# Trace Elements Supplementation: Recent Advances and Perspectives

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**Abstract:** Supplementation of essential trace elements has become an increasingly important field of research in modern pharmacology. The present review presents general aspects related to the essentiality of inorganic systems in living organisms, followed by a detailed discussion of the supplementation of iron, copper, zinc, chromium, selenium, magnesium and some other minor trace elements. Some recent advances in this field as well as future challenges and perspectives are also discussed.

**Keywords:** Trace elements, Supplementation, Iron, Copper, Zinc, Chromium, Selenium, Magnesium.

## INTRODUCTION

There are a great number of metallic elements that play a fundamental role in living organisms in which they perform a wide variety of functions, such as carrying oxygen throughout the body, transporting electrons, stabilizing important biological structures or acting as catalysts in biochemical reactions. To mention only some well-known examples: iron is involved in oxygen transport and activation; cobalt is the essential constituent of vitamin $B_{12}$; zinc provides the structural framework for the so-called "zinc fingers", involved in the transmission of genetic information, and is a natural component of insulin. On the other hand, metallic elements such as copper, iron, manganese, molybdenum and zinc are incorporated into catalytic proteins, called metalloenzymes, which facilitate a multitude of chemical reactions needed for life.

The study of these and other inorganic systems involved in metabolic processes and functions has generated a new and important field of interdisciplinary research, commonly denominated Bioinorganic Chemistry or Inorganic Biochemistry which has attained a rapid and explosive development during the last forty years [1-5]. As its name suggests, it focus on a variety of problems and systems lying at the interface between Inorganic and Biological Chemistry. Most of the results of these studies and investigations have also opened new and interesting perspectives in different applied fields of science and technology such as Ecology, Toxicology, Hydrometallurgy, Medicine and Pharmacology.

It is evident that if certain metals and other inorganic systems play important roles in the correct performance of living organisms, a better knowledge of their functions and modes of action may be useful to obtain a deeper understanding of their health effects. Besides, since Nature has made such an extensive use of metals in biological systems, an obvious consequence is to explore the possibility of incorporating metal ions into drugs and using coordination compounds as chemotherapeutic agents. The development of these ideas has generated another new field of research in modern Inorganic Pharmacology currently denominated Medicinal Inorganic Chemistry [2-12].

Although the use of different metals and their compounds in Medicine dates back to ancient times, as very old Egyptian, Chinese and Arabian writings described the use and effects some of them [8], modern Medicinal Inorganic Chemistry as an independent field of research has only existed since the discovery of the antitumoral activity of *Cisplatin*, *cis*-[Pt(NH$_3$)$_2$Cl$_2$] in 1969 [13-15]. Following this discovery an intense research activity on new antitumoral coordination compounds began all around the world and, for example, up to 1980 the U.S. National Cancer Institute had tested the antitumoral activity of more than 11000 compounds of 55 different metals [16]!

Along with the better understanding of the activity and functions of metal ions in biological systems, it has also become clear that the concentration of each element present in any living organism is subject to a continuous and strict control. This allows the perfect development of metabolic and physiological processes and an equilibrated interrelation between all the inorganic systems and species existing in this organism. Moreover, in the case of human beings, we know today a large number of disorders and diseases that can clearly be correlated with deficiency or excess of certain metal ions.

In the case of overload of an essential metal or a poisoning generated by excess of a toxic metal, different chelating agents and drugs have been developed in order to eliminate these excesses, restoring the normal biological and biochemical equilibria [5, 17,18, cf. also the chapter by O. Andersen in this volume]. On the contrary, if a disease or a disorder is generated by a deficiency of an essential element, Medicinal Inorganic Chemistry proposes different ways and methodologies to supplement them efficiently. This aspect, referred to a selected group of essential trace elements, constitutes the main subject of this review.

## GENERAL ASPECTS RELATED TO THE SUPPLEMENTATION OF ESSENTIAL ELEMENTS

As mentioned above, the deficiency of certain elements can originate different diseases or disorders. Consequently, the supplementation of essential elements constitutes a field of increasing interest and rapid development and is one of

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the preferred current research areas covered by modern Inorganic Medicinal Chemistry.

Before the presentation of some concrete examples in this field, it seems necessary to define some basic concepts, beginning with those regarding the essentiality of an element. This first task is not totally easy as it involves a number of ambiguities, formally derived from experimental difficulties and contradictions [19].

