rexresearch.com

o-Silicic Acid & Health

http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3546016/
Nutr Metab (Lond) v.10; 2013 (Jan 8, 2013)

Biological and therapeutic effects of ortho-silicic acid and some ortho-silicic acid-releasing compounds: New perspectives for therapy

Lela Munjas Jurkic, Ivica Cepanec, Sandra Kraljevic Pavelic and Krešimir Pavelic

Abstract

Silicon (Si) is the most abundant element present in the Earth's crust besides oxygen. However, the exact biological roles of silicon remain unknown. Moreover, the ortho-silicic acid (H4SiO4), as a major form of bioavailable silicon for both humans and animals, has not been given adequate attention so far. Silicon has already been associated with bone mineralization, collagen synthesis, skin, hair and nails health atherosclerosis, Alzheimer disease, immune system enhancement, and with some other disorders or pharmacological effects. Beside the ortho-silicic acid and its stabilized formulations such as choline chloride-stabilized ortho-silicic acid and sodium or potassium silicates (e.g. M2SiO3; M= Na,K), the most important sources that release ortho-silicic acid as a bioavailable form of silicon are: colloidal silicic acid (hydrated silica gel), silica gel (amorphous silicon dioxide), and zeolites. Although all these compounds are characterized by substantial water insolubility, they release small, but significant, equilibrium concentration of ortho-silicic acid (H4SiO4) in contact with water and physiological fluids. Even though certain pharmacological effects of these compounds might be attributed to specific structural characteristics that result in profound adsorption and absorption properties, they all exhibit similar pharmacological profiles readily comparable to ortho-silicic acid effects. The most unusual ortho-silicic acid-releasing agents are certain types of zeolites, a class of aluminosilicates with well described ion(cation)-exchange properties. Numerous biological activities of some types of zeolites documented so far might probably be attributable to the ortho-silicic acid-releasing property. In this review, we therefore discuss biological and potential therapeutic effects of ortho-silicic acid and ortho-silicic acid -releasing silicon compounds as its major natural sources.

Introduction

Silicon (Si) is the most abundant element (27.2%) present in the earth's crust following oxygen (45.5%) [1]. Silicon is known for a number of important chemical and physical properties, i.e. semiconductor property that are used in various scientific and technical applications. These Si features, along with structural complexity of its compounds, have attracted researchers from the earliest times [2]. In particular, silicon dioxide or silica (SiO2) is the most studied chemical compound following water, and the most important Si-containing inorganic substance [1]. Formally, silica (SiO2) is a silicic acid anhydride of monomeric ortho-silicic acid (H4SiO4), which is water soluble and stable in highly diluted

aqueous solutions. Moreover, several “lower” hydrated forms of ortho-silicic acid exist in aqueous solutions as well including meta-silicic acid (H_2SiO_3 or lower oligomers like di-silicic ($H_2Si_2O_5$) and tri-silicic acids ($H_2Si_3O_7$) including their hydrated forms pentahydro-silicic ($H_{10}Si_2O_9$), and pyro-silicic acids ($H_6Si_2O_7$) [1]. These are water soluble, formed in reversible equilibrium reactions from H_4SiO_4 and stable in diluted aqueous solutions. During a prolonged storage period, at increased concentration or in an acidic environment, these low molecular silicic acids undergo further condensation by cross-linking and dehydration. This process results in formation of poly-silicic acids chains of variable composition $[SiO_x(OH)_{4-2x}$ and complex structure [1]. The end product is a jelly-like precipitate, namely hydrated silica ($SiO_2 \cdot xH_2O$; often referred as “colloidal silicic acid” or “hydrated silica gel”). Further condensation follows which is accompanied by dehydration yielding less hydrated silicon dioxide (SiO_2) phases, also known as “silica gel” or “amorphous silicon dioxide”.

Lower molecular forms, especially the ortho-silicic acid (H_4SiO_4; Figure ?Figure1),1), play a crucial role in delivering silicon to the living organisms’ cells and thus represent major sources of silicon for both humans and animals. Most of the silica in aqueous systems and oceans is available in the form of H_4SiO_4, which makes it an important compound in environmental silicon-chemistry and biology [3]. In this paper, we critically review the most recent findings on biological effects of Si and ortho-silicic acid on animals and human beings. Moreover, we propose that previously observed positive biological effects of various colloidal silicic acids (various hydrated silica gels) as well as some zeolites [4-6], e.g. zeolite A (Figure ?(Figure2)2) and clinoptilolite (Figure ?(Figure3),3), might be, at least partially, ascribed to the ortho-silicic acid-releasing property.

Figure 1
Chemical 3D structure of Si(OH)4.

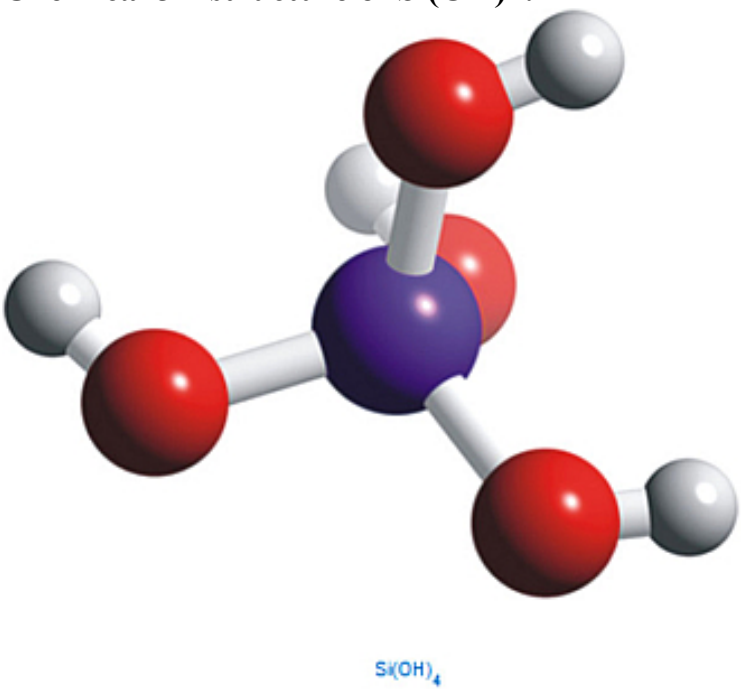


Figure 2
Zeolite A structure: an assembly of framework's cages (tiles). Centre of a tile is the centre of a void in the framework. Voids are connected with adjacent ones through the large "windows" which are faces of tiles.

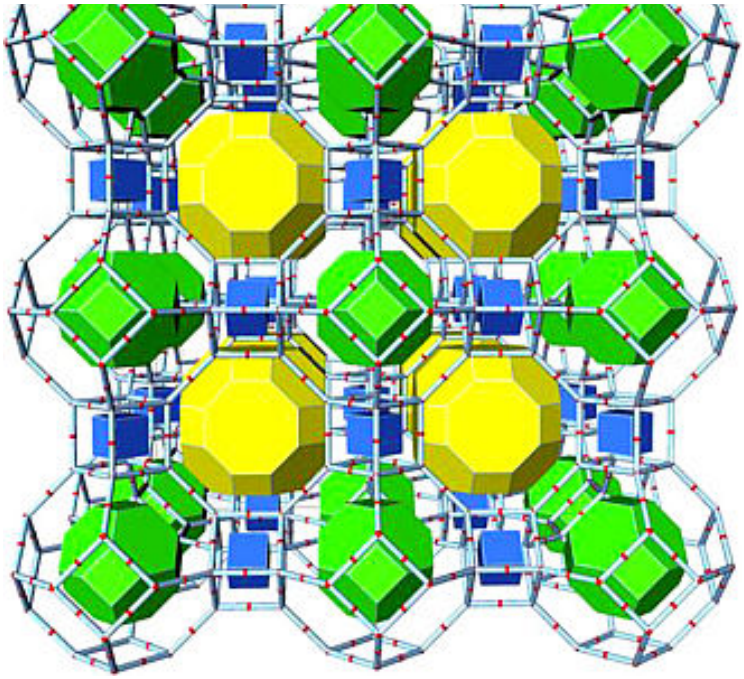
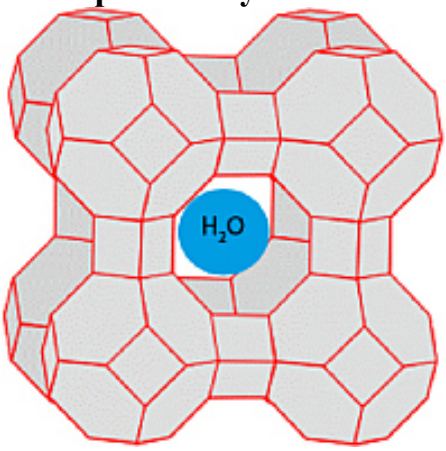


Figure 3
Microporous crystal structure of clinoptilolite.



Silicon represents the third most abundant trace element in the human body [7,8]. For example, it is present in 1–10 parts-per-million (ppm) in hair [9], nails [10], in the cornfield epidermis, and in the epicuticle of hair [11,12]. Silicon is naturally present in food as a silicon dioxide (SiO2), free ortho-silicic acid (H4SiO4), silicic acids bounded to certain nutrients, and in the silicate form. Although silicon is a life-important micronutrient mineral, in our opinion it has not received adequate attention. Considering the abundance of silicon, both in the nature and humans, it is expected that it should play an important role in human and animal health.

Silicon bioavailability and consumption

Presently, many biological roles of silicon remain unknown [13]. Consequently, the recommended daily silicon intake (RDI) has not yet been set [13,14]. Considering the risk assessment of amorphous silicon dioxide as common silicon source (e.g. food additive E551), the safe upper intake level (UIL) may be estimated as 700 mg/day for adults, that is the equivalent to 12 mg silicon/kg bw/day for a 60 kg adult [15]. These numbers refer to the amorphous silicon dioxide form and only small amounts of silicon (as H4SiO4) are actually released in the gastrointestinal (GI) tract and subsequently absorbed in the systemic circulation. Due to lack of data, it is difficult to set a recommended upper intake level for

silicon. Moreover, little information on the intake of dietary silicon by humans is available. A mean intake of daily silicon has been reported in Finland [16], (29 mg silicon/day) and in a typical British diet (20–50 mg silicon/day) [17-19]. This corresponds to 0.3-0.8 mg/silicon/kg bw/day for a 60 kg person. These data are in the same range as the estimated mean intakes of silicon in the USA (30 and 33 mg silicon/day in men, and 24 and 25 mg silicon/day in women, respectively) [8]. Silicon intake decreases with age to less than 20 mg silicon/day (18.6 ± 4.6 mg silicon/day for elderly British woman in an unrelated randomised controlled intervention study) [20].

Generally, silicon is abundantly present in foods derived from plants such as: cereals, oats, barley, white wheat flour, and polished rice. In contrast, silicon levels are lower in animal foods including meat or dairy products. Furthermore, silicon is present in drinking waters, mineral waters, and in beer as well [17]. However, Jugdaohsingh et al. [21] raised some doubt on utilisation of silicon from drinking water in an animal rat study as no significant differences were found in the silicon bone concentration when the drinking water was supplemented with silicon in the ortho-silicic acid form. Indeed, the major sources of silicon in the typical Western hemisphere diet comes from cereals (30%), followed by fruits, beverages and vegetables, which altogether comprise around 75% of total silicon intake [20]. Even though plant food contains high levels of silicon, its bioavailability from these sources is questionable, due to poor solubility of actual silicon forms present in these foods [18,19,22]. Efficient absorption in the GI tract would require their breakdown to soluble species such as ortho-silicic acid, present in drinking and mineral waters in the range of 2 to 5 mg silicon/L [23] and in beer ranging from 9 to 39 mg silicon/L [18,24]. Absorption studies indicate that the ortho-silicic acid is a main readily bioavailable source of silicon for humans, whereas its higher polymers are not of significant absorbability [25]. In a placebo-controlled study on eight volunteers, Jugdaohsingh et al. [25] showed that 53% of administered ortho-silicic acid is excreted in the urine, whereas the ingestion of polymeric silicic acid causes only a marginal increase of silicon in the urine. This result substantiates the statement that polymeric silicic acids and amorphous silicon dioxide are of poor bioavailability.

Besides the ortho-silicic acid, water soluble silicates are bioavailable silicon forms as well. For instance, pharmaceutically acceptable alkali metals silicates (M_2SiO_3; M= Na, K) in adequately diluted aqueous solutions, release ortho-silicic acid (H_4SiO_4) upon contact with stomach hydrochloric acid (HCl). Popplewell et al. [26] employed a tracer dose of radiolabelled ammonium silicate to measure total uptake and urine excretion. Their results revealed that 36% of ingested dose was absorbed and completely excreted in urine within 48h. However, elimination occurred in two steps where the major dose (90%) has been excreted within the first 2.7 hours. They suggested that excess silicon is eliminated from the body through two distinct processes, differing significantly in the duration. The ‘slower process’ is thought to include the intracellular uptake and release of silicon, whilst the ‘faster process’ probably includes retention of silicon in the extracellular fluids [26]. These data report on increased silicon levels in serum upon consumption of silicon-rich food [7,27], showing that at least some silicon is available from food as well. Indeed, selective silicon deprivation in rats showed a significant drop of urinary silicon excretion and fasting silicon serum concentration, suggesting that the rats actively regulate silicon levels via urinary conservation, perhaps through renal re-absorption [21]. Most of silicon present in the serum is filtered by the kidney [7,28] suggesting the kidney as its major excretion route; silicon levels in serum correlate with those in urine. However, it is still not clear how and if the body can efficiently retain adequate doses of silicon.

In concentrated solutions, ortho-silicic acid (H_4SiO_4) has to be stabilized to avoid its polymerization into poly-silicic acids and eventually into silica gel, resulting in a decreased silicon bioavailability. This issue has been solved in the field of pharmaceutical technology by use of choline chloride in aqueous glycerol solution. This resulted in development of a liquid formulation known as choline-stabilized ortho-silicic acid (ch-OSA). Choline chloride-stabilized ortho-silicic acid is not a new chemical entity of ortho-silicic acid, but a complex of H_4SiO_4 and choline chloride formed by several possible hydrogen bonds between these two compounds. Subsequently, from the standpoint of nutrition and pharmacology, the effects of ch-OSA must involve effects of both H_4SiO_4 and choline chloride rather than a new chemical entity. Due to a possible impact of choline chloride on the chemical stability of H_4SiO_4, certain specific biological effects different from those of a pure ortho-silicic acid or its immediate releasing compounds (e.g. sodium silicate), must be taken in account. Ch-OSA has been approved for human consumption and is known to be non-toxic. Its lethal doses (LD) exceed 5000 mg/kg bw in humans [29] and 6640 mg/kg bw in animals [30]. The ch-OSA represents the most bioavailable source of silicon [22,29]. Moreover, in a randomized placebo-controlled study [29], the bioavailability of ch-OSA during maternal transfer to the offspring was investigated in a supplementation study with pigs. The authors correlated significantly higher silicon concentrations in the serum of weanling piglets from supplemented sows and maternal transfer of absorbed silicon between sows and their offspring during lactation with high bioavailability of silicon from ch-OSA. Importantly, highly bioavailable silicon from ch-OSA did not altered calcium, phosphorus and magnesium levels in blood.

Therapeutic and biological effects of ortho-silicic acid and certain ortho-silicic acid-releasing compounds

It was reported that silicon is connected with bone mineralization and osteoporosis [31], collagen synthesis and ageing of skin [11], condition of hair and nails [32], atherosclerosis [33,34], Alzheimer disease [9,35,36], as well as with other biological effects and disorders. Trace minerals are known to generally play a vital role in the human body homeostasis [37] and the serum levels of silicon are similar to other trace elements, i.e. of iron, copper, and zinc [38]. Silicon is excreted through the urine in similar orders of magnitude as calcium. Some researches claim that silicon does not act as a protein-bounding element in plasma and is believed to exist almost entirely as un-dissociated monomeric ortho-silicic acid [28]. While early analyses showed that serum contains 50–60 μg silicon/dL [38,39], more recent analyses indicate that human serum contains 11–25 μg silicon/dL, or levels ranging between 24 and 31 μg/dL (8.5 and 11.1 μmol/L), detected by absorption spectrometry in large population groups [40]. Interestingly, pregnant women had very low serum silicon concentrations (3.3-4.3 μg/dL) in comparison with infants that have high concentrations between 34 and 69 μg/dL [27,41]. Moreover, silicon concentrations in serum showed a statistically significant age and sex dependency, as it seems that silicon concentrations decrease with age, especially in woman [40].

