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THE EFFECT OF TEMPERATURE ON THE STABILITY OF PENICILLIN-V POTASSIUM PAEDIATRIC SYRUP

BY

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A DISSERTATION PAPER SUBMITTED IN PARTIAL FULFILMENT FOR THE AWARD OF THE BACHELOR OF PHARMACY DEGREE

DEPARTMENT OF PHARMACY
FACULTY OF MEDICINE
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ACKNOWLEDGEMENTS

I wish to convey my special thanks to:-

- My project Supervisor, Mr. Ogeto whose tireless and patient guidance put me through this project.
- Lecturers, other members of staff and students at the Pharmacy department for providing the peaceful enviroment necessary for the success of such tedious work.
- Mr. Mureithi and Mr. Thuranira, Laboratory technicians in the Pharmaceutical Chemistry Section for being most helpful
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DEDICATION

To my loving mum and dad Mr. and Mrs. Machayo.

AIM:

To investigate the stability of the reconstituted penicillin - V- Potassium syrup and the effect of temperature on the rate of degradation with the view of advising Kenyans on the stability and suitability of using this syrup for upto seven days after reconstitution.

The effect of temperature on the stability of penicillin-V potassium paediatric syrup.

INTRODUCTION:-

Paediatric mediIation is a delicate issue due to the lack of development of some metabolic enzymes in infants such as glucuronyl transferase, and hence the possibility of toxic effects with relatively small doses of various drugs e.g. chloram-phenicol[1].

For this and other reasons like the inherent toxocity of various drugs has led to the wide use of penicillin-V syrup in various goverment hospitals throughout the Country. This can be attributed to the relatively wide safety margin of penicillin compared to other drugs with the same spectrum of activity also penicillin is relatively cheap. The spectrum of penicillin also has a telling effect on the frequency of its use as it is effective against many common infections caused by gram-positive cocci and bacilli: streptococci, pneumococci and gonococci are invariably sensitive save for an alarming degree of resistance developing in gonorrhea [2].

Penicillin produces its bacterialcidal activity by interfering with the cell wall development in sensitive microrganisms. Specifically by inhibition of biosynthesis of the dipeptidoglycan strand that is needed to produce strength and rigidity to the cell-wall.

Penicillins acylate the enzyme tripeptidase thus rendering it inactive for its role in forming a cross-link of the two peptidoglycan strands by transpeptidation and elimination of D-alanine [3].

It is therefore not suprising that pen-V syrup is found in many goverment hospitals such as in Nairobi at Kenyatta National Hospital, in Mombasa at the Coast General Hospital and other hospitals clinics and dispensaries in various other locations all over Kenya namely, Lodwar Mandera, Moyale, Garissa, Kitale, Eldoret Nakuru, Kisumu, Nanyuki etc.

Kenya is a country whose climate differs from place to place and for this reason, temperatures vary with different regions.

Data from the metrological Department shows that the average temperatures at various stations vary with months. Generally lower in the rainy seasons and high during the dry periods.

The Data below illustrates this more clearly.

Station	Month	Mean Temperature
Nairobi	July	20°C
Mombasa	March	28°C
Mandera	April	30.5°C
Lowdar	October	30°C
Nanyuki	October	16.1°C
Timboroa	October	13.5°C

Garissa	April	30.5°C
Eldoret	October	18°C
Kisumu	October	23°C
Endebess (Kitale)	August	17°C

From these statistics it can be seen that the temperatures range betweem 10°C to 35°C.

The majority of the Kenyan population live in the rural areas where facilities for refrigeration do not exist, hence any drug dispensed to them will be stored at the existing temperatures at which ever place they stay.

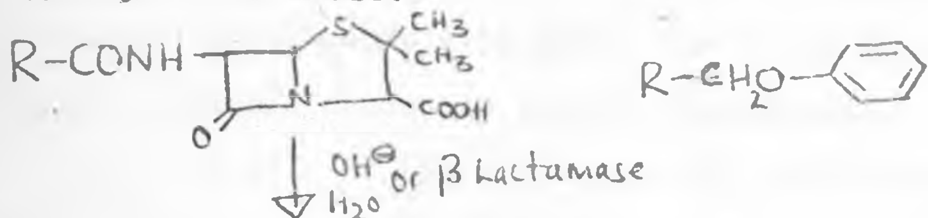
On the commercially available penicillin-V potassium syrup labels, distibuted to the various health centres through the central medical stores, the instructions state that the syrup should be used for a maximum of seven days after reconstitution regardless of the temperatures of the areas where it is being used.

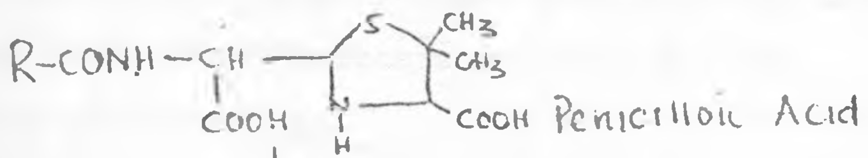
An example is the pac laboratories the label states that store in cool place Discard contents 7 days after reconstitution.

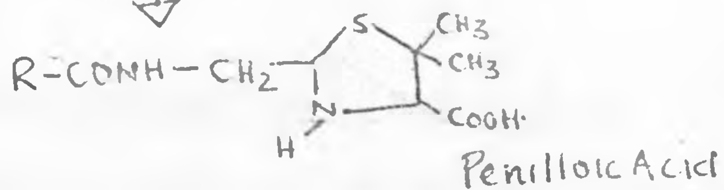
It is however believed that the rate of degradation of penicillin V syrup is temperature dependent [4]. This degradation occurs by hydrolysis and is catalysed by the presences of base or acid. An enzyme found in some microorganisms which imparts resistance to penicillin, β-Lactamase also catalyses penicillin hydrolysis:

The reaction can be represented as follows

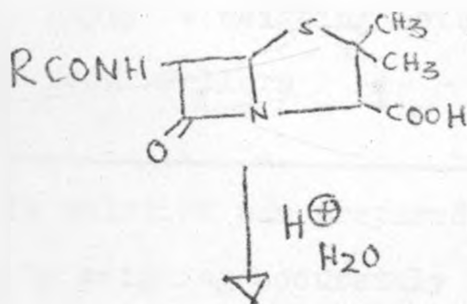
In base or B-lactamase.

