

Synthesis of Methacrylic–Ferulic Acid Copolymer with Antioxidant Properties by Single-Step Free Radical Polymerization

FRANCESCO PUOCI,* FRANCESCA IEMMA, MANUELA CURCIO, ORTENSIA I. PARISI,
GIUSEPPE CIRILLO, UMILE G. SPIZZIRRI, AND NEVIO PICCI

Dipartimento di Scienze Farmaceutiche, Università della Calabria, Edificio Polifunzionale,
Arcavacata di Rende (CS) 87036, Italia

A novel, simple, and cheap method to synthesize antioxidant methacrylic–ferulic acid copolymer (PMAA–FA) by free radical polymerization was developed by employing a hydrogen peroxide–ascorbic acid pair to produce hydroxyl radicals acting as radical initiators. FT-IR spectra were performed to verify the insertion of ferulic acid into the polymeric chain, and the antioxidant activity of PMAA–FA was compared to that of a control polymer synthesized in the absence of antioxidant molecule. Good antioxidant activity was demonstrated by obtained materials, showing the efficiency of the polymerization method. This material could be useful in the pharmaceutical field and in the food industry (food packaging).

KEYWORDS: Ferulic acid; redox initiators; antioxidant copolymer; free radical polymerization

INTRODUCTION

It is well-known that active oxygen and free radicals are involved in the pathogenesis of several human diseases, including cancer, aging, and atherosclerosis (1).

Oxidative stress, indeed, can damage lipids, proteins, enzymes, carbohydrates, and DNA in cells and tissues, resulting in membrane damage, fragmentation, or random cross-linking of molecules such as DNA, enzymes, and structural proteins and even lead to cell death induced by DNA fragmentation and lipid peroxidation (2, 3).

Active oxygen and free radicals, such as superoxide anion ($^{\ominus}\text{O}_2^{\cdot}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($^{\cdot}\text{OH}$), are constantly formed in the human body by normal activities such as immunological defenses and metabolic reactions. Their excess is opposed by a balanced system of antioxidant defenses, including antioxidant compounds and enzymes. Upsetting this balance causes oxidative stress, which can lead to cell injury and death (4). Therefore, much attention has been focused on the use of antioxidants to inhibit lipid peroxidation or to protect against the damage of free radicals (5).

Polymeric antioxidants are a particular class of systems characterized by higher stability and slower degradation rate than compounds with low molecular weight (6, 7). They could be applied in those fields in which the employment of a single molecule with antioxidant activity is prohibitive (8); for example, they can be used in hemodialysis application, in particular, by their introduction in dialysis membranes. Hemodialysis patients, indeed, are exposed to oxidative stress, which contributes to cardiovascular disease and accelerated atherosclerosis, the major causes of mortality in these patients (9). Another field of application of this kind of material could be

cosmetic formulations, to avoid the oxidation of their components, but it can also be used as a preservative agent in food packaging. For these purposes, different polymeric materials with antioxidant properties were synthesized.

Generally, to obtain this kind of material, three main strategies, involving several different steps, are used. The first one is based on the functionalization of a molecule with antioxidant properties by the insertion of a polymerizable group and its subsequent polymerization or copolymerization (10). The second strategy involves the derivatization of a preformed polymeric structure with an antioxidant (11). Finally, the third approach is the grafting of a synthesized monomeric antioxidant onto a polymeric chain via melt processing with free radical initiators (12). These strategies show some limitations. The synthesis of a monomeric antioxidant requires the purification of reaction products, whereas in the derivatization process, especially if the macromolecular system consists of a cross-linked polymer, a difficult optimization of the reaction conditions is often needed.

This work reports a novel, simple method, involving a one-step reaction, to obtain copolymers through the direct polymerization of an antioxidant molecule and a suitable monomer in the presence of water-soluble redox initiators. In particular, methacrylic acid was copolymerized with (*E*)-3-(4-hydroxy-3-methoxyphenyl)prop-2-enoic acid, commonly named ferulic acid, one of the most ubiquitous phenolic compounds in nature. Because of its ability to inhibit the autoxidation of oils, this molecule has been largely used as a food preservative. It also constitutes the active ingredient in many skin lotions and sunscreens designed for photoprotection (13). For these reasons, a polymeric device based on this compound could be very interesting from an industrial point of view.

