Chelation Therapy &
Plan of Work
Most common method for decorporation of heavy metal is known as chelation therapy. The word chelation is derived from Greek χελή, chelè, meaning claw; the ligands lie around the central atom like the claws of a lobster (Morgan and Drew, 1920). The term chelate was first applied in 1920 by Sir Gilbert T. Morgan and H. D. K. Drew.

3.1. Chelation: Concept and Chemistry

Although the concept of chelation is based on simple coordination chemistry, evolution of an ideal chelator and chelation therapy that completely removes specific toxic metal from desired site in the body involves an integrated drug design approach. Chelating agents are organic or inorganic compounds capable of binding metal ions to form complex ring-like structure called 'chelates'. Chelating agents possess "ligand" binding atoms that form either two covalent linkages or one covalent and one co-ordinate or two co-ordinate linkages in the case of bidentate chelates. Mainly atoms like S, N and O function as ligand atoms in the form of chemical groups like –SH, –S-S, –NH2, =NH, –OH, –OPO3H, or >C=O. Bi-denate or multi-dentate ligands form ring structures that include the metal ion and the two-ligand atoms attached to the metal (Andersen, 1999) (Figure 3.1). Many donors act as bi-dentate ligands. Five-membered chelate rings are specially stable and they are often formed by ligands with YCCY skeletons such as Y-CH2-CH2-Y, Y-CO-CH2-Y etc. where Y is OR, NR2, O, S, NR, etc. There are also examples of inorganic chelate ligands which form five-membered ring with metal ions. Other types of chelating ligands are possible, like EDTA4-, which is a hexadentate ligand. In the simplest case a proton (H+) that can absorb the lone pair of electrons of ligand-binding atom(s) of the chelator may be involved in the coordination complex formation. However, the positive charge on proton remains since there is no loss or gain of electrons in the process.

The latter may also be known as the 'net ionic charge' of the complex, which plays a crucial role in governing the pharmacokinetic fate and ultimately the toxicological behavior of such complexes in-vivo. In the biological environment metal cations viz. Na+, Mg+, Cu+, Cu2+, and Zn2+ especially the transition metals like Mn, Fe and Co may be involved in such complex formation. Although the stability of such complexes varies, the deciding factors are based on the properties of both the chelating agent and the chelated metal. The stability constant of a
complex can be quantitatively expressed in equilibrium equation values, which depend on the atomic structure of the chelated metals. For example, the stability constants for different metals with EDTA are on the scale (Table 3.1), where a metal with higher k constant competes for the chelating agent with a metal of lower stability value and ultimately removes the latter.

![Diagram](image)

Figure 3.1: Formation of metal-ligand complexes with mono, bi and poly-dentate ligands.

| Metal\(K\) (log) | Na \(2.5\) | Li \(1.7\) | Ba \(7.6\) | Sr \(8.7\) | Mg \(8.7\) | Ca \(10.6\) | Mn \(13.4\) | Fe \(14.4\) | Co \(16.1\) | Zn \(16.4\) | Cd \(18.3\) | Pb \(18.3\) | Ni \(18.4\) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|

However, other variables like the number of heterocyclic rings formed and relative concentration also play a role, that's why Ca\(^{2+}\), which is readily available in the body fluids, binds preferentially with Na\(_2\)EDTA in spite of the higher stability constant of Pb. Moreover, in spite of all the known properties desired in an ideal chelator the predictability of the outcome is limited. A chemical entity that qualifies as an ideal chelator in vitro might not prove so in vivo, either due to the toxicity considerations or due to the presence of endogenous substances (hemoglobin, cytochromes, etc.) that can also chelate metal ions and thus offer competition. Further, pH also is an important factor influencing complex formation and stability. Most chelating agents are unstable at low pH, whereas at high pH metals tend to form insoluble hydroxides which are less accessible to chelating agents. This feature
becomes significant in pathological conditions leading to acidosis or alkalosis. Optimally effective chelation can be achieved by virtue of some combination of the basic properties of both the metal ions, chelating agents and the resulting metal complex. A chelating agent that will occupy more of the coordination positions of a metal ion will generally (but not always) give a complex of greater stability than otherwise. Similarly, whereas the net ionic charge of the chelator defines its absorption, distribution and ability to reach the metal ion for binding; the net ionic charge of the complex decides its elimination from the specific site and excretion from the body.

