Metrohm

Application Bulletin 191/3 e

Determination of cysteine and cystine simultaneously by polarography

Summary

After the degradation of biological samples (e.g. milk, wool, etc.), it is often important to know the cystine/cysteine ratio. This Application Bulletin describes the simultaneous, polarographic determination of the two amino acids. The determination is performed in perchloric acid solution at the DME. Samples with a high protein content require that the determination is performed in an alkaline solution.

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(CH_3COOLi) = 1 mol/L (method 1) or c(KCl) = 3 mol/L (method 2)	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 or for trace analysis*).

- Cystine, for analysis, CAS 56-89-3
- Cysteine, for analysis, CAS 52-90-4
- Ultrapure water, resistivity >18 MΩ·cm (25 °C), type I grade (ASTM D1193)

For method 1

- Perchloric acid, w($HClO_4$) = 70%, for analysis, CAS 7601-90-3
- Lithium acetate dihydrate, w($LiOOCCH_3 \cdot 2\ H_2O$) ≥ 99.0%, for analysis, CAS 6108-17-4

For method 2

- Ammonium hydroxide solution, w(NH_3) = 25%, for analysis, CAS 1336-21-6
- Hydrochloric acid, w(HCl) = 30%, for analysis, CAS 7647-01-0
- Sodium hydroxide solution, c(NaOH) = 2.0 mol/L, CAS 1310-73-2

Method 1: Samples containing few proteins

Summary

Samples without proteins can be determined in perchloric acid solution. The determination limit is heavily dependent on the matrix and lies at approx. 1 mg/L for both substances.

Solutions

Electrolyte	c($HClO_4$) = 0.1 mol/L 4.16 mL perchloric acid are diluted to 500 mL with ultrapure water.
Li-acetate solution	c(CH_3COOLi) = 1 mol/L for reference electrode

Standard solutions

Cysteine standard solution	c(cysteine) = 1 g/L in perchloric acid Mix 0.1 g cysteine to a slurry in 20 mL dist. water and dissolve by adding 0.86 mL perchloric acid, then fill up to 100 mL with dist. water.
Cystine standard solution	c(cystine) = 1 g/L in perchloric acid Mix 0.1 g cystine to a slurry in 20 mL dist. water and dissolve by adding 0.86 mL perchloric acid, then fill up to 100 mL with dist. water.

