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Short communication

Synthesis and antioxidant activity evaluation of a novel cellulose hydrogel containing *trans*-ferulic acid

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ABSTRACT

In the present work, we report the synthesis of cellulose hydrogel containing ferulic moieties and the evaluation of its antioxidant and scavenger activity. Acrylic groups were inserted onto cellulose backbone by a heterogeneous synthesis to produce two cellulose monomers with different degree of substitution (DS). The radical copolymerization of acrylcellulose (AcrC) with *N*,*N*-dimethylacrylamide (DMAA) was carried out in NH_3/Urea aqueous solution, in a range of composition between 24 and 60 wt% of AcrC. The obtained hydrogels were characterized by infrared spectroscopy (FT-IR). Their equilibrium swelling degree (α%) was evaluated. They showed good swelling behavior in simulating gastric, intracellular and intestinal fluids and no more different at various pH. The ferulic moieties were directly grafted on the free hydroxylic groups of cellulose hydrogel by acylation, using dicyclohexylcarbodiimide (DCC) and 4-hydroxybenzotriazole (HBT) as condensation agents. Finally, the antioxidant activity in inhibiting the lipid peroxidation, in rat-liver microsomal membranes, induced in vitro by two different sources of free radicals, 2,2′-azobis (2-amidinopropane) (AAPH) and *tert*-butyl hydroperoxide (*tert*-BOOH), was evaluated. The effects of scavenging DPPH (1,1-diphenyl-2-picrylhydrazyl) radicals were also investigated. Hydrogel was found to be very efficient scavengers of DPPH radicals. The results strongly suggested that the antioxidant hydrogel neutralize free radicals. This biomaterial could be successfully applied in pharmaceutical field both as prodrug of *trans*-ferulic acid than as carrier for photo and thermo-degradable drugs to improve their stability.

1. Introduction

Hydrogels are presently under investigation as matrices for the controlled release of bioactive molecules. They are based on cross-linked hydrophilic matrix insoluble in water which absorb and retain large amounts of water and biological fluids, releasing the drugs by slow diffusion. There are many studies on natural (e.g. alginate and chitosan) polysaccharide based hydrogels. These materials are biocompatible, biodegradable, and non-toxic and used as drug delivery systems (Jung, Chung, & Lee, 2006; Wang et al., 2006). Recently, hydrogels based on commercial chemically crosslinked cellulose derivatives were studied for the production of dietary bulking agents (Sannino, Madaghiele, Lionetto, Schettino, & Maffezzoli, 2006). In fact, cellulose is the most abundant polymer in nature and its derivatives are very popular due to their biocompatibility with tissues and blood, non-toxicity (Alderman, 1984) and low price. They are already used in pharmaceutical formulations as inert matrix towards the incorporated drugs (Chowdary & Srinivasa Rao, 2003; Yamada, Onishi, & Machida, 2001). Over the past decade, a great deal of researches has focused on the application of antioxidants to medical treatments (Halliwell & Gutteridge, 1990; McCall & Frei, 1999; Pinchuk & Lichtemberg, 2002; Steinbrecher, 1999) to prevent free radicals formation. Antioxidant polymers have received ever-increasing attention, from both academic and cosmetic and pharmaceutical industries point of view. To confer antioxidant and free radical scavenger properties to hydrogels both acrylic or vinylic monomer bearing vitamin E as side groups than vinylic monomer binding eugenol were employed (Jung et al., 2006; Ortiz, Vàzquez, & San Romàn, 1998; Plasencia et al., 1999). Moreover, in a previous paper, we reported the synthesis and characterization of cellulose, with antioxidant efficiency, bearing ferulic, lipoic and tocopherol moieties. This study showed that the designed systems, preserve the antioxidant activity of the free substrates and that the cellulose ferulate is the best antioxidant to protect against lipid peroxidation induced by two different sources of free radicals (Trombino et al., 2008).

