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Original Article

Microparticles containing guaraná extract obtained by spray-drying technique: development and characterization

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ABSTRACT

Guaraná (*Paullinia cupana* Kunth, Sapindaceae) is well known for its dietary and pharmaceutical potential, and the semipurified extract of guaraná shows antidepressant and panicolytic effects. However, the low solubility, bioavailability and stability of the semipurified extract limit its use as a component of pharmaceutical agents. Delivery of the semipurified extract in a microparticle form could help to optimize its stability. In this study, microparticles containing semipurified extract of guaraná were obtained by the spray-drying technique, using a combination of maltodextrin and gum arabic. The raw materials and microparticles produced were characterized by particle size analysis, differential scanning calorimetry, thermogravimetric analysis, and scanning electron microscopy. The drug content and antioxidant capacity were also evaluated. *In vitro* dissolution tests using flow cell dissolution apparatus, were carried out to investigate the influence of formulation parameters on the release of semipurified extract of guaraná from the microparticles. The spray-drying technique and the processing conditions selected gave satisfactory encapsulation efficiency (80–110%) and product yield (55–60%). The mean diameter of microparticles was around 4.5 μm. The DPPH radical scavenging capacity demonstrated that microparticles can protect the semipurified extract of guaraná from the effect of high temperatures during the process maintained the antioxidant capacity. Differential scanning calorimetry results indicated an interaction between semipurified extract of guaraná and gum arabic: maltodextrin in the microparticles, and thermogravimetric analysis indicate that the profile curves of the microparticles are similar to the adjuvants used in drying, probably due to the higher proportion of adjuvants compared to semipurified extract of guaraná. *In vitro* dissolution tests demonstrate that all formulations complete dissolution within 60 min. Microencapsulation improved the technological characteristics of the powders and preserved the antioxidant properties. The study demonstrated the feasibility of producing these microparticles for a one-step process using spray drying. The composition of each formulation influenced the physical and chemical characteristics. This spray-drying technique can be used as an efficient and economical approach to produce semipurified extract of guaraná microparticles.

Introduction

Phytotherapy is making a recognized and effective contribution to public health, and the development of new herbal medicines in intermediate and final pharmaceutical forms is a contribution to this trend (Klein et al., 2009). The seeds of guaraná – *Paullinia cupana* Kunth, Sapindaceae, a Brazilian plant, are used in folk medicine as a stimulant of the central nervous system, in cases of physical and mental stress, and as an antidiarrheic, diuretic, and antineuralgic (Henman, 1982; Yamaguti-Sasaki et al., 2007). The seeds contain high amounts of methylxanthines including caffeine, theophylline, and theobromin, besides saponins, and polyphenols, especially tannins (Henman, 1982). Chemical assay of a semipurified extract of guaraná (EPA) showed the presence of caffeine, epicatechin, catechin, *ent*-epicatechin, and procyanidins B1, B2, B3, B4, A2, and C1 (Ushirobira et al., 2007; Yamaguti-Sasaki et al., 2007). The antidepressant effect has been evaluated with promising results and with a beneficial effect on cognition, without altering locomotor activity; the EPA also shows a panicolytic effect (at a dose of 8 mg kg^{-1}) (Audi and Mello, 2000; Audi et al., 2010; Otobone et al., 2005, 2007; Roncon et al., 2011). Low toxicity (Espinola et al., 1997; Mattei et al., 1998; Otobone et al., 2005, 2007), action as a chemoprophylactic in carcinogenesis (Espinola et al., 1997), and important activity against *Streptococcus mutans* (Yamaguti-Sasaki et al., 2007) have also been demonstrated.

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The demonstrated antidepressant effect of the EPA on animals may result from the condensed tannins, which can cross the blood–brain barrier and affect the central nervous system (Fukumasu et al., 2006; Johnston and Beart, 2004; Otobone et al., 2005, 2007; Ushirobira et al., 2007). The EPA caused no toxicity in rats at the dose of 30 mg kg^{-1} (Antonelli-Ushirobira et al., 2010). The high potential for using guaraná in a wide range of medicinal applications justifies the interest in the development of a pharmaceutical dosage form.

However, polyphenols are easily degradable by environmental factors such as light, temperature and the presence of oxygen. The technology of microencapsulation can stabilize these labile compounds and extend their shelf life. From an industrial perspective, microencapsulated powders are easy to handle and to use in food and pharmaceutical processing, and maintain their initial polyphenol content and bioactivity, increase solubility, and maintain safety in case of prolonged storage. Microencapsulated systems are stable because the coating material provides physical protection and forms a barrier to oxygen and small molecules (Manach et al., 2005; Sansone et al., 2011). Among the methods for preparing microparticles, the spray-drying technique is widely used in the pharmaceutical and biochemical fields, and in the food industry due to the wide availability of equipment and ease of use at an industrial scale (Bruschi et al., 2003). It is also a mild "one-step" processing operation to move from a liquid form to a powdered product. Since the rapid solvent evaporation keeps the droplet temperature far below the temperature of the drying air, spraydrying is strongly recommended for heat-sensitive materials such as polyphenols (Sansone et al., 2011).

Therefore, considering that EPA contains some labile chemical compounds, microencapsulation can increase its stability. The aim of the present study was to develop and characterize microparticles containing EPA, obtained by the spray-drying technique. The influence of formulation parameters on the yield of the process and on microparticle properties such as morphology, size, thermal behavior, and dissolution/release rate was investigated. The microparticles were also evaluated in terms of both EPA content and antioxidant capacity.

