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ORIGINAL ARTICLE

Quantification of histamine in various fish samples using square wave stripping voltammetric method

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Abstract The objective of this study was to describe a new and simple method for the determination of histamine so that it can be used in routine food analysis. A square wave stripping voltammetric (SWSV) method has been used for the indirect determination of histamine. The method is based on accumulation copper (II) - histamine complex onto a hanging mercury drop electrode and reduction of complex. The optimum conditions include an accumulation potential of −420 mV (versus Ag/AgCl), an accumulation time of 10 s. Two linear calibration graphs were obtained with slopes of 0.078 (μM/μA) and 0.014 (μM/μA), respectively. The detection limits were found to be 3×10^{-7} and 1×10^{-5} M for histamine (S/N = 3), respectively. The validated SWSV method showed good linearity as well as satisfactory repeatability and immediate precision values, for both instrument and method. The effect of common excipients and metal ions on the peak height of Cu-histamine complex peak was studied. The method was successfully, applied to the determination of histamine in canned anchovy *(Engraulis encrasicholus),* frozen *Tinca tinca (L.)* and *Cyprinus carpio* fish samples.

Keywords Determination · Voltammetry · Fish · Histamine · Trace · Food poisoning

Research highlights
✓ The new method was developed for the determination of histamine in routine food analysis using square-wave stripping voltammetry.
✓ Validation of an electrochemical analytical methodology to check fish quality and health safety.
✓ Determination of histamine in canned anchovy, frozen tinca tinca and cyprinus carpio fish samples

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Introduction

Histamine [1H-imidazole-4-ethanamine], is the most important biogenic amine which has been implicated as the causative agent in several outbreaks of food poisoning. Histamine is involved in the regulation of important neurophysiological functions, such as locomotor activity, sleep–wake cycle, attention, cognition, memory, and stress responses (Yoshitake et al. 2003). Changes in brain histamine levels have been observed in many neurological disorders, including multiple sclerosis (Jadidi-Niaragh and Mirshafiey 2010), Alzheimer's disease (Zhang et al. 2011) and febrile convulsions (Kiviranta et al. 1995). However, histamine is produced by bacterial action during food processing and storage and it can be present in substantial amounts in fermented foodstuffs and beverages. Therefore, The presence of histamine in foods is of great importance as it acts as an indicator of the state of deterioration of the product and is thus a potential public health hazard (Ladero et al. 2008). Frequently regarded as one of the biomarkers in quality control monitoring during the food production, storage and transportation. Accordingly, there is considerable interest in the determination of histamine in foods and beverages. Because of its potential risk in human health, US Food and Drug Administration (FDA) have set limits of 500 and 50 mg kg^{-1} as toxic and caution levels of histamine in fishes, respectively (U.S. FDA 2001).

Several analytical methods for the determination of histamine have been reported, involving separation techniques such as capillary electrophoresis (Ruiz-Jimenez and Luque de Castro. 2006; Simo et al. 2008; Nevado Berzas et al. 2011), gas chromatography (Cunha et al. 2011; Yoshida et al. 2012; Hwang et al. 2003), thin-layer chromatography (Romano et al. 2012; Bozina et al.

1999) and high-pressure liquid chromatography (Bueno-Solano et al. 2012; Yoshitake et al. 2012; Kose et al. 2011) using different kinds of detection.

Despite the use of electroanalytical methods for the determination of biogenic amine in general (Łuczak and Bełtowska-Brzezinska 2013; Di Fusco et al. 2011; Alonso-Lomillo et al. 2010; Chang et al. 2012; Muresan et al. 2008; Kuklinski et al. 2010), only a few studies are available for the voltammetric determination of histamine (Akbari-Adergani et al. 2010, 2012; Kim et al. 2001) and, as far as we know, no work dealing with indirect determination of histamine.

Voltammetric methods have some advantages compared with the listed techniques chromatography, i. e., their low cost and the possibility of analysis without extraction or pre-concentration, as well as the short time required for analysis. The objective of this work was to examine the electrochemical behavior of histamine and develop a suitable square-wave voltammetric method for its quantification in various fish samples. These data together with the high recoveries in the presence of ten times amounts of co-existing ions, reflect the high accuracy and precision of the proposed method.

