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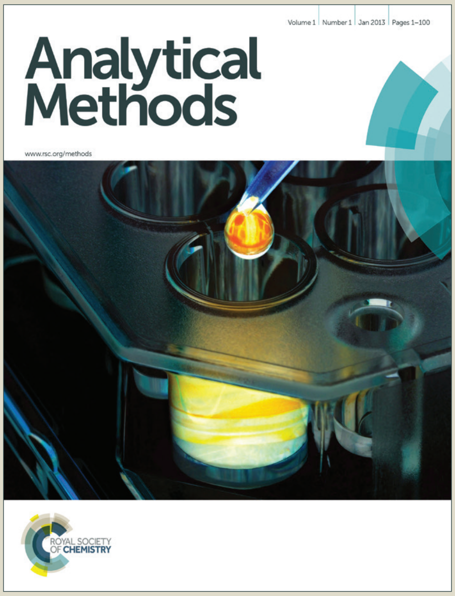

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# In-line single-phase extraction for direct determination of total iron in oils using CdTe quantum dots and a flow-batch system

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## Abstract

In this work, a novel method for direct determination of total iron in viscous samples (edible oils and biodiesel) is presented. Considering sensitivity and selectivity, the proposed method used in-line single-phase extraction and CdTe quantum dots (QDs). The method was automated employing a flow-batch system. The in-line single-phase extraction of iron consisted in the addition of a mixture of ethanol/chloroform (75:25, v/v) to dissolve the oil samples, followed by addition of an acid solution, $HNO_3$/HCl (3:1, v/v) to make the iron available. The analytical method was based on iron's capacity to establish surface interactions with CdTe QDs that result in a quenching of their fluorescent intensity proportional to the iron concentration. Various factors that may influence the fluorescence quenching of the iron, such as pH, sample volume, amount of the organic mixture, and acid solution concentration were studied. The maximum reproducibility of this fluorescence quenching occurred at pH 7.5. Phosphate buffered saline (PBS) 1.0 mol $L^{-1}$ was added before the reaction. Method validation and application to real samples showed that the analytical features of the developed method were quite satisfactory in terms of linearity ($r^2 = 0.997$), limit of detection (0.1 μg $g^{-1}$), precision (RSD < 1.6 %), and accuracy (recovery = 95.5 – 104.3 % range), when compared to others works in the literature. The proposed automatic method presented suitable robustness, a high sampling rate (79 $h^{-1}$), and lower waste generation per determination (0.900 mL), contributing to the basic principles of green chemistry.

*Keywords:* In-line single-phase extraction; Flow-batch system; CdTe quantum dots; Fluorescence determination of iron; Viscous matrices.

## 1. Introduction

Trace metallic elements are naturally present in oils due to two factors: (i) contamination from soil and fertilizers where the plant is grown, and (ii) contact with equipment during extraction, refining, and transportation. However, it is widely known that metals, although being present in low concentrations, have negative effects on the oxidative stability of both edible and non-edible oils. Iron, which has a catalytic effect on the autoxidation of both edible oils and biodiesel, is a common example of this problematic. Iron concentration is an important parameter for the quality control of oils, but due to their high organic contents, its accurate determination remains an analytical challenge.[1,2]

Several methods have been developed and applied to the determination of iron in edible oils and biodiesel, usually using atomic absorption, x-ray fluorescence, or emission spectrometry.[3,4] However, these methods have drawbacks including high cost, complex analyses, low sample throughput, and the necessary sample pretreatment methods to reduce matrix influences; these include dissolutions in organic toxic solvents, mineralization (calcination), acidic extraction, emulsification, sonication, and microwave digestion.[5]

Taking into account these disadvantages, a preferable iron determination may be explored using automated procedures based on the principles of flow analysis, which allows working with small volumes, saving both samples and reagents, and therefore contributing to green analytical chemistry.[4] However, when it comes to oils, the physic-chemical characteristics of these matrices, such as high viscosity and elevated organic load, make them difficult for analysis in flow systems, especially when using in-line pretreatments.[5,6]

An alternative to overcome such drawbacks is the use of a flow-batch system.[7] Recently, our research group has developed a procedure for automatic spectrophotometric determination of Fe(III) in oils, (without external pretreatment) using methanol-chloroform and methyl isobutyl ketone as solvents.[8] The system allowed us to obtain satisfactory figures of merit, and a high sampling frequency. However, the procedure might have been improved with respect to its sensitivity and selectivity, as well as for reducing its organic (toxic) solvents consumption.

During the last decade, several sensitive and selective methods have been developed involving the use of cadmium telluride quantum dots (CdTe QDs).[9,10] In most CdTe QD applications, the detection is based on signal quenching. More recently, attention has been focused on signal enhancement, mainly associated with QDs ability to sensitize distinct chemiluminescence systems.[11] When employing CdTe QDs in analyses involving oil matrices, it becomes necessary to solubilize the sample.

These disadvantages can be overcome by a simple strategy to transform the viscous analyte medium into a single-phase alcohol medium. Ribeiro and Rocha[12] described a simple and fast procedure for the direct determination of free glycerol in biodiesel exploiting a single-phase system. The procedure employed consists in simultaneously dissolving biodiesel and an acetyl-acetone reagent with anhydrous ethanol. A similar strategy was reported by Shishov et al.[13] where isopropyl alcohol was used to produce a single-phase system for automated determination of calcium (II), and magnesium (II) with eriochrome black T as the indicator. Both procedures are fast, simple, and inexpensive, without requiring mechanical agitation or centrifugation steps.

