Evidence of Iron Association with Emulsion Droplets and Its Impact on Lipid Oxidation

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ζ-Potential measurements were used to evaluate conditions that influence iron−emulsion droplet interactions. Fe^{2+} and Fe^{3+} associated with sodium dodecyl sulfate (SDS, anionic) but not with dodecyltrimethylammonium bromide (DTAB, cationic) and polyoxyethylene 10 lauryl ether (Brij, nonionic) stabilized hexadecane emulsions. Association of iron with SDS-stabilized emulsion droplets increased with decreasing pH and decreased in the presence of EDTA, phytate, and NaCl. Oxidation of the SDS-stabilized salmon oil emulsion increased as iron−emulsion droplet interactions increased. EDTA concentrations (≥1000 μM) that resulted in no detectable association of iron (500 μM) with the emulsion droplets were the most effective at inhibiting oxidation. The ability of iron to associate with emulsion droplets seems to be an important factor in the promotion of lipid oxidation; therefore, techniques that control iron's physical location could be effective in controlling oxidation.

Keywords: *Lipid oxidation; iron; chelators; ω-3 fatty acids; ζ-potential*

INTRODUCTION

The lipids in many foods exist in the form of small fat droplets dispersed in an aqueous matrix that may contain a variety of water-soluble components including transition metals (McClements, 1998). Among the transition metals, iron may be the most important lipid oxidation prooxidant due to its high reactivity and concentrations. The ferrous state of iron accelerates lipid oxidation by breaking down hydrogen and lipid peroxides to reactive free radicals via Fenton type reactions (Dunford, 1987). Ferric ions also produce radicals from peroxides, although the rate is ∼10-fold less than that of ferrous ions (Miller, 1996). In addition, Fe^{3+} can be reduced to Fe^{2+} by reductants such as O_2^-, ascorbic acid, and compounds containing thiol groups (Dunford, 1987), thereby increasing iron-induced lipid oxidation.

In lipid dispersions, transition metal ions accelerate lipid oxidation more effectively in the presence of negatively charged surfactants than in the presence of positively charged or nonionic lipid surfactants (Yoshida and Niki, 1992; Fukuzawa et al., 1995; Mei et al., 1998). This observation has been explained by the ability of positively charged metal ions to be attracted to the negatively charged droplet surface, resulting in generation of more free radicals at or near the droplet surface. Whereas the interactions between negatively charged surfaces and transition metals have been suggested on the basis of observations of accelerated lipid oxidation and general principles of charge interaction, little evidence has been reported to directly show the association of transition metal ions with emulsion droplet surfaces.

Measurements of the electrical charge on droplet surfaces can provide direct information about interactions between ions and emulsion droplets (Hunter, 1994; Hiemenz and Rajagopalan, 1997). Ionic emulsifiers provide a charge on emulsion droplets that attracts oppositely charged ions. These counterions are distributed from as close to the surface as allowed by their atomic radius to as far away as to where the electric influence of the surface charge has been almost totally shielded (Winkle, 1997). In the presence of an electrical field, charged emulsion droplets move toward the oppositely charged electrode. As they move, only the most strongly bound counterions are carried along with the droplets. The ζ-potential is a measure of the electrical potential at the outer limit of the bound counterion layer, called the shear plane, which is fixed to the droplets moving relative to the liquid (Hibbert and James, 1984; Winkle, 1997). Although ζ-potential is not a direct measurement of surface charge, it reflects the net charge of the particle inside the shear plane (Winkle, 1997). Thus, addition of multivalent ions (i.e., Fe^{2+} and Fe^{3+}) to an emulsion containing negatively charged droplets may result in the association of iron with unoccupied, oppositely charged sites or the displacement of univalent ions (e.g., Na^+) within the shear plane, thus causing changes in ζ-potential.

The objective of this study was to determine if ζ-potential measurements could provide evidence of the association of iron with emulsified lipid droplets and to determine if iron association was related to the oxidation of dispersed lipids. The influence of metal chelators, NaCl, and pH on iron association and lipid oxidation was investigated. Determination of the relationship between iron and lipid droplet interactions could provide insight into new techniques that could increase the oxidative stability of dispersed lipids.

MATERIALS AND METHODS

Dodecyltrimethylammonium bromide [DTAB, $CH_3(CH_2)_{11}N(CH_3)_3Br$], a cationic surfactant, was obtained from Aldrich Chemical Co., Inc. (Milwaukee, WI). $FeCl_2 \cdot 4H_2O$, polyoxyethylene 10 lauryl ether [Brij, $CH_3(CH_2)_{11}(OCH_2CH_2)_{10}OH$], a nonionic surfactant, sodium dodecyl sulfate [SDS, $CH_3(CH_2)_{11}$-

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OSO_3Na], a anionic surfactant, 2-thiobarbituric acid, and phytic acid dodecasodium salt were purchased from Sigma Chemical Co. (St. Louis, MO). Disodium ethylenetetraacetic acid (EDTA), $FeSO_4 \cdot 7H_2O$, and $FeCl_3 \cdot 6H_2O$ were from Curtin Matheson (Cinncinnati, OH). All other chemicals were of reagent grade or purer.

