



Influence of Polyvinylpyrrolidone K30 on The Complexation of Tetrahydrocurcumin with Hydroxypropyl β -Cyclodextrin

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Abstract

Tetrahydrocurcumin (THC) is a polyphenolic compound which exhibits strong antioxidant and tyrosinase inhibition activities. The use of THC in cosmetic or pharmaceutical formulations is limited because THC is slightly soluble in water. Ternary complexes consist of drug-cyclodextrin and polymer as ternary component can enhance drug solubility. The objective of this present study was to evaluate the potential synergistic effect of a ternary component, polyvinylpyrrolidone K30 (PVP K30), on the solubility and physicochemical properties of THC-hydroxypropyl β -cyclodextrin (HP β CD) inclusion complex. Phase solubility analysis was used to investigate the interaction of THC in both binary (THC-HP β CD) and ternary systems (THC-HP β CD-PVP K30). The phase solubility curves were classified as A_L -type with indicated a stoichiometry of 1:1 molar ratio between THC and HP β CD. By adding the PVP K30 (ternary system), the stability constant was enhanced. The binary and ternary inclusion complexes in 1:1 molar ratio were prepared by kneading and coevaporation methods. The solubility of THC from both systems was determined and the physicochemical properties were characterized by Fourier transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC) and powder X-ray diffractometry (PXRD) compared with pure THC and their corresponding physical mixtures. The results revealed that the solubility of THC in ternary systems was significantly greater than binary complexes, physical mixtures and pure THC. Ternary inclusion complex prepared by coevaporation method was found to be most effective in increasing the THC solubility. From FTIR, DSC and PXRD studies, the binary and ternary coevaporated samples gave THC in amorphous state and stronger complex formation than that of kneaded samples and physical mixtures.

Keywords: Tetrahydrocurcumin, Hydroxypropyl β -cyclodextrin, polyvinylpyrrolidone K30, Complexation, Solubility

Introduction

Cyclodextrins (CDs) are cyclic oligosaccharides containing 6, 7 or 8 D-(+) glucopyranose units attached by α -(1, 4) glucosidic bonds. CDs consist of hydrophilic outer surface and a hydrophobic central cavity which inner cavity of hydrophobic CDs forms non covalent inclusion complexes with a large variety of guest molecules while the hydrophilic exterior enhances CDs solubility in water (Chowdary

& Srinivas, 2006; Dos Santos *et al.*, 2011). In general, CDs has been widely used in pharmaceutical filed to improve physicochemical properties of drugs, able to reduce their irritancy and toxicity (Jullian *et al.*, 2008). Nevertheless, application of CDs is limited by its rather low aqueous solubility. Therefore, many chemical modified CDs such as 2-hydroxypropyl- β -cyclodextrin (HP β CD), methylated- β -cyclodextrin has been developed to counter the solubility limits and safety concerns of



the parent CDs. HP β CD is a hydroxyalkyl derivative of β -cyclodextrin. It is higher water solubility and safety than β -cyclodextrin. Complexation with HP β CD dramatically increased the aqueous solubility of many poorly water soluble compounds (Chowdary & Srinivas, 2006; Lavecchia & Zuurro, 2009). THC is a phenolic compound which exhibited strong antioxidative activity (Nakamura *et al.*, 1998; Khopde *et al.*, 2000). It possesses a wide range of pharmacological and biological activities (Okada *et al.*, 2001), including anti-inflammatory, antiviral, antimicrobial and anticancer. It is used in cosmetic preparations as antioxidant and skin lightening agent because of efficiently inhibit tyrosinase enzyme. THC is slightly soluble in water which appears to be limiting factor in formulation development especially in water-based formulations or hydrogels that suitable for oily or acne-prone skin. To overcome this problem, increasing THC solubility is needed. It has been reported that the combination of cyclodextrin and hydrophilic polymers such as PVP K30, hydroxypropyl methylcellulose can significantly enhance cyclodextrin complexation and increase drug solubility. To our literature reviews, there is no report available about the effect of addition of PVP K30 on HP β CD solubilization of THC. The purpose of the present study was attempted to enhance the THC solubility by preparing ternary complex consisted of THC-HP β CD-PVP K30. The binary and ternary inclusion complexes were prepared by kneading and coevaporation methods. The influence of PVP K30 on THC solubility was determined and the physicochemical properties were characterized by DSC and PXRD compared with physical mixtures and pure THC.

