Food Chemistry 122 (2010) 871–876

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

# SI lab-on-valve analysis of histamine using potentiometric detection for food quality control

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## ARTICLE INFO

*Article history:*
Received 7 June 2009
Received in revised form 15 January 2010
Accepted 19 February 2010

*Keywords:*
Potentiometry
Lab-on-valve
Miniaturisation
Histamine
Cyclodextrin

## ABSTRACT

In this work the implementation of a histamine ion-selective electrode for fish freshness control is described. The solid-contact electrode is based on a polymeric membrane incorporating, respectively, α-cyclodextrin as an ionophore, 2-fluorophenyl 2-nitrophenyl ether as a plasticiser and potassium tetrakis(*p*-chlorophenyl) borate as an ionic additive. The conventionally shaped histamine electrode responded to histamine cation in the pH operational range of 3.5–5.5 with a slope of $31.7 \pm 1.3$ mV $\text{dec}^{-1}$, and with a practical detection limit of $(1.6 \pm 0.2) \times 10^{-6}$ mol $\text{L}^{-1}$. The miniaturisation of the above-described electrode enabled its use as a detector in a sequential-injection lab-on-valve system, yet with a useful lifetime shortened from 10 months to approximately 1 month under continuous operation. The optimised flow conditions were achieved for sample injection volumes of 70 μL propelled towards the detection cell at the flow-rate of 12 μL $\text{s}^{-1}$ during 20 s followed by a flow-rate of 15 μL $\text{s}^{-1}$ during 50 s. The potentiometric analysis of histamine in different kinds of fish furnished results similar to those provided by the chromatographic method. The low cost of the analysis, the speed of the method and the use of a smaller volume and non pollutant reagents justify the use of potentiometry as an alternative analytical technique for this food control.

## 1. Introduction

Histamine [2-(4-imidazolyl)-ethylamine] is an important mediator of biological processes such as inflammation, gastric acid secretion and neural modulation. The synthesis, transport, storage, releases and degradation of histamine are carefully regulated by an organism since its pharmacological activity is significant even at very low concentrations. Both methylation of the imidazole ring promoted by histamine N-methyltransferase and oxidative deamination of primary amino group by diamine oxidase1 constitute the major routes for histamine inactivation in mammals. Histamine is also a byproduct of bacterial action during food processing so in this way it is being present in substantial amounts in fermented foodstuffs and beverages. It is enzymatically produced by means of amino acid L-histidine decarboxylation by the histidine decarboxylase enzyme. Histamine is commonly used as an indicator of food deterioration, and frequently regarded as one of the biomarkers for quality control during the food production and transportation. According to US Food and Drug Administration (FDA), fish containing less than 10 mg $\text{kg}^{-1}$ histamine is of good quality, whereas a level of 30 mg $\text{kg}^{-1}$ indicates significant deterioration. A level of about 50 mg $\text{kg}^{-1}$ is considered to be a conclusive evidence of decomposition (Rogers & Staruszkiewicz, 2000). Because of its potential risk to health, a regulation level on histamine has been set in most of the countries or organisations. In the European Union, the caution level is 100–200 mg $\text{kg}^{-1}$ according to Regulation Commission (Regulation, 2005) on microbiological criteria for foodstuffs.

In the last 10 years more than half hundred papers were published proposing different procedures for histamine determination in different foodstuffs. The methods included colorimetry (Patange, Mukundan, & Kumar, 2005), fluorimetry (Stute, Petridis, Steinhart, & Biernoth, 2002), enzyme-linked immunosorbent assay (ELISA) (Aygun et al., 1999), radio-enzymatic assay (Verburg, Bowsher, & Henry, 1988), electrochemical flow immunoassay (Lim, Ohta, & Matsunaga, 2003), amperometry (Weng, Xia, & Jin, 2001) and chromatography (Doshi & Edwards, 1979; Fernandes, Judas, Oliveira, Ferreira, & Ferreira, 2001; Kim et al., 2007; Mita, Yasueda, & Shida, 1980; Nicholls, Evans, Valic, & Zuskin, 1973; Oguri, Enami, & Soga, 2007; Peng et al., 2008; Proestos, Loukatos, & Komaitis, 2008; Soufleros, Bouloumpasi, Zotou, & Loukou, 2007; Tham, 1966; von Vietinghoff, Gabel, & Aschenbach, 2006) using different kinds of detection. However, most of them were associated with cumbersome procedures, needed expensive instrumentation, lacked adequate sensitivity and specificity or exhibited low dynamic ana-

