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Investigation on the inclusion and toxicity of acriflavine with cyclodextrins: A spectroscopic approach

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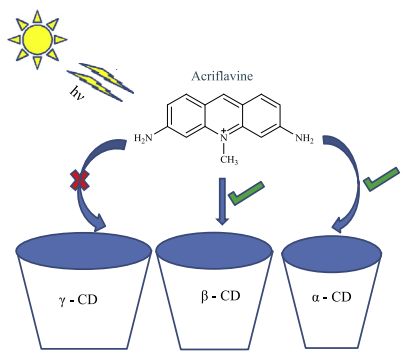
HIGHLIGHTS

- The interaction of AFN with α-, β- and γ-cyclodextrins was investigated.
- AFN forms inclusion complex with α and β-cyclodextrins.
- The inclusion phenomenon was probed via steady state measurements.
- Lifetime measurements confirm static quenching.
- The cytotoxicity was studied using arteima nauplii.

GRAPHICAL ABSTRACT



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ABSTRACT

Acriflavine hydrochloride (AFN) is a prospective drug worn in the eradication of HIV1 infection. The toxicity and adverse side effects renders the potent drug to limits its usage. However, to overcome the dilemma we have aimed to select carriers with great complexation efficiencies in different cyclodextrins (CDs) of varying cavity size. The interaction of AFN with α, β and γ-CDs were investigated using absorption and steady state as well as lifetime measurements. From the obtained data it was found that AFN fits in the cavity of α and β-CDs but unable to form inclusion complex with γ-CD. The effect of quencher molecules during the inclusion phenomena of AFN with CDs was explored via steady state measurements. The nature of binding forces responsible for the inclusion of AFN with CDs was discussed by using thermodynamic parameters. Using Benesi–Hildebrand equation the stoichiometry of AFN with CDs was predominantly found to be 1:1. To get deeper *in situ*, the *in vitro* toxicity of AFN and its complexation product were probed by *Artemia salina* sp. The toxicity of AFN was reduced when complexed with α and β-CDs.

Introduction

Acriflavine hydrochloride (AFN) (shown in Scheme 1) is an interesting molecule because it has fascinating spectroscopic and pharmacological properties. For instance, its fluorescence behavior is dependent on solvent polarity, which enables its use as an efficient laser dye [1]. Besides that combination of AFN with AZT – ellipticine is the most appropriate and efficient treatment for eradication of HIV1 infection [2]. AFN is also used as a photosensitizer for anticancer agents [3]. AFN finds to be an effective in the treatment of radiotherapy for different types of cancers. It has been observed that AFN administered orally to patients as an antiviral agent and observed with side effects [4,5]. Hence it is indispensible to improve the bioavailability and therapeutic efficiency for cancer

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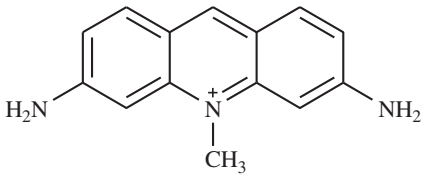

Scheme 1. Structure of acriflavine.

therapy. Therefore administration of low dosage is often required to devoid of above mentioned side effects [6].

In order to overcome the obstacles the usage of proper drug carrier becomes mandatory which would allow a reduction of drug dosage and reducing the risk of side effects. During the past decades among the many possible carriers, cyclodextrins (CDs) have attracted much attention because of their bioadaptability and capable of alleviating adverse properties of drug molecules in various routes through formation of inclusion complexes [7].

Cyclodextrins (CDs) are water soluble cyclic oligosaccharides and produced by enzymatic action on starch. CDs contain 6, 7 or 8 units of glucose connected with α-1,4 linkage and are named as α, β and γ-CDs, respectively [8,9]. This specific architecture forms a truncated cone like structure with a central hydrophobic cavity. The presence of the central hydrophobic cavity renders these molecules capable of forming inclusion complexes with many guest molecules of appropriate size and polarity in aqueous environments [10–14].

