(15) B. Chance, D. Mayer, N. Graham, and V. Legallais, *Rev. Sci. Instrum.*, **41**, 111 (1970).
(16) J. D. Defreese, T. A. Woodruff, and H. V. Malmstadt, *Anal. Chem.*, **46**, 1471 (1974).
(17) D. Harrington and H. V. Malmstadt, *Am. Lab.*, **6** (3), 33 (1974).
(18) G. Sauerbrey, *Appl. Opt.*, **11**, 2576 (1972).
(19) T. A. Woodruff and H. V. Malmstadt, *Anal. Chem.*, **46**, 1162 (1974).
(20) R. P. Gregory IV, J. Avery, B. W. Renoe, P. C. Dryden, and H. V. Malmstadt, *Clin. Chem. (Winston-Salem, N.C.)*, **20**, 950 (1974).
(21) P. C. Dryden and H. V. Malmstadt, Paper No. 29, Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, Cleveland, Ohio, March 1975.
(22) T. A. Woodruff and H. V. Malmstadt, *Anal. Chem.*, **46**, 2141 (1974).
(23) L. E. S. Mathias and J. T. Parker, *Appl. Phys. Lett.*, **3**, 16 (1963).
(24) J. J. Kirkland, *Anal. Chem.*, **40**, 391 (1968).

RECEIVED for review November 17, 1975. Accepted June 14, 1976. Presented in part at the Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, Cleveland, Ohio, March 1975. This work was supported in part by NSF Grant GP18910, and also in part by an American Chemical Society, Division of Analytical Chemistry Fellowship (to JDD) sponsored by the Procter and Gamble Company.

Spectrophotometric Determination of Hydrazides with 2,3-Dichloro-1,4-naphthoquinone

J. A. Plaizier,* J. G. Van Damme, and R. E. De Nève

Laboratorium voor Galenische en Magistrale Farmacie, Vrije Universiteit Brussel, Farmaceutisch Instituut, Paardenstraat, 67 B-1640 St. Genesius Rode, Belgium

A new colorimetric method for hydrazides, using alkaline 2,3-dichloro-1,4-naphthoquinone is tested. The method is specific for hydrazides not substituted on the β N atom, excluding hydrazine, hydrazone derivatives, ureum and semicarbazide. Aromatic hydrazides conform to Beer's law from 3.10^{-5} to 2.10^{-5} molar. Dihydrazide derivatives can only qualitatively be determined except oxalic acid dihydrazide. As the reaction mechanism, by inspecting the uv spectra, is proposed the condensation between a ketone and a hydrazide to a hydrazone, tautomerizing in alkaline medium.

The recent widespread interest in carboxylic acid hydrazides for a variety of applications, and the subsequent growing commercial importance of several acid hydrazides (*1*) led to the search for an analytical method, universally applicable, for the determination of these hydrazides. Various methods have been proposed, especially for isonicotinic acid hydrazide (INH).

Most of the proposed reactions are based on the reducing properties of the hydrazide function: one of the earliest methods was a titrimetric one with nitrous acid (*2*). Iodometric (*3, 4*) and bromometric (*5, 6*) titrations are reported, and also nonaqueous titrimetric procedures using perchloric acid in acetic acid (*2, 4, 7, 8*). These methods, however, are not specific for the hydrazide function and cannot make distinction between a hydrazide and its hydrazone derivative (*9*). A volumetric determination of nitrogen after treatment with iodate (*10*) has been used for a variety of hydrazides. Several years ago, a fluorimetric method using Rubin's (*11*) cyanogen bromide reaction, had been reported. Although sensitive, the method is not specific as it is based on the reactivity of the pyridine ring.

Colorimetric methods have been described, involving reaction between the hydrazides and an aldehyde- or keto group: a) *p*-dimethylaminobenzaldehyde forms yellow hydrazones (*12*) with hydrazine and nonsubstituted hydrazides. b) Alkaline 1,2-naphthoquinone-4-sulfonate (*13, 14*) forms orange-red reaction products with hydrazides, but also with methylene and amino groups (*15, 16*).

