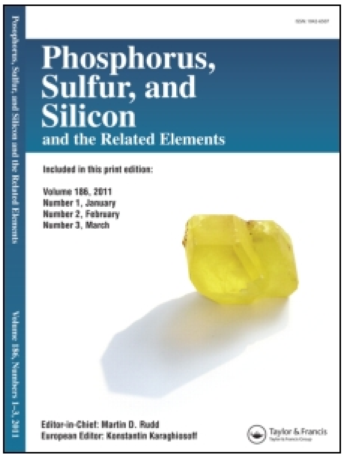

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Biocompatible Small Molecules that Enhance Silica Solubilization Under Ambient Conditions: Chemical Profile of Such Complexes, Possible Mechanism for Enhancement, and Their Effect on the Growth and Protection from Pests in the Rice Plant (*Oryza sativa* L.)

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Biocompatible Small Molecules that Enhance Silica Solubilization Under Ambient Conditions: Chemical Profile of Such Complexes, Possible Mechanism for Enhancement, and Their Effect on the Growth and Protection from Pests in the Rice Plant (*Oryza sativa* L.)

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Recent studies suggest that the depletion of silicon available to plants may be a major reason for the yield decline observed for rice cultivation. In the present article, a novel strategy is explored to identify small biocompatible molecules (carriers) that would enhance the solubility of sand ($SiO_2.\ nH_2O$) (here after named silica) in water under ambient conditions. All considerations, notably biocompatibility, make the 20 proteinous amino acids and their simplified analogs the first choice. Among the proteinous amino acids, notable enhancement of silica solubility was seen at 1000 µmole.L^{-1}: Blank: 1; glutamine: 3.5; histidine: 3; alanine: 2.9. Based on the assumption that the observed enhancement is related to proclivity for hydrogen bond formation with water, a large number of varied biocompatible substrates were examined. Among these, notable enhancement of silica solubilization at 1000 µmole.L^{-1} was seen as exemplified with Blank: 1; imidazole: 5; inositol: 4.5; mannitol: 3.9; 3, 4-dihydroxy phenylalanine (DOPA): 3.5. A complementary gravimetric protocol coupled with thermogravimetric analysis (TGA) enabled the estimation of an approximate empirical formula for few of the carrier–silica complexes. A possible mechanism for the interaction of the carrier at the silica–water interface is suggested. Field experiments (reported separately) with selected carriers, monitored in the stem extract by colorimetry and in the leaf by scanning electron microscopy (SEM) with energy dispersive X-ray (EDX) analysis showed enhanced levels of silica and were significantly beneficial for the growth and protection from predators of the rice plant.

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INTRODUCTION

The rice plant illustrates the most evolved example of the utilization of silica. Here, mechanisms for enhanced silica solubilization, which under normal conditions are available only at concentrations of 10^{-4} mole.L^{-1} and in the narrow pH range of 6–9, transport it to various parts of the plant, process it, and deposit it in all parts of the plant (stalks, leaves, and husk) at levels ranging from 10–15% in an intricate manner, make the operation unique.[1] Recent studies suggest that depletion of silicon available to the rice plant may be a reason for the yield decline observed for rice cultivation.[2] For a yield of 5000 kg of rice/ha the soil silica is depleted by as much as 230–470 kg of elemental silicon.[3–5] Agents that enhance silica uptake can, to some extent, compensate the depletion of silicon arising from excessive cultivation and at the same time provide healthy plants with increased resistance to pest and blast diseases.[6] For some years we have been examining the problem of the solubilization of silica in water under ambient conditions.[7] Silica is quite inactive, and the most general way to make it a reactive species is through penta coordination.[8–10] Indeed it has been suggested that silicon present in natural pools deviates from the usual tetra coordination.[11,12] These findings were in agreement with the reported enhancement of silica by rice plants with poly-2-vinylpyridine N-oxide.[13] The natural conclusion of this is that penta-coordinated silicon intermediates are involved in this reaction, and our recent work has confirmed that small molecules such as pyridine N-oxide enhance the solubilization of silica in water[7] and in support transformed Si(IV) to Si(V) systems by complexation with pyridine N-oxide. This led to, as stated in the Abstract, a search for small biocompatible molecules that can enhance silica solubilization. Such efforts by themselves would be rewarding and could lead to the synthesis of structures that would prove more efficient.

