Preliminary Studies on Oxidative Decomposition of Dipyrone Solutions

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Abstract □ The oxidative decomposition of dipyrone was studied in a preliminary way by utilizing hydrogen peroxide and molecular oxygen. During these studies, it was determined that hydrolysis of dipyrone will also occur and form at least two decomposition products: bis[*N*-methyl(antipyrinyl)amino]methane and *N*-methylaminoantipyrine. The structures of these two products were confirmed by studies of their TLC, melting points, and spectral characteristics compared to synthesized knowns. Stability studies conducted at pH values ranging from 4.8 to 8.8 showed that the initial rate of loss of dipyrone was very rapid regardless of the initial pH of the solution. Within the range of values examined, the dipyrone solutions showed the least decomposition at pH values above 7.8. These preliminary studies suggest that dipyrone should show optimum stability if stored completely anhydrous and excluded from the atmosphere. In liquid dosage forms, improved stability should be obtained by flushing the head-space with an inert atmosphere and by incorporating a suitable antioxidant.

Keyphrases □ Dipyrone solutions—oxidative decomposition, hydrolysis products identified □ Bis[*N*-methyl(antipyrinyl)amino]-methane—isolation, identification as dipyrone decomposition product □ *N*-Methylaminoantipyrine—isolation, identification as dipyrone decomposition product □ TLC—isolation, identification □ Hydrolysis, dipyrone—isolation, identification of decomposition products □ Oxidation, dipyrone—isolation, identification of decomposition products

Dipyrone is used therapeutically as an antipyretic analgesic and as an antirheumatic agent. Its widespread use throughout the world has led to its being known under at least 20 different trade and chemical names. It is a member of the pyrazolone group of compounds. Being a sodium sulfonate salt, it is soluble in water: 1:1.5 ml. A presently accepted chemical name (1) is: 1-phenyl-2,3-dimethyl-5-pyrazolone-4-methylaminomethanesulfonate sodium.

Formulators in the pharmaceutical industry have long known that freshly made dipyrone solutions are pale to medium yellow in color (depending upon concentration) and that the solutions deepen in color intensity with the passage of time. However, if freshly made pale-yellow solutions are subjected to autoclaving conditions (*i.e.*, 121° for 15 min.), the pale-yellow color completely disappears, only to reappear after long periods of storage.

A search of the literature dating back to the first synthesis of dipyrone (2) failed to uncover any useful information concerning its stability in solution other than to recommend the auxiliary use of suitable antioxidants (3). This study was undertaken to begin the systematic evaluation of the stability of dipyrone in solution.

It is probable that dipyrone will autoxidize in a similar manner as any other compound containing the sulfite group (4, 5). The oxygen for the reaction should be available from both the water itself and from the atmosphere over its solutions. The primary site for oxidation is the sulfite group, and the empirical equation representing sulfite oxidation may be written as:

$$SO_3^{-2} + {}^1/_2O_2 \rightarrow SO_4^{-2} \qquad \text{(Eq. 1)}$$

The specific, stepwise reactions are quite complex because they are mediated through free radical mechanisms. (See *Reference 5* for a detailed description.)

Abel (6) proposed a general scheme for anionic autoxidation:

$$O_2 + A^- \rightarrow AOO^- \qquad \text{(Eq. 2)}$$

$$AOO^- + X^0 \rightarrow 2O^- + X^+ + A\cdot \qquad \text{(Eq. 3)}$$

$$A\cdot + X^0 \rightarrow X^+ + A^- \qquad \text{(Eq. 4)}$$

where A^- is the electron donor and X^0 represents the reducing species.

Schroeter (5) described HSO_3^- ion autoxidation according to Abel's generalized scheme as follows:

$$O_2 + OH^- \rightleftarrows O_3H^- \qquad \text{(Eq. 5)}$$

$$O_3H^- + HSO_3^- \rightarrow 2O^- + HSO_3\cdot + OH\cdot \qquad \text{(Eq. 6)}$$

$$HSO_3\cdot + OH\cdot \rightarrow 2H^+ + SO_4^{-2} \qquad \text{(Eq. 7)}$$

The summation of reactions 5, 6, and 7 leads to:

$$HSO_3^- + O_2 + OH^- \rightarrow 2H^+ + SO_4^{-2} + 2O^- \qquad \text{(Eq. 8)}$$

Most autoxidations are, in general, more favored in basic rather than in acidic solutions, and they are accelerated by heat due to increased dissociation of the HSO_3^- species. The present study presents evidence that there is less overall loss of dipyrone in basic solutions, an observation that is an apparent exception to the generalization.

