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Spectrofluorimetric study on the inclusion interaction between vitamin K_3 with *p*-(*p*-sulfonated benzeneazo)calix[6] arene and determination of VK_3

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Abstract

The characteristics of host–guest complexation between *p*-(*p*-sulfonated benzeneazo) calix[6]arene (SBC6A) and vitamin K_3 (VK_3) were investigated by fluorescence spectrometry. A 1:1 stoichiometry for the complexation was established and was verified by Job's plot. An association constant of 4.95×10^3 L mol^{-1} at 20 °C was calculated by applying a deduced equation. The interaction mechanism of the inclusion complex was discussed. It was found that the fluorescence of SBC6A could be remarkably quenched by an appropriate amount of VK_3 especially when non-ionic surfactant Triton X-100 existed. According to the obtained results, a novel sensitive spectrofluorimetric method for the determination of VK_3 based on supramolecular complex was developed with a linear range of 5.0×10^{-7}–3.0×10^{-5} mol L^{-1} and a detection limit of 2.0×10^{-7} mol L^{-1}. The proposed method was used to determine VK_3 in commercial preparations with satisfactory results.

Keywords: *p*-(*p*-sulfonated benzeneazo) calix[6]arene; Vitamin K_3; Host-guest inclusion interaction; Fluorescence spectrometry

1. Introduction

Water-soluble calixarenes are a versatile family of molecules that have occupied a current interest in recent years due to their ability to form host–guest arrangements either in solution or in solid state [1]. For example, some water-soluble calix[*n*]arenes (*n* = 4, 6 and 8) and resorcinarenes towards quaternary ammonium [2,3], methylammonium cations [4–6], dyes [7,8], native amino acids [9,10] and small neutral organic molecules [11] have investigated diffusely. Among these, sulfonated azocalix[*n*]arenes are important families. Compared with other water-soluble calix[*n*]arenes, sulfonated azocalix[*n*]arenes hold chromogenic azophenol moieties and deeper cavities, which supply more binding sites and can include guest molecules more effectively [12]. However, unlike the widespread use of cyclodextrins and sulfonated calix[*n*]arenes in pharmaceutical industry or analytical chemistry [13,14], study on water-soluble sulfonated azocalix[*n*]arenes in counterpart fields is limited, only few literatures reported their natural optical properties in particular with fluorescence properties [15].

Vitamin K_3 (VK_3) (2-methyl-1,4-naphthoquinone) is a fat-soluble vitamine, which is not naturally fluorescent. Generally VK_3 plays an important role in blood coagulation, as a cofactor for the synthesis of blood-clotting factors in the liver and in bone mineralization. Recent years, VK_3 has been attracting increasing attention because it can be used as a kind of antitumor drug [16], the tube tests and the living tests showed that VK_3 can inhibit benzopyrene, quinoly aromatic compound and have been used in clinic [17]. Numerous methods for the determination of VK_3 have been described in literatures such as spectrophotometric detection [18,19], colorimetric reaction [20], chromatographic techniques [21,22], electrochemical [23,24], photochemical [25], polargraphic [26], spectrofluorimetric [27], etc. However, in most procedures above mentioned the use of an organic solvent is necessary in order to get the solubilization of the VK_3, moreover, most of methods are based on the reactivity of the cleaved molecule or expensive instruments.In this paper, the host–guest complexation of *p*-(*p*-sulfonated benzeneazo) calix[6]arene (SBC6A) and vitamin K_3 (There structures shown in Scheme 1) in aqueous solution was

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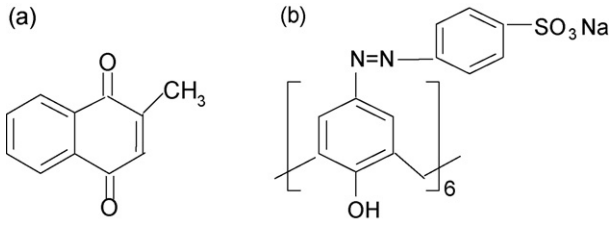

Scheme 1. Chemical structure of VK_3 (a) and SBC6A (b).

investigated using fluorescence spectroscopy. Various factors affecting the inclusion process, such as the acidity, the effect of NaCl ionic strength and the concentration of non-ionic surfactant Triton X-100 were examined in detail. Experimental results reveal that SBC6A can form 1:1 complex with VK_3, which lead to the regular decrease of fluorescence intensity of SBC6A, when non-ionic surfactant Triton X-100 existed, the quenching effect enhanced. Based on the results, a novel fluorescence spectroscopy method was developed for determination of VK_3 and was used to assay VK_3 in commercial preparations successfully.

