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ORIGINAL PAPER

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A simple and very sensitive spectrophotometric method for the direct determination of copper ions

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Abstract A sensitive spectrophotometric method for the direct determination of copper in aqueous samples without a preconcentration step has been developed. It is based on the formation of a yellow complex with the chromogenic reagent di-2-pyridyl ketone benzoylhydrazone (dPKBH) in an alkaline medium. The complex stoichiometry was 1:2 (Cu:dPKBH) and presents maximum absorbance at 370 nm. The influence of chemical variables affecting the behaviour of the system such as pH, concentration of dPKBH, buffer solution and ethanol, order of addition of the reagents and stability of the complex, were evaluated. The molar absorptivity (ε) was 3.92×10^4 L mol^{-1} cm^{-1}, and Beer's law was obeyed up to 3 mg L^{-1} of copper. The relative standard deviation was 0.46% (n=11) for a sample containing 1 mg L^{-1} Cu(II). The limit of detection was 2.5 μg L^{-1} and was therefore more sensitive than the direct methods reported previously. Finally, the method was successfully validated by analysing several real samples with different matrices, such as tap water, natural water or copper alloys, with an average relative error of 2.46%.

Keywords Copper determination · Spectrophotometry · Natural waters · Trace metals · dPKBH

Introduction

Copper is one of the metals more extensively examined in environmental studies, industrial, biological applications etc. As a consequence, many analytical techniques have been studied and are currently available to analyse copper concentrations in samples with different matrices. Thus, techniques such as flame atomic absorption spectroscopy (detection limits: 1–3 μg L^{-1}) [1], electrothermal atomic absorption spectroscopy (detection limits: 0.04–0.7 μg L^{-1}) [2], inductively coupled plasma-atomic emission spectroscopy (detection limit: 2 μg L^{-1}) [3], inductively coupled plasma-mass spectrometry (detection limit: 0.1–0.5 μg L^{-1}) [4] or differential pulse anodic stripping voltammetry (detection limit: 0.1 μg L^{-1}) [5] have been successfully applied.

Besides the well-known advantages of these instrumental techniques (precision, accuracy, sensitivity, selectivity etc.), all of them present a series of disadvantages, such as difficulty in in situ application, high investment cost, complexity etc. Therefore, low cost, simple and easily portable methods allowing the determination of copper at sub-ppm levels are desirable. With these features in mind, several spectrophotometric methods have been developed to determine copper in different samples. Some of these are based on colourless reagents, while others use reagents with chromophoric groups. Some representative examples of the former group are dithizone (which forms a violet complex with molar absorptivity 4.52×10^4 L mol^{-1} cm^{-1} at 550 nm), sodium diethyldithicarbamate (yellow-brown complex, ε=1.4×10^4 L mol^{-1} cm^{-1} at 436 nm) and cuprizone or bis-cyclohexanone-oxalyldihydrazone (blue complex, ε=1.6×10^4 L mol^{-1} cm^{-1} at 595–600 nm) [6].

On the other hand, several methods are based on the use of coloured reagents such as azo dyes. One representative example is 2-(5-bromo-2-pyridylazo)-5-(*N*-propyl-*N*-sulfopropylamino)aniline, which was employed by Kang et al. for the determination of copper with a detection limit of 50 μg L^{-1} [7]. Furukawa et al. reported a detection limit of 40 μg L^{-1} when employing 2-[2-(4-methylbenzothiazolyl)azo]-5-dimethylaminobenzoic acid as the spectrophotometric reagent [8].

Some spectrophotometric methods are based on the formation of ternary complexes, as the reported by Tarek et al. [9]. They developed a spectrophotometric method for the determination of copper in aluminium metal, aluminium and gold alloys, steels and waters, with 5-(4-hydroxy-3-methoxybenzylidene)rhodanine and polyvinylpyrrolidone reaching a detection limit of 32 μg L^{-1}. In the

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same work, the use of 5-(3,4-dihydroxybenzylidene)rhodanine and cetyltrimethylammonium bromide is also described. With this reagent, a lower detection limit, 27 μg L^{-1} was achieved.

Other representative examples of reagents allowing the sensitive spectrophotometric determination of Cu(II) are poly[allylamine-*co*-*N*-4-(8-aminoquinolyl-5-azo)benzylideneallylamine], giving a detection limit of 14 μg L^{-1} [10].

Another group of compounds frequently used as chromogenic reagents for the spectrophotometric determination of metal ions are the hydrazone derivatives. Among these, bis(cyclohexanone) oxalyldihydrazone has been applied to the determination of copper in alloys and pig feeds with a detection limit of 130 μg L^{-1} [11].

