FERMENTATION STUDIES ON PHENOBARBITONE SYRUP (PAEDRIATIC) PREPARATION AT KENYATTA NATIONAL HOSPITAL (K.N.H.)

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D E D I C A T I O N

This piece of work is dedicated

to my beloved mother FLORENCE

WANJIRU KIHOTO AND MY DEAR SISTER

ROSE NYAMBURA

A C K N O W L E G E M E N T

I am very grateful to my Supervisors Mr. K. Kuria and Mr. D. S. Karanja for their invaluable criticisms and suggestions, and also for their moral support and encouragement during the course of this work.

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SUMMARY

The Kenyatta National hospital (K.N.H.) phenobarbitone syrup and syrup simplex 150 samples were investigated for fermentation using the gasometric method.[2] Thus the amount of gas liberated as a by-product of fermentation was monitored. The phenobarbitone syrup fermented more than the syrup simplex. Factors such as tablet excipients and contamination due to extra manipulation of the syrup in preparing phenobarbitone syrup were investigated. This was done using the hospital syrup, pure phenobarbitone base and 10% starch to prepare phenobarbitone syrup. The above factors were found to contribute to fermentation.

Laboratory prepared syrup simplex and phenobarbitone syrup were tested for fermentation to see if the results would mimic those of the hospital samples. These results were qualitative replicates of the hospital results.

Official phenobarbitone B.P. and U.S.P. elixirs were also investigated for fermentation to compare with the hospital samples. Quite unexpectedly, the official preparations showed signs of fermentation. This result was unexplanable.

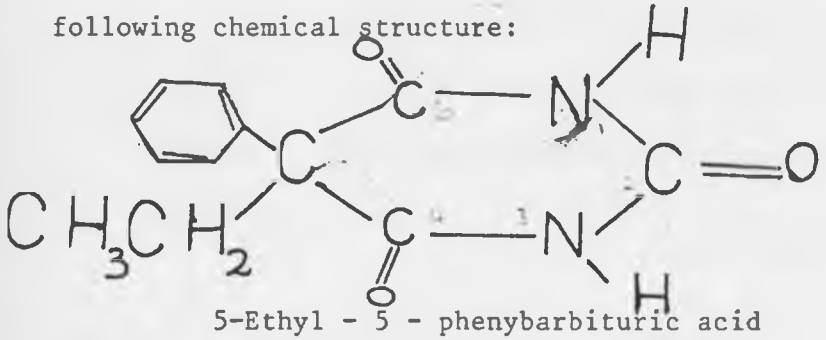
The only possibility would be that there might have been a leak through the apparatus. This should have been checked with controls of water and about 45% absolute alcohol. The official preparation had absolute alcohol as a preservative.[2] [11] Unfortunately, no controls were tested. Nevertheless, comparison of the official elixirs and hospital samples showed that the latter fermented at twice the rate of the former.

Lastly, benzoic acid preservative at concentrations ranging from 0.1-0.2% w/v were investigated for effective retardation of the fermentation of the hospital phenobarbitone syrup. It was found to be effective.

Recommendations have been made as to how problems encoutered in the phenobarbitone syrup as a dosage form with respect to dosage error (due to caking) and fermentation of the syrup can be rectified.

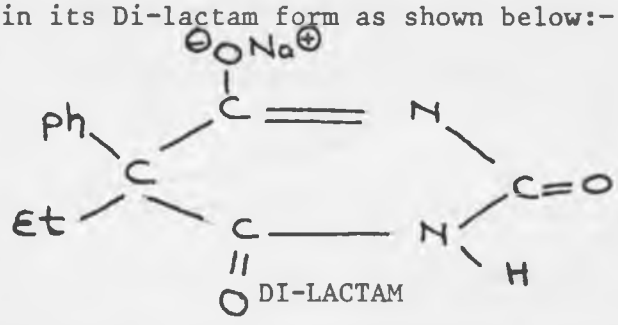
I N T R O D U C T I O N

Phenobarbitone is a long acting barbiturate.[1] It has sedative-Hypnotic and anticonvulsant actions. It has the following chemical structure:



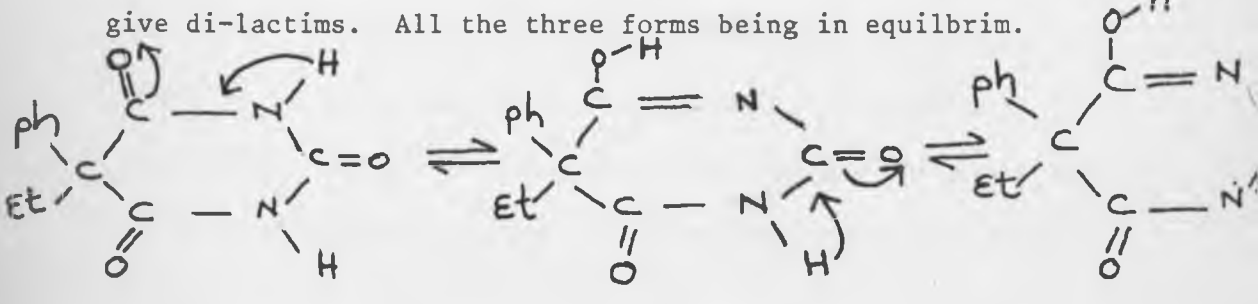
5-Ethyl - 5 - phenybarbituric acid

Phenobarbitone is weak acidic, sparingly soluble in water. It dissolves freely in solution of alkaline hydroxides, which exists in its Di-lactam form as shown below:-



DI-LACTAM

Phenobarbitone, like other barbiturates is basically a lactam and therefore is liable to undergo tautomerism to give di-lactims. All the three forms being in equilbrim.



The mono and di-lactimsare known to exist.

DOSE[1]

ADULTS: 30-125 mg thrice daily. In 24 hrs 600 mg

CHILDREN: Up to 2 years 8-15 mg thrice daily

2 to 5 years 15-30 mg thrice daily

6 to 12 years 30-120m thrice daily

INFANTS: 8-16 mg orally or intramuscularly

PREMATURE: 8 mg

Official preparation of phenobarbitones are:

1. Phenobarbitone tablets (15 mg.,30 mg. 60 mg.)
2. Phenobarbitone Sodium tablet (15 mg.30mg.60mg.)
3. Phenobarbitone Injection
4. Phenobarbitone elixir 15-20 mg. per ml.

At K.N.H. the out-patient adults are supplied with tablets. Children one supplied with either the 15 mg/5 mls teaspoonful or 15Mg Phenobarbitone tablet. phenobarbitone "Syrup". The former is an extemporaneous preparation. It is prepared by crushing tablets and suspending the powder in syrup. When 30 mg. or 60 mg. tablets are given to children, it is advised to break the tablet into two or four equal parts respectively. This is not practicable because phenobarbitone tablets are too small to be treated this way. Even when broken effectively there

is still a danger of obtaining wrong dosage due to lack of homogeneity of the tablet and variation in tablet weight. Errors in dosage are quite significant especially for potent drugs such as phenobarbitone.(3)

The extemporaneous preparation of "Syrups" from tablets in an attempt to overcome the paedriatic dosage problem has often been found necessary in developing countries like Kenya. This is due to lack of funds to ensure the availability of all strengths of tablets at all times. A literature survey shows that apparently no proprietary liquid preparations of phenobarbitone alone are available. Those proprietary preparations that contain phenobarbitone also contain other ingredients which undoubtedly make the preparations unnecessarily expensive where only the phenobarbitone is required. Furthermore, such other ingredients might even be undesirable for prolonged medication as in chronic conditions like epilepsy.

There are however, official formulae in various monographs(1), for extemporaneous preparation of suitable liquid dosage form of phenobarbitone. Due to lack of properly qualified

manpower, however, the possibility of making such preparations which are not only palatable but also relatively stable and cheap has not been realised.

