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PAPER

Determination of sotalol by fluorescence quenching method

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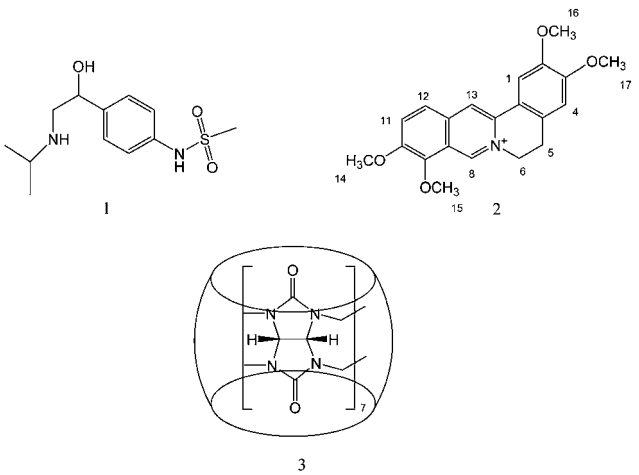
Sotalol has a weak fluorescence in aqueous solutions, so using a direct fluorescent method is difficult. The competitive reaction between sotalol and palmatine for occupancy of the cucurbit[7]uril (CB[7]) cavity was studied by spectrofluorimetry. CB[7] reacts with palmatine to form complexes with strong fluorescence. However, the presence of sotalol quenches the fluorescence intensity of the complexes. We attribute this to the competition between sotalol and palmatine for occupation of the CB[7] hydrophobic cavity. Results show that sotalol occupies the space within the CB[7] cavity, forcing palmatine molecules to reside in the aqueous environment. The quenching of the CB[7]/palmatine system is fast, and the degree of quenching is proportional to the concentration of sotalol. Therefore, a new and useful spectrofluorimetric method with high sensitivity and selectivity was developed for the determination of sotalol in aqueous solutions. The proposed method is fairly accurate, simple, rapid, reproducible, and sensitive. It is also specific for the identification of sotalol. Its detection limit is 0.033 μg mL^{-1}. This paper also discusses the competition reaction mechanism between sotalol and palmatine in the CB[7] cavity.

Introduction

Cucurbit[n]uril (CB[*n*], $n = 5$–8, 10) is a new type of highly symmetrical, barrel-shaped macrocyclic molecule. Symmetrical CB[*n*] hosts possess hydrophobic cavities and restrictive polar portals lined with ureido carbonyl groups comprised of *n* glycoluril units bridged by 2*n* methylene groups. The structural characteristics of CB[*n*] make it different from other supramolecules in terms of hydrophobic interaction, hydrogen bonding, and ion dipole bonding with a variety of organic molecules. Generally, the chemical and spectroscopic properties of guest molecules included in the CB[*n*] macrocycle are considerably altered.[1–5] In recent years, the CB[7] host has been of particular interest because of its superior solubility in aqueous solutions compared with other CB[*n*] members and because of its remarkable capability to form host–guest complexes with organic guest molecules.[5–9] However, despite the widespread interest in supramolecular systems containing CB[*n*]s, little attention has been devoted to their fluorescent properties.[11] In recent years, with the emergence of supramolecular chemistry, melon rings have been formed by research groups. It has shown a wide range of applications in supramolecular catalysis, supramolecular assembly, molecular recognition, ion channel, ultramolecular biology, nano-science, supramolecular pharmacology and biology, medicinal chemistry, and other fields.[10–12]

Palmatine (PAL) (see Scheme 1) is a kind of isoquinoline alkaloids. We investigated the supramolecular interactions of the CB[7]/PAL complex and its application in molecular recognition and determination of PAL. We also utilized its excellent optical properties.[13–15] In preliminary studies, we observed that the fluorescence of PAL in aqueous solutions is greatly enhanced by the presence of CB[7].

Sotalol (see Scheme 1) is a new class of antiarrhythmic drugs with β-adrenergic receptor blocking properties, which is a rapid non-selective receptor antagonist for all kinds of life-threatening ventricular arrhythmia.[16,17] Sotalol has been measured through various methods, with HPLC being the most common.[18–30] However, this method generally requires expensive equipment



Scheme 1 Molecular structure of the fluorescent guest molecule sotalol (1), palmatine (2) and the host molecule cucurbit[7]uril (3).