Materials and methods

Materials

Gum arabic (Synth), maltodextrin (Sigma–Aldrich), methanol (J.T. Baker, HPLC grade), acetonitrile (J.T. Baker, HPLC grade), trifluoroacetic acid (TFA) (J.T. Baker) and ultrapurified water obtained from Milli-Q (Millipore) apparatus were used.

Preparation of the EPA semipurified extract

Samples of guaraná seeds (*Paullinia cupana* Kunth, Sapindaceae) obtained in the municipality of Alta Floresta, state of Mato Grosso, Brazil, were used to prepare the acetone:water (70:30, v v^{-1}) 10% extractive solution (ES), by turbo extraction (Ultra-Turrax UTC115KT, Ika Works, Wilmington, NC, USA). The organic solvent was removed in a rotary evaporator under reduced pressure, and the remaining material was lyophilized (EBPC; Audi and Mello, 2000). The EBPC (crude extract) was partitioned with ethyl acetate, resulting in an ethyl-acetate fraction (EPA) (Audi and Mello, 2000; Antonelli-Ushirobira et al., 2010). A voucher plant specimen (#HUEM9065) was deposited at the Herbarium of Universidade Estadual de Maringá, and the species was identified by Prof. Dr. Cássia Mônica Sakuragui (UFRJ).

Table 1
Factors, the proportion of GA:MD in the mixed wall material (X1), and total solid content on total dispersion (X2), and levels of microencapsulation experiment to optimize the conditions.

Level	Factor	
	X1 (GA:MD)	X2 (%, w w^{-1})
1	30:70	10
2	40:60	20
3	50:50	30

Preparation of EPA microparticles

Dispersions of EPA/gum arabic/maltodextrin (EPA/GA/MD) in different proportions (Table 1), as a suspension, were spray-dried using a mini spray dryer (Büchi B-191, Flawil, Switzerland). The inlet temperature was 190 °C, aspiration 80%, pressure established 2 Bar, and pump% of 6%. The outlet temperature was 120–130 °C.

A mixture of GA and MD was selected as the microencapsulation wall material. A series of experiments was designed, to optimize the processing conditions (Zhang et al., 2007). The main factors selected were (1) the proportion of GA:MD in the wall material (X1), and (2) total solid content on total dispersion before spray drying (X2). Details of the experiments and formulations are described in Tables 1 and 2.

Considering the total amount of solids in the dispersion before spray drying, first 10% EPA was tested in different proportions of X1 and X2. With these results, after characterizations, the best two results were selected, and the amount of EPA was increased to 20%. 10% of the total of liquid used to prepare the dispersions was ethanol, to solubilize the EPA. The remainder was water, to disperse the proportions of GA/MD. The EPA solution and the GA/MD dispersions were stirred separately for 20 min. Then the EPA solution was added to the GA/MD dispersion, stirred for about 5 min, and spray dried.

Yield of microparticle powder

The total powder obtained after spray drying was weighed and the percentage over the initial amount of solids was taken as the yield microparticle powder.

Moisture content of spray-dried powders

The residual moisture of the EPA microparticles was measured with an infrared analytical balance (Ohaus-MB 200, Pine Brook, NJ, USA) on a 2.0 g sample at 105 °C for 30 min. Three replicates were carried out, and calculated mean and standard deviation.

Morphology and particle size analysis

The morphology of the particles produced was analyzed by means of a scanning electron microscope (SEM, Shimadzu SS-550, Tokyo, Japan) with Image Pro Plus software (Media Cybernetics, Rockville, MD, USA). The particles were coated with gold/palladium under argon atmosphere and the SEM images were recorded. Their size was determined by measuring Feret's diameter. More than 800 particles were measured and the particle size distribution was estimated.

Loading efficiency

The EPA was extracted from the microparticles using a portion of purified water and dissolved in a 20% methanol final solution. The samples in 20% methanol were submitted to solid-phase extraction (SPE cartridge, Strata C18-E, Phenomenex) prior to analysis,

Table 2
Details of formulations.

Sample	Total dispersion (g)	GA (g)	MD (g)	EPA (g)	Water (g)	Ethanol (g)
1	100	2.70	6.30	1.0	81.0	9.0
2	100	3.60	5.40	1.0	81.0	9.0
3	100	4.50	4.50	1.0	81.0	9.0
4	50	2.70	6.30	1.0	36.0	4.0
5	50	3.60	5.40	1.0	36.0	4.0
6	50	4.50	4.50	1.0	36.0	4.0
7	40	3.24	7.56	1.2	25.2	2.8
8	40	4.32	6.48	1.2	25.2	2.8
9	40	5.40	5.40	1.2	25.2	2.8
10	100	9.00	–	1.0	81.0	9.0
11	100	–	9.00	1.0	81.0	9.0
12*	100	2.40	5.60	2.0	80.0	10.0
13*	100	4.00	4.00	2.0	80.0	10.0

due to the presence of solid residues. The EPA was measured for catechin and epicatechin contents, using a HPLC method proposed and validated previously (Klein et al., 2012, 2013). The HPLC system consisted of a Thermo HPLC equipped with pumps and an integral degasser (Finnigan Surveyor LC Pump Plus), PDA spectrophotometric detector module (Finnigan Surveyor PDA Plus Detector), controller software (Chromquest), and autosampler (Finnigan Surveyor Autosampler Plus) equipped with a 10 μl loop for injection. Chromatographic separation was conducted using a Synergi POLAR – RP 80A stainless-steel analytical column (250 mm × 4.6 mm, 4 μm), and a C18 guard cartridge system (4.6 mm × 20 mm, 4 μm) (Phenomenex®). The mobile phase was a gradient system of 0.05% TFA–water (phase A) and 0.05% TFA–acetonitrile:methanol (75:25, v v^{-1}) (phase B), previously degassed using an ultrasonic bath. Gradient separation was performed at a flow rate of 0.5 ml min^{-1}, and the detection was performed at 280 nm.

