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STUDIES ON SOME FACTORS AFFECTING THE STABILITY OF RIFAMPICIN IN SOLUTION

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Rifampicin stability in relation to a number of factors namely; solvent type, pH value, type of buffering system, ionic strength and storage temperature was investigated. Kinetic treatment of the obtained data revealed first order pattern for rifampicin degradation. The degradation rate constant varied according to the type of the solvent, the pH of the solution, the type of the buffering components, the ionic strength and the storage temperature. Degradation was accelerated in solvents of higher dielectric constants in contrast to those of lower dielectric constants. The pH for minimal degradation was found to be pH 6. Rifampicin degradation proceeded at minimal rate in presence of ascorbate buffer compared to formate, acetate, phosphate, Sorensens phosphate and McIlvaine's citric acid-phosphate buffering systems . The calculated activation energy (Ea) and frequency factors of rifampicin was 15.47 K. cal/mol and 2.16 X 10^{9} day $^{-1}$ respectively

Rifampicin or rifampin is an antibiotic with a broad antibacterial spectrum which has been recommended for use in the treatment of pulmonary tuberculosis and the treatment of asymptomatic carriers of Neisseria meningitidis[1]. Rifampicin molecule[2,3] contains numerous function groups and linkages which form susceptible centers for degradation through oxidative and hydrolytic pathways. However, reports on the stability of rifampicin are scarce[3-7] and not complete. It has been reported that rifampicin undergoes rapid degradation in aqueous solution with the formation of 3-formyl rifamycin SV in acidic pH and rifampicin quinone at pH value above neutrality[3,6,7]. Berza[5] reported the formation of two degradation products other than rifampicin-quinone and 3-formyl rifamyicin SV which were separated as yellow and

orange brown compounds on TLC from degraded sample of rifampicin solution. 25-deacetyl rifampicin was reported to be formed as the major metabolic product of rifampicin in human[8].

The objective of the work hereby presented is to investigate the stability of rifampicin in relation to a number of factors namely solvent type, pH value, type of buffering system ionic strength and ambient temperature. It is assumed that information gained from this investigation will be highly valuable in predicting the stability of rifampicin in pharmaceutical formulations.

EXPERIMENTAL

Materials :

Rifampicin powder (1000 mcg/mg), provided by Lepetit laboratory. Analytical or pharmaceutical grade of; dimethylformamide, acetone, chloroform, ethyl acetate, methanol, benzene, hydrochloric acid, sodium hydroxide, ascorbic acid, acetic acid, citric acid, oxalic acid, disodium hydrogen phosphate, potassium dihydrogen phosphate, sodium chloride, ethylene diaminetetraacetic acid disodium salt, and urea. Chromatographic papers (Whatman No. 1).

Equipment :

Circulating hot air oven thermostatically controlled (± 1°C), Spectrophotometer (Spectromom 204).

pH meter (pH-meter Titrimetre U9N, Selea Lyon).

Procedure :

2 mg/ml rifampicin solution is prepared in the appropriate testing liquid. The solution is then filtered, distributed into ampoules (2ml. capacity), sealed and stored in constant temperature hot air oven at the appropriate temperature. Duplicate samples are withdrown at the begining of the experiment and at appropriate time interval and assayed for intact rifampicin applying the previously developed paper chromatographic stability indicating assay of rifampicin[9].

The testing liquids investigated include:

(A) Formamide, dimethylformamide, acetone, chloroform, ethylacetate, methanol and benzene.

(B) Water containing 20% v/v dimethylformamide, adjusted by the use of 0.1 N HCl or 0.1 N NaOH to pH values of ; 3,4,5,6,7,8,9 and 10

(C) Water containing 20% v/v dimethylformamide and adjusted to pH 6 using 0.1 N HCl and/or 0.1 N NaOH and to ionic strength values of ;0.4, 0.8, 1.2 and 1.6 using sodium chloride.

(D) 20% v/v dimethylformamide in each of; ascorbate, acetate, formate, Sørensen's phosphate [10] , McIlvaine's citric acid phosphate[10] and phospahate[11] buffers at pH 6. The ascorbate, acetate, and formate buffers were prepared by titrating 0.2 molar of sodium hydroxide against 0.2 molar of the corresponding acid until pH 6 was achieved.

