

Mg(SeO₃H)₂.4H₂O and Cu(SeO₃H)₂.2H₂O: Potentially Useful Compounds for Selenium Supplementation

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SUMMARY. The title hydrogen selenites were obtained and characterized by X-ray powder diffractometry and infrared spectroscopy. Their thermal behaviour was investigated by means of TG and DTA measurements. Several dissolution tests were also performed. The results support the potential usefulness of these salts for selenium supplementation in human and veterinary medicine and to the simultaneous reinforcement of Mg(II) and Cu(II) levels.

RESUMEN. "Mg(SeO₃H)₂.4H₂O y Cu(SeO₃H)₂.2H₂O: Compuestos Potencialmente Útiles para la Suplementación de Selenio". Los selenitos ácidos del título fueron obtenidos y caracterizados por difracción de rayos X de polvos y espectroscopía de infrarrojo. Su comportamiento térmico fue investigado a través de medidas TG y ADT. Asimismo, se realizaron diversos ensayos de disolución. Los resultados apoyan la potencial utilidad de ambas sales para la suplementación de selenio en medicina humana y veterinaria, así como para reforzar simultáneamente los niveles de Mg(II) y Cu(II).

INTRODUCTION

Supplementation of essential trace or micro-trace elements is an area of increasing interest in the field of human and veterinary pharmacology. In particular, selenium supplementation is a problem of wide and strong economic and clinical impact due to the fact that a series of well-known metabolic disorders and diseases found in both humans and animals are clearly related to selenium deficiencies¹⁻⁴.

Several different approaches have been used in an attempt to estimate human selenium requirements, including extrapolation from animal experiments, determination of the amounts needed to maintain metabolic balance, analysis of dietary intakes and measurement of functional responses during depletion-repletion studies. An estimate amount of 50-80 µg/day seems to be a reasonable figure^{3,4}, with the additional

comment that women usually requires lower doses, probably due to differences in body size between men and women³.

Regarding the selenium requirements for animals, depending on the species, the age of the animal and the criterion on deficiency selected, doses between 0.10 and 0.20 mg/Kg are probably reasonable estimates^{3,4}.

Different selenium compounds have been proposed and are currently used for supplementation of the element. They include simple inorganic species such as SeO₂, Na₂SeO₃ or Na₂SeO₄ or organic compounds such as selenomethionine and selenocystine, as well as certain yeasts incubated in selenium rich media^{1, 3-5}. In the case of animals, administration of selenium-containing heavy pellets (composed, for example, by finely divided metallic iron and up to 5% of elemental selenium) and treatment of soils with

KEY WORDS: Hydrogen-selenites, IR spectra, Thermal Behaviour, Dissolution Assays, Selenium Supplementation.

PALABRAS CLAVE: Selenitos Ácidos, Espectros IR, Comportamiento Térmico, Ensayos de Disolución, Suplementación de Selenio.

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selenium compounds have also been assayed^{3,4}.

As part of our current studies devoted to development of new forms of supplementation of essential trace elements^{6,8}, we are investigating new routes for the case of selenium. In this paper we make a new interesting proposition in this direction which may be also useful for the simultaneous supplementation of a second trace element together with selenium.

Our proposal is based on the use of hydrated divalent metal acid selenites, which can be easily prepared in a very pure form, adequate for its subsequent pharmacological use.

EXPERIMENTAL

$Mg(SeO_3H)_2 \cdot 4H_2O$ was obtained by reaction of 100 ml of a 2M aqueous solution of H_2SeO_3 (Fluka) with powdered MgO (Merck) added in slight defect over the stoichiometric calculated quantity. The resulting solution is shaken vigorously after it becomes clear, filtering if necessary. The limpid solution is left to evaporate freely at room temperature. After one week, well-formed transparent crystals are deposited. They were filtered off, washed with a little amount of cold ethanol and finally dried in air at room temperature. They were characterized by X-ray powder diffractometry as discussed in the next section.

