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Stability assessment of ketoconazole in aqueous formulations

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Abstract

Ketoconazole is an imidazole antifungal agent. It has a wide antifungal spectrum and possesses some antibacterial activity. In inappropriate formulations, especially in aqueous media, ketoconazole molecules may be unsteady. The stability of ketoconazole in aqueous media was assessed as a function of pH, antioxidant and ketoconazole concentrations. It was found that ketoconazole was least stable at pH 1 among the pH values studied (pH 1–9). Since the major degradation pathway was specific acid catalysis, based upon the transition-state theory, the entropy (ΔS) of the activation was calculated and found to be negative indicating that the activated complex was more constrained than the individual species. The free energy of activation (ΔG) was estimated to be 30 kcal mol^{-1}. The viscosity of the formulation was found to be more stable at high pH because carbopol is stable at basic pH and protected ketoconazole. It appears that the amount of ketoconazole in the formulation has a low influence on the degradation mechanisms. The increase of the butylated hydroxytoluene antioxidant levels from 0.05 to 0.4%, adversely affected the stability of ketoconazole. In conclusion, the expected shelf life of the final ketoconazole formulation (pH 7, 0.1% butylated hydroxytoluene) was 15 months.

Keywords: Ketoconazole; Stability; Antioxidant; Entropy; Free energy

1. Introduction

Ketoconazole, an imidazole antifungal agent, possesses some antibacterial activity (Shuster, 1984; McGrawth and Murphy, 1991). It may act by interfering with the permeability of the fungal cell membrane. It is a broad-spectrum anti-fungal agent formulated into a number of dosage forms through various routes of administration such as oral and topical (Shuster, 1984; McGrawth and Murphy, 1991). For example, the ketoconazole shampoo is used for antidandruff purposes in Europe and USA.

In aqueous solutions almost all of the drugs are subject to some form of chemical degradation. The most common consequence of the drug's degradation is the loss of potency but in some cases, harmful degradation products may be formed. Ketoconazole is a weak base with two

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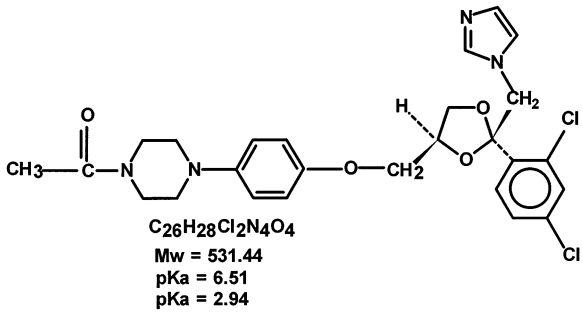

Fig. 1. Molecular structure of ketoconazole.

pK_a: 6.51 and 2.94 (Fig. 1). It may undergo degradation including oxidation and hydrolysis, especially in aqueous media, if it is not properly formulated. By investigating the intrinsic stability of the drug, it is possible to provide advice on formulations to achieve stable and desirable products.

Therefore, the objective of this study was to assess the effect of formulation factors such as pH and antioxidant levels on the stability of ketoconazole in aqueous media. The effect of pH on the viscosity of the formulation has been studied.

2. Materials and methods

2.1. Materials

The different formulations were: ketoconazole: 2, 1.5, 1, 0.5 and 0.25%; butylated hydroxytoluene (antioxidant): 0.05, 0.1, 0.2, 0.3 and 0.4%; carbopol 1342: 40%; NaOH/HCl: pH adjustment 1 to 9; purified water: q.s.p 100%.

Table 1
Stability of ketoconazole dispersion as a function of pH and butylated hydroxytoluene antioxidant levels

pH	Butylated hydroxytoluene (%)				
	0.05	0.1	0.2	0.3	0.4
9	100	100	100	100	100
8	100	100	100	100	100
7	99	99.9	100	100	100
6	98	99.6	100	100	100
5	97.3	99.7	100	100	100
4	97.2	99	100	100	100
3	90	99	100	100	100
1	80	98	100	100	100

2.2. Stability conditions

Ketoconazole aqueous formulation of various concentrations, butylated hydroxytoluene levels and pH were filled in glass bottles and stored at 25 and 50°C during 15 months. Samples were taken periodically for both physical and chemical stability evaluations.

