STUDIES ON THE NICOTINE CONTENT OF CIGARETTE SMOKE [1]

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INTRODUCTION

Nicotine has been praised and condemned ever since it was first isolated from tobacco by Posselt and Reimann as cited by Pictet (19a, *p. 159*)[2] in 1828. Both praise and condemnation have had little factual basis, however, for the physiological action of nicotine in tobacco smoke is still a controversial subject. Before investigators can establish the effect of inhaling nicotine in smoke, they must know how much of the alkaloid is present. Many conflicting statements are found on this subject.

A review of the literature shows that several factors may cause a fluctuation in the nicotine content of tobacco smoke, among which are the moisture content of the tobacco, the rate of smoking, the quantity left unburned, and the nicotine content of the tobacco itself.

In order to study these factors a satisfactory method for the determination of nicotine in smoke must be employed. The existing methods were therefore reviewed.

METHODS FOR DETERMINING NICOTINE IN TOBACCO AND TOBACCO SMOKE

A method for the determination of nicotine in tobacco smoke must have two particular qualifications. As in the case of tobacco itself, the method must not include the estimation of ammonia, pyridine and its derivatives, or other basic substances as nicotine. It is particularly important that ammonia be separated quantitatively from the nicotine, as shown by the data of Haley, Jensen, and Olson (*8*) [2] on cigar smoke. In fact, the ammonia content of smoke equals or exceeds the nicotine content in some cases.[3] Schaarschmidt (*20*) has shown that pyridine is present in much smaller amounts.

A second qualification which the method must have is the ability to measure accurately small amounts of nicotine.

Picric acid reacts with nicotine to give the yellow amorphous dipicrate which gradually changes to the crystalline form. Pfyl and Schmitt (*19*) have used this reaction as the basis of a method for the determination of nicotine. This method with modifications was used by several German investigators (*2, 6, 12, 18, 26*), in the analysis of both tobacco and tobacco smoke. Pfyl and Schmitt state that the picric acid method is excellent for the determination of nicotine in tobacco smoke because ammonia, pyridine, and other bases do not interfere. But Koperina (*12*) states that nitrogen compounds do

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[2] Reference is made by number (italic) to Literature Cited, p. 274.

[3] JENSEN, C. O. THE CHEMISTRY OF TOBACCO SMOKE. I. THE STUDY OF THE NICOTINE AND AMMONIA CONTENT OF CIGAR SMOKE. Thesis, M. S., Pa. State Col. 1930.

interfere in this determination. In a comparison of the Pfyl and Schmitt method (*19*) with Baggesgaard-Rasmussen's silicotungstic acid method (*1*), Heiduschka and Muth (*9*) showed that the two methods gave practically identical results.

At the present time Chapin's silicotungstic acid method (*7*) or modifications of it are widely used for nicotine determinations (*3, 23, 4, 11, 16, 22, 23*).

Any method in which silicotungstic acid is used as the precipitating agent solves the question of ammonia separation because ammonium silicotungstate is soluble in water (*7*). Pyridine is more likely to interfere in the precipitation of nicotine (*7, 14, 17*). However, Mach and Sindlinger (*14*) state that nicotine can be separated from pyridine in cold 0.5 percent hydrochloric acid solution, if the solution is made so dilute that no pyridine is precipitated. In this case only a very small amount of nicotine escapes precipitation. Baumberger (*3*) has stated that the nicotine content of smoke can be determined by Chapin's method without pyridine interference. Wenusch (*25*) used this method on cigar smoke and found that the results obtained were too high. On diluting the solutions to 10 times their original volume before precipitation, the results were the same as those obtained on the polarimeter. It was possible for Wenusch to determine nicotine by the polariscope for he used the smoke from 25 cigars for one determination.

Because of the possible erroneous results that might be obtained if too much pyridine is present, a series of determinations were made to find the concentration at which pyridine does not interfere with the preceipitation of nicotine in dilute solutions.

Samples containing nicotine and added amounts of pyridine were treated as outlined by Chapin (*7*). One hundred cubic centimeters of a solution of nicotine and pyridine was placed in a Kjeldahl flask, made alkaline with sodium hydroxide, and steam-distilled. The solutions obtained were made up to 500 cc, and four 100-cc aliquots were taken. The first aliquot was not diluted but the other three were diluted 1–1, 1–2, and 1–3 with distilled water, acidified with HCl, before precipitation with 5 cc of 12 percent silicotungstic acid. The precipitates were filtered through weighed Gooch crucibles, dried for 3 hours at 125° C., and weighed. The results are given in table 1.