In conclusion, it can be stated that none of the above mentioned reactions is specific for the hydrazide group.

The method presented here is based on an observation of Van Damme and De Nève (*17*) that three out of five tested naphthoquinones give a blue color in alkaline solution with isonicotinic acid hydrazide. As napthoquinones are unstable in alkaline medium, they extracted the colored reaction products with amyl alcohol. This study was undertaken to determine applicability and limitations of that method, for quantitative determination of acid hydrazides by means of a naphthoquinone.

EXPERIMENTAL

Products. All products used are commercially available: salicylic acid hydrazide (Aldrich); nicotinic acid hydrazide (Aldrich); isonicotinic acid hydrazide (Aldrich); phenylacetic acid hydrazide (Aldrich); benzoic acid hydrazide (Schuchardt); butyric acid hydrazide (Aldrich); oxamic acid hydrazide (Fluka); acetic acid hydrazide (Aldrich); oxalic acid dihydrazide (Aldrich); glutaric acid dihydrazide (Aldrich); succinic acid dihydrazide (Aldrich).

The following products are also tested: hydrazine·2 HCl (Merck); phenylhydrazine·HCl (U.C.B.); hydroxylamine·HCl (Carlo Erba); acetamide (U.C.B.); ureum (Carbo Erba); semicarbazide·HCl (Analar).

2,3-Dichloro-1,4-napthoquinone (phygon) (Fluka): a saturated solution in petroleum ether was used. The choice of the solvent will be discussed later.

Ammonia–ammonium chloride buffers were prepared at sufficiently high buffer capacity in the pH range 8–12 by neutralizing NH_3 (0.1–1 M) with HCl.

Apparatus. All spectra were recorded with a Perkin-Elmer (124) double beam spectrophotometer, and all absorbance measurements, with a Vitratron U.C. 200 S.

For pH measurements, a pH meter (Radiometer Copenhagen) was used. The aliquots were shaken in a Gerhardt horizontal shaker at 340 movements/min.

Methodology. All the hydrazides were dissolved in water and diluted to adequate concentration. To 5 ml of these solutions were added 4 ml of a saturated phygon in petroleum ether solution and 1 ml of buffer solution. The mixture was shaken. Conditions of pH, ionic strength, stability, and time, giving maximum color development were investigated. After separation of the layers, the absorption spectra were recorded. Quantitative determinations were made in 1-cm cuvettes at the appropriate wavelengths using the reagents (without hydrazides) as blanks. Conformance to Beer's law and sensitivity of the method were investigated.

RESULTS AND DISCUSSION

Qualitative Determination. *Phygon.* Naphthoquinones are unstable in alkaline medium. Phygon was chosen as it is less sensitive than the other naphthoquinones.

Solvent. If we used amyl alcohol, as investigated by Van Damme, the excess of the reagent, here phygon, a yellow re-

Table I. Maximum Color Development, Optimum pH and Absorption Maxima

	Compounds	Time, min	Optimum pH	λ_{max}, nm
1.	Acetic acid hydrazide	65	11.0	575
2.	Butyric acid hydrazide	45	11.0	575
3.	Oxamic acid hydrazide	110	10.77	590
4.	Oxalic acid dihydrazide	45	10.25	595
5.	Glutaric acid dihydrazide	35	...	575
6.	Isonicotinic acid hydrazide	15	10.77	595
7.	Nicotinic acid hydrazide	35	10.77	595
8.	Benzoic acid hydrazide	30	11.0	595
9.	Phenylacetic acid hydrazide	60	11.0	580
10.	Salicylic acid hydrazide	70	11.0	575
11.	Hydrazine·2 HCl	...	...	none
12.	Phenyl hydrazine·HCl	...	...	none
13.	Hydroxylamine·HCl	...	...	none
14.	Acetamide	...	...	none
15.	Ureum	...	...	none
16.	Semicarbazide·HCl	...	...	none

agent, was also extracted in the alcoholic solution, masking the blue color of the reaction product. It appeared that, using apolar solvents such as heptane, *n*-hexane, or petroleum ether, the excess of reagent remained in the apolar solvent and was isolated from the blue reaction product, more soluble in water. Petroleum ether was preferred to hexane or heptane on a somewhat arbitrary basis, as the three solvents separate reactant and products equally well.