For such reason the idea to link ferulic moieties to cellulose hydrogels by covalent bond (Scheme 1) to produce a carrier that protects unstable drugs against oxidative stress and then could be useful in preparations for topical and oral administrations. Particularly, in this work we reported on the antioxidant compounds

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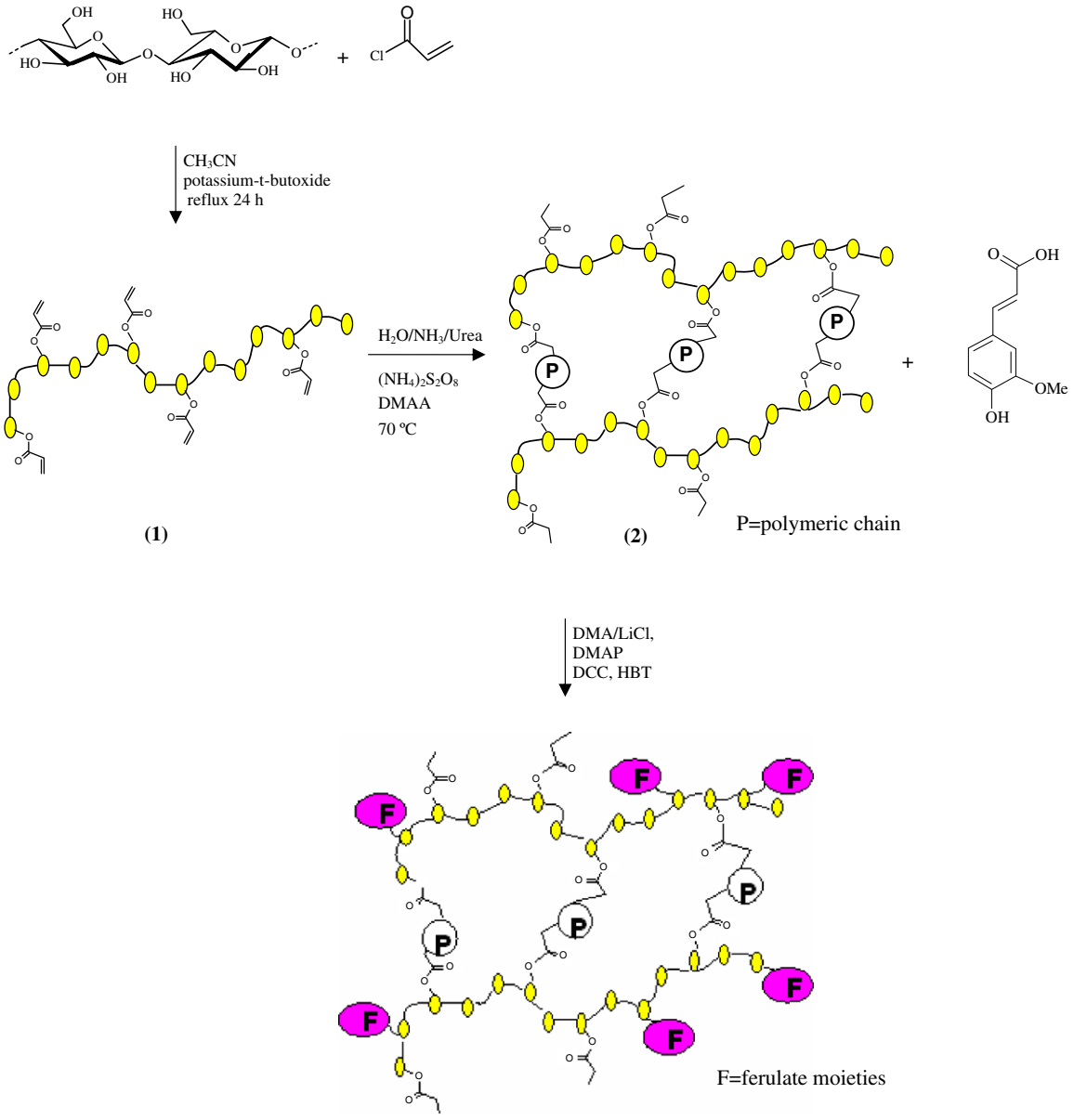

