

Application Bulletin

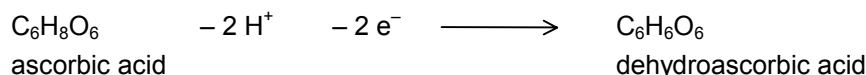
Of interest to: General analytical laboratories; Food;
Pharmaceutical industry; Biology

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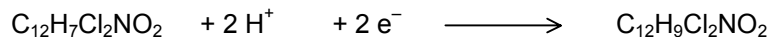
Determination of ascorbic acid (vitamin C) and its compounds

Summary

Ascorbic acid, as well as its salts and esters, can be determined by titration with 2,6-dichlorophenol indophenol (DPIP) or by means of polarography. The determination is based on the oxidation of ascorbic acid to dehydroascorbic acid:



Using 2,6-dichlorophenol indophenol as titrant, this will be reduced by the ascorbic acid:



For the titrimetric determination bi-voltametric or photometric endpoint indication can be used. Be aware, though, that only the bi-voltametric indication is independent of inherent coloration of the sample.

The polarographic method is the most selective of the described methods, since other reducing or oxidizing substances do not interfere.

Instruments and accessories

For titration:

- 702 SET/MET Titrino, 716 DMS Titrino, 736 GP Titrino, 751 GPD Titrino or 785 DMP Titrino or 726 or 796 Titroprocessor with 700 Dosino or 685 Dosimat
- 2.728.0040 Magnetic Stirrer
- 6.3014.213 or 6.3014.223 Exchange Unit
- 6.0309.100 double Pt sheet electrode with 6.2104.020 electrode cable (bi-voltametry) or
- 6.5501.01X Spectrode 610 nm (photometry)

For polarography:

- 757 VA Computrace or
- 746 VA Trace Analyzer with 747 VA Stand

Reagents

- Oxalic acid solution:
1 g oxalic acid or 1.4 g oxalic acid dihydrate is dissolved in dist. water and made up to 1 L.
- Sodium acetate solution, $w(\text{CH}_3\text{COONa}) = 10\%$ in dist. water
- Acetate buffer pH = 4.64:
8.2 g sodium acetate is dissolved in dist. water. Add 6 mL glacial acetic acid and make up to 100 mL with dist. water.
- Vitamin C standard, $\rho(\text{vitamin C}) = 0.5 \text{ g/L}$:
50.0 mg vitamin C is dissolved in oxalic acid solution and made up to 100 mL. This solution should be freshly prepared every day.
1 mL standard corresponds to 0.5 mg vitamin C
- Titrant: $c(\text{DPIP}) \approx 0.001 \text{ mol/L}$:
Approx. 330 mg 2,6-dichlorophenol indophenol sodium salt dihydrate (e.g. Merck no. 103028) is dissolved in dist. water. Add approx. 100 mg NaHCO_3 and make up to 1 L with dist. water.

Sample preparation

- Drinks, fruit and vegetable juices can be analyzed directly.
- For tablets and other vitamin preparations, a diluted solution in de-aerated dist. water is first prepared, an aliquot of which is then used for the titrimetric or polarographic determination.
- Foods, stimulants and animal feeds are extracted using the appropriate procedures. Colored substances present in the samples need not be extracted with ether, since these do not interfere with the determination of vitamin C.

Analysis

1. Titrimetric determination

Place 10 mL dist. water, 15 mL oxalic acid solution and 1 mL sodium acetate solution in the titration vessel and purge with nitrogen for 3 ... 5 min. Add the sample or standard solution (containing 0.05 ... 0.5 mg – for the photometric titration at least 0.2 mg – vitamin C), then titrate with $c(\text{DPIP}) \approx 0.001 \text{ mol/L}$ using, e.g., the MET mode.

