Stability of Morphine in Aqueous Solution III

Kinetics of Morphine Degradation in Aqueous Solution

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The degradation of morphine in aqueous solution is dependent on the pH of the solution and on the presence of atmospheric oxygen in the system. The overall reaction rate, in systems containing excess oxygen, was found to be equal to

$$\left[k_1' \left(\frac{Ka}{Ka + H^+} \right) + k_2' \left(\frac{H^+}{Ka + H^+} \right) \right] (Morphine)$$

Degradation mechanisms for morphine, based on kinetic data and previously reported data on naphthol oxidation, are presented.

MORPHINE has been used as an analgesic and sedative since its isolation in 1805. Due to the limited solubility of morphine base, the acid salts, chiefly as the sulfate and hydrochloride, have been used extensively in various pharmaceutical preparations.

The stability of morphine in aqueous solution has been studied by many investigators since such solutions, after prolonged storage, undergo decomposition, as evidenced by discoloration. This decomposition of morphine is believed to be due to an oxidation reaction resulting in the formation of pseudomorphine (oxymorphine) and morphine N-oxide in the ratio of 9:1, together with a trace of a base believed to be methylamine (1). The oxidation of morphine and subsequent condensation to the dimer pseudomorphine is assumed to involve the phenolic group, as in the oxidation of naphthols to dimolecular compounds. Morphine derivatives not possessing the free phenolic group, as in the case of codeine and diacetyl morphine, do not undergo this type reaction (2). The oxidation of morphine is catalyzed by oxygen of air (3), sunlight (4), ultraviolet irradiation (5), iron and organic impurities (6), rat liver slices (7), tissue homogenates (8), and cytochrome (9). Ionescu-Matin, et al. (10), claimed that the deterioration of morphine in presence of oxygen takes place through condensation of morphine at the phenolic hydroxy group with the formation of the dimer, pseudomorphine. However, in the absence of oxygen, and in the presence of light, they also claimed that the deterioration is due to peroxidation or dimerization which can take place through the oxygen of the hydroxy group which was thought to be activated by ultraviolet light,

resulting in the formation of bimorphine. Abood and Kun (8) reported that in the course of oxidation of morphine by tissue, one mole of morphine utilizes one-half mole of oxygen and that the phenolic hydroxy group is oxidized to a quinone. Thorn and Agren (11) reported that the pseudomorphine formed in this oxidation was extremely stable and did not undergo further decomposition. However, Balls (12) pointed out that pseudomorphine was quite unstable, that it decomposes on either oxidation or reduction, and that in alkaline solution pseudomorphine gradually decomposed to higher oxidized products.

The stability of morphine in aqueous solution is largely dependent on the hydrogen ion concentration. In alkaline or neutral solution, morphine deteriorates rapidly at room temperature, whereas acidic solutions are relatively stable (11, 13, 14). The effect of temperature on the stability of morphine has been reported to be less important than the hydrogen ion concentration (14).

Although the stability of morphine in aqueous solution has been extensively investigated, no quantitative studies have been conducted. This report deals with a kinetic study of the degradation of morphine in aqueous solution, the degradation of these solutions carried out in a light proof oven. No study was made to investigate the effect of light on morphine degradation.

EXPERIMENTAL

Reagents and Apparatus

Morphine sulfate U. S. P. recrystallized from alcoholic aqueous solution and dried under vacuum for six hours, m. p. 250°; Beckman spectrophotometer, model DU, equipped with photomultiplier, 1-cm. silica cells. The buffers used in this study are: acetate buffer: 0.2 M and 0.4 M at pH 3.0, 4.5, 5.0, and 5.5; phosphate buffer: 0.2 M and 0.4 M at pH 4.0, 5.0, 5.5, 6.0, 6.5, and 7.0. Phosphate

buffer, 0.2 M, at pH 2.0 and 2.5 were made by adjusting 0.1933 M phosphoric acid with mono-potassium phosphate. All buffer solutions were adjusted using a Beckman pH meter model H2. The phosphate buffer solutions used here are inde-pendent of the temperature (15), and the change of pH of acetate buffer at higher temperature is negligible (16).

