" INVESTIGATION OF THE EFFECTS OF EXCESS ETHYLENEDIAMINE ON THE CHEMICAL STABILITY OF AMINOPHYLLINE INJECTIONS"

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BY

UNIVERSITY. QE NAIROBI
UBRKRY

KARABA, LEONARD MWANGI

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completing this project, I am greatly indebted to
[1]1 people listed below who offered much help in many
and to all I'd like to say "Asante Mingi".

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ALL TECHNICAL STAFF,	-	Department of Pharmacy Particularly Pharmaceutical Chemistry Department.

MARK KARIUKI and LIVINGSTONE MAINA-both of the NAIROBI HILTON HOTEL, NAIROBI

DEDICATION:

This work is dedicated, and all its worth, to:-

My Dad, KARABA WA NJUGUNA,

My Late Mom, WANJIRU WA KARABA WHO TAUGHT ME PERSERVERENCE,

My Two Brothers, WACIRA NA MUNUHE. We shall overcome. My Wife-to-be, MUTHONI

ABSTRACT:

Aminophylline injections contain theophylline combined with ethylenediamine in a 2:1 ration and the injections are used for various purposes (9, 10, 12) - The official compedia specify the addition of ethylendiamine in excess of that required to form the aminophylline. This is supposedly to ensure that any slight carbon dioxide absorption does not precipitate theophilline which is only slightly/(15). A / soluble farther role to that is that the excess ethylenerii amine could in fact • chemically stabilise the aminopylline, that is decrease chemical degradition of aminophylline. This project was an investigation of that possible role.

Aminophilline injections were assayed ''^determine both the quantities of ethylendiamine and theopylline and hence the content of excess ethylenediamine. The assay included solutions made in 0.01N NaOH and in boratebuffer and percentage recovery determined. The various theophylline solutions including the injections were examined by thin layer chromatography (TLC). Differential spectroscopy work was also carried out and finally the degradation kinetics at boiling point of water (94.6^{0c}; of pure theophylline and the aminopylline injections studied.

* Reference list is provided at the end of this work and references are bracketted whenever they are mentioned.

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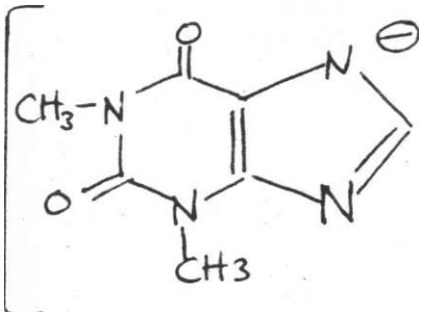
INTRODUCTION:

Aminophylline injections contain ethylenediane and theophylline which can be chemically represented thus:



Oh

I

CJfer

-MH-3

3L

ˆvibo A- dimethvlxapthine while ethylenediarTne 00

j\£cpfy|tw-ewhich is used (a, 9, 12) as a smooth muscle relaxant in treating asthma and heart failure. It also hasanother pharmacological role of a diuretic, mayfce primarily due to its myocardial stimulatatory effect. The side effects observed during its use in humans for various ailments include (8, 12) nausea, emesis and dizziness.

Theophilline, Some Vital Data (12)

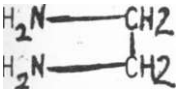
MW 180.17

Ka at 25° C 1.69 x 10″

Kb 1.9 x 10″

One gram dissolves in 120 ml. water, 80 ml. Alcohol, in alkali hydwcides and in ammonia, also soluble in dilute hydrochloric or nitric acids, sparinglysoluble in etfnr. LDgQ oral in rabbits = 350 mg. per Kg.

The official ecrpediff advocate the use of ethylenediamine excess to that required to form the aminophylline and this can be rationalised as to prevent the precipitation of theophylline because excess ethylenediamine reacts with any absorbed Carbon Dioxide in the manner shown below and since theophylline is slightly acidic (Ka = 1.69 x and would thus normally be precipitated by Carbon Dioxide, this is prevented.



?02 + 2HqC

fˆNiiˆ—CH2

HjHiii—612 + 2HCO3

Other Vital Data:

MW Anhydious Aminophy″Mine	420.43
MW Anhydious Theophylline	180.17
MW Dihydrate Aminophylline	456.46
MW Monohydrate Theophylline	198.18
MW Ethylenediamine	60 10

Survey of Official Compedia:

BP (1973): - Aminophylline injection contains not greater than 0.25 lg ethylenediamine, C ^ H ^, ner each gram of anhydrous theoDhylline, °7 ^8 N4° 2, present.

Stoichiometrically, 2 molecules anhydrous theophylline are equivalent to 1 molecule of aminophylline dihydrate. i.e. 360.34$_g$ anhydrous theophylline is equivalent to456.46$_g$ Aminophylline dihydrate and 1$_g$ anhydrous theophylline is equivalent to 1.265(5)$_g$ Aminophylline dihydrate.

The BP limit of 0.251^ ethylenediamine in eachgram of anhydrous theophylline is equivalent to 0.196$_g$ethylenediamine per each gram of Aminophylline dihydrate.

USP (XIX): - Aminophylline injections contain not less than 0.131$_g$ and not greater than 0.152$_g$ ethylenediamine for each gram of Aminophylline dihydrate.

Comparing the two limits

198

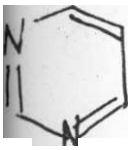
BP limit = yjy = 1.51 time USP imit

and

BP limit = 198 = 1.30 times USP upper limit
T57

Since there is such a wide range in the limits and since theophylline content of injections is below the solubility of theophylline, this prompted the investigation of whether the excess ethylenediamine, rather than just confer physical stability on theophylline against precipitation, had any effect on chemical stability of theophylline.

Theophylline is a member of a bigger chemical group called xanthines which are chemically related to purines. The purine ring, itself can be looked at as fused pyrimi'dirte and imidazole rings.



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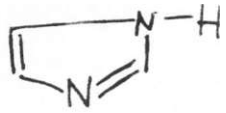
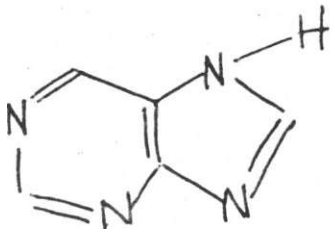
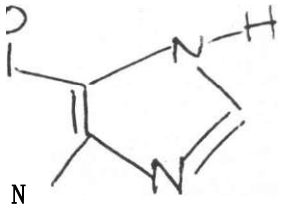


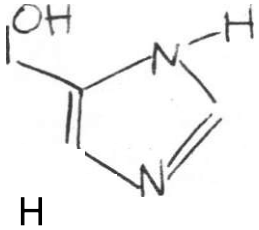
IM *WpAZOlE*
fELiN<r





XANTHINE RING

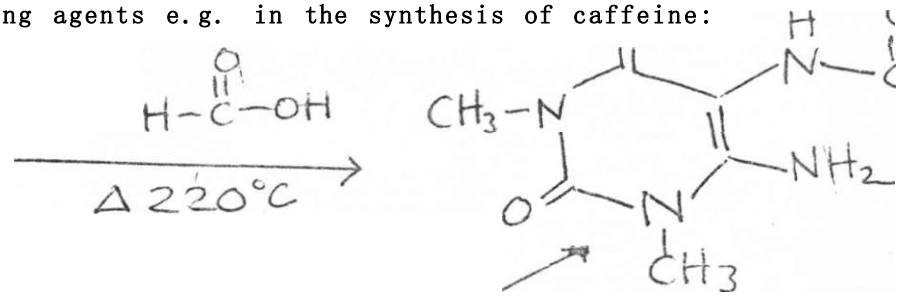
Xanthine ring may exist in various tautomeric forms such as:-

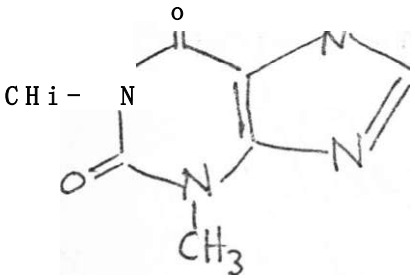


It must be mentioned here that although from the above scheme it appears that direct fussion of pyrimidine and imidazole rings would give the purine ring, that synthetic route by simultaneous ring fusions is of negligible importance in purine synthesis. The most well documented synthetic route is that of schack and waxier (see Ref 7, 11).

