On-site monitoring of fish spoilage using vanadium pentoxide xerogel modified interdigitated gold electrodes

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1. Introduction

For all kinds of fish and fishery products, freshness is essential for the quality of the final product. Freshness can be explained to some extent by some objective sensory, (bio)chemical, microbial and physical parameters, and can therefore be defined as an objective attribute \cite{1}. To date, the freshness of fish is judged by trained assessors, by evaluating various freshness attributes, such as appearance, texture, smell and color, while characteristic sensory changes of the aforementioned attributes occur when fish deteriorate \cite{2}.

Sensory evaluation is currently the most important method for freshness evaluation in the fish sector. Other approaches based on microbial methods, physical measurements (measurement of electrical parameters, monitoring of oxidation–reduction potential, instrumental monitoring of texture and color changes), and chemical methods (measurements of lipid oxidation, determination of various volatile compounds, such as C\textsubscript{6}–C\textsubscript{9} alcohols, carbonyl compounds and total volatile basic nitrogen compounds) have been also proposed. In addition, the calculation of K value, which is defined as the ratio of the sum of inosine and hypoxanthine concentration to the total concentration of ATP metabolites, is a widely used technique mostly in a research level. A detailed description of these approaches along with a comprehensive analysis on their inherent advantages and disadvantages are provided in the excellent review of Olafsdottir et al. \cite{2}. The possibility to measure and/or estimate fish freshness with a combination of instrumental techniques (electronic noses, spectroscopic methods, texture-meters, image analysers, color meters and devices measuring electrical properties) using a multi-sensor device has been also proposed \cite{3}.

Microorganisms are the major cause of spoilage of most seafood products. However, only a few members of the microbial community, the specific spoilage organisms (SSOs), give rise to the offensive off-flavors associated with seafood spoilage \cite{4,5}. Volatile compounds such as ammonia, dimethylamine, trimethylamine, which are collectively known as total volatile basic nitrogen (TVB-N), are products of microbial degradation and are considered as potential indicators of fish spoilage (index of freshness). The reference method for the determination of TVB-N, as adopted by the European community involves a rather laborious extraction–steam distillation and subsequent titration of the amines with hydrochloric acid \cite{6}, while other methods based on flow injection analysis in combination with photometric detectors \cite{7}, and solid-phase micro-extraction coupled to gas chromatography with NPD or FID detectors \cite{8} have been also proposed. Although these methods provide satisfactory results and utilize instrumentation with a high

\[ K = \frac{\text{inosine} + \text{hypoxanthine}}{\text{total ATP}} \]
degree of automation, the need of laborious steps for the preparation of the sample, cost, and the lengthiness of volatile analysis methods make them suitable only for specialized research and analytical laboratories. Recently, a direct method for the monitoring of fish spoilage, which is based on an immobilized pH sensitive dye, bromocresol green, was also proposed; however it cannot be used for on-site applications [9]. Finally, the assessment of fish spoilage using electronic noses, has been proposed by Olafsdottir and Kristbergsson [10] and Gil et al. [11].

Here, we present the construction of ammonia gas sensors, which were fabricated by dip-coating of gold interdigitated electrodes (IDEs) into aqueous solutions of vanadium pentoxide xerogel (V_2O_5·nH_2O, VXG). VXG is a highly reactive material with a diverse and rich intercalation chemistry demonstrated by numerous cation-exchange, acid–base, and redox reactions. The high affinity of VXG to 1-butylamine and other amines including ammonia has been demonstrated by Raible et al. [12]. The morphology of the sensing layer, its interaction with ammonia as well as the regeneration of the sensor with vapors of HCl were examined with scanning electron microscopy and FTIR spectroscopy. Signal changes, due to changes of the RC-product (see below) of the electrochemical cell (Au-VXG-Au), as a result of its interactions with ammonia vapors, were probed with a portable, homemade charge meter, the Multipulser [13]. The performance of the sensor was successfully tested in contact with different concentrations of ammonia vapors as well as for the on-site, real-time monitoring of fish spoilage in ambient conditions.

2. Experimental

2.1. Apparatus

Multipulser was originally designed as a cost effective alternative monitoring device to other well established commercial instruments, e.g. frequency response analyzers, for probing changes of the capacitance of an electrode/electrolyte interface, assuming that the resistance of the electrolyte (R_{cell}) is considerably lower than the device input resistance (R_{in} = 10 k\Omega), as for example, in the case of a high ionic strength aqueous solution [13]. On the other hand, if the value of R_{cell} ranges in the k\Omega level, signal output is expected to describe the RC-product of the cell. This is indeed the case of the VXG-modified gold IDEs, where C corresponds to the total capacitance of the Au-VXG interfaces, and R expresses the overall resistance of the film (VXG layer).

