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ORIGINAL PAPER

Application of a quartz crystal nanobalance and principal component analysis for the detection and determination of histidine

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Abstract The aim of the present investigation was to develop a biosensor based on a quartz crystal nanobalance (QCN) for the detection of histidine (His). A thin layer of nickel was electrochemically deposited over the gold crystal electrode and exposed to H_2O_2 to form nickel oxide. The composite electrode was then used to determine His. The frequency shifts were linear with respect to the concentration of His in solution. His can be measured in the range of 100–2000 mg L^{-1}. A lower limit of detection of 48 mg L^{-1} and a sensitivity factor of 0.0307 Hz/mg L^{-1} was obtained. Some possible interferences were checked for, and the performance of the sensor was found to be unaffected by any interference except for those from arginine, cysteine and NaH_2PO_4. Principal component analysis (PCA) was used to process the frequency response data of the single piezoelectric crystal at various times, considering the different adsorption–desorption dynamics of His and the interfering compounds. Over 85% of the variance in the data was explained by two principal components. A score plot of the data for the first two PCs showed that the modified QCN yields favorable identification and quantification performances for His and the interfering compounds.

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Introduction

Human metabolic disorders are characterized by either increased or decreased levels of special metabolites in patient serum and urine [1]. In many cases, metabolite analysis in urine is preferred because urine testing is noninvasive and it often has higher concentrations of metabolites than blood [2].

Histidinemia is a rare autosomal recessive metabolic disorder that occurs due to a deficiency of the enzyme histidine ammonia-lyase (histidase). The main symptom of histidinemia is accumulation of His in blood, urine and cerebrospinal fluid [3].

High-performance liquid chromatography (HPLC), paper and thin-layer chromatography, and gas chromatography (GC)–mass spectrometry (MS) are the techniques that are most commonly used in the area of metabolite analysis. However, these techniques often need expensive equipment such as gas chromatography, and require tedious pretreatment processes [4–6].

The quartz crystal nanobalance (QCN) is a sensing system based on the sorption of analyte onto an adsorbent material [7]. The QCN comprises a thin vibrating AT-cut quartz wafer that is sandwiched between two metal excitation electrodes. When small amounts of mass are adsorbed at the quartz electrode surface, the frequency of the quartz changes according to the well-known Sauerbrey equation [8]:

$$\Delta F = -2.26 \times 10^{-6} F_0^2 \left(\Delta m / A \right) \quad (1)$$

where ΔF is the measured frequency shift, F_0 the original oscillation frequency of the dry crystal, Δm the change in

mass, and A is the piezoelectrically active area of the excitation electrodes.

QCN has been used to measure mass changes in a variety of biological studies, such as DNA hybridization [9], immunoassay [10], protein adsorption onto a solid surface [11], and in situ cell–substrate interactions [12].

The major drawback of some sensors based on QCN is a lack of selectivity, since other compounds usually interfere along with the analyte. To overcome this shortcoming, one approach is to use a pattern recognition technique to process the signals from a nonselective sensor. Different chemometric tools can be applied for this purpose. Fuzzy clustering [13], partial least squares [14] and principal component analysis (PCA) [15] have been widely used for the classification of analytes. In recent years, considerable interest has been shown in the use of quartz crystals as sensors in conjunction with pattern recognition techniques in order to distinguish between various kinds of compounds [16].

The nickel–histidine (Ni–His) bond is a relatively strong natural bond that is commonly used in immobilized metal-affinity chromatography (IMAC). Proteins with exposed histidine and cysteine side chains have a high affinity for certain metal ions, such as nickel, cobalt, iron, zinc and copper ions [17].

It has recently been reported that His-tagged proteins can also bind tightly onto three-segment Au/Ni/Au nanorods, presumably due to the presence of nickel ions from surface oxidation, and can be separated by an applied magnetic field [18].

During the course of this work, considering the importance of determining His in body fluids, a simple, rapid, sensitive and reproducible biosensor based on QCN was developed for the determination of His in solution. Since nickel chelates have been successfully used for the immobilization and purification of His in IMAC [18], a quartz crystal electrode was coated with nickel oxide and tested for the determination of His in solution. Therefore, method based on a nickel oxide-coated QCN sensor used in conjunction with PCA was developed for determining His in the presence of interfering compounds.

