

Electrochemical Sensor for Histamine Determination Based on Zinc Oxide Thin Films Electrodeposited on Carbon Paste Electrodes

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Abstract: Histamine, a biogenic amine and important mediator of inflammation was quantitatively analysed using electrochemical techniques. The sensor designed in this context based on carbon paste (CP) as electrode material and zinc oxide (ZnO) as modifier to increase the selectivity, due to the strong complex building tendency of the imidazole ring to metals. Using an alkaline electrolyte medium the oxidation potential was shifted from + 1.20 V in neutral medium to a less positive value of + 0.750 V vs. Ag/AgCl. A logarithmic correlation for a concentration range from 5 to 500 µg/mL could be found. The developed ZnO-CP based sensor, characterized by a precision (R.S.D.) of 5.8 % and an accuracy of 5.2 % was successfully tested in complex food matrix.

Keywords: Histamine; Carbon paste electrode; Zinc oxide; Electrodeposition.

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Introduction

Histamine (2-(1H-Imidazol-4yl)ethanamine) is an paracrine hormone of the body with a heterocyclic diamine structure. It is involved in numerous physiological processes like the regulation of sleep-wake cycles, control of cardiovascular functions and gastric juice

secretion. Beside this histamine also acts as neurotransmitter in the central nervous system [1] and is an important mediator of inflammation. According to its rapid and rather aggressive effect on the microcirculation, smooth muscle cells and on pain receptors it can lead, concentration dependent, to different pathophysiological symptoms of inflammatory [2]. In this context the role of histamine in allergy should be emphasized.

Histamine is synthesized by an enzymatically catalyzed decarboxylation of the amino acid histidine [3], in the human body but also, catalyzed by bacteria, in different food [4]. Therefore histamine can also be delivered externally to the body by consumption of histamine rich food. The concentration of histamine increases in some groceries, like for example cheese or wine constantly with the ripening time. Therefore histamine may in some cases be used as a marker for food quality. As for example microorganism can grow rapidly in raw fish or meat according to the high amount of water in this tissue histamine but also other biogenic amines like cadaverine, putrescin or tyramine are enriched [5,6]. Because the concentrations of histamine found in low quality fish and meat may lead to health problems or even to intoxication (> 50 mg/100 g foodstuff [7]) freshness control concerning those is regulated by law.

Tough histamine food poisoning is quite rare, its supervision and quantitative control has gained importance. An increase of histamine intolerance symptoms provoked by the consumption of histamine rich food combined with a decreased reactivity of its gut degradation enzyme diamine oxidase was observed in the last years. The subsequent accumulation of histamine and its prolonged impact time may induce pseudo allergic reactions like diarrhea, rash, heart palpitation and others [8]. Therefore a specification according the presence of histamine and its concentration in food becomes even more important.

In this context some methods are suggested in literature. The most common method for the determination of histamine is HPLC analysis using a fluorimetric detection system [9,10,11]. Pre- or postcolumn derivatisation is done using *o*-phthalaldehyde or dansyl chloride. Other possible analytical techniques are gas chromatography [12], capillary electrophoresis [13] or ELISA [14]. These methods may be very well approved but they are, according to the derivatisation process, rather time consuming and require high priced equipment.

The most of published electrochemical methods are based on enzyme modification. These biosensors incorporate natural histamine degradation enzymes like amine oxidase [15], histamine oxidase [16], diamine oxidase [17] or methylamine dehydrogenase [18]. With

exception of Zeng et al., who use the enzyme methylamine dehydrogenase to form electron transfer reactions mediated by ferricyanide, all other biosensors are based on the production of hydrogen peroxide during the enzymatic conversion of histamine to imidazole acetaldehyde. The modification steps for the hydrogen peroxide detection vary. For the investigations without biological component mechanisms and electrode materials show a broad spectrum. Experiments using thin film mercury electrodes [19], boron doped diamond electrodes [20], glassy carbon [21, 22], and carbon paste electrode [23] or carbon nanofibre [24] are described. Unfortunately, these references show either a lack of selectivity due to a affinity towards other biogenic amines or limited application because of high working potentials.

