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STUDIES ON SOME FACTORS AFFECTING STABILITY OF ISONIAZID IN SOLUTIONS

1. Effect of pH-Value and Buffer system.

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The stability of isoniazid in solutions at various pH values in the range of 4-8 was followed up. It was found that degradation of isoniazid at the various pH-values proceeds according to first order kinetics. The degradation rate constant is pH dependent. pH 5.65 was found to be pH of maximum stability. Physical stability of the solutions did not appear in consistance with the chemical stability. Comparing the stability of isoniazid in various buffer systems, namely, Walpol's acetate, Clark and Lub's phthalate, Sørensen's phosphate, McIlvaine's citric acid-phosphate and Sørensen's citrate at pH 5.5 reveals that isoniazid is most stable in Walpole's acetate followed in order by Clark and Lub's phosphate, Sørensen's phosphate,McIlvaine's citric acid-phosphate and finally Sørensen's citrate.

Isoniazid; isonicotinic acid hydrazide, is the most potent and selective of the known tuberculostatic antibacterial agents, and it is regarded as the most effective agent in the therapy of tuberculosis[1]. In spite of the fact that any change in the structure of isoniazid leads to a decrease of potency and in most cases loss of potency[2] , proper attention to the stability behavior of this drug has not been given. Winder[3] studied the decomposition of isoniazid in presence of oxygen and found that the rate of degradation is slow at pH 6 and rapid at pH 8 and is catalyzed by hemin and some metallic ions specially manganese. Vulterin[4] reported that alkaline solutions of isoniazid decomposed rapidly while acidic solutions are comparatively more stable. Kakemi et al.[5] studied the base-catalyzed degradation of isoniazid and reported a difference in the degradation pattern of isoniazid according to whether the degradation takes place under aerobic or anaerobic conditions.

So, it was decided in these laboratories to intitiate a thorough investigation involving the different factors that could affect the stability of isoniazid in solutions. The effect of pH-value and the type of buffering systems on the stability pattern of isonizid upon autoclaving and storage form the subject matter in this communication.

EXPERIMENTAL

Materials:

Pharmaceutical or analytical grade of isoniazid, sodium acetate, disodium hydrogen phosphate, potassium dihydrogen phosphate, potassium biphthalate, sodium hydroxide, citric acid, acetic acid and methanol. 2-Methyl-3-nitropyridyl-6-carboxaldehyde was synthesized according to a repored method[6] and purified by recrystallization from petrolium ether to produce an analytical grade.

Equipment:

pH-Meter (pH-Meter Titrimeter U9 N, Solea lyon).

Spectophotometer (Specktromon 204).

Circulating hot air incubator thermostatically controled ($\pm 1^{o}$)

Procedure:

Effect of pH-value:

Solutions containing 1% w/v isoniazid in McIlvaine's citric acid-phosphate buffer[7] at pH-values covering the range of 4 to 8 were prepared. The solutions were filtered and filled in colourless neutral-glass ampoules, 5 ml each. The ampoules were sealed by fusion and sterilized by autoclaving at 116^{o} for 30 minutes and then stored at 70^{o} ($\pm 1^{o}$). The isoniazid content of the different solutions was estimated before and after autoclaving as well as after storage for appropriate time intervals at 70^{o}. The isoniazid was analyzed according to a recently developed stability indicating colorimetric procedure using 2-methyl-3-nitropyridine-6-carboxaldehyde[8].

Effect of buffer systems:

Solutions containing 1% isoniazid in five different buffer systems of similar pH-value (pH 5.5) were prepared. The investigated buffers were Walpole's acetate[7], Clark and Lub's phthalate[7], Sørensen's phosphate[7], Sørensen's citrate[7] and McIlvaine's citric acid-phosphate[7]. The solutions were filtered, filled into 5 ml colourless-glass ampoules sterilized and then stored at 70° (± 1°). The isoniazid content of the different solutions was estimated before and after autoclaving as well as periodically during storage at 70°.

