Thermodynamics of Extremely Diluted Aqueous Solutions

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An extensive thermodynamic study has been carried out on aqueous solutions obtained through successive dilutions and succussions of 1% wt/vol of some solutes up to extremely diluted solutions, (less than 1×10^{-5} mol kg^{-1}) obtained via several 1/100 successive dilution processes. The interaction of acids or bases with the extremely diluted solutions has been studied calorimetrically at 25°C. Measurements have been performed of the heat produced by the mixing of acid or basic solutions of different concentrations, with bidistilled water or with the extremely diluted solutions. Despite the extreme dilution of the solutions, an exothermic heat of mixing in excess has been found in about the 92% of the cases, compared to the corresponding heat of mixing with the untreated solvent. Here, we show that successive dilutions and succussions may permanently alter the physical–chemical properties of the solvent water. The nature of the phenomenon here described still remains unexplained, but significant experimental results are obtained.

A thermodynamic study on aqueous solutions gives interesting information about the behavior of solutes and their interactions with the solvent. The interaction of acids or bases with the extremely diluted solutions has been studied calorimetrically at 25°C. The extremely diluted solution is obtained starting from a solution at 1% wt/vol. After succussion, that solution is named 1CH preceded by the name or formula of the solute. The succussion process consists of vertical shakings of the solution by means of a mechanical apparatus. In a simple succussion process, 100 vertical strokes in six seconds are given to the glass vessel containing the solution. To prepare the successive dilution, 1 g of this solution is added to 99 g of water that again gets succussed, obtaining the 2CH solution. The iteration of this process produces the extremely diluted solutions studied. Measurements have been performed of the heats of mixing of acid or basic solutions of different concentrations with bidistilled water or with solutions, at a concentration of 0.01 mol kg^{-1}, used as reagent, whereas the concentrations of the extremely diluted solutions or with extremely diluted solutions. Procedures for the calorimetric determination of the heat of dilution or mixing are well developed.[1] The experimental results are treated according to the MacMillan-Mayer approach,[2] modified by Friedman and Krishnan.[3] The enthalpies of mixing two solutions are given by the following equations:

$$\Delta H^{\mathrm{mix}}(\mathrm{J\ kg^{-1}}) = h_{xx} m_x{}^f (m_x{}^f - m_x{}^i) + 2h_{xy} m_x{}^f m_y{}^f + h_{yy} m_y{}^f (m_y{}^f - m_y{}^i) + \text{higher terms}$$

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where in m_x^f, m_y^f, m_x^i, m_y^i are the molalities (mol kg^{-1}) after and before the mixing process, respectively, and h_{xx}, h_{yy}, and h_{xy} the enthalpy interaction coefficients, are adjustable parameters. Their values fall in the range 1×10^2–1×10^4 J kg mol^{-2}. Consequently, when the concentration of the solute y of an extremely diluted solution is of the order of 10^{-5} mol kg^{-1} or less, while the concentration of the solute x is a finite one—1×10^4–1×10^{-2} mol kg^{-1}—the previous equation reduces to the sole contribution of x. This actually happens on the third successive 1/100 dilution. Then, the extremely diluted solutions, as those described before, because of the practical absence of the solute, cannot produce any contribution to the heat of mixing via the y component. For electrolyte solutions, the powers of the molalities in the previous equation are fractionary, but the conclusions stay absolutely the same. By an extensive study it was assessed that, using aqueous solutions of acids or bases as reagent, it is possible to distinguish qualitatively the behavior of pure solvent from that of the extremely diluted solutions, whose chemical composition is the same as that of the solvent used. The interaction between acids or bases with the extremely diluted solutions has been studied calorimetrically, determining the heats of mixing at 25°C by means of a thermal activity monitor (TAM) from Thermometric (Sweden).