Materials and Methods

Experimental

Materials

Tetrahydrocurcumin was purchased from Sabinsa Corporation (Missouri, USA). HP β CD, degree of substitution per anhydroglucose unit about 0.6, was kindly provided by Wacker-chemie GmbH (Munich, Germany). PVP K30 by BASF (Bangkok, Thailand). Absolute ethanol by RIC Labscan Limited (Bangkok, Thailand). Other chemicals and solvents used in this study were of analytical reagent grade.

Phase solubility study

Phase-solubility studies were performed according to the method described by Higuchi and Connors (Higuchi & Connors, 1965). Briefly, excess amount of THC (10 mg) was added to 10 ml of aqueous solutions containing various concentrations of HP β CD (0-40 mM) with and without 2.5%w/v of PVP K30. The contents were shaken in shaking water bath at $32\pm 0.5^\circ\text{C}$ until equilibrium (48 hours). Then, the dispersions were filtered through 0.45 μm membrane filter and properly diluted with distilled water. The concentration of THC was determined by spectrophotometer at 282 nm. Each experiment was carried out in triplicate. The phase-solubility diagrams were obtained by plotting between the solubility and concentrations of HP β CD with and without adding PVP K30 concentrations.

Preparation of solid inclusion complexes

Inclusion complexes of THC-HP β CD were prepared in molar ratio of 1:1 by kneading and coevaporation methods in the absence and presence of 2.5%w/w of PVP K30.



Physical mixtures

Physical mixtures were prepared by simple mixing THC and HP β CD in molar ratio of 1:1 in the absence and presence of 2.5%w/w of PVP K30 for 3 minutes.

Kneading method

HP β CD with and without adding PVP K30 were triturated in a mortar with a small volume of distilled water. Then, THC was slowly added while grinding. The slurry formed was kneaded for 1 hour and then dried at 50°C for 2 h. The dried complexes were then powdered and stored in airtight containers, protected from light until use.

Coevaporation method

THC and HP β CD with and without adding PVP K30 were dissolved in absolute ethanol. The solvent was evaporated by rotary evaporation at 50°C. Then, the inclusion complexes were kept in vacuum oven until dry. The dried complexes were then powdered and stored in airtight containers, protected from light until use.

Aqueous solubility study

The solubility of THC, physical mixtures and the inclusion complexes were determined in distilled water. Each sample (10 mg) was added in 10 ml of the distilled water. The samples were kept at equilibrium for a period of 48 hours in shaking water bath at $32 \pm 0.5^\circ\text{C}$. Then, the contents were filtered through 0.45 μm membrane filter and analyzed by UV spectrophotometer at 282 nm. The experiment was carried out in triplicate.

Fourier transform infrared spectroscopy (FTIR)

Infrared spectra of samples were carried out using a FTIR spectrometer (Spectrum One, PerkinElmer, United Kingdom). The procedure consisted of grinding the sample with potassium bromide (KBr) into a fine powder, then placing into a pellet-forming

die and compressing the powder using a compressing gauge. The scanning range was $400\text{--}4000\text{ cm}^{-1}$.

Differential scanning calorimetry

Differential scanning calorimetry (DSC) was performed using a differential scanning calorimeter (DSC-8000, PerkinElmer). Samples (3–5 mg) were sealed into aluminum pans and hermetically sealed. The scanning rate was $10^\circ\text{C}/\text{min}$, and the scanning temperature range was between 30°C to 150°C under a nitrogen gas flow of 20 ml/min.

Powder X-ray diffractometry (PXRD)

Powder X-ray diffractograms were recorded using Powder X-ray diffractometer (Philips X'Pert MPD, Netherlands) with tube anode Cu over the interval $5\text{--}60^\circ/2\theta$ with a scan speed of $3^\circ/\text{min}$ and a step size of 0.01° .

Statistical analysis

The solubility data were expressed as means \pm standard deviation (SD). The statistical comparison was performed using Student's t-test or one-way analysis of variance (ANOVA). Differences were considered statistically significant at $p < 0.05$.