The simplest definition of an essential element is, obviously, that it is an element required for the maintenance of life; its absence results in death or a severe malfunction of the organism. Experimentally, this rigorous criterion cannot always be satisfied and has led to broader definitions of essentiality. A wider definition has been given by Frieden [20], as follows: An element is considered essential when a deficient intake produces an impairment of function and when restoration to physiological levels of that element prevents or relieves the deficiency. The organism can neither grow nor complete its life cycle without the element in question. The element should have a direct influence on the organism and be involved in its metabolism. The effect of the essential element cannot be totally replaced by any other element.

Some other, more stringent, biochemical criterion may be applied in order to complement the precedent ideas. In this sense, it is admitted that an element may be considered as essential only if it meets the following criteria: a) it is present in the healthy tissues of all living organisms; b) its concentration from one animal to the next is fairly constant; c) its withdrawal from the body induces reproducibly the same physiological and structural abnormalities, regardless of the species studied; d) its addition either reverses or prevents these abnormalities; e) the abnormalities induced by deficiencies are always accompanied by specific biochemical changes; and f) these biological changes can be prevented or cured when the deficiency is prevented or cured [19].

It is obvious that the number of elements, especially those present at the lowest levels (usually called trace or micro trace elements) depends on the sophistication of experimental procedures. Therefore, it can be expected that, with further improvement of our experimental possibilities, more elements may be demonstrated as essential.

According to our present knowledge, less than one third of the 90 naturally occurring elements are considered to be essential for life. These consist of 11 major elements (usually called bulk or constituent elements [21, 22]) namely, hydrogen, oxygen, carbon, nitrogen, calcium, phosphorus, sodium, potassium, magnesium, sulfur and chlorine. A second group (usually called trace elements) includes the first row transition metals iron, copper and zinc and the representative elements silicon and fluorine. The so-called micro or ultra micro-trace elements constitute a third group that includes vanadium, chromium, manganese, cobalt, nickel, molybdenum, selenium, boron, arsenic, and iodine. The essentiality of some other elements (mainly, lithium, rubidium, strontium, barium, cadmium, tin) is currently under discussion [21-23].

It is also important to remember that even an essential element can become toxic if its concentration exceeds certain limits. Therefore, an optimal concentration for each element is established by the organisms at which optimal function is attained [5, 20, 22]. At lower concentrations (deficient state) the organisms survive but at an absolute deficient state, death may result. At higher than the optimal concentration first, marginal toxicity and then mortal toxicity is attained.

Another important concept that may be considered is that of antagonism and/or competition between elements. This means that we need not only to know the characteristics and functional properties of any element, but also the impact of this element on other elements. These interactions are usually the origin of the so-called *secondary deficiencies,* i.e., an organism may present a deficiency in a certain element as a direct consequence of the presence of an excess of another one that limits its absorption. Therefore, this deficiency cannot be usually reversed by the simple supplementation of the deficient element, as normally occurs in the case of a *primary deficiency* [5].

Bioinorganic Chemistry has given new and valuable information on the form in which trace elements are present, transported and accumulated in living systems, and this knowledge generates new routes for the design of better and most powerful supplementation procedures.

In the case of the transition metals, different ligands may be involved in the stabilization of metalloproteins, in the complex species present in biological fluids and in the transport proteins and storage systems. On the other hand, a number of low molecular weight complexes are used to transport the metals through cell membranes and as essential building blocks in the reconstitution of metalloproteins. The stoichiometric, structural and chemical characteristics of all these systems constitute valuable starting points in the design of new chelating and supplementation systems [17, 24].

In this context it should be mentioned that chemical speciation and computer modeling studies have also contributed to a better understanding of the characteristics of the mentioned low molecular weight complexes. Speciation is the occurrence of an element in separate, identifiable forms and so the term "chemical speciation" defines the oxidation state, concentration and composition of each of the species present in a chemical sample [17, 24, 25].

Finally, it is interesting to comment that supplementation studies have also recently acquired great interest in the field of veterinary medicine, in which they often contributed in the solution of very important regional or national economic problems [26, 27].

## SUPPLEMENTATION OF TRACE ELEMENTS

In the following sections we present and discuss the most important aspects related to the supplementation of the essential transition metals iron, copper, zinc, and chromium and of the representative elements selenium and magnesium. At the end of the section some brief general comments related to other essential trace elemens are also given.