The operation of Multipulser is based on the integration of the electric charge used for the repetitive charging of the electrochemical cell capacitance after the application of a predetermined number (N) of short-duration (T = P/8, where P, the period of the applied waveform) low-amplitude (V_0 = 100 mV) voltage perturbation pulses. All packets of charge are accumulated in an analog integrator, and the output voltage is used to describe the RC-product of the electrochemical cell. Multipulser feature three user selectable operating modes each one characterized by its own particular shape of the applied perturbation voltage waveforms [13]. In this study, all measurements were performed using mode 2. In this mode, each period of the applied to the electrochemical cell waveform consists of two similar perturbation voltage pulses but of opposite polarity, in order to prevent possible polarization phenomena. FTIR spectra and SEM images were taken with a Brucker IFS66VIS spectrometer (USA) and a Fei Quanta 400 SEM (USA) instrument, respectively.

2.2. Materials

V_2O_5·nH_2O xerogel was prepared by dissolution of crystalline V_2O_5, purchased from Aldrich, in concentrated H_2O_2 (30%), according to a previous described procedure [14]. Ammonia solution 25%, dimethyamine and hydrochloric acid were purchased by Sigma and double-distilled water (DDW) was used throughout.

2.3. Preparation of the electrodes

Experiments were performed with interdigitated gold electrodes (20 digits, of 6.1 mm length × 0.2 mm width each, and an interdigit spacing of 0.2 mm), which are purchased from Gwent Electronic Materials Ltd. (UK) and comprised screen-printed gold onto a ceramic substrate 1.0 cm × 2.5 cm. Before use, electrodes were cleaned with copious amounts of isopropanol and acetone, and finally, dried under argon flow. VXG films were deposited on the substrates by dip-coating using a 1% (w/v) aqueous solution of VXG, and then were left to air-dry.

2.4. Set-up of ammonia calibration

The mixture of the ammonia vapors in an air stream with relative humidity 35–40% was achieved using two arm gas-flow set-up (Fig. 1). Two mass-flow controllers allowed the flow rate of the air that acts as the carrier gas, to be controlled from 1 to 20 cm³ min⁻¹ in one arm (D1) and from 1 to 250 cm³ min⁻¹ in the other arm (D2). In arm D1, the carrier gas passes through a spherical flask containing ammonia, thus producing a range of concentrations of the ammonia vapors [15].
3. Results and discussion

VXG is sufficient conductive even at room temperature thus allowing the direct application of the sensor electrodes without the need of an external power source. SEM images in Fig. 2 illustrates the morphology of the VXG films, which consist of flakes of the xerogel of different size with a thickness of some micrometers. The response characteristics of the sensor to volatile amines were first examined by exposing the electrode sensors over a beaker containing 10 mL of 0.10 or 1.0 M ammonia or 1.0 M dimethylamine in ambient conditions. As can be seen in Fig. 3, the interaction of both amines with the sensor electrodes results in a decrease of the signal, and indeed, a higher sensitivity was observed in the case of ammonia. The observed signal changes can be attributed to the interactions among the amine vapors with (i) the vanadium oxide framework in which partial ammonolysis reactions take place, (ii) the layered structure, as the amines, in the presence of water vapors can be intercalated within the layers of the structure, and (iii) between the film and the electrode surface. These interactions are expected to affect the overall electrical conductivity of the electrochemical cell (Au-VXG-Au) in a different manner, according to previous studies [12]. Intercalated VXG films exhibit higher conductivity compared with that of the pristine films [16], while the other two types of interactions are expected to result in a decrease of the overall conductivity of the electrochemical cell.

Due to the high affinity of vanadium pentoxide xerogel to ammonia and various organic amines [12], the design of a VXG-based sensor with enhanced selectivity to a particular amine is rather impossible. However, for the purpose of the specific application, selectivity to a particular amine is not required, as the assessment of fish spoilage is based on the monitoring of the TVB-N compounds. As a result, subsequent work was performed using ammonia vapors.

3.1. Ammonia sensing

Fig. 4A shows the Multipulser signal output changes (initial signal outputs were taken as zero, and further signal changes were accordingly corrected) for different concentrations of ammonia versus time at room temperature. Ambient conditions were simulated by adjusting the relative humidity to 35–40%. As can be seen in Fig. 4A, a linear relationship was obtained between the.

