«General pharmaceutical chemistry »

COMPLEMENTOMETRY.
DETERMINATION OF NITROGEN IN MEDICINAL PRODUCTS. KJELDAHL METHOD.

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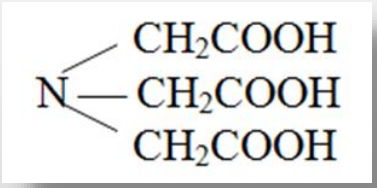
COMPLEXOMETRIC TITRATION

Complexometric titration is a method of titrimetric analysis based on the complexation reaction of metal cations with complexones – aminopolycarboxylic acids and their salts.

COMPLEXOMETRIC TITRATION

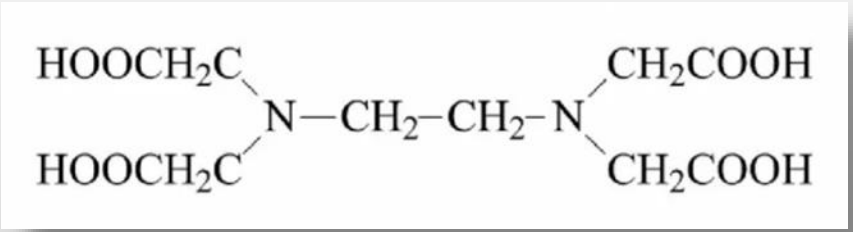
Complexons

Complexon I



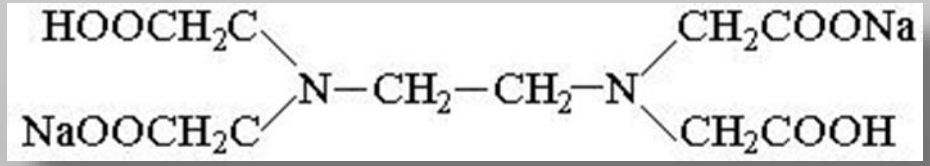
nitrilotriacetic acid (H_3Y)

Complexon II



ethylenediaminetetraaceti c acid, EDTA (H_4Y)

