



An air carrier flow system for the spectrophotometric determination of water in biodiesel exploiting bleaching of the cobalt chloride complex



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ABSTRACT

Water content is an important parameter in biodiesel quality control, as excess of this substance may lead to biofuel hydrolysis, microorganism proliferation, and alterations in the oxidative stability of the biofuel. The threshold limit is established as 200 mg kg^{-1} and the determination is usually based on Karl Fischer titration. In this work, a simple, reliable and environmentally friendly procedure is proposed for water determination in biodiesel by exploiting a multicommutated flow system with air carrier stream. The method relies on the color fading of the cobalt chlorocomplex in the presence of the analyte, which is monitored by spectrophotometry. A linear response was observed from 100 to 5000 mg kg^{-1} water, with detection limit, coefficient of variation ($n=20$) and sampling rate estimated as 25 mg kg^{-1} , 0.7% and 30 h^{-1} , respectively. The procedure consumes only $3.5 \text{ }\mu\text{g}$ of CoCl_2 and generates $750 \text{ }\mu\text{L}$ of waste per determination. Results obtained by using the standard additions method agreed with those attained by the Karl Fischer titration at the 95% confidence level.

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1. Introduction

Use of biodiesel fuel has increased worldwide due to its low impact on the environment and as a source of renewable energy to replace diesel and other petroleum derivatives. As such, this biofuel should meet the recommended parameters to ensure its quality for both use and storage [1] as well as to avoid emission of toxic substances.

Water determination is important for the quality control of biodiesel. The biofuel contains water as a result of the production process (washing to remove excess of alcohol and glycerol byproducts) and the absorption of atmospheric humidity. Excess of water may cause biodiesel hydrolysis yielding free fatty acids, which increases acidity and favors proliferation of microorganisms and corrosion of storage tanks [2]. Moreover, water may increase the formation of oxidation products during storage of the biofuel, which can damage the vehicle injection system and engine. In view of these aspects, water content in biodiesel is limited to 200 mg kg^{-1} [3,4] and this parameter should be monitored in the final product and during storage, which requires a fast and reliable procedure.

Water has been determined in different sample matrices by chromatography [5,6], spectrophotometry [7], fluorimetry [8], and mainly electroanalysis [9,10]. The Karl Fischer (KF) titration, the

most widespread method, has also been recommended for biodiesel analysis [11,12]. However, it presents some drawbacks such as the need for specialized equipment, rigorous sample manipulation, and the use of toxic reagents. Furthermore, biodiesel often contains additives and impurities that may favor side reactions during the coulometric KF titration [13].

Batch spectrophotometric procedures using alternative reagents, such as cobalt chloride [14], potassium dichromate [15], dithizone [16], and acridine orange [17] have been proposed for determination of water in various organic solvents. These procedures are time-consuming and involve several steps, thus impairing sample throughput and increasing the risks of systematic errors. Moreover, they are highly susceptible to sample contamination due to absorption of atmospheric humidity. Flow-based analytical procedures exploiting the KF reagent have been proposed to circumvent some disadvantages of the batch analogues [18–24]. In this sense, side reactions were minimized and sample throughput was increased by reducing the reaction time, while contamination from atmospheric humidity and contact of the analyst with the toxic reagents were diminished by sample processing in a closed system. On the other hand, sensitivity is usually worse than that achieved in the batch procedure and some interferences were not circumvented. The performance of flow injection spectrophotometric procedures for water determination in acetone exploiting different reagents was also evaluated [7].

In spite of the availability of some alternatives and the previously mentioned drawbacks, the KF method prevails in the analysis of biodiesel. This emphasizes the need for the

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development of rapid, reliable, and environmentally friendly procedures for biofuel analysis. The goal of the present work was therefore to develop a flow system with air carrier stream for the spectrophotometric determination of water in biodiesel based on discoloration of the cobalt chloride complex in ethanolic medium.

2. Materials and methods

2.1. Reagents and solutions

All solutions were prepared daily with anhydrous ethanol (99.9%, Merck, Germany) and analytical grade chemicals. The working standards were prepared within 100–5000 mg kg⁻¹ water, and a 5.25×10^{-3} mol L⁻¹ cobalt chloride solution was used as reagent. Air was used as a carrier to minimize the ethanol consumption and to avoid dispersion of the sample zone. Anhydrous ethanol was also used in the washing step.

