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ION-CHROMATOGRAPHIC ANALYSIS OF MIXTURES OF FERROUS AND FERRIC IRON

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Summary—Determinations of the aqueous iron species Fe(II) and Fe(III) are essential for a fully-informed understanding of redox processes involving iron. Most previous methods for speciation of iron have been based on the colorimetric determination of Fe(II) followed by reduction of Fe(III) and analysis for total iron. The indirect determination of Fe(III) and the consumption of relatively large sample volumes have limited the accuracy and utility of such methods. A method based on ion-chromatography has been developed for simultaneous direct determination of Fe(II) and Fe(III). Sample pretreatment involves only conventional filtration and acidification. No interferences with the iron(II) determination were found; in determination of iron(III) the only interference observed was an artifact peak (of unknown origin) that occurred only when iron(II) was present, and had an area that was a function of the iron(II) concentration and could hence be corrected for. Solutions of iron(II) free from iron(III) can be prepared by treatment with a mixture of hydrogen and nitrogen in the presence of palladium black as catalyst, to reduce the iron(III). Photoreduction of iron(III) in acidified samples increases the Fe(II)/Fe(III) ratio; no means of circumventing this effect is known, other than storing the samples in the dark and analysing them as soon as possible.

Iron is one of the most common elements in the Earth's crust, occurring in nearly all types of rock. Its redox and physiological properties make it an important component of the biogeochemical cycles of elements such as carbon, sulphur and oxygen.[1–3] Its reactivity also drives numerous chemical processes in natural waters, and it is a significant factor in the evaluation of water quality.[4]

There are many shortcomings in our understanding of the Fe–H_2O system that constrain the application of equilibrium thermodynamics to the solution of problems that involve iron species in water. For example, thermochemical data for aqueous Fe(II) species are especially difficult to obtain, owing to the difficulty in eliminating oxygen and Fe(III) from the system.[5] In principle, the redox potential (E) can be used to predict the equilibrium iron speciation, given the total iron concentration, but there are many problems associated with making accurate potential measurements.[6–8] Even if accurate E values are available, their interpretation is complicated by kinetic and complexation effects that can cause the observed iron speciation to differ from the ratio of Fe(II) and Fe(III) activities predicted from the measured redox potential. The value of E can also be calculated from the activity ratio of another redox couple, but that ratio may not be accurately determined and the couple's redox chemistry may not be linked to that of iron. Since predictions based on equilibria can only be accurate in certain situations, the most reliable way to acquire information on the speciation of iron is to make direct determinations of both Fe(II) and Fe(III). Unfortunately, however, finding direct analytical methods for iron species is among the problems encountered in investigation of the basic processes in the Fe–H_2O system and in studies involving iron in the environment.

Atomic absorption and emission spectrometry can only determine the total iron concentration. Though some methods of electronic or resonance spectroscopy can distinguish between Fe(II) and Fe(III), they are better suited to molecules than to ions in aqueous solution. Most practical methods of analysing for aqueous iron species involve spectrophotometric determination of Fe(II).[9–13] Fe(III) is then reduced to Fe(II) and total iron determined, yielding the concentration of Fe(III) by difference. The main drawbacks of this approach are related to sensitivity, the level of iron to be determined, and interferences.

This paper reports the development of an ion-chromatography (IC) method for directly determining both Fe(II) and Fe(III) in water samples. It requires <1 ml of sample and no sample pretreatment other than the usual filtration and acidification of the samples. Since its introduction,[14] IC has become a widely-used technique for analysis of solutions[15] (a non-exhaustive bibliography, available from Dionex Corp., 1228 Titan Way, Sunnyvale, CA 94086, USA, lists over 500 citations up to 1984). The separation and conductimetric detection of alkali-metal and alkaline-earth metal cations are well-established, but only recently has IC technology been expanded to include transition and heavy metal determination by spectrophotometric detection.[16,17]

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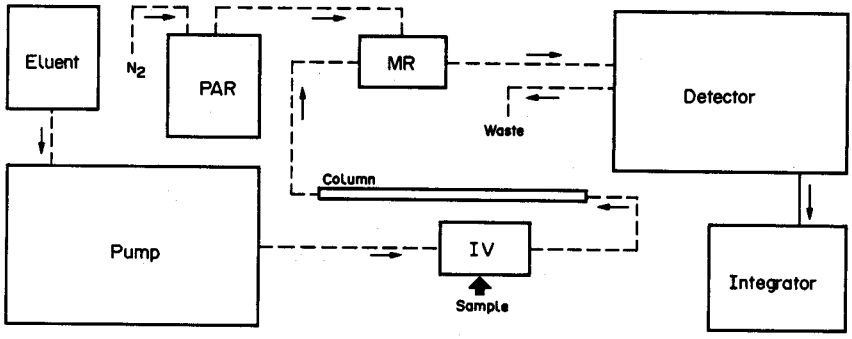

Fig. 1. Schematic of IC transition/heavy metal analytical system: IV = sample injection valve; MR = membrane reactor; dashed lines indicate fluid flow paths and the solid line indicates an electrical signal.