The picture that emerges from an examination of the Dispensing practice in most government hospitals, K.N.H. included, is a most unprofessional one, where tablets are simply crushed and suspended in syrup. In such a process, no attention is paid to the precautions and steps necessary to ensure that such products are stable both from the chemical and bacteriological viewpoint.

In the particular example of phenobarbitone, the tablets are simply crushed in a motor and suspended in syrup simplex B.P which is not necessarily freshly prepared, besides having an unsightly appearance as it is normally prepared from crude sucrose. The unattractiveness of this preparation undoubtedly has negative influence on patients compliance.

Two problems have been identified with the hospital phenobarbitone "syrup". The first problem is ironically a dosage problem due to caking of the suspended drug. This problem, however, is not the subject of the present work. The second problem associated with K.N.H. syrup is one of fermentation.

Phenobarbitone syrup (K.N.H.) has an interesting history of fermentation. This project was set out to investigate why only the phenobarbitone syrup fermented and not the other syrup mixtures prepared at K.N.H. such as piriton and phenergan syrups etc.

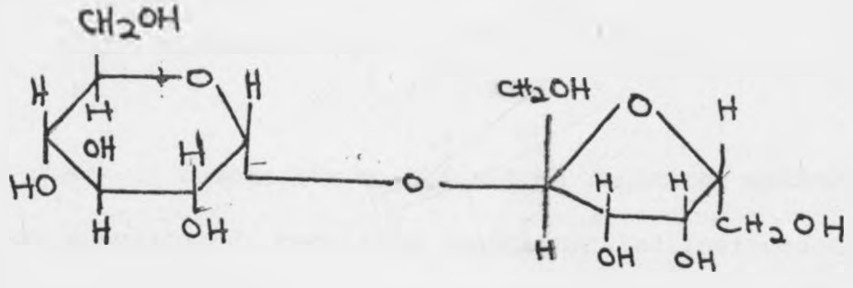
Fermentation process was thought to be taking place because of the characteristic smell of the by-products of fermentation. This smell was detected on opening of an old stock container of phenobarbitone syrup. Also the popping sound of the cork on opening of an old stock container was evidence of fermentation. The characteristic smell was that of acetic acid and the popping sound was thought to be due to carbon dioxide gas.

Fermentation was thought to occur in the phenobarbitone syrup because it was unpopular with the dispensers. It was apparent that the phenobarbitone syrup tends to be used only in the absence of the 15 mg tablets. It, therefore tends to remain on the shelf for periods much longer than the usual recommended shelf-life for extemporaneous preparations. A label with date of preparation would help to get rid of the expired preparation. Unfortunately this is

not indicated on the K.N.H. preparations. Other syrup preparations did not stay on the shelf more than one week at most. Hence no fermentation was detected.

FERMENTATION[2] "THEORY OF FERMENTATION"

The term "fermentation" with respect to carbohydrates is a term which, in its broadest sense, embraces all reactions which are brought about by the action of micro-organisms, the fungi, yeast, bacteria etc. Thousands of such reactions are known, but the mechanisms are usually incompletely understood, except that in general there are referrable to enzymes elaborated by micro-organisms. The syrup simplex B.P manufactured at K.N.H and which is used in the phenobarbitone syrup is prepared from crude sucrose. Sucrose is a dissaccharide consisting of one molecule of glucose and another of fructose. The chemical structure of sucrose can be represented as follows:



1 - α- D-Glucose pyranoside - β-Dfructose furanoside.

Sucrose is hydrolysable to glucose and fructose and it is also fermentable. Many micro-organisms possess the ability to ferment carbohydrates to simple alcohols, Ketones, acids and gases. Citric acid, 2-butanone and butyl-alcohol carbon dioxide and hydrogen gases are some of the several by-products of this process.

It is therefore very obvious that due to the diverse by-products of fermentation there is a problem in choosing the method of detecting and monitoring fermentation. Some of the various methods that have been employed or could be used in such an assessment are as follows:

1. Colorimetric method[5]
2. Manometric method[6]
3. Viscometric method[7]
4. Liquid chromatography[8]
5. Gas chromatography[9]
6. pH indicator media[4]
7. Gasometric method.[2]

With the exception of Gasometric method and pH indicator methods, the others are complicated, requiring sophisticated instruments and reagents. The pH measument was disqualified because acid

production is not the only factor that would contribute to the fall in pH in the phenobarbitone syrup. Phenobarbitone itself is an acid, moreover, benzoic acid, which was used as a preservative is an acid too.

Gasometric method had its own short-comings. Micro-organisms could be gas producers or non-gas producers. It was assumed, however, that the aerial microorganism composition would tend to remain constant. Thus, the greater the microorganism population, the more are the gas producers and the greater the degree of fermentation measured by the gasometric method would appear.

NOTE:

The word fermentation when used in microbiology means acid production.

The word "Syrup" in this context means preparation which is made by simply dissolving or suspending the ingredients in syrup simplex.

Preliminary work was carried out to check if the gasometric method would be feasible. Small amounts of phenobarbitone "syrup" and syrup simplex from K.N.H were monitored for fermentation by measuring the amounts of gas liberated. This method was found to be feasible.

PRESENT WORK

In the present work, it was intended that the following be investigated:

1. Fermentation of hospital syrup simplex B.P and phenobarbitone syrup.
2. The effects of tablet excipients and extra manipulation of syrup simplex in preparing phenobarbitone syrup.
3. The extent of fermentation of hospital phenobarbitone syrup. This would involve comparison of fermentation of the phenobarbitone syrup and the official preserved phenobarbitone syrup B.P and U.S.P.
4. Finally, Benzoic acid preservative would be used in different concentrations to check if it can effectively preserve the hospital phenobarbitone syrup. The pH of phenobarbitone syrup to be preserved with benzoic acid is crucial. It should not be more than pH 5. Hence

it was considered necessary that the pH of phenobarbitone syrup be investigated first before using benzoic acid preservative.

EQUIPMENTS, MATERIALS AND METHODS OF PREPARATION

(A) MATERIALS

(i) HOSPITAL MATERIALS

	MATERIAL	QUALITY	BRAND
1.	SUCROSE	CRUDE REFINED MIXTURE OF CRUDE AND REFINED	-
	WATER	POTABLE	-
	PHENOBARBITONE TABLETS 15 MG	-	BOEHRINGER INGELHEIM

(ii) LABORATORY MATERIALS

MATERIAL	QUALITY	BRAND
SUCROSE	PURE ANALYTICAL	BDH-CHEMICALS LTD
WATER	POTABLE	-
PHENOBRARBITONE TABLETS 15 MG.		BOEHRINGER INGELHEIM
PHENOBARBITONE BASE	PURE BASE	BDH-CHEMICALS LTD
COMPOUND TARTRAZINE	B.P.	E.T. MONKS & CO. LTD.
ORANGE OIL	B.P.	
ALCOHOL	ABSOLUTE	BDH-CHEMICALS LTD.
AMARANTH SOLUTION	1% SOLUTION B.P.C.	E.T. MONK & CO. LTD.
BENZOIC ACID	PURE	BDH-CHEMICALS LTD
STARCH	PURE ANALYTICAL	" " "
pH Tablets p^H 4 & $_pH$ 7		BDH- CHEMICALS LTD

(B) E Q U I P M E N T S

(1) Dispensing bottles of capacity 200 mls.
(2) Rubber bung, Size: No. 17
(3) Injection needle 18G½ (1.20X38 m.m)
(4) Cannular. Size: 2FG (0.63m.mX30 CMS)
(5) 25 MLS burette
(6) Retort stand and clump
(7) Thermometer
(8) 500 mls beaker
(9) pH meter. Type: Pye Unicam-PHILIPS PW 9418
(10) Rubber bands
(11) 1ml pipette
(12) Measuring cyclinders 500 mls, 250 mls.

