

Determination of Chloride in Surface and Groundwater by Centrifugal Analysis

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A procedure has been developed for the determination of chloride in environmental samples using a Cobas Fara centrifugal analyser. The procedure is based on the spectrophotometric hexacyanoferrate(III) method. Analytical variables are manipulated with ease using this instrument, thus simplifying the task of choosing reagents and reagent concentrations. Of the two commonly used iron(III) donors, iron(III) nitrate was found to be more sensitive than ammonium iron(III) sulphate, and the calibration graph for iron(III) nitrate was more linear. Assessed by comparison with reference mineral samples from the US EPA, and by results obtained using a Technicon AAI continuous flow analyser, the results obtained using the Cobas Fara procedure were found to be both accurate and precise. The following equation describes the relationship developed when surface water and groundwater samples containing chloride are analysed using the Cobas Fara and the Technicon AAI analysers: $y = 0.994x + 0.089$; $r^2 = 1.0$; $n = 79$. It is concluded that the Cobas Fara procedure produces high quality results with a considerable time (300 samples h^{-1}) and sample-size (80 μ l) saving.

Keywords: Chloride determination; Cobas Fara centrifugal analyser; hexacyanoferrate(III); groundwater; surface water

For many years centrifugal analysis has been accepted as a fast, reliable, convenient and economical method of analysis in the clinical laboratory. The technique was developed at the Oak Ridge Laboratory. Centrifugal analysers consist of computer-controlled pipettor and analyser units. Among the beneficial attributes of these analysers are ease of method development, increased speed of analysis, small sample size requirements and quickness of changeover between chemistries.¹

Centrifugal analysis could offer considerable benefit to environmental laboratories. Following a demonstration of the Cobas Fara centrifugal analyser in an adjoining clinical laboratory, we considered, and were impressed with, the adaptation of a standard procedure for the determination of chloride using the instrument. This paper reports a method that we developed for the routine determination of chloride in environmental samples.

Experimental

Equipment

A Cobas Fara centrifugal analyser was purchased from Hoffmann-La Roche in Canada. Comparisons were made using standard methodology for a Technicon AAI continuous flow analyser purchased from Technicon Canada.

Reagents

All reagents were of analytical-reagent grade and solutions were prepared with distilled, de-ionised water.

Ammonium iron(III) sulphate. A 60-g amount of ammonium iron(III) sulphate was dissolved in approximately 300 ml of water and 384 ml of concentrated nitric acid were added. The solution was diluted to 1000 ml with water.

Iron(III) nitrate. A 202-g amount of iron(III) nitrate was dissolved in approximately 200 ml of water and 21 ml of concentrated nitric acid were added. The solution was diluted to 1000 ml with water, filtered and stored in an amber bottle.

Mercury(II) thiocyanate in methanol. A 4.17-g amount of mercury(II) thiocyanate was dissolved in approximately 500

ml of methanol and the solution was diluted to 1000 ml with methanol, filtered and stored in an amber bottle.

Mercury(II) thiocyanate, aqueous. A 5-g amount of mercury(II) thiocyanate was dissolved in approximately 500 ml of water and the solution was diluted to 1000 ml with water, filtered and stored in an amber bottle.

Colour reagent [iron(III) nitrate]. Prepared fresh daily by mixing 15 ml of iron(III) nitrate and 15 ml of mercury(II) thiocyanate in methanol and diluting to 100 ml with water.

Colour reagent [ammonium iron(III) sulphate]. Prepared fresh daily by mixing 40 ml of ammonium iron(III) sulphate and 60 ml of mercury(II) thiocyanate (aqueous).

Standards and Reference Samples

Standards

A stock solution of 1000 $mg\ l^{-1}$ of chloride was prepared by dissolving 1.6482 g of sodium chloride, dried at 140 °C, in 1000 ml of water. The stock 1000 $mg\ l^{-1}$ chloride solution was then used to prepare 0, 10, 20, 40, 60, 80 and 100 $mg\ l^{-1}$ standard solutions by diluting 0, 1.0, 2.0, 4.0, 6.0, 8.0 and 10.0 ml, respectively, of the stock solution to 100 ml with water in calibrated flasks.