Experimental

Apparatus

A BAS model electrochemical analyzer (Bioanalytical Systems, Epsilon Basic Plus Potentiostat/Galvanostat, USA) was used for square wave stripping voltammetry (SWSV) and cyclic voltammetry (CV) measurements. A three electrode system was used, consisting of a platinum counter electrode, an Ag/AgCl (3 M NaCl) reference electrode and a working hanging mercury drop electrode (HMDE) as a working electrode. pH values were measured using a Thermo Scientific Orion 4-Star Plus pH/Conductivity Meter pH meter with combined glass electrode was used to measure pH of all the solutions.

Reagents

Histamine dihydrochloride was purchased from Ridel-de Haen (Merck, Darmstandt, Germany) and a primary stock solution of 1×10^{-2} M histamine was prepared in distilled water. Working solutions were then prepared by diluting the stock solution with distilled water and storing in the dark at 4 °C. Salts used for the supporting electrolyte, solvents and other reagents were of analytical reagent grade (Merck, Darmstandt, Germany). Britton-Robinson buffer (BR buffer) solutions were prepared from a stock solution containing 0.04 M phosphoric, boric and acetic acids (Sigma- Aldrich, Germany) by adding 4 M NaOH to obtain pH values ranging from 2 to 13.

Sample collection

The origin of the frozen samples was the central market of Nevsehir (Turkey) and the canned species were purchased in some of the major supermarkets in the city. All samples, with the exception of canned products, were stored in a freezer at −25 °C before analysis, that was performed in less than 9 weeks.

Sample preparation

A simple, green and efficient method was considered for extraction of histamine from various fish samples which was compatible with the proposed electrochemical detection (Akbari-Adergani et al. 2010). According to this method, a precisely weighted 10 g fish samples was finely homogenized using a domestic blender. Five grams of previously homogenized various fish samples were weighed out into test tube and 40 mL of distilled water added; the mixture was vortex, stirred for 5 min and then placed in an ultrasound bath and sonicated for 20 min. The resulted mixture then centrifuged the supernatant was then drawn off and filtered through a Whatman filter into a 50 mL flask. The resulting suspension was filtered through filter paper. Appropriate volumes of this fish samples were transferred into the voltammetric flask and diluted up to the volume with 10 mL BR buffer at pH 10.0.

Voltammetric procedure

An accurate volume of 10.0 mL of the pH 10 BR buffer was transferred to the voltammetric cell. Afterward, the electrodes were put in the solutions through which pure nitrogen gas was passed for 15 min before obtaining the voltammograms. After recording the voltammogram of the blank solution, an accurate concentration Cu (II) ions and histamine solution was added, respectively. The complex of copper(II) and histamine gives a well defined cathodic stripping peak current at −420 mV. Quantifications were performed from the peak height being measured by successively additions. The accumulation potentials from +100 to −250 mV were applied during the accumulation periods from 0.0 to 80 s. under stirring at 100 rpm. The stirring is stopped, and after waiting for 5 s equilibrium period, the square wave voltammogram was obtained by making a negative potential scan.

Optimization of instrumental conditions

The peak intensities quietly depend on the square wave voltammetric parameters. To achieve the maximum amount of square wave anodic peak, frequency (f), amplitude (ΔE), and staircase step potential (ΔEs) parameters have been optimized. The first parameter to be optimised for the indirect

determination of histamine employing SWSV was accumulation potential (E_{acc}) and time (t_{acc}). The relationship between the reduction current of the Cu-Hist complexes and the accumulation potential is shown in Fig. 1a. The dependence of the stripping peak current on the accumulation potential was studied over the range +150 to −250 mV and highest peak intensity belong to Cu-Hist complex was obtained for an E_{acc} of 0.00 mV. Maximum complex peak current was recorded at 10 s (Fig. 1b). The optimal accumulation potential and time of 0.00 mV and 10 s were chosen respectively, because the well-defined peak shape and maximum developed peak current were achieved. The peak current increased with the increments of frequency from 25 to 200 Hz, but the peak becomes ill-defined closer to 35 Hz (Fig. 1c). The peak response increased linearly with the step potential up to 4 mV. Higher peak currents were observed when the pulse amplitude was increased from 10 to 75 mV, but the background current also increased (Fig. 1d). The optimum conditions selected for the SW voltammetric indirect determination of histamine were pH 10 BR buffer as supporting electrolyte, ΔEs=4 mV, f=35 Hz, ΔE=50 mV, tacc=10 s and Eacc=0.0 mV owing to the best current responses.

