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Preparation and application of chelating multi-metal chitosan selenite

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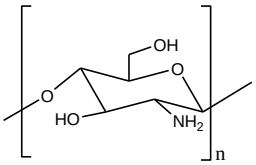
Abstract. [Objective] The aim is to introduce a production processes for the preparation of chelating multi-metal chitosan selenite and its different dosage forms, which is suitable for the fields of food and feed chemistry, fertilizer chemistry or fine chemical technology, and also belongs to the field of biomedical engineering technology. [Method] Disperse chitosan in water, and stir it in water bath at 50-70°C under heating. Add trace elements and rare earth metal salts with matching ratio to make them dissolve, where ratio of chitosan and metal salts is 1:0.1-0.3 (W/W). After 2-5 hours of stirring, chitosan chelated by multi-metal can be obtained. Chelating multi-metal chitosan selenite can be made after adding selenium oxide aqueous solution. [Result] The same molecules of target products produced according to the process contain a variety of metal elements and selenium elements and have biological synergistic effect with its biological solubility and biological activity significantly improved. [Conclusion] Chelating multi-metal chitosan selenite presented in this paper is a kind of trace element and/or rare earth preparation, which is environmentally friendly, easy to absorb and rich in selenium without toxic side effects. Therefore, it could be used as a new preparation to supplement selenium and trace elements in medicine, health care products, cosmetics and agricultural fields.

1. Introduction

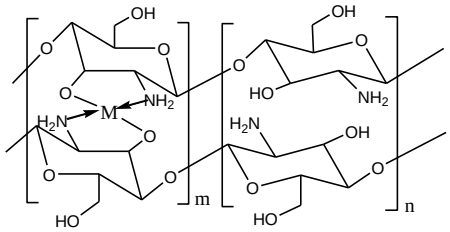
With the improvement of life quality, more attention was attracted by the relationship between trace elements and health. Selenium is more prominent and enjoys reputation of “Fire of Life”, “Guard Angle of Heart” and “King of Anti-cancer”. Medical studies have shown that low selenium intake is associated with heart and cellular diseases and with high incidence of breast, thyroid, prostate, lung, and colon cancers [1]. WHO stipulated that 50-250 micrograms of selenium should be contained in daily diet of human beings. Unfortunately, China is a big country lacking selenium, and daily selenium intake of most people per day is only about 50 micrograms [2]. Inorganic selenium has the advantages of high selenium content and low price, but absorption and utilization rate of inorganic selenium (such as Na_2SeO_3) is not ideal with low bioavailability, heavy toxicity. Besides, there is a very small gap between the poisoning amount and requisite amount; therefore, its use should be strictly limited.

Molecular structure of chitosan [3] [shortened as CTS, molecular formula as $(C_6H_{11}O_4N)_n$, also named as chitosan, scientific name as β(1,4)-2-amino-2-deoxy-D-glucose] is as follows:



Chitosan, which has no damage to the environment, people and livestock, is able to regulate plant growth, induce plant disease resistance and improve yield and quality [4-5]. Therefore, it is generally accepted that chitosan will become the leading product for the development of green agriculture in 21st century.

Chitosan molecules contain a large amount of hydroxyl and amino groups, which are good ligands to metal ions. Many researches showed that chitosan chelating metal could significantly enhance antioxidant and antibacterial activities of chitosan, and improve biocompatibility of metal ions and the absorption and utilization rate *in vivo* [4]. It has potential application prospects in industry, agriculture, medicine, food and so on. The two parallel molecules interact with each other through chelating between chitosan and metal at 2-NH_2 and 3-OH of glucose monomer. Chitosan, as a macromolecular compound, can have as high as tens of thousands or even hundreds of thousands of molecules and many glucose monomers that can connect many metal ions [6].



If we add non-monolithic metal ions, chitosan chelated by non-monolithic metal ion could be obtained[5-12]. Chitosan chelated by non-monolithic metal ions is different from compound chelated by the monolithic metal ions. However, a variety of metals exist in the same molecule; with their synergistic effects, its antioxidant & antibacterial activity, the biological compatibility of metal ions and *in vivo* rate of utilization and absorption will be greatly improved. 2-NH_2 on the glucose monomers not coordinated in the chitosan molecules and seleninic acid are combined to produce salt, which can improve the effective absorption of selenium, increase its antioxidant and anti-tumor activity, and will not produce toxicity of inorganic selenium [13-16]. Therefore, it can be used as a new kind of selenium supplement in the fields of medicine, health care, cosmetics and agriculture, etc.

This paper aims to provide a method for preparing a chelating multi-metal chitosan selenite and different dosage forms. The chelating multi-metal chitosan selenite is an environmentally friendly preparation, free of toxic and side effects, easy to absorb, rich in selenium, trace elements and/or rare earth [13]: chitosan selenite chelated by trace element can be used as a new class of preparation supplementing selenium and trace elements in the field of medicine, health products, cosmetics and agriculture[8]. For chelated chitosan selenite containing rare earth and trace elements, enzyme activity of rare earth can be used as feed additives to significant gain weight of fish, shrimp and poultry and can improve the animal's anti-bacterial and anti-virus ability [7]. High concentration of multi-element liquid fertilizer prepared by chelated chitosan selenite containing rare earth and trace elements can enhance the vitality of animals and plants with significant effect of fertilizer.

2. Materials and methods

The production processes for the preparation of chelating multi-metal chitosan selenite and its different dosage forms is as follows:

Disperse chitosan in water, stir it in water bath at 50-70 ^{o}C under heating. Add trace elements and rare earth metal salts with matching ratio to make them dissolve, where ratio of chitosan and metal

salts is 1:0.1-0.3 (W/W). After 2-5 hours of stirring, chitosan chelated by multi-metal can be obtained. chelating multi-metal chitosan selenite can be made after adding selenium oxide aqueous solution.

