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Research paper

A novel dextran hydrogel linking *trans*-ferulic acid for the stabilization and transdermal delivery of vitamin E

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ABSTRACT

Long-term exposure of the skin to UV light causes degenerative effects, which can be minimized by using antioxidant formulations. The major challenge in this regard is that a significant amount of antioxidant should reach at the site for effective photoprotection. However, barrier properties of the skin limit their use. In the present study, vitamin E (α-tocopherol) was loaded into a dextran hydrogel containing ferulic moieties, covalently linked, to improve its topical delivery, and also to increase its relative poor stability, which is due to direct exposure to UV light. Methacrylic groups were first introduced onto the dextran polymer backbones, then the obtained methacrylated dextran was copolymerized with aminoethyl methacrylate, and subsequently esterificated with *trans*-ferulic acid. The new biopolymer was characterized by Fourier transform infrared spectroscopy. The values of content of phenolic groups were determined. Its ability in inhibiting lipid peroxidation in rat liver microsomal membranes induced in vitro by a source of free radicals, that is *tert*-butyl hydroperoxide, was studied. Hydrogel was also characterized for swelling behaviour, vitamin E loading efficiency, release, and deposition on the rabbit skin. Additionally, vitamin E deposition was compared through hydrogels, respectively, containing and not containing *trans*-ferulic acid. The results showed that ferulate hydrogel was a more effective carrier in protecting vitamin E from photodegradation than hydrogel without antioxidant moieties. Then antioxidant hydrogel could be of potential use for cosmetic and pharmaceutical purposes as carrier of vitamin E that is an antioxidant that reduces erythema, photoaging, photocarcinogenesis, edema, and skin hypersensitivity associated with exposure to ultraviolet B (UVB) radiation, because of its protective effects.

1. Introduction

Dextran hydrogels have received an increased attention due to their variety of biotechnological and biomedical applications. Owing to their low tissue toxicity and high enzymatic degradability at desired sites, dextran hydrogels have been frequently considered as a potential matrix system for controlled release of bioactive agents. Several approaches to prepare dextran hydrogels have been adopted. Hydrogels were obtained by crosslinking dextran with either 1,6-hexanediisocyanate or glutaraldehyde [1,2]; reaction of dextran with glycidyl acrylate followed by polymerization of acrylated dextran [3]; methacrylation of dextran by transesterification of glycidyl methacrylate, and radical polymerization of methacrylated dextran in aqueous solution, using ammonium peroxydisulfate and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TMEDA) as initiator system [4,5]; methacrylation and acrylation of dextrans by reaction with methacrylic anhydride, and with bromoacetyl bromide and sodium acrylate, respectively [6,7]. Dextran hydrogels have been studied extensively in various areas, such as drug carriers. Due to their good tissue biocompatibility and the possibility of to transport specific drugs, they appear to be a viable alternative to the existing drug carriers [8–11]. Recently, researchers have realized and studied a variety of different dextran hydrogels as transdermal-delivery systems [12,13]. These hydrogels enhance the drug penetration improving the resulting pharmacological effects useful for the transdermal drug delivery that has always been challenged by the formidable barrier property of the intercellular lipid bilayer in the stratum corneum. For this reason, the idea is to use dextran hydrogel as a carrier of vitamin E, a topically administered antioxidant, that reduces erythema, photoaging, photocarcinogenesis, edema, and skin hypersensitivity associated with exposure to ultraviolet B (UVB) radiation [14–17]. It inhibits lipid peroxidation by preventing free radical generation, and/or reducing malondialdehyde [18]. Recently, the vitamin E is widely used in cosmetic products due to its obvious advantages for the skin [19]. However, its delivery through topical preparations such as creams, gels, lotions, and emulsion limits its effectiveness due to barrier properties of the skin, which hinder the drug deposition, and relative poor stability of vitamin due to direct exposure to UV light. Thus,

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the aim of this paper was to prepare and investigate a dextran hydrogel as a carrier to increase vitamin E deposition [20] and flux [21] on the skin. Moreover, to increase ulteriorly the stability of the vitamin E to the light, heat etc., we covalently linked *trans*-ferulic acid onto the dextran hydrogel, trapped vitamin E, and explored its topical delivery from the antioxidant carrier. 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 radical species, on the radical polymerization process. We submitted both antioxidant hydrogel and non-antioxidant hydrogel to the insult with a radical source such as *tert*-butyl hydroperoxide, and verified the stability of vitamin E after this treatment. Finally, we compared the Vitamin E deposition on the skin with that of analogous hydrogel not containing ferulic groups. In agreement with our expectations, the ferulate hydrogel was a more effective carrier in protecting vitamin E from photodegradation than hydrogel without antioxidant moieties.