In a broader sense, effective methods for solubilization of silica in water could have application in diverse domains. Colloidal silica deposits present one of the most difficult challenges of water treatment, and recently the effectiveness of small molecules to dissolve such deposits has been studied.[14]

The development of methodologies for solubilization of silica under ambient conditions could lead to more environmentally friendly procedures for the manufacture of silica-based materials such as semiconductors, glasses, ceramics, plastics, elastomers, resins, mesoporous molecular sieves and catalysts, optical fibers and coating, insulators, moisture shields, photo luminescent polymers, and cosmetics, since currently most of the methods for these materials need high temperatures or use caustic chemicals.[15] In this context, we feel a desirable objective in silicon research is to understand how nature under ambient conditions produces a remarkable diversity of structures, from nanoplanktons to higher animals, and that an obvious starting point for such a study would be the discovery of methods for dissolution of silica under ambient conditions.

As stated before, the effect of small molecules to enhance the solubilization of silica has seldom been studied under ambient conditions. However, it has been shown that polyols in aqueous media containing silica in the presence of aqueous sodium hydroxide readily form penta- or hexa-coordinated complexes. Interestingly a 1:1 ratio of silica:NaOH exclusively gives with xylitol, a penta-coordinated complex, and when the ratio is 1.1:2.8, largely hexa coordinated complexes. This is generally the case with a variety of sugar polyols.[11] The above results are in contrast to those reported in growing diatom species, where hypervalent silicon species were seen without the addition of alkali and under ambient conditions.[12]

Basic to the understanding of biosilicification are mechanisms involved in the solubilization of silicic acid in water. Enhanced solubilization of silicic acid in water can be achieved, and is supported by several indicators. In its most rudimentary form, in organic-rich environments such as swamps for example, the concentration of silicon in solution is higher than predicted.[16] In recent times much focus has been placed on the complexation of silica with polyols, notably, carbohydrates.[11] Unfortunately such complexation was demonstrated by in situ–generated sodium silicate from an admixture of silicic acid with dil. NaOH in a 1:1 or higher ratio. Thus, although there is a longstanding supposition that polyhydroxy compounds are associated with uptake of silicon and transport, this has not been proven.[16]

Physiological studies have shown that silicon uptake by rice roots is mediated by a type of transporter.[17] After it is taken up, silicon is transported in the form of monomeric silicic acid and is finally deposited on cell-wall material as a polymer of hydrated amorphous silica forming silica-cuticle double layers and silica-cellulose double layers on the surface of the leaves, stems, and hulls.[18]

The discovery of the silicon-deficient gene *Lsi1* has been most imaginatively used to bring out the roles played by the silica/silicic acid in the rice plant. Replacement of *Lsi1* has resulted in vastly reduced silicon intake, high susceptibility of the leaves to pests, the infection of the panicle, and the reduction of the yields of rice to 90%.[18] This work shows that any system that brings about enhancement of silica availability, such as pyridine N-oxide,[7] could lead to beneficial effects.

Arising from this, the present problem concerns a systematic examination of small biocompatible compounds that could exhibit enhancement of silica solubilization.

As a result, only the uptake of silicic acid/silica under ambient conditions will be discussed in the present work. Therefore, the vast amount of Si(IV) and Si(V) chemistry, from treatment of ethylene glycol with strong bases, has not been further elaborated.[19–22]

RESULTS

The major strategy in the present work is to explore small biocompatible molecules that can enhance silica solubilization. Our earlier endeavors have shown that pyridine N-oxides can enhance the solubilization of silica, and these provide protection from pests and enhance yields in the rice plant.[7,23] Since it has been established that chosen organic molecules can enhance $Si(OH)_4/SiO_2$solubilization, the choice was made to explore biocompatible small molecules from the vantage of field application.

The above consideration makes the 20 proteinous amino acids and their simplified analogs as the first choice to explore their potential to enhance silicic acid solubilization, except cystine because of its insolubility in water, by the colorimetric method.[24]

The studies with the proteinous amino acids were carried out under ambient conditions at a uniform concentration of 1000 μmoles.L^{-1} and under uniform treatment with silica in HPLC water (see the Experimental section for details). The results from this study suggested a mechanism strongly based on hydrogen bonding interactions. This in turn led to the examination of another set of biocompatible molecules that could enhance silica soublization.

The results of silica solubilization studies, based on Figure 1, on the proteinous amino acids and on compounds having potential for hydrogen bonding along with the standard deviation are presented in a tabular form in Tables I and II, respectively, and graphically in Figures 2 and 3, respectively.

