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DENSITIES, VISCOSITIES AND REFRACTIVE INDEXES OF ETHYLENE GLYCOL SOLUTIONS AND TRIS-Mg(II)-KCl BUFFERS

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SUMMARY

The densities, viscosities and refractive indexes of two Tris-Mg(II)-KCl buffers and solutions of ethylene glycol which are frequently used in various biochemical studies have been measured. These physical constants are important for the calculation of hydrodynamic and spectroscopic parameters of macromolecules. Failure to use the proper constants results in significant errors.

INTRODUCTION

In recent years two buffer systems have been widely used in various aspects of ribosome research. The TMK_{50} buffer, which contains 10 mM Tris, 20 mM Mg(II), and 50 mM KCl (pH 7.6), has been found to be the oprimal buffer for *in vitro* functional studies of protein synthesis with particular regard to the ribosome activity assay (1,2). The TMK_{360} buffer contains 10 mM Tris, 20 mM Mg(II), and 360 mM KCl (pH 7.6) and has been found by Nomura and coworkers to be the optimal medium for reconstitution of the *E. coli* 30 S and *Bacillus stearothermophilus* 50 S ribosomal subunits (1,2). Many of the structural studies of ribosomal proteins, RNAs and their mutual interactions have been performed in these buffers or similar media (3,11,16,17).

The solvent ethylene glycol, which is known to disrupt hydrophobic interactions, has been widely used in attempts to elucidate the nature of intramolecular forces involved in stabilizing macromolecular structure (4-11). Such approaches have recently been applied to the study of conformational

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stabilities of the ribosome and its subunits, including the role which the binding of ribosomal proteins to RNA plays in stabilization (11).

The physicochemical characterization of ribosomes, ribosomal subunits, and other macromolecules by hydrodynamic and spectroscopic analysis requires a knowledge of some basic physical parameters of the buffer systems concerned. For example, the conversion of an observed sedimentation coefficient (S_{obs}) obtained for a ribosome in TMK_{360} at 37°C to the density and viscosity conditions of a standard solvent (i.e. water) at 20°C requires a knowledge of the density and viscosity of TMK_{360} at 37°C. Such information is also required for calculation of the intrinsic viscosity and frictional coefficient of a macromolecule in a given buffer.

As recognized by Kawahara and Tanford (13) for urea and guanidint hydrochloride solutions, a uniformly available set of physical values for the important ribosome buffer systems is desirable for the standardization of ribosomal physical parameters thereby facilitating their confident comparison between the studies of different investigators. Hence, the densities, viscosities and refractive indexes of TMK_{50} and TMK_{360} at 5°C, 25°C, and 37°C are herein reported as well as the same three parameters for 10% to 90% (v/v) ethylene glycol in TMK_{360} solutions at 5°C.

MATERIALS AND METHODS

Tris(hydroxymethyl)aminomethane was purchased from the Sigma Chemical Co. (St. Louis, MO). Potassium chloride and $MgCl_2 \cdot 6H_2O$ were purchased from the Mallinckrodt Chemical Co. (St. Louis, MO) and ethylene glycol (Fisher certified E-178) was purchased from the Fisher Scientific Co. (St. Louis, MO). The water used to prepare all solutions was double-distilled, deionized and passed through an activated charcoal bed.

Density determinations were made with a 20 ml volume pycnometer. The empty and filled pycnometer was weighed to the nearest .00001 gram. The filled pycnometer was thermally equilibrated in a constant temperature water bath, and readings were obtained at fifteen minute intervals until no further volume changes were observed as a result of thermal expantion or contraction. Viscometry measurements of TMK_{50} and TMK_{360} were performed with a #25 Cannon semi-microviscometer (Cannon Instrument Co., State College and Boalsburg, PA) with a range of 0.5 - 2.0 centistokes (cs). A #75 Cannon semi-microviscometer (1.6 - 8.0 cs) was used for the viscometry of 20% (v/v) and 40% (v/v) ethylene glycol-TMK_{360} and viscometry of the 60%-90% (v/v) solutions was performed with a #150 Cannon semi-microviscometer (7.0 - 35 cs). The charged viscometers were placed in a constant temperature bath (± 0.1°C; Cannon

Instrument Co., State College and Boalburg, PA) and allowed to equilibrate for a minimum of fifteen minutes before readings were obtained. The viscosity was calculated via the relationship: $\eta^x = A\rho_x t$, where η^x is the viscosity of the solution at x°C, A is the viscometer constant, ρ_x is the density of the solution at x°C, and t is the capillary transit time.

