Ministry of Health of the Russian Federation

Volgograd State Medical University

Department of Pharmaceutical and Toxicological Chemistry

SPECIAL PHARMACEUTICAL CHEMISTRY

Vitamins are derivatives of pyridine. Oxy-methylpyridine vitamins

Lesson 14

VII term

Volgograd, 2023

PYRIDINE CARBONATE VITAMINS VITAMIN B5 OR PP

Representatives of the group of pyridine vitamins are nicotinic acid and its amide:

Nicotinic acid *Nicotinamide*

Nicotinic acid, or β-pyridinecarboxylic acid, and its amide have been known for a very long time (nicotinic acid was first produced synthetically by Huber in 1867-1870), but their vitamin properties were not discovered until 1937-1938. During these years, a crystalline substance was extracted from liver, which turned out to be nicotinic acid. It was used to treat pellagra in dogs.

It is known that most natural sources do not contain nicotinic acid itself, but its amide. Nicotinamide has been found to be a member of the prosthetic groups of many enzymes that carry out hydrogen transfer reactions in the body. Nicotinic acid is converted to nicotinamide as part of the body's metabolism, so it is considered a provitamin of nicotinamide.

Nicotinamide was first called PP factor (anti-pellagra factor), then vitamin PP, pellagramine and others. The name of vitamin "PP" is derived from the English word "Pellagra-Preventive". In recent years, nicotinic acid has been commonly referred to as niacin and its amide as nicotinamide.

The richest sources of nicotinic acid are yeast, wheat and rice bran, mushrooms and liver. At present, nicotinic acid and its amide are produced synthetically.

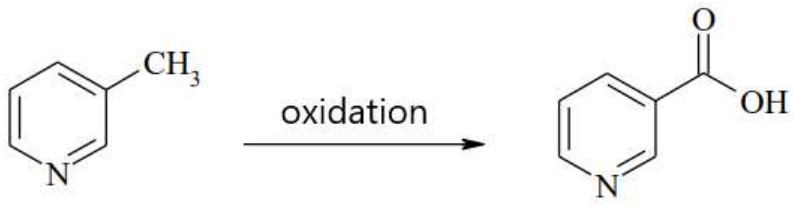
Nicotinic acid and nicotinamide are pharmacopoeial drugs.

Nicotinic acid

β-Pyridine carboxylic acid, otherwise called nicotinic acid, is known as vitamin PP. It was first was first produced back in 1867.

OBTAINING

Various ways of synthesis of nicotinic acid are known, but the method of production from β-picoline is of industrial importance. The picoline fraction is subjected to fractional separation into α- β- and γ-picoline. Then, by oxidation of β-picoline, nicotinic acid is obtained:



PHYSICAL PROPERTIES

White crystalline powder, odourless, slightly acidic taste. Melting point 234 - 238 °C.

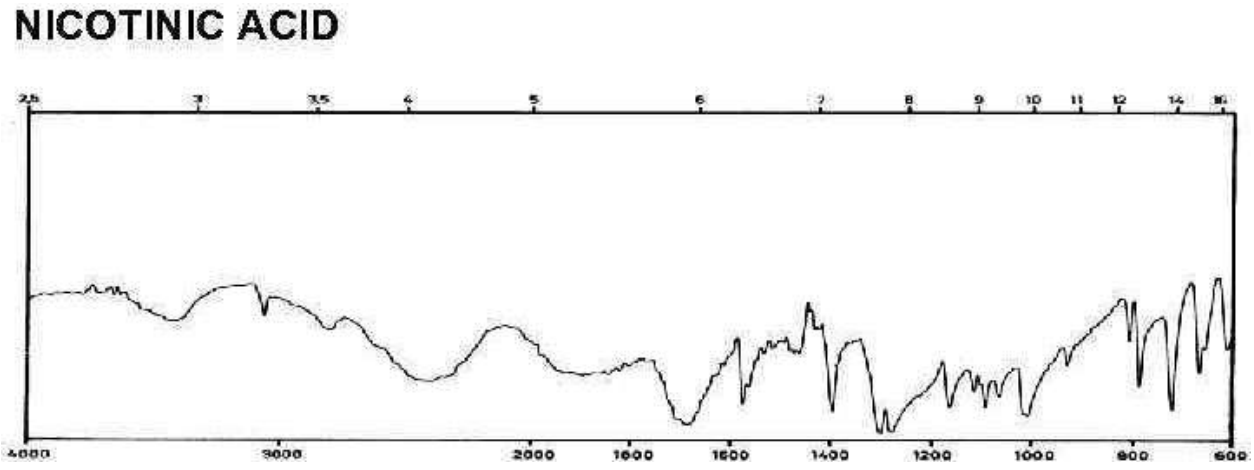
Slightly soluble in water and alcohol, soluble in hot water, very slightly soluble in ether.

IDENTIFICATION

Nicotinic acid shows amphoteric properties because of the presence of in its structure of cyclic pridinic nitrogen (basic) and carboxyl group (acidic).

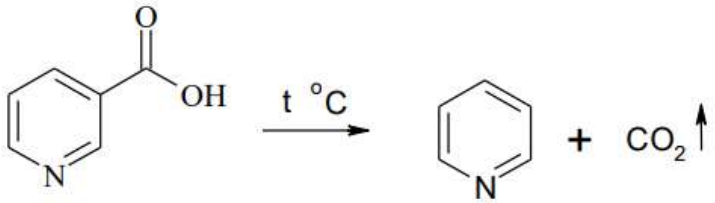
1. Infrared spectroscopy

The infrared spectrum of the substance, taken in a disc with KBr, in the region from 4000 to 400 cm^{-1}, according to the position of the absorption bands should correspond to the figure of the spectrum of nicotinic acid.



