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Highly selective spectrophotometric determination of trace cobalt and development of a reagentless fiber-optic sensor

Evangelos K. Paleologos, Mamas I. Prodromidis, Dimosthenis L. Giokas, Alexandros Ch. Pappas, Miltiacles I. Karayannis*

Laboratory of Analytical Chemistry, University of Ioannina, Ioannina 45 110, Greece

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Abstract

A highly selective spectrophotometric method for the determination of trace cobalt based upon the reaction of cobalt(III) with 1-(2-pyridylazo)-2-naphthol (PAN) in the presence of peroxodisulfate is described. Following an experimental design procedure based on a central composite design (CCD) method and a specifically orientated univariate optimization, it was found that an alkaline media of $\mathrm{pH} = 11$ and controlled peroxodisulfate concentration are crucial factors for enhancing the formation and aqueous solubility of the Co(III)–PAN chelate. Immobilization of PAN into a cellulose acetate membrane was also investigated leading to the construction of a reagentless fiber-optic sensor. According to the proposed reaction mechanism dissolved Co(II) is easily oxidized to Co(III) by peroxodisulfate and then readily complexed with PAN, either in solution or on the membrane surface giving a unique absorbance peak at 645–650 nm. Linear calibration graphs over the concentration range 0.02–0.5 mg l^{-1} with a 3σ limit of detection of 0.01 and 0.1–2 mg l^{-1} with a limit of detection of 0.07 mg l^{-1} were obtained by applying the photometric method and the fiber-optic sensor, respectively. Excellent agreement with the reference atomic absorption spectrometric method was achieved when the proposed methodology was applied for the determination of cobalt in various samples of industrial, environmental and biological importance.

Keywords: Cobalt; Spectrophotometry; PAN; Fiber-optic sensor; Central composite design; Wastewater; Pharmaceuticals; Alloys

1. Introduction

Dissolved cobalt occurs in the environment at concentrations ranging from 0.5 to 12 $\mu\mathrm{g\,l}^{-1}$ in seawater up to 100 $\mu\mathrm{g\,l}^{-1}$ in wastewater [1,2]. Calculation of the inorganic complexation of cobalt using an ion-pairing model and stability constants [3] show that it is weakly complexed by inorganic ligands, the predominant inorganic species being Co(II) and its chloride complexes. On the other hand, there is evidence that cobalt is strongly complexed by organic ligands especially in burdened waters and solids [4,5].

High sensitivity procedures for the determination of cobalt generally use graphite furnace atomic absorption spectrometry (GFAAS) after a preconcentration step, which usually involves the risk of sample contamination and analyte loss [1,6–10]. Voltammetric techniques seem to overcome most problems, proving to be very useful even at low levels of cobalt [11,12]. However, they suffer from interferences from various electroactive compounds present in real samples, which are co-oxidized at the applied potential. On

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* Corresponding author. Tel.: +30-65198406; fax: +30-65198796.
E-mail address: mkaragia@cc.uoi.gr (M.I. Karayannis).

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the other hand, spectrophotometric [13–19] and spectrofluorimetric techniques [20] have been extensively used. Most of the reagents are either not selective, with nickel and iron being the most important interferents, or the products are water insoluble and require extraction [14,21], separation [17,19] or even a computational [13,15,18] step for the determination of each species.

The analytical application of 2-pyridylazo compounds has been extensively studied. It is generally accepted that 1-(2-pyridylazo)-2-napthol (PAN) is one of the most versatile and useful reagents for the determination of trace metals. It forms chelates with a multitude of metal cations [22]. These chelates are usually colored and could be used for spectrophotometric determination; however PAN and its complexes are unchanged compounds and are therefore sparingly soluble in water so the incorporation of various time-consuming and error-introducing analytical steps in the overall analytical method is necessary. PAN has also been extensively used in numerous extraction preconcentration schemes like liquid–liquid extraction [23] and recently cloud-point methodology [24]. However, its use in direct spectrophotometric analysis is restricted due to its non-selective and hydrophobic nature. In addition, most of the PAN–metal chelates exhibit similar absorption maxima in the region of 560–580 nm.

Highly selective methods have been receiving particular attention, in that elimination of interferences would allow the precise determination of trace elements even at sub-microgram per liter levels [25,26]. Moreover, highly selective methods offer certain advantages, namely short time of the total analysis (no special sample treatment is necessary), cost-effectiveness (the majority of separation methods utilize chromatographic techniques) and performance simplicity. These characteristics make them attractive for routine analysis, or for in-field measurements in the case of reagentless schemes that are accompanied by the proper instrumentation [25].

Cobalt(III) forms a dark blue-green complex with PAN, allowing selectivity against other metals. Cobalt(II) behaves similarly after oxidation to cobalt(III) by peroxodisulfate [27]. Although the above complex is also sparingly soluble in water appropriate choice of reaction conditions, such as the pH and PAN concentration, allows enhanced solubility of the chelate in alkaline media ($\mathrm{pH} = 8$–12) and a shift of the absorbance peak to 650 nm. A multivariate study of the reaction variables revealed that, although it is necessary for oxidation, the peroxodisulfate concentration must be carefully adjusted because excessive amounts lead to signal deterioration. Finally, under the optimum conditions the calibration graph gave a linear range of 20–500 $\mu\mathrm{g\,l}^{-1}$, with a detection limit of 3 $\mu\mathrm{g\,l}^{-1}$ and a limit of quantification of 10 $\mu\mathrm{g\,l}^{-1}$. Thus, cobalt was successfully determined in cobalamin and wastewater samples and the results were in good agreement with those obtained by AAS measurements.

