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Development of an Iron Chelating Polyethylene Film for Active Packaging Applications

Fang Tian, Eric A. Decker, and Julie M. Goddard*

Department of Food Science, University of Massachusetts, 102 Holdsworth Way, Amherst, Massachusetts 01003, United States

ABSTRACT: Metal-promoted oxidation reactions are a major cause of food quality deterioration. Active packaging offers novel approaches to controlling such oxidation for the purpose of extending shelf life. Herein, we report modification of the surface of polyethylene (PE) films to possess metal chelating activity. Metal chelating carboxylic acids were introduced to the film surface using cross-linking agents [polyethylenimine (PEI) or ethylenediamine (ED)] to increase the number of available carboxylic acids. ATR-FTIR, contact angle, dye assay, and iron chelating assay were used to characterize changes in surface chemistry after each functionalization step. The chelator poly(acrylic acid) (PAA) was attached to the surface at a density of 9.12 ± 0.71 nmol carboxyl groups/cm^2, and exhibited an iron chelating activity. The results indicate that PAA-modified PE films might have a higher affinity to Fe^{3+} than Fe^{2+} with the optimum binding pH at 5.0. Such inexpensive active packaging materials are promising in food industry for the preservation of liquid and semiliquid food products and have application in heavy metal chelation therapy for biomedical materials as well.

KEYWORDS: *antioxidant, active packaging, iron chelating, polyethylene, covalent immobilization, poly(acrylic acid)*

INTRODUCTION

Oxidation, especially lipid oxidation, is one of the major causes of quality deterioration in the food industry. Oxidative deterioration is an economic concern because it leads to many food quality problems such as off-flavors, off-odors, color changes, and nutritional losses, thereby causing significant reductions in shelf life.[1] Antioxidant additives have been added into food product formulations to inhibit oxidative reactions. However, there have been questions raised regarding the safety of synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), *tert*-butylhydroquinone (TBHQ), and propyl gallate (PG).[2] Reports have suggested that synthetic antioxidants may have toxigenic, mutagenic, and carcinogenic effects.[3,4] Consumers are therefore increasingly demanding the use of natural antioxidants instead of their synthetic counterparts.[5,6] However, natural antioxidants often need to be added in larger amounts than synthetic antioxidants to achieve the same antioxidant activity, and their safety limits are mostly not known.[7] There is therefore a growing interest in development of active packaging technologies to provide alternative methods of food protection against oxidation without direct addition of food additives.

Active packaging is a class of packaging which interacts directly with the product and/or its surroundings to improve one or more aspects of the quality or safety properties of the product.[8] A number of techniques have been explored to combine active compounds into packaging systems and then impart them with antioxidant properties. The most commonly applied strategies are sachets,[9] adhesive-bonded labels,[5] and noncovalent adsorption/blending into or onto the packaging substrates.[10,11] New immobilization techniques have recently been developed to covalently bind antioxidant or other bioactive compounds to polymers or polymer films.[8,12−15] The covalent immobilization approach provides the most stable bond between the active compound and the polymer surface,[16] enabling the active packaging material to impart antioxidant activity without the direct dissolution of antioxidants into foods, a potential regulatory advantage of not requiring approval as a food additive.[17] There are four major categories of antioxidants, classified by their mechanisms of action as free radical scavengers (such as BHA, BHT, and catechins), oxygen scavengers and reducing agents (such as ascorbic acid and sulfites), singlet oxygen quenchers (such as tocopherols and carotenoids), and chelators (such as citric acid and EDTA).[1] Antioxidants, such as caffeic acid,[8] natural rosemary extracts,[5] ascorbic acid, α-tocopherol, BHA, and BHT,[18] have been incorporated into different packaging materials; however, there are limited reports of using chelators as the active agent in antioxidant active packaging materials. Transition metals (e.g., iron) are an important prooxidative factor in many foods such as oil-in-water emulsions. Chelators can bind pro-oxidant metals and inactivate or reduce their activity thus inhibiting lipid oxidation.[1] Synthetic chelators are effective; however, consumers are increasingly demanding all-natural labels. A major limitation of natural chelators is that some of them can increase oxidative reactions by increasing metal solubility and thus metal−lipid interaction.[19] If metal ions could be sequestered from the food by the packaging material, they would be unavailable to interact with lipids and other food components which are susceptible to metal ion promoted degradation. Despite the fact that chelators (e.g., EDTA) are extremely effective preservatives used by the food industry, little research has been done to investigate use of metal chelators in an antioxidant packaging system. Thus, covalently binding strong metal chelators to the food contact surface of packaging

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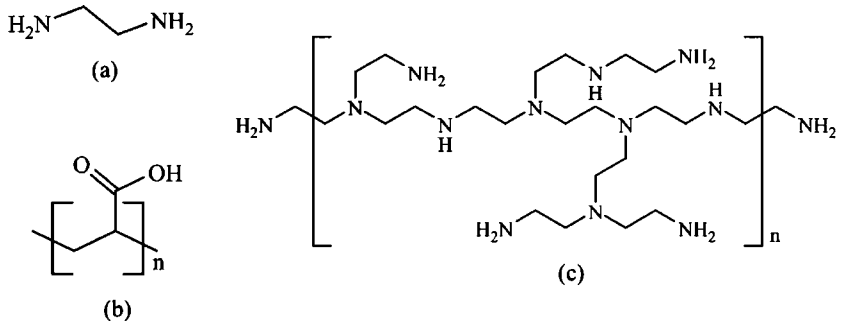

Figure 1. Chemical structure of amine compounds and iron chelator used in this study: (a) ethylene diamine (ED); (b) poly(acrylic acid) (PAA); (c) polyethylenimine (PEI).

films represents a novel, promising approach to develop antioxidant packaging materials in which the active agent does not migrate to the food. Development of active packaging which enables the elimination of additives like EDTA from product formulations would have significant industry impact as consumers are increasingly demanding all natural, additive free foods.