Principle

In analyses for iron, a small volume (10–500 μl) of sample is injected into an eluent stream, which carries the sample into a separator column (Fig. 1). The eluent contains a chelating agent that forms water-soluble complexes with metal ions, and the column is packed with a mixed-bed resin, which separates the complexes by differential dynamic sorption/desorption processes.[18] Once separated, the metal complexes are treated with a colorimetric reagent in a reactor that consists of a hollow uncharged membrane fibre bathed in the reagent. The eluent stream flows through the lumen of the fibre, where it mixes radially with reagent permeating through the membrane under pressure. Longitudinal mixing, which causes broadening of the bands, is reduced by coiling the fibre, which also enhances the radial mixing. The intensity of the resulting colour, which is proportional to the amount of metal present, is measured in a flow-through spectrophotometer cell. Since the sample volume injected is known, the original metal concentration is readily determined.

EXPERIMENTAL

Sample treatment

Samples were filtered through Gelman or Millipore membranes with pore sizes no larger than 0.45 μm. Before the sample was collected, the membrane was leached with about 500 ml of sample or demineralized water. Each filtered sample was collected in a polyethylene bottle that contained a volume of redistilled 6*M* hydrochloric acid (G.F. Smith Co.) that was 1% of that of the sample; the samples were stored at 2–5°. Analyses were completed as soon as possible after collection but always within 2 weeks (the practical length of storage is discussed below).

Reagents and standards

Fe(II) standards. A 1m*M* stock solution was prepared from $Fe(NH_4)_2(SO_4)_2.6H_2O$ (Aldrich) or from Fe wire (Baker). The solutions were made up with demineralized water that had been deaerated under reduced pressure. During storage, the salt was protected from exposure to air and light. The stock solution made from it (0.3921 g/l.) was acidified with a 1% v/v addition of redistilled 6*M* hydrochloric acid. For about 48 hr before use, the desired length (18 cm, ~56 mg) of 0.23-mm diameter Fe wire was soaked, with occasional ultrasonic treatment, in 0.18*M* ammonium oxalate to remove surface coatings of hydrous Fe(III) oxide, then quickly rinsed with demineralized water and acetone, dried on a tissue, weighed to 0.01 mg, and dissolved in 10 ml of warm redistilled 6*M* hydrochloric acid. The solution was diluted to 1 litre.

Approximately 10 mg of palladium black (Aldrich; this material will be referred to as Pd-b) was added to the stock iron(II) solutions, which were then purged for 15–20 min with a mixture of hydrogen (10–30%) and nitrogen, passed through a dispersion tube at 90–120 l./hr. This procedure ensured complete reduction of the iron(III) to iron(II). The nitrogen used was passed through a column packed with "Ascarite" to remove carbon dioxide and then through a heated glass column packed with copper turnings, to remove oxygen.

After the reduction a portion of the stock iron(II) solution was filtered through a prerinsed glass-fibre or Whatman No. 1 filter, and used for preparation of calibration standards by dilution with 60m*M* hydrochloric acid. The stock solution was always reduced before use. Calibration standards were kept for no longer than 48 hr. It is not possible to use mixed Fe(II)/Fe(III) standards (see discussion).

Fe(III) standards. Commercial AAS standards for iron, made from iron(III) chloride dissolved in hydrochloric acid, were used as Fe(III) standards not contaminated with Fe(II). A 100-mg/l. stock solution was prepared by diluting the commercial standard with 60m*M* hydrochloric acid, and further diluted with this acid to give the calibration standards.

Eluent. A 6m*M* PDCA/50m*M* sodium acetate/50m*M* acetic acid solution was made by dissolving 6.8 g of sodium acetate trihydrate (Baker) in 500 ml of demineralized water, adding 1 g of PDCA (2,6-pyridinedicarboxylic acid, Aldrich), and 3 ml of glacial acetic acid (Baker), and diluting to 1 litre. The pH of this eluent was 4.5. The eluent was purged with nitrogen (stripped of CO_2 and O_2 as above) for about 30 min before use.

Post-column reagent. A 0.2m*M* PAR/3*M* ammonia/1*M* acetic acid solution was made by dissolving 43 mg of PAR in 400 ml of 7.5*M* ammonia solution and adding 600 ml of 1.67*M* acetic acid. The solution was purged with nitrogen, and stripped of CO_2 and O_2 for about 30 min, to prevent oxidation of the PAR, which would cause a noisy chromatographic baseline.

*Sulphite solution, 0.1*M. A solution of 12.6 g of anhydrous sodium sulphite (Baker) in 1 litre of demineralized water.

