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PREPARATION OF A NOVEL CURCUMIN INSTANT GREEN TEA POWDER: OPTIMIZATION OF THE SPRAY-DRYING PROCESS BY RESPONSE SURFACE METHODOLOGY

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ABSTRACT
The objective of this work was to optimize the spray-drying conditions for the preparation of curcumin instant green tea powder (Cur-IGTP) using response surface methodology (RSM). Firstly, curcumin nanosuspension and filtrated green tea concentrate solution were prepared respectively and mixed together, and then the mixture was spray-dried to obtain Cur-IGTP. The independent variables of spray drying such as inlet air temperature, feed flow rate and drying air flow speed were optimized using a Box–Behnken design, moisture content and yield were analyzed as responses. The morphology, sensory, solubility bulk density and physical stability of spray-dried Cur-IGTP were characterized. An inlet air temperature of 147.5 ºC, a feed flow rate of 1.97 mL/min and a drying air flow speed of 62.0 L/min was selected as the optimal conditions. At the optimal conditions, the yield reached a maximum of 47.2% and moisture content reached 4.4%, which provides an efficient process for production of Cur-IGTP.

KEY WORDS: curcumin; wet milling; green tea; spray drying; optimize.

INTRODUCTION
Tea is one of the most popular flavored and healthy beverages in the world.[1, 2] Over 500,000 metric tons of dried tea is manufactured every year. The chemical composition of tea is complex including catechins, theaflavins, flavonols, flavonoids, theanine, amino acids, sugars and polysaccharides, lipids, caffeine and minerals, etc.[3] Currently, green tea gained wide spread popularity not only due to its attractive aromas and flavours, but also due to its health benefits. Numerous studies show tea has some biological functions such as anti-inflammation, anti-oxidation, anti-allergy effects.[4] It is also revealed that tea consumption may reduce the risk of cancers and many inflammatory and metabolic diseases.[5-8]

Curcumin is a naturally occurring yellow active ingredient of the curcuminoid family, extracted originally from the rhizome of turmeric. Curcumin is used in food coloring, drugs and cosmetics. Curcumin is a potential therapeutic agent for a wide variety of human diseases. Many pharmacological actions[9] have been reported for curcumin, such as anti-inflammatory, apoptotic, antioxidant[10], and antitumor effect.[11, 12] In addition, curcumin has been shown virtually no toxicity for organism.[13, 14]

However, the application of curcumin is limited due to its poor oral bioavailability, which results from its poor solubility and absorption, and its rapid metabolic elimination by reduction and conjugation.[15] In this study, We developed nanosuspension by wet milling technique in order to improve dissolution rate and bioavailability of curcumin. The nanosuspension is produced by reducing large crystals down to the sub-micron range.[16] As an efficient and convenient method, wet milling technique can sharply increase the surface area of drug particles and improve the dissolution rate, resulting in better absorption. The curcumin nanosuspension using wet milling can display a significantly high absorption.

Nowadays, dietary supplements are becoming a part of the daily diet because they could be benefit to prevent and treat some diseases. Catechins are high content of active constituents in green tea extracts. Recent studies have shown synergism between curcumin and catechins in disease prevention. curcumin and catechin combination can inhibit cell proliferation through induction of apoptosis.[17] In another study, curcumin and catechin successfully reduced colorectal aberrant crypt foci in colorectal cancer induced male Wister rats.[18]

In this study, we want to combine the tea and curcumin together to manufacture the curcumin instant green tea powder (Cur-IGTP) as a novel dietary supplements. As we all known, instant tea is extensively used nutraceuticals around the world. Instant tea contains large quantity of tea polyphenols, caffeine, saccharide, protein, vitamins, inorganic elements such as physiological active element, and is beneficial for health promotion. The drying of tea controls the moisture content by either removing the water or binding it so that the green tea becomes stable to both chemical and microbial deterioration.

