The dissolution of *p*-methoxycinnamic acid-β-cyclodextrin inclusion complex produced with microwave irradiation

Dewi Isadiartuti, Juni Ekowati, Noorma, Rosita, Nabella Rizki Amalia

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

Abstract

Background: p-methoxycinnamic acid (pMCA) is an ethyl p-methoxycinnamic derivative, which is the largest active ingredient in the rhizome of the kencur (Kaempferia galanga L) plant. Several studies reported that the compound has anti-inflammatory activity but has low solubility in water. The formation of a pMCA-β-cyclodextrin (βCD) inclusion complex with a molar ratio of 1:1 can increase its solubility. The formation of inclusion complexes with conventional methods requires a long time and the yield value is not optimal.

Objective: This study aims to evaluate the dissolution of the pMCA-βCD inclusion complex produced using the microwave irradiation method.

Methods: The product was manufactured with chloroform solvent and a power of 400 watts (power 80%). It was then evaluated using the Differential Thermal Analysis (DTA) every 2 minutes until the 8th minute. The reaction was complete after 4 minutes of treatment with a yield of 96.5%. The obtained inclusion complexes were evaluated using DTA, FTIR, and PXRD. Subsequently, a dissolution test was carried out using a type 2 apparatus in distilled water medium of pH 6.8±0.05 at 37±0.5°C.

Results: The results showed that there was a change in the melting temperature profile, infrared spectra, and crystallinity of the product. An 89.18% dissolution was also obtained within 60 minutes, which was twice that of pMCA compounds.

Conclusion: From the results of the study, it can be concluded that the formation of pMCA-βCD inclusion complexes using the microwave irradiation method is capable of providing high-yield values in a short time.

Correspondence: Dewi Isadiartuti, Department of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Airlangga, 60115, Surabaya, Indonesia.
Tel.: +62.85100684948.
E-mail: dewi-i@ff.unair.ac.id

Key words: Dissolution, *p*-methoxycinnamic acid, β-cyclodextrin, Microwave irradiation, Drug.

Acknowledgments: The authors thank to the Faculty of Pharmacy Universitas Airlangga for supporting facilities and research financial through faculty excellence research (number of funding grant 1145/UN3.1.5 /LT/2018 PUF).

Contributions: DI, JE, NR, NRA, substantial contributions to the conception or design of the work; the acquisition, analysis; DI, JE, interpretation of data for the work; DI, JE, drafting the work or revising it critically for important intellectual content; DI, JE, NR, NRA, final approval of the version to be published; DI, JE, NR, NRA, agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Conflict of interest: The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

Funding: None.

Availability of data and material: Data and materials are available by the authors.

Informed consent: The manuscript does not contain any individual person's data in any form.

Received for publication: 26 October 2022.
Revision received: 23 December 2022.
Accepted for publication: 30 December 2022.

Journal of Public Health in Africa 2023; 14(s1):2500
doi:10.4081/jphia.2023.2500

Introduction

p-methoxycinnamic acid (*p*MCA) is a cinnamic derivative compound that can be isolated from the rhizome of the kencur (*Kaempferia galanga* Linn) plant, which belongs to the Zingiberaceae family and is widely grown in most parts of Indonesia. Its rhizome is often used by Indonesians as a traditional medicine for treating various diseases. Furthermore, several studies reported that *p*MCA has a stronger analgesic effect than acetylsalicylic acid compounds. It is also known as an anti-inflammatory, antidiabetic, hepatoprotective, antihyperglycemic, and antibacterial agent.[1-3] *p*MCA is a weak acid compound with a pKa value of 4.11 and low solubility of 0.711 mg/mL at 25°C in water. This low value is a challenge in the development of pharmaceutical formulations for oral use.[4] The molecule structure of *p*MCA can be seen in Figure 1A.

The formation of inclusion complexes with cyclodextrin is one of the methods that has been widely used to the increase solubility of compounds. Cyclodextrin is a cyclic oligosaccharide consisting of 6, 7, 8, or more D-(+)-glucopyranose units. Furthermore, it is conical in shape because the primary hydroxyl group is in a narrower section than the second variant. Its outer part is hydrophilic, while the inner components are hydrophobic.[5-7] α-cyclodextrin compounds (αCD) can form inclusion complexes when combined with a molecule with low-weight or aliphatic side chains. Furthermore, β-cyclodextrin compounds (βCD) (Figure 1B) can form the product with aromatic or heterocyclic molecules, while γ-cyclodextrin compounds (γCD) can accommodate large molecules such as macrocyclic and steroids.[8,9]

Inclusion complexes are formed when the drug's components (guest) are trapped in the cyclodextrin compound (host). The condition for their formation is that part or all of the guest molecules enters the host. The products manufactured are stabilized by intermolecular bonds, such as hydrogen bonds, *van der Waals* forces, hydrophobic interactions, and high-energy water release.[4,10,11] A drug compound with low water solubility has a hydrophobic functional group in its molecular structure. If the size of the hydrophobic guest compound functional group can enter partially or completely into the cavity of the host, an inclusion complex is formed.[8,12] The products can modify the physicochemical properties of the drug, namely increasing the solubility, dissolution, bioavailability, stability, and permeability.[12,13]