RESULTS AND DISCUSSION

1- Effect of pH:

Sterilizing isonazid solutions by autoclaving seems not to be without a deleterious effect towards their stability. A pH-dependent loss of potency takes place during autoclaving (Table I). The highest loss in potency takes place at pH-values below 5 and above 7. At the pH range of 5 to 7, isoniazid solutions are rather more stable during autoclaving.

The degradation of isoniazid in the investigated solutions during storage at 70° takes place according to first-order reaction kinetics (Fig. 1). However, at the pH-values 4, 4.5, 7.5and 8, the degradation rate is not constant throughout the whole storage period. At pH 4 and 4.5, the degradation rate proceeds faster during the first two weeks of storage than during the latter periods of storage. Simillarly the degradation rate at pH 7 and 8 are faster during the first three weeks than during the latter periods of storage.

It is worthy to note that the change in the degradation rate of isoniazid during storage is observed only at the pH-values below 5 and above 7, where the degradation of the drug proceeds at remarkably high velocity compared to that at pH 5-7. This change in the degradation rate during storage can be attributed to one or both of the following postulations: (a) Accumulation of the degratation

products in relatively high concentration might afford a stabilizing effect towards the drug. A more or less similar behavior was reported by kakemi et al [9] regarding the degradation of isonicotinic acid hydrazide sodium methanesulfonate.[b] It is reported by kakemi et al [5] that there is a difference in the pattern of degradation of isoniazid according to whether the degradation takes place under aerobic or under anaerobic condition, Under aerobic condition degradation results in the formation of isonicotinic acid, isonicotinamide and 1,2 diisonicotinoyl hydrazine in addition to other unidentified products, while under anaerobic condition the degradation results principally in isonicotinic acid and 1,2-diisonicotinoyl hydrazine. Thus, under the experimental condition the degradation of isoniazid in the sealed ampoule during the initial periods of storage proceeds under aerobic condition due to the presence of oxygen dissolved in the solution and in the head space of the ampoule and as this amount of oxygen in sealed ampoule is limited, it will be totally consumed after certain time and the degradation will then proceed as under anarobic condition and the degradation rate will be thus slowed.

Table II presents the observed first-order degradation rate constant in relation to pH-value. It is quite apparent from this table that the degradation rate constant for isoniazid in solutions at pH 4 and 4.5 during the first two weeks of storage(k_1)is four times the value for the rate constant(k_2) during the next ten weeks of storage. On the other hand, the degradation rate constant of isoniazid at pH 7.5 and 8 during the first three weeks of storage k_1 is two times the k-value during the next nine weeks.

It is obvious from Table II that the degradation rate of isoniazid is highly dependent on the pH-value of the solution, the highest k-values are observed at the pH ranges of 4 to 5 and 7 to 8. A plot of long K as a function of pH of the solution in the range of pH 5 to 7 (Fig.2) reveals a minimum k-value and hence optimum stability for isoniazid at pH 5.65. Below this optimum pH, the logarithm of the degradation rate constant for isoniazid increases linearly with decreasing the pH-value. Above pH 5.65, log k increases regularly as a function of the pH.

Table III presents the colour change of isoniazid solutions upon storage at 70°. It is clear from the table that all solutions suffered from progressive colour change to variable extent during storage, except those solutions at pH values 7.5 and 8 which remained colourless throught the storage time(12weeks). The most prominent colour change was observed in solutions at pH-values of 4.5, where the solution turned brownish orange to orange in colour. Solutions of pH-values of 5 to 7 exhibited a colour change to pale yellow or yellow. It could be observed that although there is a parallism between the extent of degradation as indicated by the chemical assay and the extent of discolouration in solutions of pH-values below 5 a reverse correlation was observed in solutions of pH-values above 5, that is solutions which showed excessive degradation by the chemical assay, solutions of pH-values 7.5 and 8, remained colourless. This could indicate that there is certain difference in the pattern of isoniazid degradation in acid and alkaline solutions .This also precludes the popular use of the extent of colour change as an index of the extent of isoniazid degradation.

