mogeneous: *n*-butanol–acetic acid–water (3:1:1), 95% ethanol–water (7:3), *n*-propanol–34% ammonia (7:3), 95% ethanol–34% ammonia (7:3), and *n*-propanol–water (1:1). One additional recrystallization from aqueous ethanol gave the analytical sample (mp 178–180°, resolidifies, and then mp 253–255°); IR: λ_{max} (mineral oil) 2.86, 2.91, and 5.92 μm.

Anal.—Calc. for $C_8H_{13}N_3O_3 \cdot H_2O$: C, 44.23; H, 6.96; N, 19.34. Found: C, 44.30; H, 7.00; N, 19.44.

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Sodium Chloride Equivalents, Cryoscopic Properties, and Hemolytic Effects of Certain Medicinals in Aqueous Solution III: Supplemental Values

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Abstract □ A supplemental table of sodium chloride equivalents and freezing-point depressions at various concentrations for 44 different substances in aqueous solution is presented. Also given in the table is the isosmotic concentration of each material that can form such a solution. The degree of hemolysis of human erythrocytes was determined in 24 different isosmotic solutions, and the data are presented in a table to supplement the previously published values. Eleven isosmotic solutions prevented hemolysis, and 13 others failed to prevent hemolysis.

Keyphrases □ Sodium chloride equivalents—data for 44 drugs □ Cryoscopic properties—data for 44 drugs □ Hemolytic effects—data for 44 drugs □ Drug substances—sodium chloride equivalents, cryoscopic properties, and hemolytic effects determined for 44 drugs

The sodium chloride equivalents and freezing-point depressions for 456 substances in aqueous solution were determined experimentally and reported previously (1–4). Furthermore, the degree of hemolysis of fresh human erythrocytes in certain aqueous isosmotic solutions was determined using the hemolytic method (3–5).

The objectives of the current investigation were to study, using the same methods, some additional available substances not included in the earlier cryoscopic and hemolytic investigations and to present these data in suitable tables to supplement the previous data.

EXPERIMENTAL

Cryoscopic Measurements—The method used for the measurements of the freezing points of the solutions was the same as that already reported; all freezing-point data were obtained with a cryoscopic osmometer (4).

The freezing-point measurements were corrected for the amount of disengaged ice, and −0.52° was used as the comparative freezing point for aqueous 0.9% reagent grade sodium chloride solution, which is isotonic and isosmotic with blood and tears. The materials studied were of the official grade of purity or better, and the grade of purity of the donated specialty preparations complied with the manufacturer's specifications.

Hemolysis of Human Erythrocytes—Colorimetric hemoglobin determinations were made to indicate the degree of hemolysis for solutions that could be made isosmotic. The method, utilizing a 45-min incubation period of erythrocytes in the isosmotic solution followed by centrifugation of the erythrocytes and ghosts and determination of absorbance *versus* a standard at 520 nm in a colorimeter, was reported previously in detail (3–5).

RESULTS AND DISCUSSION

Table I lists the sodium chloride equivalents and freezing-point depressions at various concentrations for the 44 currently studied substances. To use these data, one should employ the sodium chloride equivalent that represents the concentration nearest to the desired final concentration of medicinal substance used. Because of general interest in the colligative properties of medicinal solutions, the freezing-point depressions and sodium chloride equivalents are included for several substances that are not necessarily used as isotonic or isosmotic solutions. The sodium chloride equivalents and isosmotic concentrations are reported to the nearest 0.01.

The percent of hemolysis found for the 24 compounds studied is listed in Table II in addition to the isosmotic concentration used for each and the solution's approximate pH. For solutions that developed a color or cloudiness upon the addition of blood, the proportional decrease in the volume of the packed, nonhemolyzed centrifuged erythrocytes was estimated visually. Any noticeable change in appearance of the erythrocytes or the solution is referred to in the footnotes for Table II. Of the compounds studied, 11 isosmotic solutions prevented hemolysis of human erythrocytes and 13 failed to prevent hemolysis.

