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Short communication

Mixture design applied in compatibility studies of catechin and lipid compounds

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ABSTRACT

The aim of this work was to evaluate the compatibility of (+)-catechin (CA) and excipients commonly used to prepare micro and nanoemulsions using thermal analysis along with complementary assays. Lipid compounds labrasol, plurol and ethyl oleate were combined with CA according to a simplex centroid mixture design and possible interactions between them were determined. Differential scanning calorimetry and thermogravimetric analyses were carried out together with Fourier transform infrared spectroscopy (FTIR) and morphologic characterization of the samples. A quantitative evaluation of thermal events involved in CA melting peak and initial sample decomposition temperature were performed. FTIR evaluation suggested an initial decomposition of CA mixtures exposed to a thermal aging depending on their composition corroborated by the darkening of these samples. The multiple regression analysis considering the thermal data revealed a thermal interaction compromising CA stability in multicomponent samples. Mixtures containing ethyl oleate exhibited a negative synergic action of this fatty acid with the others two lipid compounds (negative coefficients for two-factor and three-factor interaction terms). Indeed, samples decomposition was anticipated by at least 10 °C in the case of ternary and quaternary mixtures containing ethyl oleate. In conclusion, CA formulations produced with lipid components must have their stability closely monitored and production process involving heating should be avoided, especially in formulations containing ethyl oleate.

1. Introduction

(+)-Catechin (CA) is a polyphenol found in many natural products that possesses a broad spectrum of therapeutic activities, which includes anticancer, antimicrobial and anti-inflammatory activities as well as a strong antioxidant potential, even greater than that of ascorbic acid [1–3]. In addition, CA has shown to afford significant UV-protective actions against skin photo-damage and sunburn and, therefore, several cosmetic products containing CA derivatives have been placed in the market [4].

Despite the promising and broad therapeutic claims, CA has a poor oral bioavailability (< 5%) due to low membrane permeability and high biotransformation rate, restricting clinical application [5]. More importantly, CA easily decomposes during formulation, processing and storage, showing reduced stability in the presence of oxygen and alkaline pHs [6].

Several delivery systems containing this compound have been developed in the last years to circumvent these issues, as inclusion complexes with cyclodextrin, polymeric microparticles and lipid systems such as lipid nanoparticles, liposomes, multiple emulsions and nanoemulsions, demonstrating many improvements in stability and/or pharmacokinetics [3,5–7]. Lipid systems are particularly interesting for improving the bioavailability of topically applied CA because of their permeation enhancement potential trough the skin.

Lipid systems can protect the drugs against decomposition processes, as in the case of nanoemulsions in which the drug is inserted in the internal phase of the system protected from the external environment [5]. Nanoemulsions are composed by an aqueous phase, emulsifying agents and a mixture of several oils (e.g. castor oil, corn oil, coconut oil, linseed oil, mineral oil and olive oil). Contradictorily, these lipids were highly reactive and can themselves be a risk to drug stability in case of incompatibility [8,9].

The habitual protocols of thermal studies for drug-excipient compatibility determination are based on the use of binary mixtures of components [10,11], although the final formulation is usually composed of multiple components. Indeed, the preparation of lipid delivery systems involves the use of different lipid components whose complex composition easily oxidizes, which raises the risk of stability issues [9]. In this scenario, the use of experimental design as mixtures designs has proven to be a powerful tool to identify possible interactions between various formulation components

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[12,13]. Compatibility studies between drug and lipid components are still very scarce [14] and their execution in mixtures of multiple components employing statistical tools is still an unexplored field.

The aim of this work was, therefore, to evaluate CA compatibility with commonly used excipients in the preparation of micro and nanoemulsions. Such analyses were performed following a simplex centroid mixture design using thermal analysis and complementary physicochemical assays.

2. Material and methods

2.1. Chemicals and reagents

CA was purchased from Sigma-Aldrich (St Louis, MO, USA); Ethyl oleate (EO) was obtained from Merck (Darmstadt, Germany); and Labrasol (LB) and Plurol oleique CC 497 (PL) were kindly gifted by Gattefossé (Lyon, France).

2.2. Experimental mixture design

The compatibility of CA with the selected excipients was determined according to a simplex centroid mixture design, using three components without constraints. This design type allows studying possible interaction between compounds through the construction of high order polynomial models using few runs [13]. Mixtures containing 50% (w/w) of CA and 50% (w/w) of the lipid components commonly used to prepare micro and nanoemulsions were prepared as described in Table 1. The responses obtained from thermal analysis were analyzed using the software Design Expert 9.0 (Stat-Ease, Minneapolis, MN, USA). The possible mathematic models were analyzed using ANOVA one-way. The best fitting model was selected for each thermal response (variation in CA melting peak and in initial sample decomposition temperature) based on F-values, p-values and the predictive equations containing only significant terms were built from stepwise multiple regression analysis.

