Specific Colorimetric Determination of Niacinamide in Dosage Forms

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Abstract □ A chemical method for determining the amount of niacinamide in multicomponent preparations was developed. Neither niacin resulting from the degradation of niacinamide nor vitamin A, thiamine hydrochloride, riboflavin, ascorbic acid, ergocalciferol, or calcium pantothenate interferes. The method is based on the reaction of niacinamide with 1-chloro-2,4-dinitrobenzene, followed by the spectrophotometric measurement of the colored solution obtained after treatment with sodium hydroxide. The proposed method is at least as accurate as the methods currently in use. A structure is proposed for the colored product, and its route of formation is outlined.

Keyphrases □ Niacinamide—colorimetric analysis, multicomponent vitamin preparations □ Colorimetry—analysis, niacinamide in multicomponent vitamin preparations □ Vitamins—colorimetric analysis of niacinamide in multicomponent preparations

Niacinamide (I) is a common ingredient in numerous multicomponent pharmaceutical preparations. Its degradation product, niacin, is undesirable in multivitamin preparations because it produces unpleasant physiological reactions in some patients. Most analytical methods give positive results with niacin, unless it is removed prior to the determination as in some recent chromatographic procedures (1, 2).

Most photometric methods are based on the Köenig reaction (3), including the method of the Association of Official Agricultural Chemists (4). A microbiological procedure using *Lactobacillus arabinosus* (5) was described for the determination of I plus niacin. When using *Leuconostoc mesenteroides* (6), it is possible to determine the amount of niacin alone. This method has the disadvantage of poor precision.

The purpose of the present research was to find a method for the accurate determination of I in the presence of niacin and other common components in multivitamin preparations. The proposed method is simple, specific, and at least as accurate as current methods. It has at least two advantages over the Köenig reaction: (*a*) it avoids the use of the toxic, unstable, cyanogen bromide; and (*b*) it does not give positive results with niacin, the principal degradation product of I.

EXPERIMENTAL[1]

Reagents—1-Chloro-2,4-dinitrobenzene (II) (p.a. grade) was crystallized from ethanol, mp 53°. 1-Fluoro-2,4-dinitrobenzene (p.a. grade) was distilled under reduced pressure, bp 122° (1 mm Hg). A niacinamide USP reference standard was used without further purification. Acetone (p.a. grade) was distilled. Pure methanol was made anhydrous by the Lund and Bjerrum method (7).

Benzene was stored over sodium strings for at least 3 days and then distilled over them. Chloroform was shaken with concentrated sulfuric acid, washed with water, dried over calcium chloride, and distilled. Pure chloroform was kept in the dark. All other reagents were ACS grade or equivalent and were used without further purification.

Sample Preparation—In the absence of ascorbic acid, weigh, powder, and mix 20 tablets or the contents of 20 capsules. Accurately weigh a portion of the sample equivalent to approximately 10 mg of I and extract it with 50 ml of acetone (using four portions of nearly 10 ml of acetone each time and washing the residue with the remainder of the acetone). Then filter, collecting the filtrate in a 100-ml round-bottom flask. Add 1.0 g of II to the solution and evaporate the solvent under reduced pressure.

When ascorbic acid is present, proceed in the same way but extract I with 100 ml of chloroform or benzene. Add 1.0 g of II, remove about one-half of the solvent under reduced pressure, transfer quantitatively to a 100-ml round-bottom flask, and evaporate to dryness.

Syrups and elixirs also were determined by this method, with a previous partial elimination of the solvent and extraction with chloroform or benzene. Niacinamide in one-drug injectables was determined by a previous complete elimination of the solvent under reduced pressure, addition of a small amount of acetone and of 1.0 g of II, and complete removal of the acetone under reduced pressure.

Color Development—Close the flask containing the solid residue with a ground-glass stopper and heat at 80° for 2 hr with constant stirring. The holder illustrated in Fig. 1 permits the processing of six samples at the same time[2]. The holder is attached to a suitable stirrer, and the flasks are immersed in a thermostated bath.

