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The Determination of Nicotine in Particulate Matter Using Citric Acid and Bromomaleic Acid*

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INTRODUCTION

Because nicotine is the most common occurring alkaloid in tobacco smoke, there have been many methods developed to quantitate its presence. Ultraviolet readout procedures (1–4) have been documented in the literature and have the advantage of yielding precise and reproducible results. These methods, however, necessitate a preliminary distillation from acid for the removal of acidic interfering compounds (phenolics) before a final distillation from base for the removal of nicotine alkaloids. *Harvey* and co-workers (5) have eliminated the dual distillation steps by using a suspension of Darco G-60 carbon in 50 % aqueous glycerol and thereby reduced the time for the analysis without sacrificing precision and accuracy. This procedure, however, as well as gravimetric, titrimetric, and some colorimetric methods that have been developed require some form of manual manipulation and are not easily automated.

An excellent method has been developed by *Harvey* and co-workers (6) making use of the procedure as described by *König* (7). This involves the development of an orange color when nicotine alkaloids are reacted with cyanogen bromide in the presence of a primary aromatic amine. The method is simple, has a high degree of precision and accuracy and has been automated in an AutoAnalyzer. The procedure has been extended by *Harvey, Hale* and *Ikeda* (8) to estimate nicotine alkaloid content of tobacco in the field. The method, however, necessitates a clarification step – normally, a dialyzer or carbon is used. Cyanogen bromide is a highly toxic chemical and, therefore, potentially dangerous. A procedure has been developed which is relatively specific, utilizes "nontoxic" chemicals, and was easily automated.

EXPERIMENTAL PROCEDURE

Apparatus and Glassware

Absorbances were measured using a 1.0 centimeter cell in a Beckman ACTA V spectrophotometer equipped with a constant temperature water bath. All gas chromatographic separations of nicotine from extracted particulate matter were done with a Varian Aerograph Series 1200 FID (flame ionization detector) chromatograph. [The 1/8 inch × 12-foot copper column was packed with 7 % Carbowax-20M with 3 % polyphenyl ether (6-ring) on Gaschrom Q (60–80 mesh).] The injection and detector temperature was 250° C while the column temperature was isothermal at 172° C. A 7 % Carbowax-20M with 2 % KOH 1/8 inch × 9-foot column was substituted in order to reduce nicotine adsorption on the column, but no significant changes in nicotine concentration were observed. The Robot Chemist manufactured by American Optical Corporation was used for automating the procedure. The test tubes used for the Robot Chemist were Pyrex® obtained from Corning. If soda-lime glass was used, a variable blank was obtained.

Reagents

All chemicals except for nicotine were used as received from the manufacturer. The citric acid (anhydrous) was obtained from Fisher Scientific Company, while the bromomaleic acid (BMA) was purchased from Aldrich. Solvents used were 2-propanol and acetic anhydride, both from Aldrich. Nicotine purchased from Aldrich was distilled under vacuum and kept under a nitrogen atmosphere.

Analytical Procedure

Citric acid (0.35 gram, 1.82×10^{-3} mole) was dissolved in 100 ml of acetic anhydride. Bromomaleic acid (0.15 gram, 7.69×10^{-4} mole) was dissolved in 100 ml of acetic anhydride. Sample preparation was accomplished in the normal fashion (9), but one milliliter of the resulting extract was pipetted into a 10 ml volumetric flask and diluted to volume with 2-propanol. Manually, the complexation reaction was obtained in the following manner: To one milliliter of the diluted 2-propanol extract of particulate matter was added one milliliter each of the citric acid solution and bromomaleic acid solution. The mixture was allowed to stand for 25 minutes in a constant temperature water bath at 30° C. The intensity of the red complex which resulted was recorded at 505 nanometers. A suitable blank was run since there was a slight absorbance at 505 nanometers due to the extract. The blank absorbance was generally less than 0.1 absorbance units.
Nicotine was determined in a similar manner on the Robot Chemist. A portion of the diluted 2-propanol extract of particulate matter as well as portions of each

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working standard solution were submitted to the Robot Chemist where the sampling, color development, incubation and absorbance measurements were made automatically. The water bath was maintained at a constant temperature of 30° C and the wavelength of the spectrophotometer was set at 505 nm. The Robot Chemist was programmed to take 1.25 ml aliquots from the sample tube and rinse with 0.75 ml of 2-propanol which was in the sample coil. This was followed by dispensing 2 ml of each reagent into the reaction tubes. The reaction tubes were stirred and incubated for a total of 25 minutes after the addition of the reagents. Suitable blanks were obtained using reagents added to 2-propanol.

