

Spray-dried microparticles of turmeric extract for improved delivery and low toxicity

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Abstract

This study aimed to enhance the delivery of turmeric by encapsulating its extract within microparticles using chitosan and mannitol through the spray drying technique and to assess their acute oral toxicity. The resulting microparticles were spherical, with an average diameter of 4 microns, and comprised 17% curcuminoids and 4% ar-turmerone. In vitro studies demonstrated that these microparticles had a higher release rate of curcuminoids compared to raw turmeric extract and preserved antioxidant activity. In the acute toxicity study, conducted in Wistar rats with a single dose of 2,000 mg/kg, no acute toxic symptoms were observed. According to the Globally Harmonized System of Classification and Labeling of Chemicals, the microparticles were categorized as having relatively low acute toxicity (category 5). These findings support the potential utility of the microparticles in dietary supplements and pharmaceutical applications due to their effective delivery properties and safety profile.

Keywords

Chitosan, mannitol, microencapsulation, safety, turmeric extract

Introduction

Turmeric (*Curcuma longa* L., Zingiberaceae) is among the most popular medicinal herbs, known for its wide range of pharmacological activities, including antioxidant, anti-microbial, anti-inflammatory, anti-angiogenic, anti-tumor, and anti-aging properties. These activities are primarily attributed to curcuminoids and essential oils found in turmeric rhizomes. Products derived from turmeric rhizomes, such as turmeric dried powder, turmeric oleoresin, turmeric oil, curcuminoids, and curcumin, are recognized as safe and are utilized across food, dietary supplements, and pharmaceutical sectors (Bhavanishankar et al. 1980; Ganiger et al. 2007; Aggarwal et al. 2016; Soleimani et al.

2018; Sharifi-Rad et al. 2020; Pancholi et al. 2021). Specific methods have been employed to prepare the products with the required phytoconstituents and applications (Munekata et al. 2021). However, the uncertain therapeutic efficacies of turmeric products can be attributed to their water-insolubility, instability, and poor bioavailability. Consequently, high doses of turmeric products have been used, and the design of an appropriate delivery system has been proposed to achieve significant pharmacological effects.

The use of spray-drying techniques for encapsulating turmeric products has led to notable enhancements in their properties, including improved stability, increased water solubility, and controlled release profiles (Cano-Higuera et al. 2015; Goëlo et al. 2020; Lu et al. 2021). A variety of wall

materials, including maltodextrin, starch, pectin, inulin, xanthan, and gum Arabic, have been investigated to optimize the encapsulation process. The selection of a wall material is crucial, as each substance possesses unique emulsifying and film-forming properties that affect product yield, encapsulation efficiency, and the characteristics of microparticles. The combination of wall materials has been extensively investigated to meet specific requirements. Chitosan and mannitol are widely used as food and pharmaceutical additives, as well as wall materials in microencapsulation. Chitosan, a low-toxic, biocompatible, and biodegradable polymer, is extensively used to increase the oral bioavailability of drugs with poor solubility and absorption. Chitosan-based drug carriers for curcumin have been demonstrated to protect curcumin from degradation, increase drug uptake, and maintain therapeutic benefits (Akhtar et al. 2012). A topical curcumin-chitosan solution and the oral administration of a chitosan-curcumin mixture have shown superior efficacy over curcumin alone in treating oral ulcers (Mahattanadul et al. 2018) and healing indomethacin-induced gastric ulcers (Kuadkaew et al. 2022), respectively. Moreover, chitosan is of interest as a wall material for encapsulating turmeric products with a high content of turmeric oil due to its effective oil absorbent properties (Srimoon and Potipat 2021). However, there are limitations in preparing oral dry powder formulations using chitosan because of its hygroscopic properties. This issue might be diminished by blending chitosan with a non-hygroscopic carrier like mannitol. Mannitol, a sugar alcohol used as a low-calorie sweetener in food and pharmaceutical preparations, is not hygroscopic and has been shown to be a scavenger of reactive oxygen species and an effective thermoprotectant that facilitates the retention of polyphenolic compounds during spray-drying (Eldridge et al. 2014). The combination of chitosan and mannitol as wall materials is an interesting strategy for encapsulating turmeric liquid extract to produce a dry powder that improves oral delivery and is convenient to handle.

However, new formulations of turmeric, especially those with enhanced bioavailability encounter numerous challenges, particularly regarding safety concerns, which impede their application. Although the risk of liver injury is not associated with turmeric when consumed in typical dietary amounts or in rare cases with medicinal dosages, available evidence indicates that this risk may increase with higher doses and products that enhance bioavailability and contain additional ingredients (Halegoua-DeMarzio et al. 2023). Consequently, evaluating the safety profiles of new turmeric formulations is essential. Furthermore, toxicity assessments are crucial for determining dose ranges for human trials and identifying parameters for the clinical monitoring of potential adverse effects.

This study focused on developing a simple and cost-effective formulation, highlighting the synergistic effects between the sesquiterpenoids in turmeric oil and curcuminoids. This combination has been shown to improve water solubility and enhance the efficacy of turmeric products with high bioavailability (Antony et al. 2008; Antony 2011; Henrotin et al. 2013). As a result, an ethanolic standardized

extract of turmeric, containing both curcuminoids and essential oils, was selected for the preparation of microparticles. This choice was driven by the major constituents of the extract and the safety of ethanol as an extraction solvent for oral use. The aim of the present study was to employ the spray drying technique to encapsulate turmeric extract, utilizing chitosan and mannitol as wall materials to improve oral delivery and minimize toxicity. The resulting microparticles were characterized to assess their feasibility in enhancing dissolution and stability, and their antioxidant activity was evaluated *in vitro*. Additionally, the acute oral toxicity of the microparticles was investigated in Wistar rats.

