

Determination of Cu(II) in Beer by Derivative Potentiometric Stripping Analysis

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ABSTRACT

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Derivative Potentiometric-Stripping Analysis (dPSA) is described as an inexpensive and rapid method for the determination of Cu(II) in beer. Beer samples were analysed directly after degassing and addition of the analytical reagents (hydrochloric acid, mercury(II) chloride, and potassium metabisulfite). It was not necessary to digest the sample. During dPSA the metal ions are deposited on a glassy carbon-working electrode and then stripped by a suitable oxidant. Quantitative analysis was carried out by the method of standard additions. The recovery of the method was tested by adding 50 $\mu\text{g L}^{-1}$, 75 $\mu\text{g L}^{-1}$ and 100 $\mu\text{g L}^{-1}$ of Cu(II) (as Cu(II) chloride solution) to the beer. The mean recoveries of Cu(II) ranged from 95 \pm 5% to 98 \pm 2%. The reproducibility was evaluated by three repetitive analyses for each sample and the standard deviation ranged from 0.6 $\mu\text{g L}^{-1}$ to 5.5 $\mu\text{g L}^{-1}$. The detection limit was 0.8 $\mu\text{g L}^{-1}$. The Cu(II) concentration determined in seven beer samples ranged from 28 $\mu\text{g L}^{-1}$ to 48 $\mu\text{g L}^{-1}$ and the results obtained were not significantly different from those obtained by atomic absorption spectrophotometry (AAS).

Key words: Beer, copper, derivative potentiometric stripping analysis.

INTRODUCTION

The content of copper in beer is important nutritionally and technologically. Generally, copper comes from the raw materials^{6,17} and metal ions can also be introduced from substances added during brewing, such as hops, acids, bases, silica gel, other additives or stabilisers, dilution water, etc¹⁷. In commercial beer packages, the copper level can be rather high, as it is a contaminant introduced primarily by the containers. For example three-piece welded cans are generally welded with a copper-based metal that can cause elevated concentrations in beer⁷. A concentration of copper greater than 1 mg L^{-1} exerts a catalytic action in the oxidation of beer, leading to irreversible haze, in addition to a coppery, unpleasant, metallic taste¹⁷. Copper levels of 0.15 mg L^{-1} have been re-

ported to cause gushing in beer. Therefore it is recommended²⁴ that brewers not exceed copper concentrations greater than 0.05 mg L^{-1} . For these reasons copper determinations are deemed important and should be included in every beer analysis and a straightforward, fast, sensitive and inexpensive analytical method is required.

Analytical techniques frequently used for determination of trace concentrations of copper are Atomic Absorption Spectroscopy (AAS) techniques, which require previous sample digestion^{1,16}. Sample preparation is a critical and time limiting step in these techniques. The traditional dry ashing procedure used for beers⁵ is time consuming and gives imprecise results, especially for volatile elements². Other procedures for beer based on wet digestion in open vessels, has drawbacks such as risk of loss and contamination of trace elements and burner clogging, in spite of the apparent improvements obtained when H_2O_2 is added. In addition, the acid treatment of beers in a microwave oven is subject to the risk of explosion due to the rapid formation of gases, and to higher detection limits². Recently Viñas et al.²² developed a procedure for determining copper in beer without digestion, with Electrothermal Atomic Absorption Spectrophotometry (ETAAS), and the addition of nitric acid and hydrogen peroxide to the sample. Wyrzykowska et al.²⁵ determined copper and other trace elements in Polish beers with Inductively Coupled Plasma Mass Spectrometry (ICP-MS) after digestion using concentrated HNO_3 in a closed PTFE vessel and the application of microwave energy under pressure.

In this work a new, inexpensive and straightforward method to detect copper in beer by derivative potentiometric stripping analysis (dPSA) is described. PSA is an electroanalytical technique with high detection power due to the deposition (preconcentration) step in which metal ions (free or as labile complexes) in the sample solution are reduced at the negative (cathodic) potential and concentrated onto the working electrode. The deposited metals are measured in a second (stripping) step. This is achieved by the action of an oxidant present in the sample solution¹⁴.

Derivative potentiometric stripping analysis (dPSA), a variant of potentiometric stripping analysis (PSA), is utilised to facilitate evaluation of the analytical signal by using its derivative. Potential (E) and time (t) data are digitally converted into dt/dE, and E is plotted against dt/dE. This enhances the sensitivity of the method and improves resolution. The E vs dt/dE (ms/V) curve obtained exhibits a maximum at the point where the conventional PSA

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curve would show a sharp variation of the potential with time. This technique has been successfully used to determine trace concentrations of metals in matrices such as wine and oil^{12,13,19}. Since 1980 several attempts have been made to determine trace elements in beer using PSA. Jagner et al.¹⁰ were able to determine copper, lead and cadmium in wine but only lead in beer. Moreover Cerutti et al.⁴ determined lead and cadmium directly and nickel and chromium after digestion, but not copper in beer. In this paper the determination of copper in beer was carried out by dPSA without sample digestion, directly after a straightforward sample preparation.