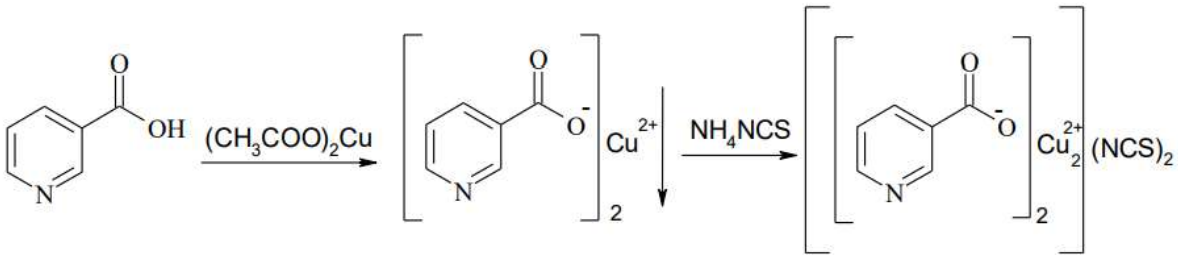
2. Decomposition reaction

When nicotinic acid is heated with crystalline potassium carbonate, it decomposes to form pyridine (characteristic odour) and carbon dioxide, which forms an opalescence when passed through lime or barite water:



3. Complexation reaction

When cupric acetate acts on a solution of nicotinic acid, copper nicotinate is formed, a blue precipitate. When ammonium thiocyanate is added, a green ternary complex is formed:



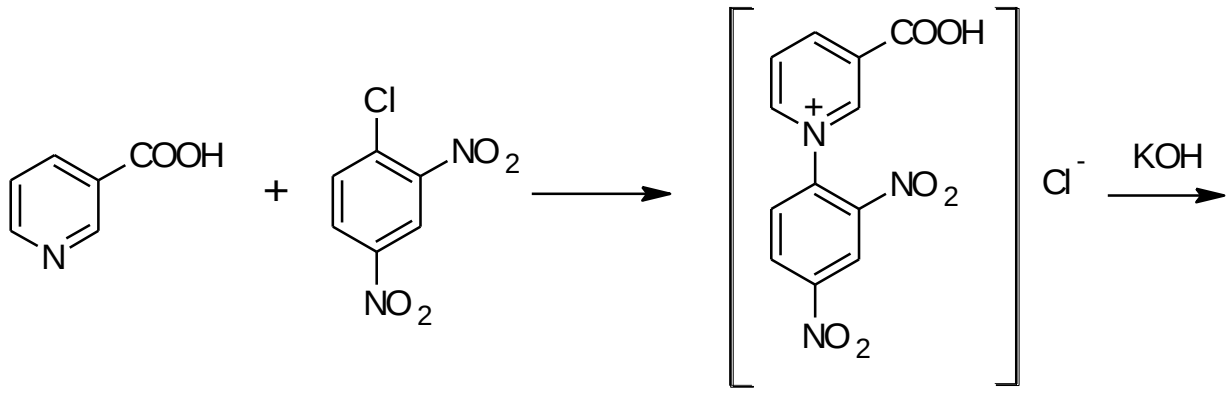
Blue *green*

4. Formation of polymethine dye (Zinke reaction)

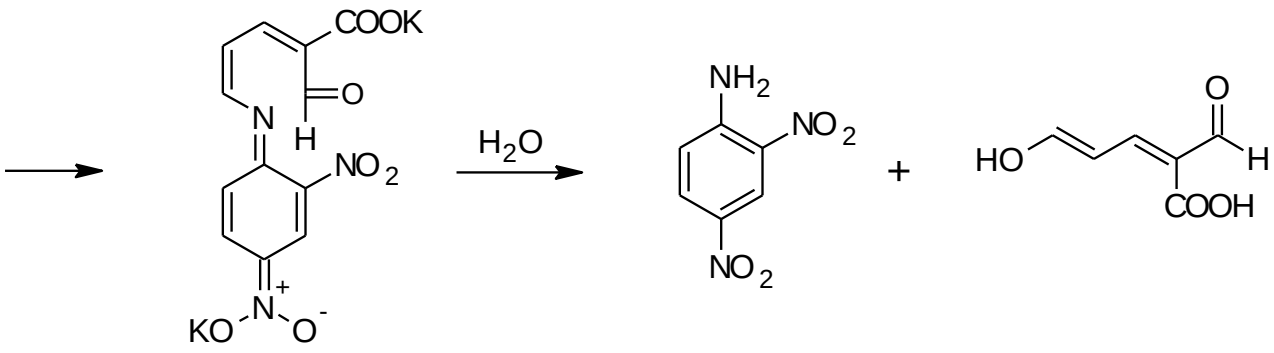
When 2,4-dinitrochlorobenzene is reacted in an alcoholic alkali solution, the pyridine cycle is cleaved.

First, a colourless pyridinium salt is formed (1), which under the action of alkali opens the pyridine cycle to form a glutaconic aldehyde derivative (polymethine base), coloured brown or red (2).

Glutaconic aldehyde derivatives are unstable compounds susceptible to hydrolysis in alkaline media. They are cleaved to glutaconic aldehyde (4) and 2,4-dinitroaniline (3):