It is also worthy to mention that in addition to the excessive discolouration experienced with solutions of pH-values 4.0 and 4.5, it was observed that these solutions, starting after the third week of storage, exhibit brownish crystal separation upon removing from the incubator and standing at room temperature for 24 hours.The period required for this type of crystallization in the sealed ampoules at room temperature is progressively decreased as the incubation period at 70° is increased. Starting after the seventh week of storage it was further observed that crystallization ceased to occur and instead a precipitate is rapidly separated upon opening the sealed ampoules. These phenomena of crystallization and precipitation are still under further investigation .

2-<u>Effect of buffer systems</u>:

Table IV comprises the value of the percentage loss in potency of isoniazid in the investigated buffer solutions at pH 5.5 during

autoclaving. Isoniazid solutions in Walpole's acetate, Sørensen's citrate and McIlvaine's citric acid-phosphate buffer system are more stable during autoclaving than solutions in Clark and Lub's phthalate and Sørensen's phosphate buffers.

Plotting the logarithm of the percentage retained isoniazid in the investigated buffer solutions as a function of storage time reveals a first-order degradation reaction (Fig.3). Table V presents the the first-order degradation rate constants k of isoniazid in the various buffer systems. It could be observed from Fig. 3 that the degradation rate of isoniazid in Sørensen's citrate buffer solution is lowered after the eight week of storage. The degradation rate constant for the drug in this buffer during the first 8 weeks of storage (K_1) is two times that during the latter storage period k_2, Table V . The change in the degradation rate takes place only in the Sørensen's citrate solution in which the degradation of isoniazid proceeds at a remarkably higher rate compared to that in the other buffer systems. This could be interpretted on similar basis as previously mentioned under effect of pH.

Table V also reveals that the stability of isoniazid in the Walpole' acetate, Clark and Lub's phthalate and Sørensen's phosphate buffer systems is much greater than in McIlvaine's citric acid-phosphate and Sørensen's citrate buffers. The Walpole's acetate buffer affords the highest stabilizing action for isoniazid followed by the Clark and Lub's phthalate buffer which is better than the sørensen's phosphat buffer. The differnce in the stability pattern of isoniazid in the various buffering systems can be attributed to a specific effect of the buffer components and or the effect of the ionic strength which is not equal in the various buffer systems used. Walpole's buffer being of the lowest ionic strength of the tested buffering system[5], showed the least extent of isoniazid degradation. However, further investigation of the effect of ionic strength will be presented in a next communication.

Table VI presents the colour changes of isoniazid solutions in the various buffering systems upon storage at 70°. From this table, it could be observed that the least discolouration occurs in Walpole's acetate and Clark and Lub's phthalate buffers. These buffers also showed the least extent of degradation as indicated by the chemical assay. However, isoniazid solution in Clark and Lub's phthalate buffer exhibits the crystal separation after ten weeks of storage.

Table I : Effect of pH on the Stability of Isoniazid Solutions During Autoclaving .

pH - Value	*Isoniazid Loss %*
4.0	7.56
4.5	4.29
5.0	1.26
5.5	3.21
6.0	1.86
6.5	2.97
7.0	3.51
7.5	4.80
8.0	5.01

Table II : Effect of pH on Degradation Rate Constants (K) of Isoniazid in Solutions Kept at 70^{o}.

pH - Value	*K Week^{-1}*
4.0	K_1 0.1819
	K_2 0.0428
4.5	K_1 0.0852
	K_2 0.0290
5.0	0.0303
5.5	0.0186
6.0	0.0196
6.5	0.0288
7.0	0.0441
7.5	K_1 0.7791
	K_2 0.0484
8.0	K_1 0.1113
	K_2 0.0473

Table III : Colour Change of Isoniazid Solutions at Different pH-Values[x] Upon Autoclaving and Storage at 70°