Therefore, the objectives of this study were to develop the spray drying procedures for manufacturing Cur-IGTP and to optimize the process parameters involved during spray drying stages. In the premiliary studies, spray drying such as inlet air temperature, feed flow rate and drying air flow speed would strongly influence moisture content and yield of tea powders. Therefore, to optimize the experiment and analyze the interactions between factors, the model by Box-Behnken design was established and assessed.

MATERIALS AND METHODS

Materials

Curcumin (Cur) was obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Green tea used in this study was purchased from Huiyou supermarket in Baoding, China. Methanol and ethanol were both provided by Beichen Founder Regent Plant (Tianjin, China). Tween 80 was purchased from Yibei Chemical Reagent (Tianjin, China). All other chemicals were of analytical grade. Distilled water was used throughout the study.

Preparation of Cur nanosuspension

Cur nanosuspension was prepared using a wet-milling technique. Pure Cur was dispersed into the aqueous stabilizer solution by a homogenizer, and then the suspension was transferred to a MiniZeta (NETZSCH Machinery and Instruments Co., Ltd., Selb, Germany) machine equipped with the grinding medium of yttrium-stabilized balls (0.6 mm in diameter). The grinding speed was 3000 rpm and the system temperature was maintained at less than 25 ℃ by passing cooling water through the outer jacket continuously.

Preparation of tea condensated extracts

The tea were extracted by the following process: Briefly, 200g of green tea was dipped in 5L water for 10 min at 80℃ in water bath and the resulting extract was filtered; the residue was added 2L water to extract again for 20 min at 80℃, and then filtered. The final extracts were combined and condensated to 1L by evaporation under vacuum.

Spray drying of Cur-IGTP

The production flowchart of Cur-IGTP is shown in Fig. 1. In brief, 200ml of tea condensated extracts was mixed with 20ml curcumin nanosuspension, then 10ml 5% mannitol solution was added, after filtrated by 120 mesh sieve, the filtrate was pray-dried using a YC-015 experimental spray drier (Yacheng experimental Co. Ltd., Shanghai, China). The Cur-IGTP powders were collected and dried for further study. A magnetic stirrer bar was used to keep the suspension homogenized at 300 rpm.

