RESEARCH PROJECT PRESENTED IN PARTIAL FULFILMENT FOR THE AWARD OF THE DEGREE OF BACHELOR OF PHARMACY OF THE UNIVERSITY OF NAIROBI.

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REFORMULATION OF ASPIRIN MIXTURE B.P.C.1963

FOR THE RURAL HEALTH CENTRES.

D E D I C A T I O N.

To My Mother, MRS. VIRGINIA WANJIKU MBATIA.

A C K N O W L E D G E M E N T S.

I am grateful to the following persons who contributed in various ways towards the success of this project.

Dr. D.G. Sixsmith, former lecturer in Pharmaceutics Department of Pharmacy for his supervision, encouragement and advice.

R.G. Mwalughu and B. Kinai, technical staff in the Department of Pharmacy.

Mr. Wambugu of the Pharmaceutics Laboratory, he unsured that my apparatus were clean and dry before I started my experiment and for his genuine concern of my experimental work.

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A B S T R A C T.

The Aspirin mixture B.P.C. 1963 is not chemically stable due to its water content. It has to be freshly prepared and this is not always easy to achieve in the rural health centres. The aim of the project was therefore to reformulate aspirin mixture B.P.C. 1963 in more stable form which is convinient to transport from the District Hospital and also more chemically stable. This was achieved by reformulating this mixture into a dry powder for reconstitution with water before use at the rural health centre.

I N T R O D U C T I O N.

One method used to provide aspirin mixture for rural health centres is to make up bulk at the apropriate District Hospital and then deliver the bulk mixture to the health centre.

This leads to several difficulties :-

a). Aspirin mixtures require to be freshly prepared as they undergo degradation in the presence of moisture (B.P.C. 1963). Using the system mentioned this is not easy to achieve.

b). Because of their high water content, the mixtures are heavy and bulky to transport.

c). The mixtures are transported in glass bottles which are in themselves heavy and require protection to prevent breakage normally in form of wooden boxes.

The combined effect of b and c above is that for the bulk mixtures, the majority of the weight is in form of packaging and water. This situation can be improved by formulating aspirin mixture as a dry powder to be reconstituted with water.

Aspirin is widely used in the rural health centres. It has the following clinical uses :-

i). Aspirin is used as an analgesic in 600 mg to 1000 mg. Up to 3.6 grams are given daily in divided doses.

The paediatric dose is :-

- One to two years : - 75 - 150 mg not more than four times daily.
- Three to five years : - 225 - 300 mg not more than three times daily.

ii). It also exhibits an anti inflammatory action. A property not shown by other mild analgexcs e.g. paracetamol or by potent analgeics e.g. pethidine (B.P.C. 1973). This property is characterized by the drug's ability to reduce shiffness and swelling of joints in rheumatoal athritis. The adult dose is :-

- In acute episodes it is 3.6 to 7.2 grams daily.
- In chronic episodes it is 300 - 900 gms every four hours over long periods.

iii). Aspirin is also used as an antipretic agent i.e. it lowers the body temperature in febrille people but it does not have this effect in normal people.

This property results in the extensive use of aspirin especially with paediatric patients who often have fevers.

Absorption of Aspirin and Elimination.

Aspirin is absorbed uncharged. The evidence for this rests on the fact that specific methods for analysis demonstrate that aspirin is present in blood for to two hours after oral administration.

Cotty and Dde ma (1966) and Rowland at all (1967) together observed that aspirin is relatively stable in GIT fluids.

The stomach is potentially an important site of aspirin absorption In the acudol conditions of the stomach, aspirin exists almost entirely in its undissociated form and in this aspect, conditions are favourable for absorption. On the other hand, aspirin is sparingly soluble in gastric P^H and the rate of absorption from the rate of absorption from the stomach is limited by the rate of dissolution of aspirin. The extent of absorption in stomach is also limited by the passage of drug from stomach to intestine (B.K. Martin. 1977).

All these factors facilities the absrophre process and its probable that absorption is more rapid the intestine than from stomach even though at higher P^H a smaller percentage of aspirin exists in undissociated form. The rate of transfer of aspirin from the stomach to the intestine probably constitute a most important and highly variable factor in determing the rate of drug absorption. The rate of drug absorption from the stomach and the rate of transfer of drug from the stomach to the intestine together determine the fraction of dose which is unabsorbed from the stomach.

Aspirin is rapidly eliminated from the body. This is almost entirely due to the rapid hydrolysis to salicylic acid by enzymes presenting many tissues including blood for the urinary excretion of aspirin accounts only I - 2 % of the dose (Cunnings & King, I966)

It was generally observed that pack blood level of aspirin are obtained very shortly after drug administration (about twenty minutes) its level then rapidly declines and simultaneously the salicylic acid level increase (Colty et al, I965). The steady decrease in proportion of aspirin to salicylic acid reflects the hydrolytic activity of the enzymes present in blood and other tissues.

The elimination half life of aspirin in man can be calculated from the data Leornards (I962) to be about I7 minutes.

Factors influencing Drug Absorption and Elimination.

Absorption :-

There are two distinct aspects of absorption.

The first concerns the physiological and biological availability of the drug from a particular oral dosage form and this reflects the extent to which the total dose is absorbed. The second is the rate of drug absorption and this determines the profile of plasma drug concentration against time.

The rate of drug absorption is determined by : -

The rate of drug absorption is determined by :-

i). The manner in which oral dosage form is administered. This modifies the rate of drug absopption by affecting the disintegration of the tablet, the dissolution of the drug, the mixing of drug with the gastric contents and the rate of transfer of drug from stomach to the intestine e.g. administration of drug before or after food.

ii). Physiological factors; GIT motility, the volume, P^H and total acidity of the gastric contents, the nature and amount of mucous and the presence, of food in the stomach are all potentially capable of modifying the rate of drug absorption. These factors lead to inter subject variation and the day - to - day variation in the same subject.

iii). Effect of formulation :- on the basis that aspirin of absorbed from solution, Edward (1951) deduced on theoretical grounds that the dissolution of aspirin in the stomach and intestine is the process that control the rate of absorption.

The rate of dissolution from the solid dosage form is related to the surface area of the drug which is affectively exposed to the gastric fluid. The intact tablet presents a small surface area but this increases considerably when the tablet disintegrates in the stomach. Rapid disintegration can therefore be seen as a normal and desirable requirement for rapid dissolution of drug (B.K. Martin (1971).

Rapid disintegration of a tablet however is certainly no indication of complete physiological availability.

-Decrease in particle size of aspirin provides a greater surface area and therefore increase rate of dissolution thereby increasing rate of drug absorption.

-Commings and Martin (I97I) compared rate of absorption of aspirin in relation to particle size.

-Formulating the drug is a suitable amount of alkali or antiacid. These formulations are the buffered aspirin type. Leornards (I963) and Morgan and Tnuitt (I965) have been shown that the formulation of drug in alkali increases its dissolution.

-Absorption os aspirin proceeds more rapidly when administered in solution since any solid dosage from passes to solution before absorption can the place. Soluble aspirin tablets or effervescent aspirin tablets enable aspirin to be administered in solutions.

The rate of absorption of aspirin can also be decreased by lestricting dissolution in GIT fluids and this is the basis of formulation of sustained release aspirin preparations.

Elimination.

Any factor in the formulation which alters distribution and elimination will reflect the drug plasma levels. This can results from the presence of certain addatives or other drugs in formulation.

-Rate of excretion of sellicylic acid is highly depends on urinary P^H. Administration of aspirin with solution bicarbonate which increases urinary P^H increases rate of elimination of salicylic acid. Macpherson et al (I955).

-Combination of aspirin with another drug may have a number of important consequences. Both aspirin and salicylic acid are bound to plasma protein and the coadministration of another drug with a high affinity for the same sites can displace some of the salicylic causing an increase in free salicylic acid. This increases the rate of elimination (B.K. Martin (I97I) other drugs sharing a vommon metabolic path with salicylic acid can competively inhabit metabolite formation and reduce rate of elimination of salicylic acid (B.K. Martin (I97I).

- Levy and Procknal (1968) have reported that salicylamide competes with salicylic acid for glucuronide formation and that the salicylamide decrease the rate of elimination of salicylic acid.

STABILITY OF ASPIRIN.

The modern trend is to formulate aspirin almost exclusively in solid dosage form. This can be commended in terms of accuracy of dosage and endorsed by virtue of the instability of aspirin in water as solutions and suspensions. However aspirin must in certain cases be formulated as a suspension.

A suspension is a two phase system of finely divided solid dispersed in a liquid. Flocculated suspensions are most stable. The particles are physically bonded together to form a loose semi rigid structure. Sedimenting particles can be easily redistributed with moderate shaking.

In non-flocculated suspensions, particles are invidiuals. They settle slowly and are hard to redisperse after settling. This type of suspensions are made more acceptable by decreasing the particle size of the suspended matter or by increasing the viscosity of the vehicle. (Remmingtons Pharmaceutical Sciences Ist Edn.).

The knowledge of stability of a product or formulation is important for the following reasons:-

i). A pharmaceutical product must appear fresh, elegant and professional no matter how long it remains on the shelf. Any changes in physical appearance such as colour fading haziness can make the patient loose confidence in the product.

ii). Some products are dispensed in multiple dose containers, uniformity of dose content of the active ingredient must be assured over a time. In other words, the active ingredient must be available to the patient throughout the expected shelf life.

-This is because with time, the activity of the product is reduced to below some arbitrary labelled content or a toxic degradation product may appear upon storage of the formulation.

iii). Most pharmaceutical products have to be legally accepted as regards strength, purity and quality. They have to comply with specifications laid down by official compendia

iv). Stability testing is useful before marketing of drug. If an unstable product is marketed it will eventually have to be withdrawn from the market and this is a bad advertisement for the manufacturer and also considerable financial loss.

As regards aspirin, some chemical and physical factors influence its stability.

The physical factors include:-

i). Temperature :- Increase in temperature results in increase in rate of reaction.

ii). Moisture :- Moisture absorbed on the surface of a solid drug often increases the rate of hydrolysis.

iii). Light.

iv). Radiation.

The chemical factors that influence stability are :-

v). Hydrolysis :- This is the major cause of instability of aspirin suspensions and solutions. The water content in these formulations enhances the hydrolysis.

Aspirin is a prodrug of salicylic acid. Salicylic acid possesed unpleasant side effects especially GIT distrubance.

To reduce this hydrolysis the PH of the suspension can be adjusted to 2 - 5.

