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A multisyringe flow-based system for kinetic–catalytic determination of cobalt(II)

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ABSTRACT

A kinetic–catalytic method for cobalt determination based on the catalytic effect of cobalt(II) on the oxidative coupling of 1,2-dihydroxyanthraquinone (alizarin) was automated exploiting multisyringe flow injection analysis (MSFIA). The proposed method was performed at pH 9.2, resulting in a discoloration process in the presence of hydrogen peroxide. The fixed-time approach was employed for analytical signal measurement. The spectrophotometric detection was used exploiting a liquid waveguide capillary cell (LWCC), of 1 m optical length at 465 nm. The optimization was carried out by a multivariate approach, reaching critical values of 124 μmol L^{-1} and 0.22 mol L^{-1} for alizarin and hydrogen peroxide, respectively, and 67 °C of reagent temperature. A sample volume of 150 μL was used allowing a sampling rate of 30 h^{-1}. Under optimal conditions, calibration curve was linear in the range of 1–200 μg L^{-1} Co, achieving a DL of 0.3 μg L^{-1} Co. The repeatability, expressed as relative standard deviation (RSD) was lower than 1%. The proposed analytical procedure was applied to the determination of cobalt in cobalt gluconate and different forms of vitamin B_{12}, cyanocobalamin and hydroxicobalamin with successful results showing recoveries around 95%.

1. Introduction

In the context of reaction-rate based methods of analysis, catalytic methods stand out by their high sensitivity and low detection limits (and also, occasionally, increased selectivity) achieved in the determination of the catalyst concerned. Kinetic-based procedures executed by means of flow injection analysis techniques have the advantage of the precise and reproducible timing of all events so that non-equilibrium conditions can be readily exploited for quantitative assays. Kinetic discrimination and kinetic enhanced approaches can be carried out by flow techniques. In kinetic-discrimination methods, the differences in the rates of reactions of the reagent with the analyte of interest and interfering components are exploited. In kinetic enhancement, the chemical reactions involved are driven in the appropriate direction to the analyte of interest [1].

Cobalt is an essential trace element in nature and has an important role in many body functions. It is essential as a component of vitamin B_{12}, and a deficiency of this vitamin can cause mild disease, neurological damage and anemia [2]. Thus, various pharmacological products involve cobalt as principal component are commercially available. However, in excess amounts it is toxic and causes pulmonary disorders, dermatitis, nausea and vomiting [3]. Hence, the quality control of cobalt concentration has to be carried out for the pharmaceutical industry in the commercialized products, since it is of great interest in nutrition researches.

In order to develop a fast, sensitive and automatic method for monitoring a target analyte, flow analysis systems are excellent tools for the manipulation of solutions, allowing environmental friendly methods.

The spectrophotometric technique has been employed in methods where cobalt acts as a catalyst for the oxidation of various colored substances [4–7]. Some procedures by catalytic effect have been proposed for spectrophotometric detection with flow injection analysis systems, using 4-benzylpiperidinedithiocarbamate [8] and tiron [7] as color developing reagents. Many 1,2-dioxy derivates of aromatic compounds (alizarin, pyrocatechin, tiron, etc.) are oxidized by hydrogen peroxide in the presence of cobalt traces. The reaction takes place in a buffer solution [9]. The reaction mechanism of alizarin dyes in combination with hydrogen peroxide in alkaline medium has been studied over a range of pH and temperature in previous work [10], in which the kinetic of oxidation between the dye and hydrogen peroxide generating bleaching of dyes is discussed in detail. One of the

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most commonly reagents used in oxidation mechanisms with hydrogen peroxide for cobalt determination is tiron [7,11–13] and to a lesser extent 3,4-hidroxybenzoic acid [9], sulfite [10] or derivates of the alizarin such as alizarin red S [14]. The affinity of alizarin towards trace metals, by configuring strong colored complexes, has been documented [15]. Nevertheless, alizarin reagent has not been exploited enough despite its lower toxicity in comparison with alizarin red S, according to their material safety data sheets.

