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Pyrenyl Carbon Nanostructures for Ultrasensitive Measurements of Formaldehyde in Urine

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Abstract

Measurement of ultra-low (e.g., parts-per-billion) levels of small-molecule markers in body fluids (e.g., serum, urine, saliva) involves a considerable challenge in view of designing assay strategies with sensitivity and selectivity. Herein we report for the first time an amperometric nano-bioelectrode design that uniquely combines 1-pyrenebutyric acid units pi-pi stacked with carboxylated multiwalled carbon nanotubes on the surface of gold screen printed electrodes for covalent attachment of NAD⁺ dependent formaldehyde dehydrogenase (FDH). The designed enzyme bioelectrode offered 6 ppb formaldehyde detection in 10-times diluted urine with a wide dynamic range of 10 ppb to 10 ppm. Fourier transform infrared, Raman, and electrochemical impedance spectroscopic characterizations confirmed the successful design of the FDH bioelectrode. Flow injection analysis provided lower detection limit and greater affinity for formaldehyde (apparent K_M 9.6 ± 1.2 ppm) when compared with stirred solution method (apparent K_M 19.9 ± 4.6 ppm). Selectivity assays revealed that the bioelectrode was selective toward formaldehyde with a moderate cross-reactivity for acetaldehyde (~ 25%) and negligible cross-reactivity toward propanaldehyde, acetone, methanol, and ethanol. Formaldehyde is an indoor pollutant, and studies have indicated neurotoxic characteristics and systemic toxic effects of this compound upon chronic and high doses of exposure. Moreover, reported chromatography and mass spectrometry methods identified elevated urine formaldehyde levels in patients with bladder cancer, dementia, and early stages of cognitive impairments compared to healthy people. Results demonstrate that pyrenyl carbon nanostructures-based FDH bioelectrode design represents novelty and simplicity for enzyme-selective electrochemical quantitation of small 30 Da formaldehyde. Broader applicability of the presented approach for other small-molecule markers is feasible that requires only the design of appropriate marker-specific enzyme systems or receptor molecules.

Graphical abstract

This is the first report of a pyrenyl carbon nanostructure based enzymatic bioelectrode for quantitation of formaldehyde marker in urine representing a non-invasive small-molecule assay methodology.

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Keywords

Nanotube-pyrene; Urine samples; Small molecules; Stirring vs. flow injection; Noninvasive analysis; Combined carboxylation

1. Introduction

Biomarkers are molecular indicators that play the crucial role in the diagnosis, prognosis, and theranostics of diseases such as cancer.¹ As a result, bioanalytical methods that allow sensitive and selective measurements of biomarkers are significant for clinical applications and therapeutics development. In view of diagnostic challenges, the molecular size of a biomarker present in complex body fluid matrices inversely affects the detection sensitivity. This is because small molecules do not yield measurable assay signal changes compared to large biomolecules. Additionally, dilution of samples to minimize clinical matrix effects can further lower the biomarker concentration and its detection. False-positive signals, tedious extraction procedures of analytes from the matrices, simplicity of the assay and detection protocol, and selectivity are other related problems.

Despite the issues described, nanotechnology-based optical and electrochemical methods have allowed detection of large proteins, receptors, antibodies, DNA, and RNA biomarkers at clinically relevant pM to aM concentrations in body fluids.² However, development of such analytical assays with simplicity, selectivity, sensitivity, and low-cost for detecting small-molecule markers of cancer and other diseases non-invasively remains to be a significant challenge. In particular, bioanalytical methods for detection of ultra-low ppb levels of formaldehyde (HCHO) in a complex clinical matrix (e.g., plasma, serum, urine) suffer by interferences from non-analyte components present in the matrix (non-specific signals), poor detection capability, and issues of enzyme instability and loss of enzyme activity. Furthermore, a non-invasive detection of biomarker levels is advantageous for routine diagnosis of these diseases at an early stage as it eliminates painful invasive procedures (e.g., biopsy, bronchoscopy), which are known to cause tissue and organ damage.³ Moreover, we can eliminate minimally invasive procedures, such as drawing of blood from patients for analysis.⁴ In this study, we demonstrate for the first time the design

of pyrenyl carbon nanostructure modified electrodes for FDH immobilization and electrochemical quantitation of HCHO in a urine matrix with a 6 ppb detection limit.

