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Explanation of Ionic Sequences in Various Phenomena. VIII. The Structure of Aqueous Urea and DMSO and Their Mechanism as Dispersing Agents

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SUMMARY

Many recent experiments have shown that urea behaves as a zwitterion. By measuring the solubility of urea in various aqueous salt solutions and by obtaining the effect of salts on the dispersing power of urea, the structure of urea and its mechanism for destroying hydrogen and hydrophobic bonds were elucidated. The zwitterion structure of urea was confirmed by means of these ionic sequence studies. The K^+ ion increases the dispersion power of urea and decreases its solubility more than any of the other monovalent cations because the K^+ ion forms a more insoluble salt bond with the negatively charged oxygen atom of the urea zwitterion. This salt bond is more insoluble than the urea-urea bond. Hence, more positively charged $-NH_2$ groups on urea are liberated by the addition of KCl. The dispersion power of the urea molecule is the result of the interaction of the negatively hydrated domain (B regions) surrounding these $-NH_2$ groups with the oxygen atoms involved in the hydrogen bonds between starch molecules. These B regions on

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urea $-NH_2$ groups are more effective in destroying hydrogen bonds than normal water molecules. That is, the anionic sequences and the solubility of benzene in aqueous urea solutions show that the hydrated $-NH_2$ groups of urea have an effective dielectric constant that is greater than that of water and is approximately equal to that of the hydrated guanidinium ion. However, the urea-urea salt complexes or polymers lower the effectiveness of urea at high urea concentrations. The charge density of the urea anion is between that of the Cl^- and F^- anions and consequently does not contribute to the dispersing power of urea. By destroying water clusters, the $-NH_2$ groups of urea increase the domain which hydrocarbons can occupy and hence destroy hydrophobic bonds. The destruction of hydrogen and hydrophobic bonds by urea therefore involves the electrostatic interactions between the urea B regions and the surrounding hydrogen bonds. It is also shown that DMSO behaves as a stronger zwitterion than urea and that it has the properties of a small anionic detergent. Anhydrous DMSO dissolves polar polymers and molecules by complexing its strongly ionized oxygen atom with the polar group. Urea and formamide form aggregates with themselves and therefore are incapable of dispersing polymers in this manner.

INTRODUCTION

Aqueous urea solutions are frequently used to dissolve, denature, or bring about reversible structural changes in many hydrophobic or hydrophilic polymers. Yet the structure of urea and the reason why it is able to bring about such manifestations is still unknown to scientists. According to Franks and Clarke [1], the general view held today is that urea lowers the tendency of nonpolar groups to form hydrophobic bonds by some modification of the structural properties of water. The evidence presented by them shows that qualitatively the urea must reduce the structuredness of water. Thus the conclusion given by Abu-Hamdiyyah [2] that urea increases the degree of hydrogen bonding in water is incompatible with the observed data [1]. Consequently, urea, just as guanidinium chloride, destroys hydrogen bonds between water molecules (or, if present, between polymer units) and by doing so increases the solubility of hydrophobic groups.

If we now apply this conclusion to the solubility of hydrocarbons in water as a function of temperature, apparently contradictory results are obtained unless the previously proposed model for water is adopted [3]. Thus the solubility of hydrocarbons such as methane, ethane, benzene, etc., decreases with an increase in temperature in going from 0 to 20°C [3]. But an increase in temperature should destroy hydrogen bonds just as the addition of urea to water at constant temperature destroys hydrogen bonds. However, in one case (addition of urea) the solubility of the hydrocarbon increases and in the other (increase in temperature) it decreases. Yet hydrogen bonds between water molecules are destroyed in both instances. With regard to the solubility of hydrocarbons in pure water, the problem is further complicated by the fact that the aqueous solubility of such compounds as benzene [3], 3-methylpyridine [4], and polyethylene oxide [5] go through minimums as the temperature is increased. That is, their solubility in water at first decreases with an increase in temperature but then at higher temperatures increases with an increase in temperature.

The minimum in the solubility of hydrocarbons in water can be explained by applying the previously proposed aggregate-cluster theory [3]. That is, at high temperatures (above approximately 60°C) only clusters of water plus unbound water exist. As the temperature is lowered these clusters of water molecules begin to aggregate. Finally, at about 20°C and lower, because of the lack of unbound water molecules, the main mechanism for the formation of hydrogen bonds is the aggregation of water clusters. Thus the solubility of a hydrocarbon decreases as the temperature is increased from 0 to 20°C because now an increase in temperature destroys the cluster aggregates and hence decreases the available space that a hydrocarbon can occupy. At higher temperatures an increase in the solubility of the hydrocarbon results as the temperature is increased, because now destruction of hydrogen bonds produces more available space by converting clusters to unbounded water molecules.

How then does urea increase the solubility of hydrocarbons? Previous results [6, 7] show that because of its large effective dielectric constant, the urea molecule can destroy the hydrogen bonds in a water cluster and hence can increase the amount of available space for the hydrocarbon. Hence the increase in the critical micelle concentration (c.m.c.) of detergent anions or the increase in the solubility of hydrocarbons with an increase in urea concentration is not due to the destruction of ordered "iceberg structures" around the detergent anions as claimed by Malik and Jain [8]. If it were, the c.m.c. and the solubility of hydrocarbons would also increase with an increase in temperature. And as we have seen, below 50 to 60°C the reverse of this is true. Rather, the increase in c.m.c. and hydrocarbon solubility with an increase in urea concentration is due to an increase in the number of unbound water molecules (an increase in free or available space for the hydrocarbon to dissolve in) because of a decrease in the number of the quite stable water clusters.

Two facets dealing with this ability of urea to dissolve hydrocarbons and destroy hydrogen bonds will be considered in this paper: (1) the structure of the urea molecule, and (2) the interaction of urea with itself and with hydrated anions and cations. It was previously concluded [6] that urea behaves as a zwitterion where the value of D_+ (effective dielectric constant) of the nitrogen end of urea is between that of the Cs^+ and guanidinium (G^+) ions and where the value of D_- for the oxygen end of urea is less than that of water. Further proof of this zwitterion model will be given here. Explanations of D_+ and D_- are given in previous papers [6, 7]. Therefore, it might suffice to say that these values were obtained from a correlation of the solubility of benzene in aqueous salt solutions with theoretically developed equations. If D_+ is greater than that of water (D_1), hydrogen bonds are destroyed, whereas if it less, these bonds remain stable in the aqueous salt solution except in certain cases, e.g. 9 M LiCl.

