



Degradation kinetics of metronidazole and its mutual prodrug with ciprofloxacin: a calorimetric analysis

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ABSTRACT

Calorimetric technique has aroused considerable interest as a versatile tool in pharmaceutical industry and academia to provide useful information about thermodynamic and kinetic aspects of drug molecules. The present paper utilizes this technique to monitor the hydrolytic degradation of metronidazole and its prodrug with ciprofloxacin, i.e. 2-(2-methyl-5-nitroimidazol-1-yl)ethyl-1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-quinoline-3-carboxylate. The synthesis of the present mutual prodrug was envisaged to combine the antiprotozoal and anaerobic antibacterial effects of metronidazole with antibacterial effects of ciprofloxacin. Heat flux microcalorimeter was used to determine the rate of heat evolved during the degradation of the drug and prodrug as a function of concentration, pH and temperature. In terms of enthalpy of hydrolysis the response is exothermic both for drug and prodrug. However, the absolute value of the enthalpy of reaction ($\Delta_r H^0$) is low for the prodrug. The degradation followed pseudo first order kinetics, showed marked stability at pH 3-7 followed by accelerated hydrolysis at higher pH, characteristic of general acid-base catalysis. The catalytic rate constant for hydrogen ion (k_H) and hydroxyl ion (k_{OH}) were found to be 0.413 and 526.1 M⁻¹h⁻¹, respectively, at 318.15 K. The hydrolysis of the prodrug was found to be approximately 50-60 times faster than that of the drug. This may be attributed to the fact that hydrolysis of ester group in prodrug is assisted by keto group on the ciprofloxacin. However, there is no effect of protonation of nitrogen in piperazine ring in ciprofloxacin on the hydrolysis due to the distance from the ester moiety.

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INTRODUCTION

Metronidazole is one of the rare examples of a drug developed against protozoa which has since gained broad use as an antibacterial agent (Freeman et al., 1997; Samuelson, 1999; O'Keefe et al., 1982). For some of the possible infections for which metronidazole might be used, there are very few alternatives. It is often prescribed along with other antibacterials which have the advantage of having broad spectrum of activity and are effective against both bacteria and protozoa (Werk et al., 1988; Shen et al., 2001; Prantera et al., 1996; Goldstein et al., 1991; Houben et al., 1999; Whiting et al., 1987; Ralph et al., 1980; Pavicic et al., 1992).

Ciprofloxacin is one of the most important members of the fluoroquinolone antibacterials having a wide range of antibacterial activity. It is advantageous to combine the antiprotozoal and anaerobic antibacterial action of metronidazole with antibacterial action of ciprofloxacin to form a broad spectrum chemotherapeutic agent (Werk et al., 1988; Mimura et al., 2002) by synthesizing a mutual prodrug. The alcoholic functional group in metronidazole is readily esterifiable and therefore, it appeared to be possible to obtain ester derivatives providing the desired improvement in water solubility (Vermeersch et al., 1990) and at the same time possessing a high susceptibility to undergo enzymatic

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hydrolysis in the body (Yang et al., 2001; Mahtouz et al., 1998; Johnansen et al., 1984; Johnansen et al., 1985).

We report here the synthesis of this prodrug and the kinetics of its hydrolysis using the technique of calorimetry. However, it is known in the literature that metronidazole itself undergo degradation (Baveja et al., 1973; Wang et al., 1993; Baveja et al., 1975). Therefore we have also studied the kinetics of degradation of metronidazole calorimetrically. This is a rapid and general technique for the determination of both thermodynamic and kinetic parameters of chemical reactions. It's indifferent to physical state of material and thus, this technique has found its niche through the high level of sensitivity and the versatility to perform a wide range of experiments for different applications (Buckton et al., 1991; Skaria et al., 2005; Angberg et al., 1988; Beezer et al., 1999; Oliyai et al., 1991; Phipps et al., 2000; Beezer, 2001; Chadha et al., 2003a, 2003b). The major advantage of the calorimetric method over other methods for measuring the rate of decomposition of organic materials is that it is the only technique, which determines the total enthalpy change associated with the degradation process. The present study was undertaken with the aim of further exploring the potential of calorimetric technique in determining the degradation of metronidazole and its prodrug and correlate the degradation enthalpy with tentative mechanism.

MATERIALS AND METHODS

Materials

Metronidazole was obtained from Rajasthan DPL, India and ciprofloxacin from Dr. Reddy's Labs., Hyderabad, India. Solvents used for synthesis and crystallization were of high purity grade and were freshly distilled before use. Buffers were prepared according to the procedure given in Indian Pharmacopoeia (TCP, 1996).

Apparatus and reagents

A C-8-calorimeter (SETARAM, France) was used to carry out calorimetric studies. Infrared and ultraviolet spectra were recorded on a Perkin-Elmer 882 infrared spectrophotometer and Perkin-Elmer 181, respectively. ¹H-NMR were recorded on Bruker AC 300F (300MHz) equipment using

trimethylsilane (TMS) as internal standard. Melting points were recorded in Veego capillary melting point apparatus in silicon oil. High sensitivity analytical balance, Mettler 433AR model was used for weighing.

Preparation of 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-quinoline-3-carboxylate

To ciprofloxacin (2.0 g, 6.35 mmol) in tetrahydrofuran (20 ml, 277.7 mmol) thionyl chloride (2 ml, 27.6 mmol) was added. This mixture was stirred for 15 minutes. The excess of thionyl chloride was removed under pressure. The product obtained was used for the next step.

