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Note

Optimal conditions for long-term storage of biogenic amines for subsequent analysis by column chromatography with electrochemical detection

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The optimal conditions for storage of biogenic amines in solutions are not known. Temperature and pH of the solvents and length of storage have dramatic effects on stabilities of amines [1–3], yet the variability in the storage conditions used by various investigators is rather striking [4–6]. These amines have been stored in a variety of weak acids and buffers, including perchloric acid, hydrochloric acid, formic acid, and acetic acid, with molarities of 0.1 to 0.5; at temperatures ranging from room temperature to −80°C; and for periods of 12 h to several weeks or months [1–3, 7–11]. Not surprisingly, the results have been highly variable. Another complicating factor has been the fact that not all biogenic amines behave uniformly under similar storage conditions. We have noticed that catecholamines remain stable for weeks in acidic solutions under refrigeration but indoleamines undergo fast degradation under the same conditions. The aims of the present study were (1) to determine stabilities of biogenic amines in Krebs–Ringer–Hensleit (KRH) saline under various combinations of pH, temperature, and length of storage, and (2) to investigate the conditions optimum for storage of catecholamines and indoleamines together in KRH which is a commonly used medium for studying release of biogenic amines.

EXPERIMENTAL

Experiment 1

This experiment was performed to determine the stabilities at room temperature of biogenic amines and the internal standard, dihydroxybenzylamine (DHBA), dissolved in KRH of various pHs. Dopamine (DA, 11.70 mg), norepinephrine (NE, 11.48 mg), epinephrine (EPI, 11.30 mg), serotonin (5-HT, 11.40 mg), and DHBA (14.96 mg) each were dissolved separately in 100 ml of 0.05 *M* perchloric acid. From each of these solutions, 50 μl were withdrawn and mixed with 99.95 ml of each of the following four solvents: (1) 0.05 *M* perchloric acid (pH 1.40), (2) KRH and 1 *M* perchloric acid in a ratio of 25:1 (v/v) (pH 1.96), (3) KRH and 1 *M* perchloric acid in a

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ratio of 50:1 (v/v) (pH 5.81), and (4) KRH (pH 7.81). KRH consisted of 117 m*M* NaCl, 4.7 m*M* KCl, 1.2 m*M* $MgSO_4$, 1.2 m*M* KH_2PO_4, 2.5 m*M* $CaCl_2$, 24.8 m*M* $NaHCO_3$ and 11.1 m*M* glucose. The solutions were kept at room temperature (22°C). From each of them, 15 μl were withdrawn at 0, 30, 60 and 90 min, and their amine content was determined by column chromatography with electrochemical detection. The percent recovery of each amine was calculated by comparison with its recovery in freshly made 0.05 *M* perchloric acid.

Experiment 2

This experiment was performed to determine the effects of refrigeration on the recovery of the above amines. They were prepared in four solutions as described above and refrigerated (4°C). Within 2 h of refrigeration on day 0 and then on days 1, 2, 4, 7, 14 and 28 after refrigeration, 15-μl aliquots were withdrawn from each solution and analyzed in duplicate.

Experiment 3

This experiment was performed to investigate the effects of freezing (−60°C) on the recovery of amines. They were prepared as described in experiment 1 and immediately frozen. Within 30 min after freezing on day 0 and again on days 1, 2, 4, 7, 14 and 28 after freezing, 15-μl aliquots were withdrawn from each solution and analyzed in quadruplicate. Before injection into the high-performance liquid chromatography (HPLC)–electrochemical detection (ED) system, each solution was thawed for 1 min at 60°C.

Column chromatography with electrochemical detection

This procedure has been described in detail previously [12]. The mobile phase (pH 3.1) included monochloroacetic acid (14.15 g/l), sodium hydroxide (4.675 g/l), EDTA (250 mg/l), octanesulfonic acid (300 mg/l), tetrahydrofuran (1.4%) and acetonitrile (3.5%). The sensitivity of the detector was 1 nA full scale, and the potential of the working electrode was 0.8 V with respect to a Ag/AgCl reference electrode. The temperatures of the column and mobile phase reservoir were maintained at 29.3–30.1°C and 43.2–44.0°C, respectively [12].

RESULTS

Effects of pH on stability of amines at room temperature

Percent recoveries of amines in KRH saline of various pH values after a 90-min storage at room temperature are shown in Fig. 1. There were no significant differences in recoveries at 0 min or after storage for 30, 60 and 90 min; therefore, results for these four time intervals were combined to calculate average percent recoveries for the 90-min period. As shown in Fig. 1, when the amines were stored in pure KRH saline (pH 7.81), marked reductions occurred in the recoveries of all catecholamines and DHBA as compared to their recoveries in 0.05 *M* perchloric acid (pH 1.40). These reductions were 46% (DA), 34% (NE), 46% (EPI), and 22% (DHBA). In contrast, this pH had no effect on the recovery of 5-HT. In acidic KRH (pH 5.81), DA, NE, and DHBA remained almost 100% stable, but EPI decreased by 10%, and 5-HT increased by 16%. In highly acidic KRH (pH 1.96), recoveries of DA, NE, and

