havior of the model organic anions employed in this study will provide further understanding, not only of the design and modification of drug molecules but also of the effects of the competitive renal tubular secretion inhibitor on the distribution pharmacokinetic parameters of the substrate compounds.

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Behavior of Erythrocytes in Ternary Solvent Systems

SHIAO-HUAN KU and DONALD E. CADWALLADER ˣ

Abstract □ The effect of ternary solvent systems on erythrocytes was investigated. Hemolysis experiments were run at 37° in solutions containing various amounts of water, two nonaqueous solvents, and 0.9% sodium chloride. The nonaqueous solvents were propylene glycol, polyethylene glycol 400, dimethyl sulfoxide, dimethylformamide, and tetramethylurea. Ternary diagrams based on the critical hemolytic compositions of the various ternary systems are presented.

Keyphrases □ Erythrocytes—behavior in ternary solvent systems, ternary diagrams, critical hemolytic compositions □ Solvents—effect of ternary solvent systems on erythrocytes, ternary diagrams, critical hemolytic compositions □ Blood—behavior of erythrocytes in ternary solvent systems, ternary diagrams, critical hemolytic compositions □ Intravenous preparations—behavior of erythrocytes in ternary solvent systems

To prepare a safe and efficacious injection, it is sometimes necessary to employ a mixed solvent system consisting of water and a nonaqueous solvent. The behavior of rabbit and human erythrocytes in aqueous solutions of propylene glycol and liquid polyethylene glycol (1–3) and the behavior of human erythrocytes in aqueous solutions of dimethyl sulfoxide, liquid amides, tetramethylurea systems, and monohydric alcohols (4–7) were reported in previous papers in this series.

Binary solvent systems are not the only systems that can be used for intravenous preparations. Since aqueous solutions containing two or more nonaqueous solvents might be necessary to prepare a suitable solution, the effects of some ternary solvent systems on red blood cells were studied. Experiments were conducted to determine the behavior of human erythrocytes in systems containing two nonaqueous solvents in 0.9% aqueous saline.

The nonaqueous solvents selected were propylene glycol, polyethylene glycol 400, dimethyl sulfoxide, dimethylformamide, and tetramethylurea. Critical hemolytic concentrations were determined for the various ternary solvent systems, and the data were used to construct ternary diagrams depicting the various concentrations of water and two nonaqueous solvents, mixed together as ternary solvent blends, which are compatible with human red cells.

EXPERIMENTAL

Materials—Propylene glycol[1] USP, polyethylene glycol 400[2] (average mol. wt. 380–420), tetramethylurea[3], and reagent grades of dimethylformamide[4] and sodium chloride[2] were used.

Preparation of Solutions—All solvent systems were volume-

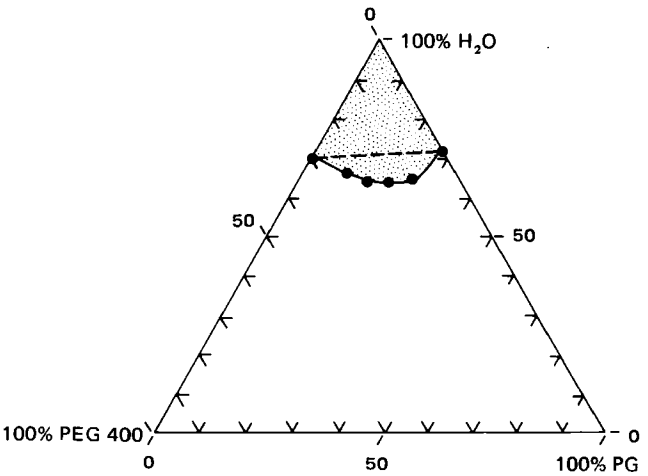

Figure 1—*Ternary diagram for aqueous propylene glycol (PG)–polyethylene glycol 400 (PEG 400) solutions containing 0.9% sodium chloride at 37°.*

[1] Fisher Scientific Co.
[2] J. T. Baker Chemical Co.
[3] Aldrich Chemical Co.
[4] Mallinckrodt Chemical Works.

