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Spectral and chromatographic characterization of fixed dose combination norfloxacin and metronidazole interacting with cetyltrimethylammonium bromide

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ABSTRACT

Surfactant molecular self-assemblies solubilize and shelter the drugs from adverse conditions. As membrane mimetic they serve as a powerful tool to understand the consequences of drug membrane interactions. In this study the interaction modulation of fixed dose combination (FDC) having norfloxacin (NOR) and metronidazole (MET) with quaternary ammonium disinfectant (cetyltrimethylammonium bromide) has been described at physiological condition (pH 7.4). The spectral and physiochemical characteristic of NOR and MET interacting with cationic micelles were investigated by electronic spectroscopy as function of cetyltrimethylammonium bromide (CTAB) concentration from pre-micellar to post-micellar region. Magnitudes of binding constant and related Gibbs free energies were optimized using differential absorption spectrometry and micellar liquid chromatography (MLC). The results indicate potential solubilization of drugs in the peripheral region of micelles that may be helpful to their control release. The values of the binding capacities of drug-micelle system have verified these results.

1. Introduction

Understanding of the biological membrane has always been a challenging assignment due to its complicated structure. Pharmacologically active molecules are hydrophobic or hydrophilic in nature and they undergo various sort of interactions with the plasma membranes. Transport of these drugs molecules through the phospholipids membranes therefore has not been fully understood. These interactions could partially be explored using simplified membrane mimetic such as micelles [1–3].These physiochemical interactions of drug molecules with the micelles can be envisioned as approximation for interaction with plasma membranes.

Micelles solubilize the sparingly soluble drugs, enhance their bioavailability, save the drugs by encapsulation and act as drug carriers to the specific site of action [4–8]. Micelles show thermodynamic stability in their aggregation and dissociation [9]. Bio-distribution, transportation and efficacy of drugs are some of the important pharmacokinetic properties of the drugs that can easily be explained by the phenomenon of drug-micelles interaction owing to the electrostatic forces of attraction at the molecular level [10–12]. Numerous biological phenomenon occurs at the membrane surfaces or within their lipophilic moiety. Lipid head groups are ionizable due to which plasma membranes frequently bear a net charge. This results into a number of diverse binding properties of uncharged and charged molecules bearing ionizable groups. In spite of large amount of research work aimed to comprehend the action mechanism of drug at the molecular level, this question still remains uncertain. For this reason more information is required from membrane model system studies [13].

Qualitative and quantitative interaction studies between micelles core and drug molecules have been described by the spectroscopic studies [14–17]. The main emphasis of the present studies is to gain in depth a better understanding of the potential assimilation of pharmacologically active drugs norfloxacin (NOR) and metronidazole (MET) into the cationic surfactant cetyltrimethylammonium bromide (CTAB) at physiological pH 7.4. The basic molecular structures of NOR and MET are given below (Fig. 1).

NOR is an antibacterial drug belongs to fluoroquinolone. It is broad spectrum in action against gram negative and gram positive bacteria [18–20]. 14% of NOR bounds to plasma protein. When taken orally, up to 40% NOR is absorbed through the gut [21]. Most fluoroquinolones solubilize in the aqueous medium. However for better bioavailability, improved efficacy and to overwhelm the lipophilic barrier, a physiological medium (pH 7.4) is overall useful for diffusion through phospholipids membranes [22]. There are two pKa values of NOR that has been reported in literature, pKa_1 6.30 and pKa_2 8.38. The first pKa value is associated with the carboxylic acid and second pKa value correlates the

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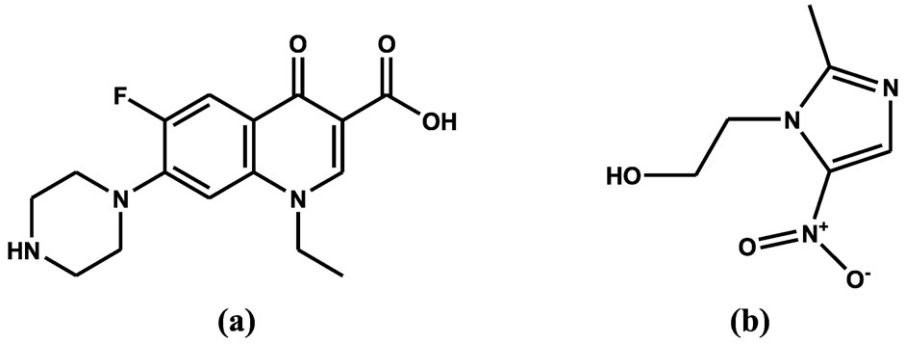

Fig. 1. Molecular structure of (a) norfloxacin (b) metronidazole.

piprazine ring of NOR [23,24]. MET has antibacterial action and has been widely used against the anaerobic infections. It acts against numerous protozoa, obligate anaerobic bacteria and facultative anaerobes. Less than 20% MET bound to plasma protein [25,26]. MET has single ionizable constant value with pKa 2.49. The ionization constant value (pKa) is an important factor in the optimization of drug design and studies. The extent of ionization has considerable influence on the disposition properties of the drugs [27]. The NOR and MET combination is commercially available in market in the form of tablets (NOR 400 mg + MET 500 mg) and syrup (NOR 100 mg + MET 100 mg).

