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Critical Appraisal of Salting-Out and Its Implications for Chemical and Biological Sciences

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1. Introduction

In simple terms, salting-out describes the precipitation of a less soluble material from a solution in which it is mixed with other substances. For at least 2 centuries, preparative chemists have known and described the process and have employed it for the isolation and purification of chemicals.[1−3] However, the application of the concept in the biological and health sciences is a relatively modern phenomenon (see section "General Significance of Salting-Out"). We have recently demonstrated that salting-out is responsible for the observation that dissolved urate promotes calcium oxalate (CaOx) crystallization in undiluted human urine *in vitro*,[4] a finding that has enormous implications for pathogenesis of urinary stones.

A higher than normal excretion of urate in the urine (hyperuricosuria) has long been proposed as a predisposing factor in the development of CaOx urolithiasis[5−7] but has lacked a firm scientific foundation for two principal reasons. First, the two theories most commonly cited to explain the association between urate excretion and urolithiasis, namely, epitaxy[8,9] and depletion of glycosaminoglycan inhibitors of CaOx crystallization,[10] are steeped in controversy and are not physiologically pertinent.[11−16] Second, there has been no unequivocal demonstration that hyperuricosuria is a common, reproducible finding in CaOx stone formers.[17−19] Nonetheless, these must be balanced against consistent reports that administration of allopurinol, a drug that decreases the synthesis and hence the urinary excretion of urate, reduces recurrence of calcium stones.[20−27] Given such empirical but persuasive evidence, it would be unreasonable to deny the existence of some connection between the level of urate and the precipitation of CaOx crystals in urine. Thus, we have demonstrated, as was originally suggested by Kallistratos et al.,[28] that the connection rests on the principle of salting-out.[4] Further, because we have also shown that the ability of urate to provoke CaOx crystal formation depends on the prevailing urinary concentrations of calcium and oxalate,[4] the credibility of our proposal does not depend on the need to explain the success of allopurinol by invoking a requirement for hyperuricosuria, which, as stated above, is not a well-documented feature of CaOx stone disease.[7,20−21,29−44] More importantly, it accommodates the possibility that urate could promote CaOx stone formation in patients with hyperuricosuria, as well as in those with normal levels of urate excretion who have relatively high urinary concentrations of calcium and oxalate. This suggests that urate may, in fact, be a bigger culprit of CaOx stone pathogenesis than has been previously thought and reported by Yu and Gutman[5,45] and others.[46−52] To

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Phulwinder K. Grover was born in India. He completed his B.S. (medical) and M.S. (biochemistry) from Panjab University in Chandigarh and Punjab Agricultural University in Ludhiana, respectively. Then, he moved to Adelaide and received his Ph.D. in Biochemistry from the Flinders University of South Australia under the supervision of Professors Rosemary L. Ryall and Villis R. Marshall. After a postdoctoral position with Professor Martin I. Resnick at the Department of Urology, at Case Western Reserve University in Cleveland, OH, he joined Professor Ryall's research group at the School of Medicine, Flinders University of South Australia. Currently, he is a Senior Hospital Scientist in the Urology Unit of the Department of Surgery at the Flinders Medical Centre and the Flinders University of South Australia. He has published extensively on various aspects of urolithiasis and has given many presentations at national and international meetings. He was invited as a visiting lecturer to the University of the Witwatersrand in Johannesburg, South Africa, in 1994. He has been awarded prizes for scientific presentations, locally (at the 27th Annual Scientific Meeting of the Australian Society for Medical Research held in Canberra in December 1988), nationally (at the 42nd Annual Scientific Meeting of the Urological Society of Australasia held in Melbourne in March 1989), and internationally: for his contributions to urolithiasis research, he was awarded the Young Investigator Award at the eighth International Symposium on Urolithiasis and Related Clinical Research held in Dallas, TX, in 1996. His major research interests are exploring various aspects of urolithiasis especially the involvement of hyperuricosuria and proteins in the development of kidney stones.

Rosemary Lyons Ryall was born in the United States when her father was working at the Australian Embassy in Washington, DC. She accompanied her parents back to Australia at the age of 2. Rose began her scientific career at the Australia National University in Canberra where she first completed an honors degree and then a Ph.D. in biochemistry. She moved to Adelaide in 1976 and was appointed to the Urology Unit at the Flinders Medical Centre, where she began her longstanding interest in kidney stone formation. Her research is presently concerned principally with exploring how proteins are involved in the development of kidney stones and the crystals from which they are built. As a result, her interests have widened to include other biominerals, especially those in higher plants such as spinach and oak trees, which use proteins to make the very same crystals but in a more salubrious and ordered fashion. She is fascinated with biomimetics. She has published numerous reviews, scientific papers, and book chapters and has given many presentations throughout the world. In 1998, her extensive contributions to kidney stone research gained her a Doctorate of Science from the Australian National University. Rose is currently Professor of Urological Research and Chief Medical Scientist in the Urology Unit of the Department of Surgery at the Flinders Medical Centre and the Flinders University of South Australia and chair of the Steering Committee of the emerging International Urolithiasis Society.

the best of our knowledge, stone formation caused by promotion of CaOx crystallization by dissolved urate is the only known pathological example of salting-out in humans.