*p*MCA has an aromatic and a methoxy group in its molecular structure.[14] Generally, the aromatic group can enter the cavity of βCD, which is a derivative of cyclodextrin with an inner cavity diameter of 6.0-6.5 Å. βCD is widely used in oral dosage formulations because it is easy to obtain, economical and its cavity size is suitable for some aromatic compounds.[7] Isadiartuti *et al*.[15] showed that it can form inclusion complexes with *p*MCA in a 1:1 molar ratio. Recently, microwave irradiation methods have been used in the manufacture of inclusion complexes with cyclodextrins in the solid state. The formation of products using the method has advantages, including shorter time, saving energy, lower costs, use of a small amount of solvent, environmentally friendly, higher yield as well as production of compounds with better physicochemical and dissolution characteristics.[16,17] Furthermore, it uses electromagnetic irradiation with a frequency range of 0.3-300 GHz and a wavelength of 1cm–1m. The microwave region of the electromagnetic spectrum lies between the infrared and radio frequencies. The ovens and reactors used for chemical synthesis are generally operated at a frequency of 2.45 GHz with a wavelength of 12.25 cm. The microwave effect is chemically based on dielectric heating, which depends on the ability of each substance to absorb energy from the radiation and convert it into heat.[18]

Several studies manufactured inclusion complexes with the microwave irradiation method using active ingredients of sodium diclofenac,[19] metformin HCl,[20] and theophylline[17] along with complex-forming compounds, such as βCD. The results showed that there was a significant increase in the dissolution of the products compared to the initial constituents. Therefore, this study aims to evaluate the dissolution of *p*MCA through the formation of inclusion complexes with βCD using the microwave irradiation method.

Materials and Methods

*p*MCA was obtained from the hydrolysis of EPMS compounds in the rhizome of the kencur (*Kaempferia galanga* Linn) plant, which was collected from the Purwodadi Botanical Gardens, East Java. The compound was produced using the method proposed by Ekowati *et al*. (2010).[14] It was then standardized using Thin Layer Chromatography, Fourier Transform Infra-Red (FTIR), Differential Thermal Analysis (DTA), ^{1}HNMR, pharmaceutical-grade βCD obtained from PT Kalbe Farma, chloroform p.a (E. Merck), ethanol p.a (E. Merck), and distilled water.

Physical mixture preparation

*p*MCA and βCD with a molar ratio of 1:1 were weighed and sieved using a 60 mesh sieve. Subsequently, they were ground in a mortar until a homogeneous mixture was obtained and then characterized using DTA, FTIR, and Powder X-Ray Diffraction (PXRD). The level of *p*MCA in the physical mixture was determined and then tested for dissolution.

Preparation of pMCA-βCD inclusion complex

*p*MCA and βCD with a molar ratio of 1:1 in chloroform solvent were placed under microwave irradiation with 80% power (400 watts), after which they were observed every 2 minutes until the 8th minute. The mixture was then filtered using the Whatman filter paper and placed in a vacuum at 40°C for 48 hours. Subsequently, the results of each observation time were determined using the melting temperature profile with DTA. The treatment with the optimal results was then characterized with FTIR and PXRD, after which *p*MCA levels were determined and tested for dissolution.

Differential thermal analysis

Approximately 5 mg of the sample was placed in a pan, after which it was transferred into a DTA (Mettler Toledo). The heating speed was then set at 10°C per minute in a temperature range of 50-300°C.

Fourier transform infrared spectroscopy (FTIR)

Potassium Bromide (KBr) powder was added to 2 mg of the sample to obtain a 300 mg mixture, after which it was observed using an infrared spectrophotometer (Jasco FTIR 5300). It was then crushed and made into pellets with a pressure of 8-9 tons to form a transparent disc. Subsequently, the sample was placed on the holder and the FTIR absorbance spectrum was observed at a wavenumber of 4000–4500 cm^{-1}.