Multicommuted flow systems have some advantages such as high accuracy, elevated sample analysis, high degree of flexibility, manipulation of micro-volumes, minimization of reagent consumption and waste generation [16,17]. Multisyringe flow-injection analysis (MSFIA) combines the advantages of employing the multichannel operation of peristaltic pumps with the constant pulseless and exactly known volume delivery achieved by piston pumps [18]. The basic element of MSFIA is a multisyringe buret that allows the simultaneous movement of four syringes, which are connected in block to the same stepper motor. Furthermore, three-way solenoid valves have been placed on the head of each syringe with the aim of increasing the versatility of the technique and reducing reagent consumption. The robustness and simplicity of the manifold together with the low consumption of reagents and samples constitute other outstanding characteristics of the multicommuted systems [18,19].

The liquid waveguide capillary cells (LWCCs) have played an important role in the measurement of chemical species at low concentrations, and have been used with a range of different detection techniques, such as spectrophotometry [20], fluorescence [21,22] and chemiluminescence [23,24]. In spectrophotometry, LWCC are illuminated axially using optical fibers attached to a light source and a detector. They can enhance the sensitivity and improve limits of detection (LD) of optical instrumentation, by detecting as much of the radiation as possible while minimizing background noise [25]. Despite the above advantages, until now the combination of multicommuted technique with a LWCC has not been used for cobalt determination by catalytic methods.

Thus, this paper describes a rapid, selective and automated system based on the combination of MSFIA technique with a LWCC for a spectrophotometric detection at 465 nm. The method for monitoring cobalt(II) exploiting the oxidative coupling of 1,2-dihydroxyanthraquinone (alizarin) reaction catalyzed by cobalt. Since this reaction implies the bleaching of the dye, net absorbance was systematically obtained as absorbance of the blank (without Co) minus absorbance of solution with Co(II) (standard or samples).

2. Experimental

2.1. Reagents and solutions

All chemicals used were of analytical reagent grade. All aqueous solutions were prepared using deionized water (resistivity $>$ 18 MW cm). Working standard solutions of Co(II) were prepared daily by dilution of a 1000 $\mu g\,L^{-1}$ Co stock solution (ultra pure, Scharlau, Barcelona, Spain) in water.

Buffered reagent was prepared with borax ($B_2Na_2O_7 \cdot 10H_2O$) (99.5% for analysis, Acros organics). The pH adjustment was done by addition of NaOH. The hydrogen peroxide solution was prepared daily (30% extra pure, Scharlau). 1–2 dihydroxyantraquinone (alizarin 97%, Sigma-Aldrich) was prepared from a 0.0033 mol L^{-1} stock solution. The study of interferences was carried out with working standard solutions prepared from stock solutions of 1000 mg L^{-1} of Ca^{2+}, Mn^{2+}, Fe^{3+}, Zn^{2+}, Ni^{2+} and Cu^{2+} (Scharlau). The materials and vessels used for samples or standards were stored in 10% (v/v) nitric acid for at least 24 h, and rinsed with deionized water prior to use.

2.2. Samples

The proposed method was applied to pharmaceutical samples. Two samples of vitamin B_{12}, cyanocobalamin and hydroxicobalamin, and a sample of cobalt gluconate were analyzed.