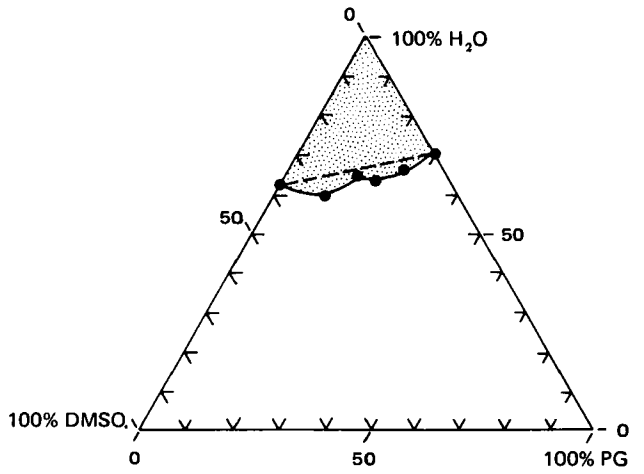

Figure 2—*Ternary diagram for aqueous propylene glycol (PG)–dimethyl sulfoxide (DMSO) solutions containing 0.9% sodium chloride at 37°.*

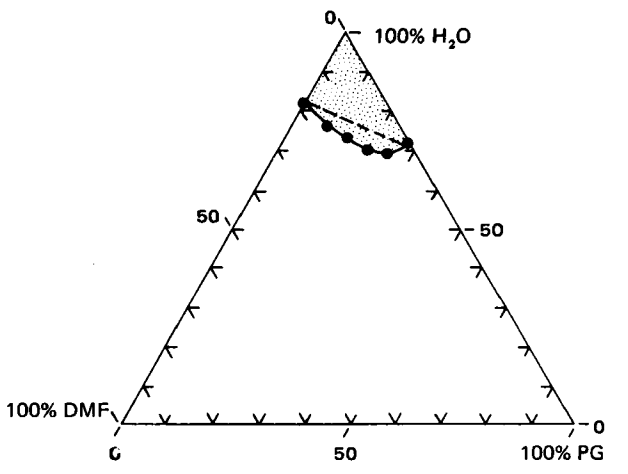

Figure 4—*Ternary diagram for aqueous propylene glycol (PG)–dimethylformamide (DMF) solutions containing 0.9% sodium chloride at 37°.*

in-volume percentage preparations, and each ternary system contained 0.9% (w/v) sodium chloride (0.15 *M*). Necessary dilutions were made from stock solutions, and distilled water was used to prepare all solutions.

Collection of Blood—Blood samples were obtained from the forearm veins of several 20–25-year-old Caucasian and Oriental subjects. Fresh blood samples were used. Approximately 10 ml of blood was obtained from each donor and placed in a 50-ml round-bottom flask containing 10–15 glass beads. The flask was rotated gently for about 5 min to separate the fibrin, and then the blood was decanted into a 50-ml conical flask and aerated by swirling the flask gently for about 5 min.

Quantitative Determination of Percent Hemolysis—In each experiment, the hemolytic method was used to determine the degree of hemolysis of erythrocytes in the various solvent systems. This quantitative method is based on the fact that a hypotonic solution liberates oxyhemoglobin in direct proportion to the number of cells hemolyzed.

Into each of two test tubes was transferred 5 ml of the standard sodium chloride solution (0.32, 0.34, . . ., 0.46, and 0.48%) or 5 ml of the mixed solvent systems. The standard sodium chloride solutions were used to ensure the osmotic normalcy of each blood sample. After the test tubes were brought to a constant temperature in a water bath (37 ± 0.5°), 0.05 ml of blood was pipetted into each tube. Each tube was shaken on a vibratory mixer to ensure thorough mixing and allowed to remain at 37° for 45 min. The tubes were centrifuged at approximately 2500 rpm, and the light absorbance of the supernatant liquid was measured using a photoelectric colorimeter[5] equipped with a No. 54 filter.