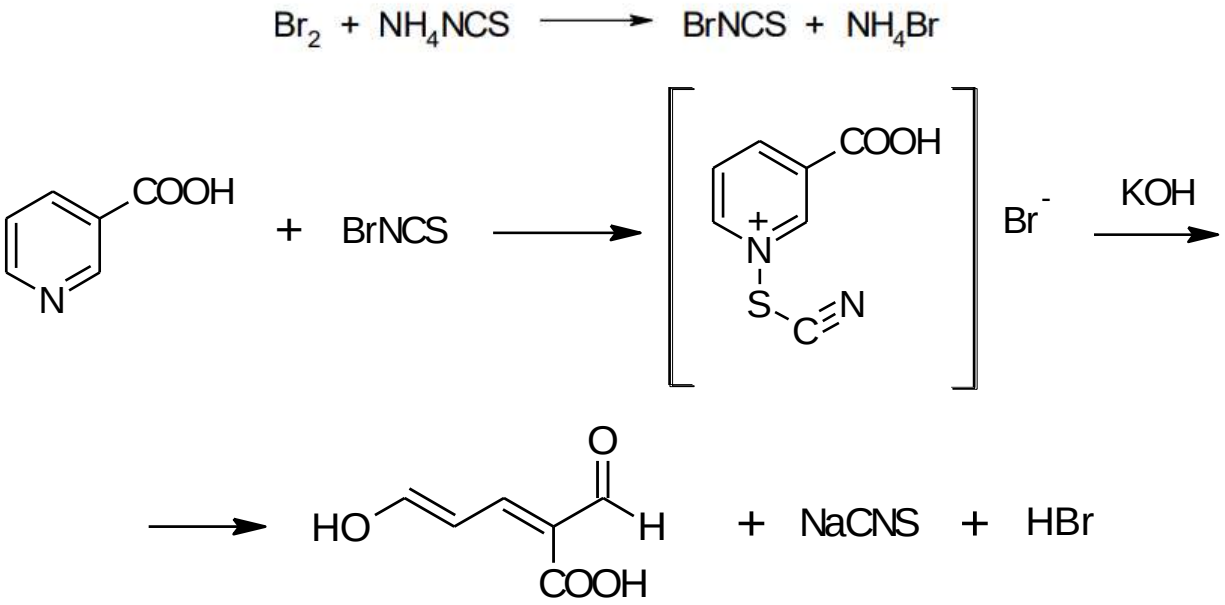
1



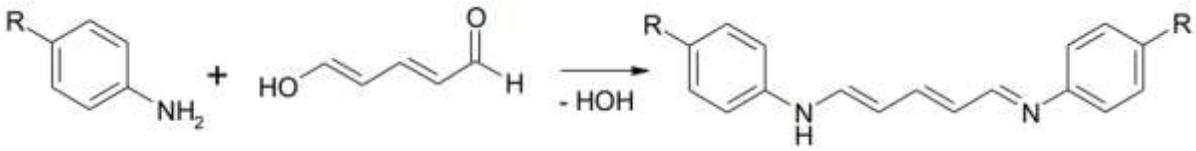
2 *3* *4*

Glutaconic aldehyde has a yellow colour in alkaline medium.

Bromine thiocyanate can be used as a cleavage agent. Bromine thiocyanate is obtained by adding to bromine water ammonium thiocyanate to bromine water until discolouration:



The subsequent addition of primary aromatic amines (aniline, novocainamide, sodium sulphacyl) leads to a condensation reaction with glutaconic aldehyde to form Schiff bases coloured intensely yellow, orange or red*:*



5. Reaction with citric acid and acetic anhydride

Interaction with citric acid and acetic anhydride produces a red-violet colouration when heated.

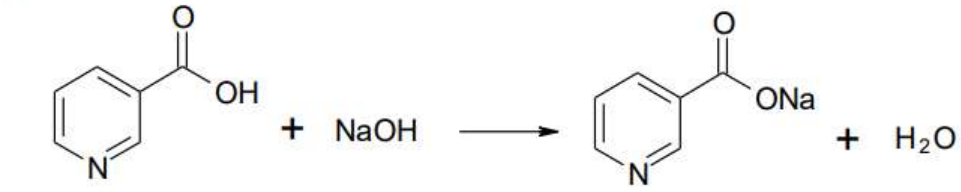
PURITY TEST

In the purity test, the permissible impurity content of the initial products of synthesis or decomposition is determined. In nicotinic acid the permissible content of 2,6- and 2,5-pyridindicarboxylic acids is determined using a standard.

QUANTIFICATION

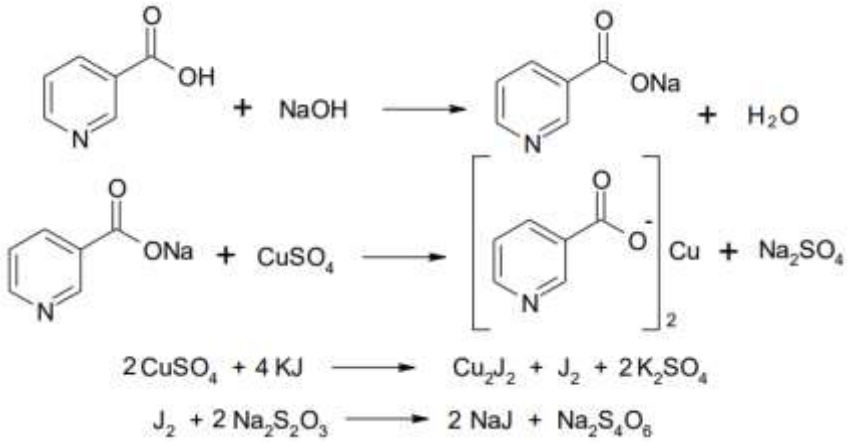
1. Alkalimetry

Aqueous solutions of nicotinic acid have acidic properties. A suspension of nicotinic acid is dissolved in hot water and titrated with 0.1 N sodium hydroxide solution to the formation of sodium salt (indicator phenolphthalein - colour change from colourless to pink):



2. Iodometric titration (after precipitation of copper nicotinate)

Nicotinic acid is treated with an excess of titrated copper (II) sulphate solution in alkaline medium. Then excess working solution is titrated iodometrically in the presence of indicator - starch:



STORAGE

Nicotinic acid is stored in well sealed containers protected from light in a dry place protected from light.

MEDICAL USE

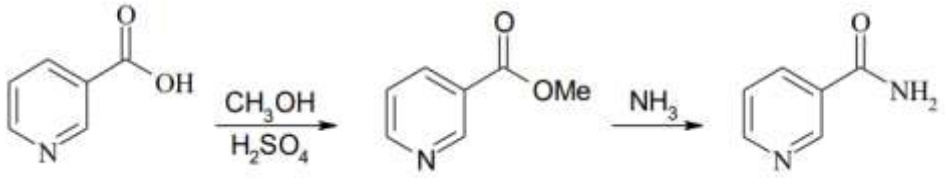
Nicotinic acid is a vitamin, hypolipidaemic and specific antipellagic agent. In the body, nicotinic acid is converted into nicotinamide, which binds to hydrogen

transfer codehydrogenase I and II coenzymes (NAD and NADPH), participates in the metabolism of fats, proteins, amino acids, purines, tissue respiration, glycogenolysis, biosynthesis processes, and, most importantly, reduces the level of lipoprotein and triglyceride, which clog blood vessels and contribute to high blood pressure.

