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The role of colloid chemistry in providing a source of iron to phytoplankton

by Mark L. Wells,[1,2] Niko G. Zorkin[1] and A. G. Lewis[1,3]

ABSTRACT

Culture experiments with the coastal marine diatom *Thalassiosira pseudonana* (WHOI clone 3H) demonstrate that, as an iron source, freshly prepared colloidal ferric hydroxide can produce better cell yield than the more crystalline goethite or hematite. Ageing or heating of the prepared ferric hydroxide stock causes a reduction in cell yield. This reduction appears to be related to increased thermodynamic stability of the colloid as suggested by thermogravimetric analysis and relative dissolution rates. The reduction in cell yield can be prevented by the addition of the chelating agent EDTA prior to, but not after, ageing or heating of the ferric hydroxide stock. These results suggest that the ability of colloidal iron to provide a source of metal for phytoplankton is related to the thermodynamic stability of the colloid.

1. Introduction

Iron is an essential micronutrient for virtually all forms of life. Phytoplankton require more iron than any other trace metal (Anderson and Morel, 1980) and iron deficiencies affect several processes associated with photosynthesis in a variety of plant types (e.g., Glover, 1977; Spiller and Terry, 1980). The effect of iron deficiencies, however, is reversible when adequate iron is made available. With phytoplankton, this occurs through chemical interaction with the various forms of iron found in the marine environment.

Iron exists in seawater as a hydrated ion, as dissolved complexes formed with organic and inorganic ligands, and also as a component of suspended particulate or colloidal organic and inorganic material. Since iron forms highly insoluble species in oxygenated seawater and the levels of dissolved organic metal complexing agents that might increase its solubility are usually low, it has been suggested that most iron in seawater is present in ferric colloidal forms (Lewin and Chen, 1973).

With phytoplankton, the biologically available species of iron appear to include dissolved ionic species (Levandowsky and Hutner, 1975; Anderson and Morel, 1982)

1. Department of Oceanography, The University of British Columbia, 6270 University Boulevard, Vancouver, British Columbia, Canada, V6T 1W5.

2. Present address: Rescan Environmental Services Ltd., Suite 1105, Board of Trade Tower, 1177 West Hastings St., Vancouver, British Columbia, Canada, V6E 2K3.

3. Author to receive reprint requests.

as well as some dissolved organic complexes (Neilands, 1974; Trick *et al.*, 1983). These biologically available species exist in equilibrium with the more dominant colloidal ferric hydroxides, oxyhydroxides and oxides in seawater. If the concentration of available soluble iron is depleted, replacement could occur through dissolution of the colloidal forms. Colloidal iron may thus form an important source of iron for phytoplankton if the nature of the colloids allows an adequate rate of dissolution.

The distinction between colloidal and soluble iron species cannot be rigorously defined. In general, a colloidal dispersion is considered one in which the discontinuous phase (in this case iron) is subdivided into units that are large compared with simple molecules but small enough that interfacial forces as well as inertial forces are significant in governing the properties of the system (Sennet and Oliver, 1965). Although there is no single accepted range, particles having diameters less than 1.0 μm have been considered to have these characteristics (e.g., Matijevic, 1973).

In seawater, iron colloids may exist in a number of crystalline and noncrystalline states. Since the internal structure of the colloid affects its thermodynamic stability (Langmuir and Whittemore, 1971), it may also influence the rate at which the colloid is able, through dissolution, to provide biologically available inorganic species of iron. However, the internal structure of a colloid may alter over time with the potential for concurrent changes in the rate of dissolution. These alterations are often so gradual that short term changes are difficult to detect with classical analytical techniques such as X-ray diffraction. Such alterations in internal colloid structure can be produced by techniques used in culturing phytoplankton and by events occurring in the natural environment.

This investigation tested the hypothesis that differences in the internal structure of iron colloids will affect their ability to supply iron for the growth of the coastal marine diatom *Thalassiosira pseudonana*. The colloidal forms used in the study were precipitated ferric hydroxide (PFH), precipitated ferric hydroxide that was treated by heating or ageing, and colloidal goethite or hematite. Cultures were grown in chelex-treated defined medium (AQUIL; Morel *et al.*, 1979) without complexing agents and either without added iron or with iron from stocks of one of the colloidal forms tested. A number of analytical techniques were used to examine the effects of heating on the structure of the colloidal iron and the results are used to relate minor changes in the colloid internal structure to the effects on final cell yield in the cultures. Levels of colloidal iron that were used were far above the levels found in nature (e.g., Gordon *et al.*, 1982). This was of some concern but it should be realized that the study was intended to examine the effects of colloid structure rather than iron concentration.

2. Materials and methods

The diatom *Thalassiosira pseudonana* (Hustedt) Hasle and Heimdal (WHOI clone 3H) was initially obtained from axenic cultures maintained in the artificial seawater medium of Harrison *et al.* (1980). Stock cultures were grown aseptically in 250 mls of

AQUIL (Morel *et al.*, 1979), without iron or EDTA (ethylenediaminetetraacetic acid), in 500 ml polycarbonate Erlynmeyer flasks at a temperature of 15 ± 1°C and an in-flask light intensity of 95 uEin · m^{-2} · s^{-1} on a 16:8 hour light-dark cycle. Three flasks were used for each test and were inoculated with enough stock culture (1–2 ml) to provide cell concentrations of 1000–2000 cells ml^{-1}. Cell concentrations were counted electronically (model Zf, Coulter Electronics) on a daily basis over the test period.