There are difficulties with dPSA analysis when the matrix is complex, as is the case with beer as the presence of electrolytically active substances interfere with the electrode^{3,15}. These compounds are inactivated during sample preparation by the addition of analytical reagents.

Quantitative analysis was carried out by the method of standard additions. The recovery of the method was tested by adding 50 $\mu\text{g L}^{-1}$, 75 $\mu\text{g L}^{-1}$ and 100 $\mu\text{g L}^{-1}$ of Cu(II) (as Cu(II) chloride solution) to the beer. The analytical procedure was automated and the time required to perform one analysis is approximately 20 min. A comparative study of the measurements of Cu(II) ions in beer by two different methods, dPSA without sample digestion, and Atomic Absorption Spectrophotometry (AAS) with wet digestion of the beer, was performed.

MATERIALS AND METHODS

Beers

All samples analysed were commercial lager beers, produced in Italy.

Reagents

All solutions were prepared with analytical reagent grade deionised water.

The dPSA reagents were concentrated hydrochloric acid, potassium metabisulfite and 2-octanol (J.T. Baker, Deventer, Holland), mercury(II) chloride for analysis and standard Cu(II) solution of 5 mg L^{-1} and 10 mg L^{-1} (Steroglass, Perugia, Italy). The AAS reagents were concentrated nitric acid, perchloric acid, and ethyl alcohol (Carlo Erba, Milano, Italia).

Instrumentation and software

Determinations were carried out using a Potentiometric Stripping Analyser, PSA ION³ (Steroglass, Perugia, Italy), connected to an IBM-compatible personal computer. The analyser operated under control of the NEOTES software package (Steroglass, Perugia, Italy). The analytical procedure was completely controlled by this program. A three-electrode system, consisting of a 3 mm diameter glassy carbon working electrode, a platinum wire counter electrode and a [silver/silver chloride//saturated potassium chloride solution] reference electrode, was used for all measurements. The electrochemical cell consisted of a 40 mL vessel fitted with an electrical spiral stirrer.

AAS analysis was carried out using a Perkin Elmer 370 instrument fitted with a graphite oven.

Table I. Analytical parameters for determination of Cu(II) in beer.

	units	Cu
Integration range	mV	-150 -350
Potential range	mV	-600 -50
Conditioning potential	mV	50 \times 5s
Plating potential	mV	-900
Plating time	180	180
Stripping time	s	10
Acquisition final potential	mV	0
Sampling time	μs	300
Discharge potential	mV	-250
Agitation speed	turns/s	2
Cycles		2
Standard additions		2

dPSA sample preparation

A 0.1% (v/v) 2-octanol solution was added to the beer samples to prevent foaming and the beer samples were degassed for 12 min using an ultrasonic bath.

One mL of potassium metabisulfite solution (8 mg/mL) was added to 100 mL of beer. The solution was maintained at room temperature for 15 min, then sonicated for 6 min and analysed immediately.

Electrode preparation (plating)

The working electrode was coated with a thin mercury film by electrolysing a mercury(II) chloride solution containing 1000 mg L^{-1} of mercury(II) ions in 1 M hydrochloric acid at -900 mV against the reference electrode for 60 sec.

The dPSA determination of copper

For subsequent determinations, 5 mL of the beer, obtained as described in the preceding section, was introduced into the electrochemical cell with 15 mL of water, 2 mL of concentrated HCl and 1 mL of a mercury(II) chloride solution equal to that used for plating. The copper concentrations were evaluated with reference to two additions of the working standard solution of 5 mg L^{-1} of copper ions to the solution. Analytical conditions for determination of Cu(II) in beer are reported in Table I.

PSA ION³ cleaning

The cleaning process does not require any particular attention. At the end of each analysis the electrochemical cell and the three electrodes are washed with deionised water. After around twenty analyses, the working electrode is cleaned with ethanol and the electrochemical cell with 10% (v/v) nitric acid solution.

AAS copper determination

The beer was filtered, degassed and 0.1 % (v/v) ethyl alcohol was added. The solution was wet digested using nitric and perchloric acid until the solution was colourless. The wet solution was subjected to AAS.