In this study for the first time, an automated method for direct determination of total iron in edible oils and biodiesel is proposed using in-line single-phase extraction, CdTe QDs, and a flow-batch system. The in-line single-phase extraction consists in the addition of an ethanol/chloroform mixture to dissolve the oil samples, followed by addition of an acid solution ($HNO_3$/HCl, 3:1) and buffer (1.0 mol $L^{-1}$ of pH 7.50 phosphate buffered saline) to bring forward the analyte. Iron's capacity to establish surface interactions with the CdTe QDs results in a quenching of the fluorescent intensity, which is proportional to the analyte concentration.

## 2. Experimental

### 2.1 Reagents, solutions and samples

All reagents were of analytical grade, and freshly distilled and deionized water ($> 18$ MΩ cm$^{-1}$) was used to prepare all solutions. The reagents were not subjected to any further purification.

Anhydrous ethanol and chloroform (Synth) were used to prepare a mixture of ethanol/chloroform (75:25 v/v) to dissolve the oil samples. To release the analyte nitric and hydrochloric acid (Merck) were used to prepare an acid solution ($HNO_3$/HCl, 3:1).

A 1.0 mol L$^{-1}$ of pH 7.50 phosphate buffered saline (PBS) solution (compound of 13.76 mol L$^{-1}$ NaCl, 0.27 mol L$^{-1}$ KCl, 0.97 mol L$^{-1}$ $Na_2HPO_4 \cdot 12H_2O$, 0.15 mol L$^{-1}$ $KH_2PO_4$) was used in the experiments.

A 100 µg g$^{-1}$ metal-organic iron standard was purchased from Quimlab (SRM 1079b NIST), and mineral oil (Sigma-Aldrich) was used for the dilutions of the metal-organic standard solution.

For the synthesis of the CdTe QDs the following reagents were used: sodium borohydride ($NaBH_4$, 99%), tellurium powder (200 mesh, 99.8%), cadmium chloride hemi (pentahydrate) ($CdCl_2 \cdot 2.5\ H_2O$, 99%), and 3-mercaptopropionic acid (MPA, 99%) purchased from Sigma-Aldrich. To adjust the alkalinity of the reaction medium, a 1.0 mol L$^{-1}$ NaOH solution was used.[11]

The methodology was applied to six commercial edible oils (olive, soybean and sunflower), and six biodiesel samples (B100) originated from different feedstocks (soybean, sunflower, maize).

### 2.2 Apparatus

Measurements in the automatic flow-batch system were carried out by a multi-channel CCD spectrophotometer (model USB4000, Ocean Optics), with a tungsten-halogen light source (LS-1-LL, Ocean Optics), and two 100 µm i.d. optical fibers.

To characterize the synthesized nanocrystals, the QD absorption spectra were obtained using a UV-Vis spectrophotometer (model 8453, Hewlett-Packard). The fluorescence measurements were performed with a spectrofluorimeter (model FL3-11, Fluorolog-3), equipped with a xenon discharge light source (450 W). The slits can vary from 1 to 20 nm. Those selected were 5 nm for excitation, and 10 nm for emission. A quartz fluorimetric cell with a 10 mm optical path, and 220 µL of internal volume (type 73.2 F-Q-10, Starna cells, USA) were used.

For comparison purposes the oil samples were analyzed using a Shimadzu AA6800 atomic absorption spectrometer with a longitudinally heated graphite tube atomizer, equipped with a Shimadzu ASC-6100 auto-sampler, and pyrolytic-coated graphite tubes. Argon 99.996% was used as purge and protective gas. A single element hollow cathode lamp for iron (Hamamatsu Photonics, $\lambda_{máx}$: 248.33 nm), operated at 4 mA was used as a light source. The monochromator spectral band pass (slit) was 0.7 nm.

### 2.3. Synthesis of CdTe QDs

CdTe QDs were synthesized as previously described[11] with some modifications. Briefly, NaHTe solution was prepared by reaction between $NaBH_4$ ($1 \times 10^{-3}$ mol), and tellurium powder ($0.4 \times 10^{-3}$ mol) in $N_2$ saturated water (20 mL). The reaction mixture was heated to 80ºC for 30 min under $N_2$ flow to get an intense clear red solution. This NaHTe solution was stored for further use at room temperature still under the protection of $N_2$.

The NaHTe solution was then transferred to another flask containing $CdCl_2$ ($4 \times 10^{-3}$ mol) and MPA ($7 \times 10^{-3}$ mol) in a 100 mL $N_2$ saturated water solution. The pH of the solution was adjusted to 11.5 by adding 1.0 mol $L^{-1}$ NaOH solution. The $Cd^{2+}$ : $Te^{2-}$ : MPA molar ratio was fixed at 1 : 0.1 : 1.7. The CdTe QD's size was tuned by varying the heating time. In order to remove the contaminants, purification of the QDs was performed by precipitation in absolute ethanol. The precipitate fractions were subsequently centrifuged, vacuum dried, and kept in a refrigerator.

The nanocrystals size for the synthesized CdTe QDs was calculated as shown in Eq. (1):[14]

$$D = (9.8127 \times 10^{-7})\,\lambda^3 - (1.7147 \times 10^{-3})\,\lambda^2 + (1.0064)\,\lambda - 194.84 \qquad (1)$$

where $D$ is the diameter or size of the nanocrystals (nm) and $\lambda$ is the wavelength of maximum absorbance corresponding to the first excitonic absorption peak of the crystal.

The CdTe QD aqueous solution molar concentration was determined by appraising the extinction coefficient ($\varepsilon$), calculated as shown in Eq. (2):[14]

$$\varepsilon = 3450\,\Delta E\,(D)^{2.4} \qquad (2)$$

where $\Delta E$ is the transition energy corresponding to the first absorption peak expressed in eV. $D$ (nm) is the size of the CdTe QDs. Knowing both $\varepsilon$ and the absorbance peak of the nanocrystal solution; the molar concentration was calculated by applying the Lambert-Beer's law.

