Metrohm

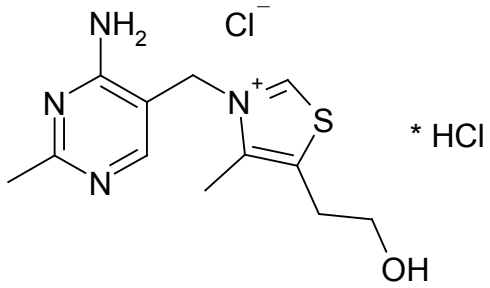
Application Bulletin 218/2 e

Determination of thiamine (vitamin B_1) by polarography

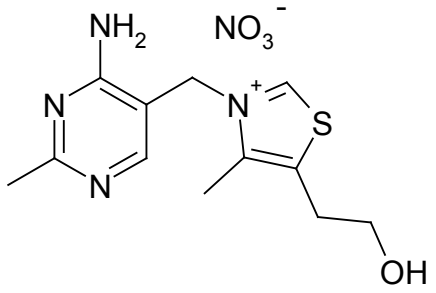
Summary

This Application Bulletins describes the polarographic determination of thiamine (vitamin B_1). The procedure allows an analysis in monovitamin preparations. The linear range of the determination is also given. The limit of detection is approx. 50 µg/L thiamine.

Vitamin B_1 is normally used in preparations in the form of thiamine hydrochloride or thiamine mononitrate:



Thiamine hydrochloride



Thiamine mononitrate

Instruments

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

Electrodes

WE	Multi-Mode Electrode pro Mercury drop capillary	6.1246.120 6.1226.030 or 6.1226.050
RE	Ag/AgCl reference electrode Ag/AgCl/KCl (3 mol/L) Electrolyte vessel Filled with c(KCl) = 3 mol/L	6.0728.x20 6.1245.010
AE	Pt rod electrode	6.0343.x00

Reagents

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

- Sodium acetate, anhydrous, for analysis, CAS 127-09-3
- Acetic acid, w(CH_3COOH) = 100%, for analysis, CAS 64-19-7
- Sodium hydroxide, for analysis, CAS 1310-73-2
- Triton X-100, (Octoxinol 9), for analysis, CAS 9002-93-1
- Thiamine hydrochloride, for analysis, CAS 67-03-8
- Thiamine mononitrate, for analysis, CAS 532-43-4
- Ultrapure water, resistivity >18 MΩ·cm (25 °C), type I grade (ASTM D1193)

Solutions

Sodium hydroxide solution	c(NaOH) = 2.5 mol/L 100 g/L NaOH in ultrapure water.
Extraction solution	c(NaOH) = 0.01 mol/L 0.4 g/L NaOH in ultrapure water.
Supporting electrolyte	c(NaAc) = 0.05 mol/L c(HAc) = 0.05 mol/L 4.10 g sodium acetate anhydrous is weighed into a beaker and dissolved in approx. 400 mL ultrapure water. After addition of 2.86 mL acetic acid, the solution is stirred and diluted with ultrapure water to approx. 950 mL and the pH value adjusted to 6.5 (6.4 ... 6.6) with c(NaOH) = 2.5 mol/L. The volume is then made up to 1000 mL with ultrapure water and the solution mixed.

TX-100 solution	w(Triton X-100) = 1% 1 g Triton X-100 is dissolved in ultrapure water and the solution made up to 100 mL.

Standard solutions

Standard stock solution	β(thiamine) = 1 g/L The stock solution is prepared from thiamine hydrochloride or thiamine mononitrate, depending on which compound is present in the preparation. The vitamin content must be taken into account when preparing the solutions. For a 1000 mg/L solution, 500 mg (or correspondingly more) are weighed into a 500 mL volumetric flask and dissolved in the supporting electrolyte. The flask is then filled to the mark with supporting electrolyte and the contents mixed. The solution can be kept for a considerable length of time in a refrigerator. Example: Vitamins B_1 99.5 %: sample weight = 500 : 0.995 = 502.5 mg
Diluted standard solution	β(thiamine) = 100 mg/L Prepared fresh every day by appropriate dilution of the standard stock solution with supporting electrolyte.

Sample preparation

Vitamin solutions

These can be used directly.

Vitamin tablets

10 tablets are weighed to obtain the average weight and then ground to a powder (grinder, mortar). An amount corresponding to the average weight of a tablet is weighed into a beaker, 30 mL c(NaOH) = 0.01 mol/L are added, the beaker covered and extraction performed for 20 min with stirring. After the insoluble fraction has settled out, the mixture is filtered through a filter paper into a 100 mL volumetric flask. The filter is washed three times with 5 mL aliquots of ultrapure water, the combined filtrate and washings made up to 100 mL with ultrapure water and the solution mixed.