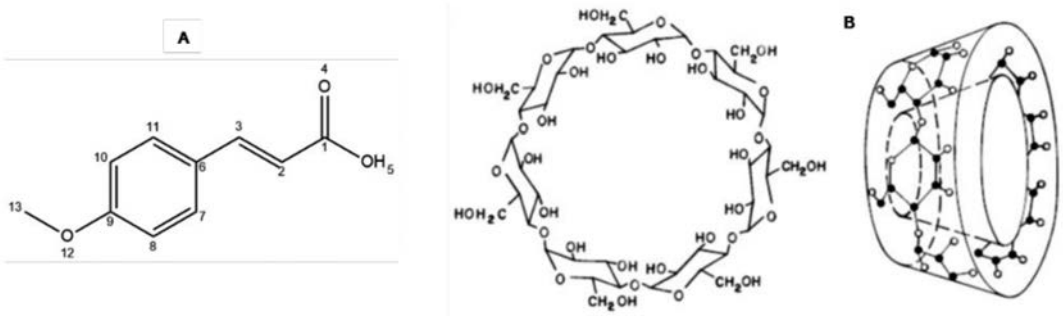

Figure 1. Molecular structure of (A) pMCA and (B) Molecular structure and cone-shaped shape of -cyclodextrin [10]

Powder X-ray diffractometry (PXRD)

The sample was ground in a mortar until a smooth and even texture was obtained. It was then transferred into a vacuum dryer containing blue silica gel, and inserted into a holder. Subsequently, observations were carried out using a PXRD (Phillips X'pert, Netherlands) at 2θ of 5–40° with an angular change rate of 0.01-0.02/second.

Dissolution test

Dissolution tests were carried out on *p*MCA, physical mixture, and inclusion complex. The physical mixture and inclusion complex were made with a molar ratio of 1:1. The results of determining *p*MCA levels in the physical mixture and inclusion complex with coefficient variation values were 107.63±0.04% and 97.59±0.02%, respectively. Based on the percentage of physical mixture and inclusion complex recovery, a sample of *p*MCA equivalent to 50 mg was weighed as a dissolution test sample. Approximately 50.0 mg of *p*MCA was placed in a dissolution vessel containing a 500 mL distilled water medium with a pH of 6.8±0.5, and the temperature was adjusted to 37±0.5 °C. The test was carried out using a type II dissolution apparatus (paddle) at a speed of 75 rpm. Subsequently, 5.0 mL of the sample was taken at 5, 10, 15, 20, 30, 45, and 60 minutes, after which the same volume was replaced after each sampling. *p*MCA levels were then determined using a UV-Vis spectrophotometer at the maximum wavelength with three replications. From the data obtained, a dissolution profile was made and the value of the dissolution efficiency in 60 minutes (ED_{60}) of *p*MCA, physical mixture, and inclusion complex was determined. The ED_{60} value was analyzed by one-way ANOVA statistic at α 0.05.

Results

Thermogram differential thermal analysis

The characterization of the inclusion complex using DTA can be seen in Figure 2, which shows the thermogram of *p*MCA, βCD, physical mixtures, and inclusion complexes.

Infrared spectrum

Figure 3 shows the infrared spectra of *p*MCA and βCD compounds as well as physical mixtures, and inclusion complexes.

Diffractogram powder X-ray diffractometry

Figure 4 shows the diffractogram of physical mixtures, inclusion complexes as well as *p*MCA and βCD compounds. X-ray diffraction of powders was used to identify the crystalline substances that determine the amorphization degree of a sample.[21]

Dissolution study

The results of the characterization with DTA, FTIR, and PXRD revealed the formation of the *p*MCA-βCD inclusion complex. To strengthen these findings, a dissolution test was carried out by comparing the dissolution of *p*MCA compounds, physical mixtures, and inclusion complexes. The concentration of the dis-