Results and Discussion

The phase solubility diagrams of THC in various concentrations of HP β CD with and without 1.5%w/v of PVP K30 are shown in **Figure 1**. It has been observed that the solubility of THC was increased linearly as a function of HP β CD concentrations in range of 0–40 mM and can be classified as A_L type according to Higuchi and Connors (1965). Because the slope of the straight lines are lower than one in each case, the increase in solubility was due to the formation of a 1:1 M complex in solution with HP β CD in the presence and absence of PVP K30. The apparent stability constant

(K_s) was calculated from the slope and intercept of straight line of the phase solubility diagram according to equation $K_s = \text{slope} / \text{Intercept} (1 - \text{slope})$, where the intercept is the intrinsic solubility of THC in the absence of HPβCD. The estimated K_s values of inclusion complexes of THC-HPβCD in the presence of PVP K30 was 514 M⁻¹ while K_s value of THC-HPβCD in the absence of PVP K30 was 455 M⁻¹. These values are within the range of 200–5,000 M⁻¹ indicated that complex formation of THC and HPβCD with and without PVP K30 are quite stable

and capable of improving the solubility and stability of THC (Sapkal *et al.*, 2010). The higher value of K_s indicated that the ternary system was more stable than binary system. The addition of PVP K30 enhanced only the K_s but did not show any change in type of phase solubility diagram. A synergistic effect on THC solubility observed in presence of PVP K30 may be due to the hydrophilic property of PVP K30 which was capable of increasing THC wettability and the formation of hydrogen bonding between THC and PVP K30.

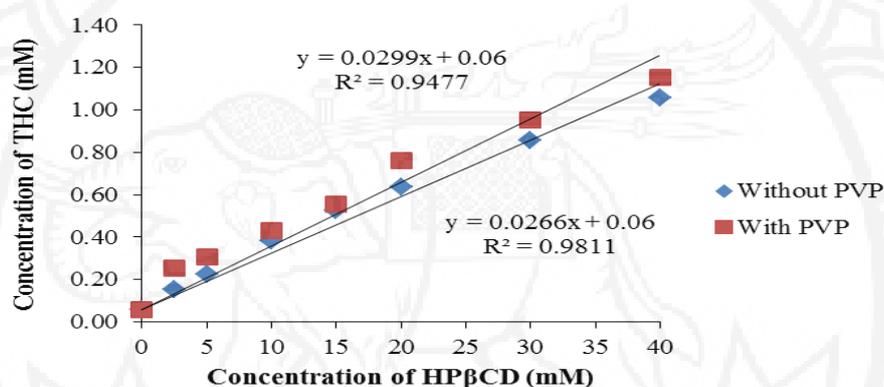


Figure 1 Phase solubility diagrams of THC in HPβCD solutions with and without adding PVP K30.

The water solubility of pure THC, physical mixtures and inclusion complexes at 32±0.5°C are shown in **Table 1**. The solubility of THC from physical mixtures and all inclusion complexes are significantly higher ($p < 0.05$) than pure THC (23.27 μg/ml). The solubility of THC from binary physical mixture (PM), ternary physical mixture (PM_PVP) was increased about 1.7 folds compared with pure THC while solubility of THC from binary kneaded (KN), ternary kneaded (KN_PVP), binary coevaporated (CO) and ternary coevaporated (CO_PVP) samples were increased in 2.4, 2.8, 2.9 and 4.9 folds, respectively, compared with pure

THC. The ternary coevaporated sample gave the highest solubility of THC and significantly higher ($p < 0.05$) solubility than other samples. The enhanced in THC solubility especially with addition of PVP K30 could be attributed to weak polymer-drug interaction such as hydrogen bonding (Patel & Vavia, 2006). In addition, amorphous state which obtained only in both binary and ternary coevaporated samples was responsible for increase solubility of THC. According to the solubility data, it can be concluded that PVP K30 and HPβCD exhibited synergistic effect in improving of THC solubility.

**Table 1** Water solubility of THC, physical mixtures, kneaded and coevaporated samples at $32 \pm 0.5^\circ\text{C}$

Samples	Solubility ($\mu\text{g/ml}$)*
THC	23.27 ± 0.51
PM	39.38 ± 1.33
PM_PVP	41.99 ± 1.61
KN	55.70 ± 3.51
KN_PVP	65.07 ± 3.92
CO	67.06 ± 3.08
CO_PVP	113.30 ± 3.08

*mean \pm SD (n = 3)