Thermal analysis

EPA, GA, MD, microparticle formulations, and physical mixtures (PM) of the substances were analyzed by differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) on a calibrated Netzsch STA 409PG/4/G Luxx thermal analyzer (Selb, Germany). Thermograms were recorded by placing accurately measured quantities of each sample in a platinum pot in analysis atmosphere with constant flow of nitrogen (N_2) at 50 ml min^{-1}. The samples were heated from ambient temperature to 500 °C at a heating rate of 10 °C min^{-1}.

DPPH radical scavenging capacity

The DPPH radical scavenging capacity was determined according to a method described previously (Chassot et al., 2011).

The free radical scavenging activities of semipurified extract EPA, the positive control Trolox, and formulations 1, 3, 12, and 13 were measured by using 2,2-diphenyl-1-picryl-hydrazyl (DPPH, Sigma). Solutions of EPA, Trolox® (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, Sigma) and formulations 1, 3, 12, and 13 at different concentrations were dissolved in 3 ml of methanol and then added to a methanolic solution of free radical DPPH (1 mM, 375 μl). The mixture was strongly shaken and maintained at room temperature for 30 min in the darkness. The absorbance of the resulting solution was read spectrophotometrically (Shimadzu UV/vis PC-1650, Japan) at 517 nm against a blank (2 mg of butylated hydroxytoluene-BHT, dissolved in 4 ml of methanol with 500 μl of the free radical DPPH solution added). The capability to scavenge the DPPH radical or to inhibit free radicals was calculated using the following equation:

$$I\% = \frac{(A_b - A_s)}{A_b} \times 10 \quad (1)$$

where $I\%$ is the capability to scavenge the DPPH radical or to inhibit free radicals, A_b is the absorbance of the control reaction (containing all reagents except the test compounds), and A_s is the absorbance of the test compound. The sample concentration providing 50% inhibition (IC_{50}), concentration required to inhibit DPPH radical formation by 50%, was calculated from the graph of $I\%$ against sample concentration. Tests were carried out in triplicate. Trolox® was used as positive control or standard. Data are presented as IC_{50} (μg ml^{-1}).

In vitro *drug release studies*

In vitro dissolution assays of EPA from the microparticles were carried out in triplicate on a Sotax® AT 7 Smart Apparatus (Manchester, USA) using a 22.6 mm flow cell, with the microparticles placed between layers of glass spheres. The release medium was purified water (pH 6.5) at 37 ± 0.5 °C, and flow at 8 ml min^{-1}. The sink conditions were determined and maintained. For evaluation of EPA release, 100.0 mg of formulations 1 and 3, and 200.0 mg of formulations 12 and 13 were used. Samples were placed on the flow cell and at predetermined time intervals (5, 10, 15, 30, 45, and 60 min), 5 ml samples of dissolution fluid were collected and the volume completed to 25 ml, and the catechin and epicatechin concentrations were analyzed by HPLC (Klein et al., 2012). None of the formulation components was found to interfere with the analysis.

Model parameters

Five different kinetic models (zero-order, first-order, Higuchi, Korsmeyer–Peppas and Baker–Lonsdale model) were used to fit the experimental data obtained in the drug release experiments.

$$Q = k \quad \text{Zero-order equation} \quad (2)$$

$$Q = 1 - e^{-kt} \quad \text{First-order equation} \quad (3)$$

$$Q = kt^{1/2} \quad \text{Higuchi's equation} \quad (4)$$

$$Q = kt^{n} \quad \text{Korsmeyer–Peppas} \quad (5)$$

$$\frac{3}{2}\left[1 - \left(1 - \frac{Q_t}{Q_\infty}\right)^{2/3}\right] - \frac{Q_t}{Q_\infty} = kt \quad (6)$$

where Q is the fraction of drug released at time t and Q_∞ at infinite time, k is the release rate constant, n is the diffusion release exponent that could be used to characterize the different release mechanism, $n \leq 0.43$ (Fickian diffusion), $0.43 < n < 0.85$ (anomalous transport), and ≥ 0.85 (case II transport) (Costa and Lobo, 2001).

Table 3
Details and results of microparticles powders characterizations experiments.