The above studies brought out the fact that selected molecules can significantly enhance the solubilization of silicic acid. This resulted in the development of a novel "quantitative assessment" of efficiency in silica solubilization (carrier efficiency). A key feature of this procedure is that it would permit the harvesting of soluble oligomers as well, since the procedure involved shaking a mixture of silica (2 g), carrier (100 μmole), and HPLC water (100 mL) for 2 days and lyophilizing the clear filtrate. The procedure results in a soluble silica–carrier complex with

TABLE I Demonstration of the Enhancement of Solubility of Silicic Acid in Water Mediated by Proteinous Amino Acids^{1,2}

Amino acid (1000 μmole.L^{-1})	Solubilization of silicic acid (μmole.L^{-1})	Amino acid (1000 μmole.L^{-1})	Solubilization of silicic acid (μmole.L^{-1})
Blank	360.25 ± 7.09	*Methionine* (M)	597 ± 6.68
***Alanine*(A)**	**1077.5 ± 44.55**	Asparagine (N)	518.66 ± 3.7
Aspartic acid (D)	854.67 ± 5.77	Proline (P)	880.0 ± 1.73
Glutamic acid (E)	986 ± 4.58	**Glutamine (Q)**	**1255.33 ± 11.7**
Phenylalanine (F)	479.67 ± 10.01	Arginine (R)	697.33 ± 4.6
Glycine (G)	404.5 ± 24.0	Serine (S)	902.5 ± 3.54
Histidine (H)	**1093.33 ± 15.37**	Threonine (T)	478.5 ± 9.53
Isoleucine (I)	682.33 ± 3.21	*Valine* (V)	891.0 ± 2.82
Leucine (L)	959.67 ± 5.68	*Tryptophan* (W)	644.0 ± 8.48
Lysine (K)	944.33 ± 4.16	*Tyrosine* (Y)	372.0 ± 6.08

Amino acids having hydrophobic side chain are italicized.
Amino acids with notable capacity for silica solubilization are in bold.

hydrogen-bonded water that is amenable for further studies. Results from "quantitative assessment of carrier efficiency" are shown in Table III computed at 1000 μmoles.L^{-1} carrier and arranged in the order of efficiency. The results are also graphically displayed in Figure 4.

DISCUSSION

The results secured from the study of proteinous amino acids (Table I) brought out several novel facets of silica solubilization. The colorimetric

TABLE II Demonstration of the Enhancement of Solubility of Silicic Acid in Water Mediated by Small Molecules

Compound (1000 μmole.L^{-1}).	Solubilized silicic acid (μmole.L^{-1})
Blank	360.25 ± 7.09
Nitrogen containing compounds	
1-N hydroxyimidazole-3-N oxide	905 ± 8.9
4-hydroxy proline	884.33 ± 14.98
Imidazole	**1865.33 ± 28.9**
1, 2,4-triazole	713.6 ± 2.88
Urea	399.33 ± 8.08
Hydroxyl containing compounds	
3,4-dihydroxy phenylalanine (L-DOPA)	**1235 ± 26.87**
myo-**inositol**	**1624.25 ± 33.06**
Mannitol	**1406.6 ± 11.37**

Compounds with notable capacity for silica solubilization are in bold.

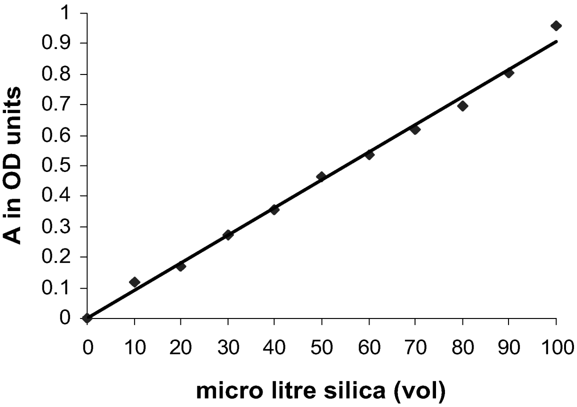

FIGURE 1 Standard Beer plot for silicon estimation.

procedure employed here largely gives values for monomeric silicic acid, since further oligomerization is stopped by the procedure developed.[24] Various factors such as size, time of contact, and agitation of the reaction mixture contribute to the solubility profile. For this reason, the results presented in Tables I and Table II are comparable, since they have been carried out under precisely standardized conditions.

