DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY, 12(4), 589-601 (1986)

The Effect of Various Additives On The Stability of Isoproterenol Hydrochloride Solutions

by

A.S. Nayak, A.J. Cutie, T. Jochsberger, and A.I. Kay

ABSTRACT

The effect of various additives on the rate of degradation of eight isoproterenol hydrochloride aqueous formulations was studied. In addition, the reaction was studied at various pH values and temperatures. From the latter data, activation parameters were determined. Rate constants increased from 10 to 100 times over a 25 degree increase in temperature. The activation energy for the reaction averaged approximately 25 kcal/mole. Ascorbic acid and sodium bisulfite appear to be the most efficient antioxidants in the system. Sequestering agents such as EDTA and citric acid do not appear to reduce the rate and, in one instance, EDTA seemed to enhance the degradation process. The reaction appears to be an oxidation of the catechol ring system of isoproterenol similar to that observed with epinephrine.

Catecholamines such as isoproterenol and epinephrine are highly subject to degradative oxidation in solutions. A number of antioxidants and other substances are added to formulations containing these substances in an effort to retard this degra-

0363-9045/86/1204-0589$3.50/0

dation. This study was undertaken to investigate the effect on the stability of the solutions. An attempt was also made to compare the interaction of isoproterenol with bisulfite and boric acid to the analogous interaction of the latter compounds with epinephrine.

EXPERIMENTAL

Isoproterenol hydrochloride, USP, and all other reagents and raw materials used in the study were either analytical or USP/NF grade. All materials were used without further purification.

Preparation of Isoproterenol Ampules - All antioxidant systems studied were formulated with 0.2mg/ml of isoproterenol hydrochloride.[1]

The pH of each formulation was adjusted to between 4 and 6 by the addition of 1.0 N sodium hydroxide. Samples were stored at 25, 37, 45, 55 and 60°. Distilled water used in the preparation of the ampules was saturated with nitrogen prior to use. All metal contacts were avoided and the solutions were not exposed to direct lighting. The antioxidants and isoproterenol hydrochloride were dissolved under a nitrogen head and rapidly filled (10ml per ampul) under a nitrogen overlayer. The contents of each formulation is given in Table 1. At designated intervals, samples were removed and assayed in duplicate for isoproterenol hydrochloride content.

Apparatus - All analyses were performed using a high-performance liquid chromatograph[2] equipped with an automatic sampler[3] having a

---

1. Greef & Company, Lot# N-136-5A, Old Greenwick, CT

2. Model 6000A Solvent Delivery System, M-Bondapak C_{18} column, Water Associates, Milford MA 01757

3. Model 838, Dupont, Wilmington, DE

TABLE I. Isoproterenol Formulations Evaluated

	F-1[a]	F-2	F-3	F-4	F-5	F-6	F-7	F-8
Isoproterenol Hydrochloride	0.2[b]	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Sodium Bisulfite	-	0.5	-	0.5	-	-	-	-
Sodium Citrate	-	2.8	-	2.8	-	-	-	-
Citric Acid Anhydrous	-	10.5	-	10.5	-	-	-	-
Sodium Chloride	7.0	4.25	-	-	-	-	-	-
Sodium Metabisulfite	1.0	-	1.0	-	-	-	1.0	-
Lactic Acid	0.12	-	0.12	-	-	-	0.12	-
Sodium Lactate	1.18	-	1.8	-	-	-	1.8	-
Boric Acid	-	-	15.0	15.0	15.0	15.0	15.0	15.0
Acetylcysteine	-	-	-	-	5.0	5.0	-	5.0
Thiourea	-	-	-	-	5.0	5.0	-	5.0
Sodium EDTA	-	-	-	-	-	-	1.0	1.0
pH	4.0	4.0	4.0	4.0	6.0	4.0	6.0	6.0

a = formula number

10 ml injection loop, a 3.9mm x 30 cm reversed-phase column, and a UV Detector[4].

