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Chelation Therapy for Metal Intoxication: Comments from a Thermodynamic Viewpoint

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Abstract: Chelation therapy plays a prominent role in the clinical treatment of metal intoxication. In this paper the principal causes of metal toxicity are exposed, and the chemical and biomedical requisites of a chelating agent are sketched. The chelating agents currently in use for scavenging toxic metal ions from humans belong to few categories: those characterized by coordinating mercapto groups, by oxygen groups, poliaminocarboxylic acids, and dithiocarbamates. Considering that the complex formation equilibria have been studied for less than 50% of chelators in use, some reflections on the utility of stability constants are presented, together with an evaluation of ligands under the stability profile. The competition between endogenous and toxic target metal ions for the same chelating agent is furthermore examined. A thorough examination of stability constant databases has allowed to select, for each toxic metal, the ligands distinguished by the best pMe values. Even though this selection does not consider the biomedical requisites of a chelating agent, it gives a clear picture both of the pMe values that can be attained, and of the most appropriate chelators for each metal ion.

Keywords: Chelating agent, Chelation therapy, Heavy metal, Speciation, Stability constant, Toxic metal.

INTRODUCTION

The poisoning action of toxic metal ions, originated by accidental, occupational/environmental and iatrogenic causes, represents one of important health and social problems both in developed and in emerging countries [1-12]. The research on this topic is nonetheless totally inadequate since the investments of pharma-industries are limited because of their poor profitability. The research on chelating agents to be used in clinical practice is based essentially on biology-driven programs, paying no attention to the chemical knowledge of the mechanism involved in the complex formation. Actually the term "chelating agent" in common sense is a drug able to bind metal ions. In this way the chelating agents used for given metal ions are not necessarily the best, but the most common drugs. Specificity and stability, as well the knowledge of the length of the treatment (limited in time or lifelong) are extremely important in the choice of a chelator. A first intent of the present paper is to remark, based on the analysis of literature data, the large extent of chelators in use for which a deep chemical basis is lacking, and to propose a strategy for the future research in this field. A second aim is the selection from stability constant databases of the best possible families of ligands for each target toxic metal ion. Speciation plots and proper parameters (pM) will allow a preliminary evaluation of the attitude of these ligand families as chelators for clinical treatments. Finally, some aspects of wide importance, as the competition between toxic and endogenous metal ions toward the same metal chelator, have been deepened.

METAL TOXICITY

Metal toxicity can be roughly sorted in three big different classes according to its sources and effects:

- Acute intoxication, deriving from direct ingestion of toxic metal (accidental, as often occurs to children, or due to a voluntary homicidal or suicidal attempt);
- Chronic intoxication, depending on environmental pollution, or related to work conditions, or to iatrogenic causes;
- Metal overload due to genetic diseases (β-thalassemia (iron)[1] and Wilson's disease (copper) [6, 13]).

This classification helps in managing clinical treatments, among which chelation therapy plays a prominent role. Chelation therapy has the intent of scavenging the toxic metal ion from the organism, or of attenuating its toxicity by converting it in a less toxic compound, or of relocating it from the position where its action is toxic to a compartment where the toxic action cannot be exploited, or of controlling its redox potential.

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[1] β-thalassemia, a genetic disease with a defect in haemoglobin synthesis, requires regular blood transfusions to prevent anaemia.

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The essential properties of a chelating agent have been better and better defined through the years. These requisites, based on both chemical and biomedical considerations, can be sketched as:

- Favorable toxicity profile of chelating agent and of its complexes;
- High stability of the formed complexes, not less than that with endogenous ligands;
- Selectivity toward the target metal ion;
- Suitable redox potential of complexes;
- Sufficient bioavailability after oral administration;
- Slow metabolism of the chelating agent, once entered into the body;
- Rapid exchange of metal between endogenous ligands and chelating agents;
- Easy excretion of the toxic metal in the complexed form;
- The ligand must not disturb the metabolic metal homeostasis in the body fluids.

The stability of the formed complex is the first necessary requisite, though not sufficient, so that the metal ion is completely transformed in the chelated species to be excreted.

