compound to get specific dissolution profiles in artificial gastrointestinal fluids.

Furthermore, a new method for the preparation of these epoxy resins in bead form in a lipophilic outer phase has been developed. Three drugs in various concentrations were embedded in these beads to prove the applicability of the method. Epoxy resins with basic curing agent are soluble in strong acidic buffers and may serve as a matrix for the initial oral dose while resins with the right amounts of acidic curing agents are soluble in weak acidic to neutral buffers and may serve as matrices for the sustained portion in long-acting oral dosage forms.

REFERENCES

(1) R. Hiltpolt, W. Fisch, and P. Speiser, Swiss pat. 364117 (1957).

(2) G. Bechmann, ETH diss. 3531, 1964, Cl. Vogt, Frankfurt am Main, W. Germany.

(3) A. Elegakey, ETH diss. 4080, 1968, Juris Verlag, Zurich, Switzerland.

(4) T. Jecklin, ETH diss. 3704, 1965, Juris Verlag, Zurich, Switzerland.

(5) H. Lee and K. Neville, in "Encyclopedia of Polymer Science and Technology," vol. 6, Interscience, New York, N. Y., 1967, p. 267.

(6) P. Speiser, APV Informationsdienst 1, 1969, in press; *Farm. Aikakauslethi*, **7**, 8(1968).

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DRUG STANDARDS

Polarographic Assay of Niacinamide in Pharmaceutical Preparations

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Abstract □ A rapid polarographic determination of niacinamide in pharmaceutical preparations had been developed and is compared with the König colorimetric assay using barbituric acid. The niacinamide is extracted from the sample using a combination of column and liquid-liquid partition chromatography. The extracted niacinamide is determined in a supporting electrolyte of 0.1 *N* sodium hydroxide using a conventional d.c. polarograph equipped with an H-cell. A wide variety of samples was analyzed repetitively by this procedure and the standard deviation was calculated. The method is rapid, specific, and accurate.

Keyphrases □ Niacinamide dosage forms—analysis □ Column, liquid-liquid partition chromatography—extraction □ Polarography—analysis

The most widely accepted analytical method for niacinamide is based on the König reaction (1). This method utilizes the reaction of pyridine or its derivatives with a cyanogen salt and an aromatic amine. A significant variation of the König reaction is the use of various amines in the reaction. Both the USP (2) and the AOAC (3) employ this assay with sulfanilic acid as the aromatic amine; the product shows an absorption maximum at 450 mμ. In both methods for multivitamin preparations, niacinamide is first hydrolyzed to nicotinic acid and then the color is formed. Other amines which have been used include barbituric acid (4, 5) and procaine hydrochloride (6). With procaine hydrochloride as the amine coupling reagent, niacinamide is hydrolyzed to nicotinic acid before the color development; with barbituric acid as the reagent, niacinamide is treated directly and a red color develops with a maximum absorbance at 550 mμ.

UV spectrophotometry has been used in the determination of niacinamide. However, the niacinamide must first be separated from complex preparations by such techniques as ion-exchange chromatography before the UV determination (7). Niacinamide has been separated from interfering substances by thin-layer and paper chromatography (8) and then determined by photometry and polarography. Microbiological (9–11) and titrimetric methods (12) have also been used.

The polarographic behavior of niacinamide is well documented (13–17) and a number of workers have reported the polarographic assay of niacinamide in simple vitamin preparations. Zuman (18) and Knobloch (19) determined niacinamide in a tablet and in an injection, respectively, by simply diluting the preparation with a 0.1 *N* sodium hydroxide supporting electrolyte and recording the polarogram. Sodium carbonate has also been used as the supporting electrolyte in the analysis of a simple tablet preparation (20). Most complex preparations, however, cannot be determined di-

rectly because of interferences with the polarographic wave. In addition to the thin-layer and paper chromatographic "clean-up" steps already mentioned, ion-exchange chromatography has been used to separate the niacinamide before polarographic assay (21). These methods are time-consuming, especially in assaying an occasional sample, and flow-rate and concentration limitations are rather strict in ion-exchange techniques.

The procedure described here utilizes liquid-liquid partition chromatography. Though niacinamide is extremely water-soluble, it is also slightly soluble in certain organic solvents, such as chloroform. Because of this characteristic, niacinamide can be quantitatively extracted from a small volume of stationary aqueous phase with chloroform by column chromatography. The solubility of the niacinamide in the mobile chloroform phase is further enhanced by saturating the stationary aqueous phase with a basic salt. The polarographic analysis was performed on a wide variety of commercial samples and the results were compared to the cyanogen bromide–barbituric acid colorimetric method (4).