Biological importance of silicon might be analysed in the context of its bio-distribution in the body. For example, the highest silicon concentration has been measured in connective tissues, especially in the aorta, tracheas, bone, and skin. Low levels of silicon in the form of ortho-silicic acid [42-44] may be found in liver, heart, muscle, and lung [45]. It is therefore plausible to assume that observed decrease of silicon concentration in the ageing population may be linked to several degenerative disorders, including atherosclerosis. Supplementation of the regular diet with bioavailable forms of silicon may therefore have a therapeutic

potential including prevention of degenerative processes. Several experiments have already confirmed this hypothesis. For example, in a controlled animal study, spontaneously hypertensive rats had lower blood pressure upon supplementation with soluble silicon [44], whilst silicon deficiency in animals has been found to be connected with bone defects and impaired synthesis of connective tissue compounds, such as collagen and glycosaminoglycans [46-48]. It is therefore reasonable to assume that silicon deficiency or lower bioavailability may be linked to problems with bone structure and collagen production. Moreover, silicon was shown to be uniquely localized in active growth areas in young bones of animals where a close relationship between silicon concentration and the degree of mineralization has been assessed [46,49]. Studies confirmed the essential role of silicon in the growth and skeletal development of chicks that during silicon deprivation showed significantly retarded skeletal development [50]. Experimental silicon deprivation in rats [51-53] and chicks [46,47] demonstrated striking effects on skeletal growth and bone metabolism as well. On the other hand, the controlled animal study of Jugdaohsingh et al. [21] showed no profound effects of a silicon-deficient diet on the bone growth and skeletal development in rats. Silicon concentrations in the tibia and soft tissues did not differ from those in rats on a silicon-deficient diet where the silicon was supplemented in drinking water. Nevertheless, silicon levels in tibia were much lower compared to the reference group fed by a silicon rich diet. Body and bone lengths were also found to be lower in comparison with the reference group, while reduction in bone growth plate thickness was found in silicon deprived rats [21].

Moreover, Reffit et al. [54] found that ortho-silicic acid stimulates collagen type 1 synthesis in human osteoblast-like cells and skin fibroblasts and enhances osteoblastic differentiation in the MG-63 cells in vitro. Ortho-silicic acid did not alter collagen type 1 gene expression, but it modulated the activity of prolyl hydroxylase, an enzyme involved in the production of collagen [55]. Similarly, Schütze et al. [56] reported that the zeolite A stimulated DNA synthesis in osteoblasts and inhibited osteoclast-mediated bone resorption in vitro. This is probably attributable to the ortho-silicic acid-releasing property of zeolite A.

The mechanism underlying observed biological effects of silicon may probably be ascribed to its interrelationships with other elements present in the body such as molybdenum [57] aluminium [9,35,58,59], and calcium [46,49,50]. For instance, it was proven that silicon levels are strongly affected by molybdenum intake, and vice versa[59]. Furthermore, silicon accelerates the rate of bone mineralization and calcification as shown in controlled animal studies, in a similar manner that was demonstrated for vitamin D [11,50]. It is well known that vitamin D increases the rate of bone mineralization and bone formation [60], and that its deficiency leads to less mature bone development. Vitamin D is known to be important in calcium metabolism, but silicon-deficient cockerels' skulls in a controlled animal study showed lower calcification and collagen levels irrespective of the vitamin D dietary levels suggesting a vitamin D-independent mechanism of action [61]. Jugdaohsingh et al. [21] found that silicon supplementation in drinking water did not significantly altered silicon concentrations in bones and suggested that some other nutritional co-factor is required for maximal silicon uptake into bone and that this co-factor was absent in rats fed with a low-silicon diet compared to the reference group fed by a silicon-rich diet. They suggested vitamin K as such co-factor, which is important in bone mineralisation through carboxylation of osteocalcin, and whose deficiency might influence incorporation of minerals such as silicon in the bones.

Osteoporosis

Osteoporosis is among leading causes of morbidity and mortality worldwide [62]. It is defined as a progressive skeletal disorder, characterised by low bone mass (osteopenia) and micro-architectural deterioration [63]. Interestingly, the administration of silicon in a controlled clinical study induced a significant increase in femoral bone mineral density in osteoporotic women [31]. Direct relationship between silicon content and bone formation has been shown by Moukarzel et al. [64]. They found a correlation between decreased silicon concentrations in total parenterally fed infants with a decreased bone mineral content. This was the first observation of a possible dietary deficiency of silicon in humans. A randomized controlled animal study on aged ovariectomized rats revealed that long-term preventive treatment with ch-OSA prevented partial femoral bone loss and had a positive effect on the bone turnover [65]. Dietary silicon is associated with postmenopausal bone turnover and bone mineral density at the women's age when the risk of osteoporosis increases. Moreover, in a cohort study on 3198 middle-aged woman (50–62 years) it was shown that silicon interacts with the oestrogen status on bone mineral density, suggesting that oestrogen status is important for the silicon metabolism in bone health [66].

Skin and hair

Typical sign of ageing skin is fall off of silicon and hyaluronic acid levels in connective tissues. This results in loss of moisture and elasticity in the skin. Appearance of hair and nails can also be affected by lower silicon levels, since they are basically composed of keratin proteins. As previously discussed, ortho-silicic acid may stimulate collagen production and connective tissue function and repair. For example, Barel et al. [67] conducted experiments on females, aged between 40–65 years, with clear clinical signs of photo-ageing of facial skin. Their randomized double-blinded placebo-controlled study illustrates positive effects of ch-OSA taken as an oral supplement on skin micro relief and skin anisotropy in woman with photo-aged skin. Skin roughness and the difference in longitudinal and lateral shear propagation time decreased in the ch-OSA group, suggesting improvement in isotropy of the skin. In addition, ch-OSA intake positively affected the brittleness of hair and nails. Oral supplementation with ch-OSA had positive effects on hair morphology and tensile strengths, as shown in a randomized placebo-controlled double blind study by Wickett et al. [68].

Alzheimer disease

Aluminium (as Al3+ ion) is a well-known neurotoxin. Aluminium salts may accelerate oxidative damage of biomolecules. Importantly, it has been detected in neurons bearing neurofibrillary tangles in Alzheimer's and Parkinson's disease with dementia as shown in controlled studies [69,70]. Amorphous aluminosilicates have been found at the core of senile plaques in Alzheimer's disease [69,71], and have consequently been implicated as one of the possible causal factors that contribute to Alzheimer’s disease. Since aluminosilicates are water insoluble compounds, the transport path to the brain is still not well understood. By reducing the bioavailability of aluminium, it may be possible to limit its neurotoxicity. Consumption of moderately high amounts of beer in humans and ortho-silicic acid in animals has shown to reduce aluminium uptake from the digestive tract and slow down the accumulation of this metal in the brain tissue [36,72]. Silicic acid has also been found to induce down-regulation of endogenous antioxidant enzymes associated with aluminium administration and to normalize tumour necrosis factor alpha (TNFa) mRNA expression [35]. Although the effect of silicic acid on aluminium absorption and excretion from human body produced conflicting results so far as shown in an open-label clinical study [7], in a controlled clinical study it was shown that silicic acid substantially reduces aluminium bioavailability to

humans [73]. In fact, it was already found that silicon reduces the aluminium toxicity and absorption in some plants and animals that belong to different biological systems [74-76]. This is possible as silicon competes with aluminium in biological systems such as fresh water, as suggested by Birchall and Chappell study perfomed on the geochemical ground [77], and later confirmed by Taylor et al. in randomized double blind study [78]. They found that soft water contains less silicic acid and more aluminium, while hard waters contain more silicic acid and less aluminium.

Removal of aluminium from the body and its reduced absorption by simultaneous administration of silicic acid was tested and proven by Exley et al. in controlled clinical study [59]. They showed reduced urinary excretion of aluminium along with unaltered urinary excretion of trace elements such as iron in persons to whom silicic acid-rich mineral water was administered. Moreover, they documented that regular drinking of a silicon-rich mineral water during a period of 3 months significantly reduced the body burden of aluminium. Similar results were obtained by Davenward et al. [79] who showed that silicon-rich mineral waters can be used as a non-invasive method to reduce the body burden of aluminium in both Alzheimer's patients and control group by facilitating the removal of aluminium via the urine without any concomitant effect. They also showed clinically relevant improvements of cognitive performances in at least 3 out of 15 individuals with Alzheimer disease. This implies a possible use of ortho-silicic acid as long-term non-invasive therapy for reduction of aluminium in Alzheimer's disease patients. The mechanism through which aluminium bioavailability reduction occurs involves interaction between aluminium species and ortho-silicic acid where highly insoluble hydroxyaluminosilicates (HAS) forms are produced [77,80]. This process makes aluminium unavailable for absorption.

Immunostimulatory effects

Quartz as a form of crystalline silicon dioxide has been connected with severe negative biological effects. However, in controlled studies on mouse and rats it was shown that sub-chronic and short-term exposure to this compound can actually have beneficial effects on respiratory defence mechanisms by stimulating immune system through the increase of neutrophils, T lymphocytes and NK cells. It also activates phagocytes and consequently additional ROS production [81-83] which can help the pulmonary clearance of infectious agents. In rats, crystalline silica caused proliferation and activation of CD8+ T cells and, to a lesser amount, of CD4+ T cells.

Recently, an “anionic alkali mineral complex” Barodon® has shown immunostimulatory effects in horses [84], pigs [85] and other animals. Barodon® is a mixture of sodium silicate (M_2SiO_3, M= Na,K) and certain metal salts in an alkaline solution (pH= 13.5), where sodium-silicate (sodium water glass) represents 60% of the total content. In a placebo-controlled experiment in pigs, the immunostimulatory effect of Barodon® was assessed by measurement of proliferation and activation of porcine immune cells, especially CD4+ CD8+ double-positive (dpp) T lymphocytes in peripheral blood and in the secondary lymphoid organ [85]. As this type of T lymphocyte cells are characterized by a specific memory cell marker CD29, they may play a role during activation of secondary immune responses as shown in a cross-sectional and longitudinal study on pigs [86]. Moreover, Barodon® acted mainly on the lymphoid organs, implying a role in antigenic stimulation of immune tissues [85]. Barodon® induced increased levels of MHC-II lymphocytes and non-T/non-B (N) cells as well along with increased stimulatory mitogen activity including the activity of PHA, concanavalin A, and pokeweed mitogen [85,87]. In a placebo-controlled experiment on pigs,

it was shown that this mineral complex exerts an adjuvant effect with hog cholera and Actinobacillus pleuropneumoniae vaccines by increasing the antibody titres and immune cell proportions [88]. Moreover, Barodon® showed nonspecific immunostimulating effects in racing horses and higher phagocytic activity against Staphylococcus equi subsp. equi and Staphylococcus aureus as well in a controlled study [84]. Administration of Barodon® in horse herds reduced many clinical complications, including stress-induced respiratory disease, suggesting activation of immune cell populations similarly to the treatment with inactivated Propionibacterium acnes[89,90]. The exact mechanism of Barodon® immunostimulatory effect is not known, although it has been suggested that sodium silicate, the main mineral ingredient, might be responsible for the observed immune-enhancing properties. Indeed, sodium silicate is known to decompose quantitatively into bioavailable ortho-silicic acid (H_4SiO_4) in the acidic gastric juice (HCl), and as such being absorbed in the body. In this manner, presumably all observed pharmacological effects of Barodon® are actually originated from the ortho-silicic acid.

Pure sodium metasilicate (Na_2SiO_3) also bears immunostimulatory effects and acts as a potent mitochondria activator [91]. Dietary silicon in the form of sodium metasilicate activates formation of ammonia by elevating mitochondrial oxygen utilisation as shown in a controlled animal experiment [91]. These findings further corroborate the hypothesis that sodium silicate might be responsible for immunostimulatory effects of Barodon®. Once again, the pharmacologically active species was ortho-silicic acid released upon the action of stomach hydrochlorid acid on sodium metasilicate.

Zeolites as a source of ortho-silicic acid

Zeolites are a class of aluminosilicates of general formula $(M^{n+})_{x/n}[(AlO_2)_x(SiO_2)_y \cdot mH_2O$, wherein M represents a positively charged metal ion such as sodium (Na^+), potassium (K^+), magnesium (Mg^{2+}), or calcium (Ca^{2+}). Zeolites are crystalline aluminosilicates with open 3D framework structures built of SiO_4 and AlO_4 tetrahedra linked to each other by sharing all the oxygen atoms to form regular intra-crystalline cavities and channels of molecular dimensions [92]. The positively charged metal ions (e.g. Na^+, K^+, Ca^{2+}, Mg^{2+}) are positioned in these cavities of aluminosilicate skeleton which are termed as micro- (2–20 Å), meso- (20–50 Å), and macro-(50–100 Å) -pores. These ions are readily exchangeable in contact with aqueous solution of other positively charged ions (e.g. heavy metal ions like Hg^{2+}). This structural characteristic of zeolites is the base of their ion (cation)-exchange property [93].

At present, 191 unique zeolite frameworks have been identified [94], while over 40 naturally occurring zeolite frameworks have been described. Zeolites have been widely employed in chemical and food industries, agriculture, and environmental technologies as adsorbents, absorbents, adsorbent filter-aids, ion-exchangers, catalysts, active cosmetic and pharmaceutical ingredients, soil improvers, etc. [95-103]. Besides, zeolites exhibit a number of interesting biological activities [5,104,105] (Figure ?(Figure4).4). For example, nontoxic natural zeolite clinoptilolite affects tumour cells proliferation in vitro and might act as an adjuvant in cancer therapy [105]. Katic et al. [106] confirmed that clinoptilolite influences cell viability, cell division, and cellular stress response that results in antiproliferative effect and apoptosis induction in vitro. Obtained results demonstrated that clinoptilolite biological effect on tumour cells growth inhibition might be a consequence of adsorptive and ion-exchange characteristics that cause adsorption of some serum components by clinoptilolite [106]. Similarly, clinoptilolite showed antiviral effects in vitro and a potential in antiviral

therapy either for local skin application against herpesvirus infections or oral treatment of adenovirus or enterovirus infections [107]. The antiviral mechanism is probably non-specific and is based on adsorption of viral particles on external cavities at the clinoptilolite surface rather than a consequence of ion-exchange properties.

Each zeolite particle acts like a large inorganic molecule and acts as a molecular sieve with a potential in molecular medicine in molecular medicine. Their pores are indeed, rather small (less than 2 nm to 50 nm) [108], and these structural similarities between the cages of zeolites and binding sites of enzymes resulted in development of zeolite structures that mimic enzyme functions [108], e.g. haemoglobin, cytochrome P450 or iron-sulphur proteins [109].

Important data on biological zeolites fate (Figure ?(Figure5)5) and effects in vivo have been widely reported so far in the scientific literature. For example, it was shown that zeolites bear detoxifying and decontaminant properties when added to animal diets, reducing levels of heavy metals (e.g. lead, mercury, and cadmium) and various organic pollutants, i.e. radionuclides (Figure ?(Figure6)6) and antibiotics [108]. Furthermore, zeolites have been successfully utilized for haemodialysis, for cartridges in haemoperfusions, for wound healing, and surgical incisions [108]. For instance, QuikClot and Zeomic formulations are already being marketed for haemorrhage control [110] and dental treatment [5], respectively.

Several toxicological studies proved that certain natural zeolite, e.g. clinoptiolite are non-toxic and completely safe for use in human and veterinary medicine [105]. In vitro and in vivo controlled animal studies have shown that clinoptilolite is an inert substance that may cause, in some instances, only moderate but not progressive fibrosis or mesothelioma [111]. This effect might be attributed to side-substances present in natural zeolites, e.g. silica or clay aluminosilicates [112]. It should be also stated that some zeolites might be extremely dangerous for human health and exert negative biological effects. For example, erionite, a fibrous type of natural zeolite, causes a high incidence of mesotheliomas and fibrosis in humans and experimental animals [113].