Micellar liquid chromatography (MLC) is one of the important alternative of the conventional high performance liquid chromatography (HPLC) regarding ecological and economic aspects. MLC consume very less quantity of the organic solvents as compared to the reversed phase liquid chromatography (RPLC). This advantage makes MLC prominent over RPLC. Brij 35, CTAB and sodium dodecyl sulphate (SDS) are often used in MLC as surfactants [28,29]. CTAB is believed to be safe as it is used as disinfectant. It is absorbed through the intestine. The undigested and unabsorbed amount comes out with feces. The absorption through the skin is very slow [30–33]. There are three fundamental types of partitioning mechanisms of solute in MLC by which retention behavior is controlled i.e. partitioning from bulk into the micelles, and/or partitioning directly from bulk into the stationary phase. They may also directly transfer from micelles present in the mobile phase into the stationary phase. Retention of highly polar molecules is described by the partitioning from aqueous phase into the micelles and alkyl stationary phase. Hydrophobic molecules are supposed to move from micelles in the mobile phase into the stationary phase [34,35]. Physiological proteins can preferably be analyzed with MLC by direct injecting them into the system owing to ability of micelles to solubilize protein, serum plasma and urine sample, which otherwise involve hectic sample preparation steps in conventional HPLC and other extraction procedures [36]. Efficiency of columns in MLC decreases with time due to very slow mass transfer from stationary phase. Monomer adsorption on the stationary phase and poor wetting alter the characteristics of alkyl stationary phases [37]. In this study the binding of NOR and MET interacting with cationic micelle has been exclusively investigated at physiological pH 7.4 using ultraviolet (UV) spectroscopy and MLC. Particularly, allocation of drug molecules in the colloidal fluid and extent of the drug-CTAB interactions were quantified as a function of different concentrations of microheterogeneous assemblies.

2. Experimental

2.1. Materials and chemicals

NOR and MET were gifted by Lahore Chemical and Pharmaceutical Works Pvt. Ltd., (Lahore, Pakistan). Acetonitrile was of HPLC grade (E. Merck).CTAB, potassium dihydrogen phosphate (KH_2PO_4) and sodium hydroxide (NaOH) were used of analytical grades (E. Merck).The nylon filter papers0.45 μm (Millipore) were used for the filtration.

2.2. Procedure

The phosphate buffer solution (0.05 M) was prepared by accurately weighing 6.8045 g KH_2PO_4 in one liter volumetric flask and volume was made up to the mark by distilled water. Dilute NaOH solution was prepared by dissolving 8 g of NaOH in 100 mL of water and was used to adjust the pH of the buffer solution.

For MLC measurements, NOR and MET stock solution (2.0 mg mL^{-1}), 500.0 mg of each drug was accurately weighed in 250 mL volumetric flask and volume was made up to mark with phosphate buffer pH 7.4. To prepare working sample solution (0.4 mg mL^{-1}), 10 mL stock solution was transferred to another 50 mL volumetric flask and volume was made up to mark with phosphate buffer pH 7.4. The nylon filter papers 0.45 μm (Millipore) were used for the filtration of final solutions. For the spectral measurements, separate stock solution were prepared for NOR and MET. For NOR stock solution (0.1596 mg mL^{-1}), 159.6 mg drug was accurately weighed in 1000 mL volumetric flask and volume was made up to mark with phosphate buffer pH 7.4. Working sample solution of NOR having concentration 2.0×10^{-5} mol dm^{-3} was prepared by diluting 20 mL of stock solution to 500 mL with phosphate buffer pH 7.4. For MET stock solution (0.0856 mg mL^{-1}), 85.6 mg drug was accurately weighed in 1000 mL volumetric flask and volume was made up to the mark with phosphate buffer pH 7.4. Working sample solution of MET having concentration 2.0×10^{-5} mol dm^{-3} was prepared by diluting 20 mL of stock solution to 500 mL with phosphate buffer pH 7.4. Surfactant stock solution (100 mM) was prepared by dissolving 3.65 g CTAB per 100 mL of phosphate buffer pH 7.4. Varying molar concentrations of surfactant (3 mM, 5 mM, 7 mM, 9 mM, 11 mM) were prepared from additive solution (as diluent) before the measurement. The stability of drug solutions was checked periodically for one week at room temperature and were found stable. However, fresh solutions were prepared every time before experiments. Both the drugs follow Beer's law for all concentrations applied in the present work. All solutions were stored at room temperature and all measurements recorded at temperature 25 ± 0.1 °C.

2.3. Micellar liquid chromatography (MLC) measurements

Development of MLC method was carried out at HPLC system Hitachi, Japan. The system consists of a pump, an auto-sampler and a UV–Visible detector. The molecular retentions of NOR-CTAB and MET-CTAB were studied on Purospher STAR RP-18 column (5 μm, 250 × 4.6 mm) and presented in Fig. 2. Varying molar concentrations of surfactant (3 mM, 5 mM, 7 mM, 9 mM, 11 mM) were manufactured from surfactant stock solution. Finally each CTAB concentration and acetonitrile was mixed in a proportion of (100:15) and filtered through 0.45 μm membrane filter (Millipore).The mobile phase flow was kept