### 2.4. Flow-batch system

A schematic diagram of the flow-batch proposed is shown in Fig. 1. The homemade mixing chamber (MC) was built in PTFE (polytetrafluoroethylene). It has a total volume of 2.0 ml and three quartz windows (W1, W2, and W3) mounted at 180º and 90° from each other (1 cm optical path).

The flow-batch (FB) consists of five three-way solenoid valves ($V_S$, $V_E$, $V_A$, $V_B$,$V_{QDs}$, and $V_W$) model EW-01540-13 (Cole Parmer); polyethylene tubing connectors with 0.8 mm id; a peristaltic pump (PP) model 78002-00 (Ismatec), Fluor-elastomer pumping tubes with 1.0 mm i.d. were used for propelling all fluids.

The additions of the sample or standard solution (S), ethanol/chloroform solution (E), acid solution (A), PBS buffer solution (B), CdTe QDs solution (QDs) into the MC were performed switching ON valves $V_S$, $V_E$, $V_A$, $V_B$, $V_{QDs}$, respectively. A stirring bar (SB) performs the homogenization of the mixture in the MC under the action of the magnetic stirrer (MS) and the MC is emptied switching ON the valve $V_W$.

Data acquisition, solenoid valves, peristaltic pump were computer controlled using an USB interface (USB6009, National Instruments) and software developed in LabVIEW 2013 (National Instruments).

## 2.5. In-line operation procedure

Before starting the analytical procedure, working solutions for each channel are pumped for continuous recirculation to their respective reservoirs. Then the valves $V_S$, $V_E$, $V_A$, $V_B$ and $V_{QDs}$ are simultaneous switched ON for a time interval of 1.50 s, and the working solutions (S, E, A, B, QDs) are pumped towards the MC to fill the channels between the valves and the chamber. Then immediately, the discard valve $V_W$ is switched ON for 5.0 s and MC is emptied using the peristaltic pump (PP). This channels filling procedure is very important and must be carried out whenever there is a change of the working solutions in reservoir.

The analyzer was operated as described in Table 1 for the direct determination of iron in edible oils and biodiesel using a portable spectrofluorimeter for detection. As the rotation speed of the peristaltic pump is computer controlled, a flow rate of 18.1 ± 1.2 μL $s^{-1}$ (n = 20) was employed for the edible oil and biodiesel sampling (S) and of 148.2 ± 1.3 μL $s^{-1}$ (n = 20) was used for the ethanol/chloroform solution (E), acid solution (A), buffer solution (B), and CdTe QDs solution (QDs) of the MC. Stirring was constant inside MC during the whole process. However, after additions of the solutions extra time of 2 s was employed for homogenization (steps 2, 4, 6, and 8). This extra time allowed for a reproducible analytical response.

The differing samples or standard solution (adding 25 μL by $V_S$), and the solvent ethanol/chloroform (adding 600 μL by $V_E$), were added simultaneously to the MC (step 1). Following, came acid, buffer, and CdTe QDs solutions (steps 3, 5, and 7, respectively), each added to the mixing chamber. Finally, the analytical signal was measured inside the MC (step 9), and all of its content was aspirated for waste (step 10, removing 900 μL by $V_W$).

Afterwards, the system carried out a cleaning cycle, simultaneously adding ethanol/ chloroform solution, and a new differing sample or standard (step 11); this procedure is necessary for cleaning of the MC, and for filling the channel between valve $V_S$ and the MC with new sample or standard. The excess of the solutions in the MC were aspirated towards waste (step 12). The cleaning cycle was completed by addition of the acid solution (step 13), homogenization (step 14), and discard (step 15).

During steps 2 through 10 shown in Table 1, the sample was manually replaced by the new sample in the intake tube (recycled to the reservoir); this period of about 11 s is sufficient for this procedure. For in-line blank preparation, mineral oil was added through the valve $V_S$, and the methodology for analysis is similar to described for the sample and standard solution.

## 2.6. Reference method

For comparison, the proposed flow-batch system was evaluated against a graphite furnace atomic absorption spectrometer (GFAAS) method. The samples were mineralized at 550 °C as previously described.[15] Ten grams (10 g) of sample (edible oils and biodiesel) were weighed directly into quartz crucibles. In sequence, 0.5 g of magnesium oxide was added. The mixture was calcinated gradually to 550 °C. (1 h at 100 °C, 1 h at 180 °C, 5 h at 250 °C, 1 h at 300 °C, and 2 h at 550 °C) in order to avoid sample loss. After cooling down carefully to room temperature, the ashes were dissolved directly in the crucible with small portions of an aqueous 1.0 mol $L^{-1}$ $H_2SO_4$ solution (total volume 25 mL), and gradually transferred to a 50.0 mL volumetric flask. Standard solutions were prepared from 1.0 to 10.0 µg $Kg^{-1}$. The analytical signals (absorbance) were measured at a maximum absorbance of around 248.3 nm. The analysis of each sample was performed in triplicate and the concentrations were calculated from the analytical curve.

## 3. Results and discussion

### 3.1. Analyte extraction

The determination method is based on the interaction of iron with CdTe QDs, reducing their photoluminescence emission by means of a quenching mechanism.[16] To eliminate the procedure of iron extraction from viscous matrices to an aqueous medium, we carried out the reactions in an organic based media, a single-phase system.[13] The criterion for the choice of the solvent and the mixing ratio was its ability to be well mixed with the edible oils and biodiesel in order to provide high repeatability.

