Drug Development and Industrial Pharmacy, 27(9), 997–1001 (2001)

COMMUNICATION

Stability of 4-DMAP in Solution

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ABSTRACT

*A stability-indicating reversed-phase performance liquid chromatographic method was developed for the detection of 4-(*N,N*-dimethylamino)phenol (4-DMAP) and its degradation products under accelerated degradation conditions. The degradation kinetics of 4-DMAP in aqueous solution over a pH range of 1.12–6.05 and its stability in solutions based on propylene glycol or polyethylene glycol 400 were investigated. The observed rate constants were shown to follow apparent first-order kinetics in all cases. The pH rate profile shows that maximum stability of 4-DMAP was observed in the pH range 2.0 to 3.0. Acid/base catalysis of 4-DMAP was not affected by systems of various ionic strengths. Incorporation of nonaqueous propylene glycol or polyethylene glycol 400 in the pH 3.05 solution of 4-DMAP showed an increase in the stability at* $55^{\circ}C \pm 0.5^{\circ}C$.

Key Words: *4-DMAP; HPLC; Kinetic; Solution; Stability*

INTRODUCTION

Cyanide (CN) is a well-known active ingredient in "blood agents," a kind of notorious chemical warfare used in World War I. Cyanide, even in minute quantity, is extremely hazardous to both humans and animals. Hospital admissions and death due to cyanide poisoning, either by accident or by suicide, have been rising steadily in the past decade. Cyanide ion is capable of penetrating cell and mitochondrial membranes freely and arrests the cellular respiratory chain by inhibition of the terminal cytochrome oxides (1,2), which prevents oxygen from arriving at the vital tissues and thereby results in tissue hypoxia. Death will occur if proper antidotes are not given immediately after poisoning as the irreversible damage to tissues is done quickly.

Several compounds have been employed for the treatment of acute cyanide poisoning (3), used either

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alone or in combination, such as nitrites, cobalt salts, sodium thiosulfate, and amyl nitrite/phenoxybenzamine (4). However, most of them suffer the drawback of being ineffective. 4-(*N*,*N*-Dimethylamino)phenol (4-DMAP) is one of the most potent and reliable antidotes in the therapy of acute cyanide poisoning in humans. The mechanism of action and pharmacokinetics of 4-DMAP have been well documented (5–8). 4-DMAP is most effective after intravenous administration, and after reacting with hemoglobin, quickly forms methemoglobin, thereby trapping cyanide within erythrocytes. However, as yet, no information concerning the chemical stability of 4-DMAP aqueous solution is available.

The purpose of this study was (1) to develop a rapid, precise, and reliable high-performance liquid chromatographic (HPLC) method with stability-indicating capacity and (2) to determine the stability of 4-DMAP in various pH buffer solutions and non-aqueous/water systems under controlled conditions.

EXPERIMENTAL

Materials

The 4-DMAP was from our laboratory (National Defense Medical Center, Taipei, Taiwan, ROC). Sodium octanesulfonate was from Sigma Chemical (St. Louis, MO). Sodium phosphate monobasic, acetic acid, and sodium acetate were from Wako Pure Chemical (Tokyo, Japan). Sodium phosphate dibasic, acetonitrile, potassium chloride, sodium hydroxide, and hydrochloric acid were from Merck (Darmstadt, Germany). Propylene glycol was from J. T. Baker Chemical (Philipsburg, NJ). Polyethylene glycol 400 was from Fisher Scientific (Fair Lawn, NJ).

Kinetic Studies

Six buffer solutions of varying buffer species with constant concentrations (0.05 M) at fixed ionic strength ($I = 0.5$) were prepared at each specific pH (pH 1.12–2.51 was hydrochloric acid, pH 3.05–5.15 was acetate buffer, pH 6.05 was phosphate buffer). The 4-DMAP was dissolved by the above buffer solutions to a concentration of 5.0 mg/ml. The solutions were then sealed in type I glass ampoules and stored in a dark oven maintained at $55^\circ C \pm 0.5^\circ C$ for up to 192 h.

Samples were removed from the oven at each time interval and stored immediately in a $-20^\circ C$ freezer until analyzed. Samples were removed from the freezer, equilibrated to room temperature, and mixed in a vortex mixer prior to assay.

