



Intercalation of the radical scavenger ferulic acid in hydrotalcite-like anionic clays

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Received 27 June 2004; received in revised form 10 January 2005; accepted 18 January 2005

Available online 31 March 2005

Abstract

Hydrotalcite is a biocompatible lamellar anionic clay formed by double hydroxide layers with a metal cation coordinating four OH groups. The different layers are held together by anionic hosts that can be replaced by a simple ion-exchange process. The synthetic Mg–Al-hydrotalcite was used to intercalate ferulic acid, a compound that shows antioxidant properties due to its free radical scavenger capacity. Analysis of the intercalated compound showed a good intercalation percentage (35.53%) accompanied by an increase of the interlayer space from 7.8 Å (chloride form) to 17.1 Å due to the presence of the ferulate. The intercalation product was stable in water, did not show any significant degradation after UV-irradiation, had a higher capacity of UV absorption in comparison to both the pure ferulic acid and ferulic acid–hydrotalcite chloride physical mixture. The intercalated compound was formulated in a siliconic cream and the ferulate in vitro release profiles determined.

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Keywords: Ferulic acid; Mg–Al hydrotalcite; Antioxidant; UV absorber; Photochemical analysis; In vitro release

1. Introduction

It is well established that UV radiation represents the most important physical attacks to which the skin is exposed. Biologically active solar radiation is mainly represented by UVB and to lesser extent by UVA. UVC

radiation from sunlight does not reach the surface of the earth and hence it is not responsible for skin damage (Graf, 1992; Saija et al., 1999, 2000). UVB radiation penetrates deeply into the skin to the basal layer while UVA radiation reaches the *chorion derma* (Coppini et al., 2001) causing modification of skin layers that may be responsible for cancer insurgence and skin ageing increase (Saija et al., 1999; Bonina et al., 1996). In particular, reactive oxygen species cause most of the deleterious effects of UV light on skin because they provoke

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a severe decrease of its natural antioxidant composition. Moreover, an overproduction of nitric oxide by the keratinocytes leads to erythema and inflammation process (Deliconstantinos et al., 1996; Romoro-Graillet et al., 1997).

Many molecules can be used to protect the skin from the sun damage and among these, ferulic acid (FERH), 4-hydroxy-3-methoxycinnamic acid, was found to be promising as active substance for sunscreen formulation (Graf, 1992; Castelluccio et al., 1995). Its antioxidant properties derive from its free radical scavenging ability (Graf, 1992). FERH acts as scavenger against hydroxyl and peroxy radicals and superoxide anions, inhibiting propagation of lipid peroxidation chain reaction. It also reduces the UVB-induced erythema, because of its high effectiveness in scavenging nitric oxide (Saija et al., 2000, 1999), and provides a high degree of skin protection acting as UV-absorber screen (Graf, 1992). FERH is already used in several countries, such as Japan, as a food preservative (Tsuchiya and Takasawa, 1975), as an active ingredient in many skin lotions, sunscreens and pharmaceutical preparations (Graf, 1992; Compton and Laszlo, 2002) although the Food and Drug Administration (FDA) did not establish its status yet. However, its usefulness is strongly limited by its low stability after exposure to air and light. For this reason, the aim of the present paper was to use a suitable inorganic matrix to protect FERH from environmentally induced degradation. In this respect, Mg–Al–hydrotalcite (HTlc) was taken into account because it could play a double role of FERH protector and potential physical sunscreen.

HTlc is a lamellar inorganic solid characterized by positively charged layers and interlayered charge balancing anions. The HTlc layers are composed of metal cations located in the center of octahedral structures and of hydroxide anions at its vertices. Each hydroxide anion is shared by three octahedral cations and the hydrogen points towards the interlayer region (Allmann, 1970). Synthetic HTlc has the general formula $[M(II)_{1-x}M(III)_x(OH)_2]^{x+}[A^{n-}]_m^{x-} mS$, where M(II) is a divalent metal cation (Mg, Zn, Co, Ni, Mn, . . .) and M(III) is a trivalent metal cation (Al, Cr, Fe, V, Co, . . .); A^{n-} is the charge compensating anion (CO_3^{2-} , SO_4^{2-} , Cl^- , NO_3^- , . . .); m is the number of moles of co-intercalated solvent (S) which normally is water (Costantino et al., 1998). The interlayer distance depends on the nature of the interlayer anions (Cavani

et al., 1991) which may be replaced by ion-exchange processes.

