Metrohm

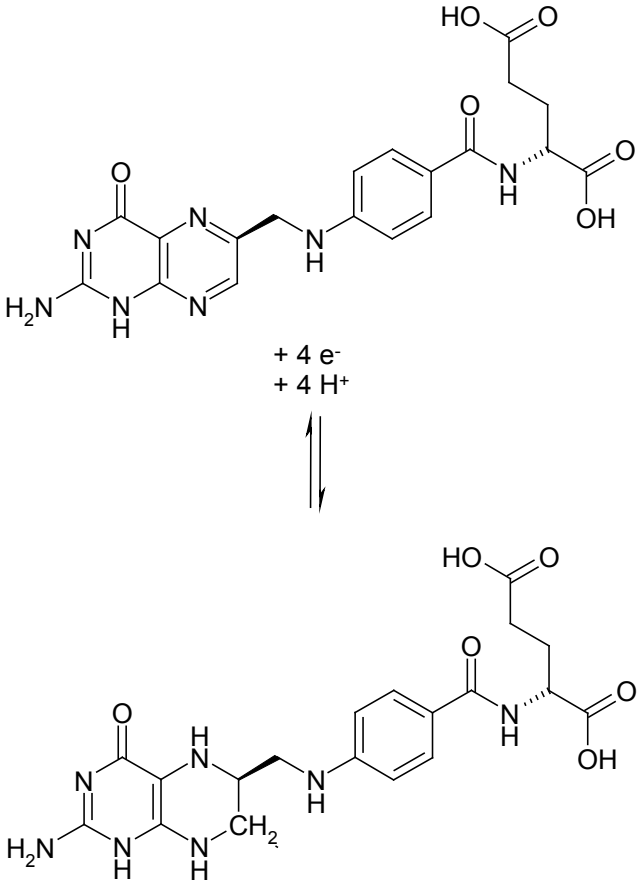
Application Bulletin 215/2 e

Determination of folic acid by polarography

Summary

This Application Bulletin describes the polarographic determination of folic acid, a vitamin of the B series, also known as vitamin B_9 or vitamin B_C. Instructions for the determination in solutions (e.g. fruit juice), vitamin capsules and multivitamin tablets are given. The linear range of the determination is also specified. The limit of detection is approx. 75 µg/L folic acid.

Folic acid is reduced in alkaline solution (pH~ 9) at the DME to tetrahydrofolic acid following the below mechanism:



The reaction has only limited reversibility.

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).

- Boric acid, for analysis, CAS 10043-35-3
- Sodium hydroxide, for analysis, CAS 1310-73-2
- Folic acid, for analysis, CAS 59-30-3
- Ultrapure water, resistivity >18 MΩ·cm (25 °C), type I grade (ASTM D1193)

Solutions

Electrolyte	c(boric acid) = 0.1 mol/L c(NaOH) = 0.05 mol/L 100 mL ultrapure water are added to 6.2 g boric acid in a beaker. After addition of 2 g NaOH, the mixture is stirred until everything is dissolved. After dilution with dist. water to 950 mL, the pH is adjusted to 11.1 - 11.2 with c(NaOH) = 2 mol/L. The solution is then made up to 1000 mL with ulrapure water. Use only CO_2 free ultrapure water!
2 mol/L NaOH solution	c(NaOH) = 2 mol/L 80 g/L NaOH in CO_2 free ultrapure water.
0.1 mol/L NaOH solution	c(NaOH) = 0.1 mol/L 4 g/L NaOH in CO_2 free ultrapure water.

Standard solutions

Folic acid standard stock solution	β(folic acid) = 0.5 g/L The stock solution of the standard is prepared with a concentration of 0.5 g/L. The actual content of the folic acid used must be taken into account here. Example: 127.5 mg 98% folic acid = 125 mg 100% folic acid are weighed into a beaker and 80 mL ultrapure water added. c(NaOH) = 0.1 mol/L is added with stirring until the solution becomes clear and a pH value of 8. 0 has been reached. An additional 120 mL ultrapure water are added and the pH value readjusted to 8.0. The solution is added to a 250 mL volumetric flask, which is then filled to the mark with ultrapure water and the contents mixed. Store solution in a brown glass bottle in a refrigerator. It is best to prepare fresh solutions daily and used CO_2 free ultrapure water.
Folic acid standard solution	This is prepared when needed from the stock solution by dilution with ultrapure water pH = 8.

