CHAPTER 3

STABILITY STUDIES

3.1 INTRODUCTION

Although the pharmaceutical industry has long been aware of the importance of stability tests in product development, it has only been with approximately the last 25 years that major studies have been made in this area. The empirical approach to stability testing has been replaced by scientific considerations. The applications of certain physicochemical principles in the performance of stability studies has proved to be of considerable advantage in the development of stable dosage forms. Only through this approach is it possible to accurately and adequately make use of data obtained from exaggerated storage conditions for the purpose of predicting the normal shelf life of the product.

The major test for validating any newly developed analytical method is to prove it to be stability indicating i.e. the method is selective for the drug alone and not its degradation products which in most cases are structurally similar.

The aim of this study was to validate the proposed analytical methods developed for 5FU, MTX and cyclophosphamide (chapter 2) by studying the pH-temperature rate profile of these drugs by the proposed methods and comparing the data obtained with the reported method.

Mathematical Model :

The stability profile of these three drugs is well established (1-3). All these chemotherapeutic agents exhibit first order degradation in aqueous solutions which is mainly

pH and temperature dependent. The first order degradation of the drug in aqueous solution is given by the equation.

$$\log C = \log Co - \frac{K_1 t}{2.303} \qquad \ldots\ldots\ldots(1)$$

Where C = Concentration of drug at time t.

Co = original concentration of the drug.

K_1 = stability constant.

The plot of log C vs t will be linear with slope = $\frac{K}{2.303}$ yielding the stability constant.

The half life (t1/2) is given by the equation

$$t\ 1/2 = \frac{0.693}{K_1} \qquad \ldots\ldots\ldots(2)$$

While the shelf life, t90 is given by the equation

$$t_{90} = \frac{0.105}{K_1} \qquad \ldots\ldots\ldots(3)$$

The effect of temperature on stability kinetics is explained on the basis of the activation energy.

This is given by the equation :

$$\log K = \log A - \frac{Ea}{2.303\ RT} \qquad \ldots\ldots\ldots(4)$$

Where K = reaction rate constant.

T = temperature in absolute scale.

A = constant.

Ea = activation energy constant.

The graph of log k vs $\frac{1}{T}$ is linear with a slope of $-\frac{Ea}{2.303\ R}$

This type of graph is known as the Arrhenius plot and can be used to determine the Ea value.

3.2 EXPERIMENTAL

3.21 Materials :

Potassium chloride, disodium hydrogen phosphate, potassium dihydrogen phosphate, ferric chloride hexahydrate, boric acid, cobalt acetate, hydrochloric acid (concentrated), chloroform, ehtylacetate, isopropanol, methanol (Qualigens, India), sodium hydroxide pellets, sulfanilic acid, sodium nitrite, ammonium thiocyanate (E. Merck, India), Folin-ciocalteau reagent, isopropylamine (s.d. fine chemicals, India), emulsifying wax, liquid paraffin, white petroleum jelly, cetomacrogol emulsifying wax, white soft paraffin (National Chemicals, India), HPMCK4M (Colorcon, USA), carbopol 941 (BASF, USA), chlorocresol, phenyl mercuric nitrate (BDH, India), purified water I.P.

3.22 Buffer Solutions :

The following I.P buffer solutions were used : Hydrochloric acid buffer pH 1.2 and 2.0, acetate buffer pH 3.9 and 5.0, phosphate buffer pH 6.0, 7.0, 7.4 and 8.0, alkaline borate buffer pH 9.0 and 10.0.

3.23 Stability Studies for 5FU :

It is reported that 5FU is quite stable in acid solutions, showing no hydrolysis. It undergoes hydrolysis in alkaline solution with the probable formation of barbituric acid which rapidly degrades to other products like urea, fluoride and an aldehyde. Some of the urea formed on hydrolysis, reacts further, giving ammonia and carbon

dioxide(1). The shelf life of aqueous solutions of 5FU is about 3 years at pH9 and room temperature.

In the present investigation stability studies have been conducted at pH 7.0, 8.0, 9.0 and 10.0 at controlled room temperature (CRT, i.e., 25°C), 45°C and 60°C.

3.231 Preparation of drug solutions :

For preparation of drug solution in all the buffers, (i.e. pH 7,8,9 and 10) a common procedure was adopted.

100mg of 5FU was carefully weighed and transferred into separate 100ml volumetric flasks. It was dissolved, made upto volume with the respective buffer solution and filtered. 5ml of drug solutions were filled in amber coloured ampoules, sealed and stored at 3 different temperatures viz. controlled room temperature (25°C), 45°C and 60°C. The samples were assayed for initial concentration by UV spectrophotometry (standard method) and by the proposed colorimetric methods viz. by complexation with metal ions (cobalt) and by coupling with diazotised primary amines (diazotised sulfanilic acid) using the procedure given under method of analysis for stability studies. At appropriate time intervals upto 90 days, samples were withdrawn and estimated for intact drug by the procedure given under method of analysis for stability studies. All sample withdrawals were carried out in triplicate.

3.232 Methods of analysis for stability studies :

a) UV Spectrophotometric method :

An aliquot of the drug solution (0.1ml) was transferred using a micropipette into separate 10ml

volumetric flasks and diluted with respective buffers to volume and the absorbance was measured at 266nm against the appropriate buffer blank.

b) Proposed colorimetric methods :

(i) Complexation with cobalt acetate :

An aliquot of the drug solution (2ml) was transferred into a separating funnel, about 2ml of water was added, pH of the solution was adjusted to 7 with a few drops of glacial acetic acid and successively extracted thrice with 10,5,5ml of ethyl acetate : isopropanol solvent mixture (7:3). The organic extract was pooled, treated with anhydrous sodium sulphate, filtered and then evaporated to dryness on a water bath. Residue was dissolved in 10ml of chloroform-methanol (3:2) mixture. Colour was developed with a suitable aliquot (1.25ml) of the drug solution as per the procedure given under preparation of calibration curve for 5FU-cobalt complex (chapter 2, section 2.224d).

(ii) Coupling with diazotised sulfanilic acid :

An aliquot of the drug solution (0.5ml). was transferred to 10ml volumetric flasks and colour was developed as per the procedure given under preparation of calibration curve for 5FU-diazotised sulfanilic acid (chapter 2 section 2.233e).

3.233 Results and discussion :

The data of the percentage drug remaining at each sampling time point obtained by various methods at different pH and temperatures are recorded in Tables 3.1-3.4. The mean

TABLE 3.1

STABILITY DATA OF 5FU AT pH 7.0

Time in days	% Drug remaining undegraded (± S.D.)								
	25°C			45°C			60°C		
	UV method at 266nm	Cobaltacetate method	Diazotised sulfanilic acid method	UV method at 266nm	Cobaltacetate method	Diazotised sulfanilic acid method	UV method at 266nm	Cobaltacetate method	Diazotised sulfanilic acid method
0	100.00 (-)	100.00 (-)	100.00 (-)	100.00 (-)	100.00 (-)	100.00 (-)	100.00 (-)	100.00 (-)	100.00 (-)
1	100.00 (0.011)	100.00 (0.132)	100.00 (0.451)	99.98 (1.259)	99.92 (1.233)	99.92 (1.549)	99.79 (1.097)	99.68 (1.578)	99.65 (1.059)
3	100.00 (0.952)	100.00 (1.234)	100.00 (1.395)	99.93 (2.017)	99.91 (0.795)	99.91 (1.934)	99.39 (1.097)	99.31 (1.386)	99.29 (1.286)
7	99.99 (0.991)	99.93 (1.354)	99.95 (0.934)	99.84 (1.485)	99.79 (1.795)	99.76 (1.605)	98.59 (1.779)	98.51 (1.342)	98.50 (1.049)
15	99.98 (1.394)	99.90 (1.054)	99.91 (1.239)	99.66 (1.654)	99.65 (0.933)	99.64 (1.469)	97.01 (1.119)	96.82 (2.013)	96.82 (1.389)
30	99.97 (1.952)	99.77 (1.709)	99.82 (1.289)	99.33 (1.395)	99.31 (0.395)	99.19 (1.113)	94.12 (2.321)	93.86 (1.836)	93.88 (2.954)
60	99.95 (1.594)	99.65 (1.569)	99.77 (1.019)	98.66 (1.323)	98.51 (1.589)	98.40 (1.391)	88.57 (1.293)	88.41 (1.001)	88.31 (1.315)
90	99.92 (1.119)	99.54 (1.495)	99.77 (1.732)	98.04 (1.045)	97.84 (2.011)	97.72 (1.393)	83.37 (1.567)	83.27 (1.017)	83.18 (1.299)

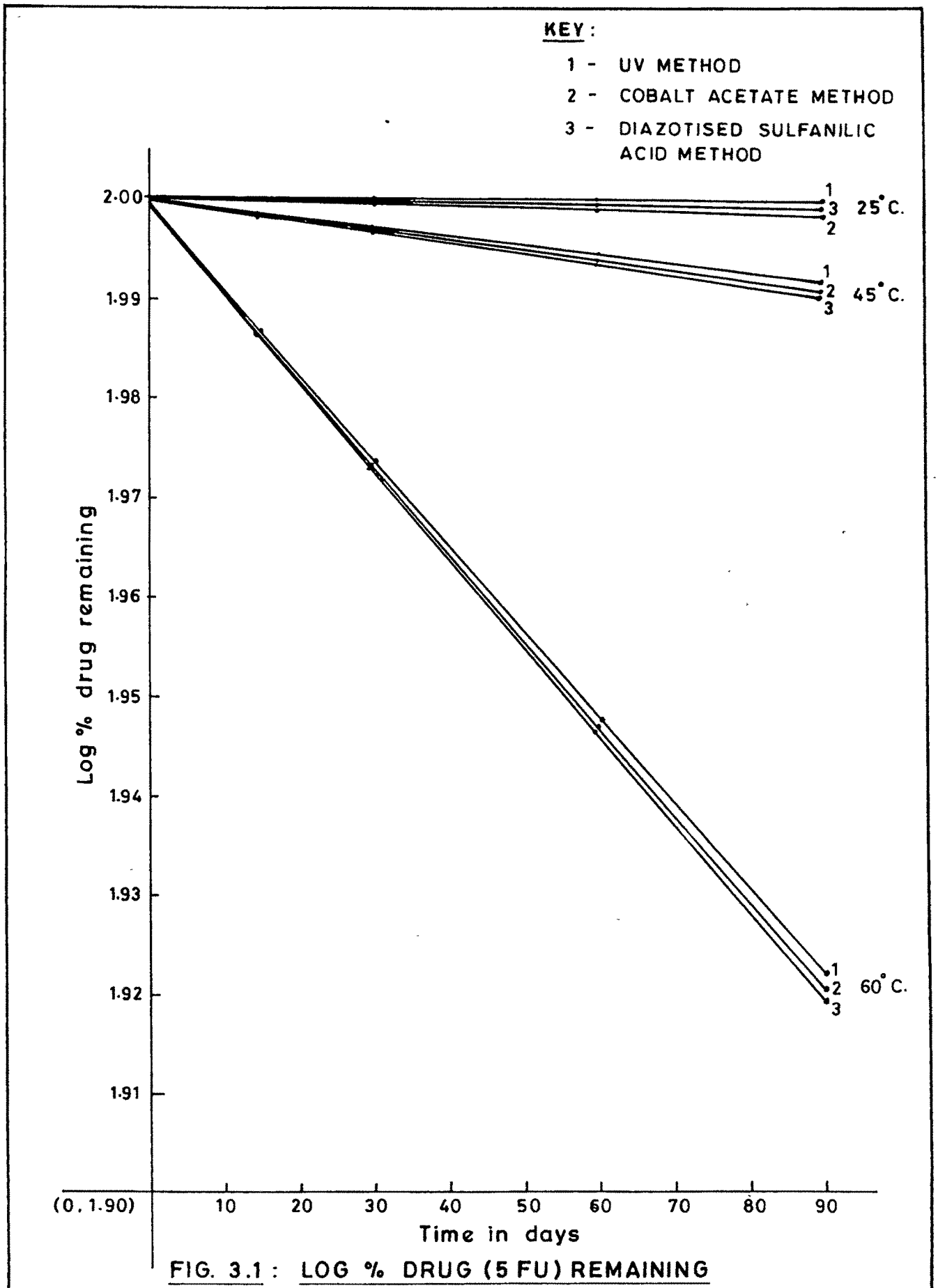

FIG. 3.1 : LOG % DRUG (5 FU) REMAINING AS A FUNCTION OF TIME AT pH 7·0

TABLE 3.2

STABILITY DATA OF 5FU AT pH 8.0

Time in days	% Drug remaining undegraded (± S.D.)								
	25°C			45°C			60°C		
	UV method at 266nm	Cobaltacetate method	Diazotised sulfanilic acid method	UV method at 266nm	Cobaltacetate method	Diazotised sulfanilic acid method	UV method at 266nm	Cobaltacetate method	Diazotised sulfanilic acid method
0	100.00 (-)	100.00 (-)	100.00 (-)	100.00 (-)	100.00 (-)	100.00 (-)	100.00 (-)	100.00 (-)	100.00 (-)
1	100.00 (0.132)	100.00 (0.079)	100.00 (0.112)	99.95 (1.312)	99.93 (0.395)	99.87 (1.333)	99.66 (0.975)	99.58 (1.853)	99.75 (0.795)
3	99.99 (0.993)	99.98 (1.012)	99.99 (0.595)	99.86 (1.935)	99.87 (1.258)	99.76 (0.955)	98.99 (1.323)	98.75 (0.879)	99.31 (1.112)
7	99.99 (0.873)	99.97 (1.378)	99.99 (1.113)	99.68 (1.878)	99.65 (1.569)	99.66 (2.011)	97.66 (1.670)	97.57 (2.232)	98.06 (1.118)
15	99.97 (1.379)	99.93 (2.121)	99.87 (0.754)	99.31 (1.394)	99.23 (0.995)	99.28 (1.392)	95.38 (1.979)	95.35 (1.591)	95.49 (1.499)
30	99.95 (1.059)	99.83 (1.389)	99.78 (1.833)	98.62 (0.789)	98.40 (1.048)	98.42 (1.249)	90.38 (1.038)	90.78 (0.778)	90.36 (1.036)
60	99.92 (1.235)	99.54 (1.452)	99.51 (1.059)	97.26 (1.623)	96.83 (1.369)	97.16 (1.116)	81.69 (1.759)	81.32 (1.323)	82.04 (1.420)
90	99.87 (1.732)	99.54 (1.054)	99.43 (1.349)	95.92 (0.882)	95.28 (2.275)	95.49 (0.995)	73.83 (1.349)	74.13 (2.113)	73.45 (0.956)