Preparation of 2-(2-methyl-5-nitroimidazol-1-yl)ethyl-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-quinoline-3-carboxylate

The above-obtained acid chloride of ciprofloxacin (1.8 g, 5.12 mmol) was dissolved in methylene chloride (40 ml). This solution was added drop wise to a mixture of metronidazole (1 g, 5.8 mmol), pyridine (1 ml) and methylene chloride (40 ml) under anhydrous conditions (4 g of 4A⁰ molecular sieves). The resulting solution was stirred at room temperature for 25 h. The solvent was removed *in vacuo*. The residue was stirred with 1M sodium carbonate solution (50 ml) for 15 minutes and extracted with chloroform three times. The chloroform extract was washed with 10% w/v hydrochloric acid (50 ml) and then with water (3 X 50 ml). The chloroform extract was dried over anhydrous sodium sulphate and solvent was evaporated *in vacuo* to give the desired product (1.5 g, 71.5%). It was recrystallized from acetone to obtain the compound, which charred at 170°C.

λ_{\max} (in water): 197.6nm, 230.6nm, 318.8nm
 R_f : 0.29 in chloroform: methanol (9.5:0.5)
IR(KBr): 1730 cm^{-1} , 1540 cm^{-1} , 1460 cm^{-1} , 1360 cm^{-1} , 1260 cm^{-1} , 1190 cm^{-1} , 820 cm^{-1}
¹H NMR (D₂O): 1.12 δ , 1.35 δ , 2.42 δ , 3.41 δ , 3.76 δ , 4.36 δ , 7.35 δ , 7.95 δ and 8.88 δ

Microcalorimetric studies

Heat flux microcalorimeter model C-80 (SETARAM, France) was used to study the degradation of 2-(2-methyl-5-nitroimidazol-1-yl)ethyl-cyclopropyl-6-fluoro-1,4-dihydro-4-

oxo-7-(1-piperazinyl)-quinoline-3 carboxylate. In accordance with the Calvet principle, two experimental vessels (reference and sample) were placed in a calorimetric block. The temperature control by the thermostat of calorimeter was within ± 0.001 K. To carry out the study, reference cell was loaded with 5 ml of buffer solution of desired pH and sample cell with a definite quantity of drug or prodrug and 5 ml of the same buffer solution partitioned by a lid. After stabilization, mixing was done in the calorimeter itself by reversing it, which homogenizes the sample with the buffer solution. The reactions were followed for 10-12 hours. The rate of heat evolution by the sample is proportional to the rate at that temperature (Chadha et al, 2003).

RESULTS

Heat evolved as a function of time has been recorded for solution of metronidazole at different concentration in the temperature range (298.15 – 318.15 K) and in aqueous solution (pH range 2-10). Similar studies are carried out on its prodrug with ciprofloxacin also. The calorimetric data of heat evolution vs. time for drug (14.035×10^{-3} M) and prodrug (6.1939×10^{-3} M) at pH 9 at 318.15 K are plotted (Figure 1).

It is assumed that total heat produced during degradation is proportional to the initial concentration of drug ($q_0 = c_0 V \Delta_r H^0$) for first order reaction (Beezer et al, 1999).

Here c_0 is the initial concentration of the drug, V is the volume of the solution taken in the calorimeter and $\Delta_r H^0$ is the enthalpy of degradation reaction. The heat evolved up to time t,

$$q_t = (c_0 - c)V\Delta_r H^0 = q_0 - cH\Delta_r H^0$$

Where, c = concentration of the drug at any time 't'

It can be shown that

$$\frac{dq_t}{dt} = k(q_0 - q_t)$$

$$dq_t / (q_0 - q_t) = k dt$$

$$\ln(q_0 - q_t) = kt + \text{constant}$$

$$\ln q_0 / (q_0 - q_t) = kt$$

From the calorimetric data we know q_t but due to slowness of reaction, only a small fraction

of the drug degrades and therefore for determining q_0 we follow the following procedure. The rate of heat evolved (dq_t/dt) as a function of time is related to the rate of reaction (Φ) at that time. Now by determining the Φ at two intervals t_1 and t_2 , q_0 was calculated.

We have at $t = t_1$, $\Phi_1 = k(q_0 - q_{t1})$

$$t = t_2, \Phi_2 = k(q_0 - q_{t2})$$

If $dq_t/dt = \Phi_1 = k(q_0 - q_{t1})$

$$\Phi_2 = k(q_0 - q_{t2})$$

$$\Phi_2 / \Phi_1 = q_0 - q_{t2} / q_0 - q_{t1}$$

or

$$q_0 = q_{t1} + \frac{q_{t1} - q_{t2}}{\Phi_2 / \Phi_1 - 1} \dots\dots\dots 1$$

where

q_0 = total heat evolved in degradation reaction starting with V litre of solution with initial concentration (c_0)

q_{t1} = heat evolved up to time t_1

q_{t2} = heat evolved up to time t_2

Φ_1 = rate of heat evolved at t_1

Φ_2 = rate of heat evolved at t_2

and $\Delta_r H^0 = q_0 / c_0 V \dots\dots\dots 2$

The q_0 is estimated using equation 1. Table 1 gives value of heat evolved up to various interval of time for drug (7.0175×10^{-5} moles) and prodrug (3.09695×10^{-5} moles) at pH 8 and temperature 318.15 K. The values for q_0 have been calculated to be 14.036 J and 4.345 J, respectively. The enthalpies of reaction ($\Delta_r H^0$) calculated from the (q_0) total heat evolved at various concentrations and pH for both drug and prodrug at different temperatures are given in tables 2, 3 and 4. It can be seen that the value differs slightly with pH and temperature but is otherwise in excellent agreement with each other for two different initial concentrations of the drug. It may be noted that q_0 is proportional to initial concentration of the drug where $(q_0 - q_t)$ is proportional to concentration of the drug at time t. For the first order reaction the rate constant is given by the equation

