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A Comparative Study of the Chelating Effect Between Textured Soya Aqueous Extract and EDTA on Fe^{3+} , Pb^{2+} , Hg^{2+} , Cd^{2+} and Ni^{2+} Ions

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1. Introduction

Metal pollution of soils, water, foods, and the environment is a grave problem. Various in-situ and ex-situ remediation techniques have been employed, e.g., solidification, stabilization, flotation, soil ashing, electroremediation, bioleaching, and phytoremediation (Mulligan, 2001). One remediation technique is ex-situ soil washing using chelating agents. The soil is removed from the site, treated in a closed reactor with the chelating agent, and returned to the site after separation of the extraction solution that now contains the extracted heavy metals (Peters & Hazard, 1999). The problem is that the used chelating agent is not a natural compound. For that reason we propose the use of the textured soya extract, which is environmentally friendly, as a natural chelating agent.

EDTA (ethylenediamine tetraacetic acid) and its salts are substituted diamines. HEDTA (hydroxyethyl ethylenediamine triacetic acid) and its trisodium salt are substituted amines. These ingredients function as chelating agents in cosmetic formulations. The typical concentration of use of EDTA is less than 2%, with the other salts in current use at even lower concentrations. The lowest dose reported to cause a toxic effect in animals was 750 mg/kg/day.

These chelating agents are cytotoxic and weakly genotoxic, but not carcinogenic. Oral exposures to EDTA produced adverse reproductive and developmental effects in animals. Clinical tests reported no absorption of an EDTA salt through the skin. These ingredients are likely, however, to affect the passage of other chemicals into the skin because they will chelate calcium. Exposure to EDTA in most cosmetic formulations, therefore, would produce systemic exposure levels well below those seen to be toxic in oral dosing studies. Exposure to EDTA in cosmetic formulations that may be inhaled, however, was a concern. An exposure assessment done using conservative assumptions predicted that the maximum EDTA dose via inhalation of an aerosolized cosmetic formulation is below that shown to produce reproductive or developmental toxicity. Because of the potential to increase the penetration of other chemicals, formulators should continue to be aware of this when combining these ingredients with ingredients that previously have been determined to be safe, primarily because they were not significantly absorbed. Based on the available data,

the Cosmetic Ingredient Review Expert Panel found that these ingredients are safe as used in cosmetic formulations.

Ethylenediaminetetraacetic acid (EDTA) is a very effective chelating agent but has the disadvantage that is quite persistent in the environment owing to its low biodegradability. For that reason different chelating agents were investigated, such as [S,S]-ethylenediaminedisuccinic acid, iminodisuccinic acid, methylglycine diacetic acid, etc. but the problem is the dependence of the pH on the extraction efficiency. (Tandy et al., 2004)

Major industrial processes involve the sequestration of metal ions in an aqueous solution. In the textile industry, this prevents metal ion impurities from modifying colors of dyed products. In the pulp and paper industry, EDTA inhibits the ability of metal ions, especially Mn^{2+} , to catalyze disproportionate amounts of hydrogen peroxide, which is used in "chlorine-free bleaching." Similarly, EDTA is added to some foods as a preservative or stabilizer to prevent a catalytic oxidative discoloration which is catalyzed by metal ions.

Oral exposures have been noted to cause reproductive and developmental effects (Elliot & Brown, 1989). The same study by Lanigan also found that both dermal exposure to EDTA in most cosmetic formulations and inhalation exposure to EDTA in aerosolized cosmetic formulations would produce systemic effects below those seen to be toxic in oral dosing studies (Lanigan & Yamarik, 2002).

A crucial factor to be considered in comparing studies on chelating agent is the pH of the extraction solution. While extraction was investigated at various pH values in some studies (Elliot & Brown, 1989 ; Pichtel, 1998; Pichtel, 1997; Kim, 2003; Ghestem, 1998), some only stated the pH of the solution (Reed, 1996; Cline, 1995; Van Benschoten, 1997), while others did not consider pH at all (Pichtel, 2001;). In general, the lower the pH of the chelating agent solution, the greater is the extraction efficiency of the toxic metals.

The history and chemistry of the industrial use of natural products and their derivatives have a rich technological tradition. Many modern products, such as plastics, fuels, chemical intermediates and fibers, find their origins in natural products derived from plants and animals. Given the recent social emphasis on the environment and resource renewability, utilizing natural materials as potential resources for industrial products receives a ready welcome. Among the most versatile of raw materials is the soybean. (Liu, 1997)

Together, the oil and protein contents of dry soybeans account for about 60% of the weight; protein being 40% and oil 20%. The remainder consists of 35% carbohydrate and about 5% ash. Most soy protein is a relatively heat-stable storage protein. This heat stability enables the manufacture of soy food products requiring high temperature cooking, such as tofu, soy milk and textured vegetable protein (soy flour).

This article focuses on the application of natural "green" textured soya extract as a substitute for EDTA in its role as a metals-sequestering agent in foods.

2. Antecedents

2.1 What is a chelating agent?

The word chelation is derived from Greek, meaning "claw." The ligands lie around the central atom like the claws of a lobster.

The IUPAC definition of chelation is the formation or presence of two or more separate bindings between a polydentate (multiple bonded) ligand and a single central atom. Usually these ligands are organic compounds and are called chelants, chelators, chelating agents, or sequestering agents. (IUPAC)

The ligand forms a chelate complex with the substrate. Chelate complexes are contrasted with coordination complexes composed of monodentate ligands, which form only one bond with the central atom. (Morgan & Drew, 1920)

The terms bidentate (or didentate), tridentate, tetradentate,... multidentate are used to indicate the number of potential binding sites of the ligand, at least two of which must be used by the ligand in forming a "chelate". For example, the bidentate ethylenediamine forms a chelate with Cu (I) in which both nitrogen atoms of ethylenediamine are bonded to copper. (The use of the term is often restricted to metallic central atoms). (Kramer, Cotter-Howells, Charnock, Baker & Smith. 1996)

Chelants, according to ASTM-A-380, are "chemicals that form soluble, complex molecules with certain metal ions, inactivating the ions so that they cannot normally react with other elements or ions to produce precipitates or scale".

2.2 The chelate effect

The increased stability of complexes containing chelating ligands over those containing comparable monodentate ligands can be envisaged as having the following physical basis. Suppose we have a metal ion in solution, and we attach to it a monodentate ligand, followed by a second monodentate ligand, figure 1. These two processes are completely independent of each other. But suppose we have a metal ion and we attach to it one end of a chelating ligand (it is reasonable to assume that when we put a chelate ligand onto a metal, it happens in a stepwise fashion, i.e. one end attaches first and then the other end). The point is that the attachment of the second end of the chelate is now no longer an independent process: once one end is attached, the other end, rather than floating around freely in solution, is anchored by the linking group in reasonably close proximity to the metal ion, and is therefore more likely to join onto it than a comparable monodentate ligand would be.

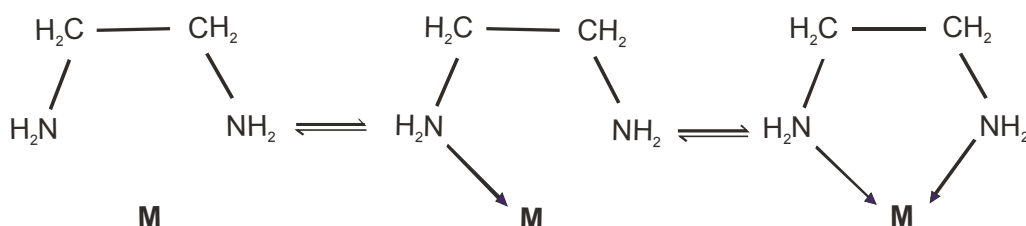


Fig. 1. Complexes formation.