Materials and methods

Materials

Chitosan, food grade (average viscosity molecular weight of 796 kDa, degree of deacetylation 92%), was purchased from Merine Bio Resources Co., Ltd., Samutsakhon, Thailand. Mannitol, USP, was purchased from P.C. Drug Center Company Limited, Bangkok, Thailand. Curcumin, demethoxycurcumin, bisdemethoxycurcumin, ar-turmerone, sodium nitroprusside, and 1, 1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich, Missouri, USA. All other chemicals used in the experiments were of analytical grade and purchased from RCI Labscan, Bangkok, Thailand. Turmeric extract, extracted with ethanol (96%), was obtained from the Pharmaceutical Laboratory Service Center, Prince of Songkla University, Songkhla, Thailand.

Preparation of spray-dried microparticles containing turmeric extract

Spray-dried microparticles containing turmeric extract were prepared following the method described by Goëlo et al. (2020) with some modifications. The microparticles were designed with a theoretical curcuminoids loading of 20% (w/w). A wall material solution was prepared by dissolving chitosan or chitosan and mannitol, at a weight ratio of 1:3, in 1% v/v of acetic acid solution. A solution of turmeric extract in ethanol (96%) was dispersed into the encapsulating agent solution prior to being fed into a spray dryer. The microparticles were produced by using a Pilotech YC-015A Mini inert loop spray dryer (Shanghai Pilotech Instrument & Equipment Co. Ltd., China) with a standard 0.7 mm nozzle. The operating conditions used include a 15 mL/min solution flow rate, a 150 °C or 160 °C inlet temperature and air blower 35 Hz. Dry particles were packaged in airtight low-density polyethylene bags, covered with aluminum foil at 4 °C before testing.

The product yield was determined as the ratio of the weight of spray-dried microparticles to the weight of raw materials (turmeric extract, chitosan, and mannitol) by Equation 1 (Goëlo et al. 2020).

$$\text{Product yield (\%)} = \frac{\text{Weight of microparticles}}{\text{Weight of raw materials}} \times 100 \quad (1)$$

Characterization of spray-dried microparticles containing turmeric extract

The characteristics of the spray-dried microparticles, including particle size and surface morphology, were analyzed using a scanning electron microscope (SEM) (Quanta 400, FEI Company, USA). By assuming the particles were spherical, their diameters were measured using ImageJ software. This analysis was conducted by randomly choosing 150 particles from three distinct SEM images per sample (O'Toole et al. 2012). In addition, the volume diameter and zeta potential of the microparticles dispersed in Milli-Q water were determined using a Spraytech instrument (Malvern Instrument, UK) and a Zetasizer (Malvern Instrument, UK), respectively. The size distribution, or SPAN, was calculated using Equation 2, with D10, D50, and D90 obtained from cumulative size distribution at 10%, 50%, and 90%, respectively.

$$\text{SPAN} = \frac{D_{90}-D_{10}}{D_{50}} \times 100 \quad (2)$$

Infrared spectra of the microparticles and ingredients were recorded using FT-IR spectrophotometry (Spectrum One, Perkin Elmer Ltd., USA) and DSC curves were analyzed using a differential scanning calorimeter (DSC 8000, Perkin Elmer Ltd., USA).

Four biomarkers, including curcumin, demethoxycurcumin, bisdemethoxycurcumin, and ar-turmerone, in the spray-dried microparticles were quantified by high-performance liquid chromatography (HPLC) following the method described by Tanmanee et al. (2023). The samples were extracted using methanol and sonicated for 30 min, then centrifuged at 6,000 rpm for 10 min. The supernatants were collected and filtered through a 0.20 µm membrane filter (Whatman, USA). The HPLC analysis was performed on a Shimadzu LC-20AD system equipped with an Inertsil® ODS-3 C18 column (4.00 mm × 3.0 mm, 5 µm, Phenomenex security guard® ODS). The column temperature was maintained at 40 °C, and the UV detector was set at 254 nm. The flow rate was 1.0 mL/min, and the injection volume was 20 µL. The mobile phase consisted of a binary eluent of 0.1% formic acid solution (A) and CH₃CN (B) under flowing gradient conditions.

The total curcuminoids content was calculated as the sum of the three individual curcuminoids, while ar-turmerone served as a biomarker representing the essential oil content in turmeric. Encapsulation efficiency was calculated using Equation 3.

$$\text{Encapsulation efficiency (\%)} = \frac{\text{Practical biomarker content}}{\text{Theoretical biomarker content}} \times 100 \quad (3)$$

The quality of the product was assured by assessing its moisture content and microbial attributes, including total aerobic microbial count, total yeast and mold count, bile-tolerant gram-negative bacteria, *Salmonella* species, *Escherichia coli*, *Staphylococcus aureus*, and *Clostridium*

species, as described by Thai Herbal Pharmacopoeia (THP) (Thai Pharmacopoeia Committee 2021). Heavy metals such as arsenic, cadmium, and lead were analyzed using an Inductively Coupled Plasma Mass Spectrometer (Perkin Elmer, NexION2000, USA), while mercury levels were measured by the Direct Mercury Analyzer (NIC MA3000, Japan). Additionally, the active phytoconstituents of the microparticles were determined after storing them at 30 °C with 75% relative humidity for 6 months in an airtight, light-resistant container.