A further point to be remarked is that redox-active metal ions as Fe^{III} and Cu^{II} exert their toxic action through the formation of reactive oxygen species (ROS), which may result in tissue or organ damage [14,15]. As pointed out in a review on iron chelation [6] the ROS defense mechanism involves glutathione peroxidase and catalase enzymes that degrade hydrogen peroxide to water and oxygen. The amount of unbound iron to prevent ROS formation is regulated by iron binding proteins, while the radical chain reaction is interrupted by the action of radical scavengers (vitamin E). Iron mobilized from proteins can take part in redox reactions with ROS generation in iron imbalance situations. When this exceeds the antioxidant protection, oxidative damage occurs. To prevent production of dangerous ROS iron redox potential has to be controlled by proper chelation. The high Fe(III) selective siderophores render redox cycling improbable at biological conditions. Nitrogen ligands, instead, are characterized by lower redox potentials so that coordinated iron is not protected and can be reduced enzymatically. Correlations between redox potential and affinity for iron(II) and iron(III), and pH effects on redox potential have been presented and discussed by Boukhalfa and Crumbliss [16]. Furthermore, the redox properties of iron complexes with hydroxypyridinones were examined by Merkofer *et al.* [17,18]: the tight binding of iron(III) by these orally active chelators prevents redox cycling.

Chelating Agents

Chelators can be classified as bidentate, tridentate and so on, according to the number of coordinating groups on the molecule able to bind the target metal ion at the same time. Ligand denticity determines the number and the stoichiometries of the formed complexes. Ligands with low denticity form multiple complexes whose speciation depends both on total ligand concentration and on metal/ligand ratio; exadentate chelators, on the contrary, form only one kind of complex. The stability constant of a complex $Me_pL_qH_r$, related to its formation equilibrium $pMe + qL + rH = Me_pL_qH_r$, is expressed as

$$\beta = [Me_pL_qH_r]/([Me]^p[L]^q[H]^r)$$

where L is the completely deprotonated form of the ligand, with charges omitted for simplicity. Since reacting molecules *in vivo* are the ligand forms prevailing at pH 7.4, according to their speciation pattern established by the protonation constants, complex formation depends on competition between proton and metal ion for the same basic sites on ligand. In addition to stability constants, a number of factors concur to the binding efficiency of a ligand toward a given metal ion, as the stoichiometry of the complex and proton competition. Different ways to express the effectiveness of a ligand have been reviewed by Bazzicalupi *et al.* [19]. In the present paper the chelating agents in use in clinical practice have been examined by means of the pMe parameter, proposed in 1981 by Harris *et al.* [20], as $-\log[M_f]$ at $[M_T]= 1x10^{-6}$ M and $[L_T] = 1x10^{-5}$ M at pH 7.4, where $[M_f]$ is the concentration of free metal ion and $[M_T]$ and $[L_T]$ are the total concentrations of metal and ligand respectively. The stronger the chelating agent, less metal ion remains unchelated (free metal ion) in solution, i.e. higher pMe values are found.

Chelating Agents in Clinical Use

The chelating agents currently in use belong to few chemical categories:

i. Poliaminocarboxylic acids,

ii. Ligands containing mercapto groups,

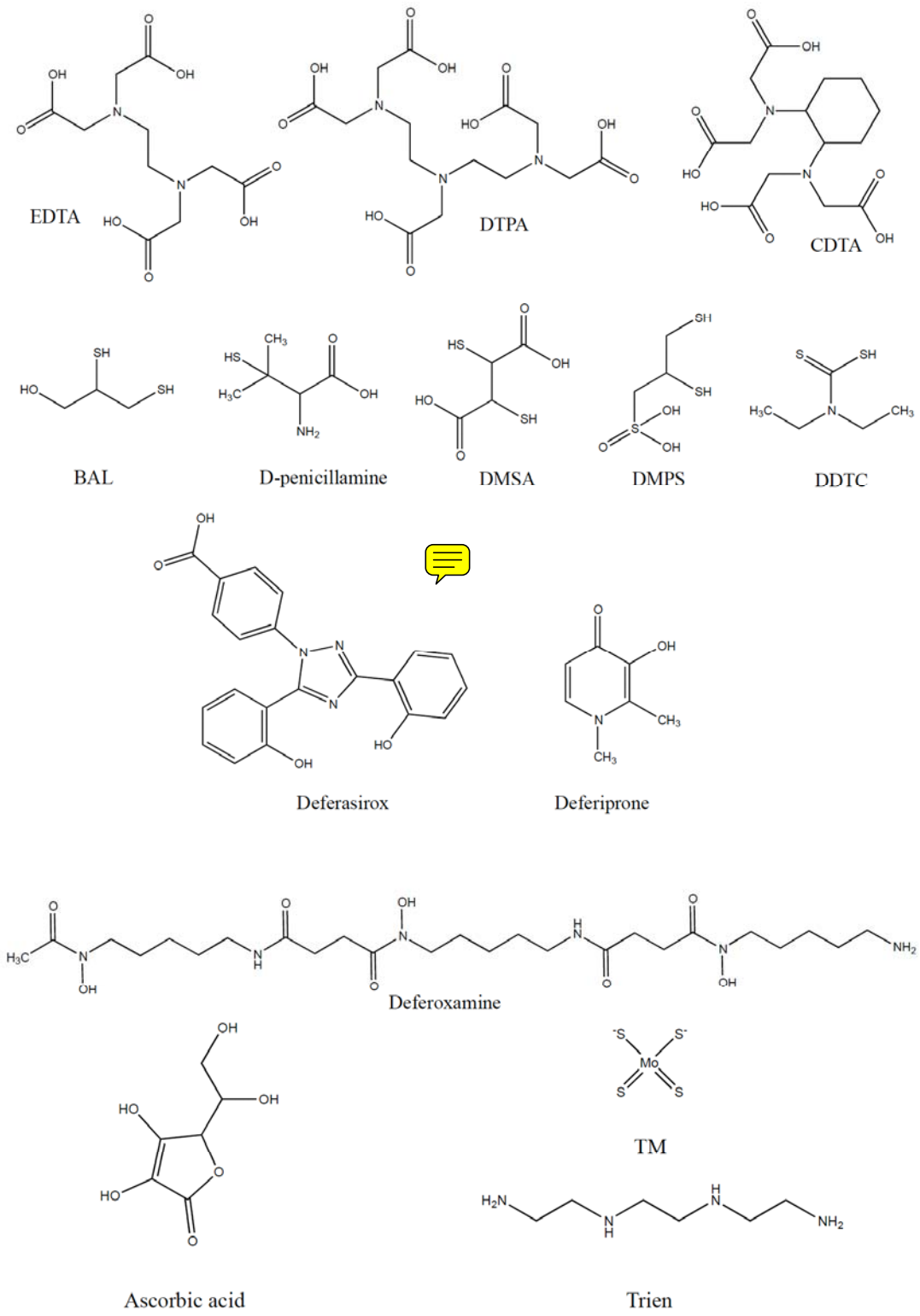
iii. Ligands with oxygen coordinating groups,

iv. Dithiocarbamates.

The most representative chelators are presented in Scheme **1**. Each chelator (or a family) can act on specific metal ions, according to the chemical characteristic of coordinating groups. The ligands allowed for chelation therapy for each metal ion are indicated in Table **1**, with their pMe values, or a symbol whenever literature does not report any equilibrium study for them.

The stability and the protonation constants used to calculate pMe values were obtained from IUPAC Stability Constant Database [21], which reports all literature data up to 2006. Table **1** gives evidence that about half of chelators are used on the basis of biomedical studies (animal or human), lacking thermodynamic characterization of the metal ligand interaction.

The diffuse utilization of soft ligands containing –SH groups, irrespective of the hard/soft character of the involved metal ion, can also be remarked; the percentage of drugs used without chemical knowledge of complex stability increases to 65% for SH-containing ligands. An examination of the pMe values shows extremely high values for mercury



Scheme (1). Representative ligands for toxic metal ions in clinical use for chelation therapy [1].

and bismuth with thiols and for iron with oxygen-based ligands.