Analysis

Measuring solution

10 mL diluted sample

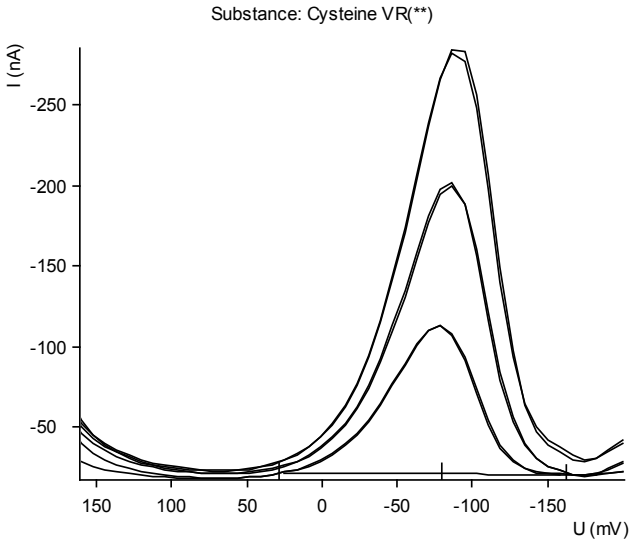
10 mL supporting electrolyte

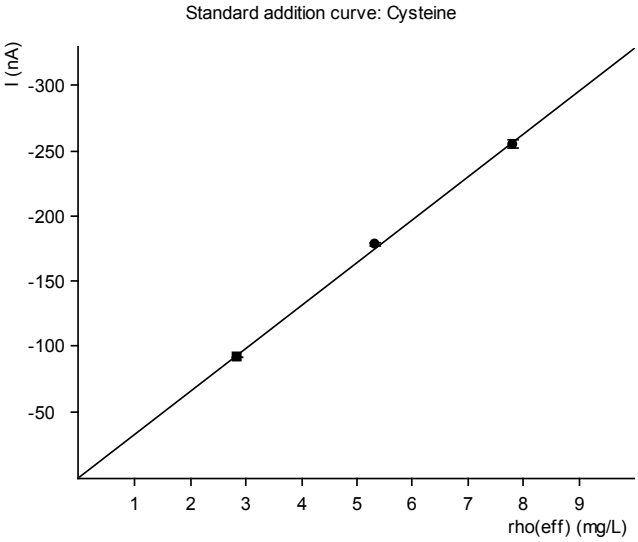
The concentrations are determined by standard addition.

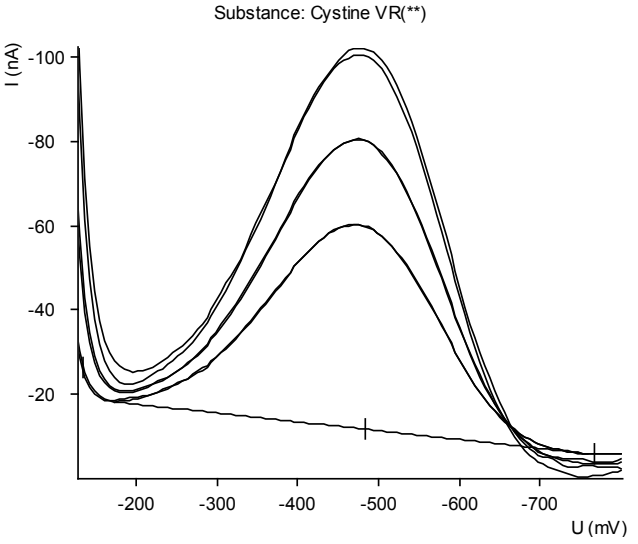
Parameters

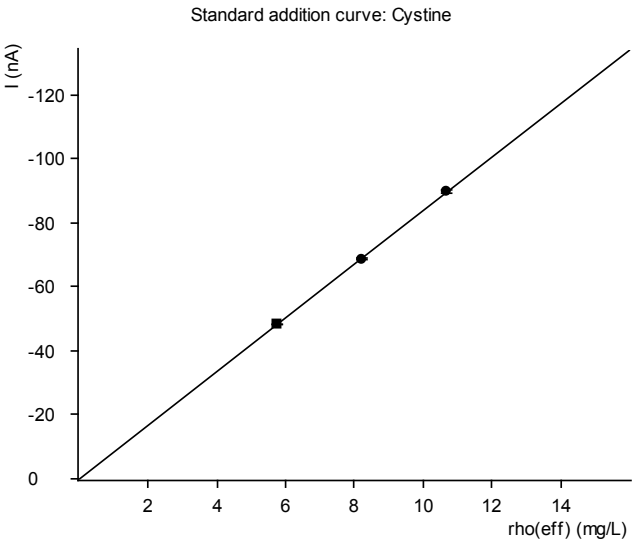
Voltammetric	
Electrode operating mode	DME
Measuring mode	DP – Differential pulse
Stirring rate	2000 min^{-1}
Equilibration time	30 s
Sweep	
Start potential	0.2 V
End potential	-0.8 V
Potential step	0.006 V
Potential step time	0.6 s
Sweep rate	0.01 V/s
Pulse amplitude	0.05 V
Substance	
Name	Cysteine
Characteristic potential	-0.08 V
Name	Cystine
Characteristic potential	-0.49 V

Example









Result

Sample size	10.0 mL
β(cysteine)	2.9 mg/L
β(cystine)	5.8 mg/L

Comments

- The peak potentials of both substances shift to slightly more negative values with increasing concentrations. The value must perhaps be corrected.
- If the concentrations of cystine and cysteine differ strongly, it is possibly necessary to perform two analyses in various dilutions for the respective substance.