If need be, a pH value of 8.0 is set by addition of diluted NaOH.

Sample preparation

Injection solutions

These are adjusted to pH = 8 with NaOH and can then be used directly.

Vitamin tablets (monovitamin tablets)

10 tablets are weighed out to obtain the average weight and then ground to a powder (grinder, mortar). 200 mg of the resulting powder are weighed into a beaker and 30 ml water added. The pH value is adjusted to 8.0 by addition of c(NaOH) = 0.1 mol/L with stirring. The solution is stirred for a further 15 min and the pH value again adjusted to 8.0 if need be. After filtration of the solution through a paper filter into a dark glass bottle, the filter is washed four times with 5 mL aliquots of dist. water pH = 8.0. The filtrate is transferred to a 100 mL volumetric flask, which is filled to the mark with ultrapure water (pH = 8.0) and the contents mixed. Use solution for the analysis immediately.

Analysis

0.50 mL of the solution obtained from the tablets are mixed in a polarographic vessel with 19.5 mL electrolyte.

Measuring solution

0.5 mL sample solution

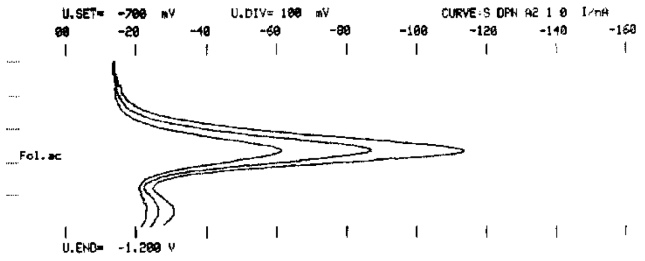
19.5 mL electrolyte

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	5 s
Sweep	
Start potential	-0.7 V
End potential	-1.2 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	Folic acid
Characteristic potential	-0.97 V

Example



Result

Sample	Folic acid vitamin tablet
β(Folic acid)	4.7 mg/tablet

Comments

- The linearity range lies between 75 µg/L and 8.75 mg/L folic acid.
- Ensure that the concentration of the folic acid in the polarographic vessel including the standard additions does not exceed the linear range of 8.75 mg/L.
- The presence of ascorbic acid, ascorbates and iron(II) compounds (e.g. iron(II) fumarate) does not disturb the determination of folic acid.
- Folic acid has to be extracted from foodstuffs and fodder.

References

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Appendix

Report for the example determination of folic acid in a vitamin tablet

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METROHM 646 VA-PROCESSOR  (5.646.6041)
Detn. of Folic Acid (Vit. Bc) in Monovitamin Tab. METHOD
MPL 1          EL.TYPE    MME

SUPP.ELEC      Borate buff.11.2pH
V.MEAS          20.000 mL
ALIQUOT         1.000

REMARK         Detn.folic acid in monovitamin tablet (5mg/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
     Niamde     A0 0 0       -968  mV      36.79 nA
                A0 1 0       -970  mV      36.97 nA
                A1 0 0       -970  mV      59.18 nA
                A1 1 0       -971  mV      58.54 nA     21.97 nA
                A2 0 0       -971  mV      79.78 nA
                A2 1 0       -972  mV      79.04 nA     20.54 nA
              m.STD     25.00 ug      SLOPE     1.175 mg/uA          43.64 ug

     rho(fol.ac  =     4.735         mg/g

     SMPL.V,m          1.00000 mg          IDENT APO folic ac.5mg/tab
     DATE  91-05-25   TIME    14:31
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Method print for the determination of folic acid

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     Detn. of Folic Acid (Vit. Bc) in Monovitamin 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                800   ms
 4c     U.SET               -700   mV
 5   SWP 0 ;                 100   s
 5a     U.END               -1.200 V
 5b     U.STEP               4   mV
        SW.RATE              5.0 mV/ s
 6   OMEAS ;
 7   REP) 1;
 8   OMEAS ;PURGE ;STIR   ;
 9   BEEP   ;ADD1]2;          30   s
10   OMEAS ;OPURGE;OSTIR ;
11   BEEP   ;END    ;
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