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On the ^{1}H NMR spectra of weak electrolytes under the influence of strong electrolytes at low concentrations

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ABSTRACT

Physical chemistry has yet to provide a convincing explanation for the many distinct ways the cosolute's properties are perturbed when under the field of action of salt ions. For that reason, a systematic and gradual approach to the problem was sought, with the present work being a step in that direction. Thus, acetic acid and n-butylamine were selected as simple models for the charged carboxyl and amine groups in more complex solutes, like proteins. The influence of the gradual addition of inorganic salts on these compounds' proton nuclear magnetic resonance spectra was analyzed. The salt concentration varied from 0.01 to roughly 100 mmol.L^{-1}.

The reported results suggest that at a low salt concentration (<c.a. 10 mmol.L^{-1}), the effect on the properties of the weak electrolyte results from an indirect action of the salt ions. More specifically, strong electrolytes perturb the auto-dissociation of water whereby H^+ or OH^- ions are released. Some salts, like NaCl, releasing H^+, suppress the dissociation of acetic acid and are neutral to n-butylamine. Other salts, like NaSCN, releasing OH^-, shift the reaction of n-butylamine with water towards the reverse direction and are neutral to acetic acid. Moreover, the required quantity of added salt to promote the effects under consideration depends, to a considerable extent, on the valency of the salt ion, the decreasing order of efficacy being as follows: trivalent > divalent > monovalent.

Therefore, the experimental facts herein reported show that the effect of salt ions at low concentrations upon the properties of other solutes can be rationalized by conventional chemical concepts, in which chemical equilibrium plays an important role. Furthermore, the results strongly support the classical interpretation of salt ions, which are charged bodies that, in solution, exert chiefly electrostatic forces.

1. Introduction

The fact that salt ions, like Na^+ or Cl^-, can be brought out of solution in stoichiometric combination with ions of opposing ionic character is the most convincing evidence that they exert electrostatic forces in solution. Therefore, it's logical to assume that the properties of compounds which contain charged groups, like proteins, are perturbed by salt ions in a manner which depends chiefly on the electrostatic forces they exert. Innumerous experiments, however, do not harmonize with the thesis [1–10]. Indeed, in an attempt to order salt ions in their ability to influence the properties of charged compounds in solution, several investigations have shown that this organization does not follow the electrostatic forces [1–10].

According to the prevailing ideas, the perturbation of the cosolute properties under the field of action of salt ions is due to the salt ions' specificities [1–10]. This term includes, besides electrostatic contributions, some direct and indirect effects [1–10] which are generally classified as weak interactions [1–10]. Direct specific interactions with particular chemical groups, indirect actions resulting from the water of hydration, or the excluded volume effect, amongst others, have been claimed to be the main driving force through which salt ions influence the properties of other solutes [1–10]. However, it is difficult to accept that some weak interactions affect a body if it is under the field of action of a more potent force.

Physical chemistry still needs to provide a compelling explanation of general acceptance for this apparent paradox question. Therefore, a systematic and gradual approach to the problem was sought to shed light on this important question.

Acetic acid and butylamine were selected as simple models for the charged carboxyl and amine groups in all amino acids, peptides and proteins. Numerous investigations have shown that the salt effects on the cosolutes' properties also depend on the salt concentration [1–13]. Therefore, the salt concentration herein studied ranged from 0.01 to roughly 100 mmol.L^{-1}. In forthcoming papers, the salt and weak