Results and discussion

Preliminary experiments and effect of pH on the peak current

For the basic study of the electrochemical behavior of histamine, square-wave stripping voltammetric (SWSV) at a static hanging mercury drop electrode (SHMDE) responses were examined. The square-wave stripping voltamograms (SWSV) of histamine were taken in 0.1 M $H_2PO_4^-$ - HPO_4^{-2} (pH 6–7), in 0.02 M SO_4^{2-} (pH 2.0–10.0), pH 6–8 acetate electrolyte and pH 2–13 B–R buffer. The peak of histamine was not observed in the acidic solutions. The voltammetric currents of histamine obtained in 0.02 M SO_4^{2-} electrolyte were very weak. The B–R buffer was chosen for its wide pH range applicability. According to this study, peak of histamine was not observed until pH 10.0. The cathodic response obtained at pH 10.0 Britton-Robinson (BR) buffer solution was observed, had a peak potential at −394 mV (versus Ag/AgCl). But this peak did not show proportional increments to standard additions. Therefore intended to indirect determination of histamine.

As can be seen from Fig. 2, at pH 10 BR buffer Cu(II) ion had one peak at −156 mV. When histamine was added copper

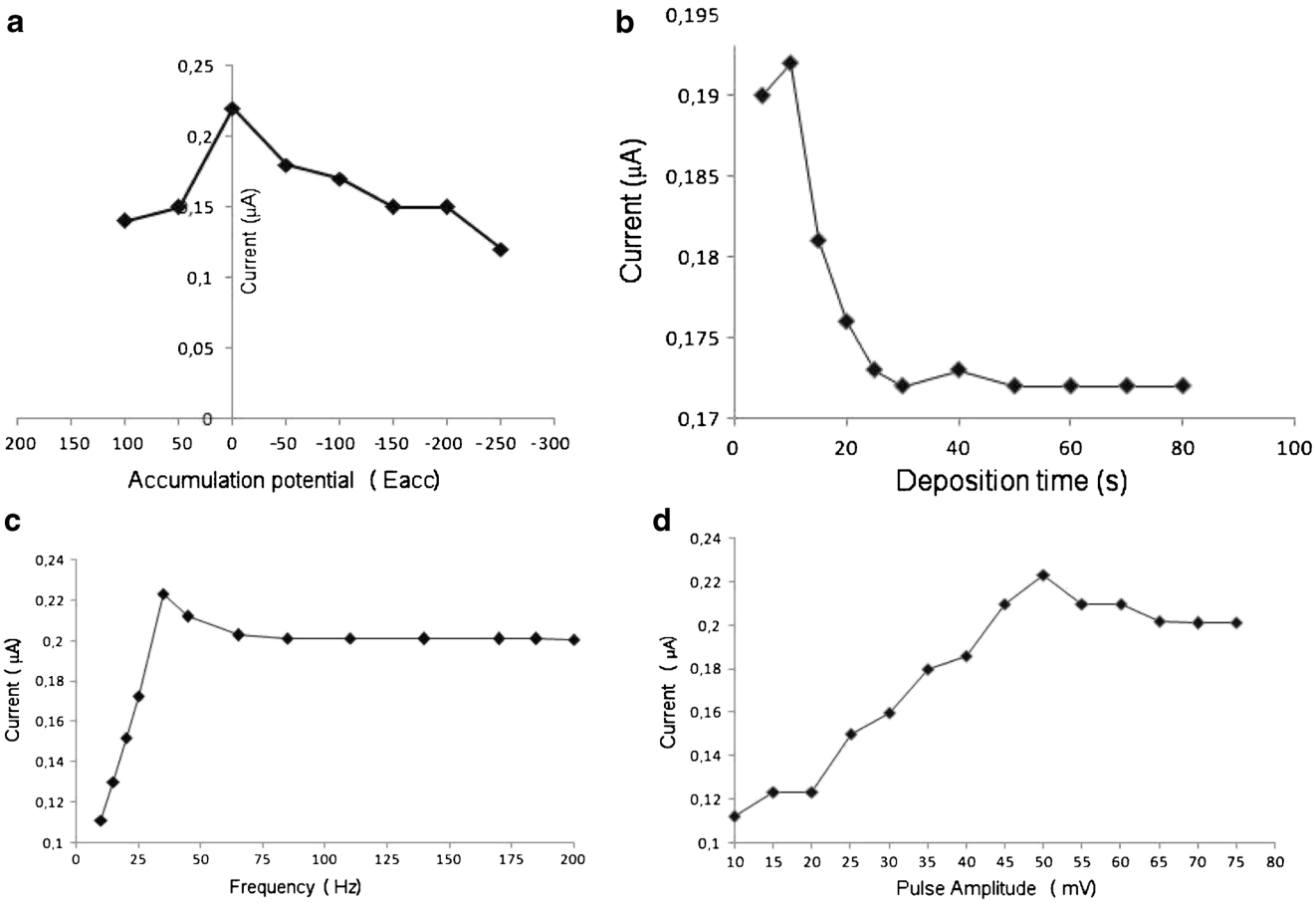

Fig 1 **a** Effect of accumulation potential (Eacc) on the peak current. **b** Effect of deposition time on the peak current. **c** Effect of frequency on the peak current **d** Effect of pulse amplitude on the peak current

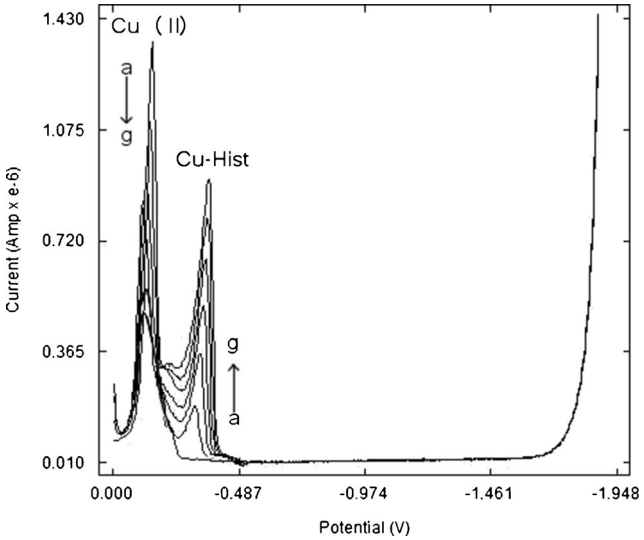

