140 ng/ml) were performed over a 31-day period (Table V). Plasma samples were stored in the freezer at −20° until the time of analysis. The results demonstrate that bumetanide can be stored frozen in plasma for at least 1 month without degradation.

Figure 3 shows a urinary excretion rate *versus* midpoint time plot of unchanged bumetanide after oral administration of 1.0 mg of bumetanide to a healthy volunteer. The biological half-life, as determined by linear regression using the last four data points from the log-linear terminal portion of the curve, was 84.5 min. The fraction of the oral dose excreted unchanged in the urine was 0.445. Both of these parameters are in agreement with values from other studies (5, 6–8, 10).

REFERENCES

(1) E. H. Østergaard, M. P. Magnussen, C. K. Nielsen, E. Eilertsen, and H.-H. Frey, *Arzneim.-Forsch.*, **22**, 66 (1972).

(2) M. J. Asbury, P. B. B. Gatenby, S. O'Sullivan, and E. Bourke, *Br. Med. J.*, **1**, 211 (1972).

(3) K. H. Olesen, B. Sigurd, E. Steiness, and A. Leth, *Acta Med. Scand.*, **193**, 119 (1973).

(4) D. L. Davies, A. F. Lant, N. R. Millard, A. J. Smith, J. W. Ward, and G. M. Wilson, *Clin. Pharmacol. Ther.*, **15**, 141 (1974).

(5) P. W. Feit, K. Roholt, and H. Sørensen, *J. Pharm. Sci.*, **62**, 375 (1973).

(6) S. C. Halladay, I. G. Sipes, and D. E. Carter, *Clin. Pharmacol. Ther.*, **22**, 179 (1977).

(7) P. J. Pentikäinen, P. J. Neuvonen, M. Kekki, and A. Penttilä, *J. Pharmacokinet. Biopharm.*, **8**, 219 (1980).

(8) W. R. Dixon, R. L. Young, A. Holazo, M. L. Jack, R. E. Weinfeld, K. Alexander, A. Liebman, and S. A. Kaplan, *J. Pharm. Sci.*, **65**, 701 (1976).

(9) L. A. Marcantonio and W. H. R. Auld, *J. Chromatogr.*, **183**, 118 (1980).

(10) P. J. Pentikäinen, A. Penttilä, P. J. Neuvonen, and G. Gothoni, *Br. J. Clin. Pharmacol.*, **4**, 39 (1977).

ACKNOWLEDGMENTS

The author thanks Hoffmann–La Roche, for graciously supplying bumetanide as well as the drug's reported metabolites.

Application of the Ammonia Gas-Sensing Electrode: Determination of Drugs Having a Carboxyamide Group by Decomposition with Acid

SHOICHIRO TAGAMI ˣ and MASAKO FUJITA

Received June 8, 1981, from the *Toyama Medical and Pharmaceutical University, Sugitani, Toyama, Japan.* Accepted for publication August 19, 1981.

Abstract □ A simple potentiometric method for the determination of drugs having a carboxyamide group is described. Ethenzamide, niacinamide, pyrazinamide, or salicylamide was refluxed with 20% HCl and the carboxyamide was hydrolyzed. The ammonia evolved at a pH >11 and was determined without separation from the decomposition solution using an ammonia gas-sensing electrode. A linear calibration plot was obtained with drugs in the range of 2×10^{-5}–1×10^{-2} *M*. This method was applied to the analysis of injection and powder-containing auxiliary compounds.

Keyphrases □ Ammonia gas-sensing electrode—analysis of drugs with a carboxyamide group by decomposition with acid □ Potentiometry—determination of ammonia using an ammonia gas-sensing electrode □ Carboxyamide groups—determination of carboxyamide moiety using ammonia gas-sensing electrode analysis

Methods for the determination of drugs having a carboxyamide group are based on the determination of ammonia liberated by refluxing the carboxyamide compounds in alkaline solution. The ammonia is distilled and determined by titration. The methods described in the United States (1), British (2), and Japanese (3) Pharmacopoeias are based on these principles. Spectrophotometric methods of analysis for the determination of carboxyamide having a pyridine ring are based on the König reaction (4) of pyridine derivatives with cyanogen bromide. These methods are sensitive and relatively free from interference; however, they have the disadvantage of using the extremely toxic cyanogen bromide. Polarographic and microbiological methods (5) have also been employed, but they are tedious and time consuming. The gas-permeable membrane electrode is advantageous because of its simplicity, accuracy of assay, and lower cost compared with the conventional methods. However, its applications to drug analysis have not been widely reported in the literature, although the analysis of *N*-unsubstituted carbamates and meprobamate has been studied (6).