Alkalinity. The reaction proceeds in alkaline medium, whether the alkalinity is produced by sodium hydroxide, ammonia, or pyridine. As the color development is strongly dependent on the pH, the use of a buffer solution with sufficiently high capacity was preferred. As the ionic strength had no effect on the color production or on the separation of the layers, ammonia–ammonium chloride solutions were used throughout the experiments. At these concentrations, the pH remains unaltered by a fivefold dilution. This is an absolute necessity as the color production is increased with increasing pH up to the point where destruction occurs. Although color intensity is reproducible and quantitative determination can be made at any particular pH, measurements were made at optimal pH values (Table I).

Color Development. Color development is time dependent. Optimum reaction time was determined by shaking the mixture and taking aliquots every 5 min at optimum pH and room temperature. The results are summarized in Table I.

Absorption Spectra. The spectra of the colored hydrazide–phygon complexes were recorded by means of a spectrophotometer. The maximum peak in the visible light is large and the top is ±10 nm broad; therefore a simple colorimeter equipped with filters can be used without loss of sensitivity. The wavelengths of the peaks are summarized in Table I.

Quantitative Determination. The color produced is directly proportional to concentration, in conformity with Beer's law except for acetic acid hydrazide. The concentration ranges are stated in Table II.

Acetic acid hydrazide follows Beer's law up to 1.7×10^{-4} M. At higher concentrations, the extinction is decreasing with increasing concentration. The reaction is most sensitive for

Table II. Concentration Range Conforming to Beer's Law, and Extinction/10 μg

Compounds	Molarity	Extinction: 10 μg/ml
Nicotinic acid hydrazide	2.4×10^{-5}–2.0×10^{-4} M	0.010
Phenylacetic acid hydrazide	2.8×10^{-5}–3.0×10^{-4} M	0.005
Isonicotinic acid hydrazide	3.0×10^{-5}–1.5×10^{-4} M	0.0093
Benzoic acid hydrazide	3.0×10^{-5}–2.0×10^{-4} M	0.0097
Salicylic acid hydrazide	3.4×10^{-5}–2.2×10^{-4} M	0.0068
Butyric acid hydrazide	4.0×10^{-5}–3.3×10^{-4} M	0.008
Acetic acid hydrazide	5.6×10^{-5}–1.7×10^{-4} M	0.0073
Oxalic acid dihydrazide	7.0×10^{-5}–5.6×10^{-4} M	0.0033
Oxamic acid hydrazide	1.6×10^{-4}–6.5×10^{-4} M	0.0023

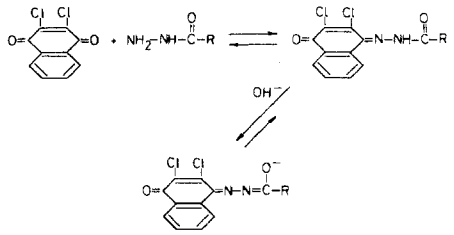


Figure 1. Reaction mechanism

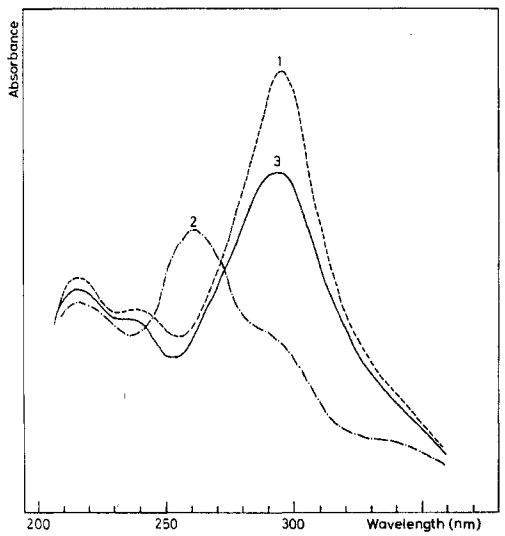


Figure 2. UV spectra of the reaction product (hydrazone) of 2,3-dichloro-1,4-naphthoquinone and butyric acid hydrazide in alkaline (1), acid (2) and alkaline (3) solution