Except for one series, all experiments were performed in modified AQUIL without EDTA. The medium was prepared with glass distilled water and both the standard ocean water (SOW) and nutrient portion were chelexed to reduce metal contamination. Iron levels were <1 ppb as determined by direct injection graphite furnace atomic absorption spectrophotometry and <0.1 ppb using the technique of Danielsson *et al.* (1978). To prevent precipitate formation during autoclaving, acid-cleaned (6N H_2SO_4), filtered (0.4 μm Nuclepore) CO_2 was bubbled through the AQUIL until the pH reached 5.5. The medium was autoclaved (30 mins., 121°C, 15 psi) and cooled in a 4-liter glass aspirator bottle. If necessary, the pH was adjusted to 8.0 ± 0.05 by bubbling with acid-cleaned, filtered air. Aliquots (250 ml) of the autoclaved stock were transferred to previously autoclaved 500 ml polycarbonate Erlenmeyer flasks fitted with polypropylene screw caps.

Reagent grade chemicals were used in the preparation of culture media and stocks. Culture flasks and glassware were pre-soaked in 6N HCl and rinsed three times with glass distilled water to reduce metal contamination. All transfers and inoculations were conducted in a class 100 laminar flow hood with all possible parts replaced with polypropylene. Due to photosynthetic removal of CO_2 in the cultures, the pH increased as the cultures grew (usually less than 0.2 pH units). No attempt was made to buffer these pH changes due to the potential effect of buffering agents on iron chemistry.

Iron was added from stocks prepared in a number of different ways. Precipitated ferric hydroxide stock (PFH; $Fe(OH)_3$, 4.5×10^{-4} M Fe) was produced by the addition of $FeCl_3$ to glass distilled water without pH adjustment. The rapid formation of polynuclear ferric colloids in such solutions is well documented (van der Giessen, 1968; Murphy *et al.*, 1976; Atkinson *et al.*, 1977). Colloid size was not determined although Murphy *et al.* (1976) estimated a size range of 2–16 nm after 24 hours of ageing at room temperatures in distilled water. The PFH was added to the cultures after various treatments (Table 1). A colloidal goethite stock was prepared following the method of Forbes *et al.* (1974) and a colloidal hematite stock by the method of Matijevic and Scheiner (1978). Confirmation of the colloidal condition was obtained through X-ray diffraction analysis of each of the stock types. The size of the polydispersed goethite (ca. 0.8 μm) and monodispersed hematite (0.1 μm) sols was determined with an Ortec scanning electron microscope. Enough goethite or hematite stock was added to the cultures to obtain the 4.5×10^{-7} M Fe concentration used. Both the goethite and hematite stocks were disaggregated by ultrasonification prior to their addition to the culture medium.

Table 1. Effect of iron stocks on cell yield of *Thalassiosira pseudonana* relative to cell yield with freshly precipitated ferric hydroxide (PFH). Duration (days) is the time after which cell yield was calculated (day 0 = the day of inoculation). The effect of PFH addition at the end of the experiment is shown as + (growth improved) or 0 (growth not improved) (NA = no addition).

Expt.	Duration (days)	Iron stock	Mean (range) of Iron stock cell yield ± 2 S.D. / PFH cell yield ± 2 S.D.	PFH addition at end of experiment	Figure number
1	6	No added iron	0.40(0.37–0.43)	+	1
		Goethite	0.23(0.12–0.34)	+	
		Hematite	0.33(0.32–0.35)	+	
2	5	PFH (aged 1 week)	0.89(0.75–1.04)	0	2
		PFH (aged 3 months)	0.16(0.12–0.21)	+	
3	7	PFH (heated)			
		5 min., 50°C	0.40(0.33–0.49)	NA	3
		5 min., 70°C	0.19(0.15–0.23)	NA	
		5 min., 90°C	0.18(0.13–0.23)	NA	
4	5	No added iron	0.41(0.36–0.46)	+	7
		PFH (autoclaved)	0.13(0.12–0.15)	+	
5	5	PFH (added to AQUIL before autoclaving)	1.04(0.95–1.13)	NA	—
6	6	PFH (stock made with AQUIL salts, stock autoclaved)	1.01(0.92–1.10)	NA	8
7	6	Fe-EDTA (fresh)	0.96(0.91–1.01)	NA	—
		Fe-EDTA (aged 14 months)	0.96(0.89–1.04)	NA	
		Fe-EDTA (autoclaved)	0.97(0.90–1.04)	NA	
8	6	Goethite + EDTA	1.08(0.94–1.22)*	NA	—
		PFH (aged 4 months) + EDTA	1.02(0.73–1.38)**	NA	

*Cell yield relative to cell yield without EDTA (compare with experiment 1)

**Cell yield relative to cell yield without EDTA (compare with experiment 2)

One series of culture experiments was run to determine the effect of the chelating agent EDTA on cell yield. An Fe-EDTA stock solution was prepared by the addition of $FeCl_3$ (4.5×10^{-4} M) and EDTA (5.0×10^{-3} M) to glass distilled water. The Fe-EDTA stock was added to the cultures after various treatments (Table 1).

