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RESEARCH PAPER

# Influence of the Preparation Method on the Physicochemical Properties of Tolbutamide/Cyclodextrin Binary Systems

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## ABSTRACT

*Tolbutamide (TBM) was found to form an inclusion complex with β-cyclodextrin (β-CD) in solution and in solid state. Inclusion complex formation between tolbutamide and β-cyclodextrin in aqueous solution was studied by phase solubility and spectral shift methods. The apparent stability constant* $K_s$ *calculated by these techniques, in water, were estimated as 195.7 and 236.5* $M^{-1}$*, respectively. The phase solubility studies revealed a* $B_S$*-type diagram with an inclusion complex of 1 : 2 molar ratio. The solid inclusion complexes of TBM and β-CD were prepared at a molar ratio of 1 : 2 by kneading, coprecipitation, freeze-drying, and spray-drying methods. In addition, the physical mixture was prepared. Characterization of TBM : β-CD inclusion was performed using differential scanning calorimetry (DSC), Raman spectroscopy, and X-ray diffractometry and by application of a so-called ether wash method. All the inclusion systems investigated led to a significant improvement in the dissolution over free TBM, and the dissolution rate of the active material was observed to be independent of the preparation method.*

***Key Words:*** *Coprecipitation; β-Cyclodextrin; Freeze-drying; Kneading; Spray-drying; Tolbutamide.*

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## INTRODUCTION

Cyclodextrins are cyclic oligosaccharides of D-glucopyranose units α-(1,4) linked in a ring formation containing a relatively hydrophobic central cavity and a hydrophilic outer surface. The most common cyclodextrins are α-cyclodextrin (α-CD), β-cyclodextrin (β-CD), and γ-cyclodextrin (γ-CD), which are formed by six, seven, and eight glucose units, respectively (1). Owing to lack of free rotation bonds connecting the glucopyranose units, the cyclodextrins are not perfectly cylindrical molecules and possess a doughnut-shaped hydrophobic cavity in which may be included ''guest'' molecules of appropriate size, shape, and polarity, resulting in a stable association without formation of any covalent bonds. Secondary forces are alone responsible for maintenance of the integrity of all inclusion complexes (2,3).

In recent years, cyclodextrins have received considerable interest in the pharmaceutical field due to their potential to entrap entirely, or at least partially, a variety of drug molecules in their cavities (1). This may lead to useful modifications of the physical and chemical properties of the inside guest molecule, allowing improvement of solubility, dissolution rate (4–6), chemical stability (7–9), and bioavailability (10–12). The complexation may also reduce volatility, unpleasant tastes or odors, local irritation, or untoward side effects associated with some drugs (13,14). In addition, cyclodextrins can avoid incompatibility problems of the inside drug with other drugs or excipients in a formulation (15).

Tolbutamide (TBM) is a sulfonylurea hypoglycemic agent that is given orally in treatment of non-insulin-dependent diabetes mellitus. This drug is a weak acid that is poorly soluble in water, and dissolution is considered to be a rate-determining step in absorption to the systemic circulation (16).

The main purpose of this study was to evaluate different methods for preparation of inclusion complexes between TBM and β-CD to improve the solubility and dissolution rate of TBM in pH 2 buffer solution. The dissolution studies were performed at this pH because the phase solubility studies showed that TBM solubility is lower at acid pH values. The experiments were carried out to define the most appropriate method for preparation of TBM:β-CD inclusion complexes for potential use in a suitable oral formulation with enhanced bioavailability.

Several methods have been proposed to obtain solid drug–cyclodextrin complexes, both in liquid and in solid medium, but there is still no general rule or a universal method, probably because each drug to be entrapped is a particular case, and the optimal conditions depend on the characteristics of both host and guest molecules (6). Selection of the most suitable preparation method for a given drug requires careful evaluation because it should take into account not only the performance of the obtained product, like the dissolution profile, but also factors such as simplicity, lower cost, high yield, swiftness, and ease of scaling up.

In the present study, the formation of TBM:β-CD complexes in solution was investigated by phase solubility and ultraviolet (UV) spectroscopy. The complexes in the solid state were prepared by coprecipitation, kneading, freeze-drying, and spray-drying. Such complexes were characterized by differential scanning calorimetry (DSC), X-ray diffraction, and Raman spectroscopy and by application of a so-called ether wash method.

## EXPERIMENTAL

### Materials

Tolbutamide was supplied by Sigma (St. Louis, MO) and β-CD by Roquette Frères (Lestrem, France). All other reagents and solvents were analytical grade, and double distilled water was used.

