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Nanoaggregation of inclusion complexes of glibenclamide with cyclodextrins

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ABSTRACT

Glibenclamide is a sulfonylurea used for the oral treatment of type II diabetes mellitus. This drug shows low bioavailability as consequence of its low solubility. In order to solve this problem, the interaction with cyclodextrin has been proposed. This study tries to provide an explanation about the processes involved in the formation of GB-βCDs complexes, which have been interpreted in different ways by several authors. Among native cyclodextrins, βCD presents the most appropriate cavity to host glibenclamide molecules showing A_L solubility diagrams ($K_{1:1} \approx 1700\,M^{-1}$).

However, A_{L^-} solubility profiles were found for βCD derivatives, highlighting the coexistence of several phenomena involved in the drug solubility enhancement. At low CD concentration, the formation of inclusion complexes can be studied and the stability constants can be calculated ($K_{1:1} \approx 1400\,M^{-1}$). Whereas at high CD concentration, the enhancement of GB solubility would be mainly attributed to the formation of nanoaggregates of CD and GB-CD complexes (sizes between 100 and 300 nm). The inclusion mode into βCD occurs through the cyclohexyl ring of GB, adopting a semi-folded conformation which maximizes the hydrogen bond network.

As consequence of all these phenomena, a 150-fold enhancement of drug solubility has been achieved using β-cyclodextrin derivatives. Thus, its use has proven to be an interesting tool to improve the oral administration of glibenclamide in accordance with dosage bulk and dose/solubility ratio requirements.

1. Introduction

Glibenclamide (GB) or glyburide is an oral hypoglycemic agent from the second generation of sulfonylureas. This drug is widely used for the treatment of type II diabetes mellitus (non-insulin dependent). In addition, recent studies have demonstrated its ability to prevent cerebral ischemia and hemorrhagic stroke (Caffes et al., 2015; Simard et al., 2014). In 2015, glibenclamide was included in the World Health Organization model List of Essential Medicines (World Health Organization Expert Committee, 2015). From a physico-chemical point of view, glibenclamide shows a lipophilic character and it is ascribed to the group II of the Biopharmaceutical Classification System (low solubility, high permeability). The main issue of its oral administration is its low bioavailability, consequence of its low solubility in physiological media. Therefore, the dissolution of glibenclamide is considered to be the rate limiting step, as its absorption after oral administration reaches 45% of the initial amount of drug (Sanghavi et al., 1994).

Different methods and strategies have been proposed to increase the solubility of this type of lipophilic drugs, including the particle size reduction (Liversidge and Cundy, 1995), incorporation of hydrophilic polymers (i.e. PEG, PVP, HPMC, alginate, starch derivatives, etc.) (Kumar et al., 2011), formation of inclusion complexes (Salústio et al., 2011) or incorporation in solid dispersions (Vasconcelos et al., 2007), nanoemulsions (Chen et al., 2011) or nanoparticles (Ponchel and Irache, 1998). In this context, the inclusion complex method has been proven to be one of the most effective when trying to increase aqueous solubility and release rate in a wide variety of drugs (Uekama, 1979). Furthermore, it allows the removal of unpleasant odors and flavors

Abbreviations: GB, Glibenclamide; αCD, α-cyclodextrin; βCD, β-cyclodextrin; HP-βCD, 2-hydroxypropyl-β-cyclodextrin; RM-βCD, randomly methyl-β-cyclodextrin; γCD, γ-cyclodextrin; DLS, dynamic light scattering; TEM, transmission electron microscopy; CE, complexation efficiency.

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while increasing chemical and physical stability of certain drugs (Saenger, 1980; Uekama, 1981).

For this purpose, inclusion complex based on the use of cyclodextrins are largely preferred. Cyclodextrins are cyclic oligosaccharides with a hydrophilic outer surface which are able to host non-polar groups of hydrophobic molecules in their internal cavity. Additionally, its oral administration has shown to be practically non-toxic (Arima et al., 2011; Luke et al., 2010), making them particularly interesting for its use in food and pharmaceutical applications. Among the different classes of cyclodextrins, oligosaccharides based on β-cyclodextrin (βCD) have shown to be the most appropriate to host molecules with aromatic rings. However, the use of βCD in solid oral dosage forms is conditioned by its low aqueous solubility. For this reason, soluble derivatives of βCD have been developed over the past few years. Some worth-mentioning examples are 2-hydroxypropyl-β-cyclodextrin (HP-βCD) (Zoeller et al., 2012) and random methylated-β-cyclodextrin (RM-βCD) (Arun et al., 2008).