Release profiles

The release profiles of spray-dried microparticles and turmeric extract were examined using the USP Dissolution Apparatus II paddle method at a rotation speed of 100 rpm, according to Wan et al. (2012). For the experiments, samples containing the equivalent of 10 mg of curcuminoids were immersed in 900 mL of 0.1 M hydrochloric acid solution with 0.05% w/v polysorbate 80, maintained at 37 ± 0.5 °C. At specific intervals, 2 mL of samples were withdrawn and immediately replaced with an equal volume of the medium. These samples were then filtered through a 0.45 µm filter. The concentration of curcuminoids in the samples was determined by measuring absorbance at 420 nm using UV-Vis spectrophotometry, following the protocol outlined by the THP (Thai Pharmacopoeia Committee 2021). The results, expressed as cumulative release of curcuminoids over time, were plotted to illustrate the in vitro release pattern, with data presented as mean ± SD (n = 6). Additionally, the release data were analyzed using the zero-order equation ($Q = k_0 t$), the first-order equation ($Q = 1 - \exp(-k_1 t)$), the Higuchi equation ($Q = k_H t^{1/2}$), and Korsmeyer-Peppas equation ($Q = k_{kp} t^n$) where Q refers to the fraction of drug released at a particular time, t. The release kinetic constants obtained from the linear curves of the zero-order, first-order, Higuchi, and Korsmeyer-Peppas models are represented by k_0 , k_1 , k_H , and k_{kp} respectively (Dash et al. 2010).

In vitro antioxidant activity

The antioxidant activity of the microparticles was assessed and compared to that of turmeric extract and curcumin. The final concentration of samples and standard ranged from 0.5 to 750 µg/mL. The nitric oxide scavenging activity of the microparticles was determined using the method described by Balakrishnan et al. (2009). For this test, 0.5 mL of 20 mM sodium nitroprusside in phosphate buffer saline (pH 7.4) was mixed with 1 mL of the sample at various concentrations. The mixture was incubated under light at 25 °C for 2.5 h. Subsequently, 100 µL of this mixture solution was mixed with an equal volume of Griess reagent and incubated at room temperature for 30 min. The amount of nitric oxide radical was quantified at 546 nm using a microplate reader. Additionally, the

DPPH radical scavenging assay was utilized to determine the antioxidant capacity of the microparticles (Pinsuwan et al. 2010). For this assay, 100 μL of 6×10^{-5} M DPPH in absolute ethanol was added to 100 μL of each concentration of the samples. The mixture was incubated for 30 min at 25 °C, and then the absorbance was measured at 517 nm by a microplate reader.

The percentage of nitric oxide radical scavenging activity and DPPH scavenging capacity was calculated using Equation 4 (Balakrishnan et al. 2009).

$$\text{Inhibition (\%)} = \left[\frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \right] \times 100 \quad (4)$$

Acute toxicity

The acute toxicity study followed the guidelines of the Organization for Economic Co-operation and Development (OECD) 425, using the up-and-down method (Organization for Economic Co-operation and Development 2008). Ten female Wistar rats (*Rattus norvegicus*), weighing 200–250 g and aged 8 weeks, were used for this study. The rats were procured from Nomura Siam International, Thailand, and housed at the Laboratory Animal Center, Prince of Songkla University, Thailand. All rats were acclimated for 7 days before the start of the experiment. The conditions for raising the rats included a temperature of 22 ± 3 °C, a relative humidity of $55\% \pm 10\%$, light intensity ranging from 130 to 325 lux, a lighting period of 12/12 h (bright/dark), and being provided with filtered water through a reverse osmosis system. After acclimation, the rats were orally administered a single dose of 2,000 mg/kg of microparticles. The microparticles were dispersed in a mixture of water and glycerol with a ratio of 3.6:1.4. The concentration of microparticles was adjustable; if rat mortality occurred, the dose would be reduced to 1,750, 550, 175, and 55 mg/kg, respectively. Following microparticle administration, rat mortality and clinical toxic signs were closely monitored for 14 days. On the final day of the experiment, all rats were euthanized using 120 mg/kg of sodium thiopental after a period of fasting. Blood samples were collected through cardiac puncture for biochemical evaluation, including complete blood count (CBC), liver function (alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST)), and kidney function (creatinine, blood urea nitrogen (BUN)). Organs, including the liver, kidney, and heart, were weighed and collected in 10% formalin for histopathological analysis using H&E staining to assess potential side effects or changes induced by the microparticles in vital organs.

Ethical approval

Ethical authorization on the use of experiment animals for this study was issued by the Institutional Animal Care and Use Committee, Prince of Songkla University, Thailand, with Ref. AR004/2023.

Results and discussion

Preparation of spray-dried microparticles containing turmeric extract

The spray-dried microparticles appeared as an orange-fine dry powder with a mild turmeric odor. The wall material and operating conditions notably influenced the product yield. Utilizing a blend of chitosan and mannitol as wall materials at an inlet temperature of 150 °C successfully encapsulated turmeric extract, resulting in a higher product yield (56.40%) compared to using chitosan alone (40%). The product yield of microparticles employing a blend of chitosan and mannitol at an inlet temperature of 160 °C was 31.72%. The decrease in yield at a higher inlet temperature could be attributed to the evaporation of turmeric essential oil during the spray-drying process. These findings corroborate previous studies indicating the product yield and characteristics of microparticles depend on the wall materials and operating parameters of spray drying (O'Toole et al. 2012; Cano-Higueta et al. 2015; Goëlo et al. 2020). In earlier investigations, the inlet temperature for the preparation of spray-dried powder containing turmeric, turmeric oleoresin, and curcumin varied in the range of 100 °C to 180 °C. Remarkably, the blending of wall materials significantly increased the percentage yield compared to using a single wall material (Cano-Higueta et al. 2015).