To find the percent hemolysis, these absorbance readings were divided by the absorbance readings for 0.05 ml of blood in 5 ml of distilled water (standard for 100% hemolysis) and multiplied by 100. A blank, made by placing 0.05 ml of blood in 5 ml of 0.9% sodium chloride solution, was used to cancel any light absorbance inherent in the blood sample. Both the standard and the blank were subjected to the same conditions of standing at 37° for 45 min followed by centrifuging (2500 rpm for 5–10 min).

Construction of Ternary Diagram—A ternary phase diagram can be constructed when three liquid components are involved in a solution at a fixed temperature. In the current studies, one apex of the triangle represents 100% of water, and each of the other two apexes represents 100% of a different nonaqueous component. The side of the triangle opposite each apex represents zero percentage of that particular component.

The points in the ternary diagram are the experimental values, and each point represents the average of at least two experiments using different blood samples. The points were connected to give an experimental curve for the particular ternary solvent system. The region within each curve (shaded area) defines the compositions of ternary solvent systems in which human red cells do not hemolyze (<5% hemolysis) under the experimental conditions. The region outside the curve describes the solvent compositions

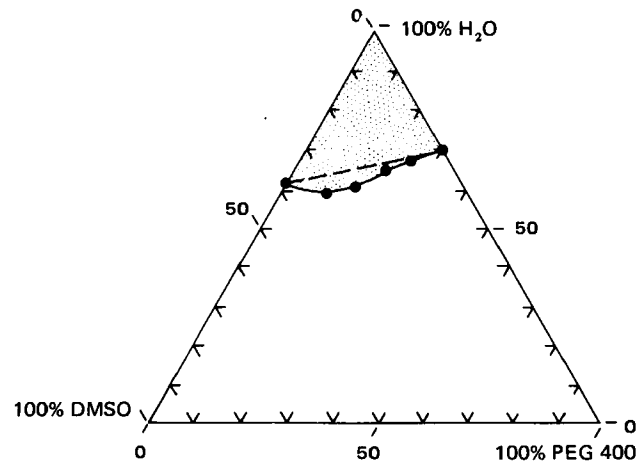

Figure 3—*Ternary diagram for aqueous polyethylene glycol 400 (PEG 400)–dimethyl sulfoxide (DMSO) solutions containing 0.9% sodium chloride at 37°.*

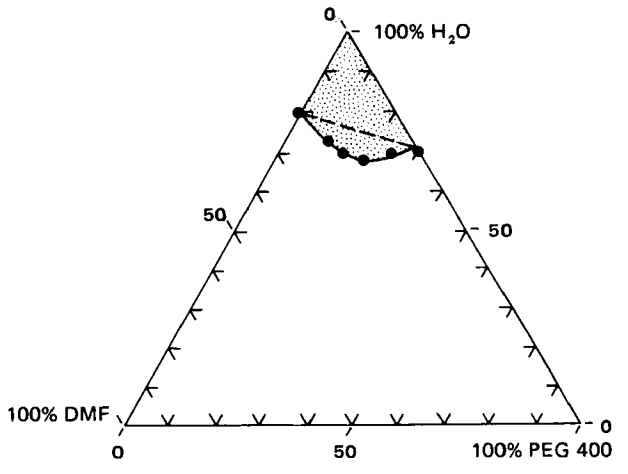

Figure 5—*Ternary diagram for aqueous polyethylene glycol 400 (PEG 400)–dimethylformamide (DMF) solutions containing 0.9% sodium chloride at 37°.*

[5] Klett–Summerson.

