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Fluid Bed Drying of Guarana (*Paullinia cupana* HBK) Extract: Effect of Process Factors on Caffeine Content

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Renata S. Pagliarussi,[1] Jairo K. Bastos,[1] and Luis A.P. Freitas[1]

[1]Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Via do Café s/n, Ribeirão Preto 14040-903, São Paulo, Brazil

ABSTRACT

The aim of this study was to study the convective drying of the hydroalcoholic extracts obtained from powdered guarana seeds in a spouted bed dryer. The influence of process variables, such as the convective airflow rate, extract feed rate, and air inlet temperature, on the quality of the dry extract was determined using the caffeine and moisture content for the process evaluation. The caffeine content in the alcoholic and dried extracts was determined by capillary gas chromatography. The experiments were performed following a 3^3 factorial design and the data analyzed by response surface. The analysis of dry extract showed that the air and extract feed rates did not significantly affect (25% level) the caffeine content, but that drying temperature is a major factor to consider when the extract is submitted to fluid bed drying. Caffeine losses were significant (1% level) for drying temperatures above 120°C, while moisture content was lower than 3% for temperatures above 120°C. The data showed that there is an optimum temperature for the drying of guarana extracts in spouted beds, and under the conditions used in this study it was 120°C.

KEYWORDS: Dry extract, convective, caffeine, capillary gas chromatography, spouted bed.

INTRODUCTION

In recent years, there has been increasing interest in the use of standardized plant extracts for medicinal purposes. Dry extracts are known to have several advantages over liquid extracts, including easier standardization, higher actives concentration, and greater stability, and can be used for different types of solid dosage forms.[1] One of the most commercialized plants in the world is the guarana (*Paullinia cupana* HBK), a shrub native to Brazil that grows in the Amazon region and has been used as a traditional medicine in many countries. The plant and its therapeutic properties have been known since the seventeenth century, when the first colonizers encountered the Brazilian indigenous tribes Mandacarus and Mauês. Guarana products have been used as stimulants, aphrodisiacs, antidiarrheals, antiaging agents, and weight loss agents.[2] In Europe, guarana was first marketed as an alternative medicinal plant from the Amazon region and purported to be beneficial to one's overall health. In Europe and the United States today, it is also used for headaches, especially for those of rheumatic nature.[3] Guarana is marketed as a syrup seed powder, in cylindrical rolls, or as an energy drink, with total production of 3500 tons/year, with total sales of $2 billion in 2003.[4] Today, the refresher and energy drink industries account for 60% of the guarana market in Brazil.[4] However, there is increasing interest in guarana dry extracts, which could be used in many pharmaceutical forms, such as capsules and tablets.

The constituents of guarana include caffeine, tannic acid, catechutannic acid, and a greenish essential oil. This plant is probably used mainly because of its high caffeine content, which varies from 3% to 6% in the dried seeds.[5] Caffeine is a purine alkaloid that is widely distributed in nature, along with theobromine,[6] and is largely consumed in analgesics and other over-the-counter drugs. It is the most widely used psychoactive drug in the Western world, particularly in the United States, with an estimated average consumption of 200 mg/day.[7] Methylxanthines, such as caffeine and its metabolite theobromine, are known to affect almost every physiological system of the body, with special stimulatory effects on the brain, the heart, gastric secretion, and urine flow.[7]

Despite guarana's commercial importance, there is not enough information on its behavior during processing and standardization. Given the pharmaceutical industry's increasing interest in the use of guarana extracts, it is important to determine the effect of process conditions on the quality of standardized dry extracts. Standardized plant extracts can be obtained by adjusting the content of the active substance, in this study the caffeine, by adding pharmaceutical adjuvants before the drying process; or by minimizing the effect of drying conditions on active content.[8] For plant extracts, drying is a crucial step since it can lead to different amorphous states for the drugs[9] and affects

Corresponding Author: Luis A.P. Freitas, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Via do Café s/n, Ribeirão Preto 14040-903, São Paulo, Brazil. Tel: 55 16 3602 4225; Fax: 55 16 3602 4879; E-mail: lapdfrei@usp.br

their stability.[10] Furthermore, the characteristics of the dry product depend on the choice of dryer type and the operational conditions.[11,12] The fluidized and spouted bed dryers with inert particles are often used for drying of heat-sensitive materials such as plant extracts[12] and have the advantage of providing the product in the form of a fine powder (smaller than 75 μm), eliminating the need for further processing, like grinding.