$$\ln q_0 - \ln(q_0 - q_t) = kt$$

or

$$k = \frac{1}{t} \ln \frac{q_0}{q_0 - q_t} \dots\dots\dots 3$$

The plots between $\ln(q_0 - q_t)$ against t are straight lines (Figure 2) at various concentrations and pH. The values of first order rate constant, k , calculated from the slope of the straight line plot at different concentration, pH and temperature for drug and prodrug are given in table 2, 3 and 4. The effect of pH on the degradation of metronidazole in aqueous solution at constant ionic strength (0.2) and at 37°C is shown in plots of $\log k$ vs pH (Figure 3). The temperature dependence of metronidazole and prodrug were studied at all the pH in the temperature range 303.15 to 318.15 K. The investigation is aimed to determine the optimum pH for the maximum stability. An Arrhenius plot of \log of k vs reciprocal of temperature is given in Figure 4.

DISCUSSION

The values of k , the rate constant calculated from the slope of plot of $\ln(q_0 - q_t)$ vs t (Tables 2, 3 and 4), for two concentrations at a particular pH and temperature indicate that the degradation follows pseudo first order kinetics for drug as well as prodrug. The absolute value of rate constant for decomposition changes with pH. Metronidazole has one ionizable group with a pKa value of 2.5. It was found that the results at different pH are in agreement with the following equation:

$$K_{obs} = \{k_H[H^+] + k_{up} + k_{OH}[OH^-]\} f^{up}$$

Where f^{up} is the fraction of unprotonated species at a particular pH and the values have been given in Table 5. The experimental k_{obs} are compared with those calculated from the above equations and presented in the table 2, 3 and 4. It can be seen that there is good agreement at all the pH. The first order rate constant for solvent assisted degradation is represented by k_{up} while k_{OH} is the second order rate constants for base catalyzed degradation. The degradation of metronidazole followed a first order reaction in both acidic and alkaline conditions and exhibited maximum stability at pH 4-6. This trend is in agreement with the earlier results published by Wang and Yeh (1993). However, direct comparison of the rate constants could not be made as the authors

have studied the degradation kinetics under accelerated conditions.

The degradation of metronidazole below pH 3 is described by catalytic effect of specific acid and water. The figure 3 shows that there is no inflection point in the pH vs rate constant of the reaction thus predicting to be a H^+ and OH^- catalyzed reaction. Not much data is available in literature about the degradation products. However, Salo and Solmies (1996) have shown the formation of ethylene diamine and corresponding acid with the breaking of the ring at higher pH. At mild pH, the degradation is governed by solvent assisted degradation while in alkaline condition there is reaction between unprotonated metronidazole and OH^- . Study by Baveja and Khosla (1975) has shown the formation of amine at lower pH also. There is a possibility that at lower pH the drug decomposes and loses its nitroimidazole structure. Calorimetric technique used in this study supports this hypothesis by comparing

Table 1: Heat evolved (q_t) at various time intervals (T) for metronidazole (7.0175×10^{-5} moles in 5ml) and its prodrug (3.09695×10^{-5} moles in 5ml) at pH 8 and 318.15K

T (min ⁻¹)	10 ² x q _t (J)		ln(q ₀ -q _t)	
	Drug	Prodrug	Drug	Prodrug
30	0.630	6.394	2.641	1.454
60	1.260	13.673	2.641	1.437
120	2.519	26.909	2.640	1.405
240	5.032	52.146	2.638	1.341
420	8.795	87.105	2.635	1.245
510	10.671	103.368	2.634	1.197
600	12.547	118.870	2.633	1.149
720	15.043	134.568	2.631	1.098
840	17.535	156.756	2.629	1.021
960	20.022	173.959	2.627	0.958

qt = heat evolved at any time 't'; q0 = total heat evolved

Table 2: Kinetic parameters of degradation of metronidazole and its prodrug at 303.15 K.

pH	10 ³ X Conc. (M)		Δ _r H ⁰ (kJ mol ⁻¹)		q ₀ (J)		10 ⁴ X k (h ⁻¹) Experimental		10 ⁴ X k (h ⁻¹) Calculated		t _{1/2} (hrs)	
	Drug	Prodrug	Drug	Prodrug	Drug	Prodrug	Drug	Prodrug	Drug	Prodrug	Drug	Prodrug
2	8.187	3.3034	222.92	129.56	9.12	2.14	3.670	164.94	3.580	164.89	1946.64	42.02
	14.03	6.1939	222.82	129.81	15.64	4.02	3.630	164.70			1985.76	42.08
3	8.187	3.3034	205.41	132.49	8.41	2.19	1.899	103.70	1.932	103.79	3664.80	49.49
	14.035	6.1939	205.22	132.39	14.40	4.11	1.897	103.84			3676.32	66.74
4	8.187	3.3034	211.09	136.89	8.64	2.27	1.244	84.62 84.99	1.267	84.7	5570.64	80.64
	14.035	6.1939	211.39	136.91	14.83	4.24	1.248	81.88 81.91			5552.88	81.49
5	8.187	3.3034	215.14	141.18	8.81	2.33	1.15	82.91 82.95	1.181	82.33	6041.76	84.64
	14.035	6.1939	214.97	140.95	15.09	4.37	1.15	90.25 90.25			6020.88	84.60
6	8.187	3.3034	216.38	144.58	8.86	2.39	1.17	186.49	1.189	83.00	5928.24	83.58
	14.035	6.1939	216.89	144.34	15.22	4.48	1.17	186.53			5912.88	83.54
7	8.187	3.3034	217.41	148.21	8.90	2.45	1.36 1.35	1107.0	1.352	92.20	5099.28	76.79
	14.035	6.1939	217.16	148.02	15.24	4.58	3.19	1107.84			5118.24	76.79
8	8.187	3.3034	211.77	148.33	8.67	2.45	3.21		3.208	184.4	2171.76	37.16
	14.035	6.1939	211.50	148.50	14.84	4.60	21.57				2162.16 321.36	37.15 6.26
9	8.187	3.3034	205.19	149.38	8.40	2.72	21.48		21.55	1106.46	322.56	6.25
	14.035	6.1939	205.08	149.79	14.39	4.67						