Materials and methods

Reagents and materials

Nickel sulfate, boric acid, sodium lauryl sulfate, urea, uric acid, ammonium chloride, sodium sulfate, sodium hydrogen phosphate, potassium chloride and hydrogen peroxide were all purchased from Merck (Darmstadt, Germany) and were used without any further purification. Analytical-grade arginine (Arg), glycine (gly), histidine (His), serine (Ser), methionine (Met), tryptophan (Try), cysteine (Cys), cystine, and tyrosine (Tyr) were from Sigma (St. Louis, MO, USA).

Instrumentation

Ten-megahertz AT-Cut quartz crystals with a gold coating on both sides were obtained commercially from International Crystal Manufacturing (ICM, Oklahoma City, OK, USA). For QCN experiments, a home-made apparatus described in our previous work was used [19]. The oscillator circuit was based on that designed by Bruckenstein and Shay [20]. However, the original design was modified somewhat to improve mass sensitivity and stability [21].

A Wenking TG 97 galvanostat/potentiostat from Bank Elektronik GmbH (Clausthal-Zellerfeld, Germany) was used in this work.

Preparation of the modified quartz electrode

Nickel was coated onto the surface of the gold crystal electrode using a constant-current electrochemical method. The electrodeposition process was carried out in a Watts nickel bath containing 240 g/l nickel sulfate, 30 g/l boric acid and 0.15 g/l sodium lauryl sulfate. A typical two-electrode cell with a nickel plate used as anode and a gold-coated crystal used as cathode was employed. To allow high-current overflow, the surface area of the anode was chosen to be at least ten times larger than that of the cathode. A constant current density of 11 mA/cm^2 was applied for 30 s at room temperature. The mass of the coating caused a frequency shift of about 7 kHz.

The nickel film that formed was then oxidized with H_2O_2 for 30 min to form the nickel oxide [22]. This process caused a frequency shift of about 120 Hz.

Data processing

Principal component analysis (PCA) is a method that is widely used to classify compounds and mixtures. PCA is defined as an orthogonal linear transformation that decomposes the data matrix by projecting the multidimensional dataset onto new orthogonal base coordinates with data maximum variance. The data matrix consists of a number of experiments, each consisting of a number of variables.

The eigenvectors of the data matrix are called the principal components and they are uncorrelated. Sensor signals can be expressed as a linear combination of eigenvectors. Generally, the k-th principal component, PC_k, is a linear combination of the n response vectors $X_{n,j}$ for the analyte under study, where n is the number of variables, j

refers to different samples, and the coefficients ($a_{n,k}$) are called the loadings (Eq. 2):

$$\mathrm{PC}_k = \sum_{n=1}^{n} a_{n,k} X_{n,j} \quad (2)$$

The magnitude of each eigenvector is expressed by its own eigenvalue, which gives a measure of the variance related to that principal component. The variance is related to the quantity of information that is supplied by the component. By eliminating the less important eigenvectors, it is possible to achieve fewer vectors without any considerable information loss. So, during data processing, the results are transformed into a plane or into a space created from the first two or three eigenvectors. The coordinates of the data in this new plane or space are called their scores. The scores plot is usually used to classify the data clusters. In this study, the data fed into the primary matrix were normalized using Eq. 3 in order to eliminate the effect of the sample concentration:

$$X_{\mathrm{std}} = \frac{x - \overline{x}}{s_{\mathrm{x}}} \quad (3)$$

where X_{std} is the normalized input data, x the frequency of the crystal at the selected time (every 10 s), $\overline{x}$ is the average crystal frequency over a certain period of time (7 min), and s_{x} the standard deviation.

Our PCA calculations used a singular-value decomposition algorithm and were performed with MATLAB software (version 6.5). The operating system used to run the software was Microsoft Windows XP.

