INVESTIGATED THE SUPRAMOLECULAR INTERACTION OF TRAMADOL HYDROCHLORIDE WITH P-SULFONATED CALIX[4,6,8]ARENE

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ABSTRACT

In this paper, the interaction behavior of p-sulfonated calix[n]arene (SCnA) with tramadol hydrochloride was investigated by fluorescence spectra, 1H NMR spectra, and theoretical calculations in aqueous solution. At the optimized conditions, the fluorescence intensity of tramadol hydrochloride showed positive correlation to the concentration of SCnA, which led to a validated, simple, and sensitive fluorescence quenching method for the determination of tramadol hydrochloride was established for the first time. Moreover, the interaction based on SCnA superstructure provided has promising potential for therapeutic monitoring and pharmacokinetics and clinical application.

Keywords: Tramadol hydrochloride; Spectrofluorometry; Supramolecular interaction; p-sulfonated calyx[n]arene

INTRODUCTION

Tramadol hydrochloride, (+)-E-2-[ (dimethylamino) methyl ]-1-(3-methoxy phenyl) cyclohexanol hydrochloride (TR, Scheme 1.b) is a centrally acting analgesic structurally related to codeine and morphine, analgesic activity in efficacy and potency that ranges between weak opioids and morphine. A number of assays have been reported for detection of TR in biological and pharmaceutical samples, including chromatographic1-4, mass spectrometric5-12, spectrophotometric13-19, and capillary electrophoresis20. However, chromatography requires the use of organic solvents, expensive instruments, and time-consuming operating steps. The reported method of spectrophotometry was based upon the oxidation reaction between the drug and alkaline potassium permanganate or was based on the reaction of tramadol hydrochloride with 4-chloro-7-nitrobenzofurazan (NBD–Cl) 11. Some other methods are showed low convenience and limited extending potential. Therefore, simple and sensitive detection method is in urgent need for determination of TR, and no fluorescence analyst method is available for the detection of TR to the best of our knowledge.

Scheme 1 The structure of p-sulfonated calix[n]arene (n=4,6,8) (b) and Tramadol hydrochloride(a)

Supramolecular chemistry provides a possibility for selective detection of TR due to supramolecular interaction. Specially, Calix[n]arenes are constructed from alternating phenol and methylene groups, such as a bowl shape which results in form a hydrophobic and electron rich cavity with excellent recognition ability, which is well-suited to the formation of inclusion complexes and have attracted considerable attentions in the host–guest chemistry14-21. The sulfonation of calix[n]arenes (Scheme 1.a) that is a kind of calixarene derivative, due to conquer the poor solubility of calixarenes in aqueous solution, showing favourable bonding properties in aqueous solution are becoming increasingly important in the fields of supramolecular chemistry. Recently, the application of p-sulfonated calix[n]arenes in inorganic ions22-24, organic molecules25, chemical dyes26-28, biological29-31 and medical32-33 aspects has occupied the current interest.
RESULTS AND DISCUSSION

Spectral characteristics

Because the same trends showed for three ScnA (i.e., Sc4A, Sc6A or Sc8A), the following research used the Sc4A as example. Fluorescent emission of Sc4A showed undetectable, nevertheless, TR exhibited very mighty native fluorescence in aqueous solution (Figure 1.Sc4A, cure 1). After the addition of Sc4A in TR solution, a dramatic quenching change of fluorescence was observed (Figure 1.Sc4A, cure 2-10). This should be attributed to the inclusion of TR by Sc4A to change the space structure or conformation of TR and produce the inclusion complexes. Furthermore, with the increasing size of the calixarene ring, Sc4A < Sc6A < Sc8A, the order of the fluorescence intensity changes was consistent (Figure 1.Sc6A, Figure 1.Sc8A).