Storage Time	*pH* 4.0	4.5	5.0	5.5	6.0	6.5	7.0	7.5	8.0
Before autoclaving	pale yellow	pale yellow	tint yellow	colourless	colourless	colourless	colourless	colourless	colourless
After autoclaving	yellow	moderate yellow	very pale yellow	"	"	"	"	"	"
1 Week	straw yellow	moderate straw yellow	pale yellow	faint yellow	faint yelloz	"	"	"	"
2 Weeks	orange	straw yellow	yellow	pale yellow	very pale yellow	very pale yellow	"	"	"
3 Weeks	brownish orange	heavy straw yellow	straw yellow	yellow	moderate yellow	pale yellow	pale yellow	"	"
4 Weeks	"	orange	"	"	"	"	"	"	"
5 Weeks	"	"	"	"	"	"	"	"	"
6 Weeks	"	"	"	"	"	"	"	"	"
7 Weeks	"	"	"	"	"	"	"	"	"
8 Weeks	"	"	"	"	"	"	"	"	"
9 Weeks	"	"	"	"	"	"	"	"	"
10 Weeks	"	"	"	"	"	"	"	"	"

x McIlvaine citric acid-phosphate buffer is used allover.

Table IV : Effect of Buffer Systems (pH 5.5) on the Stability of Isoniazid Solutions During Autoclaving.

Buffer System	*Isoniazid Loss %*
Walpole's acetate	0.51
Clark and Lub's phthalate	2.62
Sørensen's phosphate	2.01
McIlvaine's citric acid-phosphate	1.15
Sørensen's citrate	1.02

Table V : Effect of Buffer Systems (pH 5.5) on Degradation Rate Constant (K) of Isoniazid in Solutions Kept at 70°,

Buffer System	*K week^{-1}*
Walpole's acetate	0.00806
Clark and Lub's phthalate	0.0092
Sørensen's phosphate	0.0115
McIlvaine's citric acid-phosphate	0.0184
Sørensen's citrate	K_1 0.0271
	K_2 0.0121

Table VI : Colour Change of Isoniazid in Various Buffer Systems (pH 5.5) Upon Autoclaving and Storage at 70°

Storage time	*Walpole's acetate*	*Clark and Lub's pht-late*	*Sørensen"s phosphate*	*McIlvaine's citric-acid phosphate*	*Sørensen's citrate*
before auto-oclaving	colourless	colourless	colourless	colourless	colourless
after aut-oclaving	very faint yellow	pale yellow	very aaint yellow	very faint yellow	very faint yellow
1 week	pale yellow	yellow	pale yellow	moderate yellow	moderate yellow
2 weeks	"	"	pale yellow	yellow	yellow
3 weeks	"	"	moderate yellow	yellow	yellow
4 weeks	"	"	"	"	"
5 weeks	"	"	"	"	"
6 weeks	"	"	"	"	"
7 weeks	"	"	"	"	"
8 weeks	"	"	"	"	"
10 weeks	"	"	"	straw yellow	straw yellow
12 weeks	"	"	"	"	"