### Phase Solubility Studies

Solubility studies were carried out according to the method reported by Higuchi and Connors (17). Excess amounts of TBM were added to aqueous solutions containing increasing concentrations of β-CD (0.001–0.028 M). These β-CD solutions were prepared in water and pH 2 and pH 7 buffers. The flasks were sealed and shaken at 23°C for 2 weeks to ensure equilibrium. The samples were filtered through a Millipore membrane (HA 0.45 μm). The filtrates were appropriately diluted and analyzed spectrophotometrically at 229 nm (Shimadzu UV-160 spectrophotometer) to determine the total drug content. The studies were carried out in triplicate.

### Spectroscopic Studies

Complex formation between TBM and β-CD was also studied, in distilled water, by the spectral shift method (18). The concentration of TBM in these studies was $5 \times 10^{-5}$ M, while the β-CD concentration was increased from $2 \times 10^{-3}$ to $10^{-2}$ M. The mixtures were stirred before recording the UV absorption spectra in a double-beam spectrophotometer (Shimadzu). These spectra were compared with the spectrum of free TBM, and the changes in absorbance of TBM by addition of var-

ious concentrations of β-CD were measured at 229 nm to evaluate the stability constant of the complex. The absorbance measurements were carried out against appropriate blanks of β-CD, prepared in the same concentrations in water, to cancel any absorbance that may be exhibited by cyclodextrin molecules.

## Preparation of Solid Complexes

Since the phase solubility diagram was of the $B_S$ type, solid complexes could be prepared by coprecipitation, which is only possible for this type of phase solubility diagram. In addition to the coprecipitation method, the inclusion complexes were prepared by kneading, freeze-drying, and spray-drying, which are potentially suitable for industrial-scale production. These four methods are described in detail below. The physical mixture was prepared for reference by simple mixing of TBM and β-CD, previously sieved (90–160 μm), in a 1:2 molar ratio, adopting the geometric dilution method.

Furthermore, pure TBM was also separately treated by kneading, freeze-drying, and spray-drying in the same manner as in the complexes to reveal if these processes have any influence on the dissolution characteristics of free drug.

### Coprecipitation

The solid complex was obtained by mixing appropriate amounts of TBM and β-CD in distilled water. These quantities were calculated from the descending curvature of the phase solubility diagram at the point at which no solid drug existed and the solubility of β-CD was not exceeded (indicated by an arrow in Fig. 1). The mixture was stirred at room temperature for 2 weeks. After that, the complex, which precipitated as a white powder, was filtered, washed with a small amount of distilled water, and then dried under vacuum at 40°C for 24 h.

### Kneading

The β-CD was kneaded with a small amount of ethanol:water (1:3) in a mortar to obtain a homogeneous paste. TBM was subsequently added in stoichiometric proportion (1:2), and the slurry was kneaded for a further 60 min. During this process, an appropriate quantity of ethanol:water (1:3) was added to maintain a suitable consistency. The resulting paste was then dried under vacuum at 40°C for 48 h.

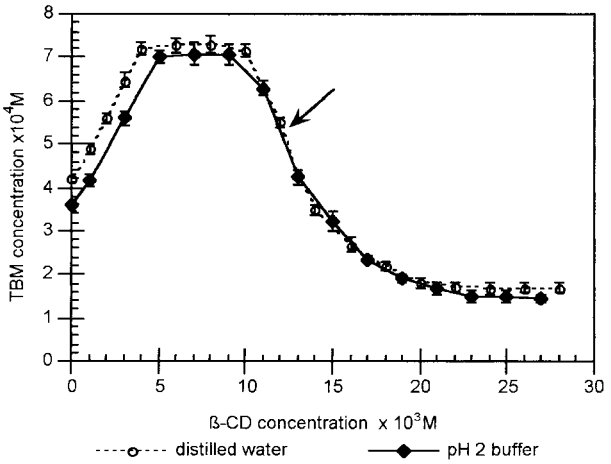


**Figure 1.** Phase solubility diagram of tolbutamide:β-cyclodextrin system in water and pH 2 buffer solution. Each point represents the mean ± SD.

### Freeze-Drying

Stoichiometric quantities of TBM and β-CD (1:2) were dissolved in distilled water with a small amount of ammonia 25% to aid dissolution of the active principle. The solution was frozen by immersion in a shell freezer and was freeze-dried over 24 h in a Lyph-lock 6 apparatus (Labconco). No trace of ammonium was detected in the resulting mixture with Nessler reagent.

### Spray-Drying

Stoichiometric quantities of TBM and β-CD (1:2) were dissolved in 95% ethanol and water, respectively. The resulting mixture was stirred and subsequently spray-dried in a Labplant SD-5 apparatus, under the following conditions: flow rate of the solution 500 ml/min; inlet temperature 165°C; outlet temperature 85°C; atomizing air pressure 1.5 bar; and airflow rate 60 m$^3$/h.

Yields of the coprecipitation, kneading, freeze-drying, and spray-drying processes were 49.6%, 84.7%, 89.8%, and 41.2%, respectively.