Degradation of Morphine Sulfate in Sealed Ampuls

Effect of pH on the Degradation of Morphine Sulfate Solution Sealed Under Atmosphere at 95°.— Five milliliters of 0.3% morphine sulfate solution in 0.2 M phosphate buffer; pH 2.0, 6.0, 6.5, and 7.0, was introduced into 5-ml. ampuls, sealed under atmosphere, and stored in an oven at 95°. These solutions were assayed for morphine content at various time intervals by a previously described chromatographic procedure (17).

The results of the study are shown in Fig. 1. The data obtained indicate that the rate and extent of decomposition of morphine is dependent on the pH of the solution. It is also apparent that after a time interval, this decomposition is halted, as evidenced by the plateaus. This behavior was probably due to the lack of oxygen in the systems. Calculation of the atmospheric oxygen present in the solutions contained in the ampuls (including the void space) was approximately 3.2×10^{-3} M/L. Since at pH 7.0 approximately 3.1×10^{-3} M/L. of morphine had undergone decomposition, it appeared that morphine and oxygen react on a mole to mole basis.
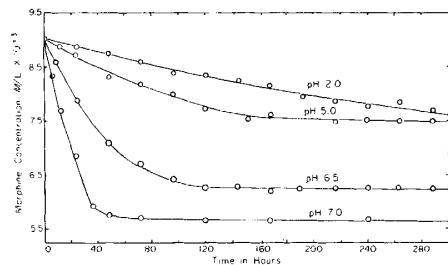

Fig. 1.—Effect of pH on degradation of mor-phine sulfate in 0.2 M phosphate buffer, sealed under atmosphere, at 95°.

In order to verify the oxygen dependency of the reaction, the following study was undertaken. Five milliliters of 0.3% morphine sulfate solution was introduced into 5-ml. ampuls and sealed under sulfur dioxide, atmosphere, commercial nitrogen, and absolute nitrogen. An additional solution of morphine sulfate containing 1% of sodium bisulfite was prepared and sealed under atmosphere. Using sulfur dioxide and commercial nitrogen, the ampuls were bubbled for one minute, three minutes for absolute nitrogen, prior to sealing. These ampuls were stored in a 95° oven and assayed for morphine at periodic time intervals. Results of this study are shown in Figs. 2 and 3. The data indicated that the rate of degradation of morphine is oxygen-dependent since solutions sealed under absolute
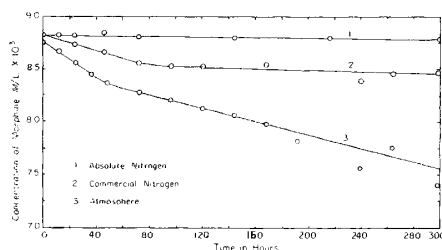

Fig. 2.—Effect of inner gas on degradation of mor-phine sulfate in deionized water, at 95°.
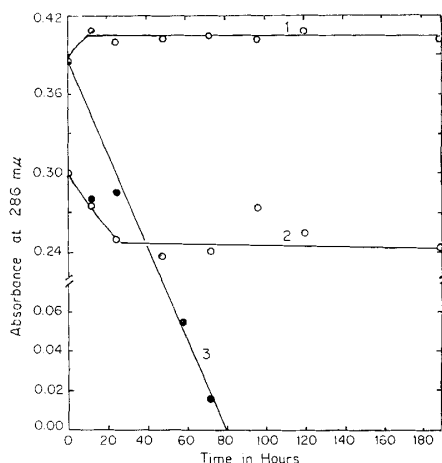

Fig. 3.—Effect of sodium bisulfite, sulfur dioxide on degradation of morphine sulfate at 95°: 1 and 2 contained 1% NaHSO₃, sealed under atmosphere; 3, M₂SO₄ in deionized H₂O, sealed under SO₂; 1, assayed by direct spectrophotometric method; 2 and 3, by the chromatographic method.

nitrogen showed no evidence of decomposition. The slight degradation noticed in systems saturated with commercial nitrogen was probably due to im-purities in the nitrogen. Absolute nitrogen was prepared by passing commercial nitrogen through three wash bottles containing Fieser's solution (18) and through a saturated solution of lead acetate.