Other attempts to increase its stability when formulated in liquid dosage forms have been worked on by many people :-

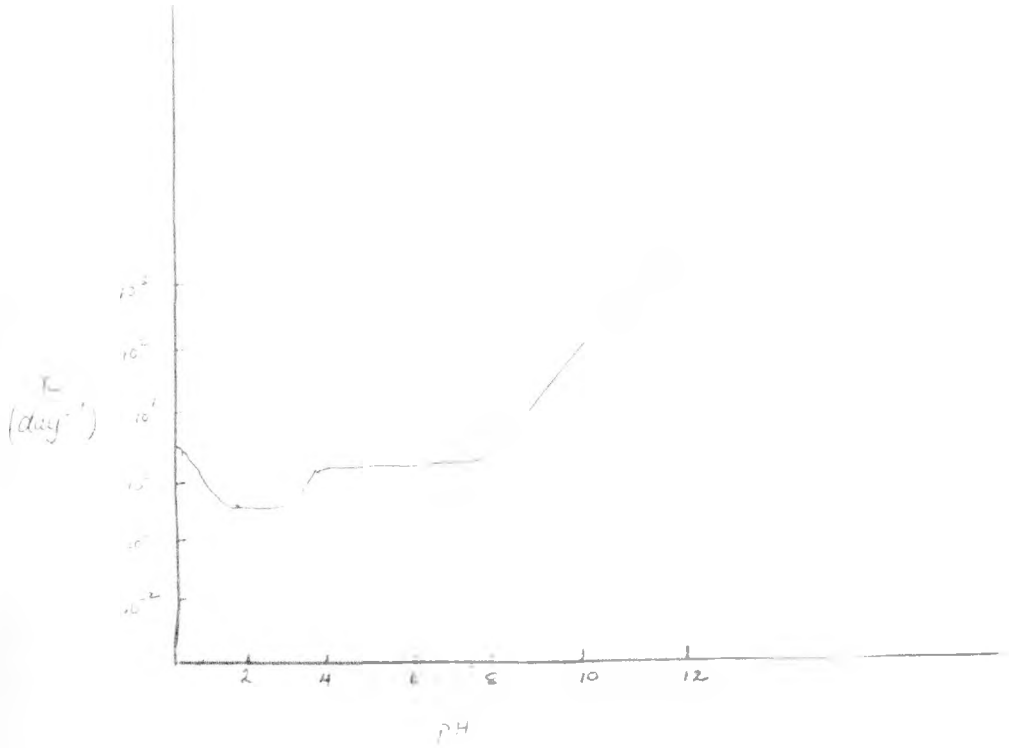
Aspirin is an acid with a PKA of 3.45. The percentage of drug ionized in a solution at any particular PH may be calculated from the equation :-

$$\text{Percentage ionized} = \frac{100}{1 + \text{antilog}\,(Pka - PH)}.$$

Thus increase in PH results in an increase in the percentage of aspirin ionized. Change in PH form 6.5 to 7.5 has little effect on the percentage ionized but it has a major effect on the percentage unionized. B.K. Martin (1971).

Aspirin is also an ester which is readily hydrolyzed in solution. The rate of hydrolysis increases with temperature and also varies with PH in a complex manner. Hydrolytic cleavage of aspirin to salicylic acid and acetic acid was divided by Edwards (I950) who obtained the PH rate profile as shown :-

FIGURE I: Variation of the rate contant K for the hydrolysis of aspirin with PH at $20^{0}C$.



The unusual PH rate profile was attributed to as a reaction of the form :-

$$-\frac{dc}{dt} = K(H^{+})(HA) + K_2\ (Ht(A^{-}) + K_3\ (HH^{-})(A^{-}) + K_0(A^{-})$$

Where HA represents undissociated aspirin and A^{-} represents aspirin (Anion).

The rate of hydrolysis is minimum in the region of PH 2.5. The PH independent anion hydrolysis indicated in the PH region of 5 - 9 has been attributed to intramolecular catalysis by ortho carboxylic anion, rather than the general acid base catalysis in water. Remmingtons pg 281.

The intramolecular catalysis which is responsible for the high instability of aqueous solutions of aspirin is in the pharmaceutically useful range.

Fersht and Kiroy (1967) represented the intramolecular carboxylated on reaction as a general base catalysis of attack of water molecule.

Whitworth et al (1973) reasoned that an aspirin solution prepared in polyethylene, glycol solvent containing no free OH groups would provide an aspirin solution of improved stability. They used acetylated PEG 400 as a solvent for aspirin and demonstrated that in such a solvent less than I % aspirin loss occured after 40 days at 45^{0} C.

W.T. Wing (I956) also showed that a I2.5% solution of aspirin in dehydrated alcohol was stable for at least two years. The presence of small amounts (up to I %) of H_2O in the alcoholic solution results in slow hydrolysis about 4 % after two years storage.

S.M. Blaug and J.W. Wesolowski (I957) showed that the addition of 50 % w/v of crystalline sorbitol had a stabilizing action on aspirin suspensions. The half life of a suspension containing 6.5 % w/v of aspirin was raised from I748 hours to 3396 hours at 25^{0}C. Solutions buffered at PH 3 were less stable than at PH 2.5.

Thus the nature of the solvent is important. Production of an insoluble drug form and the presence of surface active agents also reduces hydrolysis (Remingtons I5th Edition).

Other forms of degradation are :-

i). Oxidation; presence of antioxidants is therefore important. Examples of antioxidants are sodium metabisulphite or sulphite ion.

ii). Microbial contamination :- thus preservatives are important to inhibit growth of those fungi and other micro organisms which would be introduced during preparation and use. Preservatives used are benzoic acid, this is useful for products at a PH of less than 5, chloroform, parahydroxy benzoates.

iii). Incompatibilities, this will come about due to reaction between active ingredients and other constituents of the preparation e.g. if aspirin is preserved with hydroxybenzoates, there would be incompatibilities because hydroxybenzoates are useful at PH of 2.3

Because of all these instabilities, the method of choice of formulation of aspirin is the solid dosage form. The use of aspirin in very young children and probably very old people however makes it necessary to formulate aspirin as a suspension. The rural health centres therefore encounter the problem of instability due to the high water content in the mixture as they are dispensed from the District Hospitals. The aim of the project is an attempt to improve this situation.

To overcome the stability problem dispensing aspirin mixture as dry powders in the District Hospitals to be stored as such at the health centres should greatly increase the shelf life of the product.

The aspirin mixture B.P.C. 1963 was therefore reformulated in a suitable form for dispensing as a dry powder to be reconstituted by shaking with water at the health centre when required. These powders can be dispensed in 2 litres plastic containers readily available in large quantities from hospitals. Dispensing as a dry powder in a plastic container dramatically improves the situation in terms of product stability and transport difficulties. The practical part of the project involves two main aspects :-

a). The assessment of physical stability in term of sedimentation and also visual observation and chemical stability of normal and accerlated stability testing of a standard aspirin mixture B.P.C. 1963, an aspirin solution and a dry powder aspirin preparation.

b). Reformulation and stability testing of the dry powder preparation in an attempt to produce a dry mixture which resuspends into an elegant chemically and physically stable mixture and redisperses merely by addition of water and manually shaking.

M A T E R I A L S A N D M E T H O D S.

MATERIALS.

Chemicals.

i). Aspirin powder - B.P. Grade (E.T. Monks)

ii). Sodium carooxy methlycellulose - laboratory Reagent (Howse & MacGeorge).

iii). Tragacanth powder - Laboratory Reagent (Kobian Limited).

iv). Sodium Benzoate - Laboratory Reagent (Howse & MacGeorge).

v). Starch - Laboratory chemical (Lay & Baker Ltd).

vi). Chloroform - Laboratory Reagent (Kobian Ltd).

vii). Sodium hydroxide pellets - Laboratory Reagent (GPR). (Howse & MacGeorge).

viii). Hydrochloric acid - General purpose Reagent (B.P.H chemicals Ltd Poole England).

ix). Phenolphthalein Indicator - BOH Chemicals Ltd.

x). Phenol red indicator - BOH Chemicals Ltd.

xi). Distilled water - prepared in the laboratory.

Equipments.

i). Water baths.

ii). Mortar & Pestle

iii). conical flusks.

iv). Beakers.

v). Measuring cylinders.

vi). Volumetric flasks.

vii). Stirring rods

viii). Aluminium foil.

x). Stand.

xi). Burrettes

xii). Glass slab

xiv). Filter funnel.

xv). Filter papers.

METHODS.

Preparation of standard solutions.

I). 0. IN NaoH (B.P. I980).

The N/IO NaoH solution was prepared by weighing out accurately 4 grams of NaoH pellets and dissolving in a I000 mls of distilled water.

2). 0.5 N. NaoH (B.P. I980).

This was prepared by dissolving 20g of NaoH pellets in I000 mls of distilled water.

Assay Methods.

Assay of Aspirin (B.P. I980).

0.5g of aspirin was accurately weighed and added 30 mls of 0.5N NaoH and boiled gently for IO minutes. It was then litrated with Hel 0.5N using phenol red as indicator. The difference between the litrations represented the amount of 0.5N NaoH required by aspirin.

Each millolitre of 0.5N NaoH is equivalent to 0.04504g of aspirin.

Preparation of Aspirin formulations.

I. Aspirin suspension B.P.C. I963

	B.P.C. Amounts	Amounts prepared.
Aspirin -	34.3 g	I7.I5 g
Tragacanth -	22.9 g	II.45 g
Chloroform water -	20.0 ml.....	I0.0 ml
Water to -	I000.0 ml	500.00 ml

The method employed is given in B.P.C. I963 page which is as follows:-

The aspirin and tragacanth were weighed out and mixed together and triturated with chloroform. This mixture was poured into a measuring cyclinder and made up to volume with water. The suspension was then divided to three portions.

On one portion, the physical stability was carried out, in the other portion, the accetrated chemical stability was done and the last portion was kept at room temperature.

2. Aspirin solution.

Aspirin	9.0 g
Sodium citrate	27.0 g
Water to	500.0 ml.

Both aspirin and sodium citrate were triturated to a fine powder. The solution was prepared in about 300 ml of water, stirring vigorously in a beaker to obtain a solution. The solution was filtered through a filter paper to a 500 ml volumetric flask and made up to volume with water. -

The filtration was done to remove particulate aspirin which would markedly affect the tritution result.

The sample was divided into two, I00 mls was put in 250 ml conical flask and covered with an aluminium foil and kept at room temperature. The other sample was used for the accetrated stability tests.

3. Dry powder.

Aspirin	34.3 g
Chloroform	0.05 mls
Tragacanth	22.9 g

If added water the volume should be made to I000 mls.