The objective of this research was to develop a novel antioxidant packaging material by covalently attaching iron chelators to the surface of polyethylene (PE) films. As a covalent linkage may offer the regulatory advantage of not requiring approval as a food additive,[17] poly(acrylic acid) (PAA) (a strong metal chelator used in water purification) was chosen as the iron chelator to impart antioxidant capacity to the novel packaging material. Amine compounds [polyethylenimine (PEI) and ethylenediamine (ED)] with different molecular weights were chosen as the cross-linking agents to covalently bind PAA to the PE surface. The chemical structures of PAA, PEI, and ED used in this research are shown in Figure 1. Attenuated total reflectance/Fourier transform infrared spectroscopy (ATR-FTIR), contact angle, dye assay, and iron chelating assay were used to characterize changes in film surface chemistry after each step in the process of functionalization. The effect of the molecular weight of cross-linking agents on the covalent attachment of PAA to PE surface was investigated. The effect of pH on the iron chelating activity of PAA-modified PE was also determined.

MATERIALS AND METHODS

Materials. Low-density polyethylene (PE, pellets) and poly(acrylic acid) (PAA, M_w = 450 000) were purchased from Scientific Polymer Products (Ontario, NY); 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) was purchased from ProteoChem (Denver, CO); *N*-hydroxysuccinimide (NHS), hydroxylamine hydrochloride, ferrous sulfate heptahydrate (99+%), imidazole (99%), and 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-*p*,*p*′-disulfonic acid disodium salt hydrate (ferrozine, 98+%) were purchased from Acros Organics (Morris Plains, NJ); polyethylenimine (PEI, branched, M_w = 25 000, M_n = 10 000) was purchased from Sigma-Aldrich (St. Louis, MO); ethylenediamine (ED, 70%) was purchased from Ricca Chemical Company (Arlington, TX); toluidine blue O (TBO) was purchased from MP Biomedicals (Solon, OH); isopropanol, acetone, sodium carbonate, sodium bicarbonate, sodium acetate trihydrate, 4-(2-hyddroxyethyl)-1-piperazineethane-sulfonic acid (HEPES), hydrochloric acid, trichloroacetic acid (TCA), acetic acid glacial, ferric chloride anhydrous, and sodium hydroxide were purchased from Fisher Scientific (Fair Lawn, NJ); MES sodium salt was purchased from GenScript (Piscataway, NJ).

Preparation and Pretreatment of PE Films. PE films with the average thickness of 250 ± 50 μm were prepared with a Carver Laboratory Press (Model B, Fred S. Carver Inc., NJ). PE pellets were cleaned by sonicating in isopropyl alcohol, acetone, and deionized water (10 min per repetition, 2 repetitions per solvent) sequentially. The cleaned PE pellets were dried overnight at room temperature over anhydrous calcium sulfate. The temperature of the Carver Press was set to 130 °C to melt PE pellets for 1 min, and 9000 lbs pressure was then loaded to press pellets into films. Large pieces of films were cut to 1 × 2 cm^2 pieces and cleaned and dried by the same procedure as the PE pellets. Cleaned films were then treated for 15 min per side in an ultraviolet/O_3 cleaner (UV/O_3, Model 42, Jelight Company, Inc., Irvine, CA) to create active carboxylic acid groups on the surface of both sides.

Surface Functionalization of PE. A two-step chemical reaction was used to covalently immobilize the metal chelator PAA to the PE film surface (Figure 2). Cross-linking agent (PEI or ED) was first attached to the film surface through the formation of amide bonds between amine groups from the cross-linking agent and the carboxylic acid groups from the UV pretreated PE surface. PAA was then attached to the amine-modified PE surface also through the amide bonds to increase the number of available metal chelating carboxylic acids. PEI or ED was covalently attached to the UV treated film using a modification of the method of Goddard et al.[15] The conjugation solution was composed of 30 mg/mL PEI (or ED), 50 mM EDC, and

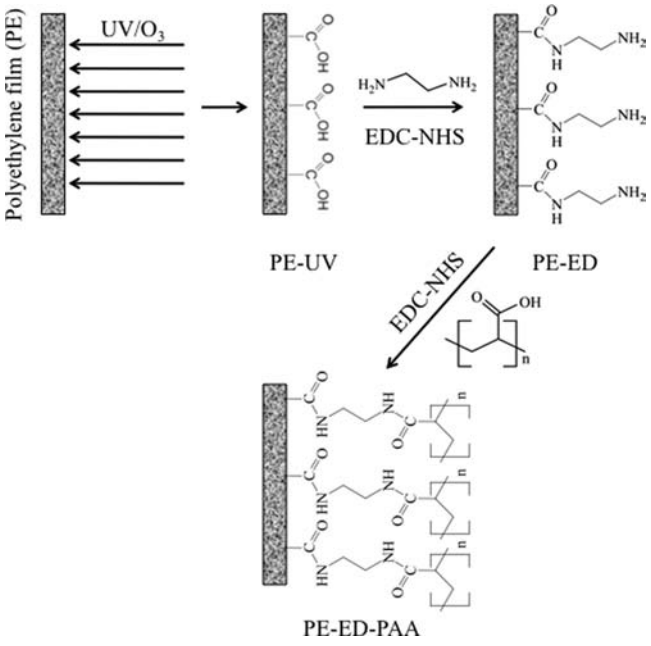

Figure 2. Representative sequential reaction steps for poly(acrylic acid) covalent immobilization on polyethylene films using ethylenediamine as the cross-linking agent.