Interaction mechanism the inclusion complex

Although the fluorescent emission of pure TR was very mighty, the fluorescence intensity greatly quenching after they interacted with the hydrophobic cavity of ScnA. The Interaction mechanism of inclusion complex were explained from the following several aspects:

1. Stoichiometry and association constant of the inclusion complex. Assuming that ScnA and TR forms a 1:1 ratio complex, the following expression can be written as

\[ TR + ScnA \leftrightarrow TR \cdot ScnA(1) \]

The formation constant of the complex (K) is given by

\[ K = \frac{C_{TR \cdot ScnA}}{C_{TR} \cdot C_{ScnA}} \]

An equation of inclusion constant K of the complex with guest–host was used to calculate the inclusion constant \[^{12}\]:

\[ \frac{1}{F \cdot F_0} = \frac{1}{(F_\infty - F_0)KCnA} + \frac{1}{F_\infty - F_0} \]

Where, \( C_0 \) is the original concentration of the TR, \( C_c \) is the original concentration of the ScnA, \( F_c \) is the fluorescence intensity of TR in the absence of ScnA, \( F_\infty \) is the fluorescence intensity when all of the TR molecules are essentially complexed with ScnA, and \( F \) is the observed fluorescence intensity at each ScnA concentration tested. \( K \) is the association constant of the complex.

In the double reciprocal plot was obtained between 1/ (\( F \cdot F_0 \)) and 1/ C ScnA (Figure 2), the fluorescence quenching values 1/ (\( F \cdot F_0 \)) show good linear relationship with ScnA concentration 1/CScnA for a certain range of concentrations, which supported the formation of a 1:1 complexes (TR and ScnA) with a binding constant at pH 7.4 were 6.84 \times 10^4, 7.5 \times 10^4, 8.72 \times 10^4 L mol\(^{-1}\) in presence of Sc4A, Sc6A and Sc8A, respectively. These values were determined by dividing the intercept by the slope of the corresponding lines. It is evident that the complex stability constant monotonically increased with the number of phenol units in the calixarene ring.
Figure 2. Plot of $1/(F-F_0)$ versus $1/[[SCnA]]$ of SCnA–TR complex.

By the Job’s plot using to calculate the association ratio, the formation of 1:1 SCnA–TR complex was also confirmed. The solutions of SCnA and TR were mixed in a different mole ratio while keeping the total concentrations of these two components to be a constant. The maximum of the relative fluorescence intensity was observed when $[SCnA]/([SCnA]+[TR]) = 0.5$. A typical Job’s plot for the inclusion complexation of SCnA with TR was shown (Figure 3).

Figure 3. Job’s plot for the complexation of TR with SCnA in Britton-Robinson buffer solution (pH 6.00) at 25℃. $([TR]+[SCnA]) = 1.0 \times 10^{-5}$ mol L$^{-1}$.

Table 1. The complexation of TR with SCnA thermodynamic parameters.

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<th>$K$(L mol$^{-1}$)</th>
<th>$\Delta H$(kJ mol$^{-1}$)</th>
<th>$\Delta G$(kJ mol$^{-1}$)</th>
<th>$\Delta S$(J/(mol K$^{-1}$))</th>
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Table 1. The complexation of TR with SCnA thermodynamic parameters.

The complexation thermodynamic parameters shown in Table 1 indicated that the negative sign for the enthalpy change and free energy meant that the interaction process was exothermal and spontaneous, but with accompanying a small entropic loss. It also indicated that higher temperature was unfavorable to the formation of inclusion complexes. Hydrogen bonding, p–p interaction, electrostatic interaction, hydrophobic interaction, dipole–dipole or Van der Waals would contribute to the favorable enthalpy change. Therefore, the present experiment should be conducted under a lower temperature, at room temperature. The complexation thermodynamic parameters indicated that the complexation of SCnA (n=4,6,8) with TR was mainly driven by the enthalpy change which mainly to the electrostatic interaction, which suggested that the electrostatic interaction and structural matching effect were the dominant host–guest complex stabilizing factor.

(3). Influence of reaction time

The effect of reaction time was studied. As shown in Figure 5, the fluorescence intensity reached a maximum within 20 min after the reagents had been added, and remained constant for at least 5 h. Hence, the reaction was carried out for 20 min, room temperature for 20 min was selected as a standard reaction condition.
Influence of reaction time on the fluorescence intensity, pH 7.4, (TR) = 1.0×10⁻⁴ mol L⁻¹ (4).