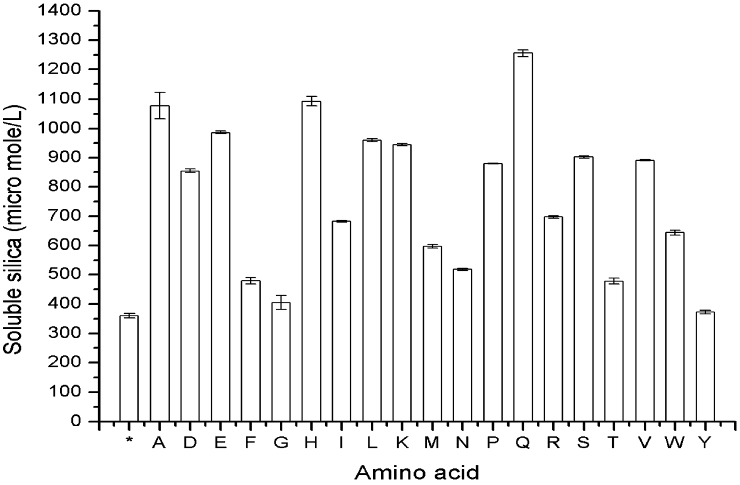

FIGURE 2 Solubilization of silicic acid (μ mole.L^{-1}) on addition of 1000 μ mole.L^{-1} of aminoacids. * = Blank. Abbreviations: A = alanine; D = aspartic acid; E = glutamic acid; F = phenylalanine; G = glycine; H = histidine; I = isoleucine; K = lysine; L = leucine; M = methionine; N = asparagine; P = proline; Q = glutamine; R = arginine; S = serine; T = threonine; V = valine; W = tryptophane; Y = tyrosine.

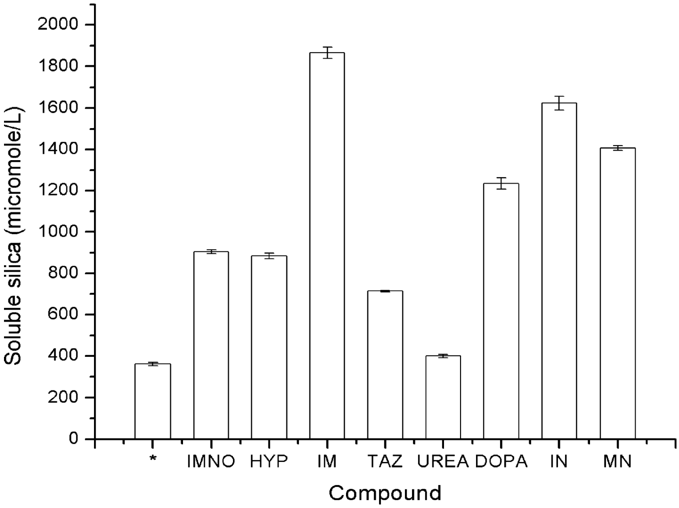

FIGURE 3 Solubilization of silicic acid (μmole.L^{-1}) on addition of 1000 μmole.L^{-1} of small molecules. * = Blank. Abbreviations: IMNO = 1-N hydroxyimidazole-3-N oxide; HYP = 4-hydroxy proline; IM = imidazole; TAZ = 1,2,4-triazole; DOPA = 3,4-dihydroxy phenylalanine; IN = *myo*-inositol; MN = mannitol.

The silica–water interface harbors an equilibrium largely consisting of silica and small amounts of the hydrolyzed product silicic acid.[25] We suggest that compounds such as imidazole, which have strong hydrogen bonding potential, can polarize the surface silica promoting hydrolysis. This factor, in addition to the fact that the silicic acid formed can be stabilized by hydrogen bonding as in imidazole (Figure 5) would promote the equilibrium in favor of silicic acid, thus leading to enhanced solubilization.

The variation of the solubility profile across the spectrum of proteinous amino acids (Table I) brings out the importance of the side chains. Therefore an examination of their influence in the process would be of interest.

Amino acids having a hydrophobic side chain are italicized in Table I. A very surprising result is that they exhibit significantly enhanced solubility. Since the studies are carried out in water, it is most likely that self-assembly of hydrophobic moieties can lead to an organized arrangement of polar ends. The trend of solubility also supports these hydrophobic associations. Alanine and leucine, for which such hydrophobic associations are well-established, showed substantial

TABLE III "Quantitative Assessment" of Carrier Efficiency in the Solubilization of Silica Under Ambient Conditions of Selected Molecules

Code	Carrier	1000 μmole.L^{-1} (mg)	Residue (per liter) (mg)	Less carrier (mg)	Efficiency[a]
A	Acetyl acetone	100	996	896	4.57
B	Formamide	45	844	799	4.07
C	*myo*-Inositol	180	828	648	3.30
D	Histidine	155	800	645	3.29
E	Diglyme	134	730	596	3.04
F	Diglyceryl silane	208	800	592	3.02
G	IMNO	100	688	588	3.0
H	EMNO	128	680	552	2.81
I	Glutamine	146	680	534	2.72
J	Lysine	146	654	508	2.59
K	Dimethyl glyoxime	116	620	504	2.57
L	Z-Ser-Ser-OMe	340	827	487	2.48
M	Glutamic acid	147	586	439	2.23
N	Imidazole	68	504	436	2.22
O	Citric acid	196	568	372	1.89
P	Tartaric acid	150	516	366	1.86
Q	Poly vinylalcohol	44	327	283	1.44

[a] Amount of silica carried (mg)/blank (196 mg).