Taking advantage of the unique selectivity of the earlier system and the limited aqueous solubility of both PAN and Co(III)–PAN complex we developed a reagentless fiber-optic sensor by immobilizing PAN in a cellulose acetate membrane. The produced sensor is highly selective towards cobalt in the presence of persulfate anions, and provides the ability for in-field measurements. The experimental conditions such as the loading of the polymeric membrane with the active compound, the working pH, the concentration of peroxodisulfate and the effect of temperature were optimized. The interference effects of various cations such as Fe^{3+}, Ni^{2+}, Mg^{2+}, Mn^{2+}, Ca^{2+}, V(V), Cu^{2+} and a mixture of these were also investigated. Under soptimized conditions, the calibration graph was linear over the range 0.1–2 $\mathrm{mg\,l}^{-1}$ Co^{2+}. The proposed method was applied to the determination of cobalt in commercial alloys (Co^{2+}–Fe^{3+}–Ni^{2+} supplied by Goodfellow). Good agreement was achieved on comparing the results obtained with the proposed sensor with the manufacture's label or with those obtained by the reference AAS method. Modified membranes showed an extended storage stability (several months) when stored sealed at +4 °C. This feature enhances the potential applicability of the sensor, because, by an easy and cost-effective procedure, we can produce a stock of such membranes, which are readily and reproducibly applicable, even without calibration.

2. Experimental

2.1. Apparatus

Batch experiments were performed, using a Shimadzu double-beam spectrophotometer connected

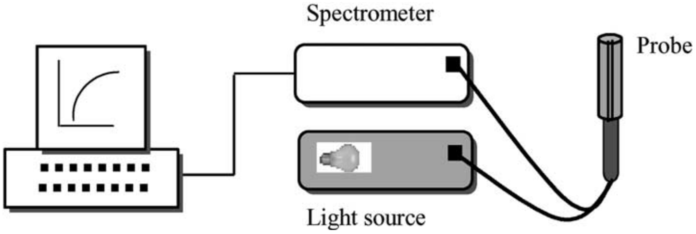


Fig. 1. Schematic representation of the measurement set-up.

to a PC equipped with the respective software. For the optrode assembly a photometric unit was employed, comprising a spectrometer equipped with a 2048 pixels charge coupled device detector (CCDD, SD2000, Ocean Optics Inc., FL) and a tungsten light source (LS-1, 360–900 nm, Ocean Optics Inc.) both connected to a reflectance fiber-optic probe. It consisted of two fibers (1000 μm o.d. each) sealed in a plastic body (8 mm o.d.), which ends in a planar surface of epoxy resin. The experimental set-up is shown in Fig. 1. Data acquisition was carried out by means of an interface card (ADC 500, Ocean Optics Inc.) between the spectrometer and an IBM compatible PC. Data evaluation was done by using the software SrectraWin 4.2 (Top Sensor System BV, The Netherlands).

2.2. Reagents

Cellulose acetate (approximately 40% acetyl) and poly(vinyl acetate) (PVA, Mr 167,000 Da) were purchased from Sigma (St. Louis, MO) and Aldrich (Gollingham, Germany), respectively.

2.2.1. Cobalt(II) stock solution 100 mg l^{-1}

Prepared by dissolving 0.0494 g $Co(NO_3)_2{\cdot}6H_2O$ in 100.0 ml distilled water.

2.2.2. Ammonium persulfate 0.25 M, pH 8.0 and 11.0

Prepared by dissolving 57.05 g $(NH_4)_2S_2O_8$ in 1 l distilled water and adjusting to the desired pH with 2 M NaOH.

2.2.3. PAN stock solution 1000 mg l^{-1}

Prepared by dissolving 0.1 g of PAN in 100 ml of ethanol due to its restricted solubility in water.

2.3. Casting of membranes: sensor assembly

Cellulose acetate membranes bearing PAN (CA-PAN) were prepared in our laboratory according to a previously described protocol [28] by dissolving 3.996 g of cellulose acetate and 4 mg of PVA in a mixture of 60 ml of acetone and 40 ml of cyclohexanone. A 10 ml of the polymeric solution was mixed with an equal volume of 2–16 mM PAN in acetone. The mixture was well homogenized in a Vortex device. Final concentrations ranging from 1 to 8 mM PAN in a 2% cellulose acetate solution were taken. CA-PAN films were cast either after the evaporation of 12 ml of the mixture in a Petri dish (110 mm diameter) or directly on the probe surface applying 7 μl of the mixture and leaving it to air dry. In both cases, the thickness of the membranes was ca. 20 μm [28]. Following the first method, a 110 mm diameter round film was peeled off, by immersing the mould in a distilled water bath, and appropriate circular pieces of 6 mm diameter were cut with the aid of a punch. Circular films were placed onto the probe surface, covered by a nylon fabric (N100, Locertext, UK) for protection with the aid of an O-ring.