The parameters used were two analyses per vial, started integration at 0 minutes and ran for a total of 6 minutes, initial flush time 21 seconds, flow rate 2.0ml/minutes UV detector at 273mm, 0.02 Absorbance Unit Full Scale (1mv) to 1 volt range and recorded input 1 volt.

Procedure for Preparation of Standard - One hundred mg of USP isoproterenol reference standard[5] were accurately weighed into a 100 ml volumetric flask and dissolved in nitrogen purged distilled water.

An appropriate volume was transferred by pipette to separate 100ml volumetric flasks and diluted to volume with 1% acetic acid which was used as the mobile phase. The assay and standard preparation were transferred into separate sampler vials. The vials were capped and loaded into the sampler tray and assayed as previously indicated. The average peak heights of the standard preparation dilutions were plotted against their concentration in mcg/ml (range to 100-250mcg/ml). The concentration of the assay preparation was determined from the average of two analyses. The boric acid chelate of isoproterenol hydrochloride was studied spectrophotometrically using 0.0002M isoproterenol hydrochloride in the presence of 0.0607M boric acid. The solutions were adjusted to varying pH values by the addition of 1.0 N sodium hydroxide. The UV absorption spectrum was evaluated at each pH.

RESULTS AND DISCUSSION

Decomposition rate constants and activation energies are summarized in Table II. It is clear that while the rates vary widely among the

4. Model LC-55, Perkin-Elmer, Elmwood Park, NJ

5. USPC Inc., Rockville, MD

TABLE II. Summary of Stability Data of Isoproterenol Formulations

Formulations	T°C	$K_1 \times 10^3$ Sec	$1/T \times 10^3$ K^{-1}	E_a (k cal/mole)
F-1[a]	37	1.04	3.20	24.5
	45	1.08	3.14	
	55	7.99	3.05	
	50	9.41	3.02	
F-2	55	4.87	3.05	19.2
	60	7.58	3.01	
F-3	37	2.52	3.20	21.0
	45	3.14	3.14	
	55	18.4	3.05	
	60	23.5	3.01	
F-4	37	1.19	3.20	27.5
	45	1.78	3.14	
	55	11.1	3.05	
	60	13.4	3.04	
F-5	37	1.02	3.22	31.6
	45	1.07	3.14	
	55	5.66	3.05	
	60	10.9	3.03	
F-6	25	1.99	3.36	22.1
	37	2.20	3.20	
	45	2.82	3.14	
	55	19.1	3.05	
	60	21.3	3.03	

(continued)

(Table II continued)

Formulations	T°C	$K_1 \times 10^3$ Sec	$1/T \times 10^3$ K^{-1}	E_a (k cal/mole)
F-7	25	1.94	3.36	26.3
	37	5.19	3.20	
	45	5.34	3.14	
	55	47.3	3.05	
	60	138.	3.03	
F-8	25	1.21	3.36	15.4
	37	2.30	3.20	
	45	2.38	3.14	
	55	5.55	3.05	
	60	8.19	3.03	

a = formula number

various formulations, the temperature effect is approximately the same in all cases. That is, the energy of activation varies slightly with the formulation.

Formulations with sodium bisulfite (F-2, F-4) had relatively low rates of decomposition. However, the presence of boric acid in the latter formulation diminished the stability by a factor of about two. The same effects can be observed in formulations with sodium metabisulfite. By comparing formulations F-1, F-3, and F-7, it can be seen that the latter two produce somewhat higher rates than the first, again due to the presence of boric acid. The addition of EDTA further diminishes the stability. Comparing F-3 and F-7, it is apparent that complexing agents, such as boric acid or EDTA, retard the stabilizing action of bisulfate.

Combinations of antioxidants are frequently used for synergistic reasons, and sodium edetate is commonly added as a sequestering agent. These antioxidants generally possess certain limitations with regard to their pH stability (5).

Although sodium metabisulfite is used extensively as an effective antioxidant, reports have indicated that the antioxidant activity of this substance is inhibited by a number of compounds (1-4). The metabisulfite has been shown to undergo degradation itself and by doing so potentiates the degradation of certain substances such as epinephrine.

