Improvement of the Solubility and Dissolution Rate of the Steroidal Drug, Mesterolone, using Cyclodextrin Complexation

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Abstract

Inclusion complexation between mesterolone (MN), a steroidal hormone, and β-cyclodextrin (βCD) or hydroxypropyl-β-cyclodextrin (HPβCD) was evaluated in aqueous environment and in solid state. Phase solubility profiles of MN with βCD or HPβCD were obtained and classified as A_L-type and B_L-type, respectively. An apparent stability constant was calculated from each phase solubility profile and was found to be higher for MN- βCD than for MN- HPβCD. Solid binary systems of MN-CD were obtained with both CDs by kneading and coevaporation at MN: CD molar ratios of 1: 1 and 1: 2. In comparison to the respective physical mixtures, the binary systems were characterized for % MN complex inclusion by chloroform extraction and for solid state by differential scanning calorimetry and X-ray diffractometry. MN inclusion was mostly dependent on MN: CD molar ratio and this dependence was higher with βCD than with HPβCD upon both kneading and coevaporation. The DSC results indicated MN inclusion and loss of MN crystallinity, which were higher at 1: 2 than at 1: 1 molar ratio for both CDs and for βCD than for HPβCD at each molar ratio. X-ray diffraction results confirmed the loss of drug crystallinity upon kneading and coevaporation particularly with βCD. The binary systems were subjected to dissolution studies in comparison to pure MN. An enhancement in MN dissolution was obtained and was explained based on local drug solubilization during dissolution, drug inclusion and loss of drug crystallinity. The enhancement in MN dissolution was found to strongly depend on MN: CD molar ratio with slight effect for CD type and preparation method.

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Keywords

Cyclodextrins; Mesterolone; Inclusion complexation; Improvement of dissolution rate.

Introduction

Mesterolone (MN) is a steroidal hormone used in the treatment of hypogonadism and of male infertility due to oligospermia. It is given by mouth in divided doses of 50 to 100 mg daily. [10] MN is practically insoluble in water, and so its dissolution is considered rate-limiting step in the gastrointestinal absorption from a solid dosage form. [11] Cyclodextrins (CDs) are cyclic carbohydrates capable of forming inclusion complexes with several poorly water-soluble compounds, thereby enhancing their solubility, stability and bioavailability. [13, 5] Several studies on the steriodal complexation with CDs are reported in litreture. Inclusion complexes of several steroid derivatives with ß-cyclodextrin were studied in dimethylsulfoxide solution. [3] The investigated molecules were ketosteroids with different functional groups on the skeleton: 3 ß-acetoxypregn-5-en-20-one (I), 3ß-acetoxypregna-5,16-dien-20-one (II), 3ß-acetoxyandrost-5-en-17-one (III), 3ß-hydroxyandrost-5-en-17-one (IV), 5α-androstane-3,17-dione (V) and 17ß-hydroxyandrost-4-en-3-one (VI). In case of inclusion complex formation, the steroid molecule penetrates the cavity of the cyclodextrin and dipole-dipole interactions (ROEs) can be detected between the glucose H-3 and H-5 protons inside the cyclodextrin cavity and the steroid skeletal protons. The complexes formed by the steroid rocuronium bromide with four different cyclodextrins were studied. [3] The diffusion coeffecients of the steroid-cyclodextrin complexes were 6-15% lower than those of the native cyclodextrins, consistent with a 1:1 stoichiometry for all of the complexes.

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The formation of inclusion complexes between epibrassinolide and ß-cyclodextrin was confirmed by their physicochemical properties and the compounds were analyzed by differential scanning calorimetry, powder X-ray diffraction, NMR spectrometry and SEM. [4] Inclusion of the side chain of the epibrassinolide molecule into the ß-cyclodextrin cavity to form a 1:1 inclusion complex, although complexes involving inclusion of the steroidal nucleus also possess a favorable interaction energy. The inclusion complexes of ß-cyclodextrin (ß-CD) with prednisolone 1, ethinylestradiol 2 and estriol 3 in aq. soln. were investigated using 1H NMR and mol. Modeling. [1] Combined approaches allow the distinction of weak nonspecific binding for 1 as compared to stronger, "through cavity", inclusion established for 2 and 3. HPLC was used to study the inclusion complexes formed between various ß- and γ-cyclodextrins and a series of corticosteroids related to betamethasone. [6] Larger apparent association constants were obtained with γ -cyclodextrin (γ -CD) than with ß -cyclodextrin (ß -CD) due to the increased diam. of the γ -CD cavity.

The purpose of this study was to improve the solubility and dissolution rate of MN through CD complexation using two types of CD: β-cyclodextrin (βCD) and hydroxypropyl-β-cyclodextrin (HPβCD). Phase solubility diagrams were constructed for MN with both CDs. MN-CD binary systems were prepared by physical mixing, kneading and coevaporation. The binary mixtures were also characterized for % MN complex inclusion and for solid state using differential scanning calorimetry and X-ray diffractometry. The effect of MN: CD molar ratio, CD type and preparation method on MN dissolution from the binary systems in comparison to pure MN was addressed.

Material and Methods

Materials: MN working standard and MN raw material were obtained from Plantaria AG, Switzerland. MN was provided as crystalline form with determined melting point of 209 ^{o}C and median particle size of 46. 545 μm. The solubility of obtained MN raw material in water was determined to be 3.44 μg/ml. This means that 1 gm of the drug needs more than 10,000 ml of water to dissolve. Accordingly, the drug can be considered practically insoluble in water according to solubility description in BP (BP, 2002). MN is also sparingly soluble in acetone, in ethylacetate and methanol (Eur Ph, 20020). MN chemical structure (Molecular weight 304.5) is shown in Figure 1. βCD (M. Weight 1135) and HPβCD (M. weight 1391) were obtained from Roquette, France. All other chemicals were of analytical grade.

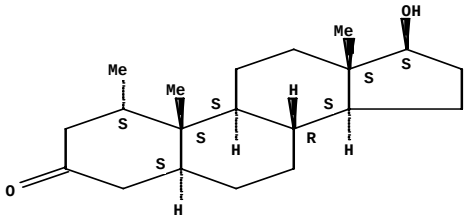


Figure 1: Chemical structure of MN.

High Pressure Liquid Chromatography (HPLC): HPLC analysis was performed using Waters 1515 isocratic HPLC pump, Waters 2487 dual λ absorbance detector, and Inertsil ODS-2 column of 4.6 × 150 mm (GL Sciences Inc.). A mobile phase mixture of methanol: acetonitrile: water (1: 2: 2) was used. The analytical method was fully validated as described in the following.

System Suitability: In order to ascertain the effectiveness of the operation system, system suitability test was performed by injecting 5 replicates of a standard solution into HPLC. The AUC values for the 5 measurements were obtained and their standard deviation was calculated.

Linearity and Range: Six standard solutions in the range of 50 to 150 % of the assay-working standard and in the range of 20 to 150 % of the dissolution-working standard were prepared and injected into the HPLC in duplicate. The AUC values were obtained and then linear regression analysis was performed on the average peak area versus concentration.