HTlc is used as catalyst, catalyst precursor and support, anionic exchanger, adsorbent, additive (Cavani et al., 1991; Meyn et al., 1990), as pharmaceutical and cosmetic formulation stabilizer (Xu et al., 2001; Ooishi et al., 1993; Ueno and Kubota, 1987) and, recently, it was also used for the preparation of modified release formulation (Ambrogi et al., 2001, 2002, 2003; Nakayama et al., 2003).

Considering the physico-chemical characteristics of FERH, its intercalation into HTlc was hypothesized.

In this paper we describe the intercalation of FER into HTlc in its chloride form. The photochemical behavior of the intercalated compound (HTlc–FER) was investigated to assess its UV absorption and its photostability. FER release from a HTlc–FER loaded formulation was monitored in a phosphate buffer solution (PBS) (pH 5.5), ultrafiltered water and artificial sea water (pH 8) in order to simulate the use conditions of a prepared cosmetic formulation.

2. Materials and methods

2.1. Materials

FERH was purchased from Fluka (Milan, Italy). Abil WE-09 (polyglyceryl-4-isostearate, cetyl dimethicone copolyol, hexyl laurate), Isolan GI-34 (polyglyceryl-3 isostearate), Abil wax 9801 (cetyl dimethicone) were purchased from Goldschmidt (Essen, Germany). Castor oil was purchased from Galeno (Prato, Italy). Ceresine wax SP 252 (solid paraffin) was purchased from Carlo Erba (Milan, Italy). Silicone DC 245 (cyclomethicone) and liquid paraffin were gifts from Dow Corning (Seneffe, Belgium). Other chemicals and solvents were of reagent grade purity and used without further purification.

2.2. HTlc–Cl preparation

The HTlc–Cl was prepared from the carbonate form. The Mg–Al–HTlc– CO_3 was prepared by adding solid urea to an aqueous 0.5 M $MgCl_2/AlCl_3$ solution. Urea/Mg + Al (molar ratio 3.3) and Al/Mg + Al (molar ratio 0.33) were used. The hydrolysis of urea, inducing slow pH increase, led to the precipitation of metals in

well crystallized HTlc carbonate form (Costantino et al., 1998). The obtained solid was recovered, washed with water to eliminate chlorides and stored in desiccator (CaCl₂) under vacuum at room temperature. The quantification of magnesium and aluminium contents was accomplished by EDTA titration of a HTlc–CO₃ aqueous solution (50 ml) obtained by dissolving the product with a proper amount of HCl 0.1N lowering the pH below 5. In these pH conditions the ion content can be measured because the matrix is soluble below pH 5.

Pure crystalline Mg–Al–HTlc–Cl was obtained by CO₃²⁻/Cl⁻ ion-exchange by titration with a dilute solution of HCl (Reichle, 1986).

2.3. Intercalation of FER into HTlc–Cl

HTlc–Cl (0.74 g) was suspended in 60 ml of a degassed aqueous solution of ferulate sodium salt (FER⁻Na⁺ 1.36 g), obtained by adding stoichiometric equivalents of carbon dioxide free 0.1N NaOH to FERH (Cl⁻:FER⁻ molar ratio 1:2), at 60 °C and 140 rpm (Ambroggi et al., 2001) for 4 days in a Gallenkamp orbital incubator INR 2000 (Gallenkamp, UK) in the dark and under nitrogen atmosphere to protect the reagents from light and air.

HTlc–FER was centrifuged in an ALC centrifuge 4236A (ALC, Milan, Italy) at 4000 rpm for 5 min and washed, first with degassed water, then with ethanol 95% and finally with degassed water. The product was dried in a desiccator (CaCl₂) under vacuum.