CAUTION. The inversion temperature of hydrogen is −80°, so at room temperature hydrogen shows an inverted Joule–Thompson effect and becomes hot on expansion.[19] The explosive limits for hydrogen are 4–75% v/v in air.[19] During the course of our work, a rich mixture of H_2 in N_2 was passed through a small glass jet into an aqueous solution. When the jet was removed from the solution, a

Table 1. IC operating conditions

Eluent flow: 1.0 ml/min
Injection volume: 50 μl
PAR delivery
pressure: 3.5–4.2 bar
flow: 0.6–0.8 ml/min
Detection wavelength: 520 nm
Recorder sensitivity: 0.16 absorbance full-scale
Time constant: 1 sec

bright orange 3-cm flame appeared at its tip, and was probably caused by a combination of heating on expansion and ignition by a static discharge as the gas passed rapidly through the small nozzle. Although the flame was easily extinguished, the incident emphasised the need for care in handling hydrogen. No problems were encountered when a gas dispersion tube was used. Palladium black is also a potential fire hazard (it is a finely divided metal, and could be pyrophoric) and quantities larger than a few mg should be stored in an inert (air-free) atmosphere. Filters containing a small amount of Pd-b (< 10 mg) can be wetted with water and placed in a glass or metal dish to dry. The Pd-b will then oxidize too slowly to ignite, and once dry can be disposed of with other dry wastes.

Potentiometric measurements

A Ross combination pH electrode (Orion 815500) was used for pH measurements and a combination Pt electrode (Orion 967800) for redox potential measurements, with a Corning 135 pH/ion-meter. The redox electrode uses a proprietary reference electrode which has a potential of 0.246 V *vs.* the normal hydrogen electrode.[20] Redox potential values (E) reported in this paper are referred to the normal hydrogen electrode.

IC methods

The IC system consisted of an APM-1 analytical pump, CG-2 precolumn, CS-5 analytical separator column, and RDM-1 reagent delivery module with membrane reactor, all from Dionex. The detector was a Knauer 87 variable-wavelength spectrophotometer with tungsten–halogen lamp, solid-state detector (photodiode), and 1-cm path-length cell (12-μl volume). The detector output was recorded on a Kipp and Zonen dual-channel strip-chart recorder and a Hewlett-Packard 3392A integrator. All parts of the system in contact with fluid were non-metallic except for the flow passages in the detector cell. From the injection loop to the detector cell, the 0.3-mm bore PTFE connections were kept as short as possible to minimize the dead volume. Figure 1 shows the system configuration and Table 1 the operating conditions.

Before a run, 0.1M sodium sulphite was pumped through the columns at 1.0 ml/min for 1–2 hr to remove oxygen from the system. Then eluent pumping was begun and the column effluent was directed to the membrane reactor, the reagent reservoir of which was then pressurized, and from there to the detector cell. When the baseline absorbance had stabilized (30–60 min after switching to the eluent), the run could be started. Standards and samples were manually loaded into the injection loop with a plastic syringe. Acid blanks (60mM hydrochloric acid prepared with demineralized water) were used to confirm that the syringe, acid and sample loop were not contaminated, but were not used in the determination.

Calculations

The peak heights were measured on the strip-chart, or the peak areas by the integrator. Sample concentrations were calculated from equations fitted to the calibration curves. Linear equations were used unless a quadratic model could be shown (by the F-test) to improve the fit significantly.

The detection limit (DL) was estimated by multiplying the standard deviation (S) of the low standard by the Student's t-value (one-tailed test) for the appropriate number of degrees of freedom at the 99% significance level ($p = 0.01$). This calculation is similar to a more formal procedure[21] proposed by the USEPA, which is a practical approach to quantifying the detection limit as defined by IUPAC and the ACS.[22]

To assess the recovery, standard additions[23] were made to samples that contained only Fe(II) or Fe(III) or a mixture of the two. Increments of standard equivalent to 30–50% of the amount of analyte already present were added to 4–10 ml portions of sample, which were then analysed in triplicate, to give a total of 12 data points. The amount found (nmoles) was normalized to a sample volume of 10 ml and plotted against the amount added. The slope indicates the recovery and the intercepts on the two axes should give the amount initially present. A difference between the two intercepts will occur owing to imprecision in making the additions and in the procedure, and can also arise if the recovery is not 100%.

RESULTS AND DISCUSSION

Preparation of Fe(II) standards

The Fe wire was the preferred source of Fe(II) because ammonium iron(II) sulphate is prone to aerial oxidation. Although the degree of oxidation normally encountered is much smaller than the experimental error in the method developed, it was considered worthwhile trying to make as pure an iron(II) solution as possible for future use by us and others.

Hydroxylamine hydrochloride is widely used for the reduction of Fe(III) but was considered unsuitable because of the possibility of damage to the resin in the columns or the fibre in the membrane reactor, and the possibility that it could be retained on the columns and cause reduction of Fe(III) in subsequently-injected samples or standards.