The strong interaction of mercury and, in a minor extent, of bismuth with the –SH groups, determines their toxicity *in vivo*; it is furthermore utilized by chelators to extract these two metal ions from the endogenous molecules. The high pMe values of oxygen containing ligands for iron(III) are instead the result of the great efforts over the last forty years by academic and industrial researchers, due to the significant health and social problems caused by iron overload diseases.

The chelators in use are the outcome of a selection of hundreds of potential molecules. The research on iron chelators has led to the proposal of a different scientific approach to obtain new ligands for metal decorporation. In fact, an increasing preponderance should be given at earlier discovery stages to the chemistry-based knowledge of the compounds, at the expenses of biology-driven programs. Thus, firstly the compounds are designed and in silico screened for the druggability and pharmacokinetic issues, and for molecular targeting ability to increase the drug concentration at the disease site. Afterwards, selected compounds are synthesized and then subjected to initial screening procedures involving chemical and basic toxicological tests to confirm their suitability for further chemical studies, before embarking on expensive biological screening. These should involve estimations of the lipophilicity and water solubility of both the chelator and its metal complex, and of the chelator affinity for the target metal ions at physiological pH as well as for other endogenous metal ions. In fact, interactions of chelators with metal ions other than the ones of interest will be crucial for the drug bioavailability and to avoid drug side effects. Therefore, the thermodynamic and kinetic parameters, governing each of these interactions, are essential to establish the level of efficacy and safety of any chelating drug or of any metal complex for clinical treatment. Consequently, there is an urgent need for the rigorous determination of the thermodynamic and kinetic stability of the complexes formed with endogenous metal ions such as the divalent Cu, Zn, Ca, Mg and the trivalent Fe, under standardized conditions that mimic the biological fluid. In some cases biological activity may be dependent on other factors due to direct or indirect metal interaction with the various intracellular or extra cellular molecules, including endogenous chelators and other bio-molecules. Interactions with metal ions other than the toxic metal ion will determine bioavailability; also, the thermodynamic and kinetic parameters governing each of these interactions are determinant for the efficiency of chelators in clinical applications. Besides, interactions with high molecular mass components of biological fluids (e.g. the serum transporters albumin or transferrin) or membrane components will highly influence their bioavailability and bio-distribution. These data are important to have a detailed picture of the species distribution *in vivo*.

Table 1. Recommended Chelators for the Clinical Treatment of Each Metal Ion Toxicity.

	Al	As	Be	Bi	Cd	Co	Cu	Fe^{III}	Hg	HgR^{+}	Mn	Ni	Pb	Pt	Sb	Sn	VO^{2+}
EDTA					14.7	14.7					10.7		16.2				16.9
DTPA					15.8	16.1					11.1						
CDTA											14.3						
BAL		✧		✧					40.3				✧		✧	6	✧
D-penicillamine		✧					x		35.3	15.6			10.0				8.5
DMSA			✧	28.1	✧	✧			✧				11.5	7.2	✧	✧	✧
DMSA esters					✧												
DMPS			✧	✧					38.3	16.6					✧		✧
Tetrathiomolybdate							x										
Ascorbic acid																	6
Deferiprone	16.4							19.4									
Deferoxamine	19.3							26.6									✧
Deferasirox	✧							23.5									
DDTC					✧							7.9	✧				
Trien							19.6										

These are indicated in the Table with the numerical value of pMe whenever the relative stability constants are reported in literature, or with the symbol ✧ when no constants were found and with x when metal ligand interaction implies different action mechanisms, as redox reactions or mixed ligand-protein-metal complex formation.