Another major synthetic route is the Traiube method which uses various cyclising agents e.g. in the synthesis of caffeine:

o h - d



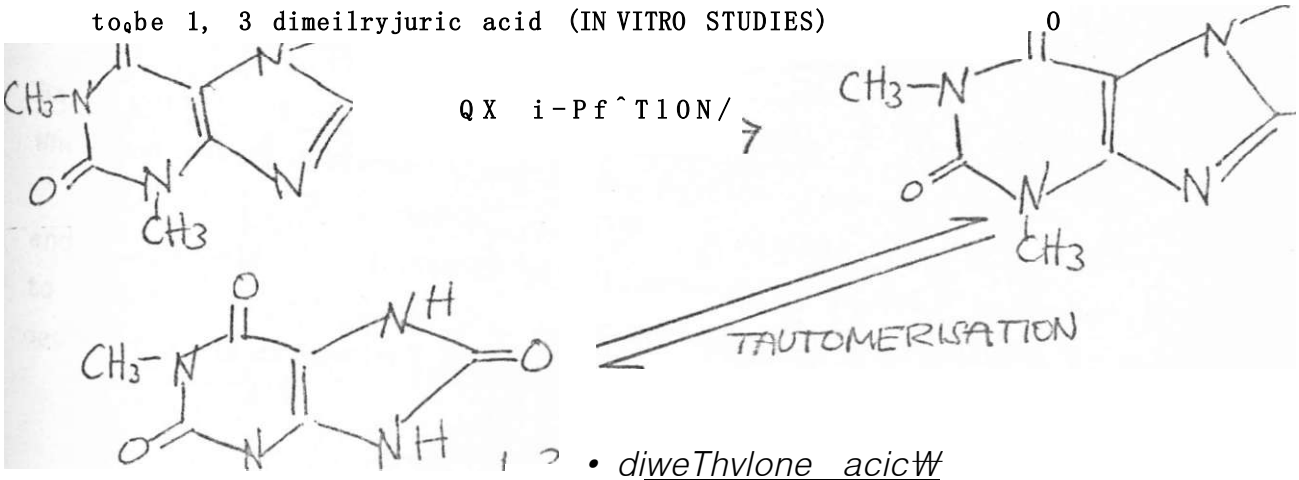


CVCUiSATfOf^

1, 3, 7 - Trimeth.ylxanthine fcaffeire)

Cyclising agents other than formic acid used include actic acid, oxalic acid, dithioformic acid etc.

The chemistry of degradation products of theophylline is complex (10) but theophylline is readily oxidised and the first product often appears to be 1, 3 dimeilryjuric acid (IN VITRO STUDIES)



61+3

• diweThvlone acic₩

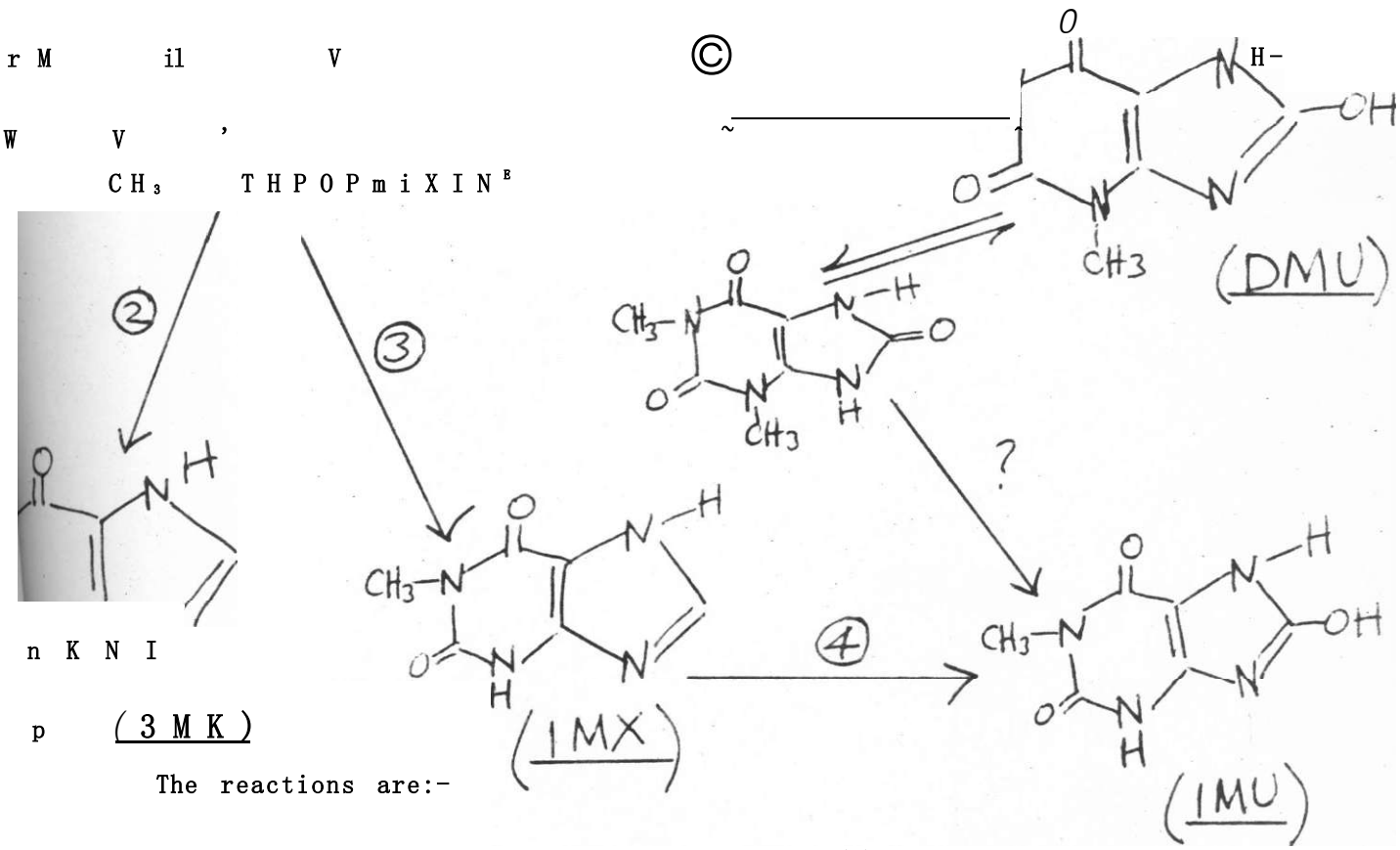
Hereafter, further oxidation occurs involving complex chemical reactions.

The reactions of theophylline following ingestion and absorption i.e. IN VITRO metabolism has been worked out by various people (13) and it appears to be converted to these products:

3 - Methyl xanthine (3mx); 1, 3- dimethyluric acid (1, 3 -DMU)

1 - mettylxanthine (IMX); 1 - Methyluric acid (1 - MU).

q The proposed scheme of IN VIVO metabolism is this:-



n K N I

p (3 M K)

The reactions are:-

Oxidation

(2) NI demeihylation

(3) N3 demetfjlation

(4) Oxidation

(?) reaction pathway is not well establi shed

BasicPrinciples of Accelerated Stability Testing:

When a compound undergoes chemical degradation thus

cpd A products

and if the rate of degradation with time represented as cW is found to be directly propational to the concentration of the remaining A; the degradation is refered to as first order that is

$$\frac{dA}{A} < (A)$$

$$\frac{dA}{T} = K\,(A)$$

Where K = degradation constant and (A) = concentration of A remaining at a given time,"fc.

The above expression is first order degradation. Higher decay orders do occur such as

$dA = K (A)^2$ (second order)

~5t

or

dA = K (A) (B) - (also second order)
it

(B) is the conc. of another compound whose presence affects the degradation rate of A.

dA = K $(A)^0$ or a constant (zero order)
eft

Higher orders are rare e.g. third orders. For simplicity, this treatment involves first order kiretics only.

As explained earlier, for first order degradation.

dA = K (A) and rearanging the equation
"Ht

dA = K (A)dt. Integrating this between times zero(to) and time t and when the concentration of A are C^a , ~A resDect and the amount of A being Ao and At respectfully. o and C .

dA = K (A) dt

At

J dA
T = K .