IMNO: 1-N hydroxyimidazole-3-N oxide; EMNO: 2-ethyl-1-N hydroxyimidazole-3-N oxide.

increase in solubility. On the other hand, where such associations are not so easy as in the case of tryptophan, the efficiency becomes low. It is possible that, particularly in the case of leucine and alanine, the side chains orient the polar end groups to promote solubilization and at the same time enhance the stability of hydrogen-bonded structures with silicic acid by hydrophobic association.

Hydroxyl-containing amino acids, particularly serine, were implicated at one time in the biosilicification of diatoms.[26] Serine alone enhances silica solubility by a factor of 2.5, ironically less than that for alanine (Table I). It is interesting to note that substitution of hydrogen in serine with a methyl group to threonine nearly eliminates its ability as a carrier compared to blank values (Table I), suggesting that steric interactions play a role in the dissolution of silica. The huge enhancement in silica solubilization seen with 3,4-dihydroxy phenylalanine (DOPA), particularly when compared to tyrosine, which lacks a vicinal hydroxyl (Table I, Table II), seems to strongly suggest that two proximate hydroxyl groups of DOPA are likely to generate efficient mechanisms, which however need to be clarified. The amino acids

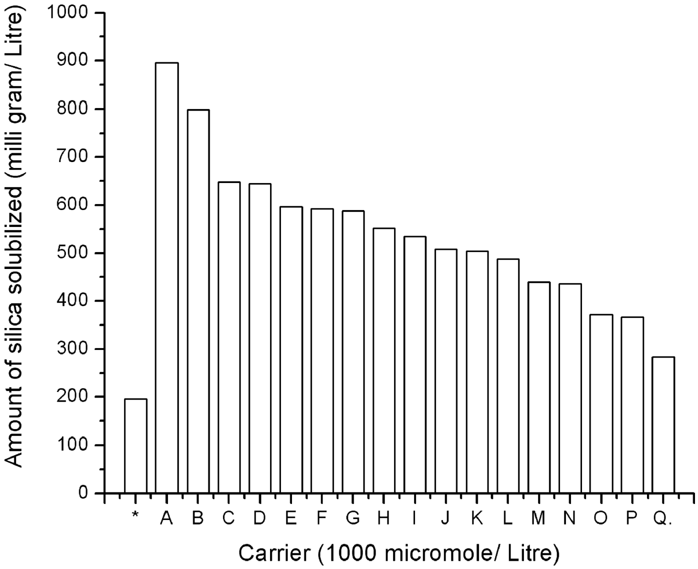

FIGURE 4 A gravimetric study of the solubilization of silica on addition of 1000 μmole.L^{-1} of carriers. * = Blank. Abbreviations: A = acetyl acetone; B = formamide; C = *myo*-Inositol; D = histidine; E = diglyme; F = diglyceryl silane; G = 1-N-hydroxyimidazole-3-N-oxide, H = 2-ethyl-1-N-hydroxyimidazole-3-N-oxide; I =glutamine; J = lysine; K = dimethyl glyoxime; L = Z-ser-ser-OMe; M = glutamic acid; N = imidazole; O = citric acid; P = tartaric acid; Q = poly vinylalcohol.

lysine and arginine are related to silaffins—a family of small proteins that have ability to polymerize silicic acid.[27] From Table I it can be seen that lysine promotes solubilization of silica and that of arginine with much less efficiency.

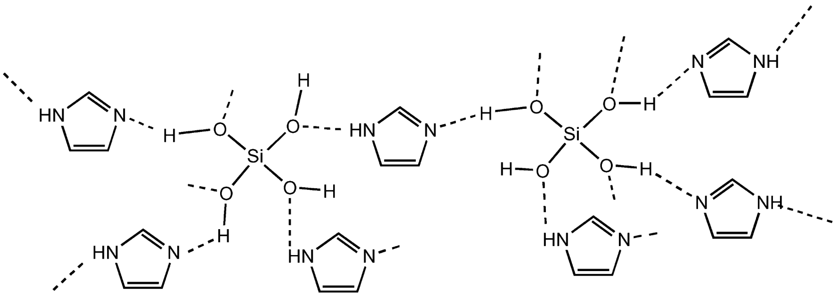

FIGURE 5 An energy minimized hypothetical profile for Si(OH)$_4$-imidazole hydrogen bonding network.