Detection and Quantitation Limit: The detection limits were determined based on the standard deviation of the response (σ) and the Slope (S) from regression analysis of linearity and range. Detection Limit (DL) was calculated as (DL)= 3.3σ/S while Quantitation Limit (QL) was calculated as (QL) = 10σ/S.

Precision

A. System precision: System precision was performed by applying 10 injections of the assay working standard or dissolution working standard into the HPLC. The standard deviation was calculated for the obtained AUC values.

B. Method precision: Six samples equivalent to 50 mg of mesterolone were prepared according to a sample preparation technique under assay method were injected into the HPLC. The standard deviation was calculated for the % assay values. Six aliquots prepared according to sample preparation technique under dissolution method were injected into the HPLC. The standard deviation was calculated for the % drug values.

Accuracy (recovery): For each excipient used in formulations, three drug solutions at concentrations of 50, 100 and 150 % of the working standard concentration for assay validation and 20, 100 and 120 % of the working standard concentration for dissolution validation were prepared in the presence of fixed amount of excipient using the same procedure described under samples preparation. The amount of each excipient used varied according to the amount used in formulation. Each concentration level was done in triplicate for each excipient i.e. a total of 9 determinations for each excipient. The samples were injected into the HPLC, the % drug recovered were obtained and the relative standard deviation of the % drug recovered was calculated.

Specificity: In order to detect the method ability to measure accurately the analyte in the presence of the excepients used in formulations, a placebo which represents each exciepent used was prepared according to samples preparation under assay and dissolution method. Each placebo was injected into HPLC and its chromatogram was recorded and compared to those obtained for working drug standard solution.

Phase Solubility Diagrams: Phase solubility diagrams of MN-CD were performed according to the method reported by Higuchi and Connors. [9] Excess amounts of MN were added to series of aqueous solutions containing various concentrations of βCD or HPβCD. The mixtures were stirred for 5 days at 37°C, filtered using 0.45 µm membrane filter and then analyzed for MN dissolved using HPLC.

Preparation of Solid Binary Systems: Three parameters for the effect of CD on MN dissolution were investigated: CD type (βCD and HPβCD), preparation method (physical mixing, kneading and coevaporation) and molar ratio of MN: CD (1: 1 and 1: 2).

Physical Mixing: Proper amounts of MN and βCD or HPβCD to give 1:1 or 1:2 guest-host molar ratio were blended in a polyethylene bag for 5 min. The mixture was passed through 355 µm sieve to remove any agglomerates and then further mixed in a polyethylene bag for another 2 min.

Kneading: Proper amounts of MN and βCD or HPβCD with guest-host molar ratio of 1:1 or 1:2 were triturated gradually in a mortar with 50% water-methanol solution until thick slurry is formed. The thick slurry was further kneaded using mortar and pestle for 45 min and then dried in an oven overnight at 45°C. The dried powder was passed through 355µm sieve and then mixed thoroughly in a polyethylene bag.

Coevaporation: Proper amounts of MN and βCD or HPβCD with guest-host molar ratio of 1:1 or 1:2 were dissolved in minimal volume of 50% alcoholic solution at 55°C using a stirrer equipped with heater. The resulting solution was further stirred for 1 h and then the solvent was evaporated in an oven at 45°C overnight to give dry powder. The obtained powder was sieved through 355 µm sieve and then mixed thoroughly in a polyethylene bag.

Assay of MN Content: Powder amount equivalent to 50 mg of MN from each binary mixture was transferred into 100 ml volumetric flask and the volume was completed with the HPLC mobile phase. The mixture was sonicated until complete drug dissolution. A filtered sample through 0.45 µm membrane filter was assayed by HPLC for MN content.

Inclusion Complex Investigation:

Percent MN complex inclusion by chloroform extraction: Preliminary studies showed that MN is freely soluble in chloroform, while MN-CD complexes are insoluble in the same solvent. These studies were based on the hydrophilic exterior of CDs, which would make CD insoluble in chloroform. FTIR result for dried chloroform extract of coevaporated Mn-CD (Figure 2) shows that the

FTIR spectrum of the dried extract completely matches that of pure MN, indicating that only free MN was extracted. This result supports the hypothesis of insolubility of CD in chloroform. In addition, the physical mixtures showed almost 100% extraction, which indicates that the drug is freely soluble in chloroform.

This difference in solubility was utilized for measuring the % MN complex inclusion. Powder amount equivalent to 50 mg of MN from each binary system was transferred into 100 ml volumetric flask and the volume was completed with chloroform. The mixture was sonicated for 5 min and the resulted dispersion was filtered through 0.45 µm membrane filter. The obtained solution was suitably diluted with methanol and assayed for free MN using HPLC. The % MN complex inclusion was calculated as the difference between total (free and complexed) MN obtained from drug assay and free MN obtained from chloroform extraction.

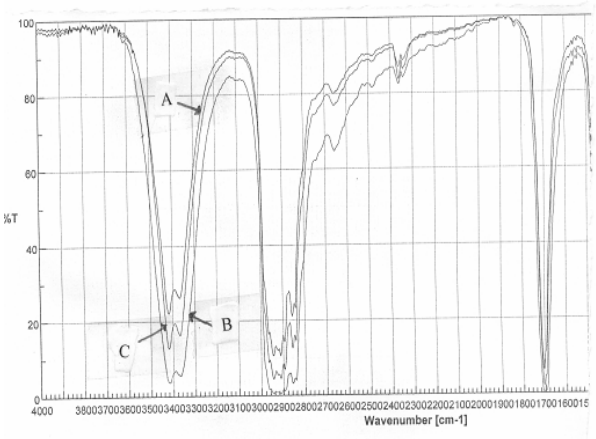


Figure 2: FTIR spectra of MN (A) and dried chloroform extracts of coevaprated MN-βCD: 1: 1 (B) and 1: 2 (C).

Differential Scanning Calorimetry (DSC): The DSC patterns were determined by thermal analyzer (Mettler TC 11, DSC 20, TA processor, Mettler instrument AG, Switzerland). Each sample (10 mg of powder in closed aluminum crucibles) was heated at a rate of 10°C/min from ambient temperature to 250°C under nitrogen supply at flow rate of 50 ml/min.

Fourier Transformation –Infrared (FTIR) Spectroscopy: The samples subjected for DSC analysis were also subjected for IR absorption spectroscopy using Nicolet Magna-IR® (USA) according to the KBr disk method .

X-ray Powder Diffraction: The X-ray diffractograms were carried out with X-ray diffractometer (X'Pert MPD, Philips, Netherlands). Operating conditions were: Cobalt radiation; angle range of (2-40) 2θ, tension of 40 kV and current of 40 mA.

Dissolution Studies: The dissolution studies were performed using type II dissolution apparatus (Pharma test, type: PTWS II, Germany). The dissolution tests were run in 600 ml distilled water at 37°C and a rotation speed of 100 rpm for 60 min. MN raw material (50 mg) or powder quantity of each binary system equivalent to 50 mg of MN was filled into a hard gelatin capsule and then the capsule was fitted with sinkers to avoid floating during dissolution. At 15, 30, 45, and 60 min, 10 ml samples were withdrawn, filtered through a 0.45 µm membrane filter and the filtrate was analyzed for % MN dissolved by HPLC. The initial volume of the vessel was maintained by adding 10 ml of the dissolution medium after each sampling.