Figure 1. Absorbance vs. mole ratio bromomaleic acid/nicotine.

1. Moles citric acid/moles nicotine = 9.1, nicotine concentration = 74.9 μg/ml.
2. Moles citric acid/moles nicotine = 14.0, nicotine concentration = 48.2 μg/ml.
3. Moles citric acid/moles nicotine = 36.8, nicotine concentration = 18.4 μg/ml.

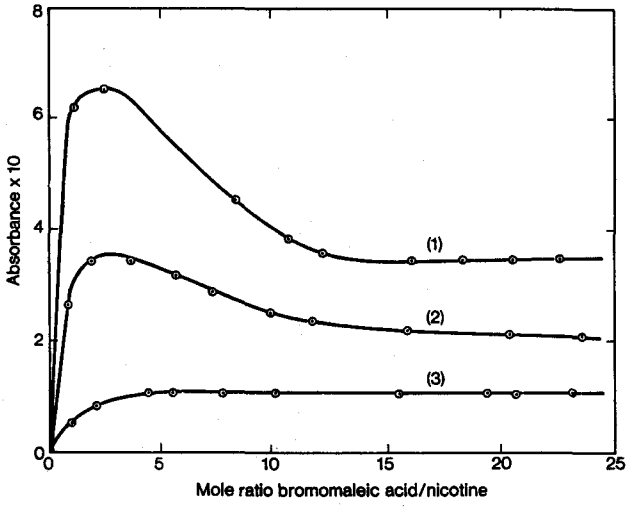
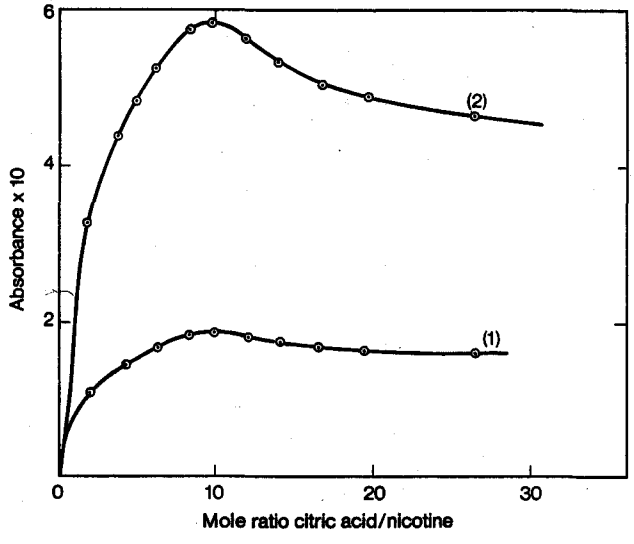


Figure 2. Absorbance vs. mole ratio citric acid/nicotine.

1. Nicotine concentration = 18.0 μg/ml.
2. Nicotine concentration = 52.4 μg/ml.

Mole ratio bromomaleic acid/nicotine = 4.0 for both nicotine concentrations.



The standard nicotine in particulate matter solution was prepared as described by *Charles* et al. (9). Suitable standard nicotine solutions were prepared from the stock solution such that a range of 20–120 μg nicotine/ml was obtained. Nicotine content in each standard was determined using gas chromatography.

Optimum Conditions for Color Development

It was necessary to determine the ratios of [citric acid/nicotine] and [BMA/nicotine] which would yield the highest sensitivity. In Figure 1, the absorbance at 505 nanometers was plotted against the mole ratio of [BMA/nicotine] at three different levels of nicotine. At each level of nicotine there was a specific [citric acid/nicotine] mole ratio. Maximum sensitivity was obtained at a [BMA/nicotine] ratio of four. The experiment as shown in Figure 1 was repeated using two different nicotine levels but at each level the citric acid/nicotine ratio was kept at 15. The maximum sensitivities obtained were identical to those shown in Figure 1. The [BMA/nicotine] ratio was kept at four while varying the [citric acid/nicotine] ratio. In this way, a maximum sensitivity of 10 was obtained for the latter. Results are shown in Figure 2. Absorbances obtained using ratios of [BMA/nicotine] $>$ 40 and [citric acid/nicotine] $>$ 100 yielded results indicative of changes in nicotine concentration rather than ratio changes.