1-o fatfmj to(j<in thms)

In At = K (t - o) = Kt
"Ao

At

2.303 log = —Kt which on rearanging and dividing both sides by 2.303 becomes

or log A_t = Log A_f

Kt

This is a useful equation sometimes *also expressed as* $A^{\wedge} = Ao\ e^{-kt}$ and when concentrations rather than amounts are known becomes

$$\text{Log } Q_A = \text{Log } c/$$

$$\frac{Kt}{2.1(73}$$

This equation is of the form y = £ - mx where y = log $_g$

the logarithm of the concentration of A at time t

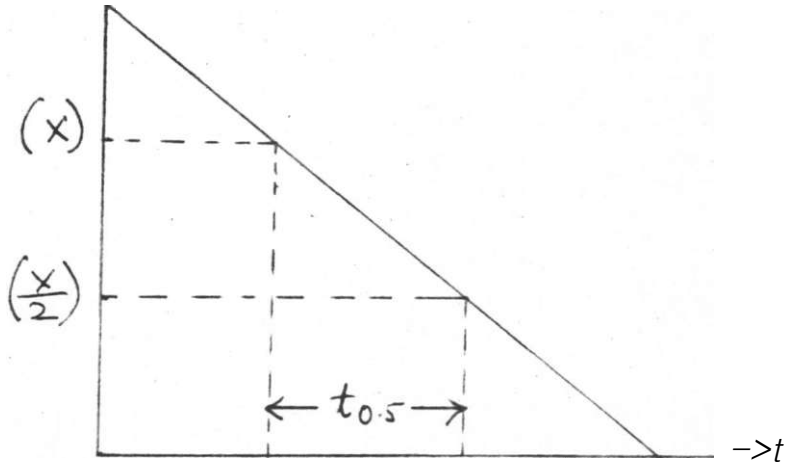
K = a constant, that is log C_{A_Q} or the logarithm of initial concentration of A.

M = a constant, that is K

Hence a plot of log C^A. against t is a straight one of gradient $-\frac{K}{77303.}$

t

$C_{\bullet t}$



For such plots, the half life (to.5), that is the time taken for the initial concentration to decay (degrade) to half, is independent of the initial concentration. It is thus a constant for a given compound undergoing first order degradation and its value can be determined either graphically or arithmetically.

(i) Graphically: if the log $C^A{}_t$ plot is on semi-logarithmic paper and taking the time for any amount (X) to degrade to x/2, the time interval f° r that is the half life, to.5, as shown above.

When the graph is not drawn on semi - log paper its easier to determine to.5 arithmetically. Alternatively, we can choose two concetrations $_cA$ t t so that their logs are within the graph. Determining the ~T two concentrations and reading the time interval between the two logs gives to.5.

(ii) Arithmetically: It was shown that log $C^a{}_{A_t}$ = loo $C^a{}_{A_o}$ $\frac{Kt}{2.303}$

or loa C t $\frac{Kt}{2.303}$ when C^A $C^A o$, t = to.5

$\frac{C^A}{C^A 0}$

The equation thus becomes

$$\text{Log } \frac{C\hat{}o}{} = \log C\hat{}o \quad \frac{K \text{ to.} 5}{2.303}$$

Log Co x - K to.5
T h 0 S I

$$. \log \backslash = - \frac{K \text{ to.} 5}{2.303} \quad \text{or } \log 2 = K \text{ to.} 5$$

Now since log 2 = 0.3010,

$$0.3010 = \frac{K \text{ to.} 5}{2.303}$$

$$\text{to.} 5 = 2.303 \times 0.3010 \qquad 0.6930$$

K

$$\text{to.} 5 \qquad 0.6930$$

K

Thus as long as the decay constant , K, 1s determined, the half life, to.5, can be easly worked out arithmetically. The decomposition rate constant, K, is constant for a compound at a particular temperature for first order reaction but varies with temperature. The way in which K varies with temperature expressed indgrees Kelvin, was shown by Arherius to be

$$K = A e^{E/RT}$$

Where K = decomposition rate constant at temperature T

A = constant

E = ' energy of the reaction

R = gas constant

T = Temperature expressed in degress Kelvin .

Taking natural logarithms on both sides of equation

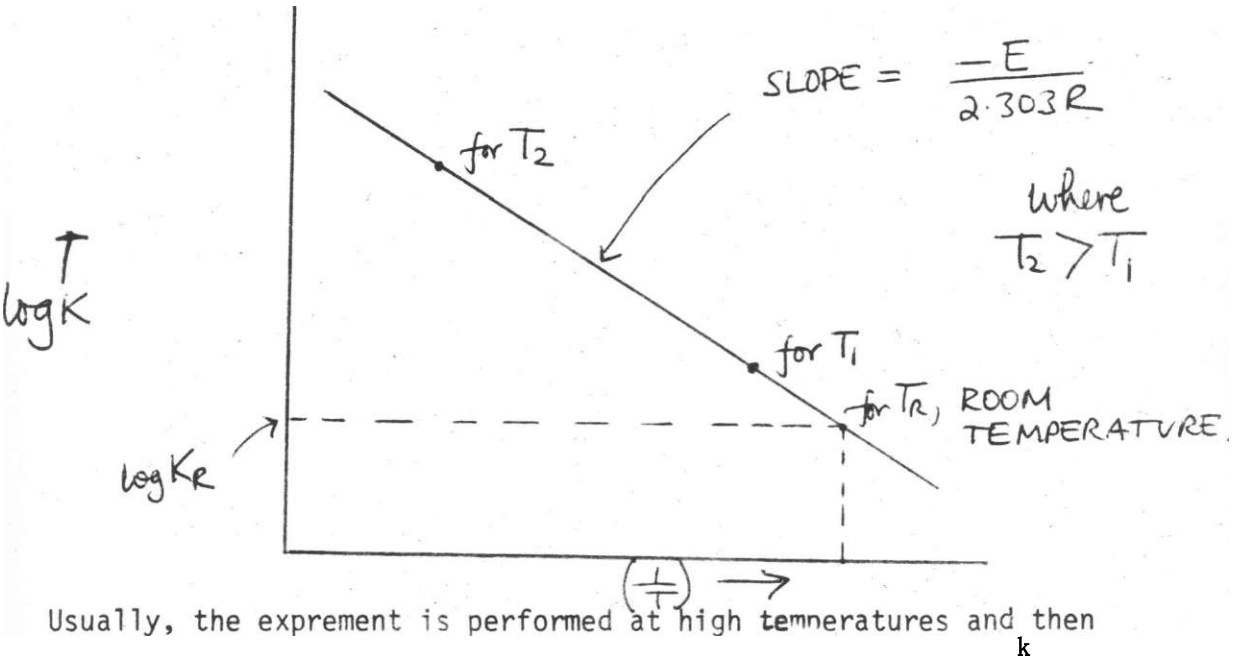
$$\text{Ink} = \text{In } A - E/RT$$

$$\text{or log } K = \text{Log } A - \frac{E}{2.303\ RT} \quad (\text{since In } A = 2.303 \log A)$$

The above equation is in the form of y = K - MX which is a straight line graph equation. Hence a plot of log K against

(j) is a straight one of u\t&rcept=- kj A.

and gradient $-\frac{\quad}{\quad}E$



Usually, the exprement is performed at high temperatures and then the graph extrapolated to room temperature, T_R This gives k_R, the room temperature decomposition constant which can be used to calculate half life at room temperature using the equation.

$$to.5 = \frac{0.6930}{K}$$

The activation energy of reaction, E, can be determined if desired from the slope of the graph.

Accelerated stability tests can be used for determining the theoretical "shelf - life" of a given drug - If the required dose is Ao and the official potency standards are 90 - 110% of the required stated dose i.e. 10% potency loss is acceptable, the time required for this 10% degradation can be worked out using first order equation $\log A = \log Ao - \frac{Kt}{2.303}$

and this becomes

$$\log 0.9\,Ao = \log Ao - \frac{Kt}{2.T0}$$

Since Ao and K are known, the theoretical shelf-life can be computed and this is equal to the value of t thus obtained.

Although this technique is useful for determining shelf life, it is not absolute but only indicative of true shelf life. True shelf life is only obtained by assaying the drug amounts at different determined times when the drug is stored under ordinary temperatures. The theoretical shelf - life calculated as explained earlier has the following limitations I

(i) When calculating it, we use the rate constant obtained by extrapolation of the graph to a constant value of room temperature, T^. In reality, room temperature is rarely constant but depends on day or night, reason etc.

(ii) The rate constant at room temperature, K_D is obtained by extrapolation of rate constants of higher temperatures but the nature and mode of the degradation reactionat these temperature need have any bearing to the nature, mode or order of the reaction at room temperature. The higher temperatures used should thus be as near as possible to room temperature so that the events would be indicative of those at room temperature.

OBJECTIVES:

The objective of the project was to carry out accelerated stability tests on various samples containing theophylline Which included - theophylline solutions in base (NaCH), Aminophylline injections, Theophylline solutions containing graded amounts of ethylenediamine. From the results, the relative degradation rate constants for these theophlline formulations would be calculated and hence establish whether in fact the excess ethylenediamine present in Aminophylline injections had any effects on chemical stability of the theophylline.

Materials Preparation of Reagents

Glassware and other conventional laboratory apparatus.

Experimental work proper and results:

PREPARATION OF REAGENTS:

H) 0.1 ($^{N}/_{10}$) Sulphuric Acid: A stock solution of 36N sulfuric acid was the starting point and 1 litre of 0.1N H2 504 was prepared thus: 2.8 ml of the stock solution was pipetted into a one litre flask and diluted to the mark with distilled water. The solution thus prepared was standardised (16) using sodium bicarbonate.