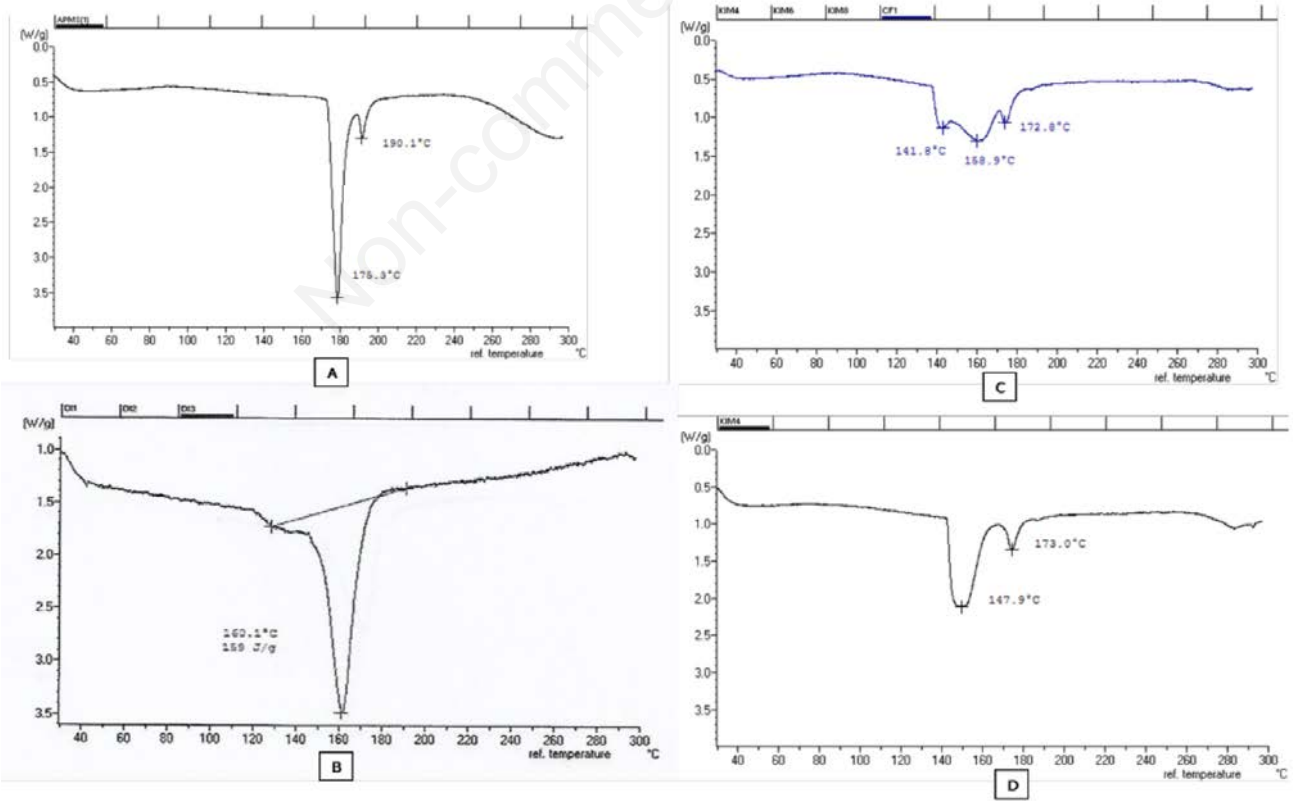

Figure 2. Thermogram of (A) *p*MCA (B) βCD (C) physical mixture, (D) inclusion complex.

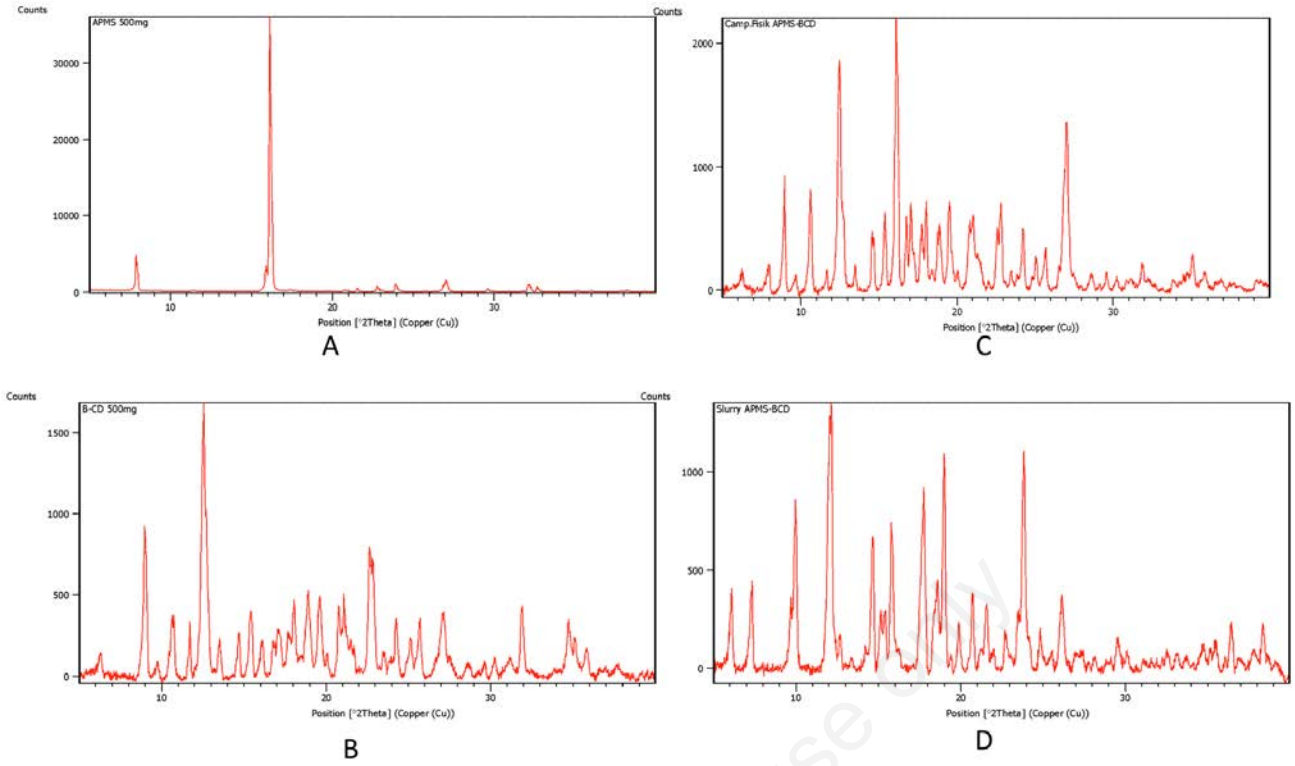

Figure 4. Diffractogram of (A) pMCA (B) βCD (C) physical mixture (D) inclusion complex.