Scheme 1. Synthesis of ferulate hydrogel.

covalently linked to a hydrogel cellulose-based. The ferulic groups do not leach out of the polymer matrix. Furthermore, the immobilization of the antioxidant also improves its long-term stability. The synthesis disclosed in this paper proceeded under mild conditions and resulted in highly selective coupling of the antioxidant compounds to hydrogels, which ensured an effective dispersion of the antioxidant throughout the materials.

The present paper reports on (i) the synthesis of hydrogels cellulose-based, carried out by radical copolymerization of AcrC with DMAA; (ii) the swelling characteristics of the hydrogels by measurements of α at various pH values; (iii) the grafting reaction of *trans*-ferulic acid on cellulose hydrogel that showed the best α; (iv) the evaluation of its antioxidant activity in inhibiting the lipid peroxidation in rat-liver microsomal membranes (Trombino et al., 2004), induced in vitro by 2,2′-azobis (2-amidinopropane) (AAPH), which exogenously produces peroxyl radicals by thermal decomposition, and *tert*-butyl hydroperoxide (*tert*-BOOH), which endogenously produces alkoxyl radicals by Fenton reactions; (v) the evaluation of its scavenging effects on DPPH radical by the decoloration method.

2. Cellulose acrylate (AcrC)

Cellulose monomer AcrC (1) was obtained by heterogeneous synthesis (Bojanic, Jovanovic, Tabakovic, & Tabakovic, 1996) with acryloyl chloride (Scheme 1). Briefly, microcrystalline cellulose was swollen in acetonitrile at room temperature for 1 h. The solution of potassium *tert*-butoxide in acetonitrile was added and the reaction mixture was allowed to react at room temperature for 4 h. An excess of acryloyl chloride in acetonitrile was added dropwise to the stirred reaction mixture at room temperature. Stirring was continued under reflux overnight. The product was filtered, washed thoroughly with water, ethanol, acetone, and diethyl ether and then dried in vacuum at 50 °C. FT-IR (KBr) spectra showed an absorption at 1721 cm^{-1} and the presence of pendant vinyl groups at 805 cm^{-1}. Varying molar ratio and reaction time (Table 1) were obtained two derivatives with different degree of substitution (DS) determined by volumetric analysis (Halliwell & Gutteridge, 1990). Briefly, a sample of 50 mg of ester derivative was dispersed in 5 ml of 0.25 M ethanolic sodium hydroxide solution under reflux for 17 h. The dosing, in return of the excess of soda, was realized by

Table 1
Reaction conditions and degree of substitution (DS) values

Molar ratio cellulose/*tert*-butoxide/acryloyl chloride	Reaction time (h)	DS
1:3:3	8	0.28
1:3:10	20	0.68

Table 2
Equilibrium swelling degree % of hydrogels prepared by AcrC with DS = 0.28

Molar ratio AcrC/DMAA	Equilibrium swelling degree % (α)		
	pH 1	pH 6.8	pH 8
1:3[a]	–	–	–
1:6	706	533	778
1:9	759	772	891

[a] No hydrogel formation.