2.3. Viscosity measurement: physical stability evaluation

The viscosity was determined by a Brookfield viscometer set at 12 rev. min^{-1} for 1 min at 25°C.

2.4. Analytical method: chemical stability evaluation

The ketoconazole content was determined after extraction in the supernatants by an HPLC method in the following conditions: μBondapak C_{18} column (150 × 5 mm, Waters, USA); mobile phase, ammonium acetate 0.2%/isopropylamine in methanol (27:73); flow rate, 1.5 ml min^{-1}; detection at 254 nm.

The effect of the concentration on the stability of ketoconazole at pH 7 was investigated. Following pseudo first-order kinetics, the degradation rate constants observed (K_{obs}) and activation energies (E_a) of various formulations were calculated and the shelf life (90% remaining at 25°C) of each batch was estimated.

3. Results and discussion

3.1. Effect of pH and temperature on ketoconazole stability

Ketoconazole in dispersion studies was to determine the hydrolytic stability of 0.5% ketoconazole as a function of pH (1–9) and butylated hydroxytoluene levels (0.05, 0.1, 0.2, 0.3 and 0.4%).

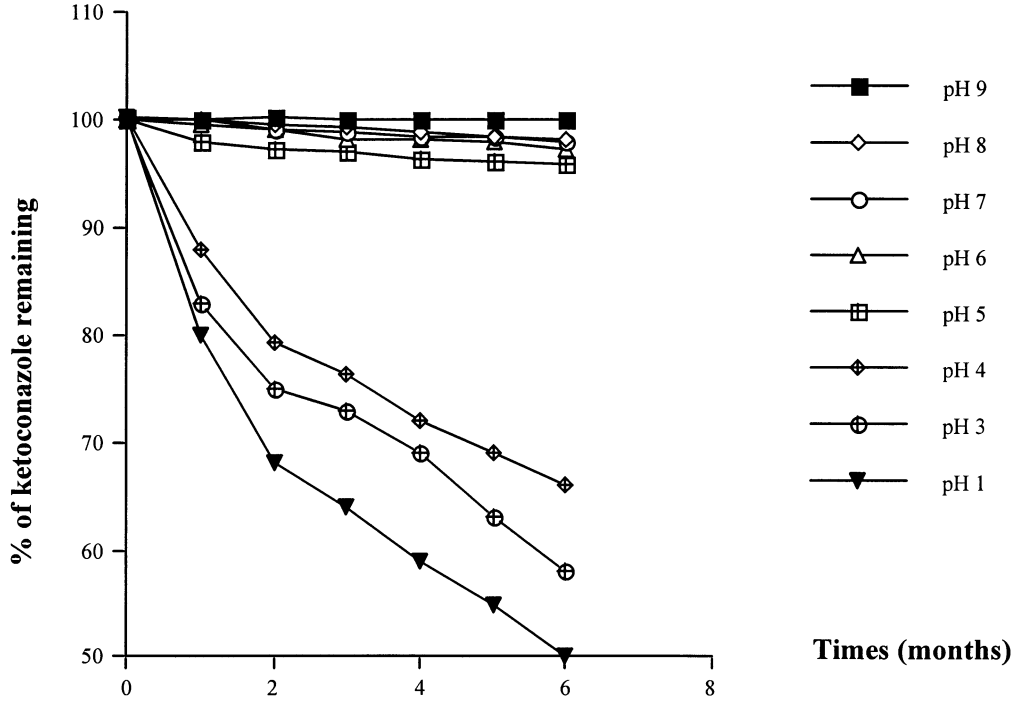

Fig. 2. Chemical stability of ketoconazole in aqueous solution: effect of pH.

