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

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Abstract □ A supplemental table of NaCl equivalents and freezing point depressions at various concentrations for 30 different medicinal substances in aqueous solution is presented. Also given in the table is the isosmotic concentration of each of the materials which can form a solution. The degree of hemolysis of human erythrocytes was determined in 63 different isosmotic solutions and the data are presented in a table to supplement the previously published values. Freezing point depression graphs for two substances—fluphenazine dihydrochloride and methotrimeprazine hydrochloride—are presented because they showed discontinuities caused by some type of association or aggregation in their solutions.

Keyphrases □ Sodium chloride equivalents—drugs □ Cryoscopic properties—drugs □ Hemolytic effects—drugs □ Colorimetric analysis—hemolysis determination

The NaCl equivalents and freezing point depressions for many aqueous medicinal solutions have been determined experimentally and reported (1–3). Furthermore, the degree of hemolysis of fresh human erythrocytes in certain aqueous isosmotic solutions has been determined using the hemolytic method and reported previously (3, 4).

The objectives of the present investigation were to study a number of additional substances not included in the earlier cryoscopic and hemolytic investigations and to present the data in suitable tables to supplement the previous data—these earlier cryoscopic data for 426 substances have been combined and published as a single table in the 8th edition of *The Merck Index* (5). In addition, since it has been reported by various workers (3, 6–8) that some amine salts tend to form aggregates in aqueous solution above a certain concentration, data are presented for two more substances which have been found to show this behavior.

EXPERIMENTAL AND RESULTS

Cryoscopic Measurements—The general method used for the measurements of the freezing points of the solutions was similar to that already reported in detail (1, 3). The main difference is that all data reported in this current study were obtained by employing a cryoscopic osmometer[1] to measure freezing point depressions rather than a differential thermometer (Beckman).

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% NaCl solution which is isotonic with blood and tears. The materials used were of official grade of purity or better, and for those nonofficial substances, the grade of purity of each complied with the manufacturer's specifications. The NaCl equivalents and isosmotic concentrations are reported to the nearest 0.01.

Table I lists the NaCl equivalents and freezing point depressions at various concentrations for all of the currently studied substances. To use these data one should employ the NaCl equivalent which 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 NaCl equivalents are included for several substances which are not necessarily used as isotonic or isosmotic solutions.

The term "isotonic solution" is used in its customary sense in this report—meaning that the solution freezes at the same temperature as normal saline solution, blood, and tears. However, the term isosmotic is more proper when only colligative properties are being studied and no biological membrane is being considered.

Hemolysis of Human Erythrocytes—The method made use of colorimetric hemoglobin determinations to indicate the degree of hemolysis. It was essentially the same as that employed by Husa *et al.* (9, 10) as modified by Hammarlund and Van Pevenage (3). The percent of hemolysis found for the 63 compounds studied is listed in Table II in addition to the isosmotic concentration used for each and the solution's approximate pH. For those solutions which developed a color or cloudiness upon the addition of blood, the proportional decrease in volume of the packed, nonhemolyzed, centrifuged erythrocytes was estimated visually. Any change in appearance of the erythrocytes or the solution was referred to in the footnotes for Table II.

Cryoscopic Behavior of Certain Aggregate-Formers—When the freezing point depression data were plotted for the various substances, it was noted that the characteristics of the graphs for two of them were considerably different. This observation suggested that there could be some type of association or aggregation of the solute or solvent taking place above a particular concentration of the solute at the freezing temperature of the solution. In order to ascertain the solute concentration above which aggregation took place, the freezing point depression was plotted *versus* the molar concentration of the solutions, thus yielding freezing point depression graphs for the two substances. These are shown in Fig. 1, and the concentrations of the substances at their apparent points of discontinuity in the figure were found to be approximately 0.04–0.05 *M* (2% w/v) for fluphenazine dihydrochloride and 0.029 *M* (1.05% w/v) for methotrimeprazine hydrochloride. A comparison of the two slopes of the methotrimeprazine HCl freezing point curve indicates that this substance has an apparent aggregation number of four above 0.029 *M* concentration.

All of the fluphenazine dihydrochloride solutions required a much longer time than usual to freeze and for their temperatures to reach the normal "plateau" of the cryoscopic osmometer. The reasons for such prolonged freezing times for this substance and whether unusual supercooling was involved remain to be investigated.

DISCUSSION

Isosmotic solutions of 36 substances prevented hemolysis of human erythrocytes and solutions of 27 other substances failed

[1] Advanced Instruments, Inc., Newton Highlands, Mass.