In this work, the hydrazone derivative di-2-pyridylketone benzoylhydrazone (dPKBH) has been used as a reagent for the direct determination of Cu(II) at sub-ppm levels in aqueous samples. dPKBH has been previously described as a bidentate or tridentate ligand for several heavy metals [12]. Besides this, it has been applied to the spectrophotometric determination of iron [13, 14, 15], palladium [16], cobalt [17, 18, 19] and nickel [20, 21].

Experimental

Reagents and solutions

Commercial standard solutions of 1000 mg L^{-1} Cu(II) were purchased from Merck (Darmstadt, Germany). All the chemicals used were of analytical-reagent grade and were purchased from Merck. All the solutions were prepared with doubly distilled water. The spectrophotometric reagent dPKBH was synthesized according to the procedure outlined by García Vargas et al. [12]. Thus, equimolar amounts of di-2-pyridyl ketone and benzoylhydrazide were mixed in ethanol, and a few drops of concentrated hydrochloric acid were added. The mixture was heated at reflux for 1 h, and after cooling, doubly distilled water was added to a 1:3 volume ratio (V_{org}:V_{aq}). Several drops of a sodium hydroxide solution were added to obtain a slightly acidic solution. The solid obtained was recrystallized twice, dissolved in ethanol, and reprecipitated with water.

Stock solutions of dPKBH (0.4% *w*/*v*) were prepared by dissolving the synthesized reagent in ethanol. Finally, a 0.33 mol L^{-1} phosphate buffer solution with pH 8.3 was prepared by dissolving the appropriate amounts of Na_2HPO_4 and KH_2PO_4 in water.

Instruments

Spectrophotometric measurements were carried out by using a PU 8750 UV/vis spectrophotometer (Philips, Netherlands) and a quartz cell with 10 mm pathlength (Hellma, Mulheim, Germany).

Acidity was measured with a Model 2001 pH meter provided with a combined glass-Ag/AgCl electrode (Crison, Alella, Spain).

Procedure

To ensure the highest sensitivity of the determination method, the optimisation of the chemical variables controlling the behaviour of the system was performed by varying the pH of the solutions, concentration of phosphate buffer solution, concentration of dPKBH and concentration of ethanol. The influence of other variables such as the order of addition of the reagents and the reaction time were also studied. The optimisation studies were carried out with solutions containing 1 mg L^{-1} Cu(II) prepared under different conditions, but always with a final volume of 10 mL, and by measuring the absorbance of the Cu-dPKBH complex against its corresponding blank at 370 nm. Once these parameters were optimised, the interferences caused by several species were studied and the proposed method was applied to the determination of copper in several real samples.

Results and discussion

To develop the absorption spectrum of the coloured complex, Cu(II)-dPKBH, several solutions containing 1 mg L^{-1} Cu(II) were prepared in excess of dPKBH and 10% *v*/*v* ethanol, at different acidities. Corresponding blank solutions were prepared in the same way, but in the absence of Cu(II). The maximum absorbance of the Cu(II)-dPKBH complex, measured against its blank, was always found at 370 nm as shown in Fig. 1, in which the spectra of both blank reagent and complex are given.

Optimisation of the system

To study the effect of the solution pH on the absorbance, several samples containing 1 mg L^{-1} Cu(II), 3.25×10^{-4} mol L^{-1} dPKBH and 10% *v*/*v* ethanol were prepared. Their pH values were varied between three and eleven by adding a few drops of nitric acid or sodium hydroxide solution. The absorbances of these samples were measured at 370 nm against their corresponding blanks, prepared by following the same procedure but in the absence of copper. The results obtained (Fig. 2) had a pH range of 7–9, over which formation of the complex proceeded in opti-

