Principle and Mode of Action Common Anticoagulants.

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Introduction

- Anticoagulants are the chemicals used in the lab. that when combined with blood specimens at a proper concentration, prevents the blood from clotting
- Some anticoagulants occur naturally in blood-eating animals such as leeches and mosquitoes,
- Anticoagulants are used as therapy for **thrombotic disorders**.

  This can be oral anticoagulants (OACs) usually in pill or tablets form, and various intravenous anticoagulant dosage form.

- Some anticoagulants are used in medical equipment, such as **sample tubes**, **blood transfusion bags**, **heart-lung machines**, and **dialysis** equipment.[

Mode of actions of anticoagulants

Anticoagulants inhibit specific pathways of the coagulation cascade, which happens after the initial platelet aggregation but before the formation of fibrin and stable aggregated platelet products.

Qualities of a good anticoagulant used in the lab

- Must not alter the size of the cells
- Must not cause haemolysis
- Must minimize platelet aggregation
- Must minimize distribution of staining and morphology of leukocytes
- Must be readily soluble in water
- Must keep blood in fluid state

Color code tube selection of anticoagulants commonly used

Stopper color	Additive	Notes
Gray 	•**Sodium fluoride & potassium oxalate**: inhibits enolase (phosphopyrovate dehydrogenase) •**Sodium iodoacetate**: inhibits glocose-3-phosphate dehydrogenase	•For glucose determination in chemistry (stabilize glucose in plasma)
	Acid citrate dextrose (ACD)	•For use in blood bank studies, HLA phenotyping, DNA and paternity testing (preserves red cells)

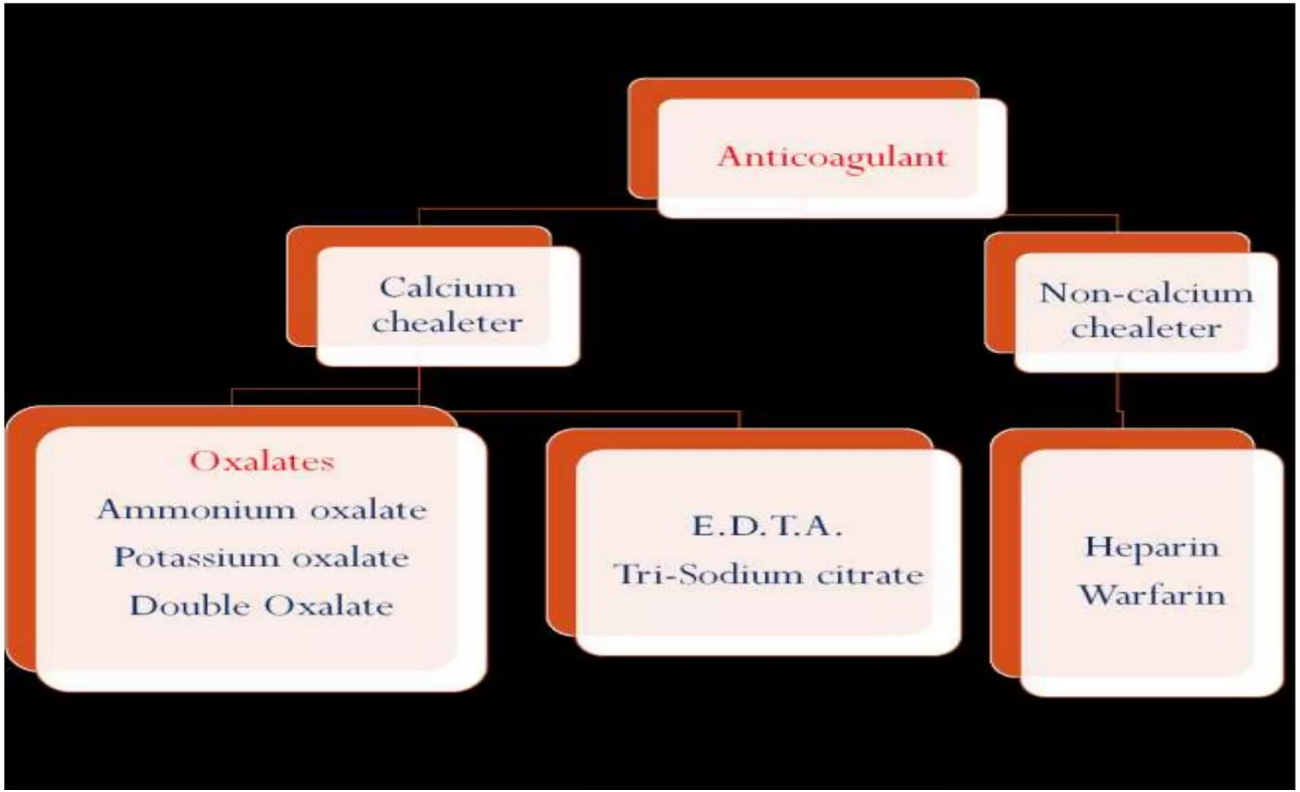
Color code tube selection of anticoagulants commonly used