Δ_rH⁰ = Enthalpy of reaction; q₀ = Total heat evolved; k = rate constant

Table 3: Kinetic parameters of degradation of metronidazole and its prodrug at 310.15 K.

pH	10 ³ X Conc. (M)		Δ _r H ⁰ (kJ mol ⁻¹)		q ₀ (J)		10 ⁴ X k (h ⁻¹) Experimental		10 ⁴ X k (h ⁻¹) Calculated		t _{1/2} (hrs)	
	Drug	Prodrug	Drug	Prodrug	Drug	Prodrug	Drug	Prodrug	Drug	Prodrug	Drug	Prodrug
2	8.187	3.3034	219.97	124.08	9.00	2.05	6.448	217.46	6.594	217.63	1035.87	31.87
	14.035	6.1939	220.43	126.41	15.47	3.27	6.522	217.57			1062.88	31.85
3	8.187	3.3034	200.44	128.54	8.21	2.12	3.385	138.32	3.486	138.38	2093.04	50.10
	14.035	6.1939	200.84	128.42	14.09	3.98	3.420	138.47			2026.32	50.05
4	8.187	3.3034	200.20	133.91	8.19	2.21	2.165	113.69	2.233	113.74	3192.00	60.98
	14.035	6.1939	205.08	134.25	14.39	4.16	2.163	113.77			3174.48	60.91
5	8.187	3.3034	208.16	136.88	8.52	2.26	1.980	110.21	2.072	110.69	3499.92	62.88
	14.035	6.1939	209.87	136.71	14.73	4.23	1.982	110.20			3496.56	62.89
6	8.187	3.3034	209.53	140.56	8.58	2.32	2.021	111.32	2.085	111.53	3430.80	62.25
	14.035	6.1939	209.63	140.53	14.71	4.35	2.079	111.45			3331.68	62.18
7	8.187	3.3034	211.01	143.44	8.64	2.37	2.292	122.78	2.337	123.15	3026.16	56.44
	14.035	6.1939	210.78	143.51	14.79	4.44	2.338	123.68			2961.60	56.03
8	8.187	3.3034	204.57	144.70	18.37	2.40	5.15	239.65	5.292	239.68	1345.92	28.92
	14.035	6.1939	205.00	145.17	14.39	4.50	5.24	240.11			1323.60	28.86
9	8.187	3.3034	198.81	145.85	8.14	2.41	34.162	1405.56	34.47	1405.0	202.80	4.93
	14.035	6.1939	199.11	146.91	13.97	4.5	34.778	1405.38			199.20	4.93

Δ_rH⁰ = Enthalpy of reaction; q₀ = Total heat evolved; k = rate constant

Table 4: Kinetic parameters of degradation of metronidazole and its prodrug at 318.15 K.

pH	10 ³ X Conc. (M)		$\Delta_r H^0$ (kJ mol ⁻¹)		q ₀ (J)		10 ⁴ X k (h ⁻¹) Experimental		10 ⁴ X k (h ⁻¹) Calculated		t _{1/2} (hrs)	
	Drug	Prodrug	Drug	Prodrug	Drug	Prodrug	Drug	Prodrug	Drug	Prodrug	Drug	Prodrug
2	8.187	3.3034	217.31	123.51	8.89	2.044	10.922	294.04	10.828	293.86	637.593	23.568
	14.035	6.1939	217.26	124.39	15.25	3.852	10.934	293.58			642.261	23.605
3	8.187	3.3034	196.33	126.54	8.04	2.091	5.796	189.46	5.980	189.47	1361.28	36.577
	14.035	6.1939	197.21	126.65	13.84	3.922	5.798	189.48			1353.6	36.551
4	8.187	3.3034	202.89	126.31	8.31	2.158	3.901	158.02	4.0257	157.11	1753.2	43.58
	14.035	6.1939	202.79	130.41	14.23	4.039	3.894	156.14			1781.52	44.948
5	8.187	3.3034	204.62	139.83	8.38	2.309	3.620	152.43	3.774	153.1	1912.8	45.463
	14.035	6.1939	204.79	134.57	14.37	4.167	3.620	152.50			1963.2	45.442
6	8.187	3.3034	205.75	136.83	8.42	2.260	3.711	154.16	3.789	154.17	1867.92	44.95
	14.035	6.1939	205.81	134.26	14.44	4.159	3.770	154.11			1838.16	44.967
7	8.187	3.3034	206.61	144.13	8.46	2.381	4.062	166.04	4.267	169.09	1706.88	41.735
	14.035	6.1939	206.76	139.16	14.51	4.309	4.518	171.04			1533.12	40.516
8	8.187	3.3034	199.50	140.59	8.17	2.322	8.667	320.09	9.001	318.69	799.2 771.6	21.656
	14.035	6.1939	200.02	140.29	14.04	4.345	8.984	318.58			122.88	21.753
9	8.187	3.3034	194.14	142.88	7.95	2.366	56.350	1794.4	56.35	1814.8	121.68	3.862
	14.035	6.1939	194.45	140.90	13.65	4.364	56.920	1835.3				3.7393

