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Comparison of the Solubility and Dissolution Rate Between Gliclazide Solid Complex and Its Nanospheres

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ABSTRACT The solid complex of gliclazide and β-cyclodextrin was prepared by neutralization method and the precipitation solvent evaporation method was used to prepare gliclazide nanospheres. Fourier-transform infrared spectroscopy and differential scanning calorimetry were used to examine whether gliclazide solid complex and gliclazide nanospheres were successfully formed in this study. The dissolution rate of gliclazide from its nanospheres was faster than its solid complex and pure drug. The morphology of particles for nanospheres showed no crystal character of gliclazide. In summary, the results indicate that nanotechnology provides better effects in solubility and dissolution rate of gliclazide than neutralization method.

KEYWORDS Gliclazide, Cyclodextrin, Neutralization, Nanospheres, Solubility

INTRODUCTION

Cyclodextrins (CDs), as know as cyclic oligosaccharides, have six to eight glucose units linked by α-1,4-glucosidic bonds, which are crystalline and non-hygroscopic. The molecules of CDs are of distinguished cylinder shape, with a macro ring structure and a large internal axial cavity. Contrary to the character of hydrophobicity on the internal cavity, the outer surface of CDs is hydrophilic (Saenger et al., 1980; Szejtli et al., 1998). CDs are able to form the inclusion complexes with many drugs due to the possession of its cavity by either the whole or partial drug molecule (Loftsson et al., 1996). With the aim of improving pharmaceutical interest, such as solubility in aqueous media, dissolution rate, chemical stability and bioavailability, the application of complexation in different drugs with CDs has been extensively studied in recent years (Andersen & Bundgaard, 1984; Chow & Karara, 1986; Pitha et al., 1986; Bekers et al., 1991; Blanco et al., 1991; Ammar et al., 1999).

Although the inclusion complex made with CDs can improve the dissolution rate of many insoluble drugs, the safety aspects of CDs for various routes of administration are not yet completely clarified (Irie et al., 1997). CDs could induce changes in the shape of cellular membrane invagination on human erythrocytes and even cause lysis under high concentration rate of CDs

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(Leroy-Lechat et al., 1994). About parenteral safety issue, CDs could induce nephrotoxicity and some hepatic disorder (Uekama et al., 1991). Due to lack of absorption through the gastrointestinal tract, all toxicity studies of orally administered CDs were shown as nearly nontoxic. Nevertheless, the inhibition of mammalian amylases by β-CD potentially converts the digestible starches into indigestible materials and causes an increased occurrence of soft stools and diarrhea (Pitha & Pitha, 1985). In the ophthalmic use of CDs, the increase in stability and/or solubility could avoid irritation and discomfort of drug, however, high concentration of CDs could have opposite effect of irritating the conjunctival and corneal surface and causing reflex tearing and blinking (Jansen et al., 1990). Some reports indicated that nasal preparation with CDs could cause the hemolytic activity of the nasal mucous membrane or ciliotoxicity (Krishnamoorthy et al., 1995). When using CDs as rectal absorption enhancers, the potential for systemic absorption of pathogenic substances and the irritating effects of CDs on rectal mucosa need to be considered, because it might cause severe irritation with erosion of the rectal mucosa (Mesens et al., 1991). Finally, CDs may interact with some elements of the skin, which may reduce the function of skin as a barrier and provide for the enhancement of drug absorption (Uekama et al., 1987).

Nanospheres are solid colloidal particles with the size range between 10–1000 nm that have been employed to ameliorate solubility and dissolution rates for water insoluble drug (Müller et al., 2001; Eerikäinen & Kauppinen, 2003). Reducing the size of drug particle can increase its particle surface area, which can also improve its solubility and dissolution rate. Different techniques have been used to manufacture nanosized drug particles, such as dry and wet milling and solvent-based techniques (e.g., emulsification-solvent evaporation, emulsification-solvent diffusion and precipitation- solvent evaporation) (Bodmeier & Chen, 1990; Couvreur et al., 1995; Leroux et al., 1995). As one of new alternative device for drug manufacturing, nanospheres have been used for parenteral injection and oral administration. For clinical application, nanospheres are not only to increase the therapeutic efficiency of drug but also to reduce the quantity of drug administrated and to minimize undesirable side effects (Jung et al., 2003).