Table I—Sodium Chloride Equivalents and Freezing Point Depressions, °C.

Chemical	Concn. of Solution, NaCl Equivalents						
	0.5%	1%	2%	3%	5%	At Isotonicity	
Acetrizoate methylglucamine	0.09	0.08	0.08	0.08	0.08	0.07	12.12%
	0.024°	0.047°	0.093°	0.137°	0.222°	0.52°	12.12%
Aminoacetic acid NF	0.42	0.41	0.41	—	—	0.41	2.20%
	0.119°	0.235°	0.470°	—	—	0.52°	2.20%
Calcium lactobionate	0.08	0.08	0.08	0.07	0.07	—	—
	0.022°	0.043°	0.085°	0.126°	0.197°	—	—
Cephaloridine	0.09	0.07	0.06	0.06	0.05	—	—
	0.023°	0.041°	0.074°	0.106°	0.145°	—	—
Congo red	0.05	0.05	0.05	0.05	0.05	—	—
	0.015°	0.030°	0.059°	0.092°	0.151°	—	—
Diphenidol HCl	0.16	0.16	0.16	—	—	—	—
	0.045°	0.090°	0.180°	—	—	—	—
Doxapram HCl	0.12	0.12	0.12	0.12	—	—	—
	0.035°	0.070°	0.140°	0.210°	—	—	—
Fluphenazine di-HCl	0.14	0.14	0.12	0.09	—	—	—
	0.041°	0.082°	0.145°	0.155°	—	—	—
Glycopyrrolate	0.15	0.15	0.15	0.14	0.13	0.12	7.22%
	0.042°	0.084°	0.166°	0.242°	0.381°	0.52°	7.22%
Hexamethylenamine sodium acetaminosalicylate	0.18	0.18	0.17	0.17	0.16	0.16	5.48%
	0.049°	0.099°	0.199°	0.297°	0.485°	0.52°	5.48%
Lyapolate sodium	0.10	0.09	0.09	0.09	0.09	0.09	9.96%
	0.025°	0.051°	0.103°	0.157°	0.263°	0.52°	9.96%
Magnesium sulfate, anhydrous	0.34	0.32	0.30	0.29	—	0.28	3.18%
	0.093°	0.184°	0.345°	0.495°	—	0.52°	3.18%
Methotrimeprazine HCl	0.12	0.10	0.07	0.06	0.04	—	—
	0.034°	0.060°	0.077°	0.094°	0.125°	—	—
Novobiocin sodium	0.10	0.08	0.08	0.07	0.07	—	—
	0.025°	0.046°	0.086°	0.122°	0.190°	—	—
Oleandomycin phosphate NF	0.08	0.08	0.08	0.08	0.08	0.08	10.82%
	0.017°	0.038°	0.084°	0.129°	0.255°	0.52°	10.82%
Pentazocine lactate	0.15	0.15	0.15	0.15	0.15	—	—
	0.042°	0.085°	0.169°	0.253°	0.420°	—	—
Phenylethyl alcohol NF	0.25	0.25	0.25	—	—	—	—
	0.070°	0.141°	0.283°	—	—	—	—
Prilocaine HCl	0.22	0.22	0.22	0.22	—	0.22	4.18%
	0.062°	0.125°	0.250°	0.375°	—	0.52°	4.18%
Proparacaine HCl	0.16	0.15	0.15	0.14	0.13	0.12	7.46%
	0.044°	0.086°	0.169°	0.247°	0.380°	0.52°	7.46%
Rose bengal	0.08	0.07	0.07	0.07	0.07	0.06	14.9%
	0.020°	0.040°	0.083°	0.124°	0.198°	0.52°	14.9%
Rose bengal B	0.08	0.08	0.08	0.08	0.08	—	—
	0.022°	0.044°	0.087°	0.131°	0.218°	—	—
Sodium acetrizoate	0.10	0.10	0.10	0.10	0.10	0.09	9.64%
	0.027°	0.055°	0.109°	0.163°	0.273°	0.52°	9.64%
Sodium ampicillin	0.16	0.16	0.16	0.16	0.16	0.16	5.78%
	0.045°	0.090°	0.181°	0.272°	0.451°	0.52°	5.78%
Sodium diatrizoate	0.10	0.09	0.09	0.09	0.09	0.09	10.55%
	0.025°	0.049°	0.098°	0.149°	0.248°	0.52°	10.55%
Sodium methiodal NF	0.24	0.24	0.24	0.24	—	0.24	3.81%
	0.068°	0.136°	0.274°	0.410°	—	0.52°	3.81%
Sparteine sulfate	0.10	0.10	0.10	0.10	0.10	0.10	9.46%
	0.030°	0.056°	0.111°	0.167°	0.277°	0.52°	9.46%
Trisodium edetate (monohydrate)	0.29	0.29	0.28	0.27	—	0.27	3.31%
	0.079°	0.158°	0.316°	0.472°	—	0.52°	3.31%
Tromethamine	0.26	0.26	0.26	0.26	—	0.26	3.41%
	0.075°	0.152°	0.305°	0.458°	—	0.52°	3.41%
Trypan blue	0.26	0.26	—	—	—	—	—
	0.075°	0.150°	—	—	—	—	—
Uridine	0.12	0.12	0.12	0.12	0.12	0.11	8.18%
	0.035°	0.069°	0.138°	0.208°	0.333°	0.52°	8.18%

