Study of Tolbutamide–Hydroxypropyl- γ -cyclodextrin Interaction in Solution and Solid State

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Tolbutamide–hydroxypropyl- γ -cyclodextrin (TBM–HPGCD) interaction has been investigated in an aqueous environment and in the solid state. The solubility of TBM was increased in accord with the amount of HPGCD added to the aqueous medium forming a soluble inclusion compound. The phase solubility diagram obtained was of A_L type. Physical mixtures and kneaded systems of the drug and cyclodextrin derivative were prepared in 1 : 1 and 1 : 2 drug/cyclodextrin mol/mol ratio. All solid binary systems were characterised by hot-stage microscopy (HSM), differential scanning calorimetry (DSC), thermogravimetry (TG) and X-ray powder diffractometry (XRD). An inclusion complex was formed in both of the kneaded systems. In the 1 : 2 kneaded system, the entire drug was included in the cyclodextrin cavity, while, in the 1 : 1 kneaded system only a part of the drug formed an inclusion complex with the cyclodextrin. A significant improvement in the dissolution of the drug was obtained from the kneaded systems in comparison with that of the pure TBM and physical mixtures. However, there was no significant difference between the dissolution profiles of the two kneaded systems. The study suggests that an inclusion complex was obtained both in aqueous solution and in solid state.

Key words tolbutamide; hydroxypropyl- γ -cyclodextrin; solubility; inclusion complex; dissolution; characterisation tolbutamide–hydroxypropyl- γ -cyclodextrin inclusion compound in solid state

Hydroxypropyl-cyclodextrins have found considerable interest as parenteral drug carriers because of their low toxicity and high tolerance. Their excellent solubilizing capacity enables poorly water-soluble drugs to dissolve fully and rapidly in water and in consequence improves the bioavailability significantly. Among the hydroxypropyl-cyclodextrins, hydroxypropyl- β -cyclodextrin (HPBCD) has been studied extensively.^{1–3)} However, hydroxypropyl- γ -cyclodextrin (HPGCD) has been studied to a lesser extent, although its parent compound γ -cyclodextrin (GCD) is a potent solubilizer. Because of its large cavity size, GCD can effectively encapsulate bulky drug molecules such as prostaglandins, steroid hormones, and benzodiazepines.⁴⁾ Like GCD, which has been used as bonding materials of the stationary phase of chiral chromatography, especially to separate enantiomers containing three benzene rings,⁵⁾ HPGCD has been used as a chiral resolving agent in enantioseparation by capillary electrophoresis. In a recent study, 30 racemic drugs were separated by electrophoresis using HPGCD as a chiral resolving agent.⁶⁾ As HPGCD is much more soluble than its parent molecule, it has also been found promising in enhancing solubility and dissolution of poorly water soluble drugs. These properties of HPGCD have been exploited by several authors. The poor aqueous solubility of carbamazepine, an anti-convulsant agent, was dramatically increased *via* complexation with HPGCD.⁷⁾ An enhanced dissolution of spironolactone was obtained from its inclusion complex with HPGCD prepared by spray drying.⁸⁾ However, studies about the interaction of this cyclodextrin derivative with poorly watersoluble drugs, especially in the solid state, have not been extensive in nature.

Tolbutamide (TBM) is well known for its inclusion compound with cyclodextrins. Its molecular geometry allows it to form inclusion compound with β -cyclodextrin (BCD) and HPBCD.⁹⁾ The drug forms a 1 : 2 inclusion compound with BCD¹⁰⁾ and a 1 : 1 inclusion compound with HPBCD.⁹⁾ The interactions of TBM with BCD and HPBCD, both in solution

and solid state, were studied by several authors.^{9–11)}

In this paper, TBM was selected as a substrate to study its interaction with HPGCD in solution and in the solid state. Hot stage microscopy (HSM), differential scanning calorimetry (DSC) and X-ray powder diffractometry (XRD) were employed in studying the interaction between TBM and HPGCD in the solid state.

Experimental

Materials TBM, 1-butyl-3-*p*-tolylsulphonylurea, was purchased from Sigma Chemicals Co. (St. Luis, MO, U.S.A.) and 2-hydroxypropyl- γ -cyclodextrin (HPGCD), with a substitution degree of 0.6, was a gift from Wacker-Chemie GmbH (München, Germany).