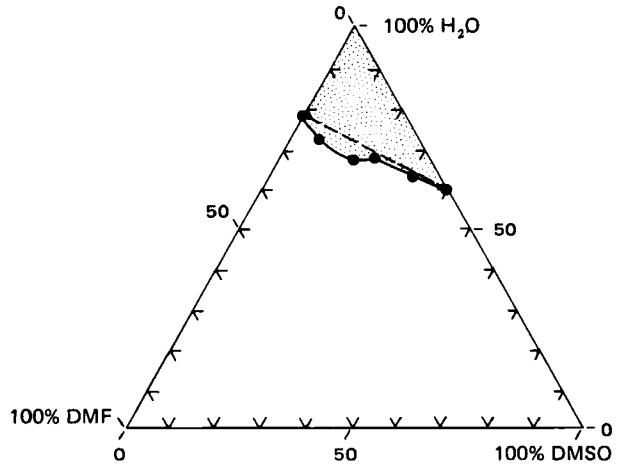

Figure 6—*Ternary diagram for aqueous dimethyl sulfoxide (DMSO)–dimethylformamide (DMF) solutions containing 0.9% sodium chloride at 37°.*

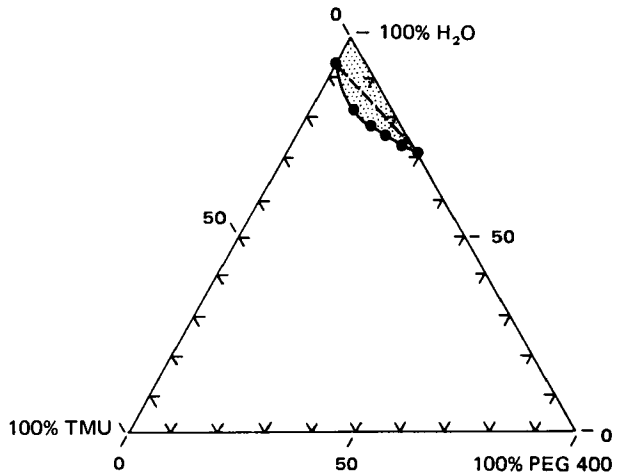

Figure 8—*Ternary diagram for aqueous polyethylene glycol 400 (PEG 400)–tetramethylurea (TMU) solutions containing 0.9% sodium chloride at 37°.*

where hemolysis and/or discoloration and precipitation were not prevented.

Each ternary diagram has a tie-line (dashed line) connecting the critical concentrations of the two nonaqueous solvents. If the hemolytic effect of each individual component is strictly an additive property of the solvent, this tie-line should represent the theoretical curve delineating nonhemolytic from hemolytic compositions.

RESULTS AND DISCUSSION

Binary Solvent Systems—Experiments were carried out to determine the critical hemolytic concentration of the individual nonaqueous solvents in 0.9% saline solution. These data were utilized for the baseline points in the construction of the individual ternary diagrams. The critical hemolytic concentrations (% v/v) of the solvents were: propylene glycol, 30%; polyethylene glycol 400, 31%; dimethyl sulfoxide, 38%; dimethylformamide, 20%; and tetramethylurea, 8%. Each critical concentration is the average of four experiments with different blood samples and is in good agreement with the data reported previously (2–6).

Ternary Solvent Systems—To determine the effect of ternary solvent systems on erythrocytes, hemolysis experiments were run at 37° in solutions containing various amounts of water, two nonaqueous solvents, and 0.9% sodium chloride. Ternary diagrams showing the critical concentrations of each ternary solvent system are shown in Figs. 1–10. These curves were constructed as described under *Experimental*, utilizing the experimental data. In each diagram, the experimental curve separates the nonhemolytic from the hemolytic compositions of the ternary systems. The transition from nonhemolytic to hemolytic concentrations was very abrupt.

All ternary solvent systems had experimental curves that were convex with reference to the theoretical tie-lines and penetrated into higher nonaqueous solvent concentration areas. This finding indicated that one or both of the nonaqueous components in the solvent system contributed somewhat to the tonicity of the extracellular solvent. Since there was no radical departure of the experimental curves from the theoretical tie-lines, the hemolytic effect of the nonaqueous combinations apparently was essentially dependent on their total concentration in solution and did not depend on one solvent being at or near its critical concentration.