Animal studies have also shown the possibility of zeolite A (sodium aluminosilicate) as a viable source of silicon [4,6,114]. The latter is one of known zeolites that breaks down into bioavailable ortho-silicic acid (H_4SiO_4) in the digestive system. This property arises from the structure of zeolite A which is characterized by the same number of aluminium and silicon atoms in zeolite A [115]. Zeolite A is hydrolysed at low pH (stomach hydrochloric acid) into ortho-silicic acid (H_4SiO_4) and aluminium ions (Al^{3+}). These are combined back to the amorphous aluminosilicate. Such process readily provides additional source of bioavailable silicon to the organism [114,116]. Indeed, randomized placebo-controlled studies on dogs [114] proved that silicon is absorbed upon oral administration of zeolite A. Comparable results have been obtained in a randomized placebo-controlled research on horses as well [6]. Addition of zeolite A to the diet of young racing quarter horses have resulted in decreased skeletal injury rates and better training performance [117]. However, increased bone formation was found in randomized controlled studies on broodmare horses [118], but not in yearling horses [119]. Food supplementation with zeolite A in calves showed no changes in bone architecture or mechanical properties [120]. However, in a controlled study Turner et al. [120] showed increased aluminium content in the bone and cartilage of zeolite A-fed calves which is an important safety issue for the zeolite A therapeutic usage.

Conclusion

In conclusion, we believe that ortho-silicic acid (H_4SiO_4) might be a prominent therapeutic agent in humans. Some potential therapeutic and biological effects on bone formation and bone density, Alzheimer disease, immunodeficiency, skin, hair, and nails condition, as well as on tumour growth, have already been documented and are critically discussed in the presented paper. Acid forms of ortho-silicic acid include: choline-chloride-stabilized ortho-silicic acid (ch-OSA) as a specific pharmaceutical formulation of H_4SiO_4, simple water soluble silicate salts such as sodium silicate (E550; Na_2SiO_3) or potassium silicate (E560; K_2SiO_3), and certain water-insoluble forms that, upon contact with stomach juice (HCl), release small, but biologically significant amounts of ortho-silicic acid. The latter involves: colloidal silicic acid (hydrated silica gel), amorphous silicon dioxide (E551), certain types of zeolites such as zeolite A (sodium aluminosilicate, E554; potassium aluminosilicate, E555; calcium aluminosilicate, E556), and the natural zeolite clinoptilolite. However, for some of the above-proposed therapeutic perspectives of both ortho-silicic acid and ortho-silicic acid - releasing derivatives, additional insights into biological mechanisms of action and larger studies on both animals and humans are required.

Acknowledgements

This work was supported by the Croatian Ministry of Science, Education and Sports (grants number 335-0982464-2393 and 335-0000000-3532).

References

Greenwood NN, Earnshaw A. Chemistry of the elements. 2nd. Oxford: Butterworth-Heinemann; 1997.

Martin KR. The chemistry of silica and its potential health benefits. J Nutr Health Aging. 2007;11(2):94–97.

Treguer P, Nelson DM, Van Bennekom AJ, DeMaster DJ, Leynaert A, Queguiner B. The silica balance in the world ocean: A reestimate. Science. 1995;268(5209):375–379. doi: 10.1126/science.268.5209.375.

Frey KS, Pottery GD. Plasma silicon and radiographic bone density in weaning quarter horses fed sodium zeolite A. Equine Vet Science. 1991;12:292–296.

Pavelic K, Hadzija M. In: Handbook of zeolite science and technology. 1st. Auerbach SM, Carrado KA, Dutta PK, editor. New York: Marcel Dekker; 2003. Medical applications of zeolites; pp. 1143–1174.

O'Connor CI, Nielsen BD, Woodward AD, Spooner HS, Ventura BA, Turner KK. Mineral balance in horses fed two supplemental silicon sources. J Anim Physiol Anim Nutr (Berl) 2008;92(2):173–181. doi: 10.1111/j.1439-0396.2007.00724.x.

Reffitt DM, Jugdaohsingh NR, Thompson RPH, Powel JJ. Silicic acid: its gastrointestinal uptake and urinary excretion in man and effects on aluminium excretion. J Inorg Biochem. 1999;76(2):141–147. doi: 10.1016/S0162-0134(99)00126-9.

Jugdaohsingh R, Anderson SHC, Tucker KL, Elliot H, Kiel DP, Thompson RPH, Powell JJ. Dietary silicon intake and absorption. Am J Clin Nutr. 2002;75(5):887–893.

Smith BL. In: Trace elements in man and animals, TEMA 8. Anke M, Meissner D, Mills CF, editor. New York: Kluwer; 1993. Analysis of hair element levels by age, sex, race, and hair color; pp. 1091–1093.

Austin JH. Silicon levels in human tissues. Nobel Symp. 1997. pp. 255–268.

Carlisle EM. Silicon: an essential element for the chick. Science. 1972;178:619–621. doi: 10.1126/science.178.4061.619.

Fregert S. Studies on silicon in tissues with special reference to skin. J Invest Dermatol. 1958;31(2):95–96.

European Food Safety Authority. Opinion of the scientific panel on dietetic products, nutrition and allergies on a request from the commission related to the tolerable upper intake level of silicon. The EFSA Journal. 2004;60:1–11.

Scientific Committee for Food. Nutrient and energy intakes for the European Community. Reports of the Scientific Committee for Food (Thirty-first series) Luxembourg: European Commission; 1993.

Institute of Medicine, Food and Nutrition Board. Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. Washington: National Academy Press; 2000.

Varo P, Koivistoinen P. Mineral element composition of Finnish foods. Acta Agric Scand. 1980;22:165–171.

Bowen HJM, Peggs A. Determination of the silicon content of food. J Sci Food Agric. 1984;35:1225–1229. doi: 10.1002/jsfa.2740351114.

Bellia JP, Birchall JD, Roberts NB. Beer: a dietary source of silicon. Lancet. 1994;343(8891):235.

Pennington JAT. Silicon in food and diets. Food Addit Contam. 1991;8(1):97–118. doi: 10.1080/02652039109373959.

McNaughton SA, Bolton-Smith C, Mishra GD, Jugdaohsingh R, Powell JJ. Dietary silicon intake in post-menopausal women. Br J Nut. 2005;94:813–817. doi: 10.1079/BJN20051548.

Jugdaohsingh R, Calomme MR, Robinson K, Nielsen F, Anderson SHC, D'Haese P, Geusens P, Loveridge N, Thompson RPH, Powell JJ. Increased longitudinal growth in rats on a silicon-depleted diet. Bone. 2008;43(3):596–606. doi: 10.1016/j.bone.2008.04.014.

Van Dyck K, Van Cauwenbergh R, Robberecht H, Deelstra H. Bioavailability of silicon from food and food supplements. Fresenius J Anal Chem. 1999;363:541–544. doi: 10.1007/s002160051243.

Barnett PR, Skougstad MW, Miller KJ. Chemical characterization of a public water supply. J Am Water Works Assoc. 1969;61:61–68.

Sripanyakorn S, Jugdaohsingh R, Elliott H, Walker C, Mehta P, Shoukru S. The silicon content of beer and its bioavailability in healthy volunteers. Br J Nutr. 2004;91(3):403–409. doi: 10.1079/BJN20031082.

Jugdaohsingh R, Reffitt DM, Oldham C, Day JP, Fifield LK, Thompson RPH. Oligomeric but not monomeric silica prevents aluminum absorption in humans. Am J Clin Nutr. 2000;71(4):944–949.

Popplewell JF, King SJ, Day JP, Ackrill P, Fifield LK, Cresswell RG. et al. Kinetics of uptake and elimination of silicic acid by a human subject: a novel application of 32Si and accelerator mass spectrometry. J Inorg Biochem. 1998;69(3):177–180. doi: 10.1016/S0162-0134(97)10016-2.

Calomme R, Cos P, D'Haese PC, Vingerhoets R, Lamberts LV, De Broe ME, In: Metal ions in biology and medicine. Volume 5. Collery P, Brätter P, Negretti De Brätter V, Khassanova L, Etienne JC, editor. Paris: John Libbey Eurotext; 1998. Absorption of silicon in healthy subjects; pp. 228–232.

Berlyne GM, Adler AJ, Ferran N, Bennett S, Holt J. Silicon metabolism. I. Some aspects of renal silicon handling in normal man. Nephron. 1986;43(1):5–9. doi: 10.1159/000183709.

European Food Safety Authority. Scientific opinion of the panel on food additives and nutrient sources added to food. The EFSA Journal. 2009;948:1–23.

Budavari S. Merck index: An encyclopaedia of chemicals, drugs, and biologicals. 11th. Rahway: Merck & Co; 1989. pp. 342–343.

Eisinger J, Clairet D. Effects of silicon, fluoride, etidronate and magnesium on bone mineral density: a retrospective study. Magnes Res. 1993;6(3):247–249.

Lassus A. Colloidal silicic acid for oral and topical treatment of aged skin, fragile hair and brittle nails in females. J Int Med Res. 1993;21(4):209–215.

Schwarz K. Silicon, fibre, and atherosclerosis. Lancet. 1977;1(8009):454–457.

Schwarz K, Ricci BA, Punsar S, Karvonen MJ. Inverse relation of silicon in drinking water and atherosclerosis in Finland. Lancet. 1977;1(8010):538–539.

Candy JM, Edwardson JA, Klinowski J, Oakley AE, Perry EK, Perry RH. In: Senile dementia of the Alzheimer type. Traber J, Gispen WH, editor. Heidelberg: Springer; 1985. Co-localisation of aluminum and silicon in senile plaques: implications for the neurochemical pathology of Alzheimer's disease; pp. 183–197.

Gonzalez-Munoz MJ, Meseguer I, Sanchez-Reus MI, Schultz A. Beer consumption reduces cerebral oxidation caused by aluminum toxicity by normalizing gene expression of tumor necrotic factor alpha and several antioxidant enzymes. Food Chem Toxicol. 2008;46(3):1111–1118. doi: 10.1016/j.fct.2007.11.006.

Maehira F, Ishimine N, Miyagi I, Eguchi Y, Shimada K. Anti-diabetic effects including diabetic nephropathy of anti-osteoporotic trace minerals on diabetic mice. Nutrition.

2010;27(4):488–495.

Dobbie JW, Smith MJB. The silicon content of body fluids. Scott Med J. 1982;27:17–19.

Carlisle EM. In: Biochemistry of the essential ultratrace elements. Frieden E, editor. New York: Plenum Press; 1984. Silicon; pp. 257–291.

Bissé E, Epting T, Beil A, Lindinger G, Lang H, Wieland H. Reference values for serum silicon in adults. Anal Biochem. 2005;337(1):130–135. doi: 10.1016/j.ab.2004.10.034.

Van Dyck K, Robberecht H, Van Cauwenbergh R, Van Vlaslaer V, Deelstra H. Indication of silicon essentiality in humans. Serum concentrations in Belgian children and adults, including pregnant women. Biol Trace Elem Res. 2000;77(1):25–32. doi: 10.1385/BTER:77:1:25.

Adler AJ, Berlyne GM. Silicon metabolism II. Renal handling in chronic renal failure patients. Nephron. 1986;44:36–39.

D' Haese PC, Shaheen FA, Huraid SO. Increased silicon levels in dialysis patients due to high silicon content in the drinking water, inadequate water treatment procedures, and concentrate contamination: a multicentre study. Nephrol Dial Transplant. 1995;10:1838–1844.

Maehira F, Motomura K, Ishimine N, Miyagi I, Eguchi Y, Teruya S. Soluble silica and coral sand suppress high blood pressure and improve the related aortic gene expressions in spontaneously hypertensive rats. Nutr Res. 2011;31(2):147–156. doi: 10.1016/j.nutres.2010.12.002.

Carlisle EM. The nutrition essential of silicon. Nutr Rev. 1982;40:193–198.

Carlisle EM. Silicon: a possible factor in bone calcification. Science. 1970;167:279–280. doi: 10.1126/science.167.3916.279.

Carlisle EM. In vivo requirement for silicon in articular cartilage and connective tissue formation in the chick. J Nutr. 1976;106:478–484.

Carlisle EM. Biochemical and morphological changes associated with long bone abnormalities in silicon deficiency. J Nutr. 1980;110:1046–1055.

Carlisle EM. Silicon localization and calcification in developing bone. Fed Proc. 1969;28:374.

Carlisle EM. A relationship between silicon and calcium in bone formation. Fed Proc. 1970;29:565.

Schwarz K, Miline DB. Growth promoting effects of silicon in rats. Nature. 1972;239(537):333–334. doi: 10.1038/239333a0.

Seabron CD, Nielsen FH. Dietary silicon affects acid and alkaline phosphatase and 45calcium uptake in bone of rats. J Trace Elem Exp Med. 1994;7:1–11.

Seabron CD, Nielsen FH. Dietary silicon and arginine affect mineral element composition of

rat femur and vertebra. Biol Trace Elem Res. 2002;89:239–250. doi: 10.1385/BTER:89:3:239.

Reffitt DM, Ogston N, Jugdaohsingh R, Cheung HFJ, Evans BAJ, Thompson RPH. et al. Orthosilicic acid stimulates collagen type 1 synthesis and osteoblastic differentiation in human osteoblast-like cells in vitro. Bone. 2003;32(2):127–135. doi: 10.1016/S8756-3282(02)00950-X.

Keeting PE, Oursler MJ, Wiegand KE, Bonde SK, Spelsberg TC, Riggs BL. Zeolite A increases proliferation, differentiation, and transforming growth factor production in normal adult human osteoblast like cells in vitro. J Bone Miner Res. 1992;7(11):1281–1289.

Schutze, Oursler MJ, Nolan J, Riggs BL, Spelzberg TC. Zeolite A inhibits osteoclast mediated bone resorption in vitro. J Cell Biochem. 1995;58(1):39–46. doi: 10.1002/jcb.240580106.

Carlisle EM. A silicon-molybdenum interrelationship in vivo. Fed Proc. 1979;38:553.

Carlisle EM, Curran MJ. Effect of dietary silicon and aluminum on silicon and aluminum levels in rat brain. Alzheimer Dis Assoc Dis. 1986;1(2):83–89.

Exley C, Korchazhkina O, Job D, Strekopytov S, Polwart A, Crome P. Non-invasive therapy to reduce the body burden of aluminium in Alzheimer's disease. J Alzheimers Dis. 2006;10(1):17–24.

Muller SA, Posner AS, Firschein HE. Effect of vitamin D deficiency on the crystal chemistry of bone mineral. Proc Soc Exp Biol Med. 1966;121(3):844–846.

Carlisle EM. Silicon: a requirement in bone formation independent of vitamin D. Calcif Tissue Int. 1981;33:27–34. doi: 10.1007/BF02409409.

Spector TD, Calomme MR, Anderson SH, Clement G, Bevan L, Demeester N. et al. Choline-stabilized orthosilicic acid supplementation as an adjunct to calcium/vitamin D3 stimulates markers of bone formation in osteopenic females: a randomized, placebo-controlled trial. BMC Musculoskelet Disord. 2008;9:85–95. doi: 10.1186/1471-2474-9-85.

Marcus R. Clinical review 76: the nature of osteoporosis. J Clin Endocrinol Metab. 1996;81(1):1–5. doi: 10.1210/jc.81.1.1.

Moukarzel AA, Song M, Buchman AL, Ament ME. Silicon deficiency may be involved in bone disease of parenteral nutrition. J Am Coll Nutr. 1992;11:584.

Calomme M, Geusens P, Demeester N, Behets GJ, D'Haese P, Sindambiwe JB. et al. Partial prevention of long-term femoral bone loss in aged ovariectomized rats supplemented with choline-stabilized orthosilicic acid. Calcif Tissue Int. 2006;78(4):227–232. doi: 10.1007/s00223-005-0288-0.

Macdonald HM, Hardcastle AC, Jugdaohsingh R, Fraser WD, Reid DM, Powell JJ. Dietary silicon interacts with oestrogen to influence bone health: Evidence from the Aberdeen Prospective Osteoporosis Screening Study. Bone. 2012;50(3):681–687. doi:

10.1016/j.bone.2011.11.020.

Barel A, Calomme M, Timchenko A, De Paepe K, Demeester N, Rogiers V, Clarys P, Vanden Berghe D. Effects of oral intake of choline-stabilized orthosilicic acid on skin, nails, and hair in women with photodamaged skin. Arch Dermatol Res. 2005;297:147–153. doi: 10.1007/s00403-005-0584-6.

Wickett RR, Kossmann E, Barel A, Demeester N, Clarys P, Vanden Berghe D. et al. Effect of oral intake of choline-stabilized orthosilicic acid on hair tensile strength and morphology in woman with fine hair. Arch Dermatol Res. 2007;299(10):499–505. doi: 10.1007/s00403-007-0796-z.

Perl DP, Brody AR. Alzheimer's disease: X-ray spectrometric evidence of aluminium accumulation in neurofibrillary tangle-bearing neurons. Science. 1980;208(4441):297–309. doi: 10.1126/science.7367858.

