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Effect of hydroxyl radicals on the stability of metronidazole in buffer solution at pH 9.2

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Summary

Metronidazole in buffer solution (pH 9.2) undergoes degradation very slowly at 40° and 70°C on irradiation with sonic energy. Irradiation with light (360 nm) at 20°, 48°, and 63°C produces more rapid degradation, the reaction following zero order kinetics at 20°C and first order kinetics at both 48 and 63°C. The addition of hydrogen peroxide solution increases the reaction rate constant, having a less significant effect at the higher temperatures. The addition of glucose (5% w/v) as well as hydrogen peroxide retards the reaction at 20 and 48°C but accelerates it to above the rate of the photolysed reaction without additives at 63°C.

Introduction

Metronidazole (1-hydroxyethyl-2-methyl-5-nitroimidazole) is an antibacterial agent with potent action against Gram negative bacteria and protozoa. This drug has been shown to be subject to hydrolysis in aqueous media, a reaction which is often accelerated by the presence of hydroxyl radicals (Parkinson et al., 1989). Barnes and Sugden (1986) have shown that metronidazole is decomposed by photolytically generated hydroxyl radicals. The object of the present work is to compare the decomposition rates of metronidazole in aqueous buffer at pH 9.2 under the effects of photolysis and sonic energy.

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Materials and Methods

Metronidazole powder (B.P.), buffer solution (pH 9.2) (B.D.H. buffer tablets), hydrogen peroxide (20 vol.) (Boots p.l.c.) and D-glucose (B.D.H., G.P.R. grade) were used.

Apparatus included a Perkin Elmer 550 uv/vis spectrophotometer, sonic bath and generator (Analytical Supplies, Derby, U.K.) and a temperature control unit (Grant Instruments, U.K.).

Methods

Sonication. The uv/vis spectrum of metronidazole was run in pH 9.2 buffer against a buffer blank and the absorption maximum was found at 320 nm. A series of dilutions were made containing 3.72, 7.44, 14.9, 29.8 and 37.2 μg/ml and a calibration graph constructed (correlation coefficient, $r = 0.9998$, standard deviation ± 0.0139). Samples of metronidazole solution (37.2 μg/ml) were irradiated with sonic energy at 0, 20, 40, and

70 °C. The absorbance was measured at time zero and subsequently at time intervals, the percentage drug remaining was calculated in each case. A second experiment was carried out in which hydrogen peroxide 1 ml (20 vol.) was added prior to irradiation with sonic energy. In a third experiment 5% w/v D-glucose was added as well as the hydrogen peroxide prior to irradiation with sonic energy and measurements were taken at 0, 20, and 70 °C. In each experiment control samples were subjected to the same conditions of temperature but were not irradiated with sonic energy.

Photolysis. The experimental procedures described above were used but the solutions were irradiated with light (360 nm) (intensity 400 $\mu W/cm^2$) at 20, 48, and 63 °C.

Treatment of results. The order of reaction was determined by conducting a linear regression analysis on a Casio FX 7300 microcomputer on plots of percentage drug remaining vs. time and on $\log_{10}$ percentage drug remaining vs. time. The order of reaction was decided on which analysis gave the higher correlation coefficient (Florence and Attwood, 1981).

Discussion

In the sonic energy experiments it was clear that there was no reaction below 40 °C; even at this temperature after 5.5 h of irradiation only 7.5% of the metronidazole had been degraded; at 70 °C, 11.3% of the drug had been degraded after 6 h (Tables 1 and 2). This result was unusual, since sonic energy has its maximal effect at low temperatures where the vapour pressure is minimal since cavitation, which causes the decomposition of water into radicals, one of which is the hydroxyl radical, is inversely related to the vapour pressure and thus to the temperature (Suslick et al., 1983). The addition of hydrogen peroxide at both 40 and 70 °C markedly increased the reaction rate constant; at 40 °C the increase was from 0.0149 to 0.0347, whilst at 70 °C the increase was from 0.0171 to 0.041. The change in rate constant at 40 °C was 0.0198 whilst at 70 °C the change had risen to 0.0239, this was unexpected since the formation of hydroxyl radicals would be expected to decline as the temperature was increased. It must be recognised that there is probably more

TABLE 1

Sonication of metronidazole in buffer (pH 9.2, 37.2 μg/ml at 40 °C)