The heats of mixing with the solvent (bidistilled water) and those with the extremely diluted solutions were determined. Sodium hydroxide (NaOH) or hydrochloric acid (HCl) aqueous solutions, at a concentration of 0.01 mol kg^{-1}, used as reagent, whereas the concentrations of the extremely diluted solutions were less than 1×10^{-5} mol kg^{-1}. Despite the extreme dilution of the solutions used, an excess exothermic heat of mixing has been found in nearly all the cases, with respect to the heat of mixing of the same reagents with the solvent. The excess heat of mixing, namely, the difference between the heat of mixing of the reagent (a solution at finite concentration) with the extremely diluted solutions and the heat of mixing of the same reagent with the solvent, is of the same order of magnitude or higher than the heat of mixing of the reagent with the solvent. To explain this heat in excess, we are forced to focus our attention on the solvent and, in particular, on possible chemical–physical changes induced by the procedure employed in preparing the solutions.

The excess heats of mixing of about 300 experimental measurements, using as reagent aqueous solutions of NaOH or HCI 0.01 mol kg^{-1}, are reported in columns 1–5 of TABLE 1, together with the heats of mixing of the same reagent with the solvent. The reported heats in excess are detectable for some weeks. From this table it clearly appears that a new phenomenon occurs and, because of the absence of solute, it can be inferred that the physical–chemical properties of the solvent must be permanently altered by the procedure of successive dilutions (1/100) and succussions used to prepare the extremely diluted solutions. Thus, we can firmly state that it is now easily possible to measure a chemical–physical property, the heat of mixing with acids or bases, characterizing this new state of the water,[4–6] using a commercial microcalorimeter.

To confirm these very surprising findings, but otherwise "objective" instrumental responses, and to get a deeper insight into this new behavior, a calorimetric titration procedure was adopted. The excess heats of mixing , thus produced in about 300 experimental measurements are reported in columns 6–13 of TABLE 1. A "titration" of the extremely diluted solutions implies the determination of the heat of mixing in ex-

TABLE 1. Excess heats of the mixing for extremely diluted solutions with sodium hydroxide and hydrochloric acid solutions

System	$-Q^g$ NaOH 5×10^{-3} m[e]	N[d]	$-Q^g$ HCl 5×10^{-3} m[e]	N[d]	$-Q^g$ NaOH 2.5×10^{-3} m[e]	N[d]	$-Q^g$ NaOH 1×10^{-3} m[e]	N[d]	$-Q^g$ NaOH 5×10^{-4} m[e]	N[d]	$-Q^g$ NaOH 2×10^{-3} m[e]	N[d]
H_2O bidisi[a]	2.1±0.1[a]	30	0.85±0.01[a]	30	1.7±0.1[a]	30	1.4±0.1[a]	30	1.0±0.1[a]	30	0.5±0.2[a]	30
H_2O 1 CH[b,c]	0[f]–10	21	0.4–0.6	12	0[f]–9.8	11	0[f]–7.9	11	0[f]–3.3	11	0[f]–2.1	11
H_2O 3 CH[b,c]	0[f]–38	52	0[f]–3.2	35	0[f]–35	18	0[f]–11	18	0[f]–5.9	18	0[f]–3.6	18
H_2O 30 H[b,c]	0.8–16	35	0[f]–5.8	26	1.5–3.4	4	1.4–3.1	4	1.1–2.9	4	0.5–1.7	4
NaCl 3 CH[c]	0[f]–17	31	0[f]–3.3	19	1.3	1	1.3	1	1.2	1	0.8	1
NaCl 30 CH[c]	0[f]–16	25	0[f]–1.5	19	—	—	—	—	—	—	—	—
IAA 7 CH[c]	2.4–3.8	2	—	—	2–3.6	2	2–3.1	2	1.8–2.7	2	1–1.6	2
IAA 8 CH[c]	3.1–4.5	2	—	—	3–4	2	2.7–3.7	2	2.4–3.1	2	1.4–1.6	2
IAA 9 CH[c]	1.4–3.1	2	—	—	1.4–3	2	1.3–2.6	2	1–2.2	2	0.4–1.2	2
IAA 10 CH[c]	8.3–9.3	2	—	—	8–9	2	7.1–7.9	2	5.7–6.2	2	2.7–2.9	2
IAA 11 CH[c]	4.5–7.9	2	—	—	4.3–7.6	2	3.8–6.6	2	3.2–5.5	2	1.6–2.9	2
IAA 12 CH[c]	0[f]–4.9	12	—	—	3.8–4.8	12	3.3–4.1	12	2.9–3.4	12	1.7–1.8	12