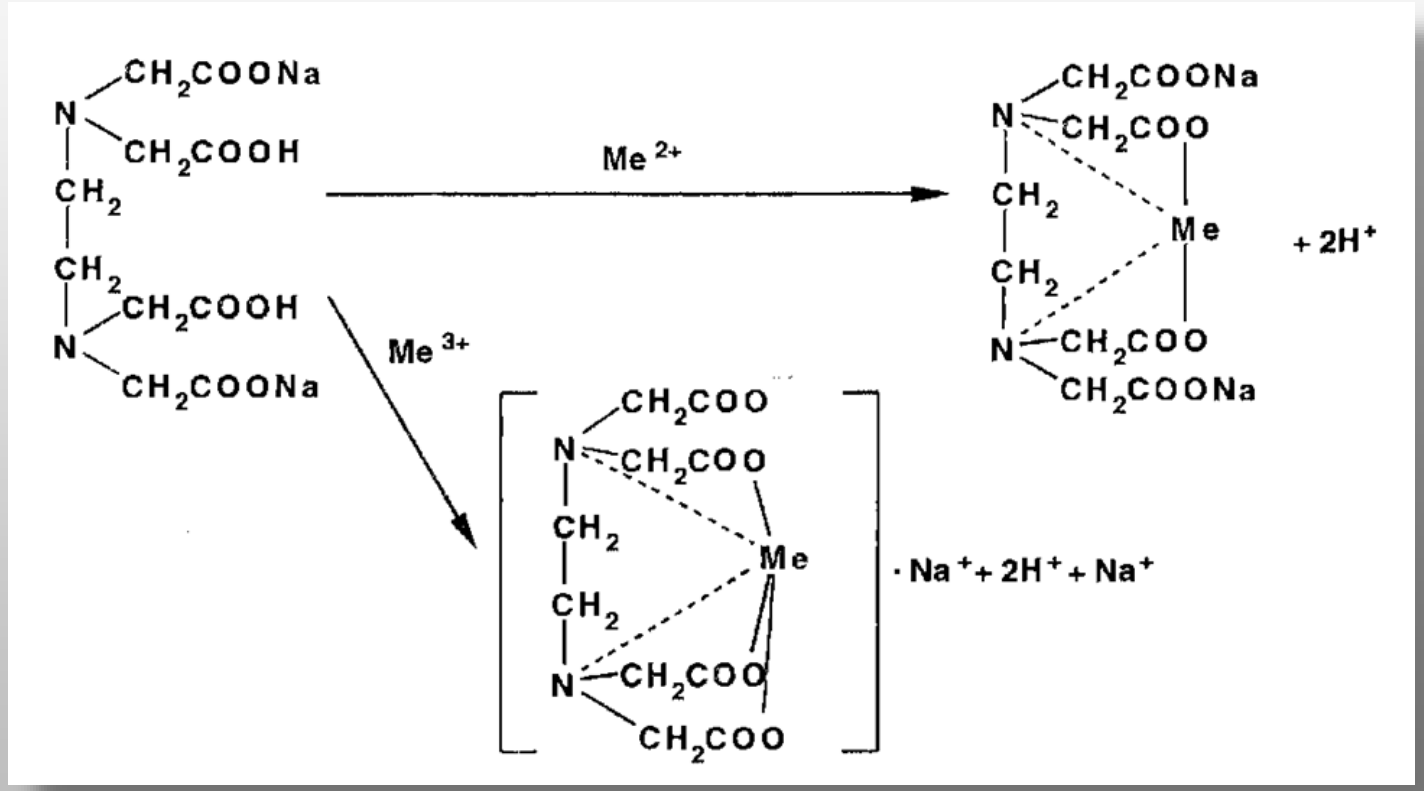
Complexon III



Ethylenediaminetetraacetic acid diatrium salt, Na_2EDTA, trilon B (Na_2H_2Y)

COMPLEXOMETRIC TITRATION

Chemistry



the composition of the resulting complexes is, with few exceptions, 1:1

COMPLEXOMETRIC TITRATION

Specific features of the reaction

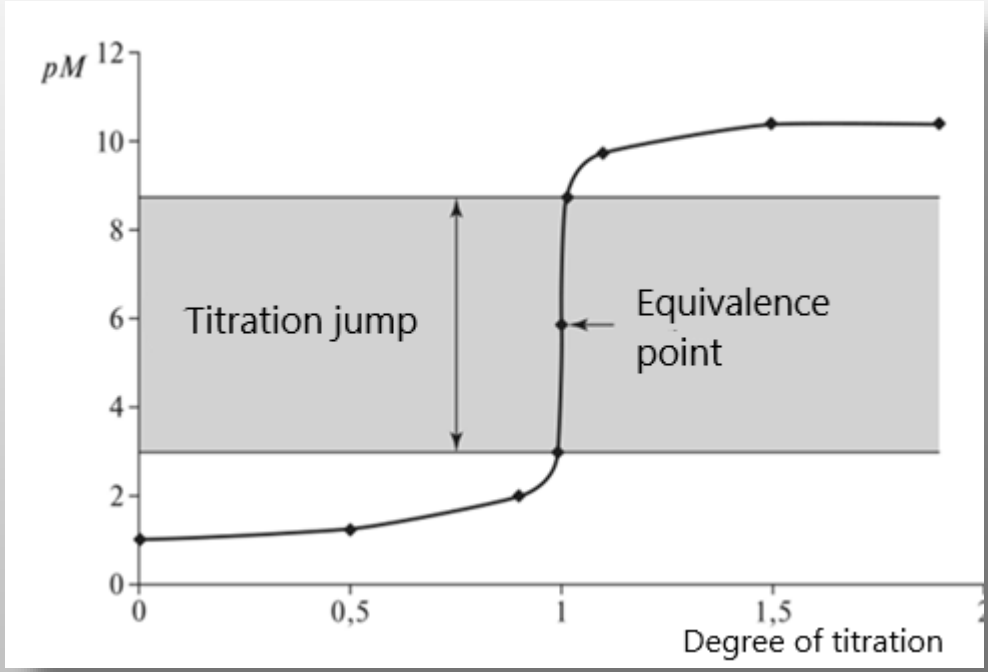
1. the reaction produces complexes of only one composition with a metal-complexing - ligand-complexon (M:L) component ratio of 1:1.
2. The complexonates are colourless, water-soluble and highly stable, as the central metal atom is firmly bound to the polydentate chelate ligand.
3. The reaction is a reversible process and can be shifted both towards the formation and destruction of the complexonate. This is easily achieved by varying the pH of the solution: acidification shifts the equilibrium to the left towards the parent reagents, while alkalinisation promotes the formation of the complexonate:

$$M^{2+} + Na_2H_2Y \rightleftarrows Na_2[MY] + 2H^+$$

4. The reaction generates hydrogen ions, so it should be carried

COMPLEXOMETRIC TITRATION

Titration curves



The magnitude of the jump in the titration curve is affected:

- initial concentration of the ion to be determined in the solution
- the stability constant of the resulting compound with EDTA

The higher the concentration of the ion to be determined and the stronger the complex, the greater the jump.

COMPLEXOMETRIC TITRATION

Preparation of Trilon B standard solution

The diisodium salt of ethylenediamine-tetraacetic acid is hygroscopic, so a **secondary standard solution** is prepared from it.

Standardised by

standard substances – chemically pure ZnO, $CaCO_3$.

standard solutions of $ZnSO_4$ or $MgSO_4$.

COMPLEXOMETRIC TITRATION

Fixing the titration end point

The titration end-point is set with metal indicators – indicators that change colour depending on the concentration of the metal ion.

These are organic compounds that contain chromophore groups in their molecules and are therefore coloured. Such indicators form complexes with metal ions less strong than the titrant and the colour of these complexes differs from the colour of the indicators themselves.