(2) Alkaline Borate Buffer pH

12.4g Boric acid and 14.9 g Potassium Chloride powders were dissolved in water and more water added to make a litre. 0.2M NaOH was then prepared (1 litre) by weighing 9.2 g pellets and disolving inwaterto make one litre. 150 cm^3 of the Boric acid and pot. Chloride solution was placed in a 200 ml volumetric flask and then 36.9 ml of the 0.2N NaOH added then finally water to the 200ml mark. The PH of the solution was then read as 8.6.

(3) Preparation of Tris - Chloride Buffers '^{ta}

0.6 g Tromethamine and 2.34 g sodium Chloride were weighed accurately and disolved in one litre of distilled water. The PH of solution thus made was read and then adjusted to the appropriate pH using 0.1N Hcl By this method buffers of pH9.0 and pH 7.0 were prepared.

(4) Buffer solutions pH 9.0 and pH 7.0

One buffer tablet was dissolved in 100ml distilled water to make standard pH buffers.

(5) Stationary phase for TLC Plates

50 grams Kielsegel 60 G F ^ were weighed into a dry bottle with a nicely fitting lid. 100 ml distilled water was added with vigorous shaking and slury was ready for spreading onto TLC plates.

(6) 70 jjj per ml Standard Theophylline Stock Solutions:

50 mg Theophylline powder was accurately weighed into a 500 ml volumetric flask and volume made to the mark with distilled water. Shaking the flask ensured dissolution to give a solution of 10Cpq per ml. 7.0ml of this |fn9asurpJ with 2.0 and 5.0 bulb pipettes) was poured into a 10 ml volumetric flask and the volume-make to the mark with the appropriate buffers giving two 70 pg per ml Theophylline solutions, one in pH 7.0 and the other in pH 9.0

(7) Dilution of Aminophylline injection tc 70 eg per ml.

The injection used contained 250 mg Theophylline in 2 ml solution ampoules. 1.0ml solution was pipetted into 100 ml flask' and the volume made to mark with distilled water givino a solution of 2.5 mq per ml (2500 per ml) . This was further to give a solution containing 100-uj p°r nil 7.0 ml of this was diluted to 10 ml with the appropriate buffers to give twow'ruof 70 ug per ml Theophyline at pH 9.0 and pH 7.0.

(8) Standard Stock of 50 ug per ml Theophylline soln

0.5 g Theophylline, accurately weighed, was disolved in warm distilled water to make 100 ml solution, strength 0.5% (5 mg per ml) 1.0 ml of that soln was then further diluted to 100 ml with water to give 0,05 mg per ml Theophylline soln or 50 ug per ml.

(9) Soln of 100 Ug per ml Theophlline:

50 mg Theophlline powder was accurately weighed into a 500 ml flask and disolved in some distilled water and the volume then made to the mark with more distilled water giving a solution of 0.lmg per ml or 100 ug per ml.

EXPERIMENTAL WORK PROPPER:

ASSAY OF ETHYLENEC-LAMIRE CONTENT OF AMINOPHYLLINE AMPOALLS BY B.P. MEHTOD:

10 ml amponles containing 2.4% aminophylline were assayed for theophylline and ethylenediamine content using the BP method with a slight modification. The modification involved the determination of ethylennediamine and want thus:

1) 1.0. ml injection was pipetted into a conical flask
2) Water was added to make about 20 ml.
   The rest was as set in the BP (8)
   (see also Figure 1)
   The results are represented in table I

Detamination	Ethylenediamine Content (mg/mlinjection) (X)	Theophylline content (mg/ml) (Y)	Ratio of (X/Y)
	6.97	28.304	0.246
	6.66	30.152	0.221
	6.66	31.380	0.212

The results of the last column in Table 1 imply that the contents of the aminophylline injection passed the BP test. This is because the PB (8) states that the ratios of ethylenediamine to theophylline contents should not exceed 0.251. The label claim on the injection was 25mg theophylline per ml and hence the values of percentage label claim are admittedly way off the B.P limits and this cannot be readily explained.

Assay of Theophylline using two methods:

(i) Spretrophotometric using Theophylline in buffer 8.6

(ii) A?say as in BP method, that is theophylline dissolved in 0.01N NaOH.

Theophylline powder (0.5 g) was weighed out accurately and then dissolved in the appropriate solution and then diluted with the same solution using this scheme.

TABLE TWO

Vol sample (ml)	Vol of diluting solution	Final volume	Final concentration
	(ml)	(ml)	(ug/ml)
0.5g powder	100	100.0	5000
1.0 ml	99	100.0	50
1.0	4.0	5.0	10

The diluting solutions were (i) Potassium Borate Buffer pH 8.6

(ii) 0.01N NaOH

The total dilutions were thus:1 in 100 x 5

= 1 in 500. The absorbances of the two solutions were then read off sp 8000 tracing (see figure 2)

(i) In Alkalive Borate Buffer : A = 0.53 - 0.04 = 0.49

Thus C = 0.49 = 0.000753 g per 100 ml

Correcting for dilution, C = 0-375 g per 100 ml

(ii) Similarily for Theophylline in 0.0IN NaOH

A = 0.65 - 0.04 = 0.61

$\overset{A}{r}$ = $\frac{0.61}{SSfT}$ - = 0.000938 g per 100 ml

Correcting for I in 500 dilution

= 0.469 g per 100 ml

The accuracy of determination can be tabulated thus - Table 2 (iJL)

Solvent	Assay Value (g Theophylline per 100 ml)	Weighedout Powder (g per 100 ml)	% Theophylline detected
Buffer, Alkaline	FICRTT?		
0.01N NaOH	A 0.377	0.4342	86.83
	0.469	0.4893	95.85

From the results, it can be observed that even with pure theophylline the assay of Theophylline in 0.01N NaOH and as outlined in BP does not give 100% detection although it does in fact approach that. I think it can safely be assumed that the analytical reagent was not pure or had undergone some degradation, the later being more probable. TLC results carried out later confirmed this.

Thin Layer Chromatography: Identification and impurity detection of various theophylline samples.
Five TLC plates were dipped into theslurry and activated at 110ˆ in the oven for one hour and then spotted with the following solutions:-

1. Aminophylline injection- unheated
2. Theophylline solution, unheated.
3. Theophylline solution, boiled for one hour
4. Aminophylline injection boiled for three hours
5. Aminophylline injection boiled for two hours.

Two mobile solvents were tried in the chromatogram development based on literature survey and these were:-

(a) n - propanol (CH, CH_2 ChˆDHJ
(b) n - propanol mixed with liquid ammonia in 1.1 ratio

Using two large develpment tanks.ˆ the large TLC plates (20 x 20 cm^2) were dipped and development carried out. The developed plates were then removed, viewed under ultraviolet (U.V.) light and later sprayed with Dragend offs reagent.

The separation using n - propanol was poor due to overloading but incficated at least three different types of compounds. The separation using a mixture of n-propanol and liquid ammonia gave good separation of three different compounds.

All the samples showed the slowest moving spot which was due to Theophylline.Solutions of Theophylline made from Thephylline powder gave a further spot which was assumed due to an impurity in it because that same spot did not appear with the injection separation.

The heated samples gave a t^ird ªP° t which was assumed to be due to the presence of a degradation product. No attempt was made to identify the powder impurity or the nature of the degradation product.

TABLE 3.

Spot	Distance Spot moved (cm)	Solvent Front (cm)	Rf	HRf
Theophylline	2.4	10.6	0.226	22.6
Powder impurity	5.9	10.6	0.562	56.2
Degradation product	8.7	10.6	0.828	82.8

(see also FIGURES 3 and 4)

Differential Spectroscopy Work:

In view of the fact that the Theophylline powder contained an impurity and that in the accelerated rate studies we must differentiate the Theophylline from its degradation products, literature survey pointed to use of differential spectroscopy in accelerated rate analysis of Theophylline.

Work performed (14) had brought out the following facts. Theophylline had its maximum absorption peak at 285 nanometers but that least interference with other xanthines or degradation products occured at 290 nm. The literature also suggested use of two samples one at pH 9.0 and the other at pH 7.0 and to record the absorbance of PH 9.0 sample using pH 7.0 samples as reference. The buffer recommended for this type of *work* was Tris - *Chloride buffer* system (Tromethamine buffer system).