EXPERIMENTAL

Apparatus—A recording polarograph (Sargent model XV) equipped with a dropping mercury electrode and H-cell with a saturated calomel reference electrode; a glass chromatographic column 250 mm. long by 22 mm. i.d., with one end constricted to a stem 60 mm. long.

Reagents—All chemicals were reagent grade. Mercury, triple-distilled[1]; sodium bicarbonate; saturated aqueous solution of sodium bicarbonate; NaOH, 0.1 *N* aqueous solution; diatomaceous earth[2]; water-saturated chloroform, prepared by vigorously mixing equal volumes of chloroform and water and allowing the phases to separate and settle for several minutes; gelatin, 0.2% aqueous solution.

Sample Preparation—*Solid Dosage Preparations*—Weigh 20 tablets to determine the average tablet weight; then grind them to a powder to pass a 60-mesh sieve. Mix the powder until uniform. In the case of capsules, empty the contents of 20 units and determine the average content weight. Accurately weigh a portion of the powdered sample equivalent to about 10 mg. of niacinamide into a 100-ml. beaker. Add 3.0 ml. of the saturated aqueous solution of $NaHCO_3$ and swirl until the powder is completely wetted. Add 5 g. of diatomaceous earth and mix with a spatula until fluffy. Place a plug of glass wool in the bottom of the chromatographic column and transfer the sample in several portions to the column with moderate packing after each transfer. Wash the beaker with about 0.5 g. of diatomaceous earth wetted with a few drops of water and transfer to column. Pack moderately and overlay with a plug of glass wool.

Liquid Preparations—Dilute preparation with water, if necessary, to obtain a final niacinamide concentration of 6–14 mg./3.0 ml. (If the preparation is more dilute than 6 mg./3.0 ml., use 3.0 ml. of the undiluted sample.) Pipet 3.0 ml of the final solution into a 100-ml. beaker, add 0.5 g. of sodium bicarbonate, and let stand for several minutes, with frequent swirling, to dissolve as much of the salt as possible. Proceed as with solid dosage preparations, beginning with "add 5 g. of diatomaceous earth and mix with a spatula. . . ."

Procedure—Place a 250-ml. beaker directly beneath the prepared chromatographic column. Elute the niacinamide from the column with 100 ml. of water-washed chloroform. (In the case of liquid preparations containing less than 6 mg./3.0 ml., elute the niacinamide with 200 ml. of water-washed chloroform.) After the elution is complete, wash the stem of the chromatographic column with chloroform. Evaporate the chloroform extract on a steam bath to a volume of about 15 ml. with a current of air directed on its surface. Remove the beaker from the steam bath and complete the evaporation to dryness with air. Disperse the residue in the beaker with a small volume of 0.1 *N* NaOH and swirl for several minutes to dissolve the niacinamide. Quantitatively filter into a volumetric flask through cotton wetted with 0.1 *N* NaOH. Wash the beaker with several portions of 0.1 *N* NaOH and pass through the cotton filter into the flask. Add a volume of the 0.2% gelatin solution to the flask and dilute with 0.1 *N* NaOH so that the final niacinamide concentration is 5–15 mg./100 ml. and the gelatin concentration is 0.002%. At the time the NaOH is added to the sample, prepare a standard solution by dissolving an accurately weighed amount of niacinamide USP in 0.1 *N* NaOH, adding 2% gelatin, and diluting with 0.1 *N* NaOH to give a final niacinamide and gelatin concentration equivalent to that of the sample. The standard and sample polarograms should be recorded within 1 hr. (Niacinamide hydrolyzes about 1% during the first hour after the addition of the base.)

Polarographic Determinative Step—Instrument parameters: adjust the mercury column height to between 50 and 80 cm. so that the drop time is between 2 and 5 sec.; sensitivity, adjust μamp./mm. so that the height of the diffusion current of the standard and sample will be at least 60% full scale; voltage range, 0 to −3 v.; applied voltage, begin each polarogram at −1.5 v. and record to about −2.1 v.