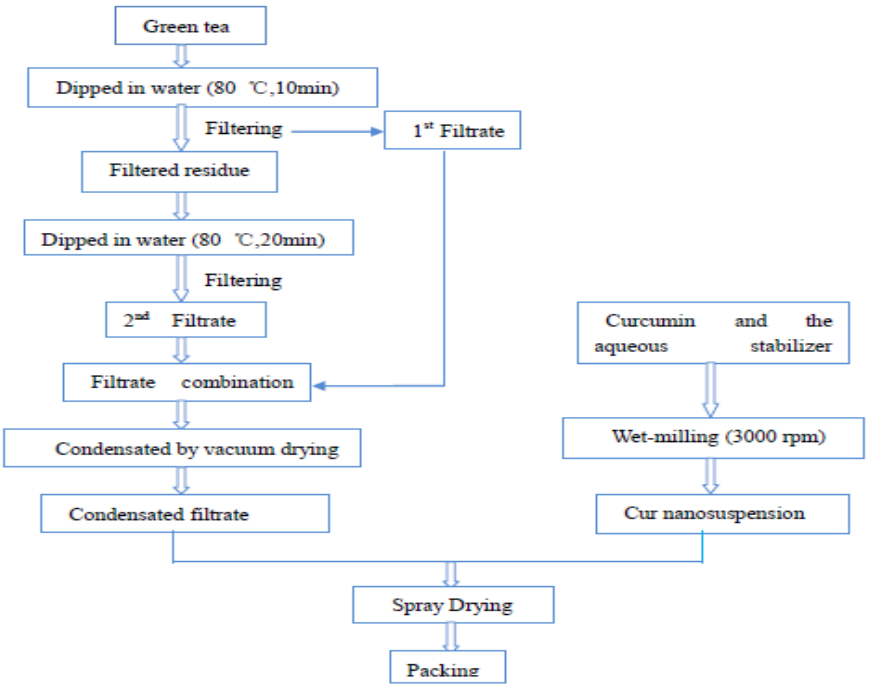


Figure. 1. The production flowchart of Cur-IGTP

Optimization of the spray-drying process for Cur-IGTP

Box-Behnken design was used to evaluate the effects of the processing variables on the quality of spray dried Cur-IGTP. A three-factor, three-level design within 17 experimental runs was employed in this research. The design parameters and the experimental design are given in Table 1 along with their low, medium and high levels. The independent variables are inlet air temperature (A), feed flow rate (B), air flow speed (C). Besides, the dependent variables are selected to be the yield of the product (Y_1) and moisture content (Y_2).The design consists of 17 experimental runs, and analyzed by the statistical software package Design-Expert 8.0.7.1 (Stat-Ease Inc., Minneapolis, Minnesota, USA). The values of the product yield and the fine particle fraction for each trial of the experimental design are shown in Table 2.

Table 1: Experimental parameters for Box–Behnken Design.

Factors	Process parameters	Levels		
		Low	Medium	High
		(-1)	(0)	(+1)
Independent variables				
A	inlet temperature (℃)	135	145	155
B	feed flow rate (ml/min)	1	3	5
C	air flow speed (L/min)	50	65	80
Dependent variables				
Y_1	Yield (%)			
Y_2	Moisture content (%)			

Physico-chemical properties of spray-dried Cur-IGTP

Sensory and solubility evaluation of Cur-IGTP

The sensory properties of Cur-IGTP were scored by six volunteers. Different taste attributes, including bitterness, astringency, sweet aftertaste, and overall acceptability was evaluated. Each volunteer taste 5ml sample solution and kept in the mouth for 10 s the intensity of bitterness was recorded, then the solution was spit out, and the intensity of astringency was recorded. After washing oral cavity with water, the intensity of the sweet aftertaste was recorded in 5 s. They were asked to rate the taste of Cur-IGTP using a scale of 0–10. i.e. 8–10 'very good', 6–8 'good', 4–6 'neutral', 2–4 'bad', and 0–2 'extremely bad'. Each evaluation was repeated twice.

For solubility analysis, 1 g of the Cur-IGTP samples was added to 100 mL of distilled water and the mixture was stirred at 300 rpm for 3 min. The solution was then filtered with the pre-weighed 0.45 µm filter membrane and dried in an oven at 80 °C until constant weight. Weight difference was used to calculate the percent solubility.

Morphology of Cur-IGTP

The shape and surface morphology of Cur-IGTP were evaluated by a scanning electron microscopy (SEM). The Cur-IGTP was initially fixed on stubs using a double-sided sticky and coated with gold under a high-vacuum atmosphere and subsequently observed by SEM (JSM-7500F, Japan Electronics Co., Ltd., Japan) at an acceleration voltage of 10 kV.

Product yield

Yield was calculated as ratio of the weight of the resultant powder after spray drying in collecting bottle and the weight of all solids in the feeding suspension, any powders adhering to the walls of drying chamber or cyclone were not considered.

Moisture content: The moisture content was determined by drying of 1 g samples at 75°C in an oven until a constant weight was obtained. The moisture content was calculated as: the loss weight of water / weight of powder sample×100%. All experiments were performed in triplicate.

Bulk density: To determine the bulk density, the spray-dried powder was gently added into an empty 10 mL graduated cylinder and bulk density was calculated as the ratio between the mass of the powder and the volume occupied in the cylinder.

Physical stability: Physical stability is a crucial issue in formulating dry powders. Cur-IGTP was sealed in vials and stored for 3 months at 4 ℃ in a refrigerator. The stability study was examined after 3 months storage. Samples were withdrawn to measure moisture content, bulk density and solubility.