## Free Drug Determination

The ''ether wash'' method is probably the simplest method to detect inclusion complex formation in the solid state and to determine the free drug content. This method is based on the fact that the β-CD and its complexes are completely insoluble in diethyl ether. Since the pure drug is soluble in diethyl-ether, the amount of drug that dis-

solves provides an estimation of the noncomplexed drug fraction in the powder (19,20).

An exactly weighed amount (20 mg) of each powder was shaken with 50 ml of diethyl-ether (dried on anhydrous magnesium sulfate) for 10 min. The particles were removed by filtration, and the ether was evaporated. The remaining drug was then dissolved in methanol and determined spectrophotometrically. Each experiment was carried out in triplicate.

### Differential Scanning Calorimetry

Thermal analyses were carried out with a Shimadzu model 50. Indium (99.98%, mp 157.6°C; Aldrich) was used to calibrate the apparatus. Each sample, corresponding to 1 mg of TBM, was placed in a pierced aluminum pan and heated at a rate of 10°C/min in the 25°C to 250°C temperature range under a nitrogen stream (20 ml $min^{-1}$).

### X-Ray Diffraction

X-ray diffraction patterns of different samples were obtained using an Enraf-Norius powder diffractometer equipped with a horizontal mounted INEL CPS120 curved position-sensitive detector. Cu-$K_{\alpha 1}$ radiation was selected by a bent quartz crystal monochromator, and each pattern was collected over about 24 h.

### Raman Spectroscopy

The Raman spectra were recorded on a Spex 1403 double spectrometer. The 514.5-nm line of an argon ion laser (Spectra Physics, model 2020-03) was used as the Raman excitation.

### Dissolution Studies

The dissolution studies were performed according to the USP 23 rotating paddle method using 1000 ml of pH 2 buffer as the dissolution medium. The stirring speed employed was 75 rpm, and the temperature was maintained at 37°C ± 0.5°C. Powdered samples of each preparation (sieve fraction 90–160 μm), equivalent to 25 mg of TBM (corresponding to about 20% of saturated concentration), were spread over the dissolution medium. Dissolution rates were measured in an apparatus (Hanson Research) connected to the spectrophotometer by a peristaltic pump so that the absorbance was monitored automatically at 229 nm. All samples were analyzed at least six times.

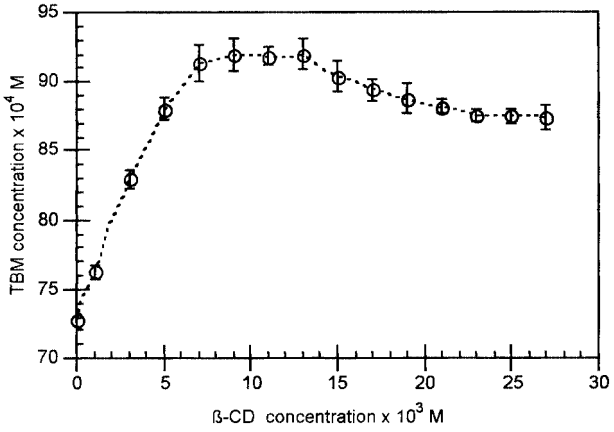


**Figure 2.** Phase solubility diagram of tolbutamide:β-cyclodextrin system in pH 7 buffer solution. Each point represents the mean ± SD.

## RESULTS AND DISCUSSION

### Phase Solubility Studies

The phase solubility diagrams of TBM:β-CD were obtained by plotting the changes in guest solubility as a function of CD concentration and are shown in Figs. 1 and 2. The solubility curves were classified as the $B_s$ type according to Higuchi and Connors (17), indicating the limited solubility of the complex, for which the initial rising portion was followed by a plateau, and finally the concentration of TBM decreased with the precipitation of solid microcrystalline complex. The stoichiometry of the complexes in the solid phase was analyzed on the basis of data in the plateau region of the solubility diagrams and was estimated as 1:2 (TBM:β-CD). These results were confirmed by chemical analysis of the solid complex. Similar results were obtained by Chow and Karara (21) and Kedzierewicz et al. (22).

The extent of complexation in aqueous media (i.e., the stability of the formed complex) is characterized by the stability constant $K_s$. In the present study, the $K_s$ determination was based in the solubility diagrams, which required calculations involving drug solubility. Hence, $K_s$ values were calculated according to the equation of Higuchi and Connors (17) (Eq. 1) from the initial straight-line portion of the solubility diagrams by assuming that a 1:1 complex was initially formed (the slope was smaller than 1) and were found to be 195.7, 212.1, and 50.2 $M^{-1}$ in water and pH 2 and pH 7 buffer solutions, respectively.

$$K_s = \text{Slope}/S_0(1 - \text{Slope}) \quad (1)$$

where $K_s$ is the stability constant for the complex, and $S_0$ is the solubility of TBM in the absence of β-CD (intercept of the solubility diagram).

