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General Director's Word

In the name of Allah The Most Gracious The Most Merciful

Praise be to Allah and peace and Blessings be upon the Prophet Muhammed

Sirte University Scientific Journal is considered to be an academic, refereed that publishes in various areas of humanities and applied Sciences.

As scientific research has become a primary measure that keeps universities and research centers presence and lifting their scientific credit. For that this journal is an important indicator of scientific products as of quality, and quantity, also presents the contributions of researchers from the faculties members and graduate students within or from outside the university to publish their research and achievements.

The release of this issue is to be what can be done towards our community, in order to actively contribute to their services, and generally to present basic research devoted to serve science and knowledge.



I am pleased to present this second issuance of the fifth volume. I hope that the editorial board has succeed in the right selection and the publication that fulfills the journal terms. I call upon all researchers to participate, as we would be also willing to receive any feedback about it.

Lastly, I extend all my thanks and appreciation to the editorial board, and who are charged with the issuing, also extend thanks to all researchers, reviewers and those who contributed to this work.

May Pease be Upon You

The General Director

Editor in Chief's Word

In the name of Allah The Most Gracious The Most Merciful

It is my pleasure and on behalf of my colleagues, I would like to extend my sincere gratitude to Sirte University, the journal authority, for their trust and confidence in us to do such an important task, I also appreciate the efforts make by all parties in the university in making this journal continue going forward. I also wish to express my appreciation to the scholars, who have contributed with their research papers in the second issuance of the fifth volume, peer reviewers who helped us to select the valuable papers, and of course, to our dear respected readers.

I would like to take this opportunity to call upon all colleagues, researchers to submit their best work for publication in the next editions of the *SUSJ*, according to the journal's rules of publication which are available on the University Research Center website (www.su.edu.ly/rcc).

We look forward to your most serious and effective efforts, which aspire members of the editorial board and its general director, also aspire to the university in the dissemination of scientific research in all fields of human and applied sciences.



It is again my pleasure to offer my thanks to the President of the University for his encouragement to researchers, and editorial board.

Lastly, although every effort has been made to make this journal errors-free and as scientific as possible, no work is perfect and this journal is not an exception. Therefore, we appreciate it if you could give us your feedback, suggestions and ideas in order to improve the next issues.

May Pease be Upon You

Editor in Chief

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Spectrophotometric Determination of Nicotine in Cigarette Tobacco in Libyan Market Using Iron (III) and Potassium Ferricyanide

Abstract

A new simple, accurate, sensitive and economical procedure for the estimation of nicotine is described. In the presence of potassium ferricyanide, it has been demonstrated that Fe^{3+} is reduced to Fe^{2+} by nicotine in acidic medium. In addition, soluble Prussian blue ($\text{KFe}[\text{Fe}(\text{CN})_6]$) was produced by the reaction between the formed Fe^{2+} and potassium ferricyanide. The absorbance of soluble Prussian blue is measured at the absorption maximum of 736 nm. Beer's law is obeyed in the concentration range 0.1-4.4 $\mu\text{g}/\text{ml}$. The molar absorptivity is $3.05 \times 10^4 \text{ l}/\text{mol}\cdot\text{cm}$. Sandell sensitivity is 5.32 ng/cm^2 . The limits of detection as well as quantification are reported. Six replicate analyses ($n=6$) of solutions containing three different concentrations of nicotine was carried out. The percent error and the RSD values have been reported. The proposed method was applied to the determination of nicotine in cigarette tobacco present in Libya and the results demonstrate that the method is equally accurate and precise as found from the t - and F -values. The reliability of the method was established by recovery studies using standard-addition technique.

Keywords: Nicotine; Spectrophotometry; Oxidation reaction; Ferric chloride; Potassium ferricyanide; Cigarette tobacco in Libyan markets.

1. Introduction

Nicotine (pyridine, 3-(1-methyl-2-pyrrolidiny), (S) is one of the highly toxic tobacco alkaloids present in tobacco leaves and cigarette smoke ^[1]. Nicotine appears to be a promising tracer for environmental tobacco smoke (ETS) because of its specificity for tobacco ^[2]. It is also a systemic and contact insecticide and is also used as a drug and in

chemical ^[3]. The threshold limit value reported for nicotine is 0.05 mg/m³ ^[1]. The most important symptoms of exposure to nicotine are bronchitis, emphysema, cyanosis, and exenation of the central nervous system. Excessive smoking has been implicated in lung cancer, bladder cancer, and cancer of the larynx and oesophagus ^[4].

