
Application Bulletin

Of interest to: General analytical laboratories, Biology, Foodstuff

B 1, 3, 4, 7, 8, 13

Polarographic determination of cystine and cysteine simultaneously

Summary

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

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.

Apparatus and accessories

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

Electrodes

- Working electrode (WE):
Multi Mode electrode 6.1246.020
- Reference electrode (RE):
Ag/AgCl/lithium acetate:
6.0728.020 electrode Ag/AgCl (c(KCl) = 3 mol/L)
+ 6.1245.010 electrolyte vessel
with **lithium acetate** solution c(CH₃COOLi) = 1 mol/L
- Auxiliary electrode (AE): platinum:
6.0343.000 platinum rod electrode

Reagents

All of the used reagents must be of purest quality possible (p.a. or suprapur). Only high purity water should be used.

- Perchloric acid, $w(\text{HClO}_4) = 70\%$
- Cystine, puriss p.a., CAS 56-89-3
- Cysteine, puriss p.a., CAS 52-90-4
- Lithium acetate dihydrate, MicroSelect, $w(\text{LiOOCCH}_3 \cdot 2 \text{H}_2\text{O}) \geq 99.0\%$, CAS 546-89-4

Ready to use solutions

- **Supporting electrolyte:**
 $c(\text{HClO}_4) = 0.1 \text{ mol/L}$
- **Cystine standard solution:** $\beta(\text{cystine}) = 1 \text{ g/L}$ 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.
- **Cysteine standard solution:** $\beta(\text{cysteine}) = 1 \text{ g/L}$ 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.

Analysis

Measuring solution:

10 mL (diluted) sample
+ 10 mL supporting electrolyte

The polarogram is recorded with the following parameters:

working electrode	DME
stirrer speed (rpm)	2000
mode	DP
purge time	300 s
equilibration time	30 s
pulse amplitude	50 mV
start potential	200 mV
end potential	-800 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 cystine	-490 mV
peak potential cysteine	-80 mV

The concentration is determined by standard addition. Remarks

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

Figures

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

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Method: AB191_1          SEGMENT
                        POL
    
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	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	OMEAS		U.standby mV	
5	END			

Fig. 1 Method for the determination of cystine and cysteine acc. to method 1 with the 746 VA Trace Analyzer

===== 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	Mass conc.:	MC.dev.:	Cal.dev.:	Mass	Add.mass	V0.sample:	Comments
Cysteine	2.873 mg/L	0.121 mg/L (4.22%)	-	28.73 µg	25 µg	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	Mass conc.:	MC.dev.:	Cal.dev.:	Mass	Add.mass	V0.sample:	Comments
Cystine	5.798 mg/L	0.090 mg/L (1.55%)	-	57.98 µg	25 µg	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	

Fig. 2 Example of a determination of cystine and cysteine acc. to method 1 with the 746 VA Trace Analyzer

