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Physicochemical investigation of the complexation between γ-cyclodextrin and doxorubicin in solution and in solid state

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ABSTRACT

The importance of Doxorubicin (Dox) as anti-cancer drug is well recognized. Dox side effects are however a major drawback in an efficient medicinal utilization. Cyclodextrin inclusion is an effective approach to enhance drug delivery and stability. In this research host − guest complex formation of Dox cytotoxic drug with gamma-cyclodextrin (γCD) in aqueous solutions and in solid state was investigated by differential scanning calorimetry (DSC), thermogravimetry (TG), Fourier transform infrared spectroscopy (FT-IR) analysis, scanning electron microscopy (SEM), ultraviolet-visible (UV–vis) spectroscopy, isothermal titration calorimetry (ITC) and pH measurements. The thermodynamic parameters were discussed considering the weak interactions between γCD and Dox molecules. UV–vis and ITC gave comparable results regarding the formation constant and thermodynamic parameters values. The data indicated that the γCD/Dox complex in the aqueous solution is formed in a 1:1 stoichiometry ratio and the binding process of γCD with Dox is exothermic and enthalpy controlled, but entropy driven. The solid state characterization of the γCD/Dox complex indicated the occurrence of complexation by encapsulation of the hydroxyanthraquinonic rings of Dox into γCD cavity and come to support and complete the data resulted from liquid state investigation.

1. Introduction

Doxorubicin (Dox) is an antimitotic and cytotoxic agent and it is obtained by partial chemical synthesis from cultures of Streptomyces peucetius var. caesius bacterium [1,2]. Dox belongs to antracyclines class of drugs from the aromatic polyketides group [1]. It occurs in several different forms due to the structural modifications in the aglycone part and the various sugar residues linked [3,4]. The cytotoxic activity of Dox is influenced by the presence of electron donating groups in its structure, thus Dox acting as a powerful inhibiter of DNA and RNA synthesis [2,3–5]. Its therapeutic applications include the treatment of various cancers but these important therapeutic properties are associated with a high toxicity, especially the cardiotoxicity [1,6,7]. However, the side effects depend on higher or lower cumulative treatment doses of Dox which are responsible for immunosuppressive activity of the body, allergic reactions and also active oxygen species generation [1]. Dox is a hydrophobic drug and Doxorubicin hydrocloride was formulated as a Dox salt to get higher water solubility and maintain the same therapeutic effect as free Dox [8]. Dox hydrochloride is a weak chemical base and a strong fluorescent dye depending upon the available light [9]. At various pH values Dox can present many prototropic forms from which depends its therapeutic efficiency [3,4]. The chemical structure of main prototropic form of Dox (98.42%) at pH of 6.2 and temperature of 298.15 K is shown in Fig. 1.

Cyclodextrins are macrocyclic oligosugars, shaped like an intrusive truncated cone which is a relatively hydrophobic in the middle and relatively hydrophilic on the outside because of the lack and presence, respectively, of the hydroxyl groups. Thus, CDs are designed to have a high ability to form inclusion complexes in solid state and in aqueous solution with many types of molecules or parts of large molecules depending upon their size, shape and hydrophobicity. When a guest molecule fit properly within the CD's cavity it can have highly different properties compared to that of the guest molecule alone. The stability of CDs inclusion complexes is determined by the size of guest molecules and geometric properties of CD cavity which is related with the number of glycosidic units [10,11]. According to literature, the main forces involved in the inclusion of guest molecules into CD cavity are van der Waals, hydrophobic interactions, hydrogen bonding, electrostatic interactions, charge-transfer interactions, release of conformational strain and release of bond water from CD cavity [10–13]. The association of Dox with various macromolecules was investigated in different interaction media and the main purpose of these studies was focused on the development of carriers for site specific Dox delivery having the aim to increase Dox clinical efficacy and to decrease its side effects [14–17].

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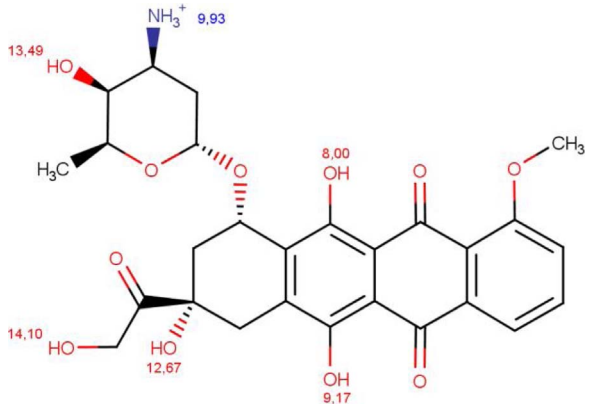

Fig. 1. The chemical structure of main prototropic form of Dox (98.42%) at pH of 6.2 and temperature of 298.15 K and pKa values.

The least toxic CD for the human body is the natural γCD which is composed of eight glucopyranose units and is more appropriate to complexate a great number of molecules or large sized molecules [10–16]. γCD is able to reduce the undesirable properties of Dox such as prevention of side effects through the formation of the γCD/Dox complex [6,15]. There are several techniques used to investigate the host–guest complexation between CDs and the Dox molecule. Regarding the properties of the γCD/Dox complex it was shown that in buffered media Dox binds to γCD with higher affinity than for other types of CDs. For γCD/Dox complex, often 1:1 stoichiometry was reported when diluted solutions of Dox were used and was established that only Dox monomer has antitumor efficiency [16–18].

