Abstract: The present invention disclosed a stabilized solution of ortho-silicic acid that serves as highly bioavailable silicon (Si) source consisting of: (i) ortho-silicic acid (H4Si04), from 0.01-8% w/w; (ii) carnitine salt (1) of pharmaceutically acceptable acids, formula (1), X= Cl, H2PO4, HSO4, NO3, CH3SO3, CH2=CH2SO3, and 4-CH3CH2H2SO3, from 7-40% w/w; (iii) pharmaceutically acceptable acid, from 0.05-4 molar equivalents to H4Si04; (iv) auxiliary stabilizer of H4Si04, selected from the group comprising: glycerol, 1, 2-propylene glycol, d-panthenol, glucosamine, or their mixtures, from 10-50% w/w; and (v) diluent, selected from the group consisting of purified water, ethanol, or their mixtures, in amounts of up to 100% w/w of overall formulation. The present invention discloses the preparation and the use of the formulation that provides all known positive therapeutic effects of ortho-silicic acid in human and animals, and benefits of use for plants.
STABILIZED SOLUTION OF ORTHO-SILICIC ACID, ITS PREPARATION AND USE

DESCRIPTION

Field of the invention

The present invention relates to a stabilized solution of ortho-silicic acid (H₄SiO₄), which is used as nutritional and therapeutic source of silicon (Si) in nutrition, medicine, cosmetic, veterinary and agriculture.

Summary of the invention

The present invention solves the technical problem of stabilization of ortho-silicic acid (H₄SiO₄) solution, and procedure for production of such stabilized solution.

The solution is consisting of:

(i) ortho-silicic acid (H₄SiO₄), from 0.01-8% w/w;
(ii) carnitine salt (1) of pharmaceutically acceptable acids,

\[
\left(\frac{\text{H}_3\text{C}}{\text{N}}\right)^{+}\text{OH} \rightarrow \text{COOH} \rightarrow X^{-}
\]

\[X=\text{Cl}, \text{H}_2\text{PO}_4, \text{HSO}_4, \text{NO}_3, \text{CH}_3\text{SO}_3, \text{C}_3\text{H}_5\text{SO}_3, 1,4-\text{CH}_3\text{C}_6\text{H}_4\text{SO}_3\]

from 7-40% w/w;

(iii) pharmaceutically acceptable acid, from 0.05-4 molar equivalents to H₄SiO₄;

(iv) auxiliary stabilizer of H₄SiO₄, selected from the group comprising glycerol, 1,2-propylene glycol, d-panthenol, glucosamine, or their mixtures, from 10-50% w/w; and

(v) diluent, selected from the group consisting of purified water, ethanol or their mixtures, in amounts of up to 100% w/w of overall formulation.
Prior art

Silicon (Si) is important biogenic microelement which exhibits several roles in human and animal organism:


(iv) stimulates immune system [A. Schiano, F. Eisinger, P. Detolle: Silicium, tissu osseux et immunite, Revue du Rhumatisme 46 (1979) 483];


calves with stabilised orthosilicic acid effect on the Si, Ca, Mg and P concentration in serum and the collagen concentration in skin and cartilage, Biol. Trace Element Res. 56 (1997) 153;

(viii) stimulates growth and improves strength and shine of hair and nails [A. Lassus: Colloidal silicic acid for oral and topical treatment of aged skin, fragile hair and brittle nails in females, J. Int. Med. Res. 21 (1993) 209]; and


At plants, silicon exhibits the following effects [H. A. Currie, C. C. Perry: Silica in Plants: Biological, Biochemical and Chemical Studies, Ann. Botany 100 (2007) 1383-1389]:

(i) stimulates photosynthesis process and increases utility of nutrients what results in enhanced crop yields;

(ii) improves water management and thus enhances resistance to stress conditions like drought; and

(iii) enhances resistance to insect attacks and fungal diseases.

Biologically available source of silicon is ortho-silicic acid ($\text{H}_4\text{SiO}_4$).
It is known to those skilled in the art that silicic acid, in its monomeric form, ortho-silicic acid (\(\text{H}_3\text{SiO}_4\)) is not stable, but at higher concentrations undergoes polymerization yielding dimeric (\(\text{H}_6\text{Si}_2\text{O}_7\)), trimeric (\(\text{H}_8\text{Si}_3\text{O}_{10}\)), as well as linear unbranched oligomers (SI) which are all water soluble. Linear polymers of silicic acid (SI) undergo further polymerization giving tridimensional, branched polymers (S2) which are of very low water solubility and give opalescent gel. The process of polymerization proceeds further with generation of hydrated silicon dioxide (silica gel; \(\text{SiO}_2-\text{xH}_2\text{O}\)). The course of polymerization of silicic acid is given in Scheme 1, at the end of the specification.

Beside monomeric ortho-silicic acid (\(\text{H}_3\text{SiO}_4\)), biologically available forms are also its lower oligomers that are soluble in water; they release starting \(\text{H}_3\text{SiO}_4\) by hydrolysis (oligomerization is reversible). In other words, at certain concentrations, an equilibrium between monomeric ortho-silicic acid and its lower oligomers is established.


The use of natural, as low as possible refined food (e.g. whole grain cereals), usually provides sufficient intakes of silicon to organism. However, at the use of highly refined and unhealthy food, deficiency of silicon can take place. Such conditions, with eventual accompanied factors, often can cause development of diseases or disorders connected with silicon deficiency.

Because of this reason, development of stabilized form of ortho-silicic acid, wherein its polymerization is inhibited, and
consequently increased its bioavailability, is of a great importance.

Such products can be used as effective food supplements or therapeutic agents at such diseases or disorders. For the use in nutrition, medicine, and cosmetic, there are included only pharmacologically acceptable forms of silicic acid.

In agriculture, therein also only non-toxic forms of silicic acid of high bioavailability can be employed.

The most known product which is used as food supplement for silicon supplementation is "BioSil®" which is based on choline chloride (2)-stabilized ortho-silicic acid [S. R. Bronder, WO 95/21124; V. Berghe, D. A. Richard, EP 1 371 289 A1 (2002), BioPharma Sciences B.V., Belgium].

\[
\frac{\text{(H}_3\text{C})_3\text{N}}{\text{OH}} \text{Cl}^- \quad 2
\]

Additionally, in patent literature there are disclosed other, mainly as auxiliary, stabilizers that inhibit polymerization of ortho-silicic acid such as: boric acid (H₃B₅O₇) or sodium tetraborate natrijev tetraborat (Na₃B₅O₇·10H₂O) [L. J. Clapsdale, M. G. Syracuse: Nongelling aqueous silica sols stabilized with boron compounds, US 2,630,410 (1953); Union Carbide Co.]; H₃B₅O₇ in the presence of humectants like polyethylene glycol, urea, sorbitol; then polysorbates; pectin; ethoxylated higher fatty acids; acetylated or hydroxypropyl-starch; starch phosphate; maltitol; vitamins [W. A. Kros, US Patent application 2006/0178268 A1]; amino acids proline, serine, lysine, arginine, glycine or their mixtures; polypeptides or protein hydrolyzates [V. Berghe, D. A. Richard, WO 2004/016551 A1, BioPharma Sciences B.V.]; M.-C. Seguin, J. Gueyne: Complex containing biologically assimilable orthosilicic acid, which is under solid form, stable and concentrated, and a process for preparation of said complex, US Patent 6,335,457 B1 (2002) Exsymol S.A.M., Monako]; and calcium chloride (CaCl₂) in combination with
choline chloride or betaine [V. Berghe, D. A. Richard, WO 2003/077657 Al, Bio Pharma Sciences B.V.].

Partially polymerized forms of silicic acid (of nano-sized particles) were stabilized with strong inorganic acids in the presence of methyl sulfonilmethane (CH₃SO₂CH₃) or dimethylsulf oxide (CH₃SOCH₃) and humectants like 1,2-propylene glycol or polyethylene glycol 400 (PEG-400) [I. Suvee, G. Tourgis: Hydronium stabilized and dissoluble silicic acid nanoparticles : Preparation, stabilization and use, WO 2009/127256 Al (2008)].


Beside choline chloride-stabilized ortho-silicic acid (H₄SiO₄), on the market there can be find various food supplements containing silicon in the forms of amorphous or colloidal silicon dioxide (SiO₂)- The latter is also called „silicic acid“, despite the fact that it is actually an anhydride of silicic acid. Such products are characterized by very low bioavailability [R. Jugdaohsingh : Silicon and bone health, J. Nutr. Health Aging 11 (2007) 99].

Alternative and slightly more effective (bioavailable) sources of silicic acid are various plant drugs like extracts of horsetail (Equisetum arvense), nettle (Urtica dioica), and some other plants. However, it is known that portion of soluble (and thus bioavailable) silicic acid from these healing plants usualy does not exceed 10% w/w. All other silicic acid is insoluble and thus not biologically

In agriculture, the products based on silicon are used for increasing of resistance to stress (at drought and hail) and fungal diseases. Widely known products contain extract of horsetail (Equisetum arvense) or milled quartz sand (silicon dioxide; SiO₂) in organic, and solution of potassium silicate (30% w/w K₂SiO₃) in conventional agriculture (most often in wine growing: e.g. „Sil-Matrix®”). Such products are usually employed by foliar application.