The most convex curves were obtained for ternary systems containing polyethylene glycol 400 (Figs. 1, 3, 5, and 8), while the least deviation from the theoretical tie-line was observed for the aqueous dimethylformamide–tetramethylurea system (Fig. 10). The results indicated that the hemolytic effect of the aqueous dimethylformamide–tetramethylurea system was additive, since the experimental curve was very similar to the theoretical tie-line. The relatively greater convex experimental curves of the ternary systems containing polyethylene glycol 400 indicated that these systems offered more protection to red cells than did solvent systems without polyethylene glycol 400.

Previous workers (3) reported that polyethylene glycol 400 has the ability to contribute to the tonicity of extracellular solutions, and they calculated a hemolytic i value (isotonic coefficient) of 0.6

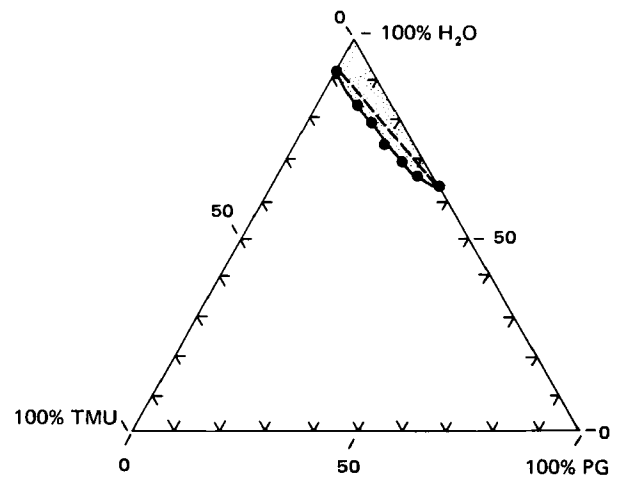

Figure 7—*Ternary diagram for aqueous propylene glycol (PG)–tetramethylurea (TMU) solutions containing 0.9% sodium chloride at 37°.*

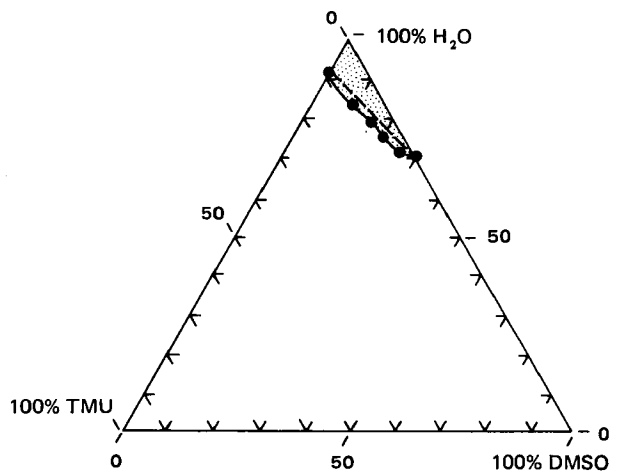

Figure 9—*Ternary diagram for aqueous dimethyl sulfoxide (DMSO)–tetramethylurea (TMU) solutions containing 0.9% sodium chloride at 37°.*

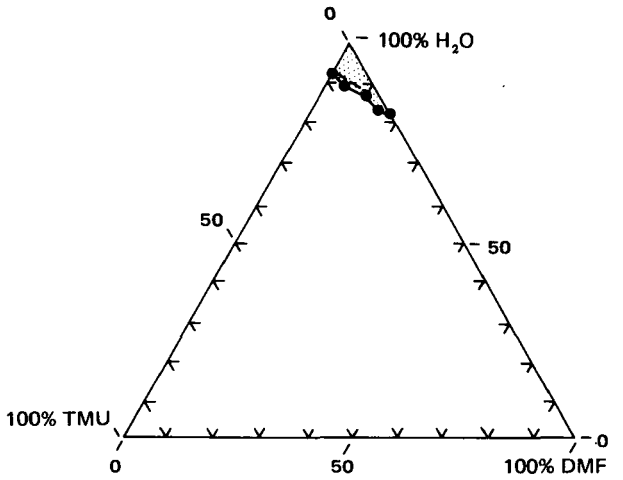

Figure 10—*Ternary diagram for aqueous dimethylformamide (DMF)–tetramethylurea (TMU) solutions containing 0.9% sodium chloride at 37°.*

