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Online Monitoring of Aluminium in Drinking Water with Fluorimetric Detection

A. Alonso-Mateos · M. J. Almendral-Parra ·
Y. Curto-Serrano · F. J. Rodríguez-Martín

Abstract We report a procedure for the online monitoring of aluminium in drinking water by flow injection analysis. The reaction used is the formation of a complex with morin. Under the working conditions, this can be accomplished in an ethanol-rich hydroalcoholic medium, which modifies the fluorescent characteristics of the complex, allowing the determination of aluminium concentrations higher than 3.1 μgl^{-1}, with a linear application range between 2 and 250 μgl^{-1}, an R.S.D. of 2.3% ($n=10$, 120 μgl^{-1}) and a sampling frequency of 90 h^{-1}. The method can thus be considered one of the most sensitive and fastest for the continuous determination of aluminium. In the presence of anionic surfactants, the sensitivity of the determination is increased. In this form, aluminium is detected at concentrations higher than 2.8 μgl^{-1}, with a linear application range of 2–50 μgl^{-1}. The procedure was applied to the analysis of aluminium in drinking, river, and underground water. Under the proposed working conditions, only Fe(III), fluoride and phosphates interfere. The interference of Fe(III) can be avoided with hydroxylamine and that of phosphates and polyphosphates by acid digestion of the samples.

Keywords Aluminium · Morin · Flow injection analysis · Fluorimetry · Drinking water

A. Alonso-Mateos · M. J. Almendral-Parra (✉) ·
Y. Curto-Serrano · F. J. Rodríguez-Martín
Departamento de Química Analítica, Nutrición y Bromatología,
Faculty of Chemistry, University of Salamanca,
Plaza de la Merced s/n,
37008 Salamanca, Spain
e-mail: almendral@usal.es

Introduction

Aluminium is the third most abundant element at the Earth's crust, and forms part of minerals, clays and rocks. As a result of its widespread distribution, aluminium is present, variable concentrations, in nearly all natural waters in different forms: salts, colloid species, or insoluble compounds.

However, humans are also responsible for the presence of aluminium in water. Acid rain favours increases in its concentrations in natural waters. It is also found in the waste waters from certain processes and even in water treated for drinking; in this case as a result of coagulation treatment, in which aluminium salts are used. Water filtered at modern treatment plants with rapid sand filters may contain up to 50 μgl^{-1} of total aluminium.

For a long time it was believed that the aluminium content in water, in particular drinking water, was of little relevance because it was considered a non-toxic element. However, since 1973, certain clinical disorders were observed in renal patients subjected to dialysis. These were attributed to high aluminium concentrations in their bodies [1–3]. Currently, aluminium toxicity is associated not only with dementia and neurodegenerative disorders but also with osteodystophagia [4, 5], anaemia [6, 7] gastrointestinal disturbances [8], and cardiotoxicity [9]. Recently, some epidemiological studies have investigated the relationship between the ingestion of aluminium from drinking water and Alzheimer's disease [10]. All these findings have caused great concern in the field of public health [11], meaning that accurate determination of this metal ion at trace and sub-trace levels is crucial.

Among the different methods available for the determination of Al(III), there are many that use spectrophotometric determination with chromogenic reagents, such as

eriochromocyanin R [12], pyrocatechol violet [13], pyrogallol [14] xylenol orange [15, 16] and chromoazurol S [17–19]. There is also an abundance of continuous flow methods [20–22]. The classical techniques, such as atomic absorption or emission spectrometry, are not normally used in the average laboratory and do not allow real-time or even on-site determinations. Therefore, much interest has been sparked as regards the development of analytical methods that can be adapted to online determinations with a suitable range of sensitivity. In this sense, fluorimetry is a well known ultra-trace analytical technique, but only few methods have been reported for the fluorimetric determination of aluminium [23, 24]. Among them, those that employ lumogalion [25], chromotropic acid [26], and 8-hydroxyquinoline-5-sulphonic acid [27] are of greatest interest, some of them having been automated by flow analysis [28].

The reaction between morin and aluminium to generate a fluorescent product has traditionally been carried out in slightly acid medium, in an aqueous solution with 5% ethanol for the determination of the metallic cation [29–32]. The reaction kinetics is slow, such that 20 min are required for the maximum signal to be achieved. Moreover, in discontinuous mode many interferences arise [31]. In the present paper we report a procedure for the determination of aluminium with morin in continuous flow mode under conditions such that the luminescent properties of both the metallic chelate and of the morin are not modified at concentration ranges low enough to allow application in the control of aluminium in drinking water, whose content should not exceed 200 μgl^{-1}.

With the proposed methodology it was possible to minimise the time of analysis and reduce or remove the interferences in comparison with other methods.

It is well known that surfactants modify the molar absorptivity coefficient and the fluorescence proportionality constant owing to their ability to form micelles in aqueous medium containing a given analyte in complex form. They have a concentrating effect, either on charged surface or hydrophobic interior [33–37]. Accordingly, with a view to increasing sensitivity in the determination of aluminium by the formation of a complex with morin, the effect of dodecylbenzenesulphonate as a conditioning reagent was used.