Cell yield at the end of log phase growth of the control was used to indicate the ability of colloidal iron to provide a source of biologically available iron. Differences between tests were considered to be significant when there was no overlap between the means ± 2 standard deviations. The cell yield of each test was compared only with those values from the same experimental series so as to reduce the effect of minor variations in the cell stock and experimental conditions between tests.

The relationship between colloid structure and the ability to provide iron for the test organism is of primary importance to the study. Physical and chemical analyses of the fresh and autoclaved colloidal iron stocks included X-ray diffraction, thermal gravimetric analysis, Mossbauer spectroscopy, gel filtration and comparison of colloid dissolution rates.

For X-ray diffraction, thermal gravimetric analysis and Mossbauer spectroscopy, iron stocks were adjusted to their experimentally determined isoelectric pH (ca. 7.5) and rapidly mixed to produce aggregates by colloidal coagulation. After the aggregates had settled they were extracted by pipette. For X-ray diffraction, the aggregates were deposited on an X-ray slide and dried at room temperature. X-ray diffractograms were generated on a Phillips diffractometer using Ni-filtered Cu K_α radiation and a 1° 2 theta min^{-1} scan speed. X-ray powder photographs were also obtained using a Debye-Scherrer camera and a 6-hour rotation period. For thermal gravimetric analysis the aggregates were initially held at 115°C for 4 minutes to remove free moisture; the temperature was then raised at a rate of 40°C min^{-1} to 700°C. For Mossbauer spectroscopy, the aggregates were rinsed with distilled water to remove chloride ions and then centrifuged. The pellets were transferred to Mossbauer cells which then were sealed with epoxy and quick frozen in liquid nitrogen. Each sample was measured for 24 hours at liquid nitrogen temperatures.

For gel filtration, a 60 cm polycarbonate column was filled with Sephadex G-10 that had been rinsed with a 1.0 M zirconium solution to block the available adsorption sites and permit the passage of the iron (Murphy *et al.*, 1975). A 1.0 ml aliquot of the iron stock was introduced at the top of the gel and eluted with a 1.0 M zirconium solution at a flow rate of 6.0 ml · hr^{-1}. Fractions (3 ml) were collected and, after acidification to pH 1.0 (6N HCl), iron concentrations were determined by direct injection graphite furnace atomic absorption spectrophotometry.

The colourimetric method of Langford *et al.* (1977) was employed to determine the difference in the rate of dissolution between fresh and autoclaved iron. Mononuclear ferric ion concentrations in aliquots of the stock were measured over time with the colourimetric chelating agent sulphosalicylic acid. The pH of the sulphosalycylic acid solution was adjusted to that of the iron stocks and then mixed (1:1) with the stock in a

10 cm spectrophotometer cell which was immediately placed in a spectrophotometer (Bausch and Lomb 2000) and the absorbance at 500 nm measured for 30 minutes.

3. Results and discussion

Results of the culture experiments are shown in Table 1. These are experiments with *T. pseudonana* in AQUIL medium (Morel *et al.*, 1979) without any metal complexing agents and either without iron or with iron added from a colloidal stock. The effect of the various forms of iron on cell numbers only becomes evident 2–3 days after culture inoculation. Because of this delay, experiments were run for a minimum of 5 days. The absence of immediate effect suggests the possibility of some iron carryover in the cell inoculum. Since iron levels in the stock medium were <0.1 ppb, cell growth can occur at very low levels of iron in the medium.

a. Relationship between final cell yield and major types of iron colloids. Cell yield is significantly higher in the presence of newly precipitated ferric hydroxide (PFH) than without added iron or with iron added from the colloidal goethite or hematite stocks (Table 1, Expt. 1; Fig. 1). Furthermore, cell numbers in flasks containing colloidal goethite or hematite can be increased by the addition of PFH at the end of the experiment but not by the addition of AQUIL levels of nutrients (NO_3, PO_4, $Si(OH)_4$) or metals (Cu, Mo, Co, Mn, Zn). This suggests not only that iron is limiting in these cultures but also that, of the three types of colloidal iron, PFH provides the best source of biologically available metal.