### Iron

Iron is the most abundant transition metal in the human body (about 4-5 g in a normal human adult of 70 Kg weight [5]) and the importance of well-defined amounts of this

element for the survival, replication and differentiation of almost all organisms is well established. Iron deficiency is the most frequent nutritional problem in the world. It affects 24% of the population: its incidence in the developed countries is approximately between 4 and 10%, whereas in the developing countries this figure rises dramatically to about 40%. Studies carried out by WHO demonstrated that in the developing countries, 47 % of the women in reproductive age are at least slightly anemic [28].

Classical sources for oral iron supplementation are some simple Fe(II) or Fe(III) salts of organic or inorganic acids, such as ferrous or ferric sulfate, ferric ammonium citrate, ferrous fumarate, ferrous gluconate, ferric ammonium sulfate, ferrous carbonate, etc. [17, 29, 30]. Besides, the use of elemental iron has attained renewed interest in recent years. A very fine Fe-powder (2-6 μm particle size) can be obtained by thermal decomposition of the $Fe(CO)_5$ carbonyl complex. It appears as an ideal form for the iron enrichment of white flour (and subsequently of baked bread) and cereals. In this form iron is easily absorbed and does not present undesirable side effects [29, 30].

The NaFe[EDTA] complex (EDTA = ethylenediamine-tetraacetic acid, Fig.**1**) has recently been postulated as another appropriate system for iron supplementation [31]. It has shown very promising results in animal studies, presenting a very good bioavailability of iron [32]. Notwithstanding, its use is currently restricted to experimental trials, as it still remains unclear if it alters the metabolism of other essential elements or, eventually, enhances the absorption of toxic metals [32, 33].
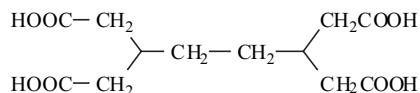


**Fig. (1).** Schematic structure of EDTA.

Most patients will respond promptly to an orally administered soluble iron salt. Ferrous salts are absorbed approximately three times as well as ferric salts when administered in the fasting state [34]. Optimal benefit is derived from iron salts taken between meals with water or a fruit juice. Food, tea and coffee usually reduce absorption [34]. The concurrent administration of ascorbic acid (vitamin C) is sometimes recommended because it enhances the iron absorption [31, 34, 35]. Ascorbic acid reduces Fe(III) to Fe(II), inhibiting the formation of poorly soluble iron compounds [35].

Oral iron is usually well tolerated even by patients with gastrointestinal disorders. However, certain of the mentioned salts present some side effects (abdominal cramps, nausea, mild diarrhea, etc.) that affect mainly the gastrointestinal system. Attempts have been made to design preparations with fewer side effects, using delivery systems that allow a slower release of iron [34].

Parenteral iron supplementation is rarely necessary since most patients respond rapidly to oral preparations. This way of supplementation was only introduced in 1947 by Nissam [36]. So far, the most widely used system is iron/dextran which is a complex of $Fe(OH)_3$ with dextran that can be administered intramuscularly or intravenously [29, 30, 34]. Other similar systems are those conformed by solutions of colloidal $Fe(OH)_3$ with partially hydrolyzed dextrin, with polyisomaltose or with a citric acid/sorbitol mixture [29].

In the design of new iron-containing drugs, its biological activity has been tested on the basis of a set of general physico-chemical criteria, as follows [29, 37]: a) The chelate must be stable and monomeric at biological pH-values in order that it may cross various cell membranes; b) It should be able to exchange iron rapidly with apotransferrin (transferrin is the iron transport protein); c) The chelating ligand should be able to extract iron fairly rapid from ferritin (the iron storage protein). An experimental study with anemic rats [37] has shown that the simple iron(III) complexes of the type $FeL_3$, with L = acetohydroxamate and glycinehydroxamate, exchange iron very rapidly pointing to the usefulness of monohydroxamic acids as ligands in the synthesis of new supplementation systems [29, 30, 37].

## Copper

Like iron, copper participates in a wide range of biological functions and systems and is involved in the activity of a wide range of enzymatic systems (i.e., cytochrome oxidase, tyrosinase, ceruloplasmin, lysine oxidase, ascorbate oxidase, superoxide dismutase, amine oxidase, etc.) [1-5, 38]. Thus, its deficiency generates a lot of physiological and health problems. Different well-known diseases can clearly be correlated with copper deficiencies. For example, patients with rheumatoid arthritis, gastric ulcers, cancers or epileptic episodes usually present elevated serum or plasma copper concentrations [39, 40]. Another well-known problem clearly related to copper deficiency is Menkes'disease [40-42]. It is an X-linked genetic disorder, which causes rapidly progressive cerebral degeneration, usually accompanied by convulsions, growth retardation, hypothermia, skeletal anomalies and gross abnormality in elastic fibers in the arterial wall.

