Flow injection determination of aluminium by spectrofluorimetric detection after complexation with N-o-vanillidine–2-amino-p-cresol: The application to natural waters

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ABSTRACT

An on-line flow injection spectrofluorimetric method for the direct determination of aluminium in water samples is described. The method is based on the reaction of aluminium with N-o-vanillidine–2-amino-p-cresol (OVAC) in acidic medium at pH 4.0 to form a water-soluble complex. The excitation and emission wavelengths were 423.0 and 553.0 nm, respectively, at which the OVAC–Al complex gave the maximum fluorescence intensity at pH 4.0 in a 50% methanol–50% water medium at 50 °C. An interference from fluoride ions was minimised by the addition of Be^{2+}. Other ions were found not to interfere at the concentrations likely to be found in natural waters. The proposed methods were validated in terms of linearity, repeatability, detection limit, accuracy and selectivity. Under these conditions, the calibration was linear up to 1000 $\mu g\,L^{-1}$ ($r = 0.999$). The limit of detection (3σ) for the determination of Al(III) was 0.057 $\mu g\,L^{-1}$ and the precision for multiple determinations of 3 $ng\,mL^{-1}$ Al(III) prepared in ultra-pure water was found to be 0.62% ($n = 10$).

The Schiff base ligand could be used to determine ultra-trace aluminium from natural waters. Analysis of environmental certified reference materials showed good agreement with the certified values. The procedure was found to be equally applicable to both freshwater and saline solutions, including seawater.

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1. Introduction

During the last few decades aluminium and its distribution in the environment has attracted much attention. The concentrations of its dissolved species in most natural waters are below the ppb level because of the relatively low solubility of aluminium minerals. However, a considerable release of this element has occurred because of the increased input of acids into the environment [1]. Increased concentration of aluminium in natural waters and soil solutions has been reported as being poisonous to fish and marine bacteria [2] as well as to plants [3]. After entering the blood, aluminium accumulates in tissues such as bone, liver and the central nervous system with toxic consequences [4]. When it reaches a threshold concentration in the human body it is believed to cause either renal failure in patients undergoing treatment with peritoneal dialysis and haemodialysis [5] or diseases from the nervous system such as dementia and

encephalopathy [5], Alzheimer's disease [6] and Parkinson's disease [7].

Determination of aluminium is generally performed using atomic spectrometry. Such techniques are very selective, but they do not provide sufficiently low determination ranges. Moreover, they can be very time consuming (especially electrothermal AAS) and expensive in terms of both purchase and operating costs. In addition, they do not allow real-time or even on-site determinations. In order to obtain better detection limits, a pre-concentration or solid-phase extraction step is often required prior to the analysis. Molecular fluorescence spectrometry is an important analytical technique for quantitative determination of trace and ultra-trace inorganic substances since it is inherently more sensitive than many other molecular spectroscopic methods. In general, not only is the sensitivity of spectrofluorimetry two or three orders of magnitude higher than that of UV–vis spectrophotometry for example, but its selectivity is also much better than that of the latter method. However, because of the lack of specific methods, a prior separation step may be required [8].

An ideal analytical method for routine analysis and quality assurance should be precise and accurate, robust, automatic, simple and cost-effective, as well as having a high sample analysis frequency. Flow injection (FI) and sequential injection (SI) analysis are well-established analytical techniques that fulfil the above-mentioned demands. These readily automated techniques offer significant advantages for the determination of one selected analyte and can therefore be applied to routine analysis. Furthermore, SI over FI additionally offers a simpler flow manifold, reduced consumption of sample and reagents, the easier and more convenient variation of the experimental parameters and the greater potential for fluidic handling. There have been a few FI methods reporting the determination of aluminium in water samples [8–13].

N-o-vanillidine–2-amino-p-cresol was used first to detect aluminium using a spectrofluorimetric method developed by this research group [14]. The previous study reported the development of an off-line method for the determination of very low concentrations of aluminium in water samples. In this work, the method has been modified for use with flow injection analysis since it was noted that in the off-line method the reaction between N-o-vanillidine–2-amino-p-cresol and aluminium was very fast.

