Hofmeister Series

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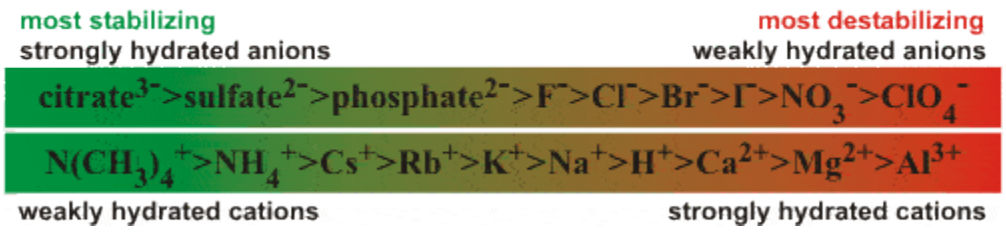
The effects of ions on biological and chemical processes in solution usually depend on the particular ions involved. These specific ion effects make up the Hofmeister phenomena [1827]. The Hofmeister series [85], below, originates from the ranking of various ions toward their ability to precipitate a mixture of hen egg white proteins.[a]

Anions: $SO_4^{2-} > HPO_4^{2-} > acetate^- > Cl^- > NO_3^-$

Cations: $Mg^{2+} > Li^+ > Na^+ = K^+ > NH_4^+$

Simplistically, this protein precipitation can be explained in terms of the extent of the ions binding to water (see also salting-out).[b] Thus the effective concentration of the proteins increases (in the remaining 'free' water) and they precipitate, so releasing low entropy surface water. The series has been shown to have a much more general utility with at least 38 observed phenomena (given in the comprehensive review [139], with the current state of play recently reviewed [671,1132]) including showing the graduated effects on the structuring or denaturation of biological macromolecules, effects on interfacial hydration [1302] and affecting pH measurements [481]. Nowadays the Hofmeister series are usually given in terms of the ability of the ions to stabilize the structure of proteins. A similar effect has been found with the salt-induced activation of lyophilized enzymes [204]. They show opposite correlations for anions and cations with their degree of strong hydration. The relative positions (mostly corresponding to the degree of strong hydration [250]) in the series should be thought of as indicative only as there will be variation with protein, pH and temperature, with acetate ions showing pronounced cation-specific effects. The relative order of cations may reverse with different anions (for example, NO_3^- rather than Cl^-) under some circumstances due to ion pair effects [431]. The relative order of anions may reverse dependent on the hydrophobicity/hydrophilicity and charge on the interacting surfaces; with $I^- > Cl^- > F^-$ on hydrophilic negatively charged and hydrophobic positively charged surfaces but $F^- > Cl^- > I^-$ on hydrophilic positively charged and hydrophobic negatively charged surfaces [1659]. Depending on the circumstances, NH_4^+ may occur out of order amongst the monovalent cations due to its hydrogen bonding capacity [1834]. Also, Li^+ often appears out of order as the water molecules within its first hydration shell are so tightly bound that its effective surface charge density is decreased by its hydration water [1834].



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Rationale

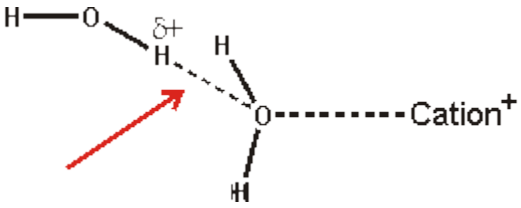
Although some data indicates that ions destroy the natural hydrogen bonded network of water, having effects similar to increased temperature or pressure [40] (for example, by lowering the pressure required to form high pressure ice VII[705]), other methodology indicates that ions cause negligible change to water's bulk structure [514, 1132]; these differences may be due to the ionic concentration used, the sensitivity of the methods to the scale of potential structural changes in the bulk water, the difficulty in separating coexisting but opposite effects (that is, chaotropic and kosmotropic, see later), the precise meaning of 'bulk' water, and the importance of the presence of surfaces in stabilizing effects [2091]. In particular, effects of salts at lower concentrations may be smothered (in many studies) by the relatively large amount of unaffected 'bulk' water present whereas at high concentrations there may be insufficient water to properly show any specific effects. Some techniques do pick up the more extensive clustering effects expected; for example, Fourier transform infrared studies have shown four well-defined hydration spheres around a proton with an additional outer hydration layer and further more loosely bound water molecules [1133].