Goethite and hematite are probably the most stable ferric oxyhydroxide and oxide, respectively, under earth surface conditions (Langmuir and Whittemore, 1971). PFH, in contrast, consists of aggregates of hydrated ferric ions that have a much lower thermodynamic stability. Because cell yield was less in cultures containing goethite or hematite than those with PFH, the thermodynamic stability may be important in controlling the supply of biologically available iron. Since biologically available iron is believed to consist of ionic species in some mononuclear organic or inorganic complexed form (Anderson and Morel, 1982), dissolution of colloidal iron must occur to provide biologically available metal. As the thermodynamic stability of the colloid increases so does the activation energy required for dissolution. Therefore, an increase in thermodynamic stability may reduce the rate of dissolution and hence the supply of biologically available metal.

b. The effect of ageing and heating of PFH stocks on cell yield. Three months of ageing of PFH stock in distilled water at room temperature and a pH of 2.5 resulted in a significant decrease in cell yield relative to that of fresh or one week old stock (Table 1, Expt. 2; Fig. 2). (The one week old stock gave a slightly lower cell yield than PFH although the difference was not significant.) Cell numbers in flasks containing three month old PFH can be increased by the addition of new PFH at the end of the test period but not by the addition of nutrients or other trace metals of the AQUIL metal

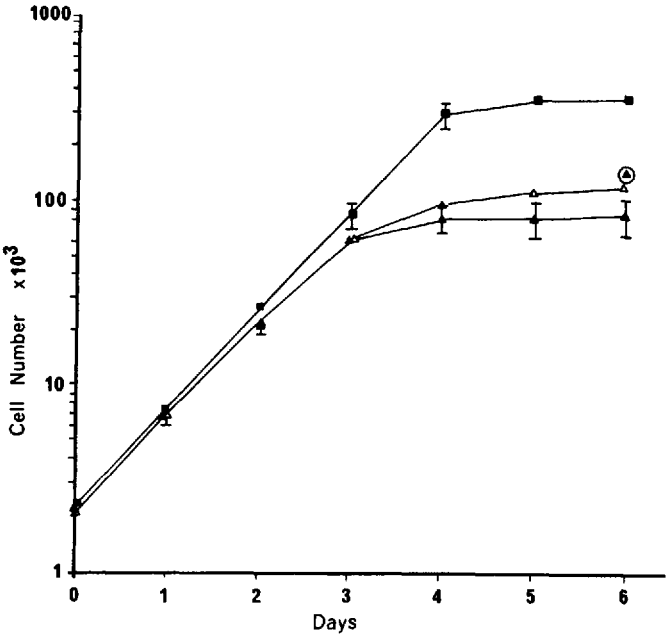


Figure 1. Cell numbers in cultures supplied with various iron stocks—PFH (■), colloidal goethite (▲), colloidal hematite (△). Cell numbers in cultures without iron (◎) were similar to those supplied with colloidal hematite. The vertical bar represents one standard deviation where it is larger than the symbol diameter.

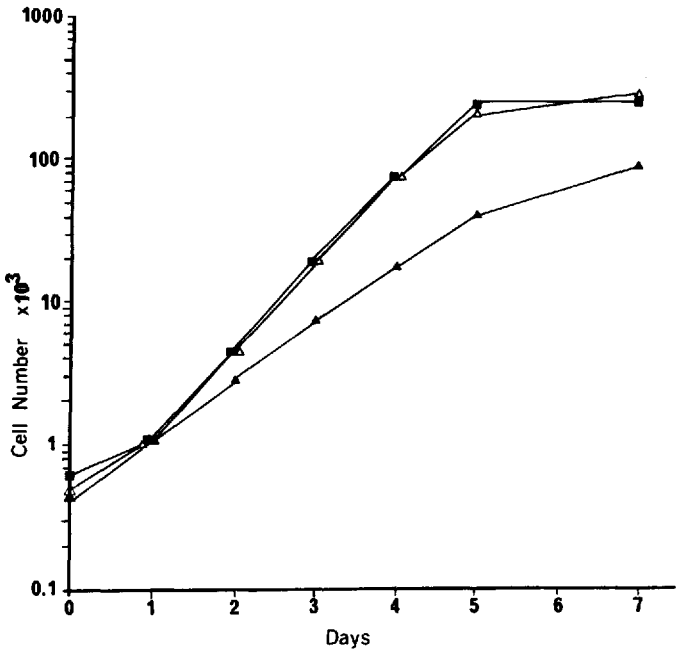


Figure 2. Cell numbers in cultures supplied with PFH stocks of various ages—freshly prepared (■), one week old (△), and three month old (▲). One standard deviation is less than the symbol diameter in all cases.

