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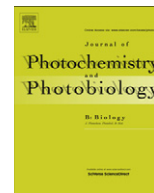

A fluorescence parameter based analysis on the solubilization of carvedilol by bile salt media

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ABSTRACT

Carvedilol (CVL), a β-adrenergic receptor, is insoluble under physiological conditions and hence its oral administration is difficult. Bile salts are widely used as drug delivery media for many hydrophobic drugs. CVL has a strong intrinsic fluorescence with a carbazole moiety. When excited at 320 nm, it exhibits dual emission at 347 and 357 nm. As the concentration of bile salt is increased there is an overall increase in the fluorescence intensity and also the ratio of the intensities at 347 and 357 nm (I_1/I_2) increases. This indicates that the CVL molecule can sense the non-polar nature of bile salts with increasing concentrations. It is showed that the new parameter (I_1/I_2) can be used as an indicative tool in determining the polarity of the medium. The increase in fluorescence anisotropy and fluorescence lifetimes is observed, indicating the micro-heterogeneity of the bile salt solutions as experienced by CVL molecules. The possibility of electrostatic interactions between CVL and bile salt micelles is ruled out in view of the results obtained here and their interaction is purely hydrophobic in nature. The results suggest that the CVL molecule associates with the steroidal moiety of bile salts through its carbazole moiety.

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1. Introduction

Carvedilol (CVL) (Fig. 1A) is a drug molecule, primarily used in clinical practice for the treatment of cardiovascular diseases (hypertension, congestive heart failure or myocardial infarction) [1]. The pharmaceutical importance of carvedilol and related β-blockers is that they lengthen the survival duration of a patient with heart failure and hence they are called as '*life-saver*' drugs. However, it could not be used as a drug for oral administration due to its poor water solubility, which prevents it from being absorbed well in the body. Hence various drug delivery systems have been evaluated to associate with CVL [2].

There are few number of drug delivery systems, associated with CVL and the prominent being liposomes [1,3]. Another class of well known drug delivery media are bile salts, which have been used as emulsifiers for a wide range of drugs from earlier times [4]. Bile salts are synthesized from cholesterol and have biological importance such as lipid solubilization. The structure of bile salts consist of a hydrophobic steroidal backbone, where one to three hydroxyl groups are present and a carboxyl side chain is attached along the same plane of hydroxyl groups (Fig. 1B and C). Owing to this structure, bile salts undergo an aggregation of unique pattern, which is responsible for their solubilization property of both hydrophobic and hydrophilic solutes [3,4]. The aggregation pattern of bile salts follows a two step process; initially dimeric primary aggregates are formed through the hydrophobic interaction between steroidal domains and the increasing concentration of bile salts leads to the formation of larger secondary aggregates, formed primarily due to the hydrogen bonding between hydroxyl and carboxyl groups of different dimeric bile salt units [3–6]. There are two different types of bile salts, namely primary (unconjugated) bile salts and secondary (conjugated) bile salts, derived from the primary bile salts by conjugating to either taurine or glycine amino acids through a peptide linkage [3].

CVL has a strong intrinsic fluorescence property owing to the presence of carbazole moiety, which exhibits excited state proton transfer (ESPT) and used in probing micro-heterogeneous media in the alkaline pH [8]. Accordingly, the fluorescence property of CVL is found to be dependent on the micro-environment surrounding it. This leads to the utility of the photophysical property of CVL for analyzing its association with a variety of drug delivery media. For the present work, four bile salts namely, sodium cholate (NaC), sodium deoxycholate (NaDC), sodium taurocholate (NaTC) and sodium taurodeoxycholate (NaTDC) are used. Among these four bile salts, NaC and NaDC are primary (unconjugated) bile salts and NaTC and NaTDC are secondary (conjugated) bile salts. The structural modifications of these bile salts not only vary the hydrophobic–hydrophilic balance, but also the aggregation pattern. The bile salt aggregates are considered pseudo-micellar as they do not have sharp monomer to aggregate transition, characterized by well defined critical micellar concentrations (cmc), instead they have a

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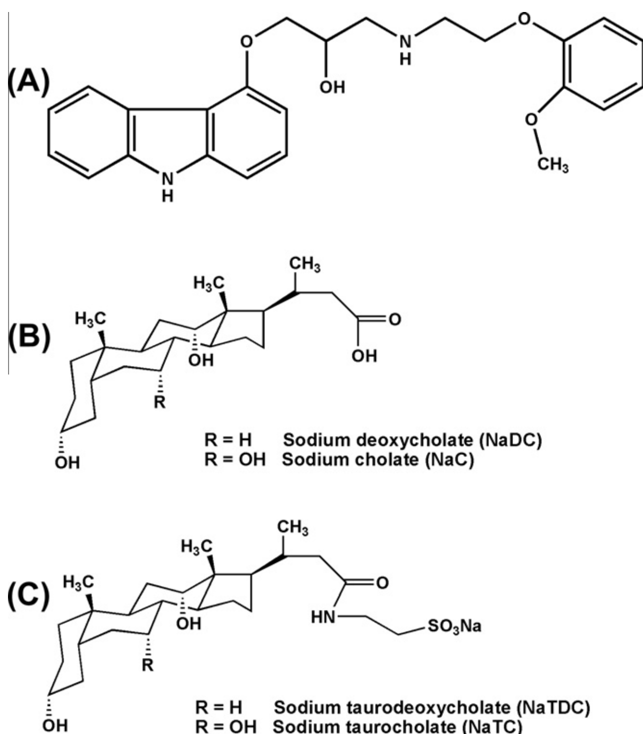

Fig. 1. Molecular structure of (A) Carvedilol (CVL), (B) and (C) bile salts.

cmc range, *viz.,* NaC and NaTC = 12–16 mM and NaDC = NaTDC = 4–6 mM [6]. Thus, in the present study, our objective is to understand the nature of interaction between CVL and bile salts. Therefore, evaluate the solubilization efficiency of bile salts in solubilizing CVL along with the temperature dependence.