RESULTS AND DISCUSSIONS

The stability of rifampicin solution in different solvents is illustrated in Figure 1. The solvents were chosen to represent a wide spectrum of polarity or dielectric constant [12]. The figure depicts that the degradation of rifampicin in all the tested solvents proceeds according to first-order reaction kinetic However, the degradation rate differs according to the type of the solvent. Table I presents the calculated first-order reaction rate constants and half-life times of rifampicin degradation in the various solvents. It is obvious from both Figure 1 and Table I that the degradation of rifampicin is more rapid in dimethylformamide followed in order by; formamide, methanol, 1,2 propylene glycol, acetone, ethyl acetate, chloroform, and the slowest degradation rate accurred in benzene. It could be observed that degradation of rifampicin is more accelerated in solvents of higher dielectric constant in contrast to that in solvents of lower dielectric constant and the arrangement of solvents according to their effect on the reaction rate constant of rifampicin degradation could be demonstrated to have same pattern of arrangement according to their dielectric constants. This indicates that the degradation rate of rifampicin could be correlated to the dielectric constant of the solvent.

Figure 2 illustrates the stability of rifampicin in aqueous solutions containing 20% dimethylformamide and adjusted to different pH values. It is obvious from this figure that the degradation of rifampicin in these solutions follows

first order kinetics and the degradation rate is dependent on the specific pH of the solution. Figure 3 illustrates the pH-rate profile of rifampicin degradation. It is obvious from both figures, 2 and 3, that rifampicin degradation is catalyzed by both hydrogen and hydroxyl ions. The pH of minimal degradation appears to be pH 6, while extreme pH values on both sides are associated with excessive rifampicin.

The stability of rifampicin solutions in blends of 20% v/v of dimethylformamide with buffer solution at pH 6 is illustrated in Figure 4. The buffering systems used comprise, ascorbate , acetate, formate, McIlvaine's citric acid phosphate, Sørensen's phosphate and phosphate buffers. It could be observed from this figure that the first order degradation rate of rifampicin is quite affected by the composition of the buffering system used in the preparation of the solution. Table II presents the reaction rate constants and half-life times for rifampicin degradation. It is quite apparent that rifampicin degradation proceeds with comparatively rapid rate in McIlvaine's citric acid-phosphate followed in order by Sørensen's phosphate, phosphate, acetate, formate and finally ascorbate buffer which showed the slowest degradation rate. However, it is worthy to note that the degradation rate in the control solution in which the pH was adjusted merely with sodium hydroxide and/or hydrochloric acid, was much slower compared to all solutions with the buffering systems tested. This could be attributed to the comparatively high ionic strength in buffered solutions which resulted in accelerated rifampicin degradation.

The variable pattern of stability of rifampicin solutions in relation to the type of the buffering system could be attributed to a specific effect of the buffering components and/or the effect of ionic strength of the buffering solution which could be shown to be different among the buffering systems used.

As it was reported that rifampicin molecule is a zwitter ion that is it can be positively or negatively charged according to the pH of the solution, it was expected that the ionic strength of the solution could influence the degradation rate of such charged molecule. To varify this assumption, sodium chloride was used

to adjust rifampicin solutions to various ionic strengths and the stability was then follwed up. Figure 5 illustrates the effect of ionic strength in the solution upon the stability of rifampicin. Table III presents the first order reaction rate constants and half-life times of rifampicin degradation in relation to the ionic strength of the solution. It is quite apparent from Figure 5 and Table III that the ionic strength in the solution markedly affects the degradation rate of rifampicin. Increasing the ionic strength in the solution resulted in an acceleration of rifampicin degradation.

The stability of rifampicin in solution in relation to the storage temperature is illustrated in Figure 6. The first order reaction rate constants and half-life times of rifampicin degradation in solution of pH 6 upon storage at different temperatures are presented in Table IV. It is obvious from both the figure and the table that rifampicin degradation is accelerated as the temperature of storage is increased. This indicates that the degradation of rifampicin is an endothermic reaction. Applying the Arrhenius equation on the obtained data, it was possible to calculate the activation energy(Ea) and the frequency factor for rifampicin degradation and were found to be 15.47 K Cal./mol^{-1} and 2.16×10^{9} day $^{-1}$ respectively.

Table I: Degradation Rate Constants and Half-Lives of Rifampicin in Different Solvents Stored at 40°.

SOLVENT	*K(day^{-1})*	*t 1/2 (day)*
Benzene	$710X10^{-5}$	97.60
Chloroform	$819X10^{-5}$	84.61
Ethyl acetate	$914X10^{-5}$	75.82
Acetone	$1405X10^{-5}$	49.32
Propylene Glycol	$2164X10^{-5}$	32.02
Methanol	$2465X10^{-5}$	28.11
Formamide	$3934X10^{-5}$	17.59
Dimethylformamide	$6015X10^{-5}$	11.52

Table II : Degradation Rate Constants and Half-Lives of Rifampicin in Solutions at pH 6 in Presence of Different Buffer Systems, Stored at 40°.