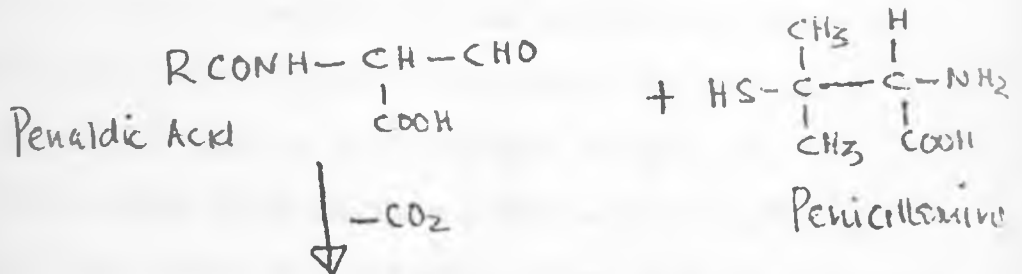






In Acid





RCONH - CH2 CHO

Penilloaldehyde

An examination of the penicillin structure shows it contain a fused ring system of unusual design, the B lactam thiazolidine structure. The B lactam is highly unstable hence the ease of hydrolysis.

The products of hydrolysis are not active anti-microbial agents therefore with hydrolysis the activity of the suspensions decreases and with this the therapeutic efficacy.

Reagents

1N SODIUM HYDROXIDE V.S. NaOH = 40.00

Wt of Sample + weighing bottle	25.5555g
Wt of Empty + weighing bottle	15.0134g
Wt of NaOH Pellers	10.5421

This solution was prepared by the B.P. (1980) method by weighing accurately by difference 10.5421g of sodium hydroxide pellets and dissolving them in sufficient carbon dioxide free water to produce a volume of 250 mls in a volumetric flask.

The carbon dioxide free water was prepared by boiling the water and allowing it to cool in a closed container.

The exact strength of the solution was not determined as this was necessary for the expriment.

IM Hydrochloric Acid VS Hcl=36.46

This solution was prepared by diluting a stock solution of 32% Hcl.

From this solution each millilitre contains 0.32 g of Hcl.

A solution of I molar Hcl contains 36.46 g of Hcl in 100o mls of water and $\left(\frac{36.46 \times 250}{1000}\right)$g in 250 mls

= 9.115 g in 250 mls.

The volume of the stock solution required to produce 9.115 g is given by

$$\left(9.115 \times \frac{100}{32}\right) = 28.48 \text{ mls.}$$

28.5 mls of this stock solution was therefore measured in aburette and diluted to 250 mls with water in a volumetric flask to produce a one molar Hcl solution.

0.02 M Sodium Thiosulphate VS $Na_2S_2O_3\ 5H_2O$= 248.2

This solution was prepared by diluting a 0.1M solution.

Sodium Thiosulphate

Wt. of sample + weighing bottle	23.1591 g
Wt. of empty weighing bottle	16.8819 g
Wt. of the sodium thiosulphate	6.2772 g

Sodium Carbonate

Wt. of sample + weighing bottle	16.7457 g
Wt. of empty weighing bottle	16.6933 g
Wt. of sodium carbonate	0.0524 g

6.2772 g of sodium thiosulphate and 0.0524 g of sodium carbonate were dissolved in sufficient carbon dioxide free water to 250 mls in a volumetric flask. 200 mls of the resulting solution was then diluted to one litre in a volumetric flask using carbon dioxide free water to produce a 0.02 m solution.

The exact strength was asacertained using this 0.02 m solution by dissolving an accurately weighed postassium bromate as follows.

Potassium Bromate

Wt. of sample + weighing bottle	16.1808 g
Wt. of empty weighing bottle	15.9722 g
Wt. of sample (K Bro_3	0.2086 g

The 0.2086 g of $KBro_3$ was dissolved in sufficient water to produce 250 ml. to 50 ml of this solution 2g of KI and 3 ml of 2m hydrochloric acid were added.

10 ml aliquots of this solution were then titrated with the 0.02 M sodium thiosulphate using starch mulitage as indicator.

	1st sample	2nd sample
2nd Burrette Reading	13.4	33.4
1st Burrette Reading	0.0	20.0
Volume of $Na_2S_2O_3$	13.4	13.4

From the B.P (1980) we know that I ml of 0.1 M sodium thiosulphate is equivalent to 0.02784 g $KBrO_3$

Therefore I ml of 0.02 M $Na_2S_2O_3$= 5.5568×10^{-4} $KBro_3$

The amount of $KBro_3$ weighed was 0.2086 g. This was dissolved in 250 mls and 50 mls of the resulting solution KI and 2M Hcl was added. 10 ml of this was what was titrated with the thiosulphate.

Therefore amount of $KBro_3$ present in the 10 mls

$$= \frac{0.2086}{25} = 8.344 \times 10^{-3} g.$$

This was equivalent to 13.4 ml of the thiosulphate solution.

Therefore I ml of the 0.02 M thiosulphate is equivalent to

$$\frac{8.344}{13.4} \times 10^{-3} = 6.226 \times 10^{-4} g \text{ of } KBro_3$$

The standardization factor is therefore given by

$$F = \frac{6.226 \times 10^{-4}}{5.5568 \times 10^{-4}} = 1.118$$

0.01 M Iodine VS I = 253.8

Iodine

Wt. of sample + weighing bottle	19.6736 g
Wt. of empty weighing bottle	16.4181 g
Wt. of Iodine	3.2755 g

Potassium Iodine

Wt. of sample + weighing bottle	21.6459 g
Wt. of empty weighing bottle	16.6321 g
Wt. of Iodine	5.0135 g

The 0.01 M was made by diluting a 0.05 M solution.

The Ki was dissolved in a minimum amount of water to this 3.2755 g of Iodine were added and allowd to dissolve the solution was then made to the 250 ml mark using distilled water.

The resulting solution is 0.05 molar from the relation ship

$$C_1V_1 = C_2V_2$$

were C = Concentration and V= volume

$$V_2 = \frac{C_1V_1}{C_2}$$

$$V_2 = \frac{0.05 \times 100}{0.01} = 500 \text{ mls.}$$

That is 100 mls of the 0.05 M solution was diluted to 500 mls using distilled water to produce the 0.01 M solution.

The exact strenght of the resulting solution was determined by titrating 10 mls against the standardized 0.02 m sodium thiosulphate solution using starch Mucilage added towards the end as the indicator.

	1st sample	2nd sample	3rd sampl
2nd Burrette reading	9.6	19.5	29.5
1st Burrette reading	0.0	10.6	20.0
Volume of thiosulphate	9.6	9.5	9.5

Average volume = 9.5

The standardization factor of the thiosulphate solution was 1.118

Using the relationship

$N_1 V_1 F_1 = N_2 V_2 F_2$.

where F = factor

N = Normality

V = Volume

The $F_2 = \frac{V_1 F_1}{V_2} = \frac{9.5 \times 1.118}{10}$

$= 1.0621$

Buffer Solution

The Buffer that was used was the acetate buffer as in the B.P.C (1973). This was freshly prepared each day of analysis. Made up of 5.44% $W/_V$ sodium acetate and 2.40% $W/_V$ glacial acetric acid. The sodium acetate was first dissolved in a small amount of water, the glacial acetic acid added and the resulting solution made up to volume with water.

Starch Indicator Solution.