The effect of ions has been successfully approximated by the equivalent osmotic pressure [224] and by the equivalent effect on water activity (4 molal NaCl is equivalent to 0.14 GPa [457]). Ions that have the greatest such effect (exhibiting weaker interactions with water than water itself) are known as structure-breakers or chaotropes, whereas ions having the opposite effect are known as structure-makers or kosmotropes (exhibiting strong interactions with water molecules). Strongly hydrated ions considerably increase the difference between the hydrogen bond donating and accepting capacity of the linked water molecules resulting in the breakdown of the tetrahedral network. Anions hydrate more strongly than cations for the same ionic radius as water hydrogen atoms can approach (about 0.8 Å) more closely than the water oxygen atoms (but note that most anions are larger than most cations), giving rise to greater electrostatic potential. Also, anions are far more polarizable than cations (compare Na^+ 0.12; K^+ 0.78; Cl^- 4.00 [742]) due to their more diffuse extra electron(s) and breaking hydrogen bonds round anions is relatively slow due to the difficulty in finding a new hydrogen-bonding partner [190]. Anions are also thought more likely to promote the salting-out of amphiphiles. Although we put forward the surface charge density as being the important determinant of Hofmeister effects (as does [1469], others state it is the polarizability that is important [1113]. However, a comprehensive study has shown the dominant role of charge density but no correlation of polarizability with thermal effects on either of an acidic or basic protein [1525].

Small ions are strongly hydrated, with small or negative entropies of hydration, creating local order and higher local density. Small cations do not bind directly to polar surfaces but small anions, which have lower surface charge density than the cations, may bind through ion pairing [1535]. Large singly charged ions such as I^-, SCN^- or Cs^+, with more positive entropies of hydration, act like hydrophobic molecules, binding to surfaces dependant not only on charge but also on van der Waals forces [521]. The large anions interact with the polypeptide backbone via a hybrid binding site that consists of the peptide nitrogen and the adjacent α-carbon [1914]. These amide backbone binding sites for weakly hydrated anions are the most significant locations for the salting-in of uncharged polypeptides. They may additionally be pushed on by strong water-water interactions and certainly induce a change in the surface hydration and interfacial aqueous clustering [1575]. Such large ions possess low surface charge density (*e.g.* for comparison, F^-, Cl^-, Br^- and I^- have surface charge densities of -8.98×10^{-21}, -4.42×10^{-21}, -3.64×10^{-21}, and -2.84×10^{-21} Coulombs/Å^2 respectively [139]) and are able to sit comfortably within dodecahedral water clathrate shells and produce the lowest apparent[c] density for the solution water [223]. Less large ions (for example, Rb^+, K^+, Br^-) cause the partial collapse of such clathrate structures through puckering. These ions allow rotations of the

water molecule dipole towards the oppositely charged ions, through weak interactions, that would be prevented at truly hydrophobic surfaces and hence produce greater localized water molecule mobility ('negative' hydration). Larger ions, such as the tetramethylammonium cation, form clathrate structures but do not allow these rotations in the surface water surface ('hydrophobic' hydration). The collapse, through puckering, of the water clathrate structures surrounding the smallest ions (for example, Na^+, Li^+, F^-) is tightly formed as these ions hold strongly to the first shell of their hydrating water molecules and hence there is less localized water molecule mobility [1358] (strong or 'positive' hydration) and higher apparent[c] density for the solution water [223]. There is also a less complete cluster structure, due to the hydrogen-bonding defects[d] caused by the inward-pointing primary hydrogen-bonding to anions or disoriented lone-pair electrons and electrostatic repulsion together with weakened hydrogen bonding reducing inward-pointing secondary hydrogen bond donation near cations or acceptance near anions. Generally, the water surrounding anions tends to retain favorable water-water hydrogen bonding whereas that surrounding small cations does not [741]. Higher charge density anions, such as sulfate, are exceptions to this generalization.

