Mongolian Academy of Sciences

Mongolian Journal of Chemistry

The Institute of Chemistry & Chemical Technology

Separation and purification of curcumin preparation of morphology controlled micro particles

Ts.Tsedendorj[1*], Hong Hai-long,[2] Han Li-min[2], N.Lkhagvajav,[3] B.Darkhijav[1]

[1]*Institute of Chemistry and Chemical Technology, MAS, Peace ave., Ulaanbaatar 13330, Mongolia*
[2]*Chemical engineering college, Inner Mongolia University of Technology, Hohhot 010051, China*
[3]*Plant Protection Research Institute, Ulaanbaatar 210153, Mongolia*

ARTICLE INFO: Received 15 November 2014; revised 01 December 2014; accepted 05 December 2014

Abstract: Curcumin was extracted from turmeric plants which is the most commonly used natural pigments, and possess a variety of pharmacological functions except for using pigment. The morphology and particle size of curcumin are main factors affecting the application. Therefore, the morphology and particle size distribution of curcumin were effectively controlled by advanced technology, which is significant for expanding the application and added value of curcumin. The curcumin crystal was obtained from curcumin pigments by using column chromatography and recrystallization techniques. The composition and structure of curcumin were characterized by elementary analysis, UV-Vis, IR and NMR. Micronization of curcumin was carried out the Solution Enhanced Dispersion by Supercritical Fluids (SEDS) technology. In the process, supercritical carbon dioxide was used as anti-solvent and acetone/dichloromethane (1:4, v:v) was used as solvent. The curcumin crystals with PSs of about 378 μm were successfully micronized by the SEDS process to micro particles with PSs of about 2.6-10 μm. The acicular, leaves, dendritic and tubular micro particles were obtained through controlling parameters such as pressure, temperature, solution concentration and solution flow rate.

Keywords: Curcumin; supercritical fluid; micro particle; morphology

INTRODUCTION

Curcumin is a highly safety natural food coloring mainly extracted from Araceae plants irises and ginger turmeric, curcuma, turmeric rhizomes, etc., including curcumin (about 77%), norepinephrine curcumin group (about 17%), double demethoxyhaleniaside curcumin (about 3%) [1]. Curcumin is the active ingredient of which is important, but also the plant kingdom pigment having extremely rare β-diketone structure, molecular formula $C_{21}H_{20}O_6$, molecular weight 368.37, soluble in methanol, acetone, chloroform, ethanol and propylene glycol, and other organic solvents [2], and glacial acetic acid and alkali solution. Curcumin has several specific features including anti-inflammatory, anti-bacterial, anti-viral, anti-cancer, anti-AIDS, liver, stop spasms [3, 4], anti-oxidation, gallbladder, lowering blood pressure, lowering cholesterol, anticoagulant, and other pharmacological functions [5]. However, it has low solubility in water (less than 50 μmol L^{-1} [6]). Present study shows that the morphology, particle size and size distribution of the particles are a major factor affecting the solubility and bioavailability of drugs, so by the high-tech control particle morphology and particle size can effectively improve the water solubility of the drug. Supercritical fluid forced dispersion solution (Solution Enhanced Dispersion by Supercritical fluids, SEDS) [7] technology, as ultrafine particle preparation technique developed in recent years has attracted researchers attention, has been applied to drug ultrafine [8], biodegradable polymeric carrier ultrafine [9], natural medicinal active ingredients ultrafine [10], the natural pigment of ultrafine [11] and many other fields. In this work, column chromatography, recrystallization, isolated and purified from commercial curcumin crystals obtained by elemental analysis, UV, IR, NMR and other analytical tools were used to characterize its characteristic properties. Curcumin ultrafine crystals were studied by changing the operating parameters of the Supercritical fluid technology solution enhanced dispersion technology. We found different morphologies such us needles, willow-shaped, petal-like, dendritic and square tubular ultrafine particles. The particle diameter of the above mentioned morphologies are greatly reduced.