Characteristics of spray-dried microparticles containing turmeric extract

The optimized spray-dried microparticles, utilizing a blend of chitosan and mannitol as wall materials, exhibited a spherical shape, a non-smooth surface, and an average size of 4.02 ± 0.24 μm , as shown in Fig. 1.

The volume diameter and size distribution (SPAN) of the optimized microparticles (18.62 ± 0.76 μm and 3.31 ± 0.70 , respectively) were smaller than those of the microparticles made with chitosan alone (23.32 ± 1.10 μm and 4.46 ± 0.68 , respectively). A lower SPAN value indicates a more uniform particle size distribution. These findings suggest that the optimized microparticles have a broad size distribution, though it is narrower than that of microparticles using only chitosan. The microparticles had a zeta potential of $+30.5 \pm 0.2$ mV, indicating a significant energy barrier against particle coalescence, thereby ensuring stability.

The FT-IR spectra of the microparticles and their ingredients are shown in Fig. 2. The FT-IR spectra of the microparticles exhibited strong peaks in the 1500–1600 cm^{-1} range and a weak peak near the 1400 cm^{-1} region, attributed to asymmetric and symmetric carboxylate anion stretching, respectively. The disappearance of the carbonyl stretching peak at 1651 cm^{-1} (amide I peak) of chitosan and the appearance of a new peak at 1625 cm^{-1} , attributed to asymmetric NH_3^+ bending, were observed (Parize et al. 2012). The FT-IR spectra of the turmeric extract revealed the hydroxyl group stretching region between 3200–3600 cm^{-1} . The change in this absorbing band in the

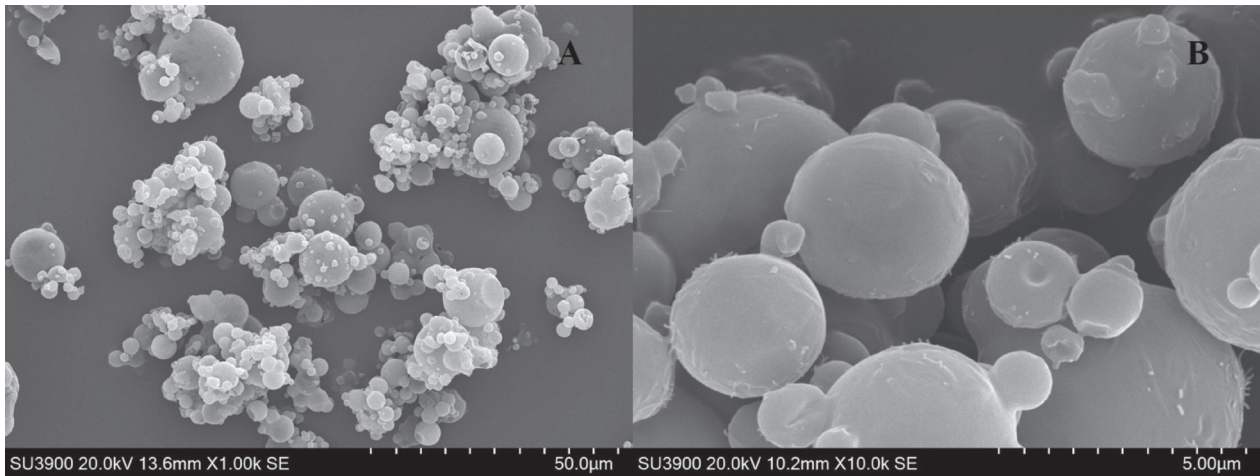


Figure 1. Morphology of spray-dried turmeric extract powder, SEM images 1,000× (A); 10,000× (B).

