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Thermal characterization of usnic acid/collagen-based films

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Abstract The purpose of this study was to evaluate the physical–chemical properties of collagen (CL) and usnic acid/collagen-based (UAC) films, using differential thermal analysis (DTA), thermogravimetry (TG/DTG), infrared spectroscopy (FTIR), and scanning electron microscopy (SEM). Both films were prepared by casting process using polyethylene glycol 1500 (PEG 1500) as plasticizer. In the spectrum of UAC, similar bands of the usnic acid are observed, indicating that the polymerization (film formation) did not affect the stability of the drug. Distinctly, DTA curve of UAC did not show an endothermic peak at 201 °C, indicative that the drug was incorporated into the polymeric system. These results were corroborated by the scanning electron microscopy (SEM). The TG/DTG curves of UAC presented a different thermal decomposition profile compared to the individual compounds and CL. These findings suggest the occurrence of molecular dispersion or solubilization of the drug in the collagen film.

Keywords Casting · Biodegradable films · Usnic acid · Collagen · Thermal characterization

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Introduction

Natural polymers have been increasingly studied for controlled-release applications due to their biocompatibility and biodegradability. Materials such as hyaluronic acid [1], fibrin [2], fibrinogen [3], and collagen [4] have been tested as carries for drug delivery systems [5]. Collagen is a potentially useful biomaterial since it is a major constituent of connective tissue. The main applications of collagen as drug delivery systems are collagen-shields in ophthalmology, sponges for burns/wounds, and liposomes–collagen associations for transdermal and sustained drug delivery [6–8].

Formulations such as ointments and wound dressings have been developed for the treatment of severe skin wounds or ulcers including bedsores and burn wounds [9, 10]. These studies generally involved the development and/or the physical characterization of the materials [11, 12]. In this regard, a better understanding of the physical properties of films is of great importance for subsequent applications of these materials. Physical properties are strongly affected by the state of the material: for example, in the glass state the material will be hard and rigid, but in the rubbery state it will be flexible and extendible. Besides, some studies have been carried out in order to incorporate bioactive compounds into collagen-based films, so that such films could work as drug controlled release within the target tissues [13, 14].

As previously reported, usnic acid is one of the most common and abundant lichen metabolites with interesting antibiotic [15], anti-inflammatory [16], antiprotozoal [17], antitumoral [18], larvicidal [19], antipyretic and analgesic [20] activities. The aim of this study was to prepare a collagen-based film constituted of usnic acid-loaded liposomes and to evaluate its physico-chemical properties using differential thermal analysis (DTA), thermogravimetry/derivative thermogravimetry (TG/DTG) and infrared spectroscopy

(FTIR). Films were also characterized regarding microstructure using scanning electron microscopy (SEM).

Experimental

Materials

Usnic acid, 2,6-diacetyl-7,9-dihydroxy-8,9b-dimetyl-1,3 (2H,9bH)-dibenzeno-furandione, was isolated from *Cladonia substellata* Vainio collected in March, 2006, in the Itabaiana county, Sergipe-SE, Brazil. Lichen sample was identified by M. P. Marcelli (Botanical Institute of São Paulo-SP, Brazil), where a voucher specimen was deposited (Deposit #SP393249). All chemicals were of reagent grade. The collagen was prepared according to the method proposed by 21o, 2005 [21].

Extraction and purification of usnic acid

Air-dried lichen (300 g) was extracted with diethyl ether in a Soxhlet apparatus and the precipitate formed on cooling collected, recrystallised from ethanol, yielding 330-mg usnic acid [22].

Films preparation

Collagen-based (CL) films were prepared by casting method using collagen dispersion (2%) in 0.5 M acetic acid with 20% of plasticizer (polyethylene glycol-PEG 1500 Isofar Lot. 021423) in relation to the polymer dry weight. This dispersion was casted onto a clean rimmed perspex plate and allowed to dry at room temperature in order to obtain the films.

Usnic acid-loaded liposomes were prepared by conventional rotary evaporation method. Briefly, phosphatidylcholine (Lipoid GMBH 75% Lot 776095-1) was dissolved in an appropriate volume of chloroform according to the ratio phosphatidilcoline/usnic acid 18:1 w/w. The mixture was dried to a thin lipid film using a rotary evaporator. This film was kept in desiccator for at least 24 h until the organic solvent was totally eliminated. Then, the lipid film was hydrated and dispersed in water, under vigorous magnetic agitation, promoting the formation of the multilamellar vesicles (MLV). Small unilamellar vesicles (SUV) were prepared by probe sonication of the MLV dispersion. After this procedure, the usnic acid-loaded liposomes were mixed with collagen dispersion (2% in 0.5 M acetic acid with 20% of collagen:PEG1500 w:w) in the ratio 1:4 (v/v) in order to obtain the usnic acid/collagen-based films (UAC).