THERMODYNAMIC CORRELATIONS

Some considerations related to the interaction between chelators and toxic metal ions will be developed in the following. A fundamental work by Hancock and Martell [22] gave evidence that many thermodynamic relationships between stability constants can be revealed, of great utility both in explaining the chemical behaviour of metals and ligands, and in forecasting the chemical behavior from related information. The high correlation between the stability constants of Fe^{III} and Al^{III} towards the same ligands has been demonstrated [23]. This correlation can help in the selection, based on the large amount of knowledge acquired for iron, of proper drugs for the treatment of aluminium toxicity. A similar approach can be applied to study the competition between endogenous and toxic metal ions for the same chelating agent. Actually, chelating agents can perturb the homeostatic equilibrium of essential metal ions: the effects of EDTA on calcium homeostasis, which in the early applications of this chelator produced unexpected consequences due to calcium depletion, are paradigmatic.

A speciation analysis of chelators for cadmium intoxication can give answer to a number of questions: is there competition between cadmium, soft metal ion, and essential zinc, borderline metal ion? What effects can this competition produce? In order to answer the first question, 54 stability constants for 1: 1 different cadmium complexes were compared to the corresponding ones for zinc. The IUPAC stability constants database allows to easily find all the literature complex formation constants and to select those determined in homogeneous experimental conditions (solvent, temperature, ionic strength). These data often present a high variability among different authors, mainly when measured by non-specialists of solution equilibria. Nevertheless the use of a huge amount of data allows to gain statistically reliable correlations, which can be furthermore favored by limiting for each ligand the comparison to results by the same author. The stability constants of cadmium ($\log K_{Cd}$) *vs* those of zinc are reported in (Fig. **1**). The data points are roughly distributed around a straight line through origin with slope 0.979, and more than 90% of data points are included between the two dashed straight lines with slope 0.979 x (1±0.15), i.e. 1.13 and 0.832.

This implies that ratios between the formation constants for cadmium and zinc complexes range between the values of these two slopes, i.e. the constants for the two metal ions are almost similar and each chelator for toxic cadmium acts also on endogenous zinc. To answer the second question on the effects produced by the competition between two metal ions for the same ligand, 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) was chosen to exemplify the behavior of toxic cadmium in the presence of physiological zinc.

DOTA, a macrocyclic chelator with four pendant acetate groups, is characterized by comparable formation constants for the two metal ions ($\log K_{Zn}$ = 18.9 and $\log K_{Cd}$ = 19.0 according Stetter and Frank [24]). The more composite complex formation scheme presented by Chaves *et al.* [25] for the same systems allows to calculate similar pM values

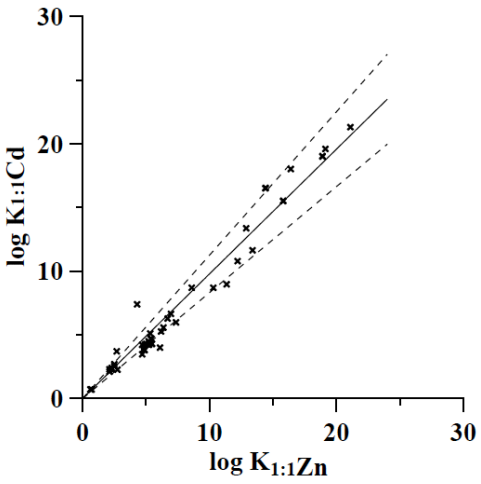


Fig. (1). Stability constants of cadmium 1:1 complexes with different ligands vs. those of the corresponding zinc complexes.

15.2 and 15.0 for Cd and Zn, respectively. To estimate the behaviour of DOTA as a chelator for cadmium intoxication, the following concentrations were chosen as representative of those in the blood stream: cadmium 10^{-6} M, corresponding to an extremely toxic situation, i.e. 112 μg/L, ligand 10^{-5} M (20 mg of assumed drug, completely adsorbed and circulating in 5.2 litres of blood) and zinc 10^{-4} M, as reported in Reference Man [26]. Using the program HYSS[2] it was possible to draw the speciation plots reported in the left part of (Fig. **2**). A tenfold excess of DOTA binds less than 20% of cadmium, being the major part of chelator involved in zinc coordination. TETA, 1,4,8,11-tetraazacyclododecane-1,4,8,11-tetraacetic acid, a chelator similar to DOTA with a larger macrocyclic cavity, presents for both metal ions lower stability constants [25]. The complex formation curves (Fig. **2**, right part) are shifted to higher pH values than those of the corresponding curves for DOTA, being this behaviour more marked for zinc. The values pCd 13.1 and pZn 11.5 for TETA are indicative of a lower binding capacity towards both metal ions, deriving from the larger macrocyclic cavity of TETA. The difference of 1.6 log units between pCd and pZn implies instead a marked selectivity of TETA for cadmium. This is clearly shown by the speciation plot in the right lower part of (Fig. **2**), where the formed CdL complex at physiological pH 7.4 now reaches the 80% of total cadmium with respect to the 20% with DOTA. These results point out that TETA, despite the lower stability of its cadmium complexes, is a better chelator for cadmium than DOTA thanks to its selectivity.