Isolation of Fish Oil. Fresh salmon was obtained from a local store. The salmon was hand chopped into small pieces and minced for 2−3 min using a food processor. The mince was centrifuged at 10000*g* for 20 min at room temperature. The centrifuge tubes containing the salmon mince were cooled to −15 °C overnight followed by storage in the dark at room temperature for 10 min. While the muscle fraction was still frozen, the melted salmon oil was decanted and centrifuged for 5 min at 2000*g* to remove contaminating solids and water. The resulting oil was stored at −40 °C. The isolated salmon oil contained 99.5 ± 0.2% triacylglycerols [using the method of Zhou and Ackman (1996)], 4 μg of α-tocopherol/g of oil [using the method of Liu et al. (1996)], 0.034 μmol of thiobarbituric acid reactive substances (TBARS)/g of oil [using the method of McDonald and Hultin (1987)], and 0.12 μmol of lipid peroxides/g of oil [using the method of Shantha and Decker (1994)].

Preparation of Emulsions. Emulsions of *n*-hexadecane (a nonoxidizable lipid) were used for ζ-potential measurements to avoid potential alterations in interfacial properties by lipid oxidation products, which could be present or produced in emulsions containing polyunsaturated fatty acids. Salmon oil was used to make emulsions for lipid oxidation experiments. Oxidized salmon oil for lipid peroxide studies was produced by storing the oil at −15 °C for ∼4 months. To make a 5% lipid emulsion, 1.5 g of *n*-hexadecane or salmon oil was added to 28.5 g of a 0.017 M solution of selected surfactants and the mixture was sonicated as described in Mei et al. (1998).

ζ-Potential Measurement. Ninety-nine parts of 0.017 M solution of selected surfactant was mixed with 1 part each of 1.0 M acetate and 1.0 M imidazole buffer. The solution was adjusted to pH 3.0−7.0 with HCl and NaOH and was used to dilute the hexadecane emulsion 500-fold. Following dilution, NaCl, chelators, and/or iron were added. The diluted emulsion was injected into a ZEM5002 Zetamaster unit (Malvern Instruments, Ltd., Worcstershire, U.K.), and the ζ-potential was measured. Data were averages of eight readings of four injections from two separate trials. Performance of the instrument was monitored by using a DTS5050 standard colloidal suspension obtained from Malvern Instruments.

Determination of Lipid Oxidation. Ninety-nine parts of salmon oil emulsion (5% lipid) was mixed with 1 part each of acetate (1.0 M) and imidazole (1.0 M) buffer. The pH of the emulsion was adjusted to 3 and then chelators, NaCl, and $FeSO_4$ were added. Oxidation time began immediately after $FeSO_4$ addition, and samples were incubated at 22 °C. Ferrous ions were used instead of an iron−ascorbate redox cycling system to avoid the potential complicating effects of the antioxidant properties of ascorbate. TBARS were measured according to the method of McDonald and Hultin (1987), using 0.1 mL of emulsion, 0.9 mL of H_2O, and 2 mL of TBA reagent. To minimize error due to the potential of light scattering by sample cloudiness, TBARS were calculated as $\text{absorbance}_{532nm} - \text{absorbance}_{580nm}$. $\text{Absorbance}_{580nm}$ was used as a correction for light scattering because 580 nm represents the closest non-TBARS-absorbing wavelength to 532 nm. Concentrations of TBARS were determined from a standard curve prepared using 1,1,3,3-tetraethoxypropane.

Headspace propanal was measured in selected samples as a verification of secondary lipid oxidation products. Analysis was performed with a Hewlett-Packard (HP) 19395A headspace sampler and an HP 5890 gas chromatograph coupled with an HP 3392A integrator. Headspace sampler conditions were as follows: sample incubation temperature, 27 °C; incubation time, 0 min; sample loop and transfer line temperature, 100 °C; pressurization, 10 s; venting, 10 s; injection, 1 min. For the HP 5890 gas chromatograph, the oven, injector, and detector temperatures were 70, 180, and 200 °C, respectively. Volatile compounds were separated on an HP methyl silicon (DB-1) fused silica capillary column (50 m, 0.31 mm i.d., 1.03 μm film thickness) and were detected using a flame ionization detector (FID). A propanal standard curve was used to determine concentrations.

Table 1. ζ-Potential of SDS-, Brij-, and DTAB-Stabilized Hexadecane Emulsions in the Presence of Iron Salts at pH 3.0