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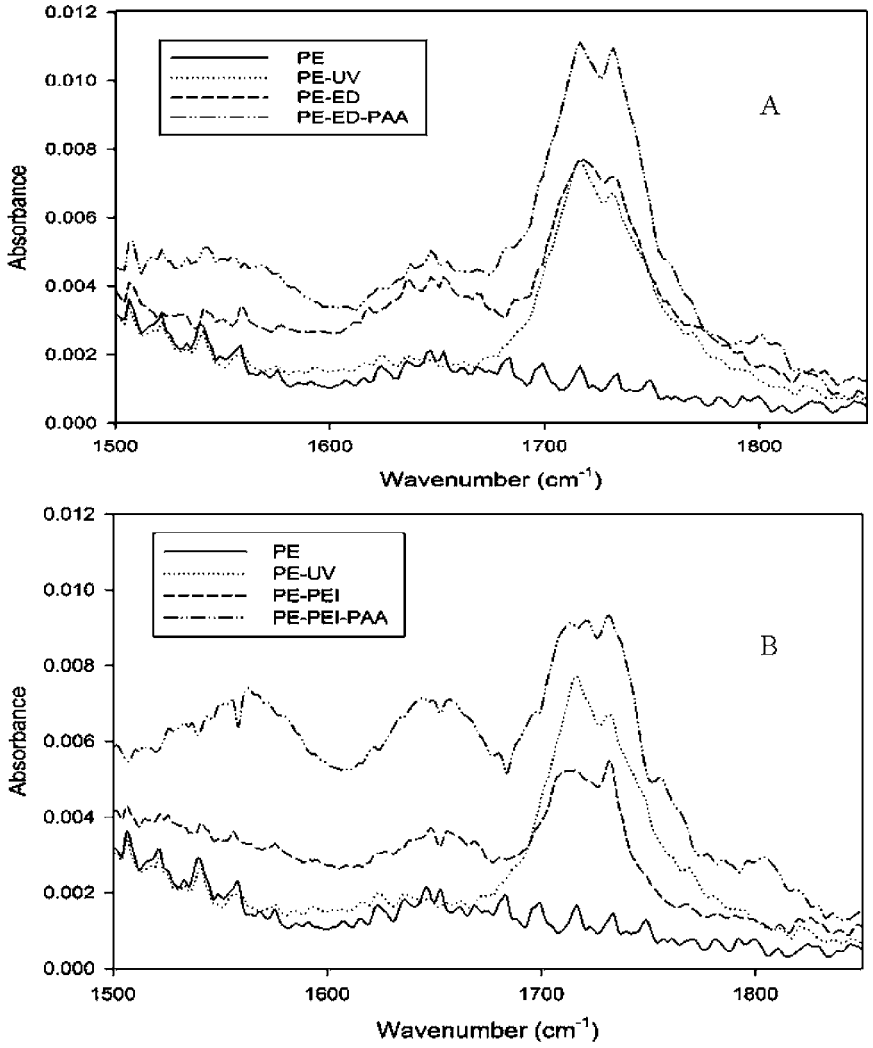

Figure 3. ATR-FTIR spectra of PE and modified PE films from 1500 to 1850 cm^{-1}: (A) the spectra of PE, PE-UV, and PAA modified PE using ED as the cross-linking agent; (B) the spectra of PE, PE-UV, and PAA modified PE using PEI as the cross-linking agent. Spectra shown here are representative of four replications collected from two independent films per treatment.

5 mM NHS in 0.1 M pH 9.6 sodium carbonate buffer. Films were shaken in the conjugation solution for 1 h at 25 °C, followed by rinsing in copious purified water 3 times. Surface amine groups were modified with PAA to generate chelating carboxylic acid groups through the formation of amide bonds.[16] The conjugation solution was composed of 1 mg/mL PAA, 50 mM EDC, and 5 mM NHS in 0.1 M pH 6.5 MES buffer. Films were shaken in the conjugation solution for 1 h at 25 °C, followed by rinsing in copious purified water 3 times.

ATR-FTIR Analysis. Changes in surface chemistry before and after the modification were determined using ATR-FTIR spectroscopy, which was conducted on an IRPrestige-21 FTIR spectrometer (Shimadzu Scientific Instruments, Inc., Kyoto, Japan) using a diamond ATR crystal. Each spectrum represents 32 scans at a 4 cm^{-1} resolution taken against a reference spectrum of an empty ATR crystal. The resultant spectra were processed with SigmaPlot 10.0 (Systat Software, Inc., Chicago, IL) and analyzed with KnowItAll Informatics System 8.1 (BioRad, Hercules, CA).

Contact Angle Analysis. The hydrophilicity of polymer surfaces was also measured after each step in the modification. To measure surface hydrophilicity, the static sessile drop method was used to measure the water contact angles of PE and modified PE films (n = 6, two measurements on each of three separate films). Measurements were conducted on a DSA 100 (Kruss, Hamburg, Germany) with HPLC grade deionized water.

TBO Dye Assay. TBO dye complexes with available carboxylic acids under basic conditions, and can be desorbed in acidic solution to be quantified spectrophotometrically. The carboxylic acid density on PE and modified PE surface was determined using the methods reported by Kang et al.[20] and Uchida et al.[21] with some modifications. Control and modified PE films were shaken for 2 h in 0.5 mM TBO solution (in deionized water adjusted to pH 10.0 by NaOH) at 25 °C. The films were then rinsed with NaOH solution (pH 10.0) 3 times to remove noncomplexed dye. Complexed dye on the film surface was desorbed by submerging films in 50 wt % acetic acid for 15 min. The absorbance of the acetic acid solution was then detected at 633 nm and compared to a standard curve made of TBO in 50 wt % acetic acid. The determination of each sample was quadruplicate.