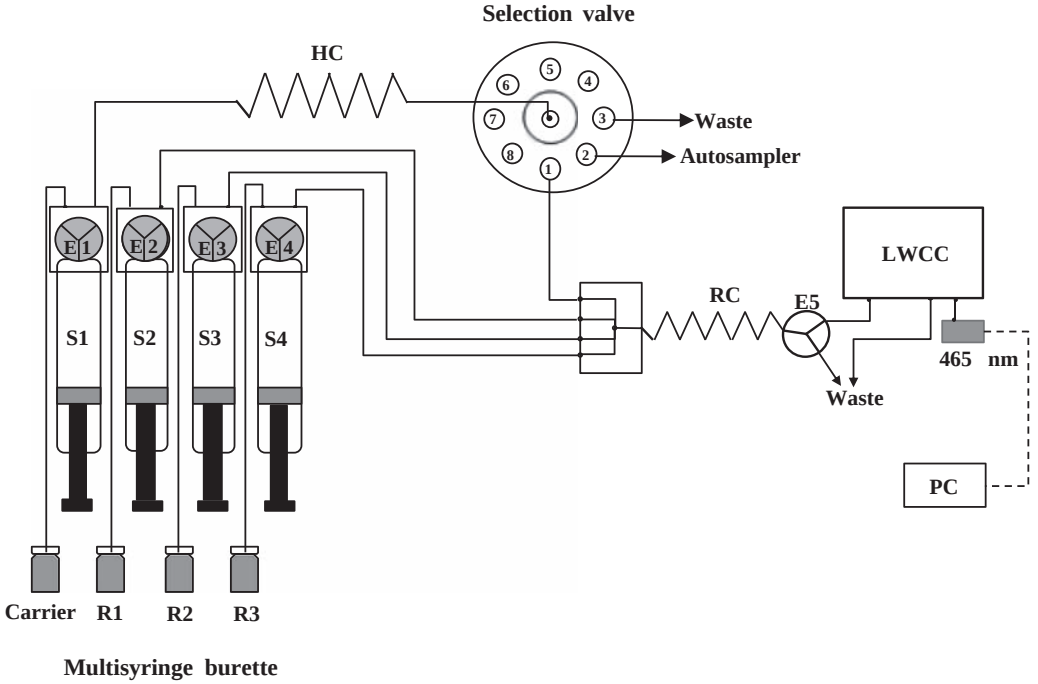

Fig. 1. Kinetic–catalytic MSFIA system for cobalt determination. LWCC: Liquid Waveguide Capillary Cell; HC: Holding Coil; RC: Reaction Coil; Ex: Solenoid valve; Sx: Glass syringes; R1: Alizarin; R2: H_2O_2; R3: Buffer solution.

Samples of cyanocobalamin commercialized as injectable solution, cobalt gluconate as drinking vial of 2 ml, and hydroxicobalamin in tablets, were subjected to a microwave digestion. 1 ml of injectable solution and drinking vial samples was mixed with 9 ml of concentrated HNO_3 and later digested. 0.6 g of the tablet sample was previously crushed and homogenized. 9 ml of concentrated HNO_3 was added, followed of a digestion procedure. The used program for microwave digestion was as following: 6 min at 250 W, 6 min at 400 W, 6 min at 650 W, 6 min at 250 W and finally 10 min for ventilation. After digestion, each extract was taken to complete dryness on the grid, and the residue was dissolved in deionized water. A suitable aliquot solution was used for cobalt analysis by the developed method. Three replicates per sample as well as a blank sample for every pharmaceutical product were performed. The procedure was similar than the proposal in a previous work for pharmaceutical samples [26].

2.3. Manifold and software

A scheme of the MSFIA-LWCC-UV–vis system is shown in Fig. 1. The system is constituted by a multisyringe buret with programmable flow rate (MicroBU 16A, Crison, Barcelona, Spain), a selection valve module (VA 2 SW, Crison) and an auto-sampler (Crison). The syringes S1, S2, S3 and S4 (Hamilton, Bonaduz, Switzerland) have the following capacities: 2.5, 1, 1 and 1 ml, respectively. Each syringe has a three-way solenoid valve on each head: E1, E2, E3, E4 (N-Research, Caldwell, NJ, USA). The "off" position (solenoid disable) of the valves connects syringes to a right channel and "on" position (solenoid enabled) to a left one. The MSFIA system has also one additional independent three-way solenoid valve (E5) (Takasago, Electric Inc., Tokyo, Japan). The syringe S1 is connected to the central port of the selection valve module for loading and dispensing the liquids. Syringe S2 dispenses the alizarin solution. The syringe S3 is employed to dispense hydroxide peroxide and syringe S4 contains the buffer solution. Valve E5 leads flow to LWCC, which is connected to the spectrophotometer for the measurement, or to the waste.

The manifold was built with PTFE (polytetrafluoroethylene) tubing of 0.8 mm i.d. to transport reagents and sample to the system, and 1.5 mm i.d. for picking up reagents. Holding coil (HC) and reaction coil (RC) were constructed of 0.8 mm i.d. and 3.8 m and 1.3 m long PTFE tube, respectively. A five-way connector of PMMA (polymethylmetacrylate, Sciware Systems, Palma de Mallorca, Spain) has been used for mixing reagents and sample and directing them to the reaction coil.

Instrumental control, acquisition and processing of data were performed using the software AutoAnalysis 5.0, (Sciware Systems).

2.4. Apparatus

A pH-meter Crison model 2002 was used for pH measurements. A sand thermostatic bath (Techno Test, Germany) was maintained at the desired temperature to enhance the coloration and accelerate the reaction. A microwave digestion oven (Milestone, Italy) with a vessel's volume of 100 ml was utilized for sample digestion.