EXPERIMENTAL

Materials, reagents and equipment: Curcumin powder, (Xi'an, Biotechnology Ltd), petroleum ether, ethyl acetate, acetone, methanol and other reagents

*corresponding author: e-mail: *tse_shuurkhai@yahoo.com*

DOI: http://doi.dx.org/10.5564/mjc.v15i0.314

with analytical grade (Tianjin Chemical Reagent Factory; CO_2 99.9%, Beijing Millennium Beijing gas sales center) supercritical fluid precipitation equipment, laboratory-made; elemental analyzer (VarioELIII, Germany Elemental company), UV-visible spectroscopy (UV-3101), Shimadzu Corporation; Fourier transform infrared spectroscopy (NEXUS 670), KBr pressure films, American Nicolet company; NMR (AVANCE DRX-500), US Bruker company CD_3OD as solvent; scanning electron microscope (S-3400), Shanghai Hitachi Electrical Appliances Co., Ltd.

***Separation and Purification of curcumin*:** 1.5 g of Curcumin powder was dissolved with a small amount of acetone and loaded to silica gel (200-300 mesh) column as a stationary phase. The column was washed with petroleum ether-ethyl acetate (1:1), following with petroleum ether-ethyl acetate (1:2), and ethyl acetate, acetone and methanol as the mobile phase with the gradient elution. The fraction of 1:2 petroleum ether-ethyl acetate was concentrated and recrystallized from the methanol and gave an orange-yellow rod-like crystals of curcumin yielding 0.2442 g.

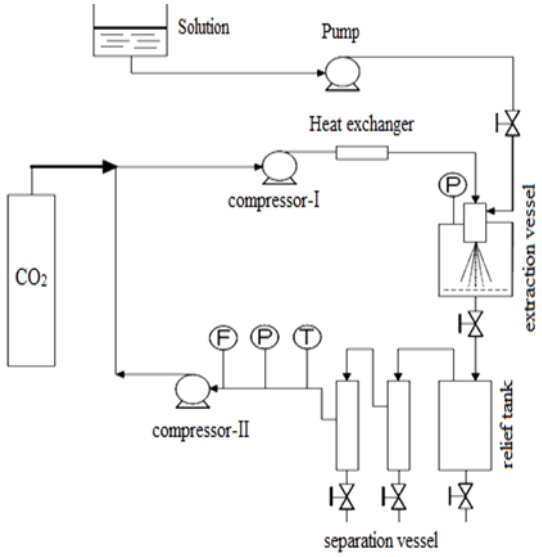

Fig. 1. Flow chart of SEDS process

Preparation of ultrafine particles of curcumin: A flow diagram of SEDS process is shown in Fig 1. CO_2 from the cylinder through diaphragm compressors and heat exchangers I pressure are injected to the precipitation reactor after preheating. When the precipitation vessel reaches to predetermined temperature and pressure under the influence of advection pump, the curcumin dissolved solution was sprayed to nozzle of the precipitation reactor where SC-CO_2 existed as anti-solvent to supersaturate and precipitate the ultrafine particles. Detailed experimental procedure is shown in previous work [11]. Ultrafine particles can be prepared with different morphologies with respect to controlling parameters.

RESULTS AND DISCUSSION

Characterization of curcumin: The curcumin crystals were characterized by using elemental analysis, UV, IR and NMR spectra and obtained data was compared with the literature [12]. The morphology of characterized crystals of curcumin was determined by scanning electron microscopy.

Elemental Analysis: An elemental analysis (C, H and N) data for orange-yellow crystal rod curcumin resulted from separation and purification under melting point of 178-180^oC are shown in Table 1.

Table 1. Elemental analysis data of compound

Element	C	H	N
Theoretical value, (%)	68.47	5.47	0
Measured values, (%)	68.40	5.90	0.22

As seen from the Table 1, the curcumin was well purified,

therein, the experimentally determined values were almost consistent with those of theoretical values in allowable error of 0.5%).

UV-visible spectroscopy: The UV-visible absorption spectra of the curcumin in a pure ethanol are presented in Figure 2. The adsorption wavelength was ranged in 200 ~ 600 nm. There are two peaks were appeared at the wavelength of 263 nm and 425 nm suggesting that two kind of substituents **(1)** on benzene band of curcumin absorption, and **(2)** on the phenyl ring contained benzene with p-π and π-π-conjugated which resulted to red-shifted absorption band. Therefore, the phenyl ring is absorbed into the visible region at 425 nm [12].