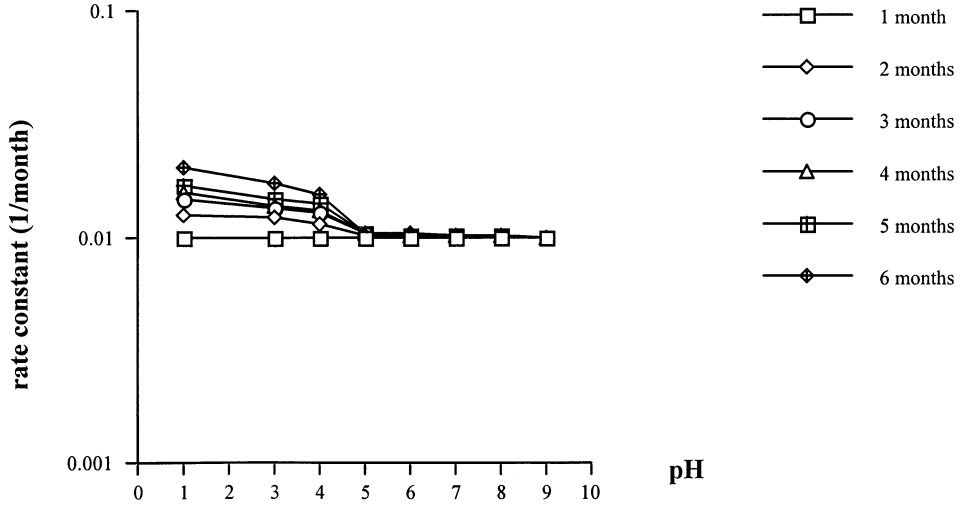

Fig. 3. pH/stability profiles of ketoconazole in aqueous solution.

The pH stability studies of ketoconazole formulation (0.5% ketoconazole and 0.4% butylated hydroxytoluene) were conducted for 6 months at room temperature and accelerated conditions and the results are listed in Table 1.

Fig. 2 indicates that the amount of ketoconazole in the aqueous formulation decreased more quickly as the pH decreases. The pH rate profiles

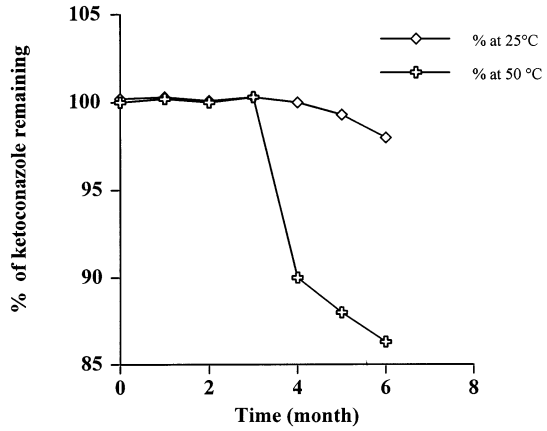

Fig. 4. Chemical stability of ketoconazole in aqueous formulation: effect of temperature.

Table 2
Effect of pH and butylated hydroxytoluene levels on shelf life (T_{90})

pH	Butylated hydroxytoluene (%)				
	0.05	0.1	0.2	0.3	0.4
9	60.7	57.14	54	50	48
8	56.3	53.2	50	40	41.99
7	34.1	32.8	32.1	30	27.1
6	26.3	25.8	25.5	25	24.6
5	17.9	17.7	17.3	17	18
4	14.8	14.3	14.1	13	12.46
3	13.7	12	12.7	11.27	11.1
1	12	11.3	10.33	10.1	9.39

Table 3
Effect of pH and butylated hydroxytoluene levels on activation energy [E_a (kcal mol^{-1})] of ketoconazole

pH	Butylated hydroxytoluene (%)				
	0.05	0.1	0.2	0.3	0.4
9	17.5	17	17.6	17.4	17.7
8	16.9	16.9	17	17.54	17.8
7	17.1	16.1	16.1	16.99	17.6
6	16.3	16	17.5	17.5	17.9
5	17.4	15.8	17.3	17	17.6
4	17	17.2	17.1	17	17.5
3	16.7	15.9	16.7	16.27	17.6
1	16.8	16.6	17.33	17.1	17.4

of ketoconazole at various pH are shown in Fig. 3.

