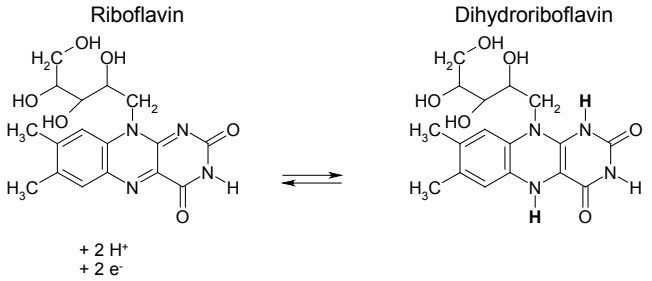
Metrohm

Application Bulletin 219/3 e

Determination of riboflavin (vitamin B_2) by polarography

Summary

This Application Bulletin describes the polarographic determination riboflavin (vitamin B_2). The procedure allows an analysis in monovitamin preparations. The limit of detection is approximately 100 µg/L.



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
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). Only oxygen-free ultrapure water should be used.

- Potassium chloride, for analysis, CAS 7447-40-7
- Potassium carbonate , for analysis, CAS 584-08-7
- Potassium hydroxide, for analysis, CAS 1310-58-3
- Riboflavin, for analysis, CAS 83-88-5
- Ultrapure water, resistivity >18 MΩ·cm (25 °C), type I grade (ASTM D1193)

Solutions

Supporting electrolyte	c(KCl) = 0.05 mol/L c(K_2CO_3) = 0.1 mol/L 6.9 g potassium carbonate and 1.86 g potassium chloride are weighed in a 500 mL calibrated flask and made up to the mark with oxygen-free high purity water.
KOH solution	c(KOH) = 0.2 mol/L 1.12 g KOH is weighed into a beaker and dissolved in 100 mL deaerated ultrapure water. After transfer to a plastic bottle, the solution is purged for 3 min with nitrogen and the bottle closed. Before use, purging is performed with nitrogen for 5 min.

Standard solutions

Riboflavin standard solution	β(Riboflavin) = 1 g/L 100 mg (or a correspondingly greater amount) riboflavin are weighed into a beaker and 8 mL KOH solution (oxygen-free) is added. The vitamin content of the starting material must be taken into account. Example: Content of vitamin B_2 98.8 % → sample weight 100 : 0.988 = 101.2 mg Nitrogen is passed through the mixture, which is then stirred to dissolve the vitamin. After addition of 50 mL oxygen-free ultrapure water, the solution is rinsed into a 100 mL volumetric flask, which is then filled to the mark with ultrapure water. The flask is wrapped in aluminum foil and stored in a cool place in the dark. Only 100 mL of standard solution are prepared at once. The solution must be prepared freshly every day.

Sample preparation

Solutions

These can be used directly.

Tablets

10 tablets are weighed exactly to obtain the average weight and then ground to a powder as quickly as possible (mortar, grinder). An amount corresponding to the average weight of a tablet is weighed into a 125 mL conical flask, 8 mL c(KOH) = 0.2 mol/L (oxygen-free) added and extraction performed for 15 min in the dark with stirring and bubbling through nitrogen. Towards the end of the extraction time, 50 mL oxygen-free ultrapure water are added. After the solid fraction has settled, the mixture is filtered through a paper filter into 100 mL volumetric flask. The filter is washed three times with 3 mL aliquots of KOH solution and the filtrate plus washings made up to 100 mL with oxygen-free ultrapure water. The volumetric flask is wrapped in aluminum foil and stored in a cool place in the dark. Analyze as quickly as possible!

