RESEARCH ARTICLE

The Milling Method in Formation of Inclusion Complex p-Methoxycinnamic Acid-Hydroxypropyl-β-Cyclodextrin

Dewi Isadiartuti*, Helmy Yusuf, Dwi Setyawan, Juni Ekowati

Department of Pharmaceutical Sciences, Faculty of Pharmacy Universitas Airlangga Surabaya, Indonesia.

Received: 20th February, 2024; Revised: 27th March, 2024; Accepted: 05th August, 2024; Available Online: 25th September, 2024

ABSTRACT

p-methoxycinnamic acid (*p*MCA) is a substance with several pharmacological activities, derived from the rhizome of *Kaempferia galanga* Linn. The solubility of *p*MCA can be increased by the formation of inclusion complexes (IC) using hydroxypropyl-β-cyclodextrin (HPβCD) compounds. IC are obtained through the milling method, which is a simple, environmentally friendly, and low-cost manufacturing method. The process involves the pounding of material with a certain speed and impact force. This research aims to characterize the *p*MCA-HPβCD IC made using the milling method. Milling the mixture of *p*MCA and HPβCD using a ball mill is a common technique to enhance the formation of the inclusion complex. The IC's physical characteristics were analyzed in terms of their morphology, particle size, crystallinity, and thermal properties. The dissolution profile was also performed using paddle-typed dissolution apparatus (Type II) with 500 mL distilled water as dissolution medium. The amount of *p*MCA dissolved was determined using UV spectrophotometry. The dissolution parameter was determined as dissolution efficiency at 60 minutes (DE_{60}). The IC particles were irregular in shapes and porous. The size was reduced as compared to the individual components. The XRD revealed that the ICs were amorphous. The DE_{60} of IC increased by a factor of 4.3 compared to the pure pMCA. In conclusion, the milling method successfully formed complex of *p*MCA-HPβCD with different characteristics from the initial compound and was significantly increased the percentage of the dissolved *p*MCA.

Keywords: Dissolution, Inclusion complex, Milling method, *p*-methoxycinnamic acid, Hydroxypropyl-β-cyclodextrin.

International Journal of Drug Delivery Technology (2024); DOI: 10.25258/ijddt.14.3.38

How to cite this article: Isadiartuti D, Yusuf H, Setyawan D, Ekowati J. The Milling Method in Formation of Inclusion Complex p-Methoxycinnamic Acid-Hydroxypropyl-β-Cyclodextrin. International Journal of Drug Delivery Technology. 2024;14(3):1526-1530.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

p-Methoxycinnamic acid (*p*MCA) is a biologically active substance from the rhizome of *Kaempferia galanga* Linn.[1] *p*MCA has several pharmacological activities and can be developed into pharmaceutical preparations for oral use.[2,3] Oral preparations are preferred because of their ease of use[4] and one of the important parameters in the development of the dosage forms is the solubility of the active compound. The low solubility of the compound in water on oral use will result in dissolution and uncertain bioavailability to reduce the therapeutic.[5]

*p*MCA is a weakly acid compound with low aqueous solubility (0.712 mg/mL at 25°C). The solubility of substances in water can be increased through the formation of IC with cyclodextrin compounds.[6] IC are a type of monomolecular complex that can be formed when macrocyclic compounds with intramolecular cavities interact with small molecules at molecular dimensions. Generally, cyclodextrin compounds as the host can trap hydrophobic guest molecule. The interaction between guest and host molecules occurs at the supramolecular level involving non-covalent bonds such as *van der Waals* and hydrophobic bonds.[7] Furthermore, the IC formed can increase solubility and dissolution, and furthermore bioavailability guest compound.[8-14]

Isadiartuti's research (2021) showed that the formation of IC with complex-forming compounds HPβCD can increase the solubility of *p*MCA more than βCD[1]. HPβCD is a βCD partially substituted by a hydroxypropyl group and it is a hydrophilic derivative of βCD which has the same cavity size as βCD. HPβCD has greater solubility in water (50 g/100 mL) than βCD (1.85 g/100 mL), and with the use of high concentrations, it does not form crystal complex deposits in the kidney because of decrease nephrotoxicity. In addition, HPβCD has a lower hemolytic effect on erythrocytes than βCD, therefore, it can be administered through parenteral method.[7,15]

