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RESEARCH ARTICLE

SYNTHESIS OF WATER SOLUBLE COMPLEX OF METHYL-PIOGLITAZONE WITH β-CYCLODEXTRIN

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Abstract

Thiazolidinediones (TZD), have been approved for the treatment of type 2 diabetes mellitus either as monotherapy for patients with intolerance. The potential usefulness as antidiabetic drugs is however limited by the poor bioavailability, thus necessitating administration of a higher dose regime. To augment bioavailability, we prepared an inclusion complex of methyl-pioglitazone in b-cyclodextrin (β-CD) and evaluated its physicochemical characteristics. Our phase-solubility analysis shows a 1:1-complexation of methyl-pioglitazone with β-CD that offers better dissolution properties. We confirmed complex formation by powder X-ray diffractometry, Fourier-transform infrared spectroscopy, ^{1}H NMR spectroscopy and by molecular modeling methods. Based upon theoretical calculations in gas phase, we propose phenyl part of methyl-pioglitazone is oriented in the β-CD cavity. Our studies propose for the first time a stable methyl-pioglitazone-β-CD inclusion complex as an effective approach to enhance the solubility and bioavailability of methyl-pioglitazone for antidaibetic therapy.

Keywords: TZDs, β-CD, antidiabetic, complexation.

Introduction

Thiazolidine-2,4-diones (TZDs) are a new class of antidiabetic agents as well 3rd generation antidiabetic agents and include DRF-2189, ciglitazone, pioglitazone (**Figure 1**) etc. However, the developments of oral controlled-release formulations of TZDs are severely hampered due to short biological half-life, large dose, poor absorption, low dissolution or aqueous solubility and extensive first pass metabolism (Arii et Al., 2005; Singh et al., 2008; Singh et al., 2008; Kumar et al., 2008; Gupta et al., 2005). Rather than using co-solvents to enhance the solubility of the drug, which can be irritant or toxic, supramolecular hosts, such as cyclodextrins (CDs) could be an attractive option to overcome these limitations. β-CD, having an inner cavity diameter of 6.0–6.5A° and a depth of inclusion complexing agents to increase the aqueous solubility, bioavailability and stability of poorly water-soluble drugs (Loftsson T, Brewster, 1996; Loukas et al., 1996; Djedaini and Perly, 1991; Wilson and Verral, 1998; Gandhi and Karara, 1988; Wong and Yuen, 2001; DeAraujo et al., 2007). Therefore, beta-CD is valuable for engineering the novel host-guest molecular complexes (Pascall et al., 2005; Szejtli and Szente, 2005; Ali and Upadhyay, 2008; Martin Del Valle, 2004; deAraujo et al., 2007). Hence, in the present investigation, complexation of methyl-pioglitazone with β-CD was attempted and their interaction was assessed by the application of molecular modeling using semiempirical methods.

Figure 1 Structure of methyl-pioglitazone

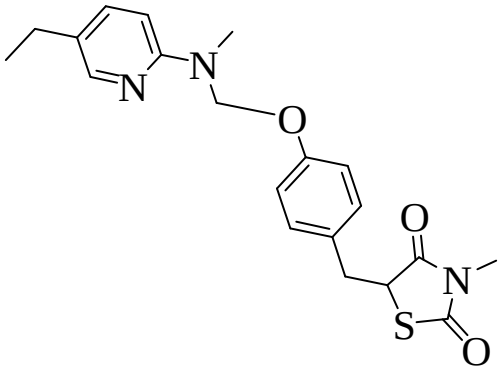
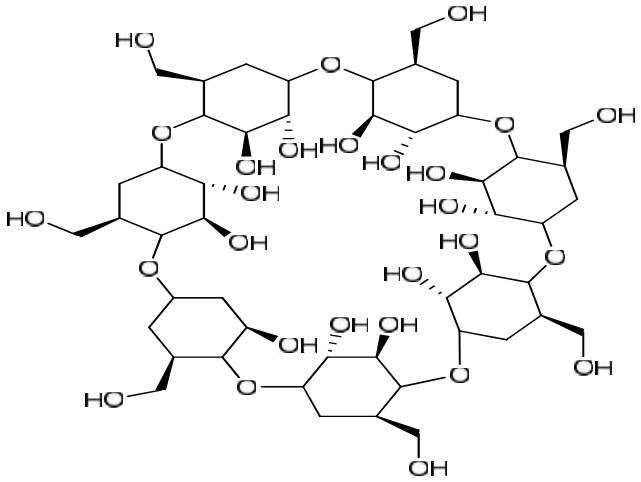