The design of a selective but facile sensor system for the determination of histamine would help to simplify the supervision of food quality and the diagnostic of inflammatory diseases. Therefore this work focuses on the design of an electrochemical pathway for the analysis of histamine to be used in food quality management. Carbon paste was used as electrode material to form the cheapest and easiest to handle foundation. Using metal modifiers, selectivity towards other amines was aspired.

Experimental

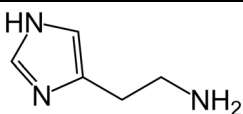
Chemicals and Materials

The electrode material consisted of spectral carbon powder purchased from Ringsdorff-Werke GmbH (RW-B type, Bad Godesberg, Germany) and paraffin oil Uvasol® from Merck KGaA (Vienna, Austria) as binding agent.

Histamine dihydrochloride standard (Table I) was ordered from Sigma-Aldrich GmbH (Vienna, Austria) as well as zinc chloride (ZnCl_2) for the electrodeposition solution. Potassium chloride (KCl), used as electrolyte during the electrodeposition procedure, was purchased from Merck KGaA (Vienna, Austria). *o*-Phthalaldehyde (OPA) for the precolumn derivatisation has been purchased from Sigma-Aldrich.

Supporting electrolytes for the cyclic voltammetric measurements were $\text{Na}_2\text{HPO}_4/\text{NaOH}$; pH 12 or phosphate buffer $\text{Na}_2\text{HPO}_4/\text{NaH}_2\text{PO}_4$; pH 7 both with concentrations of 0.1 M.

Table I: Characteristics of the histamine molecule.

| IUPAC name | Molecular formula | Molar Mass | Structure |
|--------------------------------|--|----------------------------|---|
| 2-(1H-imidazol-4-yl)ethanamine | C ₅ H ₉ N ₃ | 111.15 g mol ⁻¹ |  |

The solution for the film electrodeposition consisted of 1 M KCl and 1.5 M ZnCl₂ aqueous solution. Histamine stock solution as well as all solutions used for the interference tests were prepared freshly before measurement using water as solvent. The water therefore was deionized and purified using a Millipore® water purification system.

For the extraction of the cheese samples phosphate buffer saline (PBS) was used: NaCl (140 mM), Na₂HPO₄ (10 mM), KCl (2.7 mM), KH₂PO₄ (1.8 mM).

Apparatus

Cyclic voltammetry (CV) as well as differential pulse voltammetry (DPV) measurements were performed on a 797 VA Computrace (Metrohm AG, Herisau, Switzerland) in a three electrode arrangement. The system was equipped with a carbon paste (CP) working electrode, a Ag/AgCl (3M KCl) reference and a platinum auxiliary electrode. The potential range for the CV was from 0.00 to +1.50 V. Scan rate of 50 mV/s was used. For the DPV experiments a pulse time of 0.10 s, a voltage step of 6 mV and a voltage step time of 0.40 s measurements were performed. The explored potential window was between 0.00 and +1.0 V.

Construction of CP Electrode and Electrodeposition Procedure

For the preparation of the CP, spectral carbon powder and binding agent were mixed in a ratio of 7:3 (m/m) in a mortar and stirred to produce a smooth paste. A portion of this paste was transferred into a special Teflon holder and smoothed on a paper sheet to prepare a plane surface 1.5 mm in diameter.

The thin film deposition was performed following the instructions of Li et.al. [25]. To modify the pure CP electrode with a thin film layer of ZnO, the system was dipped into an aqueous solution of ZnCl₂ (1.5 M) and KCl (1 M) blended in ration of 3:5 (V/V). Using CV technique at cathodic potentials from -0.70 to -1.00 V over 30 cycles a layer of ZnO was

formed among the sensing area (ZnO-CP based sensor). For the formation of the incorporated paste, 4 % (m/m) of modification material was mixed into the plain paste.

Measuring Procedure

Before the analysis of histamine, the plain CPE had to undergo the standard deposition procedure described before. This modified electrode was air-dried and transferred into a system containing 5 mL of supporting electrolyte (phosphate/NaOH buffer 0.1 M; pH 12). After the blank control, aliquots of the histamine stock solution were added to the cell and distributed using a magnetic stirrer. By applying the aforementioned instrumental parameters CV or DPV techniques were performed. The data was evaluated using the tangential method.