		ζ-potential[a] (mV)		
treatment	concn (μM)	SDS	Brij	DTAB
$FeSO_4$	0	−105.7 ± 2.1	−2.8 ± 1.2	74.7 ± 1.6
	50	−105.4 ± 1.7	−4.4 ± 0.6	74.2 ± 1.4
	150	−103.1 ± 1.8	−4.5 ± 0.9	74.0 ± 0.9
	500	−95.9 ± 1.2	−5.2 ± 0.7	71.6 ± 1.2
$FeCl_2$	0	−105.3 ± 1.8	−2.7 ± 0.8	75.8 ± 0.4
	5	−104.8 ± 0.1	nd[b]	nd
	50	−103.6 ± 4.1	−2.6 ± 0.8	76.0 ± 0.3
	150	−97.9 ± 1.0	−2.8 ± 0.6	75.6 ± 0.9
	500	−94.6 ± 0.9	−2.9 ± 1.0	74.6 ± 0.7
$FeCl_3$	0	−105.8 ± 0.6	−3.2 ± 0.7	75.5 ± 0.3
	50	−101.3 ± 0.2	−3.6 ± 0.7	76.0 ± 0.2
	150	−93.9 ± 0.4	−3.0 ± 0.6	75.5 ± 0.8
	500	−82.9 ± 0.7	−3.3 ± 1.2	74.3 ± 0.7

[a] Data represent means ± standard deviations. [b] nd, not determined.

To measure lipid peroxides, a mixture of 0.2 mL of emulsion, 10 mg of NaCl, and 1 drop of a saturated aqueous solution of propyl gallate was vortexed for 10 s to break the emulsion. Two milliliters of isooctane was added, and the sample was covered tightly with Parafilm and was vortexed vigorously for 10 s, a total of three times. After centrifugation for 5 min at 2000*g*, 0.1 mL of the clear upper phase was taken for peroxide measurement. Peroxides were measured using a modified method of Shantha and Decker (1994). Methanol/1-butanol (2:1, v:v; 2.8 mL), the lipid extract (0.1 mL), H_2O (0.1 mL), and thiocyanate/Fe^{2+} solution (30 μL) were mixed together by vortexing. The thiocyanate/Fe^{2+} solution was made immediately before use by mixing 1 volume of 3.94 M ammonium thiocyanate with 1 volume of Fe^{2+} solution (obtained from the supernatant of a mixture of 3 mL of 0.144 M $BaCl_2$ in 0.4 M HCl and 3 mL of 0.144 M $FeSO_4$). Twenty minutes after Fe^{2+} had been added, the absorbance was measured at 510 nm. Peroxide concentrations were obtained from a hydrogen peroxide standard curve.

All oxidation data represent averages of four measurements of two samples in two separate trials.

RESULTS AND DISCUSSION

Iron−Droplet Interactions and Lipid Oxidation. ζ-Potential measurements show that hexadecane emulsion droplets stabilized by SDS are strongly negative, droplets stabilized by Brij are weakly negative, and those stabilized by DTAB are strongly positive at pH 3 (Table 1). Addition of $FeCl_2$, $FeCl_3$, and $FeSO_4$ reduced the net negative charge of SDS emulsion droplets. $FeCl_2$ and $FeCl_3$ had no effect on the DTAB and Brij emulsion droplets. $FeSO_4$ was capable of causing a small decrease in the ζ-potential of both the Brij and DTAB emulsion droplets. Ion-induced changes in ζ-potential can occur through displacement of univalent ions such as Na^+ and Cl^- by multivalent ions. This explains why iron altered the charge of SDS emulsion droplets and SO_4^{2-} altered the charge of Brij and DTAB droplets while Cl^- ions from $FeCl_2$ and $FeCl_3$ were ineffective. Ion displacement also explains why Fe^{3+}, which carries an additional positive charge, reduced the ζ-potential >2-fold more than Fe^{2+}. The inability of both $FeCl_2$ and $FeSO_4$ to reduce the net negative charge of the Brij emulsions suggests that iron was not capable of strongly

Table 2. Estimated Concentrations of Iron Associated with SDS-Stabilized Hexadecance Emulsion Droplets

Fe concn (μM)	$\Delta\zeta$-potential (mV)	ΔN Fe/droplet	% droplet surface area occupied by Fe
Fe^{2+}			
5	0.4	1.6×10^3	0.06
50	1.4	5.2×10^3	0.19
150	3.6	1.3×10^4	0.48
300	6.8	2.5×10^4	0.91
500	11.2	4.1×10^4	1.48
Fe^{3+}			
5	0.3	6.8×10^2	0.05
50	4.3	1.1×10^4	0.86
150	11.9	2.9×10^4	2.38
300	20.0	4.9×10^4	3.98
500	25.1	6.2×10^4	4.99

associating with the slightly negative charged groups on the droplet surface.

Estimations of the magnitude of iron interactions with the emulsion droplets are shown in Table 2. The relationship between net surface charge and ζ-potential (Hiemenz and Rajagopalan, 1997) can be expressed as

$$q = 4\pi\epsilon\zeta R(1 + \kappa R) \quad (1)$$

where q = net charge enclosed by the surface of the shear plane, ϵ = dielectric constant = 6.95×10^{-10} C^2 J^{-1} m^{-1}, ζ = ζ-potential in volts, R = particle radius at the shear plane in meters, and κ = the Debye−Huckle parameter = $2.32 \times 10^9 \times (2I)^{1/2}$ m^{-1}, where I is the ionic strength (27 mM in hexadecane emulsion model).