Absorbances of between 2 - 10 ug per ml using 50 ug/ml and diluted thus.- (TABLE 4)

TABLE 4

Volume of 50 ug per ml STOCK SOLN Taken (ml)	Made up to this Final Volume with the Appropriate pH Buffer	Final Theophylline Cone.(ug per ml)
1.0	25.0	2
2.0	25.0	4
0.5	5.0	5
3.0	25.0	6
4.0	25.0	8
1.0	5.0	10

The solutions were then placed in cuvettes and their absorbances at 290 nm read using sp 21 spectrophotometer. The readings are tabulated below (Table 5) The differences between pH 9.0 and pH.7.0were calculated and another plot (Fig 5) made. This graph was linear anticipated but did not pass through the origin, a seriously bad observation

Table 5

Concentration (ug per ml)	Absorbance at 290 nm pH 9.0	pH 7.0	
2.0	0.020	0.050	
4.0	0.055	0.042	
5.0	0.065	0.055	
6.0	0.082	0.068	
8.0	0.112	0.099	
10.0	0.135	0.115	

After this, the supervisor demonstrated the correct way to read absorbances from SP 21 in differential spectroscopy work. The SP 21 would be zeroed with the pH 7.0 solution and then the absorbance of the corresponding pH 9.0 sample read, minimising errors. Fresh solutions of between 10 and 100 ug per ml Theophylline were prepared using 100 ua per ml.

Stock solution diluted as shown (Table fi) below and the absorbances read. This was in an attempt to make a standard curve which would be used thereafter to study the degradation rates.

TABLE 6

Volume stock solution taken (100 ug per ml)	Final volume with appropriate buffer (ml)	Final conc (ug per ml)
1.0	10	10.0
5.0	25	20.0
3.0	10	30.0
10.0	25	40.0
12.5	25	50.0
15.0	25	60.0
17.5	25	70.0
20.0	25	80.0
25.0	25	100.0

A plot of absorbance against concentrations (Fig 7) was made using the absorbances read as:-

TABLE 7

Cone (ug per ml)	Absorbance
10	0.089
20	0. 146
30	0.204
40	0.307
50	0.292
60	0.300
70	0.317
80	0.340
90	0.412
100	0.392

The graph obtained (Fig 7) was not good and ir'act two straight lines could be drawn ver

the reproductibity and reliability of differential spectroscopy tested. This involved preparing 7.0 ug ner ml standard Theophylline solutions at the two usual pH's and dilution of the Aminophylline injection to two 70 ug per ml solutions at the two buffer solutions and hence determination of the percentage label claim of theophylline injection.

TABLE 8

Concentration (ug per ml)	Absorbance at 290 nm		
	standard solution	diluted injection	
		Trial 1	Trial 2
70.0 ug per ml		i	j
	0.32	0.35	0.348

Theophylline content in the injection was calculated as follows:-

Average absorbance of diluted injection = $\frac{0.34 + 0.348}{2}$ = 0.349

Absorbance of standard 70 ug per ml solution = 0.32

Cone of Theophylline in injection = $\frac{0.349 \times 70}{0.32}$

= 76.343 ug per ml

The total dilutions of the injection (see reagent 7) were

1 in 100 x 25 x 10/7 = J571.4

Cone of Theophylline in the undiluted injection

= 76.343x3571.4

272651 . 4 ug per ml

272.65 mg per ml

The lable claim was 250 mg per ml hence percentage lable claim was

= $\frac{272.7 \times 100}{250.0}$

= 108.8%

The reproducibity of results was testsd by a repeat of the above determination of the % label claim and the reslults were as tabulated here:"

(TABLE 9)

TABLE 9

Cone Theophylline (ug per ml)	Absorbance read at 290	
	Standard Solution	Diluted injection (results of 3 exps)
70.0	0.32	0.319, 0.318 0.320

The calculated percentage label claims were(Table 10)

TABLE 10

Injection Absorbance	Percentage label claim
0.319	99.68
0.318	99.36
0.320	100

The determination of Theophylline by differential spectroscopy was thus accurate and the reliability was estimated by calculation of the standard deviation of all the percentage label claims in this fashion (TABLE 11)

TABLE 11

Theophylline content of injection (mg per ml) X	Mean (m)	(x - m)	(x - m i^2
273.44	258.58	+14.86	220.82
271.82		+13.29	176.62
249.20	M	- 9.38	87.98
248.40	ii	-10.18	103.63
250.0	ii	- 8.58	73.62
			662.67

The standard duration given by

$$SO - \sqrt{\frac{[x - nr}{N - 1}}$$

ACCELERATED STABILITY TESTS ON THEOPHYLLINE:

70 ug per ml Theophylline solutions were prepared (reagents 6 and 7) These were then boiled for specified times at the boiling point of oc water (94.6) and with the solutions completely immersed in the boiling water. After heating for different times, 5 ml alinuots were withdrawn from the bulk solutions and the absorbance read as usual.

(1) Stability studies on heophylline solution:absorbance at 290 nm

TABLE 12

A&SGLQfl+JCBS

Time (hrs)	Trial One	Trial Two
0	0.319	0.298
0.5	0.315	0.320
1.0	0.308	0.278
1.5	0.303	
2.0	0.280	0.260
3.0	0.290	0.270

(ii) Stability studies on Aminophylline injection

TABLE 13

	Absorbance at 290 nm
0	0.290
1.0	0.270
2.0	2.281
3.0	2.269

Semi - logauthmic graphs (fig. 8, 9, 10) *of* absorbance against time were made for all of these readings. Since absorbance is proportional to concentration the graphs can be used to determined the relative degradation rates of

(i) Theophylline solutions

(ii) Aminophylline injection

As shown earlier, the cuNfcs are straight and the graph can thus be expressed as

$$\text{Log } A = \log Ao - \frac{Kt}{27103}$$

where, A = absorbance at a certain time^t

Ao = initial Absorbance. Hence the gradient of the curves = $-\frac{K}{2.303}$

The degradation rate constants, K, were calculated as shown be low:-

(i) Theophylline solution: (Fig. 8)

gradient = $\frac{\log 0.26 - \log 0.32}{190 - 0}$

$\frac{0.26}{3-30\ 3}$ $\log$ = $\frac{-0.090}{190}$

$k = 0.09 \times 2.303 = 1.09 \times 10^{-3}$

190

for the repeat experiment, i.e. trial two (fig 9)

$\frac{K}{2.303} = \frac{\log 0.25 - \log 0.30}{190} = \frac{-0998}{190}$

$K - \frac{2\text{-}303 \times 0.798}{190}$

$= 0.97 \times 10^{-3}$

(ii) Similarily for Aminophylline injection (Fig 10)

$\frac{\log 0.25 - \log 0.29}{IW}$

$= -0..0645$

$is = 2.303 \times 0.0645$

$= 6.19 \times 10^{-4}$

$= 0.62 \times 10^{-3}$

The average value of K for the Theophylline solutions (i)

$= \frac{(0.97 + 1.09) \times 10^{-3}}{2}$

1.03×10^{-3}.

DISCUSSION

It would appear that the degradation rate constant for aminophylline injection is less than that of Theophylline solution and by a significant factor. This in real terms implies that Theophylline is more stable in presence of excess athylenediamine at least the temperature of this experiment, 94.6 °c. This would seem to justify the addition of excess ethylenediamine even when the toxicity of ethylenediamine is well documented. (?) This role of chemically stabilising Theophylline is in addition to that of confering physical dissolution of Theophylline.

Theophylline is not very soluble in water (15) and hence in presence of atmospheric carbon Dioxide and Theophylline being less soluble in acidic solutions, the excess ethylenediamine would react with the Carbon Dioxide in solution and hence prevent the solution acidijucation and hence theophylline precipitation.

In reaching that conclussion about ethylenediamine giving chemical stability to Theophylline solutions as in injection, it was assumed that no other substances such as any preservatives added by the manufacturer had such a role.

