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Thermodynamic study of β-cyclodextrin-dye inclusion complexes using gradient flow injection technique and molecular modeling

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ABSTRACT

Gradient flow injection technique-diode array spectrophotometry was applied for β-cyclodextrin (β-CD)-dye inclusion complex studies. A single injection of a small amount of mixed β-CD-dye solution (100 μl) into the carrier solution of the dye and recording the spectra gave the titration data. The mole ratio data were calculated by calibrating the dispersion pattern using a calibrator dye (rose bengal). Model-based multivariate methods were used to analyze the spectral-mole ratio data and, as a result, estimate stability constants and concentration-spectral profiles. Reliability was tested by applying this method to study the β-CD host-guest complexes with several dyes as guest molecules. Singular value decomposition (SVD) was used to select the chemical model and reduce noise. Molecular modeling provided the ability to predict the guest conformation-orientation (posing) within the cavity of β-CD and the nature of the involved interactions. Among those dyes showing observable spectral variation, the stoichiometric ratio of β-CD: dye (and log K_f) of methyl orange, fluorescein, phenol red, 4-(2-pyridylazo) resorcinol (PAR), and crystal violet were calculated to be 1:1 (4.26 ± 0.01), 1:1 (1.53 ± 0.08), 1:1 (3.11 ± 0.04), 1:1 (1.06 ± 0.12), and 2:1 (5.27 ± 0.03), respectively. Compared with the classical method of titration, this method is simple and fast and has the advantage of needing reduced human interference. Molecular modeling facilitates a better understanding of the type of interactions and conformation of guest molecules in the β-CD cavity. The details of the proposed method are discussed in this paper.

1. Introduction

Naturally occurring α-, β-, and γ-cyclodextrins and their higher homologues are truncated cone-shaped molecules with a cavity of 7.9 Å depth. The top and bottom diameters of the cavity are 4.7 and 5.3 Å for α-cyclodextrin, 6.0 and 6.5 Å for β-cyclodextrin, and 7.5 and 8.3 Å for γ-cyclodextrin [1]. CDs have a hydrophobic central cavity which is able to include guest molecules to form inclusion complexes [2–4]. The inclusion process alters the photochemical and photophysical properties of the guest molecules. Thus, the physical, chemical, biochemical, and application capabilities of guest molecules can be modified through inclusion complexation. Because of this specific capability, CDs are used in drug delivery technology [5–8], separation and food industries [9], biotechnology [10], analytical chemistry [11–13], textile industry [14], and many other industries [15]. Also, interaction of CDs with various molecules serves as excellent models for understanding general inclusion phenomena [16].

The magnitude of stability constants is an important factor for application of CDs in different fields of analytical chemistry [1]. In textile industry, cyclodextrins form complexes with detergent molecules, and can act as defoaming agents. The formation of inclusion complexes may influence the performance of surfactants and CDs and it is important to have a measure of strength of formed complexes by stability constants [14]. Also CDs are used for removal of various dyes from aqueous solutions. The mechanism of absorption and desorption of these dyes on cyclodextrin polymers is directly related to the complexation strength of cyclodextrins [17]. The molecular encapsulation of lipophilic food ingredients with cyclodextrin improves the stability of flavors, vitamins, colorants and unsaturated fats, etc., both in physical and chemical sense leading to extended product shelf-life. Stability constants of molecular encapsulation are important for evaluating the performance of CDs in this context [9]. The most common pharmaceutical application of cyclodextrin is to enhance the solubility, stability, safety and bioavailability of the drug molecules. Since the changes of physicochemical and biological properties of a drug are dependent on the magnitude of the stability constant of CD complexes, it is important for prediction or simulation of the property change to determine accurately this parameter [18].

In the usual titration procedure for calculation of stability constants of CD complexes, a series of mixed cyclodextrin-guest solutions are prepared. The concentration of one component (usually the guest) is kept constant, while the concentration of the other (usually cyclodextrin) is

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varied. A comprehensive study of the phenomenon requires a wide range of concentration ratios, and manipulation is necessary in each step of titration [19,20].

Various instrumental analysis methods, including UV–Vis [21], spectrofluorometry [22], chromatography [23], circular dichroism and NMR [24], FT-IR [25], polarography [26], and surface tension measurements [27], have been applied to detect and study cyclodextrins inclusion complexes. Diode array spectrophotometry is a highly sensitive and suitable method for studying chemical equilibria in solutions because of the multivariate nature of its data. Besides of exploratory aspect, multivariate data in comparison with univariate data has advantages such as noise reduction, proper handling of interferents, and outlier control [28]. Using multivariate data in chemical equilibrium and kinetic studies provides extra tools for scientists that univariate data can't afford [29], including (a) the determination of the pure spectra and concentration profiles for all reacting species, (b) the possibility to apply a wide range of model free analyses, and (c) elimination of the need to determine a "good" wavelength for following the reaction.

Model-based nonlinear curve fitting is an invaluable instrument in the hand of any scientist interested in the quantitative analysis of measured data. The first and central component of any data analysis is the collection of measured data. The second component of data fitting is the model that is used to quantitatively describe the data, and the third aspect is the fitting algorithm, which determines the most likely values for the parameters of the model [30]. The most prominent algorithms are based on the Newton–Gauss–Levenberg–Marquardt algorithm [31].