It should also be noted that Colacicco [9] had previously proposed the zwitterion model for urea by using other evidence. But it should be emphasized that the "zwitterion" proposed by Colacicco [9] was rather arbitrary and inexplicit in meaning. In this paper the term "zwitterion" means an ionic model such as found in amino acids where there is a definite positive and negative electrostatic charge. And it will be shown that the positive charge on urea (which can be designated as the "urea cation") has a charge density equivalent to that of the guanidinium ion, and the negative charge (or the "urea anion") has a charge density equivalent to that between the F^- and Cl^- ion. Consequently, the urea molecule behaves as if the G^+ and F^- (or Cl^-) ions had been tied or attached together without cancelation of their charges, just as in the case of molecules such as amino acids where the charge groups are separated by one or more neutral atoms. In a sense, the urea charges are also separated by neutral atoms. That is, the positive charge may be essentially distributed to the hydrogen atoms of the two $-NH_2$ groups, leaving the nitrogen less charged. Also the carbon atom of urea may be neutral. Hence the electron distribution on urea may be such that the positive and negative charges are separated by a distance of one or two atoms.

But if these charges exist, urea should form salt bonds. That such bonds are formed with urea has been shown by Saito et al. [10] and others cited by them. Their studies have shown that urea forms stoichiometric compounds with alkaline salts, alkaline earth salts, tetraalkylammonium halides, and many other salts of metallic elements. In other words, these stoichiometric complexes illustrate that urea behaves as a zwitterion as in the case of amino acids.

This conclusion is emphasized by the recent results of Radell et al. [11]. They observed that dicarboxylic acids form stoichiometric complexes with urea rather than urea inclusion compounds. Thus when the pK of the carboxylate group is sufficiently low, a 1:1 complex is formed with each carboxylate group except in the case of malonic acid, where a stable six-membered malonic acid ring occurs. Their results confirm the infrared results and conclusions of Kutzelnigg and Mecke [12] who state that urea in these complexes exists as $[(H_2N)C(OH)NH_2]^+$ rather than $(H_2N)CONH_3^+$. Thus if urea were a neutral molecule, the $-NH_2$ group and not the $\rangle C{=}O$ group of urea would bind to the H^+ ion obtained from the carboxylic acid. But as a zwitterion, the positively charged $-NH_2$ group would repel the H^+ ion and the negatively charged oxygen atom would attract it—which it does. Consequently, this work again adds proof that urea behaves as a zwitterion and not as a neutral or polar molecule.

Further proof that urea behaves as a zwitterion is seen from the observation that addition of salt markedly increases the conductivity of saturated urea solutions [13] or increases the dispersing power of urea solutions [14]. This synergistic effect of added salt suggests that urea-urea salt complexes exist in aqueous solutions between urea zwitterions and that other ions can compete for or reduce these ion-ion interactions. Also the fact that the urea concentration must be approximately 4 M to be effective as a dispersing agent [14, 15] shows that urea behaves as a normal hydrated salt which has an effective dielectric constant greater than that of water [14]. Further evidence to support the urea zwitterion structure will now be given.

EXPERIMENTAL

A sample of high-amylose hybrid corn starch granules containing an apparent amylose content of 70% was isolated as described previously [14, 16]. That is, the protein on the isolated starch granules was removed by shaking the starch granules in a Pentasol*-water mixture containing 20% Pentasol, according to the method of Maywald et al. [17]. The starch granules were then washed several times with ethanol to remove the amyl alcohols and was then air dried on a glass plate. The salts used were the best grade that could be purchased. They were obtained mainly from K & K Laboratories, Inc., and a few from other companies cited previously [14]. The amount of moisture in each sample was obtained from the refractive index of the aqueous salt solution according to the previously [14] developed equation.

The dispersing power of the various aqueous salt and urea solutions was determined as previously described [14]. In general, the method involves placing enough starch in a Virtis homogenizer to produce a 1% solution in the final volume assuming that all the starch will dissolve. Five to ten times as much water as starch granules was added to the Virtis bowl and the aqueous mixture was

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*Pentasol is a mixture of amyl alcohols. Mention of firm names or trade products does not imply that they are endorsed or recommended over other firms or over similar products not mentioned.

ground for about 5 min to remove any water solubles from the starch and to wet the starch thoroughly. If the starch is not initially wet, the solvent cannot penetrate sufficiently into the starch sample before gelatinization of the outer surface occurs and lumps result. This water-starch slurry was sedimented in an International centrifuge bottle to remove excess water. The solvent was then added to the centrifuged pad and the mixture was transferred quantitatively to an Erlenmeyer flask. After the sample in the stoppered flask had been swirled on a shaker for 24 h, the concentration of starch in the final solution was obtained by centrifuging the mixture for 15 min at 18000 rpm (39,000 × g) in a Sorvall centrifuge and by determining the optical rotation of the resulting starch solution assuming a specific rotation $[\alpha]_D^{25^\circ C} = 198.8$ for all solvents.

The maximum solubility of urea in water and in aqueous salt solutions was determined by recording the amount of salt and urea added to the water and determining the final volume of the saturated urea solution. Also, in obtaining the 8 M urea plus 1 or 2 M salt solutions, enough urea and salt were added so that in the final volume the correct molarity was obtained. In preparing the saturated urea solutions, the salt was added first and then the urea was added until a small amount of urea remained undissolved after vigorous shaking on a mechanical shaker. This solution with a small amount of excess urea was maintained overnight (about 12 h) to ensure that no more urea could dissolve. Sufficient water (approximately 0.2 to 0.5 ml) was added to the 30 or 40 ml of saturated urea solution to dissolve the remaining insoluble urea. The final molarity of the salt and urea was then calculated from the amount of urea and salt added and from the final volume. In all cases the urea was more insoluble than the added salt. Thus addition of a small amount of the particular salt to the final solution did not produce a precipitate. Only two salts, KCl and GCl (guanidinium chloride), decrease the solubility of urea with an increase in the concentration of salt. All the others tested salt-in urea.