The detection system is composed of a deuterium halogen light source (Top Sensor Systems, Eerbeek, Netherlands), and a USB 2000 miniaturized CCD spectrophotometer (Ocean Optics, USA), which was accomplished using a LWCC (World Precision Instruments, Inc., FL USA), model 3100 with 100.0 ± 0.5 cm optical length, internal volume 240 μl, internal diameter 550 μm. Two optical fibers of 600 μm internal diameter (Ocean Optics) were used between light source, LWCC and spectrophotometer. The absorbance was measured at 465 nm using an integration time of 10 ms. Statistical calculations and experimental design have been performed by Statistica®.

2.5. Procedure

Due to the effect of cobalt(II) on the oxidative coupling of 1,2-dihydroxyanthraquinone implies the bleaching of dye, net absorbance was systematically obtained as absorbance of the blank (reaction without cobalt) minus absorbance of the Co(II) standard/sample, using the peak height as analytical signals.

The operational sequence for determining cobalt is described in Table 1 and summarized as follows: the procedure of sample injection begins with sample loading by selection valve. Sample is aspired with the valve E1 in On position, loading the sample into the HC. These instructions are included into a procedure for sample changing when is requested, which involves the loading of a sample volume of each replica and the rising with the next sample to avoid cross-contamination. Then S2, S3 and S4 containing alizarin, hydrogen peroxide and buffer solutions respectively, were activated allowing the simultaneous injection of sample and reagents plugs through the reaction coil (RC). In the next step, the mixture is dispensed toward detector with Milli-Q water as carrier.

Table 1
Automatic procedure for determination of cobalt.

Description	Step	Volume (ml)	Flow rate (ml min^{-1})	Operation	Valve position				
					E1	E2	E3	E4	E5
Loop 1	1			Start: sample change					
Multisyringe	2	1.00	2.5	Dispense	Off	Off	Off	Off	Off
Selection valve	3			Move to position 2					
Multisyringe	4	1.00	2.5	Pick up	On	Off	Off	Off	Off
Selection valve	5			Move to position 3					
Multisyringe	6	1.20	2.5	Dispense	On	Off	Off	Off	Off
Loop 2	7			Start: for replicate					
Multisyringe	8	0.15	2.5	Dispense	Off	Off	Off	Off	Off
Selection Valve	9			Move to position 2					
Multisyringe	10	0.15	2.5	Pick up	On	Off	Off	Off	Off
Selection valve	11			Move to position 1					
Multisyringe	12	0.21	2.5	Dispense	On	Off	Off	Off	On
Multisyringe	13	Fill	5	Priming	Off	Off	Off	Off	Off
Ocean optics	14			Start measure					
Multisyringe	15	0.15	1	Dispense	On	On	On	On	On
Multisyringe	16	0.15	1	Dispense	On	Off	Off	Off	Off
Ocean optics	17			Stop measure					
Multisyringe	18	Fill	5	Priming	Off	Off	Off	Off	Off
Loop 2	19			End: repeat 3 times					
Autosampler				Position change					
Multisyringe	20	1.50	5	Dispense	On	Off	Off	Off	Off
Selection valve	21			Move to position 2					
Multisyringe	22	1.00	5	Pick up	On	Off	Off	Off	Off
Selection valve	23			Move to position 1					
Multisyringe	24	2.00	5	Dispense	On	Off	Off	Off	Off
Multisyringe	25	Fill	5	Priming	Off	Off	Off	Off	Off
Loop 1	26			End: repeat *n* times					

2.6. Variable optimizations

In order to achieve the most efficient performance in terms of highest analytical sensitivity and lowest deviation of signals, some experimental parameters were investigated. Since there were numerous variables to be optimized, variables were divided in two groups: two variables optimized by univariate approach and the rest by multivariate approach. This decision was adopted in order to avoid the difficult interpretation of results of experimental design when several variables are optimized together. Thus, taking into account results of preliminary assays, the pH effect and the dispensing flow rates of sample and reagents were optimized by univariate approach. The catalytic reaction for cobalt determination should be carried out at basic media. So, the univariate approach was performed for refining the precise pH value. The flow rate was evaluated in order to reach the maximum value for high sampling rate and at the same time avoiding an overpressure. So, the conditions for the best response were established and therefore the interpretation of results was simplified.