The change in net charge (Δq) can be calculated from the change in ζ-potential ($\Delta\zeta$)

$$\Delta q = 4\pi\epsilon\Delta\zeta R(1 + \kappa R) \quad (2)$$

thus allowing a calculation for the increase in number of iron per droplet (ΔN)

$$\Delta N = \Delta q/ze \quad (3)$$

where z is the number of charges per ion (i.e., $Fe^{2+} = 2$) and e is the elementary charge (1.602×10^{-19} C). Using this mathematical model, the increase in Fe^{2+} associated with the SDS-stabilized droplet ranged from 1.6×10^3 to 4.1×10^4 per droplet, whereas the increase in Fe^{3+} ranged from 6.8×10^2 to 6.2×10^4 per droplet. At iron concentrations ≥ 50 μM, Fe^{3+} was estimated to bind more readily to the droplet surface than Fe^{2+}. This difference is most likely due to the greater charge density of Fe^{3+} (Israelachvili, 1992). Assuming an emulsion droplet diameter of 1.0 μm, the surface area of a single droplet would be 3.14×10^{-12} m^2. Using an effective radius of iron in aqueous solution of 0.6 and 0.9 nm for Fe^{2+} and Fe^{3+}, respectively (Dean, 1979), the calculated coverage of the droplet surface by iron was $<1.5\%$ for Fe^{2+} and $<5.0\%$ for Fe^{3+} when the molar ratio of Fe (5−500 μM) to emulsion droplets was 1.2×10^7 to 1.2×10^9.

Previous results (Mei et al., 1998) have shown that iron-induced oxidation of corn oil emulsions was much greater in SDS emulsions than in Brij and DTAB emulsions, suggesting that iron association with the emulsion droplet surface influences lipid oxidation rates. Figure 1 shows that increasing Fe^{2+} (from $FeSO_4$) concentration increases the change in ζ-potential (i.e., the droplets become less negative) in SDS-emulsified hexadecane and increases TBARS formation in SDS-emulsified salmon oil. Oxidation was also observed to

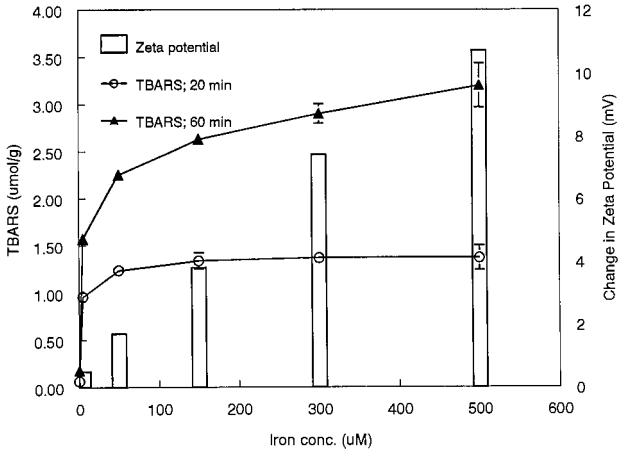

Figure 1. Ability of Fe^{2+} to alter ζ-potential and accelerate lipid oxidation (as measured by TBARS) in SDS-stabilized emulsions at pH 3. Change in ζ-potential was calculated as ζ-potential of SDS emulsion − ζ-potential of SDS emulsion with Fe^{2+}.

Table 3. Influence of Fe^{2+} ± EDTA on Propanal Formation and Lipid Peroxide Concentrations in SDS-Stabilized Salmon Oil Emulsions[a]

		oxidation time		
Fe^{2+} (μM)	EDTA (μM)	5 min	20 min	60 min
Propanal (μmol/g of Oil)				
0	0	nd[b]	0.06 ± 0.01	0.07 ± 0.01
5	0	nd	0.12 ± 0.02	0.24 ± 0.01
500	0	nd	0.63 ± 0.03	1.29 ± 0.01
500	500	nd	0.53 ± 0.00	0.89 ± 0.12
500	1000	nd	0.37 ± 0.08	0.29 ± 0.02
Lipid Peroxides (mmol/g of Oil)				
0	0	19.0 ± 0.4	20.4 ± 0.4	23.1 ± 0.8
5	0	22.3 ± 0.0	23.7 ± 0.7	26.1 ± 0.1
150	0	11.6 ± 0.3	10.0 ± 1.0	15.1 ± 0.4
500	0	7.5 ± 0.1	7.9 ± 0.6	9.6 ± 0.3

[a] Propanal concentration in emulsions prior to Fe^{2+} addition was 0.04 ± 0.01 μmol/g of oil. Emulsions for lipid peroxide measurements were made with oxidized oil. [b] nd, not determined.