This was prepared by the B.P (1980) method by titurating 0.5g of soluble starch with 5 mls of water. To this sufficient water was added to produce about 100ml with continuos stirring.The solution was then boiled for a few minutes , cooled and filtered.

A fresh solution was prepared on each day it was required.

EXPERIMENTAL

The method used in the analysis was based on the fact that the average mean temperatures in Kenya were found to be in the range of 10°C to 35°C with this in mind an upper temperature limit of 40°C was chosen and a lower limit of 5°C. Two other intermidiate temperatures were used these were room temperature which averaged at about 20°C and the second temperature of 35°C.

The syrup after reconstruction was divided into three samples, in the first experiment the 5°C sample was stored at the bottom of a refrigerator the room: temperature sample was stored on a bench while the 40°C regulated thermostatically at 40°C throughout the whole period of analysis.

In the second experiment the 5°C and room temperature sample were stored as before and the 35°C was stored in the same water bath this time regulated at 35°C.

Small amount were removed from each sample for analysis without disturbing the incubation temperatures as much as possible.

The assay was carried out using the Iodometric method for penicillin. This method originally described by Alicino has been generally accepted as a dependable analytical method for the determination of penicillin potency.

The assay is probably one of the most rapid, accurate and specific chemical test available for penicillins. It compares favourably with the microbiological cup-plate method in accuracy and it has the advantages of speed, precision and economy.

It is based on the fact that the alkaline hydrolysed penicillin molecule reduces between six and nine moles of Iodine per mole of penicillin molecule depending upon the penicillin being assayed. The intact molecule does not reduced Iodine and so the difference between the number of moles reduced by a control sample and a fully hydrolysed sample would give the amount of intact biologically active phenoxymethyl penicillin potassium.

Finholt et al [6] used the assay in the Kinetic analysis of penicillin (benzyl penicillin) the assay was also used by Hon and Poole in the Kinetic analysis of ampicillin [7] as well as the determination of the amino acid nature of ampicillin. In their work on chemical reactions involved in penicillin allergy, Kinetics and mechanism of penicillin aminolysed. Akira Tsuji et al [8] used the same Iodine method. The procedure has been also used by savello et al [9] in their work on the stability of sodium ampicillin solutions in the frozen and liquid states.

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The procedure used for the assay in this study is that found in the B.PC (1973). The gramles for reconstitution for this study were supplied by PAC laboratories

PAC PEN

Dry gramles for reconstitution as syrup

Mfg. PaC laboratories Ltd.

Batch no 20633

Date of Manufacture 6/82

Expiry date October 85

Label claim each 5 ml of syrup when freshly prepared contains penicillin V potassium equivalent to 125 mg penicillin V. B.P

The granules were reconsituted as recommeded by the manaufactures by adding 1950 ml of distilled water in stages with shaking until all was dissolved.

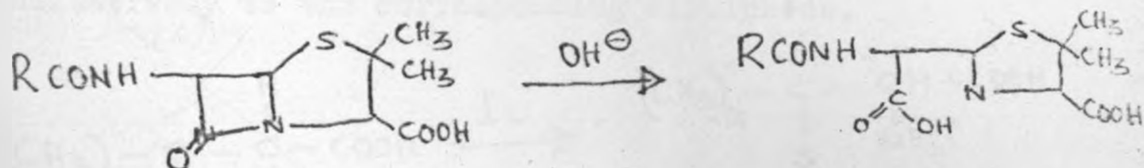
The white powder when reconstituted gave a pinkish viscous syrup with the characteristic odour of penicillins.

The reconstituted syrup was then divided into three conical flasks which were tightly stoppered and stored at 5°, room temperature and 40°C as explained earlier the second sample was treated like wise and stored at 5°C, room temperature and 35°C as explained earlier.

These samples where then analysed at intervals as shown in the results for the total penicillins over a period of seven days which is the period recommeded by the manufactures as the number of days for which the reconstituted syrup may be used.

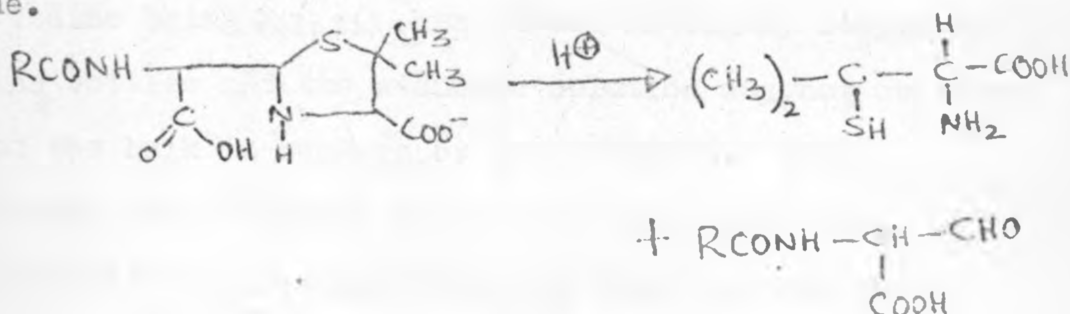
This long term storage was chosen instead of an accelerated stability test as this provides a better approximation to normal condition as it has been reported that some reactions occur at elevated temperature which would otherwise not occur at normal storage temperatures(10). Also a period of seven days was reasonably short and convinient.

Immediately after reconstitution the syrup was analysed to determine the penicillins present this was done by accurately weighing a quality of the syrup equivalent to about 0.06 g phenoxy methyl penicillin and diluting it to 50 mls with distilled water in a volumetric flask 10 mls were transfered to a wet stoppered 'Iodine flask' and 5 ml of N/I sodium hydroxide added and allowed to stand for 20 minutes. This is the alkaline hydrolysis whichcleaves the B- lactam ring to produce the corespoding penicillioc acid



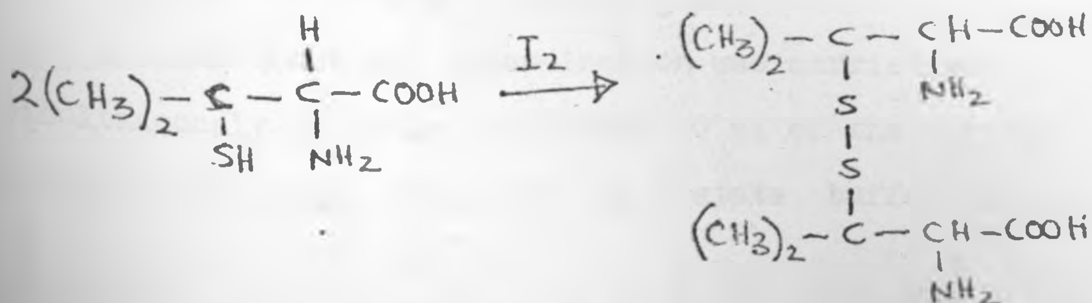
The time of 20 minutes was important to ensure that the same period was given for the hydrolysis for comparative purposes and is thought to be sufficient for complete hydrolysis to occur.