HPLC Analysis

HPLC analysis of histamine has been performed according to Salazar et al. [26]. A precolumn derivatisation using the fluorimetric reagent OPA has been performed as described. Using an elution gradient of 11 % 2-propanol, 0.9 % potassium phosphate dibasic, 0.3 % acetic acid and 88 % water, the histamine peak could be found after a retention time of 9 minutes.

ZnO-CP Based Sensor in Food Analysis

To test the functionality of the ZnO-CP based sensor, different food from local supermarkets was chosen. Samples like fish sauce, soy sauce, and vinegar were injected into the system without further dilution. For the sample analysis, DPV was selected as standard technique to obtain the analytical data.

Results and Discussion

Optimization of the Operating Conditions

In the last years, the analysis of biogenic amines has gained intensive interest not only because of their undeniable importance in biochemical processes but also because of their rising role in food quality management. Due to the huge similarity in structure the difficulty in amine analysis became selectivity. For this reason, the aim of this work was to enable

selective quantitative evaluation of histamine in complex matrix especially in the presence of other amines.

The characterization of 100 $\mu\text{g/mL}$ histamine on unmodified CP electrodes using a neutral electrolyte media shows an oxidation peak at +1.24 V (Fig. 1) matching the results described elsewhere [23,27]. Potentials higher than +1.00 V can lead to interferences of the main oxidation process with the oxygen evolution when using CPE. Therefore the first necessary step in the sensor development was to shift the peak position to less positive values. As the oxidation mechanism of histamine, mostly explained as a radical formation process resulting into dimeric structures [28,29] is pH dependent, the peak position relocation was achieved by exchanging the electrolyte to use a more alkaline media. Using phosphate/NaOH buffer of pH 12 the histamine signal appeared at + 0.75 V (Figure 1).

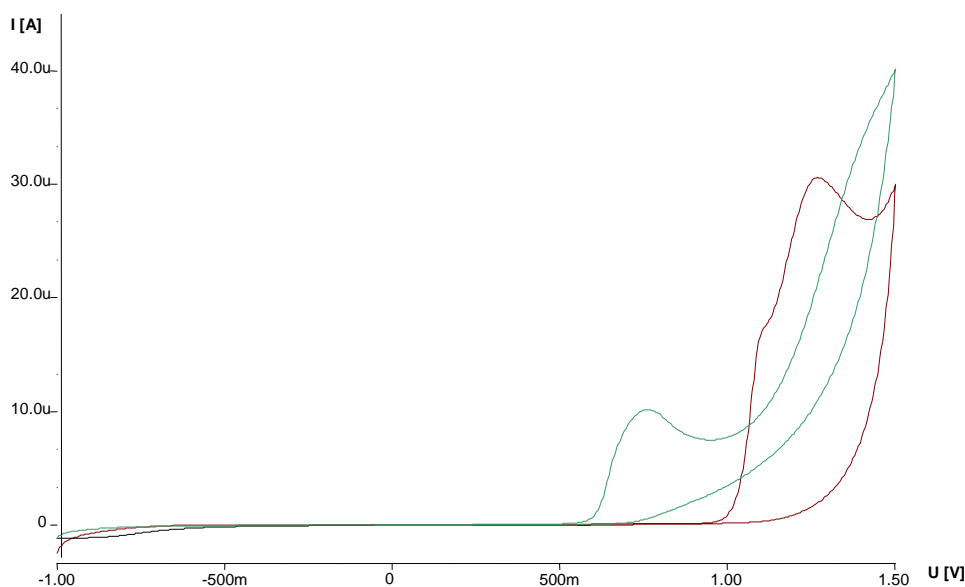


Fig. 1: Comparison of histamine signals (100 $\mu\text{g/mL}$) obtained at CPE using — neutral medium and — alkaline medium.

Sensor Modification and Refining

Due to former reports [29], histamine, respectively imidazole molecules, tend to form high stability and low solubility complexes with different metals. According to Weitzel et al. the strong alkaline amino group forms a ring system with the imidazole nitrogen and the metal ion as centre. Therefore different metal oxides (MnO_2 , PbO_2 , HgO , ZnO , As_2O_3 , and RuO_2)

but also phthalocyanines and cyclodextrines with metallic centres were tested as possible modifiers to increase the selectivity and the sensitivity of the system. In this context especially the zinc compositions showed interesting results. Referred to those, ZnSO_4 , ZnCl_2 and ZnO were selected for more detailed testing.

