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Spectral and theoretical study on complexation of sulfamethoxazole with β- and HPβ-cyclodextrins in binary and ternary systems

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ABSTRACT

The inclusion complexes of sulfamethoxazole (SMX) with β-cyclodextrin (βCD) and (2-hydroxypropyl) β-cyclodextrin (HPβCD) were prepared. Fluorescence spectroscopy and electrospray mass spectrometry, ESI-MS, were used to investigate and characterize the inclusion complexation of SMX with cyclodextrins in solutions. Whereas in the solid state the complexes were characterized by Fourier transform infrared spectroscopy (FTIR), powder X-ray diffraction (PXRD) and Raman techniques. Enhanced twisted intramolecular charge transfer (TICT), emission as well as local excited (LE) bands were observed upon addition of HPβCD indicate that SMX enters deeper into the cyclodextrins cavity. The stoichiometries and association constants of these complexes have been determined by monitoring the fluorescence data. The effect of presence of ternary components like arginine and cysteine on the complexation efficiency of SMX with cyclodextrins was investigated. Molecular Dynamic simulations were also performed to shed an atomistic insight into the complexation mechanism. The results obtained showed that complexes of SMX with both cyclodextrins are stabilized in aqueous media by strong hydrogen bonding interactions.

1. Introduction

The glorious history of sulfonamides began with fame as the first class of true antimicrobial agents with life-saving potency and these molecules are still considered as an outstanding group having the potential to cure severe bacterial infections. Nevertheless, the introduction of newly safer antibiotics reduces its attractiveness, sulfonamides still account for ~10–15% of the total number of antimicrobial agents used worldwide. Sulfamethoxazole (SMX) is one of the most widely prescribed drugs from sulfonamide family, which is frequently used in human medicine to treat bronchitis and urinary tract infections. The pharmacological applications of SMX in humans, animals and even in the aquaculture industry to control fish diseases have been reviewed extensively, so there is a wealth of data on their activity as bacteriostatic antibiotics [1–6]. Chemically, SMX is one example of an electron donor-acceptor system in which SO_2 group connecting the aniline moiety to the isoxazole ring through an amide S—N bond. The mechanism of antimicrobial action of these drugs is highly influenced by the presence of these two moieties and is also believed to be central to the pathogenesis of hypersensitivity reactions.

Cyclodextrins (CDs) occupy a prominent place in supramolecular chemistry and these cyclic oligosaccharides have a rigid, well-defined ring structure and an ability to incorporate in their hydrophobic cavity various inorganic and organic molecules *via* hydrophobic and van der Waals interactions and its applications have been discussed in myriads of publications [7–15]. It was reported that the inclusion complexation with cyclodextrins significantly enhanced the solubility, dissolution rate, stability and bioavailability of SMX. All these studies applied UV–visible spectrometry, circular dichroism, FT-IR spectrometry, molecular mechanics and Molecular Dynamics as well as thermodynamic methods to investigate the host-guest interaction [16–19].

Recently, there have been increasing interests in the effect of ternary agents on inclusion complexation and the effect of co-guest on the properties of cyclodextrin inclusion complexes [20–26]. Amino acids especially arginine has been widely studied as a ternary agent to enhance binding stability, solubility and bioavailability of several pharmaceuticals [20–23,27]. On the other hand, low molecular mass hydroxyl-acids such as maleic, tartaric and citric acids have also been used as synergistic ternary agents [23,28]. The mode of interaction of these ternary agents is still not clear, however, from molecular modeling studies it is inferred that compounds like arginine and citric acid may participate in strong hydrogen bond interaction with the cyclodextrin or with the protruding motifs of the guest molecule resulting in enhanced binding of the complex. It is also worth noting here, that the use of polymers in pharmaceutical formulations over the years has inspired many

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authors to use soluble polymers such as poly(vinylpyrrolidine) (PVP) and poly(ethyleneglycol) (PEG) as well as surfactants as ternary complexing agents [24–26,29–32]. It has also been reported that addition of βCD to poly(acrylic acid) (PAA) and PEG facilitated controlling the size of inter-polymer complexes which improved the drug carrier effect for the anticancer agent doxorubicin [33].

Despite being a proven SMX-cyclodextrin complexation, none of the above mentioned works studied extensively the geometric fitness of SMX inside the β-cyclodextrin (βCD) and (2-hydroxypropyl) β-cyclodextrin (HPβCD) cavity using fluorescence spectroscopy. Therefore, the main purpose of this work is to study the effect of inclusion of SMX within the cyclodextrins hydrophobic interior on its fluorescence intensity. Moreover, freeze-drying methods were employed to prepare inclusion complexes of SMX with βCD and HPβCD, which were characterized by ESI-MS, FTIR, RXRD and Raman techniques.

It is generally considered that the "loose" binding environment inside the CD cavity causes the solvent molecules to occupy the void space and leads to decrease in complexation efficiency. Therefore, it seemed of interest to extend our studies on the multicomponent complex formation as a strategy for improving the SMX-CDs interaction and to investigate the effectiveness of amino acids like arginine and cysteine as ternary components on the fluorescence of SMX. Finally, host-guest interaction in the complex was simulated by Molecular Dynamics to explain the inclusion interactions and therein the inclusion model could be proposed. In this work we focused on hydrogen bond interactions between the hosts and the guests to highlight the differences in stabilities between the two complexes.

