

Application Bulletin



Of interest for:
Pharmacy
Foodstuffs

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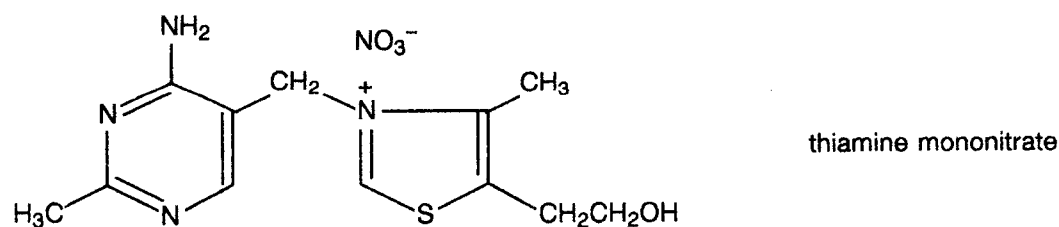
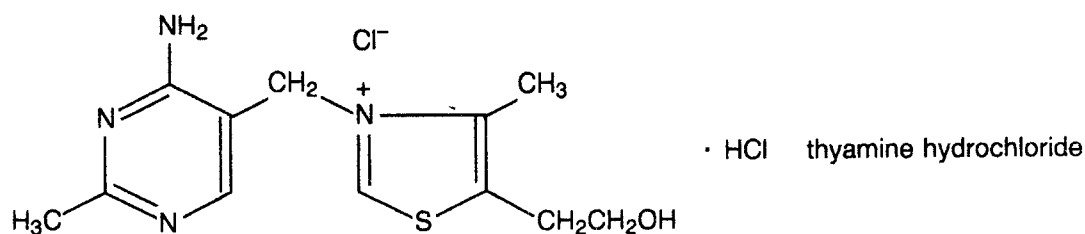
Polarographic determination of thiamine (vitamin B₁)

Summary

- ▶ This work is part of a series of Application Bulletins published at varying intervals for the polarographic determination of vitamins. The determination of thiamine (vitamin B₁) is detailed. The procedure allows an analysis in monovitamin preparations. The linearity range of the determination is also given. The limit of determination is ca. 1 µg thiamine / 20 mL cell volume.

Theory

- ▶ Vitamin B₁ is normally used in preparations in the form of thiamine hydrochloride or thiamine mononitrate:



Apparatus

- ▶ 2.646.003X VA Processor with 2.647.0020 VA Stand or 2.506.0010 Polarecord or 2.626.0010 Polarecord with 2.663.002X VA Stand

Polarographic determination of thiamine (vitamin B₁)

Reagents	<p>Only ultrapure water and the purest chemicals should be used for preparation of the reagents.</p> <ul style="list-style-type: none"> ▶ Acetate primary solution: Sodium acetate (4.10 g, anhydrous p.a.) is weighed into a beaker and dissolved in ca. 400 mL ultrapure water. After addition of 2.86 mL acetic acid, the solution is stirred and diluted with ultrapure water to ca. 950 mL and the pH value adjusted to 6.5 (6.4 ... 6.6) with $c(\text{NaOH}) = 2.5 \text{ mol/L}$. The volume is then made up to 1000 mL with ultrapure water and the solution mixed. ▶ Triton X-100 1%: Triton X-100 (1 g) is dissolved in ultrapure water and the solution made up to 100 mL. ▶ Standard, stock soln: Stock solution (500 mL) 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 ppm solution, 500 mg (or correspondingly more) are weighed into a 500 mL volumetric flask and dissolved in the primary solution. The flask is then filled to the mark with primary solution and the contents mixed. The solution can be kept for a considerable length of time in a refrigerator. Example: vitamine B₁ 99.5 % sample weight = $500 : 0.995 = 502.5 \text{ mg}$ ▶ Standard, working soln: Prepared fresh every day by appropriate dilution of the primary solution.
Sample preparation	<ul style="list-style-type: none"> ▶ Solutions These can be used directly. ▶ 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 $c(\text{NaOH}) = 0.01 \text{ mol/L}$ 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.
Method	<ul style="list-style-type: none"> ▶ Primary solution (18 mL) together with 0.4 mL sample solution in a polarographic vessel is deaerated with nitrogen for 5 min. After addition of 0.8 mL Triton X-100, deaeration is performed for a further 30 s and the DP polarogram then recorded (amplitude -50 mV) at the DME between -1.10 V and -1.48 V. ▶ The peak potential of thiamine is at ca. -1.38 V. ▶ The content is determined by double standard addition. It must be ensured that the concentration of thiamine in the polarographic vessel including that due to the standard additions does not exceed the linearity range. ▶ Figs 1 to 5 show the parameter settings of the 646 VA Processor.