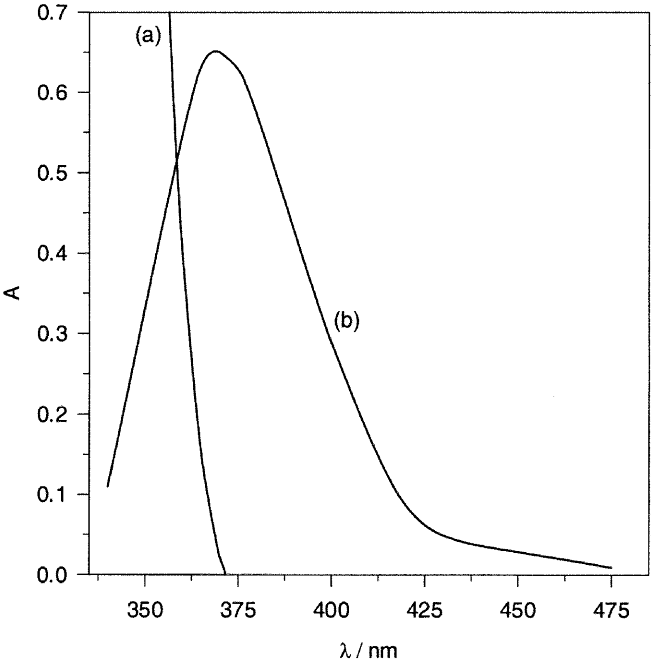


Fig. 1 Absorption spectra of *a* reagent blank ([dPKBH]=3.25×10^{-4} mol L^{-1}; pH 8; 10% ethanol) and *b* Cu(II)-dPKBH complex ([Cu(II)]=1 mg L^{-1}; [dPKBH]=3.25×10^{-4} mol L^{-1}; pH 8; 10% ethanol)

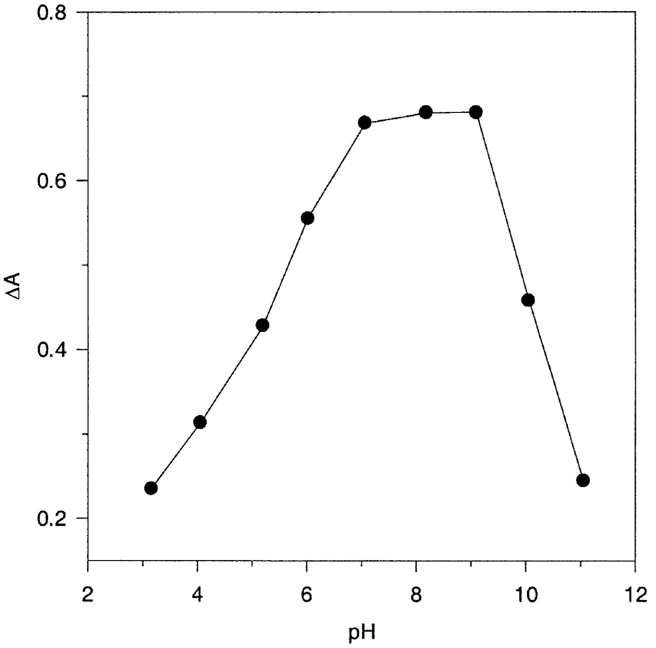


Fig. 2 Effect of the pH on the absorbance of the Cu(II)-dPKBH complex. 1 mg L^{-1} Cu(II); 3.25×10^{-4} mol L^{-1} dPKBH; 10% ethanol. $\Delta A=A_{complex}-A_{blank}$

mum conditions and, as a consequence, the highest absorbance was obtained; pH 8 was chosen as an optimum value.

To ensure the formation of the complex at pH 7–9, a phosphate buffer solution was used. To select the optimum buffer concentration, several samples and their corresponding blanks were prepared by following the same procedure described above. Phosphate buffer solution was added and its final concentration was varied within the range 3.33×10^{-3}–3.33×10^{-2} mol L^{-1}. Buffer concentration had no influence on the absorbance of the complex, and the most diluted buffer solution was enough to keep the pH of the sample within the optimum range. For further studies, a 1.65×10^{-2} mol L^{-1} phosphate buffer solution was used to allow the correct formation of the complex with acidified samples.

The effect of the concentration of dPKBH was studied between 1.3×10^{-4}–1.3×10^{-3} mol L^{-1}. Thus, several samples containing 1 mg L^{-1} Cu(II), and their corresponding blank solutions, were prepared at optimum acidity conditions, and their absorbances measured at 370 nm. Under the conditions studied, dPKBH was always in excess, and the absorbance became practically independent of its concentration. For further studies, 3.25×10^{-4} mol L^{-1} dPKBH was used.

The addition of ethanol was required to dissolve the reagent and to ensure the absence of precipitate in the solutions. To study the effect of its concentration, several samples containing 1 mg L^{-1} Cu(II), 3.25×10^{-4} mol L^{-1} dPKBH and 1.65×10^{-2} mol L^{-1} phosphate, and their corresponding blank solutions, were prepared by varying its final ethanol concentration within the range 2.5–50%, and were then spectrophotometrically measured at 370 nm. Although in the range studied the absorbance was almost independent of ethanol concentration, less precision in the measurement was observed for ethanol concentrations higher than 20% due to the presence of turbidity in the solutions. To form the Cu-dPKBH complex under optimum conditions, 10% ethanol was used henceforth.