SELECTION OF BEST LIGANDS FOR A NUMBER OF TOXIC METAL IONS

The influence of coordinating groups on the chelating properties of the ligands has been quantitatively evaluated. In this respect, for a number of toxic metal ions the 10-20 ligands characterized by the highest stability constants were looked for in the database. The protonation constants of these ligands were then acquired and the corresponding pMe

[2] www.hyperquad.co.uk/hyss.htm

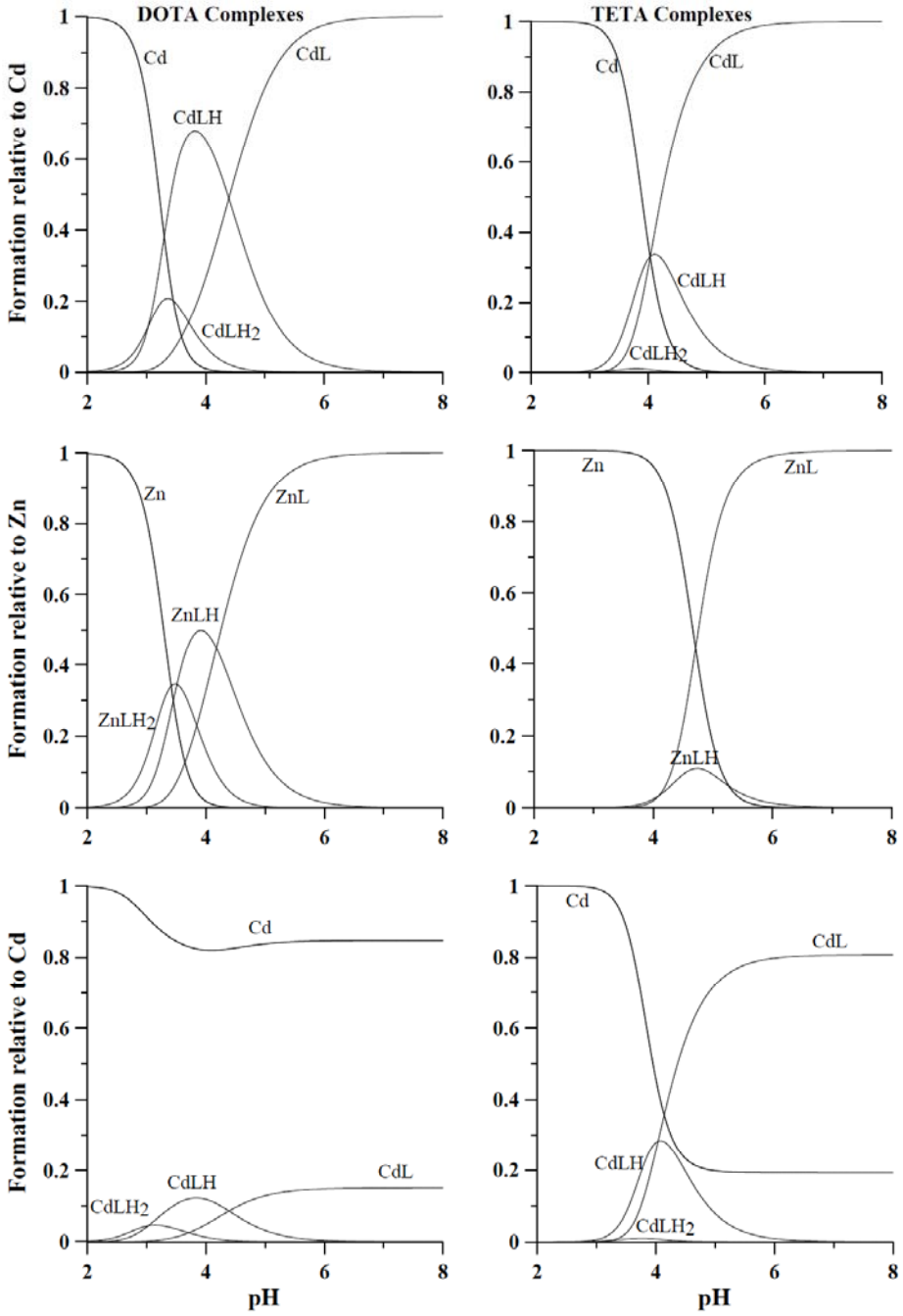


Fig. (2). Speciation plots of (left part) cadmium and zinc in presence of DOTA $1x10^{-5}$ M at the concentration Cd $1x10^{-6}$ M and no Zn (upper), Zn $1x10^{-6}$ M and no Cd (medium) and Cd $1x10^{-6}$ M and Zn $1x10^{-4}$ M (lower); (right part) cadmium and zinc in presence of TETA $1x10^{-5}$ M at the concentration Cd $1x10^{-6}$ M and no Zn (upper), Zn $1x10^{-6}$ M and no Cd (medium) and Cd $1x10^{-6}$ M and Zn $1x10^{-4}$ M (lower).

values calculated. The five highest pMe values for each metal ion are reported in Table **2**, together with ligand acronyms, indicating their coordinating groups. This procedure identifies the strongest ligands for each metal ion, not necessarily the best chelators for metal intoxication. No evaluation was in fact carried out on the multiple requisites that a metal chelator must possess for clinical use. Nonetheless, the entity of pMe which can be reached for each metal ion can be evaluated from these results. They furthermore identify the most apt families of ligands for any given metal ion.

