International Journal of Pharmaceutics, 46 (1988) 267–270
Elsevier

IJP 01577

Short Communication

Dielectric constant effects on degradation of azathioprine in solution

Saranjit Singh and R.L. Gupta

Department of Pharmaceutical Sciences, Panjab University, Chandigarh (India)

(Received 4 January 1988)
(Accepted 20 March 1988)

Key words: Azathioprine; Dielectric constant effect; Reaction mechanism

Azathioprine (6-[(1-methyl-4-nitroimidazol-5-yl)thio]purine) is marketed as a freeze-dried preparation (Imuran, Burroughs Wellcome) to be reconstituted at the time of injection. This type of formulation has been necessitated by the insolubility of the drug base and the relative instability of the sodium salt in aqueous solutions.

We wished to develop a liquid injectable preparation of azathioprine using a suitable water-organic solvent mixture. In this context, preliminary investigations were carried out in our laboratory to observe the effect of solvent dielectric constant on the degradation rate of azathioprine.

In this communication we discuss the nature, hitherto unknown, of dielectric influence on the rate of azathioprine hydrolysis under acidic and basic conditions. That azathioprine degrades in the two pH regions by different reaction pathways was reported by us recently (Gupta et al., 1987; Singh and Gupta, 1988).

The reactions were carried out at 80 °C in acid (0.1 N HCl, pH 1.11) and alkali (0.02 N NaOH, pH 10.84) solutions containing various amounts of ethanol. The drug strength was maintained at 2.54×10^{-4} M. Residual azathioprine was analyzed by HPLC method reported earlier (Singh and Gupta, 1988). Pseudo-first-order rate constants for the overall degradation of azathioprine were calculated from the slopes of log(peak area) vs time plots ($r^2 > 0.98$).

The dielectric constant values (D) at 80 °C for the used water–alcohol binary systems are due to Åkerlöf (1932). These were related to the observed rate constants by plotting the logarithm of the rate constant against $1/D$ according to the Bronsted–Christiansen–Scatchard equation

$$\log k = \log k_0 - \frac{Z_A Z_B e^2}{2.303 D r k_B T} \quad (1)$$

where k_0 is the rate constant at $D = \infty$, Z_A and Z_B are the numbers of electronic charges on the ion, r is the radius of the activated complex, k_B is the Boltzmann constant and T is the absolute temperature. The plot in Fig. 1 shows that the rate of degradation of azathioprine decreases with decrease in dielectric constant of acidic solutions and remains unchanged with decreasing dielectric constant in alkaline medium.

Below pH 3.0, azathioprine essentially exists as an undissociated species and the rate of degradation is attributed exclusively to the proton-catalyzed reaction of undissociated azathioprine (Mitra

Correspondence: R.L. Gupta, Department of Pharmaceutical Sciences, Panjab University, Chandigarh 160 014, India.

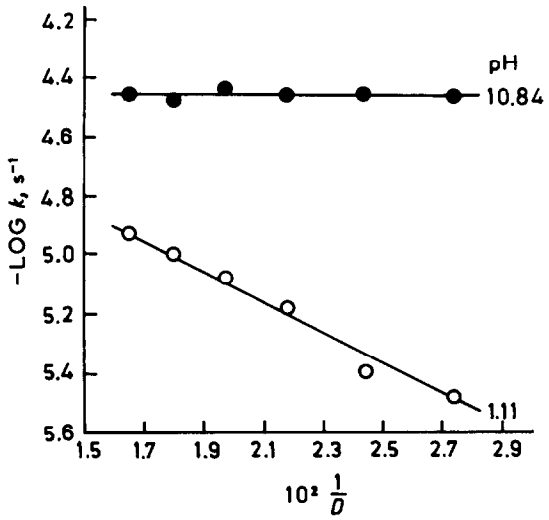


Fig. 1. Influence of solvent dielectric constant on the observed rate constants for azathioprine hydrolysis at indicated pH and 80 °C.

and Narurkar, 1986). The observed behaviour of dielectric influence at pH 1.11 is in conformity to this. A similar dielectric constant effect has been reported for acid hydrolysis of neutral intrazole (Prasad et al., 1972).

The observed absence of dielectric influence at pH 10.84 is, however, unexpected for a reaction between azathioprine anions and hydroxyl ions (Mitra and Narurkar, 1986). For interaction between equal charges, the theory of electrostatic effects predicts a lowering of rate with decrease in dielectric constant of the medium (Laidler, 1950).

It is found that no standard text on chemical kinetics principles carries a discussion on the circumstances under which dielectric influence may be totally absent for reaction between ions. An in-depth literature survey, however, reveals that similar deviations from electrostatic theory have been reported though rarely. The effect has been postulated to arise when either there is simultaneous increase in keto–enol tautomerization constant (Hansen and Bundgaard, 1981) or when a dielectric component of the solvent participates in the reaction process (Michoel and Kinget, 1977).