Metal indicators can be divided into two groups:

- **Indicators which are not themselves coloured, but form coloured complexes with metal ions.**
- **metallochromic indicators** – organic substances with chromophore groups that form intensely coloured complexes with metal ions, which are less stable than

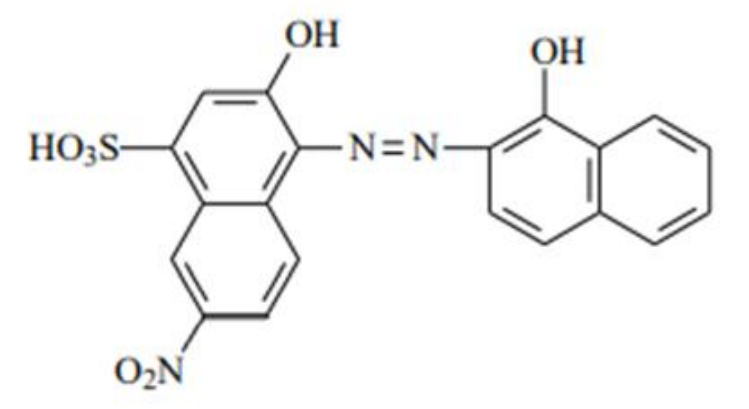
COMPLEXOMETRIC TITRATION

Requirements for metal indicators

1. In the selected pH region they should form sufficiently stable complexes with metal ions with the ratio M:Ind=1:1.
2. The complex of the metal ion with the indicator must be kinetically labile and rapidly break up on addition of the complexons;
3. The colour change of the solution in the titration end-point should be contrasting.

COMPLEXOMETRIC TITRATION

Eriochrome black T

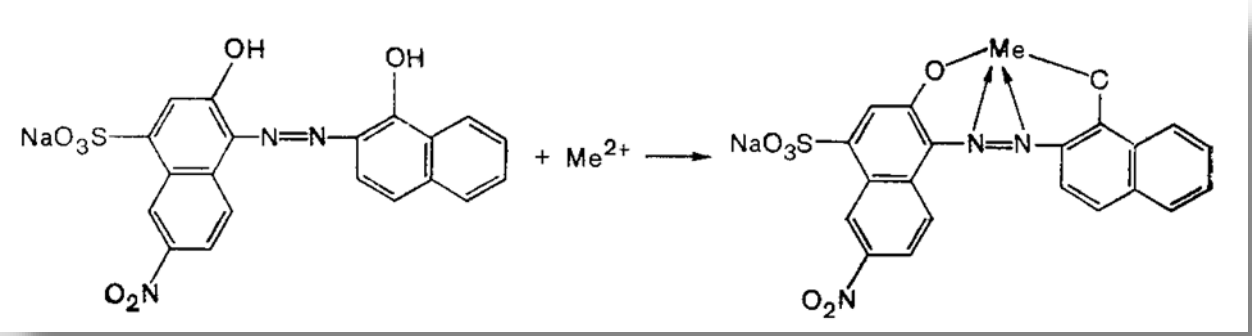


$H_2Ind^- \longleftrightarrow HInd^{2-} \longleftrightarrow Ind^{3-}$

pH < 6	6 < pH < 11	pH > 11
Red	*Blue*	*Orange*

COMPLEXOMETRIC TITRATION

Eriochrome black T



COMPLEXOMETRIC TITRATION

Eriochrome black T

During titration with a complexon III solution near the point of equivalence, the indicator is displaced by the complexon with the formation of a colourless complexonate and a blue coloured form of the free indicator $HInd^{2-}$:

$$MInd^{n-3} + [H_2Y]^{2-} \leftrightarrow [MY]^{n-4} + HInd^{2-} + H^+$$

Wine red — Colourless — Blue

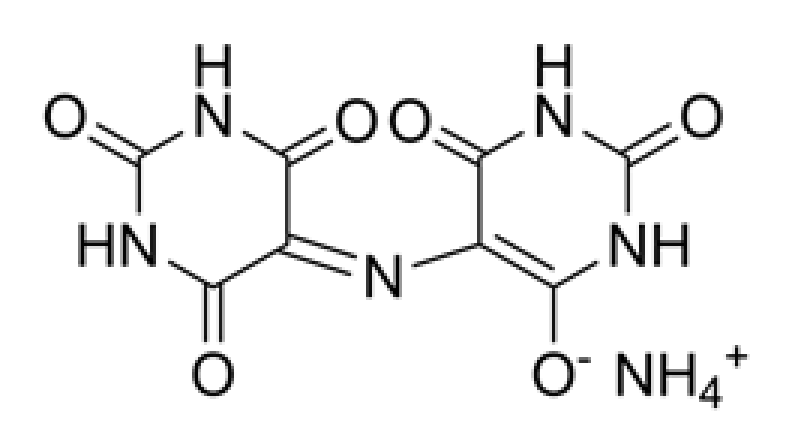
The change in colour of the indicator from **wine red** to **blue** indicates the end point of the titration

Colouring at the beginning of the titration

At the end of the titration

COMPLEXOMETRIC TITRATION

Murexide



COMPLEXOMETRIC TITRATION

Murexide

$H_4Ind^- \longleftrightarrow H_3Ind^{2-} \longleftrightarrow H_2Ind^{3-}$

pH < 9	9 < pH < 11	pH > 11
Red-Purple	**Purple**	**Blue**

COMPLEXOMETRIC TITRATION

Murexide

Murexide forms stable coloured complexes with calcium, nickel, cobalt, copper and other cations.
Calcium complexes are **red**,
complexes of nickel, cobalt and copper are **yellow**.