Component	*K(day)$^{-1}$*	*t 1/2 (day)*
Blank	$3680X10^{-5}$	18.83
Ascorbate Buffer	$4513X10^{-5}$	15.35
Formate Buffer	$6162X10^{-5}$	11.24
Acetate Buffer	$7982X10^{-5}$	8.68
Phosphate Buffer	$9574X10^{-5}$	7.23
Sørensen's Buffer	$12588X10^{-5}$	5.50
McIlvaine's Buffer	$14435X10^{-5}$	4.80

Table III : Degradation Rate Constants and Half-Lives of Rifampicin Solutions at pH 6 in Presence of Different Molar Concentrations of Sodium Chloride and Stored at 40°

Molar concentration of sodium chloride	*K (day^{-1})*	*t ½ (day)*
0.0	3182 X 10^{-5}	21.77
0.4	10248 X 10^{-5}	6.76
0.8	13638 X 10^{-5}	5.08
1.2	16319 X 10^{-5}	4.24
1.6	19653 X 10^{-5}	3.52

Table IV : Degradation Rate Constants and Half-Lives of Rifampicin in Solutions at pH 6 Stored at Different Temperatures.

Rifampicin solution stored at	*K (day^{-1})*	*t ½ (day)*
5°	1623 X 10^{-5}	42.68
15°	2386 X 10^{-5}	29.02
40°	3417 X 10^{-5}	20.27
70°	30081 X 10^{-5}	2.30

