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Interactions of phenothiazine drugs with bile salts: Micellization and binding studies

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ABSTRACT

An evaluation of the interactions of phenothiazine tranquilizer drugs (promazine hydrochloride; PMZ and promethazine hydrochloride; PMT) with bile salts viz., sodium cholate (NaC) and sodium deoxycholate (NaDC) in aqueous medium, investigated through different physicochemical measurements is presented in this work. The mixed micellization behavior and surface properties of the phenothiazine–bile salt systems have been analyzed by conductivity and surface tension measurements. Application of different theoretical approaches to all the phenothiazine–bile salt mixtures shows a non-ideal behavior. Further, the spectroscopic techniques such as UV–visible and steady state fluorescence have been employed to study the binding of phenothiazines with bile salts. The stoichiometric ratios, binding constants (K), and free energy change (ΔG) for the phenothiazine–bile salt complexes were estimated from the Benesi–Hildebrand (B–H) double reciprocal plots obtained by using the changes in spectral intensities of phenothiazines on addition of bile salts. The results are discussed in the light of use of bile salts as promising drug delivery agents for phenothiazines and hence improve their bioavailabilty.

1. Introduction

Oral route is the most convenient, economic, and frequently used route of drug administration, but it suffers from a major drawback of poor gastrointestinal membrane permeability. Penetration enhancers may be incorporated into various formulations in order to overcome the problem of low permeability and bioavailability of drugs across the biological membranes. They include surfactants, fatty acids, bile salts, medium chain glycerides, calcium chelators such as ethylenediaminetetraacetic acid (EDTA), acyl carnitine and alkanoylcholines, *N*-acetylated α-amino acids and *N*-acetylated non-α-amino acids, chitosans, and other mucoadhesive polymers [1,2]. Bile salts have been extensively used as penetration enhancers and are also the most important biosurfactants of anionic type that are biosynthesized from cholesterol in the liver, stored in the gall bladder, and then secreted through the bile duct into the small intestine [3]. The micellization behavior of bile salts is very controversial. The most accepted primary-secondary micelle model known as Small's model [4] proposed that micellization in bile salts is a stepwise aggregation process. At low concentrations, the bile salt monomers aggregate to form primary aggregates containing 2–10 monomers through hydrophobic interactions between the nonpolar side of the monomers. As the concentration of bile salt monomer is raised, these aggregates further interact to form larger secondary aggregates via hydrogen bonding among the hydroxyl groups located on the surface of primary micelles. Due to the unusual self-assembly behavior of bile salts, their critical micelle concentration (*cmc*) is considerably lower, and the micelles formed just above the *cmc* are characterized by much smaller aggregation numbers than in the case of simple surfactants [5]. Bile salts are involved in a variety of important physiological functions like solubilization and transport of fats and lipids, assistance to hydrolysis of triglycerides by pancreatic enzymes along their transport, cholesterol homeostasis and in the formulation of food, cosmetics, and several other chemicals [4,6,7]. Owing to these widespread applications and unique facial amphiphilic structure, the physicochemical properties of bile salts have been extensively investigated [3,8,9].

In addition to these applications, bile salts have also been used as drug delivery media for the transport of some drugs through the intestine mucous membrane due to their biocompatibility and solubilizing property [10]. The surfactants are useful in drug delivery as they minimize drug degradation and loss, increase the bioavailability, and protect the body from any unwanted side effects of the drug and at the same time, achieving the desired concentration of drug at the target site [11,12]. For instance, colloidal suspensions of the bile salt, sodium deoxycholate have been commercially used as a medium for Amphotericin B delivery [13]. Phenothiazines represent a group of biologically important heterocyclic compounds endowed with dopamine receptor antagonistic activities in the central nervous system (CNS), which are commonly employed as

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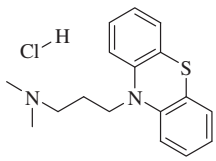
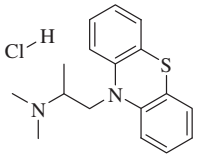
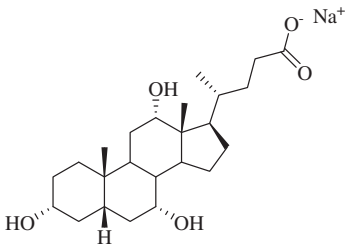


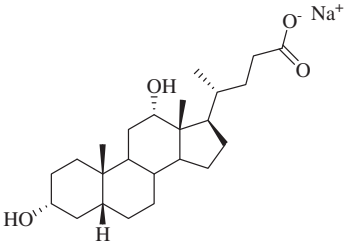
(a) Promazine hydrochloride (PMZ)