RESULTS

Results reported previously (Fig. 4 of [14]) showed that the solubilization of high-amylose starch increased linearly from about 7% in 2 M LiSCN to about 30% in 2 M LiSCN + 6 M urea. Yet the same studies showed that 6 M urea dispersed this starch by only about 3%. If the two reagents were additive in their dispersing abilities, then instead of solubilizing the starch by 30%, the amount of starch solubilized should only be 15% if one considers dehydration effects of the urea. This dramatic increase or synergistic effect suggested [14] that the LiSCN was interacting with the urea. The interaction somehow caused an increase in the dispersing power of the urea.

To further verify and examine this synergistic effect, the solubilization of high-amylose starch was studied in various aqueous salt and urea solutions. The results are reported in Table 1. As shown previously [14], the solubility of this starch in urea reaches

Table 1. Solubility of High-Amylose (70%) Corn Starch Granules in Various Aqueous Solvents[a]

Solvents	Solubility, %	Solvent	Solubility, %
8 M urea + 3 M KCl	7.8	8 M urea	2.5
8 M urea + 2 M KCl	5.9	6 M KCl	0
8 M urea + 2 M GCl	4.5	8 M urea + M $BaCl_2$	8.1
8 M urea + 2 M NaCl	3.3	8 M urea + M $SrCl_2$	3.1
8 M urea + 2 M CsCl	2.5	8 M urea + M $CaCl_2$	0
8 M urea + 2 M LiCl	0	8 M urea + M $MgCl_2$	0
8 M urea + 2 M LiBr	2.0		

[a] Solubility is expressed in grams per 100 ml (per cent) and was obtained as described under Experimental.

a maximum or plateau at approximately 4 M urea. Hence the 2.5% value for 8 M urea substantiates the earlier results. Table 1 also shows that 6 M KCl (almost saturation) does not dissolve any starch (0% solubility). This result is to be expected since the value of D_+ and D_- for the K^+ and Cl^- ions is less than that of water. However, the synergestic effect noted earlier [14] is substantiated in the solvent 8 M urea + 2 M KCl. The effect must be entirely due to interactions of KCl with urea because, as noted, KCl cannot dissolve starch because of its low value of D_+ and D_-. Moreover, when an additional amount of the nondispersing salt KCl is added to give a solvent of 8 M urea + 3 M KCl, the dispersing power is further increased to 7.8%, as shown in Table 1. In addition, when one examines a salt such as CsCl, where $D_+ > D_1$ (and hence the Cs^+ ion is capable of dissolving the starch), the synergistic effect does not occur because the solubility remains at 2.5% for the 8 M urea + 2 M CsCl solvent as in the case of 8 M urea. Also, the value of D_+ for Ba^{2+} is less than that of water (D_1) whereas $D_+ > D_1$ for the Mg^{2+} ion [7]. Thus the Mg^{2+} ion is capable of dissolving starch but the Ba^{2+} ion is not. Yet the solubility of high-amylose starch is increased from 2.5% in 8 M urea to 8.1% in 8 M urea + M $BaCl_2$, whereas it is decreased to 0% in 8 M urea + M $MgCl_2$. The data in Table 1 therefore clearly show that the addition of salt to urea solutions alters the dispersing ability of the urea molecule.

To study this effect further, the interaction of urea with various salts was examined by determining the maximum solubility of urea in the presence of different salts as outlined under Experimental. The results are recorded in Table 2. These values become clearer when the molarity of the saturated urea is plotted versus the molarity of the added salt as shown in Figs. 1, 2, and 3. In Fig. 1 it is seen that at all concentrations the ability of the salt to salt out urea increases according to the sequence: LiCl $<$ CsCl $<$ NaCl $<$ KCl. However, the solubility of urea in GCl decreases dramatically with an increase in concentration of GCl.

Table 2. Maximum Solubility of Urea in Monovalent and in Divalent Aqueous Salt Solutions[a]

Salt	M of salt +	M of urea	M of salt +	M of urea
NaCl	1.56	10.41	2.04	10.46
KCl	1.61	9.90	2.13	9.83
LiCl	1.34	11.11	2.08	11.45
CsCl	0.10	10.40	0.48	10.48
GCl	1.56	10.15	2.15	9.75
LiBr	1.00	10.85	1.53	11.42
LiBr	2.31	12.17	—	—
LiSCN	1.65	11.34	2.10	11.99
$MgCl_2$	0.90	11.59	1.03	11.95
$CaCl_2$	0.79	11.40	1.02	11.68
$SrCl_2$	1.05	11.61	0.92	11.27
$BaCl_2$	0.70	11.02	1.33	12.22
No salt	—	10.5	—	—

[a] Concentrations (M) are in moles per liter. Solubility studies were carried out at 25°C. The data given here are plotted in Figs. 1-3.

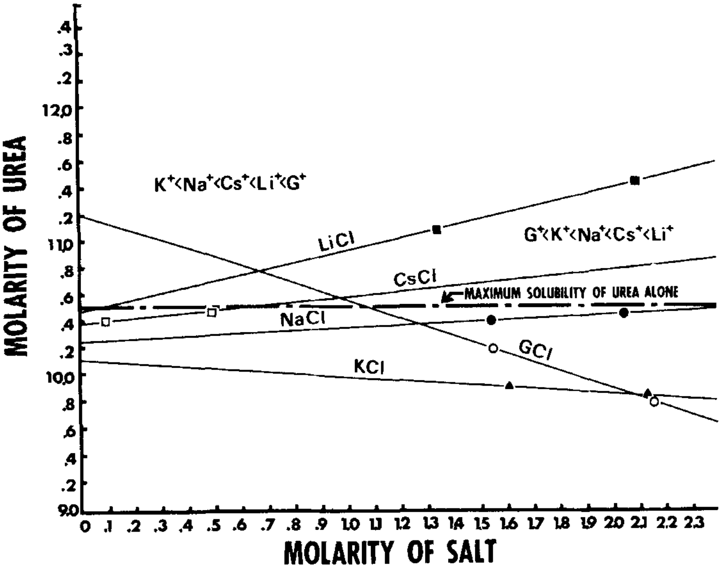

Fig. 1. Maximum solubility of urea in water in the presence of monovalent cations with a common anion (Cl$^-$). Molarity (moles per liter) of urea is plotted versus molarity of salt. The salts are represented by the points: ▲, KCl; ●, NaCl; □ CsCl; ■ LiCl; and ○, GCl.