2.4. Characterization of the intercalated product

HTlc–FER was analyzed by X-ray powder diffraction (XRPD) with a computer controlled PW 1710 Philips diffractometer (Philips, Netherland), using the Ni-filtered Cu K α radiation. Thermal behavior of HTlc–FER was studied by differential scanning calorimetry (DSC) with an automatic thermic analyzer Mettler Toledo DSC 821e (Mettler, Milan, Italy) at heating rate of 10 °C min⁻¹. Quantitative determination of intercalated FER was performed by thermogravimetric analysis (TGA) with a Stanton-Redcroft STA 780 thermoanalyzer (Stanton-Redcroft, UK) at heating rate of 5 °C min⁻¹ in airflow. FER content in HTlc was confirmed by a UV-Vis spectrophotometer Jasco-V-520 (Jasco, Tokyo, Japan) at 310 nm after de-

struction with HCl of a known amount of HTlc–FER and its further dissolution in PBS pH 7.4.

2.5. Scanning electron microscopy (SEM)

Morphological evaluation was carried out by SEM using a Philips XL30 microscope. The intercalated clay was suspended in acetone and was placed on an aluminum specimen stub. After drying, the sample was coated with gold using an EMITECH K-550X sputter coater (Emitech, Ashford, Kent, UK). Coating was done at 20 mA for 4 min.

2.6. Photochemical studies

FERH, HTlc–FER and a physical mixture of HTlc–Cl and FERH were analyzed by spectrophotometric method to obtain information on their spectral properties and on their relative photochemical stability. UV–vis absorption spectra were measured by a home-made spectrophotometer that uses a Deuterium-Halogen lamp (DH-2000-FHS) as source, a CCD as detector (200–1100 nm range, 2048 pixel, 86 photons/counts) and it was equipped with an integration sphere for reflectance spectra recording. A barium sulphate bar was used as reference to calibrate the spectrophotometer. The absorption spectra of the three samples were recorded before and after different irradiation times. The solid samples were irradiated using a Mercury lamp. A band pass filter selected the excitation wavelength at 366 nm.

For comparison, aqueous solution of FERH was irradiated at 313 nm and spectrophotometrically investigated by use of a Perkin-Elmer Lambda 800 spectrophotometer.

2.7. HTlc–FER stability

The stability of HTlc–FER was performed in ultra-filtered water in sink and static conditions at 25 °C. At predetermined time intervals the mixture was centrifuged with a 4236A ALC centrifuge (ALC, Milan, Italy) at 4000 rpm for 5 min. The FER content in the supernatant was determined by UV absorption at 310 nm. The withdrawn supernatants were always replaced with the same amount of fresh medium. The test was performed in triplicate and the error was expressed as standard deviation.

Table 1
Creams composition

	Ingredients	Cream 1 ^a (%)	Cream 2 ^a (%)
Phase A	Abil WE-09	5	5
	Isolan GJ-34	1	1
	Silicone DC-245	7.50	7.50
	Abil wax 9801	3	3
	Castor oil	0.50	0.50
	Ceresine wax SP 252	1	1
	Liquid paraffin	2	2
Phase B	Ferulic acid	3.49	0
	HTlc-FER	0	10
	Water	76.51	70

^a Both creams contained the same sunscreen moles.

2.8. Cream preparation

Two silicone-based waterproof creams were prepared according to the compositions reported in Table 1 (Cosmetics and Toiletries, 1997). All the components of the lipidic phase (Table 1, phase A) were mixed together at 60 °C until obtaining a clear solution. Phase B (Table 1), with the active compounds, was then added to the initial mixture under slow and continuous agitation until cooling.

2.9. In vitro FER release from HTlc-FER powder and HTlc-FER loaded cream

In vitro FER release studies were performed using HTlc-FER powder not formulated, HTlc-FER loaded cream and FER in acidic form loaded cream. The tests were carried out in sink conditions by a dissolution apparatus for semi-solid preparation using the Petri dish method (Poulsen et al., 1968). The cream (300 mg) was carefully spread on the Petri dish (50 mm diameter), to produce a uniform surface. The dish was placed in the bottom of a vessel filled with 100 ml of the dissolution medium (Chowhan and Pritchard, 1975) and kept at constant temperature (32 °C) while stirred (60 rpm). The release media were: (i) ultrafiltered water; (ii) 0.1N PBS pH 5.5 and (iii) artificial sea water (Cavanaugh, 1964).