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Linearity	<p>▶ Fig. 8 shows a plot of nA/μg thiamine. It is not intended to be a calibration curve, but simply to illustrate the approximate slope of the curve.</p>
Remarks	<p>▶ The content of thiamine in the polarographic vessel should not be less than 5 μg. Further, the total content including that due to the standard additions should not exceed 250 μg / 20 mL.</p> <p>▶ The SMDE can not be used as a working electrode for these determinations.</p> <p>▶ Nicotinamide and Fe(II) ions interfere with the determination.</p>
Literature	<p>[1] Tichomirova, G.P. / Balenkaja, S.L. <i>Der Einfluss von Aluminiumsalzen auf das polarographische Verhalten von Thiamin.</i> Zh. Anal. Khim. 17, (1962) 767-769 Ref: Fresenius, Z. Anal. Chem. 202, (1964) 381</p> <p>[2] Tichomirova, G.P. / Benenkaja, S.L. <i>Polarographic determination of thiamine.</i> Ukrain. Chim. Z. 29, (1963) 97-99 (in Russian) Ref: Fresenius, Z. Anal. Chem. 201, (1964) 300</p> <p>[3] Dewjatnin, W.A. / Kusnetzowa, L.A. <i>Polarographic determination of vitamin B-1, B-2, PP and nicotinamide in mono- and polyvitamin preparations.</i> Med. Prom. USSR 58, (1964) 58-60 (in Russian) Ref: Electroanal. Abstr. 5, (1967) 43</p> <p>[4] Göbbeler, K.H. / Breinlich, J. <i>Quantitative wechselstrompolarographische Simultanbestimmung von Vitaminen der B-Gruppe.</i> Pharm. Ztg. 48, (1972) 1859-1862</p> <p>[5] Söderhjelm, P. / Lindquist, J. <i>Electrochemical assay of thiamine, riboflavine, pyridoxine, nicotinamide and ascorbic acid in pharmaceutical preparations.</i> Acta Pharm. Suec. 13, (1976) 201-212</p> <p>[6] Matsunaga, T. / Karube, I. / Suzuki, S. <i>Electrochemical microbioassay of vitamin B-1.</i> Anal. Chim. Acta 98, (1978) 25-30</p> <p>[7] Kishore, K. / Moorthy, P.N. / Rao, K.N. <i>Thiamine assay by differential-pulse polarography.</i> Indian J. Chem. (1979), 206-208 Ref: Fresenius, Z. Anal. Chem. 301, (1980) 83</p> <p>[8] Vergara, T. / Marin, D. / Vera, J. <i>Polarographic determination of thiamine and its monophosphate and pyrophosphate esters.</i> Anal. Chim. Acta 120, (1980) 347-351</p>

Polarographic determination of thiamine (vitamin B₁)

Fig. 1 Example program page 2, 646 VA Processor

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1 Detn. of Thiamine Mononitrate in Monovit. Tab.      METHOD 4 PAGE 2
2 MPL 1          EL.TYPE MME                          GEN.SPECIFICATIONS

PARAMETERS
3 IR.MODE          N
4 SPEED            5
5 D.SIZE          5
6 N.DROPS         3

RECOGNITION
7 SPIKE THRESH    4
8 H.THRESH        3
9 U.TOL           9
10 W.TOL          9
11 ASYM.TOL       9

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Fig. 2 Example program page 3, 646 VA Processor

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

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 ;

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Fig. 3 Example program page 4, 646 VA Processor

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Detn. of Thiamine Mononitrate in Monovit. Tab.      METHOD 4 PAGE 4
MPL 1          EL.TYPE MME                          ALLOCATIONS
a              b              c              d              e              f
SOLUTE        U.VERIF        DOS            V.SOLN          m.CONC          m.BLANK
Subst         Ux              Soln       c, v           rho.x           bx
1 Vit.B1      -1.375 V        1          c 40 uL        1.000 g /L     0.000 g
2
3
4
5
6
7
8
9 SUPP.ELEC   0.05M acet. pH 6.5
10 V.MEAS     20.000 mL
11 ALIQUOT    1.000
12 DATE       91-06-24
13 TIME       13:54

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Polarographic determination of thiamine (vitamin B₁)

Fig. 7 Result block of above determination (Fig. 6)

METROHM 646 VA-PROCESSOR (5.646.6041)
 Detn. of Thiamine(C1).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(C1).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

Fig. 8 Linearity curve 50 300 µg thiamine / 20 mL