Table 3
Equilibrium swelling degree % of hydrogels prepared by AcrC with DS = 0.68

Molar ratio AcrC/DMAA	Equilibrium swelling degree % (α)		
	pH 1	pH 6.8	pH 8
1:3	364	266	570
1:6	680	557	646
1:9	652	562	700

titration with 0.1 N HCl (first equivalent point). The moles of chloride acid used between the first and second equivalence correspond to the moles of free esters. The degree of substitution (DS) was determined by the Eq. (1).

$$DS = \frac{MM_{glucose\ unit}}{(g_{sample}/n_{free\ ester}) - (MM_{free\ ester} - MM_{H_2O})} \quad (1)$$

In Eq. (1) $n_{free\ ester} = (V_{2\ e.p.} - V_{1\ e.p.}) \times [HCl]$; $MM_{glucose\ unit}$ is the molecular mass of a glucose unit; g_{sample} is the weight of the sample; $n_{free\ ester}$ is the number of moles of free ester; $MM_{free\ ester}$ is the molecular mass of free ester; and MM_{H_2O} is the molecular mass of water.

3. AcrC/DMAA hydrogel

Preparation of hydrogels (2) was carried out by radical polymerization of AcrC, the crosslinking agent, with DMAA (Scheme 1). Cellulose monomer (AcrC) was swollen under stirring for 10 min in 2.5 ml of NH_3 (30%)/Urea (12%) aqueous solvent, cooled at −5 °C. Comonomer (DMAA) and ammonium persulfate, the polymerization initiator, were added. The mixture was heated to 70 °C for few minutes, until the crosslinking took place and the mixture became like gel. The hydrogels were washed with several aliquots of hot water under stirring, filtered, deswollen with acetone, dried under vacuum at 50 °C and then investigated by FT-IR spectroscopy that confirmed the copolymerization. Precisely, was observed a decrease of peak intensity in the double bonds region at 805 cm^{-1} and the appearance of a new ester band at 1732 cm^{-1} due to a formation of hydrogel between AcrC and DMAA. Carrying out the polymerization in *N*,*N*-dimethylacetamide and lithium chloride (DMA/LiCl) solvent system were obtained hydrogels with lower α.

4. Swelling studies

The swelling behavior of hydrogels was determined in order to check their hydrophilic affinity. Typically, aliquots (40–50 mg) of materials dried to constant weight were placed in a tared 5-ml sintered glass filter (Ø 10 mm; porosity G3), weighed, and left to swell by immersing the filter in a beaker containing the swelling media (acidic solution pH 1, simulated gastric fluid; phosphate buffer pH 6.8, simulated intercellular fluid; basic solution pH 8, simulated intestinal fluid). At predetermined times (1, 4 and 24 h), the excess of water was removed by percolation and then the filter was centrifuged at 3500 rpm for 15 min and weighed. The filter tare was determined after centrifugation with only water. The weights recorded at different times were averaged and used to give the equilibrium swelling degree (α%) by the Eq. (2). In Eq. (2) W_s and W_d are the weights of swollen and dried hydrogels, respectively. Each experiment was carried out in triplicate and the results were in agreement within ±4% standard error. The amount of monomer and crosslinking agent used for hydrogels preparation and the relative α (%) values were reported in Tables 2 and 3. All prepared hydrogels showed no more different swelling behaviors at various pH values. By changing the amount of comonomer hydrogels with different swelling behavior were obtained. Particularly, hydrogels synthesized by AcrC with higher DS showed lower α due to formation of a network with higher density. The larger amount of DMAA can increase the length of PDMAA segments which decreases the network density, respectively.