The aim of this study was to verify the effect of drying conditions on caffeine and moisture content, Cc and Mc, of guarana extracts obtained in a spouted bed dryer. This information is essential for the standardization of the dry extract processing. Response surface methodology (RSM) was applied to analyze the significance of the effects of process factors on product quality (eg, caffeine and moisture content) and to obtain fitted equations to predict dry powder properties.

Materials and Methods

Extract Preparation

Certified guarana seeds were purchased from Herba Química (São Paulo, Brazil). The seed preparation process involved grinding in a disk mill and mixing. The content of caffeine in the ground seeds was 2.47%, determined by capillary column gas chromatography (GC)[13,14] using the same method described in this article for the dry extract analysis. The extraction was performed using a solution of ethanol:water 7:3 wt/wt and 20% wt/vol of the seed powder in a shaker at 40°C and 200 rpm for 2 hours. The extracts obtained were clarified in a lab scale continuous centrifuge, which retained most of the insoluble solids. The resulting extract had a total solids content of 4.08% and a density of 0.89 g/cm^3.

Spouted Bed Drying

The drying experiments were performed in a lab scale spouted bed dryer SBD-1.0 (Labmaq Ltd, Ribeirão Preto, Brazil) loaded with polytetrafluoroethylene beads as inert particles. The equipment was built in borosilicate glass and stainless steel, allowing for the drying of liquid feeds up to a rate of 1.0 L/h. The dryer bowl volume was 2.5 L, and its internal diameter was 9.3 cm, with a single air inlet orifice of 2.5 cm in diameter. The dry powder was collected at the dryer outlet by a cyclone separator. Temperatures could be measured and controlled (up to 200°C) at the bowl entrance by a digital controller. A digital temperature indicator measured exit temperatures. Extract feed rates were set by a variable speed peristaltic pump, allowing feed rates from 1.0 to 17.0 mL/min. Some important operational conditions were varied for the study of the drying process, such as the airflow rate, V; the extract feed rate, Ve; and the air inlet temperature, Ti. In all runs, the load of inert particles was kept at 600 g. The minimum spouting airflow rate, Vms, for this load of inert bodies was determined to be 0.36 m^3/min, following a classical procedure based on visual observation of the particle flow regime.[15] Additional information on the characteristics of the dryer, such as geometry and dimensions, can be found elsewhere.[16] Extract feed rates were limited by safety requirements, for instance, the upper and lower inflammability limits for the ethanol-air mixture.[17,18] The dryer had explosion-proof parts and electricals for hazardous applications and was dotted with a vent system. The drying system was gas-tight to prevent vapor leakage out or air in, ensuring that no explosive mixtures could be created.

Capillary Column GC

The chromatographic methodology used[14] was recently proposed for quantification of caffeine in guarana products. The analysis was performed in a Hewlett-Packard 6890 plus GC (Palo Alto, CA) equipped with a split/splitless injector inlet and a flame ionization detector (FID). A 100% methylsilicon chromatographic capillary column, DB-1, with a length of 30 m, a 0.25-mm internal diameter, and a 0.25-μm film thickness was used. The carrier gas was hydrogen at a flow rate of 1.0 mL/min, and the increasing temperature gradient was 170°C to 210°C at 6.0°C/min and 210°C to 250°C at 3.0°C/min. Injector port and detector temperatures were held constant at 250°C. The injector was operated in the split mode (1/10). For the quantification methodology, caffeine and 7-β-hydroxyethyltheophylline were used as external and internal standards, respectively. Under this set of analysis conditions, the retention times were 13.2 minutes for 7-β-hydroxyethyltheophylline and 5.9 minutes for caf–feine. All chemicals used, absolute ethanol (Merck), caffeine (Merck), and 7-β-hydroxyethyltheophylline (Sigma-Aldrich), were AR grade. Peaks were assigned according to their retention times and by co-chromatography of authentic standards.