Thus, it is important that a chelator satisfy criteria that allow it to:

I. Transport across physiological barriers into compartments where a toxic metal ion is concentrated

II. Form a stable complex with the metal after removing it from the biological chelator, if required at the site and

III. Form a chelation complex whose properties render it non-toxic and facilitate its excretion, not only from the site of deposition, but also from the body (Jones, 1994).

3.1.1. Limitations of Current Chelation Therapy

Most of the currently used chelating agents have serious side effects (Angel et al., 1996). Since possible adverse effects and risk associated with conventionally used chelators has already been highlighted in the previous sections, mechanistic limitations are addressed here. Ca-Na$_2$EDTA is a general chelating agent that complexes a wide variety of metal ions and is used clinically despite associated risks. Ca-Na$_2$EDTA cannot pass through cellular membranes and therefore its use is restricted in removing metal ions from their complexes in the extracellular fluid. Similarly conventionally used succimer, DMSA although is considered safer, it shares the limitation of extracellular distribution. The latter renders the drug effective in cases of slow, low dose, chronic metal poisoning (especially lead and arsenic) since metal reaches the cellular compartments behind the physiological barriers including the blood brain barrier.
Benefits and drawbacks of chelation therapy

The reason to look for newer antidotes are that: (i) there is no effective and safe pre-treatment available which could be instituted as preventive measure against possible arsenic exposure, (ii) the recommended treatments have serious limitations like side-effects or are contraindicated for various instances of heavy metal poisoning, (iii) most of the available treatments are to be given intravenously by a medical practitioner and under no circumstances victim can resort to self-aid, (iv) there is no safe and effective oral treatment available and (v) there is no fast acting antidote available which could immediately remove toxic metal from blood and soft tissues. It is thus clear from above that most of the conventional chelators are compromised with many side effects and drawbacks and there is no safe and effective treatment available for heavy metal poisoning.

EDTA cannot easily chelate metallic ions when they are tightly bound within metal-containing enzymes or to specific metal-binding proteins. On the other hand, when metals accumulate in unbound form, and are able to act as catalysts of uncontrolled lipid peroxidation or metabolic poisons, EDTA can easily bind and inactivate them. Iron accumulates with age and in abnormal locations, where it greatly accelerates free radical damage (Halstead et al., 1978). EDTA binds much more tightly to iron and other
potential free radical catalysts than it does to calcium. EDTA will only bind calcium if those other metal ions are not present (Halstead et al., 1978).

The affinity of EDTA to bind various metals at physiologic pH, in order of decreasing stability, is listed below. In the presence of a more tightly bound metal, EDTA releases metals lower in the series and binds to the metal for which it has a greater affinity. In clinical practice, chromium, mercury and copper are not removed in any significant amount by EDTA—evidence that those essential metals are more tightly bound by natural ligands in the body than by EDTA. Magnesium is a calcium antagonist and is relatively deficient in many chelation patients. Magnesium is the metallic ion least likely to be removed by EDTA. In fact, EDTA is usually administered as magnesium-EDTA, which provides an efficient delivery system that increases magnesium stores and reduces likelihood of pain at the infusion site.

Lasting inhibition of disease-causing free radicals by EDTA offers an explanation for data from Switzerland, which documented a 90 percent reduction in deaths from cancer in a large group of patients who were chelated and then carefully followed over an eighteen-year period. Chelation patients were compared with a statistically matched control group. Death rate from cancer was ten times greater in the untreated group, compared to the death rate of patients who had previously been treated with EDTA chelation (p=0.002). (Cranton and Frackelton; 2001) A greatly reduced incidence of cardiovascular deaths was also observed following chelation. One common denominator of both cancer and atherosclerosis is free radical oxidative damage to molecules (Cranton and Frackelton; 2001). Calcium-EDTA was administered in that study, which precludes any direct effect on calcium metabolism as an explanation for outcomes. Removal of free radical catalysts seems one likely explanation. Demopoulos first proposed that chelation be used to control free radical pathology. He also pointed out that many antioxidants have chelating properties.