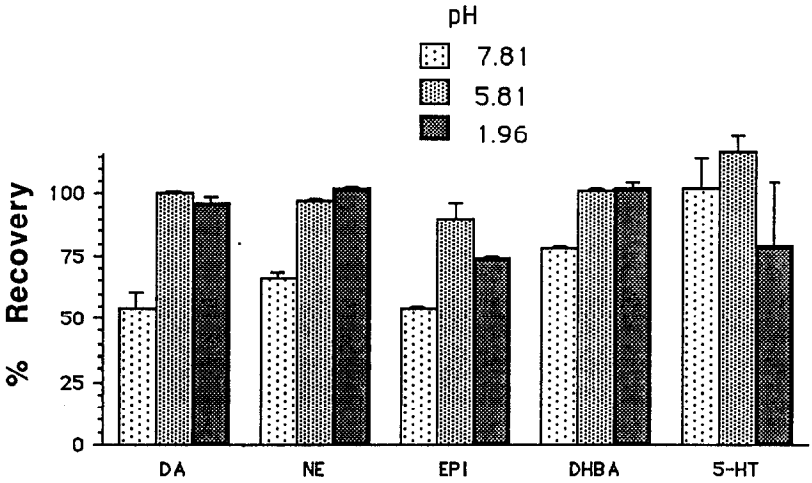

Fig. 1. Percent recoveries of dopamine (DA), norepinephrine (NE), epinephrine (EPI), dihydroxybenzylamine (DHBA) and serotonin (5-HT) after 90 min at room temperature (22°C) in Krebs–Ringer–Hensleit (KRH) saline of pH 7.81, 5.81 and 1.96.

DHBA were again almost 100%, but EPI and 5-HT decreased by 26% and 21%, respectively.

Effects of pH on stability of amines under refrigeration

In alkaline KRH saline (pH 7.81), all the catecholamines and DHBA suffered 100% loss by day 2 (Fig. 2). On the other hand, 5-HT decreased more gradually, until it became undetectable by day 28. In acidic KRH saline (pH 5.81), the decline in the recoveries remained essentially the same, except that 5-HT became undetectable earlier (day 14) than before. In highly acidic KRH saline (pH 1.96), recoveries of all the amines improved considerably and more than 50% of DA, NE and DHBA and more than 20% of EPI and 5-HT were recovered on day 28. In 0.05 *M* perchloric acid (pH 1.4), 5-HT declined rapidly and was undetectable by day 14, whereas DA and EPI declined more gradually and NE and DHBA suffered only minor losses by day 28.

Effect of pH on stability of amines under freezing

Freezing had remarkable stabilizing effects on all the catecholamines and DHBA. They remained completely stable up to 28 days under all the pHs tested (Fig. 2). However, the losses in 5-HT were very significant and very rapid under acidic conditions. Recoveries of this amine decreased by more than 75% in acidic KRH (pH 1.96) and by more than 50% in 0.05 *M* perchloric acid (pH1.4) within 1 day. However, like catecholamines, 5-HT remained completely stable for up to 28 days in KRH solutions of pHs 5.81 and 7.81.

DISCUSSION

The results presented above demonstrate that the stabilities of biogenic amines in solutions are affected not only by pH, temperature and length of storage, but also