EXPERIMENTAL

Dipyrone[1] Solutions—The concentration selected for study was held constant at 3.5% w/v. The solvent used was distilled water except as noted. For all studies in which air was excluded, the solutions were prepared as follows. Freshly distilled water was heated to boiling to remove the dissolved gases and then cooled while nitrogen gas was bubbled constantly through the solution. During all manipulations, nitrogen was continually utilized. The solutions, so protected, were transferred to 10-ml. tip-sealed ampuls; then the head-space was flushed with nitrogen, and the ampul was sealed.

Accelerated Studies—Appropriate numbers of ampuls of 3.5% w/v dipyrone solutions were autoclaved at 121° for 1, 3, 5, and 7 hr. At each time interval, three ampuls were selected at random from the container for assay.

Hydrogen Peroxide Studies—Dipyrone solutions in deoxygenated water, protected with nitrogen gas, were combined with measured

[1] Dipyrone, Lot RMR6600515, provided by the Sterling-Winthrop Research Institute, Rensselaer, N. Y., through the courtesy of Morris E. Auerbach, formerly Head of the Analytical Section. The dipyrone was further purified by soxhlet extraction using chloroform and benzene over a 34-hr. period.

amounts of standardized hydrogen peroxide solution (2.12% w/v) theoretically to induce serial oxidation amounting to 10–60% of the dipyrone content.

Studies in Buffered Solutions—Standard USP XVIII buffer solutions (0.2 *M*) were prepared. These were used to prepare 250 ml. of 3.5% w/v dipyrone solutions. All pH measurements were made on an expanded scale pH meter[2].

TLC Studies—The stationary phase consisted of precoated special silica gel plates[3]. The mobile phase consisted of a mixture of freshly distilled chloroform and isopropylamine (97:3). For each determination, 0.005 ml. of solution was spotted using a microsyringe. The location of migrating spots was found by UV illumination.

Preparation of Bis[*N*-methyl(antipyrinyl)amino]methane—The procedure reported by Wagner (7) was followed; m.p. 171–174.5° [lit. (7) m.p. 171–175°]; R_f 0.34 in system described.

Preparation of *N*-Methylaminoantipyrine—Formylaminoantipyrine[4] was methylated using dimethyl sulfate according to the well-known procedure. *N*-Methylaminoantipyrine is a thick oily liquid (8) at room temperature. Purification by vacuum distillation at 5 μ of Hg was not successful: thermal decomposition occurred. IR and NMR data provided confirmatory data; R_f 0.51 in the system described.

Extraction Studies—Following the oxidation of dipyrone solutions by standardized hydrogen peroxide solutions, the reaction products in aqueous solution were serially extracted with chloroform. The chloroform-soluble extractives were combined and determined gravimetrically. The extractives varied from 2% from the solution representing 10% oxidation to 25% for 60% oxidation.

Molecular Oxygen Study—Dipyrone solution in deoxygenated water was charged into a pressure reaction bomb[5]. Under constant agitation, oxygen was fed to the bomb at a constant pressure of 4 atm. Samples of 10 ml. were taken from the reaction vessel every 48 hr.

Iodometric Assay of Dipyrone[6]—To an accurately measured aliquot of 7 ml. of the sample were added, in rapid succession, 50 ml. of distilled water, 1 ml. of starch indicator solution, and 5 ml. of 1 *N* sulfuric acid. The prepared sample was rapidly titrated against standardized 0.1 *N* iodine solution. Each milliliter of 0.1 *N* iodine is equivalent to 17.57 mg. of dipyrone monohydrate.