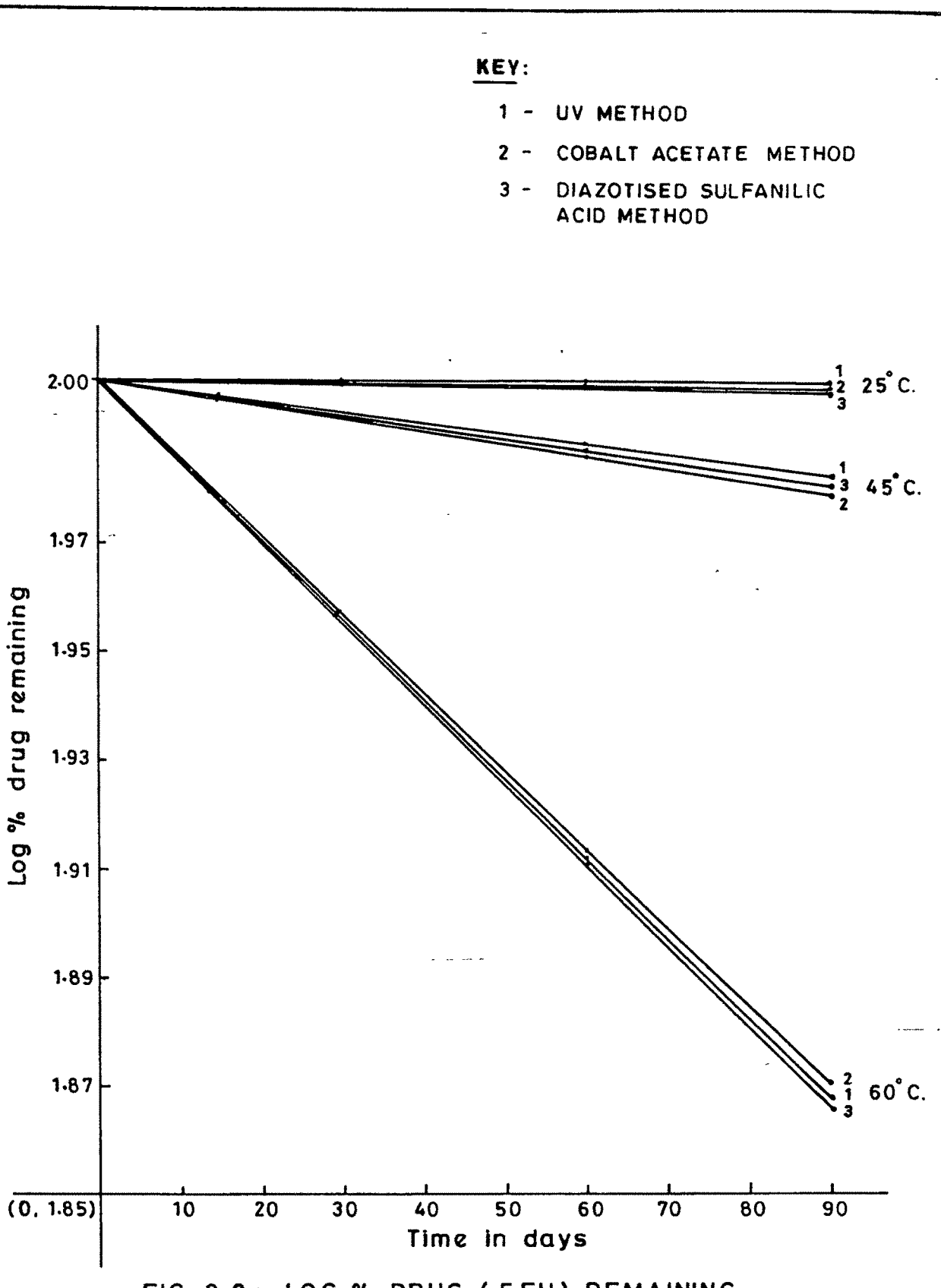

FIG. 3.2: LOG % DRUG (5FU) REMAINING AS A FUNCTION OF TIME AT pH 8·0

TABLE 3.3

STABILITY DATA OF 5FU AT pH 9.0

Time in days	% Drug remaining undegraded (± S.D.)								
	25°C			45°C			60°C		
	UV method at 266nm	Cobaltacetate method	Diaz. Sulfanilic acid method	UV method at 266nm	Cobaltacetate method	Diaz. Sulfanilic acid method	UV method at 266nm	Cobaltacetate method	Diaz. Sulfanilic acid method
0	100.00 (-)	100.00 (-)	100.00 (-)	100.00 (-)	100.00 (-)	100.00 (-)	100.00 (-)	100.00 (-)	100.00 (-)
1	99.99 (1.352)	100.00 (0.105)	99.92 (2.102)	99.85 (1.531)	99.78 (0.512)	99.76 (1.712)	99.15 (1.052)	98.27 (3.102)	97.12 (2.432)
3	99.97 (2.105)	99.98 (0.952)	99.85 (1.305)	99.55 (2.112)	99.25 (1.012)	99.24 (2.213)	97.47 (1.056)	96.82 (1.491)	95.95 (1.232)
7	99.92 (1.573)	99.45 (1.352)	99.37 (1.112)	98.95 (1.978)	98.62 (1.932)	98.40 (0.559)	94.19 (1.315)	94.11 (0.775)	93.97 (1.753)
15	99.83 (1.405)	99.42 (1.567)	99.32 (1.539)	97.77 (1.652)	97.72 (1.671)	96.92 (1.312)	87.96 (0.953)	87.52 (1.456)	87.49 (3.112)
30	99.66 (3.162)	99.21 (1.731)	99.15 (1.561)	95.59 (2.132)	95.50 (1.095)	94.40 (2.179)	77.37 (1.212)	77.62 (1.253)	76.91 (1.506)
60	99.32 (2.932)	99.11 (2.011)	98.54 (1.232)	91.37 (3.012)	91.20 (0.961)	89.95 (1.112)	59.86 (1.301)	60.53 (0.854)	59.70 (1.732)
90	98.99 (3.171)	99.08 (2.352)	98.17 (1.392)	87.35 (2.432)	86.69 (1.735)	85.90 (1.561)	46.99 (1.011)	46.31 (2.052)	45.70 (1.562)

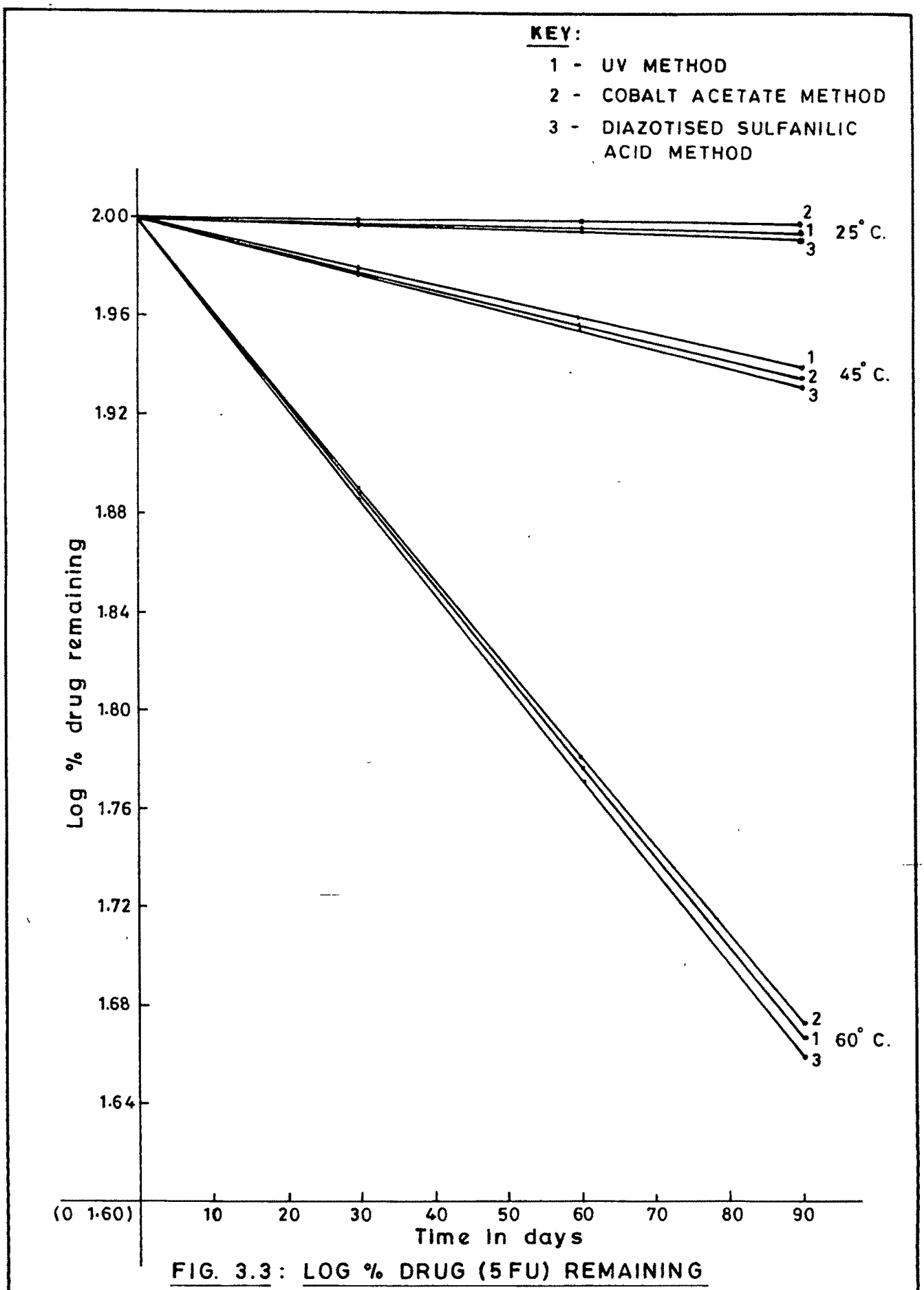

FIG. 3.3: LOG % DRUG (5FU) REMAINING AS A FUNCTION OF TIME AT pH 9.0

TABLE 3.4

STABILITY DATA OF 5FU AT pH 10.0

Time in days	% Drug remaining undegraded (± S.D.)								
	25°C			45°C			60°C		
	UV method at 266nm	Cobaltacetate at 570 nm	Diaz. Sulfanilic acid at 520 nm	UV method at 266nm	Cobaltacetate at 570 nm	Diaz. Sulfanilic acid at 520 nm	UV method at 266nm	Cobaltacetate at 570 nm	Diaz. Sulfanilic acid at 520 nm
0	100.00 (-)	100.00 (-)	100.00 (-)	100.00 (-)	100.00 (-)	100.00 (-)	100.00 (-)	100.00 (-)	100.00 (-)
1	99.98 (0.105)	99.98 (1.113)	99.75 (0.952)	99.79 (1.752)	99.82 (0.955)	99.35 (0.652)	98.86 (1.856)	98.92 (1.532)	98.35 (1.321)
3	99.95 (1.025)	99.94 (2.105)	99.72 (1.975)	99.37 (1.532)	99.45 (1.452)	99.28 (1.395)	96.64 (0.693)	97.57 (1.812)	96.11 (1.119)
7	99.87 (1.113)	99.85 (1.392)	99.69 (0.511)	98.56 (0.594)	98.85 (1.532)	97.92 (0.452)	92.33 (1.553)	93.45 (1.233)	91.23 (1.235)
15	99.74 (2.032)	99.56 (1.506)	99.61 (0.971)	96.94 (1.553)	97.52 (0.653)	95.23 (1.162)	84.38 (1.062)	85.21 (1.523)	84.21 (0.951)
30	99.48 (1.978)	99.52 (2.151)	99.45 (1.532)	93.96 (3.012)	94.12 (1.352)	93.82 (2.011)	71.02 (1.952)	71.75 (2.096)	70.05 (1.009)
60	98.97 (1.025)	98.99 (1.572)	98.53 (1.532)	88.30 (1.751)	89.35 (1.545)	87.51 (1.115)	50.85 (0.591)	50.92 (1.754)	49.99 (1.543)
90	98.45 (2.131)	98.42 (2.032)	97.42 (1.211)	82.97 (1.115)	83.01 (1.219)	81.59 (2.532)	35.83 (0.753)	36.31 (3.12)	35.07 (1.952)

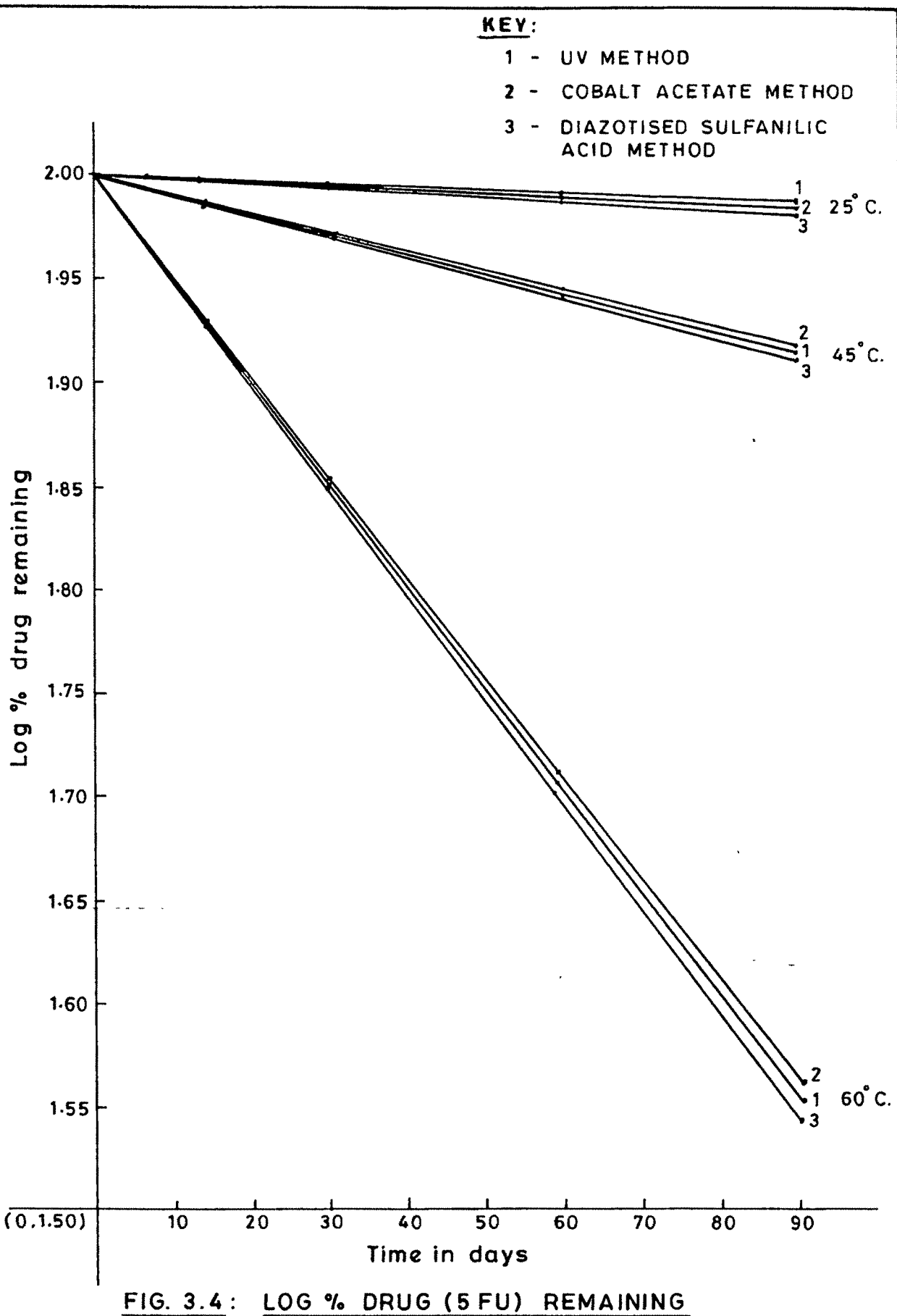

FIG. 3.4: LOG % DRUG (5 FU) REMAINING AS A FUNCTION OF TIME AT pH 10·0

TABLE 3.5

STABILITY PARAMETERS OBTAINED FOR 5FU AT VARIOUS pH AND TEMPEATURES

		25^0C			45^0C			60^0C		
pH	Stability parameter	UV method	Cobaltace-tate method	Diaz. Sulfa-nilic acid	UV method	Cobaltace-ate method	Diaz. Sulfa-nilic acid method	UV method	Cobaltace-tate method	Diaz. Sulf-anilic acid method
7.0	$K(S^{-1})$	$9.76x10^{-11}$	$9.84x10^{-11}$	$9.79x10^{-11}$	$2.67x10^{-9}$	$2.86x10^{-9}$	$2.95x10^{-9}$	$2.32x10^{-8}$	$2.34x10^{-8}$	$2.39x10^{-8}$
	t1/2(days)	82180.66	81512.53	81928.84	3004.06	2804.49	2718.93	345.74	342.77	335.59
	t90(days)	12451.62	12350.38	12413.46	455.16	424.93	411.96	52.38	51.93	50.85
8.0	$K(S^{-1})$	$1.62x10^{-10}$	$1.61x10^{-10}$	$1.62x10^{-10}$	$5.00x10^{-9}$	$5.21x10^{-9}$	$5.17x10^{-9}$	$3.97x10^{-8}$	$3.91x10^{-8}$	$3.99x10^{-8}$
	t1/2(days)	49511.3	49818.84	49511.32	1604.17	1539.51	1851.42	202.04	205.14	201.02
	t90(days)	75017.7	7548.31	7501.71	243.06	233.26	235.06	30.61	31.08	30.46
9.0	$K(S^{-1})$	$1.303x10^{-9}$	$1.29x10^{-9}$	$1.35x10^{-9}$	$1.57x10^{-8}$	$1.63x10^{-8}$	$1.74x10^{-8}$	$9.69x10^{-8}$	$9.42x10^{-8}$	$9.93x10^{-8}$
	t1/2(days)	6155.60	6217.70	5941.35	510.88	492.08	460.97	82.77	85.15	80.77
	t90(days)	932.67	942.21	900.21	77.41	72.56	69.84	12.54	12.90	12.24
10.0	$K(S^{-1})$	$1.98x10^{-9}$	$2.01x10^{-9}$	$2.02x10^{-9}$	$2.41x10^{-8}$	$2.52x10^{-8}$	$2.60x10^{-8}$	$1.38x10^{-7}$	$1.32x10^{-7}$	$1.40x10^{-7}$
	t1/2(days)	4016.40	4010.42	4008.61	330.20	318.29	308.49	58.12	60.76	57.29
	t90(days)	646.42	607.64	589.95	50.63	48.23	46.74	8.806	9.206	8.681

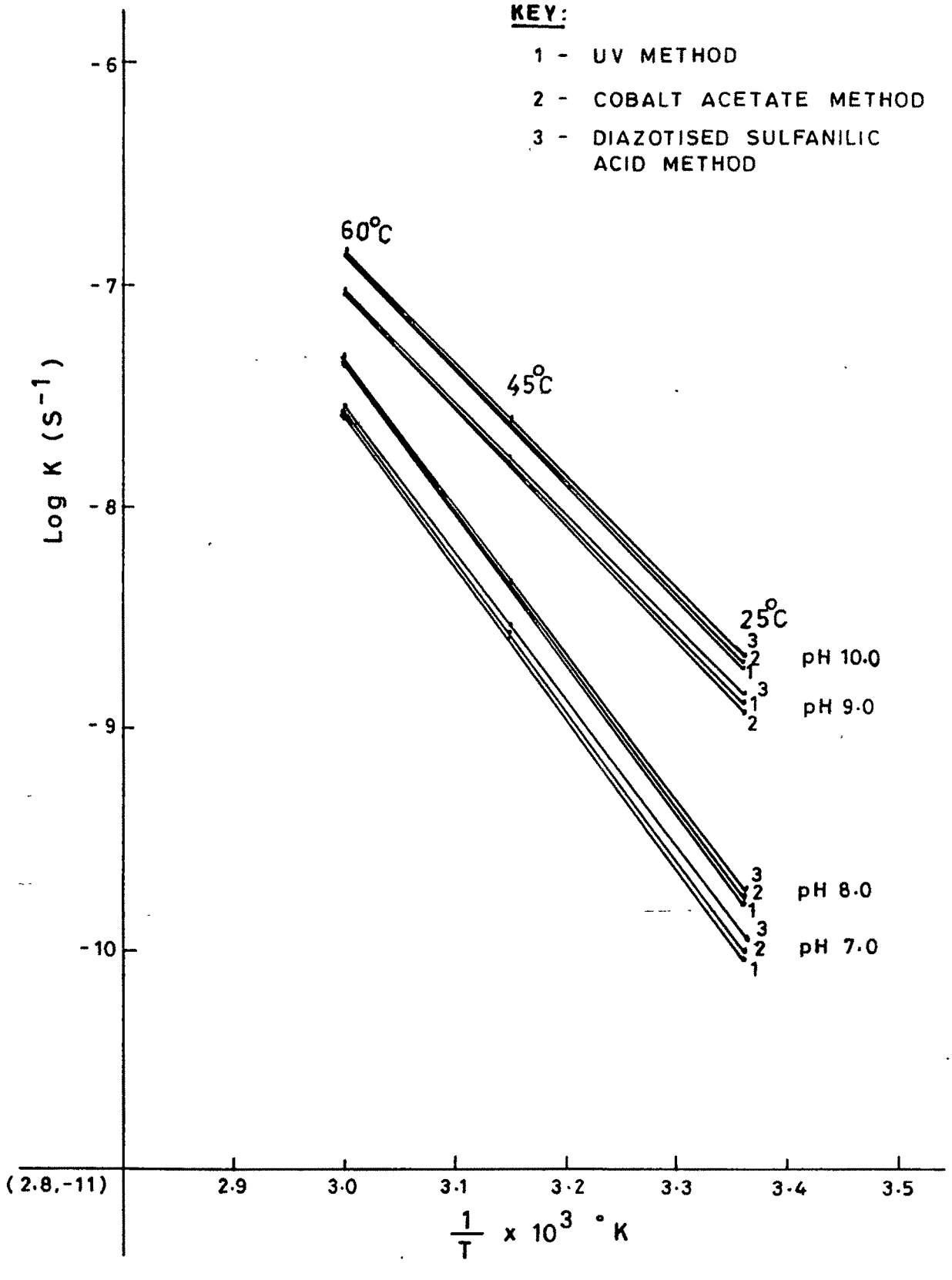

FIG. 3.5 : ARRHENIUS PLOTS FOR 5FU AT DIFFERENT pH VALUES

TABLE 3.6

ACTIVATION ENERGIES OF 5FU AT DIFFERENT pH VALUES

pH	Ea(Kcal/mol)		
	UV method	Cobaltacetate method	Diazotised Sulfanilic acid method
7.0	30.33	29.49	29.35
8.0	29.06	29.27	28.67
9.0	24.53	24.62	24.43
10.0	24.48	24.23	23.62

log percentage drug remaining vs time curves for the first order degradation of 5FU were constructed for each pH at the different temperatures (Fig 3.1-3.4).