Therefore, each modifier was incorporated into the carbon paste to form a 4 % modified electrode but also the modification process of thin film development by electrodeposition was tested [25]. All the zinc salts lead to an increase of selectivity but also sensitivity when used in the described alkaline system (Fig. 2). As can be seen in Fig. 2, ZnCl_2 as an incorporated modifier enlarged the histamine current signal by approximately 100 %, the ZnSO_4 by 70 % and ZnO by 160 %. The thin film modification with ZnCl_2 on the other hand showed an increase by 180 %. However a certain leakage of the incorporated modifier and the low tendency of ZnSO_4 to form ZnO thin films resulted in the selection of ZnCl_2 as modifier applied to the CPE by the electrodeposition procedure described above.

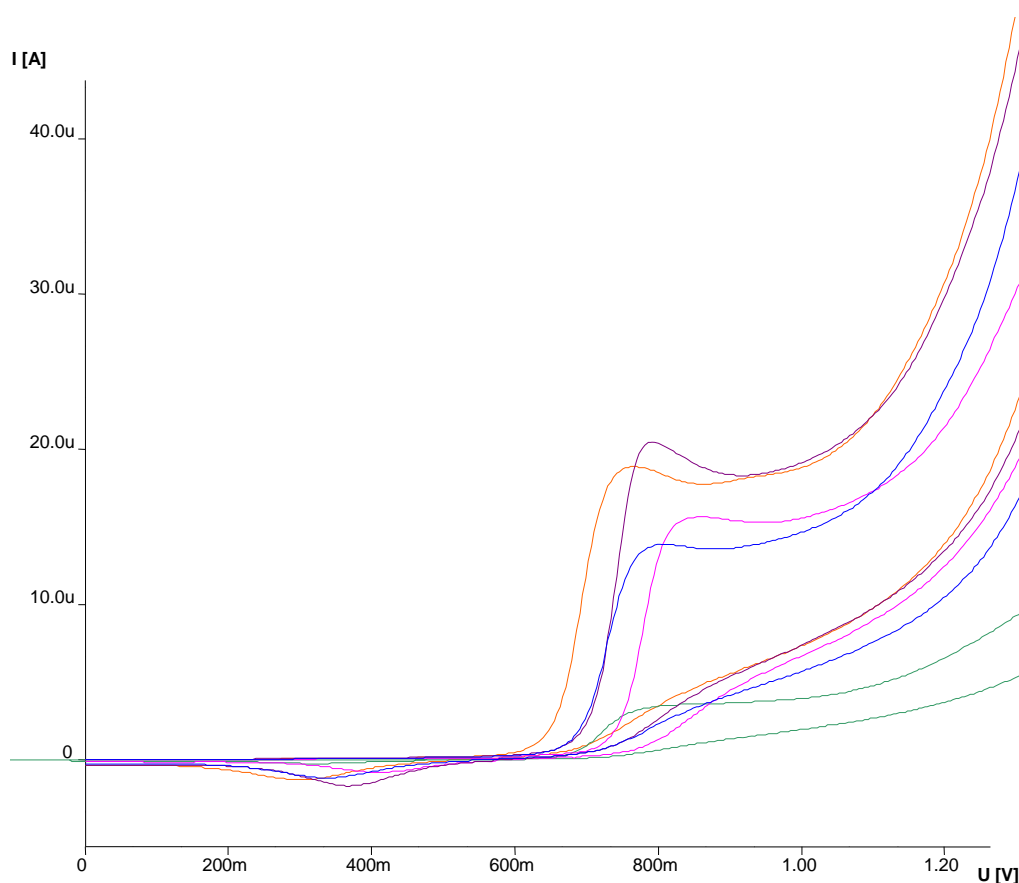


Fig. 2: Comparison of the signal intensity increase of $100 \mu\text{g/mL}$ histamine caused by different modifiers. — Unmodified CPE, — 4 % ZnCl_2 , — 4 % ZnSO_4 , — 4 % ZnO , — ZnCl_2 thin film and their influence on the stability of the system.