RESULTS AND DISCUSSION

Study I, reported in Table I, shows that analytical loss of dipyrone in distilled water sealed in an ampul ceased after 5 hr. at 121°. About 22% of the label claim of dipyrone (3.5% w/v) was lost. It appears that the decomposition of dipyrone was limited by the amount of air (*i.e.*, oxygen) entrapped in the sealed ampul. Study II, also reported in Table I, reveals that if air (*i.e.*, oxygen) is excluded from the system, the extent of decomposition is markedly reduced. Only 6% decomposition was observed over 7 hr. at 121°. These studies indicate that temperature *per se* has no effect on the decomposition of dipyrone other than to accelerate the rate of oxidative loss.

Table II presents the composite results of several experiments in which the oxidative decomposition of dipyrone in solution was mediated by standardized solutions of hydrogen peroxide at room temperature. On comparing the values obtained, it is observed that at most of the time periods sampled, the desired theoretical extent of oxidation was not achieved while in some samples the decomposition measured exceeded the degree anticipated. It is, therefore, possible that "decomposition" might not be due only to oxidation. Other factors such as hydrolysis and pH changes could influence the overall decomposition. The data in this table also show that hydrogen peroxide rapidly oxidizes dipyrone early in time and that the overall rate of loss of dipyrone slows down as oxidation proceeds. The data presently available are insufficient to make any approximation of either the order or the overall rate constant for this reaction.

Table I—Autoclaved Dipyrone Samples in Distilled Water (I) and Nitrogen-Treated Distilled Water (II), Showing Percent Remaining Values

Sample	Milliliters of 0.1136 *N* I_2 Consumed		Percent Remaining	
	I[a]	II[a]	I[a]	II[a]
Control	11.40	11.20	100	100
1 hr.	9.84	10.90	86.32	97.31
3 hr.	9.30	10.50	81.58	93.76
5 hr.	8.85	10.50	77.62	93.76
7 hr.	8.85	10.60	77.62	94.64

[a] Average of two readings.

Additional samples from Study III, Table II, were assayed at the end of storage for 7 days at room temperature. At this time interval, the 50% sample showed completion of reaction. At the end of 3 weeks at room temperature, the 60% sample had not yet gone to completion.

TLC studies were done on samples of the solutions reported in Table II. In the system described previously, two compounds (having R_{f1} 0.34 and R_{f2} 0.51) were separated. The latter component was not, however, detected in those solutions designed to yield 30% or less decomposition. Upon exposure of these TLC plates to air for 48 hr., the 0.51 spot then became visible.

Oxidation of the sulfonate species in dipyrone should result in measurable decreases in solution pH. The changes in solution pH as a result of hydrogen peroxide-induced oxidation were examined and are reported in Column 2 of Table III. The data raised the question: why should the 40% solution show a *less* acid pH than the solution designed for 10% decomposition? To aid in the resolution of this question, the oxidized solutions were serially extracted with chloroform until the aqueous solution pH stabilized. These data comprise the remaining columns in Table III. It was determined that the chloroform-soluble extractive varied from 2 to 25% w/w of the starting dipyrone content. It would thus appear that considerable decomposition to form amine bases was also occurring during the oxidative decompositions. The amount and type of amines created would affect the final solution pH's of their sulfate salts. TLC resolution of the chloroform-soluble products produced three spots visible under UV illumination: R_{f1} 0.06, R_{f2} 0.32, and R_{f3} 0.53.

Since a low level of oxidation produced measurable amounts of chloroform-soluble extractive, the starting dipyrone sample was