(b) Promethazine hydrochloride (PMT)



(c) Sodium cholate (NaC)



(d) Sodium deoxycholate (NaDC)

Fig. 1. Chemical structures of phenothiazines (a and b) and bile salts (c and d).

antipsychotics, neuroleptics, and antihistamines [14]. Promazine hydrochloride (PMZ) and promethazine hydrochloride (PMT) are important phenothiazine derivatives with the sole difference being the additional secondary methyl group on the side chain of PMT (Fig. 1a and b). For the antipsychotic activity of the phenothiazines, there should be three carbon atoms between both the nitrogen atoms of the alkylamino chain (in the case of PMZ), whereas the phenothiazine derivatives with chains consisting of only two carbon atoms, as in PMT are very powerful antihistaminic, antiallergic and sedative drugs, but have no antipsychotic activity. Because of their potentially useful pharmacological and biological applications, it is very important to investigate the physicochemical aspects of the interactions of phenothiazine derivatives with model membranes such as micelles of surfactants and phospholipid bilayers, and accordingly, several literature studies have been reported in this field [15–18]. Caetano and Tabak [18] reported the interactions of two phenothiazine derivatives, chlorpromazine (CPZ), and trifluoperazine (TFZ) with anionic surfactant, sodium dodecyl sulfate (SDS), by means of electronic absorption and fluorescence spectroscopy and evaluated the binding constants of these drugs in the micelles by using the red[1] shifts of the maximum absorption band and absorbance changes upon alkalization or in the presence of SDS.

Recently, our research group reported the detailed physicochemical investigation of interactions between pyridinium gemini surfactants, that is, [12-(S-2-S)-12], [14-(S-2-S)-14], [16-(S-2-S)-16], and PMT by employing conductivity, surface tension, steady state fluorescence, UV–visible, and NMR measurements [17]. In continuation of our interest in phenothiazines, we have now studied the interactions of bile salts with phenothiazines (PMZ and PMT). Since bile salts are very often used as penetration enhancers, so their binding with phenothiazines will improve their bioavailability due to which they can reach the systemic circulation and hence become available for distribution to the intended site of action. Bile salts possess a rigid steroid backbone with polar hydroxyl groups on the concave α-face and hydrophobic methyl groups on the convex β-face. The bile salts chosen for the present study are sodium cholate (NaC) and sodium deoxycholate (NaDC), which differ only in the number of hydroxyl groups (Fig. 1c and d). The hydrophobic bile salts such as NaDC promote absorption more effectively than NaC, which has more polar surface area [19]. Further, the trihydroxy bile salt, that is, NaC has higher *cmc* value as compared to the dihydroxy bile salt, NaDC.

Various bulk, interfacial, and thermodynamic parameters for the mixtures of phenothiazines (PMZ and PMT) with bile salts (NaC and NaDC) have been evaluated from conductivity and surface tension techniques. Moreover, the photophysical properties of phenothiazines have been utilized to study their binding behavior with bile salts by using the UV–visible and steady state fluorescence spectroscopy. The Benesi–Hildebrand (B–H) equation has been used to calculate the binding constants (K), stoichiometric ratios, and free energy change (ΔG) for the drug–bile salt complexes. These parameters predict the extent of binding between the phenothiazines and the bile salts.

2. Experimental section

2.1. Materials

The phenothiazines, promazine hydrochloride (PMZ, purity $\geqslant$ 98%) and promethazine hydrochloride (PMT, purity $\geqslant$ 98%), and bile salt, sodium deoxycholate (NaDC, purity $\geqslant$ 97%), were purchased from Sigma–Aldrich. The bile salt, sodium cholate (NaC, purity $\geqslant$ 99%), was purchased from Alfa Aesar, UK. All the chemicals were used as received. Sartorius analytical balance with a precision of ±0.0001 g was used for weighing the amount of different substances. The deionized double distilled water having conductivity in the range of 1–2 μS cm^{-1} was used for preparing all the solutions. Different mole fractions of drug–bile salt mixed systems were prepared from the stock solutions of different concentrations of drugs and bile salts.

2.2. Methods

2.2.1. Conductivity measurements

Conductometric measurements were taken on an EQUIP-TRONICS auto temperature conductivity meter model EQ.661 equipped with a dip-type conductivity cell having a cell constant of 1.01 cm^{-1}. Temperature was maintained constant at 25.0 ± 0.1 °C using a constant temperature bath. The conductivity values were recorded when their fluctuation was less than 1% within 2 min. The reproducibility of these measurements was within ±0.2%.

[1] For interpretation of color in Figs. 2, 3, 5 and 6, the reader is referred to the web version of this article.