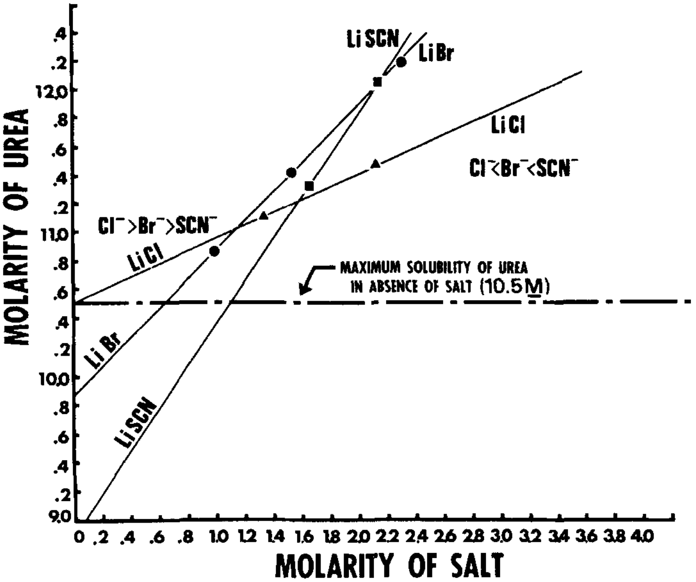

Fig. 2. Maximum solubility of urea in water in the presence of monovalent anions with a common cation (Li$^+$) in moles per liter. The points are ▲, LiCl; ●, LiBr; and ■, LiSCN.

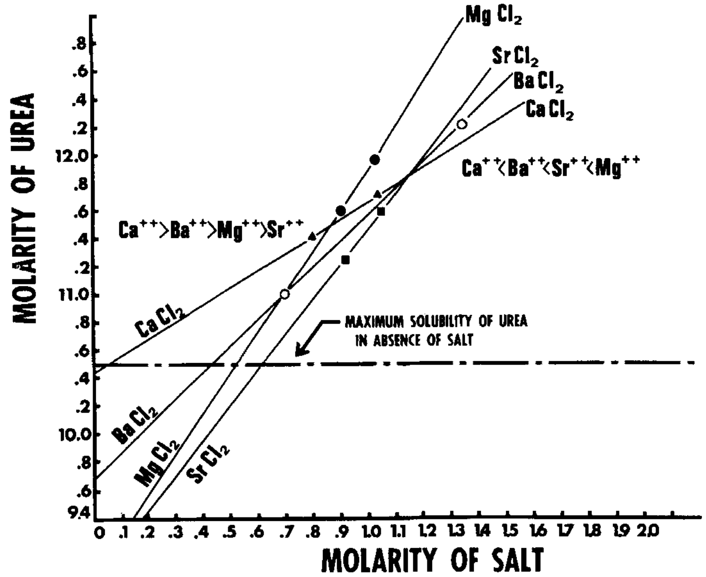

Fig. 3. Maximum solubility of urea in water in the presence of divalent cations with a common anion (Cl^-), in moles per liter. The points are ▲, $CaCl_2$; ○, $BaCl_2$; ●, $MgCl_2$; and ■, $SrCl_2$.

With regard to the effect of the anion, it is seen in Fig. 2 that the effect of the anions on the solubility of urea completely reverses itself in going from lower (< 1.1 M salt) to higher (> 2.1 M salt) salt concentrations. A similar reversal occurs for the divalent cations, as shown in Fig. 3. It should also be noted that some salts salt in urea, whereas others such as NaCl, KCl, and GCl (at high concentrations) in the present study salt out urea.

The three points obtained for LiBr-urea samples (Fig. 2) illustrate that these lines are linear. Theoretically one would expect that by extrapolating to zero concentration of salt, the solubility of urea should be the same in all cases and all lines should meet at a common point. However, the results indicate that any such convergence must occur below 0.1 M salt (see the CsCl results of Fig. 1). Because of the limited number of points presented in this study, no definite conclusions can be made as to when such a convergence occurs. The points obtained in this study show, however, that the various cations and anions effect the solubility of urea to different degrees. Moreover, the position of the experimental points in Fig. 1-3 leaves no doubt that these effects may reverse themselves for some salts as the concentration of the particular salt is increased. The results on LiBr suggest that the effect of the salt is a linear function of the salt concentration in the range of concentrations studied. However, the primary purpose of the figures is to show that different cations or anions do not have the same effect on the urea solubility. Consequently, even if some of these functions may not be exactly linear, the comparisons are still valid and therefore conclusions can be drawn from the resulting ionic sequences.

DISCUSSION

Proof of the Existence of Urea as a Zwitterion

To analyze why the addition of salt increases the dispersing ability of urea, one must also study why the same salt decreases the solubility of urea. The two phenomena are related to one another because, as seen in Table 1 and Fig. 1, the ability of a salt to increase the dispersion power of urea goes according to the sequence KCl > NaCl > CsCl > LiCl and the ability of a salt to decrease the solubility of urea is KCl > NaCl > CsCl > LiCl. The position of the Cs^+ ion in these two identical sequences shows that these sequences are "basic" according to the classification given previously [18, 19]. That is, if urea is a nonionic molecule, it would behave as a basic-type molecule such as NH_3 or trimethylamine [18]. If urea is an ionic zwitterion, the basic salting-out sequence shows that the negative oxygen atom behaves as if $D_- < D_1$ and as if no A region is present, such as in the case of Cl^- [19, 20]. Also, Table 3 shows that a different type of sequence (an "acidic" sequence [19]) is obtained with regard to the ability to destroy intermolecular hydrogen bonds in starch granules or retrograded starch. Consequently, the basic sequence obtained from Table 1 cannot be due to the interaction of these salts with starch but must be due to the salt's interaction with urea.