The $K_s$ values obtained reflect that β-CD in pH 2 buffer form inclusion complexes with TBM that are more stable than in pH 7 buffer. This finding may demonstrate that the interaction of β-CD with the un-ionized form of TBM is stronger than with the ionized one (TBM $pK_a$ = 5.4), which suggests that chemical parameters are involved in the complexation. Although β-CD demonstrated lower affinity to the ionized form of TBM, both forms can be complexed.

## Spectroscopic Studies

The effect of different molar concentrations of β-CD on the absorption spectrum of TBM is illustrated in Fig. 3. A bathochromic shift was evident in the absorption maximum of TBM, with the peak at 229 nm, with diminution in the absorbance. These induced changes in absorbance are attributed primarily to the formation of inclusion complexes. The changes in peak intensity are assumed to be from changes in the solvent microenvironment on inclusion of the solute. The observed reduction in peak intensity may result from the loss of hydrogen bonding accompanying the transfer of the guest molecule from water to the cyclodextrin cavity. This is reasonable in light of the fact that there are no proton-donating groups inside the cavity of the cyclodextrin molecule (23).

Calculation of the stability constant of the complex from these spectral changes of TBM by addition of various concentrations of β-CD was possible because molar absorptivities of the complex and the drug differ at the same wavelength (18).

The apparent stability constant $K_s$(1:1) was determined according to the Benesi-Hildebrand equation (24):

$$1/\Delta A = 1/[D]K_s\Delta\varepsilon \times 1/[\text{CD}] + 1/[D]\Delta\varepsilon$$

where $\Delta A$ is the difference of absorbance at 229 nm, [CD] is the concentration of cyclodextrin, [$D$] is the total drug concentration (constant), and $\Delta\varepsilon$ represents the difference in molar absorptivities between the free and complexed drug.

The apparent stability constant obtained from the intercept : slope ratio of the double reciprocal plot of $1/\Delta A$ versus 1/[β-CD] (Fig. 4) was 236.5 $M^{-1}$, in good agreement with the one estimated from the solubility study (195.7 $M^{-1}$). Several methods have been reported for $K_s$ determination, and it is of extreme importance to calculate $K_s$ at least by two methods.

The above $K_s$ values fell within the range 100–1000 $M^{-1}$ considered by some authors (25) to be adequate for the formation of an inclusion complex, which may contribute to improve the bioavailability of drugs that are poorly water soluble.

## Free Drug Determination

The results of the ether wash procedure (Table 1) revealed a free drug fraction below 12.8% in all the complexes investigated. These small values suggest inclusion complexation between TBM and β-CD when compared with the higher percentage of free TBM in the physical mixture (99.2%).

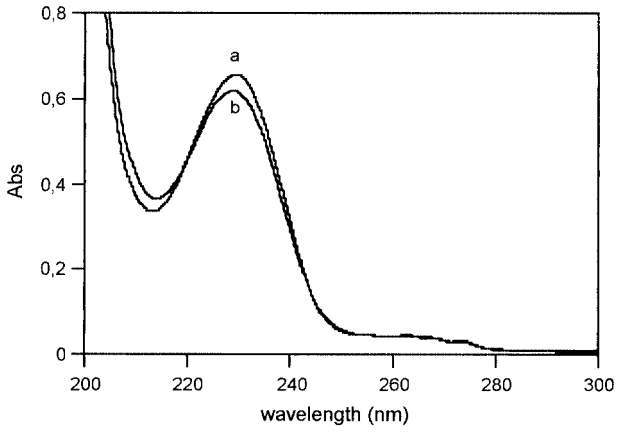


**Figure 3.** Effect of β-CD concentration on ultraviolet absorption spectra of TBM in water. The concentration of tolbutamide was $5 \times 10^{-5}$ M: (a) TBM alone; (b) TBM in the presence of $1.0 \times 10^{-2}$ M of β-CD.

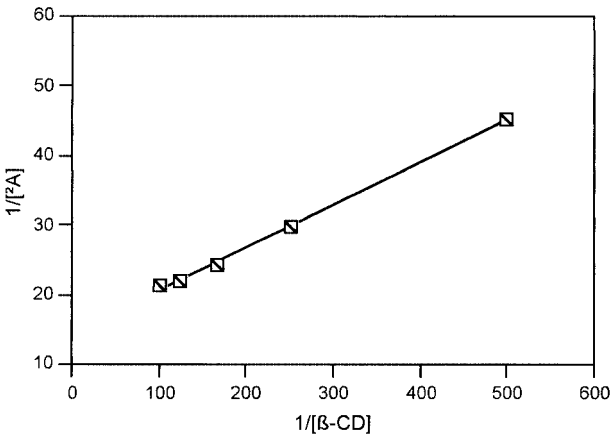


**Figure 4.** Benesi-Hildebrand plot for the effect of β-CD on the absorbance of TBM at 229 nm.

***Table 1***

*Free Tolbutamide (TBM) Fraction in TBM:β-CD (β-Cyclodextrin) Systems*

| | Percentage (w/w) of Free Fraction of TBM[a] |
|---|---|
| Physical mixture | 99.2 ± 0.4 |
| Coprecipitated | 10.5 ± 5.8 |
| Kneaded | 12.8 ± 3.7 |
| Freeze dried | 7.7 ± 0.7 |
| Spray-dried | 8.2 ± 0.5 |

[a] Each point represents the mean ± SD.