The aspirin and tragacanth were trilurated together and then added the chloroform. The mixture was left in the open, spread on a glass slab for the chloroform to evaporate. After the mixture was completely dry, the powder was trilurated in a mortar to a fine powder.

I.I4 g of the dry powedr was resuspended in 20 mls water and the physical stability done. The rest of the powder was used for the accetrated stability tests and storage at room temperature.

New formulations.

Preparation I.

Aspirin	3.43 g
Tragacanth	2.29 g
Sodium Benzoate ...	0.I0 g
Water to	I00. mls

Preparation 2.

Aspirin	3.43 g
Sodium carboxy methyl cellulose.....	0.74 g
Sodium Benzoate	0.I0 g
Water to	I00.00 ml

Preparation 3.

		Amount prepared.
Aspirin	I7.I5 g.....	3.43 g
Sodium carboxy methly cellulose..	3.70 g.....	0.74 g
Sodium Benzoate	0.50 g	0.I0 g
Starch	5.00 g	I.00 g
Water to	500.00 ml.....	I00.0 ml

Preparation 4.

		Amount prepared
Aspirin	I7.I5 g	3.43 g
Sodium Benzoate.....	0.50 g	0.I0 g
Sodium carboxy methyl cellulose...	2.00 g	0.40 g
Tragacanth	I0.00 g	2.00 g
Water to	500.00 ml	I00.00 ml

Preparation 5.

Preparation 4 + starch 5.00 g.

For all the preparations, the powders were mixed together in a mortar with a pestle. After mixing to fine powders, the powder was transferred to a dispensing bottle and shaken thouroughly and then made to a I00 mls with water and shaken again.

The physical stability was carried out for all the preparations. Chemicals stability was done only on preparation 5. The other preparations I - 4 did not look acceptable from the physical stability point of view and thus there was no need of carrying out the chemical stability tests.

Preparation 5.

A volume of 500 mls was prepared for carrying out the chemical stability tests. I50 mls was stored at room temperature and the rest of the suspension was used for accelerated chemical stability tests. Volumes of I00 mls, 500 mls, I litre, 2 litres were also prepared for "scale up" physical stability tests. For I litre and 2 litres, the powders were mixed in a ball mill since mixing manually was difficult because of the increased bulk of the powders. The dry powder of preparation 5 was also tested for chemical stability by storing room temperature and by doing accelerated stability tests.

PHYSICAL STABILITY TESTS.

I). First method.

This is visual observation. Lump formation and tendency of caking was looked for.

2). Second method.

(Remmington's pharmaceutical sciences page 332. I4th Edition). The suspension which was to be used for this test was shaken thoroughly and poured into a 20 ml measuring cyclinder. The equilibrium volume of sediment was measured after every minute for 5 minutes.

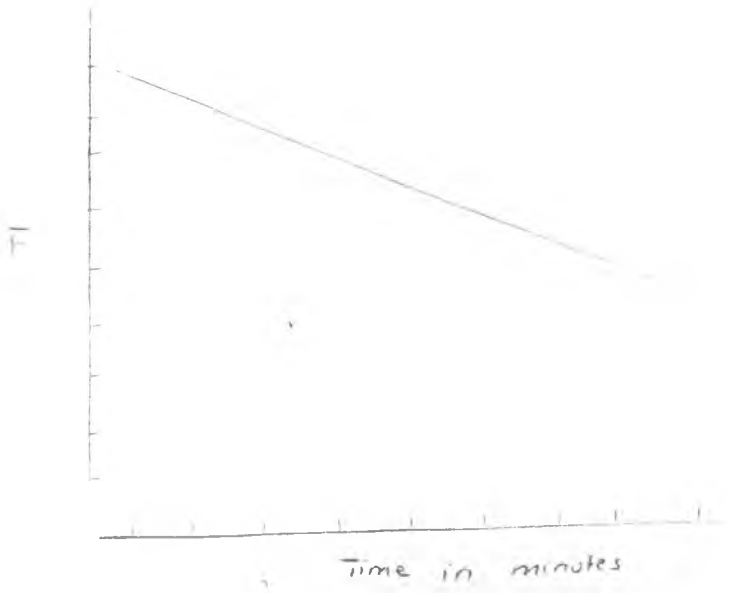
The volume was compared with the total volume of the suspension and the ration in the sedimentation volume.

If the sedimentation volume is 7, the equilibrium volume % sediment Vu & total volume of suspension Vo then

$$F = \frac{Vu}{Vo}$$

The value of F ranges from o to I

Figure I, plot of 7 versus time.



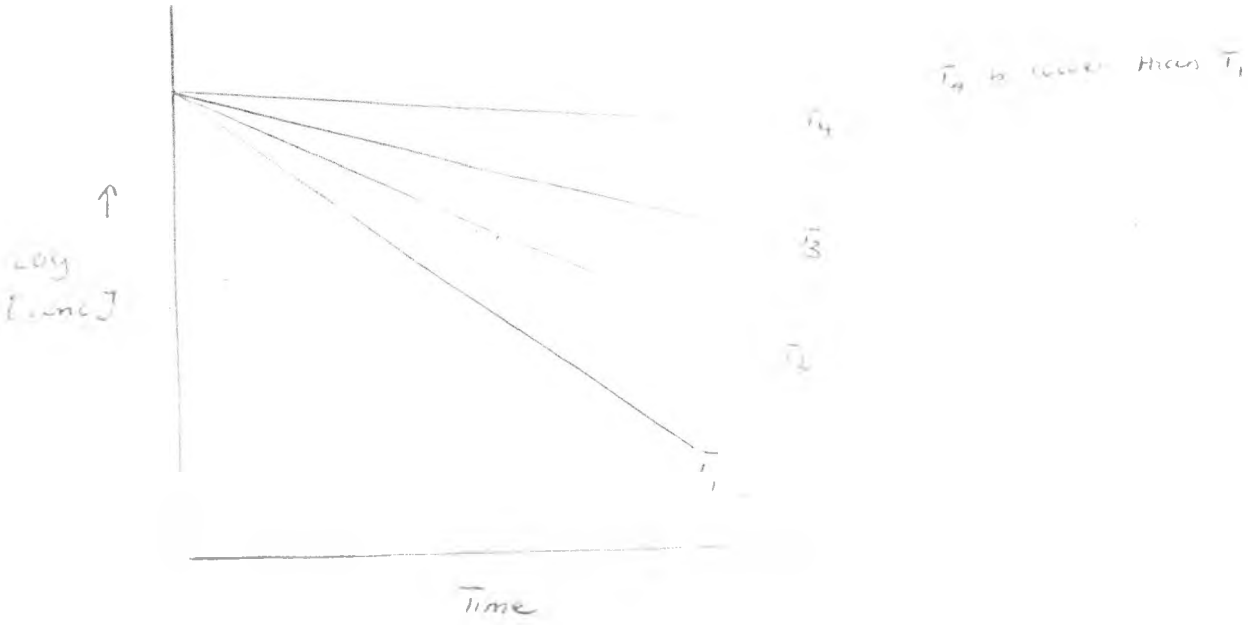
CHEMICAL STABILITY TESTS.

I). Storing samples at room temperature; this is done by storing samples at room temperature and carrying out assays until potency drops to 90 %.

2). Accelrated stability testing.

Accelurated staoility studies involve the determination of the concentration of the drug remaining as a function of time. This was put forward by Carret and Carper (I955).

Figure II.



The effect of temperature on the rate of reaction id described by Arrheniuzm equation (Garret and Carper (I955).

$$K = AC^{-Ea/RT} \quad \text{.....(I)} \qquad K = A_{C^{-Ea/RT}}$$

Where A = the frequency factor.

K = reaction rate constant.

Ea= energy of activation.

R = gas constant (I.987 cal/degree mole)

T = Absolute temperature.

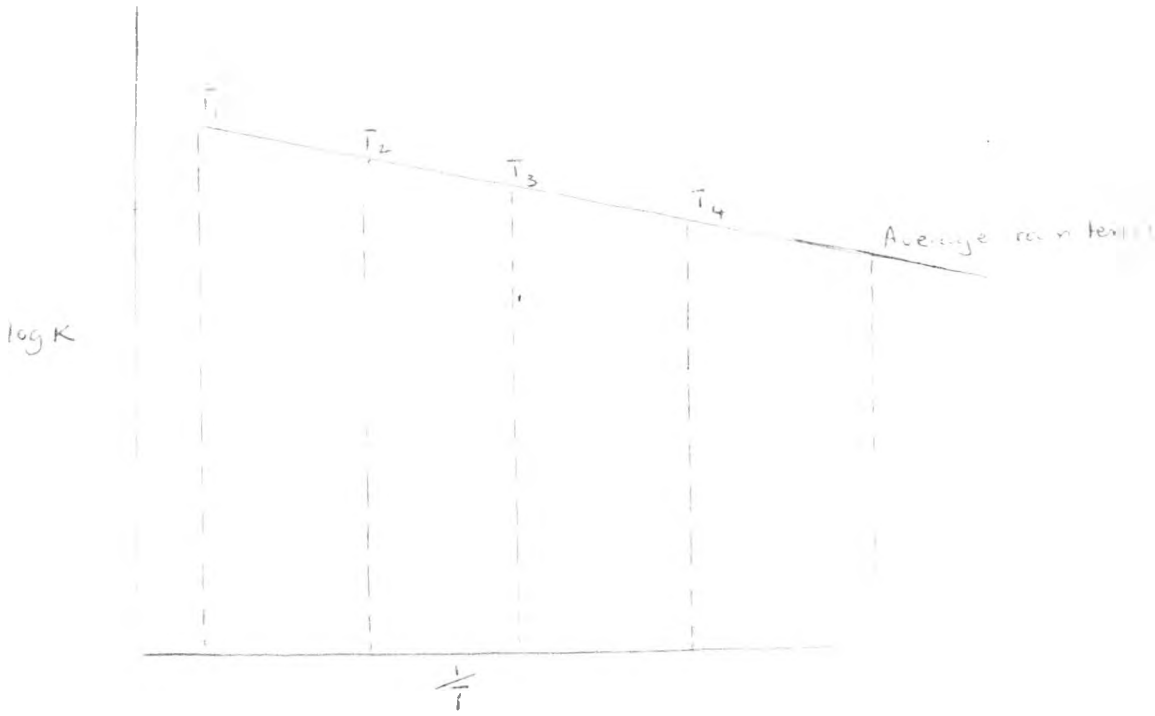
Equation I can be rewritten as :-

$$\log K = \log A - \frac{Ea}{2.303RT} \quad \text{.....(2)}$$

Log K is proportional to I/T. A straight line is obtained (figure III) which can be extrapolated to room temperature and the corresponding value of K obtained.

