Sodium Chloride Equivalents, Cryoscopic Properties, and Hemolytic Effects of Certain Medicinals in Aqueous Solution

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A supplemental table of NaCl equivalents and freezing point depressions at various concentrations for 21 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 such a solution. The degree of hemolysis of human erythrocytes was determined in 45 different isosmotic solutions and the data are presented in a table to supplement the previously published values. Freezing point depression graphs for five substances—bromodiphenhydramine HCl, dexchlorpheniramine maleate, hydroxyzine HCl, sodium nafcillin, and valethamate bromide—are presented because they showed one or more discontinuities caused by some type of association or aggregation in their solutions.

THE NaCl equivalents and freezing point depressions for various aqueous medicinal solutions have been determined experimentally and reported (1, 2). Likewise, the amount of hemolysis of fresh human erythrocytes in certain aqueous isosmotic solutions was determined using the hemolytic method and has been reported previously (3).

The objective of the present investigation was to study in a similar manner 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. Furthermore, since it has been reported by Hammarlund and Pedersen-Bjergaard (4) and by Johnson, Goyan, and Tuck (5) and others that certain amine salts and other substances tend to aggregate in aqueous solution, data are presented for five such substances which have been found to show this behavior.

EXPERIMENTAL

Freezing Point Measurements.—The method used for most of the measurements of the freezing points of the solutions was the same as that reported previously in detail (1). The freezing point depression measurements were made directly on aqueous solutions of the compounds at selected concentrations by means of a Beckman differential thermometer. For several of the compounds used in the latter portion of the study, a cryoscopic osmometer[1] was employed for the freezing point depression measurements. Several substances were determined by both instruments and the data correlated almost exactly.

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 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 concentration of medicinal substance used.

Because of interest in the colligative properties of some medicinal solutions, the freezing point depressions and NaCl equivalents are included for several preparations which are not used necessarily as isotonic 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.

Hemolysis of Human Erythrocytes.—The method used was essentially the same as that employed by Husa and co-workers (6, 7) and is a modification of the method by Hunter (8). The principal deviations from Husa's method were that only isosmotic concentrations were employed, and an aqueous saponin solution, 100 mg./L., was used as the 100% hemolyzing solution for the erythrocytes. A fresh sample of human venous blood was used daily, and each investigation was completed within 3 hr. from the time the blood was drawn. The method employed in this study was identical with that previously described by Hammarlund and Pedersen-Bjergaard (3) except that the absorbance of each centrifuged supernatant liquid was determined in a Spectronic[2] 20 photoelectric colorimeter at 540 mμ instead of the previously used instrument employing a standard green filter.

The per cent of hemolysis found for the 45 compounds studied is listed in Table II including the isosmotic concentration used for each and its approximate pH. The few experimental solutions which were colored before the addition of blood were handled in the same manner as described previously (3). For those solutions which developed a color or cloudiness upon the addition of blood, the proportional decrease in volume of the packed, unhemolyzed, 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 graphs for five of them had a definite discontinuity at certain concentrations. This observation suggested that there could be some type of association of the solute or aggregation taking place above a

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

[2] Bausch & Lomb, Rochester, N. Y.