that is, these H-bonds possess reduced strength



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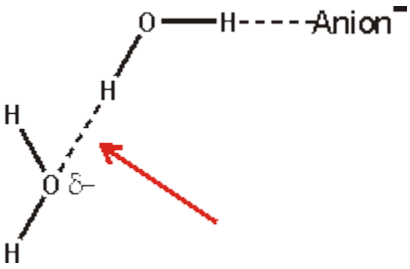

It can be noted that such strong hydration round both anions and cations costs the equivalent of two hydrogen bonds (~ 46 kJ mol^{-1}; that is, one replaced by the ion plus one chain of H-bonding with severely reduced strength) and is only found to form round ions where at least this enthalpic contribution is released [63]. [**Back to Top**]

Thermodynamic properties

Water cluster binding studies have shown that the ions possessing high charge density bind larger water clusters more strongly [306], see molar ionic volumes below. The entropies of hydration correlate with the tendency for the ion to accumulate in low-density water (LDW, for example, ES; see values below) such that a gain in entropy of the ion on solution is countered by a loss in entropy of the water [4]. Another correlation is with the Jones-Dole viscosity ***B*** coefficient [277] (see below).[e] Ions that are weakly hydrated exhibit a smaller change in viscosity with concentration, having negative ***B*** coefficients (chaotropes), than strongly hydrated ions that have positive ***B*** coefficients (kosmotropes). Such negative ***B*** coefficients are not shown by ions in any

other solvent, except D_2O where they are even more negative [304]. They lose this effect under pressure [299].

Similar effects are seen with the ionic surface tension increments, $k_i = d\gamma/dc_i$ (mN m^{-1} M^{-1}) (see table below [1981]), where more positive values indicate ions most repelled from the aqueous interface. Some exceptions exist where ions have ion-specific interfacial effects, such as the hydrogen ion.

Key properties of aqueous ions

Cations	Ionic volume[i]	ΔS[ii]	Jones-Dole[iii]	k_i[iv]	Anions	Ionic volume[i]	ΔS[ii]	Jones-Dole[iii]	k_i[iv]
Al^{3+}	-58.7	-396	+0.67	2.65	$Citrate^{3-}$	+77[v]	na	+0.27[vi]	na
Mg^{2+}	-32.2	-174	+0.385	2.25	SO_4^{2-}	+25	-126	+0.206	0.55
Ca^{2+}	-28.9	-132	+0.298	2.10	HPO_4^{2-}	na	na	+0.382	0.70
H^+	-5.5	na	+0.068	-1.05	F^-	+4.3	-70	+0.127	0.80
Na^+	-6.7	-5	+0.085	1.20	Cl^-	+23.3	+6	-0.005	0.90
K^+	+3.5	+34	-0.009	1.10	Br^-	+30.2	+28	-0.033	0.55
Rb^+	+8.6	+52	-0.033	0.95	I^-	+41.7	+55	-0.073	-0.05
Cs^+	+15.8	+59	-0.047	0.80	NO_3^-	+34.5	+9	-0.045	0.15
NH_4^+	+12.4	+5	-0.008	0.70	ClO_4^-	+49.6	+30	-0.061	-0.70
$N(CH_3)_4^+$	+84.1	na	+0.123	-0.10	Data from	[128]	[128]	[128]	[1981]