$Cu(SeO_3H)_2 \cdot 2H_2O$ was prepared by reaction between aqueous solutions of copper nitrate and selenous acid, and acidifying the resulting mixture with HNO_3 up to pH = 1.5, as described earlier⁹. The blue crystals, which precipitate after a few hours, were filtered, washed, dried and characterized in the same way as the magnesium salt.

X-ray powder diagrams were obtained with a Rigaku Miniflex CN 2005 diffractometer using Ni-filtered $Co-K\alpha$ radiation. The instrument was calibrated in the usual way, using some of the characteristic reflections of α -quartz, Al and NaCl.

The FTIR spectra of the salts were recorded on a Bruker IF66 spectrophotometer in the 4000-250 cm^{-1} range, using the KBr pellet technique.

Thermogravimetric (TG) and differential thermal analysis (DTA) were performed with a Shimadzu thermoanalytical system (TGA-50 and DTA-50 models). Measurements were done under a constant oxygen flow (50 ml/min) and at a heating rate of 10 $^{\circ}C/min$, using Pt-crucibles. Al_2O_3 was used as a DTA standard.

Dissolution tests were performed following the recommendations and procedures of the *United States Pharmacopoeia* (XXII. Edition)¹⁰. The simulated gastric juice was obtained using NaCl/pepsine/HCl mixtures (final pH = 1.4)¹⁰.

RESULTS AND DISCUSSION

Crystal structures

Both compounds were characterized by X-ray powder diffractometry. The obtained powder diagrams were compared with the theoretical diagrams generated by the program POWDERCELL¹¹ on the basis of the known single crystal data^{9,12}. The salts are not isostructural, but present closely related structures. Our structural analysis confirmed that $Mg(SeO_3H)_2 \cdot 4H_2O$ crystallizes in the monoclinic C2/c space group with $Z = 4$ as reported by Engelen *et al.*¹² (a different space group (I2/a) was determined in another crystallographic study)¹³. For $Cu(SeO_3H)_2 \cdot 2H_2O$ we also confirmed the previously reported structure (space group $P2_1/c$ and $Z = 2$)⁹.

In both compounds the divalent metal cation presents a distorted MO_6 - octahedral environment constituted by four coplanar oxygen atoms of the selenite moieties and two oxygen atoms of two water molecules located in trans-positions. These building blocks are bridged to other similar units by the selenite oxygens, generating an extended sheet structure, further stabilized by multiple hydrogen bondings, involving the OH-groups attached to selenium, the cation bonded water molecules and, in the case of the Mg(II) compound, also the free crystal water molecules.

Infrared spectra

In order to have an easy characterization procedure for these compounds, we have recorded its IR spectra, and proposed an assignment of them, on the basis of the classical papers of Simon and Paetzold^{14,15} and some other general references^{16,17}, and compatible with the commented structural characteristics.

The FTIR spectrum of $Mg(SeO_3H)_2 \cdot 4H_2O$ is shown in Figure 1. That of the copper salt is similar. The assignment proposed for both salts is shown in Table 1 and briefly commented, as follows:

a) One of the O-H stretching bands of the water molecules appears as very broad absorption, suggesting the overlapping of different components. The position of these bands, points to the existence of a variety of H-bonds of medium