The slopes of these lines gave the stability constant values 'K'. The t 1/2 and t 90 values were calculated from the K values using equations 2 and 3 respectively. The K values, t 1/2 and t 90 values obtained by the various methods are recorded in Table 3.5 and were compared statistically using the student's 't' test. From this data it may be inferred that 5FU in aqueous solutions follows first order degradation which is pH and temperature dependent.

From Tables 3.1-3.4 and Figs. 3.1-3.4 it may be inferred that no statistically significant difference exist in the degradation patterns observed by the standard spectrophotometric method and the proposed analytical methods. The difference in the stability rate constant (K), t1/2 and t 90 values (Table 3.5) obtained by the standard and the proposed analytical methods is also not statistically significant ($P < 0.01$). The activation energy (Ea) values for each pH were calculated graphically (Fig. 3.5) and are recorded in Table 3.6. The values so obtained were compared statistically with that of the UV spectrophotometric method and they were found to be comparable.

The results of the above experiments indicate that the proposed analytical methods are stability indicating.

3.24 Stability Studies for MTX :

It is reported that under strongly acidic conditions, MTX gets hydrolysed yielding N^{10}-methyl-4amino-4deoxy pteroic

acid. Under strongly alkaline aqueous conditions, especially at elevated temperature, the principal decomposition products are N^{10} - methyl folic acid, N^{10} - methyl pteroic acid. The reported method for analysis of stability of MTX is HPLC(2). The present investigation was undertaken to study the decomposition rate of aqueous solutions of MTX at different pH viz. 1.2, 3.9, 5, 6, 7, 8 and 10 and at different temperatures viz controlled room temperature (25°C), 45°C and 60°C by UV spectrophotometric method and by proposed colorimetric method (Folin - Ciocalteau method).

3.241 Preparation of drug solution :

A common procedure was adopted for preparation of drug solution in different buffers.

100mg of chromatographically purified MTX (section 2.31) was accurately weighed and transferred to different 100ml volumetric flasks. The drug was dissolved in and diluted to volume with the respective buffers and filtered.

5ml of drug solution was filled in amber coloured ampoules and sealed. The initial concentration of MTX in each buffer was determined in triplicate by the UV spectrophotometric method and the proposed colorimetric method as described under methods of analysis for stability studies of MTX. The ampoules were then placed at 3 different temperatures viz 25°C, 45°C and 60°C. The stability studies were conducted over a period of 90 days. At specified time intervals samples were withdrawn and analysed by the procedure given under methods of analysis for stability studies of MTX. All sample withdrawals and analysis were carried out in triplicate.

3.242 Methods of analysis for stability studies of MTX :

5ml of drug solution was allowed to run slowly into the DEAE cellulose column and eluted with ammonia - ammonium bicarbonate buffer as given under chapter 2. (Section 2.31) The MTX fraction was collected. The concentration of the drug was determined with a suitable aliquot of the drug solution, spectrophotometrically at 305nm and colorimetrically with F.C. reagent by the procedure given under preparation of calibration curve for MTX with F.C. reagent (chapter 2, section 2.333d).

3.243 Results and discussion :

The data of the percentage drug remaining at each sampling time point determined by both the methods at different pH are recorded in Tables 3.7-3.13. The mean log percentage of drug remaining vs time curves (Fig 3.6-3.12) for the degradation of MTX were plotted for each pH at three different temperatures for the values obtained by the UV spectrophotometric method and the proposed analytical method. The slopes of these lines gave the stability constant values 'K'. The t1/2 and t90 values were calculated using equation 2 and 3 respectively. The K values, t1/2 and t 90 values by reported standard method, UV spectrophotometric method and proposed colorimetric method are recorded in Table 3.14. These values obtained by various methods were compared statistically using student's 't' test. From this data it may be inferred that MTX in aqueous solutions undergoes first order degradation which is pH and temperature dependent.

TABLE 3.7

DATA FOR STABILITY OF MTX AT pH 1.2

Time in hours	% Drug remaining undegraded (± S.D.)					
	25°C		45 C		60°C	
	UV method at 305nm	Folin ciocalteau at 760nm	UV method at 305nm	Folin ciocalteau at 760nm	UV method at 305nm	Folin ciocalteau at 760nm
0	100.00 (-)	100.00 (-)	100.00 (-)	100.00 (-)	100.00 (-)	100.00 (-)
1	98.32 (0.912)	98.34 (0.910)	98.09 (0.865)	98.11 (0.679)	90.57 (0.852)	92.12 (0.591)
2	96.86 (0.595)	96.70 (0.695)	96.24 (1.011)	96.26 (2.012)	82.04 (1.112)	84.21 (1.062)
3	95.33 (0.792)	95.09 (1.011)	94.41 (1.325)	94.45 (1.219)	74.31 (2.01)	75.41 (2.134)
5	92.34 (1.072)	91.96 (1.097)	90.35 (0.977)	90.92 (0.895)	60.96 (1.591)	62.32 (1.321)
10	85.26 (0.972)	84.55 (0.875)	82.55 (1.023)	82.66 (1.375)	37.16 (1.021)	38.14 (0.793)
24 (1 day)	67.36 (2.113)	67.42 (1.247)	63.19 (0.495)	63.09 (0.395)	9.29 (0.951)	11.52 (1.115)
72 (3 days)	30.72 (1.006)	29.51 (1.195)	25.18 (1.134)	25.12 (0.866)	-	-
168 (7 days)	6.32 (1.125)	5.881 (1.213)	4.38 (1.391)	4.07 (1.232)	-	-

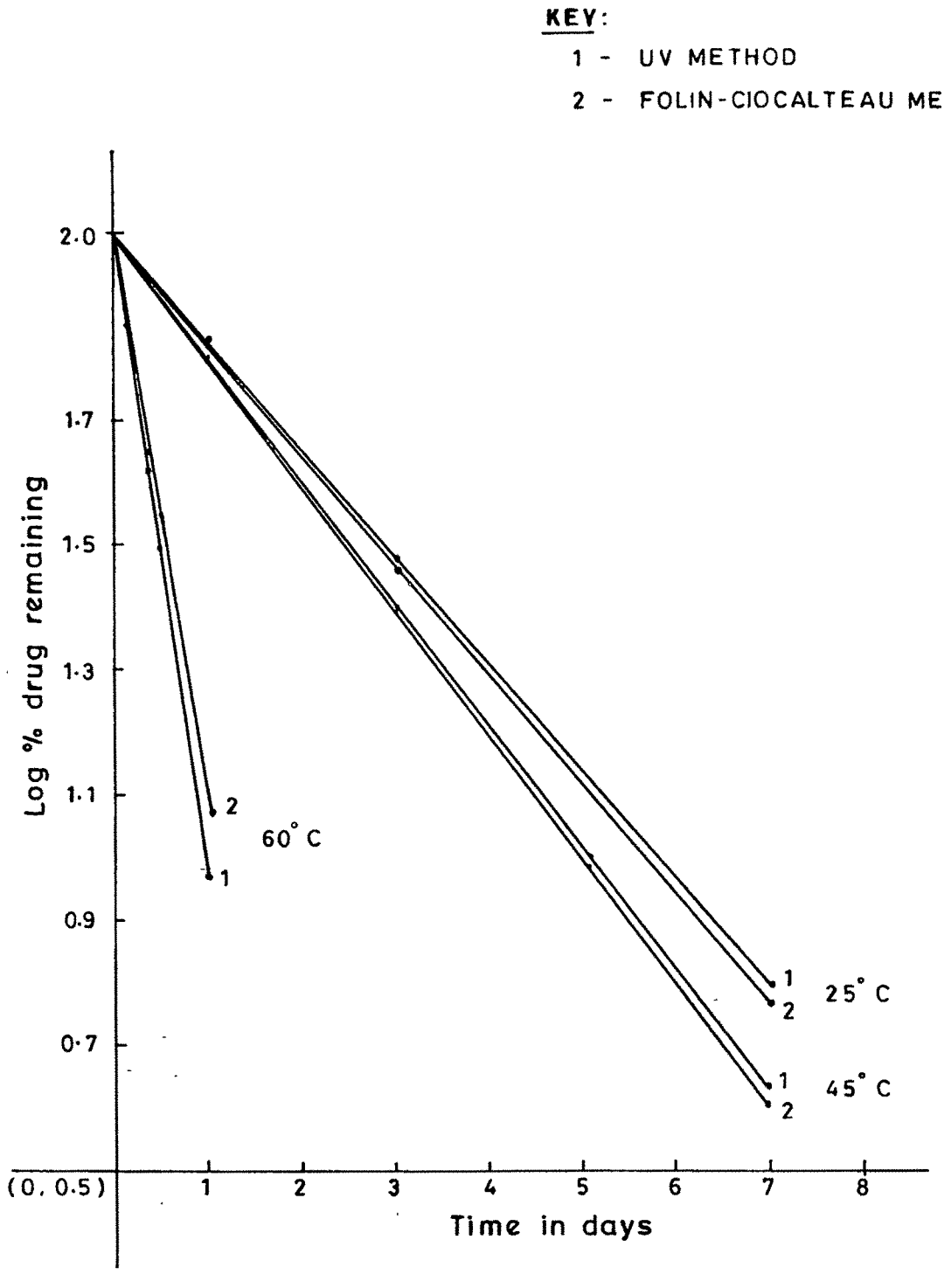

FIG. 3.6: LOG % DRUG (MTX) REMAINING AS A FUNCTION OF TIME AT pH 1·2

TABLE 3.8

DATA FOR STABILITY OF MTX AT pH 3.9

Time in days	% Drug remaining undegraded (± S.D.)					
	25°C		45°C		60°C	
	UV method at 305nm	Folin ciocalteau method	UV method at 305nm	Folin ciocalteau method	UV method at 305nm	Folin ciocalteau method
0	100.00 (-)	100.00 (-)	100.00 (-)	100.00 (-)	100.00 (-)	100.00 (-)
1	99.30 (1.031)	99.24 (1.243)	92.03 (1.375)	91.20 (0.932)	65.57 (1.512)	65.13 (1.725)
3	97.92 (0.975)	97.85 (1.735)	78.27 (1.027)	77.62 (1.762)	28.16 (0.975)	27.92 (0.879)
7	95.21 (1.212)	95.0 (1.075)	56.10 (1.635)	54.95 (0.975)	5.19 (1.025)	4.786 (0.995)
15	89.94 (0.979)	87.09 (0.972)	29.02 (1.235)	28.18 (1.018)	-	-
30	80.97 (1.053)	79.43 (1.342)	8.416 (1.321)	8.318 (1.335)	-	-
60	65.36 (1.367)	63.09 (1.921)	-	-	-	-
90	53.05 (1.529)	50.11 (0.423)	-	-	-	-

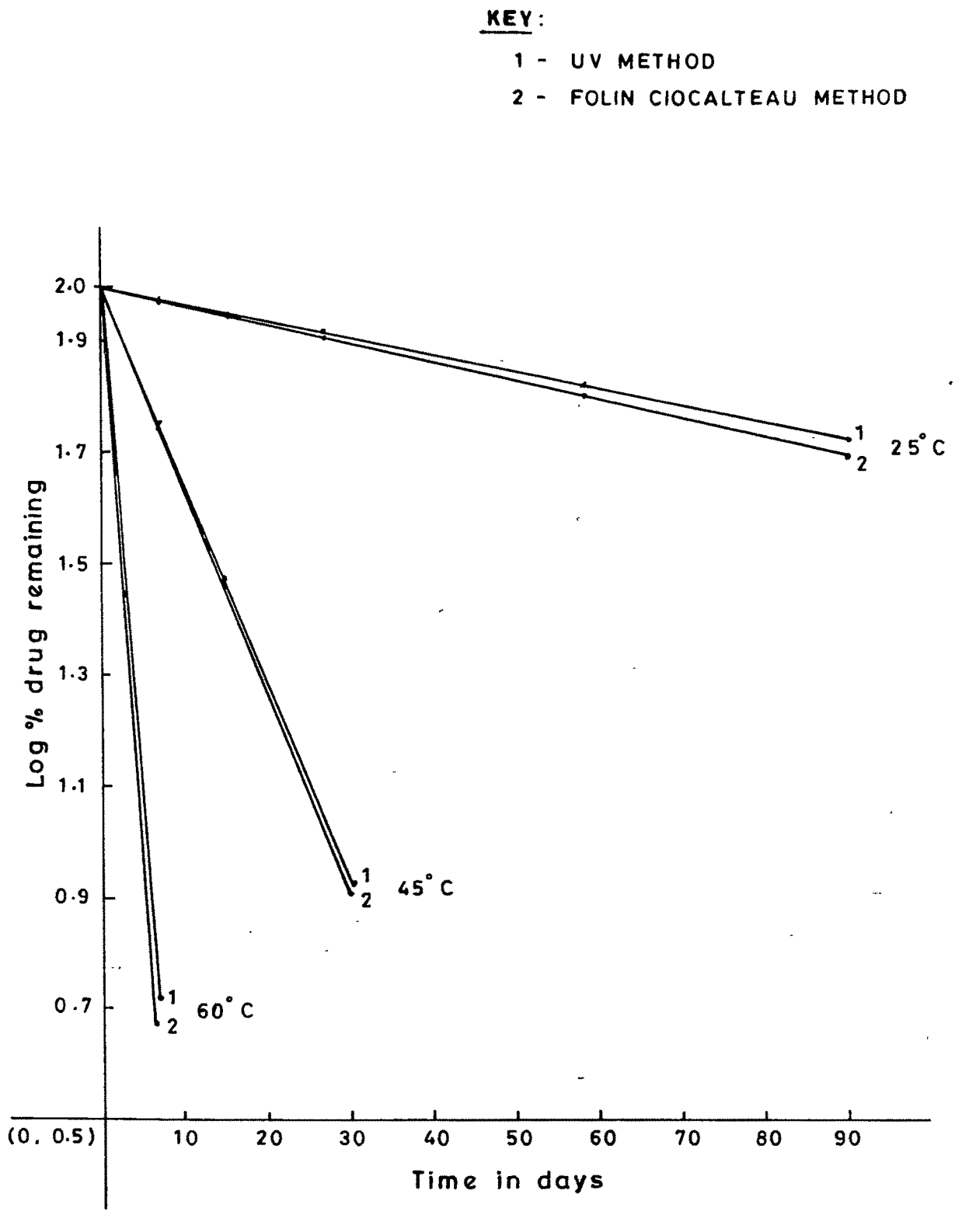

FIG. 3.7: LOG % DRUG (MTX) REMAINING AS A FUNCTION OF TIME AT pH 3·9

TABLE 3.9

DATA FOR STABILITY OF MTX AT pH 5.0

Time in days	% Drug remaining undegraded (± S.D.)					
	25°C		45°C		60°C	
	UV method at 305nm	Folin ciocalteau at 760nm	UV method at 305nm	Folin ciocalteau at 760nm	UV method at 305nm	Folin ciocalteau 760nm
0	100.00 (-)	100.00 (-)	100.00 (-)	100.00 (-)	100.00 (-)	100.00 (-)
1	99.55 (1.05)	99.35 (0.953)	94.83 (1.595)	93.33 (1.054)	76.18 (2.812)	76.18 (2.745)
3	98.71 (1.217)	99.23 (1.293)	85.3 (1.359)	85.11 (1.596)	44.22 (1.235)	44.07 (1.047)
7	96.95 (1.632)	97.72 (1.759)	69.22 (1.777)	69.18 (1.117)	14.88 (1.561)	14.79 (1.025)
15	93.52 (1.595)	93.83 (1.611)	45.43 (1.859)	44.67 (1.735)	1.69 (1.932)	1.548 (2.017)
30	87.67 (1.692)	89.13 (2.313)	20.64 (1.596)	19.95 (0.932)	-	-
60	76.41 (1.782)	79.43 (1.645)	4.251 (1.634)	4.073 (2.012)	-	-
90	66.69 (0.959)	70.79 (0.834)	-	-	-	-