It worth highlighting that CdTe QDs are insoluble in ethanol and chloroform, and when precipitating they exhibit a pronounced decrease of fluorescence. However, we observe that the addition of $HNO_3$/HCl 3:1 (v/v) and PBS buffer solutions, and homogenization in the sequence presented in Table 1 (1 to 8 steps) avoid CdTe QDs precipitation and a concomitant decrease of fluorescence.

The results shown in Fig. 2a suggest that anhydrous ethanol and chloroform mixing 75:25 (v/v) is the most suitable solvent for in-line dilution of viscous samples. The acid solution of $HNO_3$/HCl 3:1 (v/v) was the most effective for the extraction of all iron remnants in the sample (Fig. 2b). Using acid solution without direct organic extraction is not effective or reproducible due to formation of emulsions and/or non-mixing of the phases. In fact, the joining of the two extractions allowed greater data reproducibility.

### 3.2. Optimization of the reaction conditions

As previously reported in the literature,[16] the larger CdTe QDs are not sufficiently sensitive to iron. However, in the study, smaller nanocrystals were clearly more sensitive to the metal, providing a significant decrease in fluorescence intensity. Thus, the size of 1.65 nm CdTe QDs was selected as the most adequate for iron determination.

When the CdTe QDs concentration was too low, the slope was gentle because the QDs did not quantitatively complex in the given concentration range of iron, in other words, the limited

CdTe cannot occupy all non-specific binding sites of coexisting iron in the system. In addition, it was found that at higher QD concentrations, the relative effect of quenching magnitude decreased by reason of the lower concentration, that is, a relatively higher concentration of iron would be needed to quench the luminescence of the CdTe QDs, a reduced sensitivity towards probing iron. Considering these factors, a QD concentration of $2.5\times10^{-4}$ mol $L^{-1}$ was adopted.

The effect of solution pH value on the fluorescent intensity was studied, and the results are shown in Fig. 3. It can be seen that the optimum range of pH was 7.0 to 8.0. If the pH is too low or too high, the relative fluorescence intensity is lower. The reason may be explained as follows: in acid medium, the fluorescence intensity decreases as a possible result of the deconstruction of the Fe(III)-MPA complex's annulus due to the protonation of the surface-binding thiolates.[17] When the pH increases to above 8, the fluorescence intensity decrease may be due to the precipitation product ($Fe(OH)_3$). In this work, in order to avoid iron precipitation, a pH of 7.5 was chosen to run the assay. This result is in agreement with other studies available in the literature.[16,17]

### 3.3. Optimizatization of the proposed flow-batch method

The parameters of the proposed flow-batch method were evaluated in order to improve the sensitivity and reproducibility of the analytical signal. All the optimization studies were performed automatically in the proposed FB. The studied range and selected values as the best compromise between reproducibility and sensitivity are presented in Table 2. If needed, modifications of parameters may be re-studied and carried out by simply changing the operational parameters in the FB system control software.

### 3.4. Evaluation of interferences

The selectivity of the spectrofluorimetric method using CdTe QDs was investigated. Samples containing a fixed amount of iron (50 µg $g^{-1}$), and increasing concentrations of the species under evaluation were analyzed using the developed methodology. A compound was considered "non-interfering" if the analytical signal variation was ± 5 % as compared to that obtained in its absence. Under the reaction parameters used, the results (Table 3) show that no significant interfering effect for the majority of the tested compounds was found. Under the system operating conditions, no interfering effect was observed.

## 3.5. Analytical features

For the determination of iron in viscous matrices using the proposed method, the regression equation was $A = -0.0797 + 0.0087\ C$, where $A$ is the analytical response for the fluorescence intensity signal (Eq. 3), and $C$ is the iron concentration in μg $g^{-1}$ in the measuring solution. The squared linear correlation coefficient, $r^2$ was 0.997 (n = 5) in the range between 10.0 to 100.0 μg $g^{-1}$.

$$A = -\log P / P_0 \qquad (3)$$

where $P$ and $P_0$ are fluorescence intensity of standard solutions and blank, respectively.

The analytical curve was statistically validated by analysis of variance (ANOVA), showing no significant lack of fit in the proposed models at a 95% confidence level. The limit of detection (LOD), and the limit of quantification (LOQ) for both methods were estimated based on the criteria established by the International Union of Pure and Applied Chemistry (IUPAC).[18] LOD and LOQ were evaluated as $3s_b / S$ and $10s_b / S$, respectively, where $s_b$ is the standard deviation for 20 measurements of the blank and $S$ is the slope of analytical curve. For the determination of iron the LOD and LOQ were 0.10 μg $g^{-1}$ and 0.34 μg $g^{-1}$, respectively.

Table 4 presents the results for the proposed FB system, and the reference method for the iron in viscous matrices. No statistically significant differences were observed between the results at a confidence level of 95% when applying the paired *t*-test. The relative standard deviation (RSD %) was less than 1.5 %, for iron, respectively, and it were obtained from five replicates.

Recovery tests were also performed using four real samples of edible oils and biodiesel with mean values of analyte concentration equal 4.02 and 5.03 μg $g^{-1}$ for edible olive and soybean oil, respectively; and 1.05 and 2.98 μg $g^{-1}$ for soybean and sunflower biodiesels, respectively (see Table 5). For this purpose, 1.0 mL of standard solution with known concentrations of 10.0, 25.0, and 50.0 μg $g^{-1}$ of iron was added to 9.0 mL of each real sample, and spiked samples were analyzed using the proposed FB system. As can be seen in Table 5, good recoveries values (within the 95.5 – 104.3 % range) were obtained for the three analyzed samples.

Table 6 presents selected analytical features of the proposed and other methods.[4,6,8,16,19] Compared to other procedures, the proposed FBA system presents, in general, satisfactory parameters, such as; limit of detection, working range, smaller sample volumes, and relative standard deviation. Moreover, this method is fully automated; a simple in-line single-phase extraction is carried out for sample pretreatment.