Fig. 2 The formation of an complex compound formed between histamine and the Cu(II) ions. a) 10 mL pH 10 BR and 10 μM Cu(II) ions b) a+0.1 mL 10^{-4} M Histamine (Hist) c) b+0.1 mL 10^{-4} M Hist d) c+0.1 mL 10^{-4} M Hist e) d+0.1 mL 10^{-4} M Hist f) e+0.1 mL 10^{-4} M Histamine g) f+0.1 mL 10^{-4} M Histamine

peak at −156 mV decreased and a new peak appeared at more negative potential (−420 mV) and this peak increased with the standard additions of histamine. Continuous additions of histamine, the copper peak at −156 mV decreased. The new peak formation at −420 mV was attributed to the formation of an complex compound (Mikulski et al. 2012), formed between histamine and the Cu(II) ions (Fig. 2). This procedure was repeated at pH 4, 6, 8, 10, 12 BR buffer. A series of supporting electrolytes were tested (acetate, Britton Robinson and phosphate buffers). Both the peak height and the peak shape were taken into consideration when choosing the supporting electrolyte. Of these, BR buffer gave the best response.

Stability of the complex largely depends on the pH of the system. A system may become unstable with a small variation in the pH. So far the optimization of a stable complex of Cu–histamine was concerned, a large variation in pH values, from 2 to 12 was studied. Reduction peak of the complex was observed only at pH 8 and 10. At pH more than 10.0, the precipitation of copper as $Cu(OH)_2$ occurred resulting complex peak was not observed. The voltammetric reduction corresponding to the Cu(II)–histamine complex peak at pH 10 (BR buffer solution) showed quantitative increments with the additions of standard histamine solution under the optimal conditions (Fig. 2). Thus, it was decided to use the complex peak for the indirect determination of histamine.