Results and Discussion

Validation of the HPLC Method: The % RSD for the system suitability was less than 1% for the assay and dissolution method, which complies with the USP acceptance criteria that states the % RSD should not be more than 2 %. The plot of AUC versus concentration showed R-squared of more than 0.99 which indicates a good linearity. The detection limits were calculated as 5.65 µg/ml and 0.54 µg/ml for assay method and dissolution method, respectively. The quantitaition limits were calculated as 17.12 µg/ml and 1.64 µg/ml for assay and dissolution method, respectively. These limits are acceptable, since in case of dissolution this means lower than 1% drug release can be detected. The RSD for precison for the assay and dissolution was less than 2%. The recovery results with each studied excepient showed % RSD less than 2%. The specifity results (obtained chomatograms of the drug and excepients) showed no interference of the excipients with the mesterolone principal peak for both assay and dissolution method.

Phase Solubility Diagrams: Figures (3 & 4) show the phase solubility diagram obtained for MN with βCD, and HPβCD, respectively. The solubility of MN increased as a function of βCD concentration until a concentration of 0.27 mM. Beyond this point, further addition of βCD did not lead to a significant increase in MN solubility. On the other hand, the solubility of MN increased linearly with the increase of HPβCD concentration over the whole range of the used concentrations. These results could be explained as due to the limited water solubility of 1.85 gm/100 ml for βCD and the high water solubility of >50 gm/100 ml for HPβCD. [2] According to the Higuchi and Connors method, [9] B_L-type curve was observed with βCD and A_L-type was obtained with HPβCD. An apparent stability constant (K_s) with the assumption of 1:1 stoichiometry was calculated using simple linear regression of the initial linear portion of the curve obtained with βCD or the whole line obtained with HPβCD and the apparent water solubility of MN (S_0) according to the following equation:

$$K_s = \frac{\text{slope}}{S_0\,(1\text{-slope})}$$

K_s was found to be 5.39 $\times 10^4$ M^{-1} for MN-βCD and 2.63 $\times 10^4$ M^{-1} for MN-HPβCD, which indicates higher MN affinity for βCD than for HPβCD. These results could reflect a steric hindrance of the hydroxypropyl groups of HPβCD, which hamper the inclusion of guest molecules within CD cavity. [12]

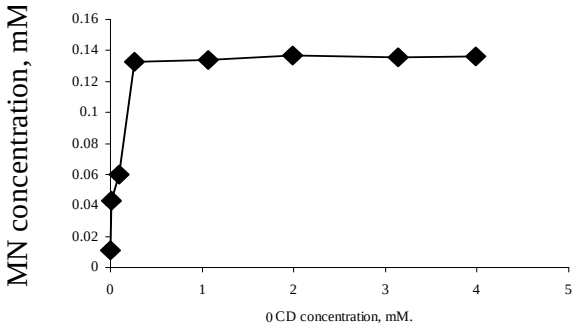


Figure 3: Phase solubility diagram of MN-βCD system. Each data point is the mean of two determinations.

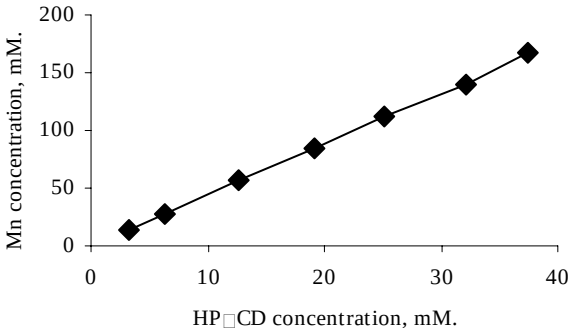


Figure 4: Phase solubility diagram of MN-HPβCD system. Each data point is the mean of two determinations.

Inclusion Complexes Investigation: MN content assay and % MN complex inclusion by chloroform extraction MN content in the binary mixtures, % MN extracted with chloroform from the mixtures and the calculated % MN complex inclusion are listed in Table (1). All the assay results were in the range of 97-106%, which indicates acceptable drug content. Since the chloroform extractable MN is only the free form, the higher the MN amount that was extracted in chloroform, the lower the MN complex inclusion. MN complex inclusion in the kneaded and coevaporated mixtures was dependent on CD type and molar ratio with an interaction between the two parameters. With βCD, % MN inclusion at 1: 2 molar ratio was 1.7 times and 2.0 times that obtained at 1: 1 molar ratio using kneading and coevaporation, respectively. On the other hand and with HPβCD, the effect of molar ratio was not significant with kneading (< 0.5%) and slight with coevaporation, which is represented by 1.3 fold increase in MN inclusion with doubling the MN: CD molar ratio. The higher increase in MN inclusion in βCD than in HPβCD with doubling the MN: CD molar ratio is consistent with the higher K_s for MN-βCD than for MN- HPβCD as obtained from the phase solubility diagrams. Slight differences between the kneaded mixtures and the coevaporated mixtures can be concluded, which can be mostly represented for HPβCD at 1: 2 molar ratio as 12 %. Other differences were less than 7%. Both CDs showed similar MN inclusion with kneading and coevaporation at 1:1 molar ratio, as the differences were less than 8%. However, at 1: 2 molar ratio, higher MN inclusion was obtained with βCD

than with HPβCD by 33% and 23% for kneading and coevaporation, respectively. This interaction effect between CD type and molar ratio could also be considered a reflectance of the higher K_s obtained for MN-βCD than for MN-HPβCD.

Table 1: MN assay and % MN complex inclusion by chloroform extraction for MN- βCD and MN-HPβCD binary systems prepared by physical mixing, kneading and coevaporation at MN: CD molar ratios of 1: 1 and 1: 2.

Preparation method	*MN assayed (%)*		*MN extracted via chloroform (%)*		*Calculated MN complex Inclusion (%)*	
(MN: CD molar ratio)	*βCD*	*HPβCD*	*βCD*	*HPβCD*	*βCD*	*HPβCD*
Kneading (1:1)	*97.3*	*101.4*	*49.1*	*50.6*	*48.2*	*50.8*
Kneading (1: 2)	*98.7*	*101.3*	*15.1*	*50.2*	*83.6*	*51.1*
Coevaporation (1:1)	*104.7*	*101.1*	*62.7*	*52.1*	*42.0*	*49.0*
Coevaporation (1:2)	*106.4*	*100.5*	*20.5*	*37.4*	*85.9*	*63.1*

Differential Scanning Calorimetry (DSC): To investigate for the effect of the process of CD preparation on possible polymorphic transformation of MN alone, kneading and coevaporation were done on MN alone and the products were run for DSC and compared to that of MN untreated (Figure 5). The treated and untreated MN showed the same melting behavior with peak temperatures of 207.0, 208.8 and 206.9 for the untreated, kneaded and evaporated MN, respectively. Accordingly, it can be concluded that process (kneading or evaporation) did not lead to polymorphic transformation of MN in the absence of CD. The DSC results for the pure components (MN, βCD and HPβCD), and the corresponding binary systems are presented in Figures (6 & 7). MN showed an endothermic melting peak at 209°C. Broad endothermic peaks were obtained between 30°C and 130°C for both βCD and HPβCD, which can be attributed to loss of moisture. The thermograms for the physical mixtures contained the peaks obtained for the pure components. However, MN-HPβCD showed an extra exothermic peak after MN melting, which is possibly due to oxidative degradation. In comparison to the respective physical mixtures, there was partial or complete disappearance of MN melting peak upon kneading and coevaporation, which was dependent on CD type and MN: CD molar ratio. The coevaporated and kneaded MN-βCD systems did not show the melting peak of MN at 1:2 molar ratio, which indicates the loss of MN crystallinity as a result of inclusion complexation. On the other hand and at 1:1 molar ratio, partial complexation can be concluded, which was higher for coevaporation than for kneading, as it can be seen from the decrease in peak magnitude of MN melting.