Calculation

1 mL $c(\text{DPIP}) = 0.001 \text{ mol/L}$ corresponds to 0.176 mg vitamin C

Figures

```
'pa
785 DMP Titrino      02287  785.0010
user
date 1999-05-04     time 10:26      22
MET Ipol           *****
parameters
>titration parameters
  V step            0.10 ml
  dos.rate          max. ml/min
  signal drift      OFF mV/min
  equilibr.time     8 s
  start V:          OFF
  pause            0 s
  I(pol)           1 µA
  electrode test:   OFF
  temperature       25.0 °C
>stop conditions
  stop V:          abs.
  stop V           5 ml
  stop U           OFF mV
  stop EP         9
  filling rate     max. ml/min
>statistics
  status:          OFF
>evaluation
  EPC              5 mV
  EP recognition:  greatest
  fix EP1 at U    OFF mV
>preselections
  req.ident:       OFF
  req.smpl size:   OFF
  limit smpl size: OFF
  activate pulse:  OFF
-----
```

```
'fr
785 DMP Titrino      02287  785.0010
user
date 1999-05-04     time 10:16      21
U(init)            141 mV MET Ipol *****
smpl size          2 ml
EP1                4.179 ml                28 mV
RS1                0.368 g/l
stop V reached
=====

'cu
785 DMP Titrino      02287  785.0010
user
date 1999-05-04     time 10:16      21
start V           0.000 ml MET Ipol *****
2.0 ml/div        dU=50.0 mV/div
```

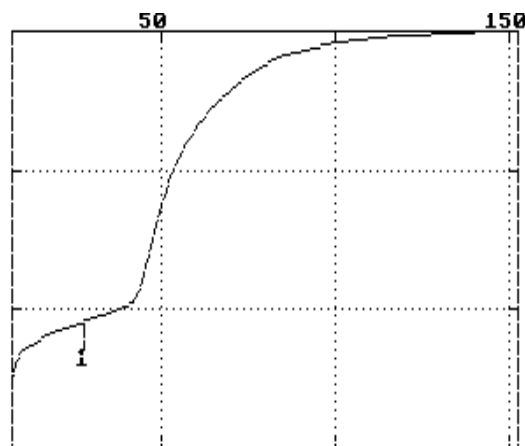


Fig. 1: Parameter settings on the 785 DMP Titrino for the titration of vitamin C with bi-voltametric indication.

Fig. 2: Result block and titration curve for the determination of vitamin C in orange juice (bi-voltametric indication).

```
'pa
785 DMP Titrino      02287  785.0010
user
date 1999-05-04    time 08:28      9
DET U *****
parameters
>titration parameters
  meas.pt.density      4
  min.incr.            100 µl
  dos.rate             max. ml/min
  signal.drift         30 mV/min
  equilibr.time        32 s
  start V:             OFF
  pause                0 s
  meas.input:          1
  temperature          25.0 °C
>stop conditions
  stop V:              abs.
  stop V               15 ml
  stop U               OFF mV
  stop EP              9
  filling rate         max. ml/min
>statistics
  status:              OFF
>evaluation
  EPC                  5
  EP recognition:      greatest
  fix EP1 at U        OFF mV
  pK/HNP:              OFF
>preselections
  req.ident:           OFF
  req.smpl size:       OFF
  limit smpl size:     OFF
  activate pulse:      OFF
=====
```

```
'fr
785 DMP Titrino      02287  785.0010
user
date 1999-05-04    time 08:45      11
U(init)            164 mV DET U *****
smpl size          1.3 ml
EP1                3.820 ml          106 mV
Vit C              0.52 g/l
manual stop
=====

'cu
785 DMP Titrino      02287  785.0010
user
date 1999-05-04    time 08:45      11
start V            0.000 ml DET U *****
2.0 ml/div         dU=50.0 mV/div
```

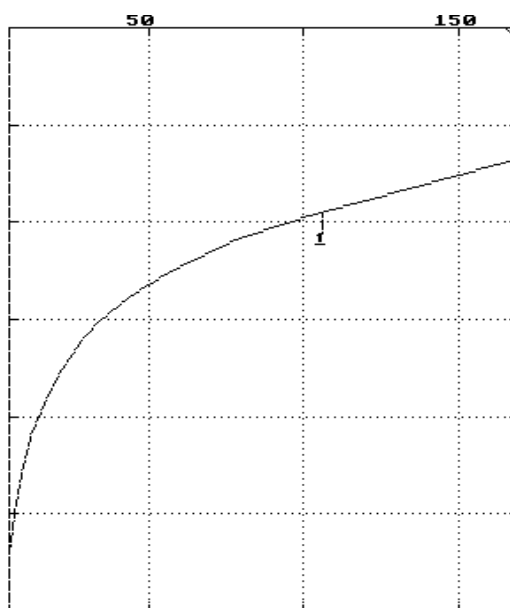


Fig. 3: Parameter settings on the 785 DMP Titrino for the titration of vitamin C with photometric indication.