It is interesting to point out here that morphine solutions underwent a color change to yellow when sulfur dioxide was introduced. This color intensity was dependent on the length of time used to saturate the solution with sulfur dioxide. Chromatographic analysis of these freshly prepared sulfur dioxide-saturated solutions gave low recoveries for morphine. Direct spectrophotometric analysis, however, of these same samples, using deionized water as the diluent, gave only slight absorbance difference when compared to the original solutions indicating that some reaction had probably taken place. These sulfur dioxide solutions become highly colored after three to four days' storage at 95° and developed a yellow precipitate after one month. An investiga-tion of this phenomenon is, at present, continuing.

It was also noted that the chromatographic

analysis of the morphine sulfate solution containing 1% sodium bisulfite prior to sealing and storage at 95° resulted in low recovery of morphine (70%). Direct spectrophotometric analysis of the freshly prepared solution, using deionized water as the diluent gave, on the other hand, higher values for morphine. Further study dealing with this aspect showed there was no appreciable change in pH of the solution, although the peak of the spectrogram was shifted slightly to the lower wavelength from 286 to 284 mμ and a stronger absorbance was noted, indicating that some reaction had taken place between morphine and sodium bisulfite. This phenomenon will be discussed in a future communication (19).

This initial investigation confirmed previous studies and showed that the decomposition of *morphine sulfate in aqueous solution was dependent* on the hydrogen ion and oxygen concentration of the system. Attempts to employ a manometric procedure using a Warburg manometer in the study of the oxygen dependency regarding the rate of degradation of morphine were unsatisfactory in that morphine sulfate, in 0.2 M phosphate buffer pH 7.0 at 60°, underwent only slight color change after twelve hours, the amount of oxygen uptake being negligible. However, it was felt that valuable information with respect to the kinetics of this reaction could be obtained by maintaining a relatively constant oxygen concentration in the system. To this end, the rate of degradation of morphine was studied as a function of hydrogen ion concentration, molarity of buffers used, ionic strength, and concentration of morphine in oxygen-saturated systems.

Degradation of Morphine Sulfate in the Presence of Excess Oxygen

Effect of pH.—Approximately 0.15-Gm. portions of anhydrous morphine sulfate were accurately weighed, transferred to 100-ml. vaccine bottles, and dissolved in 50 ml. of the following buffers: 0.2 M phosphate buffer, pH 2.5, 6.0, 6.5, and 7.0; 0.2 M acetate buffer, pH 4.0, 4.5, 5.0, and 5.5. Three milliliters of each solution was withdrawn and assayed chromatographically for the original morphine concentration. These solutions were bubbled with oxygen for two minutes, rubber stoppered, sealed with aluminum caps, and placed in a 95° oven. At various time intervals the bottles were removed, chilled, and 3-ml. aliquots of solution were withdrawn. After each sample removal, the remaining solution was again saturated with oxygen for two minutes, and stored in the oven. The process was repeated for a total of ninety-six hours. The data obtained, as shown in Fig. 4, indicated that the rate of degradation of morphine was hydrogen ion-dependent. A plot of the log of the concentration of undecomposed morphine against time gives a straight line, indicating that the reaction is pseudo first order with respect to morphine at constant hydrogen ion and oxygen concentration. A plot of the log of the specific rate constant of these reactions as a function of pH resulted in an "S"-shaped curve (Fig. 5). This plot resembles a dissociation curve and indicates that the rate of degradation of morphine is dependent on the type of morphine species present in the solution. Data obtained from studies conducted in 0.2 M phosphate buffer at pH 3.0, 4.0, 4.5, 5.0, 5.5, and 0.4 M phos-
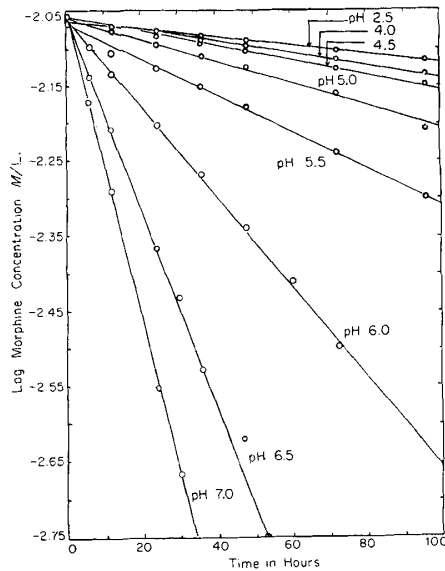