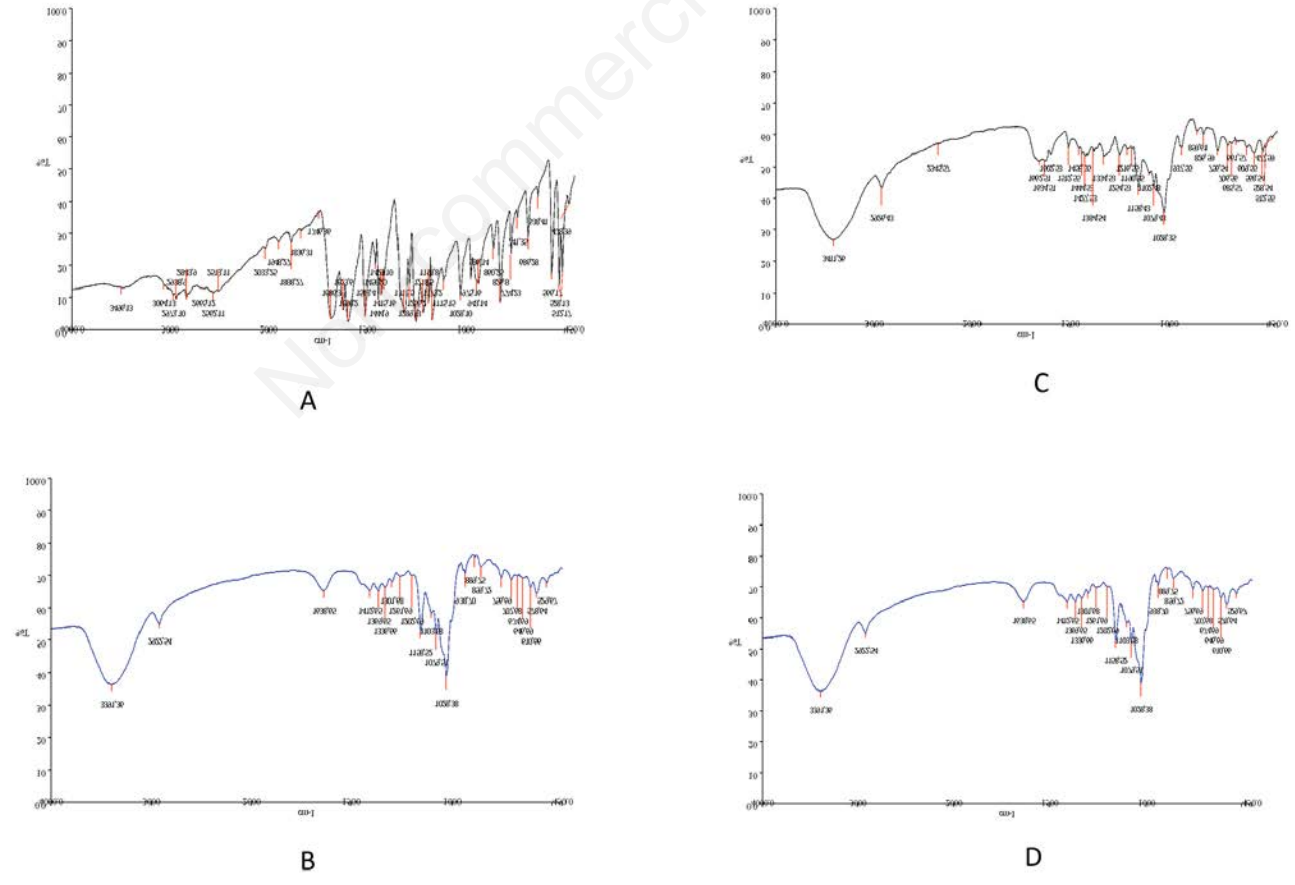

Figure 3. FTIR spectra of (A) *p*MCA (B) βCD (C) physical mixture, (D) inclusion complex.

solved compounds in the medium was determined using a UV spectrophotometer at λ_{max} of 286 nm. The standard *p*MCA curve in the range levels of 0.5-8.0 µg/mL gave the regression equation Y=0.11726 X+0.01530 as well as an R-value of 0.99928. The results of the determination of its levels in the physical mixture and inclusion complex were 107.63±0.04 % and 97.59±0.02 %, respectively can be seen at Figure 5.