The determination of total nicotine alkaloids is of particular importance to the tobacco industry and in the area of toxicology. Various analytical methods such as GC ^[5], TLC ^[6], HPLC ^[7-9], ASS ^[10], capillary electrophoresis ^[11], radio immuno assay ^[12], GC-MS ^[13] and circular dichroism spectropolarimetry ^[14] have been reported. Most spectrophotometric methods reported are based on cleavage of the pyridine ring with cyanogen bromide or chloride and condensation of the resulting compound with *p*-amino benzoic acid ^[15] and diethyl thiobarbituric acid ^[16] and other spectrophotometric methods ^[17-24], determination of outdoor Tobacco smoke exposure by distance From a smoking source ^[25], an electrochemically ^[26].

Many of these methods involve a prolonged extraction procedure prior to the determination of nicotine and the use of highly toxic cyanide ^[15, 16]. These methods were sophisticated to perform and/or time consuming. Potassium ferricyanide-Fe (III) which is has applied in the determination of some drugs ^[27-31].

2. Experimental

Apparatus

All the spectral measurement were made using double-beam UV/Vis spectrophotometer (Biotech Engineering Ltd., UK), with wavelength range 190 –1100 nm, spectral bandwidth 2.0 nm, with scanning speed 400 nm/min, equipped with 10 mm matched quartz cells. A thermostat water bath, Buchi 461 water bath, Schwiz (France) was used to carry out the temperature studies and Magnetic Mix. 100, Thermo Scientific, UK.

Materials and reagents

- All chemicals used were of analytical reagent grade of the best available quality. and all solutions were freshly prepared in doubly distilled water.
- Standard nicotine solution (Merck) was prepared by dissolving 0.01 g of pure nicotine

in 50 ml of bi-distilled water and complete to 100 ml with bi-distilled water to obtain the working standard solution of 100 µg/ml.

- Aqueous solutions of 0.2 % Fe (III) chloride was purchased from Merck (Darmstadt, Germany) was prepared immediately before use by dissolving 0.2g salt in an appropriate weight and completed to 100 ml water.
- Aqueous solutions of (0.2 %) potassium ferricyanide [$K_3Fe(CN)_6$] was purchased from Merck (Darmstadt, Germany) was prepared immediately before use (freshly prepared) by dissolving an appropriate weight and completed to 100 ml bi-distilled water.
- A solution of 10.0 M H_2SO_4 , was prepared by adding exact volume from stock (98%)concentrated acid to bi-distilled water in 500 ml measuring flask, and standardized as recorded ^[32].

General procedure

Up to 0.44 ml of the standard or sample solution was transferred into a 10.0 ml calibrated flask. A 1.4 ml of 0.2 % $FeCl_3$ and 1.1 ml of 10.0 M sulphoric acid was added and warmed in a thermostat water bath at $35 \pm 1^\circ C$ for 2.0 min subsequent interaction of produced iron (II) with 1.6 ml of 0.2 % potassium ferricyanide, to form Prussian blue ^[33]. The volume was completed to 10.0 ml with bi-distilled water, which measurable at λ_{max} 736 nm, against acidic Fe (III)-ferricyanide similarly prepared as a blank.

Extraction and determination of nicotine in cigarette tobacco ^[34]

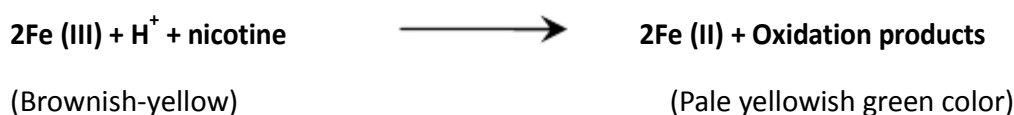
Weigh 10 g of cigarettes leaves in beaker. Add 100 ml NaOH solution and stir very well for 15 min, filter in Buchner using glass wool and press the cigarettes very well by using other beaker, transfer the cigarettes again to beaker, add 30 ml distilled water and stir and filter again, collect the filtrate together, (if there is any impurities re-filter), transfer the filtrate to the separating funnel and extract by 25 ml ether, repeat the extraction 3 times, gather the 4 filtrates in conical flask, dry by using 1.0 teaspoon anhydrous potassium carbonate, filter. evaporate ether on water bath, (avoid extra heat because nicotine is hydrolyzed by extreme heating), after evaporation of ether add 4.0 ml methanol to dissolve the resulted oil, to the filtrate 14 ml of 1.0 mol/L HCl was added and the solution was made up to 100 ml with water. An aliquot of this solution was taken and analysed as

described above. Results are shown in Table 2.