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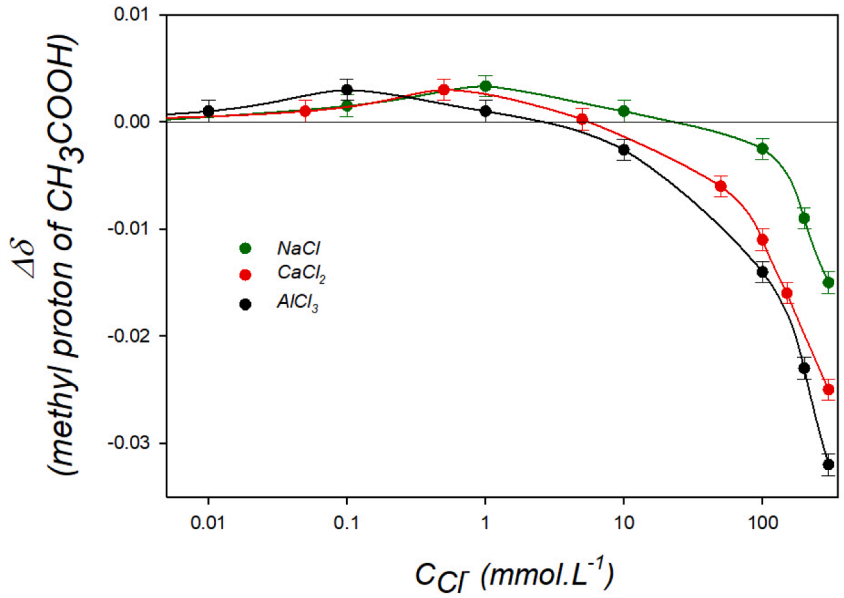

Fig. 1. Influence of the chloride salts of sodium, calcium and aluminum upon the ^{1}H NMR spectra of the methyl proton of acetic acid. The concentration of acetic acid in this Figure is *c.a.* × ($n_{CH3COOH}/n_{Total}$) = 0.085. Herein and elsewhere, the lines have no physical meaning and are intended to aid in the visualization of the data.

electrolyte concentrations, as well as the complexity of the cosolute, will vary.

In this way, it is hoped that a precise clarification of the role of salt ions in modulating the properties of other solutes will be reached. Their effects are ubiquitous in chemistry and biology. Therefore, it would have implications in the various scientific domains involved, ranging from simple separation processes, or our comprehension of their effects on protein solubility, protein denaturation or protein stability, to the complexity of the maintenance of life.

2. Experimental

2.1. Materials

2.1.1. Reagents

The salts used were NaCl (from LabKem, Extra Pure), $CaCl_2$ (from Panreac, 95 %), $AlCl_3$ (from Merck, >98%), $NaSCN{\cdot}2H_2O$ (from Sigma, >98%), Na_2SO_4 (from Sigma-Aldrich, 99.9%), $Na_3PO_4{\cdot}12H_2O$ (from Sigma, 98%), and KOH (from EKA, >99%). Acetic acid glacial was from Honeywell (>99.99 %), 1-Butylamine from Alfa Aesar (>99%), and hydrochloric acid (37%) from Fisher Scientific. The water was ultrapure, double distilled, passed by a reverse osmosis system, and further treated with a Mili-Q plus 185 water purification apparatus. All the reagents were used without further purification.

2.2. Methods

2.2.1. ^{1}H NMR experiments

As previously described [14], NMR chemical shifts (δ) were obtained in ppm using a Bruker Avance 300 spectrometer (operating at 300.13 MHz for ^{1}H NMR). More specifically, solutions containing each sample to be characterized, and a solution of Trimethylsilylpropanoic acid (TSP) in pure deuterated water (99.9% D) as internal standard, were used in NMR tubes adapted with coaxial inserts. The TSP/D_2O solution was used as the inner part of the concentric tubes, while each sample was used in the outer part of the NMR tube. Therefore, it was possible to guarantee that the TSP standard and D_2O were not in direct contact with the sample, avoiding potential interferences in the ^{1}H NMR chemical shifts. At least three measurements were performed for each instance. Replicas on different days were also undertaken. The values amount to the one presented ±0.0015 ppm. The measurements were undertaken within a period of 24 h after the preparation of the salt solutions.

2.2.2. pH experiments

A Metter Toledo Seven Excellence pH meter was used for the pH measurements. After calibration, according to manufacturer instructions, the electrode was inserted in a sample for at least 3 min. After this period, the pH was measured (at least three measurements were performed). The measurements were undertaken at room temperature (~23 °C). The dissociation constant of acetic acid was often measured using this method. Comparison with typical values consistently gave an error below 1.0 %, thus validating the methodology. The uncertainty on the pH values presented is ±0.015. The measurements were undertaken within a period of 24 h after the preparation of the salt solutions.