Although our interest in salting-out stemmed from our interest in pathogenesis of urinary calculi, the phenomenon has much wider applications ranging from fundamental to applied science (see section "General Significance of Salting-Out"). A full comprehension of salting-out necessitates a sound knowledge of the hydration and thermodynamic properties of ions, which, in turn, requires at least a basic understanding of physical chemistry, electrochemistry, and mathematics. Because application of the principle of salting-out in industry and in the chemical and biological sciences is rising dramatically, interest in understanding the phenomenon, particularly the basic mechanisms involved, is at its highest. Although there have been excellent reviews on the subject,[53−57] they were all written either before or in the early 1960s. Furthermore, they were aimed at chemical engineers and/or physical chemists and thus are beyond the easy comprehension of most investigators in the biological and health sciences. Therefore, our aims were to provide a critical and updated review of the phenomenon of salting-out for chemists and biologists, particularly those working in the health sciences, and to highlight the ramifications of its use in chemistry, biochemistry, and molecular biology.

2. Definition

The change in solubility of a nonelectrolyte in an aqueous solution, which results from the addition of an electrolyte, is known as the salting effect. Thus, there can either be an increase or a decrease in solubility of a nonelectrolyte with increasing concentrations of added electrolyte. They are known as salting-out and salting-in, respectively.[56,57] For the purpose of the definition, electrolytes and nonelectrolytes are salts that have high and low solubilities, respectively. Mathematically, the influence of an electrolyte on the aqueous solubility of a nonelectrolyte can be expressed by the physical equation for gases, commonly known as the Setschenow equation,[56] given below

$$\log s_0/s = \log fc = kc_s \qquad (1)$$

where s_0 and s are the solubilities of the nonelectrolyte in water and electrolyte in solution, respectively, c_s is the concentration of the electrolyte in moles per liter, fc is the activity coefficient of the nonelectrolyte (expressed in concentration units), and k is the salting constant. A positive value for this constant indicates salting-out, and a negative value indicates

salting-in. Because s_0 is constant in an aqueous solution, it follows from eq 1 that the amount of electrolyte added is directly proportional to the amount of nonelectrolyte precipitated and *vice versa*.

Initial studies by Brønsted[58] showed the numerical value of the salting constant, k, to be equal to the product of its cationic and anionic components, but later work by Larsson[59] and Gross[54] proved it to be additive, that is

$$k = k^+ + k^- \quad (2)$$

where k^+ and k^- are the cationic and anionic salting constants, respectively. Furthermore, Randall and Failey[60,61] showed that, while k is largely dependent on the electrolyte, it is also dependent to a lesser extent on the nonelectrolyte.

Because different cations and anions have different ionic salting constants, their different combinations (such as in different salts) differ in their propensity to cause salting-out. This is attributable, as will be discussed later, to their varying structure, size, charge density, hydration, and dielectric constant (also known as polarizability) as salts, as well as the polarizability of the supporting solvent. The efficiency of some common cations as salting-out agents, in decreasing order, is sodium > potassium > lithium > barium > rubidium > calcium > nickel > cobalt > magnesium > ferrous > zinc > cesium > manganous > aluminum > ferric and chromic > ammonium > hydrogen.[60] For anions, the sequence is hydroxide > sulfate and carbonate > chlorate > bromate > chloride > acetate > iodate and perhalide > bromide and iodide > nitrate.[60]

It must be noted that the Setschenow's equation holds good only for solutions containing high concentrations of electrolyte. In dilute solutions (containing an electrolyte concentration equal to or less than 0.5 mol per liter), however, additional errors are introduced in the calculation and hence the limiting form of Setschenow's equation, that is

$$v_a = k_1 c_s \quad (3)$$

is applicable.[56] In eq 3, v_a is the additional volume (in liters of solution) that would be required to hold 1 mol of the nonelectrolyte in solution in the presence of the added salt concentration (c_s), in excess of the volume of water (v_0) required for its solution in the absence of the salt. k_1 is a constant, which is given by

$$k_1 = 2.303 v_0 k \quad (4)$$

3. Mechanisms of the Salting Effect

The effect of the addition of salts on solutions of nonelectrolytes is very complex, primarily because a large number of different types of intermolecular interactions come into play between the ion and solvent, ion and nonelectrolyte, and nonelectrolyte and solvent. This is further complicated by the fact that the extent of the interactions varies in relation to the types of ions, nonelectrolytes, and solvents involved. When this is kept in view, it is not surprising that from time to time various qualitative and quantitative theories, emphasizing different intermolecular interactions, have been put forward to explain the mechanisms of salting-out. For the sake of clarity and convenience of presentation, however, the theories presented in this review have been grouped into five sections. There are no clear-cut distinctions between these theories: the artificial divisions simply reflect the different approaches adopted to explain the concept. Moreover, the thermodynamics of the salting effect is beyond the scope of this paper and interested readers are referred to several excellent papers on the subject.[62−64]