MATERIALS AND METHODS

Materials. Ferulic acid (FA), methacrylic acid (MAA), *N,N*-dimethylformamide (DMF), hydrogen peroxide (H_2O_2), ascorbic acid

* Author to whom correspondence should be addressed (e-mail francesco.puoci@unical.it).

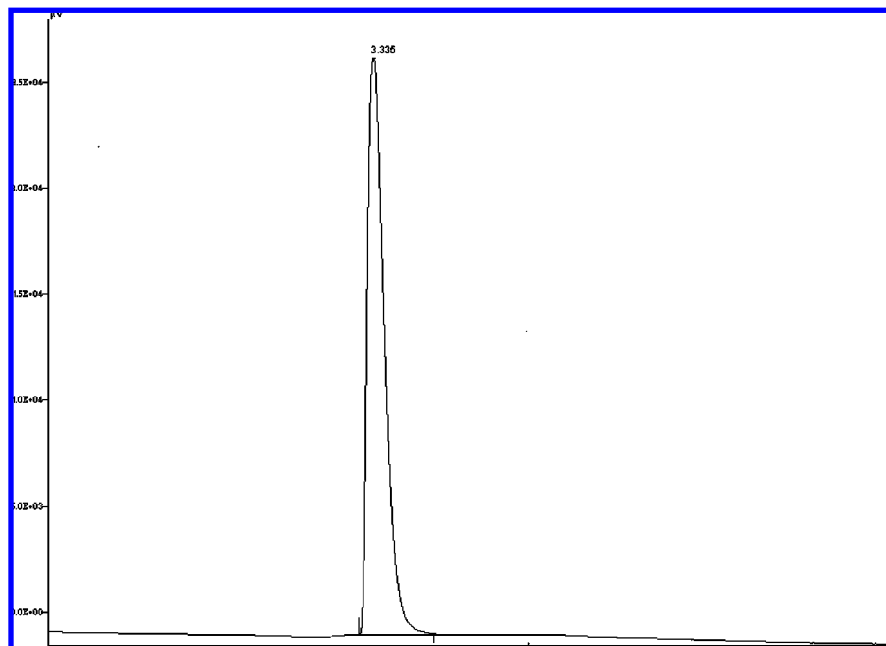


Figure 1. HPLC chromatogram of the FA standard.

(AA), linoleic acid, disodium hydrogen phosphate, sodium hydrogen phosphate, Tween 20, 2,2'-azobis(2-methyl)propionamide dihydrochloride (AAPH), ammonium thiocyanate, ferrous chloride, hydrochloric acid (37% w/w), ethanol, 2,2'-diphenyl-1-picrylhydrazyl radical (DPPH), sulfuric acid (96% w/w), trisodium phosphate, ammonium molybdate, methanol, Folin–Ciocalteu reagent, sodium carbonate, ethyl acetate, and acetic acid were obtained from Sigma-Aldrich. All solvents were of reagent grade or HPLC grade. MAA was purified before use by distillation under reduced pressure.

Synthesis of MAA–FA Copolymer (PMAA–FA). The polymerization of methacrylic acid with ferulic acid by ascorbic acid–hydrogen peroxide redox initiators was carried out as follows: in a 10 mL glass tube, 0.50 g of FA was dissolved in 5.50 mL of DMF, and then MAA (3.50 g) and 2 mL of distilled water containing 10 mM ascorbic acid and 7 mM hydrogen peroxide were added. The mixture was maintained at 25 °C for 3 h under atmospheric air.

The obtained polymer was precipitated by adding the polymeric solutions to an excess volume of diethyl ether (5:1), under agitation at room temperature. The suspensions were filtered by sintered glass filter funnel (Pyrex, Ø30 mm; porosity 3) and washed with diethyl ether, and the recovered polymer was dried in a vacuum oven at 40 °C. They were then further purified by dissolution in water and precipitation in diethyl ether (5:1) three times.

The copolymer was checked to be free of unreacted FA and any other compounds by HPLC analysis after each purification step. In Figure 1 the chromatogram of a 0.1 mM FA standard solution in ethanol is reported. Retention time = 3.34 min.

Blank polymer (PMAA) was prepared under the same conditions without using FA.