3. Results and discussion

3.1. The carboxyl group

Fig. 1 shows how the gradual addition of the chloride salts of sodium, calcium and aluminium influences the ^{1}H NMR spectra of the methyl proton of acetic acid.

It can be seen in Fig. 1 that on adding to aqueous acetic acid the studied salts, an initial deshielding (Δδ > 0), followed by a monotonic increasing shielding (Δδ < 0) of its methyl proton by still further addition of salt, is observed. Also noteworthy is that the salt-induced non-monotonic behaviour illustrated in Fig. 1 depends, to a considerable extent, on the valency of the salt cation. Indeed, if the cations are organized in their decreasing efficacy to promote the inversion of the behaviour illustrated in Fig. 1, the following series is obtained: trivalent > divalent > monovalent.

We might be tempted to interpret the results shown in Fig. 1 in light of the prevailing ideas [1–10,15–19], according to which the salt-induced non-linear behaviour of the ^{1}H NMR spectra is due to some weak specific interactions the salt ions exert [15–19]. However, several investigations [11–12,20–26] indicates that the molecules in solution are under the field of action of electrostatic forces. In these circumstances, it's not plausible that some weak forces have a critical role in the observations if any.

Therefore, to interpret the results shown in Fig. 1, it is required to

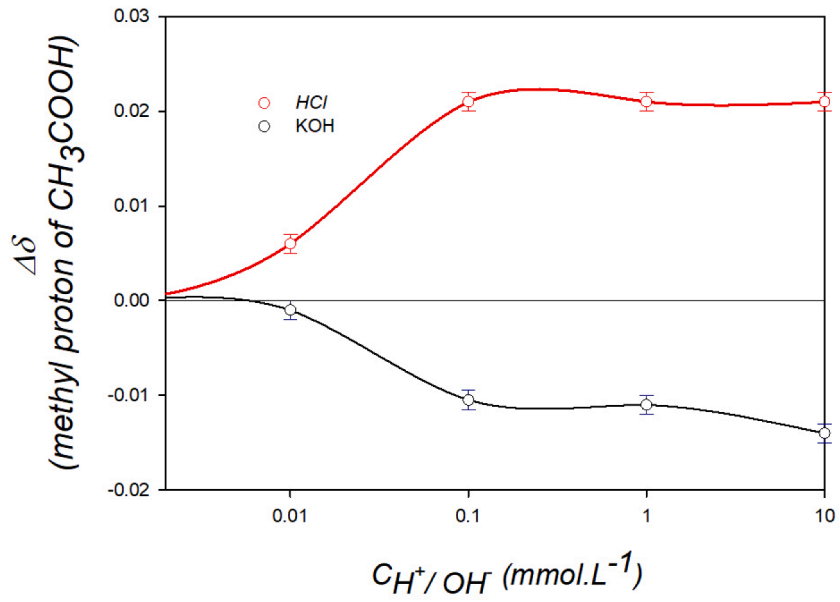

Fig. 2. Influence of HCl or KOH upon the ^{1}H NMR spectra of the methyl proton of acetic acid. The concentration of acetic acid in this Figure is *c.a.* × ($n_{CH3COOH}$/n_{Total}) = 0.085.