Particle size distribution and ζ-potential measurement

The particle size and surface change potential of usnic acid-loaded liposomes were determined using Zetasizer® (Nano-ZS90, Malvern Instruments, United Kingdom), at 25 °C. Samples of liposomes were diluted in water (1:10) for an effective particle count. The distribution and the mean diameter of particles, as well as their standard deviation and poliydispersity index (PDI) were assessed. The surface change of usnic acid-loaded liposomes was determined by the measurement of the zeta potencial (ζ) by electrophoresis after dilution of liposomes in water, using the same apparatus.

Thermal analysis

TG/DTA curves were obtained in a TA instruments model SDT 2960 Simultaneous using platinum crucibles with about 5 mg of samples, under dynamic nitrogen atmosphere (100 mL min^{-1}) and heating rate of 10 °C.min^{-1} in the temperature range from 25 to 900 °C. The DTA cell was calibrated with indium (m.p. 156.6 °C; $\Delta H_{fus.} = 28.54$ J g^{-1}) and zinc (m.p. 419.6 °C). TG/DTG was calibrated using a $CaC_2O_4{\cdot}H_2O$ standard in conformity to ASTM.

Infrared spectroscopy

The infrared absorption data was obtained in the range 4,000–400 cm^{-1} in KBr pellets using a FTIR-Bomen spectrophotometer model MB-120 at room temperature.

Scanning electron microscopy

The dried films were mounted on aluminum stubs, coated with a thin layer of gold and visualized with a JEOL Model JSM-6360-LV scanning electron microscope, at an accelerated voltage of 20 kV.

Results and discussion

Collagen and UA-loaded liposome showed to be a very interesting material for the development of films. The mean of the vesicles was 70 nm and the surface charge 11.8 ± 7.19 mV. The process developed here, produced films slightly yellowish with moderate opacity and good flexibility. The presence of phosphatidylcholine and collagen of its formulation provide it with better properties if compared with other films.

DTA curve of usnic acid showed an endothermic event between 192 and 230 °C with a well-defined peak at 201 °C corresponding to the melting point of the drug (Fig. 1a). Furthermore, the DTA curve showed an

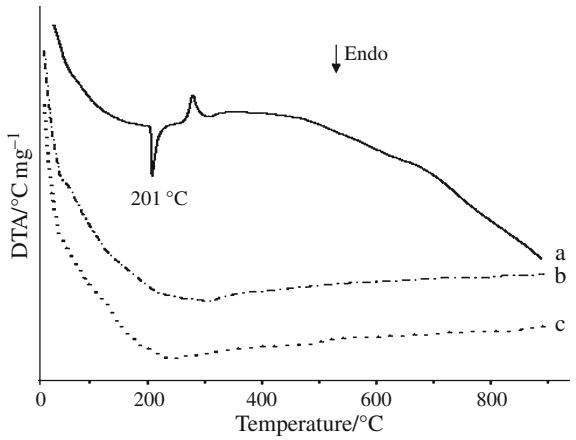

Fig. 1 DTA curves of *a* usnic acid, *b* collagen film and *c* usnic acid–collagen film obtained in heating rate of 10 °C min^{-1} under dynamic nitrogen atmosphere (50 mL min^{-1})

exothermic event caused by thermal decomposition of the examined sample. This second event had a peak at 273.3 °C. TG curves showed an event of fast mass loss occurring in the range of 230 to 350 °C (Fig. 2a), as well as further slow mass loss in the range of 350 to 900 °C, which is usually attributed to the elimination of carbonaceous material.

As seen in Fig. 1b, the CL sample showed a major endothermic transition in the DTA curve between 30 and 80 °C corresponding to its dehydration (unbound water). However, this event was also seen in the TG curve (Fig. 2b) where a humidity loss of about 10.24% was detected. This film showed a thermal stability region between 80 and 175 °C, and an endothermic event indicated by a broad peak at 303 °C, corresponding to the thermal decomposition of this material. This result was confirmed by TG curve.

TG/DTG curve of UAC showed four weight loss events at the following temperature ranges and weight loss