Influence of pH. The complexation of TR with SCnA was also studied in acidic, neutral and basic media. The pH dependence of association constants was carried out in the pH range of 4.0–11.0, which suggest that large affinity of TR to SCnA in the fluorescence spectra was found irrespective of pH. As seen in Table 2. The binding constants were a little sensitive to pH with increasing the size of SCnA cavity, which imply that the dominant host–guest complex stabilizing factor was not Coulomb force but the electrostatic interaction and structural matching effect.

Only take into consideration the optimal inclusion condition of SCnA (6.0–8.5) and physiological environment of drug action, the buffer of pH 7.4 was chosen in the following study.

From the Supporting information, with increasing the pH from pH 7.4 to 9.0 or more basic condition the electrostatic interaction between TR and SCnA is becoming much more weaker, it meant that the binding constant of pH 7.4 is larger than other pH. With increasing the pH, deprotonation of the phenolic OH groups of SCnA could be further strengthened which lead to the hydrogen bonds strengthened among the phenolic hydroxyl groups allowing conformation flexibility for the calixarene ring. So, in experimental condition pH 7.4, the electrostatic interaction of TR and the negatively charged sulphonyl groups of SCnA is existed and the association constant is the maximum.

Table 2. Complex association constants for 1:1 intermolecular complexation of TR with SCnA in different pH.

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<td>SC4A</td>
<td>K</td>
<td>2.97×10⁴</td>
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<td>R²</td>
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<td>SC6A</td>
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<td>1.15×10⁵</td>
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<td>7.38×10⁴</td>
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<td>R²</td>
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(5). ¹H NMR study. The formation of TR-SCnA inclusion complex could be confirmed by ¹H NMR spectroscopy in D₂O at room temperature. (Figure 6). Compared with the proton resonance of the unbound TR molecule (Figure 6B), the resonance of protons H3, H4, H5, and H6 of the bound TR in the ¹H NMR spectrum of TR-SC4A complex experienced a progressively up-field shift (Figure 6C), indicating that SC4A binds selectively to the cyclohexanol group residues due to cooperative hydrophobic and ion-dipole interactions, but because of the excellent matched size and morphology, cyclohexanol group residues was enclosed in the cavity of SC4A more tightly and was shown obviously. The chemical shift of the methoxy protons is practically unchanged, indicating this part of the molecule was located just outside the SC4A host. All the H signals of N-methylene protons and N-methyl significantly shifted upfield. This feature shows that TR part of the molecule with the p-sulfonated calix[n]arenes along with a negatively charged sulfonate and electron interactions on the benzene ring.

Figure 7 (A→D) shows the ¹H NMR spectra of the formation of a SC6A-TR complex and a SC8A-TR complex. Compared with the proton resonances of the unbound TR molecules (Figure 7B), all the H signals of the bound TR significantly shifted upfield, and with the increase of the number of phenolic units of the calixarene ring, the interaction more strongly and the molecular distance more close.

(6). Molecular modeling calculations were optimized at the B3LYP/6-31G(d) level of density functional theory using the Gaussian 03 program. The results confirmed the partial inclusion of TR in the hydrophobic cavity of SC4A (Figure 8). From molecular simulation, it can be seen that cyclohexanol group of the molecule located inside the SC4A host, however, the heterocyclic nitrogen interacted with the negatively charged sulphonyl groups in the SC4A vicinity.
In this work, the supramolecular interaction of TR with p-sulfonated calix[4,6,8]arene were investigated for the first time, and characterized via fluorescence spectroscopy and 1H NMR, moreover the stability constants, binding ratio, enthalpy and entropy of complexation were studied and analyzed respectively. The inclusion stoichiometric ratio of 1:1 was verified, and the inclusion constant was estimated. Density functional theory calculations were carried out to propose the possible molecular inclusion model of TR with SC4A. These research results were consistent with the fluorescence discussion carried out to propose the possible molecular inclusion model of TR with SCnA. Moreover, the stability constants, binding ratio, enthalpy and entropy of complexation were studied and analyzed for the first time, and characterized via 1H NMR results. The complex between TR and SCnA may be used as a fluorescence probe and a fluorescence sensor for the detection of non-fluorescent or weakly fluorescent substances, this studies are in progress in our laboratory.

CONCLUSIONS

ACKNOWLEDGEMENTS

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