$\Delta_r H^0$ = Enthalpy of reaction; q₀ = Total heat evolved; k = rate constant

Table 5: Fraction of different species of metronidazole and its prodrug at different pH

pH	f ^p	f ^{up}	f ^{pp}	f ^p	f ^{up}
Drug			Prodrug		
2	0.7597	0.2403	0.7597	0.2403	3.808 X 10 ⁻⁸
3	0.2403	0.7597	0.2403	0.7597	1.204 X 10 ⁻⁶
4	0.0307	0.9693	0.03065	0.9693	1.536 X 10 ⁻⁵
5	0.0032	0.9968	0.00315	0.99669	0.00016
6	0.0003	0.9997	0.00032	0.99810	0.00158
7	-	-	3.1128x10 ⁻⁵	0.98437	0.01560
8	-	-	2.7296x10 ⁻⁶	0.86319	0.13681
9	-	-	1.2234x10 ⁻⁷	0.38686	0.61314

f^p = fraction of protonated species at particular pH; f^{up} = fraction of unprotonated species at particular pH; f^{pp} = fraction of the total prodrug when both nitrogens are unprotonated.

Table 6: Calculated value of overall enthalpy change

Total bond broken	Enthalpy (kJ mol ⁻¹)	Bond form	Enthalpy (kJ mol ⁻¹)
One H ₂ C-O	426.36	One N-H	351.12
One N=C	936.32	One NH-H	372.02
One HO-H	497.42	One H-NC	351.12
One C-N	330.22	One OH bond attached to CH ₃	334.40
Total	2190.32	One C=O	1070.08
		Total	2478.74
Therefore overall enthalpy = -288.42 kJ mol ⁻¹			

Table 7: Various rate constants for the degradation of metronidazole and prodrug

Temperature	k _H (M ⁻¹ h ⁻¹)	K _p (h ⁻¹)	K _{up} (h ⁻¹)	k _{OH} (M ⁻¹ h ⁻¹)
Drug				
303.15	0.1373	-	0.000117	203.8
310.15	0.2539	-	0.000205	324.4
318.15	0.4132	-	0.000374	526.1
Prodrug				
303.15	0.2032	0.01708	0.008195	10245.1
310.15	0.2681	0.02248	0.01102	12948
318.15	0.3569	0.03024	0.015246	16623.4

k_p and k_{up} = first order rate constants for solvent assisted degradation; k_H = second order rate constant for acid catalyzed degradation; k_{OH} = second order rate constant for base catalyzed degradation.

the enthalpy of reaction at various pH (Tables 2, 3 and 4). Much higher value of degradation enthalpy at lower pH indicates the decomposition of the drug. We have tried to correlate our enthalpy of reaction with the tentative mechanism. According to the figure 5 certain bonds are broken while new bonds are formed leading to the exothermic enthalpy of reaction (Table 6). The calculated value has been found to be 288.42, which agrees well with the molar enthalpy of degradation (222.92-203 over pH 2-9).

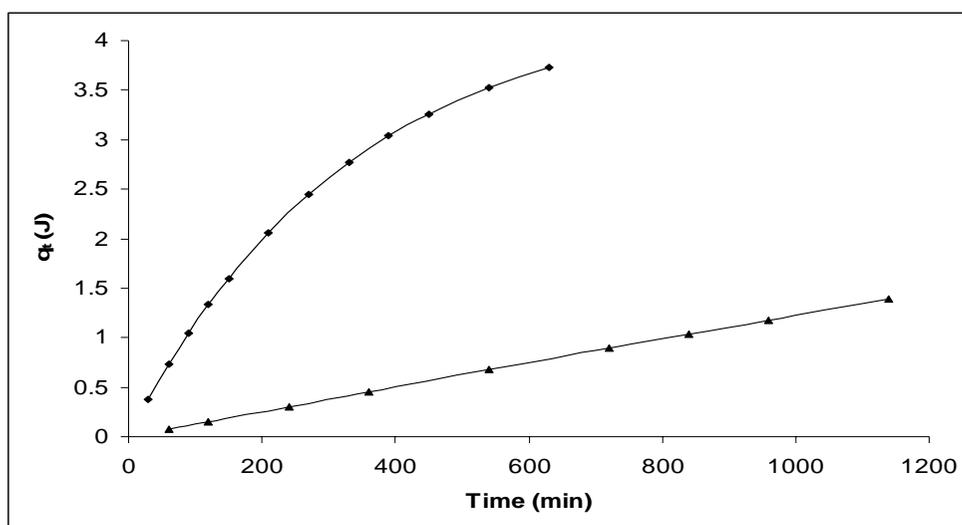
The rate of hydrolytic reaction leading to the liberation of metronidazole and ciprofloxacin from mutual prodrug of

metronidazole is much higher as compared to the degradation of metronidazole (Tables 2, 3 and 4). The observation of significantly higher value of rate constant is an evidence for the operation of intramolecular catalysis assisted by keto group on the ciprofloxacin as shown in Figure 6 (Bowden, 1995). Therefore under the experimental conditions the enthalpy involved in the hydrolysis of prodrug does not involve the enthalpy associated with the degradation of metronidazole. Literature survey has revealed that ciprofloxacin is also stable over this pH range. Tables 2, 3 and 4 indicate that the hydrolysis of the prodrug is also pH dependent and can be accounted for