Place the standard solution in the sample compartment of the H-cell and deaerate with nitrogen for 5 min.[3] Record the polaro-

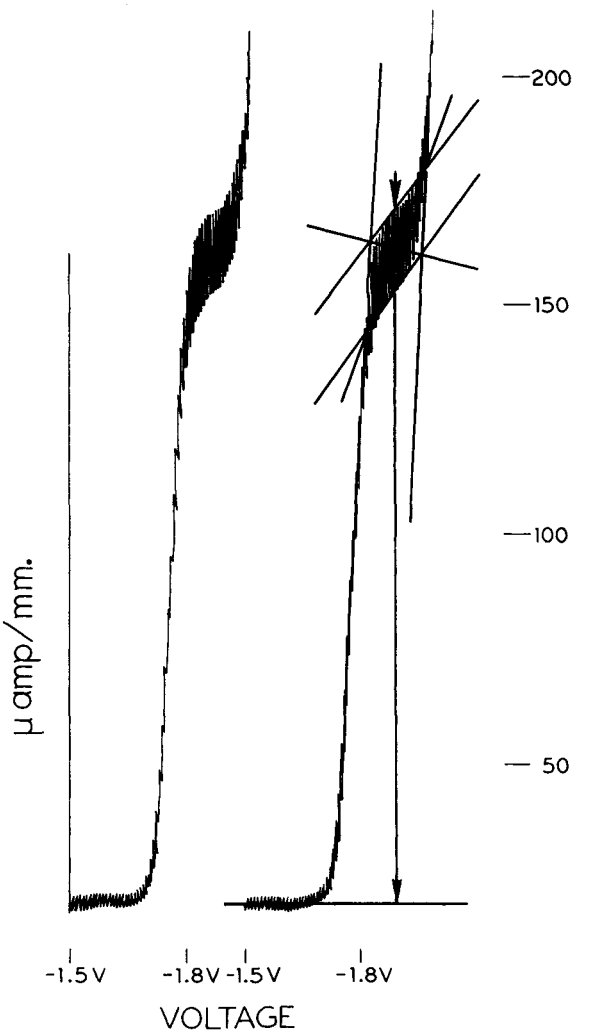

Figure 1—*Typical polarogram of niacinamide in 0.1* N *NaOH; recommended method of calculation.*

[1] Obtained from Bethlehem Apparatus Co., Inc., Hellertown, Pa.
[2] Celite 545, acid-washed, Johns-Manville, New York, N. Y.
[3] Trace amounts of oxygen in the nitrogen are removed by bubbling the nitrogen through two scrubber solutions. The first consists of 100 g. of lightly amalgamated zinc, 100 ml. of 0.1 *M* vanadyl sulfate, and some free H_2SO_4. The second contains water (22).

gram of the solution twice from −1.5 v. to about −2.1 v. Treat the sample solution in the same manner, beginning with the deaeration step. The temperature of the standard and sample polarographic solutions are held constant at room temperature during the determinations.

Calculations—Figure 1 shows the recommended method of measuring the height of the standard and sample diffusion currents. The amount of niacinamide in a sample unit can be calculated by the following equation:

$$\text{Niacinamide, mg./unit dosage} = \frac{\text{av. height, sample diffusion current (mm.)}}{\text{av. height, standard diffusion current (mm.)}} \times \text{concentration of standard} \times \text{dilution} \times \frac{\text{av. unit weight or volume}}{\text{sample weight or volume}}$$

RESULTS AND DISCUSSION

Assay of Commercial Preparations—The results of the assays by both the polarographic and colorimetric methods are given in Table I. The mean and standard deviations are also given for the polarographic analysis.

The flexibility and ruggedness of the polarographic method were tested by assaying a wide variety of commercial preparations a minimum of five times each; the majority of assays were run on different days. In addition to niacinamide, the preparations also contained vitamins A, D, B_1, B_2, C, B_6, B_{12}, choline, sorbitol, and salts of cobalt, iron, calcium, magnesium, manganese, copper, and potassium. Other components included liver, yeast, inositol, methionine, folic acid, calcium pantothenate, sodium fluoride, phenobarbital, and alcohol. Table I shows that for Liquid Preparation *E* the colorimetric analysis is about 6% higher than that obtained by polarography. In the colorimetric assay of this preparation a slight turbidity was noted in the final solution and was impossible to remove. This turbidity was not present in the sample blank. Some inactive ingredient in the preparation apparently reacted with the cyanogen bromide to cause the turbidity and the higher result. Tablet *A* yielded the greatest amount of residue after chloroform elution and evaporation because of a liver substance in the tablet. In this case, it was necessary to aid dispersion of the residue by breaking it up with a glass rod and allowing it to stand for several minutes; otherwise losses of niacinamide occurred. In all cases the polarographic waves for the samples were free from interferences.