The pH values were checked (Vision 607 JENCO Electron LTD, CA) for each sample to detect any significant pH change at each designated time. Concentrations of 4-DMAP were determined in triplicate by the stability-indicating HPLC method.

High-Performance Liquid Chromatographic Analysis

The HPLC instrument was equipped with a single piston pump (model LC-6A, Shimadzu, Kyoto, Japan) set at 220 nm and a LichroCART C18 column 3.9×15 cm with 5-μm packing (Merck). The mobile phase contained 20% acetonitrile (containing 2% sodium octanesulfonate); pH was adjusted to 3.0 by acetic acid. Its flow rate was maintained at 1.0 ml/min. The absorbance of 4-DMAP and its degradation products was recorded using a strip chart (model C-R6A, Shimadzu) at a chart speed of 0.1 cm/ml. The linearity of the calibration curve of peak height versus concentration (μg/ml) for the analytical range between 2.0 and 100.0 μg/ml was excellent, with a correlation coefficient of 0.999.

The intra- and interday precision of this HPLC method at a 4-DMAP concentration of 20.0 μg/ml were 0.34% and 0.72% ($n = 3$), respectively. The stability-indicating nature of this assay is depicted by the chromatogram (Fig. 1) of the samples of 4-DMAP (50 μg/ml) in pH 3.05 acetate buffer solution after degradation at $55^\circ C \pm 0.5^\circ$ for 15 h. The degradation products eluted separately and were detected without apparent interference with the peak of interest. The relative retention time of 4-DMAP is 9.0 min. The homogeneity of the 4-DMAP peak was examined by performing a diode array (model SPD-m6A, Shimadzu) spectral overlay analysis in the ultraviolet (UV) range of 191.0 to 401.0 nm. No difference (curve fit > 0.99) in the UV spectra between the eluted and pure drug samples suggests the absence of any degradate or exogenous impurities eluting under the peak of interest. The sensitivity of the reported procedures was 50 ng/ml. The stability of 4-DMAP at each designated storage time interval in this study was expressed as a

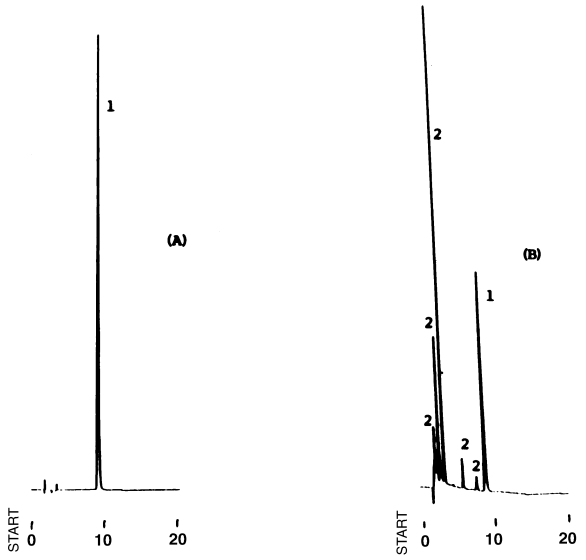

Figure 1. HPLC chromatogram of 4-DMAP (5 μg/ml) in pH 3.05 acetate buffer solution (A) immediately after preparation and (B) after 15 h of storage at 55°C ± 0.5°C. Key: (1) solvent, (2) degradation products, (3) 4-DMAP.

percentage of its initial concentration (100.0% at time zero).

Buffer Effect Studies

Three buffer solution of different buffer concentrations (0.01, 0.05, and 0.1 M) and $I = 0.5$ were used to study the catalysis effect of buffer species on the degradation of 4-DMAP at each species pH and temperature (55°C ± 0.5°C). Acetate buffer at pH 3.05, 4.01, and 5.15 and phosphate buffer at pH 6.05 were evaluated. The final concentration of 4-DMAP was 5.0 μg/ml.

Salt Effect Studies

To test the effect of ionic strength on the degradation of 4-DMAP (5.0 μg/ml), buffer solutions of various total strengths (0.05, 0.3, 0.5, and 0.7) with constant buffer species concentration at fixed pH were prepared. A stability study of these solutions at55°C ± 0.5°C was conducted. A pH 3.05 acetate buffer with 0.05 M total buffer concentration was studied.