Many of the advantages of ship-board determination of aluminium are discussed in the excellent review article by Tria et al. [15]. These authors discussed a large number of methods reported in the literature for the determination of Al in the marine environment. Of these, very few discuss using a flow injection method with fluorescence detection. A report by Resing and Measures, however, discussed the use of lumogallion as the fluorophore whilst also using 8-hydroxyquinoline as a means of pre-concentrating the analyte [11]. Although an impressively low detection limit was observed (0.15 nM) and the procedure was used successfully aboard a ship, a few problems were noted. Amongst these was the use of a purified seawater as a carrier stream. The authors stated that achieving the necessary purity of the seawater was both troublesome and time consuming. Other papers that have used this pre-concentration method reported problems of irreproducible peak shapes and peak splitting [16]. In addition, the 8-hydroxyquinoline functionalised resins reportedly do not retain Al from freshwater matrices, thus limiting the potential for other sample types.

The potential advantages of modifying the procedure developed previously into an on-line flow injection method are that it may be automated more readily, it is fully enclosed and hence is less prone to contamination, it may be performed on-board ship because it does not require heavy, complex or cumbersome detection techniques and it requires less sample manipulation, again leading to a decreased chance of contamination. Since the analysis may be performed on-site, there is no need to transport samples back to the laboratory, hence the time interval between sampling and analysis is minimised, decreasing the possibility of analyte losses and facilitating the chance of re-sampling.

2. Experimental

2.1. Apparatus

Fluorescence measurements were made using a commercial HPLC fluorescence detector (Varian model 9070) (Wokingham, UK) for both excitation and emission. pH adjustments of the solutions were monitored using a Jenway 3305 pH meter (Essex, UK). The FI system consisted of a Gilson Minipuls 3 peristaltic pump (Anachem Ltd., Luton, UK), a HPLC pump (Varian 9001), two 4-way valves (Rheodyne 5040), a thermostatted water bath (Grant Instruments, Cambridge, UK) and a mixing coil.

2.2. Reagents and solutions

A stock solution containing $100\,mg\,L^{-1}$ of Al was prepared from a $10{,}000\,mg\,L^{-1}$ stock standard solution (BDH, Aristar, Poole, UK). Working standards of between 1 and $10\,\mu g\,L^{-1}$ were prepared on a daily basis by dilution from $1\,mg\,L^{-1}$ solution prepared from the stock standard. Buffer solution, at a concentration of $1.0\,M$ and at pH 4.0, was prepared using pure acetic acid and sodium acetate (BDH).

All reagents used were of the highest available purity. Doubly de-ionized water ($18.2\,M\Omega\,cm$) obtained from a Maxima water system (Elga, Buckinghamshire, UK) was used throughout. O-vanillin, 2-amino-p-cresol and methanol were purchased from Fluka (Gillingham, Dorset, UK).

2.3. Synthesis of N-o-vanillidine–2-amino-p-cresol (OVAC)

N-o-vanillidine–2-amino-p-cresol (OVAC) was synthesized according to the procedure given in the literature [17]. The structure of this molecule is shown in Fig. 1.
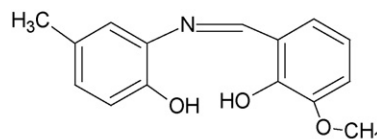

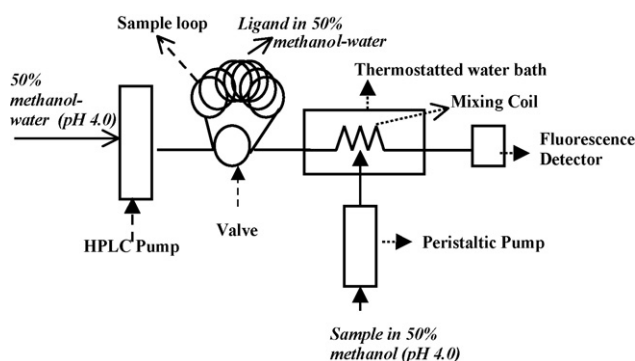**Fig. 1 – Structure of the OVAC molecule.**

Fig. 2 – A schematic diagram of the flow injection system to determine aluminium using spectrofluorimetric detection.