Gliclazide [GL; 1-(1-azabicyclo(3,3,0)octyl)-3-(*p*-tolylsulfonylurea)], as a second generation sulfonylurea, is widely used in the treatment of noninsulin dependent diabetes mellitus (NIDDM). Because of its short-term acting, GL has been considered suitable for diabetic patients with renal impairment and for elderly patients that have reduced renal function and follow a sulphonylurea treatment, which may increase the risk of hypoglycemia (Tessier et al., 1994). The molecule of GL represents the following characters as low solubility in gastric fluids, low dissolution rate and interindividual variability in its bioavailability (Kahn et al., 1990).

The present study was to compare the differences between GL solid complex and GL nanospheres in order to discover a new possible safer alternative while improving GL's solubility and dissolution rate. The GL solid complex was prepared by neutralization method, while the GL nanospheres were made by precipitation-solvent evaporation method. Differential scanning calorimetry (DSC), Fourier transform- infrared spectroscopy (FT-IR), the dissolution rate, the size and the morphology of particles from GL solid complex and its nanospheres were then evaluated. Besides, the use of GL nanospheres has not yet been reported (Moyano et al., 1997a,b; Özkan et al., 2000; Aggarwal et al., 2002). The results suggest that GL nanospheres could be used to increase its solubility and enhance dissolution rate for the clinical application.

MATERIALS AND METHODS

Materials

Gliclazide was from Lotus Pharmaceutical Co., Ltd. (Taipei, Taiwan). β-CD was purchased from Roquette Frères (Lestrem, France) Röhm GmbH & Co. KG (Darmstadt, Germany). Eudragit® E100 was a gift from Röhm GmbH & Co. KG (Darmstadt, Germany). Tolbutamide and Polyvinyl alcohol were purchased from Sigma Chemical Co. (Louis). All other materials were of either reagent or analytical grade.

Preparation of Solid Complex

GL was weighed and poured into 50 mL of 1*N* sodium hydroxide solution, and then β-CD was added and stirred until a clear solution was obtained. 50 mL of 1*N* hydrochloric acid was dripped into the solution and stirred for 2 hr. The precipitate obtained was then separated by vacuum filtration and washed repeatedly

for three times by cold alcohol and dried at room temperature (Moyano et al., 1997a,b; Özkan et al., 2000; Aggarwal et al., 2002).

Preparation of Nanospheres

GL nanospheres were prepared by a precipitation-solvent evaporation method. GL (10 mg) and Eudragit® E100 (100 mg) were dissolved entirely in a mixture of acetone (2 mL) and methanol (1 mL). The solution was poured into 25 mL of aqueous polyvinyl alcohol solution (1.0%, w/v) and stirred at 400 rpm for 5 min. The mixed solution was then centrifuged (14,000 rpm for 10 min; St Herblain, France), washed and redispersed in distilled water. Procedure repeated three times and the final dispersion was dried using a freeze drying method.

Determination of Drug Loading

The loading efficiency of the GL solid complex and its nanospheres were measured. Before using a freeze drying method, GL nanospheres were separated from the mixing solution by ultracentrifugation at 14,000 rpm for 10 min. At the same time, the solid complex of GL was dissolved in distilled water to obtain a homogenous solution. The amount of GL presented in the nanospheres and the solid complex were calculated from Eqs. (1) and (2).

$$\textit{Drug recovery}\,(\%) = \frac{\textit{weight of drug in nanospheres/solid complex}}{\textit{weight of drug loaded in the system}} \times 100 \quad (1)$$

$$\textit{Drug content}\,(\%) = \frac{\textit{weight of drug in nanospheres/solid complex}}{\textit{weight of nanospheres/solid complex recovered}} \times 100 \quad (2)$$

The contents of GL were assayed using the high-performance liquid chromatography (HPLC) method with Waters 2690D separations module (Milford, Massachusetts, USA) and Supelco Discovery® C18 column (5 μm, 250 × 4.6 mm I.D.) (Bellefonte, Pennsylvania, USA). The mobile phase consisted of methanol/water (65:35, v/v) adjusted to pH 3.0 with phosphoric acid at the flow rate of 1.0 mL/min. A 20 μL sample and tolbutamide as internal standard were injected together and absorbed at 225 nm with Waters 2996 photodiode array detector.