2.4. Synthesis of PAN–Co(II) and PAN–Co(III) salts

2.4.1. PAN–Co(III)

A 0.1 g of PAN (0.4 mmol) and 0.12 g of $Co(NO_3)_2{\cdot}6H_2O$ (0.4 mmol) were mixed under stirring in a beaker containing 100 ml of 0.25 M $(NH_4)_2S_2O_8$, pH 8.0, and left to react overnight. Quantitative precipitation of the salt was achieved by adding 5 ml of 4 M NaOH. The precipitate, a deep-green powder, was isolated by filtering the mixture through sintered glass G3, washed with warm distilled water and dried at 100 °C for 2 h.

2.4.2. PAN–Co(II)

Following the same protocol as above in an acetone medium and in the absence of the peroxodisulfate a dark-red powder of PAN–Co(II) was isolated.

2.5. Sample preparation: performance of measurements

Wastewater samples from the local wastewater treatment plant were filtered through a Whatman no. 40 filter paper to remove suspended solids. Sewage sludge samples were dried in an oven at 150 °C, and pulverized and homogenized with a handmill. One gram of each sample were acid digested and the residue was dissolved in 100 ml of 1 M nitric acid and filtered as above to remove any residual solids.

For the determination of cobalt in cobalamin, five tablets of a dietary supplement were acid digested to destroy the vitamin structure and dissolved in 25 ml of 1 M nitric acid. In 10 ml solutions of each sample 50 μl of PAN solution and 100 μl of persulfate were added and the mixture was vigorously shaken at 40 °C for 20 min. The absorbance was measured at 650 nm against a reagent blank.

As far as the alloy is concerned, 0.1–0.25 g of the alloy was dissolved in 5 ml of warm 6 M HNO_3 and diluted to 100.0 ml with distilled water. Using the same alloy, three different samples solutions were prepared.

Measurements were carried out in an amber colored vessel of 20 ml at room temperature under mild stirring. The fiber-optic sensor bearing the PAN-modified polymeric membrane was immersed in a $(20 - x)$ ml solution of 0.25 M ammonium peroxodisulfate, pH 8, and the absorbance was adjusted to zero (blank). A normalized performance of the spectrometer was established by adjusting the focal distance of the illuminating fiber-optic from the light source and by setting an integration time so that the reflectance spectrum reaches a value of ca. 3500–3600 counts. Using the SpectraWin software differences in the light intensity were recorded in the absorbance mode. The reaction was initiated by the addition of (x) ml (0.02–1.00) ml of cobalt solution and the increase of the absorbance at 645 nm was recorded after a 10 min interval. Absorbance values referring to the optimization experiments were taken after 30 min.

3. Results and discussion

3.1. Optimization of working parameters

3.1.1. Photometric method

In order to investigate the effect of pH, and PAN and $(NH_4)_2S_2O_8$ concentrations on the absorbance of the Co–PAN complex an optimization method based on a central composite design (CCD) procedure was applied. This approach was preferred against the univariate optimization designs due to the distinct advantages that it offers, the most important being that it allows for the identification of interactions between the variables [29–31].

The CCD method allows for all the selected variables to be investigated individually as squared terms and considers two-component interaction effects. The results of the CCD were evaluated by an automated STATISTICA algorithm, using multiple regression analysis. Each variable was assigned limits depending upon their effect on the Co–PAN absorbance resulting from the implementation of the preceding study. The assigned values at coded levels −2, −1, 0, +1, +2 are given in Table 1. The corresponding design matrix was then constructed for a Co(II) concentration of 200 $\mu g\,l^{-1}$. The multiple regression and ANOVA results are collected in Table 2. At 95% confidence level certain variables are statistically significant while for others a weaker significance is observed. The coefficient for $(NH_4)_2S_2O_8$ and all the interaction terms are not significant. The lack of significance of the cross-product terms (interactions) suggests non-significant interactions between the variables in the zone studied.

For the data set at hand, a reduced model was formulated which takes into account only the significant contributions. The intercept is the response at zero coded levels of the parameters. As illustrated in Table 2, the

Table 1
CCD used for Co absorbance signal

Coded levels	pH	PAN ($mg\,l^{-1}$)	$(NH_4)_2S_2O_8$ ($mg\,l^{-1}$)
−2	5	2.5	10
−1	7	5	50
0	9	10	250
+1	11	20	500
+2	13	50	1000

Table 2
Multivariate and ANOVA results of the CCD

Term	Coefficient	Sum of squares	d.f.	*P*-value
Intercept	0.093		1	
pH	0.00675		1	0.0124
PAN	−0.00988		1	0.0331
pH·pH	−0.00789		1	0.0556
PAN·PAN	−0.01376		1	0.0033
$S_2O_8 \cdot S_2O_8$	−0.00564		1	0.0154
Regression		0.006101	5	
Linear			2	
Square			3	
Residual		0.002894	11	
Total			16	

$R = 0.824$, $R^2 = 0.678$.

main effect (coefficient) of the pH of the reaction has a positive contribution, signifying an increase in the Co–PAN absorbance with increasing values of the respective variable. On the other hand, the main effect of PAN concentration is a negative contribution, which indicates that the Co–PAN absorbance decreases on increase of PAN concentration. This behavior can be attributed to the shift of the absorbance peak of the complex towards the PAN peak at very high concentrations. Furthermore, the main effects are of the same order of magnitude, which indicate that both variables influence the model equally (but adversely).