Method 2: Samples with a high protein content

Summary

Samples with a high protein content cannot be determined using perchloric acid as electrolyte, since proteins are precipitated in acidic solutions. Therefore the determination is carried out in ammonium buffer at pH 9.6.

The limit of detection for cysteine is approx. 0.05 mg/L, for cystine approx. 1 mg/L. The determination runs linear for cysteine up to 180 mg/L, for cystine up to 300 mg/L.

Solutions

Ammonia buffer pH 9.6	c(NH_4Cl) = 1 mol/L c(NH_3) = 2 mol/L (pH 9.6) 112.5 mL NH_3 + 53 mL HCl, filled up to 500 mL with ultrapure water.

Standard solutions

Cysteine standard solution	c(cysteine) = 1 g/L Dissolve 0.100 g cysteine in 20 mL ammonium buffer and fill up to 100 mL with dist. water.
Cystine standard solution	c(cystine) = 1 g/L Dissolve 0.100 g cystine in 5 mL sodium hydroxide and fill up to 100 mL with dist. water.

Analysis

Measuring solution

10 mL diluted sample

1 mL ammonia buffer pH 9.6

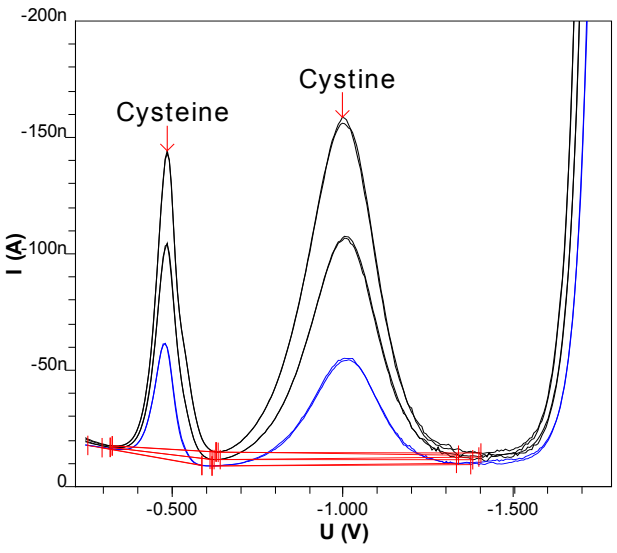
The concentrations are determined by standard addition.

The peak potential of cystine is strongly dependent on the pH value and shifts in negative direction with increasing pH value.

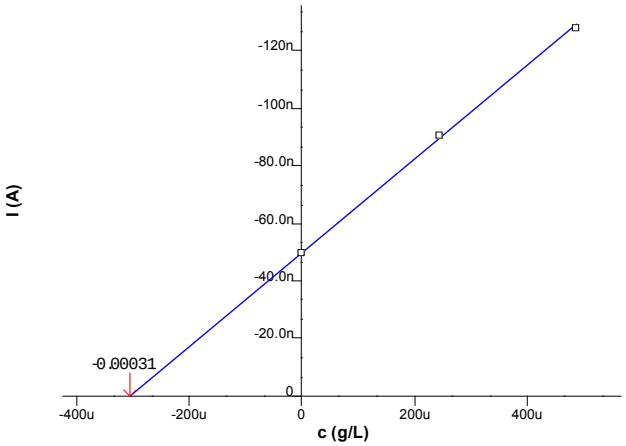
Parameters

Voltammetric	
Electrode operating mode	DME
Measuring mode	DP – Differential pulse
Stirring rate	2000 min^{-1}
Equilibration time	30 s
Sweep	
Start potential	-0.25 V
End potential	-1.75 V
Potential step	0.006 V
Potential step time	0.8 s
Sweep rate	0.0075 V/s
Pulse amplitude	0.05 V
Substance	
Name	Cysteine
Characteristic potential	-1.0 V
Name	Cystine
Characteristic potential	-0.5 V