Instrumentation. The liquid chromatograph consisted of a Jasco BIP-I pump and a Jasco UVDEC-100-V detector set at 240 nm. A 250 mm × 4 mm C-18 Hibar column, particle size = 5 μm (Merck, Darmstadt, Germany) was employed. As reported in the literature (14), the mobile phase was methanol at a flow rate of 0.5 mL min⁻¹ and at room temperature. IR spectra were recorded as films or KBr pellets on a Jasco FT-IR 4200. UV–vis absorption spectra were obtained with a Jasco V-530 UV–vis spectrometer.

Evaluation of the Antioxidant Activity. *Determination of Scavenging Effect on DPPH Radicals.* Synthesized PMAA–FA was allowed to react with a stable free radical, DPPH, with the aim of evaluating the free radical scavenging properties of these materials (15). One hundred milligrams of PMAA–FA was dissolved in 12.5 mL of distilled water in a volumetric flask (25 mL), and then 12.5 mL of an ethanol solution of DPPH (200 μM) was added to obtain a solution of

DPPH with a final concentration of 100 μM. The sample was incubated in a water bath at 25 °C and, after 30 min, the absorbance of the remaining DPPH was determined colorimetrically at 517 nm. The same reaction conditions were applied for the blank polymer to evaluate the interference of polymeric material on DPPH assay. The scavenging activity of the tested polymeric materials was measured as the decrease in absorbance of the DPPH, and it was expressed as percent inhibition of DPPH radicals calculated according to the equation

$$\text{inhibition \%} = \frac{A_0 - A_1}{A_0} \times 100 \quad (1)$$

where A_0 is the absorbance of a standard that was prepared in the same conditions, but without any polymers, and A_1 is the absorbance of polymeric samples.

A calibration curve was recorded by using five different ferulic acid standard solutions. Half a milliliter of each solution was added to DPPH system to obtain the final concentrations of 2.0, 6.0, 12.0, 24.0, and 42.0 μM, respectively. DPPH assay was performed, and percent inhibition of DPPH radicals was calculated to record the calibration curve. The correlation coefficient (R^2), slope, and intercept of the regression equation were obtained by the method of least-squares.

Linoleic Acid Emulsion System–Thiocyanate Assay. The antioxidant properties of synthesized PMAA–FA were evaluated through measurement of percent inhibition of peroxidation in the linoleic acid system by using the thiocyanate method (15) with some modification. A linoleic acid emulsion (0.2 M, pH 7) was prepared by mixing 0.2804 g of linoleic acid, 0.2804 g of Tween 20 as emulsifier, and 50 mL of phosphate buffer (0.2 M, pH 7.0), and then the obtained mixture was homogenized. Subsequently, 2.5 mL of the prepared emulsion was mixed with 100 mg of PMAA–FA, and AAPH (final concentration = 25 mM) was added to start the peroxidation of linoleic acid (16). Then the reaction mixture was incubated at 40 °C for 3 days to accelerate the oxidation process, and after this period, the degree of oxidation was measured according to the ferric thiocyanate method as following. Subsequent to the incubation, the sample was centrifuged and, in a 10 mL volumetric flask, 0.2 mL of sample solution, 0.2 mL of an aqueous solution of ammonium thiocyanate (30%), 0.2 mL of ferrous chloride (FeCl₂) solution (20 mM in 3.5% HCl), and ethanol (75% v/v) to 10 mL were added in sequence. After 3 min of stirring, the absorbance was measured at 500 nm to determinate the peroxide content. Linoleic assay was performed in the same conditions using 100 mg of the blank polymer to establish eventual interferences of polymeric material.

Ferulic acid was employed to record a calibration curve. Volumes of 0.1 mL of five different ferulic acid standard solutions were added

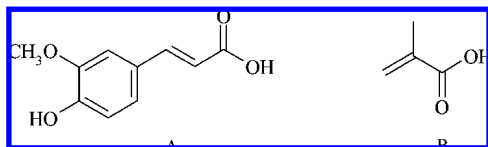


Figure 2. Structures of ferulic acid (A) and methacrylic acid (B).

to 25 mL of linoleic system to raise the final concentrations of 0.12, 0.36, 0.60, 0.84, and 1.08 mM, respectively. The peroxidation protocol was applied and, after peroxide content measurement, the calibration curve was recorded. Percent inhibition of linoleic acid peroxidation was calculated according to eq 1.