Figure 2 Structure of β-cyclodextrin



Experimental

Phase-solubility analysis

Phase-solubility assay for determination of stoichiometry of methyl-pioglitazone–β-CD binary system was performed according to the method of Higuchi and Connors. An amount of methyl-pioglitazone (15 mg) was added to the dissolved β-CD in phosphate buffer saline, PBS (10 ml; pH 7.4) at various concentrations (2–16 mM). The concentration range of β-CD was set based on the maximum solubility of β-CD in water at 37 ^{0}C (1.85 g/ 100 ml & 16 mM/L at 25 ^{0}C). The contents were stirred for 72 h at 37 ± 1 ^{0}C. After equilibrium, the samples were filtered and absorbance was read at 311.2 nm. The apparent stability constant was calculated from the slope of the phase-solubility diagram using Equation 1.

$$Kc = Slope/S_o (1\text{-slope}) \quad (1)$$

Where S_o is the solubility of drug in the absence of β-CD.

Preparation of inclusion complexes

The physical mixture (PM) of methyl-pioglitazone and β-CD in 1:1 M ratio was obtained by mixing individual component that had previously been passed through ~100 µm sieve. Freeze drying method with slight modification was used to synthesize the inclusion complex by dissolving methyl-pioglitazone and β-CD in water in 1:1 mM and pH was adjusted up to 4.5 with 0.02 M hydrochloric acid. The resultant solution was stirred for 24 h in orbit shaker at room temperature. Subsequently, the solution was lyophilized and sample obtained was collected and passed through ~100 µm sieve.

Physico-chemical characterization of solid binary systems

Fourier-transform infrared spectra (FTIR)

Fourier-transform infrared spectra (FTIR) were recorded for methyl-pioglitazone, β-CD, and methyl-pioglitazone–β-CD inclusion complex.

Powder X-ray diffraction (p-XRD) Powder

Powder X-ray diffraction (p-XRD) of methyl-pioglitazone–β-CD inclusion complex was performed using a RIGAKU, Rotaflex in the range of 20-80^0.

Molecular modeling

Geometry optimization and calculation of the transition energies, molecular modeling studies were carried out to understand the inclusion orientation of methyl-pioglitazone in β-CD. The minimum energy structure was calculated in gas phase using semiempirical AM1 (Austin model 1) method implemented in Hyperchem 8.0 software.

Nuclear magnetic resonance (NMR) measurements

^{1}H-NMR spectra were recorded with BRUKER 300 MHz spectrometer. ^{1}H NMR of methyl-pioglitazone was performed in DMSO-d_6 at 0.04 mg/ml, whereas β-CD and inclusion complex (methyl-pioglitazone–β-CD; 1:1) in D_2O solution.

Determination of solubility

An excess amount of methyl-pioglitazone was added to 10 ml of PBS (pH 7.4) and shaken vigorously for 24 h at 37 ^{0}C. Subsequently, the contents were centrifuged and supernatant liquid

was filtered through 0.22-µm membrane filter and analyzed in UV-Visible Spectrophotometer. The above procedure was repeated for the methyl-pioglitazone-β-CD inclusion complex and a mean of three observations were calculated.

Results

Phase-solubility analysis

Figure 3 represents the phase-solubility diagram for methyl-pioglitazone–β-CD binary system and shedow a linear increase in solubility of methyl-pioglitazone with increasing concentration of β-CD. The aqueous solubility of methyl-pioglitazone increased linearly (slope 0.047) as a function of β-CD concentration. The stability constant (Kc) of methyl-pioglitazone and β-CD complex (1:1) was calculated as 0.1 mM^{-1} from the linear plot of the phase solubility diagram.