Fig. 4.—Effect of pHs on degradation of morphine sulfate in excess oxygen, at 95°.

phate buffer at pH 5.0 were unsatisfactory in that the capacity of these buffers was inadequate.

Effect of Buffer Molarity and Ionic Strength.—Data obtained in this study using 0.2 M and 0.4 M acetate buffer at pH 5.0 and 0.2 M acetate buffer at pH 5 containing 1 and 3% sodium sulfate indicate that the rate of degradation of morphine is independent of the molarity of buffer and of the ionic strength present, as shown in Figs. 6 and 7.

Effect of Morphine Concentration.—Although data already showed that the overall rate of degradation of morphine at constant hydrogen ion and oxygen concentration is pseudo first order with respect to the concentration of morphine present, this first-order reaction was further verified by a study of 0.3 and 0.15% morphine sulfate in 0.2 M acetate buffer, pH 5.0, as shown in Fig. 8.

Effect of Temperature.—Solutions of morphine sulfate in 0.2 M phosphate buffer, pH 6.0, 6.5, 0.2 M acetate buffer, pH 5.0, were subjected to degradation at 85, 90, and 95°. Results of this investigation are shown in Figs. 9, 10, and 11. By plotting the log of specific rate constant, k, against the reciprocal of temperature, T, as shown in Fig. 12, the apparent energy of activation, E_a, under the condition employed in this study, was calculated from the slopes of these lines and was found to be of the order of 22.8 Kcal.

DISCUSSION

Oxidation of Morphine.—As has already been pointed out, morphine undergoes *decomposition* resulting in the discoloration of the solution and the formation of precipitates. Our present study indicates that this degradation of morphine is chiefly dependent on the pH of the solution and the pres-
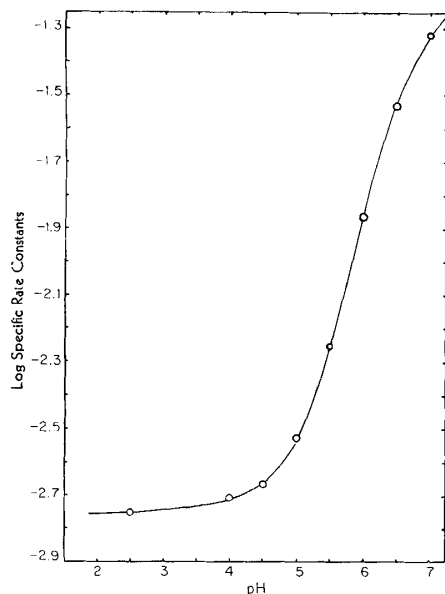
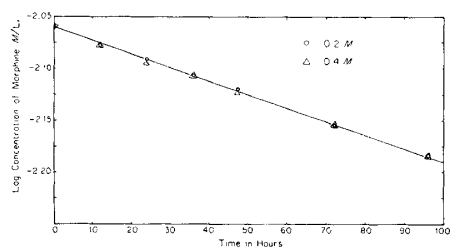Fig. 5.—Plot of the log of specific rate constants as a function of pH.