20 ml of a freshly prepared buffer solution containing 5.44% W/$_V$ sodium acetate and 2,40% W/$_V$ glacial acetic acid were added this in effect stopped further hydrolysis by the base. 5 ml of N/$_1$ hydrochlolic acid were then added and this converts the penicilloic acid to D- penicilliamine.



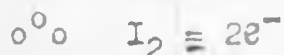
25 mls of 0.02 N Iodine solution where then added and the flask stoppered using a wet stopper and allowed to stand for 20 minutes protected from light to avoid light catalysed free radical reaction of Iodine.

The Iodine oxidises the D-penicillamine almost quantitatively to the corresponding disulphide.



the oxidizing power of Iodine can be denoted as follows

$I_2 + 2e^- \longrightarrow 2I^-$



$\therefore \quad I_2 \equiv 2e^-$

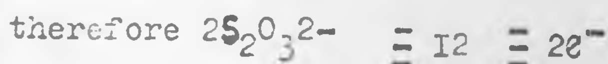
Because Iodine is practically insoluble in water, use was made of the fact that it dissolves in solutions of potassium Iodine to form potassium Iodine which behaves in solution as free Iodine (B.P method for preparation of Iodine solution).

Care was taken in handling the Iodine solution as Iodine being volatile was stored in tighly stoppered glass bottles and the standard solution was not collected from the bulk in beakers or open vessels. When measured from suitable containers into burrettes, It was titrated without delay as these precautions have to be observed if satisfactory results are to be obtained.

The excess Iodine was then titrated with 0.02 N sodium thiosulphate using starch mucilage as an indicator added towards the end of the titration.

$2S_2O_3^{2-} \longrightarrow S_4O_6^{2-} + 2e^-$

$I_2 + 2e^- \longrightarrow 2I^-$



therefore $2S_2O_3^{2-} \equiv I_2 \equiv 2e^-$

A blank (control) determination was carried out simultaneonsly by taking a further 10 ml of the diluted solution and adding 10 mls of the acetate buffer solution

to oxidized any of the penicillins that had already hydrolysed in solution. The flask was stoppered with a wet stopper and allowed to stand for 20 minutes protected from light.

The excess Iodine was the back titrated with the 0.02 N sodium thiosulphate solution using starch micilage added towards the end of the titration.

These determination were done in duplicate.

The total penicillins calculated as phenoxymethyl penicillin potassium were determined by repeating the procedure with a standard (BCRS) phenoxymethyl penicillin potassium obtained from the drug research and analysis unit.

RESULTS

Standard BPCRS sample from DARU

for this sample each mg of pen V. potassium BPCRS is equivalent to 0.9019 mg total penicillins calculated

as $C_{16} H_{18} N_2O_5S$

% wt of standard equivalent to 0.06 g pen V. potassium

$$= 0.06 \times \frac{1}{0.9019} = 0.0665 \text{ g}$$

Sample taken for analysis

wt. of empty 50 ml vol. flask + standard	38.2023 g
wt. of empty flask	38.1356 g
wt. of sample	0.0667 g

Titres:

	Blank		sample	
	1	2	1	2
2nd Reading	24.15	24.1	11.3	31.3
1st Reading	0.0	0.0	0.0	20.0
Titre	24.15	24.1	11.3	11.3
Average	24.1		11.3	

Vol. of 0.02 N Thiosulphate used = 24.1 - 11.3 = 12.8 mls the standardization factor for 0.02 N thiosulphate F = 1.118.

F for Iodine 0.02N solution = 1.062

$\therefore$ amount of Iodine used = $12.8 \times \frac{1.118}{1.062} = 13.5$ ml

Since only 10 mls of the solution was used in the analysis this was equivalent to

$$66.7 \times 0.9019 \times \frac{10}{50} = 12.0313 \text{ mg of}$$

total penicillins.

$\therefore$ 135 ml of Iodine is $\equiv$ 12.0313 mg total penicillins

Each ml of 0.02N Iodine $\equiv$ 0.8930 mg.

Results for syrup analysis.

After reconstitution the weight per ml of the syrup was determined.

wt. of Pyknometer + syrup	50.2205 g
wt. of empty Pyknometer	22.3182 g
wt. of syrup	27.9023 g

Vol. of Pyknometer is 25.0 ml.

$\therefore$ wt. of syrup per ml = $\frac{27.9023}{25} = 1.1161$ g.

Weight of sample taken for analysis.

Label claim states each 5 ml contains 0.125 g of pen V B.P.

$\therefore$ amount equivalent to 0.06 g is given by

$\frac{0.06}{0.125} \times 5 = 2.4$ mls

a weight equivalent to 3 mls was used in this case i.e

3 ml x 1.1161 = 3.3483 g.

This weight was used for all the samples analysed.

Assay on day of reconstitution to determine the total penicillins in the syrup intitally.

	Blank		Test	
	1	2	1	2
2nd Reading	23.8	48.8	10.0	20.05
1st Reading	0.0	25.0	0.0	10.00
Titre	23.8	23.8	10.0	10.05
Average Titre	23.8		10.0	

Vol. of 0.02 N thiosulphate used = 23.8 -10.0= 13.8 ml.

this is equivalent to $\frac{13.8 \times 1.118}{1.062}$ mls of Iodine

= 14.53 ml.

This is equivalent to 14.53 x 0.8930 = 12.972 mg total penicillins .(B.P.CRS)

Total penicillins in the 3.3483 g taken initially

$= 12.972 \times \frac{50}{10} = 64.86$ mg

This was equivalent to 3 mls of the syrup

∴ Total penicillins in 5 ml = 64.86 x $5/_3$ =108.1 mg

in 1 ml = 21.62 mg

Percentage of label claim = $\frac{108.1 \times 100}{125}$ = 86.4%

Room temperature sample

Day 1

Room temperature 20.1°C

	Blank		Test	
	1	2	1	2
2nd Reading.	23.3	48.2	9.8	20.0
1st Reading	0.0	25.0	0.0	10.0
Titre	23.3	23.2	9.8	10.0
Average Titre	23.25		9.9	

Vol. of thiosulphate used = 13.35

this is ≡ $\frac{13.35 \times 1.118}{1.062}$ = 14.05 mls Iodine

Total penicillins present = $\frac{14.05 \times 21.62}{14.53}$ = 20.91 mg

Penicillins present in 5 ml = 104.53

Percentage of original penicillins present = 104.53

$\frac{104.53 \times 100}{108.1}$

= 96.7%

Day 3

Room temperature 20°C

	Blank		Test	
	1	2	1	2
2nd Reading	22.6	47.55	9.9	29.9
1st Reading	0.0	25.0	0.0	10.0
Titre	22.6	22.55	9.9	9.9
Average Titre	22.6		9.9	

Vol. of thiosulphate used = 22.6 - 9.9 = 12.7 mls

$$\equiv 12.7 \times \frac{1.118}{1.062} = 13.37 \text{ mls}$$

Total penicillins in 1 ml

$$= \frac{13.37 \times 21.62}{14.53} = 19.89 \text{ mg}$$

Penicillins in 5 ml = 99.47 mg

Percentage of original penicillins in the syrup

$$= \frac{99.47 \times 100}{108.1} = 92.02\%$$

Day 5

Room temperature 20°C

	Blank		Test	
	1	2	1	2
2nd Reading	21.9	46.9	10.0	20.0
1st Reading	0.0	25.0	0.0	10.0
Titre	21.9	21.9	10.0	10.0
Average titre	21.9		10.0	

Vol. of thiosulphate used = 11.9 mls.