The Co–PAN absorption signal as a function of the two studied variables was investigated keeping the rest of the variables at the medium level ('0' coded level) of the experimental domain. The three-dimensional response surfaces are illustrated in Fig. 2. As can be seen, optimum experimental conditions correspond to the following uncoded values: PAN concentration = $10\,\mathrm{mg\,l^{-1}}$ and pH = 11. Fluctuation of $(NH_4)_2S_2O_8$ was insignificant at coded pH levels higher than 0 (and below +2) indicating that if the $(NH_4)_2S_2O_8$ concentration is adequate to oxidize Co(II) to Co(III), it has no significant influence on the absorbance of the Co–PAN complex at the optimum range of the pH value. Based on the results obtained, even at the lower level of the studied zone, $(NH_4)_2S_2O_8$ was adequate to completely oxidize $200\,\mu\mathrm{g\,l^{-1}}$ Co(II) to Co(III) whereas a slight decrease was observed with increasing amounts up to the value assigned as the highest in the CCD ($1000\,\mathrm{mg\,l^{-1}}$ uncoded value). As far as the PAN concentration is concerned, a decrease on the absorbance was observed at both higher and lower levels beyond the optimum value due to either a shift of the complex peak towards the reagent peak or due to insufficient reagent (PAN) for the realization of the complexation reaction, respectively. The pH-absorbance signal pattern exhibits an increase in the range of 0 to +1 coded levels yielding a maximum at +1 (11 uncoded value) and decreasing at higher pH values. This is in agreement with the pH dependent

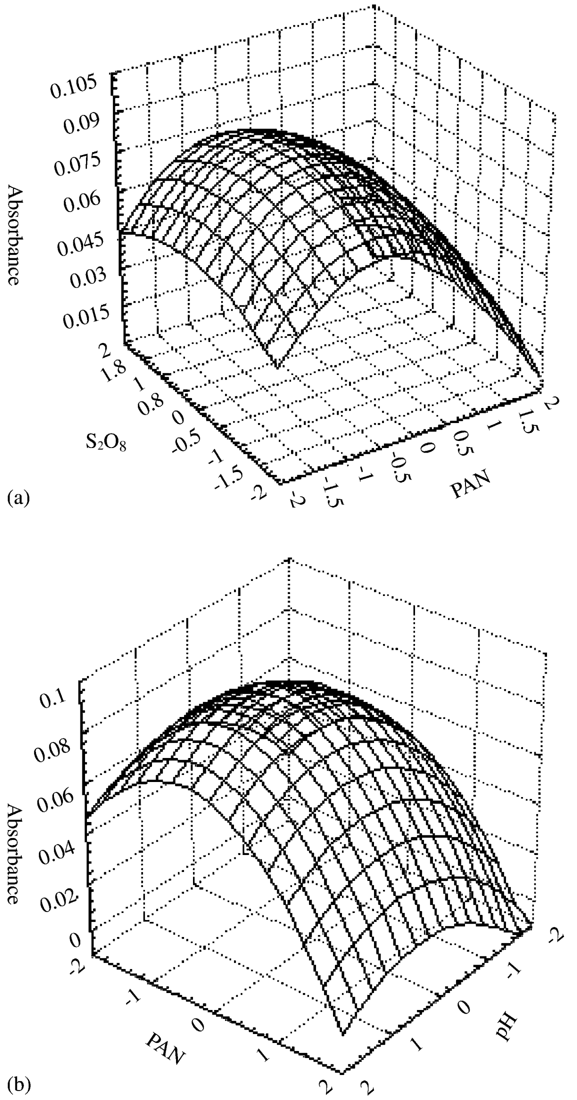


Fig. 2. Response surfaces of the multivariate optimization for total Co: (a) absorbance as a function of $(NH_4)_2S_2O_8$ and PAN concentration; (b) absorbance as a function of pH and PAN concentration.

behavior of PAN at such pH values. The decrease of the absorbance at lower pH values also justifies the fact that the Co(II)–PAN reaction is favored at higher pH values.

The effect of peroxodisulfate concentration was further investigated by means of univariate experiments, as the CCD reveals the tendency but does not define the time dependence of the absorption signal. Absorbance was measured for 100 $\mu g\,l^{-1}$ solutions of cobalt in the presence of PAN and increasing peroxodisulfate concentration, at 2 min intervals and recording the final value after 20 min. The results show that the reaction rate increases upon increase of peroxodisulfate concentration from 10 to 50 $mg\,l^{-1}$, remaining almost constant over the range 50–250 $mg\,l^{-1}$. For higher concentrations though, the reaction rate decreases and after completion of the reaction ($t = 20$ min) the absorbance is also less. It becomes clear that peroxodisulfate has an inhibitory effect when present in a 2500-fold excess over cobalt and therefore, it must be carefully adjusted.