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and complicated procedures. Spectrophotometry[28,29] and thin-layer chromatography[28] have also been employed to assay sotalol. The former method is not sensitive enough and has poor selectivity, and the latter method has poor reproducibility. Spectrofluorimetry has the advantages of both sensitivity and simplicity, so it has found extensive use for the measurement of inorganic, organic, and bioactive materials.[31–33] Based on the ternary complex formation of sotalol and on the separation and spectrofluorimetric assay of the β-adrenergic blocker sotalol from blood and urine, Garrett and Schnelle[34] developed a fluorimetric method to determine sotalol. However, the method does not possess sufficient sensitivity, and the detection limit is only 2.5 μg mL^{-1} in urine, so it cannot be widely used.

To our knowledge, the use of fluorimetry for the measurement of sotalol has never been reported. We aim to find a method that is simple, rapid, accurate, and sensitive. The proposed procedures are tested for the determination of drugs in their pharmaceutical dosage forms, in urine and plasma samples.

Experimental

Instruments

Fluorescence spectra and intensity measurements were obtained with a Hitachi F-4500 spectrofluorimeter equipped with a 150 W xenon lamp (Japan). The slit width of both the excitation and emission monochromators was set at 5 nm. The fluorescence spectra were recorded at a scan rate of 1200 nm min^{-1}. All measurements were performed in a standard 10 mm path–length quartz cell set to a temperature of 25.0 ± 0.5 °C. The pH values were measured with a pHS-3TC digital precision pH meter (Shanghai, China). CB[7], PAL, and their complex be monitored by ^{1}H-NMR. Spectra were recorded on a Bruker DRX-300MHz spectrometer at 20 °C using deuterated water as the solvent.

Reagent and chemicals

The sotalol, palmatine (PAL) used in the experiment were obtained from the Chinese National Institute for the Control of Pharmaceutical and Biological Products. A sotalol stock solution of 100 μg mL^{-1} was prepared with double-distilled water. Working solutions were prepared by diluting appropriate amounts of the stock solution before use. PAL was used as received without further purification. Stock solutions of these were prepared with double-distilled water to a final concentration of 1.0×10^{-3} mol L^{-1}. CB[7] was prepared and characterized according to recently reported procedures.[35] A CB[7] stock solution of 1.0×10^{-3} mol L^{-1} was prepared by dissolving CB[7] in double-distilled water. Working solutions were obtained by the dilution of the stock solution. All stock standard solutions were stable for several weeks at room temperature. All chemicals were of analytical reagent grade, and double-distilled water was used throughout the procedures.

Pharmaceutical formulation

The following available commercial preparations were analyzed:

(1) Weite® Sotalol Hydrochloride tablets (lunan beite–pharmaceutical, Ltd. Batch No. 081102), labeled to contain 80 mg sotalol per tablet.

(2) Shitaike® Sotalol Hydrochloride tablets® (Shanghai shiguibao–pharmaceutical, Ltd. Batch No. 080801), labeled to contain 80 mg sotalol per tablet.

Experimental procedure

A total of 1 mL of the 1.0×10^{-4} mol L^{-1} CB[7] solution was placed into a 10 mL colorimetric flask, to which 1.4 mL of 1.0×10^{-4} mol L^{-1} PAL solution and 1.0 mL of 0.001 mol L^{-1} hydrochloric acid were also added. The mixture was shaken for 15 min at room temperature, and a suitable amount of sotalol solution was added sequentially to the flask. The solution was diluted to volume with double-distilled water and shaken thoroughly. The fluorescence intensity of the solution with CB[7]/PAL was measured at 495 nm using an excitation wavelength of 343 nm against a reagent blank. A calibration graph of CB[7]/PAL was constructed in the same way as those of studied drug solutions of known concentration.