An Arhenious plot for predicting stability at Room Temperature.

(Figure III)



Subsitution in the appropriate rate equation for the decomposition being studies together with the degree of decomposition to be permitted enables the time for this to occur to be calculated which is the shelf life.

Amorjahed (1977) suggested that the shelf life or t 0.9 i.e. the time required for the concentration of a drug to decrease to 90 % of its value at zero time could determined at elevated temperatures can be calculated by extraporation of the resultant straight line. This approach was suggested to be applicable to all orders of reaction since the initial decay of up to 10 % could be fitted by a first order equation regardless of the actual order of reaction.

The specific reaction rate constants at a particular temperature may be determined by assaying the amount of drug remaing at various time intervals and plotting the results graphically.

- For a first order reaction a plot of log C against time gives a straight line with a slope equal to $\frac{K}{2.303}$ (figure IV). This is seen from the equation.

$$K = -\frac{2.303}{t_I\ t_0} \log \frac{CO}{C_I}$$

$$\text{or } \log C_I = \log C_0 - K\,(t_I - t_0) / 2.303$$

CO = concentration at time t_0 (usually 100 %)

C = concentration at time t_I

K = Specific reaction rate constant at room temperature extrapoluted from graph of log K vs time.

Figure IV. A plot of log concentration versus time.

Half life of a drug is the period of time required for it to decompose to one half of its original concentration. For a first order reaction, $t_{\frac{1}{2}}$ can be calculated as follow :-

$$\log C = \log C_0 - \frac{Kt}{2.303}$$

$$K = \frac{2.303}{t} \log \frac{CO}{C}$$

If t = half life ($t_{\frac{1}{2}}$)

$$C_0 = X$$

$$C = \tfrac{1}{2} X$$

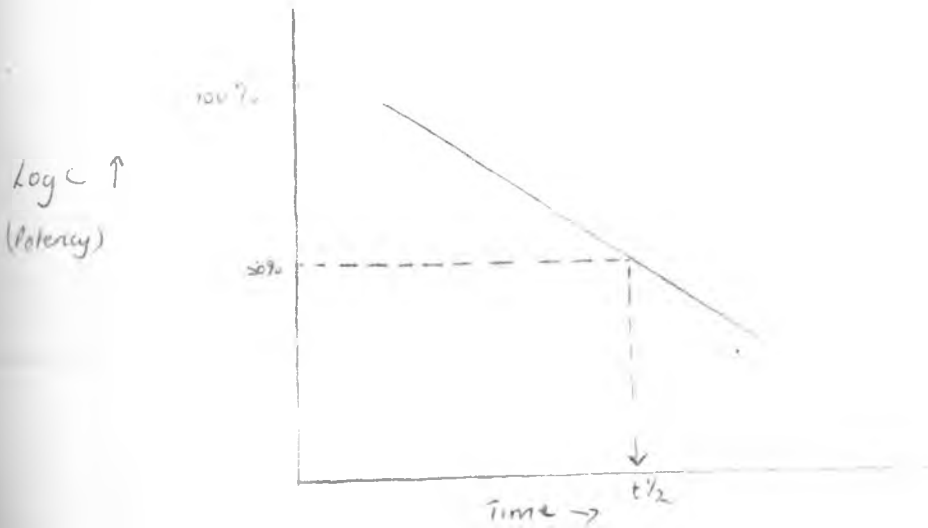
$$\text{Then } K = \frac{2.303}{t_{\frac{1}{2}}} \log \frac{2X}{X}$$

$$t_{\frac{1}{2}} = \frac{2.303}{K} \log 2$$

$$= \frac{0.693}{K}$$

The value of $t_{\frac{1}{2}}$ can also be used for the calculation of the reaction rate constant K.

Figure V. A plot of log C_0 versus time to show how $t_{\frac{1}{2}}$ can be obtained from this plot.



Experimental.

A 100 ml sample of aspirin solution was put into each of five 250 ml concial flasks and each flask was covered with aluminium foil. The samples were kept at $70^{0}C$, $60^{0}C$, $50^{0}C$, $40^{0}C$ and at room temperature.

The aspirin content of the solution in the flasks was determined at 0, 1, 2, 3, 4 hours for the samples at $70^{0}C$, $60^{0}C$, $50^{0}C$, $40^{0}C$. The room temperature samle was assayed at 0 hour, 6 hours and 24 hours or until the potency dropped to 90 %.

The aluminium foil was replaced highly over the neck of the flask after withdrawing the sample. If this was not done, water would be lost from the solution especially at higher temperatures leading to concentration of the solution and inaccuracies.(The experiment was set in duplicate).

Assay.

10 ml sample from the flask was titrated with N/10 NaoH solution using phenolphthalein as indicator. All the samples were titrated at the same temperature thus all the flasks were cooled under the tap before titration.

The initial titration figure X ml represented 100 % potency. When aspirin was completely hydrolyzed to salicylic acid and acetic and 2X mls of alkali was required. The amount of aspirin remaining after partial hydrolysis was equivalent to 2X - y ml N/10 NoaH solution where y was the titration figure for partially hydrolyzed sample.

Therefore potency (%) = $\frac{2X - y}{X} \times 100$

ii). Suspensions

The same procedure was replaced but instead of solution, suspension was used.

iii). Dry powder.

10 g of powder was put in a conical flask. The flask was covered with a cork and aluminium foil to exclude any moisture which would interfere with the results.
Assay was done by weighing out 0.693 g of powder into a conical flask and suspending it with 10 mls of water. The rest of the test was carried out as for aspirin solution.
(0.693 g of powder was weighed because by calculation from the suspensions , a 10 ml sample contained 0.693 g of solids).

RESULTS.

The results of the physical stability experiments on the standard formulations and the new preparations are as shown in Appendix page 2 Table I.

The results for the chemical stability experiments for both the room temperature samples and for the accelrated stability tests are as shown in Appendix page 4 - 5 Table II and Table III.

Treatment of Results (Physical stability).

The value of F is obtained by

$$F = \frac{Vu}{V0}$$

The value of F is plotted against time and the graphs drawn are as in Appendix page 13 Graph No. I

An average of 3 readings for Vu is taken and F is calculated from this average.

Values for F are as shown in Appendix page 11 - 12 Table IX.

I). Calculation of percentage potency.

$$\text{Percentage potency} = \frac{2x - y}{X} \times 100$$

X ml = initial lilrated figure at 0 hour for every temperature,

Y ml = Tilrated figure for partially hydrolyzed sample at various hourly intervals.

(2x - y)ml = Amount of aspirin remaining after partial hydrolysis.

Thus for example aspirin suspension B.P.C. 1963 at 1 hour for 40^{0}C.

$$\% \text{ potency} = \frac{34.2 \text{ ml} - 17.30 \text{ ml}}{17,1 \text{ ml}} \times 100$$

$$= 98.17\ \%$$

The percentage potencies were thus calculated and the results are tabulated in Appendix page 6 & 7 Table IV & V. Graphs of log potency versus time were plotted and from this the reaction rate constant were determined.

From plots of log % potency against time Appendix page 14 - 16 the hydrolysis of aspirin by NaOH follows first order kinetics.

2). Calculation of K.

K can be calculated from the graphs .....

using the equation

$$K = - \frac{2.303}{t_I - t_0} \log \frac{C_o}{C_I}$$

Since the hydrolysis follows order kinetics the reaction rate constant K can be calculated the equation.

$$t_{\frac{1}{2}} = \frac{0.693}{K} \quad : \quad K = \frac{0.693}{t_{\frac{1}{2}}}$$

$t_{\frac{1}{2}}$ is half life.

$t_{\frac{1}{2}}$ values are obtained from the graphs of log concentration versus time Appendix page

Thus the calculated K from the t½ is shown in Appendix page 8 & 9 Table VI & VII for the various preparations.

3. Calculation of values of K at room temperature ($25^{0}C$).

The temperature is converted to absolute temperature and the values of $\frac{I}{T}$ are worked out do as to plot the graph of log K versus $\frac{I}{T}$

The graphs are in Appendix page I6 - I8. The value of K is calculated at room temperature from extrapolation of the graph to room temperature.

$T = 25^{0}C = 298\ K.$

$\frac{I}{T} = 3.36 \times I0^{3}$

From the graphs for the various preparations this corresponds to values K shown in Appendix page 8 & 9 Tables VI & VII.

4). Calculation of shelf life.

This is calculated at a potency limit of 90 %. The hydrolysis of aspirin has been established as a first order reaction, the shelf life can be calculated using the equation.

$$\log C_I = \log C_0 - \frac{K\,(t_I - t_0)}{2.303}$$

C_0 = concentration at time to (I00 %)

C_I = cooncentration at time t, (90 %)

$t_I - t_0$ = shelf life

K is as calculated in No. 3 above for the various preparation at room temperature.

$$K\,(t_I - t_0) = \log C_0 - C_I$$

$$2.303$$

$$= \log \frac{C_0}{C_I}$$

$$t_I - t_0 = \log \frac{C_0}{C_I} \times \frac{2.303}{K}$$

$$= \log \frac{100}{90} \times \frac{2.303}{K}$$

The shelf lives were thus calculated for all the preparations. Values are as shown in Appendix page 10 Table VIII.

Aspirin degrades to salicylic acid and acetic acid. The breakdown is enhanced by moisture and this was the reason why aspirin mixture B.P.C. 1963 had to be reformulated as a dry powder for reconstitution with water before use.

The standard aspirin mixture contains aspirin, chloroform, tragacanth and water. It has to be freshly prepared since it degrades very fast. Its shelf-life was calculated to be about 18 hours from the physical stability data, it was physically stable because there were no lumps formed and even the rate of settling was not very high. Because of its short shelf-life due to the water content, the attempt was to formulate it as a dry powder and the first thing taken into consideration was the chlofoform contained in the preparation. Chloroform acts as a flavour as well as a preserving agent. Chloroform was in liquid form and thus a preservative which was in powder form but soluble in water was looked for. The preservative had to be active within a low P^H range since aspirin is also at a low P^H range of 2.3. Sodium benzoate was thus used. Its active at a P^H range of less than 5 (marbrindale, Marbrindale) The solubility of the preservative was required so as to ensure uniform distribution in the reconstituted suspension.

The formulation of the aspirin mixture was eliminated by the chloroform. The solids were triturated together, put in a dispensing bottle and added water and shaken. The resulting suspension had a lot of lumps and thus is the second preparation tragacanth was eliminated and Nacarboxy cellulose used as the suspending agent. This preparation (prep. 2) did not produce better results. Though there were no lumps, the preparation did not look elegant.

