

Cryoscopic properties of aqueous solutions of heptacaine and some its analogues

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Received 28 October 1976

*Paper published on the occasion of the 25th anniversary of the foundation
of the Faculty of Pharmacy, Komenský University, Bratislava*

Freezing point depressions of the diluted aqueous solutions of a new local anaesthetic heptacaine (*N*-[2-(2-heptyloxyphenylcarbamoyloxy)-ethyl]piperidinium chloride) and four its analogues were measured, as well as some of their mixed solutions with urea. The derived osmotic data pointed out various degrees of self-association of the studied substances and their interaction with urea in aqueous solutions.

Были измерены понижения температур замерзания разбавленных водных растворов нового местного анестетика гептакаина (*N*-[2-(2-гептил-оксифенилкарбамоилокси)-этил]пиперидиниум хлорида), четырех его аналогов и некоторых смешанных растворов с мочевиной. Полученные осмотические данные указывают на различную степень самоассоциации исследуемых веществ и их взаимодействие с мочевиной в водных растворах.

Heptacaine was recently described by Čižmárik and Borovanský [1, 2] originally under the name substance *XIX*. The pharmacologic studies [2—4] revealed its typical local anaesthetic properties as well as a significant antiarrhythmic activity. The analytical profile of heptacaine was also worked out [5], however, it did not cover information on its cryoscopic properties which are necessary both for the preparation of its isoosmotic aqueous solutions and its further study. In this paper, therefore, we report the measured freezing point depressions of the aqueous solutions of heptacaine and the relative compounds with a shorter alkoxy substituent (substances *XVI* and *XIII* [1]), the parent compound without the alkoxy

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substituent (substance *IR-1*) and the perhydroazepine analogue of heptacaine (substance *XIXA* [6]), all of them in the form of hydrochlorides. Mixed aqueous solutions of heptacaine and the substance *XIII* with urea were also measured, with the intention to reveal the eventual mutual interaction of the solutes.

Experimental and methods

Chemicals

The studied substances were synthesized and recrystallized as described earlier: substance *XIX* (heptacaine, *N*-[2-(2-heptyloxyphenylcarbamoxyloxy)-ethyl]piperidinium chloride), substance *XIII* (2-pentyloxy), and *XVI* (2-hexyloxy) according to [1], the unsubstituted parent compound *IR-1* [*N*-(2-phenylcarbamoxyloxyethyl)piperidinium chloride] according to Häring [7]. The preparation of the different substance *XIXA* (*N*-[2-(2-heptyloxyphenylcarbamoxyloxy)-ethyl]perhydroazepinium chloride) is described in [6]. Other necessary chemicals, NaCl, KCl, urea, and HCl were anal. grade. The measured solutions of the required molalities were prepared from weighed samples of the dried substances and redistilled freshly boiled water.

Relationships

The freezing point depression (Θ) of the aqueous solution is given by a difference of the temperature (T_0) of the equilibrium system water—ice and that (T) of the system solution—ice, *i.e.* $\Theta = T_0 - T$. A simple approximate relationship can be derived [8, 9] for the diluted solution with a single solute

$$\Theta = E_c \varphi \nu m \quad (1)$$

In eqn (1), $E_c = 1.860 \text{ K kg mol}^{-1}$ is the cryoscopic constant, φ is the molal osmotic coefficient at the freezing point, ν is the number of ions into which a stoichiometric molecule of the dissolved substance is split in the solution (for nonelectrolytes $\nu = 1$), and m is the stoichiometric molality (mol kg^{-1}) of the dissolved substance. The molal (practical) osmotic coefficient is here defined as $\varphi = -55.51/(\nu m) \ln a_w$, where a_w is the water activity in the solution.

For the freezing point depression of the aqueous solution with several solutes it can be analogously written

$$\Theta = E_c \varphi \sum \nu_i m_i \quad (2)$$

Eqns (1) and (2) are valid for solutions with $\Theta \leq 1 \text{ K}$ with the precision at least 0.001 K [8, 9].