Hydrogen is used with a catalyst to remove oxygen from the atmosphere of glove boxes or growth chambers in studies of anaerobic bacteria,[24] so the possibility of using this procedure was examined. In the work with anaerobic bacteria, the hydrogen, carried in nitrogen or a CO_2/N_2 mixture, was passed over Pd-coated alumina or charcoal pellets, where it reacted with any oxygen present in it, to form water. In addition, Pd-b was suspended in the bacteriological media, which were placed in similar atmospheres. By the catalysed reaction the oxygen concentration in the atmosphere of a glove box was reduced to 0.001%, and a medium containing Pd-b and buffered at pH 7 had an E value of −0.29 V.[24] The first step in adapting the latter procedure to preparation of Fe(III)-free solutions of Fe(II) was to determine whether hydrogen in the presence of catalyst would reduce dissolved oxygen to water in an abiotic system.

The E value of a 0.1M sodium chloride/60mM hydrochloric acid solution in equilibrium with air, measured with a combination Pt electrode, was very unstable, as expected for a solution with no reactive

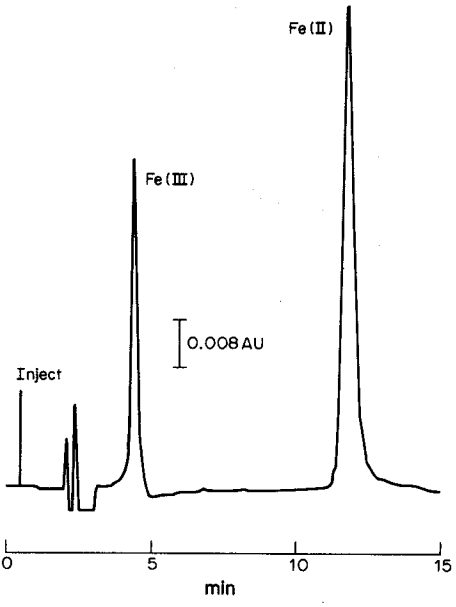

Fig. 2. Chromatogram obtained from 50 μl injection of 4.48μM Fe(III)/20.0μM Fe(II).

redox couple, and varied between +0.3 and +0.4 V. When about 10 mg of Pd-b was suspended in the solution and this was purged with 20% hydrogen in nitrogen, the E value rapidly dropped to a steady value of −0.059 V. An E of −0.068 V would be predicted for a hydrogen partial pressure of 0.2 atm in equilibrium with water at pH 1.5. An E value of −0.059 V would correspond to a hydrogen partial pressure of 0.1 atm. The E measurements were precise to ±0.002 V, but the accuracy of the mixing control for the gases was probably very poor. In addition, the oxidation of hydrogen on a Pt electrode tends to attain equilibrium only slowly unless the electrode surface area is very large (which requires a platinized electrode surface). It was concluded that the H_2/Pd-b reduction procedure was successful for removal of dissolved oxygen.

The ability of H_2 to reduce Fe(III) in the presence of Pd-b was then investigated. A steady E value of −0.03 V was attained for a solution of 2mM Fe(II)/0.1M NaCl/60mM HCl purged for 15 min with a 20% H_2/N_2 mixture in the presence of Pd-b. Similar results were obtained with a corresponding Fe(III) solution, with purging for 30 min. The computer program WATEQF[25] was used to calculate the equilibrium distribution of the iron under these conditions. The activity ratio Fe(II)/Fe(III) at pH 1.5 and E −0.03 V was $10^{13.5}$, and the concentration ratio $10^{12.9}$. These results show that Pd-b is a suitable catalyst for the reduction of Fe(III) by hydrogen.

However, Pd also catalyses the oxidation of Fe(II),[26] so it is essential to filter it off from the stock iron(II) solution before mixing the calibration standards. Without the catalyst, the hydrogen that remains dissolved in solution is no longer an effective reducing agent, and can neither damage the IC columns nor interfere with subsequent iron analyses. Since the Pd-b catalyst loses effectiveness with time, it is occasionally necessary to suspend fresh Pd-b in the solution.

Method performance

A typical chromatogram of a mixture of Fe(III) and Fe(II) is shown in Fig. 2. Table 2 summarizes the retention times, sensitivities and detection limits for Fe and several other metals.

Calibrations for Fe(III) were always linear. Peak-area calibrations were superior to peak-height calibrations, especially at the lowest concentrations. The calibrations for Fe(II) were usually linear but occasional large negative intercepts and curved plots of residuals indicated that a non-linear calibration model would improve the fit, especially for low concentrations, but use of quadratic models for the calibration curve improved accuracy over most of the calibration range by only about 1% or less.

