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Transport properties in aqueous ethambutol dihydrochloride

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ABSTRACT

Mutual diffusion coefficients, densities and viscosities are reported for aqueous solutions of ethambutol as its dihydrochloride (EMBDHC) at finite concentrations and at 298.15 K. From these experimental results and by using the appropriate models (Stokes–Einstein and Hartley), the hydrodynamic radii R_h, the diffusion coefficient at infinitesimal concentration D^0 and the thermodynamic factors, F_T, have been estimated, permitting us to have a better understanding of the transport behavior of ethambutol dihydrochloride in solution. Elucidation of lack of any possible drug–drug interactions in these systems was obtained by complementary ^{1}H nuclear magnetic resonance (NMR) spectroscopy data.

1. Introduction

Ethambutol as its dihydrochloride (EMBDHC) is a first-line antituberculosis drug with a high specificity toward *Mycobacterium tuberculosis*, and is administered in an association protocol with isoniazid, rifampicin and pyrazinamide, as currently recommended by international guidelines (Tomioka, 2006; Scior and Garces-Eisele, 2006; Zhao et al., 2007; Banerjee et al., 2008; Ranguelova et al., 2008; Crofts et al., 2008; Golub et al., 2008). However, the global health problems of tuberculosis (TB) on this infectious deadly disease and the high rate of co-infection with persons suffering from the human immunodeficiency virus (HIV) (e.g., Golub et al., 2008) have greatly contributed to the need of developing new, affordable, anti-tuberculous drugs without cross-resistance with known antimycobacterial agents. In fact, tuberculosis was declared a global emergency by WHO in 1993. We live, therefore, in the confluence of a double challenge, expeditious and pragmatic treatment of infected populations, and control of resistance to treatment, bound to break the cycle of infection. However, knowledge of the mechanisms involved is still limited, both in terms of bacillary defenses and the action of drugs, and the properties and behavior of such chemical systems are poorly known, even though this is a prerequisite to obtain an adequate understanding and solve these problems of health. In fact, few researchers have taken into account the transport behavior of these anti-tuberculous drugs in aqueous solutions (e.g., Ribeiro et al., 2009, 2010), although this is an important property for their in vivo behavior. Concerning EMBDHC, no data on mutual diffusion coefficients are available, namely, at 298.15 K – relevant data for in vivo pharmaceutical application – as far as careful literature searches have shown. This paper reports experimental data for mutual diffusion (interdiffusion) coefficients D, measured by the Taylor dispersion method. These studies were complemented by some density and viscosity measurements for aqueous solutions of EBMDHC at concentrations from (0.00 to 0.10) g dm^{-3} at 298.15 K, in addition to NMR spectral studies on solutions.

From the experimental data it is possible to estimate important parameters, such as the hydrodynamic radius, R_h, apparent molar volumes, ϕ_V, and diffusion coefficient at infinitesimal concentration, D^0.

In addition, the Hartley equation (Erdey-Grúz, 1974; Tyrrell and Harris, 1984) and the measured diffusion coefficients are used to estimate activity coefficients for aqueous EMBDHC, contributing to a better understanding of their thermodynamic behavior in aqueous solution at different concentrations.

We intend to both contribute to the body of knowledge of this critical disease, and concurrently defend the important role that

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studies of transport properties may have on current and future drug development pipelines.

2. Experimental

2.1. Reagents and solutions

Ethambutol dihydrochloride (EMBDHC) (Sigma–Aldrich, pure >99.9%, SLBF2556V, $M = 277.23\ g\ mol^{-1}$) (Table 1 and Scheme 1) was used as received without further purification. The solutions for the diffusion measurements were prepared in calibrated volumetric flasks using Millipore-Q water (18.2 MΩ cm). The solutions were freshly prepared and de-aerated for about 30 min before each set of runs. The uncertainty concerning their compositions was usually within ±0.1%. For the density and viscosity measurements, solutions were prepared by direct weighing both the solute and distilled water in a Mettler AE 240 balance with a precision of ±0.0001 g (the uncertainty concerning composition was less than ±0.07%).

2.2. Density measurements

The densities of EMBDHC aqueous solutions at 298.15 K were determined with an Anton Paar DMA5000M densimeter (precision of $1 \times 10^{-6}\ g\ cm^{-3}$ and accuracy of $5 \times 10^{-6}\ g\ cm^{-3}$ in the ranges of 0–90 °C of temperature and 0–10 bar of pressure). The uncertainty of the results obtained is estimated to be less than 0.001%.

2.3. Viscosity measurements

Viscosity measurements of these solutions were performed with an Ostwald type viscometer, calibrated from water, immersed into a water-thermostat bath which temperature was controlled within ±0.02 K by using a digital thermometer. The arithmetic mean value of four flow times for each solution was taken to calculate such viscosity values. The measurement of the efflux time was carried out with a stopwatch with a resolution of 0.2 s. The uncertainty of these values was less than ±0.1%.