The technical problem of effective stabilization of ortho-silicic acid (H₄SiO₄):

(i) at low pH value (stabilization of solution from the present invention) ; as well as

(ii) at physiological conditions (close to pH= 7; where the rate of its polymerization is drastically reduced, and consequently increases its bioavailability) ; is solved on a new and effective manner as will be demonstrated in detailed description of the invention.

**Detailed description of the invention**

The present invention involves improved formulation of stabilized solution of ortho-silicic acid (H₄SiO₄) which is used in nutrition, medicine, cosmetic, veterinary, or agriculture as effective source of highly bioavailable silicon (Si).

The solution is consisting of the following ingredients:

(i) ortho-silicic acid (H₄SiO₄), from 0.01-8% w/w;

(ii) carnitine salt (1) of pharmaceutically acceptable acids,
X = CI, H₂PO₄, HSO₄, NO₃, CH₃SO₃, C₆H₅SO₃, 1,4-CH₃C₆H₄SO₃
from 7-40% w/w;
(iii) pharmaceutically acceptable acid, from 0.05-4 molar equivalents to H₄SiO₄;
(iv) auxiliary stabilizer of H₄SiO₄, selected from the group comprising: glycerol, 1,2-propylene glycol, D-panthenol, glucosamine, or their mixtures, from 10-50% w/w; and
(v) diluent, selected from the group consisting of purified water, ethanol or their mixtures, in amounts of up to 100% w/w of overall formulation.

Since enantiomeric form of carnitine does not have any impact on stabilization of H₄SiO₄, herein mentioned salts can be derivatives of racemic DL-carnitine or enantiomerically pure L- or D-carnitine.

Carnitine salt is selected from the group consisting of: carnitine hydrochloride (la; X = CI), carnitine dihydrogenphosphate (lb; X = H₂PO₄), carnitine hydrogensulfate (lc; X = HSO₄), carnitine nitrate (Id; X = NO₃), carnitine methanesulfonate (le; X = CH₃SO₃), carnitine benzenesulfonate (lf; X = C₆H₅SO₃), carnitine p-toluenesulfonate (lg; X= 1,4-C₆H₄SO₃), or mixtures of these substances.

Pharmaceutically acceptable acid which is used in the solution from the present invention is selected from the same group, where the advantage is given to phosphoric acid (H₃PO₄), because it was found that H₃PO₄ additionally stabilizes ortho-silicic acid (H₄SiO₄), presumable by inhibition of its polymerization (see Table 1).

Glucosamine as auxiliary stabilizer is used either in the form of free base, or corresponding salt of pharmaceutically acceptable acid such as sulphuric (H₂SO₄), phosphoric (H₃PO₄), hydrochloric (HCl), or other above-mentioned acid.
Unexpectedly, it was found that carnitine salts like carnitine hydrochloride (Ia), effectively act as stabilizers of ortho-silicic acid (H₄SiO₄) at low pH values (acidic medium).

\[
\text{[CH₃C₃N(OH)COOH]⁺ Cl}^-
\]

In this manner, solutions of ortho-silicic acid of concentration of 2-4% w/w, prepared by hydrochloric acid (HCl)-catalysed hydrolysis of tetraethyl orthosilicate [TEOS; Si(OCH₂CH₃)₄], are stable at room temperature (20-25 °C) for 2-3 months. During storage, slow polymerization occurs (as given in Scheme 1) with formation of opalescent gel.

In contrast, carnitine hydrochloride (Ia) in concentration from 7-40% w/w does act as stabilizer of ortho-silicic acid (H₄SiO₄) due to the fact that solutions of analogous concentrations do not undergo gelling, e.g. polymerization, even after 2 years storage at room temperature.

Mechanism of stabilizing action of carnitine salts such as hydrochloride Ia on H₄SiO₄ in acidic medium is presumably analogous to the same action of choline chloride, which is known stabilizer from the literature; this effect obviously includes the impact of "deep eutectic liquid" property of these compounds when they are in mixture with suitable hydrogen bond donors like glycerol [S. R. Bronder, US Patent 5,922,360 (1999)].


However, choline chloride (2) destabilizes ortho-silicic acid ($\text{H}_4\text{SiO}_4$) at pH conditions that are close to physiological (around 7). Moreover, choline chloride in these conditions does catalyze polymerization of $\text{H}_4\text{SiO}_4$ (see Table 1), what actually decreasing its bioavailability.

Completely unexpected, it was found that carnitine hydrochloride (la), in contrast to choline chloride (2), under physiological conditions close to pH=7, does not destabilize ortho-silicic acid significantly, and thus represents an important improved version:

(i) of „deep eutectic liquid” which does stabilize $\text{H}_4\text{SiO}_4$ in acidic pH medium of the solution from the present invention; as well as

(ii) in the same time does not influence negatively (does not destabilize) the stability of $\text{H}_4\text{SiO}_4$ under physiological conditions (pH values around 7), and in this manner does not decrease its bioavailability.

The effect was found and studied on a model solution of DL-($\pm$)-carnitine hydrochloride (la) and ortho-silicic acid ($\text{H}_4\text{SiO}_4$), prepared by hydrolysis of tetraethyl orthosilicate [TEOS; Si(OC$_2$H$_5$)$_4$] in the presence of phosphoric acid ($\text{H}_3\text{P}0_4$). The hydrolysis reaction of TEOS with formation of the complex of $\text{H}_4\text{SiO}_4$ and la in molar ratio of 1:1, compound 3a, is given in Scheme 2, end of specification.

For the purpose of study of the effect of DL-($\pm$)-carnitine hydrochloride on stabilization of $\text{H}_4\text{SiO}_4$, as controls, the following samples are prepared:

(i) a standard solution of $\text{H}_4\text{SiO}_4$ of concentration of 1% w/w Si; and

(ii) a solution of analogous complex of $\text{H}_4\text{SiO}_4$ with choline chloride (2) with the same molar ratio (1:1).
The study of stabilizing effect was carried out under conditions that are known to lead fast polymerization of ortho-silicic acid (H₄SiO₄), and these are at pH values close to neutral. At such conditions, pH= 6-7, relatively fast polymerization of H₄SiO₄ takes place, with formation of its polymers in the form of opalescent gel. In more concentrated systems, this change, from the phase of solution (which is, at the beginning, clear and then opalescent) to the phase of (opalescent) gel is relatively fast, so it can be employed for analytical purpose for determination of gelling (polymerization) rate of H₄SiO₄.

Test solutions are prepared by mixing equal volume of solution of complex 3a (or complex of choline chloride or standard solution of pure H₄SiO₄ of the same concentration) and 1.32M phosphate buffer of pH= 7. For these test solutions it was determined the time required for change from the moment of mixing (clear solution) to the formation of opalescent gel. This time was termed as gelling (or polymerization) time (tₓ). Longer gelling time (tₓ) means slower polymerization what suggests to more stable complex. Results are given in Table 1.

Table 1. The effect of choline chloride (2) and DL-(±)-carnitine hydrochloride (1a) on stabilization of ortho-silicic acid (H₄SiO₄) in solution at pH= 6.5.¹

<table>
<thead>
<tr>
<th>No.</th>
<th>Composition of sample solution</th>
<th>Gelling time tₓ [min]²</th>
<th>Relative stability³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>H₄SiO₄ (1% w/w Si)⁴</td>
<td>170</td>
<td>1</td>
</tr>
<tr>
<td>Prior art, complex with choline chloride:⁵</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>H₄SiO₄ (1% w/w Si) + 1 mol. equiv. choline chloride</td>
<td>35</td>
<td>0.21</td>
</tr>
<tr>
<td>The present invention, complex with carnitine hydrochloride:⁶</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>H₄SiO₄ (1% w/w Si) + 1 mol. equiv. DL-(±)-carnitine hydrochloride</td>
<td>165</td>
<td>0.97</td>
</tr>
</tbody>
</table>
a Test solution was prepared by mixing 2 mL of sample solution and 2 mL of 1.32 M phosphate buffer of pH= 7. Final pH value of all test solutions after mixing of corresponding sample solution with the buffer was 6.5.

b Time from the moment of mixing the sample solution with phosphate buffer (clear solution) until the formation of opalescent gel, expressed in minutes.

c „Relative stability“ expressed as numerical parameter, coefficient which describes stability of ortho-silicic acid in the given sample in comparison with the standard (solution of $H_4SiO_4$). Shows stabilizing or destabilizing effect on ortho-silicic acid, and its polymerization (gelling).

d The standard was prepared by addition of TEOS (1.2 mL; 1.12 g; 0.0054 mol) to a solution of 85% phosphoric acid (0.2 mL; 0.34 g; 0.289 g $H_3PO_4$; 0.0029 mol; 0.55 mol. equiv.) in distilled water (10.00 g) followed by stirring at room temperature for 3 h, with subsequent dilution with distilled water up to the total weight of 15.00 g [contains 150 mg (1% w/w) of Si].

e Sample solutions were prepared by addition of 0.0054 mol of choline chloride (2; 0.75 g), or DL- (2S) -carnitine hydrochloride (1a; 1.06 g), and then TEOS (1.2 mL; 1.12 g; 0.0054 mol) to a solution of 85% phosphoric acid (0.2 mL; 0.34 g; 0.289 g $H_3PO_4$; 0.0029 mol; 0.55 mol. equiv.) in distilled water (10.00 g). Reaction mixture was stirred at room temperature for 3 h, and then diluted with distilled water to overall weight of 15.00 g [contains 150 mg (1% w/w) of Si].