Fig. 4: Result block and titration curve for the determination of vitamin C in the standard (photometric indication).

2. Polarographic determination

Place 10 mL dist. water and 1 mL acetate buffer pH = 4.64 in the polarographic vessel and purge with nitrogen. Add 1 mL sample solution, mix and purge briefly with nitrogen once again. Afterwards record the DP polarogram using the following parameters:

working electrode	DME
stirrer speed	2000 rpm
mode	DP
purge time	300 s
equilibration time	5 s
pulse amplitude	50 mV
start potential	-50 mV
end potential	+200 mV
voltage step	6 mV
measure time	20 ms
pulse time	40 ms
voltage step time	0.6 s
sweep rate	10 mV/s
peak potential	+100 mV

The concentration is determined by standard addition.

Figures

```

===== METROHM 746 VA TRACE ANALYZER (5.746.0101) =====
Method: AB98      .mth      OPERATION SEQUENCE
Title : Determination of Vitamin C. AB98
    
```

```

-----
Instructions  t/s  Main parameters  Auxiliary parameters
-----
1  SMPL/M
2  DOS/M      V.fraction      mL      V.total      L
3  PURGE      V.added        11.000 mL
4  STIR      300.0  Rot.speed      2000 /min
5  (ADD
6  PURGE
7  STIR      10.0  Rot.speed      2000 /min
8  SEGMENT   Segm.name      pol
9  ADD>M    Soln.name      AscStd      V.add      0.100 mL
10 ADD) 3
11 END
    
```

```

Method: AB98      SEGMENT
                  pol
    
```

```

-----
Instructions  t/s  Main parameters  Auxiliary parameters
-----
1  OPURGE
2  OSTIR      5.0
3  (REP
4  DME
5  DPMODE    U.ampl      50 mV      t.meas      20.0 ms
6  SWEEP     18.0  t.step      0.40 s      t.pulse     40.0 ms
7  REP) 1    U.start     -50 mV     U.step      6 mV
8  PURGE    U.end       200 mV     Sweep rate  15 mV/s
9  STIR      Rot.speed   2000 /min
10 OMEAS    U.standby   mV
11 END
    
```

Fig. 5: Method for the determination of vitamin C with the 746 VA Trace Analyzer.

Method: AB98

 SUBSTANCES
 Ascorb - pol

Recognition		Display / Plot	
U.verify	100 mV	I.scale	auto
U.tol (+/-)	50 mV	U.div	50.00 mV/cm
U.width min	10 mV	U.begin	0 mV
U.width max	200 mV	U.end	200 mV
I.threshold	200 pA		
Baseline		Evaluation	
Type	linear	Mode	VA
Scope	whole	Quantity	I.peak
dU.front	auto	Sign. digits	4
S.front	auto		
dU.rear	auto		
S.rear	auto		
Calibration	2000-08-22 17:24:39	Coefficients	
Technique	std.add.	Y.reg	4.103e-07
Curve type	linear	Slope	3.836e-05
		Nonlin.	
		Mean dev.	9.826e-09
Additions			
Soln.name	AscStd		
Mass conc.	1 g/L	g/L	g/L
Range min	g/L	g/L	g/L
Range max	g/L	g/L	g/L
M.conc./cm	g/L	g/L	g/L
Method: AB98	CALCULATION max. 15 lines		
Quantity	Formula (R##, C##, A##)	Res.unit	Sig.dig.
Ascorb	R1000=MC:Ascorb	#g/L	5

Fig. 6: Method (continued).

```

===== METROHM 746 VA TRACE ANALYZER (5.746.0101) =====
Determ.      : 08221608          User:          Date: 2000-08-22
Modified     : no                Run : 0          Time: 16:08:59
Sample table: -
    
```

```

-----
Pos.  Ident.1/S1  Ident.2/S2  Ident.3/S3  Method.call  Sample size/S0
      O_Saft                                0.5 mL
-----
Method : AB98
Title  : Determination of Vitamin C. AB98
Remark1 : 10 ml water + 1 ml buffer
Remark2 : + 0.5 ml sample
-----
    
```

```

Substance : Ascorb
Mass conc.: 246.1 mg/L          Mass      : 123 ug
MC.dev.   : 5.15 mg/L (2.09%)  Add.mass  : 100 ug
Cal.dev.  : -                  V0.sample: 500 uL
Comments  : -----
    
```