Evaluation of Disposable Phenolic Groups by Folin–Ciocalteu Procedure. The amount of total phenolic groups was determined using Folin–Ciocalteu reagent procedure, according to the literature with some modifications (17).

PMAA–FA (100 mg) was dissolved in distilled water (6 mL) in a volumetric flask. Folin–Ciocalteu reagent (1 mL) was added, and the contents of the flask were mixed thoroughly. After 3 min, 3 mL of Na_2CO_3 (2%) was added, and then the mixture was allowed to stand for 2 h with intermittent shaking.

The absorbance was measured at 760 nm against a control prepared using the blank polymer under the same reaction conditions. The amount of total phenolic groups in polymeric materials was expressed as ferulic acid equivalent concentration by using an equation that was obtained from a ferulic acid calibration curve. This one was recorded by employing five different ferulic acid standard solutions. Half a milliliter of each solution was added to the Folin–Ciocalteu system to raise the final concentrations of 0.03, 0.06, 0.09, 0.12, and 0.15 mM, respectively. After 2 h, the absorbance of the solutions was measured to record the calibration curve, and the correlation coefficient (R^2), slope, and intercept of the regression equation obtained were calculated by the method of least-squares.

Determination of Total Antioxidant Activity. The total antioxidant activity of polymeric materials was evaluated according to the method reported in the literature (18). Briefly, 100 mg of PMAA–FA was mixed with 1.2 mL of reagent solution (0.6 M sulfuric acid, 28 M sodium phosphate, and 4 M ammonium molybdate) and 0.3 mL of methanol, and then the reaction mixture was incubated at 95 °C for 150 min. After cooling to room temperature, the absorbance of the mixture was measured at 695 nm against a control prepared using blank polymer in the same reaction. The total antioxidant activity of polymeric materials was expressed as ferulic acid equivalent concentration.

By using five different ferulic acid standard solutions, a calibration curve was recorded. A volume of 0.2 mL of each solution was mixed with 0.8 mL of reagent solution to obtain the final concentrations of 1.0, 1.5, 2.0, 2.5, and 3.0 mM, respectively. After 150 min of incubation, the solutions were analyzed by using a UV–vis spectrophotometer, and the correlation coefficient (R^2), slope, and intercept of the regression equation obtained by the method of least-squares were calculated.

RESULTS AND DISCUSSION

Synthesis of Antioxidant PMAA–FA. PMAA–FA was synthesized by direct employment of ferulic acid as comonomer (without any other derivatization reactions) and ascorbic acid– H_2O_2 as redox initiator at room temperature.

Ferulic acid (4-hydroxy-3-methoxycinnamic acid) (Figure 2) has received much attention in the study of medicine. In recent years, there have been an increasing number of reports on the physiological functions of ferulic acid and its derivatives in human. Free ferulic acid is a good antioxidant because it forms a resonance-stabilized phenoxy radical. It showed high scavenging activity for hydrogen peroxide, superoxide, hydroxyl, and nitrogen dioxide free radicals. In addition, many applications of ferulic acid in the food and cosmetic industries have also been discovered (19). It is usually found as ester cross-links with polysaccharides in the cell wall and also with proteins (20). The cross-linking property of ferulic acid with both polysac-

charides and proteins suggests that it can be used in the preparation of complex gels in food applications.

The choice of methacrylic acid as comonomer is related to the broad application field of methacrylate polymers in biomedicine and biotechnology. Acrylate and methacrylate polymers have been applied in drug delivery systems, contact lenses, food technology, quality control systems, and synthetic membranes for biosensors (21).

In a radical polymerization process, another parameter to be considered is the initiator system. Conventional initiators including azo compounds, peroxides, and thermal iniferters require a relatively high polymerization temperature to ensure rapid decomposition of the initiator. By employing a redox initiation system it is possible to perform polymerization processes at lower temperatures, with all of the polymer chains initiated almost instantaneously because of the reduction of the induction time (22). Furthermore, the lower polymerization temperature reduces the risks of antioxidant degradation.

In this work, an AA– H_2O_2 redox pair was employed to avoid the generation of any kind of toxic reaction products.