3. Results and Discussion

Ferricyanide is the name for the anion $[\text{Fe}(\text{CN})_6]^{3-}$. Its systematic name is hexacyanoferrate (III) ion. The most common salt of this anion is potassium ferricyanide, a red crystalline material that is used as an oxidant in organic chemistry. The method is based on the reduction of iron (III) by the nicotine in acidic medium and subsequent interaction of iron (II) with ferricyanide to form Prussian blue. The product exhibits λ_{max} 735 nm. The color remains constant for at least 24 h. The method involves two steps namely:

- Reduction of nicotine with Fe (III) in acidic medium by heating in water bath $35 \pm 1^\circ\text{C}$ for 2.0 min.



- Determination of Fe (II) by potassium ferricyanide to form deep blue color (Prussian blue) at a suitable λ_{max} .



Reproducibility of the method

The reproducibility of the method was assessed by carrying out seven replicate analyses of a solution containing 3.0 $\mu\text{g}/\text{ml}$ of nicotine in a final solution volume of 25 ml. The standard deviation and relative standard deviation of absorbance values were found to be ± 0.011 and 0.66 %, respectively.

Optimization

The influence of each of the following variables on the reaction was tested.

Effect of Fe (III) concentration

The influence of Fe (III) concentration was studied using different volumes of 0.2% FeCl_3 . The optimum results were obtained with 1.4 ml of 0.2% Fe (III) chloride; higher concentration of Fe (III) not affected absorbance (Fig. 1).

Effect of acid medium

Different types of acids were examined HCl, HClO₄, H₂SO₄, CH₃COOH and HNO₃. The most suitable acid to achieve maximum yield of redox reaction was found to be H₂SO₄. Moreover, various volumes of 10 M acid were found to be 1.1 ml is the optimum volume due to highly concordance results (Fig. 2).

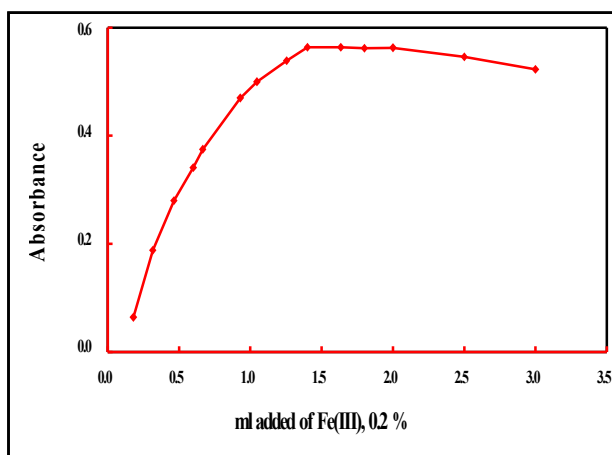


Figure 1. Effect of ml added of 0.2% FeCl₃; nicotine (3.0 µg/ml), 1.6 ml of 0.2% ferricyanide, 1.1 ml of 10 M sulphuric acid, reaction temperature 35 °C; reaction time: 2.0 min

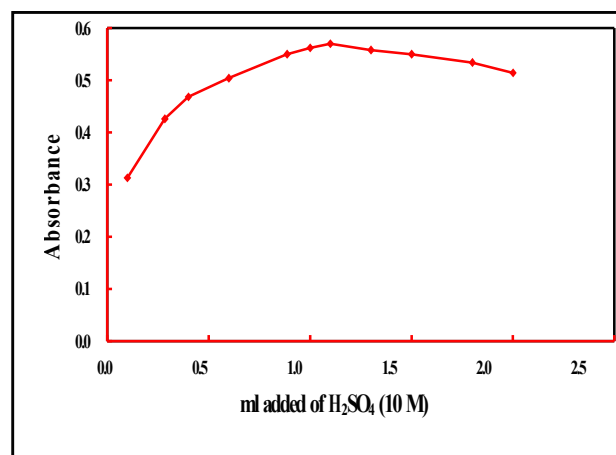


Figure 2. Effect of ml added of H₂SO₄ (10 M) on 3.0 µg/ml nicotine, FeCl₃ (1.4 ml, 0.2 %), K₃[Fe(CN)₆] (1.6 ml, 0.2 %) reaction temperature 35 °C; reaction time: 2.0 min