Analysis

Measuring solution

18 mL supporting electrolyte

+ 0.4 mL sample solution

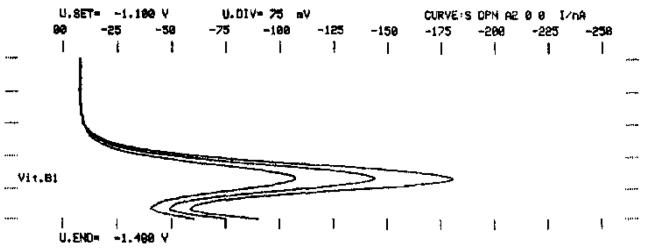
+ 0.8 ml TX-100 solution

The concentration is determined by standard addition.

Parameters

Voltammetric	
Electrode operating mode	DME
Measuring mode	DP – Differential pulse
Stirring rate	2000 min^{-1}
Equilibration time	5 s
Sweep	
Start potential	-1.1 V
End potential	-1.48 V
Potential step	0.004 V
Potential step time	0.7 s
Sweep rate	0.006 V/s
Pulse amplitude	0.05 V
Substance	
Name	Thiamine
Characteristic potential	-1.38 V

Example



Result

Sample	Vitamin B1 tablet
Sample size	1 tablet
β(thiamine)	49.48 mg/tablet

Comments

- The content of thiamine in the polarographic vessel should not be less than 0.25 mg/L. Further, the total content including that due to the standard additions should not exceed 12.5 mg/L.
- It must be ensured that the concentration of thiamine in the measuring vessel including that due to the standard additions does not exceed the linear range.
- The SMDE cannot be used as a working electrode for these determination.
- Nicotinamide and Fe(II) ions interfere with the determination.

References

- Tichomirova, G. P., Balenkaja, S. L., Der Einfluss von Aluminiumsalzen auf das polarographische Verhalten von Thiamin, Zh. Anal. Khim. 17, (1962) 767-769
- Tichomirova, G. P., Benenkaja, S. L., Polarographic determination of thiamine, Ukrain. Chim. Z. 29, (1963) 97-99 (Russian)
- Dewjatnin, W. A., Kusnetzowa, L. A., Polarographic determination of vitamin B-1, B-2, PP and nicotinamide in mono- and polyvitamin preparations, Med. Prom. USSR 58, (1964) 58-60 (Russian)
- 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
- Matsunaga, T., Karube, I., Suzuki, S., Electrochemical microbioassay of vitamin B-1, Anal. Chim. Acta 98, (1978) 25-30
- Kishore, K., Moorthy, P. N., Rao, K. N., Thiamine assay by differential-pulse polarography, Indian J. Chem. (1979), 206-208
- Vergara, T., Marin, D., Vera, J., Polarographic determination of thiamine and its monophosphate and pyrophosphate esters, Anal. Chim. Acta 120, (1980) 347-351

Appendix

Report for the example determination of thiamine in a monovitamin tablet

```
METROHM 646 VA-PROCESSOR  (5.646.6041)
Detn. of Thiamine(Cl).HCl(Vit.B1) in Monovit.Tab.      METHOD 24
MPL 1          EL.TYPE    MME

SUPP.ELEC      0.1M LiCl/Li2CO3 adj
V.MEAS          20.000 mL
ALIQUOT         1.000

REMARK         Thiamine(Cl).HCl(Vit.B1) in Monovit.Tab. (50mg/tab)
               Ag/AgCl (3M KCl) reference electrode
NAME           Prof.J.G.Dick
RUN#            1

     ANALYTE     L R S        U.SUBST      EV.VALUE     DELTA        m.ANALYTE
     Vit.B1     A0 0 0       -1.375 V      78.05 nA
                A0 1 0       -1.376 V      78.64 nA
                A1 0 0       -1.375 V      110.2 nA
                A1 1 0       -1.376 V      109.9 nA     31.74 nA
                A2 0 0       -1.375 V      141.6 nA
                A2 1 0       -1.376 V      140.7 nA     31.12 nA
              m.STD     40.00 ug      SLOPE     1.272 mg/uA          99.84 ug

     rho(vitB1)  =     49.48         mg/g

     SMPL.V,m          1.51000 mg          IDENT Monovit.tab. 50mg/tab
     DATE  91-06-11   TIME    14:25
```

Method print for the determination of thiamine

```
     Detn. of Thiamine Mononitrate in Monovit. Tab.      METHOD  4  PAGE 3
     MPL 1           EL.TYPE MME                         OPERATION SEQENCE

     OPERATIONS/PARAMETERS                OPERATIONS/PARAMETERS
  1  PURGE ;STIR   ;          5   s
  2  [ADDL ;OPURGE;OSTIR ;    5   s
  3  (REP   ;
  4  DME    ;MEAS  ;          5   s
  4a     M.MODE       DPN    -50   mV
  4b     T.STEP              700   ms
  4c     U.SET              -1.100 V
  5  SWP 0 ;                 66   s
  5a     U.END              -1.480 V
  5b     U.STEP              4   mV
         SW.RATE             5.7 mV/ s
  6  REP) 1;
  7  OMEAS ;PURGE ;STIR   ;
  8  BEEP   ;ADD1]2;          30   s
  9  OMEAS ;OPURGE;OSTIR ;
 10  BEEP   ;END    ;
```