In the case of the azathioprine molecule, the position where tautomerization occurs is not considered as significant to influence the reaction rates. The other explanation, i.e. influence of dielectric saturation, can be considered if a reasonable mechanism, involving a highly charged transition state having high field strength at the collision site, can be hypothesized for the reaction between azathioprine anion and hydroxyl ion.

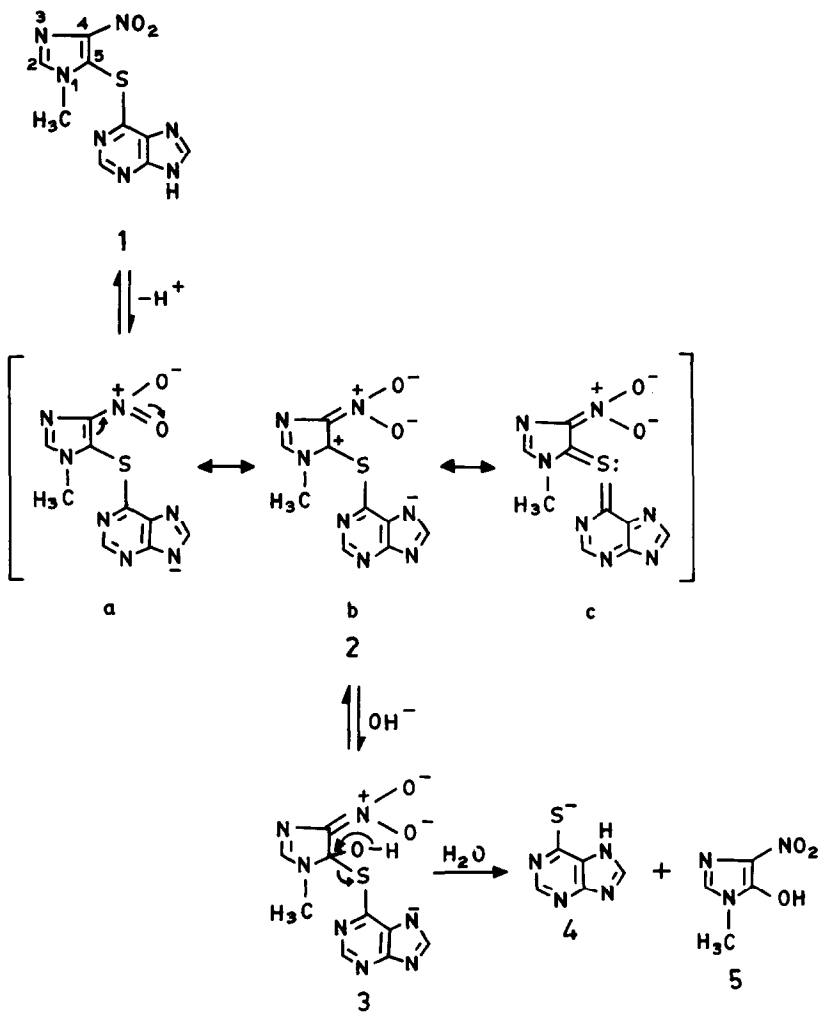
A mechanism for hydrolysis of azathioprine to 6-mercaptopurine under neutral and slightly alkaline conditions has been described earlier (Mitra and Narurkar, 1986). It was postulated that the degradation reaction is initiated by the nucleophilic attack by water molecules or by the hydroxide ion on the carbon atom at the 5-position of the methylnitroimidazole ring of azathioprine with simultaneous delocalization of π electrons of the C_4 and C_5 bond towards the electron-withdrawing nitro group. In the transition state electronic rearrangements occur leading to the double bond formation between C_4 and C_5 with simultaneous breakdown of the C_5–S bond leading to 6-mercaptopurine.

A plausible mechanism for hydrolysis of azathioprine to 6-mercaptopurine under high alkalinity conditions is described in Scheme 1. At high pH, the anionic form of azathioprine (**1**) can be more appropriately represented as a resonance hybrid of structures **2a–c** rather than structure **2a** alone. The existence of canonical structure **2b** is rationalized on the basis that the nitro group at C_4 in the imidazole moiety withdraws electrons from the adjacent unsaturated system and is fully ionized at high pH (Dumanović et al., 1975). The resonating structure **2c** can result in consequence to the possible transmission of electronic effects through the sulfide bridge (Hyne and Greidanus, 1968). Of the 3 resonating structures, the reaction is expected to proceed via **2b** as this is the one that will have a greater contribution under these polar conditions, due to its ionic character (March, 1977). It is interesting to note that this structure has a positive charge at C_5 in the imidazole moiety, which position is considered as a seat of reaction by Mitra and Narurkar (1986). The hydroxyl ion in such a case, therefore, easily combines with the oppositely charged positive center in the drug anion in a rate-determining step. An unstable intermediate, **3**, is produced which upon subse-

quent water attack rapidly breaks down to the monoanion of 6-mercaptopurine (**4**) and 1-methyl-4-nitro-5-hydroxyimidazole (**5**).