IC in the solid-state can be prepared by various methods, including the milling method. The IC obtained using this method are simple, environmentally friendly, and low cost.[16,17] The manufacture of *p*MCA-HPβCD IC by milling method has not been conducted. Therefore, this research aims to

Author for Correspondence: dewi-i@ff.unair.ac.id

characterize the *p*MCA-HPβCD IC through the milling method.

MATERIALS AND METHODS

Materials

The *p*MCA used was extracted from *K. galanga* Linn. rhizome obtained from Purwodadi Botanical Garden, Pasuruan, Indonesia. *p*MCA made according to the method used by Ekowati *et al*. (2010)[18] and the identification was conducted with TLC, FTIR, DTA and ^{1}H-NMR. The results are in accordance with *p*MCA standards. The pharmaceutical-grade HPβCD was obtained from Sigma Aldrich (Darmstadt, Germany) and distilled water was also used.

Formation of inclusion complex by milling method

The *p*MCA-HPβCD IC was prepared by milling method using *p*MCA and HPβCD with a 60 mesh size sieve. *p*MCA and HPβCD equimolar mixed and put into a grinding jar containing a ball mill made of alumina. The mixture was grounded using a high energy milling machine at a speed of 1430 rpm for 3 hours. Furthermore, the inclusion complex formed was stored in a desiccator for 96 hours and then characterized.

Preparation of Physical Mixture Non Milling (PMNM) and Pysical Mixture Milling (PMM)

*p*MCA and HPβCD (PMM) were sieved using a 60 mesh size sieve and each material was milled using a high energy device under the same conditions as in the manufacture of IC. Next, *p*MCA and HPβCD were mixed by tumbling for 5 minutes. For PMNM made with the same molar ratio but not milled.

Characterization

Morphological analysis

Scanning electron microscope (SEM Jeol JSM-7900F) was used to morphological examination with 1000 magnification.

Particle size analysis

Particle size examination was conducted using an Olympus Japan Model XSZ-107 Series light microscope and the observations were made on 300 particles. The sample was dispersed in liquid paraffin and then placed under a microscope equipped with an oculomicrometer. Furthermore, the size of the samples was observed at 100 to 400 times magnification.

Crystallographic analysis

The crystallinity was observed using an X-ray diffractometer Philip X'Pert, Netherland and observed in the range of 2θ:5–40°.

Thermal analysis

Thermal analysis was conducted using a thermal differential analysis tool Mettler Toledo FP99A Cell, USA with a heating speed of 10°C with in a range of 30 to 300°C.

Dissolution

Dissolution tests were performed using paddle-typed dissolution apparatus (Type II) with 500 mL distilled water as dissolution medium with pH 6.5 ± 0.5 and temperature at 37 ± 0.5ºC. About 50 mg *p*MCA was put into the vessel and constant stirring was applied at speed of 75 rpm. The samples were taken every time point up to 60 minutes and then filtered with 0.45 µm (Millipore-USA). The absorbance was analyzed using a UV spectrophotometer at a wavelength of 286 nm. The dissolution test was caried out in triplicates each samples. From the data obtained, the dissolution efficiency of 60 minutes (DE_{60}) of each samples was calculated and one-way ANOVA with α 0.05 was used for statistical analysis.

RESULT AND DISCUSSION

Morphology

The IC made using the milling method with a duration of 3 hours showed different morphologies of their constituent components. The SEM image of the constituent compounds, PM, and IC at 1000 magnification are presented in Figure 1. The morphology of *p*MCA is similar to blade sheet, HPβCD looks like a hollow spherical shape, PMNM showed a mixture of the two components, and in PMM, shapes of both *p*MCA and HPβCD have changed to irregular. Furthernore, SEM showed that the IC compound made was fused between *p*MCA and HPβCD, and there was no visible surface picture of the constituent components.