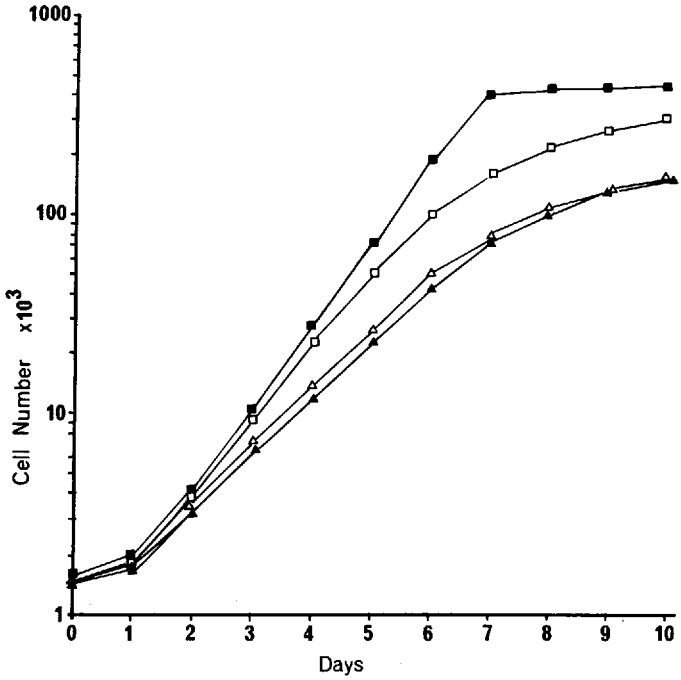


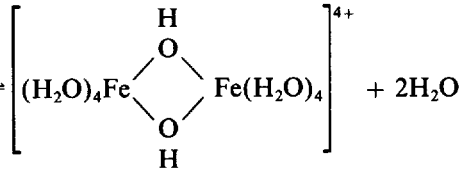
Figure 3. Cell numbers in cultures supplied with PFH stock prepared at room temperature (21°C; ■), and then heated for 5 minutes at 50°C (□), 70°C (△) and 90°C (▲). One standard deviation is less than the symbol diameter in all cases.

mix. It thus appears that colloids from an aged stock do not contribute as much available iron as does the fresh PFH.

Heating of the PFH stocks for short periods of time also causes a decrease in cell yield. Stock heated for 5 minutes at 50°C produces a significant decrease in cell yield relative to fresh stock prepared at room temperature (21°C; Table 1, Expt. 3; Fig. 3). The decrease is more pronounced with PFH heated for 5 minutes at 70°C. Use of stock that has been heated for 5 minutes at 90°C or autoclaved for 15 minutes or longer (121°C, 15 psi) produces little additional change (Table 1, Expts. 3, 4). Thus some change in the colloidal ferric hydroxide stock between room temperature (21°C) and 70°C reduces its ability to readily provide a source of biologically available iron. As with ageing, cell numbers could be increased at the end of the experiment by adding new PFH to the cultures containing the heated stock but not by adding nutrients or other trace metals.

The chemical changes occurring in PFH stocks are those described for ferric salt solutions (van der Giessen, 1968; Dousma and de Bruyn, 1976; Murphy *et al.*, 1976). With the treatments of heating and ageing, ferric salt solutions undergo hydrolysis and precipitation processes that ultimately result in the formation of crystalline products. The ferric salts initially dissociate, the Fe^{+3} ions rapidly being hydrated with the

coordination sphere containing six H_2O molecules. This complex reacts as an acid and releases H^+ to form $\{Fe(H_2O)_5(OH)\}^{+2}$ (van der Giessen, 1968) which may polymerize with other such complexes by the formation of OH bridges:



This reaction is known as olation. These ol bridges can split off protons to form Fe—O—Fe bonds (or oxo bridges) by a reaction termed oxolation. The final result of these processes is the formation of ferric colloids consisting of a network of polynuclear complexes linked up with —O— and —OH bridges. Over time, the proportion of —O— linkages will increase and they may re-orientate to form crystalline structures. Although these reactions are reversible, the oxo bridge is believed to be particularly difficult to break (van der Giessen, 1968; Dousma and de Bruyn, 1966). As a consequence, the thermodynamic stability of the colloid will increase as the hydrolytic (or maturation) processes continue.

While the initial hydrolysis and polymerization reactions proceed rapidly, the subsequent oxolation is very slow and may take hundreds of hours for completion at room temperature (van der Giessen, 1968). However, this rate is greatly increased at higher temperatures (e.g., Matijevic, 1973).

The culture experiments suggest that both ageing and heating of PFH decrease the rate of colloid solubilization as a consequence of enhanced thermodynamic stability. In order to determine the effect of heating on stability we used X-ray diffraction, Mossbauer spectroscopy, thermal gravimetric analysis and gel filtration to characterize the mineralogy, degree of ordering, degree of hydration and colloid size in fresh and autoclaved PFH stocks. (Unfortunately, the quantity of three month old PFH stock available was insufficient for a similar analysis of aged PFH.) No crystallinity was detectable in the fresh or autoclaved (30 mins.) PFH stocks either by X-ray scanning or Debye-Scherrer camera techniques. However, microcrystallites were observed in autoclaved stock under crossed nicols with a petrographic microscope. These results indicate that although some dehydration may have occurred in the colloids it was inadequate to produce sufficient crystallization to be detectable with the X-ray diffraction equipment used. When PFH stocks were autoclaved for different lengths of time (15 mins., 30 mins., 1 hr., 4 hrs., 12 hrs., and 24 hrs.) crystal structures were detected with the X-ray powder camera only after 24 hours of autoclaving.

Mossbauer spectroscopy was used to investigate changes in the bonding within the autoclaved PFH colloids. While there was no significant difference in the chemical isomer shift or quadrapole splitting there was a slight increase in the peak width of the autoclaved iron. However, this difference was not enough to draw any meaningful conclusions.