Reference samples

Reference, quality control samples for mineral analysis were obtained from the United States Environmental Protection Agency (EPA) in Cincinnati. Solutions were prepared from concentrates labelled 1A, 1B and 1C (Lot No. WP384) by transferring 20 ml of each into 900 ml of water and diluting to volume with water in a 1-l calibrated flask labelled Mineral No. 9. Additional samples of concentrates labelled A and B (Lot No. WF1185) were used to prepare a solution by transferring 10 ml of each into 1800 ml of water and diluting to volume with water in a 2-l calibrated flask labelled Mineral No. 10.

Methods

Reference 2 gives four methods for the determination of chloride, including a titrimetric method, an argentimetric method, a potentiometric method and a spectrophotometric hexacyanoferrate(III) method. The last method has been

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designated as standard in this text, and also in publications of the US EPA,³ the Environment Canada's Inland Waters Directorate,⁴ ASTM publications⁵ and the Illinois State Water Survey.⁶ The spectrophotometric hexacyanoferrate(III) procedure results in the liberation of thiocyanate ion from mercury(II) thiocyanate by chloride, and the formation of iron(III) thiocyanate, a coloured complex, in proportion to the chloride concentration in the sample. Different sources of iron(III) may be used, such as iron(III) nitrate²⁻⁴ and ammonium iron(III) sulphate.^{3,5,6}

It was decided to study these different colour reagents using the Cobas Fara centrifugal analyser. The instrument was set up and operated in accordance with the manufacturer's specifications. Samples or standards and controls, in sealed 700- μ l plastic cups, were placed with the appropriate colour reagent in the sample, calibration or reagent rack and then loaded into the instrument. A plastic cuvette rotor was placed in the instrument. The appropriate test parameters, listed in Table 1, were called up by pressing the designated chloride test key, and the analysis was initiated by depressing the start key. All remaining steps were carried out by the instrument.

The Technicon AAI continuous flow analyser was also set up and operated in accordance with the manufacturer's specifications. The manifold described in reference 2 was used with the colour reagent ammonium iron(III) sulphate - mercury(II) thiocyanate. The analyses were carried out at a rate of 60 h⁻¹. The results obtained with this instrument were compared with those obtained with the Cobas Fara centrifugal analyser.

Results and Discussion

General Observations

Cobas Fara instrument settings for the various test parameters in the procedures involving iron(III) nitrate or ammonium iron(III) sulphate are shown in Table 1. Samples or standards and controls were added concurrently with the colour reagent, in a technique known as parallel pipetting, to compartments in the plastic cuvette rotor. This operation took approximately 5.5 min to complete. The mixing cycle was initiated as the rotor began to spin causing the samples to be propelled from their individual wells into the common optical compartment. An absorbance at 480 nm was measured 5 s after completion of mixing. The results were automatically calculated from a logarithmic calibration model fitted to the standard data.

Choice of Colour Reagent

Standard calibration graphs are presented in Fig. 1 for both of the procedures developed, one using iron(III) nitrate and the other using ammonium iron(III) sulphate. The iron(III) nitrate procedure was found to be more sensitive and more linear than the ammonium iron(III) sulphate procedure.

Effect of Carry-over

The effect of sample to sample contamination in the Cobas Fara analyser during the pipetting sequence was investigated. The maximum sample and diluent (wash-out) volume for the Cobas Fara procedure is 90 μ l. A number of runs consisting of five sets of an 80 mg l⁻¹ standard followed by two 5 mg l⁻¹ standards were carried out using the following sample and diluent volume ratios: 90:0, 85:5, 80:10, 75:15 and 70:20 μ l. The results are summarised in Table 2. The results show that the effect of carry-over was statistically significant only when a sample to diluent ratio of 90:0 μ l was used. Nonetheless, to provide a margin of safety, a sample to diluent ratio of 80:10 μ l was chosen for all subsequent experiments.

