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Significant fluorescence enhancement by supramolecular complex formation between berberine chloride and cucurbit(n = 7)uril and its analytical application

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ABSTRACT

The supramolecular interaction of cucurbit(n = 7)uril (Q[7]) with berberine chloride (BER) has been studied in aqueous solution at pH 2.0 and room temperature by spectro-fluorimetry. The association constant of the complex was 2.07×10^6 L mol^{-1} calculated by using a nonlinear least squares method. ^{1}H NMR spectra confirmed that a 1:1 stable complex is formed between Q[7] and BER. This work proposes a possible interaction mode, in which the guest BER is incorporated inside the hydrophobic cavity of the host Q[7] via the isoquinoline ring part of the guest molecule. Based on a significant enhancement of the fluorescence intensity of this supramolecular complex, a spectrofluorimetric method with high sensitivity and selectivity has been developed for the determination of BER in aqueous solution in the presence of Q[7]. The linear range of the method was from 7.43 to 11.2×10^3 ng mL^{-1} with the detection limit 4.2 ng mL^{-1}. There was no interference from the compounds normally used in tablets, serum or urine constituents. The proposed method was applied to the determination of BER in tablets, serum and urine samples with satisfactory results and good consistency with those obtained by the pharmacopoeia method. This shows that it has promising potential for therapeutic drug monitoring and pharmokinetics and for clinical application.

1. Introduction

Fluorescent sensors are advantageous for the analysis and detection of various organic and biological molecules due to their high sensitivity and selectivity [1,2]. Meanwhile, supramolecular transducing systems have attracted enormous research interest in recent years for the development of chemical sensors [3]. A variety of receptor molecules, such as cyclodextrin [4,5], crown ethers [6], calixarenes [7], and porphyrin [8], as typical host compounds, can selectively recognize organic species. Cucurbituril and its derivatives (Fig. 1) form a significant new kind of host compound, with a series of barrel-shaped molecules containing a hydrophobic cavity accessible through two identical carbonyl-fringed portals [9]. They are capable of interacting with a variety of organic or inorganic molecules through cavity encapsulation or portal ion–dipole interaction [10]. The encapsulation and release of guest molecules are controlled by the size of the carbonyl portal and cavity [11]. Noncovalent intermolecular forces, including van der Waals interaction, hydrophobic interaction, electrostatic interaction, hydrogen bonding, are believed to play a key role in the complex formation and its stabilization [12]. In addition, the formation of inclusion complexes is often able to effect enhancements or perturbations of the photophysical and photochemical properties of the included guest molecules [13].

Berberine chloride (BER), a kind of isoquinoline alkaloid, is the basic active ingredient of widely used traditional Chinese medicine *Coptis Chinensis* with antibacterial and anticonvulsant activities. It is commonly used for the treatment of diseases such as bacillary dysentery, lobar pneumonia and pertussis. At present, the analytical methods applied to the determination of BER are HPLC [14], capillary electrophoresis [15], electrochemical analysis [16], fluorophotometry [17] and chemiluminescence [18]. Most of the methods need a complicated extraction process [19], and also use organic solvents. BER can emit strong fluorescence in organic solvents but in aqueous solution, it possesses a low fluorescence quantum yield. Therefore, we need to develop a simple, highly sensitive and selective method, without an extraction process, for the determination of BER in aqueous solution.

In this paper, we report the supramolecular interaction between Q[7] and BER. A 1:1 stable complex and possible interaction model between host and guest were investigated by ^{1}H NMR. These investigations showed that the fluorescence intensity of BER was dramatically enhanced in the presence of Q[7], and was clearly associated with the formation of the inclusion complex between Q[7] and BER. To our knowledge, usage of Q[7] as the sensitiz-

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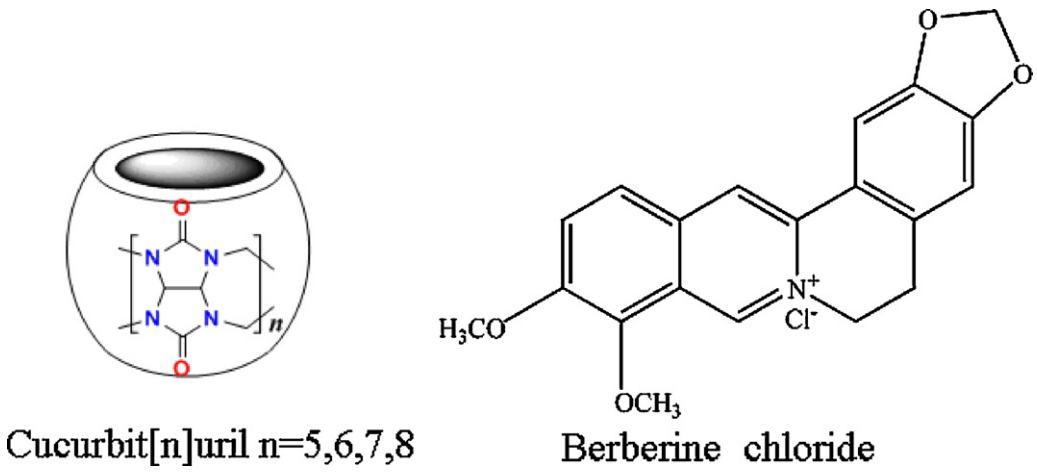

Fig. 1. The structures of cucurbit[*n*]uril and berberine chloride.

ing agent for the determination of BER in the aqueous solution by spectrofluorimetry has not previously been reported.