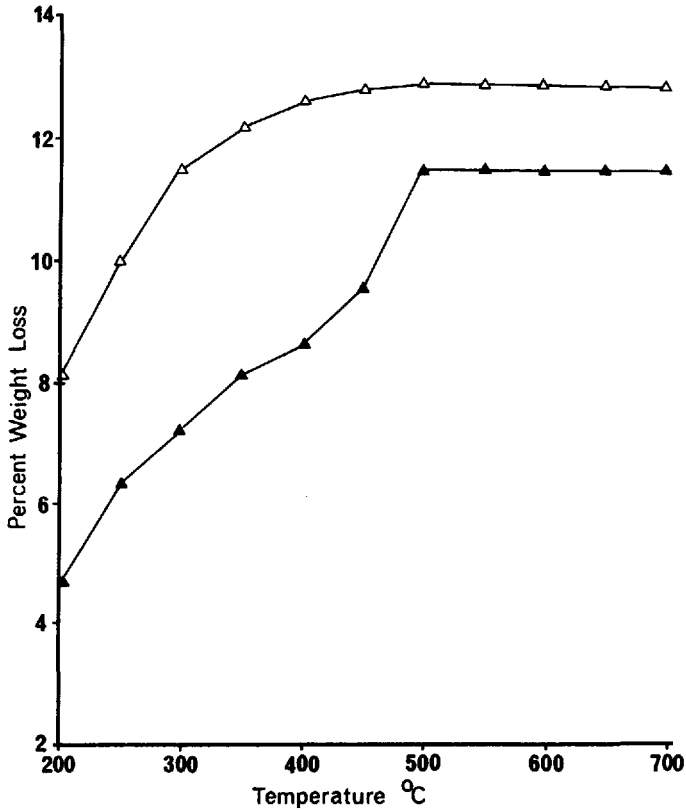


Figure 4. Thermal gravimetric analysis. The percent weight loss through the temperature range used is shown for fresh (△) and autoclaved (▲) PFH stocks.

Thermal gravimetric analysis of autoclaved and nonautoclaved PFH stocks was used to characterize colloid dehydration caused by autoclaving. Nonautoclaved PFH stock had the highest percent weight loss (Fig. 4) until a constant maximum was reached at 500°C. Autoclaved PFH stock had a rapid weight loss between 450°C and 500°C which could be due to a sudden release of loosely incorporated water molecules or a release of chemically bound (—OH, —H) water by a simultaneous shift in bonding configurations within the colloids. In either case, these results suggest that autoclaving caused dehydration of the colloids, an indication of maturation.

Gel filtration was used to measure changes in colloid size in order to estimate the potential change in surface area (Fig. 5). The results suggest that colloid size increased with autoclaving. This increase in size could be due either to coagulation of the existing colloidal aggregates or a dissolution-reprecipitation reaction. While simple coagulation of the PFH would not effectively reduce the colloid surface area (due to the porous nature of hydrated ferric ion aggregates), a dissolution-reprecipitation reaction could

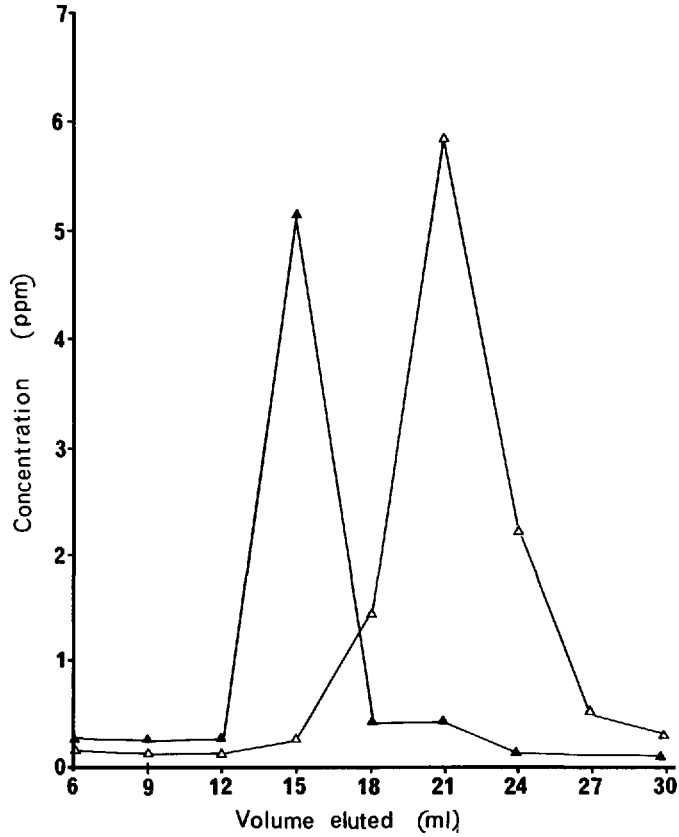


Figure 5. Iron concentrations in 3 ml fractions of freshly prepared (△) and autoclaved (▲) PFH stocks after passage through Sephadex G-10.

substantially reduce the surface area. Murphy *et al.* (1976) suggest that such dissolution-reprecipitation reactions do occur but only after a period of many hours. We believe that such a reaction would not occur to any great extent during the 15 minutes of autoclaving used in these experiments and thus coagulation appears as the most likely explanation for the increase in colloid size.