The powders obtained by coevaporation with βCD showed an endothermic peak at 150°C, which was not obtained for the physical mixtures. This peak was investigated by thermogravimetric analysis (Figure 8), which showed loss of weight at that peak temperature probably as a result of desolvation. No complete disappearance of MN melting peak can be seen in the thermograms of the kneaded and coevaporated MN-HPβCD products, indicating that a true inclusion complex had not formed. However, partial disappearance of MN melting peak was apparent, which was in descending order of coevaporation (1:2), kneading (1:2), coevaporation (1:1), and then kneading (1:1). Accordingly, higher MN complexation was obtained with HPβCD at 1:2 than at 1:1 molar ratio and with coevaporation than with kneading at both molar ratios. By comparing the thermograms of the kneaded and coevaporated MN-βCD mixtures with those of MN-HPβCD mixtures using the magnitude of MN endothermic peak, higher MN complexation with βCD than with HPβCD can be concluded for each preparation method and at each molar ratio.

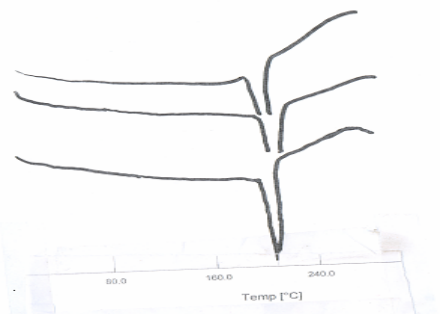


Figure 5: DSC of Kneaded and evaporated MN in the absence of CD.

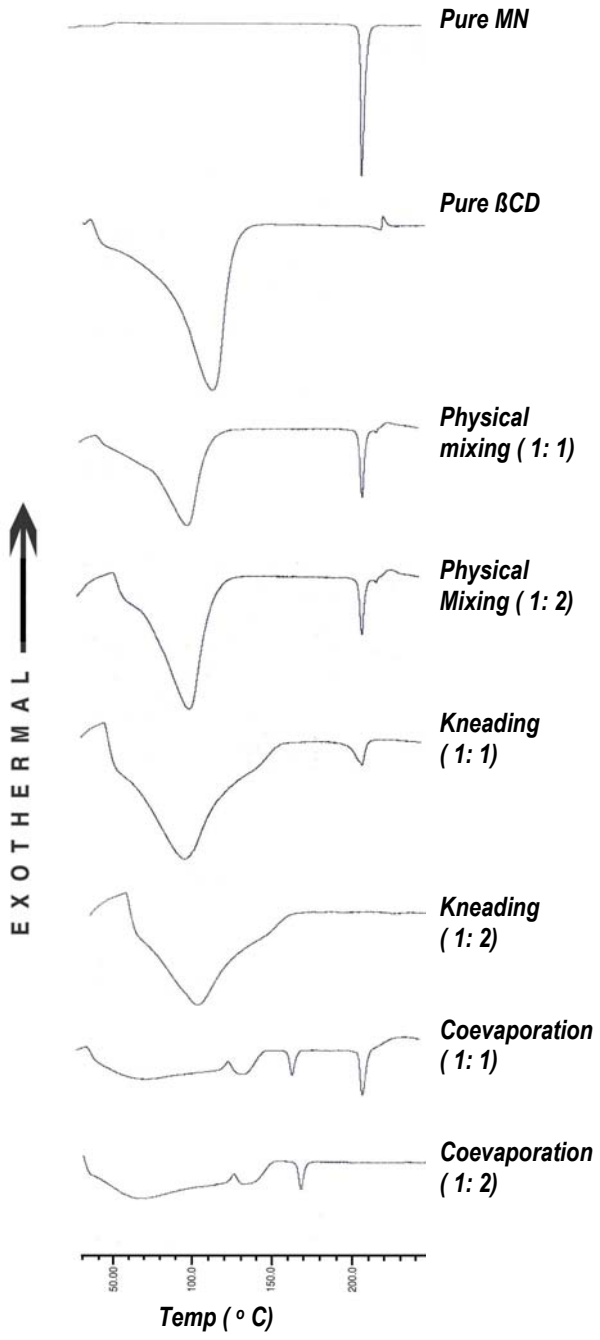


Figure 6: DSC thermograms of pure MN, pure βCD, and MN-βCD binary systems prepared by physical mixing, kneading, and coevaporation at MN-CD molar ratios of 1: 1 and 1: 2.