The FTIR spectra of pure THC, physical mixtures, kneaded and coevaporated samples are given in **Figure 2**. Pure THC is showing peaks at 3414 cm^{-1} (aromatic and aliphatic-CH stretching vibration), 1614 and 1602 cm^{-1} ($\text{C}=\text{C}_{\text{ring}}$), 1515 cm^{-1} (C-H in aromatic ring), 1277 cm^{-1} (stretching vibrations of enolic C-O), 1155 and 1114 cm^{-1} (C-O-C stretching vibration), 1032 cm^{-1} (C-O-H stretching vibration), etc. There were not presence of any peaks in the region of $1650\text{--}1800\text{ cm}^{-1}$, carbonyl group (C=O) region (Mohan *et al.*, 2012), indicated that THC still presence in the keto-enol tautomeric form (Pan *et al.*, 1998). The FTIR spectrum of HP β CD exhibited absorption bands at 3402 cm^{-1} (OH), 1084 cm^{-1} (C-O), 2929 cm^{-1} (C-H). The H-O-H deformation bands of water present in cyclodextrins are shown at 1650 cm^{-1} . Absorption band in the region of $1300\text{--}700\text{ cm}^{-1}$ assigned to skeletal C-C vibrations and that at 1156 cm^{-1} refer to C-O-C vibrations. The FTIR spectrum of PVP K30 exhibited characteristic peaks at 2955 cm^{-1} (C-H stretching vibration), 1662 cm^{-1} (C=O stretching vibration) and 1463 cm^{-1} (N-H band). A very broad peak was also visible at 3436 cm^{-1} that was attributed to the presence of water (Sammour

et al., 2006). The binary system and ternary system of THC-HP β CD complexation, namely physical mixtures and kneaded sample with and without PVP K30 (PM, PM_PVP, KN, KN_PVP), THC still presence in the keto-enol tautomeric form. These FTIR spectrums was summation of THC and HP β CD spectra, and the intense peak of THC located at 3414 cm^{-1} (O-H groups) was shifted to the lower frequency. These results may be due to the intermolecular hydrogen bonding between THC and HP β CD or this band was hidden from the broad OH peak of HP β CD. The low intensity of the absorption peaks at 1616 and 1602 cm^{-1} for physical mixtures, kneaded sample and coevaporated sample assumed the aromatic ring ($\text{C}=\text{C}_{\text{ring}}$) of THC might be entrapped inside the hydrophobic cavity of HP β CD, while the other part may remain outside of the HP β CD. However, the stretching vibration of ketone (C=O) groups were observed in coevaporated sample both of binary and ternary system. This result indicated the formation of intermolecular hydrogen bonding between ketone group and hydroxyl group of HP β CD.

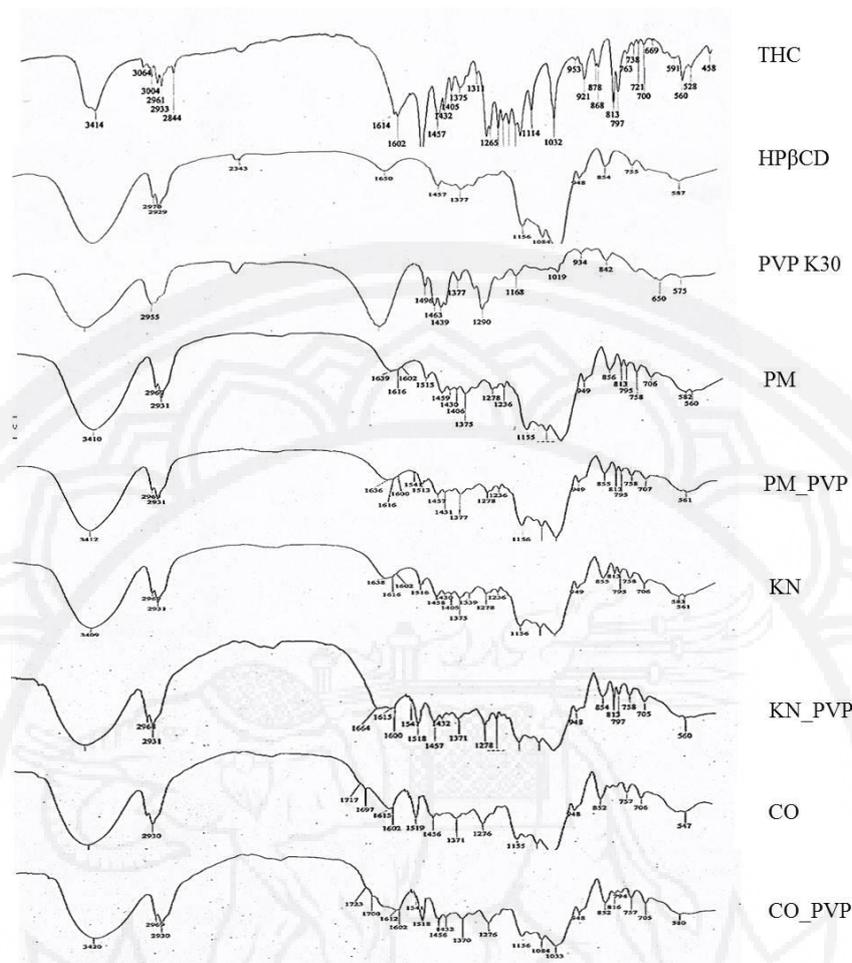


Figure 2 The FTIR spectrum of THC, HPβCD, binary and ternary physical mixtures (PM, PM_PVP), binary and ternary kneaded samples (KN, KN_PVP) and binary and ternary coevaporated samples (CO, CO_PVP).

