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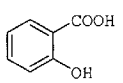

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(57) Abstract: The present invention discloses a formulation that serves as a highly bioavailable silicon (Si) source consisting of: (i) ortho-silicic acid (H_4SiO_4), from 0.01-8% w/w; (ii) salicylic acid (1), from 1-2 molar equivalents to H_4SiO_4; (iii) pharmaceutically acceptable acid, from 0.1-4 molar equivalents to H_4SiO_4; or pharmaceutically acceptable base, in amounts of 2 molar equivalents to salicylic acid (1); and (iv) diluent, selected from the group consisting of: purified water, 1, 2-propylene glycol, glycerol, ethanol, or their mixtures, in amounts of up to 100% w/w of the formulation. The present invention discloses the preparation and the use of the formulation that provides all known positive therapeutic effects of ortho-silicic and salicylic acid in human and animals, and benefits of use for plants.

STABILIZED SOLUTION OF ORTHO-SILICIC ACID BASED ON SALICYLIC ACID AS EFFECTIVE INHIBITOR OF ITS POLYMERIZATION, ITS PREPARATION AND USE

DESCRIPTION

Field of the invention

The present invention relates to the composition of highly bioavailable silicon (Si) which is used in medicine, cosmetics, veterine and agronomy.

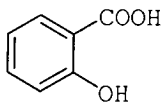
Summary of the invention

The present invention solves technical problem of effective stabilization of ortho-silicic acid (H_4SiO_4), which is used as nutritional and therapeutic source of highly bioavailable silicon (Si).

Formulation of the product is in the form of a solution comprising:

(i) ortho-silicic acid (H_4SiO_4), from 0.01-8% w/w;

(ii) salicylic acid (**1**),



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from 1-2 molar equivalents to H_4SiO_4;

(iii) pharmaceutically acceptable acid, from 0.1-4 molar equivalents to H_4SiO_4; or pharmaceutically acceptable base, in amounts of 2 molar equivalents to salicylic acid (**1**); and

(iv) diluent, selected from the group consisting of: purified water, 1,2-propylene glycol, glycerol, ethanol, or their mixtures, in amounts of up to 100% w/w of the formulation.

The use of the formulation provides all positive therapeutic effects of silicon in human, animal or plant organism.

Prior art

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

(i) helps resorption of calcium and takes part in its metabolism; stimulates osteoblasts; stimulates bone mineralization; in traumatic cases, influences faster bone healing; helps in prevention of osteoporosis [E. M. Carlisle: A requirement for silicon for bone growth in culture, *Fed. Proc.* **37** (1978) 1123; E. M. Carlisle: A relation between silicon and calcium in bone formation, *Fed. Proc.* **29** (1970) 265; E. M. Carlisle: Silicon: a requirement in bone formation independent of vitamin D, *Calcif. Tissue Int.* **33** (1981) 27; D. M. Reffitt, N. Ogston, R. Jugdaohsingh: Orthosilicic acid stimulates collagen type I synthesis and osteoblast-like cells *in vitro*, *Bone* **32** (2003) 127; S. Spripanyakorn, R. Jungdaohsingh, R. P. H. Thompson, J. J. Powell: Dietary silicon and bone health, *Nutr. Bull.* **30** (2005) 222];

(ii) takes part in the structure of connective tissue and formation of functional tertiary structure of building proteins of soft organs such as liver, lung, and brain; takes part in structure of arterial, vein, and capillary walls, increases elasticity and hardness of blood vessels, decreases their permeability [E. M. Carlisle, D. L. Garvey: The effect of silicon on formation of extra-cellular matrix components by chondrocytes in culture, *Fed. Proc.* **41** (1982) 461; E. M. Carlisle, C. Suchil: Silicon and ascorbate interaction in cartilage formation in culture, *Fed. Proc.* **42** (1983) 398];

(iii) acts as cross-linking agent for glucosaminoglycans and mucopolysaccharides in joints, ligaments, and sinovial fluid [K. Schwartz: A bound form of silicon in glycosaminoglycans and polyuronides, *Proc. Nat. Acad. Sci. USA* **70** (1973) 1608; A. Lassus: Colloidal silicic acid for the treatment of psoriatic skin lesions, arthropathy and onychopathy. A pilot study. *J. Int. Med. Res.* **25** (1997) 206];

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

(v) exhibits antiinflammatory effect; e.g. helps at various inflammatory diseases like rheumatoid arthritis, muscle inflammation, skin disorders such as psoriasis, seborrheic dermatitis, neurodermitis, skin irritations, accelerates wound healing, soothes decubitus and other skin disorderds and diseases [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; A. Lassus: Colloidal silicic acid for the treatment of psoriatic skin lesions, arthropathy and onychopathy. A pilot study. *J. Int. Med. Res.* **25** (1997) 206];

(vi) in oligomeric form, silicic acid inhibits resorption of aluminum (Al^{3+}) from gastrointestinal tract, and beside antioxidative action, preventively influences on development of neurodegenerative diseases like Alzheimer disease [J. D. Birchall, J. S. Chappell: The chemistry of aluminium and silicon in relation to Alzheimer's disease, *Clin. Chem.* **34** (1980) 265; R. Jugdaohsingh: Soluble silica and aluminium bioavailability, PhD Thesis (1999) University of London; R. Jugdaohsingh, S. H. Anderson, K. L. Tucker: Dietary silicon intake and absorption, *Am. J. Clin. Nutr.* **75** (2002) 887; R. Jugdaohsingh, D. M. Reffitt, C. Oldham: Oligomeric but not monomeric silica prevents aluminium absorption in human, *Am. J. Clin. Nutr.* **71** (2000) 944; D. M. Reffitt, R. Jugdaohsingh, R. P. H. Thompson: Silicic acid: its gastrointestinal uptake and urinary excretion in man and effects on aluminium excretion, *J. Inorg. Biochem.* **76** (1999) 141];

(vii) stimulates biosynthesis of skin building proteins: collagen and elastin [C. D. Seaborn, F. H. Nielsen: Silicon deprivation decreases colagen formation in wounds and bone, and ornithine transaminase enzyme activity in liver, *Biol. Trace Element Res.* **89** (2002) 251; M. R. Calomme, D. A. V. Berghe: Supplementation of 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]; and

(viii) stimulates growth 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].

At plants, silicon shows 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 enhances utility of nutrients, what results in increased crop yields;
(ii) improves water management, thus increases resistance to stress events like drought; and
(iii) enhances resistance to attacks of insects and fungal diseases.

Biologically available form of silicon is ortho-silicic acid (H_4SiO_4). However, in literature, there is described that too large doses of silicic acid can cause damages of liver and kidney which is the most important organ for excretion of silicon [J. W. Dobbie, M. J. Smith: Silicate nephrotoxicity in the experimental animal: the missing factor in analgesic nephropathy, *Scotish Med. J.* **27** (1982) 10].

A person skilled in the art knows that silicic acid in its monomeric form, ortho-form (H_4SiO_4), is not stable and at higher concentration, but undergoes polymerization with formation of dimer ($H_6Si_2O_7$), trimer ($H_8Si_3O_{10}$), and linear chain oligomers (**S1**) which are still water soluble. Linear chain polymers of silicic acid (**S1**) undergo further polymerization yielding tridimensional, branched polymers (**S2**) which are not of significant water solubility but form opalescent gel. The polymerization process proceeds further with formation of hydratized silicon dioxide (silica gel; $SiO_2 \cdot xH_2O$). The course of polymerization of silicic acid is given in Scheme 1 (at the end of the specification).

Beside monomeric ortho-silicic acid (H_4SiO_4), biologically available forms are also its lower oligomers soluble in water, due to partial hydrolysis that release starting H_4SiO_4 (oligomerization is reversible). In other words, under certain conditions of concentration, the equilibrium between ortho-silicic acid and its lower oligomers is established.

Branched polymers of silicic acid are not biologically available [H. Yokoi, S. Enomoto: Effect of degree of polymerization of silicic acid on the gastrointestinal absorption of silicate in rats, *Chem. Pharm. Bull.* **27** (1979) 1733; K. Van Dyck, R. Van Cauwenbergh, H. Robberecht: Bioavailability of silicon from food and food supplements, *Fresenius J. Anal. Chem.* **363** (1999) 541].

By using natural, as less as possible refined food (e.g. whole grain cereals), usual intakes of silicon in organism are sufficient. However, at use of highly refined and unhealthy food, silicon deficiencies occur quite often. Such conditions, with eventual other factors, often can cause development of diseases or disorders where silicon plays important role.

Because of this reason, it is of a great importance development of stabilized form of silicic acid where its polymerization is inhibited and, in this way, lost its bioavailability. Such products can be used as effective food supplements or therapeutic agents at diseases and disorders caused by silicon deficiency.

For application in pharmacy, cosmetics, and veterinary, only pharmaceutically acceptable forms of silicic acid can be employed. For use in agriculture, also, only non-toxic forms of silicic acid of high bioavailability can be applied.

The most known product used as food supplement for silicon supplementation is „BioSil^{R}", based on choline chloride-stabilized ortho-silicic acid [S. R. Bronder, U.S. 5,922,360 (1999); V. Berghe,

D. A. Richard, E.P. 1 371 289 A1 (2002), the holder is BioPharma Sciences B.V., Belgium].

Except choline chloride, in the patent literature there are mentioned also other stabilizers that prevent (inhibit) polymerization of ortho-silicic acid such as humectants like polyethylene glycol, polysorbates, plant gums, substituted cellulose, 1,2-propylene glycol, pectin, ethoxylated derivatives of higher fatty acids, acetylated or hydroxypropyl-derivatized starch, starch phosphate, urea, sorbitol, maltitol, vitamins [W. A. Kros, U.S. 2006/0178268 A1], as well as proline, serine, lysine, arginine, glycine, their mixtures, polypeptides or protein hydrolyzates [V. Berghe, D. A. Richard, WO 2004/016551 A1 (Bio Pharma Sciences B.V.)].