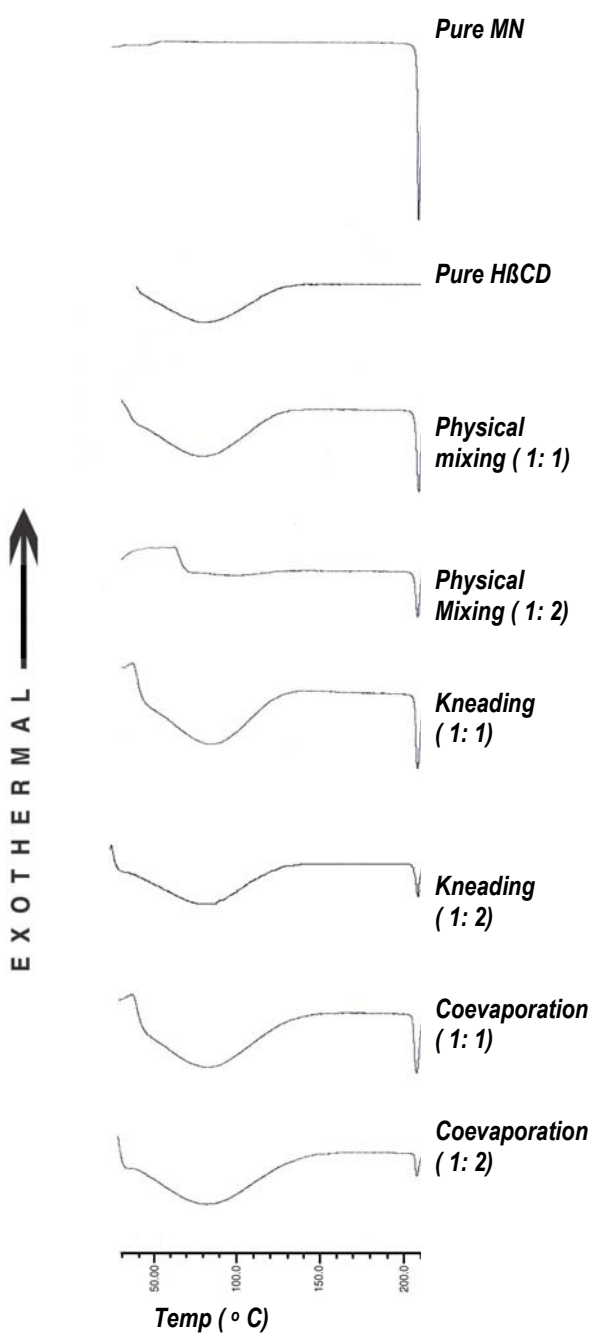


Figure 7: DSC thermograms of pure MN, pure HPβCD, and MN-HPβCD binary systems prepared by physical mixing, kneading, and coevaporation at MN: CD molar ratios of 1: 1 and 1: 2.

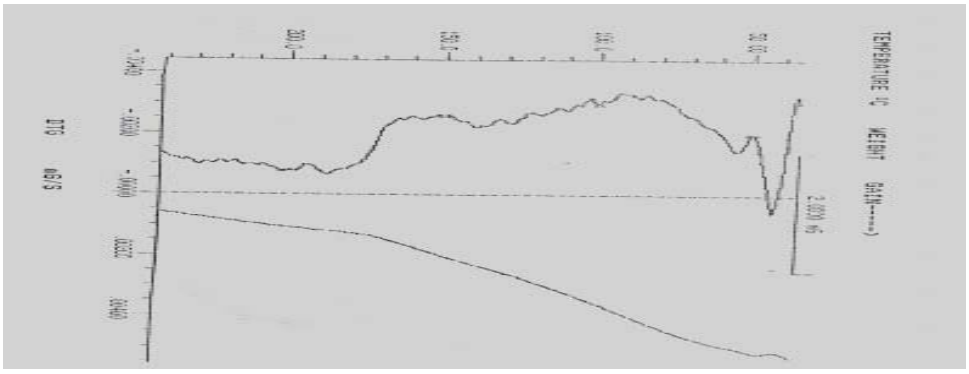


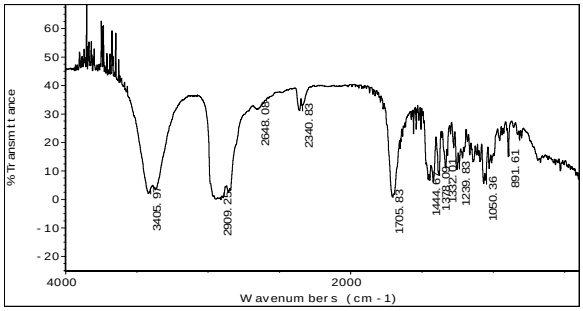
Figure 8: TGA analysis of coevaporated MN- βCD.

Fourier Transformation –Infrared (FTIR) Spectroscopy:

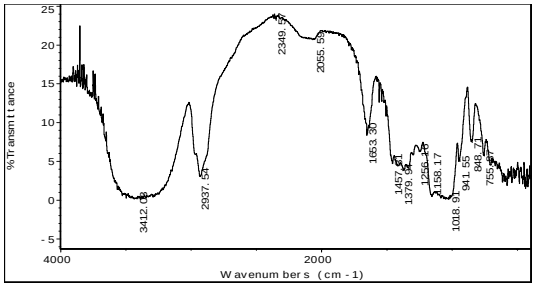
The FTIR spectra for MN, HPβCD, and MN-HPβCD binary systems are reported in (Figure 9). MN showed the following absorption peaks characteristic: OH stretching free and bonded, between 3350 cm^{-1} and 3430 cm^{-1}, C—H multiple stretching of aliphatic between 2830 cm^{-1} and 2950 cm^{-1}, C=O stretch at 1706 cm^{-1}, C—O bending at about 1070 cm^{-1} and 1250 cm^{-1}, C—H aliphatic bending between 1380 cm^{-1} and 1460 cm^{-1}. The IR spectra of HPβCD are characterized by a wide band between 3000-4000 cm^{-1}, which corresponds to absorption by hydrogen-bonded OH groups. The band corresponding to vibration of the -CH and $–CH_2$ groups appears at 2800-3000 cm^{-1}. Accordingly, both MN and HPβCD showed OH and C-H bands in the same region, however, the OH band was wider for HPβCD and the C-H band was wider for MN. Due to this overlaping, the physical mixtures showed only two peaks above 2800 cm^{-1} with an OH band narrower than that of HPβCD, but wider than that of MN. This overlaping makes it almost impossible to reveal any involvment of hydorgen bond formation inside CD cavity as a result of complexation. This is supported by following the reported ability of FTIR to show drug-CD complexation according to Wei-Qin. 2000.: "in most cases, no change due to complex foramtion can be observed. Bands due to the included part of the guest molecule do shift or their intensities are altered, but since the mass of the guest molecule do not exceed 5-15 percent of the mass of the complex, these alterations are usually obscurred by the host. Therefore, no usefull information can be obtained". However, comparison of the OH band for the kneeded and coevaporated mixture and their corresponding physical mixtures at each molar ratio versus that of HPβCD reveals that the intensity of the bands of the coevaporated and kneaded mixture have higher intensity than the physical mixtures. This is particularly true for the coevaporataed mixtures with wide OH bands similar to that of HPβCD. This could be explained by the fact that the more MN inclusion the more the FTIR spectrum will be determined by HPβCD with less impact of MN as it will be hidden inside the CD cavity. These comparisons could be taken as an evidence of complexation between MN and HPβCD. Similar FTIR results were obtained for MN-βCD.

X-ray Powder Diffraction: The X-ray powder diffractograms of the pure components and the binary systems are shown in Figures (10 & 11). The diffractograms of MN and βCD exhibited intense peaks, which is indicative of their crystalline character. The diffraction pattern of the MN-βCD physical mixture is simply the superposition of each component. The kneaded mixtures showed fewer peaks and lower intensity for the diffraction pattern than the respective physical mixtures. The coevaporated mixtures exhibited fewer peaks than the kneaded mixtures. Accordingly, loss of MN crystallinity upon kneading and coevaporation with βCD, which was higher for coevaporation than for kneading, can be concluded. HPβCD had no distinctive X-ray diffraction pattern due to its amorphic nature, which also seemed to make the x-ray diffraction of its physical mixtures with MN with few distinctive peaks mainly between 15 and 20 (2θ).

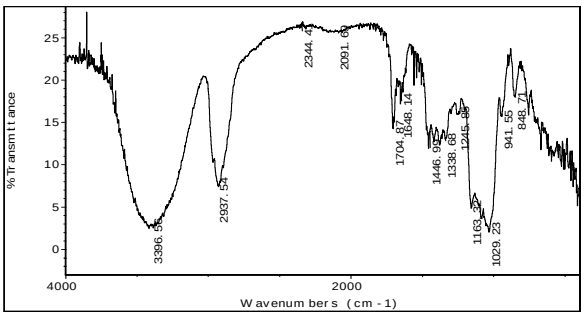
Figure 9: FTIR analysis of pure MN, pure HPβCD, and MN-HPβCD binary systems prepared by physical mixing, kneading, and coevaporation at MN: CD molar ratios of 1: 1 and 1: 2.