## Differential Scanning Calorimetry

The thermal behavior of β-CD inclusion compounds was studied using DSC to confirm the formation of the solid complexes. When guest molecules are incorporated in the CD cavity or in the crystal lattice, their melting, boiling, and sublimation points usually shift to a different temperature or disappear within the temperature range at which the CD lattice is decomposed (26).

The DSC curves for all the systems assayed are represented in Fig. 5. TBM exhibited two endothermic peaks, one at 39.6°C and the major one at 129.2°C, concerning the reversible solid/solid transformation and melting point, respectively (27). The DSC thermogram of β-CD shows a very broad endothermic peak around 80°C, corresponding to the release of water molecules, in accordance with findings of other authors (28).

In the thermal curve of the physical mixture, the melting endothermic peak characteristic of pure TBM and the broad peak corresponding to the dehydration of β-CD were also evident, as if this thermogram was the superposition of those components analyzed separately. Thus, we can consider the absence of interaction between TBM and β-CD in such a system.

Thermograms of TBM:β-CD complexes prepared by kneading and coprecipitation displayed a broad endothermic band between 50°C and 100°C due to the dehydration of the complexes, and they had two peaks near 126°C and 155°C. The peak at 126°C can still reflect the presence of a few drug crystals in the preparation. However, this thermal effect appeared more broadened and reduced in intensity, which suggests some drug-cyclodextrin interaction (29). The peak near 155°C was due to the shift of melting point of TBM to a higher temperature as a consequence of inclusion of TBM in the β-CD cavity or in the crystal lattice (30). Changes in the broad peak of β-CD dehydration provided a further indication of the existence of inclusion complexes in these systems. However, the kneading and coprecipitation methods do not provide complete encapsulation, and TBM was dispersed in the free state between inclusion complexes.

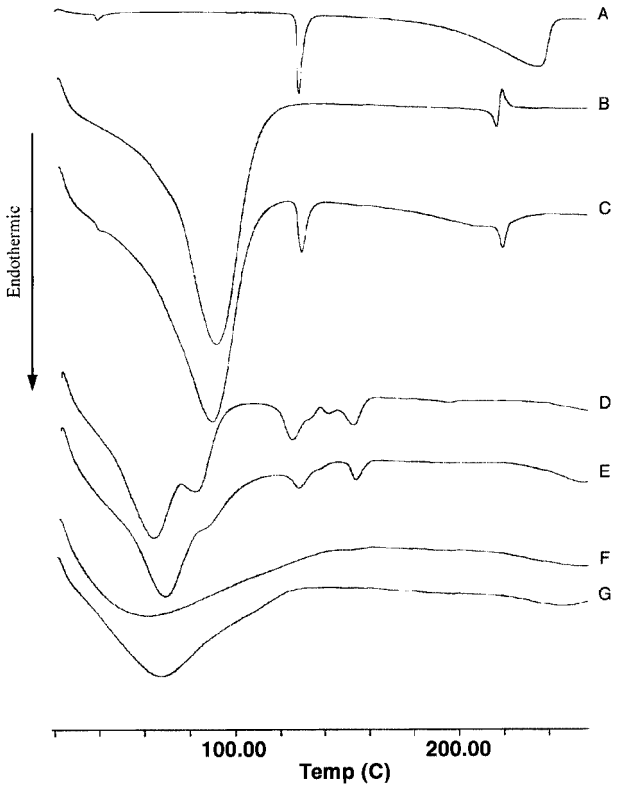


**Figure 5.** DSC thermograms: (A) TBM; (B) β-CD; (C) TBM:β-CD physical mixture; (D) TBM:β-CD coprecipitated complex; (E) TBM:β-CD kneaded complex; (F) TBM:β-CD freeze-dried complex; (G) TBM:β-CD spray-dried complex.

The complete disappearance of the endothermic effect of pure TBM was instead observed for freeze-drying and spray-drying complexes and is attributed to the formation of an amorphous solid product, the encapsulation of the drug inside the β-CD cavity, or both (26,31).