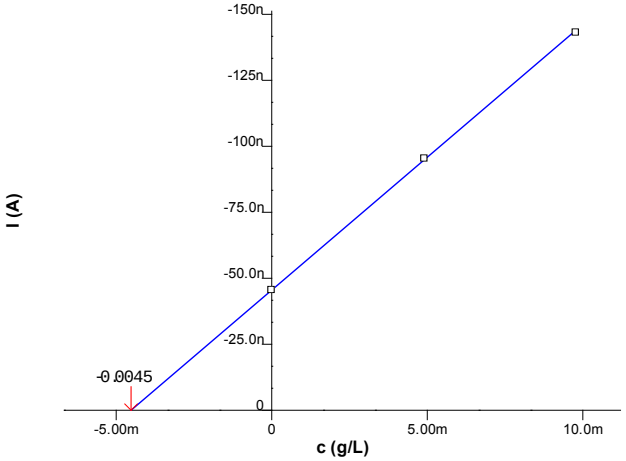
Example



Cysteine
c = 0.031 g/l
+/- 0.001 g/l (1.92%)



Cystine
c = 0.456 g/l
+/- 0.006 g/l (1.22%)



Result

Sample	Sodium caseinate
Sample size	0.1 mL
β(Cysteine)	0.031 g/L
β(Cystine)	0.456 g/L

Comments

- If the pH value is over 10, the cystine peak becomes very flat and insensitive.
- If the concentrations of cystine and cysteine differ greatly, it will possibly be necessary to perform two analyses in various dilutions for the respective substance.

Appendix

Report for the example determination of cysteine and cystine according to method 1

```
================== METROHM 746 VA TRACE ANALYZER (5.746.0101) ==================
 Determ.     : 06111056             User:  mj                  Date: 1999-06-11
 Modified    : 1999-06-11 11:12:06  Run :  18                  Time: 10:56:21
 Sample table: -
--------------------------------------------------------------------------------
  Pos.  Ident.1/S1    Ident.2/S2    Ident.3/S3    Method.call   Sample size/S0
        cysHClO4                                                      10.0 mL
--------------------------------------------------------------------------------
 Method  :  AB191/1
 Title   :  Determination of Cystine and Cysteine
 Remark1 :  10 mL supporting electrolyte + 10 mL sample
 Remark2 :  supporting electrolyte: HClO4 c = 0.1 mol/L
--------------------------------------------------------------------------------
  Substance : Cysteine                                          Comments
  Mass conc.:    2.873 mg/L           Mass     : 28.73 µg   ------------------
  MC.dev.   :    0.121 mg/L (4.22%)   Add.mass :    25 µg
  Cal.dev.  :    -                    V0.sample:    10 mL

              VR   U/mV    I/nA   I.mean  Std.dev. I.delta   Comments
              ---  -----  ------  ------  -------  -------   ------------------
              00   -80    -92.07  -92.09   0.0219
              01   -79    -92.11
              10   -88    -175.9  -176.9    1.432   -84.84
              11   -87    -177.9
              20   -92    -254.5  -252.3    3.016   -75.41
              21   -91    -250.2

  Substance : Cystine                                           Comments
  Mass conc.:    5.798 mg/L           Mass     : 57.98 µg   ------------------
  MC.dev.   :    0.090 mg/L (1.55%)   Add.mass :    25 µg
  Cal.dev.  :    -                    V0.sample:    10 mL

              VR   U/mV    I/nA   I.mean  Std.dev. I.delta   Comments
              ---  -----  ------  ------  -------  -------   ------------------
              00   -483   -48.28  -48.25   0.0341
              01   -482   -48.23
              10   -487   -68.13  -68.30   0.2406   -20.05
              11   -487   -68.47
              20   -489   -89.29  -88.88   0.5862   -20.58
              21   -492   -88.46

 Substance    Techn.     Y.reg/offset   Slope        Nonlin.      Mean deviat.
 ---------    --------   ------------   ----------   ----------   ------------
 Cysteine     std.add.     -9.352e-08   -3.284e-05                 3.016e-09
 Cystine      std.add.     -4.813e-08   -8.373e-06                 3.830e-10

 Final results                        +/- Res.dev.   %      Comments
 ---------------------------------    -----------------     ------------------
   Cysteine =   2.8726 mg/L              0.121    4.22
    Cystine =   5.7979 mg/L              0.090    1.55
```