The standard fresh GL solutions for preparation of calibration curve was measured at 8:00 and 10:00 a.m., and 12:00, 2:00, 4:00, and 6:00 p.m. for 6 consecutive days. The average, standard deviation, variation and relative difference of GL contents were calculated to evaluate the stability of analytic condition, as well as the precision and the recurrence of measured data.

Particle Size Measurement

The average particle size of GL nanoparticles, GL solid complex, β-CD and pure drug were measured by the method of photon correlation spectroscopy (PCS) using Malvern Zetasizer 3000HSA (Malvern Worcestershire, UK). Prior to measurements, about 50 mg of each sample were diluted with 100 mL of ethylene glycol. The four particle size distributions were estimated by setting the intensity of the scattered light at $\lambda = 750$ nm and the scattering angle at $\theta = 90°$. The background medium was ethylene glycol ($\eta = 16.1$ mPas) and refractive index was 1.431.

Morphological Analysis of Particles

The morphology of the GL solid complex and pure drug were observed using a Jeol JSM 5300 scanning electron microscope (SEM, Tokyo, Japan). A little amount of these two samples was placed in turn on a copper support with an adhesive carbon foil. The samples were then coated with gold at 10 mA for 3 min by Jeol JEC-1100E ion sputter (Tokyo, Japan) and observed at 10 kV.

A total of 0.6 μL of nanosphere solution containing 0.01% of phosphotungstic acid was deposited on a transmission electron microscope (TEM) copper grid coated with carbon film and then was dried at 25°C. Samples were observed at 100 kV with a JEM-2000 EX II (Tokyo, Japan).

DSC Thermogram Studies

DSC scans of GL, its solid complex and its nanospheres were recorded on a Perkin-Elmer model DSC 7 (Norwalk, Connecticut, USA). The instrument was calibrated with indium and zinc prior to analyzing the samples under nitrogen at the flow rate of 20 mL/min.

Each sample (4 mg) was scanned in sealed aluminum pans at the heating rate of 20°C/min over the temperature range of 50–200°C.

FT-IR Spectroscopic Studies

The FTIR spectra were performed on Perkin-Elmer Spectrum System 2000 FT-IR spectrometer (Norwalk, Connecticut, USA). GL, its solid complex and its nanospheres were prepared by the KBr disc method and scanned at resolution of 4 cm^{-1} over the wavenumber region 4000–400 cm^{-1}. Air was used as the background and the data were averaged from 10 scans.

Dissolution Test

According to the USP 24 paddle apparatus, in vitro dissolution tests were performed with Hanson SR8 Plus (Chatsworth, California, USA). Three samples containing 80 mg of GL, equivalent amount of its solid complex and its nanospheres were poured separately into 900 mL of deionized water at 37 ± 0.5°C with stirring rate at 100 rpm. Afterwards, 0.2 mL of each sample was withdrawn at 1, 3, 5, 10, 15, 30, 60, and 120 min, respectively by the Waters transfer module (Milford, Massachusetts, USA). Subsequently, the concentration of the samples was assayed using the HPLC method.

The dissolution profiles were evaluated by the dissolution efficiency (DE) parameter (Khan, 1975) and the dissolved percentage (DP). The DE of GL, its nanospheres and its solid complex were calculated from Eq. (3).

$$\textit{Dissolution efficiency } (DE) = \frac{\int_{t_1}^{t_2} y \cdot dt}{y_{100} \cdot (t_2 - t_1)} \times 100\% \quad (3)$$

y: the percentage of dissolved product
t_1 and t_2: two time points
y_{100}: maximum percentage of the dissolved product

RESULTS AND DISCUSSION

Determination of Drug Loading

Calibration curve of GL, $Y = 0.0198X - 0.0079$ ($r^2 = 0.9999$), showed the excellent linear relationship with concentration range between 10.0 and 100.0 μg/mL. Precision and accuracy of chromatography are listed in Table 1. The lowest measurable concentration was 10.0 μg/mL and CV % was from 0.31–3.50, so the result was sufficiently acceptable.

The loading efficiency of GL solid complex and its nanospheres was calculated from Eqs. (1) and (2) (see Table 2). The loading efficiency between GL solid complex (60.50 ± 1.42%) and GL nanospheres (56.86 ± 2.06%) had no obvious differences. Consequently, nanospheres could provide the similar loading efficiency than the solid complex.