The figure 2 shows the EDTA ligand binding to a central copper ion.

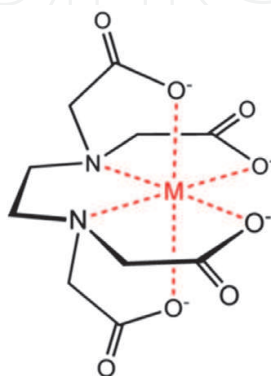


Fig. 2. Copper ion complexes with EDTA.

Amino acids are classified into different ways based on polarity, structure, nutritional requirement, metabolic fate, etc.

Generally used classification is based on polarity. Based on polarity amino acids are classified into four groups.

- *Non-polar amino acids.- They have equal number of amino and carboxyl groups and are neutral. These amino acids are hydrophobic and have no charge on the 'R' group. The amino acids in this group are alanine, valine, leucine, isoleucine, phenyl alanine, glycine, tryptophan, methionine and proline.*
- *Polar amino acids with no charge.- These amino acids do not have any charge on the 'R' group. These amino acids participate in hydrogen bonding of protein structure. The amino acids in this group are - serine, threonine, tyrosine, cysteine, glutamine and asparagine.*
- *Polar amino acids with positive charge.- Polar amino acids with positive charge have more amino groups as compared to carboxyl groups making it basic. The amino acids, which have positive charge on the 'R' group, are placed in this category. They are lysine, arginine and histidine.*
- *Polar amino acids with negative charge.- Polar amino acids with negative charge have more carboxyl groups than amino groups making them acidic. The amino acids, which have negative charge on the 'R' group are placed in this category. They are called as dicarboxylic mono-amino acids. They are aspartic acid and glutamic acid.*

Chelates of glycine with cations such as iron, zinc and copper have been fully studied. The chelates usually contain two moles of ligand (glycine) and one mol of metal as demonstrated in the figure 3.

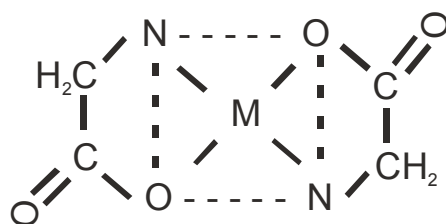
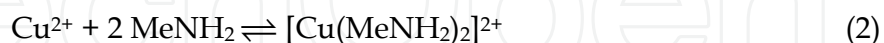


Fig. 3. Chelate of glycine with some metal M.

Consider the two equilibriums, in an aqueous solution, between the copper (II) ion, Cu^{2+} and ethylenediamine (en) on the one hand and methylamine, MeNH_2 on the other.



In (1) the bidentate ligand ethylene diamine forms a chelate complex with the copper ion. Chelation results in the formation of a five-member ring. In (2) the bidentate ligand is replaced by two monodentate methylamine ligands of approximately the same donor power, meaning that the enthalpy of formation of $\text{Cu}-\text{N}$ bonds is approximately the same in the two reactions. Under conditions of equal copper concentrations and when the concentration of methylamine is twice the concentration of ethylenediamine, the concentration of the complex (1) will be greater than the concentration of the complex (2). The effect increases with the number of chelate rings so the concentration of the EDTA complex, which has six chelate rings, is much higher than a corresponding complex with two monodentate nitrogen donor ligands and four monodentate carboxylate ligands. Thus, the phenomena of the chelate effect are a firmly established empirical fact.

The thermodynamic approach to explaining the chelate effect considers the equilibrium constant for the reaction: the larger the equilibrium constant, the higher the concentration of the complex.

The formation of a chelant compound is an equilibrium reaction as shown in the reaction (3)



Metal Ligand Metal-chelate

The reaction rates of the forward and reverse reactions are generally not zero but, being equal; there are no net changes in any of the reactant or product concentrations. Since forward and backward rates are equal:

$$k_1 [\text{M}^{n+}]^a [\text{L}]^b = k_2 [\text{ML}]^c \quad (4)$$

and the ratio of the rate constants is also a constant, now known as an equilibrium constant.

$$K = \frac{[\text{ML}]^c}{[\text{M}^{n+}]^a [\text{L}]^b} \quad (5)$$

The concentration of ligand does not change during the reaction. For that reason the equilibrium constant can be expressed only in function of metal ion and metal-complex, as showing in the equation 6.

$$K = \frac{[\text{ML}]^c}{[\text{M}^{n+}]^a} \quad (6)$$

2.3 Common chelating agents

There are many chelating agents used in the industry as Na, Ca-ethylenediaminetetraacetic (EDTA), diethylenetriaminepentaacetic acid (DTPA), nitriloacetic acid, ethylene glycol-bis(8-aminoethyl)tetraacetic acid (EGTA), D,L-mercaptosuccinic acid (MSA), meso-2,3-dimercaptopropanesuccinic acid (DMSA), D,L-2,3-dimercaptopropane-1-sulfonic acid (DMPS), penicillamine (PA), N-acetylpenicillamine (NAPA), vitamins as: thiamine (B1), pyridoxine (B6), cobalim (B12) and ascorbic acid, and many more. The most common is EDTA.

2.4 Natural chelating agents

Virtually all biochemicals exhibit the ability to dissolve certain metals cations. Thus, proteins, polysaccharides, and polynucleic acids are excellent polydentate ligands for many metal ions. In addition to these adventitious chelators, several biomolecules are produced to specifically bind certain metals. Histidine, malate and phytochelatin are typical chelators used by plants. (U Kramer, 1996; Jurandir, 2006 & Suk-Bomg Há, 1999)

Virtually all metalloenzymes feature metals that are chelated, usually to peptides or cofactors and prosthetic groups (Lippard & Berg, 1994). Such chelating agents include the porphyrin in hemoglobin and chlorophyll. Many microbial species produce water-soluble pigments that serve as chelating agents, termed siderophore. For example, species of *Pseudomonas* are known to secrete pyocyanin and pyoverdine that bind iron. Enterobactin, produced by *E. coli*, is the strongest chelating agent known.

In earth science, chemical weathering is attributed to organic chelating agents, *e.g.* peptides and sugars that extract metal ions from minerals and rocks. (Michael) Most metal complexes in the environment and in nature are bound in some form of chelate ring, *e.g.* with a humic acid or a protein. Thus, metal chelates are relevant to the mobilization of metals in the soil, the uptake and the accumulation of metals into plants and micro-organisms. Selective chelating of heavy metals is relevant to bioremediation *e.g.* removal of ^{137}Cs from radioactive waste. (Prasad, 2001)

2.5 Applications

Chelators are used in chemical analysis as water softeners, and are ingredients in many commercial products such as shampoos and food preservatives. Citric acid is used to soften water in soaps and laundry detergents. A common synthetic chelator is EDTA. Phosphonate are also well known chelating agents. Chelators are used in water treatment programs and specifically in steam engineering, *e.g.*, boiler water treatment system.

Chelation therapy is the use of chelating agents to detoxify poisonous metal agents such as mercury, arsenic, and lead by converting them to a chemically inert form that can be excreted without further interaction with the body, and was approved by the U.S. Food and Drug Administration in 1991. In alternative medicine, chelation is used as a treatment for autism, though this practice is controversial due to an absence of scientific plausibility, lack of FDA approval, and its potentially deadly side-effects. (Doja & Can, 2006).