Effect of Cu(II) concentration

The effect of concentration of Cu(II) ions on the peak current (Fig. 3) was studied (Abu-Zuhri et al. 1999; Mahajan et al. 2005) at pH 10.0 for the range 1.0×10^{-5} to 7.0×10^{-5} M. The peak current of the histamine-copper complex increased when the concentration of copper was increased in Fig. 3. At a higher concentration of copper (4.0×10^{-5} M), however, the peak height of the complex remained unchanged. So an increase in peak current up to 4×10^{-5} M and then leveled off, was observed. At the break point (4×10^{-5} M), the Cu(II) concentration is equal to histamine concentration (4×10^{-5} M). The change in concentration of Cu (II) ion showed that the complex formed had a stoichiometry of 1:1 (copper: histamine). This was confirmed by changing the concentration of histamine, with constant concentration of Cu(II) ion.

Calibration, accuracy, precision and detection limits

The voltammetric method used to detect and quantify the histamine was validated for linearity, precision (assays performed for repeatability and intermediate precision) and accuracy (absolute recovery study).

According to the afore-mentioned results, the optimum conditions for the analytical determination of histamine by square wave voltammetry at HMDE are the following: pH 10.0, accumulation time 10 s, accumulation potential 0.0 V

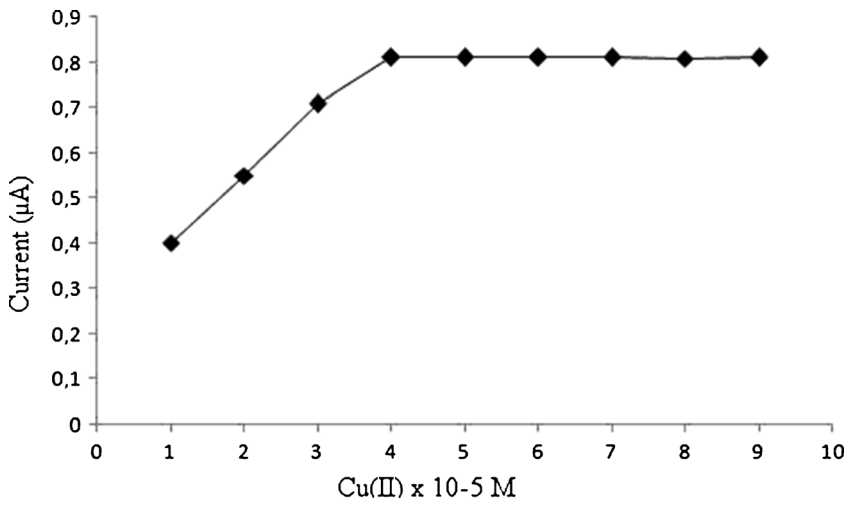

Fig 3 Effect of Cu(II) ion concentration. Accumulation for 10 s at 0.0 mV in pH 10 BR buffer containing 4.0×10^{-5} M histamine