Perl DP. Relationship of aluminium to Alzheimer's disease. Environ Health Perspect. 1985;63:149–153.

Candy JM, Oakley AE, Klinowski J, Carpenter TA, Perry EK, Blessed G. et al. Aluminosilicates and senile plaque formation in Alzheimer's disease. Lancet. 1986;1(8477):354–357.

González-Muñoz MJ, Pena A, Meseguer I. Role of beer as a possible protective factor in preventing Alzheimer’s disease. Food Chem Toxicol. 2008;46(1):49–56. doi: 10.1016/j.fct.2007.06.036.

Edwardson JA, Moore PB, Ferrier IN, Lilley JS, Newton GWA, Barker J. et al. Effect of silicon on gastrointestinal absorption of aluminium. Lancet. 1993;342(8865):211–212. doi: 10.1016/0140-6736(93)92301-9.

Birchall JD, Exley C, Chappell JS, Phillips MJ. Acute toxicity of aluminium to fish eliminated in silicon-rich acids waters. Nature. 1989;338:146–148. doi: 10.1038/338146a0.

Hammond KE, Evans DE, Hodson MJ. Aluminium/silicon interactions in barley (Hordeum vulgare L.) seedlings. Plant Soil. 1995;173(1):89–95. doi: 10.1007/BF00155521.

Exley C. A biogeochemical cycle for aluminium. J Inorg Biochem. 2003;97(1):1–7. doi: 10.1016/S0162-0134(03)00274-5.

Birchall JD, Chappell JS. Aluminium, water chemistry, and Alzheimer's disease. Lancet. 1989;1(8644):953.

Taylor GA, Newens AJ, Edwardson JA, Kay DW, Forster DP. Alzheimer's disease and the relationship between silicon and aluminium in water supplies in northern England. J Epidemiol Community Health. 1995;49(3):323–324. doi: 10.1136/jech.49.3.323.

Davenward S, Bentham P, Wright J, Crome P, Job D, Polwart A, Exley C. Silicon-rich mineral water as a non-invasive test of the ‘aluminum hypothesis’ in Alzheimer's disease. J Alzheimers Dis. 2012. to be published.

Exley C, Schneider C, Doucet FJ. The reaction of aluminium with silicic acid in acidic solution: an important mechanism in controlling the biological availability of aluminium. Coord Chem Rev. 2002;228(2):127–135. doi: 10.1016/S0010-8545(02)00077-2.

Antonini JM, Roberts JR, Yang HM, Barger MW, Ramsey D, Castranova V. et al. Effect of silica inhalation on the pulmonary clearance of a bacterial pathogen in Fischer 344 rats. Lung. 2000;178(6):341–350. doi: 10.1007/s004080000038.

Antonini JM, Yang HM, Ma JY, Roberts JR, Barger MW, Butterworth L. et al. Subchronic silica exposure enhances respiratory defense mechanisms and the pulmonary clearance of Listeria monocytogenes in rats. Inhal Toxicol. 2000;12(11):1017–1036. doi: 10.1080/08958370050164635.

Kumar RK. Quantitative immunohistologic assessment of lymphocyte populations in the pulmonary inflammatory response to intratracheal silica. Am J Pathol. 1989;135(4):605–614.

Koo HC, Ryu S-H, Ahn HJ, Jung WK, Park YK, Kwon NH. et al. Immunostimulatory effects of the anionic alkali mineral complex BARODON on equine lymphocytes. Clin Vaccine Immunol. 2006;13(11):1255–1266. doi: 10.1128/CVI.00150-06.

Yoo BW, Choi SI, Kim SH, Yang SJ, Koo HC, Seo SH. et al. Immunostimulatory effects of anionic alkali mineral complex solution Barodon in porcine lymphocytes. J Vet Sci. 2001;2(1):15–24.

Zuckermann FA, Husmann RJ. Functional and phenotypic analysis of porcine peripheral blood CD4/CD8 double-positive T cells. Immunology. 1996;87(3):500–512.

Yoo BW, Choi SI, Kim SH, Yang SJ, Koo HC, Kown NH. et al. Immunostimulatory effects of anionic alkali mineral complex solution Barodon in porcine lymphocytes. J Swine Health Prod. 2002;10:265–270.

Park BK, Park YH, Seo KS. Lymphocyte subpopulations of peripheral blood in pigs treated with an ionized alkali mineral complex. J Vet Sci. 1999;24:67–74.

Moore BR, Krakowska S, Robertson JT. Evaluation of an immunostimulant in preventing shipping related respiratory disease. J Equine Vet Sci. 1996;16:78. doi: 10.1016/S0737-0806(96)80160-2.

Flaminio MJ, Rush BR, Shuman W. Immunologic function in horses after non-specific immunostimulant administration. Vet Immunol Immunopathol. 1998;63(4):303–315. doi: 10.1016/S0165-2427(98)00111-1.

Oner G, Cirrik S, Bakan O. Effects of silica on mitochondrial functions of the proximal tubule cells in rats. Kidney Blood Press Res. 2005;28(4):203–210. doi: 10.1159/000086006.

Libau F. Structural chemistry of silicates. Berlin: Springer; 1985.

Cejka J, Heyrovsky J, editor. Zeolites and ordered mesoporous materials: progress and prospects. Amsterdam: Elsevier; 2005. (Stud Surf Sci Catal).

Baerlocher C, Meier WH, Olson DH. Atlas of zeolite framework types. 6th. Amsterdam: Elsevier; 2007.

Flaningen EM. In: Proceedings of the 5th international conference of zeolites. Rees LV, editor. London: Heyden; 1980. Molecular sieve zeolite technology – the first five years; pp. 760–780.

Sersale R. Natural zeolites: processing, present and possible applications. Stud Surf Sci Catal. 1985;24:503–512.

Naber JE, De Jong KP, Stork WHJ, Kuipers HPCE. Post industrial application of zeolite catalysis. Stud Surf Sci Catal. 1994;84:2197–2220.

Pavelic K. Medical News. 1998. pp. 21–22.

Colella C. Natural zeolites in environmentally friendly processes and applications. Stud Surf Sci Catal. 1999;125:641–655.

Garaces JM. In: Proceedings of the 12th international conference of zeolites. Treacz MMJ, Marcus BK, Misher ME, Higgins JB, editor. Warrendale: Materials Research Society; 1999. Observations on zeolite applications; pp. 551–566.

Mumpton FA. La roca magica: uses of natural zeolites in agriculture and industry. Proc Natl Acad Sci. 1999;96(7):3463–3470. doi: 10.1073/pnas.96.7.3463.

Yang RT. Adsorbents, fundamentals and applications. New York: John Wiley & Sons Inc; 2003.

Loidelsbacher T. Process for manufacturing fertilizers or soil amendments from mineral and organic components. Munich: European Patent Publication (EP 0444392 B1); 1993.

Rodriguez-Fuentes G, Barrios MA, Iraizoz A, Perdomo I, Cedre B. Enterex: Anti-diarrheic drug based on purified natural clinoptilolite. Zeolites. 1997;19:441–448. doi: 10.1016/S0144-2449(97)00087-0.

Pavelic K, Hadzija M, Bedrica L, Pavelic J, Dikic I, Katic M. Natural zeolite clinoptilolite: new adjuvant in anticancer therapy. J Mol Med. 2001;78(12):708–720. doi: 10.1007/s001090000176.

Katic M, Bosnjak B, Gal-Troselj K, Dikic I, Pavelic K. A clinoptilolite effects on cell media and the consequent effects on tumor cells in vitro. Front Biosci. 2006;11:1722–1732. doi: 10.2741/1918.

Grce M, Pavelic K. Antiviral properties of clinoptilolite. Microporous and Mesoporous Materials. 2005;79:165–169. doi: 10.1016/j.micromeso.2004.10.039.

Kralj M, Pavelic K. Medicine on a small scale. EMBO Rep. 2003;4(11):1008–1012. doi: 10.1038/sj.embor.7400017.

Bedioui F. Zeolite-encapsulated and clay-intercalated metal porphyrin, phthalocyanine and

Schiff-base complexes as models for biomimetic oxidation catalysts: an overview. Coord Chem Rev. 1995;144:39–68.

Rhee P, Brown C, Martin M, Salim A, Plurad D, Green D. et al. QuikClot use in trauma for hemorrhage control: case series of 103 documented uses. J Trauma. 2008;64(4):1093–1099. doi: 10.1097/TA.0b013e31812f6dbc.

Adamis Z, Tatrai E, Honma K, Six E, Ungvary G. In vitro and in vivo tests for determination of the pathogenicity of quartz, diatomaceous earth, modernite and clinoptilolite. Ann Occup Hyg. 2000;44(1):67–74.

Kogan FM, Nikitina OV. Solubility of chrysotile asbestos and basalt fibres in relation to their fibrogenic and carcinogenic action. Environ Health Perspect. 1994;102(5):205–206. doi: 10.1289/ehp.94102s5205.

Baris YI, Sahin AA, Ozemi M, Kerse I, Ozen E, Kolacan B. et al. An outbreak of pleural mesothelioma and chronic fibrosing pleurisity in the village of Karani/Urgup in Anatolia. Thorax. 1978;33:181–192. doi: 10.1136/thx.33.2.181.

Cefali EA, Nolan JC, McConnell WR, Lowe Walters D. Bioavailability of silicon and aluminium from zeolite A in dogs. Int Journ Pharm. 1996;127(2):147–154. doi: 10.1016/0378-5173(95)04110-9.

Hartman RL, Fogler HS. Understanding the dissolution of zeolites. Langmuir. 2007;23(10):5477–84. doi: 10.1021/la063699g.

Thilsing-Hansen T, Jorgensen RJ, Enemark JM, Larsen T. The effect of zeolite A supplementation in the dry period on periparturient calcium, phosphorus, and magnesium homeostasis. J Dairy Sci. 2002;85(7):1855–1862. doi: 10.3168/jds.S0022-0302(02)74259-8.

Nielsen BD, Potter GD, Morris EL, Odom TW, Senor DM, Reynolds JA. Training distance to failure in young racing quarter horses fed sodium zeolite a. J Equine Vet Sci. 1993;13:562–567. doi: 10.1016/S0737-0806(06)81526-1.

Lang KJ, Nielsen BD, Waite KL, Hill GM, Orth MW. Supplemental silicon increases plasma and milk silicon concentrations in horses. J Anim Sci. 2001;79(10):2627–2633.

Lang KJ, Nielsen BD, Waite KL, Link J, Hill GM. Increased plasma silicon concentrations and altered bone resorption in response to sodium zeolite A supplementation in yearling horses. J Equine Vet Sci. 2001;21:550–555. doi: 10.1016/S0737-0806(01)70161-X.

Turner KK, Nielsen BD, O'Connor CI, Robison DS, Rosenstein DS, Marks BP. et al. Sodium zeolite a supplementation and its impact on the skeleton of dairy calves. Biol Trace Elem Res. 2008;121(2):149–159. doi: 10.1007/s12011-007-8040-4.

---

http://worldwide.espacenet.com/advancedSearch?locale=en_EP

PATENTS

WO2012035364
STABILIZED SOLUTION OF ORTHO-SILICIC ACID, ITS PREPARATION AND USE

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= C1, H2PO4, HSO4, NO3, CH3SO3, C6H5SO3, 1, 4-CH3C6H4SO3 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.

DESCRIPTION

Field of the invention

The present invention relates to a stabilized solution of ortho- silicic acid (H4Si04) , 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 (H4Si04) solution, and procedure for production of such stabilized solution.

The solution is consisting of:

(i) ortho-silicic acid (H4Si04) , from 0.01-8% w/w;
(ii) carnitine salt (1) of pharmaceutically acceptable acids,
OH
(H3C) 3N OOH
X= CI, H2P04, HS04, N03, CH3S03, CfiH5S03, 1 , -CH3C6H4S03 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. Prior art
Silicon (Si) is important biogenic microelement which exhibits several roles in human and animal organism:
(i) helps resorption of calcium and takes part in its transport, stimulates osteoblasts; stimulates bone mineralization; in traumatic cases provides faster bone healing; prevents 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 structure of connective tissue and formation of functional tertiary structure of building proteins of soft organs like 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. . 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 immunite, Revue du Rhumatisme 46 (1979) 483] ;
(v) exhibits antiinflammatory action at various inflammatory diseases like arthritis, osteoarthritis, skin disorders like psoriasis, seborrheic dermatitis, neurodermitis , skin irritations; hastes wound healing, soothes decubitus, etc. [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 decreases resorption of aluminum (Al<3+>) from gastrointestinal tract, thus beside antioxidative action, preventively acts on development of neurodegenerative diseases connected with prolonged exposure to aluminum, 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 collagen 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];
(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
(ix) antioxidative action; by this way preventively acts on development of atherosclerosis and other diseases caused by prolonged oxidative stress condition such as diabetes and diabetes complications [J. Loeper, J. Goy-Loeper, L. Rozensztajn, M. Fragny: The antiatheromatous action of silicon, Atherosclerosis 33 (1979) 397-408; J. Loeper, J. Emerit, J. Goy, L. Rozensztajn, M. Fragny:
[Fatty acids and lipid peroxidation in experimental atheroma in the rabbit. Role played by silicon (in French)], Pathol. Biol. (Paris) 32 (1984) 693-697; J. Loeper, J. Goy, M . Fragny, R.

Troniou, O. Bedu: Study of fatty acids in atheroma induced in rabbits by an atherogenic diet with or wihout silicon i.v. treatment, Life Sci. 42
(1988) 2105-2112; J. Loeper, J. Goy-Loeper, L. Rozensztajn, M. Fragny: [The antiatheromatous action of silicon (in French)], Bull. Acad. Natl. Med. 163 (1979) 530-534].

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 (H4Si04) . It is known to those skilled in the art that silicic acid, in its monomeric form, ortho-silicic acid (H Si04) is not stable, but at higher concentrations undergoes polymerization yielding dimeric (H6Si207) , trimeric (H8Si3Oi0) , 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; Si02-xH20) . The course of polymerization of silicic acid is given in Scheme 1, at the end of the specification.

Beside monomeric ortho-silicic acid (H Si04) , biologically available forms are also its lower oligomers that are soluble in water; they release starting H4Si0 by hydrolysis (oligomerization is reversible) . In other words, at certain concentrations, an equilibrium between monomeric 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] .

The use of natural, as low as possible refined food (e.g. whole grain cereals), usually provides sufficient intakes od 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(R)" 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 Al (2002) , BioPharma Sciences B. V. , Belgium] .

Additionally, in patent literature there are disclosed other, mainly as auxiliary, stabilizers that inhibit polymerization of ortho- silicic acid such as: boric acid (H3B03) or sodium tetraborate natrijev tetraborat (Na2B407 . 10H2O) [L. J. Clapsdale, M. G. Syracuse: Nongelling aqueous silica sols stabilized with boron compounds, **US 2,630,410** (1953); Union Carbide Co.]; H3B03 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 Al]; amino acids proline, serine, lysine, arginine, glycine or their mixtures; polypeptides or protein hydrolyzates [V. Berghe, D. A. Richard, WO 2004/016551 Al , 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 Bl (2002) Exsymol S.A.M., onako] ; and calcium chloride (CaCl3) 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 (CH3S02CH3) or dimethylsulfoxide (CH3SOCH3) and humectants like 1 , 2-propylene glycol or polyethyene glycol 400 (PEG-400) [I. Suvee, G. Tourgis: Hydronium stabilized and dissoluble silicic acid nanoparticles : Preparation, stabilization and use, WO 2009/127256 Al (2008)].

Somewhat similar products are based on silicic acid stabilized by alkali hydroxides [J. M. Rule: Process of making stable silica sols and resulting composition, US Patent 2,577,485 (1951) E.I. DuPont de Nemours Co.] or as basic complexes with amino acid arginine in the presence of inositol [M. F. McCarty, J. Zielinski: Arginine silicate complex and use thereof, US Patent 5,707,970 (1998); V. Juturu, J. R. Komorowski: Arginine silicate inositol complex and use thereof, WO2004/017913 A2 (2002) and US Patent 7,576,132 B2 (2005)] . However, in these cases, silicic acid is present in anionic form (as silicate; Si (OH) 30<"> or Si03<2">) .

Beside choline chloride-stabilized ortho-silicic acid (H4Si04) , on the market there can be find various food supplements containing silicon in the forms of amorphous or colloidal silicon dioxide (Si02) - 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 available [D. Kustrak: Pharmacognosy and phytopharmacy , (in Croatian) Golden marketing-Tehnicka knjiga, Zagreb, Croatia (2005)] .