2. Materials and methods

2.1. Materials

The drug molecule, CVL was generously donated by Sun pharmaceuticals, India. Sodium taurodeoxycholate (NaTDC) and Sodium taurocholate (NaTC) were purchased from Sigma Chemical Company, USA. Sodium deoxycholate (NaDC) and Sodium cholate (NaC) were purchased from S.D. Fine Chemicals, India. Spectroscopy grade solvents were obtained from Sisco Research Laboratories, India. Analytical grade ethanol was obtained from Merck, USA. The stock solutions of CVL for the homogeneous media study were prepared by dissolving it in required solvents separately.

2.2. Measurements

The absorption spectra were recorded using Jasco – V550 UV–Visible spectrophotometer and fluorescence spectra using Hitachi F4500 model spectrofluorimeter. The excitation and emission slits were set to a bandwidth of 5 nm. Temperature control was attained by using a double walled cuvette holder; connected to an INSREF thermostat with an accuracy of ±0.1 °C. For steady state fluorescence anisotropy measurements polacoat grating polarisers and Glan–Thompson polarisers were used. The steady state fluorescence anisotropy is defined as [9]:

$$r_{ss} = \frac{I_{VV} - GI_{VH}}{I_{VV} + 2GI_{VH}}$$

where I_{VV} and I_{VH} are the fluorescence intensities and the subscript indicates the vertical (V) and horizontal (H) orientations of the excitation and emission polarizer. G is the instrumental correction factor:

$$G = I_{HV}/I_{HH}$$

The fluorescence decays are collected using Horiba Jobin Yvon TCSPC Lifetime system with FluoroHub single-photon counting controller module and equipped with the TBX single-photon detection module, typically of 180 ps FWHM. The NanoLED-16 nanosecond source with UV output at 340 nm, typical optical pulse duration of 800 ps FWHM and a repetition rate of 1 MHz was used. The decay curves are further analysed using the DAS6 analysis software. A fitted value of $0.99 \leqslant \chi^2 \leqslant 1.4$ was considered as a good fit.

2.3. Preparation of CVL–bile salt solutions

CVL was first dissolved in ethanol and further diluted with bile salt solutions having concentrations above their cmc (20.0 mM for NaDC and NaTDC; 48.0 mM for NaC and NaTC). In these solutions, the final CVL concentration is maintained constant at 10 μM and varying bile salts concentrations were prepared by appropriate addition of bile salt solution and the ethanol contamination was kept at 2%. The range of concentration used for each bile salt is, NaDC, NaTDC = 2.0–18.0 mM and NaC, NaTC = 4.8–43.2 mM. To maintain the physiological condition, pH was kept constant at 7.4 with 50 mM sodium phosphate buffer for all bile salt experiments.

3. Result and discussions

3.1. Interaction between CVL and sodium cholate micelles

3.1.1. Fluorescence spectra of CVL in NaC micelles

The emission spectra (λ_{ex} = 320 nm) of CVL (10 μM) in pH 7.4 buffer solution and gradual addition of NaC solution is given in Fig. 2. There is an enhancement of CVL emission with an increase in NaC concentration. The emission spectra become more structured and emission peak at 347 nm becomes predominant. The vibrational structure of pyrene fluorescence spectrum has been used to sense the polarity of solvent environment [10]. The intensity ratio of two vibronic bands (I_1/I_3) of pyrene is recognized as a solvent polarity parameter in determining the polarity of microheterogeneous media [11,12]. In the case of CVL, as the NaC concentration increases, the overall emission intensity and the relative intensities of peaks at 347 nm (I_1) and 357 nm (I_2) are increased. Thus, in a manner similar to the pyrene vibronic band ratio, it is pro-

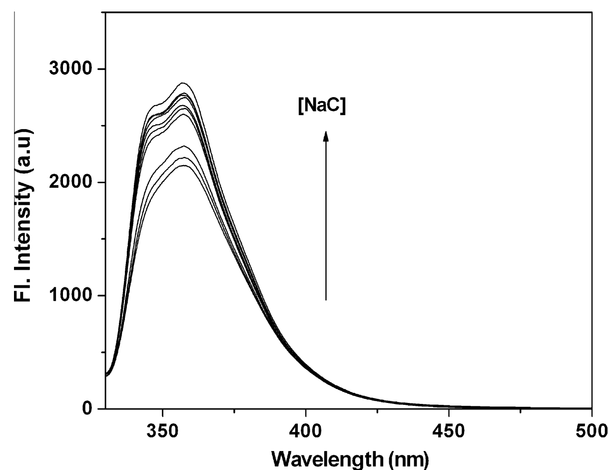

Fig. 2. Emission spectra of CVL in increasing concentration of NaC (λ_{ex} = 320 nm). [NaC] = 0–48.0 mM. T = 25 °C and pH = 7.4.

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