EDTA increases the efficiency of mitochondrial oxidative phosphorylation and improves myocardial function, quite independently of any effect on arterial blood flow. Treatment
with deferoxamine, an iron chelator, has been shown to improve cardiac function in patients with increased iron stores (Cranton and Frackelton; 2001). In addition, removal of iron with deferoxamine reduces inflammatory responses in animal experiments. Sullivan has suggested that periodic donation of blood be studied as a way to reduce the risk of atherosclerosis in men and postmenopausal women. By reducing damage from free radicals, EDTA chelation therapy can support normal healing. The time required for healing of damaged tissues gives us another explanation for the time lapse of several months following chelation before full benefit is achieved. By correcting an underlying cause of the disease process, and allowing time for subsequent healing, tretment with EDTA seems far superior to the mere suppression of symptoms achieved with so many other therapies.

3.2. Characteristics of an ideal chelator
An ideal chelator should have high solubility in water, resistance to biotransformation, ability to reach the sites of metal storage, retain chelating ability at the pH of body fluids and the property of forming metal complexes that are less toxic than the free metal ion.

- Greater affinity and low toxicity
- Ability to compete with natural chelator
- Ability to penetrate cell membranes
- Rapid elimination of toxic metal
- High water solubility
- Capacity to form non-toxic complexes
- Same distribution as the metal

3.3. Rationale of the study
In a radioactivity fallout scenario, a number of radionuclides with long half-lives and harmful ionizing radiation would be released. Medically relevant dose of the radionuclide would be inhaled and ingested by exposed population for several weeks post events, causing morbidity and mortality in the affected population. In real time situation, the number of people exposed to 20-200 rad of radiation by incorporated radionuclides would be of the order of tens of
thousands. These shall require decorporation therapy to reduce the incidence of cancer and early death by systemic tissue damage. Same thing apply for heavy metal exposure which is due to leakage or waste products discharge from heavy metals industries. Exposure to these toxicants is not confined only to occupational workers but also to general population at large (Passos et al., 2008). Another major concern with respect to heavy metal toxicity that cannot be overlooked is the increased threat perception of a radiological device being used by rouge states or terrorist organizations that may lead to significant radio-metal contamination of the affected population, mainly through inhalation, ingestion or topical exposure. The challenge of removing such contaminants from the body assumes greater significance considering that in vivo radio-metal deposition even in miniscule quantities may impart significant radiation dose to body’s target organs depending upon their half-lives.

Although use of chelating agents against metal toxicity was employed decades back, introduction of ethylene diamine tetraacetic acid (EDTA) as a chelator is considered as a breakthrough in chelation therapy (Kalia et al., 2005). Use of EDTA and other chelating agents is the medically accepted treatment for lead and other heavy metal poisoning (James et al., 2007; Henge et al., 2000 and Goyer 1995). As already mentioned, inhalation, ingestion and topical surfaces is a major route through which heavy metal toxicity can occur. Therefore it seemed logical to us to formulate various formulation, which traps the metal at the portal of entry so that it can be excreted without absorption, thereby helping in minimizing its toxicity. Therefore, there is a need to develop novel decorporation or radio-protective formulations that are more effective than the existing ones for localize action and minimize the total burden of chelating agent to body and are also cost-effective to ensure availability at a mass scale.

3.4. Selection of chelator

Calcium disodium ethylenediamine tetraacetic acid (Ca-Na₂EDTA) is the most commonly used chelating agent. It is a derivative of ethylenediamine tetraacetic acid (EDTA); a synthetic polyamino-polycarboxylic acid and since 1950s has been one of the mainstays for the treatment of childhood lead poisoning (Klaassen et al., 2006).

Ca-Na₂EDTA is chosen as chelator of choice on following parameters:

- Cost effective
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• Easily available
• Good chelation efficacy
• Water soluble
• Stable

Calcium-disodium EDTA chelation is approved by the U.S. Food and Drug Administration (FDA) for treating lead poisoning and heavy metal toxicity.