2. Experimental

2.1. Apparatus

Fluorescence spectra and intensity measurements were made on a Hitachi F-4500 spectro-fluorometer (Tokyo, Japan) with a 1.0 cm quartz cell, excitation and fluorescence emission wavelengths of 320 and 450 nm, respectively. Slit widths of monochromators were set at 10.0 and 5.0 nm, respectively. All measurements were carried out at 20 °C by use of a thermostated cell holder and a thermostatically controlled water bath. A model pHS-3C (Dazhong Analytical Instruments Factory, Shanghai, China) pH meter was used for accurate adjustment of pH.

2.2. Chemicals

All reagents used were of analytical-reagent grade or the best grade commercially. Doubly distilled water was used throughout. SBC6A was synthesized according to the literature [28] and identified by IR, ^{1}H NMR and element analysis. Stock solution of SBC6A was prepared directly with distilled water as 2.0×10^{-3} mol L^{-1}. VK_3 (biochemical reagent) was purchased from Shanghai Reagent Factory, and was used without purification. Britton–Robinson buffer solution was prepared using a mixed acidic solution that contained 0.04 mol L^{-1} H_3PO_4, HAc and H_3BO_3, respectively, and then was adjusted to accurate values by using 0.2 mol L^{-1} NaOH.

2.3. Procedures

2.3.1. Inclusion process

A 1 mL aliquot of the SBC6A solution (1.0×10^{-4} mol L^{-1}) was transferred into a 10 mL volumetric flask, then an appropriate amount of 1.0×10^{-3} mol L^{-1} VK_3 was added. The mixed solution was diluted to final volume with doubly distilled water and stirred thoroughly. After 20 min at 20 °C, fluorescence intensities were determined.

In order to study the various factors affecting the inclusion process, certain concentration of Britton–Robinson buffer solution (pH 2.0–12.0), NaCl solution (0.5 mol L^{-1}) and 0.5% (v/v) Triton X-100 solution were added into the flask, in respective order. The mixed solution was diluted to final volume with doubly distilled water and stirred thoroughly. After incubating for 20 min, fluorescence intensities were determined.

2.3.2. Analytical procedures

Into a 10 mL volumetric flask were placed in turn an appropriate volume of working or sample solution, 1.0 mL of 1.0×10^{-4} mol L^{-1} SBC6A solution, 0.5 mL of 0.5% (v/v) Triton X-100 solution and 1.0 mL pH 7.0 Britton–Robinson buffer solution. The mixture was diluted to 10 mL with doubly distilled water and mixed thoroughly. After incubation for 20 min at 20 °C, the fluorescence intensities were measured.

2.4. Sample preparation

The contents of 10 tablets of VK_3 (Huayuan Century Pharmaceutical Industries Co. Ltd., Hubei, China) with a labeled content of 4 mg were grinded into fine powder by agate mortar, and then transferred into a eremeye flash. Adding a small amount of water into the flask, disturbing it about 2 h, then filtered into a 100 mL calibrated flask. The residue was washed several times with water and also filtered into the flask, and then the solution was diluted to final volume and the sample solution was retained in the refrigerator.

3. Results and discussion

3.1. Fluorescence spectra characteristics of the system

In order to study the fluorescence properties of the system, different pH values of Britton–Robinson buffer solution (pH 2.0–12.0) was used (Fig. 1). It can be seen that the maximum and constant fluorescence quenching occupied at pH 7.0. Therefore, in this work, all the data were measured in a

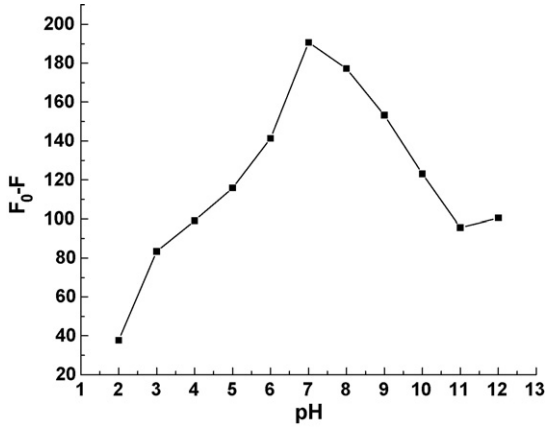

Fig. 1. Influence of pH value on the fluorescence intensity of SBC6A–VK_3 system; $C_{VK_3} = 1.0 \times 10^{-4}$ mol L^{-1}, $C_{SBC6A} = 2.0 \times 10^{-5}$ mol L^{-1}.