Stopper color	Additive	Notes
Green 	Sodium or lithium heparin	•Inhibits thrombin activation. •chemistry studies
Light blue 	Sodium citrate	•Coagulation studies (bind calcium) (PT &PTT) (ESR).

Color code tube selection of anticoagulants commonly used

Stopper color	Additive	Notes
Red 	No additive	•Used for blood bank, some biochemistry Invst. •Collection of serum •10-15 min is required to allow blood to clot before centrifugation
Lavender (purple) 	EDTA	•Collection of whole blood (binds calcium)

CLASSIFICATION OF ANTICOAGULANTS



Classification of anticoagulants used in haematology.

Calcium chelators

- ❖ Ammonium oxalate
- ❖ Potassium oxalate
- ❖ Double oxalate
- ❖ EDTA
- ❖ Citrates
  - ➢ Sodium citrate
  - ➢ Acid citrate dexrose

Non-calcium chelators

- ❖ Sodium heparin
- ❖ Warfarin

Commonly used anticoagulants in the lab.

- EDTA
- Oxalate
- Sodium citrate
- Sodium fluoride
- Potassium oxalate

Ethylene Diamine Tetra Acetic Acid (EDTA)

- It is the most frequently used anticoagulant
- It is an amino carboxylic acid and a colourless, water soluble solid
- Also known as sequestrene or versenate
- It is the best option for haematological testing since it best preserves blood cell morphology and cellular components.
- EDTA most important advantage is that it does not distort blood cells, making it ideal for the most hematological tests.

Types of EDTA

There are 3 different formulations of EDTA used as anticoagulants: The choice of used is mostly dependent on the type of analyses to be performed.

- Na2EDTA (Di- sodium EDTA)
- K2EDTA
- K3EDTA (Tri potassium salt)

Example 3

- Causes significant shrinking of the red cells with a decrease of 1–2% in the MCV.
- K_2EDTA in a concentration of 1.5–2.2 mg/ml (4.55 ± 0.8 mmol/ml) as this cause less cellular change[5].

K_3EDTA

K_2EDTA

Reference:
(5) Bachmann F. Molecular aspects of plasminogen, plasminogen activators and plasmin, in Bloom EGD (eds): Haemostasis and Thrombosis. Edinburgh, Churchill Livingstone, 1994, p 575-613

QC at Pre-analytical stage

EDTA salts

K3EDTA

- Causes significant shrinking of red cells with a decrease of 1-2% in MCV

K2EDTA

In a conc. Of 1.5 - 2.2mg/ml gives less cellular changes

- K2EDTA as the anticoagulant of choice for hematological testing in Europe and Japan.

- K3EDTA is more commonly used in the US and the UK

- K2EDTA comes in a spray-dried form that does not dilute small sample volumes. It has a less pronounced osmotic effect on blood cells than K3EDTA.

Mode of action of EDTA

- EDTA inhibits clotting by removing or chelating calcium from the blood.
- EDTA forms insoluble calcium salt by chelation

Concentration

0.5 -2.0mg EDTA/ml of blood will preserve blood excellently for 6hrs.