ABBREVIATIONS: Sodium chloride, NaCl; Indole-3-acetic acid, IAA; 2,4-dichloro phenoxyacetic acid, 2,4-D; *N*-(phosphonomethyl)-glycine, GLP.
[a]Heats of dilution (mean + SD) of sodium hydroxide solutions.
[b]In these cases the procedure of preparation starts with pure solvent. Succussion and dilution is performed just as in the cases of 1% solutions.
[c]Because of the quantitative variability of the excess heats of mixing for these systems, the range of values obtained is reported.
[d]Number of experiments performed.
[e]Concentration (mol kg^{-1}) of the reagent after the mixing process. In these experiments the final concentration is half of the initial one.
[f]Percentage of experiments that give null excess: 8%.
[g]Excess heats of mixing (J/kg) of the extremely diluted solution.

TABLE 1 — *continued*

System	$-Q^g$ NaOH 5×10^{-3} m[e]	N[d]	$-Q^g$ HCl 5×10^{-3} m[e]	N[d]	$-Q^g$ NaOH 2.5×10^{-3} m[e]	N[d]	$-Q^g$ NaOH 1×10^{-3} m[e]	N[d]	$-Q^g$ NaOH 5×10^{-4} m[e]	N[d]	$-Q^g$ NaOH 2×10^{-3} m[e]	N[d]
H_2O bidisi[a]	2.1±0.1[a]	30	0.85±0.01[a]	30	1.7±0.1[a]	30	1.4±0.1[a]	30	1.0±0.1[a]	30	0.5±0.2[a]	30
2,4-D 3 CH[c]	2.6	1	—	—	2.5	1	2.1	1	1.7	1	1	1
2,4-D 5 CH[c]	0[f]	1	—	—	0[f]	1	0[f]	1	0[f]	1	0[f]	1
2,4-D 7 CH[c]	1.6	1	—	—	1.2	1	1.2	1	0.8	1	0.3	1
2,4-D 8 CH[c]	1.6	1	—	—	1.5	1	1.2	1	0.9	1	0.6	1
2,4-D 12CH[c]	0.5–2.6	6	—	—	0.2–2.4	6	0.2–2.4	6	0.2–1.8	6	0.2–1.1	6
GLP 4 CH[c]	0[f]	1	—	—	0[f]	1	0[f]	1	0[f]	1	0[f]	1
GLP 5 CH[c]	0.6	1	—	—	0.6	1	0.4	1	0.3	1	0.1	1
GLP 6 CH[c]	0.3	1	—	—	0.3	1	0.2	1	0.1	1	0.1	1
GLP 7 CH[c]	0[f]	1	—	—	0[f]	1	0[f]	1	0[f]	1	0[f]	1
GLP 8 CH[c]	0.4	1	—	—	0.4	1	0.4	1	0.3	1	0.1	1
GLP 9 CH[c]	1.1	1	—	—	1	1	1	1	0.8	1	0.4	1
GLP 10 CH[c]	4.9	1	—	—	4.6	1	4.2	1	3.4	1	1.8	1
GLP 11 CH[c]	3.5	1	—	—	3.3	1	3.1	1	2.5	1	1.2	1
GLP 12 CH[c]	0[f]	1	—	—	0[f]	1	0[f]	1	0[f]	1	0[f]	1