A compilation of the 293 substances whose isosmotic solutions were studied using the same hemolytic method in this laboratory

Table I—Sodium Chloride Equivalents and Freezing-Point Depressions[a]

Chemical	Concentration of Solution, Sodium Chloride Equivalents						
	0.5%	1%	2%	3%	5%	At Isosmotic Concentration	
Acetylcysteine	0.20	0.20	0.20	0.20	—	0.20	4.58%[b]
	0.055°	0.113°	0.227°	0.341°	—	0.52°	4.58%
Alphaprodine hydrochloride	0.19	0.19	0.18	0.18	—	0.18	4.98%
	0.053°	0.105°	0.212°	0.315°	—	0.52°	4.98%
Aminohippuric acid	0.13	0.13	—	—	—	—	—
	0.035°	0.075°	—	—	—	—	—
Anileridine hydrochloride	0.19	0.19	0.19	0.18	0.18	0.18	5.13%
	0.052°	0.104°	0.212°	0.316°	0.509°	0.52°	5.13%
Arginine glutamate	0.17	0.17	0.17	0.17	0.17	0.17	5.37%
	0.048°	0.097°	0.195°	0.292°	0.487°	0.52°	5.37%
Bupivacaine hydrochloride	0.17	0.17	0.17	0.17	0.17	0.17	5.38%
	0.048°	0.096°	0.193°	0.290°	0.484°	0.52°	5.38%
Butabarbital sodium	0.27	0.27	0.27	0.27	—	0.27	3.33%
	0.078°	0.155°	0.313°	0.470	—	0.52°	3.33%
Capreomycin sulfate	0.04	0.04	0.04	0.04	0.04	—	—
	0.011°	0.020°	0.042°	0.063°	0.106°	—	—
Carbenicillin disodium	0.20	0.20	0.20	0.20	—	0.20	4.40%
	0.059°	0.118°	0.236°	0.355°	—	0.52°	4.40%
Carboxymethylcellulose sodium	0.03	0.03	—	—	—	—	—
	0.007°	0.017°	—	—	—	—	—
Chloroquine phosphate	0.14	0.14	0.14	0.14	0.13	0.13	7.15%
	0.039°	0.082°	0.162°	0.242°	0.379°	0.52°	7.15%
Chlortetracycline hydrochloride	0.10	0.10	0.10	—	—	—	—
	0.030°	0.061°	0.121°	—	—	—	—
Clindamycin phosphate	0.08	0.08	0.08	0.08	0.08	0.08	10.73%
	0.022°	0.046°	0.095°	0.144°	0.242°	0.52°	10.73%
Colistimethate sodium	0.15	0.15	0.15	0.15	0.14	0.13	6.73%
	0.045°	0.085°	0.170°	0.253	0.411°	0.52°	6.73%
Cytarabine	0.11	0.11	0.11	0.11	0.11	0.10	8.92%
	0.034°	0.066°	0.134°	0.198°	0.317°	0.52°	8.92%
Deferoxamine mesylate	0.09	0.09	0.09	0.09	0.09	—	—
	0.023°	0.047°	0.093°	0.142°	0.241°	—	—
Dicloxacillin sodium (monohydrate)[c]	0.10	0.10	0.10	0.10	—	—	—
	0.030°	0.061°	0.122°	0.182	—	—	—
Doxycycline hyclate	0.12	0.12	0.12	0.11	0.09	—	—
	0.035°	0.072°	0.134°	0.186°	0.264°	—	—
Dyphylline	0.10	0.10	0.09	0.09	0.08	—	—
	0.025°	0.052°	0.104°	0.155°	0.245°	—	—
Floxuridine	0.14	0.13	0.13	0.12	0.12	0.12	8.47%
	0.040°	0.076°	0.147°	0.213°	0.335°	0.52°	8.47%
Gentamicin sulfate	0.05	0.05	0.05	0.05	0.05	—	—
	0.015°	0.030°	0.060°	0.093°	0.153°	—	—
Glycine	0.41	0.41	0.41	—	—	0.41	2.19%
	0.118°	0.235°	0.470°	—	—	0.52°	2.19%
Hetacillin potassium	0.17	0.17	0.17	0.17	0.17	0.17	5.50%
	0.048°	0.095°	0.190°	0.284	0.474°	0.52°	5.50%
Indigotindisulfonate sodium	0.30	0.30	—	—	—	—	—
	0.085°	0.172°	—	—	—	—	—
Isometheptene mucate	0.18	0.18	0.18	0.18	—	0.18	4.95%
	0.048°	0.095°	0.196°	0.302°	—	0.52°	4.95%
Isoproterenol sulfate	0.14	0.14	0.14	0.14	0.14	0.14	6.65%
	0.039°	0.078°	0.156°	0.234°	0.389°	0.52°	6.65%
Levallorphan tartrate	0.13	0.13	0.13	0.12	0.12	0.10	9.40%
	0.036°	0.073°	0.143°	0.210°	0.329°	0.52°	9.40%
Levorphanol tartrate	0.12	0.12	0.12	0.12	—	—	—
	0.033°	0.067°	0.136°	0.203°	—	—	—
Mesoridazine besylate	0.10	0.07	0.05	0.04	0.03	—	—
	0.024°	0.040°	0.058°	0.071°	0.087°	—	—
Methocarbamol	0.10	0.10	—	—	—	—	—
	0.030°	0.060°	—	—	—	—	—
Methylergonovine maleate	0.10	0.10	—	—	—	—	—
	0.028°	0.056°	—	—	—	—	—
Methylprednisolone sodium succinate	0.10	0.09	0.09	0.08	0.07	—	—
	0.025°	0.051°	0.102°	0.143°	0.200°	—	—
Minocycline hydrochloride	0.10	0.10	0.09	0.08	—	—	—
	0.030°	0.058°	0.107°	0.146°	—	—	—
Naloxone hydrochloride	0.14	0.14	0.14	0.13	0.13	0.11	8.07%
	0.042°	0.083°	0.158°	0.230°	0.367°	0.52°	8.07%
Novobiocin sodium	0.12	0.10	0.07	—	—	—	—
	0.033°	0.057°	0.073°	—	—	—	—
Orphenadrine citrate	0.13	0.13	0.13	0.12	0.10	—	—
	0.037°	0.074°	0.144°	0.204°	0.285°	—	—
Paraldehyde	0.25	0.25	0.25	0.25	—	0.25	3.65%
	0.071°	0.142°	0.288°	0.430°	—	0.52°	3.65%
Pyridostigmine bromide	0.22	0.22	0.22	0.22	—	0.22	4.13%
	0.062°	0.125°	0.250°	0.377	—	0.52°	4.13%