Cryoscopic apparatus and procedures

With respect to the previous experience [10], a simple cryoscopic apparatus was set up. Its cooling unit was based on a thermoelectric thermostat TT-1 (Tesla, Lanškroun). In this unit, a conical probe with 1.5 ml sample of pure water or the measured solution was cooled to the freezing temperature, by the final rate *ca.* 0.5 K min⁻¹. The sample was stirred by a stainless coil to reduce the effect of supercooling. The course of its temperature was followed by a thermistor (Knauer) sealed in the glass tube immersed in the sample. The resistance of the thermistor was measured by the Universal-Temperatur-Meßgerät (Knauer). Its values R_0 and R at the freezing point of pure water and the measured solution, respectively, were read off from the cooling curves as the lowest thermistor resistances (the highest temperatures) on the start of freezing, after the end of supercooling. The thermistor was calibrated by the measurements of aqueous solutions of NaCl (0.02—0.4 mol kg⁻¹) the freezing point depressions of which are known, so as to enable to evaluate from the resistance difference $\Delta R = R - R_0$ values of Θ of the studied solutions. The reliability of both the apparatus and the adopted calibration was verified with solutions of KCl.

The necessary Θ values of the aqueous solutions of NaCl and KCl at the used molalities were obtained from mathematical processing of the very precise (up to 10⁻⁴ K) older cryoscopic results of *Scatchard* and *Prentiss* [11]. Their data were published as the values of the function $j = 1 - \Theta/(E_c \nu m)$ (practically $1 - \varphi$), however, with an older value of E_c . For our use we processed the dependence of the experimental j values upon molality into the semiempirical equation

$$j = 0.375\sqrt{m}/(1 + 1.7\sqrt{m}) + a_1 m + a_2 m^2 + \dots + a_5 m^5 \quad (3)$$

From the empirical coefficients $a_1 - a_5$, obtained by the least squares method, we recalculated the smoothed j values and the Θ 's of the solutions of NaCl and KCl listed in Table 1.

Repeated measurements of the NaCl solutions given in Table 1 yielded the calibration function $\Delta R = f(\Theta)$ of the used thermistor; this being numerically processed as the second-degree polynomial. The standard deviation of the experimental ΔR 's from the smoothed function was $\pm 2.4 \Omega \sim \pm 0.0023$ K while the standard deviation of the thermistor resistance R_0 (*ca.* 23 k Ω) at the freezing point of pure water was only $\pm 0.5 \Omega \sim \pm 0.0005$ K. By means of the determined calibration function, the measured ΔR 's of the control KCl solutions as well as those of the studied solutions were converted into the corresponding Θ values.

The deviations between the measured and literature values of Θ of the control KCl solutions increase only slightly with their molality, the standard deviation is ± 0.0027 K in the range of Θ up to 1.3 K (Table 1). Besides, the KCl solutions were worse measurable (higher supercoolings, prevalence of $\Theta_3 > \Theta_2$ in Table 1) and, therefore, they were not chosen as the calibration standard. Since the Θ 's of the studied solutions are rather small, their uncertainties are estimated as ± 0.002 K which is adequate to the used procedure. Higher precision of the literature values of Θ of NaCl and KCl [11] resulted from a different, so-called equilibrium (adiabatic) measuring procedure [11—12] which requires an analysis

Table 1

Calibration data (NaCl) and control measurements of the freezing point depressions of aqueous KCl

