A COMPARATIVE STUDY OF BIOAVAILABITY OF BRANDS OF ACETYLSALICYLIC ACID TABLETS FROM DIFFERENT MANUFACTURERS.

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D E D I C A T I O N

To the memory of my beloved grandmother, Mrs Hannah W.Miano who was a constant source of encouragement and inspiration throughout my studies.

To my parents, Mr and Mrs D.K.Raga, for their love, encouragement ,patience and support both morally and financially, without whom this project would not have been possible. To my brothers and sisters for their overwhelming companionship, moral support and love.

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ABSTRACT

The project was intended to compare relative bioavailabilities f 4 brands of acetylsalicylic acid tablets as well as to establish hether a correlation exists between in-vitro dissolution rate and rinary excretion analysis.

The official chemical analysis of the tablets showed that l the tablets contained amounts of acetylsalicylic acid within ie required official limits.

The B.P. method of obtaining disintegration times of the blets was adopted and the brands had a rank order of: C astest rate), D,B,A.

The dissolution profiles of the 4 brands was obtained employing the rotating basket method (U.S.P). The rank order sed on the rate of drug release ran as: B(most superior), D,C,

The in-vivo(urinary excretion) analysis was conducted urine samples. These samples were periodically obtained from catheterized male rabit. The rank order of bioavailability was tained after (by) computing the area under the curve in the plot cumulative amount of drug excreted versus time. The order s: B(highest% bioavailability),D,C,A.

The in-vitro dissolution rates were found to correlate th the in-vivo results. No correlation could be established tween in-vitro dissolution rates and disintegration times.

INTRODUCTION

Through out its history pharmacy has been primarily concerned h the dosage forms in which drugs are administered. ffessionalism in pharmacy used to be intimately related to the ertise of the pharmacist in formulating the prescriptions he ained from the physcian for the patient. The present=day rmacist will normally rely on products already formulated by pharmaceutical manufacturer.

The pharmaceutical manufacturer will normally desigh an ortment of dosage forms to suit various needs of the patient. a given drug entity, there will often be differences in siological availability and presumably in clinical responses ng various dosage forms of the same drug given in the same te of administration or among same type of dosage form made two or more manufacturers. The same differences are met n within lots of a drug product made by apparently same ufacturer.

A large body of scientific evidence has now clearly ablished that bioavailability, i.e. the rate and extent absorption of a drug from its dosage form into the systemic culation may vary for a number of reasons. (WAGNER J.G.1971). h variations have been identified as causative factors in ain failures in drug therapy. For many years it was assumed t if a drug product contained the amount of drug claimed on label, its performance could be taken for granted. Therapeutic lures were seldom attributed to the product, but rather to some ormality of the patient.

Variations in bioavailability occur for a number of reasons. se falls under two categories, namely. Patient=related and age-form-related factors. Among the patient-related factors those over which the physcian and/or the patient can exert some trol. These include the time of drug administration relative meals,i.e, the effect of stomach-emptying time, the concommitant inistration of other drugs, which may affect absorption and the pliance of the patient with the instructions of the physcians rmacist or nurse.

Dosage-form-related factors which can produce profound differences drug bioavailability include formulation and manufacturing iables such as the particle size, particle size distribution,

the chemical form and solubility of the drug, the nature and quantity of excipient used, the compaction pressure, etc.

The release of a drug from its dosage form is of utmost importance. The release pattern should be reliable and predictable from batch to batch as well as from product to product since the patient's therapy may involve a change in the brand being taken. Such brand interchanges can lead to either an underdosing of the patient if the new brand of the drug releases, its active ingredient less efficiently than the original product or an overdosing if the converse is true. Either happening is undesirable since at bes it will result in the physcian having to modify the dosage regimen of the second of the drug so as to bring the patient's condition under control, and at worst the therapeutic failure may ultimately result in the death of the patient. Thus, it is highly desirable to develop rapid, efficient, and reliable techniques for monitoring the release of the active ingredients from the various brands that are available. This is particularly so for high-riskagents which are used to treat life-threatening or serious diseases, or those drugs which exhibit a step log-dose response curve and/or a low therapeutic index, or those drugs which have relatively low aqueous solubility i.e. less than 1% over the PH range 1-8. Example of drugs which fall into this category include digoxin, phenytoin, warfarin, quinidine, prednisone, and theophylline(ANON,1973).

In the initial development of appropriate techniques for monitoring the release of the active ingredients from various brands of a drug product disintegration test was considered to be predictive of physiological bioavailability. Around the same period, there began to appear studies involving a number of different tests performed under varying conditions, suggesting that disintegration times <u>in-vitro</u> may not give a clear indication of physiological availability. This in effect increased awareness of the potential of dissolution rate studies as indicators of physiological availability.

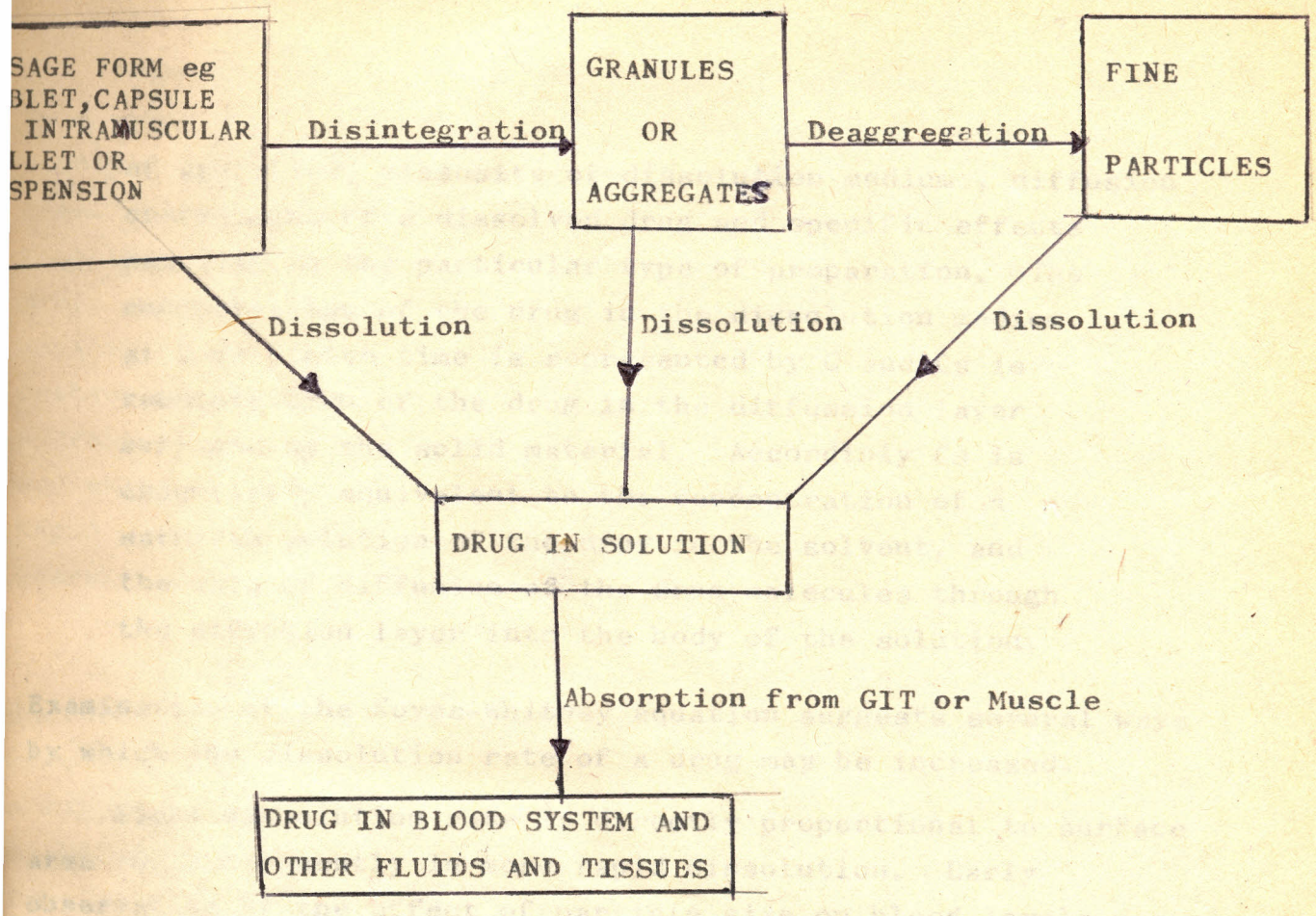
Dissolution testing will only assure of drug availability if a correlation can be established between in-vivo and in-vitro data. Where such relationship exists dissolution testing becomes an important tool in assessing a drug product quality and also it can be used as an indicator of drug bioavailability. There are several advantages associated with in-vitro dissolution testing, i.e. unlike in-vivo analysis, the in-vitro tests are easier, less tedious and are run over a shorter duration of time.