From thus obtained results it is clear that choline chloride (2) drastically destabilizes ortho-silicic acid ($H_4SiO_4$) at pH values close to physiological (pH= 6.5), because the gelling time was approx. 5.5 times shorter than in the case of the standard (Experiment 2 versus Experiment 1). This suggests approx. 5.5 times faster polymerization affected by choline chloride; it not only destabilizes $H_3SiO_4$, but moreover does act as catalyst of its polymerization.
Choline chloride can be obviously considered as "stabilizer" of silicic acid in a formulation with very low pH, lower than pH = 3, in technological sense (as excipient), helping stabilization of final product based on H₄SiO₄, to ensure prolonged shelf life.

In contrast, DL-(±) -carnitine hydrokloride (1a) does not influence significantly on rate of polymerization of ortho-silicic acid (H₄SiO₄), where observed gelling time was only 3% shorter than for the standard (Experiment 3 against Experiment 1), what can be considered as acceptable difference within the limits of experimental error which are normally for this method up to approx. 5%.

In continuation of the study, it was found that not all strong mineral acids influence in the same manner to the stability of ortho-silicic acid (H₄SiO₄). Despite that in initial experiments, hydrochloric acid (HCl) was employed as classical agent for acidification and regulation of pH in pharmaceutical products, phosphoric acid (H₃PO₄) appeared to exhibit significant additional stabilization effect against polymerization of H₄SiO₄ (Table 2).

**Table 2.** Study of influence of pharmaceutically acceptable acids on stability of ortho-silicic acid (H₄SiO₄) at pH = 6.5. a

<table>
<thead>
<tr>
<th>No.</th>
<th>Composition of sample solution</th>
<th>Gelling time t₀ [min] b</th>
<th>Relative stability c</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>H₄SiO₄ (1% w/w Si) + HCl d</td>
<td>115</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>H₄SiO₄ (1% w/w Si) + H₃PO₄ c</td>
<td>160</td>
<td>1.4</td>
</tr>
</tbody>
</table>

a Test solution was prepared by mixing 2 mL of sample solution and 2 mL of 1M phosphate buffer of pH = 8.5; pH values of test solutions after mixing of the sample solutions and buffer were the same (pH = 6.5).

b The time from the moment of mixing of the test solution with phosphate buffer (clear solution) until the formation of opalescent gel, expressed in minutes.
"Relative stability" expressed as numerical parameter, coefficient which describes stability of ortho-silicic acid in the given sample in comparison with the standard (solution of \( \text{H}_4\text{SiO}_4 \)). Shows stabilizing or destabilizing effect on ortho-silicic acid, and its polymerization (gelling).

These solutions were prepared by addition of TEOS (1.2 mL; 1.12 g; 0.0054 mol) to a solution of 85% phosphoric acid (0.2 mL; 0.34 g; 0.289 g \( \text{H}_3\text{PO}_4 \); 0.0029 mol; 0.55 mol. equiv.) or 37% hydrochloric acid (0.25 mL; 0.30 g; 0.11 g HCl; 0.003 mol; 0.55 mol. equiv.) in distilled water (10.00 g) at room temperature during 3 h, with subsequent dilution with distilled water up to total weight of 15.00 g [contains 150 mg (1% w/w) of Si].

From thus obtained results it was concluded that phosphoric acid (\( \text{H}_3\text{PO}_4 \)) in the same concentration provides approx. 40% longer gelling time, this means slower polymerization than analogous solution where hydrochloric acid (HCl) was employed.

Furthermore, effect of humectants, which were described in the literature like 1,2-propylene glycol, glycerol, sorbitol, and polyethylene glycol (PEG)-400, and also substances which have not been described (as stabilizers) : d-panthenol (4) and glucosamine (5), on stability of ortho-silicic acid (\( \text{H}_4\text{SiO}_4 \)) in the presence of DL- (±) -carnitine hydrochloride.

![Chemical structures](image)

The study was conducted in analogous manner with the use of 1M phosphate buffer of pH= 8.5. Results are presented in Table 3.

**Table 3.** The study of influence of auxiliary stabilizer on polymerization (gelling) rate of ortho-silicic acid (\( \text{H}_4\text{SiO}_4 \)) in
solution at pH= 6.5 in the presence of DL-(+)-carnitine hydrochloride.

<table>
<thead>
<tr>
<th>No.</th>
<th>Auxiliary stabilizer (20% w/w)</th>
<th>Gelling time $t_c$ [min]$^b$</th>
<th>Relative stability$^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-$^d$</td>
<td>70</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>glycerol</td>
<td>80</td>
<td>1.14</td>
</tr>
<tr>
<td>3</td>
<td>1,2-propylene glicol</td>
<td>120</td>
<td>1.71</td>
</tr>
<tr>
<td>4</td>
<td>sorbitol</td>
<td>70</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>PEG-400</td>
<td>47</td>
<td>0.67</td>
</tr>
<tr>
<td>6</td>
<td>glucosamine$^e$</td>
<td>95</td>
<td>1.36</td>
</tr>
<tr>
<td>7</td>
<td>d-panthenol</td>
<td>110</td>
<td>1.57</td>
</tr>
</tbody>
</table>

$^a$ Composition of the sample solutions (% w/w): 3.5% $\text{H}_4\text{SiO}_4$ (1% of Si), 7% carnitine hydrochloride (1 mol. equiv. / $\text{H}_4\text{SiO}_4$), 6.6% ethanol, 20% auxiliary stabilizer, 1.9% $\text{H}_3\text{PO}_4$ (0.55 mol. equiv. / $\text{H}_4\text{SiO}_4$), and up to 100% distilled water.

Test solution was prepared by mixing 2 mL of sample solution with 2 mL of 1M phosphate buffer of pH= 8.5; pH values of all test solutions after mixing a corresponding sample solution and buffer were the same (6.5).

$^b$ Time from the moment of mixing of sample solution with the phosphate buffer (clear solution) until the formation of opalescent gel, expressed in minutes.

$^c$ "Relative stability" expressed as numerical parameter, coefficient which describes stability of ortho-silicic acid in the given sample in comparison with the standard (solution of $\text{H}_4\text{SiO}_4$). Shows stabilizing or destabilizing effect on ortho-silicic acid, and its polymerization (gelling).

$^d$ Instead auxiliary stabilizer, in this sample solution was added 20% w/w more distilled water.

$^e$ Since pH value is in acidic region, as source of glucosamine was employed glucosamine sulfate.

The results showed that the claim "humectants (as such) do additionally stabilize ortho-silicic acid ($\text{H}_4\text{SiO}_4$) because of

It was observed that polyethylene glycol (PEG-400) does destabilize H₄SiO₄, where observed gelling time was more than 30% shorter than that for the standard (Table 3; Experiment 5 / Experiment 1).

Sorbitol does not exhibit any significant influence on stability of H₄SiO₄ in the presence of carnitine hydrochloride; gelling time (t₀) was the same as for the standard (Table 3; Experiment 4 / Experiment 1).

At other auxiliary stabilizers activity increases in the following order (see Table 3):

(i) glycerol (+14%; Experiment 2 / Experiment 1);
(ii) glucosamine (+36%; Experiment 6 / Experiment 1);
(iii) d-panthenol (+57%; Experiment 7 / Experiment 1);
(iv) 1,2-propylene glycol (+71%; Experiment 3 / Experiment 1).

Finally, it appeared that the combination of:

(i) DL- (±) -carnitine hydrochloride (1a);
(ii) pharmaceutically acceptable acid, among them phosphoric acid (H₃PO₄) is preferred; and
(iii) auxiliary stabilizer: glycerol, glucosamine, d-panthenol, and 1,2-propylene glycol;

does stabilize ortho-silicic acid (H₄SiO₄), both at low pH values (acidic range) of the solution from the present invention, as well as in physiological conditions (pH around 7), in unexpected manner.

Study of effect of whole formulation of the present invention [combination of (i)-(iii)] was performed on analogous manner, by determination of gelling (polymerization) time with the use of 1M phosphate buffer of pH= 8.5. As the control probe, the solution from the prior art based on mixture of choline chloride and glycerol was
studied, however, with the same concentration of silicon, in order to provide comparable results. Results are given in Table 4.

Table 4. The study of solution composition on inhibition of gelling (polymerization) of ortho-silicic acid ($\text{H}_4\text{SiO}_4$) in solution at pH = 6.5.

<table>
<thead>
<tr>
<th>No.</th>
<th>Composition of sample solution</th>
<th>Gelling time $t_g$ [min]$^b$</th>
<th>Relative stability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>The concept from the prior art: $^d$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>choline chloride + glycerol</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>The present invention: $^e$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>DL-$(\pm)$-carnitine hydrochloride + glycerol</td>
<td>150</td>
<td>50</td>
</tr>
<tr>
<td>3</td>
<td>DL-$(\pm)$-carnitine hydrochloride + glucosamine sulfate</td>
<td>60</td>
<td>20</td>
</tr>
<tr>
<td>4</td>
<td>DL-$(\pm)$-carnitine hydrochloride + d-panthenol</td>
<td>120</td>
<td>40</td>
</tr>
<tr>
<td>5</td>
<td>DL-$(\pm)$-carnitine hydrochloride + 1,2-propylene glycol</td>
<td>210</td>
<td>70</td>
</tr>
</tbody>
</table>

$^a$ The test solutions were prepared by mixing 2 mL of each of sample solution with 2 mL of 1M phosphate buffer pH = 8.5; pH values of all test solutions were corrected to pH = 6.5 by addition of small amounts of anhydrous sodium carbonate.