Validation of analytical parameters

Due to the standardized electrodeposition procedure, a constant concentration of ZnO is bound to the sensing area. The complex formation tendency of the histamine molecule with zinc leads to certain saturation phenomena that result into a logarithmic calibration curve with the following calibration equation: $I[A] = 3 \times 10^{-6} \cdot \ln(c[\mu\text{g/mL}]) - 8 \times 10^{-6}$ corresponding to a regression coefficient of $R = 0.996$. The limit of detection (LOD) was estimated to be $5 \mu\text{g/mL}$ and the limit of quantification (LOQ) $15 \mu\text{g/mL}$ was determined according to the ICH Q2(R1) guideline. The relative standard deviation (RSD) was estimated to be 5.89 % ($n = 6$) in average for five different concentrations along the calibration range while the accuracy was found to be 5.23 %.

Specificity

The most important tests during the development of the ZnO-CP based sensor were selectivity tests to guarantee the sole responsiveness to histamine. Fig. 3 shows the response of eight selected amines on unmodified CPE and on the new ZnO-CP based sensor. Fig. 4 summarizes the matching signal positions and the induced current signals.

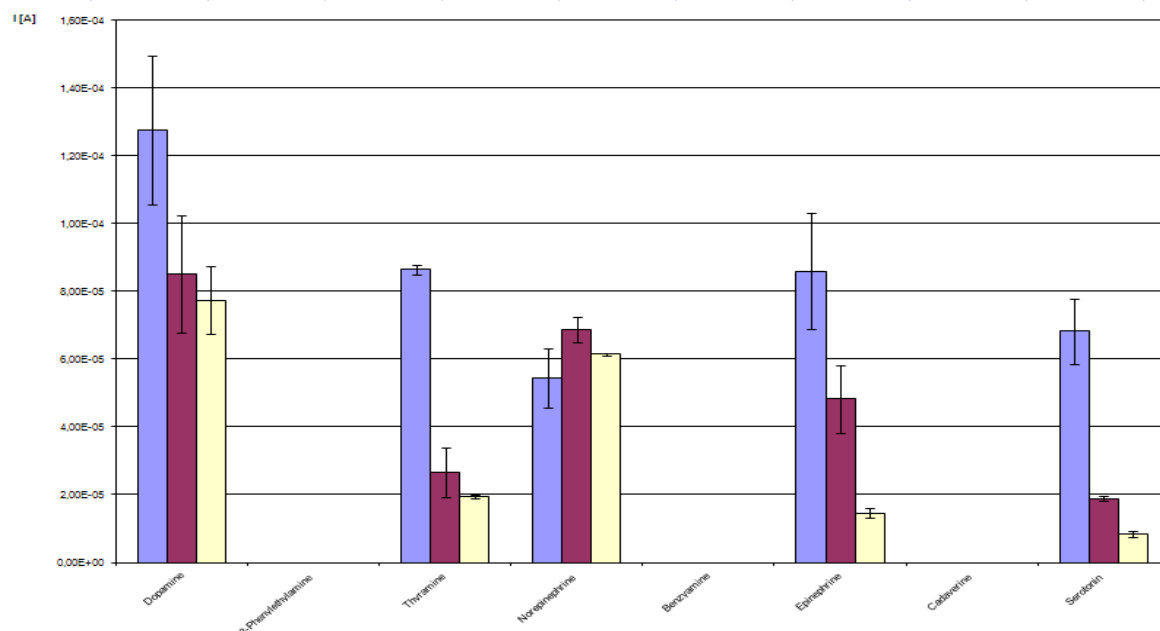


Fig. 3: Response of $500 \mu\text{g/mL}$ different interference substances. — CPE unmodified in neutral electrolyte medium, — CPE unmodified in alkaline electrolyte medium, — ZnO-CP based sensor in alkaline medium ($n = 4$).

As expected, 2-phenylethylamine, benzylamine, and cadaverine showed no signals along the scanned potential window according to the low oxidation attendance of the monoamine. The other amines (dopamine, tyramine, norepinephrine, epinephrine, and serotonin) clearly show decreased responses when ZnO-CP based sensor is used, probably according to the fact that the oxidation peaks induced by these molecules can be traced back not to the amine- but the hydroxide-group. Anyway they can clearly be separated from the histamine signal because of their much lower oxidation potentials (Fig. 4).