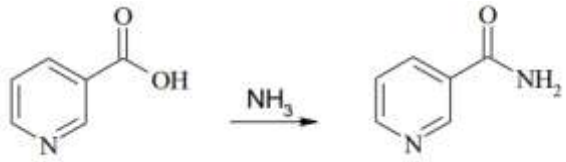
Nicotinamide

OBTAINING

Nicotinamide is synthesised from nicotinic acid. The intermediate product of the synthesis is nicotinic acid methyl ester:



A better and more economical way to synthesise nicotinamide is by passing ammonia gas into a mixture of nicotinic acid and aqueous ammonia solution at 180-185 °C:



PHYSICAL PROPERTIES

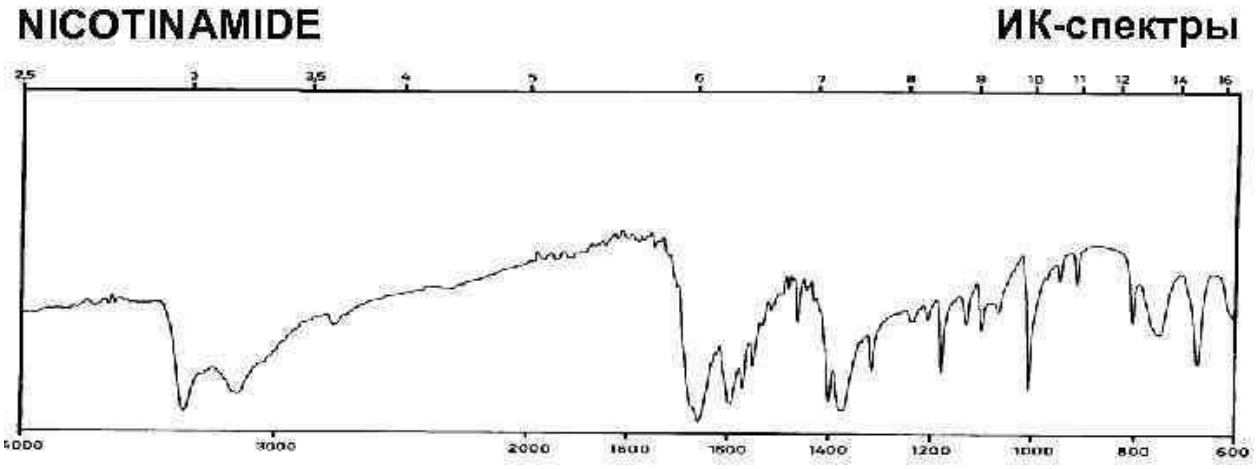
A white, fine crystalline powder, with very faint odour, bitter taste. Melting point 128 - 131 °C.

Easily soluble in water and in alcohol, soluble in glycerine, very slightly soluble in ether and chloroform.

IDENTIFICATION

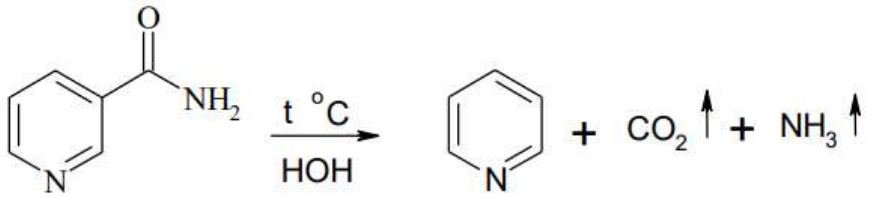
1. Infrared spectrometry

The infrared spectrum of the substance, taken in a disc of potassium bromide, in the region from 4000 to 400 cm-1 in the position of the absorption bands shall correspond to the spectrum of a standard sample of nicotinamide or to the following figure.



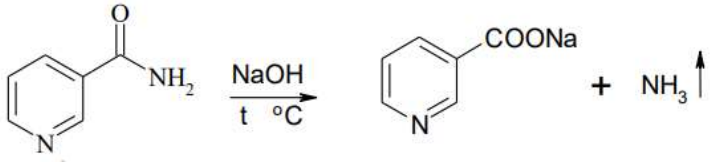
2. Decomposition reaction

When nicotinamide is heated with crystalline potassium carbonate, it decomposes to form pyridine (characteristic odour), ammonia (blueing of wet red litmus paper) and carbon dioxide, which forms an opalescence when it is passed through lime or barite water:



3. Alkaline hydrolysis

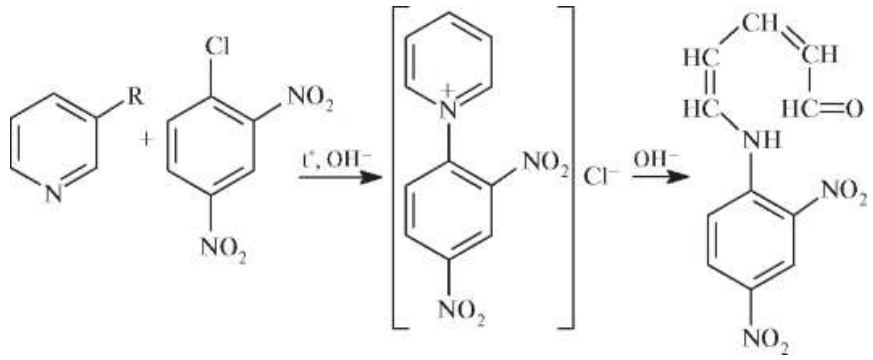
When nicotinamide is heated in solutions of alkali metal hydroxides, hydrolysis products are formed: nicotinic acid and ammonia. The effect of the reaction: sharp odour and blueing of wet red litmus paper.



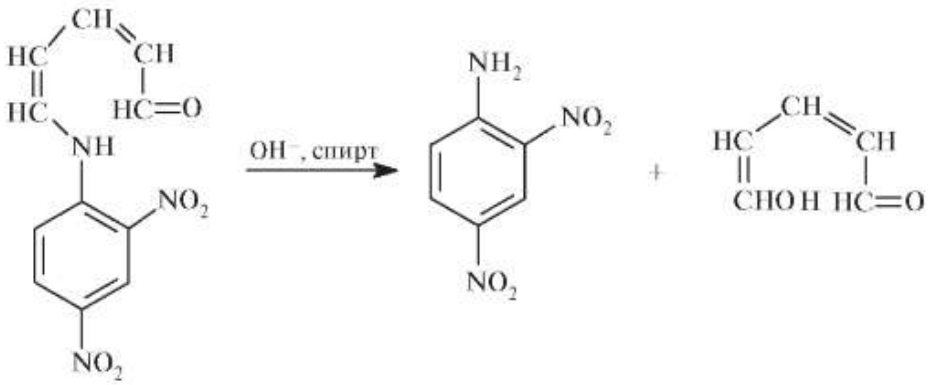
4. Formation of a polymethine dye (Zinke reaction)

The reaction proceeds similarly to nicotinic acid.