2. Experimental

2.1. General

All reagents and solvents used in this study were obtained from Sigma-Aldrich Chemical Company (USA) and used without further purification. Ultrapure water was obtained in the laboratory using a Milli-Q water purification system (Millipore, Billerica, MA, USA). IR spectra were recorded by using Cary 660 FTIR (Agilent technologies) spectrometer. A Shimadzu (model multispec-1501) UV–Vis spectrophotometer (Shimadzu, Japan) and a Perkin Elmer (model LS 55) Luminescence spectrometer (Perkin Elmer, USA) were used to collect absorption and fluorescence spectra, respectively. All measurements were done repeatedly, and reproducible results were obtained. Crystallographic studies were done in X Pert PRO (PANalytical, USA) X-ray diffractometer using Cu Kα (λ = 1.54 Å) on powder samples. The Raman spectra were excited with a laser of 532 nm and recorded on samples using a Horiba XploRA spectrometer (HORIBA scientific, USA). Mass spectra were measured with Micromass Quattro Ultima Pt (Waters Corp. MA, USA) equipped with an ESI source and operated in the negative and positive ion mode.

2.2. Preparation of Complexes

Inclusion complexes for the fluorescence study were prepared by mixing appropriate solutions of SMX with cyclodextrins. In all experiments SMX concentration was fixed at 6.0×10^{-6} M while the concentration of cyclodextrins was varied between 0 and 16 mM. The solid inclusion complexes of SMX with βCD and HPβCD were prepared by the freeze drying methods. In all cases SMX solid was added to 25 mL of 15 mM cyclodextrin solutions in stoppered vials. The mixture was sonicated for 30 min and then placed in an incubator-shaker at 40 °C for 72 h. The reaction mixture was filtered and the filtrate was freeze dried. The product from the freeze dryer was stored in a desiccator for further characterization. The physical mixtures were prepared by grinding solid CDs and SMX at a mole ratio of 1:1 in a pestle-mortar and also the resultant mixtures were kept in desiccator for further analysis.

2.3. Molecular Modeling

ChemBio3D-Ultra [34] and MOPAC 2012 [35] were used to perform all calculations. The initial geometry of drugs were obtained from the crystal structure and optimized using DFT-B3LYP method using 6-31G* basis set. On the other hand, the structures of β-cyclodextrin (βCD) was extracted from the crystallographic parameters provided by the Structural Data Base System of the Cambridge crystallographic data center and optimized using PM7 semiempirical method [36]. The structure of the 2-hydroxypropyl-β-cyclodextrin (HPβCD) was built on the structure of βCD by substitution of 2-hydroxypropyl moieties randomly at O2 and O6 positions on the glucose units of βCD as a representation of HPβCD mixtures.

Autodock program (version 4.2) was used to generate the inclusion complexes by docking guest into the cyclodextrins nanocavities [37]. *Autodock* tools were then utilized to perform cluster analysis for all inclusion complexes generated using a cutoff of 1.0 Å root mean square deviation (RMSD) [38]. The lowest energy structures which correspond to the cluster with maximum number of conformations were obtained for guest-CD complex where the guest molecule enters through secondary hydroxyl rim of the CD. The structure of the ternary complex, SMX$(\beta CD)_2$ was based on the binary complex obtained from the docking experiment. Different orientations for the two βCD host molecules were investigated. The energies of all complexes were minimized using PM6 method and the minimum energy complex was found to be the head-to-head complex where the two host molecules face each other through the secondary hydroxyl group rims. This conformation allows for the formation of the maximum number of hydrogen bonds and thereby stabilizing the complex.

Molecular Dynamics (MD) simulations were carried out using the Desmond molecular simulations package, as distributed by Schrodinger-2015 suite of programs [39–41]. The OPLS_2005 all-atom force field with explicit solvent (TIP3P water model) were used throughout the calculations. Simulations were run with periodic boundary conditions, in an orthorhombic box with the solutes placed in the middle at 20 Å distance from each of the boxes edges. The SHAKE algorithm was used to constrain covalent bonds between hydrogen and heavy atoms. Long-range electrostatic interactions were dealt with the Ewald smooth particle mesh (PME) method [42]. The solvated molecules were subjected to sequential restraint solvent-solute minimization and short MD simulations on NVT-NPT ensembles (as implemented in the default relaxation protocol in Desmond) coupled to Brendsen thermostat. Finally the production run was NPT run at 300 K and 1 bar. The simulations were then analyzed by the "*simulation event analysis*" module in Schrodinger 2015 suit.

3. Results and Discussions

3.1. Fluorescence Spectroscopy

In aqueous solution, it is observed that SMX shows a strong locally excited (LE) band around 350 nm along with a shoulder peak at 320 nm and both bands are believed to be essentially π-π* in nature. SMX serves as a proper model of electron donor- acceptor (D-A) system in which the aniline moiety and an isoxazole ring are positioned at the end of the structure (Fig. 1); meanwhile the two main parts are connected with the SO_2 moiety through the NH bond. From these considerations it is clear that there is a chance for TICT state in SMX which depends on the twisting of molecular fragments at SO_2—NH bond and is reported in some literature [43]. Due to the very efficient non-radiative bond twisting process in its excited state, the fluorescence spectra of SMX recorded in pure water show the absence of CT emission band. On the emission profile of SMX, an increasing concentration of βCD is found to introduce significant changes in emission intensity along with a slight blue shift denotes the movement of SMX from polar environment to a nonpolar, hydrophobic cyclodextrin cavity as