Reagent Optimisation

The ease with which analytical variables can be manipulated using the Cobas Fara analyser simplified the task of reagent

optimisation. Proportions of each reagent, that is, mercury(II) thiocyanate and iron(III) nitrate or ammonium iron(III) sulphate, were optimised by adjusting the volume of mercury(II) thiocyanate in 10 μ l increments above and below the standard volume usually quoted. Volumes of iron(III) nitrate or ammonium iron(III) sulphate were kept constant. The results obtained are presented in Figs. 2 and 3.

Standard procedures using the iron(III) nitrate colour reagent prescribe a 1:1 ratio of iron(III) nitrate to mercury(II) thiocyanate. The results of these tests suggest a slight improvement in absorbance with increasing amounts of mercury(II) thiocyanate. The absorbance increase was marginal; therefore, taking into consideration the ease of preparation of a 1:1 ratio, a colour reagent using equal volumes was chosen. For the ammonium iron(III) sulphate colour reagent, the EPA suggests a ratio of mercury(II) thiocyanate to ammonium iron(III) sulphate of 0.375:1.³ The results given in Fig. 3 show that the absorbance increases as the proportion of mercury(II) thiocyanate increases, then begins to level off at ratios greater than 1. Hence, we chose a mercury(II) thiocyanate to ammonium iron(III) sulphate ratio of 1:1.5 as the optimum.

Tests were also performed to determine the optimum volume of the iron(III) nitrate colour reagent relative to the volume of sample or standard. The reagent volume was adjusted in 20 μ l increments while the sample or standard (plus diluent) volume was held constant. The results are presented in Fig. 4. A reagent to sample or standard ratio of 3.3:1.0 was chosen. Other possible combinations used in the determination of chloride are also plotted in Fig. 4. They

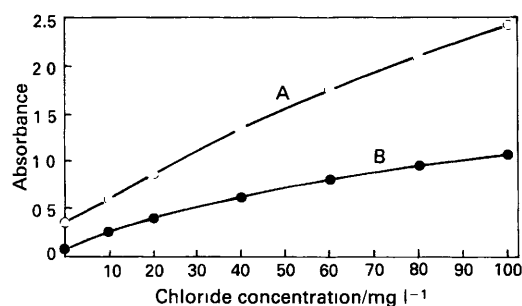


Fig. 1. Standard calibration graphs for chloride using two different colour reagents. (A) Iron(III) nitrate - mercury(II) thiocyanate; and (B) ammonium iron(III) sulphate - mercury(II) thiocyanate

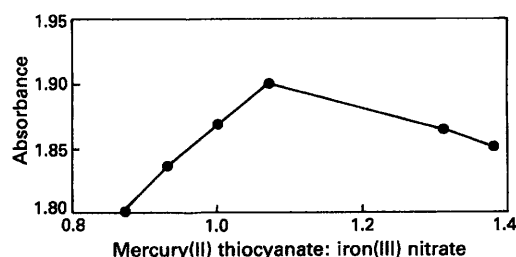
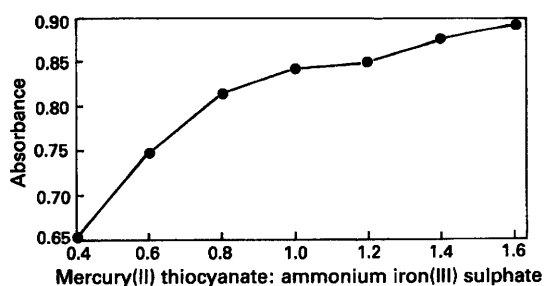
Table 1. Instrument settings used for the Cobas Fara chloride method

Measurement mode	Absorb
Reaction mode	II-P-A0
Calibration mode	Std. non-linear
Reagent blank	No blank
Wavelength	480 nm
Temperature	25 °C
Unit	mg l ⁻¹
Incubation time	5 s
Sample volume	80 μ l
Diluent volume	10 μ l
Reagent volume*	300 μ l
Time of 1st reading	5 s
Number of readings	1
Standard 1 concentration	0 mg l ⁻¹
Standard 2 concentration	10 mg l ⁻¹
Standard 3 concentration	20 mg l ⁻¹
Standard 4 concentration	40 mg l ⁻¹
Standard 5 concentration	60 mg l ⁻¹
Standard 6 concentration	80 mg l ⁻¹
Standard 7 concentration	100 mg l ⁻¹
Calculation model	LOGIT/LOG 4

* Either ammonium iron(III) sulphate or iron(III) nitrate colour reagent can be used.