The pH has also a great influence on the absorbance. Although for a wide range of pH color development is sufficient, at low pH values the complex is readily precipitated even for cobalt concentration of 0.20 $mg\,l^{-1}$. On the other hand, alkaline media up to pH 12 allow for dissolution of the complex for a cobalt concentration as high as 0.5 $mg\,l^{-1}$. The alkaline pH also seems to favor the oxidation reaction and the formation of the complex, by lowering the oxidation potential of cobalt through precipitation. Apparently, Co(II) is oxidized to Co(III) which, in turn, is complexed in situ by PAN.

Batch experiments were also performed to investigate the effect of the solvent on the proposed system in order to examine the possibility of increasing the solubility of the product. Ethanol and acetone solutions, which were employed in the study, revealed that 5% ethanol or acetone inhibits and at higher ratios stops the formation of the complex. The same effect was also encountered in the attempt to increase the solubility by adding a surfactant. In the presence of as little as 0.2 $g\,l^{-1}$ of the non-ionic surfactant Triton-X100, reaction rate and final absorbance values deteriorate, allowing for the color to develop only in the presence of cobalt concentrations >0.4 $mg\,l^{-1}$. In all the cases, a hypsochromic shift of the absorption peak occurs, shifting maxima towards 620 nm (light-green).

3.1.2. Fiber-optic sensor

Membrane loading was tested by measuring the increase of the absorbance of a standard solution of 1.5 $mg\,l^{-1}$ Co^{2+}. The response of the probe was tested for different loadings in the range 1–8 mM PAN and the relative responses are illustrated in Fig. 3. The sensor exhibits highest sensitivity for membranes bearing 8 mM PAN, however further experiments

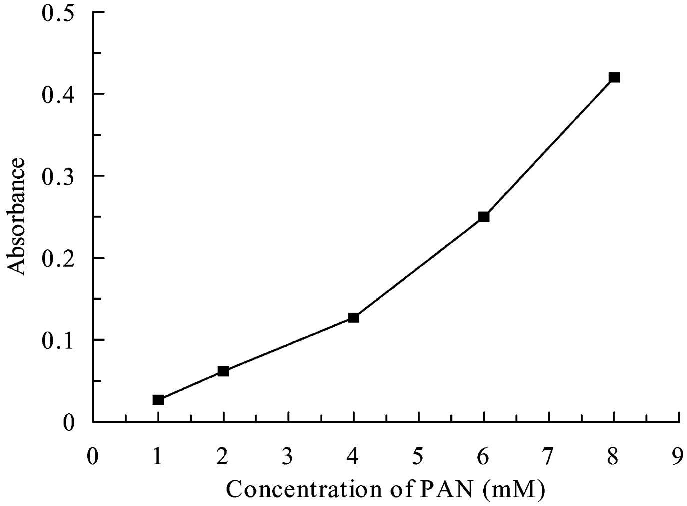


Fig. 3. Effect of the concentration of PAN on the response of the sensor. Two percent cellulose acetate films bearing (x) mM PAN. Parameters: 0.25 M peroxodisulfate; pH 8.0; 1.5 $mg\,l^{-1}$ Co^{2+}; data collection after 30 min.

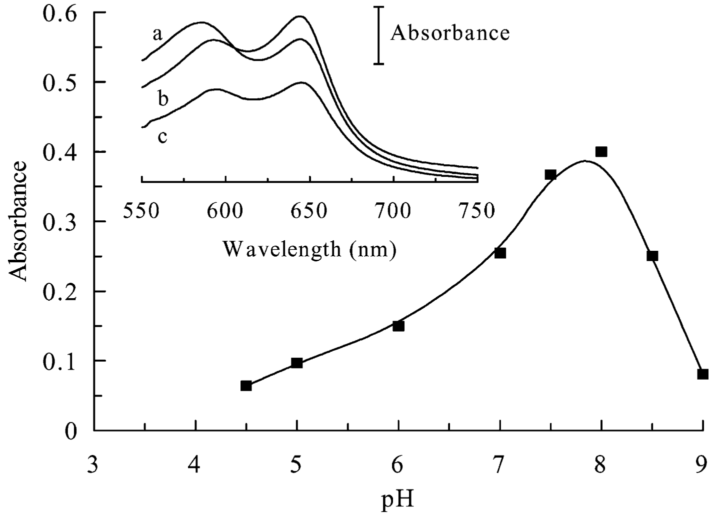


Fig. 4. The pH profile of the sensor. Inset graph depicts the spectra at pH 7.0, 7.5 and 8.0. Parameters: 2% cellulose acetate films bearing 6 mM PAN; 0.25 M peroxodisulfate; pH 8.0; 1.5 mg l^{-1} Co^{2+}; data collection after 30 min.

were performed at lower loadings since at concentrations >6 mM PAN, cellulose films are not uniform resulting in poor reproducibility of the sensor. Lack of uniformity at loadings higher than 6 mM is probably due to the formation of stacks of PAN on the polymeric backbone thus resulting in the blocking of some binding sites.