(*continued*)

Table I—*Continued*

Chemical	Concentration of Solution, Sodium Chloride Equivalents						
	0.5%	1%	2%	3%	5%	At Isosmotic Concentration	
Rolitetracycline	0.11	0.11	0.10	0.09	0.07	—	—
	0.032°	0.064°	0.113°	0.158	0.204°	—	—
Spectinomycin hydrochloride	0.16	0.16	0.16	0.16	0.16	0.16	5.66%
	0.045°	0.092°	0.185°	0.280	0.460°	0.52°	5.66%
Thiotepa	0.16	0.16	0.16	0.16	0.16	0.16	5.67%
	0.045°	0.090°	0.182°	0.278	0.460°	0.52°	5.67%
Tridihexethyl chloride	0.16	0.16	0.16	0.16	0.16	0.16	5.62%
	0.047°	0.096°	0.191°	0.280	0.463°	0.52°	5.62%
Triflupromazine hydrochloride[c]	0.10	0.09	0.05	0.04	0.03	—	—
	0.031°	0.051°	0.061°	0.073	0.092°	—	—
Tromethamine	0.26	0.26	0.26	0.26	—	0.26	3.45%
	0.074°	0.150°	0.300°	0.450	—	0.52°	3.45%

[a] Top value is sodium chloride equivalent, and bottom value is freezing-point depression. [b] Isosmotic concentration, % w/v. [c] Solution foams readily.

shows that 136 failed to prevent hemolysis while 157 prevented hemolysis completely. One must be careful not to equate isotonicity and isosmoticity without knowledge of the corresponding data whenever a biological membrane is being utilized. This aspect was discussed in detail previously (2, 4–8).