[i] Molar aqueous ionic volume, cm^3 mol^{-1}, 298.15 K; negative values indicates contraction in volume. [Back]
[ii] Entropy of hydration, kJ mol^{-1}, 298.15 K; standard molar entropy less the entropy of the primary hydrating water (that is, its immobilization). [Back]
[iii] Viscosity Jones-Dole *B*-coefficient,[e] dm^3 mol^{-1}, 298.15 K; results from the degree of water structuring by the ions. The accuracy and precision of this data may be overstated but the values are indicative. [Back]
[iv] Ionic surface tension increments, $k_i = d\gamma/dc_i$ (mN m^{-1} M^{-1}) over the range 0.1 M - 1.0 M , values from [1981]. [Back]
[v] Trisodium citrate value from [743] less Na^+ values from this table. [Back]
[vi] Calculated from [743]. [Back]
na not available

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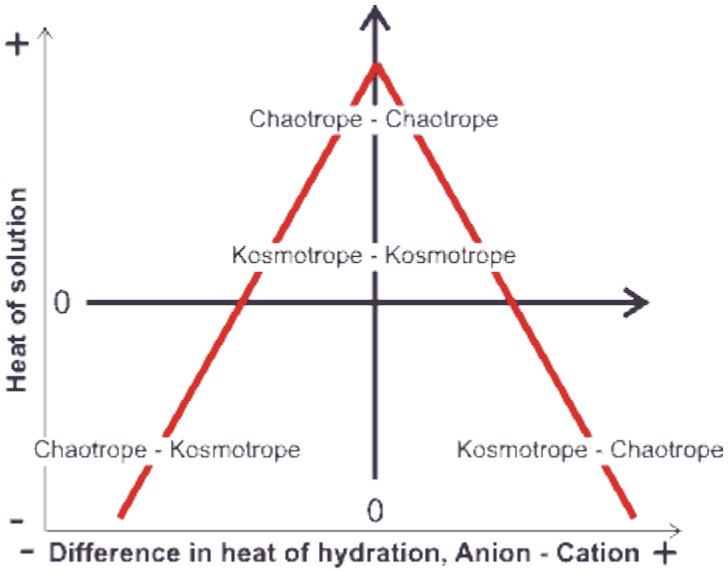
Effect on physical properties

As weakly hydrated ions accumulate in low-density (ES) water, ions can fit into the icosahedral network without affecting its hydrogen bonding and, therefore, with less effect on its viscosity as the concentration increases. Pressure shifts the ES⇌CS equilibrium towards CS formation, so reducing this viscosity effect. It is clear from the experimental data that although the weakly hydrated ions encourage ES formation the resultant structure is not more-strongly hydrogen bonded (as expected in supercooled water) or else the viscosity would be expected to be higher (larger ***A*** coefficients), which is not found [304]; the low viscosity being at least partially due to the lower density reducing non-bonded inter-molecular attractions. Similar correlations are seen with surface tension changes (more strongly hydrated ions increasing the surface tension more [671]) and dissolved gas concentrations (more strongly hydrated ions reducing the solubility more) [512]; both effects are due to the shift to CS formation. The increased van der Waals dispersion forces of large ions also increase the pull on the gas/liquid surface [513] and confuse the surface tension effects. As such dispersion forces correlate well with lower charge density for similarly charged ions, it may be difficult to separate these effects. A theory has been presented which takes into account both the ionic hydration and the polarizability to allow calculation of the surface tensions of various sodium salts [1663]. [**Back to Top**]

Effect on solubility

Arrangement of water around ions is not restricted to the first hydration layer (for example, magnesium ions [253, 378], see also the ion puckered clusters) except at high concentrations (the apparent number of hydrating water molecules and their rates of exchange depending not only on the ion but also the method of determination and the ionic concentration [1064]; a review is available [936]). Oppositely charged salt ions generally show two energy minima in their radial separation, one of which will be the global minimum. These involve close contact and solvent separated contact. The relative energy of these minima depends on the ions, their concentration, other solutes and the prevailing physical conditions. The type of ions present in solution control their overall properties; inner sphere ion pairs (that is, close contact) may be formed between two small ions of high charge density (for example, CaF_2), where the strong ionic attraction overcomes the hydration shells, or between two large ions of small charge density (for example, AgI; monovalent cations >1.06 Å, monovalent anions > 1.78 Å), where there are no strong hydration shells (i.e. when the ions have similar water affinities [667]).