Disintegration Test: (B.P.1980)

Disintegration may be defined as the process by which the tablet breaks up into small fragments and aggregates of powder. Deaggregation of the aggregates normally result in formation of fine particles of the drug. In routine production, disintegration test is carried out as a quality control rather than a test for physiological availability of the drug in the body and cannot be correlated with in-vivo urinary excretion as is pointed out under dissolution.

Dissolution Test

It is axiomatic that a drug preparation shall be therapeutically effective yet only in the recent past has it been realized how wide may be the margin between potential activity as inferred from the known drug content and the actual activity observed in clinical use. Many variations of biological activity of a given drug substance are brought about by the rate at which it becomes available to the organism. In many instances, dissolution rate, or the time it takes for the drug to dissolve in the fluids at absorption site, is the rate limiting step in the absorption process. This is true for drugs administered orally in solid forms such as tablets, capsules or suspensions, as well as drugs administered intramuscularly in the form of pellets or suspensions. The scheme shown below shows the various processes occuring prior to absorption of a drug from its dosage form (S.KARGER,1979).



From the above scheme it can be concluded that dissolution of drug from its formulation occurs not only from the fine particles of the drug that are ultimately produced, but also to a small extent from the intact dosage form and from fragments and agglomerates produced after disintegration.

When dissolution rate is the rate-limiting step, anything which affects it will also affect absorption. Consequently, dissolution rate can affect the onset, intensity and duration of response, and control the overall bioavailability of the drug from the dosage form.

The dissolution of a substance in a non-reacting solvent may be described by the Noyes-Whitney equation shown below (NOYES et al, 1897):

$$\frac{dc}{dt} = K S (Cs - C)$$

Where the term $\frac{dc}{dt}$ is the rate of solution, S is the surface area of a dissolution solid and K is the dissolution rate constant, which includes several factors, such as intensity

of agitation, vissosity of dissolution medium , diffusion coefficient of a dissolved drug and specific effects peculiar to the particular type of preparation. The concentration of the drug in the dissolution medium at some finite time is represented by C and Cs is concentration of the drug in the diffussion layer surrounding the solid material. Accordinly Cs is essentially equivalent to the concentration of a saturate solution of the drug in the solvent, and the rate of diffusion of the drug molecules through the diffusion layer into the body of the solution.

Examination of the Noyes-Whitney equation suggests several ways by which the dissolution rate of a drug may be increased.

Since dissolution rate is directly proportional to surface area and subsequently in more rapid dissolution. Early observation of the effect of particle size on blood levels was made with several sulfa drugs, specifically, sulfadiazine and sulfaethylthiadiazole. Modern insulin preparations provide a further example of importance of particle size and polymorphic form as factors controlling the biological availability of a drug. An insulin-Zinc complex can be precipitated either as 10-40 Um crystals or as amorphous particles upto 2 Um in size. If a mixture of both types is injected the amorphous particles dissolve rapidly for prompt response which is maintained by the slower dissolution of the crystals. The proportions of each type of particle determine the release profile of the preparation and may be varied to suit individual needs of the patient.

Griseofulvin is a drug which provides one of the most striking examples of the role dissolution rate and effect of surface area play in biological availability. It was shown that the amount of griseofulvin absorbed increase linearly with an increase in specific surface area of this drug. These observations led to marketing of micronized preparation of this drug which permit a dosage reduction of 50% compared to the original forms. (ATKINSON Et al, L962). Particle size reduction can be obtained in several ways. Historically, this was accomplished by grinding or micronization. More recently, particle size reductions have been accomplished in a number of instances by the preparation of microcrystalline molecular dispersions of poorly soluble drugs

Although reducing particle size will influence dissolution in a positive direction, use of this technique to enhance absorption is limited to those cases in which absorption is dissolution-rate limited. Normally, an increase in dissolution rate is of little value for water soluble drugs or weakly basic drugs administered as their salts since their absorption is usually not dissolution-rate limited. In addition, there are instances in which particle size reduction may result in an overall decrease in bioavailability. For example, a situation may exist in which a drug is unstable in the gastric fluids. Rapid dissolution results in a more rapid degredation and an overall reduction in bioavailability.

The dissolution rate of drugs may also be increased by increasing the solubility in the diffusion layer. The most effective means of obtaining higher dissolution rates is to use a highly water soluble salt of the parent substance. Although a soluble salt of a weak acid will subsequently precipitate as the free acid in the bulk phase of an acidic solution,(such as gastric fluid) it will do so in the form of very fine particles with a large surface area. High solubility is one of the several factors affecting the dissolution rate, but this property is not always necessarily associated with a high dissolution rate. This is so since solubility refers to an equilobrium condition while dissolution is a kinetic process. Saturation is seldom reached in gastrointestinal fluids, and, since absorption and distribution processes constantly remove dissolved drug, the important factor is how rapidly the solid drug appears in solution in an absorbable and diffusable form. In case where a drug present itself in form of several polymorphs, the metastable ones will have a greater dissolution rate compared to the most stable one. The same applies to amorphous form which tends to be more soluble than the corresponding crystalline form, The state of hydration of the drug will normally have a bearance on solubility and it is normally found that anhydrous form is more soluble that hydrated form. The chemical form of the substance, that is, free acid or base or salt or ester form, can also result in significant differences in dissolution rate.

The mode of formulation and method of manufacture can effect dissolution rate. Thus for instance, the use of hydrophobic lubricants such as magnesium stearate in tablet manufacture may confer to the tablets non-wettability property, thus preventing

an adequate contact between gastrointestinal fluids and drug particles and hence the subsequent decrease in dissolution rate. The use of hydrophillic lubricants such as sodium lauryl sulfate has an opposite effect for thereis a reduction in angle of contact thus promoting wettability of the particles. A notable example of the influence of formulation aspects on dissolution rate is provided by phenytoin, the antiepileptic drug. It was found that incorporation of lactose as a diluent in capsulation of the drug resulted in attainment of toxic blood level of the drug indicating that lactose indeed influenced the rate of dissolution (TYRER Et,al,1970).

In-Vivo Urinary Excretion:

This can be used as a method of determining bioavailability of various brand of a drug product. The term bioavailability as applied to a formulation of a drug means in its limited form, the relative absorption efficiency of the formulation. A broader definition (suggested by METZLER C.M. I974) refers to the study of the factors which influence and determine the amount of active drug which gets from the administered dose to the site of pharmocologic action as well as the rate at which it gets there. In general practise, bioavailability refers both to the relative amount of drug that reaches the circulatory blood and the rate at which the drug appears to blood stream and hence in the urine. A knowledge of bioavailability of a drug is important during the development, formulation, evaluation of dosage forms as well as in drug product selection.

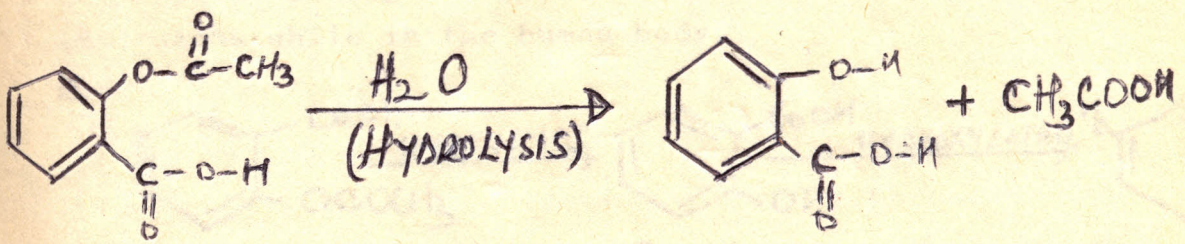
The concentration of the drug in the immediate vicinity of the receptor sites can rarely be measured direct but usually an equilibrium is established between this concentration and that of the drug circulating in the blood stream. After attainment of this equilibrium, measurements of plasma level of the drug reflects both the drug concentration at the receptor sites and the pharmacological activity of the drug. It can be shown using pharmacokinetic data from the rate of urinary excretion of a drug that the rate at which unchanged drug is excreted in urine is directly proportional to the concentration or amount of drug present in the body, and

that changes in urinary excretion reflect changes in the concentration of drug in plasma or amount of drug in the body(CHULSKI T Et al, 1963).

Since the largest proportion of the aspirin is excreted in urine as salicylic acid(see elsewhere), the chemical assay of the urine can be based on the measurement of the amount of salicylic acid in urine. Being phenolic,salicylic acid complexes with ferric ion producing a stable colored complex which can be measured spectrophotometrically.