## X-Ray Diffraction

The X-ray diffraction patterns of pure TBM, β-CD, and their physical mixture and coprecipitated, kneaded, freeze-dried, and spray-dried inclusion complexes are represented in Fig. 6. The diffractograms of TBM and β-CD exhibited a series of intense peaks, which are indicative of their crystallinity. The diffractogram of the physical mixture was constituted practically by simple superposition of each component, indicating the presence of

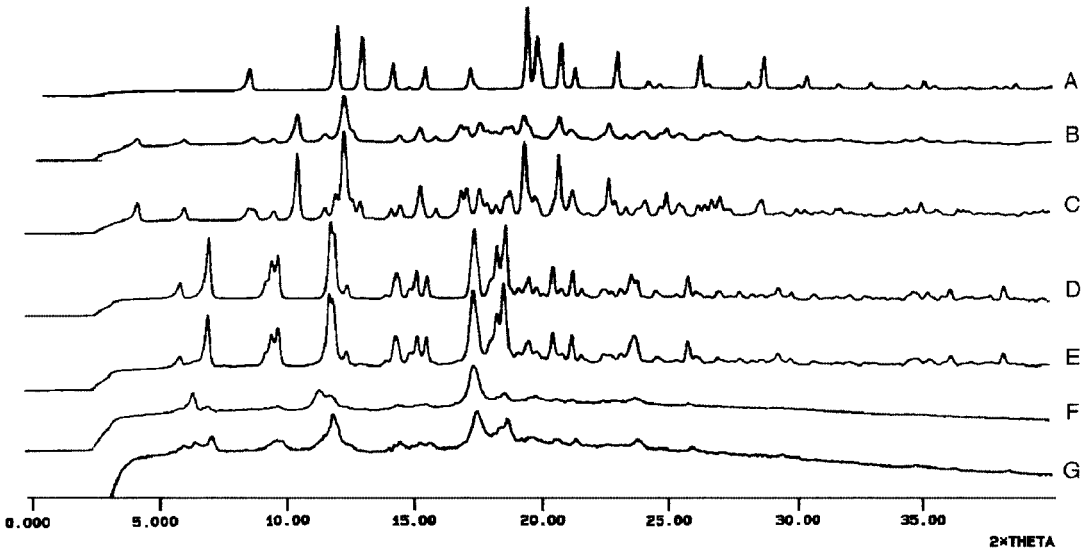


**Figure 6.** X-ray diffraction patterns: (A) TBM; (B) β-CD; (C) TBM:β-CD physical mixture; (D) TBM:β-CD coprecipitated complex; (E) TBM:β-CD kneaded complex; (F) TBM:β-CD freeze-dried complex; (G) TBM:β-CD spray-dried complex.

TBM in the crystalline state and no formation of a new structure.

The inclusion complexes prepared by coprecipitation and kneading had a crystalline structure, while those obtained by freeze-drying and spray-drying exhibited considerable diminution in crystallization state, as evidenced by fewer and broader diffraction peaks of lower intensity. The differences in the diffraction patterns of these compounds (coprecipitated, kneaded, freeze-dried, and spray-dried systems) from each isolated constituent seem to indicate the formation of a new solid phase as a result of formation of inclusion compounds, which is in good agreement with DSC studies.

***Table 2***

*Carbonyl Stretching Raman Frequencies in Tolbutamide (TBM) and TBM:β-CD (β-Cyclodextrin) Systems*

| | νC = O/cm$^{-1}$ | | |
|---|---|---|---|
| TBM (solid) | 1670 | 1712 (w) | |
| Physical mixture of TBM and β-CD | 1674 | 1712 (w) | |
| TBM/β-CD (coprecipitation) | 1673 (vw) | 1718 | 1734 |
| TBM/β-CD (kneading) | | 1718 | 1735 |
| TBM/β-CD (freeze-drying) | | ≈1712 | 1731 |
| TBM/β-CD (spray-drying) | | 1711 | 1732 |

## Raman Spectroscopy

Since β-CD has no Raman bands in the region of carbonyl stretching vibration (1650–1780 cm$^{-1}$), the following discussion focuses on the region of TBM carbonyl stretching bands near 1670 cm$^{-1}$.

The carbonyl stretching Raman frequencies for TBM, TBM:β-CD physical mixture, and TBM:β-CD inclusion complexes are presented in Table 2. For convenient interpretation of these Raman frequencies, canonical structures of TBM should be taken into account (Fig. 7).

An increased contribution of structure II is associated with a diminution of electronic density of the C═O stretching bond, which is consequently accompanied by an increase in the bond length and a shift to lower frequency. Hence, the spectral feature occurring at the highest frequencies (i.e., at about 1734 cm$^{-1}$) should correspond to the carbonyl group in a predominantly apolar environment (structure I of Fig. 7). In contrast, the spectral features occurring at lower frequencies (i.e., at about