An equivalent but alternative way of looking at this (see 'volcano plot' opposite) is that when the anion and cation have similar affinities for water they are able to remove the water from each other most easily, to become ion-paired [1190]. A small ion of high charge density plus a large counter-ion of low charge density forms a highly soluble, solvent-separated hydrated but clustered ion pair [407] as the large ion cannot break through its counter-ion's hydration shell (for example, CaI_2, AgF and LiI [1547]) but prefers to sit within the disturbed hydrogen bonding at intermediate distance between the ordered but poorly hydrogen bonded strongly-held first hydration shell and the more disordered but strongly hydrogen bonded bulk phase water; an effect that also enhances strongly-hydrating ion-hydrophobe interactions [522].

The tendency to form inner-sphere ion pairs between is reflected in the salt's solubility; the solubility of CaF_2, AgI, CaI_2 and AgF being 0.016, 0.0000016, 2090 and 1820 g/kg water respectively [63].[f] Such ion pair effects also cause partial raising (Cl^- > Br^- > I^-) of the homogeneous nucleation temperature of ice that has been lowered by increasingly hydrated (smaller) alkali cations (Cs^+ < Rb^+ < K^+ < Na^+ < Li^+) [341]. [**Back to Top**]

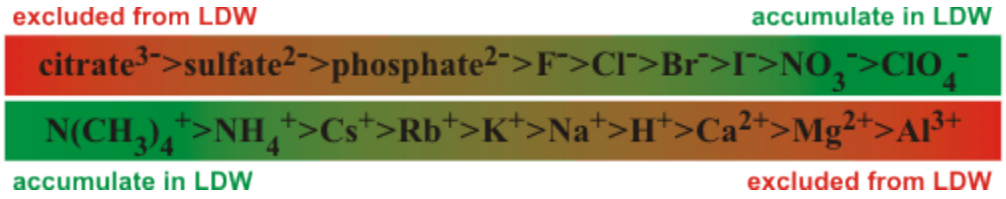
Stabilization of proteins

Proteins are most stable in solution when they are surrounded by fully hydrogen-bonded water molecules, as water molecules with spare hydrogen bonding capacity have higher entropy and are

more aggressive. Such reactive water behaves in a similar way to that on raising the temperature and is the cause of the denaturing behavior of proteins on heating. Salt ions must be evenly distributed in solution as their distribution is controlled by osmotic gradients. Thus thechaotropic ions (with their weak aqueous interactions) should be closer to the protein and the kosmotropic ions (with their strong aqueous interactions) in the bulk. Thus ammonium sulfate is often a good salt for stabilizing protein structure and bioactivity. [**Back to Top**]

Hydrophobic and hydrophilic associations

The Hofmeister series also shows the promotion of hydrophobic associations. The ions are in order of stabilizing structured low-density water that, in turn, stabilizes both the hydrophobic interactions of large molecules and the solubility of small hydrophobic molecules [1324]. Note that ionic chaotropes prefer low-density water, which is not required to break hydrogen bonds to accommodate them, but ionic kosmotropes are attracted to aqueous environments providing more-available hydration sites (CS like) and are excluded from low-density water (and hence also much of the protein's surface [1158]). Ionic chaotropes therefore tend to higher concentrations next to a protein's hydrophobic surfaces whereas ionic kosmotropes tend to higher concentrations near protein residues that break up the local water structure such as typical carboxylates.