Pharmacokinetics and stability of Aspirin

Aspirin finds clinical uses owing to its analgesic, non-inflammatory and antipyretic properties. Being an ester, Aspirin is susceptible to hydrolysis yielding salicylic acid and acetic acid:

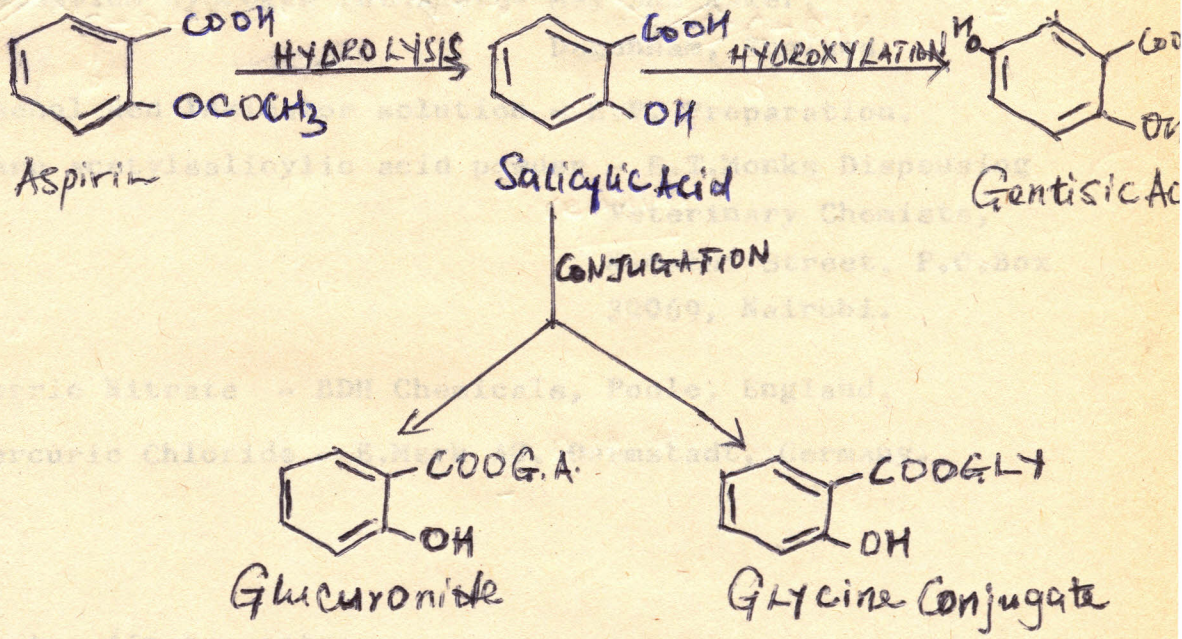


The rate of hydrolysis increases with temperature and also varies with PH in a complex manner, being minimum at PH 2-3 then increasing upto PH 5.0 but virtually remains constant from PH 5-8. When it again increases rapidly with further rise in PH (EDWARBS L.J,1952).

The absorption of aspirin can be explained by the PH-partition hypothesis of Brodie, Hogben Schanker,Shore and Tocco (1957-1964). The hypothesis holds that:- the gastrointestinal membrance acts like a lipoid sieve barrier, the unionized form of drug is preferentially absorbed, absorption occurs by passive diffusion and the rate of absorption and amount absorbed in a given time are related to O/W partition coefficient. On the basis of this hypothesis the stomach is potentially the most important site of aspirin absorption since the acidic conditions favour existence of the unionized form of the drug, whose absorption is favoured. On the other hand, aspirin is sparingly soluble at gastric PH and the rate of absorption is limited by the rate of dissolution of aspirin. Other factors that influence absorption of aspirin from the stomach include gastric emptying time and the nature of stomach contents, thus if neutralised or alkaline it is noted that absorption is greatly reduced due to conversion of aspirin to its, ionized form(CARNOT P. 1932).

In the intestines the conditions are less acidic but aspirin is more soluble and aspirin will thus dissolve more rapidly. The extended mucosal surface together with peristathic movements are further factors which facilitate most of absorption to be in the intestines.

Aspirin undergoes the following metabolic conversions while in the human body.



Aspirin is rapidly eliminated from the body. This is almost entirely due to its rapid hydrolysis to salicylic acid by enzymes present in many tissues including blood, for the urinary excretion of aspirin accounts for only 1-2% of the dose taken (CUMMINGS Et al,1966). The rate of excretion of aspirin varies with the PH of urine, increasing as the PH rises and being greatest at PH 7.5 and above. Aspirin is excreted in urine in the following forms and proportions: Salicylic acid 10% Gentisic acid 5% Clycine conjugate 60% and Glucuromide conjugate 25%.

EXPERIMENTAL WORK

Materials and Methods

I. O.INHCL & O.5NHCL, prepared from II.8NHCL Supplied by May and Baker Ltd, Dagenham, England,

2. Potassium Hydrogen Phthalate- May and Baker, Dagenham, England

3. Phenol Red Indicator solution - B.P. Preparation.

4. Pure acetylsalicylic acid powder - E.T.Monks Dispensing Veterinary Chemists, Kimathi Street, P.O.Box 30069, Nairobi.

5. Ferric Nitrate - BDH Chemicals, Poole, England.

6. Mercuric Chloride - E.Merk AG. Darmstadt, Germany.

Apparatus /Instruments:

I. Spectronic 20 - Arthur H.Thomas Co. Scientific Apparatus, Philadelphia, PA, U.S.A.

2. Spectronic 21 - Bausch & Lomb, U.S.A.

3. Dissolution apparatus - Erweka Apparatebau, G.M.B.H, W.Germany.

Materials

I. Acetylsalicylic acid tablets.

2. Adult male rabbits.

Analysis to determine Acetyl salicylic Acid content in the four Brands A,B,C AND D

(a) Preparation of analytical reagents

(i) 0.5N HaOH (1000ml)

I Mole NaOH = 40g

$\frac{1}{2}$Mole NaOH = 20g

Thus to produce 0.5N NaOH solution, 20g of NaOH pellets were dissolved in distilled water and the resulting solution made up to 1 litre in a I000ml volumetric flask.

(ii) 0.1 N HCL (I000ml)

$N_1V_1 = N_2V_2 \quad \therefore \quad V_1 = \frac{N_2V_2}{N_1}$

Where V_1=Vol of conc acid of normality N_1 required to be diluted to produce a dilution of volume V_2 having a normality of N_2.

Normality of conc. Hcl used N_1 = 11.8N

Expected volume of dilution, V_2 = 1000mls

Expected normality of dilution, N_2 = 0.5N

$\therefore$ Vol of conc. Hcl required $V_1 = \frac{0.5 \times 1000\text{mls}}{11.8}$

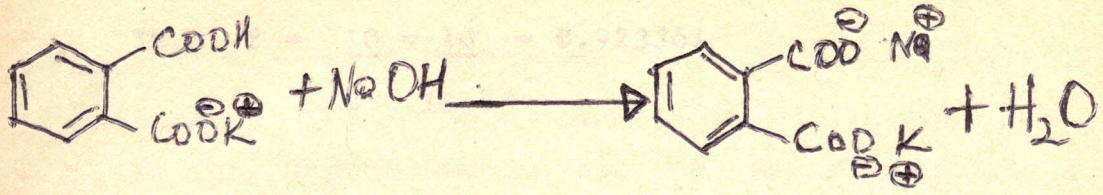
= 42.37mls

Thus 42.37mls of the acid were diluted to 1000mls with distilled water in a 1000ml volumetric flask.

(b) Standardization of the analytical reagents.

The primary standard against which the two reagents prepared as above were standardized was Potassium Acid Phthalate.

The reaction between NaOH and the latter can be depocted as follows:-



Thus stoichiometrically,

I mole of potassium acid phthalate(KAPh)= Imole of NaOH
or 204.23g of KAPh = 1000mls of IN NaOH
or 102.115g of KAPh = 1000mls of 0.5N NaOH
Thus 1.02115g of KAPh = 10mls of 0.5N NaOH

I.02115g of KAPh was accurately weighed out and dissolved in 50 ml of distilled water in a conical flask. Using phenophthalein as the indicator, the solution in the flask was titrated against 0.5N NaOH solution to be standardized. Titration results:

		I	II
Final Burette Reading	(Mls)	IO.80	22.85
Initial Burette Reading	(Mls)	0.00	12.00
0.5N NaOH titre	(Mls	10.80	10.85

∴ mean titre = $\frac{(10.80 + 10.85)\ \text{mls}}{2}$ = 10.83mls

Volume correction factor (F) for 0.5N NaOH = $\frac{1.02115 \times 10}{1.02115 \times t} = \frac{10}{t}$

where t is experimentally obtained titre.