increase with increasing Fe^{2+} concentration when headspace propanal was used as an oxidation marker (Table 3). Concentrations of Fe^{2+} (5 μM) that had little influence on ζ-potential increased TBARS formation >14-fold compared to the control (no added Fe^{2+} after 20 min of oxidation). Increasing Fe^{2+} from 5 to 50 μM and from 50 to 500 μM increased TBARS formation 1.3- and 1.1-fold, respectively (Figure 1). TBARS concentrations after 60 min of oxidation show a similar trend, with 5.0 and 50 μM Fe^{2+} increasing oxidation 8.9- and 12.5-fold, respectively, compared to the control, whereas increasing Fe^{2+} to 500 μM increased TBARS only an additional 1.4-fold compared to 50 μM Fe^{2+}. Iron-induced lipid oxidation is likely to occur through the decomposition of preexisting peroxides in the oil. The ability of low concentrations of Fe^{2+} to dramatically increase oxidation rates suggests that small amounts of iron are needed at the droplet surface to promote peroxide breakdown. Increasing iron concentrations in the emulsion system would also increase its concentration in the aqueous phase. However, the observation that interactions between Fe^{2+} and Brij and DTAB emulsion droplets are undetectable (Table 1) and previous results that show that iron only weakly promotes oxidation in DTAB and Brij emulsions (Mei et al., 1998) suggest that iron not strongly associated with the

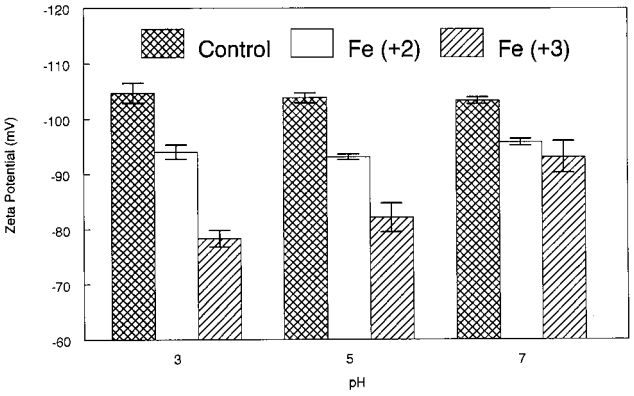

Figure 2. Ability of Fe^{2+} and Fe^{3+} (500 μM) to alter ζ-potential of an SDS-stabilized hexadecane emulsion over the pH range of 3−7.

emulsion droplets is not an important prooxidant in these emulsion model systems.

The ability 500 μM Fe^{2+} to decrease ζ-potential 9 mV more than 50 μM Fe^{2+} (increasing the estimated number of Fe^{2+}/emulsion droplet >7-fold) without dramatically increasing lipid oxidation is likely due to low initial lipid peroxide concentrations, which would limit reaction rates. To determine the effect of iron on lipid peroxide concentrations, an SDS emulsion containing >17 μmol of lipid peroxide/g of oil was prepared from oxidized oil (Table 3). In controls and samples containing 5 μM Fe^{2+}, lipid peroxide concentrations were found to gradually increase during the 60 min incubation time. However, in samples containing higher concentrations of Fe^{2+} (≥150 uM), lipid peroxides initially decreased and then increased during the latter stages of oxidation. This initial depletion in peroxides by high Fe^{2+} concentrations suggests that peroxide concentrations could be limiting reaction rates.

Figure 2 shows that pH had no effect on the charge of SDS emulsion droplets. Decreasing pH (from 7 to 3) had little effect on the association of Fe^{2+} to the SDS emulsion droplets, whereas Fe^{3+} association increased with decreasing pH. Fe^{3+} changed the ζ-potential of the SDS-stabilized emulsion 10.2 mV at pH 7.0 and 26.4 mV at pH 3.0. Differences in the ability of pH to alter the association of Fe^{2+} and Fe^{3+} with SDS emulsion droplets can be explained by the effect of pH on iron solubility. Ferric ions have very low solubility at pH 7 (4×10^{-17} M), and their solubility increases $>10^{12}$ from pH 7 to 3, whereas Fe^{2+} is more soluble at pH 7 (0.18 M) with a solubility increase of 10^8 from pH 7 to 3 (Zumdahl, 1989). These observations and previous results which show that iron(III) ascorbate-catalyzed lipid oxidation of SDS-emulsified corn oil increases with decreasing pH (Mei et al., 1998) again suggest that increased iron association with emulsion droplets can result in increased lipid oxidation. Decreasing pH from 7 to 3 did not increase oxidation in the DTAB- and Brij-stabilized emulsion, suggesting that increasing lipid oxidation rates were not simply due to increases in iron solubility but were due to specific iron−droplet interactions (Mei et al., 1998).

Effect of Chelators on Iron−Droplet Interaction and Lipid Oxidation. EDTA and phytate (1000 μM) had no effect on the ζ-potential of Brij emulsions (Table 4). In the DTAB emulsion, phytate (1000 μM) caused destabilization of the DTAB emulsion, whereas ETDA (1000 μM) decreased the ζ-potential 5 mV, presumably through the displacement of univalent ions by the multivalent EDTA. Both EDTA (500−2000 μM) and phytate (500−1000 μM) caused a small decrease in the ζ-potential of the SDS emulsion, suggesting that they were capable of removing endogenous cations associated with the droplet surface. The ability of high concentrations of phytate (2000 μM) to increase the ζ-potential of droplets stabilized by SDS is likely due to changes in ionic strength resulting from the 6 times higher sodium levels in the phytate than in the EDTA.