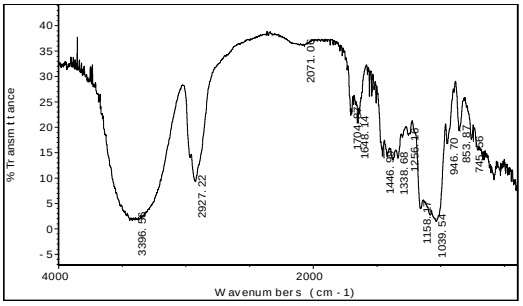
Hp-ß- CyD



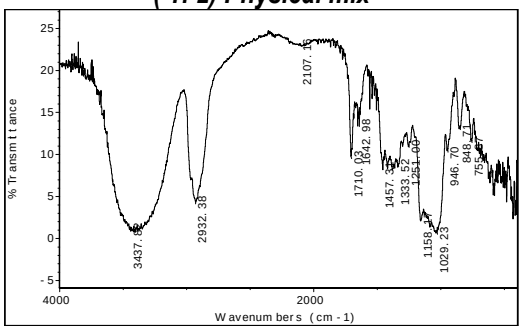
MN



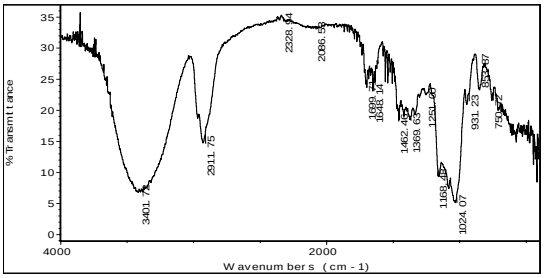
(1: 2) Physical mix



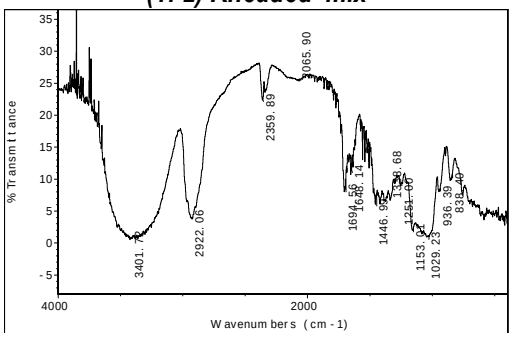
(1: 1) Physical mix



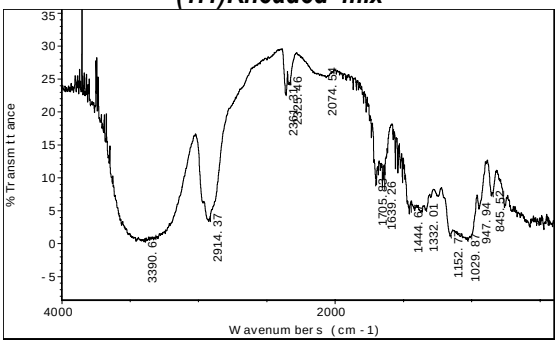
(1: 2) Kneaded mix



(1:1)Kneaded mix



(1: 2) Coevaporated mix



(1:1) Coevaporated mix

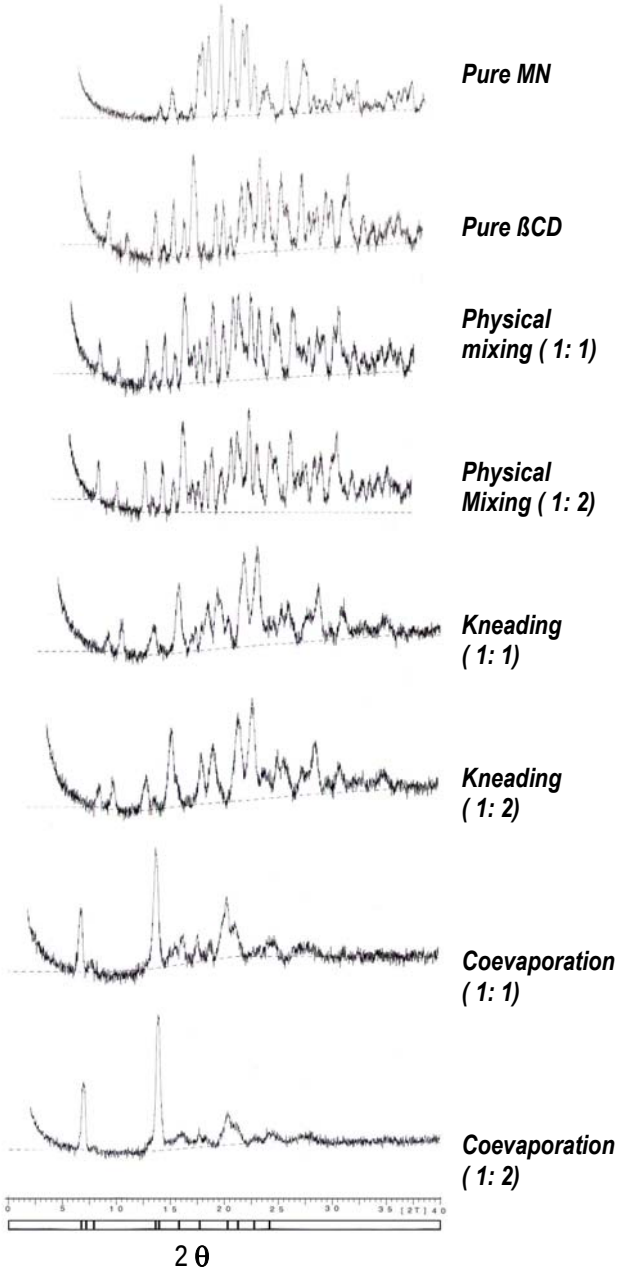


Figure 10: X-ray diffractograms of pure MN, pure βCD, and MN-βCD binary systems prepared by physical mixing, kneading, and coevaporation at MN: CD molar ratios of 1: 1 and 1: 2.