Temperature Effect Studies

Solutions of 0.5 μg/ml of 4-DMAP acetate buffer at pH 3.05 and $I = 0.5$ were prepared. The temperature dependence of the degradation of 4-DMAP was studied at 35°C, 45°C, and 55°C ± 0.5°C.

Solvent Effect Studies

Solutions of 5.0 μg/ml of 4-DMAP in different propylene glycol/water or polyethylene glycol 400/water systems were prepared. All solutions were buffered to pH 3.05 with a 0.05 M acetate buffer at $I = 0.5$. These solutions were then stored in a constant temperature oven at 55°C ± 0.5°C.

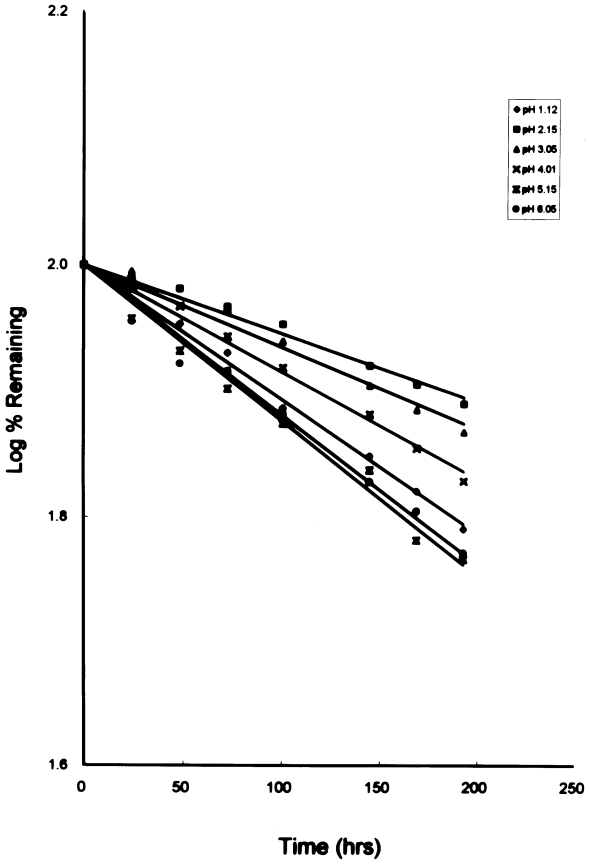

Figure 2. Pseudo-first-order degradation kinetics of 4-DMAP in various buffer solutions (0.1 M) of different pH at 50°C ± 0.5°C and $I = 0.5$.

Table 1

Degradation Rate Constants[a] of 4-DMAP HBr in Different Buffer Concentrations at Various pH Under Constant Ionic Strength (I = 0.5) and Storage Temperature (55°C ± 0.5°C) Conditions

	K_{obs} (h)		
pH	0.01 M	0.05 M	0.1 M
1.12	0.002543 ± 0.00011	0.002532 ± 0.00009	0.002541 ± 0.00016
3.05	0.001675 ± 0.00020	0.001682 ± 0.00014	0.001676 ± 0.00015
5.15	0.002526 ± 0.00017	0.002523 ± 0.00023	0.002525 ± 0.00022
6.05	0.002568 ± 0.0001	0.002565 ± 0.00019	0.002571 ± 0.00014

*Mean ± SD ($n = 3$).

RESULTS AND DISCUSSION

Degradation Kinetics

Stability profiles for 4-DMAP in 0.1 M buffer solution at 55°C ± 0.5°C are shown in Fig. 2. The linear relationship between logarithmic percentage remaining and storage time indicated pseudo-first-degradation kinetics for 4-DMAP in aqueous solution; the degradation rate constants were determined from the slope of the semilog plot by statistical regression with correlation coefficient r greater than 0.98.