The response for both species was linear up to 200μM. Higher concentrations were not examined, but smaller injection volumes, a higher concentration of PAR (*e.g.*, 0.4mM) and a less sensitive detector range, can be used to extend the linear range and, in some cases, avoid sample dilution. Concentrations lower than 1μM can be determined by increasing the injection volume (up to 500 μl) and using a more sensitive detector range. With fresh PAR, a properly-maintained membrane reactor, and a good-quality detector, the noise in the chromatographic baseline is low enough to permit detection of concentrations at

Table 2. Method performance

Species	Retention time*, *min*	Sensitivity†, *peak area*/μM	DL‡, μM
Fe(III)	3.9	5.60×10^5 (1.3%)	1.22
Fe(II)	11.8	4.50×10^5 (3.5%)	1.46
Cu	6.1	2.86×10^5	
Ni	6.8	7.00×10^4	
Zn	7.5	1.00×10^5	
Co	8.3	4.66×10^5	
Cd	8.9	1.42×10^4	
Mn	10.1	1.08×10^5	
Pb	§	—	

*Characteristic retention time for a 20μM injection.

†Slope of calibration graph over 2–100μM range. For non-ferrous metals, sensitivities were estimated from 1 or 2 analyses and are presented for comparison only. Number in parentheses is the relative standard deviation of the slope (7 replicates). Peak area expressed in integrator counts.

‡Detection limit for 50 μl injection volume and detector cell with 1-cm path-length; not determined for non-ferrous metals.

§No peak detected in 20 min.

least as low as 50nM ($\sim$3 μg/l.). Analyses at this level were not examined beyond determination of their feasibility, because they require clean-laboratory conditions and great care in the preparation of reagents and standards.

The sensitivity of the method is quite high (Table 2). PAR was introduced as a colorimetric reagent when it was realized that thanks to the chromatographic separation high specificity of the reagent was no longer as important as high sensitivity.[27] PAR is useful for determination of cadmium, lead, uranium, and all first-row transition metals except scandium, and is well-suited to the detection of metals in IC methods, provided the metals can be separated. Besides the sensitivity, the rapidity of PAR reactions is an advantage in conjunction with the low-volume, short path-length detector cells that are desirable for chromatographic methods.

Separation and interferences

The CS-5 column and PDCA eluent were essential for the determination of Fe(III). Fe(II) can be determined with other combinations of column and eluent, but most other transition/heavy metal IC eluents contain components (*e.g.*, oxalate, tartrate or citrate) which can form neutral complexes with Fe(III) that are eluted in the column void volume, whereas PDCA forms an anionic complex with Fe(III) which thus undergoes retention in the CS-5 column.

Solutions of several other metals were injected to identify potential interferences. None of the metals tested showed any interference with the iron determination (Table 2), but some modification of this method may be necessary before all of them can be simultaneously resolved.

The only significant interference is a peak that overlaps that for Fe(III). Early in the development of this method, it was thought that this peak was due to Fe(III) present in the Fe(II) standards. Efforts were made to eliminate the peak by adding reductants for Fe(III), but the peak area could not be reduced below a constant value for a given Fe(II) concentration and the peak shape was different from that for pure Fe(III). The size of the peak was the same (within 3%) whether the Fe(II) standards were made from ammonium iron(II) sulphate, iron wire, a non-stoichiometric iron(II) sulphate, or a reduced Fe(III) standard, or whether the reducing agent was hydrogen (with Pd-b) or hydroxylamine hydrochloride. The cause of this peak is unknown, but it is evidently not Fe(III).

Attempts to eliminate the interference by separating the artifact from the Fe(III) peak by using eluents with less PDCA, lower pH, or both, were tried, but the main effects were merely to increase the retention time of Fe(III) slightly and that of Fe(II) considerably (to >18 min).

The interference was finally dealt with by applying an empirical correction based on the observation that the artifact area was proportional to the square root of the Fe(II) concentration and the assumption that the areas of the artifact and Fe(III) peaks were additive. The Fe(II) concentration is determined, then the peak area of the artifact is calculated and subtracted from the combined artifact–Fe(III) peak area, and the concentration of Fe(III) determined from the remaining area.

Table 3. Analyses of mixtures

	A	B	C
	Fe(II)		
expected*	4.00	8.00	20.0
found*	4.72	8.63	19.6
rsd†, %	(4.2)	(1.9)	(0.2)
recovery, %	118.0	107.9	98.0
	Fe(III)		
expected	17.9	8.95	4.48
found	16.4	7.96	3.12
rsd†, %	(0.9)	(1.9)	(2.4)
recovery, %	91.6	88.9	69.6
	ΣFe		
expected	21.9	17.0	24.5
found	21.1	16.6	22.7
recovery, %	96.4	97.9	92.7
	Fe(II)/Fe(III)		
expected	0.223	0.894	4.464
found	0.288	1.084	6.282
recovery, %	128.8	121.3	140.7

*All concentrations expressed in μM.
†Relative standard deviations ($N = 3$).