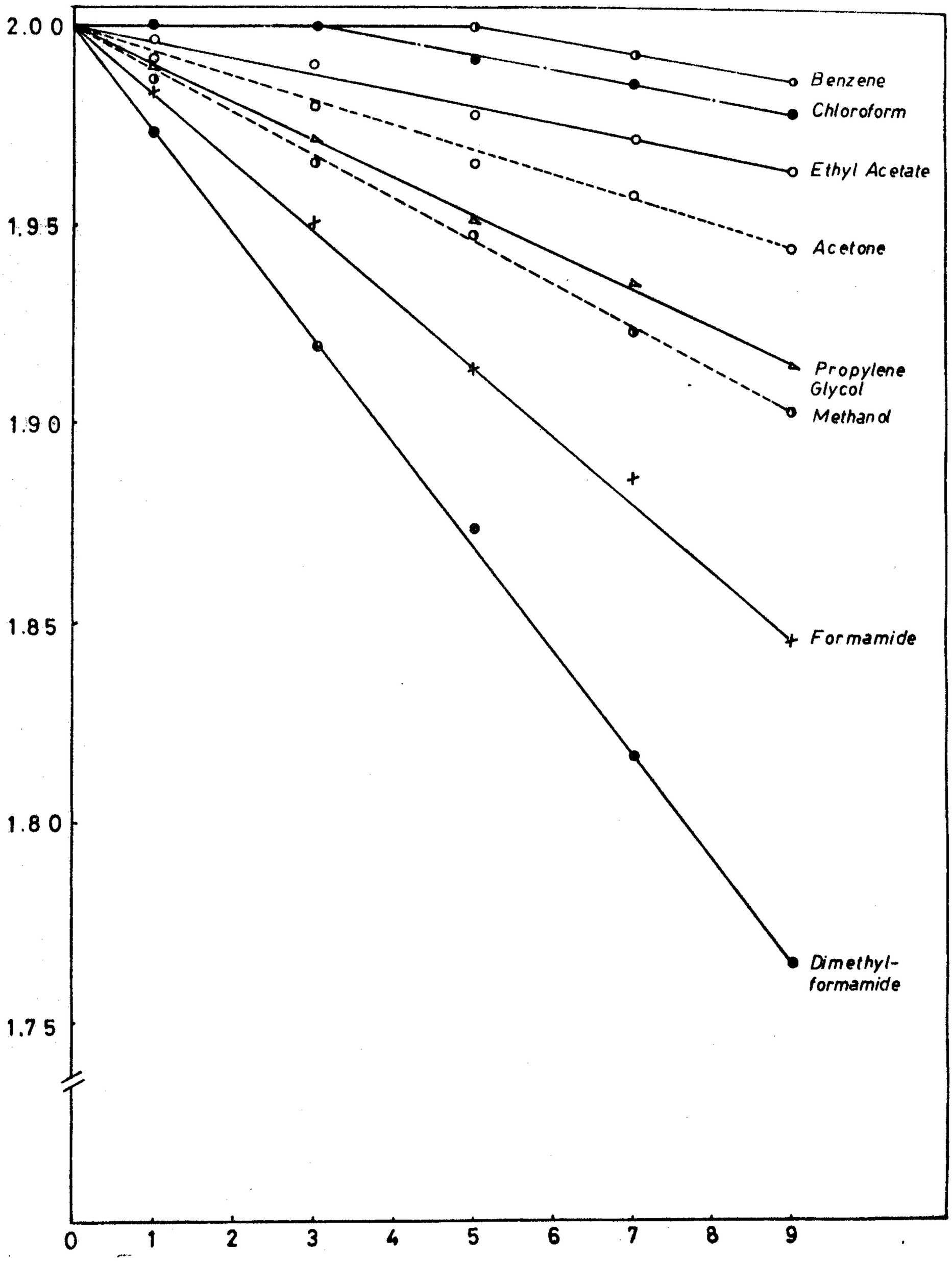

Fig. 1 Stability Of Rifampicin In Different Solvents At 40°

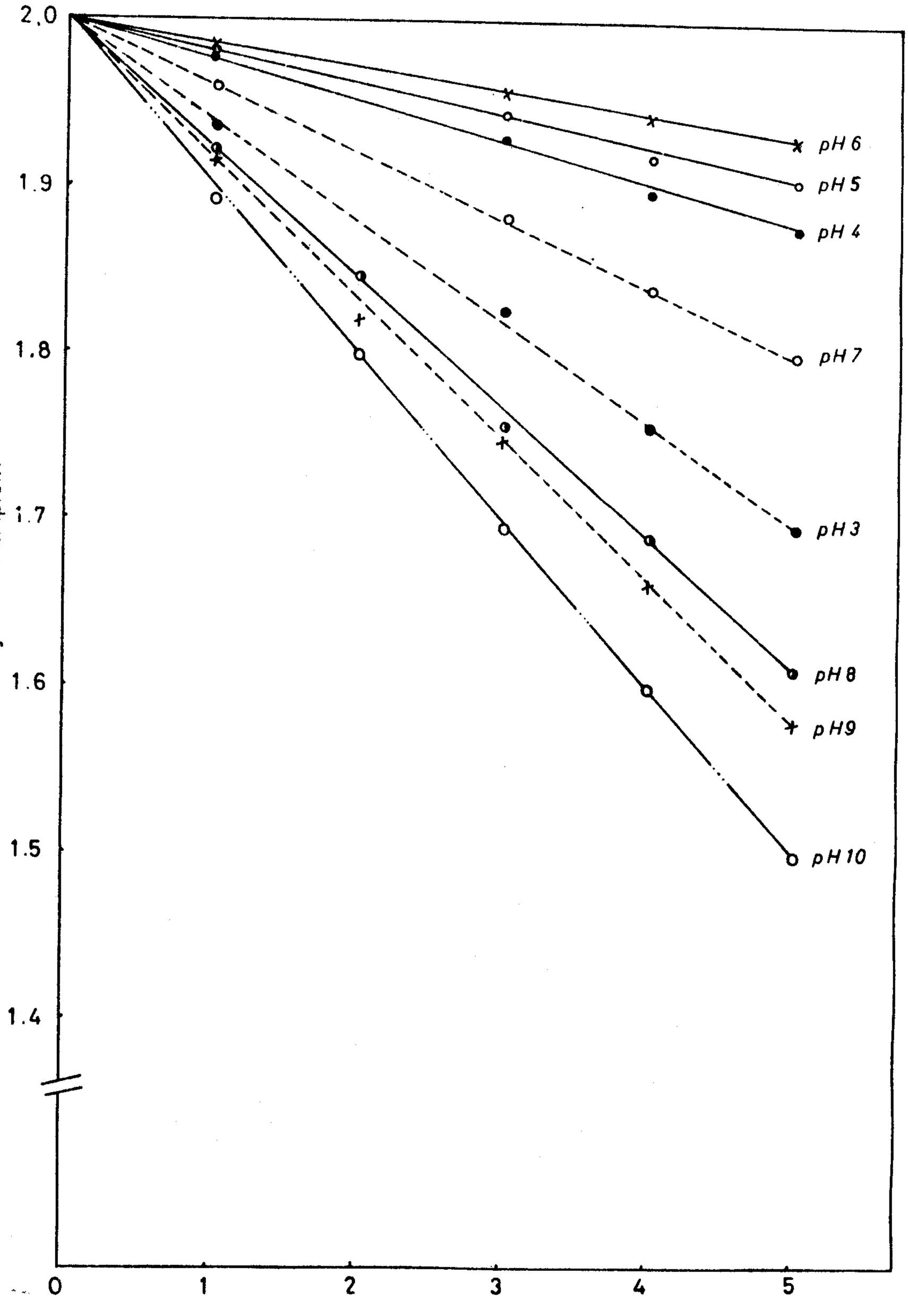

Fig. 2 Stability Of Rifampicn In Aqueous Solution Containing 20% v/v Dimethylformamide At Different pH Values