the aromatic hydrazides, i.e., INH, nicotinic acid hydrazide, benzoic acid hydrazide; somewhat less for the substituted derivatives, salicylic acid hydrazide and phenylacetic acid hydrazide. From the data of aliphatic monohydrazides (acetic acid hydrazide, butyric acid hydrazide), we can predict with sufficient certitude that reaction will also take place with the other derivative in the homologue series, i.e., propionic acid hydrazide (this product is not commercially available). For the dihydrazides, the results are somewhat less: the color from oxalic acid dihydrazide is stable but the method is less sensitive. The colors from succinic acid dihydrazide and glutaric

Table III. UV Absorption Maxima (nm) from the Reaction Product in Acid and Alkaline Solution

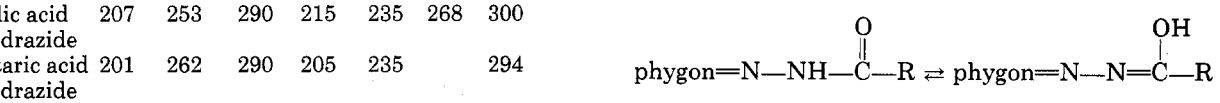
Compounds	Acid media			Alkaline media		
	Chief max	Shoulder		First max		Second max
1. Acetic acid hydrazide	216	260	290	216	232	290
2. Butyric acid hydrazide	215	263	290	215	238	296
3. Oxamic acid hydrazide	...	...	305	...	...	318
4. Oxalic acid dihydrazide	207	253	290	215	235	268 300
5. Glutaric acid dihydrazide	201	262	290	205	235	294
6. Isonicotinic acid hydrazide	214	260	290	213	235	290
7. Nicotinic acid hydrazide	212	260	290	212	236	298
8. Benzoic acid hydrazide	232	264	294	228	...	312
9. Phenylacetic acid hydrazide	208	260	288	207	240	295
10. Salicylic acid hydrazide	238	261	301	215	238	315

acid dihydrazide are not stable. However the reaction can be used as a qualitative test for these compounds.

Oxamic acid hydrazide is somewhat analogous in structure with oxalic acid dihydrazide, both products possessing an α, β dicarboxyl function. Both are also comparable with regard to sensitivity.

Reaction Mechanism. The following reaction mechanism is proposed: the hydrazide condenses with a ketone, forming a hydrazone. The hydrazone tautomerizes in alkaline medium to the enolate ion, which accounts for the color (Figure 1). The mechanism proposed is based on the following considerations: 1) The color disappears upon acidification, reversibly. 2) A reaction mechanism similar to the one proposed has been described for 1,2-naphthoquinone sulfonate (*14*). 3) Inspection of the spectral data. The uv spectra of the hydrazide and the hydrazone are recorded in neutral, alkaline, acid, and again in alkaline medium (spectra for one hydrazide are presented in Figure 2). The wavelengths of maximum absorbance are summarized in Table III. All the hydrazones show a maximum at 260 nm in acid solution, splitting upon alkalinization into 2 maxima at 230 and 300 nm. This splitting is reversible on reacidification and is not given by the reactants separately: INH and nicotinic acid hydrazide present a bathochromic shift but only at pH > 12 (*18*). Salicylic acid hydrazide shows a bathochromic shift somewhat displaced (298 to 325 nm) due to the phenolic group present in the molecule (*19*).

It is concluded that the splitting of the band is due to the amidoimidol tautomerism.