2.4. Diffusion measurements

The Taylor dispersion method for measuring diffusion coefficients is well described in the literature (e.g., Barthel et al., 1996; Callendar and Leaist, 2006; Ribeiro et al., 2005, 2006; Tyrrell and Harris, 1984), and consequently we only indicate some of its most relevant points on the experimental determination of binary diffusion coefficients.

This technique is based on the dispersion of a very small amount of solution injected into a laminar carrier stream of solvent or solution of different composition flowing through a long capillary tube of length and radius $3.2799\ (\pm 0.0001) \times 10^3$ cm and 0.05570 (±0.00003) cm, respectively, at $T = 298.15$ K (±0.01 K) in a carefully home-made air thermostat.

At the start of each run, a 6-port Teflon injection valve (Rheodyne, model 5020) was used to introduce $0.063\ cm^3$ of solution into the laminar carrier stream of slightly different composition. A flow rate of $0.23\ cm^3\ min^{-1}$ (corresponding to 3.5 rpm of the peristaltic pump head) has been used, and was controlled by a metering pump (Gilson model Miniplus 3) to give retention times of about 8×10^3 s. The dispersion tube and the injection valve were kept at 298.15 K (±0.01 K) in an air thermostat.

Dispersion of the injected samples was monitored using a differential refractometer (Waters model 2410) at the outlet of the dispersion tube. Detector voltages, $V(t)$, were measured at accurately timed 5 s intervals with a digital voltmeter (Agilent 34401A) with an IEEE interface. Binary diffusion coefficients were evaluated by fitting the dispersion equation

$$V(t) = V_0 + V_1 t + V_{max}\left(\frac{t_R}{t}\right)^{1/2} \exp\left[-12D\frac{(t-t_R)^2}{r^2 t}\right] \quad (1)$$

to the detector voltages. The additional fitting parameters were the mean sample retention time t_R, peak height V_{max}, baseline voltage V_0, and baseline slope V_1.

The concentrations of the injected solutions ($\bar{c} + \Delta c$) and the carrier solutions ($\bar{c}$) differed by $\pm 0.150\ mol\ dm^{-3}$ or less. Solutions of different composition were injected into each carrier solution to confirm that the measured diffusion coefficients were independent of the initial concentration difference and therefore represented the differential value of D at the carrier-stream composition.

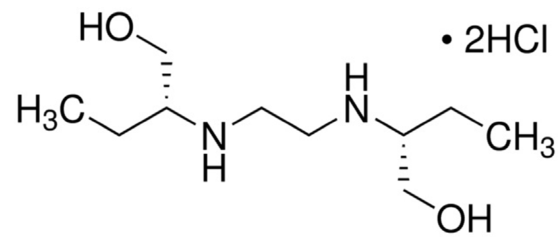
2.5. ^{1}NMR experiments

A solution of EMBDHC $0.20\ mol\ dm^{-3}$, pH* 4.0, was prepared in D_2O (99.9%, Aldrich) and was used as a stock solution. The additional solutions of concentrations 0.10, 0.050, 0.025, 0.010 and 0.005 $0.20\ mol\ dm^{-3}$ were prepared by dilution of this. The pH* values quoted are the direct pH-meter readings (room temperature) after standardization with aqueous (H_2O) buffers. The ^{1}H spectra were obtained on a Bruker Avance III 400 at an operating frequency of 400 MHz. The methyl signal of *tert*-butyl alcohol was used as external reference for ^{1}H (δ 1.3).

3. Results and discussion

3.1. Concentration dependence of density and molar volume at finite concentrations

The experimental density and viscosity values of EMBDHC aqueous solutions at 298.15 K are indicated in Table 2. The data of



Scheme 1. Ethambutol dihydrochloride ($C_{10}H_{24}N_2O_2{\cdot}2HCl$).

Table 1
Sample description.

Chemical name	Source	Purity
Ethambutol dihydrochloride ($C_{10}H_{24}N_2O_2{\cdot}2HCl$)	Sigma–Aldrich	Mass fraction > 0.99 (SLBF2556V, $M = 277.23\ g\ mol^{-1}$)

Table 2
Binary mutual diffusion coefficients of (EMBDHC) in aqueous solutions at different concentrations, c, and at 298.15 K.

$c/(mol\ dm^{-3})$	$D \pm \sigma/(10^9\ m^2\ s^{-1})$
0.0010	1.055 ± 0.003
0.0040	0.956 ± 0.003
0.0100	0.907 ± 0.002
0.0200	0.844 ± 0.003
0.0500	0.772 ± 0.001
0.1001	0.738 ± 0.002