Iron Chelating Assay. Ferrozine is a specific reagent that forms a red complex (absorption maximum at 562 nm) with ferrous ions.[22] The ability of PE and modified PE films to bind Fe^{2+} or Fe^{3+} iron was determined using the method reported by Bou et al.[23] with some modifications. A solution of ferrous sulfate heptahydrate (20 mM, in 0.05 M HCl) was added into 0.05 M sodium acetate/imidazole buffer (pH 3.0, 5.0, and 7.0) to make the iron buffer mixture with the final Fe^{2+} concentration of 1 mM (0.01 mM for ferric chloride). Four pieces of PE and the modified PE films (PE-UV, PE-ED, PE-ED-PAA, PE-PEI, PE-PEI-PAA; two-side treated) were put into a centrifuge tube with 20 mL of iron buffer solution and rotated for 30 min. The films were then taken out, and rinsed in deionized water 3 times to wash off

the unbound iron. Films were then submerged in hydroxylamine hydrochloride (0.72 M) and TCA (0.61 M) to both release iron from films, and also to reduce Fe^{3+} to Fe^{2+} iron. After 2.5 h rotation, the solution was reacted with ferrozine solution (9.0 mM in 50 mM HEPES buffer, pH 7.0) with the ratio 1:1 (v/v) at room temperature (25 °C) for 1 h. The absorbance of the solution was then detected at 562 nm and normalized to a hydroxylamine hydrochloride, TCA, ferrozine solution negative control. The results were compared to a ferrous iron calibration curve, and the determination of each sample was in quadruplicate.

Statistical Analysis. The data presented are means ± standard deviation (SD) of four determinations. Statistical analyses were conducted using SPSS Release 17.0 (SPSS Inc., Chicago, IL). One-way analysis of variance (ANOVA) followed by Duncan's pairwise comparison was conducted to determine differences ($P < 0.05$).

RESULTS AND DISCUSSION

ATR-FTIR Analysis of Surface Chemistry. The ATR-FTIR spectra of PE and modified PE (PE-UV, PE-PEI, PE-ED, PE-PEI-PAA, and PE-ED-PAA) are shown in Figure 3. The spectra can be separated into three regions corresponding to the following: (1) the intense C═O band of carboxylic acid groups at 1725−1700 cm^{-1}; (2) the C═O band of amide groups at 1680−1630 cm^{-1}; (3) the C—N and N—H combination band of amide groups at 1570−1515 cm^{-1}.

The PE surface did not have significant absorbance in the range 1500−1850 cm^{-1} (Figure 3A,B), representing a clean native PE film. After the functionalization by UV/O_3 treatment, the absorbance band at 1725−1700 cm^{-1} was observed in the spectra of PE-UV surface (Figure 3A,B) due to the creation of carboxylic acid groups. After the grafting of cross-linking agent ED or PEI, amide groups were formed between amine and carboxylic acid groups, and absorbance at 1680−1630 cm^{-1} and 1570−1515 cm^{-1} was observed in the ATR-FTIR spectra of PE-ED (Figure 3A) and PE-PEI (Figure 3B). The spectra of PE-PEI also showed a decrease in the absorbance at 1725−1700 cm^{-1} compared to that of PE-UV and PE-ED. This may be due to the fact that PEI contains many more amine groups than ED, and more carboxylic acid groups were consumed on the surface of PE-UV to react with PEI. After the grafting of PAA, a significant increase of the absorbance at 1725−1700 cm^{-1} was evident in the spectra of both PE-ED-PAA (Figure 3A) and PE-PEI-PAA (Figure 3B) compared to PE-ED and PE-PEI. This indicates that PAA was successfully immobilized to the surface of PE-ED and PE-PEI. The spectra also showed that many more amide groups were formed on the surface of PE-PEI-PAA than that of PE-ED-PAA, further confirming successful modification of the material.

Surface Hydrophilicity. The surface hydrophilicity of PE and modified PE films was evaluated by their water contact angles using the static sessile drop method (Figure 4). It was noted that the UV/O_3 functionalization brought the contact angle from 102.40 ± 2.41° of PE films down to 72.61 ± 1.08° of PE-UV films indicating that the hydrophilicity was significantly increased ($P < 0.05$). This is likely a result of the introduction of a range of polar groups, including ionizable carboxylic acids, after UV/O_3 treatment. In comparison to UV/O_3 functionalized films, the contact angle was not changed after the grafting of ED but increased after the grafting of PEI. One possible reason would be the higher hydrophilicity of carboxylic acids, present as a result of UV/O_3 treatment, than that of PEI.[24] This observation is supported by work by Coupe et al.[25] which demonstrated that carboxylic acids may contribute more to wettability than primary amines. Since ED is a small

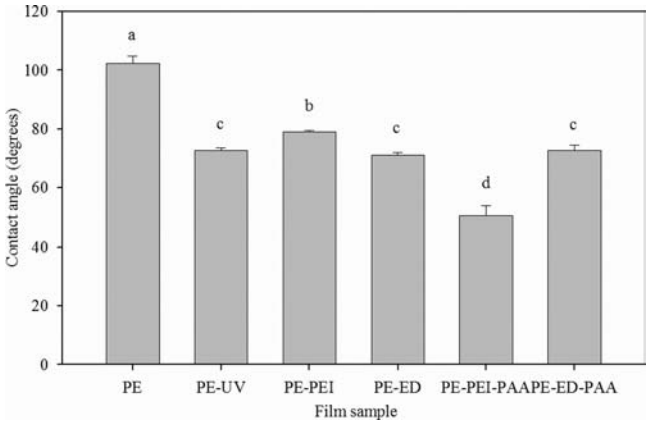

Figure 4. Water contact angle of PE and modified PE films. Values are means of six replications collected from three independent films (n = 6, ±SD). Different letters indicate significant differences ($P < 0.05$).