TABLE 1.—*Determination of nicotine as influenced by various concentrations of pyridine*

Solution analyzed	Volume of solution precipitated	Dilution	Concentration of added pyridine	Concentration of nicotine	Weight of precipitate	Nicotine calculated on basis of original sample
	cc		*Percent*	*Percent*	*Grams*	*Percent*
0.060 g nicotine in 100 cc, no pyridine	100	None.	0	0.012	0.1164	0.059
	100	None.	0	.012	.1168	.059
	200	1–1	0	.006	.1141	.058
	300	1–2	0	.004	.1105	.056
	500	1–4	0	.002	.1070	.054
0.060 g nicotine and 0.20 pyridine in 100 cc	100	None.	.04	.002	.1649	.083
	200	1–1	.02	.006	.1188	.060
	300	1–2	.013	.004	.1104	.056
	500	1–4	.008	.002	.1075	.054
0.060 g nicotine and 0.060 g pyridine in 100 cc	100	None.	.12	.012	.5666	.286
	200	1–1	.06	.006	.3014	.153
	300	1–2	.04	.004	.1649	.083
	500	1–4	.024	.002	.1052	.053

These results show that in solutions containing less than 0.02 percent of pyridine, there is little interference in the estimation of nicotine. However, it is also evident that as the solutions are diluted a small amount of nicotine escapes precipitation. In order, therefore, to be certain that pyridine does not interfere with the determination of nicotine, the solutions must be diluted by trial until there is no sharp drop in the weight of the precipitate.

Solutions obtained from the absorption of cigarette smoke were so treated, and there was no significant difference between the weight of the precipitates from the diluted and the undiluted samples. This agrees with the results of Baumberger (*3*) and Schaarschmidt and his coworkers (*20*). Furthermore, after 18 hours the precipitates were crystalline and settled out rapidly after stirring, which is not the case when more than 0.02 percent of pyridine is present.

Since ammonia did not interfere with the results of Chapin's silicotungstic acid method (*7*) and pyridine did not interfere under the conditions of the writers' tests with rather dilute smoke solutions, this method was used for the present work.

APPARATUS AND PROCEDURE

The machine shown in figure 1 was devised to smoke cigars, cigarettes or pipes with a constant length and strength of puff at the same intervals.

The cigarette *a* is held in a small calcium chloride tube *b* and the smoke is absorbed in the gas-absorption tubes *c*, *d*, *e*, and *f*. The pump *p* creates a partial vacuum in the flask *j*, and this puffs the cigarette whenever the slowly rotating valve *h* is open.

The reducing gear *g* is connected by a short piece of garden hose to the valve *h*. The reducing gear is turned by a synchronous motor *i*. In order to prevent an increase of the vacuum in *j* as measured by the water manometer *m*, air is allowed to bubble through *l* into the bottle *k* which contains oil. The amount of vacuum can be regulated by the height of the oil in *k* and also partly by the valve *n*. The calcium chloride tower *o* is used to prevent water vapor from entering the pump *p*.

Cigarettes were stored for at least 2 weeks in containers of known humidity so that the moisture content could be controlled. Five cigarettes were weighed to the nearest hundredth of a gram and smoked with a "puff" 1.6 seconds long with an interval of 6.1 seconds between puffs. The difference between atmospheric pressure and the pressure in the vacuum bottle was 15.25 inches of water, unless otherwise stated. This allowed 20 cc of air, as measured by a gas meter, to pass through the cigarette with every puff. The smoke was collected in a train of four gas-absorption tubes each containing 12.5 cc of chloroform and 12.5 cc of 0.1 N sulphuric acid.

After the smoke from the five cigarettes had been collected the chloroform-acid solution was placed in a separatory funnel together with the chloroform and water washings from the cigarette holder and the absorption tubes. The liquid was agitated and the lower chloroform layer was removed and discarded, since nicotine sulphate is insoluble in chloroform. The acid solution containing the nicotine sulphate was placed in a Kjeldahl flask and steam-distilled after the addition of 35 cc of sodium hydroxide and a few pieces of porous plate.