Table 2. Effect of carry-over

Sample/ diluent volume/ μ l	Mean absorbance (x) of 5 mg l ⁻¹ following 80 mg l ⁻¹	Mean absorbance (y) of 5 mg l ⁻¹ following 5 mg l ⁻¹	Mean absorbance (x - y)	Paired <i>t</i> value	Probability (2-tail)
90/0	0.4785	0.4775	0.001	2.8178	0.0226
85/5	0.4672	0.4663	0.0009	1.0973	0.3044
80/10	0.4635	0.4635	0.00003	0.0505	0.961
75/15	0.4607	0.4612	-0.0004	-0.3515	0.7343
70/20	0.4548	0.4543	0.0005	0.7743	0.461

Fig. 2. Reagent optimisation. Effect of varying the ratio of mercury(II) thiocyanate (methanol) to iron(III) nitrate using a chloride solution of 100 mg l⁻¹Fig. 3. Reagent optimisation. Effect of varying the ratio of mercury(II) thiocyanate (aqueous) to ammonium iron(III) sulphate using a chloride solution of 100 mg l⁻¹

include the ratio of 0.8:1.0 used by Environment Canada,⁴ the ratio of 3.1:1.0 cited in reference 2 and the ratio of 0.9:1.0 used by the EPA.³

Time Optimisation

A principal advantage of the Cobas Fara analyser is the speed with which results can be generated. The total test time for the procedure was kept to a minimum by choosing a short incubation time and parallel pipetting.

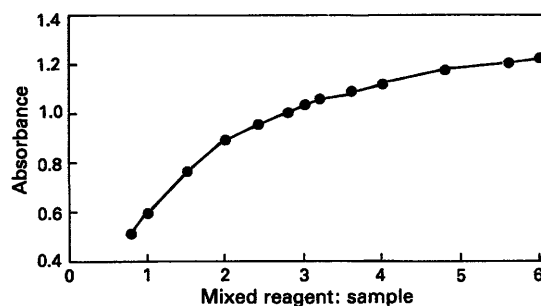
Complete colour development was found to occur immediately as shown in Table 3. This table was compiled by taking absorbance readings every 5 s beginning 1 s after reagent and sample union. Although complete colour development occurred immediately, it was decided to standardise the incubation time at 5 s as a compromise between optimum time and good analytical technique was desired.

Parallel pipetting, that is, adding sample and colour reagent to the cuvette simultaneously rather than sequentially, saved approximately 3 min for each rotor of 30 cuvettes. The Cobas Fara software requires that the initial absorbance measurements be taken to satisfy subsequent calibration models. The initial absorbance measurement is taken with an empty cuvette.

The total time required to analyse 30 samples for chloride

Table 3. Absorbances for a standard 60 mg l⁻¹ sample taken every 5 s beginning 1 s after mixing

Time/s	Absorbance	Time/s	Absorbance	Time/s	Absorbance
1	1.6121	81	1.6037	161	1.5981
6	1.6119	86	1.6032	166	1.5977
11	1.6106	91	1.6022	171	1.5984
16	1.6107	96	1.6018	176	1.5977
21	1.6099	101	1.6022	181	1.5975
26	1.6093	106	1.6013	186	1.5964
31	1.6087	111	1.6012	191	1.5969
36	1.6081	116	1.6008	196	1.5963
41	1.6079	121	1.6009	201	1.5952
46	1.6071	126	1.6003	206	1.5957
51	1.6069	131	1.5998	211	1.5959
56	1.6058	136	1.6001	216	1.5943
61	1.6053	141	1.5986	221	1.5947
66	1.6049	146	1.5989	226	1.5949
71	1.6039	151	1.5987	231	1.5947
76	1.6048	156	1.5993	236	1.5938

Fig. 4. Reagent optimisation. Effect of varying the ratio of colour reagent to sample using a 100 mg l⁻¹ chloride solution. Sample and reagent volumes halved to compensate for volume restriction

by this procedure was 6 min; this was directly extrapolated to an analysis rate of 300 samples h⁻¹.