TABLE I.—SODIUM CHLORIDE EQUIVALENTS AND FREEZING POINT DEPRESSIONS

Chemical	Concn. of Soln., NaCl Equivalents 0.5%	1%	2%	3%	5%	At "Isotonicity"	
Amantadine HCl	0.31[a]	0.31[a]	0.31[a]	...	...	0.31[a]	(2.95)[c]
	0.090°[b]	*0.180°*[b]	*0.354°*[b]	...	...	*0.52°*[b]	(2.95)[c]
Chloroprocaine HCl U.S.P.	0.20	0.20	0.18	...	...	...	...
	0.054°	*0.108°*	*0.210°*	...	...	...	...
Diethanolamine	0.31	0.31	0.31	...	...	0.31	(2.90)
	0.089°	*0.177°*	*0.358°*	...	...	*0.52°*	(2.90)
Dimethylsulfoxide	0.42	0.42	0.42	...	...	0.42	(2.16)
	0.122°	*0.245°*	*0.480°*	...	...	*0.52°*	(2.16)
Echothiophate iodide U.S.P.	0.16	0.16	0.16	...	...	...	...
	0.045°	*0.090°*	*0.179°*	...	...	...	...
Gold sodium thiomalate U.S.P.	0.10	0.10	0.10	0.09	0.09	...	...
	0.032°	*0.061°*	*0.111°*	*0.159°*	*0.250°*	...	...
Lincomycin HCl	0.16	0.16	0.15	0.14	0.14	0.14	(6.60)
	0.045°	*0.090°*	*0.170°*	*0.247°*	*0.400°*	*0.52°*	(6.60)
Monoethanolamine N.F.	0.53	0.53	...	...	...	0.53	(1.70)
	0.154°	*0.306°*	...	...	...	*0.52°*	(1.70)
Oxymetazoline HCl	0.22	0.22	0.20	0.19	...	0.18	(4.92)
	0.063°	*0.124°*	*0.232°*	*0.335°*	...	*0.52°*	(4.92)
Polyethylene glycol 300 N.F.	0.12	0.12	0.12	0.12	0.13	0.13	(6.73)
	0.034°	*0.069°*	*0.141°*	*0.216°*	*0.378°*	*0.52°*	(6.73)
Polyethylene glycol 400 U.S.P.	0.08	0.08	0.09	0.09	0.09	0.11	(8.50)
	0.022°	*0.047°*	*0.098°*	*0.153°*	*0.272°*	*0.52°*	(8.50)
Polyethylene glycol 1500	0.06	0.06	0.07	0.07	0.07	0.09	(10.00)
	0.015°	*0.036°*	*0.078°*	*0.120°*	*0.215°*	*0.52°*	(10.00)
Polyethylene glycol 1540 N.F.	0.02	0.02	0.02	0.03	0.03	...	...
	0.005°	*0.012°*	*0.028°*	*0.047°*	*0.094°*	...	...
Polyethylene glycol 4000 U.S.P.	0.02	0.02	0.02	0.02	0.02	...	...
	0.004°	*0.008°*	*0.020°*	*0.033°*	*0.067°*	...	...
Polyvinyl alcohol (99% hydrol.)	0.02	0.02	0.02	0.02	0.03	...	...
	0.004°	*0.008°*	*0.020°*	*0.035°*	*0.075°*	...	...
Polyvinylpyrrolidone	0.01	0.01	0.01	0.01	0.01	...	...
	0.003°	*0.006°*	*0.010°*	*0.017°*	*0.035°*	...	...
Sodium cephalothin	0.18	0.17	0.16	0.15	0.14	0.13	(6.80)
	0.050°	*0.095°*	*0.179°*	*0.259°*	*0.400°*	*0.52°*	(6.80)
Sodium methicillin U.S.P.	0.18	0.18	0.17	0.16	0.15	0.15	(6.00)
	0.050°	*0.099°*	*0.192°*	*0.281°*	*0.445°*	*0.52°*	(6.00)
Sodium succinate	0.32	0.32	0.31	...	...	0.31	(2.90)
	0.092°	*0.184°*	*0.361°*	...	...	*0.52°*	(2.90)
Sodium tartrate	0.33	0.33	0.33	...	...	0.33	(2.72)
	0.098°	*0.193°*	*0.385°*	...	...	*0.52°*	(2.72)
Triethanolamine U.S.P.	0.20	0.21	0.22	0.22	...	0.22	(4.05)
	0.058°	*0.121°*	*0.252°*	*0.383°*	...	*0.52°*	(4.05)

[a] The values first listed for the chemical substances are NaCl equivalents. [b] The second values, in *italics*, are freezing point depression values in ° C. [c] The percentage concentration (w/v) at isotonicity (isosmotic) is given in parentheses in the last column.