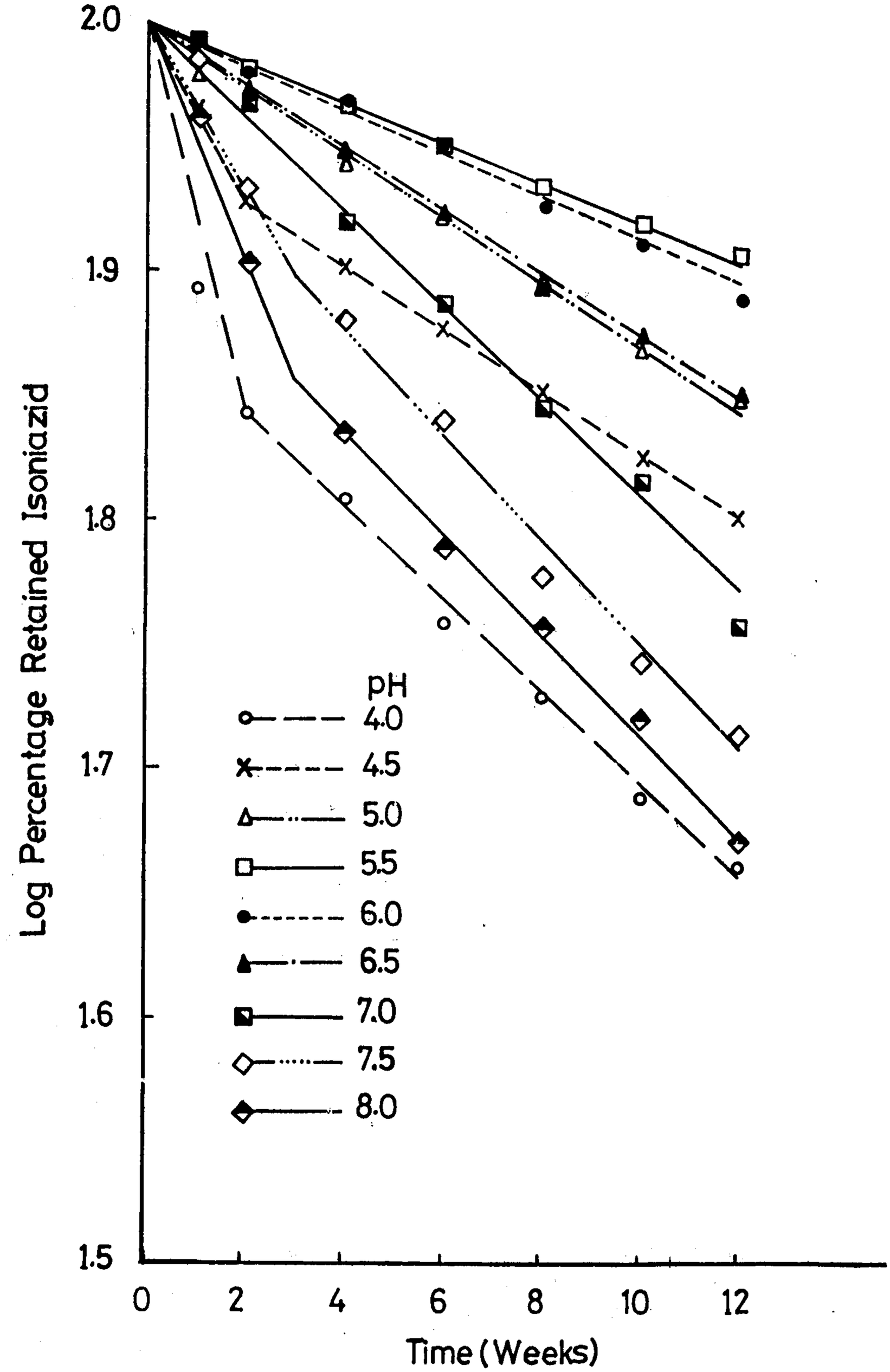