In Figure 3 is reported the mechanism of redox reagents interaction: the AA is oxidized by H_2O_2 , forming hydroxyl radical and ascorbate radical intermediates that initiate the polymerization (23).

The ratio 1:7 w/w between FA and MAA in the prepolymerization feed represents the optimal value to obtain the polymer with the highest antioxidant efficiency. A higher amount of the ferulic acid, indeed, carries out to the formation of oligomers that are difficult to purify by the conventional purification technique.

The obtained polyacrylic systems were characterized by high antioxidant activity, higher stability, and slower degradation than natural antioxidants with low molecular weight.

On the basis of the chemical structure of ferulic acid, characterized by a carbon–carbon double bond in styrenic position and a phenolic group, a polymerization mechanism could be hypothesized. It is known, indeed, that the styrenic group of cinnamic acid can undergo free radical polymerization in a wide range of conditions (24). Phenolic group compatibility with this kind of polymerization was also proved in different research work (25): monomers with active functional groups (phenolic groups) as side substituents, indeed, were used for the preparation of chelating (26) or grafted polymeric systems using free radical initiators. On the other hand, the phenolic group could be directly involved in the polymerization process; it is indeed reported that phenolic radical undergoes dimerization processes by reaction between hydroxyl radical and aromatic ring (27).

On the basis of these considerations, our hypothesized polymerization mechanism involves the reaction of both styrenic carbon–carbon double bond and phenolic oxygen for ferulic acid insertion in the polymeric chain.

Characterization of PMAA–FA. PMAA–FA and the respective control polymer were characterized by Fourier transform IR spectrophotometry and UV analysis.

By comparison of two IR spectra, it is clear that in the spectrum of the PMAA–FA (trace B) a new peak, which is absent in the spectrum of PMAA (trace A), can be ascertained (Figure 4). The new peak at 1544 cm^{-1} , indeed, is awardable to carbon to carbon stretching within the aromatic ring of ferulic acid.

The incorporation of FA in the polymeric structure is also clear in a comparison of the UV spectra of FA (25 μM) and PMAA–FA in ethanol (4 mg/mL) (Figure 5). In the PMAA–FA spectrum,

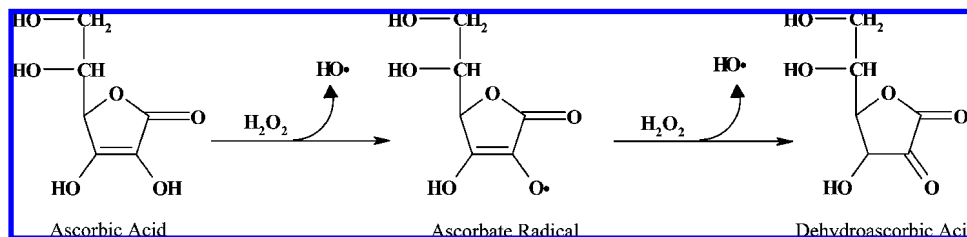


Figure 3. Interaction between ascorbic acid and hydrogen peroxide.

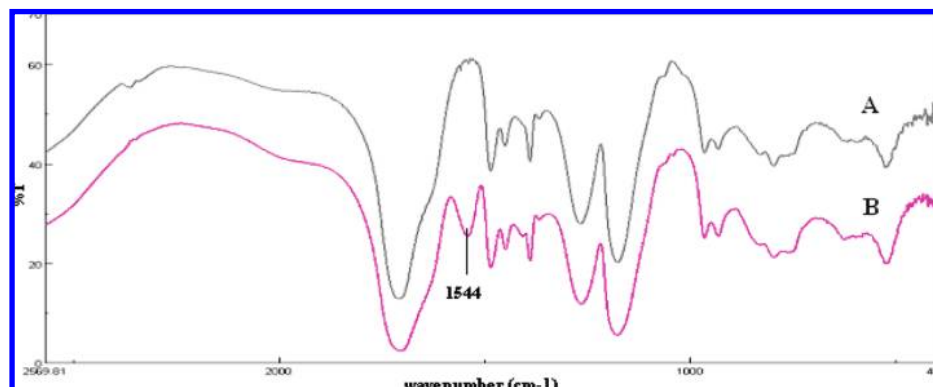


Figure 4. FT-IR spectra of PMAA (A) and PMAA–FA (B).