Table 8: Activation parameters for the overall degradation reaction and individual rate constants of metronidazole and its prodrug over the pH range 2-9.

pH	E_a (kJ mol ⁻¹)		$\ln A$ (h ⁻¹)		ΔG^\ddagger (kJ mol ⁻¹)		ΔS^\ddagger (J mol ⁻¹ K ⁻¹)		ΔH^\ddagger (kJ mol ⁻¹)	
	Drug	Prodrug	Drug	Prodrug	Drug	Prodrug	Drug	Prodrug	Drug	Prodrug
2	59.05	30.81	15.52	15.029	132.33	105.28	-188.76	-245.648	57.63	30.80
3	53.24	32.03	12.58	15.05	133.96	106.43	-210.586	-245.449	52.69	32.025
4	61.28	32.26	15.32	14.945	134.99	106.93	-184.34	-246.333	60.958	32.250
5	60.70	33.13	15.01	15.25	135.20	107.03	-188.35	-243.811	61.183	33.123
6	62.09	33.05	15.59	15.226	135.15	107.00	-183.59	-243.98	61.440	33.039
7	61.58	33.23	15.52	15.39	134.78	106.79	-184.08	-242.674	60.642	33.223
8	54.26	28.67	13.48	14.298	132.62	104.96	-201.06	-251.695	53.059	28.658
9	51.70	26.59	14.38	15.258	127.81	100.47	-193.65	-243.742	51.480	26.579
Individual rate constants										
k_H	58.76	30.10	21.36	10.35	117.34	116.35	-135.81	-227.11	58.757	30.09
k_p	-	30.62	-	8.08	-	122.59	-	-245.97	-	30.62
k_{up}	62.11	33.19	15.59	8.36	135.15	124.44	-183.52	-243.62	62.106	33.18
k_{OH}	50.68	25.87	25.43	19.50	98.93	89.06	-101.76	-151.04	50.673	25.86

k_H and k_{up} = first order rate constants for solvent assisted degradation; k_H = second order rate constant for acid catalyzed degradation; k_{OH} = second order rate constant for base catalyzed degradation; E_a = free energy of activation; $\ln A$ = frequency factor; ΔG^\ddagger = free energy of activation; ΔS^\ddagger = entropy of activation; ΔH^\ddagger = enthalpy of activation

**Figure 1:** Calorimetric data of heat evolution (q_t) vs time for degradation of metronidazole and its prodrug at pH 9 at 318.15K. (Drug (\blacktriangle), prodrug (\blacklozenge)).

by general acid/base catalysis at different temperatures. The influence of pH on the rate of hydrolysis at 37°C is shown in figure 3 in which the logarithm of the pseudo first order rate constants (k_{obs}) is plotted against pH. For the prodrug the stability occurs at pH values about 4-6 characteristic of general acid/base catalysis. In the pH region investigated the prodrug occurs in three forms with the nitrogen in imidazoline ring as well as

nitrogen in piperazine ring in protonated and unprotonated forms. The shape of the pH rate profile indicates that the unprotonated and protonated nitrogen forms of prodrug undergo hydrolysis at different rates. The hydrolysis can be described in terms of specific reactions involving both species and a spontaneous and specific acid and base catalyzed reaction.

The best agreement at all the pH was obtained from the following equation:

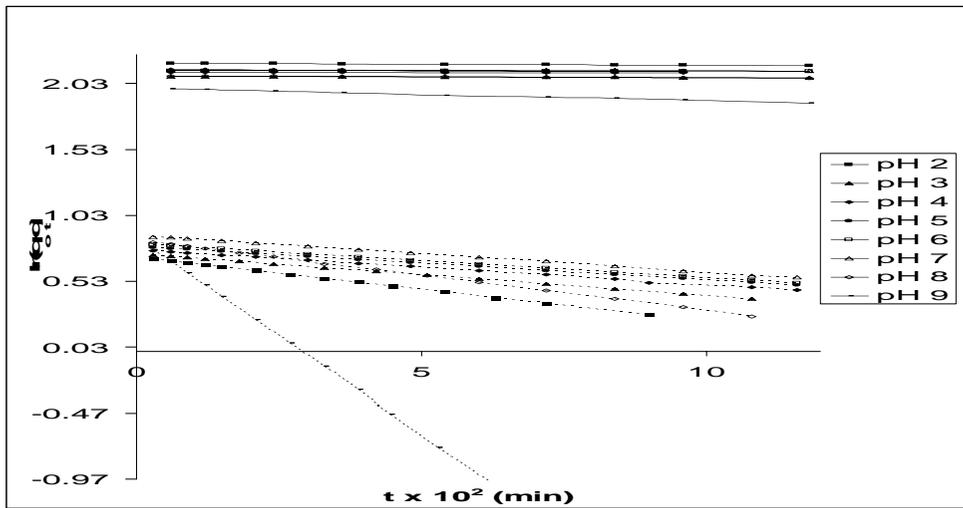


Figure 2: First order plot of degradation of metronidazole (—) (8.187×10^{-3} M) and its prodrug (- -) (3.3034×10^{-3} M) over the range of pH 2-9 at 318.15K.