RESULTS AND DISCUSSION

Design of experiments: A total of 17 experiments were carried out to study the effect of experimental parameters on the product yield and moisture content (Table 2). A second-order polynomial equation was used to express the response variables as a function of the independent variables as follows

$$Yield=51.28+1.37\times A+2.89\times B-1.89\times C-0.80\times A\times B+4.80\times A\times C+0.83\times B\times C-5.25\times A^2-3.23\times B^2-5.73\times C^2$$

Moisture content
$=4.64-0.1\times A+0.51\times B+0.11\times C+0.4\times A\times B+0.35\times A\times C-0.075\times B\times C+0.39\times A^2+0.067\times B^2+0.32\times C^2$

Where A, B, C are inlet air temperature, feed flow rate, air flow speed, respectively.

Table 2 Experimental runs, independent variables, and measured responses of the Box–Behnken Design.

Run	A	B	C	Y_1	Y_2
	Inlet temperature (℃)	feed flow rate (ml/min)	Air flow speed (L/min)	Yield (%)	moisture content (%)
F1	155	1	65	38.7	3.9
F2	145	3	65	51.5	4.3
F3	145	3	65	52.3	4.6
F4	145	3	65	50.9	4.7
F5	135	3	50	44.3	5.7
F6	135	3	80	30.4	5.2
F7	145	3	65	51.2	4.8
F8	145	1	50	43.7	4.5
F9	155	3	80	45.9	5.7
F10	145	1	80	38.8	4.9
F11	155	5	65	46.5	6.1
F12	135	1	65	37.5	4.9
F13	145	3	65	50.5	4.8
F14	155	3	50	40.6	4.8
F15	145	5	50	44.2	5.3
F16	135	5	65	48.5	5.5
F17	145	5	80	42.6	5.4

Table 3: Analysis of variance of regression coefficients calculated for curcumin instant green tea powder.

Independent variable	Yield		Moisture Content	
	Coefficient	p-value	Coefficient	p-value
A	1.3700	0.0048	-0.100	0.0062
B	2.8900	0.1832	0.5100	0.3037
C	-1.8900	0.0173	0.1100	0.0007
AB	-0.8000	0.0822	0.4000	0.2519
AC	4.8000	0.5626	0.3500	0.0164
BC	0.8300	0.0082	-0.0750	0.0286
A^2	-5.2500	0.5507	0.3900	0.5746
B^2	-3.2300	0.0046	0.0670	0.0159
C^2	-5.7300	0.0401	0.3200	0.6036
Constant	51.2800	0.0029	4.6400	0.0000
R^2	0.9653		0.8471	

Inlet air temperature (A), feed flow rate (B), air flow speed (C).

Response surface analysis

Product yield: Analysis of variance (ANOVA) was performed to evaluate the significance of the coefficients of the quadratic polynomial models (Table 3). The estimated regression coefficients for yield and moisture content with their corresponding R^2, p value of regression were also shown in Table 3. The coefficients of multiple determinations, R^2, of 0.9653 and 0.8471 all indicate that the models fit the experiment data points. For any of the terms in the models, a small p value would indicate a more significant effect on the respective response variables. The linear terms of inlet air temperature ($p < 0.01$), air flow speed ($p < 0.05$), quadratic terms of feed flow rate ($p < 0.01$), air flow speed ($p < 0.05$) and interactive terms of feed flow rate and air flow speed ($p < 0.01$) had a significant effect on the product yield. Figure 2a-2c is the response surface plot showing the effect of different independent variables on the product yield. As observed in Figure 2a and 2c, it is clear that the yield of spray drying is improved with increasing feed flow rate. As seen in Figure 2a and 2b, results showed that increasing drying temperature resulted in improving the product yield first and then decreasing the yield. For each inlet temperature, the outlet air temperature is mainly dependent on the feed rate and less on the aspirator rate, air humidity and spray air flow. The outlet temperature cannot be too high as it affects the product quality or too low as it corresponds to undesirably high residual moisture in the product. At first, the yield of spray drying increased as the outlet air temperature increased. This was caused by the lower moisture content of the powder at higher outlet

temperatures, which reduced its stickiness. Then higher outlet temperatures resulted in a lower yield, it can be attributed to partially sticking of the insufficiently dry particles on the dryer walls at higher temperatures. The response surface plots show that the increase of air flow speed decreases product yield.