## Conclusion

A novel method for direct determination of total iron in edible oils and biodiesel using in-line single-phase extraction, CdTe QDs and a flow-batch system was presented. The in-line single-phase extraction consisted in the addition of an ethanol/chloroform mixture (75:25) to dissolve the oils samples, followed by addition of an acid solution ($HNO_3$/HCl, 3:1) to make the iron available. The iron analyte establishes a surface interaction with the nano-crystals which results in a concentration proportional quenching of fluorescent intensity.

When compared to other works reported in the literature[4,6,16,19], the proposed method presents satisfactory limits of detection, quantification, precision, and accuracy. Moreover, it also presented significant reductions in consumption of reagents (0.275 mL), organic solvents

(0.600 mL), and samples (0.025 mL). Therefore, it was possible to developed a good automatic method with high sample throughput (79 $h^{-1}$) and lower waste generation per determination (0.900 mL), contributing to the basic principles of green chemistry. All this permits suggesting the proposed method as a potentially useful alternative for determination of other analytes in viscous matrices.

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## FIGURE CAPTIONS

**Figure 1**. The flow-batch system manifold. Mixing-chamber (MC); quartz windows (W1, W2, W3); light source (tungsten lamp); spectrofluorimeter (USB4000); optical fiber (OP); three-way solenoid valves ($V_S$, $V_E$, $V_A$, $V_B$, $V_{QDs}$, and $V_W$); magnetic stirrer (MS); stirring bar (SB); sample or standard solution (S); ethanol/chloroform solution (E), acid solution (A), PBS buffer solution (B), CdTe QDs solution (QDs).

**Figure 2**. Effect of the solvent extraction ratio.

**Figure 3.** Effect of pH on the relative fluorescence intensity of system CdTe QDs $2.5\times10^{-4}$ mol $L^{-1}$; 50 µg $L^{-1}$ of iron solution.

**Table 1**

Operation steps of the flow-batch system for the direct determination of iron in edible oils and biodiesel.

| Step | Event | Time (s) | Volume (µL) |
|---|---|---|---|
| 1 | Addition of the sample ($V_S$) [a] | 1.4 [b] | 25 |
| | Addition of the solvent ethanol/chloroform ($V_E$) [a] | 4.0 | 600 |
| 2 | Homogenization | 2.0 | – |
| 3 | Addition of the acid solution ($V_A$) | 0.7 | 100 |
| 4 | Homogenization | 2.0 | – |
| 5 | Addition of the buffer solution ($V_B$) | 0.7 | 100 |
| 6 | Homogenization | 2.0 | – |
| 7 | Addition of the CdTe QDs solution ($V_{QDs}$) | 0.5 | 75 |
| 8 | Homogenization | 2.0 | – |
| 9 | Measurements of the analytical signal | 1.0 | – |
| 10 | Waste ($V_W$) – empting of MC | 6.1 | – |
| 11[c] | Addition of the ethanol/chloroform solution ($V_E$) [a] | 4.0 | 600 |
| | Addition of the new sample or standard ($V_S$) [a,d] | 2.8 | 50 |
| 12[c] | Waste ($V_W$) – empting of MC | 6.1 | – |
| 13[c] | Addition of the acid solution ($V_A$) | 6.1 | 900 |
| 14[c] | Homogenization | 2.0 | – |
| 15[c] | Waste ($V_W$) – empting of MC | 6.1 | – |

[a] Simultaneous addition.

[b] Addition of edible oils or biodiesel.

[c] Steps 11 to 15 belong to the cleaning cycles.

[d] Sometimes necessary for filling the feed channel from valve VS to MC with the new sample or standard.

**Table 2**

Selected parameters of the FB system procedure for direct determination of iron in edible oils and biodiesel.

| **Parameter** | **Range** | **Selected value** |
|---|---|---|
| Sample volume (µL) [a] | 10 – 100 | 25 |
| Ethanol/chloroform (75:25, v/v) (µL) | 200 – 1000 | 600 |
| $HNO_3$/HCl (3:1, v/v) (µL) | 50 – 200 | 100 |
| PBS buffer solution (µL) | 50 – 200 | 100 |
| CdTe QDs solution (µL) | 50 – 200 | 50 |
| Homogenization between additions (s) | 1 – 10 | 2 |
| Cleaning numbers | 1 – 4 | 2 |
| Total volume (µL) | 500 – 1200 | 875 |

[a] For both viscous samples (edible oils and biodiesel).

**Table 3**

Summary of the interference effects of possible ions on the peak height obtained from 50 µg $g^{-1}$ of iron solution.

| **Foreign ions** | **Tolerable concentration ratio**[a] (µg $L^{-1}$) |
|---|---|
| $Cd^{2+}$ | 85 |
| $Zn^{2+}$ | 65 |
| $Pb^{2+}$ | 75 |
| $Cr^{2+}$ | 80 |
| $Cu^{2+}$ | 85 |
| $Ca^{2+}$ | 70 |
| $Mg^{2+}$ | 70 |

[a] The concentration of an ion is considered to be interfered with when causing a relative error of more than ± 5% with respect to the signal of iron alone.