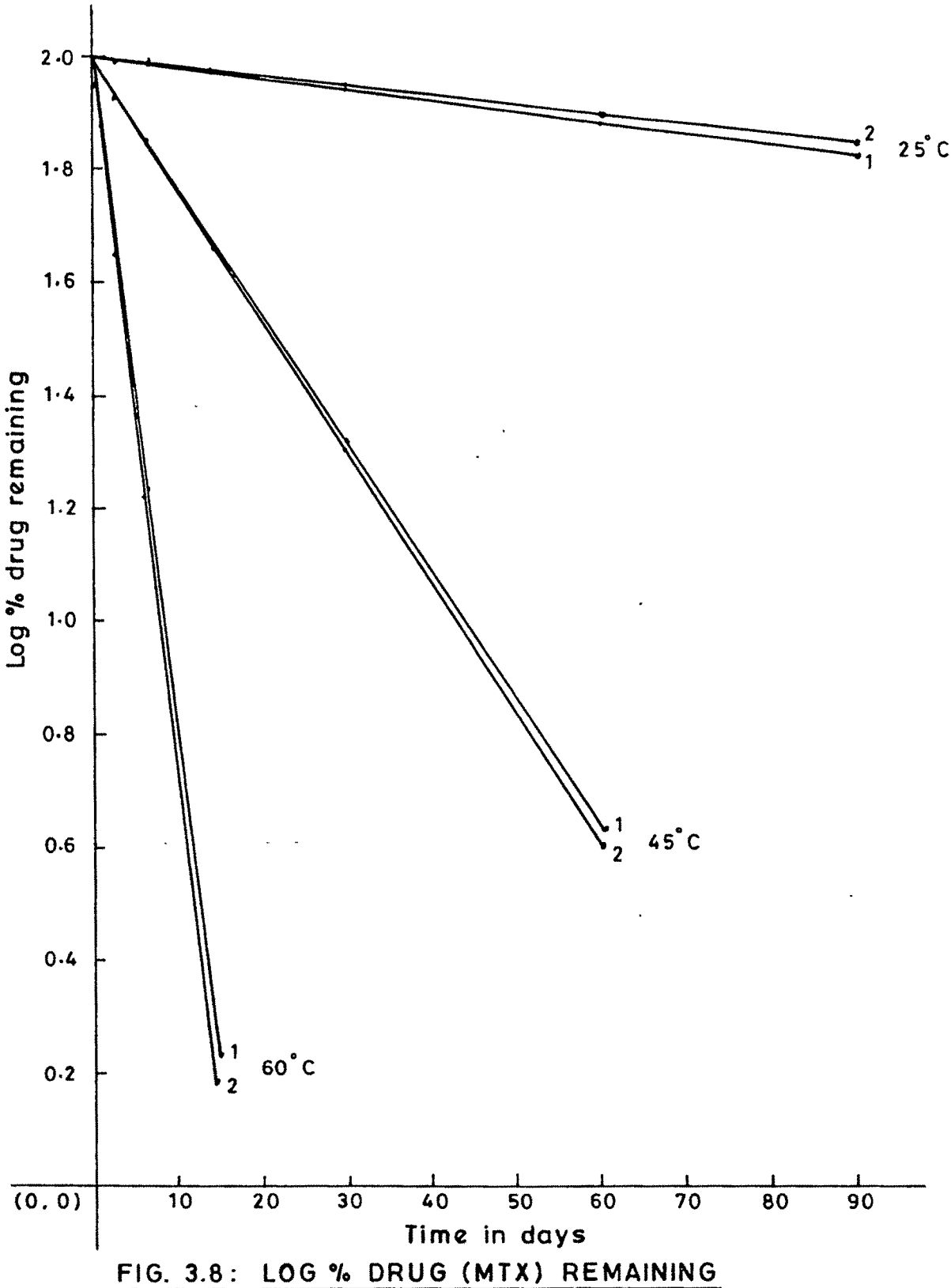

FIG. 3.8: LOG % DRUG (MTX) REMAINING AS A FUNCTION OF TIME AT pH 5·0

TABLE 3.10

DATA FOR STABILITY OF MIX AT pH 6.0

Time in days	% Drug remaining undegraded (± S.D.)					
	25°C		45°C		60°C	
	UV method at 305nm	Folin ciocalteau at 760nm	UV method at 305nm	Folin ciocalteau at 760nm	UV method at 305nm	Folin ciocalteau at 760nm
0	100.00 (-)	100.00 (-)	100.00 (-)	100.00 (-)	100.00 (-)	100.00 (-)
1	99.82 (1.282)	99.81 (1.183)	97.92 (1.792)	98.85 (1.589)	89.94 (0.956)	89.93 (1.475)
3	99.47 (1.342)	99.35 (1.432)	94.14 (1.235)	95.49 (1.073)	72.76 (1.177)	79.43 (1.349)
7	98.87 (1.072)	97.72 (1.273)	86.49 (1.097)	87.09 (1.329)	47.52 (1.257)	53.10 (0.979)
15	97.43 (2.121)	95.49 (0.935)	73.41 (1.341)	75.86 (1.022)	20.39 (1.075)	24.55 (1.155)
30	94.83 (1.384)	91.20 (1.354)	53.85 (1.472)	56.23 (1.321)	4.12 (1.735)	5.012 (1.007)
60	89.94 (0.795)	87.10 (1.012)	28.96 (1.759)	31.62 (0.975)	-	-
90	85.29 (1.732)	81.26 (0.975)	15.59 (1.752)	17.38 (1.852)	-	-

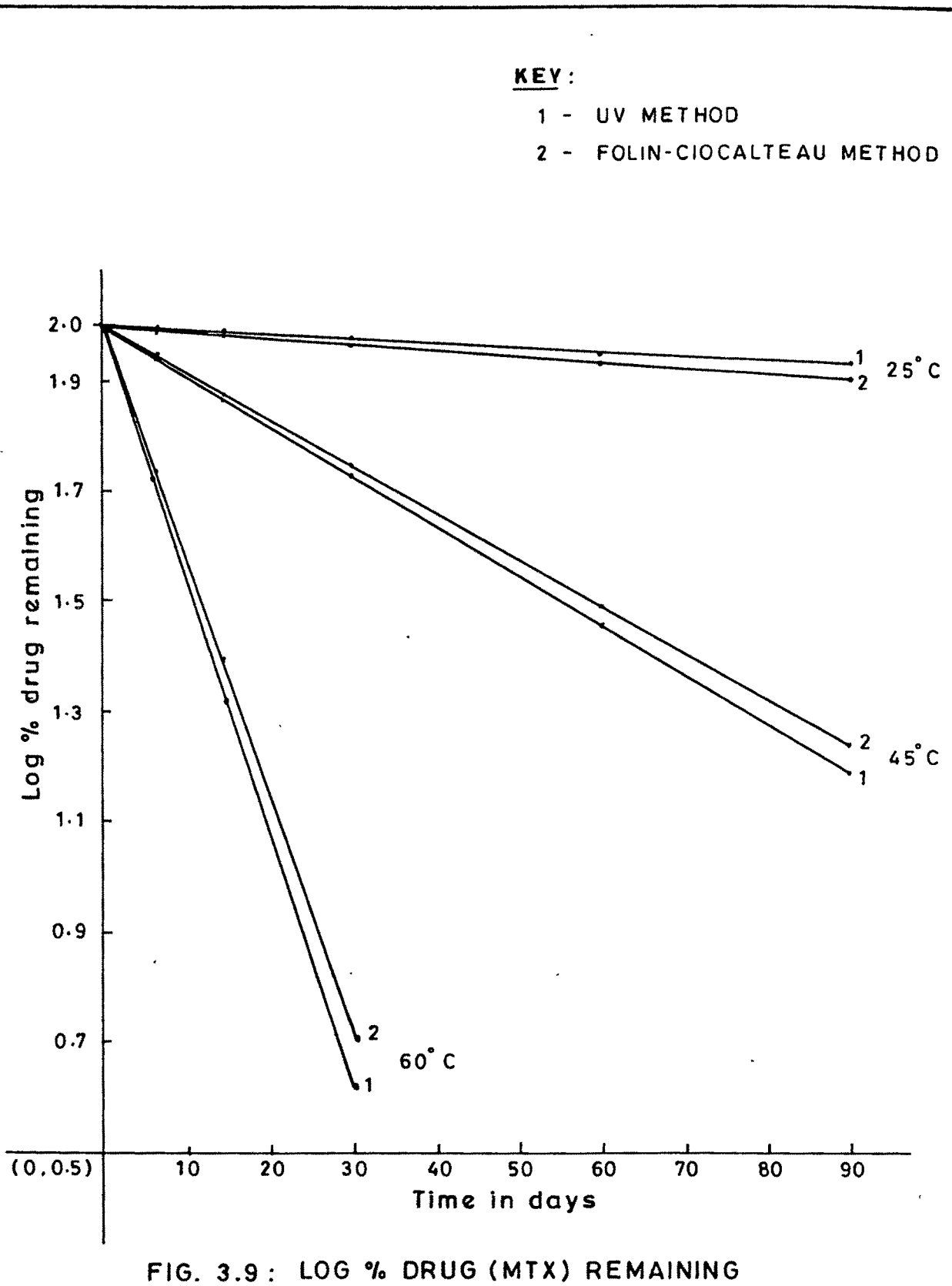

FIG. 3.9 : LOG % DRUG (MTX) REMAINING AS A FUNCTION OF TIME AT pH 6·0

TABLE 3.11

DATA FOR STABILITY OF MTX AT pH 7.0

Time in days	% Drug remaining undegraded (± S.D.)					
	25^0C		45^0C		60^0C	
	UV method at 305nm	Folin ciocalteau at 760nm	UV method at 305nm	Folin ciocalteau at 760nm	UV method at 305nm	Folin ciocalteau at 760nm
0	100.00 (-)	100.00 (-)	100.00 (-)	100.00 (-)	100.00 (-)	100.00 (-)
1	99.86 (1.692)	99.82 (1.298)	98.41 (1.481)	98.54 (1.368)	91.94 (1.432)	91.96 (1.725)
3	39.58 (1.252)	99.52 (2.012)	95.22 (1.375)	90.16 (0.975)	77.72 (1.277)	84.14 (1.414)
7	99.10 (1.009)	99.01 (1.113)	89.14 (1.003)	39.13 (1.117)	55.48 (1.481)	59.57 (1.045)
15	97.92 (1.217)	97.87 (1.234)	78.19 (1.175)	76.79 (0.975)	28.31 (0.992)	31.26 (1.957)
30	95.89 (1.325)	95.49 (1.732)	60.65 (1.037)	60.26 (0.976)	8.01 (1.013)	8.913 (2.132)
60	91.94 (0.975)	90.16 (1.345)	37.53 (1.275)	37.52 (0.579)	-	-
90	88.16 (1.652)	84.14 (1.424)	22.83 (1.312)	22.38 (1.325)	-	-

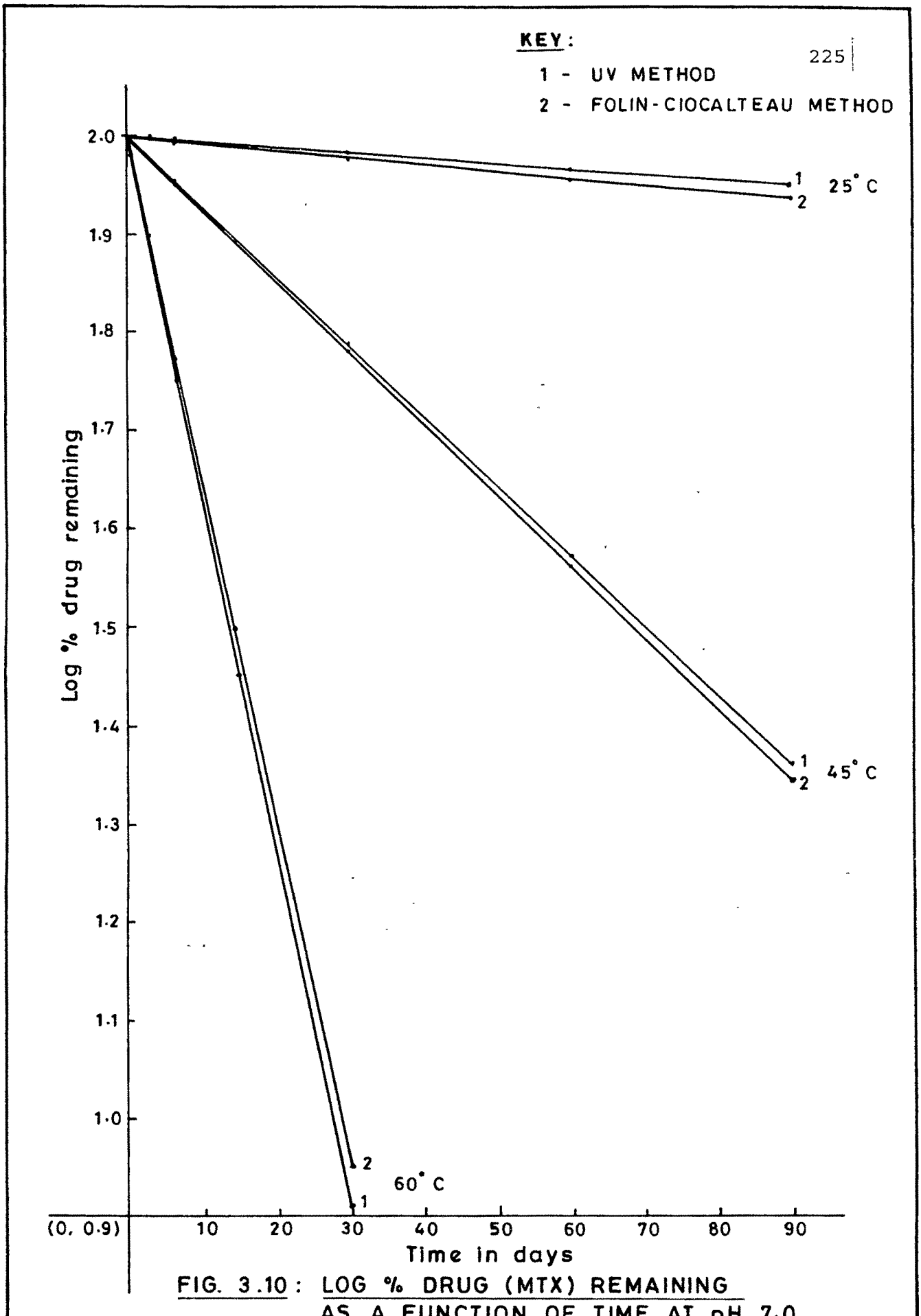

FIG. 3.10 : LOG % DRUG (MTX) REMAINING AS A FUNCTION OF TIME AT pH 7.0

TABLE 3.12

DATA FOR STABILITY OF MTX AT pH 8.0

Time in days	% Drug remaining undegraded (± S.D.)					
	25°C		45°C		60°C	
	UV method at 305nm	Folin ciocalteau at 760nm	UV method at 305nm	Folin ciocalteau at 760nm	UV method at 305nm	Folin ciocalteau at 760nm
0	100.00 (-)	100.00 (-)	100.00 (-)	100.00 (-)	100.00 (-)	100.00 (-)
1	99.75 (1.023)	99.45 (1.432)	97.14 (1.042)	97.02 (0.975)	86.16 (0.959)	87.09 (1.092)
3	99.25 (1.525)	99.12 (1.231)	91.66 (1.002)	90.21 (1.012)	63.89 (1.112)	67.61 (1.113)
7	98.31 (1.317)	97.61 (1.121)	81.55 (0.972)	81.28 (1.111)	35.13 (1.312)	39.81 (1.325)
15	96.36 (0.992)	95.33 (0.795)	64.53 (1.327)	61.28 (1.278)	8.15 (0.975)	13.18 (1.072)
30	92.77 (0.995)	89.13 (0.541)	41.65 (1.153)	40.74 (1.074)	1.13 (0.972)	1.59 (1.212)
60	86.16 (0.872)	84.20 (0.972)	17.34 (1.042)	16.59 (1.313)	-	-
90	79.93 (1.023)	76.86 (1.001)	7.22 (1.213)	6.761 (1.217)	-	-