A better knowledge of copper absorption and transport appears as indispensable for the design of adequate supplementation systems. It is known that ionic forms of copper have particularly high affinities for organic ligands in biological systems and, as a consequence of this, all measurable amounts of copper exist as complexes or chelates in biological systems [39]. On the other hand, it has been established that when the pharmacological activity of copper complexes of different ligands (amino acids, penicillamine, anthranilic acids, salicylic acids, and a variety of other amines and carboxylic acids) is compared with that of the free ligands, the complexes are always more active than the parent ligands [40, 43, 44].

It has also been well established that copper complexes of amino acids, small peptides and other low molecular weight ligands often present an important anti-inflammatory, antiulcer, anticonvulsant and anticarcinogenic activity [39, 40, 43, 44]. Evidently, these low molecular weight compounds may serve as transport forms of copper that allow the activation of copper-dependent enzymes or may have chemical reactivity that facilitates the correction of the chemical problem that originally led to the disease state. In this context, the activation or regeneration of the copper-dependent superoxide dismutase (SOD) appears as especially

important. Also, different low molecular weight copper-complexes present SOD-like activity *per se* [40, 44-50].

Most of the Cu(II) complexes of amino acids present comparable copper environments. As an example, the structure of the complex of L-alanine is schematized in Fig.**2**. In this case the metal center interacts with other two double bonded O-atoms of neighboring complexes, generating two additional longer Cu-O bonds, producing a strongly distorted octahedral environment. In other complexes coordination remains directly square planar whereas in some others the geometry is square pyramidal with the vertex of the pyramid occupied by a water molecule or by an O-atom of a neighboring complex.
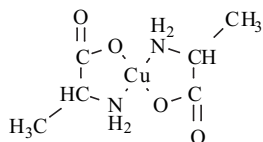
**Fig. (2).** Schematic structure of bis (L-alaninato)copper(II) (adapted from A. Dijkstra, *Acta Crystallogr.* **1966**, *20*, 588).

The presence of a 1:2 Cu(II)/histidinate complex in human serum was firstly reported in 1966 [51] and, subsequently, the existence of several ternary complexes involving histidine and other amino acids were also reported. On the other hand, computer model studies of blood plasma show that the administered copper complexes release their copper to serum albumin, suggesting that low molecular copper complexes are effective for copper supplementation as they increase the concentrations of complexes which are able to pass through membranes thereby distributing copper into tissue [52]. These facts suggest the usefulness of copper (II) complexes of amino acids, small peptides and other low molecular weight ligands for copper supplementation. In relation to this proposal the general physicochemical properties of a great number of Cu(II) complexes of dipeptides [48,49] and amino acids [53-58] have been investigated in detail. Also, some mixed cation EDTA complexes appear as interesting ways for copper supplementation [59].

Although it is accepted that Menkes' disease cannot be treated by copper supplementation since there appears to be a serious impairment of copper utilization in several tissues [51,60], treatment of patients suffering this disorder with the histidinate complex [Cu(L-His)$_2$], which seems to be a physiological form of copper, appears up to now as the most effective therapy when started early in life. The efficacy of early treatment may be the result of making copper available during a critical period of myelination and development of the central nervous system [51].

Detailed studies on the copper metabolism in mottled mutant mice, an animal model for this disease, demonstrated that treatment with cuprous salts in the presence of sebacic acid (Fig. **3**) resulted in nearly normal growth rates and survival of these animals [61]. A human patient has also shown a good response to this treatment [62]. However, and as the copper complex or complexes actually present are not well characterized, we have performed some general physicochemical studies of this system [63-65]. The pure complex of Cu(II) with sebacic acid could be prepared and thoroughly characterized [63,64] and used for further studies

that suggested that the active species may be a mixture of this complex and a fraction of "free" Cu(II) dissolved in the employed solvent [65].
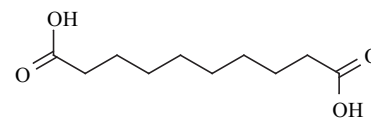
**Fig. (3).** Schematic structure of sebacic acid.

## Zinc

Zinc is an essential micronutrient for all forms of life, from microbes to man. It appears to have a greater variety of biochemical functions than any other trace element, as shown by the wide variety of zinc enzymes that are known [2, 5, 66, 67]. It presents catalytic, regulatory and structural functions.