Beside choline chloride-stabilized silicic acid, on the market exist various food supplements which contain silicon in the forms of amorphous or colloidal silicon dioxide (SiO_2). However, such products are characterized by very low bioavailability [R. Jugdaohsingh: Silicon and bone health, *J. Nutr. Health Aging* **11** (2007) 99].

Somewhat effective (bioavailable) sources of silicic acid are also various plant drugs like extracts of horsetail (*Equisetum arvense*), nettle (*Urtica dioica*), and some other plants. However, it is known that portions of soluble (and thus bioavailable) silicic acid from these healing plants usually do not exceed 1/10 of total amounts. All remained silicic acid is not soluble and, as such, not bioavailable [D. Kuštrak: Pharmacognosy and phytopharmacy (in Croatian) Golden marketing-Tehnička knjiga, Zagreb, Croatia (2005)].

In agriculture, silicon based products are used for only a few years. They are used for increasing resistance of plants to stress (at drought or hail) and against fungal diseases. It seems that they also pasively protect from insect attacks by forming thin hard barrier of silicon dioxide on the plant leaves. The most known

product are those based on horsetail (*Equisetum arvense*) extract or finelly milled quartz sand (silicon dioxide; SiO_2) in organic, and solution of potassium silicate (30% K_2SiO_3) in conventional agriculture (mainly at grape; e.g. „Sil-Matrix"). These products are usually applied by foliar spraying.

Salicylic acid (**1**) is a well known pharmaceutically active substance which, as such, or in forms of its derivatives (e.g. salicylamide, acetylsalicylic acid), is widely used as antiinflammatoric, analgesic, and antipyretic for decades. At topical application in higher concentrations (>5%) acts as keratolytic (removes dead top skin layers) what is used both in medicine and cosmetic (peeling). In lower concentrations (1-2%), it acts as keratoplastic. Beside this, exhibits topical microbiocidal action.

Technical problem of production of improved product with effects of bioavailable silicon based on effective stabilization of ortho-silicic acid (H_4SiO_4) is solved by the present invention on a new [with salicylic acid (**1**)] and significantly better way, as will be demonstrated in detailed description of the invention.

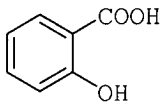
Detailed description of the invention

The present invention represents improved pharmaceutical, cosmetic, veterinary or agrochemical composition which is effective source of highly bioavailable silicon.

The formulation is consisting of:

(i) ortho-silicic acid (H_4SiO_4), from 0.01-8% w/w;

(ii) salicylic acid (**1**),



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from 1-2 molar equivalents to H_4SiO_4;

(iii) pharmaceutically acceptable acid, from 0.1-4 molar equivalents to H_4SiO_4; or pharmaceutically acceptable base, in amounts of 2 molar equivalents to salicylic acid (**1**); and

(iv) diluent, selected from the group consisting of: purified water, 1,2-propylene glycol, glycerol, ethanol, or their mixtures, in amounts of up to 100% w/w of the formulation.

In the present formulation the following pharmaceutically acceptable acids can be used: hydrochloric (HCl), sulfuric (H_2SO_4), nitric (HNO_3), phosphoric (H_3PO_4), methanesulfonic (CH_3SO_3H), benzenesulfonic ($C_6H_5SO_3H$), salicylic (1,2-C_6H_4(OH)COOH) or sulfosalicylic [C_6H_3(3-COOH)(4-OH)SO_3H] acid, mixtures of these acids, or other acids which are not of significant toxicity for human, animal, or plant organism.

The use of salicylic acid as pharmaceutically acceptable acid represents the special case of the present invention, because then it is in the same time:

(i) a stabilizer of ortho-silicic acid at pH values closed to neutral (and physiological);

(ii) agent for acid-catalyzed hydrolysis of precursor or silicic acid (**PSA**); and

(iii) pH-regulating agent of the present formulation.

Pharmaceutically acceptable base is selected from the group comprising sodium hydroxide (NaOH), potassium hydroxide (KOH), ammonium hydroxide (NH_4OH), tetramethylammonium hydroxide [$N(CH_3)_4OH$], tetraethylammonium hydroxide [$N(C_2H_5)_4OH$], mixtures of these bases, or other bases characterized by:

(i) negliable toxicity to human, animal or plant organism; and

(ii) which do not precipitate insoluble silicates in aqueous medium.

Completely unexpectable, it was found that salicylic acid (**1**) acts as effective stabilizer of ortho-silicic acid (H_4SiO_4) at pH values closed to neutral. In this manner, it inhibits its polymerization into biologically unavailable polymers of silicic acid. Consequently

increases its bioavailability after oral administration of the formulation from the present invention.

The effect was found and studied on a model complex **2**, disodium salicylate-H_4SiO_4, prepared from sodium silicate (Na_2SiO_3) and salicylic acid at molar ratio of 1:1. Chemically pure sodium silicate was prepared by base-catalyzed hydrolysis of tetraethyl orthosilicate [TEOS; $Si(OC_2H_5)_4$] with sodium hydroxide (NaOH). Hydrolysis reaction and formation of the complex **2** with salicylic acid is given in Scheme 2 (at the end of the specification).

Since pH values of solutions of complexes like compound **2** are in basic region, and are between 10-13, these are termed as „basic complexes of salicylic and ortho-silicic acid".

The study of stabilizing effect of salicylic acid was carried out in conditions that are known to result in fast polymerization of ortho-silicic acid (H_4SiO_4), and these are at pH values close to neutral. At these conditions, pH= 6-7, relatively fast polymerization of H_4SiO_4 takes place with formation of its polymers what is accompanied with generation of opalescent gel. In more concentrated systems, the change from the phase of solution (which is, at the begining, clear and afterwards opalescent) to the moment of formation of (opalescent) gel is relatively fast, and can be used in analytical purpose for determination of gelling (polymerization) rate (time) of ortho-silicic acid (H_4SiO_4).

The test solution was prepared by mixing equal volumes of the solution of compound **2** (sample solution) and 1.5M phosphate buffer pH= 4.5. The time required for conversion of thus prepared clear test solution until the formation of opalescent gel was determined. This time was called gelling or polymerization time (t_G). Longer gelling (t_G) time means slower polymerization, this suggests on more stable complex.

Beside the complex **2** from the present invention, as control probes, by the same manner the followings are studied:

(i) sodium silicate solution (Na_2SiO_3) as standard;

(ii) solution of complex with choline chloride [$(CH_3)_3N^+(CH_2CH_2OH)$ Cl^-]; and

(iii) solution of complex with L-serine ($HOOCCH(NH_2)CH_2OH$);

which are described in the prior art as H_4SiO_4 stabilizers [S. R. Bronder: Stabilized orthosilicic acid comprising preparation and biological preparation, WO95/21124 (1994)]. Results are given in Table 1.

Table 1. Basic complexes of salicylic and ortho-silicic acid: Stabilizing effect of salicylic acid (**1**) on polymerization of ortho-silicic acid (H_4SiO_4) at pH= 6.5.

No.	**Composition of sample solution**[a]	**Gelling time t_G [min]**[b]	**Relative stability**[c]
Standard:[d]			
1	(i) Na_2SiO_3 (1% w/w Si)	105	1
Prior art:[e]			
2	(i) Na_2SiO_3 (1% w/w Si) (ii) 1 mol. equiv. choline chloride	25	0.24
3	(i) Na_2SiO_3 (1% w/w Si) (ii) 1 mol. equiv. L-serine	115	1.1
The present invention, basic complexes of H_4SiO_4:			
4	(i) Na_2SiO_3 (1% w/w Si) (ii) 1 mol.equiv. salicylic acid[f]	230	2.2
5	(i) Na_2SiO_3 (1% w/w Si) (ii) 15% w/w 1,2-propylene glycol[g]	140	1.3
6	(i) Na_2SiO_3 (1% w/w Si) (ii) 40% w/w 1,2-propylene glycol[g]	140	1.3
7	(i) Na_2SiO_3 (1% w/w Si) (ii) 1 mol. equiv. salicylic acid (iii) 15% w/w 1,2-propylene glycol[h]	430	**4.1**

[a] In all test solutions as diluent was employed distilled water, except otherwise noted. All solutions of complexes contained 6.5 w/w of ethanol which was generated as side-product of hydrolysis of tetraethyl orthosilicate (TEOS). Stability tests were carried out by mixing 2 mL of each of sample solution or standard with 2 mL of 1.5M phosphate buffer of pH= 4.5; pH values of all solutions after mixing with the buffer were the same (pH= 6.5).

[b] The time from the moment of mixing the sample solution and phosphate buffer (clear solution) until the formation of opalescent gel, expressed in minutes [min].

[c] „Relative stability" is expressed as numerical parameter, coefficient, which describes stability of ortho-silicic acid in the given sample in comparison with the standard [pure solution of sodium silicate (Na_2SiO_3)]. It shows stabilizing or destabilizing effect on ortho-silicic acid, in other words on its polymerization (gelling).

[d] This was prepared by addition of TEOS (1.2 mL; 1.12 g; 0.0054 mol) to a solution of sodium hydroxide (NaOH; 0.44 g; 0.011 mol; 2.05 equiv.) in distilled water (6.00 g) with stirring during 6 h, and subsequent dilution with distilled water (7.44 g) up to the total weight of 15.00 g [contains 150 mg (1% w/w) of Si].