The linearity of response was determined by injecting the mixture of caffeine, 7-β-hydroxyethyltheophylline, and benzophenone at 10 different concentrations. The original mixture containing 1.0 mg/mL of each compound in absolute ethanol was diluted and injected at concentrations ranging from 50 μg/mL to 1000 μg/mL.

The precision was determined by analyzing 6 samples of a mixture of ground seeds added with 2.5% wt/wt of pure caffeine. The accuracy was determined by analyzing in quadruplicate 3 mixtures with nominal concentrations of 1.0, 3.0, and 5.0% wt/wt, followed by 2 injections of each sample on 3 different days.

The reproducibility of the method was determined by 5-fold extraction and GC analysis of 1 commercial sample of guarana on 5 different occasions. The repeatability of the

method was evaluated by injecting a solution containing both caffeine and 7-β-hydroxyethyltheophylline 6 different times and observing the ratios between the areas obtained for the compounds. The relative standard deviation (RSD) of the drying procedure was determined by running 5 different drying experiments at the same operational conditions and injecting the samples to analyze caffeine content.

Sample Preparation

A sample of the dried extract (0.05 g) and 10 mL of absolute ethanol containing 1.0 mg/mL of the internal standard (7-β-hydroxyethyltheophylline) was prepared in a 50-mL Erlenmeyer flask at room temperature. The mixture was filtered through an analytical paper, 0.45 μm pore size, under vacuum, and an aliquot of 2 mL was transferred to a chromatography vial for GC analysis. The expected caffeine content of the dry extract, estimated by mass balance and neglecting any losses, was 130.75 mg/g.

Moisture Content

Dry extract moisture content was gravimetrically assayed by mass loss in a microprocessed tray oven (model M330, Nova Etica, São Paulo Brazil) at 60°C until it reached a constant weight in an analytical balance (±0.1 mg, model Explorer, Ohaus Co, Pine Brook, NJ).

RESULTS AND DISCUSSION

To verify the validity of the conditions of the chromatographic method,[13,14] preliminary runs were made with the injection of samples of the ground seeds, liquid extract, and dry extract. In all cases the chromatograms showed distinct, clear peaks for both caffeine and the internal standard, as expected based on a previous study.[14] The study on linearity of responses showed linear correlations of 0.9998 for both caffeine and 7-β-hydroxyethyltheophylline. The quantification limit for caffeine was 15 μg/mL and the linear response was up to 1000 μg/mL. For caffeine and 7-β-hydroxyethyltheophylline the FID responses were linear from 30 to 2200 μg/mL. The highest RSD found in precision tests was 2.0%, and the highest average accuracy was (% bias) 2.1%. The RSDs of the reproducibility and repeatability tests were 1.0% and 0.9%, respectively. The RSD for the drying procedure was 1.1%.

Table 1. Levels of Factors Studied in the Fluid Bed Drying Experiments

	Levels		
Factors	+	0	−
Airflow rate, V (m^3/min)	0.57	0.50	0.43
Air inlet temperature, Ti (°C)	180	120	60.00
Extract feed rate, Ve (mL/min)	7.00	5.00	3.00

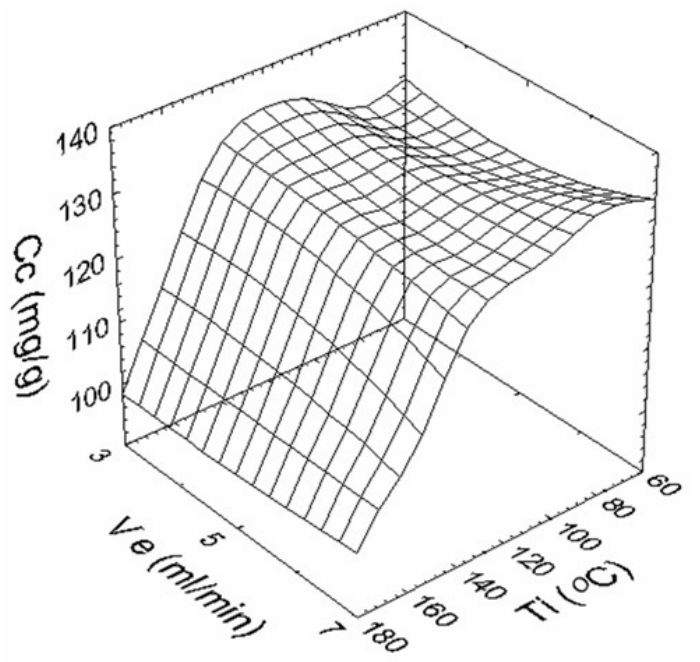

Figure 1. Effect of inlet air temperature and extract feed rate on caffeine content of dry powder; V = 0.43 m^3/min.