- Preparation 3, which was added starch as a dispensing agent did not look elegant either and thus, in prep. 4, tragacanth and Nacarboxy methyl cellulose were mixed together. The amounts were used in prep. I incase of tragacanth and prep. 2 incase of Nacarboxy methyl cellulose were reduced. This produced better results but the suspension had a problem of having a high rate of settling and also having small lumps.

Starch was therefore added (prep. 5). The final preparation produced better results. It was more physically stable than the official suspension. Its shelf-life however was almost the same with that of the standard mixture. This preparation had an advantage of the standard mixture because it was in dry form to be reconstuted with water when required for use. The dry powder had a shelf-life I28.I0 hours. This time was more than that of the B.P.C. 1963 Aspirin mixture.

When B.P.C. I963 Aspirin mixture was made in dry form, it had a shelf life of about I26 hours. It could not be used for reconstituting with water because its was not physically stable. It had alot of lumps when reconstuted with water.

The amounts of starch, tragacanth and sodium carboxy methyl cellulose added were all by trial and error. No interval was followed as to how much of which substance was added.

Reducing the particle zise helped to achieve more physically stable suspension. Thus when mixing the powders, the best results were achieved when a ball mill was used for mixing. The particle size reduction was better than inhand mixing.

When the results of 500 ml suspension which was manually mixed are compared with those of I000 ml & 2000 ml suspensions where the mixing and particle size reduction was carried out with a ball mill, these suspension are more physically stable. The I00 ml suspension which was manually mized had better results than 500 mls since in I00 mls. the amount of ingredients was less and hence the mixing and particle size reduction was more efficient.

Thus, since 2000 mls of suspensions was to be dispensed in the rural health centres, the ball mill mixing is therefore recommended.

The shelf lifes obtained however are short and do not look very practical. The calculated shelf lives from the accelerated stability test are shorter than the actual shelf- lives. This could have been so due to the following reasons.

i). In the case of dry powder, moisture could have been incorporated which would result in degradation and hence a shorter shelf life than what would be expected.

ii). When popetting I0 mls of the suspension for assay bubbles were also being taken in the pipette. The volume occupied by the bubbles was not the same all the time. This meant that different values of original concentrations were obtained every time since the volume pipetted was not exactly I0.0 mls. This probably is the reason for a difference in the litres obtained for the same sample at same temperature.

iii). The temp - time program for the accelerated stability test were chosen according to the convinience especially to fit into a working day. Assays were carried out at I hour intervals for 4 hours. Longer periods would have been better for conclusive results.

iv). The exp assumed a constant room temperature of $25^{0}C$ which was not the case. Sometimes the temperature was as high as $26.5^{0}C$.

v). The order of reaction may change during the study. This could have happened to the sample under investigation but it was difficult to investigate whether the order of the reaction really changed.

vi). At certain temps autocatalysis may occur to make the room temperature stability predictions impractical. This possibility cannot be ruled our because the system was not a closed one and anything from the atmosphere could have intefered with the reaction.

It is not possible to extend the predictions to all climatic conditions especially those found in the tropics where there are large diurnal variations in temp. It has already been commended that the room temp. was not always constant and the average temperature was sometimes higher than the assumed room temperature ($25^{0}C$). It was not possible to establish a system where the surroundings temperature was constant and the assumption had to be accepted.

vii). There is usually a time log before the product reaches eqm storage temperature and also before it returns to IOO m temperature upon removal from the test environment. This means that the exact times of storage at elevated temps can be considerably erroneous. The bulk of the products may also be the determinant factor to prevent rapid equilibivation $\bar{\theta}$ the test environment.

These problems were encountered during the determination of shelf life and this could have to led to the short shelf lifes obtained.

C O N C L U S I O N.

From the shelf lives obtained the powder is most stable but it is experimentally impractical to conclude that the figure obtained for the shelf life is the right one because many similar exps were not carried out to control experimental errors in the determination K (the reaction rate constant) and hence in the claculation of the shelf life.

What has been achieved in this project is that the dry powder formulation of aspirin is most stable and thus one of the ways of sending the aspirin mixtures to the rural Hospitals from the District Hopsitals.

R E S U L T S.

Physical Stability.

I). Visual Observation.

A). Official preparations.

i). Suspensions B.P.C. 1963.

- No lumps were formed on the suspension.
- On shaking it redispensed well.

ii). Dry powder.

- On adding water, alot of lumps were formed.
- There was tendency of caking on settling

B). New preprarations.

i). Preparation I.

- There was lump formation
- Rate of settling was high.

ii). Preparation 2.

- There was no sedimentation, the suspension looked viscous but it was not elegant.

iii). Preparation 3.

- The preparation looked viscous and very unpalatable.

iv). Preparation 4.

- Small lumps formed. It redispensed fast on shaking.

v). Preparation 5.

- The suspension settled slowly. No lumps were observed.

TABLE I.

Preparation.	Time (min)	1st reading(ml)	2nd reading(ml)	Average(mls)
Official Suspension.	0	20	20	20
	I	19.00	I9.02	I9.0I
	2	I7.85	I7.95	I7.90
	3	I6.33	I6.35	I6.34
	4	I5.20	I5.20	I5.20
	5	I4.I5	I5.45	I4.30
Preparation 4.	0	20	20	20
	I	I8.I5	I7.85	I8.00
	2	I6.00	I6.20	I6.I0
	3	I4.30	I4.20	I4.25
	4	I3.36	I3.32	I3.34
	5	I2.25	I2.55	I2.40
Preparation 5. For I00 mls. (Hand-mixing)	0	20	20	20
	I	I9.50	I9.30	I9.40
	2	I9.0I	I8.99	I9.00
	3	I8.50	I8.70	I8.60
	4	I8.00	I8.20	I8.I0
	5	I8.65	I8.55	I7.60

	Time(min)	Ist reading(ml)	32nd reading(ml)	Average (mls)
Preparation 5.				
for 500 mls.	0	20	20	20
(Hand-mixing)	*I*	*I9.45*	*I9.55*	*I9.50*
	2	I9.I0	I8.90	I9.00
	3	I8.32	I8.48	I8.40
	4	I7.85	I7.95	I7.90
	5	I7.30	I7.I0	I7.20
Preparation 5.	0	20	20	20
For I000 mls	I	I9.85	I9.75	I9.80
(Ball mill mixing)	2	I9.I5	I9.25	I9.20
	3	I9.I0	I8.70	I8.90
	4	I8.65	I8.55	I8.60
	5	I8.I0	I8.I0	I8.I0
Preparation 5.	0	20	20	20
For 2000 mls	I	I9.90	I9.80	I9.85
(Ball mill mixing)	2	I9.30	I9.30	I9.30
	3	I8.98	I8.92	I8.95
	4	I8.50	I8.60	I8.55
	5	I8.50	I8.00	I8.25

C H E M I C A L S T A B I L I T Y R E S U L T S.

Aspirin solution (Official)

Tritration with N/IO NaoH solution.

TABLE II.

A).

	25^0C	40^0C	50^0C	60^0C	70^0C
0 Hr	I2.Iml	I2.5Iml	I0.50ml	9.65ml	I2.00 ml
I Hr	-	I2.65"	II.90"	I2.20"	I6.90 "
2 Hr	-	I3.05"	I3.I5"	I4.I0"	I9.77 "
3 Hr	-	I3.40"	I4.05"	I5.55"	2I.25"
4 Hr	-	I3.I7"	I5.00"	I6.60"	22.08 "
6 Hr	I2.8 ml	-	-	-	-
24 Hr	I3.9 ml	-	-	-	-

B). Aspirin suspension (Official)

	25^0C	40^0C	50^0C	60^0C	70^0C
0 Hr	I7.I ml	I7.Iml	I7.I2ml	I7.00ml	I7.00ml
I Hr	-	I7.30"	I8.50"	22.60"	26.26"
2 Hr	-	I7.75"	I9.04"	23.44"	29.50"
3 Hr	-	I8.40"	I9.38"	26.60"	3I.03"
4 Hr	-	I9.38"	2I.72"	27.20"	36.05"
6 Hr	I7.28 ml	-	-	-	-
24 Hr	20.16 ml	-	-	-	-

Dry powder (Official).

C).

	40°C	50°C	60°C	70°C
0 Hr	I7.0I ml	I7.0I ml	I7.64 ml	I7.04 ml
I Hr	I7.0I "	I7.0I5 "	I7.I7 "	I7.3I "
2 Hr	I7.03 "	I7.03 "	I7.I2 "	I7.59 "
3 Hr	I7.035"	I7.I0 "	I7.49 "	I8.32 "
4 Hr	I7.04 "	I7.25 "	I7.5I "	I8.68 "

Room temperature (25°C).

0 Hr	I7.02 ml	I4 Hr	I7.65 ml
6 Hr	I7.03 "	I24 Hr	I8.I6 "
24 Hr	I7.I0 "	I47 Hr	20.00 "

NEW PREPARATIONS.

TABLE III.

Preparation 5 (Suspension).

A).

	25°C	40°C	50°C	60°C	70°C
0 Hr	I7 mls	I7 mls	I7.I mls	I7.I mls	I7.I2 mls
I Hr	-	I7.32"	I7.68 "	I8.34 "	20.06 "
2 Hr	-	I8.65"	I8.I6 "	I9.02 "	20.74 "
3 Hr	-	I8.50"	I9.07 "	20.42 "	23.03 "
4 Hr	-	I9.0I"	20.03 "	20.90 "	24.40 "
6 Hr	I7.68 mls	-	-	-	-
24 Hr	I9.33 mls	-	-	-	-

Dry powder.

B).

	40^0C	50^0C	60^0	70^0C
0 Hr	I7.02 mls	I7.02 mls	I7.02 ml	I7.0I mls
I Hr	I7.02 "	I7.I0 "	I7.24 "	I7.60 "
2 Hr	I7.03 "	I7.20 "	I7.63 "	I8.50 "
3 Hr	I7.I2 "	I7.48 "	I7.98 "	I8.82 "
4 Hr	I7.32 "	I7.59 "	I8.I7 "	I9.62 Mls

Room temperature (25^0C).

o Hr	I7.0I mls	I24 Hr	I8.33 mls
6 Hr	I7.02 "	I47 Hr	I9.96 Mls
24 Hr	I7.26 "		
74 Hr	I7.68 "		

Percentage potency values.

Official preparations.

a). Aspirin solution.

TABLE IV.