Remove the flasks from the bath, add 10 ml of benzene to each, shake to assure dissolution of the unreacted II, and extract with four portions of about 20 ml of distilled water, collecting the aqueous extracts in a 100-ml volumetric flask. Dilute to volume with water. Transfer a 2.0-ml aliquot to a 50-ml volumetric flask, add about 45 ml of 96% ethanol and then 2.0 ml of aqueous 1 *N* sodium hydroxide, and dilute to volume with ethanol.

Monitor the absorbance of the sample at 504 nm, using 96% eth-

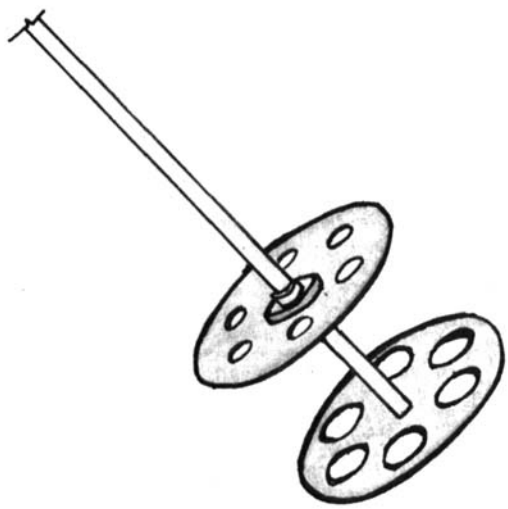
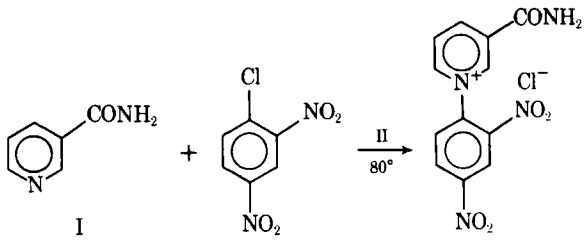


Figure 1—*Holder that allows six determinations of niacinamide at the same time (Scheme I).*

[1] A Beckman DU-2 spectrophotometer with 1-cm path length quartz cells was used for spectrophotometric measurements.

[2] Neoprene filter adapters, tapered collar type, are inserted in each hole of the plates to assure firm support of the flasks.



Scheme I

anol as a reference, until the highest absorption value is reached, *i.e.*, 5–6 min after the addition of the alkaline solution. The concentration of I present in the sample is calculated by comparison with the absorbance of a known amount of I treated in the same way. This value is then converted by the usual calculation to the basis of I declared in the sample and expressed as a percentage of the declared value.

RESULTS AND DISCUSSION

A procedure similar to the one described is given in NF XIII (8) as a qualitative assay for niacin but not for niacinamide (I). The NF procedure is more useful for the identification of I since it produces an unmistakable deep-violet color instead of the red one obtained in the assay with niacin. With the conditions described under *Experimental*, niacin does not give a measurable reaction.

Investigation of Parameters—Different experimental conditions were tested for the reaction of I with II (Scheme I) and for the reaction of the preceding reaction mixture with sodium hydroxide (Scheme II).

The influence of each of the following variables on the reaction shown in Scheme I was tested: solvent, temperature of the reaction, reagent (by using 1-fluoro-2,4-dinitrobenzene instead of II, no better results were obtained), time of reaction, and temperature and time of elimination of the solvent. The following solvents were used: anhydrous methanol, methanol–water (1:1), acetone, acetone–water (1:1), benzene, hexane, and dimethylformamide. No reaction was observed in any case, except for acetone in which about 5% of the reaction occurred. Acetone must be removed prior to the reaction in Scheme II to avoid the known reaction between II and acetone in alkaline media (9).

It is possible that most of the reaction in Scheme I occurred while the solvent was removed, and this may be also the reason that the temperature and the time of evaporation of the solvent seemed to influence the extent of the reaction. After several trials, it was concluded that fused II was by far the best solvent for the Scheme I reaction, since the reaction was less than 5% complete in all other media investigated.