particular concentration of the solute at the freezing temperature of the solution. In order to obtain a more definite estimation of the various points of discontinuity, the freezing point depression graphs for the five substances were plotted as the log of the freezing point depression *versus* the log of the molar concentration of the solutions. These are shown in Figs. 1 and 2 and the concentrations of the substances at their points of discontinuity in the figures were found to be as follows: bromodiphenhydramine HCl, 0.058 *M* (2.15% w/v) and 0.248 *M* (9.19% w/v); dexchlorpheniramine maleate, 0.088 *M* (3.44% w/v); hydroxyzine HCl, 0.055 *M* (2.48% w/v); sodium nafcillin, 0.062 *M* (2.52% w/v); and valethamate bromide, 0.098 *M* (3.80% w/v).

DISCUSSION

Isosmotic solutions of 20 substances prevented hemolysis of erythrocytes and solutions of 25 other substances failed to prevent hemolysis, the degree of hemolysis varying from slight to complete. This type of result has been discussed previously in considerable detail (3).

Figures 1 and 2 show that five of the substances studied undergo a solute association or aggregation similar to that found for dibucaine HCl, tetracaine HCl, and pramoxine HCl and which was discussed previously by Hammarlund and Pedersen-Bjergaard (4) and by Johnson, Goyan, and Tuck (5).

The freezing point depression data for bromodiphenhydramine HCl (Fig. 2) presented an anomaly as at 0.248 *M* concentration the freezing point curve broke sharply back to exactly the same slope that it had prior to its point of the initial aggregation at 0.058 *M*.

One objection to the use of the freezing point depression method for the sole determination of aggregation is that it cannot reveal any temperature dependence of the aggregation. This is so, since, for a given concentration, observations are restricted to only one temperature, the freezing temperature. A substance might exhibit a freezing point depression curve similar to that of any other aggregate-forming substance; however, in this instance a break in its curve would not indicate some critical concentration, but would indicate rather a particular temperature

below which some other change takes place in the system such as the occurrence of a solute–solvent interaction. In order to determine whether aggregation depends significantly upon temperature, it is necessary to resort to other analytical techniques in which measurements are made on corresponding solutions over a range of temperatures. Johnson, Goyan, and Tuck (5) have done this for various substituted amine salts by employing a thermoelectric vapor-phase osmometer at 25°, and they found evidence of aggregation at this temperature for several substances. Likewise, Farhadieh (9) in a study of the aggregation of dibucaine HCl, tetracaine HCl, diphenhydramine HCl, procaine HCl, tripelennamine HCl, pyrilamine HCl, and bromodiphenhydramine HCl employed both a vapor pressure osmometer and a Dike-Jones[3] con-