Analytical Variables

Accuracy

The reference mineral samples obtained from the EPA for quality control (QC) purposes were analysed daily for several weeks. The results are summarised in Table 4. Both the low and high chloride QC samples had a slight positive bias. The differences between the assayed values and the stated or certified values were found to be significant; however, the assayed value for each QC sample fell within the range quoted by the EPA. The bias detected is possibly a calibration variance that would probably have gone undetected had it not been for such a low coefficient of variation typical of the Cobas Fara analyser.

The results obtained by the Cobas Fara chloride procedure were compared with the results obtained by the Technicon AAI procedure. Parallel analyses by the Cobas Fara and Technicon AAI procedures, in duplicate, for 40 surface water (mean 8.0 mg l⁻¹, SD 4.5 mg l⁻¹) and 39 groundwater (mean 46.4 mg l⁻¹, SD 26.0 mg l⁻¹) samples were run and the average chloride concentrations obtained for each procedure were plotted. The results from both procedures agree well throughout the analytical range. The statistical data describing the agreement of the Cobas Fara procedure with the Technicon AAI procedure are as follows: $y = 0.994x + 0.089$; $r^2 = 1.0$; $n = 79$; and the 95% confidence interval for the slope is 0.97–1.03.

Variations in the differences between replicates for each procedure were calculated and the results are given in Table 5.

Table 5. Although the replicate population variances were found to be statistically different, for all practical purposes the two methods were considered to be similar as the variances obtained were at such low levels as to be practically insignificant. The mean and standard deviations of the differences between the individual average Cobas Fara and average Technicon AAI results were also calculated (Table 5). Statistically, no difference was detected between the Cobas Fara and the Technicon AAI procedures.

Recovery

The mean recoveries for the Cobas Fara chloride procedure were determined from spiked samples and the results are summarised in Table 6. The mean recovery was 101.6% for a sample with a chloride concentration of 16.1 mg l⁻¹ spiked with an additional 10 mg l⁻¹ of chloride. In a second sample, with a chloride concentration of 3.9 mg l⁻¹ spiked with an additional 2 mg l⁻¹ of chloride, the mean recovery was 102.6%.

Precision

Table 7 gives precision data for chloride determined by the Cobas Fara procedure using the EPA QC samples. Both within-run and between-day precision were considered. In all instances, the coefficient of variation was less than 1%. Although the standard deviation was slightly larger within the run, the differences on a practical scale were very small.

Interferences

Humic acids are occasionally present in surface water samples commonly analysed for chloride in our laboratory. Humic acids impart a slight brown colour to the samples, which causes positive interference in the spectrophotometric chloride procedure. For example, 10 mg l⁻¹ of humic acid will increase the chloride concentration by approximately 0.8 mg l⁻¹. This interference can be reduced 4-fold, from 0.8 to 0.2 mg l⁻¹, by allowing the sample to serve as its own blank. Chloride interference from increasing concentrations of humic acids, with and without sample blanking, is shown in Fig. 5.

This step is easily accommodated by sequential addition of the sample and colour reagent as opposed to simultaneous addition or parallel pipetting. In addition, this will increase the analysis time by 3 min per rotor, thus decreasing the theoretical analysis rate from 300 to 200 samples h⁻¹.

This interference effect is similar for both the Cobas Fara and the Technicon AAI procedures. Both instruments have the capability of allowing each sample to serve as its own blank.