The FER release profile was determined by cumulative release at predetermined time intervals over a period of 8 h. Supernatants were withdrawn and replaced by the same amount of fresh medium. The FER content was determined spectrophotometrically at 310 nm after

filtration (13-mm Filter UNIT 0.45 µm NY PP, Lida, WI).

Release tests were performed in triplicate and the errors were expressed as standard deviation.

3. Results and discussion

3.1. Preparation and characterization of the intercalated compound

The HTlc matrix was prepared as chloride form (Reichle, 1986) to facilitate the ion exchange process (Miyata, 1983).

From the performed analysis, the HTlc-Cl composition was the following: $Mg_{0.61}Al_{0.39}(OH)_2 \cdot Cl_{0.39} \cdot 0.56H_2O$. HTlc-Cl was then converted into HTlc-FER form by following the procedure described above (Section 2).

X-ray diffraction was used to gain structural information on the arrangement of the FER anions in the interlayer region of the matrix. The X-ray spectrum changes with the nature of the anions intercalated in the matrix and allowed the determination of the space between layers. Fig. 1 shows the patterns of the original

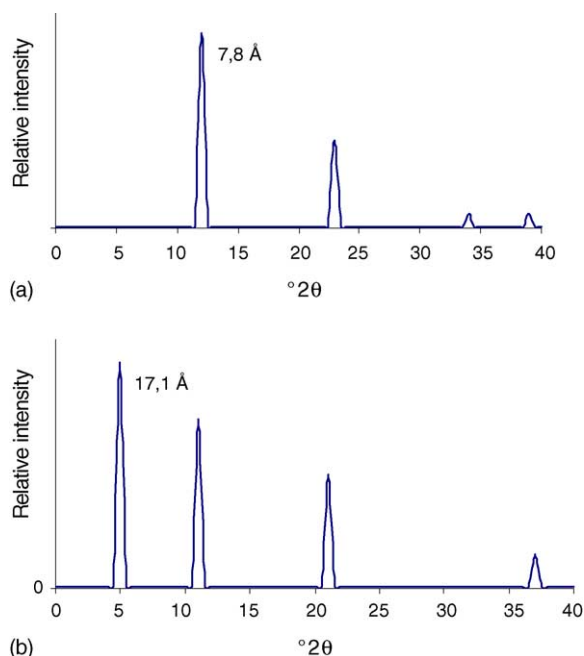


Fig. 1. Powder XRD patterns for HTlc-Cl (a) and HTlc-FER (b).

HTlc–Cl (a) and of HTlc–FER (b). As a consequence of FER intercalation, the interlayer space increased from 7.8 Å of the HTlc–Cl (Miyata, 1983) to 17.1 Å. As the layer thickness is 4.8 Å, the gallery height was 12.3 Å (Whilton et al., 1997). These data are in agreement with previous studies that showed interlayer spaces ranging from 12.0 to 13.0 Å for anions like succinate, tartrate, maleate and from 14.3 to 15.4 Å for anions like phthalate, salicylate or anthranilate (Meyn et al., 1990). A gallery height of 12.3 Å suggested that FER anions were arranged in the interlayer region as a monolayer with the main axes perpendicular to the layer plane of the matrix and with the carboxylic functions ionically bonded to the aluminium cations of the double hydroxide layers. A parallel study was carried out to generate a theoretical model of HTlc–FER that could correlate with the model provided by experimental observation. A geometrical structure of HTlc–FER (Fig. 2) was built by considering the stereochemical rules and the van der Waals forces that characterize this kind of system. According to this approach, the contribution of every OH bond present either in the matrix layers or in the ferulate molecule is 1.4 Å. Since the ferulate molecule has 8.5 Å size, its disposition in the interlayer space was in agreement with the model proposed in Fig. 2. In fact, the interlayer distance calculated in the model well correlated with the experimental value obtained by X-rays.