Thus $f = \frac{10}{t} = \frac{10}{10.83} = 0.923361$

In a similar titration, 10mls of 0.5N Hcl were found to consume 10.45mls of the 0.5N NaOH solution.

Using the formula $f_1 N_1 V_1 = f_2 N_2 V_2$, the volume correction factor (f_2) for the 0.5N Hcl can be calculated.

Thus, $f_2 = \frac{f_1 N_1 V_1}{N_2 V_2}$

where f_1 = volume correction factor for the 0.5N NaOH

$N_1 V_1$ = Normality of NaOH and its volume, respectively,

and

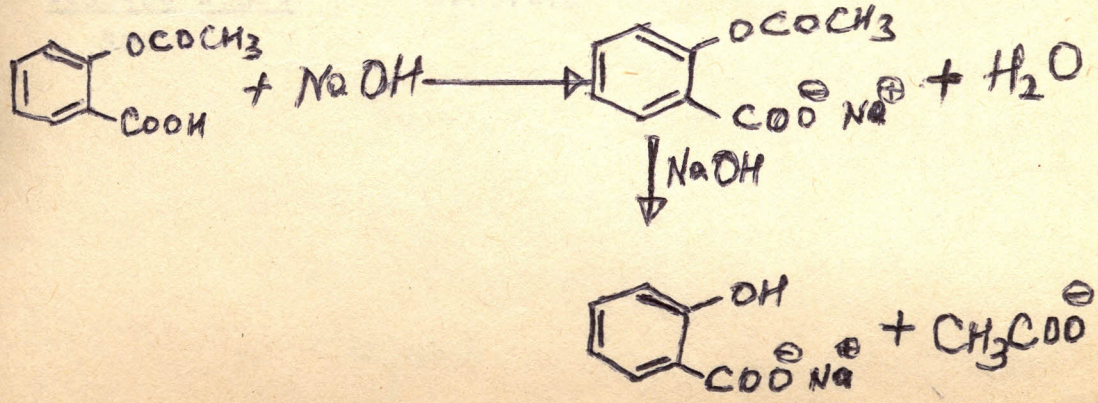
N_2 and V_2 = Normality of HCL and its volume, respectively.

$$f_2 = \frac{0.923361 \times 0.5N \times 10.45mls}{0.5N \times 10mls}$$

$$= 0.964912.$$

(c) Assay of acetylsalicylic acid content by B.P method - Hydrolysis followed by Backtitration

Acetyl salicylic acid dissolves readily in dilute alkaline solution and it is completely hydrolysed by boiling or heating for a few minutes on a water-bath in the presence of an excess of base,



Thus every one mole of aceylsalicylic acid is stoichiometrically uivalent to two moles of NaOH. For a given brand, 20 tablets re randomly selected, weighed and powdered by use of a pestle and rtar. To a quantity of the powder equivalent to 0.5g of etylsalicylic acid, 30 mls of 0.5N NaOH solution were added. e flask contents were boiled gently over a water-bath for some minutes. The cold flask contents were titrated with 0.5N Hcl ing phenol red as the indicator.

The operation was repeated without the powder, the difference tween the titrations representing the amount of 0.5N NaOH lution required by the acetylsalicylic acid.

From the chemical equation given earlier, it can be shown at,

IML OF 0.5N NaOH = 0.04504g of acetylsalicylic acid

determination were made for each of the 4 brands.

lay out of calculation as exemplified by results obtained

brand A

Wt of 20 tablets + container	g	7.4227
Wt of 'Empty ' Container	g	0.7355
Wt of the 20 tablets	g	6.6872

Wt of each tablet = 6.6872/20 = 0.334369 g

Manufacturer's label claimed each tablet contained 300mg of acetylsalicylic acid.

amount of powder with approximately 0.5g of acetylsalicylic acid = $\frac{0.33436 \times 500}{300}$ = 0.55727g

		I	II
Wt of container + Powder sample	(g)	11.2940	11.2942
Wt of 'Empty' container	(g)	10.7361	10.7359
∴ Wt of powder used	(g)	0.5579	0.5583
Final Burette Reading	(mls)	17.20	34.25
Initial Burette Reading	(mls)	0.00	17.20
∴ 0.5N Hcl Titre	(mls)	17.20	17.05

In addition, 0.5N Hcl titre for 30mls of the 0.5N NaOH was found to be 28.71 mls.

For brand A sample I:

Vol of 0.5N NaOH solution required = (28.71 -17.20)mls = 11.51 mls).

∴ corrected volume of 0.5N NaOH = 11.51 X 0.923361 mls

= 10.63 mls.

∴ Acetylsalicylic acid content per tablet =

$$\frac{10.63 \times 0.04504 \times 0.33436g}{0.5579}$$

$$= 0.28689$$

Thus % label claim = $\frac{286.8 \text{ mg} \times 100}{300\text{mg}} = 95.61\%$

Similarly:

% label claim for brand A sample II

$$= \frac{(28.71-17.05) \times 0.923361 \times 0.04504 \times 0.33436\%}{0.5583 \times 0.5}$$

$$= 96.8\%.$$

∴ mean % label claim for brand A

$$= (95.61 + 96.8)\%/2$$

$$= \frac{192.41\%}{2}$$

The other 3 brands B,C and D were treated in the same way and the results entered in table I cited under "RESULTS".

In Vitro Drug Dissolution

The official dissolution method of N.P. and U.S.P was used. In this method, otherwise known as the Rotating Basket Method, the operating conditions were as follows:

(i) One litre of 0.1N Hcl, employed as the dissolution medium.

(11) The temperature of the dissolution medium was kept at $37 \pm 0.5^{o}C$.

(111) The basket was set to rotate at 50 R.P.M.

After switching on the machine following introduction of a tablet inside the basket, 5 mls samples of dissolution medium were withdrawn after 5min, 10min, 20 min, 30 min, 40 min..... and so on. This withdrawal was facilitated by use of a syringe and each was accompanied by introduction of 5 mls of fresh dissolution medium to replace the 5 mls that had been drawn out.

The samples were assayed for acetylsalicylic acid content spectrophotometrically. The assay involved diluting the 5 mls sample with 0.1N Hcl to give readings within the sensitive range for the spectrometer (spectronic 21). The absorbance of the solution were read at $\lambda = 277nm$, the wavelength at which acetylsalicylic acid has an absorption maximum. The absorbance values were converted to amounts by use of calibration curve.

In the construction of the calibration curve, a stock solution containing 0.2g of acetylsalicylic acid in 1000mls of 0.1 N Hcl was prepared by dissoving 0.2g of pure acetylsalicylic acid powder in the 0.1N Hcl, marking up to 1000mls in a 1000mls volumetric flask.

The stock solution was diluted with 0.IN Hcl to produce the following dilutions.

Final dilution mg acetylsalicylic acid per 100ml	Volume (mls) of stock solution Taken	Volume (mls) of 0.IN Hcl required to mark upto 100mls
1	5	95
3	15	85
6	30	70
9	45	55
12	60	40
15	75	25

The absorbance of each dilution was read at 277nm using spectonic 21.

The results are shown in table 3.

A plot of absorbance versus concentration yield a calibration curve(fig.1).

In Vivo Dissolution

A day before the experiment, urine samples were collected over a period of six hours. The samples were pooled together to give the blank sample. After an overnight fasting of the rabbit 40 mls of warm water(37^{o}c) was used to make a suspension of a crushed tablet and the latter was introduced into the stomach with the aid of a syringe and a piece of rubber tubing. Further 30mls of warm water in portions of 10mls was used to wash into the stomach any left-over particles in the syringe and the rubber tubing. The time for the last washing was taken to be the reference time zero. For effective hydration of the animal a further 20mls was administered at hourly interval.

Through the marginal vein of the ear 50% urethane solution was administered gradually until the depth of anaesthesia was sufficient enough to allow catheterization of the bladder. At half-hour interval, urine samples were collected and after noting the volume collected 10mls were transfered to bottles pending analysis. The 10ml, samples werestored in a refrigerator until all samples were collected.

Chemical Analysis of Salicylate from Urine:

Aspirin is rapidly metabolized in the body and excreted into the urine, in the form of salicylic acid (10%),gentisic acid (5%), glycine conjugate (60%) and glucuronide (25%) (see elsewhere).

The analysis of the phenolic group which reacts with the ferric ion and does not include the determination of the glucuronide.