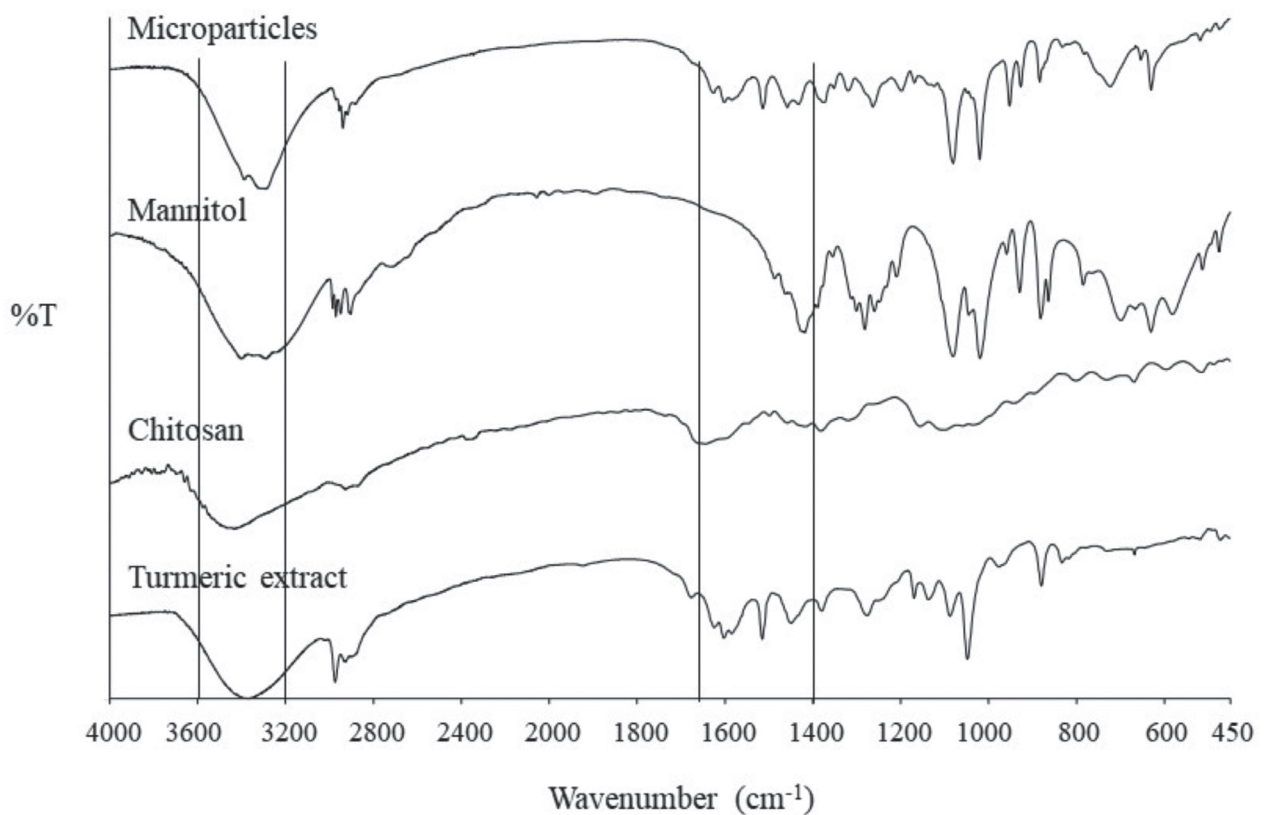


Figure 2. FT-IR spectrum of turmeric extract, chitosan, mannitol and spray-dried microparticles of turmeric extract.

microparticles suggested hydrogen bonding interactions (Azkia et al. 2020), confirming the successful encapsulation of the turmeric extract within the microparticles.

The DSC thermograms of the microparticles and their ingredients are shown in Fig. 3. The glass transition temperature of chitosan was not observed in the thermogram. The curve of the turmeric extract showed two endothermic peaks at 112 °C and 155 °C, which might be attributed to curcuminoids and other phytoconstituents present in the turmeric extract. Endothermic peaks of curcuminoids around 166–176 °C have been reported (Opustilová et al. 2023). Mannitol exhibited a sharp peak at 170 °C due to its α and β polymorphs (Milenkova et al. 2024). The

appearance of a small peak at 165 °C in the DSC curve of the microparticles indicated mannitol in a low percent crystallinity form. The disappearance of the turmeric extract peaks suggested that the encapsulated phytoconstituents in the microparticles exist in an amorphous state.

The microparticles were produced with encapsulation efficiencies of 85.25% for curcuminoids and 38.71% for ar-turmerone. The blending of chitosan and mannitol as wall materials successfully encapsulated both curcuminoids and ar-turmerone in the microparticles at a ratio of approximately 4:1. The physical, chemical, and microbial analysis results for the spray-dried microparticles containing turmeric extract used in the present study are

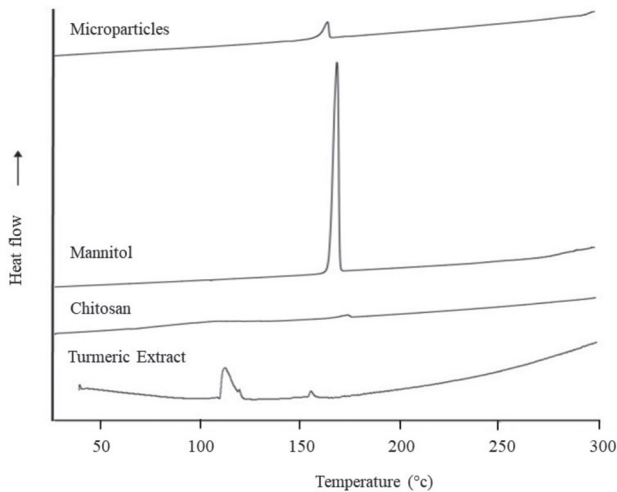


Figure 3. DSC curves of turmeric extract, chitosan, mannitol and spray-dried microparticles of turmeric extract.

presented in Table 1. The rising global consumption of natural food products and herbal medicines has heightened safety concerns in several regions. These concerns are primarily related to microbial contamination and the presence of heavy metals. They may be exposed to microorganisms from the soil, water, and inappropriate handling or storage. Heavy metals are commonly found in herbal medicine products in concentrations exceeding the permitted limits (Alharbi et al. 2024). Notably, lead, a potent neurotoxin, was detected as a contaminant in turmeric from Bangladesh and South Asia due to the addition of lead chromate (PbCrO₄), a yellow pigment used to enhance brightness (Forsyth et al. 2019). In this study, the moisture content, microbial levels, and heavy metal contamination in the microparticles were found to be within the acceptable limits for herbal preparation for oral administration as per the standard of the Thai FDA.

The microparticles were physicochemically stable after storage at 30 °C with 75% relative humidity for 6 months, with the remaining percentage of curcuminoids, and ar-turmerone at $93.54 \pm 1.73\%$ and $94.98 \pm 4.43\%$, respectively. These findings suggest that using a blend of chitosan and mannitol as wall materials enhances the stability of turmeric extract more effectively than using chitosan alone, where the content of curcuminoids was lower than 90%.