2.4. Procedures

A schematic diagram of the on-line flow injection system is presented in Fig. 2. The HPLC pump transported the 50% methanol–water buffered to pH 4.0 at a flow rate of 0.7 mL min^{-1}. The valve had a sample loop (0.7 mL) fitted that could be filled with ligand solution (1×10^{-4} mol L^{-1}) in a 50% methanol–water mixture. The second valve was connected to a peristaltic pump uptaking sample solution in 50% methanol at pH 4.0. Standard Al solutions were prepared using sufficient Al to produce calibration standards in the range 1–10 μg L^{-1}. Then, 1 mL of acetate buffer (1.0 M, pH 4.0) and 0.2 mL of 100 mg L^{-1} Be solution were added to pre-cleaned 10 mL volumetric flasks in 50% methanol–water mixture. The outlet tubes coming from the two valves were connected by a mixing T and a mixing coil (1 mL) in a thermostatted water bath (50 °C). The outlet of the mixing coil was connected to a fluorescence detector (the excitation and emission wavelengths were 423.0 and 553.0 nm, respectively). The flow rate of the system was optimized and found to be 0.7 mL min^{-1}. The aluminium concentrations in the samples were determined from these calibration graphs. To achieve this, the sample was dispensed into the 10 mL volumetric flasks, prior to the other reagents being added. It is important to note that the concentration of methanol in all of the flow streams be kept the same. If there were differences in methanol concentration between the different flow streams, then bubbles were produced causing instability of the fluorescence signal.

3. Results and discussion

3.1. Fluorescence spectrum of the Al–OVAC complex

Previous work determined the optimum analytical wavelengths and the spectral characteristics of the Al(III)–OVAC complex at various pH values. The excitation and emission spectra of the Al(III)–OVAC complex indicated that the most intense excitation and emission wavelengths were at 423.0 and 553.0 nm, respectively. The un-complexed ligand OVAC gave very low intensity at these wavelengths [14]. These optimal wavelengths were therefore chosen for this present study.

3.2. Optimization

The experimental conditions such as the wavelengths of excitation and emission, pH, solvent–water ratio and concentration of OVAC were optimized in previous work. A pH of 4.0, fixed by an acetate buffer, was used as optimum for all subsequent work. All solutions were prepared in a methanol–water mixture because the ligand is not soluble in pure water. In addition, if some solutions contained methanol and others did not, bubbles were produced in the flow system causing instability of the fluorescence intensities. Fifty percent methanol concentration was therefore selected as a compromise between greatest sensitivity and the ability to introduce all the relevant reagents/sample. For seawater samples, a 50:50 mixture of methanol and water led to precipitation. The method was therefore modified slightly so that a methanol concentration of 40% was used in both the ligand solution and the sample. Results indicated that the fluorescence of the complex solution containing 1 μM of Al reached a maximum when the amount of OVAC exceeded 100 μM at the optimized pH and time. Therefore 100 μM was used for all subsequent work.

In this work, some other parameters needed to be optimized such as the flow rates of the FI system and the reaction temperature. In our previous work, the OVAC ligand was used to develop an off-line spectrofluorimetric detection method at room temperature. In that method, the time required to obtain a stable fluorescence intensity was about 20 min. This is not suitable for use in a flow injection system. In this work the effect of temperature have been studied to investigate any increase in the reaction kinetics between 20 and 60 °C in flow injection system at the flow rate of 0.7 mL min^{-1} (Fig. 3). The temperature was found to affect the kinetics of the reaction greatly, with the fluorescence signal increasing more than twofold between 20 and 37 °C. After that temperature, the signal increased further but at a reduced rate. The optimal experimental temperature was selected as 50 °C, because at this temperature the fluorescence intensity was almost its maximum whilst the temperature was sufficiently low so as not to boil the solutions (causing bubbling and instability). In addition, evaporation of water from the thermostatted water bath was also minimized.