Infrared Spectroscopy: The infrared spectra of

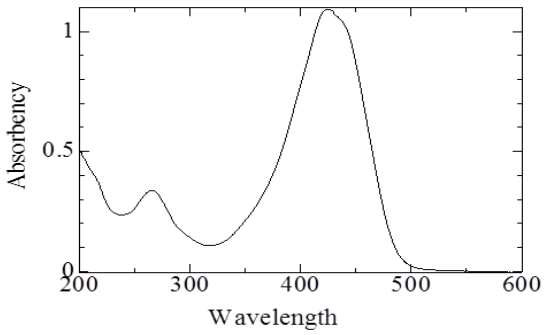

Fig. 2. The UV-vis absorption spectra of curcumin in ethanol

curcumin was showed a sharp peak at 3501 cm^{-1} indicate the presence of free O-H, stretching vibration absorption peak at 3420 cm^{-1} was assigned to associative intermolecular or intramolecular hydrogen bonds caused 3011, 859 and 812 cm^{-1}, respectively [13]. Benzene spectra of C-H bond stretching vibration absorption peak attributed due to the ring and the outer surface of the bending vibration absorption

peak located at 2972 and 2937 $cm^{-1.}$

The 1505 cm^{-1} peak is assigned to the C=O, while enol C-O peak was obtained at 1271 cm^{-1}, C-O-C peak at 1030 cm^{-1}, benzoate trans-CH vibration at 960 cm^{-1} and cis CH vibration of aromatic ring at 713 cm^{-1} [12].

NMR analysis: Curcumin ^{1}H NMR (CD_3OD) data are as follows: δ: 7.573 (d, J = 15.5Hz, 2H, C_1-H, C_7-H), 7.215 (d, 2H, J = 1.5Hz, C_2'-H, C_2''-H), 7.108 (dd, J1 = 8.0Hz, J2 = 1.5Hz, 2H, C_6'-H, C_6''- H), 6.824 (dd, J1 = 8.0Hz, J2 = 2.5Hz, 2H, C_5'-H, C_5''- H), 6.626 (d, J1 = 16.0Hz, 2H, C_2-H, C_6-H), 5.964 (s, 1H, C_4-H), 3.894 (s, 6H, C_7'-3H, C_7''- 3H).

Curcumin ^{13}C NMR (CD_3OD) data are as follows: δ: 184.77 (C_3, C_5), 150.49 (C_4, C_4''), 149.43 (C_3', C_3''), 142.14 (C_1, C_7), 128.60 (C_1', C_1''), 124.12 (C_6', C_6''), 122.30 (C_2, C_6), 116.58 (C_5', C_5''), 111.75 (C_2', C_2''), 101.97 (C_4), 56.46 (C_7', C_7''). Results obtained from elemental analysis, UV, IR and NMR were nearby with reported values. The molecular structure of curcumin with two keto and enol tautomers is shown in Figure 3.

The ultra-fine curcumin crystals were performed by SEDS process using supercritical carbon dioxide (SC-CO_2) as an anti-solvent and acetone/dichloromethane (1:4) as a solvent under different controlling parameters including pressure (P), temperature (T), concentration of solution (C_0), solution flow rate (V_L) and other parameters. The curcumin crystals were successfully purified by needle-like or rod-like shape with the length of 1778 μm and cross-sectional diameter of 378 μm (Fig 4a). Its shape has been changed into a willow with cross-sectional diameter