Phase Solubility Study A phase solubility study was performed according to the method reported by Higuchi and Connors.¹²⁾ An excess (20 mg) of TBM was weighed out into a series of test tubes. A constant volume of demineralized water or aqueous solutions of cyclodextrin containing increasing concentration of HPGCD (0.002–0.02 M) were added to each test tube. Test tubes were then closed and placed in an oscillating water bath (15 oscillation per minute) and solutions were brought to solubility equilibrium at room temperature (25 °C), with constant shaking for 5 d. After attainment of equilibrium, the contents of the test tubes were filtered through Whatman filter paper (type 42). The drug concentration in the filtered solutions was determined from the absorbance at 228 nm using a Beckman DU-6 spectrophotometer and three replicates were made for each assay. To nullify the absorbance due to the presence of the cyclodextrin, each time the apparatus was calibrated with the corresponding blank.

Preparation of Binary Systems Two types of binary systems were prepared: physical mixtures and kneaded systems. In both physical mixtures and kneaded systems, drug–HPGCD ratios were 1 : 1 (14.64% w/w of TBM and 85.36% w/w of HPGCD) and 1 : 2 mol/mol (7.32% w/w of TBM and 92.68% w/w of HPGCD). Physical mixtures were prepared by homogeneous intermingling of previously sieved and weighed drug and cyclodextrin in a suitable container.

Kneaded systems were prepared from physical mixtures by adding a small volume of distilled water. After moistening, the resultant systems were kneaded to produce a homogeneous dispersion. Once a homogeneous slurry was obtained, samples were dried at 40 °C for 24 h; all of the dried systems were crushed and sieved, and fractions smaller than 100 μ m were collected for further study. Pure TBM and HPGCD have also been kneaded in the same manner to reveal if the kneading method has any influence on the physical characteristics of the pure substances.

Drug concentration in physical mixtures and kneaded systems was deter-

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mined by UV spectrometry (228 nm), to confirm that the drug–HPGCD ratios were not changed during the treatment.

HSM Analysis About 1 mg of sample was placed on a microscopic slide with cover and heated at a rate of 2 °C/min on a Kofler stage and samples were carried out by using a Thermogalen microscope fitted with the Kofler stage.

DSC Analysis DSC curves of pure materials (kneaded and without any treatment) and all binary systems were recorded on a Mettler TA 3000 differential scanning calorimeter (model DSC 20). About 5–10 mg of sample was placed in a pinholed aluminum sample pan with lid and heated at a rate of 10 °C/min between 30 and 350 °C. The instrument was periodically calibrated with a standard sample of indium.

Thermogravimetry (TG) Analysis TG curves were obtained by using a Mettler TA 3000 thermogravimetric analyzer (TG 50). Samples (10 mg) were placed in a crucible of alumina and heated at a rate of 10 °C/min between 30 and 600 °C.

XRD Analysis The powder X-ray diffraction patterns of pure ingredients, their kneaded counterparts, and all binary systems were recorded by using an automated Philips X'Pert X-ray diffractometer. Samples were irradiated with monochromatized CuK α radiation and analysed between 2 θ angles of 5 and 40°. The voltage, current, and time per step used were 40 kV, 55 mA, and 1 s, respectively.

Dissolution Study A Sotax AT-7 dissolution apparatus with paddles was employed to carry out all of the tests. The volume of the dissolution medium, experimental temperature, and paddle speed were 1000 ml, 37 $\pm$ 0.1 °C, and 75 rpm, respectively. Previously powdered and sieved (particle size lower than 100 μ m) samples (250 mg of TBM or equivalent amount of binary systems) were used for all dissolution studies. All of the systems were assayed in demineralized water and the duration of assay was 3 h, the samples were withdrawn at measured time interval and filtered with a Whatman filter paper (type 42). Dissolved drug was assayed at a wavelength of 228 nm in a Beckman DU-6 spectrophotometer and three replicates of each dissolution assay were carried out.

Results and Discussion

Phase Solubility Study The phase solubility diagram (Fig. 1) indicates that the drug solubility increases linearly in accord with the amount of cyclodextrin added. It seems that in the range of concentration studied, at least, a soluble TBM–HPGCD was formed giving rise to a phase solubility diagram of A_L type.¹²⁾ The apparent stability constant of the inclusion complex can be calculated from the slope and intercept of the linear phase solubility diagram by using the equation.¹³⁾

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

The stability constant (K_s) obtained was 38.12 M⁻¹. The value for the stability constant was lower in comparison with that obtained for the TBM–HPBCD inclusion complex (144.8 M⁻¹).⁹⁾ This lower value of stability constant indicates that although HPGCD can form an inclusion complex with TBM, the complex between HPBCD and TBM is more stable. This maybe due to the larger cavity size of HPGCD and the binding forces between the guest and host are not sufficient to hold firmly the TBM molecule in the cavity of the cyclodextrin.