Based on the supramolecular interaction, the concentration of BER in aqueous solution was determined with high sensitivity and selectivity by spectrofluorimetry. The linear range of the method was from 7.43 to 11.2×10^3 ng mL^{-1} with a detection limit of 4.2 ng mL^{-1}. The proposed method has been applied to the determination of BER in tablets, serum and urine with satisfactory results consistent with those determined by the Chinese pharmacopoeia promulgation method. The method shows potential for therapeutic drug monitoring, pharmokinetics and for clinical applications.

2. Experimental

2.1. Apparatus and reagents

All the spectrofluorimetric measurements were carried out on a Cary Eclipse (Varian, Australia) spectrofluorimeter equipped with a xenon lamp and 1.0 cm quartz cells. Absorption spectra were obtained from a UV-1700 (Shimadzu) UV–vis spectrophotometer. pH measurements were made with a pH 3 digital pH-meter (Shanghai Lei Ci Device Works, Shanghai, China) with a combined glass-calomel electrode. ^{1}H NMR spectra were performed on a VARIAN INOVA-400 spectrometer in D_2O with DCl (pH = 2.0, 0.01 M). All ^{1}H MMR spectra are referenced in ppm with respect to a TMS standard.

BER (99%) (purchased from WuHan Yuancheng coll) was used as received without further purification. Q[7] was prepared at the Applied Chemistry Institute of Guizhou University, according to the literature [20]. Other chemicals used were analytical reagent grade. Doubly distilled water was used throughout.

2.2. Experimental procedure

2.2.1. Calibration graph

Into a series of 10 mL flasks were added different aliquots of the BER stock solution containing 0–11.2×10^3 ng mL^{-1} of BER and 0.9 mL of 1.00×10^{-3} mol L^{-1} Q[7]. The mixture was diluted to mark with 0.01 mol L^{-1} hydrochloric acid solution, shaken thoroughly and equilibrated at room temperature for 5 min. Then the fluorescent intensity of the solution was measured at 345/498 nm against a reagent blank. The excitation and emission band width were 5 nm.

2.2.2. Determination of the apparent association constant

Into a series of 10.0 mL flasks were added 0.1 mL of 1.00×10^{-4} mol L^{-1} BER and variable amounts of 2.00×10^{-4} mol L^{-1} Q[7]. The mixture was diluted to mark with 0.01 mol L^{-1} hydrochloric acid solution, shaken thoroughly and equilibrated at room temperature for 5 min. Then the fluorescent intensity of the solution was measured at 345/498 nm against a reagent blank. Again, the excitation and emission band width were 5 nm.

3. Results and discussion

3.1. Excitation and emission spectra

The excitation and emission spectra were scanned as described above (Fig. 2.). The maximum excitation and emission wavelengths of BER were 345 and 515 nm, respectively. However, when Q[7] was added to the aqueous solution of BER, a significant increase of the fluorescence intensity was observed, accompanied by a blue shift from 515 nm to 498 nm.

3.2. Influence of pH and reaction time

BER is a quaternary ammonium salt, so its fluorescence emission intensity should be unrelated to pH value in aqueous solution. The experimental results also indicated that there is no significant change of the fluorescence intensity over the pH range 1–7 for Q[7]-BER system. Considering that samples were prepared under acid condition (pH ≈ 2.0), a pH of 2.0 was fixed using 0.01 mol L^{-1} hydrochloric acid solution throughout experimental process.

In addition, the fluorescence intensity quickly reached a maximum after the reagents had been added and remained constant for at least 120 min. Hence, after the inclusive reaction was carried out for 5 min, the subsequent fluorescence measurement was made at room temperature.

3.3. Influence of Q[7] concentration

The influence of Q[7] concentration on the fluorescence intensity is shown in Fig. 3. In order to compare with Q[7], the influence of β-CD on the fluorescence intensity of BER was also tested. The results showed that with increasing concentration of Q[7], the fluorescence intensity of the complex increased and finally remained approximately constant. When the concentration of Q[7] was 3 times that of BER, the fluorescence intensity of BER was increased by a factor of nearly 140 compared to only a 3-fold increase of the fluorescence intensity with the same concentration of β-CD (Fig. 3). Thus, Q[7] shows a strong enhancement of the fluorescence inten-