It is important to realize that the basic salting-out sequence $K^+ > Na^+ > Li^+$, if applied to a nonionic or polar urea molecule, means that the K^+ ion is the most effective in salting out the basic $-NH_2$ groups on urea. On the other hand, when this basic sequence is applied to a urea zwitterion, the sequence means that the K^+ ion is the most effective in salting out the negatively charged oxygen atom of urea. But as seen in Table 1, the K^+ ion increases the dispersing power of urea. Consequently, the K^+ cannot be salting out any nonionic $-NH_2$ group on urea. If it were, the K^+ ion would decrease, not increase, the dispersing power of urea. In other words, if it salted out the $-NH_2$ group, the K^+ ion would inhibit, not increase the dispersing power of urea. Therefore, the cationic sequence for the dispersing power of urea when combined with that for the salting-out sequence of urea shows that urea must be a zwitterion. Thus in

Table 3. Ionic Sequences for the Salting Out of Urea and Solubilization or Retrogradation of Starch[a]

	Anion	Monovalent cation	Divalent cation
Urea (at 2.1 M salt)	$Cl^- > Br^- > SCN^-$	$G^+ > K^+ > Na^+ > Cs^+ > Li^+$	$Ca^{2+} > Ba^{2+} > Sr^{2+} > Mg^{2+}$
Urea (at 0.5 M salt)	$Cl^- < Br^- < SCN^-$	$K^+ > Na^+ > Cs^+ > Li^+ > G^+$	$Ca^{2+} < Ba^{2+} < Mg^{2+} < Sr^{2+}$
Starch (ability to retrograde)[b]	$Cl^- > Br^- > I^- > SCN^-$	$Li^+ > Na^+ > K^+$	
Starch (ability to solubilize)[b]	$Cl^- < Br^- < SCN^-$	$Li^+ < K^+ < G^+$	
Starch + 8 M urea[c] (ability to solubilize)	$Cl^- < Br^-$	$K^+ > G^+ > Na^+ > Cs^+ > Li^+$	$Ca^{2+}, Mg^{2+} < Sr^{2+} < Ba^{2+}$

[a] The salting-out sequences for urea are the reverse of its solubility sequences. Hence the salting-out sequence for urea at low salt concentration ($Cl^- < Br^- < SCN^-$) corresponds to the solubility sequence of a cation such as G^+ or Cs^+ having $D_+ \geqslant D_1$ or $Cl^- > Br^- > I^-$ in solubility.

[b] Data on starch retrogradation were obtained from Morsi and Sterling [21] and data on starch solubilization were obtained from Erlander and Tobin [14].

[c] Sequences for starch in 8 M urea were obtained from Table 2.

saturated urea solutions in the presence of KCl the K^+ ions forms an insoluble salt complex with the negatively charged oxygen atom. Consequently, the monovalent cationic sequences of Table 1 and Fig. 1 verify the results and conclusions of those scientists referred to in the Introduction; that is, urea is a zwitterion in aqueous solutions.

Existence of Urea-Urea Salt Bonds and the Salting In of Urea

The results given in Fig. 1 show another important aspect of the urea molecule. It is clearly seen that the K^+ ion salts out whereas the Li^+ ion salts in the urea molecule. That is, the Li^+ ion increases the maximum solubility (10.5 M) of urea, whereas the K^+ ion decreases it. Presumably the Na^+ ion would also salt in urea if the concentration of NaCl were great enough. These salting-in and salting-out effects illustrates that the urea solubility is governed by ion-ion interactions between urea molecules. That is, the positively charged $—NH_2^+$ groups of one urea zwitterion and the negatively charged oxygen atom $\rangle C—O^-$ of another interact to form an insoluble salt complex. The K^+—urea salt complex is more insoluble than the urea-urea complex, whereas the Li^+—urea complex is more soluble. Consequently, the more soluble Li^+ ions are able to interfere with and partially destroy urea-urea interactions without lowering the solubility of urea by forming a Li^+—urea salt bond that is more insoluble than the urea-urea salt bond. Thus the Li^+ ion increases the solubility of urea by decreasing the urea-urea interactions. But since the K^+ ion produces a more insoluble salt bond with the oxygen atom of urea than the Li^+ ion, the K^+ ion is more effective in destroying urea-urea salt bonds. Because of this greater solubility, the K^+ ion is therefore more effective in liberating the positively charged $—NH_2^+$ groups on urea. And the greater the number of $—NH_2^+$ groups which are liberated, the greater will be the number of hydrogen bonds that can be destroyed. Hence K^+ increases the hydrogen-bond-destroying properties of urea but decreases its solubility.

The ionic sequences obtained from Figs. 1-3 are compiled in Table 3. They show that the divalent cationic sequences reverses itself when the molarity of the salt goes above 1.2 M salt. Thus at low salt concentrations (<0.7 M salt), the Ca^{2+} ion forms the most soluble salt bond with urea, whereas the reverse of this appears to be true at higher salt concentrations.

The ionic solubility sequences for various anions and cations is given in Table 4. There it is seen that for all types of monovalent, monoatomic anions, the Ca^{2+} ion is always more soluble than the Ba^{2+} ion or any other divalent ion. Consequently, the results given in Table 3 at low ionic strength show that the Ca^{2+} salts out urea less than the other divalent cations because the Ca^{2+} ion is the most soluble.