[e] Samples are prepared by addition of 0.0054 mol of choline chloride (0.75 g) or L-serine (0.57 g) in hydrolyzed solution of sodium silicate (6.00 g distilled water + 0.44 g NaOH + 1.2 mL TEOS), with subsequent dilution with distilled water up to the total weight of 15.00 g [contains 150 mg (1% w/w) of Si].

[f] The solution of the complex was prepared by addition of salicylic acid (0.75 g; 0.0054 mol) in previously prepared solution of sodium silicate (6.00 g distilled water + 0.44 g NaOH + 1.2 mL TEOS), with subsequent dilution with distilled water up to the total weight of 15.00 g [contains 150 mg (1% w/w) of Si].

[g] Solutions are prepared by mixing previously prepared solution of sodium silicate (6.00 g distilled water + 0.44 g NaOH + 1.2 mL TEOS) and 2.25 g (15% w/w) or 6.00 g (40% w/w) of 1,2-propylene glycol with subsequent dilution with distilled water, up to the total weight of 15.00 g [contains 150 mg (1% w/w) of Si].

[h] The solution of the complex was prepared by addition of salicylic acid (0.75 g; 0.0054 mol) to previously prepared solution of sodium silicate (6.00 g distilled water + 0.44 g NaOH + 1.2 mL TEOS). Reaction mixture was stirred at room temperature during 1 h. Then, 1,2-propilene glycol (2.25 g; 15% w/w) was added, and subsequently diluted with distilled water, up to the total weight of 15.00 g [contains 150 mg (1% w/w) of Si].

Solutions like those of the complex **2** are clear and colourless solutions, stable to the occurence of gelling at room temperature (17-25 °C), and at temperatures <30 °C, during minimally 2 years.

Alternatively, the formulation from the present invention can be prepared as complex with ortho-silicic acid (H_4SiO_4) with salicylic acid salts (like disodium salicylate) in molar ratio of 1:2.

Beside basic complexes like compound **2**, the formulation from the present invention can be prepared as stabilized solution of ortho-silicic acid (H_4SiO_4) also in acidic medium, by the influence of one or more above-mentioned pharmaceutically acceptable acid (0.1-4 molar equivalents) in the presence of 1-2 molar equivalents of salicylic acid, calculated to H_4SiO_4.

Complex of salicylic acid and ortho-silicic acid, compound **3**, was prepared *in situ*, by phosphoric acid-catalyzed hydrolysis of tetraethyl orthosilicate (TEOS) in the presence of salicylic acid. The reaction is given in Scheme 3 (at the end of the specification).

Since pH values of solutions of the complexes like compound 3 are in acidic region, between 1-2.5, these are called „acidic complexes of salicylic and ortho-silicic acid".

The study of stability of acidic complexes of salicylic and ortho-silicic acid (H_4SiO_4) was performed with 1.32M phosphate buffer of pH= 7. As the control, complexes with choline chloride and L-serine,

described in the prior art as stabilizers of H_4SiO_4, were used. Results are given in Table 2.

Table 2. Acidic complexes of salicylic and ortho-silicic acid: Stabilizing effect of salicylic acid (**1**) on polymerization of ortho-silicic acid (H_4SiO_4) at pH= 6.5.

No.	Composition of sample solution[a]	Gelling time t_G [min][b]	Relative stability[c]
Standard:[d]			
1	(i) H_4SiO_4 (1% w/w Si) (ii) 0.55 mol. equiv. H_3PO_4	195	1
Prior art:[e]			
2	(i) H_4SiO_4 (1% w/w Si) (ii) 0.55 mol. equiv. H_3PO_4 (iii) 1 mol. equiv. choline chloride	18	0.09
3	(i) Na_2SiO_3 (1% w/w Si) (ii) 0.55 mol. equiv. H_3PO_4 (iii) 1 mol. equiv. L-serine	210	1.08
The present invention, acidic complexes of H_4SiO_4:[f]			
4	(i) H_4SiO_4 (1% w/w Si) (ii) 1 mol.equiv. salicylic acid (iii) ad 100% 1,2-propylene glycol	430	**2.2**
5	(i) H_4SiO_4 (1% w/w Si) (ii) 1 mol.equiv. salicylic acid (iii) 0.55 mol. equiv. H_3PO_4 (iv) ad 100% 1,2-propylene glycol	390	**2.0**

[a] In all test solutions, as diluent was used distilled water, except otherwise noted. All solutions contained 6.5% w/w of ethanol which was formed as side-product during hydrolysis of tetraethyl orthosilicate (TEOS). Stability tests were performed by mixing 2 mL of each of sample solution with 2 mL of 1.32M phosphate buffer of pH= 7.0; pH values of all test solutions after mixing with buffer were the same (6.5).

[b] The time from the moment of mixing the given sample solution and phosphate buffer (clear solution) until the formation of opalescent gel, expressed in minutes [min].

[c] „Relative stability" is expressed as numerical parameter, coefficient, which describes stability of ortho-silicic acid in the given sample in comparison with the standard [pure solution of silicic acid (H_4SiO_4)]. It shows stabilizing or destabilizing effect on ortho-silicic acid, in other words on its polymerization (gelling).

[d] This 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; 0.00295 mol; 0.55 mol. equiv.) in distilled water (13.54 g) with stirring for 6 h [total wight 15.00 g; contains 150 mg (1% w/w) of Si].

[e] Samples are prepared by addition of 0.0054 mol of choline chloride (0.75 g) or L-serine (0.57 g) to a solution of ortho-silicic acid (H_4SiO_4; 10.00 g destilirana voda + 1.2 mL TEOS + 0.2 mL 85% H_3PO_4; 3 h-stirring / room temperature) with subsequent dilution with distilled water, up to the total weight of 15.00 g [contains 150 mg (1% w/w) of Si].

[f] Samples are prepared by addition of salicylic acid (0.75 g; 0.0054 mol) to a solution of tetraethyl orthosilicate (TEOS; 1.2 mL; 1.12 g; 0.0054 mol) in 1,2-propylene glycol (10.00 g). Distilled water (0.4 mL; 0.022 mol; 4.1 mol. equiv.) was added to the reaction mixture, and stirred at room temperature during 5 h. Then, 1,2-propylene glycol was added to the solution up to the total weight of 15.00 g [contains 150 mg (1% w/w) of Si].
To the solution from the Experiment 5, also 85% phosphoric acid (0.2 mL) was added.

From thus obtained results, it was concluded that choline chloride, which is in the literature described as „stabilizer" of ortho-silicic acid, actually acts as catalyst of its polymerization under physiological conditions where pH value is close to 7. Solutions which contained choline chloride showed 5-10x faster polymerization process accompanied with formation of silica gel in comparison to

the solution of the standard (Experiments 2; Table 1 and 2). Choline chloride can be obviously considered as „stabilizer" of silicic acid in a formulation with very low pH, lower than pH= 3, due to its property of „deep eutectic liquid" in mixture with polyols like glycerol. In fact, it is „stabilizer" in technological sense (as excipient) which helps stabilization of final product, solution of H_4SiO_4, providing long term shelf life of the product.

However, in contrast to this, under physiological conditions, at pH values close to 7, it destabilizes ortho-silicic acid catalyzing its polymerization, and thus decreases their bioavailability. This finding is in accordance with literature data wherein it was described that bioavailability of choline chloride-stabilized ortho-silicic acid at oral administration is <50% [R. Jugdaohsingh: Silicon and bone health, *J. Nutr. Health Aging* **11** (2007) 99].

Additionally, amino acid serine, which is also described in the literature as stabilizer of ortho-silicic acid, does exhibit slight stabilizing effect, indeed. However, this effect is almost negliable because observed increase of gelling time was only 8-10% prolonged against that for the standard (Experiments 3; Tables 2 and 3).

In contrast, salicylic acid (**1**) exhibits significant effect of stabilization of ortho-silicic acid (H_4SiO_4) where observed polymerization time was 2.2x longer (Experiments 4; Tables 2 and 3), what suggest on high stability of the complex H_4SiO_4-salicylic acid (compound **3**).

It was found that application of 1,2-propylene glycol as humectant which acts as auxiliary stabilizer, in accordance to the literature statements, does increase polymerization time of H_4SiO_4, indeed, for approx. 30% (Experiments 5 and 6; Table 1). Determination of optimal weight percentage of 1,2-propylene glycol, where concentrations of 15% w/w (Experiment 5) and 40% w/w (Experiment 6) were studied, showed that the use of higher concentration fail to result in further positive effect on stability of H_4SiO_4. In conclusion,

optimal concentration of 1,2-propylene glycol in the formulation was 15% w/w.

In continuation of the research, it was found a synergistic effect of 1,2-propylene glycol (in optimal concentration of 15% w/w) on the basic stabilizing effect of salicylic acid.

The formulation of the present invention based on combination of salicylic acid (1 mol. equiv. to H_4SiO_4) and 15% w/w of 1,2-propylene glycol showed 4.1x longer polymerization time than at the standard (Experiment 7; Table 1). This result represents increase of almost 100% from the result obtained with the use of salicylic acid (Experiment 4; Table 1) as sole stabilizer. These results clearly suggest to those skilled in the art an unexpected additional synergistic effect on stabilization of ortho-silicic acid.

By the use of a version of the formulation from the present invention with 1,2-propylene glycol as sole diluent, this additional synergistic effect onto basic stabilizing effect of salicylic acid is lost. In this manner, in Experiments 4 and 5 (Table2), obtained gelling times are 2-2.2x longer than at standard, what is also a very good result, but in the same range as with salicylic acid only (Experiment 4; Table 1).

However, such versions of the formulation of the present invention exhibit adequate stability in real time at acidic acomplexes of salicylic and ortho-silicic acid.

Except 1,2-propylene glycol, as humectant can be also used glycerol. Additionally, as alternative diluent, beside purified water, can be employed ethanol, or mixtures of these substances.