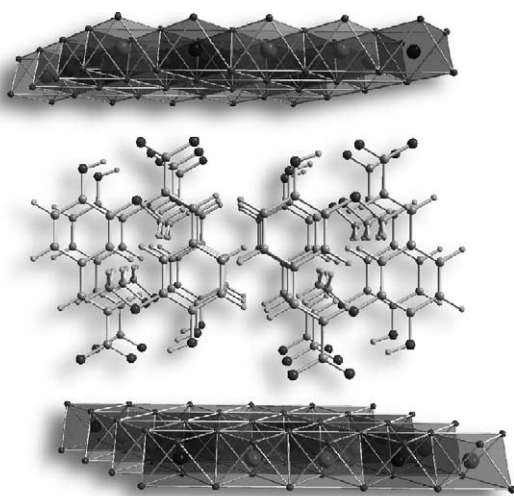


Fig. 2. Geometric model of HTlc–FER.

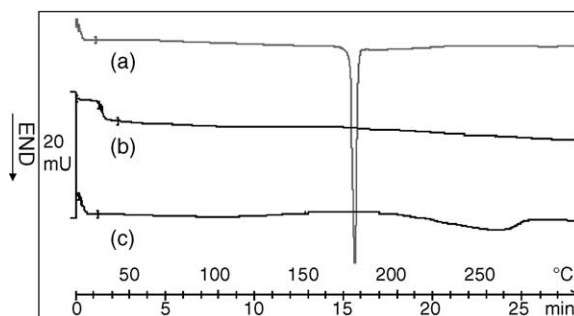


Fig. 3. Thermogram of FERH (a), HTlc–Cl (b) and HTlc–FER (c).

A further confirmation for the ion-exchange process occurrence was provided by DSC analysis. Fig. 3 compares the thermograms of FERH (a), HTlc–Cl (b) and HTlc–FER (c) in the temperature range of 20–300 °C. FERH thermal curve was typical of crystalline anhydrous substance characterized by a sharp endothermic peak, due to drug melting at 175.2 °C followed, at temperatures higher than 300 °C, by an exothermic effect due to decomposition phenomena (data not shown). A different pattern was observed in HTlc–FER thermogram where the disappearance of the FERH melting peak denoted a fully intercalation of the compound in the matrix.

Thermogravimetric analysis (Fig. 4) shows first a reduction in mass from 25 to 120 °C that can be attributed to the loss of co-intercalated water. A second step occurred between 120 and 550 °C due to intercalated ferulate decomposition followed by dehydroxylation of the inorganic layers. Taking into account that the product obtained at 1000 °C is composed of MgO and Al₂O₃, the exact formula of HTlc–FER was: [Mg_{0.61} Al_{0.39} (OH)₂] FER_{0.22} Cl_{0.17}·0.66H₂O. From these data the

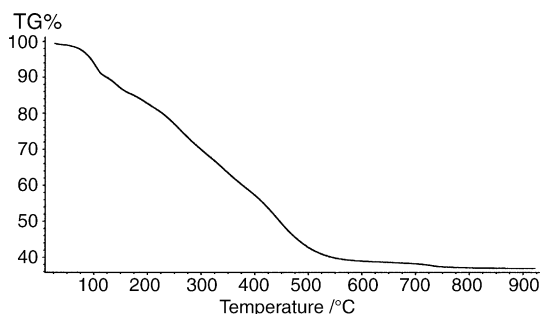


Fig. 4. Thermogravimetric analysis of Mg–Al–HTlc–FER.

FER loading in the HTlc–FER was found to be 35.53% (w/w). The amount of intercalated FER was confirmed by the UV–spectrophotometric analysis after HTlc destruction with HCl.

3.2. Scanning electron microscopy (SEM)

Scanning electron micrographs (Fig. 5) of both HTlc–Cl (a) and HTlc–FER (b and c) show regular well-formed crystals with diameter around 4 μm that appeared as intact discs piled up one on the other. Moreover, neither aggregation process or crystal breakdown were noted after FER intercalation in the HTlc matrix.

3.3. Photochemical tests

To gain information on FERH protection against UV–vis radiations, absorption spectra of different samples were recorded (Fig. 6). The spectrum of HTlc–FER (Fig. 6(1)) was reported and compared to those of pure solid FERH (Fig. 6(2)), FERH/HTlc–Cl physical mixture (Fig. 6(3)) and inorganic matrix alone (Fig. 6(4)). The spectra obtained from HTlc–FER and FERH were similar in the range 250–400 nm even though a shifting of the maximum from 390 to 300 nm was observed. However the HTlc–FER spectrum was slightly broader above 390 nm suggesting that in the intercalated sample specific interactions occurred between the chromophore and the matrix. This observation was further confirmed by comparing the spectra of the matrix alone, that did not present any remarkable absorption contribution, and of the physical mixture where no broadening was observed.