Fig. 6.—Effect of molarity of acetate buffer at pH 5 on degradation of morphine sulfate, in excess oxygen, at 95°.
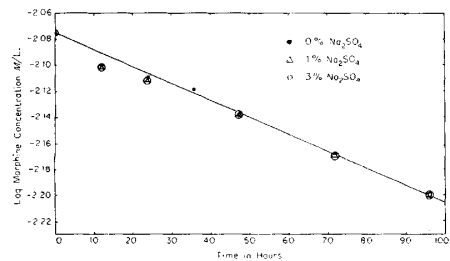

Fig. 7.—The effect of ionic strength on degradation of morphine sulfate in 0.2 M acetate buffer, at pH 5.0 and in excess oxygen, at 95°.
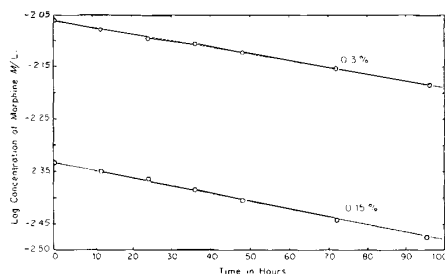

Fig. 8.—Effect of morphine concentration on degradation of morphine sulfate, in excess oxygen, at 95°.

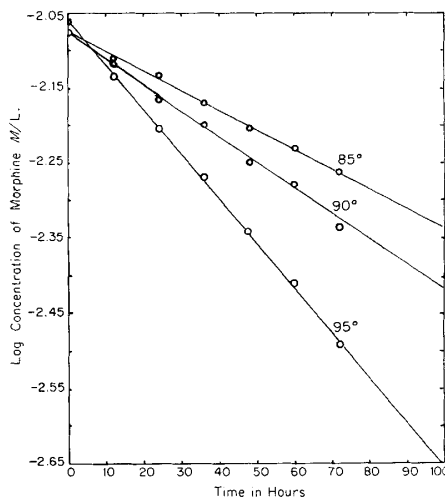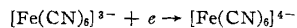Fig. 9.—Effect of temperature on degradation of morphine sulfate in 0.2 M phosphate buffer pH 6.0, in excess oxygen.

ence of atmospheric oxygen. It has been reported (1) that the degradation products of morphine are pseudomorphine and morphine N-oxide together with traces of the base said to be methylamine, and that the deterioration of morphine takes place through a condensation process at the phenolic hydroxyl group with the formation of pseudomorphine (10). Derivatives of morphine in which this phenolic group is alkylated, as in codeine, do not undergo this type of oxidation (2). The fact that deterioration of morphine increases rapidly in the presence of ultraviolet light (5) and decreases in a more acidic solution (11) gives additional support to the oxidation reaction. This oxidation of morphine to pseudomorphine may be somewhat analogous to the oxidation of phenols to dimolecular compounds and is presented here in some detail.

The ferricyanide oxidation method commonly used to prepare pseudomorphine involves one electron transfer which is common in free radical systems. This reaction can be illustrated as follows:

$$[Fe(CN)_6]^{3-} + e \rightarrow [Fe(CN)_6]^{4-}$$

Pummerer, *et al.* (20–23), in their studies of the oxidation of naphthols with alkaline potassium ferricyanide reported that the oxidizing agent attacks
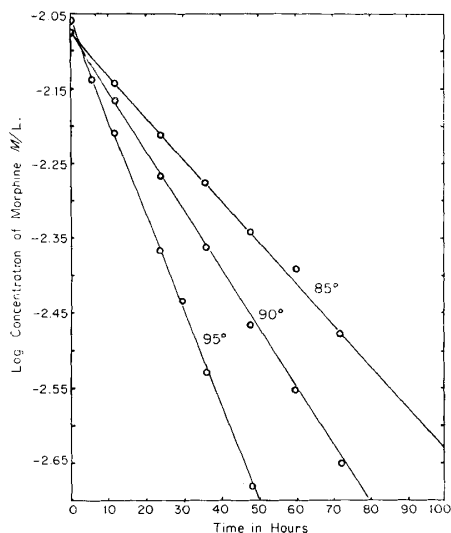
Fig. 10.—Effect of temperature on degradation of morphine sulfate in 0.2 M phosphate buffer solution, pH 6.5, in excess oxygen.
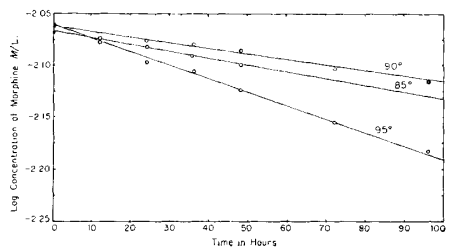