Though they can be beneficial in cases of heavy metal poisoning, chelating agents can also be dangerous. The U.S. CDC reports that use of disodium EDTA instead of calcium EDTA has resulted in fatalities due to hypocalcemia. (U. S. Center for Disease Control)

Homogeneous catalysts are often chelated complexes. A typical example is the ruthenium (II) chloride chelated with BINAP (a bidentate phosphine) used in *e.g.* Noyori asymmetric hydrogenation and asymmetric isomerization. The latter has the practical use of manufacture of synthetic mentol.

Products such as Evapo-Rust are chelating agents sold for the removal of rust from iron and steel.

2.6 Chemical composition of the soybean seed

Together, oil and protein content account for about 60% of dry soybeans by weight; protein at 40% and oil at 20%. The remainder consists of 35% carbohydrate and about 5% ash. Soybean cultivars comprise approximately 8% seed coat or hull, 90% cotyledons and 2% hypocotyl axis or germ.

Most soy protein is a relatively heat-stable storage protein. This heat stability enables soy food products requiring high temperature cooking, such as tofu, soy milk and textured vegetable protein (soy flour) to be made.

The principal soluble carbohydrates of mature soybeans are the disaccharide sucrose (range 2.5–8.2%), the trisaccharide raffinose (0.1–1.0%) composed of one sucrose molecule connected to one molecule of galactose, and the tetrasaccharide stachyose (1.4 to 4.1%) composed of one sucrose connected to two molecules of galactose. While the oligosaccharides raffinose and stachyose protect the viability of the soy bean seed from desiccation (see above section on physical characteristics) they are not digestible sugars and therefore contribute to flatulence and abdominal discomfort in humans and other monogastric animals; compare to the disaccharide trehalose.

Since soluble soy carbohydrates are found in the whey and are broken down during fermentation, soy concentrate, soy protein isolates, tofu, soy sauce, and sprouted soy beans are without flatus activity. On the other hand, there may be some beneficial effects to ingesting oligosaccharides such as raffinose and stachyose, namely, encouraging indigenous bifidobacteria in the colon against putrefactive bacteria.

The insoluble carbohydrates in soybeans consist of the complex polysaccharides cellulose, hemicellulose and pectin. The majority of soybean carbohydrates can be classed as belonging to dietary fiber.

The following Table 1 shows the composition of mature, raw soybean seeds.

Energy	1,866 kJ (446 kcal)
Carbohydrates	30.16 g
Sugars	7.33 g
Dietary fiber	9.3 g
Fat	19.94 g
Saturated	2.884 g
monounsaturated	4.404 g
polyunsaturated	11.255 g
Protein	36.49 g
Tryptophan	0.591 g
Threonine	1.766 g
Isoleucine	1.971 g
Leucie	3.309 g
Lysine	2.706 g
Methionine	0.547 g
Phenylalanine	2.122 g
Tyrosine	1.539 g
Valine	2.029 g
Arginine	3.153 g
Histidine	1.097 g
Alanine	1.915 g
Aspartic acid	5.112 g
Glutamic acid	7.874 g
Glycine	1.880 g
Proline	2.379 g
Serine	2.357 g
Water	8.54 g
Vitamin A equiv	1 µg
Vitamin B ₆	0.377 mg
Vitamin B ₁₂	0 µg
Vitamin C	6.0 mg
Vitamin K	47 µg
Calcium	277 mg
Iron	15.70 mg
Magnesium	280 mg
Phosphorus	704 mg
Potassium	1797 mg
Sodium	2 mg
Zinc	4.89 mg

Source: USDA Nutrient database.

Table 1. Composition of soybean, mature, rawNutritional value per 100 g (3.5 oz)

2.7 How soybeans are used

When the farmer sells soybeans to a grain dealer, the beans may then go to a number of ultimate destinations. When processed, a 60-pound bushel will yield about 11 pounds of crude soybean oil and 47 pounds of soybean meal. Soybeans are about 18% oil and 38% protein. Because soybeans are high in protein, they are a major ingredient in livestock feed. Most soybeans are processed for their oil and protein for the animal feed industry. A smaller percentage is processed for human consumption and made into products including soy milk, soy flour, soy protein, tofu and many retail food products. Soybeans are also used in many non-food (industrial) products.

Fuel for diesel engines can be produced from soybean oil with simple processing. Soy biodiesel is cleaner burning than petroleum-based diesel oil. Its use reduces particulate emissions, and it is non-toxic, renewable and environmentally friendly. Soy crayons made by the Dixon Ticonderoga Company replace the petroleum used in regular crayons with soy oil making them non-toxic and safer for children. Candles made with soybean oil burn longer but with less smoke and soot.

Soy oil produces an environmentally friendly solvent that safely and rapidly removes oil from creeks, streams and shorelines without harming people, animals, and the environment. Soy is an ingredient in many industrial lubricants, solvents, cleaners, and paints. Soy ink is superior to petroleum-based inks because soy ink is not toxic, is renewable and also environmentally friendly. Furthermore, it cleans up easily. Soy-based lubricants are as good as petroleum-based lubricants, but can withstand higher heat. More importantly, they are non-toxic, renewable and environmentally friendly. Soy-based hydraulic fluid and rail flange lubricants are among the more recent products developed with check-off funds.

Soy-based foams are currently being developed for use in coolers, refrigerators, automotive interiors and even footwear. Beginning in October 2007, Ford Mustangs rolled off the production line with soy flexible foam in the seats. (2009 Annual Report of the North Carolina Soybean Producers Association)

2.8 Textured soya

Textured or textures vegetable protein (TVP), also known as textured soya protein (TSP), soy meat, or soya meat is a meat analogue or nutritious meat extender made from defatted soy flour, a by-product of extracting soybean oil. It is quick to cook, with a protein content equal to that of the meat, and contains no fat. (Riaz, 2006)

TVP is made from a mixture of proteins extracted primarily from soybeans, but also cotton seeds, wheat, and oats. It is extruded into various shapes (chunks, flakes, nuggets, grains, and strips) and sizes, exiting the nozzle while still hot and expanding as it does so. (Foote, 1996)

TVP can be made from soy flour or concentrate, containing 50% and 70% soy protein respectively, and is relatively flavorless. Both require rehydration before use, sometimes with flavoring added in the same step. TVP is extruded, causing a change in the structure of the soy protein which results in a fibrous spongy matrix that is similar in texture to meat. In its dehydrated form TVP has a shelf life of longer than a year, but will spoil within several days after being hydrated. In its flaked form TVP can be used similarly to ground meat. (Hoogenkamp & Wallingford, Oxon, 2005; Endres, 2001)

3. Materials and synthesis

3.1 Reagents

$\text{Fe}(\text{NO}_3)_3$, NiCl_2 , CuSO_4 , HgCl_2 , CdSO_4 , $\text{Pb}(\text{NO}_3)_2$ and EDTA (Etilenediaminetetraacetic acid), purchased from Sigma-Aldrich, were used without any further purification. Textured soya was purchased. Mili-Q water (18.2 Ω) was used throughout the experiment.

3.2 Characterization

The amount of metallic ions present in the solutions was determined by using a conductivity meter.