2. Experimental

2.1. Materials

Dextran (Dex) (Fluka) (M_r = 15000–25000), vitamin E (α-Tocopherol (αT)) (Sigma), ferulic acid (FA) (Sigma), Folin-Ciocalteu reagent (FC reagent) (Fluka), and ammonium peroxydisulfate (APS) (Sigma) were used as received without further purification. *N*,*N*-dimethylformamide (DMF), pyridine (Py) aminoethyl methacrylate (AEMA), and methacryloyl chloride were supplied by Sigma, and were purified by the standard procedures. Lithium chloride (LiCl), dicyclohexylcarbodiimide (DCC), 4-hydroxybenzotriazole (HBT), *N*,*N*-dimethylaminopyridine (DMAP), potassium chloride (KCl), ethylenediaminetetraacetic acid (EDTA), sucrose, 4-2-hydroxyethyl-1-piperazineethanesulfonic acid (HEPES), trichloroacetic acid (TCA), hydrochloric acid, butylated hydroxytoluene (BHT), *tert*-butyl hydroperoxide (*tert*-BOOH), and 2-thiobarbituric acid (TBA) were supplied by Sigma (Sigma Chemical Co, St. Louis, MO), and were used as received. Methanol, ethanol, dihydrogen sodium phosphate, phosphoric acid, and acetonitrile were obtained from Fluka Chemika-Biochemika (Buchs, Switzerland) and Carlo Erba Reagents (Milan, Italy), and were used as received.

2.2. Measurements

^{1}H NMR and ^{13}C NMR spectra of dextran, dextran-methacrylate, FA, and AEMA were acquired on Bruker VM-300 ACP. Dextran, dextran methacrylate, and hydrogels, containing and not containing ferulic acid, were analyzed by FT-IR spectroscopy (Jasco 4200) using KBr disks. Estimation of Vitamin E was carried out using Hewlett Packard GC-MSD 5972, UV–Vis spectrophotometer (V-530 JASCO), and HPLC (Jasco BIP-I pump and Jasco UVDEC-100-V detector). Digital Micrometer Carl Mahr D7300 Essilingen A.N. was used for measurement of the membranes thickness.

2.3. Synthesis of dextran-methacrylate (Dex-MA)

A fixed amount (0.5 g) of dextran (**1**) was added to a LiCl/DMF (4% w/v) solvent mixture inside a reaction flask maintained under a dry nitrogen environment. The temperature of the oil bath was raised from room temperature to 120 °C over a period of 2 h, and the resultant mixture became a homogeneous gold-colored solution. The solution was cooled to room temperature, and pyridine was added as an acid acceptor. After 15 min, a calculated amount of methacryloyl chloride in a DMF solution was added slowly into the flask with constant stirring. The reaction was conducted at room temperature until completion. The reaction mixture was precipitated in an excess amount of cold ethanol. The product was filtered, washed several times with cold ethanol, dried at 40 °C in a vacuum oven for 2 days [22], and was characterized by FT-IR and ^{13}C NMR spectroscopy. Yield 0.64 g; substitution degree (DS) = 0.28. Varying the reaction conditions (time, temperature, and concentration), hydrogels with higher DS were obtained.

2.4. Preparation of the Dex-MA/AEMA hydrogel

The Dex-MA/AEMA hydrogel was obtained by free-radical polymerization of dextran-methacrylate (**2**) (0.500 g) and AEMA (0.209 g) using APS as an initiation system in an aqueous solution of NH_3/urea 2/12% w/w (2.5 g), a good solvent for poly-glucosidic systems, at 60 °C [23–25]. Briefly, dextran-methacrylate and AEMA were dissolved in an aqueous solution of NH_3/urea to obtain a final homogenous solution. Then, APS as an initiator was added to this solution, and mixed well for few minutes until a tacky hydrogel was obtained. The hydrogel was first washed with deionized water, and after with acetone to remove the unreacted dextran-methacrylate, AEMA, and solvents. Finally, the hydrogel was dried under vacuum at 50 °C for several days, and characterized by FT-IR spectroscopy. Yield 0.67 g.

2.5. Preparation of dextran hydrogels containing trans-ferulic acid

The dry hydrogel (0.5 g) was swollen in DMF/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, heated to 100 °C for 4 h, and then to room temperature overnight. The solid was filtered and washed with hot methanol, then with tetrahydrofurane, and acetone to remove the reaction sub-products as dicyclohexylurea and unreacted FA. The removal of all impurities was confirmed by HPLC and GC/MS analysis of the washing solvents. The antioxidant hydrogel was dried under vacuum at 50 °C, and characterized by FT-IR spectroscopy. Yield: 1.32 g. Amount of ferulic groups: 2.28×10^{-5} moles/g polymer.

2.6. Vitamin E loading by soaking procedure

Dex-MA/AEMA (0.1 g) hydrogels, containing and not containing ferulic moieties, were soaked, for 3 days at room temperature, and under magnetic stirring, in a drug solution. Vitamin E was solubilized in ethanol/water (4/1). The amount of drug solubilized was chosen in order to have a drug loading of 20% (w/w). After filtration, the hydrogels were dried at 10.1 mmHg until a constant weight was reached.