The following points can be remarked, based on data presented in Table **2**:

- The highest pMe values for the soft Hg^{2+} are always observed with SH-containing ligands, confirming the results in Table **1**. No problem of selectivity is thus presented in the chelation therapy of mercury intoxication;

Table 2. The Five Highest pMe Values for Each Metal Ion are Reported, Together with the Acronyms of the Corresponding Ligands. The Capital Letter of Acronyms are Indicative of the Ligand Coordinating Properties, the Number Allows to Identify the Ligands, whose Structures are Reported in the Supporting Material.

	pMe					Ligand[a]
Be	16.5	12.0	11.7	11.6	11.2	P3 - A1 - A2 - C3 - P2
Bi	22.9	20.4	16.6	16.4	15.8	C1 - T3 - E4 - E1 - T2
Cd	19.5	17.7	16.8	16.1	15.5	M7 - S7 - E9 - S8 - E8
Co	20.6	19.0	17.4	17.0	16.2	M3 - M5 - E7 - T4 - E9
Hg	40.6	38.5	37.4	35.3	35.0	S1 - S4 - S6 - S2 - S5
Mn	21.0	19.1	16.0	14.2	13.2	M2 - M1 - E9 - M6 - M4
Ni	22.7	21.5	20.2	20.0	17.5	Q1 - E7 - Q2 - T5 - E3
Pb	17.3	16.6	16.0	15.6	15.2	E3 - M6 - E1 - E2 - S6
Sn	17.9	15.4	14.1	13.7	12.7	P6 - O2 - P4 - P5 - P6
Th	26.0	23.0	18.0	17.4	17.2	P1 - E2 - P7 - O3 - E5
VO^{2+}	16.9	16.5	16.1	15.7	15.7	E1 - Q3 - E6 - C2 - Q4

[a] A = aminoacid; C = catechol-containing ligand; E = polyamino-carboxylic ligands; M = macrocycles; O = oxygen-containing ligands: P = disphosphonic acids; Q = quinolones; S = SH-containing ligands; T = polyamides.