Method print for the determination cysteine and cystine according to method 1

```
================== METROHM 746 VA TRACE ANALYZER (5.746.0101) ==================
 Method: AB191_1 .mth         OPERATION SEQUENCE
 Title : Det of cystine and cysteine (low protein content)
--------------------------------------------------------------------------------

      Instructions   t/s   Main parameters            Auxiliary parameters
      ------------- -----  ------------------------  ------------------------
 1    SMPL>M               V.fraction          mL     V.total              L
 2    DOS>M                Soln.name   electrol          V.add         10 mL
 3    PURGE
 4    STIR          300.0  Rot.speed      2000 /min
 5    (ADD
 6      PURGE
 7      STIR        30.0   Rot.speed      2000 /min
 8      0PURGE
 9      0STIR       20.0
10      (REP
11        SEGMENT          Segm.name   POL
12      REP)1
13      PURGE
14      ADD>M              Soln.name   Cystine        V.add         0.025 mL
15      ADD>M              Soln.name   Cysteine       V.add         0.025 mL
16    ADD)2
17    END
```

```
 Method: AB191_1               SEGMENT
                                    POL
-------------------------------------------------------------------------------

      Instructions   t/s   Main parameters            Auxiliary parameters
      ------------ -----  ------------------------  ------------------------
 1    DME
 2    DPMODE               U.ampl          -50 mV     t.meas         20.0 ms
                           t.step         0.60 s      t.pulse        40.0 ms
 3    SWEEP         101.4  U.start         200 mV     U.step            6 mV
                           U.end          -800 mV     Sweep rate       10 mV/s
 4    0MEAS                U.standby           mV
 5    END
```

Report for the example determination of cysteine and cystine according to method 2

```
========== METROHM 797 VA COMPUTRACE (Version 1.0.0.1) (Serial No. 0) ==========
 Determination  : 06150859_Na-caseinat.dth
 Sample ID      : Na-caseinat
 Creator method :                  Date :                      Time:
 Creator determ.:                  Date : 1999-06-15           Time:  08:59:33
 Modified by    :                  Date : 2017-07-20           Time:  16:46:51
-------------------------------------------------------------------------------
 Method         : AB191_Det of Cystin and Cystein in alkaline solution.mth
 Title          : Bestimmung von Cystin und Cystein
 Remark1        : background electrolyte: NH3/NH4Cl pH 9.6
 Remark2        :
-------------------------------------------------------------------------------
 Sample amount  :   0.100 mL
 Cell volume    :  10.100 mL
-------------------------------------------------------------------------------
 Substance      : Cystine
 Conc.          :   4.513 mg/L
 Conc.dev.      :   0.055 mg/L     ( 1.22%)
 Amount         :  45.583 ug
 Add.amount     :  50.000 ug

 VR     V       nA      I.mean  Std.Dev.  I.delta   Comments
 -----  ------  ------  ------  --------  --------  ----------------------
 1 - 1  -1.018   -45.3   -45.5     0.251       0.0
 1 - 2  -1.006   -45.7
 2 - 1  -1.006   -95.2   -95.3     0.166     -49.8
 2 - 2  -1.006   -95.4
 3 - 1  -1.000  -141.9  -143.0     1.527     -47.7
 3 - 2  -1.000  -144.1

 Substance      : Cysteine
 Conc.          : 305.007 ug/L
 Conc.dev.      :   5.841 ug/L     ( 1.92%)
 Amount         :   3.081 ug
 Add.amount     :   2.500 ug

 VR     V       nA      I.mean  Std.Dev.  I.delta   Comments
 -----  ------  ------  ------  --------  --------  ----------------------
 1 - 1  -0.482   -49.7   -49.5     0.249       0.0
 1 - 2  -0.482   -49.4
 2 - 1  -0.488   -90.1   -90.3     0.346     -40.8
 2 - 2  -0.488   -90.6
 3 - 1  -0.488  -128.1  -127.5     0.746     -37.2
 3 - 2  -0.488  -127.0

 Substance    Calibr.      Y.reg/offset        Slope Mean deviat. Corr.Coeff.
 ------------ ------------ ------------ ------------ ------------ ------------
 Cystine      std.add.      -4.553e-008  -1.009e-005   7.436e-010      0.99983
 Cysteine     std.add.      -4.968e-008  -1.629e-004   1.092e-009      0.99968

 Final results                        +/- Res. dev.   %     Comments
-----------------------------------    ------------------    -------------------

 Cystine:
 default          =    0.456   g/l    0.006        1.215

 Cysteine:
 default          =    0.031   g/l    0.001        1.915
```