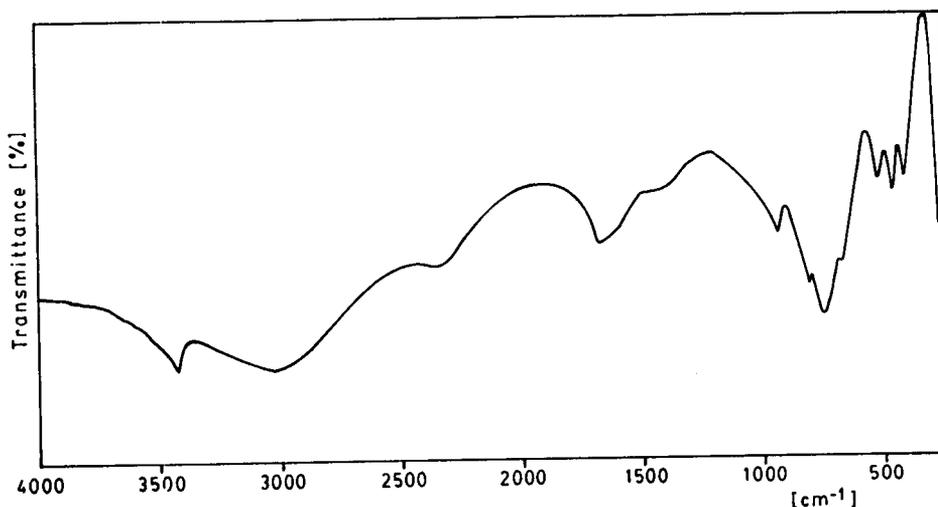


Figure 1. FT Infrared spectrum of $\text{Mg}(\text{SeO}_3\text{H})_2 \cdot 4\text{H}_2\text{O}$.

| $\text{Mg}(\text{SeO}_3\text{H})_2 \cdot 4\text{H}_2\text{O}$ | $\text{Cu}(\text{SeO}_3\text{H})_2 \cdot 2\text{H}_2\text{O}$ | Assignment |
|---|---|--|
| 3425 s | 3497 vs/3184 s | $\nu(\text{O-H}) (\text{H}_2\text{O})$ |
| 3047 s,br | 2870 vs, br | |
| 2336 m | 2368 m | $\nu(\text{O-H}) (\text{Se-OH})$ |
| 1666 m/1604 sh | 1650m/1552 m | $\delta(\text{H}_2\text{O})$ |
| | 1180 vw | $\delta(\text{Se-OH})$ |
| 933 w | 933 m | $\gamma(\text{OH})$ |
| 808 w | 776 m | $\nu_s(\text{SeO}_2)$ |
| 739 vs | 713 vs | $\nu_{as}(\text{SeO}_2)$ |
| 656 w | 564 s | $\nu(\text{Se-OH})$ |
| 515 m | | $\rho(\text{H}_2\text{O}) (?)$ |
| 457 m | 458m/431m | $\delta_s(\text{SeO}_2)$, see text |
| 413 m | 391 w | $\delta_{as}(\text{SeO}_2)$ |
| | 348 s | $\rho(\text{H}_2\text{O}) (?)$ |
| 265 vs | 270 vs | $\delta_s(\text{SeO}_2)$ |

Table 1. Assignment of the IR spectra of the two investigated compounds (band positions in cm^{-1}); vs: very strong; s: strong; m: medium; w: weak; vw: very weak; sh: shoulder; br: broad.

strength¹⁶, in agreement with the structural data^{9,12}. The $\delta(\text{H}_2\text{O})$ vibration appears with a doublet structure, also in agreement with the involvement of the water molecules in different types of hydrogen bonding^{9,12}.

b) Also the $\nu(\text{O-H})$ band related to the Se-OH group appears somewhat broadened. This is in agreement with the behaviour of the hydroxyl groups of other acid salts like hydrogen phosphates, arsenates, sulfates and selenates, which IR spectra often exhibit a multiple band structure for this vibration^{12,18}. These bands

usually can be clearly seen in the low temperature IR spectra.

c) The in-plane $\delta(\text{Se-OH})$ vibration is seen only as a very weak band in the case of the Cu(II) salt whereas it could not be identified in the Mg(II) compound. This band often presents great intensity differences in different compounds of the same type¹⁸. On the contrary, the out-of-plane deformation of this group could be clearly identified, and at the same position, in both compounds.

d) Two librational modes of the water mole-

cules, one in each salt, are only tentatively assigned. One of the components of the 458/431 cm^{-1} doublet in the copper compound eventually belongs also to a vibrational mode of this type.

e) The three assigned deformational modes, although lying in the usual ranges, are not identified with certainty. In this spectral range also metal-to-ligand vibrations can be expected ¹⁹.