The colour developing reagent was prepared in accordance to the following formula:

Mercuric Chloride	8.0g
Ferric Nitrate	8.0g
n Hcl	24.0mls
Distilled water to	200.0mls

I ml of urine was pipetted into a centrifuge tube and to this was added 5 mls of colour developing reagent. The tube was centrifuged at 1000g for 10 minutes. The supernatant was

transferred to a tube and its absorbance read against the blank spectronic 20 at 540nm. Too concentrated samples were diluted to reach an acceptable absorbance value (0.15 to 0.7), taking note of the dilution factor.

RESULTS

Table I : Chemical assay of acetylsalicylic acid content in thetablets by B.P.method.

Brand	% Label claim Ist Determination	2nd Determination	mean
A	95.61	96.80	96.21
B	94.52	95.05	94.79
C	95.22	96.14	95.68
D	94.62	94.84	94.74

Table 2: Disintegration times of the tablets obtained by the official B.P.method.

Brand	Disintegration time in seconds Ist Determination	2nd Determination	mean
A	64.0	61.0	62.5
B	20.0	24.0	22.0
C	11.0	13.0	12.0
D	14.0	12.5	13.5

Table 3: Concentration of pure acetylsalicylic acid an corresponding absorbance. The data gave the calibration curve for In-Vitro dissolution determination.

Concentration of pure Acetylsalicylic(mg/100ml)	Absorbance at 277nm
1	0.060
3	0.160
6	0.340
9	0.520
12	0.800
15	0.830

Table 4: In Vitro Dissolution Data for Brand A

ng	Dilution factor	Absorbance λ = 277nm 1st Determination	Absorbance λ = 277nm 2nd Determination	Mean Absorbance	Conc Aspirin (mg/100ml)	Conc Aspirin X Dilution factor (mg/100ml)	Amount of Aspirin in 5mls sample (mg)	Amount of Aspirin in the flask (mg)	Cumulative Aspirin Release (mg)
	1	0.120	0.105	0.110	2.0	2.0	0.10	20.0	20
	1	0.200	0.220	0.210	3.7	3.7	0.19	37.0	37.10
	2	0.265	0.260	0.260	4.6	9.2	0.46	92.0	92.29
	2	0.405	0.400	0.400	7.1	14.2	0.71	142.0	142.75
	2	0.435	0.430	0.435	7.7	15.4	0.77	154.0	155.46
	2	0.505	0.490	0.500	8.8	17.6	0.88	176.0	178.23
	2	0.510	0.500	0.505	8.9	17.8	0.89	178.0	181.11
	2	0.515	0.520	0.515	9.1	18.2	0.91	182.0	186.00
	2	0.525	0.520	0.520	9.2	18.4	0.92	184.0	188.91
	2	0.535	0.525	0.530	9.3	18.6	0.93	186.0	191.83
	2	0.540	0.545	0.540	9.5	19.0	0.95	190.0	196.76
	2	0.535	0.545	0.540	9.5	19.0	0.95	190.0	197.71
	2	0.545	0.550	0.545	9.6	19.2	0.96	192.0	200.67
	2	0.540	0.550	0.545	9.6	19.2	0.96	192.0	201.63

TABLE 5: IN VITRO DISSOLUTION DATA FOR BRAND D

ING E)	DILUTION FACTOR	ABSORBANCE IST DETERMINATION	λ = 277nm DETERMINATION	MEAN ABSORBANCE	CONC ASPIRIN mg/100ml	CONC ASPIRIN X DILUTION FACTOR mg/100ml	AMOUNT OF ASPIRIN IN 5MLS SAMPLE(mg)	AMOUNT OF ASPIRIN IN FLASK (MG)	CUMULATIVE ASPIRIN RELEASE (mg)
	1	0.210	0.200	0.205	3.6	3.60	0.18	36	36.0
	1	0.310	0.290	0.295	5.20	5.20	0.26	52	52.18
	3	0.190	0.195	0.190	3.40	10.20	0.51	102	102.44
	3	0.225	0.220	0.230	4.1	12.20	0.61	122	122.95
	3	0.27	0.260	0.265	4.7	14.10	0.705	141	142.56
	3	0.290	0.280	0.285	5.0	15.00	0.75	150	152.27
	3	0.300	0.295	0.295	5.2	15.60	0.78	156	150.02
	3	0.350	0.340	0.345	6.1	18.30	0.915	183	186.80
	3	0.400	0.390	0.395	7.0	21.0	1.05	210	214.71
	3	0.430	0.420	0.425	7.5	22.5	1.125	225	230.76
	3	0.460	0.460	0.465	8.1	24.3	1.225	243	249.89
	3	0.495	0.480	0.485	8.5	25.5	1.275	255	263.11
0	3	0.500	0.490	0.495	8.7	26.1	1.305	261	270.39

DILUTION FACTOR	ABSORBANCE λ = 277nm		MEAN ABSORBANCE	CONC ASPIRIN (mg/100ml	CONC ASPIRIN X DILUTION FACTOR (mg/100ml	AMOUNT OF ASPIRIN IN 5MLS SAMPLE (mg)	AMOUNT OF ASPIRIN IN THE FLASK (mg)	CUMULATIVE ASPIRIN RELEASE (mg)
	IST DETERMINATION	2 ND DETERMINATION						
1	0.140	0.140	0.140	2.5	2.5	0.125	25	25.00
3	0.095	0.090	0.095	1.8	5.4	0.270	54	54.13
3	00135	0.145	0.140	2.5	7.5	0.375	75	75.40
3	0.190	0.180	0.185	3.3	9.9	0.495	99	99.77
3	0.230	0.230	0.230	4.1	12.3	0.615	123	124.27
3	0.230	0.250	0.240	4.2	12.6	0.630	126	127.88
3	0.260	0.265	0.275	4.8	14.4	0.720	144	146.51
3	0.285	0.290	0.295	5.3	15.9	0.795	159	162.23
3	0.300	0.305	0.305	5.4	16.2	0.810	162	166.03
3	0.370	0.360	0.365	6.4	19.2	0.96	192	196.84
3	0.360	0.390	0.375	6.6	19.8	0.99	198	203.80
3	0.400	0.410	0.405	7.1	21.3	1.07	213	219.87
3	0.410	0.420	0.415	7.3	21.9	1.10	219	226.94

TABLE 7: IN VITRO DISSOLUTION DATA FOR BRAND B

DILUTION FACTOR	ABSORBANCE λ = 277nm		MEAN ABSORBANCE	CON ASPIRIN (mg/100ml)	CONC ASPIRIN X DILUTION FACTOR (mg/100ml)	AMOUNT OF ASPIRIN IN 5ml SAMPLE (mg)	AMOUNT OF ASPIRIN IN THE FLASK (mg)	CUMULATIVE ASPIRIN RELEASE (mg)
	IST DETERMINATION	2ND DETERMINATION						
1	0.185	0.175	0.180	3.2	3.2	0.16	32	32.0
3	0.120	0.120	0.120	2.1	6.3	0.32	63	63.16
3	0.200	0.205	0.205	3.6	10.8	0.54	108	108.48
3	0.305	0.310	0.310	5.5	16.5	0.83	165	166.02
3	0.415	0.400	0.410	7.2	21.6	1.08	216	217.85
3	0.480	0.460	0.470	8.3	24.9	1.25	249	251.93
3	0.500	0.490	0.495	8.8	26.4	1.32	264	268.18
3	0.510	0.505	0.510	9.0	27.0	1.35	270	275.50
3	0.510	0.510	0.510	9.0	27.0	1.35	270	276.85
3	0.515	0.520	0.520	99.2	27.6	1.38	276	284.20
3	0.525	0.525	0.525	9.25	27.8	1.39	278	287.58
3	0.530	0.530	0.530	9.30	27.9	1.40	279	289.97
3	0.530	0.535	0.535	9.4	28.2	1.41	282	294.37

TABLE IO: DATA FOR IST DETERMINATION OF URINARY EXCRETION FOR BRAND B

TIME (min)	VOL OF URINE COLLECTED (mls)	DILUTION FACTOR	ABSORBANCE AT 540nm	AMOUNT OF SALICYLATE IN THE URINE SAMPLE (mg)	CUMULATIVE AMOUNT SALICYLATE EXCRETED (mg)
30	12.7	1	0.185	2.35	2.35
60	15.4	2	0.490	15.09	17.44
90	36.3	5	0.530	96.20	113.64
120	24.9	5	0.675	84.04	197.68
150	14.3	5	0.295	21.09	218.77
180	9.0	5	0.260	11.70	230.47
210	3.5	5	0.400	7.00	237.47