3.3 Determination of the chelating agent in textured soya extract

The Biuret test is a chemical test used for detecting the presence of peptide bonds. In the presence of peptides, a copper (II) ion forms a violet-colored complex in an alkaline solution. Several variants on the test have been develop

In order to find the chelating component in the textured soya extract, first a textured soya extract was prepared by heating to boiling point 1000 ml of deionizer water with 30 grams of textured soy for 20 minutes. 10 ml of textured soya extract is treated with an equal volume of 1% strong base (sodium or potassium hydroxide most often) followed by a few drops of aqueous copper (II) sulfate. The solution turns violet, for that reason we can affirm that proteins are present in the textured soya extract and these are the chelating agents.

A Fehling test was made too, and the Fehling reaction was positive, in which the green color characteristic of mono-saccharides was obtained. For that reason we can affirm that mono-saccharides present in the textured soya extract are present but the amount is not significant (< 3%).

3.4 Experimental method

First, it was necessary to find a concentration of textured soya equivalent to an EDTA solution 5×10^{-4} (the maximum concentration permitted in foods). For that reason we prepared six solutions of CuSO_4 with concentrations 0.05M, 0.1M, 0.15M, 0.2M, 0.25M and 0.3M. The conductivity of each one was then measured. Next, we mixed 1 ml of each CuSO_4 solution with 10 ml of an EDTA solution 5×10^{-4} M and we measured the conductivity of each one. Several textured soya aqueous solutions were prepared by dissolving 1 grams, 2 grams, 3 grams, and 5 grams of textured soya, each one in 100 ml of water, and heating them to boiling point for 10 minutes. The fiber was then separated by filtration. Afterwards, we mixed 1 ml of each solution of CuSO_4 with 10 ml of each prepared textured soya extract solution and measured the conductivity of each sample.

With the aim of studying the comparative chelating effect between textured soya extract and EDTA on some metals, we prepared five different aqueous solutions of each metal ion, Fe^{3+} , Pb^{2+} , Hg^{2+} , Cd^{2+} , Ni^{2+} and Cu^{2+} with different concentrations, with 0.01, 0.03, 0.05, 0.07 and 0.1 grams of each salt dissolved in 10 ml of deionizer water. Then we measured their conductivity. An EDTA aqueous solution of 5×10^{-4} M was prepared. A solution of 15 grams of textured soya in 500 ml of deionizer water was heated to boiling point for 10 minutes. Afterwards, we measured the conductivity and ppm (parts of million) of each ion solution, chelant solution of textured soya extract and EDTA. In order to determine the chelating capacity of EDTA and textured soya extract, we added 0.01, 0.03, 0.05, 0.07 and 0.1 grams of each salt in 10 ml of EDTA solution, and then in the same form in 10 ml of textured soya

extract solution and we measured the conductivity of each one, using a conductivity meter. All measurements were made at room temperature and at average room pressure, and pH 7.

4. Results and discussion

Table 2 shows the conductivity and parts per million of EDTA solution (5×10^{-4} M), measured with the conductivity meter, and the four different solutions of textured soya extract prepared.

	1 gram textured soya	2 grams textured soya	3 grams textured soya	5 grams textured soya	EDTA (5×10^{-4} M)
Conductivity (μ s)	459.1	454.7	836.6	1191	63.6
ppm	308.5	303	564.6	820.3	40.51

Table 2. Conductivity y ppm of EDTA and textured soya extract solutions

Table 3 shows the resulting conductivity after mixing each of the CuSO_4 solutions with the EDTA solution and the four textured soy extract solutions. These results are the differences between the measurement of the mixture of the CuSO_4 solution with the soy chelating solution and the pure extraction solution.

Concentration CuSO_4 (M)	1 gram textured soya (μ s)	2 grams textured soya (μ s)	3 grams textured soya (μ s)	5 grams textured soya (μ s)	EDTA (μ s)
0.025	481.8	402	401.4	443	820
0.05	958.9	645.3	853.4	835	1350.4
0.1	2157.9	1857.3	1619.4	1685	2172.4
0.15	2569.9	2969.3	2479.4	2614	3057.4
0.2	3195.9	3503.3	2876.4	3340	3682.4
0.25	3407.9	4310.3	3968.4	3952	4200.4
0.3	3780.9	4836.3	4590.4	4631	4919.4

Table 3. Conductivity of EDTA solution and textured soya extract solutions with the prepared CuSO_4 solutions

Figure 4 shows graphically the results of the Table 3.

From these results, we can conclude that is necessary to prepare the textured soya extract solution by using 2 or 3 grams of textured soy in 100 ml of water, heating it to boiling point for 10 minutes and separating out the fiber by filtration.

Table 4 contains the conductivity and ppm of the aqueous EDTA solution and the aqueous textured soya extract using 3 grams of textured soya in 100 ml of deionizer water we prepared.

	Textured soya extract	EDTA (5×10^{-4} M)
Conductivity (μ s)	1414	150.3
ppm	981	67.06

Table 4. conductivity and ppm of EDTA and textured soy extract solutions.

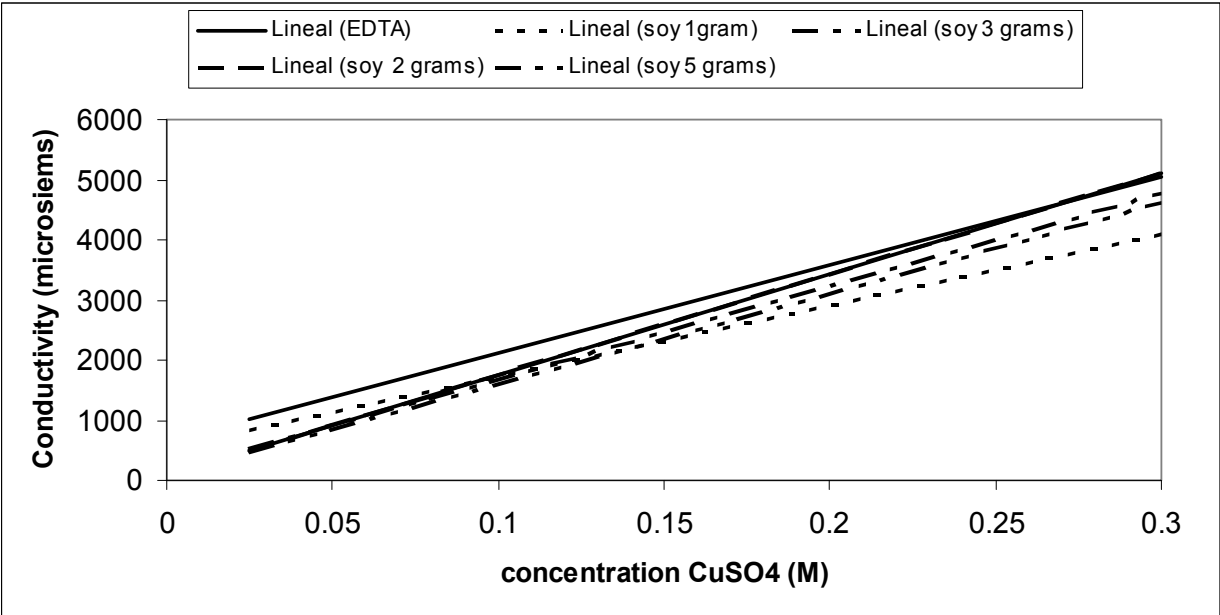


Fig. 4. Conductivity of the mixture of different textured soya extracts solutions with EDTA solution with the CuSO_4 solutions .