For the second group, the optimization was carried out using a multivariate approach since it is an efficient tool in the process of optimizing analytical methods, allowing the simultaneous study of the relationship between responses and factors, and constructing a mathematical model through response surface methods [27]. Firstly, a screening for three variables (alizarin and hydrogen peroxide concentrations and reagent temperature) was carried out applying a 2^k full factorial design in order to study the effects of individual variables and their second order interactions. Finally, the critical values of the variables which affect significantly to the system were obtained by a face centered central composite design.

3. Results and discussion

3.1. Analytical response

Previous assays were performed to choice the best analytical response between alizarin and alizarin red S. For this purpose, batch tests were conducted following a procedure described elsewhere [28]. 1 ml of 50 μg L^{-1} Co solution, 1 ml of buffer at pH 9.2, 1 ml of 0.35 mol L^{-1} H_2O_2 and 1 ml of 0.0033 mol L^{-1} alizarin and alizarin red S solutions, both stabilized with 0.03 mol L^{-1} NaOH, were employed to carry out the assay. The obtained results showed a stronger analytical signal (in terms of coloration intensity) and a faster reaction when alizarin reagent was used. In addition, taking into account the lower toxicity of alizarin, it was selected for further analysis.

Later assays were accomplished to establish the mode to carry out the measurement, i.e. the initial-rate mode and the fixed time mode. In the first approach, once the sample-reagent plug was in the LWCC, the flow was stopped and the analytical signal was registered each second. In the case of fixed time, the absorbance measurements were made when the absorbance was maximum. In order to establish this measuring time, previous assays were carried out varying the length of the reaction coil and the flow rate of the mixture plug towards the detector. Best results were obtained with the fixed time mode, since sampling rate and precision were higher. In this sense, other authors reported that the use of flow analysis on fixed time mode by a catalytic kinetic reaction offered stable reaction conditions, speedy solution handling and a higher precision [29]. Thus, the further assays were made in this way.

3.2. Effect of pH and dispensing flow rates

Based on previous works [9,10], the effect of pH was examined over the range 9.0–9.6 by using 0.025 mol L^{-1} sodium tetraborate and adjusted with 0.1 mol L^{-1} of NaOH. The assays were carried out using 50 μg L^{-1} Co(II), 100 μmol L^{-1} alizarin in 0.03 mol L^{-1} of NaOH and 0.35 mol L^{-1} hydrogen peroxide. Fig. 2a shows that the maximum net absorbance obtained was at pH 9.2. It confirmed that the reaction takes place at basic media and also that the pH range is very narrow.

The influence of dispensing flow rates for a simultaneous injection of sample, alizarin, buffer and hydrogen peroxide on analytical signal of cobalt was investigated within the range 0.5–3 ml min^{-1}. As can be seen in Fig. 2b, above 1 ml min^{-1}, as flow rate increases, the analytical signal decreases. It can be due to there is not enough time for an optimum mixture of reagents. A total flow rate of 1 ml min^{-1} for simultaneous injection of the four syringes was chosen for further experiments.

3.3. Optimization of the variables using experimental design

Firstly, a screening (2^k full factorial) was performed, involving the following variables (studied range in brackets): alizarin concentration (70–100 μmol L^{-1}), H_2O_2 concentration (0.2–0.5 mol L^{-1}) and reagent temperature (60–90 °C). For these experiments a 50 μg L^{-1} of Co(II) solution was used. The Pareto diagram and the ANOVA table including curvature, significance of variables and pure error were evaluated. The factorial design demonstrated that the effects of alizarin concentration and temperature were statistically significant in the studied experimental domain, while hydrogen peroxide concentration not showed significant effects. However, since significant interactions were observed between hydrogen peroxide concentration and the others variables, we decided also included in the response-surface method. Then, a face centered central composite design was performed with the above-mentioned variables changing their ranges following the trends of the screening assay. i.e., the ranges were set between 90 and 150 μmol L^{-1} for alizarin; 50 and 80 °C for reagent temperature, and 0.15 and 0.35 mol L^{-1} for hydrogen peroxide. In this case, ANOVA table including curvature,