TABLE II.—HEMOLYSIS OF ERYTHROCYTES IN ISOSMOTIC SOLUTIONS

Substance	Isosmotic Concn., % w/v	Hemolysis, %	Approx. pH
Amantadine HCl	2.95	91	5.7
Aminocaproic acid	3.52	0	7.2
Bethanechol chloride U.S.P.	3.05	0	6.0
Calcium disodium edetate U.S.P.	4.50	0	6.1
Chloramphenicol sodium succinate U.S.P.	6.83	Partial[a]	6.1
Chlordiazepoxide HCl N.F.	5.50	66[b]	2.7
Dexamethasone sodium phosphate N.F.	6.75	0[c]	8.9
Diethanolamine	2.90	100	11.3
Dimethylsulfoxide	2.16	100	7.6
Edathamil disodium	4.44	0	4.7
Furtrethonium iodide	4.44	0	5.4
Hydroxyzine HCl N.F.	6.32	100[d]	1.3
Lincomycin HCl	6.60	0	4.5
Mafenide HCl	3.55	0	5.0
Mepivacaine HCl N.F.	4.60	45	4.5
Metaraminol bitartrate U.S.P.	5.17	59[d]	3.8
Methapyrilene HCl N.F.	6.00	99	6.2
Methitural sodium	3.85	78[e]	9.8
Methoxyphenamine HCl	3.47	96	5.4
p-Methylaminoethanol-phenol tartrate	5.83	0	6.2
Methyldopate HCl	4.28	Partial[f]	3.0
N-Methylglucamine	5.02	4	11.3
Methylphenidate HCl N.F.	4.07	66	4.3
Monoethanolamine N.F.	1.70	100[g]	11.7
Nalorphine HCl U.S.P.	6.36	63	4.1
Oxymetazoline HCl	4.92	86[f]	5.7
d-Pantothenyl alcohol	5.60	92	6.8
Pargyline HCl	3.18	91	3.8
Phentolamine mesylate U.S.P.	8.23	83[e]	3.5
Polyethylene glycol 300 N.F.	6.73	53	3.8
Polyethylene glycol 400 U.S.P.	8.50	0	4.4
Polyethylene glycol 1500	10.00	4	4.1
Potassium acetate N.F.	1.53	0	7.6
Pralidoxime chloride	2.87	0	4.6
Sodium bismuth thioglycollate	5.29	0	8.3
Sodium cephalothin	6.80	Partial[a]	8.5
Sodium colistimethate U.S.P.	6.85	0[h]	8.4
Sodium methicillin U.S.P.	6.00	0	5.8
Sodium oxacillin U.S.P.	6.64	0[i]	6.0
Sodium succinate	2.90	0	8.5
Sodium tartrate	2.72	0	7.3
Sodium warfarin U.S.P.	6.10	0	8.1
Theophylline sodium glycinate N.F.	2.94	0	8.9
Triethanolamine U.S.P.	4.05	100	10.7
Xylometazoline HCl	4.68	88[j]	5.0

[a] Solution becomes very cloudy. [b] Solution darkens and brown sediment forms; solution foams when shaken. [c] Solution turns light yellow—*no* hemolysis. [d] Solution turns dark brown. [e] Solution turns light brown. [f] Solution darkens and brown sediment forms. [g] Solution turns red-brown. [h] Solution foams readily. [i] Cells turn purple and solution foams readily. [j] Solution turns light brown and foams readily.

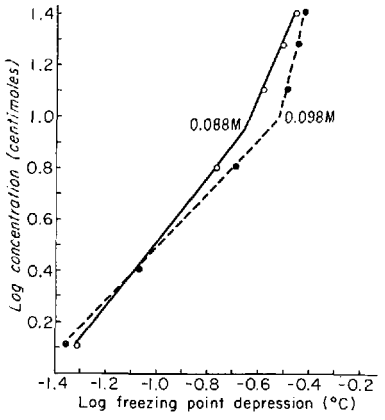

Fig. 1.—Cryoscopic behavior of certain aggregate-formers. Key: O, dexchlorpheniramine maleate; ●, valethamate bromide.

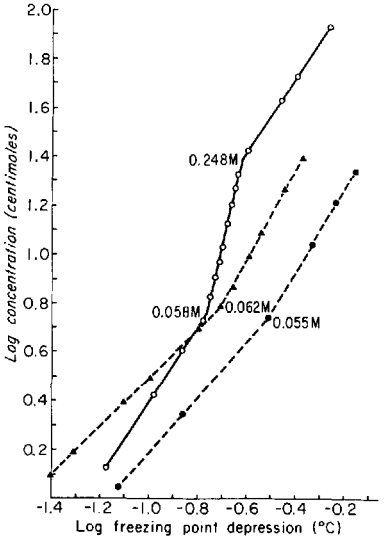

Fig. 2.—Cryscopic behavior of certain aggregate-formers. Key: O, bromodiphenhydramine hydrochloride; ▲, sodium nafcillin; ●, hydroxyzine hydrochloride.

