Automatic determination of cobalt at the submicrogram per millilitre level using a flowthrough spectrophotometric sensor

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Abstract

A flowthrough spectrophotometric sensor for the determination of cobalt at the nanogram per millilitre level using pyridoxal 4-phenylthiosemicarbazone as reagent and integrated preconcentration and detection in the flow cell is proposed. The method is highly selective for cobalt(II); it features detection and determination limits of 0.02 and 0.06 μg ml⁻¹ respectively, and a linear range of at least 0.04–18 μg ml⁻¹. The method is subject to very few interferences because the strongly acidic medium used prevents the formation of most complexes of the reagent with other metal ions. The method was applied to the determination of cobalt in pharmaceutical preparations.

Keywords: Cobalt; Flow injection; Flowthrough sensor; Pharmaceutical preparations; Spectrophotometry

1. Introduction

Several procedures for the flow-injection (FI) determination of cobalt, many of which use spectrophotometric or chemiluminescence detection, have been reported in the last few years. The spectrophotometric technique has been used in methods where cobalt acts as a catalyst for the oxidation of various coloured substances [1–5]. Differential kinetics have also been used for this purpose, particularly in the simultaneous determination of cobalt and nickel with salicylaldehyde thiosemicarbazone as reagent [6,7]. pH gradients have also been exploited for this purpose, in conjunction with 4-(2-pyridylazo) resorcinol [8]. However, the determination of cobalt usually involves gallic acid [9–11] or luminol [12,13], which are oxidized by hydrogen peroxide in the presence of the metal.

Both types of method are generally highly sensitive, with detection limits of a few nanograms or picograms per millilitre, which allows the determination of cobalt in biological samples and drinking waters. However, they are subject to a number of interferences which call for prior separations that normally make them time-consuming and unreliable.

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Recently, a very selective method for the FI spectrophotometric determination of cobalt was developed. Based on the formation of a highly stable complex between cobalt and pyridoxal 4-phenyl-3-thiosemicarbazone (PPT) in a strongly acidic medium, the method is not very sensitive [14]. In this work, we improved its sensitivity and selectivity by using a flow manifold integrating preconcentration and detection in a sorbent material packed in a flow cell for the determination of
Table 1
Optimum values of FI variables

<table>
<thead>
<tr>
<th>Sample flow rate (ml min⁻¹)</th>
<th>Reagent flow rate (ml min⁻¹)</th>
<th>3 M HClO₄ flow rate (ml min⁻¹)</th>
<th>Eluent flow rate (ml min⁻¹)</th>
<th>Length of reactor R₁ (cm)</th>
<th>Length of reactor R₂ (cm)</th>
<th>Length of reactor R₃ (cm)</th>
<th>Injection loop volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.6</td>
<td>0.4</td>
<td>0.6</td>
<td>0.6</td>
<td>116 (0.5 mm i.d.)</td>
<td>150 (0.5 mm i.d.)</td>
<td>35 (0.5 mm i.d.)</td>
<td>2.4 (in order to ensure approximately 30 s of constant absorbance before elution)</td>
</tr>
</tbody>
</table>


cobalt. This modified method provides better selectivity and sensitivity, with detection and determination limits of 0.02 and 0.06 μg ml⁻¹ respectively. The method relies on the recently developed flowthrough sensor technology [15,16] by which retention and detection are integrated in a FI system [17,18]. In this case, successive passage of the complex (previously formed in the flowing stream) and eluent through the flow cell and continuous photometric monitoring of the process provides the analytical information needed to determine the cobalt. The proposed method was used for the automatic on-line determination of this ion in vitamins contained in pharmaceutical preparations (it is present in the essential vitamin B₁₂) [19]. This vitamin takes part in a wide variety of metabolic processes involving factors that affect growth, homopoiesis and preservation of the integrity of nerve cells.

2. Experimental

2.1. Reagents

All chemicals were of at least analytical-reagent grade, and bidistilled, deionized water was used throughout.

A stock solution of Co(II) was prepared from the nitrate (Merck P.A.) and standardized complexometrically. Standards of working strength were made by appropriate dilution as required.

A 0.05% solution of PPT was prepared by dissolving 0.05 g of the reagent in 30 ml of N,N-dimethylformamide (DMF) and diluting to 100 ml with water. The reagent was synthesized according to a procedure described elsewhere [20].