For the study of the influence of drying conditions on dry extract quality (eg, caffeine and moisture content), a full 3^3 factorial design was applied. Runs were performed by varying air inlet flow rates, air inlet temperatures, and extract feed rates. Airflow rates 20%, 40%, and 60% above the minimum spouting flow rates were chosen. Extract feed rates were kept under two thirds of the lower limit of inflammability for an ethanol-air mixture.[17,18] The air inlet temperatures chosen were 60°C, 120°C, and 180°C. The selected levels of factors (low, medium, and high, denoted as −, 0, and +) are shown in Table 1.

The effects of air inlet temperature and extract feed rate on the dry extract caffeine content, Cc, for the airflow rate of 0.43 m^3/min can be seen in Figure 1. The surface plot in this figure shows that caffeine losses are negligible for temperatures up to 120°C, but there is a clear and sharp decrease in caffeine content for temperatures above 120°C. Another effect that can be seen is the influence of extract feed rates, which is very low for the data presented. A possible explanation for the marginal effect of extract feed rates on caffeine content is that the dryer was being operated well under its maximum evaporative capacity, so it had an excess of thermal energy as well as little effect on the bulk bed temperature. The same trends as in Figure 1 were observed for other airflow rates in this study.

Figure 2 shows a surface plot of caffeine content in dry extract as a function of dimensionless airflow rate, V/Vms,

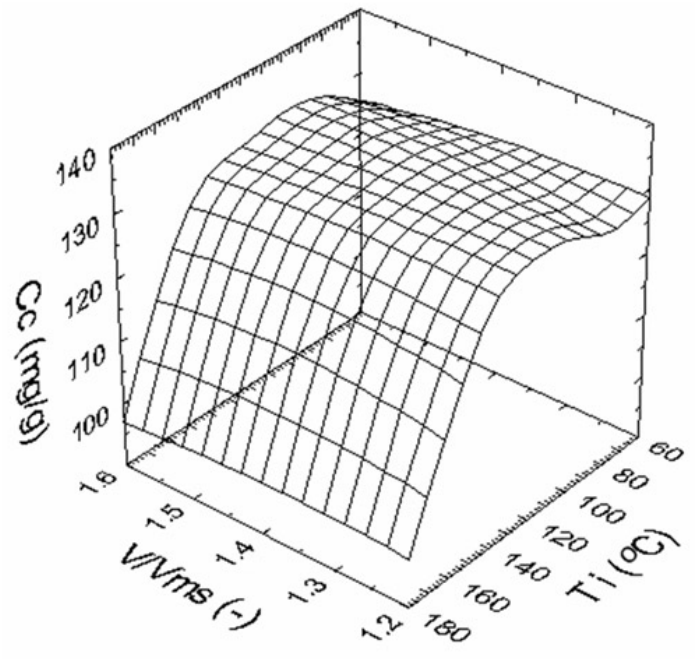

Figure 2. Effect of dimensionless airflow rate and inlet temperature on caffeine content of dry powder; Ve = 3 mL/min.

and temperature for the extract feed rate of 3 mL/min. For temperatures up to 120°C, the caffeine content was very close to the one expected in the case of no loss (130.8 mg/g), but a sharp decrease in Cc was seen for temperatures above 120°C. The airflow rate appeared not to affect Cc throughout the conditions shown. The same trends were observed for the data obtained for the other 2 extract feed rates (5 and 7 mL/min).