LE 11: DATA FOR 2ND DETERMINATION OF URINARY EXCRETION FOR BRAND B

IME nin)	VOL OF URINE COLLECTED (mls)	DILUTION FACTOR	ABSORBANCE AT 540nm	AMOUNT OF SALICYLATE IN THE URINE SAMPLE (mg)	CUMULATIVE S AMOUNT SALICYLATE EXCRETED (mg)
)	14.7	1	0.125	1.84	1.84
)	13.5	2	0.620	16.74	18.58
)	20.0	10	0.450	90.00	108.58
)	16.0	10	0.495	79.20	187.78
)	14.0	10	0.180	25.20	212.98
)	9.1	5	0.240	10.92	223.90
)	3.8	5	0.450	8.55	232.45

TABLE 12 DATA FOR IST DETERMINATION OF URINARY EXCRETION FOR BRAND C

TIME (min)	VOL OF URINE COLLECTED (mls)	DILUTION FACTOR	ABSORBANCE AT 540nm	AMOUNT OF SALICYLATE IN THE URINE SAMPLE (mg)	CUMULATIVE AMOUNT SALICYLATE EXCRETED (mg)
30	15.0	1	0.040	0.60	0.60
60	40.0	1	0.110	4.40	5.00
90	26.0	5	0.475	61.75	66.75
120	19.5	10	0.555	108.23	174.98
150	7.0	5	0.645	22.58	197.56
180	4.5	5	0.440	9.90	207.46
210	2.5	5	0.315	3.94	211.40

BLE 13: DATA FOR 2ND DETERMINATION OF URINARY EXCRETION FOR BRAND C

IME IN)	VOL OF URINE COLLECTED (mls)	DILUTION FACTOR	ABSORBANCE AT 540nm	AMOUNT OF SALICYLATE IN THE URINE SAMPLE (mg)	CUMULATIVE AMOUNT SALICYLATE EXCRETED (mg)
30	13.5	1	0.00	0.00	0.00
60	11.0	2	0.470	10.34	10.34
90	31.8	5	0.370	58.83	67.17
20	21.5	10	0.490	105.35	174.52
50	9.0	10	0.280	25.20	199.72
80	4.8	10	0.250	12.00	211.72
10	1.6	10	0.340	5.44	217.16

ABLE I4: DATA FOR IST DETERMINATION OF URINARY EXCRETION FOR BRAND D

IME nin)	VOL OF URINE COLLECTED (mls)	DILUTION FACTOR	ABSORBANCE AT 540nm	AMOUNT OF SALICYLATE IN THE URINE SAMPLE (mg)	CUMULATIVE AMOUNT OF SALICYLATE EXCRETED (mg)
30	22.0	1	0.050	1.10	1.10
60	37.2	2	0.470	35.00	36.10
90	23.5	5	0.600	70.50	106.60
20	15.3	10	0.585	89.51	196.11
50	10.0	5	0.375	18.75	214.86
30	4.0	5	0.590	11.80	226.66
10	3.0	5	0.315	4.73	231.39

BLE 15: DATA FOR 2ND DETERMINATION OF URINARY EXCRETION FOR BRAND D

TIME (min)	VOL OF URINE COLLECTED (mls)	DILUTION FACTOR	ABSORBANCE AT 540nm	AMOUNT OF SALICYLATE IN THE URINE SAMPLE (mg)	CUMULATIVE AMOUNT OF SALICYLATE EXCRETED (mg)
30	16.0	1	0.055	0.88	0.88
60	17.0	5	0.385	32.79	33.67
90	25.3	5	0.580	72.37	107.04
20	14.7	10	0.620	91.14	198.18
50	10.2	10	0.195	19.89	218.07
80	6.4	10	0.155	9.92	227.99
10	2.5	10	0.160	4.00	231.99

TABLE 16: AREA UNDER THE CURVE OF CUMULATIVE AMOUNT SALICYLATE EXCRETED VERSUES TIME FOR THE INITIAL 210 MINUTES

	AREA UNDER THE CURVE.UNITS mg.Min			
BRAND	A	B	C	D
1st Determination	24330	27435	22800	26550
2nd Determination	19200	27570	23700	26370
Mean 1st/2nd Determination	21765	27503	23250	26460
% Relative Bioavailability	79.14	100.00	84.54	96.21

TABLE 17: A SUMMARY OF VARIOUS PARAMETERS THAT RELATES TO THE 4 BRANDS OF TABLETS

BRAND	%LABEL CLAIM	MEAN DISINTE-GRATION TIME (SECONDS)	IN-VITRO DISSOLUTION CUMULATIVE AMOUNT DISSOLVED AFTER 120MINUTES (mg)	CUMULATIVE AMOUNT EXCRETED AFTER 210 MINUTES (mg)	AREA UNDER THE CURVE EXCRETION VERSUS TIME (mg.Min)
A	96.21	62.5	201.63	206.52	21765
B	94.79	22.0	295.79	234.96	27503
C	95.68	12.0	234.04	214.28	23250
D	94.74	13.5	274.69	231.69	26460

DISCUSSION AND CONCLUSION

The chemical assay of the aspirin tablets shows that all the brands contained amounts of aspirin per tablet which are within official (B.P) requirements, i.e. 95-105% of the stated amount on the label (Table 1). The order of % label claim was 96.21, 95.68, 94.79,94.74 for brand A,C,B and D respectively.

Generic equivalents are of essense supposed to contain same amount of the same therapeutically active ingredients in the same dosage forms and must meet official requirements. The chemical assay of the tablets established that the brands contained comparable amounts of acetylsalicylic acid and this formed an important departure as any difference in bioavailability could not be explained by variation in tablets' aspirin content.

The disintegration time rank order ran as: 12.0 sec, 13.5secs, 22.0 secs and 62.5 secs for brand C,D,B and A, respectively. Thus brand C showed the fastest rate of disintegration while brand A had the slowest rate. Disintegration times are measured for a number of reasons. Thus for control purposes, disintegration time once measured can ensure that a given pharmaceutical product is essentially uniform from lot to lot. Evaluation of disintegration time may be of value in developmental aspects in guiding the pharmaceutical formulator in the preparation of optimum dosage forms of drugs for clinical trials. Thirdly but not least absorption disintegration times may be of predictive value in estimating rate(s) of absorption in-vivo and availability of drug for in man,provided a correlation of In-vitro and In-vivo results can be established as is the case where disintegration process constitute the rate-limiting step in the absorption of the drug.

Tables 4,5,6 and 7 shows that there is a significant variation in dissolution rates among the brands. These variations are well manifested in fig 2 in which cumulative amount of acetylsalicylic acid released by each of the brand is plotted against time. Brand B had an exceptionally high rate of dissolution compared to the other 3 brands. The release order was: 295.79mg,274.67mg,234.04mg and 201.63mg for brand B,D,C and A, respectively.

A comparison of this order with that obtained on % label claim i.e. A,C,B and D with 96.21%,95.68% , 94.79% and 94.74% respectively, shows that the brand with most acetylsalicylic acid per tablet did not necessarily correspond to the one having the highest dissolution rate. An explanation for this could be that all the 4 brands contained almost same amount of aspirin per tablet and the small differences in amount were statistically insignificant. The dissolution rate order does not correlate with that of disintegration time i.e. D,D,B and A with times 12.0 secs,13.5secs, 22.0secs respectively. This is not unexpected as there is no unequivocal relationship between the two processes. A number of variables that affect both disintegration and dissolution of compressed tablets include, type and concentration of disintegrant, type and concentration of lubricant, compression force, particle size, amount and type of binder, crystal form, method of incorporating the various additives and processing of the final formulation. Rapid disintegration does not necessarily imply rapid dissolution because use of large amounts of disintegrant may cause disintegration while release of drug from the aggregates of granules resulting from disintegration may be slow due to strong intragranular forces or presence of a film of hydrophobic binder around the granules.

The urinary excretion data is presented in tables 8-16. Fig 3 and 4 shows how cumulative amount of acetylsalicylic acid excreted varies with time. From the figures, all the brands appear to share a common profile and distinction among the brands was arrived at after considering the area under the curve of rate excretion for each of the brand. Table 16 shows the areas under the curve were: 27503, 26460,23250 and 21765-mg min for brand B,D,C and A respectively. Thus the order of bioavailability was B,D,C and A with IOO.00%, 96.21%, 84.54% and 79.14% bioavailability respectively. This order correlates with that of dissolution rate i.e. B,D,C and A. However, it does not correlate with that obtained for % label claim i.e. A,C,B and probably for same reasons considered under In-Vitro dissolution.

It has been shown that aspirin absorption in dissolution rate controlled and thus the correlation obtained dissolution rate and in-vivo bioavailability can be expected (Levy G,1961). The correlation may appear simple in some instances but at times it is necessary to analyse both the In-Vitro and in-vivo data statistically and select parameters for date correlation. The advantage with such treatment is that a quantitative measure of correlation can be obtained.