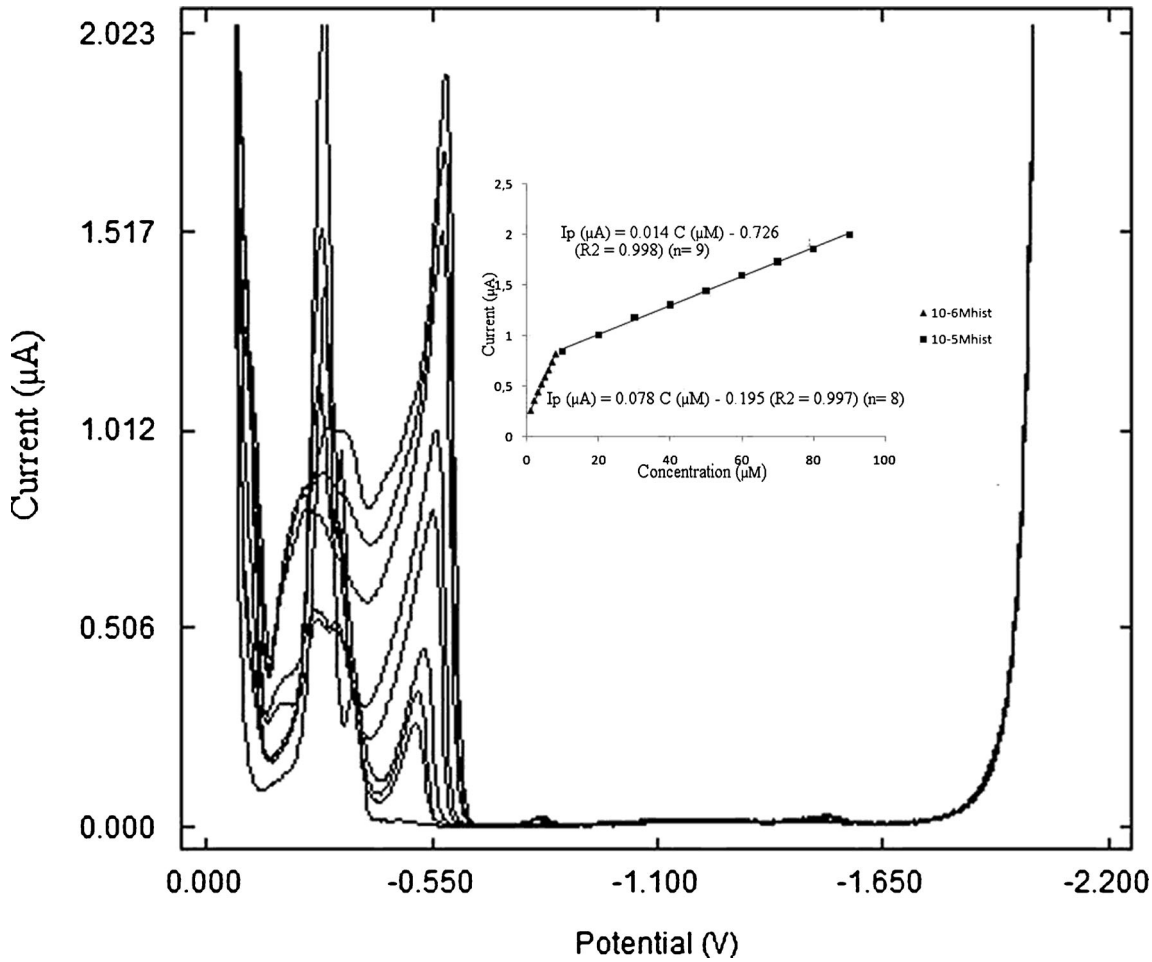

Fig 4 SWS voltammograms of different concentrations of histamine and plot of I_p vs. $C_{histamine}$. Conditions: Cu(II) 200 μM; 10 mL pH 10 BR; E_{acc} 0.0 mV; t_{acc} 10 s; ΔEs 4 mV; f=35 Hz; ΔE=50 mV. (a) 200 μM Cu(II) (b) 1 μM (c) 2 μM (d) 3 μM (e) 10 μM (f) 20 μM (g) 50 μM (h) 70 μM (i) 90 μM

in the presence of 200 μM Cu(II) ions, respectively. Under the above conditions, the dependence of the height of the complex reduction peak on the concentration of histamine is linear. Two linear calibration graphs were obtained with slopes of 0.078 (μM/μA) and 0.014 (μM/μA), respectively (Fig. 4). A good linear calibration curve was constructed for the first linear section in the range from 1 to 8 μM with the analytical equation given by:

$$I_p(\mu A) = 0.078\ C(\mu M) - 0.195\left(R^2 = 0.997\right)(n = 8)$$

The second linear section in the range from 30 to 90 μM with the analytical equation given by:

$$I_p(\mu A) = 0.014\ C(\mu M) - 0.726\left(R^2 = 0.998\right)(n = 9)$$

The detection limits were found to be 3×10^{-7} and 1×10^{-5} M for histamine (S/N=3), respectively. The reproducibility of the proposed method was checked by five successive measurements on 1×10^{-6} M histamine. The average relative standard deviation (RSD) of 2 % was obtained.

Interference effect

The selectivity of the proposed method for histamine was investigated in the presence of some inorganic ions found mostly in fish samples. The influence of some electroinactive ions such as Ca(II), Al(III) or electroactive ions such

Table 1 Degree of recovery of histamine (1 μM) in the presence of interfering species (10 μM) using the optimized conditions

Interfering species	Recovery (%)	Interfering species	Recovery (%)
Co^{+2}	97	Zn^{+2}	96
Cr^{+3}	94	Cd^{+2}	100
Fe^{+3}	97	Mn^{+2}	100
Hg^{+2}	96	Al^{+3}	103
Ni^{+2}	100	Ba^{+2}	105
NO_2^-	94	Ca^{+2}	93
Se^{+4}	100	Mg^{+2}	94
SO_3^{-2}	104	NO_3^-	93
Pb^{+2}	104	Cl^-	100