Degradation rate constants (K_{obs}) were higher at pH 1. This suggests that ketoconazole is subjected to specific acid catalysis. Parallel degradation phenomena were obtained for both room temperature and accelerated conditions. In addition, thermal energy plays an important role (Figs. 3 and 4). There were about 99 and 85% of ketoconazole left in a pH 7 formulation after 6 months at 25 and 50°C respectively.

3.2. Effect of antioxidant level

By experiments (data not published), we showed that one of the degradation pathways of ketoconazole is oxidation. The role of antioxidant level on the stability of ketoconazole is presented in Table 1. The increase in butylated hydroxytoluene antioxidant levels from 0.05 to 0.4% seems to improve the stability of ketoconazole in the aqueous formulation particularly at pH 1. The effect of butylated hydroxytoluene levels on the shelf life (T_{90}) is compared in Table 2. The result indicated that formulations containing 0.4% of butylated hydroxytoluene have a shorter shelf life. No difference was found in the energy of activation (Table 3).

3.3. Effect of ketoconazole concentration

The effect of concentration on the stability of ketoconazole at pH 7 was investigated. Following pseudo first-order kinetics, the degradation rate constants observed (K_{obs}) and activation energies (E_a) of various formulations were calculated and the shelf life (90% remaining at 25°C) of each batch was estimated (Table 4). In general, all of the batches have a shelf life of about 15 months. The activation energy was calculated to be approximately 18 kcal. The 1% ketoconazole solution appears to have a longer shelf life. The data indicate that the change of the ketoconazole concentration from 0.25 to 2% in the solution has a negligible effect on the degradation kinetics.

Table 4
Chemical kinetics of ketoconazole in aqueous formulation based on pseudo first-order kinetics

Kinetic data		Ketoconazole (%)				
		0.25	0.5	1	1.5	2
K (month^{-1})	25°C	0.0025	0.0032	0.0028	0.0030	0.0029
	50°C	0.0541	0.0504	0.0408	0.0400	0.0360
T_{90} (months)	25°C	41.5	33	37	35	36
E_a (kcal)		22.9	20.6	20.1	20	20.3

3.4. Thermodynamic parameters of ketoconazole at a quasi thermodynamic equilibrium

It appears in Fig. 3 that the degradation of ketoconazole is mainly catalyzed by the hydronium ion present in the solution.

Therefore, the degradation rate of ketoconazole $[-\mathrm{d[keto]}/\mathrm{d}t]$ can be described by the following equation:

$$-\mathrm{d[keto]}/\mathrm{d}t = K_{obs}[\mathrm{keto}] = K_{H+}[H^+][\mathrm{keto}] \quad (1)$$

where [keto] is the total concentration of ketoconazole in the solution and K_{obs} is the observed rate constant

$$K_{obs} = K_{H+} + [H^+] \quad (2)$$

Taking the logarithm of Eq. (2) yields the relationship:

$$\log K_{obs} = \log K_{H+} - \mathrm{pH} \quad (3)$$

and K_{H+} was thus obtained from the intercept of log K_{obs} vs pH. K_{H+} at 25°C was calculated to be 0.02872 l mol^{-1} month^{-1}.

According to Eyring's transition state theory (Eisenberg and Crothers, 1979), the rate constant can be expressed by the following equation:

$$K_{obs} = [K_B TK/h] \exp(-\Delta G/RT) \quad (4)$$

where K_B is Boltzmann's constant (1.38066×10^{-23} K J^{-1}), T is the absolute temperature of reaction, K is the transmission constant (always assumed to be unity) and h is Plank's constant (6.6262×10^{-34} J s).