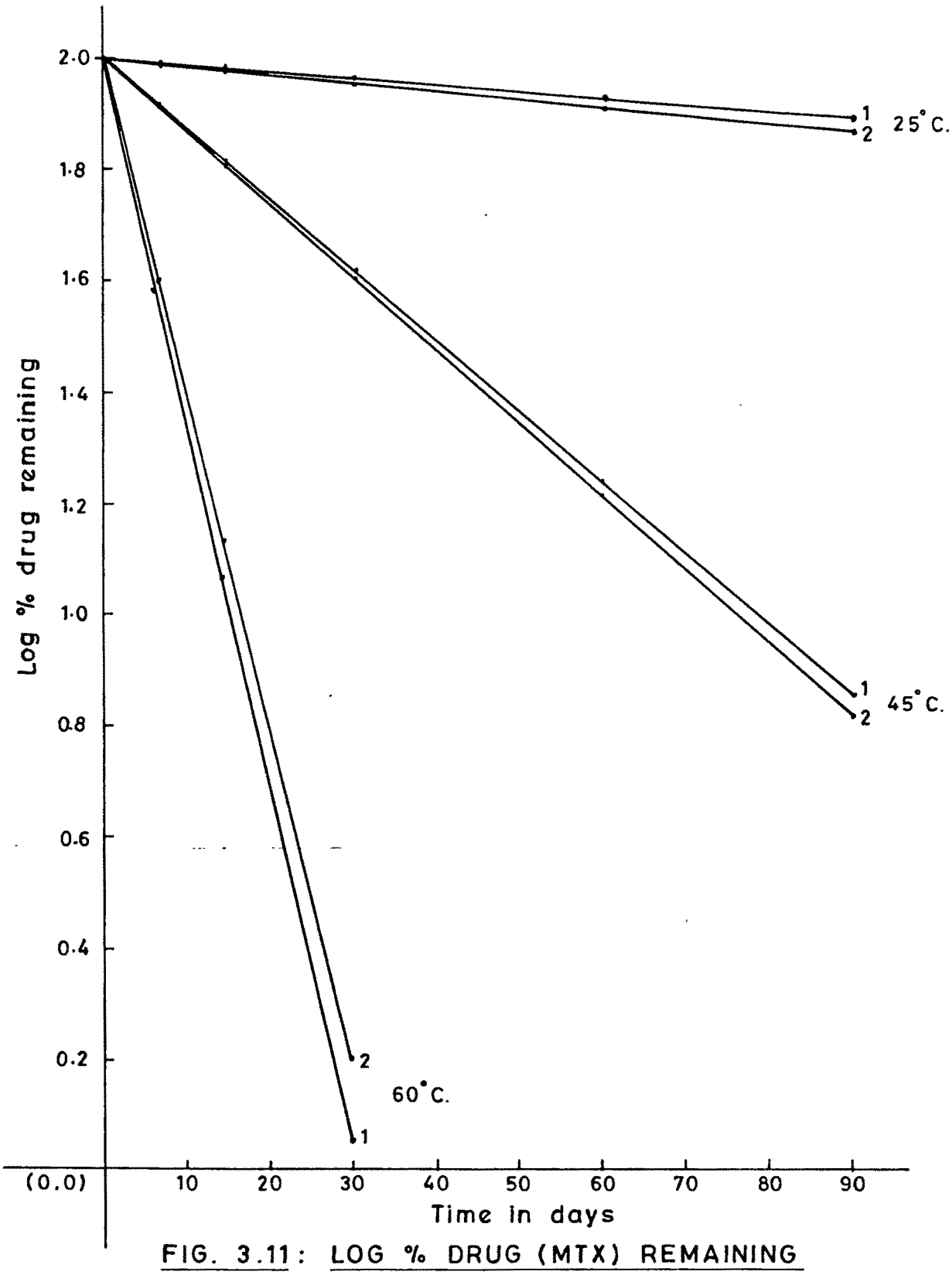

FIG. 3.11: LOG % DRUG (MTX) REMAINING AS A FUNCTION OF TIME AT pH 8·0

TABLE 3.13

DATA FOR STABILITY OF MTX AT pH 10.0

Time in days	% Drug remaining undegraded (± S.D.)					
	25°C		45°C		60°C	
	UV method at 305nm	Folin ciocalteau at 760nm	UV method at 305nm	Folin ciocalteau at 760nm	UV method at 305nm	Folin ciocalteau at 760nm
1	2	3	4	5	6	7
0	100.00 (-)	100.00 (-)	100.00 (-)	100.00 (-)	100.00 (-)	100.00 (-)
0.042 (1 hr)	99.88 (1.035)	99.88 (1.135)	98.67 (1.234)	98.68 (1.321)	93.25 (1.213)	93.38 (1.213)
0.083 (2 hrs)	99.76 (0.776)	99.75 (0.159)	97.35 (1.137)	97.42 (1.024)	86.90 (1.014)	87.20 (1.012)
0.125 (3 hrs)	99.65 (0.977)	99.52 (0.897)	96.06 (1.623)	96.10 (1.021)	81.01 (1.312)	81.43 (0.932)
0.208 (5 hrs)	99.41 (0.759)	99.35 (1.321)	93.52 (1.232)	93.62 (1.075)	70.40 (1,114)	71.01 (1.112)
0.416 (10 hrs)	98.83 (1.132)	98.12 (0.525)	87.47 (1.042)	87.57 (1.212)	49.56 (1.231)	50.43 (1.113)
1	97.24 (1.275)	96.89 (1.375)	71.96 (1.321)	73.42 (1.213)	19.57 (1.115)	24.70 (1.117)

contd...

1	2	3	4	5	6	7
3	91.94 (1.213)	90.26 (1.236)	37.24 (1.421)	39.21 (1.011)	-	-
7	82.20 (0.973)	81.78 (1.327)	9.98 (1.234)	11.02 (1.113)	-	-
15	65.64 (1.463)	65.06 (1.213)	-	-	-	-
30	43.08 (1.072)	42.67 (1.132)	-	-	-	-
60	18.54 (1.452)	17.85 (1.237)	-	-	-	-
90	7.99 (1.013)	7.08 (1.024)	-	-	-	-

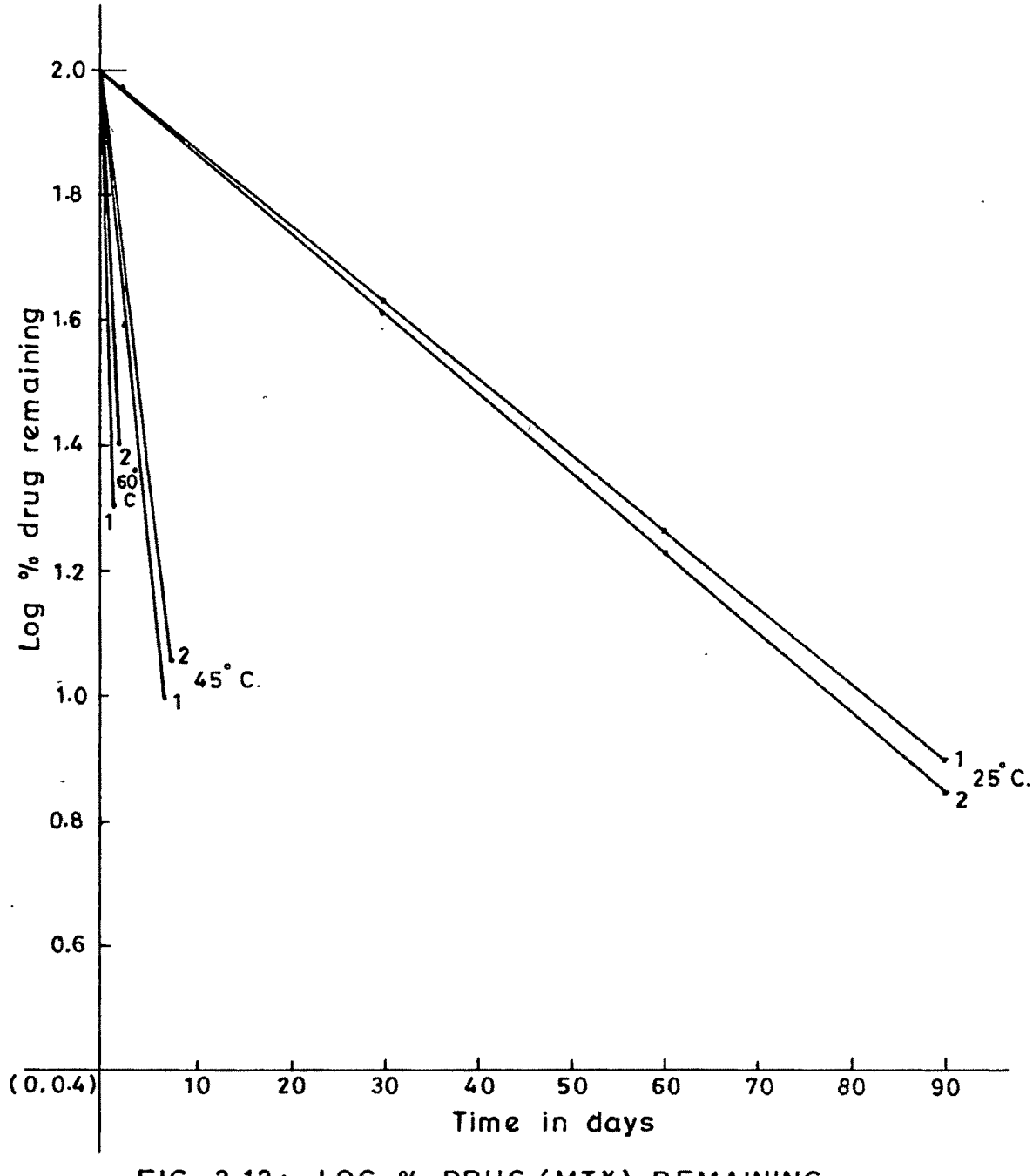

FIG. 3.12: LOG % DRUG (MTX) REMAINING AS A FUNCTION OF TIME AT pH 10.0

TABLE 3.14

STABILITY PARAMETERS OBTAINED FOR MIX AT VARIOUS pH AND TEMPERATURES

pH	Stability Parameters K-(s^{-1}) t1/2and t90 = days	25°C Reported data	25°C UV method	25°C Folin-ciocal-teau Method	45°C Reported data	45°C UV method	45°C Folin-ciocal-teau method	60°C Reorted data	60°C UV method	60°C Folin-ciocal-teau method
1.2	K	4.57×10^{-7}	4.43×10^{-7}	4.66×10^{-7}	5.32×10^{-6}	5.33×10^{-6}	5.29×10^{-6}	2.75×10^{-5}	2.71×10^{-5}	2.60×10^{-5}
	t1/2	17.55	18.11	17.22	1.51	1.505	1.516	0.292	0.296	0.308
	t90	2.66	2.743	2.61	0.228	0.228	0.229	0.044	0.045	0.046
4.0	K	8.15×10^{-8}	7.91×10^{-8}	7.99×10^{-8}	9.55×10^{-7}	9.85×10^{-7}	9.87×10^{-7}	4.89×10^{-6}	4.89×10^{-6}	4.92×10^{-6}
	t1/2	98.42	101.40	100.39	8.39	8.14	8.13	1.64	1.64	1.63
	t90	14.91	15.36	15.21	1.27	1.234	1.231	0.24	0.25	0.25
5.0	K	5.11×10^{-8}	4.99×10^{-8}	4.83×10^{-8}	6.09×10^{-7}	6.15×10^{-7}	6.16×10^{-7}	3.15×10^{-6}	3.15×10^{-6}	3.17×10^{-6}
	t1/2	154.5	160.48	166.06	13.17	13.04	13.01	2.55	2.55	2.53
	t90	23.42	24.32	25.16	1.99	1.98	1.97	0.386	0.386	0.383
6.0	K	2.04×10^{-8}	2.18×10^{-8}	2.35×10^{-8}	2.39×10^{-7}	2.29×10^{-7}	2.46×10^{-7}	1.23×10^{-6}	1.33×10^{-6}	1.16×10^{-6}
	t1/2	393.17	367.93	341.31	33.56	35.10	32.61	6.52	6.02	6.93
	t90	59.57	55.75	51.72	5.08	5.32	4.94	0.990	0.912	1.05
7.0	K	1.62×10^{-8}	1.54×10^{-8}	1.55×10^{-8}	1.91×10^{-7}	1.99×10^{-7}	2.01×10^{-7}	9.74×10^{-7}	9.33×10^{-7}	9.29×10^{-7}
	t1/2	495.11	520.83	517.47	42.24	40.31	39.90	8.23	8.59	8.63
	t90	75.01	78.91	78.41	6.39	6.11	6.05	1.25	1.30	1.31
8.0	K	2.88×10^{-8}	2.88×10^{-8}	2.92×10^{-8}	3.38×10^{-7}	3.46×10^{-7}	3.53×10^{-7}	1.73×10^{-6}	1.72×10^{-6}	1.67×10^{-6}
	t1/2	278.50	278.50	274.68	23.73	23.18	22.72	4.64	4.66	4.80
	t90	42.19	42.19	41.62	3.590	3.51	3.44	0.701	0.71	0.73
10.0	K	3.25×10^{-7}	3.27×10^{-7}	3.21×10^{-7}	3.81×10^{-6}	3.72×10^{-6}	3.78×10^{-6}	1.95×10^{-5}	1.95×10^{-5}	1.90×10^{-5}
	t1/2	24.68	24.53	24.99	2.11	2.16	2.12	0.411	0.411	0.422
	t90	3.74	3.72	3.79	0.31	0.33	0.32	0.069	0.062	0.063

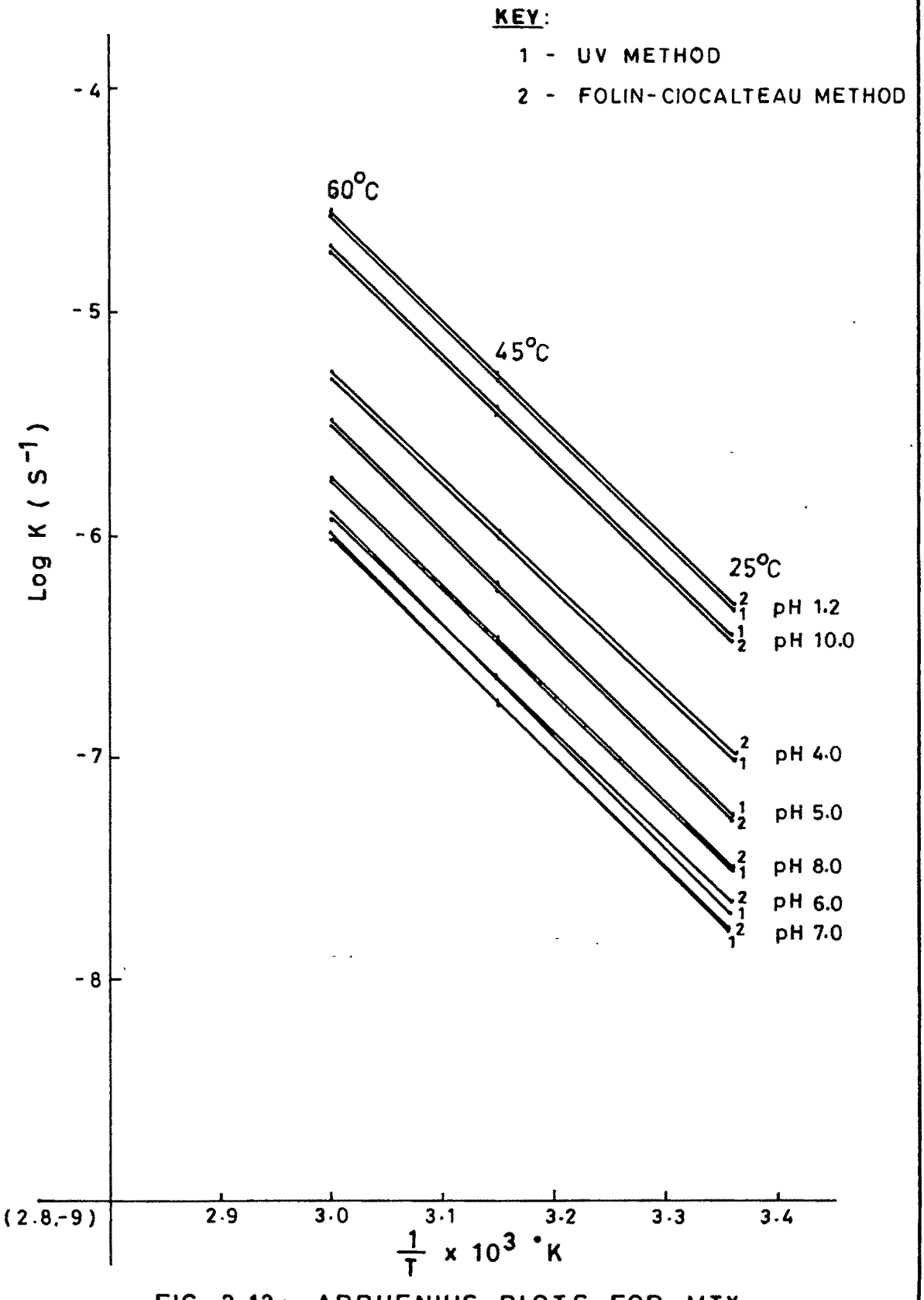

FIG. 3.13: ARRHENIUS PLOTS FOR MTX AT DIFFERENT pH VALUES

TABLE 3.15

ACTIVATION ENERGIES OF MTX AT DIFFERENT pH VALUES OBTAINED BY DIFFERENT METHODS

pH	Ea(Kcal/mol)		
	Reported data	UV method	Folin-ciocalteau method
1.2	23.05	22.79	22.34
3.9	22.91	22.91	22.54
5.0	23.06	22.92	22.98
6.0	22.98	22.92	22.98
7.0	22.93	22.67	22.47
8.0	22.91	22.49	22.08
10.0	22.91	23.24	22.65

From the Tables 3.7-3.13 and Figs 3.6-3.12 it may be seen that there is no statistically significant difference in the degradation pattern obtained by the UV spectrophotometric method and proposed colorimetric method. The difference in the stability rate constant (K), t 1/2 and t 90 values (Table 3.14) obtained by the reported method, UV spectrophotometry and proposed colorimetric method are not statistically significant ($P<0.05$ by student's t test).

The Ea values at different pH were calculated graphically (Fig 3.13) and are recorded in Table 3.15. No statistically significant differences were observed between the reported values, and those obtained by UV spectrophotometry and the proposed colorimetric methods.

The results of these experiments indicate that the UV spectrophotometric method and the proposed analytical method are stability indicating.