During the last decades a wide spectrum of clinical deficiency of zinc in humans has been recognized including severe to mild or marginal effects. Severe deficiency has been observed in patients with acrodermatitis enteropathica, following total parenteral nutrition without zinc, following excessive consumption of alcohol or following penicillamine therapy. Moderate deficiency of zinc has been observed in a variety of clinical disorders, including nutritional problems due to dietary factors, malabsorption syndrome, alcoholic liver disease, sickle cell anemia and chronic debilitated condition [68, 69]. Low zinc levels usually have an important impact on growth, wound healing, immune response and reproduction, as well as on disorders of taste, vision and smell. Most recently, also anorexia nervosa has been related to low zinc levels [67]. In summary, zinc deficiency appears in humans as a common problem but not easily recognized. It leads to neurosensory, hormonal, cell-mediated immunological disorders, and skin problems. In severe cases, mental irritability, emotional disorders, and chronic diarrhea may also be found [68].

Therefore, zinc supplementation has become an important aspect of modern inorganic pharmacology. The Food and Nutrition Board of the U.S. National Academy of Sciences have recommended a daily zinc intake of 15 mg for adults, with an additional 5 mg during pregnancy and 10 mg during lactation [69, 70]. The usually employed zinc compounds for supplementation are the inorganic salts sulfate or chloride, and the organic salts acetate, gluconate and stearate [70-72]. Zinc oxide has been used for food fortification, too [72].

A major disadvantage of zinc sulfate, the most widely used salt for supplementation purposes, especially during prolonged administration, is the effect of its acidity, which often causes gastrointestinal disorders, nausea and vomiting [70]. In order to reduce these secondary side effects, the use of basic zinc salts has recently been suggested. The first compound proposed as useful in this context was the basic zinc chloride, of composition $Zn_5(OH)_8Cl_2 \cdot H_2O$ [73]. Most recently, the basic carbonate $Na_2Zn_3(CO_3)_4 \cdot 3\,H_2O$ has also shown a rapid Zn(II) liberation in dissolution assays, together with a certain buffering capacity [74] showing its excellent potential for zinc supplementation.

Finally, it must be commented that there are clear evidences that high levels of dietary zinc may cause copper

deficiency, probably generated by a competitive mechanism for the absorption of both cations in the gastrointestinal tract [70, 71]. Therefore, during long-term zinc supplementation it is usual to add to the diet low levels of any copper salt, such as sulfate, in order to compensate that deficiency [70, 71].

## Chromium

In the last 15 years, a wide number of nutritional studies suggested that chromium (III) may have an essential role in mammals and is required for proper carbohydrate and lipid metabolism [75, 76]. The bioinorganic system containing Cr(III) has been usually known as *glucose tolerance factor* (GTF) [4,5, 77, 78]. Most recently, a naturally occurring oligopeptide, called *chromodulin* has been proposed as a candidate for the biologically active form of chromium [75, 76].

In the initial clinical studies chromium(III) chloride, administered in solution or as a pill, was used [78]. The utilization of Cr(III) complexes with nicotinic acid and the amino acids-glycine, cysteine and glutamic acid (the constituents of the tripeptide glutathione) as ligands, also generate chemical species which in their composition and activity resembles that of GTF [78, 79]. Notwithstanding, a great part of the performed supplementation studies has used directly brewer's-yeast which is high in total and biologically active chromium [78].

Tris(2-carboxypyridine)chromium(III) (chromium picolinate, Fig. **4**) is now one of the most widely used, commercially available, therapeutic forms of chromium. It is sold as a dietary supplement for fat loss and muscle building, and is also recommended as a therapy for type II diabetes and glucose intolerance. However, some adverse effects in humans, apparently related to the use of high doses of the complex, have recently been established [80-82].
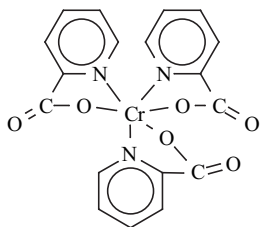


**Fig. (4).** Schematic structure of tris(picolinato)chromium(III).

Finally, it is interesting to mention that all the current chromium-containing nutritional supplements, only serve as sources of absorbable chromium. But, on the other hand, the existence of a multinuclear Cr(III) carboxylate in the active biomolecule *chromodulin* has generated great interest in the synthesis and characterization of multinuclear oxo (or hydroxo)-bridged Cr(III) carboxylate assemblies [83, 84]. Some of these complexes apparently interact directly with the insulin receptor, activating its kinase activity [84] and generating a totally different way of action of chromium supplements.