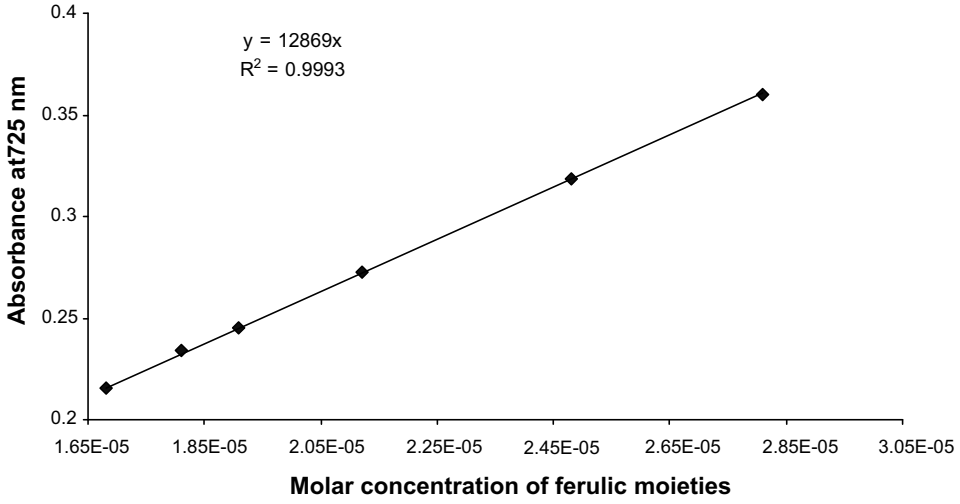

Fig. 1. Calibration curve obtained with six varying concentrations of *trans*-ferulic acid.

$$\alpha(\%) = \frac{(W_s - W_d)}{W_d} \cdot 100 \quad (2)$$

5. Cellulose hydrogel containing *trans*-ferulic acid

With the aim of taking advantage of the antioxidant properties of the *trans*-ferulic acid, an hydrogel containing this residue has been synthesized. The linking of antioxidant groups on the preformed hydrogel rather than on its precursor was effected to avoid the inhibitory action of *trans*-ferulic acid, a scavenger of radicals species, on the radical polymerization process. The grafting reaction of ferulic acid (FA) (Scheme 1) was carried out on hydrogel, synthesized by ArcC (DS 0.28)/DMAA with 1:9 molar ratio, that showed higher α value. The dry hydrogel (0.4 g corresponding to 3.3 mmol of free hydroxylic groups) was swollen in DMA/LiCl solvent system at 130 °C for 2 h. The mixture was cooled to room temperature then a catalytic amount of *N*,*N*-dimethylaminopyridine, an excess of FA and condensation agents (DCC, HBT) were added under stirring, heating to 100 °C for 4 h and to room temperature overnight. The solid was initially filtered and washed with water, methanol, tetrahydrofurane and acetone to remove the reaction sub-products as dicyclohexylurea, and unreacted FA. After that, it was dried under vacuum at 50 °C and characterized by FT-IR that confirms the formation of a new ester bond (1698 cm^{-1}).

6. Determination of FA content in ferulate AcrC/DMAA hydrogel

DS value of ferulic portion was determined by the Folin-Ciocalteu (FC) method (Singleton, Orthofer, & Lamuela-Raventós, 1999; Vinson, Hao, Su, & Zubík, 1998; Wildenradt & Singleton, 1974), a colorimetric assay that requires few reagents and relies on the use of the free ferulic acid as standard compound. Fig. 1 shows the calibration curve used to obtain DS value of the ferulic moieties onto hydrogel. The calibration curve was obtained with *trans*-ferulic acid solutions ranging from 1.68×10^{-5} to 2.81×10^{-5} mol/L, and the results are given as moles of phenolic groups per grams of hydrogel. The results furnished a value of 2.36×10^{-5} mol of ferulic moieties.

7. Radicals scavenging activity of ferulate hydrogel

The ability of prepared hydrogel to act as radical scavengers was considered. The radical scavenging ability of ferulate hydrogel was assessed through their reaction with stable DPPH radicals, using the methodology of Wang et al. (1998). DPPH typically extracts a proton to form DPPH during the reaction (Brand-Williams, Cuvelier, & Berset, 1995). Briefly, in an ethanol solution of DPPH radical (final concentration 1.1×10^{-4} M), hydrogels was added, and their concentrations were 5, 10, 20, and 40 mg/ml. The reaction mixtures were soaken vigorously and then kept in the dark for 30 min. Their absorbances were then measured in 1 cm cuvettes using a UV–Vis spectrophotometer (V-530 JASCO) at 516 nm