2.7. In vitro skin permeation

Permeation study (n = 3) was performed on the excised skin of sacrificed rabbit (New Zealand rabbits of 2.9–3.1 kg), which was obtained from a local slaughter's house, using Franz Diffusion cells. Ear skin of rabbit was shaved, and the skin was carefully separated. Subcutaneous fat was cautiously removed using a bistoury, and the skin samples were washed with saline solution (NaCl 0.9%), and frozen at −20 °C. The skin thus obtained was allowed to equilibrate with dissolution medium (ethanol/distilled water 20/80 solution) for 12 h before using it for permeation studies, and was mounted on Franz Diffusion cells having a surface area of 0.4614 cm^2, and on receptor compartment having a capacity of 5.5 ml. Epidermal side of the skin was exposed to ambient condition while dermal side was kept facing the receptor solution. The receptor compartment was filled with double distilled water containing ethanol (ethanol/double distilled water 20/80 solution) as diffusion medium (37 ± 0.5 °C). Reservoir solution was stirred, and the diffusion

cells were protected from light. Skin was saturated with dissolution medium for 1 h before the application of sample.

2.8. Swelling studies

Hydrogel without Vitamin E was placed on the stratum corneum side of the skin. The donor compartment of the cell was covered with laboratory film (Parafilm®) to prevent dehydration of the gel. At predetermined times, the swelling degree (W_t) was obtained by withdrawing the hydrogel, lightly drying it with filter paper, and by weighing it quickly in a tared sample bottle by means of an electronic balance ($\pm 10^{-4}$ g), then the hydrogel was placed back in the receiver. W_t was calculated by using Eq. (1) [26,27], where Ws and Wd are the weights of the swollen, and initial dry hydrogels, respectively. The extent of equilibrium swelling of the hydrogel was reached when the weight of the swollen hydrogel was constant. The fluid volume of the receiver department was maintained constant by the addition of double distilled water/ethanol 80/20. Each experiment was carried out in triplicate ($n = 3$).

$$W_t = \frac{(\mathrm{Ws} - \mathrm{Wd})}{\mathrm{Ws}} \cdot 100 \quad (1)$$

2.9. Permeability of Vitamin E

Vitamin E (0,045 g, $1{,}04 \cdot 10^{-4}$ mol) that was adequately dissolved in double distilled water/ethanol 80/20 solution was applied on the donor compartment, and was covered with laboratory film (Parafilm®). At specific intervals of time (0.5, 1.5, 2.5, 3.5, 4.5, 5.5, 6.5, and 24 h), 100 μl of receiver solution was withdrawn from the receiver compartment, and replaced with fresh distilled water/ethanol 80/20 solution in order to maintain the chemical gradient of the vitamin E between the two compartments, and to favour the spread (samples were kept in a freezer (−20 °C) until analyzed by UV–Vis spectrophotometer). Vitamin E amount released on the skin was assayed by UV–Vis spectrophotometry. Permeability measurements were performed in triplicate ($n = 3$).

2.10. Skin permeation of Vitamin E from hydrogels

Vitamin E-loaded hydrogels, containing and not containing ferulic acid, were placed on the stratum corneum side of the skin, and the donor compartment was covered with laboratory film (Parafilm®). The surface area of hydrogel was the active diffusion area. At specific intervals (1, 2, 3, 4, 6, and 24 h), 100 μl of receiver solution was withdrawn from the receiver compartment, and replaced with fresh double distilled water/ethanol (80/20). After 24 h, the hydrogels were recovered, and the still present vitamin E that was still present extracted in ethanol under sonication, and its amount was estimated by UV–Vis spectrophotometer. The drug concentration in the receiver solution samples was assayed by UV–Vis spectrophotometry. The concentration of Vitamin E in the receiver medium was always less than 15% of the maximum solubility of Vitamin E in the solvent system (22 mg/ml in 20/80 ethanol/water), thus sink conditions were maintained. All experiments were performed in triplicate ($n = 3$).

2.11. Prooxidant test

Antioxidant and non-antioxidant vitamin E-loaded hydrogels (0.1 g) were soaked for 2 h at room temperature, and under magnetic stirring, in 5 ml of water containing 500 μl of *tert*-BOOH (0.25×10^{-3} M). After filtration, the excess of solvent was removed by evaporation under reduced pressure, and the hydrogels were dried at 10.1 mmHg until a constant weight was reached. After freezing and lyophilization, the effect of prooxidant was evaluated. Precisely, insulted vitamin E-loaded hydrogels, linking and not linking ferulic moieties, were divided into three equal portions and were placed in three round-bottomed flask, containing a solution of water/ethanol 80/20, under stirring for 30, 60, and 120 min, respectively. After the selected times, the solvent contained in the different flasks was recovered by filtration, and analyzed through mass spectrometry and HPLC that was effected using as stationary phase a C18 3 μ SUPELCOSIL™ column (150 mm × 4.6 mm), and as eluent 0.01 M dihydrogen sodium phosphate/0.01 M phosphoric acid with acetonitrile (88:12 v/v), pH 2.3. The flow rate was set at 0.5 ml/min, and the detector wavelength was 292 nm.