Table 5 shows the results of the conductivity and ppm of five aqueous Pb^{2+} solution prepared dissolved 0.01, 0.03, 0.05, 0.07 and 0.1 grams of $\text{Pb}(\text{NO}_3)_2$ in 10 ml of deionizer water, each one. In a similar process were prepared five aqueous solutions EDTA- Fe^{3+} dissolved 0.01, 0.03, 0.05, 0.07 and 0.1 grams of $\text{Pb}(\text{NO}_3)_2$ in 10 ml of aqueous EDTA solution and in the same way were prepared five aqueous solutions of textured soya extract- Pb^{2+} dissolved 0.01, 0.03, 0.05, 0.07 and 0.1 grams of $\text{Pb}(\text{NO}_3)_2$ in 10 ml of textured soya extract.

Grams $\text{Pb}(\text{NO}_3)_2$	Pb^{2+} aqueous solutions ppm (μs)		Textured soya extract- Pb^{2+} Ppm (μs)		EDTA- Pb^{2+} ($5\times10^{-4}\text{M}$) ppm (μs)	
0.01	472.45	704.54	116	160	629	907.7
0.03	1193.35	1703.34	572	762	933	1328.7
0.05	2020.35	2764.34	1332	1720	2363	2323.7
0.07	2759.35	3687.34	2016	2569	2519	3354.7
0.1	4031.35	5203.34	3961	3815	3896	5013.7

Table 5. Conductivity and ppm of aqueous solutions: Pb^{2+} , EDTA- Pb^{2+} and textured soy extract- Pb^{2+} .

The conductivity and ppm of the mixture of EDTA and textured soya extract with aqueous Pb^{2+} solution, shown in the Table 5, are a result of subtracting the conductivity or ppm of the mixtures and conductivity and ppm from the EDTA and texture soya extract with $\text{Pb}(\text{NO}_3)_2$.

Figure 5 is a graphic representation of Table 5 results.

Table 6 shows the results of the conductivity and ppm of five aqueous Fe^{3+} solution prepared dissolved 0.01, 0.03, 0.05, 0.07 and 0.1 grams of $\text{Fe}(\text{NO}_3)_3$ in 10 ml of deionizer water, each one. In a similar process were prepared five aqueous solutions EDTA- Fe^{3+} dissolved 0.01, 0.03, 0.05, 0.07 and 0.1 grams of $\text{Fe}(\text{NO}_3)_3$ in 10 ml of aqueous EDTA solution

and in the same way were prepared five aqueous solutions of textured soya extract-Fe³⁺ dissolved 0.01, 0.03, 0.05, 0.07 and 0.1 grams of Fe(NO₃)₃ in 10 ml of textured soya extract.

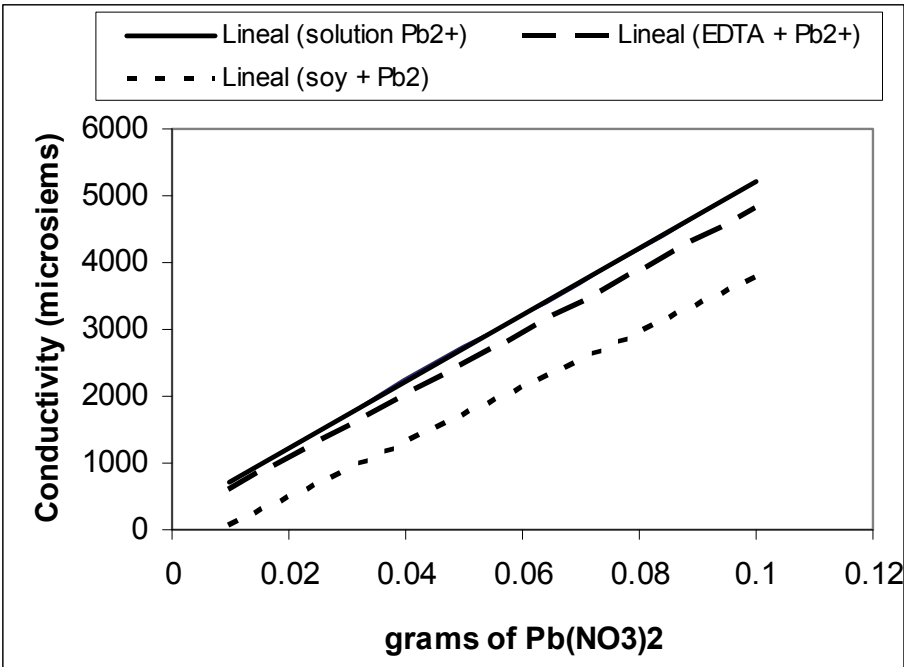


Fig. 5. Conductivity of aqueous solutions: Pb²⁺, EDTA- Pb²⁺ and textured soya extract-Pb²⁺ .

Grams Fe(NO ₃) ₃	Fe ³⁺ aqueous solutions ppm (μs)		Textured soya extract-Fe ³⁺ Ppm (μs)		EDTA- Fe ³⁺ (5x10 ⁻⁴ M) ppm (μs)	
0.01	932.64	1351.34	138	177	902.1	1286.7
0.03	2335.34	3159.34	995	1261	2097	2826.7
0.05	3910.34	5072.34	2785	3421	3573	4620.7
0.07	5078.34	6446.34	4431	5336	5008	6302
0.1	7329.34	8964.34	6065	7141	6900	8442

Table 6. Conductivity and ppm of aqueous solutions: Fe³⁺ , EDTA- Fe³⁺ and textured soya extract-Fe³⁺.

The conductivity and ppm of the mixture of EDTA and textured soya extract with aqueous Fe³⁺ solution, shown in the Table 6, are a result of subtracting the conductivity or ppm of the mixtures and conductivity and ppm from the EDTA and texture soya extract with Fe(NO₃)₃. Figure 6 is a graphic representation of the Table 6 results.

Table 7 shows the results of the conductivity and ppm of five aqueous Cd²⁺ solution prepared dissolved 0.01, 0.03, 0.05, 0.07 and 0.1 grams of CdSO₄ in 10 ml of deionizer water, each one. In a similar process were prepared five aqueous solutions EDTA-Cd²⁺ dissolved 0.01, 0.03, 0.05, 0.07 and 0.1 grams of CdSO₄ in 10 ml of aqueous EDTA solution and in the same way were prepared five aqueous solutions of textured soya extract-Cd²⁺ dissolved 0.01, 0.03, 0.05, 0.07 and 0.1 grams of CdSO₄ in 10 ml of textured soya extract.

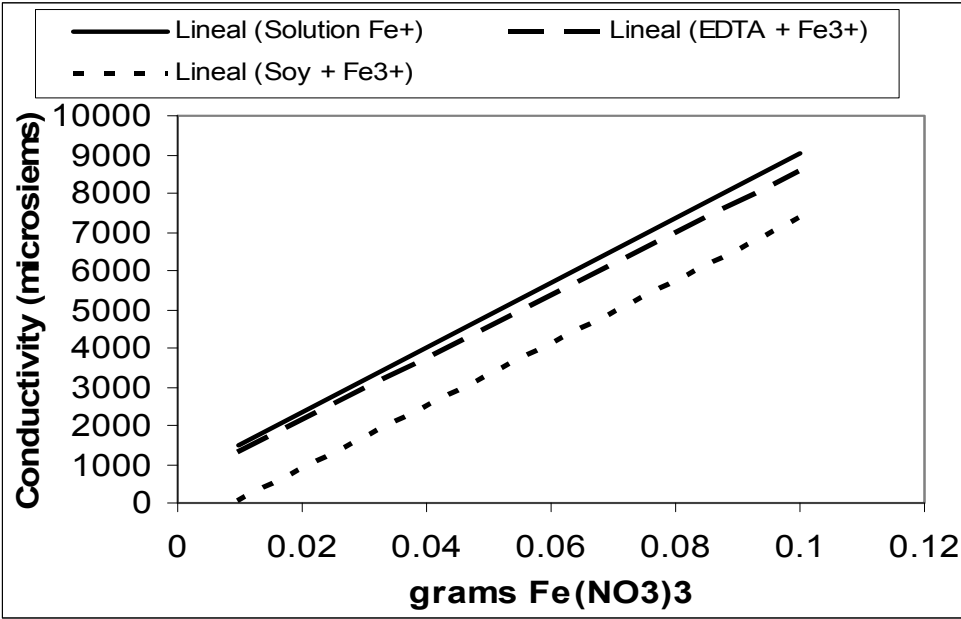


Fig. 6. Conductivity of the aqueous solution of: Fe^{3+} , EDTA-Fe^{3+} and textured soya extract- Fe^{3+} .