Analysis

Measuring solution

10mL (diluted) sample

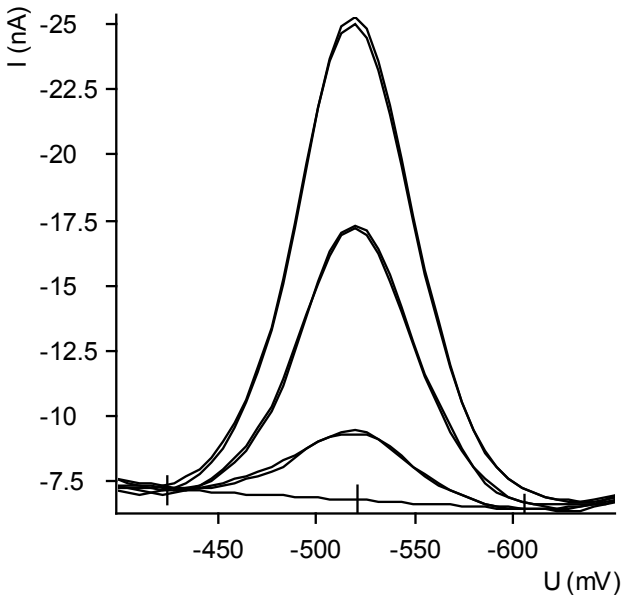
10mL supporting electrolyte

The concentration is determined by standard addition.

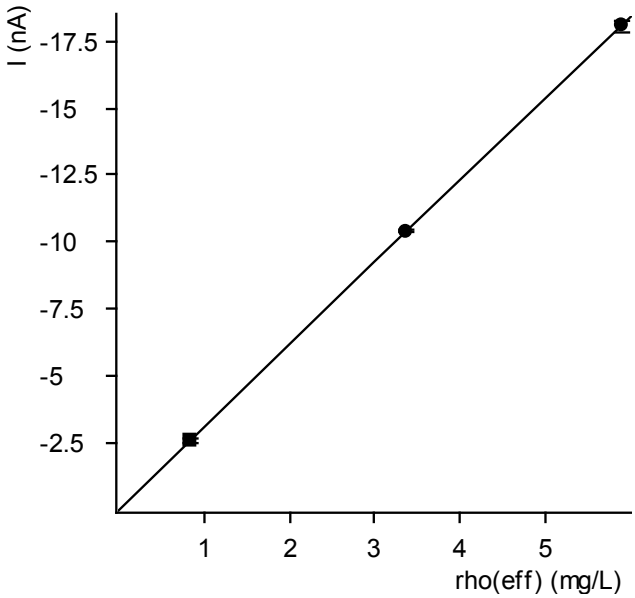
Parameters

Voltammetric	
Electrode operating mode	DME
Measuring mode	DP – Differential pulse
Stirring rate	2000 min^{-1}
Equilibration time	10 s
Sweep	
Start potential	-0.35 V
End potential	-0.9 V
Potential step	0.006 V
Potential step time	0.6 s
Sweep rate	0.01 V/s
Pulse amplitude	0.05 V
Substance	
Name	Riboflavin
Characteristic potential	-0.53 V

Example



Standard addition curve: VitB2



Result

Sample	Vitamin tablet
Sample size	0.4853 g
β(Riboflavin)	352 µg/g

Comments

- Riboflavin solutions, especially alkaline solutions, are rapidly destroyed by atmospheric oxygen, light and heat. It is thus absolutely essential to work with darkened vessels under nitrogen. Samples and

standards must be analyzed rapidly and stored in a cool place.

- Fe(II) ions do not interfere with the determination.
- The SMDE cannot be used for this determination.
- Very small amounts of riboflavin can be determined by adsorptive voltammetry using the method described by Wang.
- The determination is linear until approx. 10 mg/L.