Aldehydes, including HCHO, have received considerable attention as a key class of volatile organic compound markers that exhibit toxic effects in humans, and are suggested to be relevant for cancer and neural diseases.^{5–8} Many techniques have been developed to detect HCHO, including spectrophotometric,⁹ electrochemical,^{10,11} optical,¹² electronic,¹³ and colorimetric¹⁴ methods. Sensitive detection of urine HCHO by chromatography-mass spectrometry methods is known. However, these methods involve expensive instrumentation, and time consuming laborious sample preparation, measurement, and analysis steps.⁵ In contrast, electrochemical methods are straightforward and cost effective.^{15,16} In particular, electrochemical enzymatic amperometric biosensors offer the advantages of direct and quick detection of analytes with simplicity, and by appropriate design of surface strategies, high sensitivity, robustness, and excellent reproducibility can be attained.

FDH (~ 170 kDa), an enzyme of the oxidoreductase family,^{17,18} is a homotetrameric enzyme containing 398 amino acids per subunit. Ali *et al.* developed a biosensor made from NAD⁺ and glutathione-dependent recombinant FDH immobilized on the surface of a Si/SiO₂/Si₃N₄ structure.¹¹ A mediator-based amperometric biosensor was constructed on disks of woven graphite gauze coated with NAD⁺ and glutathione-independent FDH from *Hyphomicrobium zavarzinii* strain ZV 58.¹⁹ Demkiv *et al.* modified platinized graphite electrodes with NAD⁺ and glutathione-dependent FDH isolated from a genetically engineered strain *H. polymorpha*. In that study, electron transfer between the enzyme and the electrode was established using low molecular weight redox mediators or positively charged cathodic electrodeposition paints modified with Os-bis-N,N-(2,2'-bipyridyl)-chloride ([Os(bpy)₂Cl]).²⁰ FDH from *Pseudomonas* sp. immobilized on mesoporous silica materials²¹ and multiwalled carbon nanotube-modified screen printed electrodes²² for HCHO detection in buffer solution have been developed.

Herein, we report for the first-time 1-pyrenebutyric acid (PBA) pi-pi stacked with carboxylated multiwalled carbon nanotubes (MWNTs) on the surface of the gold screen printed electrodes (AuSPEs) for covalent FDH immobilization offering highly sensitive and selective ultra-low detection HCHO concentration in a 10-times buffer diluted urine. We combined here the –COOH groups of MWNTs with the non-covalently attached PBA because this combination approach, discovered by us recently, offered 3-fold improved sensitivity for a serum insulin immunosensor compared to the use of only carboxylated MWNTs.²³ HCHO was quantitated using a quinone compound (Q: 1, 2-naphthaquinone-4-sulfonic acid sodium salt) as an electron transfer mediator to obtain electrochemical signals in proportion to HCHO concentration. To our knowledge, this is the first report on pyrenyl carbon nanostructure based enzymatic bioelectrode for urine HCHO quantitation.

2. Experimental section

2.1 Materials and chemicals

Gold screen printed electrodes (AuSPEs) with a three-electrode cell configuration integrated on a ceramic substrate (Model: 250 BT, 4 mm diameter Au working electrode, Pt counter

electrode, and Ag pseudo reference electrode) were purchased from DropSens Inc. (Spain). Multiwalled carboxylic acid functionalized carbon nanotubes (MWNTs, > 8% carboxylic acid functionalized, avg. diam. 9.5 nm, length 1.5 μm), β -Nicotinamide adenine dinucleotide sodium salt (NAD^+), FDH from *Pseudomonas* sp., PBA, 1,2-naphthaquinone-4-sulfonic acid sodium salt (Q), HCHO solution (36.5 – 38% in water), 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), and formaldehyde-2,4-dinitrophenylhydrazone analytical standard (HCHO-DNPH) for liquid chromatography were purchased from Sigma and were used as received. Vivaspin 6 ultrafiltration cartridges (molecular weight cut off: 3 kDa, GE Healthcare, Little Chalfont, Buckinghamshire, UK) were used for filtration of urine. All aqueous reagents were prepared in deionized water ($\text{DI H}_2\text{O}$) using a Milli-DI water purification system (Millipore Ltd., Billerica, Massachusetts, USA). All other chemicals were high purity analytical grade.