Particle Size

The milling process generates mechanical energy in the form of a collision between the device and the compound being grounded to change the morphology of the initial compound. In the formation of IC, where *p*MCA and HPβCD compounds are inserted into the grinder and intensively collided for a certain time, an interaction will occur to move part or all of the *p*MCA compounds into the HPβCD cavity.[7,14,17]

Comparison of the particle size of the constituent compounds, PM and IC can be seen in Figure 2. Furthermore, the milling process can reduce the particle size since *p*MCA has a much larger particle size than HPβCD, and PMNM

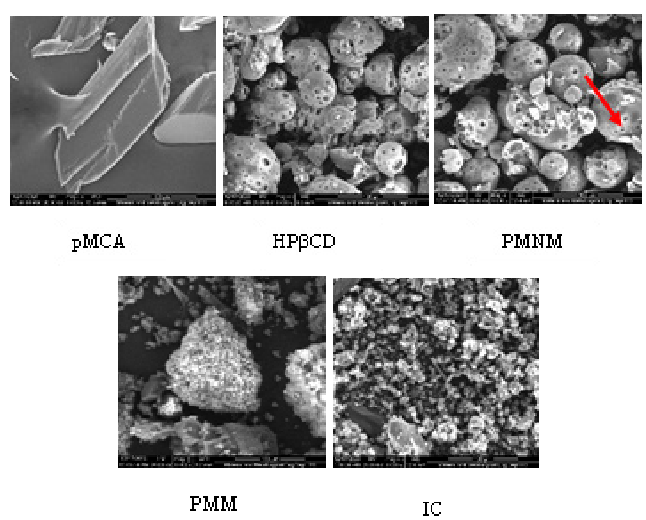

Figure 1: SEM image of *p*MCA, HPβCD, PMNM, PMM and IC with 1000 magnification.

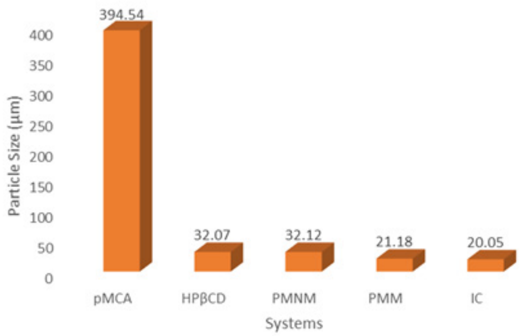

Figure 2: Particle size histograms of *p*MCA, HPβCD, PMNM, PMM and IC

shows a size close to HPβCD. The particles are observed per field of view in the sample glass object using the microscope. The 1:1 molar ratio between *p*MCA and HPβCD compounds gives HPβCD a much greater weight than *p*MCA. The PMNM particle size observation, more HPβCD particles were observed than *p*MCA particles. The PMM shows a smaller particle size than PMNM and the IC is the smallest among the samples measured.

Crystallography

Figure 3 showed the comparison of diffractograms of constituent compounds, PM and IC. From the diffractogram image, the crystallinity of the *p*MCA compound with sharp peaks are shown at 2 θ: 7.84, 16.01, 16.27, and 23.85°. Meanwhile, the HPβCD diffractogram showed an amorphous form without peaks with sharp intensity. The difractogram PMNM showed a crystalline pattern of *p*MCA mixed with an amorphous pattern of HPβCD. The PMM diffractogram showed a sharp peak shift at 10.34, 16.12, 18.67, and 26.87° with decreasing intensity from PMNM. In addition, the IC diffractogram pattern still showed sharp peaks of pMCA at 2θ: 7.89, 16.1. and 26.8° with lower intensity than PMM.