(C) M E T H O D S OF P R E P A R A T I O N

1. METHOD OF PREPARATION OF HOSPITAL (K.N.H.) SYRUP SIMPLEX B.P.

F O R M U L A :	QUANTITIES FOR 40 LITRES
Sucrose 667G	Sucrose 26.68Kg.
Water to 1000G	Water to 40.0 Ltrs.

C A L C U L A T I O N S

Density of syrup [10] at 20°C is 1.315 to 1.333 GML-1

average density of syrup is 1.324 GML-1

i.e.

1ml syrup = 1.324G syrup

40 liters syrup = $1.324 \times 40 \times 10^3 = 5.296 \times 10^4$G

40 liters syrup = 52.96Kg. syrup

Concentration of sucrose required in syrup simple B.P. is 66.7% W/W

amount of sucrose required for 40 liters syrup is $52.96 \times \frac{66.7}{100}$ = 35.32Kg. sucrose

Weight of sucrose required = 35.32Kg.
Weight of water required = (52.96-35.32)Kg.
= 17.64Kg. (Liters)

Density of water is 1 GML-1 at 20°C.

PROCEDURE

1. 35.32Kg. Sucrose is weighed and placed in a large 40 liter metal vessel.
2. 17.64 liters of water is added to the sucrose.
3. The sucrose is dissolved by heating at a very slow rate with stirring. The preparation is coveved to limit evaporation.
4. After all the sucrose has dissolved, the syrup is left to cool. Then it is strained through gauze and transfered to official stock container. Finally the label is fixed.

LABEL

SYRUP SIMPLEX B.P.

METHOD

2. METHOD OF PREPARATION OF K.N.H. PHENOBARBITONE SYRUP

FORMULA		QUANTITIES FOR 5 LITERS
Phenobarbitone	15mg	Phenobarbitone 15G
Syrup simplex B.P.	5mls	Syrup simplex B-P 5liters

CALCULATION

Phenobarbitone 15mg tablet = 5mls syrup

5 liters syrup = 1000 tablets

PROCEDURE

1. 1000 tablet 15 mg phenobarbitone are soaked in a mortar with a little water for about 10-15 mins.
2. A smooth paste is obtained from the soaked tablets. A little syrup is added and the smooth paste is transferred to a tared stock container.
3. The mortar and pestle are rinsed with syrup and the rinsings added into the stock container.
4. The preparation is made to volume with syrup simplex B.P. the label is finally fixed.

LABEL

PHENOBARBITONE SYRUP 15 MG PHENOBARBITONE IN 5 ml

2. METHOD OF PREPARATION OF LABORATORY SYRUP SIMPLEX B.P.

FORMULA	QUANTITIES FOR 1 LITER
Sucrose 667 G	Sucrose 0.896Kg.
Water to 1000G	Water to 1000mls.

CALCULATION:

Weight of syrup per 1m is 1.315 to 1.333G at 20°C

Average density of syrup is 1.324 GML-1

i.e.

1ml Syrup = 1.324G Syrup

1liters syrup = 1.324Kg. Syrup

Amount of sucrose required is $\frac{66.7 \times 1.324}{100}$ = 0.896Kg.

Amount of water required is (1.324−0.896)= 0.428Kg.

PROCEDURE

1. 0.896Kg. Sucrose was weighed and put in1litre beaker
2. 01428Kg. of water was added to dissolve the sucrose
3. The mixture was heated at a very slow rate with occasional stirring. The mixture was covered to limit evaporation
4. After all the Sucrose had dissolved, the syrup was left to cool, then strained through gauze.
5. The syrup was transferred to stock container and label fixed.

LABEL

SYRUP SIMPLEX B.P. DATED CODE SS_L

4. METHOD OF PREPARATION OF LABORATORY PHENOBARBITONE SYRUP

F O R M U L A	QUANTITIES FOR 500 mls
Phenobarbitone 15MG	Phenobarbitone (15MGtabs)100tabl
Syrup to 5ml	Syrup to 500 ml.

C A L C U L A T I O N S

5 ml = 1 table (15MG phenobarbitone)

500mls =100 tablets

P R O C E D U R E

1. 100X15MG phenobarbitone tablets were crushed in a mortar to a powder.
2. A smooth paste was obtained by adding a little syrup to the the powder.
3. The paste was transferred to a measuring cylinder. The mortar and pestle were rinsed and the rinsing added into the cylinder.
4. The preparation was made to volume with syrup simplex B.P. prepared in the laboratory.
5. The label was fixed after transfering to stock container.

L A B E L

PHENOBARBITONE SYRUP
15MG Phenobarbitone / 5ml.
Dated Code:PP_L

5. METHOD OF PREPARATION OF LABORATORY PHENOBARBITONE SYRUP USING PURE PHENOBARBITONE BASE

F O R M U L A	QUANTITIES FOR 500 mls
Phenobarbitone base 15MG	Phenobarbitone base 1.5G
Syrup to 5 mls	Syrup to 500mls

Cont'd..... .....

P R O C E D U R E

1. 1.5G phenobarbitone pure base was weighed and put in mortar.
2. A little syrup (prepared in the laboratory) was added to the base and a smooth paste obtained.
3. The paste was transferred into a measuring cylinder
4. The mortar and pestle were rinsed and the rinsing added into the measuring cylinder
5. The preparation was made to volume with syrup prepared in the laboratory and the label fixed after transfering to stock container.

L A B E L

PHENOBARBITONE SYRUP
15mg phenobarbitone/5mls
Dated Code: BB

6. METHOD OF PREPARATION OF PHENOBARBITONE SYRUP WITH 10% STARCH

F O R M U L A		QUANTITIES FOR 500 mls	
Phenobarbitone pure base	15G	Phenobarbitone pure base	=1.5Kg.
Starch	1.5G	Starch	=0.15Kg.
Syrup to	5mls	Syrup to	500mls.

PROCEDURE

1. 1.5Kg of pure phenobarbitone base was weighed and put in a mortar.
2. 0.15Kg. starch was weighed and triturated with the base (phenobarbitone).
3. A little syrup from the laboratory was added to the mixture of powder in the mortar and mixed to obtain a smooth paste.
4. The paste was transferred to a measuring cylinder.
5. The mortar and pestle were rinsed and the rinsing added into the measuring cyclinder.
6. The preparation was made to volume with syrup simplex from the laboratory and the label fixed after transfer to stock container.

L A B E L

PHENOBARBITONE SYRUP
15mg phenobarbitone/5mls
Dated Code:- BS

7. METHOD OF PREPARATION OF PHENOBARBITONE ELIXIR B.P.

FORMULA		QUANTITIES FOR 500 mls	
Phenobarbitone	3G	Phenobarbitone	1.5G
Compound		Compd tartrazine	5.0mls
Tartrazine	10 mls	Orange oil	12.0 mls
Compond		Glycerol	200.0mls.
Orange sprit	24mls	Alcohol (abs)	200.0mls.
Glycerol	400mls	Water to	500.0mls
Alcohol (abs)	400 mls		
Water to	1000mls		

PROCEDURE

1. Phenobarbitone pure base was weighed and put in a mortar
2. Alcohol was added to dissolve the base.
3. Compound tartrazine, orange oil were added.
4. The mixture was transferred to a measuring cylinder and Glycer added.
5. The mortar was rinsed with water and the rinsing added to the mixture in the cylinder
6. The preparation was made to volume with distilled water and th preparation transferred to the stock container and the label f

L A B E L

PHENOBARBITONE ELIXIR B.P.
15 mg phenobarbitone/5mls
Dated Code: P.E.-B.P.