## Selenium

Increasing evidences about the essentiality of selenium have been accumulated in the last decades. In humans its deficiency has been mainly linked to heart diseases and bone structure and articulations problems. It has also been related to the immunity system and to cancer and, most recently, to AIDS. Most of the diseases originated in selenium deficiencies may be related to glutathione- peroxidase and to cellular defense mechanisms against oxidative (free-radicals) attack [85-87].

Therefore, selenium supplementation has attained wide and continuous interest and different selenium species has been incorporated to food and nutritional fortification systems. Different simple inorganic salts, such as $SeO_2$, $Na_2SeO_3$ or $Na_2SeO_4$ have been shown physiologically active [87, 88]. The use of sodium selenite or selenate has demonstrated to be very useful also for animal supplementation, which are also affected by low selenium levels [86, 87]. Recently, we have also suggested the use of divalent-metal acid selenites of the type $M^{II}(SeO_3H)_2 \cdot nH_2O$ for selenium supplementation and for an additional reinforcement of the metallic species ($M^{II}$ = Mg, Cu) [89].

A great number of organic Se-containing compounds have also been tested for supplementation purposes and some of these, especially L(+)-selenomethionine (Fig. **5**), seem particularly useful. Studies conducted on selenium metabolism in humans showed that this compound is more effective than selenite in rising blood selenium levels [90] and produces an increased retention of the element [91].
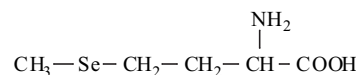
$$CH_3-Se-CH_2-CH_2-\overset{\overset{\displaystyle NH_2}{|}}{CH}-COOH$$

**Fig. (5).** Schematic structure of L-selenomethionine.

From the pharmacological point of view, organic compounds appear as more appropriate than inorganic ones [87, 88]. Selenites and related oxoanions are much more toxic than organic compounds and, on the other hand, inorganic selenium compounds tend to reduce to elemental selenium or to selenide, inactivating the effect of the element. Also some yeasts, grown in selenium-rich medium, present very useful supplementation properties [87,88]. In this same context, the usefulness of naturally selenium-enriched wheat has recently been pointed out [92].

## Magnesium

The alkaline and alkaline-earth elements sodium, potassium, magnesium and calcium are the four most abundant metals in biological systems [1-5]. Consequently, deficiency syndromes associated with these elements have been rare phenomena for many years, as virtually all standard diets appeared to guarantee a sufficient supply of them. This situation suffered changes in modern society, where, for example, animals are raised in controlled environments with special growth-regulated diets, and humans may be subject to rapid loss of electrolytes due to stress, excessive recreative and sport activities, or highly irregular and selective drinking and eating habits. This situation is particularly true for magnesium, which is most likely to become deficient owing to its lower abundance compared with that of sodium, potassium, or calcium. For this reason, magnesium supplementation has become an important issue in contemporary medicine and pharmacology [93, 94].

One of the most relevant questions arising in this context is to find optimal routes for magnesium administration.

This implies a selection of complex compounds or salts which lead to a rapid and efficient resorption of Mg(II) without upsetting the pH or the ionic equilibria in body fluids. Possible side effects arising from the anioinic and/or ligand components of the proposed systems should also be minimized.

Among the many possible candidates for the generation of such systems, the natural amino acids and their derivatives are preferred candidates [93, 94], and different compounds of these types have been described and characterized during the last years [93-95]. One of them, derived from L-aspartic acid, of composition Mg(L-HAsp)Cl.3H$_2$O, which can be orally administered, has awaken great medical interest because it presents a potentially broad range of therapeutic activity [93-95]. The compound has a layer structure, where the chloride anions and one of the water molecules are found between sheets of composition [Mg(L-HAsp)(H$_2$O)$_2^+$]$_n$. As shown in Fig. **6**, the Mg(II) cation presents octahedral coordination. The hydrogen aspartate anions function as chelating ligands through their α- and β-carboxylate groups; the amino group is protonated and has no metal contact. Two O-atoms of two water molecules and two carbonyl oxygen atoms of neighboring complex units occupy the remaining coordination sites of the octahedron.
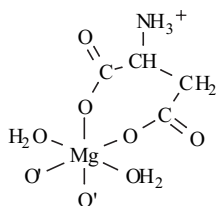