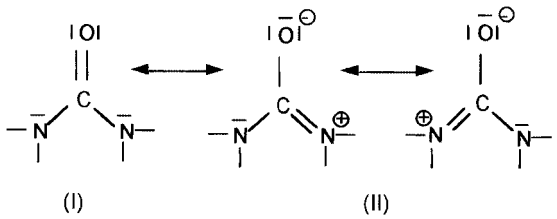


**Figure 7.** Canonical structures of carbonyl group.

1718 and 1670 $cm^{-1}$ should be attributed to differently polarized carbonyl groups (structure II of Fig. 7), with the lowest frequency being ascribed to a more strongly polarized C═O bond. Considering these general findings, we may conclude that

1. Assuming that the β-CD cavity offers a predominantly apolar environment for the included guest molecules (TBM), the weak spectral feature occurring at 1734 $cm^{-1}$ should be considered as a spectroscopic ''signature'' for the inclusion complexation. In fact, this spectral band was exhibited by all the inclusion complexes, in contrast to free TBM and the physical mixture.
2. The spectral bands at about 1674 and 1718 $cm^{-1}$ are attributed to the C═O group of TBM being differently polarized, probably by hydrogen bonds.

Thus, the frequencies of the C═O group in the physical mixture were similar to those of pure TBM, and in the other TBM:β-CD systems, there was an evident shift of the carbonyl stretching bands of TBM to a higher wavenumber, indicating the occurrence of a true interaction between drug and β-CD. In addition, intensity changes of the Raman peaks of TBM at 804 and 818 $cm^{-1}$, assigned to the C–H vibration of the phenyl group, were observed in the inclusion complexes, which suggests that the phenyl group was directly involved in the inclusion complexation.

## Dissolution Studies

In the present study, the dissolution profiles were evaluated by means of three parameters: dissolution efficiency over the first 15 min $DE_{15}$, dissolution efficiency over the first 60 min $DE_{60}$, and dissolution half-life time $t_{50\%}$.

The dissolution rate profiles and dissolution parameters of commercial TBM and TBM treated by kneading, freeze-drying, and spray-drying are reported in Fig. 8 and Table 3, respectively. There was no significant influence of these treatments on the dissolution behavior of pure drug since the various dissolution curves were practically superimposable, and obviously, the several dissolution parameters did not significantly differ. Even the reduction of TBM crystallinity after the spray-drying and freeze-drying processes did not increase the drug dissolution, which seems to indicate that the poor wettability of TBM was the determining factor for the reduced dissolution characteristics.

The dissolution profiles and dissolution parameters of commercial TBM and TBM:β-CD systems are presented in Fig. 9 and Table 4, respectively. It was clearly observed that all TBM:β-CD systems exhibited a higher dissolution rate than pure drug. The dissolution rate, expressed by $t_{50\%}$, of complexes was much faster ($t_{50\%} < 3$ min) than for TBM alone ($t_{50\%} = 94$ min), while the physical mixture presented an intermediate value ($t_{50\%} = 29$ min).

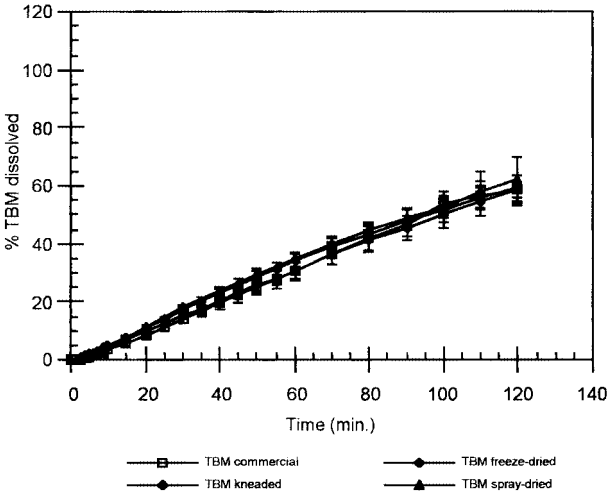


**Figure 8**. Dissolution profiles of commercial, kneaded, freeze-dried, and spray-dried TBM in pH 2 buffer at 37°C ± 0.5°C. Each point represents the mean ± SD.

Considering the $DE_{15}$ values, the dissolution rate of TBM was increased about 30-fold as an inclusion complex and nearly 9-fold as a physical mixture with β-CD. Taking into account the $DE_{15}$ values for the inclusion complexes, higher drug dissolution was achieved by the spray-dried product ($DE_{15} = 99.21\%$), followed by freeze-dried, kneaded, and coprecipitated products, respectively. However, after 60 min, these slight differences become insignificant, with $DE_{60}$ values above 93%

***Table 3***

*$DE_{15}$ (%), $DE_{60}$ (%), and $t_{50\%}$ (min) of Commercial, Kneaded, Freeze-Dried and Spray-Dried Tolbutamide in pH 2 Buffer at 37°C ± 0.5°C*

| | $DE_{15}$ (%)[a] | $DE_{60}$ (%)[a] | $t_{50\%}$ (min)[b] |
|---|---|---|---|
| Commercial TBM | 2.55 ± 0.09 | 14.35 ± 0.07 | 94.0 |
| Kneaded TBM | 3.36 ± 0.23 | 14.96 ± 0.22 | 96.5 |
| Freeze-dried TBM | 3.42 ± 0.52 | 16.88 ± 0.39 | 95.5 |
| Spray-dried TBM | 3.54 ± 0.43 | 17.60 ± 0.48 | 93.5 |

[a] Each point represents the mean ± SD ($n = 6$).
[b] Each point represents the mean ($n = 6$).