$\equiv 11.9 \times \frac{1.118}{1.062} = 12.53$ mls Iodine

Total penicillins in 1 ml = $\frac{12.53 \times 21.62}{14.53} = 18.64$ mg .

In 5 ml of syrup = 93.22 mg

% of the original penicillins in the syrup

$= \frac{93.22 \times 100}{108.1} = 86.2\%$

Day 7

Room temperature = 19°C

	Blank		Test	
	1	2	1	2
2nd Reading	20.5	45.5	9.5	19.5
1st Reading	0.0	25.0	0.0	10.0
Titre	20.5	20.5	9.5	9.5
Average Titre	20.5		9.5	

Vol. of thiosulphate used = 11.0 ml

$$\equiv \frac{11.0 \times 1.118}{1.062} = 11.58 \text{ mls of Iodine}$$

$$\text{Total penicillins in 1 ml} = \frac{11.58 \times 21.62}{14.53} = 17.23 \text{ mg}$$

Penicillins in 5 ml = 86.15 mg

$$\% \text{ of original } \frac{86.15}{108.1} \times 100 = 79.7\%$$

Summary of room temperature results.

Day	0	1	3	5	7
Amount of Total Penicillins (Mg)	108.1	104.53	99.47	93.22	86.15
Percentage of original Penicillins in syrup	100	96.7	92.02	86.2	79.7

Summary of results for the sample stored at 5°C

Day	0	1	3	5	7
Amount of Total Penicillins (Mg)	108.1	107.6	103.9	101.9	99.22
Percentage of Original Penicillins in syrup	100	99.5	96.13	94.3	91.79

Summary of results for the sample stored at 40°C

Day	0	1	3	5	7
Amount of Total penicillins (Mg)	108.1	98.38	83.89	62.7	42.40
Percentage of Original penicillins in syrup	100	91.01	77.6	58.0	39.22

A second sample of dry granles for reconstitution as syrup was reconstituted and the results were as follows.

Summary of results for the sample stored at 5°C

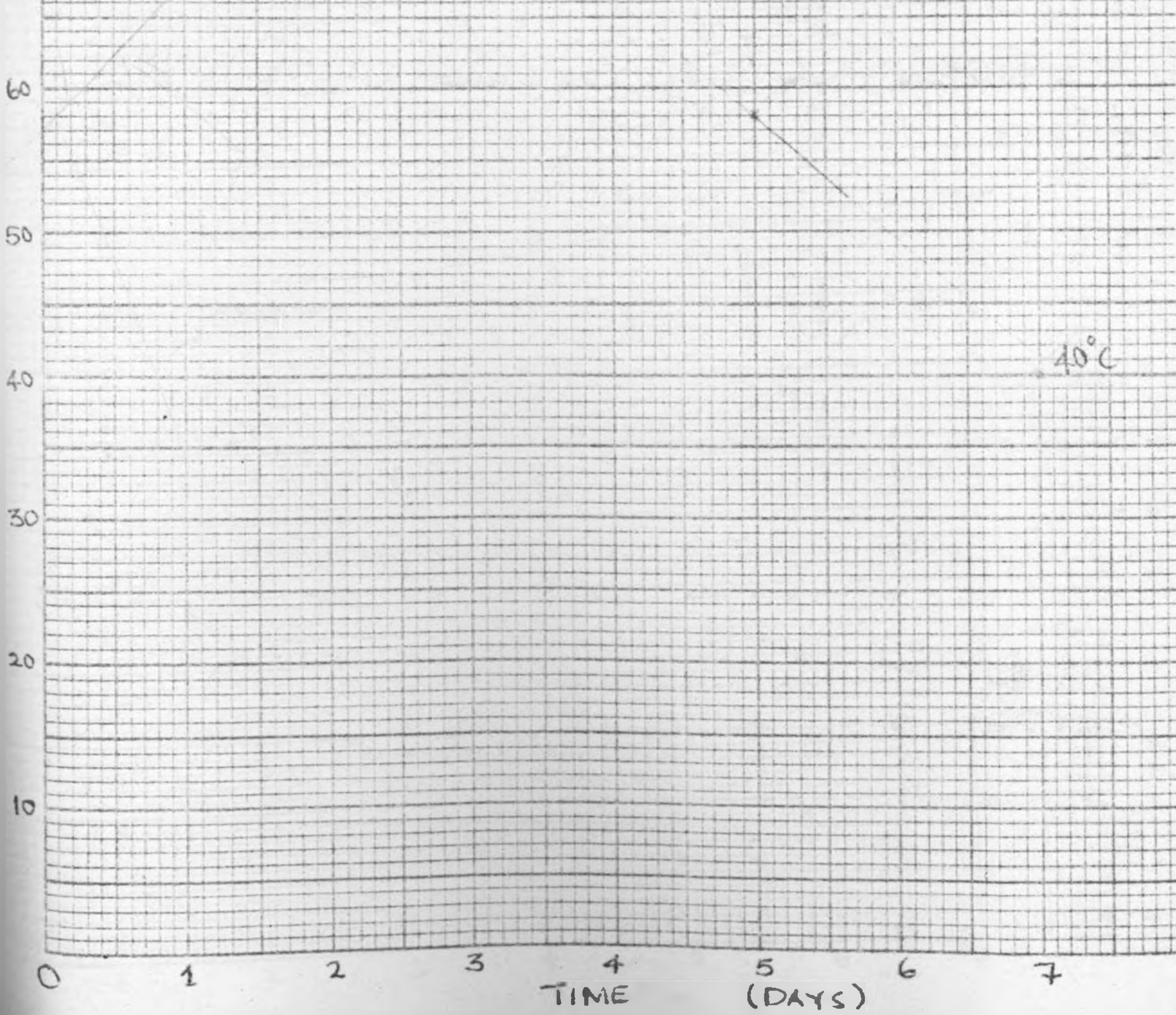
Day	0	2	4	6	7
Amount of total penicillins (mg)	123.6	120.92	117.4	[illegible].1	110.42
Percentage of original penicillins in syrup	100	97.83	94.98	91.5	89.34