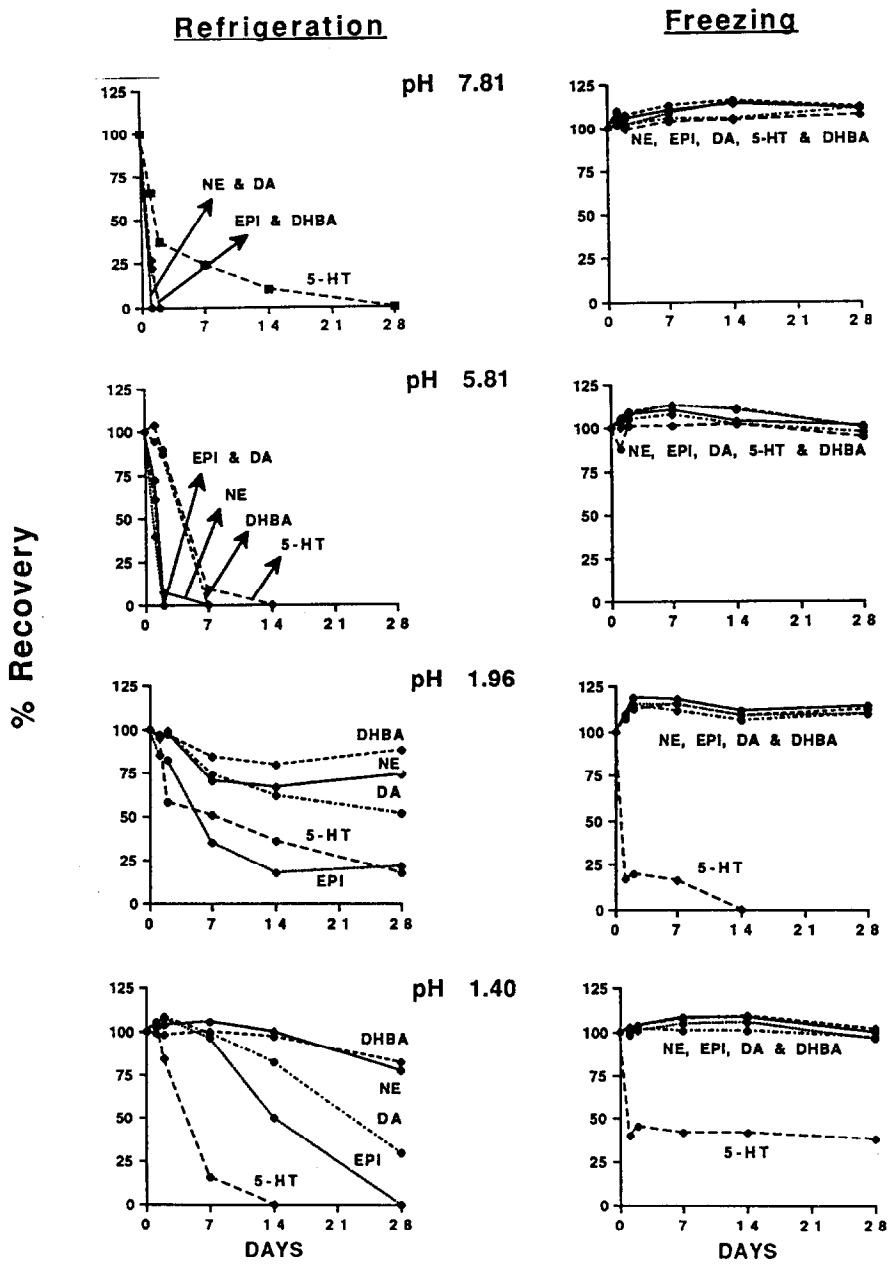

Fig. 2. Percent recoveries of dopamine (DA), norepinephrine (NE), epinephrine (EPI), dihydroxybenzylamine (DHBA) and serotonin (5-HT) under refrigeration (4°C) and under freezing (−60°C) for variable periods of time in Krebs–Ringer–Hensleit (KRH) saline of pH 7.81, 5.81 and 1.96 and in 0.05 *M* perchloric acid (pH 1.4).

by slight differences in structures of amines. At room temperature, DA and NE remained stable for at least 90 min in acidic KRH saline (pH 1.96). But, this was not true for the other catecholamine, EPI, indicating that minor differences in structure can lead to major differences in stabilities. Interestingly, the loss of this catecholamine was almost identical to the loss of the indoleamine, 5-HT, under the same conditions, making it difficult to relate stability to chemical structure with confidence.

The results indicate that catecholamines are more stable in acidic solutions. However, acid solutions do not confer stability for indefinite periods of time [1,3,5,6]. In 0.4 *M* perchloric acid, NE recovery decreases by 50% in 4 months [1]. Also, there is a limit to acidity of the solvents before it becomes detrimental to stability of catecholamines. According to one report, at room temperature, NE was stable for several hours in the pH range of 4–5, but losses occurred below pH 3 [3].

Our observation that 5-HT is not stable in strong acidic solutions is consistent with earlier reports [1,3]. Acidic solutions are also not suitable for storage of 5-HT metabolites. In dilute hydrochloric acid, 5-hydroxyindoleacetic acid (5-HIAA) suffered losses at room temperature, 4°C, and −20°C, and these losses were greater in stronger acidic solutions [3].

Our results give a clear demonstration of the advantages of low temperature for storing amines. In alkaline KRH (pH 7.81), DA and NE decreased by 30–45% at room temperature but remained stable for up to 28 days at −60°C. In acidic KRH (pH 1.96), EPI decreased by more than 25% in 90 min at room temperature, but remained more than 80% stable for 2 days at 4°C and was 100% stable for 28 days at −60°C. Other investigators also have recognized the beneficial effects of low temperatures on the stability of amines [1–3].

Low temperatures, however, do not always guarantee stability. The behavior of 5-HT is a case in point. In acidic KRH (pH 1.96), it decreased by 25% in 90 min at room temperature, remained 50% stable for 7 days at 4°C, but suffered a loss of more than 75% in 1 day at −60°C. In 0.05 *M* perchloric acid (pH 1.4), it decreased by 84% in 7 days at 4°C but lost more than 60% of its content within 1 day at −60°C. Taken together, these examples indicate that for each amine, there is a combination of pH and low temperature that is most conductive to its stability.

The results of this study demonstrate that it is possible to devise conditions that maintain stability of catecholamines and indoleamines in the same solution. DA, NE, EPI, and 5-HT remained 100% stable for 28 days at −60°C if stored in KRH saline of pH 5.81 or 7.81. We feel that pH of 5.81 is more suitable, because at that pH all of the amines also remained stable for at least 90 min at room temperature, whereas at pH 7.81 catecholamines decreased by more than 25% in 90 min at room temperature. Thus, pH 5.81 would permit short delays in freezing without any losses.

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