The refractive indexes of the above solutions were obtained with a Bausch and Lomb refractometer. Temperature regulation was achieved at 5°C ± 0.1°C, 25°C ± 0.1°C, and 37°C ± 0.1°C via circulation of fluid from a Lauda K2/R (Brinkmann Instruments, W. Germany) bath through the refractometer prism blocks. Prior to each refractive index determination, the refractometer was calibrated with water, using the refractive index for water at the appropriate temperature (14,15).

RESULTS

The densities, viscosities and refractive indexes of TMK_{50} and TMK_{360} at 5°C, 25°C and 37°C are given in Table I. Graphs of the same three parameters as functions of the concentration of ethylene glycol in TMK_{360} at 5°C are presented in Figures 1-3 with specific experimental values cited in Table II. Equations representing Figures 1-3 were obtained with a computer regression technique and are given by:

TABLE I

Physical Constants of TMK_{50} and TMK_{360} Buffers at Various Temperatures

Temp. (°C)	Density (g/ml)	Viscosity (centipoise)	Refractive Index
	TMK_{50} Buffer		
5	1.00461	1.547	1.3343
25	1.00167	0.9138	1.3337
37	0.9976	0.7100	1.3328
	TMK_{360} Buffer		
5	1.01995	1.525	1.3370
25	1.01606	0.9078	1.3371
37	1.01200	0.7135	1.3355

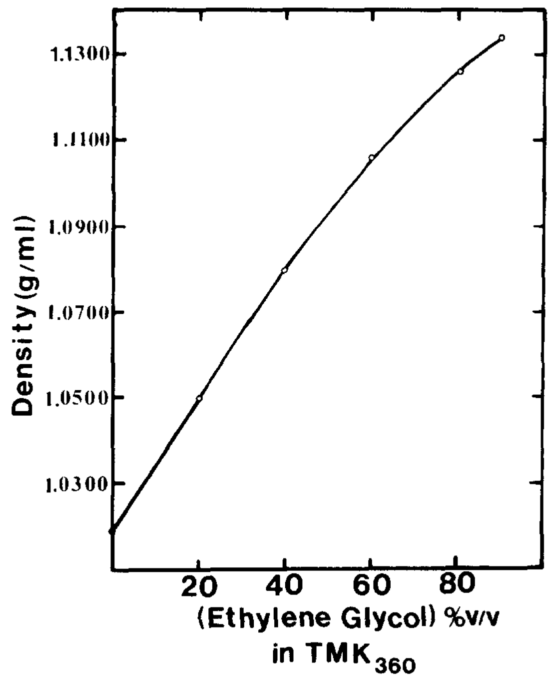

Figure 1. The densities of aqueous solutions of ethylene glycol in 10 mM Tris, 20 mM $MgCl_2$, 360 mM KCl (pH 7.6) at 5°C as a function of increasing concentration of ethylene glycol.

$$\text{Density (g/ml)} = 1.0194 + 0.00142X + 0.00001X^2 \quad \text{(Eqn. 1)}$$

$$\text{Viscosity (cp)} = 1.46 + 0.048764X + 0.0011701X^2 \quad \text{(Eqn. 2)}$$

$$\text{Refractive Index} = 1.3381 + 9.1993 \times 10^{-4}X + 9.7788 \times 10^{-6}X^2 \quad \text{(Eqn. 3)}$$

where X is the percent v/v concentration of ethylene glycol in TMK_{360} at 5°C.

DISCUSSION

Important structural information about macromolecules has ofted been obtained as a result of monitoring various physicochemical parameters as functions of the concentration of a denaturant. Spectroscopic techniques utilizing ethylene glycol as a denaturant have revealed important structural information about protein and nucleic acid secondary structure (e.g. 4,10,11,12).