Please cite this article in press as: S. Helali, et al., On-site monitoring of fish spoilage using vanadium pentoxide xerogel modified interdigitated gold electrodes, Electrochim. Acta (2009), doi:10.1016/j.electacta.2009.01.027
response and the concentration of the ammonia vapors in the range 46–187 ppm NH₃ with a correlation coefficient, r = 0.9941 (n = 5). Data fitted the equation: signal change (V) = \((0.28 \pm 0.02) + (0.0024 \pm 0.0002) \times C_{NH_3} \) (ppm). Based on the confidence limits of the regression equation and for a factor of 3, the limit of detection (LOD) was calculated, and found to be 25 ppm NH₃. The reproducibility between different sensor electrodes was checked by comparing the responses of four different electrodes for a concentration of 112 ppm NH₃ and it found to be 7.7%. This deviation is satisfactory and eventually can be further improved if the procedure for the application of the sensing films being automated.

Even though the sensor electrodes are intended to be used as disposable sensors, the ability to be reused, after the regeneration of their surfaces with HCl vapors (Fig. 4B), is an interesting feature, and thus it was further investigated by IR spectroscopy.

3.2. IR spectra

The interaction of the vanadium oxide xerogel film with ammonia was probed with infrared spectroscopy (IR). In particular, we recorded the IR spectrum of the film at representative working stages: (a) as-made film, (b) film exposed to ammonia vapors and (c) the same film exposed to HCl vapors. The results are shown in Fig. 5. The IR spectrum of the pristine VXG (black line) shows the characteristic vibrations originating from the V–O–V and V=O vibrations, which are observed in the range 1050–450 cm⁻¹ in full agreement with values reported in literature [17]. In addition, the characteristic OH stretching bands at 3580 and 3233 cm⁻¹ as well as the H₂O scissoring band at 1613 cm⁻¹ are observed. The ammonia exposed film (red line) shows new relatively intense bands at 3212, 3007 and 2820 cm⁻¹ that corresponds to NH stretching vibrations of NH₄⁺ adsorbed cations. The intense and sharp band at 1423 cm⁻¹ corresponds to the scissoring vibration of NH₄⁺ cations. The IR spectrum of the ammonia treated film exposed in HCl vapors (blue line) shows reduced intensities of the NH₄⁺ related bands in agreement with the reduced ammonia content. These results are in fully agreement with previous reports and suggest that ammonia is protonated by adsorption in the interlayer space of the vanadium xerogel structure through a Brønsted acid–base mechanism [18,19]. Moreover, the differences observed in the range 500–1000 cm⁻¹ suggest a partial ammonolysis of the V₂O₅ framework with a possible formation of V–NH₃ bonds upon exposure to ammonia vapors.

In addition, a simultaneously redox reaction, taking place in a minor extent, cannot be excluded, as has been reported in the past. It is important to note that the ammonia exposed material shows a characteristic blue color as a result of a partial reduction of VV⁵⁺ to VV⁴⁺ whereas the as-made solid and the HCl-treated material shows a green-orange and a orange-red color respectively, typical of VV¹⁻-oxo species.

3.3. Application to on-site fish spoilage trials

The practical usefulness of the proposed sensors is clearly shown in Fig. 6. Fresh and frozen samples of bream fish (about 300 g) were put in a Plexiglas box (30 cm × 20 cm × 20 cm), electrodes were held atop the box and signal changes versus time were recorded. The formation of ammonia and other organic (trimethylamine, dimethylamine) amines, due to the bacterial growth in fish samples gave rise to analytical useful signal changes, indicating that the proposed sensors can be potentially used for the on-site, real-time monitoring of spoilage. Based on the kinetics of the signal decay, it seems that is possible to judge for the freshness of a fish sample, or to predict its shelf time. It is important to mention that the above trial was made in ambient conditions, without the need of any sample pretreatment or the establishment of any special experimental conditions. This trial was designed in accordance with previous works [9]; however, no special precautions were taken to avoid sample contamination.

Further evaluation of the observed signal changes are beyond the scope of this work. In order useful conclusions about the freshness or the general condition of the tested products to be drawn, the received analytical signals should be correlated with bacterial population, and, under specific measuring conditions, to define exact freshness and spoilage thresholds for different fish species.

4. Conclusions

This work employs functional chemical sensors for the detection of volatile inorganic and organic amines produced during bacterial growth in fish samples. Films of vanadium pentoxide xerogel, which were used as sensing layers, were deposited over gold interdigitated electrodes using a simple method that enables the mass production of low-cost sensors.

If proper correlation studies are made, the proposed sensors could offer an extra diagnostic tool to both the fish industry and retailers who are interested in approaches and devices that are...
cost effective, rapid, non-destructive and able to be used for on-site applications.

Acknowledgements

This work was financially supported by a NATO Collaborative linkage grant (contract no. CBP.MD.CLG.982802). Authors wish to thank Prof. L. Vonna (ICSI, Mulhouse, France) for SEM photo.

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Please cite this article in press as: S. Helali, et al., On-site monitoring of fish spoilage using vanadium pentoxide xerogel modified interdigitated gold electrodes, Electrochim. Acta (2009), doi:10.1016/j.electacta.2009.01.027