Time (h)	Sonicated		Control	
	Remaining (%)	Log remaining	Remaining (%)	Log remaining
0	100.0	2.0000	100.0	2.00000
0.5	99.2	1.9966		
1.0	98.75	1.9945		
1.5	98.0	1.9911	99.8	1.9993
2.0	97.2	1.9878	99.3	1.9970
2.5	96.35	1.9838		
3.0	95.8	1.9813		
3.5	95.5	1.9800		
4.0	93.6	1.9715		
5.0	93.0	1.9683	98.7	1.9945
5.5	92.4	1.9658		
log % vs. t first order $k = 0.015$ $r = -0.9956$			log % vs. t first order $k = 0.00253$ $r = -0.9583$	

TABLE 2

Sonication of metronidazole in buffer (pH 9.2, 37.2 μg/ml at 70 °C)

Time (h)	Sonicated		Control	
	Remaining (%)	Log remaining	Remaining (%)	Log remaining
0	100.0	2.0000	100.0	2.0000
0.5	99.0	1.9955	99.8	1.9991
1.5	98.2	1.9921	98.9	1.9951
2.0	96.4	1.9841		
3.0	95.4	1.9752		
4.0	94.5	1.9752	98.7	1.9946
4.5	93.6	1.9713	98.5	1.9935
5.0	93.3	1.9681		
6.0	88.7	1.9480	98.3	1.9927
log % vs. t first order			log % vs. t first order	
$k = 0.0171$			$k = 0.0025$	
$r = -0.9675$			$r = -0.9233$	

than one reaction taking place, hydrolysis and photolysis, but from the control experiments and the work of Baveja and Rao (1973), the hydrolysis would appear to be a minor component. It must be acknowledged that the photolysis may consist of other reactions besides the action of the hydroxyl radical.

In a third experiment 5% w/v D-glucose was added as well as the hydrogen peroxide prior to light irradiation stopped the reaction totally with no detectable degradation of metronidazole occurring after 6 h. Examination of Table 5 shows that the photolysis of metronidazole in buffer pH 9.2 follows zero-order kinetics at 20 °C (correlation coefficient $r = -0.9951$ whereas the value for first-order kinetics is $r = -0.9903$). However, at 48 °C the reaction follows first-order kinetics, ($r = -0.9995$) and the rate constant is 0.269 (Table 6). The addition of hydrogen peroxide results in a very small change in rate constant to 0.259 but the

TABLE 3

Sonication of metronidazole in buffer (pH 9.2, 37.2 μg/ml at 40 °C) with added hydrogen peroxide (1.0 ml; 20 vol./100 ml)

Time (h)	Sonicated		Control	
	Remaining (%)	Log remaining	Remaining (%)	Log remaining
0	100.0	2.0000	100.0	2.0000
0.5	98.6	1.9940		
1.0	96.9	1.9861	99.7	1.9988
1.5	94.4	1.9749	99.3	1.9970
2.0	93.2	1.9693		
2.5	90.8	1.9580	99.2	1.9963
3.0	88.8	1.9486	99.0	1.9955
3.5	87.2	1.9405		
4.0	85.9	1.9339		
5.0	84.6	1.9276		
5.5	83.4	1.9211		
log % vs. t first order			log % vs. t first order	
$k = 0.0347$			$k = 0.0032$	
$r = -0.9898$			$r = -0.9707$	

TABLE 4

Sonication of metronidazole in buffer (pH 9.2, 37.2 μg/ml, 70 °C) with added hydrogen peroxide (1 ml; 20 vol./100 ml)

Time (h)	Sonicated		Control	
	Remaining (%)	Log remaining	Remaining (%)	Log remaining
0	100.0	2.0000	100.0	2.0000
0.5	95.4	1.9797		
1.0	93.4	1.9705	99.7	1.9988
1.5	90.4	1.9563		
2.0	89.8	1.9532		
3.0	84.9	1.9290	99.23	1.9966
5.0	80.2	1.9041	99.13	1.9962
log % vs. t first order			log % vs. t first order	
$k = 0.041$			$k = 0.0$	
$r = -0.9864$			$r = -0.9799$	

TABLE 5

Photolysis of metronidazole in buffer (pH 9.2, 37.2 μg/ml at 20 °C)

Time (h)	% remaining	Log remaining
0	100	2.0000
0.4	98.8	1.9950
0.95	96.2	1.9830
1.60	93.5	1.9708
2.72	84.7	1.9277
3.40	78.9	1.8972
4.13	73.1	1.8644
4.83	67.8	1.8313
5.30	64.75	1.8112
Zero-order, $r = -0.9915$; first-order, $r = -0.9903$.		