2.3. Thermal characterization

Differential scanning calorimetry (DSC) analyzes were carried out in samples of approximately 3 mg placed in aluminum-sealed pans. The samples were analyzed under a heating rate of 10 °C min^{-1} from 30 to 300 °C in a DSC-60 (Shimadzu, Kyoto, Japan) using argon as inert atmosphere, which flowed at 100 mL min^{-1}.

Differential thermal analysis (DTA) and thermogravimetric analysis (TGA) were simultaneously performed using a DTG-60H (Shimadzu, Kyoto, Japan) operating under argon atmosphere with a gas flow of 100 mL min^{-1}. Samples of approximately 3 mg were analyzed in a platinum pan in the range of 25–450 °C using a heating rate of 10 °C min^{-1}. Dehydration, evaporation and decomposition events were monitored based on mass loss. Events integrations were performed according to the first derivative of mass loss curves of the TGA and the DTA thermograms.

All components were analyzed in duplicate, both individually and in combination, according to the mixture design. Thermal measurements were determined using the TA-60 Shimadzu® software.

2.4. Molecular characterization

Fourier transform infrared spectroscopy (FTIR) spectra were recorded at wavenumbers ranging from 4000 to 400 cm^{-1} with a resolution of 4 cm^{-1} using a Varian 640-IR FTIR spectrometer with an imaging ATR accessory (Varian Inc., Palo Alto, CA, USA). Individual compounds and mixtures of CA with the excipients were evaluated before and after a thermal stressing produced by heating samples until 180 °C. The resulting spectra were analyzed considering the correlation coefficient (r) between samples before and after thermal stress based on the bands corresponding to the functional groups of CA using the Essential FTIR software operating with the normal set sensitivity (Operant LLC, Madison, WI, USA).

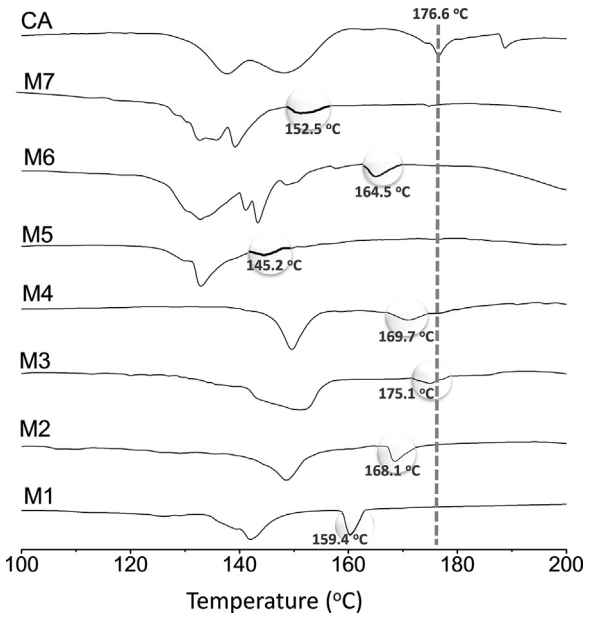

Fig. 1. DSC curves of catechin (CA) and its mixtures. Drug melting peak as supplied is indicated by the dashed line and this event in each mixture is displayed with magnification.

2.5. Morphological analysis

Morphological characteristics of the samples before and after de thermal treatment described in section 2.4 were analyzed using an SZ-SZT stereomicroscope connected to a video camera (Laborana, São Paulo, Brazil). Image processing was performed using an ISCapture software, version 2.2.1.

3. Results and discussion

3.1. Thermoanalytical characterization

CA presented a DSC thermal profile with two unresolved peaks, corresponding to crystal water loss in the range of 130–160 °C followed by anhydrous drug melting (t_{peak} = 176.6 °C and enthalpy = 14.2 J g^{-1}), which is in agreement with previously published characterizations of this compound [15,16].

CA was partially dissolved in the mixtures produced with lipid solvents, which considerably reduced the heat transitions expected for this drug in DSC curves. Indeed, CA melting peak presented very reduced enthalpies in all mixtures, nevertheless the peak was still distinguishable from the baseline and displaced to lower temperatures (Fig. 1). The interaction with lipid solvents caused pronounced changes in CA thermal profile with displacements of dehydration and drug melting peaks, as for example in sample M5, showing a reduction of more than 30 °C in the drug melting peak.

The thermal interactions identified by DSC may be related to chemical reactions between the components of the mixtures or to innocuous physical interactions as observed in the CA solubilization processes. The interpretation of samples containing lipids presents high complexity and multiple physicochemical evaluations are necessary to trace a more precise diagnosis about components compatibility [14].