3.5. Objectives of the study

The objectives of the present study are:

➢ Development of analytical method for Ca-Na$_2$EDTA (U.P.L.C., U.V. and Radiometry)
➢ Development of some novel formulation/s for heavy metal/radio-isotope decorporation from the body. (Dry powder inhaler (DPI) for lung, Eye drop for eye surface and floating tablet for stomach).
➢ Optimization and characterization of developed formulation/s
➢ Safety and efficacy studies of optimized formulation/s
➢ Clinical trials (Phase -0) of optimized formulation/s

3.6. Plan of work

I. Literature and patent survey

II. Selection and procurement of Chelator.

III. Selection and procurement of excipients, chemicals and solvents.

IV. Analytical methodology of the Chelator-

➢ U.V. method for analysis like solubility studies, dissolution studies and in-vitro studies.
➢ UPLC method for plasma analysis and stability studies.
➢ Radiometry method (radiolabelling of the chelator) for in-vivo, scintigraphy studies.

V. Design of appropriate method for in vitro drug release testing for formulation/s

Appropriate bio-relevant dissolution media will be prepared/designed for performing in vitro release methods.

VI. Optimization of formula for formulation/s:

On the basis of yield, solubility, stability and dissolution characteristics the formulations will be selected for optimization. They will be optimized on the basis of

➢ Fourier transforms infrared spectroscopy
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- Differential scanning calorimetric
- Surface morphology by scanning electron microscopy
- Drug release profile

The formulation/s which will be give satisfactory /promising result would be selected as optimized formulation.

VII. Interaction studies:
These studies will be performed to ascertain that no interaction has occurred between the drug and polymer or any other additive/excipients, or due to condition of the formulation processing.

VIII. Comparison of drug release from conventional/ modified products with optimized formulation

IX. Accelerated stability studies
Stability studies will be carried out to determine the effect of the presence of polymer, excipient and also determine the physical stability of the formulation under accelerated storage condition of the temperature and humidity.

X. Pharmacokinetic studies
*In-vivo* studies in suitable animal model shall be conducted and various pharmacokinetic parameters will be calculated.

XI. Acute toxicological studies
*Sub-acute* toxicological studies in suitable animal model (Rat/Mice/Rabbit) will be conducted and various pharmacokinetic parameters will be calculated.

XII. *In-vivo* & *in-vitro* correlation
Based on the result obtained, *in-vitro* & *in-vivo* correlation would be established using suitable statistical methods.

XIII. Clinical trials
On the basis of result obtained, permission from ethical committee for conducting clinical trials has been obtained for carrying out phase-I clinical trials of the developed formulations to evaluate its safety and safety and efficacy.
3.7. Drug profile

Chemical names : Calcium disodium ethylenediaminetetraacetate;
Calcium disodium (ethylenedinitrilo) tetraacetate
Sodium calcium edetate;

Nonproprietary Names : BP: Calcium Disodium edetate,
JP: Disodium edetate
PhEur: Dinatrii edetas
USP: Edetate Calcium Disodium

Synonyms : Calcium Disodium EDTA, Versene CA
Calcium disodium versenate
Morpholinobenzene
N-Phenylmorpholine
Phenyl morpholine

Empirical formula : C₁₀H₁₂CaN₂Na₂O₈·2H₂O

Structural formula :

Molecular weight : 410.31
CAS No. : 62-33-9

Physical state & appearance: Solid. (Powdered solid)

Odor : Odorless.

Taste : Saline. (Slight.)

Color : White.

pH (1% soln/water) : 6.5-7.5 (1 wt % solution)

Solubility : Soluble in cold water. 30 wt % at 25°C/77°F

Bulk Density : 640 kg/m3 or 40 lb/cu ft

Use : As a sequestrant.

Functional Category : Chelating agent.
There are six sites for the binding with the electron deficient molecule by $=O$ & $-OH$. It shows nucleophilic conjugation or addition reaction with electron deficient element or compound.

3.7.1. Indications

Edetate calcium disodium is indicated for the reduction of blood levels and depot stores of lead in lead poisoning (acute and chronic) and lead encephalopathy, in both pediatric populations and adults.