Surprisingly, the efficiency of glutamic acid and lysine to dissolve silica is almost the same (Table I). A finding of potential interest is that among all the proteinous amino acids, glutamine proved to be most efficient, particularly in the context of nor-analog asparagine, showing a much lower capacity. Among the homologous proteinous amino acid pairs, aspartic acid–glutamic acid, serine–threonine, and asparagine–glutamine, there is a consistent demonstration of the steric effects in silica solubilization.

Among the proteinous amino acids, histidine harboring an imidazole ring showed exceptional ability to promote silica solubilization (Table I). Indeed the imidazole ring itself proved to be most efficient in solubilization of silica of all the compounds studied thus far (Table II). It may be noted that in the mechanism proposed for solubilization using imidazole as an example (Figure 5), this ring plays the role in solubilizing silica as an efficient hydrogen bond donor/acceptor in the stabilization of silicic acid at equilibrium at the silica–water interface. Imidazole and other efficient carriers promote the equilibrium in favor of silicic acid by a dual mechanism involving favoring silica hydrolysis and its subsequent stabilization by hydrogen bonding. Several other nitrogen-harboring compounds showed limited ability to solubilize silica (Table II).

A significant outcome of this study is the notable ability of poly hydroxy compounds to solubilize silica, as could be exemplified with *myo*-inositol and mannitol (Table II). The solubilization of silica with efficiency by poly hydroxy compounds deserves further examination.

It may be noted that, whereas the colorimetric method provided a comparison of uptake of largely silicic acid by carrier, in the "quantitative procedure" the uptake is assessed as silica, to which the soluble carrier bound silicic acid, and its oligomers get transformed upon lyophilization.

The mechanical shaking for 2 days is expected to augment the silicic acid solubilization. During this period the carrier–silicic acid complex can condense to carrier–silica oligomers. When the reaction is terminated, the clear aqueous medium will have this milieu to a saturation point. These factors would increase the carrier efficiency (Table III).

An important criterion that needs to be established here for application of the carriers is optimum concentration under defined conditions, if any, in silica uptake. As could be seen from Table IV, there is an optimum value arrived at by the "quantitative assessment" procedure, which in the present set of cases lies in the range of 500–1000 μmole.L^{-1} in terms of efficiency (mg SiO_2 carried per μmole of carrier).

The quantitative assessment enables the delineation of possible empirical formula of such complexes. In a sample secured from

TABLE IV The Demonstration of Optimum Carrier Efficiency Under Specified Conditions

Carrier	μmole of carrier.L^{-1} 500	1000	1500	2000
	Amount of silica carried (mg)			
Imidazole	294	436	302	344
myo-Inositol	286	648	350	492
Histidine	393	645	637.5	600
IMNO	330	588	600	580
Acetyl acetone	490	896	950	950
Formamide	417.5	799	852.5	860
EMNO	356	552	528	494

IMNO: 1-N hydroxyimidazole-3-N oxide; EMNO: 2-ethyl-1-N hydroxyimidazole-3-N oxide.

lyophilization, there would be present the carrier, silica, and, in all probability, hydrogen-bonded water. The procedure involves at first the determination of water in a given sample of the complex by thermogravimetric analysis by monitoring the loss of water at 110°C. Since the carrier present in the total complex is known, the amount used in the thermogravimetric analysis can be computed. Therefore in a given sample, the amounts of carrier and water are known, so the difference comes out as the amount of silica. The steps described thus far leading to empirical formula are illustrated below.

Composition of complex:

$$\text{I. Carrier used} = \frac{\text{Total carrier added X sample used}}{\text{Total residue}} = \times \text{ mg}$$

$$\text{II. Water content} = \frac{\text{Sample used X water loss (\%)}}{100} = \text{y mg}$$

$$\text{III. Silica content} = \text{total sample used} - \text{x} - \text{y} = \text{z mg}$$

$$\therefore \text{mmoles of carrier used} = \frac{\text{x}}{\text{Molecular weight (mmole)}} = \text{x}'$$

$$\therefore \text{mmoles of water} = \frac{\text{y}}{\text{Molecular weight (mmole)}} = \text{y}'$$

$$\therefore \text{mmoles of silica} = \frac{\text{z}}{\text{Molecular weight (mmole)}} = \text{z}'$$

$$\therefore \text{Percentage molar composition of carrier} = \frac{\text{x}' \text{ X } 100}{(\text{x}'+\text{y}'+\text{z}')} = \text{x}''$$

$$\therefore \text{Percentage molar composition of water} = \frac{\text{y}' \text{ X } 100}{(\text{x}'+\text{y}'+\text{z}')} = \text{y}''$$

$$\therefore \text{Percentage molar composition of silica} = \frac{\text{z}' \text{ X } 100}{(\text{x}'+\text{y}'+\text{z}')} = \text{z}''$$

Division of the smallest quantity with the others would lead to the empirical formula.