Grams CdSO_4	Cd^{2+} aqueous solutions ppm (μs)		Textured soya extract- Cd^{2+} Ppm (μs)		EDTA-Cd^{2+} ($5 \times 10^{-4}\text{M}$) Ppm (μs)	
0.01	393.75	591.54	82	110	439	643.4
0.03	948.55	1373.34	528	704	521.4	757.2
0.05	1346.35	1906.34	861	1135	1458	1947.7
0.07	1760.35	2442.34	1326	1301	2078	2807.7
0.1	2189.35	2979.34	1760	2256	2704	3584.7

Table 7. Conductivity and ppm of aqueous solutions: Cd^{2+} , EDTA- Cd^{2+} and textured soya extract- Cd^{2+} .

The conductivity and ppm of the mixture of EDTA and textured soya extract with aqueous Cd^{2+} solution, shown in the Table 7, are a result of subtracting the conductivity or ppm of the mixtures and conductivity and ppm from the EDTA and textured soya extract solutions with CdSO_4 .

Figure 7 is a graphic representation of Table 7 results. Table 8 shows the results of the conductivity and ppm of five aqueous Hg_2^{2+} solution prepared dissolved 0.01, 0.03, 0.05, 0.07 and 0.1 grams of HgCl_2 in 10 ml of deionizer water, each one. In a similar process were prepared five aqueous solutions EDTA-Hg_2^{2+} dissolved 0.01, 0.03, 0.05, 0.07 and 0.1 grams of HgCl_2 in 10 ml of aqueous EDTA solution and in the same way were prepared five textured soya extract- Hg_2^{2+} dissolved 0.01, 0.03, 0.05, 0.07 and 0.1 grams of HgCl_2 in 10 ml of textured soya extract.

The conductivity and ppm of the mixture of EDTA and extract of soybeans with aqueous Hg_2^{2+} solution, shown in Table VII, is a result of subtracting the conductivity or ppm of the mixtures from the EDTA and textured soya extract with HgCl_2 .

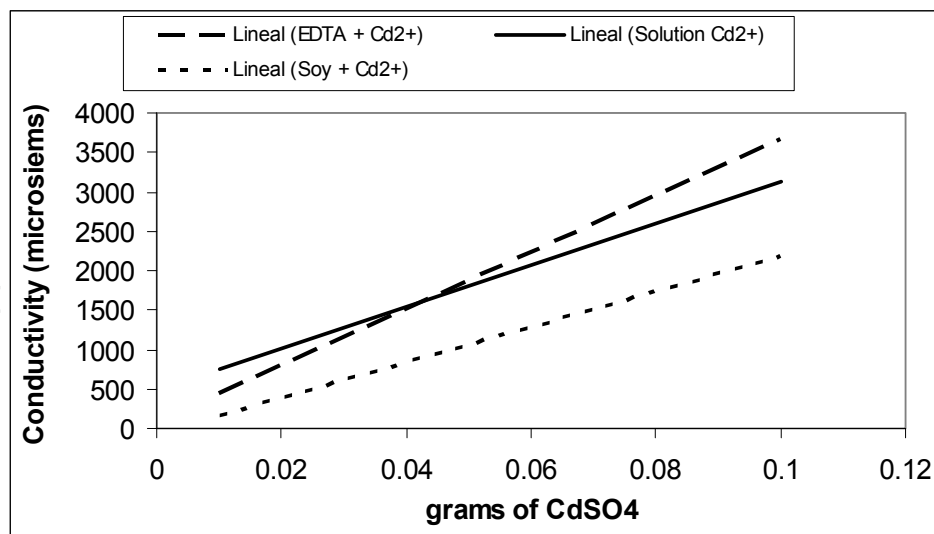


Fig. 7. Conductivity of the aqueous solution of: Cd²⁺, EDTA-Cd²⁺ and textured soya extract-Cd²⁺.

Grams HgCl ₂	Hg ₂ ²⁺ aqueous Solutions		Textured soya extract-Hg ₂ ²⁺ Ppm		EDTA-Hg ₂ ²⁺ (5x10 ⁻⁴ M) Ppm (μs)	
	ppm (μs)		μs)			
0.01	25	39.41	46	5	113	171
0.03	26.92	42.28	58	74	107.2	163.7
0.05	33.82	53.24	78	94	100	151.7
0.07	37.56	59.09	79	100	107	160.7
0.1	48.58	76.74	100	134	113.7	173

Table 8. Conductivity and ppm of aqueous solutions: Hg₂²⁺ , EDTA- Hg₂²⁺ and textured soya extract-Hg₂²⁺.

Figure 8 is a graphic representation of Table 8 results.

Table 9 shows the results of the conductivity and ppm of five aqueous Ni²⁺ solution prepared dissolved 0.01, 0.03, 0.05, 0.07 and 0.1 grams of NiCl₂ in 10 ml of deionizer water, each one. In a similar process were prepared five aqueous solutions EDTA-Ni²⁺ dissolved 0.01, 0.03, 0.05, 0.07 and 0.1 grams of NiCl₂ in 10 ml of aqueous EDTA solution and in the same way were prepared five aqueous textured soya extract-Ni²⁺ dissolved 0.01, 0.03, 0.05, 0.07 and 0.1 grams of NiCl₂ in 10 ml of textured soya extract.

Grams NiCl ₂	Ni ²⁺ aqueous solution		Textured soya extract-Ni ²⁺ solution		EDTA-Ni ²⁺ (5x10 ⁻⁴ M) ppm (μs)	
	ppm (μs)		Ppm (μs)			
0.01	720.25	1052.34	210	273	450.5	716.7
0.03	1849.35	2553.34	1108	1404	997	1413.7
0.05	2677.35	3585.34	1845	2306	2653	3519.7
0.07	4093.35	5287.34	3225	3940	4088	5194.7
0.1	6069.35	7575.34	4658	5599	5607	7006.7

Table 9. Conductivity and ppm of aqueous solutions: Ni²⁺ , EDTA- Ni²⁺ and textured soya extract-Ni²⁺.