Summary of results of sample stored at room temperature

Day	0	2	4	6	7
Amount of total penicillins (mg)	123.6	117.21	105.18	103.21	96.66
Percentage of Original penicillins in syrup	100	94.83	85.1	83.5	78.2

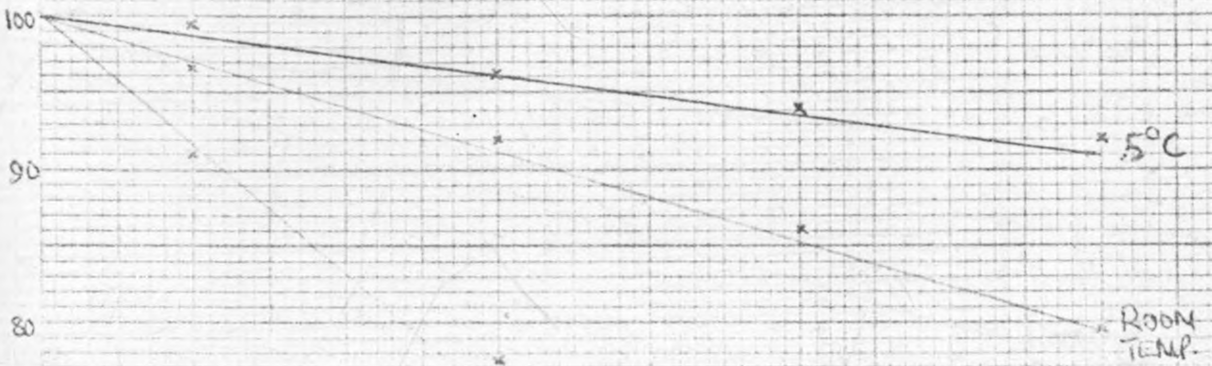
Summary of results of sample stored at 35°C

Day	0	2	4	6	7
Amount of total penicillins (mg)	123.6	109.02	92.58	80.83	74.75
Percentage of Original penicillins in syrup	100	88.2	74.9	65.4	60.48


60
50
40
30
20
10
0
1
2
3
4
5
6
7
40°C
TIME
(DAYS)

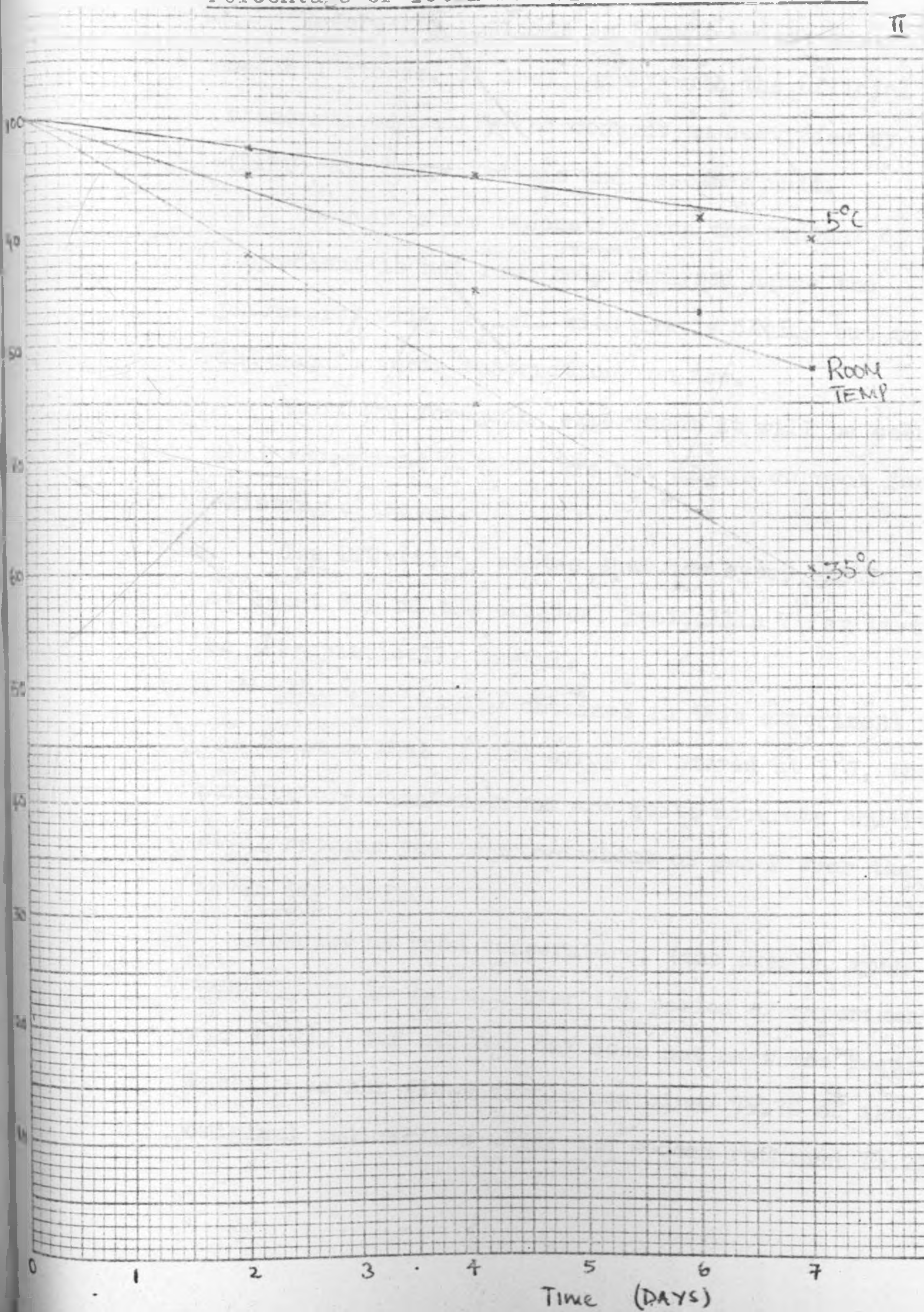
Sample 1

Percentage of total penicillins Vs Time (Days)



Sample II

Percentage of Total Penicillins Vs Time (Days)



Discussion:-

Before any medicine is bought by the central medical stores, it must comply with the official standards of purity and content as certified by (DARU) the drug analysis and research unit.

However after reconstitution the content of active ingredient is not routinely analysed hence the pharmacist will be left with little choice but to follow the manufactures instructions on use.

From the foregoing experiment it will be seen that those instructions are not always correct for all existing climates.

The BPC (1973) states that the syrup should contain at least 80% of the original penicillin V for it to be therapeuticaily useful.

From the results obtained in this experiment it can be seen that if the syrup is stored at 5°C, the manufuctures instructions are quite accurate and it still retains the required about of pen V over the seven day period.

However at room temperature, (the average room temperature in Nairobi over the period of experiment was 20°C). The seventh day does not meet the requirements exactly as the first sample contained 79.7% of the original penicillins while the second retained 78.2%.