For the Scheme II reaction, the following parameters were studied: solvent, sodium hydroxide concentration, ionic strength, time, and wavelength at which the maximum of absorbance is reached. Table I shows some solvents tested. Each increment of water in the ethanol–water mixture used as solvent had a hyperchromic effect accompanied by a small hypsochromic shift (Table I) and by an increase in the rate of the reaction. This effect became a disadvantage at over 12% of water because of the instability of the color. Changes in the concentration of sodium hydroxide from 0.02 to 0.2 *N* and of the ionic strength from 0.05 to 1 *N* did not modify the development of the color.

The linearity of the method was examined by performing the assay with different amounts of I, from 0.010 g (0.083 mmole) to 0.126 g (1 mmole). In all cases, 1.0–1.045 g of II was used (Table II). In addition, 12 determinations were performed with 10.0 mg of I and the standard deviation of the method was obtained; the mean value was 9.92 ± 0.06 mg.

It is possible to determine as little as 2 mg of I using the method described by extracting the Scheme I reaction mixture with 50 ml of water, taking a 4.0-ml aliquot, and using absolute ethanol for dilution prior to the Scheme II reaction.

Influence of Niacin (III)—Because niacin is the principal degradation product of I, the reaction of niacin under the same conditions used to determine I was performed. There was no develop-

Table I—Solvents for Reaction Shown in Scheme II

Solvent	λ_{max}, nm	t_{max}, min	Absorbance
Anhydrous methanol	510	70	—
96% ethanol–4% water	508	30	0.402
92% ethanol–8% water	507	20	0.438
88% ethanol–12% water	504	5	0.483
80% ethanol–20% water	502	~1	0.522
Water	490	≪1	

Table II—Determination of Niacinamide

Niacinamide Added, mg[a]	Absorbance	Niacinamide Found, mg
10.0	0.485	10.0
20.0	0.980	20.2
31.5	0.605	31.2
35.0	0.684	35.3
40.0	0.780	40.2
45.0	0.870	44.8
47.5	0.920	47.4
50.0	0.970	50.0
63.0	1.220	61.9
126.0[b]	1.235	127.3

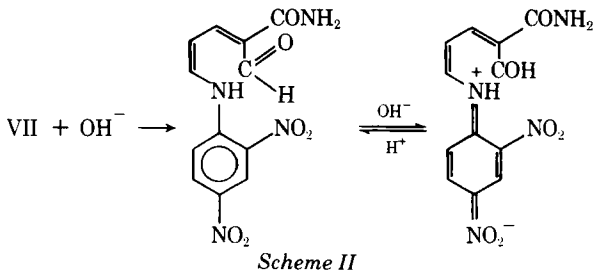
[a] When the amount of niacinamide was ≥31.5 mg, the aqueous extracts were collected in a 250-ml volumetric flask. [b] An aliquot of 1.0 ml was used instead of 2.0 ml.

Table III—Influence of Niacin

Niacinamide Added, mg	Niacin Added, mg	Niacinamide Found, mg	Recovery Values, %
50.0	—	50.0	100
47.5	3.5	47.4	99.8
47.5	4.4	47.4	99.8
45.0	6.3	44.9	99.8
40.0	11.0	40.2	100.5
35.0	17.0	35.2	100.6
—	47.0	0.0	0.0
—	100.0	0.0	0.0

Table IV—Effects of Various Vitamins on Recovery of Niacinamide

Niacinamide Added, mg	Vitamin Added, mg	Niacinamide Found, mg
50.0	Niacin, 100	50.5
50.0	Vitamin A, 5000 IU	49.5
50.0	Thiamine hydrochloride, 59.0	49.8
50.0	Riboflavin, 20.0	51.0
50.0	Ascorbic acid, 50.0	17.5
50.0	Ergocalciferol, 5000 IU	51.0
50.0	Calcium pantothenate, 50.0	50.0



Scheme II

Table V—Recovery of Niacinamide from Synthetic Formulations

Mixture	Added Vitamins, mg							Niacinamide	
	Vitamin A	Thiamine Hydrochloride	Riboflavin	Pyridoxine	Ascorbic Acid	Ergocalciferol	Calcium Pantothenate	Added	Found
1	—	10	5	5	50	—	10	10.0	10.0
2	—	100	10	10	100	—	50	10.0	9.80
3	5000 IU	10	5	5	10	5000 IU	10	10.0	9.95
4	—	10	5	5	—	—	10	10.0	10.05