Table 4. ζ-Potential of SDS-, Brij-, and DTAB-Stabilized Hexadecane Emulsions in the Presence of Ferrous Chloride ± Chelators at pH 3.0[a]

	ζ-potential (mV)		
treatment	SDS	Brij	DTAB
control	−105.0 ± 1.0	−2.9 ± 0.1	75.0 ± 0.9
Fe^{2+} (500 μM)	−94.0 ± 0.6	−2.6 ± 0.2	73.2 ± 0.5
EDTA (500 μM)	−106.1 ± 0.6	nd[b]	nd
EDTA (1000 μM)	−106.3 ± 0.5	−3.1 ± 0.2	70.0 ± 1.0
EDTA (2000 μM)	−106.9 ± 0.6	nd	nd
EDTA (50 μM) + Fe^{2+} (500 μM)	−94.1 ± 0.5	nd	nd
EDTA (200 μM) + Fe^{2+} (500 μM)	−94.1 ± 0.5	nd	nd
EDTA (500 μM) + Fe^{2+} (500 μM)	−105.5 ± 0.6	−3.2 ± 0.2	71.2 ± 0.4
EDTA (1000 μM) + Fe^{2+} (500 μM)	−106.9 ± 0.4	nd	nd
EDTA (2000 μM) + Fe^{2+} (500 μM)	−106.9 ± 0.3	nd	nd
phytate (500 μM)	−106.1 ± 0.4	nd	nd
phytate (1000 μM)	−106.8 ± 0.5	−3.2 ± 0.2	creaming[c]
phytate (2000 μM)	−99.5 ± 0.8	nd	nd
phytate (50 μM) + Fe^{2+} (500 μM)	−95.8 ± 0.6	nd	nd
phytate (200 μM) + Fe^{2+} (500 μM)	−95.4 ± 0.2	nd	nd
phytate (500 μM) + Fe^{2+} (500 μM)	−96.3 ± 0.4	nd	nd
phytate (1000 μM) + Fe^{2+} (500 μM)	−99.9 ± 0.9	−3.4 ± 0.0	creaming
phytate (2000 μM) + Fe^{2+} (500 μM)	−96.7 ± 0.7	nd	nd

[a] Data represent means ± standard deviations. [b] nd, not determined. [c] Creaming = destabilization of emulsion by creaming.

At concentrations of 50 and 200 μM, EDTA had little effect on the ability of Fe^{2+} (500 μM) to associate with the SDS emulsion droplets (Table 4). At 500 μM EDTA, 97% of the iron was removed from the SDS droplets when the ζ-potential was compared to that of EDTA alone. At higher EDTA concentrations (≥1000 μM), ζ-potentials were lower than those of no iron controls and equal to those of samples containing only EDTA, suggesting that high concentrations of EDTA were capable of removing both added and endogenous iron and other cations from the droplet surface. Phytic acid was also capable of removing Fe^{2+} from the SDS emulsion droplets. Phytic acid had more influence on Fe^{2+}−SDS emulsion interactions than EDTA at low concentrations (50 and 200 μM) but was less effective at higher concentrations. The greatest reduction in iron binding by phytic acid was 46% when phytate concentration was 1000 μM. High phytate concentrations (2000 μM) decreased changes in ζ-potential in the presence of iron (i.e., the droplets became less negative) in a manner similar to that shown by phytate alone. EDTA and phytate removal of iron from the SDS emulsion droplets was not influenced by the order of addition (e.g., EDTA and then Fe^{2+} or Fe^{2+} and then EDTA; data not shown).

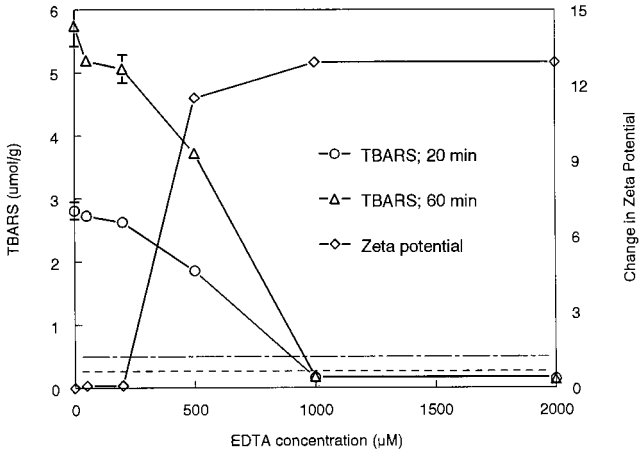

Figure 3. Ability of EDTA to alter Fe^{2+}-induced changes in ζ-potential and Fe^{2+}-accelerated lipid oxidation (as measured by TBARS) in SDS-stabilized emulsions at pH 3. Change in ζ-potential was calculated as ζ-potential of SDS emulsion with Fe^{2+} − ζ-potential of SDS emulsion with Fe^{2+} and EDTA. Lines represent TBARS of control (no added Fe^{2+}) at 20 (- - -) and 60 (— - —) min.