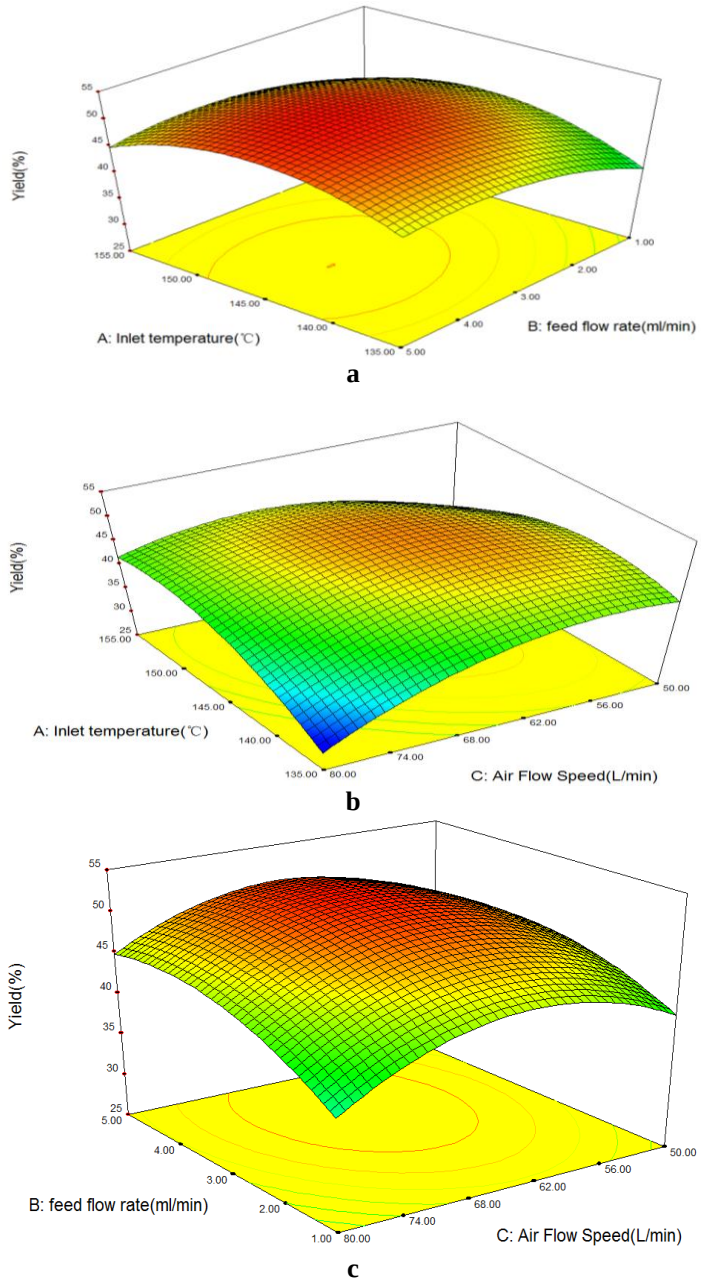
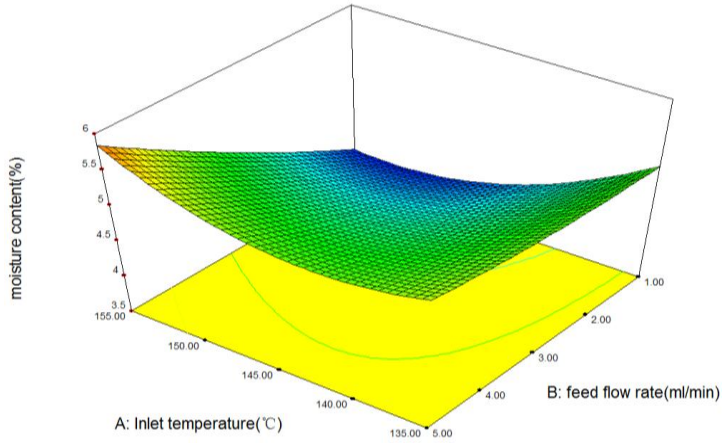


