Determination of Cyanide at Trace Levels by Computational Scanning Densitometry

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Abstract:

Background: Cyanide is one of the most commonly present anions in industrial effluents, highly toxic to human and animal life. Therefore, its determination in aqueous media by simple, portable, and quick methods is required.

Objective: This study aims to develop a simple and quick method to determine this anion at the micro level in aqueous media without using any expensive instrument.

Method: The method is based on treating the microliter sample of aqueous cyanide with the classical Lassaigne's reagents on a TLC plate. After heating in an oven for a few minutes, a deep blue spot of ferric ferrocyanide complex appeared on the plate. The color depth of the spots was measured by scanning the TLC plate and analyzing the image with an indigenous software package.
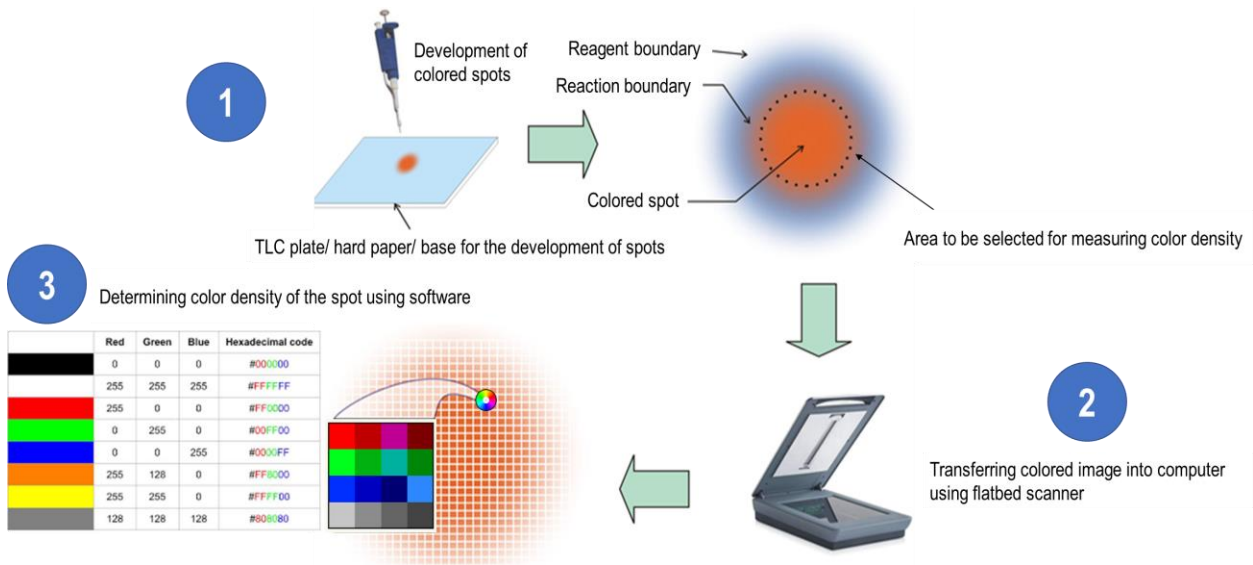
Conclusion: From the comparison of results obtained by the proposed method and standard ion-selective electrode method, it can be concluded that the former method for determining micro quantities of cyanide in aqueous samples using computational densitometry is a simple, accurate, and adequately precise method without the involvement of sophisticated instrumentation.

37   **Graphical Abstract:**

	Red	Green	Blue	Hexadecimal code
	0	0	0	#000000
	255	255	255	#FFFFFF
	255	0	0	#FF0000
	0	255	0	#00FF00
	0	0	255	#0000FF
	255	128	0	#FF8000
	255	255	0	#FFFF00
	128	128	128	#808080

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39   Determination of cyanide at trace levels in aqueous samples using computational
40   scanning densitometry, i.e., developing colored spots of cyanide on support (TLC
41   plate), scanning, and transferring image to a computer for measuring the color den-
42   sity of spots with a software

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Introduction:

Industrial and domestic water pollution is a major global problem for environmental and health authorities. It has been found that some anions in the water can be toxic and harmful to humans and aquatic life. For instance, cyanide, fluoride, and nitrite are harmful agents in the aquatic environment. Industrial units are the primary sources of these toxic agents [1]–[3]. Cyanides in soil and water come from industrial plants like electroplating, metallurgy, mining, organic chemicals, plastics production, and photographic development [4], [5]. Cyanide may enter the air, water, and soil from natural and industrial processes. In the aquatic compartment of the environment, cyanides occur through the action of certain bacteria and algae or human activities [6].