The volume of liquid at this point was about 300 to 400 cc. In order to keep the volume of the solution from increasing the flask was heated gently. The distillate was collected in water acidified with 15 cc of dilute HCl (1–4) until 500 cc had distilled over. After the distillation was completed the nicotine was precipitated with 5 cc of 12 percent silicotungstic acid, stirred well, and allowed to stand overnight. It was then filtered through a weighed Gooch crucible, heated in an oven at 125°C. for 3 hours, and weighed. The weight of the precipitate multiplied by the factor 0.1012 gave the weight of nicotine. This was divided by the weight of tobacco smoked to get the weight of nicotine secured from 1 g of tobacco. The data given in this paper are averages of two or more results.

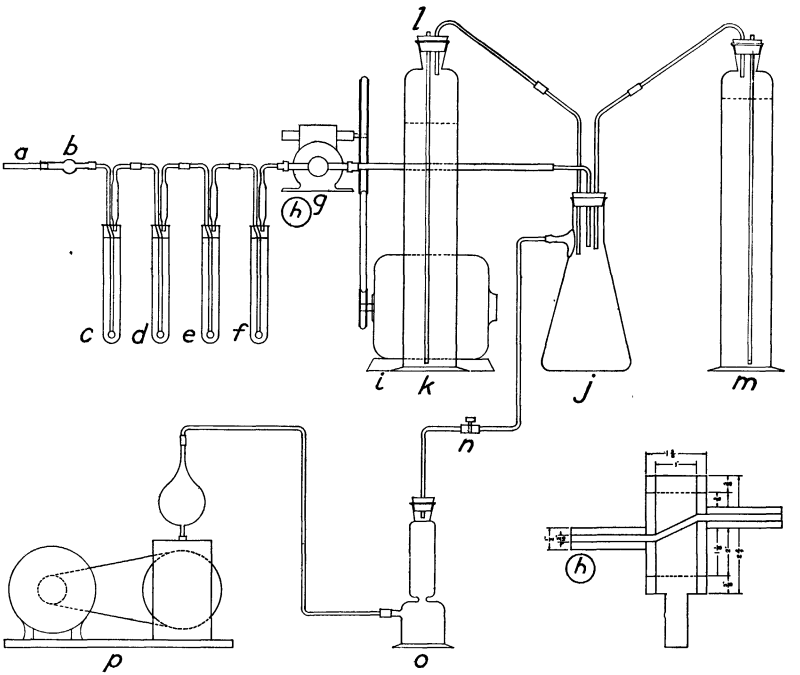

FIGURE 1.—Apparatus used to smoke cigars, cigarettes, or pipes with constant length and strength of puff at same time intervals: *a*, Cigarette; *b*, small calcium chloride tube used as cigarette holder; *c-f*, glass-stoppered gas-absorption tubes, 1 by 8 inches; *g*, reducing gear, 48 to 1, with 8-inch pulley; *h*, brass valve; *i*, synchronous motor, one-sixteenth horsepower, 1,200 revolutions per minute; *j*, 2-liter suction flask; *k*, tall (24-inch) bottle containing motor oil, S. A. E. 30, to a height of 17 inches; *l*, 3.5-mm glass air-inlet tube; *m*, tall (24-inch) bottle used as a water manometer; *n*, Hoke needle valve from oxygen tank; *o* calcium chloride tower; *p*, high-vacuum pump.

The experimental conditions did not of course duplicate actual cigarette smoking conditions.

NICOTINE CONTENT OF CIGARETTE SMOKE AS AFFECTED BY THE MOISTURE CONTENT OF THE TOBACCO

In 1923 Heinz (*10*) stated that smoke from a moist cigar contained from 50 to 75 percent more nicotine than smoke from a dry cigar. But Schöller's results (*21*) in 1928 showed practically no difference between the nicotine content of the smoke of dry and moist cigars.

Winterstein and Aronson (*29*) published results in 1929 which differed from the findings of both of these workers. They state that from a dry cigarette 30 percent more nicotine goes into the mouth of the smoker than from a moist one. The water content of the dry tobacco was 4.8 percent and that of the moist tobacco was 16.5 percent. Waser and Stähli (*24*) and Molinari (*15*) also found more nicotine in the smoke from dry cigarettes than from moist ones. Results which differed from any yet reported were given by Kovalenko (*13*) in 1931, who stated that an increase in the moisture content of cigarettes from 9 to 11 percent increases the nicotine content of the smoke but a further increase in the moisture content decreases the nicotine in the smoke.