3.1. Hydration Theories

These theories, which constitute the oldest and simplest explanation of salting-out, postulate that ions in solution attract and are consequently surrounded by a layer of solvent molecules—a process commonly referred to as ionic hydration. This effectively immobilizes solvent molecules and quenches their role as solvents.[65−68] When an electrolyte is added to a solution of a nonelectrolyte, they compete with each other for solvent molecules. As expected, the competition is won by the electrolyte ions (or ions with a relatively strong affinity for the solvent), and those of the nonelectrolyte (or ions with a relatively less affinity for the solvent) lose. This causes preferential movement of the solvent molecules away from the ions of the nonelectrolyte to those of the electrolyte, which, in turn, decreases hydration and hence the solubility of the ions of the nonelectrolyte. As a consequence, the nonelectrolyte precipitates from the solution. Thus, according to the hydration theories, salting-out is attributable to the preferential movement of solvent molecules from their role as the solvent for ions of the nonelectrolyte.[65−68] These theories are depicted in Figure 1.

Generally, cations have a higher degree of hydration than anions. This led Gross[54] to propose that cations and anions are responsible for salting-out and salting-in, respectively, and that the net salting effect of an electrolyte depends on the balance of these two opposing forces. While Gross's model of the salting effect explains most observations, more recent studies reveal that it is by no means universally applicable.

It is worth mentioning that the nature of ion−solvent interactions is very complex when water is the solvent. For instance, such interactions can be classified as hydrophobic, hydrophilic, structure breaking, or polarization type.[69] In addition, as mentioned above, ions in aqueous solution attract water molecules and become enveloped in a layer of them commonly known as the hydration or coordination shell. The number of water molecules in the hydration shell is equal to the coordination number of the ion in water. Except for some multivalent ions, water molecules in the hydration shell surrounding the ions are in continuous exchange with those comprising the bulk of the solution.[70] All water molecules in the shell are influenced by the ionic field and fluctuate continuously, and it is only on a time average basis that a number of them are immobilized by the ion. That number is known as the hydration number. Thus, the hydration number of an ion is an empirical parameter

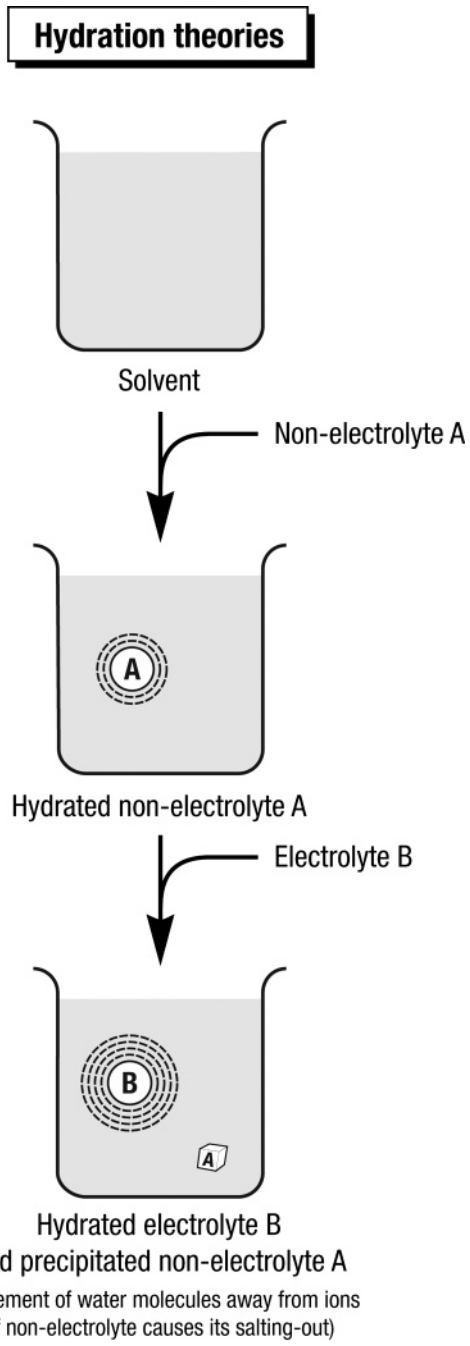

Figure 1. Diagrammatic representation of the hydration theories. According to them, salting-out is the result of preferential movement of water molecules away from their role as the solvent from ions of nonelectrolyte to those of electrolyte.