[3] Leeds and Northrup Co., Philadelphia, Pa.

ductivity bridge at 25°. The data from both of the analytical procedures demonstrated that there was some aggregation at 25° for each substance which had shown aggregation previously at its freezing temperature, except for pyrilamine HCl which did not aggregate at 25° in the concentration range studied. Moreover, with both the conductivity and vapor pressure methods bromodiphenhydramine HCl data showed an initial aggregation point at 0.052 *M* which agreed quite well with the freezing depression data of the initial aggregation concentration (0.058 *M*), but it did not show the second break in the curve at 0.248 *M* which was disclosed by the freezing point method. The fact that this second break was not found at 25° could mean that it was temperature dependent or resulted from some other condition not controlled in the study. This point needs further investigation.

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Attempted Mannich Condensation with Indanedione-1,3

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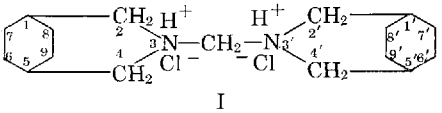
All attempts to prepare Mannich bases of indanedione failed. In every instance the end product was an amorphous solid with a high melting point and insoluble in most organic solvents. In one instance, while using 3-azabicyclo (3.2.2)nonane as the secondary amine component, a small amount of white crystalline substance was isolated from the mother liquor and identified as methylenebis-3-azabicyclo (3.2.2)nonane dihydrochloride with the help of infrared and NMR spectra. The structure of this compound was confirmed by unambiguous synthesis.

THE CONDENSATION between an amine (primary or secondary) or its salt with formaldehyde and a compound having an active hydrogen is known as the Mannich reaction.

Numerous Mannich bases are recorded in the literature (1–9); these have been prepared for pharmacological screening as antispasmodics, analgesics, chemotherapeutics, and local anesthetics.

In an effort to prepare Mannich bases of indanedione-1,3 for pharmacological testing, the condensation reaction was attempted several times utilizing dimethylamine, diethylamine, morpholine, piperidine, and 3-azabicyclo (3.2.2)nonane (AZBN) as the secondary amine component. Formaldehyde was used either as its aqueous solution or paraformaldehyde. Each synthesis resulted in a high yield of amorphous solid which was insoluble in most organic solvents. This solid material was washed several times with ether and ethanol, dried, and analyzed for elemental content. The analytical data did not correspond with the desired Mannich base. In one instance while using AZBN the mother liquor was refrigerated after adding acetone. This gave a white crystalline solid in small amounts. A pure sample was prepared after three recrystallizations from ethanol. The analytical values corresponded with methylenebis-3-azabicyclo(3.2.2)-nonane dihydrochloride (I). The infrared spectrum showed no carbonyl absorption. This ruled out the possibility of its being an indanedione Mannich base. The infrared spectrum was somewhat similar to that of AZBN.



I

The NMR spectrum was consistent with the proposed structure (I). Bands at δ = 1.95 (16H, singlet) are due to methylene protons at 6,7,8,9,-6′,7′,8′,9′ and those at δ = 2.17 (6H, multiplet) correspond to protons at 1,5,1′,5′ and the methylene group between the two nitrogens. Bands at δ = 3.36 (8H, triplet) may be assigned to protons at 2,4 and 2′,4′. Bands at δ = 7.47 (2H, singlet) represent protons at 3 and 3′. When D_2O was added, the peak at δ = 7.47 disappeared due to the exchange of the protons on the nitrogen atoms.

Methylenebis-3-azabicyclo(3.2.2)nonane dihydrochloride was synthesized by utilizing another route. The melting point and infrared spectrum of the resulting product were identical in every respect to those relative to the Mannich (AZBN) mother liquor product. The mixed melting point showed no depression. On the basis of the above evidence the structure (I) of methylenebis-3-azabicyclo(3.2.2)-nonane dihydrochloride is assigned to the product isolated from the mother liquor. Isolation of similar types of by-products are recorded in the literature (10, 11). Thus *N,N′*-tetraethylmethylenediamine (10) and methylenedipiperidine (11) have been obtained when using diethylamine and piperidine, respectively.

The mechanism of the Mannich reaction has been investigated by Hellmann and Opitz (12) and Cummings and Shelton (13). It is proposed (14) that the reaction is initiated by a condensation between the amine and formaldehyde to yield an amino-

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