Earlier reports in the series contained data on substances that foamed in aqueous solution and apparently aggregated above certain critical concentrations. These critical concentrations can be determined readily from the abrupt change in their cryoscopic graphs (3, 4, 9). The following behaved in this manner: benzethonium chloride, chlorpheniramine maleate, chlorpromazine hydrochloride, bromodiphenhydramine hydrochloride, dexchlorpheniramine maleate, dibucaine hydrochloride, fluphenazine hydrochloride, hydroxyzine hydrochloride, methotrimeprazine hydrochloride, pramoxine hydrochloride, pyrilamine maleate, nafcillin sodium, tetracaine hydrochloride, and valethamate bromide. Later, Altwood (10) studied micelle formation of some antihistamines in aqueous solution with a light-scattering photometer and reported their critical micelle concentrations (CMC), micellar weights, and aggregation numbers, which agreed well with our cryoscopic data.

Because of interest in the apparent aggregation of some substances in aqueous solution, the authors were especially looking for this characteristic discontinuity in the cryoscopic graphs from any of the 44 new compounds in this study, but none behaved in this manner. Triflupromazine hydrochloride was the only one whose graph even slightly suggested the possibility of any aggregation in solution. Its graph showed a slight discontinuity at a concentration of 0.019 *M* and approximately −0.05°, which corresponded to an apparent aggregation number of six. However, the discontinuity in the graph was not as distinct as it was with all of the before-mentioned substances.

A sufficient number of compounds demonstrated that they tend to aggregate in solution to suggest that this interfacial phenomenon may play a role in drug action by affecting biological activity. Associations taking place in the complex biological medium undoubtedly affect the thermodynamic activity of a given drug at the molecular level and, therefore, warrant increased study.

Table II—Hemolysis of Erythrocytes in Isosmotic Solutions

Substances	Isosmotic Concentration, % w/v	Hemolysis, %	Approximate pH
Acetylcysteine	4.58	100[a]	2.0
Alphaprodine hydrochloride	4.98	100	5.3
Anileridine hydrochloride	5.13	12	2.6
Arginine glutamate	5.37	0	6.9
Bupivacaine hydrochloride	5.38	83	6.8
Butabarbital sodium	3.33	0	6.8
Carbenicillin disodium	4.40	0	6.6
Chloroquine phosphate	7.15	0	4.3
Clindamycin phosphate	10.73	58[b]	6.8
Colistimethate sodium	6.73	0	7.6
Cytarabine	8.92	0	8.0
Floxuridine	8.47	3[c]	4.5
Glycine	2.19	0[d]	6.2
Hetacillin potassium	5.50	0	6.3
Isometheptene mucate	4.95	0	6.2
Isoproterenol sulfate	6.65	Trace	4.5
Levallorphan tartrate	9.40	59[b]	6.9
Naloxone hydrochloride	8.07	35	5.2
Paraldehyde	3.65	97	5.3
Pyridostigmine bromide	4.13	0	7.2
Spectinomycin hydrochloride	5.66	3	4.4
Thiotepa	5.67	10[e]	8.2
Tridihexethyl chloride	5.62	97	5.4
Tromethamine	3.45	0	10.2

[a] Solution turned dark green-brown. [b] Solution and cells turned brown. [c] Cells clumped. [d] Cell volume increased slightly, darkened in color, and clumped. [e] Solution became turbid.

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