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Perspective

Polarity of Aqueous Solutions

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Abstract: This short review describes the expansion of the solvatochromic approach utilizing water-soluble solvatochromic dyes to the analysis of solvent features of aqueous media in solutions of various compounds. These solvent features (polarity/dipolarity, hydrogen bond donor ability (HBD acidity), and hydrogen bond acceptor ability (HBA basicity)) vary depending on the nature and concentration of a solute. Furthermore, the solvent features of water (the solvent dipolarity/polarizability and hydrogen bond donor ability) in solutions of various compounds describe multiple physicochemical properties of these solutions (such as the solubility of various compounds in aqueous solutions, salting-out and salting-in constants for polar organic compounds in the presence of different inorganic salts, as well as water activity, osmotic coefficients, surface tension, viscosity, and the relative permittivity of aqueous solutions of different individual compounds) and are likely related to changes in the arrangement of hydrogen bonds of water in these solutions.

Keywords: water; solvent properties; hydrogen-bonds; solubility; physicochemical properties of aqueous solutions

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1. Introduction

Unusual and important physical and chemical properties of liquid water, such as surface tension and high permittivity, are based on its ability to form hydrogen bonds. The strength of these bonds depends, in part, on the mutual positions of the interacting molecules. For that and possibly other reasons, the conventional use of thermodynamics in many studies of liquid water properties may be viewed as debatable or at least requiring more complex models. The conventional use of thermodynamics is based on the ideal gas laws, but this simple approximation seems rather questionable in the case of liquid water.

Conclusions based on various thermodynamic properties of aqueous electrolyte solutions could not be used to explain the effects of salts on the structure of water. The attempts to use Flory–Huggins theory (see in [1]) to explain liquid–liquid phase separation have been inadequate, especially for aqueous two-phase systems [2]. For liquid–liquid phase separation to occur in water, the necessary and sufficient condition is the emergence of an interfacial tension. The interfacial tension between organic solvent and water two-phase systems is well-known [3] to increase with increasing dissimilarity of the two solvents. However, in any of these organic solvent–water systems, the value of interfacial tension is not affected by the increase in the organic solvent concentration. On the other hand, in aqueous two-phase systems formed by two polymers or a single polymer and salt, it is well-established experimentally [4–7] that the interfacial tension increases with increasing concentrations of the polymers, i.e., with increasing differences between the polymer concentrations in the two phases.

According to the recently reported [8] model of phase separation in aqueous mixtures of two polymers, the phase diagram may be described in terms of the polymers' effects on

the solvent features of water. The different properties of the coexisting phases in aqueous two-phase systems are successfully described by different solvent properties of water in the phases [9–11]. It should be emphasized that the Flory–Huggins theory of the incompatibility of polymers in solution considers the solvent solely as a diluent of unfavorable contacts between polymers and cannot be used to explain the liquid–liquid phase separation in aqueous media. This is unsurprising, as the theory was stated [12] as inapplicable to polar systems. Therefore, attempts to use this theory for the explanation of liquid–liquid phase separation (LLPS) in biological systems seem to be counterproductive.

It is well-established that the high overall concentration of biological macromolecules, occupying up to 40% of the cellular volume and typical for different biological systems [13,14], may influence different properties of proteins and nucleic acids in vivo, such as conformational stability, folding mechanisms, aggregation propensity, interactions with partners, etc. This effect was initially ascribed to the restriction of the volume accessible to a query protein or nucleic acid by the excluded volume effects induced by macromolecular components. Additional weak or "soft" nonspecific interactions between the target protein or nucleic acid and surrounding macromolecules have been suggested to explain numerous experimental observations [15–19]. We showed [20] that most of the crowding effects can be attributed to the effects of proteins or nonionic crowding polymers on the solvent properties of water. The same effects are also displayed by various polyols and other osmolytes [21].

The solvent properties of the aqueous solutions of individual phase-forming polymers describe the binodal line for such systems much better [8]. The two-phase distribution of solutes, from small organic compounds to large proteins, depends on the solvent properties of the two aqueous phases and not on the type and concentrations of the phase-forming polymers [22,23].