Release profiles

The correlation coefficients for the zero-order, first-order, and Higuchi models were 0.8688, 0.8794, and 0.9320, respectively. The release profile of the spray-dried microparticles was fitted to the Korsmeyer-Peppas equation, with a correlation coefficient of 0.9598, indicating a diffusion and erosion mechanism. Analysis of the release profile in simulated gastric fluid revealed that the microparticles increased the release rate and curcuminoid content by about 6 times compared to the turmeric extract, as shown in Fig. 4. This finding indicates that curcuminoids within the microparticles were in an amorphous state, consistent with the DSC findings. This finding aligns with previous research emphasizing the synergistic effects between turmeric oil and curcuminoids in turmeric formulations with enhanced bioavailability (Antony et al. 2008; Henrotin et al. 2013; Aggarwal et al. 2016). Specifically, the inclusion of turmeric oil has been shown to increase the solubility of curcumin in duodenal conditions by about 7.5 times compared to native curcumin (Henrotin et al. 2013).

In vitro antioxidant activity

The antioxidant capacities of the spray dried powder, turmeric extract, and curcumin, expressed as half maximal inhibitory concentration (EC₅₀) values, are shown in

Table 1. Certificate of analysis of spray dried microparticles containing turmeric extract.

Parameters	Specifications	Results
Physical characteristics		
Color	Orange	Conform
Appearance	Free flowing powder	Conform
Chemical characteristics		
Total curcuminoids content	Not less than 10%	16.96%
Ar-turmerone	Not less than 1%	4.06%
Moisture content	Not more than 7.0% w/w	2.56%
Heavy Metals		
Arsenic	Less than 5 ppm	Conform (0.12 ppm)
Cadmium	Less than 0.3 ppm	Conform (< 0.04 ppm)
Lead	Less than 10 ppm	Conform (1.04 ppm)
Mercury	Less than 0.5 ppm	Conform (Not detected)
Microbiology		
Total Aerobic Microbial Count	Not exceed 2×10^4 cfu/g	Conform (<10 cfu/g)
Total Yeast and Mold Count	Not exceed 2×10^2 cfu/g	Conform (<10 cfu/g)
Bile-tolerant gram-negative bacteria	Not exceed 10^2 cfu/g	Conform (<10 cfu/g)
<i>Salmonella</i> spp.	Absence/10 g	Conform
<i>Escherichia coli</i>	Absence/g	Conform
<i>Staphylococcus aureus</i>	Absence/g	Conform
<i>Clostridium</i> spp.	Absence/g	Absence

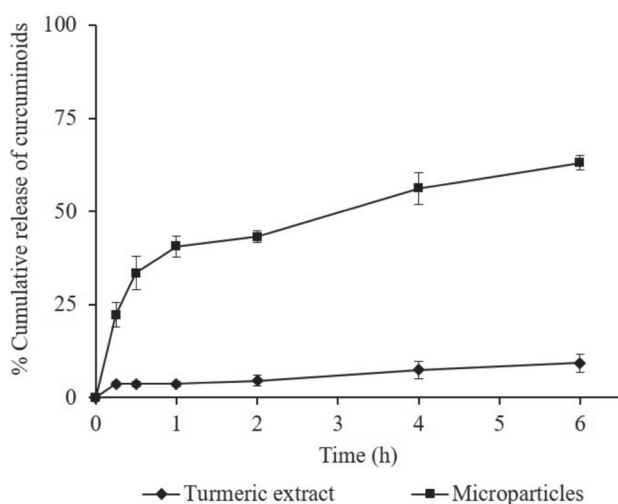


Figure 4. Release profiles of spray-dried microparticles containing turmeric extract and turmeric extract. (Mean \pm SD, $n = 6$).

Table 2. The scavenging of nitric oxide and DPPH by the spray-dried microparticles and turmeric extract increased in a dose-dependent manner and demonstrated lower inhibitory efficacy compared to pure curcumin. These may be attributed to individual curcuminoids, essential oils, and other constituents in turmeric and microparticles that affect free radical scavenging activity (Sreejayan and Rao 1997; Zhang et al. 2017).

Acute toxicity activity

Although chitosan and mannitol are generally used as excipients in oral pharmaceutical formulations and food supplements, the spray-dried microparticles encapsulated with turmeric extract possess new properties such as increasing the dissolution of curcuminoids, which may impact their safety.

Table 2. Antioxidant activity of spray-dried turmeric extract powder, turmeric extract, and curcumin (Mean \pm SD, $n = 3$).

Samples	EC ₅₀ (μ g/mL) of samples (curcuminoids, ar-turmerone)	
	Nitric oxide	DPPH
Spray-dried microparticles	189.96 \pm 16.82 (32.22 \pm 2.85, 7.71 \pm 0.68)	19.17 \pm 0.46 (3.25 \pm 0.08, 0.78 \pm 0.02)
Turmeric liquid extract	77.97 \pm 7.98 (15.49 \pm 1.58, 7.31 \pm 0.75)	32.46 \pm 0.93 (6.45 \pm 0.18, 3.04 \pm 0.09)
Curcumin	19.25 \pm 0.48	3.32 \pm 0.02

EC₅₀ = Effective concentration of sample requires scavenging the free radical by 50%.

The body weight, growth rate, and organ weight

The weights of rats on days 1, 3, 7, and 14, which were 204.50 \pm 2.83 g, 213 \pm 3.00 g, 230 \pm 4.41 g, and 245 \pm 4.59 g, respectively (Fig. 5A). The average growth rate over the 14 days increased by 8.89% (Fig. 5B), and there were no significant differences in the variance of the average body weight among the rats ($p > 0.05$). A comparison of the growth rate data with the data from Nomura Siam International, Thailand, showed a growth rate of 6.34%. This indicates that the administered microparticles did not induce abnormalities in the growth rate.