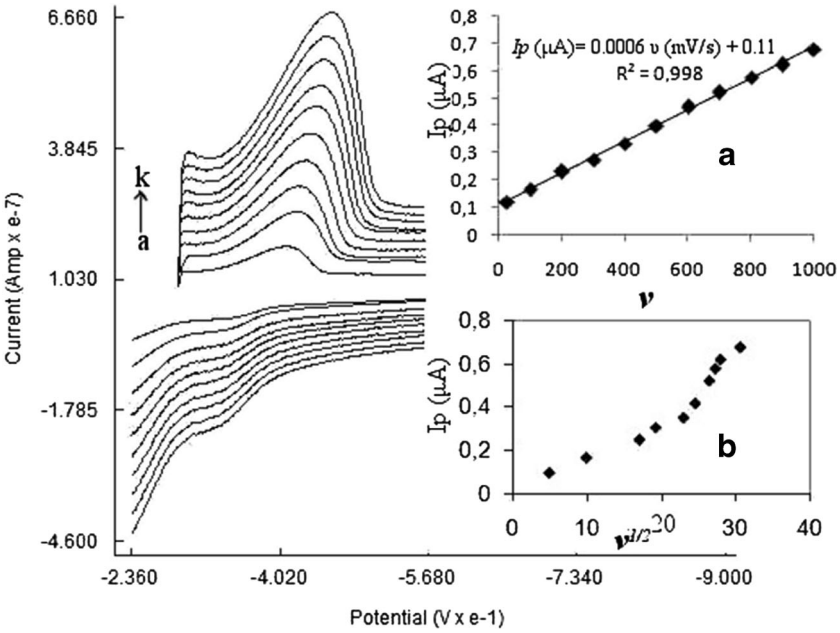

Fig 5 Cyclic voltammogram of 6 μM histamine in 10 mL pH 10 BR and 20 μM Cu(II) solution with different scan rates, between 100 and 1000 mV s^{-1}. a) 100, b) 200, c) 300, d) 400, e) 500, f) 600, g) 700, h) 800, j) 900 and k) 1000 mV s^{-1} (*A*) Peak current (Ip; μA) versus scan rate (υ; mVs-1) for 6 μM histamine (*B*) Peak current (Ip; μA) versus square root of scan rate ($\upsilon^{1/2}$) for 6 μM histamine

as Co(II), Ni(II) on the assay of histamine was evaluated by the proposed analysis method. The degree of interference effects was treated as the recoveries of 1 μM histamine in the presence of 10 times larger concentration of interfering ions. The degree of interference effects were evaluated by comparing the assay results in the presence of the interfering compounds to that in their absence (Table 1). They did not show serious interfering effects on the determination of histamine. The recovery of histamine in the presence of interfering species showed that the degree of the peak current did not deviate by more than ±6 %, correting that the improved Square Wave Voltammetric method is free from serious interferences and for this reason can be suggested as a selective method (Švarc-Gajic and Stojanovic 2011).

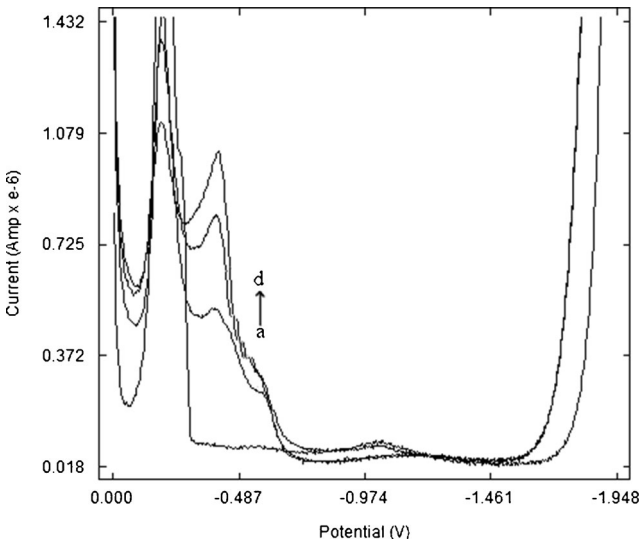

Fig 6 Determination of histamine in canned anchovy *(Engraulis encrasicholus)*. (a) BR buffer 10.0 mL, pH 10.0+0.5 mL 10^{-2} M Cu(II) ions, (b) a+0.1 mL 10^{-3} M histamine (c) b+0.1 mL 10^{-3} M histamine

Cyclic voltammetry

Cyclic voltammetry is the most convenient ones for clarifying the oxidation– reduction behavior of the organic compounds. Therefore cyclic voltammetric records resulting from the oxidation – reduction properties of electrochemically active compounds might have marker effects on the comprehension of the redox mechanism. In cyclic voltammetric studies a single well defined reduction peak was observed at a potential of about −430 mV (vs. Ag/AgCl) at pH 10.0 and peak intensity increases linearly with increasing concentration of histamine, showing that this reduction peak is due to the reduction of Cu-Histamine complex.