The optimization of the sample flow rate is very important to get higher fluorescence intensity and a lower limit
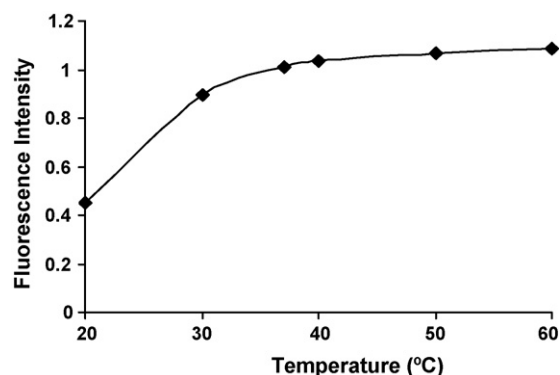

Fig. 3 – The effect of temperature on the fluorescence intensity of aluminium using the flow injection system.
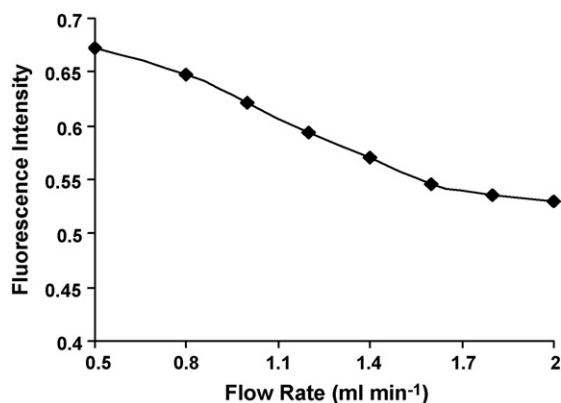
Fig. 4 – The effect of flow rate on the fluorecsence intensity of the aluminium complex using the flow injection system.

of detection for aluminium. Higher sample flow rates and higher reaction yield are desired in flow injection experiments because this will give the highest fluorescence intensity in the shortest time. The sample flow rate was evaluated between 0.5 and 2 mL min^{-1} and the signal was recorded with the fluorescence detector without any pre-concentration step. The effects of sample flow rate are shown in Fig. 4. As the flow rate increases, the fluorescence intensity decreases slowly. A compromise between speed of analysis and signal intensity was therefore required. A sample flow rate of 0.7 mL min^{-1} was selected as optimum for all subsequent experiments.

3.3. Precision and detection limit

Using the optimized conditions, the limit of detection (LOD) (3σ) for Al(III) was 2.11 nM (0.057 μg L^{-1}), determined from the analysis of 10 different solutions of 2 μg L^{-1} Al(III). From our previous work, the limit of detection using the off-line procedure was found to be 7.40 nM (0.19 μg L^{-1}), determined from the analysis of 11 different solutions of 3 μg L^{-1} Al(III). The LOD obtained using the flow injection system at 50 °C is therefore over three times lower than that obtained using the off-line system. In addition, this LOD is much lower than that reported for the original Al-lumogallion method (37 ± 2 nM) and its recent applications [18]. The improved lumogallion method has been reported to have a LOD of 0.7 nM [19]. This method is, however, very time consuming, since the complexation time for Al(III) and lumogallion is 24 h at room temperature, and in excess of an hour even at 80 °C. The task of modifying the procedure into an automated FI method would, therefore, not be easy. Despite this, the paper by Resing and Measures did

manage to report the use of lumogallion in a FI mode [11]. As described in Section 1, there were, however, some problems associated with their procedure. The method developed in this work enables the Al to be determined using a FI system in less than 2 min.

The calibration for this method was found to be linear up to at least 1000 μg L^{-1} and the regression equation was $Y = 0.020C_{Al} + 0.054$ ($r = 0.999$). A standard of 3 μg L^{-1} of Al(III) prepared in ultra-pure water was analyzed in replicate ($n = 10$) and the relative standard deviation was calculated to be 0.62%.