Discussion

Chloroform solvent was used as a medium in the manufacture of inclusion complexes using the microwave irradiation method at a power of 400 watts (80%). Microwaves are electromagnetic waves, which travel at the speed of light in a vacuum. Yaqin *et al*.[22] showed that the use of high-power levels can shorten the reactions involved in the synthesis of methyl-ortho-methoxycinnamate. The formation of the inclusion complexes with microwave irradiation was observed using DTA every 2 minutes. The observation results revealed a different endothermic peak for the constituent components of *p*MCA and βCD at 4 minutes with a temperature of 56 °C. The formation of products was then carried out under these conditions. The shortest reaction time and production temperatures close Das[19] were also considered. The production process under the selected experimental conditions gave a 96.5% recovery of *p*MCA in the complex.

Analysis of thermal using DTA

*p*MCA had an endothermic peak at 175.3°C, while βCD occurred at 137.5 and 171.8 °C. Furthermore, the physical mixture thermogram showed that there was a superposition between both components at 141.8; 158.9; and 172.8 °C. The inclusion complex showed a wide endothermic and a sharp peak with low intensity at 147.9°C and 173°C, respectively.

Thermogram changes, such as shifting, widening as well as the appearance or disappearance of endothermic peaks indicate the formation of the complexes during characterization using DTA or DSC. This is due to the entry of guest molecules into the cavity of the βCD host to ensure the melting, boiling or sublimation point shifts to a different temperature or disappears into the decomposed βCD range.[23] Thermograms of several products manufactured using the microwave irradiation method showed that they all experienced a shift or decrease in endothermic peaks compared to guest compounds. This finding indicates that there was an exchange between the external components and water molecules in the βCD cavity. With the loss of water, the guest compounds can enter and bind to the groups in the cavity. The loss of several peaks indicates a strong interaction between the guest compounds and βCD. A decrease or shift in the endothermic peak also indicates a decrease in crystallinity or ability to become more amorphous in the drug.[21,24]

Analysis using FTIR

The analysis results showed the loss of aromatic, -OH carboxylates, and -CO groups of *p*MCA compounds as well as their physical mixtures at 1650-1450 cm^{-1}, 3600-2500 cm^{-1}, and 1060-1040 cm^{-1}, respectively.

Furthermore, the loss of the -OH and -CO groups was caused

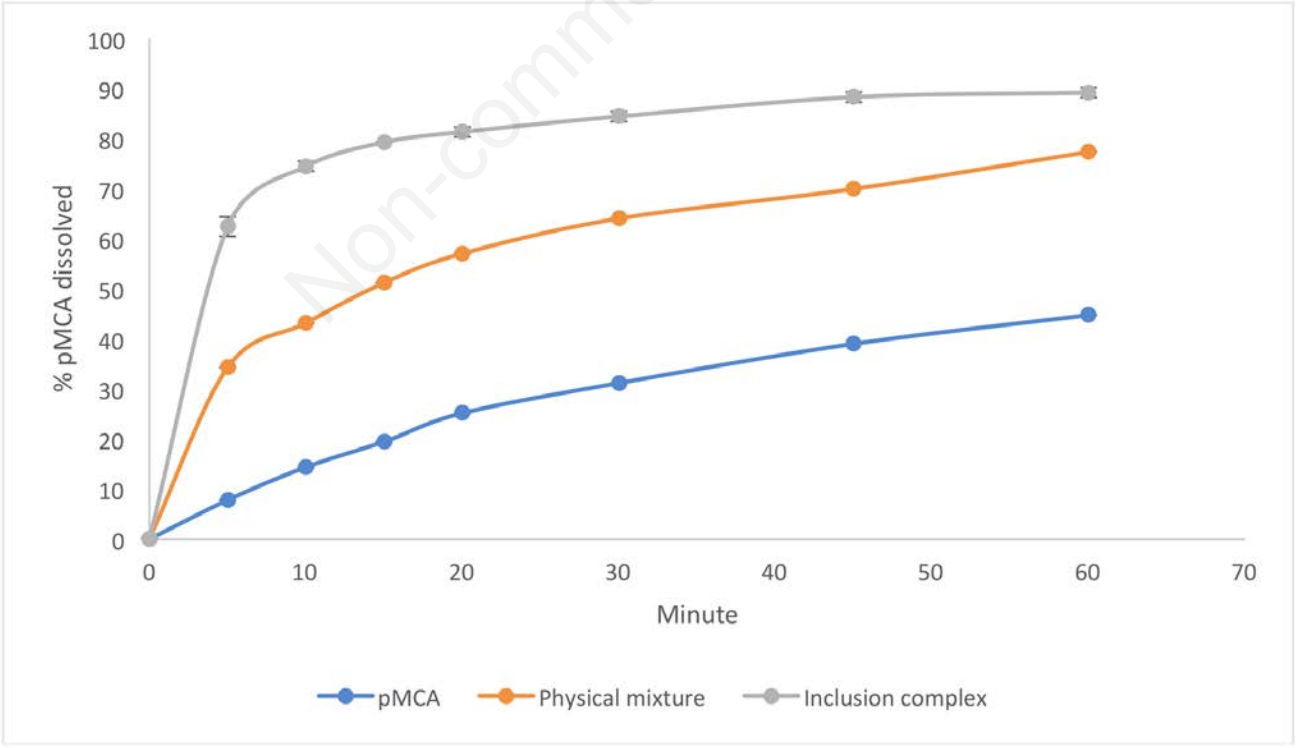

Figure 5. Dissolution profile of pMCA, physical mixture, and inclusion complex in distilled water pH 6.8±0.5 at temperature 37±0.5 °C (n=3).