Another indicator of dry extract quality is the final moisture content, Mc. In general, it is accepted that for a moisture content under 5% the microbial growth and chemical degradation reactions are significantly diminished,[8] providing longer stability to the powder. The effects of air inlet temperature and dimensionless airflow rate on the final moisture content for an extract feed rate of 3.0 mL/min are shown in Figure 3. The surface plot presented shows that moisture content was around 7% for temperatures ~60°C and decreased monotonically with air inlet temperature for values above 80°C. Also, the surface indicates that moisture content slightly decreased with airflow rate increase. Figure 4 shows the moisture surface plots as a function of extract feed rates and dimensionless airflow rates at 120°C. For this plot one can observe the same trends seen in Figure 3: the moisture content decreased with an increase in airflow rate, but it grew with an increase in extract feed rate. Generally, moisture content was under 7% and reached values under 3% at temperatures above 120°C, as can be seen in Figures 3 and 4.

To develop a response surface equation to predict the caffeine and moisture content in the dry extract, the data were statistically analyzed using the module Visual General Linear Model from the software Statistica '99 Edition (StatSoft, Inc, Tulsa, OK).

A 3^3 full factorial design was used for statistical and response surface analysis.[19,20] This design was chosen because

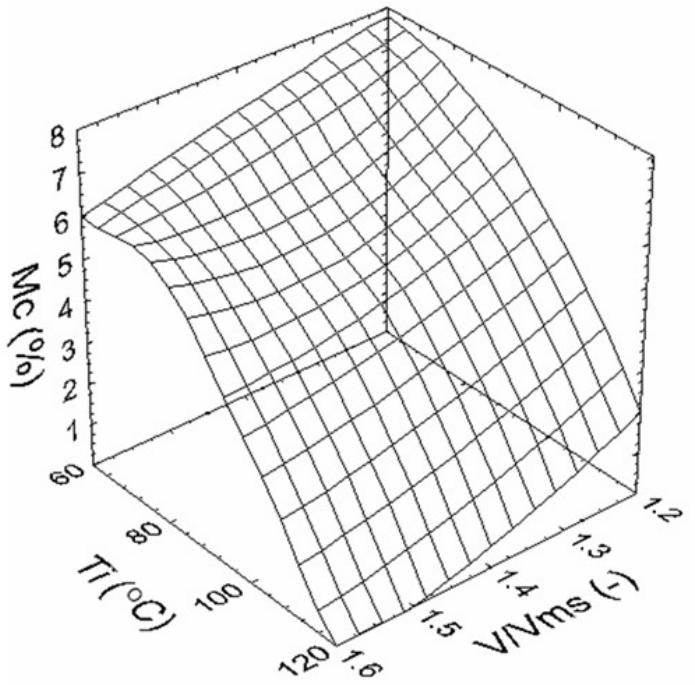

Figure 3. Effect of dimensionless airflow rate and inlet temperature on moisture content of dry powder; Ve = 3.0 mL/min.

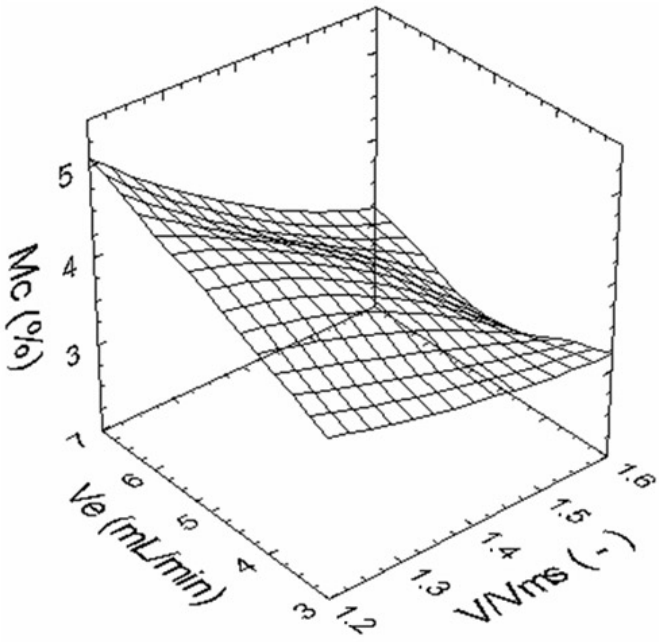

Figure 4. Effect of dimensionless airflow rate and extract feed rate on moisture content of dry powder; Ti = 120°C.