RESULTS AND DISCUSSION

In Fig.1 the stripping curve relative to the copper determination in beer is reported and the potential E (mV) is plotted vs dt/dE (ms/V). The resulting curve has the form of a Gaussian curve and the peak, symmetrical with re-

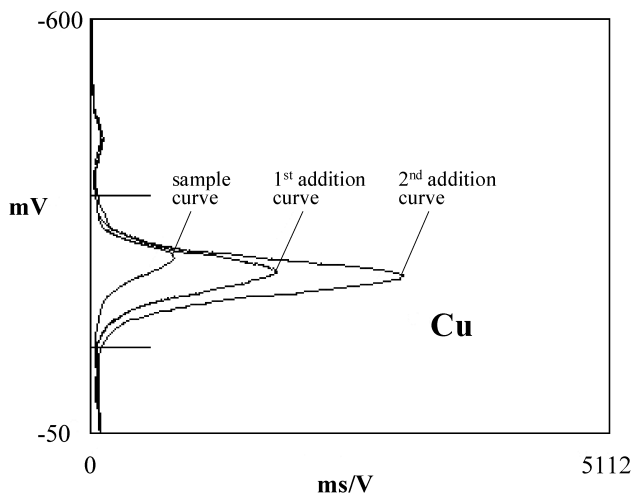


Fig. 1. Stripping curves relative to copper (II) determination in a sample of beer: Copper oxidation potential -250 mV.

spect to the abscissa, has an area normally proportional to the concentration of the analyte. As each element has its specific oxidation potential, the resulting stripping curves do not overlap and interfere. The copper is oxidized at approximately -250 mV.

Initially, difficulties were encountered in determining copper levels in beer. The results were not reproducible and after a short time the mercury film of the working electrode was oxidised. This was probably due to the presence of metal binding proteins. The lager beers contain approximately 4.5 g L^{-1} of beer proteins/polypeptides. The beer polypeptides are distributed over a wide range of relative molecular mass and more than 82.5% are lower than 13000 Da ^{11,21}. They generally originate from barley and are sulphur-rich. Recently Jégou et al.¹¹ purified and characterized the structures of the 9000 Da lipid transfer protein (LTP1) and protein Z, two barley albumins that survive the malting and brewing processes. LTP1 was found to be rich in cysteine and had four disulfide bridges. They became increasingly reduced during malting. Thus malt proteins contain a high level of free thiol groups and oxidative cross-linking of these occur during mashing^{9,20}. Matsui et al.¹⁵ found that beer samples contained disulfide bridges in a much higher quantity than sulphhydryl groups. The disulfide bridges were adsorbed on mercury and electrolytically reduced, forming a surface bound mercuric cysteine thiolate and destroying the mercury coating of the glassy-carbon electrode^{3,8}. The use of potassium metabisulfite solution, during sample preparation, is fundamental for the dPSA without digestion since the disulfide containing compounds are reduced by the potassium metabisulfite solution to sulphhydryl groups²³. The sulphhydryl groups do not interfere with the working electrode.

In Fig. 2 the regression line for a single analysis is shown. The measured peak areas in milliseconds (ms) of 5 mL analysed beer and of the two standard additions are plotted vs total quantity of copper. A straight line was obtained. The Neotes software program determined that the equation of the regression line was $Y = 7.2 \times 10^4 X + 1.4 \times 10^4$; the determination coefficient 99.8 % and the concentration in $\mu\text{g L}^{-1}$. The curve intercepts the X axis at

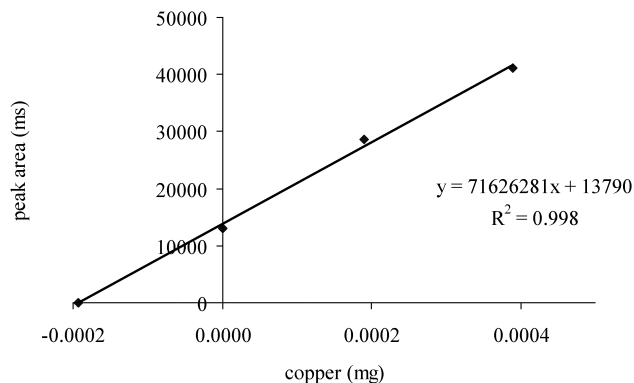


Fig. 2. Regression line for analysis of a beer sample. Beer sample prepared as described in the text (5 mL of the beer, 15 mL of water, 2 mL of HCl conc. and 1 mL of a mercury(II) chloride solution containing 1000 mg L^{-1} of mercury(II) ions in 1 M HCl). The electrolysis time was 180 sec .

the point $(-X, 0)$, where X is the value of the amount of the copper in the sample. The determination coefficient and the equation line confirm a good linearity of the method in the range of concentrations examined.