that gives the effective number of water molecules that have undergone some constant critical change in property while surrounding the ion.[69] Generally, all four types of interactions between any ion and solvent, as mentioned above, coexist. It is, however, the extent of those interactions that varies in relation to the ion and solvent. It therefore follows that the hydration number of an ion (a) depends on more than one type of hydration such as hydrophobic, hydrophilic, structure breaking, or polarization type; (b) may change from one property examined to another; and (c) can be compared only if the property studied depends on the same kinds of ion−solvent interactions.[69] When this modern concept of ionic hydration is kept in view, researchers can appreciate why previous workers[71,72] divided the process into two main types: primary (also known as near hydration) and secondary (also known as far hydration). While the former referred to the water molecules immobilized by the ion and moving as one unity with it, the latter denoted other ion−solvent interactions beyond the primary hydration shell. It is not our intention to present a detailed discussion of the calculation of coordination and hydration numbers of ions, and interested readers are referred to a review on the subject.[69]

The major drawback of the hydration theories is that they imply that each ion ties up a finite share of water molecules and has no effect on the solvent properties of the rest of them. From this, it can be inferred that the hydration numbers calculated from salting-out experiments should be independent of the nonelectrolyte salted out—a conclusion that is not true.[56] The theories also provide no explanation for the observed dependence of the salting constant, k, on the size of the nonelectrolyte[69,73] and the fact that hydration numbers deduced from salting-out experiments do not correspond with the degree of hydration obtained from other related experiments.[56] Finally and most importantly, the hydration theories are unable to explain the salting-in effect.[56]

3.2. Water Dipole Theories

To surmount the issue of salting-in, Kruyt and Robinson[74] in 1926 suggested that the solvent structure should play a major role in determining salt effects, a factor that received very little further attention until 1945.[75] The theory was largely ignored until 1981, when it was further developed by Treiner,[76] but was later extended in the 1990s by other researchers.[77,78] They showed that variations in specific effects of salts on different nonelectrolytes might arise from the fact that the water dipoles in the hydration shell around an ion are *oriented*. Thus, if there is a preferred orientation of water molecules toward a polar solute, then ions of one sign should have a tendency to promote its solubility (salting-in), while those of the opposite sign, which should orient water molecules unfavorably, should have a tendency to decrease its solubility (salting-out). This model is illustrated in Figure 2. It is also possible that the structure of the electrolyte itself could play a pivotal role in determining the salting effect. It has been suggested that, if the structure of the electrolyte is such that it affects the field beyond its hydration shell, then it will affect the water dipoles,[57,79] which, in turn, will determine whether salting-out or salting-in will occur, as explained above. Hydrophilic hydration near the ion, as well as polarization of nonelectrolyte and water molecules, has also been proposed as a primary determinant of the salting effect.[69]

Although the dipole model of salting-out is quite helpful in interpreting some of the observed relative effects on different polar solutes, it does not explain observed variations in the effects of different nonpolar solutes.[56,80]

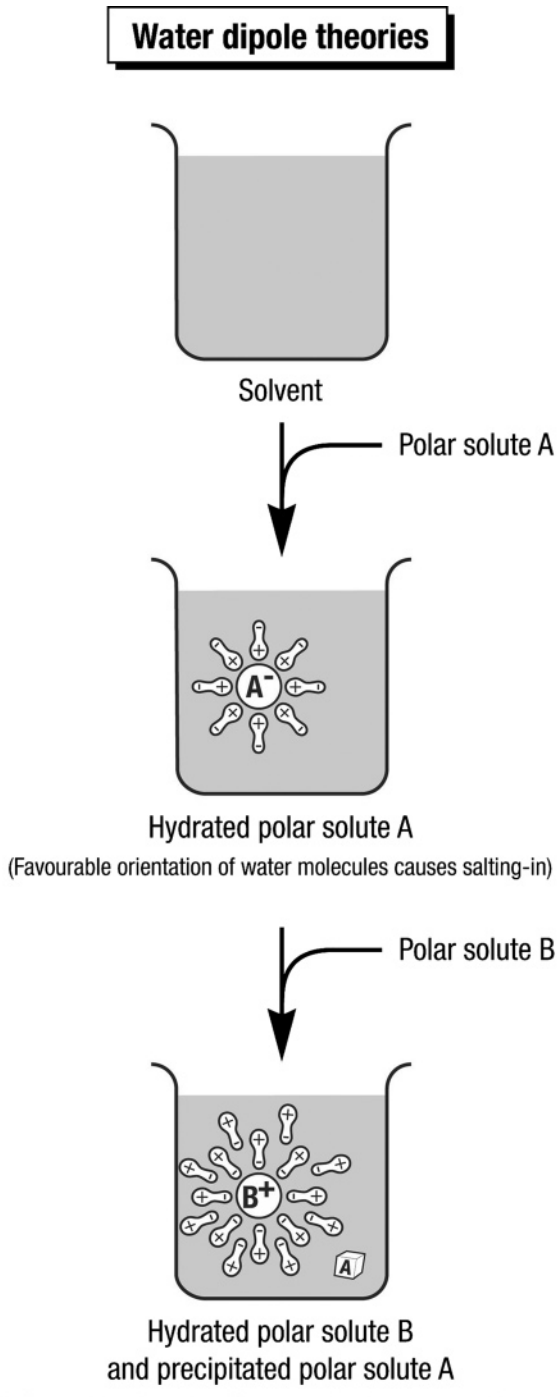

Figure 2. Diagrammatic representation of the water dipole theories. These theories suggest that favorable orientation of water molecules around polar solutes causes salting-in and vice versa.