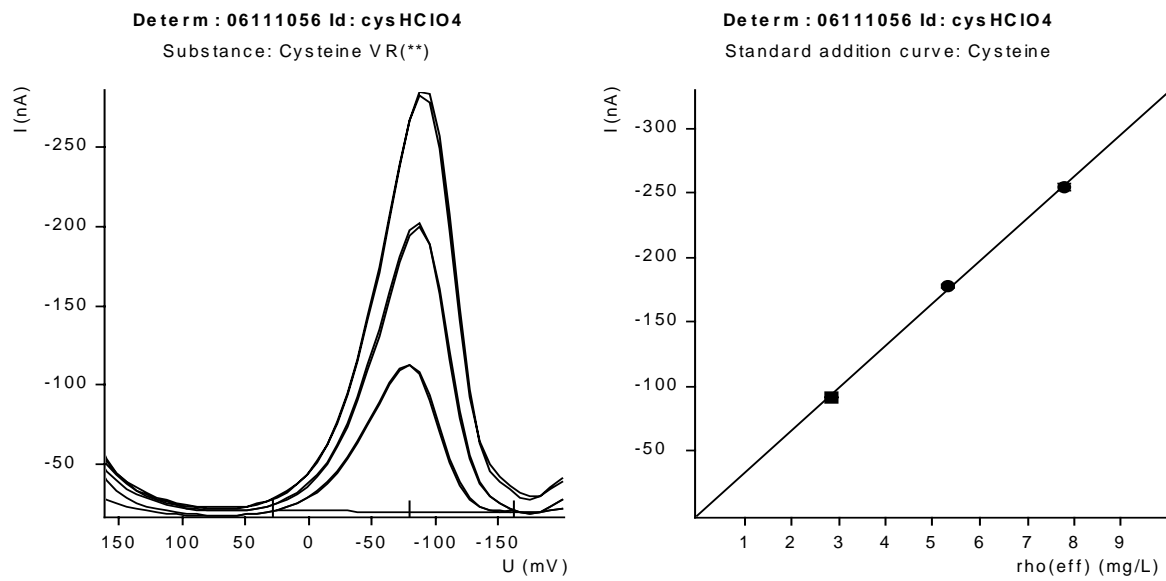


Fig. 3 Example of curves for cysteine

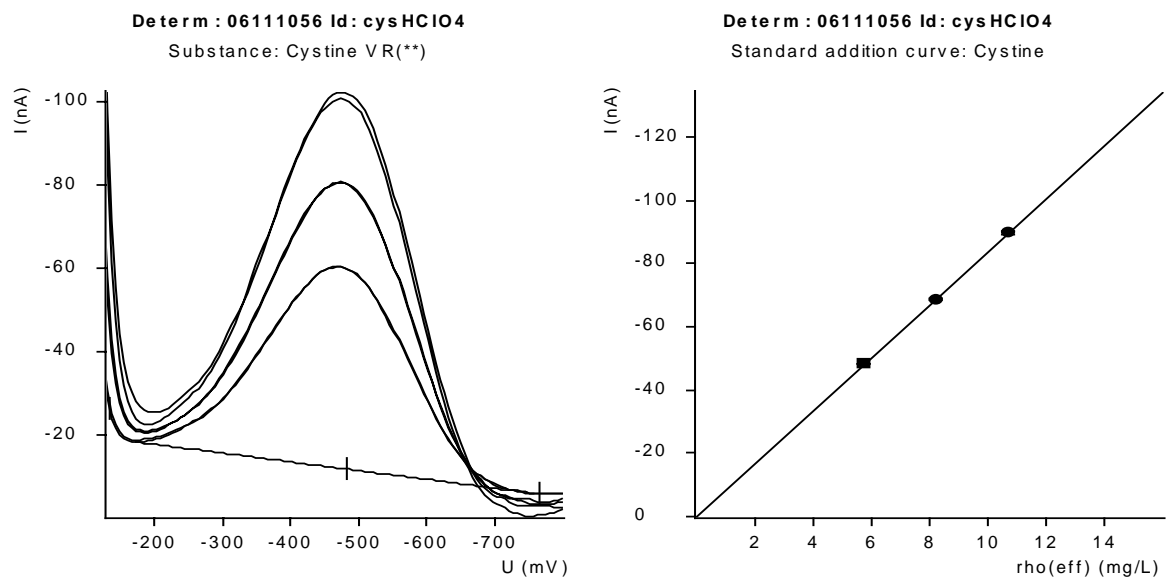


Fig. 4 Example of curves for cysteine

Method 2: Samples exhibiting a high protein content

Summary

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

The determination limit for cysteine is approx. 50 µg/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.

Apparatus and accessories

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

All of the used reagents must be of purest quality possible (p.a. or suprapur). Only high purity water should be used.

- Ammonia solution, suprapur, $w(\text{NH}_3) = 25\%$
- Hydrochloric acid, suprapur, $w(\text{HCl}) = 30\%$
- Cystine, puriss. p.a., CAS 56-89-3
- Cysteine, puriss. p.a., CAS 52-90-4
- Sodium hydroxide, $c(\text{NaOH}) = 2.0 \text{ mol/L}$

Ready to use solutions

- **Supporting electrolyte:**
Ammonium buffer $c(\text{NH}_4\text{Cl}) = 1 \text{ mol/L}$ and $c(\text{NH}_3) = 2 \text{ mol/L}$ (pH 9.6)
 - **Cystine standard solution:** $\beta(\text{cystine}) = 1 \text{ g/L}$
Dissolve 0.100 g cystine in 5 mL sodium hydroxide and fill up to 100 mL with dist. water.
 - **Cysteine standard solution:** $\beta(\text{cysteine}) = 1 \text{ g/L}$
Dissolve 0.100 g cysteine in 20 mL ammonium buffer and fill up to 100 mL with dist. water.
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Analysis

Measuring solution:

- 10 mL (diluted) sample
- + 1 mL supporting electrolyte ($\text{NH}_3/\text{NH}_4\text{Cl}$ buffer pH 9.6)

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

The polarogram is recorded with the following parameters:

working electrode	DME
stirrer speed (rpm)	2000
mode	DP
purge time	300 s
equilibration time	30 s
pulse amplitude	50 mV
start potential	-250 mV
end potential	-1750 mV
voltage step	6 mV
measure time	20 ms
pulse time	40 ms
voltage step time	0.8 s
sweep rate	7.5 mV/s
peak potential cystine	-1000 mV
peak potential cysteine	-500 mV

The concentration is determined by standard addition.