VR	U/mV	I/uA	I.mean	Std.dev.	I.delta	Comments
00	103	0.4100	0.4096	0.0006		
01	103	0.4091				
10	104	0.7410	0.7419	0.0013	0.3323	
11	104	0.7428				
20	104	1.039	1.056	0.0243	0.3139	
21	105	1.073				
30	105	1.348	1.373	0.0359	0.3176	
31	106	1.399				

Substance	Techn.	Y.reg/offset	Slope	Nonlin.	Mean deviat.
Ascorb	std.add.	4.103e-07	3.836e-05		9.826e-09

```

Final results
-----
Ascorb = 246.06 mg/L          +/- Res.dev.  %      Comments
                               5.15    2.09
-----
    
```

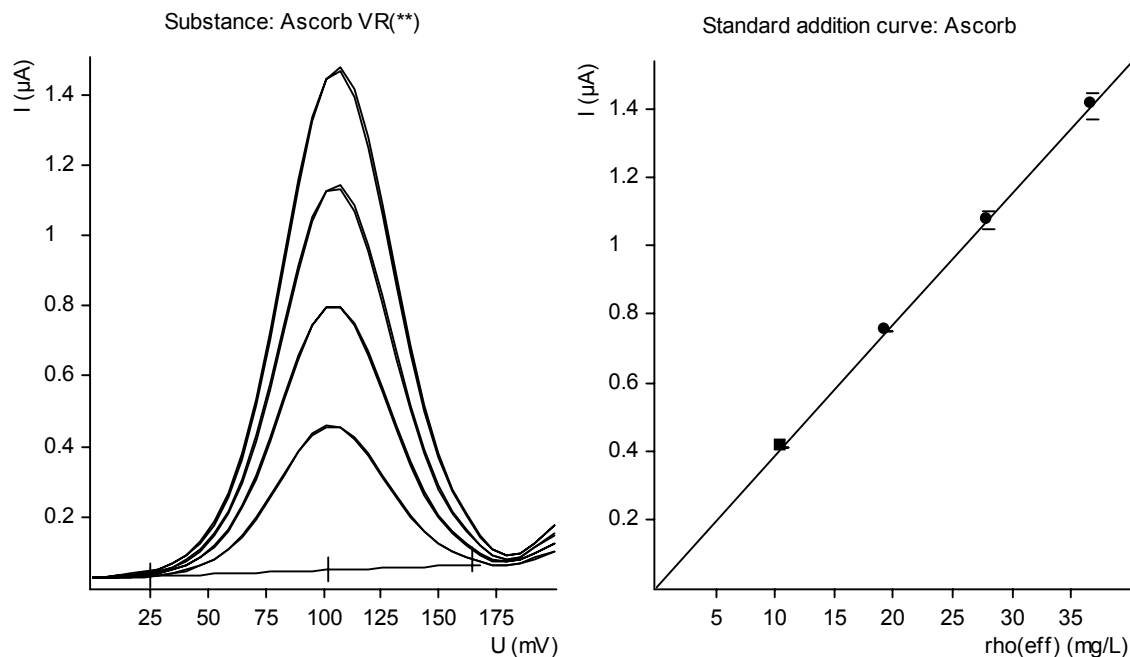


Fig. 7: Results, polarograms and standard addition curve for the determination of vitamin C in orange juice.

Remarks

- Instead of 2,6-dichlorophenol indophenol, other oxidizing titrants can be used as well, e.g. iodine, iodide/iodate, chloramine-T, Fe(III). Here, however, the danger is greater that apart from ascorbic acid other oxidizable compounds present in the sample will also be determined.
- Larger quantities of chloride ions (e.g. in sauerkraut) interfere with the polarographic determination of vitamin C. They are removed from the sample solution by precipitation with silver nitrate and subsequent filtration.

Literature

- Metrohm Application Note T-30
Ascorbic acid (vitamin C) in fruit juices
Metrohm Ltd., Herisau.
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Vitamin C (ascorbic acid) by photometric titration
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Verlag Akademiai Kiado, Budapest, 1973.
- G. Pongracz
Neue potentiometrische Bestimmungsmethoden für Ascorbinsäure und dessen Verbindungen
Fresenius Z. Anal. Chem. 253 (1971) 271–274.
- K. K. Verma, A. K. Gulati
Determination of vitamin C with chloramine T
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