The debate between those accepting the salt classification as water structure-making and structure-breaking [24] and its opponents [25,26] remains unsettled. The issue is important because water structure is commonly ignored in molecular biology even though it affects protein folding and LLPS leading to the formation of membrane-less organelles and impacting multiple other biological processes. Computer simulations and predictions of protein structures usually ignore the presence of water in protein solutions, while the results of such simulations are often considered [27] as experimental evidence. Our attempts to produce a suitable theoretical model for phase separation in aqueous two-phase systems were only partially successful [28]. It has been shown [28] that interfacial tension in aqueous two-phase systems may be described by the linear combination of differences between the solvent properties of the coexisting phases.

We found that such proteins as heat-shock protein HspB6 [29], plant stress dehydrins [30], and crystallins affect the solvent properties of water in their solutions as strongly as other solutes. For such studies to succeed it must be proven, first, that there are no direct protein interactions with the solvatochromic dyes [29,30]. Most of the proteins are inclined to bind aromatic compounds; therefore, only a few proteins have been studied so far. Serum albumin has been shown by NMR [31,32] to alter the hydrogen bond donor acidity of water. These studies need to be extended to explore various chaperones and other proteins.

The discovery [33] of disordered proteins with enlarged water-exposed surfaces indicated, at least indirectly, the role of water in the regulation of protein functions but did not hinder computer modeling of the protein characteristics.

The role of different water properties in various tissues [34] as a factor in the distribution of drugs and viruses is generally neglected because the distribution is often viewed solely as a function of binding to specific receptors or other tissue-specific structures. The possibility of water properties in the vicinity of the receptor altering local drug concentration and thus affecting the equilibrium of the drug–receptor binding is commonly ignored.

All of the above considerations allow us to hypothesize that the emergence of interfacial tension and resulting liquid–liquid phase separation in biological systems may be the consequence of different effects of phase-forming proteins and RNAs on the properties of

water in the cytoplasm, nucleoplasm, mitochondrial matrix, or stroma of chloroplasts. The further studies of interfacial tension might be more productive for gaining deeper insights into the molecular mechanism of liquid–liquid phase separation in biology than those of unarguably important structural details of the macromolecules participating in and/or driving such phase separation.

Physicochemical properties of aqueous solutions, such as water activity, osmotic coefficient, surface tension, relative permittivity, and viscosity, are well-known to differ in solutions of various compounds depending on the compound nature and concentration. Water in all these solutions is generally viewed as a media maintaining essentially the same bulk properties. It established, however, that solubility of various compounds change quite significantly in aqueous solutions of different salts [35], polyols [36], and other osmolytes [37], amino acids [38,39], and polymers [40] due to possible rearrangement of the hydrogen bonds.

Different solvents are often classified according to their polarity [41] generally considered an overall measure of all specific and non-specific solute–solvent interactions (electrostatic, dipole–dipole, H-bonding). There is a large number of different polarity scales based on different probes and spectroscopic techniques (NMR, IR, UV/visible absorption and emission spectroscopy) [41]. According to Ab Rani et al. [42], there is no absolute correct measure of polarity; different polarity values provide different estimates for the same solvent. There is no useful concept of "right" or "wrong". The usefulness of an empirical polarity scale is its ability to describe and predict solvent dependent phenomena.

The set of multi-parameter polarity scales pioneered by Kamlet and Taft [43–45] includes three separate scales. One is based on the ability of the solvent to serve as a hydrogen bond donor (HBD) acidity (α) [45], the other based on the ability to serve as a hydrogen bond acceptor (HBA) basicity (β) [44], and the scale based on the ability of the solvent to participate in dipole–dipole interactions (dipolarity/polarizability, π*) [43]. The combination of these three parameters describes the ability of a given solvent to participate in solute–solvent interactions much better than any single parameter.

In our studies we used a set of three solvatochromic dyes; 4-nitroanisole for estimation of dipolarity/polarizability of aqueous media, π*-value; 4-nitrophenol for the solvent HBA basicity, β-value; and Reichardt's carboxylated betaine dye sodium {2,6-diphenyl-4-[4-(4-carboxylato-phenyl)-2,6-diphenylpyridinium-1-yl])phenolate} for solvent HBD acidity, α-value.

Solvent features of aqueous media in solutions of over 60 various solutes including inorganic salts [46,47], free amino acids [47,48], small organic compounds [49–51], polymers [38,52–54], and a few proteins [52,54] were estimated.