Since the rate of colloid dissolution is expected to covary with changes in thermodynamic stability, relative dissolution rates of autoclaved and nonautoclaved PFH stocks were determined. Using the colourimetric technique of Langford *et al.* (1977) in a qualitative manner, nonautoclaved PFH stock had a higher initial absorbance and a greater rate of increase in absorbance than the autoclaved stock (Fig. 6). This suggests that, under the conditions of the test, the initial monomeric ferric iron concentration was greater and dissolution occurred more rapidly in the nonautoclaved stock.

While no single technique provides unequivocal evidence of the changes produced by autoclaving of the PFH stock, the combined results suggest that the thermodynamic

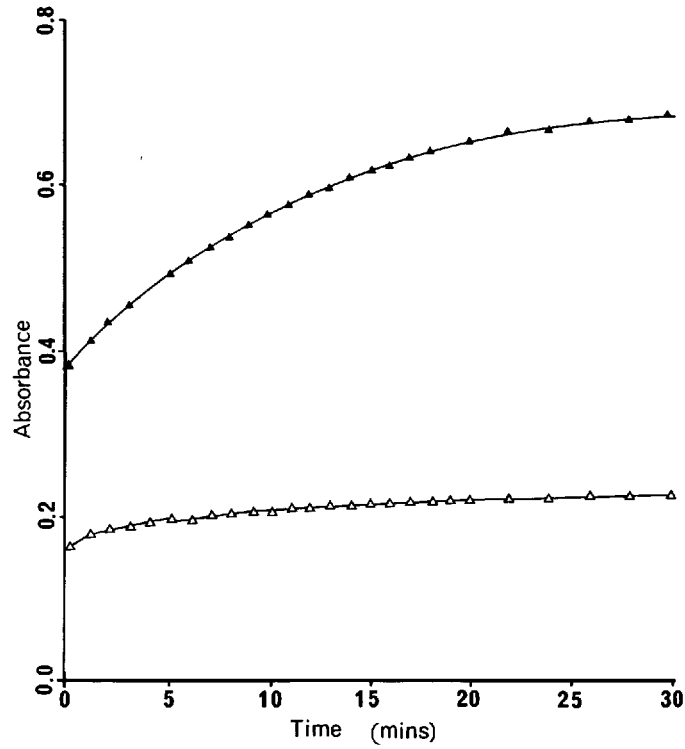


Figure 6. Dissolution of freshly prepared (▲) and autoclaved (△) stocks as shown by absorbance at 500 nm after treatment with sulphosalicylic acid (Langford *et al.*, 1977).

stability of the colloidal iron increases. More importantly, however, the effect of these changes is to decrease the dissolution rate of the colloids.

Cell yield in cultures supplied with autoclaved (Fig. 7) (as well as heated and three month old) PFH stocks was not only less than cultures with new PFH but was also lower than that of cultures without added iron (Table 1, Expt. 4). A possible explanation for this is the adsorption of residual available iron by the added heated and aged PFH; the metal scavenging abilities of colloidal hydrous oxides are well documented (Kim and Zeitlin, 1971). Fresh PFH will also do this but its lower thermodynamic stability will allow faster dissolution.

c. The relationship between ionic strength and the effect of autoclaving of PFH on cell yield. While autoclaving of the PFH stock before addition to the AQUIL reduces cell yield, no deleterious effect occurs if the PFH stock is added to the AQUIL and then autoclaved (Table 1, Expts. 5, 6). Although increased ionic strength may result in an increased precipitation rate (Dousma and de Bruyn, 1976), foreign ions reduce the rate of colloid maturation (Langmuir and Whittemore, 1971). Thus a hydrous oxide phase

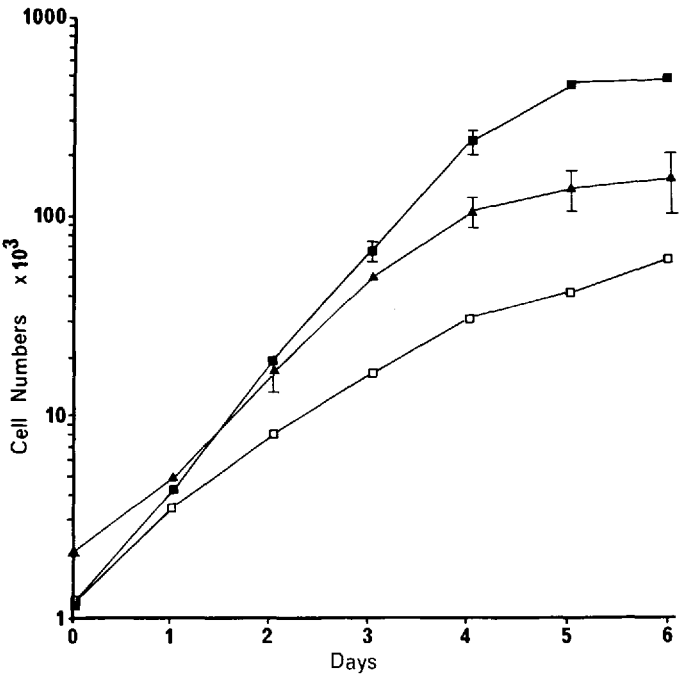


Figure 7. Cell numbers in cultures without iron (▲) and supplied with fresh (■) and autoclaved (□) PFH stocks. The vertical bar represents one standard deviation where it is larger than the symbol diameter.