EDTA
(Ethylene Diamine Tetra Acetic Acid)

- **Advantages :**
- Making a blood smear for cell morphology studies.
- used for Tests for CBC, microfilaria, coombs test.
- EDTA preserves the staining and morphology of Leukocytes

EDTA
(Ethylene Diamine Tetra Acetic Acid)

- **Disadvantages :**
- Excessive conc% of EDTA will cause shrinkage of RBC's and erroneous PCV, MCV, and MCHC results.
- EDTA interferes with blood chemistry tests as follows Falsely decreases alkaline phosphates by binding Mg^{++}
- Decreases CO_2 combining power of blood.
- Interferes with jaffes reaction for creatinine test
- Decreases or alters Na+. K+, and Ca^{2++}con % in plasma

OXALATES

- **Mode of Action :**
- These acts by chelating calcium . Calcium oxalate is formed as insoluble precipitate , these are used for blood chemistry and hematocrit.

Types

- **Potassium oxalate**: 2mg/ml of blood often used for chemical analysis
  Disadvantage: shrink rbc by about 8%.
  Not recommended for PCV and ESR test

- Ammonium oxalate

- Double oxalate
  Combination of potassium oxalate and ammonium oxalate in ratio 2:3
  This is done to counter the swelling effect of ammonium oxalate and the shrinking effect of potassium oxalate.
  Can be used for PCV and ESR
  Disadvantages
  Leukocytes morphology not well preserved
  The calcium chelated is precipitated as calcium oxalate which is toxic
  Cannot be used in transfusion

HEPARIN

- It is a natural anticoagulant in the body, found in the liver, and may also be with in basophils and mast cells, heparin also called anti thromboplastin or antithrombin.
- It is available in a liquid or dry form as.....
- sodium, calcium, ammonium and lithium salt, Each of these will interfere with determination of their respective ions in the plasma

HEPARIN

- **Mode of Action :**
- It interferes with the formation and or activity of thrombin and the activity of clotting factors IX, X, XI, XII

Conc 0.1 to 0.2mg/ml of blood

HEPARIN

- **Advantages :**
- Heparin is the choice of Anticoagulant for blood pH,and blood gas Analysis. Acid base balance.
- It may be used for special trace elements studies and some cytology .
- Excessive heparin does not alter the RBC volume

HEPARIN

- **Disadvantages :**
- It causes clumping of leukocytes
- It interferes with staining of leukocytes.
- It is the most expensive of the anticoagulant
- Blood clot in 8-12 hrs because clotting is only delayed and not prevented.
- It is not suitable for agglutination tests , and coagulation studies
- It may interfere with some automated biochemical analysis of plasma.

SODIUM CITRATE

- The formal citrate solution (Dacies solution) is used as diluent in the counting of RBCs and PLT's
- **Concentration :**
- 3.13 grms of Trisodium citrate is dissolved in 100 ml of water, 1 ml of formaldehyde is added to every 99 ml of the solution.

- **Mode of action :**
- It combines with calcium to form insoluble salt of calcium citrate

- **Advantages** :
- Sodium citrate is the anticoagulant for choice for studies of PLTs function and morphology

- **Concentration**:
- The standard concentration 1 part (3.8%) for 9 parts of blood

- **Disadvantages** :
- It interferes with many chemical tests
- Used alone it preserves blood for only few min.
- It has a tendency to shrink cells. Because of 10% dilution of blood – sodium citrate is generally not used for CBC

ACID CITRATE DEXTROSE (ACD)

- Is prepared from disodium hydrogen citrate and is the anticoagulant of choice for blood transfusion.

Eg; 2 grms of Na2 hydrogen citrate and 3 grms dextrose are added to 120 ml of water autoclaved for 30 min at 20 PSI and used the ratio 1 part acid to 4 parts of blood

SODIUM FLUORIDE AND POTASSIUM OXALATE MIXTURE

- **Mode of Action** :
- Sodium fluoride inhibits the glycolytic enzymes responsible for the break down of glucose in the blood.
- (At RT. About 10% glucose is lost per hour from an untreated sample)
- The potassium oxalate is the primary anticoagulant as sodium fluoride has a poor anticoagulant effect.

SODIUM FLUORIDE AND POTASSIUM OXALATE MIXTURE

- **Concentration**:
- The optimum concentration : 1 mg of mixture per 1 ml of blood

- Uses: Glucose determination