Biodiesel samples were analyzed by the standard additions method. Solutions were prepared from 3.75 mL of biodiesel with the addition of 10–50 µL of water (two spikes per sample). Volumes were made up to 5.00 mL with anhydrous ethanol, which is miscible with both water and biodiesel. Samples with the same dilution in ethanol, but without water addition, were taken as reference.

2.2. Apparatus

The flow system was built up with four three-way solenoid valves (NResearch, USA), a Teflon[®] confluence connector, and 0.8-mm i.d. polyethylene tubes. A peristaltic pump (model CP 78017-10, Ismatec IPC, Switzerland) was equipped with Tygon[®] and Viton[®] tubes for propelling aqueous and ethanolic solutions, respectively. The solenoid valves were computer-controlled through a parallel interface coupled to a current drive based on the ULN2803 integrated circuit [25]. The detection system consisted of a multi-channel CCD spectrophotometer (USB 2000, Ocean Optics, Dunedin, FL) with a tungsten-halogen light source and optical fibers for radiation transport. A lab-made glass flow-cell (30-cm optical path and 1.7 mm i.d.) with glass waveguides [26] was used for the spectrophotometric measurements. The control software was developed in Visual Basic 6.0 (Microsoft, USA), and data acquisition was performed with the software supplied by the spectrophotometer manufacturer.

2.3. Flow system

The flow diagram showed in Fig. 1 was operated according to the switching course in Table 1. The multicommutation approach was exploited for solutions management by binary sampling [27] and air was used as the carrier stream [28]. The fluids were propelled at 0.6 mL min⁻¹ (S, R and E) and 1.0 mL min⁻¹ (air). In the position showed in Fig. 1, all liquids are recycled to the corresponding vessels and only air is flowing to the system. Each solution is sampled by switching the corresponding valve simultaneously to V₄, aiming to interrupt the air flow through the manifold and avoid segmentation of the sample zone. Volumes are defined by the flow rate and the switching times (see Table 1). The analytical cycle started (steps 1 and 2) by inserting 10 sample and reagent aliquots alternately (i.e. 10 sampling cycles). This corresponds to total sample and reagent volumes of 600 and 100 µL, respectively. The sample zone was transported by air through to reaction coil toward the detection cell (step 3). The analytical path was washed with ethanol by the actuation of valve V₃ before sample replacement (step 4).

The coiled reactor and the reagent solution were maintained inside a water bath with temperature controlled at 25.0 ± 0.1 °C

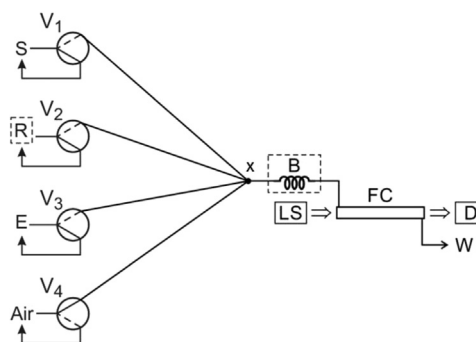


Fig. 1. Flow diagram of the proposed procedure. S=sample; R=reagent (5.25×10^{-3} mol L⁻¹ CoCl₂ in anhydrous ethanol); E=anhydrous ethanol; Air=carrier stream; V₁–V₄: three-way solenoid valves; x=Teflon[®] joint point; B=50-cm coiled reactor; LS=tungsten-halogen light source; FC=30-cm long flow cell (1.7 mm i.d.); D=detector; W=waste. Dashed lines indicate components inserted in a temperature-controlled water bath (25.0 ± 0.1 °C).

Table 1
Valve switching course for water determination in biodiesel.

Step	Description	Valves	Time (s)	Volume (µL)
1 ^a	Sample insertion	V ₁ , V ₄	6.0	60
2 ^a	Reagent insertion	V ₂ , V ₄	1.0	10
3	Measurement	0	10	–
4 ^b	Washing	V ₃ , V ₄	5.0	50

^a Ten sampling cycles.

^b Step required only for sample replacement.

due to the thermochromic properties of the reagent [29]. Off-set adjustment of the detection system was performed with ethanol inside the flow cell. Absorbance values were measured as peak height at 650 nm; all measurements were carried out in triplicate. The analytical signal was based on the difference between reference and sample signals, measured in the absence and presence of water, respectively.