$^b$ The time from the moment of mixing of the given sample solution with the phosphate buffer (clear solution) until the formation of opalescent gel, expressed in minutes.

$^c$ "Relative stability" expressed as numerical parameter, coefficient which describes stability of ortho-silicic acid in the given sample in comparison with the standard (solution of $\text{H}_4\text{SiO}_4$). It shows stabilizing or destabilizing effect on ortho-silicic acid, and its polymerization (gelling).
The solution of analogous composition like the product "BioSil®" was prepared by addition of TEOS (1.2 mL; 1.12 g; 0.0054 mol) to a solution of 85% phosphoric acid (0.2 mL; 0.34 g; 0.289 g H₃PO₄; 0.0029 mol; 0.55 mol. equiv.) and choline chloride (7.05 g; 47% w/w) in a mixture of distilled water (3.00 g) and glycerol (2.85 g; 19% w/w) with stirring at room temperature for 3 h, with subsequent dilution with distilled water (0.64 g) up to the total weight of 15.00 g [contains 150 mg (1% w/w) of Si].

The solutions were prepared by addition of TEOS (1.2 mL; 1.12 g; 0.0054 mol) to a solution of 85% phosphoric acid (0.2 mL; 0.34 g; 0.289 g H₃PO₄; 0.0029 mol; 0.55 mol. equiv.) and DL-(+)-carnitine hydrochloride (3.75 g; 25% w/w) in a mixture of distilled water (6.00 g) and auxiliary stabilizer (3.00 g; 20% w/w) followed by stirring at room temperature for 3 h, and subsequently diluted with distilled water (0.79 g) up to the total weight of 15.00 g [sadrzi 150 mg (1% w/w) Si].

From thus obtained results, it is clear to those skilled in the art that the solution from the present invention exhibits drastically enhanced effects of stabilization of ortho-silicic acid (H₄SiO₄) at physiological pH values. Observed gelling (polymerization) times of H₄SiO₄ were 20-70x longer than the same value for analogous solution from the prior art.

The best version of the solution from the present invention is based on the combination of carnitine salt (like carnitine hydrochloride), 1,2-propylene glycol and phosphoric acid (Table 4, Experiment 5). Analogous solution with glycerol and d-panthenol showed somewhat weaker stabilizing effect. The version of the formulation with glucosamine (as sulfate) showed the weakest effect, but even this was 20x stronger than is the case at the solution based on choline chloride from the prior art.

Finally, it was found that the kind of carnitine salt, which is used in the formulation from the present invention, does not have significant effect. The study of the kind of anion of carnitine salt
on stability of H₄SiO₄ was carried out analoguously, by determination of gelling (polymerization) time with the use of 1M phosphate buffer of pH = 8.5. Results are given in Table 5.

**Table 5.** Study of the kind of carnitine salt on inhibition of gelling (polymerization) of ortho-silicic acid (H₄SiO₄) in solution at pH = 6.5.

<table>
<thead>
<tr>
<th>No.</th>
<th>Composition of sample solution</th>
<th>Gelling time tₛ [min]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>carnitine hydrochloride + HCl</td>
<td>95</td>
</tr>
<tr>
<td>2</td>
<td>carnitine dihydrogenphosphate + H₃PO₄</td>
<td>140</td>
</tr>
<tr>
<td>3</td>
<td>carnitine hydrogensulfate + H₂SO₄</td>
<td>100</td>
</tr>
<tr>
<td>4</td>
<td>carnitine hydrochloride + H₃PO₄</td>
<td>120</td>
</tr>
</tbody>
</table>

*The composition of the sample solutions (% w/w): 3.5% H₄SiO₄ (1% Si); 5.8% carnitine base; 1.5 molar equiv. / Si of corresponding acid (Experiment 1: HCl; Experiment 2: H₃PO₄; Experiment 3: H₂SO₄); 6.6% ethanol; and up to 100% distilled water.

The composition of the sample solution in the Experiment 4 (% w/w): 3.5% H₄SiO₄ (1% Si); 7% carnitine hydrochloride; 1.9% H₃PO₄ (0.5 mol. equiv. / H₄SiO₄); 6.6% ethanol, up to 100% distilled water.

Test solutions were prepared by mixing 2 mL of each of sample solution with 2 mL of 1M phosphate buffer pH = 8.5; pH values of all test solutions after mixing of each of the sample solutions and the phosphate buffer were the same, pH = 6.5.

The time from the moment of mixing of a given sample solution with the phosphate buffer (clear solution) until the formation of opalescent gel, expressed in minutes.

From thus obtained results it is clear that the kind of anion from carnitine salt has some effect on stability of H₄SiO₄, however, none of them did not exhibit significant negative (destabilizing) effect.
The best effect showed carnitine dihydrogenphosphate, or alternatively, the combination of equimolar quantities of carnitine base and phosphoric acid (Table 5; Experiment 2).

In the case where the work with large amounts of acids wants to be avoided, for industrial purpose, the most convenient version is the use of carnitine hydrochloride (commercially available) and phosphoric acid (Table 5; Experiment 4).

**Explanation of stabilizing effect of the formulation from the present invention on ortho-silicic acid (H₅SiO₄).** Unexpected effect of the formulation from the present invention.

A key unexpected effect of carnitine salt on stability of ortho-silicic acid is obviously based on possibility of forming relatively stable complexes like compound 3a (Scheme 2).

In the patent application WO 95/21124, which discloses stabilized solution of ortho-silicic acid (H₅SiO₄) based on choline chloride (2) as stabilizer in strongly acidic medium, there are mentioned quaternary ammonium salts (where belongs choline chloride itself) as stabilizers of H₅SiO₄. The person skilled in the art can consider carnitine salts from the present invention also as “quaternary ammonium salts” that are mentioned “generically” in the prior art, indeed.

However, until the studies described in the present invention, the person skilled in the art could not know that stabilizing effect of carnitine salts (like carnitine hydrochloride) would be substantially different than at choline chloride as sole really disclosed stabilizer of H₅SiO₄ from the category of “quaternary ammonium salts”.

Precisely, in the present invention it has been disclosed that choline chloride obviously does stabilize H₅SiO₄ in acidic solution as is in the formulation of the solutions from the prior art (e.g.
at product "BioSil®", however, choline chloride at physiological pH values not only destabilizes, but moreover catalyzes its polymerization. In this manner, it decreases its bioavailability, because, bioavailability of polymeric silicic acids are drastically lower. This was confirmed in independent study published in the literature, where, for corresponding product based on choline chloride ("BioSil®") determined bioavailability was at a level of 30% [R. Jugdaohsingh : Silicon and bone health, J. Nutr. Health Aging 11 (2007) 99-110].

In contrast, carnitine salts, like DL- (±) -carnitine hydrochloride, not only act as stabilizers of ortho-silicic (H₄SiO₄) in acidic pH medium of the formulation from the present invention, but they do not cause its destabilization at physiological conditions. In contrast to choline chloride, carnitine salts do not catalyze polymerization of H₄SiO₄ under physiological conditions, and subsequently, cannot exhibit negatively on (decreasing) of its bioavailability.

The reason of this presumably lies behind the fact that both choline chloride from the prior art and carnitine salts from the present invention, as well as many other quaternary ammonium salts are able to stabilize H₄SiO₄ in acidic medium by the way of solvation mechanism as "deep eutectic liquids" in combinations with hydrogen-bond donors (e.g. polyols like glycerol). However, this effect is lost at physiological conditions wherein pH is close to 7. In these conditions differences in the structure of quaternary ammonium salts become important, and where not all of them can be considered as the same.

Presumably the key factor of stabilization / destabilization of ortho-silicic acid (H₄SiO₄) is ability or disability to form stable complex with the given molecule. Whereas choline chloride can act as bidentate ligand for H₄SiO₄, carnitine salts act as tetradeutate ligands. Because of this, carnitine hydrochloride (1a) forms far more stable complex 3a than choline chloride (2) which gives the
corresponding complex with less hydrogen bonds. Also, in the case of the use of carnitine salts with acids which can additionally stabilize $\text{H}_4\text{SiO}_4$, as in the case of phosphoric acid (complex 3b), stability is additionally increased (compare results from Table 5; Experiment 2/Experiment 1) (Scheme 3, end of specification).

In this case, less stable complex (e.g. with choline chloride) means higher equilibrium concentration of free $\text{H}_4\text{SiO}_4$, (because the formation of the complex is a reversible process), and consequently its faster polymerization. Unwanted polymerization causes shift of the equilibrium of formation of the complex into the left (to the degradation direction).

In short, less stable complex finally results in faster polymerization process, what directly leads to decreased bioavailability of silicon (Si) at in vivo conditions.