molecule, most of the carboxylic acids present on the surface of the UV/O_3 functionalized polyethylene would be still exposed after ED conjugation, explaining the lower contact angle of PE-ED than PE-PEI. This conclusion is also supported by the ATR-FTIR spectra of PE-UV and PE-ED which both show strong absorbances associated with carboxylic acids (Figure 3A). After the grafting of the high molecular weight branched polymer PEI, more carboxylic acids were involved in the conjugation reaction and covalently attached to PEI, which could be confirmed by the ATR-FTIR spectra of PE-UV and PE-PEI (Figure 3B), and the increase in hydrophobicity of the PE-PEI film compared to both PE-UV and PE-ED is likely a result of the relatively more hydrophobic PEI polymer layer. The grafting of PAA decreased the contact angle of PE-PEI from 78.92 ± 0.56° to 50.41 ± 3.47°, which might be attributed to the formation of the hydrophilic matrix network between cationic polyelectrolyte PEI and anionic polyelectrolyte PAA. Notably, PE-PEI-PAA films exhibited a more hydrophilic surface than both PE-PEI and PE-ED-PAA films. This somewhat counterintuitive observation may be due to a synergistic effect between the two layers of polyelectrolytes, which when deposited in conjunction may have a stronger effect on hydrophilicity than when deposited individually. Indeed, Coupe et al.[25] reported that sequential deposition of two functional polymers (PEI and PAA) has a synergistic effect on increasing the hydrophilicity of a hydrophobic substrate compared to the deposition of each individual polyelectrolyte (PEI or PAA).

Carboxylic Acid Density. The density of available carboxylic acid groups on the surface of PE and modified PE films was measured using the TBO dye assay, and the results were shown in Figure 5. After the functionalization with UV/O_3 treatment, a small amount (1.19 ± 0.12 nmol/cm^2) of available carboxylic acid groups were created on PE surface, which can be used to conjugate with cross-linking agents. After the conjugation of PEI, the available carboxylic acid groups on the PE surface significantly decreased ($P < 0.05$). The loss of the available carboxylic acids was not significant ($P > 0.05$) after the conjugation of ED. These were consistent with the results shown in the ATR-FTIR spectra. The significant increase of available carboxylic acid density on the surface of PE-PEI-PAA (5.86 ± 0.47 nmol/cm^2) and PE-ED-PAA (9.12 ± 0.71 nmol/cm^2) confirmed the successful immobilization of PAA. In addition, the available carboxylic acid density on the PE-ED-

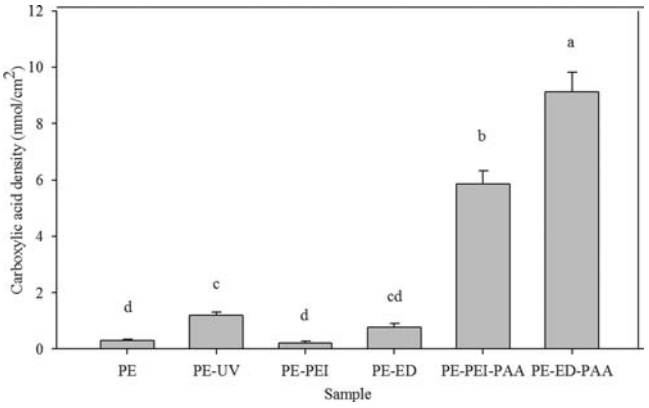

Figure 5. Carboxylic acid density on the surface of PE and modified PE films. Values are means of four independent films (n = 4, ±SD). Different letters indicate significant differences (P < 0.05).

PAA surface was higher than that on the PE-PEI-PAA surface (P < 0.05). It is possible that the high number of primary amines present on PEI reacted with a larger number of carboxylic acids on PAA to form amide bonds, resulting in fewer available carboxylic acids in PE-PEI-PAA films compared PE-ED-PAA films.

Iron Chelating Activity. The two most common forms of iron are ferrous (Fe^{2+}) and ferric (Fe^{3+}). Ferric is the oxidized form of ferrous. In terms of lipid oxidation, ferrous is the stronger pro-oxidant of the two due to its higher water solubility and higher reactivity in decomposing lipid hydroperoxides into free radicals. Ferric is less reactive, but it can be reduced to ferrous to promote lipid oxidation. Both ferrous and ferric iron chelating activities of PE and modified PE films were measured by an iron chelating assay at pH 5.0, and the results were shown in Figures 6 and 7.

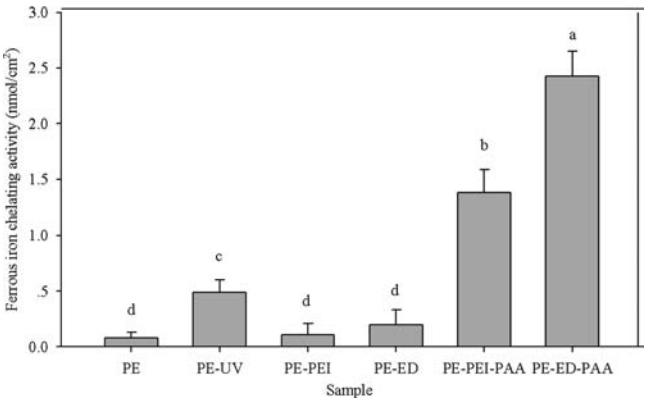

Figure 6. Ferrous iron chelating activity of PE and modified PE films. Values are means of four independent films (n = 4, ±SD). Different letters indicate significant differences (P < 0.05).