8. METHOD OF PREPARATION OF PHENORBARBITONE ELIXIR U.S.P

FORMULA		QUANTITIES FOR 500 mls	
Phenobarbitone	4G	Phenobarbitone	2G
Amaranth solution	10mls	Amaranth solution	5mls.
Orange oil	0.75mls	Orange oil	0.375mls
Glycerol	450mls	Glycerol	225mls
Alcohol(abs)	150mls	Alcohol	75mls
Water to	1000mls	Water to	500mls

PROCEDURE

1. Phenobarbitone base was weighed and put in a mortar
2. Alcohol was added to dissolve the base
3. The Amaranth solution, orange oil and Glycerol were added to the mixture.
4. The mixture was transferred to a measuring cylinder
5. The mortar and pestle were rinsed with distilled water and the rinsing added to the mixture in the cyclinder.
6. The preparation was made to volume with water, transferred to the stock container and the label fixed.

L A B E L

PHENOBARBITONE ELIXIR U.S.P.
20 mg phenobarbitone/5mls
Dated Code: P.E.-U.S.P.

9. METHOD OF PREPARATION OF PHENOBARBITONE SYRUP WITH 0.1% BENZOIC ACID

FORMULA		QUANTITIES FOR 500 mls	
Phenobarbitone	15mg	Phenobarbitone	15mgX100 tablets
Benzoic acid	0.015mg	Benzoic acid	1.5mg
Syrup to	5mls	Syrup to	500 mls.

PROCEDURE

1. 15mgX100 phenobarbitone tablets were crushed in a mortar.
2. Benzoic acid powder was weighed and triturated with the powdered tablets.
3. A little syrup simplex B.P. from the hospital was added to the mixture of powder and a smooth paste formed.
4. The paste was transferred to a measuring cyclinder
5. The mortar and pestle were rinsed and the rinsings added into the cyclinder.
6. The preparation was made to volume with syrup simplex B.P. from the hospital and then transferred to stock container and the label fixed.

L A B E L

PHENOBARBITONE SYRUP
(with 0.1% w/v Benzoic acid)
15 mg phenobarbitone/5 mls
Dated Code:PSO.1%

10. Phenobarbitone Syrup with 0.15% w/v and 0.2% w/v Benzoic acid were prepared as (9) above.

The labels are as shown below.

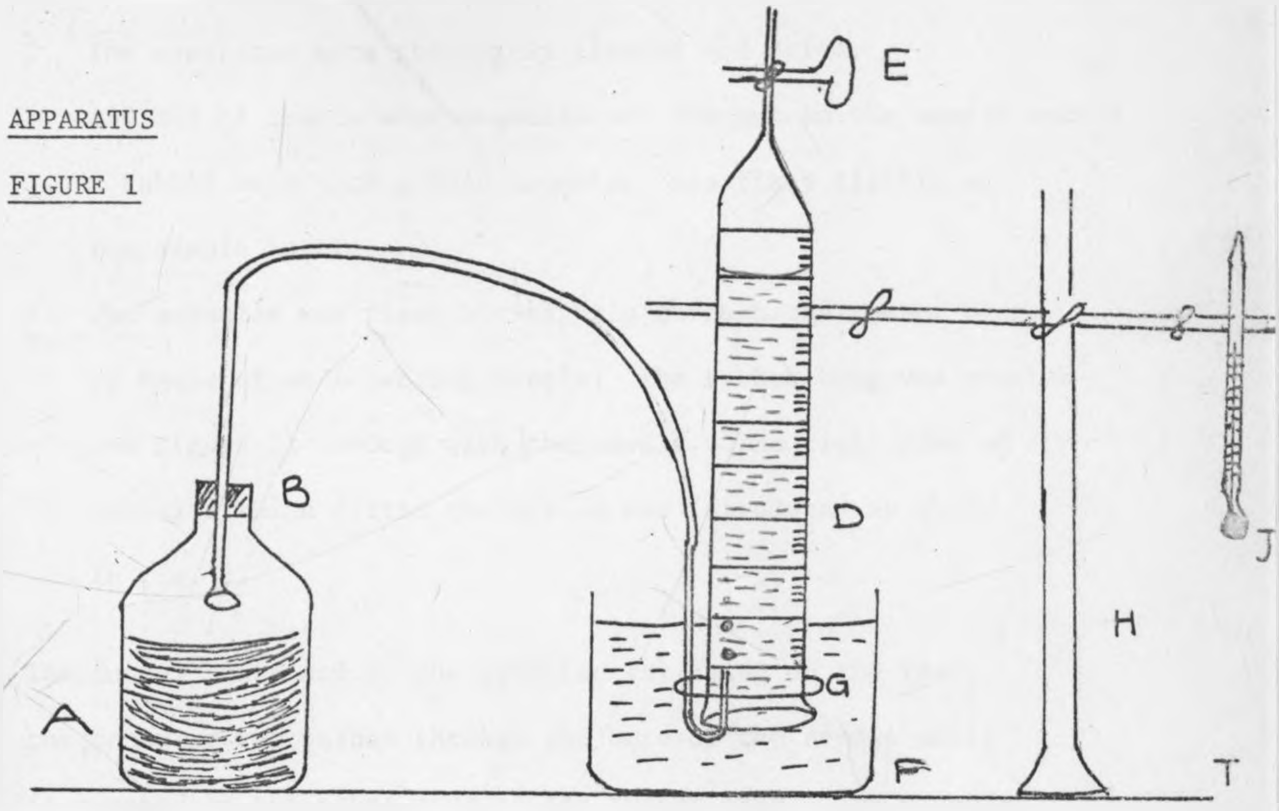
PHENOBARBITONE SYRUP
(0.15% w/v Benzoic acid)
15mg. phenobarbitone/5ml
Dated Code: PS 0.15%

PHENOBARBITONE SYRUP
0.2% w/v Benzoic Acid
15 mg phenobarbitone/5mls
Dated Code:PSO.2%

EXPERIMENTAL

APPARATUS

FIGURE 1



A - Sample bottle (Dispensing bottle) Capacity 200 mls.