Reagent Stability

The bromomaleic acid reagent was stable for at least 96 hours at room temperature. The citric acid reagent deteriorates with time if allowed to stand at room temperature for an extended period of time. The following procedure yielded reproducible results: the reagent was allowed to stand at room temperature for 24 hours and refrigerated overnight. The reagent was ready to use in the morning and could be used all day with very little change in sensitivity. At the end of the day, it was refrigerated again. Following this procedure, the citric acid reagent may be used for five days without sensitivity loss.

Figure 3. Effect of water on color formation.

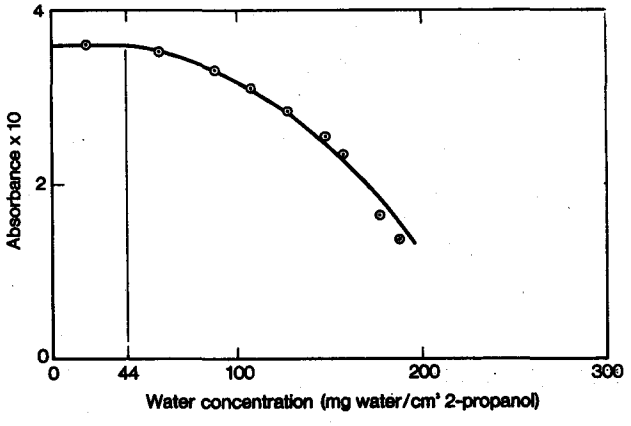
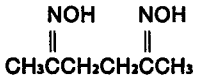
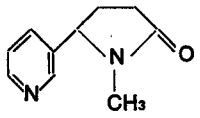
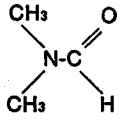
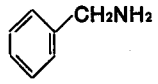
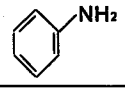
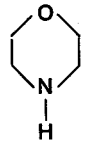
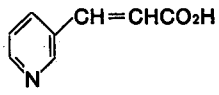
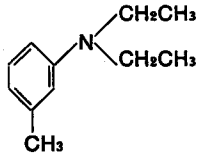
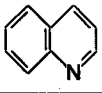
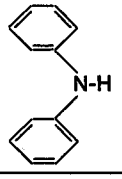
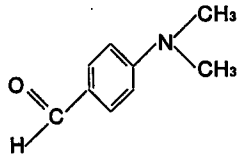
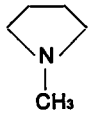


Table 1. Interferences.

Compound	Class[a]	Structure	Concentration (μg/ml)	Absorbance
Pyridine	3° aromatic		50.0	0.08
2,5-Hexanedione oxime	oxime		43.0	N.A.
Cotinine	amide		50.0	.01
Dimethylformamide	amide		50.0	N.A.
Benzylamine	1° amine		50.0	N.A.
Ethylamine	1° amine	$CH_3CH_2NH_2$	50.0	N.A.
Aniline	1° amine (aromatic)		50.0	N.A.
Morpholine	2° amine		50.0	N.A.[b]
β-(3-Pyridyl) acrylic acid	3° amine (aromatic)		50.0	N.A.
N,N-Diethyl-m-toluidine	3° amine		50.0	.04
3,4-Benzoquinoline	3° amine		46.0	N.A.
Diphenyl amine	2° amine (aromatic)		50.0	N.A.
N,N-Diethylethylene diamine	1°+3° amine	$Et_2NCH_2CH_2NH_2$	50.0	.36
Triethylamine	3° amine	$(Et)_3N$	50.0	.29
p-Dimethylamino benzaldehyde	3° amine (aromatic)		50.0	N.A.
N-Methyl pyrrolidine	3° amine		60.0	.62

a: 1° = primary, 2° = secondary, 3° = tertiary. b: N.A. = no absorbance.