Results and discussion

The fluorescence enhancement of PAL by cucurbit[7]uril

The spectral characteristics of PAL and its complexes were studied. As can be seen in Fig. 1, aqueous solutions of PAL has weak native fluorescence. However, significant increases in fluorescence intensity were observed when CB[7] was added to the alkaloid solutions. This modification in the features of the fluorescence spectra of the solutions is considered to be the result of the formation of an inclusion complex between the isoquinoline alkaloid and CB[7].[13–15]

The fluorescence quenching of CB[7]/PAL by sotalol

The dramatic quenching of the fluorescence intensity of the CB[7]/PAL complexes with the addition of sotalol was observed. The fluorescence spectra of the CB[7]/PAL complexes in the presence of different concentrations of sotalol are shown in Fig. 2. Fluorescence intensity decreases with increasing sotalol concentration. Furthermore, the fluorescence quenching (ΔF)

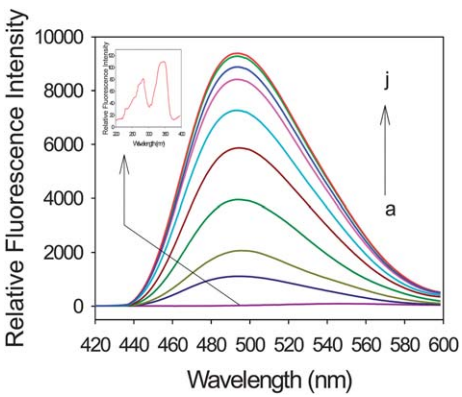

Fig. 1 Fluorescence spectra of CB[7] with different concentration of PAL: from (a) to (j). (a) 0.00; (b) 0.10; (c) 0.20; (d) 0.40; (e) 0.60; (f) 0.80; (g) 1.00; (h) 1.20; (i) 1.40; (j) 1.80×10^{-5} mol L^{-1}. $C_{CB[7]} = 1.0 \times 10^{-5}$ mol L^{-1}. a. PAL, $\lambda_{ex}/\lambda_{em}$ = 240/372 nm; b → j. CB[7]/PAL, $\lambda_{ex}/\lambda_{em}$ = 343/495 nm.

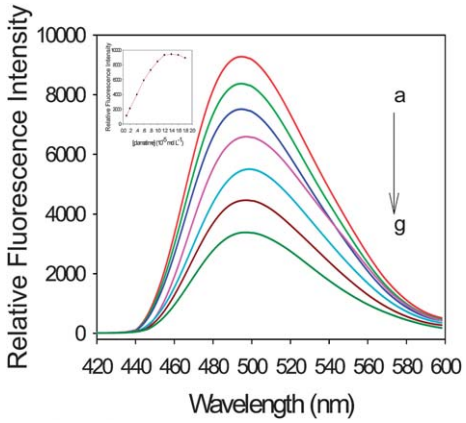

Fig. 2 The fluorescence spectra of CB[7]/PAL in different concentrations of sotalol in aqueous solutions with λ_{em} = 495 nm. The concentrations of sotalol (μg mL^{-1}): (a) 0; (b) 0.8; (c) 2.4; (d) 3.6; (e) 5.0; (f) 6.0; (g) 7.0. $C_{CB[7]} = 1.0 \times 10^{-5}$ mol L^{-1}, $C_{PAL} = 1.4 \times 10^{-5}$ mol L^{-1}.

values show good linear relationship with sotalol concentration for a certain range of concentrations. The linear regression equation of the sotalol concentration using CB[7]/PAL as the probe was determined to be $\Delta F = 0.4650 + 418.864C$. In addition, compared with the PAL, as coptisine[14] and sanguinarine[13] can react with CB[7] to form more stable inclusion complex than PAL respectively. Squeezing out these molecules from the CB[7] cavity are very difficult through the introduction of sotalol. So we choose the PAL/CB[7] to be the fluorescent probes of sotalol. For this reason, further studies using CB[7]/PAL as a fluorescent probe for drug determination may be required.

These results are likely due to the competition between sotalol and the PAL for occupancy of the CB[7] cavity. Portions of the PAL molecule can be expelled from CB[7] cavities by the introduction of sotalol, thereby causing a decrease in the fluorescence intensity of the PAL and resulting in the formation of an inclusion complex between CB[7] and sotalol.