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; SiCb) in organic, and solution of potassium silicate (30% w/w K2Si03) in conventional agriculture (most often in wine growing: e.g. "Sil- Matrix<R>") . Such products are usually employed by foliar application.

The technical problem of effective stabilization of ortho-silicic acid (H4Si04) :

(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 (H4Si04) 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 (H4Si04), from 0.01-8% w/w;
(ii) carnitine salt (1) of pharmaceutically acceptable acids,
X= CI, H2P04, HSO", N03, CH3SO3, C6H5S03, 1 , 4 -CH3C5H4S03 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.

Since enantiomeric form of carnitine does not have any impact on stabilization of H4Si0 , 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= H2P0), carnitine hydrogensulfate (lc; X= HS0) , carnitine nitrate (Id; X= N03) , carnitine methanesul fonate (le; X= CH3S03) , carnitine benzenesul fonate (If; X= C6H5S03) , carnitine p-toluenesulfonate (lg; X= 1 , 4-C6H4S03) , 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 (H3P04) , because it was found that H3PO4 additionally stabilizes ortho-silicic acid (H4Si04), presumably 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 (H2S04) , phosphoric (H3P04) , hydrochloric (HC1) , or other above-mentioned acid. Unexpectedly, it was found that carnitine salts like carnitine hydrochloride (la) , effectively act as stabilizers of ortho-silicic acid (H4Si0) at low pH values (acidic medium) .

In this manner, solutions of ortho-silicic acid of concentration of 2-4% w/w, prepared by hydrochloric acid (HC1) -catalysed hydrolysis of tetraethyl orthosilicate [TEOS; Si(OC2H5)4], are stable at room temperature (20-25 [deg.]C) for 2-3 months. During storage, slow polymerization occurs (as given in Scheme 1) with formation of opalescent gel.

In contrast, carnitine hydrochloride (la) in concentration from 7- 40% w/w does act as stabilizer of ortho-silicic acid (H4S04) 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 la on H4Si04 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)] .

In the prior art there are described deep eutectic mixtures and their use as solvents or as reaction mediums [A. P. Abbott, D. Boothby, G. Capper, D. L. Davies, R. K. Rasheed: Deep Eutectic Solvents Formed between Choline Chloride and Carboxylic acids: Versatile Alternatives to Ionic Liquids, J. Am. Chem. Soc. 126 (2004) 9142-9147; M. Figueiredo, C. Gomes, R. Costa, A. Martins, C. M. Pereira, F. Silva: Differential capacity of deep eutectic solvent based on choline chloride and glycerol on solid electrodes, Electrochim. Acta 54 (2009) 2630-2634] .

However, choline chloride (2) destabilizes ortho-silicic acid (H4Si04) at pH conditions that are close to physiological (around 7) . Moreover, choline chloride in these conditions does catalyze polymerization of H Si0 (see Table 1), what actually decreasing its bioavailability .

Completely unexpectable , 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 H4Si04 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 H Si0 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- (+-) - carnitine hydrochloride (la) and ortho-silicic acid (H4Si04) , prepared by hydrolysis of tetraethyl orthosilicate [TEOS; Si(OC2H5)4] in the presence of phosphoric acid (H3P0) . The hydrolysis reaction of TEOS with formation of the complex of H4Si04 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- (+-) -carnitine hydrochloride on stabilization of H4Si04, as controls, the following samples are prepared:
(i) a standard solution of H4Si0 of concentration of 1% w/w Si; and
(ii) a solution of analogous complex of H4Si04 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 (H4Si04) , and these are at pH values close to neutral. At such conditions, pH= 6-7, relatively fast polymerization of H4Si04 takes place, with formation of its poylmers in the form of opalescent gel. In more concentrated systems,

this change, from the phase of solution (which is, at the begining, 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 H4Si04.

Test solutions are prepared by mixing equal volume of solution of complex 3a (or complex of choline chloride or standard solution of pure H4Si04 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 (tG) . Longer gelling time (tG) 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 (la) on stabilization of ortho-silicic acid (H4Si04) in solution at pH= 6.5.<a>

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"jSi04) . 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 H3P04; 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- (+-) -carnitine hydrochloride (la; 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 H3P04; 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 (H4Si04) 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 Si04, but moreover does act as catalyst of its polymeri zation . 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 H4Si0 , to ensure prolonged shelf life.

In contrast, DL- (+-) -carnitine hydrokloride (la) does not influence significantly on rate of polymerization of ortho-silicic acid (H4S1O4) , 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 (H4Si04) . Despite that in initial experiments, hydrochloric acid (HC1) was employed as classical agent for acidification and regulation of pH in pharmaceutical products, phosphoric acid (H3P04) appeared to exhibit significant additional stabilization effect against polymerization of H4Si04 (Table 2) .

Table 2. Study of influence of pharmaceutically acceptable acids on stability of ortho-silicic acid (H4Si04) at pH= 6.5.<a>
<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) .

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. <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 H4Si04) . Shows stabilizing or destabilizing effect on ortho-silicic acid, and its polymerization (gelling) .

d 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 H3P04; 0.0029 mol; 0.55 mol. equiv.) or 37% hydrochloric acid (0.25 mL; 0.30 g; 0.11 g HC1; 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 (H3PO4) in the same concentration provides approx. 40% longer gelling time, this means slower polymerization than analogous solution where hydrochloric acid (HC1) 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 (H Si04) in the presence of DL- (+-) -carnitine hydrochloride.

The study was conducted in analogous manner with the use of 1 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 (H4Si04) in solution at pH= 6.5 in the presence of DL- (+) -carnitine hydrochloride . <a>

<a> Composition of the sample solutions (% w/w) : 3.5% H4Si04 (1% of Si), 7% carnitine hydrochloride (1 mol . equiv. / H4Si04), 6.6% ethanol, 20% auxiliary stabilizer, 1.9% H3P04 (0.55 mol. equiv. / H4Si04) , 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 H4Si04) . 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 (H4Si04) because of retaining water (hygroscopic) action, and thus inhibit its polymerization (gelling) [W. A. Kros : Aqueous solution of non- colloidal silicic and boric acid, US Patent Appl . 2006/0178268 Al]" is not valid.

It was observed that polyethylene glycol (PEG-400) does destabilize H4Si04, 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 H4S1O4 in the presence of carnitine hydrochloride; gelling time (tG) 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 (la) ;
(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 (H4Si04) , 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 (H4Si04) in soluton at pH=

<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 H4Si0) . It shows stabilizing or destabilizing effect on ortho-silicic acid, and its polymerization (gelling) . <d> The solution of analogous composition like the product "BioSil<R>" 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 H3P04; 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].
e 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 H3P04; 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 (H4Si04) at physiological pH values. Observed gelling (polymerization) times of H4Si04 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 H4Si0 was carried out analoguously, by determination of gelling (polymerization) time with the use of 1M phosphate buffer of pH= 8.5. Result are given in Table 5.

Table 5. Study of the kind of carnitine salt on inhibition of gelling (polymerization) of ortho-silicic acid (H4Si04) in solution at pH= 6.5.<a>

<a> The composition of the sample solutions (%, w/w) : 3.5% H4Si04 (1% Si); 5.8% carnitine base; 1.5 molar equiv. / Si of corresponding acid (Experiment 1: HC1; Experiment 2: H3P04; Experiment 3: H2S04); 6.6% ethanol; and up to 100% distilled water.

The composition of the sample solution in the Experiment 4 (%, w/w) : 3.5% H4Si04 (1% Si); 7% carnitin hydrochloride; 1.9% H3P04 (0.5 mol. equiv. / H4Si04); 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 1 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.
b 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 H4Si04, 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 (H4SiQ4) . 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 (H4Si04) 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 H4Si04. 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 H4Si04 from the category of "quaternary ammonium salts".

Precisely, in the present invention it has been disclosed that choline chloride obviously does stabilize H4Si04 in acidic solution as is in the formulation of the solutions from the prior art (e.g. at product "BioSil<R>") , 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<R>") 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 (H4Si04) 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 H4Si0 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 H4Si04 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 (H4Si04) is ability or disability to form stable complex with the given molecule. Whereas choline chloride can act as bidentate ligand for H4Si04, carnitine salts act as tetradentate ligands. Because of this, carnitine hydrochloride (la) 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 H4Si04, 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 H Si04 (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 (H4Si04) is known, among them, there is "generically" mentioned also phosphoric acid (H3P0) [I. Suvee, G. Tourgis: Hydronium stabilized and dissoluble silicic acid nanoparticles : Preparation, stabilization and use, WO 2009/127256 Al (2008); W. A. Kros, US Patent 2006/0178268 Al], there is no a single and clear evidence or study of kind of acid on stability of H4Si04.

In the present application, clear additional stabilizing effect of phosphoric acid on stability of H4Si04 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 acceptabe acid, expecially phosphoric acid; and (iii) auxiliary stabilizers, glycerol, glucosamine, d-panthenol, and 1 , 2-propylene glycol; on stability of H4Si04 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)] in previously prepared solution of carnitine salt like DL- (+-) -carnitine hydrochloride (la) , pharmaceutically acceptable acid, and auxiliary stabilizer of H4Si04 according to the invention with vigorous stirring at temperatures between -10 [deg.]C to +40 [deg.]C, preferably at +15 [deg.]C to +30 [deg.]C (room temperature conditions) during 1-24 h.

Alternatively, as PSA the followings can be employed:
(i) sodium or potassium silicate (common composition xM2OySi02; 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 (HC1) released during its hydrolysis (4 mol of HC1 per single mol of SiCl4) .
Excess of HC1 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, CaC03, MgC03] , etc. In this case, corresponding salts of used bases are generated, e.g. NaCl, KC1, CaCl2, MgCl2. These salts do not alter stability of ortho- silicic 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 [deg.]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 (C2H5OH) 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. H3P04) 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 , eczema, 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 (Al<3+>) 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; strengthes 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 [deg.]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 (HjSiO^) and its complexes with choline chloride (2) and PL- (+-) -carnitine hydrochloride (la) . Influence of 2 and la on stability of H Si04 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 H3P04; 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 H3P04; 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 H3P04; 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, tG, of ortho- silicic acid (H4Si04) 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 (t0) until the formation of opalescent (and thick) gel was determined. This time interval is termed as "gelling (polymerization) time", tG, and expressed in minutes. Thus obtained results for tG were given in comparison with the value obtained for the standard solution of H4Si04 (as the standard) . The results are given in Table 1.

Preparation of 1.32M phosphate buffer of pH= 7 for the testing:

Sodium dihydrogenphosphate (NaH2P04; 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 (H^SiO^) 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 (HC1) (Table 2, Experiment 1): To a solution of 37% hydrochloric acid (0.25 mL; 0.296 g; 0.11 g HC1; 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, tG, of ortho- silicic acid (H4Si04) 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 (tD) until the formation of opalescent (and thick) gel was determined. This time interval was termed as "gelling (polymerization) time", tG, 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 (NaH2P04; 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 (H4Si04) in solution at pH= 6.5.

(i) Preparation of solutions of complexes of DL- (+-) -carnitine hydrochloride (la) with different auxiliary stabilizers, in concentration of 1% w/w of silicon (Si) (Table 3, Experiments 2-7) . General procedure: To a solution of DL- (+) -carnitine hydrochloride (la; 1.06 g; 0.0054 mol; 1 mol. equiv.) and auxiliary stabilizer
(3.00 g; 20% 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 H3P04; 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. After this, distilled water (2.48 g) was added up to the total weight of reaction mixture of 15.00 g. Content of silicon (Si) in thus prepared solutions was 10 mg/g (1% w/w) .
(ii) Determination of gelling (polymerization) time, tG, of ortho- silicic acid (H4Si04) in the presence of DL- (+) -carnitine hydrochloride (la) 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 (tQ) until the formation of opalescent (thick) gel was determined. This time interval is termed as "gelling (polymerization) time", tG, 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 (H SiQ4) in solution at pH= 6.5.
(i) Preparation of the control solution of analogous composition as the product "BioSil<R>" (Table 4, Experiment 1): To a solution of 85% phosphoric acid (0.2 mL; 0.34 g; 0.289 g H3P04 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 H3P04; 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-panthenol (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, tG, of ortho- silicic acid (H4Si04) 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 (Na2C03) . For thus prepared test solutions, the time from the moment of mixing with the phosphate buffer (tD) until the formation of opalescent (thick) gel was determined. This time interval was termed as "gelling (polymerization) time", tG, 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 (H^SiO^) in solution at pH= 6.5.

(i) Preparation of complexes of H4Si0 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 accepatable acids were added:
(a) 37% hydrochloric acid (0.70 mL; 0.83 g; 0.31 g HC1; 0.0084 mol; 1.5 mol. equiv.) (Table 5, Experiment 1);
(b) 85% phosphoric acid (0.55 mL; 0.935 g; 0.795 g H3P04; 0.0081 mol; 1.5 mol. equiv.) (Table 5, Experiment 2);
(c) 96% sulfuric acid (0.45 mL; 0.828 g; 0.795 g H2S0"; 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 (la) complex in the presence of phosphoric acid was described in Example l/(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, tG, of ortho- silicic acid (HSi0) 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 (tD) until the formation of opalescent (thick) gel was determined. This time interval was termed as "gelling (polymeri zaton) time", tG, 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 H4Si04 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 mg; 0.00106 mol), 85%

phosphoric acid (182 mg; 155 mg H3P0 ; 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% H4Si04 (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 H4S1O4 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 H3P04; 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% H4S1O4 (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 H4SiC>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 coolod to -10 [deg.]C, and then silicon tetrachloride (SiCl4; 24 mL; 35.59 g; 0.209 mol) was added dropwise. The reaction mixture was stirred at temperatures between -10 [deg.]C to - 5 [deg.]C during 1 h. Then, solid calcium carbonate (CaC03; 37.00 g; 0.37 mol) was added in several portions during 30 minutes. The reaction mixture was stirred at temperatures between -5 [deg.]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% H4Si04 (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 H4SiQ4 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 H3P04; 0.52 mol; 2.5 mol. equiv.) were added. The reaction mixture was cooled to 0 [deg.]C, and then sodium silicate (Na2Si03; 25.40 g; 0.208 mol) was added in portions during 30 minutes. The reaction mixture was stirred at temperatures from 0 [deg.]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% H4S1O4 (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 H4SiQ4 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 H3P04; 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% H4Si04 (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 H4SiQ4 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 H3P04; 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. 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% H4Si04 (2.34% Si) ;
- 2% phosphoric acid;
- 20% DL- (+-) -carnitine hydrochloride;
- 15% 1 , 2-propylene glycol;
- 10% glycerol; and
- up to 100% purified water.

Example 12

Preparation of solution of ortho-silicic acid of 2% w/w concentration of H SiQ4 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 [deg.]C . Then, 85% phosphoric acid (84.00 g; 71.40 g H3P04; 0.73 mol; 0.53 mol. equiv. / H4Si04) 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 wight 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% H4S1O4 (0.58% Si) ;
- 1% phosphoric acid;
- 16% L-carnitine dihydrogenphosphate;
- 30% 1 , 2-propylene glycol;
- 3.8% ethanol; and
- up to 100% purified water.

WO2012032364
STABILIZED SOLUTION OF ORTHO-SILICIC ACID BASED ON SALICYLIC ACID

The present invention discloses a formulation that serves as a highly bioavailable silicon (Si) source consisting of: (i) ortho-silicic acid (H4SiO4), from 0.01-8% w/w; (ii) salicylic acid (1), from 1-2 molar equivalents to H4SiO4; (iii) pharmaceutically acceptable acid, from 0.1-4 molar equivalents to H4SiO4; 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.