**Fig. (6).** Environment of the Mg(II) cation in Mg(L-HAsp)Cl.3H$_2$O (adapted from refer.[93]).

Other similar and potentially useful complexes may be magnesium-L-glutamate tetrahydrate [93, 94, 96], the magnesium citrates [93-95] and the hydrates of magnesium bis(L-pyroglutamate) [93, 94, 97]. Other interesting complexes are those of orotic (6-carboxyuracil) and iso-orotic (5-carboxyuracil) acids. The first of them is not a "true" magnesium complex, as it is constituted by a [Mg(H$_2$O)$_6$]$^{2+}$ cation associated with two hydrogenorotate anions, only through hydrogen bonds [93, 95, 98] whereas in the iso-orotato complex the ligand acts as bidentate and is directly bound to the cation, as shown in Fig. **7** [99]. Dissolution assays have shown that this complex presents better dissolution properties than the orotate compound [99].
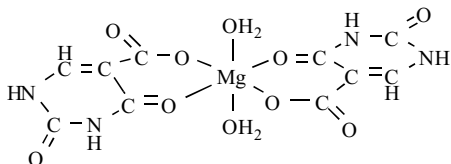


**Fig. (7).** Schematic structure of the Mg(II)/isoorotato complex of composition [Mg(isoor)$_2$(H$_2$O)$_2$].2H$_2$O (adapted from refer.[99]).

### Other Elements

In this section brief comments on the supplementation of some other essential bioelements are presented, although for most of them only little experience and information have been gathered.

### Cobalt

Vitamin B$_{12}$ (Fig. **8**) is the first vitamin found to contain a metal and the only cobalt-containing compound present in the human body. It is one of the most complicated naturally occurring coordination compounds and participates as a coenzyme in an important series of biological reactions and transformations [1-5]. In monogastric animals and man it is synthesized by bacteria in the large intestine but is only absorbed in the lower part of the small intestine. In humans, most of the vitamin B$_{12}$ is obtained from dietary sources. In well-balanced diets, the obtained amounts are normally quite sufficient and meet the recommended daily allowance, which for adults is 3 μg/day [100].
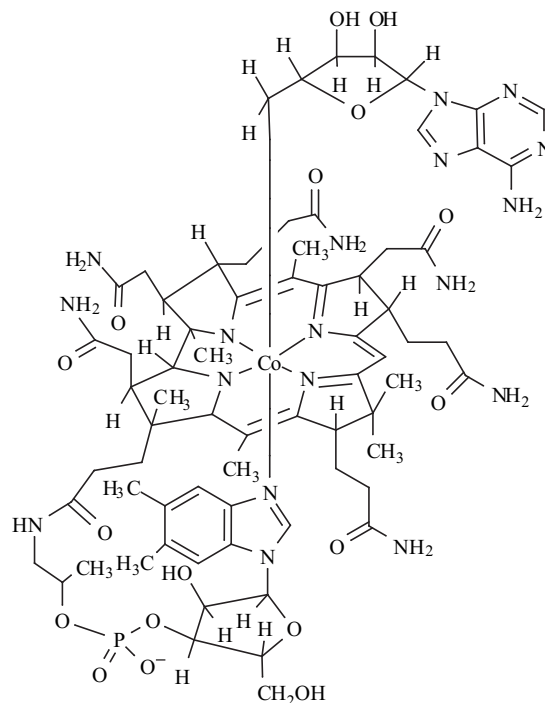


**Fig. (8).** Schematic structure of vitamin B$_{12}$.

A deficiency of vitamin B$_{12}$ can develop if its uptake is impeded through lack of production of the so-called *intrinsic factor*, a glycoprotein that specifically binds the vitamin and is required for its absorption. Vitamin B$_{12}$ deficiency primarily gives rise to *pernicious anemia*. Symptoms of the disease develop only in adults as the body stores of the vitamin are depleted slowly. Initial symptoms are extreme weakness, fatigue, and soreness of tongue and paresthesias of the extremities. Besides, enlarged erythrocytes are observed and the overall number of erythrocytes is diminished (megaloblastic anemia). In some elderly patients, neural and mental symptoms may develop prior to the appearance of hematological problems [100].

Vitamin B$_{12}$ deficiency can usually be controlled by intramuscular cyanocobalamin injections. A typical dosage is 100 μg, 1-3 times per week [100].