Table 4. Solubility Sequences for Various Theoretical Zwitterions[a]

Effective dielectric constant	Zwitterion model	Anion solubility	Monovalent cation solubility	Divalent cation solubility
$D_{\pm} \geqslant D_1$	$G^+—I^-$ $Cs^+—Br^-$	$Cl^- > Br^- > I^-$	$Li^+ > Na^+ > K^+ > Cs^+$	$Ca^{2+} > Sr^{2+} > Mg^{2+} > Ba^{2+}$
$D_{\pm} < D_1$	$NH_4^+—Cl^-$ $K^+—Cl^-$	$Cl^- < Br^- < I^-$	$K^+ < Na^+ < Li^+ < Cs^+$	$Ca^{2+} > Mg^{2+} > Sr^{2+} > Ba^{2+}$
A regions; $D_{\pm} < D_1$	$Na^+—F^-$	$Cl^- < Br^- < I^-$	$Li^+ < Na^+ < K^+ < Cs^+$	$Ca^{2+} > Ba^{2+} > Mg^{2+} > Sr^{2+}$

[a] The ionic sequences for a particular anion or cation were obtained from solubility studies [20]. The value of D_+ or D_- of a cation or anion was calculated as shown previously [7]. Other combinations of anions and cations to make different theoretical zwitterions can also, of course, be made from the above ions.

At higher ionic strengths, the most insoluble cation-urea salt bond (the Mg^{2+}—urea salt bond) increases the solubility of urea the most. This reverse effect on solubility is most likely due to the fact that the divalent cation does not cancel an electrostatic charge on the urea molecule as in the case of monovalent cations. Rather, it changes the negative charge to a positive charge. The ability of the divalent cation to reverse the charge will increase with an increase in the cation's concentration. But this increase in the solubility of urea with an increase in the concentration of divalent cation should increase faster for the more insoluble M^{2+}—urea complexes. Consequently, the slope of the lines in Fig. 3 should be a function of the equilibrium constant of the M^{2+}—urea complexes. That is, a greater increase in the solubility of urea with an increase in the concentration of M^{2+} would be due to a greater insolubilization of the M^{2+}—urea complex. Hence, in summary, the increase in the solubility of urea with an increase in concentration of the divalent cation is due to the imparting of a positive charge on the urea molecule by the cation. Moreover, the inversion of the divalent cationic solubility sequence is due to differences in the equilibrium constants between the divalent cations and the urea.

It should also be pointed out that eventually the salt will salt out the M^{2+}—urea complex. This salting out will depend on the salt's ability to reverse the electrostatic charge of the M^{2+}—urea ion. Consequently, the salting-out concentration will be a function of the type of anion employed. The more soluble the anion, the more effective it will be in reversing the charge [22].

Determination of the Charge Density of the Urea Anion

As seen in Table 3 and the above discussion, the K^+ ion forms the most insoluble salt with the urea zwitterion. From Table 4 it is also seen that the only anion which has this basic sequence is the Cl^- ion (or any other anion not shown which has $D_- < D_1$ and which has no A regions or tightly bound water) [7, 8]. Consequently, it is immediately seen from the monovalent cationic sequence that the oxygen atom of urea has a charge per unit surface area which is comparable to that of the Cl^- ion.

Examination of the divalent cationic sequences of Tables 3 and 4 shows that both the solubility of the Cl^- ion and the ability of the divalent cation to increase the dispersion power of urea go according to the sequences

Dispersion sequence in 8 M urea:	$Ba^{2+} > Sr^{2+} > Mg^{2+}, Ca^{2+}$
Cl^- solubility sequence:	$Ba^{2+} < Sr^{2+} < Mg^{2+} < Ca^{2+}$

Consequently, these two sequences agree with the above conclusion that the oxygen atom on urea behaves as if it had a charge per unit surface area equal to that of the Cl^- ion. On the other hand, a comparison of the solubility sequence of urea (as obtained at low ionic strength) with the divalent cationic solubility sequences of F^- and Cl^- gives

Urea solubility sequence:	$Ca^{2+} > Ba^{2+} > Mg^{2+} > Sr^{2+}$
F^- solubility sequence:	$Ca^{2+} > Ba^{2+} > Mg^{2+} > Sr^{2+}$
Cl^- solubility sequence:	$Ca^{2+} > Mg^{2+} > Sr^{2+} > Ba^{2+}$

The fact that the divalent cationic solubility sequence of the negatively charged oxygen atom of urea is the same as that for the fluoride ion rather than the Cl^- ion suggests that the electrostatic charge per unit surface area on the urea oxygen atom is greater than that on the Cl^- atom. In other words, based on the monovalent cationic sequences and the divalent cationic sequence from dispersion studies, it is concluded that A regions are not present on the urea oxygen atom. But according to the divalent cationic sequence from solubility studies, it must be concluded that in comparison to the Cl^- ion the charge density of the urea anion must approach more closely the charge density of a positively hydrated anion such as F^-. Hence it is concluded that the urea anion has a charge density between that of the Cl^- anion and that of the F^- anion.

Determination of the Charge Density of the Urea Cation

The anionic sequences are the result of the positive electrostatic charge on the $-NH_2$ groups of urea. As seen in Table 4, a different solubility will be obtained for cations having $D_+ > D_1$ when compared to cations having $D_+ < D_1$ (with or without A regions). As shown above, the value of D_- for the negatively charged oxygen atom is less than that of water ($D_- < D_1$). But previous results [6] show that urea behaves as if its effective dielectric constant were greater than that of water. Hence the value of D_+ for the positively charged $-NH_2$ groups must be greater than that of water ($D_+ > D_1$). Consequently, the charge density of the $-NH_2$ groups must be in the range of the Cs^+ and G^+ ions.

In comparing the ability of urea and GCl to disperse starch, it was observed that the dispersing power of GCl rises sharply after 4 M GCl, whereas that of urea remains constant or even declines slightly (see Fig. 3 of [14]). Similar results were obtained by Wetlaufer et al. [23] in their studies on the solubility of butane in concentrated urea and GCl solutions and also by Erlander and McGuire [6] with regard to the solubility of benzene in these two aqueous solvent systems. From the above data concerning the addition of various salts to urea solutions, it is apparent that these effects must be due to urea-urea interactions. Hence the declining ability of urea to destroy hydrogen or hydrophobic bonds it not due to a low value of D_+ but is due to the aggregation of urea molecules. This aggregation results in a masking or inactivation of the $-NH_2$ groups of urea. Consequently, the value of D_+ for these groups on urea may approach that on the guanidinium ion. The greater dispersing power of the thiourea molecule [6] indicates, however, that the $-NH_2$ groups on urea has not reached the maximum value [7] of D_+ which is attainable by cations.