a) With 2,4-dinitrochlorobenzene

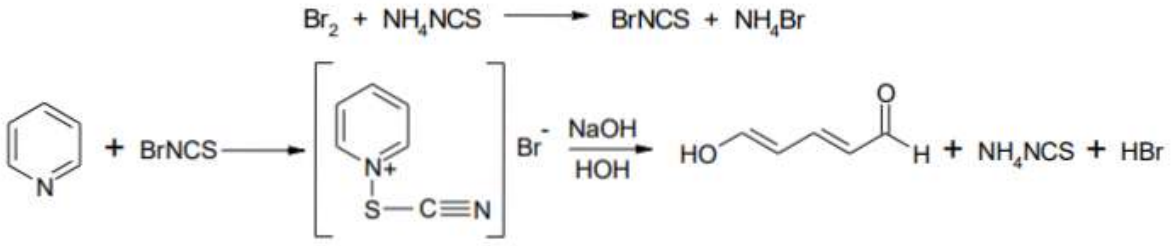


brown or red colour

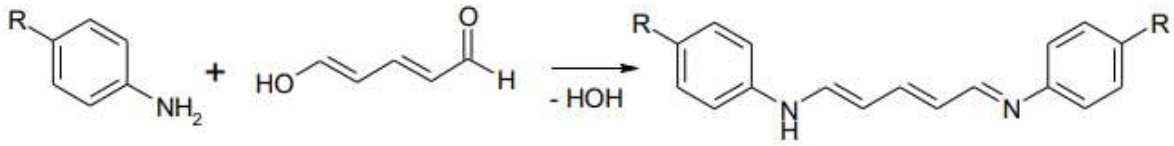


yellow colour

(b) with bromine thiocyanate



yellow colour



intense yellow, orange or red colour

5. Reaction with citric acid and acetic anhydride

Interaction with citric acid and acetic anhydride produces a red-violet colouration when heated.

PURITY TEST

In the purity test, the permissible impurity content of the initial products of synthesis or decomposition is determined. Related impurities are determined by HPLC method.

QUANTIFICATION

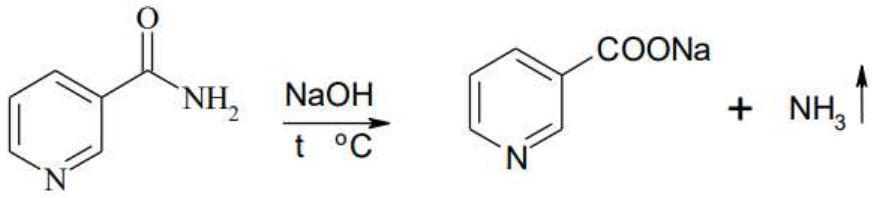
1. Kjeldahl method

The Kjeldahl method is used to quantify nicotinamide in two ways:

(a) after alkaline hydrolysis;

(b) after boiling in 50% sulphuric acid solution.

(a) Alkaline hydrolysis of nicotinamide yields ammonia, which are quantitatively distilled off into a receiver containing boric acid solution:



Boric acid in water forms tetrahydroxyboronic acid, which interacts with ammonia. The forming ammonium salts are titrated with 0.1 M hydrochloric acid solution in the presence of methyl red or methyl orange indicator:

$$H_3BO_3 + H_2O \longrightarrow H[B(OH)_4]$$

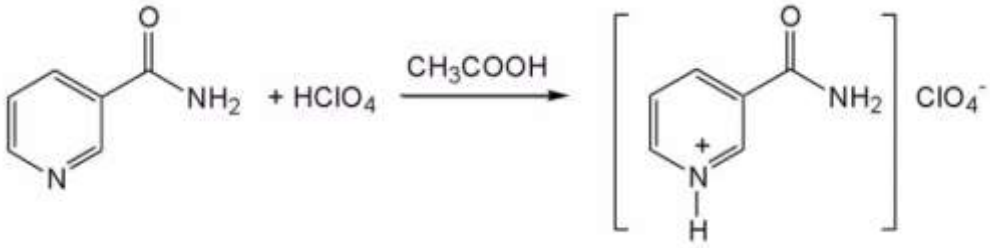
$$NH_3 + H[B(OH)_4] \longrightarrow NH_4[B(OH)_4]$$

$$NH_4[B(OH)_4] + HCl \longrightarrow NH_4Cl + H_3BO_3 + H_2O$$

(b) After boiling nicotinamide in 50% sulphuric acid solution, all organically bound nitrogen is converted to ammonia. It is distilled off into a receiver containing boric acid solution. The resulting ammonium tetrahydroxoborate is titrated with 0.1 M hydrochloric acid solution in the presence of methyl red or methyl orange indicator (chemistry above).

2. Non-aqueous titration

The basic properties of nicotinamide are enhanced in the presence of glacial acetic acid or acetic anhydride. Titrant 0.1 M is chloric acid solution. The indicator is crystal violet.



STORAGE

Nicotinamide is stored in well sealed containers protected from light in a dry place protected from light.

MEDICAL USE

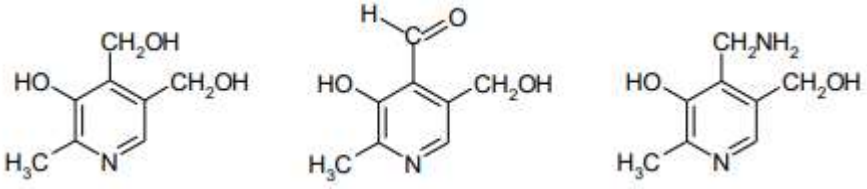
Nicotinamide is used as a vitamin preparation (vitamin PP). It is a specific antipellagic agent and also has a vasodilating effect.

OXYMETHYLPYRIDINE VITAMINS. VITAMINS B6

The work of Burch, Giorgi and Harris (1935) showed that pellagra in rats was not only due to a deficiency of nicotinic acid, as had previously been thought, but also to a deficiency of another substance called vitamin B6. Its necessity was soon established not only in rats but also in other higher animals and humans.

In 1937, Soviet researchers obtained vitamin B6 in pure crystalline form from yeast, and then in the United States it was obtained from rice bran (1938).

In 1939, the structure of vitamin B6 (pyridoxine) was determined and confirmed by synthesis. Its chemical structure was found to be 2-methyl-3-oxy-4,5-(oxymethyl)-pyridine. It was also established that vitamin B6 is a group of three related substances: *pyridoxol (pyridoxine), pyridoxal* and *pyridoxamine*.



pyridoxol *pyridoxal* *pyridoxamine*

The main biological role of pyridoxine vitamins in the animal organism is that, as part of pyridoxal and pyridoxamine phosphate proteins, they act as catalysts of the main processes of nitrogen metabolism.