Characterization of solid complexes

FTIR spectra were recorded to analyze the stretching bands FTIR spectrum peaks of methyl-pioglitazone, β-CD and the inclusion complex are shown in **Table 1**. The FTIR spectrum of methyl-pioglitazone shows a characteristic peak at 1,755 cm^{-1}, indicating the presence of carbonyl. Other peaks of methyl-pioglitazone observed are 1,425 and 1,388 cm^{-1} for N–CH_3 bending vibrations and 2,944, 2,882, 2,842, 2,798 cm^{-1} due to CH_3 groups. The spectrum of pure β-CD reveals the vibration of free –OH groups between 3,300 - 3,500 and 2,925 cm^{-1} for C–H stretching as in CH_2 and for CH_3. However, insertion by means of inclusion complexation in 1:1 completely masked the characteristic peak such as 2,898 cm^{-1} indicating the absence of phenyl group or insertion of phenyl in the β-CD cavity. We next attempted to define the crystalline structure of the drug in the inclusion complex using pXRD technique and peaks were intense and sharp indicating its crystalline structure while the inclusion complex of methyl-pioglitazone with β-CD showed peaks of diminished intensity.

Molecular modeling

We have listed the heat of formation of both orientations derived by semi-empirical methods (AM1 and PM3) in **Table 2**. Molecular structures of methyl-pioglitazone–β-CD inclusion complex (-phenyl) in orientation. The results of molecular modeling showed peak of phenyl protons indicated the insertion of phenyl protons of methyl-pioglitazone in β-CD cavity was formed.

Nuclear magnetic resonance

NMR spectroscopy provides the most direct evidence for true inclusion complex formation. There is a significant chemical shift in protons of methyl-pioglitazone when encapsulated in β-CD as the host molecule. All the aromatic protons of the methyl-pioglitazone are deshielded upon complexation. However, the phenyl protons of methyl-pioglitazone upon complexation with β-CD are shielded. (**Table 3**)

Powder X-ray diffraction

X-ray diffraction of the inclusion complex of the methyl-pioglitazone with beta cyclodextrin clearly indicates the amourphous nature and showed the presence of the methyl-pioglitazone and beta cyclodextrin as in **Figure 4**.

Solubility study

To express the potential of β-CD in solubilizing the guest molecule, the apparent solubility of methyl-pioglitazone in the aqueous solution was measured, which was significantly increased by methyl-pioglitazone complexation with β-CD, unlike the pure methyl-pioglitazone that has more affinity for the non-aqueous phase. Methyl-pioglitazone–β-CD inclusion complex has a solubility of 30.5 mg/L in the aqueous phase, whereas drug alone showed solubility of 3.90 mg/L in the aqueous phase. This represent about 9-fold increase in aqueous solubility of methyl-pioglitazone.

Discussion

Complex determination in solution state

It is well appreciated that phase-solubility studies are widely accepted for evaluating drug solubility behavior. The determination of Kc between drug and CD is based on the measurement of an index of changes in physico-chemical properties of a drug upon inclusion. Most methods for determining the Kc values are based on the determination of concentration of drug by titration experiments. Hence, the pharmaceutical applications of CD are to increase drug solubility and stability in aqueous phase. We thus choose this method for the evaluation of the effect of CD complexation on methyl-pioglitazone solubility. The 1:1 drug/CD complex is the most common type of association where a single drug molecule is included in the cavity of one CD molecule, with a stability constant,

Table 1 Peaks in FTIR spectra of methyl-pioglitazone, β-CD, and methyl-pioglitazone–β-CD inclusion complex

Sample	Infra-red stretching (¬=cm^{-1})
Methyl-pioglitazone	2945 (Aliphatic CH), 2889 (Aliphatic CH), 1705 (Carbonyl group), 1465 (Ether group), 1248 (C-C bond)
β-CD	3296 (O-H), 2910 (Aliphatic CH), 1142 (C-C bond), 1035 (C-O-C)
Methyl-pioglitazone-β-CD	3205 (O-H), 2942 (Aliphatic C-H), 1715 (Carbonyl group), 1462 (Ether group), 1240 (C-C bond), 1098 (C-O-C)