Niacinamide Recoveries—Table II lists the recoveries for various amounts of niacinamide from the column. In all cases a volume of 3.0 ml. was used. For the lower concentrations, *i.e.*, 1 and 3 mg./3.0 ml., the recoveries are lower when 100 ml. of chloroform eluant was used. The amount recovered for these lower concentrations can be increased by increasing the chloroform elution to 200 ml. This larger volume is necessary for only those liquid preparations containing less than 6 mg./3 ml. of niacinamide. The chromatographic column can accommodate relatively large amounts of niacinamide without seriously affecting the recovery.

Flow rate studies showed that the recovery of niacinamide was not affected when rates as low as 1 ml./min. were used. However, to keep the analysis time at a minimum a flow rate of 3–4 ml./min. was found to be optimum.

Factors Affecting Niacinamide Stability—If the chloroform extract was evaporated completely to dryness while on the steam bath, losses of niacinamide occurred. These losses were probably due to the hydrolysis to nicotinic acid, which does not exhibit a polarographic wave under the conditions of the method, and increased with increasing time of the dry residue on the steam bath. The problem was eliminated by evaporating the last 15 ml. of chloroform to dryness at room temperature with a current of air. Although the saturated sodium bicarbonate had no hydrolytic effect on the niacinamide, a gradual degradation was noted after the 0.1 *N* NaOH supporting electrolyte was added; hydrolysis was only about 1% after the first hour but was complete in about 24 hrs.

Table I—Polarographic and Colorimetric Assays of Commercial Pharmaceutical Preparations

Preparation	Niacinamide Declared, mg./unit	Niacinamide Found, mg./unit—Polarographic Assay— No. of Assays	Mean ± *SD*[a]	Colorimetric Assay[b]
Tablet *A*	20	7	21.5 ± 0.26	22.0
Tablet *B*	20	6	20.5 ± 0.39	21.2
Tablet *C*	20	6	20.2 ± 0.39	21.1
Tablet *D*[c]	20	7	4.89 ± 0.16	5.1
Tablet *E*	50	6	49.4 ± 1.09	49.3
Capsule *A*[d]	10	7	12.3 ± 0.30	12.6
Capsule *B*	50	5	49.7 ± 0.84	50.7
Liquid *A*, ml.	13.3	6	13.1 ± 0.34	12.5
Liquid *B*, ml.	2.0	7	1.95 ± 0.03	2.0
Liquid *C*, ml.	50.0	6	54.5 ± 0.84	54.5
Liquid *D*, ml.	4.0	7	3.73 ± 0.10	3.7
Liquid *E*, ml.	0.45	11	0.406 ± 0.010	0.43

[a] Standard deviation. [b] Average of two determinations. [c] Product subpotent. [d] Product superpotent.

Table II—Recoveries of Various Concentrations of Niacinamide on Chromatographic Column with 100 ml. of Eluant

Niacinamide, mg./3.0 ml.	Recovery, %
1[a]	95
3	96
6	97
10	99
12	98
16	99
20	99
30	98

[a] Recovery was 98% with 200 ml. of eluant.

CONCLUSIONS

Although some simple pharmaceutical preparations can be determined directly by polarography, most complex mixtures must be purified before quantitation is possible. The method described here is suitable for the analysis of a wide variety of complex samples. It is equally as specific and rapid as existing methods, compares favorably in accuracy, and eliminates the use of objectionable chemicals such as cyanogen bromide.

REFERENCES

(1) W. König, *J. Prakt. Chem.*, **69**, 105(1904); **70**, 19(1904).

(2) "United States Pharmacopeia," 17th rev., Mack Publishing Co., Easton, Pa., 1965, p. 411.

(3) "Official Methods of Analysis," Association of Official Agricultural Chemists, 10th ed., Washington, D. C., 1965, pp. 763–764.

(4) O. Pelletier and J. A. Campbell, *J. Pharm. Sci.*, **50**, 926 (1961).

(5) "Changes in Methods," *J. Assoc. Offic. Anal. Chemists*, **51**, 506(1968).

(6) R. Strohecker and H. M. Henning, in "Vitamin Assay—Tested Methods," Verlag Chemie, G.m.b.H., Weinheim/Bergstr., Germany, 1965, pp. 193–195.

(7) *Ibid.*, pp. 189–192.

(8) *Ibid.*, pp. 203–207.