Toxic cyanide compounds include hydrogen, potassium, and sodium cyanides [7]. At pH less than 8, cyanide exists in water as hydrogen cyanide, which is more dangerous to aquatic life than free cyanide [8]. Cyanide can also be found in cyanogenic glycosides in certain plants' seeds, such as apricots, peaches, and plums [9]. Hydrogen cyanide gas is also a chemical warfare weapon [10]. The toxic effect of cyanide is due to its reaction with the trivalent iron in the cytochrome oxidase to inhibit electron transport and thus prevent the cells from consuming oxygen, leading to rapid impairment of vital functions [8]. The half-life of cyanide ion in the body is about 2 hours, followed by its metabolization to a less toxic form and excretion with body fluids [4], [6]. USEPA has set aquatic-life water quality criteria for free cyanide at 22 µg/L acute and 5.2 µg/L chronic in freshwater, 1 µg/L acute and 1 µg/L chronic in saltwater, and health criteria for organisms at 140 µg/L as total cyanide (TCN). The maximum contaminant level (MCL) for drinking water was set at 200 µg/L in 1992 [11].

71   One of the reasons for the presence of cyanide ions in the air is car exhaust

72   and fires [12]. Several cyanide poisonings in fire cases, which led to death, have

73   been reported [12]. Human exposure to cyanide is also associated with tobacco

74   smoking – active and passive. Prolonged exposure to the ETS (Environmental To-

75   bacco Smoke) components, including cyanide, leads to the weakening of the body,

76   many diseases, and even death [12]. Most of the cyanide in surface water will form

77   hydrogen cyanide and evaporate, but the amount of hydrogen cyanide generally

78   formed is not harmful to humans. It is estimated that the threshold of cyanide for

79   humans is 1.5 mg/kg [13], [14]. The primary source of cyanide exposure is drinking

80   water and fruits and vegetables irrigated with contaminated water. Therefore, it is

81   important to monitor the concentrations of cyanides in the aquatic compartments of

82   the environment.

83   Several methods have been developed for determining cyanide concentration,

84   including mass spectrometry, ion chromatography, Raman spectrometry, electro-

85   chemistry, flow injection, fluorescent probe, and colorimetry [1], [15]–[19]. In the

86   present work, spot testing has been used to determine the trace level concentration

87   of cyanide. Despite the availability of sophisticated modern instrumental analytical

88   techniques, spot tests are still frequently used in many areas, including quality con-

89   trol in food and pharmaceutical analysis, environmental and water analysis, forensic

90   science, and screening tests in clinical examination [15], [18]–[20]. Commercial

91   analysis kits based on spot tests are also available to identify and/or quantify sub-

92   stances such as glucose and nitrites [20]. The most frequent method for determining

93   the analyte concentration is measuring the change in the color yielded in the test.

94   Color values and color and luminous intensity of the spots can be obtained by spe-

95   cific apps available for computers and smartphones [20]. The answer is in the form

96　of hex color codes that display what color it is. The values are a unique code repre-

97　senting color values from 0 to 255. If red, green, and blue are all at the minimum 0

98　or "00" in the code, the color expressed is black, whereas red, green, and blue are all

99　at the maximum 255 or "ff" in the code, the color represented is white. Hex colors

100　are based on the RGB color model. The theory behind the model is that you can

101　create virtually any color the eye can see by assigning different combinations of red,

102　green, and blue color values [21]. In the present work, an indigenous computer soft-

103　ware has been used to determine the color value of spots of cyanide with ferrous

104　sulfate reagent. Previously, the so-called "computational scanning densitometry"

105　has been successfully employed for the micro-determination of metals like Fe, Ni,

106　Hg, Pb, and As [21], [22], sulfide [23], and formaldehyde [24].

Experimental:

Materials and Method:

109　Micropipette (Pipettman, 0.2–2 μL) was used for developing the spots on TLC plates

110　(Merck, Aluminum sheets, 20×20 cm, silica gel 60 F254). A reflective flatbed scan-

111　ner (HP-3670) was used to scan and transfer the images of the spots to the computer

112　(Windows XP, Service Pack 2). Visual Basic 6-based computer graphic software

113　program with a circular picture box, selection marquee, and flex grid control was

114　used to measure the color density of the spot. In the reference method, an ion-selec-

115　tive electrode (EDT Instruments and Electrodes) was used to determine cyanide. So-

116　dium cyanide (NaCN), sodium hydroxide (NaOH), ferrous sulfate ($FeSO_4$), and hy-

117　drochloric acid (HCl), all from Merck, and deionized water were used for this work.

Standard Solutions:

Sodium or potassium cyanide (NaCN or KCN) is usually used to prepare stock and standard cyanide solutions, also proposed in the standard EPA method 9014 to determine cyanide in waters and extracts using titrimetric and spectrophotometric procedures. The stock solution of cyanide (1000 ppm) was prepared by dissolving 1.90 g of sodium cyanide in deionized water and diluting the volume to one liter. Then, using this stock solution, 10 to 100 ppm cyanide solutions were prepared in 100 mL flasks by making appropriate dilutions. Solutions of ferrous sulfate (11% to 19%), sodium hydroxide (0.5% to 2.5%), and hydrochloric acid (2% to 10%) were prepared by dissolving the respective compound in the appropriate volume of water in 100 mL flasks. Moreover, three synthetic aqueous samples of different cyanide concentrations were prepared.