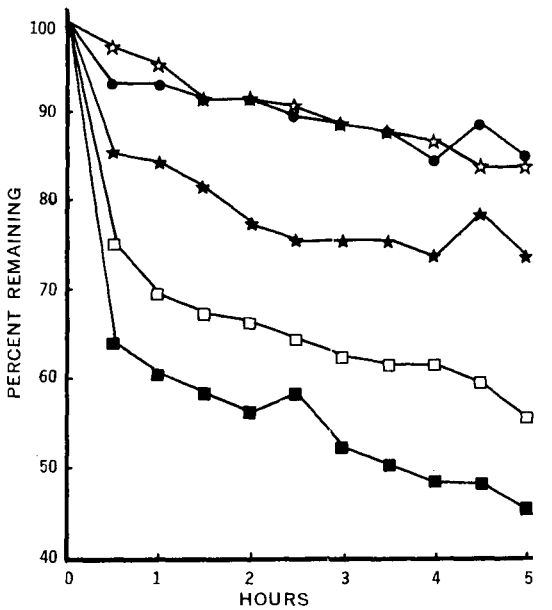

Figure 1—*Percent of label claim* versus *time at different pH's. Key:* ☆, *pH 8.85;* ●, *pH 7.75;* ★, *pH 6.75;* □, *pH 5.85; and* ■, *pH 4.85.*

[2] Beckman model 76.

[3] Silica gel F_{254}, supplied by EM Reagents Division, Brinkmann Instruments, Inc., Westbury, CT 11590. These plates were donated to the College of Pharmacy, Wayne State University, through the generosity of Walter Wiehler, Sterling-Winthrop Research Institute, Rensselaer, N. Y.

[4] The authors are indebted to John Flynn, Plant Manager, Winthrop Laboratories, Rensselaer, N. Y., for providing this intermediate.

[5] Supplied by Parr Instrument Company, Inc., Moline, IL 61265

[6] Method supplied through the courtesy of Morris E. Auerbach, Sterling-Winthrop Research Institute.

Table II—Percent of Dipyrone Remaining Values following Oxidative Decomposition with Hydrogen Peroxide for 24, 48, and 72 hr.[a]

	I		II		III	
	Milliliters of 0.1099 N I_2 Consumed	Percent Remaining	Milliliters of 0.1099 N I_2 Consumed	Percent Remaining	Milliliters of 0.1099 N I_2 Consumed	Percent Remaining
Control (fresh)	11.1	100	11.1	100	11.1	100
Control (after 24, 48, and 72 hr.)	11.1	100	11.1	100	11.1	100
10%	10.5	94.59	9.65	86.8	9.7	87.38
20%	9.5	85.58	8.8	79.2	8.5	76.57
30%	8.6	77.47	7.85	70.6	7.7	69.36
40%	7.7	69.37	7.1	63.9	6.8	61.26
50%	6.9	62.16	6.5	58.6	6.2	55.85
60%	6.4	57.65	5.6	50.4	5.6	50.45

[a] I, II, and III are the three different studies wherein the samples were assayed after allowing them to stand at room temperature (25.1°) for 24, 48, and 72 hr., respectively.

Table III—Solution pH's following Extraction with Chloroform Samples[a]

	pH					
	At the Start	First Extraction	Second Extraction	Third Extraction	Fourth Extraction	Fifth Extraction
Control	6.95	—	5.35	5.62	5.75	5.3
10%	4.55	3.18	3.15	3.05	3.05	3.05
20%	4.3	2.95	2.65	2.65	2.65	—
30%	4.45	2.92	2.65	2.45	2.55	—
40%	5.45	2.95	2.65	2.52	2.52	—
50%	4.20	—	2.65	2.82	2.68	—

[a] pH of deoxygenated water = 7.25; pH of dipyrone solution (3.5% w/v) = 6.95; pH of hydrogen peroxide solution (2.12% w/v) = 4.95; and pH of deoxygenated water plus varying amounts of hydrogen peroxide solution (2.12% w/v) = 5.3.

extracted in a soxhlet extractor with chloroform. The soluble extractive (2% w/w) yielded two spots in the TLC system described: R_{f_1} 0.057 and R_{f_2} 0.335. It thus appears that the R_{f_1} 0.06 compound is a contaminant in the sample as received. The R_{f_2} 0.335 product may be the same product as the R_{f_2} 0.32 compound already described or a very closely related product.

The data show that, as expected, the pH of oxidized dipyrone solutions decreases. Extraction of the oxidized solutions causes a further decrease in aqueous pH because the basic compounds partition even at an acidic pH. At this stage it is not possible to identify at what pH the hydrolytic degradation of dipyrone begins to be of major importance.