A 30% solution of perchloric acid in DMF–water (3:10, v:v) was used as eluent.

A 3 M perchloric acid solution was also used. C₃₀₂ in a Sep-Pak cartridge (Waters, Millipore Division), which demonstrated its resistance to the eluent, was used for retention–detection of cobalt in the flow cell.

2.2 Apparatus

A Hewlett-Packard 8452A diode-array detector interfaced to a Vectra ES:12 computer which delivered results through an HP Think-Jet printer was used. The flow manifold consisted of a Gilson Minipuls-3 peristaltic pump, a Rheodyne Type 50 six-port rotary valve and a Hellma OS 0.200 flow cell.

2.3. Manifold and procedure

Three different configurations were tested (Fig. 1). In the first configuration, the reagent was merged with 3 M HClO₄ to prevent the formation of complexes of other cations along reactor R₁; in this way, the reagent was retained first on the sorbent material (preconcentration step). When a preset volume of reagent had passed through the flow cell, Vₐ was switched in order to introduce the sample. In this way, the reaction took place in the flow cell (reaction step) and the detector continuously monitored the absorbance increase (detection step). After the reaction was complete, Vₐ was switched, thereby allowing the reaction product to be eluted from the sorbent material, which was thus made ready for a new sample.

In the second configuration, the sample was merged with the reagent along reactor R₁, and the
complex formed was merged with 3 M HClO₄ along reactor R₂, where a strongly acidic medium destroyed the complexes of other metal ions. The cobalt complex was retained on the sorbent material packed in the flow cell (preconcentration step) and the detector continuously monitored the signal increase up to a constant value. As the injection valve V₁ was switched to the "inject" position, the eluent passed through the flow cell to remove the retained complex and make the sorbent material ready for a new sample.

The third configuration was similar to the previous one but the sample was used to fill the injection loop instead of being continuously circulated as the carrier, thus reducing sample consumption.

3. Results and discussion

Cobalt forms a yellow 1:2 complex with PPT (λ = 430 nm, ε = 1.35 × 10⁴ 1 mol⁻¹ cm⁻¹). The complex is stable in perchloric acid up to a concentration of 1.2 mol ¹⁻ (12%) [14], conditions under which most of the complexes of PPT with other cations are not formed. This reaction can be carried out with the reagent or complex retained on a sorbent material. Thus in preliminary assays several sorbent materials were tested in order to find the most appropriate for this purpose. In an acid medium, the cobalt complex is in cationic form, so cationic exchange resins (Dowex, Amberlite, SP-Sephadex) were assayed: however, neither the reagent nor the complex was retained. Other sorbent materials, such as aluminium oxide, silicagel and C₁₈, were tested, but only the latter effectively retained both the reagent and the complex. The maximum absorption wavelength of the cobalt-PPT complex retained on C₁₈ was 276 nm.

Of the buffered eluents tested, 30% perchloric acid in DMF-water proved to be the most efficient for removing the complex from the C₁₈ without damaging it.

The next task was to select the most advantageous manifold among those shown in Fig. 1. The manifold depicted in Fig. 1a provided a poor response because the reagent was packed onto C₁₈ phase before the passing of the sample, causing the saturation of the C₁₈ phase, so that subsequent passage of the sample through the sensor did not give a sensitive enough signal. The manifolds of Figs. 1b and 1c provided appropriate responses so that complex formed was passing directly through the sensor and the difference between blank and complex signals was better than the response provided by manifold 1a. Comparing manifolds 1b and 1c, the former was approximately 10 times more sensitive than the latter and so configuration 1b was finally selected.

3.1. Optimization of variables

The optimum reagent concentration was found to be 0.05% in DMF-water (3:10, v/v). The amount of DMF used was the minimum ensuring complete dissolution of the reagent, and DMF was also used in the eluent which also served as the carrier, in order to maximize peak height and ensure complete elution of the complex.

Based on the influence of the concentration of perchloric acid on the complex formation, 3 mol ¹⁻ was found to be the optimum concentration as it produced the lowest standard deviation even though the peak height was somewhat greater with 1.5 mol ¹⁻ perchloric acid. Obviously, once the different streams have merged, the acid concentration in the reaction plug will have been decreased by dilution.