EXPERIMENTS

Cigarettes were stored for a period of at least 2 weeks in desiccators having known humidities. The desired relative humidities were obtained from the data of Wilson (*27*) on humidity control by means of sulphuric-acid solution.

The moisture content of the cigarettes was determined by drying in a vacuum over concentrated sulphuric acid for 2 weeks. The procedure described in the previous section was used to determine the nicotine content of the smoke from these cigarettes. The results are shown in table 2.

TABLE 2.—*Nicotine content of cigarette smoke as affected by the moisture content of the tobacco*

Moisture content of cigarette (percent)	Nicotine found in smoke per gram of dry tobacco	Nicotine found in the smoke as compared to nicotine in the original tobacco	Moisture content of cigarette (percent)	Nicotine found in smoke per gram of dry tobacco	Nicotine found in the smoke as compared to nicotine in the original tobacco
	Milligrams	*Percent*		*Milligrams*	*Percent*
0.0	9.2	42.4	11.10	5.7	26.3
3.99	7.8	35.9	14.34	5.1	23.5
6.43	7.3	33.6	24.44	4.6	21.2
9.22	6.5	30.0			

These results clearly show that an increase in the moisture content of cigarettes decreases the nicotine content of the smoke. Contrary to the findings of Kovalenko (*13*), the nicotine found in the smoke does not increase with the moisture content from 9 to 11 percent.

NICOTINE CONTENT OF CIGARETTE SMOKE AS AFFECTED BY THE STRENGTH OF PUFF

Bogen (*5*), Wenusch (*26*), and Kovalenko (*13*) all state that the nicotine content of cigarette smoke increases with the rate of smoking.

EXPERIMENTS

Cigarettes stored at four different humidities were smoked under conditions identical with those reported in the previous section, except that the vacuum used was equal to 14 inches of water instead of 15.25 inches. This allowed 16 cc of air to pass through the cigarette at

each puff instead of 20 cc as in the previous series. The results are given in table 3.

TABLE 3.—*Nicotine content of cigarette smoke as affected by the strength of puff, cigarettes of different moisture content being used* [1]

Moisture content of cigarette (percent)	Nicotine found in the smoke per gram of dry tobacco		Nicotine found in the smoke as compared to nicotine in the original tobacco	
	16 cc of air puff	20 cc of air puff	16 cc of air puff	20 cc of air puff
	Milligrams	*Milligrams*	*Percent*	*Percent*
0.0	8.1	9.2	37.3	42.4
3.99	7.0	7.8	32.3	35.9
6.43	6.6	7.3	30.4	33.6
31.5	2.3	----------	10.6	----------

[1] In these trials 16 cc of air passed through the cigarette at each puff instead of 20 cc as in series reported in table 2.

The results show that an increase in the volume of air going through the cigarette at each puff increases the amount of nicotine in the smoke. This agrees with the findings of others (*5*, *13*, *26*).

NICOTINE CONTENT OF CIGARETTE SMOKE AS AFFECTED BY THE LENGTH OF BUTT

Heiduschka and Muth (*9*) determined the amount of nicotine in the smoke when four-fifths of a cigarette was moked. Using a 4-second puff at 6-second intervals, they found an average of 0.19 percent of the weight of the cigarettes as nicotine in the smoke. The original nicotine content of the cigarettes used was 1.19 percent.

EXPERIMENTS

Cigarettes of 7-cm length with a nicotine content of 2.17 percent and a moisture content of 11.1 percent were smoked until butts of 1-, 2-, or 3-cm lengths remained. The cigarettes were given a light coating of paraffin near the end and inserted in a warm glass tube which was only slightly larger than the cigarettes. Only the part to be burned remained outside the tube. The paraffin solidified on cooling, forming an air-tight joint. The cigarettes were smoked until the burning zone reached the glass tube. The results are given in table 4.

TABLE 4.—*Nicotine content of cigarette smoke as affected by the length of butt*

Length of butt (length of unsmoked cigarettes, 7 cm) (centimeters)	Fraction of cigarette smoked (F)	Amount of nicotine in smoke per cigarette whose dry weight equals 1 g (A)	Nicotine found in the smoke as compared to nicotine in the original tobacco	Nicotine condensed in butt $\left(\frac{5.7F-A}{5.7F}\times 100\right)$
		Milligrams	*Percent*	*Percent*
3	4/7	1.3	6.0	60.1
2	5/7	2.5	11.5	38.6
1	6/7	3.8	17.5	22.3
0	7/7	5.7	26.3	0.0

The percentage of nicotine condensed in the butt was calculated from the difference between the amount of nicotine actually found and the nicotine which theoretically should have been found in the smoke $\left(\frac{5.7\ F-A}{5.7\ F}\times 100\right)$.