After administering the microparticles to the rats and raising them for 14 days, internal organs including the heart, kidneys, liver, spleen, and ovaries did not exhibit any abnormalities. The organ-to-bodyweight ratio (relative organ weight) is presented in Table 3. There were no significant differences in the variance of the organ-to-bodyweight ratio among the rats ($p > 0.05$). Therefore, the microparticles did not induce abnormalities in internal organs. On the final day, no mortality was observed among the rats. The microparticles were classified

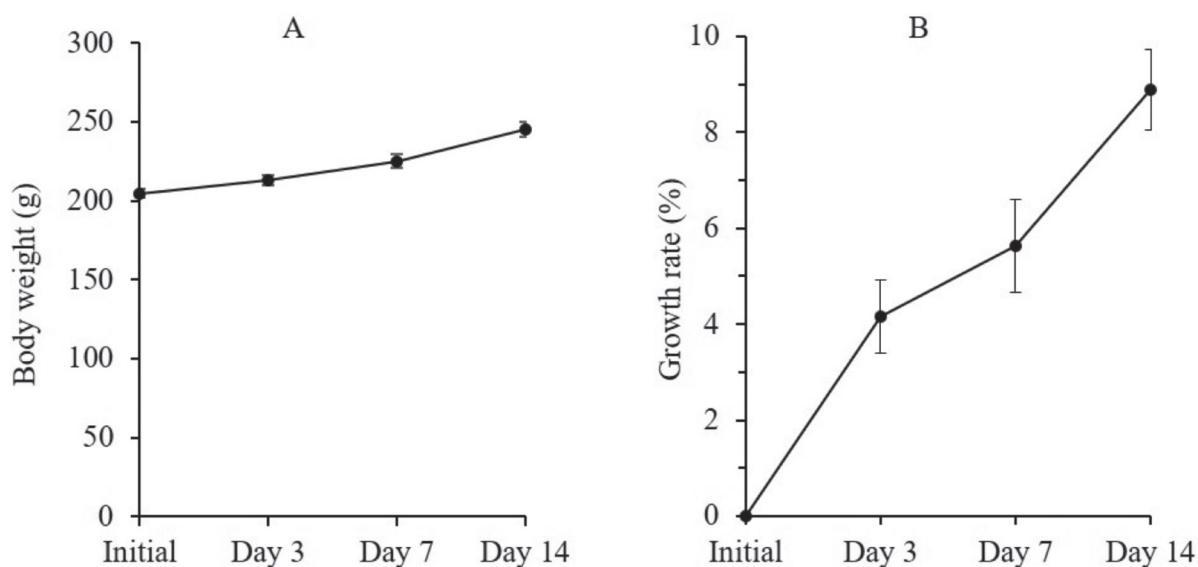


Figure 5. Effects of spray-dried microparticles containing turmeric extract on bodyweight (A) and growth rate (B). (Mean \pm SEM, $n = 10$).

Table 3. Effects of spray-dried microparticles containing turmeric extract on relative organ weight.

Organ	Relative organ weight (g)
Heart	0.75 ± 0.01
Liver	9.52 ± 0.19
Kidney	1.87 ± 0.03
Spleen	0.53 ± 0.02
Ovary	0.56 ± 0.03

Note: The data showed mean ± SEM (n = 10).

as category 5, indicating low-dose toxicity according to the Globally Harmonized System for Classification and Labeling of Chemicals (GHS). The 50% lethal dose (LD50) of the microparticles was estimated to be between 2,000 and 5,000 mg/kg, suggesting their safety.

Biochemical and histopathological

Liver injury associated with turmeric appears to increase when combined with other ingredients, such as black pepper, that enhance the absorption of curcumin (Halegoua-DeMarzio et al. 2023). Importantly, turmeric products with high bioavailability may potentiate liver injury. Table 4 illustrates the liver and kidney functions of rats treated with microparticles. Microparticles did not affect liver function, as indicated by ALT, ALP, and AST levels within the reference range, according to the study by Giknis and Clifford (2008). However, at a dose of 2,000 mg/kg, microparticles affected the creatinine level, which was 0.52 ± 0.04 mg/dL, exceeding the reference range by 4.0% (Giknis and Clifford 2008). The histopathology results of rats administered 2000 mg/kg of microparticles (Fig. 6) were observed un-

Table 4. Effects of spray-dried microparticles containing turmeric extract on biochemical levels (Mean ± SEM, n = 10).