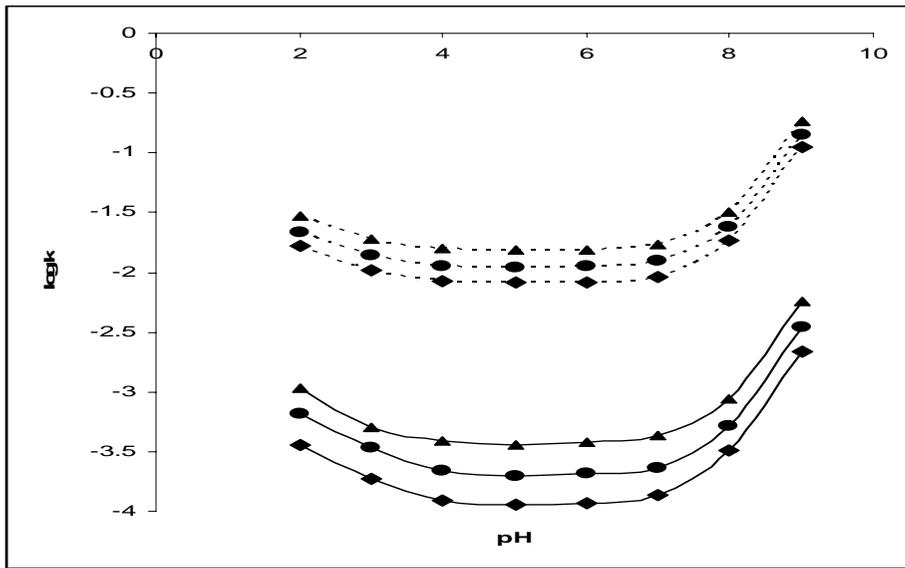


Figure 3: Plot of log k vs pH profile in the temperature range of 303.15-318.15 K. Solid lines represent the drug and broken lines the prodrug (303.15 (♦), 310.15 (●), 318.15 (▲))

$$k_{obs} = k_H[H^+]f^{PP} + k_p f^{PP} + k_{up}(f^P + f^{UP}) + k_{OH}[OH^-](f^P + f^{UP}) \dots\dots\dots 5$$

f^{PP} = fraction of the total prodrug when both nitrogens are protonated (form I);
 f^P = fraction of total prodrug when N in imidazoline ring is unprotonated but N in piperazine ring is protonated (form II);
 f^{UP} = fraction of the total prodrug when both nitrogens are unprotonated (form III);
 k_H = second order rate constant for acid catalyzed degradation;

k_{OH} = second order rate constant for base catalyzed degradation.

f^{PP} , f^P and f^{UP} were calculated from the ionisation constant pK_a 2.5 for the nitroimidazole and pK_a 8.8 for the piperazine nitrogen.

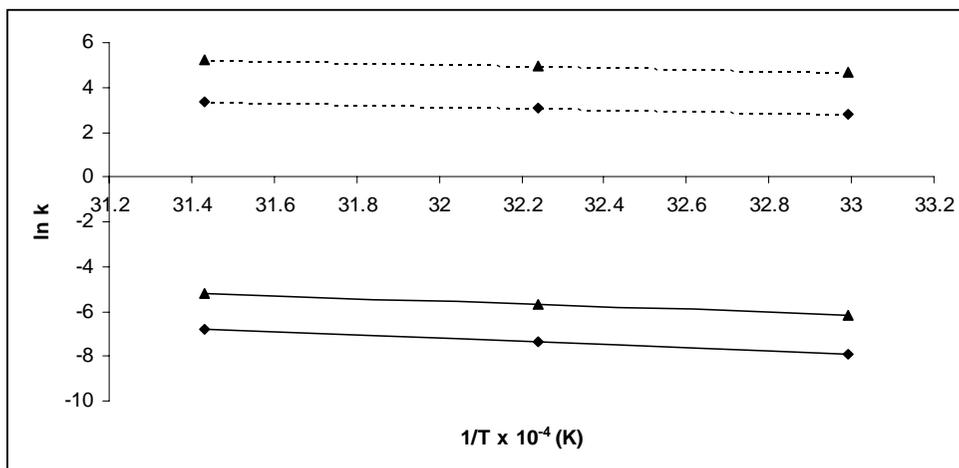


Figure 4: Arrhenius plot for degradation of metronidazole and prodrug at pH 2 (◆) and pH 9 (▲). Smooth lines are for drug and broken for prodrug.

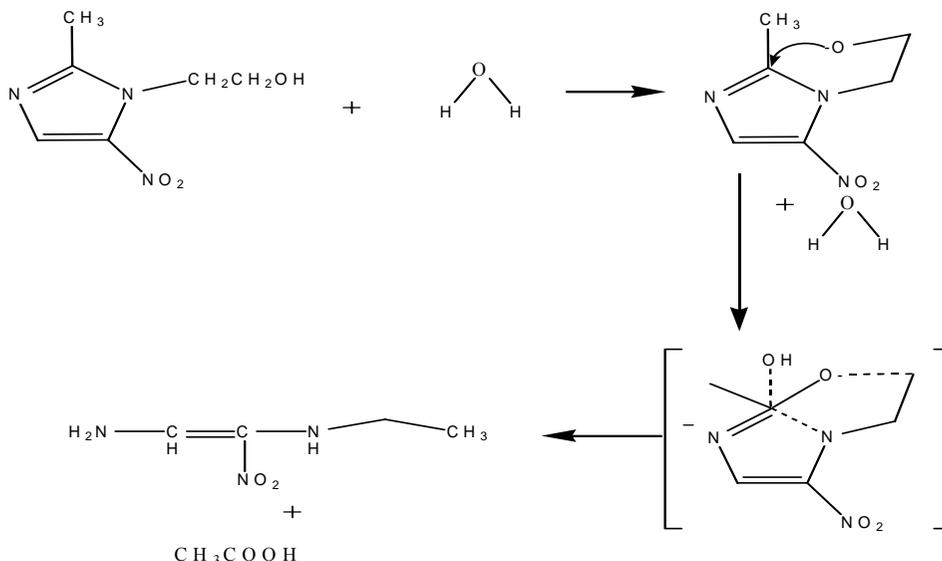


Figure 5: Scheme depicting degradation/hydrolysis of metronidazole.