Thermal behaviour

The TG and DTA traces of $\text{Mg}(\text{SeO}_3\text{H})_2 \cdot 4\text{H}_2\text{O}$ are shown in Figure 2. As it can be seen, the thermolysis occurs in four well-defined steps. The thermal decomposition is expected to occur through the following events, with the theoretically expected mass losses given within brackets:

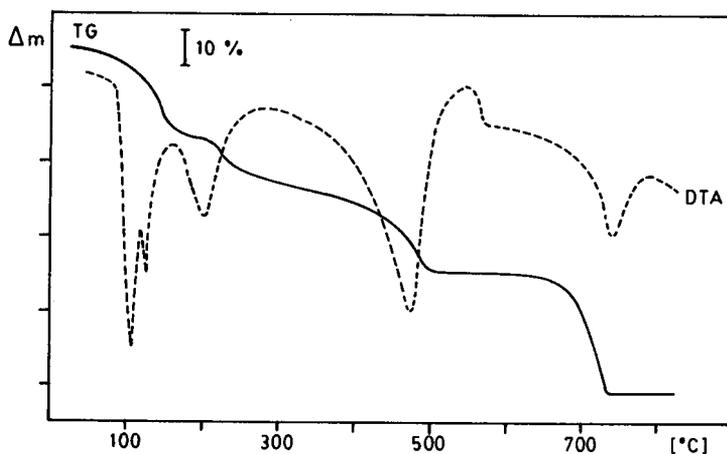
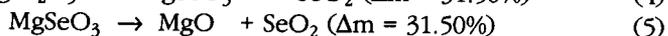
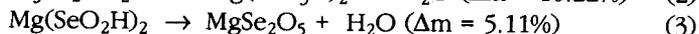
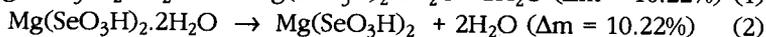
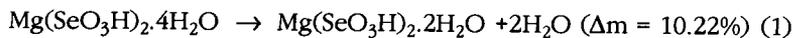


Figure 2. Thermogram of $\text{Mg}(\text{SeO}_3\text{H})_2 \cdot 4\text{H}_2\text{O}$.

For the first three steps experimental weight losses of 13.05, 9.04 and 35.00% were found. The total found loss (57.09%) agrees with the summation of the theoretical values of steps 1-4 (57.05%), suggesting that one or more of the predicted steps may be superimposed. This presumption is confirmed by the DTA behaviour, which shows a first endothermic doublet at 107/127 °C surely related with steps (1) and (2) followed by another endothermic peak located at 203 °C, related with the third step. The broader peak centered at 478 °C can be related with process (4). This means that the two crystal water molecules together with the two molecules linked to the cation are given off consecutively without a clear separation, covering the first two observed TG steps and partially the beginning of the third one. The intramolecularly generated water and the SeO_2 loss (theoretical steps 3 and 4) also occur on this third part of the thermogram. The last experimental weight loss (31.50%) coincides exactly with the theoretical expected value, and is related to the last endothermic DTA-peak at 736 °C.

The DTA trace shows another weak endothermic shoulder at about 570 °C, which may be probably related to a phase transition of the anhydrous MgSeO_3 , which is stable between 500 and 650 °C.

The total observed mass loss (88.59%) is in perfect agreement with the theoretical value (88.55%), and the generation of MgO as the final solid residue was confirmed by means of IR spectroscopy.

The thermolysis of the copper salt has been previously investigated ⁹. It shows a similar but not identical behaviour. In this case, five steps are evident. The coordinated water is given off between 85 and 95 °C, and the generation of CuSe_2O_5 occurs above 200 °C. This behaviour supports the analysis made above for the $\text{Mg}(\text{II})$ compound: if coordinated water is lost at relatively low temperatures it can be given off in the same range as the coordinated water molecules and these processes can be partially superimposed with the generation of the intramolecular water molecule. Interestingly, in this case the degradation of CuSe_2O_5 occurs in

three steps, generating first CuSeO_3 and then $\text{Cu}_2\text{O}(\text{SeO}_3)$, which finally degrades to CuO between 550 and 600 °C.