Effect of PAL concentration on the fluorescence intensity of CB [7]/PAL complexes

The effect of varying PAL concentrations on the fluorescence intensity of CB[7]/PAL complexes was investigated. The concentration of PAL was varied from 1×10^{-6} mol L^{-1} to 1.8×10^{-5} mol L^{-1} (Fig. 1). The fluorescence intensity of the CB[7]/PAL complexes was gradually enhanced with increasing PAL concentration until the maximum inclusion equilibrium at CB[7] saturation. In this paper, PAL served as a fluorescent probe. Thus, the selection of its concentration was crucial. If the PAL concentration selected is too low, the sensitivity of the probe is likewise low. On the other hand, a concentration that is too high may not be beneficial for establishing the optimum detection limit of the analyte. Considering the reasons mentioned above, the optimal PAL concentration was selected to be 1.4×10^{-5} mol L^{-1}.

Influence of pH

The pH dependence of ΔF was studied over the pH range of 2.0–12.0. The results indicate that the ΔF value remains at a maximum and constant over the pH range 2.0–7.0. However, the fluorescence quenching dramatically decreased with any further increases in pH, as seen in Fig. 3.

This is due to the stability of the CB[7]/PAL complex significantly decreases in the presence of buffer solution with alkali cations. This is in accordance with the previous findings that alkali cations are readily coordinated to the carbonyl-fringed portals of CB[7], thereby hindering the confinement of an organic guest.[36] Binding of sodium cation decreases the rate constant of the organic guest ingression. Our results show that the presence of sodium salts in the buffer solution lessens not only the equilibrium constant of PAL binding to CB[7] but also the fluorescence quantum yield. Therefore, hydrochloric acid was used to adjust the acidity of the system in this experiment.

The optimum amount of hydrochloric acid buffer was investigated in the range of 0.1–2.0 mL. The results indicated that the ΔF value increased with increasing amount of hydrochloric acid solution. The ΔF value remains at a maximum when the amount of hydrochloric acid solution was >1.0 mL. Therefore, in a final 10 mL volume 1.0 mL of 0.001 mol L^{-1} hydrochloric acid solution is recommended and was used for all subsequent experiments.

Influence of temperature and reaction time

The effect of temperature on the ΔF of the CB[7]/PAL complexes with sotalol was investigated in the temperature range of 10–80 °C. All formed complexes were stable up to 35 °C. At temperatures above 35 °C, the fluorescence intensity greatly decreased due to the dissociation of the complexes at high temperatures. Based on these findings, all further measurements were performed at room temperature.

ΔF was reached at a maximum of 10 min after the reagents were added and remained constant for at least 5 h. For this reason, at room temperature for 10 min was selected as a standard reaction condition.

The response mechanism of the fluorescent probe

PAL itself exhibits very weak fluorescence emissions in aqueous solutions. The isoquinoline ring and substituted benzene ring is not a plane due to their connection through a hexatomic ring. Therefore, the two rings can not form a conjugated system and result in a complete loss of fluorescence. When CB[7] was added

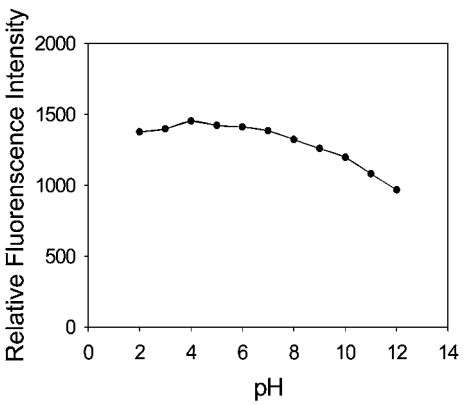

Fig. 3 Influence of pH on the fluorescence intensity.

into the aqueous solution of isoquinoline, the methoxy parts of isoquinoline entered into the host system, there is a static attraction between the positive charge of heterocyclic nitrogen of guest system and the high density of electron cloud of carbonyl oxygen. Eventually, there is a formation of a conjugated system between isoquinoline ring and substituted benzene ring. the fluorescene of isoquinoline intensified with the spread of the conjugated system. An additional factor favorable for fluorescence enhancement is that the degree of freedom of motion of the isoquinoline molecule in the CB[7] cavity is reduced, thus reducing the probability of radiationless transition. At the same time, the cavity can shield the excited single state of isoquinoline from probable quenching processes that usually occur in an aqueous solution.