	25^0C	40^0C	50^0C	60^0C	70^0C
0 Hr	I00 %	I00 %	I00 %	I00 %	I00 %
I Hr	-	98.80	86.67	73.38	59.I7
2 Hr	-	95.60	74.76	53.89	35.2I
3 Hr	-	92.80	66.67	38.86	22.92
4 Hr	-	94.40	57.38	26.68	I6.04
6 Hr	94.2I	-	-	-	-
24 Hr	85.I2	-	-	-	-

b). Aspirin suspension.

	25^0C	40^0C	50^0C	60^0C	70^0C
0 Hr	I00 %	I00 %	I00 %	I00 %	I00 %
I Hr	-	98.I7	9I.20	67.00	45.5
2 Hr	-	85.60	88.00	62.I0	26.6
3 Hr	-	92.I0	86.00	43.50	I7.5
4 Hr	-	86.00	72.22	40.0I	-
6 Hr	98.35	-	-	-	-
24 Hr	8I.36	-	-	-	-

c). Aspirin Dry powder.

	40^0C	50^0C	60^0C	70^0C
0 Hr	I00 %	I00 %	I00 %	I00 %
I Hr	I00 %	99.97	99.57	98.20
2 Hr	99.97	99.80	99.32	96.52
3 Hr	99.86	99.40	97.I2	92.26
4 Hr	99.8I	98.50	96.95	90.0I

TREATED RESULTS.

Values for $\frac{I}{T}$ and $t\frac{1}{2}$

(K is calculated from $t\frac{1}{2}$)

Official preparations.

TABLE VI.

a). Aspirin solution.

Temp ^{O}C	*Temp. T*	$\frac{I}{T}$	$t\frac{1}{2}$	K
$40^{O}C$	313	3.2×10^{-3}	18.90 Hr	0.0366
$50^{O}C$	323	3.1×10^{-3}	5.54 Hr	0.1250
$60^{O}C$	333	3.0×10^{-3}	2.28 Hr	0.3039
$70^{O}C$	343	2.9×10^{-3}	1.54 Hr	0.4497

The value of K at Room temperature.

Room Temp = $25^{O}C$ = 298 K $\frac{I}{T}$ = 3.36×10^{-3}

This is used to calculate the shelf life.

b). Aspirin suspension.

Temp. ^{O}C	Temp. T	$\frac{I}{T}$	$t\frac{1}{2}$	K
$40^{O}C$	313	3.2×10^{-3}	23.10 Hr	0.03
$50^{O}C$	323	3.1×10^{-3}	9.90 "	0.07
$60^{O}C$	333	3.0×10^{-3}	2.31 "	0.31
$70^{O}C$	343	2.9×10^{-3}	1.05 "	0.66

K at Room temperature = 0.0058.

Shelf lives.

The calculated shelf lives from the accetrated stability studies for all the preparations and at room temperature.

TABLE VIII.

a). Official preparations.

	t_{90}	t_{90}
Aspirin solution	II.03 Hr	I4 Hrs
Aspirin suspension	I8.I8 "	I6.20 Hrs.
Aspirin Dry powder	I26.20 "	I24.0 Hrs

b). New preparations.

	t_{90}	t_{90} at $25^{0}C$
Aspirin suspension	I8.25 Hr	I6 Hrs
Aspirin Dry powder	I28.I0 "	I26 Hrs

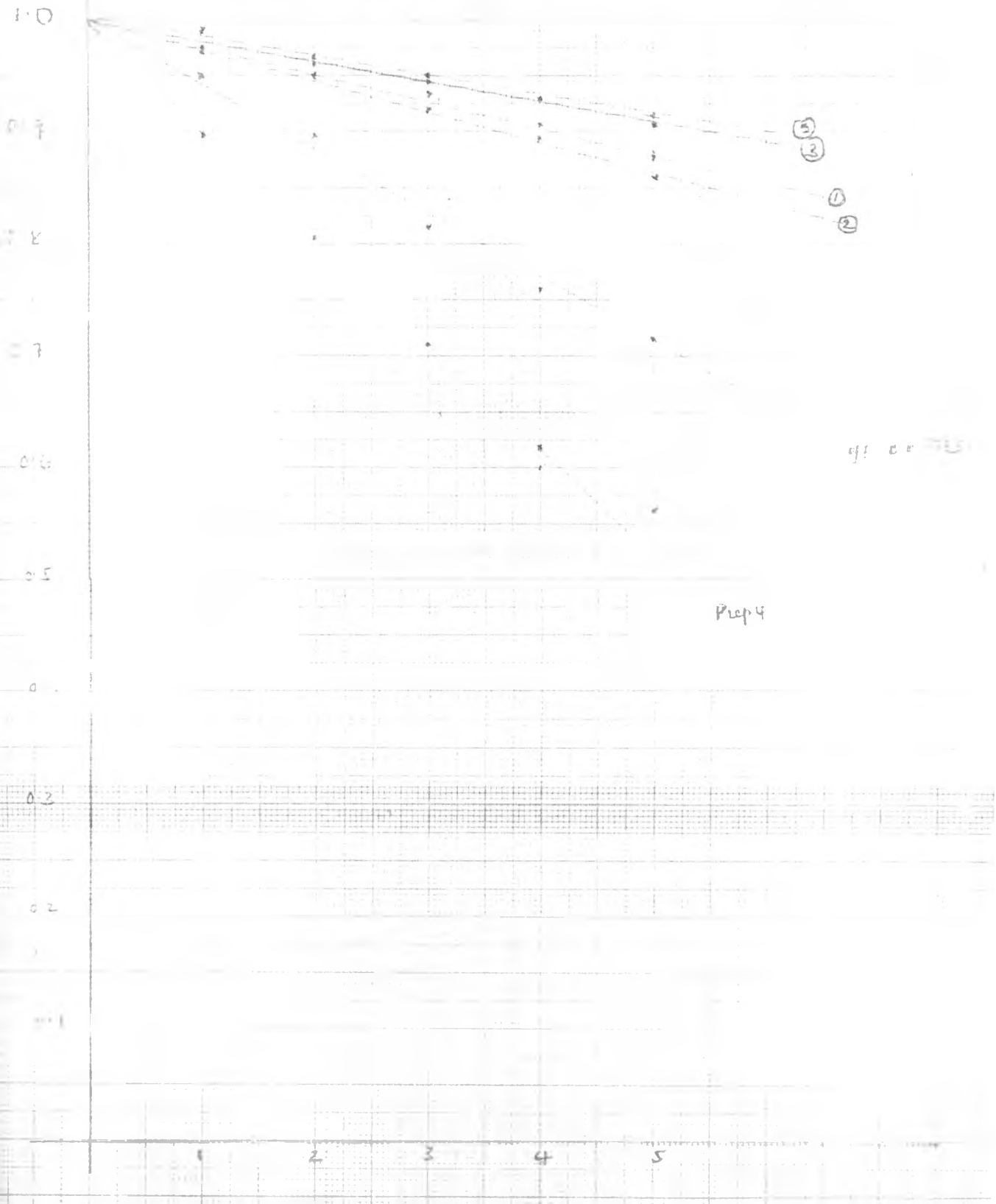
The shelf lifes are calculated as a potency limit of 90 %

Treated Results (Physical).

Preparation	Time	Average Reading(ml)	$Vu/_{VO}$ (F)
Official	0	20.00	I.0
suspension	I	I9.0I	0.9505
B.P. I963	2	I7.90	0.895
	3	I6.34	0.8I7
	4	I5.20	0.76
	5	I4.30	0.7I5

	Time	Average Reading(ml)	$Vu/_{VO}$ (F)
Preparation 4.	0	20.00	I. 0
	I	I8.00	0.90
	2	I6.I0	0.805
	3	I4.25	0.7I3
	4	I2.40	0.62
	5	I0.I0	0.5I
Preparation 5.	0	20.00	I.0
(I00 ml,	I	I9.40	0.97
Hand -mixing)	2	I9.00	0.95
	3	I8.60	0.93
	4.	I8.I0	0.905
	5	I7.60	0.88
Preparation 5	0	20.00	I.0
(500 ml, Hand -	I	I9.50	0.975
mixing)	2	I9.00	0.95
	3	I8.40	0.92
	4	I7.90	0.895
	5	I7.20	0.860

Physical Stability ; F vessus Time (min)