Effect of potassium ferricyanide concentration

Various amount of 0.2 % ferricyanide was studied in 10 ml calibrated flask. The optimum results were obtained with 1.6 ml of 0.2 % ferricyanide; higher concentration of ferricyanide has no effect on the absorbance, the color remains constant for at least 24 h (Fig. 3).

Effect of temperature and time

The reaction takes place completely after 5.0 min at room temperature 25±1°C. The oxidation process of nicotine with Fe (III) is catalyzed by heating in a thermostat water bath at 35±1°C for 2.0 min, in acid medium and subsequent interaction of produced iron (II) with ferricyanide to form deep blue color.

Effect of sequence of additions

The effect of sequence of additions on the oxidation process of nicotine by measuring the absorbance of solution prepared by different sequence of additions against a blank solution prepared in the same manner. Experiments showed that [(Fe (III)-Acid-Nicotine)-Ferricyanide] gave the best results.

Analytical data

Beer's law was verified up to (0.1-4.4 $\mu\text{g/ml}$) as shown in (Fig. 4) and Ringbom limits, molar absorptivities, Sandell sensitivities, regression equations and correlation coefficients were calculated and recorded in (Table 1). The limits of detection ($K=3$) and quantitation ($K=10$) were established according to IUPAC definitions^[35] are recorded in (Table 1).

In order to determine the accuracy and precision of the methods, solution containing three different concentrations of nicotine were prepared and analyzed in six replicates (Table 2).

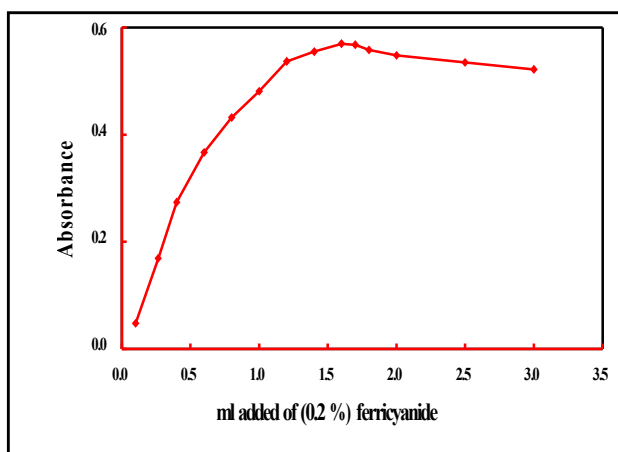


Figure 3. Effect of ml added of 0.2 % ferricyanide; nicotine (3.0 $\mu\text{g/ml}$), 1.4 ml of 0.2% FeCl_3 , 1.1 ml of 10 M sulphuric acid, reaction temperature 35 $^\circ\text{C}$; reaction time: 2.0 min

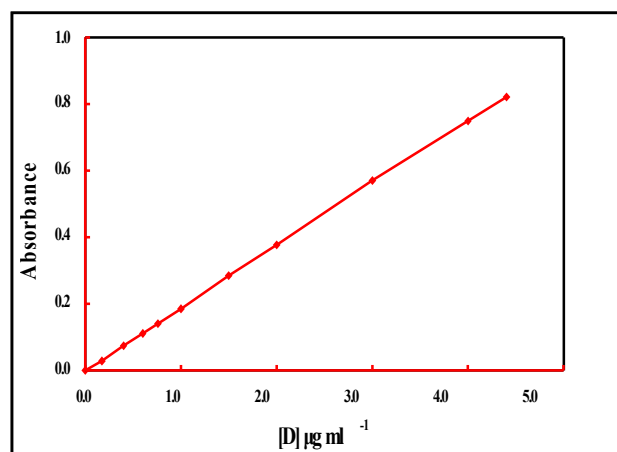


Figure 4. Calibration curve of 3.0 $\mu\text{g/ml}$ nicotine FeCl_3 (0.2 %), 2.0 ml $\text{K}_3[\text{Fe}(\text{CN})_6]$ (0.2 %) 1.5 ml reaction temperature 35 $^\circ\text{C}$; reaction time:

Stability of color

The Prussian blue color formed was stable for at least 24 h under optimum conditions.