The results in Figure 6 indicate that PE-UV, PE-PEI-PAA, and PE-ED-PAA all had ferrous iron chelating activity. Both PE-PEI-PAA and PE-ED-PAA had much stronger ferrous iron chelating activity than PE-UV. After being covalently immobilized to the PE surface, PAA could still participate in iron chelating. For the PAA-modified PE films, PE-ED-PAA showed stronger ferrous iron chelating activity than PE-PEI-PAA (P < 0.05). These findings were consistent with the results of dye assay. It was observed that the more available carboxylic

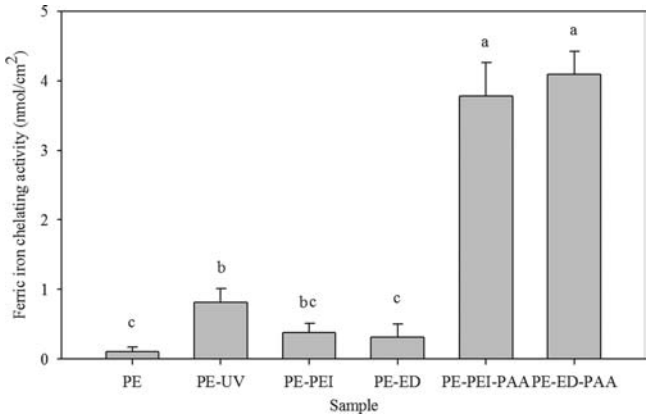

Figure 7. Ferric iron chelating activity of PE and modified PE films. Values are means of four independent films (n = 4, ±SD). Different letters indicate significant differences (P < 0.05).

acids on the surface, the stronger the ferrous iron chelating activity. In addition, according to the results of the dye assay and the ferrous iron chelating assay for PE-PEI-PAA and PE-ED-PAA, the ligand to metal ratio was about 4. This meant four carboxylic acid ligands were needed to chelate one ferrous ion to form the ligand–metal complex on the PAA-modified PE surface. According to theoretical chelation chemistry,[26] two parts of carboxylic acids bind with one part of divalent metal ion to form a $(COOH)_2$:Fe^{2+} complex. The increase of the ligand–metal ratio may due to the diffusion limitation of iron coming to react with ligands on film surfaces. The steric hindrance effect from the surrounding groups may also contribute to the loss of activity. Even though amide groups formed between cross-linking agents and PAA have oxygen and nitrogen atoms, which could donate a pair of electrons for the formation of a bond with ferrous iron, they may not have been involved in the complexation reaction. The research of Masuda et al.[27] indicated that the abilities of polymethacryloylglycine and poly(acrylic acid) to chelate divalent metal ions had no difference, and no additive effect of amide and carboxyl groups was found on the complexation.

The ferric iron chelating activities of PE and modified PE films are depicted in Figure 7. In comparison to the virgin PE, PE-UV, PE-PEI-PAA, and PE-ED-PAA also showed significant ferric iron chelating activity. The activity of PE-PEI-PAA and PE-ED-PAA was also stronger than PE-UV. However, the two kinds of PAA-modified PE films using different cross-linking agents presented similar ferric iron chelating activity (P > 0.05). Moreover, their ferric iron chelating activities were significantly stronger than their ferrous iron chelating activities (P < 0.05). The $(COOH)/Fe^{3+}$ ratio was 1.5 for PE-PEI-PAA and 2 for PE-ED-PAA. Ferric ions have six coordination sites, and ligands with six coordinating atoms surrounding them would provide three negative charges to balance the three positive charges of Fe^{3+} to form stable iron complexes.[26] The lower limit of coordinated carboxylic acids should be 3. However, ferric ion is ruled out in this study to be involved into the complexation with carboxylic acid groups by the ligand–metal ratio of 1.5 or 2. Presumably, this is either because of the formation of cationic polymer–metal complexes, or because of the precipitation of ferric hydroxide in the cross-linked polymer matrix on the surface. Blçak et al.[28] reported the free amine groups were capable of chelating with various transition metal ions such as Co^{2+} and Cu^{2+} to form cationic polymer–metal complexes. The

solubility of Fe^{3+} is very low in aqueous solution with high pH, and ferric hydroxide would be possibly generated to form precipitation. The pH 5.0 sodium acetate/imidazole buffer was used to do the ferric iron chelating assay, and at this pH Fe^{3+} is not expected to be fully soluble.

Effect of pH on the Chelation of Fe^{3+}. The chelation of iron by anionic polymeric ligands is ionic in nature and therefore highly pH dependent.[29] As expected our initial studies indicated that ferric ions complexed to a greater degree than ferrous ions (Figures 6 and 7). We therefore performed the pH optimum study using ferric ions in order to best observe the influence of pH on iron ion complexation by our films. The ferric iron chelating activity of PE-ED-PAA was measured with sodium acetate/imidazole buffer (0.05 M) at pH 3.0, 5.0, and 7.0. From the results shown in Figure 8, it can be seen that PE-ED-PAA had the highest ferric iron chelating activity at pH 5.0. The activity at pH 7.0 was slightly lower than that at pH 5.0. However, the unmodified PE film also had apparent ferric iron chelating activity at pH 7.0. Solubility of ferric ions decreases with increasing pH, so it is likely that the observed decrease in ferric ion concentration in the control (unmodified) PE films at pH 7.0 may be a result of precipitation, not chelation. The chelating activity at pH 3.0 was much weaker than at pH 5.0 ($P < 0.05$). The results indicated that the optimum pH for ferric iron complexation of PE-ED-PAA was 5.0. Sebastian et al.[29] reported that the optimum pH for the complexation of ferric iron by sodium salt of PAA was 5.4. Our results indicate that PAA immobilized on a PE surface has a similar pH optimum for ferric iron complexation as free PAA.