The pH of the working buffer was investigated in a peroxodisulfate buffer system covering the pH range 4.5–9. The optimum pH for the cobalt fiber-optic sensor is 8.0 as shown in Fig. 4. The inset graph depicts the spectra of the species involved in the reaction 10 min after the addition of the cobalt solution, at pH values of 7.0, 7.5 and 8.0. In addition to the higher absorbance recorded at pH 8 the peak appearing at 645 nm (characteristic of cobalt presence) seems to dominate the peak appearing at 575 nm (characteristic of the presence of various metals, including cobalt) so further experiments were carried out at this pH. Though the batch reaction performs better in a more alkaline medium, pH 8 was selected since at greater pH values hydrolysis of the membrane occurs [28].

The sensitivity of the cobalt fiber-optic sensor is also increased with increase of the temperature within the tested range of 20–40 °C (data not shown). No maximum response was recorded within the tested range. The behavior of the sensor was not tested, however, at higher values since at elevated temperatures the mechanical stability of the films is questionable [28].

4. Interferences

The interference effect of various metals was investigated comparing the absorbance values recorded at 645 nm in the presence of foreign cations in the measuring cell. As can be seen in Fig. 5, the proposed reaction is highly selective to cobalt cations since other metals (Fe^{3+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, V(V), Mg^{2+} and Ca^{2+}), at concentrations of 5 mg l^{-1}, gave no signal at the measurement wavelength under the optimum conditions. At the applied pH value some of the tested metal ions appear in the form $M^{x+}(OH)_x$, and their low signals can be attributed to this phenomenon. Fe(III), Cr(III) and Cu(II) cations, which form hydroxides at pH 3, 5 and 6.7 respectively, are precipitated when they are present at concentration of 5 mg l^{-1} at pH 8. At the same acidity, Co(II) cations form hydroxides at concentrations >14 mg l^{-1}, whereas Co(III) with a Ksp value of 2.5×10^{-43}, exists in a soluble form only in a strongly acidic medium. The same behavior is expected for Sn^{2+} and Al^{3+} ($Ksp_{(Sn(OH)_2)} = 3\times10^{-27}$, $Ksp_{(Al(OH)_3)} = 5\times10^{-33}$). This effect is more pronounced at pH = 11, which is

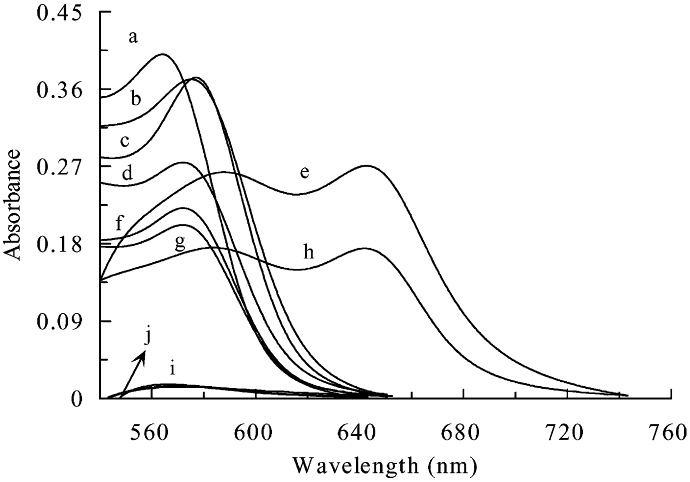


Fig. 5. Spectra of some foreign metalions with the proposed sensor: (a) Ni^{2+}, (b) Cd^{2+}, (c) Zn^{2+}, (d) V(V), (e) Co^{2+}, (f) Mg^{2+}, (g) Ca^{2+}, (h) Co^{2+}, (i) Cu^{2+} and (j) Fe^{3+}. Parameters: 2% cellulose acetate films bearing 6 mM PAN; 0.25 M peroxodisulfate; pH 8.0; [metalion] = 5 mg l^{-1}; data collection after 5 min; but for (e) after 10 min.

the optimum for the batch experiments. In a 50 μg l^{-1} cobalt solution no interference was observed by even a 200-fold excess of the above mentioned ions and more than a 2000-fold excess of EDTA, fluoride, citrate, phosphate, nitrate or chloride.

The interference effect of nickel and iron, at concentrations of 2 and 4 mg l^{-1}, respectively, was also investigated applying the method of mixed solutions in the presence of 1 mg l^{-1} cobalt. Fig. 6 illustrates the resulting spectra and can be seen that at the measuring wavelength of 645 nm no interference was observed.