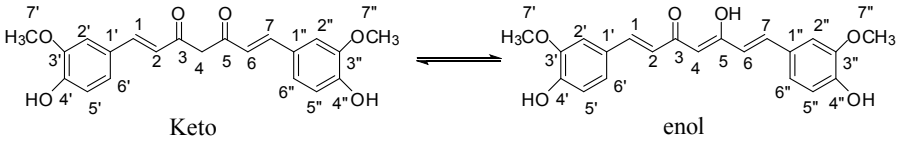

Fig. 3. The chemical structure of curcumin

The morphology of ultrafine curcumin particles

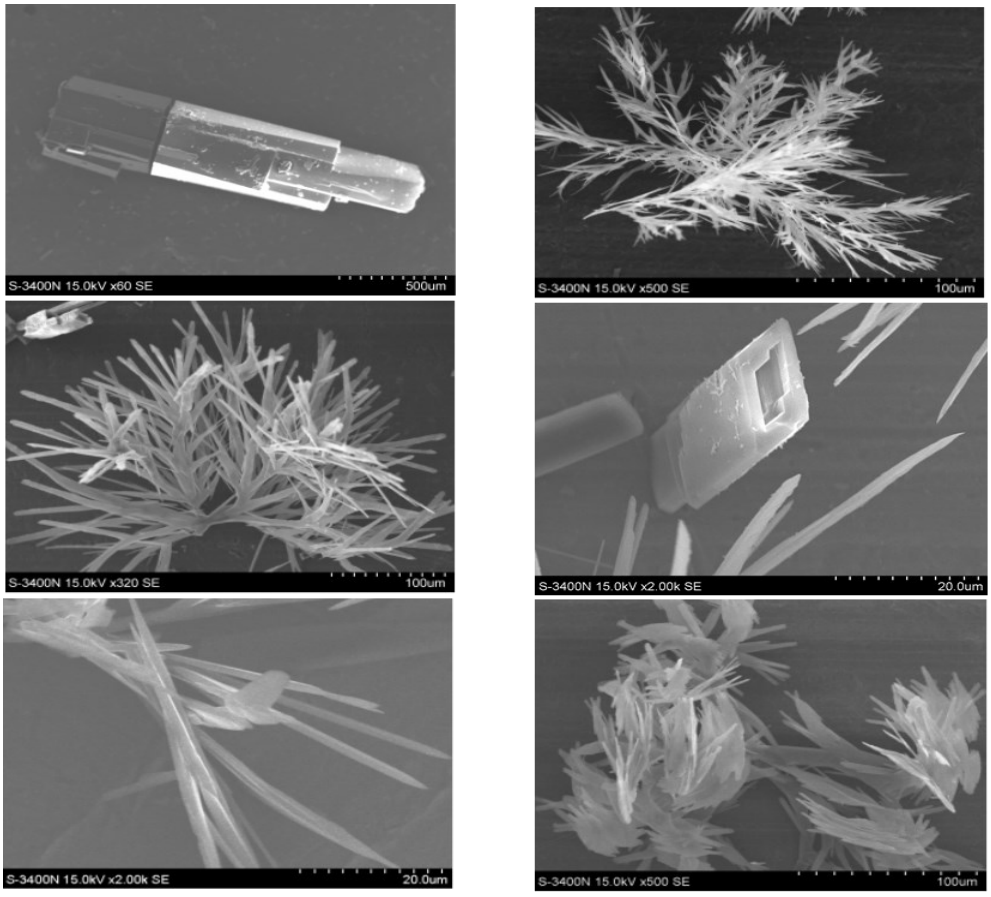

Fig. 4. The SEM micrographs of unprocessed and processed curcumin crystal by SEDS process
(a) Separating purified crystals of curcumin
(b) T = 309 K, C_0 = 2.0 mg / mL, V_L = 2.0 mL / min, P = 12 MPa;
(c) T = 309 K, C_0 = 3.0 mg / mL, V_L = 2.0 mL / min, P = 12 MPa;
(d) T = 313K, C_0 = 2.0 mg / mL, V_L = 2.0 mL / min, P = 12 MPa;
(e) T = 309 K, C_0 = 2.0 mg / mL, V_L = 1.0 mL / min, P = 12 MPa;
(f) T = 313 K, C_0 = 2.0 mg / mL, V_L = 2.0 mL / min, P = 10 MPa;

of 2.6-10μm, dendritic, square tube, needle and a petal-shaped during factors controlling experiments. (Figs. 4b-f).

The particle size of ultrafine particles was increased with increase in solution concentration as seen by comparison between Figure 4(b) and 4(c) where morphology is changed from the lancet-shaped dendrimers. This can be caused by intensified solubility of solution, amount of solution at the moment of supersaturation, and/or precipitation of increased solute, which are resulted to the formation of inter-particle collisions promoted for the possibility of large particles. Moreover, the particle size can also be increased by the blade-like dendritic.