Amperometric measurements were performed at room temperature (23°C) using a CHI 6017E electrochemical workstation coupled to a faraday cage and a picoamp booster (Austin, TX, USA). The flow-cell was connected to a syringe pump system (New Era Pump Systems Inc., NY, USA) and a sample injector valve (Rheodyne model 9725i PEEK injector, IDEX Health & Science LLC, CA, USA). The sample loop volume was 200 μL . Spectroscopic characterizations of the bioelectrode fabrication steps were carried out using Fourier transform infrared spectroscopy (FTIR, Thermo Scientific Nicolet iS50) in the attenuated total reflectance (ATR) mode. The bioelectrodes were placed directly on the ATR diamond crystal and 32 scans were taken and averaged to obtain a good signal-to-noise ratio. In addition, Raman spectroscopy (Nicolet NXR FT-Raman module, Nd:YVO₄ laser, 1064 nm, 0.2 W, resolution 4 cm^{-1}) and electrochemical impedance spectroscopy (Interface 1000 potentiostat/galvanostat/ZRA, Gamry instruments, Warminster, PA) were used for the characterization of the designed bioelectrode. Liquid chromatography-mass spectrometry (LC-MS) with an electrospray ionization source (Shimadzu LCMS-2010EV) was used for identification of HCHO-DNPH derivative, methanal 2,4-dinitrophenylhydrazone.

2.2 Surface modification of AuSPE

Briefly, on the surface of disposable AuSPEs, 5 μL dispersions of 2 mg mL^{-1} MWNTs in dimethylformamide (DMF) were added to cover the entire AuSPE surface and allowed to dry at room temperature. The electrodes were washed well with deionized H_2O and dried under nitrogen prior to adding 3 μL of 3 mg mL^{-1} PBA solution in DMF. The electrodes were kept in a cold and moist environment for 1 h to allow the formation of strong pi-pi interactions between MWNTs and PBA. The electrodes then were washed well with deionized H_2O and dried under nitrogen.

FDH enzyme was covalently linked via its free surface lysine residues (PDB: 4JLW, Figure S1) to the $-\text{COOH}$ terminal groups of both the MWNT and PBA assembly by the established carbodiimide chemistry.²⁴ To carry out this reaction, MWNT/PBA-modified AuSPEs were treated with 5 μL solution of a freshly prepared mixture of 0.35 M EDC and 0.1 M NHS for 15 min to activate the surface carboxylic acid groups of MWNTs and PBA into lysine (Lys) amine reactive N-succinimidyl esters. The electrodes were then washed with deionized H_2O to remove unreacted reagents and subsequently introduced to 5 μL of

200 µg/mL FDH solution prepared in phosphate buffered saline (PBS) and incubated for 1 h to obtain covalently linked FDH films. The prepared electrodes were used immediately for measuring HCHO in urine.

2.3 Chronoamperometric detection of formaldehyde in urine samples

Urine samples were collected from a healthy male adult volunteer in a 50 mL sterile polyethylene vial. Samples were then immediately vortexed and divided into 10 mL aliquots and stored at -20°C for up to 30 days to avoid any storage loss until further use. Before use, the frozen samples were thawed at room temperature. Prior to spiking with formaldehyde, 6 mL aliquot of the urine sample was subjected to centrifugal filtration using Vivaspin-6 cartridges at a speed of 4000 rpm for 30 min at room temperature.²⁵ Filtered urine samples were diluted 10-times in PBS. Various concentrations of formaldehyde were spiked in the 10-times diluted urine and were used for analysis.