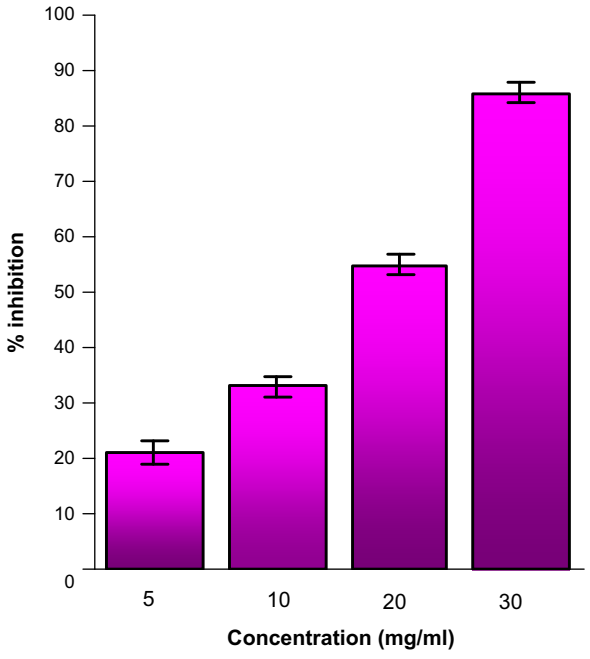

Fig. 2. Scavenging effects of ferulate hydrogel on the DPPH free radical. The results represent means ± SEM of three determinations.

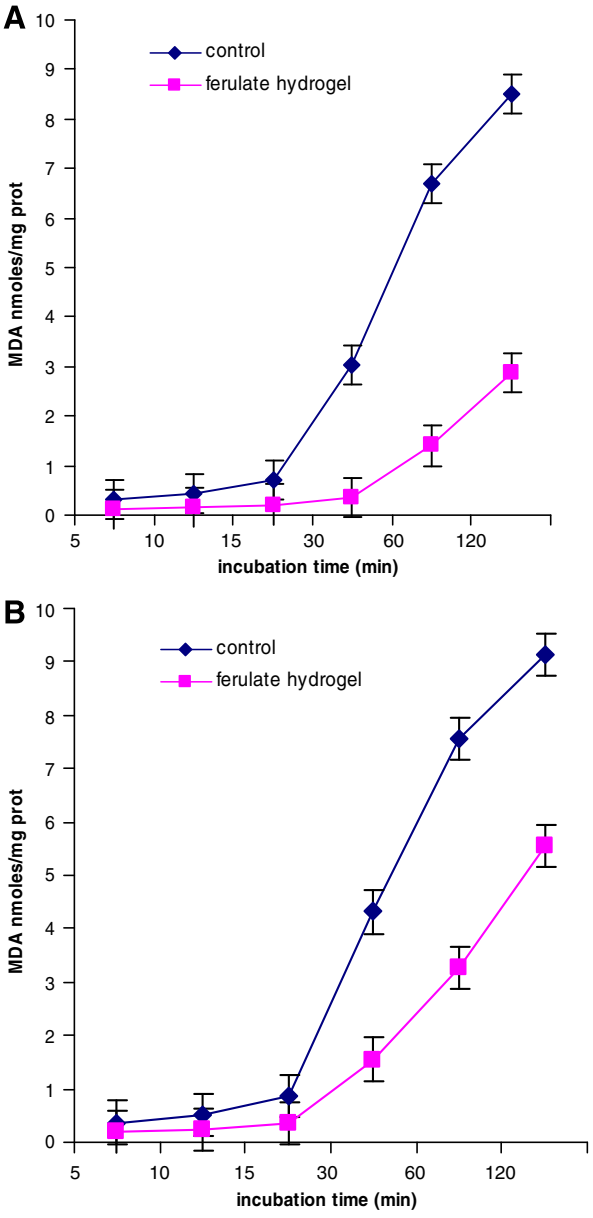

Fig. 3. Effects of ferulate hydrogel on MDA production induced by (A) *tert*-BOOH and (B) AAPH in rat-liver microsomal membranes. The microsomal membranes were incubated with 0.25×10^{-3} M *tert*-BOOH or 25×10^{-3} M AAPH at 37 °C under air in the dark. The results represent means ± SEM of six separate experiments.