HSM Analysis The examinations of both pure TBM and its kneaded counterpart revealed that the samples were constituted of laminar crystalline particles and both of the samples appeared as coloured particles even without polarised light. No change in the particle aspect was observed until the melting of the substance (115–118 °C); the melting process was followed by the decomposition of the material. Under microscopic view, the sample of pure HPGCD was constituted of spherical particles, while all the particles of the

Fig. 1. TBM–HPGCD Phase Solubility Diagram

Each point is the average of three measurements; error bars indicate $\pm$ standard deviation.

kneaded HPGCD sample were of irregular shape. The thermal behaviour of both HPGCD (pure and kneaded) was similar. Upon heating, melting was the first change detected (250–255 °C). Immediately after the fusion, decomposition of the melted particles begins, which was detected from the darkening of the droplets.

In the physical mixtures (1:1 and 1:2), TBM and HPGCD particles were easily distinguishable from each other. The drug melted in the range of 115–120 °C; afterwards, the melted mass of the drug mixed with the HPGCD particles and a partial dissolution of the cyclodextrin in the melted TBM was observed. The decomposition of melted TBM was not observed. The melting of undissolved HPGCD particles begins at 220 °C. The completion of the fusion of the system was observed at 250 °C and this process was followed by the decomposition of the system.

The 1:1 kneaded system sample consisted of irregular particles. In polarised light some coloured particles, similar to that of pure TBM, were observed and upon heating, melting of the coloured particles took place at 125 °C. This melt mixed with the rest of the particles of the system. The sample melted completely in the range 220–250 °C and immediately afterwards, the decomposition of the system started (darkening of the droplets). The 1:2 kneaded system appeared as a homogeneous sample formed by particles of irregular shape. In this system, no isolated particles of TBM were observed as in the 1:1 kneaded system. Unlike the sample of the 1:1 kneaded system, the entire sample of the 1:2 kneaded system melted in the range of 220–250 °C without any prior transition. After the sample melted it decomposed.

DSC Analysis The analysis of pure and kneaded TBM by DSC gives rise to a curve of three endotherms. The first endotherm is for melting of the drug and the remaining two could indicate the decomposition of the melted drug (Fig. 2, Graphs A and B); this behaviour is in accord with the HSM observation of the drug. DSC traces of pure and kneaded HPGCD consists of two peaks: a dehydration endotherm and a decomposition endotherm (Fig. 2, Graphs C and D). The melting process of this cyclodextrin derivative was not detected by DSC. Because of its amorphous nature, the transition for fusion takes place with the absorption of a lower

Fig. 2. DSC Curves of TBM and 2-HPGCD

(A) Pure TBM, (B) kneaded TBM, (C) pure HPGCD and (D) kneaded HPGCD.

Table 1. Thermal Data from DSC Analysis of pure TBM and HPGCD and Their kneaded Counterparts

Transition	Pure TBM	Kneaded TBM	HPGCD	Kneaded HPGCD
Dehydration	—	—	76 ^{a)} 91 ^{b)}	93 ^{a)} 157 ^{b)}
Melting	126 ^{a)} 86 ^{b)}	125 ^{a)} 87 ^{b)}	—	—
Decomposition	251 ^{a)} , 318 ^{a)} 249 ^{b)} , 109 ^{b)}	246 ^{a)} , 315 ^{a)} 313 ^{b)} , 114 ^{b)}	308 ^{a)} 193 ^{b)}	316 ^{a)} 293 ^{b)}

a) Peak temperature (°C). b) Enthalpy (J g⁻¹).

amount of energy, as it was observed with other cyclodextrins of amorphous nature.¹⁴⁾ The thermal data from the DSC analysis of TBM and HPGCD, and their kneaded counterparts, presented in Table 1, indicate that the possible changes produced by the kneading process were insignificant, and these minor changes may be attributed to the partial dissolution followed by the re-crystallisation of the materials.