by the interaction with the -OH of βCD, namely the -CO carboxylic group in *p*MCA forms hydrogen bonds with the -OH group of βCD. The infrared spectrum of the physical mixture and the inclusion complex are similar but can be distinguished from the loss of the -OH carboxylate group in the inclusion complex. Meanwhile, the methoxy from *p*MCA did not show infrared spectra due to their entrance into the βCD cavity. The loss of most of the pMCA peaks and the shift in the peak wavenumber of the pMCA indicates the formation of inclusion complexes made by the microwave irradiation method. Bekers *et al*.[8] reported that the interaction between guest compounds and cyclodextrins led to the production of different infrared spectra compared to the initial constituent. Their entry into the cyclodextrin cavity caused the loss of several peaks.

Analysis Using PXRD

*p*MCA diffractogram shows sharp peaks at 2θ of 7.98; 16.12; 16.23; 15.92; and 27.04°, while βCD occurred at 8.97; 8.99; 12.43; 12.71; 22.61; and 22.61°. This finding indicates that they are crystalline with regular molecular structures, hence, the distance between parallel planes can be measured. The physically mixed diffractogram shows a superposition pattern between that of *p*MCA and βCD at 2θ of 12.41; 12.54; 16.11; 16.24; 26.99; and 27.5° with a lower peak intensity than the constituent compounds. The pattern of the *p*MCA-βCD inclusion complex produced by the microwave irradiation method shows a lower intensity compared to the physical mixture and some of their peaks were not visible. The formation of inclusion complexes generally results in a dramatic change in the results of the diffractogram in the form of the appearance or disappearance of the characteristic peaks of the inclusion complex.

The characterization of the products using powder X-ray diffraction revealed the appearance or disappearance of characteristic peaks or a decrease in sharpness. Their diffractogram profile shows a decrease in the crystallinity of the material, and this was indicated by a decrease in the intensity of the peak or the disappearance or appearance of some of them. A decrease in the intensity indicates that the product has undergone changes to a more amorphous form.[21,23-25] The reduction in the peak of the pMCA-βCD inclusion complex diffractogram shows that the material was more amorphous than the initial compound. With the inclusion of some *p*MCA molecular structure in the cavity, its diffractogram pattern is covered with βCD.

Dissolution test

Figure 5 shows the comparison between the dissolution test of *p*MCA compounds, the physical mixture, and the inclusion complex, in a distilled water medium (pH 6.8±0.5) at 37±0.5 °C. The percentage of *p*MCA dissolved for 60 minutes was 44.78±0.01%, while in the physical mixture and inclusion complex, there was a 77.34±0.03 and 89.18±1.00% increase, respectively. The dissolution efficiency (DE_{60}) of the 3 components was 13.63%; 29.51%; and 47.95%, respectively. Based on the one-way ANOVA test with α 0.05, the DE_{60} value was significantly different between the treatments. The increase in the value obtained for the inclusion complex was 3.49 and 2.16 times greater than *p*MCA compound and physical mixture, respectively. *p*MCA had a lower dissolution compared to the physical mixture because it was dispersed in hydrophilic βCD. However, the dissolution of the mixture was still smaller than the inclusion complex.

A microwave is an electromagnetic wave, which has both electric and magnetic components. The formation of inclusion complexes using the microwave method was caused by electromagnetic radiation exposure, which led to the vibration of the compound as well as the production of heat.[18] Extensive heating in the material occurred at the core of the reaction mixture, which led to the increase in friction between the molecules, hence, the process of trapping *p*MCA compounds into the βCD cavity occurred more quicker. The heat energy changed the crystalline nature of the drug into an amorphous state and it also lowered the melting point.[7] This led to a significant increase in *p*MCA dissolution in the inclusion complex compared to the starting compound and its physical mixture.

Conclusions

During the formation of the *p*MCA-βCD inclusion complex using the microwave irradiation method, the constituent compound is exposed to electromagnetic radiation, which causes vibration and the production of heat. This energy source causes the entry of *p*MCA hydrophobic groups into the βCD cavity. It also lowers its melting point and the compound becomes more amorphous. Changes in the physicochemical properties of the *p*MCA-βCD complex significantly increased the dissolution of *p*MCA. The process requires a short time and provides a large yield value; hence, it can prospectively be used for the manufacture of inclusion complexes.