Experimental

Apparatus and materials

Minipuls HP4 (Gilson, France) peristaltic pumps with silicone or vinyl pump tubes. PTFE simple-injection valve (Rheodine). Detection was performed with an RF-5000 spectrofluorimeter (Shimadzu, Japan) fitted with a DR-15 data processor and an FDU-13 data storage unit, to which a sensitization unit was coupled, (Shimadzu, 200-26841-01). A 25 µl flow-cell (Hellma, Germany, 176.052) with an optical pathway of 0.150 cm and a rectangular quartz flow-cell (Shimadzu, 204-05566), of 1 cm optical pathway and 12 µl volume. PTFE tubing of 0.5 mm internal diameter with standard tube fittings and connectors (Upchurch Scientific, Inc.) was used. A Crison 501 potentiometer and a Digiterm 3000542 (Selecta) water bath thermostatted at 27°C were also used.

Reagents and solutions

All chemicals used in this work were of analytical grade and were prepared with ultra-high quality deionized water. 50 and 100 mgl^{-1}, stock solutions of aluminium, from solid $AlK(SO_4)_2.12H_2O$ (Schering-Kahlbaum). The purity of the solid (98.92±0.02%) was determined by gravimetry. All aluminium solutions were prepared in 1.0×10^{-2} M HCl, adjusting pH to 3. Further dilutions were prepared daily as required. Morin solutions of different concentrations were prepared by weighing the solid product (Sigma) and later dissolution in absolute ethanol or in ethanol–water mixtures. A standard concentrated solution of 100 mgl^{-1} of NaF (Probus) was prepared by weighing the previously dried reagent at 120°C and dissolution in distilled water. Working standard solutions were prepared after suitable dilutions of the stock solution. The TISAB II buffer solutions were prepared by dissolving 102.6 g of sodium acetate trihydrate, $CH_3COONa.3H_2O$ (Panreac), 15 ml of glacial acetic acid (Panreac), 58.5 g of NaCl (Probus) and 0.3 g of sodium citrate dihydrate (Probus) in (1L) deionized water. A solution of sodium dodecylbenzenesulphonate, $NaC_{18}H_{29}SO_3$ (Acros organics), was prepared in distilled deionized water. Solutions of a large number of inorganic ions, prepared from their water-soluble salts (generally nitrates or sulphates) at pH=2 were prepared. Certified QC Materials for water analysis -LGC6010 and LGC6011 from Promochem- were also used.

Flow system

The flow systems used in the experiments are shown in Fig. 1. In general, flow systems should meet two basic requirements: they should be as simple as possible and should be able to monitor aluminium concentrations for the control of drinking water. Monitoring counsels use of an inverse Flow Injection (FI) system, in which the sample flow through a channel into which a defined volume of the morin reagent solution is injected, able to detect both the concentrations of Al(III) usually found in surface waters (<50 μgl^{-1}) and those close to 200 μgl^{-1}, the maximum

Fig. 1 Two-channel flow system with normal (**a**) or reverse (**b**) injection after confluence of the C_1 and C_2: channels; *I*, injection valve; *R*, coiled reactor; *D*, detector; *W*, waste
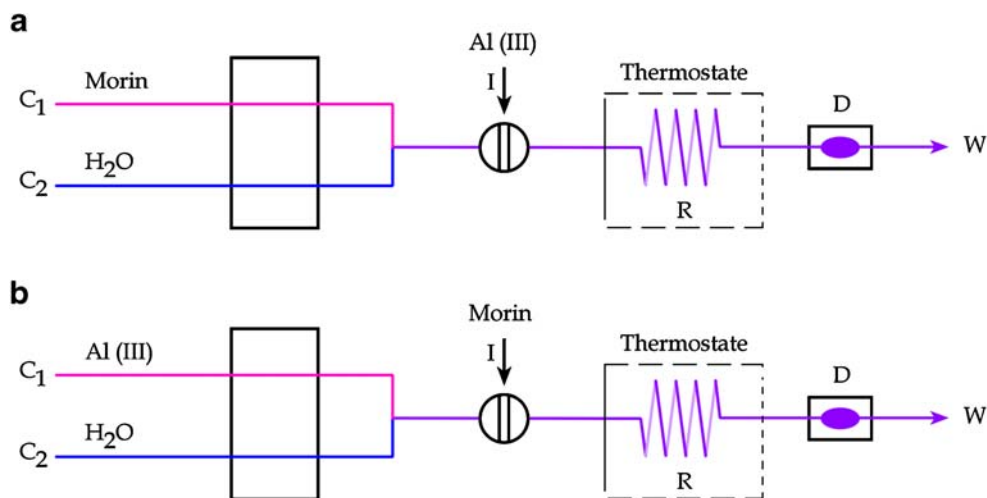
permitted level in drinking water. However, traditional FI systems, in which the reagent solution flows through the channel into which the analyte is injected, allows the effect of the different variables affecting the Al-morin reaction in continuous flow mode to be known, and hence the analytical characteristics of the proposed method. Additionally, it is possible to add a second channel to both flow systems; this merges with the first one before the injection site, enabling the working pH to be modified without having to manipulate the solution of morin or the samples themselves. The configuration of the dual-channel in normal FI systems allows study of the possible existence of a matrix effect deriving from the fluorescence of morin at the selected wavelengths.