The vitamin E standards of 1–1000 μg/ml were run for the external standardisation, and linear curves, with a correlation coefficient of 0.999, were generated from the area under the peak measurements. The Vitamin E retention time was 11.13 ± 0.2 min.

2.12. Determination of FA contents in ferulate Dex-MA/AEMA hydrogel

Folin-Ciocalteu (FC) method, based on the reduction of a phosphowolframate-phosphomolybdate complex by phenolics to blue reaction products, was used to determine phenolic compounds [28–30]. The sample was allowed to react with Folin-Ciocalteu's reagent and sodium carbonate solution at room temperature for 48 h in the dark. Absorption at 725 nm was measured, and the total phenolic content calculated as ferulic acid equivalents. An absorbance was measured twice for our sample against blank using 2×10^{-2} mmol/L ferulic acid as the standard ($R^2 = 0.9916$).

2.13. MA contents in Dex-MA by volumetric analysis

A sample of 50 mg 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 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 Eq. (2) [31], where $n_{\text{free ester}}$ is $(V_{2^\circ\ \text{e.p.}} - V_{1^\circ\ \text{e.p.}}) * [\text{HCl}]$; $\mathrm{MM_{glucose\ unit}}$ is the molecular mass of glucose unit; g_{sample} is the weight of sample; $n_{\text{free ester}}$ is the mol of free ester; $\mathrm{MM_{free\ ester}}$ is the molecular mass of free ester, and $\mathrm{MM_{H2O}}$ is the molecular mass of water.

$$\mathrm{DS} = \frac{\mathrm{MM_{glucose\ unit}}}{(g_{\text{sample}}/n_{\text{free ester}}) - (\mathrm{MM_{free\ ester}} - \mathrm{MM_{H_2O}})} \quad (2)$$

2.14. Microsomal suspensions preparation

Liver microsomes were prepared from Wistar rats by tissue homogenization with 5 volumes of ice-cold 0.25 M sucrose containing 5 mM Hepes, 0.5 mM EDTA, pH 7.5 in a Potter–Elvehjem homogenizer [32]. Microsomal membranes were isolated by the removal of the nuclear fraction at 8000*g* for 10 min, and by the removal of the mitochondrial fraction at 18,000g for 10 min. The microsomal fraction was sedimented at 105,000g for 60 min, and the fraction was washed once in 0.15 M KCl, and was collected again at 105,000g for 30 min [33]. The membranes, suspended in 0.1 M potassium phosphate buffer, pH 7.5, were stored at −80 °C. Microsomal proteins were determined by the Bio-Rad method [34].

2.15. Addition of Dex-MA/AEMA hydrogel to microsomes

Aliquots of hydrogel containing and not containing FA in the range of 0.5–6 mg/ml were added to the microsomes. The microsomes were gently suspended by a Dounce homogenizer, and then

the suspensions were incubated at 37 °C in a shaking bath under air in the dark.

2.16. Malondialdehyde formation

Malondialdehyde (MDA) was extracted and analyzed as indicated [35]. Briefly, aliquots of 1 mL of microsomal suspension (0.5 mg proteins) were mixed with 3 mL of 0.5% TCA and 0.5 mL of TBA solution (two parts 0.4% TBA in 0.2 M HCl and one part distilled water), and 0.07 mL of 0.2% BHT in 95% ethanol. Samples were then incubated in a 90 °C bath for 45 min. After incubation, the TBA-MDA complex was extracted with 3 mL of isobutyl alcohol. The absorbances of the extracts were measured by the use of UV spectrophotometry at 535 nm, and the results were expressed as mmol per mg of protein, using an extinction coefficient of 1.56×10^5 l mmol^{-1} cm^{-1}.

3. Results and discussion

3.1. Synthesis of dextran-methacrylate

With the aim of taking advantage of the antioxidant properties of the FA, a hydrogel containing this residue has been synthesized. The synthesis of hydrogel precursor was carried out with dextran and methacryloyl chloride in the presence of pyridine as catalyst (Fig. 1). The methacrylated dextran was isolated, purified, and characterized by FT-IR spectroscopy. Depending on the reaction conditions (time, temperature, and concentration), methacrylic derivatives with different DS were obtained (from 0.28 to 0.82) and evaluated by volumetric analysis, and the derivative with lower DS was used. In fact, the literature data show that the highly substituted hydrogels reach equilibrium swelling faster than the other hydrogels. Swelling ratios depend very much upon the DS of the dextran-methacrylate. As the DS of the dextran-methacrylate hydrogels increases, their swelling ratios decreased in all pH ranges [36].

