Reactive & Functional Polymers 73 (2013) 1030–1037

Contents lists available at SciVerse ScienceDirect

Reactive & Functional Polymers

journal homepage: www.elsevier.com/locate/react

Stabilization of oxidable vitamins by flavonoid-based hydrogels

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ARTICLE INFO

Article history:
Received 7 January 2013
Received in revised form 10 April 2013
Accepted 1 May 2013
Available online 9 May 2013

Keywords:
Hydrogels
Oxidative damage
Drug stability
Folic acid
Thiamine

ABSTRACT

This study has the goal to evaluate the stability of B complex vitamins, loaded into catechin-based cross-linked hydrogels, in aqueous medium at accelerated conditions of oxidative stress. The formulations were prepared by coupling polyacrylic acid with catechin in the presence of dicycloexylcarbodiimide. Folic acid (FA) and Thiamine (TH) were chosen within the B complex vitamins because of the well-known oxidative degradation characteristics. The protective role of the hydrogels against the oxidation damage was monitored inducing oxidative stress, by means of UV irradiation and *t*-butyl hydroperoxide treatment for FA and TH, respectively. The characterization of the hydrogels was obtained by morphological, calorimetric, FT-IR analyses, antioxidant assays and evaluation of the swelling behavior in aqueous media at different pH. The experimental results confirmed the suitability of the hydrogels as pH-responsive devices, furthermore the presence of oxidative condition proved the protective effect of the hydrogels. Catechin-based polymers gave a pronounced improvement in the stability of vitamins compared to hydrogels prepared by the coupling reaction of polyacrylic acid in the presence of 1,6-hexandiol. Under oxidative conditions, FA and TH were preserved by 96% and 60% respectively.

1. Introduction

Hydrogels have received considerable attention in many biomedical fields such as drug delivery system, tissue engineering, and biomedical devices [20,1]. This versatile applicability is attributable to their biodegradability and biocompatibility [15], but also to the tunable physic-chemical properties tailoring by the use of a wide variety of available synthetic monomers ([10,8,25,29].

An ideal polymeric carrier should improve the therapeutic performance of a specific bioactive molecule [13]. Despite the many efforts done in the last decade to improve the drug-protecting ability of polymeric carriers, some therapeutics are easily denatured or degraded during storage, topical administration or circulation in the body, and they lose their therapeutic efficacy before reaching the target site [24]. Degradation phenomena can be easily caused by heat, oxygen, pH, radical species, irradiation or the combination of these factors [28]. Among the easily degradable bioactive molecules, the B complex vitamins, and Folic acid (FA) and Thiamine (TH) in particular, occupies a relevant position because of the well-known oxidative degradation characteristics [3,19].

Folic acid is a vitamin playing a central role in DNA synthesis and repair. It exists in a number of different naturally occurring forms: as folate in different food, while Folic acid represents the synthetic form of the vitamin used in the supplements [22]. Many evidences indicate that an adequate folate intake is crucial for a variety of physiological processes and that folates are essential for human health in the prevention of megaloblastic anemia and neural tube birth defects and play an important role in cardiovascular diseases and cancer. Furthermore, it has been hypothesized that skin cells might benefit from an exogenous FA supplementation [14]. From a chemical point of view, FA consists of a pterin ring system, a p-aminobenzoic acid portion, and the aminoacid glutamic acid. Upon irradiation, FA decomposes through cleavage of the bond between the p-aminobenzoic acid portion and pterin ring system [17].

Thiamine is involved in numerous body functions, including nervous system and muscle functioning, and it is found at high concentrations in cereals, yeast, beef, pork, nuts, whole grains, etc. Deficiency of TH can develop beriberi, a disease characterized by heart, nerve and digestive disorders [16]. The structure of TH consists of substituted pyrimidine and thiazole rings linked by a methylene bridge. Thiamine can be oxidized by both weakly oxidant agents (e.g. dilute hydrogen peroxide, iodine, and air) carrying out to the opening of the thiazole ring, and more vigorous agents (e.g. potassium permanganate or manganese dioxide, potassium ferricyanide or hydrogen peroxide) producing the thiochrome compound [6].

Various methods have been proposed to protect the vitamins against the oxidative damages. Appropriate correct storage conditions can help to protect the B complex vitamins [2], and a pronounced improvement in the stability of water soluble vitamins was observed at proper pH and in the presence of maltitol [23]. Dif-

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http://dx.doi.org/10.1016/j.reactfunctpolym.2013.05.001

ferent polymeric systems were proposed as vitamins protecting agents, in particular, solid-state polyester nanoparticles were prepared to improve the thermal and chemical stability of retinol [4,9]. Alternatively, casein micelles were proposed as natural nanovehicles for vitamin D against thermal and oxidative degradation [11].

The aim of the present study was the preparation and characterization of catechin-based hydrogels, potentially useful in the stabilization of FA and TH under conditions of oxidative stress in aqueous media at different pH values. The hydrogels were prepared by coupling of poly acrylic acid (PA) and catechin (CT) in the presence of *N*,*N*′-dicyclohexylcarbodiimide (DCC), as condensing agent (Fig. 1).

After the characterization of the materials in term of physic-chemical and antioxidant properties, their protective role against the vitamins oxidation damages was monitored performing experiments in different oxidative conditions. In particular, FA was stressed by UV irradiation at a suitable wavelength, while an oxidative stress was simulated by treatment of TH with *t*-butyl hydroperoxide (*t*-BOOH). Results showed that the CT-based polymers carried out to a pronounced improvement in the stability of vitamins in comparison to the hydrogels prepared in the same experimental conditions, but using 1,6-hexandiol as crosslinker in place of CT.