2.4. Reference procedure

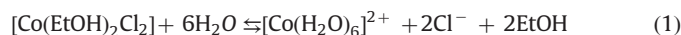
Coulometric KF titration for determining the water content in engine fuels, as recommended by ASTM [3], was employed as the reference procedure for accuracy assessment. A biodiesel aliquot was injected into the titration vessel of a KF apparatus in which iodine was coulometrically generated at the anode for the KF reaction. After titration of the water content, excess of iodine indicated the end point.

3. Results and discussion

The proposed procedure was based on discoloration of the CoCl₂ complex, which shows thermochromic and solvatochromic properties [29], i.e., the solution color is affected by the temperature and solvent nature. The aqueous solution of cobalt chloride is pink due to the formation of the octahedral hexaaquacobaltate complex, [Co(H₂O)₆]²⁺. On the other hand, anhydrous CoCl₂ is a blue solid and its solutions in different organic solvents (e.g. ethanol) are also blue due to the formation of chlorocomplexes. The reversible changes in color of this system caused by temperature are also known [29]. Despite being widely investigated, bleaching of the cobalt chloride complex was not still exploited for water determination.

The addition of low amounts of water into a CoCl₂ ethanolic solution causes fading of the blue color, simultaneously with the formation of the pink aquocomplexes. Thus, the hexaaquacobaltate

and chlorocomplexes coexist, and the equilibrium between them is shifted by increasing the amount of water in ethanolic medium (Eq. (1)). The tetrachlorocobaltate complex presents higher molar absorptivity than the hexaaquacobaltate, and thus the fading of the blue complex can yield higher sensitivity than the formation of the pink complex.



The solvent polarity affects the absorption spectrum of the cobalt chloride and blue solutions are also formed in other organic solvents, such as acetone [30]. Aiming to evaluate the effect of the solvent on the color fading in the presence of water, absorption spectra were obtained for $1.3 \times 10^{-3} \text{ mol L}^{-1}$ CoCl_2 solutions with and without 3000 mg kg^{-1} water in ethanol, acetone and a 1:1 ethanol/acetone mixture at room temperature (Fig. 2). Low water amounts effectively decrease the solution absorbance in anhydrous ethanol due to the displacement of the equilibrium (Fig. 2a). It was observed that the molar absorptivity of the reagent was higher in acetone ($294 \text{ L mol}^{-1} \text{ cm}^{-1}$) and in the solvent mixture ($346 \text{ L mol}^{-1} \text{ cm}^{-1}$) than in ethanolic medium ($126 \text{ L mol}^{-1} \text{ cm}^{-1}$), but the absorption spectra did not significantly change with water addition (Figs. 2b and c), thus indicating that formation of the octahedral complex is not favored in these media. Indeed, the blue complex formed in aprotic solvents, such as acetone, is quite stable to chromotropic changes [30]. Aiming to improve sensitivity, the reagent was prepared in ethanol for the implementation of the flow-based procedure.

In preliminary experiments with a 1-cm optical path cell, a linear response was observed from 1000 to 10000 mg kg^{-1} water ($A = 0.05 + 0.06 C_{\text{H}_2\text{O}}$, $r = 0.994$). Although the quantification limit was five-fold better than that reported previously for water determination in acetone [7], it is still unsuitable for biodiesel analysis, by considering the

threshold limit of 200 mg kg^{-1} [3,4]. Therefore, a 30-cm optical path flow cell was selected to improve sensitivity of the proposed procedure.

The blue color of the CoCl_2 ethanolic solution did not change significantly upon heating [29]. However, the measurement repeatability was affected by temperature changes. Coefficients of variation (500 mg kg^{-1} water, $n=20$) with and without temperature control were 0.7% and 3.6%, respectively. The reagent solution and the reaction coil were then kept in a temperature-controlled water bath to improve precision.

Procedure optimization was carried out by the univariate method aiming to improve analytical sensitivity. Both the magnitude of the reference signal relative to the cobalt chloride complex and that after bleaching by water were taken into account.

The effect of the reagent concentration (Fig. 3a) was evaluated by keeping the total volumes of sample and reagent at $350 \mu\text{L}$ (divided in 35 sampling cycles, i.e., 35 pairs of $10 \mu\text{L}$ aliquots of sample and reagent). This volume was required to completely fill the long path-length cell, whose internal volume was ca. $700 \mu\text{L}$. The analytical response increased up to 1.5 mmol L^{-1} , which is five-fold lower than that used in the flow system for water determination in acetone [7].