Further verification that the urea cation behaves as the guanidinium can be obtained by examining the anionic sequence for the salting in of urea. At low ionic strength (<1.1 M salt) this sequence is $Cl^- > Br^- > SCN^-$. That is, the solubility sequence is the reverse of the salting out sequence of Table 3. Similarly, the solubility sequence for those cations having $D_+ \geqslant D_1$ is $Cl^- > Br^- > I^-$, whereas the opposite of this is true for cations having $D_+ < D_1$ (see Table 4). Hence the solubility sequence at low ionic strength verifies the conclusion that $D_+ > D_1$ for the $-NH_2$ groups of urea.

At high concentrations of salt the reverse of this solubility sequence for urea is obtained (see Table 3 and Fig. 2). The only possible explanation of this reversal is that at high ionic strengths the reversal of charge phenomenon is more important than the insolubility. In other words, it has been shown previously [19, 22] that the greater the solubility of the counter ion, the greater will be its ability to reverse the electrostatic charge of the ion.

As seen in Tables 1 and 3, 2 M LiBr increases the effectiveness of urea as a dispersing solvent more than 2 M LiCl (or $Br^- > Cl^-$). At this molarity of salt, the Br^- ion is more effective in increasing the solubility of urea than the Cl^- ion (Fig. 2). Consequently, the greater ability of 8 M urea + 2 M LiCl is due to the greater ability of the Br^- ion to destroy urea-urea interactions. Another contributing factor is that the Br^- ion has a higher value of D_- than the Cl^- ion and hence is capable of destroying hydrogen bonds (see starch retrogradation and solubilization sequences in Table 3).

Chelation Mechanism of the Guanidinium Ion

The change in the position of the guanidinium ion in the cationic sequence with a change in concentration of the G^+ ion deserves some consideration. All the other monovalent cations studied do not change their position (see Fig. 1 and Table 3). The G^+ ion changes position in the cationic sequence most likely because of its chelating properties. That is, the positive electrostatic charge on the G^+ ion is spread over all of its three $-NH_2$ groups. Consequently, as the G^+ ion approaches the negative oxygen atom of the urea molecule (the urea anion), it can form a salt bond which is more stable at high concentrations of salt than the other cation-urea complexes. Hence the solubility of urea is diminished by addition of sufficient GCl.

At low concentrations of GCl, the interaction of the hydrated shells of the G^+ and the negatively charged oxygen atom is the most important factor. That is, as shown previously [20] and also recently by Petrucci [24], the interaction of the hydrated water surrounding the anion and cation is the initial determining factor for ion-ion stability. As the concentration of salt increases and the amount of water of hydration diminishes, the formation of the stable chelate structure as in the case of the G^+—urea complex will become more important. Hence the position of G^+ shifts from its correct position at low ionic strength to a position signifying a more insoluble G^+—urea complex at higher concentrations of urea.

Mechanism of Urea as a Dispersing Agent

The above studies show that urea is a zwitterion. The positive charge on the $-NH_2^+$ groups of urea have an effective dielectric constant in aqueous solutions which is greater than that of water ($D_+ > D_1$), whereas the value of D_- for the negatively charged oxygen atom is less than that of water ($D_- < D_1$). The ionic sequences given in Tables 3 and 4 therefore show that urea can be represented as the theoretical zwitterion G^+—Cl^- or G^+—F^-. That is, the negative oxygen atom has a charge density between that of the F^- and Cl^- ions. The inability of aqueous urea to disperse starch to the same extent as aqueous GCl was shown to be due to the formation of urea-urea dimers or polymers in solution.

By expanding on the discussion given in the Introduction, the mechanism by which urea can destroy hydrogen or hydrophobic bonds can now be specifically explained. The positively charged $-NH_2^+$ groups of urea produce negatively hydrated water (B shell) which is capable of destroying hydrogen bonds ($D_+ > D_1$) because of the ability of the negatively hydrated water molecules to effectively compete with the groups involved in the hydrogen bonds [7]. This means that clusters of water molecules are also destroyed by the $-NH_2^+$ groups of urea. Hence, by destroying such clusters, the solubility of hydrocarbons is increased because now the potential space that a hydrocarbon can occupy has been increased. Consequently, the hydrocarbon bond is "broken." Aqueous urea, therefore, has the same effect as an increase in temperature from about 60 to 100°C (see the Introduction).

The above mechanism readily explains the enigma that scientists have been confronted with concerning the mechanism by which urea and similar salts can increase the solubility of hydrocarbons in aqueous solutions. Némethy [25] has recently admitted that this mechanism was hitherto unknown. But the mechanism has remained unknown because scientists have stubbornly clung to the concept that hydrocarbons are surrounded by a clathrate water structure. Such a concept was previously shown to be false [3]. Thus urea and salts such as guanidinium chloride destroy water structures. Such destruction of water clusters has recently been confirmed by studying the velocity of sound in urea-water systems [15].

Attempts have been made to explain the increase in solubility of hydrocarbons in aqueous urea solutions on the basis of a proposed clathrate structure. For example, Malik and Jain [8] state that any salt or molecule which increases the orderness of water will increase the formation of the clathrate structure. With such an increase in clathrate structure, they then state that the hydrophobic bond is stabilized because of greater entropy changes in going from clathrates surrounding individual chains to clathrates surrounding the aggregated hydrocarbon chains. But as pointed out in the Introduction, why does an increase in temperature above 20°C increase the aqueous solubility of hydrocarbons such as benzene [3], 3-methylpyridine [4], and polyethylene oxide [5]? If the formation of clathrate structures is important in the production of the hydrophobic bond as claimed by Malik and Jain [8], then the reverse should happen; that is, the solubility of hydrocarbons should decrease, not increase, as the temperature is increased. But the enigma that both an increase in water structure (caused by lowering the temperature) and a decrease in water structure (caused by addition of urea or an increase in temperature) cause the destruction of the hydrophobic bond is explainable when one discards the clathrate theory, as done in the above mechanism.