It has been established that the solvent features estimated for aqueous solutions of over 60 different compounds are linearly related to each other as reported in [39]. This relationship may be described as follows:

$$\pi^*_{ij} = k_{\pi^*j} + k_{\alpha j}\alpha_{ij} + k_{\beta j}\beta_{ij}, \quad (1)$$

where i denotes the solute concentration; j denotes the particular solute; and k_{π^*j}, $k_{\alpha j}$, and $k_{\beta j}$ are constant coefficients.

It was found [39] that the above coefficients are linearly interrelated, as illustrated graphically in Figure 1 for all examined compounds:

$$\begin{gathered} k_{\pi^*j} = 1.096_{\pm 0.002} - 1.235_{\pm 0.002}k_{\alpha j} - 0.5956_{\pm 0.0003}k_{\beta j}, \\ N = 61;\ r^2 = 0.99991;\ SD = 0.014;\ F = 3{,}369{,}994, \end{gathered} \quad (2)$$

where N—number of solutes; r^2—correlation coefficient; SD—standard deviation; F—variation ratio.

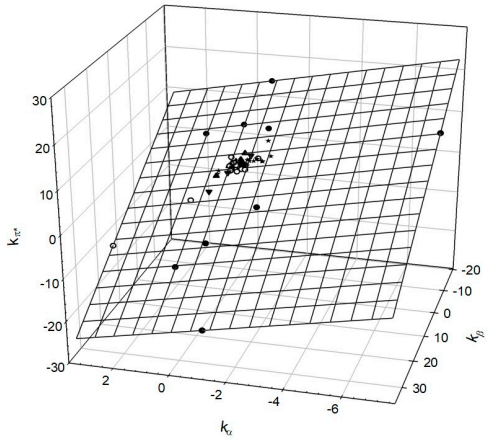

Figure 1. Linear interrelationship between the solvent properties of the solutes (data from [39]).

The above relationship seems to be generally applicable, and it confirms that the aqueous solutions of various compounds can be viewed as different solvents of the same aqueous nature. It also indicates that dipole–dipole interactions in water depend on hydrogen bonding. The permanent dipole moment of water is known to be 1.85 D in the gas phase, while it increases to 2.9 ± 0.6 D [55] in the liquid phase. This difference is generally attributed to polarization of water molecules due to the hydrogen bonding in liquid water. Theoretical consideration and discussion of this effect may be found in [53]. Hence, it seems reasonable to suggest that all three solvent features—π*, α, and β—in water depend on properties of hydrogen bonds, and that they already embody dispersion forces.

2. Solvent Features Characterize the Physicochemical Properties of Aqueous Solutions

The solubility of various compounds in aqueous solutions often depends on the nature and concentration of co-solutes exemplified by salting-out and salting-in effects induced by inorganic salts [46].

The solubility of different amino acids and their derivatives vary with the concentration of co-solutes glucose, sucrose, and other polyols [56] in the co-solute-specific manner [57]. Similar effects were observed in the presence of urea [58]. All the solubility data in the presence of different co-solutes have been found to correlate strongly with the solvent features, dipolarity/polarizability, π*, and hydrogen bond donor ability, α, of water in the corresponding co-solute solution [39].

Carbohydrates have been shown to exert a long-range influence on water solvation dynamics [59] and alter the dielectric properties of solutions [60].

Certain free amino acids, such as arginine and lysine, enhance solubility of various compounds [59,61,62]. The solubility of gluten and coumarin in arginine solutions [61], for instance, is strongly associated with the solvent dipolarity/polarizability, π*, and HBD acidity, α, of water in arginine solutions [63]. This contradicts the assumption [63] that the increased solubility of these two solutes is solely due to their direct interactions with arginine. Similarly, the solubility of a series of alkyl gallates in aqueous solutions of arginine and lysine has been shown [64] to be influenced by the differential effects of the two amino acids on the water's dipolarity/polarizability, π*. This finding contradicts the notion [39] that solubility enhancement occurs through direct interactions with or hydrophobic interactions with lysine.

The aqueous solubility of alanine, valine, leucine, and isoleucine decrease while those of phenylalanine and tryptophan increase in the presence of 3 to 7 wt.% PEG-20,000 in water [56]. Additionally, the solubility of sucrose in aqueous solutions of PEG-200 and PEG-400 decreases with increasing PEG concentration [65]. Conversely, highly lipophilic compounds generally exhibit increased solubility in water with a higher concentration of PEG-400 [58]. The addition of PEG commonly precipitates proteins from aqueous solutions, with PEG's precipitating efficiency escalating with the polymer's molecular weight [66].

