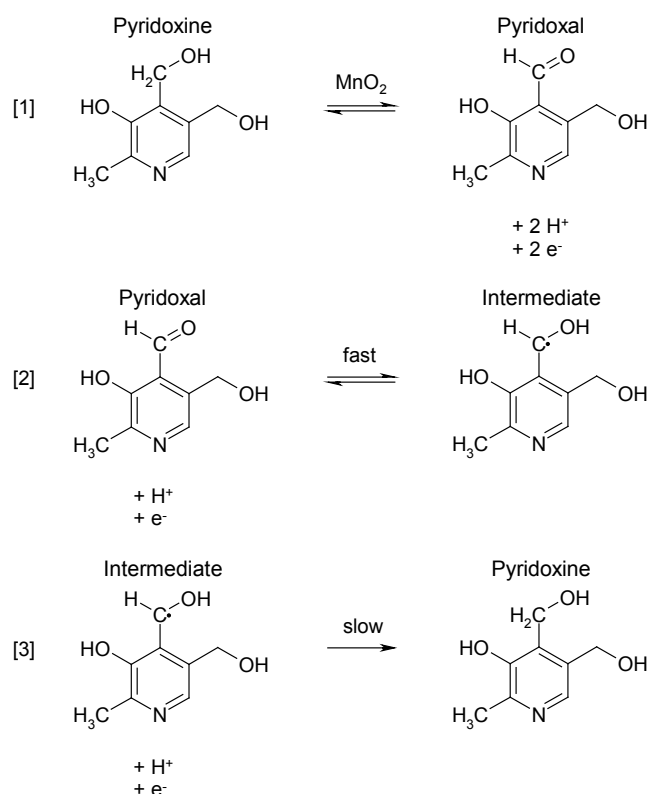


Determination of pyridoxine (vitamin B₆) by polarography

Summary

This Application Bulletin describes the polarographic determination of pyridoxine (vitamin B₆). The method given allows determination in monovitamin and in some multivitamin preparations. The linear range of the analysis is also specified. The limit of detection is approx. 100 µg/L pyridoxine · HCl.

Pyridoxine is oxidized to pyridoxal by treatment with «active» manganese dioxide in a phosphate buffer pH= 6.8 ... 7.0 [1]. At pH 13 the electrochemical reduction at the DME or SMDE occurs in two steps. The first, reversible and fast reaction step leads to an intermediate and is used for the determination [2]. The second, slow and irreversible reaction step leads back to pyridoxine [3].



Instruments

VA instrument
capable of operating a Multi-Mode Electrode and
supporting differential pulse (DP) measuring mode

Electrodes

WE	Multi-Mode Electrode pro	6.1246.120
	Mercury drop capillary	6.1226.030
RE	Ag/AgCl reference electrode	6.0728.x20
	Ag/AgCl/KCl (3 mol/L)	
	Electrolyte vessel Filled with c(KCl) = 3 mol/L	6.1245.010
AE	Pt rod electrode	6.0343.x00

Reagents

All of the used reagents must be of purest quality possible (for analysis).

- Manganese(IV) oxide (active), MnO₂, for synthesis, CAS 1313-13-9
- Sodium hydroxide, for analysis, CAS 1310-73-2
- Disodium hydrogen phosphate heptahydrate, Na₂HPO₄ · 7 H₂O, for analysis, CAS 7782-85-6
- Potassium dihydrogen phosphate, KH₂PO₄, for analysis, CAS 7778-77-0
- Phosphoric acid, w(H₃PO₄) = 85%, for analysis, CAS 7664-38-2
- Pyridoxine hydrochloride, C₈H₁₁NO₃ · HCl, for analysis, CAS 58-56-0
- Ultrapure water, resistivity >18 MΩ·cm (25 °C), type I grade (ASTM D1193)

Solutions

NaOH solution	c(NaOH) = 2.5 mol/L 25 g NaOH is dissolved in ultrapure water and after cooling the solution is made up to 250 mL.
Phosphate buffer pH 6.8 ... 7.0	c(Na ₂ HPO ₄) = 0.027 mol/L c(KH ₂ PO ₄) = 0.021 mol/L 7.16 g Na ₂ HPO ₄ · 7 H ₂ O and 2.8 g KH ₂ PO ₄ are weighed into a beaker and dissolved in 950 mL ultrapure water. The pH value is then adjusted to 6.8 ... 7.0 by addition of c(H ₃ PO ₄) = 2 mol/L or

c(NaOH) = 2.5 mol/L. The solution made up to 1 liter with ultrapure water.

Standard solutions

Pyridoxine standard stock solution $\beta(\text{pyridoxine} \cdot \text{HCl}) = 3000 \text{ mg/L}$
The stock solution is freshly prepared every day in quantities not exceeding 50 mL. The content of the starting material must be taken into account. Pyridoxine hydrochloride (150 mg or correspondingly more) is dissolved in 30 mL phosphate buffer, the solution made up to 50 mL with ultrapure water and stored in a refrigerator.