Chitosan (Degree of deacetylation 99%) was provided by Shanghai Zhanyun Chemical Co., Ltd. (Shanghai, China). Another chemical reagent (AR) was provided by Sinopharm chemical reagent Co., Ltd. (Shanghai, China).

*2.1. Preparation of chelating multi-metal chitosan **1a***

Firstly, the chitosan (10g) is dispersed in water (1000mL) and stirred at 50-70℃. 3g salts, including $CaCl_2$, $FeCl_2$ and $ZnCl_2$ with appropriate ratio that is 10: 1: 1 (W/W), are added to the solution of the chitosan. Centrifugation is conducted after stirring for 2-5 hours. The supernatant is discarded to obtain chitosan crude chelated by multi-metal. The crude product is washed with distilled water until the supernatant is colorless. The pure product is obtained after being dried.

The product of chelating multi-metal chitosan **1a**, which is easily absorbed and metabolized, can be used in medicine, health products, cosmetics and agriculture and other fields. Synergistic absorption of three ions of calcium, iron and zinc in the same molecule is better than the absorption effect of single ion preparation, and a variety of ions do not affect absorption due to mutual restraint because it is a simple compound preparation.

*2.2. Preparation of chelating multi-metal chitosan selenite **2a***

Chelating multi-metal chitosan **1a** is added to an aqueous solution of seleninic acid, wherein the ratio of chitosan chelated by calcium iron zinc to seleninic acid is 1:0.01-0.1 (W/W). The aqueous solution of chelating multi-metal chitosan selenite is obtained after stirred for 1.5-3, and the mass concentration is 10-45%. Ethanol is added to precipitate, filter and wash with ethanol, then dry to obtain the pure product of chelating multi-metal chitosan selenite **2a**.

*2.3. Preparation of chelating multi-metal chitosan selenite **2b***

The steps are the same as point 2.1, while salts including calcium iron zinc are replaced with salts of calcium magnesium iron zinc manganese rare earth. It consists of $CaCl_2$, $MgCl_2$, $ZnCl_2$, $FeCl_2$, $MnCl_2$ and rare earth chlorides including $LaCl_3$ and $CeCl_3$, whose mass ratio is 10: 2: 1: 1: 0.1: 0.1 (W/W). The other steps are the same, so chelating multi-metal chitosan **1b** is obtained. The steps are the same as in point 2.2, the pure product of chelating multi-metal chitosan selenite **2b** is obtained.

*2.4. Preparation of water quality suspension agent of chelating multi-metal chitosan selenite **2b***

The pure product of chelating multi-metal chitosan selenite **2b** is dispersed in water to obtain an aqueous solution of 10-45%. Xanthan gum and diatomaceous are added in proportion, of which the ratio of chitosan selenite chelated by calcium, magnesium, iron, zinc, manganese and rare earth, xanthan gum and diatomaceous earth is 10: 1: 1 (W/W). The water quality suspension agent of chitosan selenite chelated by calcium, magnesium, iron, zinc, manganese and rare earth is obtained after being stirred for 2 hours.

3. Results and discussion

Chelating multi-metal chitosan selenite **2b** can be used as raw material of feed additive. The effects of chelating multi-metal chitosan selenite **2b** as feed additive are revealed in Table 1. The synergistic effect is significant. It supplements trace elements, rare earth and selenium elements, improves animal antibacterial and anti-viral capacity and selenium content of animal products; the enzymatic activity of rare earth can significantly gain weight of fish, shrimp and poultry.

Table 1. The effects of chelating multi-metal chitosan selenite **2b** as feed additive

No.	area of shrimp pond	Mass concentration in fodder	Effects

1	10 acres of land	0	Gaining weight commonly and containing selenium generally.
2	10 acres of land	1.0 %[a]	Gaining weight quickly, and containing slightly more selenium[b].

[a]. Mixing the feedstuff and chelating multi-metal chitosan selenite **2b** by mass ratio; [b]. Just compare with example 1.

The function mechanism is as follows [5, 7]:

1. Promote endogenous growth hormone (GH), thyroxine (T4), insulin (ISN) synthesis and release in animal body.
2. Activate growth factors in animal body; promote calcium influx; activate calcium channels.
3. Improve activity of intestinal protease, lipase and amylase; promote nutrient digestion and absorption in animal body.
4. Enhance the activity of superoxide dismutase (SOD); inhibit and remove free radicals in animals; prevent and inhibit cancers.
5. Significantly increase the natural killer vitality of immune cell and cell vitality; prevent coagulation and promote tissue repair; promote harmful toxins excreted from the liver and intestinal canal.
6. Chitosan chelated by calcium, magnesium, iron, zinc, manganese and rare earth in the chitosan selenite chelated by calcium, magnesium, iron, zinc, manganese and rare earth is positively charged. It can combine with negative bacteria, inhibit pathogenic bacteria and promote the growth of beneficial bacterium such as bifidobacterium and lactobacillus. It has the effect of class antibiotics without pollution and monomer.
7. Regulate cholesterol metabolism; reduce lipid deposition; improve animal product quality; improve product quality.
8. Chelate heavy metal ions; purify water quality.

In conclusion, chelating multi-metal chitosan selenite presented in this paper is a kind of trace element and/or rare earth preparation. It is environmentally friendly, easy to absorb and rich in selenium without toxic side effects. Therefore; it can be used as a new preparation to supplement selenium and trace elements to be used in medicine, health care products, cosmetics and agricultural fields.

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