Sample	AG:MD	% Solids	Yield (%)	Moisture (% ± SD)	Loading efficiency (% ± SD)	
					Catechin	Epicatechin
1	30:70	10	62.39	5.60 ± 0.14	99.15 ± 2.07	97.95 ± 8.70
2	40:60	10	57.62	7.44 ± 0.27	85.02 ± 0.10	89.02 ± 0.59
3	50:50	10	58.59	5.27 ± 0.39	87.14 ± 1.18	91.31 ± 8.09
4	30:70	20	45.88	6.10 ± 0.09	92.94 ± 1.58	89.62 ± 4.96
5	40:60	20	57.50	6.24 ± 1.19	81.53 ± 0.75	90.73 ± 6.57
6	50:50	20	55.70	6.00 ± 0.70	97.94 ± 0.58	108.35 ± 2.70
7	30:70	30	37.19	5.66 ± 0.25	88.36 ± 0.84	91.39 ± 0.33
8	40:60	30	36.66	5.26 ± 0.55	81.73 ± 1.04	90.66 ± 2.83
9	50:50	30	34.70	6.10 ± 0.52	81.55 ± 1.82	82.10 ± 10.15
10	100:0	10	42.12	6.03 ± 0.20	87.04 ± 0.97	89.22 ± 11.79
11	0:100	10	51.90	6.03 ± 0.11	84.05 ± 1.09	86.79 ± 3.14
12[a]	30:70	10	60.00	8.40 ± 0.50	95.61 ± 1.69	84.81 ± 7.39
13[a]	50:50	10	55.54	5.73 ± 0.15	96.34 ± 1.62	84.16 ± 10.56

[a] EPA percentage increased to 20% in total solids.
SD, standard deviation; AG:MD, proportion of arabic gum:maltodextrin.

Linear regression was performed and the model parameters calculated.

Statistical analysis

All experiments were performed at least in triplicate. The data were compared by one-way analysis of variance (ANOVA) and Tukey post hoc test using the Statistica 8 software (StatSoft, Tulsa, OK, USA). A p-value <0.05 was considered statistically significant in all cases.

Results and discussion

Results of microparticles powders characterizations experiments

The microparticle formulations, and the details and results of the microparticle powder characterizations (yield, moisture, and loading efficiency to catechin and epicatechin) are described in Table 3.

The ANOVA indicated $p < 0.05$ for the variable yield. Therefore, at least one sample showed a yield different from the others, and to determine which sample(s) differed we applied the test for multiple comparisons. Samples with 10 and 20% solids gave equivalent yields, which were higher than the samples with 30% solids.

For the variable catechin encapsulation content, no sample showed a catechin encapsulation content significantly different from the others ($p > 0.05$). The %GA and the percentage of solids in each sample did not significantly affect the catechin encapsulation content. For the variable epicatechin encapsulation content, the results were $p > 0.05$, so the conditions for epicatechin are the same as for catechin.

Therefore, we chose 10% of solids in dispersion the reference for the production of microparticles, and the formulations with 30% solids were excluded from subsequent characterizations. The 20% solids was initially an option for the production of microparticles, but although the yield of the microparticles was equivalent to the microparticles produced with 10% solids, in practice the dispersion is very viscous and difficult to spray dry.

The 10% solids percentage was chosen to test the extreme concentration of %GA, in order to better characterize these formulations (formulations with 30% and 50% of GA). We increased the EPA concentration to 20% (samples 12 and 13) only in the formulations that provided the best results (samples 1 and 3).

According to the results of the initial characterization of powders of microparticles, we concluded that 10% solids is most suitable concentration for the production of microparticles, in the working conditions. Formulations 1, 3, 12, and 13 obtained the best results for the tests performed and were used in subsequent studies.

Table 4
Particle size of microparticles formulations.

Sample	Particle size (μm) ± SD	Size interval (μm)
1	4.22 ± 1.79	1.31–12.46
2	4.72 ± 1.98	1.64–14.45
3	4.92 ± 2.15	1.39–17.39
4	4.81 ± 2.53	1.05–18.20
5	4.97 ± 2.10	1.14–15.75
6	6.71 ± 3.41	1.03–20.48
7	4.74 ± 2.27	1.05–15.75
8	4.48 ± 2.16	1.35–16.48
9	7.51 ± 4.03	1.01–30.46
10	5.64 ± 2.37	1.85–15.50
11	5.78 ± 2.17	1.24–14.98
12	4.34 ± 1.97	1.36–13.67
13	4.67 ± 1.85	1.43–13.32

SD, standard deviation.

Particle size analysis and morphology

The size of the microparticles was very similar in all formulations (Table 4). Only samples 6 and 9 had a larger particle size, probably due to the higher content of GA combined with the higher percentage of solids in the initial dispersion. The microparticles, with a mean diameter around 4.5 μm, indicated that the arrangements of both polymers and EPA in the micrometric particles containing the drug were homogeneously distributed, showed well formed, small, spherical in shape microparticles, presence of surface depression (or collapse) and absence of particles aggregates and crystals (Sansone et al., 2011).

For the microparticle morphology analysis, SEM micrographs were used. Fig. 1 shows the morphology of samples 1, 3, 12, and 13 and the formulations of the samples without EPA. These formulations were chosen because they gave the best results in the characterization tests, and were used for the dissolution tests.