B - Rubber bung

C - Cannular

D - Burette

E - Burette tap

F - Beaker of water

G - Rubber band

H - Retort stand and clumps

J - Themormeter

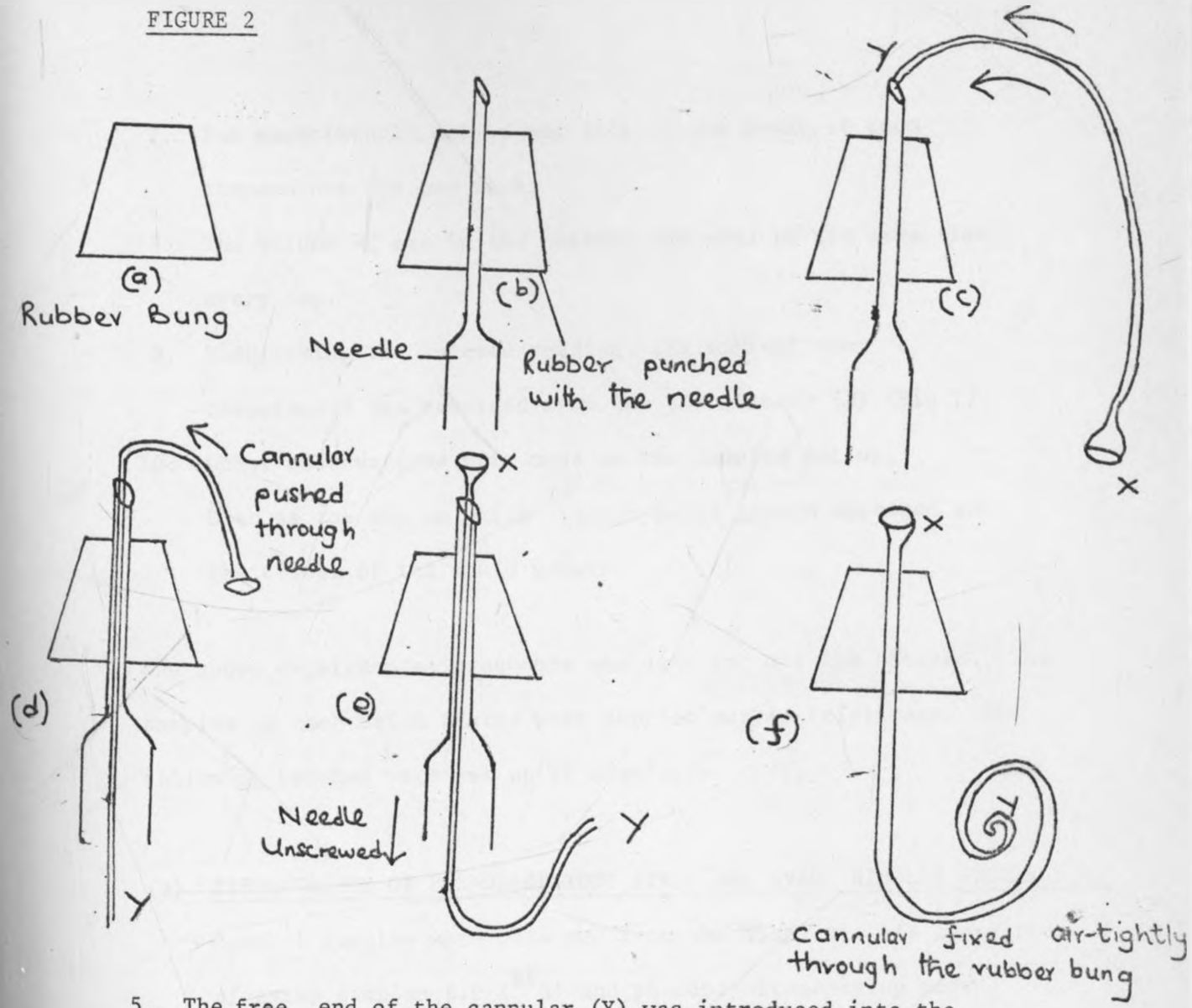
T - Bench

EXPERIEMENTAL PROCEDURE

1. The apparatus were thoroughly cleaned and dried.
2. 150 mls of sample were measured out and put in the sample bottle.
3. A rubber bung with a thin cannular was fixed tightly on the sample bottle.
4. The cannular was fixed air-tightly through the rubber bung by means of an injection needle. The rubber bung was punched (see figure 2) through with the needle. The right size of cannular which fitted the needle was introduced as shown in fig. 2.

The funnel shape end of the cannular (x) being on the rear, the cannular was pushed through the bore of the needle until it emerged on the other side of the rubber bung. The cannular was then pulled leaving the funnel shaped end sticking out as shown in fig. 2. The needle was then unscrewed carefully to avoid occlusion of the thin cannular. (This was a difficult task, most of the thin cannulars were easily occluded during this operation). On removal of the needle the cannular was left in place fixed air-tight through the rubber bung.

FIGURE 2



5. The free end of the cannular (Y) was introduced into the 25 mls burette full of water (fig.1). The cannular was held in place by means of a rubber band. The burette was then inverted over a beaker of water and the volume of the burette adjusted to read 25 mls with the burette tap (E)

6. The burette tap and mouth of sample bottle were greased properly to check any gas leak.

7. The experimental set up was left on the bench at room temperature for one week.
8. The volume of gas in the burette was read at the same time every day.
9. When taking the burette reading, the ambient room temperature was recorded from the thermometer (J) (Fig 1).
10. Other observations were made on the samples set up. Such as the day on which visible mould growth appeared and the colour of the mould growth.

The above experimental procedure was done for all the batches. The samples in each batch tested were carried out in triplicate. The following batches were set up to examine:-

(a) FERMENTATION OF PHENOBARBITONE SYRUP AND SYRUP SIMPLEX FROM K.N.H.

Batch 1 samples were obtained from the hospital. It consisted of syrup simplex B.P ($^{SS}_{H}$) and phenobarbitone syrup made from tablets ($^{PP}_{H}$). The results in table 2 showed that $^{PP}_{H}$ fermented more than the syrup $^{SS}_{H}$. Another batch, Batch 2 was tested containing identical samples from the hospital ($^{SS}_{H}$) and ($^{PP}_{H}$) to see if batch 1 results would be reproduced. Batch 2 (Table 3) were replicates of the first batch qualitatively.

From the above results, it was apparent that two possibilities would have contributed to the fermentation of phenobarbitone syrup. These are

1) The tablet excipients
2) Contamination due to extra manipulation of Syrup simplex B.P in preparing the phenobarbitone Syrup.

b) EFFECTS OF TABLETS EXCIPIENTS AND EXTRA MANIPULATION OF SYRUP ON FERMENTATION OF PHENOBARBITONE SYRUP:

In this batch 3, syrup simplex and phenobarbitone syrup were prepared in the laboratory and their fermentation compared with phenobarbitone syrup prepared using pure phenobarbitone base instead of tablets and syrup simplex prepared in the laboratory (BB), and phenobarbitone syrup (BS) prepared using pure phenobarbitone base and laboratory prepared syrup with 10% starch. Starch was the only excipient which was easily detected by iodine test.

The results in table 4 were as follows:-

1) $^{PP}_{L}$ fermented more than $^{SS}_{L}$, thus the same trend of fermentation like one obtained with the hospital samples ($^{PP}_{H}$ and $^{SS}_{H}$).
2) BS fermented more than BB. This indicated that other tablet excipients and possible contamination during the preparation of phenobarbitone syrup from syrup simplex contributed to the fermentation of phenobarbitone syrup.

c) COMPARISON OF FERMENTATION OF K.N.H. PHENOBARBITONE SYRUP AND SYRUP SIMPLEX WITH OFFICIAL PHENOBARBITONE ELIXIRS (B.P AND U.S.P

A comparison of fermentation of the hospital samples and the official elixirs was carried out to "gauge" the extent of fermentation of the hospital samples. This was necessary because previous results of the hospital samples showed fermentation. but the fermentation could not be quantified.

This batch no.4 consisted of:

1. SS_H and PP_H
2. Phenobarbitone elixir B.P(P.E.-B.P)
3. Phenobarbitone elixir U.S.P. (P.E.- U.S.P).

Results one shown in table 5.

d) EFFECTS OF BENZOIC ACID ON THE FERMENTATION OF HOSPITAL PHENOBARBITONE SYRUP

From batch 4 results (Table 5), the K.N.H. phenobarbitone syrup fermented more compared to the official elixirs.

Batch 5 was therefore set to investigate the preservative effects of benzoic acid or the fermentation of phenobarbitone syrup. Benzoic acid was used in three different concentration to find out the most effective concentration. These concentrations are $0.1\%^{w/}v$, $0.15\%^{w/}v$ and $0.2\%^{w/}v$ benzoic acid.

This batch consisted of:

1. SS_H and PP_H
2. P.E.- B.P and P.E.- U.S.P
3. Phenobarbitone syrup from the hospital with
   - (a) 0.1%^{W}/v
   - (b) 0.15%^{W}/v
   - (c) 0.2%^{W}/v

   } -Benzoic acid preservative

Results are shown in table 6.

e) p^H of HOSPITAL PHENOBARBITONE SYRUP WITH BENZOIC ACID PRESERVATIVE

Benzoic acid acts effectively as a preservative at p^H not more than p^H5. The p^H of hospital phenobarbitone syrup with the different concentration of benzoic acid was recorded to check p^H requirement. (table 7). The pH of other samples in this batch were recorded too.

Results are shown in table 7.