FDH immobilized AuSPE/MWNT/PBA electrodes were attached to an in-house designed flow cell that was connected to a syringe pump and a sample injector (Figure 1A). The running buffer was made up of PBS containing 5 mM NAD^{+} and 1 mM Q. Various concentrations of HCHO prepared in 10-times diluted urine in PBS were injected via a manual injector valve onto the bioelectrode surface. Real-time measurements of the oxidation currents of QH_2 formed from NADH as a result of FDH catalyzed HCHO oxidation were performed at an applied constant potential of + 0.35 V (Figure 1B).^{21,26} The current versus HCHO concentration was plotted and the resulting curve was fit by the Michaelis-Menten non-linear regression equation available with the K-graph software used.

3. Results and Discussion

3.1 Characterization of the modified electrodes

Figure 2 shows the baseline corrected FTIR-ATR results for the modified bioelectrode surface that confirmed the covalent immobilization of FDH onto the $-\text{COOH}$ groups of AuSPE/MWNT/PBA electrodes. A strong vibrational peak at 1714 cm^{-1} indicated the presence of carbonyl stretching from the $-\text{COOH}$ groups of MWNTs. Pi-pi stacking of PBA with MWNTs red shifted the $\text{C}=\text{O}$ stretching vibration to 1698 cm^{-1} , which has been attributed to the partial electron transfer from PBA to MWNT resulting in a relatively weaker C-C bond strength in the MWNT/PBA complex than the MWNT alone.²⁷ Additionally, a broad peak at 3136 cm^{-1} for the O-H stretching was observed.²⁸ Following covalent FDH attachment via coupling of Lys amines with surface carbodiimide activated (EDC/NHS) $-\text{COOH}$ groups of PBA and MWNTs forming amide bonds, typical amide-I and amide-II bands from the peptide backbone of FDH were observed at 1683 and 1603 cm^{-1} , respectively.²⁹ The disappearance of the prior O-H stretching and appearance of a new broad band at 3472 cm^{-1} arising from N-H stretching of FDH indicated amide bond formation and thus the enzyme immobilization. Furthermore, it is possible that a partial non-covalent adsorption of the FDH onto the MWNT/PBA surface cannot be ruled out. Despite this, based on the observed FTIR spectral changes of the surface carboxylic acids the covalent FDH attachment is evident.

Additionally, Raman spectroscopy and Faradaic impedance measurements confirmed successful AuSPE sensor surface fabrication and FDH immobilization (Figures S2 and S3, and associated text of discussion of results, Supporting Information). The enzyme fabricated SPEs were assessed for HCHO oxidation in a 10-times diluted urine matrix. The ability of the enzyme to function well in the urine matrix was very important, thus adequate NAD⁺ cofactor (5 mM) was supplied. Ten times dilution of urine provided the optimum performance of the FDH bioelectrode with a minimal non-specific signals in the working potential region as discussed below in Section 3.2. FDH-catalyzed detection of HCHO was performed according to the reaction sequence shown in Figure 1B. Q was used as the electron transfer mediator to transfer electrons to the electrode from NADH formed in solution as a result of the FDH-catalyzed HCHO oxidation.²¹ This in turn regenerates NAD⁺ to receive subsequent protons upon HCHO oxidation.

3.2 Amperimetric response for the stirred vs flow injection analysis

Upon dilution of the urine matrix, an increase in the amperometric current was observed (Figure S4, tested using 1 ppm of HCHO for method optimization). This could be attributed to the reduction in high salt concentration and other interfering agents for the optimum performance of the sensor and/or the FDH enzyme.³⁰ Diluting the urine samples more than ten times did not significantly increase the amperometric response (at 95% confidence level). Hence, ten times dilution of the urine was selected for further studies.