With the participation of vitamin B6 in the reactions of amino acids in the body, over-amination with ketocarboxylic acids, as well as decarboxylation, racemisation, desulphurisation and other transformations of amino acids are carried

out. Therefore, vitamin B6 deficiency in the body leads to diseases associated with disruption of vital biochemical processes.

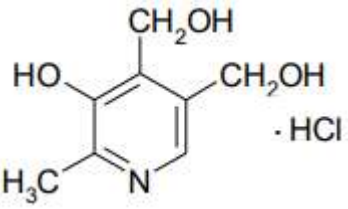
Pyridoxol should be considered a provitamin, as it does not manifest its vitamin properties as such, but is converted in the body to pyridoxal and pyridoxamine.

B6 vitamins are thermally stable and resistant to acids and bases. However, they are unstable to oxidising agents and their solutions are unstable to light.

PYRIDOXINE HYDROCHLORIDE

Pyridoxini hydrochloridum

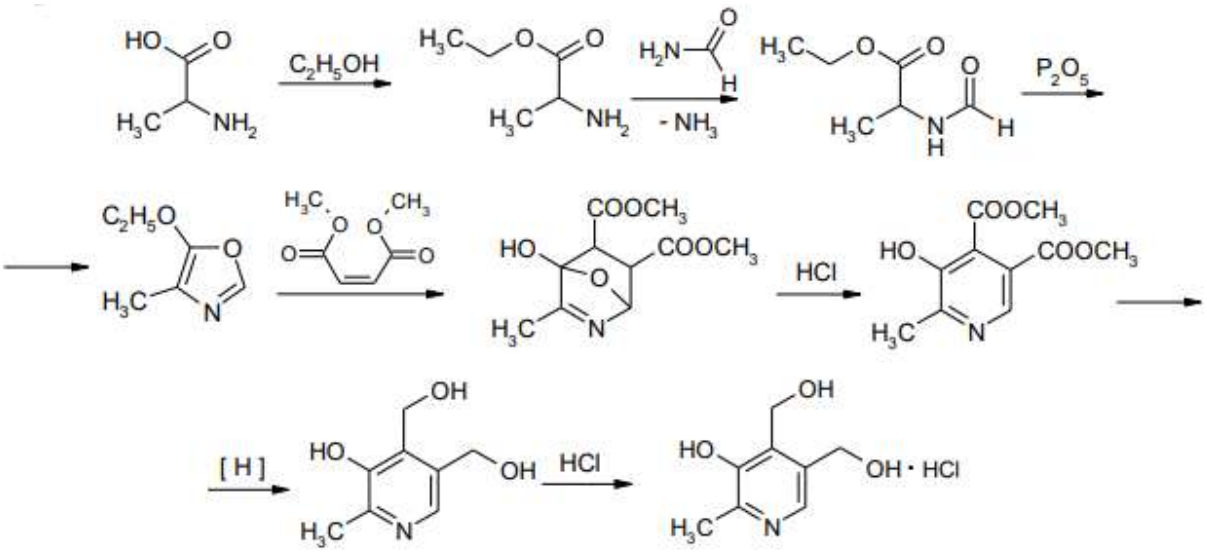
2-Methyl-3-oxy-4,5-di(oxymethyl)-pyridine hydrochloride



OBTAINING

The production of vitamin B6 is reduced to the production of pyridoxine. The latter was originally obtained from natural sources (yeast, rice bran), but this process is very long and labour-intensive, requires large quantities of raw materials and the yield of pure pyridoxine is very low. Therefore, pyridoxine is currently produced synthetically.

The most effective method of obtaining pyridoxine hydrochloride is based on the cyclization of N-formyl-D,L-alanine to an oxazole derivative and its subsequent cyclocondensation with the ester of 1,4-butenedione (succinic) acid. The resulting bicyclic compound is cleaved in an acidic medium and dehydrated to a pyridine derivative, which is hydrogenated to pyridoxine:



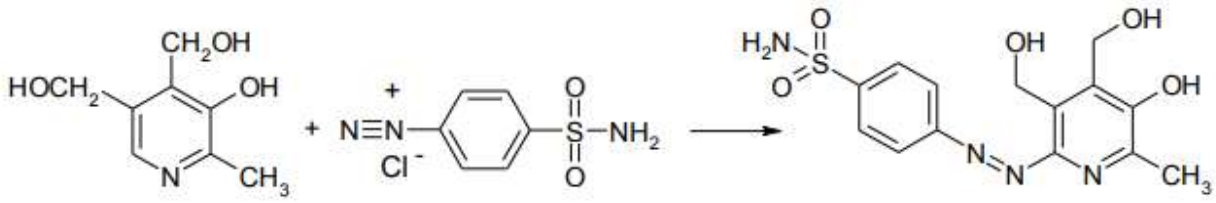
PHYSICAL PROPERTIES

Pyridoxine hydrochloride is a white, fine crystalline powder with a bitter-sour taste. It is easily soluble in water, slightly soluble in alcohol and insoluble in ether. Aqueous 1% solutions have a pH of 3.0 - 3.2. Like the base, pyridoxine hydrochloride is sublimable.

IDENTIFICATION

1. ***UV spectrophotometry***
2. ***Formation of azo dyes with diazonium salts***

   The phenolic hydroxyl in the pyridoxine molecule offers the possibility of azo dye formation when it interacts with various diazonium salts:

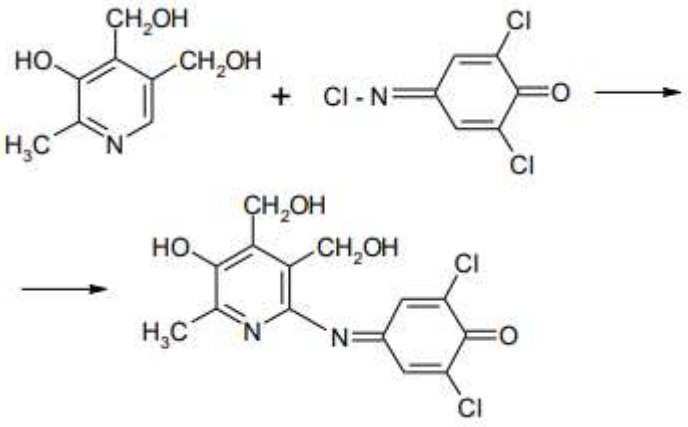


   However, this reaction is non-specific as many other substances of phenolic nature also give this reaction.