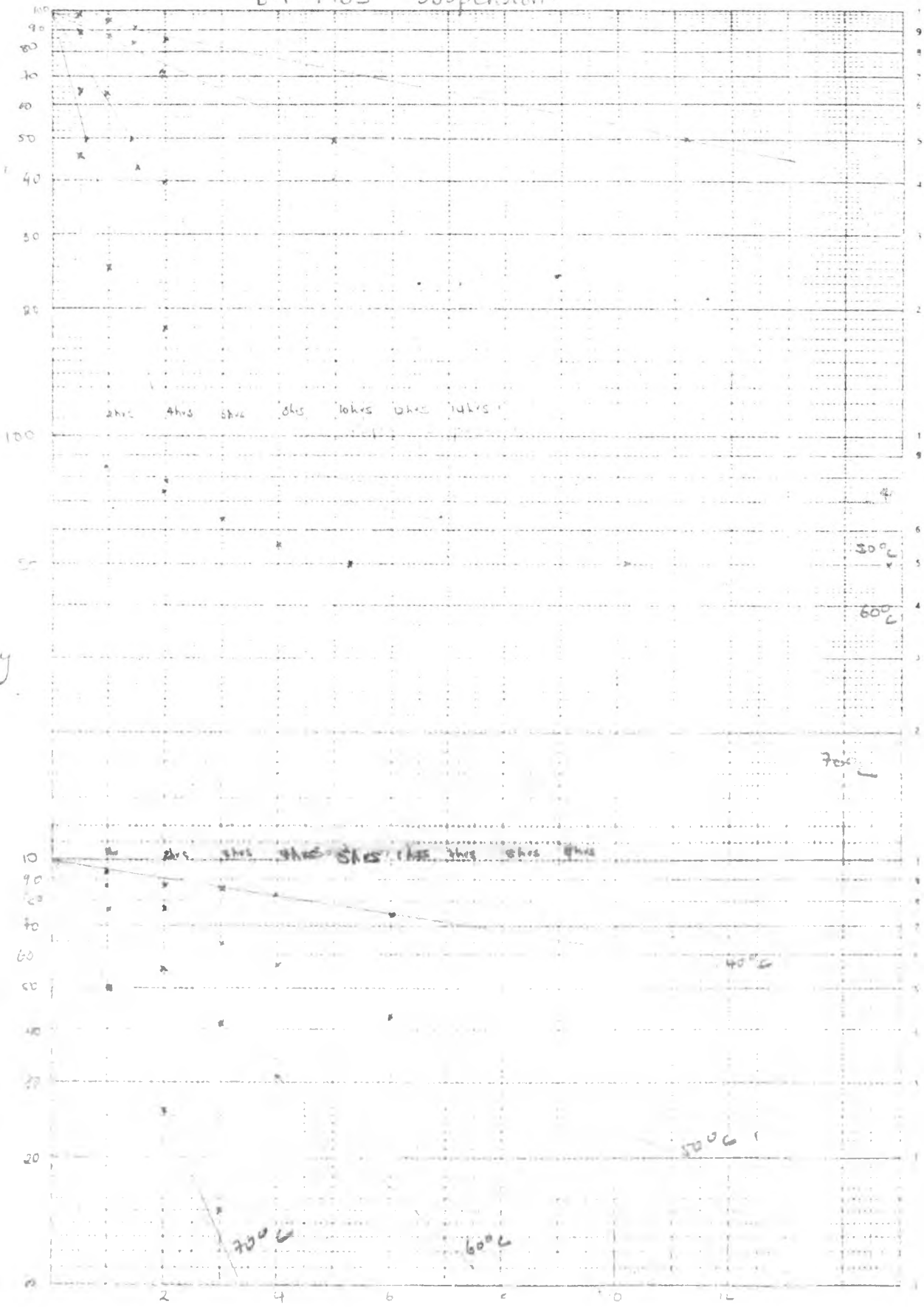
Prep 4

① Prep 5, 100 ml Hamel-mixing

② Prep 5, 2000 ml Ball-mill mixing

③ Prep 5 1000 ml Ball mill mixing

B·P·1963 suspension.

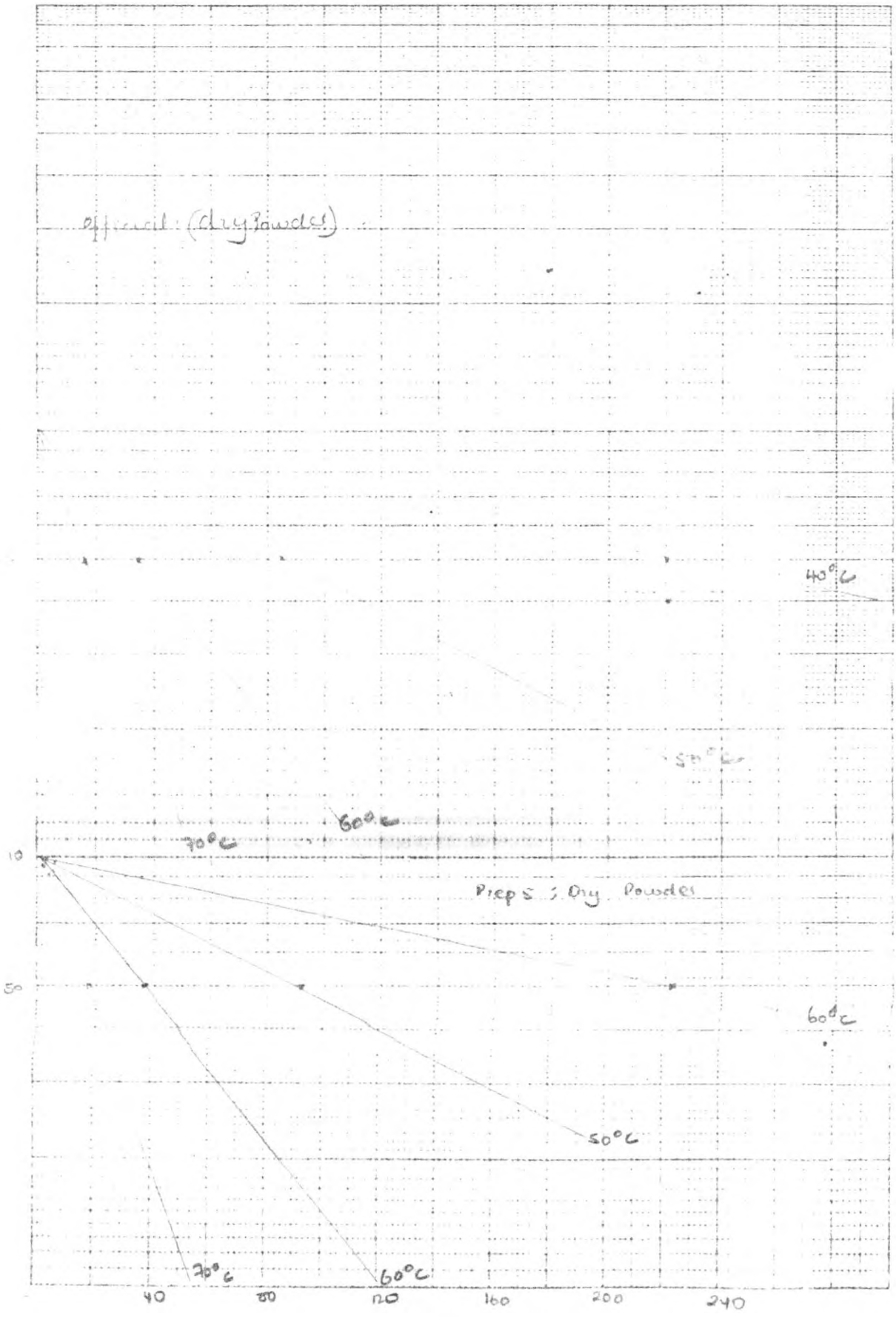


Time

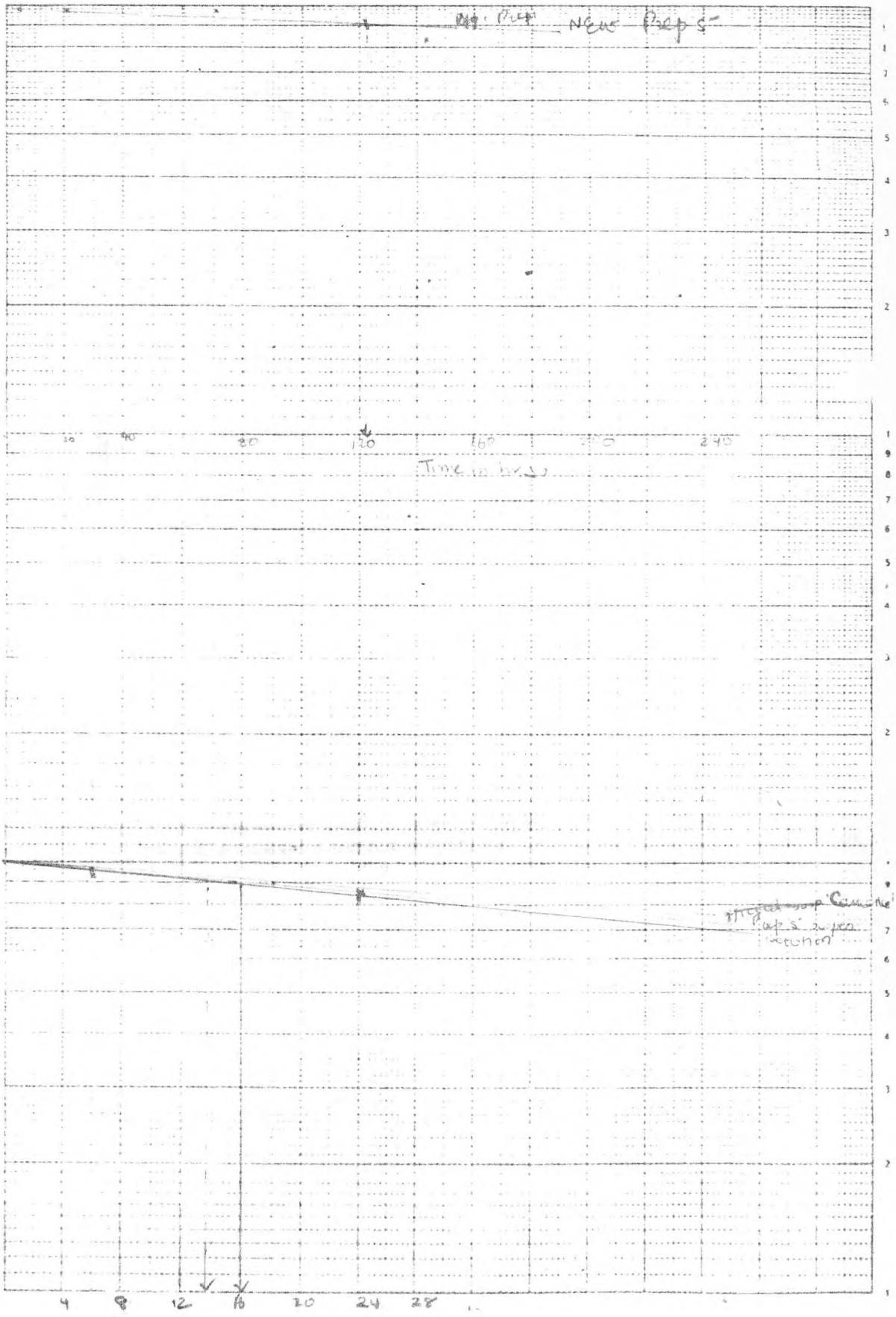
(3)

pg 14

Log Potency vs time (Dry Powders)