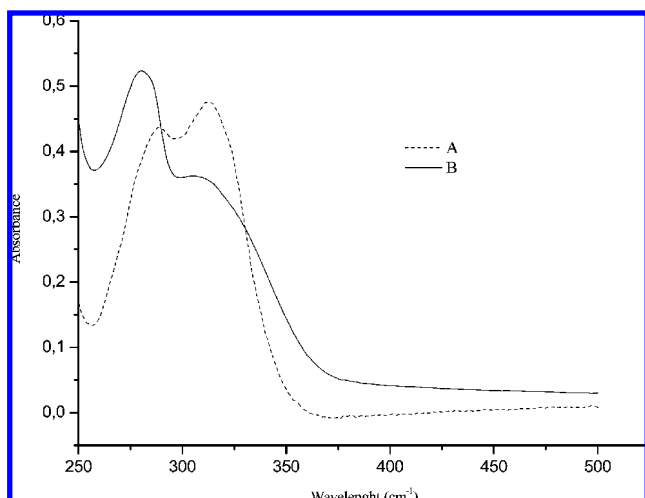


Figure 5. UV spectra of FA (A) and PMAA–FA (B).

the presence of absorption peaks in the aromatic region is related to the presence of FA in the sample. In addition, the wavelength of the aromatic peaks is shorter in PMAA–FA than in FA.

Measurement of Antioxidant Activity. *Determination of Scavenging Effect on DPPH Radicals.* The DPPH radical is a stable organic free radical with an absorption maximum band around 515–528 nm and, thus, it is a useful reagent for evaluation of the antioxidant activity of compounds (15).

In the DPPH test, the antioxidants reduce the DPPH radical to a yellow compound, diphenylpicrylhydrazine, and the extent of the reaction will depend on the hydrogen-donating ability of the antioxidants. It has been documented that cysteine, glutathione, ascorbic acid, tocopherol, and polyhydroxy aromatic compounds (e.g., ferulic acid, hydroquinone, pyrogallol, gallic acid) reduce and decolorize 1,1-diphenyl-2-picrylhydrazine by their hydrogen-donating capabilities.

Polymer scavenger ability was evaluated in terms of DPPH reduction using ferulic acid as reference compound, and data are expressed as inhibition (percent).

Table 1. Inhibition Percentages of DPPH Radical and Linoleic Acid Peroxidation by PMAA–FA and PMAA^a

polymer	inhibition (%)	
	DPPH	linoleic acid peroxidation
PMAA–FA	80 ± 1	50 ± 1
PMAA	30 ± 1	7 ± 1

^a Inhibition percentage = [1 – (absorbance of sample)/(absorbance of control)] × 100. Results are means ± SD of three parallel measurements.

Antioxidant polymer showed high scavenging activity, and blank polymer did not significantly interfere with the scavenger process (Table 1).

Linoleic Acid Emulsion System–Thiocyanate Assay. The antioxidant activity of synthesized material was measured using the ferric thiocyanate test, which determines the amount of peroxide produced at the initial stage of lipid peroxidation.

Linoleic acid, an unsaturated fatty acid, is usually used as a model compound in lipid oxidation and antioxidation-related assays in which carbon-centered, peroxy radicals and hydroperoxides, etc., are involved in the oxidation process. During the linoleic acid oxidation, peroxides are formed. These compounds oxidize Fe²⁺ to Fe³⁺. The latter Fe³⁺ ions form complexes with SCN[–], which have maximum absorbance at 500 nm. Therefore, high absorbance indicates high linoleic acid oxidation, whereas lower absorbance indicates a higher level of antioxidant activity.

In Table 1, the effects of polymeric particles (100 mg) on linoleic acid peroxidation compared to ferulic acid after 72 h are shown. As it is possible to note, ferulic acid-containing polymers are very effective in peroxidation process inhibition.

The lowest inhibition value obtained from the experiments performed with blank polymer clearly show that absorbance reduction is ascribable to ferulic acid moieties in the polymeric structure.

Evaluation of Disposable Phenolic Groups by Folin–Ciocalteu Procedure. Because the antioxidant activity of PMAA–FA is derived from phenolic groups, it is useful to express the antioxidant potential in terms of phenolic content (17).