The optimum working conditions in the flow system proposed for monitoring (System B) are summarised in Table 1. For water samples with $4.5 \leq pH \leq 8$, a range much higher than is usual, we propose an inverse FI system in B-1 mode. The sample is mixed with a stream of 0.1 M NaCl before injection of the fluorogenic reagent for online adjustment of the ionic strength. This mode is also suitable for samples with elevated contents of orthophosphates, which must be subjected to acid digestion and brought to a suitable pH before determination, and/or fluorides, in which

case the standards must be spiked with an appropriate amount of NaF.

When the samples have extreme pHs, <4.5 or >8, we propose mixing, prior to injection, with a stream of acetic acetate buffer, which adjusts the pH and the ionic strength of the resulting stream (mode B-2).

Results and discussion

Fluorescence spectra

In preliminary experiments it became necessary to determine the fluorescence behaviour of the morin and of the complex this forms with Al(III). Additionally, since morin is sparingly soluble in aqueous medium, its solubility in other solvents and the behaviour of these solutions in the flow system were assayed.

The spectra of morin and of the morin-Al(III) complex in water and in ethanol ($\lambda_{ex}=410$ nm and $\lambda_{em}=497.6$, slit width 5 nm) were recorded, observing that they were similar in both media, although sensitivity was greater in alcoholic medium as a result of the better solubility of morin in that medium.

Table 1 Optimum working conditions for the determination of Al(III) using the proposed method

Variable	Mode B1		Mode B2	
Channel C_1	2.30 ml min^{-1}	Al(III) Samples or standards	2.00 ml min^{-1}	Al(III) Samples or standards
Channel C_2	0.30 ml min^{-1}	NaCl 0,1 M NaF	0.60 ml min^{-1}	4 M Acetic/acetate buffer pH 5.5
Q_t	2.60 ml min^{-1}			
Injection	30 μl of a solution of 50 mg l^{-1} of morin in ethanol/water 3:1			
Reactor	100 cm			
Thermostat	Room temperature—30 or 35°C			
Detection	$\lambda_{ex}=410.0$ nm $\lambda_{em}=497.6$ nm			

Both morin and its chelate with aluminium showed a lower emission of fluorescence at acid or basic pHs. Moreover, at pH>9, the spectra of both species pointed to a modification of the maximum excitation and emission wavelengths, as a result of the different structure of morin at those pH values [28].

Optimisation of experimental conditions

The main variables affecting the formation and detection of the aluminium–morin complex in the proposed flow systems were considered. First, the effect of the chemical variables (proportion of ethanol, pH, morin concentration) was investigated, followed by investigation of the geometric and hydrodynamic variables (flow rate, injection volume and length of the reactor) in order to optimise the process.

Except for those cases in which other values are specified, the following general working conditions were employed.

Proportion of ethanol:water: 1:1 (normal FI system), 3:1 (reverse FI system); morin concentration: 3.9 mgl^{-1}; pH of the solution of Al(III): 4.5; flow rates: $F_1=F_2=0.83$ ml min^{-1}; Vi=30 μl, R:155 cm, Temperature 22°C, $\lambda_{ex}=$ 410 nm, $\lambda_{em}=497.6$ nm.

Proportion of ethanol

In preliminary studies it was observed that the analytical signal increased in hydroalchoholic medium, attributable to the higher concentration of morin in that medium, although the role of the solvent as a fluorescence-sensitising agent cannot be ruled out. Accordingly, bearing in mind that the ethanol–water mixture initially passes through a phase of microemulsion formation, it appeared appropriate to use this mixture to dissolve the morin reagent. However, given the critical effect of the hydroalcoholic medium of the morin solution on the sensitivity of the method, a study of this was addressed systematically as one more variable to be optimised. To do so, in the flow system depicted in Fig. 1a, solutions of morin at 52 mgl^{-1} in different ethanol–water mixtures between 1:10 and 2:1 (v/v) at pH values between 5.5 and 6.6 were passed through channel C$_1$. A solution of Al(III) of 5.0 mgl^{-1} was injected into channel C$_2$, such that the proportion of ethanol: water in the resulting stream varied between 1:21 and 1:2.

The experimental results revealed what was expected: for hydroalcoholic media rich in ethanol the fluorescence intensity increased, corroborating the sensitising effect of the solvent. From the practical point of view, it may be considered that for ethanol: water media of 2:1, or even richer in ethanol, a constant signal is obtained. Nevertheless, under these working conditions the microemulsions formed during solvent mixing were more persistent,

strongly affecting the reproducibility of the analytical signal. It therefore seemed appropriate to choose a proportion of 1:1 for later studies.