R E S U L T S

TABLE 1

RELATIONSHIP BETWEEN FERMENTATION AND TEMPERATURE VARIATION AND GRADE OF SUCROSE IN K.N.H. SAMPLES IN BATCHES 1,2 AND 3

BATCH	GRADE OF SUCROSE	TEMP. RANGE	AVERAGE VOL. OF GAS AT 25°C AND 630 m.mHg in mls/week	
			SS_H	PP_H
1	CRUDE SUCROSE	19-26°C	3.80	4.40
2	CRUDE SUCROSE	25-30°C	0.0	19.15
3	MIXTURE OF CRUDE AND REFINED - SUCROSE	19-25°C	4.0	10.60

SS_H = K.N.H. Syrup Simplex

PP_H = K.N.H. Phenobarbitone Syrup.

NOTE

1. The volume of gas collected in the following batches (table 2 to 6) is the volume of gas collected from the beginning of testing to the time (day) on which the reading was recorded. The volumes are already converted to 25°C and 630 m.m. Hg using the gas equation PV = RT.

2. Blank indicates no readings were recorded.

FERMENTATION OF PHENOBARBITONE SYRUP AND SYRUP SIMPLEX FROM K.N.H.

1. BATCH 1 TABLE 2

DAY	AVERAGE VOLUME OF GAS LIBERATED IN MLS AT 25°C AND 630 M.M. Hg		ROOM TEMP.
	SS_H	PP_H	
1	0.86	2.05	25°
2	1.60	3.05	26°C
3	2.15	3.25	24°C
4	2.70	3.25	22°C
5	-	-	-
6	-	-	-
7	3.80	4.40	21°C

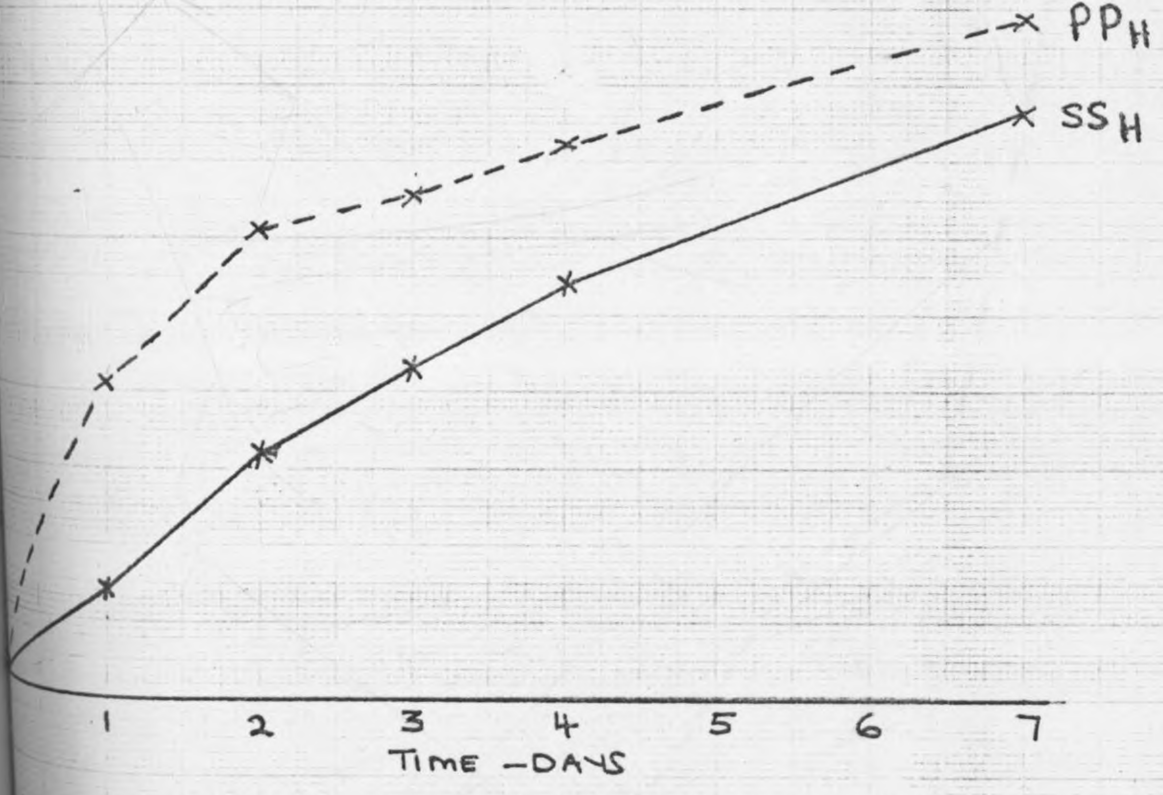
2. BATCH 2 TABLE 3

DAY	AVERAGE VOLUME OF GAS LIBERATED IN MLS AT 25°C AND 630 M.M. Hg.		ROOM TEMP.
	SS_H	PP_H	
1	0.0	0.0	30°C
2	-	-	-
3	0.0	3.0	25°C
4	0.0	5.9 * MG (GREY)	28°C
5	0.0	8.9	27°C
6	0.0	10.2	25°C
7	0.0	19.15	28°C

* MG (GREY) - DAY OF MOULD GROWTH APPEARANCE AND COLOUR

GRAPH 1 (TABLE 2)

RATE OF FERMENTATION OF K·N·H SYRUP SIMPLEX AND PHENOBARBITONE SYRUP IN TERMS OF GAS LIBERATED IN MLS



K·N·H SYRUP SIMPLEX ———— SS$_H$

K·N·H PHENOBARBITONE SYRUP - - - - - PP$_H$

GRAPH 1 (TABLE 5)