it allows the estimation of complex response functions, up to the quadratic order.[20] The design of selected experiments as well as the caffeine and moisture content values is shown in Table 2. To follow the levels adopted in this design, the factors studied needed to be decoded. The decoding formula was as follows:

$$Coded\ Variable = \frac{\left(uncoded\ value - 0.5 \times (high\ value + low\ value)\right)}{0.5 \times (high\ value\ low\ value)} \quad (1)$$

The response function applied was a quadratic polynomial equation:

$$Y_i = A_o + A_1X_1 + A_2X_2 + A_3X_3 + A_4X_1X_2 + A_5X_1X_3 + A_6X_2X_3 + A_7X_1^2 + A_8X_2^2 + A_9X_3^2 \quad (2)$$

where Y_i = dependent variable = caffeine or moisture content; X_1 = coded airflow rate; X_2 = coded air inlet temperature; X_3 = coded extract feed rate; and A_i = polynomial coefficients.

Table 2. Design for Statistical and Response Surface Analysis and Respective Results on Caffeine and Moisture Content

	Coded Variables			Results	
Treatment Number	X_1	X_2	X_3	Cc (mg/g)	Mc (%)
1	1	1	1	99.00	0.5
2	1	0	1	99.60	0.5
3	1	–1	1	97.00	0.3
4	1	1	0	127.8	2.3
5	1	0	0	127.1	2.9
6	1	–1	0	126.9	2.7
7	1	1	–1	130.8	6.0
8	1	0	–1	130.8	5.4
9	1	–1	–1	125.1	6.1
10	0	1	1	99.70	0.5
11	0	0	1	101.8	0.3
12	0	–1	1	97.50	0.3
13	0	1	0	126.8	3.7
14	0	0	0	126.3	2.5
15	0	–1	0	129.3	2.1
16	0	1	–1	130.8	7.0
17	0	0	–1	130.8	6.9
18	0	–1	–1	128.2	5.6
19	–1	1	1	98.70	0.5
20	–1	0	1	99.40	0.6
21	–1	–1	1	98.60	1.1
22	–1	1	0	130.5	4.1
23	–1	0	0	127.6	3.2
24	–1	–1	0	130.8	2.1
25	–1	1	–1	130.8	7.8
26	–1	0	–1	127.6	7.1
27	–1	–1	–1	130.1	7.3

Table 3. Analysis of Variance for Caffeine Content

	Sum of Squares	Degrees of Freedom	Mean Square	F_{calc}
Factor				
V	3.040	1	3.040	0.530
Ve	13.52	1	13.52	2.350
Ti	4487	1	4487	778.0*
V^2	0.860	1	0.860	0.150
Ve^2	0.500	1	0.500	0.010
Ti^2	1052	1	1052	182.6*
Interaction				
V × Ve	8.670	1	8.670	1.500
V × Ti	0.160	1	0.160	0.030
Ve × Ti	10.08	1	10.08	1.750
Error	97.97	17	5.760	
Total	5674	26		

*Term is significant at $P = .01$.

Table 3 shows the results of the statistical analysis (analysis of variance) for the caffeine content and the effect of the 3 factors studied, their quadratic terms and interactions. As can be seen by the F values in Table 3, only the terms related to the coded air inlet temperature were significant. Both the linear and the quadratic terms of Ti were significant at the 1% level. None of the other factors and derived terms were significant, even at the 25% level. The analysis of variance confirms the observed trends in Figures 1 and 2, where only Ti was shown to affect the caffeine content of the dry extract. As discussed above, a possible explanation for this result is that the dryer was operated well under its maximum evaporative capacity, so that the bulk temperatures inside the dryer and the outlet air temperatures were unaffected by V and Ve.

Table 4. Analysis of Variance for Moisture Content in Drying Experiments

	Sum of Squares	Degrees of Freedom	Mean Square	F_{calc}
Factor				
V	2.810	1	2.810	12.65*
Ve	0.140	1	0.140	0.61
Ti	1.280	1	1.280	5.78†
V^2	0.030	1	0.030	0.12
Ve^2	165.6	1	165.6	747.93*
Ti^2	2.940	1	2.940	13.28*
Interaction				
V × Ve	0.400	1	0.400	1.82
V × Ti	1.200	1	1.200	5.43†
Ve × Ti	0.330	1	0.330	1.51
Error	3.760	17	0.220	
Total	178.5	26		

*Term is significant at $P = 0.01$.
†Term is significant at $P = 0.05$.