for this compound. All other solvents have been reported to offer very little protection to red blood cells from hemolysis at all concentrations (1, 4–6). Therefore, the convex deviation from the theoretical tie-line can be explained by the fact that polyethylene glycol 400 contributed to the tonicity of the ternary solvent by virtue of its intrinsic property of incomplete penetration into the red blood cell.

The components of ternary solvent systems not containing polyethylene glycol 400 do contribute to the tonicity of the system but to a lesser degree than the polyethylene glycol 400 systems. Hemolytic isotonic coefficients (i values) were reported previously for sodium chloride in all solvents used in this study (1, 4–6). These i values for sodium chloride in aqueous solutions containing approximately one-half the critical hemolytic concentrations of the solvents were 1.71 for 4% tetramethylurea (6), 1.90 for 10% dimethylformamide (5), 1.95 for 15% propylene glycol (1), and 2.10 for 20% dimethyl sulfoxide (4).

When the isotonic coefficients are used as an indicator of the individual solvent's contribution to the tonicity of extracellular solutions, tetramethylurea would have the least contribution and dimethyl sulfoxide the most. Therefore, according to these hemolytic i values, the aqueous tetramethylurea–dimethylformamide system (average i value 1.80) should contribute very little, if at all, to the extracellular tonicity and should display little deviation from the theoretical tie-line. The experimental data (Fig. 10) are in agreement with this explanation.

The experimental data for the other systems also concur with this explanation, since the rank order of the average sodium chloride i values of the two nonaqueous components in a particular ternary system was approximately the same as the rank order of the degree of deviation of the experimental curves from their theoretical tie-lines. The rank order for the amount of deviation from the theoretical tie-line was propylene glycol–dimethyl sulfoxide (i = 2.03) > dimethylformamide–dimethyl sulfoxide (i = 2.00) > dimethylformamide–propylene glycol (i = 1.93) = tetramethylurea–dimethyl sulfoxide (i = 1.92) > tetramethylurea–propylene glycol (i = 1.83) > tetramethylurea–dimethylformamide (i = 1.80).

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Analysis of *Duboisia myoporoides* R. Br. and *Duboisia leichhardtii* F. Muell.

W. J. GRIFFIN [x], H. P. BRAND, and J. G. DARE

Abstract □ *Duboisia* samples were analyzed for scopolamine and hyoscyamine using GLC, by a slope ratio method, and by methods employing homatropine, tetraphenylethylene, and phenylacetyltropine as internal standards. Limits of precision were determined by the inverse prediction method. Phenylacetyltropine was the preferred internal standard. The alkaloids were silanized with either hexamethyldisilazane or *N,O*-bis(trimethylsilyl)acetamide to prevent dehydration to the apo forms. Samples from a commercial bale of *Duboisia myoporoides* were assayed. The alkaloid content varied considerably, depending on the sample position within the bale.

Keyphrases □ *Duboisia myoporoides* and *Duboisia leichhardtii*—GLC analysis for scopolamine and hyoscyamine, slope ratio method □ Scopolamine and hyoscyamine—GLC determination in *Duboisia* samples, slope ratio method □ Phenylacetyltropine—internal standard in GLC analysis of scopolamine and hyoscyamine in *Duboisia* samples

Two species of *Duboisia, Duboisia leichhardtii* and *Duboisia myoporoides,* are commercial sources of the mydriatic tropane alkaloid scopolamine, 1000 tons being grown annually. They are cultivated together with their hybrids, and it was essential that an accurate analytical method for the major alkaloids, scopolamine and hyoscyamine, be established for cultivation trials. The assay is complicated by the wide spectrum of tropane alkaloids the genus offers (Table I). Coulson and Griffin (1) found that aerial parts of