Developing Colored Cyanide Spots:

A TLC plate was dipped in a 1% sodium hydroxide solution and dried in a microwave oven for 2 minutes. With the help of a graduated micropipette, 1–µL of each standard cyanide solution was applied to the TLC plate. After drying at ambient temperature, 1–µL of ferrous sulfate solution was applied on each standard solution spot of cyanide, followed by placing the TLC in an oven at 75ºC for 60 seconds. As the spots on the TLC plate turned brown, 1–µL of 10% hydrochloric acid was dropped on each spot. Blue-colored spots of varying color density depending upon cyanide concentration were produced.

Scanning of Spots:

Spots on the TLC plates were scanned at 600 dpi on a flatbed scanner in a reflective mode and transferred to a computer as an image, and color densities of these spots were digitalized with the help of the purposely built software. A linear calibration

143 (10 to 100 ppm) was obtained when the system plotted color density against the

144 cyanide concentration. The concentration of "unknown" synthetic samples was de-

145 termined by finding their color density and fitting it into the calibration line.

Effect of Reagents Concentration:

147 The effects of the concentrations of reagents on the color densities of the spots were

148 investigated by taking the fixed quantity of cyanide solution but varying concentra-

149 tions of the reagents. The concentration of sodium hydroxide varied from 0.5% to

150 2.5%, hydrochloric acid strength was changed from 2% to 10%, and ferrous sulfate

151 concentration was altered from 0.5% to 2.5%. The effect of the concentration of each

152 reagent was investigated by keeping the concentrations of other reagents constant.

Effect of Heat and Time:

154 The effect of heating and reaction time on the color density of the spot was checked

155 by placing the prepared TLC plates for different contact times between reagents and

156 cyanide spots or temperatures in a temperature–controlled oven. Calibration graphs

157 were plotted to find optimum time and temperature conditions where the maximum

158 color density of the spots can be obtained.

Effect of Interfering Ions:

160 Some cations and anions were added to the cyanide spots to check the effect of in-

161 terfering ions. The anions were added as sodium salts, and cations as chlorides. The

162 solutions of ions to be studied were added onto the TLC plate before applying the

163 reagents. Afterward, the spots were developed and scanned using the abovemen-

164 tioned procedure. The color density of each spot was measured and compared with

165 the blank (cyanide without any interfering ion).

Analysis of Real Samples:

Actual aqueous samples containing cyanide were collected from a local electroplating industry outlet daily at the same time for a few days. The industry uses free cyanide in electroplating baths to facilitate anode corrosion, help to maintain a constant metal ion level, and contribute to conductivity. Five samples of the light brown effluent were filtered twice through Whatman filter paper 41 and a 4-inch column of activated carbon. Afterward, the proposed new and reference methods determined cyanide content in the colorless filtrate.

Results and Discussion:

Chemical reactions employed in this method are the same as those in classical Lassaigne's test for detecting nitrogen in organic compounds. As a result of fusion with metallic sodium, carbon and nitrogen of the organic compound combine to form cyanide, which first reacts with Fe(II) to form hexacyanoferrate ion $[Fe(CN)_6]^{4-}$ that further combines with Fe(III) to create a neutral deep blue colored coordination complex, ferric ferrocyanide $Fe_4[Fe(CN)_6]_3$. The compound is a blue pigment with a commercial name, Prussian blue or Paris blue. Different sets of reactions are given in the literature to explain the formation of this complex compound. The addition of sodium hydroxide at the start and hydrochloric acid at the end play an important role in forming the blue-colored ferric ferrocyanide. A little sodium in the form of hydroxide is added to keep the cyanide in solution form. Adding acid at the end at elevated temperature oxidizes Fe(II) to Fe(III) and converts the media to acidic [25]. It is mentioned in the literature that the addition of ferric chloride can also provide Fe(III) ions which are required to form Prussian blue [26].

$$FeSO_4 + 2\ NaCN \rightarrow Fe(CN)_2 + Na_2SO_4$$

$$Fe(CN)_2 + 4\ NaCN \rightarrow Na_4[Fe(CN)_6]$$

191     $3\ Na_4[Fe(CN)_6] + 4\ FeCl_3 \rightarrow Fe_4[Fe(CN)_6]_3 + 12\ NaCl$

192   The technique, computational scanning densitometry, used for determining

193   cyanide in micro quantities in this work, combines spot tests and colorimetry. Spot

194   tests have been used in analytical chemistry for over a hundred years, mainly for

195   detection/ qualitative purposes. No satisfactory method was available for the accu-

196   rate quantification of spots. However, it changed after the development of computers

197   and image analysis software. Our research group has employed an indigenous soft-

198   ware package for micro-determining metals, anions, and organic compounds in care-

199   fully developed colored spots [21], [22]. In the present work, the classical Las-

200   saigne's test reaction was used to develop the colored spots of cyanide in aqueous

201   media, then scanning the spots and calculating their color densities utilizing the soft-

202   ware capable of calculating the red, green, and blue components of the digital image

203   of the spots.