Sensing procedures

The nickel and nickel oxide-coated sides of the quartz electrode were exposed directly to histidine solutions. His solutions of various concentrations were prepared by dissolving His in Milli-Q water. A flow of dried air was passed through the cell to recover the electrode. All solutions were filtered using a syringe filter (0.2 μm) before injecting them into the cell. All experiments were carried out at room temperature (25 °C).

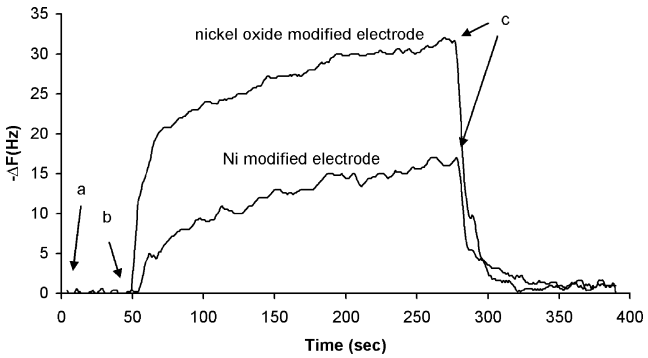

Fig. 1 Typical frequency changes in the nickel and nickel oxide-modified quartz crystal electrode recorded upon the exposure of the electrode to a solution of His (500 mg L^{-1}). *a*, Distilled water; *b*, His solution (500 mg L^{-1}); *c*, flow of dried air

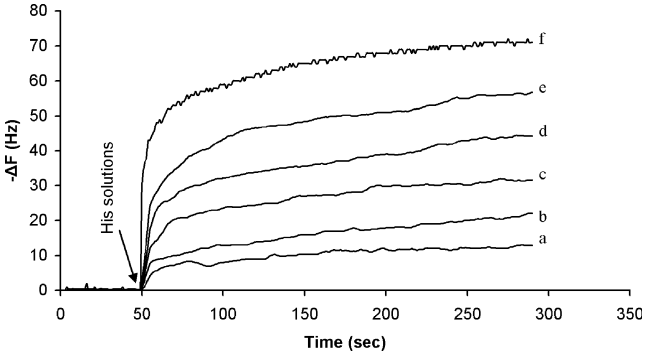

Fig. 2 Frequency changes in the nickel oxide-modified quartz crystal electrode as a function of the time that it was exposed to various concentrations of His solutions: (*a*) 100 mg L^{-1}; (*b*) 250 mg L^{-1}; (*c*) 500 mg L^{-1}; (*d*) 1000 mg L^{-1}; (*e*) 1500 mg L^{-1}; (*f*) 2000 mg L^{-1}

Results and discussion

Typical frequency changes in the nickel and nickel oxide-modified quartz crystal electrode were recorded upon exposure to 500 mg L^{-1} His solution (Fig. 1). The frequency of the crystal decreased due to the adsorption of His onto the surface of the modified electrode, according to Eq. 1.

However, when the Ni-modified quartz electrode was exposed to various concentrations (100–2000 mg L^{-1}) of His solution, a maximum frequency shift of 15–20 Hz was obtained. This response is presumably due to the presence of restricted numbers of adsorption sites (nickel ions) on the

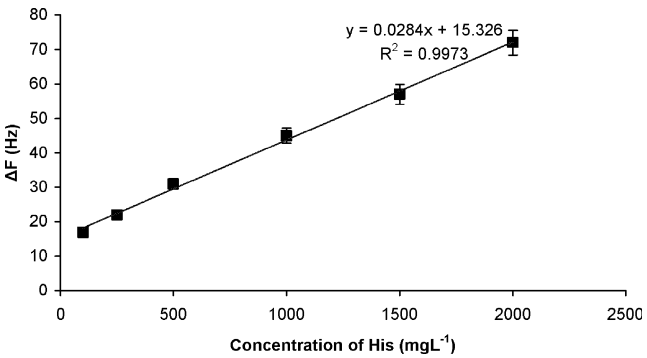

Fig. 3 Calibration graph for the nickel oxide modified quartz crystal electrode exposed to His solutions. Exposure time: 5 min

Table 1 Reproducibility of the nickel oxide-modified quartz crystal electrode

Sample	1	2	3	4	5	6	7	8	9	RSD %
Frequency shift (Hz)	32	29	30	32	31	30	35	33	28	2.15

surface of the nickel film. To solve this problem, the nickel layer was exposed to H_2O_2 to form nickel oxide.