Solutions of the complex like compound **3** are also clear, colourless and relatively viscous solutions, stable to occurrence of gelling at room temperature (17-25 °C), and at temperatures <30 °C, during minimally 2 years.

Explanation of inhibition effect of salicylic acid on polymerization of ortho-silicic acid (H_4SiO_4)

From obtained results, it can be concluded that salicylic acid acts stabilizing to ortho-silicic acid presumably due to formation of relatively stable complexes with it.

In the basic medium, as is the case with the complex **2** (Scheme 2), in solution are present 2 molar equivalents of strong base (e.g. NaOH) which reacts with salicylic acid yielding its disodium salt, disodium salicylate [1,2-C_6H_4(ONa)COONa]. Acidity of ortho-silicic acid [$pK_a(H_4SiO_4) = 2,2 \cdot 10^{-10}$] is similar to that of hydroxyl group of simple phenol [$pK_a(C_6H_5OH) = 1,3 \cdot 10^{-10}$]. However, due to electron-attracting properties of carboxylic group in the ortho-position, acidity of phenolic group of salicylic acid is higher than that of ordinary phenol or ortho-silicic acid (H_4SiO_4). Because of this, the compound **2** is not correct to name silicate, but it can be rather considered as the complex of disodium salicylate and ortho-silicic acid (H_4SiO_4).

Since in the solution of complex **2** in (predominantly) aqueous medium, due to hydrolysis, is present also significant concentration of hydroxide anions (OH^-), what is the reason of why the solution is basic, subsequently, certain amounts of ortho-silicic acid is present in the form of ortho-silicate anion $Si(OH)_3O^-$, indeed.

However, this fact does not have any negative consequences in final use of the formulation from the present invention, because, upon dilution with water at oral administration, it provides ortho-silicic acid exclusively in its monomeric form. This ensures maximal level of bioavailability, what is not the case at choline chloride-stabilized H_4SiO_4 where some significant amounts of the same is already polymerized, and thus corresponding product is of lowered bioavailability.

In acidic medium salicylic acid also forms complex with ortho-silicic acid, like complex **3** (Scheme 3). Completely the same (analogous) complex is generated by addition of basic complex like compound **2** into acidic or neutral (physiological) medium. From this follows complete analogy between the complex **2** and complex **3** because:

(i) compound **2** in physiological conditions gives the complex **3** (Scheme 4, at the end of specification);

(ii) whilst the compound **3** exists both in more acidic medium as well as under physiological conditions (at pH values closed to 7).

Finally, stablizing effect of salicylic acid is obviously consequence of its structure, where two functional groups are present, carboxylic (as bidentate ligand) and phenolic hydroxyl group (as monodentate ligand). Due to their neighbouring, ortho-position, salicylic acid acts as very effective tridentate ligand for ortho-silicic acid (H_4SiO_4). Stability of such complex is significant, what is visible from drastically increased polymerization (gelling) time at pH= 6.5. This actually means that the stability constant of the complex **3** is very high; this result in very low equilibrium concentration of free H_4SiO_4 in the solution of the complex, what consequently leads to drastically slower polymerization process (high values of t_G).

Additional synergistic effect of 1,2-propylene glycol (**PG**) on the basic stabilizing effect of salicylic acid is presumably consequence of additional formation of hydrogen bonds between molecules of **PG** and the complex **3**. It can be shown by calculation that (roughly) estimated optimal amounts of 1,2-propylene glycol of 15% w/w in the formulation corresponds to the value of approx. 5.5 molar equivalents of **PG** to H_4SiO_4. Probably, minimal molar excess of 4 equivalents of **PG** to H_4SiO_4 does act positively in a synergistic manner, due to the formation of hydrogen bonds between molecules of **PG** and the complex **3**.

Use of the formulation from the present invention

Application of the formulation of the present invention provides all known positive therapeutic effects of silicic acid on human, animal or plant organism, which are known to those skilled in the art.

At humans and animals, the present formulation is used in the following medicinal, cosmetic, and veterinary indications:

(i) helps in resorption of calcium; takes part in its transport, stimulates osteoblasts, stimulates bone mineralization, accelerates wound healing; in prevention of osteoporosis;

(ii) takes part in structure of arterial, vein, and capillary walls, increases elasticity and hardness of blood vessels, decreases its 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 at all diseases and disorders which develop upon weak immune system like various allergic diseases;

(iv) antiinflammatory effect of silicon and silicic acid; therapy of various acute and chronic inflammatory diseases, e.g. positively acts at various inflammations of locomotive system such as muscle inflammations, rheumatoid arthritis, etc; skin diseases like psoriasis, seborrheic dermatitis, neurodermitis, eczema, skin irritations, burns, wound healing, at dandruff, and at other skin disorders and diseases; also positively acts at other inflammatory diseases;

(v) acts as cross-linking agent for glucosaminoglycans and mucopolysaccharides, and thus helps function of joints, ligaments, and production of synovial fluid;

(vi) inhibits resorption of aluminum (Al^{3+}) from gastrointestinal tract, thus preventively acts on development of neurodegenerative diseases like Alzheimer or Parkinson diseases;

(vii) stimulates biosynthesis of skin building proteins: collagen and elastin; in treatment of wrinkles and prevention of their development; thus helps in slowing-down skin ageing;

(viii) stimulates growth of hair and nails; for strengthening of hair and nails; also hair becomes shinier.

Due to the presence of salicylic acid which, beside antiinflammatory action, exhibits also analgesic and antipyretic effects, the formulation from the present invention is used as adjuvant in treatment of pain and decreasing of increased body temperature. This is expecially recommended at indications where basic patological condition is consequence of silicon deficiency.

As example, herein is given the treatment of strong pain at bone fractures, joints and/or ligaments. The silicon therapy in these cases is essential for fast mineralization process and healing, and in the same time can provide (due to the content of salicylic acid):

(i) soothing of inflammation process; and

(ii) calming pain; which are formed due to given traumatological changes.

At topical application (e.g. in cosmetics), the formulation of the present invention, due to the content of salicylic acid, shows:

(i) keratoplastic effect, at concentrations of salicylic acid <2% w/w;

(ii) keratolytic (peeling) effect, at concentrations of salicylic acid >5% w/w in the final formulation; and

(iii) microbiocidal effect.

The latter effects of salicylic acid are excellently supplemented with basic actions of silicon, where effects of refreshing of the skin are achieved through combination of wrinkle reducing (biosynthesis of collagen and elastin), keratolytic/keratoplastic, and microbiocidal effects.

Moreover, due to microbiocidal effect of salicylic acid and fungistatic action of ortho-silicic acid, the formulation from the present invention at topical application provides positive effects in conditions like:

(i) acne;
(ii) problematic skin;
(iii) seborrheic dermatitis; and
(iv) dandruff.

It is known to those skilled in the art that analogous biological effects of silicon (in the form of H_4SiO_4) exhibits also at animals, in this manner, the formulation of the present invention is applied in veterinary in all mentioned indications.

At plants, the formulation of the present invention provides:

(i) increased crop yields (due to stimulation of photosynthesis through better utility of nutrients which are added by common fertilization; silicon effects);
(ii) resistance to stressful events (e.g. during drought or after hail; silicon effects); and
(iii) resistance to fungal diseases (effects of silicon and salicylic acid).

The formulation of the present invention intended for medicinal, cosmetic, veterinary, and agrochemical applications is in the dosing form of solution (concentrate). Before use, the solution is diluted with water and administered orally in a dosage which corresponds to the following daily intakes of silicon (Si):

(i) 5-25 mg of Si at humans; and

(ii) 5-250 mg of Si at animals; 5-50 mg at small animals like cats or dogs, 50-250 mg at large ones like horses and cows.

In agriculture, the present formulation is also diluted with water up to the final concentration od silicon from 0.005-0.1% w/w, and applied by foliar application by using all common spraying equipments.

Lower concentrations (0.005-0.05% w/w of Si) are used preventatively for stimulation of growth and against occurrence of fungal diseases (e.g. at grape), whilst higher concentrations (0.05-0.1% w/w of Si) are applied in urgent conditions of drought or after hail. Dosage rates are from 10-100 g of silicon per hectare (ha) or 1-10 L of the present formulation in concentration of 1% w/w of Si per single tank of 200-400 L of water, applied to the area of 1 ha.

Finally, the formulation of the present invention can be used as starting material (intermediate) for production of other pharmaceutical products, cosmetics, then veterinary or agrochemical products with content of silicon (Si) of high bioavailability.

For instance, the version of the formulation from the present invention of the composition:

- 3.8% w/w H_4SiO_4 [corresponds to 1% w/w of Si]
- 5% w/w salicylic acid;
- 6.5% w/w ethanol;
- ad 100% w/w 1,2-propylene glycol;

in the form of colourless viscous solution, serves as suitable concentrate (intermediate) for production of various oral and topical final dosage forms for human or veterinary use, such as: oral solution, oral suspension, shampoo, lotion, cosmetic mask, cream, ointment, gel, therapeutic patch for human use; or concentrate for solution intended for use in agriculture.

Preparation of the formulation from the present invention

Basic complexes of ortho-silicic (H_4SiO_4) and salicylic acid are prepared by hydrolysis of precursor of silicic acid (**PSA**) tetraethyl orthosilicate (TEOS):

(i) in the presence of 2 molar equivalents of pharmaceutically acceptable base in a diluent, with subsequent addition of salicylic acid; or alternatively,

(ii) in previously prepared solution of salt of salicylic acid with pharmaceutically acceptable base in a diluent.

Alternatively, the following **PSA** can be used:

(i) sodium or potassium silicate (common composition $xM_2O \bullet ySiO_2$; M= Na,K, x:y= 1:1 do 1:3,5); or

(ii) silicon tetrachloride ($SiCl_4$).