Sample preservation and storage

Filtration is an essential step in the collection of samples. To exclude bacteria and colloidal Fe(III) species, pore sizes of 0.2 μm or less should be used. Certain bacteria, common in environments containing Fe(II), can catalyse its oxidation.[28–30] Any colloidal material will partly dissolve when the sample is acidified and hence give a time-dependent and increasing value for dissolved Fe(III).[31] Any colloids that remain in suspension when the sample is injected into the IC are more strongly retained on the column than the dissolved Fe(III), and this may cause substantial broadening of the Fe(III) peak and eventually clogging of the column.

Oxidation of Fe(II) was quenched by acidifying samples with hydrochloric acid immediately after collection, since the rate of Fe(II) oxidation is minimal and is independent of acidity at pH <3.[26,32] Re-analysis of 7 lake-sediment pore-water samples containing 100–2900μM Fe(II) and no detectable Fe(III) showed an average loss of 1.6% of the Fe(II) originally present, after 31 days of storage. Shortage of sample precluded detailed analysis of the variability in Fe(II) loss but the variability did not appear to be correlated with the original Fe(II) concentration and could not be distinguished from the imprecision of the technique. The concentration of hydrochloric acid used in the standards and samples, 60mM, gives a pH of 1.4–1.5 (on the activity scale, with correction for ionic strength). This is probably more than necessary to quench Fe(II) oxidation, but gives samples

Table 4. Standard-additions tests

Sample	Recovery*, %	Initial†, *nmole*	Actual‡, *nmole*
Fe(II) additions (increment = 39.4 nmole)			
8.0μM Fe(II)	93.0 (4.8)	80.0	86.1
Mixture B§	98.9 (4.8)	80.0	80.9
ΣFe in mixture	94.6 (2.6)	153.4	162.2
Fe(III) additions (increment = 17.6 nmole)			
4.48μM Fe(III)	97.7 (0.5)	43.5	44.6
Mixture B§	82.1 (2.9)	79.4	96.8
ΣFe in mixture	92.8 (6.0)	157.4	169.6

*Slope of the line fitted to the graph of amount found (y) *vs.* amount added (x), expressed as per cent of ideal slope (1.0); numbers in parentheses are relative standard deviations ($N = 12$).

†Initial amount: intercept on the y-axis, *i.e.*, amount recovered without addition, normalized to a 10-ml sample.

‡Actual amount: modulus of the intercept on the x-axis, *i.e.*, amount present calculated from intercept and recovery factor, and normalized to a 10-ml sample.

§See Table 3.

and standards having the same ionic strength and major-ion composition, which experience has shown to improve the analytical accuracy and precision in IC methods.

Analysis of mixtures

The chief advantage of the method is the simultaneous direct determination of Fe(II) and Fe(III). To demonstrate its performance, three mixed standards were made and analysed in triplicate (Table 3). One of these mixtures was also analysed by the standard-additions method (Table 4). In addition, a USEPA quality-control sample and a lake-sediment pore-water sample,[33] both of which were mixtures, were analysed by this method and the Ferrozine method[13] (Fig. 3).

The recoveries of Fe(II) and Fe(III) from the known mixtures sometimes differed considerably from 100%, but the absolute differences were all less than the value of the detection limit. The recovery of total iron was practically 100%, however, which in conjunction with the consistently biased value of the Fe(II)/Fe(III) ratios suggests that the distribution in the mixtures was altered and that the errors in recovery of the individual species cannot be attributed solely to the random errors of the method.

However, the standard-additions analyses (Table 4) showed that the over-recovery of Fe(II) only occurred in mixtures with Fe(III). The high ratios for the mixtures might be attributed to an under-recovery of Fe(III) caused by some error in the procedure for correcting for the artifact peak, but no such error could be demonstrated and there was some real over-recovery of Fe(II), which would have been unaffected by the artifact. Furthermore, the comparison with the Ferrozine method (Fig. 3) showed that the IC method had no more tendency to over-recover Fe(II) or under-recover Fe(III) than did the Ferrozine method. In fact, the comparison validated the artifact correction procedure, without which the two methods would have grossly disagreed on both the Fe(III) and ΣFe concentrations.

The consistent positive error in the Fe(II)/Fe(III) ratio appears to be real and not peculiar to the IC method, but is difficult to explain. The bathophenanthroline method uses an extraction procedure to separate the two species and thus avoids any alteration of the Fe(II)/Fe(III) distribution.[11] An excess of ammonium fluoride has also been used with 1,10-phenanthroline,[34] presumably to avoid the same problem by masking the Fe(III).