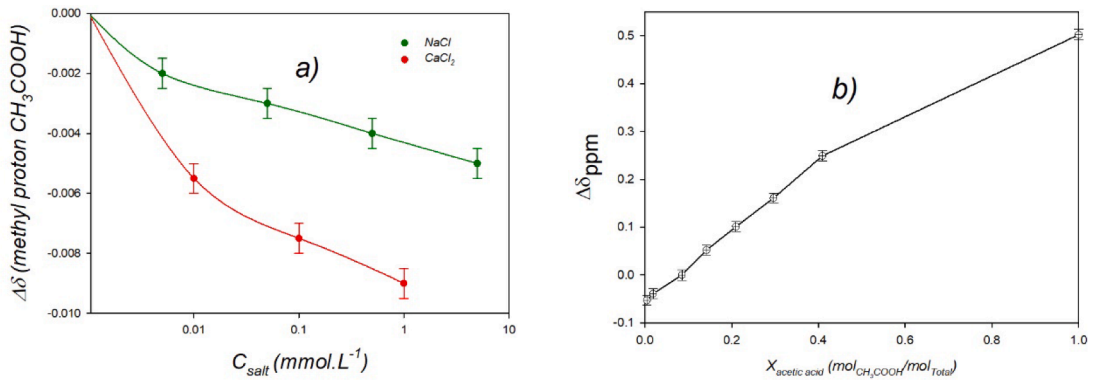

Fig. 3. Influence of (a) the chloride salts of sodium and calcium upon the ^{1}H NMR spectra of the methyl proton of neat acetic acid (the addition of aluminum chloride to neat acetic acid promoted visible precipitation) and (b) of concentration, upon the ^{1}H NMR spectra of the methyl proton of acetic acid. As reference in Fig. 3b the concentration of x = 0.085 was used.

understand the meaning of the deshielding/shielding of the methyl proton of acetic acid.

The ^{1}H NMR spectroscopy is a standard technique to measure the dissociation constants of weak electrolytes [27–29]. The underlying principle is that the change in the chemical shifts while varying the solution pH result from the molecular proportions in which the dissociated and the non-dissociated forms are in chemical equilibria in the solution [27–29]. Therefore, to better understand the results shown in Fig. 1, HCl or KOH were added to acetic acid. Fig. 2 shows the results.

The results in Fig. 2 show that the addition of HCl or KOH induces a monotonic deshielding or shielding, respectively, of the methyl proton of acetic acid. Since H^{+} suppresses acetic acid's dissociation, while OH^{-} promotes its further dissociation, the increasing deshielding or shielding of the methyl proton of acetic acid corresponds to an increase in the molecular proportion in which their undissociated or dissociated forms, respectively, are found in the solution.

While therefore, the addition to acetic acid of NaCl, $CaCl_2$ or $AlCl_3$ at moderate to high concentrations brings about, as expected, further

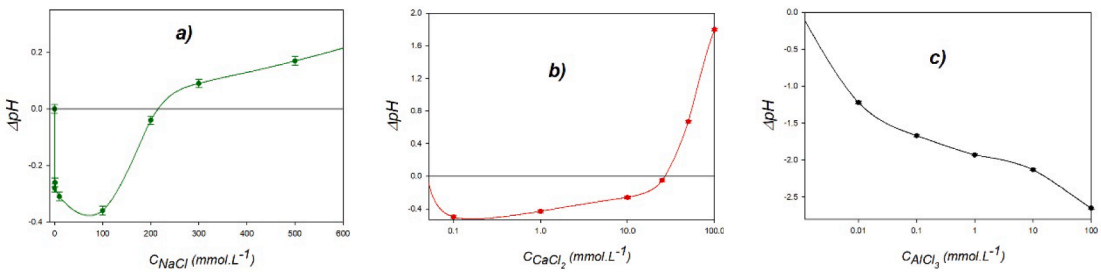

Fig. 4. Influence of (a) sodium chloride, (b) calcium chloride, and (c) aluminium chloride upon the pH of water.