Method print for the determination cysteine and cystine according to method 2

```
-------------------------------------------------------------------------------
 Method parameters
-------------------------------------------------------------------------------
```

```
Method          : AB191_2 Det of Cystine and Cysteine (high protein content).mth
Title           : Determination of Cystine and Cysteine (high protein content)
Remark1         : 10 mL sample + 1 mL ammonia buffer (pH 9.6)
Remark2         :

Calibration     : Standard addition
Technique       : Batch
Addition        : Manual

Sample ID       : Na-caseinat
Sample amount (mL): 10.000
Cell volume (mL): 11.000

Voltammetric parameters
-------------------------------------------------------------------------------
Mode                            : DP - Differential Pulse

Highest current range           : 1   mA
Lowest current range            : 100 nA

Electrode                       : DME
Stirrer speed (rpm)             : 2000

Initial electr. conditioning    : No

No. of additions                : 2
No. of replications             : 2

Measure blank                   : No
Addition purge time (s)         : 30

Initial purge time (s)          :      300

Sweep
Equilibration time (s)          :    5.000
Start potential (V)             :   -0.250
End potential (V)               :   -1.750
Voltage step (V)                :    0.006
Voltage step time (s)           :    0.800
Sweep rate (V/s)                :    0.007
Pulse amplitude (V)             :    0.050
Pulse time (s)                  :    0.040

Cell off after measurement      :      Yes

Peak evaluation
-------------------------------------------------------------------------------
Regression technique            : Linear Regression
Peak evaluation                 : Height
Minimum peak width (V.steps)    : 5
Minimum peak height (A)         : 1.000e-010
Reverse peaks                   : No
Smooth factor                   : 4
Eliminate spikes                : Yes

Substances
-------------------------------------------------------------------------------
Cystin                  : -1.000 V   +/- 0.050 V

Standard solution       : 1   1.000 g/L
Addition volume (mL)    : 0.050

Cystine                 : Final result (Cystin) =
                          Conc * (11 / 10) * (1000 / 1) + 0 - 0

Cystei                  : -0.480 V   +/- 0.050 V

Standard solution       : 2   0.100 g/L
Addition volume (mL)    : 0.025

Cysteine                : Final result (Cystei) =
                          Conc * (11 / 10) * (1000 / 1) + 0 - 0

Baseline
-------------------------------------------------------------------------------
Substance Addition    automatic start (V) end (V) type        scope
-------------------------------------------------------------------------------
Cystin    Sample      yes       ---       ---     linear      wholePeak
          Addition 1  yes       ---       ---     linear      wholePeak
          Addition 2  yes       ---       ---     linear      wholePeak
-------------------------------------------------------------------------------
Cystei    Sample      yes       ---       ---     linear      wholePeak
          Addition 1  yes       ---       ---     linear      wholePeak
          Addition 2  yes       ---       ---     linear      wholePeak
```