to prevent hemolysis, the degree of hemolysis varying from slight to complete. This type of result has been discussed previously in considerable detail (4, 11).

Fluphenazine and methotrimeprazine both foam in solution and apparently undergo association or aggregation similar to that previously found for dibucaine HCl, tetracaine HCl, pramoxine HCl, bromodiphenhydramine HCl, dexchlorpheniramine maleate, hydroxyquine HCl, sodium nafcillin, and valethamate bromide (3, 6). Since fluphenazine and methotrimeprazine are both phenothiazines and are somewhat similar in structure, their aggregation in solution was not unexpected. However, the apparent difference in the shape of their cryoscopic graphs was surprising. Methotrimeprazine exhibited essentially a straight line graph with a sharp break in the curve at 0.029 *M* concentration; whereas the apparent aggregation of fluphenazine was much more gradual over a wider range in concentration in the vicinity of 0.04 to 0.05 *M*. Each of these substances appeared to be completely soluble at the freezing temperatures of the most concentrated solutions tested.

The size of the aggregates and the aggregation number of these drugs and the aforementioned substituted amines in solution vary somewhat, and with some of them the size has been found to depend upon temperature and the presence of NaCl, in addition to chemical structure and variations among the several analytical methods employed. For example, in 1956 Hammarlund and Pedersen-Bjergaard (6) using a cryoscopic method reported an aggregation number of 10 for the phenothiazine, chlorpromazine HCl. In the present study methotrimeprazine was found to have an apparent aggregation number of only 4 while the aggregation of the similar compound, fluphenazine, appears to occur over too broad a range of concentration for accurate estimation. In 1956 Farhadieh (12), employing a conductometric method, found that dibucaine HCl had an aggregation number of 8 at 25°. Recently Johnson and Lud-