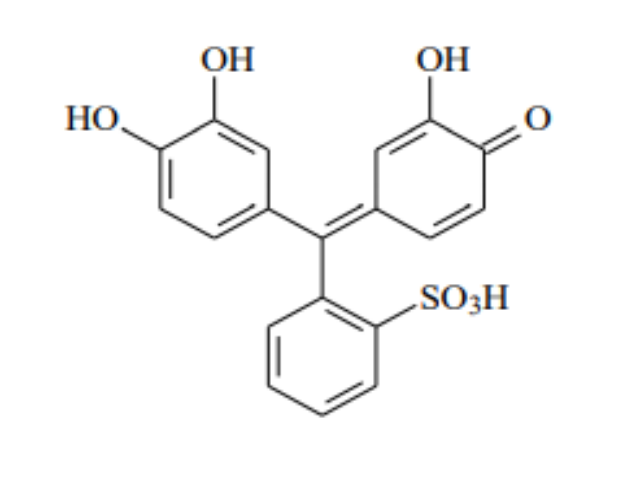
Murexide is used in the complexometric determination:

- Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+} (pH 8–9),
- Ca^{2+} (pH >12).

The indicator is used as a 1% aqueous solution or as a dry mixture with sucrose (or sodium chloride) in a 1:500 ratio.

COMPLEXOMETRIC TITRATION

Pyrocatechin purple



H_4Ind	$\longleftrightarrow$	H_3Ind^-	$\longleftrightarrow$	H_2Ind^{2-}	$\longleftrightarrow$	$HInd^{3-}$	$\longleftrightarrow$	Ind^{4-}
pH < 1		1 < pH < 8		8 < pH < 10		10 < pH < 11		pH > 11
Red		**Yellow**		**Purple**		**Red-purple**		**Blue**

COMPLEXOMETRIC TITRATION

Complexometric titration conditions

1. The complexation reactions should be fast, quantitative and stoichiometric so that near the equivalence point the determined cations are almost completely bound to the complex.
2. Detectable ions should form less strong complexes with the metalindicator than their complexes with trilon B.
3. The complexometric titration must be conducted at a certain pH value (pH < 10), because precipitation of hydroxides of determined cations or their basic salts can form in an alkaline environment.

In order to maintain a certain pH value the titration should be carried out in the presence of buffer solutions having a certain pH value. Most cations are titrated with Trilon B in the

COMPLEXOMETRIC TITRATION

Direct titration

Ammonia buffer solution and the metal indicator are added to the test solution and titrated with a standard trilon B solution. At the point of equivalence the colouring of the solution changes from the colouring of the cation complex with the metal indicator to the colouring of the free metal indicator. The cations Cu^{2+}, Co^{2+}, Pb^{2+}, Ni^{2+}, Zn^{2+}, Fe^{3+}, Ba^{2+}, Cr^{3+},Ca^{2+}, Mg^{2+} etc. are determined by direct titration.

COMPLEXOMETRIC TITRATION

Reverse titration

To the analysed solution add

- ✓ Ammonia buffer solution,
- ✓ Exactly measured double of the minimum volume of Trilon B standard solution, which reacts with the determined ions,
- ✓ Excess Trilon B is titrated with a standard solution of magnesium sulphate or zinc sulphate in the presence of a metal indicator.

In the proces

$$Me^{2+} + H_2Y^{2-} \leftrightarrow [MeY]^{2-} + 2H^+$$

$$\text{изб. } H_2Y^{2-} + Zn^{2+} \leftrightarrow [ZnY]^{2-} + 2H^+$$

COMPLEXOMETRIC TITRATION

Reverse titration

Change from free indicator colouring to colouring of the metal indicator complex with the titrant cation.

The reverse titration method is used:

- ✓ when the complexation reaction is slow;
- ✓ there is no suitable indicator for fixing the titration end-point in the direct titration method;
- ✓ The indicator forms a very strong complex with the determined ion, which is not destroyed by the complexon;
- ✓ for the determination of cations in water-insoluble sediments for example Ca^{2+} in CaC_2O_4, Mg^{2+} in $MgNH_4PO_4$, Pb^{2+} in $PbSO_4$.

COMPLEXOMETRIC TITRATION

Substitution titration

The method is based on the fact that most ions form more stable complex compounds with trilon B than the complex of Mg^{2+} cations with trilon B $[MgY]^{2-}$. After the addition of the $[MgY]^{2-}$ complex to the analysed solution, an exchange reaction takes place:

$$[MgY]^{2-} + Me^{2+} \rightarrow [MeY]^{2-} + Mg^{2+}$$

The released Mg^{2+} ions are titrated with a standard solution of Trilon B in the presence of a metal–chromium indicator:

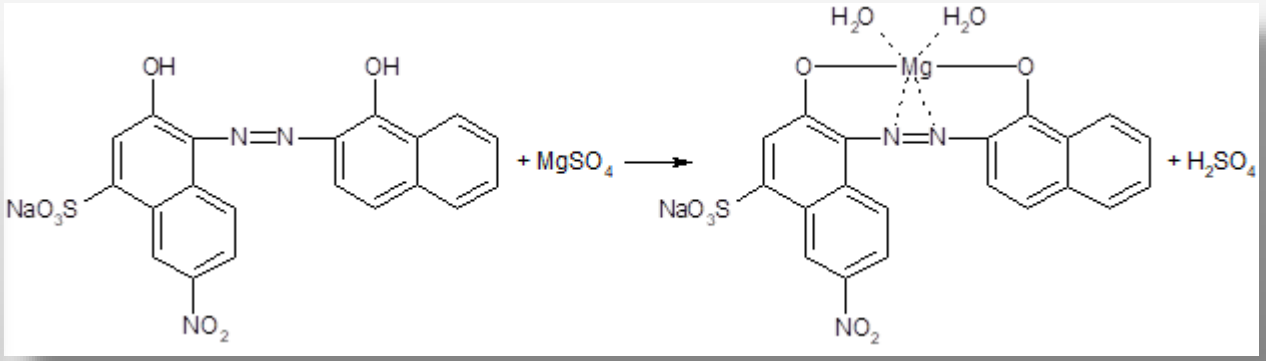
$$Mg^{2+} + H_2Y^{2-} \rightarrow [MgY]^{2-} + 2H^{+}$$

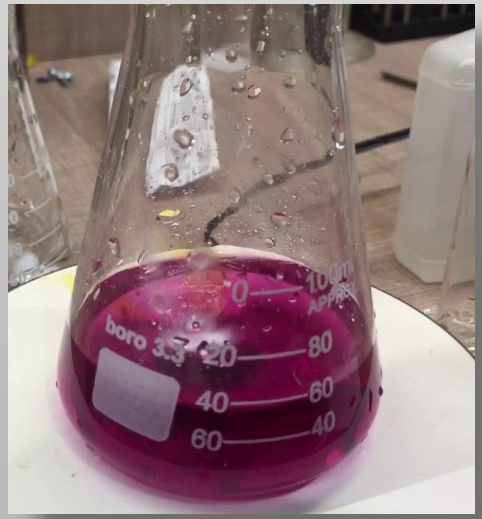
At the point of equivalence, the colouring of the solution changes to the colouring of the free metal indicator.

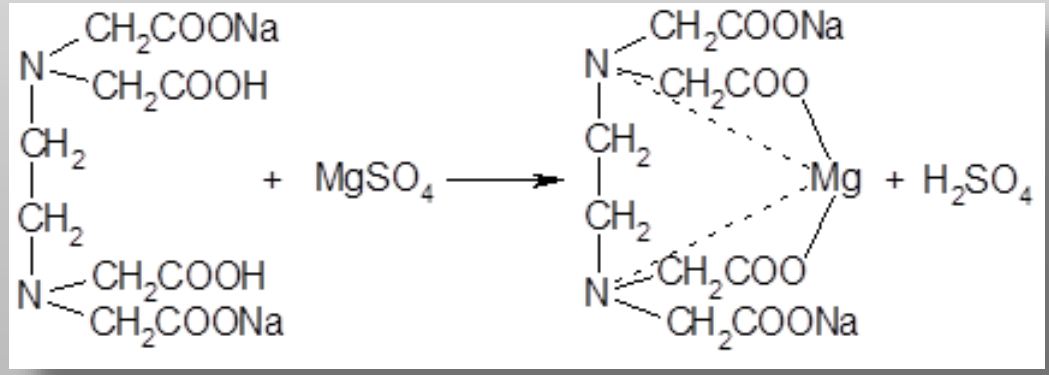
COMPLEXOMETRIC TITRATION

Applications in pharmaceutical analysis

$MgSO_4$
Titrant: **EDTA**
Indicator:
Eriochrome black T







COMPLEXOMETRIC TITRATION

Applications in pharmaceutical analysis

$MgSO_4$
Titrant: **EDTA**
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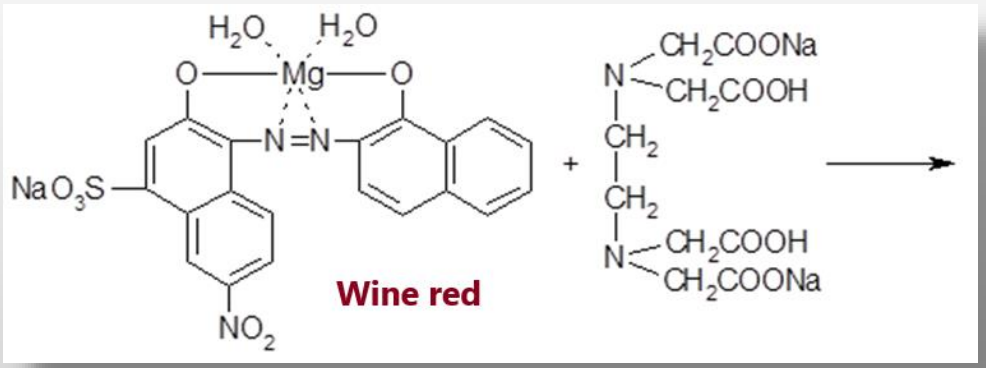
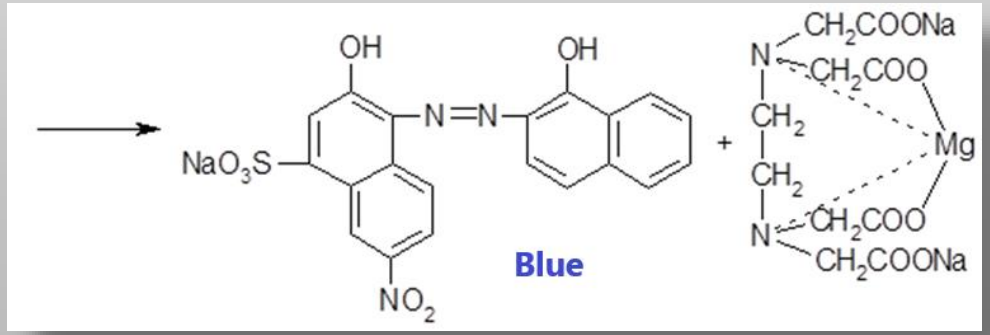