It has been suggested that Fe(III) tends to be photochemically reduced in acidic systems.[11] Photochemical reduction of Fe(III) has been discussed previously, especially for Fe(III)–dye complexes in-

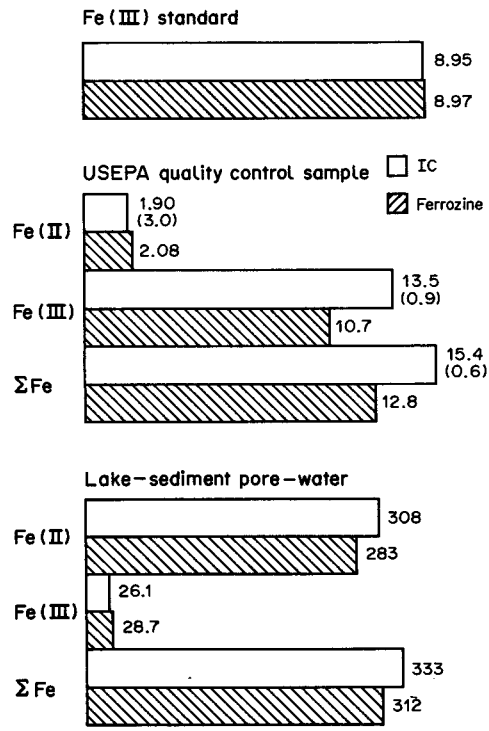

Fig. 3. Comparison of the IC method with the Ferrozine method. Concentrations in μM. Note the use of different scales. The USEPA quality control sample is Trace Metal I, sample 2, vial number WP481. USEPA reports the true value (for ΣFe only) as 14.3μM and the mean recovery from performance evaluation studies as 14.1μM, with s.d. = 0.8μM. The numbers in parentheses are relative standard deviations ($N = 3$). The lake-sediment pore-water sample was collected from Contrary Creek, Louisa County, Virginia, U.S.A., on 2 May 1986 and was diluted tenfold for analysis.

volving nitrogen donor atoms[35] or the initiation of polymerization of certain plastics.[34,36] It has also been observed to affect the Fe(II)/Fe(III) ratio in samples of lake water.[37] Since acidified Fe(III) solutions that initially contain no Fe(II) can be stored without detectable reduction, the presence of Fe(II) must be required for the photochemical reduction of Fe(III). Furthermore, since the effect has been observed in essentially pure solutions of iron(III) chloride, the process does not require agents found in natural waters, such as organic species. Simple boiling of acidified natural water samples containing Fe(II) and Fe(III) has also been shown to result in rapid conversion of Fe(III) into Fe(II),[38] but it is not clear from the published results whether the reaction involves species found in natural waters or is the same as the reaction observed in pure solutions. Radiolysis of aqueous solutions does produce reducing agents, chiefly electrons and hydrogen atoms,[39] but no experimental results are available from which to predict the possible effects of these agents on Fe(II)/Fe(III) rearrangements. The reduction of Fe(III) also proceeds in the dark,[37] but the effect of hydrochloric acid on the reaction is controversial, since it is stated (*a*) to increase the effect,[37] and (*b*) *not* to affect it.[40]

The prevention of rearrangements of Fe(II)/Fe(III) would improve the reliability of any analytical method for speciation of iron. It has been shown that $FeOH^{2+}$ is more susceptible to reduction than Fe^{3+},[40,41] but since at pH 0.5 the activity of $FeOH^{2+}$ is 2 orders of magnitude lower than the activity of Fe^{3+}, this does not seem to be a likely reason for the problem. Until the rearrangement can be reliably prevented, the conclusion of this study is the same as that of an earlier report,[37] namely to avoid exposure of samples to light and to analyse them very quickly after collection.

CONCLUSIONS

An IC method has been developed for the simultaneous direct determination of Fe(II) and Fe(III). Sample treatment involves only filtration and acidification at the time of collection and storage at 2–5° until analysis. Detection limits for the analytical conditions considered were 1.5μM Fe(II) and 1.2μM Fe(III). Increasing the sample injection volume (from 50 μl) and the sensitivity of the spectrophotometric detector should lower the detection limit.

A means of preparing Fe(III)-free solutions of Fe(II) has also been developed. Hydrogen is shown to reduce both dissolved oxygen and aqueous Fe(III) in the presence of palladium black as catalyst. This procedure is useful for making accurate Fe(II) standards free from Fe(III), and should prove useful in the determination of thermodynamic data for Fe(II) and kinetic investigations of Fe(II) oxidation initiated in the absence of Fe(III).

In analysis of Fe(II)/Fe(III) mixtures, an increase in this ratio was observed and was attributed to Fe(III) being reduced, possibly photochemically. This effect apparently requires the initial presence of Fe(II), since solutions of Fe(III) that contained no Fe(II) showed no evidence of reduction. At present, the only means of avoiding this problem is to protect samples from light and analyse them as soon as possible, preferably within a few minutes.