The Folin–Ciocalteu phenol reagent is used to obtain a crude estimate of the amount of disposable phenolic groups present in the polymer chain. Phenolic compounds undergo a complex redox reaction with phosphotungstic and phosphomolybdic acids present in the Folin–Ciocalteu reactant. The color development is due to the transfer of electrons at basic pH to reduce the phosphomolybdic/phosphotungstic acid complexes to form chromogens in which the metals have lower valence.

Disposable phenolic groups in the samples were expressed as milligram equivalents of ferulic acid, and this value was 2.77 mg/g of dry polymer. Control experiments were also performed with blank polymer, and no activity was recovered.

Determination of Total Antioxidant Activity. The assay is based on the reduction of Mo(VI) to Mo(V) by ferulic acid and subsequent formation of a green phosphate–Mo(V) complex at acid pH (18). The total antioxidant activity was measured and compared with that of ferulic acid and the control, which contained no antioxidant component. The high absorbance values indicated that the sample possessed significant antioxidant activity. According to the results, synthesized materials had significant antioxidant activities, and the ferulic acid equivalent concentration was found to be 0.59 mM for 1 g of PMAA–FA. Control experiments were also performed with blank polymer, and no activity was recovered.

PMMA–FA Antioxidant Efficiency. In this work, MAA–FA copolymer was successfully synthesized by employing redox water-soluble initiators. IR and UV spectra performed on the copolymer testified to the success of the procedure.

Antioxidant properties of obtained materials were evaluated by different assays. In particular, determination of the scavenging activity on DPPH radicals, the linoleic acid emulsion system–thiocyanate assay, determination of disposable phenolic groups in polymeric matrices, and determination of total antioxidant capacity were performed. Good antioxidant properties were recorded in all of the tested conditions, confirming that ferulic acid activities were maintained after introduction onto the polymeric chain.

The adopted synthetic strategy is thus a very simple approach for preparing macromolecular systems with high antioxidant power that may have potential for use as preservatives in food and cosmetics.