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doi:10.1016/j.foodchem.2010.02.055

lytical range (Weng et al., 2001). Due to simplicity, low cost and miniaturisation ability, potentiometric procedures can provide an expeditious alternative for food quality control. A histamine electrode based on a polymeric membrane containing tetrakis(*p*-fluorophenyl)borate as an ion-exchanger and *o*-nitrophenyl phenyl ether as a membrane solvent was firstly proposed (Katsu, Kanamitsu, Hirota, Tasaka, & Fujita, 1986). Its performance was limited mainly by both the poor selectivity and the limit of detection ($5 \times 10^{-4}$ mol $L^{-1}$) presented. Later the same authors proposed a new electrode based on prothiofos as a membrane solvent and sodium tetrakis[3,5-bis(2-methoxyhexafluoro-2-propyl)phenyl] borate as an ion-exchanger. The electrode response to histamine in the concentration range of $5 \times 10^{-5}$ to $1 \times 10^{-2}$ mol $L^{-1}$ was almost Nernstian (56 mV $dec^{-1}$) in physiologically balanced salt solution, with a response time lower than 10 s (Katsu & Hirodo, 1999). Yet the electrodes response to lipophilic quaternary ammonium ions such as $(CH3)_4N^+$ and acetylcholine was much greater than that to histamine. A histamine sensitive liquid ion-exchanger, composed by tetrafluorophenylboron histamine plus 3-nitro-*o*-xylol, was also proposed (Bi, 1989) for the implementation of microelectrodes for histamine determinations in the working range of $10^{-4}$ to $10^{-3}$ mol $L^{-1}$ and with the slope of 48 mV $dec^{-1}$. Another ion-selective membrane based on a thiopyrilium derivative as a charged carrier has been reported by Javanbakht, Ganjali, Norouzi, Abdouss, and Riahi (2008) for application into synthetic human serum although requiring toxic solvents and tedious synthesis of the ionophore. Its figures of merit were the sub Nernstian slope (55.6 mV $dec^{-1}$), the linear response in the $2.5 \times 10^{-6}$ to $1.0 \times 10^{-1}$ histamine interval and the detection limit of $1.0 \times 10^{-6}$ mol $L^{-1}$. Furthermore, the electrode showed low potentiometric selectivity coefficients for several aminoacids and common inorganic anions. Iron(III) and manganese(III) tetraphenylporphyrins were also explored by (Amini, Shahrokhian, & Tangestaninejad, 1999) as electroactive materials for preparing polymeric membrane-based sensors selective to histamine. These sensors exhibited near-Nernstian responses in the concentration range from $1 \times 10^{-6}$ to $1 \times 10^{-1}$ mol $L^{-1}$ with detection limits of about $5 \times 10^{-7}$ mol $L^{-1}$ and response times lower then 30 s. However, the main conditions to obtain these figures were the higher content of porphyrins (~10%) in the membrane and the conditioning time of 24 h in a histamine solution (0.05 mol $L^{-1}$). Moreover, the life time of the electrodes under batch conditions' evaluations was 1 month. Recently, the incorporation in membranes of compounds with molecular recognition ability (ionophores) rendered electrodes with more reproducible responses, longer working lifetime and higher selectivity when compared with membranes based only on ionic exchangers (Amorim, Araújo, Montenegro, & Silva, 2007b, 2008; Pimenta, Araújo, & Montenegro, 2002). With them, the potentiometric response mechanism was governed by the ability of cyclodextrins to establish lipophilic interactions and to form inclusion complexes. In the following, an electrode based on α-cyclodextrin as histamine ionophore is evaluated in order to provide a simpler procedure for histamine control in fish. Simultaneously, the new electrode is evaluated in a miniaturised configuration, according to Amorim, Araujo, and Montenegro (2007a), and coupled to a sequential-injection lab-on-valve system (SI-LOV) in order to allow automatic and miniaturised determinations. Similarly to sequential-injection analysis, SI-LOV technique allows sample and reagent solutions to be selected, mixed and diluted automatically by means of its sequential aspiration from the core stream selecting valve, into a holding coil (Ruzicka, 2000). Through down scaling histamine-selective electrode, it was possible to benefit from the advantages recognised in SI-LOV-based systems, namely regarding equipment portability, reduced consumption of sample and reagents and reduction of effluent waste. The reduced volume of the sensor cocktail used in the proposed configuration allows for the preparation of an increased number of electrodes with modest costs. Electrical noise, which is often regarded as a major drawback of potentiometric flow-based procedures, is herein significantly reduced by the adopted detection cell configuration.