A similar study was carried out in the same FI system with Al(III) injection (Fig. 1b). 30 μl of the 52 mgl^{-1}-morin solution in ethanol: water between 1:10 and 3:1 was injected into the stream resulting from the merging of channels C$_1$—solution of Al(III) of 5.0 mgl^{-1}, pH=4.8— and C$_2$: bidistilled water. The experimental results were qualitatively similar to those obtained with the previous system: fluorescence intensity increased with the increase in the proportion of ethanol in the morin until values almost identical to those attained for ethanol:water ratios of 3:1 were reached. This mode has the advantage that the formation of microemulsions due to the dissolution of ethanol in water is minimised.

pH

The acid–base properties of morin (pK$_a$ 3.7 and 8.9) not only determine its fluorescence characteristics but also its chelation capacity. Accordingly, the pH of the medium in which the morin–Al(III) chelate is formed will largely determine the extent of the complexing reaction and hence will decisively affect the sensitivity of the method under study. In the flow system depicted in Fig. 1, a 52 mgl^{-1} solution of morin in ethanol: water 1:1 flowed through channel C$_1$ and bidistilled water, with a pH varying between 0.8 and 10.5, flowed through C$_2$ such that the pH of the resulting stream, into which solutions of Al(III) of 1.5 mgl^{-1} and pH 4.8 were injected, varied between 1.05 and 10.0. The results obtained (Fig. 2) show that the maximum fluorescence emitted by the morin–Al(III) chelate was obtained at pH values between 5 and 8. Outside this range, fluorescence intensity decreased sharply.
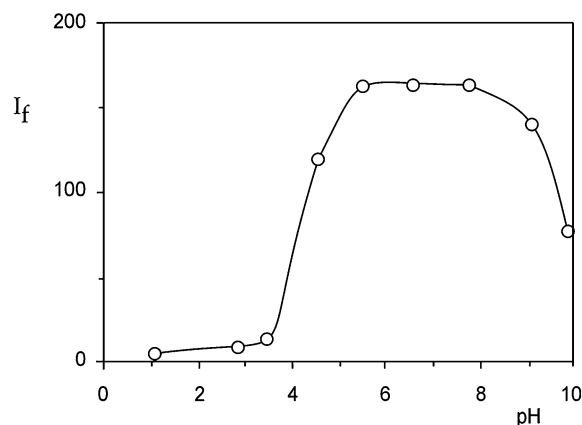
Fig. 2 Effect of pH on the analytical signal. C$_1$: solution of morin of 52 mgl^{-1} in ethanol–water 1:1; C$_2$: bidistilled water at different pHs; I: 30 μl of a standard solution of Al(III) of 1.5 mgl^{-1} at pH=4.8; $Q_1=Q_2=0.83$ mL min^{-1}; R: coiled tube 155 cm in length and 0.5 mm i.d.; $\lambda_{ex}=410.0$ nm; $\lambda_{em}=497.6$ nm

Additionally, at more alkaline pH slight displacements were observed in the excitation and emission maxima, undoubtedly due to the different fluorescence properties of the more alkaline form of the morin (28).

In a similar study carried out in the reverse FI system (Fig. 1b), similar results were observed. Maximum fluorescence intensity was emitted for $5 \leq pH \leq 8$, although a higher value was obtained.

Morin concentration

The optimum concentration of morin is affected by the concentration of Al(III), hence the need study at least two relatively disparate concentrations of the analyte—60 and 600 μgl^{-1}—of Al(III), in both cases varying the concentration of morin within the same range. The results are shown in Fig. 3. In both cases, a sharp increase in the signal is observed when the concentration of morin is increased, maximum fluorescence intensities being reached at concentrations of 4 and 6 mgl^{-1} for both aluminium standards respectively. At higher morin concentrations, at which almost constant fluorescence intensities would be expected, a new decrease in the signal occurs, attributable to a quenching effect or a decrease in the quantum fluorescence yield as a result of self-absorption phenomena.

A similar study was carried out in the reverse flow system (Fig. 1b) an increase in the fluorescence emitted being observed upon increasing the morin concentration up to values of 100 mgl^{-1}, after which a similar decrease in fluorescence was observed, corroborating the initial hypothesis of a quenching effect [36, 37].