3.25 Stability Studies of 5FU and MTX in Semisolid Dosage Forms :

The aim of this study was to determine the stability of 5FU and MTX in four different bases viz hydrous emulsifying base (I.P), cetomacrogol cream (B.P), HPMC K4M gel base and carbopol gel base.

3.251 Preparation of semisolid bases :

a) Hydrous emulsifying base (I.P) :

Hydrous emulsifying base was prepared as per the procedure given in I.P. 1966.

b) Cetomacrogol cream (B.P) :

Cetomacrogol cream was prepared as per the procedure given in B.P 1980.

c) HPMC K4M gel base : (4)

25g of HPMC K4M was added to 500ml of water with constant stirring taking care to prevent lump formation. 0.001% phenylmercuric nitrate was added as a preservative.

d) Carbopol gel base : (4)

4g of carbopol 941was added to 500 ml of water with constant stirring. After complete dispersion of the powder, 10ml of 20% sodium hydroxide solution was added; the stirring was continued till complete gel formation occured.

3.252 Preparation of the drug cream :

A common procedure was followed for the preparation of all the creams for both the drugs.

150mg of 5FU and purified MTX were carefully weighed and levigated with 1.0g of each of the base on a pill tile. This was then diluted in a geometric proportion. The final weight was made to 100gm with the base. The cream so obtained was passed through a triple roller mill. The creams were assayed for initial concentration of 5FU and MTX in triplicate by the spectrophotometric method and proposed analytical methods.

5g of each of the creams were filled in suitable lacquered aliminium tubes. These were stored at refrigeration ($4^{o}C$) and controlled room temperature ($25^{o}C$). The stability studies were conducted over a period of 90 days. At each

sampling time point, three tubes were withdrawn and assayed for the drug in triplicate by the UV spectrophotometric method and the proposed analytical methods by the procedure given in method of analysis from creams as described below.

3.253 Method of analysis of 5FU and MTX from creams :

A common procedure was adopted for extraction of the drug from the cream base.

A quantity of cream equivalent to 5mg of drug was transferred to 25 ml volumetric flask. 10ml of pH7.4 phosphate buffer I.P was added and the flask was shaken by mechanical means for 5 minutes, and the volume was made up with the buffer. The solution was filtered and 10 ml of clear filtrate was transferred into a separating funnel and extracted thrice with 10,5,5ml portions of ether followed by chloroform (10,5,5ml). The organic layer was discarded and any residual solvent was evaporated under stream of nitrogen. The aqueous layer was diluted to 25ml with pH 7.4 buffer. The same operations were carried out for plain cream bases without drug. Concentration of the drug in aqueous extract was analysed by the following methods.

a) For 5FU :

(i) UV spectrophotometric :

An aliquot of the drug solution (1.25ml) was suitably diluted with pH7.4 phosphate buffer and absorbance was measured at 266 nm against the appropriate cream blank solution.

(ii) Cobalt acetate method :

5ml of aqueous drug solution was transferred into a separating funnel and drug was extracted with

TABLE 3.16 (a)

STABILITY DATA OF 5FU IN DIFFERENT BASES AT $4^{o}C$.

	% drug remaining undegraded (± S.D.)											
	Hydrous emulsifying base			Cetomacrogol cream			HPMC K4M base			Carbopol base		
Time in days	UV method	Cobalt acetate method	diazoti-sed sulfani-lic acid method	UV method	Cobalt acetate method	diazoti-sed sulfani-lic acid method	UV Method	Cobalt acetate method	diazoti-sed sulfani-lic acid method	UV method	Cobalt acetate method	diazoti-sed sulfani-lic acid method
0	100.00 (-)	100.00 (-)	100.00 (-)	100.00 (-)	100.00 (-)	100.00 (-)	100.00 (-)	100.00 (-)	100.00 (-)	100.00 (-)	100.00 (-)	100.00 (-)
1	99.99 (0.975)	99.98 (1.239)	99.89 (1.192)	99.98 (0.875)	99.92 (1.093)	99.85 (1.587)	99.97 (1.792)	99.97 (0.932)	99.89 (1.981)	99.99 (1.045)	99.98 (1.879)	99.96 (1.679)
3	99.99 (0.572)	99.98 (0.977)	99.87 (0.792)	99.97 (0.975)	99.92 (1.237)	99.79 (1.113)	99.97 (1.043)	99.96 (0.977)	99.87 (1.732)	99.97 (1.324)	99.96 (1.692)	99.93 (1.392)
7	99.98 (1.021)	99.98 (0.752)	99.85 (1.277)	99.97 (1.412)	99.92 (1.021)	99.79 (1.007)	99..97 (1.792)	99.95 (0.879)	99.87 (0.932)	99.96 (1.679)	99.96 (0.932)	99.93 (1.313)
15	99.98 (1.237)	99.98 (0.975)	99.85 (1.892)	99.97 (1.431)	99.91 (1.739)	99.76 (1.697)	99.97 (1.777)	99.95 (1.345)	99.85 (1.578)	99.95 (1.597)	99.94 (1.497)	99.93 (1.303)
30	99.96 (1.213)	99.96 (1.001)	99.83 (1.342)	99.97 (0.895)	99.91 (1.021)	99.76 (1.692)	99.95 (1.632)	99.93 (1.431)	99.84 (0.475)	99.95 (1.503)	99.93 (0.975)	99.93 (1.379)
60	99.95 (0.978)	99.95 (0.919)	99.80 (1.087)	99.96 (0.999)	99.91 (1.075)	99.75 (1.097)	99.93 (1.301)	99.93 (1.379)	99.84 (1.087)	99.94 (1.432)	99.93 (1.321)	99.91 (1.179)
90	99.85 (0.978)	99.91 (0.919)	99.80 (1.087)	99.95 (0.999)	99.89 (1.075)	99.72 (1.097)	99.93 (1.301)	99.85 (1.379)	99.83 (1.087)	99.93 (1.432)	99.91 (1.321)	99.89 (1.179)

TABLE 3.16 (b)

STABILITY DATA OF 5FU IN DIFFERENT BASES AT $25^{o}c$.

	% drug remaining undegraded (± S.D.)											
	Hydrous emulsifying base			Cetomacrogol cream			HPMC K4M base			Carbopol base		
Time in days	UV method	Cobalt acetate method	diazoti-sed sulfani-lic acid method	UV method	Cobalt acetate method	diazoti-sed sulfani-lic acid method	UV Method	Cobalt acetate method	diazoti-sed sulfani-lic acid method	UV method	Cobalt acetate method	diazoti-sed sulfani-lic acid method
0	100.00 (-)	100.00 (-)	100.00 (-)	100.00 (-)	100.00 (-)	100.00 (-)	100.00 (-)	100.00 (-)	100.00 (-)	100.00 (-)	100.00 (-)	100.00 (-)
1	99.98 (0.975)	99.98 (1.239)	99.95 (1.192)	99.98 (0.875)	99.92 (1.093)	99.85 (1.587)	99.97 (1.792)	99.97 (0.932)	99.89 (1.981)	99.99 (1.045)	99.98 (1.879)	99.96 (1.679)
3	99.99 (0.572)	99.98 (0.977)	99.87 (0.792)	99.97 (0.975)	99.92 (1.237)	99.79 (1.113)	99.97 (1.043)	99.96 (0.977)	99.87 (1.732)	99.97 (1.324)	99.96 (1.692)	99.93 (1.392)
7	99.98 (1.021)	99.98 (0.752)	99.85 (1.277)	99.97 (1.412)	99.92 (1.021)	99.79 (1.007)	99..97 (1.792)	99.95 (0.879)	99.87 (0.932)	99.96 (1.679)	99.96 (0.932)	99.93 (1.313)
15	99.98 (1.237)	99.98 (0.975)	99.85 (1.892)	99.97 (1.431)	99.91 (1.739)	99.76 (1.697)	99.97 (1.777)	99.95 (1.345)	99.85 (1.578)	99.95 (1.597)	99.94 (1.497)	99.93 (1.303)
30	99.96 (1.213)	99.96 (1.001)	99.83 (1.342)	99.97 (0.895)	99.91 (1.021)	99.76 (1.692)	99.95 (1.632)	99.93 (1.431)	99.84 (0.475)	99.95 (1.503)	99.93 (0.975)	99.93 (1.379)
60	99.95 (0.978)	99.95 (0.919)	99.80 (1.087)	99.96 (0.999)	99.91 (1.075)	99.75 (1.097)	99.93 (1.301)	99.93 (1.379)	99.84 (1.087)	99.94 (1.432)	99.93 (1.321)	99.91 (1.179)
90	99.85 (0.978)	99.91 (0.919)	99.80 (1.087)	99.95 (0.999)	99.89 (1.075)	99.72 (1.097)	99.93 (1.301)	99.85 (1.379)	99.83 (1.087)	99.93 (1.432)	99.91 (1.321)	99.89 (1.179)

TABLE 3.17 (a)

STABILITY DATA OF MIX IN DIFFERENT BASES AT $4^{O}C$

Time in days	% drug remaining undegraded (± S.D.)							
	Hydrous emulsifying base		Cetomacrogol cream		HPMC K4M base		Carbopol base	
	UV method	Folin-cio -calteau method	UV method	Folin-cio -calteau method	UV Method	Folin-cio -calteau method	UV method	Folin-cio -calteau method
0	100.00 (-)	100.00 (-)	100.00 (-)	100.00 (-)	100.00 (-)	100.00 (-)	100.00 (-)	100.00 (-)
1	99.99 (1.313)	99.98 (1.002)	99.99 (0.993)	99.98 (0.972)	99.98 (0.932)	99.99 (0.932)	99.95 (0.931)	99.99 (0.399)
3	99.99 (0.952)	99.96 (0.634)	99.96 (0.875)	99.96 (1.672)	99.97 (0.992)	99.97 (0.793)	99.94 (0.911)	99.98 (0.875)
7	99.98 (1.895)	99.95 (1.005)	99.93 (0.312)	99.95 (0.523)	99.94 (0.932)	99.96 (0.931)	99.93 (0.917)	99.97 (0.932)
15	99.95 (1.521)	99.95 (1.593)	99.93 (1.323)	99.93 (1.319)	99.93 (0.975)	99.94 (0.499)	99.90 (1.091)	99.95 (1.001)
30	99.95 (0.932)	99.93 (1.391)	99.91 (1.112)	99.93 (0.975)	99.92 (1.213)	99.93 (0.993)	99.90 (0.993)	99.95 (0.932)
60	99.92 (1.212)	99.91 (1.007)	99.89 (1.231)	99.91 (0.991)	99.89 (0.931)	99.85 (0.913)	99.89 (0.897)	99.90 (1.002)
90	99.87 (1.793)	99.89 (1.721)	99.75 (1.514)	99.81 (0.892)	99.87 (0.732)	99.79 (0.792)	99.87 (1.002)	99.91 (1.140)

TABLE 3.17 (b)

STABILITY DATA OF MIX IN DIFFERENT BASES AT 25°C

Time in days	% drug remaining undegraded (± S.D.)							
	Hydrous emulsifying base		Cetomacrogol cream		HPMC K4M base		Carbopol base	
	UV method	Folin-cio -calteau method	UV method	Folin-cio -calteau method	UV Method	Folin-cio -calteau method	UV method	Folin-cio -calteau method
0	100.00 (-)	100.00 (-)	100.00 (-)	100.00 (-)	100.00 (-)	100.00 (-)	100.00 (-)	100.00 (-)
1	99.99 (0.975)	99.97 (1.003)	99.95 (1.212)	99.97 (1.732)	99.98 (1.112)	99.99 (1.132)	99.97 (1.313)	99.95 (0.993)
3	99.91 (0.832)	99.89 (1.192)	99.89 (0.975)	99.94 (1.431)	99.87 (1.732)	99.91 (1.007)	99.89 (0.977)	99.87 (1.212)
7	99.87 (1.312)	99.82 (1.021)	99.79 (1.323)	99.92 (1.032)	99.67 (1.329)	99.54 (1.000)	99.75 (2.001)	99.69 (0.932)
15	99.53 (0.995)	99.63 (1.325)	99.54 (0.592)	99.92 (0.993)	99.45 (1.400)	99.29 (1.292)	99.43 (1.313)	99.53 (0.993)
30	99.25 (0.936)	99.10 (1.112)	99.23 (0.475)	99.90 (0.912)	98.99 (0.999)	99.10 (0.999)	99.11 (1.312)	99.23 (0.542)
60	98.91 (0.679)	99.01 (1.091)	98.85 (0.572)	99.89 (0.735)	98.99 (1.003)	98.97 (0.932)	98.79 (0.932)	99.07 (1.009)
90	98.81 (1.095)	98.92 (0.975)	98.79 (0.912)	98.81 (0.817)	98.71 (1.072)	98.81 (0.875)	98.60 (1.067)	98.95 (0.932)

ethylacetate : isopropanol mixture (70:30) and colour was developed as per the procedure given under estimation of 5FU from cream by cobalt acetate method (chapter 2. section 2.224d).

(iii) Diazotised sulfanilic acid method :

Colour was developed with a suitable aliquot (2.5ml) of the aqueous solution containing 5FU by the procedure described under preparation of calibration curve for 5FU with diazotised primary amines (chapter-2. section 2.233e).

b) For MTX :

(i) UV spectrophotometric method :

An aliquot (1.25ml) of the drug solution was diluted to 10ml with pH7.4 buffer and absorbance was measured at 305nm against the appropriate cream blank solution.

(ii) Folin-ciocalteau method :

Colour was developed with a suitable aliquot (2.5ml) of the drug solution, by the procedure given under preparation of calibration curve for MTX with Folin-ciocalteau reagent. (chapter 2 section 2.333d)

3.254 Results and discussion :

The percentage of drug remaining at each sampling time point obtained by the UV spectrophotometric method and the proposed analytical methods were estimated for 5FU and MTX and the data is presented in Tables 3.16a to 3.17b respectively. It may be observed that 5FU and MTX are stable over a 90 days period in all the four bases at both the temperatures.

The data obtained by the UV and colorimetric methods are statistically similar ($P<0.05$ as per student's 't' test).

From these studies it may be concluded that 5FU and MTX are compatible with all the four bases and that the proposed analytical methods can be used for estimating the drug from semisolid dosage form.

3.26 Stability Studies of Cyclophosphamide :

Cyclophosphamide is susceptible to spontaneous hydrolysis in aqueous solution and undergoes both specific acid and specific base catalysis at extreme pH(3). The reported methods of analysis are high resolution NMR and HPLC(5,6). The aim of the present study was to evaluate whether or not the proposed thiocyanate method was stability indicating. The stability studies were carried out at pH 1.2,2.0,3.9, 7.0,8.0 and 10.0 at temperature of 25°C, 45°C, and 60°C.

3.261 Preparation of drug solution :

A common procedure was used for preparation of drug solution in all buffers. 100 mg of cyclophosphamide was transferred to 100 ml volumetric flask, dissolved, and the volume made up with the respective buffer and filtered.

5ml of drug solutions were filled in amber coloured ampoules and sealed. The initial concentration of cyclophosphamide was determined in triplicate by the proposed analytical method. The ampoules were then placed at 3 different temperatures 25°C, 45°C and 60°C. At specified time intervals upto 30 days, samples were withdrawn in

TABLE 3.18

STABILITY DATA OF CYCLOPHOSPHAMIDE AT pH 1.2 BY FERRO THIOCYANATE METHOD

Time in hours	%Drug remaining undegraded (±S.D.)		
	25^0C	45^0C	60^0C
0	100.00 (-)	100.00 (-)	100.00 (-)
1	99.65 (1.521)	94.19 (1.291)	70.53 (1.945)
2	99.31 (0.934)	88.73 (1.357)	49.74 (0.632)
3	98.95 (1.239)	83.58 (1.253)	35.07 (1.777)
5	98.27 (0.752)	74.17 (0.975)	17.45 (2.052)
7	97.59 (0.811)	65.82 (1.851)	3.041 (0.192)
10	96.57 (2.015)	55.01 (1.355)	-
24 (1 day)	91.96 (1.679)	23.83 (0.547)	-
72 (3 day)	77.76 (1.021)	1.351 (0.329)	-
168 (7 days)	55.62 (0.775)	-	-
360 (15 days)	28.45 (1.923)	-	-