4.1. Some preliminary mechanistic studies

As revealed so far the high selectivity of the proposed method is based on the presence of the peroxodisulfate anions. Initially, the possibility of the formation of an intermediate compound between PAN

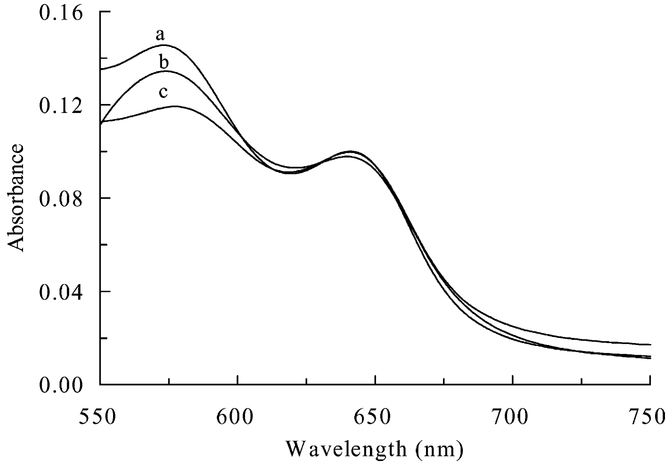
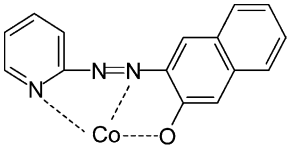


Fig. 6. Interference effect of nickel (2 mg l^{-1}) and iron (4 mg l^{-1}) in a mixed solution containing 1 mg l^{-1} cobalt: (a) Co(II), (b) Ni(II) + Co(II), (c) Ni(II) + Co(II) + Fe(III). Parameters: 2% cellulose acetate films bearing 6 mM PAN; 0.25 M peroxodisulfate; pH 8.0; data collection after 10 min.



Scheme 1. Tentative coordination of cobalt with PAN.

and peroxodisulfate, selectively reacting with cobalt cations, was investigated. In order to verify this hypothesis we isolated PAN–Co(II) in the presence and absence of peroxodisulfate. IR spectra (Perkin-Elmer RX FTIR spectrum) of these salts, however, do not support this possibility since the comparison of the spectra with that of the pure ligand (PAN) showed no significant interaction of the characteristic peaks. The absence of a strong absorbance peak in the region of 1650 cm^{-1} indicates that there is no oxidation of the quinolinic hydroxyl, which would have resulted in the formation of a quinone. In the area between 750 and 850 cm^{-1} there is a shift of the absorption peaks, which suggests a probable coordination of cobalt with the nitrogen atom of the pyridine ring (Scheme 1). Furthermore, the appearance of sharp absorption peaks in the region of 1150 and 1410 cm^{-1} suggests a further coordination of the quinolinic oxygen and one of the azo-nitrogens. This assumption is further enhanced by the translocation of the N=N absorption peaks in the region of 1500–1550 cm^{-1}. Therefore, the formation of one or even two coordination spheres is evidenced leading the respective stable five-member rings.

Excluding, by the earlier experiment, the formation of an intermediate compound, the possibility of the oxidation of cobalt(II) to cobalt(III) due to the presence of peroxodisulfate, was examined. This was confirmed by adding a small quantity of peroxodisulfate to the red acetone solution of PAN–Co(II) (Fig. 7A, spectrum b) precipitated in the absence of peroxodisulfate, which turned green giving an identical UV-spectrum (Fig. 7A, spectrum c) to that obtained from an acetone solution of PAN–Co(II) precipitated in the presence of peroxodisulfate (Fig. 7A, spectrum a). The peak at 625 nm is therefore attributed to PAN–Co(III) and this is the beak appearing at 645 nm in the experiments with the fiber-optic sensor. The shift of 20–25 nm is due to the nature of the solvent, since dilution of the green acetone solution in water (80/20 in water) gave a peak at 645 nm (Fig. 7A, spectrum d).

More evidence for this possibility is also provided by the cyclic voltammograms (CVs) (Fig. 7B). CVs were made in a three-electrode voltammetric cell (BAS) using a computer-controlled potentiostat (Autolab, Eco Chemie) [28]. A platinum electrode (BAS) and a platinum wire were used as working and auxiliary electrodes, respectively. All the experiments were carried out in an acetone solution, at a scan rate of 0.2 $V\,s^{-1}$. PAN (0.01 mM) seems to be inert to any redox reaction (scan a). No oxidation or reduction currents were developed during the scanning over the potential window from –1 to +2 V versus a non-aqueous Ag/Ag^{+} reference electrode (BAS). On the other hand, PAN–Co(III) (0.01 mM) gave rise to