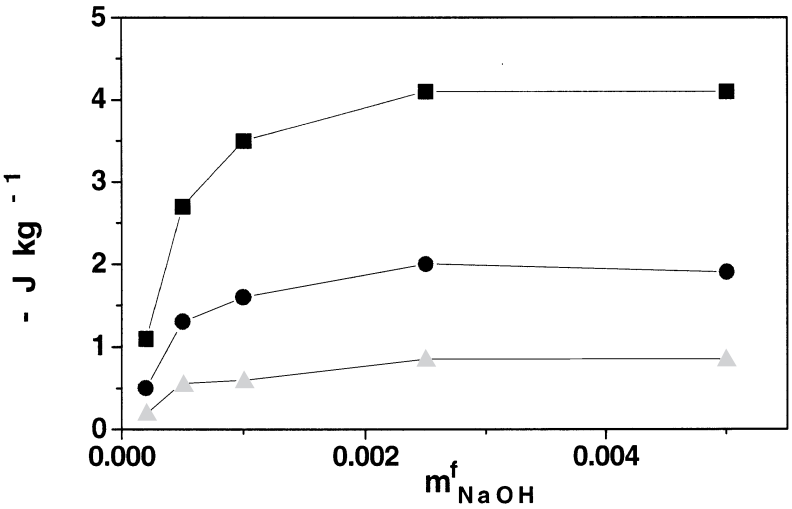

FIGURE 1. Calorimetric titration curves. Extremely diluted solutions of IAA 12CH (■) and its 1:1 (●) and 1:2 (▲) normally diluted solutions with bidistilled water.

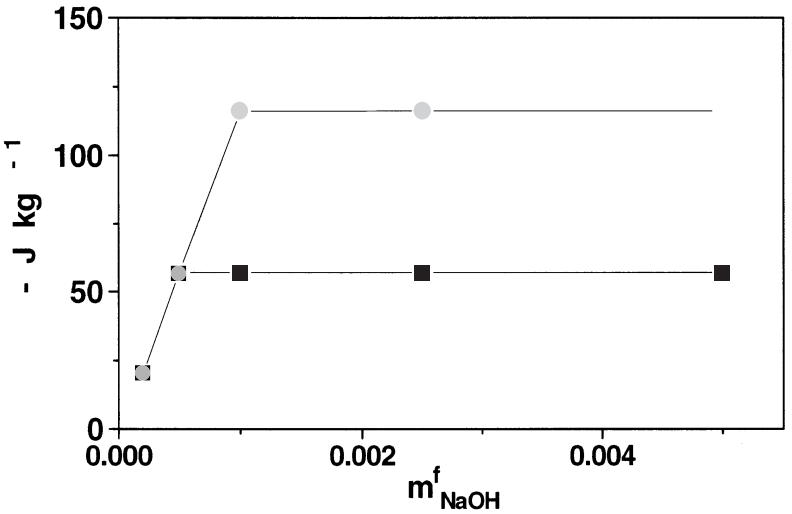

FIGURE 2. An example of calorimetric titration of at 2×10^{-3} (●) mol kg^{-1} HCl solution and its 1:1 (■) diluted solution with bidistilled water.

cess, compared to the heat are obtained with the reference bidistilled water, whereas solutions of NaOH at different concentrations are mixed with the samples under examination. About 60 titrations have been performed with about 40 different samples. These titration curves present two peculiar features (see FIG. 1). First, a plateau appears at the highest concentrations of the titrant, and, second, there's a "break" point

TABLE 2. Excess heats of mixing in the titration of the extremely diluted solutions with sodium hydroxide

M_{NaOH}[a]	$-Q$[b] 1AA[c] 12 CH	$-Q$[b] 1AA[c] 12 CH diluted 1 : 1	$-Q$[b] 1AA[c] 12 CH diluted 1 : 2
2×10^{-4}	1.1	0.5	0.2
5×10^{-4}	2.7	1.3	0.55
1×10^{-3}	3.5	1.6	0.6
2.5×10^{-3}	4.1	2.0	0.85
5×10^{-3}	4.1	1.9	0.85

[a]Concentration (mol kg^{-1}) of the reagent after the mixing process. In these experiments the final concentration is half of the initial one.
[b]Excess heats of mixing (J/kg) of the extremely diluted solution.
[c]Indole-3-acetic acid, IAA.

at a concentration of about 0.001 mol kg^{-1} of the reagent used (NaOH) in the final solution. This latter feature, particularly the fact that it appears exactly at the same concentration, is common to all experiments (with different samples of the extremely diluted solutions used). On the other hand, the magnitude of the excess heat, characterizing the plateau, depends on the nature of the solutions.