The DSC profiles of the physical mixtures (1:1 and 1:2) show identical traces (Table 2; Fig. 3, Graphs A and B). In both traces, there are three endotherms and these can be attributed to dehydration of HPGCD, melting of TBM and decomposition of the system. The DSC scan of the 1:1 kneaded system (Fig. 3, Graph C) shows similar behaviour to that of the physical mixtures: the peak of the dehydration from the system, peak of TBM melting and the peak of the decomposition of the system. Conversely, the DSC curve of the 1:2 kneaded system (Fig. 3, Graph D) exhibits only two

Table 2. Thermal Data from DSC Analysis of TBM-HPGCD Physical Mixtures (PM) and Kneaded Systems (KS)

Transition	PM (1:1)	PM (1:2)	KS (1:1)	KS (1:2)
Dehydration from the system	84 ^{a)} 107 ^{b)}	78 ^{a)} 103 ^{b)}	92 ^{a)} 67 ^{b)}	97 ^{a)} 160 ^{b)}
Melting of TBM	127 ^{a)} 8 ^{b)}	127 ^{a)} 5 ^{b)}	126 ^{a)} 3 ^{b)}	—
Decomposition of the system	297 ^{a)} 485 ^{b)}	311 ^{a)} 399 ^{b)}	303 ^{a)} 482 ^{b)}	298 ^{a)} 140 ^{b)}

a) Peak temperature (°C). b) Enthalpy (J g⁻¹).

Fig. 3. DSC Curves of TBM-HPGCD Binary Systems

(A) TBM-HPGCD physical mixture 1:1, (B) TBM-HPGCD physical mixture 1:2, (C) TBM-HPGCD kneaded system 1:1 and (D) TBM-HPGCD kneaded system 1:2.

endothermic peaks corresponding to the dehydration from the system and decomposition of the system. Like that of pure HPGCD, the fusion process corresponding to the complex was not detected in the kneaded system. Instead, the decomposition of the system was detected.

The absence of the TBM melting peak indicates that in the 1:2 kneaded system the entire drug forms a complex with the cyclodextrin. Comparing the scan of the 1:2 kneaded system with that of the 1:1 kneaded system (in which a small peak is observed in the melting range of TBM) it can be deduced that in the latter system, only a part of the drug forms a complex with the cyclodextrin and the rest of the drug remains free. This free part of the drug melts when the sample reaches the melting point of TBM.

The absence of the two endothermic peaks, corresponding to the TBM decomposition, in all binary systems could indicate that during heating an inclusion complex could have

Table 3. Data from Thermogravimetric Analysis of TBM and HPGCD, Their Kneaded Counterparts, TBM-HPGCD Physical Mixtures (PM), and Kneaded Systems (KS)

Sample	Ranges (°C)	Weight loss (%)	Inference
Pure TBM	121–294	100	Decomposition
Kneaded TBM	127–324	100	Decomposition
Pure HPGCD	52–94	6	Dehydration
	288–481	83	Decomposition
Kneaded	49–122	8	Dehydration
HPGCD	260–489	88	Decomposition
PM 1 : 1	49–92	6	Dehydration from HPGCD
	268–501	88	Decomposition of system
PM 1 : 2	41–99	8	Dehydration from HPGCD
	283–487	82	Decomposition of system
KS 1 : 1	48–105	7	Dehydration from system
	255–505	78	Decomposition of system
KS 1 : 2	46–99	8	Dehydration from system
	264–486	79	Decomposition of system

formed; the melted drug could have occupied the cyclodextrin cavity at higher temperature¹⁵ and in this way the complexed TBM remained protected from the decomposition. This theory is in agreement with the observation of HSM and indicates that the inclusion of TBM into the HPGCD cavity has a stabilizing effect for tolbutamide.

Thermogravimetry Results of the thermogravimetric study are shown in Table 3. Data from both pure and kneaded TBM reveals that both materials lose 100% of their weight in a single step, which begins at 121–127 °C. Both pure HPGCD and its kneaded counterpart lose their weights in two steps. The first step corresponds to the loss of water of hydration and the second step is due to the decomposition of the sample (Table 3).

Thermogravimetric behaviour of all solid binary systems is similar, showing two steps of weight loss attributed to dehydration from HPGCD and decomposition of the system. The temperature at which the decomposition process begins is higher than that of the pure TBM. This behaviour could confirm the hypothesis suggested in the explanation of the results obtained from DSC curves. The formation of a TBM-HPGCD inclusion compound during the kneading process or heating process (thermal analysis) impedes the characteristic decomposition of TBM at temperatures above its melting point.