It should be noted that in the different cases studied, the concentration of morin necessary for maximum florescence emission to be reached did not coincide with the expected aluminium–morin stoichiometric concentration, 3:1. This is
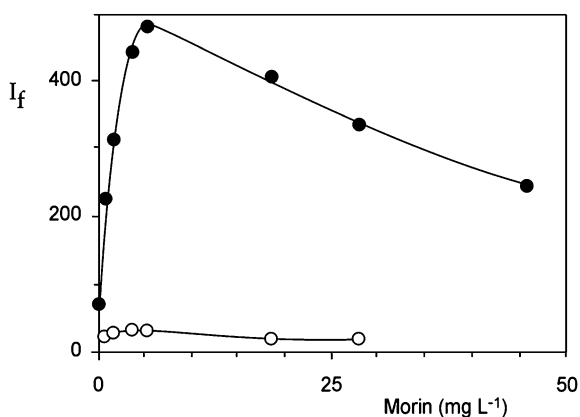

Fig. 3 Effect of morin concentration on the analytical signal upon injecting standards of 60(*open circle*) and 600(*filled circle*) μgl^{-1} of Al(III). C_1: morin solutions of between 0.02 and 46.1 mgl^{-1} ethanol–water 1:1; C_2: bidestilled water; I: 30 μl of a standard solution of Al(III) at pH=4.5; $Q_1=Q_2=0.83$ ml min^{-1}; R: coiled tube 155 cm in length and 0.5 mm i.d.; λ_{ex}=410.0 nm; λ_{em}=497.6 nm

due to the dilution undergone by the bolus injected as a result of the dispersion phenomena typical of the FI and the slow reaction kinetics prevailing under the working conditions.

Reactor length

The reactor comprised a coiled Teflon tube of 0.5 mm i.d. to favour axial mixing of the stream and minimise the dispersion of the bolus injected. When the length of the reactor was increased, the analytical signal was also increased until constant values were attained for reactor lengths between 100 and 155 cm. As from this latter length, the signal decreased. Although for short reactor lengths the reaction time was not optimum, it should be noted that the intensities of fluorescence emitted were significantly high, showing that the reactions kinetics was not as slow as expected, at least in the initial phases. For lengths greater than 155 cm a decrease in the signal was noted; this was due to the dispersion undergone by the bolus before arriving at the detector.

A similar study carried out in the reverse flow system afforded analogous conclusions. The maximum analytical signal was obtained for reactor lengths of around 150 cm, thereafter decreasing as a result of the dispersion of the bolus of injected sample.

Flow rates

The effect of the flow rates of streams C_1 and C_2 was studied through the total flow rate $F_t = F_1 + F_2$, in all cases maintaining the flow rates of both streams equal. The results obtained differed for both types of FIA systems and are shown in Fig. 4. In the FI system depicted in Fig. 1a, at low flow rates the analytical signal was less intense as a result of the strong dispersion undergone by the bolus of the injected sample (Fig. 4a); the FI signals are broader, have elevated Δt and are Gaussian in shape. At the highest flow rates studied, smaller analytical signals were obtained, in these cases because the residence time was not sufficient for the chelation reaction to be fully completed. The optimum working flow rates must be 2.0 ml min$^{-1} \leq F_t \leq 2.7$ ml min^{-1}. Under these conditions, the maximum rate of determination would range between 90 and 100 samples h^{-1}.

A similar study carried out with the reverse flow system, Fig. 1b, provided qualitatively similar results (Fig. 4b). In this flow system, the maximum signal was obtained at between 2 and 3 ml min^{-1}, conditions in which the reaction times coincided with the optimum ones in the normal FI system. However, this time the fiagrams were broader, indicating greater dispersion of the bolus of morin with respect to that undergone by the Al(III). This must be attributed to the greater ease with which the ethanol:water
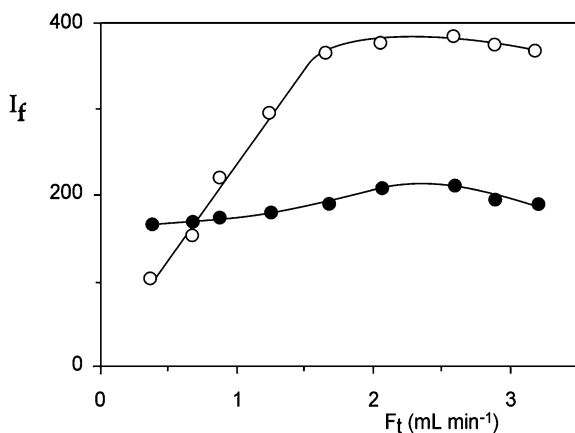
Fig. 4 Effect of flow rate on the intensity of maximum fluorescence (*filled circle*) flow system as in Fig. 1a, C_1: morin solution of 3.9 mgl^{-1} in ethanol–water 1:1; C_2: bidistilled water; I: 30 µl of a Standard solution of Al(III) de 350 µgl^{-1} at pH=4.5; (*open circle*) flow system as in Fig. 1b; C_1: standard solution of Al(III) of 500 µgl^{-1} at pH=4.5; C_2: bidistilled water ; I: 30 µl of morin solution of 3.9 mgl^{-1} in ethanol–water 3:1; $Q_1=Q_2$=variable; R: coiled tube 155 cm in length and 0.5 mm i.d.; λ_{ex}=410.0 nm; λ_{em}=497.6 nm

3:1, the morin solvent, disperses in the aqueous carrier medium. Nevertheless, the maximum rate of determination would also be in the 100 samples h^{-1} range.