The *p*MCA diffractogram pattern provides sharp peaks and high intensity at the typical 2θ position. This describes the material in a crystalline state, while the HPβCD diffractogram

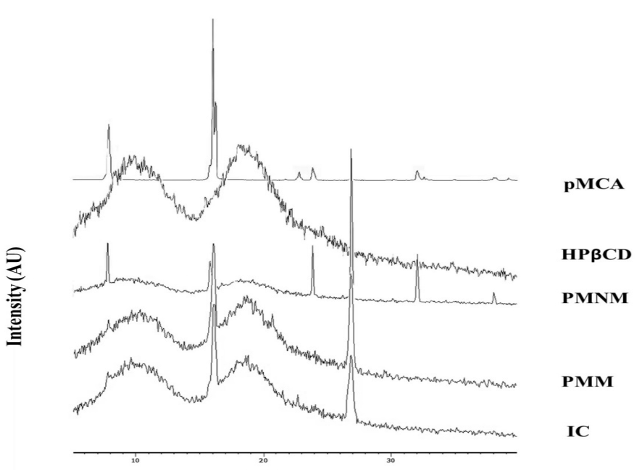

Figure 3: Diffractogram of *p*MCA, HPβCD, PMNM, PMM and IC

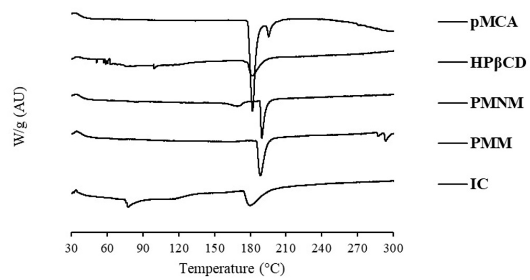

Figure 4: Thermogram of *p*MCA, HPβCD, PMNM, PMM and IC

gives a different pattern, such as the absence of sharp peaks indicating an amorphous state of the material. Meanwhile, the PMNM diffractogram pattern is a combination of the *p*MCA and HPβCD. The PMM diffractogram is similar to PMNM but there is a sharp peak shift at the 2θ position with a lower intensity. Therefore, the milling process on the two constituent components can change the crystallinity of the compound to be more amorphous. In the IC diffractogram pattern, there was one missing *p*MCA peak and the visible peaks still had lower intensity. The disappearance of *p*MCA peaks in the IC diffractogram pattern indicates the formation of a new compound. However, the *p*MCA peaks that still appear on the IC profile indicate that the formation is not yet complete. This is caused by grinding conditions such as milling time, grinding speed, and ball ratio as well as the material used.[8,17]

Thermal Analysis

Figure 4 showed the comparison of the thermograms of constituent compounds, PM and IC. Meanwhile, The *p*MCA thermogram showed large and small endothermic peak at 174.8 and 190.1°C, respectively. The *p*MCA thermogram showed the melting point with the typical endothermic peak of the compound following the literature 173 to 175°C.[19-21] The HPβCD thermograms showed an endothermic peak at 191.6°C, PMNM at 185.8°C, PMM at 183.5°C and two endothermic peaks above 200°C. The IC thermogram provided two wide endothermic peaks before 100 and at 175.8°C.

PMNM and PMM thermograms provided endothermic peaks with melting temperatures between *p*MCA and HPβCD. Milling treatment shifted the melting temperatures to a lower direction, and the IC thermogram provided two broad endothermic peaks that differ from the constituent components and the physical mixture. Furthermore, the endothermic peak widened before 100°C indicating the formation of water crystals. The wide endothermic peak at 175.8°C indicated that compounds with more amorphous properties had been formed.[7]

Dissolution

The percent recovery of *p*MCA in the PM sample and the IC was determined before the dissolution test. The percent recovery for PMNM, PMM, and IC were 93.21 ± 1.02%, 93.71 ± 1.85%, and 100.50 ± 0.06%, respectively, still meets the content requirements between 90-115%. The result of the low PMNM recovery percentage is due to the small particle size of HPβCD with a smooth surface causing electrostatic forces. This reduces the mixing process between *p*MCA and