Additionally, despite the fact that in the prior art the use of strong pharmaceutically acceptable mineral acids in the formulations of ortho-silicic acid ($\text{H}_4\text{SiO}_4$) is known, among them, there is "generically" mentioned also phosphoric acid ($\text{H}_3\text{PO}_4$) [I. Suvee, G. Tourgis: Hydronium stabilized and dissoluble silicic acid nanoparticles: Preparation, stabilization and use, WO 2009/127256 A1 (2008); W. A. Kros, US Patent 2006/0178268 A1], there is no a single and clear evidence or study of kind of acid on stability of $\text{H}_4\text{SiO}_4$.

In the present application, clear additional stabilizing effect of phosphoric acid on stability of $\text{H}_4\text{SiO}_4$ in solution, is clearly demonstrated.

Finally, in development of the solution from the present invention, significant synergistic effect of the formulation of:

(i) carnitine salts;
(ii) pharmaceutically acceptable acid, especially phosphoric acid; and
auxiliary stabilizers, glycerol, glucosamine, d-panthenol, and 1,2-propylene glycol; on stability of H₄SiO₄ under physiological conditions (pH values closed to 7) has been clearly shown, what is the key prerequisite for high level of bioavailability under in vivo conditions.

Preparation of the solution from the present invention

The solution from the present invention is prepared by addition of precursor of silicic acid (PSA) of tetraethyl orthosilicate [TEOS; Si(OC₂H₅)₄] in previously prepared solution of carnitine salt like DL-(±)-carnitine hydrochloride (Ia), pharmaceutically acceptable acid, and auxiliary stabilizer of H₄SiO₄ according to the invention with vigorous stirring at temperatures between -10 °C to +40 °C, preferably at +15 °C to +30 °C (room temperature conditions) during 1-24 h.

Alternatively, as PSA the followings can be employed:

(i) sodium or potassium silicate (common composition xM₂O·ySiO₂; M=Na, K, x:y= 1:1 to 1:3.5); or
(ii) silicon tetrachloride (SiCl₄).

However, the use of tetraethyl orthosilicate (TEOS) provides advantage, since it is neither toxic nor corrosive like SiCl₄. Moreover, commercially available products are of very high purity because TEOS is readily purified by distillation. This provides very pure final product with the content of unwanted heavy metals (Pb, Cd, Hg, As) far under limits usual for pharmaceutical products and food supplements.

In contrast, purification of sodium or potassium silicate from heavy metals is difficult and commercial products are not of so high level of chemical purity.

In the case of the use of silicon tetrachloride (SiCl₄), as pharmaceutically acceptable acid is usually employed hydrochloric
acid (HCl) released during its hydrolysis (4 mol of HCl per single mol of SiCl₄). Excess of HCl is neutralized by addition of corresponding amounts of pharmaceutically acceptable base such as sodium or potassium hydroxide (NaOH, KOH), calcium or magnesium hydroxide or carbonate [Ca(OH)₂, Mg(OH)₂, CaCO₃, MgCO₃], etc. In this case, corresponding salts of used bases are generated, e.g. NaCl, KCl, CaCl₂, MgCl₂. These salts do not alter stability of orthosilicic acid in the solution from the present invention; these are leaved in the final product or, if precipitate, removed by filtration.

In the case of the use of sodium or potassium silicate and silicon tetrachloride (SiCl₄), the reaction is strongly exothermic, and intensive cooling of the reaction mixture is necessary. At the use of tetraethyl orthosilicate, the reaction is only slightly exothermic, temperature raises for only a few °C, and the reaction can be conducted with only a mild external cooling, without special difficulties.

In the cases of the use of SiCl₄ or sodium/potassium silicate, the reaction is complete almost instantly, whilst hydrolysis of tetraethyl orthosilicate is far more slower; it tooks from 1.5-2 h at room temperature.

In any case, generated ortho-silicic acid in status nascendi forms complex with carnitine (e.g. carnitine hydrochloride).

As side-products, in reactions with sodium or potassium silicate, equivalent amounts of sodium or potassium salt of pharmaceutically acceptable acid is generated; these are eventually removed by filtration after completion of the reaction.

In the case of the use of tetraethyl orthosilicate, four molar equivalents of ethanol (C₆H₁₂O₆) is formed. Since ethanol is completely non-toxic in this concentration, the latter is not subjected to removing, but leaved in the final product as diluent.
It is known to those skilled in the art that ethanol is usual and widely employed pharmaceutical excipient - diluent.

Hydrolysis of tetraethyl orthosilicate can be carried out in the presence of pharmaceutically acceptable acid in purified water (only), whereas all other ingredients (carnitine salt, auxiliary stabilizer) can be added afterwards.

Alternatively, the solution from the present invention can be prepared by the same manners, with the use of free carnitine ("carnitine base" or its zwitter-ionic form), however, then pharmaceutically acceptable acid (e.g. \( \text{H}_3\text{PO}_4 \)) is used in excess of one molar equivalent; this is because one equivalent is spent on neutralization of carnitine base.

At the end, the product is subjected to dilution with water up to declared concentration of silicon (Si), filtration, and packaging into plastic bottles.

The course of reaction is given in Scheme 2.

The use of the solution from the present invention

The use of the solution from the present invention provides all known positive therapeutic effects of silicic acid on human, animal, or plant organism which are known to a person skilled in the art.

The solution from the present invention is employed as food supplement or as therapeutic agent for humans and animals, and for plant nutrition.

Before the application, the solution is diluted with water to a concentration suitable for application:

(i) at humans in doses from 5-15 mg of silicon (Si);

(ii) in animals in doses from 5-50 mg; and
(iii) at plants, by foliar application in concentrations from 0.005-
0.01% w/w of silicon (Si), in amounts from 10-30 g per hectare
(ha).

At humans and animals, the solution is used in all medicinal,
cosmetic, and veterinary indications wherein it is known that
silicon (Si) acts positively:
(i) helps in resorption of calcium; takes part in its transport,
stimulates osteoblasts, stimulates bone mineralization, hastes bone
fracture healing; in prevention of osteoporosis;
(ii) takes part in structure of arterial, vein, and capillary walls,
increases their elasticity and hardness of blood vessels, decreases
their permeability; also takes part in structure of connective
tissue and formation of functional tertiary structure of building
proteins of soft organs like liver, lung, and brain;
(iii) stimulates immune system; thus increases natural ability of
organism to fight against microorganisms at infective diseases, and
in all other disorders and diseases which develop in conditions of
weak immune system, e.g. allergies;
(iv) antiinflammatory effect; the therapy of various acute and
chronic inflammatory diseases, e.g. positively acts at various skin
diseases such as psoriasis, seborrheic dermatitis, neurodermitis,
eckema, skin irritations, burns, wound healing, soothes decubitus,
then at dandruff, and other skin diseases and disorders; helps also
in other inflammatory diseases like rheumatoid arthritis;
(v) acts as cross-linking agent for glucosaminoglycans and
mucopolysaccharides and thus helps in function of joints, ligaments
and formation of sinovial fluid;
(vi) inhibits resorption of aluminum (Al³⁺) from gastrointestinal
tract, and beside antioxidative action, preventively acts on
development of neurodegenerative diseases such as Alzheimer or
Parkinson diseases;
(vii) stimulates biosynthesis of skin building proteins: collagen
and elastin; in treatment of wrinkles and prevention of its
formation; thus helps in slowing-down of skin ageing;
(viii) stimulates growth of hair and nails; strengthens hair and nails, hair becomes even shinier; and
(ix) acts as antioxidant; prevents development of atherosclerosis and other diseases which are caused by prolonged exposure to oxidative stress like diabetes and diabetes complications.

It is known to those skilled in the art that analogous biological effect silicon exhibits also in animals, and therefore the formulation from the present invention is also used in veterinary in all mentioned indications.

In plants, silicon nutrition provides:

(i) increased crop yields (due to stimulated photosynthesis caused by better utility of nutrients added by common fertilization);

(ii) resistance to stress conditions (e.g. during drought or after hail); and

(iii) resistance to insects attacks and fungal diseases.

In agriculture, the present solution is diluted with water to the final concentration of 0.005-0.1% and used by foliar application by all common spraying equipments. Lower concentrations (0.005-0.05% of Si) are used preventively for stimulation of growth and against development of fungal diseases (e.g. at grape), whilst higher concentrations (0.05-0.1% of Si) are employed in stressful conditions at drought or after hail. Dosing rates are between 10-100 g of silicon per hectare (ha), or 1-10 L of the formulation from the present invention in concentration of 1% w/w of Si to the sprayer with 200-400 L of water, applied on area of 1 ha.

Finally, the solution from the present invention can be used as raw material (intermediate) for production of other pharmaceutical products, cosmetics, food supplements, veterinary, and agrochemical products with content of highly bioavailable silicon (Si).
Examples

General remarks

The term room temperature means the temperature interval: 20–25 °C. All portions (%) of ingredients are expressed as weight percentages (w/w). Relative ratio of reactants in reaction mixtures are expressed as molar equivalents (mol. equiv.).

Example 1

Preparation of the standard solution of ortho-silicic acid (\(\text{H}_4\text{SiO}_4\)) and its complexes with choline chloride (2) and PL- (±) -carnitine hydrochloride (1a). Influence of 2 and 1a on stability of \(\text{H}_4\text{SiO}_4 \) in solution at pH= 6.5.