As the temperatures increase, the rate of degradation also increases as shown by the samples stored at 35°C and 40°C. These cross the 80% limit after about 3½ and 2½ days respectively which denotes that if used on the fourth and third days respectively they do not contain the penicillins required that is the dose prescribed by the doctor is not dispensed by the pharmacist as what the pharmacist would dispense at this period would be an underdose as per the doctors instructions.

In conclusion it is therefore evident that in those regions where the average temperatures are greater than or equal to 30°C the manufuctures instructions are not compartible with official B.PC (1973) standards.

Pharmacists at places like Mandera, Mombasa, Lowdar and Garissa should be made aware of this fact with the view of advising the patients on how best to use the medicine.

Some suggestions of overcoming this problem are:-

Usually the pharmacy, hospital or clinic is located in a region where a refrigerator is available if not these should be installed using generators or the more portable paraffin using refrigerators.

This would enable the reconstituted syrup to be stored at the low temperatures with the concommitant slower degradation rate with this possible, the patients in such areas should only be given a three day dose of the syrup and if this is not enough for cure the patients should be instructed to come back for a second dose after three days.

Alternatively the dry granles which have a much longer self-life should be dispensed in such areas and the patients should be shown how to reconstitute the granules of course they should be dispensed in three day batches so that the patient is given two packages of granules for six days therapy one containing only an average three day dose. These smaller batches could be obtained from the manufucturer on order (more expensive as more packaging material is required) or the pharmacy could be used to do the repackaging for such areas.

Chemically derivatives with a slower rate of degradation than pen V or different types of drugs which do not suffer from hydrolysis to the same extent as penicillins but have the same spectrum of activity and efficacy could be employed in such areas. An example being the Depot injections of procaine penicillin and Benzathine penicillin.

ADDENDUM

An investigation on the effect of the Acetate and phosphate buffers on Benzyl-penicillin analysis.

Introduction:

The recommendation of the buffer to be used in the analysis of benzyl-penicillin using the Iodometric method differs from the manufacturer - Beecham, and the British Pharmacopoeia Commission the publishers of the British Pharmacopoeia.

The manufacturers recommend the phosphate buffer and claim that the use of the acetate would yield different results. The pharmacopoeia commission on the other hand use the acetate buffer and claim that the phosphate buffer gives similar results.

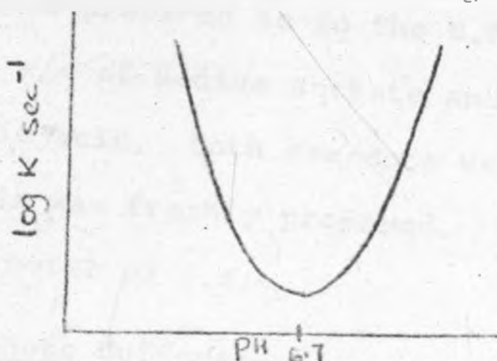
A literature survey shows that some buffers have an effect on the results obtained.

In the J. Pharm. Sci Vol 65 August 1976 some workers have shown using the citrate buffer that the rate of degradation of penicillin was directly proportional to the buffer concentration increasing with increase in concentration. This they explain to be a result of the citric acid at pH 2.7 being present as the dihydrogen citrate anion which is known to have a catalytic effect on the degradation of penicillins.

It has also been shown that the effect of the buffer may also be influenced by the pH at which it is used. This is evident by the pH-rate profile determined by Brodersen at 30°C and more recently by Finholt, Jurgensen

et al in the J.Pharm Sci 1965.

From their work the pH of maximum stability was found to be pH 6.75 with a narrow range near the maximum



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The calculate half-life at pH 6.75 and 30°C was $3.3X10^{6}$ sec. or 38 days with a computed shelf-life of six days

Further work has shown that the dihydrogen citrate ion, the mono-hydrogen phosphate ion and the Borate iion have a catalytic effect on the benzylpen-icellate ion whereas acetic acid catalyses the degradation of benzyl-penicillinic acid. Metal ions have also been shown to catalyse the hydrolysis whose overall kinetics is observed to be first order (chemical stability of pharmaceuticals by Kenneth A. (Connors).

With this in mind, the following experiment work was carried out to access the practical effect on benzyl-penicillin analysis using two buffers. That is the phosphate buffer and the acetate buffer.

Reagents:

The Acetate Buffer

Was prepared as in the B.P. (1980) using 5.44 per cent w/v of Sodium acetate and 2.40 percent w/v of glacial acetic acid. Both reagents were of analar grade and the buffer was freshly prepared. The pH was determined using a pH meter pH 5.4.

Phosphate Buffers:-

Were prepared according to the USP XIX by using appropriate quantities of dibasic potassium phosphate and mono basic Potassium phosphate both of analar grade and adjusting the pH using 18N phosphoric acid or 10N Potassium hydroxide pH $\pm$ 0.05.

The buffers prepared were of pH 5.4, 6.2 and 7.6.

The other reagents used were prepared in the same manner as in the previous experiment namely:-

0.02N Iodine Vs F= 1.000

0.02N Sodium thiosulphate Vs F=0.9634

1N Sodium Hydroxide

1N Hydrochloric acid

Starch mucilage

EXPERIMENTAL

The analysis was carried out on crystalline benzyl-penicillin Sodium B.P. Batch Number F570 EL cordially supplied by MAC'S Pharmaceuticals. The standard was also supplied by the same company.

The method used in the analysis was that found in the B.P.(1980) for assay of benzylpenicillin Sodium injection

Two determinations were carried out for each buffer and each pH value for the phosphate buffers.

0.1g was accurately weighed into a 100ml flask and sufficient distilled water added to volume.

10ml were then transfered to a stoppered flask and 5ml of molar sodium hydroxide added and allowed to stand for twenty minutes.

20ml of the freshly prepared appropriate buffer was then added, 5ml of molar hydrochloric acid and 25ml of 0.01M Iodine VS. The flask was then closed with a wet stopper and allowed to stand for twenty minutes protected from light.

The excess Iodine was then titrated with 0.02M Sodium thiosulphate VS using starch mucilage as indicator added towards the end of the titration.

To a further 10ml of the initial solution 20ml of the appropriate buffer was assed and 25ml of 0.01M Iodine VS and allowed to stand for twenty minutes. protected from light. The excess Iodine was then titrated with 0.02M Sodium thosulphate VS using starch mucilage as indicator added towards the end of the titration.

The difference between the two titrations represents the volume of 0.01M Iodine VS equivalent to the total penicillins present.

The total penicillins content was calculated as $C_{16}H_{17}N_2NaO_4S$ from the difference obtained by simultaneously carring out the assay using benzyl penicillin sodium standard instead of the penicillin being assayed.