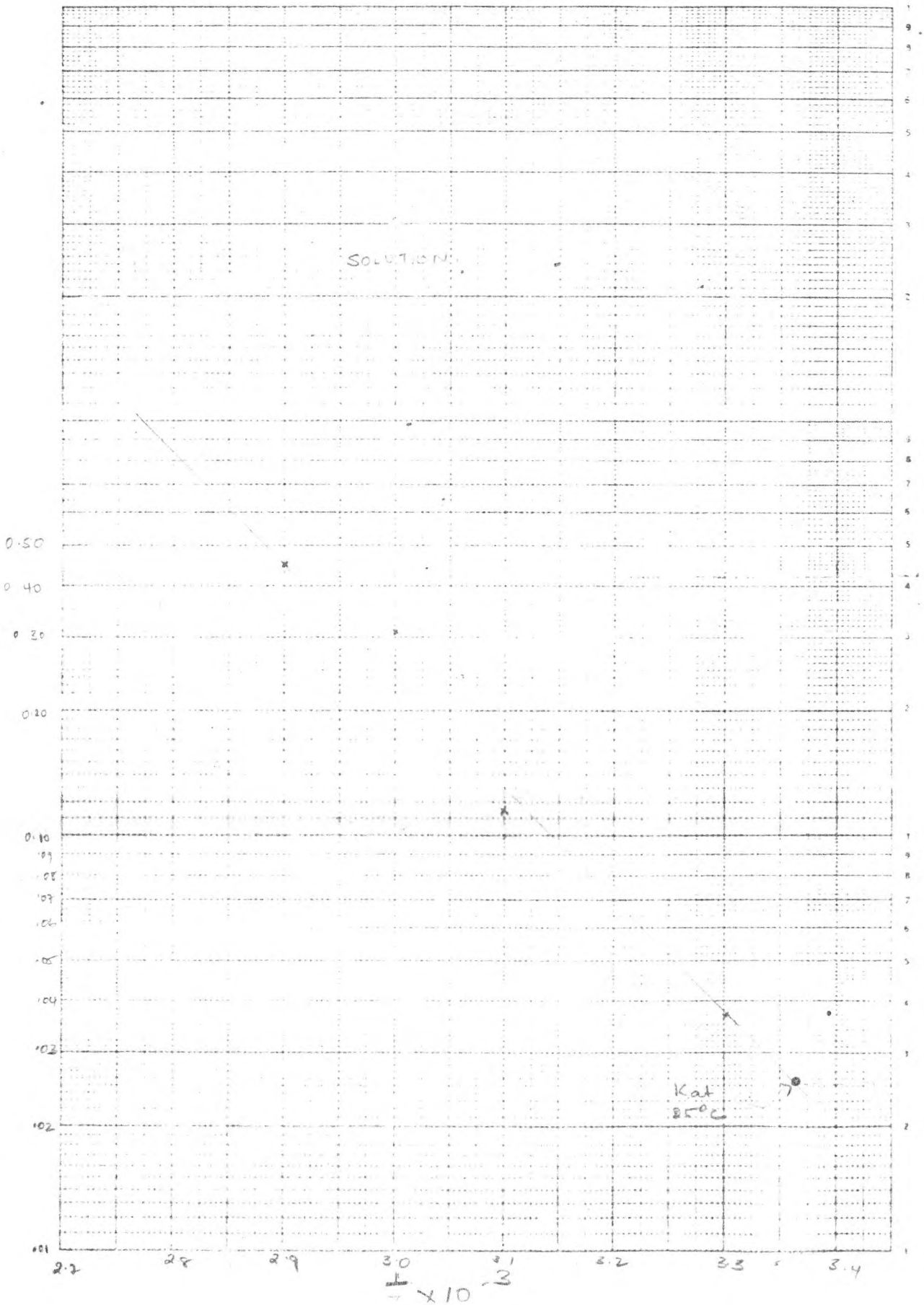


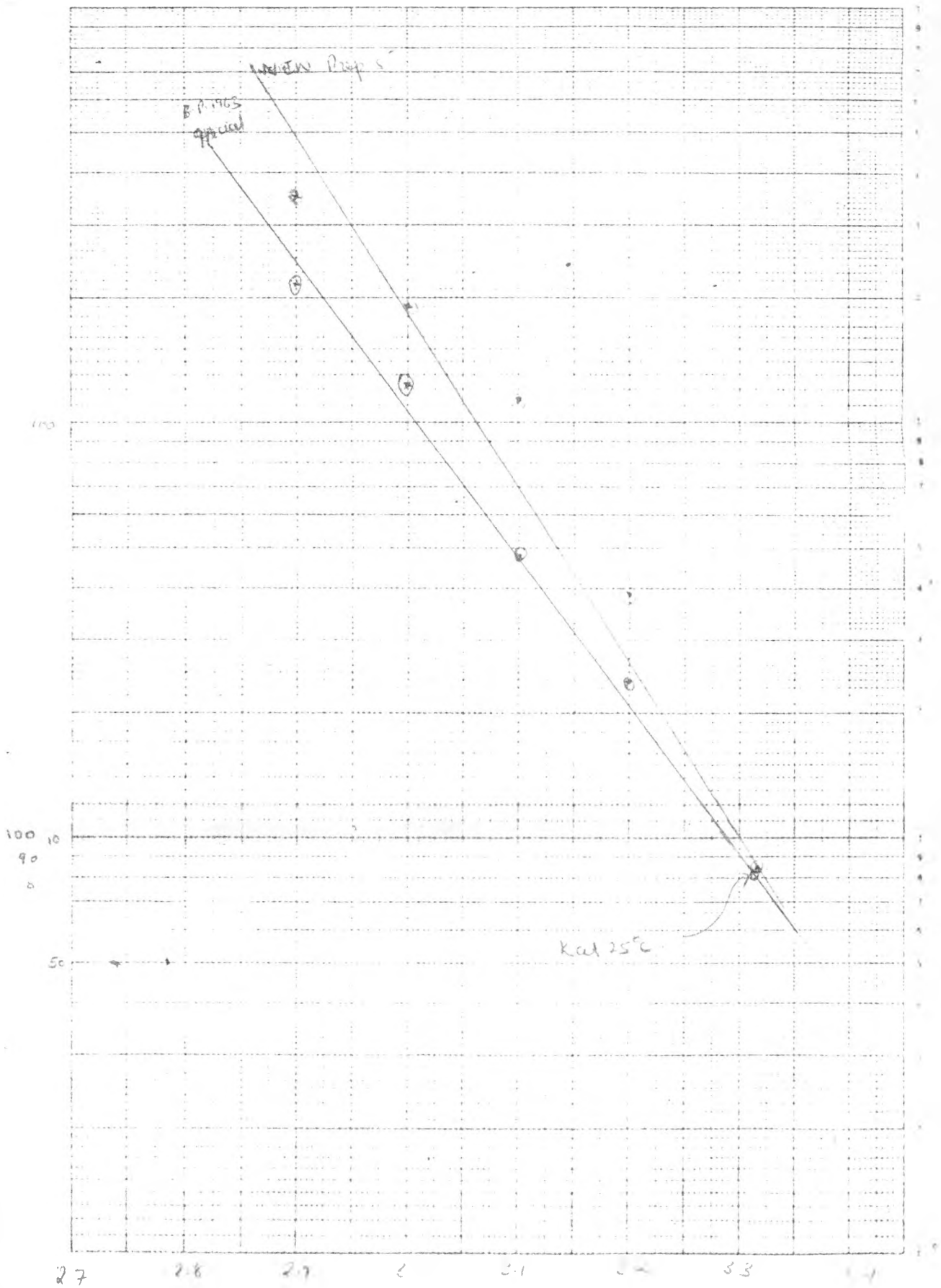
B15 (F) Graphs of Log Potency vs Time at 25°C.



(5)

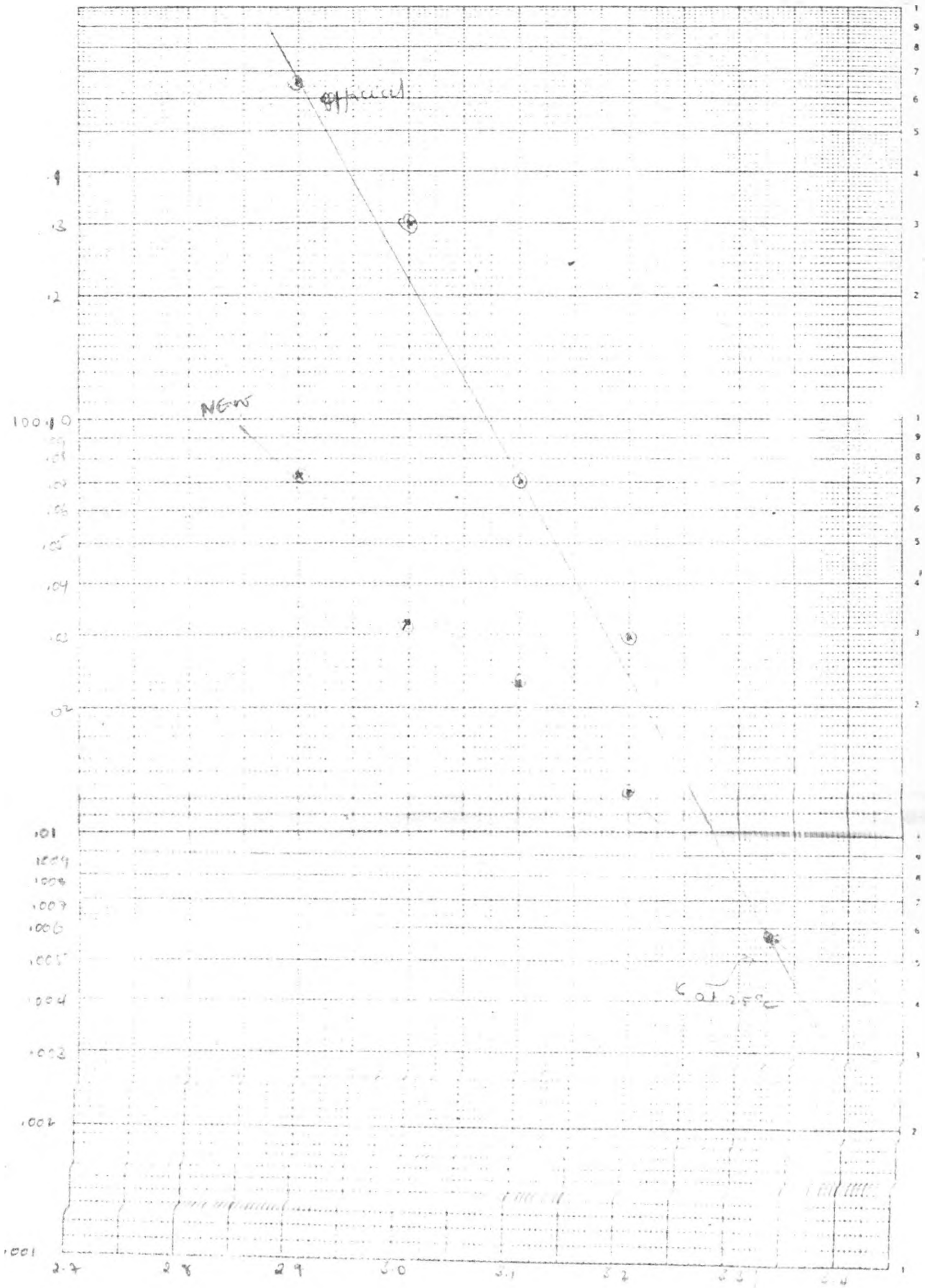
Log K vs. Time




Water Prop's
B.P. 1965
Kal 25°C
100
100
90
50
10
2.7
2.8
2.9
3.1
3.3

Pg 15 (7) Dry Powders

$\frac{1}{T}$



Km temp.

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