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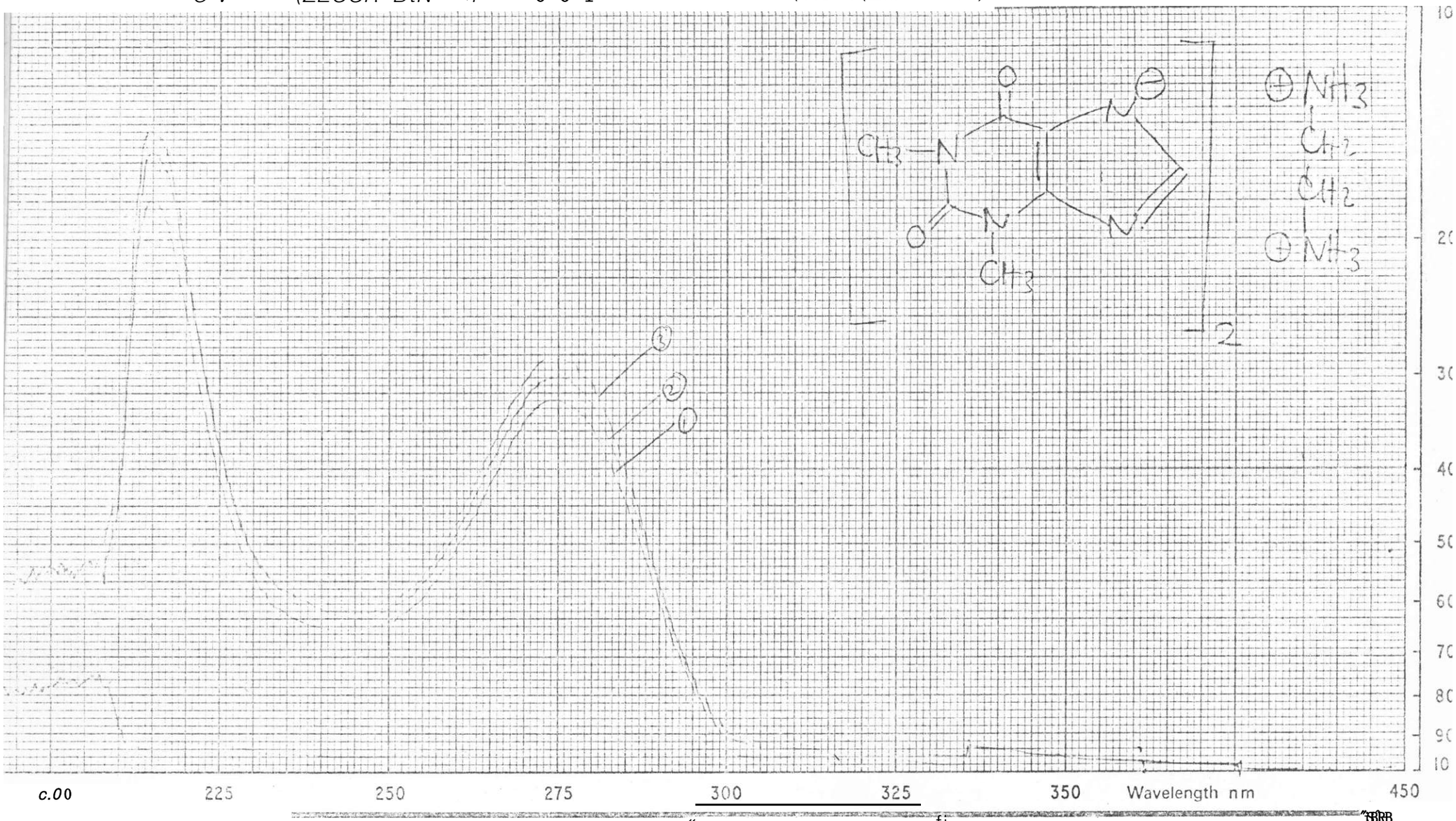

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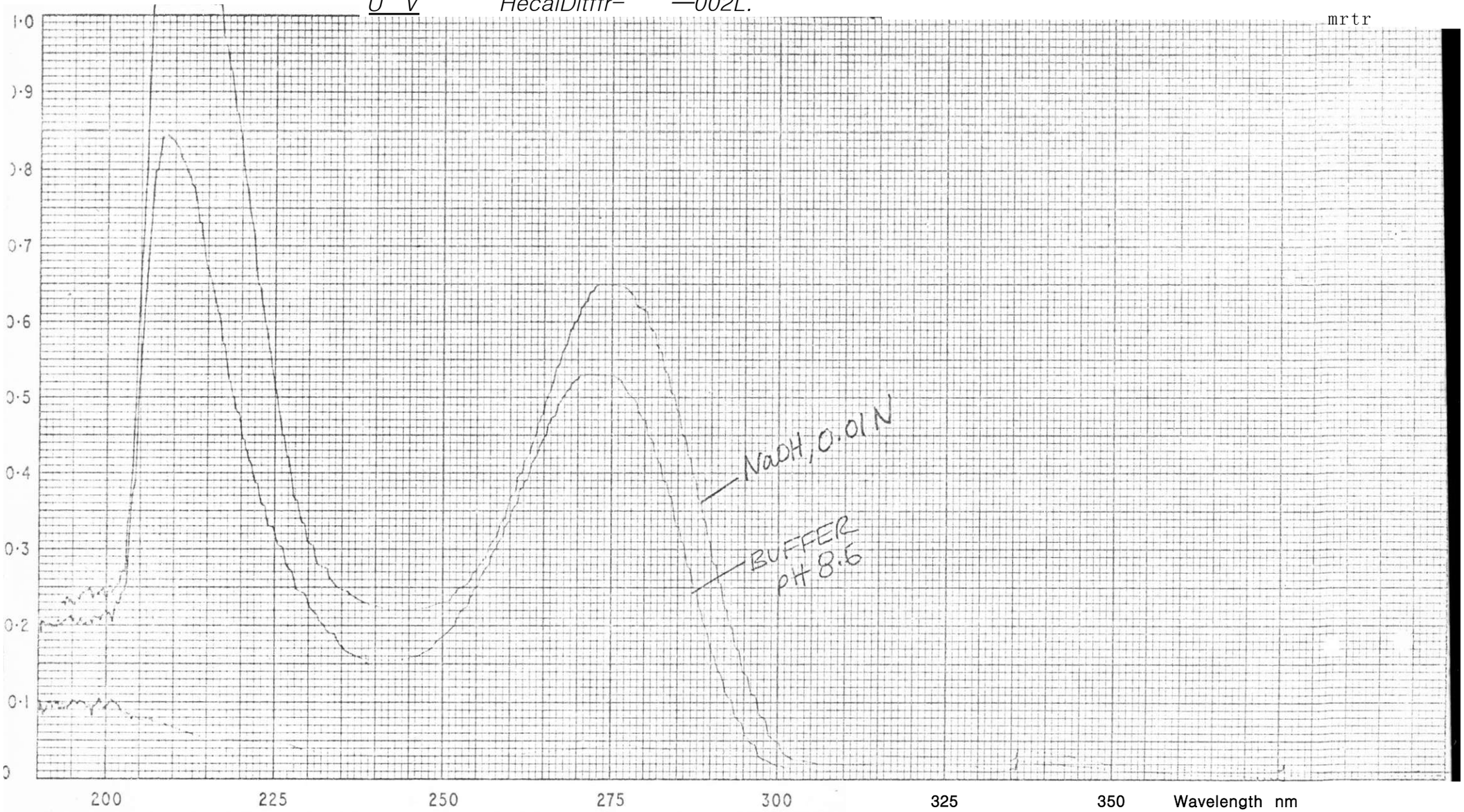