RESULTS

Standard Sample

Each 1.0mg of benzylpenicillin standard sample contains 1.00mg of total penicillins calculated as $C_{16}H_{17}N_2NaO_4S$

Wt. of 100ml flask plus std.	44.9320
Wt. of empty flask	44.8317
Wt. of std.	0.1003g.

After titration the volume of 0.02N sodium thiosulphate used were as follows

Using the phosphate Buffer pH 5.4 std	16.2
Blank	6.0
Volume used	10.2mls

This is equivalent to 10.2 X 0.9634mls of 0.02N Iodine

= 9.827mls

Since only 10ml of the original sample was used, the amount of Std present $= \frac{10}{100} \times 0.1003g$

= 10.03mg

Therefore 1ml of the 0.02 Iodine $= \frac{10.03}{9.827}$

= 1.021mg

Using the Acetate Buffer pH 5.4

std 16.3

Blank 6.1

10.2mls

Therefore again 1ml of Iodine 0.02N = 1.021mg of total penicillins.

Calculations for total penicillins in sample

Wt. of 100ML flask plus sample	45.1847g
Wt. of empty flask	45.0754g
Wt. of sample	0.1093g

10ml of the resulting solution was analysed by the Iodometric method the litres obtained were as follows:

Using the phosphate Buffer pH 5.4

Test	16.3	16.3
Blank	5.1	5.0
Volume	11.2	11.3

Average litre = 11.25mls

This was equivalent to 11.25 X 0.9634mls of 0.02N Iodine

= 10.838mls

Each 1.0ml of 0.02N Iodine is $= \frac{10.93}{10.838}$

= 1.0085mg of sample.

Content of benzylpenicillin as a percentage of the std

$$= \frac{1.0085}{1.021} \times 100$$

= 98.78%

Using the acetate Buffer pH 5.4

Titres	test	16.5	16.5
	Blank	5.3	5.2
Volume used		11.2	11.3

Average titre 11.25ms

This is equivalent to 10.838mls of 0.02N Iodine

Each 1.0ml of 0.02N Iodine is = 1.0085mg of sample.

Therefore percentage of label claim = 98.78%

Using the phosphate Buffer pH 6.2

Test	15.7	15.7
Blank	4.3	4.3
	11.4	11.4

Volume of 0.02N thiosulphate used = 11.4mls

= 11.4mls

= 11.4 X 0.9634 mls of Iodine

= 10.983

Therefore each 1.0ml of 0.002N Iodine

$$= \frac{10.93}{10.983} = 0.9956\text{mg of sample}$$

Percentage of label claim $= \frac{0.9956}{1.021} \times 100 = 97.52\%$

Content of benzylpenicillin as a percentage of the std

$$= \frac{1.0085}{1.021} \times 100$$

= 98.78%

Using the acetate Buffer pH 5.4

Titres test	16.5	16.5
Blank	5.3	5.2
Volume used	11.2	11.3

Average titre 11.25ms

This is equivalent to 10.838mls of 0.02N Iodine

Each 1.0ml of 0.02N Iodine is = 1.0085mg of sample.

Therefore percentage of label claim = 98.78%

Using the phosphate Buffer pH 6.2

Test	15.7	15.7
Blank	4.3	4.3
	11.4	11.4

Volume of 0.02N thiosulphate used = 11.4mls

= 11.4mls

= 11.4 X 0.9634 mls of Iodine

= 10.983

Therefore each 1.0ml of 0.002N Iodine

$$= \frac{10.93}{10.983} = 0.9956\text{mg of sample}$$

$$\text{Percentage of label claim} = \frac{0.9956}{1.021} \times 100 = 97.52\%$$

Using phosphate Buffer pH 7.6

Titres		
Test	15.5	15.5
Blank	3.8	3.6
	11.7	11.9

Average 11.8mls

Volume of thiosulphate = 11.8mls

= 11.8 X 0.9634mls of Iodine

= 11.63mls

Each 1.0ml of 0.2N Iodine = $\frac{10.93}{11.63}$ = 0.9394 mg of sample

Percentage label claim = $\frac{0.9394}{1.021}$

= 92.06%

Summary of Results

Phosphate Buffer pH 5.4

Total penicillins in sample = 98.78%

Acetate Buffer pH 5.4

Total penicillins in sample = 98.78%

Phosphate Buffer pH 6.2

Total Penicillin in sample = 97.52%

Phosphate Buffer pH 7.6

Total penicillins in sample = 92.06%

Discussion and Conclusion

The results obtained above were done on the same day at room temperature and carried out using a method that most analysis in our analytical lab such as the Drug analysis and research unit (DARU) or a quality control lab in an industry would use. Therefore any temperature flactuation effect would mimic normal procedures.

The results show that when the two buffers are used at pH 5.4 similar results are obtained and the buffer effect is therefore negligable.

However when the phosphate buffer is used at different pH that is 6.2 and 7.6 the results do not show good correlation with the B.P, method results.

It can therefore be seen that the pH of the buffer may significantly affect the results obtained. This may be due to the buffer existing in different ionic species at different pH.

The acetic acid sodium acetate buffer exerts its buffering effect from the fact that the acid which exists largely in molecular (non-ionized) form combines with hydroxyl ions that may be added to form the acetate ion and water. $CH_3COOH + OH^- \rightarrow CH_3COO^- + H_2O$ while the acetate ion which is a base combines with the hydronium ions (H_3O^+) that may be added to form essentially non-ionized acetic acid and water. $CH_3COO^- + H_3O^+ \rightarrow CH_3COOH + H_2O$

The change in pH is slight so long as the amount of hydroxyl or hydronium ion added does not exceed the capacity of the buffer system to neutralize.

The phosphate buffer on the other hand is composed of two salts namely monobasic potassium phosphate (KH_2PO_4) and the dibasic Potassium phosphate (K_2HPO_4) when hydroxyl ions are added.

$$H_2PO_4^- + OH^- \longrightarrow HPO_4^{2-} + H_2O$$

and when hydronium ions are added

$$HPO_4^{-2} + H_3O^+ \longrightarrow H_2PO_4^- + H_2O$$

It is therefore apparent that the mechanisms of these two buffers are essentially the same.

At different pH however the concentration of the ions vary as in acid pH, the phosphate buffers contains the monobasic ions ($H_2PO_4^-$) in higher concentration than the diabasic while in alkaline pH the dibasic species occur in higher concentration.

It therefore appears that the pH is more determinant than the buffer per se. as it has an effect on the buffer ionic composition. It has also been shown by other workers that the monohydrogen phosphate ion and acetic acid catalyse benzylpenicillin degration.

Another possibility is the varing stability of the benzylpenicillin molecule itself at different pH values this may therefore lead to different results being obtained.

In conclusion therefore, the author has shown by this work that the use of the phosphate and acetate buffer may result to different results in the assay of benzylpenicillin injection if the pH of the two buffers are varied. However at pH 5.4, the results obtained show good correlation with either buffer.

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