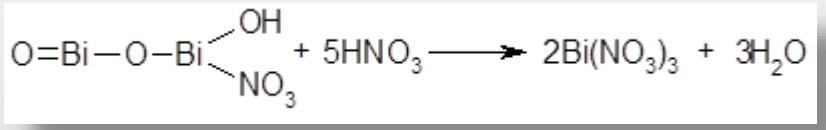


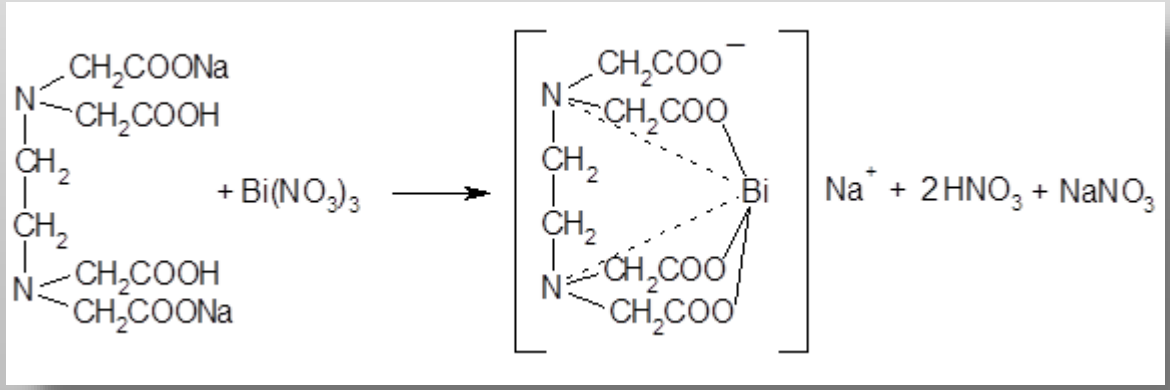
COMPLEXOMETRIC TITRATION

Applications in pharmaceutical analysis

Bismuth nitrate basic

Titrant: **EDTA**
Indicator: **Pyrocatechin purple**





COMPLEXOMETRIC TITRATION

Applications in pharmaceutical analysis

Bismuth nitrate basic

Titrant: **EDTA**
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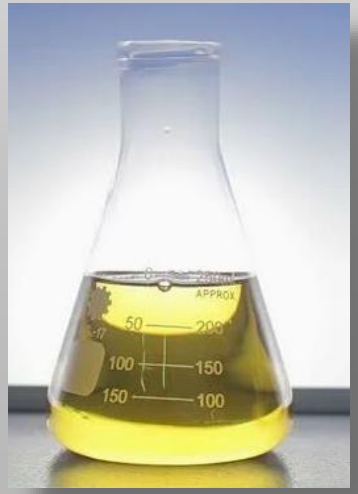
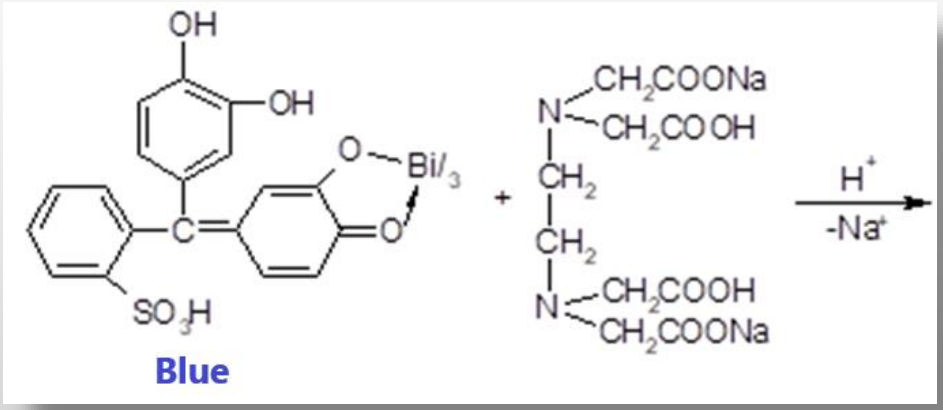
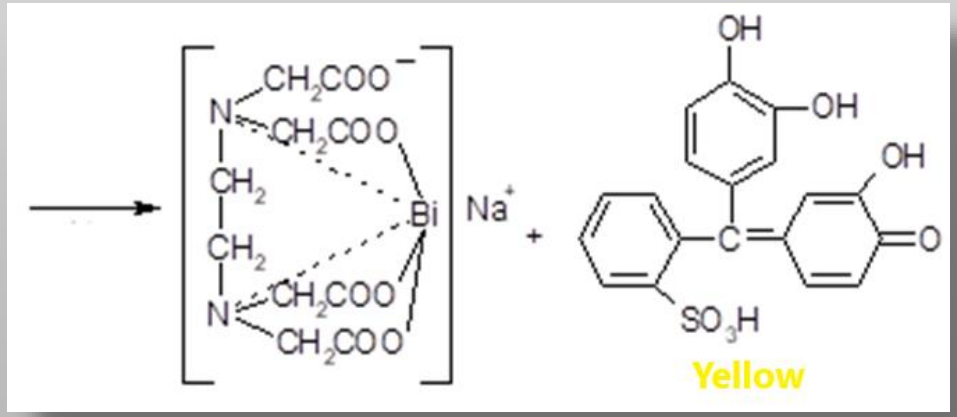