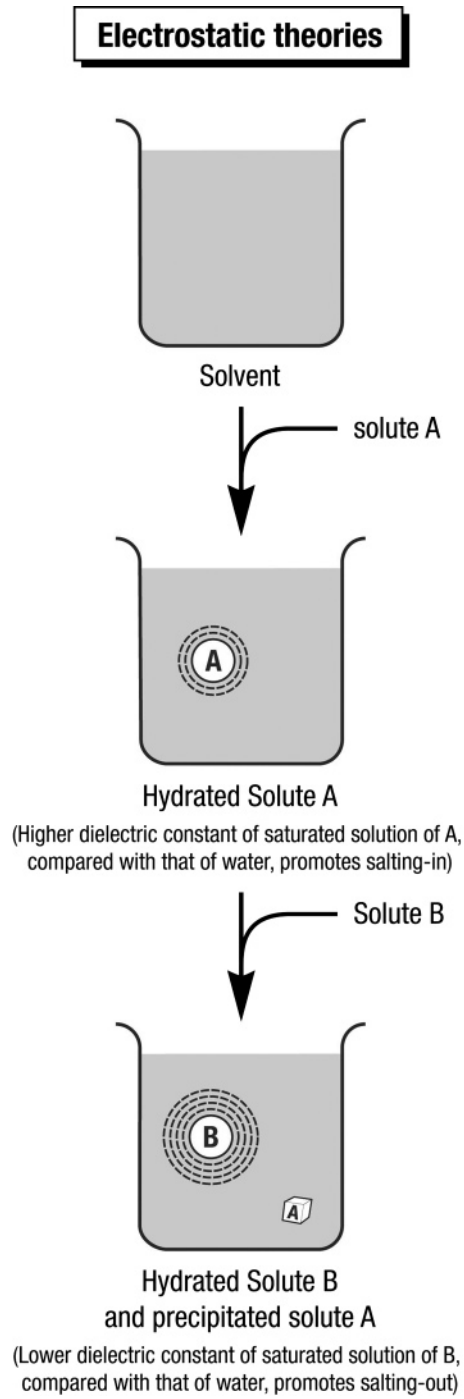

Figure 3. Diagrammatic representation of the electrostatic theories. The tenet of these theories is that if the saturated solution of a solute has a higher dielectric constant compared with that of water, it causes salting-in and vice versa.

3.3. Electrostatic Theories

To explain the observed variations in salting effects of different nonpolar solutes, Debye and McAulay[81] and Debye[82] suggested an electrostatic explanation of the salting effect. Their theory was further developed by various workers to give qualitative and quantitative dimensions.[83−86] The tenet of the theories is that the amount of work necessary to discharge the ions in pure solvent is different from that required in a solution containing a solute. They therefore related both salting-out and salting-in to the influence of the solute on the dielectric constant of the solvent. On that basis, if the saturated solution of solute has a dielectric constant less than water, then salting-out occurs, and if the saturated solution of solute has a dielectric constant more than water, then salting-in occurs. This model is shown in Figure 3.

The major advantages of the electrostatic theories are that they provide an explanation for and the correct order of magnitude of the salting effects of ordinary neutral electrolytes such as sodium and potassium chlorides.[56] They also predict reasonably

well the dependence of the salting constant, k, on the molecular size of the nonelectrolyte;[57] that is, the degree of salting-in of nonpolar solutes increases with ionic size, although there are some notable exceptions to this generalization.[56] These theories can also be used to estimate the volumes of hydrated ions from their hydration numbers.[57] However, they cannot reasonably account for marked discrepancies in the ranking sequences of similar electrolytes, and they are completely unable to account for observed shifts from salting-out to salting-in with particular nonelectrolytes.[56] For example, while the theories predict salting-out by salts of smaller ions (such as sodium and potassium chlorides), they provide no explanation of salting-in caused by salts of large ions (such as tetramethylammonium, naphthalenesulfonate, and long-chain fatty acids). These drawbacks are not unexpected because they take into account only the primary electrical effect and not "displacement" and "structural" effects.[56]