When sotalol, which contains a hydrophobic chain, was added to the host–guest system of CB[7]/PAL, sotalol and PAL competed to occupy the CB[7] cavity. Not only the electrostatic ion-dipole attraction between sotalol and the carbonyl groups at the portal of CB[7] but also hydrophobic interactions contribute to the driving force of complexation. Some portions of the PAL molecule were expelled from the CB[7] cavity due to the introduction of sotalol. The photochemical property of PAL is strongly dependent on its local micro-environment, so the addition of sotalol causes PAL to lose its protection in the CB[7] hydrophobic cavity, thus resulting in the decrease of the fluorescence intensity of PAL.

CB[7], PAL, and their complex can be monitored by ^{1}H-NMR. Assignments for CB[7], PAL, and their complex are as follows:

PAL (D_2O, ppm), 2.981 (t, 2H, H_5), 3.723 (s, 3H, H_{14}), 3.856-(s, 3H, H_{15}), 5.865 (s, 6H, H_{16} and H_{17}), 6.690 (s, 1H, H_4), 7.135 (s, 1H, H_1), 7.754 (d, 1H, $J = 11$ Hz, H_{12}), 7.644 (s, 1H, $J = 11$ Hz, H_{11}), 8.123 (s, 1H, H_{13}), 9.393 (s, 1H, H_8) (Scheme 1(2));

CB[7] (D_2O, ppm), 4.077 (d, 14H, $J = 5.2$ Hz, CH_2), 5.378 (s, 14H, CH), 4.077(d, 14H, $J = 5.2$ Hz, CH_2);

CB[7]/PAL (D_2O, ppm), 3.12 (s, 2H, H_5), 3.641 (s, 3H, H_{14}), 3.909 (s, 3H, H_{15}), 4.008 (d, 14H, $J = 4.9$ Hz, CH_2), 5.239 (s, 14H, CH), 5.338 (s, 1H, H_{11}), 5.419 (s, 1H, H_{12}), 5.77 (d, 14H, $J = 4.7$ Hz, CH_2), 5.947 (s, 6H, H_{16} and H_{17}), 6.839 (s, 1H, H_4), 7.046 (s, 1H, H_1), 7.757 (s, 1H, H_{13}), 8.518 (s, 1H, H_8).

The formation of 1 : 1 complexes with PAL guests and CB[7] can be conveniently monitored by ^{1}H NMR spectroscopy. With the addition of CB[7] to a PAL solution, induced upfield chemical shifts in the ^{1}H NMR spectrum of the resonances of the methoxy-isoquinoline portion of the guest molecule are observed. The H_8, H_{11}, H_{12}, and H_{13} protons significantly shift to higher fields. The most remarkable upfield displacements were detected for the aromatic H_{11} and H_{12} protons.

In the presence of CB [7], the chemical shifts of the H15, H16, and H17 protons are hardly changed, thus suggesting that there is no interaction between the proton and CB[7]. The signals of the H1 and H4 protons from the O-dimethoxyphenyl phenyl moiety exhibited slight chemical shifts, indicating that the carbonyl groups of CB[7] affect this portion of the guest molecule less. These observations suggest that a 1 : 1 CB[7]/PAL complex is formed and that the methoxy-isoquinoline moiety in the PAL molecule is embedded in CB[7], while the heterocyclic nitrogen is located in the vicinity of the carbonyl-laced portal. The other end of the PAL molecule is not encapsulated because its smaller size does not allow a tight fit with CB[7].

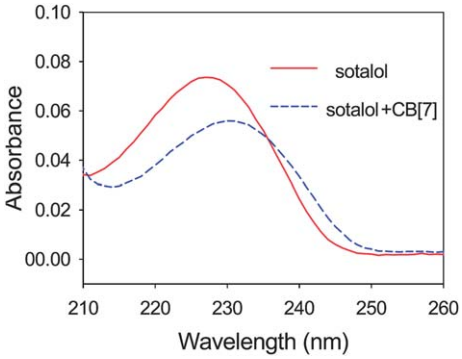

Fig. 4 Absorption spectrum of 1.6 μg mL^{-1} sotalol in water (red line) and in an aqueous solution of 1.0×10^{-5} mol L^{-1} CB[7] (blue line).