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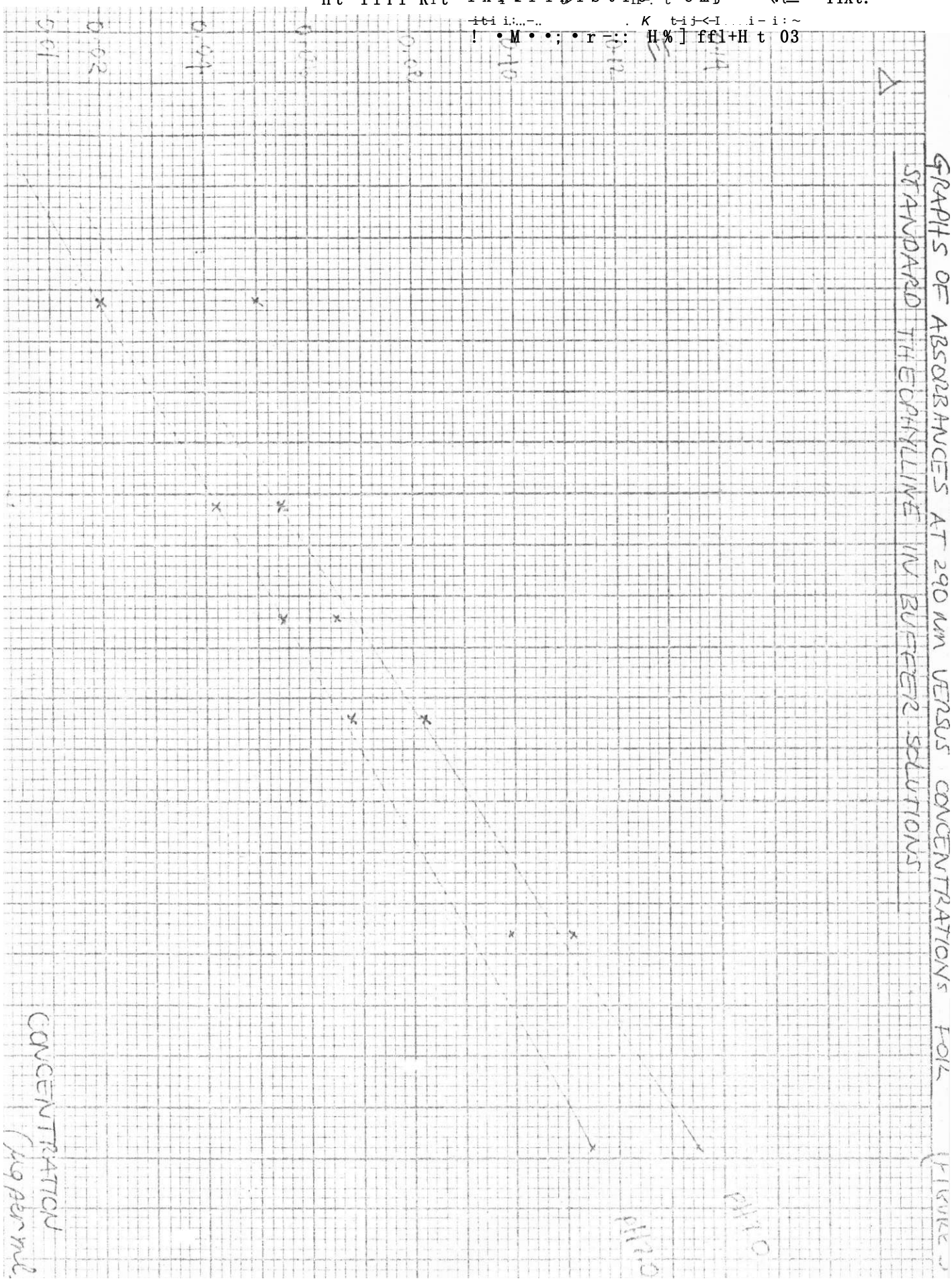
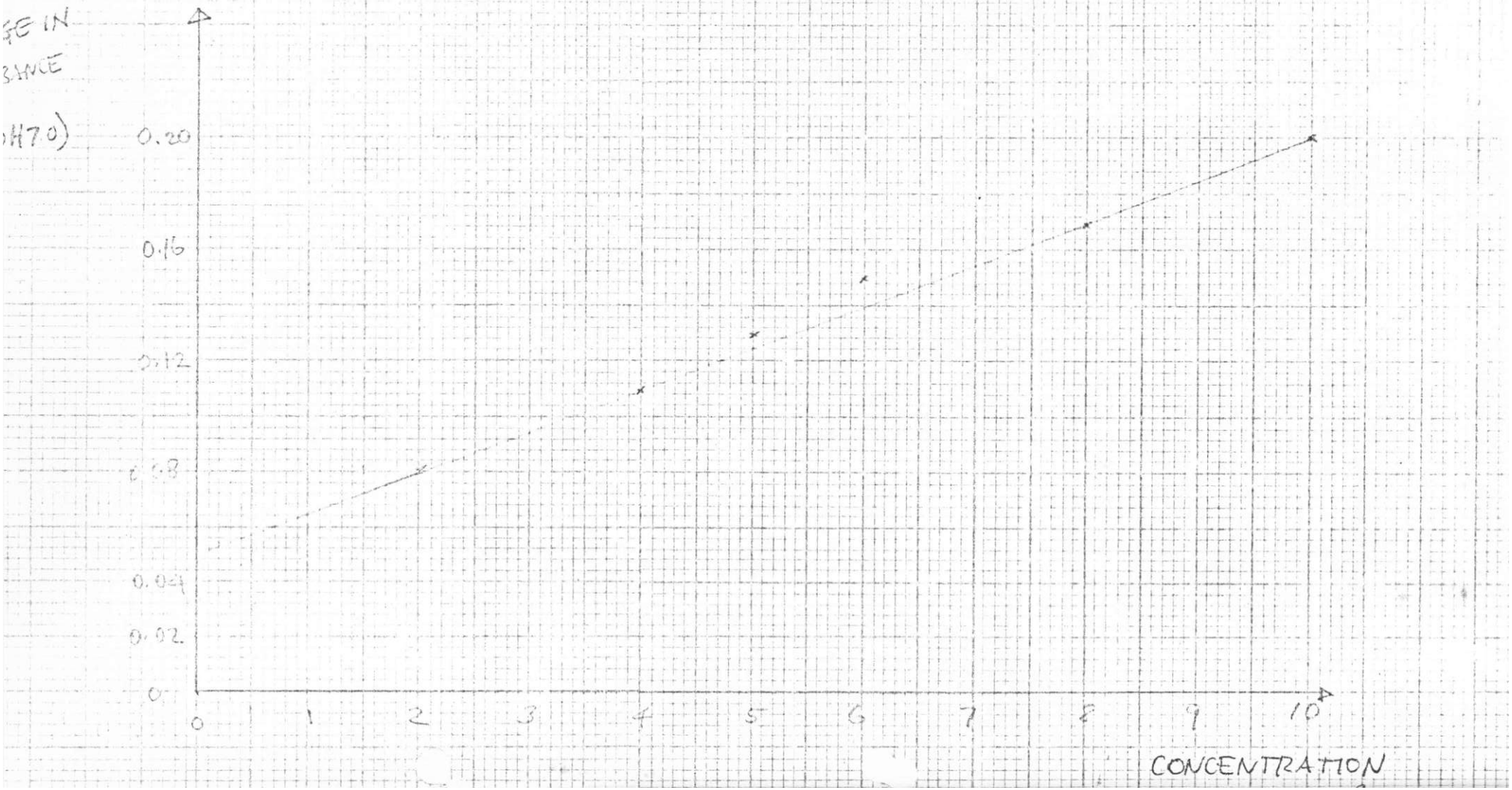
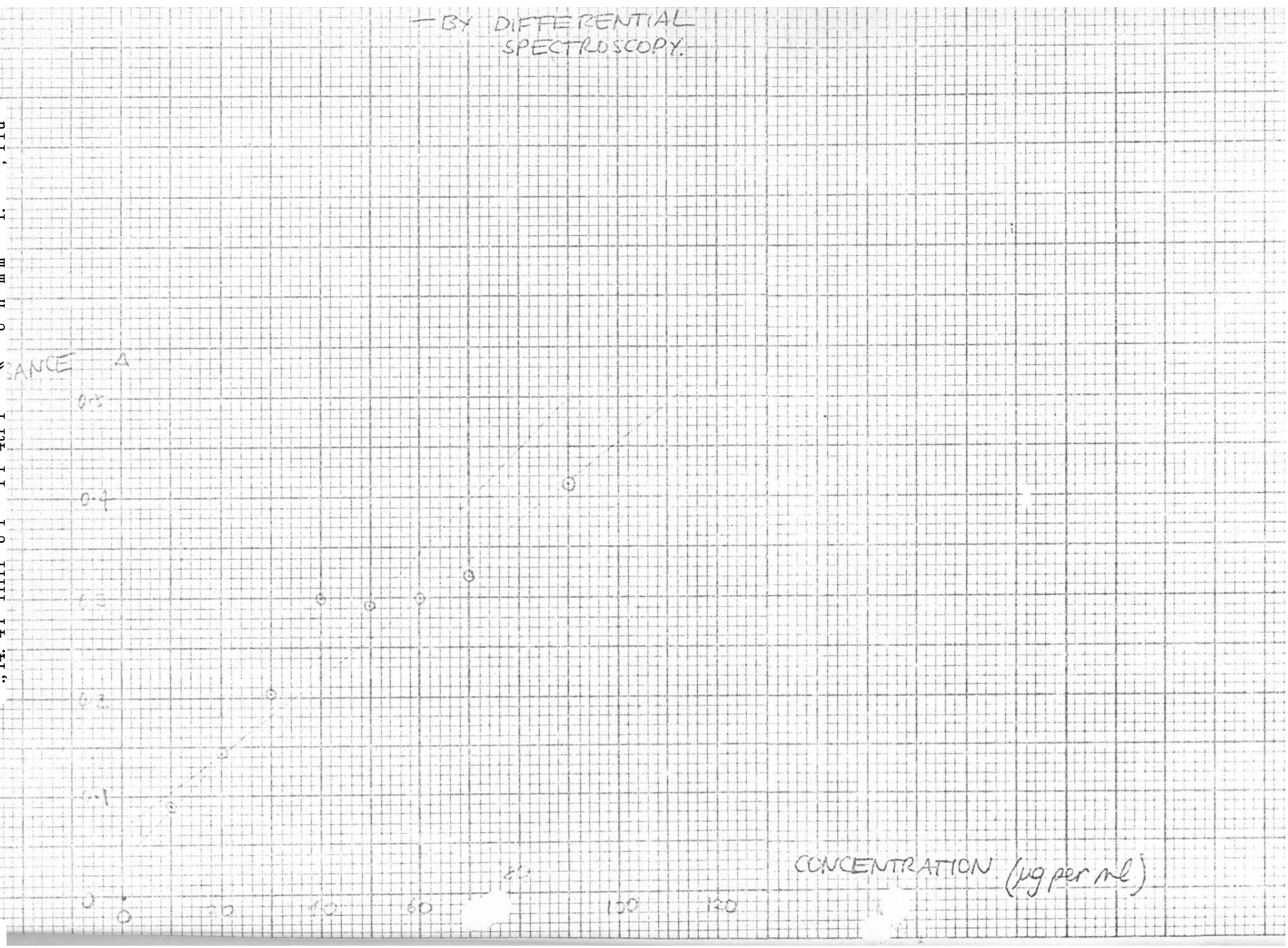