The calculation of such physcial parameters as the standardized sedimentation coefficient ($S^{\circ}_{20,w}$) and intrinsic viscoeity ([η]) requires these physical constants. Further examination of such parameters as they are reported in

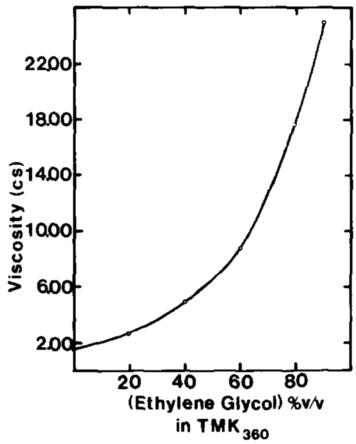

Figure 2. The viscosities of aqueous solutions of ethylene glycol in 10 mM Tris, 20 mM $MgCl_2$, 360 mM KCl (pH 7.6) at 5°C as a function of increasing concentration of ethylene glycol.

Tables I and II reveals that: 1) one cannot assume these values to be the same as those for water at the corresponding temperature without introducing significant error into the calculations; 2) even small differences in the physical buffer values used by different investigators can produce significant differences in standardized physical values and hence structural interpretations of identical experimental results; and 3) linear interpolation of ethylene glycol-TMK_{360} viscosity and density values in order to obtain the viscosities and densities of solutions of intermediate concentrations may generage erroneous values.

For example, the calculation of the $S_{20,w}$ of a particle with an S_{obs} of 40.0 in TMK_{360} at 25°C using the density and viscosity of TMK_{360} at 25°C, gives a $S_{20,w}$ value of 37.0. If one were to assume the density and viscosity of the TMK_{360} at 25°C to be virtually the same as those for water at 25°C, the $S_{20,w}$ decreases to 34.8, i.e. a 6% error.

The distinctly nonlinear natures of the density (especially at higher ethylene glycol concentrations) and viscosity plots of ethylene glycol-TMK_{360} solutions as functions of ethylene glycol concentration are evident in Figures 1 and 2. Clearly, linear interpolation of the physical values for high and

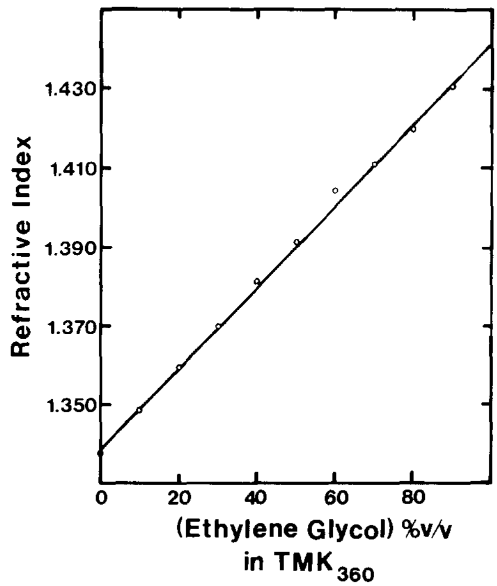

Figure 3. The refractive indexes of aqueous solutions of ethylene glycol in 10 mM Tris, 20 mM $MgCl_2$, 360 mM KCl (pH 7.6) at 5°C as a function of increasing concentration of ethylene glycol.

low ethylene glycol concentration solutions in order to obtain the corresponding values for intermediate concentrations of ethylene glycol may generate grossly inaccurate values. Utilization of equations 1, 2 and 3 may avoid error introduced from extracting data from the published graphs.

As mentioned earlier, transition curves expressing macromolecular physical parameters as functions of denaturant concentration may yield information

TABLE II

Physical Constants For Ethylene Glycol-TMK_{360} Solutions at 5°C

Ethylene Glycol in TMK_{360} (%v/v)	Density (g/ml)	Viscosity (centistoke)	Refractive Index
0	1.0194	1.47	1.3379
20	1.0496	2.75	1.3595
40	1.0796	4.93	1.3814
60	1.1060	8.79	1.4041
80	1.1260	17.7	1.4200
90	1.1339	25.1	1.4306

important to understanding the structure of a macromolecule. The refractive index plot (Figure 3) and equation 3 may be helpful in the precise determination of the ethylene glycol concentration of a ethylene glycol-TMK_{360} solution simply from the convenient and precise measurement of the solution's refractive index.

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