The influence of the FI variables (stream flow rates, reactor lengths and injection volume) was studied and the optimum values found are summarized in Table 1.

Scans were performed in the kinetic mode at 276 nm for 150 s, in cycles of 2 s with an
Table 2

Analytical figures of merit for the determination of cobalt by the proposed method

<table>
<thead>
<tr>
<th>Linear range (µg ml⁻¹)</th>
<th>Calibration equation</th>
<th>Regression coefficient</th>
<th>RSD (%) (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.04 – 1</td>
<td>( y = 1.25 \times 10^{-4}x + 0.554 )</td>
<td>0.994</td>
<td>± 3.2 (0.2)</td>
</tr>
<tr>
<td>1 – 18</td>
<td>( y = 2.80 \times 10^{-5}x + 0.663 )</td>
<td>0.994</td>
<td>± 3.6 (2.0)</td>
</tr>
</tbody>
</table>

* v. Absorbance; x, cobalt concentration (µg ml⁻¹).

Table 3

Determination of cobalt in several synthetic samples using the proposed method

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Concentration added (µg ml⁻¹)</th>
<th>Concentration found (µg ml⁻¹) ± standard deviation (concentration, µg ml⁻¹) (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.080</td>
<td>0.079 ± 0.001</td>
</tr>
<tr>
<td>2</td>
<td>0.20</td>
<td>0.19 ± 0.02</td>
</tr>
<tr>
<td>3</td>
<td>0.50</td>
<td>0.56 ± 0.05</td>
</tr>
<tr>
<td>4</td>
<td>1.00</td>
<td>0.96 ± 0.02</td>
</tr>
<tr>
<td>5</td>
<td>2.0</td>
<td>2.2 ± 0.5</td>
</tr>
<tr>
<td>6</td>
<td>5.0</td>
<td>5.1 ± 0.5</td>
</tr>
</tbody>
</table>

integrating time of 1.5 s. The maximum signal measured corresponded to the time at which the analyte started to be eluted. Fig. 2 shows typical recordings obtained and the point for analytical measurements (marked with arrowheads). Under these final experimental conditions, a sampling rate of 15 samples h⁻¹ was reached.

3.2. Calibration curve and precision

In order to establish the optimal range for the determination of cobalt by the proposed method, several standard solutions of cobalt(II) were injected into the flow system under the optimum experimental conditions. From the data obtained, two linear calibration curves were obtained from 0.04 – 1 µg ml⁻¹ and from 1 – 18 µg ml⁻¹ cobalt (Table 2).

The detection and determination limits, defined as the concentrations of analyte giving signals equivalent to three and ten times respectively the standard deviation of the blank plus the net blank intensity, were calculated to be 0.02 and 0.06 µg ml⁻¹ respectively.

Various synthetic samples were readily resolved using pertinent calibration graphs (Table 3).

3.3. Interferences

The effect of various ions on the determination of cobalt by the proposed method was examined under the optimum working conditions. For this purpose, variable amounts of the ionic species tested were added to a 0.2 µg ml⁻¹ solution of Co(II) up to a maximum interferent:cobalt ratio of 225:1 mM; if any compound was found to interfere, the ratio was gradually lowered until the interference disappeared. The tolerated limits, defined as the interferent concentration (µg ml⁻¹) resulting in a deviation of less than ±5% in the analytical response, are shown in Table 4.

3.4 Application of the proposed method: determination of cobalt in pharmaceutical preparations

In a reaction flask 0.5 – 2 ml of vitamin ampoules and 3 – 6 ml of concentrated nitric acid were placed, and the mixture was refluxed until a colorless or pale yellow solution was obtained. Then, 0.5 1 ml of hydrogen peroxide was added and the mixture evaporated to a small volume by heating on a hot-plate to remove nitric acid. Next, the mixture was adjusted to pH 2 with NaOH solution and diluted to an appropriate volume (100 ml). Aliquots (10 – 20 ml) of this solution were determined using the recommended procedure. The standard-addition method was used and the results were obtained by extrapolation. Such results are given in Table 5 as the averages of three separate determinations. As can be seen, the cobalt concentrations determined by the proposed method are quite consistent with the specified values.
Influence of foreign species on the determination of cobalt(II) by the proposed method

