IN THE SALIVA OF INDIVIDUAL VOLUNTEERS FOLLOWING THE INGESTION OF 1g IN SOLUTION

(a)Calpol

	VOLUNTEER NUMBER				
PARAMETER	I	II	III	IV	MEAN(±S.D)
AUC_{0-4hr} (µg/hr)	54,21	57,65	54,28	55,89	55,5(1,6)
C_{max} (µg/ml)	21,08	24,84	22,81	22,91	22,9(1,5)
t_{max} (hr)	0,33	0,33	0,33	0,33	0,33
Ke (hr^{-1})	0,19	0,22	0,21	0,19	0,20(0,01)
$t_{\frac{1}{2}\beta}$ (hr)	3,63	3,19	3,28	3,64	3,4(0,23)
Ka (hr^{-1})	17,94	14,34	18,20	17,39	17,0(1,8)
$t_{\frac{1}{2}\alpha}$ (hr)	0,039	0,048	0,038	0,040	0,041(0,01
t_{lag} (min)	1,56	0,855	1,073	1,043	1,13(0,30)

(b) Painax

	VOLUNTEER NUMBER				
PARAMETER	I	II	III	IV	MEAN(±S.D)
AUC_{0-4hr} (µg/hr)	44,29	47,08	56,24	55,08	50,7(5,7)
C_{max} (µg/ml)	21,82	23,06	29,73	23,11	24,4(3,6)
t_{max} (hr)	0,25	0,25	0,25	0,25	0,25
Ke (hr^{-1})	0,239	0,270	0,192	0,160	0,228(,06)
$t_{\frac{1}{2}\beta}$ (hr)	2,90	2,56	3,61	4,34	3,4(0,08)
Ka (hr^{-1})	62,85	98,79	25,28	66,50	60,9(26,3)
$t_{\frac{1}{2}\alpha}$ (hr)	0,011	0,008	0,027	0,010	0,014(,003
t_{lag} (min)	4,21	4,52	2,07	4,32	3,78(1,1)

(c) Remidol

	VOLUNTEER NUMBER				
PARAMETER	I	II	III	IV	MEAN(±S.D)
AUC_{0-4hr} (µg/hr)	73,92	65,25	75,67	66,38	70,3(5,3)
C_{max} (µg/ml)	23,31	20,38	23,80	24,99	23,14(2,0)
t_{max} (hr)	2,0	3,0	2,0	1,5	2,1(0,6)
Ke (hr^{-1})	0,169	-7,93	0,199	0,147	2,1(3,9)
$t_{\frac{1}{2}\beta}$ (hr)	4,11	-8,7	3,49	4,72	5,3(2,4)
Ka (hr^{-1})	0,658	7,13	0,578	1,44	2,45(3,1)
$t_{\frac{1}{2}\alpha}$ (hr)	1,053	0,097	1,199	0,483	0,71(0,5)
t_{lag} (min)	-18,0	4,49	-19,68	-5,27	11,9(8,1)

APPENDIX 11

Apparatus for the Disintegration of Tablets

Appartus

The apparatus consists of

(a) a rigid basket-rack assembly supporting 6 cylindrical glass tubes 77.5 mm long, 21.5 mm in internal diameter, and with a wall thickness of about 2 mm.

(b) a cylindrical disc for each tube, each 20.7 mm in diameter and 9.5 mm thick, made up of transparent plastic with a relative density of 1.18 to 1.20, and pierced with five holes, each 2 mm in diameter, one in the centre and the other four spaced equally on a circle of radius 6 mm from the centre of the disc. Four equally-spaced grooves are cut in the lateral surface of the disc in such a way that at the upper surface of the disc they are 9.5 mm wide and 2.55 mm deep and at the lower surface 1.6 mm square.

(c) The tubes are held vertically by 2 superimposed transparent plastic plates 90 mm in diameter and 6 mm thick, perforated by 6 holes having the same diameter as the tubes. The holes are equidistant from the centre of the plate and are equally spaced from one another. Attached to

the underside of the lower plate is a piece of wooven gauze made from stainless steel wire 635 µm in diameter and having nominal mesh apertures of 2.00 mm. The upper plate is covered with a stainless steel disc perforated by six holes each about 22 mm in diameter, which fits over the tubes and holds them between the plastic plates. The holes coincide with those of the upper plastic plate and the upper open ends of the glass tube.

(d) The plates are held rigidly in position and 77.5 mm apart by vertical metal rods at the periphery and a metal rod is also fixed to the centre of the upper plate to enable the assembly to be attached to a mechanical device capable of raising and lowering it smoothly at a constant frequency of between 28 and 32 cycles per minute, through a distance of 50 to 60 mm.

(e) The assembly is suspended in the liquid medium in a suitable vessel, preferrably a 1000-ml beaker. The volume of liquid is such that the wire mesh at its highest point is at least 25 mm below the surface of the liquid, and at its lowest point is at least 25 mm above the bottom of the beaker.

(f) A suitable device maintains the temperature of the liquid at 36° to 38°.