It was next of interest to observe the effect of the pH of the initial dipyrone solution upon the rate of oxidation. The study of the decomposition of dipyrone in buffer solutions at 121° is summarized in Fig. 1. This preliminary study indicates that under the very specific conditions utilized, the rate of loss of dipyrone appears to be pH dependent. However, since it is now becoming evident that the overall loss of dipyrone from solutions is exceedingly complex, the added complexity introduced by the buffer constituents cannot yet be properly evaluated. On the other hand, these data indicate that the initial rate of loss of dipyrone is quite rapid regardless of starting solution pH.

Attention was turned to the identification of the chloroform-soluble, basic products of reaction. The work of Wagner (7) showed that under strongly acidic conditions, dipyrone yields bis[*N*-methyl-(antipyrinyl)amino]methane. This compound was made according to his method and it possessed the proper melting point. In his proposed reaction pathway, Wagner indicated that *N*-methylaminoantipyrine was an intermediate. This compound was synthesized by methylating formylaminoantipyrine by use of dimethyl sulfate to yield a viscous brown oil as previously reported (8). Attempts to purify this compound further through vacuum distillation and by salt exchange have been unsuccessful so far. NMR examination showed the proper number of hydrogens.

These two known compounds were utilized in individual and in mixed sample TLC chromatograms, as previously described, and the results are presented in Table IV. These data demonstrate that bis-[*N*-methyl(antipyrinyl)amino]methane and *N*-methylaminoantipyrine are two major hydrolytic products which form during the concurrent oxidative decomposition of dipyrone in solution.

Since dipyrone is rapidly oxidized by hydrogen peroxide, a study was conducted on the ability of molecular oxygen to degrade dipyrone in solution. The study was carried out on an unbuffered solution of dipyrone in deoxygenated distilled water charged into a pressure reactor (Parr)[7] under 4 atm. of oxygen at room temperature. The data obtained are summarized in Fig. 2. Under these conditions, the dipyrone showed about 10% decomposition within 38 hr. at room temperature. The TLC studies (Table IV) on samples removed for assay showed that the bis-compound and the unknown component R_f 0.057 were formed under these conditions. It is probable that the latter component is the same R_f 0.06 component found in the starting unextracted dipyrone sample. No detectable *N*-methylaminopyrine was found under these conditions.

The data presented in Fig. 2 was plotted on semilogarithmic paper as percent remaining *versus* time to yield a reasonably linear plot. However, since this study terminated before more than three half-lives had occurred, no preliminary judgment on the order of reaction is warranted.

SUMMARY

1. As long as molecular oxygen is freely available to dipyrone solutions, oxidative loss of sulfite is relatively rapid, at least initially.

2. Dipyrone solutions also undergo decomposition to yield at least three compounds: R_{f_1} 0.06, structure unknown; R_{f_2} 0.34, bis-[*N*-methyl(antipyrinyl)amino]methane; and R_{f_3} 0.53, *N*-methylaminoantipyrine.

3. The overall decomposition of dipyrone in solution is quite complex.

APPLICATION OF DATA

From the standpoint of the acceptability of a "stable" pharmaceutical dosage form, the formulator is concerned with only the rate of loss of the first 10% of the label claim. Thus, the findings do offer guidance which should lead to improved overall product stability for the following:

[7] While under constant agitation with two turbine stirrers.

Table IV—Mixed R_f Study to Identify the Number of Degradation Products of Dipyrone

Samples	R_f Values
Bis[*N*-methyl(antipyrinyl)amino]-methane	0.34
N-Methylaminoantipyrine	0.51
Dipyrone oxidized by hydrogen peroxide	
First spot	0.34
Second spot	0.51
Dipyrone oxidized by molecular oxygen	
First spot	0.057
Second spot	0.34
Dipyrone oxidized by hydrogen peroxide + dimer + *N*-methylaminoantipyrine	
First spot	0.34
Second spot	0.51
Dipyrone oxidized by molecular oxygen + dimer + *N*-methyl-aminoantipyrine	
First spot	0.057
Second spot	0.34
Third spot	0.51