<table>
<thead>
<tr>
<th>Foreign species</th>
<th>Tolerated ratio (M M⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pb(II), Ba(II), Ca(II), Be(I), Li(I), Sr(II), Na(I), K(I), Zr(IV), U(VI), W(VI), Ag(I), Fe(II), thiosulphate, sulphate, bromide, thiocyanate, oxalate, iodide, tartrate</td>
<td>( &gt; 725 )</td>
</tr>
<tr>
<td>Br(III), Th(I), tetraborate</td>
<td>150</td>
</tr>
<tr>
<td>Ni(II), Cd(II), Zn(II), Mn(II), Hg(I), Cr(III), Sb(III), phosphate, nitrite, arsenite, citrate</td>
<td>100</td>
</tr>
<tr>
<td>V(V), Mo(VI), arsenite</td>
<td>50</td>
</tr>
<tr>
<td>Hg(II), Al(III), Cu(II), Fe(III)</td>
<td>25</td>
</tr>
</tbody>
</table>

Maximum concentration tolerated for the interferent with respect to the analyte (µg ml⁻¹ in each case).

Table 5
Determination of cobalt in vitamin B₁₂

<table>
<thead>
<tr>
<th>Sample</th>
<th>Co content stated (µg ml⁻¹)</th>
<th>Co added (µg ml⁻¹)</th>
<th>Co found (µg ml⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nervobion “5000”</td>
<td>0.22</td>
<td>0.3</td>
<td>0.23 ± 0.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.6</td>
<td>0.86</td>
</tr>
<tr>
<td>B₁₂ Latino depot</td>
<td>0.22</td>
<td>0.3</td>
<td>0.19 ± 0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.6</td>
<td>0.78</td>
</tr>
<tr>
<td>Neuromade</td>
<td>0.22</td>
<td>0.3</td>
<td>0.217 ± 0.006</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.6</td>
<td>0.826</td>
</tr>
<tr>
<td>Optovite</td>
<td>0.35</td>
<td>0.3</td>
<td>0.35 ± 0.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.6</td>
<td>0.92</td>
</tr>
<tr>
<td>Neurodavur plus</td>
<td>0.34</td>
<td>0.3</td>
<td>0.34 ± 0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.6</td>
<td>0.93</td>
</tr>
</tbody>
</table>

Composition of the pharmaceutical preparations:

*a* Composition of the pharmaceutical preparations:

*a* Nervobion “5000” (Merck). Vitamin B₁₂ (cyanocobalamin), equivalent to 0.2174 mg of cobalt, cocarboxylase (100 mg), pyridoxal-5-phosphate (100 mg), excipient (lidocaine) up to 2 ml.

*b* B₁₂ Latino depot (Syntex Latino, S.A.). Vitamin B₁₂ (cyanocobalamin), equivalent to 0.04348 mg of cobalt, excipient up to 1 ml.

*c* Neuromade fuerte (Knoll). Vitamin B₁₂ (cyanocobalamin), equivalent to 0.2174 mg of cobalt, pyridoxine CLH (100 mg), thiamine CLH (100 mg), lidocaine (6 mg), excipient up to 3 ml.

*d* Optovite B₁₂ (Normon, S.A.). Vitamin B₁₂ (cyanocobalamin), equivalent to 0.04348 mg of cobalt, excipient up to 2 ml.

*e* Neurodavur plus (Belmac, S.A.). Vitamin B₁₂ (hydroxycobalamin), equivalent to 0.2098 mg of cobalt, thiamine·HCl (50 mg), pyridoxine·HCl (50 mg), dexamethasone phosphate sodium (1.6 mg), lidocaine·HCl (12.5 mg), excipient up to 2.5 ml.

4. Conclusions

Automatic methods based on flowthrough sensors offer interesting assets such as simplicity, rapidity, low cost and flexibility. The proposed method exhibits selectivity (iron being the main interference, at a 25-fold level with respect to cobalt) and high sensitivity, with a detection limit of 0.02 µg ml⁻¹. It is more selective and simple than other continuous FI spectrophotometric
methods, because it requires neither separation nor masking of foreign species. The proposed method was successfully applied to the determination of cobalt in vitamins.

Acknowledgement

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References