Fig. 11.—Effect of temperature on degradation of morphine sulfate in 0.2 acetate buffer solution at pH 5.0, in excess oxygen.

Fig. 12.—Plot of the log specific rate constants as a function of the reciprocal of the absolute temperature.

the OH group and has a direct dehydrogenating action and that the primary oxidation product is a resonant aroxyl radical. They confirmed this by isolating and identifying the possible dimeric forms. The oxidation of naphthol gives binaphthol which is further oxidized to oxy-binaphthylene-oxide and binaphthylene dioxide as illustrated in Diagram I.

Oxidation of p-cresol was found to give dicresol and an ether (24, 25). If the resonant forms of p-cresol are examined, it becomes evident that these are all cogent in the transition of the resonant aroxyl radical as illustrated in Diagram II.

Considering such a reaction and by direct analogy, morphine could undergo a similar type of process in its degradation and subsequent production of pseudomorphine as shown in Diagram III.

Mechanism of Degradation of Morphine.—Since data obtained from this investigation showed that the rate of decomposition of morphine in solution was dependent on the presence of oxygen and that no decomposition occurred in systems void of oxy-
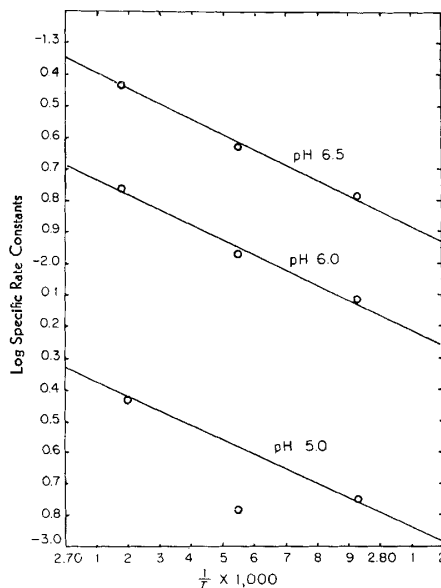
gen, it was concluded that a free radical reaction was involved in this process. It was also found that the rate of degradation was dependent on the hydrogen ion concentration of the solution since the rate was considerably greater at the higher pH. It is interesting to note here that an "S"-shaped curve, similar to the typical dissociation curve, was obtained when the log of the specific rate constant of these reactions was plotted as a function of pH. This indicated that the degradation was dependent on the type of morphine species present in solution. Based on this information, it appeared that the undissociated morphine molecules undergo oxidation more easily. At the pH values employed in this study and considering the pKa and pKb of morphine, only the protonated and undissociated morphine species are involved. The amount of anionic species of morphine present at these hydrogen ion concentrations is negligible and has been included in the concentration of the undissociated species. Since the rate of decomposition of morphine at lower pH's, i. e., at pH 2.5 and pH 4.0, is nearly the same, this indicated that the protonated morphine species also undergoes oxidation, but at a different rate to that of the free undissociated morphine base.

The undissociated (free base form) and protonated morphine are both oxidized by atmospheric oxygen to give a semiquinone (MO) and a free radical peroxide ($HO_2\cdot$). This semiquinone is further transformed to a free radical quinone ($MO\cdot$), which can undergo coupling with: (a) itself, (b) the undissociated morphine, and (c) the protonated morphine. Since the amount of activated or free radical morphine species present in the system is small compared to the protonated or free base forms, interaction or union of two such activated species is un-

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