Therefore, the free energies of activation (ΔG) for each condition can be obtained. In addition, the enthalpies (ΔH) of activation can be calculated from the relationship:

$$\Delta H = E_a - RT \quad (5)$$

where E_a is the activation energy and R is the gas constant. The entropies can be calculated from

$$\Delta G = \Delta H - T\Delta S \quad (6)$$

The thermodynamic parameters (ΔH, ΔG and ΔS) are listed in Table 5. Both ΔG and ΔH are positive in all the conditions studied indicating that activated complex always has higher energy levels than that of the reactants and because

Table 5
Thermodynamic parameters of ketoconazole at a quasi-thermodynamic equilibrium

	ΔH (kcal mol^{-1})	ΔG (kcal mol^{-1})	ΔS (kcal K^{-1} mol^{-1})
25°C	16.7	28	−0.038
50°C	16.6	29.2	−0.038

Table 6
Effect of pH on the physical stability of aqueous ketoconazole formulation (50°C/0.5% ketoconazole/0.1% butylated hydroxytoluene)

pH	Time (months)						
	0	1	2	3	4	5	6
9	9	9	8	7.7	7.2	6.3	6.1
8	8	8	7.5	7	6.5	5.8	6
7	7	7	6.8	6.74	6.3	5	5
6	6	6	5.8	6	5.9	5.1	5.2
5	5	5	5.1	5	5.2	5.08	5
4	4	4	4.5	4.8	5.1	5.09	5
3	3	3	3.5	4.3	4.8	5.1	5.3
1	1	1	2	3.5	4.6	4.9	5.2

Table 7
Effect of pH on the viscosity of ketoconazole formulation

	pH							
	9	8	7	6	5	4	3	1
Viscosity (mPa s)	4.000	5.000	8.000	25.000	20.000	5.000	4.000	3.000

energy is required to create the partially broken bonds of the activated complex. The entropies of all the reactions are negative since molecules are more constrained in the activated complex than when they are able to move separately in solution. This is in agreement with the results in Fig. 2, degradation is faster at pH 1–4.

3.5. Effect of pH on the physical stability of ketoconazole solution

The role of pH on the physical stability of the formulation was also assessed. As is demonstrated in Table 6, the pH of low pH formulations (pH 1, 3, 4) increase with time and that of high pH formulations (pH 6, 7, 8, 9) decrease with time, i.e. the pH values of all formulations approach between 5 and 6.

The initial viscosities of formulations of various pH values is shown in Table 7. The viscosity of formulation peaked at pH 5 ($<25\,000$ mPa s) and dropped drastically as pH was adjusted away from this value. This could be attributed to the characteristics of the viscosity inducing agent (carbopol 1342) used in the system. Carbopol 1342 shows the highest viscosity at around pH 5. Since the pH values of all the formulations are moving toward pH 5–6 range, which is the pH exhibiting the highest viscosity, the viscosities of all the formulations increase over time.

The results also indicate that the variability in viscosity is less in high pH formulations (pH 7, 8 and 9). The pH values of the formulations became dark at low pHs or high temperatures.

4. Conclusion

In this performulation phase, ketoconazole was found to undergo less hydrolysis at alkaline pH.

The viscosity of the formulation was found to be more stable at high pH values. The amount of ketoconazole (ranging from 0.25 to 2%) in the formulation has little influence on the degradation mechanism. The increase in the antioxidant level, i.e. increasing butylated hydroxytoluene from 0.05 to 0.4%, affected the stability of ketoconazole particularly at pH 1. The pH value of the final formulation was chosen to be pH 7 based on the optimization of physical and chemical stability.

In conclusion, the final formulation developed (pH 7, 0.1% butylated hydroxytoluene) is stable with a shelf life of around 15 months.

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