In this study, DSC is used to characterize interaction between THC-HPβCD inclusion complexes in the presence and absence PVP K30. The DSC thermograms of THC, physical mixtures, kneaded and coevaporated samples are shown in **Figure 3** The DSC thermogram of THC showed a single sharp endothermic peak at 99.8°C, corresponding to its melting point while HPβCD showed a very broad endotherm in the temperature range of study. The DSC thermograms of physical mixtures and kneaded samples showed the melting endothermic peaks which shifted to lower

temperature, indicating the interaction between THC and polymer in physical mixtures and kneaded samples but THC from these samples remained in crystalline form. On the other hand, the completely disappeared of endothermic peak of THC in binary and ternary coevaporated samples was observed, indicating the change of crystalline form to amorphous form and a strong interaction between THC and polymer. This result may indicate molecular encapsulation of THC inside the HPβCD cavities (Chowdary & Srinivas, 2006).

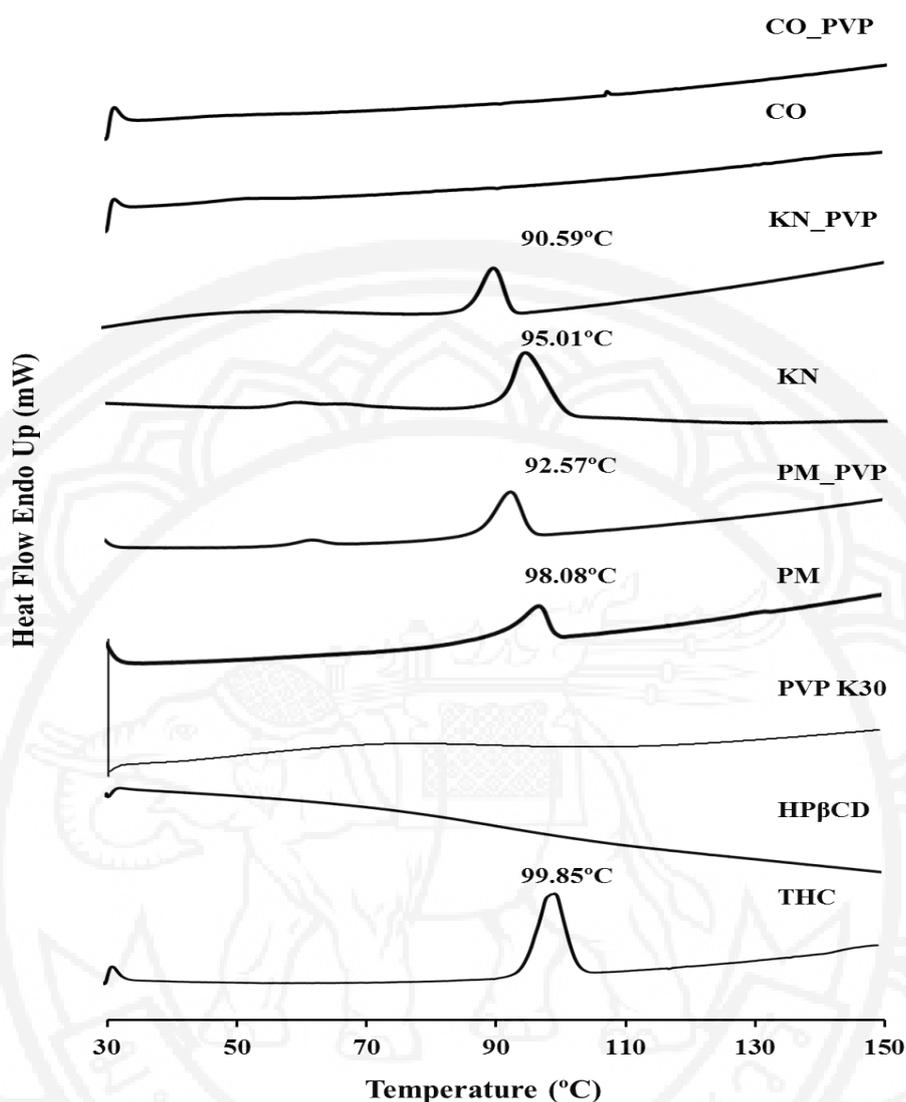


Figure 3 The DSC thermograms of THC, HPβCD, binary and ternary physical mixtures (PM, PM_PVP), binary and ternary kneaded samples (KN, KN_PVP) and binary and ternary coevaporated samples (CO, CO_PVP).