### Table 4

Results for the direct determination of iron in edible oils and biodiesel using the proposed flow-batch system, and atomic absorption as the comparative method (μg $g^{-1}$). Mean values and uncertainties are based on five analytical determinations.

| Samples | | Proposed method | | Reference method | |
|---|---|---|---|---|---|
| | | Fe (III) ± $t_{n-1}$ S/$\sqrt{n^a}$ | RSD % [b] | Fe (III) ± $t_{n-1}$ S/$\sqrt{n^a}$ | RSD % [b] |
| Edible oils | Olive | 3.96 ± 0.06 | 1.45 | 4.02 ± 0.05 | 0.88 |
| | | 4.63 ± 0.05 | 1.51 | 4.59 ± 0.05 | 0.91 |
| | Soybean | 4.98 ± 0.04 | 1.42 | 5.03 ± 0.04 | 0.76 |
| | | 3.41 ± 0.05 | 1.36 | 3.38 ± 0.03 | 0.81 |
| | Sunflower | 2.55 ± 0.06 | 1.15 | 2.59 ± 0.04 | 1.09 |
| | | 5.11 ± 0.05 | 1.32 | 5.14 ± 0.03 | 0.95 |
| Biodiesel | Soybean | 1.09 ± 0.05 | 1.23 | 1.05 ± 0.04 | 0.79 |
| | | 4.58 ± 0.04 | 1.51 | 4.61 ± 0.03 | 0.63 |
| | Sunflower | 2.21 ± 0.05 | 1.15 | 2.26 ± 0.04 | 0.94 |
| | | 2.94 ± 0.05 | 1.32 | 2.98 ± 0.03 | 0.85 |
| | Maize | 3.03 ± 0.05 | 1.23 | 2.96 ± 0.04 | 0.92 |
| | | 2.05 ± 0.04 | 1.51 | 1.99 ± 0.05 | 0.65 |

[a] Where $n$ is the number of replicate measurements, $t_{n-1}$ is the statistic parameter often called Student's $t$ (with $n = 5$, at 95% level of confidence), and S is the standard deviation.

[b] RSD: relative standard deviation.

**Table 5**

Recoveries of iron in edible oils and biodiesel (n = 5).

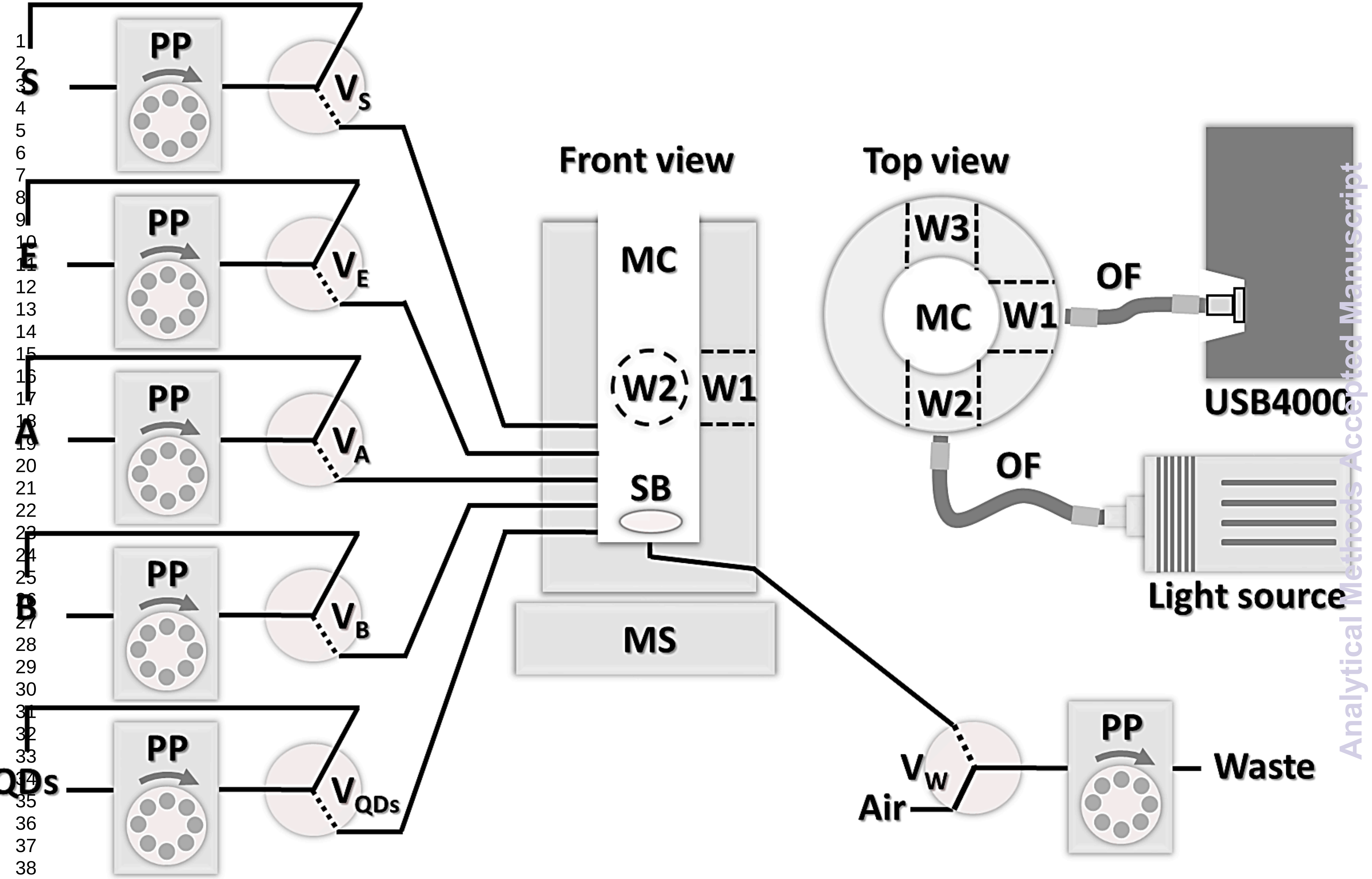
| Samples (µg $g^{-1}$) | | Recovery % | | |
|---|---|---|---|---|
| | | 10.0 (µg $g^{-1}$) ± $t_{n-1}$ S/$\sqrt{n^{a}}$ | 25.0 (µg $g^{-1}$) ± $t_{n-1}$ S/$\sqrt{n^{a}}$ | 50.0 (µg $g^{-1}$) ± $t_{n-1}$ S/$\sqrt{n^{a}}$ |
| Edible oil | Olive oil (4.02) | 102.4 ± 2.1 | 98.5 ± 2.3 | 104.3 ± 2.4 |
| | Soybean oil (5.03) | 99.3 ± 2.4 | 95.5 ± 2.2 | 101.6 ± 2.2 |
| Biodiesel | Soybean (1.05) | 103.5 ± 2.2 | 102.5 ± 2.1 | 95.9 ± 2.3 |
| | Sunflower (2.98) | 98.7 ± 2.5 | 101.6 ± 2.4 | 103.4 ± 2.3 |