3.2. Preparation of ferulate hydrogel

NH_3/urea aqueous solution of dextran-methacrylate (Dex-MA) with DS of 0.28 has been crosslinked in the presence of aminoethyl methacrylate (AEMA) by free-radical polymerization using APS, to initiate the formation of radicals, as reported in Fig. 2. To make the obtained hydrogel antioxidant, characterized by FT-IR, FA was bonded. The synthesis was carried out from *trans*-ferulic acid and hydrogel, by condensation in the presence of *N*,*N*-dimethylaminopyridine as catalyst using DCC and HBT as condensing agents (Fig. 3). After purification, antioxidant hydrogel was characterized by FT-IR spectroscopy. A detailed study of its content of ferulic moieties was performed by the FC test, a colorimetric assay that requires few reagents and relies on the use of the free ferulic acid as the standard compound. Our results showed a value of ~0.4% that corresponds to 2.36×10^{-5} mol of ferulic groups.

3.3. Characterization

The Dex, Dex-MA, Dex-MA/AEMA hydrogel, and hydrogel containing FA were analyzed by FT-IR spectroscopy. All samples were tested as compressed KBr pellets (polymer: KBr 51: 10 w/w). Dex-MA with DS of 0.28 showed an ester FT-IR band at 1735 cm^{-1}. Clearly, this ester band increased sharply with an increase in the degree of substitution of the methacrylate group to dextran. The presence of pendant vinyl groups in dextran-methacrylate was confirmed by the FT-IR bands at 1636 cm^{-1} (C=C) and 803 cm^{-1}

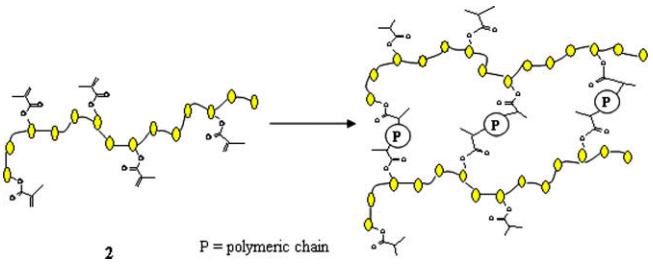

Fig. 2. Schematic representation of the polymerization of Dex-MA with AEMA.

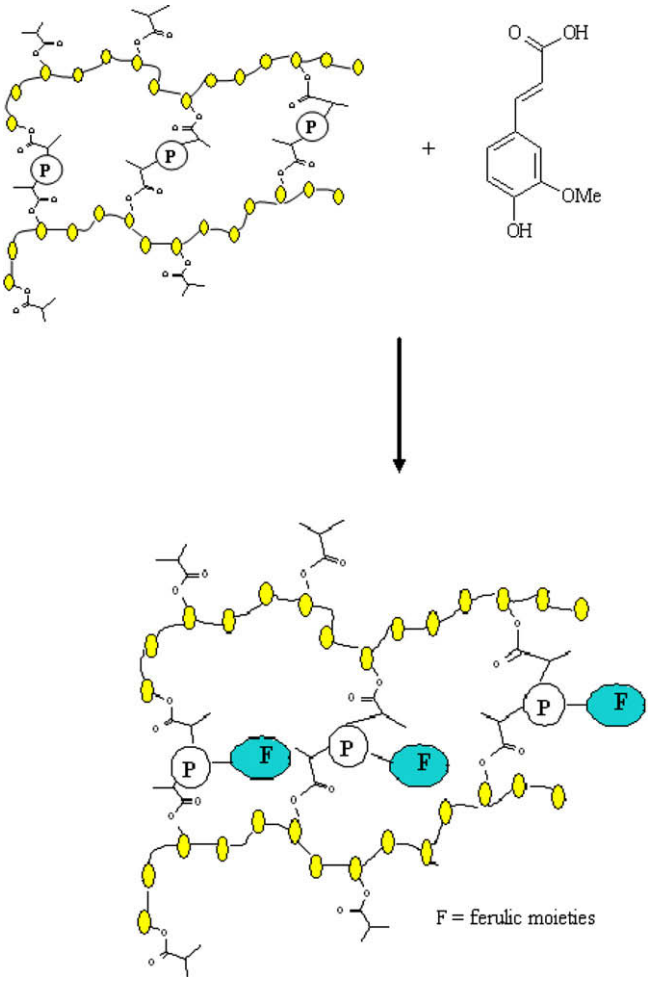

Fig. 3. Synthesis of ferulate hydrogel.