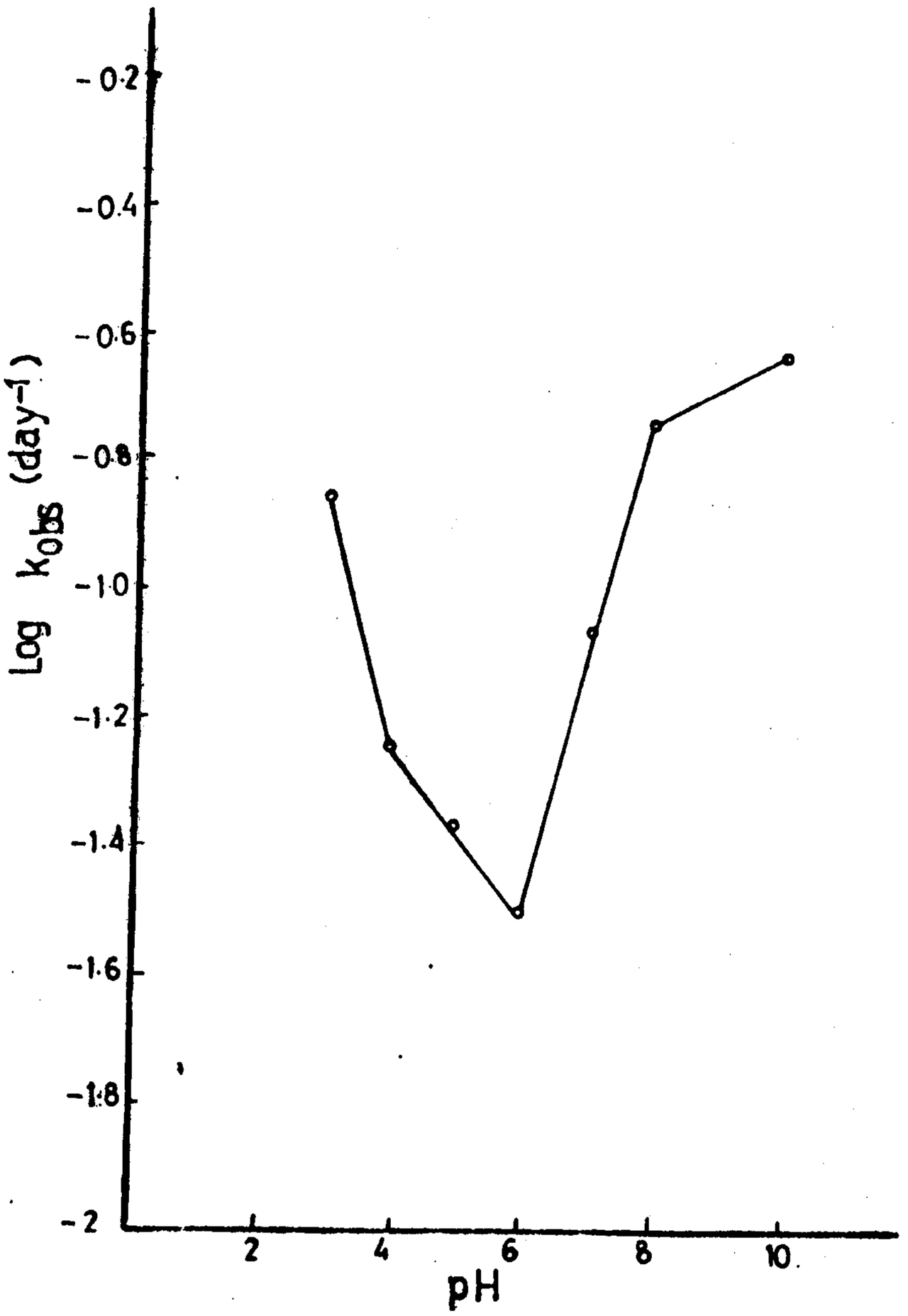

Fig. 3 pH-Rate Profile Of Rifampicin Degradation at 40°

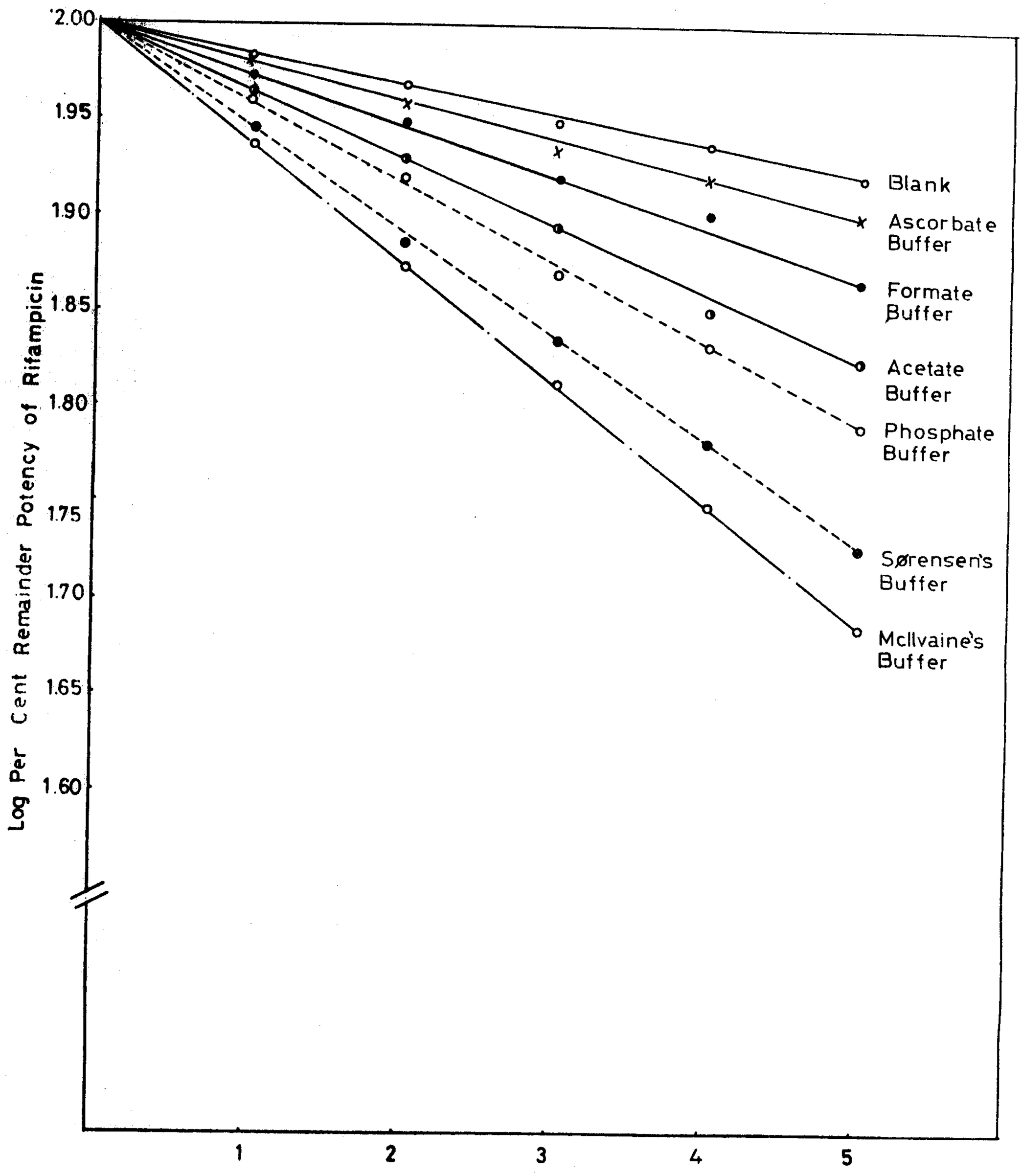

Fig. 4 Stability Of Rifampicin In Different Buffer Systems Containing 20% v/v Dimethylformamide At pH 6 and 40°