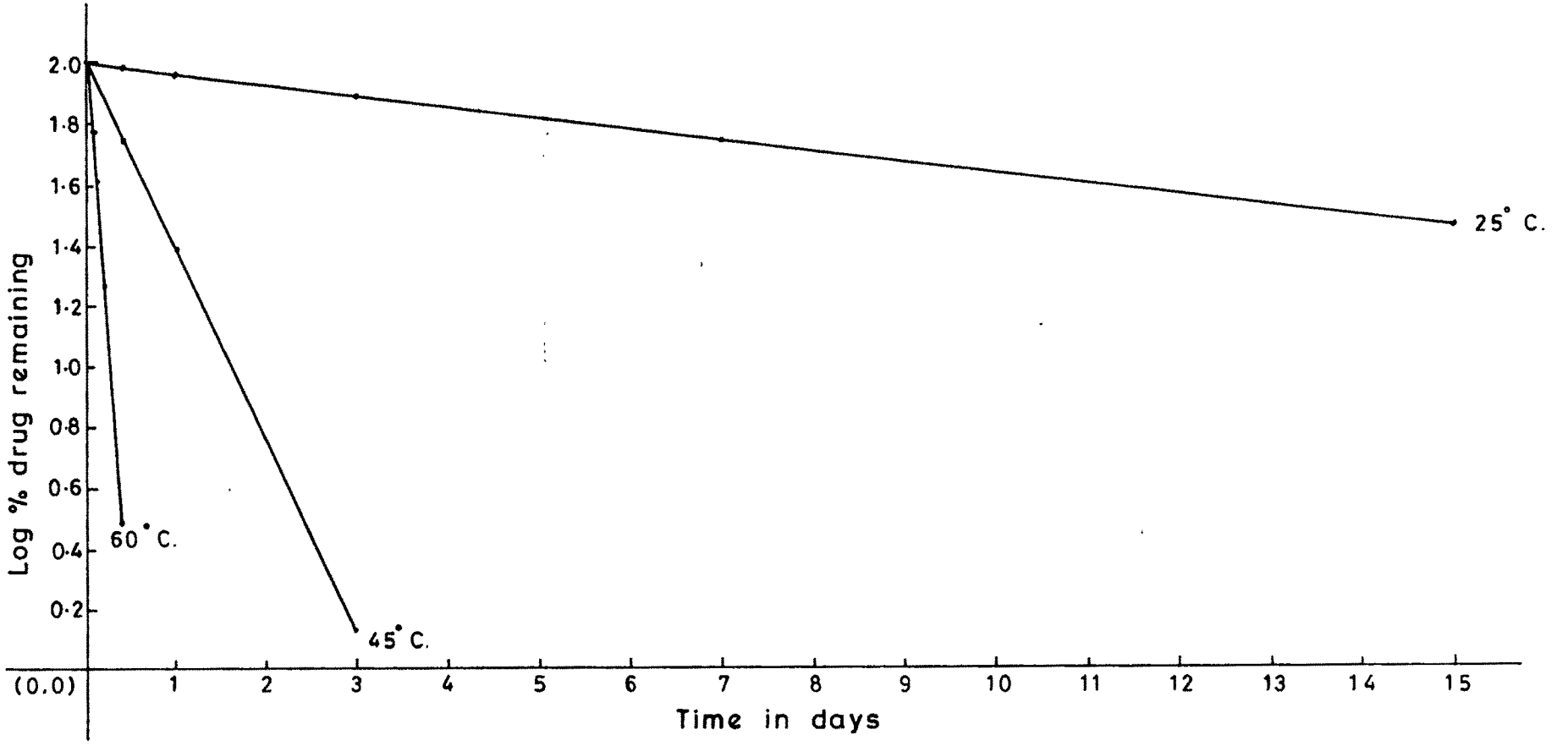

FIG. 3.14: LOG % DRUG (CYCLOPHOSPHAMIDE) REMAINING AS A FUNCTION OF TIME AT pH 1·2

TABLE 3.19

STABILITY DATA OF CYCLOPHOSPHAMIDE AT pH 2.0 BY FERROTHIOCYANATE METHOD

Time in hours	%Drug remaining undegraded (±S.D.)		
	25^0C	45^0C	60^0C
0	100.00 (-)	100.00 (-)	100.00 (-)
1	99.89 (1.219)	98.12 (1.287)	89.50 (1.055)
2	99.77 (0.756)	96.27 (0.963)	80.05 (0.807)
3	99.67 (1.321)	94.46 (1.649)	71.70 (1.275)
5	99.34 (1.456)	90.95 (1.035)	58.27 (1.334)
7	99.15 (1.151)	87.56 (1.678)	46.02 (1.027)
10	98.86 (1.635)	82.72 (1.257)	32.99 (0.999)
24 (1 day)	97.36 (0.534)	63.43 (1.665)	6.96 (1.453)
72 (3 days)	92.28 (2.154)	25.51 (0.517)	-
168 (7 days)	82.90 (1.029)	4.13 (0.422)	-
360 (15 days)	66.91 (0.541)	-	-

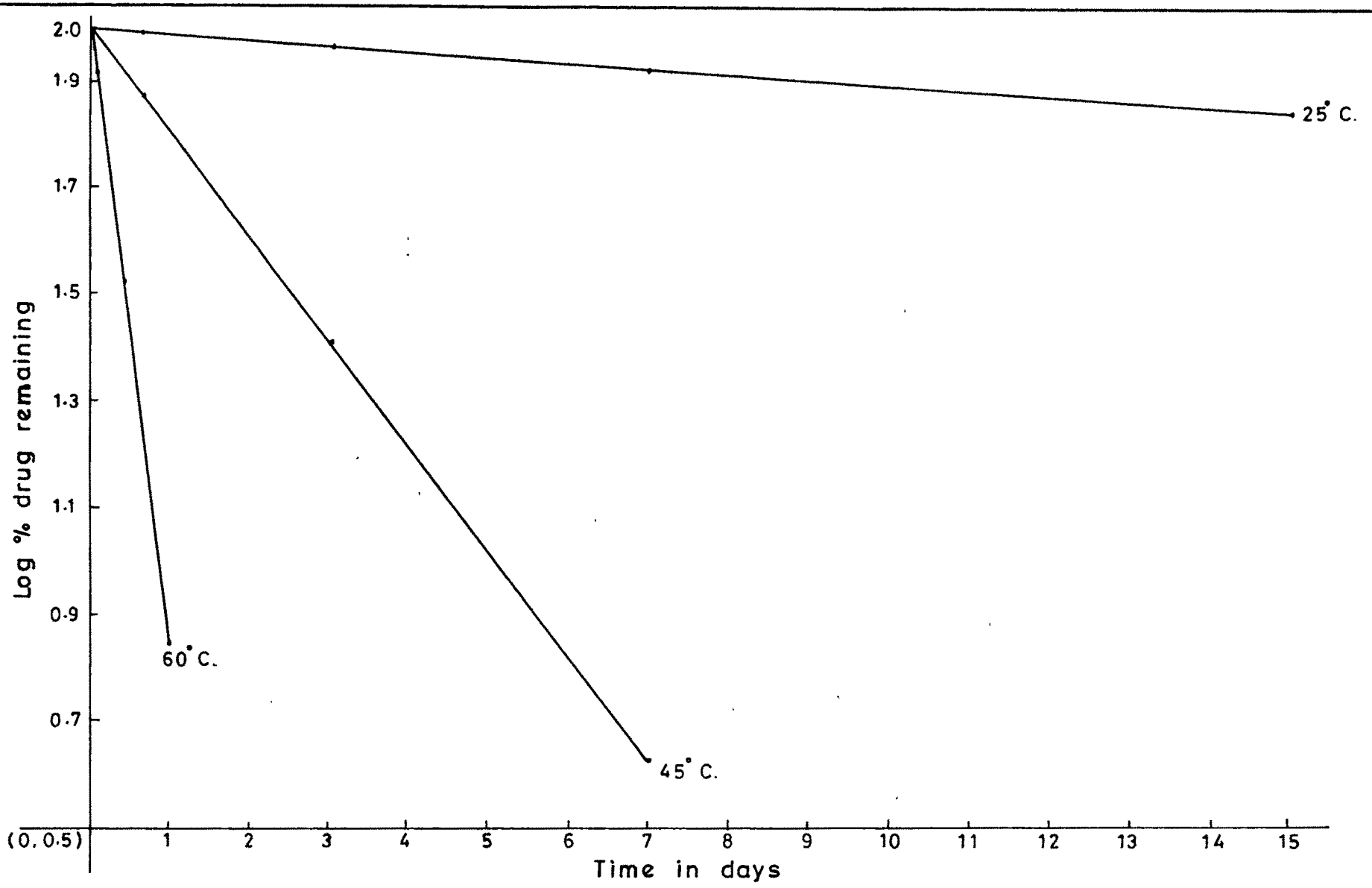

FIG. 3.15: LOG % DRUG (CYCLOPHOSPHAMIDE) REMAINING AS A FUNCTION OF TIME AT pH 2·0

TABLE 3.20

STABILITY DATA OF CYCLOPHOSPHAMIDE AT pH 3.9 PROPOSED ANALYTICAL METHOD

Time in hours	%Drug remaining undegraded (±S.D.)		
	25^0C	45^0C	60^0C
0	100.00 (±)	100.00 (-)	100.00 (-)
1	99.88 (1.879)	98.36 (1.368)	90.44 (1.453)
2	99.65 (1.659)	96.73 (1.543)	81.80 (1.081)
3	99.57 (1.733)	95.15 (0.954)	73.98 (1.625)
5	99.45 (1.493)	92.05 (1.052)	63.49 (1.945)
7	99.22 (1.215)	89.05 (1.119)	49.51 (1.223)
10	98.88 (3.021)	84.73 (1.473)	36.63 (1.366)
24 (1 day)	97.28 (1.825)	67.18 (1.118)	8.98 (1.052)
72 (3 days)	92.18 (1.298)	30.33 (1.034)	-
168 (7 days)	82.70 (1.087)	6.18 (1.618)	-
360 (15 days)	66.57 (1.732)	-	-

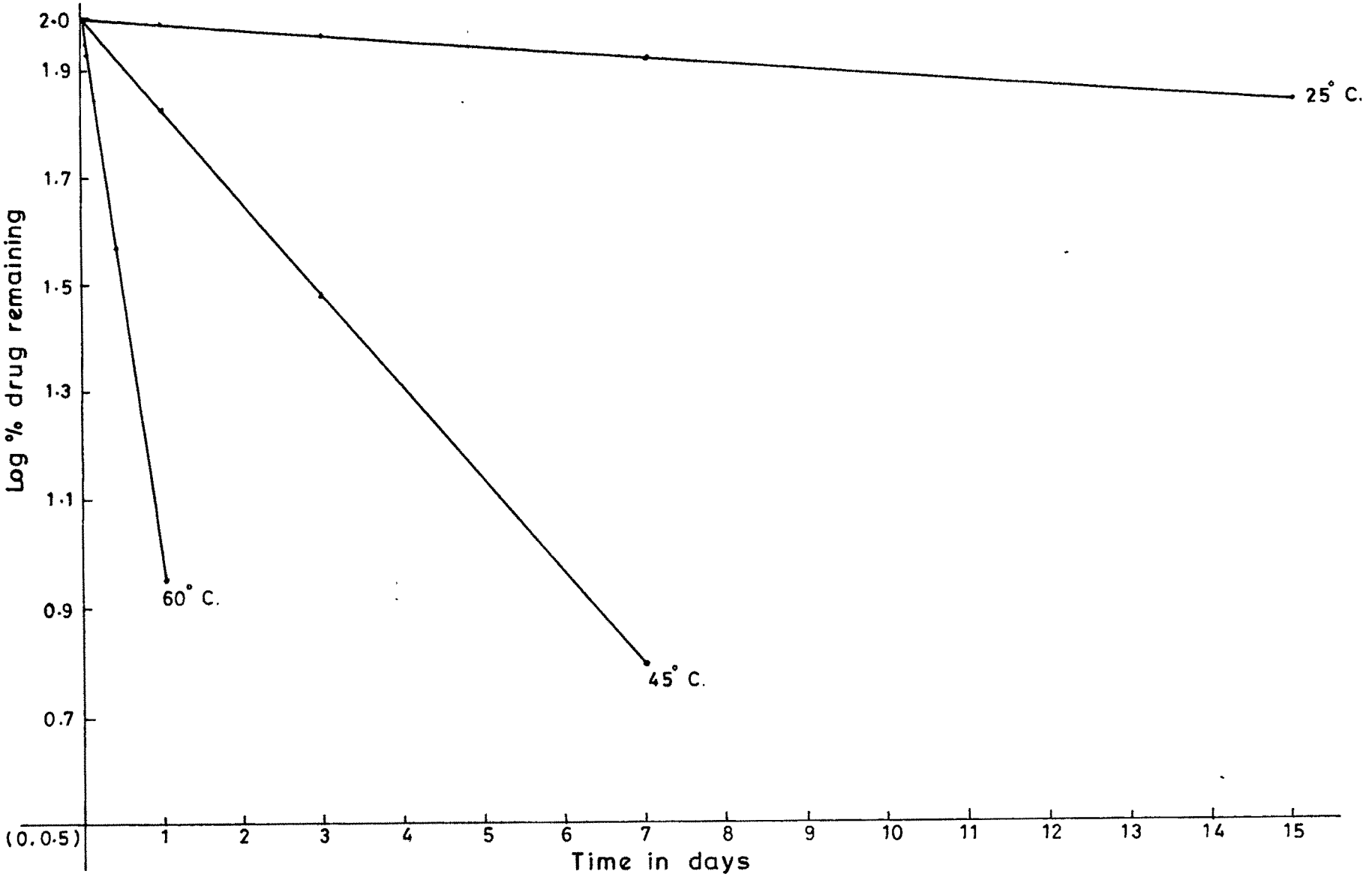

FIG. 3.16 : LOG % DRUG (CYCLOPHOSPHAMIDE) REMAINING AS A FUNCTION OF TIME AT pH 3·9

TABLE 3.21

STABILITY DATA OF CYCLOPHOSPHAMIDE AT pH 7.0 BY FERRO -THIOCYANATE METHOD

Time in hours	%Drug remaining undegraded (±S.D.)		
	25^0C	45^0C	60^0C
0	100.00 (-)	100.00 (-)	100.00 (-)
1	99.92 (1.532)	98.65 (1.562)	91.53 (1.352)
2	99.83 (1.389)	97.34 (1.347)	83.76 (1.673)
3	99.76 (1.345)	96.03 (1.011)	76.67 (1.233)
5	99.62 (0.975)	93.47 (1.002)	64.22 (1.407)
7	99.45 (1.743)	90.78 (0.835)	53.79 (1.389)
10	99.22 (1.378)	87.37 (1.738)	41.25 (1.252)
24 (1 day)	98.13 (1.387)	72.32 (1.242)	11.94 (1.049)
72 (3 days)	94.51 (1.564)	37.83 (1.235)	-
168 (7 days)	87.65 (2.954)	10.35 (1.532)	-
360 (15 days)	75.38 (0.969)	-	-

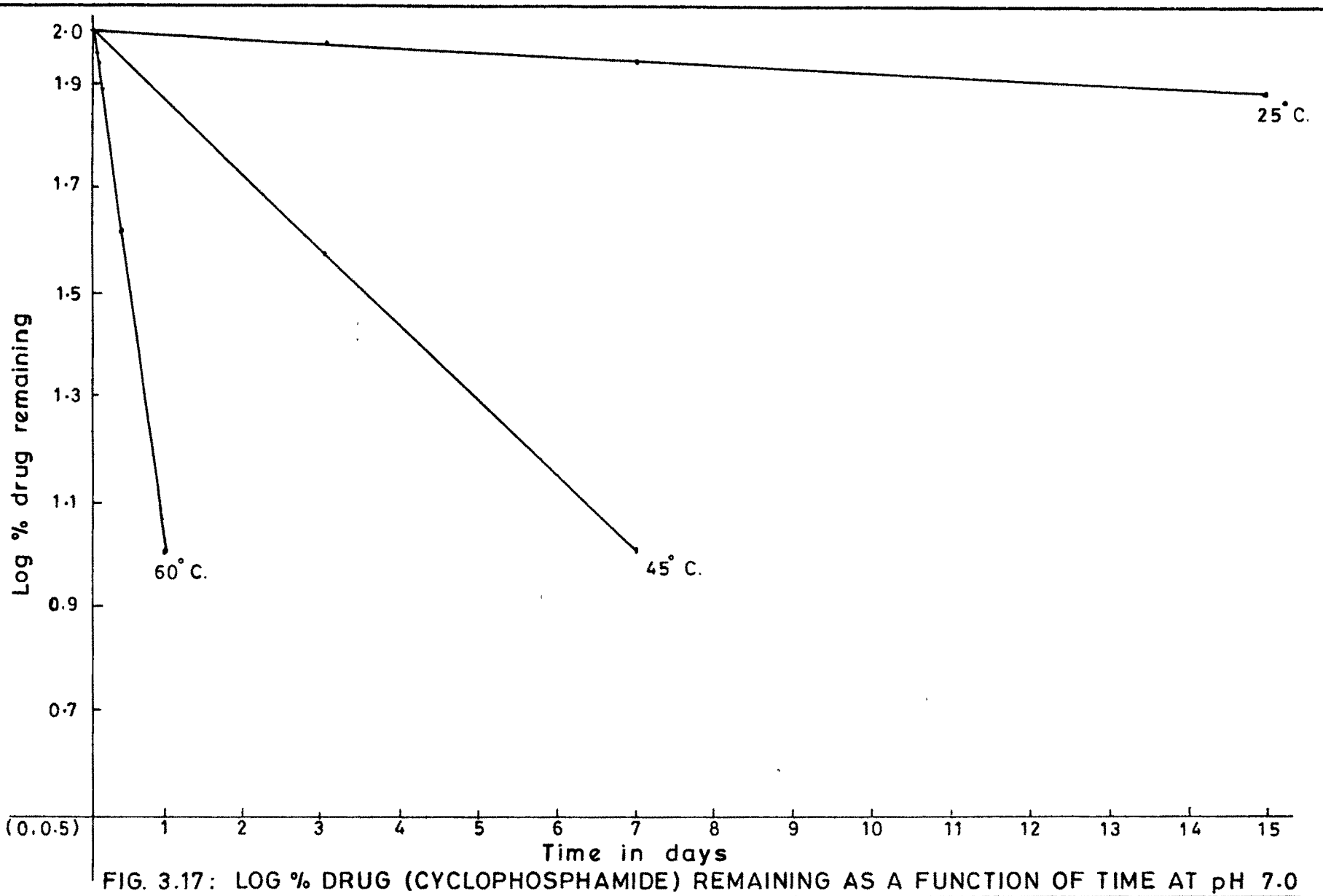

FIG. 3.17: LOG % DRUG (CYCLOPHOSPHAMIDE) REMAINING AS A FUNCTION OF TIME AT pH 7.0

TABLE 3.22

STABILITY DATA OF CYCLOPHOSPHAMIDE AT pH 8.0 BY FERRO-THIOCYANATE METHOD

Time in hours	%Drug remaining undegraded (±S.D.)		
	25^0C	45^0C	60^0C
0	100.00 (-)	100.00 (-)	100.00 (-)
1	99.95 (1.575)	98.88 (1.568)	91.53 (1.352)
2	99.75 (1.075)	97.55 (1.725)	84.62 (1.233)
3	99.65 (1.659)	96.23 (1.363)	75.92 (1.263)
5	99.59 (1.352)	94.33 (1.429)	63.49 (1.057)
7	99.46 (1.649)	90.33 (1.752)	52.65 (1.265)
10	99.12 (1.129)	85.33 (0.956)	41.11 (0.915)
24 (1 day)	97.95 (1.579)	70.23 (1.329)	10.69 (1.133)
72 (3 days)	93.67 (1.763)	35.85 (1.632)	-
168 (7 days)	86.92 (1.269)	11.01 (1.571)	-
360 (15 days)	74.57 (1.747)	-	-