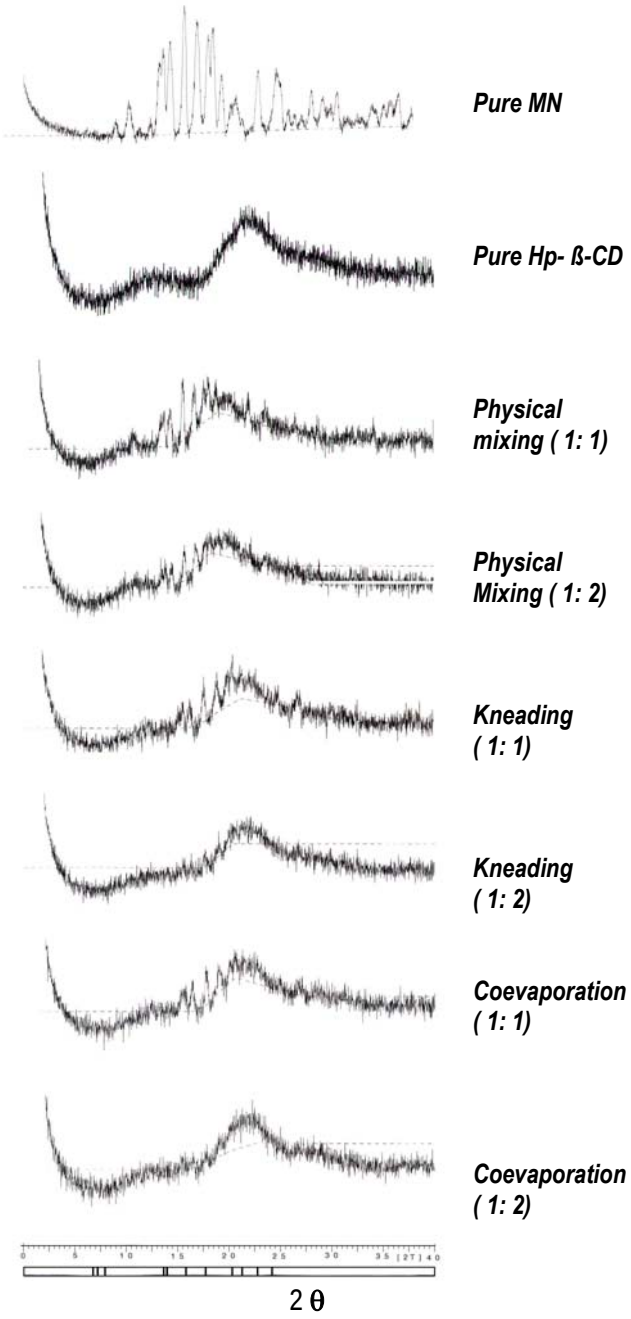


Figure 11: X-ray diffractograms of pure MN, pure HPβCD, and MN-HPβCD binary systems prepared by physical mixing, kneading, and coevaporation at MN: CD molar ratios of 1: 1 and 1: 2.

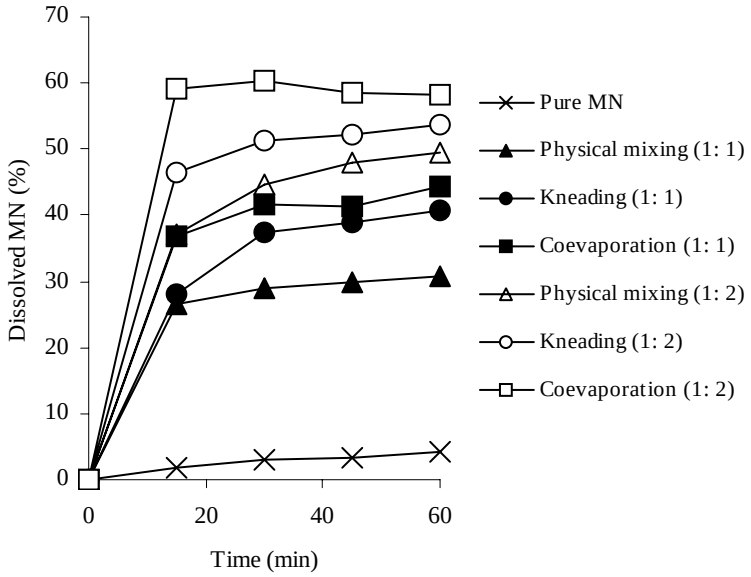


Figure 12: Dissolution profiles of pure MN, and MN-βCD binary systems prepared by physical mixing, kneading, and coevaporation at MN: CD molar ratios of 1: 1 and 1: 2. Each data point is the mean of three determinations with SD not more than 5%.

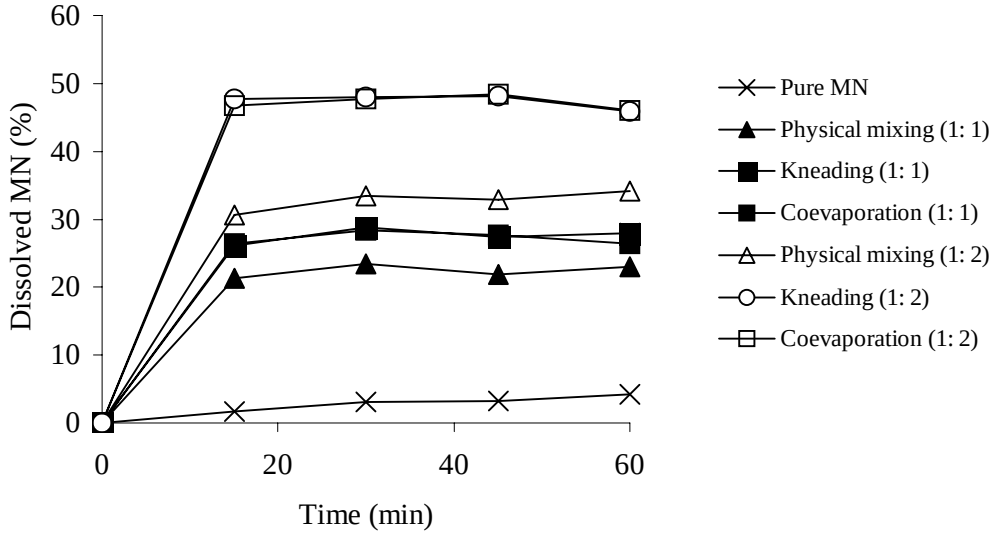


Figure 13: Dissolution profiles of pure MN, and MN-HPβCD binary systems prepared by physical mixing, kneading, and coevaporation at MN: CD molar ratios of 1: 1 and 1: 2. Each data point is the mean of three determinations with SD not more than 5%.

This effect of amorphic nature of HPβCD made the X-ray diffractometry less powerful in investigating amorphic transformation of MN upon kneading and coevaporation with HPβCD. However, compared to physical mixing, MN amorphization can be seen upon kneading and coevaporation as reduction in the intensity and disappearance of the peaks between 15 and 20 (2θ) at 1: 1 and 1: 2 molar ratio, respectively. However, no differences were apparent between the kneaded mixture and coevaporated mixture at each molar ratio.