CB[7]/sotalol and their complex might be monitored by UV. Addition of CB[7] to sotalol aqueous solution leads to marked alteration in the absorption spectrum. The hypochromicity of the bands prove complex formation, as seen in Fig. 4.

Stoichiometry and association constant of the inclusion complex

The magnitude of the association constant is usually taken as a measure of the strength of the interaction between the two molecules forming the complex. For this study, 1×10^{-3} mol L^{-1}of PAL solution and varying amounts of 1.0×10^{-3} mol L^{-1} CB[7] solution were prepared. Aliquots of each solution were added in different ratios to a series of 10 mL calibrated flasks, then the general procedure was followed for determination of the association constant.

The stoichiometry and association constant of the inclusion complex were studied under the established experimental conditions by the following method:

Assuming that the composition of the complex was 1 : 1, the expression can be written as follows:

$$\text{PAL} + \text{CB[7]} \leftrightarrow \text{CB[7]} - \text{PAL} \tag{1}$$

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

$$K = \frac{C_{\text{CB[7]-PAL}}}{C_{\text{PAL}} \times C_{\text{CB[7]}}} \tag{2}$$

where C_{PAL}, $C_{CB[7]}$, and $C_{CB[7]/PAL}$ are equilibrium concentrations. The association constant value for the inclusion complex can be determined by the typical double reciprocal (or Benesi–Hildebrand) plots:

$$\frac{1}{F - F_0} = \frac{1}{(F_\infty - F_0)KC_{\text{CB[7]}}} + \frac{1}{F_\infty - F_0} \tag{3}$$

where F_0 is the fluorescence intensity of PAL in the absence of CB[7], F_∞ is the fluorescence intensity when all of the PAL molecules are essentially complexed with CB[7], and F is the observed fluorescence intensity at each CB[7] concentration tested.

A good linear relationship was obtained when $1/(F - F_0)$ was plotted against $1/C_{CB[7]}$, which supported the existence of a 1 : 1 complex. The apparent association constants for this 1 : 1 complex was determined to be 1.13×10^5 L mol^{-1} in presence of

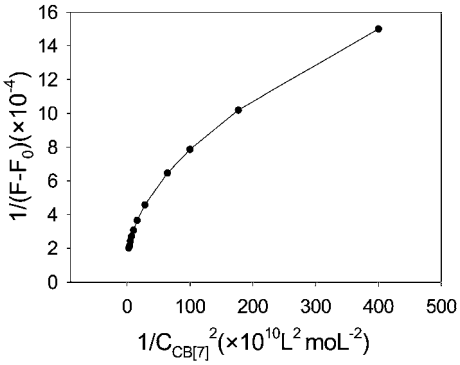

Fig. 5 Plot of $1/(F - F_0)$ *vs.* $1/C_{CB[7]^2}$ of CB[7]/PAL complex. Experiment conditions–PAL: 1.0×10^{-5} mol L^{-1} PAL.

CB[7], respectively. These values were determined by dividing the intercept by the slope of the corresponding lines.

Assuming that PAL and CB[7] form a 1 : 2 complex, the reaction can be written as,

$$\text{PAL} + 2\text{CB[7]} \leftrightarrow \text{CB[7]}_2 - \text{PAL} \quad (4)$$

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

$$K' = \frac{C_{\text{CB[7]}_2\text{-PAL}}}{C_{\text{PAL}} \times C_{\text{CB[7]}^2}} \quad (5)$$

If $C_{CB[7]} \gg C_{CB[7]^2\text{–PAL}} \gg C_{CB[7]\text{–PAL}}$, the following expression is obtained:

$$\frac{1}{F - F_0} = \frac{1}{(F_\infty - F_0)K'C_{CB[7]^2}} + \frac{1}{F_\infty - F_0} \quad (6)$$

When making a plot of $1/(F - F_0)$ against $C_{CB[7]^2}$, no linear relationship could be observed (Fig. 5), which indicated that the composition of the complex was not 1 : 2. In conclusion, the composition of the complex was 1 : 1.