Table 2 Heats of formation of methyl-pioglitazone, β-CD, and methyl-pioglitazone–β-CD inclusion complex

Sample	aH_f (Kcal/mol)	
	^ j N	**PM3**
Methyl-pioglitazone	-132.54	-140.87
β-CD	-1418.65	-1425.05
Methyl-pioglitazoneβ-CD	-1621.45	-1687.12

Table 3 ^{1}H-NMR data of pioglitazone, β-CD, and pioglitazone–β-CD inclusion complex

Sample	^{1}H-NMR data (¢)
Methyl-pioglitazone	^{1}H-NMR (δ , d_6-DMSO): 3.91 (CH_2CH_2), 6.50-6.62 (ArH), 7.49–7.55 (ArH), 7.78 (C_6H_4CH═C)
β-CD	^{1}H-NMR (δ , d_6-DMSO): 3.42-4.15 (Aliphatic C-H), 2.78-3.25 (O-H);
Methyl-pioglitazone-β-CD	^{1}H-NMR (δ , d_6-DMSO): 7.05-7.12 (ArH), 2.12-3.43 (Aliphatic C-H) 165.5, 167.9

Figure 3 Phase-solubility diagram of pioglitazone in β-CD solution.

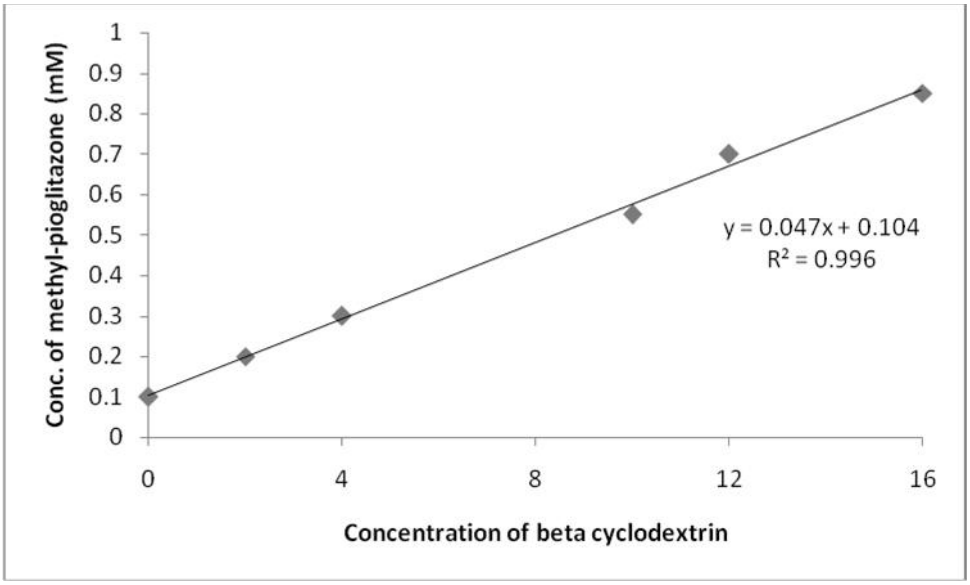
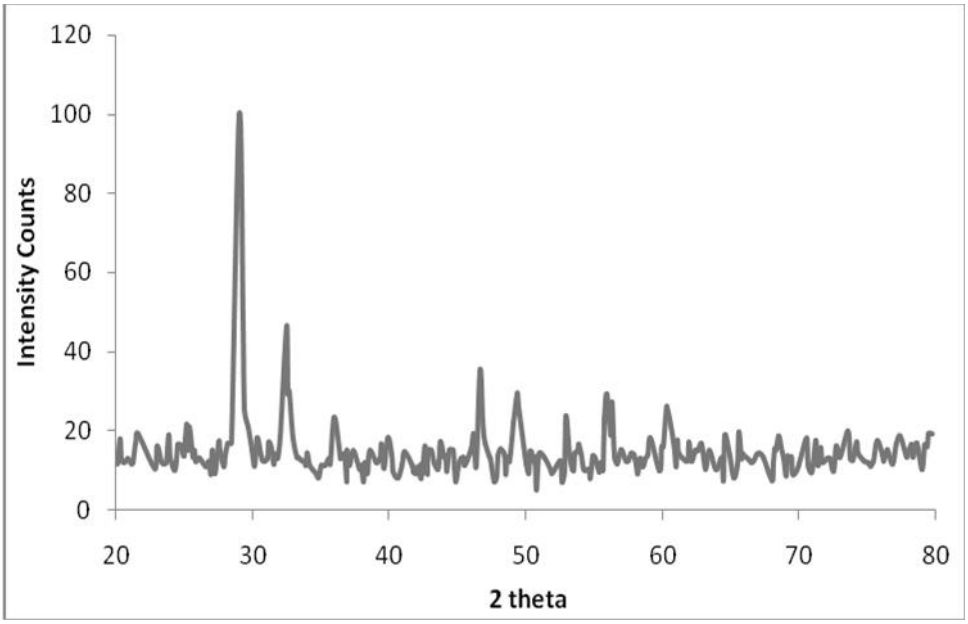