Injection volume

We next studied the effect of this variable on the analytical signal, modifying it between 30 and 200 µl. The maximum analytical signal was obtained for an injection volume between 60 and 100 µl. For larger volumes, the signal decreased as a result of the appearance of multiple peaks. This can be prevented by increasing the time of residence of the bolus in the system, working at lower flow rates or with longer reactors. However, in these cases not only would the rate of determination be reduced but also a less intense and broader signal would be obtained as a result of the increase in dispersion.

Upon injecting the morin reagent into a carrier stream containing the Al(III) (Fig. 1b), the signal was maximum between 60 and 135 µl, although as already observed in this system dispersion was slightly greater.

It may therefore be concluded that after setting F_1 at 2.60 ml min^{-1} and the reactor length between 100 and 150 cm, the injection volume should be between 60 and 90 µl when Al(III) is injected and between 60 and 135 µl when morin is injected.

Temperature

For the study of this variable, the flow system depicted in Fig. 1a was used, but with only one channel, C_1 carrying the morin solution. The reactor, R, was submerged in a water bath thermostatted at the desired temperature. The

variation in the signal with time was recorded, stopping the flow when the reacting bolus was inside the reactor for a thermostatting time (t_T) of 1 min and also without performing the stop. The results are shown in Fig. 5. As may be seen, in the series under thermostatted conditions a clear increase in the signal can be observed up to temperatures of the order of 35°C. The reaction was favoured by the increase in temperature. The decrease that occurred when temperature was greater than 35°C is due to the volatilisation of the ethanol.

When a 1 min stop was implemented, the maximum values of the signal were obtained for the lowest temperatures of the series (close to room temperature). At higher values, the signal gradually decreased as a result of the loss of ethanol, also favoured by the longer thermostatting time.

On comparing both series, it was observed that at the lowest temperatures the reaction did not reach its full extent during the time it remained in the system without flow stopping. As a practical conclusion, it may be inferred that in both flow systems the greatest sensitivity is obtained by working in continuous flow mode at 30≥T≥35°C or with flow stopping for one min (for longer times the signal did not increase) and thermostatting at 20°C.

Influence of ionic strength

In order to demonstrate that the flow system did allow the analysis of samples with non-neutral pHs and hence that it could be applied to samples other than drinking water, a study was made of the effect of the ionic strength of the solution. Sodium chloride was used at different concentrations in the $1.0.10^{-4}$–$1.2.10^{-1}$ M range, with the observation that upon increasing the salt concentration the analytical signal remained almost constant up to values
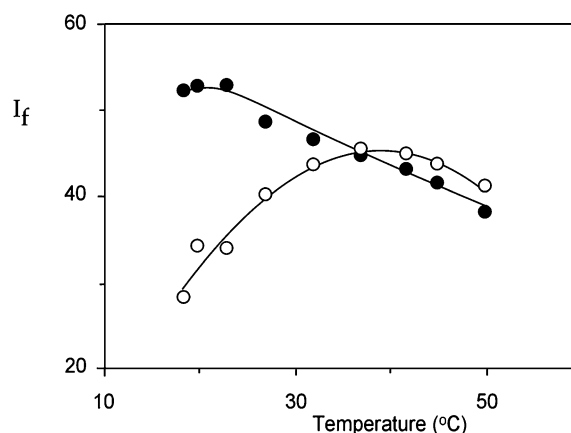
Fig. 5 Effect of temperature on the analytical signal. C_1: morin solution of 3.9 mgl^{-1} in ethanol–water 1:1; I: 85 µl of standard solution of Al(III) de 40 mgl^{-1} at pH=4.5; Q_1=2.60 mL min^{-1}; R, coiled tube 100 cm in length and 0.5 mm i.d.; T, variable; (*open circle*) continuous mode; (*filled circle*); stopping the reacting bolus in the thermostatted reactor for 1 min. λ_{ex}=410.0 nm; λ_{em}=497.6 nm

Table 2 Characteristics of the equations of the calibration straight lines in a sing-channel flow system with reagent injection: linear range of concentrations, number of standards (n), equation and correlation coefficient (r^2)

Range	n	VI:30µl			
µg Al(III) l^{-1}		Equation	r^2	RSD %; $n=10$;µgl^{-1}	Detection limit
2–250	11	If $= (-39.5 \pm 0.1) + (3.2 \pm 0.1)$C	0.993	2.3 (120)	3.1
2–50	6	If $= (2.70 \pm 3.8) + (1.54 \pm 0.06)$C	0.979	2.8 (50)	4.2
40–250	7	If $= (-76.0 \pm 8.1) + (3.42 \pm 0.07)$C	0.999	2.1 (200)	

I_F, intensity of emitted fluorescence; C, concentration of Al(III) expressed in µgl^{-1}

close to 10^{-2} M, decreasing for higher concentrations. Accordingly, the ionic strength must be adjusted in standards and samples to a constant value, the above value of 10^{-2} M being considered optimum.

Analytical characteristics

Under the optimum working conditions obtained, a study was made of the effect of the Al concentration on the analytical signal, using mode B, for values between 2 and 250 µgl^{-1}.