This paper describes a potentiometric method for the determination of drugs having a carboxyamide group. Assay methods for ethenzamide (o-ethoxybenzamide), salicylamide, niacin (nicotinamide), and pyrazinamide were developed. The samples were refluxed with 20% HCl and the carboxyamide was hydrolyzed, liberating an equivalent amount of ammonium ion. After alkalinization, the ammonia was determined with an ammonia gas-sensing electrode.

EXPERIMENTAL

Apparatus—The ammonia gas-sensing electrode[1] consisted of an ammonia gas-permeable membrane, a pH glass-electrode, and a silver–silver chloride reference electrode. The potential measurement system consisted of a pH/mV meter[2] and recorder[3].

All measurements were carried out at 20° in an 80-ml cell equipped with a magnetic stirrer.

Reagents—Ethenzamide[4], salicylamide[4], and pyrazinamide[4] were purified twice by recrystallization from water and then dried *in vacuo* at room temperature for 5 hr. Niacinamide[5] was dried *in vacuo* at room temperature for 4 hr. Other chemicals used were reagent grade.

A stock solution of 0.1 *M* NH_4Cl was prepared for testing the ammonia response of the electrode, and 5 *M* NaOH was employed to adjust the pH of the solution to within the operating range of the electrode.

[1] Model 5002-05 T, Horiba, Co., Kyoto, Japan.
[2] Model F-7ss, Hitachi-Horiba Instruments, Horiba Co., Kyoto, Japan.
[3] Model EPR-22A, Toa-Denpa Co., Tokyo, Japan.
[4] Tokyo Kasei Co., Tokyo, Japan.
[5] Japanese Pharmacopoeia Reference Standard.

0022-3549/82/0500-0523$01.00/0

© 1982, American Pharmaceutical Association

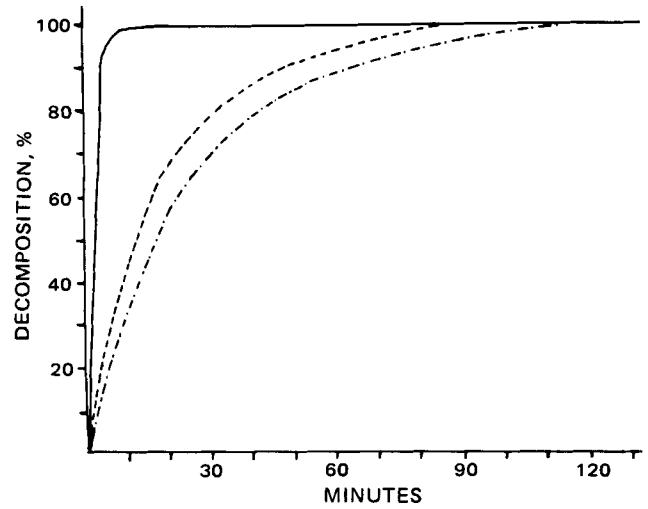

Figure 1—*Effect of 20% HCl concentration on drug decomposition. Key: —, pyrazinamide, niacinamide; - - -, ethenzamide; and — · —, Salicylamide.*

The internal filling solution supplied with the ammonia electrode was used.

An electrode storage solution of 0.01 *M* NH_4Cl for storage overnight or a weekend was prepared and the electrode tip was immersed in this solution. In 10^{-5}–10^{-4} *M* ammonia measurements, the use of the electrode immersed in 0.01 *M* NH_4Cl led to erroneous results because the gas-permeable membrane absorbed ammonium chloride and did not regenerate when washed with water. The electrode tip was therefore immersed in a buffer solution[6], pH 4, between measurements.