The solubility of alkyl bromides in aqueous solutions of nonionic polymers increases linearly with increasing polymer concentration in solutions of PVP-24,000 and PEG-6000 [67]. Solubility of trimethoprim increases tenfold in 70% wt. solution of PVP-40,000 [40], while that of naproxen increases six-fold in the presence of 10% wt. PVP-25,000 [63].

It is well-established that certain physicochemical properties of aqueous solutions of various compounds in the presence of different salts are linearly related. As an example, the optical rotation of different enantiomeric amino acids and glucose in the presence of various sodium salts [68,69] are linearly related. The solubility of amino acids in aqueous solutions of urea [39] and sorbitol [40] are linearly related as well. The so-called Collander solvent regression relationship between logarithms of partition coefficients of compounds of the same chemical nature in different organic solvent–water biphasic systems [70] is well-known and is widely used [71].

It has been recently shown that specific physicochemical properties, such as the salting-out and salting-in constants for polar organic compounds in the presence of different inorganic salts [72] he same interrelationships may be observed between logarithms of distribution coefficients of drugs in octanol–buffer systems with buffers of various ionic compositions [73]. Logarithms of partition coefficients of different solutes in aqueous two-phase systems of various ionic compositions may be described by similar three-dimensional linear relationships [43].

These linear relationships may be described as follows:

$$Y_1 = k_o + k_1 Y_2 + k_2 Y_3, \quad (3)$$

where Y_1, Y_2, and Y_3 are solute properties in aqueous solutions in the presence of solutes 1, 2, and 3; and k_o, k_1, and k_2 are constants.

Analysis of water activity, osmotic coefficients, surface tension, viscosity, and relative permittivity of aqueous solutions of different individual compounds at the same concentrations has shown [43] that the above relationship is generally applicable. The observed [43] relationships typically hold for significant ranges of concentrations—up to 70 mass% in polymer solutions or up to 7 M in solutions of small compounds. Similar relationships are observed for all physicochemical properties. It is also valid for describing different physicochemical properties of the same compound.

Various types of water–water interactions probably define all the physicochemical properties of aqueous solutions; hence, these properties are interrelated.

All physicochemical properties of aqueous solutions of different compounds may be described via a linear combination of the solvent dipolarity/polarizability and hydrogen bond donor ability.

3. Solvent Properties of Aqueous Solutions Are Due to Rearrangement of Hydrogen Bonds

Attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR) is one of the most readily available experimental methods for the analysis of the rearrangement of H-bonds in aqueous solutions of various compounds [74–76].

Analysis of arrangement of H-bonds in aqueous solutions is based on inspecting the OH stretching band. This band may be viewed as composed of several components, represented by a set of Gaussian curves. Each Gaussian curve is assigned to water molecules existing in different H-bonded environments [74,75,77–79]. These assignments are based on the rather uncertain models of water structure. The Gaussian curves positioned at lower optical frequencies are generally assigned to water molecules forming strong, ice-like, hydrogen bonds, while those at higher frequencies are assigned to water molecules in an environment with weaker and/or distorted hydrogen bonds. Fitting the OH stretching band in water and all the aqueous solutions of various compounds with one to five Gaussian components showed [80] that the satisfactory fit was always obtained with exactly four components positioned at 3080 cm^{-1}, 3230 cm^{-1}, 3400 cm^{-1}, and 3550 cm^{-1} in agreement

with the data reported by Kitadai, et al. [76]. The optimal fit to experimentally observed OH stretching band in pure water and all solutions examined so far was obtained [80] with four Gaussian curves assigned as follows: (I) 3080 cm^{-1}—water molecules with four tetrahedrally arranged hydrogen bonds; (II) 3230 cm^{-1}—water molecules with four distorted hydrogen bonds; (III) 3400 cm^{-1}—water molecules with loosely arranged four and three hydrogen bonds; and (IV) 3500 cm^{-1}—water molecules with three, two, and single hydrogen bonds. This assignment is only a rough approximation of the complex hydrogen bond network existing in water [81] based on the internally consistent empirical measurements. The corresponding subpopulations or clusters of water with different properties may be distributed throughout, and the ratio of these subpopulations/clusters, existing in pure water, may vary in solutions of different solutes.

Analysis of the OH stretching band in aqueous solutions is performed by decomposition of the band into four Gaussian components and the estimation of the relative percentage area for each component as the function of the solute concentration. The area sum of all four Gaussian distributions is normalized to unity to better indicate their relative contributions, i.e., fractions of different water subpopulations.