The observed values are compared with the calculated values in table 3. The values of various macro-constants are given in table 7.

The increased rate of reaction at pH 2 may be attributed to the inductive effect of protonated nitrogen of imidazole ring (pKa 2.5) in form I. However, protonation of nitrogen in piperazine ring in ciprofloxacin reveals no effect due to its long distance from phenyl group and its reduced electron withdrawing effect. Therefore, forms II and III contribute equally towards degradation of prodrug. To our knowledge there is no literature for the rate constants under these

experimental conditions but, however, the pH rate profile follows the same pattern as for other prodrugs of metronidazole (Bowden et al., 1997). The temperature dependence of drug and its prodrug was aimed to determine the optimum pH for the maximum stability. The linearity ($r = 0.997$) of the plot of $\log k$ vs $1/t$ shows a good indication of invariant activation energy for degradation of metronidazole and prodrug in this temperature range. We have also calculated the activation energy for individual rate constants along with other Arrhenius parameters which are given in table 8. This table indicates small enthalpy of

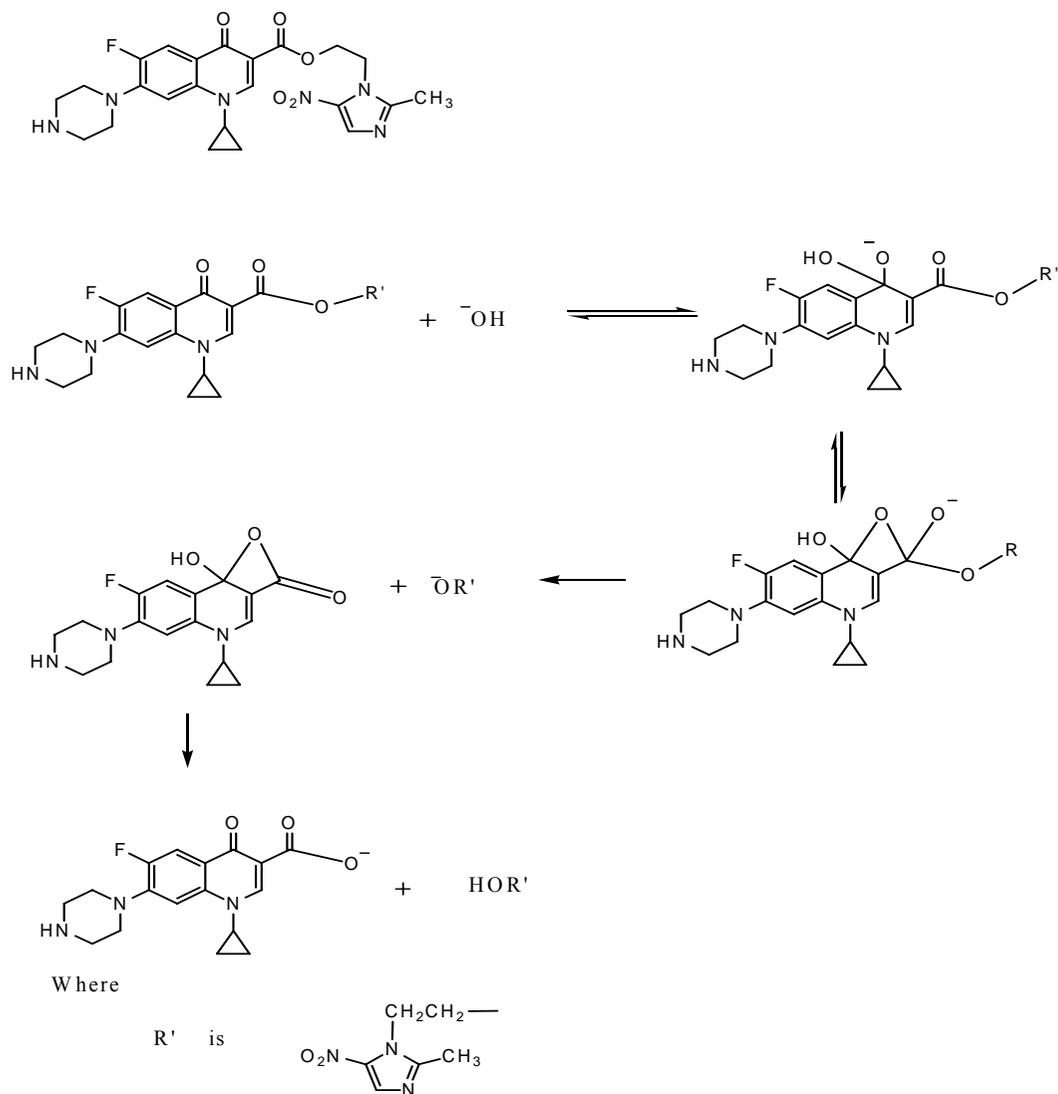


Figure 6: Scheme depicting hydrolytic degradation of prodrug.

activation for the hydrolysis of prodrug as compared to drug. This small ΔH^\ddagger accompanied by large negative entropy of activation ΔS^\ddagger is associated with facile intramolecular catalysis by keto group in prodrug (Bowden, 1995).

The present results reveal that utilization of calorimetric technique to study degradation and hydrolytic kinetics is a successful approach. The drug was found to most stable between pH 4-6. The proposed mechanism for degradation of metronidazole at higher pH is supported by the magnitude of exothermic enthalpy accompanying the degradation.

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