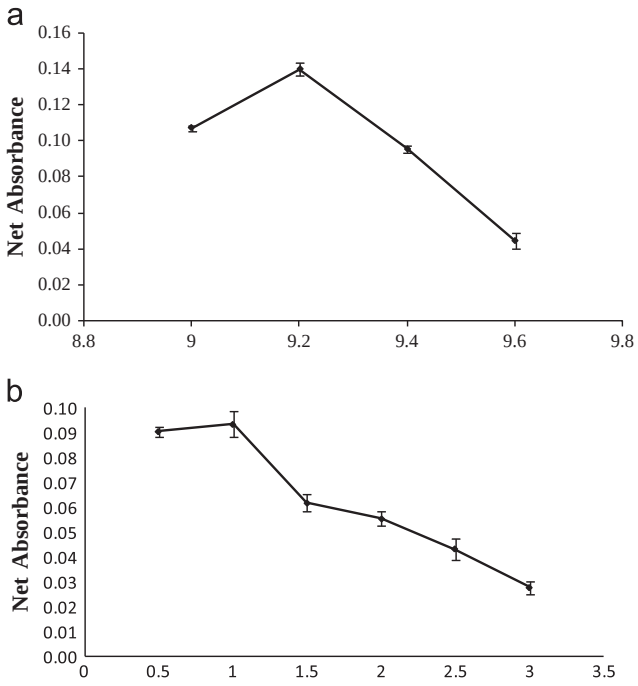

Fig. 2. Effect of changing (a) pH; (b) flow rate of sample and reagents. Operating conditions: (a) 50 μg L^{-1} Co(II), alizarin 100 μmol L^{-1} in 0.03 mol L^{-1} of NaOH and H_2O_2 0.35 mol L^{-1} and 0.025 mol L^{-1} of $Na_2B_4O_7 \cdot 10H_2O$. (b) 50 μg L^{-1} Co(II), alizarin 100 μmol L^{-1} in 0.03 mol L^{-1} of NaOH, H_2O_2 0.35 mol L^{-1}, 0.025 mol L^{-1} of $Na_2B_4O_7 \cdot 10H_2O$ at a pH of 9.2 adjusted with 0.1 mol L^{-1} of NaOH.

significance of variables, pure error and lack of fit, histogram of residuals and the adjust coefficient of the selected model (linear/quadratic main effects +2 ways interactions) were evaluated. Thus, the critical values of 124 μmol L^{-1} for alizarin, 0.22 mol L^{-1} for H_2O_2, and a temperature of 67 ± 2 °C were established. The optimal operation conditions are summarized in Table 2.

3.4. Analytical parameters

The analytical parameters were evaluated under the optimal experimental conditions. The calibration curve for cobalt determination was $y=0.0013x+0.004$ (net absorbance vs. Co concentration in μg L^{-1}, $r^2=0.9994$, $n=11$ within the range 1–200 μg L^{-1} Co). The absorbance value of the blank was 0.7186 ± 0.0003 and its repeatability was 0.04% (RSD), $n=17$.

Detection limit (DL) was calculated from $3\sigma b/S$, where σb is the blank standard deviation for ten replicates, and S is the slope of the calibration curve, while the quantification limit was calculated from $10\sigma b/S$. Thus, the detection limit for cobalt was 0.3 μg L^{-1} (Table 3). The repeatability and reproducibility were expressed as relative standard deviation (RSD). The repeatability was evaluated from 10 successive injections of 50 μg L^{-1} of cobalt, reaching a RSD of 0.4%. A reproducibility of 1.3% (RSD) was calculated from results obtained on different working days ($n=5$), using the same standard solution above-mentioned. Thus, the results obtained with the proposed system have a good level of precision. The sampling rate calculated for the proposed system being 30 h^{-1}.

The obtained detection limit is similar than that obtained by other authors with spectrophotometric detection by a FIA system (DL of 0.1 μg L^{-1} for cobalt), but its linear working range is more narrow (0.6–100 μg L^{-1}) [11]. A DL of 2.4 μg L^{-1} of cobalt (12-times higher) has been reported even exploiting atomic absorption spectrometry as detector [30]. Recently, a manual FIA-spectrophotometric system reaches a low detection limit (0.05 μg L^{-1} cobalt), although it has a linear working range extremely narrow (up to 2 μg L^{-1}) [7].

Table 2
Optimized experimental conditions for cobalt determination[a].