Finally, it should be mentioned that the preparation and structural characterization of $\text{Mg}(\text{HSeO}_3)_2$ has been recently reported ²⁰, but the generation of this anhydrous form could not be established from our TG/DTA study. This means that the hydrated salt cannot be used as a precursor for the synthesis of this anhydrous modification.

Dissolution assays

They were performed in simulated gastric juice and in 0.1 N HCl in order to verify the solubility and the speed of dissolution of both compounds. To supply the lower-limit selenium dose to a normal human adult (50 µg/day), 0.12 mg of any one of the two compounds must be given. In order to avoid errors, during the assays a tenfold quantity of this dose was used.

A sample containing 1.2 mg of each compound was introduced into 250 ml of the simulated gastric juice and slowly stirred. After 2 min a totally clear solution was observed in the case of the magnesium compound whereas for the copper salt, 20 min were necessary. These assays were duplicated, with the same results. This test confirms the dissolution of the compound and the release of selenite, magnesium and copper into the gastric juice.

The same sample quantities as above were introduced into 50 ml of 0.1 N HCl, maintained at 37 °C and stirred at a constant speed of 60

rpm. The dissolution times, obtained from two independent experiments for each compound, were similar to those obtained above, i.e., 2 min for $\text{Mg}(\text{HSeO}_3)_2 \cdot 4\text{H}_2\text{O}$ and about 20 min for $\text{Cu}(\text{HSeO}_3)_2 \cdot 2\text{H}_2\text{O}$. This test accepted by the U.S.P. as an assay for dissolution of powders allows a maximum dissolution time of 30 min for one dose ¹⁰.

The commented results confirm a rapid release of selenite and of the divalent metal cations and show that both compounds are potentially useful for selenium supplementation. They could be used as a part of solid mixtures of micronutrients, in the form of aqueous solutions, or as an injectable solution.

Supplementation of magnesium and copper

The investigated compounds are also useful to supplement small quantities of Mg(II) and Cu(II) with each selenium administered dose. For adult humans, the requirements of these two elements are in the order of about 300 mg/day (Mg(II)) ^{4,21} and 1.5-3 mg/day (Cu(II)) ⁴. For animals the doses range between 10-15 mg/Kg (Mg(II)) ^{4,21} and 6-8 mg/Kg (Cu(II)) ⁴.

In Table 2 the available quantities of each of the two metals are shown, related to the supplemented doses of selenium. As it can be seen the supplemented metal doses lie far away from the minimum requirements. Notwithstanding, one or other compound may be selected to ameliorate, simultaneously with the selenium supplementation, copper or magnesium levels, as needed in particular cases.

| | a | b | | c | |
|---------|-----------|---|---|--------|--------|
| | Se | $\text{Mg}(\text{SeO}_3\text{H})_2 \cdot 4\text{H}_2\text{O}$ | $\text{Cu}(\text{SeO}_3\text{H})_2 \cdot 2\text{H}_2\text{O}$ | Mg(II) | Cu(II) |
| humans | 50 µg/d | 0,12 mg | 0.12 mg | 8.3 µg | 21 µg |
| animals | 0.1 mg/Kg | 0.22 mg | 0.22 mg | 15 µg | 40 µg |

Table 2. Selenium requirements. (a) minimum Se requirements for humans and animals; (b) quantities of the two salts to be administered to cover these minimum Se levels; (c).concentration of the simultaneously administered levels of Mg(II) and Cu(II).

Acknowledgements. This work was supported by the "Consejo Nacional de Investigaciones Científicas y Técnicas de la República Argentina" (CONICET) and the "Agencia Nacional de Promoción Científica y Tecnológica" (PICT 06-06148). C.C.W. benefits of a research fellowship from FOMEC and E.J.B. is a member of the Research Career from CONICET.

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