RATE OF FERMENTATION OF K·N·H SYRUP SIMPLEX AND PHENOBARBITONE SYRUP IN TERMS OF GAS LIBERATED IN MLS

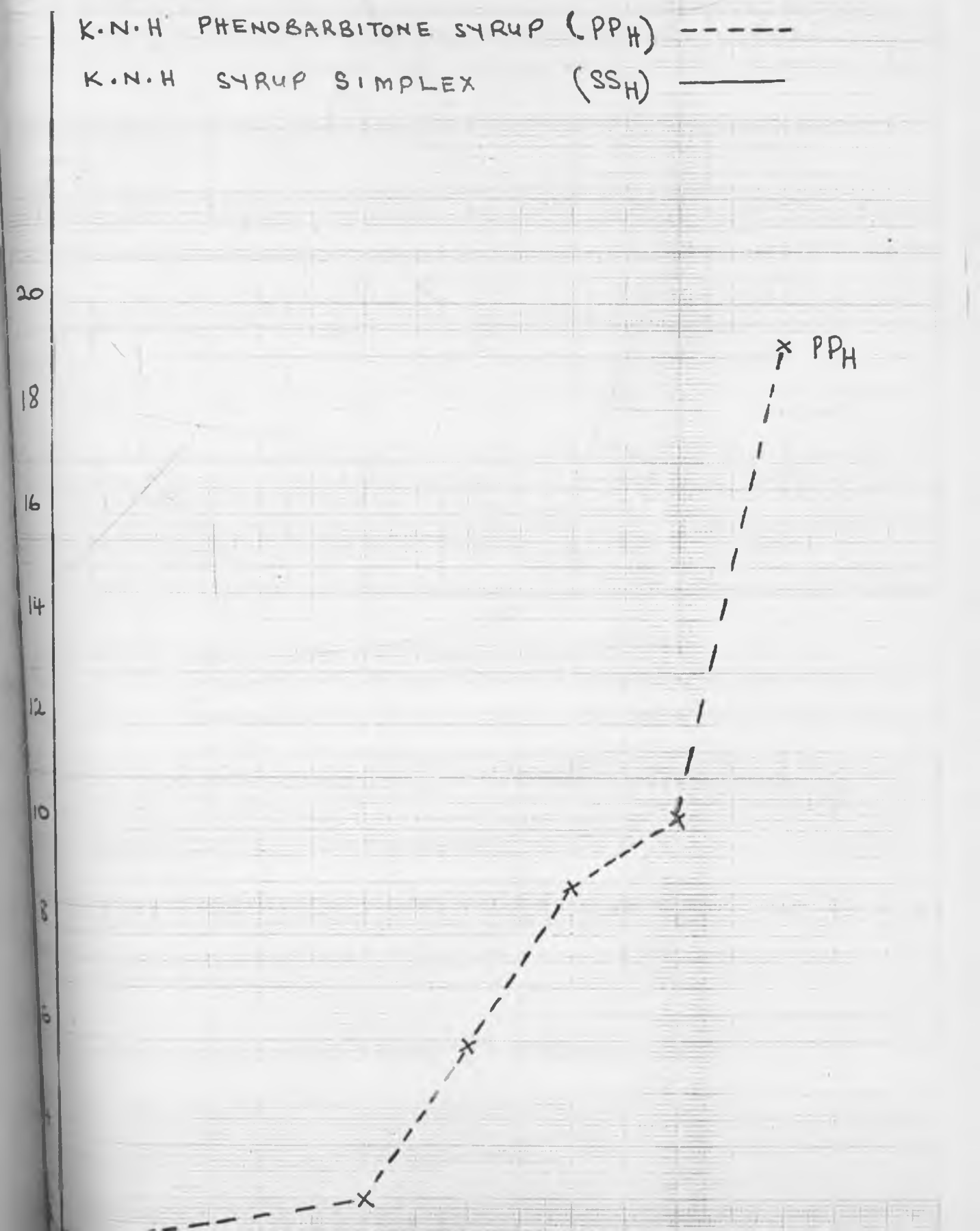


TABLE 4 (BATCH 3)

EFFECT OF TABLET EXCIPIENTS AND EXTRA MANIPULATION ON FERMENTATION OF PHENOBARBITONE SYRUP

DAY	AVERAGE VOLUME OF GAS LIBERATED AT 25°C AND 630 m.m. Hg in mls.				ROOM TEMP.
	SS_L	PP_L	BB	BS	
1	-	-	-	-	-
2	-	-	-	-	-
3	0.0	4.0	0.0	3.1	19°C
4	0.0	5.1	0.0	4.1	22°C
5	0.0	7.6	2.0	6.1	23°C
6	0.9	8.1	2.2	6.8	22°C
7	3.7	10.6	5.4	9.8	25°C

SS_L = Syrup simplex prepared in the laboratory

PP_1 = Phenobarbitone (tablets) syrup prepared in the laboratory

BB = Phenobarbitone syrup prepared from pure phenobarbitone base and laboratory syrup simplex B.P. (SS_L above).

BS = Phenobarbitone syrup as (BB) above with 10% starch.

TABLE 5 (BATCH 4)

COMPARISON OF FERMENTATION OF K.N.H. PHENOBARBITONE SYRUP AND SYRUP SIMPLEX WITH OFFICIAL PHENOBARBITONE ELIXIRS (B.P. AND U-S.P.)

DAY	AVERAGE VOL. OF GAS LIBERATED AT 25°C AND 630 m.m. Hg IN mls.				ROOM TEMP.
	SS_H	PP_H	P.E.-B.P.	P.E-U.S.P.	
1	2.9	3.4	1.9	1.55	20°C
2	5.4	6.0	3.4	3.0	27°C
3	9.4	10.1	5.4	4.1	30°C
4	-	-	-	-	-
5	-	-	-	-	-
6	17.4	10.9 *mg(Pink)	10.0	9.5	27°
7	18.9	22.0	13.0	10.5	28°C

P.E.-B.P. = Phenobarbitone elixir B.P.

P.E.-U.S.P= Phenobarbitone elixir U.S.P.

TABLE 6 (BATCH 5)

EFFECTS OF BENZOIC ACID ON THE FERMENTATION OF HOSPITAL PHENOBARBITONE SYRUP

DAY	AVERAGE VOL. OF GAS IN MLS PRODUCED AT 25°C AND 630 m.m. Hg.							ROOM TEMP
	SS_H	PP_H	P.E-B.P	P.E.U-SP	PSO.1%	PSO.15%	PSO.2%	20°C
1	2.9	3.4	1.9	0.5	2.6	3.0	1.2	20°C
2	5.4	6.0	3.6	1.9	3.0	4.1	2.0	25°C
3	9.2	*MG(Pink) 10.1	4.9	3.3	3.6	5.2	4.3	30°C
4	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-
6	*MG (Pink) 12.9	14.7	9.0	8.0	9.3	9.3	7.7	27°C
7	13.6	22.0	9.4	8.0	9.7	10.1	8.6	27°C

PS 0.1% = PP_H with 0.1% $^{w}/_{v}$ Benzoic Acid preservative

PS.0.15%= PP_H with 0.15% $^{w}/_{v}$ " " "

PS.0.2% = " " 0.2% $^{w}/_{v}$ " " "

TABLE 7 (BATCH 6)

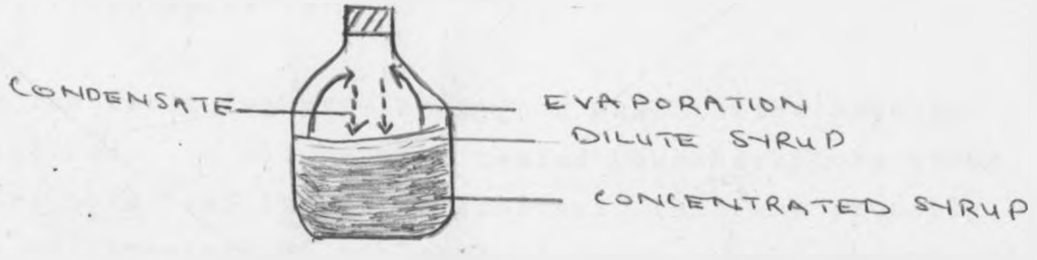
P^H OF HOSPITAL PHENOBARBITONE SYRUP WITH BENZOIC ACID PRESERVATIVE

SAMPLE	INITIAL P^H	FINAL P^H
SS_H	5.6	5.5
PP_H	5.4	5.0
PSO.1%	4.4	3.9
PSO.15%	4.3	4.0
PSO.2%	4.3	3.7

D I S C U S S I O N

In carrying out the determination and monitoring of fermentation, several problems were encountered. These problems resulted in obtaining only qualitative rather than both qualitative and quantitative results. The main problem was lack of suitable water-baths. The samples were left at room temperature which fluctuated within the range of 19°C to 30°C. Table 1 shows the relationship between gas liberated, grade of sucrose and the temperature in the first three batches. Had the temperature been controllable, then the effect of the grade of sucrose would have been obtainable. Favourable temperature range of the second batch (25°C-30°C) resulted in a higher rate of fermentation of phenobarbitone syrup than that for the first or the third batch. The volume of gas collected at ambient temperature was corrected to 25°C and 630 m.m. Hg using the Gas Equation PV=RT. This correction was that of expansion and contraction of the volume of gas with temperature.

Fluctuation in room temperature would tend to cause a lowering of the sucrose concentration in the top layers of the syrup in a container. An increase in temperature would lead to evaporation of the water. If this is followed by a decrease in temperature, the condensate would full on top of the syrup. This would result in a decrease in concentration of the syrup at the top.