The effectiveness of bisulfite as an antioxidant in typical pharmaceutical systems depends on the ease with which this compound is oxidized in comparison with the drug it is supposed to protect. Substances that inhibit bisulfite oxidation may exert important effects on the overall stability of the product by decreasing the antioxidant effect of bisulfite. It has been postulated that the mechanism by which these substances inhibit bisulfite activity is through the formation of coordination compounds between inhibitor and bisulfite. Typical substances that can inhibit the oxidation of bisulfite are mannitol, phenol, inorganic anions, aldehydes, ketones, and alkaloids (7).

A study of the stabilization of epinephrine (4) against sulfite catalyzed degradation revealed that when boric acid was added to the solution a marked stabilization of the catecholamine took place. The authors postulated that the stabilizing effect of boric acid was due to chelate formation between boric acid and the catechol moiety of epinephrine. Epinephrine is able to form a 1:2 chelate via its dihydroxy structure (Figure 1).

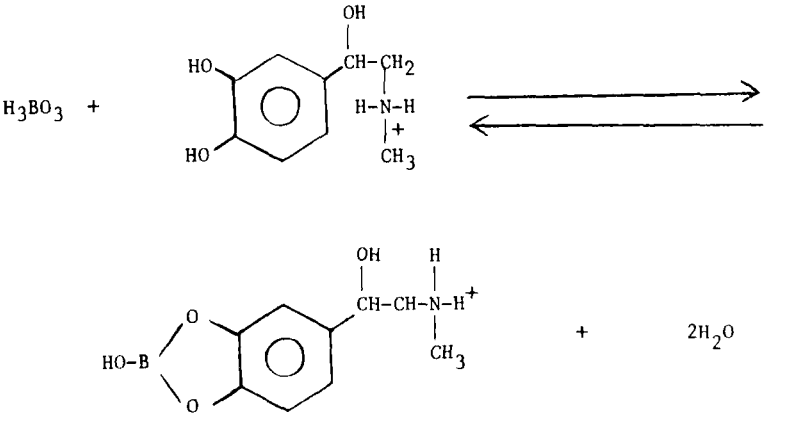
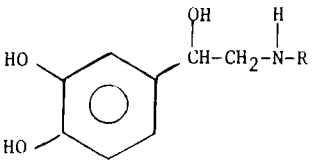


Figure 1. Chelate Formation between Boric Acid and the Catechol Moiety of Epinephrine.

In the presence of boric acid, the rate of sulfite attack is reduced as the hydrogen ion concentration decreases (4). The half-life for epinephrine under the reaction conditions of pH 6.0 in the absence of boric acid was found to be 195 hours, whereas in the presence of boric acid, the half-life was found to be 267 hours. At pH 7.5, however, the half-life of epinephrine was found to be 74 hours in the absence, and 1270 hours in the presence of boric acid. It was postulated (4) that epinephrine is increasingly chelated by the boric acid molecules as the pH is made more alkaline and that the chelated epinephrine is far less susceptible to sulfite attack than epinephrine. Isoproterenol, which is structurally similar to epinephrine, (Figure 2), shows the same behavior with bisulfite and boric acid (Figure 3).

Figure 4 represents the ultraviolet absorption spectra of isoproterenol (0.0002M) in the presence of 0.0607M boric acid at varying hydrogen ion concentrations. Since two isosbestics were observed, the solutions contained only two absorbing species. The absorption of these two species as a function of pH is provided in Figure 5.



ISOPROTERENOL R = $CH(CH_3)_2$

EPINEPHRINE R = CH_3-

Figure 2. Structural Similarity between Isoproterenol and Epinephrine.