In particular it has been demonstrated that the advantage of the drug molecule to form IC with CDs have been widely reported in the scientific literature viz., improving bioavailability, accompanied by reducing the toxicity effects and hence more easily dispensed from the body [15–17]. Moreover, CDs act as drug carriers and deliver the required amount of drug to the targeted site for a necessary time period, both efficiently and precisely [18–21]. CDs have been tested for transport of many anticancer drugs [23,23]. The effectiveness of CDs can be significantly depends on the size of the cavity and the size of the incoming guest molecule [25,25]. Hence selection of suitable cyclodextrin (CD) for complexation with a given drug is an essential step for successive development of pharmaceutical formulations [27]. Furthermore design of pharmaceutical formulations requires determination of the inclusion complex geometry, which is a thorny step and signify one of the highest challenge in the host–guest chemistry. When a guest molecule is incorporated into CD cavity its physiochemical properties serves as a parameter to confirm the IC formation, since products obtained by mixing drug and the CD are not necessarily lead to the formation of IC [28,28].

As a part of our quest to investigate the inclusion of AFN with CDs, the present work mainly highlights how the spectroscopic behavior of AFN is affected upon complexation with CDs. It is one of our prime intentions to study the interaction of AFN with appropriate biological targets and in this perspective the study of interaction of biologically potent AFN with host molecules like CDs is pharmacologically as well as chemically relevant.

Therefore, in this work, as the first phase of a wider study intending at the progress of a new buckle formulation of the drug, we have investigated the inclusion complexation of AFN with a series of native CDs, in order to select the carriers with the greatest complexing efficacies and reducing the toxicity towards the drug. Previously we have reported the inclusion behavior of 9-aminoacridine with cyclodextrins as evidenced from spectroscopic and theoretical studies [30].

In the present work, the inclusion complexation of AFN with α, β and γ-CDs were evaluated by using UV–Vis absorption, steady state fluorescence and lifetime measurements. The association constant (K) and the thermodynamic parameters of the IC were also determined. The buffer solution plays an imperative role in the inclusion phenomena of host–guest complexation. So we aimed to study the inclusion of AFN in the cyclodextrins at different pH buffer solution.

Murphey et al. suggested that the aquatic exposure of the compounds is a viable itinerary of drug administration for evaluating the toxicity in aquatic organisms [31]. Thus we have probed the *in vitro* cytotoxicity of AFN and its complexation product with the insecticidal activity of compounds using *Artemia salina* nauplii.

Experimental section

Materials

Acriflavine hydrochloride, α-CD, β-CD and γ-CD were purchased from Sigma–Aldrich. Potassiumdichromate, sodiumhydroxide, dimethylsulfoxide were purchased from Hi-Media. Artemia salina was purchased from Ocean Star International Inc., USA. Sodium dihydrogen phosphate and disodium hydrogen phosphate were purchased from Merck. All reagents and solvents were of analytical grade. Water was doubly distilled and MilliQ water was used throughout the experiments. All chemicals were used without further purification.

Apparatus

Absorption spectra were recorded using JASCO V-630 UV–Vis spectrophotometer. Fluorescence measurements were acquired by the use of a JASCO FP-6500 spectrofluorimeter. ELCO Li-120 pH meter was used to maintain the pH of buffer solution. Each sample was deoxygenated by purging with nitrogen for 20 min prior to analysis. Fluorescence emission spectra were acquired with an excitation wavelength of 449 nm. Excitation and emission bandwidths were set at 5 nm. All measurements were performed at ambient temperature, unless otherwise indicated. Fluorescence lifetimes were measured using time-correlated single photon counting (TCSPC) spectrometer which comprised of a diode laser pumped milenia V (spectra physics) CW Nd-YVO_4 laser that was used as the excitation source for a titanium-sapphire rod locked laser. The emitted photos were detected by a MCP-PMT (Hamamtsu R3809U) after passing through the monochromator (f/3).The laser source was operated at 4 MHz and the signal from the photodiode was used as a stop signal. The data analysis was carried out by the software provided by IBH (DAS-6). The kinetic trace was analysed by non-linear square fitting of mono exponential.

Methods

Approximately 0.1 mM dye solution was prepared. From this 0.2 mL was added to different volumes of CDs and then made up to 5 mL with the triple distilled water. The sample were shaken with a wrist action shaker for 20 min and allowed to equilibrate for at least 12 h, prior to measurement. These samples were used for lifetime measurements as well. The individual AFN, α-CD, β-CD and γ-CD samples were prepared for absorbance measurements as previously mentioned. The blanks were prepared with appropriate concentrations of CD and deionized water for absorbance measurements (corresponding concentrations of the CDs were used as a reference for the absorption measurements).

Brine shrimp toxicity test (BST)

The BST was carried out according to Vanhaecke et al. [32] with slight modifications. *A. salina* cysts were hatched out under