Hydrophilic polymers in solution are surrounded by water with varying LDW content; for example, proteins may be surrounded by LDW out to about 15 Å from the surface [89], equivalent to the radius of a complete icosahedral water cluster. Generally this LDW acts to separate such molecules but this process is also dependent on the ions present. Ions that only weakly interact with water (ionic chaotropes) partition into LDW, so stabilizing it and structures that depend on it [4]. However, LDW is labile and may be abolished by solutes it accumulates due to the micro-osmotic gradients that may be fleetingly established. If both ions accumulate then micro-osmosis destroys the LDW and oscillations may occur. NH_4I and NH_4NO_3 destabilize LDW because both ions favor LDW. $MgSO_4$ destabilizes LDW because both ions favor unstructured normal-density water (followed by a tendency to form ion-pairs). $(NH_4)_2SO_4$ stabilizes LDW, as the ions are distributed evenly, and hence also stabilizes proteins and hydrophobic junction zones. This behavior has been shown to also enhance the activity of lyophilized enzymes for use in organic liquids [204], where the formation of enhanced clathrate structuring may encourage a more fluid surface environment given the restricted water content. Net LDW formers increase the viscosity of poly-electrolyte solutions due to the increased LDW created between the polymers whereas netLDW destroyers (for example, NaCl, $MgCl_2$) reduce this viscosity. It should be noted that strongly hydrated salts alone, without polymer, have the opposite effect and increase viscosity, due to their tightly bound high-density ordered water.

Ions distribute in aqueous two-phase systems according to their affinity for the more-LDW phase. Thus iodide ions prefer the polyethylene glycol (PEG) phase so producing a charged interface in PEG/dextran systems. [**Back to Top**]

Footnotes

[a] Note (i) that the ability to precipitate protein(s) depends on the protein(s) present, (ii) that some proteins exhibit opposite Hofmeister series behavior to others (which may be due to the protein's net charge [812] and the ionic strength of the solution [1606]; ovalbumin being negatively charged in the key experiments) and (iii) the order of some of the ions may be reversed in some series depending on the application, the counter ions used, the pH and any ion-specific factors present. Always note the application, conditions and method of comparison when examining published 'Hofmeister series'. Experiments into Hofmeister (ion-specific) effects have been criticized in that they often neglect the (possibly) confounding effects of the concentration of the ions (including counter-ions) present, the presence of other ions such as buffers, pH and the temperature [1463]. Clearly such effects must be taken into account if the relative effectiveness of the ions in any process is to be compared. The complete original series [85], given below in terms of molar effect, used a mixture of egg white proteins and did not control for pH changes.

Anions: $citrate^{3-} > SO_4^{2-} = tartrate^{2-} > HPO_4^{2-} > CrO_4^{2-} > acetate^{-} > HCO_3^{-} > Cl^{-} > NO_3^{-} > ClO_3^{-}$

Cations: $Mg^{2+} > Li^{+} > Na^{+} = K^{+} > NH_4^{+}$

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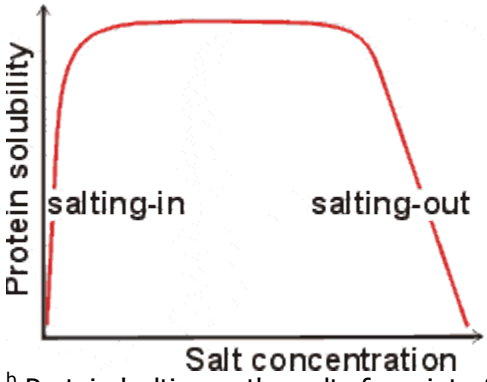


[b] Protein 'salting out' results from interfacial effects of strongly hydrated anions near the protein surface so removing water molecules from protein solvation and dehydrating the surface. The greatest effect is due to the most strongly hydrated anions. Protein 'salting in' (solubility increase on the addition of low levels of salt) results from protein-counter ion binding and the consequent higher net protein charge and solvation [667]. Salting-in occurs where the protein has little net charge near its isoelectric point [1554]. In this case the greatest effect is due to the most weakly hydrated anions.