(1) Preparation of the standard solution of ortho-silicic acid of concentration of 1% w/w of silicon (Si) (Table 1, Experiment 1; Table 2, Experiment 2; Table 3, Experiment 1): To a solution of 85% phosphoric acid (0.2 mL; 0.34 g; 0.29 g \(\text{H}_3\text{P}_0_4\); 0.003 mol; 0.55 mol equiv.) in distilled water (10.00 g), tetraethyl orthosilicate (TEOS; 1.2 mL; 1.12 g; 0.0054 mol) was added. The reaction mixture was stirred at room temperature for 3 h. Then, distilled water (3.54 g) was added up to the overall weight of the reaction mixture of 15.00 g. Content of silicon (Si) in such prepared solution was 10 mg/g (1% w/w).

(ii) Preparation of control solution of complex of choline chloride (2) and ortho-silicic acid in concentration of 1% w/w of silicon (Si) (Table 1, Experiment 2): To a solution of choline chloride (2; 0.75 g; 0.0054 mol; 1 equiv.) in distilled water (10.00 g), 85% phosphoric acid (0.2 mL; 0.34 g; 0.29 g \(\text{H}_3\text{P}_0_4\); 0.003 mol; 0.55 mol equiv.) was added. Then, tetraethyl orthosilicate (TEOS; 1.2 mL; 1.12 g; 0.0054 mol) was added, and reaction mixture was stirred at room temperature for 3 h. After this, distilled water (2.79 g) was
added up to the overall weight of the reaction mixture of 15.00 g. The content of silicon (Si) in thus prepared solution was 10 mg/g (1% w/w).

(iii) Preparation of complex of DL- (+) -carnitine hydrochloride (la) and ortho-silicic acid of concentration of 1% w/w of silicon (Si) (Table 1, Experiment 3): To a solution of DL- (+) -carnitine hydrochloride (la; 1.06 g; 0.0054 mol; 1 equiv.) in distilled water (10.00 g), 85% phosphoric acid (0.2 mL; 0.34 g; 0.29 g H₃PO₄; 0.003 mol; 0.55 mol. equiv.) was added. Then, tetraethyl orthosilicate (TEOS; 1.2 mL; 1.12 g; 0.0054 mol) was added, and the reaction mixture was stirred at room temperature for 3 h. Finally, distilled water (2.48 g) was added up to the total weight of 15.00 g. Content of silicon (Si) in such prepared solution was 10 mg/g (1% w/w).

(iv) Determination of gelling (polymerization) time, t₉, of ortho-silicic acid (H₅SiO₄) in the presence of choline chloride (2) and DL- (+) -carnitine hydrochloride (la): In a test tube, 2 mL of freshly prepared 1.32M phosphate buffer of pH= 7, and 2 mL of sample solution or standard solution was mixed. pH values of all thus prepared test solutions were 6.5. To such prepared test solutions, the time from the moment of mixing with phosphate buffer (tᵭ) until the formation of opalescent (and thick) gel was determined. This time interval is termed as "gelling (polymerization) time", t₉, and expressed in minutes. Thus obtained results for t₉ were given in comparison with the value obtained for the standard solution of H₅SiO₄ (as the standard). The results are given in Table 1.

Preparation of 1.32M phosphate buffer of pH= 7 for the testing: Sodium dihydrogenphosphate (NaH₂PO₄; 16.00 g; 0.132 mol) and sodium hydroxide (3.14 g; 0.0785 mol) were quantitatively transferred into a 100 mL measuring flask and dissolved in 80-85 mL of distilled water by shaking at room temperature. Thus obtained solution was carefully diluted to 100 mL mark with distilled water. Measured pH value of thus prepared solution was 7.0.
Example 2

The study of influence of pharmaceutically acceptable acid on stability of ortho-silicic acid (H₄SiO₄) in solution at pH = 6.5.

(i) Preparation of the standard solution of ortho-silicic acid of concentration of silicon (Si) of 1% w/w in the presence of hydrochloric acid (HCl) (Table 2, Experiment 1): To a solution of 37% hydrochloric acid (0.25 mL; 0.296 g; 0.11 g HCl; 0.003 mol; 0.55 mol. equiv.) in distilled water (10.00 g), tetraethyl orthosilicate (TEOS; 1.2 mL; 1.12 g; 0.0054 mol) was added. The reaction mixture was stirred at room temperature for 3 h. Then, distilled water (3.59 g) was added up to the overall weight of the reaction mixture of 15.00 g. Content of silicon (Si) in thus prepared solution was 10 mg/g (1% w/w).

(ii) Determination of gelling (polymerization) time, t₀, of orthosilicic acid (H₄SiO₄) prepared with hydrochloric and phosphoric acid: To a test tube, 2 mL of freshly prepared 1M phosphate buffer of pH = 8.5 and 2 mL of sample or standard solutions were mixed. pH values of all test solutions were 6.5. To thus prepared test solutions, the time from the moment of mixing with the phosphate buffer (t₀) until the formation of opalescent (and thick) gel was determined. This time interval was termed as "gelling (polymerization) time", t₀, and expressed in minutes. The results are given in Table 2.

Preparation of 1M phosphate buffer of pH = 8.5 for the testing: Sodium dihydrogenphosphate (NaH₂PO₄; 12.00 g; 0.1 mol) and sodium hydroxide (4.00 g; 0.1 mol) were quantitatively transferred into a 100 mL measuring flask and dissolved in 80-85 mL of distilled water by shaking. Thus prepared solution was carefully diluted up to the 100 mL mark with distilled water. Measured pH value of this solution was 8.5.

Example 3
The study of influence of auxiliary stabilizer on stability of ortho-silicic acid \((\text{H}_4\text{SiO}_4)\) in solution at pH= 6.5.

(i) Preparation of solutions of complexes of DL-\((\pm)\)-carnitine hydrochloride \((1a)\) with different auxiliary stabilizers, in concentration of 1\% w/w of silicon \((\text{Si})\) (Table 3, Experiments 2-7). General procedure: To a solution of DL-\((+)\)-carnitine hydrochloride \((1a;\ 1.06 \ g; \ 0.0054 \ \text{mol; \ 1 \ mol. equiv.) and auxiliary stabilizer (3.00 \ g; \ 20\% \ \text{w/w)}:

(a) glycerol (Table 3, Experiment 2);
(b) 1,2-propylene glycol (Table 3, Experiment 3);
(c) sorbitol (Table 3, Experiment 4);
(d) PEG-400 (Table 3, Experiment 5);
(e) glucosamine sulfate (Table 3, Experiment 6);
(f) d-panthenol (Table 3, Experiment 7);

in distilled water (7.00 \ g), 85\% phosphoric acid (0.2 mL; 0.34 \ g; 0.29 \ g \ \text{H}_3\text{P}_0_4; \ 0.003 \ \text{mol; \ 0.55 \ mol. equiv.}) was added. Then, tetraethyl orthosilicate \((\text{TEOS; 1.2 mL; 1.12 g; 0.0054 mol})\) was added, and the reaction mixture was stirred at room temperature for 3 h. After this, distilled water (2.48 \ g) was added up to the total weight of reaction mixture of 15.00 \ g. Content of silicon \((\text{Si})\) in thus prepared solutions was 10 \ mg/g \ (1\% \ \text{w/w}).

(ii) Determination of gelling (polymerization) time, \(t_o\), of ortho-silicic acid \((\text{H}_4\text{SiO}_4)\) in the presence of DL-\((+)\)-carnitine hydrochloride \((1a)\) and various auxiliary stabilizers: In a test tube, 2 mL of freshly prepared 1M phosphate buffer pH= 8.5 and 2 mL of sample or standard solutions were mixed. pH values of all prepared test solutions were 6.5. For thus prepared test solutions, the time from the moment of mixing with the phosphate buffer \((t_o)\) until the formation of opalescent (thick) gel was determined. This time interval is termed as "gelling (polymerization) time", \(t_o\), and expressed in minutes. The results are give in Table 3. Preparation of 1M phosphate buffer of pH= 8.5 required for this testing is described in Example 2.
Example 4

Stabilizing effect of the formulation from the present invention in comparison with the solution based on choline chloride, analogous to the prior art, on ortho-silicic acid (Si₄O₄) in solution at pH= 6.5.

(i) Preparation of the control solution of analogous composition as the product „BioSil®“ (Table 4, Experiment 1): To a solution of 85% phosphoric acid (0.2 mL; 0.34 g; 0.289 g H₃PO₄; 0.0029 mol; 0.55 mol. equiv.) and choline chloride (7.05 g; 47% w/w) in a mixture of distilled water (3.00 g) and glycerol (2.85 g; 19% w/w), TEOS (1.2 mL; 1.12 g; 0.0054 mol) was added. The reaction mixture was stirred at room temperature for 3 h, and subsequently diluted with distilled water (0.64 g) up to the total weight of the reaction mixture of 15.00 g [contains 10 mg/g (1% w/w) of Si].

(ii) Preparation of versions of the formulation from the present invention. General procedure (Table 4, Experiments 2-5): To a solution of 85% phosphoric acid (0.2 mL; 0.34 g; 0.289 g H₃PO₄; 0.0029 mol; 0.55 mol. equiv.) and DL-(-)-carnitine hydrochloride (3.75 g; 25% w/w) in a mixture of distilled water (6.00 g) and auxiliary stabilizer (3.00 g; 20% w/w):
   (a) glycerol (Experiment 2);
   (b) glucosamine sulfate (Experiment 3);
   (c) d-pantenol (Table 4, Experiment 4); or
   (d) 1,2-propylene glycol (Experiment 5);
TEOS (1.2 mL; 1.12 g; 0.0054 mol) was added. The reaction mixtures were stirred at room temperature for 3 h, and then diluted with distilled water (0.79 g) up to the total weight of the reaction mixtures of 15.00 g [contain 10 mg/g (1% w/w) of Si].