<i>m</i>	NaCl Θ_1	KCl		<i>m</i>	NaCl Θ_1	KCl	
		Θ_2	Θ_3			Θ_2	Θ_3
0.02	0.0714	0.0712	0.072	0.22	0.7519	0.7430	0.741
0.04	0.1412	0.1407	0.140	0.24	0.8192	0.8087	0.811
0.06	0.2102	0.2092	0.212	0.26	0.8864	0.8743	0.876
0.08	0.2788	0.2771	0.277	0.28	0.9536	0.9398	0.942
0.10	0.3470	0.3446	0.343	0.30	1.0208	1.0053	1.002
0.12	0.4149	0.4117	0.410	0.32	1.0880	1.0706	1.068
0.14	0.4826	0.4784	0.482	0.34	1.1552	1.1360	1.140
0.16	0.5501	0.5449	0.548	0.36	1.2223	1.2013	1.204
0.18	0.6174	0.6111	0.614	0.38	1.2894	1.2666	1.263
0.20	0.6847	0.6772	0.675	0.40	1.3564	1.3318	1.335

Θ [K] — freezing point depressions of aqueous solutions, *m* [mol kg⁻¹] — molality.

Θ_1 (NaCl, calibration solutions) and Θ_2 (KCl) calculated from the data of *Scatchard* and *Prentiss* [11] according to eqn (3), Θ_3 measured for KCl solutions. The standard deviation between Θ_2 and Θ_3 is ± 0.0027 K.

of the equilibrium mixture solution—ice. Because of the lack of sufficiently accurate and rapid analytical methods for the studied organic substances, we used the described procedure based on the dynamic cooling of the solutions with a known composition.

Measurements of the studied solutions and results

Since the studied substances are hydrochlorides of weak bases, their measured aqueous solutions were prepared with a small admixture of HCl, amounting to 0.001 mol kg⁻¹, to suppress the hydrolysis. The corresponding value of Θ was always subtracted from the results. The mixed solutions of the substances *XIX* and *XIII* with urea as well as the solutions of urea alone were prepared and measured in the same way. Especially these solutions were therefore measured immediately after the preparation.

The resulting Θ 's of the aqueous solutions of the studied substances are summarized in Table 2. Substances *XIX*, *XIXA*, and *XVI* are so poorly soluble that their solutions with the highest used molalities are already slightly supersaturated at the freezing temperature. The solubility of the substances *XIII* and *IR-1* exceeds the measured range. For the purpose of the practical preparation of isoosmotic solutions, smoothed values of Θ at round mass concentrations of the studied substances are given in Table 3. They were calculated from the experimental results in Table 2 using the least squares technique and the polynomial of the second or third (substance *XIII*) degree. As the determined densities of the measured

Table 2

Measured freezing point depressions (Θ) of aqueous solutions
of the studied substances

<i>m</i>	Θ , K			<i>m</i>	Θ , K	
	XIX	XIXA	XVI		XIII	IR-1
0.01	0.032	0.025	0.036	0.04	0.134	0.139
0.015	0.048	0.035	0.054	0.05	0.163	0.166
0.02	0.058	0.044	0.069	0.06	0.191	0.198
0.025	0.070	0.054	0.085	0.07	0.215	0.232
0.03			0.100	0.08	0.236	0.256
0.035		0.074		0.09	0.249	0.285
				0.10	0.257	0.308
0.01	0.036	0.037		0.11	0.262	0.334
0.02	0.072	0.071		0.12	0.267	0.356
0.03	0.103	0.108		0.13	0.275	0.382

Table 3

Freezing point depressions (Θ) of aqueous solutions of the
studied substances at round mass concentrations (*w*)

<i>w</i>	Θ , K			<i>w</i>	Θ , K	
	XIX*	XIXA	XVI		XIII	IR-1
0.2	0.017	0.012	0.019	0.5	0.047	0.063
0.4	0.033	0.023	0.038	1.0	0.094	0.123
0.6	0.047	0.034	0.056	1.5	0.138	0.180
0.8	0.059	0.041	0.072	2.0	0.178	0.234
1.0	0.070	0.54	0.089	2.5	0.213	0.284
1.2		0.063	0.104	3.0	0.239	0.331

w — concentration g/100 g or g/100 ml of solution.