3.4. Analysis of real samples

The proposed method was used to determine Al in different environmental matrices such as river water and seawater. River water was collected from the River Dart, Devon, UK and the seawater was collected from Plymouth Hoe, Devon, UK. Both of those samples were filtered immediately on return to the laboratory using 0.45 μm cellulose acetate filters and then acidified with HNO$_3$ to a pH of 1.6. Before analysis, the pH of the solution was adjusted to pH 4 by the addition of 0.1 M NaOH. A sample aliquot (3.5 mL), 1 mL of acetate buffer (1.0 M, pH 4.0), 0.2 mL of 100 mg L^{-1} Be solution (to remove interferences from fluoride) and 5 mL of methanol were added to pre-cleaned 10 mL capacity volumetric flasks. After addition of all the reagents, the final volume of the solutions was adjusted to 10 mL and the fluorescence signal measured using the flow injection system. Precision for the natural concentration in the waters was found to be in the range 0.60–2.35% R.S.D. As can be seen from Table 1, excellent precision was obtained for the analysis of the same sample ($n = 5$) during the same run. In addition, the results obtained are also in close agreement with those using the off-line method and from the lumogallion method. Overall, it can be concluded that the technique is reliable, reproducible and repeatable.

When a comparison of more than two treatment or sample means is required, the null hypothesis to be tested is usually that the t treatment or sample means are the same, and that the alternative hypothesis is that they are not. Analysis of variance (ANOVA) is a useful technique for making decisions about hypotheses. In ANOVA it is actually the variation in the t treatment or sample responses that is used to decide whether or not treatment effects are significant. The one-way ANOVA statistical method was applied to the results obtained using the different methods to determine whether or not there were any significant differences between them [20]. To accomplish this, the SPSS 10.0 for windows program (Woking, Surrey, UK) was therefore used as a chemometric package. Results from the ANOVA analysis indicated that there was no signifi-

Table 1 – The comparison of the results obtained from the analysis of natural waters using the on-line flow injection fluorimetric method, the off line fluorimetric method using OVAC and the standard lumogallion method

Sample	Found value (μg L^{-1}) for the lumogallion method (X_1)	Found value (μg L^{-1}) for the off-line method	Found value (μg L^{-1}) for the on-line method
Seawater	27.49 ± 0.61	26.82 ± 0.73	25.40 ± 0.08
River water	36.75 ± 0.77	37.15 ± 1.15	38.50 ± 0.50

$N = 5$.

Table 2 – Analysis of certified reference materials ($n = 5$)				
Sample	Certified value ($\mu g\,L^{-1}$)	Found value ($\mu g\,L^{-1}$)	$|x - \mu|$	$\frac{ts}{\sqrt{N}}$
High purity standards trace metals in drinking water	120	119.3 ± 2.8	0.7	3.23
SLRS-2 river water	84.4 ± 3.4	84.0 ± 0.5	0.4	3.92
SLRS-4 river water	54 ± 4	51.5 ± 0.5	2.5	4.61
NIST 1643c trace elements in water	114.6 ± 5.1	114.9 ± 0.8	0.3	5.88

Table 3 – Characteristics of fluorimetric flow injection methods for the determination of aluminium in water samples					
Method	LODs ($\mu g\,L^{-1}$)	Detection method	Interferences	Samples	Reference
Morin	10.0	Fluorimetric	Fe(III), Mg(II), Cu(II), Ca(II), Mn(II) and Zn(II)	Tap and river water	[8]
Chromotropic acid	2.6	Fluorimetric	Fe(III) and Mg(II)	Drinking water	[9]
8-Hydroxyquinoline	0.2–6	Fluorimetric	Ca(II), F$^-$, Cu(II), Mg(II) and Fe(III)	Tap, river and wastewater	[10]
Lumogallion (pre-concentration on a column of resin-immobilized 8-hydroxyquinoline)	0.004	Fluorimetric	Fe(III) and F$^-$	Seawater	[11]
Salicylaldehyde picolinoylhydrazone	1.9	Fluorimetric	Cu(II), Fe(III) and Zn(II)	Drinking water	[12]
8-Hydroxyquinoline-5-sulphonic acid	0.5	Fluorimetric	Ca(II), Mg(II), Zn(II), Cu(II) and Fe(III)	Drinking water	[13]
This study	0.057	Fluorimetric	F$^-$	River and seawater	This work

cant difference between the results obtained from any of the methods.