### Manganese

Supplementation systems for this important biometal have so far not been investigated in detail. Moreover, the

common foods in human diets are highly variable in manganese concentration. Nuts and whole cereals normally contain the highest levels of this element and also tea is found to be exceptionally rich in manganese [101].

## Vanadium

Although it essentiality remains controversial [102-105], great interest in the pharmacological activity of certain vanadium compounds has arisen in the last years due to its important insulino-mimetic activity and in order to find a better form of diabetes control [106-110]. Simple salts, such as oxovanadium (IV) sulfate and sodium vanadate have shown this type of activity. Most recently, some oxovanadium (IV) and oxovanadium (V) chelates as well as a number of peroxovanadates have been assayed. One interesting example of this type of drugs is bis(maltolato)oxovanadium (IV) (Fig. **9**) [108-110]. It can be readily synthesized by reaction of vanadyl sulfate and maltol [111]. It is well soluble in water and in a number of organic solvents and presents a good lipophilicity. It can be administered orally or by intraperitoneal injection.
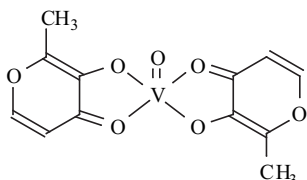


**Fig. (9).** Schematic structure of bis(maltolato)oxovanadium (IV).

## Boron

Boron has been known to be essential for higher plants since 1923 but only recently evidence has been accumulated indicating that it may be an essential nutrient for humans and animals [5, 112-114]. It apparently has an essential function that somehow regulates parathyroid hormone action, and therefore, indirectly influences metabolism of calcium, phosphorus, magnesium and cholecalciferol.

Fruits and vegetables are especially rich in boron [113, 114]. An analysis of both human and animal data suggests that an acceptable safe range of mean intake of boron for adults could well be 1-13 mg/day [114]. Notwithstanding, recent findings indicate that a significant number of people do not consistently consume more than 1 mg/day, suggesting that boron could be a practical nutritional or clinical concern [114].

Boric acid and some of the common inorganic borates may be useful for boron supplementation [115, 116]. Boric acid given orally is readily and completely absorbed. It is rapidly distributed throughout the body water via passive diffusion. Following administration, the ratio of blood: soft tissue concentration of boron in humans is approximately 1.0; in contrast, concentration of boron in bone exceeds those in blood by a factor of around four [116]. Boric acid is not metabolized owing to the high energy required to break the B-O bonds and it has been hypothesized that the mechanism of its biological activity is related to the well-known affinity of boric acid for *cis*-hydroxo groups [116].

Finally, it is worth commenting that it has been found that, when orally administered, inorganic borates have low toxicity, even when supplied during long periods of time [117].

## CONCLUSIONS AND PERSPECTIVES

In this brief review we have tried to show the relevance and some recent advances in essential trace elements supplementation, illustrated for some of the most important bioelements. The most important advances are derived from a better knowledge on the form in which these elements are absorbed, transported, accumulated and metabolized in the organism emphasizing the impact of the development of the field of Bioinorganic Chemistry.

There are a number of aspects which merit further exploration and analysis in order to ensure a wider expansion of this field of modern pharmacology. Some of them are briefly commented, as follows:

-    Design of new systems that preferably allow oral supplementation with the aim of increasing the flow of metal complexes from intestine to blood by increasing the concentration of lipid-soluble, low molecular weight complexes present in the intestinal fluids.

-    The search for such systems should be based on careful speciation and model studies to attain a clearer insight into the complex species that are physiologically active and to predict and understand possible competencies of biologically active ligands and systems.

-    The minimization of possible side effects of the proposed formulations must also be considered.

-    It is very important to develop new forms that allow a controlled drug release. These systems may improve the effectiveness of supplementation increasing therapeutic activity, and reducing the number of administrations required during treatment [118, 119].

-    In the case of Zn(II) compounds, it is necessary to develop systems that enhance the absorption of this cation in order to increase its levels in plasma [71]. Besides, the search for new basic salts, useful for supplementation, seems to be a promising area of research.

-    The search for new systems adequate for cobalt and manganese supplementation seems also an area of interest. In the last case, it is important to remember the important and promising SOD-like activity of some Mn (II) and Mn(III) macrocycles [120, 121]. A better characterization and the possible use of new organic species containing selenium (cf., for example [122]) appear to be relevant, too.

-    Further investigations on chromium and vanadium complexes possessing insulino-mimetic activity are also highly desirable, as are the study of compounds of other metals with similar properties (for example, copper [123] or zinc [124] complexes).

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