Size and Morphology of Particles

The mean particle size of GL, β-CD, GL solid complex and its nanospheres are listed in Table 3. We found that the mean particle size of β-CD was 161.03 ± 3.12 μm, however the mean particle size of GL solid complex was 68.29 ± 2.76 μm (from neutralization). Because of hydrogen bonding, the molecules of β-CD was bonded together and formed bigger particles than those in solid complex. In addition, the mean particle size of nanospheres (58.38 ± 2.08 nm) was one thousands times smaller than GL solid complex. Thereby, it demonstrated that GL nanospheres had more outer surface area comparing to the GL solid complex under the same amount of drug.

TABLE 1 Intra-day and Inter-day Analytical Precision and Accuracy of GL Determination

	Intra-day (n = 6)		Inter-day (n = 6)	
Concentration (μg/mL)	Precision mean ± SD (CV %)	Accuracy RE (%)	Precision mean ± SD (CV %)	Accuracy RE (%)
10.00	9.97 ± 0.32 (3.21)	–0.30	10.01 ± 0.35 (3.50)	0.10
20.00	19.95 ± 0.29 (1.45)	–0.25	20.12 ± 0.48 (2.39)	0.60
40.00	40.19 ± 0.23 (0.57)	0.48	39.89 ± 0.36 (0.90)	–0.28
80.00	80.09 ± 0.35 (0.44)	0.11	81.03 ± 0.29 (0.36)	1.29
100.00	100.28 ± 0.31 (0.31)	0.28	99.98 ± 0.32 (0.32)	–0.02

TABLE 2 **Encapsulation Efficiency of GL From its Solid Complex and its Nanospheres (n = 10)**

Materials	Loading amount (%)
Solid complex	60.50 ± 1.42
Nanospheres	56.86 ± 2.06

TABLE 3 **Mean Particle size of GL, β-CD, its Solid Complex and its Nanospheres (n = 3)**

Materials	Mean size ± SD
GL	47.56 ± 2.11 μm
β-CD	161.03 ± 3.12 μm
Solid complex	68.29 ± 2.76 μm
Nanospheres	58.38 ± 2.08 nm

The morphology photos of three samples are presented in Fig. 1. The scanning electron microscopy (SEM) was used to observe the shape of particle. The SEM image of GL showed many irregular flakes revealing its crystal character (Fig. 1a). And the SEM image of GL solid complex indicated that many masses gathered and lost its crystal character of pure drug (Fig. 1b). The transmission electron microscopy (TEM) was used to examine the internal formation of the nanospheres. From the TEM image of GL nanospheres, the sample showed no crystal character of GL (Fig. 1c). The particles of nanospheres were rather shown as a homogeneous matrix without any sign of phase separation between the drug and the polymer.

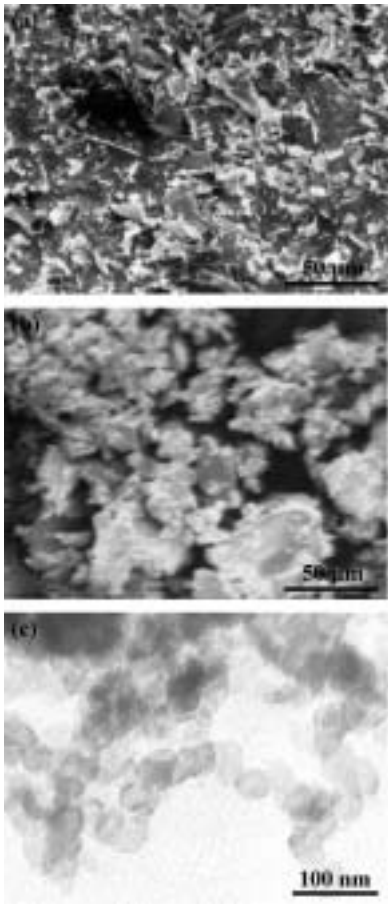

FIGURE 1 **SEM Images of GL (a) at 350 Times of Amplification and GL Solid Complex (b) at 350 Times of Amplification; TEM Image of GL Nanospheres (c) at 40,000 Times of Amplification.**