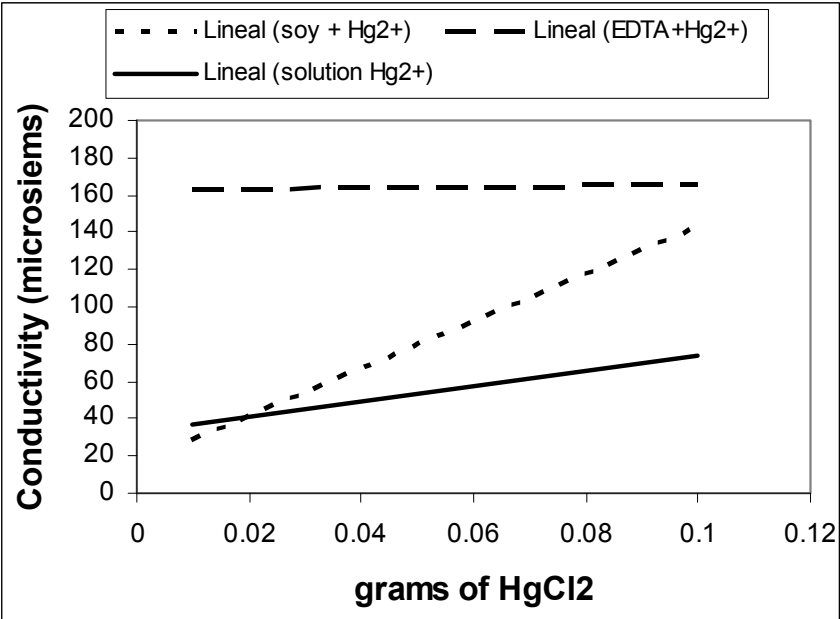


Fig. 8. Conductivity of the aqueous solution of: Hg_2^{2+} , EDTA-Hg^{2+} and textured soya extract- Hg^{2+} .

The conductivity and ppm of the mixture of EDTA and textured soya extract with aqueous Ni^{2+} solution, shown in the Table VIII, is a result of subtracting the conductivity or ppm of the mixtures from the EDTA and textured soya extract with NiCl_2 . Figure 9 is a graphic representation of Table 9 results.

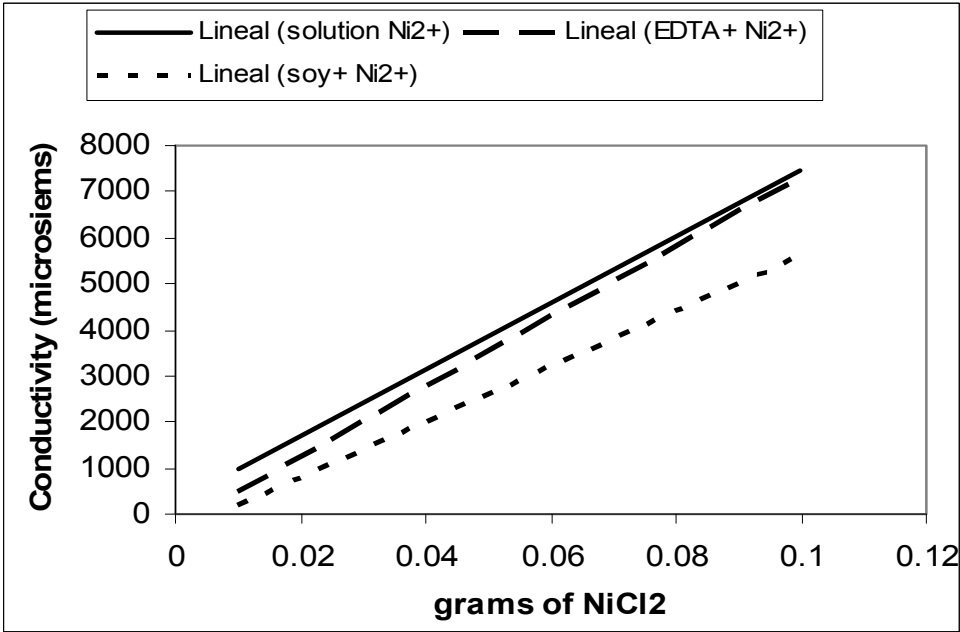


Fig. 9. Conductivity of the aqueous solution of: Ni^{2+} , EDTA-Ni^{2+} and textured soya extract- Ni^{2+} .

In Table 10 we can see the amount of metal ion sequestering for the textured soya extract in five different amounts of each salt: 0.01, 0.03, 0.05, 0.07 and 0.1 grams.

	0.01 grams	0.03 grams	0.05 grams	0.07 grams	0.1 grams
Pb ²⁺	0.0076	0.008	0.019	0.018	0.027
Fe ³⁺	0.0087	0.017	0.016	0.0089	0.021
Cd ²⁺	0.0082	0.013	0.020	0.017	0.025
Hg ²⁺	-----	-----	-----	-----	-----
Ni ²⁺	0.0075	0.012	0.018	0.014	0.027

Table 10. Amount of metal ion sequestering using textured soya extract

Figure 10 is a graphic representation of Table 10 results.

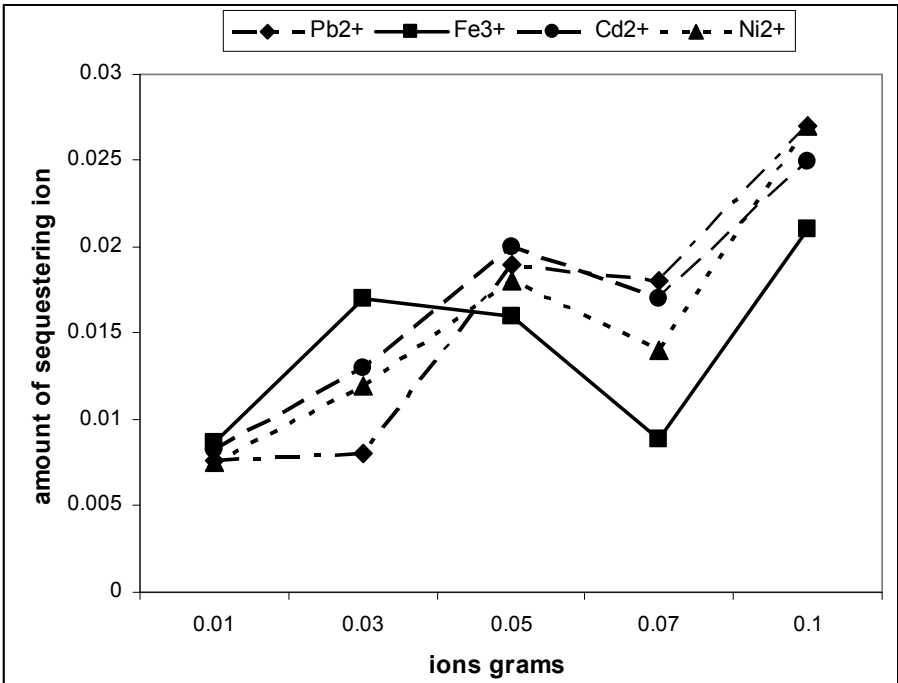


Fig. 10. Amount of metal ion sequestering using textured soya extract.

In Table 11 we can see the amount of metal ion sequestering for the EDTA in five different amounts of each salt: 0.01, 0.03, 0.05, 0.07 and 0.1 grams.