The influence of sample volume was evaluated by keeping CoCl_2 concentration in the sample zone to avoid reagent dilution due to changes in the volumetric fractions. Reagent volume was maintained at $10 \mu\text{L}$, and its concentration was varied from 1.5 to 6.8 mmol L^{-1} while the sample volume was varied from 10 to $80 \mu\text{L}$ (Fig. 3b). The analytical response increased with the sample volume up to $60 \mu\text{L}$, and then the sample:reagent volumetric fraction of 6:1 was selected for further experiments. The corresponding cobalt chloride concentration was 5.25 mmol L^{-1} to assure the same amount established in the previous study. Under these conditions, 10 sampling cycles ($70 \mu\text{L}$ each) were enough to

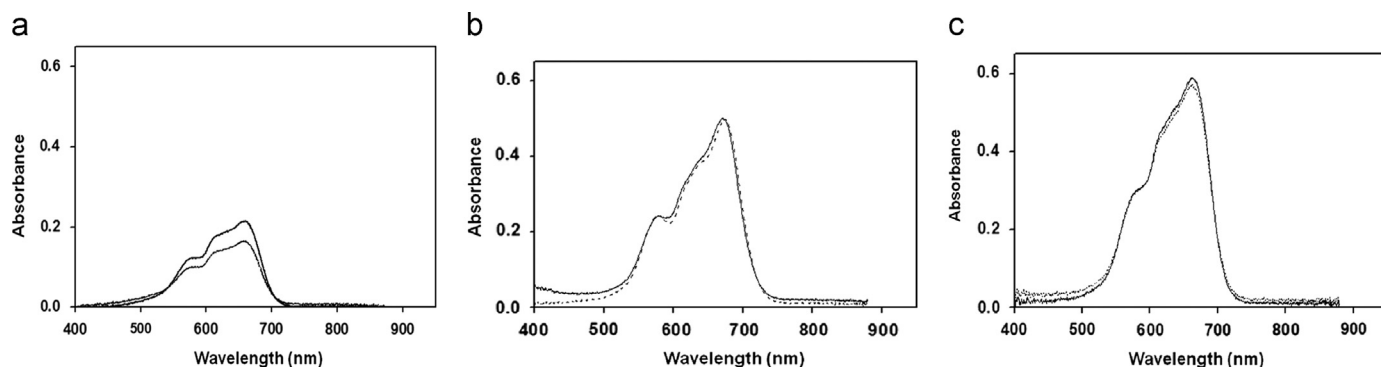


Fig. 2. Absorption spectra of $1.3 \times 10^{-3} \text{ mol L}^{-1}$ CoCl_2 in (a) ethanol, (b) acetone, (c) 1:1 ethanol:acetone solutions in the absence (upper curve) and presence (lower curve) of 3000 mg kg^{-1} water.

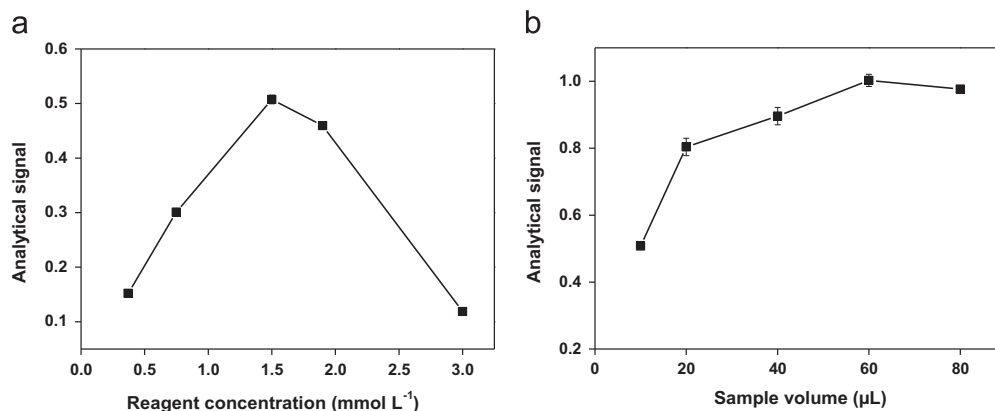


Fig. 3. Influence of (a) reagent concentration and (b) sample volume on the analytical signal. Error bars refer to the estimative of standard deviations from triplicate measurements.

completely fill the flow cell. As the reaction (Eq. (1)) is relatively fast (steady state is achieved in less than 5 s) and suitable mixing conditions were provided by the binary sampling approach, the reactor was maintained as short as possible (50 cm).