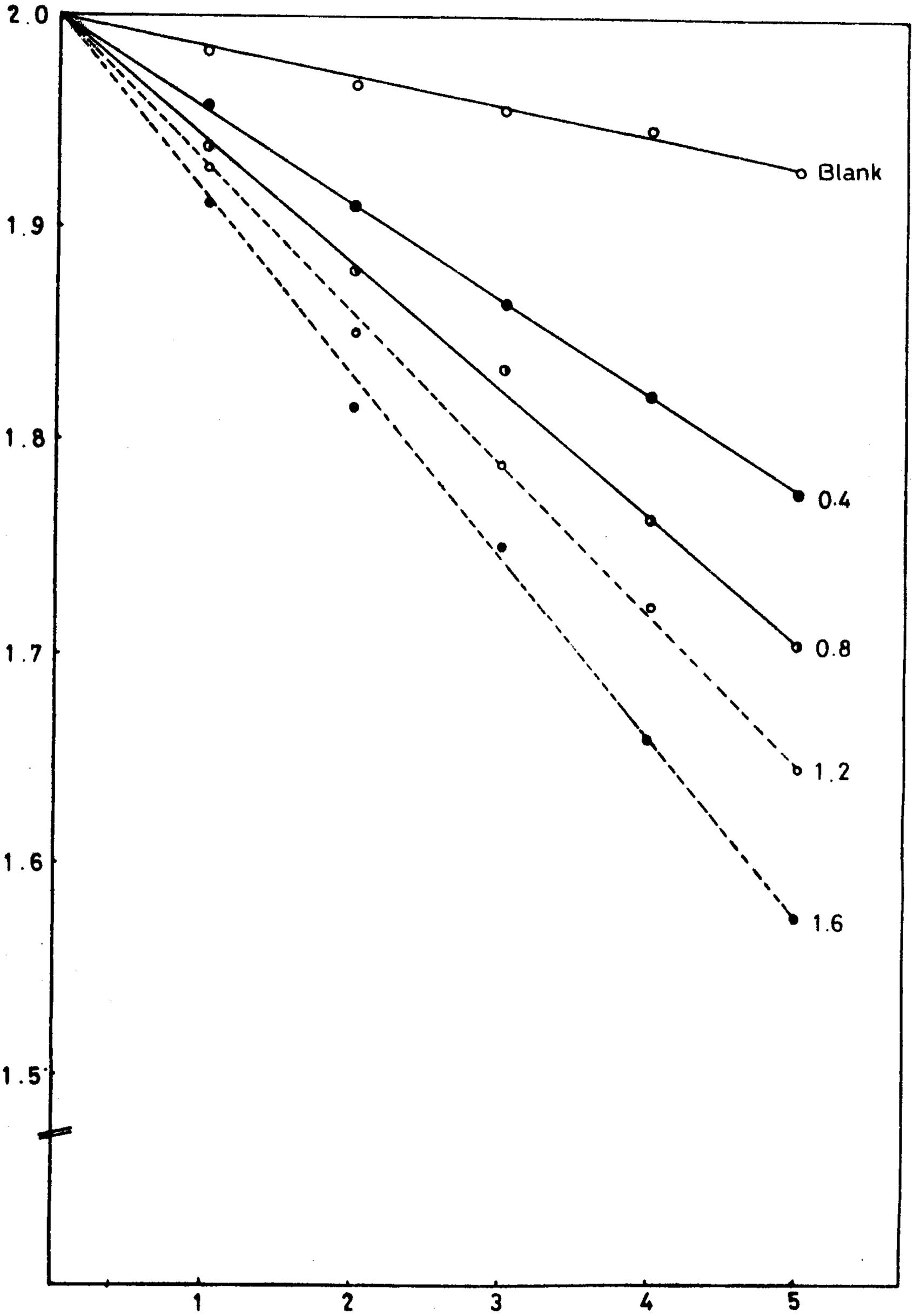

Fig. 5 Effect Of Ionic Strength On The Stability Of Rifampicin Aqueous Solution Containing 2o% v/v Dimethylformamide At pH 6 and 40^{o}