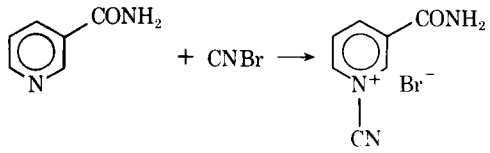
ment of color. To prove that this method could be used to follow hydrolytic degradation of I, mixtures containing it and small amounts of niacin in proportions slightly greater than expected from various degrees of deterioration of I were determined. Table III shows that the recovery of I was unaffected by the presence of niacin in the simulated degraded mixtures.

Other Interferences—Because I is usually formulated with other vitamins, the influence of vitamin A, thiamine hydrochloride (vitamin B_1), riboflavin (vitamin B_2), ascorbic acid (vitamin C), ergocalciferol (vitamin D_2), and calcium pantothenate (IV) was determined (Table IV). Only ascorbic acid (V) interfered. No exact correlation was observed between the content of ascorbic acid and the response. Ascorbic acid also interfered in the Scheme I reaction, because when it was added after the reaction was performed, the absorbances corresponded to the expected value in its absence. Oxidation to dehydroascorbic acid or neutralization with ammonia did not eliminate the interference. Extraction of I with chloroform or benzene, instead of acetone, gave satisfactory results since ascorbic acid was insoluble in these solvents. A 99.5% recovery was found using chloroform, and a 98.8% recovery was found using benzene.

Finally, Table V shows the amounts of I determined by the proposed method in synthetic mixtures. In the absence of ascorbic acid, extraction with acetone was performed; but when ascorbic acid was present, I was extracted with chloroform. The results shown are the averages of at least four determinations.

The proposed method was applied to authentic commercial multivitamin samples containing I with satisfactory results.

Mechanism—Niacinamide seems to be similar to pyridine in its reaction with II. When pyridine is added to an acetone solution of II, slightly colored crystals of 2,4-dinitrophenylpyridinium chloride, mp 195–195.5°, are formed (10). This compound, when treated with sodium hydroxide, develops a violet color which has a maximum absorbance at 540 nm (11) in 90% ethanol. The color is probably due to the anionic form of 5-(2′,4′-dinitrophenyl)amino-2,4-pentadienal (VI). Compound VI was prepared by an independent method (12), mp 179°, and its UV and visible spectra in the same media were identical with those obtained from the reaction of 2,4-dinitrophenylpyridinium chloride with sodium hydroxide.



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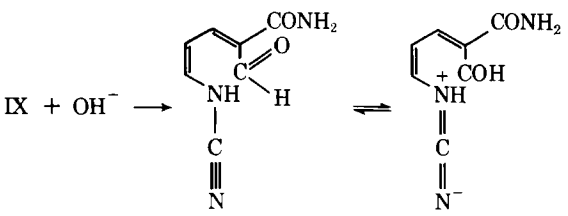
Scheme III



Scheme IV

In the case of I, the deactivation of the heterocyclic nucleus by the amido group is responsible for the more drastic conditions required to produce the pyridinium compound (VII), although it is not clear why it is formed more rapidly in the absence of solvent. When a carboxyl group is located at position 3, as in niacin, the nucleus is so deactivated that formation of the pyridinium complex is not possible under the conditions described.

The reaction in Scheme II probably occurs in the same way as that with the 2,4-dinitrophenylpyridinium chloride, since the complex formed has UV–visible spectra very similar to its pyridine analog, and its dependence on the ethanol–water ratio is also similar to that reported for the pyridine derivative (11).

Finally, it may be useful to point out that the parallelism between the reactions involved in this method and the mechanism accepted for the Köenig reaction (13) (Scheme III) can be postulated for the addition of cyanogen bromide (VIII), and it has been shown (14) that the condensation product (IX) of I with VIII has an open chain structure at pH 9 in water (Scheme IV) instead of the usual formulation as *N*-cyanopyridone.

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