The interaction of GB with different cyclodextrins has been previously reported and the use of βCD has shown to increase *in vivo* bioavailability of GB (Gerlóczy et al., 1996). However, discrepancies arise when interpreting solubility diagrams and regarding the mechanism of drug inclusion into the cyclodextrin cavity for GB:βCD and GB:HP-βCD systems. A_L type (Savolainen et al., 1998; Radi and Shimaa, 2010; Zerrouk et al., 2006; Obaidat and Ababneh, 2009; Cirri et al., 2009) and *B* type (Prasad et al., 2014) solubility diagrams were reported for GB:βCD systems, whereas A_L type (Sanghavi et al., 1994; Zerrouk et al., 2006; Cirri et al., 2009; Esclusa-Díaz et al., 1994) and A_P type (Savolainen et al., 1998) were reported for GB:HP-βCD systems. As the type of solubility diagram indicates the possible stoichiometry of the inclusion complexes (Higuchi and Connors, 1965), different interpretations arise about the mechanism of complexation between cyclodextrins and glibenclamide. Moreover, the aforementioned studies reported inconsistencies in stability constants, which is common when assessing drugs with very low aqueous solubility.

The aim of this investigation was to study in depth the complexation process between glibenclamide and different cyclodextrins (native and derivatives) and to establish the different processes involved in the enhancement of glibenclamide solubility, that could be explained by inclusion and non-inclusion processes simultaneously.

2. Materials and methods

2.1. Materials

Glibenclamide was supplied by Sigma-Aldrich (Spain). Native cyclodextrins (α, β and γCD), random methylated-β-cyclodextrin (RM-βCD) and 2-hydroxypropyl-β-cyclodextrin (HP-βCD), with an approximate substitution degree of 12 and 4.5 respectively, were supplied by Cyclolab (Hungary). All aqueous solutions were prepared with deionized water obtained from a commercial Millipore Elix 3 system. (0.1 mS/cm conductivity).

2.2. Phase solubility studies

The effects of cyclodextrins on the solubility of glibenclamide were studied in phosphate buffer solutions (pH 7.4). An excess of GB was added to different solutions containing increasing amounts of αCD, βCD, γCD (from 1 to 12 mmol/L for native CDs), HP-βCD and RM-βCD (from 1 to 80 mmol/L for βCD derivatives). Sealed glass containers were magnetically stirred at constant temperature (37 °C) until equilibrium was reached (4 days). After equilibrium, an aliquot of solution (3 mL) was withdrawn with a syringe filter (pore size 0.45 μm) and GB concentration was determined at 300 nm by UV–vis molecular absorption spectrophotometry (Hewlet Packard 8452A diode-array spectrophotometer). Each experiment was performed in triplicate (coefficient of variation CV <4.5%).

2.3. Molecular docking

As complementary data and to verify the experimental results, a molecular modeling study was conducted in order to determine the most favourable conformation of the inclusion complexes, by means of a docking protocol, taking the analyzed cyclodextrin as the receptor (host) and glibenclamide as the ligand (guest).

The calculations were performed on SGI Virtu VS100 workstation, provided with MOE2015.1001 software package. The reference models LILLUM (Caira et al., 1998) and XUCMEP (Trollope et al., 2014) were obtained from the Cambridge Structural Database (CSD System version 5.35: search and information retrieval with ConQuest (Bruno et al., 2002) version 1.18, structure visualization with Mercury (Macrae et al., 2006)) were used as templates for building the initial models for the analyzed β-cyclodextrins. The substitution degree and the location of the 2-hydroxypropyl chain were selected according to the data obtained in recently published studies (Tang et al., 2016).

The 3D model of glibenclamide was constructed in a Born implicit solvent model (dielectric constant = 80), using atoms from the corresponding Builder module of the MOE2015 suite and using the implemented MMFF94× force field. A preliminary optimization was carried out with a root mean square gradient of 0.001 Kcal mol^{-1} $Å^{-2}$ as completion criterion.

2.4. ^1H and 2D NMR analysis

Monodimensional ^1H NMR and 2D NOESY spectra were recorded at 298 K on a Bruker Advance DMX 400 spectrometer at a proton resonance frequency of 400 MHz. Samples were prepared in D_2O (99.99% in deuterium purchased from Sigma Aldrich) and DMSO using the residual HDO signal as reference. For monodimensional analysis 256 scans were carried out. NOESY experiments were performed on 32 scans with presaturation of the solvent signal by using an optimal mixing time of 500 ms.

2.5. Dynamic light scattering

The size distribution of the different particles was determined by dynamic light scattering (DLS) using a DynaPro photon correlation spectrometer at 25 ± 0.1 °C, equipped with a 248 channel multi-tau correlator. The wavelength of the laser was 825.2 nm. Saturated solutions of glibenclamide using CD concentration of 1, 3, 5, 12, 30 and 80 mmol/L in pH 7.4 were used after filtration through a 0.45 μm filter. The intensity of size distribution was calculated by the method of regularization with DynaLS 1.0 software, expressed in terms of the hydrodynamic radius (R_h) calculated from the Stokes-Einstein equation (Eq. (1)):

$$R_h = \frac{kT}{6\pi\eta_0 D_0} \quad (1)$$

where T is the absolute temperature, k the Boltzmann constant, η_0 the viscosity of the solvent and D_0 the diffusion coefficient at infinite dilution.

2.6. Transmission electron microscopy analysis

The morphology and size of the aggregates in solution were analyzed using a transmission electron microscope. The samples