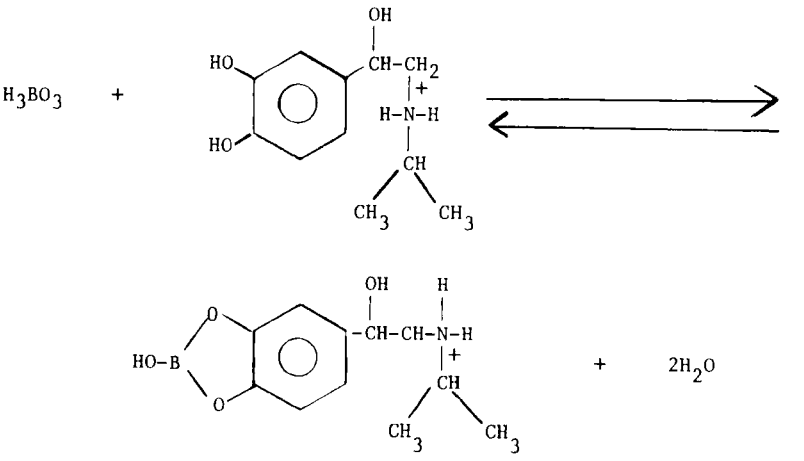


Figure 3. Chelate Formation between Boric Acid and the Catechol Moiety of Isoproterenol.