The Effect of Water on Color Development

It was necessary to determine if the water present in the 2-propanol extract of particulate matter would retard color formation or if there was any loss in color intensity due to water. Figure 3 shows that the absorbance remained constant until the water concentration exceeded 40 mg per milliliter of 2-propanol. Since the normal water concentration in the extract was generally less than 1 mg per milliliter of 2-propanol, there was no loss in color intensity due to water.

Interferences

Table 1 lists the different classes of compounds that have been analyzed, their concentrations in 2-propanol and their relative absorbances. Neither primary nor secondary amines (cyclic and acyclic) yield a colored complex.

RESULTS AND DISCUSSION

The major problem encountered during the investigation of the method was the stability of the citric acid reagent. If the refrigeration step was not followed, the results became erratic and the sensitivity of the test was greatly reduced. During each six-hour run, a set of five nicotine in particulate matter standards were run between each twenty-sample run. Figure 4 shows the variation in absorbance of the standards over a period of 72 hours using a single batch of the citric acid reagent. Even though the variation during this time period was observed to be small, other batches of the reagent yielded varying intercepts with no change in the slope of the line.

Table 2.

Citric acid-BMA system*		CNBr system	Griffith distillation**
Day 1	Day 3		
1.40	1.36	1.37	–
1.50	1.53	1.44	–
1.48	1.52	1.40	–
1.36	1.40	1.35	–
1.14	1.18	1.10	–
1.52	1.44	1.42	–
1.39	1.45	1.40	–
1.46	1.47	1.41	–
1.41	1.36	1.44	–
1.20	1.12	1.10	–
1.29	–	1.27	1.27
1.28	–	1.19	1.21
1.29	–	1.27	1.20
1.22	–	1.24	1.25
1.17	–	1.16	1.15

All nicotine values are reported as mg of nicotine/cigarette and represent the average of two determinations.

* The first ten values reported were obtained from the automated procedure on the Robot Chemist. The last five are from the manual procedure using the Beckman ACTA V spectrophotometer.

** UV readout. See reference 9 for sample preparation.

Figure 4. Absorbance of the standard nicotine solution (from particulate matter) vs. concentration of nicotine using aged nitric acid reagent.

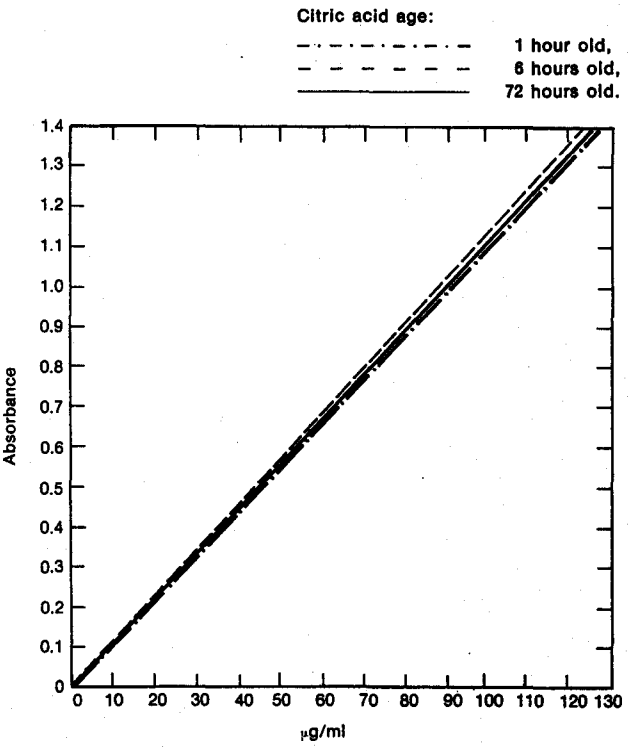


Table 2 shows typical comparison values of nicotine concentrations between the established cyanogen bromide method, the *Griffith* distillation procedure, and the citric acid-bromomaleic acid method. Using the same batch of the citric acid reagent (with refrigeration) no deterioration or loss in sensitivity was observed, even after five days.