(iii) Determination of gelling (polymerization) time, t₆, of ortho-silicic acid (Si₄O₄) at various versions of the formulation from the present invention in comparison with the solution based on choline chloride analogous to the prior art: In a test tube, 2 mL of freshly prepared 1M phosphate buffer of pH= 8.5 and 2 mL of sample or
standard solutions were mixed. pH values of all test solutions were corrected to the same value of 6.5 by addition of minimal amounts of solid sodium carbonate (Na₂CO₃). For thus prepared test solutions, the time from the moment of mixing with the phosphate buffer (t₀) until the formation of opalescent (thick) gel was determined. This time interval was termed as "gelling (polymerization) time", tₛ, and expressed in minutes. The results are given in Table 4.

Preparation of 1M phosphate buffer of pH= 8.5 for this testing was described in Example 2.

Example 5

The study of influence of various carnitine salts on stability of ortho-silicic acid (H₄SiO₄) in solution at pH= 6.5.

(i) Preparation of complexes of H₄SiO₄ and carnitine salts with different pharmaceutically acceptable acids. General procedure

(Table 5, Experiments 1-3): To a solution of L-carnitine base (0.87 g; 0.054 mol) in distilled water (10.00 g) the following pharmaceutically acceptable acids were added:

(a) 37% hydrochloric acid (0.70 mL; 0.83 g; 0.31 g HCl; 0.0084 mol; 1.5 mol. equiv.) (Table 5, Experiment 1);
(b) 85% phosphoric acid (0.55 mL; 0.935 g; 0.795 g H₃PO₄; 0.0081 mol; 1.5 mol. equiv.) (Table 5, Experiment 2);
(c) 96% sulfuric acid (0.45 mL; 0.828 g; 0.795 g H₂SO₄; 0.0081 mol; 1.5 mol. equiv.) (Table 5, Experiment 3); followed by tetraethyl orthosilicate (TEOS; 1.2 mL; 1.12 g; 0.0054 mol). The reaction mixture was stirred at room temperature for 3 h. Then, distilled water was added up to the overall weight of the reaction mixtures of 15.00 g.

Preparation of the solution of carnitine hydrochloride (1a) complex in the presence of phosphoric acid was described in Example 1/(iii) (Table 5, Experiment 4).

Content of silicon (Si) in thus prepared solutions was 10 mg/g (1% w/w).
(ii) Determination of gelling (polymerization) time, \( t_\text{g} \), of orthosilicic acid \((\text{H}_4\text{SiO}_4)\) at complexes with carnitine salts with different pharmaceutically acceptable acids: In a test tube, 2 mL of freshly prepared 1M phosphate buffer of pH= 8.5 and 2 mL of sample or standard solutions were mixed. pH values of all thus prepared test solutions were the same (6.5). For thus prepared test solutions, the time from the moment of mixing with the phosphate buffer \((t_\text{g})\) until the formation of opalescent (thick) gel was determined. This time interval was termed as "gelling (polymerization) time", \( t_\text{g} \), and expressed in minutes. The results are given in Table 4.

Preparation of 1M phosphate buffer of pH= 8.5 for this study is described in Example 2.

Example 6

Preparation of solution of ortho-silicic acid of concentration of 0.01% w/w of \( \text{H}_4\text{SiO}_4 \) stabilized with carnitine hydrochloride according to the invention (0.0029% w/w of Si); 1 kg-scale.

To a solution of DL-carnitine hydrochloride (70.00 g; 7% w/w) in a mixture of distilled water (400.00 g) and glycerol (500.00 g; 50% w/w), L-carnitine base (170 g; 0.00106 mol), 85% phosphoric acid (182 mg; 155 mg \( \text{H}_3\text{PO}_4 \); 0.0016 mol; 1.5 mol. equiv.), and tetraethyl orthosilicate (TEOS; 220 mg; 0.00106 mol) were added. The reaction mixture was stirred at room temperature for 24 h. Then, purified water (29.43 g) was added up to the total weight of the reaction mixture of 1000.00 g. The silicon (Si) content in thus prepared solution was 0.028 mg/g (0.0029% w/w of Si).

Composition of the solution (% w/w):
- 0.01% \( \text{H}_4\text{SiO}_4 \) (0.0029% Si);
- 7% DL-(±)-carnitine hydrochloride;
- 50% glycerol; and
- up to 100% purified water.
Example 7

Preparation of solution of ortho-silicic acid of 2% w/w concentration of H$_4$SiO$_4$, stabilized with carnitine hydrochloride according to the invention (0.58% w/w of Si); 1 kg-scale.

To a solution of DL- (+)-carnitine hydrochloride (la; 300.00 g; 30% w/w) in purified water (300.00 g), 1,2-propylene glycol (250.00 g; 25% w/w) and 85% phosphoric acid (12.00 g; 10.20 g H$_3$PO$_4$; 0.1 mol; 0.52 mol. equiv.) were added. Then, tetraethyl orthosilicate (TEOS; 43.40 g; 0.208 mol) was added, and the reaction mixture was stirred at room temperature for 3 h. Afterwards, distilled water (94.60 g) was added up to the total weight of the reaction mixture of 1000.00 g. Content of silicon (Si) in thus prepared solution was 5.84 mg/g (0.58% w/w of Si).

Composition of the solution (% w/w):
- 2% H$_4$SiO$_4$ (0.58% Si);
- 1% phosphoric acid;
- 30% DL- (+)-carnitine hydrochloride;
- 25% 1,2-propylene glycol;
- 3.8% ethanol; and
- up to 100% purified water.

Example 8

Preparation of solution of ortho-silicic acid of 2% w/w concentration of H$_4$SiC$_4$, stabilized by carnitine hydrochloride according to the invention (0.58% w/w of Si); 1 kg-scale.

To a solution of DL- (+)-carnitine hydrochloride (la; 350.00 g; 35% w/w) in purified water (200.00 g), glycerol (350.00 g; 35% w/w) was added. The reaction mixture was cooled to -10 °C, and then silicon tetrachloride (SiCl$_4$; 24 mL; 35.59 g; 0.209 mol) was added dropwise. The reaction mixture was stirred at temperatures between -10 °C to -5 °C during 1 h. Then, solid calcium carbonate (CaCO$_3$; 37.00 g; 0.37
mol) was added in several portions during 30 minutes. The reaction mixture was stirred at temperatures between -5 °C and room temperature during 1 h. Afterwards, purified water (approx. 28-30 g) was added up to the total weight of the reaction mixture of 1000.00 g. Content of silicon (Si) in thus prepared solution was 5.84 mg/g (0.58% w/w of Si).

Composition of the solution (% w/w):
- 2% $H_4SiO_4$ (0.58% w/w Si);
- 35% DL- (+) -carnitine hydrochloride;
- 35% glycerol;
- 3.8% ethanol; and
- up to 100% purified water.

**Example 9**

**Preparation of solution of ortho-silicic acid of 2% w/w concentration of $H_4SiO_4$ stabilized with carnitine hydrochloride according to the invention (0.58% w/w of Si); 1 kg-scale.**

To a solution of DL- (+) -carnitine hydrochloride (la; 300.00 g; 30% w/w) in purified water (250.00 g), 1,2-propylene glycol (300.00 g; 30% w/w), d-panthenol (50.00 g; 5% w/w), and 85% phosphoric acid (60.00 g; 51.00 g $H_3PO_4$; 0.52 mol; 2.5 mol. equiv.) were added. The reaction mixture was cooled to 0 °C, and then sodium silicate ($Na_2SiO_3$; 25.40 g; 0.208 mol) was added in portions during 30 minutes. The reaction mixture was stirred at temperatures from 0 °C to room temperature during 1 h. Then, purified water (14.60 g) was added up to the total weight of the reaction mixture of 1000.00 g. The content of silicon (Si) in thus prepared solution was 5.84 mg/g (0.58% w/w of Si).

Composition of the solution (% w/w):
- 2% $H_4SiO_4$ (0.58% Si);
- 1% phosphoric acid;
- 30% DL- (+) -carnitine hydrochloride;
- 30% 1,2-propylene glycol;
• 5% d-panthenol;
• 3.8% ethanol; and
• up to 100% purified water.

Example 10

Preparation of solution of ortho-silicic acid of 4% w/w concentration of H₄SiO₄ stabilized by carnitine hydrochloride according to the invention (1.17% w/w of Si).

To a solution of DL-(±)-carnitine hydrochloride (la; 300.00 g; 30% w/w) in purified water (300.00 g), 1,2-propylene glycol (200.00 g; 20% w/w), d-panthenol (100.00 g; 10% w/w), and 85% phosphoric acid (12.00 g; 10.20 g H₃PO₄; 0.104 mol; 0.25 mol. equiv.) were added. Then, tetraethyl orthosilicate (TEOS; 87.00 g; 0.42 mol) was added, and the reaction mixture was stirred at room temperature for 3 h. Afterwards, purified water (1.00 g) was added up to the total weight of the reaction mixture of 1000.00 g. Content of silicon (Si) in thus prepared solution was 11.7 mg/g (1.17% w/w of Si).