References

1. Sharma P. Cinnamic acid derivatives: A new chapter of various pharmacological activities. J Chem Pharm Res. 2011;3:403–23.
2. Ekowati J, Diyah NW. Antinociceptive activity and in silico test against cyclooxygenase from p-methoxycinnamic acid and m-methoxycinnamic acid. BIKF. 2013;2:33–40.
3. Guzman JD. Natural cinnamic acids, synthetic derivatives and hybrids with antimicrobial activity. Molecules, 2014;19:19292–349.
4. Isadiartuti D, Rosita N, Syahrani A, et al. pH Adjustment and Inclusion Complex Formation With Hydroxypropyl-β-Cyclodextrin To Increase p-Methoxycinnamic Acid Solubility. J Chem Technol Metall. 2022;57:723-9.
5. Cheirsilp B, Rakmai J. Inclusion complex formation of cyclodextrin with its guest and their applications. Biol Eng Med, 2016;2:1-6.
6. Loftsson T, Brewster ME. Cyclodextrins as a Functional Excipients: Methods to Enhance Complexation Efficiency, J Pharm Sci. 2012;101:3019-32.
7. Alshehri S, Imam SS, Altamimi MA, et al. Host-guest complex of β-cyclodextrin and pluronic F127 with Luteolin: Physicochemical characterization, anti-oxidant activity and molecular modeling studies. J Drug Deliv Sci Technol [Internet]. 2020;55:101356.
8. Bekers O, Uijtendaal EV, Beijnen JH, et al. Cyclodextrins in the pharmaceutical Field. Drug Dev Ind Pharm. 1991;17:1503–49.
9. Jambhekar SS, Breen P. Cyclodextrins in pharmaceutical formulations I: Structure and physicochemical properties, formation of complexes, and types of complex. Drug Discov Today [Internet]. 2016;21:356–62.
10. Isadiartuti D, Martodiharjo S. The formation of inclusion complex of phenobarbital with hydroxypropyl-β-cyclodextrin. MFI. 2005;16:28–37.
11. Kulkarni AS, Dias RJ, Ghorpade VS, Mali KK. Freeze-dried multicomponent inclusion complexes of curcumin for

enhancement of solubility and anti-inflammatory activity. Trop J Nat Prod Res. 2020;4:887–94.
12. Borba PAA, Pinotti M, Andrade GRS, et al. 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;133:373–83.
13. Isadiartuti D, Martodiharjo S. The thermodynamics of inclusion complex of phenobarbital with hydroxypropyl-β-cyclodextrin. MFI. 2007;18:57–62.
14. Ekowati J, Rudyanto M, Sasaki S, et al. Structure Modification of Ethyl p-methoxycinnamate Isolated from Kaempferia galanga Linn. and Citotoxicity Assay of The Products on WiDr Cells. ISCC. 2010;1:12.
15. Isadiartuti D, Rosita N, Ekowati J, et al. The thermodynamic study of p-methoxycinnamic acid inclusion complex formation, using β-cyclodextrin and hydroxypropyl- β-cyclodextrin. J Basic Clin Physiol Pharmacol. 2021;32:663–7.
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. Das S, Maharana J, Mohanty S, Subuddhi U. Spectroscopic and computational insights into theophylline/β-cyclodextrin complexation: inclusion accomplished by diverse methods. J Microencapsul [Internet]. 2018;35:667–79.
18. Kappe CO, Dallinger D. The impact of microwave synthesis on drug discovery. Nat Rev Drug Discov. 2006;5:51–63.
19. Das S, Subuddhi U. Studies on the complexation of diclofenac sodium with β-cyclodextrin: Influence of method of preparation. J Mol Struct [Internet]. 2015;1099:482–9.
20. Kaur KP, Upadhyaya T, Palandoken M, Gocen C. Ultrathin dual-layer triple-band flexible microwave metamaterial absorber for energy harvesting applications. Int J RF Microw Comput Eng. 2019;29:1–7.
21. Mura P. Analytical techniques for characterization of cyclodextrin complexes in the solid state: A review. J Pharm Biomed Anal [Internet]. 2015;113:226–38.
22. Yaqin IN, Ekowati J, Nofianti KA, Suzana S. Utilization of Microwaves Irradiation on Synthesis of Methyl ortho-Methoxycinnamate. J Sains Farm Klin. 2021;8:157.
23. Ghosh A, Biswas S, Ghosh T. Preparation and evaluation of silymarin β-cyclodextrin molecular inclusion complexes. J Young Pharm. 2011;3:205–10.
24. Rachmawati H, Edityaningrum CA, Mauludin R. Molecular inclusion complex of curcumin-β-cyclodextrin nanoparticle to enhance Curcumin skin permeability from hydrophilic matrix gel. AAPS PharmSciTech. 2013;14:1303–12.
25. Gao S, Bie C, Ji Q, et al. Preparation and characterization of cyanazine-hydroxypropyl-β-cyclodextrin inclusion complex. RSC Adv. 2019;9:26109–15.