TABLE 6

Photolysis of metronidazole in buffer (pH 9.2, 37.2 μg/ml at 48 °C)

Time (h)	% remaining	Log remaining
0	100.0	2.0000
0.95	78.3	1.8937
1.60	65.2	1.8144
1.88	60.0	1.7782
2.08	56.5	1.7522
2.47	51.3	1.7101
Zero order, $r = -0.9965$; first order, $r = 0.9995$, $k = 0.2696$		

TABLE 7

Photolysis of metronidazole in buffer (pH 9.2, 37.2 μg/ml at 48 °C) with hydrogen peroxide (1.0 ml; 20 vol./100 ml) and D-glucose (5% w/v)

Hydrogen peroxide			Hydrogen peroxide and glucose		
Time (h)	% remaining	Log remaining	Time (h)	% remaining	Log remaining
0	100.0	2.0000	0	100.0	2.0000
0.27	94.7	1.9765	0.5	97.9	1.9907
0.38	92.1	1.9642	1.36	90.4	1.9561
0.63	85.7	1.9343	1.56	86.5	1.9372
0.93	78.95	1.8973	2.4	82.7	1.9174
1.13	73.7	1.8673	2.82	79.8	1.9020
1.38	68.4	1.8352	3.32	76.0	1.8808
1.72	63.2	1.8004	3.85	73.1	1.8639
1.93	60.5	1.7819	4.25	70.4	1.8474
2.25	57.0	1.7560	4.58	69.0	1.8391
2.50	53.5	1.7284			
First-order kinetics			First-order kinetics		
$r = -0.9975$			$r = -0.9975$		
$k = 0.259$			$k = 0.083$		

TABLE 8

Photolysis of metronidazole in buffer (pH 9.2, 37.2 μg/ml at 63°C) and hydrogen peroxide (1.0 ml; 20 vol./100 ml) and D-glucose (5% w/v)

Time (h)	Buffer		Time (h)	Hydrogen peroxide		Time (h)	Hydrogen peroxide and glucose	
	Remaining (%)	Log remaining		Remaining (%)	Log remaining		Remaining (%)	Log remaining
0	100.0	2.0000	0	100	2.0000	0	100.0	2.0000
0.61	96.15	1.9829	0.917	74.1	1.8699	0.3	95.0	1.9777
0.73	92.3	1.9652	1.333	65.3	1.8150	0.967	76.8	1.8852
1.033	88.84	1.9486	1.617	59.9	1.7710	1.417	68.15	1.8334
1.667	82.7	1.9174	1.817	55.5	1.7439	1.633	61.8	1.7910
2.0	80.1	1.9072	2.25	51.1	1.7080	2.25	59.1	1.7288
2.70	77.0	1.8860	2.55	47.7	1.6786	3.317	43.4	1.6377
2.83	74.2	1.8706	3.68	40.5	1.6074	3.633	41.8	1.6216
3.33	72.7	1.8615				4.133	38.8	1.5885
First order				First order		4.55	37.6	1.5754
$r = -0.9972$				$r = -0.9806$		5.08	36.5	1.5619
$k = 0.099$				$k = 0.249$		First order; $r = -0.9868$, $k = 0.207$		

addition of both hydrogen peroxide and 5% w/v D-glucose gives a rate constant of 0.086, (Table 7); this suggests that the glucose retards all the reactions. At 63°C the reaction follows first-order kinetics ($k = 0.099$), the addition of hydrogen peroxide produced the expected rise in rate constant to 0.249, whilst the addition of 5% D-glucose results in a decrease in the reaction rate constant to 0.207 (Table 8). This suggests that the glucose is acting as a hydroxyl radical scavenger and having an accelerating effect on the other components of the reaction. Such an effect is not uncommon; Ashwin and Lynn (1975) reported that dextrose accelerated the decomposition of ampicillin. Further work on this reaction by Bundgaard and Larsen (1979) indicated that the glucose promoted the decomposition of the ampicillin by means of a nucleophilic reaction.

In conclusion, it can be seen that metronidazole is subject to photochemical degradation and that this reaction is not solely mediated by hydroxyl radicals. The degradation rates due to hydroxyl radicals and to hydrolysis are relatively slow under the conditions examined.

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