204   A scanned image of the TLC from a scanner digitalizes the colors of the spots.

205   This process converts real-world colors into numeric computer data consisting of

206   rows and columns of pixels. Each pixel will consist of three numeric components,

207   i.e., red, green, and blue. The pixel's color will be one of 16.8 million possible color

208   combinations (256 shades of red, green, and blue each). But any and every pixel is

209   always only one color computationally with a particular value of red-, green-, and

210   blue-component, making spot colors quantitative. A calibration curve (Fig. 1) was

211   obtained for cyanide in the range of 10 to 100 ng by applying the optimum concen-

212   trations of reagents and performing the procedure under optimum conditions (details

213   for optimum conditions are described later).

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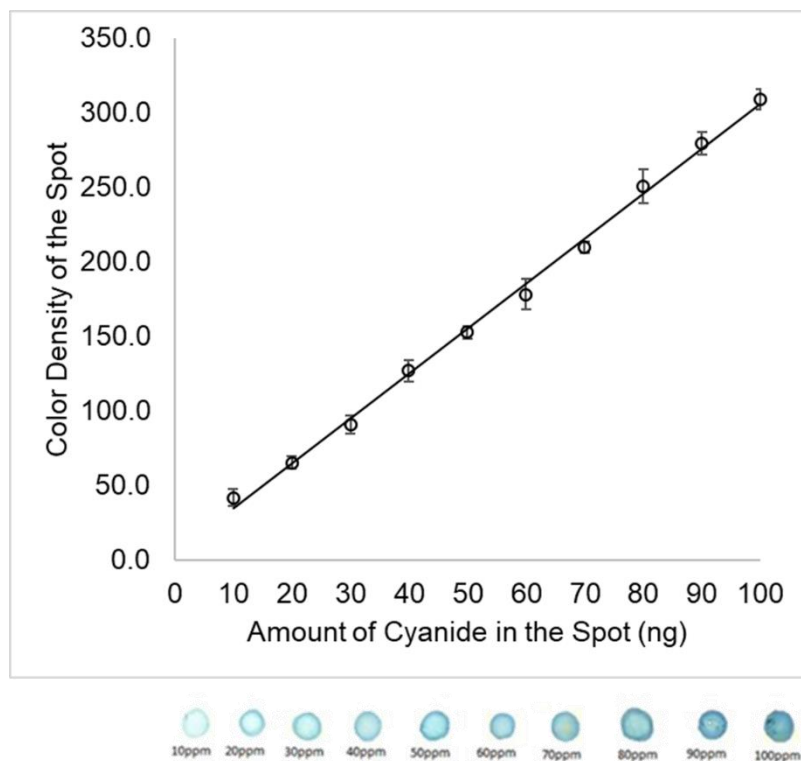
Fig. 1: Calibration line (color density as a function of cyanide concentration in the colored spot on the TLC plate); $y = 3.01\ x + 4.73$; $R^2 = 0.99$

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218 Reaction conditions and procedures to develop Prussian blue spots were opti-
219 mized to get the best results from the minimum amount of cyanide in the spot. As
220 shown in Figs. 2–4, 1.0% NaOH, 17% $FeSO_4$, and 8–10% HCl gave maximum color
221 density to the spots, thus, increasing the sensitivity of the method. The color density
222 decreased if the concentration of any reagent increased or decreased from the opti-
223 mum value. It was further observed that the blue color of spots developed in the first
224 forty seconds and remained stable for the next 30 minutes. Heating the TLC plate
225 before adding the hydrochloric acid in an oven at 75°C seems essential because the
226 color does not fully develop without heating. Probably heating facilitates the oxida-
227 tion of Fe(II) to Fe(III) – the necessary step for developing the blue color.