The use of sodium (Na_2SiO_3) or potassium silicate (K_2SiO_3) as **PSA** represents a special case of performance of the present invention, because these are in the same time:

(i) pharmaceutically acceptable bases, as sources of sodium (NaOH) or potassium (KOH) hydroxide; and

(ii) sources of silicic acid (**PSA**).

In these cases, no additional pharmaceutically acceptable base is used, since equimolar amounts of these silicates and salicylic acid do directly give salicylate salts like disodium or dipotassium salicylates which, in the same time act as:

(i) basic agent for hydrolysis of TEOS; and as

(ii) ligand for complexation of *in status nascendi* formed H_4SiO_4.

In the case of the use of $SiCl_4$ as **PSA** in this synthesis, 6 molar equivalents of pharmaceutically acceptable base (e.g. NaOH) is employed, because, 4 equivalents is spent on neutralization of hydrochloric acid (HCl) generated during hydrolysis of $SiCl_4$, whilst 2 remained equivalents serve for neutralization reaction of salicylic acid yielding salicylate salt (e.g. disodium salicylate)

which forms the complex with liberated H_4SiO_4 (complex 2; analogously to Scheme 2).

Acidic complexes of salicylic and ortho-silicic acid, such as compound **3**, are prepared by addition of 0.1-4 molar equivalents of pharmaceutically acceptable acid into previously prepared solution of precursor of silicic acid (**PSA**) and salicylic acid in the diluent.

In the preparation of the formulation of the present invention, no matter of the kind of either basic or acidic complex of ortho-silicic and salicylic acid, the following molar ratios of salicylic acid and precursor of silicic acid (**PSA**; expressed through the molar portion of silicon in the **PSA**) is used:

salicylic acid : Si = 1:1 to 2:1

As the diluent or solvent 1,2-propylene glycol, purified water, glycerol, ethanol, or mixtures of these substances can be employed.

Reactions are conducted by vigorous stirring at temperatures from -10 °C to +40 °C, preferably from +15 °C to +30 °C (conditions of room temperature) during 0,5-6 h.

In the case of the use of sodium or potassium silicate or silicon tetrachloride ($SiCl_4$) reaction is very exothermic. At the use of tetraethyl orthosilicate (TEOS), the reaction is only mildly exothermic, however, with mild cooling; the reaction is conducted without special difficulties.

In the case of the use of $SiCl_4$ or sodium/potassium silicate, the reaction is almost instantly finished, whereas the hydrolysis reaction of TEOS tooks 1.5-2 h at room temperature.

The use of tetraethyl orthosilicate (TEOS) is preferred because it is neither toxic nor corrosive like $SiCl_4$, and available commercial

products are of very high purity due to the fact that TEOS is readily purified by distillation. In this manner, final product of very high purity with the content of unwanted heavy metals (Pb, Cd, Hg, As) far under common limits for pharmaceutical products and food supplements can be produced. In contrast, sodium or potassium silicate are difficult to purify from heavy metals, so, commercial products are not of so high level of chemical purity.

In every case, ortho-silicic acid (H_4SiO_4), *in status nascendi* generated in the reaction, forms the complex with:

(i) salicylate salt (in basic medium; example is the complex **2**, Scheme 2); or with

(ii) salicylic acid (in acidic medium; example is the complex **3**, Scheme 3).

In all cases, the formulation of the present invention is clear, colourless, more or less viscous solution.

As side-products in reactions of sodium or potassium silicate, equivalent amounts of sodium or potassium salts of pharmaceutically acceptable base are formes, which, after completion of the reaction can be eventually removed by filtration. For instance, at the use of sodium silicate and hydrochloric acid (HCl), the side-product is sodium chloride (NaCl) which is not soluble in 1,2-propylene glycol, and after synthesis is removed by filtration.

In the case of the use of tetraethyl orthosilicate (TEOS), four molar equivalents of ethanol (C_2H_5OH) are generated. Since ethanol in this concentration is completely harmless and does not influence negatively on the stability of the present solution, it is not removed but kept in the final product as auxiliary solvent or diluent. It is known to those skilled in the art of pharmaceuticaly technology that ethanol is widely used as pharmaceuticaly excipient, diluent. Alternatively, ethanol can be removed from the final solution of the present invention by evaporation under high vacuum at temperatures <40 °C, without negative effect upon its stability.

Finally, the reaction product, the solution, is only diluted with water or 1,2-propylene glycol up to the nominal concentration of silicon (Si), filtered, and paked into plastic bottles.

The course of the reaction is given in Schemes 2 and 3.

Examples

General remarks

The term room temperature refers to the temperature interval: 20-25 °C. All percentage (%) portions of ingredients are expressed as weight (w/w) portions.

Example 1

Preparation of standard solutions of sodium silicate and ortho-silicic acid, as well as solution of the control complexes with stabilizers choline chloride and L-serine from the prior art

(i) Preparation of standard solution of sodium silicate (Na_2SiO_3) of concentration of 1% w/w of silicon (Si) (Experiment 1; Table 1): To a solution of sodium hydroxide (NaOH; 0.44 g; 0.011 mol; 2.05 mol. equiv.) in distilled water (6.00 g), tetraethyl orthosilicate (TEOS; 1.2 mL; 1.12 g; 0.0054 mol) was added. The reaction mixture was stirred at room temperature for 6 h. Then, distilled water (7.44 g) was added up to the total weight of 15.00 g. Silicon content in such prepared standard solution is 150 mg (1% w/w of Si). Colourless clear solution, pH= 13-14.

(ii) Preparation of standard solution of ortho-silicic acid (H_4SiO_4) of 1% w/w concentration of silicon (Si) (Experiment 1; Table 2): To a solution of 85% phosphoric acid (0.2 mL; 0.34 g; 0.289 g H_3PO_4; 0.00295 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, destilled water (3.54 g) was added up to the total weight of reaction mixture of 15.00 g. Content of silicon in such prepared standard solution is 150 mg (1% w/w of Si). Clear colourless solution, pH= 1.5.

(iii) Preparation of basic complexes of choline chloride and L-serine with ortho-silicic acid of 1% w/w concentration of silicon (Si) (Experiments 2 and 3; Table 1). General procedure: To a solution of sodium hydroxide (NaOH; 0.44 g; 0.011 mol; 2.05 mol. equiv.) in distilled water (6.00 g), tetraethyl orthosilicate (TEOS; 1.2 mL; 1.12 g; 0.0054 mol) was added. The reaction mixture was stirred at room temperature for 6 h. Afterwards, to the reaction mixture that contains sodium silicate in amounts equivalent to 150 mg (0.0054 mol) of silicon (Si), choline chloride (0.75 g; 0.0054 mol) or L-serine (0.57 g; 0.0054 mol) as literature described „stabilizers" of ortho-silicic acid was added. Each solution was stirred at room temperature for 30 minutes, and then, in each of them, distilled water was added up to the total weight of 15.00 g. The silicon content in each of solution of complex was 150 mg (1% w/w of Si). pH of solutions was 12.0-12.5.

(iv) Preparation of solution of acidic complexes of choline chloride and L-serine with ortho-silicic acid of 1% w/w concentration of silicon (Si) (Experiments 2 and 3; Table 2). General procedure: To a solution of 85% phosphoric acid (0.2 mL; 0.34 g; 0.289 g H_3PO_4; 0.00295 mol; 0.55 mol. equiv.) in distilled water (10.00 g):

(a) choline chloride (0.75 g; 0.0054 mol; 1 mol. equiv.) was added in one solution; whilst to another,

(b) L-serine (0.57 g; 0,0054 mol; 1 mol. equiv.) was added.

In each reaction mixture, tetraethyl orthosilicate (TEOS; 1.2 mL; 1.12 g; 0.0054 mol) was added. The reaction mixtures was stirred at room temperature for 3 h. Then, distilled water was added in each solution up to the total weight (of each) of 15.00 g. Silicon content in each of the solution of complex is 150 mg (1% w/w of Si).

Example 2

Preparation of basic complexes of ortho-silicic and salicylic acid according to the present invention

(i) Preparation of the solution of complex 2, disodium salicylate / ortho-silicic acid of 1% w/w concentration of silicon (Experiment 4; Table 1): To a solution of sodium hydroxide (NaOH; 0.44 g; 0.011 mol; 2.05 mol. equiv.) in distilled water (6.00 g), tetraethyl orthosilicate (TEOS; 1.2 mL; 1.12 g; 0.0054 mol) was added. The reaction mixture was stirred at room temperature for 6 h. Then, salicylic acid (0.74 g; 0.0054 mol) was added to the reaction mixture in portions during 10 minutes with vigorous stirring. The reaction mixture was stirred at room temperature for 1 h. Afterwards, distilled water (6.70 g) was added up to the total weight of the reaction mixture of 15.00 g. Clear colourless solution; content of silicon in such prepared solution is 150 mg (1% w/w of Si). pH of the solution was 12.0-12.5.

(ii) Preparation of control solution of sodium silicate with 15% and 40% concentrations of 1,2-propylene glycol of 1% w/w concentration of silicon (Experiments 5 and 6; Table 1): Two analogous experiments of preparation of sodium silicate from tetraethyl orthosilicate were conducted: To a solution of sodium hydroxide (NaOH; 0.44 g; 0.011 mol; 2.05 mol. equiv.) in distilled water (6.00 g), tetraethyl orthosilicate (TEOS; 1.2 mL; 1.12 g; 0.0054 mol) was added. The reaction mixture was stirred at room temperature for 6 h. Then, to the reaction mixtures, 1,2-propylene glycol (**PG**) was added:

(a) 2.25 g for the contet of 15% **PG**; and

(b) 6.00 g for the content of 40% **PG**.