The detection of correlation between dissolution rates of various brands showing different dissolution rates with the In-Vivo availability shows that dissolution process is the rate-limiting step in-Vivo. Lack of correlation may mean that the in-Vitro dissolution method is incorrect or that the in-Vitro differences obtained are not significant enough to be detected in In-Vivo analysis.

In conclusion it can be said that brand B was more superior to the other 3 brands since it had the highest bioavailability. The project has illustrated that dissolution rate unlike disintegration time can be a good predictor of bioavailability of potential and existing drug products. The use of anaesthetised animal compromised the length of time over which urine was collected. This was a major drawback as not all the drug administered could be reclaimed from the urine. None-the-less the animal model provided an appropriate alternative to human-beings who indeed proved hard to present themselves as volunteers for the exercise.

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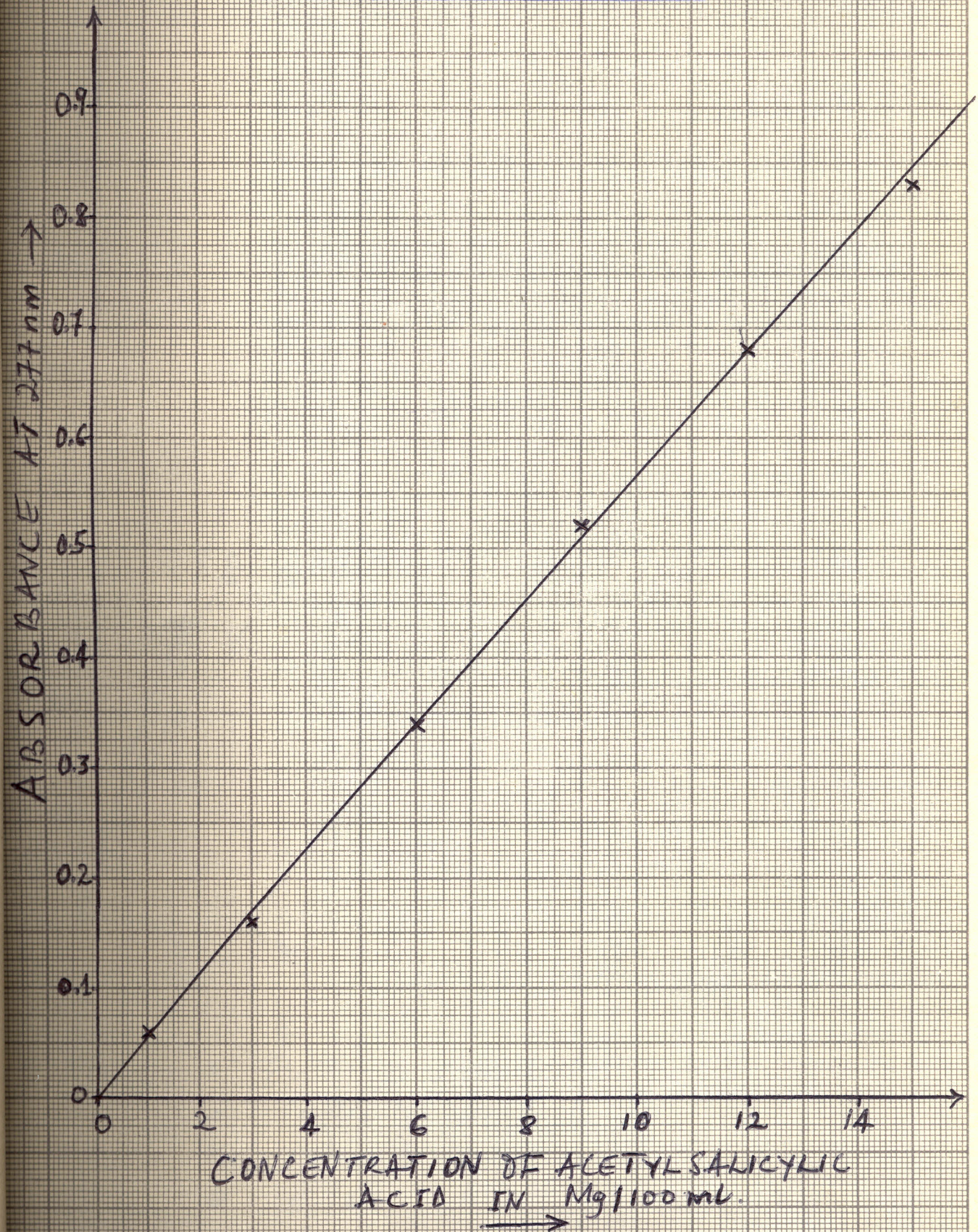
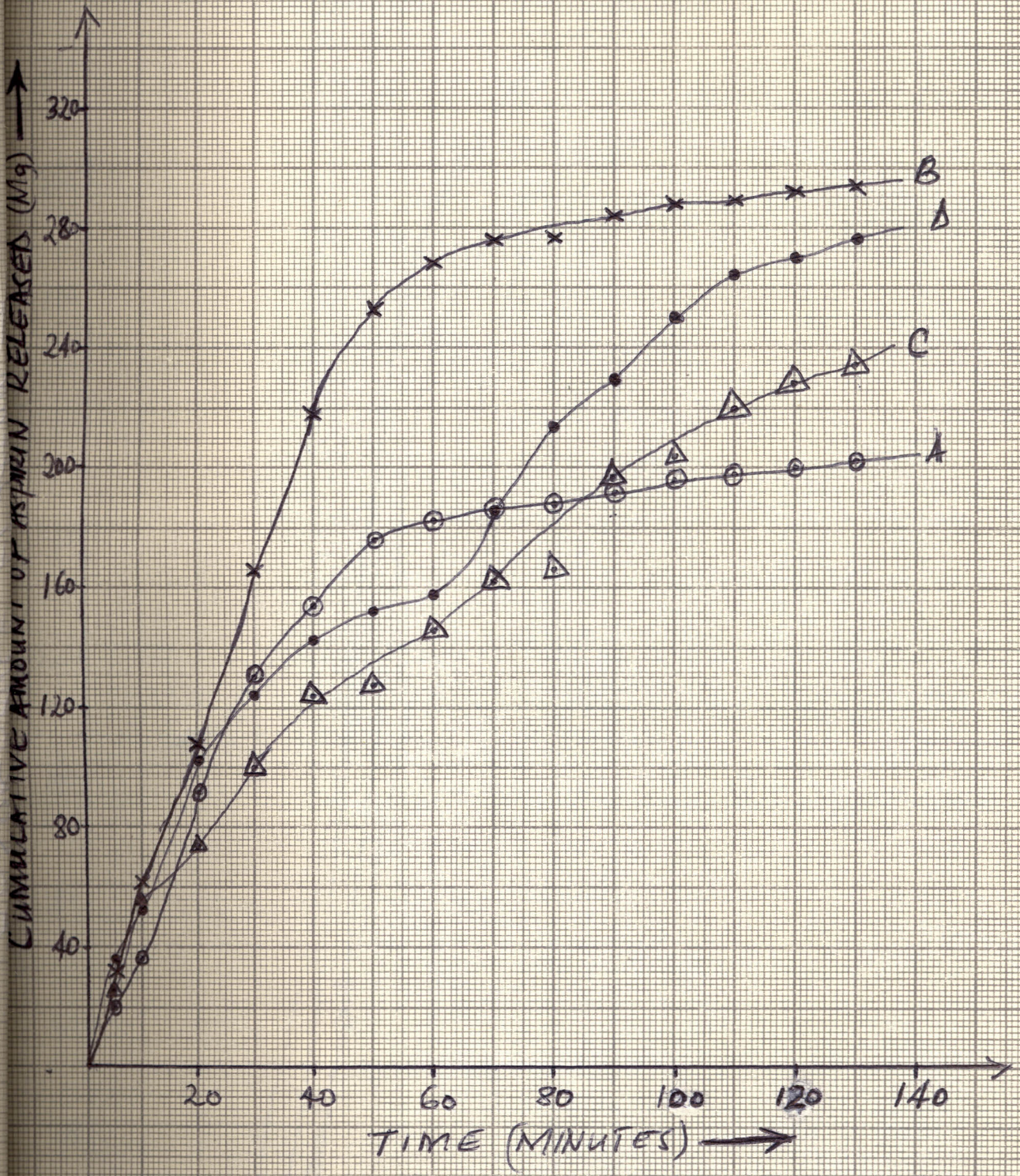
Fig. 1. CALIBRATION CURVE: ABSORBANCE AT λ=277nm VERSUS CONCENTRATION OF PURE ACETYL SALICYLIC ACID.