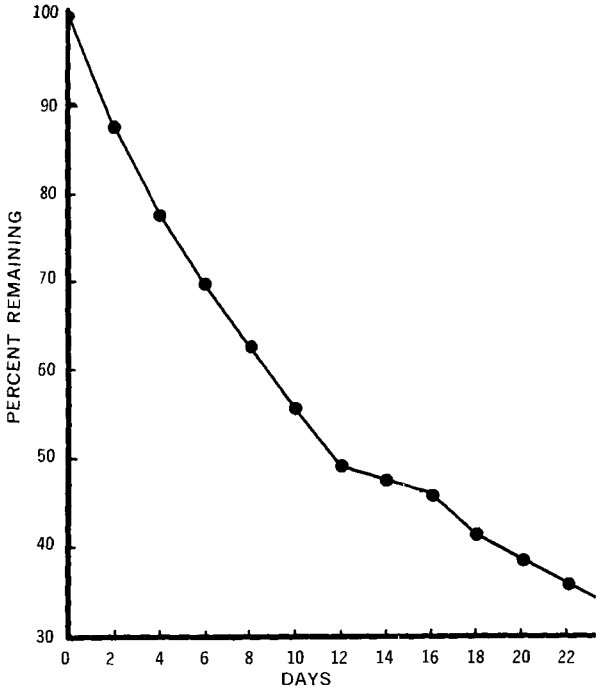

Figure 2—*Percent dipyrone remaining* versus *time using molecular oxygen.*

Dipyrone Solutions—

1. It may be advantageous to study a formulation buffered to pH 6.7 or above in addition to the formula ultimately selected for stability study.

2. The head-space of the container should be flushed with an inert gas before closing, and the water used in the formula should be deoxygenated.

3. The formula should contain an acceptable antioxidant such as was recently patented by Erbe (3), who found that solutions containing ethylenediaminetetraacetic and citric acids remained colorless after several days of exposure to light (whereas pure solutions of the pyrazolone turned lemon yellow within 18 hr.). Erbe also studied butylated hydroxyanisole and butylated hydroxytoluene as antioxidants for this compound. Sodium bisulfite would not be acceptable if the above assay is used for dipyrone because it is equally oxidized by iodine.

4. A "use test" on the solution may be indicated for the solution in its selected container. The air-space volume keeps increasing as the product is consumed, and it is likely that whatever the atmosphere is in the initial head-space, it would be largely replaced during the first use by the patient.

Dipyrone Dry Dosage Forms—

1. All granulations of dipyrone should be prepared "dry" because the large surface area exposed to both heat and moisture as a result of wet granulation would be conducive to both oxidation and hydrolysis of the dipyrone content.

2. Bulk containers (more than 100 tablets per bottle) should be airtight and provided with a packaged dessicant to help protect the last few tablets dispensed.

3. A stability study in unit dose aluminum foil packets appears to be warranted.

Suppositories—It appears that the nonaqueous suppository bases may provide maximal protection to dipyrone due to their hydrophobic character. However, biologic availability and stability must be confirmed. Daskel and Raik (9) showed that dispersions of dipyrone and sodium salicylate in anhydrous lanolin or cocoa butter were more rapidly released than when incorporated in emulsion bases.

Voigt and Falk (10) showed that dipyrone and other compounds were released quite rapidly from fatty bases. The addition of 2% w/w additives such as a sorbitan fatty acid ester[8], aluminum stearate, or a dialkyl sodium sulfosuccinate[9] to cetyl phthalate had an inhibiting effect on the release of soluble drugs but only a slight effect, or none, on water-insoluble drugs. The addition of 2% w/w bentonite did not reduce the drug release rate for either class of compounds.

The data developed in this preliminary study clearly indicate that additional investigation of the overall decomposition of dipyrone in solution is warranted. Of prime importance is the development of a complete analytical procedure to detect and quantify *all* of the products formed during the complex degradation. The study of the reaction of dipyrone with molecular oxygen in the pressure reactor indicates that it offers promise for the determination of the kinetic and thermodynamic parameters of the oxidative reaction.

[8] Arlacel 161.
[9] Aerosol.

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