3.7.2. Mechanism of Action

The pharmacological effects of Ca-Na$_2$EDTA result from formation of chelates with divalent and trivalent metals in the body. Accessible metal ions (both exogenous and endogenous) with an affinity for Ca-Na$_2$EDTA that is higher than that of Ca$^{2+}$ will be chelated, mobilized, and usually excreted. Because EDTA is charged at physiological pH, it does not significantly penetrate cells; its volume of distribution approximates extracellular fluid space. Experimental studies in mice have shown that administration of Ca-Na$_2$EDTA mobilizes several endogenous metallic cations, including those of zinc, manganese, and iron. The main therapeutic use of Ca-Na$_2$EDTA is in the treatment of metal intoxications, especially lead intoxication Ca-Na$_2$EDTA is available as edetate Calcium disodium (Calcium Disodium Versenate) (Klaassen et al., 2006).

Intramuscular administration of Ca-Na$_2$EDTA results in good absorption, but pain occurs at the injection site; consequently, the chelator injection often is mixed with a local anesthetic or administered intravenously. For intravenous use, CaNa$_2$EDTA is diluted in either 5%
dextrose or 0.9% saline and is administered slowly by intravenous drip. A dilute solution is necessary to avoid thrombophlebitis. To minimize nephrotoxicity, adequate urine production should be established prior to and during treatment with Ca-Na$_2$EDTA. However, in patients with lead encephalopathy and increased intracranial pressure, excess fluids must be avoided. In such cases, conservative replacement of fluid is advised, and intramuscular administration of Ca-Na$_2$EDTA is recommended.

3.7.3. Pharmacological profile

At the pH of the body fluid, edetate calcium disodium combines with polyvalent metallic ions to form a non-ionized water-soluble complex or chelate which is comparatively stable (Leckie et al 1958). When the complex given either by injection or orally, the calcium is exchanged for metallic ions and the chelate enters body fluids. Edetate calcium disodium does not enter erythrocytes. Edetate calcium disodium will instantly chelate the metals circulating in the extravascular compartment and those in the parts of the extracellular compartment that it can reach. The rest of intracellular compartment will then gradually decrease metallic ions as they migrate back into the extracellular fluid across the gradient. The chelate is rapidly and almost completely excreted via the kidneys. Heavy metals can be removed in this way from the plasma, the gastrointestinal tract, soft tissues and bone deposits.

In biological systems, Ca ion will usually be most accessible to EDTA. In general, zinc seems to be next most accessible. About 80 percent of the zinc is freely available to EDTA. The over-all availability of the other physiologically important metals is probably in the order: Cu$>$Fe$>$Mn$>$Co (Chenoweth, 1961). EDTA removes about 1.4 per cent of the total iron from ferritin at pH 7.4 to form an iron chelate. Transfer of Fe from Fe-transferrin to EDTA in vitro occurs at a rate of less that 1% in 24 hours. In vivo studies in rabbits demonstrated transfer of Iron only from FeEDTA to transferrin and not the reverse. It appeared that tissue iron beams available to chelating agents including EDTA only when an excess of iron was present (Candela et al., 1984).

Ca-Na$_2$EDTA enhanced the excretion of Co, Hg, Mn, Ni, Pb, Tl and W (Foreman, 1961). The treatment of heavy metal poisoning with Ca-Na$_2$EDTA has become so well established.
that its use for more commonly seen metal poisonings, e.g. lead, is no longer reported in the literature (Foreman, 1961). EDTA could prevent the accumulation of $^{90}$Sr, $^{106}$Ru, $^{141}$Ba and $^{226}$Ra in the skeleton. $^{91}$Y, $^{239}$Pu and $^{238}$U responded fairly well to EDTA, the excretion being accelerated (Forbes, 1961). EDTA had a lowering effect on serum cholesterol level when given orally or intravenously. It may have acted by decreasing the capacity of serum to transport cholesterol (Perry & Perry, 1959). Disodium EDTA had a pyridoxin-like effect on the tryptophan metabolism of patients with porphyria or scleroderma, due to a partial correction of imbalance of polyvalent cations (Alsmeyer et al 1976).