3.4. Internal Pressure Theories

In 1899, Euler[87] made an empirical observation that the aqueous dissolution of ethyl acetate caused shrinkage in the volume of water. He also noted that the increasing order of these volume contractions upon the dissolution of different salts was related, in the same sequence, to an increase in salting-out. Later, Geffcken[88] and Tammann[89,90] showed a similar correlation between salting effects and the relative effects of salts in decreasing the compressibility of the solution, which prompted Tammann[90] to suggest a theory commonly known as the "internal pressure" concept of salting-out. Later, McDevit and Long[91] proposed an explicit model of the theory to study qualitative and quantitative aspects of the salting effect. According to that model, neutral solute molecules in solution merely occupy volume. Their presence exerts internal pressure on solvent molecules, which, in turn, modifies the ion−solvent interactions and causes precipitation of the nonelectrolyte. Thus, according to these theories, the degree of salting-out or salting-in of a nonpolar solute is determined by the extent to which the solvent medium is compressed or expanded when the ions are present. Generally, as the compressibilities of the solutions increase, the salting-out effect diminishes and vice versa.[56] These theories, which are schematically represented in Figure 4, are supported by the fact that predicted and observed salting effects for nonpolar nonelectrolytes correlate quite well with the corresponding volume changes that occur when the salts are dissolved in water. However, they cannot reasonably account for marked variations in the order of ranking of the salting effects of similar electrolytes and vice versa. For instance, according to these theories, the *predicted* order of salting-out for various salts is essentially the same for such disparate species as hydrogen, nitrous oxide, and benzene, which, in fact, is not true.[56] Their major drawback, therefore, is that they hold well strictly for nonpolar nonelectrolytes[91] but give no explanation for the effects of polar nonelectrolytes.

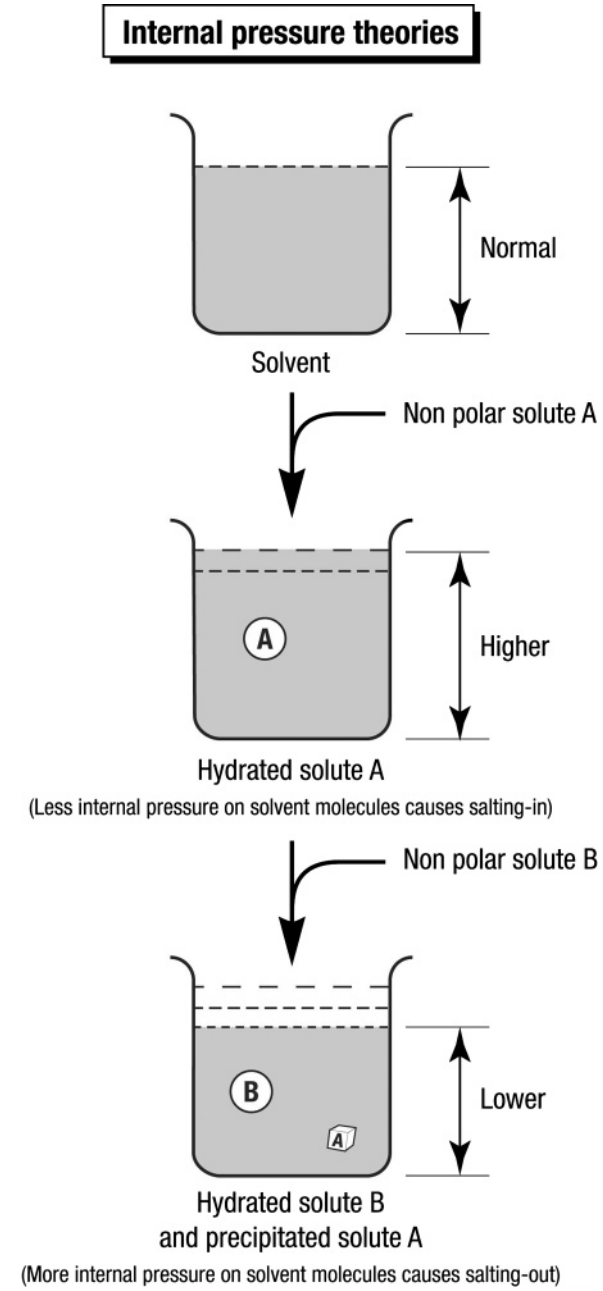

Figure 4. Diagrammatic representation of the internal pressure theories. According to them, if the presence of a nonpolar solute decreases the internal pressure on solvent molecules, it causes salting-in and vice versa.

3.5. Theories Based on van der Waals Forces

The tenet of these theories is that short-range electrostatic interactions, other than those mentioned in section 3.1, occur between ions and neutral molecules. Such intermolecular interactions, which are collectively known as van der Waals forces, can be of two types: attractive and dispersive. Of these, the latter have been suggested to play an appreciable role in salting effects of ions.[56] From time to time, various workers have proposed explicit models for the quantification of these dispersive forces.[92] A diagrammatic representation of the theories of van der Waals forces is presented in Figure 5.