of relatively low thermodynamic stability could occur in the AQUIL after autoclaving. To test the effects of foreign ion interference, PFH stock was prepared using the AQUIL salt medium (4.5×10^{-4} M Fe). Autoclaving this stock did not cause the reduction in cell yield found with distilled water PFH stock (Table 1, Expt. 6; Fig. 8) suggesting that the rate of colloid maturation is reduced in the presence of salts. In addition to the effects of the higher ionic strength, the lower iron concentration in the medium during autoclaving (4.5×10^{-7} M Fe) would also decrease the rate of hydrated iron aggregation and thus the rate of maturation (Murphy *et al.*, 1976).

d. Comparison of cell yield with new PFH and Fe-EDTA. The chelating agent EDTA is commonly used to provide an improved source of biologically available metal in phytoplankton cultures. EDTA prevents the precipitation of ferric colloids by the chelation of the aquo-ions, maintaining higher levels of iron in solution. Cell yields with new PFH stock, Fe–EDTA stock and autoclaved and aged (14 months) Fe-EDTA stock were similar (Table 1, Expt. 7) if the EDTA was added prior to heating or ageing of the PFH. This did not occur when EDTA was added after heating or ageing (Table 1, Expt. 8). In addition, cell yield was not improved when EDTA was added to goethite supplied cultures (Table 1, Expt. 8). Increased colloid stability following heating or

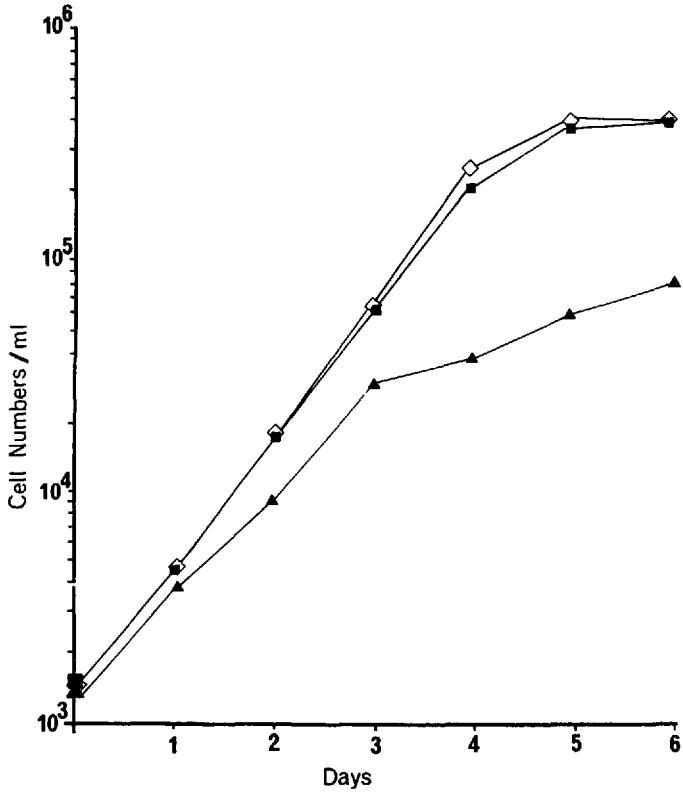


Figure 8. Cell numbers in cultures supplied with PFH stocks prepared in distilled water (◇), prepared in distilled water and autoclaved (▲) and prepared in the AQUIL salt medium and autoclaved (■). One standard deviation is less than the symbol diameter in all cases.

ageing appears to reduce the ability of the EDTA to solubilize the iron and enhance the supply of biologically available metal. Thus the state of the colloid is important in providing a source of metal to *T. pseudonana,* with or without the organic complexing agent EDTA.

4. Conclusions

The internal chemistry of colloidal iron controls its ability to provide a source of available iron for the coastal marine diatom *Thalassiosira pseudonana.* Ageing and heating of freshly precipitated ferric hydroxide is associated with a reduction in available iron as a result of a decrease in the rate of colloid dissolution. The occlusion of salts into the colloidal iron extends the time that it takes for this to occur as does the use of the chelating agent EDTA. Once the change has taken place, however, the addition of EDTA does not improve the ability of the colloidal iron to provide a source of biologically available metal. It is beginning to appear that naturally occurring iron

complexing agents such as siderophores (Trick *et al.*, 1983) play an important role in controlling the biological availability of iron. However, complexing agents may have reduced benefit as the thermodynamic stability of the colloid increases. Information is needed on the equilibration rates between the various types of colloidal iron and important iron complexing agents such as siderophores.

Three applications of this work should be considered. First, the effect on iron stocks used in culturing phytoplankton, heating and ageing can cause biologically important changes to take place in the stock. Second, since estuaries are areas of increasing ionic strength, changes in river-introduced precipitated ferric hydroxide will be controlled by the interaction of the iron with salts. Ultimately, maturation will occur in the colloidal iron with a reduction in dissolution rate and the ability to provide a source of iron for phytoplankton. Third, the colloidal state should be considered when examining the effects of natural complexing agents (e.g., siderophores, humic materials) on phytoplankton in estuarine and oceanic environments.

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