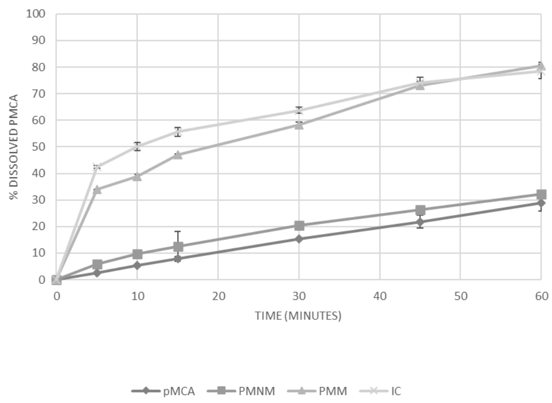

Figure 5: The dissolution profiles of all samples: initial substances *p*MCA, PMNM, PMM and IC (n = 3)

HPβCD materials. In this situation, *p*MCA segregation from HPβCD can occur since the percentage recovery value is low. The milling process of PMM increases the roughness of the the HPβCD surface, as shown in the morphology of Figure 1. This situation is advantageous because an interactive mixture can occur between *p*MCA and HPβCD. However, the *p*MCA and HPβCD components can still be segregated so that the percent recovery is not as high as IC.

Furthermore, the dissolution profile of the four treatments can be seen in Figure 5, and percent dissolved for 60 minutes of *p*MCA, PMNM, PMM, and IC compounds were 28.85 ± 2.99%; 32.18 ± 0.13%; 80.48 ± 1.32%; and 78.40 ± 2.74%, whereas DE_{60} values were 13.87 ± 1.36%; 17.78 ± 0.13%; 52.45 ± 0.56%; and 56.28 ± 1.42% respectively which were significantly different each other ($p< 0.05$). The IC dissolution significantly increased compared to *p*MCA and PMNM compounds. However, the PMM dissolution is almost the same as the IC. Based on the calculation of the dissolution efficiency 60 minutes (DE_{60}), the IC has the highest DE_{60} value among the treatments.

Percent dissolution for 60 minutes from IC was lower than PMM, but the DE_6 value was significantly greater. The formation of an interactive mixture between *p*MCA and HPβCD which is a highly soluble carrier in water can achieve higher dissolution of PMM than PMNM. In addition, the formation of IC with the entry of *p*MCA into the HPβCD cavity provided greater dissolution among all treatments.[7,14] The percent yield of dissolution for 60 minutes of IC compounds of around 78% can be increased by optimizing the grinding conditions such as milling time, grinding speed and the ratio of materials to grinding balls.

CONCLUSION

The milling method successfully formed IC of *p*MCA-HPβCD with different characteristics from the initial compound and was significantly increased the percentage of the dissolved *p*MCA. The characteristics of IC showed that the particles were irregular in shapes, reduced in size and amorphous. The formation of the *p*MCA-HPβCD IC was indicated by the increasing DE_{60} *p*MCA value up to 4.3 times compared to the initial compounds.

ACKNOWLEDGMENTS

The authors would like to thank the Faculty of Pharmacy Universitas Airlangga for supporting the 2020 Faculty Leading Research Fund with SK Rektor number 346/UN3/2020. Thanks to Anindya Ramadhanti Yufinanda and Galuh Primadani who have assisted in this research.