2. Materials and methods

2.1. Materials

Polyacrylic acid (PA) (10^6 g mol^{-1}), (+)-Catechin hydrate (CT), *N*,*N*′-dicyclohexylcarbodiimide (DCC), 1,6-hexanediol (ED), Folin–Ciocalteu reagent, 2,2′-diphenyl-1-picrylhydrazyl radical (DPPH), 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid), ammonium molybdate, Folic acid (FA), Thiamine (TH), *t*-butyl hydroperoxide (*t*-BOOH), trisodium phosphate, disodium hydrogen phosphate, sodium dihydrogen phosphate, potassium dihydrogen phosphate, potassium persulfate, hydrochloric acid, sulfuric acid were obtained from Sigma–Aldrich (Sigma Chemical Co., St. Louis, MO, USA). Acetone, methanol, water, ethanol, *N*,*N*-Dimethylformamide (DMF), diethyl ether, were reagent-grade or HPLC-grade and provided by Carlo Erba reagents (Milan, Italy).

2.2. Instrumentation

FT-IR spectra were recorded as pellets in KBr in the range 4000–400 cm^{-1} using a Jasco FT-IR 4200 spectrophotometer (resolution 1.0 cm^{-1}). The morphology of the samples was analyzed by utilizing a Scanning Electron Microscope (NOVA NanoSEM 200 [0–30 kV], Comm. FEI Company, Hillsboro, OR, USA). Samples were prepared by deposition onto self-adhesive, conducting carbon tape (Plano GmbH, Wetzlar, Germany). The calorimetric analyses were performed using a Netzsch DSC200 PC. The analyses were performed on the dry samples (20 mg) from 80 to 300 °C under constant purge of nitrogen with a flow rate of 25 mL min^{-1} and a heating rate of 10 °C min^{-1}. The HPLC analyses were carried out using a Jasco PU-2080 liquid chromatography equipped with a Rheodyne 7725i injector (fitted with a 20 μL loop), a Jasco UV-2075 HPLC detector setted at 280 nm for FA and 254 nm for TH, and a Jasco–Borwin integrator. A reversed-phase C_{18} column (μBondapak, 10 μm of 150 × 4.6 mm internal diameter obtained from Waters) was used as stationary phase, while the mobile phases for both the vitamins consist of a mixture of 0.1 mol L^{-1} KH_2PO_4 (pH 7.0)-methanol, 90:10, running isocratically at 0.7 mL min^{-1} [7]. UV–Vis absorption measurements were performed with a Jasco V-530 UV/Vis spectrometer. A freeze drier apparatus Micro Modulyo, Edwards was employed.

2.3. Synthesis of hydrogels

The synthesis of CT-based hydrogels was performed as follows: 125 mg of PA were dissolved in 10.0 mL of DMF in a thich-walled glass tube, and then CT and DCC were added. The tubes were sealed and incubated at 60 °C for 24 h. After the reaction time, the polymers were extensively washed with DMF and ethanol (3 × 50 mL), dried under vacuum at 40 °C overnight, then grounded into powder and sieved through a 63 μm stainless steel sieve. The sieved materials were collected and washed with ethanol, distilled water and diethyl ether (3 × 50 mL) to remove unreacted species, frozen and dried with "freezing–drying apparatus" to afford vaporous solids.

The same experimental conditions were used for the synthesis of blank hydrogels, where ED was used instead of CT. The experimental conditions used for the synthesis of each hydrogel were reported in Table 1.

2.4. Water content measurements

The swelling characteristics of microparticles were determined to check hydrophilic affinity of hydrogels. Typically, aliquots (40–50 mg) of the microparticles, dried to constant weight, were placed in a tared 5-mL sintered glass filter (∅10 mm; porosity, G4), weighted, and left to swell by immersing the filter plus support in a beaker containing the swelling media (HCl 0.1 mol L^{-1} and PBS solution, pH = 7.4, at 37 °C). After 24 h, the excess water

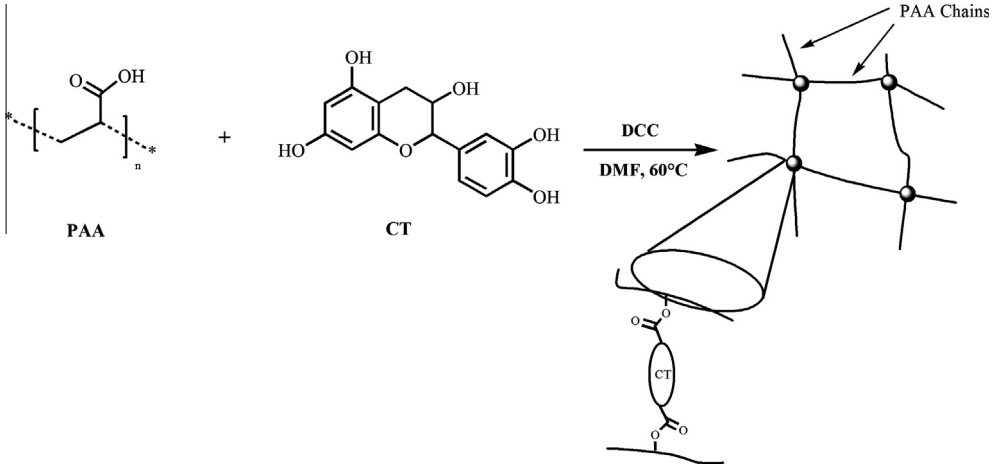

Fig. 1. Synthesis of catechin based hydrogels.