The i.p. injection of 4.2mmol/kg body-weight (equivalent to 1722mg/kg body-weight) Ca-Na$_2$EDTA caused in rats an inhibition of the alkaline phosphatase of liver, prostate and serum up to 4 days depending on the dose administered; zinc restored the activity (Swenerton & Hurley, 1971). In vitro, EDTA inhibited blood coagulation by chelating Ca$^{2+}$. The complete coagulation inhibition of human blood required 0.65-1.0mg/ml. The i.v. injection of 79-200mg EDTA/rabbit had no effect on blood coagulation (Jugo et al 1975).

I.V. injections of Na$_2$EDTA and Ca-Na$_2$EDTA had some pharmacological effect on the blood pressure of cats; 0-20mg/kg body-weight Ca-Na$_2$EDTA (as Ca) produce a slight rise; 20-50mg/kg, a biphasic response; and 50mg/kg, a clear depression (Brownie et al 1986). One percent Na$_2$EDTA enhances the absorption of $^{14}$C-labelled acidic, neutral and basic compounds (mannitol, inulin, decamethonium sulfanilic acid and EDTA itself) from isolated segments of rat intestine, probably due to an increased permeability of the intestinal wall (Schanker & Johnson, 1961).

### 3.7.4. Dosage and administration

Blood lead levels 20–70micrograms/dL: 1g/m$^2$ per day. IV: infuse over 8–12 hours. IM: divided doses every 8–12 hours. Treat for 5 days then stop for 2–4 days; may repeat. If levels over 70micrograms/dL: give with BAL (British anti lewisite);

### 3.7.5. Side effects

The following adverse effects have been associated with the use of edetate calcium disodium:

**Body as a Whole**

Pain at intramuscular injection site, fever, chills, malaise, myalgia, arthralgia.
Cardiovascular
Hypotension, cardiac rhythm irregularities.

Renal
Acute necrosis of proximal tubules (which may result in fatal nephrosis), infrequent changes in distal tubules and glomeruli.

Urinary
Glycosuria, proteinuria, microscopic hematuria and large epithelial cells in urinary sediment.

Nervous System
Tremors, headache, numbness, tingling.

Gastrointestinal
Cheilosis, nausea, vomiting, anorexia, excessive thirst.

Hepatic
Mild increases in SGOT and SGPT are common, and return to normal within 48 hours after cessation of therapy.

Immunogenic
Histamine-like reactions (sneezing, nasal congestion, lacrimation), rash.

Hematopoietic
Transient bone marrow depression, anemia.

Metabolic
Zinc, hypercalcemia.

3.7.6. Drug Interactions
There is no known drug interference with standard clinical laboratory tests. Steroids enhance the renal toxicity of edetate calcium disodium in animals. Edetate calcium disodium interferes with the action of zinc insulin preparations by chelating the zinc [Drug Evaluations, 1986].

3.7.7. General Precautions
Edetate calcium disodium may produce the same renal damage as lead poisoning, such as proteinuria and microscopic hematuria. Treatment induced nephrotoxicity is dose-dependent and may be reduced by assuring adequate
diuresis before therapy begins. Urine flow must be monitored throughout therapy which must be stopped if anuria or severe oliguria develop. The proximal tubule hydropic degeneration usually recovers upon cessation of therapy. Edetate calcium disodium must be used in reduced doses in patients with pre-existing mild renal disease. Patients should be monitored for cardiac rhythm irregularities and other ECG changes during intravenous therapy.

3.7.8. Overdose

**Symptoms**

Inadvertent administration of 5 times the recommended dose, infused intravenously over a 24 hour period, to an asymptomatic 16 month old patient with a blood lead content of 56 mcg/dl did not cause any ill effects. Edetate calcium disodium can aggravate the symptoms of severe lead poisoning; therefore, most toxic effects (cerebral edema, renal tubular necrosis) appear to be associated with lead poisoning. Because of cerebral edema, a therapeutic dose may be lethal to an adult or a pediatric patient with lead encephalopathy. Higher dosage of edetate calcium disodium may produce a more severe zinc deficiency.

**Treatment**

Cerebral edema should be treated with repeated doses of mannitol. Steroids enhance the renal toxicity of edetate calcium disodium in animals and, therefore, are no longer recommended. Zinc levels must be monitored. Good urinary output must be maintained because diuresis will enhance drug elimination. It is not known if edetate calcium disodium is dialyzable.