The possible ranges of application and the equations obtained are shown in Table 2. The detection limit for the calibration at low concentrations proved to be 4.2 µgl^{-1}, meaning that the proposed method is one of the most sensitive of those reported in the literature. The reproducibility of the analytical signal was studied for 50 µgl^{-1} of Al(III), proving to be 3.2%. Moreover, since the procedure allowed the determination of 90 samples h^{-1}, the method in continuous mode can be considered one of the fastest for the determination of Al.

The precision of the method was studied for low and high concentrations, injecting 8 standard solutions of 20, 120 and 200 µgl^{-1} respectively, all of them at pH=4.5. Bearing in mind that some samples must be digested, the same study was carried out boiling each solution with 5 ml of 3 M H_2SO_4 per 100 ml of sample for 90 min and adjusting the final pH to 4.5 before bringing up to the initial volume. The results reveal satisfactory precision even for samples with relatively low concentrations and subjected to acid digestion.

Interferences

Under the same calibration conditions, we next studied the interfering capacity of ionic species that, due to a reaction with morin or Al, might affect the analytical signal and cause certain errors. All the metal cations with which morin is able to form complexes, fluorescent or not, interfered in the determination of Al. This is because they compete for the reagent, decreasing its free concentration. Additionally, the cations that form fluorescent chelates also interfere

because they are able to be excited or emitted at wavelengths close to those of the morin–Al(III) complex. Likewise, all anions able to react with Al(III) interfered in its determination with morin.

The criterion adopted to consider that a given species interfered as from a certain concentration was a decrease of ≥5 in the analytical signal.

The possible interfering cations studied were Ca(II), Co(II), Cr(III), Cu(II), Fe(III), Mg(II), Mn(II), NH$_4^+$ and Zn(II). Other possible interfering cations such as Zr(II), Sn(II), Ga(III) and In(III) were not studied because they are not normally present in drinking water.

The study was carried out independently for each of the species. A series of Al standards of 100 µgl^{-1} at pH=4.5 was prepared with increasing concentrations of the interfering species and these were injected in triplicate, averaging the signal obtained. Table 3 shows the minimum interfering concentrations of each ion.

When the concentration of morin injected was increased, the minimum interfering concentrations of certain cations increased, confirming that these did form complexes with morin that were less stable than that formed with Al. Furthermore, some of the interfering species, such as Cu(II), which interfered as from 0.8 mgl^{-1}, are not very frequent, and less so at such concentrations, in water fit for human consumption.

Table 3 Interferents in the continuous-mode fluorimetric detection of Al(III) with morin under calibration conditions

Interfering species	Concentration ≥ mg l^{-1}
F$^-$	0.16
Phosphates	0.50
Cu^{2+}	0.80
Fe^{3+}	1.5
Zn^{2+}	1.5
Cr^{3+}	1.5
NH$_4^+$	1.5
Mn^{2+}	3.0
Co^{2+}	20
Mg^{2+}	50
Ca^{2+}	80

Table 4 Analytical characteristics of the calibration of aluminium in the presence of SDBS: linear concentration range, number of standards (n), correlation coefficient (r^2) and limiting concentration (CL)

Range µg Al(III) l^{-1}	n	Equation	r^2	Detection limit µg Al(III) l^{-1}
2–50	6	If $= (1.7 \pm 3.5) + (3.81 \pm 0.13)$C	0.990	2.8

If, intensity of fluorescence emitted; *Ci*, concentration of Al(III), expressed in µg l^{-1}

Cations such as Fe(III) and Ca(II) are found in many treated waters. The presence of Fe(III), which interfered as from 1.5 mgl^{-1} may be masked due to reduction with hydroxylamine. The determination of Al in waters with a certain hardness—a Ca(II) content ≥80 mgl^{-1}—was achieved with higher concentrations of morin, although sensitivity was lost in the calibration.

Among the anionic species, the interference due to Cl$^-$, F$^-$, NO$_3^-$, PO$_4^{3-}$, SiO$_3^-$ and SO$_3^{2-}$ was assayed. With the exception of fluoride and phosphate, the remaining species did not interfere when they were present at concentrations ≤ 500-fold the concentration of Al(III). Fluoride and phosphate, which are usual interferents in photometric and fluorimetric methods for the determination of Al because they form fairly stable complexes with it, generated a clear decrease in the analytical signal. Although the minimum interfering concentrations were not very low—0.16 and 0.50 mgl^{-1} respectively—most drinking water contains amounts of the order, or even higher, of one or both species. The presence of phosphates or polyphosphates in amounts that interfere in the determination of Al with the proposed method was resolved by acid digestion of the samples, which destroys the complexes that Al forms with such species. From the point of view of selectivity, it may therefore be concluded that the proposed method is better than most spectrophotometric or spectrofluorimetric procedures reported in the literature, especially those most commonly used involving Eriochromocyanin R, pyrocatechol violet and, of course, lumigalion. Regarding interference by phosphates and fluoride, only the method proposed by Reis et al. [32] has lower interference levels with respect to both species.