Figure 4. Powder X-ray diffraction of the inclusion complex of the methyl-pioglitazone with beta cyclodextrin



K 1:1 for the equilibrium between the free and associated species. This relationship suggests a first order kinetics on complex formation between methyl-pioglitazone and β-CD. K_c value was 0.1 mM^{-1}, at pH 7.4, indicating the formation of a stable complex with low affinity. Our data suggest that the increase in drug solubility observed was possibly due to the formation of a 1:1 inclusion complex.

Complex determination in solid states

The phase-solubility analysis of methyl-pioglitazone–β-CD inclusion complex indicated the formation of 1:1 complexation. However, to explore the molecular mechanism in the inclusion mode, spectroscopic techniques were used to elucidate the structure of the inclusion complex and correlated with the molecular modeling. However, FTIR data indicated that CH_3 moiety of methyl-pioglitazone is involved in the formation of inclusion complex. Phase-solubility analysis confirmed the stoichiometry (1:1) of complex in solution state. Furthermore, PXRD analysis was conducted to express the crystalline structure of drug in inclusion complexes. Therefore, PXRD pattern of methyl-pioglitazone, β-CD and inclusion complexes were recorded. Methyl-pioglitazone–β-CD inclusion complex (1:1) exhibited the peaks of diminished intensity in comparison with methyl-pioglitazone showed the sharp peaks. This suggested that methyl-pioglitazone is present in the high energetic amorphous state in methyl-pioglitazone–β-CD inclusion complex (1:1). This feature of methyl-pioglitazone in inclusion complex would favor the enhancement of solubility in aqueous phase. Stability study analysis supported that the complexes were stable at 40 ^{0}C/75% after 3 months storage.

Structure elucidation by molecular modeling and NMR spectroscopy

Physical characterization of solid complexes confirmed the formation of methyl-pioglitazone–β-CD inclusion complex (1:1). However, to elucidate the structure of the inclusion complex, molecular modeling and NMR spectroscopy were used. Molecular mechanics calculations showed the lower minimum energy structure calculated by AM1 and PM3 methods and proposed that phenyl moiety fit better in to hydrophobic vicinity of the β-CD. NMR techniques have been widely used to investigate supramolecular assemblies in solution and structure of the resulting complexes. It is well known that the chemical shift of a given nucleus depends on its shielding constant and in turn is sensitive to medium effects. Therefore, changes in δ (ppm) values of the host and guest nuclei can provide a measure of the degree of complex formation since significant changes in the microenvironment are known to occur between free and bound states. As the chemical environment of some protons changes upon complexation, there is a consequent variation in the chemical shifts of ^{1}H-NMR data.

Conclusion

The molecular structure of the inclusion complex of methyl-pioglitazone in β-CD was elucidated. It was observed that phenyl protons of the methyl-pioglitazone turned to non-equivalent state after inclusion in the β-CD cavity, whereas these protons were equivalent in the chemical structure of methyl-pioglitazone. Hence, we propose that the inclusion mode involves phenyl in orientation in agreement to AM1 optimized structure.

Acknowledgments

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