DSC Thermogram Studies

DSC is a fast and relatively inexpensive technique to examine the absence of the drug melting endotherm and to verify if the drug is successfully complexed or completely dispersed into the polymeric matrix (Otagiri et al., 1983; Pignatello et al., 2002). The results of DSC thermogram for these three samples are shown in Fig. 2. The DSC curve of GL showed an endothermic event of a melting peak with the onset temperature of 175°C. From the thermogram, we found that the GL solid complex and GL nanospheres had obvious different reactions. The GL solid complex from neutralization showed a very small melting peak at temperature of 175°C, which may be caused by remains of GL. This also indicates that most of GL molecules were dispersed into the β-CD cavities and their recrystallizations were almost

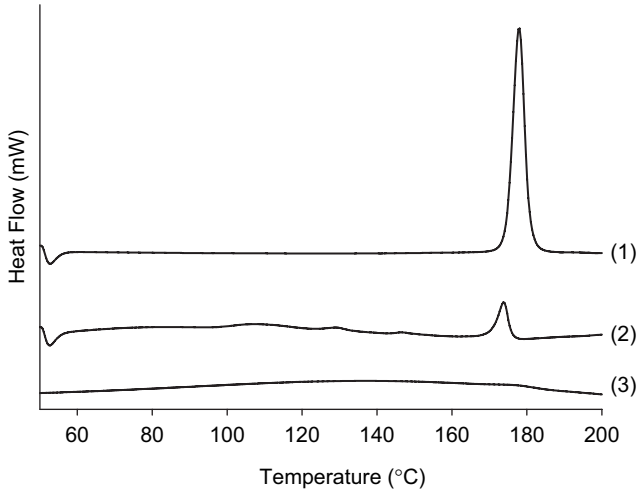

FIGURE 2 **DSC Curves of (1) GL, (2) its Solid Complex, and (3) its Nanospheres.**

restrained (Redenti et al., 1996). The thermal behavior of GL nanospheres demonstrated no reaction from any temperature intervals. This occurrence could be

attributed to the molecules of GL successfully dispersed into the polymeric matrix.

FT-IR Spectroscopy Studies

FT-IR spectra were employed to confirm the complexation of GL/β-CD from neutralization and the dispersion of GL in polymeric matrix of nanospheres (Fig. 3 and Table 4). Accordingly, the spectrum of GL for carbonyl group showed a sharp concave curve at 1709 cm^{-1}. The band of GL solid complex, however, was broader with less frequency and shifted from 1709 to 1716 cm^{-1}. This reaction seems to result from the interaction between the hydroxyl groups of β-CD and the carbonyl groups of GL that is in good agreement with the published researches (Winters et al., 1997; Özkan et al., 2000). The band of GL nanospheres was similar than the one of GL solid complex, which was also broader with less frequency, and shifted from 1709 to 1729 cm^{-1}. To cause this phenomenon, Eudragit® E100 was supposed to interact with GL.

For the sulphonyl group bands, a symmetric stretching peak at 1164 cm^{-1} and an antisymmetric stretching peak at 1350 cm^{-1}, characterizes the spectra of GL. The symmetric vibration peak of the GL solid complexes appeared at less frequency and shifted from 1164 to 1156 cm^{-1}, whereas the antisymmetric vibration peak performed the same frequency reduction and shifted from 1350 to 1345 cm^{-1}. Based on the previous reports, the reasons for this phenomenon might be the interaction between the oxygen atoms of GL and the hydrogen atoms of β-CD (Winters et al., 1997; Özkan et al., 2000). The symmetric vibration peak of the GL nanospheres also had less frequency and shifted from 1164 to 1143 cm^{-1}; however, the antisymmetric vibration peak appeared more frequency and shifted from 1350 to 1377 cm^{-1}. Therefore, we supposed that the interaction between GL and Eudragit® E100 caused this reaction.

For the amino group, GL demonstrated an evident peak at 3272 cm^{-1}. Nevertheless, a slight curve shifting from 3272 to 3368 cm^{-1} appeared on the inclusion complex band. This phenomenon corresponds with the previous reports as well (Özkan et al., 2000). In addition, the band of GL nanospheres presented the similar upward movement with a peak shifted from 3272 to 3389 cm^{-1}. The reason of this reaction may be attributed to the interaction between GL and Eudragit® E100.