We have recorded the voltammograms of a solution containing 6 μM of histamine, 20 μM Cu(II) solution in pH 10 BR with different scan rates, between 25 and 1000 mV s^{-1}. The scan rate increased the peak potential (Ep) shifted in the

Table 2 Determination of histamine in canned anchovy *(Engraulis encrasicholus)*, frozen *Tinca tinca* and *Cyprinus carpio* fish samples

Fish samples	[a] $\overline{X}$ (mg histamine/100 g fish)	s	$\overline{X} \pm ts/\sqrt{N}$	% RSD
Canned anchovy *(Engraulis encrasicholus),*	50.1	3,5	50.1±3.2	7
Frozen *Tinca tinca (L.)*	18.1	1.5	18.1±1.4	8
Frozen *Cyprinus carpio*	41.2	2.2	41.2±2.0	5

[a] 90 % confidence *interval* (*N*=5)

negative direction (Fig. 5). This behavior shows the irreversible character of electrode reaction.

A potential scan rate was carried out to assess whether the processes diffusion or adsorption controlled. The dependence of peak current (I_p) on scan rate (ν) was studied in the range 25–1000 mVs^{-1}. While a linear relationship was observed between peak current Ip (μA) and scan rate ν ($mV\ s^{-1}$) (Fig. 5, inset A), a linear relationship was not observed between peak current (I_p) and square root of the scan rate ($\nu^{1/2}$) (Fig. 5, inset B). The linear relationship between peak current and scan rate confirms an adsorption controlled mechanism (Lund and Hammerich 2001). Also a plot of the logarithm of the peak current versus the logarithm of the scan rate ($mV\ s^{-1}$) was studied. This relationship was found to be linear with a slope of 0.604. This value being close to that theoretically expected (0.5) which has been accepted for an diffusion-controlled electrode process (Laviron 1980). Therefore, some extra studies were carried out to understand whether the electrode process is diffusion or adsorption controlled according to literature (Wopschall and Shain 1967).

As a result, it is found that the value of the ratio of cathodic peak current to concentration (Ip/C) decreases with increasing concentration and value of the ratio of cathodic peak current to multiplication of concentration and square root of scan rate ($Ip/C, \upsilon^{1/2}$) increases with increasing scan rate. Results of all these experimental investigations suggest that electroreduction of complex molecules on the hanging mercury drop electrode (HMDE) is mainly controlled by diffusion with some adsorption contribution.

Determination of histamine in various fish samples

In order to investigate the analytical potential of the proposed method was applied for the determination of histamine in canned anchovy *(Engraulis encrasicholus),* frozen *Tinca tinca (L.)* and *Cyprinus carpio* fish samples. For the determination of histamine in 0.1 mL fish samples was added into a voltammetric cell containing 10.0 mL pH=10.0 BR buffer, 5×10^{-4} M Cu(II) ions and voltammogram was taken (Fig. 6). As can be seen from Fig. 6 histamine in various fish samples was determined by standard additions (Table 2). The considerably low standard deviations provided evidence for the high accuracy and precision of the recommended square wave voltammetric method. SWSV method offers high sensitivity, low limit of determination, easy operation, and simple instrumentation.

Conclusions

A fast, very sensitive and quite inexpensive electroanalytical method for the indirect determination of histamine was assessed and validated. Also the electrochemical behavior of histamine was investigated at HMDE using cyclic voltammetry. The reduction peak belong to Cu-Histamine complex was appeared at −420 mV and quantifications were performed on the basis of this peak by successive standard additions. The recommended voltammetric method was successfully applied to various fish samples. The sufficiently low standard deviations for the data reflect the high accuracy and precision of proposed square stripping voltammetric method. This method has some other advantages such as the low costand possibility of analysis without the need of extraction or pretreatment, as well as the short time required for analysis.

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