It follows that the investigated CB[7] exhibited stronger binding ability according to the determination of the association constants of the complexes.

Calibration graph and sensitivity

Under the optimum experimental conditions described above, a linear relationship between ΔF intensity and the concentration of sotalol was obtained in the range of 0.1–3.6 μg mL^{-1}. A correlation coefficient of 0.9995 and a detection limit of 0.033 μg mL^{-1} were also determined. The linear regression equation obtained was $\Delta F = 0.4650 + 418.864C$. The level of sensitivity found using the proposed method was substantial in comparison with other reported methods for the determination of some studied drugs. The proposed method appeared to have higher sensitivity than HPLC[26] and other spectrofluorimetry methods,[34] as described in the literature (Table 1).

Influence of usual excipients

Before the proposed spectrofluorimetric method was applied to real samples, the influence of the commonly used tablet excipients on the determination of 2.0 μg mL^{-1} sotalol was studied. A 3000-fold mass excess of each excipient over sotalol was tested first. If interference occurred, the ratio was reduced progressively until the interference ceased. The criterion for interference was fixed at a ±5% variation of the average fluorescence intensity calculated for the established level of sotalol. The results are shown in Table 2 and it is obvious that the determination is free from the interference of the usual excipients. In the detection of real samples, for the determination of pharmaceutical preparations, there are no other interfering substances having a similar structure with sotalol in excipients. So there is no background interference. In the determination of biological fluids, heterocyclic compounds containing nitrogen atoms, certain amino acids (cysteine, cystine, alanine, phenylalanine, valine) have slight interference for determination of the drug. The drugs and interfering substances have been separated by column chromatography with different eluent in advance.

Table 2 Influence of excipients normally used in the tablet formulation on the determination of 2.0 μg mL^{-1} sotalol (tolerance error ±5%)

Tolerance ratio in mass	Excipient
3000	Starch, glucose, sucrose, lactose, sorbitol, mannitol, boracic acid, hexane diacid, sodium acetate, urea, Mg^{2+}, K^+, Cl^-, SO_4^{2-}
2000	Methyl cellulose, glycin, Zn^{2+}, HPO_4^{2-}, PO_4^{3-}
1500	Gelatin
1000	Sodium hydroxymethyl cellulose, gum acacia power, tryptophan, Na^+, Ca^{2+}
500	Gum acacia power, NH_4^+, sodium carboxymethyl cellulose
300	Fe^{3+}
0.3	L-Cysteine
0.2	Alanine

Table 1 Comparison with other proposed methods for the determination of sotalol

Technique	Linear range (μg mL^{-1})	Detection limit (μg mL^{-1})	Application	Reference
HPLC	3.25–45	1.08	Human urine samples	[26]
	5.0–45	1.67	Pharmaceutical tablets	[27]
UV	4.0–24.0	0.60	Pharmaceutical tablets	[29]
Capillary zone electrophoresis	2.85–23.0	0.94	Pharmaceutical tablets and human serum.	[30]
Spectrofluorimetry		2.50	Human urine samples	[34]
Spectrofluorimetry	0.10–3.60	0.033	Pharmaceutical tablets and human urine samples.	This paper

Table 3 Determination of sotalol in commercial tablets ($n = 5$)[a,b]

	Fluorescent probe method				Spectrophotometry[28]	
Drugs	Label claim (mg/grain)	Found (mg/grain)	Equivalent nominal content (%) ± S.D.[b]	Recovery (%) ± S.D.[b]	Found (mg/grain)	Equivalent nominal content (%) ± S.D.[b]
1	80	79.408	99.26 ± 0.91 ($t = 0.25$; $F = 1.11$)	98.90 ± 0.69	79.031	98.79 ± 1.12
2	80	78.848	98.56 ± 0.73 ($t = 0.61$; $F = 1.01$)	98.8 ± 0.96	79.246	99.06 ± 0.76

[a] The tabulated values of t and F at the 95% confidence limit are $t = 2.78$ and $F = 6.39$. [b] Average of five determinations.