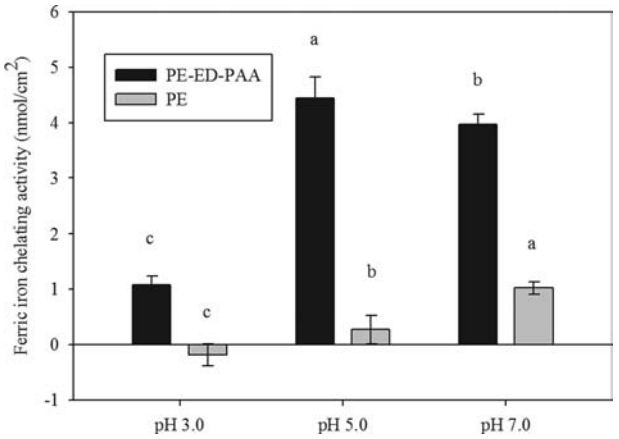

Figure 8. Ferric iron chelating activity of PE and PE-ED-PAA at different pH values. Values are means of four independent films ($n = 4$, ±SD). Different letters indicate significant differences for each series ($P < 0.05$).

In addition to the effects of oxygen, free radicals, and other factors, the presence of trace metal ions in foods can promote oxidative deterioration such as lipid oxidation. Metal chelators such as EDTA are commonly included in food product formulations to prevent metal ion promoted oxidative degradation reactions. A novel metal chelating active packaging was successfully developed by the covalent immobilization of PAA to a functionalized PE surface. As cross-linking agents, low molecular weight homobifunctional amine compound ED could introduce more available carboxylic acids to PE surface than the high molecular weight branched amine compound PEI. After covalent immobilization onto the PE surface, PAA still exhibited iron chelating capacity, and PE-ED-PAA exhibited an iron chelating activity of 2.43 ± 0.22 nmol ferrous iron per cm^2 and 4.44 ± 0.38 nmol ferric iron per cm^2, respectively. As the ligand−metal ratio for Fe^{3+} was lower than that for Fe^{2+}, PAA-modified PE films may have a higher affinity to Fe^{3+} with the optimum binding pH at 5.0. Our study demonstrates the potential for an alternative approach to prepare antioxidant active packaging films using an inexpensive polymeric metal chelator. Such films would be best suited for liquid food products which are not a nutritionally important source of iron. The application of this economical metal chelating active packaging should be very promising to control iron-catalyzed lipid oxidation.

On a macromolecular scale, polymer packaging films are thick (on the order of 100 μm); yet, the portion of the film that directly interacts with the food is just the top several nanometers. In this work, we have covalently immobilized ED-PAA and PEI-PAA onto UV activated polyethylene. While ellipsometry could not be used to quantify the thickness of the immobilized polymer due to similar refractive indices, prior work on silicon wafers has confirmed that the thickness of a deposited PEI-PAA multilayer is approximately 3 nm.[30] As such, the impact of the nanoscale surface modification on the bulk film properties (e.g., mechanical, thermal, or barrier properties) is expected to be minimal. Nevertheless, a thorough evaluation of final film bulk properties as well as a study on the impact of the active packaging film on the physicochemical properties of the packaged food would be necessary prior to commercial adoption of the developed film. The complex nature of food would likely impact the ability of the developed materials to chelate iron. Therefore, additional studies are ongoing to evaluate the impact of counterions and other food components on the ability of the developed materials to complex iron and to demonstrate the effect of iron chelation on prevention of oxidative degradation in a food matrix.

AUTHOR INFORMATION

Corresponding Author

*E-mail: goddard@foodsci.umass.edu. Phone: 413-545-2275. Fax: 413-545-1262.

Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Chaiyasit, W.; Elias, R. J.; McClements, D. J.; Decker, E. A. Role of physical structures in bulk oils on lipid oxidation. *Crit. Rev. Food Sci. Nutr.* **2007**, *47*, 299−317.

(2) Qader, S. W.; Abdulla, M. A.; Chua, L. S.; Najim, N.; Zain, M. M.; Hamdan, S. Antioxidant, total phenolic content and cytotoxicity evaluation of selected Malaysian plants. *Molecules* **2011**, *16*, 3433−3443.