LITERATURE CITED

- Moskovitz, J.; Yim, M. B.; Chock, P. B. Free radicals and disease. *Arch. Biochem. Biophys.* **2002**, *397*, 354–359.
- Nordberg, J.; Arner, E. S. J. Reactive oxygen species, antioxidants, and the mammalian thioredoxin system. *Free Radical Biol. Med.* **2001**, *31*, 1287–1312.
- Aruoma, O. I. Nutrition and health aspects of free radicals and antioxidants. *Food Chem. Toxicol.* **1994**, *32*, 671–683.
- Halliwell, B.; Gutteridge, J. M. C.; Aruoma, O. I. The deoxyribose method: simple “test tube” assay for determination of rate constants for reactions of hydroxyl radicals. *Anal. Biochem.* **1987**, *165*, 215–219.
- Vendemmiale, G.; Grattagliano, I.; Altomare, E. An update on the role of free radicals and antioxidant defense in human disease. *Int. J. Clin. Lab. Res.* **1999**, *29*, 49–55.
- Kurisawa, M.; Chung, J. E.; Uyama, H.; Kobayashi, S. Enzymatic synthesis and antioxidant properties of poly(rutin). *Biomacromolecules* **2003**, *4*, 1394–1399.
- Pan, J. Q.; Liu, N. C.; Lau, W. W. Y. Preparation and properties of new antioxidants with higher MW. *Polym. Degrad. Stab.* **1998**, *62*, 165–170.
- Iemma, F.; Cirillo, G.; Puoci, F.; Trombino, S.; Castiglione, M.; Picci, N. Iron(III) chelation and antioxidant properties of myo-inositol phosphorylated polymeric microspheres. *J. Pharm. Pharmacol.* **2007**, *59*, 597–601.
- Calo, L. A.; Naso, A.; Pagnin, E.; Davis, P. A.; Castoro, M.; Corradin, R.; Riegler, R.; Cascone, C.; Huber, W.; Piccoli, A. Vitamin E-coated dialyzers reduce oxidative stress related proteins and markers in haemodialysis—a molecular biological approach. *Clin. Nephrol.* **2004**, *62*, 355–361.
- Ortiz, C.; Vazquez, B.; Roman, J. S. Hydrophilic acrylic biomaterials derived from vitamin E with antioxidant properties. *J. Biomed. Mater. Res.* **1999**, *45*, 184–191.
- Atkinson, D.; Lehrle, R. A model polymer-bound antioxidant system for lubricants. *Eur. Polym. J.* **1992**, *28*, 1569–1575.
- Al-Malaika, S.; Suharty, N. Reactive processing of polymers: mechanisms of grafting reactions of functional antioxidants on polyolefins in the presence of a coagent. *Polym. Degrad. Stab.* **1995**, *49*, 77–89.
- Trombino, S.; Cassano, R.; Bloise, E.; Muzzalupo, R.; Tavano, L.; Picci, N. Synthesis and antioxidant activity evaluation of a novel cellulose hydrogel containing trans-ferulic acid. *Carbohydr. Polym.* **2009**, *75* (1), 184–188.
- Chakravartula, S. V. S.; Guttarla, N. Identification and characterization of phenolic compounds in castor seed. *Nat. Prod. Res.* **2007**, *12*, 1073–1077.
- Ardestani, A.; Yazdanparast, R. Antioxidant and free radical scavenging potential of *Achillea santolina* extracts. *Food Chem.* **2007**, *104*, 21–29.
- Dufour, C.; Loonis, M.; Dangles, O. Inhibition of the peroxidation of linoleic acid by the flavonoid quercetin within their complex with human serum albumin. *Free Radical Biol. Med.* **2007**, *43*, 241–252.
- Pan, Y.; Zhu, J.; Wang, H.; Zhang, X.; Zhang, Y.; He, C.; Ji, X.; Li, H. Antioxidant activity of ethanolic extract of *Cortex fraxini* and use in peanut oil. *Food Chem.* **2007**, *103*, 913–918.
- Prieto, P.; Pineda, M.; Aguilar, M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Anal. Biochem.* **1999**, *269*, 337–341.
- Ou, S.; Kwok, K.-C. Ferulic acid: pharmaceutical functions, preparation and applications in foods. *J. Sci. Food Agric.* **2004**, *84*, 1261–1269.
- Figueroa-Espinoza, M. C.; Morel, M. H.; Surget, A.; Asther, M.; Moukha, S.; Sigoillot, J. C.; Rouau, X. Attempt to cross-link feruloylated arabinoxylans and proteins with a fungal laccase. *Food Hydrocolloids* **1999**, *13*, 65–71.
- Herveás Pérez, J. P.; López-Cabarcos, E.; López-Ruiz, B. The application of methacrylate-based polymers to enzyme biosensors. *Biomol. Eng.* **2006**, *23*, 233–245.
- Simms, R. W.; Cunningham, M. F. High molecular weight poly(butyl methacrylate) by reverse atom transfer radical polymerization in miniemulsion initiated by a redox system. *Macromolecules* **2007**, *40*, 860–866.
- Kitagawa, M.; Tokiwa, Y. Polymerization of vinyl sugar ester using ascorbic acid and hydrogen peroxide as a redox reagent. *Carbohydr. Polym.* **2006**, *64*, 218–223.
- Esen, H.; Küsefoğlu, S. H. Photolytic and free-radical polymerization of cinnamate esters of epoxidized plant oil triglycerides. *J. Appl. Polym. Sci.* **2002**, *89*, 3882–3888.
- Kim, T. H.; Oh, D. R. Melt grafting of maleimides having hindered phenol antioxidant onto low molecular weight polyethylene. *Polym. Degrad. Stab.* **2004**, *84*, 499–503.
- Nanjundan, S.; Selvamalar, C. S. J.; Jayakumar, R. Synthesis and characterization of poly(3-acetyl-4-hydroxyphenyl acrylate) and its Cu(II) and Ni(II) complexes. *Eur. Polym. J.* **2004**, *40*, 2313–2321.
- Larsen, E.; Andreasen, M. F.; Christensen, L. P. Regioselective dimerization of ferulic acid in a micellar solution. *J. Agric. Food Chem.* **2001**, *49*, 3471–3475.

Received for review July 25, 2008. Revised manuscript received September 18, 2008. Accepted September 29, 2008. This work was financially supported by university funds.