	0.01 grams	0.03 grams	0.05 grams	0.07 grams	0.1 grams
Pb ²⁺	0.0025	0.013	0.008	0.006	0.004
Fe ³⁺	0.0005	0.003	0.045	0.001	0.006
Cd ²⁺	0.0026	0.013	----	----	----
Hg ²⁺	----	----	----	----	----
Ni ²⁺	0.0032	0.013	0.001	0.0085	0.008

Table 11. Amount of metal ion sequestering using EDTA

Figure 11 is a graphic representation of Table 11 results

From the results obtained in Table 10 and Table 11, we can say that the amount of metal ion chelating increases with the increase of the concentration but the amount of salt chelated with textured soya extract is considerable major in comparison to the EDTA. In the case of Hg₂²⁺ ions, the textured soya extract and EDTA is not effective as a chelating agent. Another

difference is that EDTA is effective as chelating agent of Cd²⁺ only with low concentrations (less to 0.04 g of CdSO₄ in 10 ml of EDTA solution 5x10⁻⁴M) but without exception the textured soya extract is a good chelating agent.

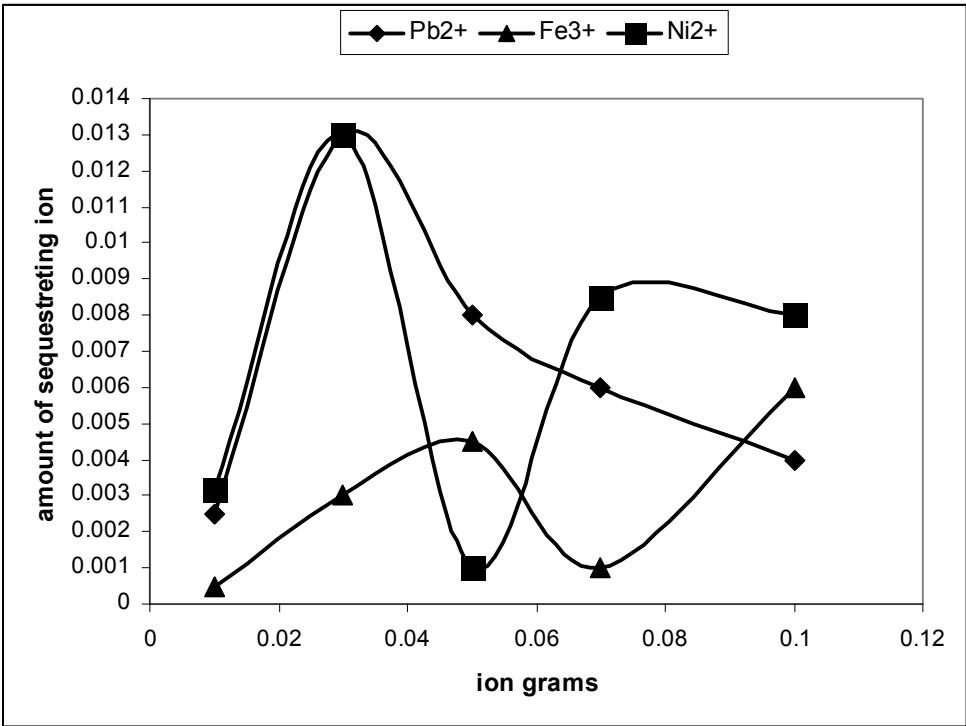
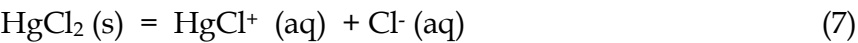


Fig. 11. Amount of metal ion sequestering using EDTA

In the case of HgCl₂ it is a weak electrolyte, the ionization is partial, as showing in the reaction 7.



Maybe for this reason the EDTA and textured soya extract are not effective as chelating agents. But the textured soya extract is effective as chelating agent at low concentrations; nevertheless, the EDTA is not an effective chelating agent even in low concentrations. From the results of Table 11, we are able to calculate the equilibrium constant for the textured soya extract, using the equation 6, and the results being shown in Tables 12.

	0.01 grams	0.03 grams	0.05 grams	0.07 grams	0.1 grams
	Textured soya extract	Textured soya extract	Textured soya extract	Textured Soya extract	Textured soya extract
Pb ²⁺	0.76	0.11	0.38	0.25	0.27
Fe ³⁺	0.87	0.56	0.32	0.12	0.21
Cd ²⁺	0.82	0.43	0.4	0.24	0.25
Hg ₂ ²⁺	-----	----	-----	----	-----
Ni ²⁺	0.75	0.4	0.36	0.2	0.27

Table 12. Equilibrium constant of metal ion sequestering

From the results of Table 11, we are able to calculate the equilibrium constant for the EDTA, using the equation 6, and the results being shown in Tables 13.

	0.01 grams	0.03 grams	0.05 grams	0.07 grams	0.1 grams
	EDTA	EDTA	EDTA	EDTA	EDTA
Pb ²⁺	0.25	0.2	0.16	0.08	0.04
Fe ³⁺	0.05	0.1	0.09	0.014	0.06
Cd ²⁺	0.26	0.43	-----	----	----
Hg ₂ ²⁺	-----	----	-----	----	----
Ni ²⁺	0.32	0.43	0.02	0.12	0.08

Table 13. Equilibrium constant of metal ion sequestering

5. Conclusions

In the case of the ion Pb²⁺, it can be seen that the solution of the complex EDTA with the ion P²⁺ gives a line which is very close to the reference line of the ionic solution Pb²⁺. This indicates that the amount of Pb²⁺ ion chelated is small in comparison to the solution of the chelate formed from the textured soya extract and the Pb²⁺ ion which has a line that is way below the reference line and the EDTA.

A similar conclusion for the study with the Fe³⁺ ion can be given.

With respect to the Cd²⁺ ion, the EDTA only acts as a chelate in concentrations lower than 0.04 grams of CdSO₄ per 10ml of deionized water. On the other hand, the textured soya extract is a good chelate in a wider concentration range (between 0.01 and 0.1 grams of CdSO₄ per 10ml of deionized water).

The chelate solution of EDTA for the Hg₂²⁺ ion does not have any effect on the Hg₂²⁺ ion in the test range from 0.01 up to 0.1 grams of HgCl₂ per 10ml of water. However, the textured soya extracts act as a chelate only in concentrations lower than 0.15 grams of HgCl₂ per 10ml of deionized water. The problem presented by this salt rests on the fact that it is a weak electrolyte and when it is placed in the water, it decomposes into two ions. Since the conductivity of the solution is measured in this study, the formation of two ions has a negative effect on the measurements obtained.

Just as in the case of Pb²⁺, Fe³⁺ and Ni²⁺ ions, the textured soya extract is a much better chelate than EDTA.

There is normally a low concentration (parts per million) of heavy ions in food. Thus our proposal of using the textured soya extract as the chelate for heavy ions instead of EDTA. In addition, as a result of the low concentration of ions, a solution with a low concentration of the textured soya extract will be used in order not to change the color, scent or taste of the food.

A problem to be considered in this application is that food is prepared with water which has salts that are ionized, and the textured soya extract will chelate some of these ions also. It will be necessary to perform tests on the food sample to determine if the application will be practical or not.

Another application possible is the extraction of heavy metals in water, cosmetics, and soils employing textured soya extract as the chelate for heavy ions instead of EDTA.

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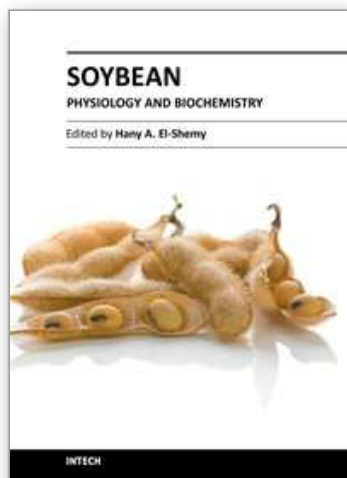
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