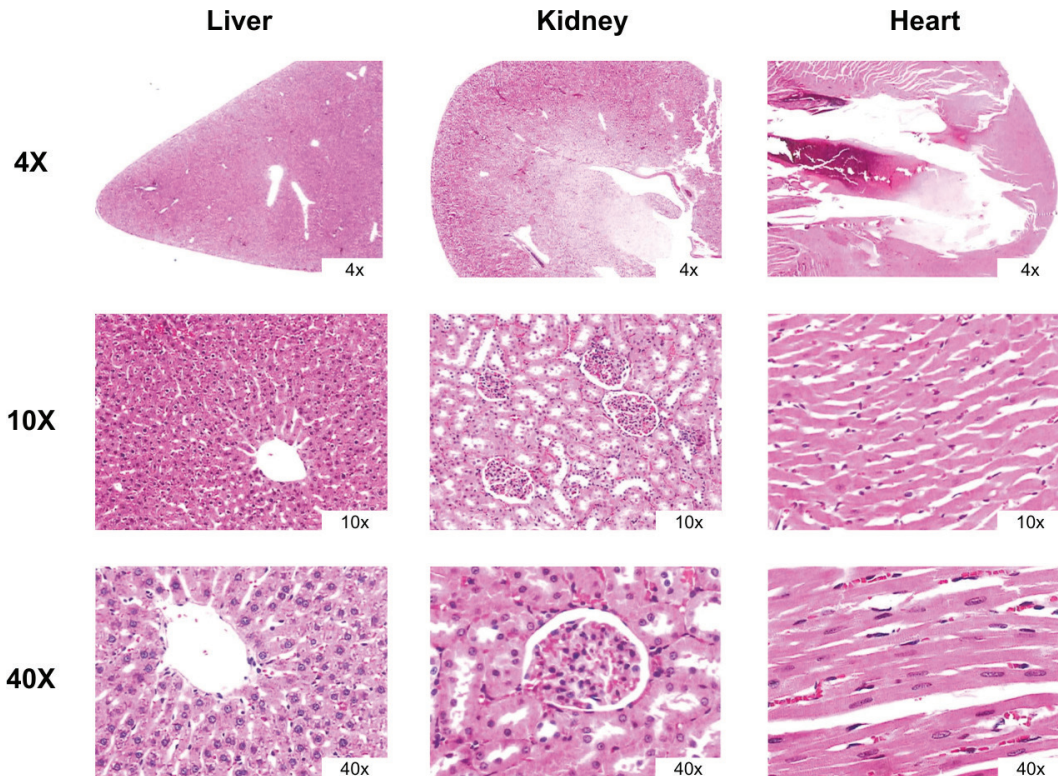
Biochemical levels	Unit	Treated rats	Reference*
Alanine aminotransferase (ALT)	IU/L	35.80 ± 0.87	18.00–45.00
Alkaline phosphatase (ALP)	IU/L	145.72 ± 11.72	62.00–230.00
Aspartate aminotransferase (AST)	IU/L	97.62 ± 5.83	74.00–143.00
Blood urea nitrogen (BUN)	mg/dL	23.88 ± 1.18	12.30–24.60
Creatinine	mg/dL	0.52 ± 0.04	0.20–0.50

* Giknis et al. (2008).

der a light microscope at magnifications of 4×, 10×, and 40×. The liver, kidney, and heart tissues were stained using H&E staining, and no abnormalities were found in any of these tissues. Creatinine level is a normal waste product that accumulates in the blood due to muscle use, indicating the kidney's healthy status (Khorsandi and Orazizadeh 2008). Kidney issues lead to increased creatinine levels and decreased urine output. Upon considering both the creatinine levels and the renal histopathology of rats, no abnormalities were observed in the renal architecture, including the glomeruli and tubules. Therefore, the administration of 2000 mg/kg of microparticles mildly increased creatinine levels but did not significantly impact renal architecture. It is suggested to further focus on kidney function and extend the study period for more comprehensive insights.

Hematology

Table 5 illustrates that rats administered 2000 mg/kg of microparticles did not significantly affect hematology, except for a mild reduction in platelet count to $672.63 \pm 52.07 \times 10^{12}/L$, which decreased by approximately 1.17% from the

**Figure 6.** The histology and specimen of the organ changed in rats treated with spray-dried microparticles.

reference range. A decrease in platelet count may result from bone marrow dysfunction or other causative factors, leading to decreased platelets in specific diseases such as idiopathic thrombocytopenic purpura (ITP) and systemic lupus erythematosus (SLE) (Rasmy et al. 2015). The severity of thrombocytopenia is classified into five levels according to the National Cancer Institute Common Terminology Criteria for Adverse Events (2010), ranging from 1 to 5, representing normal to the most serious conditions. In the treated rats, the platelet count was at level 2, indicating a condition that does not pose significant harm to the body (Sekhon and Roy 2006). Hence, it is recommended to further investigate the platelet count in subsequent studies.

Table 5. Effects of spray-dried microparticles containing turmeric extract on hematological levels (Mean \pm SEM, n = 10).

Hematological levels	Unit	Treated rats	Reference*
White blood cells (WBC)	$\times 10^9/L$	2.38 \pm 0.27	1.13–7.49
Red blood cell (RBC)	$\times 10^{12}/L$	7.46 \pm 0.15	7.07–9.03
Hematocrit (HCT)	%	43.75 \pm 0.65	37.90–49.90
Hemoglobin (Hb)	g/dL	15.40 \pm 0.22	13.70–16.80
Mean cell volume (MCV)	fL	56.26 \pm 0.48	49.90–58.30
Mean cell hemoglobin (MCH)	Pg	20.70 \pm 0.26	17.80–20.90
Mean cell hemoglobin concentration (MCHC)	%	36.81 \pm 0.30	33.20–37.90
Platelet count	$\times 10^3/L$	672.63 \pm 52.07	680.00–1200.00

* Giknis et al. (2008).

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Conclusion

Encapsulating turmeric liquid extract with a high turmeric oil content in a blend of chitosan and mannitol using the spray drying technique successfully yielded a stable, dry micronized powder for oral delivery. The spray-dried microparticles, encapsulated with turmeric extract and formulated using a blend of chitosan and mannitol as wall materials, demonstrated superior release profiles compared to turmeric extract alone and retain their free radical scavenging activity. The estimated lethal dose (LD50) values for the microparticles ranged from over 2,000 to 5,000 mg/kg in rats, suggesting low toxicity. These findings support the potential for using spray-dried microparticles containing turmeric extract for treating gastrointestinal disorders in future studies. Nonetheless, it is advisable to monitor kidney function and extend the duration of the test in subsequent studies.

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