All the samples displayed an absence of crystals and aggregates. These results indicate a possible interaction and solubilization of EPA in the GA:MD microparticles (Sansone et al., 2011). The photomicrographs of samples 12 and 13 showed small and well-formed spherical microparticles, and those of samples 1 and 3 revealed microparticles with a more irregular shape and the presence of partially collapsed particles. These studies indicate that the amount of EPA had a significant influence on the microparticles, altering

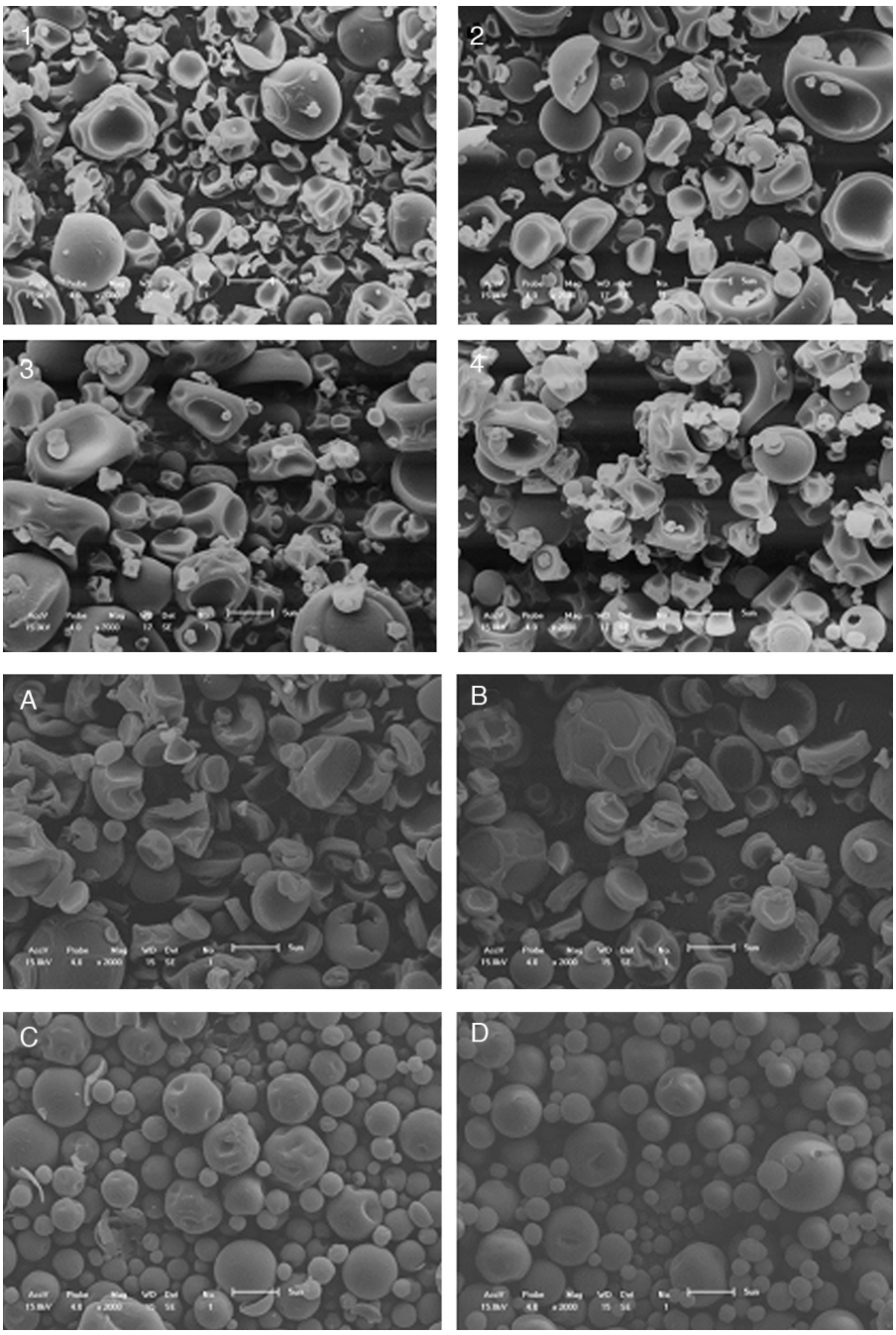

Fig. 1. SEM photomicrographs of microparticles (2000×). 1–4: formulations of samples 1, 3, 12, and 13, respectively without EPA; A–D: samples 1, 3, 12, and 13, respectively with EPA.

the morphology, as observed by with chitosan microspheres for encapsulation of α-lipoic acid (Weerakody et al., 2008).

Thermal analysis

Thermal analysis may provide information on the solid state and extract–polymer interactions as well as on physical stability of materials after the technological process. Differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) were performed to evaluate the stability of the microparticles after thermal oxidation (temperature increase) (Weerakody et al., 2008).

Overall, two thermal events were observed for EPA and for GA, MD, microparticles, and the physical mixtures in the DSC curves (Figs. 2–4). The first endothermic event was between room temperature and 150 °C, depending on the sample, and corresponded to loss of absorbed water. The difference of several °C up or down can be related to the binding mode of water in different samples, characteristic of each substance, which required more or less energy to release it. As observed by in previously study (Borghetti et al., 2006), samples of quercetin from different suppliers had different temperatures of water loss, caused by differences in morphology and particle size. The second endothermic event observed for all samples corresponds to the melting point, with possible degradation of these compounds due to the high temperatures involved in this process (over 240 °C).

These results can be confirmed by analysis of the TGA curve, in which a mass loss can be observed at these temperatures, probably related to degradation of these substances (Figs. 5–7).