Table 4. Bias of the Cobas Fara chloride procedure determined from quality control samples

Stated level/ mg l ⁻¹	Stated range/ mg l ⁻¹	Assayed level/ mg l ⁻¹	<i>n</i>	Bias/ mg l ⁻¹	Bias, %	<i>t</i> value	Probability level
80.8	75.5–86	81.0	30	0.24	0.30	3.151	0.004
25.9	22.3–28	26.0	30	0.1	0.39	3.177	0.004

Table 5. Comparison of results obtained using the Cobas Fara and Technicon AAI methods

Group	Variance between replicates			Replicate mean difference
	Cobas Fara	Technicon AAI	Variance ratio	Cobas Fara – Technicon AAI
All samples	0.074	0.109	0.679	0.069
Surface water	0.005	0.139	0.036	0.009
Groundwater	0.146	0.086	1.698	0.116

Linearity and sensitivity

Model choice. Under the standard non-linear calibration mode, the Cobas Fara analyser offers a choice of six calculation models. The chloride standard curve was run a number of times to obtain statistically significant data for each point. Because the chloride method is not linear, we decided not to use the linear interpolation or regression models. Of the four mathematical algorithms, a log *versus* log match appeared to give a reasonable fit with our calibration graph data. A comparison of LOGIT/LOG 4 and LOGIT/LOG 5 calculation models showed better agreement using the LOGIT/LOG 4 model. The standard points were closer to the true values of the standards with a lower average coefficient of variation (CV = 0.45 for LOGIT/LOG 5 *versus* CV = 0.35 for LOGIT/LOG 4).

Detection limit. The minimum concentration of chloride, above zero, that can be reported with a 99% confidence was determined. The standard deviation of a solution containing a chloride concentration of approximately 2 mg l⁻¹, together with the Student's *t* value for a one-tailed test appropriate for a 99% confidence level, allowed us to operationally define the method detection limit as 0.3 mg l⁻¹. Approximating the graph of absorbance *versus* chloride concentration as linear allowed us to roughly calculate an absorbance sensitivity for the Cobas Fara method of 0.02 A l mg⁻¹.

Conclusion

Centrifugal analysis has proved to be of benefit to an environmental laboratory for the determination of chloride in surface water and groundwater samples. Chief among the attributes of this mode of analysis is the ease with which analytical variables can be manipulated.

Iron(III) nitrate and ammonium iron(III) sulphate adaptations of the spectrophotometric hexacyanoferrate(III) method

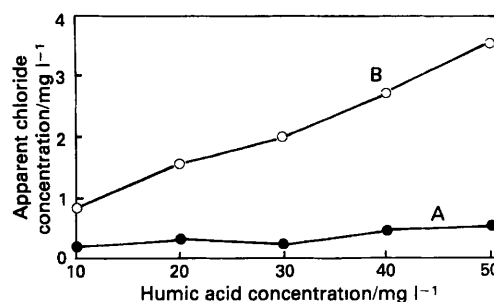


Fig. 5. Interference caused by humic acids. (A) With sample blanking and (B) without sample blanking

Table 6. Precision of the Cobas Fara chloride procedure determined from spiked samples

Spike amount/ mg l ⁻¹	<i>n</i>	Mean recovery, %	Standard deviation/ mg l ⁻¹	Coefficient of variation, %
10	10	101.6	0.12	0.48
2	10	102.6	0.15	2.5

Table 7. Precision of the Cobas Fara chloride procedure determined from quality control samples

Precision	Assayed level/ mg l ⁻¹	<i>n</i>	Standard deviation/ mg l ⁻¹	Coefficient of variation, %
Between-day	81.0	30	0.42	0.52
	26.0	30	0.18	0.67
Within-run	80.8	10	0.56	0.68
	26.1	10	0.21	0.80

were developed for centrifugal analysis; the procedure using iron(III) nitrate was found to be approximately twice as sensitive.

The Cobas Fara procedure was found to be both accurate and precise. Results obtained for reference mineral samples agreed with certified values within the ranges cited. Within-run and between-day precision were acceptable, and no difference was detected when surface water and groundwater samples were analysed by means of the Cobas Fara centrifugal analyser and the Technicon AAII continuous flow analyser.

The blanking capabilities of the Cobas Fara centrifugal analyser were shown to be capable of correcting the interference caused by humic acids.

It is concluded that the Cobas Fara procedure produces high quality results with a considerable time and sample-size saving. In terms of throughput, it was shown that the Cobas Fara procedure could analyse samples at a rate of 300 samples h^{-1} . The total amount of sample used was 80 μl .

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