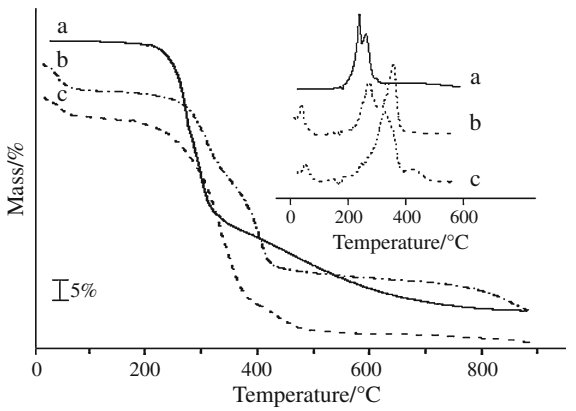

Fig. 2 TG curves of *a* usnic acid, *b* collagen film and *c* usnic acid–collagen film obtained in heating rate of 10 °C min^{-1} under dynamic nitrogen atmosphere (50 mL min^{-1})

percentages: 25–96, 150–381, 381–516, and 516–880 °C (Fig. 2c). The first event is related to the superficial water releasing. The second and third events correspond to the thermal decomposition process followed by carbonization. The last event is related to the carbon material elimination. DTA curve showed an endothermic peak between 147 and 371 °C, corresponding to the first step of thermal degradation of usnic acid–collagen (Fig. 1c). In addition, the melting peak of usnic acid at 201 °C was not observed, indicating absence of free usnic acid. It was noted that UAC has a different thermal decomposition profile compared to the CL indicating that usnic acid was effectively loaded into the membrane.

The FTIR spectra of free usnic acid, CL and UAC are shown in Fig. 3a–c, respectively. The usnic acid spectrum (Fig. 3a) showed a 1,690 cm^{-1} band corresponding to a conjugated cyclic ketone group. Weak bands at 1,715 and 1,678 cm^{-1} in the infrared spectrum are assigned to the *v*(C=O) non-conjugated cyclic ketone and the non-aromatic methyl ketone, respectively. Conjugation, electron donating ring substituents and possible intra-molecular hydrogen-bonding, all contribute to the lower wavenumber position of the aromatic methyl ketone to 1,628 cm^{-1}. It is also possible to assign the antisymmetric and symmetric *v*(COC) aryl alkyl ether modes to bands at approximately 1,283 and 1,072 cm^{-1}, respectively. In the spectrum of UAC (Fig. 3c), similar bands of the usnic acid are observed, indicating that the film formation did not affect the stability of the drug.

The microstructures of CL and UAC analyzed by SEM are presented in Fig. 4. Both films presented a dense structure, typical of protein films, as has been previously reported for films prepared with amaranth proteins [23]. The microstructure of the cross-sectional area of the films revealed that CL structure was dense, with a fibrous formation parallel arranged in relation to the film surface. Notwithstanding, some porous formation was also detected (Fig. 4 and 5), although these cavities seem to be closed

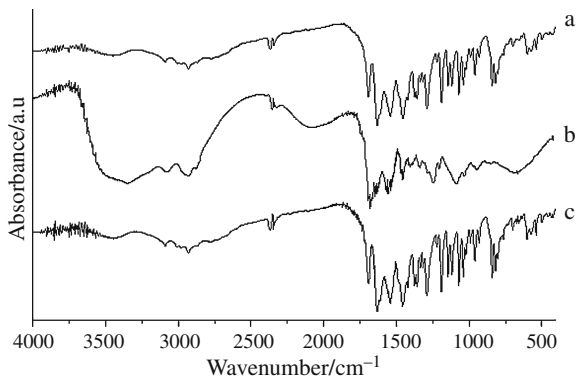

Fig. 3 IR spectra of *a* usnic acid, *b* collagen film, and *c* usnic acid–collagen film

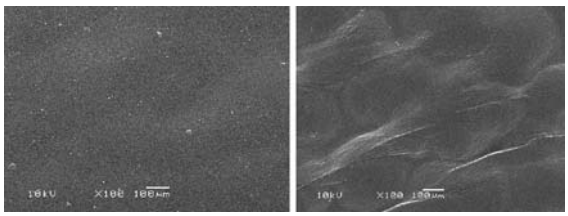

Fig. 4 SEM photographs of surface of **a** collagen membrane and **b** usnic acid–collagen

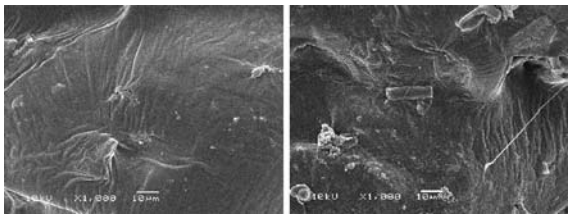

Fig. 5 SEM micrographs of cross-sections of of **a** collagen membrane and **b** usnic acid–collagen films

porous. The surface and cross-sections of UAC film shows non-homogeneities when to compare with CL.

Conclusions

According to the data presented in this study, it was possible to assert that the usnic acid molecules were encapsulated by liposomes and incorporated into the polymeric system as demonstrated in the analysis of the DTA and TG/DTG curves. Furthermore, similar bands of the usnic acid were observed in the IR spectrum of UAC, indicating the presence of the drug in the system. Nevertheless, further studies are required in order to evaluate whether these in vitro models can be used to characterize usnic acid release from collagen-based films.

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