Calibration in the presence of anionic surfactants

In order to increase sensitivity in the determination of Al by means of the formation of the complex with morin, a study was made of the effect of the presence of sodium dodecylbenzenesulfonate (SDBS) in the 50–500 µgl^{-1} range.

In the inverse flow system (Fig. 1b), 30 µl of a solution of 50 mgl^{-1} of morin in 3:1 ethanol/water and SDBS at varying concentrations was injected. Channel C$_1$ carried the standard solutions of Al at pH 4.5 at a flow rate of 2.30 ml min^{-1}. Channel C$_2$ carried an aqueous solution of 0.1 M NaCl at a flow rate of 0.30 ml min^{-1}. The emitted fluorescence intensity recorded when measuring at the usual wavelengths increased with the concentration of surfactant, a constant value being reached for concentrations of ≥250 µgl^{-1}.

Table 5 Aluminium content of the samples analysed

SAMPLE	PH	F$^-$(mg l^{-1})	Phosphates (mg PO$_4^{3-}$ l^{-1})	Al^{3+} (µg l^{-1})			
				Added	Proposed method		ETAAS
					Untreated	Treated	
Real samples							
[a]Outflow from plant-1	6.8	0.08			30±5	37±5	34
[a]Outflow from plant-2	6.9	0.08			58±5	57±5	52
[b]Rural well-1	7.0	0.13			14±5	17±5	16
[b]Rural well-2	6.6	0.16			25±5	28±5	30
[c]River Tormes-1	6.7	0.09	1.20		10±5	22±5	26
[c]River Tormes-2	6.6	0.10	3.05		12±5	20±5	24
Aqueous standard	6.8			110	110±10	117±10	120
Blank				<C$_L$	<C$_L$	5	

[a] Purification plants
[b] Rural wells at different localities
[c] Two different points of the river

The analytical characteristics of the method proposed for the determination of Al in the presence of SDBS are shown in Table 4.

Application to the determination of the Al content in water samples

The procedure was applied to the determination of the Al content in surface and ground waters and water subjected to purification processes. The results were compared with those obtained with electrothermal atomic absorption spectrometry with a graphite furnace.

The samples were from two different stretches of the River Tormes (Salamanca, Spain), from a treatment plant and, from a well in the rural setting. Some of the samples were filtered through a 0.45 μm pore membrane before being introduced into the flow system. Their pH and fluoride contents were also determined, the latter with electrometry with a selective fluoride electrode. Determination of the Al(III) content was performed on original untreated samples and on samples previously treated in acid medium to remove polyphosphates, in some cases also determining the contents of these (using the vanadomolybdophosphoric spectrophotometric method). The results are shown in Table 5 and show the mean values of three determinations. The differences observed between the treated and untreated samples are due to interference by phosphates, since in no case did the fluoride content surpass the minimum interfering concentration of the method. These results are in agreement with those obtained with electrothermal atomic absorption spectrometry (ETAAS). To validate these results, aluminium was also analysed in two Certified Quality Control Materials for water analysis (LGC6010 and LGC 6011, from Promochem) containing this and other trace elements (Ag(I), As(III), Ba(II), Ca(II), Cr(III), Fe(III), K(I), Mg(II), Mn(II), Na(I), Ni(II), Pb(II), Sb(III), Se(IV) and Zn(II)). The results found were not significantly different from the certified values.

Conclusions

The reaction between aluminium and morin, which leads to the formation of a fluorescent complex and allows the determination of the metal cation, has traditionally been performed in acid aqueous medium, pH 3, with a 5% volume of ethanol. Under these conditions the reaction shows slow kinetics at room temperature, such that the maximum signal is only obtained at 25 min after starting. Another important pitfall is the large number of interfering species, especially metal cations.

Fortunately the flow injection technique allows the reaction to be accomplished in a hydroalcoholic medium richer in ethanol. The strongly ethanolic aqueous medium modifies the fluorescence characteristics of the morin and of the complex with Al, allowing its determination at $5 \leq pH \leq 8$, with a detection limit 3.1 μgl^{-1}. Additionally, working with flow injection the kinetics of the reaction is modified, the maximum analytical signal being obtained 11 s after injection.

Accordingly, recalling that the maximum value of Al that can be present in drinking water is 200 μgl^{-1}, the calibrations are valid for the monitoring of this analyte in public drinking water, the only interference coming from fluoride and phosphates, at concentrations higher than 160 and 800 μgl^{-1} respectively.

In sum, the fluorescence reaction between morin and Al carried out in a flow system such as the one proposed here could be used in the design of a continuous flow system for monitoring aluminium concentrations, mainly at the exit of water purification systems, in which aluminium sulphate is used as a coagulant. The concentration of fluoride and phosphates is analysed first, because the former will be constant and the latter normally varies very little at the inflow of water-purifying plants. Sampling can be direct and injection of the morin solution can be automated periodically, thereby proving to be simple and economic.

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