To test the stability of these chemical–physical changes in water "structure," the extremely diluted solutions were further diluted (without the succussion procedure) in different proportions (e.g., 1 : 1, 1 : 2, …) with bidistilled water. These "simply diluted" solutions were "titrated" with the NaOH solutions. The resulting curves are characterized by plateaux that are proportional to the degree of the "simple" dilution (i.e. for the proportions just cited as examples, 1/2, 1/3 of the plateaux obtained with the original samples of the extremely diluted solutions), but showing the "break" points at the same concentration of the reagent used (TABLE 2, FIG. 1). This means that the modifications in water "structure" induced by the preparation procedure are stable with respect to a normal dilution process and that the reagent interacts via a destroying mechanism, revealing a pHdependent phenomenon. FIGURE 1 reports titration curves for a highly diluted sample. For the sake of comparison, TABLE 3 and FIGURE 2 show typical calorimetric titration curves for an acid–base reaction, obtained by titrating two solutions of the acid, whose concentrations are in the 1 : 2 ratio. As can be seen, two different plateaux are reached, and two different equivalent points are identified, both in the 1 : 2 ratio, thus showing a sharp difference, either in the amount of heat, slope of the curves, and equivalent point positions, with respect to the behavior of the extremely diluted solutions for which each "titration" curve reaches its own plateau at the same "break" point. It is very interesting to look at the heat of mixing versus pH diagrams (see FIG. 3): they reveal an extraordinary similarity with the normally reported ones for a two-state, pH-induced denaturation process of proteins.[7]

The exact nature of the phenomena here described still remains unexplained, but significant experimental results have been obtained. The mixing process of acids or bases reveals a statistically significant exothermic excess heat with respect to the same process carried on the untreated solvent, bidistilled water, despite the physical absence of solute molecules in the solution obtained after just a few dilution/succus-

TABLE 3. Excess heats of mixing in the titration of hydrochloric acid with sodium hydroxide

M_{NaOH}[a]	$-Q$[b] HCl 1×10^{-3} mol kg^{-1}	$-Q$[b] HCl 2×10^{-3} mol kg^{-1}
2×1^{-4}	19.7	19.7
5×10^{-4}	57	57
1×10^{-3}	57	116.2
2.5×10^{-3}	57	116.2
5×10^{-3}	57	116.2

[a]Concentration (mol kg^{-1}) of the reagent after the mixing process. In these experiments the final concentration is half of the initial one.
[b]Excess heats of mixing (J/kg) of the extremely diluted solution.

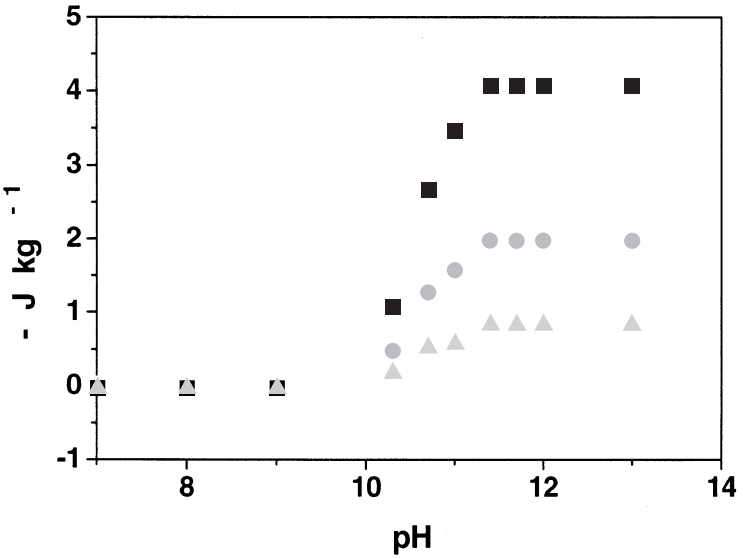

FIGURE 3. Calorimetric titrations curves: Extremely diluted solutions of IAA 12CH (■) and its 1 : 1 (●) and 1 : 2 (▲) normally diluted solutions with bidistilled water, as a function of the pH in the final solutions.

sion procedures. All that is not, at present, in agreement with current theories concerning the properties of liquid water at room temperature,[8] and consequently, the need for appropriate new theoretical studies is urgent.

A hypothesis of disorder–order transition could be proposed, based on the exothermic excess heat and its pH dependence, induced by the addition of acid and/or basic reagents.

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