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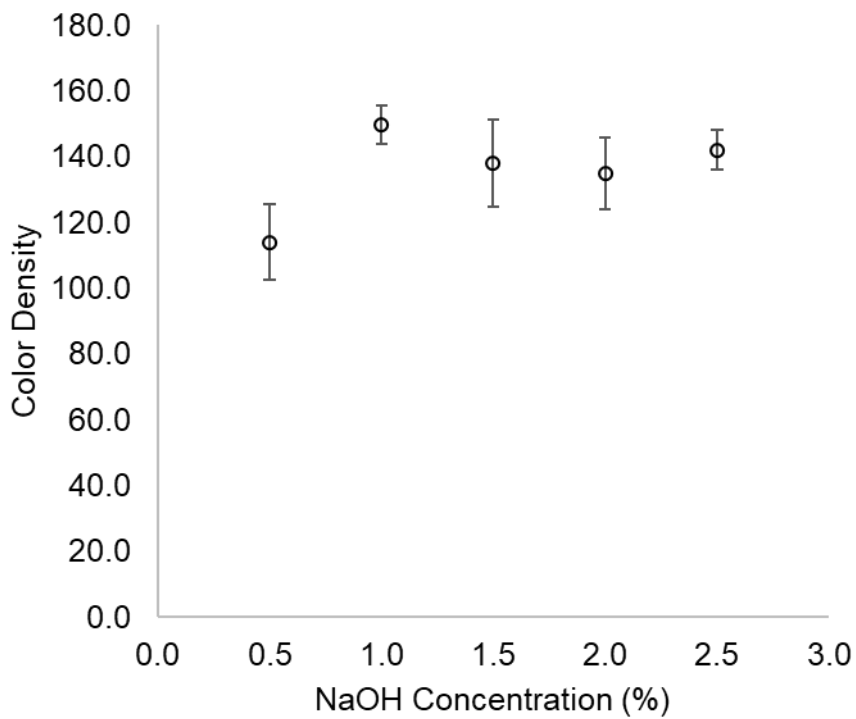
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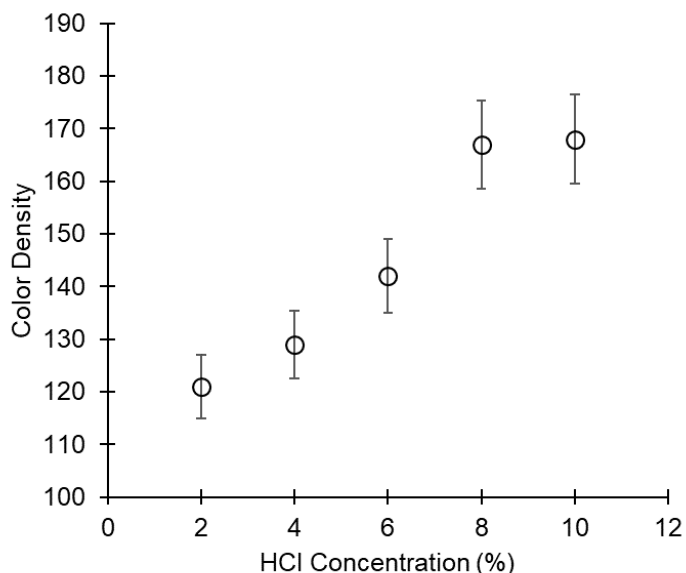229   Fig. 2: Effect of NaOH concentration on the color density of cyanide (50 ng) spot

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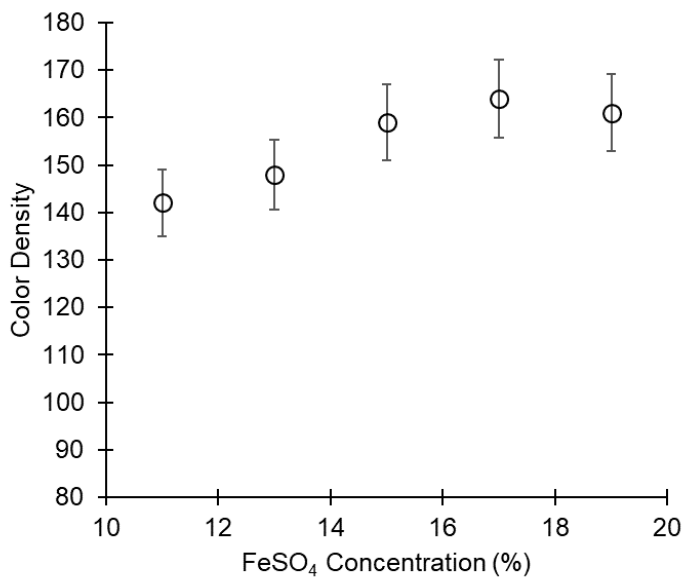
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232   Fig. 3: Effect of $FeSO_4$ concentration on the color density of cyanide (50 ng) spot

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234

235   Fig. 4: Effect of HCl concentration on the color density of cyanide (50 ng) spot

236

237   Although the reaction and blue color complex are specific for the cyanide

238   ions, experiments were carried out to check the effect of certain cations (Group I and

239   II) and anions (Group VII) on the development of spots. Among the anions, only

240   fluoride suppressed the intensity of the blue spots of the iron complex to some extent,

241   probably because of having a strong coordination affinity for iron.

242   To check the validity of the proposed method, the results obtained for syn-

243   thetic samples and the respective standard deviations were compared with those ob-

244   tained from a reference method using an ion-selective electrode system. Both meth-

245   ods also analyzed five actual samples collected from a local electroplating industry.