The responses show that the nickel oxide-modified electrode is sensitive to the analyte concentration. The frequency of the crystal was back-shifted to its initial value by dried air, indicating the full desorption of His from the electrode surface.

Linearity of the response

In any sensor, a plot of response against the concentration of analyte should exhibit a linear relationship. This property was evaluated by exposing the nickel oxide quartz crystal electrode to various concentrations of His solutions ranging from 100 to 2000 mgL^{-1} (Fig. 2).

The calibration curve was created by plotting the frequency change against to the concentration of His. The frequency shift of each sample was recorded after 4 min of exposure to the modified electrode (Fig. 3). According to the calibration curve, there is a linear relationship between the His concentration and the response obtained. Therefore, this graph can be used to determine His concentration in the range of 100–2000 mg L^{-1}.

Reproducibility of the response

In order to investigate the repeatability of the results, three different nickel oxide-modified electrodes were tested. Each electrode was exposed to a solution of 500 mg L^{-1} His three times (Table 1). A relative standard deviation (RSD) of 2.15% was obtained. This indicates that the sensor performs in a reproducible manner.

Sensitivity of the sensor

The slope of the calibration graph is defines the sensitivity of the sensor [23]. The sensitivity of the nickel oxide-modified quartz crystal electrode was calculated with respect to His and a value of 0.0284 Hz/mg L^{-1} was obtained, which is comparable with a previously reported value [24].

Lower limit of detection (LLD) of the sensor

The LLD is defined as the lowest concentration of analyte that produces a signal that is distinguishable from a noise signal. The LLD was calculated from the calibration graph according to the method described by Miller and Miller [23]. In this study, an LLD value of 48 mg L^{-1} was obtained for the sensor.

Since the average clinical levels of His in human blood and urine are respectively 55 and 600 mg L^{-1} for people with histidinemia [3, 25], the LLD value of 48 mg L^{-1} obtained by the QCN method in this work is especially suitable for His detection in urine.

Lifetime

Further experiments were carried out in order to investigate the lifetime of the sensor. The response of the nickel oxide-modified quartz electrode to a His solution (500 mg L^{-1}) was recorded ten times within a period of four months (Table 2).

The results showed that there is no significant difference between the recorded responses over this period of time. Between each set of measurements, the crystal (cell) was stored in a medium free of moisture and contamination. It can be concluded that the sensor can be stored at ambient temperatures for many months without any loss of its recognition capabilities.

Interference

The potential for interference from some compounds in urine, such as urea, uric acid, ammonium chloride, sodium sulfate, sodium hydrogen phosphate and potassium chloride (which is expected to exist in urine as a potential interference) and a number of amino acids (Cys, cystine, Arg, Gly, Ser, Try and Tyr), was examined. All of the compounds were present in a fivefold excess (2500 mg L^{-1}) over His, except for Tyr, cystine and uric acid, which were present at saturated concentrations due to their low solubilities in

Table 2 Durability test of the frequency change in a nickel oxide-modified quartz crystal electrode exposed to 500 mg L^{-1} His

Duration from the start of the durability test	1 h	5 h	1 day	2 days	1 week	2 weeks	1 month	2 months
Frequency shift (Hz)	32	35	33	31	30	32	29	30

Table 3 Frequency changes of a nickel oxide-modified quartz crystal electrode exposed to a solution of His (500 mg L^{-1}) in the presence of interfering compounds (2500 mg L^{-1})

Compound	His	NaH_2PO_4	Arg	Cys
Frequency shift (Hz)	32	47	72	76

water. The frequency changes of the nickel oxide-modified quartz crystal electrode upon exposure to 500 mg L^{-1} His solution with a background of various compounds (urine compounds and amino acids) were recorded.