Operation conditions	
Dispensing flow rate	1 ml min^{-1}
Holding coil, HC	21 cm (0.8 mm i.d.)
Reaction coil, RC	100 cm (0.8 mm i.d.)
Sample volume	0.150 ml
Alizarin volume	0.06 ml
H_2O_2 volume	0.06 ml
Buffer volume	0.06 ml
Alizarin concentration (in 0.03 mol L^{-1} NaOH)	124 μ mol L^{-1}
H_2O_2 concentration	0.22 mol L^{-1}
Buffer pH	9.2
Temperature of reagents	67 °C

[a] The values are per assay.

Table 3
Analytical parameters of the proposed method.

Parameter	Value
Linear working range (μg L^{-1})	1–200
Detection limit (μg L^{-1})	0.3
Limit of quantification (μg L^{-1})	1
Sampling rate (h^{-1})	30
Repeatability (RSD, %) ($n=10$)	0.4
Reproducibility (RSD, %) ($n=5$)	1.3

Table 4
Results of the analysis of pharmaceutical products using the developed method.

[a]Sample	Co in pharmaceutical preparation (μg L^{-1})	Co found (μg L^{-1})	Recovery (%)
[b]Cyanocobalamin	86.4	83.1 ± 0.9	95
[c]Cobalt gluconate	145	137.1 ± 0.3	94
[d]Hydroxicobalamin	84.6	80.9 ± 0.4	95

[a] Three replicates, and four peaks for each replicate.
[b] Cyanocobalamin from Optovite B_{12} injectable solution (Normon S.A.), containing 0.0434 mg of cobalt in 2 ml of excipient.
[c] Cobalt gluconate from Labcatal drinking vial, containing 0.0725 mg of cobalt and niquel gluconate (0.5 mg) in 2 ml of excipient.
[d] Hydroxicobalamin HCl from Hidroxil B_{12}–B_6–B_1 tablet (Almirall S.A.), containing 0.021 mg of cobalt, pyridoxine HCl (250 mg), thiamine HCl (250 mg).

3.5. Interferences

The effect of the presence of other ions on the trace determination of Co(II) by the proposed method was studied. It has been assumed that an element does not interfere when the absorbance variation is lower than ± 10%. For this purpose were prepared a series of solutions containing 20 μg L^{-1} of cobalt, adding the following chemical species: Ca^{2+}, Mn^{2+}, Fe^{3+}, Zn^{2+}, Ni^{2+} and Cu^{2+}, which were selected as potential interferents. The sample of cobalt gluconate contents also nickel gluconate. Thus, nickel was considered as potential interfering ion. Moreover, multivitamin and mineral supplements can content the studied foreign ions, constituting potential interferences. On the other hand, alizarin reagent possesses a strong affinity with cooper, nickel and calcium [18].

The tolerated concentrations of the studied elements were 1000 μg L^{-1} for Mn and Ca, whereas for Ni was 600 μg L^{-1}, 500 μg L^{-1} Fe, 400 μg L^{-1} Zn and 350 μg L^{-1} Cu.

3.6. Determination of Co(II) in pharmaceutical samples

The proposed analytical procedure was satisfactorily applied to the determination of cobalt in cobalt gluconate and in different forms of vitamin B_{12}, cyanocobalamin and hydroxicobalamin. The t-test for comparison of means revealed there were no significant differences at the 95% confidence level between the referenced value and the results obtained with the proposed method. In order to make possible this statistical comparison, we assume that the precision of the cobalt concentration in pharmaceutical samples is 5%. The results of analysis of the three pharmaceutical samples are shown in Table 4. As can be seen, recoveries were fairly good, higher than 90% in all cases.

4. Conclusions

An automatic procedure based on kinetic–catalytic reaction of oxidative coupling of alizarin for cobalt determination becomes in attractive and economical for routine analysis, due to the minimum consumption of reagents and its high frequency of analysis.

The coupling of MSFIA technique together with a LWCC–UV–vis demonstrated that the determination of cobalt(II) in samples of pharmaceutical products was satisfactory performed. The use of alizarin–hydrogen peroxide for the kinetic–catalytic reaction with temperature provided a rapid and sensitive determination without require a pre-concentration step, i.e. the determination of cobalt is carried out directly. This method has the advantage of an elevated precision (RSD < 1%) as well as a high sample throughput (30 h^{-1}). Furthermore, the high tolerance levels of interfering ions

on the analytical determination of cobalt enhance the ability to measure concentrations at trace levels.

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