Standard Drug Solutions—A mixture of 307.78 mg (2.50×10^{-3} moles) of pyrazinamide and 50 ml of 20% HCl was placed in a 100- ml round-bottom flask and boiled gently in an oil bath for 20 min. The flask was then cooled, the solution poured into a 250-ml beaker, and diluted with ~150 ml of water. A drop of methyl orange was added and, while cooling the beaker continuously, the acid was carefully neutralized with 10 *N* NaOH solution until the indicator began to change color. Using a pH meter, the solution was adjusted to pH 6.5 with diluted sodium hydroxide solution, and the solution was poured into a 250-ml volumetric flask and diluted to full volume with water. The concentration of the final pyrazinamide solution was 1×10^{-2} *M*, corresponding to 1×10^{-2} *M* ammonia. Standard solutions for calibration were obtained by diluting this stock solution with water.

Other standard solutions of ethenzamide, salicylamide, and niacinamide were prepared in a similar manner as described previously according to the corresponding drug decomposition time shown in Fig. 1.

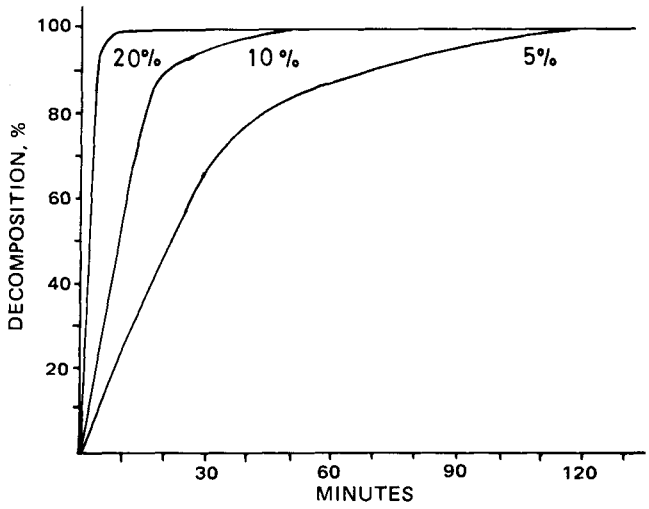

Figure 2—*Effect of various HCl concentrations on niacinamide decomposition.*

[6] Orion Research, Inc., Cambridge, U.K.

Table I—Determination of Drugs Having a Carboxyamide Group

Drug	Number	Taken, mg	Found, mg	Recovery, %
Ethenzamide	1	4.13	4.12	99.8
	2	4.13	4.16	100.7
	3	4.13	4.15	100.5
	4	41.30	41.21	99.8
	5	41.30	41.30	100.0
	6	41.30	41.34	100.1
			Mean	100.15
			± *SD*	0.37
Salicylamide	1	3.43	3.46	100.9
	2	3.43	3.44	100.3
	3	3.43	3.42	99.7
	4	34.28	34.08	99.4
	5	34.28	34.40	100.4
	6	34.28	34.60	100.9
			Mean	100.27
			± *SD*	0.62
Niacinamide	1	3.05	3.02	99.0
	2	3.05	3.07	100.7
	3	3.05	3.06	100.3
	4	30.53	30.72	100.6
	5	30.53	30.33	99.3
	6	30.53	30.41	99.60
			Mean	99.92
			± *SD*	0.71
Pyrazinamide	1	1.23	1.24	100.8
	2	1.23	1.22	99.2
	3	12.31	12.40	100.7
	4	12.31	12.34	100.2
	5	12.31	12.28	99.8
	6	12.31	12.39	100.6
			Mean	100.22
			± *SD*	0.62

Assay Procedure—A mixture of 123 mg (~1×10^{-3} mole) of pyrazinamide and 20 ml of 20% HCl was boiled for 20 min. The resultant solution was adjusted to pH 6.5, diluted to 100 ml in a volumetric flask, and then 20 ml of this solution was diluted to 100 ml with water. A 50-ml portion of the sample was transferred to an ~80-ml cell (3.5 × 9-cm), 1 ml of 5 *M* NaOH was added, and the mixture was incubated for 30 min at 20°. Finally, the ammonia electrode was immersed in the solution, and the potential measurements were carried out. The ammonia concentration in the sample solution was determined from the calibration curve.

Assay procedures of ethenzamide, salicylamide, and niacinamide were carried out in a similar manner. The ammonia concentration was determined from the calibration curve prepared from the corresponding standard drug solutions.

Because of the temperature dependence of the ammonia electrode, the sample solution was maintained at 20 ± 0.1° and stirred during the measurement. Since the potential may vary as a result of changes in the internal filling solution, the solution was replaced with fresh solution before subsequent use.