* Heptacaine.

solutions ranged from 0.997 to 1.002 g cm⁻³ (25°C), the data in Table 3 are practically valid for the concentration both g/100 g and g/100 ml of the solution.

The measured freezing point depressions of the aqueous solutions of urea and its mixed solutions with substance XIX or XIII are given in Table 4. The Θ values for urea agree with the literature data [12] within ± 0.002 K, although they were measured in the acidified aqueous solution.

Table 4

Freezing point depressions of aqueous urea and its mixed solutions with substances XIX and XIII

Urea		XIX + Urea				XIII + Urea			
<i>m</i>	Θ	<i>m</i> -XIX	<i>m</i> urea	Θ	Θ_{calc}	<i>m</i> -XIII	<i>m</i> urea	Θ	Θ_{calc}
0.04	0.073		0.04	0.122	0.121		0.20	0.592	0.604
0.08	0.146		0.08	0.197	0.194	0.08	0.32	0.800	0.821
0.12	0.222	0.015	0.12	0.270	0.270		0.20	0.609	0.617
0.16	0.295		0.16	0.347	0.343	0.09	0.32	0.818	0.834
0.20	0.368		0.04	0.136	0.131		0.20	0.615	0.625
0.24	0.440	0.02	0.08	0.213	0.204	0.10	0.32	0.823	0.842
0.28	0.514		0.12	0.289	0.280		0.20	0.623	0.630
0.32	0.585		0.16	0.363	0.353	0.11	0.32	0.834	0.847

 Θ [K] — measured depression. Θ_{calc} [K] — the sum of the depressions of individual solutions of urea and the substance XIX or XIII (Table 2) at corresponding molalities.

Discussion

The osmotic coefficients φ of the aqueous solutions of the studied substances at the freezing point were calculated from the experimental Θ values (Table 2) according to eqn (1). Since the substances are salts of the BH^+Cl^- type, the value $\nu = 2$ was inserted. Such calculated osmotic coefficients are outlined in Figs. 1 and 2 in the form of the dependences of $1 - \varphi$ upon molality. For the more soluble substances *XIII* and *IR-1*, the dependences of the Θ/E_c ratio upon m are furthermore seen in Fig. 3. For the sake of comparison, the $1 - \varphi$ values for NaCl are also marked in Figs. 1 and 2, thus representing a simple electrolyte of the same formal charge type (1:1) as the studied substances.

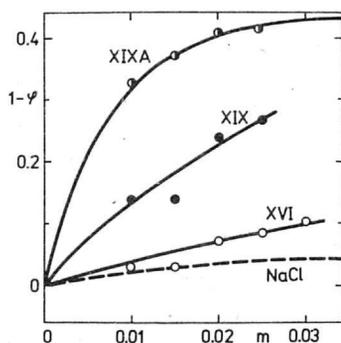


Fig. 1. Dependence of the $1 - \varphi$ values of aqueous solutions of the substances XVI, XIX, and XIXA upon their molality (m). φ is the molal osmotic coefficient at the freezing point.

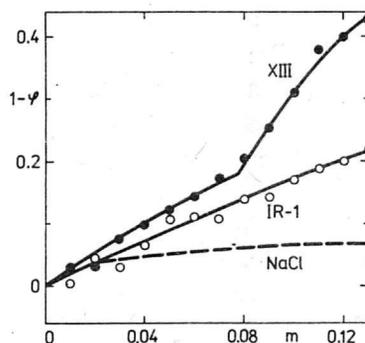


Fig. 2. Dependence of the $1 - \varphi$ values of aqueous solutions of the more soluble substances XIII and IR-1 upon their molality.

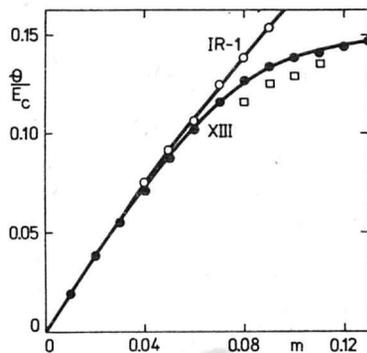


Fig. 3. Dependence of the Θ/E_c ratio of aqueous solutions of the substances XIII (●) and IR-1 (○) upon their molality. □ Substance XIII in the medium of 0.32 m urea.