Changes in frequency of more than 5% were considered to be the result of interference. Gly, Met, Ser, Try, Typ, urea, uric acid, ammonium chloride, sodium sulfate, cystine and potassium chloride had no effect at the tested concentrations. However, Cys, Arg and sodium hydrogen phosphate interfered with the response (Table 3). These interfering effects are probably due to the ability of nickel to bind electron-rich molecules such as His. Nevertheless, the sensor shows very significant responses to His in comparison to other interfering compounds present at similar concentrations (Fig. 4).

Identification of His using principal component analysis

To evaluate the ability of the nickel oxide-coated QCN to discriminate between His and other interferences, a PCA was carried out. Since the shape of the transient response of the sensor is different for each compound (Fig. 4), this characteristic was used to distinguish between compounds using principal component pattern recognition analysis.

The input variable is the frequency of the crystal every 10 s for 7 min after the compound has been injected into the cell. All compounds were exposed to the cell for three different concentrations, including 500, 1000 and 1500 mg L^{-1}. To account for the different response magnitudes from the different concentrations, the responses were normalized and standardized to give a response vector of unity in each case, and subjected to PCA.

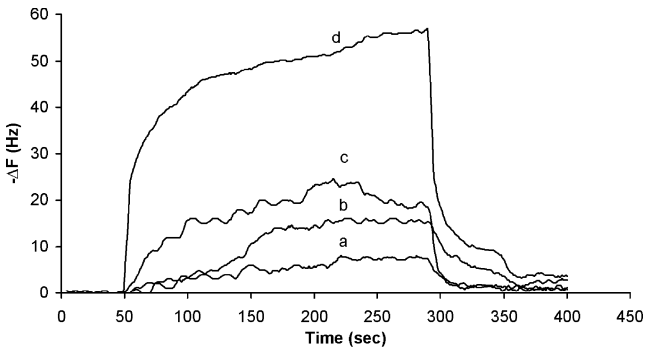

Fig. 4 Transient frequency response of the nickel oxide-coated QCN sensor upon detecting compounds. *a*, NaH_2PO_4; *b*, Arg; *c*, Cys; *d*, His

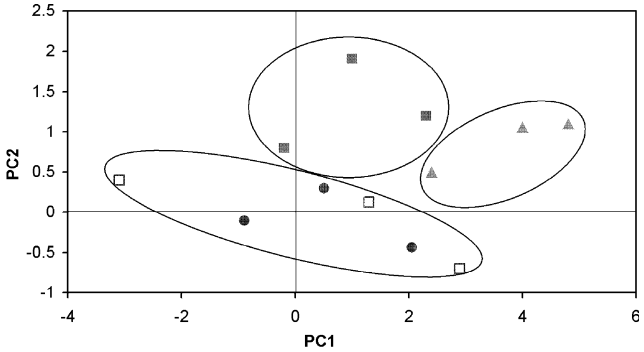

Fig. 5 Identification of His (*triangles*), Cys (*filled squares*), NaH_2PO_4 (*circles*) and Arg (*open squares*) using PCA

The results of the principal component analysis indicated that most of the information (close to 85% of it) is provided by PC1 and PC2. Figure 5 is a plot of the PCA data for different concentrations of compounds. As shown, easy discrimination of the His from other compounds was possible because the data were clustered in three different regions, independent of their concentrations.

Conclusions

A simple and inexpensive method of achieving a rapid quantitative determination of His in solution was presented. The method, based on a nickel oxide-modified quartz crystal electrode used in conjunction with PCA, demonstrated a high sensitivity and selectivity toward His solutions. A linear response was obtained for the sensor in the His concentration of 100–2000 mg L^{-1}. The RSD. of 2.15% obtained for the results indicated that they are reproducible. An LLD of 48 mg L^{-1} was obtained for the sensor. The possible interference from a number of amino acids and urine compounds was investigated. Except for Cys, Arg and sodium hydrogen phosphate, no major interference with the performance of the sensor was observed. A principal component analysis was performed based on the frequency response of the sensor every 10 s as input data. The results showed that the data for His and other (interfering) compounds were clustered in three different regions, independent of their concentrations.

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