Pyridoxine standard solution $\beta(\text{pyridoxal}) = 180 \text{ mg/L}$
3.00 mL of the pyridoxine standard stock solution is pipetted into a 50 mL conical flask and 17 mL phosphate buffer are added. After addition of 2.1 g MnO_2 , the flask is stoppered and shaken for 1 h. The contents are then filtered through a filter paper into a 50 mL volumetric flask and the conical flask and filter paper washed 6 times with 3 mL aliquots of phosphate buffer. The purified filtrates are made up to the mark (50 mL) with phosphate buffer. This standard must also be freshly prepared every day and stored in a refrigerator.
1 mL = 180 μg pyridoxine \cdot HCl as pyridoxal

Analysis

Measuring solution

14.5 mL phosphate buffer
+ 500 μL clear sample solution
+ 5 mL c(NaOH) = 2.5 mol/L

The content is determined by standard addition, each with 200 μL standard solution.

Parameters

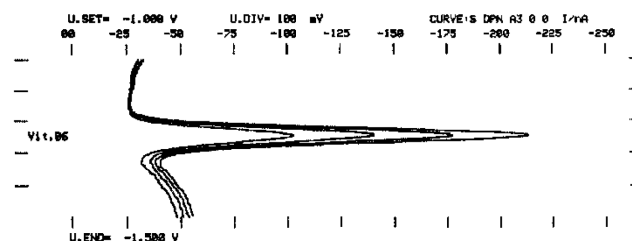
Voltammetric

Electrode operating mode	DME
Measuring mode	DP – Differential pulse
Stirring rate	2000 min^{-1}
Equilibration time	5 s
Sweep	
Start potential	-1.0 V
End potential	-1.5 V
Potential step	0.004 V
Potential step time	0.8 s
Sweep rate	0.005 V/s
Pulse amplitude	0.05 V

Substance

Name	Pyridoxine
Characteristic potential	-1.25 V

Example



Result

Sample	Vitamin tablet
Sample size	1 tablet
$\beta(\text{pyridoxine})$	3.098 mg/g

Sample preparation

Tablets and capsules

10 tablets or the contents of 10 capsules are weighed exactly to determine the average weight and then pulverized (mortar or grinder). The average weight of one tablet or the content of a capsule is weighed into a centrifuge tube, which can be stoppered, and 20.0 mL phosphate buffer are added. After addition of 2.1 g manganese dioxide, the tube is stoppered and shaken for one hour. MnO_2 is separated out in a centrifuge at 5000 rpm.

Comments

- The curve is linear up to 85 mg/L.
- After the determination of pyridoxine, nicotinamide could also be analyzed in the same solution between -1.4 V and -1.9 V with appropriate standard additions (see also Application Bulletin 213).
- Not only tablet constituents such as methyl cellulose or polyvinyl pyrrolidone, but also the actual tablet coatings can adversely affect the sensitivity of the determination. In several cases it is advisable to remove the tablet coatings before pulverizing.

References

- Jacobsen, E., Tommelstad, T. M., Differential pulse polarographic determination of pyridoxine in multivitamin tablets, *Anal. Chim. Acta* 162, (1984) 379-383
- Göbbeler, K. H., Breinlich, J., Quantitative wechselstrompolarographische Simultanbestimmung von Vitaminen der B-Gruppe, *Pharm. Ztg.* 48, (1972) 1859-1862
- Söderhjelm, P., Lindquist, J., Electrochemical assay of thiamine, riboflavine, pyridoxine, nicotinamide and ascorbic acid in pharmaceutical preparations, *Acta Pharm. Suec.* 13, (1976) 201-212

Appendix

Report for the example determination of pyridoxine in vitamin tablets

METROHM 646 VA-PROCESSOR (5.646.6041)

Detn. of Pyridoxine.HCl in Vitamin Preparations

METHOD

MPL 1 EL.TYPE MME

SUPP.ELEC Buffer 6.8-7 adj.13

V.MEAS 20.000 mL

ALIQUT 1.000

REMARK Pyridoxine.HCl (Vitamin B₆) in Monovitamin Prepn.
Ag/AgCl (3M KCl) reference electrode

NAME Prof.J.G.Dick

RUN# 3

ANALYTE	L R S	U.SUBST	EV.VALUE	DELTA	m.ANALYTE
Vit.B1	A0 0 0	-1.247 V	72.06 nA		
	A1 0 0	-1.246 V	108.1 nA	36.07 nA	
	A2 0 0	-1.246 V	143.8 nA	35.67 nA	
	A3 0 0	-1.246 V	179.5 nA	35.78 nA	
m.STD	36.00 ug	SLOPE	1.004 mg/uA		72.51 ug

rho(vitB6) = 3.098 mg/g

SMPL.V,m 7.02500 mg

IDENT B6 Multi.Vit.cap.bas

DATE 91-11-25 TIME 17:07

Method print for the determination of ...

Detn. of Pyridoxine.HCl in Vitamin Preparations

METHOD

PAGE 3

MPL 1 EL.TYPE MME

OPERATION SEQUENCE

OPERATIONS/PARAMETERS

OPERATIONS/PARAMETERS

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1 PURGE ;STIR ; 5 s
2 [ADDL ;OPURGE;OSTIR ; 5 s
3 DME ;
4 MEAS ; 5 s
4a M.MODE DPN -50 mV
4b T.STEP 800 ms
4c U.SET -1.000 V
5 SWP 0 ; 100 s
5a U.END -1.500 V
5b U.STEP 4 mV
SW.RATE 5.0 mV/ s
6 OMEAS ;PURGE ;STIR ;
7 BEEP ;ADD1]3; 25 s
8 OMEAS ;OPURGE;OSTIR ;
9 BEEP ;END ;
    
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