NB: Each single point on the graph correspond to a single determination.

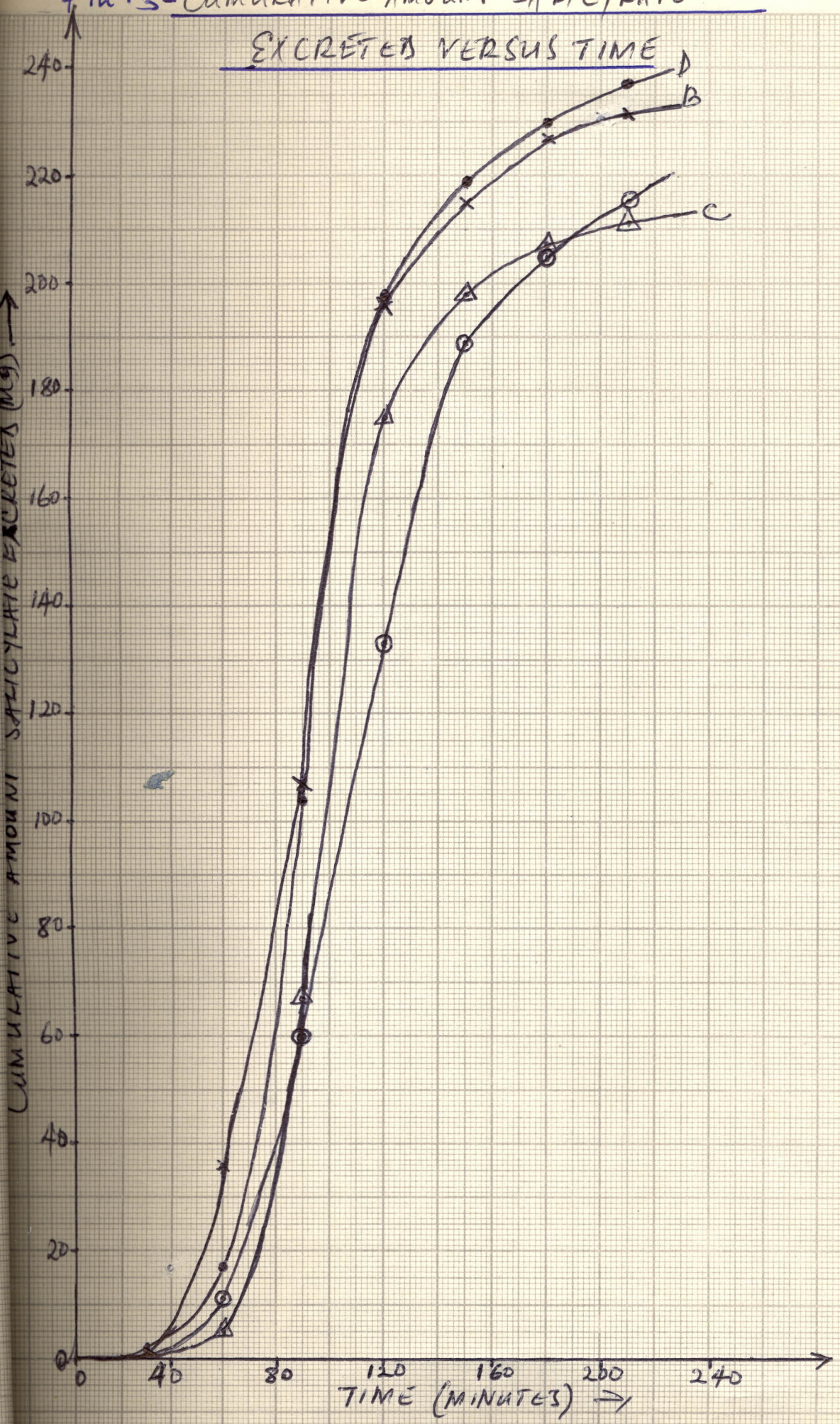
Fig 2 CUMULATIVE AMOUNT OF ACETYL SALICYLIC ACID RELEASED VERSUS TIME (FROM DISSOLUTION DATA)



NB: 1) × Refers to Brand B
● Refers to Brand D
△ Refers to Brand C and
◎ Refers to Brand A

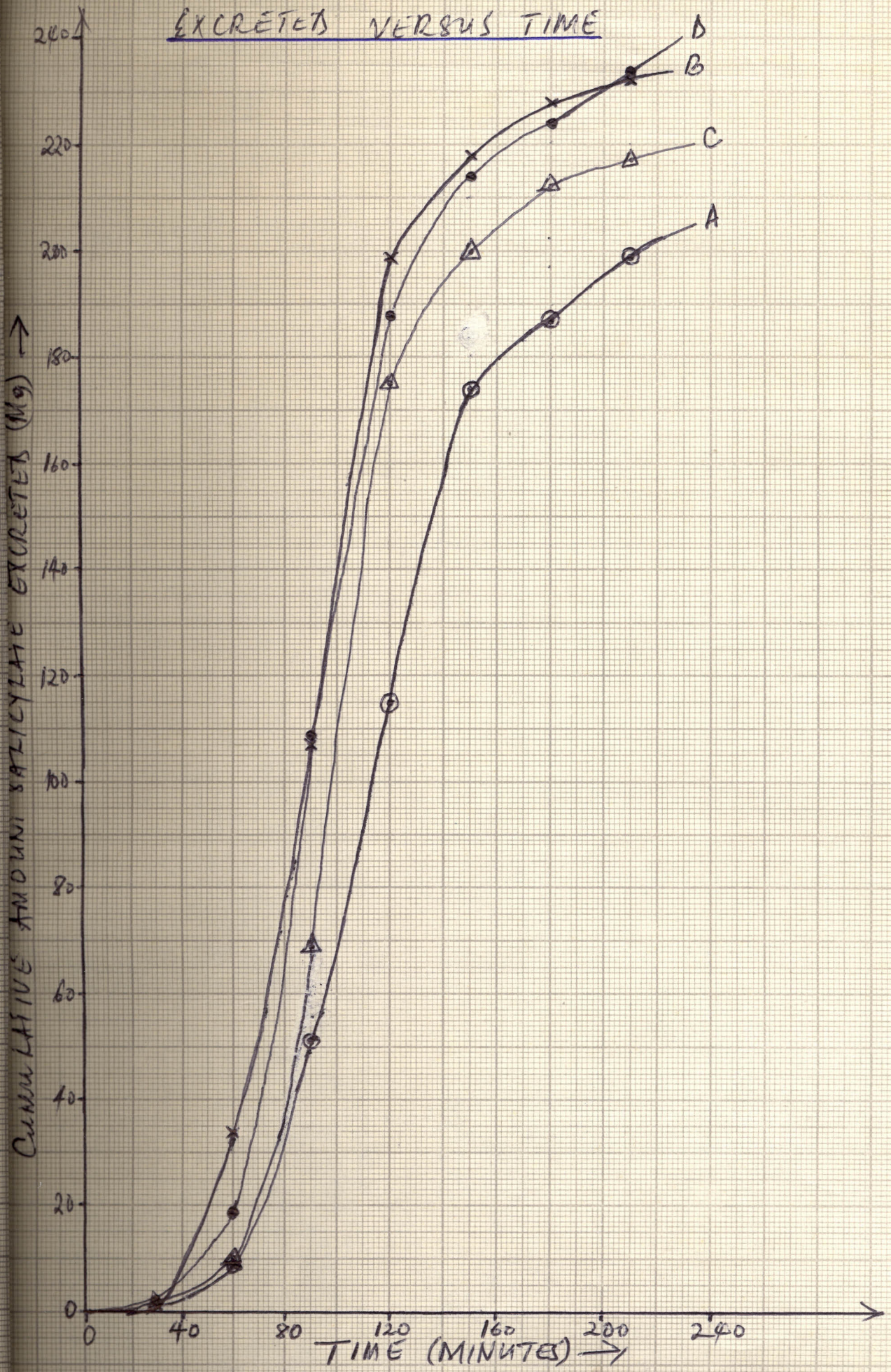
2) Each point on the graph represent a mean value for two determinations

Fig. 3 - CUMULATIVE AMOUNT SALICYLATE EXCRETED VERSUS TIME



NB: 1) ⊙ Refers to Brand A
× Refers to Brand B
△ Refers to Brand C Qud

Fig 4:- CUMULATIVE AMOUNT SALICYLATE EXCRETED VERSUS TIME



NB:1) ⊙ Refers to Brand A
x Refers to Brand B
△ Refers to Brand C and