3. ***Formation of indophenol dye***

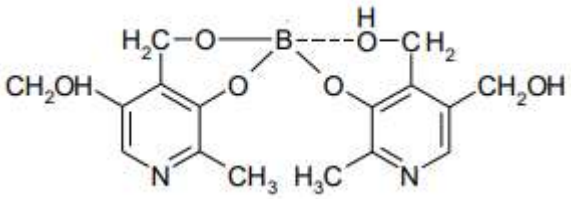
   A more specific reaction is the formation of indophenol dyes when the vitamin interacts with 2,6-dichloroquinone chlorimide.

   The latter reacts only with those phenols in which the para position is unsubstituted:



   The resulting blue-colored product is extracted with butyl alcohol.

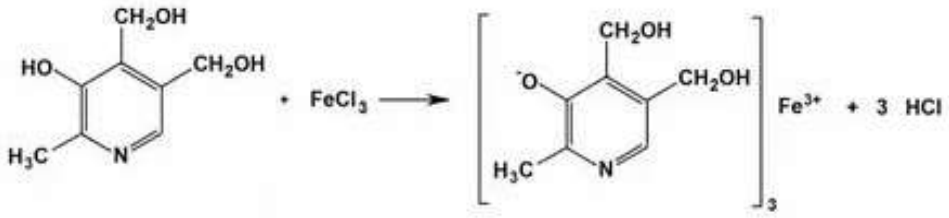
   If boric acid is added to the solution, no colouration occurs (in the presence of boric acid, no indophenol dye is formed because pyridoxine binds to the borate complex):



This is used to detect impurities of other compounds of phenolic character - not substituted in the para position of phenols, such as pyridoxal and pyridoxamine, which give an indophenol dye, unlike the boron complex of pyridoxine.

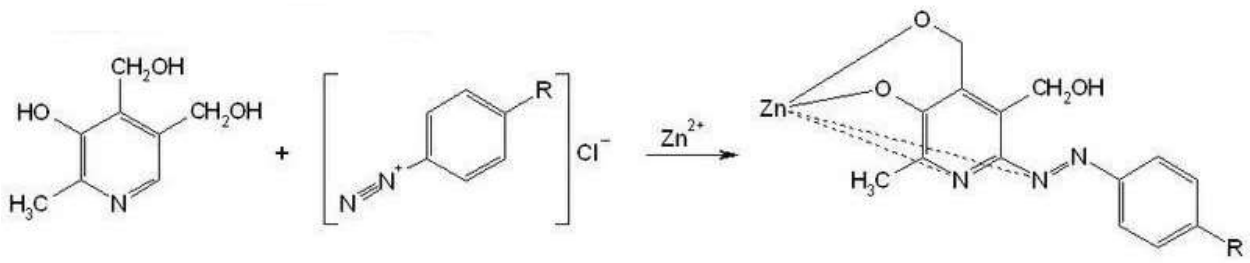
4. *Interacting with ferric chloride*

When $FeCl_3$ solution is added to the drug, a red coloration appears, which disappears when a few drops of dilute sulfuric acid are added.



5. *Formation of metallocomplex of pyridoxine with azo dye*

A proposed reaction for pyridoxine involves the use of zinc chloride stabilized diazonium salt formed by norsulfazole in an aqueous-alcoholic medium at pH 6.5-7.0:



A consistent red-purple colouration is present. This reaction is specific to the vitamin B_6 group, allowing differentiation of each vitamin by a distinct colour.

6. *Interaction with common alkaloid reagents*

Pyridoxine can be easily precipitated from solutions by phosphorus-tungstate, silicon-tungstate, and sulphuric acid because of the tertiary nitrogen present in its molecule.

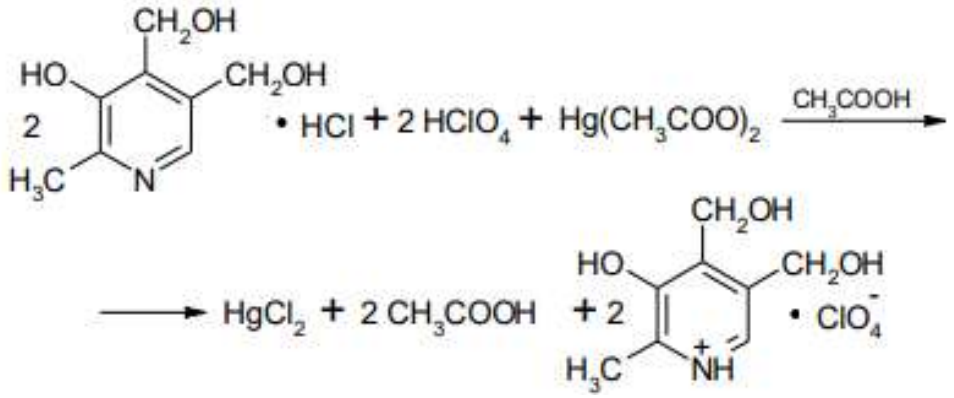
7. *Detection of chloride ions*

When silver nitrate is added, a white amorphous precipitate is formed.

$$AgNO_3 + HCl \leftrightarrow AgCl\downarrow + HNO_3$$

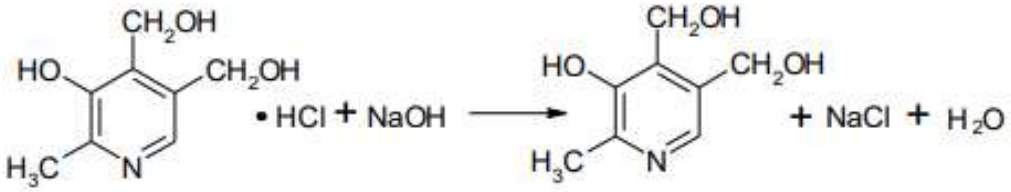
QUANTIFICIFICATION

1. *Method of acid-base titration in non-aqueous media*



The titration is carried out in glacial acetic acid using chloric acid as titrant and crystal violet as indicator.

2. *Neutralisation*



Titrant: Nitrium hydroxide solution. Indicator: Bromthymol blue.

3. *Reverse Argentometric titration (Volgard method)*

Add twice the minimum accurately measured volume of the standard silver nitrate solution (1st working solution), which reacts with chloride ions, to the analysed solution.

$$AgNO_3 + HCl \leftrightarrow AgCl\downarrow + HNO_3$$

The silver nitrate residue that has not reacted is titrated using a second standard solution of ammonium thiocyanate in the presence of iron-ammonium alum indicator.