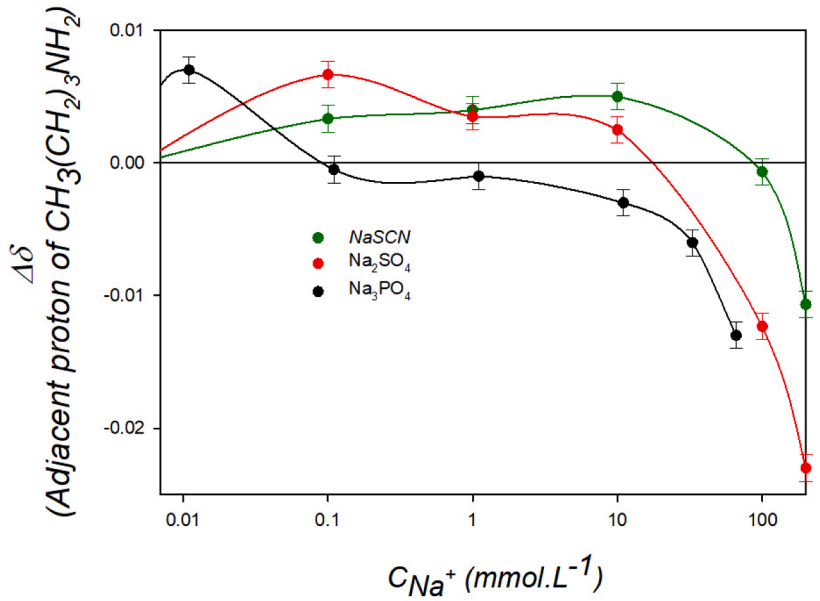

Fig. 5. Influence of the sodium salts of thiocyanate, sulfate and phosphate upon the ^{1}H NMR spectra of the adjacent proton of n-butylamine. The concentration of n-butylamine in Fig. 5 is *c.a.* × ($n_{n\text{-butylamine}}/n_{Total}$) = 0.10.

dissociation of the acid (see Fig. 1), their addition at low concentrations (<c.a. 10 mmol.L^{-1}) brings about, somewhat surprisingly, the suppression of the ionization of the acid. Or in other words. The effect of the studied inorganic salts at low concentrations upon aqueous acetic acid is what would be expected if acid was added to the solution.

The ^{1}H NMR spectra of acetic acid in the absence of water or salt were next studied to clarify which of the perturbing agents, the salt or the water, is responsible for suppressing the dissociation of acetic acid. Fig. 3 shows the results.

Fig. 3a and 3b show that the gradual addition of a strong electrolyte or water, respectively, to neat acetic acid, brings about a monotonic shielding of its methyl proton, consistent with the promotion of a higher degree of dissociation of the acid by the gradual addition therein of any of the studied perturbing agents.

Taking all the previous facts together, it is logical to conclude that the non-monotonic effect shown in Fig. 1 results from a joint action, of the salt and the water, upon acetic acid. For that reason, the effect of the studied chloride salts on the pH of the water was next investigated. Fig. 4 shows the results.

In conformity with earlier observations [30–31], Fig. 4 shows that inorganic salts perturb, to a considerable extent, the autodissociation of water. And although the studied inorganic salts reported in Fig. 4 affect the dissociation of water in different ways, in all cases, a decrease in pH or, instead, a release of H^+ is observed at low salt concentrations.

The previous facts could explain the non-monotonic effect shown in Fig. 1 in the following manner. Acetic acid dissociates when dissolved in water, according to Eq. (1).

$$CH_3COOH \rightleftarrows CH_3COO^- + H^+ \quad (1)$$

The solute's macroscopic properties result from the molecular proportions in which the protonated and the non-protonated forms are in chemical equilibria in the solution. Salt ions influence the chemical equilibrium between these forms to a considerable extent. On the one hand, salt ions perturb the autodissociation of water, whereby H^+ could be released in the solution, suppressing the acid's dissociation. On the other hand, salt ions induce further acid dissociation.

At low salt concentrations, the effect resulting from the dissociation of water prevails. Therefore, equilibrium (1) shifts in the reverse direction. Consequently, the ^{1}H NMR peak of the methyl proton of acetic acid moves downfield ($\Delta\delta > 0$). Above a particular salt concentration, the effect of the salt, which leads to further dissociation of the acid, prevails. Therefore, by still further addition of salt, additional dissociation of the acid takes place. Eq. (1) shifts forward, and the ^{1}H NMR peak moves upfield ($\Delta\delta < 0$).