Validation method

The proposed method was successfully applied to determine nicotine in cigarettes tobacco. The accuracy of the proposed methods is evaluated by applying standard addition technique, in which

variable amounts of the nicotine were added to the previously analyzed portion of extracted nicotine from cigarettes tobacco. The results are recorded in (Table 3), The results are in good agreement with those obtained by the reported method ^[17-19], by Student's *t-test* (for accuracy), and variance ratio *F-test* (for precision) ^[36], at 95% confidence level as recorded in (Table 4). The results showed that the *t*- and *F*- values were lower than the critical values indicating that there was no significant difference between the proposed and reported methods. The proposed method was more accurate with high recoveries.

Table 1. Optical and regression characteristics of nicotine for the proposed method.

Parameters	Values
λ_{\max} (nm)	736
Stability / h	24
Beer's law limits ($\mu\text{g/ml}$)	0.1 – 4.4
Ringbom limits ($\mu\text{g/ml}$)	0.3 – 4.1
Molar absorptivity ($\text{L mol}^{-1} \text{cm}^{-1}$)	3.05×10^4
Sandell sensitivity (ng/cm^2)	5.32
Detection limits ($\mu\text{g/ml}$)	0.049
Quantitation limits ($\mu\text{g/ml}$)	0.163
Regression equation ^a :	
Slope (b)	0.188
Intercept (a)	9.4×10^{-4}
Correlation coefficient (r)	0.9999
RSD ^b %	0.49

^a $A = (a + bC)$ where C is concentration of nicotine in $\mu\text{g/ml}$ and A is absorbance.

^b Relative standard deviation for six determinations.

Table 2. Evaluation of the accuracy and precision of the proposed procedures for nicotine.

Reagent	Taken $\mu\text{g/ml}$	Found $\mu\text{g/ml}$	Recovery %	RSD ^a %	RE ^b %	Confidence Limits ^c
Ferric –	1.0	1.01	101.0	0.59	1.19	1.02 ± 0.0121
Ferricyanide	2.5	2.48	99.20	0.61	1.27	2.48 ± 0.0317
	4.0	4.01	100.25	0.66	0.81	4.01 ± 0.0324

^a Relative standard deviation for six determinations.

^b Relative error.

^c 95% confidence limits and five degrees of freedom

Table 3. Determination of nicotine in cigarette tobacco in Libyan markets using standard addition technique.

Cigarette Type	Taken µg/ml	Proposed method		
		Added µg/ml	Found* µg/ml	Recovery %
American Legend ^a	1.0	0.0	1.02	102.00
		1.0	2.01	100.50
		2.0	2.98	99.33
		3.0	4.01	100.25
Marlloborro ^b	1.0	0.0	0.99	99.00
		1.0	2.02	101.00
		2.0	2.97	99.00
		3.0	3.98	99.50

* Average of six determinations.

^a Cigarette smoke, made in EU, under the authority of the Trade mark Owners, by KTG, INC.

^b Cigarette smoke, Karelia light, Karelia tobacco company INC, made in Greece, nicotine content 0.7 mg.

Table 4. Determination of nicotine in cigarette tobacco in Libyan markets and using the proposed and reported methods.

Samples	Proposed method			Reported method ^[17]
	Recovery %	t-test	F-value	
American Legend ^a	99.87	1.22	2.44	95.57
Marlloborro ^b	100.08	0.89	1.69	99.70

Theoretical value for t- and F- values for five degrees of freedom and 95% confidence limits are 2.57 and 5.05, respectively.

^a Cigarette smoke, made in EU, under the authority of the Trade mark Owners, by KTG, INC.

^b Cigarette smoke, Karelia light, Karelia tobacco company INC, made in Greece, nicotine content 0.7 mg..

4. Conclusion

The proposed method was advantageous over other reported visible spectrophotometric and colorimetric methods, related to their high reproducibility, high sensitivity, less time

consuming and using simple and inexpensive reagents. Moreover, these methods allowed the determination of nicotine up to 0.1 µg/ml, in “addition to simplicity, rapidity, precision and stability of colored species for more than 24 h at room temperature”.

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