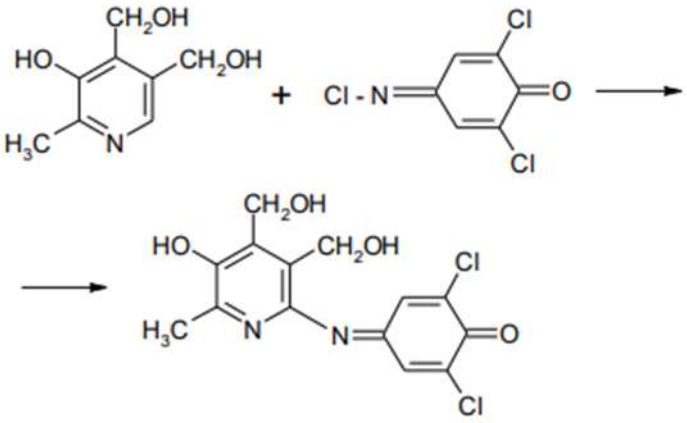
$$AgNO_3 + NH_4NCS \leftrightarrow AgNCS\downarrow + NH_4NO_3$$

At the end of the titration, an excess drop of NH_4NCS solution reacts with the indicator and the solution turns red.

$$NH_4[Fe(SO_4)_2] + 3NH_4NCS \leftrightarrow Fe(NCS)_3 + 2(NH_4)_2SO_4$$

4. Photometric method

a) The method is based on the reaction of indophenol dye formation through the interaction of the vitamin with 2,6-dichloroquinone chlorimide.



b) The method is based on forming a metal complex of pyridoxine with an azo dye. Mercury ions can be used as a complexing agent. First, the pyridoxine azo-colouring agent is obtained, and then it is bound to mercury ions, with the azo-complexing agent being the diazonium salt of norsulfazole (as described above).

STORAGE

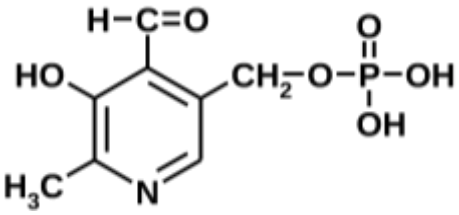
The drug should be stored in well-corked orange glass jars in a cool place. Tablets and ampoules should be stored in a place protected from light.

MEDICAL USE

Pyridoxine hydrochloride is used therapeutically for various diseases, including toxicosis of pregnancy, pellagra (in combination with nicotinic acid), various types of parkinsonism, acute and chronic hepatitis, and other diseases.

The drug can be administered orally, subcutaneously, intramuscularly, or intravenously.

Pyridoxal phosphate



PHYSICAL PROPERTIES

Odourless light yellow crystalline powder. Unstable in light. Slowly and slightly soluble in water, but practically insoluble in ethanol and chloroform.

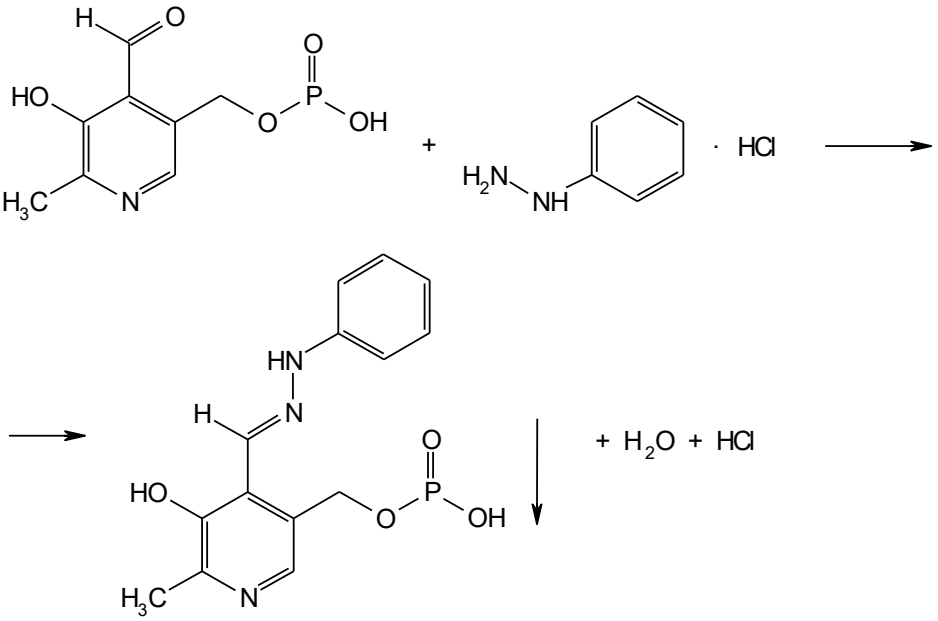
IDENTIFICATION

1. ***UV spectrophotometry***
2. ***Interaction with iron chloride***

   The phenolic hydroxyl is detected by means of iron (III) ion (red colouration disappearing on addition of dilute sulphuric acid). *Write a reaction.*
3. ***Formation of azo dyes with various diazo compounds***

   A yellow-orange staining is formed. *Write a reaction.*
4. ***Interaction with phenylhydrazine hydrochloride (reaction with aldehyde group)***

   On addition of phenylhydrazine hydrochloride a yellow flaky precipitate of phenylhydrazone is formed:



5. ***Phosphate detection***

   After destruction by boiling in the presence of nitric acid, pyridoxal phosphate gives a positive reaction for phosphate. Ammonium molybdate (yellow precipitate) is used as a reagent for phosphate ion.

$$H_3PO_4 + 12(NH_4)_2MO_4 + 21HNO_3 \longrightarrow 21\,NH_4NO_3 + 12H_2O +$$
$$+ (NH_4)_3PO_4 \cdot 12\,MoO_3\downarrow$$

QUANTIFICIFICATION

Method of acid-base titration in non-aqueous media

The solvent used is a mixture of acetic anhydride and formic acid, with Sudan III solution as an indicator, and chloric acid as a titrant. *Write a reaction.*

STORAGE

In a dry place protected from light at room temperature.

MEDICAL USE

Used in toxicosis in pregnant women, various types of parkinsonism, chorea, pellagra, acute and chronic hepatitis, some skin and other diseases.