(9) *Ibid.*, pp. 207–212.

(10) "Methods of Vitamin Assay," Association of Vitamin Chemists, Inc., Interscience Publishers, New York, N. Y., 1966, pp. 172–176.

(11) "Official Methods of Analysis," Association of Official Agricultural Chemists, 10th ed., Washington, D. C., 1965, pp. 771–772.

(12) *Ibid.*, p. 772.

(13) R. Cerntăescu, M. Poni, and R. Ralea, *Studii Cercetari Stiin.*, **4**, 117(1953).

(14) W. Kemula and J. Chodkowski, *Roczniki Chem.*, **29**, 839 (1955).

(15) P. C. Tompkins and C. L. Schmidt, *Univ. Calif. Berkeley Publ. Physiol.*, **8**, 247(1943).
(16) J. J. Lingane and O. L. Davis, *J. Biol. Chem.*, **137**, 567(1941).
(17) F. Sorm and Z. Sormova, *Chem. Listy*, **42**, 82(1948).
(18) P. Zuman, "Organic Polarographic Analysis," Macmillan, New York, N. Y., 1964, pp. 189–190.
(19) E. Knobloch, *Chem. Listy*, **39**, 54(1945); *Collection Czech. Chem. Commun.*, **12**, 581(1947).
(20) K. Wenig and M. Kopecký, *Časopis Československov Lékarnictva*, **56**, 49(1943).
(21) R. Strohecker and H. M. Henning, "Vitamin Assay—Tested Methods," Verlag Chemie, G.m.b.H., Weinheim/Bergstr., Germany, 1965, pp. 201–203.
(22) L. Meites and T. Meites, *Anal. Chem.*, **20**, 984(1948).

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TECHNICAL ARTICLES

A Critical Analysis of a Capsule Dissolution Test

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Abstract □ A suitably modified USP disintegration apparatus has been used to obtain dissolution rate data for chloramphenicol capsules. Differences between various commercial and laboratory formulations of this drug were reflected in their dissolution profiles. Several shortcomings of this apparatus are described.

Keyphrases □ Capsule dissolution testing—methodology □ Chloramphenicol capsules—dissolution □ Particle size effect—capsule dissolution rates □ Lactose effect—capsule dissolution rates □ Diagram—dissolution apparatus □ UV spectrophotometry—analysis

The desirability of an *in vitro* test which adequately reflects the physiological availability of solid dosage forms of drugs is now well recognized. The inadequacy of disintegration times in this context has been pointed out (1). The measurement of a parameter which is related to the rate of dissolution of a solid has been suggested as a more realistic variable and this has led to an abundance of papers [see for example (2–6)] describing different methods and equipment for following dissolution rates. One of the more widely used methods (7) adapts, with suitable modifications, the apparatus recommended by either the USP (8) or FDD (9) for the measurement of disintegration time of tablets.

Important requirements for an adequate dissolution test include:

(*a*) The design of the equipment and protocol should allow a rapid evaluation of some specified dissolution parameter by using equipment or components that are either commercially available or readily fabricated. The dimensions and geometry of the individual components of the apparatus should be rigidly specified, together with tolerances, so that inter- and intra-laboratory variations are kept to a minimum.

(*b*) Analysis of the dissolution medium, in order to establish the dissolution profile,[1] should be rapid, sensitive, and simple.

(*c*) The procedure used should rank different formulations of the same drug in the same order as their *in vivo* availability.

(*d*) A detailed description of the procedure used in the kinetic analysis and derivation of suitable dissolution parameters is essential.

(*e*) Enough specimens of each formulation should be examined to permit a significant statistical analysis. The resulting statistical parameters should reflect inter-vehicle formulation differences and permit differentiation of formulation and manufacturing variables.

In this paper one dissolution test, which involves the use of a modified version of the USP disintegration apparatus (8), was used to obtain dissolution profiles of a variety of encapsuled formulations of one drug (chloramphenicol).

EXPERIMENTAL

Equipment—The USP disintegration apparatus, without disks or plungers, was used. Since the specifications (8) for the dimensions and geometry of this apparatus allow some variation, the apparatus which was used is illustrated in Fig. 1.

Procedure—Eight hundred milliliters of simulated gastric juice solution was allowed to equilibrate with a thermostat whose temperature was controlled at 37 ± 0.5°. Two methods were used to follow the dissolution of a given formulation:

[1] In this paper, the term is used to mean the curve obtained when percent dissolved is plotted against sampling time.