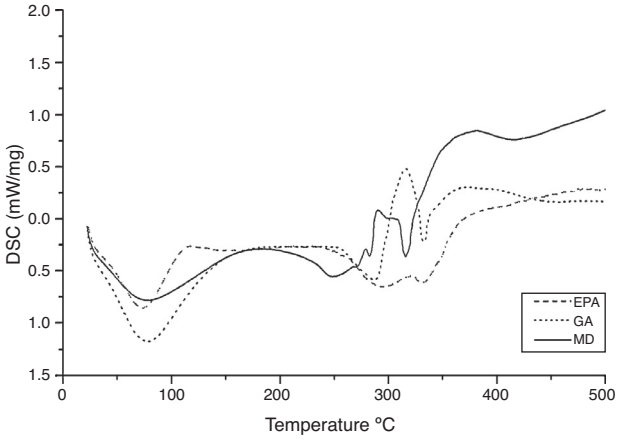

Fig. 2. DSC curves for EPA, GA and MD.

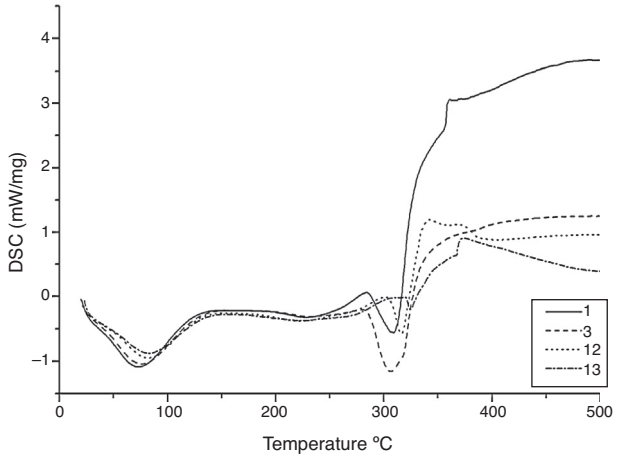

Fig. 3. DSC curves for microparticles for samples 1, 3, 12, and 13.

The DSC curves indicated an interaction between EPA and GA:MD in the microparticles, because in the second endothermic event there was a mixture of profiles of the individual substances.

Analyzing the data for TGA revealed two characteristic mass losses, of EPA as well as GA, MD, and microparticles. The first mass loss corresponds to water desorption, which began almost at room temperature and continued up to 120 °C. This loss was around 5–12% of the total mass. The second loss corresponds to the melting with degradation discussed above, and progressed to a structural collapse.

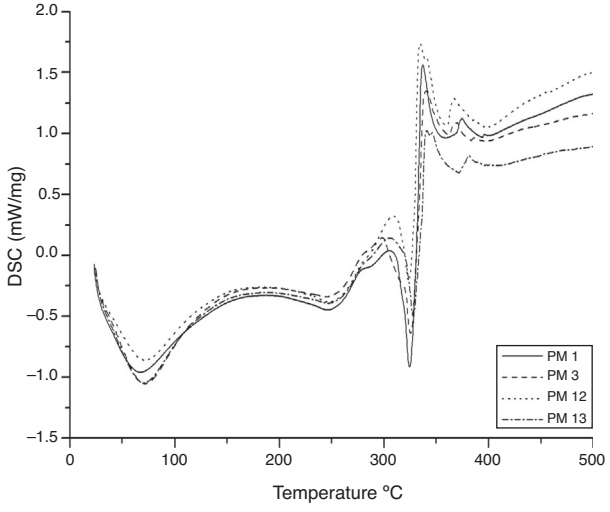

Fig. 4. DSC curves for physical mixtures (PM) 1, 3, 12, and 13.

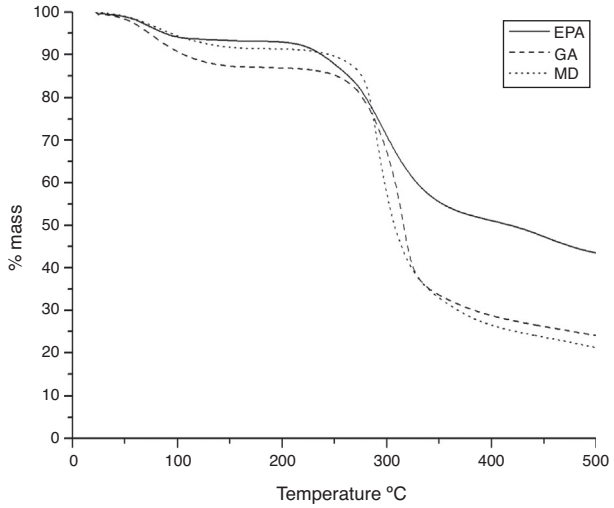

Fig. 5. TG curves for EPA, GA, and MD.

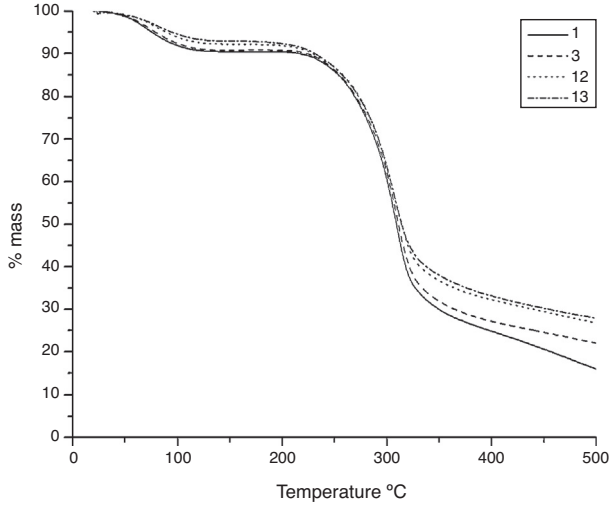

Fig. 6. TG curves of microparticles for samples 1, 3, 12, and 13.