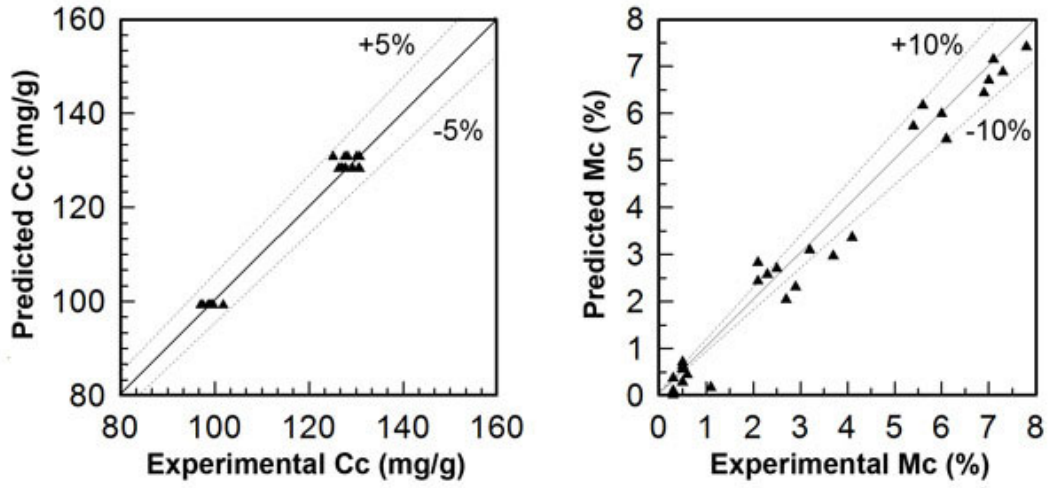

Figure 5. Experimental versus predicted (response surface methodology) values for caffeine and moisture content.

The response surface analysis resulted in the following equation for the prediction of Cc:

$$Cc = 128.26 - 15.79\left(\frac{Ti-120}{60}\right) - 13.24\left(\frac{Ti-120}{60}\right)^2 \quad (3)$$

with a squared correlation coefficient $R^2 = 0.9827$.

Table 4 presents the results from the statistical analysis of moisture content for all effects up to the quadratic terms. In this table, the linear term related to the airflow rate and the terms corresponding to the squared extract feed rate and the squared air inlet temperature are significant at the 1% level. Terms related to the inlet temperature and its interaction with airflow rate were significant at the 5% level. The results show the complex mechanisms affecting moisture content during the drying of pastes in spouted beds with inert bodies. Moisture content results from a combination of factors, such as the kinetics of deposition, drying, and removal of the paste from the surface of the inert bodies.

An equation was fitted by response surface analysis, taking into account only the significant terms and with correlation coefficient $R^2 = 0.9789$, as follows:

$$Mc = 2.70 - 0.394.\left(\frac{V-0.50}{0.07}\right) + 0.267.\left(\frac{Ve-5.0}{2.0}\right) - 3.033.\left(\frac{Ti-120}{60}\right) + 0.700.\left(\frac{Ti-120}{60}\right)^2 + 0.317.\left(\left(\frac{V-0.50}{0.07}\right) \times \left(\frac{Ti-120}{60}\right)\right) \quad (4)$$

The comparison of the experimental values of Cc and Mc with the values predicted by Equations 3 and 4 is shown in Figure 5. In Figure 5a, Cc data versus predicted Cc is plotted, showing that all data were predicted with a deviation below ±5%. The moisture content data were predicted with a deviation below ±10%, as shown in Figure 5b.

CONCLUSIONS

The spouted bed dryer proved to be adequate for the processing of this plant extract, allowing the drying to be done with or without the addition of pharmaceutical excipients. The caffeine content of the dry extract depended on the air inlet temperature, showing a quick and sharp drop for temperatures above 120°C. The moisture content of the dry extract depended on such operational variables as airflow rate, extract feed rate, and air inlet temperature, and on the squared Ti and the interaction of V and Ti. The optimum temperature for the convective drying of guarana extracts in spouted beds was 120°C for the conditions used in this study.

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