The flow injection gradient technique is suggested by the authors as a suitable method for metal complexation titration and the study of the thermodynamics of these reactions [32]. Although the idea of application of flow injection analysis for calculation of stability constants has been reported in the past [33–36], gradient flow injection technique is simple, time-saving, and purely experimental without complex mathematics behind it. Furthermore, in comparison with the batch titration data, more precise results are achieved because of the intrinsic improved precision of the automated titration methods in comparison with non-automated titration methods.

Molecular modeling (docking of small molecules to the binding sites) was pioneered during the early 1980s [37], yet it remains a highly active area of research today [38–41]. The docking process involves the prediction of ligand conformation and orientation (posing) within the targeted binding [42]. In general, there are two aims of docking studies: accurate structural modeling and correct prediction of activity [43]. Molecular modeling has been applied in the study of interaction of macromolecules with different ligands as a complementary tool for understanding the nature of their interactions [42,44–48].

In this research, flow injection gradient technique was adapted for studying the thermodynamics of β-CD inclusion complexation and calculating the inclusion constants. The stability constant of complexation of β-CD: dye complexes were determined and compared with the literature values. Each run of the gradient flow injection technique provides duplicate titration data. Multivariate approaches were used for selecting the chemical model and calculating the thermodynamic constants. Under equilibrium conditions, the orientation and posing of the dye molecules in the β-CD cavity (the most likely structure of the complex) were predicted by molecular docking. The nature and degree of effectiveness of the involved chemical interactions were evaluated by molecular modeling.

2. Experimental

2.1. Materials and chemical reagents

All of the used reagents were analytical grade. Crystal violet, sodium fluorescein, methyl orange, phenol red, and sodium dihydrogen phosphate were purchased from Merck. β-CD, phloxine B, PAR, and rose bengal were purchased from Fluka. The stock 0.2 mol l^{-1} of the phosphate buffer solution was prepared in distilled water and further diluted for use in the preparation of β-CD and dye solutions. Phosphate buffer was used, because it doesn't form inclusion complexes with β-CD [49]. The dye solutions were prepared daily in the lowest possible concentrations to minimize dimerization [50]. The stock solutions of the β-CD were prepared in the phosphate buffer. Fresh dye solutions were prepared daily in the phosphate buffer.

2.2. Apparatus, instrumentation and software

The FIA manifold used for titration is schematically shown in Fig. 1. All tubes employed were made of silicon (0.80 mm internal diameter and 250.0 cm length). Injection was carried out using a six-way Rheodyne injection port. A multi-channel Heidolph peristaltic pump was used. The absorption spectra with a 1.0 nm spectral bandpass were recorded using an Agilent-8453 UV–Vis diode-array spectrophotometer. Agilent UV–Visible Chem-Station software for data acquisition was used throughout the study. A 1.0 ml flow cell with a 2.0 mm path length was used. All absorption spectra were recorded in the wavelength range of 350–650 nm. The pH values were measured and adjusted by an AMTAST pH-meter equipped with an Ag/AgCl electrode. A home-written Excel-VBA macro was used to prepare the required input file format of the SQUAD computer program [51]. Further data treatments were done using MATLAB ver. 7.11 [52].

2.3. Calibration and titration procedures

Dispersion calibration was done in two steps: first, a solution of rose bengal (4×10^{-3} mol l^{-1}) was used as a carrier phase, and the absorbance of this solution at λ_{max} was recorded ($A_{o,Cal}$). In the next step, a 0.01 mol l^{-1} of the phosphate buffer solution (pH of 7.40) was chosen as carrier stream and 100 μl of the same solution of rose bengal (used above) was injected into the carrier phase. Then, 100 s after injection, the spectrophotometer started recording the absorbance at λ_{max} of the rose bengal ($A_{t,cal}$) every 5 s for the next 500 s, thus giving a vector of 1×100 for absorbance values. Plotting the absorbance values versus the time steps gave the dispersion curve (Fig. 2). The injection produced a gradient which indicated that the concentration of rose bengal was maximum at the center of the injected plug and decreased gradually by moving toward the edges. Dispersion (D_t) of the rose bengal was calculated from Eq. (1):

$$D_t = {}^{A_{o,\mathrm{cal}}}\Big/_{A_{t,\mathrm{cal}}}. \quad (1)$$

Titration was carried out by injecting 100 μl of the mixed β-CD: dye solution into the carrier phase of the dye solution to neutralize the dilution effect of the colorless β-CD solution. Beginning 100 s after injection, the spectrophotometer scanned every 5 s for the next 500 s (the pattern of spectra sampling was the same as calibration step). Fig. 3 shows the absorption spectra of the crystal violet and methyl orange dyes. These spectra were obtained from the second half of the dispersion curve (equivalent to spectra 29–56 of Fig. 2). Another set of absorption spectra can be obtained from first half of the dispersion curve (spectra 12–28 of Fig. 2). Thus, for each titration, there are two sets of titration data which gives the advantage of repetition for each injection. It can be clearly observed that the addition of the β-CD caused changes in the spectral behavior of the dyes. In the case of crystal violet dye, injection of the β-CD caused the absorption spectra to shift to a higher absorbance. In the case of methyl orange, phenol red, and fluorescein, however, the opposite trend was observed. In comparison, the PAR ligand showed the lowest amount of spectral change.