In addition to chemical conditions where a coloured complex is formed, the mixing order of reagents often has a marked effect on the colour reaction and on the rate of development. Therefore, once the chemical conditions were selected, several samples were prepared by using different orders of reagent addition. The absorbances of the complex Cu(II)-dPKBH obtained with four different orders of addition are shown in Table 1. It can be seen that the absorbance was practically independent of the order of addition. Slightly higher absorbance was obtained with the order: Cu (II), phosphate buffer, dPKBH and ethanol, which was used henceforth.

The composition of the complex was then studied by applying Job's method of continuous variations. The results obtained suggested the formation of a complex with

Table 1 Absorbance of Cu-dPKBH complex for different orders of reagent addition[a]

Order of addition	ΔA
Cu-buffer-dPKBH-ethanol	0.677±0.001
Cu-buffer-ethanol-dPKBH	0.635±0.001
Cu-ethanol-dPKBH-Buffer	0.664±0.001
Cu-dPKBH-ethanol-buffer	0.654±0.001

[a] 1 mg L^{-1} Cu(II); pH 8; 1.65×10^{-2} mol L^{-1} phosphate; 3.25×10^{-4} mol L^{-1} dPKBH; 10% ethanol. $\Delta A=A_{complex}-A_{blank}$.

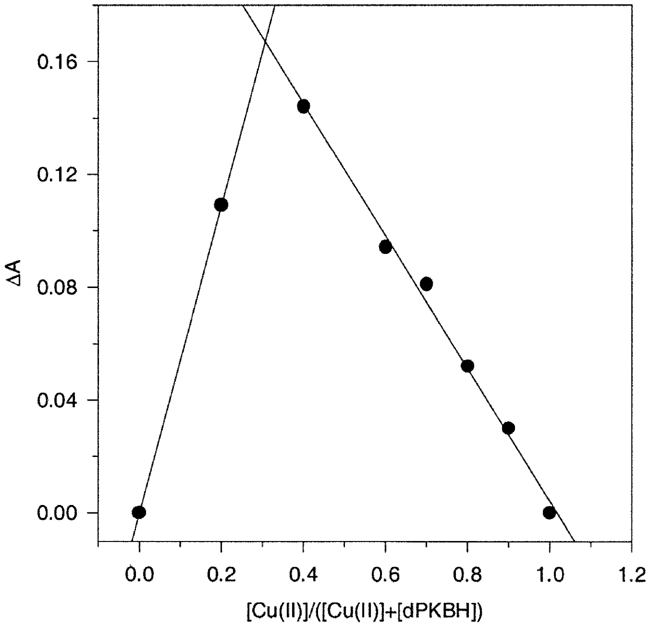
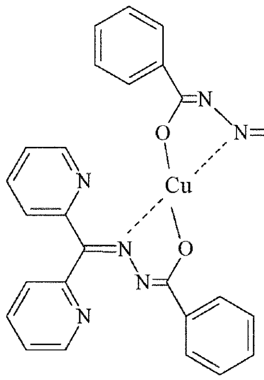


Fig. 3 Dependence of absorbance (370 nm) of Cu-dPKBH complex on Cu(II) molar ratio. pH 8; 1.65×10^{-2} mol L^{-1} phosphate; 10% ethanol. $\Delta A=A_{complex}-A_{blank}$

a stoichiometric ratio 1:2 of Cu(II) to dPKBH (see Fig. 3), which is probably formed by dPKBH acting as a bidentate ligand as follows:



Finally, the stability of the Cu(II)-dPKBH complex under the optimum conditions was determined by measuring the absorbance at 370 nm as a function of time. The results obtained demonstrated that maximum absorbance is reached immediately, and it remains stable during at least 24 h.

Calibration curve

Once all the chemical variables controlling the determination system were optimised, calibration plots were obtained by applying the developed procedure to several samples containing different copper concentrations. At 370 nm, a linear relationship between the absorbance and the concentration of Cu(II) was obtained; Beer's law was obeyed up to a concentration of 3 mg L^{-1} Cu(II). Under these conditions, the equation of the straight line was $y=0.6169(\pm 0.0060)x+3.9(\pm 1.0)\times 10^{-3}$ ($r^2=0.9996$). The confidence intervals for slope and y-intercept were calculated as ts_a and ts_b, where s_a and s_b are the standard deviations of slope and y-intercept, respectively, and t is the Student-t ($p=0.05$, $n-2$). This calibration gave a molar absorptivity of 3.92×10^4 L mol^{-1} cm^{-1}, and a limit of detection of 2.5 µg L^{-1}, calculated as $3s_b/m$, where s_b represents again the standard deviation of the y-intercept and m is the slope of the straight line. It can be noted that this method provides a sensitivity of the same order of magnitude than that provided by some atomic methods, and better than the spectrophotometric methods previously reported, which have been summarized in the introduction section of this work. The precision of the method was established by measuring the absorbance of eleven samples containing 1 mg L^{-1} Cu(II). Under these conditions, a relative standard deviation of 0.46% was obtained.