Assay of Niacinamide Injection—A mixture of 3 ml (equivalent to 150 mg of niacinamide) of the injection and 25 ml of 20% HCl was boiled for 30 min. The resultant solution was poured into a 250-ml volumetric flask and diluted to full volume with water. The ammonia concentration in the 50-ml portion of the sample was determined using an ammonia gas-sensing electrode.

Assay of 10% Niacinamide Powder—A portion of the powder (equivalent to ~153 mg of niacinamide) was accurately weighed out, and 25 ml of acetone was added. The solution was swirled for 5 min to extract the niacinamide and allowed to stand for ~3 min. The supernatant acetone solution was removed and 25 ml of acetone was added to the residue. The extraction procedure was carried out four times and the solution was finally filtered through a dry filter into a dry flask. The collected acetone fractions were evaporated to dryness. The residue was then refluxed for 30 min with 25 ml of 20% HCl. The same procedure as described previously was followed.

RESULTS AND DISCUSSION

The dissolved ammonia in the sample solution diffused through the hydrophobic gas-permeable membrane and dissolved in the internal filling solution. The resulting pH change was then measured.

A mixture of ethenzamide, salicylamide, niacinamide, or pyrazinamide,

Table II—Determination of Niacinamide Injection [a]

Number	Taken, mg	Found, mg	Recovery, %
1	30.00	32.36	107.8
2	30.00	32.20	107.3
3	30.00	32.34	107.8
4	30.00	32.14	107.1
5	30.00	32.35	107.8
6	30.00	32.23	107.4
		Mean	107.53
		± *SD*	0.31

[a] 50 mg in 1 ml.

Table III—Determination of 10% Niacinamide Powder

Number	Taken, mg	Found, mg	Recovery, %
1	30.94	35.79	115.7
2	30.62	35.63	116.4
3	30.61	35.55	116.1
4	30.94	35.87	116.0
5	30.68	35.63	116.1
6	30.72	35.53	115.8
		Mean	116.0
		± *SD*	0.24

and 20% HCl was boiled gently and the resultant ammonia in the decomposition solution was determined at various boiling times. In the case of ethenzamide and salicylamide, the electrode potentials reached a maximum at boiling times of 90 and 120 min, respectively. However, the electrode potentials of pyrazinamide and niacinamide reached a maximum at the same boiling times of 10 min. In the case of decomposition with 10 and 5% HCl, the electrode potentials of niacinamide reached a maximum at boiling times of 60 and 90 min, respectively (Figs. 1 and 2).

In the potential measurements, the level of ions in the decomposition solution (osmotic strength) is not equal to that of the internal filling solution. Water vapor as well as ammonia can move across the gas-permeable membrane, modifying the concentration of the internal filling solution. Since such changes lead to errors in potential measurements, standards and samples should have approximately the same level of ions in solution (7). Therefore, the standard solution of drug used the corresponding pure drug instead of ammonium chloride.

When the potential *versus* the logarithm of drug concentration was plotted, a linear calibration plot was obtained in the drug concentration range of 2×10^{-5}–1×10^{-2} *M*.

The amount of drug was determined in the pure drug powder. According to the Pharmaceopoeia, ethenzamide (8), salicylamide (9), and niacinamide (10, 11) determined on a dry weight basis should be over 98, 98, and 98.5% pure, respectively. Pyrazinamide (1) contains not less than 99% and not more than 101.0%. The recoveries of the drugs are given in Table I. The amounts of ethenzamide, salicylamide, niacinamide, and pyrazinamide can be determined with average errors of 0.15, 0.27, 0.08, and 0.22%, respectively.

The determination of commercially available niacinamide injection was carried out on six 3-ml samples (equivalent to ~150 mg niacinamide) of niacinamide injection, which is a sterile solution of niacinamide in water. A mixture of 3 ml of niacinamide injection and 25 ml of 20% HCl was boiled for 20 min and the potential measurements were carried out as described previously. According to the United States (10) and Japanese (11) Pharmacopoeias, niacinamide injection contains not less than 95% and not more than 110% of the labeled amount of $C_6H_6N_2O$. The recoveries of niacinamide injection are given in Table II. The mean recovery was 107.5 ± 0.31%. The accuracy of the proposed method was checked by the König method. The recoveries of niacinamide injection from four samples were 107.3, 106.4, 106.4, and 108.7%, respectively; the mean value was 107.2 ± 1.09%.