References

- Cheng H. Y., Falat L., Li R. L., Preconcentration of phenanthrenequinone-like compounds for electrochemical determination at a carbon paste electrode via spontaneous adsorption, Anal. Chem. 54, (1982) 1384-1388
- Dryhurst G., Electrochemistry of Biological Compounds, Academic Press, New York 1982, p.365 (Book)
- Pergola F., Foresti M. L., Aloisi G., Guidelli R., The riboflavin-dihydroriboflavin system in acidic medium on mercury, J.Electroanal.Chem. 137, (1982) 355-356
- Arizan S., Popa M., Sterescu M., Polarographic determination of cobalt, vitamin B-2 and vitamin C mixtures in tablets and ampoules, Electroanal. Chem. 2, (1961) 177
- Göbbeler K.H., Breinlich J., Quantitative wechselstrompolarographische Silmultanbestimmung von Vitaminen der B-Gruppe, Pharm. Ztg. 48, (1972) 1859-1862 (German)
- Maslowska J., Malicka M., Determination of riboflavin in injection by voltammetric method on graphite electrode, Chem. Analit. Warsaw 33, (1989) 903-909 (Polish)
- Wang J., Luo D. B., Farias P. A. M., Mahmoud J. S., Adsorptive stripping voltammetry of riboflavin and other flavin analogues at the static mercury drop electrode, Anal. Chem. 57, (1985) 158-162
- Zimmer A. J., Huyck C. L., Eine lichtabsorptiometrische und eine polarographische Bestimmungsmethode für Riboflavin, J. Am. Pharm. Ass. Sci. 44, (1995) 344-348

Appendix

Report for the example determination of riboflavin in vitamin table

```
================== METROHM 746 VA TRACE ANALYZER (5.746.0101) ==================
 Determ.     : 09081452             User:                       Date: 1995-09-08
 Modified    : 1995-09-12 10:47:18  Run :  0                    Time: 14:52:22
 Sample table: -
--------------------------------------------------------------------------------
  Pos.  Ident.1/S1    Ident.2/S2    Ident.3/S3    Method.call   Sample size/S0
        VitTab    B2          1.0                                      0.4853 g
--------------------------------------------------------------------------------
 Method  :  VitaminB
 Title   :  Bestimmung von Vitamin B2 in Getraenken
 Remark1 :  Bestimmung von Vitamin B2 in Vitamntabletten
 Remark2 :  1 ml Probe + 19 ml Grundloesung KCl/K2CO3
--------------------------------------------------------------------------------

  Substance : VitB2                                          Comments
  Mass conc.:    1.708 mg/L            Mass     : 17.08 ug   ----------
  MC.dev.   :    0.079 mg/L (4.64%)    Add.mass : 50.35 ug
  Cal.dev.  :    -                     V0.sample:    10 mL

              VR   U/mV    I/nA   I.mean  Std.dev. I.delta   Comments
              ---  -----  ------  ------  -------  -------   ----------
              00   -520   -2.661  -2.591   0.0998
              01   -521   -2.520
              10   -519   -10.41  -10.38   0.0425   -7.794
              11   -519   -10.35
              20   -518   -18.11  -17.95   0.2245   -7.569
              21   -518   -17.80


 Final results                        +/- Res.dev.   %      Comments
 ---------------------------------    -----------------     ----------
      VitB2 =   352.03 ug/g               16.3    4.64
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Method print for the determination of riboflavin

```
================== METROHM 746 VA TRACE ANALYZER (5.746.0101) ==================
 Method: VitaminB.mth         OPERATION SEQUENCE
 Title : Bestimmung von Vitamin B2 in Getraenken
--------------------------------------------------------------------------------

      Instructions   t/s   Main parameters            Auxiliary parameters
      ------------ -----  ------------------------  ------------------------
 1    DOS/M               V.added      10.000 mL
 2    SMPL/M              V.fraction   10.000 mL     V.total          0.1 L
 3    STIR                Rot.speed      2000 /min
 4    PURGE         300.0
 5    (ADD
 6      SEGMENT           Segm.name    pol
 7      ADD>M             Soln.name    VitB2Std      V.add         0.050 mL
 8    ADD)2
 9    END

 Method: VitaminB             SEGMENT
                                   pol
--------------------------------------------------------------------------------

      Instructions   t/s   Main parameters            Auxiliary parameters
      ------------ -----  ------------------------  ------------------------
 1    NOP           10.0
 2    0PURGE
 3    0STIR         10.0
 4    (REP
 5      DME
 6      DPMODE            U.ampl          -50 mV     t.meas         20.0 ms
                          t.step         0.30 s      t.pulse        40.0 ms
 7      SWEEP       28.5   U.start        -350 mV     U.step            6 mV
                          U.end          -900 mV     Sweep rate       20 mV/s
 8    REP)1
 9    PURGE
10    STIR                Rot.speed      2000 /min
11    END
```