The results for the TGA curves indicate that the profile curves of the microparticles are similar to the adjuvants used in microencapsulation process (GA and MD), including the mass losses. This is probably due to the higher proportion of adjuvants compared to EPA, which was present in a proportion of 10 or 20%.

DPPH radical scavenging capacity

The IC_{50} expresses the amount of EPA microparticles necessary to scavenge 50% of free radicals present in the medium, and shows the antioxidant capacity. Fig. 8 shows the results for the antioxidant capacity of the semipurified extract EPA, the positive control Trolox, and formulations 1, 3, 12, and 13.

It is expected that EPA will lose some antioxidant capacity after the spray-drying process (Fu et al., 2011). As shown in Fig. 8, samples 1, 3, and 12 showed smaller antioxidant capacities than Trolox (positive control) and EPA. Only sample 13 demonstrated a similar antioxidant capacity to the control and EPA. This suggests that microparticles can protect the EPA from the effect of high

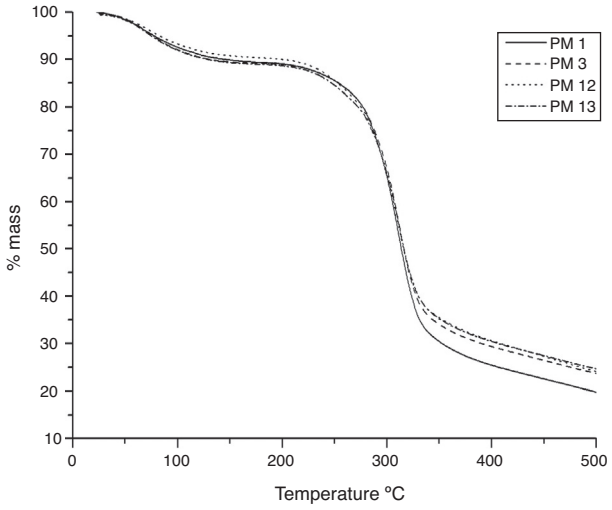

Fig. 7. TG curves for physical mixtures (PM) 1, 3, 12, and 13.

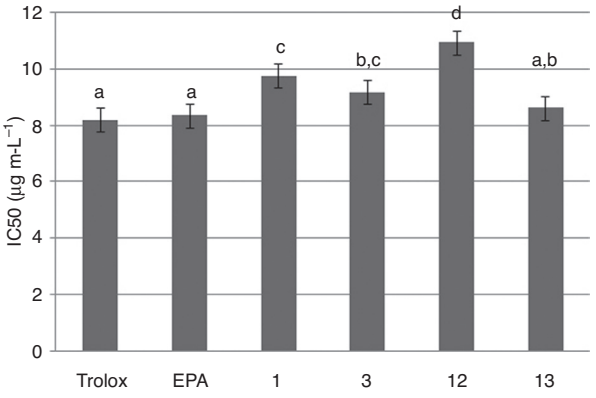

Fig. 8. Antioxidant capacity of semipurified extract EPA, the positive control, Trolox, and the formulations 1, 3, 12, and 13 (a–d: same letters indicate values with the same statistical significance).

temperatures during the process (Fu et al., 2011; Sansone et al., 2011). The antioxidant activity was maintained in epigallocatechin gallate microparticles that were spray dried at temperatures ranging from 95 to 130 °C (Fu et al., 2011). The microencapsulation improved stability, preventing the loss of the quercetin antioxidant capacity during 12 months of storage (Sansone et al., 2011).

This study demonstrated significant retention of the antioxidant capacity of EPA after the microparticle production.

In vitro *dissolution tests*

First, to evaluate the dissolution/release profile of EPA from microparticles, their solubility in the medium was assessed. The solubility value of EPA was 0.92 mg ml^{-1} in water. Then, the sink conditions, which describe a dissolution system sufficiently dilute so that the dissolution process is not impeded by saturation of the solution, were evaluated. The dissolution profiles of EPA from the microparticles are shown in Fig. 9.

All formulations evaluated in the dissolution test started the release at the beginning of the test and showed complete dissolution within 60 min. Among the compounds tested, only sample 1 had a different profile, releasing all the contents in the first 15 min. All formulations released the total content of EPA, suggesting that no interactions occurred between the polymers and EPA that were able to retain the active compounds in the polymeric mixture.

The microparticles dissolve through a process of erosion of the matrix together with swelling (Aulton, 2007), with a large contact area. It was expected that the microparticles would release the active compounds more rapidly with immediate release, but this was not observed. A plausible explanation for this release profile is that the fraction is microencapsulated, it has hygroscopic characteristics and good solubility; however in aqueous dispersion it is different, and may tend to release more slowly (Klein et al., 2013).