XRD Analysis The X-ray profiles of TBM and HPGCD (pure and kneaded) are shown in Fig. 4. There was no change in the profiles of the two X-ray patterns of TBM and its kneaded counterparts suggesting that the kneading process has no influence on the crystal structure of pure TBM (Fig. 4, Graphs 1 and 2). On the other hand, both X-ray profiles of HPGCD were of halo pattern indicating its amorphous nature. There was no change in the amorphous nature of HPGCD due to the kneading process (Fig. 4, Graphs 3 and 4).

The X-ray patterns of the two physical mixtures show the characteristic peaks of TBM, although they were of lower intensity in the 1 : 2 physical mixture in comparison with that of the 1 : 1 physical mixture (Fig. 5, Graphs 1 and 3). However, compared with that of pure TBM, the intensities of some of the important peaks of TBM were lower in the profiles of the physical mixtures. This behaviour is due to the

Fig. 4. X-Ray Diffraction Profiles of TBM and HPGCD

(1) Pure TBM, (2) kneaded tolbutamide, (3) pure HPGCD and (4) kneaded HPGCD.

Fig. 5. X-Ray Diffraction Profiles of TBM-HPGCD Binary Systems

(1) TBM-HPGCD physical mixture 1 : 1, (2) TBM-HPGCD kneaded system 1 : 1, (3) TBM-HPGCD physical mixture 1 : 2 and (4) TBM-HPGCD kneaded system 1 : 2.

presence of proportionately (% w/w) higher amount of the carrier in the systems.

The X-ray patterns of the two kneaded systems are of dissimilar type. In the X-ray pattern of the 1 : 1 kneaded system (Fig. 5, Graph 2), some of the TBM peaks are present and this behaviour indicates that some of the drug particles remained in uncomplexed form. In other words, a complete inclusion was not obtained in this system. On the other hand, like that HPGCD, the profile of the 1 : 2 kneaded system is of halo pattern (Fig. 5, Graph 4). The absence of any peaks corresponding to the drug indicates that the entire drug was included in the cavity of this cyclodextrin.

Dissolution Assay The results of the dissolution assay are presented in Table 4 and Fig. 6. Both the 1 : 1 and 1 : 2 kneaded systems showed significant improvement in dissolution in comparison with the physical mixtures and the drug alone. The release of the drug from the kneaded systems was almost instantaneous. Because the formation of TBM-HPGCD inclusion complex increases solubility and dissolution of the drug (Fig. 6). The profiles from the physical mixtures were similar to those obtained from the pure drug. Comparable dissolution profiles from the physical mixtures and pure TBM indicate that the rate of the formation of the TBM-HPGCD inclusion complex in solution was not so fast as to give rise to an *in situ* inclusion complex during the period of assay. This is because the dissolution profiles from

Table 4. Dissolution Efficiency Obtained from Pure TBM and TBM-HPGCD Binary Systems

Systems	Dissolution efficiency (%)		
	30 min	90 min	180 min
Pure TBM	14.94	23.43	29.27
TBM-HPGCD physical mixture 1 : 1	12.61	22.59	30.25
TBM-HPGCD physical mixture 1 : 2	21.36	28.71	34.83
TBM-HPGCD kneaded system 1 : 1	42.05	47.56	48.85
TBM-HPGCD kneaded system 1 : 2	45.72	50.04	51.37

Fig. 6. Dissolution Profiles of TBM and TBM-HPGCD Binary Systems

(x) Pure TBM, (●) TBM-HPGCD physical mixture 1 : 1, (○) TBM-HPGCD physical mixture 1 : 2, (■) TBM-HPGCD kneaded system 1 : 1 and (□) TBM-HPGCD kneaded system 1 : 2.

the physical mixtures cannot reach the profiles from the kneaded system during the three hours of the assay.

The results of the physical characterisation and dissolution studies demonstrate that a TBM-HPGCD inclusion compound was formed due to the kneading process. The improvement in the dissolution of TBM from this inclusion complex was not, however, as promising as those from the TBM-BCD and TBM-HPBCD inclusion compound.⁹⁾ Nevertheless, by the preparation of a TBM-HPGCD kneaded

system, a dissolution rate better than that from the solid dispersion with PEGS 6000¹¹⁾ was obtained. Analysing the results of this study along with other studies about complexation phenomena between TBM and different cyclodextrins,^{9,16)} it can be concluded that the preparation of a TBM-cyclodextrin inclusion compound using any cyclodextrin (BCD, HPBCD and HPGCD) could be the best procedure to obtain an adequate drug dissolution from a solid dosage form of TBM.

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