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 Si04) , 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 (H4Si04) , from 0.01-8% w/w;
(ii) salicylic acid (1) ,
from 1-2 molar equivalents to H4Si04;
(iii) pharmaceutically acceptable acid, from 0.1-4 molar equivalents to H4Si04; 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 [. 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 immunite, 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. . 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 Si04) . 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 (H4Si04) , is not stable and at higher concentration, but undergoes polymerization with formation of dimer (H6Si207) , trimer (H8Si3O10) , and linear chain oligomers (SI) which are still water soluble. Linear chain polymers of silicic acid (SI) 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; Si02'xH20) . The course of polymerization of silicic acid is given in Scheme 1 (at the end of the specification) . Beside monomeric ortho-silicic acid (HSi04) ,

biologically available forms are also its lower oligomers soluble in water, due to partial hydrolysis that release starting HSi0 (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 Al (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 Al] , as well as proline, serine, lysine, arginine, glycine, their mixtures, polypeptides or protein hydrolyzates [V. Berghe, D. A. Richard, WO 2004/016551 Al (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 (Si02) . 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. Kustrak: Pharmacognosy and phytopharmacy (in Croatian) Golden marketing-Tehnicka 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; Si02) in organic, and solution of potassium silicate (30% K2Si03) 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 Si0) 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 (H4Si04), from 0.01-8% w/w;
(ii) salicylic acid (1) ,

from 1-2 molar equivalents to H Si04; (iii) pharmaceutically acceptable acid, from 0.1-4 molar equivalents to H Si04; 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 (HC1) , sulfuric (H2S04) , nitric (HN03) , phosphoric (H3P04) , methanesulfonic (CH3SO3H) , benzenesulfonic (C6H5S03H) , salicylic (1 , 2-C6H4 (OH) COOH) or sulfosalicylic [C6H3(3- COOH) (4-OH)S03H] 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 OH) , tetramethylammonium hydroxide [N(CH3)4OH], tetraethylammonium hydroxide [N (C2H5) 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 Si0) 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-HSi0 , prepared from sodium silicate (Na2Si03) and salicylic acid at molar ratio of 1:1. Chemically pure sodium silicate was prepared by base-catalyzed hydrolysis of tetraethyl orthosilicate [TEOS; Si(OC2H5)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 (H4Si0) , and these are at pH values close to neutral. At these conditions, pH= 6-7, relatively fast polymerization of HSi0 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 (H4Si04) .

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 (tG) . Longer gelling (tG) 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 (Na2Si03) as standard;
(ii) solution of complex with choline chloride [(CH3) 3N<+> (CH2CH2OH) CI<">] ; and
(iii) solution of complex with L-serine (HOOCCH (NH2) CH2OH) ;
which are described in the prior art as HSi04 stabilizers [S. R. Bronder: Stabilized orthosilicic acid comprising preparation and biological preparation, W095/21124 (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 (HSi0) at pH= 6.5.

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) .

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] .

"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 (Na2Si03)] . It shows stabilizing or destabilizing effect on ortho-silicic acid, in other words on its polymerization (gelling) .

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].

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].

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].

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]. 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 [deg.]C) , and at temperatures <30 [deg.]C, during minimally 2 years.

Alternatively, the formulation from the present invention can be prepared as complex with ortho-silicic acid (HSi04) 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 (H4Si04) 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 H4Si0 .

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 (H4Si04) 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 Si0 , 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 (H4Si04) at pH= 6.5.

<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 (HSi04)] . 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 H3P04; 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 (H4Si04; 10.00 g destilirana voda + 1.2 mL TEOS + 0.2 mL 85% H3P04; 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 Si0 , 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 (H4Si04) where observed polymerization time was 2.2x longer (Experiments 4; Tables 2 and 3), what suggest on high stability of the complex H4Si04-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 Si04, 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 H4Si04. 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 H4Si04) and 15% w/w of 1 , 2-propylene glycol showed . lx 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 [deg.]C) , and at temperatures <30 [deg.]C, during minimally 2 years. Explanation of inhibition effect of salicylic acid on polymerization of ortho-silicic acid (HSiQ)

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-C6H4 (ONa) COONa] . Acidity of ortho-silicic acid

[pKa (H4Si04) = 2,2.10<"10>] is similar to that of hydroxyl group of simple phenol [pKa (C6H5OH) = 1,3.10<~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 (H4Si04) . 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 (H4Si04) .

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)30<">, 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 HSi0 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 (HSi04) . 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 H4Si0 in the solution of the complex, what consequently leads to drastically slower polymerization process (high values of tG) .

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 H4Si04. Probably, minimal molar excess of 4 equivalents of PG to H4Si04 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 HSi0) 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 HSi04 [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 (H4Si04) 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 xM2OySi02; M= Na,K, x:y= 1:1 do 1:3,5); or
(ii) silicon tetrachloride (SiCl4) .
The use of sodium (Na2Si03) or potassium silicate (K2Si03) 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 H4Si04.
In the case of the use of SiCl4 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 (HC1) generated during hydrolysis of SiCl4, whilst 2 remained equivalents serve for neutralization reaction of salicylic acid yielding salicylate salt (e.g. disodium salicylate) which forms the complex with liberated H4Si04 (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 [deg.]C to +40 [deg.]C, preferably from +15 [deg.]C to +30 [deg.]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) 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 SiCl4 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 SiCl4, 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 (HSi0) , 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 (HC1) , 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

(C2H5OH) 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 [deg.]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 [deg.]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 (Na2Si03) 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 (H4Si04) 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 H3P04; 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 H3P04; 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 (H4Si04) 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 HSi04 [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 H3P04; 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 H4Si0 [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 ([Eta]43[iota]04) in solution. Influence of salicylic acid on stability of H4Si04 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 (tc; 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", tG, and expressed in minutes. Obtained results for tG are expressed in comparison with results obtained for the standard solution of sodium silicate (Na2Si03) 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 (NaH2P04; 18.00 g; 0.15 mol) was quantitatively transferred into a 100 inL 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 (tD; 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", tG, and expressed in minutes. Obtained results for tG are expressed in comparison with results obtained for the standard solution of ortho-silicic acid (HSi04) 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 (NaH2P04; 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 (H4Si04) with dipotassium salicylate of 0.5% w/w concentration of H4Si04 (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

Form of solution of the complex of ortho-silicic acid (H4Si04) with disodium salicylate of [epsilon] !% w/w concentration of HSi04 (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^SiO^) (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 H4Si04 [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 (HqSiO (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 H2S0 ; 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 H4Si04 [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^SiOj) (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 H3P04; 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 H4S1O4 [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 (H4Si04) of 2% w/w concentration of H4Si0 (or 0.58% w/w of Si) with the use of sodium silicate as precursor of silicic acid To a solution of sodium silicate (Na2Si03; 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 H4Si04 [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^SiC (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 [deg.]C, under vigorous stirring, silicon tetrachloride (SiCl ; 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 [deg.]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 H4S1O4 [or 0,58% w/w of silicon (Si)];
- 2.9% w/w salicylic acid;
- up to 100% w/w 1 , 2-propylene glycol.

IL150370
METHOD FOR PREPARING ORTHO SILICIC ACID

The invention relates to a method for preparing ortho silicic acid, to the ortho silicic acid obtainable by this method and to its use as a silicon preparation as formed in the production of animal feed, food or food supplement, and of pharmaceutical or cosmetic preparation.

[0001] The present invention relates to a method for preparing ortho silicic acid, to the ortho silicic acid obtainable by this method and to its use as a silicone preparation in the production of animal feed, food, food or feed supplement, and for the production of a pharmaceutical or cosmetic preparation.

Silicon (Si) has been recognized as an essential trace element for diatoms, Si accumulating plants and higher animals. The best documented function of silicon in vertebrates is its

regulatory action in bone calcification and its chemical association with several constituents of the extracellular matrix in connective tissues (Carlisle E. (1989), Silicon, in : Handbook of Nutritionally Essential Mineral Elements, ed. B.L. O'Dell and R.A. Sunde, Marcel Dekker Inc., New York, pp. 603-618). This matrix consists primarily of fibrous proteins such as collagen, embedded in a hydrated polysaccharide gel. Silicon being bound to components of this matrix is regarded to be important for the structural integrity, the development and the regulatory functions of connective tissue. Gastro-intestinal absorption of Si is only possible after hydrolysis of dietary Si-compounds into ortho silicic acid. The solubility of silicon compounds in the diet is low and consequently these compounds have a limited bioavailability. Organic compounds comprising Si-C bounds are not found in biological systems and several classes of synthetized products were found to have an unacceptable high toxicity. The natural soluble silicon compound, ortho silicic acid also called monomeric silicic acid is present both in fresh and sea water but only at very low concentrations (<1 mmol l<-1> [Sullivan C. (1986) Silicification by diatoms, in : Silicon Biochemistry, CIBA Foundation Symposium 121, John Wiley and Sons, New York, pp. 24-39].) Higher concentrations in aqueous media initiates a polymerization reaction of into non-bioavailable colloids and ultimately gels. A method for the preparation of a stabilized formulation of ortho silicic acid is disclosed US 5,922,360.

[0002] The present invention has for its object to provide a method for preparing ortho silicic acid starting from relatively inexpensive and market available starting materials while polymerisation of formed ortho silicic acid is substantially avoided.

[0003] This is obtained with the method according to the invention for preparing ortho silicic acid wherein an acid hydrolysable silicon compound is hydrolysed in an acid solution in the presence of a solvent agent under the formation of ortho silicic acid, such as a acid aqueous solution. Due to the use of an acid solution and to the presence of a solvent agent the afore mentioned polymerisation reaction is substantially suppressed and the ortho silicic acid formed is sufficiently stabilized.

[0004] The starting material, which is an acid hydrolizable silicon compound, may be selected from a silicate, such as a monomeric silicate such as silicon halogenide, methyl ortho silicate, sodium or magnesium orthosilicates, or from hydrated silicate such as crystalline sodium silicate.

[0005] According to another embodiment the acid hydrolizable silicon compound has the general formula
EMI3.1
wherein R1, R2, R3 and R4 are independently selected from H, C1-C12 alkyl, C1-C12 alkoxy which are optionally substituted by an hydroxyl group, under the proviso that R1, R2, R3 and R4 are not simultaneously H. Preferably, R1, R2, R3 and R4 are selected from H, C1-C4 alkyl, C1-C4 alkoxy optionally substituted by an hydroxylgroup. It is noted that R1, R2, R3 and R4 are preferably selected such that the compound split off from the hydrolisable silicon compound is removable using traditional techniques such as evaporation and distillation, and most preferably is non-toxic (LD50 orally in rat higher than 1g/kg bodyweight). The most preferred silicon compound is tetra-ethoxy-silanol.

[0006] Other preferred examples for R are C2H5, CH3CO, HCO, C3H7, C4H9 and CH3CH(OH)CHCO. The solution may comprise 1-80%, preferably 10-70%, more preferably 40-60% solvent agent.

[0007] The solvent agent used in the acid solution for stabilizing the formed ortho silicic acid may be selected from the group comprising glycol, glycerol, (poly)alkylene glycol, DMSO and polysorbate 80. The (poly) alkylene glycol may be polypropylene glycol or polyethylene glycol. The alkylene glycol may be ethylene glycol or propylene glycol. A common set of properties for all solvent agents are a high solubility in water (more than 30%), a boiling point higher than 130 DEG C, a liquid state between -10 DEG C and 40 DEG C and a stability at an acid pH of generally 0-4.

[0008] The formed ortho silicic acid stabilized by the solvent agent, may be stabilized further by contacting the ortho silicic acid with a particulate carrier.

[0009] Surprisingly, it is experienced that this particulate carrier adsorbed ortho silicic acid has a bioavailability which is comparable or even improved over the stabilized formulation, as disclosed in US 5,922,360. The bioavailability is a critical issue since it was recently shown in comparative human supplementation studies that solid silicon supplements such as colloidal silica and phytolytic silicates are not bioavailable whereas a solution of stabilized ORTHO SILICIC ACID in a HCl-choline matrix has a high bioavailability [Calomme M., Cos P., Vingerhoets R., Van Hoorebeke C., Vanden Berghe D. (1998) Comparative bioavailability study of silicon supplements in healthy subjects, Journal of Parenteral and Enteral Nutrition, 22, S12, (abstract #47) .Van Dyck K., Van Cauwenbergh R., Robberecht H., Deelstra H. (1999), Bioavailability of silicon from food and food supplements, Fresenius Journal of Analytical Chemistry, 363, 541-544.]
Accordingly, the present invention also provides a silicon preparation, comprising ortho silicic acid adsorbed on a particulate carrier, obtainable by the process comprising the steps of:

i) providing a solution, comprising ortho silicic acid stabilized with said acid solvent agent; and
ii) contacting the ortho silicic acid comprising solution with the particulate carrier.
In order to avoid to an additional extent the polymerization of ortho silicic acid, it is preferred that the ortho silicic acid is formed in situ. The handling and the formation of dosing forms of the silicon preparation are further improved when the carrier, after contact with ortho silicic acid, is extruded.

[0010] The skilled person will appreciate that the silicon preparation according to the invention may contain ortho silicic acid over a broad silicon content range depending on the contemplated use of the silicon preparation. Generally, the silicon content of the silicon preparation is within the range of 0.01-50 wt.%, preferably within the range of 0.01-10 wt.%, more preferably within the range of 0.1-10 wt.%, and most preferably within the range of 0.1-5 wt.%. Accordingly, the silicon preparation may be used in a dosing regime which is suitable for most contemplated food, feed, pharmaceutical and cosmetic utilities. In this respect it is noted that the pharmaceutical and cosmetic preparation will have a positive effect on nails, hair, skin, teeth, collagen, connetive tissue, bones, encourages cell generation, stimulates the immune system against infections and toxins and inhibits degenerative (ageing)-process.

[0011] Experimental use of silicon preparations according to the invention have shown, that the silicon preparation has a desired high bioavailability expressed as the total silicon absorption by an organism such as a human being. Over a period of 0-8 hours the relative bioavailability was much improved over the afore mentioned colloidal and phytolytic silica preparations. In other words the total silicon absorption over 8 hours is more than 250 mu g

Si.h/l, preferably more than 500 mu g Si.h/l, more preferably more than 600 mu g Si.h/l, such as 250-700 mu g Si.h/l, preferably 300-700 mu g Si.h/l.

[0012] The silicon preparation according to the invention adsorbed on a carrier may be used as such or in combination with any acceptable carrier material, excipient or diluent.

[0013] The silicon preparation according to the invention may be administared orally or in any other suitable fashion. Oral administration is preferred and the silicon preparation may have the form of a tablet, aqueous dispersion, dispersable powder or granule, emulsion, hard or soft capsule, syrup, elixir or gel. The dosing forms may be prepared using any method known in the art for manufacturing these pharmaceutical or cosmetic compositions and may comprise as additives sweeteners, flavoring agents, coloring agents, preservatives and the like. Carrier materials and excipients may include calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents, binding agents and the like. The silicon preparation may be included in a gelatin capsule mixed with any inert solid diluent or carrier material, or has the form of a soft gelatin capsule, in which the ingredient is mixed with a water or oil medium. Aqueous dispersions may comprise the silicon preparation in combination with a suspending agent, dispersing agent or wetting agent. Oil dispersions may comprise suspending agents such as a vegetable oil. A gel formulation may be prepared following the teaching given in US 5,922,360.

[0014] It is now possible to make dry mixtures of carrier-bound ortho silicic acid with other components such as trace elements, vitamins, amino acids, sugars, plant extracts, and other ingredients used in the manufacturing of food and food supplements. As an explanation it is considered that the ortho silicic acid remains in its monomeric form in carrier-bound ortho silicic acid and is therefore different from non-bioavailable polymerized forms of ortho silicic acid such as in colloidal or solid silicic acid and silicates.

Ortho silicic acid is for instance prepared in the presence of the acid solvent agent and in situ by (a) hydrolysis of monomeric silicon compounds such as silicon halogenide or methyl orthosilicate [Iler R. (1979) Monosilicic acid, in : The Chemistry of Silica, John Wiley and Sons, New York, pp. 178-180.], (b) by reacting monomeric silicates such as sodium or magnesium orthosilicates or hydrated crystalline sodium silicate with dilute acid (Iler 1979), (c) by hydolyzing organic alkylsilanol compounds. It is noted that next to the formed ortho silicic acid the other hydrolization reaction compounds should be non-toxic and if desired should be removed from the reaction mixture. Preferably, the alkylsilanol compound is an ethoxysilanol compound and the formed ethanol may be separated without difficulty. The freshly prepared ortho silicic acid is bound to the carrier or a combination of carriers. A second method is to bind first a organic silicon compound on a carrier and thereafter hydrolyzing the organic silicon compound into ortho silicic acid for instance at a pH of lower than 4, such as 0.2-2.5, more preferably 0.8-1.0.