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REFERENCES

1. H. Lepp (ed.), *Geochemistry of Iron*, in *Benchmark Papers in Geology*, Vol. 18. Dowden, Hutchinson, and Ross, Stroudsburg, PA, 1975.
2. H. D. Holland, *The Chemistry of the Atmosphere and Oceans*, Wiley-Interscience, New York, 1978.
3. H. D. Holland, *The Chemical Evolution of the Atmosphere and Oceans*, Princeton University Press, Princeton, 1984.
4. J. D. Hem, *U.S. Geol. Surv., Water-Supply Paper*, 2254, 1985.
5. D. K. Nordstrom, S. D. Valentine, J. W. Ball, L. N. Plummer and B. F. Jones, *U.S. Geol. Surv., Water Resources Investigations Rept.*, 84–4186, 1984.
6. M. Whitfield, *Limnol. Oceanog.*, 1970, **19,** 857.
7. J. D. Hostettler, *Am. J. Sci.*, 1984, **284,** 734.
8. R. D. Lindberg and D. D. Runnels, *Science*, 1984, **225,** 925.
9. APHA, *Standard Methods for the Examination of Water and Wastewater*, 14th Ed., American Public Health Association, Washington, D.C., 1976.
10. M. L. Moss and M. G. Mellon, *Ind. Chem. Eng., Anal. Ed.*, 1942, **14,** 862.
11. G. F. Lee and W. Stumm, *J. Am. Water Works Assoc.*, 1960, **52,** 1567.
12. M. M. Ghosh, J. T. O'Connor and R. S. Engelbrecht, *ibid.*, 1967, **59,** 897.
13. L. L. Stookey, *Anal. Chem.*, 1970, **42,** 779.
14. H. Small, T. S. Stevens and W. C. Bauman, *ibid.*, 1975, **47,** 1801.
15. C. Pohl and E. L. Johnson, *J. Chromatog. Sci.*, 1980, **18,** 442.
16. J. M. Riviello, A. Fitchett and E. Johnson, *Proc. Int. Water Conf., 43rd*, p. 458. Eng. Soc. West. Pa., 1982.
17. R. Slingsby and J. M. Riviello, *LC Mag.*, 1983, **1,** 354.
18. J. M. Riviello, in *Ion Exchange Technology*, D. Naden and M. Streat (eds.), p. 585. Horwood, Chichester, 1984.
19. MCA, *Guide for Safety in the Chemical Laboratory*, 2nd Ed., Manufacturing Chemists' Association, Van Nostrand–Reinhold, New York, 1972.
20. D. K. Nordstrom, *Geochim. Cosmochim. Acta*, 1977, **41,** 1835.
21. J. A. Glaser, D. L. Foerst, G. D. McKee, S. A. Quave and W. L. Budde, *Environ. Sci. Technol.*, 1981, **15,** 1426. See also letters, *ibid.*, 1982, **16,** 430A–431A.
22. G. L. Long and J. D. Winefordner, *Anal. Chem.*, 1983, **55,** 712A.
23. R. Klein, Jr. and C. Hach, *Am. Lab.*, 1977, **9,** No. 7, 21.
24. A. Aranki, S. A. Syed, E. B. Kenney and R. Freter, *Appl. Microbiol.*, 1969, **17,** 568.
25. L. N. Plummer, B. F. Jones and A. H. Truesdell, *U.S. Geol. Surv., Water-Resources Investigations*, 76-13, 1976.

26. P. C. Singer and W. Stumm, *Oxygenation of Ferrous Iron*, Federal Water Quality Administration, Report 14010-06/69, 1970.
27. F. H. Pollard, P. Hanson and W. J. Geary, *Anal. Chim. Acta*, 1959, **20**, 26.
28. T. D. Brock, S. Cook, S. Petersen and J. L. Mosser, *Geochim. Cosmochim. Acta*, 1976, **40**, 493.
29. N. Lazaroff, W. Sigal and A. Wasserman, *Appl. Environ. Microbiol.*, 1982, **43**, 924.
30. S. J. Onysko, R. L. Kleinmann and P. M. Erickson, *ibid.*, 1984, **48**, 229.
31. V. C. Kennedy, G. W. Zellweger and B. F. Jones, *Water Resour. Res.*, 1974, **10**, 785.
32. P. C. Singer and W. Stumm, *Science*, 1970, **167**, 1121.
33. A. T. Herlihy and A. L. Mills, *Appl. Environ. Microbiol.*, 1985, **49**, 179.
34. M. G. Evans, M. Santappa and N. Uri, *J. Polymer Sci.*, 1951, **7**, 243.
35. G. K. Oster and G. Oster, *J. Am. Chem. Soc.*, 1959, **81**, 5543.
36. M. G. Evans and N. Uri, *Nature*, 1949, **164**, 404.
37. J. W. McMahon, *Limnol. Oceanog.*, 1967, **12**, 437.
38. J. Shapiro, *ibid.*, 1966, **11**, 293.
39. A. Appleby, G. Scholes and M. Simic, *J. Am. Chem. Soc.*, 1963, **85**, 3891.
40. P. G. David, *J. Chem. Soc., Chem. Commun.*, 1972, **23**, 1294.
41. R. Broszkiewicz and S. Minc, *Nukleonika*, 1963, **8**, 165.