3.7.9. Contraindications

Edetate calcium disodium should not be given during periods of anuria, nor to patients with active renal disease or hepatitis. Anuria Renal disease and hepatitis.

3.7.10. Pharmacokinetics

**Absorption**

Oral edetate calcium disodium is poorly absorbed from the gastrointestinal tract with over 90% usually eliminated in feces (Leckie et al 1958; Lilis et al 1976). At the low pH of the
stomach the calcium chelate is dissociated with subsequent precipitation of the free acid, and this is only slowly re-dissolved in the intestines (Foreman et al., 1953).

**Distribution**
Edetate calcium disodium administered intravenously, intramuscularly, or subcutaneously becomes rapidly distributed throughout the extracellular fluid. Red cells are impervious to edetate calcium disodium and it seems likely that many other cell membranes are also (Leckie 1958; Foreman 1959). After initial distribution, ethylenediaminetetraacetic acid appears to become preferentially bound to bone, with delayed release subsequently. It passes slowly into spinal fluid compartment (Foreman et al 1954).

**Metabolism**
Edetate calcium disodium is not metabolized and the unchanged drug being excreted in the urine.

**Elimination**
It is almost entirely via the kidneys by glomerular filtration and tubular secretion. About 60 to 70% of the drug is excreted within 2h, and about 90% within 6h when given intravenously (Foreman et al., 1954). Estimated half-life is 20-60min. Renal clearance is approximately 1.5 ml/min but is dependent on renal function.

3.7.11. **Pharmaceutics**
Edetate calcium disodium is available from several manufacturers. Ledelair injection (Sinclair UK) contains edetate calcium disodium hydrate equivalent to anhydrous edetate calcium disodium 200 mg/ml in 5 ml ampule. For intravenous infusion 1 g of edetate calcium disodium should be diluted with 250 ml to 500 ml of 5% dextrose or 0.9% sodium chloride intravenous infusions, as appropriate: dilutions should be used immediately. Edetate calcium disodium is incompatible with 10% dextrose, 10% invert sugar in 0.9% sodium chloride, Lactated Ringer's injection. Ringer's solution for injection, sodium lactate M/6 and also amphotericin and hydralazine injections. These injections should be stored between 15°C and 30°C. In the USA and UK, the injections have shelf-lives of 5 years.
3.7.12. Stability and Storage Conditions

Edetate salts are more stable than edetic acid. However, disodium edetate dihydrate loses water of crystallization when heated to 120°C. Aqueous solutions of disodium edetate may be sterilized by autoclaving, and should be stored in an alkali-free container. Disodium edetate is hygroscopic and is unstable when exposed to moisture. It should be stored in a well-closed container in a cool, dry place.

3.7.13. Incompatibilities

Disodium edetate behaves as a weak acid, displacing carbon dioxide from carbonates and reacting with metals to form hydrogen. It is incompatible with strong oxidizing agents, strong bases, metal ions, and metal alloys.

3.7.14. Acute toxicity

The oral LD₅₀ in rats is not affected by the presence of food in the stomach or by pre-existing deficiency in Ca, Fe, Cu or Mn (Oser et al., 1963). Oral doses of over 250mg/animal cause diarrhea in rats (Foreman et al., 1953). There are many reports in the literature on kidney damage by parenteral over-dosage of Ca-Na₂EDTA. A review was given by Lachnit (1961). Lesions simulating "versene nephrosis" in man have also been produced in rats. Disodium EDTA in doses of 400-500mg i.p. for 21 days caused severe hydropic degeneration of the proximal convoluted tubules of the kidneys. Ca-Na₂EDTA produced only minimal focal hydropic changes in 58% of animals, disappearing almost 2 weeks after stopping the injections (Reuber & Schmieller, 1962).

LD₅₀ (mouse, IP) : 0.26 g/kg
LD₅₀ (mouse, IV) : 0.056 g/kg
LD₅₀ (mouse, OP) : 2.05 g/kg
LD₅₀ (rabbit, IV) : 0.047 g/kg
LD₅₀ (rabbit, OP) : 2.3 g/kg
LD₅₀ (rat, OP) : 2.0 g/kg