Therefore the spectrophotometric analysis indicated that the intercalation process led to a new material with a slightly broader protection range.

Fig. 7 shows the spectra recorded before and after irradiation (dotted line) at 366 nm for 210 min of HTlc–FER (a) and FERH/HTlc–Cl physical mixture (b). It can be noted that the spectra of HTlc–FER sample did not present any significant change after irradiation. On the other hand irradiation of the physical mixture led to decreased absorbance all over the spectral range indicating a degradation of the chromophore material. These observations suggested that the solar screen was protected from photodegradation when intercalated in the matrix.

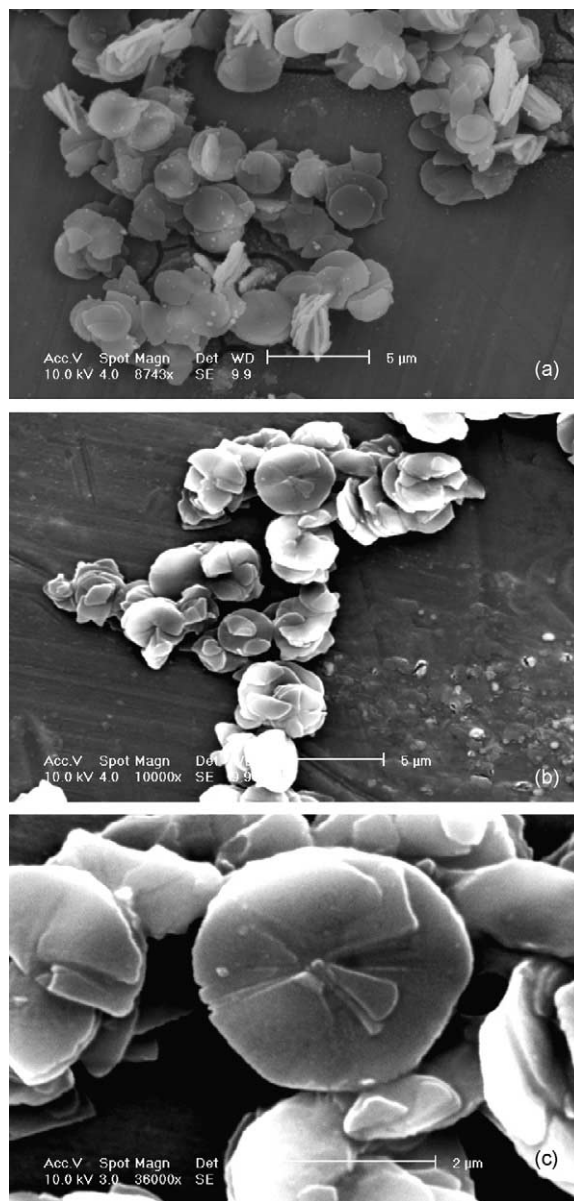


Fig. 5. Scanning electron micrographs of HTlc–Cl (a) and HTlc–FER (b and c).

For comparison Fig. 7(c) shows the spectra of a FERH aqueous solution before and after irradiation (dotted line) at 313 nm for 90 min. In these conditions the irradiation led to the modification expected for a *trans*–*cis* isomerization (Berger et al., 1997).

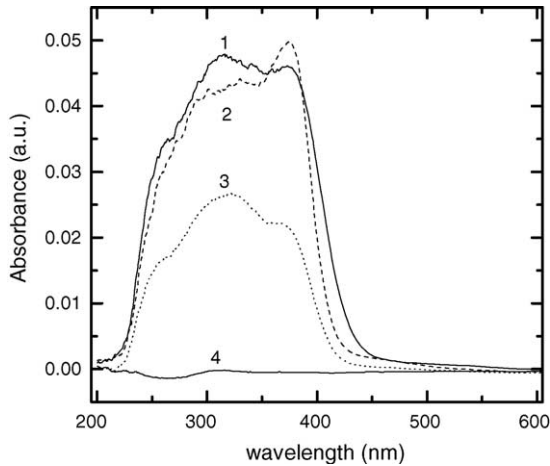


Fig. 6. Absorption spectrum of (1) HTlc-FER, (2) FERH, (3) FERH/HTlc mixture and (4) HTlc-Cl.