246   The comparison of results and the standard deviations are given in Table 1. It can be

247   seen from the table that the results of the proposed method are statistically compa-

248   rable ($p = 0.05$) with those obtained by the reference method. Synthetic samples were

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249 prepared in deionized water, and the recoveries were between 99–101% for both the
250 reference and proposed methods. It is important to note that the standard deviations
251 mentioned in Table 1 indicate repeatability for the same solution. In Table 2, the
252 detection limit of the present method ($3 \times$ standard deviation of blank) is compared
253 with those of some other methods reported in the literature. The detection limit of
254 the present method is higher than most of the spectrophotometric methods reported
255 in the literature. However, the use of commonly available reagents, simplicity, ac-
256 curacy, and precision without any involvement of costly instrumentation make this
257 method useful for regular laboratory analysis of cyanide ions in aqueous samples.

258 Table 1: Sample analysis by proposed and reference methods

Cyanide sample	Proposed method (ng $\pm s$)	Reference method (ng $\pm s$)
Sample A ($\mu = 15$ ng, $n = 3$)	15.2 ± 2.10	14.9 ± 1.09
Sample B ($\mu = 35$ ng, $n = 3$)	34.9 ± 1.12	34.9 ± 1.10
Sample C ($\mu = 60$ ng, $n = 3$)	59.7 ± 2.15	60.8 ± 1.13
Real sample 1	50.7 ± 3.18	52.4 ± 2.14
Real sample 2	59.7 ± 2.17	59.9 ± 3.16
Real sample 3	62.7 ± 3.19	60.1 ± 3.15
Real sample 4	55.7 ± 3.17	53.6 ± 2.15
Real sample 5	63.2 ± 2.19	59.7 ± 2.16

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260 Table 2: Comparison of detection limits of the present method with some other meth-
261 ods reported in the literature

Technique	Main Chemicals/ Reagents	Detection Limit	Reference
Ion chromatography with electrochemical detection	Sodium carbonate, sodium hydroxide, ethylenediamine, sodium tetraborate	0.038 µmol L^{-1}	[27]
Spectrophotometer ($\lambda_{max} = 520$ nm)	p-Nitrobenzaldehyde, tetrazolium blue	0.192 µmol L^{-1}	[14]
Spectrophotometer ($\lambda_{max} = 590$ nm)	Phenolphthalein, Cu(II)	0.192 µmol L^{-1}	[28]
Spectrophotometer ($\lambda_{max} = 560$ nm)	p-Nitrobenzaldehyde, o-dinitrobenzene	0.192 µmol L^{-1}	[29]

Naphthalene-based ratiometric fluorescent probe	Potassium phosphate buffer, dimethylformamide (DMF)	0.231 μmol L^{-1}	[16]
Spectrophotometer ($\lambda_{max} = 586$ nm)	Chromogenic disulfide, cetyltrimethylammonium chloride	0.269 μmol L^{-1}	[30]
Spectrophotometer ($\lambda_{max} = 600$ nm)	Isonicotinic acid, barbituric acid	0.385 μmol L^{-1}	[31]
Spectrophotometer ($\lambda_{max} = 553$ nm)	Ninhydrin	0.769 μmol L^{-1}	[32]
Computational colorimetry for colored spot tests	p-Nitrobenzaldehyde, o-dinitrobenzene	0.385 mmol L^{-1}	[18]
Computational colorimetry for colored spot tests	Ferrous sulfate, sodium hydroxide, hydrochloric acid	0.055 mmol L^{-1}	Present

Conclusion:

A method is proposed to determine cyanide ions in aqueous samples by treating a microliter quantity with the classical Lassaigne's reagents on a TLC plate. The color depth of the developed deep blue spots of the ferric ferrocyanide complex was measured by scanning the TLC plate and analyzing the image with an indigenous software package. From the results obtained in the present study, it can be concluded that the proposed method for determining micro quantities of cyanide in aqueous samples using computational densitometry is a simple, accurate, and adequately precise method without any involvement of sophisticated instrumentation.

References:

[1]   J. Qi, J. Hu, Y. Sun, and J. Li, "Cyanide detection using azo-acylhydrazone in aqueous media with high sensitivity and selectivity," *Curr. Anal. Chem.*, vol. 12, no. 2, pp. 119–123, Mar. 2016, doi: 10.2174/1573411011666150827194259.

[2]   S. Sharma and A. Bhattacharya, "Drinking water contamination and treatment

15

techniques," *Appl. Water Sci.*, vol. 7, no. 3, pp. 1043–1067, Jun. 2017, doi: 10.1007/s13201-016-0455-7.

[3] M. B. Tahir *et al.*, "Role of Nano-Photocatalysts in Detoxification of Toxic Heavy Metals," *Curr. Anal. Chem.*, vol. 17, no. 2, pp. 126–137, Feb. 2021, doi: 10.2174/1573411016666191230151455.