The concept of van der Waals forces is supported by the fact that predicted salting-in effects of large

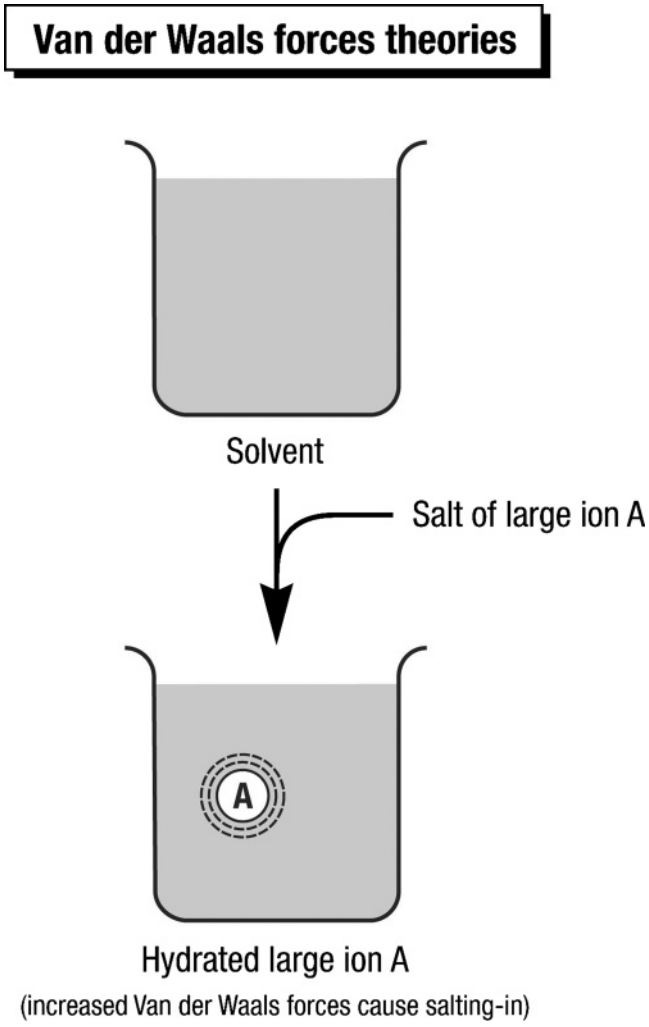

Figure 5. Diagrammatic representation of the theories of van der Waals forces. These theories suggest that increased van der Waals forces between ions and neutral molecules cause salting-in.

ions (also known as hydrotropism), such as quaternary ammonium ions and long-chain fatty acids, are observed experimentally. To further explain salting-in, Desnoyer et al.[93] demonstrated that the dissolution of salts of large ions increases the structure of water, which decreases the entropy of the system, increases the solubility, and hence causes salting-in. The theories of van der Waals forces explain only salting-in, which is not the primary focus of this paper but is discussed in detail elsewhere.[56,69]

Despite the success of the theories based on van der Waals forces in explaining some observed solubility data, their quantitative application in the calculation of dispersion potential of complex molecules is questionable.[94] The theories also predict much less specificity in the effects of different ions than is actually observed,[56] and they fail to account for the anomalously low salting-out effects generally caused by lithium and hydrogen ions, as well as some nonelectrolytes.[56] On the basis of these shortcomings, along with the theoretical limitations of the concept, it has been argued[56] that van der Waals forces may play only a secondary role, if any, in determining the relative effects of a series of ions. Hence, the concept must remain speculative until more exhaustive, convincing evidence becomes available.

4. General Significance of Salting-Out

The phenomenon of salting-out is of both fundamental and applied interest. Its study can provide a wealth of information of theoretical importance to understand the complex nature of interactions between ions and solvent molecules, which, in particular, allows an appreciation of the unique nature of water as a solvent.[56] Data obtained from experimental investigations can also have direct implications for studying kinetic salt effects,[56] elucidating mechanisms of reactions,[56,95] determining protein microheterogeneity,[96,97] and estimating the relative surface hydrophobicity of proteins[98] and bacterial cells.[99] From a practical perspective, it has already proved useful to differentiate virulent strains of *Yersinia pseudotuberculosis*[100] and inhibit proteolytic enzymes.[101] Salting-out is especially useful for quantifying proteins[102] and active metabolites of drugs and toxins in blood and other body fluids,[103−107] because it improves recovery. Consequently, it is not surprising that the concept is used in broad-spectrum drug screening.[108]

Data obtained from salting-out experiments are also invaluable for the colorimetric assays of free fatty acids[109] and for high-grade purification of chemicals, pharmaceuticals, proteins,[110−115] and DNA from fresh[116−119] and stored samples,[120,121] which is particularly important in forensic science, because most specimens are generally available in very limited amounts. Macromolecules required for research and/or molecular (health and forensic) diagnostic purposes[122−131] are commonly isolated using the salting-out principle, which is also invaluable for the routine synthesis of membrane vesicles of different compositions.[132] Such vesicles, which are representative of different pathological conditions, can then be used as models for studying transport systems across biomembranes.