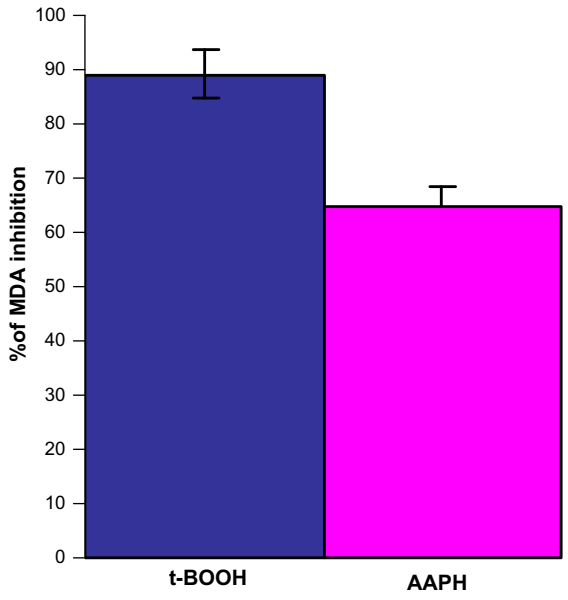

Fig. 4. Percentage of inhibition of *tert*-BOOH– and AAPH-induced MDA formation in the presence of ferulate hydrogel in rat-liver microsomal membranes after 30 min of incubation. The microsomal membranes were incubated with 0.25×10^{-3} M *tert*-BOOH or 25×10^{-3} M AAPH at 37 °C under air in the dark. The results represent means ± SEM of six separate experiments.

against a blank, in which DPPH was absent. All tests were run in triplicate and averaged. Ferulate hydrogel was found to be very efficient scavengers of DPPH radicals as showed in the inhibition curve (Fig. 2).

8. Antioxidant properties of hydrogels

Finally, we evaluated the antioxidant activity of cellulose hydrogels in inhibiting the lipid peroxidation in rat-liver microsomal membranes (Trombino et al., 2004), during 120 min of incubation, induced in vitro by two different sources of free radicals including AAPH and *tert*-BOOH. The same experiment was performed on a non-derivatized hydrogel and on a commercial *trans*-ferulic acid (data not shown). The results revealed that the hydrogel without ferulic groups has no antioxidant activity. The effects of cellulose derivatives on the lipid peroxidation were time-dependent and brought as nmol/mg proteins of MDA inhibition (Fig. 3). Ferulate hydrogel was a stronger antioxidant in protecting the membranes from *tert*-BOOH– than from AAPH-induced lipid peroxidation, showing in either case higher efficiency at 30 min of incubation (Fig. 4) and the preservation of antioxidant activity up to 2 h confirming results found previously (Trombino et al., 2008).

9. Conclusion

Antioxidant cellulose hydrogel was successfully prepared introducing FA moieties onto cellulose backbone. Two in vitro tests, the DPPH test, for direct free radical scavenging action, and the lipid peroxidation assay, for antioxidant activity, were used to assess its antioxidant properties. In fact, together both tests provide a better assessment of antioxidant properties.

The results suggested that ferulate material posses an excellent antioxidant and radical scavenger activity. For this reason, it could be well suited and sound approach to obtain carrier that could preserve drugs that tend to be unstable after prolongated exposure to air and light, during their vesiculation and release. On the other hand, our antioxidant hydrogel could also act as prodrug allowing a delivery of the *trans*-ferulic acid by means specific esterase. The so obtained antioxidant biopolymer could be used in cosmetic and pharmaceutical fields and would substantially reduce free radical damage and oxygen depletion.

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