The relative contributions of the four components estimated from analysis of ATR-FTIR spectra and the solvent features of aqueous solutions of inorganic salts, urea, trimethylamine N-oxide, and several nonionic polymers show that these features are strongly correlated with the relative fractions of certain solute specific water subpopulations. Each correlation for solutions of every examined compound j at the i^{th} concentration may be described in the general form as follows:

$$SF_{ji}(\pi^*_{ji}; \alpha_{ji}; \beta_{ji}) = k_{0j} + k_{mj}A_{ji}{}^{m} + k_{nj}A_{ji}{}^{n}, \quad (4)$$

where SF_{ji} is a solvent feature (dipolarity/polarizability, π^*, solvent HBD acidity, α, solvent HBA basicity, β); A_{ji} is the fraction of water subpopulation (I, II, III, or IV), denoted as I and n (I, II, III, or IV correspondingly); subscript I denotes the solute concentration; subscript j denotes the solute; and k_{0j}, k_{mj}, and k_{nj} are constants.

While the relative contributions of Gaussian components corresponding to the fractions of the water subpopulations I, II, III, and IV may not be totally independent, an analysis of the cross-terms in the covariance matrix suggests that this set of four appears to be both necessary and sufficient, and was demonstrated [80] to be superior to a fit with either three or five Gaussian components. The observed correlations described by Equation (4) imply that the particular water subpopulations or clusters cannot be localized within the hydration layer of a solute because solvatochromic probes are too big to fit in such a layer.

The only other rational explanation seems to be that the relative amounts of the water subpopulations/clusters in bulk water change in the presence of a solute. This explanation agrees with the fact that, typically, all three solvent features for solutions of a given solute may be described by Equation (3) with both fractions of two solute-specific water subpopulation $A_{ji}{}^{m}$ and $A_{ji}{}^{n}$, or with either $A_{ji}{}^{m}$ and $A_{ji}{}^{n}$. As an example, the solvent dipolarity/polarizability, π^*, in aqueous solutions of NaSCN may be described as follows:

$$\pi^*_I = 2.34_{\pm 0.03} - 2.62_{\pm 0.06}{}^{a}{}_{i}{}^{II},$$
$$N = 7;\ r^2 = 0.9976;\ SD = 0.004;\ F = 1973, \quad (5a)$$

the solvent acidity as follows:

$$\alpha_I = 25.1_{\pm 5.7} - 19.8_{\pm 5.2}A_i{}^{II} - 130.2_{\pm 29.1}A_i{}^{IV},$$
$$N = 7;\ r^2 = 0.9901;\ SD = 0.006;\ F = 199, \quad (5b)$$

and the solvent basicity as follows:

$$\beta_I = 0.4_{\pm 0.01} - 1.08_{\pm 0.08}A_i{}^{IV},$$
$$N = 7;\ r^2 = 0.9709;\ SD = 0.001;\ F = 167, \quad (5c)$$

where A_i^{II} is the fraction water subpopulation II (3230 cm^{-1}); and A_i^{VI} is the fraction water subpopulation IV (3550 cm^{-1}) in aqueous solutions of NaSCN at concentration I; all the other parameters are as defined above.

Qualitatively similar relationships were valid for aqueous solutions of all nine solutes examined [80].

By comparing the relationship of different components of the OH stretching band with previously reported solvent features of water, we have confirmed that such relationships may describe the origin of the solute effects on the solvent properties of water.

4. Conclusions and Future Directions

Analysis of solvent properties of aqueous solutions of small organic compounds, such as protective osmolytes, provides information leading to explanation of important biological phenomena. One example is the so-called volume exclusion effect, where it was recently established that the initial theoretical considerations cannot describe the increased protein stability or function change. There is no experimental evidence that has been suggested to explain the observed disagreements between the theory and experimental observations; multiple soft nonspecific interactions have not been shown to exist. The results of the solvatochromic analysis of the osmolyte solutions, however, provided experimental evidence that the explanation is in the changes in the solvent properties of water.

Future applications of the described approach may lead to improvement in our understanding of multiple biological processes, from the function of chaperones and protein folding to the mechanism of liquid–liquid phase separation involved in the formation of biological condensates and the development of new aqueous two-phase systems for better clinical testing of various diseases.

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