[a] Where $n$ is the number of replicate measurements, $t_{n-1}$ is the statistical parameter often called Student's $t$ (with $n$ = 5, at 95% level of confidence), and s is the standard deviation.

**Table 6**

Comparison of the proposed method with previously reported methods for determination of iron in edible oils and biodiesel.

| **Detection technique** | **Analyte** | **Sample** | **Sample preparation** | **Fully automated technique** | **Sample amount** | **Working range (mg $L^{-1}$)** | **LOD (µg $g^{-1}$)** | **RSD%** | **Ref.** |
|---|---|---|---|---|---|---|---|---|---|
| UV-Vis (with multivariate calibration) | Si, P, Fe, Al | Biodiesel | Microwave digestion | No | 1.0 g | 2 – 20 | 0.6 | 5.0 | 4 |
| UV-Vis | Fe(III) | Edible oil | Organic solvents | Yes | 150 µL | 0.5 – 25 | 0.31 | < 3.5 | 6 |
| UV-Vis | Fe(III) | Edible and Mineral oils | Organic solvents | Yes | 100 µL | 0.1 – 1.0 | 0.02 | < 1.6 | 8 |
| Fluorescence | Fe | Biodiesel | Ultrasound-assisted | No | 200 µL | 6 – 100 | 1.25 | < 2.6 | 16 |
| AAS | Cu, Fe | Edible oil | Dry ashing | No | 4 – 20 g | 0 – 10 | 0.6 | 2 | 19 |
| Fluorescence | Fe | Edible and biodiesel | In-line single phase extraction | Yes | 25 µL | 0.1 – 1.0 | 0.1 | < 1.6 | This work |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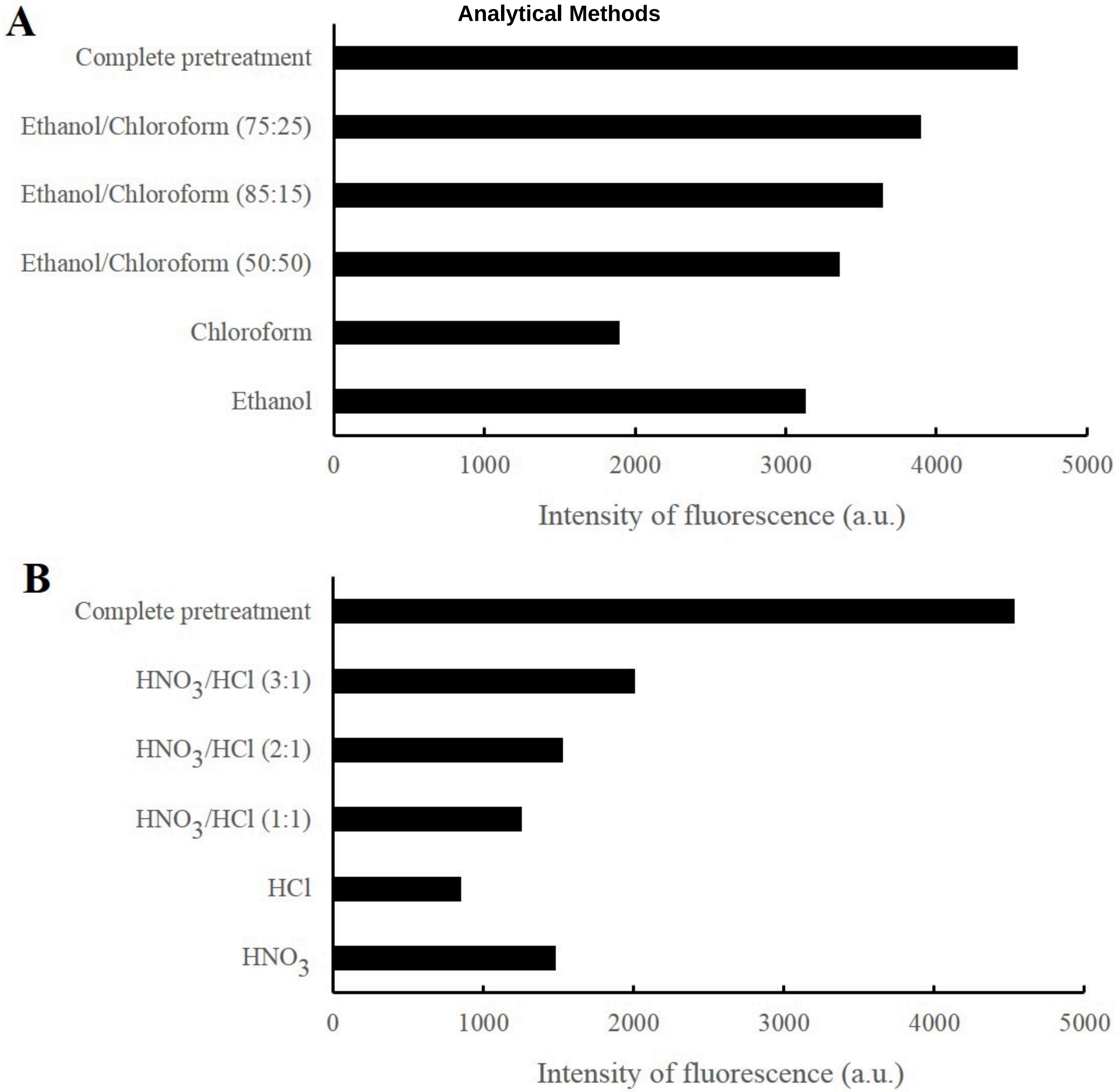
V_S
E
PP
V_E
A
PP
V_A
B
PP
V_B
QDs
PP
V_QDs
Front view
MC
W2
W1
SB
MS
Top view
W3
MC
W1
W2
OF
USB4000
OF
Light source
V_W
Air
PP
Waste