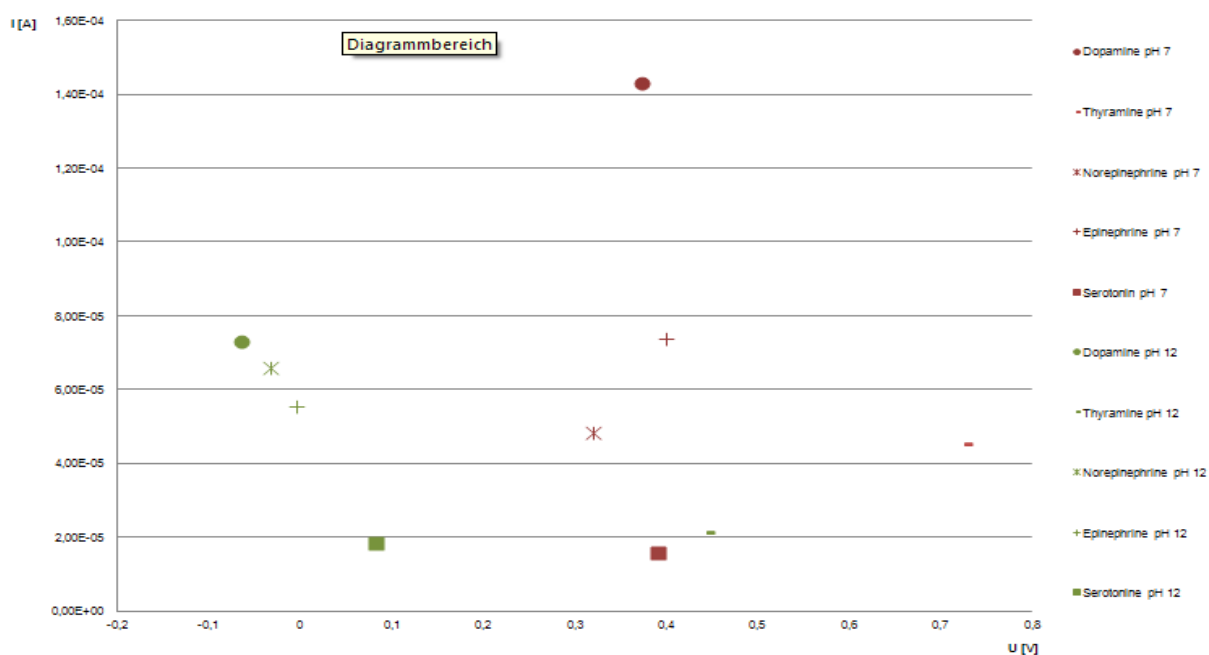


Fig. 4: Summary of peak positions and signal intensities of the interference substances performed at — pH 7 and — pH 12 using plain CPE. ● Dopamine, - tyramine, * norepinephrine, + epinephrine, ■ serotonin.

Application of the ZnO-CP Based Sensor in Food Matrix

To ensure the functionality of the ZnO-CP based sensor in complex matrix different food from local markets was chosen. The sauces and the vinegar were injected into the system without any purification. The results of these analyses are summarized in table 2 and were successfully reproduced using HPLC analysis.

Table II: Results of the histamine analysis using the ZnO-CP based sensor in food matrix (n = 4).

| Food | Electrochemical determination ($\mu\text{g/mL}$ histamine) | HPLC determination ($\mu\text{g/mL}$ histamine) |
|----------------------------------|--|---|
| Vinegar balsamico | 43.0 ± 2.1 | 41.4 ± 1.9 |
| Fishsauce | 70.2 ± 3.5 | 73.2 ± 3.1 |
| Fishsauce flavoured with chillis | 60.6 ± 3.0 | 57.8 ± 2.4 |

Conclusion

Using electrodeposition, the attachment of a ZnO thin film to the surface of a classic carbon paste electrode was successfully achieved. The so modified sensor was used to determine histamine in a more selective way than described before using the classical complex affinity of histamine. The ZnO-CP based sensor is a new, simple but efficient method for standard histamine analysis but also to be used in complex food matrix as could be shown.

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