Composition of the solution (% w/w):
• 4% H₄SiO₄ (1.17% Si);
• 1% phosphoric acid;
• 30% DL-(±)-carnitine hydrochloride;
• 20% 1,2-propylene glycol;
• 10% d-panthenol;
• 7.7% ethanol; and
• up to 100% purified water.

Example 11

Preparation of solution of ortho-silicic acid of 8% w/w concentration of H₄SiO₄ stabilized by carnitine hydrochloride according to the invention (2.34% w/w of Si).
To a solution of DL-(-) -carnitine hydrochloride (la; 200.00 g; 20% w/w) in purified water (250.00 g), 1,2-propylene glycol (150.00 g; 15% w/w), glycerol (100.00 g; 10% w/w), and 85% phosphoric acid (24.00 g; 20.40 g H₃PO₄; 0.208 mol; 0.25 mol. equiv.) were added. Then, tetraethyl orthosilicate (TEOS; 175.00 g; 0.84 mol) was added, and the reaction mixture was stirred at room temperature for 2 h. Upper ethanol layer was removed by separatory funnel. Afterwards, purified water was added up to the total weight of the remained reaction mixture of 1000.00 g. Content of silicon (Si) in such prepared solution was 23.4 mg/g (2.34% w/w of Si).

Composition of the solution (% w/w):
- 8% H₄SiO₄ (2.34% Si);
- 2% phosphoric acid;
- 20% DL-(-) -carnitine hydrochloride;
- 15% 1,2-propylene glycol;
- 10% glycerol;
- up to 100% purified water.

Example 12

Preparation of solution of ortho-silicic acid of 2% w/w concentration of H₄SiO₄ stabilized by carnitine dihydrogenphosphate according to the invention (0.58% w/w of Si); 1 kg-scale.

Solution of L-carnitine base (100.00 g; 10% w/w; 0.62 mol) in purified water (450.00 g) and 1,2-propylene glycol (300.00 g; 30% w/w) was cooled with stirring to 0 °C. Then, 85% phosphoric acid (84.00 g; 71.40 g H₃PO₄; 0.73 mol; 0.53 mol. equiv. / H₄SiO₄) was added dropwise during 1 h. Afterwards, tetraethyl orthosilicate (TEOS; 43.40 g; 0.208 mol) was added, and the reaction mixture was stirred at room temperature during 3 h. Then, purified water (22.60 g) was added, up to the total weight of the reaction mixture of 1000.00 g. Content of silicon (Si) in such prepared solution was 5.84 mg/g (0.58% m/m Si).

Composition of the solution (% w/w):
• 2% $H_4SiO_4$ (0.58% Si);
• 1% phosphoric acid;
• 16% L-carnitine dihydrogenphosphate;
• 30% 1,2-propylene glycol;
• 3.8% ethanol; and
• up to 100% purified water.
A stabilized solution of ortho-silicic acid (H₄SiO₄) as source of biologically available silicon, that consists of:
(i) ortho-silicic acid (H₄SiO₄), from 0.01-8% w/w;
(ii) carnitine salt (I) of pharmaceutically acceptable acids,

\[
\left(\text{H}_3\text{C} \right)_3\text{N} - \text{CH} - \text{COOH} \right]^{+}X^{-}
\]

where \( X = \text{Cl, H}_2\text{PO}_4, \text{HSO}_4, \text{NO}_3, \text{CH}_3\text{SO}_3, \text{C}_6\text{H}_5\text{SO}_3, 1,4-\text{CH}_3\text{C}_6\text{H}_4\text{SO}_3 \)

from 7-40% w/w;
(iii) pharmaceutically acceptable acid, from 0.05-4 molar equivalents to H₄SiO₄;
(iv) auxiliary stabilizer of H₄SiO₄, selected from the group comprising: glycerol, 1,2-propylene glycol, d-panthenol, glucosamine, or their mixtures, from 10-50% w/w; and
(v) diluent, selected from the group consisting of purified water, ethanol, or their mixtures, in amounts of up to 100% w/w of overall formulation.

2. A stabilized solution of ortho-silicic acid (H₄SiO₄) as source of biologically available silicon according to claim 1, characterized by that the carnitine salt is selected to be carnitine hydrochloride.

3. A stabilized solution of ortho-silicic acid (H₄SiO₄) as source of biologically available silicon according to claims 1 and 2, characterized by that the pharmaceutically acceptable acid is selected from the group comprising of: hydrochloric (HCl), sulfuric (H₂SO₄), phosphoric (H₃PO₄), nitric (HNO₃), methanesulfonic (CH₃SO₃H), benzenesulfonic (C₆H₅SO₃H), p-toluenesulfonic acid (1,4-CH₃C₆H₄SO₃H); or mixtures of these substances.
4. A stabilized solution of ortho-silicic acid ($\text{H}_4\text{SiO}_4$) as source of biologically available silicon according to claims 1 and 2, characterized by that the pharmaceutically acceptable acid is selected to be phosphoric acid ($\text{H}_3\text{PO}_4$).

5. A process for preparation of the solution of ortho-silicic acid ($\text{H}_4\text{SiO}_4$) as source of biologically available silicon according to claims 1-4, characterized by the reaction of precursor of silicic acid (PSA) with previously prepared solution of carnitine salt like DL-(+)-carnitine hydrochloride, auxiliary stabilizer, and pharmaceutically acceptable acid, where the said reaction is carried out at temperatures from -10 °C to +40 °C, during 1-24 h.

6. A process for preparation of the solution of ortho-silicic acid ($\text{H}_4\text{SiO}_4$) as source of biologically available silicon according to claim 5, characterized by that the precursor of silicic acid (PSA) is selected from the group consisting of: tetraethyl orthosilicate [TEOS; Si(OC$_2$H$_5$)$_4$], sodium silicate, potassium silicate or silicon tetrachloride (SiCl$_4$).

7. A process for preparation of solution of ortho-silicic acid ($\text{H}_4\text{SiO}_4$) as source of biologically available silicon according to claim 5, characterized by that the precursor of silicic acid (PSA) is selected to be tetraethyl orthosilicate [Si(OC$_2$H$_5$)$_4$].

8. A stabilized solution of ortho-silicic acid ($\text{H}_4\text{SiO}_4$) according to claims 1-4 for use as source of silicon (Si) for realization of physiological and therapeutic effects of silicon in human or animal organism.

9. A stabilized solution of ortho-silicic acid ($\text{H}_4\text{SiO}_4$) according to claims 1-4 for use as a therapeutic agent for:
- stimulation of immune system;
- treatment of allergies;
- strengthening structure and elasticity of arterial, vein, and capillary walls; decreasing their permeability; for improving structure of connective tissue, and building proteins of soft organs such as liver, lung, and brain;
- stimulation of function of joints and ligaments;
- stimulation of osteoblasts, mineralization of bones, and prevention of osteoporosis;
- stimulation of resorption and transport of calcium, as adjuvant at diseases where calcium resorption brings positive effects;
- decreasing resorption of aluminum from gastrointestinal tract; thus preventively on development of neurodegenerative diseases like Alzheimer or Parkinson diseases which are commonly connected with resorption of aluminum;
- treatment of dermatoses such as: skin irritations, eczema, seborrheic dermatitis, neurodermitis, and psoriasis;
- treatment of dandruff;
- treatment of decubitus;
- treatment of burns;
- wound healing;
- stimulation of biosynthesis of collagen and elastin;
- treatment of wrinkles and prevention of their development;
- slowing-down skin ageing;
- stimulation of growth, strength, and shine of hair;
- stimulation of growth and strength of nails;
- adjuvant treatment of infective diseases;
- treatment of acute and chronic inflammatory diseases (as anti-inflammatory agent); and
- as antioxidant for prevention of: diseases which are caused by prolonged oxidative stress condition; development of atherosclerosis; diabetes and diabetes complications.

10. A stabilized ortho-silicic acid-based formulation according to claims 1-4, for use as an agent for realization of physiological effects of silicon (Si), and increasing resistance to stress and fungal diseases in plants.
Scheme 1
Scheme 2

STABILITY OF COMPLEXES

Scheme 3
### INTERNATIONAL SEARCH REPORT

**International application No**

PCT/HR2011/000036

#### A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K31/047 A61K31/16 A61K31/205 A61K31/44 A61K31/7008

A61K33/00 A61K47/14 ...

P.B. 5818 Patentlaan 2

N L - 2280 H V Rijswijk

Tel. (+31-70) 340-2040,

Fax: (+31-70) 340-3016

** bayrak, Timur

#### B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal , BIOSIS, EMBASE, WPI Data

#### C. DOCUMENTS CONSIDERED TO BE RELEVANT

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**Date of the actual completion of the international search**

7 December 2011

**Date of mailing of the international search report**

14/12/2011

**Name and mailing address of the ISA**

European Patent Office, P.B. 5818 Patentlaan 2

NL - 2280 HV Rijswijk

Tel. (+31-70) 340-2040,

Fax: (+31-70) 340-3016

**Authorized officer**

Al bayrak, Timur

Form PCT/ISA/210 (second sheet) (April 2005)
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