As seen from Figure 4(b) and 4(d), it is clear that slightly high temperature has been influenced to the particle size. The morphology with square tubular shape of lancet might be due to molecular thermal motion affected by increased temperature. Because the nuclei nucleation speed is reduced while the growth rate is accelerated that resulted in enlarged particles.

In Figs. 4(b) and 4(e), the change in particle size of ultrafine particles was small showing a needle-like willow morphology at increased solution flow rate condition. Furthermore, the particle size was increased with willow-like petals shape at the conditions for high temperature and low pressure.

CONCLUSIONS

The curcumin crystal was obtained from curcumin pigments by using column chromatography and recrystallization techniques. The composition and structure of curcumin were characterized by elemental analysis, UV-Vis, IR and NMR. Isolation of curcumin crystals was performed by SEDS technology using supercritical CO_2 as anti-solvent and acetone/ dichloromethane (1:4) as solvent, respectively. The curcumin crystals with PSs of about 378 μm were successfully micronized to micro particles with PSs of about 2.6-10 μm. The acicular, willow-shaped, petal-like, leaves, dendritic and tubular micro particles were obtained at different conditions with controlling parameters such as pressure, temperature, solution concentration and solution flow rate.

REFERENCES

1. Ramsewak R.S., De Witt D.L., Nair M.G. (2000) Cytotoxicity, antioxidant and anti inflammatory activities of curcumins I-III from Curcuma longa. *J. Phytomedicine,* **7**(4), 303-308.
2. Liu Shu xing., Hu Xiao jun. (2003) A review on research advancement of (Research Curcumin). J. *Curcumin Journal of Shaanxi University of Science & Technology*, **21**(4), 37-39.
3. Hatcher H., Planalp R., Cho J., et al. (2008) Curcumin: From ancient medicine to current clinical trials. *J. Cellular and Molecular Life Sciences*, **65**(11), 1631-1652.
4. Goel A., Kunnumakkara A.B., Aggarwal B.B. (2008) Curcumin as "Curecumin": from kitchen to clinic. *J. Biochemical Pharmacol.*, **75**(4), 787-809.
5. Park E.J., Jeon C.H., Ko G., et al. (2000) Protective effect of curcumin in rat liver injury induced by carbon tetrachloride. *J. Journal of Pharmacy and Pharmacology*, **52**(4), 437-440.
6. Wang Q.I., Wang Kui. (2003) Metabolism of curcumin (curcumin metabolic studies). *J. Chinese Pharmacological Bulletin (Chinese Pharmacological Bulletin)*, **19**(10), 1097- 1011.
7. Hanna M., York P. (1994) Method and Apparatus for the formation of particles. P. Patent Application PCT / GB94 / 01426.
8. Pyo D.J. (2009) Two different shapes of insulin micro particles produced by solution enhanced dispersion supercritical fluid (SEDS) process. *J. Bull Korean Chem Soc.*, **30**(5), 1215-1217.
9. Kang Y.Q., Yin G.F., Ou Y.P., *et al*. (2008) Preparation of PLLA /PLGA microparticles using solution enhanced dispersion by supercritical fluids (SEDS). *J. Colloid. Interf. Sci*., **322**(1), 87-94.
10. Jin H.Y., Hemingway M., Xia F., *et al*. (2011) Production of β-Carotene Nanoparticles by the Solution Enhanced Dispersion with Enhanced Mass Transfer by Ultrasound in Supercritical CO_2 (SEDS-EM). *J. Ind. Eng. Chem. Res.,* **50**, 13475-13484.
11. Hong H.L., Suo Q.L., Han L.M. (2009) Study on Precipitation of Astaxanthin in Supercritical Fluid Powder Technology, **191**(3), 294-298.
12. Suo Quanling, Huang Yanchun, Weng Linhong, *et al*. (2006) Study on Purification and Molecular Crystal Structure of Natural Curcumin (purification and molecular and crystal structures of Natural curcumin). *J. Food Science,* **27**(3), 27-30.
13. Mohanty C., Sahoo S.K., (2010) The in vitro stability and in vivo pharmacokinetics of curcumin prepared as an aqueous nano particulate formulation. *J. Biomaterials*, **31**(25), 6597-6611.