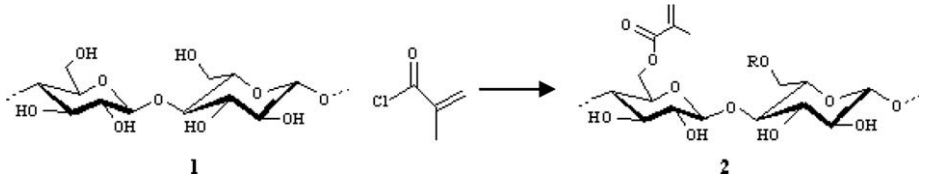

Fig. 1. Representative route to Dex-MA.

(C=C–H). The presence of pendant vinyl groups in dextran-methacrylate, was confirmed further by the ^{1}H NMR spectrum. There were two distinctive peaks in the double bond region (5.690 and 6.115 ppm) which correspond with the two hydrogens adjacent to double bond (C=CH_2). These two peaks were not present in the spectrum of the original dextran. The hydrogens of the methyl substitute (CH_3) in the methacrylate group also were observed as a single peak, at 1.903 ppm.

The polymerization of Dex-MA with AEMA was confirmed by FT-IR spectra that showed the consumption of double bonds as a result of crosslinking. Precisely, a decrease in peak intensity in the double bond region at 1636 and 803 cm^{-1} and the appearance of a new ester band at 1722 cm^{-1} due to a formation of hydrogel between Dex-MA and AEMA was observed.

The functionalization of amino groups of the hydrogel with FA gave a material that when analyzed by infrared spectroscopy showed an additional characteristic peak of carboxylic group of amide at 1650 cm^{-1}.

3.4. Swelling behaviour

Swelling behaviour of Dex-MA/AEMA hydrogel, through the ear skin of rabbits, was measured at a constant temperature of 37 °C. In this kind of experiment, Franz diffusion cells are mainly used, and the skin was mounted on the receptor compartment with the stratum corneum side facing upwards into the donor compartment containing water/ethanol solution 80/20. Fig. 4 shows the sorption of solution with the time of treatment. The curve plot clearly indicated that the swelling degree of the hydrogel slowly increased until 24 h.

3.5. Antioxidant properties of Hydrogels

The ability of ferulate hydrogel to protect against lipid peroxidation induced by the *tert*-BOOH, a source of free radicals, was examined in rat-liver microsomal membranes during an incubation period of 120 min [37]. In order to evaluate the antioxidant properties of the non-derivatized polymeric structure, the same experiment was performed on hydrogel without ferulic moieties. The data revealed that this last material has no antioxidant activity. The effects of antioxidant hydrogel on the lipid peroxidation were time-dependent and effected as the MDA production (in nmol mg^{-1} protein) (Fig. 5). Our ferulate material was a strong antioxidant in protecting the membranes from tert-BOOH-induced lipid peroxidation showing a higher efficiency at 30 min of incubation, and the preservation of antioxidant activity up to 2 h.

3.6. Prooxidant test remarks

We exposed hydrogels bearing vitamin E and bonding and not bonding ferulic acids to *tert*-BOOH a free radical generator.

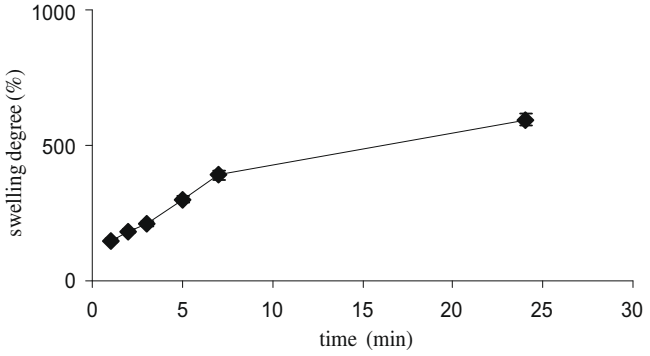

Fig. 4. Swelling degree of Dex-MA/AEMA hydrogel.

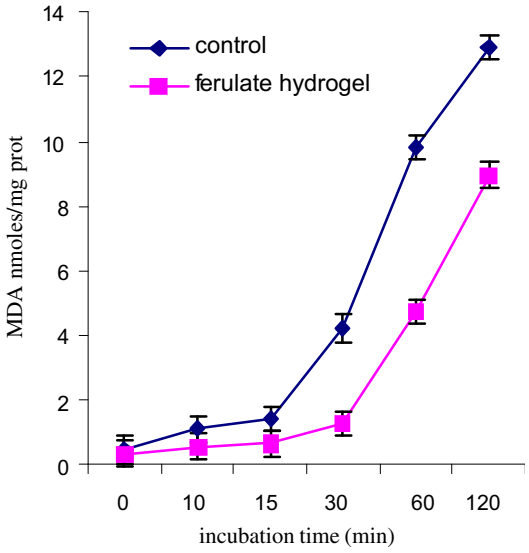

Fig. 5. Effects of ferulate hydrogel on malondialdehyde (MDA) production induced by tert-BOOH in rat liver microsomal membranes. The microsomal membranes were incubated with 0.25×10^{-3} M tert-BOOH at 37 °C under air in the dark. The results represent the mean ± SEM of six separate experiments.