Table II—Hemolysis of Erythrocytes in Isosmotic Solutions

Substance	Isosmotic Concn., % w/v	Hemolysis, %	Approx. pH
Acetrizoate methylglucamine	12.12	0	7.1
Adrenalone HCl	4.24	68	4.5
Aminoacetic acid NF	2.20	0[a]	6.2
Amprotropine phosphate	5.90	0	4.2
Antazoline phosphate NF	6.05	90	4.0
Atropine methylnitrate	6.52	0	5.2
Bismuth sodium tartrate	8.91	0	6.1
Calcium aminosalicylate NF	4.80	0	6.0
Calcium levulinate	3.58	0	7.2
Carbachol USP	2.82	0	5.9
Diphenhydramine HCl USP	5.70	88[b]	5.5
Ethylenediamine USP	2.08	100[c]	11.4
Ferric ammonium citrate, green	6.83	0	5.2
D-Glucuronic acid	5.02	48[d]	1.6
Glycopyrrolate	7.22	92[c]	4.0
Hexamethylenamine sodium acetaminosalicylate	5.48	0[e]	4.0
Hexylcaine HCl NF	4.30	100	4.8
Histamine phosphate USP	4.10	0	4.6
Histidine HCl	3.45	40	3.9
4-Homosulfanilamide HCl	3.69	0	4.9
Hydromorphone HCl NF	6.39	64	5.6
8-Hydroxyquinoline sulfate	9.75	59[d]	2.5
Hyoscyamine HBr	6.53	68	5.9
Lidocaine HCl USP	4.42	85	4.3
Lyapolate sodium	9.96	0	6.5[f]
Magnesium sulfate, anhydrous	3.18	0	7.0
Menadiol sodium diphosphate USP	4.36	0	8.2
Neostigmine bromide USP	4.98	0	4.6
Neostigmine methylsulfate USP	5.22	0	5.9
Oleandomycin phosphate NF	10.82	0	5.0
Pentolinium tartrate	5.95	55[c]	3.4
Phenylephrine tartrate	5.90	58[d]	5.4
Pilocarpine nitrate USP	4.60	88	3.9
Piperocaine HCl	5.22	65	5.7
Prilocaine HCl	4.18	45	4.6
Procainamide HCl USP	5.17	88	5.9
Propoxycaine HCl NF	6.40	16	5.3
Pyridoxine HCl USP	3.05	31[d]	3.2
Quinine di-HCl	5.07	Trace[g]	2.5
Scopolamine methylnitrate	6.95	0	6.0
Sodium acetrizoate	9.64	0	6.9[f]
Sodium ampicillin	5.78	0	8.5
Sodium bromide NF	1.60	0	6.1
Sodium diatrizoate	10.55	0	7.9
Sodium hypophosphite	1.60	0	7.3
Sodium iodohippurate	5.92	0	7.3
Sodium mercaptomerin USP	5.30	0	8.4
Sodium methiodal NF	3.81	0	5.9
Sodium penicillin G NF	5.90	18[h]	5.2
Sodium pentobarbital USP	4.07	0	9.9
Sodium secobarbital USP	3.90	Trace	9.8
Sodium sulfamerazine	4.53	0	9.8
Sodium sulfapyridine	4.55	5	10.4
Sodium sulfite, anhydrous	1.45	0	9.6
Sodium thiopental USP	3.50	74	10.3
Sparteine sulfate	9.46	19[c,d]	3.5
Stibophen USP	6.18	0	6.3
Tetrahydrozoline HCl NF	4.10	60[i]	6.7
Tripelennamine HCl USP	5.50	100	6.3
Trisodium edetate (monohydrate)	3.31	0	8.0
Tromethamine	3.41	3	10.3
Uridine	8.18	0[j]	6.1
Zinc phenolsulfonate	5.40	0[j]	5.4

[a] Cell volume increased, darkened, and clumped. [b] Cells turned lighter red. [c] Solution turned brown. [d] Cells turned dark brown. [e] Solution turned gray color. [f] pH Determined after addition of blood. [g] Cells turned black and increased in volume. [h] Solution turned red-tan color. [i] Solution foams readily. [j] Cells clumped.

lum (13), using a light-scattering photometer (Brice-Phoenix), found that dibucaine "micelles" contained 15 monomers in pure aqueous solution, and that they contained 35 monomers in an aqueous NaCl medium.

The increasing evidence that interfacial phenomena play a role in drug action suggests that the aggregation of drug molecules may affect their biologic activity. Associations taking place in the complex biological medium will naturally affect the thermodynamic activity of a given drug at the molecular level and warrant increased study.

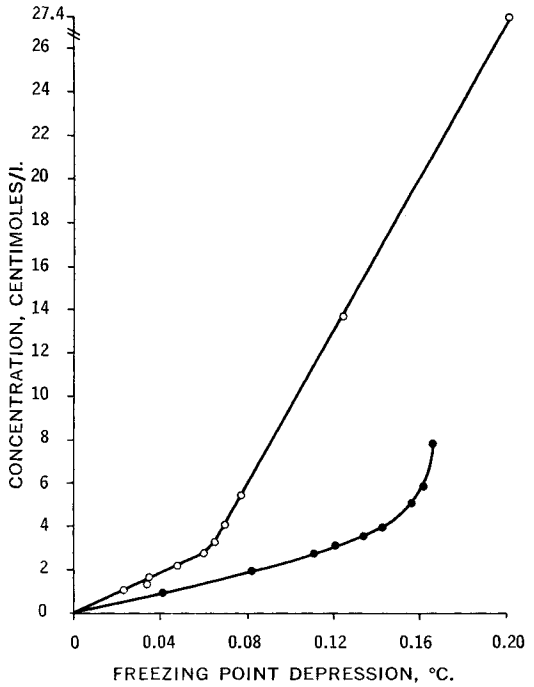

Figure 1—*Cryoscopic behavior of fluphenazine and methotrimeprazine. Key:* ●, *fluphenazine dihydrochloride;* ○, *methotrimeprazine hydrochloride.*

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