Figure 2. Response surface plots for product yield. (A) inlet temperature, (B) feed flow rate, (C) air flow speed

Moisture content

The linear terms of inlet temperature and air flow speed had a significant effect ($p < 0.01$) on the moisture content (Table 3). According to response surface plots from Figure 3a and 3c, the increased feed flow rate gradually enhances the value of moisture content. As seen in Figure 3b and 3c, the effect of the air flow speed in the range studied is not statistically significant. Figure 3a shows the effect of inlet temperature and feed flow rate on moisture content. The use of a low level of feed flow rate and the increase in the inlet temperature decreases the moisture content. The higher flow rate results in short contact time between the feed and the drying air, making the heat transfer less efficient and resulting in lower water evaporation.



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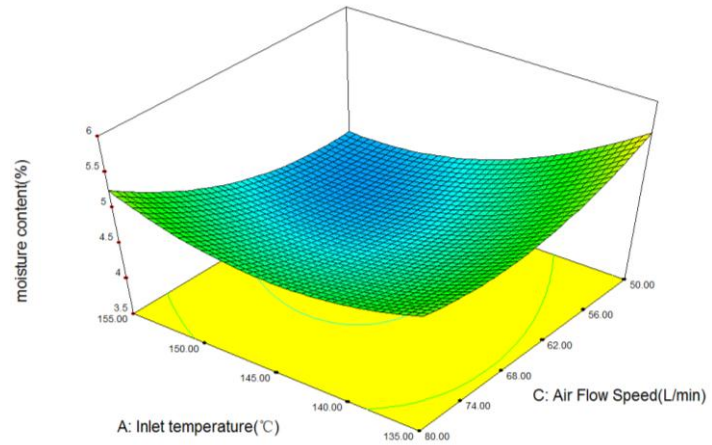

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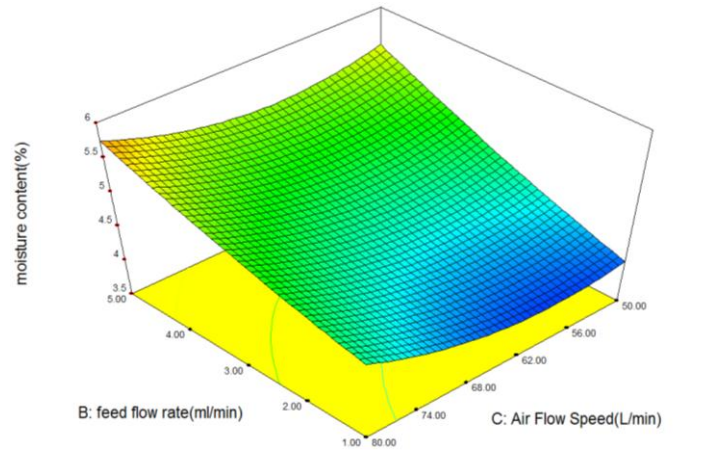

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Figure 3. Response surface plots for moisture content. (A) inlet temperature, (B) feed flow rate, (C) air flow speed

Optimization of spray-dried formulation: To verify the validity of the design, the responses of the optimized formulations were evaluated (Table 4) to ensure the product yield and moisture content. The composition, predicted and observed responses of the optimized film formulation are presented in Table 4. Results show that the observed values of the prepared optimized spray-dried formulation were mostly similar with predicted values.