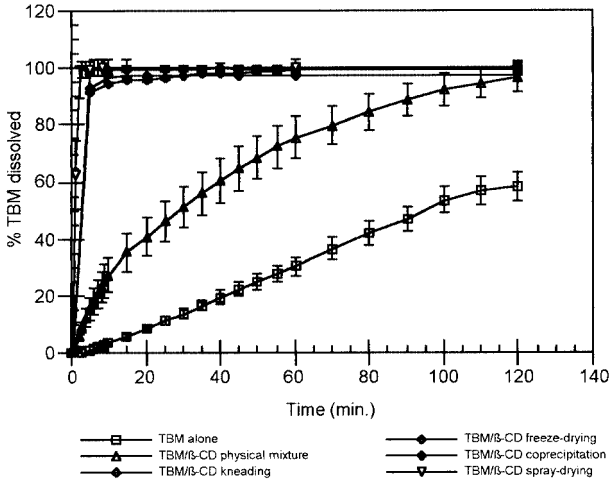


**Figure 9.** Dissolution profiles of TBM and TBM:β-CD systems in pH 2 buffer at 37°C ± 0.5°C. Each point represents the mean ± SD.

for all the inclusion complexes. These results allow us to conclude that the extent of the enhancing effect is not strongly affected by the complex preparation method.

The significant improvement in dissolution characteristics of the complexes was justified through the concurrence of several factors: formation of a readily soluble inclusion complex in the dissolution medium, increased drug particle wettability, and reduction of crystallinity degree of the product (23,32). For spray-dried and freeze-dried products, this enhancement might also be ascribed to the high-energy amorphous state (33). On the other hand, the small dissolution rate increase for the physical mixture compared to the other TBM:β-CD systems was probably due to the rapid formation of inclusion complexes in the dissolution medium or due to the wetting effect of β-CD. In fact, β-CD has surfactantlike properties owing to the hydrophilicity of its exterior surface, which can reduce the interfacial tension between water-insoluble drugs and the dissolution medium, leading to a higher dissolution rate.

***Table 4***

*$DE_{15}$ (%), $DE_{60}$ (%) and $t_{50\%}$ (min) of Tolbutamide (TBM) and TBM:β-CD (β-Cyclodextrin) Systems in pH 2 Buffer at 37°C ± 0.5°C*

| | $DE_{15}$ (%)[a] | $DE_{60}$ (%)[a] | $t_{50\%}$ (min)[b] |
|---|---|---|---|
| TBM | 2.55 ± 0.18 | 14.35 ± 0.21 | 94.0 |
| TBM:β-CD physical mixture | 21.68 ± 0.11 | 48.95 ± 0.15 | 29.0 |
| TBM:β-CD coprecipitated | 82.84 ± 0.73 | 93.39 ± 0.68 | <3 |
| TBM:β-CD kneaded | 85.74 ± 0.51 | 95.36 ± 0.45 | <3 |
| TBM:β-CD freeze-dried | 88.64 ± 0.62 | 96.47 ± 0.49 | <3 |
| TBM:β-CD spray-dried | 97.97 ± 0.49 | 99.21 ± 0.36 | <3 |

[a] Each point represents the mean ± SD ($n = 6$).
[b] Each point represents the mean ($n = 6$).

## CONCLUSION

β-CD was found to form an inclusion complex with TBM in aqueous solution and in the solid state. In solution, the ability of β-CD to include TBM was found to be pH dependent. The increase in ionization of TBM resulted in a decrease in the $K_s$ value.

All four techniques for the preparation of solid complexes (coprecipitation, kneading, freeze-drying, and spray-drying) revealed the formation of a true inclusion complex. The dissolution of TBM was significantly enhanced by complex formation. In addition, the extent of the dissolution rate–enhancing effect was found to be independent of the method used for the preparation of the inclusion complexes. As a result of this study, it may be concluded that TBM:β-CD inclusion complexation led to an improvement of drug solubility and dissolution rate, which suggests possible enhancement of TBM oral bioavailability. The higher yield of the freeze-drying method, together with the fact that it is a process potentially suitable for industrial-scale production, allowed us to select this method as the most adequate to obtain inclusion complexes of TBM and β-CD. Hence, in vivo studies in rabbits are in progress to evaluate the effect of freeze-dried TBM:β-CD inclusion complex on the bioavailability and pharmacological activity of TBM.

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