The condensation of nicotine in the unburned tobacco is a very important factor governing the amount of the alkaloid that the smoker will receive. Under the conditions of this experiment the condensation amounts to 60 percent of the nicotine which ordinarily appears in the smoke, when the length of unburned cigarette is 3 cm or three-sevenths of the original product. With a length of butt of 1 cm, only 22 percent of the nicotine which ordinarily appears in the smoke was held in the unburned portion. The actual amount of nicotine found in the smoke of 1 cigarette burned to a length of 1 cm will be approximately equal to the nicotine found in the smoke from 3 cigarettes whose butts are 3 cm in length. One cigarette smoked to a 2-cm butt will have as much nicotine in its smoke as 2 cigarettes smoked to a length of 3 cm. Although the above exact relationships will hold true only under the conditions of this experiment, it is apparent that the length of butt is one of the most important factors governing the nicotine content of cigarette smoke.

THE NICOTINE CONTENT OF THE "SIDE STREAM"

Bogen (*5*) states that the side stream ordinarily constitutes the greater part of the smoke as shown by the carbon-dioxide content. Winterstein and Aronson (*28*) measured the nicotine and reported that 43 to 62 percent of the total nicotine goes into the side stream.

EXPERIMENTS

Cigarettes were placed in the machine and smoked as usual, but instead of allowing the side stream to escape, it was trapped and passed through four gas-absorption tubes. In order to trap the side stream the burning cigarette was placed in a bulb with two small openings at opposite sides. Through one of these openings the cigarette was placed. This opening had a diameter 1 cm greater than that of the cigarette holder, which allowed air to enter the bulb at such a rate that none of the smoke was lost. The air and smoke were pulled through the other opening into the gas-absorption tubes by means of a continuously running water pump.

The results are given in table 5.

TABLE 5.—*Nicotine content of the side stream smoke as modified by the moisture content of the cigarette*

Moisture content of cigarette (percent)	Main stream (amount of nicotine as compared to the nicotine in tobacco)	Side stream (amount of nicotine as compared to the nicotine in tobacco	Amount of nicotine in tobacco not found in smoke
	Percent	*Per cent*	*Percent*
11.13	26.3	31.8	41.9
0.0	42.4	28.6	29.0

Contrary to the results of the study of the relation between the moisture content of the tobacco and the amount of nicotine in the main stream, the side stream shows a decrease of nicotine when the moisture content decreases. This may be partly explained by the fact that dry cigarettes burn more quickly than moist ones. Only 8 puffs are required to burn 1 cm of the dry cigarettes, while 15 puffs are required for those with a moisture content of 11.13 percent. In the more rapidly burning dry cigarettes there is evidently greater distillation and less destruction of the nicotine. This accounts for the increased amounts of the alkaloid in the main stream. But the rate at which the tobacco burns when air is not passing through the dry cigarette may not be increased to the extent that it is when air is drawn through it. Thus the shortened time of burning will cause a smaller amount of nicotine to be found in the side stream. A study of the carbon-dioxide content of the two streams of smoke and the temperature of the burning zone might help to explain the foregoing facts.

SUMMARY

A study of methods of determining nicotine, applicable to cigarette-smoke solutions, has shown (1) that pyridine does not interfere in the precipitation of nicotine by silicotungstic acid in concentrations below 0.02 percent, and (2) that the concentration of pyridine in cigarette-smoke solutions is not high enough to interfere with the precipitation of nicotine by silicotungstic acid.

A machine is described which will smoke cigarettes, cigars, or pipes with puffs of constant volume and unvarying length at constant intervals.

The nicotine content of cigarette smoke varies inversely as the moisture content of the cigarettes.

The amount of nicotine in the smoke is directly proportional to the strength of the puff.

There is a marked condensation of nicotine in the short unburned portion of a cigarette.

Under the conditions of these experiments cigarettes with a moisture content of 11.13 percent contained more nicotine in the side stream than in the main stream; cigarettes with a moisture content of 0 contained less nicotine in the side stream than in the main stream.

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