Benzoic acid and salicylic acid hydrazone show a first maximum in absorbance at 232 and 238 nm, respectively, in acid medium, masking the splitting of the 260 nm to 230 nm band upon alkalinization. The oxalic hydrazone deviates from the general behavior, showing in alkaline solution a supplemental absorbance at 268 nm. This phenomenon can tentatively be explained as one carbonyl group remaining in the amide configuration. Oxamic acid hydrazone shows no absorbances at 230 and 260 nm either in acid or in alkaline solution.

LITERATURE CITED

(1) Patai, "The Chemistry of Amides", Wiley-Interscience, New York, N.Y., 1970, pp 516–589.
(2) P. G. W. Scott, *J. Pharm. Pharmacol.*, **4**, 681 (1952).
(3) T. Canback, *J. Pharm. Pharmacol.*, **4**, 407 (1952).
(4) J. F. Alicino, *J. Am. Pharm. Assoc., Sci. Ed.*, **41**, 401 (1952).
(5) H. Wojahn, *Arzneim.-Forsch.*, **2**, 324 (1952).
(6) J. Vulterin and J. Lyka, *Chem. Listy*, **48**, 1745 (1954).
(7) E. Kuhni, M. Jacob, and H. Grossglauser, *Pharm. Helv. Acta*, **29**, 233 (1954).
(8) M. B. Devani and C. J. Shishov, *J. Pharm. Sci.*, **59**, 90 (1970).
(9) M. I. Blake, D. Bode, and H. Rhodes, *J. Pharm. Sci.*, **8**, 1303 (1974).
(10) H. McKennis, J. Weatherly, and E. Dellis, *Anal. Chem.*, **30**, 499 (1958).
(11) S. H. Rubin et al., *Dis. Chest*, **21**, 439 (1952).
(12) J. Kelly and R. B. Poet, *Am. Rev. Tuberc.*, **65**, 484 (1952).
(13) E. J. Short, *Lancet*, **266**, 656 (1954).
(14) E. L. Pratt, *Anal. Chem.*, **25**, 814 (1953).
(15) F. Feigl, "Spot Tests", Nordemann Publishing Co., New York, 1937, pp 291–3.
(16) P. Ehrlich and C. A. Herter, *Z. Physiol. Chem.*, **41**, 329 (1904).
(17) J. Van Damme and R. De Nève, *Farm. Tijdschr. Belg.*, **4**, 327 (1973).
(18) G. C. Belles and M. L. Littleman, *Anal. Chem.*, **32**, 720 (1960).
(19) R. M. Silverstein, C. G. Bassler, and T. C. Morril, "Spectrophotometric Identification of Organic Compounds", 3rd ed., Wiley-Interscience, New York, 1974, p 248.

RECEIVED for review January 30, 1976. Accepted April 26, 1976. The authors are indebted to the "Nationaal Fonds voor Geneeskundig Onderzoek" for financial support.

Spectrophotometric Determination of Micro Amounts of Arsenic

Gerald J. Kellen[1] and Bruno Jaselskis*

Department of Chemistry, Loyola University of Chicago, Chicago, Ill. 60626

The determination of submicro amounts of arsenic is based on the reduction of silver and iron(III) ions by arsine in the presence of Ferrozine. Amounts as low as 0.1 μg and as high as 1.5 μg in 5 ml of solution have been determined with a relative precision of 2% at levels above 0.8 μg and of 20% at the 0.1-μg level.

[1] Present address, Krafco R & D, 801 Waukegan Rd., Glenview, Ill. 60025.

There are a number of spectrophotometric methods for the determination of micro amounts of arsenic. The Gutzeit method (*1*) is the most sensitive chemical method known. In this method, arsine reacts with mercuric bromide sensitized paper to form a yellow spot. Obtaining reproducible spots and measuring the intensity of the color quantitatively are difficult. The molybdenum blue method (*2*) avoids these difficulties by producing the color in solutions; however, the sensitivity is about one tenth that of the Gutzeit method. A