[4] F. P. Simeonova, L. Fishbein, W. H. Organization, and I. P. on C. Safety, "Hydrogen cyanide and cyanides: human health aspects." World Health Organization, Geneva PP - Geneva, 2004. [Online]. Available: https://apps.who.int/iris/handle/10665/42942

[5] R. R. Dash, A. Gaur, and C. Balomajumder, "Cyanide in industrial wastewaters and its removal: A review on biotreatment," *J. Hazard. Mater.*, vol. 163, no. 1, pp. 1–11, Apr. 2009, doi: 10.1016/j.jhazmat.2008.06.051.

[6] E. Jaszczak, Ż. Polkowska, S. Narkowicz, and J. Namieśnik, "Cyanides in the environment—analysis—problems and challenges," *Environ. Sci. Pollut. Res.*, vol. 24, no. 19, pp. 15929–15948, Jul. 2017, doi: 10.1007/s11356-017-9081-7.

[7] S. C. Curry and M. B. Spyres, "Cyanide: Hydrogen cyanide, inorganic cyanide salts, and nitriles," in *Critical Care Toxicology*, Cham: Springer International Publishing, 2015, pp. 1–21. doi: 10.1007/978-3-319-20790-2_101-1.

[8] S. Mousavi, M. Balali-Mood, B. Riahi-Zanjani, and M. Sadeghi, "Determination of cyanide and nitrate concentrations in drinking, irrigation, and wastewaters," *J. Res. Med. Sci.*, vol. 18, pp. 65–69, Jan. 2013.

[9] I. F. Bolarinwa, C. Orfila, and M. R. A. Morgan, "Determination of amygdalin

in apple seeds, fresh apples and processed apple juices," *Food Chem.*, vol. 170, pp. 437–442, Mar. 2015, doi: 10.1016/j.foodchem.2014.08.083.

[10] K. Ganesan, S. Raza, and R. Vijayaraghavan, "Chemical warfare agents," *J. Pharm. Bioallied Sci.*, vol. 2, no. 3, p. 166, 2010, doi: 10.4103/0975-7406.68498.

[11] M. F. Delaney and C. Blodget, "Reliable Determination of Cyanide in Treated Water," *J. Am. Water Works Assoc.*, vol. 108, pp. E87–E98, Feb. 2016, doi: 10.5942/jawwa.2016.108.0006.

[12] E. Jaszczak, S. Narkowicz, J. Namieśnik, and Ż. Polkowska, "Determination of cyanide in urine and saliva samples by ion chromatography with pulsed amperometric detection," *Monatshefte für Chemie - Chem. Mon.*, vol. 148, no. 9, pp. 1645–1649, Sep. 2017, doi: 10.1007/s00706-017-1977-x.

[13] O. S. Oluwole, "Incidence of endemic ataxic polyneuropathy and its relation to exposure to cyanide in a Nigerian community," *J. Neurol. Neurosurg. Psychiatry*, vol. 74, no. 10, pp. 1417–1422, Oct. 2003, doi: 10.1136/jnnp.74.10.1417.

[14] V. Pitschmann, Z. Kobliha, and I. Tušarová, "A Simple Spectrophotometric Determination of Cyanides by P-nitrobenzaldehyde and Tetrazolium Blue," *Adv. Mil. Technol.*, vol. 6, no. 2 SE-Research Paper, pp. 19–27, Feb. 2011, [Online]. Available: https://www.aimt.cz/index.php/aimt/article/view/1601

[15] H. B. Singh, N. Wasi, and M. C. Mehra, "Detection and Determination of Cyanide—A Review," *Int. J. Environ. Anal. Chem.*, vol. 26, no. 2, pp. 115–136, Aug. 1986, doi: 10.1080/03067318608077109.

324   [16] L. Long *et al.*, "Determination of Cyanide in Water and Food Samples Using
325   an Efficient Naphthalene-Based Ratiometric Fluorescent Probe," *ACS Omega*,
326   vol. 4, no. 6, pp. 10784–10790, Jun. 2019, doi: 10.1021/acsomega.9b01308.

327   [17] P. Virbickas, A. Valiūnienė, D. Baryševa, G. Popkirov, and A. Ramanavičius,
328   "Determination of cyanide concentration by chronoamperometry, cyclic
329   voltammetry and fast Fourier transform electrochemical impedance
330   spectroscopy," *J. Electroanal. Chem.*, vol. 895, p. 115449, Aug. 2021, doi:
331   10.1016/j.jelechem.2021.115449.

332   [18] J. A. D. Fávero and M. Tubino, "Semi-Quantitative 'Spot-test' of Cyanide,"
333   *Anal. Sci.*, vol. 19, no. 8, pp. 1139–1144, Aug. 2003, doi:
334   10.2116/analsci.19.1139.