[0015] The solid carrier or combination of solid carriers may be selected from the group comprising:

i) natural and semi-synthetic fibers,
ii) plant metabolites such as polyfenols, lignans, flavonoid,
iii) fatty acids and esters thereof such as stearates, palmitates, linoleates, oleates, adipates, caprylates, caprates, cocoates,
iv) phosholipids and derivates thereof,

v) polyalcohols such as inositol, trehalose,
vi) hydrogenated and sulfated compounds,
vii) salts such as chlorides, sulfates, nitrates, etc.,
viii) pectines and alginates,
ix) sugars or sugar alcohols and derivatives thereof such as lactose, sucrose, mannitol, sorbitol, sorbitolesters,
x) poly- and oligosaccharides silicic acharides and derivatives thereof such as dextran, fructans, inulin, oligofructose,
xi) gelatine or derivatives thereof such as gelatine hydrolysate
xii) cellulose er derivatives thereof such as microcrystalline cellulose,hydroxypropylcellulose, hydroxypropyl methylcellulose,carboxymethylcellulose, cellulose gum
xiii) peptides and polypeptides such as collagen, soy proteins, mays protein and derivates thereof
xiv) glucans and derivatives thereof such as proteoglycans, glycosaminoglycans, hyaluronic acid, chondroitin sulfate, heparin, heparan sulfate, keratan sulfate, dermatan sulfate,
xv) starch and derivatives thereof,
xvi) lecithin and derivatives thereof, and
xvii) byproducts of foodproduction, such as fermented byproducts from cheese, beer and mays, and cheese whey as an example
xviii) foodproducts such as dried animal food, substrates for plants such as natural peat for plant production, dried plant extracts are dried plant homogenates and cosmetic powders such as talc.

Example A

[0016] Ortho silicic acid is prepared as followed. Two liters of a fresh solution of cold sodiumsilicate (27 % SiO_2 in 14 % NaOH) is mixed with 2 - 4 liters of glycerol (pro analyse, 100 %) until a homogeneous solution is obtained. To decrease the pH, one liter of cold, concentrated hydrochloric acid is added and the mixture is stirred strongly at a temperature between 0 - 10 DEG C. During continuous mixing, solid or a suspension of calcium carbonate is added until a pH of 1-3 is obtained. During mixing CO_2 gas will be formed.

[0017] Half a liter of freshly prepared combination of concentrated ortho silicic acid is mixed with 0.5 kg of gelatine, or 0.5 kg of cheese whey, or 200 g of cellulose, or 1 kg of galactose, or 1 kg of saccharose. The resulting paste is mixed until a homogeneous paste is obtained. The paste is dried in vacuo. The final product contains minimum 0.1 % elemental silicon and preferably between 1 - 5 % elemental silicon.

[0018] A daily intake of 0.5 g during 2 months resulted in improved nail and hair quality in four different persons. This improvement was equivalent as observed using the formulations mentioned in US 5,922,360.

Example B

[0019] The carrier (65 %) microcrystalline cellulose is mixed with 35 % of a combination of concentrated ortho silicic acid with glycerol (see example A). Demineralized water is added during continuous mixing to obtain an appropiate quality of the granulated material. The plastic mass is extruded with a basket extruder (Caleva Model 10, Sturminster Newton, Great Britain) at 750 rpm. The extruded strands are spheronized (Caleva Model 120 spheronizer).

The resulting pellets are dried to a final water content of lower than 5 %. Typical pellet size is between 800 and 1200 mu m. The pellets are encapsulated in hard gelatine capsules size OO. Each capsule contains 0.54 g pellets equal to 5 mg elemental silicon in the form of carrier-bound ortho silicic acid. The loading capacity of the microcrystalline cellulose can be increased to 45 % ortho silicic acid.

Example C

[0020] The carrier, a mixture (1:1) of soy proteins and mays proteins (70 %) are mixed with 30 % of a combination of ortho silicic acid with glycerol (see example A). Demineralized water is added during continuous mixing to obtain an homogenous plastic mass. The mixture is dried by lyophilization. Following granulation the protein-bound ortho silicic acid is directly encapsulated or used as a raw material in the manufacturing of animal feed, food, food supplements, cosmetics or pharmaceutical preparations.

Example D

[0021] The carrier (65 %) a mixture (3:1) of microcrystalline cellulose and fructans is mixed with 35 % of a combination of concentrated ortho silicic acid with glycerol (see example A). Demineralized water is added during continuous mixing to obtain an appropiate quality of the granulated material. The plastic mass is extruded with a basket extruder (Caleva Model 10, Sturminster Newton, Great Britain) at 750 rpm. The extruded strands are spheronized (Caleva Model 120 spheronizer). The resulting pellets are dried to a final water content of lower than 5 %. Typical pellet size is between 800 and 1400 mu m. The pellets are pressed to tablets or used as a raw material in the manufacturing of animal feed, food, food supplements, cosmetics or pharmaceutical preparations.

Example E

[0022] 100 ml icecold tetra-ethoxy-silanol is dropped slowly in 1 liter of 50% solution icecold glycerol in water pH 1,0. After 8 h at 0 DEG C the silanol compound is completely hydrolysed. Ethanol is removed by quick evaporation under vacuum.

The remaining OSA solution is mixed with 2 - 3 kg lactose as a paste and further dried under vacuum. The final product contains minimum 0.1 % Si and preferably between 0.3 abd 2% Si.

[0023] Dissolution assays of the preparations of Examples A-E prove that ortho silicic acid is released within 30 minutes into the dissolution medium. This is demonstrated by measuring the silicon content of the dissolution medium at fixed time-points with Zeeman corrected Electrothermal Atomic Absorption Spectometry (Perkin Elmer). The fact that ortho silicic acid is released during dissolution demonstrates clearly that binding of ortho silicic acid to the carrier will not result in polymerization of ortho silicic acid but remains in a dissociatable form. Dissolution assays were repeated at 3, 6 and 12 months after the production date without difference in results demonstrating that carrier-bound ortho silicic acid is chemically stable over a long period of time.

Example F

[0024] Three healthy subjects (2 females, 1 male, aged 22-34 y) were included after

informed, written consent. None had taken Si supplements within 3 months before the start of the study. Each fasting subject received in a cross-over protocol Si p.o. as follows:10 mg of Si in the form of stabilized ortho silicic acid (ortho silicic acid, 0.5 ml of BioSil containing 20 g Si/l, as in US 5, 922,360), 10 mg of Si in the form of carrier-bound ortho silicic acid (capsules of the preparation of Example D), 20 mg of Si in the form of colloidal silica (polymerized ortho silicic acid) 20 mg of Si in the form of phytolytic silica (a standarized dry extract of the Si-accumulating plant Equisetum arvense) or a placebo (10 ml mineral water) within 1 week washout period between each supplement or the placebo. Blood samples were collected in Si free polypropylene tubes prior to supplementation and after 1, 2, 4, 6 and 8 hours post partem. Identical meals were consumed during the experiment after 2 and 6 hours supplementation. The Si concentration in serum and urine was determined for each subject in one batch with AAS. A Zeeman/3030 Atomic Absorption Spectrometer equipped with a HGA-600 graphite furnace was used in combination with an AS-60 autosampler (Perkin-Elmer Corp. Norwalk CT). The area under the time concentration curve (A.U.C.) was calculated using the linear trapezoidal rule as an objective parameter of the total Si absorption. The serum silicon concentration increases significantly from the baseline value after supplementation of both liquid ortho silicic acid and carrier-bound ortho silicic acid (fig. 1 ortho silic acid = OSA) but not after supplementation of polymerized ortho silicic acid forms such as colloidal silica or phytolytic silica. The kinetic absorption profile for carrier-bound ortho silicic acid indicates a slower-release effect compared to liquid ortho silicic acid. The total bioavailability is similar for carrier-bound ortho silicic acid and liquid ortho silicic acid whereas the polymerized forms of ortho silicic acid are not bioavailable since no significant difference is seen for these products compared to the placebo (fig. 2 ortho silicic acid = OSA). Bioavailability experiments were repeated one year after the production date of the carrier-bound ortho silicic acid without differences in results, demonstrating that carrier-bound ortho silicic acid is chemically stable over a long period of time.

Fig. 1 Increase in silicon concentration in serum from the baseline value in healthy subjects after supplementation of respectively 10 mg Si in the form of carrier-bound OSA, 10 mg Si in the form of liquid OSA, 20 mg Si in the form of colloidal silica, 20 mg of Si in the form of phytolytic silica.
EMI13.1

Fig. 2 Total absorption of silicon in serum over a period of 0-8 hours p.p. measured in healthy subjects after supplementation of respectively 10 mg Si in the form of carrier-bound OSA, 10 mg Si in the form of liquid OSA, 20 mg Si in the form of colloidal silica, 20 mg of Si in the form of phytolytic silica.
EMI13.2

US5922360
Stabilized orthosilicic acid

Preparation comprising ortho silicic acid which is stabilized with a stabilizing agent and is substantially free of organic silicon compounds, preferably a nitrogen-containing stabilizing agent such as choline, to a method for preparing such a preparation, comprising: i) providing a solution containing a stabilizing agent; ii) dissolving an inorganic silicon compound in the solution containing the stabilizing agent; and iii) hydrolyzing the silicon compound to ortho silicic acid, and to the obtained biological preparation.

Silicon is an essential trace element for plants, animals and humans. In a watery environment silicon is initially present as ortho silicic acid which is quickly converted by polycondensation to polysilicic acid, which transposes into a colloidal solution and gels. Ultimately, insoluble silicates are formed.

In the same way as carbonic acid for compounds comprising carbon, ortho silicic acid is the most important metabolite for organic silicon compounds. Water glass (sodium ortho silicate) is the usual source of ortho silicic acid, which however hydrolyses after oral administration to mammals and forms insoluble and non-absorbable gels through polycondensation.

Organic silicon compounds such as alcohol esters, such as ethyl ortho silicate and glycol ortho silicate, cannot be used in biological systems because of the poor solubility and the low resistance to hydrolysis, but above all because of the unacceptable toxicity.

There therefore exists a need for a silicon-comprising preparation not possessing the above stated drawbacks, because silicon has a positive biological effect on nails, hair, skin, teeth, collagen, connective tissue, bones, encourages cell generation, stimulates the immune system against infections and toxins and inhibits degenerative (ageing)-processes.

The present invention is based on the insight that if ortho silicic acid is formed in the presence of a stabilizing agent, polycondensation is inhibited and even avoided and, furthermore organic silicon compounds substantially do not occur.

A first aspect of the present invention therefore relates to a preparation comprising ortho silicic acid which is stabilized with a stabilizing agent and is substantially free of organic silicon compounds.

A second aspect of the present invention relates to a method for preparing a preparation as according to claims 1-7, which comprises of:
i) providing a solution containing a stabilizing agent;
ii) dissolving an inorganic silicon compound in the solution containing the stabilizing agent; and
iii) hydrolyzing the silicon compound to ortho silicic acid.

A third aspect of the present invention relates to a biological preparation containing a preparation according to claims 1-7, and/or a preparation prepared according to claims 8-13, and a pharmacologically acceptable diluent.

The biological preparation according to the invention is can be used for:
chronic infections with destruction of the mucous membranes: forms of sinusitis and ulcers.
problems with connective tissues, arteriosclerosis, bone and tendon problems, gynaecology (fibroids, polycystic adenopathy); and
the growth of children: children with recurrent infections with overload of the lymphatic system.

The stabilization using a stabilizing agent preferably takes place with stabilizing agents containing a nitrogen atom with a free electron pair which forms a complex with the silanol groups of the ortho silicic acid. Quaternary ammonium compounds are preferably used, for instance tetra-alkyl compounds, wherein each alkyl group contains for instance 1-5 carbon atoms, in particular methyl and ethyl groups. Very highly recommended are

trialkylhydroxyalkyl compounds, wherein the hydroxy group is preferably methanol or ethanol. Choline has been found very suitable, which is further recommended in that it provides the option of the stabilizing agent also forming the solution for the ortho silicic acid, and an inert solvent can therefore be omitted.

Another or additional type of stabilizing agent is an amino acid, such as proline and serine. Serine enhances uptake in the stomach and gives additional stability.

Starting point for the preparation of the ortho silicic acid-comprising preparation is a solution containing the stabilizing agent, wherein an inert solvent can be used. Incorporated in -This solution is an inorganic silicon compound which hydrolysis under the influence of water to ortho silicic acid, which is immediately stabilized by the stabilizing agent that is present. The solution containing the stabilizing agent can initiate the hydrolysis immediately after addition of the inorganic silicon compound. Usually recommended is a solution containing a stabilizing agent in which no hydrolysis can take place until after the addition of a hydrolyzing agent, such as water.

If choline is used as stabilizing agent it can be converted to choline hydrochloride using dry hydrochloric acid. In this liquid stabilizing agent can be incorporated the inorganic silicon compound, such as a silicon halogenide, particularly silicon tetrachloride.

Simultaneously with the addition of the inorganic silicon compound, or following the addition of the hydrolyzing agent, the hydrolysis of the inorganic silicon compound to ortho silicic acid takes place. The silicic acid formed in situ is subsequently stabilized by forming a complex with the stabilizing agent. It is of great importance herein that the stabilizing agent only forms a complex and does not enter into a reaction, particularly an esterifying reaction, with the ortho silicic acid. Then achieved is that no organic silicon compounds are created which have an inherent toxicity, are absorbed in the stomach and enter the blood circulation.

After forming a complex the ortho silicic acid-comprising solution can if desired be partially neutralized by adding a base, such as a lye, particularly sodium hydroxide. Neutralization can take place to a pH smaller than 4, in particular smaller than 3, in general to a pH lying in the range of 1-3, whereby any polycondensation of ortho silicic acid is substantially avoided.

If desired, a further purification of the preparation can take place, for instance through absorption of contaminants on active carbon, optionally followed by filtration.

If desired, the content of hydrolyzing agent, particularly water, can be reduced by removing the hydrolyzing agent, for instance through distillation, whereby a constant viscosity is achieved if use is made of choline as the stabilizer.

Preparations then result with a silicon content generally of 1% by weight, preferably of about 4% by weight, such as 8% by weight. A very acceptable preparation contains 3-5% by weight of silicon, 70s by weight of choline hydrochloride and the rest water. The pH of this preparation lies within the range 1-3.

Biological preparations can be-manufactured from this prepared preparation for the purpose of administering ortho silicic acid to plants, animals and humans, whereby the bio-availability of silicon is greatly improved. The above prepared solution can be administered as biological preparation as such, for instance as nail tincture. A usage of 0.5 ml of a 2% Si-solution per

day for three weeks caused a fungal infection to disappear (3 patients), where treatment with ketonazols did not render any improvement. If for instance an edible acid, such as malic acid, is added a preparation results which is very suitable for administering to horses.

If a solid carrier is added, for instance cattle feed, cattle feed pellets can be pressed therefrom which contain ortho silicic acid in stabilized form for administering silicon to cattle. If sugar/maltose is used as solid carrier, tablets and gels can be formed therefrom.

Through use of a glucuronic acid buffer a preparation on a cream basis can be formed wherein the pH is less than 4, which creams are suitable for local cutaneous application.

It will be apparent that all kinds of diluents can be used in order to obtain a preparation for biological application. Such diluents contain lower alkanols, such as ethanol, dichloromethane, ethyl acetate, glycerine and polyalcohols.

PREPARATION EXAMPLE

Choline hydrochloride (UCB) is dried under vacuum (100 DEG C./6 hours). The choline hydrochloride is treated with dry hydrochloric acid. Silicon hydrochloride (1 mol per mol) is added to the formed choline solution at a temperature which is kept below 40 DEG C.

For hydrolysis, water (ice/ice water) is added to the solution while cooling, wherein the temperature is held within the range of -20 DEG C. to -30 DEG C.

The solution containing the ortho silicic acid is subsequently neutralized by adding sodium hydroxide wherein cooling takes place to a temperature below 0 DEG C. The pH neutralization amounts to about 1.3.

A purification over active carbon is then performed, followed by filtering off the formed precipitate and the active carbon.

After distillation under vacuum a preparation is obtained which contains 3% by weight of silicon, 70% by weight choline hydrochloride and the rest water.

FAB/MS with glycerol as liquid matrix provides a spectrum with a molecular cation at M/Z 104 (C@+) and an MC@+ adduction at M/Z243/245, typical for chloride isotropy. This spectrum is the same as the spectrum for choline.

NMR-SPECTRUM OF THE PREPARATION SHOWING CHOLINE/ALCOHOL GROUPS

Element analysis produces 24.+-.2% by weight chlorine and 9.+-.1% by weight N. This points to a ratio of chloride to nitrogen of 1:1.

Neutralization is subsequently carried out to a pH of 2.7-3.0.

The preparation is stable for more than two years when stored at room temperature.

FORMULATION EXAMPLES

Formulation Example A

The biological preparation contains 3% by weight silicon in the form of ortho silicic acid, 70% by weight choline hydrochloride, the rest water and a pH of 2.7-3.0. This liquid is suitable for oral and cutaneous administering.