GRAPHS OF ABSORBANCES AT 290 nm VERSUS CONCENTRATIONS FOR
STANDARD THEOPHYLLINE IN BUFFER SOLUTIONS
CONCENTRATION

THEOPHYLLINE CONCENTRATIONS

(FIGURE 6)

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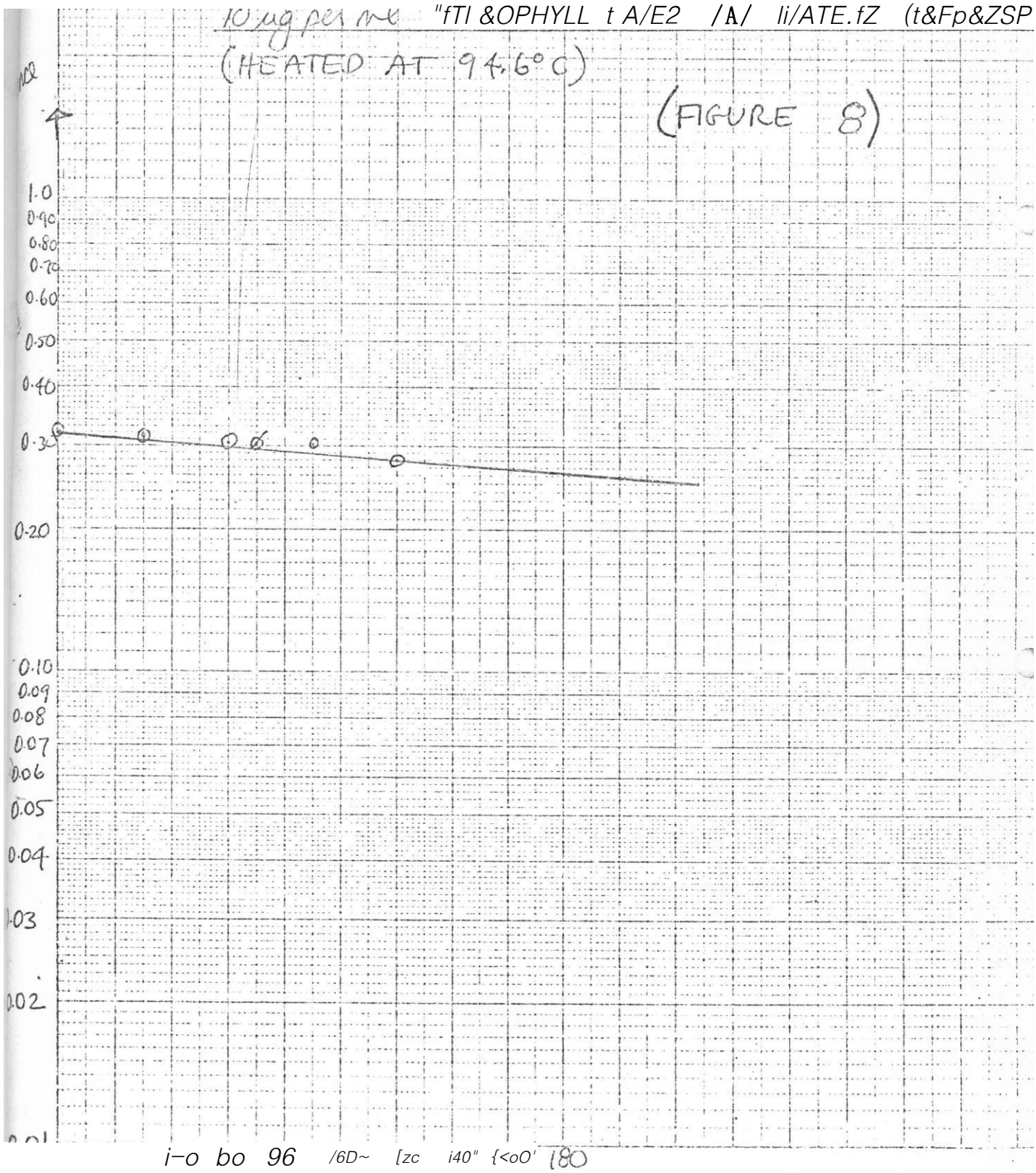

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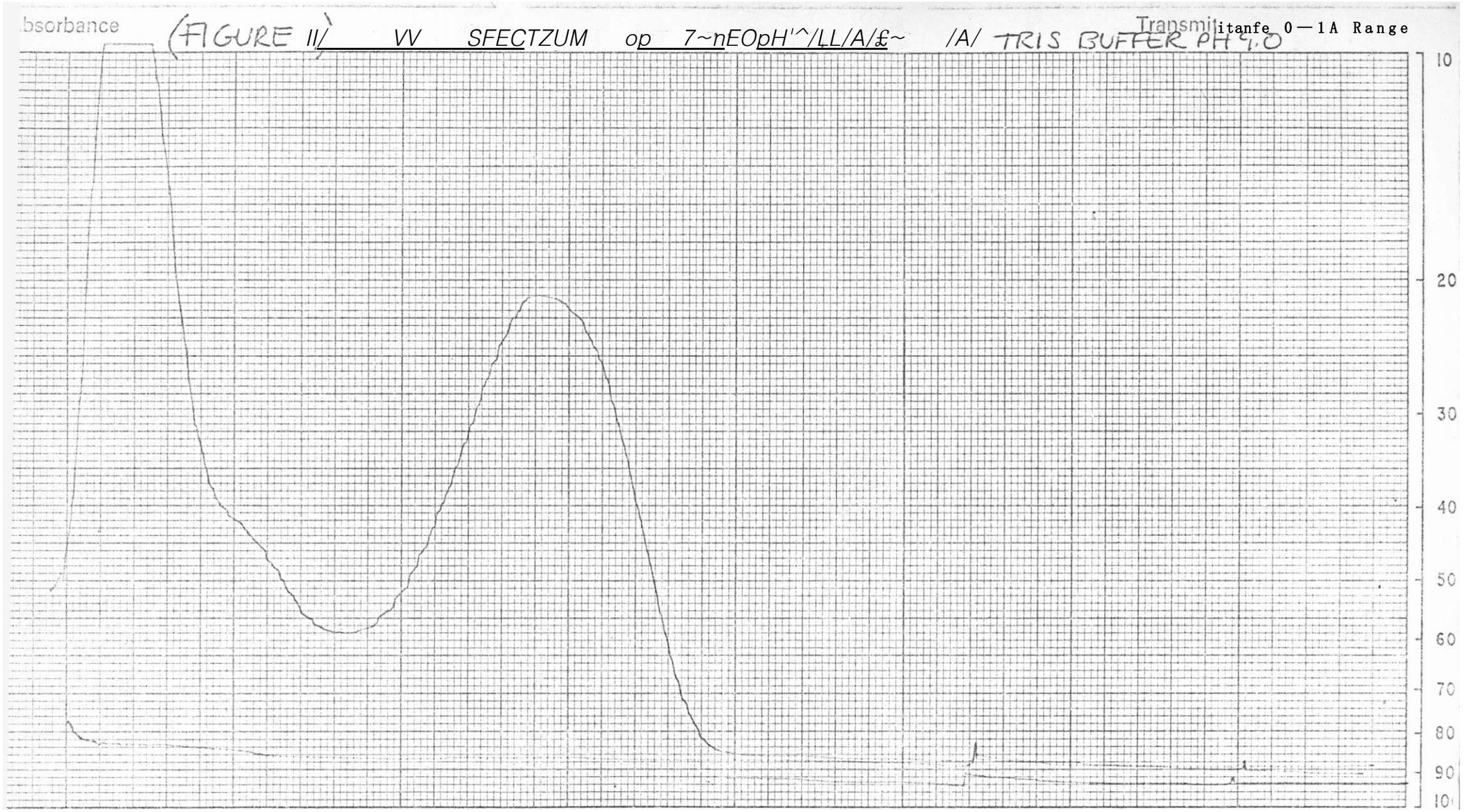

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