In the measured molality range, the $1 - \varphi$ values of the solutions of the studied substances are positive and mostly substantially higher than the corresponding values for sodium chloride or other nonassociated electrolytes of the same type [11], which indicates self-association of the studied substances in aqueous solutions. Below 0.03 *m*, the order of magnitude of the $1 - \varphi$ values increases in the series *IR-1* < *XIII* < *XVI* < *XIX* < *XIXA*. The substance *IR-1* is here quite similar to the nonassociated electrolytes, while the high $1 - \varphi$ values of the poorly soluble substances *XIX* (heptacaine) and *XIXA* (Fig. 1) corroborate their appreciable self-association (aggregation) even at such dilution. Though the mechanism of the considered association cannot be derived unambiguously from the osmotic data, it is indicated by an accordance of the above-mentioned order of the $1 - \varphi$ values with the order of the number of CH₂ groups in the BH⁺ cations of the studied substances. It suggests the aggregation of these amphiphilic cations through the hydrophobic interaction of their CH₂ groups, e.g. those in the alkoxy substituent and in the piperidine or perhydroazepine (*XIXA*) ring.

The more soluble derivative *XIII* furthermore exhibits in the area of ca. 0.08 *m* solution a sharp upturn of the dependence of $1 - \varphi$ upon molality (Fig. 2) or, reversally, a progressively reduced slope of the Θ/E_c values (Fig. 3). In that area, the substance *XIII* apparently reaches its critical micellar concentration which is also characteristic of the hydrophobic interaction of amphiphilic ions, especially of those with a longer noncyclic hydrocarbon chain [13]. On the other hand, the parent substance *IR-1* with no alkoxy substituent on the benzene ring does not exhibit c.m.c. in the studied range, however the steady increase of the $1 - \varphi$ values (Fig. 2) indicates its stepwise association at higher molalities.

The hydrophobic interaction of the nonpolar groups of organic compounds in aqueous solutions is thought to be weakened by the presence of urea [14, 15], due to its structure-breaking effect on the liquid water [16]. This is also confirmed by the freezing point depressions of the mixed solutions of the substance *XIX* with urea, given in Table 4. The measured Θ 's are here slightly higher than the sum of the corresponding depressions of the individual solutions of *XIX* and urea which is denoted as Θ_{calc} . For example, in the mixed solution 0.02 *m*-*XIX* + 0.16 *m* urea the measured Θ is higher than Θ_{calc} by 0.01 K, thus indicating a weakened self-association of *XIX* due to the effect of urea. At the same time, the measured Θ of the individual solution of 0.02 *m*-*XIX* (Table 2) is only by 0.013 K lower than that of the nonassociated electrolytes, NaCl or KCl, at the same molality (Table 1). However, measurements at higher molality of the substance *XIX* are not feasible because of its poor solubility, its 0.025 *m* solution given in Table 2 is in fact already slightly supersaturated at the freezing point and after addition of urea it is practically unmeasurable.

The mixed aqueous solutions of the substance *XIII* with urea show quite opposite behaviour, the measured Θ 's are lower than Θ_{calc} 's by as much as 0.02 K

(Table 4). The ratios Θ/E_c calculated for *XIII* from the measured Θ 's of the mixed solutions after subtracting the corresponding Θ values of urea are thus substantially lower than those for the individual solutions of *XIII* (Fig. 3). It may be explained by a marked hetero-association with urea, i.e. binding of the BH^+ cations of the substance *XIII* to the amidic group. Since both self-association and the interaction with amides can influence the biological activity of the studied substances, the presented results point out the necessity of further studies of their behaviour in aqueous solutions.

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Translated by F. Kopecký