335   [19] G. G. Guilbault and D. N. Kramer, "Specific detection and determination of
336   cyanide using various quinone derivatives," *Anal. Chem.*, vol. 37, no. 11, pp.
337   1395–1399, Oct. 1965, doi: 10.1021/ac60230a027.

338   [20] M. T. Doménech-Carbó and A. Doménech-Carbó, "Spot tests: past and
339   present," *ChemTexts*, vol. 8, no. 1, p. 4, Feb. 2022, doi: 10.1007/s40828-021-
340   00152-z.

341   [21] J. Anwar, Waheed-uz-Zaman, M. U. Shafique, and M. Salman,
342   "Computational Quantification of Spot Tests by Image Scanning-A New
343   Analytical Technique for Micro Samples," *Anal. Lett.*, vol. 43, no. 2, pp. 367–
344   371, Jan. 2010, doi: 10.1080/00032710903325898.

345   [22] M. Salman *et al.*, "Micro-Determination of Arsenic in Aqueous Samples by
346   Image Scanning and Computational Quantification," *Anal. Methods*, vol. 4,
347   no. 1, pp. 242–246, Jan. 2012, doi: 10.1039/c1ay05569k.

348 [23] A. Dar, J. Anwar, and U. Shafique, "Estimation of Sulfur by Gas-Phase
349 Molecular Absorption Spectroscopy (GPMAS) and Use in Pharmaceutical
350 Analysis," *J. Sulfur Chem.*, vol. 31, no. 1, pp. 13–18, Feb. 2010, doi:
351 10.1080/17415990903480379.

352 [24] A. Dar, U. Shafique, J. Anwar, Waheed-uz-Zaman, and A. Naseer, "A Simple
353 Spot Test Quantification Method to Determine Formaldehyde in Aqueous
354 Samples," *J. Saudi Chem. Soc.*, vol. 20, pp. S352–S356, Sep. 2016, doi:
355 10.1016/j.jscs.2012.12.002.

356 [25] A. M. Jones, P. J. Griffin, and T. D. Waite, "Ferrous iron oxidation by
357 molecular oxygen under acidic conditions: The effect of citrate, EDTA and
358 fulvic acid," *Geochim. Cosmochim. Acta*, vol. 160, pp. 117–131, Jul. 2015,
359 doi: 10.1016/j.gca.2015.03.026.

360 [26] J. Liu and G. Jiang, "Spectrophotometric flow injection determination of total
361 reducing sugars in tobacco based on oxidation by ferricyanide and formation
362 of Prussian blue," *Anal. Lett.*, vol. 34, no. 11, pp. 1923–1934, Aug. 2001, doi:
363 10.1081/AL-100106122.

364 [27] W. F. Koch, "The Determination of Trace Levels of Cyanide by Ion
365 Chromatography with Electrochemical Detection," *J. Res. Natl. Bur. Stand.*
366 *(1934).*, vol. 88, no. 3, p. 157, May 1983, doi: 10.6028/jres.088.008.

367 [28] D. Cacace, H. Ashbaugh, N. Kouri, S. Bledsoe, S. Lancaster, and S. Chalk,
368 "Spectrophotometric Determination of Aqueous Cyanide using a Revised
369 Phenolphthalin Method," *Anal. Chim. Acta*, vol. 589, no. 1, pp. 137–141, Apr.
370 2007, doi: 10.1016/j.aca.2007.02.004.

371 [29] G. G. Guilbault and D. N. Kramer, "Ultra Sensitive, Specific Method for

372   Cyanide Using p -Nitrobenzaldehyde and o -Dinitrobenzene.," *Anal. Chem.*,

373   vol. 38, no. 7, pp. 834–836, Jun. 1966, doi: 10.1021/ac60239a009.

374 [30] A. A. Druzhinin, I. A. Lozanov, M. Y. Prosvetova, and K. V. Veksler,

375   "Spectrophotometric Determination of Cyanide by Chromogenic Disulfide in

376   Water using Micellar Catalysis," *J. Anal. Chem.*, vol. 65, no. 7, pp. 704–706,

377   Jul. 2010, doi: 10.1134/S1061934810070087.

378 [31] S. Nagashima and T. Ozawa, "Spectrophotometric Determination of Cyanide

379   with Isonicotinic Acid and Barbituric Acid," *Int. J. Environ. Anal. Chem.*, vol.

380   10, no. 2, pp. 99–106, Oct. 1981, doi: 10.1080/03067318108071535.

381 [32] P. Nagaraja, M. S. Hemantha Kumar, H. S. Yathirajan, and J. S. Prakash,

382   "Novel Sensitive Spectrophotometric Method for the Trace Determination of

383   Cyanide in Industrial Effluent," *Anal. Sci.*, vol. 18, no. 9, pp. 1027–1030, Sep.

384   2002, doi: 10.2116/analsci.18.1027.

385