In analyzing the results of the release profile, different values of n (release exponent), which characterizes the release mechanism, were obtained. The n values and correlation coefficients (r) of catechin and epicatechin in the samples are summarized in Table 5. For spherical shapes, the ranges of n values are: Fickian diffusion ($n \leq 0.43$), non-Fickian diffusion ($0.43 < n < 0.85$), and transport mechanism type II ($n > 0.85$). Therefore, the microparticles of

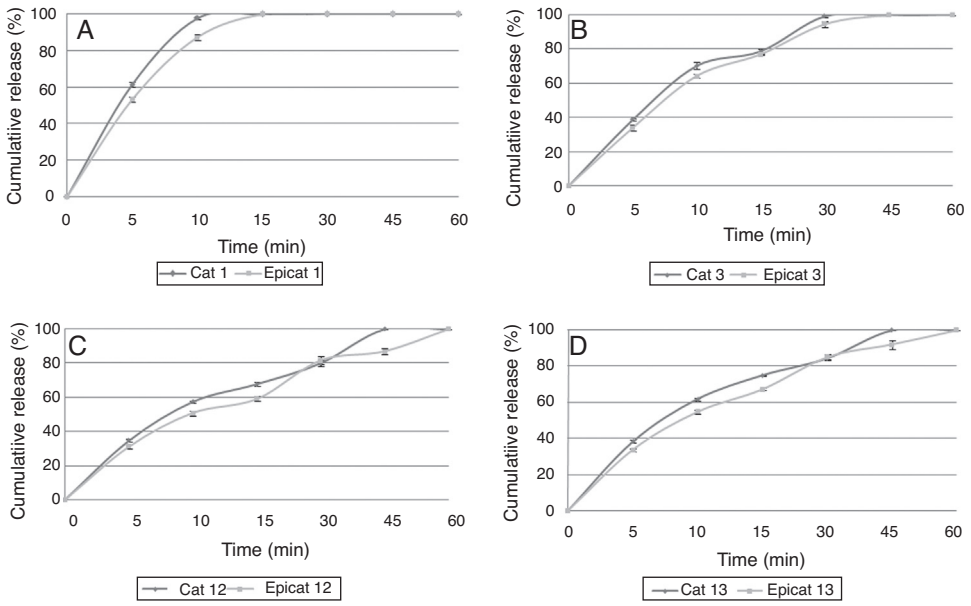

Fig. 9. Dissolution profile of the markers catechin (Cat), and epicatechin (Epicat) from samples 1 (A), 3 (B), 12 (C), and 13 (D), determined by HPLC according previously study (Klein et al., 2012).

Table 5
Catechin and epicatechin release exponent (n) values and correlation coefficients (r) from dissolution profiles obtained by Korsmeyer–Peppas model (*) and by Baker–Lonsdale model (**).

Sample	n^*		r^*		r^{**}	
	Catechin	Epicatechin	Catechin	Epicatechin	Catechin	Epicatechin
1	0.2316	0.3133	0.8812	0.9309	0.8167	0.8804
3	0.4853	0.5540	0.9882	0.9934	0.9768	0.9850
12	0.5290	0.5690	0.9929	0.9909	0.9930	0.9942
13	0.4828	0.5428	0.9963	0.9982	0.9940	0.9955

sample 1 showed release with an influence of Fickian diffusion. In this context, the microparticles of sample 3 would show anomalous behavior, with a greater influence of Fickian diffusion and less relaxation of the polymer chains. The microparticles of sample 12 would show Higuchi's model profile with anomalous behavior, with a greater influence of Fickian diffusion and less relaxation of the polymer chains. The microparticles of sample 13 would show release profile very close to the Higuchi model, and anomalous behavior, with a greater influence of Fickian diffusion and less relaxation of the polymer chains (Aulton, 2007). However, it was also observed a biphasic behavior for the release of epicatechin and catechin from samples 3, 12, and 13. Therefore, it was applied the model of Baker–Lonsdale, which describes the drug release from a non-homogeneous spherical matrix by diffusion, and has been utilized to analyze the results of drug release from microspheres and microcapsules. The correlation coefficients (r) of epicatechin and catechin release from samples 3, 12, and 13 were higher than 0.97 (Table 5), confirming the diffusion is the main phenomenon involved in the release of the active agents from the formulations.

The aqueous medium was chosen as a first step in assessing the release of markers in the dosage form, because freshly purified water lacks interfering attributes, *i.e.*, has a pH close to neutral and contains no ions. For future studies, we intend to evaluate acidic media, simulating the environment of the gastric or the intestinal tract (Klein et al., 2013).

Conclusions

This study investigated the use of the spray-drying technique to produce microparticles containing a semipurified extract of guaraná (EPA). The study demonstrated the feasibility of producing these microparticles using gum arabic and maltodextrin polymers for a one-step process using spray drying. The composition of each formulation influenced the physical and chemical characteristics. The results of the *in vitro* dissolution test may be related to the structure of each formulation of microparticles, which hinders the delivery of the markers within 60 min. This spray-drying technique shows great potential as an efficient and economic process for the production of EPA microparticles for drug delivery.

Conflicts of interest

The authors declare no conflicts of interest.

Authors' contributions

TK performed the laboratory work, analyzed the data and drafted the paper. RL contributed to chromatographic analysis and discussed the results. MLB contributed to designing the study, supervised the laboratory work, and critically read the manuscript. JCPM contributed to designing the study, supervised the laboratory work, and helped to write the manuscript. All the authors have read the final manuscript and approved the submission.

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