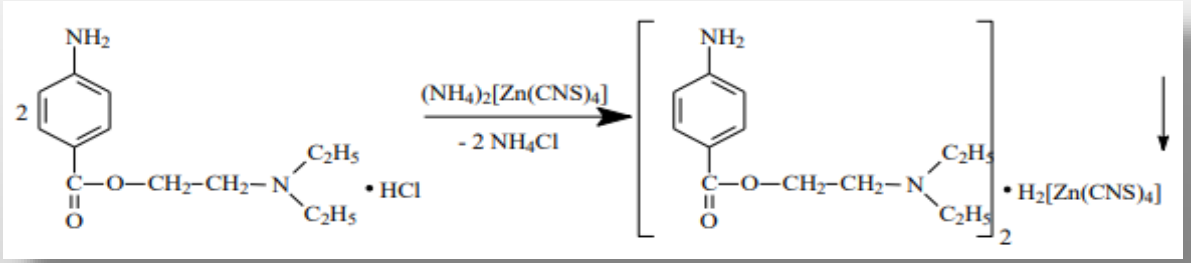


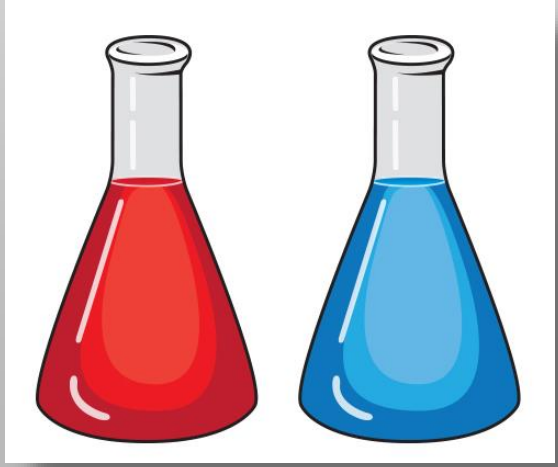
COMPLEXOMETRIC TITRATION

Applications in pharmaceutical analysis

Novocaine hydrochloride

Titrant: **EDTA**
Indicator: **Eriochrome black T**

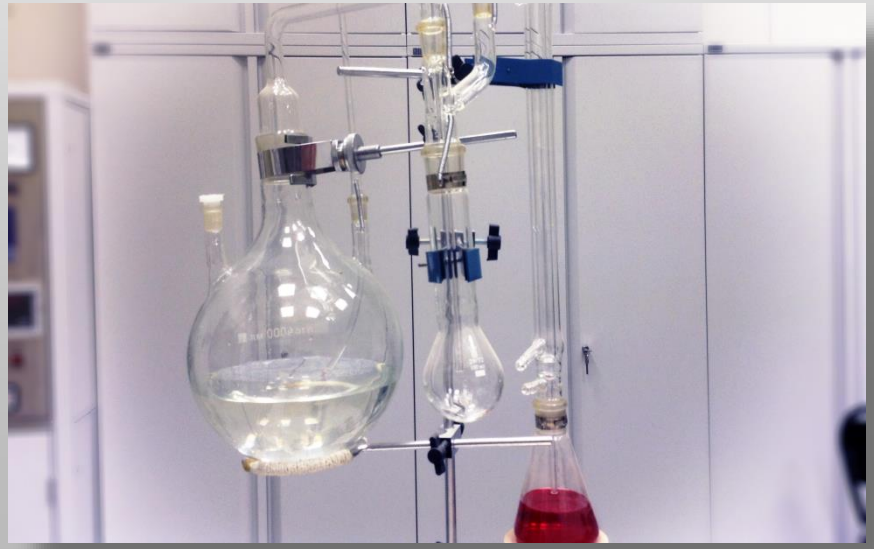




$$(NH_4)_2[Zn(CNS)_4] + Na_2[H_2Y] = Na_2[ZnY] + 2NH_4CNS + 2HCNS$$

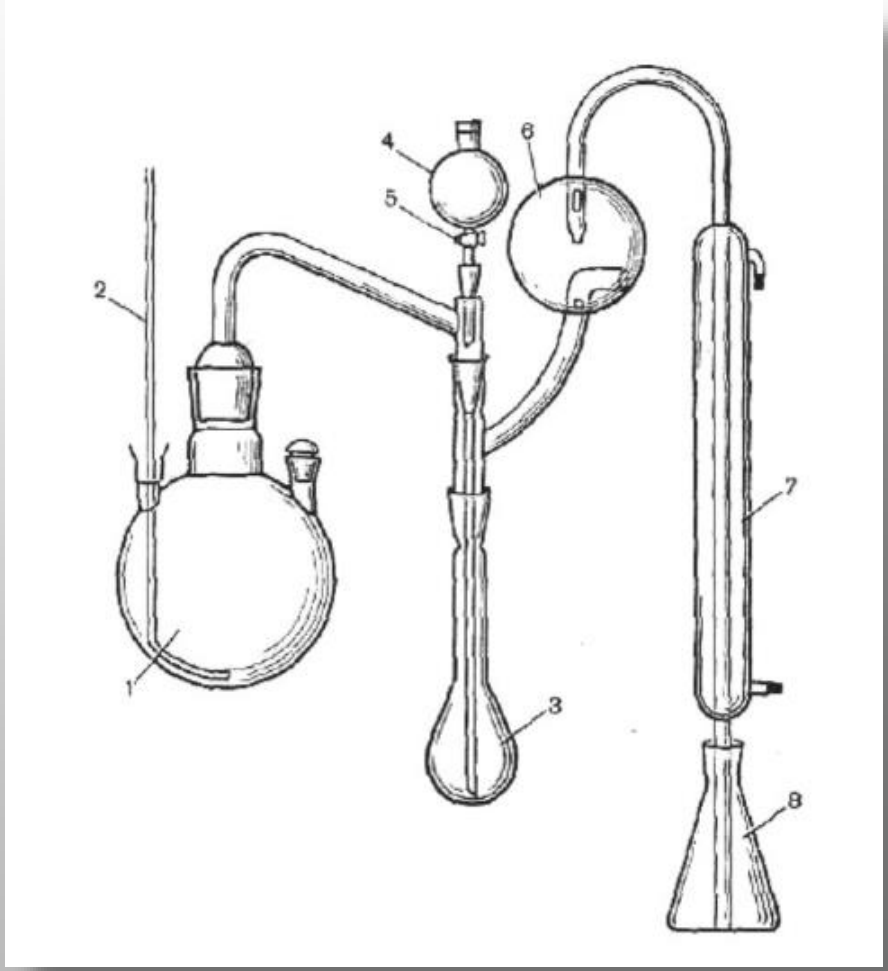
DETERMINATION OF NITROGEN IN MEDICINAL PRODUCTS. KJELDAHL METHOD.

The method is based on the mineralisation of a drug by exposure to concentrated sulphuric acid when heated in the presence of catalysts.



A mixture of potassium sulphate, copper sulphate and/or selenium and/or titanium dioxide can be used as catalysts

DETERMINATION OF NITROGEN IN MEDICINAL PRODUCTS. KJELDAHL METHOD.



1. Steam generator – flask with a volume of 3 litres
2. Safety tube
3. Long-necked Kjeldahl exchange flasks for condensing water vapour and protecting against loss of substance,
4. Drop funnel
5. Clamp or tap for adding sodium hydroxide