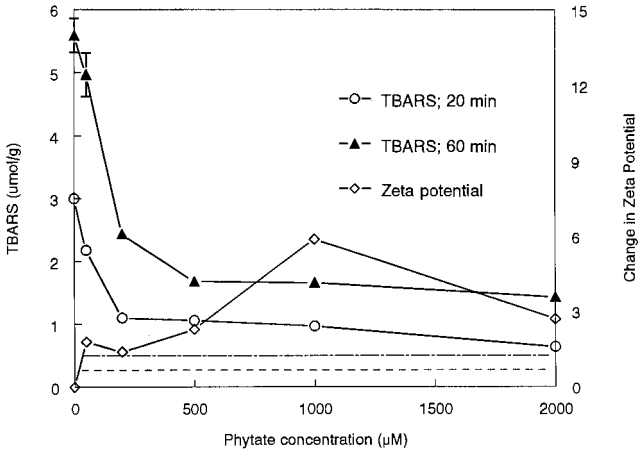

Figure 4. Ability of phytate to alter Fe^{2+}-induced changes in ζ-potential and Fe^{2+}-accelerated lipid oxidation (as measured by TBARS) in SDS-stabilized emulsions at pH 3. Change in ζ-potential was calculated as ζ-potential of SDS emulsion with Fe^{2+} − ζ-potential of SDS emulsion with Fe^{2+} and phytate. Lines represent TBARS of control (no added Fe^{2+}) at 20 (- - -) and 60 (— - —) min.

EDTA (50−2000 mM) decreased Fe^{2+} (500 μM) accelerated TBARS formation in the SDS−salmon oil emulsion in a dose-dependent manner (Figure 3). At an EDTA concentration of 500 μM, iron-induced changes in ζ-potential were decreased 97% compared to EDTA alone, whereas lipid oxidation was decreased only 35% after 60 min of incubation. Higher EDTA concentrations (1000 and 2000 μM) resulted in no detectable association of iron with the SDS emulsion droplets and decreased TBARS concentrations to levels lower than the no added iron controls. When lipid oxidation was measured by headspace propanal, 500 μM EDTA inhibited oxidation 31% compared to 77% for 1000 μM EDTA after 60 min of oxidation (Table 3). The inability of EDTA to strongly inhibit lipid oxidation at concentrations which removed >97% of the iron from the emulsion droplet surface suggests that only small amounts of iron are needed at the droplet surface to promote oxidation. The large decrease in lipid oxidation observed at EDTA concentrations ≥1000 mM could be due to a combination of complete iron removal from the droplet surface and more effective control of iron activity if iron not associated with the droplet surface is actively promoting oxidation in this model. At EDTA concentrations ≥1000 mM, formation of TBARS was less than that in no-Fe^{2+} controls, suggesting that transition metals were active prooxidants in the control samples. These transition metals could originate from numerous sources including water, oil, surfactants, and buffers.

Phytate (50−1000 μM) reduced Fe^{2+}-induced lipid oxidation from 11 to 74% (Figure 4). At low concentrations (50−500 μM), phytate inhibited oxidation more effectively than EDTA, possibly because phytate effectively decreases iron's catalytic activity at phytate-to-Fe ratios of 0.25 (Graf and Eaton, 1990) compared to a 1:1 ratio for EDTA. Higher concentrations of phytate (1000 and 2000 μM) were less effective at inhibiting oxidation than EDTA, which could be due to phytate's inability to completely remove iron from the emulsion surface.

Effect of NaCl on Iron−Droplet Interaction and Lipid Oxidation. Addition of NaCl up to 3.0 mM had no effect on ζ-potential of SDS, DTAB, and Brij emulsion droplets (Table 5). The inability of NaCl at concentrations <3.0 mM to influence the net charge of the SDS and DTAB emulsion droplets suggests that the droplet interfaces were already saturated with counterions such as Na^+ and Cl^- originating from the surfactants, buffer, and water. At NaCl concentrations ≥17 mM, the ζ-potential of the SDS emulsion became less negitive. This is predictable because as the ionic strength increases, the counterions are more tightly compacted near the droplets, which results in a greater shielding of the surface charge (Hunter, 1993). Changes in ζ-potential of the SDS emulsion caused by 500 μM Fe^{2+} were decreased by NaCl (17−170 mM) when ζ-potentials of NaCl alone were compared to those of NaCl + Fe^{2+} (Table 4). The ability of NaCl to decrease Fe^{2+} association with SDS emulsion droplets could be due to the far in excess amounts of Na^+, which would compete for surface binding sites and make it difficult for Fe^{2+} to displace Na^+. Additionally, the great excess of Cl^- could also cause formation of $[FeCl_4]^{-2}$ (Chipper-

Table 5. ζ-Potential of SDS-, Brij-, and DTAB-Stabilized Hexadecane Emulsions in the Presence of Ferrous Chloride ± NaCl at pH 3.0[a]