In accordance with this reaction scheme it is reasonable to assume that a transition state with exceptional multiplicity of charges originates when the azathioprine anion **2b** interacts with the hydroxyl ion. It is reported in the literature that the mesomeric effect is a permanent one which may even exist outside this reaction (Laidler, 1950). Since the transition state is highly charged, it shall be strongly solvated by solvent (water) molecules even when no organic solvent has been added to the system. By virtue of this hydration shell, the microdielectric constant in the vicinity of activated complex does not change despite a decrease in macrodielectric constant of bulk media upon addition of alcohol. The rate constants, therefore, correspond to these at all lower dielectric constants, as is the case in media of high dielectric constant.

It is pertinent to mention here that, while azathioprine exists predominantly in an *N*(1)H–*N*(9)H tautomeric form, 6-mercaptopurine is more stable as the *N*(1)H–*N*(7)H tautomer (Chenon et al., 1975).



Scheme 1

Mitra and Narurkar (1986) have reported a near 6000 times increase in rate constant with increase in pH beyond 10.0. The occurrence of coulombic attraction among the otherwise counter ions, in accordance with Scheme 1, is the determinant of facile hydrolysis of azathioprine in basic solutions.

The study highlights the importance of investigating dielectric constant effects during studies on kinetics and mechanisms of degradation of drugs, which are mostly complicated molecules. It stresses the need for giving careful consideration to the activated complexes together with their surrounding shells (Amis, 1966).

It is shown here that addition of organic solvent to aqueous alkaline solutions of azathioprine does not improve drug stability. However, at acidic pH one may get both increased solubility and stability if part of water as solvent medium is replaced by a suitable organic solvent. The latter approach is being tried in future studies.

References

Åkerlöf, G., Dielectric constants of some organic solvent-water mixtures at various temperatures. *J. Am. Chem. Soc.*, 54 (1932) 4125–4139.

Amis, E.S., *Solvent Effects on Reaction Rates and Mechanisms*, Academic, New York, 1966.

Chenon, M-T., Pugmire, R.J., Grant, D.M., Panzica, R.P. and Townsend, L.B., Carbon-13 magnetic resonance, XXVI. A quantitative determination of the tautomeric populations of certain purines. *J. Am. Chem. Soc.*, 97 (1975) 4636–4642.

Dumanović, D., Maksimović, R., Ćirić, J. and Jeremić, D., Simultaneous determination of *N*-unsubstituted and *N*-substituted nitroazoles and criteria for their identification. III. Chromatographic separation and polarographic determination of halo-nitroimidazoles. *Talanta*, 22 (1975) 811–818.

Gupta, R.L., Kumar, M., Singla, R.K. and Singh, S., Degradative behaviour of azathioprine in aqueous solutions. *Indian J. Pharm. Sci.*, 49 (1987) 169–171.

Hansen, J. and Bundgaard, H., Studies on the stability of corticosteroids VII. The effect of the dielectric constant on the rate of degradation of hydrocortisone in alcohol–water mixtures. *Arch. Pharm. Chemi, Sci. Ed.*, 9 (1981) 55–60.

Hyne, J.B. and Greidanus, J.W., Transmission of electronic effects in certain sulfides. In A.V. Tobolsky (Ed.) *The Chemistry of Sulfides.* Interscience, New York, 1968, pp. 83–104.

Laidler, K.J., *Chemical Kinetics*, McGraw-Hill, New York, 1950, pp. 127–130, 137.

March, J., *Advanced Organic Chemistry: Reactions, Mechanisms and Structure*, 2nd edn., McGraw-Hill, London, 1977, p. 37.

Michoel, A. and Kinget, R., Kinetic study of the hydrolysis of pancuronium bromide III. Media effects. *Acta Pharm. Suec.*, 14 (1977) 255–266.

Mitra, A.K. and Narurkar, M.M., Kinetics of azathioprine degradation in aqueous solution. *Int. J. Pharm.*, 35 (1986) 165–171.

Prasad, V.K., Ricci, R.A. and Granatek, A.P., Kinetics of solvolysis of intrazole. *J. Pharm. Sci.*, 61 (1972) 1579–1585.

Singh, S. and Gupta, R.L., A critical study on degradation of azathioprine in aqueous solutions, *Int. J. Pharm.*, 42 (1988) 263–266.