Because salting-out increases recovery, it has quite a lot of industrial applications as well. For instance, it is used for large-scale purification of chemicals (synthetic and semisynthetic) and pharmaceuticals.[133−137] Also, it is used for large-scale purification of petroleum-based products and enzymes.[138,139] The latter have applications in industry (e.g., amylase for starch processing used in the sugar industry, cellulase and pectinase for wood processing used in the pulp and paper industry, and zymase for fermentation used in brewing beer, wine making, and the baking industry), diagnostics, and biochemical research.[140−141]

It is remarkable that salting-out is an entirely physical phenomenon and does not affect properties of molecules or macromolecules (polyelectrolyte, neutral, or hydrophobic in nature) including RNA, DNA, and proteins. This is evidenced, as mentioned above, by the fact that pharmaceuticals, nucleic acids, and proteins purified by salting-out are used routinely in research, molecular health diagnostics, and, more importantly, in molecular forensic diagnostics.

5. Particular Significance: Kidney Stones

From the foregoing discussion, it is easily seen that the phenomenon of salting-out has much to offer for industry, biology, and the health sciences in general. Human urolithiasis, however, provides an example of its potential usefulness in understanding the processes underpinning a human disease and, more importantly, in developing methods for its treatment. As mentioned earlier, recently, we demonstrated that enhanced crystallization of CaOx in urines spiked with dissolved urate is attributable, as was originally suggested by Kallistatos et al.,[28] to the principle of salting-out.[4] This finding has enormous ramifications for understanding pathogenesis and treatment of urinary stones. This is because it provides a sound scientific explanation for the occurrence of CaOx calculi in hyperuricosuric patients and it also provides a justification for the administration of allopurinol commonly used to reduce recurrence of the disease in these patients. It must be noted that, once instituted, allopurinol treatment must be continued indefinitely if stone prevention is to be guaranteed, thus raising the cost of therapy in the long term. Although the drug is tolerated reasonably well by most patients, it can cause serious allergic reactions, particularly in the elderly,[142−145] and a rare fatal systemic vasculitis.[146,147] Also, its dosage needs to be carefully monitored in patients with renal impairment.[148] There is thus a need for developing better tolerated and more cost-effective modalities for the treatment of allopurinol-sensitive hyperuricosuric or normouricosuric CaOx stone formers. Although the use of allopurinol in the treatment of hyperuricosuric patients might appear obvious, there are good reasons for using the drug for preventing stone recurrence in those who excrete normal amounts of uric acid in their urine. We have shown that the induction of CaOx precipitation by dissolved urate, although dependent upon the ambient urate concentration, is also influenced by the prevailing concentrations of calcium and oxalate.[4] The possibility cannot be excluded, therefore, that a reduction in urinary urate excretion might be beneficial in patients in whom a decrease in oxalate or calcium excretion has proved difficult or unhelpful. Results of further studies with large numbers of subjects and multiple urine samples may allow the possible definition of an index relating urinary calcium, oxalate, and urate concentrations to the risk of crystal formation, thus enabling the identification of patients likely to benefit from allopurinol treatment and those in whom a reduction in calcium and oxalate excretion might also be advantageous.

Furthermore, studies investigating the phenomenon may identify other agent(s) that may attenuate the effects of urate. For instance, pyrophosphate, magnesium, and citrate have been reported to inhibit the growth of CaOx crystals in undiluted human urine *in vitro*, and magnesium has been shown to raise the metastable limit of urine samples.[149] The real value of these agents is that their concentrations can be increased relative to therapeutic levels. A detailed study of the effects of these low-molecular-weight inhibitors, especially citrate and magnesium, on the urate-induced salting-out of CaOx is therefore warranted. Urinary pH can also be altered relatively easily, and it is well-known that pH has a profound effect on the solubility of CaOx and urate.[28] Further investigations, such as those performed by Kallistratos et al.,[28] would provide sound data confirming whether alterations in urinary pH affect the degree of salting-out of CaOx by urate, and therefore, whether this might be a useful approach for preventing stone recurrence in selected individuals.

6. Summary

Although several qualitative and quantitative theories have been advanced to explain the salting effect, our complete understanding of the phenomenon is still far from satisfactory. This is mainly because a large spectrum of intermolecular forces comes into play between electrolyte, nonelectrolyte, and solvent molecules and because different theories simply emphasize different intermolecular interactions and do not preclude the possibility of other types of forces that may be more relevant. On the basis of the information currently available, the salting effect depends mainly on the properties of the electrolyte, nonelectrolyte, and solvent. These include the size, structure, charge density, polarizability, and hydration of the electrolyte and nonelectrolyte, as well as the dielectric constant (or polarizability) of the solvent. Generally, it is widely accepted that, while salting-out is essentially an electrostatic effect, salting-in is primarily a structural effect.

The phenomenon of salting-out has applications in almost all facets of life. These include from fundamental to applied chemistry, biology, and molecular biology.

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