- The second soft metal ion, cadmium, is characterized by pMe values about one half than those of mercury. Two of the best ligands for cadmium are SH-containing ligands, two polyaminocarboxylic ligands, and the best is a macrocyclic ligand. Macrocyclic ligands give the opportunity of modulating the selectivity toward a target metal ion both with the cavity dimensions, and with the addition of coordinating groups with the proper hard/soft characteristics;
- The hard thorium is favoured by hard ligands as bisphophonates, deferoxamine and polyaminocarboxylic acids;
- Beryllium complexation, with significantly lower pMe values, is also favoured by hard bisfosfonates, cathecols, and by the two bicarboxylic aminoacids glutamic and aspartic acids;
- Thin exhibits a hard character, preferring bisphophonates and O-containing ligands;
- Borderline metal ions present more diversified situations with a variety of possible coordinating groups. Among them, the behaviour of manganese drastically prefers macrocyclic ligands.

These results, even though indicative of the metal ion behaviour, can be influenced by different factors, as the experimental complexity that prevented the measurement of reliable stability constants for a specified metal with a given class of ligands.

CONCLUSIONS

Chelation therapy, started at the end of the Second World War with the introduction of BAL, can still be considered a field of research that can be largely developped. In fact, the officially allowed drugs in major use, apart BAL nowadays of minor application, are DMSA and DMPS (introduced in fifties in China and in Soviet Union) and EDTA. These drugs, excellent for various toxic metals, are surely a compromise in most situations.

The large research efforts devoted to iron overload disease, a syndrome largely diffuse all over the world, have led to excellent results regarding the drugs in use and the route of their administration, and have improved the knowledge on the role of iron and chelators in living organisms. A variety of features that can be of the greatest utility in chelation therapy are now clarified, as the target organs of chelators, the thermodynamic and kinetic competition with endogenous ligands, the metabolism, and the factors affecting oral absorption and bioavailability. The large development of chelators for iron overload has opened new clinical treatments for different pathologies, as an example in cancer therapy [27,28]. On the contrary, Wilson's disease, due to copper overload, has had a lower research interest, due to its lower diffusion, and the three chelators in use (D-penicillamine, triethylenetetramine and tetrathiomolybdate) have all been introduced over the years thanks to the constant dedication of J. M. Walshe [29-31]. The interest for copper chelation is increasing thanks to its use in the treatment of neurodegenerative diseases [32-33] and to the promising applications in the cure of diabetes [34]. Large research efforts are moreover exerted towards sequestering agents for plutonium and other actinide elements, due to their large military and strategic interest; one-third of the world's electrical power is produced using nuclear fuels, and the challenge of limiting the environmental and health effects from accidental or sabotage contamination is of increasing concern [35]. The remaining elements causing critical problems in different populations require large research efforts, using the most up to date possibilities presented by *in silico* calculations, as far as the chemical research is implied. These methods allow to design

proper chelating molecules on the basis of different restraints as high stability, correct lipophilicity for oral administration and bioavailability, toxicity of ligands and metal complexes. The best molecules obtained by this sort of selection have to be synthesized and tested in a joined effort of chemical and biomedical researchers.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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SUPPLEMENTARY MATERIALS

Supplementary material is available on the publisher's web site along with the published article.

Structures and acronyms of the ligands that give the best pMe values, reported in Table **2**, for the different metal ions.

ABBREVIATIONS

BAL stands	=	British anti-Lewisite (2,3-dimercaptopropanol)
CDTA	=	cyclohexanediaminetetraacetic acid
DTPA	=	diethylenetriaminepentaacetic acid
DDTC	=	sodium diethyldithiocarbamate
DMPS	=	2,3-dimercaptopropane-1-sulphonate
DMSA	=	meso-2,3-dimercaptosuccinic acid
DMPS	=	sodium 2,3-dimercaptopropane-1-sulphonate
EDTA	=	ethylenediaminotetracetic acid
Trien	=	triethylene tetraamine

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