Table 4 Composition, predicted, and observed responses of the optimized spray-dried formulation

Variables	Values	Responses	Predicted values	Observed values
Inlet temperature (°C)	147.5	Yield (%)	49.1%	47.2%
Feed flow rate (mL/min)	1.97	Moisture Content (%)	4.3%	4.4%
Air flow speed (L/min)	62.0			

Physico-chemical properties of Cur-IGTP

The physico-chemical properties of the spray dried Cur-IGTP were investigated. The bulk density was not significantly influenced by different spray dried conditions (0.45-0.47 g/cm^3). Water solubility of Cur-IGTP was found to between 95% and 98%.

After 3 months of storage, the appearance, moisture content, bulk density and solubility of Cur-IGTP slightly changed with increased storage, indicating a good stability of Cur-IGTP under sealed condition.

In addition, the SEM micrograph showed that the particle size of spray-dried Cur-IGTP was in the range of 3 to 8 μm, and no holes or cracks were found in the surface of powders (Fig 4). The powders were irregularly spherical shaped particles, not aggregated together and some roughness and shrinkages on the surface was also found.

The sensory results of Cur-IGTP are shown in Table 5, it was found that the standing time influenced the taste attributes of Cur-IGTP, the Cur-IGTP exhibits little astringency and bitterness with relatively good sweet aftertaste at 5 min. and no significant differences were found between 5 and 15 min. After standing for over 30 min the bitterness of Cur-IGTP enhanced while the sweet aftertaste decreased.

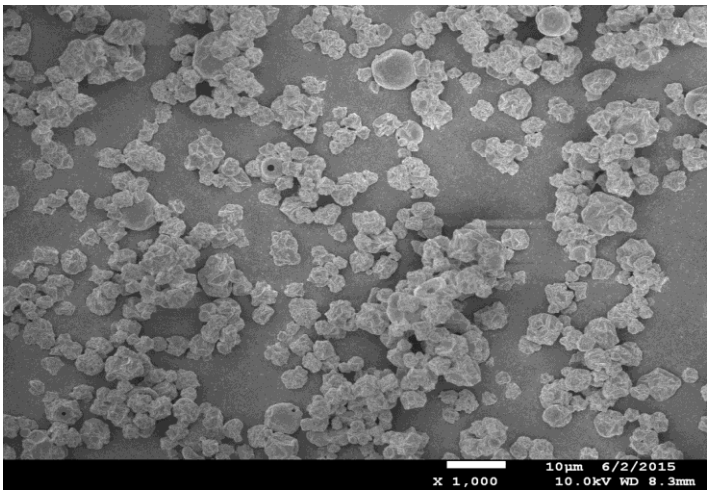

Figure 4. SEM images of the optimized spray-dried Cur-IGTP

Table 5 Sensory evaluation of Cur-IGTP (n=6)

Time(min)	Taste intensity		
	Bitterness	Astringency	Aftertaste
5	6.3±0.3	4.9±0.5	6.9±0.4
15	6.1±0.4	5.0±0.5	7.0±0.5
30	4.8±0.5	4.8±0.6	6.0±0.5
60	4.0±0.3	4.6±0.9	5.8±0.9

CONCLUSIONS

In this study Cur-IGTP could be successfully prepared by spray drying of the mixture of curcumin nanosuspension and green tea condensated extracts. The spray drying conditions were optimized using moisture content and process yield as responses. Furthermore, other characteristics such as the appearance, moisture content, bulk density and solubility of Cur-IGTP were also conducted. Experimental results proved that the optimum process conditions for obtaining spray-dried Cur-IGTP were inlet air temperature of 147.5 ºC, a feed flow rate of 1.97mL/min and a drying air flow speed of 62.0 L/min. The Cur-IGTP developed using the optimized spray-drying process showed a small particle size and uniform particle shapes. Solubility analysis also verified that Cur-IGTP had the good solubility in water with good stability. Therefore, spray-dried Cur-IGTP might have potential for applications in foods and beverages.

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