The antioxidant activity of both ferulate and not hydrogels vitamin E-loaded was evaluated after their prooxidation with *tert*-BOOH and vitamin E release in water/ethanol 80/20. The results suggested that after the drug release (120 min), non-ferulate hydrogel possesses 2% antioxidant activity, perhaps due to the presence of small vitamin E traces. On the contrary, the ferulate hydrogel showed a higher antioxidant activity analogous to non-ferulate hydrogel but a minor activity than non-loaded antioxidant hydrogel (Fig. 6) confirming that the ferulic moieties protected the vitamin E during the prooxidation, and its release, as unequivocally confirmed by GC/MS analysis. In fact, as shown in the mass spectrum of Fig. 7a, vitamin E (retention time ~29.9) was almost integrally released already after 30 min from the hydrogel containing ferulic moieties. Analogous behaviour was observed at 60 and 120 min. On the contrary, the hydrogel without antioxidant groups released, in the same conditions, only a small amount of vitamin E and a great deal of unidentified products (Fig. 7b). The same results (not shown) were observed through HPLC.

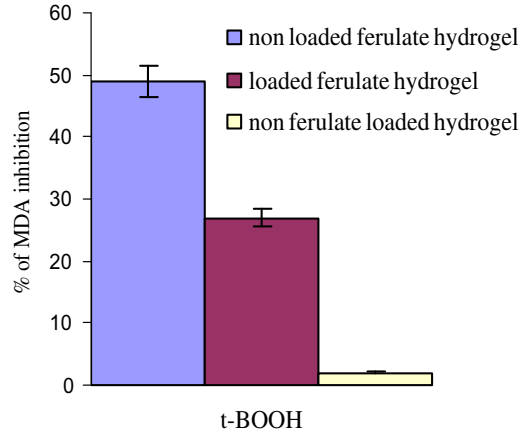

Fig. 6. Antioxidant activities of hydrogels after prooxidant test.