Fig. 1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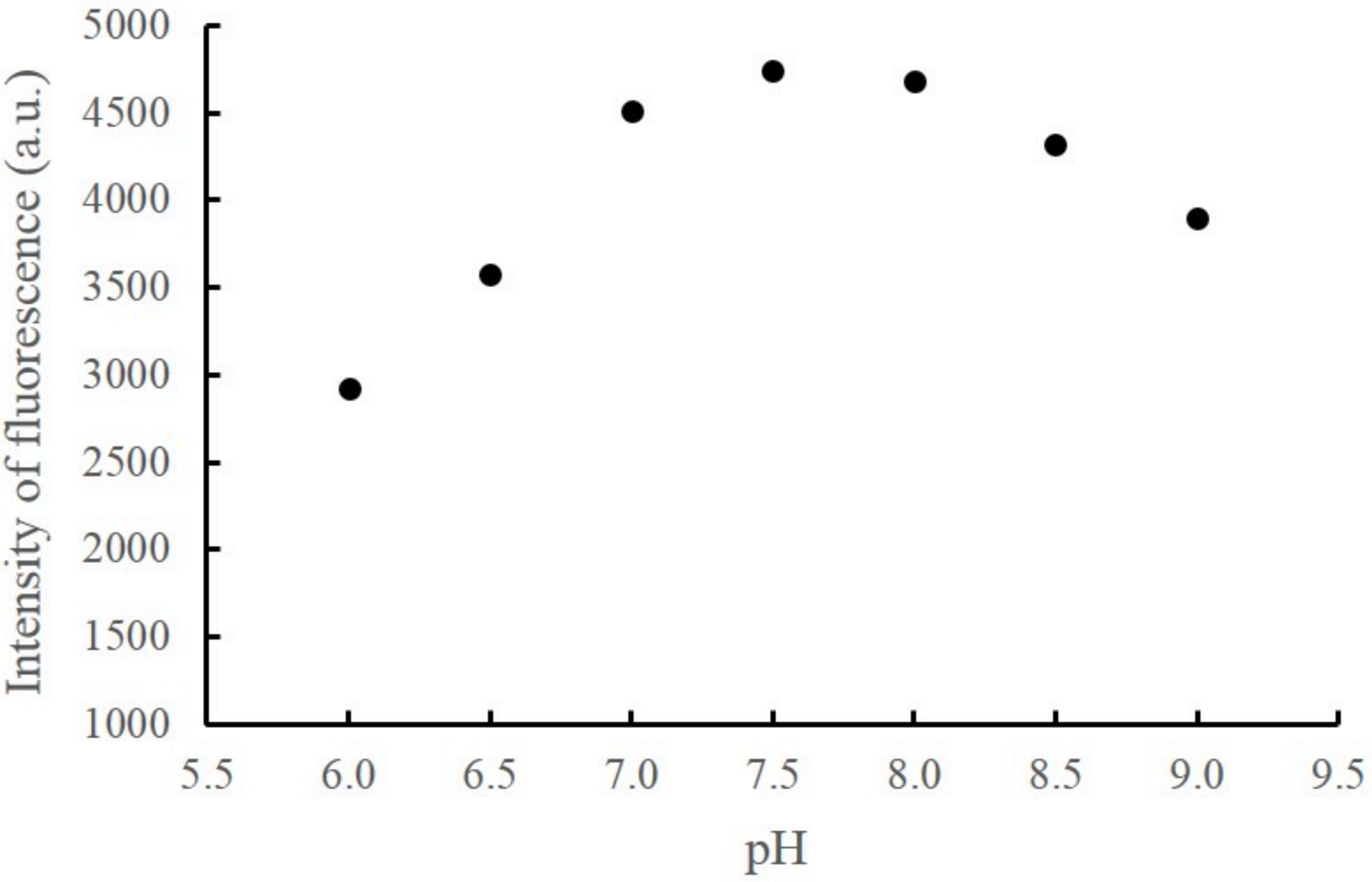
Complete pretreatment
Ethanol/Chloroform (75:25)
Ethanol/Chloroform (85:15)
Ethanol/Chloroform (50:50)
Chloroform
Ethanol
0
1000
2000
3000
4000
5000
Intensity of fluorescence (a.u.)
B
Complete pretreatment
HNO3/HCl (3:1)
HNO3/HCl (2:1)
HNO3/HCl (1:1)
HCl
HNO3
0
1000
2000
3000
4000
5000
Intensity of fluorescence (a.u.)

Fig. 2


Intensity of fluorescence (a.u.)
5000
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4000
3500
3000
2500
2000
1500
1000
5.5
6.0
6.5
7.0
7.5
8.0
8.5
9.0
9.5
pH

Fig. 3