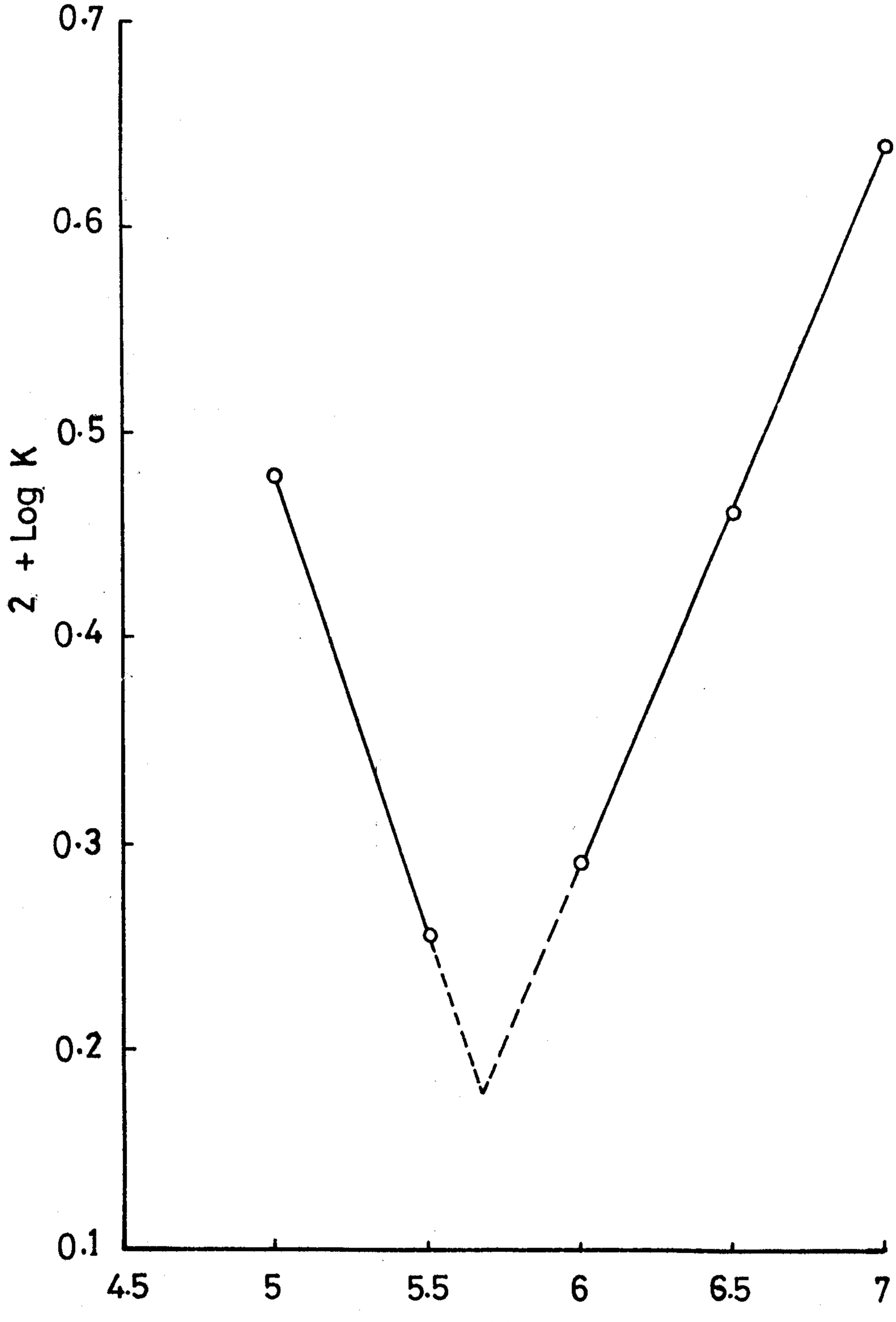