REFERENCES

1. Isadiartuti D, Rosita N, Ekowati J, Syahrani A, Ariyani T. The thermodynamic study of p -methoxycinnamic acid inclusion complex formation, using β-cyclodextrin and hydroxypropyl-β-cyclodextrin. J Basic Clin Physiol Pharmacol. 2021;32:663–667.
2. Sharma P. Cinnamic acid derivatives: A new chapter of various pharmacological activities. J Chem Pharm Res. 2011;3:403–423.
3. Guzman JD. Natural cinnamic acids, synthetic derivatives and hybrids with antimicrobial activity. Molecules. 2014;19:19292–19349.
4. Wijiani N, Isadiartuti D, Rijal MAS, Yusuf H. Characterization and dissolution study of micellar curcumin-spray dried powder for oral delivery. Int J Nanomedicine. 2020;15:1787–1796.
5. Isadiartuti D, Budiati T, Martodihardjo S. Bioavailability study of physical mixture of carbamazepine and amino acids. Asian J Pharm Clin Res. 2015;8:92-95.
6. Isadiartuti D, Ekowati J, Rosita N, Amalia NR. The dissolution of p-methoxycinnamic acid-β-cyclodextrin inclusion complex produced with microwave irradiation, JPHA. 2023; 14(s1):2500.
7. Bekers O, Uijtendaal EV, Beijnen JH, Bult A, Underberg WJM. Cyclodextrins in the pharmaceutical Field. Drug Dev Ind Pharm. 1991;17:1503–1549.
8. Borba PAA, Pinotti M, Andrade GRS, Jr. NB da C, Junior LRO, Fernandes D, Campos CEM, Stulzera HK. The effect of mechanical grinding on the formation, crystalline changes and dissolution behaviour of the inclusion complex of telmisartan and β-cyclodextrins. Carbohydr Polym. 2015;133:373–383.
9. Li S, Yuan L, Chen Y, Zhou W, Wang X. Studies on the inclusion complexes of daidzein with β-cyclodextrin and derivatives. Molecules. 2017;22:1–11.
10. Verma U, Naik JB, Patil JS, Yadava SK. Screening of process variables to enhance the solubility of famotidine with 2-HydroxyPropyl–β-Cyclodextrin & PVP K-30 by using Plackett–Burman design approach. Mater Sci Eng C. 2017;77:282–292.
11. Jug M, Mura PA. Grinding as solvent-free green chemistry approach for cyclodextrin inclusion complex preparation in the solid state. Pharmaceutics. 2018;10:1-22.
12. Gao X, Wei M, Shan W, Liu Q, Gao J, Liu Y, Zhu, S, Yao H. An oral 2-hydroxypropyl-β-cyclodextrin-loaded spirooxindole-pyrrolizidine derivative restores p53 activity via targeting MDM2 and JNK1/2 in hepatocellular carcinoma. Pharmacol Res. 2019;148:1-10.
13. Jagdale SK, Dehghan MH, Paul NS. Enhancement of dissolution of fenofibrate using complexation with hydroxy propyl β-cyclodextrin. Turkish J Pharm Sci. 2019;16:48–53.
14. Inoue Y, Shinohara I, Murata I, Kanamoto I. Study on the molecular stability, solubility, and diffusibility of guaiazulene included in β- and γ-cyclodextrin. J Mol Struct. 2019;1186:50–59
15. Zhang L, Liu M, Lu C, Ren D, Fan G, Liu C, Liu M, Shu G, Peng G, Yuan Z, Zhong Z, Zhang W, Fu H. The hydroxypropyl–β-cyclodextrin complexation of toltrazuril for enhancing bioavailability. Drug Des Devel Ther. 2018;12:583–589.

16. Patil PH, Belgamwar VS, Patil PR, Surana SJ. Solubility enhancement of raloxifene using inclusion complexes and cogrinding method. J Pharm. 2013;2013:1–9.
17. Inoue Y, Yamazoe T, Watanabe S, Murata I, Kanamoto I. Examination of intermolecular interaction as a result of cogrinding actarit and β-cyclodextrin. J Incl Phenom Macrocycl Chem. 2014;78:457–464.
18. Ekowati J, Rudyanto M, Sasaki S, Budiati T, Sukardiman, Hermawan A, and Meiyanto E. Structure modification of ethyl p-methoxycinnamate isolated from *Kaempferia galanga* Linn and cytotoxicity assay of the products on WiDr cells. IJCC. 2010; 1(1):12-18.
19. Fisher Scientific [Internet]. [cited 2020 Sep 17]. Available from: www.fishersci.com
20. Sweetman SC. Martindale: The Complete Drug Reference (36th ed). London; Pharmaceutical Press; 2009;3:2291.
21. Tang P, Li S, Wang L, Yang H, Yan J, Li H. Inclusion complexes of chlorzoxazone with β- and hydroxypropyl-β-cyclodextrin: Characterization, dissolution, and cytotoxicity. Carbohydr Polym. 2015;131:297–305.