The resultant decrease in syrup concentration would render the syrup incapable of retarding micro-organism growth. Syrup at concentration of 67% ^{w}/w Sucrose has the Intrinsic capacity

to preserve itself from any microbial growth. Thus it exerts an osmostic pressure which is intolerable by the micro-organism. This argument was supported by the fact that visible mould growth appeared only on the top layer of the syrup samples. This was further supported by the fact that there was no apparent fermentation in syrup (^{SS}H) in the second batch probably because the temperature range ($25^{\circ}C$-$30^{\circ}C$) did not result in any significant evaporation and condensation. (TABLE 3 AND GRAPH 2)

The B.P. Specification for syrup simplex is 67% $^{w}/w$ sucrose. It also states the storage condition- " should not be exposed to undue fluctuation in temperature [11]". In concentration of 65%$^{w}/v$ sucrose the syrup would retard the growth of micro-organisms. In dilute solutions, sucrose provides an excellent nutrient for moulds, yeast and other micro-organisms.

Other aspects of the project that contributed to lack of quantitative results were:-

1. Variation in grade of sucrose used in different batches. Crude, refined or a mixture of these were used to prepare syrup simplex.

2. Problems encountered in biological assay, such as phase of micro-organism growth (vegetative or spore forms).

Despite the foregoing problems, good qualitative results were obtained. In all batches tested phenobarbitone syrup fermented more than the syrup simplex. This was probably because of: Presence of tablet excipients in the phenobarbitone tablet which was used in preparing the syrup or due to contamination of syrup simplex during the extra manipulation when preparing the phenobarbitone syrup. The former possibility was not anticipated at the beginning

of the project. It was thought that the micro-organisms would prefer to utilise simple sugar rather than complex sugars.

Phenobarbitone syrup showed possible contamination because visible mould growth appeared more frequently and faster than that of syrup simplex.

Tests were carried out to investigate the effect of tablet excipients and extra manipulation of the syrup in preparing phenobarbitone syrup. Syrup simplex B.P. prepared in the laboratory (SS_L) using pure analytical sucrose was used. Starch was the only excipient that was easily identified in the tablet by the iodine test.

Results in tablet 4 showed that phenobarbitone syrup prepared from pure phenobarbitone base and laboratory prepared syrup simplex (BB) fermented more than the laboratory prepared syrup simplex SS_L. This is probably due to extra manipulation of the syrup simplex SS_L. It was noted that in both cases (BB) and (SS_L) no fermentation was shown in the first four days. This could be looked at as the maximum shelf-life of the unpreserved laboratory phenobarbitone syrup. This however needs further investigation to be conclusive.

The phenobarbitone syrup prepared from pure phenobarbitone base, syrup simplex (SS_L) and 10% starch (BS) fermented more than (BB). The possible explanation is probably due to starch and extra manipulation.

From table 4 results, the following quantitative results were obtained:-

(a) Effect of table excipients = $PP_L - (SS_L - BB)$

= 10.6(3.7+5.4)

= 10.6-9.1

= 1.5 mls gas/150 mls sample/week

(b) Effect of 10% starch= $BS-(SS_L-BB)$

= 9.8-9.1

= 0.7 mls gas/150 mls sample/week

(c) Effect of Un-identified tablet excipients

= (a)-(b)

= 1.5-0.7

= 0.8 mls gas/150 mls sample/week

(d) Effect of contamination due extra mainpulation

= $BB-SS_L$

= 5.4-3.7

= 1.7 mls gas/sample 150mls/week

Thus the tablet excipients and possible contamination due to extra manipulation of the syrup simplex contributed to the rise in fermentation. The effects of these were approximately the same i.e. 1.5 mls and 1.7 mls gas/150 mls sample/week respectively.

Comparison of the hospital samples (SS_H and PP_H) with the official phenobarbitone elixirs B.P.", and U.S.P. [2], was carried out to see the extent of fermentation of the hospital syrup. The official elixirs, quite unexpectedly showed signs of fermentation. These results could not be interpreted since these official elixirs are preserved with high concentrations of alcohol. 12% alcohol is minimum concentration required to retard micro-organism growth [2]. The elixir B.P. has 45% alcohol and Elixir U.S.P. has 15% alcohol.

This unexpected observation can only be interpreted as a probable gas leak into the experimental set up. Possibly

through the burette tap despite the greasing that was done. This possibility should have been tested by carrying out controls with water and water with 45% alcohol. Unfortunately the necessity for these controls was not recognised in time and hence the controls were therefore not examined.

However, despite the discrepancy above, the un-official hospital preparation fermented approximately twice as much as the official elixirs. Oral preparations are not required to be sterile but should contain minimal microbial contamination. No pharmacological investigations were carried out to find if the apparent fermentation of the un-official unpreserved hospital preparation (^{PP}H) was detrimental to the patient or not. This would be seen if the preparation would be capable of causing gastro-enteritis expecially in children.

Further tests were carried out to investigate the effects of benzoic acid preservative on K.N.H. phenobarbitone syrup (^{PP}H). Benzoic acid was used in three concentrations 0.1% $^{w}/v$ 0.15% $^{w}/v$ and 0.2% $^{w}/v$ benzoic acid, to find the most effective preservative concentration. Benzoic acid was chosen rather than sodium benzoate because phenobarbitone is incompatible with the latter. Benzoic acid acts only in acid media of not more than $_{p}H$ 5. It has to be in its non-ionised form so as to penetrate the micro-organism membrane to be effective. The $_{p}H$ of the hospital samples were within the required $_{p}H$ (Table 7). Solubility of benzoic acid is 1 G in 300 mls. (2).

The three concentrations of benzoic acid effectively retarded micro-organism growth to that level of the official elixirs (Table 6).

The mould growth colour on crude sucrose tended to be grey,

while that of refined sucrose tended to be pink. This could be looked at in view of the assumption made initially that aerial microbial composition would tend to be constant.

N.B. When preparing syrup simplex B.P. in the laboratory excess evaporation resulted in crystallisation of the sucrose. Hence evaporation was minimised by covering the syrup all the time apart from when occasional stirring was required. The rate of heating was controlled at minimum.

However crystallisation of sucrose can be retarded by inco-orperation of polyols such as Glycerol or sorbitol[2]. This was not attempted in the project.

Graph 1 and 2 shows comparative rate of fermentation of phenobarbitone syrup and syrup simplex from the hospital in batch 1 and 2.

C O N C L U S I O N

Gasometric method gave fairly good qualitative results of fermentation in terms of gas production.

A good correlation of fermentation and micro-organism growth is obtainable. Phenobarbitone syrup showed persistently more fermentation than the syrup simplex. The tablet excipients and contamination during extra manipulation of the syrup were shown to have contributed to fermentation of phenobarbitone syrup.

The unpreserved hospital sample (^{PP}H) showed twice as much fermentation as the official elixirs. The hospital sample was preserved with 0.1-0.2% $^{w}/v$ benzoic acid and fermentation was retarded effectively to that level of the official preserved elixirs.

The problem of caking, however, still persisted. This would most likely lead to errors in dosage. It is difficult to evenly distribute the solid in the vehicle long enough to ensure uniformity of the measured dose of a caked solid. The dosage error problem is quite ironical in that the hospital phenobarbitone "Syrup" was made to eliminate the possibility of wrong dosage in splitting up tablets. This problem would be eliminated if the suspension

was formulated to obtain a flocculated system.[12] An elegant flocculated system could be made by inco-orporation of a suspending agent. Hence a possible problem of incompatibility between the flocculating agent and suspending agent should be considered.

The K.N.H. phenobarbitone syrup formula is not acceptable. It is not preserved and it also cakes leading to wrong dosage. It is most un-economical since most of the tablets used in the syrup end up thrown down the drain.

This is because the dispensing of this preparation is unpopular. It is unfortunate for such a waste to occur in a developing country like Kenya.

It would be recommended that phenobarbitone liquid preparation (extemporaneous) be in solution. The solution would be obtained using phenobarbitone sodium and the compatible preservative, Sodium benzoate. It would also be a good pharmaceutical practice and of help to include the date of preparation of the preparation on the label.

A suitable alternative to the extemporaneous paedriatic phenobarbitone syrup would be to ensure that the paedriatic strength tablets (15 mg) are always available at all times.

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