Powder X-ray diffractograms of THC, HPβCD, physical mixtures and inclusion complexes are illustrated in **Figure 4**. The complete absence of any diffraction peaks in PXRD diffractogram of HPβCD, indicating its amorphous nature. THC exhibited several diffraction peaks revealing its crystalline nature. Some diffraction peaks due to THC crystals are still detectable in physical mixtures and kneaded

samples. On the other hand, all of THC diffraction peaks are completely disappeared in the diffractograms of coevaporated samples. These findings demonstrated that the amorphous inclusion complexes are formed in both binary and ternary inclusion complexes. The results can be verified by DSC analysis.

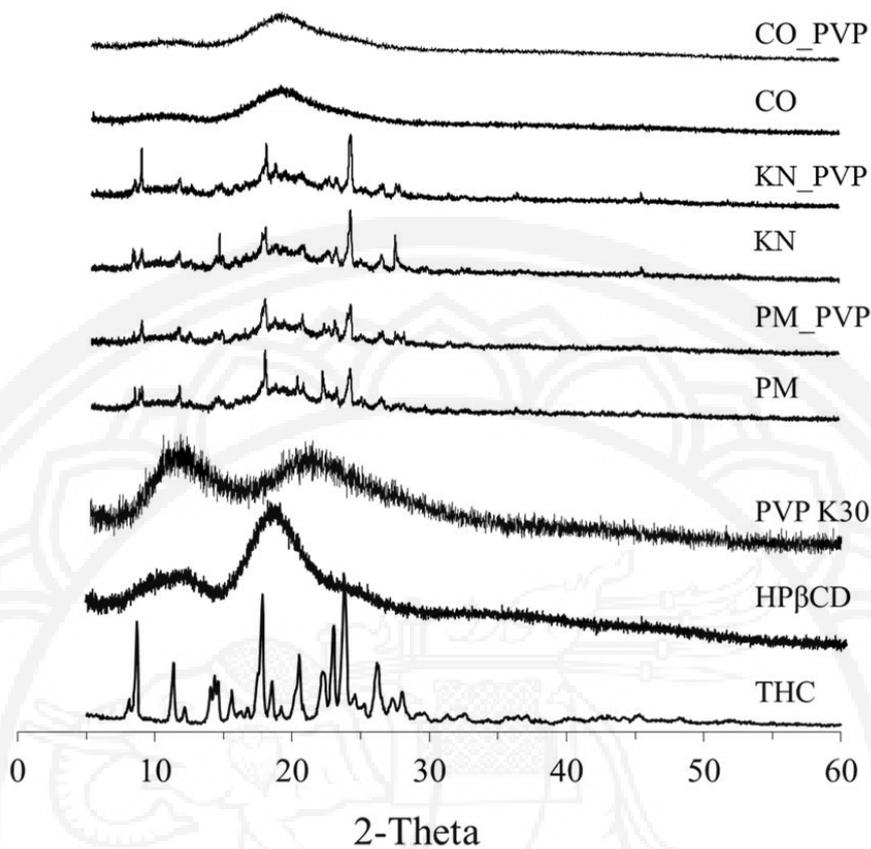


Figure 4 Powder X-ray diffractograms of THC, binary and ternary physical mixtures (PM, PM_PVP), binary and ternary kneaded samples (KN, KN_PVP) and binary and ternary coevaporated samples (CO, CO_PVP).

Conclusions

The solubility of THC was increased as the function of HPβCD concentration in range of 0–40 mM in the presence and absence of PVP K30 and classified as A_L -type phase solubility diagrams. THC is able to form more stable inclusion complex with HPβCD in the presence of PVP K30. The ternary inclusion complex prepared by coevaporation method demonstrated a superior water solubility of THC. In conclusion, the ternary system of THC and PVP K30 is a promising alternative for increasing the aqueous solubility of THC that lead to be valuable application in pharmaceutical and cosmetic formulations especially water-based formulations and hydrogels.

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