6. Splashguard
7. Condenser
8. Trapping flask

DETERMINATION OF NITROGEN IN MEDICINAL PRODUCTS. KJELDAHL METHOD.

1. Mineralisation (heating with concentrated H_2SO_4):

$$R{-}CH_2{-}NR_2 \xrightarrow{[O]} CO_2\uparrow + H_2O + (NH_4)HSO_4$$

2. Decomposition of $(NH_4)HSO_4$ with sodium hydroxide and distillation of the resulting ammonia into the trapping flask:

$$(NH_4)HSO_4 + 2OH^- \rightarrow NH_3\uparrow + 2H_2O + SO_4^{2-}$$

3. Interaction of NH_3 in a trapping flask with boric acid to form ammonium tetrahydroxiborate:

$$B(OH)_3 + H_2O \rightarrow H[B(OH)_4]$$

$$NH_3 + H[B(OH)_4] \rightarrow NH_4[B(OH)_4]$$

4. Titration of the distillation with 0.1M hydrochloric acid solution:

$$NH_4[B(OH)_4] + HCl \rightarrow NH_4Cl + B(OH)_3 + H_2O$$

the colour change of the mixed indicator from green to red-violet

DETERMINATION OF NITROGEN IN MEDICINAL PRODUCTS. KJELDAHL METHOD.

Equipment package for protein/nitrogen determination by the Kjeldahl method

Kjeldahl apparatus

Thank you for attention!