Thus the mechanism of urea denaturation is apparent from the above results. Urea does not complex with or form hydrogen bonds with various groups on proteins. Rather, it behaves as any salt having a value of D_+ for its cation which is greater than that of water. Consequently, if a foreign salt is added to aqueous urea solutions, that salt will increase or decrease the dispersion power of urea, depending on how the added salt interacts with urea and on the value of D_+ for this salt. Thus, as indicated by the data of Colacicco [9], the addition of sulfates decreases the dispersion power of both urea and guanidinium ions because it blocks the action of the G^+ ion and the $-NH_2^+$ groups of urea by forming insoluble salt complexes with these positive ions. Furthermore, the value of D_- for the sulfate ion is very low [7]. Consequently, all the known properties of urea are explainable on the basis of the approximate zwitterion structure G^+-Cl^-.

Comparison of Urea with DMSO and Formamide

Because of the increasing importance of dimethyl sulfoxide (DMSO) and because of its similar structure to urea, the elucidation of the DMSO structure and its comparison to urea would be profitable. DMSO strongly interacts with water to give off heat [26]. This negative heat of hydration shows that positively hydrated water molecules (A regions) are formed around the DMSO molecule. Experimental results [26, 27] indicate that this strong interaction consists of the complex $2H_2O \cdot DMSO$. In order to form such strong water-DMSO interactions, the DMSO must act as a zwitterion just as in the case of urea.

But if DMSO is a zwitterion, what are the electrostatic charge densities on its atoms? Payne [28], in studying the electrocapillary properties of the mercury-DMSO interface, showed that the cathodic capacity of DMSO-salt solutions decreased in the order $Li^+ > Na^+ > K^+$, which was the exact reverse of the order found with water-salt solutions. Examination of Table 4 shows that if water were considered as a dipolar ion, it should have the approximate "solubility" sequence of $Li^+ > Na^+ > K^+$, since $D_\pm = D_1$ by definition for water. Therefore, the negative charge on the DMSO molecule must be approximately equivalent to that on the F^- ion, since the only Li^+, Na^+, K^+ solubility sequence which is the reverse of that for $D_\pm \geqslant D_1$ is the sequence for ions having A regions and having $D_\pm < D_1$.

Examination of the absorption of anions by electrocapillary curves [28] for 0.1 M solutions of various salts in DMSO shows that as in the case for water-salt solutions, the anions are specifically adsorbed according to the sequence $I^- > Br^- > Cl^-$. Consequently, unlike the above result for the cationic sequence, there is no reversal of the anionic sequence. Hence the more insoluble I^- ion is adsorbed more strongly. Examination of Table 4 shows that if the positive charge on DMSO is larger than that on a water zwitterion, the anionic adsorption sequence would reverse itself from $Cl^- < Br^- < I^-$ for $D_- \geqslant D_1$ to $Cl^- > Br^- > I^-$ for $D_- < D_1$ (with or without A regions). Consequently, it is concluded that the positive charge on DMSO is similar to that of the G^+ or Cs^+ ion, where $D_+ \geqslant D_1$.

It can therefore be surmised from the above experimental findings that in comparison to urea, the negative charge on the oxygen atom of DMSO is slightly greater than that on the oxygen atom of urea. Moreover, the positive charge on the sulfur atom of DMSO behaves as if it were approximately equal to that on the $-NH_2$ groups of urea. This may be due to the inability of H_2O molecules to approach the sulfur atom because of the steric interference of the methyl groups on DMSO, and consequently the positive charge on the sulfur atom may be smaller than it actually appears to be. Therefore, it is the negatively charged oxygen atom of DMSO to which the water molecules become strongly hydrated (positive hydration).

It was concluded in another study [14, 29, 30] that DMSO disperses starch in a manner similar to that of 9 M LiCl [31]. That is, the unhydrated Cl^- ion in 9 M LiCl and the highly charged oxygen atom of DMSO can become hydrated or complexed with the hydroxyl groups of starch and hence can destroy intermolecular hydrogen bonds between starch molecules [14]. Therefore, when either the

Cl^- in LiCl or the oxygen ion of DMSO becomes hydrated, its dispersing action is destroyed. Thus hydroxyethyl cellulose is soluble in both DMSO and in water but produces maximum aggregation when the molar ration of H_2O to DMSO is 2:1 [at 65% (v/v) DMSO] [32]. But this ratio is exactly the same as that obtained for the hydration complex of DMSO [26, 27]. Hence these results confirm the conclusion that it is the unhydrated oxygen atom of DMSO which disperses the polar polymer, just as in the case of the unhydrated Cl^- ion in 9 M LiCl [31].

Such complexing in concentrated urea solutions does not occur because of the urea-urea interactions, as described above. Hence DMSO becomes more effective than urea because the methyl groups on DMSO prohibit such ion-ion interactions. Furthermore, the methyl groups provide a site for hydrophobic bonding. Yet hydrophobic bonding between DMSO units does not occur to any great extent because of the close proximity of the positive charge to each methyl group. The DMSO zwitterion therefore resembles a small detergent molecule and consequently has unique dispersing properties.

It should be noted that formamide ($HCONH_2$) which has a similar structure to urea, does not become a good solvent for hydroxyethylcellulose until water is added (<80% formamide) [27]. It is well known that in the absence of water formamide forms chain aggregates [27]. The degree of association is on the average about 8-14 formamide molecules. The chain (rather than three-dimensional aggregates as in the case of water) indicates that the positive charge on formamide is concentrated only on the $-NH_2$ group. Ionic sequence studies would have to be done to verify if formamide is a zwitterion and if this zwitterion is stronger or weaker than that of urea. However, properties of formamide indicate that it is a zwitterion. Unlike DMSO, both urea and formamide are therefore incapable of dispersing polymers in concentrated solutions because of the formation of aggregates. In diluted aqueous solutions, the B region of the $-NH_2$ groups of urea or formamide must have a value of $D_+ > D_1$ in both cases.

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