Probing Location of Anti-TB Drugs Loaded in Brij 96 Microemulsions Using Thermoanalytical and Photophysical Approach

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ABSTRACT: The aim of this work is to monitor the changes in microstructure in nonionic Brij 96 microemulsions and to locate the solubilization loci of antituberculosis drugs (of variable solubility using photophysical and thermoanalytical properties. Using properties such as spectral shift, Stroke's shift, and anisotropy for two dyes, that is, Nile red (NR) and tris(2,2′-bipyridine)ruthenium(II) dichloride (RC), the structure of microemulsions has been investigated. With the help of spectral and deconvoluted analysis, it has been seen that rifampicin (RIF) shows a strong interaction with NR and isoniazid (INH) and pyrazinamide (PZA) with RC. It has been concluded that RIF molecules are mainly present at the interface toward oil side and INH toward hydrophilic side, whereas PZA remains in free water. The findings have been correlated with aqueous solubility drugs and partition coefficients. Differential scanning calorimetry elucidates the state of water in microheterogeneous environment and variation of different states, that is, free, bound, interphasal, and nonfreezable water with dilution. In addition, it confirmed the stability and location of the drugs in the prepared Brij 96 microemulsion formulations. A good agreement between both the studies has been achieved. These findings will help in elucidating the drug delivery properties of anti-TB drugs-loaded microemulsion formulations in future. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 103:937–944, 2014

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INTRODUCTION

Microemulsions are isotropic in nature and are believed to have domains of water entrapped in a hydrocarbon dispersion phase (or vice versa) usually stabilized by a monolayer of surfactant.[1,2] For a nonionic surfactant-based microemulsion, the change in the content of water gives a peculiar transformation in structure from w/o to o/w via a bicontinuous phase.[3,4] Microemulsions act as super solvents for drugs. They can solubilize hydrophilic and lipophilic drugs. This is because of the existence of microdomains of different polarity within the same single-phase solution. The drug partitions between dispersed and continuous phase, and when the system comes into contact with a semi-permeable membrane, the drug can be transported through the barrier.[5–8]

The understanding of internal distribution of water is of great help in efficient utility of these systems in various chemical and biological reactions. Internal distribution of water, that is, bulk water, bound water, and free water, has been very well dealt in the past using methods such as nuclear magnetic resonance (NMR) and Fourier-transformed infrared spectroscopy (FTIR).[9,10] Among number of methods available for probing the changes in microstructure in the microemulsion that occur either because of the change in composition or temperature, our interest has been in uncovering information using photophysical and thermoanalytical methods. The use of solvochromatic probe has attracted many researchers working in the field of microemulsions as they give a direct correlation between the changing composition and microenvironment of microemulsions.[11–13]

Differential scanning calorimetry (DSC) is a powerful technique and is widely used for studying low-temperature behavior of multicomponent microemulsion systems.[14–18] This method has been used by Senatra et al.[19] for the investigation of thermal properties of percolative microemulsions. Whereas, Garti group[20] have utilized this technique for understanding the structure of food grade microemulsions. The measurements are beneficial for describing the melting behavior of water in restricted domain and are very well studied. However, there is a rare mention of its utilization in position location of hydrophobic as well as hydrophilic external entities.[21]

The objective of the present work is twofold: first is to understand the changes in microstructure of Brij 96 microemulsion and their dependence on ω ($[H_2O]/[surfactant]$) by probing the solubilization sites of optical probes. Second is to understand the percolation behavior and states of water in this nonionic microemulsion, to find out the probable location of antituberculosis drugs of variable solubilities [i.e., rifampicin (RIF) (hydrophobic) and isoniazid (INH) and pyrazinamide (PZA) (hydrophilic)] in this microheterogeneous media.

The photophysical properties of solvatochromic probes [i.e., Nile red (diethylamino-5H-benzo[a]-phenoxazin-5-one) (NR) and tris(2,2′-bipyridine)ruthenium(II) dichloride, $[Ru(bpy)_2]Cl_2$ (RC)] of variable solubility have been utilized not only to trace the changes in microstructures but also as markers to find out location of anti-TB drugs. To get the complete picture of the

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continuous transition of internal structure on dilution with water and to understand the heterogeneous nature of entrapped water, DSC has been carried out. The present work very well highlights the correlation and utility of thermophysical and photophysical techniques in elucidating the position of both hydrophobic and hydrophilic drugs in a microemulsion formulation. The findings of the work will help in increasing the basic understanding of a pseudo-ternary system for their use as a drug delivery vector in a better and more efficient way.