3.2. The amine group

Fig. 5 below shows how the gradual addition of the sodium salts of thiocyanate, sulfate and phosphate influence the ^{1}H NMR spectra of the adjacent (A) proton of n-butylamine. Figure S1 in the Supplementary information indicates the $CH_3(CH_2)_3NH_2$ proton assignments.

It can be seen in Fig. 5 that below a particular salt concentration, the ^{1}H NMR spectra of the A proton of n-butylamine shift downfield ($\Delta\delta > 0$), which changes upfield ($\Delta\delta < 0$) by further addition of salt. Figure S2 shows that the $CH_3(CH_2)_3NH_2$ terminal proton behaves similarly.

The required quantity of added salt to promote the inversion of the behaviour illustrated in Fig. 5 depends, to a considerable extent, on the valency of the salt anion. This quantity follows approximately the proportion x, x^2, x^3 for mono-, di- and trivalent ions, respectively, suggesting that the ions participate in some chemical equilibria of electrostatic nature [12–13].

Therefore, the gradual addition of certain salt ions to aqueous n-butylamine brings about a non-monotonic effect upon the ^{1}H NMR spectra of their methyl protons. Experiments have been undertaken to clarify this effect, similar to those used for acetic acid.

Firstly, the effect of the gradual addition of acid or base was analysed. Figure S3 shows that adding base to n-butylamine increases deshielding while acid shields its adjacent methyl proton. Therefore, the effect of the studied inorganic salts at low concentrations (<*c.a.* 10 mmol.L^{-1}) upon aqueous n-butylamine is what would be expected if a base had been added therein.

Next, the effect of water and salt separately was studied. Figure S4 shows that adding water to neat butylamine, similar to the effect observed in acetic acid, increases the shielding of its adjacent methyl proton, consistent with promoting a higher degree of dissociation of the base by the gradual addition therein of water.

The study of the influence of salt ions upon the ^{1}H NMR spectra of neat butylamine is more complex than that of acetic acid and requires closer consideration. The underlying reason is that butylamine is not a dissociation molecule like acetic acid. Its aqueous behaviour is more

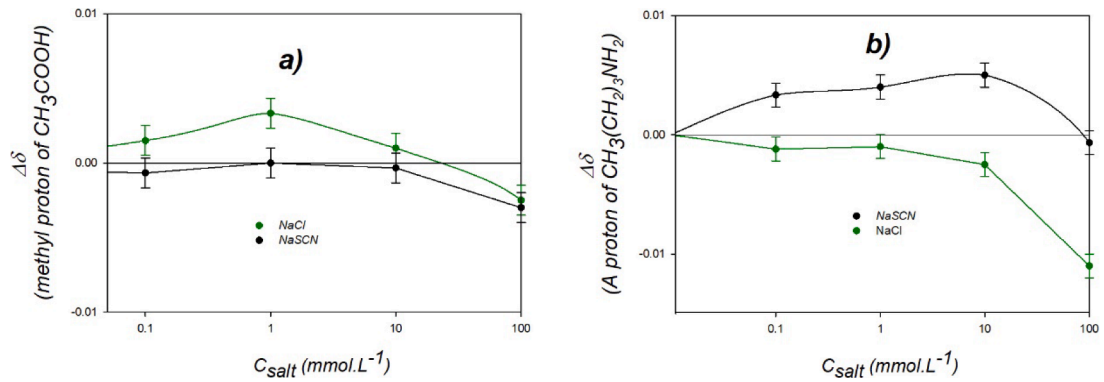

Fig. 6. Influence of NaCl and NaSCN upon the ^{1}H NMR spectra (a) acetic acid and (b) the adjacent proton of n-butylamine.

akin to a chemical reaction with water. That's probably why small quantities of water considerably affect the overall ^{1}H NMR spectra of butylamine (see figure S5).