Then, distilled water was added up to the total weight of each reaction mixture of 15.00 g. Clear, colourless, and slightly viscous solutions were obtained; the silicon content in such prepared solutions is 150 mg (1% w/w of Si).

(iii) Preparation of complex 2, disodium salicylate and ortho-silicic acid (H_4SiO_4) with 15% 1,2-propylene glycol, according to the present invention, of 1% w/w concentration of silicon (Experiment 7; Table 1): To a solution of sodium hydroxide (NaOH; 0.44 g; 0.011 mol; 2.05 mol. equiv.) in distilled water (6.00 g), tetraethyl orthosilicate (TEOS; 1.2 mL; 1.12 g; 0.0054 mol) was added. The reaction mixture was stirred at room temperature for 6 h. Then, distilled water (4.45 g) and 1,2-propylene glycol (2.25 g) were added to the reaction mixture. Afterwards, salicylic acid (0.74 g; 0.0054 mol) was added in portions during 10 minutes with vigorous stirring. The reaction mixture was stirred at room temperature during 1 h. Then, the product was filtered. Colourless, clear, and slightly viscous solution was obtained; the silicon content was 150 mg (1% w/w of Si). pH value of the solution was 12.0-12.5.

The results of stability tests at pH= 6.5 and also the influence of salicylic acid on stability of ortho-silicic acid for basic complexes are given in Table 1.

Example 3

Preparation of acidic complexes of ortho-silicic and salicylic acid according to the present invention

(i) Preparation of solution of the complex 3 of ortho-silicic and salicylic acid of 1% w/w concentration of silicon (Experiment 4; Table 2): To a solution of salicylic acid (0.74 g; 0.0054 mol) in 1,2-propylene glycol (10.00 g), distilled water (0.40 g; 0.022 mol; 4.1 mol. equiv.) followed by tetraethyl orthosilicate (TEOS; 1.2 mL; 1.12 g; 0.0054 mol) were added. The reaction mixture was stirred at room temperature for 5 h. Then, 1,2-propylene glycol (2.74 g) was added to the reaction mixture up to the total weight of 15.00 g, and the product is filtered. Colourless, clear, and viscous solution of the following composition was obtained:

- 3.8% w/w H_4SiO_4 [or 1% w/w of silicon (Si)];
- 5% w/w salicylic acid;

- 6.6% w/w ethanol;
- up to 100% w/w 1,2-propylene glycol.

(ii) Preparation of the complex 3 of ortho-silicic and salicylic acid in the presence of phosphoric acid of 1% w/w concentration of silicon (Experiment 5; Table 2): To a solution of salicylic acid (0.74 g; 0.0054 mol) in 1,2-propylene glycol (10.00 g), distilled water (0.40 g; 0.022 mol; 4.1 mol. equiv.) and tetraethyl orthosilicate (TEOS; 1.2 mL; 1.12 g; 0.0054 mol) were added. Then, 85% phosphoric acid (0.2 mL; 0.34 g; 0.289 g H_3PO_4; 0.003 mol; 0.55 mol. equiv.) was added and stirred at room temperature for 3 h. To the solution, 1,2-propylene glycol (2.40 g) was added up to the total weight of 15.00 g, and the product was filtered. Colourless, clear, and viscous solution of the following composition was obtained:

- 3.8% w/w H_4SiO_4 [or 1% w/w of silicon (Si)];
- 5% w/w salicylic acid;
- 2% w/w phosphoric acid;
- 6.6% w/w ethanol;
- up to 100% w/w 1,2-propylene glycol.

The results from stability tests at pH= 6.5, and the effect of the influence of salicylic acid on stability of ortho-silicic acid, for acidic complexes of ortho-silicic acid are presented in Table 2.

Example 4

The study of influence of choline chloride and L-serine on stability of silicic acid (H_4SiO_4) in solution. Influence of salicylic acid on stability of H_4SiO_4 in solution.

(i) General procedure for basic complexes: In a test tube, 2 mL of 1.5M phosphate buffer of pH 4.5 and 2 mL of sample solution or solution of standard were mixed. pH values of all resulting test solutions after mixing with the buffer were the same (6.5). To such prepared mixtures (test solutions), the time from the moment of

mixing with phosphate buffer (t_o; all solutions in the moment of preparation were clear) to the formation of opalescent (thick) gel was determined. This time interval was termed as „gelling (polymerization) time", t_G, and expressed in minutes. Obtained results for t_G are expressed in comparison with results obtained for the standard solution of sodium silicate (Na_2SiO_3) of the same concentration of 1% w/w of silicon (the standard for basic complexes). The results are given in Table 1.

(ii) Preparation of 1.5M phosphate buffer of pH= 4.4 required for the testing of basic complexes: Sodium dihydrogenphosphate (NaH_2PO_4; 18.00 g; 0.15 mol) was 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 diluted with distilled water up to the mark of 100 mL. Colourless clear solution, pH= 4.5.

(iii) General procedure for acidic complexes: In a test tube, 2 mL of 1.32M phosphate buffer pH 7 and 2 mL of sample solution or solution of standard were mixed. pH values of all resulting test solutions after mixing with the buffer were the same (6.5). To such prepared mixtures (test solutions), the time from the moment of mixing with phosphate buffer (t_o; all solutions in the moment of preparation were clear) to the formation of opalescent (thick) gel was determined. This time interval was termed as „gelling (polymerization) time", t_G, and expressed in minutes. Obtained results for t_G are expressed in comparison with results obtained for the standard solution of ortho-silicic acid (H_4SiO_4) of the same concentration of 1% w/w of silicon (the standard for acidic complexes). The results are given in Table 2.

(iv) Preparation of 1.32M phosphate buffer of pH= 7 required for study of acidic complexes: Sodium dihydrogenphosphate (NaH_2PO_4; 16.00 g; 0.132 mol) and sodium hydroxide (NaOH; 3.14 g; 0.0785 mol) were quantitatively transferred into a 100 mL measuring flask and dissolved in about 80 mL of distilled water by shaking at room

temperature. Thus obtained solution was diluted with distilled water up to the mark of 100 mL. Colourless clear solution, pH= 7.0.

Example 5

Preparation of the formulation from the present invention in the form of solution of complex of ortho-silicic acid (H_4SiO_4) with dipotassium salicylate of 0.5% w/w concentration of H_4SiO_4 (or 0.15% w/w of Si)

To a solution of potassium hydroxide (KOH; 0.31 g; 0.0055 mol; 2.04 mol. equiv.) in distilled water (8.00 g), 1,2-propylene glycol (2.25 g; 15% w/w) was added, followed by salicylic acid (0.37 g; 0.0027 mol; 1 mol. equiv.). The reaction mixture was stirred at room temperature for 1 h. Then, to this clear colourless solution containing dipotassium salicylate, tetraethyl orthosilicate (TEOS; 0.6 mL; 0.56 g; 0.0027 mol) was added. Reaction mixture was stirred at room temperature for 5 h. Then, distilled water (3.51 g) was added up to the total weight of 15.00 g, and the product is filtered. Colourless, clear, and slightly viscous solution was obtained; the silicon content was 0.15% w/w of Si; pH= 12.0-12.5.

Example 6

Preparation of the formulation from the present invention in the form of solution of the complex of ortho-silicic acid (H_4SiO_4) with disodium salicylate of 8% w/w concentration of H_4SiO_4 (or 2.27% w/w of Si)

To a solution of sodium hydroxide (NaOH; 1.00 g; 0.025 mol; 2 mol. equiv.) in distilled water (7.00 g), tetraethyl orthosilicate (TEOS; 2.8 mL; 2.62 g; 0.0126 mol) was added. The reaction mixture was stirred at room temperature for 6 h. Then, salicylic acid (1.74 g; 0.0126 mol; 1 mol. equiv.) was added to the reaction mixture during 30 minutes with vigorous stirring. The reaction mixture was stirred at room temperature for 1 h. Afterwards, 1,2-propylene glycol (2.25

g) and distilled water (0.39 g) were added up to the total weight of 15.00 g. Finally, the reaction mixture was filtered. Colourless, clear, and viscous solution was obtained; content 2.27% w/w of Si; pH= 12.0-12.5.

Example 7

Preparation of the formulation from the present invention in the form of 1% w/w solution of ortho-silicic acid (H_4SiO_4) (or 0.29% w/w of Si)

To a solution of salicylic acid (0.43 g; 0.0031 mol; 2 mol. equiv.) in a mixture of 1,2-propylene glycol (7.50 g) and glycerol (3.00 g), tetraethyl orthosilicate (TEOS; 0.35 mL; 0.33 g; 0.00157 mol) was added. The reaction mixture was stirred at room temperature for 5 h. Then, distilled water (3.74 g) was added up to the total weight of 15.00 g. After filtration, colourless, clear, and viscous solution of the following composition was obtained:

- 1% w/w H_4SiO_4 [or 0.29% w/w of silicon (Si)];
- 2,9% w/w salicylic acid;
- 1.9% w/w ethanol.

Example 8

Preparation of the formulation from the present invention in the form of 2% w/w solution of ortho-silicic acid (H_4SiO_4) (or 0.58% w/w of Si)

To a solution of salicylic acid (0.43 g; 0.0031 mol; 1 mol. equiv.) in 1,2-propylene glycol (10.00 g), distilled water (0.23 g; 0.0128 mol; 4.1 mol. equiv.) and tetraethyl orthosilicate (TEOS; 0.7 mL; 0.65 g; 0.0031 mol) were added. Then, sulfuric acid (0.1 mL; 0.18 g; 0.177 g H_2SO_4; 0.0018 mol; 0.58 mol. equiv.) was added dropwise to the reaction mixture, and stirred at room temperature during 3 h. Afterwards, 1,2-propylene glycol (3.51 g) was added up to the total

weight of 15.00 g. After filtration, colourless, clear, and voscous solution was obtained with the following composition:

- 2% w/w H_4SiO_4 [or 0.58% w/w of silicon (Si)];
- 2.9% w/w salicylic acid;
- 3.8% w/w ethanol;
- up to 100% w/w 1,2-propylene glycol.