3.4. HTlc-FER stability

Since a variable amount of water is always present in cosmetic products, HTlc-FER should be stable in the water phase of the formulation. Thus the FER release from HTlc-FER was studied in water at 25 °C. As shown in Fig. 8, HTlc-FER was very stable in this medium because less than 1.5% of ferulic acid was released in 50 days, since no anions, exchangeable with the ferulate in the matrix, were present. The low amount

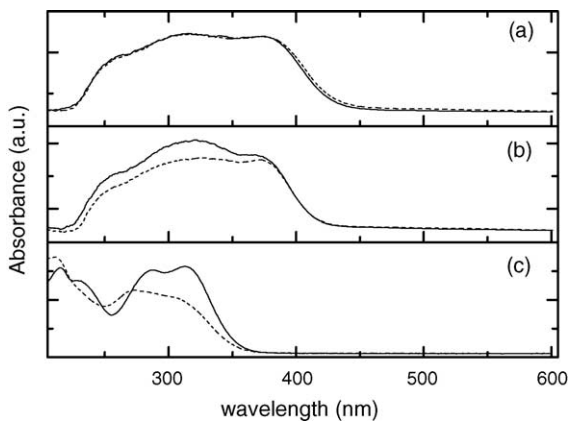


Fig. 7. Absorption spectra of HTlc-FER (a), FERH/HTlc mixture (b) and FERH in aqueous solution (c), recorded before (full line) and after (dashed line) irradiation (see text for the experimental conditions, $\lambda_{\text{exc}} = 366$ nm for solid samples and 313 nm for the aqueous solution).

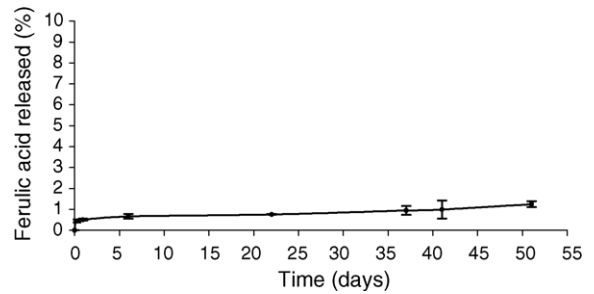


Fig. 8. FER release from HTlc-FER in ultrafiltered water at 25 °C.

of FER found in the medium probably arose from the FER adsorbed on the HTlc outer layers.

3.5. In vitro FER release from HTlc-FER powder and HTlc-FER loaded cream

FERH and HTlc-FER were formulated into water resistant siliconic creams (both containing the same sunscreen moles) and the release of the active ingredient was investigated in different media over a period of 8 h in order to simulate the utilization condition of the formulation (Fig. 9). The release profiles showed that free FERH was released from the cream almost completely after 2 h in all media considered. This behavior can be explained by the fact that at the pH used FERH was mostly in dissociated form ($pK_a = 4.04$) that diffused rapidly in the aqueous media. On the contrary, when FER was intercalated in the matrix, no significant release (Fig. 9a and c) or a very slow release (Fig. 9b) was observed. These findings can be explained by the different ionic compositions of the media. In water, no significant release was observed as noted above. In artificial sea water, only 5% of FER was released after 8 h, probably due to the very slow diffusion of the anions (HCO_3^- , SO_4^{2-} , Cl^-) into the hydrophobic outer phase of the cream. Similar behavior was observed in PBS, but the much higher affinity of H_2PO_4^- and HPO_4^{2-} for the matrix with respect to HCO_3^- , SO_4^{2-} and Cl^- (Reichle, 1986) could explain the greater FER release that nevertheless did not exceed 35%.

In order to evaluate the influence of the chosen silicone cream, the release from unformulated HTlc-FER powder was also tested in the same media and conditions.