TABLE V Calculation of Empirical Formulae Based on Thermogravimetric Analysis

Carrier	Total carrier used (mg)	Bulk residue (mg)	Sample used (mg) in TGA	% Water loss	Composition of complex
Blank	—	19.6	3.8	2.97	$(SiO_2)_{10}.\ H_2O$
Imidazole	6.8	50.4	1.5	11.76	$(SiO_2)_6.$ (IM).$(H_2O)_3$
Histidine	15.51	80.0	1.2	16.83	$(SiO_2)_{8.5}$.His. $(H_2O)_{7.5}$
Inositol	18	82.8	5.1	3.74	$(SiO_2)_{10}.$ (Inositol).$(H_2O)_2$
IMNO	10	68.8	10.6	0.47	$(SiO_2)_{54}$.(IMNO)$_6$ (H_2O)

IMNO = 1-N hydroxyimidazole-3-N oxide.

The amounts of silica carried in Table III also contain the amount of water taken up. In the calculation of empirical formulae, these have been separately identified and therefore the gravimetric ratio has higher value.

Table V gives details of the carrier used, bulk residue, sample used, % water loss, and computed composition of complex following the methodology outlined above.

The empirical formula represents the composition of the lyophilized product. The aqueous clear precursor solutions when lyophilized afford materials that are practically insoluble. Therefore, during the lyophilization, considerable oligomerization could have taken place. For empirical formulae presented in Table V, a large number of structures could be written based on the nature of silica polymers. The empirical formulae presented in Table V seem to support the view that silica solubilization is enhanced by hydrogen bonding association between silicic acid, the carrier, and water molecules.

TABLE VI IR Spectra (nujol) of Carrier–Silica Complexes[1]

S No	Compound	IR bands (cm^{-1})
1.	Blank	3514 (Si-OH), 3402 (Si-OH-H_2O)
2.	Imidazole	3143, 3120 (H-bonded imidazole complex)
3.	SiO_2. Imidazole Complex	3371 (SiOH—-t-Nitrogen), 3158 $(\mathrm{Si}\underset{\underset{\mathrm{H}}{|}}{\mathrm{O}}\text{—-}\mathrm{H}\underset{/}{\mathrm{N}}\text{—})$
4.	Histidine	3428 $(\text{—}\underset{\|}{\mathrm{N}}\text{—H—O—H})$
5.	SiO_2. Histidine Complex	3388 (SiOH——t-Nitrogen of Histidine)
6.	Inositol	3378 br 3236 br, 3166 br –OH absorption
7.	SiO_2 Inositol complex	3390 br Si—OH $\text{–}\overset{\overset{\mathrm{H}}{|}}{\mathrm{O}}$—inositol, Si—$\underset{\underset{\mathrm{H}}{|}}{\mathrm{O}}$—-H-O —inositol

[1] IMNO-Silica gave a complex set of peaks in the region 3406.23 cm^{-1}–3104.2 cm^{-1}

FTIR studies in nujol mull of silica complexes arising from histidine and *myo*-inositol in comparison with blank has been carried out. The interaction between carrier and silica takes place on the silica–water interface and most likely involves the Si–OH bond on the surface. Therefore the examination is confined to the 4000–3000 cm^{-1} region. Assignments have been made in accordance with a large number of data available relating to the IR spectra of the Si–OH grouping with compounds containing oxygen and nitrogen.[28–32] The IR spectra of the complexes presented in Table VI suggest strong hydrogen bonding between the carrier and silicic acid/silica/water molecules.

CONCLUSION

The major objective of this study, namely to identify small biocompatible molecules that enhance the solubilization of silica, has been accomplished, and has led to the application of promising carriers such as imidazole, histidine, lysine, glutamic acid, glycine, glutamine, 1 N-hydroxy imidazole 3 N-oxide, and *myo*-inositol in field studies in the growth of the rice plant leading to very promising results. Indeed this study is responsible for the large scale application of glycine and imidazole in field studies.

Within the limitations imposed relating to problems associated with chemistry of $Si(OH)_4$ and SiO_2, limited gains have been made based on the profile of molecules that enhance the silica solubilization. A novel mechanism for this process has been suggested. It is proposed that carriers not only promote the hydrolysis of silica but also stabilize the silicic acid formed by hydrogen bonding, as exemplified with imidazole. In terms of chemistry, only an empirical formula could be derived in certain cases, which clearly shows that a small number of carriers and water molecules can lead to complexation with a much larger number of silica molecules. Finally we feel that silica solubility studies of proteinous amino acids has provided data that could be useful.

