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Complexation and Antimicrobial activities of β sitosterol with trace metals. (Cu (II), Co (II), and Fe (III))

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Abstract:

The purpose of the present work is to develop a plant-based method to minimize the toxicity caused by high levels of some essential trace elements in the body. The present work is related to investigating the complexation and antimicrobial activities of β-sitosterol with trace elements (Cu (II), Co (II), and Fe (III)). The β-sitosterol chelating with above metals to form a complex.The complex formation has been determined by Potentiometric titrations. The metals after the formation of complexes unable to absorb in the body and then excreted out from the body in the form of complex [9]. β-sitosterol is the most common plant sterol (phytosterol) and by eating vegetables toxic metal may be eliminated out from the body. Antimicrobial result indicates that all the complexes have potential to kill some of highly pathogenic bacterial and fungal species that has a critical role in number of cutaneous, pyogenic and urinary tract infection. The complex could be a good therapeutic candidate for the sake of treatment if fully explored from all parameters.

Key words: Phytosterol, ß-sitosterol, Trace element, Chelation, Antimicrobial

Introduction

The most common plant sterol is The ß-sitosterol and is structurally similar to cholesterol [Oja 2009; Matsuoka et al. 2008; Law 2000]. B-sitosterol is a 4-dismethyl sterol (containing no methyl group at C-4) it has a double bond at C-5 and hydroxyl group at C-3 [Plat, Kerckhoffs, and Mensink 2004]. Scientific research has proven that ß-sitosterol is a safe and nontoxic plant nutrient for maintaining health and for protection against many serious health disorders and diseases. ß-sitosterol is used as a cholesterol lowering agent through the introduction of phytosterol-fortified margarine. Scientific research has shown that patients on diets devoid of plant sterols quickly became free of ß-sitosterol [Oja 2009] which implies that the nutrients should be taken daily for the most excellent functioning of the immune system and health generally [Matsuoka et al. 2008]. ß-sitosterol posse's antihyperglycemic, antidiabetic, antibacterial, and antimicrobial, anti inflammatory and antipyretic acridity. It is also used to improve blood parameters beneficial for the uterus, anti cancers [Normen et al. 2001].

In humans and other mammals, 23 elements have known for physiological activities. From these elements eleven (Fe, Cr, Cu, V, Co, Zn, Mn, Se, Ni, Sn and Mo) can be classified as "trace elements" (TE) because of their essentiality and very limited quantity in humans [Murray et al. 2009]. TE is essential components of biological structures, but at the same time they can be toxic at concentrations beyond those necessary for their biological function. For example, iron (Fe) toxicity can damage the intestinal lining and may cause abnormalities in body pH, shock and liver failure [Fraga 2005].

Excessive copper (Cu) intake may cause headache, nausea, abdominal pain and diarrhea. Acute copper toxicity may result in heart problems, liver damage, jaundice, kidney failure, coma and death [Mudgal et al. 2010]. Large doses of cobalt (Co) might stimulate the thyroid and bone marrow fraction [Fraga 2005]. Metal toxicity is more severe than organic toxicity because metals retain their identity in the body; whereas organic compounds may decompose.

The most common technique which is used to treat heavy metal toxicity is chelation therapy. It involves administration of chelating agents to remove heavy metals from the body [Marsha 1996].

In the present paper we are reporting the complication of ß-sitosterol with TE such as Cu, Co and Fe. The complex formation has been determined by Potentiometric titration. Along with this we are also presenting the antibacterial and antifungal activities of these complexes.

Material and Method

Reagent and glassware: The entire reagents used were of analytical grade purchase from Merck and Bio Basic Inc. All glassware used was of standard quality. They were properly cleaned and rinsed with distilled deionized water and finally dried in oven before used. For potentiometric study salts of ferric chloride, cobalt nitrate and copper nitrate were used.

Instrumentation

Electrical balance: Denver Instrument, TP- 214 was used for weighing.

pH meter: Jenway, model 3510 was used for pH metric titration.

Stirrer: Hot plate stirrer (lab Tech) with bead was used for stirring.

Potentiometric titration: All Potentiometric titration was done at 25 ± 5^0C. Sodium hydroxide solution was standardized using standard oxalic acid every time before titration of sample solution. All Potentiometric titration was carried out in the conical flask cover with rubber stopper. The rubber stopper has four holes, one for burette for the addition of standard base, and another for purging inert gas (Nitrogen), third hole for removal of oxygen and fourth for glass electrode. The solution completely inert the atmosphere by passing Nitrogen gas for 30 minutes.

Potentiometric titration of ligand (β-sitosterol): For this purpose, 40 mL of ß-sitosterol solution (10^{-4}) and 10mL chloroform were taken in a conical flask containing magnetic bead. Purified nitrogen gas was purged in the solution for half

an hour. Then the ß-sitosterol solution was titrated against 0.1 M standard NaOH solution. Sodium hydroxide solution was prepared in methanol. Sodium hydroxide solution was standardized using 0.05M oxalic acid solution prior to the pH metric titrationof ß-sitosterol.

During titration regular stirring was maintained by means of magnetic stirrer. Standard NaOH was added in sufficiently small increments of 0.1 ml with the help of the burette and after each increment pH of the reaction mixture was recorded till pH was not affected by further addition of standard NaOH.pH values were plotted against the added volume of standard NaOH.

Potentiometric titration of metal{Fe (III) Co (II)and Cu (II)} withβ-sitosterol:

In order to obtain metal- ligand complex Potentiometric titration of metal (Fe,Co,Cu) with ß-sitosterol were performed by taking 40mL ß-sitosterol solution (10^{-2}M) prepared in methanol: chloroform(1:1) and 10mL of metalsolution (10^{-2}M) prepared in methanol to give 1:4 metal ligand solutions. The mixture obtained was subjected to titration by using NaOH solution (0.1M) as a standard and under the same condition as used for previously mention titration. The complex formation was confirmed by a change in color at different pH.