Example 9

Preparation of the formulation from the present invention in the form of 6% w/w solution of ortho-silicic acid (H_4SiO_4) (or 1.75% w/w of Si)

To a solution of salicylic acid (1.30 g; 0.0094 mol; 1 mol. equiv.) in 1,2-propylene glycol (10.00 g), distilled water (0.70 g; 0.039 mol; 4.1 mol. equiv.) and tetraethyl orthosilicate (TEOS; 2.1 mL; 1.96 g; 0.0094 mol) were added. Then, to the reaction mixture, 85% phosphoric acid (0.16 mL; 0.27 g; 0.23 g H_3PO_4; 0.0024 mol; 0.25 mol. equiv.) was added, and stirred at room temperature during 6 h. Afterwards, 1,2-propylene glycol (0.77 g) was added up to the total weight of 15.00 g. After filtration, colourless, clear, viscous solution of the following composition was obtained:

- 6% w/w H_4SiO_4 [or 1.75% w/w of silicon (Si)];
- 8.7% w/w salicylic acid;
- 1.5% w/w phosphoric acid;
- 11.5% w/w ethanol;
- up to 100% w/w 1,2-propylene glycol.

Example 10

Preparation of the formulation from the present invention in the form of solution of the complex of disodium salicylate and ortho-silicic acid (H_4SiO_4) of 2% w/w concentration of H_4SiO_4 (or 0.58% w/w of Si) with the use of sodium silicate as precursor of silicic acid

To a solution of sodium silicate (Na_2SiO_3; 0.38 g; 0.0031 mol) in distilled water (10.00 g), salicylic acid (0.43 g; 0.0031 mol; 1 mol. equiv.) was added in portions during 30 minutes under vigorous stirring. The reaction mixture was stirred at room temperature for 1 h. Then, 1,2-propylene glycol (2.25 g) and distilled water (1.94 g) were added up to the total weight of the reaction mixture of 15.00 g. After filtration, colourless, clear solution of the following composition was obtained:

- 2% w/w H_4SiO_4 [or 0.58% w/w of silicon (Si)];
- 2.9% w/w salicylic acid;
- 15% w/w 1,2-propylene glycol;
- up to 100% water.

Example 11

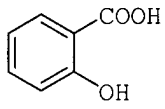
Preparation of the formulation from the present invention in the form of 2% w/w solution of ortho-silicic acid (H_4SiO_4) (or 0.58% w/w of Si) with the use of silicon tetrachloride as precursor of silicic acid

To a solution of salicylic acid (0.43 g; 0.0031 mol; 1 mol. equiv.) and sodium hydroxide (NaOH; 0.46 g; 0.0115 mol; 3.7 mol. equiv.) in mixture of 1,2-propylene glycol (12.00 g) and distilled water (2.00 g) cooled to -5 to -10 °C, under vigorous stirring, silicon tetrachloride ($SiCl_4$; 0.36 mL; 0.53 g; 0.0031 mol) was added dropwise during 15 minutes. The reaction mixture was stirred at this temperature during 1 h, then, for 1 h at temperatures from -5 °C to room temperature. Afterwards, 1,2-propylene glycol (0.25 g) was added to the reaction mixture, and stirring was continued for additional 15 minutes at room temperature. After filtration where a precipitate of sodium chloride (NaCl; approx. 0,67 g) was removed, colourless, clear, and viscous solution of the following composition was obtained:

- 2% w/w H_4SiO_4 [or 0,58% w/w of silicon (Si)];
- 2.9% w/w salicylic acid;
- up to 100% w/w 1,2-propylene glycol.

CLAIMS

1. A formulation of stabilized ortho-silicic acid (H_4SiO_4) as source of biologically available silicon (Si), that consists of:
   (i) ortho-silicic acid (H_4SiO_4), from 0.01-8% w/w;
   (ii) salicylic acid (**1**),



1

   from 1-2 molar equivalents to H_4SiO_4;
   (iii) pharmaceutically acceptable acid, from 0.1-4 molar equivalents to H_4SiO_4; or pharmaceutically acceptable base, in amounts of 2 molar equivalents to salicylic acid (**1**); and
   (iv) diluent, selected from the group consisting of: purified water, 1,2-propylene glycol, glycerol, ethanol, or their mixtures, in amounts of up to 100% w/w of the formulation.

2. A formulation of stabilized ortho-silicic acid (H_4SiO_4) as source of biologically available silicon (Si), according to claim 1, **characterized by that** the pharmaceutically acceptable acid is selected from the group comprising: hydrochloric (HCl), sulfuric (H_2SO_4), nitric (HNO_3), phosphoric (H_3PO_4), metanesulfonic (CH_3SO_3H), benzenesulfonic ($C_6H_5SO_3H$), salicylic (1,2-C_6H_4(OH)COOH) and sulfosalicylic [C_6H_3(3-COOH)(4-OH)SO_3H] acid, or mixtures of these substances.

3. A formulation of stabilized ortho-silicic acid (H_4SiO_4) as source of biologically available silicon (Si), according to claim 1, **characterized by that** the pharmaceutically acceptable base is selected from the group consisting of: sodium hydroxide (NaOH), potassium hydroxide (KOH), ammonium hydroxide (NH_4OH), tetramethylammonium hydroxide [$N(CH_3)_4OH$], tetraethylammonium hydroxide [$N(C_2H_5)_4OH$], or mixtures of these substances.

4. Process for preparation of the formulation according to claims 1-3, **characterized by** the reaction of salicylic acid (**1**) with precursor of silicic acid (**PSA**) of tetraethyl orthosilicate [TEOS; $Si(OC_2H_5)_4$] in which:
   (i) the molar ratio of salicylic acid and **PSA**, expressed on the content of silicon (Si) is given by the range:

   salicylic acid : Si = 1:1 to 2:1;

   (ii) a diluent is selected from the group comprising: 1,2-propylene glycol, purified water, glycerol, ethanol, or mixtures of these substances;
   (iii) the said reaction is carried out in the presence of pharmaceutically acceptable acid or pharmaceutically acceptable base; and
   (iv) the said reaction is carried out at the temperatures from -10 °C to +40 °C, during 0.5-6 h.

5. A process for preparation of the formulation according to claim 4, **characterized by that** the precursor of silicic acid (**PSA**) is selected to be sodium or potassium silicate.

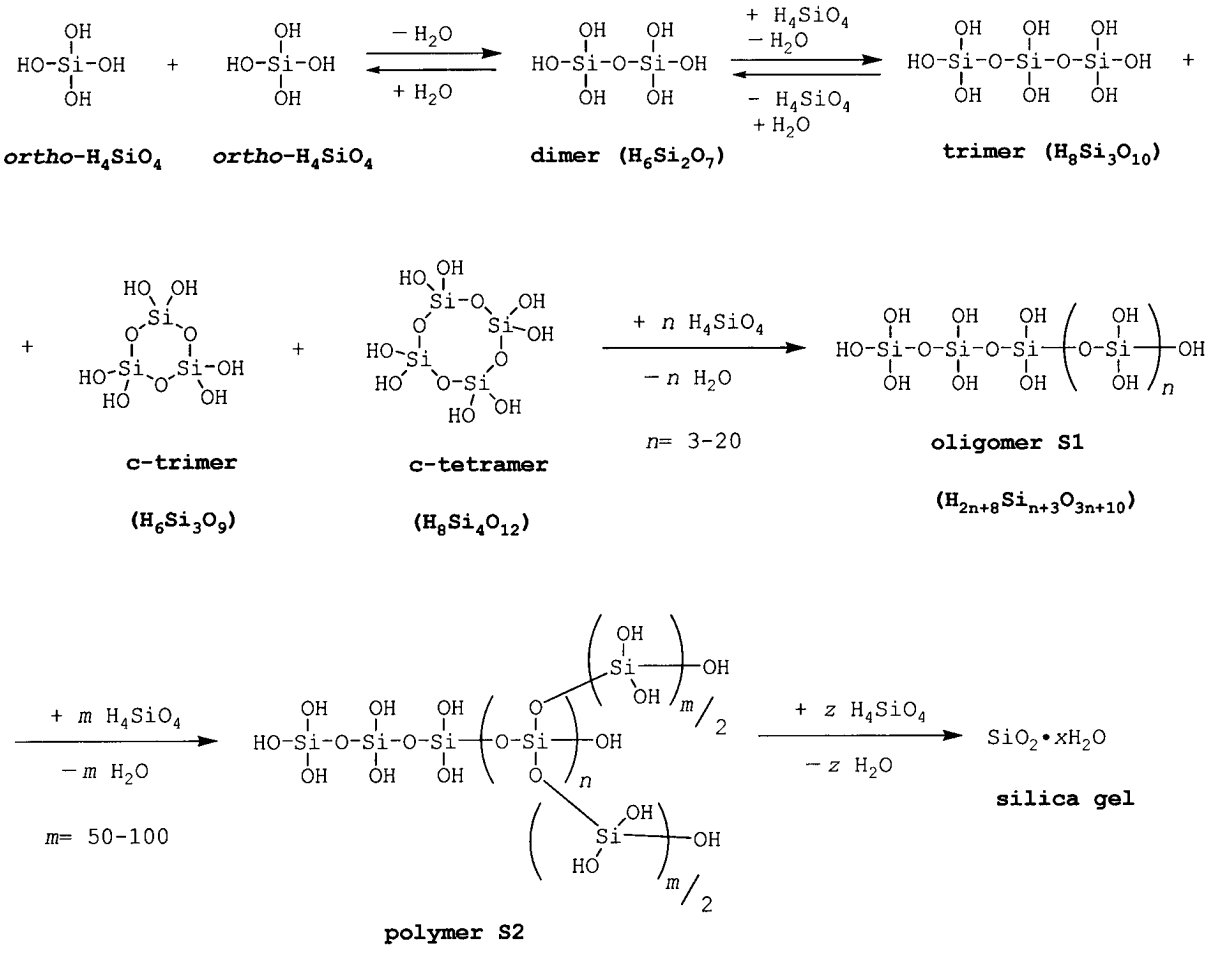
6. A process for preparation of the formulation according to claim 4, **characterized by that** the precursor of silicic acid (**PSA**) is selected to be silicon tetrachloride ($SiCl_4$).