In dPSA the detection limit depends on the determined element and matrix as well as on electrodeposition time. It is inversely proportional to the electrodeposition time, when no other modification has been made to the procedure²⁰. The theoretical detection limit of the instrument was evaluated using the expression $3\sigma S^{-1}$, where σ is the base noise (setting the peak threshold at 200) and S is the sensitivity obtained from the regression line of each analyte¹³. The copper detection limit value was $0.8 \mu\text{g L}^{-1}$, permitting the detection of very small quantities of the metal.

To determine the recoveries of copper(II), different volumes of a copper solution (10 mg L^{-1}) were added to a sample of beer to increase the concentration ($50, 75$ and $100 \mu\text{g L}^{-1}$). This range of concentrations was selected because it represented the amounts of copper in beers analysed. The results are reported in Table II. The recoveries ranged from 95 to 98% and increased as the amount of copper added was increased. This may be due to a slight matrix effect. Reproducibility of the adopted method was evaluated by analysing each sample three times. The standard deviation ranged from $0.6 \mu\text{g L}^{-1}$ to $5.5 \mu\text{g L}^{-1}$, as shown in Table III.

The results obtained were also compared with those obtained by AAS. The standard deviation of the AAS method ranged from $3.4 \mu\text{g L}^{-1}$ to $9.0 \mu\text{g L}^{-1}$ as confirmed by Bellido-Milla et al. 2000². Table III shows that the standard deviation of the dPSA method was always lower than the AAS method, due to the reproducibility of the dPSA method for trace metal analysis.

Table II. Recovery ($\mu\text{g L}^{-1}$) of copper added to a sample of beer in dPSA.

Original Cu	Added	Found	Recovery (%)
37.1	50.0	82.5 ± 4.8	95 ± 5
37.1	75.0	106.3 ± 4.2	95 ± 4
37.1	100.0	133.8 ± 2.9	98 ± 2

$n = 3$

Table III. Concentration of Cu(II) in beer with dPSA (Derivative Potentiometric Stripping Analysis) and AAS (Atomic Absorption Spectrophotometry)

Sample	dPSA		AAS		P
	$\mu\text{g L}^{-1}$	sd*	$\mu\text{g L}^{-1}$	sd*	
1	31.33 ^(a)	5.5	47.50 ^{(a)**}	6.9	0.06
2	33.91 ^(b)	1.6	44.93 ^{(b)**}	8.9	0.17
3	35.51 ^(c)	1.7	36.99 ^(c)	3.4	0.52
4	36.00 ^(d)	3.5	40.20 ^{(d)**}	7.6	0.44
5	27.91 ^(e)	1.6	30.32 ^(e)	6.1	0.59
6	39.30 ^(f)	0.6	33.80 ^(f)	8.3	0.32
7	48.33 ^(g)	0.7	54.04 ^(g)	9.0	0.39

$n = 3$

Value in the same row followed by the same superscripts are not statistically different ($P < 0.05$)

*Standard deviation

** $n = 2$

A t-test was performed between the two data sets (dPSA vs AAS). The difference in the mean values of the dPSA and AAS results were not great enough to reject the possibility that the difference was due to random sampling variability. This result was found for all samples, therefore there is no statistical difference between the two groups ($P < 0.05$).

CONCLUSIONS

The proposed method provides a sensitive procedure for the determination of trace amounts of copper in beer by dPSA, with a straightforward sample treatment, short time of analysis and without the need to eliminate oxygen. The addition of potassium metabisulfite solution inactivates the protein, thus avoiding the destruction of the coating of the glassy-carbon electrode. The use of potassium metabisulfite solution permits the analysis to be carried out without sample digestion, avoiding metal contamination or loss. The elimination of the digestion step reduces the total time of analysis. The sample preparation and the instrumental determination are rapid, and for these reasons dPSA can be used for the routine analysis of copper in beer. The advantages of dPSA analysis are low cost and the small size of instrumentation and extensive and flexible software support that permits the immediate presentation of results digitally and graphically and allows storage for possible future processing and statistical treatment. Moreover, the standard deviation data of the dPSA method was lower than the AAS method giving more reproducible results. The method is less expensive than AAS and ICP or ICP-MS because of a lower equipment cost and easier maintenance. ICP and ICP-MS methods can easily be automated, but they suffer from higher costs, need for digestion of the sample and require a skilled operator. The dPSA analysis is simple and direct and can be performed directly in the brewing industry laboratory.

As La Pera has confirmed¹² the main advantage of this analytical method is the storing of metals on the working electrode, which allows the recovery of enough quantities, even if the concentration is very low, in the order of $\mu\text{g L}^{-1}$. However, the sensitivity of the method may be improved by increasing the deposition time. Work is in progress towards the determination of lead, zinc and cadmium in beer by dPSA.

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