Biological Assay

Preparation of media for antimicrobial activity:

Muller Hinton agar and Muller Hinton broth [10] was used as the media for culturing bacterial strains and Sabourd dextrose agar (SDA) [Smyth et al.] was used as the media for fungal strains.

Screening of antibacterial activity:

Antibacterial activity was determined by using the agar-well method. The Autoclaved Muller Hinton broth was used to refresh the bacterial culture, later well were punched into Muller Hinton Agar and 10 microliters of culture were poured into the wells [Perez and Bazerque 2009]. All plates were incubated at 28±2oC for 24-48 h and after the incubation diameter of zone of inhibition was noted by Vernier caliper. Gentamicin antibiotic was used as a standard.

Screening of antifungal activity:

Antifungal activity was determined by using the agar-well method. Autoclaved distilled water was used for the preparation of fungal spore suspension and transfer aseptically into each SDA plates [Wuthi-udomlert and Vallisuta 2011]. All plates were incubated at 28±2oC for 24-48h and after the incubation diameter of zone of inhibition was measured by vernier caliper. Gresiofulvin antifungal agent was used as a standard.

Result and discussion

From the present study it is revealed that the ß-sitosterol could be helpful in removing toxicity of heavy metals from the body. The ß- sitosterol binds trace elements that are present in toxic concentration so they can be excreted usually in urine from the body [Marsha 1996]. Initially ß- sitosterol titration was performed as a reference. In a plot of pH against the added volume of NaOH only one curve was observed near pH 12.2. Remarkable changes in titration curves of ß- sitosterol and its complexes were observed (Fig.1), which indicates Complexation between metals and the ß- sitosterol. Complexation of ß- sitosterol with Cu (11) was confirmed by a change in color from Blue to green at pH 5.21.In plot a slight twist was observed at 5.21, which may be due to the formation of metal-ligand complex. In case of Co (11) complex two curves were observed. First one near pH 6.54 which may be due to the formation of the complex and it was further confirmed by a change in color from Pink to Peach at pH 6.54., whereas the second curve indicates the neutralization of excess ß- sitosterol by NaOH. Titration curves of Fe (111) show two cures in the plot, first near pH 3.6, whereas the second near 11.81. Complexation of ß- sitosterol with Fe was further confirmed by a change in color from yellow to brown at pH 11.81.

It was concluded that out of all metals Fe(111) formed complex at low pH. Cu (11) formed complex at moderate pH, whereas Co(11) formed complex at relatively high pH. As far as stability of these complexes is concerned Fe (111) complex seems more stable, Co(11) complex has moderate stability, whereas Cu (11) complex has relatively lower stability and

these complexes are effective against antifungal and antibacterial infection. The results of antibacterial and antifungal activity are presented in Table 1.

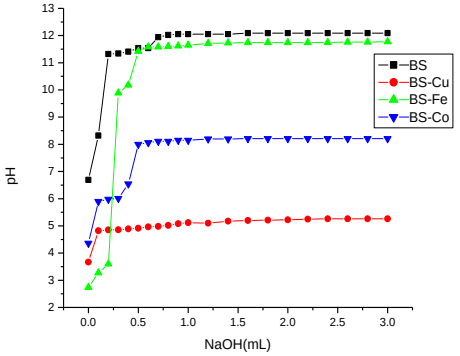

Fig 1 βs = β- Sitosterol, βs-Cu = Complex of β- Sitosterol with copper, βs-Fe = Complex of β- Sitosterol with iron, βs-Co = Complex of β- Sitosterol with cobalt.

Gram positive bacteria	Zone of inhibition in mm (mean± S.D)				Fungal isolates	Zone of inhibition in mm (mean± S.D)			
	Cu-βs	Fe-βs	Co-βs	Standard Gentamicin		Cu-βs	Fe-βs	Co-βs	Standard Gresiofulvin
Bacillus cereus	14 ± 0.6	-	-	>15	*Aspergillus flavus*	15 ± 0.6	20 ± 0.6	-	>12
Bacillus subtilis	15 ± 0.6	-	-	>15	*Aspergillus Niger*	17 ± 0.6	18 ± 0.1	-	>12
Bacillus thruingiensis	15 ± 0	-	-	>15	*Trichophyton rubrum*	-	-	14 ± 1.2	>12
Gram negative bacteria					*Trichophyton mentagrophytes*	-	-	13 ± 1.5	>12
Proteus mirabilis	-	19 ± 1.0	-	>15	*Trichophyton tonsurans*	-	-	16 ± 0	>12
Pseudomonas aeroginosa	-	12 ± 2.1	-	>15					
Pseudomonas aeroginosa ATCC	-	15 ± 1.0	-	>15					

Table1: Antibacterial and Antifungal Activity of complexes of β-sitosterol
Values are zones of inhibition (mm and an average of triplicate. Cu-βs = Complex of β- Sitosterol with copper. Fe-βs = Complex of β-Sitosterol with iron.Co-βs = Complex of β- Sitosterol with Cobalt.

Conclusion

From the above observation it is concluded that change in color of reaction mixture confirmed the formation of the complex and their antimicrobial activity shows that these complexes are effective against antifungal and antibacterial infection. Simple deposition of toxic metals may also increase fungal, bacterial and viral infections that are not easy to destroy until this cause is removed. Hence ß- sitosterol is present in most vegetables; by eating vegetables the above essential metal toxicity could be beneficial without any side effects.

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