Study of interferences

Potential interferences caused by several cations in the form of chlorides, nitrates or sulfates, and anions such as potassium, sodium or ammonium salts were studied by quantifying the variation experimented by the absorbance of a solution containing 50 µg L^{-1} Cu(II) when the interfering ion was added. The results obtained are shown in Table 2, in the form of maximum concentration ratio accepted with a variation in the absorbance lower than 5%. As expected, several cations interfered at similar or even lower molar concentration than the copper concentration, especially those previously reported to form coloured complexes with dPKBH [13, 14, 15, 16, 17, 18, 19, 20, 21].

Table 2 Effect of foreign ions on the absorbance of Cu-dPKBH complex[a]

Ion	Mole ratio (Ion/Cu)
Cl^-, Br^-, F^-, I^-, NO_3^-, SO_4^{2-}, ClO_4^-, Na^+, K^+, NH_4^+, citrate, oxalate, acetate	>20,000
Tartrate	5000
Sb^{3+}	2000
As^{5+}, Ba^{2+}	1000
NO_2^-, Sr^{2+}	500
$B_4O_7^{2-}$, Ca^{2+}, Be^{2+}	200
Se^{4+}, Si^{4+}	175
Mg^{2+}, Mo^{6+}, V^{5+}	100
Ti^{4+}	50
Fe^{3+}, Sn^{2+}, Cr^{3+}	20
Al^{3+}, Bi^{3+}	10
Pb^{2+}, Ag^+	5
Mn^{2+}	1
Zn^{2+}, Ni^{2+}, Co^{2+}, Cd^{2+}, Hg^{2+}	0.25

[a] 0.05 mg L^{-1} Cu(II). Other conditions as in Table 1.

Although in many real samples most of the interfering species appear at lower concentration than copper, we studied the use of masking agents to avoid the potential interferences caused by these cations. Thus, among the masking agent studied, the best results were obtained with the addition of 0.8 M ammonium fluoride, which decreased or even removed the interferences of Mg(II), Al(III) and Mn(II). Trisodium citrate (1 M) was an appropriate masking agent for Zn(II), Cd(II) and Ni(II).

Application

With the aim of validating the new method, it was applied to the determination of copper in several real samples with different matrices. First, two Certified Reference Materials (Silicon Brass C31X WSB 10 and Cu/Be/Co alloy C36X CBC 40) were analysed. Both materials were dissolved by heating in a mixture HNO_3:HCl (3:1) and finally, diluted with doubly distilled water. The addition of few drops of HF was also needed to dissolve the former material. Each sample was diluted and analysed in triplicate.

On the other hand, two water samples were analysed. The first one was a tap water sample containing 13.21 µg L^{-1} Cu, 30.90 µg L^{-1} Zn, 41.07 µg L^{-1} Fe, 146.66 µg L^{-1} Al and 2.56 µg L^{-1} Mn (measured by flameless atomic ab-

Table 3 Analysis of copper in different real samples[a]

Sample	Reference method	This method	ε_r(%)
Silicon brass C31XWSB10	0.919	0.929 (±0.005)	1.09
Cu/Be/Co Alloy C36XCBC40	1.097	1.122 (±0.011)	2.28
Tap water	$13.21(\pm0.44)\times10^{-3}$	$13.53\ (\pm0.87)\times10^{-3}$	2.42
River water	0.542(±0.042)	0.564 (±0.017)	4.06

[a] Results expressed as concentration±standard deviation (in mg L^{-1}). ε_r: relative error.

sorption spectroscopy). This sample was directly analysed in triplicate with the proposed method. The last sample was river water containing 542 μg L^{-1} Cu, 590 μg L^{-1} Zn, 604 μg L^{-1} Fe and 63.8 μg L^{-1} Pb (measured by inductively coupled plasma-atomic emission spectroscopy). This sample was diluted by tenfold and analysed with the proposed method in triplicate. In all cases, fluoride and citrate were added as masking agents.

Table 3 shows the average concentration measured for each sample and their corresponding standard deviations. As can be observed, the results obtained are in good agreement with those values used as reference, with an average standard error of 2.46%. The accuracy of the results was tested by applying the paired *t*-test, which confirmed the absence of systematic errors at the 0.05 level.

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