The results indicate that the citric acid-bromomaleic acid system is specific to tertiary aliphatic amines. Complexation of nicotine, therefore, occurred at the N-methylpyrrolidine nitrogen as opposed to the pyridine nitrogen. Primary and secondary amines did not yield a color; e.g., alkaloids, such as anabasine and nornicotine, did not interfere. The method as presented is an excellent procedure for the qualitation and quantitation of any tertiary amine in the presence of any nonreacting nitrogenous compounds. Its high specificity is important since it can complement the *Hinsberg* method and other procedures which distinguish between primary, secondary, and tertiary amines.

SUMMARY

It was found that a system consisting of citric acid and bromomaleic acid each dissolved in acetic anhydride may be used to quantitatively determine nicotine, colorimetrically, in a 2-propanol extract of smoke particulate matter.

The colored complex between nicotine and the above reagents formed in 25 minutes (at 30° C) and had a λ_{max} at 505 nanometers. With nicotine, complexation occurred preferentially at the N-methylpyrrolidine nitrogen as opposed to the pyridine nitrogen. Out of the classes of compounds that were tested, which included primary, secondary and aliphatic and aromatic amines, oximes and amides, only tertiary aliphatic amines yielded a red colored complex. The coefficient of variation at the 2-sigma level was 11 %.

ZUSAMMENFASSUNG

Es wurde gefunden, daß es mit Hilfe eines aus Zitronensäure und Brommaleinsäure (jeweils in Essigsäureanhydrid gelöst) bestehenden Systems möglich ist, Nikotin kolorimetrisch in einem 2-Propanol-Extrakt der Partikelphase des Rauches quantitativ zu bestimmen.
Der gefärbte Komplex aus Nikotin und den oben erwähnten Reagenzien entsteht innerhalb von 25 Minuten (bei 30° C) und hat ein Absorptionsmaximum bei 505 nm. Beim Nikotin vollzog sich die Komplexbildung vorzugsweise am N-Methylpyrrolidinstickstoff im Gegensatz zum Pyridinstickstoff. In der Reihe der untersuchten Verbindungsgruppen, zu der primäre, sekundäre sowie aliphatische und aromatische Amine, Oxime und Amide gehörten, ergaben lediglich tertiäre aliphatische Amine einen roten Komplex. Bei einer doppelten Standardabweichung ($\sigma = 2$) belief sich der Variationskoeffizient auf 11 %.

RESUME

On a trouvé une méthode permettant la détermination quantitative de la nicotine à l'aide d'acide citrique et bromomaléique dissous séparément dans l'anhydride acétique, et de façon colorimétrique dans un extrait au 2-propanol de matière particulaire de fumée.
Le complexe coloré obtenu après 25 minutes à 30 °C par la nicotine et les réactifs mentionnés démontre un λ max à 505 nanomètres. La formation de complexe avec la nicotine a lieu de préférence avec le N-méthylpyrrolidine azoté plutôt qu'avec le pyridine azoté. Parmi les groupes de composés testés, tels que les amines primaires, secondaires, aliphatiques et aromatiques, les oximes et les amides, seules les amines aliphatiques tertiaires donnent un complexe rouge. Le coefficient de variation au niveau sigma-2 est de 11 %.

REFERENCES

1. Pillsbury, H. C., C. C. Bright, K. J. O'Conner, and F. W. Irish: J. Ass. Offic. Anal. Chem. 52 (1969) 458.
2. Ogg, C. L., and E. F. Schultz: J. Ass. Offic. Anal. Chem. 53 (1970) 659.
3. Willits, C. O., M. L. Swain, J. A. Connelly, and B. A. Brice: Anal. Chem. 22 (1950) 430.
4. Griffith, R. B.: Tobacco Science 1 (1957) 130.
5. Harvey, W. R., C. E. Badgett, and F. E. Resnik: Tobacco Science 11 (1967) 84.
6. Harvey, W. R., H. M. Stahr, and W. C. Smith: Tobacco Science 13 (1969) 13.
7. König, W.: J. Prakt. Chem. N. F. 69 (1904) 105.
8. Harvey, W. R., R. W. Hale, and R. M. Ikeda: Tobacco Science 14 (1970) 141.
9. Charles, J. L., H. M. Stahr, and R. M. Ikeda: Tobacco Science 13 (1969) 54.

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