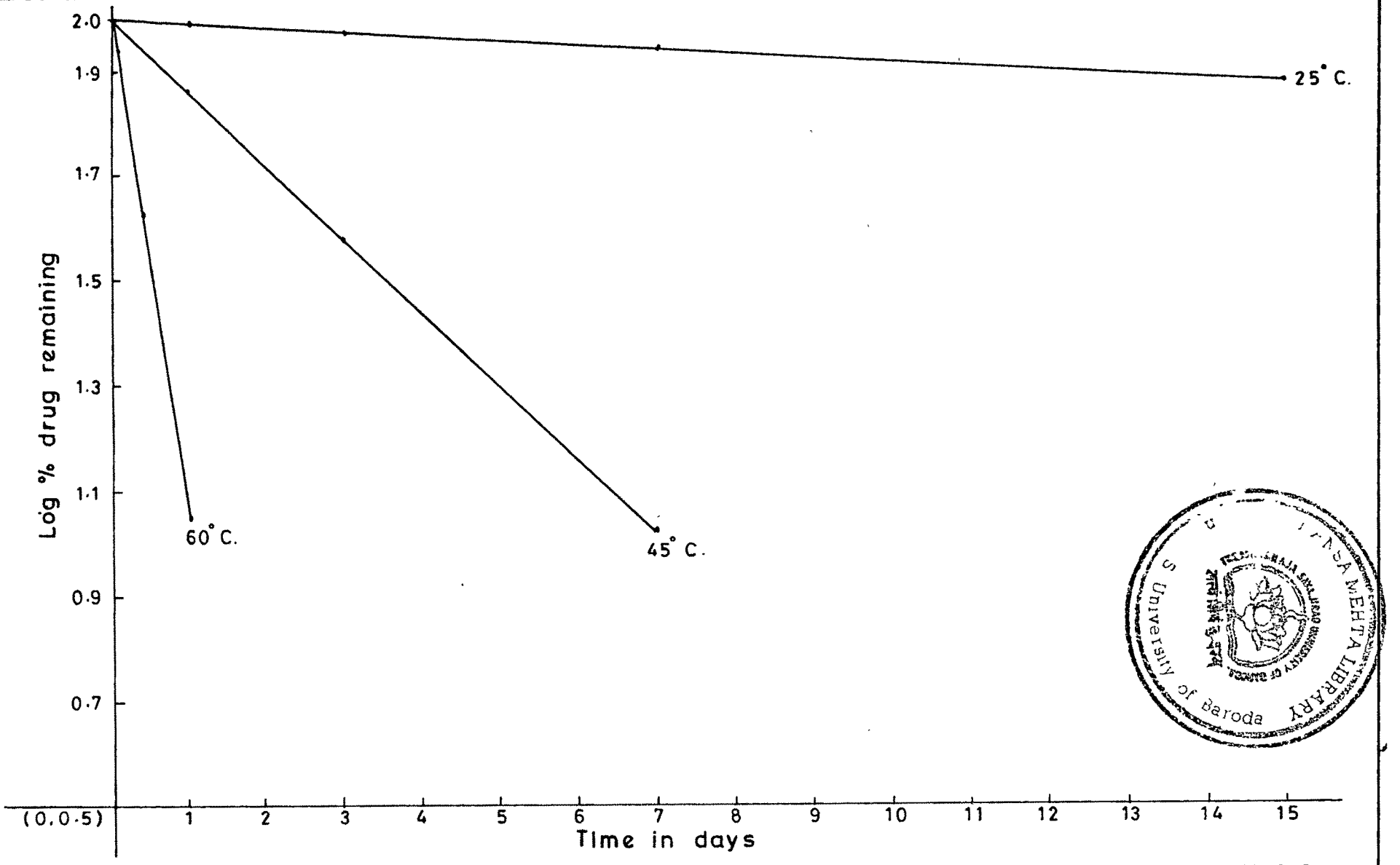

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FIG. 3.18 : LOG % DRUG (CYCLOPHOSPHAMIDE) REMAINING AS A FUNCTION OF TIME AT pH 8·0

TABLE 3.23

STABILITY DATA OF CYCLOPHOSPHAMIDE AT pH 10.0 BY FERRO-THIOCYANATE METHOD

Time in hours	%Drug remaining undegraded (±S.D.)		
	25^0C	45^0C	60^0C
0	100.00 (-)	100.00 (-)	100.00 (-)
1	99.85 (1.573)	98.56 (1.687)	91.47 (1.723)
2	99.75 (1.673)	97.33 (1.322)	83.68 (1.658)
3	99.64 (0.953)	96.11 (1.093)	76.64 (1.466)
5	99.57 (1.095)	92.55 (1.523)	63.95 (1.393)
7	99.51 (1.593)	89.75 (1.598)	52.89 (1.278)
10	99.23 (1.329)	85.45 (1.698)	40.93 (3.013)
24 (1 day)	98.32 (1.893)	72.13 (2.059)	10.97 (1.791)
72 (3 days)	95.01 (1.009)	36.89 (1.965)	-
168 (7 days)	86.68 (0.867)	10.53 (0.327)	-
360 (15 days)	74.56 (1.037)	-	-

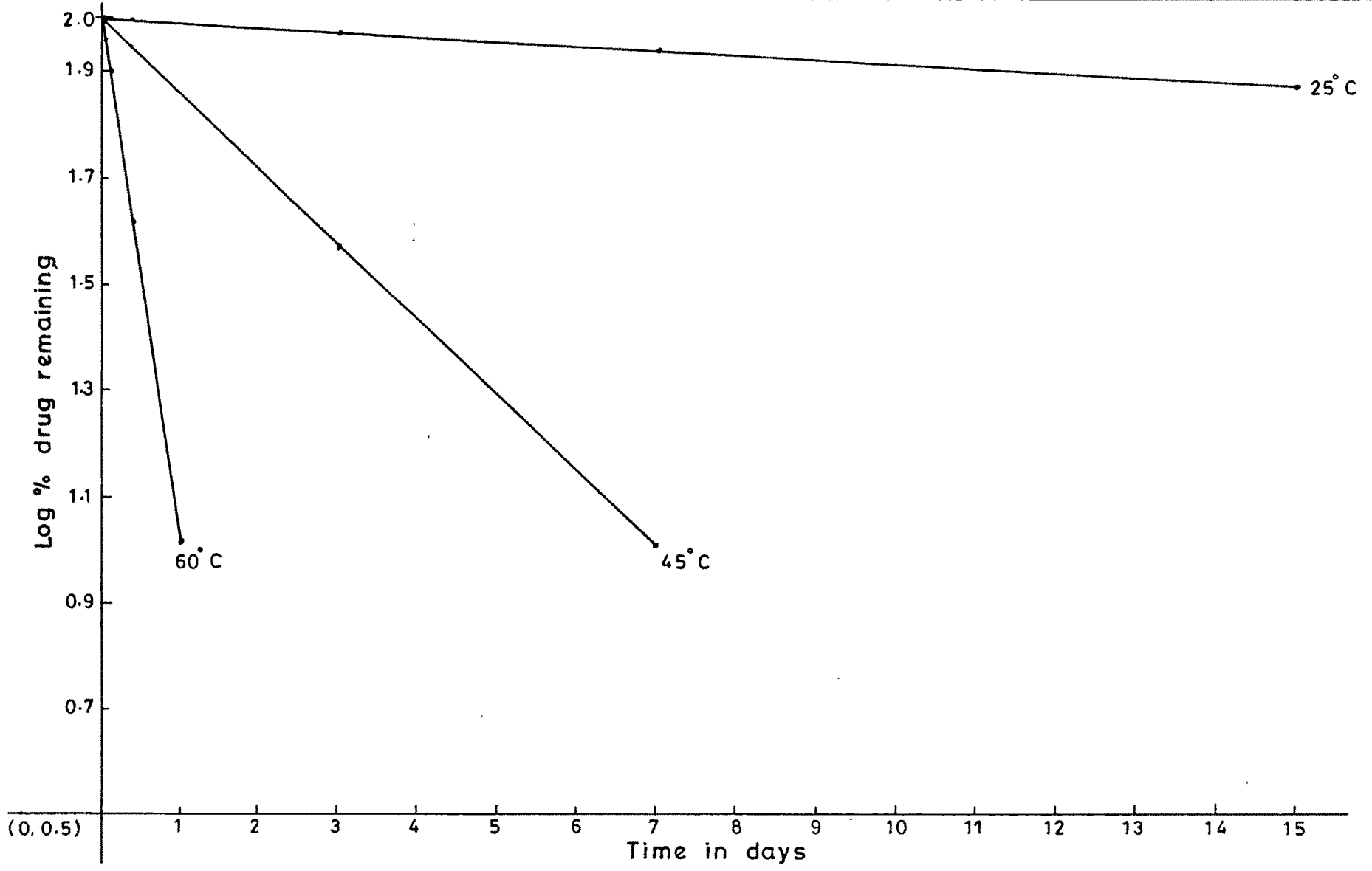

FIG. 3.19: LOG % DRUG (CYCLOPHOSPHAMIDE) REMAINING AS A FUNCTION OF TIME AT pH 10.0

TABLE 3.24

STABILITY PARAMETERS FOR CYCLOPHOSPHAMIDE AT VARIOUS pH AND TAMPERATURES

pH	Stability parameter $K = s^{-1}$ t1/2 and t90=days	60^0c		45^0c		25^0c	
		Reported data	Ammonium ferro-thiocyanate method	Reported data	Ammonium ferro-thiocyanate method	Reported data	Ammonium ferro-thiocyanate method
1.2	K	9.70×10^{-5}	9.10×10^{-5}	1.66×10^{-5}	1.66×10^{-5}	9.78×10^{-7}	9.30×10^{-7}
	t1/2	0.083	0.088	0.483	0.486	8.201	8.62
	t90	0.0125	0.013	0.073	0.074	1.243	1.307
2.0	K	2.98×10^{-5}	2.80×10^{-5}	5.27×10^{-6}	5.24×10^{-6}	3.10×10^{-7}	2.96×10^{-7}
	t1/2	0.269	0.286	1.522	1.531	25.874	28.04
	t90	0.041	0.043	0.231	0.232	3.920	4.249
4.0	K	2.79×10^{-5}	2.84×10^{-5}	4.60×10^{-6}	4.66×10^{-6}	3.14×10^{-7}	2.99×10^{-7}
	t1/2	0.287	0.282	1.743	1.721	25.54	26.83
	t90	0.0435	0.043	0.264	0.261	3.870	4.064
7.0	K	2.46×10^{-5}	2.49×10^{-5}	3.75×10^{-6}	3.68×10^{-6}	2.18×10^{-7}	2.28×10^{-7}
	t1/2	0.326	0.322	2.139	2.179	36.79	35.18
	t90	0.049	0.049	0.324	0.330	5.575	5.330
8.0	K	2.46×10^{-5}	2.51×10^{-5}	3.75×10^{-6}	3.70×10^{-6}	2.18×10^{-7}	2.29×10^{-7}
	t1/2	0.326	0.319	2.139	2.168	36.793	35.03
	t90	0.049	0.048	0.324	0.328	5.574	5.307
10.0	K	2.47×10^{-5}	2.51×10^{-5}	3.74×10^{-6}	3.67×10^{-6}	2.19×10^{-7}	2.26×10^{-7}
	t1/2	0.325	0.319	2.145	2.186	36.62	35.49
	t90	0.049	0.048	0.325	0.331	5.549	5.377

triplicate and concentration of the drug was determined by the proposed analytical method.

3.262 Method of analysis for stability studies of cyclophosphamide :

Colour was developed with a suitable aliquot (0.5ml) of the drug solution by the procedure given under calibration curve for cyclophosphamide with ammonium ferrothiocyanate (chapter 2. section 2.423d).

3.263 Results and discussion :

The data of percentage drug remaining at each sampling time point obtained by the proposed analytical method at different pH values are recorded in Tables 3.18-3.23. The mean log percentage concentration of drug remaining vs time curves were plotted for each pH value at three different temperatures (Figs 3.14-3.19). The slopes of these lines gave stability constant values K. The K, t1/2 and t90 values obtained by the proposed analytical method along with the reported data are recorded in Table 3.24 and these values were compared statistically using student's 't' test.

From this data it may be inferred that cyclophosphamide in aqueous solutions undergoes first order degradation which is temperature and pH dependent.

From Table 3.24 it may be inferred that no statistically significant difference ($P<0.05$) exists in the stability rate constant K, t1/2 and t90 values obtained by proposed analytical method and reported data.

The activation energy (Ea) values for each pH were calculated graphically (Fig. 3.20) and the data is recorded

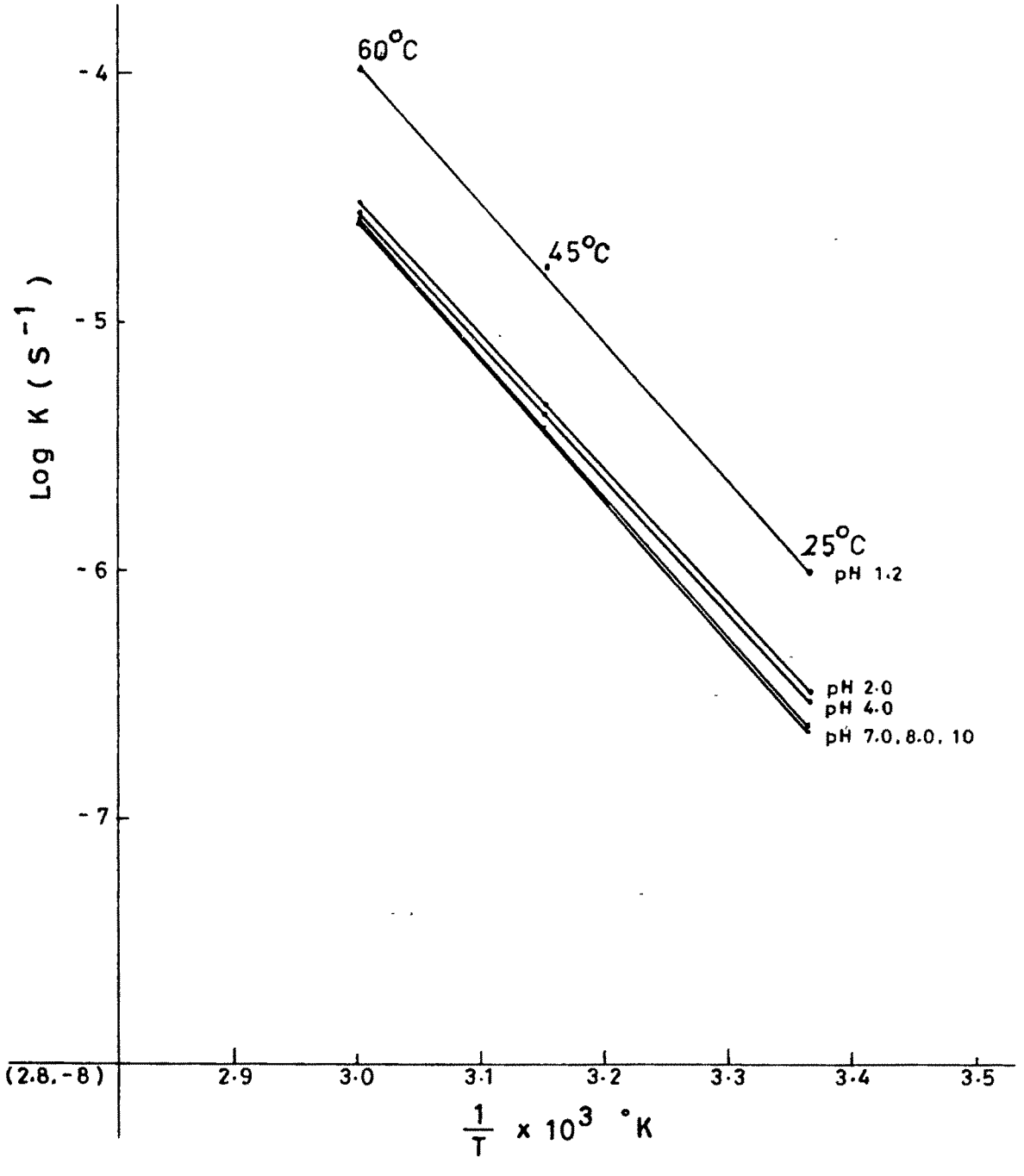

FIG. 3.20: ARRHENIUS PLOTS FOR CYCLOPHOSPHAMIDE AT DIFFERENT pH VALUES

TABLE 3.25

ACTIVATION ENERGIES FOR CYCLOPHOSHAMIDE AT DIFFERENT pH VALUES

pH	Ea (Kcal/mol)	
	Reported data	Ammonium ferrothiocyanate method
1.2	24.77	23.96
2.0	24.51	24.21
3.9	25.28	25.35
7.0	26.39	26.82
8.0	26.39	26.86
10.0	26.48	26.97

in Table 3.25. These values were statistically compared with the reported data (Table 3.25) and they were found to be comparable.

The results of the above experiment indicate that the proposed analytical method is stability indicating.

3.3 REFERENCES

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