Salting-in and salting-out processes are reviewed [926]. Salting out follows the Hofmeister series above the isoelectric point for a number of proteins including lysozyme, but follows the reverse order below the isoelectric point [1535]

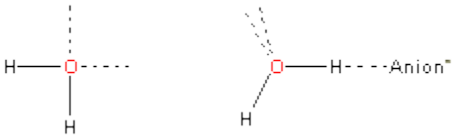
Many 'insoluble' proteins can be dissolved in pure water so long as the pH is away from the isoelectric point [1554]. Here the charged protein molecules repel each other, with hydrophobic areas covered with an organised low density water network so avoiding protein-protein interactions. In these cases, addition of ions reduce the repulsions and water organisation, so resulting in protein precipitation [1554]. [Back]

[c] The apparent density for the solution water is the weight of the water in the solution (that is, the

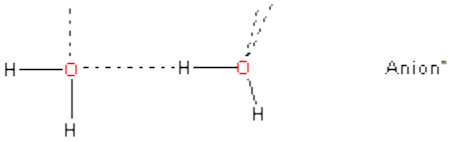
weight of the solution less the weight of the salt) divided by the volume of the water in the solution (that is, the volume of the solution less the volume of the salt). The conclusion is irrespective of whether the 'dead space' around the ions is included (as in [224]) or not (as in [223]). [Back, 2]

[d] Cluster defects are water molecules, (labeled b-type previously) within the icosahedral cluster structure, with only 3 rather than 4 hydrogen bonds as the fourth site cannot accept/donate a hydrogen bond from/to either (a) a water molecule already possessing 4 hydrogen bonds;

that is, the fourth site has the arrangement



rather than



or (b) a bound atom that already possesses 3 hydrogen bonds such as the oxygen atoms in SO_4^{2-}. [Back]

[e] The viscosity (η) of an aqueous salt solution (up to about 0.1 M), relative to the viscosity of water at the same temperature (η_0), varies with the salt concentration (c) according to the Jones-Dole expression:

$$\eta/\eta_0 = 1 + \boldsymbol{A}c^{0.5} + \boldsymbol{B}c$$

A is always positive and is greater for strongly hydrated ions (except H^+ and OH^-). It is associated with counter ion screening and only appreciably affects the viscosity at low concentrations (< 0.05 M) [304]. The ***B*** coefficient results from the degree of water structuring by the ions relative to bulk water interactions; also understood as the relative strength of the ion water interactions and varying linearly with the solute-water distance for monovalent electrolytes [1764]. Ions with negative ***B*** coefficients have appreciably more negative ***B*** coefficients in D_2O but those with positive ***B*** coefficients show little change in D_2O [304]. At higher concentrations (that is, above about 0.5 M), a further term ($\boldsymbol{D}c^2$), or terms, may be added to the right hand side as the viscosity increases more rapidly with concentration. ***A***, ***B*** and ***D*** all depend on temperature; ***B*** particularly so with ***B*** generally increasing with increasing temperature for the ionic chaotropes and reducing with increasing temperature for the ionic kosmotropes [1165]. Salts where both *Jones-Dole* ***B*** coefficients are negative show a reduction in viscosity with increasing concentrations at higher concentrations; for example, KI solutions at 20 °C show reductions in viscosity with increasing concentrations above 2 mM reaching a minimum relative viscosity of ~0.888 at ~2.7 M [70]. When used with non-electrolytes both the ***A*** and ***D*** coefficients are often neglected and ***B*** depends on the shape and solvated molar volume. [Back]

[f] An equivalent but alternative viewpoint is that salts that are exclusively kosmotropic or chaotropic (that is, kosmotropiccation with kosmotropic anion or chaotropic cation with chaotropic anion [277]) are less soluble than salts formed from ions with differing properties (for example, CsI (1.7 M, 0 °C) and LiF (0.1 M, 18 °C) are both less soluble than CsF (24 M, 18 °C) despite Li^+ and F^- ions generally hydrating most strongly) [276], due to the energy cost of hydration shifting the ES⇌ CS equilibrium. [Back]

Source:http://www1.lsbu.ac.uk/water/hofmeist.html