Amongst the studied salts, Na_2SO_4 was in the non-hydrated form, whose effect on neat butylamine was the only one explored. Figure S6 shows the results. It can be seen in this figure that the gradual addition of the inorganic salt to neat butylamine brings about a monotonic deshielding of its adjacent proton, consistent with the promotion of a higher degree of dissociation of the base by the gradual addition therein of the perturbing agent. In this regard, several investigations have emphasized the striking parallelism between the general properties of liquid amines and water [32–37]. It is beyond the scope of the present consideration to get further insights into the behaviour of pure amines, and the interested reader may find helpful information elsewhere [32–37].

Based on the previous considerations, it is logical to suggest that the non-monotonic effect observed in Fig. 5 is from a joint action of the salt and the water upon n-butylamine. Consequently, the impact of the sodium salts of thiocyanate, sulfate and phosphate on the pH of the water was next investigated. Figure S7 shows that these salts perturb, to a considerable extent, the autodissociation of water. Moreover, pH increases are observed in all cases at low salt concentrations.

Following a similar reasoning undertaken for acetic acid, an explanation for the salt-induced non-monotonic effect shown in Fig. 5 could be as follows: n-Butylamine, when dissolved in water, reacts according to equation (2):

$$CH_3\,CH_3(CH_2)_3NH_2 + H_2O \rightleftarrows CH_3\,CH_3(CH_2)_3NH_3^+ + OH^- \quad (2)$$

The solute's macroscopic properties in water result from the molecular proportions of the protonated and non-protonated forms in chemical equilibrium. Salt ions influence the chemical equilibrium between these forms to a considerable extent. On the one hand, salt ions perturb the autodissociation of water, whereby OH^- could be released in the solution, shifting the reaction (2) in the reverse direction. On the other hand, salt ions move the response forward.

At low salt concentrations, the effect resulting from the dissociation of water prevails. Therefore, the reverse reaction in (2) is promoted—consequently, the ^{1}H NMR peak of the methyl proton of n-butylamine moves downfield. Above a particular salt concentration, equation (2) shifts forward, and the ^{1}H NMR peak moves upfield.

The results herein discussed suggest that at a low salt concentration (<*c.a.* 10 mmol.L^{-1}), the effect on the cosolute properties results from an indirect action of the salt ions. If this is so, then the effect of NaCl on the properties of n-butylamine should be similar to that of HCl, while that of NaSCN on acetic acid should be similar to that of KOH. Therefore, as a final test to the suggested conjecture, the effect of NaSCN and NaCl upon the ^{1}H NMR spectra of acetic acid and n-butylamine, respectively, was also studied. Fig. 6 shows the results.

The results shown in this Figure confirm that at low salt concentrations, NaSCN and NaCl have practically no influence upon the ^{1}H NMR spectra of acetic acid and n-butylamine, respectively, which is as striking as could be expected in support of the herein advocated ideas.

4. Conclusions

Weak electrolytes, like acetic acid or n-butylamine, manifest themselves in distinct forms in chemical equilibria between them when dissolved in water. The solute's macroscopic properties result from the molecular proportions of these forms. Adding a strong electrolyte, like NaCl, to an aqueous solution of a weak electrolyte will perturb the chemical equilibria of their distinct molecules, and their macroscopic properties change accordingly.

At low salt concentrations (<c.a. 10 mmol.L^{-1}), the results herein discussed indicate that the properties of weak electrolytes change as the result of indirect action. More specifically, salt ions perturb the autodissociation of water whereby H^+ or OH^- can be released. Some salts, like NaCl, releasing H^+, suppress the dissociation of weak acids, like acetic acid, and are neutral to weak bases, like n-butylamine. Other salts, like NaSCN, releasing OH^-, suppress the dissociation of weak bases and are neutral to weak acids.

Moreover, the required quantity of added salt to promote the effects under consideration depends, to a considerable extent, on the valency of the salt ion. This quantity follows, within experimental uncertainty, the proportion x, x^2, x^3 for mono-, di- and trivalent ions, respectively, suggesting that the ions participate in some chemical equilibria of electrostatic nature.

CRediT authorship contribution statement

Pedro P. Madeira: Conceptualization, Methodology, Validation, Investigation, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.molliq.2023.121932.

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