MATERIALS AND METHODS

Brij 96 (polyoxyethylene-10-oleoyl ether) was purchased from Fluka (MO, USA). Purity and composition of Brij 96 were elucidated with ^{1}H NMR, ^{13}C NMR, and MS (Figures S1a–S1e, Supporting Information). Butanol (purity >99.8%) was purchased from Spectrochem (Maharashtra, INDIA). RC (purity > 99%), ethyl oleate (EO), NR (purity > 99%), RIF (purity >98.0%), PZA (purity >98.0%), and INH (purity >98.0%) were supplied by Sigma (MO, USA). The chemical structures of NR, RC, RIF, INH, and PZA are given in Figures S2a–S2e (Supporting Information). The chemicals were used as received. Doubly distilled water (specific conductance, 2–4 μS cm^{-1} at 303.15 K) was used in all the preparations. Absorption and fluorescence spectra of microemulsions were recorded by using JASCO-530 UV–visible spectrophotometer (Japan) and Hitachi spectrofluorimeter (Japan) with a wavelength accuracy of ±1 nm. The temperature was kept constant at 30°C by circulating water through the cuvette holder. DSC measurements were carried out using TA (Q20) (USA) with blank pan as reference (maintained at same temperature). All the compositions are given in w/w. All the experiments were carried out in duplicate.

Microemulsion Preparation

Microemulsions consisting of oil (EO), surfactant (Brij 96), butanol, and double distilled water were prepared with a constant surfactant: cosurfactant mass ratio (K_m) of 1.5 in screw-cap glass vials at 303.15 ± 0.01 K using a RE320 Ecoline thermostat. The compositions were monitored visually every day for a month. Transparent, single-phase mixtures stable for over 6 months were designated as microemulsions. The microemulsion systems of different weight fractions at various ω (molar ratio of water:surfactant) were selected from the phase diagram from previous studies at oil–surfactant mass ratio of 1.2:2.[22] The phase diagram is given in Figure S3 (Supporting Information). The was varied from 1.3 to 13.0. The upper was selected keeping in mind the dilution limit of the microemulsion system (phase separation occurs beyond these values).

Probe Insertion

The NR incorporation was carried out by dissolving in EO to obtain stock solutions for absorbance (6.28×10^{-5} M) and emission (0.069×10^{-5} M) measurements, respectively. RC was dissolved in water to obtain stock solutions (0.055×10^{-5} M) for absorbance and emission measurements. For quenching analysis, concentration of RIF was varied from 0.08 to 0.75 mM.

The measurements were carried out using blank microemulsion in the reference cell for the absorption measurements. For the emission measurements, the background fluorescence was adjusted before the measurements.

Steady-State Anisotropy

For steady-state fluorescence anisotropy measurements, the spectrofluorimeter was equipped with polarizers in the excitation and emission paths and I_{II} and $I_{\perp}$ were recorded. Steady-state fluorescence anisotropy (r) was calculated using following equations

$$r = \frac{I_{II} - I_{\perp}}{I_{II} + 2GI_{\perp}} \quad (1)$$

where, G is a correction factor for the polarization due to the optics of the instrument. The samples were run at scan speed of 240 nm/min, with emission and excitation slit width of 10.0 nm.

Data Analyses Via Peak Fit

Fit analysis of spectroscopy data of Brij 96 microemulsions as a function of values in absence or presence of anti-TB drugs were performed with Peak fit 4.0 (Sigma). In Peak fit, nonlinear least-squares fitting routine was used to analyze the emission/absorbance spectra. The individual spectrum either absorbance or emission (at fixed excitation wavelength and at a particular) was deconvoluted into a sum of overlapping Gaussian functions with frequency as the independent variable. All fits represent the minimum number of components required to achieve a fit (R^2 of 0.99) and a random scattering of residuals. The reproducibility of the curve fitting was tested by varying the starting positions and amplitudes of the constituent curves. For each final fit of a spectrum, area of each Gaussian function was characterized.

Drug Incorporation

To incorporate anti-TB drugs in prepared Brij 96 microemulsions, INH and PZA were dissolved into the known quantity of water (individually), whereas RIF was dissolved into the preweighed hydrophobic component of the microemulsion system (i.e., EO) in required amounts under stirring followed by addition of remaining components.

DSC *Measurements*

To verify the accuracy of the caloric data before experiments, the instrument was calibrated using indium. For measurements, 5–15 mg microemulsion samples of different (with or without drug) were weighed in aluminum pans and were instantly sealed. The samples were rapidly cooled in liquid nitrogen up to −100°C. After equilibration, the samples were heated at 10°C min^{-1} to reach the ambient temperatures. An empty pan was used as a reference. DSC temperatures were reproducible to ±0.5°C.

Oil/Buffer Partition Coefficients

Oil/buffer partition coefficients of drugs were determined by dissolving 10 mg of each drug in respective solvents. RIF was dissolved in 1 mL EO, whereas INH and PZA were dissolved in 1 mL water. Final mixture of EO:water was made in 1:1 ratio (v/v). Each oil water mixture of individual drug was shaken for 5 min and centrifuged for 1 h. The two layers were separated and the content of each drug RIF, INH, and PZA in aqueous layer was assayed separately by UV–visible spectrophotometer at characterstic wavelength of 475, 262, and 268 nm, respectively.