Formulation Example B

The biological preparation as prepared above is mixed with cattle feed which ultimately contains silicon as ortho silicic acid in a concentration of 0.001-0.005% by weight. This mixture can be pressed to pellets which are administered to cattle.

Formulation Example C

The preparation A is mixed with sugar and/or maltose which is pressed to tablets containing silicon in the form of ortho silicic acid at a content of 0.1-0.2% Si by weight.

Formulation Example D

A silicon-comprising cream is prepared as follows. A fat phase containing Imwitor 960 (Huls) 7%, Miglyol 812 10%, Softigon 701 (Huls) 2%, Marlowet TA 25 (Huls) 2%, Lanette N (Henkel) 4%, Isopropylmyristate 3%, a water phase containing Inositol 0.2%, Gluconate buffer 0.05 M, pH 3.8 ad 100, Glycerol 10% and the preparation A, as well as a perfume.

The fat phase is melted at 80 DEG C., whereafter the water phase, also heated to 80 DEG C., is admixed, followed by cooling. Shortly before solidifying, the preparation A and perfume (4 drops) are added. The cream eventually contains 0.01-0.05% by weight silicon as ortho silicic acid.

Flavourings can be added if desired, for instance by dilution (1:30) in a 0.01 M citrate buffer (pH 3.5-3.8) and by adding a flavouring (raspberry and the like).

FR2936712
Composition, useful to stimulate the manufacture and repair of cartilage to treat osteoarthritis

Composition comprises silicon and chondroitin, which are present in a form that is soluble in water, the chondroitin has a molecular weight of = 25000 g/mole and the silicon is present in a complexed form, e.g. with sugar, or in a form of silicic acid or ortho-silicic acid. - ACTIVITY : Antiarthritic; Osteopathic.

The present invention relates to a composition combining silicon and chondroitin, particularly advantageous in the prevention and / or treatment of osteoarthritis.

Osteoarthritis, or arthropathic chronic degenerative cartilage degeneration is a c.0 joints which leads to its dest_: uction more or less rapid.

The cartilage surface cracking, crumbling and eventually disappear.

Then, bone growths in form of the joint and hinder movement.

Symptoms include joint pain and difficulty performing 11 movements.

This is the most common joint disease.

The first symptoms usually appear from 40 to 50 years, but the disease often begins much earlier in life.

The most commonly used in the treatment of osteoarthritis treatments are simple analgesics (paracetamol), nonsteroidal anti-inflammatory drugs (aspirin, diclofenac), anti-inflammatory drugs (corticosteroids).
A number of molecules are proposed as chondroprotective (protective cartilage) also called antiarthrosiques (glucosamine, chondroitin).

They have not shown that they were "pushing" destroys cartilage, but they slow down the evolution of malad = e.

Indeed, the "sodium chondroitin" for example is a molecule used in the long term (6 month treatment renewable), which inhibits elastase (an enzyme involved in the degradation of cartilage).

The clinical effects are possible after pl-zsieurs weeks of treatment, but they are inconsistent.

In the prior art, the chondroitin has also been proposed in combination with silicon to form silica, for the treatment of osteoarthritis (US2007122473), or with compositions comprising chondroitin and plant extracts, for relieve the pain of osteoarthritis US6579543).

These compounds have the drawback of being difficult to solubilize and be of very low bioavailability.

This implies the use of high doses of these compounds to observe an effect on osteoarthritis, this effect is associated with side effects.

The side effects seen with the compositions of the prior art include skin reactions (erythema, urticaria, eczema, maculopopuleuse rash with or without pruritus and / or edema) and gastrointestinal effects (Nausea, vomiting) (Vidal 2008 Page 406 ISBN 978-2-85091-156-9).

Thus, treatment of osteoarthritis of the known prior art are not satisfactory enough.

They have a limited and inconsistent efficacy.

They act primarily on the symptoms, pain, but do not allow cartilage repair.

They require long-term treatment.

In addition, they have no preventive action.

Given the above, a technical problem to be resolved is one invention to provide an improved method for the treatment and. / Or prevention of osteoarthritis, which does not present the

aforementioned drawbacks composition.

The solution proposed by the inventon to this problem for a first object compound: itiDn combining silicon and chondroitin, chondroitin and silicon being present in soluble form in water, characterized in that the chondroitin has a lower or equal to 25 000 g / mol molecular weight and the silicon is present in a form complexed, for example sugars, or in a form of silicic or orthosilicic acid.

The invention secondly relates to a composition combining silicon and chondroitin, wherein the silicon and chondroitin are present in soluble form in water, has a chondroitin is less than or equal to 25 000 g / mol molecular weight and the silicon is present in a form complexed, for its application as a medicament in the treatment and / or prevention of osteoarthritis.

Indeed, the Applicant has found, surprisingly, that the combination of silicon and chondroitin, chondroitin and the silicon being present in soluble form in water, chondroitin having a molecular weight below or equal to 25 000 g / mol, permits treatment and more efficient and faster osteoarthritis without the drawbacks observed in one prior art prevention.

Advantageously, in the framework of the present invention, has a chondroitin or less molecular weight 0000 g / mol, preferably less than or equal to 16 000 g / mol; - The silicon is present at a concentration between 0.0001 and 5% by weight, based on the total weight of the composition; - Chondroitin is present at a concentration between 0.5% and 6C by weight relative to the total weight of the composition; - The silicon is derived from the envelope of cereals, rice, or plants such as nettle; - The composition is applied as a drug to stimulate the production and repair of cartilage in] e = reatment and / or prevention of osteoarthritis..

The invention will be better understood on reading the following non-limiting description.

The composition according to the invention. Ion present improved preventive action against osteoarthritis.

It combines silicon and chondroitin which are present in a soluble form in water and which act synergistically against osteoarthritis.

The compositions of the invention can improve the solubility and bioavailability of silicon and chondroitin.

The dDses necessary to observe an effect are thus reduced, which improves the risk / benefit ratio.

Thus, the compositions of the invention are more effective and have fewer side effects than those of the prior art.

The silicon is preferably in a form complexed, for example with sacred, that is to say, with one or more carbohydrates, or the silicon is present as silicic acid raw orthosilicic acid.

These forms have better bioavailability and more advantageous than the silicon in the form of silica plarmacocinétiques properties.

Carbohydrates which may be complexed silicon include the monosacch6rides, di-, tri-, oligo-

and polysaccharides and glycosides.

These carbohydrates may comprise one or more aldoses, such as glucose, or ketoses such as fructose, one or more tr: monosaccharides, such as dihydroxyacetone and glyceraldehyde one or more tetroses as erythrose, and the threose Erythrulose a pertoses or more, such as ribose, arabinose, xylose, lycose, ribulose and xylulose, one or more hexoses such as allose, altrose, glucose, mannose, gulose, idose , galactose, talose, psicose, fructose, sorbose and tagatose, heptoses one or more, such as sedoheptulose, and / or one or more octoses, alone or in combination.

THE include disaccharides preferably sucrose, trehalose, lactose, maltose, isomaltose, cellobiose 2t.

Trisaccharides include raffinose and preferably gentianose.

In the context of the invention, preferred sugars include glucose, fructose, sucrose, e galactose, mannose and other natural sugars.

Preferably silicon can originated envelope cereal, rice, or plants comne nettle, in which p is preferably in a larger proportion :: ESENT soluble form in water.

As against, silicon or horsetail bamboo is not preferred because it is present in a greater proportion in a form The recrystallized plant and is therefore slightly soluble in water.

Silicon as silica or SiO2 called Silicon dioxide or silica in English only suitable PES either in the compositions according to the invention because it is very assimilated by the body.

Silicon according to the invention Fulani. be obtained by applying known extraction methods such as those described in the Treaty of clinical herbal medicine.

Endobiogénie and Medicine (Christian Luraffourd, Jean-Claude Lapraz Editions Massonn; 1: uly 2002 ISBN-10: 2294005961 pages 4-25).

Chondroitin has a lower or equal to 25 000 g / mol molecular weight Éftre more soluble in water.

A less than or equal to 20 000 g / mol molecular weight is preferred, and in particular less than or equal to 1700 molecular weight, 16000, 15000, 10000, 5000 or 3000 g / mol are. particularly preferred.

A preferred range of molecular weight is between 15000 and 16000 g / mol, a value of t e molecular weight is preferably 15 900 g / mol.

For this, the chondroitin is preferably hydrolyzed.

Chondroitin comes from various known sources, such as cartilage, bone or cornea, fish, marine mammals, birds, ducks, swine, sheep, cattle or goats.

Chondroitin according to the invention can be obtained by application of known extraction methods, such as those described in Technical Bulletin Biocatalysts n [deg.] 106 p 1-4.

For example, the method described in this document comprises the steps of: - the cartilage is used intact or cut into pieces; - It is hydrolyzed enzymatically, preferably with proteases, for 16 to 24 hours, at a pH between 5 and 7 and a temperature between 55 and 70 [deg.

] C, stirring, for example in 7-10 mL of medium Promod648L (marketed by B_ocatalysts [deg.]) Per kg of cartilage.

In most cases, this results in a complete solubilization of the cartilage; - The mixture is cooled to 40 [deg.] C and gra3 and solid wastes are separated; - Soluble peptides and proteins are removed from the aqueous solution; and - chondroitin sulphate is precipitated in the aqueous solution, then dried and reduced to powder.

Chondroitin thus obtained comprises a mixture of molecules of chondroitin moléculaLres whose weight can be between 620 000 g / mol and 25 000 g / mol.

The classic has a chondroitin: 7oids molecular average of about 100,000 to 3_0000 g / mol.

Chondroitin is then hydrolyzed, preferably enzymatically.

Proced {s of enzymatic hydrolysis used to obtain chondroitin according to the invention are described in "Extraction and purification of chondroitin sulfate from cartilage stripe by enzymatic hydrolysis and ultrafiltration" (Lignot B., et al; Sow Congress French Process Engineering, L9 :7-October 2001, Nancy, France).

In another example of the prior art, an extraction process chondreït = not from cartilage, such as sharks, little ': Understanding the steps of: -. Cartilace the previously cleared of muscle tissue residual and frozen at - 20 [deg.] C, is supplied and maintained throughout his treatment at a temperature near or below 4 [deg.] C; - The minced cartilage in pure water (amount equivalent weight / volume); - Chopped mixture was homogenized in a blender hung twenty minutes.

The resulting mixture Contains particles whose size is less than 500 mm; - The mixture is centrifuged at 13600 g for 15 minutes and then the supernatant was filtered on Whatman 24 filter, or the mixture is centrifuged at 3000-4000 g in a CEPA centrifuge equipped with a nylon bag that porosity of 1 m , which allows to obtain 30 L of supernatant of starting / water mixture cartilage 25 kg/25 L; then - the recovered solution undergoes ultrafiltration tangential flow columns known whose porosity is chcis_.e depending on the molecular weight of chondroitin sought.

The liquid extract may be used as such or after concentration, for example by na: iofiltration which favors the maintenance of biological activity.

However, this method presents a method to lower extraction efficiency of any chondroitin followed by enzymatic hydrolysis.

In the compositions according .. to the invention, the chondroitin is present at a concentration: 0.01 ration and 90% by weight. the total weight of the composition, preferably between 0.1 and 85%, more preferably between 0.5% and 80% wt%, and particularly 80%, 70%, 60% oi 40%.

Preferably, the concentration of a composition in choridrDïtine liquid cotprise is between 0.5 and 1% by weight of the total weight of the composition, while it is preferably of the order of 50% in a composition in solid as a capsule.

Indeed, the total weight of composition per day is conventionally absorbed substantially infér_eur with a solid composition (in the order of 500mg/jour) relative to a liquid composition (in the order of 30g/jour).

In the compositions according to the invention, according to the invention is silicon) resent at a concentration between 0.0001 and 5% by weight, based on the total weight of the composition preferably comprises between 0.0005 and 2 % or between 0.001 and 1%, in particular 0.001%, 0.005%, 0.01 `-: and 1%.

Particularly preferably, the combination comprises silicon at a concentration of between 0.001 and 1% by weight, based on the total weight of the composition comprising, chondroitin and present at a concentration: ration between 0, 5 and 60% by weight relative to the total weight of the composition.

The concentration ratio that chondroitin / silicon is advantageously between 10 and 200 and preferably it is L60.

The combination according to the invention offers the following advantages compared to the prior art, in the treatment or prevention of osteoarthritis, particularly in relation to treatment with silicon, in particular as silica, and chondroitin greater than 25,000 g / mol, comparable concentrations molecular weight - the curative and preventive treatment 10 are substantially more effective; - The combination according to the invention can shorten the latency period before obtaining therapeutic results of curative or preventive order 15 - the combination according to the invention reduces side effects and increasing the tolerance of the treatment; - A synergistic effect is observed related compounds according to the invention which extends beyond the sum of effects of these 20 compounds indivic'Luellement taken.
Indeed, surprisingly, the Applicant has found that the silicon acts as a catalyst in the manufacture of chondroitin novo by the treated organism.

It appears that this action 25 catalyst silicon SENSIB =. Ement increased when administered in combination with chondroitin molecular weight = _Lnf than or equal to 25000 g / mol, particularly with chondroitin molecular weight : equal to or Lnfrieur 30 20000 g / mol.

Advantageously, the invention is effectively used as a medicament for:. Th treatment of osteoarthritis, and in particular the treatment of cartilage degeneration, to slow or stop the formation of cracks on cartilage, erosion of cartilage, loss of cartilage, bone formation excroissanc = _s on the joint, bone and joint pain and difficulty performing movements reshuffle.

Advantageously, the compositions according to the invention are used to allow and stimulate the production of cartilage and réparaticn.

Advantageously, the invention is effectively used as a medicament for the prevention of osteoarthritis, and in particular to reduce the incidence of osteoarthritis and its characteristic symptoms, decrease the intensity of the symptoms and reduce the age of onset of first symptônes.

Compositions of the Invention are intended for administration topically From transcutaneous, perlingual or enteral or oral, parenteral, anal, nasal, ocular or oral route is preferred.

Furthermore the combination according to the invention, the compositions may comprise other suitable compounds to optimize their effectiveness.

These other compounds may be selected from the glucosanine the méthylesulfonyl-methane (MSM), the curcuminDïdes, piperine, bromelain, iridoids, gingerols, prodelphinidins, boswellic acid and its salts, salicylic acid and its salts and esters, or extracts Vegete. ux rich in these components alone or in combination.

The compositions comprise a pharmaceutically cutre oi cosmetically or nutritionally acceptable, that is to say a vehicle suitable for use in contact with human cellLles and animals without excessive toxicity, irritation, undue allergic response, and the like and proportionate to a benefit / risk ratio.

The compositions of the present invention may be in any pharmaceutical form normally used for adminis: ration orally, especially in anhydrous form (preferably the tablet, capsule, powder or lyophilized or liquid form , preferably in the form of oral solution.

The compositions of the present invention are preferably anhydrous.

For example, a capsule according to invention may be formulated from a mix of turmeric extract, glucosamine and maltodextrin wherein the silicon is added and chondroitin according to the invention.

For example, an oral solution of the invention can be prepared by dissolving silicon and chondroitin according to the invention in water or an aqueous composition.

A preferred method of preparing an oral solution according to the invention comprises me process step that is solubilized in water with gentle agitation, the following compounds: preservatives such as potassium sorbate and sodium benzoate, and . an antioxidant such as ascDrbique acid; and a step that is added with vigorous stirring, and then hydrolyzed chondroitin eg tréLit nettle titrated silicon.
Examples of compositions according to the invention 30, there may be mentioned: 1 / capsule comprising (percent by weight of the total composition) - 40% glucosamine; - 8% extract of turmeric; - 1.7% maltodextrin; - 0.3% of silicon complexed with sugars from nettle; - 50% 16 000 g of chondroitin / Raol.

A process for preparing such capsules includes a step in which the ingredients introduced oz powder form in a mixer, in order of increasing weight; and one step in which the mixture of these ingredients is homogenized under moderate agitation. 2 / a drinkable solution comprising (in percentage by weight of the total composition): - 0.6% ascorbic acid; - 0.1% of sodium benzoate; - 0.1% potassium sorbate; - 0.02% silicon complexed with sugars from nettle; - 10% aqueous extract of turmeric; - 1% chondroitin hydrolyzed 1E 000 g / mol; - Qs 100% RO water.
Of course, the invention is not limited to the embodiments described above and those skilled in the art can, by routine, adapt these embodiments.