EXPERIMENTAL

HPLC pure water and poly carbonate apparatus were used in all experiments. The compounds listed in Tables I–III, except for 1N-hydroxy imidazole 3N-oxide (IMNO) and 2-ethyl 1N-hydroxy imidazole 3N-oxide (EMNO), were prepared by known procedures.[33] Sand ($SiO_2 . nH_2O$), ammonium molybdate, L-tartaric acid, 1-amino-2 naphthol-4 sulfonic acid, Na_2SO_3, and $Na_2S_2O_3$ were purchased commercially. Sodium metasilicate ($Na_2SiO_3 . 9H_2O$), used in the construction of the Beer's plot, was also purchased commercially. UV measurements were carried out in Cintra 5 GBL scientific equipment at 800 nm. Aqueous samples were lyophilized using a Heto Power dry LL 3000 Lyophylizer using dry ice. IR spectra were recorded using a Thermo Nicolet nexus 670 spectrometer in nujol matrix. Thermogravimetric analysis was carried out in a Mettler Toledo Star System in the range of 25–800°C.

Colorimetric Estimation of $Si(OH)_4$ in Solution

A standardized procedure was followed for the estimation of silicic acid in water, based on the well-established method developed by Hartley and Jones.[24] The steps followed are briefly outlined below.

Stock solutions were used.[24]

A: Ammonium molybdate (7.58 g, 6.068 mmol) in 4 N H_2SO_4 (100 mL). **B:** L-tartaric acid (25 g, 166.66 mmol) in HPLC grade water (100 mL). **C:** 1-Amino-2-naphthol-4-sulfonic acid (0.2 g, 0.836 mmol), Na_2SO_3 (2.4 g, 19.04 mmol), and sodium meta bisulfate $Na_2S_2O_5$ (12 g, 63.11 mmol) in HPLC grade water (100 mL).

Standard Beer Plot

A standard solution was prepared by adding 0.050 g of $Na_2SiO_3.9H_2O$ in 10 mL HPLC grade water (containing 17.6 μmole silicon/1000 μL). Aliquots of 10 μL, 20 μL, 30 μL, 40 μL, 50 μL, 60 μL, 70 μL, 80 μL, 90 μL, and 100 μL were transferred to 10 volumetric flasks (25 mL), in each case diluted with 15 mL of HPLC grade water. Aliquots of 2.5 mL stock solution **A** were added to each of the flasks, left aside for 10 min, and admixed in each case with stock solution **B** (2.5 mL) followed by stock solution **C** (0.5 mL). The volumes were made up to 25 mL, and O.D. after 10 min recorded at 800 nm (Figure 1). From Figure 1, an average value of 0.510 O.D. units was found equivalent to 1 μmole of silicon.

Solubilization Studies of Potential Carriers

A mixture of sand (2 g) and HPLC grade water (100 mL) was hand shaken for 1 h, admixed with 100 μmole of solid carrier (except in blank run), and from the clear supernatant, 200 μL, 400 μL, 600 μL, 800 μL, and 1000 μL was withdrawn to separate 25 mL volumetric flasks and diluted with HPLC water (15 mL). Each of flasks was treated with 2.5 mL of stock solution **A**, left aside for 10 min, and admixed within each case stock solution **B** (2.5 mL) followed by stock solution **C** (0.5 mL).

The volumes were made up to 25 mL and O.D. were recorded after 10 min at 800 nm. In each case, a blank run with 100 μmole of carrier in the absence of sand was done, and the resulting O.D. blanks at all data points were subtracted for the entire run. The solubilization of silicic acid in water in terms of mean values from five readings is computed and is presented as μmoles/L (Tables I and II)[34]; the results are also presented graphically in Figures 2 and Figure 3.

A Novel Method for "Quantitative Assessment" of Carrier Efficiency

Sand (2 g) and HPLC grade water (100 mL) were mechanically shaken for 1 day and admixed with 100 μmoles of the carrier. The shaking was continued for another day, filtered, and in each case, aliquots of 25 mL of the clear filtrate was withdrawn and freeze-dried. A blank run was made without the added carrier. The carrier efficiency was computed in terms of enhancement of solubilized silica compared to the control (Table III and Figure 4).

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[34] The solubility in water of 31 potentially biocompatible carriers including blank has been carried out in the present study. The mean readings are only presented in the Tables; a full set of readings is available on request.