	ζ-potential (mV)		
treatment	SDS	Brij	DTAB
control	−105.0 ± 1.0	−2.9 ± 0.1	75.0 ± 0.9
Fe^{2+} (500 μM)	−93.5 ± 0.4	−2.6 ± 0.2	73.2 ± 0.5
NaCl (0.05 mM)	−104.7 ± 0.5	nd[b]	nd
NaCl (0.1 mM)	−105.9 ± 0.2	nd	nd
NaCl (0.3 mM)	−105.9 ± 0.2	nd	nd
NaCl (0.5 mM)	−105.0 ± 0.4	nd	nd
NaCl (1.0 mM)	−106.0 ± 0.2	nd	nd
NaCl (3.0 mM)	−106.9 ± 0.1	−3.0 ± 0.0	72.5 ± 0.6
NaCl (3.0 mM) + Fe^{2+} (500 μM)	−95.8 ± 0.1	−2.7 ± 0.2	72.6 ± 0.3
NaCl (17.0 mM)	−98.7 ± 1.6	nd	nd
NaCl (17.0 mM) + Fe^{2+} (500 μM)	−91.5 ± 0.6	nd	nd
NaCl (87.0 mM)	−94.8 ± 2.3	nd	nd
NaCl (87.0 mM) + Fe^{2+} (500 μM)	−91.4 ± 3.0	nd	nd
NaCl (170.0 mM)	−79.5 ± 2.3	nd	nd
NaCl (170.0 mM) + Fe^{2+} (500 μM)	−74.4 ± 2.6	nd	nd

[a] Data represent means ± standard deviations. [b] nd, not determined.

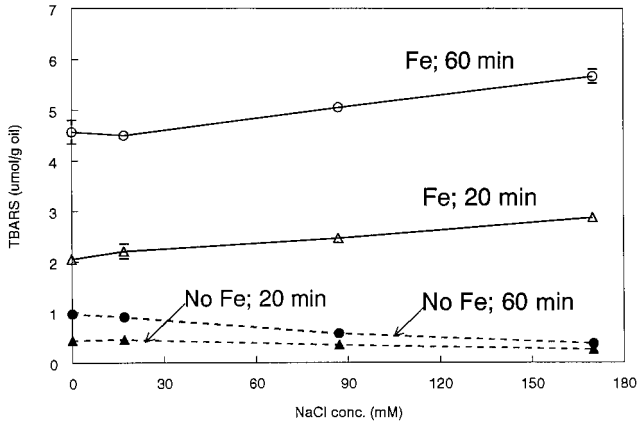

Figure 5. Fe^{2+}-accelerated formation of TBARS in SDS-stabilized salmon oil emulsion at pH 3.0 in the presence of various concentrations of NaCl.

field, 1993), which would be less likely to associate with the negatively charged SDS droplets.

NaCl (17 mM) had no effect on Fe^{2+} (500 μM) catalyzed TBARS formation in the SDS−salmon oil emulsion at pH 3.0 (Figure 5). However, higher NaCl concentrations (87 and 170 mM) increased lipid oxidation in emulsions containing added Fe^{2+}. NaCl stimulation of oxidation occurred even though these same concentrations of NaCl caused displacement of iron from the emulsion droplet surface. NaCl stimulation of oxidation could be due to the ability of chloride ions to increase the catalytic activity of iron (Osinchak et al., 1992) or to NaCl-induced changes in the physical properties of the emulsion droplets such as reduction in the thickness of the double layer (Hunter, 1993; Winkle, 1997).

When Fe^{2+} was not added to the SDS−salmon oil emulsion, NaCl was observed to inhibit lipid oxidation. As seen by the observation that EDTA inhibited autoxidation in the salmon oil emulsion (Figure 3), it is likely that low concentrations of contaminating metals accelerate oxidation in the non-Fe^{2+}-added samples. It is possible that when iron concentrations are low, NaCl can more effectively remove iron from the emulsion surface, thus resulting in an inhibition of lipid oxidation. This observation is supported by our previous work (Mei et al., 1998) in which oxidation of SDS-emulsified corn oil was catalyzed by Fe^{3+} (50 μM) and ascorbic acid (150 μM) at pH 6.5. In that system, where iron was added at a concentration 10 times lower than in this study, NaCl was observed to inhibit oxidation. Osinchak et al. (1992) also found that NaCl inhibited oxidation of phosphatidylcholine liposomes accelerated by low iron concentrations (15 μM) and ascorbate. These observations suggests that NaCl can be both antioxidative and prooxidative depending on iron concentrations.

Conclusions. Both Fe^{2+} and Fe^{3+} can associate with negatively charged SDS emulsion droplets but not with nonionic Brij- and cationic DTAB-stabilized emulsions as determined by ζ-potential. The degree of iron association with SDS-stabilized emulsion droplets is increased by decreasing pH and decreased by chelators and NaCl. Oxidation of an SDS-stabilized salmon oil emulsion by Fe^{2+} increased with increasing iron concentrations and decreased in the presence of chelators. The combination of ζ-potential and lipid oxidation data suggests that iron associated with the emulsion droplets is an active prooxidant and that only small amounts of emulsion droplet associated iron are needed to accelerate oxidation. Chelators were able to slow oxidation rates at all concentrations. However, the most effective inhibition of lipid oxidation was observed at chelator concentrations that completely removed iron from the droplet surface (EDTA/Fe^{2+} $\geq$ 2:1), suggesting that ζ-potential is an effecive method of determining the potential of chelators to inhibit oxidation.

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