Remarks

- 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.

Figures

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===== METROHM 746 VA TRACE ANALYZER (5.746.0101) =====
Method: AB191_2 .mth          OPERATION SEQUENCE
Title : Det of cystine and cysteine (high protein content)
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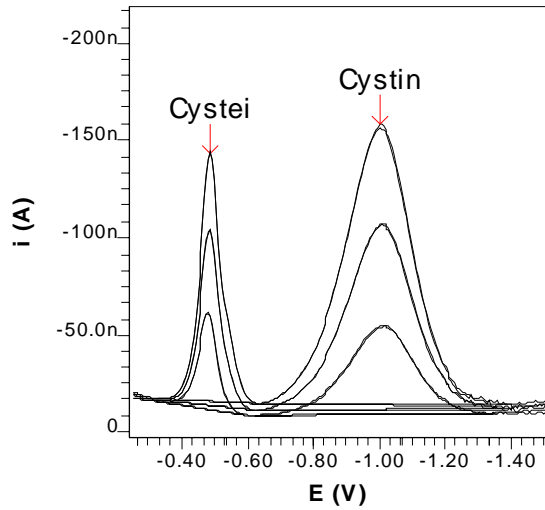
	Instructions	t/s	Main parameters	Auxiliary parameters
1	SMPL>M		V.fraction	mL
2	DOS>M		Soln.name	buffer
3	PURGE			
4	STIR	300.0	Rot.speed	2000 /min
5	(ADD			
6	PURGE			
7	STIR	30.0	Rot.speed	2000 /min
8	0PURGE			
9	0STIR	20.0		
10	(REP			
11	SEGMENT		Segm.name	POL
12	REP)1			
13	PURGE			
14	ADD>M		Soln.name	Cystine
15	ADD>M		Soln.name	Cysteine
16	ADD)2			
17	END			

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Method: AB191_2          SEGMENT
                        POL
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	Instructions	t/s	Main parameters	Auxiliary parameters
1	DME			
2	DPMODE		U.ampl	-50 mV
			t.step	0.80 s
3	SWEEP	201.6	U.start	-250 mV
			U.end	-1750 mV
4	OMEAS		U.standby	mV
5	END			
			t.meas	20.0 ms
			t.pulse	40.0 ms
			U.step	6 mV
			Sweep rate	7.5 mV/s

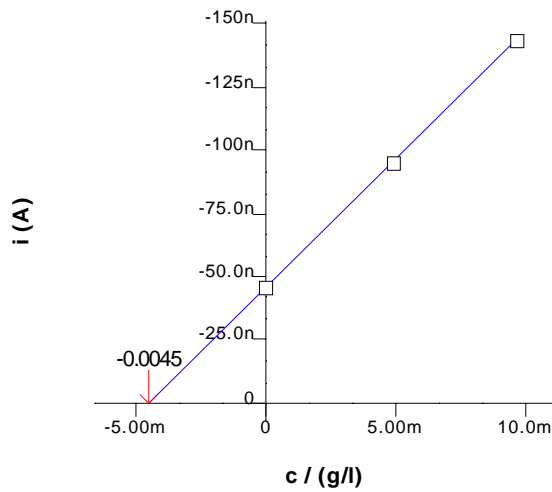
Fig. 5 Method for the determination of cystine and cysteine acc. to method 2 with the 746 VA Trace Analyzer

Bestimmung von Cystin und Cystein
Na-caseinat



Cystin

c = 453.062 mg/l
+/- 5.435 mg/l (1.20%)



Cystei

c = 30.728 mg/l
+/- 0.392 mg/l (1.28%)

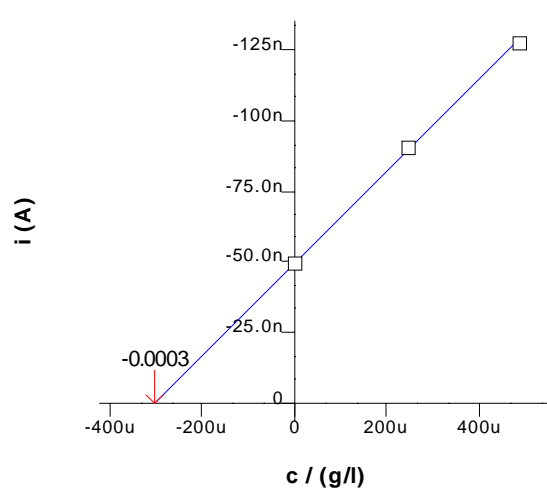


Fig. 6 Example of a determination of cystine and cysteine acc. to method 2 with the 757 VA Computrace