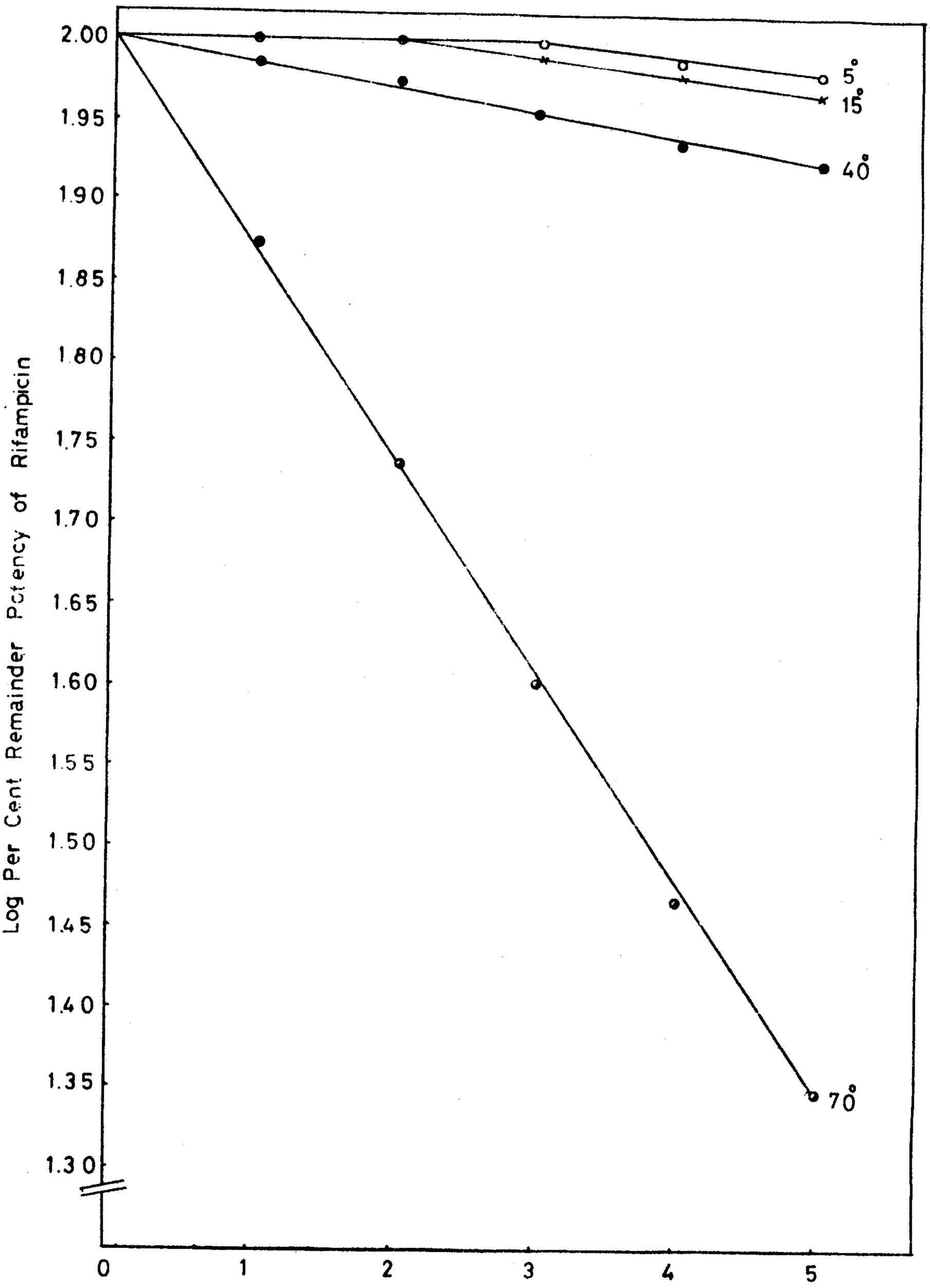

Fig. 6 Effect Of Storage Temperature On The Stability Of Rifampicin In Aqueous Solution Containing 20% v/v Dimethylformamide At pH 6

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دراسات على بعض العوامل المؤثرة على ثبات الريفاميسين فى المحلول

السيد على ابراهيم ـ على على قاسم ـ اسماعيل عطيه ـ سيد اسماعيل محمد

قسم الصيدلانيات ـ كلية الصيدلة ـ جامعة اسيوط

تمت دراسة ثبات الريفاميسين تحت تاثير عدد من العوامل وهى نوع المذيب وقيمة الاس الايدروجينى ونوع النظام الفرملى والقوة الايونية ودرجة حرارة التخزين •

وعند معالجة النتائج حركيا وجد ان تحلل الريفاميسين يخضع للنظام الاول للتفاعل فى كل الحالات وان ثابت معدل التفاعل يتغير طبقا لنوع المذيب وقيمة الاس الايدروجينى للمحلول ونوع المكونات للنظام الفرملى والقوة الايونية بالمحلول ودرجة حرارة التخزين •

وقد كان تحلل العقار اسرع فى المذيبات ذات ثابت العزل الكهربائى العالى بالمقارنة بالمذيبات ذات ثابت العزل الكهربائى المنخفض كذلك فقد وجد ان الاس الايدروجينى لاقل معدل تحلل يكون عند قيمة قدرها ٦ وأن معدل التحلل يكون اقل فى وجود نظام الاسكوربات المفرمل بالمقارنة بنظام الخلات والفوسفات وفوسفات سورانس وحمض ليمونيك وفوسفات ماك الفان وقد تم حساب قيمة الطاقة المنشطة وعامل التردد للريفاميسين وجد انه ١٥٫٤٧ ك يسعر للجزئى ٢٫١٦ × ١٠ فى اليوم على التوالى •