7. A stabilized ortho-silicic acid-based formulation according to claims 1-3, for use as source of silicon (Si) for realization of physiological and therapeutic effects of silicon in human or animal organism.

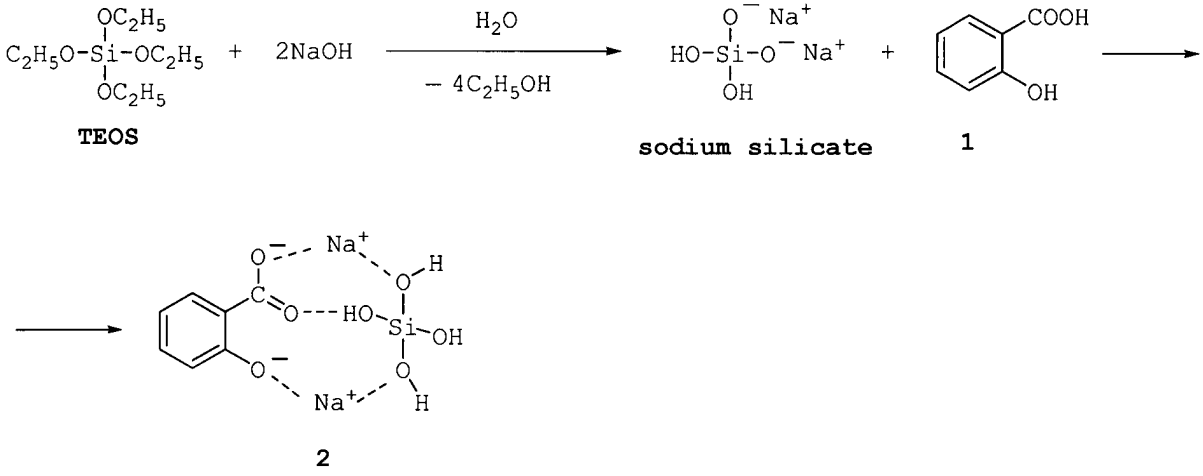
8. A stabilized ortho-silicic acid-based formulation according to claims 1-3, for use as a therapeutic agent for:
   - stimulation of immune system;
   - treatment of allergies;
   - strengthening structure and elasticity of arterial, vein, and capillary walls; decreasing their permeability; for improving

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;
- 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;
- lowering of body temperature (antipyretic);
- relieving pain (analgetic);
- treatment of acne;
- adjuvant treatment of infective diseases;
- treatment of acute and chronic inflammatory diseases (as anti-inflammatory agent); as well as
- topical keratoplastic agent (epitelization); and
- topical keratolytic agent.

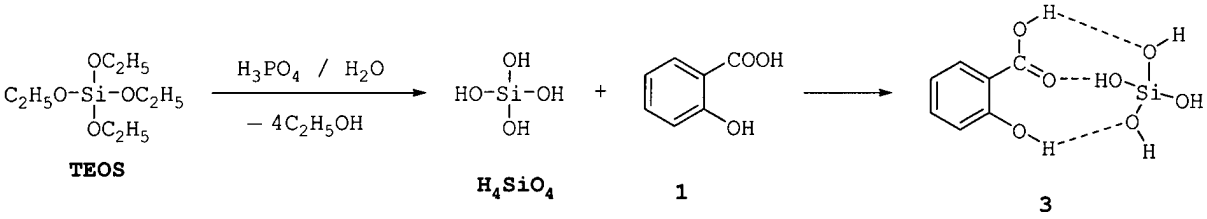
9. A stabilized ortho-silicic acid-based formulation according to claims 1-3, for use as an agent for realization of physiological effects of silicon (Si) and increasing resistance to stress and fungal diseases in plants.


ortho-H4SiO4
ortho-H4SiO4
dimer (H6Si2O7)
trimer (H8Si3O10)
c-trimer
(H6Si3O9)
c-tetramer
(H8Si4O12)
+ n H4SiO4
− n H2O
n= 3-20
oligomer S1
(H2n+8Sin+3O3n+10)
+ m H4SiO4
− m H2O
m= 50-100
polymer S2
+ z H4SiO4
− z H2O
SiO2•xH2O
silica gel

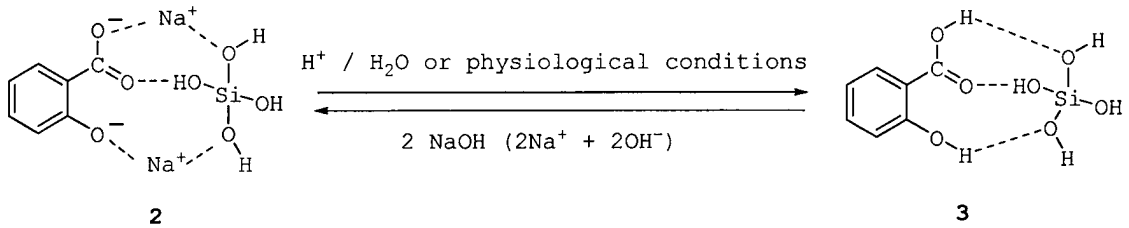
Scheme 1



Scheme 2



Scheme 3



the form in basic medium

the form in acidic and neutral (physiological) medium

Scheme 4

INTERNATIONAL SEARCH REPORT

International application No
PCT/HR2011/000034

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K9/08 A61K47/02 C01B33/113 A61K9/00 A01P3/00
A01P21/00 A61K8/25 A61K47/12
ADD.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K C01B

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

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2006/178268 A1 (KROS WILLEM A [ZA] KROS WILLEM ADRIANUS [ZA]) 10 August 2006 (2006-08-10) cited in the application claims 54,59,61 claims 66,74,75 paragraphs [0027], [0031]	1-9
Y	WO 95/21124 A1 (BIO PHARMA SCIENCES BV [NL]; BRONDER STEFAN RAYMOND [BE]) 10 August 1995 (1995-08-10) cited in the application claims 1-14	1-9

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
8 November 2011	16/11/2011

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Giacobbe, Simone

INTERNATIONAL SEARCH REPORT

International application No
PCT/HR2011/000034

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 6 197 986 B1 (SEGUIN MARIE-CHRISTINE [FR] ET AL) 6 March 2001 (2001-03-06) column 1, line 61 claim 5 column 2, line 64 - column 3, line 7 -----	1-9
Y	GB 1 388 330 A (GUEYNE J) 26 March 1975 (1975-03-26) page 1, line 55 - line 60 page 2, line 16 - line 20 page 2, line 31 example VII -----	1-9

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/HR2011/000034

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2006178268 A1	10-08-2006	AT 361267 T	15-05-2007
		AU 2003273570 A1	19-12-2003
		BR 0311383 A	15-03-2005
		CA 2486514 A1	11-12-2003
		CN 1656044 A	17-08-2005
		DE 60313610 T2	31-01-2008
		EP 1517874 A1	30-03-2005
		ES 2286445 T3	01-12-2007
		HK 1081521 A1	26-09-2008
		MA 27595 A1	01-11-2005
		NZ 536880 A	26-05-2006
		PL 206314 B1	30-07-2010
		UA 80969 C2	26-11-2007
		US 2006178268 A1	10-08-2006
		WO 03101915 A1	11-12-2003
		ZA 200409637 A	11-10-2005
WO 9521124 A1	10-08-1995	AT 168662 T	15-08-1998
		AU 698236 B2	29-10-1998
		AU 1545995 A	21-08-1995
		CA 2181825 A1	10-08-1995
		CN 1143354 A	19-02-1997
		DE 69503604 D1	27-08-1998
		DE 69503604 T2	26-11-1998
		DK 743922 T3	26-04-1999
		EP 0743922 A1	27-11-1996
		ES 2119388 T3	01-10-1998
		JP 3808499 B2	09-08-2006
		JP H09508349 A	26-08-1997
		NL 9400189 A	01-09-1995
		US 5922360 A	13-07-1999
		WO 9521124 A1	10-08-1995
US 6197986 B1	06-03-2001	AT 225794 T	15-10-2002
		BR 9800936 A	08-02-2000
		CA 2234284 A1	24-09-1998
		DE 69808539 D1	14-11-2002
		DE 69808539 T2	26-06-2003
		EP 0867445 A1	30-09-1998
		ES 2185133 T3	16-04-2003
		FR 2761074 A1	25-09-1998
		JP 4121604 B2	23-07-2008
		JP 10279467 A	20-10-1998
		US 6197986 B1	06-03-2001
GB 1388330 A	26-03-1975	CH 562255 A5	30-05-1975
		DE 2252064 A1	03-05-1973
		ES 407914 A1	16-11-1975
		FR 2158068 A1	15-06-1973
		GB 1388330 A	26-03-1975
		JP 48049915 A	14-07-1973
		JP 53001811 B	23-01-1978
		MC 945 A	12-10-1973