Fig.(1) Effect of pH on the Stability of Isoniazid Solutions Kept at 70°.



Fig(2) Degradation Rate Constant for Isoniazid as a Function of PH

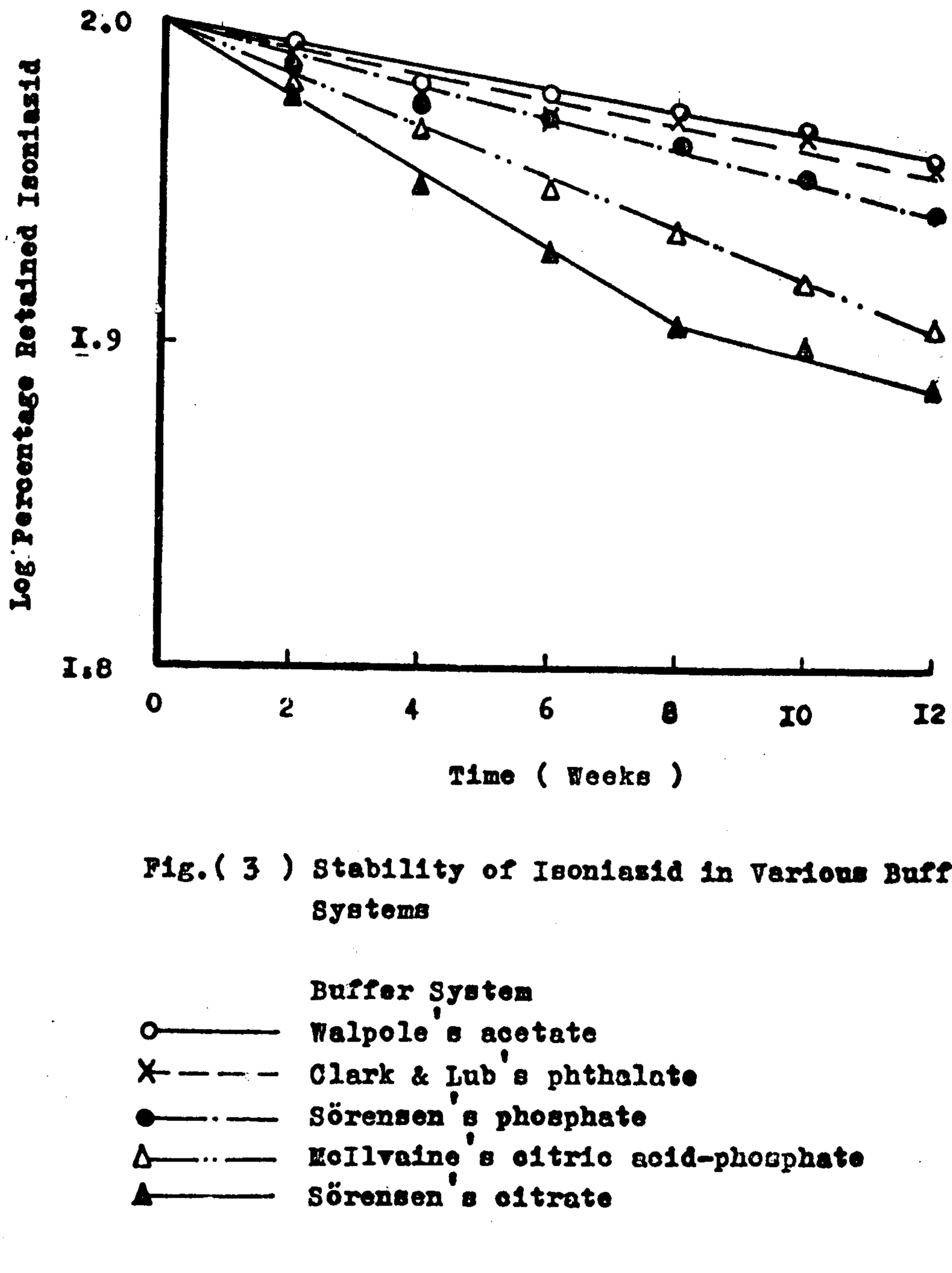


Fig.(3) Stability of Isoniasid in Various Buffer Systems

Buffer System

○——— Walpole's acetate

✕— — — Clark & Lub's phthalate

●—·— Sörensen's phosphate

△—··— McIlvaine's citric acid-phosphate

▲——— Sörensen's citrate

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دراسات على العوامل المؤثرة على نبات الايزونيازيد فى المحلول

١ــ تأثير قيمة الاس الايدروجينى ونظام الفرملة

السيد على ابراهيم ــ حسين عثمان حسن عطار ــ محمد جمال عبد المحسن
قسم الصيدلانيات ــ كلية الصيدلة ــ جامعة اسيوط

تم تتبع ثبات الايزونيازيد فى المحلول عند قيم مختلفة للاس الايدروجينى فى المدى من ٤ ــ ٨ وقد وجد أن تحلل الايزونيازيد عند القيم المختلفة للاس الايدروجينى تسير وفقا للنظام الاول المعدل التفاعل.

وأن الاس الايدروجينى ٦٥ر٥ هى القيمة لذرة ثبات الايزونيازيد هذا وقد لوحظ ان نمط الثبات الطبيعى للمحاليل لا يتوازى مع نمط الثبات الكيميائى.

ومقارنة ثبات الايزونيازيد فى انظمة فرملية مختلفة وهى خلات " والبول " ، وفيثالات كلارك ولوب ، وفوسفات سورانس ، وحمض الليمونيك ، فوسفات مالك الفان ، وليمونات سورانس عند أس ايدروجينى قيمته ٥ر٥ أتضح ان الايزونيازيد يكون اكثر ثباتا فى نظام خلات والبول الفرملى تتبعه حسب ورودها انظمة : فوسفات كلارك ولوب وفوسفات سورانس ، وحمض ليمونيك فوسفات مالك الفان ، ثم فى النهاية نظام ليمونات سورانس.