## 2. Experimental

### 2.1. Reagents and solutions

Distilled, deionised water (conductivity <0.1 μS $cm^{-1}$) and analytical grade chemicals were used without further purification, unless otherwise stated. Polyvinyl chloride (PVC), carboxylated polyvinyl chloride (PVC-COOH), potassium tetrakis(*p*-chlorophenyl)borate (KTpClPB), citric acid, lithium hydroxide and lithium chloride, chloridric acid were from Fluka; tetrahydrofuran (THF), methanol and isopropanol were from Riedel-de-Haën; 2-fluorophenyl 2-nitrophenyl ether (FNDPE), α-cyclodextrin (α-CD) and histamine hydrochloride were from Sigma.

A stock solution of histamine was daily prepared by weighing about 92 mg of the reagent and afterwards transferred into a 50 mL volumetric flask where subsequent dilution to the mark was proceeded either with a LiCl solution ($I = 0.1$ mol $L^{-1}$) or with an acidic buffer solution (HCOOH/LiOH) at pH 4 and with ionic strength adjusted to 0.5 mol $L^{-1}$. The working calibrating solutions were daily prepared by rigorous dilution with the same solvent.

Fish of local usual consumption was purchased at the local market and histamine was extracted by acidic digestion. Therefore, each kind of fish was firstly ground in a domestic mill and further macerated in a porcelain mortar in order to increase the surface area in contact with extraction solvent. About 5 g of macerate was transferred to 50 mL volumetric flask half full with deionised water acidified with 0.5 mL of HCl 5 mol $L^{-1}$. The final mixture was heated in a water bath to 100 °C for 20 min and after cooling, the volume settled up to 50 mL with deionised water. Afterwards, centrifugation at 5000*g* for 10 min was performed to eliminate the suspended denaturised proteins and tissue fragments. The supernatants were further purified by filtering through a membrane filter unit (Spin-X HPLC Micro Centrifuge Filter, 0.2 μm Nylon filter). In turn, the samples to be analysed by SI-LOV system were alternatively cleaned-up using a solid-phase extraction cartridge LiChrolut-SCX according to Mahendradatta and Schwedt (1998). The reference method for histamine determination was based on HPLC-fluorimetric detection after derivatisation with the *o*-phthalaldehyde adduct in alkaline medium (Kalligas, Kaniou, Zachariadis, Tsoukali, & Epivatianos, 1994; Rönnberg, Hansson, & Håkanson, 1984).

### 2.2. Apparatus

A Crison 2002 pH potentiometer (sensitivity: ±0.1 mV) coupled to an Orion 605 electrode switcher was used to provide the potential differences between an Orion 90-02-00 doubled junction AgCl/Ag reference electrode (with LiCl 0.1 mol $L^{-1}$ as an external reference solution) and the histamine-selective electrodes. The pH values of all solutions and the operational pH range characteristics of the electrodes were controlled by means of a Phillips GAH 110 glass electrode.

The schematic representation of the computer-controlled SI-LOV system used is depicted in Fig. 1a. It comprises a Minipuls 3 Gilson (Viliers-le-Bell, France) peristaltic pump with a PVC pumping tube ($\varnothing_{int} = 0.90$ mm) of the same brand, a VICI C25-3118E, eight-port stream selecting valve (Valco Instruments, Houston, TX), a 161T031 NResearch three-way solenoid valve (Stow, MA) and a Crison MicropH-2002 potentiometer to which a Metrohm electrode of Ag/AgCl (KCl 3 mol $L^{-1}$), model 6.0727.000 was con-