Under optimized conditions, a linear response was observed from 100 to 5000 mg kg⁻¹ water described by the equation $A = 0.007 + 0.118C_{H_2O}$ ($r = 0.998$, $n = 5$), with sensitivity 20-fold higher than that achieved with a 1-cm optical path flow cell. The discrepancy in relation to the theoretical value expected from Beer's law is due to the higher relative response provided by lower reagent concentration used in the experiments with the 1-cm cell. The coefficient of variation and sampling rate were estimated at 0.7% ($n = 20$; 1000 mg kg⁻¹) and 30 h⁻¹, respectively. The detection limit (25 mg kg⁻¹) was estimated as the lowest concentration that yielded an absorbance value significantly different of the reference signal at the 95% confidence level. For one determination, 60 µg of CoCl₂ and 600 µL of sample were required.

Comparison of the analytical features of the proposed procedure with those achieved in previously described flow-based ones is presented in Table 2. All procedures were applied to water determination in pure organic solvents, and the present work is the first application for determining this parameter in biodiesel, which is a considerably more complex sample. In spite of the lower sampling rate, the proposed procedure stands out because of better precision, lower detection limit, and by avoiding the use of toxic, expensive or hazardous reagents. For comparison, the coulometric Karl Fischer procedure designed for petroleum products, lubricating oils, and additives allowed water determination from 10 to 25000 mg kg⁻¹ [3]. As previously mentioned, the main drawbacks of this procedure are consumption of toxic reagents and solvents as well as risks of interferences by side reactions.

The analytical features obtained with the proposed procedure allowed for determination of water in biodiesel without any

sample pretreatment. However, the slopes of the analytical curves obtained in the presence of biodiesel were from 25 to 75% lower than that attained in ethanol, thus indicating a matrix effect. The standard addition method was then applied for sample analysis and the results (Table 3) agreed with those obtained by the Karl Fischer titration recommended by ASTM at the 95% confidence level. Results obtained by both procedures also showed comparable variances at 95% of significance.

4. Conclusions

A simple, precise and sensitive flow-based procedure was developed for the spectrophotometric determination of water in biodiesel, based on an inexpensive and low-toxic reagent. Sample and reagent consumptions, as well as waste generation, were minimized. The low detection limit achieved is an attractive aspect, as this parameter tends to become increasingly restrictive in biodiesel quality control (e.g. Brazilian legislation has reduced the threshold limit from 500 to 200 mg kg⁻¹ with a perspective to reduce to 100 mg kg⁻¹ in the near future). In addition, the achieved analytical features indicate that the proposed procedure can be applied to other kinds of samples, such as organic solvents. Moreover, contamination caused by atmospheric humidity was minimized by sample processing in a closed system. The proposed procedure is thus an inexpensive and reliable alternative for water determination in routine analysis of biodiesel.

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Table 2

Analytical features of flow-based procedures for the spectrophotometric determination of water in different samples.

Sample	Reagent	Linear Range (mg kg ⁻¹)	LD (mg kg ⁻¹)	CV (%)	SR (h ⁻¹)	Reference
Acetone	K ₂ Cr ₂ O ₇	500–50,000	300	2.4	–	7
Fugacillin	Karl Fischer	100–50,000	–	0.5	120	20
Organic solvents	Karl Fischer	100–50,000	–	0.5	120	21
Methanol	Karl Fischer	100–10,000	20	3.0	120	22
Organic solvents	Karl Fischer	100–2,000	–	1.0–2.0	120	23
Ethanol	Karl Fischer	200–5,000	–	3.0–9.0	–	24
Biodiesel	CoCl ₂	100–5,000	60	0.7	30	This work

LD: limit of detection; CV: coefficient of variation; SR: sampling rate.

Table 3

Mean values and uncertainties ($n = 3$) for determination of water in biodiesel by the proposed and reference procedures.

Sample	Water (mg kg ⁻¹)	
	Proposed procedure	Reference procedure [3]
1	118 ± 1	112 ± 1
2	272 ± 2	285 ± 3
3	337 ± 3	323 ± 5
4	197 ± 2	184 ± 2
5	214 ± 2	213 ± 2
6	296 ± 3	294 ± 3

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