In determining other commercially available 10% niacinamide powders, a suitable extraction was sought. (Extraction of niacinamide is necessary to avoid interference from auxiliary compounds.) Niacinamide is very soluble in water (1 g in 1 ml) as are the auxiliary compounds; however, methanol does not dissolve the auxiliary compounds and separation of the extract from the powder is difficult. Niacinamide powder (10%) was extracted with acetone, since acetone does not inhibit the assay of pure niacinamide powder. After dissolution in acetone, the supernate was removed. The extraction was carried out four times and the extract filtered from the powder. The auxiliary compound was very hygroscopic, and it is thought that the niacinamide in the auxiliary compound was not homogeneous. Therefore, the recovery of the assay varied from 98 to >100%. The 10% niacinamide powder was mixed thoroughly in a mortar with a pestle and was dried *in vacuo* at room temperature for 24 hr (Table III). The mean recovery was 116.0 ± 0.24%. According to the Japanese Pharmacopoeia, niacinamide tablets contain not less than 95.0 and not more than 115.0% of the labeled amount of $C_6H_6N_2O$, but the description as a percentage of niacinamide powder is not noted. The accuracy of the proposed method was checked using the König method. The assay was performed on three samples of the 10% powder. The recoveries were 115.7, 118.5, and 115.3%, respectively, with a mean value of 116.5%.

CONCLUSIONS

In the Pharmacopoeial assay methods for ethenzamide, pyrazinamide, and salicylamide, the ammonia gas liberated by decomposition with sodium hydroxide is determined by acid titration (1, 8, 9). Using this method, the separation of the ammonia gas is difficult and tedious. The following procedures have been described previously for the analysis of niacinamide: (*a*) the sodium hydroxide decomposition method, (*b*) the nonaqueous titration method (12), (*c*) the colorimetric method for pure powder (10, 11), and the König method for injections. The sodium hydroxide decompositon method is identical to the method discussed. In the nonaqueous titration method, the normality factor is unstable. The colorimetric method is nonspecific, and the König method is adopted for the determination of injections in Japanese Pharmacopoeia IX (11) and USP XIX (13). The latter method is influenced by the experimental conditions (14, 15) and has the disadvantage of using extremely toxic cyanogen bromide. The present method using an ammonia gas-sensing electrode, beside being simple, has high specificity and precision.

REFERENCES

(1) "The United States Pharmacopoeia," 20th rev., Mack Publishing, Easton, Pa., 1980, p. 692.

(2) "The British Pharmacopoeia," University Printing House, Cambridge, U.K., 1973, p. 288.

(3) "The Japanese Pharmacopoeia," 9th rev., Hirokawa Publishing Co., Tokyo, Japan, 1976, p. 468.

(4) "Official Method of Analysis of the Association of Official Analytical Chemists," W. Horwity, Ed., 12th ed. Association of Official Analytical Chemists, Washington, D.C., 1975, p. 828.

(5) R. Strohencker and H. M. Henning, "Vitamin Assay Tested Methods," 1965, pp. 195, 201, and 207.

(6) S. Van-Vlasselaer and F. Cruke, *Ann. Pharm. Fr.*, **31**, 769 (1973).

(7) "Instruction Manual," Orion Research Inc., Cambridge, U.K., 1978, p. 23.

(8) "The Japanese Pharmacopoeia," 7th rev., Nankodo Publishing Co., Kyoto, Japan, 1965, p. 154.

(9) *Ibid.*, p. 508.

(10) "The United States Pharmacopeia," 20th rev., Mack Publishing, Easton, Pa., 1980, p. 548.

(11) "The Japanese Pharmacopoeia," 9th rev., Hirokawa Publishing Co., Tokyo, Japan, 1976, p. 436.

(12) *Ibid.*, 8th rev., Nankodo Publishing Co., Kyoto, 1973, p. 317.

(13) "The United States Pharmacopeia," 19th rev., Mack Publishing, Easton, Pa., 1975, p. 341.

(14) H. Makino, *Iyakuhin Kenkyu*, **3**, 175 (1972).

(15) I. Utsumi and M. Sameshima, *Yakugaku Kenkyu*, **29**, 980 (1957).