Dissolution Studies: The dissolution profiles of pure MN and the binary systems are illustrated in Figures 12 and 13. The mean values of % MN dissolved at 60 min from the binary systems are listed in Table (2). MN-CD physical mixtures exhibited much higher drug dissolution than the pure drug; increases of 6 to 12 folds in % MN dissolved at 60 min were observed. This enhancement in drug dissolution could be attributed to a local solubilization action operating in the microenvironment or the hydrodynamic layer surrounding the drug particles in the early stages of the dissolution process as CD dissolves in a short time. This improves the wetability and hence dissolution of the drug particles. [8] It is worth mentioning that the drug release was leveled off after 15 minutes. The constant drug release after 15 minutes would be due to saturation of the medium with the drug i.e. when the drug concentration exceeded the solubilizing power of CD. Accordingly, CD should provide higher drug release by providing higher sink condition as a result of higher drug solubilizing power. It can be seen from the figures that there is a remarkable effect for MN: CD molar ratio on MN dissolution. For all the binary systems, MN dissolution was higher at 1: 2 molar ratio than at 1: 1 molar ratio. The ratio of % MN dissolved at 60 min with 1: 2 molar ratio to that with 1: 1 molar ratio was 1.5, 1.3 and 1.3 in case of βCD, and 1.5, 1.6, and 1.8 in case of HPβCD for the physical mixture, kneaded mixture and coevaporated mixture, respectively. There was also an effect for the molar ratio on the differences in MN dissolution between the physical mixtures on one hand and the kneaded and coevaporated mixtures on the other. Higher MN dissolution from the physical mixtures at 1:2 molar ratio than from kneaded or coevaporated mixtures at 1:1 molar ratio is apparent regardless the CD type. This suggests a stronger effect of more local drug solubilization with higher CD amount in the physical mixture than the effect of complexation due to kneading or coevaporation at lower CD amount. At the same molar ratio and for both CDs, MN dissolution was higher for the kneaded and coevaporated mixtures than for the physical mixtures, which could be attributed to loss of MN crystallinity upon kneading and coevaporation as shown in the DSC and X-ray diffraction results. At each molar ratio, higher MN dissolution was obtained for coevaporation than for kneading in case of βCD and almost the same dissolution extent was obtained for the two methods in case of HPβCD. If linked to loss of MN crystallinity, this comparison between kneading and coevaporation is in agreement with the X-ray diffraction discussion. By comparing the values in Table (2) for the effect of CD type, higher MN dissolution from MN-βCD mixtures than from MN-HPβCD mixtures is apparent regardless the molar ratio or preparation method. This comparison is in agreement with the higher K_s for MN with βCD than with HPβCD as obtained from the phase solubility diagrams, with the DSC results and with the % drug inclusion obtained from chloroform extraction at 1:2 molar ratio. Multiple regression analysis was performed for % MN dissolved at 60 min versus molar ratio, CD type, preparation method and their interaction terms. The results of the regression analysis (Table 3) indicate significant effect for the three variables at α = 0.05 with no interaction. The effect was the strongest for the molar ratio (P = 0.0001) and slight for the preparation method (P = 0.0272) and CD type (P = 0.0265).

Table 2: Percentages of MN* dissolved at 60 min for MN-βCD and MN-HPβCD binary systems prepared by physical mixing, kneading, and coevaporation at MN: CD molar ratios of 1: 1 and 1: 2.

Preparation method (MN: CD molar ratio)	Average MN dissolved at 60 Min (mean ± SD)	
	BCD	HPβCD
Physical mixing (1:1)	30.9±0.42	23.06±0.12
Physical mixing (1: 2)	49.48±0.84	34.10±0.47
Kneading (1:1)	40.7±0.29	28.02±0.73
Kneading (1: 2)	53.63±0.65	45.82±0.96
Coevaporation (1:1)	44.47±0.32	26.39±2.88
Coevaporation (1:2)	58.23±4.24	46.03±1.64

* *The value for pure MN was 4.2±0.38.*

Table 3: Regression analysis for % MN dissolved at 60 min versus CD type, preparation method, and MN: CD molar ratio.

Parameter	Coefficient	Standard error	t Stat	P-value
Intercept	32.87	7.34	4.48	0.0001
Preparation method (P)	5.90	2.55	2.33	0.0272
CD type (T)	-17.59	7.53	-2.34	0.0265
Molar ratio (M)	14.97	3.24	4.62	0.0001
P × T	-1.77	1.17	-1.51	0.1417
P × M	0.95	1.17	0.81	0.4257
T × M	-1.77	3.99	-0.44	0.6617

In conclusion, MN was found to form inclusion complex in solution with βCD and HPβCD with higher Ks for MN-βCD than for MN-HPβCD. DSC analysis showed that upon kneading and coevaporation, MN could make true inclusion complex with βCD at MN: CD molar ratio of 1: 2. True MN- HPβCD inclusion complex could not be obtained upon kneading or coevaporation at MN: CD molar ratios of 1: 1 and 1: 2. The X-ray diffractometry showed a high loss of MN crystallinity upon kneading and coevaporation with βCD, but was less powerful in detecting polymorphic changes of MN upon kneading and coevaporation with HPβCD, which could be attributed to the amorphic character of HPβCD.

Dissolution enhancement of MN with CD could be attributed to local drug solubilization in the dissolution medium attained by physical mixing, drug inclusion and loss of drug crystallinity upon kneading and coevaporation. These mechanisms were mostly affected by MN: CD molar ratio making the effect of local drug solubilization to dominate the other mechanisms with increasing the molar ratio. The effect of drug inclusion and loss of drug crystallinity was shown at the same molar ratio and was higher for βCD than for HPβCD.

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Improvement of the Solubility and Dissolution Rate of the Steroidal Drug, Mesterolone, using Cyclodextrin Complexation

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ملخص:

تم دراسة تكوين معقدات بين الهرمون السستروديدي مسترولون (MN) والهيدروكس– بيتا–سيكلوديكسترين(HPβCD) او بيتا– سيكلوديكسترين (βCD) في الحالة الصلبة والسائلة. تم الحصول على منحنيات الدائبية وقسمت إلى نوع A_L ل βCDو نوع B_L ل HPβCD ووجد أن معامل الثبات أعلى ل βCD منة ل HPβCD. تم تحضير خلائط ثنائية ما بين MN و كل سيكلوديكسترين (CD) بواسطة الخلط الفيزيائي والعجن والتبخير المتزامن على نسب مولية من : MN:CD: 1:1 و 1:2 . تم فحص الخلائط لنسبة MN المحو صل عن طريق الاستخلاص بواسطة الكلوروفورم. كما تم تحليل الخلائط بواسطة مسح السعرات الفرقي (DSC) و انعكاس أشعة اكس (XRD).

وجد أن نسبة التحوصل يعتمد على النسبة المولية وان الاعتماد كان بدرجة اكبر ل βCD منة من HPβCD لكل من العجن والتبخير المتزامن.

نتائج أل DSC أظهرت تحوصل أل MN وفقدان لتبلوره والتي كانت أعلى على نسبة مولية 1:1 من 1:2 و ل βCD من HPβCD على كل نسبة المولية. نتائج XRD قدمت دليلا أخر لفقدان التبلور بالأخص ل βCD. تم أخيرا تعريض الخلائط لدراسات التحرر الدوائي في الماء ووجد أن هنالك زيادة في التحرر الدوائي للخلائط مقارنتا مع الدواء وحدة وتم تفسير دلك للادابة المحلية خلال الانحلال بواسطة أل CD و تحوصل الدواء داخل أل CD و فقدان التبلور للدواء. ووجد أيضا أن الزيادة في الانحلال تعتمد بشكل كبير على النسبة المولية مع وجود اثر ل نوع CD و طريقة التحضير.