Table 4 Fluorimetric determination of sotalol in spiked urine and plasma ($n = 5$)

	Spiked urine			Spiked plasma		
Samples	Amount added (μg mL^{-1})	Amount found (μg mL^{-1})	Recovery (%) ± S.D.[a]	Amount added (μg mL^{-1})	Amount found (μg mL^{-1})	Recovery (%) ± S.D.[a]
Urine 1 plasma 1	0.8	0.81	101.25 ± 0.98	0.48	0.47	97.92 ± 0.96
Urine 2 plasma 2	1.60	1.57	98.13 ± 1.10	0.8	0.78	97.50 ± 0.98
Urine 3 plasma 3	2.40	2.29	95.42 ± 0.95	1.6	1.54	96.25 ± 1.30
$\bar{X}$ ± S.D.			98.27 ± 1.01			97.22 ± 1.08

[a] Average of five determinations.

Analysis of pharmaceutical formulations

The proposed fluorescent probe was further applied to the determination of sotalol in pharmaceutical tablets and biological fluids. The contents of 10 tablets of a sotalol drug were carefully pulverized. A portion of this powder equivalent to 10 mg sotalol was accurately weighed, dissolved with double-distilled water in a 100 mL volumetric flask, and sonicated for 3 min. The solution was then diluted to the mark with double-distilled water. The first 10 mL of the filtrate was discarded, after which 10 mL of the remaining filtered sample solution was diluted to 100 times its volume with double-distilled water. Further dilutions were made to obtain sample solutions using the same detection methods as described in Pharmaceutical Formulation. The results showed that the concentrations of sotalol determined by the proposed fluorescent probe were in agreement with the obtained by the method of spectrophotometry.[28] The relative standard deviations obtained from the proposed method were less than 1.00%, and *t*-tests and *F*-tests showed that it had better precision and accuracy compared with other official and reported methods (Table 3).

Analysis of spiked human urine and plasma

The proposed optical fluorescent probe was also tested in recovery studies of sotalol in urine samples, where varying amounts of sotalol were added to diluted (100-fold) urine samples (Table 4). The results suggest that the probe can be used for the determination of sotalol with satisfactory recoveries of 95.42–101.25%. For plasma only a deproteination process was carried out using acetonitrile as a sample pretreatment; an extraction procedure was not necessary.[37] For urine, an extraction procedure was necessary, since urine is miscible with acetonitrile and no phase separation occurs.

The proposed procedure is so sensitive (Table 1); that 0.033 μg mL^{-1} of some of the studied drugs could be determined with satisfactory accuracy and precision. This characteristic permits an accurate analysis of the drug in biological fluids, urine or plasma (Table 4).

The cucurbit[7]urils hosts possess hydrophobic cavities and restrictive polar portals lined with ureido carbonyl groups. CB[7] can only form inclusion complexes with guest molecules with nitrogen that possess suitable polarity and dimensions. The molecular structure and size of sotalol is suitable for CB [7] cavity. So sotalol can easily occupy the CB [7] cavity. We have also carried on with the analysis of drugs having a similar structure with sotalol, such as propranolol, metoprolol, bisoprolol and atenolol, but we have not observed obvious fluorescence quenching phenomenon. Furthermore, there is no other interfering substance having a similar structure with sotalol in pharmaceutical preparations and biological fluids. Therefore, the fluorescent probe has a good selectivity.

Conclusions

In this paper, the competitive reaction between sotalol and fluorescent probes palmatine for occupancy of the CB[7] cavity was studied by spectrofluorimetry. The results show that sotalol can quench the fluorescence of the fluorescent probes with CB[7]. The quenching of the fluorescence intensity of the CB[7]/PAL system is fast, and the degree of quenching is proportional to the concentration of sotalol. A new simple, sensitive, fast, and low-cost fluorimetric method for the determination of sotalol was developed. The method can be successfully applied in determining sotalol in tablets and urine samples. The new fluorescence quenching processes enable a better understanding of CB[*n*]-based chemistry and the exploration of a novel analysis method for organic molecules. The proposed method can also be applied for the detection of biologically relevant cations. This method can be used in a fluorescence sensor for the detection of non-fluorescent or weakly fluorescent substances. Related studies are in progress in our laboratory.

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