(3) Vermeiren, L.; Devlieghere, F.; van Beest, M.; de Kruijf, N.; Debevere, J. Developments in the active packaging of foods. *Trends Food Sci. Technol.* **1999**, *10*, 77−86.
(4) Yu, R.; Mandlekar, S.; Kong, A. Molecular mechanisms of butylated hydroxylanisole-induced toxicity: Induction of apoptosis through direct release of cytochrome c. *Mol. Pharmacol.* **2000**, *58*, 431−437.
(5) Nerin, C.; Tovar, L.; Salafranca, J. Behaviour of a new antioxidant active film versus oxidizable model compounds. *J. Food Eng.* **2008**, *84*, 313−320.
(6) Nanditha, B.; Prabhasankar, P. Antioxidants in bakery products: A review. *Crit. Rev. Food Sci. Nutr.* **2008**, *49*, 1−27.
(7) Pokorny, J. Are natural antioxidants better and safer than synthetic antioxidants. *Eur. J. Lipid Sci. Technol.* **2007**, *109*, 629−642.
(8) Arrua, D.; Strumia, M.; Nazareno, M. Immobilization of caffeic acid on a polypropylene film: Synthesis and antioxidant properties. *J. Agric. Food Chem.* **2010**, *58*, 9228−9234.
(9) Ozdemir, M.; Floros, J. Active food packaging technologies. *Crit. Rev. Food Sci. Nutr.* **2004**, *44*, 185−193.
(10) Lee, C.; An, D.; Lee, S.; Park, H.; Lee, D. A coating for use as an antimicrobial and antioxidative packaging material incorporating nisin and alpha-tocopherol. *J. Food Eng.* **2004**, *62*, 323−329.
(11) Gucbilmez, C. M.; Yemenicioglu, A.; Arslanoglu, A. Antimicrobial and antioxidant activity of edible zein films incorporated with lysozyme, albumin proteins and disodium EDTA. *Food Res. Int.* **2007**, *40*, 80−91.
(12) Curcio, M.; Puoci, F.; Iemma, F.; Parisi, O.; Cirillo, G.; Spizzirri, U.; Picci, N. Covalent insertion of antioxidant molecules on chitosan by a free radical grafting procedure. *J. Agric. Food Chem.* **2009**, *57*, 5933−5938.
(13) Gao, X.; Hu, G.; Qian, Z.; Ding, Y.; Zhang, S. Immobilization of antioxidant on nanosilica and the antioxidative behavior in low density polyethylene. *Polymer* **2007**, *48*, 7309−7315.
(14) Maeda, S.; Nonaka, T.; Ogata, T.; Kurihara, S. Synthesis of macroreticular copolymer beads having various phenolic derivatives immobilized via different bond and their radical scavenging activity. *J. Appl. Polym. Sci.* **2006**, *102*, 4791−4800.
(15) Goddard, J. M.; Talbert, J. N.; Hotchkiss, J. H. Covalent attachment of lactase to low-density polyethylene films. *J. Food Sci.* **2007**, *72*, E36−E41.
(16) Goddard, J. M.; Hotchkiss, J. H. Tailored functionalization of low-density polyethylene surfaces. *J. Appl. Polym. Sci.* **2008**, *108*, 2940−2949.
(17) Anonymous. Code of federal regulations. Government Printing Office: Washington, DC, 2005; Title 21, parts 170−199.
(18) Day, B. P. F. Active packaging of food. In *Smart Packaging Technologies for Fast Moving Consumer Goods*, 1st ed.; Kerry, J., Butler, P., Eds.; John Wiley and Sons: New York, 2008; pp 1−18.
(19) Decker, E. A. Antioxidant mechanisms. In *Food lipids: Chemistry, Nutrition, and Biotechnology*, 2nd ed.; Akoh, C. C., Min, D. B., Eds.; Marcel Dekker: New York, 2002; pp 517−542.
(20) Kang, E.; Tan, K.; Kato, K.; Uyama, Y.; Ikada, Y. Surface modification and functionalization of polytetrafluoroethylene films. *Macromolecules* **1996**, *29*, 6872−6879.
(21) Uchida, E.; Uyama, Y.; Ikada, Y. Sorption of low-molecular-weight anions into thin polycation layers grafted onto a film. *Langmuir* **1993**, *9*, 1121−1124.
(22) Mladenka, P.; Macakova, K.; Filipsky, T.; Zatloukalova, L.; Jahodar, L. In vitro analysis of iron chelating activity of flavonoids. *J. Inorg. Biochem.* **2011**, *105*, 693−701.
(23) Bou, R.; Guardiola, F.; Codony, R.; Faustman, C.; Elias, R.; Decker, E. Effect of heating oxymyoglobin and metmyoglobin on the oxidation of muscle microsomes. *J. Agric. Food Chem.* **2008**, *56*, 9612−9620.
(24) Jiang, Y.; Wang, W. Functional membranes prepared by layer-by-layer assembly and its metal ions adsorption property. *Polym. Adv. Technol.* **2010**, *22*, 2509−2516.
(25) Coupe, B.; Evangelista, M.; Yeung, R.; Chen, W. Surface modification of poly(tetrafluoroethylene-co-hexafluoropropylene) by adsorption of functional polymers. *Langmuir* **2001**, *17*, 1956−1960.
(26) Kontoghiorghes, G.; Pattichis, K.; Neocleous, K.; Kolnagou, A. The design and development of deferiprone (L1) and other iron chelators for clinical use: Targeting methods and application prospects. *Curr. Med. Chem.* **2004**, *11*, 2161−2183.
(27) Masuda, S.; Miyahara, T.; Minagawa, K.; Tanaka, M. Polymerization and complexation of methacryloylglycine. *J. Polym. Sci., Part A: Polym. Chem.* **1999**, *37*, 1303−1309.
(28) Blcak, N.; Koza, G.; Atay, T. Metal chelating resins by condensation of ethylene diamine with p-dichloromethyl benzene. *J. Appl. Polym. Sci.* **1996**, *61*, 799−804.
(29) Sebastian, N.; George, B.; Mathew, B. Metal complexes of poly(acrylic acid): Synthesis, characterization and thermogravimetric studies. *Polym. Degrad. Stab.* **1998**, *60*, 371−375.
(30) Mandal, S.; Goddard, J.; Erickson, D. A multiplexed optofluidic biomolecular sensor for low mass detection. *Lab Chip* **2009**, *9*, 2924−2932.