Buffer Species

No significant difference was observed (Table 1) for the degradation rate constants of 4-DMAP under three different concentrations (0.01, 0.05, and 0.1 M) of the same buffer species (acetate, phosphate) at each species pH solution over the range of 1.12 to 6.05. The general acid/base catalysis of acetate and phosphate buffers on the degradation of 4-DMAP was not significant.

pH-Rate Profile

The effect of pH on the degradation of 4-DMAP in aqueous solution under zero buffer concentration and $I = 0.5$ at 55°C ± 0.5°C is shown as the plots of log K_{obs} versus pH and is depicted in Fig. 3. In the pH range 2.0 to 3.0 under these study conditions, 4-DMAP was found to be more stable than in other regions.

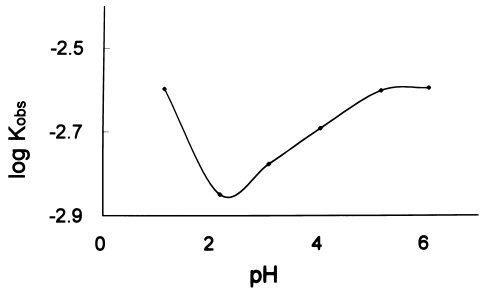

Figure 3. The pH-rate profile of 4-DMAP HBr in aqueous solution under zero buffer concentration and constant ionic strength ($I = 0.5$) at 55°C ± 0.5°C.

Table 2

Degradation Rate Constants of 4-DMAP HBr in Different Ionic Species Concentrations in pH 3.05 Solution Under Constant Buffer Species Concentration (0.1 M) and Storage Temperature (55°C ± 0.5°C)

Ionic Strength (I)	K_{obs} (hr^{-1})[a]
0.05	0.001578 ± 0.00012
0.3	0.001665 ± 0.00009
0.5	0.001676 ± 0.00015
0.7	0.001681 ± 0.00009

[a]Mean ± SD ($n = 3$).

Salt Effect

The results of different ionic strength effects on the stability of 4-DMAP in the pH 3.05 acetate buffer solutions at 55°C ± 0.5°C are listed in Table 2. No significant difference ($P > .05$) was observed

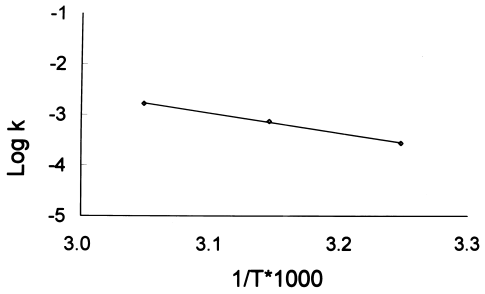

Figure 4. Arrhenius plot of the degradation of 4-DMAP (5 μg/ml) in pH 3.05 acetate buffer (0.1 M) solution and at a constant ionic strength of 0.5.

for the degradation rate constants of 4-DMAP at different ionic strengths.

Temperature Effect

The temperature dependence of the degradation of the rate of 4-DMAP (0.5 μg/ml) in pH 3.05 acetate buffer (0.05 M) at $I = 0.5$ was determined by plotting the log of the degradation rate constants versus 1/temperature (K), as seen in Fig. 4. The energy of activation in this solution was determined to be 17.65 kcal/mol from the slope.

Solvent Effect

The results of propylene glycol/polyethylene glycol 400 effects on the stability of 4-DMAP in the pH 3.05 acetate buffer solutions at $55^{\circ}C \pm 0.5^{\circ}C$ are listed in Table 3. It was observed that the degradation rate of 4-DMAP in propylene glycol and polyethylene glycol 400/water systems decreased as the content of propylene glycol and polyethylene glycol 400 increased. No explanation is proposed related to the degradation mechanism of 4-DMAP in these solvent systems due to complicated factors, such as dielectric constant, surface tension, viscosity, activity coefficient of the 4-DMAP and its transition products, and so on.

Table 3

Stability of 4-DMAP HBr in Mixed Solvent Systems at pH 3.05 and ($55^{\circ}C \pm 0.5^{\circ}C$)

Solvent	% (w/w)	K_{obs} (hr^{-1})[a]
pH 3.05 buffer	100	0.001676 ± 0.00015
Polyethylene glycol 400	25	0.000980 ± 0.00044
Propylene glycol	25	0.001285 ± 0.00016

[a]Mean ± SD ($n = 3$).

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