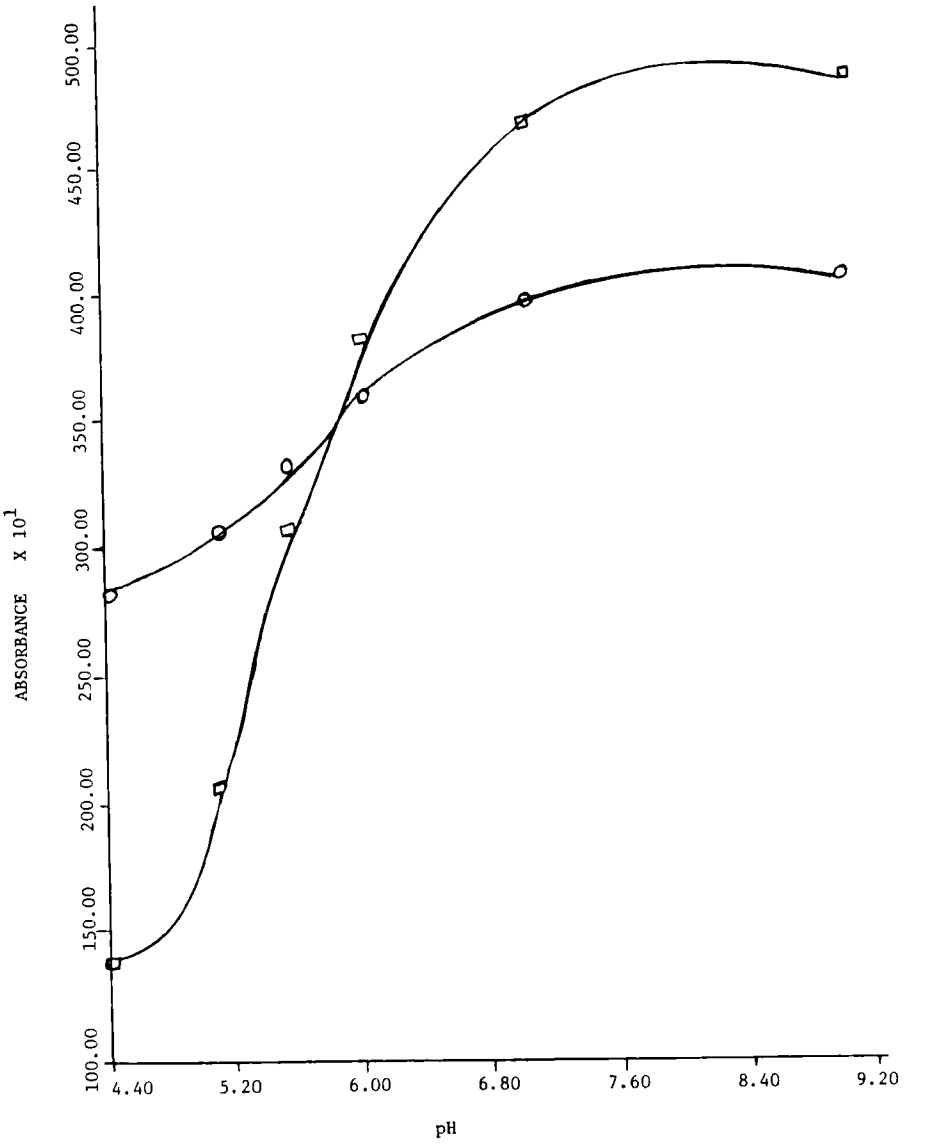


Figure 4. Ultraviolet Absorption Spectra of 0.0002M Isoproterenol in the Presence of 0.0607M Boric Acid at Various Hydrogen Ion Concentrations.

□ Absorbance at 240 nm

o Absorbance at 280 nm

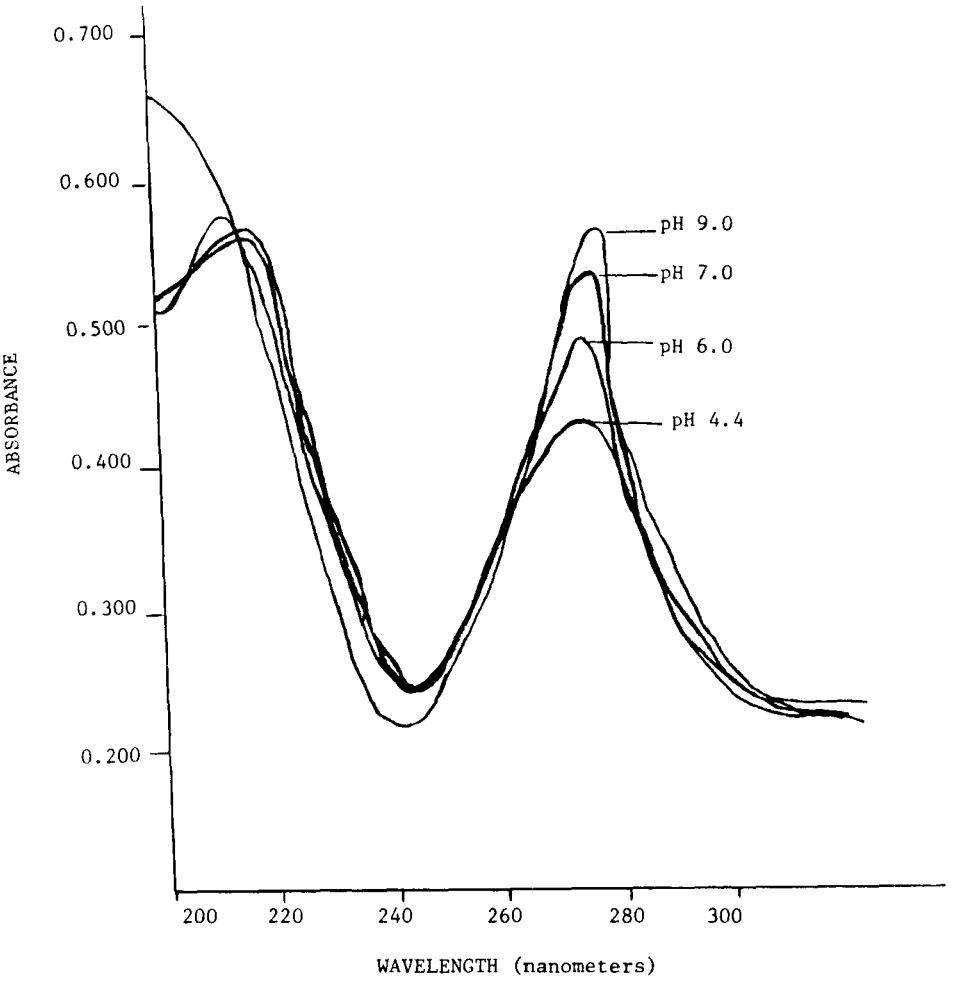


Figure 5. Isosbestic Species of Isoproterenol as a function of pH.