3.5. Analysis of certified reference materials

In order to evaluate the accuracy and precision of the proposed method further, four certified reference materials (CRMs), a high purity standard (CRM–TMDW, trace metals in drinking water), a water (NIST 1643c, trace elements in water) and river waters (SLRS-2 and SLRS-4) were analyzed using the proposed method under optimal conditions. Each of the CRMs were analyzed in replicate ($n = 5$) and the results for the aluminium obtained are shown in Table 2. The results obtained by the proposed method were in good agreement with the certified values. The Student's t-test was applied to the results of the proposed method and certified values. The results show that $|x - \mu|$ differences are smaller than $ts/\sqrt{N}$ for all certified samples, indicating that there is no evidence of systematic error in the proposed method.

3.6. Comparison with other flow injection fluorimetric determination methods of aluminium in water samples

Comparative data for figures of merit from some recent papers reporting the flow injection fluorimetric determination of aluminium are summarized in Table 3. The method presented in this study is clearly one of the most promising for the flow injection determination of aluminium. The detection limit routinely achieved in this work is lower than all of the other flow injection methods except for the lumogallion method developed by Resing and Measures [11]. All the methods cited in Table 3 report a significant interference from iron but as demonstrated in previous work, iron does not cause interference until present in excess of $250\,\mu g\,L^{-1}$; a level not usually

found in natural water samples. The seawater analyses in particular can be problematic because of the high salinity. Only one of the flow injection methods in the literature could be applied for the determination of aluminium in seawater samples. This paper by Resing and Measures used a pre-concentration procedure utilizing 8-hydroxyquinoline (that had the added effect of removing iron from the samples). Since this paper used a pre-concentration system, the LOD reported was approximately 10 times superior to that reported here. However, as described previously, this pre-concentration procedure was not without problems. In addition, purified seawater was used as sample carrier. Since it is both time consuming and problematic to produce this purified seawater, the authors identified the need for a method that could simply use ultra-pure water as sample carrier. The method proposed in this work has achieved this and has been used successfully to determine aluminium in either sea or in freshwater samples. It is possible that, had a pre-concentration system been used, the LODs reported in this current work could have been improved to a level comparable with that reported by Resing and Measures.

4. Conclusions

The OVAC ligand, a Schiff base that is easily synthesised in the laboratory, forms a highly fluorescent complex with aluminium. This material was used to develop a simple yet very versatile on-line flow injection spectrofluorimetric method for the determination of aluminium in natural water samples. The work reported here developed an easy and fast flow injection system using the OVAC ligand. The off-line method, developed previously [14] had been used at room temperature and required about 20 min to obtain a stable fluorescence intensity. In the flow injection application of the

method, the effect of reaction temperature was investigated and it was shown that the reaction kinetics between OVAC and aluminium increased as the temperature increased with the fluorescence signal increasing about twofold at 50 °C. The limit of detection of the proposed flow injection method was substantially lower than the other flow injection methods and was even lower than the off-line procedure using OVAC. The method was also reliable and reproducible, is relatively fast and requires few reagents or solvents. These properties have enabled it to be used in routine analysis and monitoring experiments and it offers clear advantages over the alternative lumogallion off-line method and other FI methods. The only significant interference identified was by fluoride, but this could readily be overcome by the addition of beryllium ions to the sample. The successful analysis of certified reference materials and of natural water samples demonstrates the robust character and reliability of the method.

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