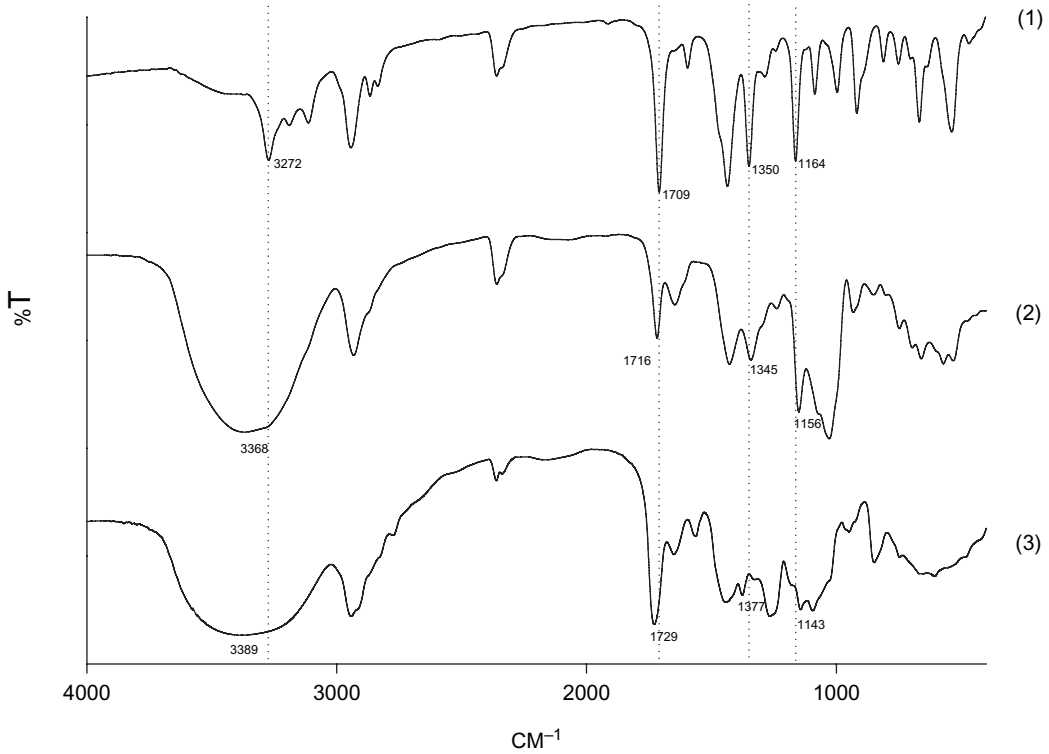

FIGURE 3 **FT-IR Spectra of (1) GL, (2) its Solid Complex, and (3) its Nanospheres.**

TABLE 4 **Comparison of FT-IR Spectra From GL, its Solid Complex and its Nanospheres (n = 10)**

GL (cm^{-1})	Solid complex (cm^{-1})	Nanospheres (cm^{-1})	Comment
1164	1156	1143	Change in S=O symmetric stretching
1350	1345	1377	Change in S=O antisymmetric stretching
1709	1716	1729	Change in C=O stretching
3272	3368	3389	Change in N—H stretching

In short, the GL solid complex prepared from neutralization had no significant characters of GL in the FT-IR spectrum, suggesting that the complex was well formed. Besides, the GL nanospheres from the FT-IR spectrum examination showed no significant character of GL and suggested that the molecules of GL might disperse completely into its polymeric matrix.

Dissolution Behavior

The dissolution profiles of GL, GL solid complexes and GL nanospheres are shown in Fig. 4 and Table 5. The dissolution rate for the GL nanospheres was greater than its solid complexes and GL alone. The dissolution rates of GL solid complex in this study were different from previous reports (Moyano et al., 1997a; Özkan et al., 2000), which may be caused from the variation of dissolution condition and materials.

From Table 5, the DP of GL was 1.44%, while 5.44% for GL solid complex and 82.66% for GL nanospheres after 5 min. Even after 60 min, the DP of GL was only 8.23% and 48.54% for GL solid complex. This result indicated that GL's nanospheres had