Comparison of formulation F-5 and F-6 indicates that the degradation rate decreases with increasing pH. This would indicate the participation of some sort of acid catalyzed reaction. It might also account, in part, for the role of boric acid in decreasing the stability. The equilibrium constant for the reaction shown in Figure 3 may be represented as follows:

$$K = \frac{\{H^+\}\ \{IpB^-\}}{\{Ip\}\ \{HB\}} \qquad \text{(Eq. 1.)}$$

where {HB}, {Ip} and $\{IpB^-\}$ represents the equilibrium concentration of boric acid, isoproterenol and boro-isoproterenol chelate respectively.

Under the conditions of this study where the concentration of boric acid is equal to or exceeds the concentration of isoproterenol, one can expect 1:1 chelation. This was verified by the fact that one mole of base was required to react per mole of isoproterenol added. Adopting a modification of the method of Rosenblatt (6), a stability constant for the boric acid-isoproterenol complex can be calculated from the absorption data.

$$K_1 = \{HB\}K = \frac{\{H^+\}\ \{IpB^-\}}{\{Ip\}} \qquad \text{(Eq. 2.)}$$

This can be solved by the use of a third order determinant (equation 3).

$$\{HB\}K = K_1 \frac{\begin{vmatrix} \{H^+\}_1 & \varepsilon_1\{H^+\}_1 & 1 \\ \{H^+\}_2 & \varepsilon_2\{H^+\}_2 & 1 \\ \{H^+\}_3 & \varepsilon_3\{H^+\}_3 & 1 \end{vmatrix}}{\begin{vmatrix} \varepsilon_1\{H^+\}_1 & 1 \\ \varepsilon_2\{H^+\}_2 & 1 \\ \varepsilon_3\{H^+\}_3 & 1 \end{vmatrix}} \qquad \text{(Eq. 3.)}$$

Solution of the determinant at the two wavelengths of interest yields pK values of 4.34 and 4.20 at 280 and 240 mm respectively.

These values are quite similar to those observed for the epinephrine-boric acid chelate reported by Riegelman et al (4) and indicate, therefore, a parallel stability pattern. This is not surprising considering the structural similarity of the two molecules, particularly in the area of interaction.

The values of the stability constants calculated in this manner indicate that the reaction illustrated in Figure 3 is predominantly shifted to the left at low pH. Both the ratio of boric acid to iso-

proterenol and the hydrogen ion concentration affect the concentration of free isoproterenol in the system. Since there is a preactical limit to which the concentration of boric acid can be increased, the most important variable in the reaction is the hydrogen ion concentration. As the hydrogen ion concentration is decreased, the boro-isoproterenol chelate is stabilized to bisulfite attack.

While there is no evident change in stability of isoproterenol solutions in the presence of boric acid at pH 4, boric acid enhances isoproterenol stability at pH 7 or higher because of the formation of the complex. However, this effect may be negated, in part, by the presence of competing complexing agents, such as EDTA.

REFERENCES

1. L.C. Schroeter, J. Pharm. Science, 52:564 (1963)

2. T. Higuchi and L.C. Schroeter, J. Am. Pharm. Assoc., Sci. Ed. 48:535 (1959)

3. L.C. Schroeter, T. Higuchi and E. Schuler., J. Am. Pharm. Assoc., Sci. Ed. 47:724 (1958

4. S. Riegelman and E.Z. Fisher, J. Pharm. Sciences, 51:206 (1962)

5. M.J. Akers, J.P.D.A., 33:346 (1979)

6. D.M. Rosenblatt, J. Phys. Chem. 58:40 (1959)

7. "The Theory and Practice of Industrial Pharmacy," L. Lachman, H.A. Lieberman, and J.L. Kanig, Eds., Henry Kimpton Publishers, London, 2nd Edition, 1976, p. 57.