In the present work, the comlpexation of Dox with γCD in aqueous solution was analyzed by means of ITC, UV–vis spectroscopy and pH measurements. The stoichiometry, apparent binding constants and thermodynamic parameters of the aqueous solutions of the γCD/Dox complex were calculated. Also, the γCD/Dox complex obtained by co-precipitation method and the solid powders of the pure substances were investigated by DSC, TG/DTG, FT-IR in attenuated total reflectance mode and SEM techniques. This work provides new data on γCD/Dox interaction which may be useful to make clear the relationship between the thermodynamic stability and the structural factors and to prove the complexation efficiency between Dox and γCD.

2. Experimental

2.1. Source and purity of samples

Doxorubicin hydrochloride (Dox) and gamma-Cyclodextrin (γCD) were purchased from Sigma Aldrich Chemical Company and used without further purification. The chemical identifiers and purity of the used chemicals are given Table 1. The purity of the purchased reagents was verified by Purity Application from Pyris software. The purity values obtained are 99.98 ± 0.12% for Dox and 99.42 ± 0.99% for γCD.

2.2. Preparation of the samples

The solid state inclusion compound γCD/Dox was prepared in a 1:1 molar ratio of the host and guest using “melting in solution” method, procedure previously reported in literature [19]. The solid powder of γ-CD was dissolved in double distilled water (10^{-5} mol/L) then the corresponding quantity of solid Dox hydrocloride was added in reaction. The red-orange mixture was firstly sonicated for 5 min.5 min at 45 kHz, and then stirred (600 rpm) for 24 h at room temperature. The γCD/Dox solid powder was resulted after dehydration under vacuum at 60 °C.

In order to avoid a predominance of the dimer over the monomer, small concentration of aqueous Dox were used in UV–vis and pH experiments, (concentrations of 10^{-5} mol/L for Dox and of 0–2 × 10^{-3} mol/L for γCD aqueous solutions). Considering the previous attempts, aqueous solutions with concentrations of 10^{-3} mol/L for Dox and 40.4 × 10^{-3} mol/L for γCD were suitable to accomplish the ITC measurements.

2.3. Apparatus and methods

2.3.1. Ultraviolet-visible (UV–vis) spectroscopy

All records of UV–vis spectra were carried out on a Carry 300 Bio spectrophotometer equipped with a temperature controlled cell holder in the range of 200–600 nm using 1 × 1 × 4 – cm micro quartz cells with teflon stopper. The stoichiometry of the inclusion complexes was assessed by continuous variation method (Job's method) by varying the mole fraction of each component (R = [γCD]/([Dox] + [γCD])) from 0 to 1 and the total molar concentration of the species is kept constant (10^{-5} mol/L). After 24 h the absorption spectra were recorded at 25 °C. The difference in absorbance (ΔA) measured at 473 nm between solutions containing only guest and the γCD/Dox mixtures, multiplied by the molar ratio of Dox was plotted as a function of the R of guest [20,21]. The stoichiometric ratio of the inclusion complex is corresponding to the point where the derivative of the curve is zero [22]. The quantitative determination of the apparent formation constant (*K*) of the inclusion complexes was done spectrophometrically at four different temperatures (298.15 ± 0.5 K, 303.15 ± 0.5 K, 308.15 ± 0.5 K and 313.15 ± 0.5 K) and pressure p = 0.1 MPa using a method based on Benesi – Hildebrand equation [22,23]. In order to obtain the *K* values the Dox concentration was kept constant (10^{-5} mol/L) and the γCD concentration was varied from 0 to 2 × 10^{-3} mol/L. The γCD solutions of corresponding concentrations were used in the reference cuvette [22,23].

2.3.2. pH measurements

A Thermo Scientific Orion 5-Star Plus benchtop meter with model 9107BN Triode 3-in–1 pH/automatic temperature compensation probe was used to measure the pH of the Dox, γCD solutions and γCD/Dox mixture solutions. All the measurements were made in a fixed volume of sample (2 mL) placed in a flask in a water bath with thermostat maintaining the temperature at 298.15 ± 0.5 K and p = 0.1 MPa.

2.3.3. Isothermal titration calorimetry (ITC)

Isothermal Titration Calorimetry (ITC) experiments were performed using an iTC200 high-sensitivity microcalorimeter (MicroCal Inc., Northampton, MA, USA) at ambient temperature and pressure (temperature of 298.15 ± 0.5 K and a pressure of 0.1 MPa). A calibration of the calorimeter was carried out electrically and the $CaCl_2$–EDTA titration was performed to ensure that the apparatus was operating correctly. All samples were degassed under vacuum, prior to the titration. The reference cell was filled with deionized and degassed water. The sample cell was filled with 280 μL of Dox solution and the titrant syringe was filled with 40 μL of γCD solution. In incremental ITC, 19 injections of 2 μL each (except 0.5 μL for the first injection) were

Table 1
Chemical identifiers and purity of the materials studied.

Chemical Name	CAS Number	Molecular weight [g/mol]	Source	Initial mass fraction purity, %[a]	Purification method
Doxorubicin Hydrochloryde	25316 – 40-9	579.98	Sigma-Aldrich	98.0 – 102.0%	None
Gamma-cyclodextrin	17465 – 86-0	1297.12	Sigma-Aldrich	≥ 98%	None