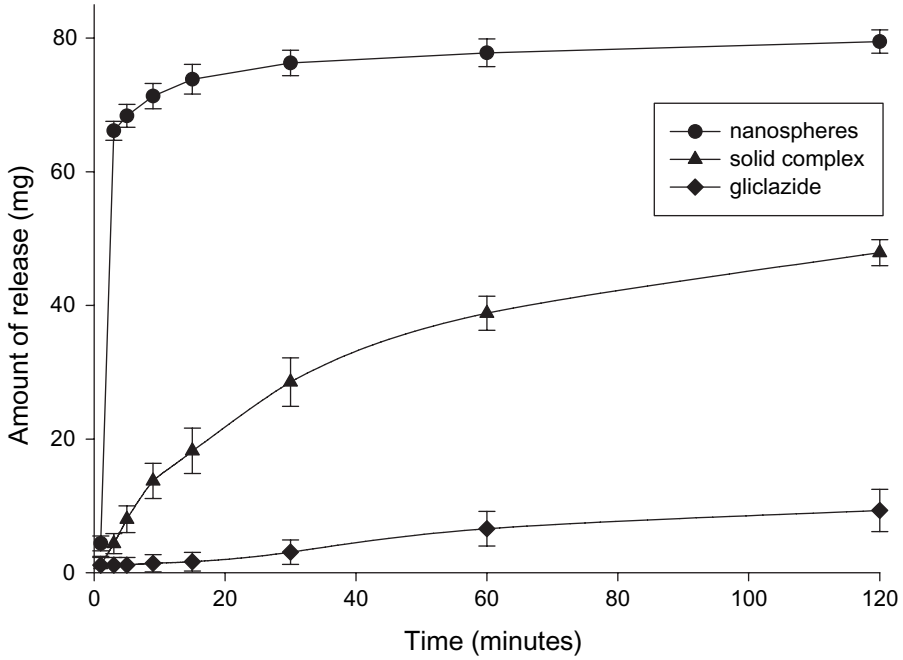

FIGURE 4 **The Dissolution Profile of GL Nanospheres (•), GL Solid Complex (▲), and GL (♦).**

TABLE 5 **Comparison of the DE and DP From GL, its Solid Complex and its Nanospheres (n = 6)**

	5 min		30 min		60 min	
Materials	DE_5	DP_5	DE_{30}	DP_{30}	DE_{60}	DP_{60}
GL	0.0129	1.44	0.0229	3.84	0.0416	8.23
Solid complex	0.0463	5.44	0.2119	35.67	0.3165	48.54
Nanospheres	0.5180	82.66	0.8533	95.34	0.9081	97.25

superior drug release percentage in a very short period. Moreover, the DE of GL was 0.0129 at 5 min and 0.0416 at 60 min. The DE of GL solid complex had a better performance at 5 min (0.0463) and at 60 min (0.3165) than GL alone. Nevertheless, the DE of GL's nanospheres demonstrated the highest dissolution efficiency 0.5180 at 5 min and 0.9081 at 60 min.

From both results of DP and DE of three samples, we found that GL's nanospheres had a greater performance of dissolution profiles than its solid complex and pure drug. Although the exterior of cyclodextrin is hydrophilic, it has limited effect on increasing the dissolution rate of GL inclusion complex, which formed a slow upward curve (Fig. 4). This reaction might be due to the hydrogen bonding of β-CD, causing the particle aggregation of GL inclusion complex and the decrease of the dissolution rate. This finding is very important and indicates that nanotechnology provides a higher performance on the dissolution rate and solubility than the complexation method. In the future, nanotechnology could be a fast and safe way in clinical therapy.

CONCLUSION

From our studies, the GL solid complexes and its nanospheres were confirmed being formed by FT-IR analysis and DSC thermograms. FT-IR analysis indicated the interaction of hydrogen bonding between the molecules of GL and β-CD. For GL nanospheres, FT-IR analysis demonstrated that GL and Eudragit® E100 interacted, thus the molecules of GL dispersed completely into the polymeric matrix. DSC thermograms showed the absence of GL melting peak from the curve of GL nanospheres, while the convex curve of GL solid complex from neutralization remained very small.

In regard to the dissolution study, drug releases from GL nanospheres was faster than other two samples. The measurement of particle size also found that the smallest particle was GL nanospheres, which also attained the scale of nanosize. From TEM observation, the particles of nanospheres had no crystal character of pure drug. The result of SEM for GL solid complex appeared without any irregular crystal flake characters of GL. In short, using nanotechnology could reduce the particle size of drug and increase the total particle surfaces, thus enhancing drug wettability and preventing its aggregation.

From above experiments, the GL nanospheres demonstrated a better effect on the dissolution rate than on the inclusion complex of GL-βCD. Therefore, the future suggestion is that other insoluble drugs could employ nanotechnology for immediate dosage release in clinical therapy.

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