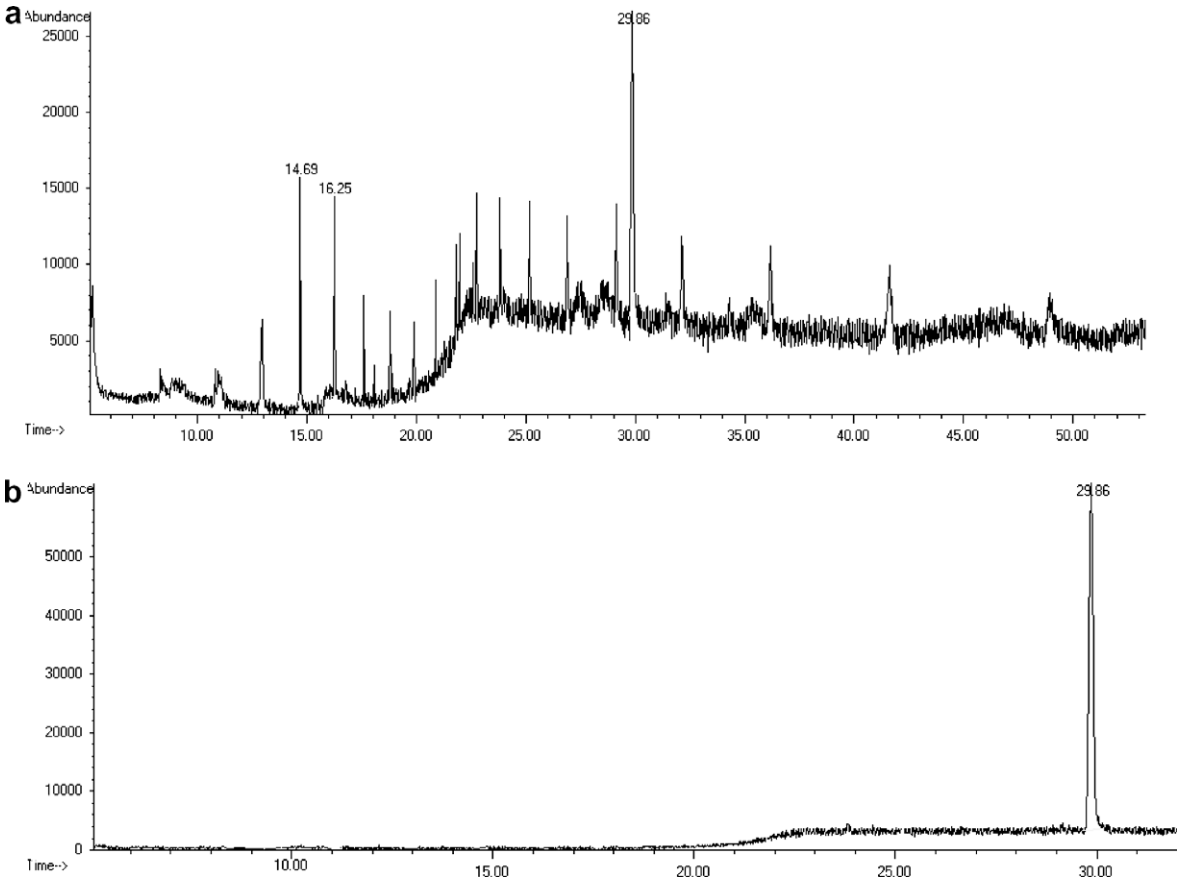

Fig. 7. GC-MS spectra of the 30 min stirred solution containing (a) insulted non-ferulate vitamin E-loaded hydrogel; (b) insulted ferulate vitamin-E loaded hydrogel.

3.7. Drug release

Permeation of vitamin E from vitamin E-loaded hydrogels through the rabbit ear skin was studied for hydrogels containing and not containing ferulic loaded with 22 mg of the drug. In both cases, the vitamin E flux value is lower when the drug is included in the hydrogels than when it is dissolved in a ethanol/water (20/80) solution, since the drug must be first released from the gel and then permeate the skin. Particularly, the results indicated that when vitamin E was released from drug-loaded hydrogel without ferulic groups, only a small amount of vitamin E remained intact. In fact, UV–Vis analysis revealed that the most part of products in the receiver compartment was unidentified. On the other hand, permeation studies of antioxidant hydrogel showed that almost the total amount of vitamin E that permeated unchanged into and through the rabbit ear skin was of 65%, and it took place in 24 h. In particular, we observed that the vitamin E was released in decreasing way until 3 h. After that, the release increased with time. This behaviour was probably due to the initial release of the vitamin E-loaded hydrogel in proximity of the hydrogel surface, followed by the release of that one loaded in depth. The release of the inner-loaded portion of vitamin E is determined by the swelling rate of the hydrogel.

The amount of vitamin E from antioxidant hydrogel permeated per diffusion area as a function of time was determined by UV–Vis spectrophotometer is shown in Fig. 8.

This result has demonstrated that ferulic groups on the polymeric matrix prevent the effects of oxidative degradation preserving the drug.

4. Conclusions

Antioxidant dextran hydrogel was successfully prepared introducing FA moieties onto Dex-MA crosslinked with AEMA. Its antioxidant activity was evaluated through rat liver microsomal membranes. The results suggested that ferulate material possesses an excellent antioxidant activity. Moreover, preparation of ferulate hydrogel-based dextran was found to be well suited and a sound

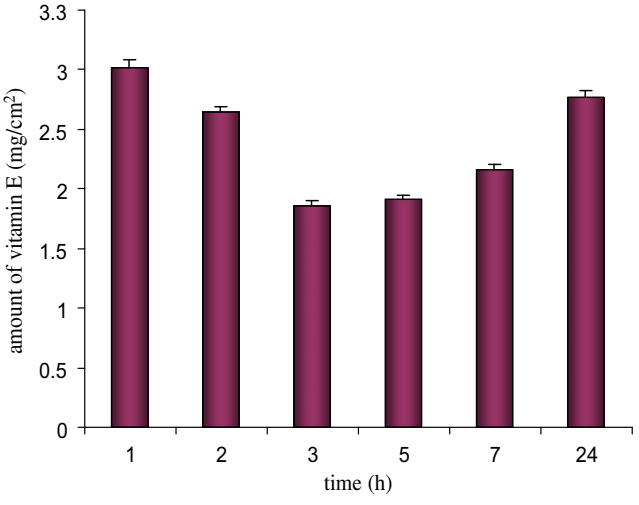

Fig. 8. Release of entrapped vitamin E from ferulate hydrogel through a rabbit ear skin.

approach to obtain carrier that preserves Vitamin E during its release. Linking FA in the hydrogel greatly influenced the drug deposition on the ear skin of rabbit. In fact, we observed increased drug deposition suggesting that the ferulic groups protect the drug during the deposition with respect to the analogous hydrogel without antioxidant moieties. Therefore, it can be said that our antioxidant hydrogel could be an adequate system for the controlled release of vitamin E in the human skin. Regarding that it is acknowledged that a photodamaged and/or age-damaged skin is characterized not only by the presence of radical oxygen species, but also by a lower quantity of lipids. For this reason, it could be advisable to use preparations that contribute to reestablishing the lipidic equilibrium of the stratum corneum as well as having some kind of molecules, such as vitamin E, with an antioxidant effect.

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