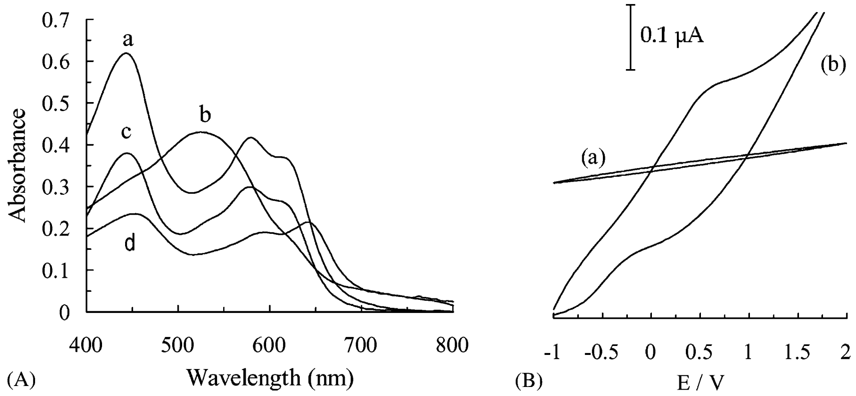


Fig. 7. (A) Spectrum (a), PAN–Co(III) in acetone; spectrum (b), PAN–Co(II) in acetone; spectrum (c), PAN–Co(II) in acetone after its oxidation by persulfate; spectrum (d), as (c) in a 80/20 water/acetone mixture. (B) CVs of (a) 0.5 mM PAN in acetone and (b) 0.5 mM PAN–Co(III) in acetone. Scan rate 0.1 $V\,s^{-1}$.

a pair of redox waves with a cathodic peak potential E_{pc} of 0.5 V ($Co^{3+} \rightarrow Co^{2+}$) and an anodic peak potential E_{pa} of 0.65 V ($Co^{2+} \rightarrow Co^{3+}$) versus a non-aqueous Ag/Ag^{+} reference electrode (scan b). The potential peak separation, ΔE_p, of 146 mV (calculated using the software GPES3 developed by Eco Chemie) indicates a diffusion-limited, one-electrode semi-reversible process.

4.2. Application to real samples

PAN is well known to form complexes of very high absorptivity with various kinds of metal ions and it has been used for the spectrophotometric determination of several metals, such as nickel [17]. Azobenzenes (or substituted azobenzenes) can chelate metal ions using *o*-hydroxy or *o*-amino azo groups to give square planar, tetrahedral or octahedral complexes. The majority of them shift the main peak of the azobenzene due to a $\pi \rightarrow \pi^*$ transition at ca. 450 nm, some 100 nm further into the visible to give purple and red complexes with Co(II) and Ni(II), Cu(II) and Pb(II) [32]. Selectivity in the proposed methods is achieved utilizing masking agents such as hexametaphosphate for iron and manganese, or thioglycollate and EDTA for cobalt, zinc, copper and chromium [17]. The present work explores a selective reaction of PAN with cobalt in the presence of peroxodisulfate ions that gives an absorbance peak at 645 nm.

4.2.1. Fiber-optic sensor

Under the optimum conditions a linear calibration graph of, $A_{(645)} = f$ (Co^{2+}, $mg\,l^{-1}$), over the concentration range 0.1–2 $mg\,l^{-1}$ Co^{2+} was constructed. Experimental data fit the equation $A_{(645)} = 0.007(\pm 0.002) + 0.087(\pm 0.002)\,mg\,l^{-1}\,Co^{2+}$, with a correlation coefficient $r = 0.998 (n = 7)$. The detection limit (signal to noise ratio of 3) was calculated as 0.07 $mg\,l^{-1}$ Co^{2+}. The relative standard deviation (R.S.D.) for a set of five measurements of a standard solution of 1 $mg\,l^{-1}$ Co^{2+} was found to be 1.5%.

The proposed method was applied to a commercial alloy of Fe(III)–Co(II)–Ni(II) (glass sealing alloy, Goodfellow). According to the manufacturer's label the content of Fe(III)–Co(II)–Ni(II) is 54–17–29%, respectively. Results obtained with the proposed fiber-optic sensor were compared with that obtained with the reference AAS method and excellent agreement was achieved (Table 3). The storage stability of the CA-PAN membranes was tested by performing single measurements over several time periods no loss of activity was observed over a period of 6 months. Between measurements membranes were kept sealed at +4 °C.

Table 3
Determination of Co(II) in real samples[a]

Sample	Proposed method (% w/w)	AAS, (% w/w)	Mean error (%)	Manufacturer labeling (% w/w)
1	16.6	16.4	1.2	17.0
2	16.8	16.7	0.6	17.0
3	17.5	17.4	0.6	17.0

[a] The standard error of the means ranges from 0.6 to 1.7% ($n = 3$).

Table 4
Determination of Co(II) in real samples.

Sample	GFAAS method	Proposed method[a]	Relative error (%)
Wastewater[b]	32.8	32.0 ± 0.5	2.4
Sludge[c]	6.6	6.5 ± 0.2	1.5
Tablet[d]	180	175 ± 4	2.7

[a] Triplicate measurements.
[b] In $mg\,l^{-1}$.
[c] In $\mu g\,g^{-1}$ dry weight.
[d] In ng per tablet.

4.2.2. Bulk solution

Batch experiments reveal a calibration graph that was linear over the range 0.02–0.5 $mg\,l^{-1}$ cobalt. The respective equation is $A_{(645)} = 0.009(\pm 0.003) + 0.63(\pm 0.005)\,mg\,l^{-1}\,Co^{2+}$. The detection limit (signal to noise ratio of 3) was calculated as 0.01 $mg\,l^{-1}$ Co^{2+}. The method was applied for the determination of cobalt in wastewater, wastewater sludge and a dietary supplement rich in cobalamin. The samples were also analyzed by FAAS and GFAAS and an excellent match was found (Table 4).

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