In PBS, 58% of FER was released after one hour and 86% after six hours whereas in artificial sea water

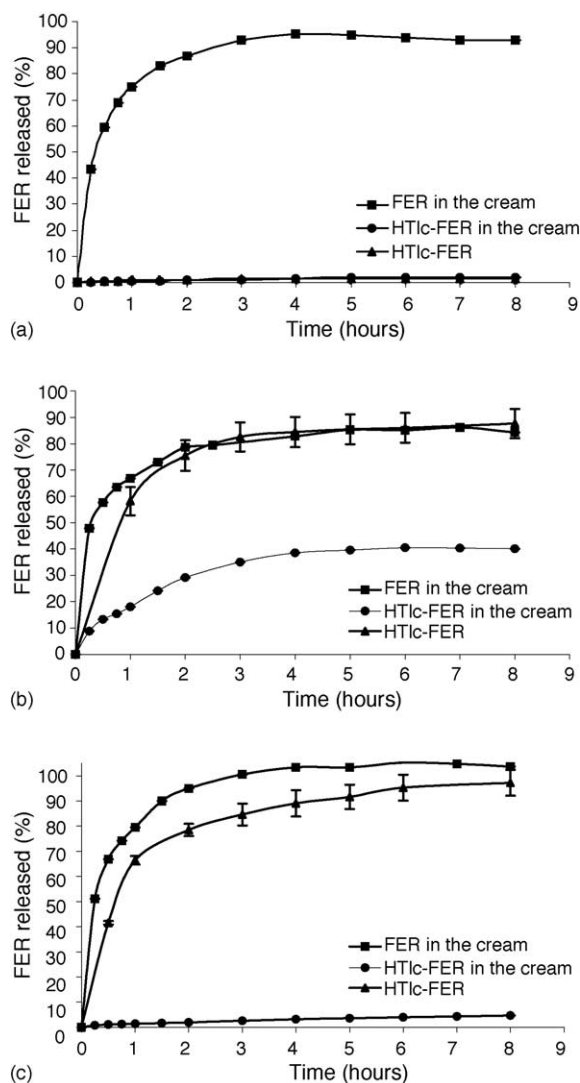


Fig. 9. FER release from creams and from HTlc-FER in ultrafiltered water (a), in phosphate buffer solution at pH 5.5 at the concentration of 0.1 N (b) and in artificial sea water (c).

pH 8, 41% of FER was released in the media after one hour and 91% after six hours. In both media, the rate of release was faster at the beginning and progressively slower after two hours. The initial burst effect was due to the small amount of FER adsorbed on the matrix surface and to the intercalated ferulate released from the more external part of the crystals. The further slowing down of ferulate release was explained considering the semi-rigidity of the layers and the diffusion path length

that controlled the rate of ferulate diffusion out of the matrix (Ambrogio et al., 2001). When anions smaller than ferulate took place in the interlayer space, a resulting decrease of the interlayer distance occurred. The initial exchange of anions from the external part of the crystals led to the formation of phase boundary (Alberti and Costantino, 1996) between inner zones containing ferulate and external ones where smaller anions have replaced the ferulate (Ambrogio et al., 2001). As the exchange proceeded, the phase boundary moved towards the central part of the crystal slowing release.

The results obtained demonstrated that HTlc-FER incorporated in a silicone cream reduced drastically the release of FER in the media and hence its absorption through the skin, thus avoiding all the possible problems related to FER topical use. In fact, the active molecule in sunscreen preparation remains on the skin for a reasonable period of time, conserving at the same time its activity despite perspiration and bathing.

4. Conclusion

In the present work, FER has been successfully intercalated into a HTlc-like anionic clay by a simple ion-exchange process. HTlc was able to protect FER from the degradation due to irradiation and moreover to potentially improve FER sunscreen properties in the region around 300 nm. The HTlc clay protected FER even when formulated into a silicone cream by preventing its release from the matrix. Hence, it was demonstrated that HTlc-FER can be formulated into a potentially useful silicone-based cream for sunscreen formulation.

Acknowledgements

Authors wish to thank the MIUR (Ministry for Education, University and Research)— research programs of national interest.

Special thanks are due to Professors Umberto Costantino and Riccardo Vivani for helpful discussion.

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