Figure 3A-a shows the increase in currents of the bioelectrode in stirred solutions containing various concentrations of HCHO from ppb to ppm levels in 10-times diluted urine matrix at an optimum solution stirring rate of 150 rpm. Linearity of the currents vs. concentration of HCHO was observed between 100 ppb and 16 ppm (initial range in Fig. 3A-b), which is a wide dynamic range useful for clinical assay of such markers. A deviation from the linearity was observed at higher concentrations of HCHO (i.e., > 16 ppm) and followed typical electrochemical Michaelis-Menten enzyme kinetics (Fig. 3A-b).³¹ The estimated apparent Michaelis-Menten constant (K_M^{app}) using KaleidaGraph software (version 4.1) was 19.9 ± 4.6 ppm. The limit of detection (LOD) (the signal at three times the standard deviation of the mean of the HCHO unspiked control urine sample³²) of the described stirred solution-based HCHO detection was 73 ppb.

To examine improvements on the detection sensitivity of the stirred solution method by facilitating better mass transport of HCHO and other assay reagents, we used flow injection analysis.³³ The cofactor and electron transfer mediator containing solution mixture (5 mM NAD⁺ and 1 mM Q in PBS, pH 7.4) was delivered at an optimum flow rate of $100 \mu\text{L min}^{-1}$ onto the FDH bioelectrode surface using a syringe pump. Various concentrations of HCHO spiked in 10-times diluted urine in PBS (pH 7.4) then were injected via a manual injection valve, and the resulting oxidation currents were measured (Figure 3B-a).

The sensitivity of HCHO detection was only moderately enhanced (by two times) in the flow injection method compared to the stirred solution (calculated from the slopes of the initial linear range in Fig. 3A-b and 3B-b). Possible reason could be that the HCHO and mediators used are small molecules. As a result the mass transport by convection (stirring vs fluid flow) does not seem to be greatly different in view of sensitivity (i.e., the slope of

response plots covering the linear range of HCHO concentrations). Nevertheless, the flow injection analysis significantly decreased the LOD to 6 ppb, which is 12-fold smaller than the stirred-solution method. This is likely because diffusion of reactants to products (cofactor, urine matrix, and mediator reaching the electrode and the product diffusing away) becomes much more prominent at the lowest concentration corresponding to the LOD. As a result, the flow injection method for the designed bioelectrode seems to be better than the stirred-solution analysis in facilitating effective electronic communication of the low levels of urine HCHO with the surface of the AuSPE/MWNT/PBA enzyme electrode.

Reported studies based on chromatography and mass spectrometry methods identified increased levels of HCHO present in the urine samples of patients with dementia and bladder cancer conditions compared to healthy individuals. Thus, the obtained LOD of the FDH bioelectrode for measuring HCHO in urine meets the reported clinically relevant range [e.g., bladder cancer (> 85 ppb),³⁴ dementia (> 5.8 ppm),^{8a} and early stages of cognitive impairments in older adults (> 330 ppb)^{8b}]. The lower LOD illustrates the advantages of flow injection analysis in improving analyte mass transport and minimizing noise levels, resulting in enhanced signal-to-noise ratio and thus an improved detection limit. The linear dynamic range of our flow analysis for HCHO in 10-times diluted urine was from 10 ppb to 10 ppm (Figure 3B-b). The relationship between current signal and HCHO concentration was fit by the Michaelis-Menten non-linear regression curve (Figure 3B-b) using KaleidaGraph software (version 4.1).

The estimated K_M^{app} of flow injection method was 9.6 ± 1.2 ppm, which is better than the stirred-solution method and likely due to enhanced substrate mass transport facilitating kinetically faster steady state conditions in the flow system. The observed difference between the affinity constants was calculated to be significant at 95% confidence level. Previously reported K_M values of HCHO solution bioassays were 5.4 ppm for FDH from genetically engineered *H. polymorpha*,²⁰ 7.5–8.7 ppm for a homologous enzyme from *Candida boidinii*,³⁵ 6.3 ppm for FDH from *H. polymorpha*,³⁶ and 12.9 ppm for FDH from *Pichia pastoris*.³⁷ Results indicate that the K_M^{app} values obtained in the present work are comparable with the reported bioassays.

The presence of HCHO in the prepared urine samples was independently verified by 2,4-dinitrophenylhydrazine (DNPH) derivatization of HCHO and identification of the resulting methanal 2,4-dinitrophenylhydrazone derivative (HCHO-DNPH) product using the liquid chromatography-mass spectrometry method (LC-MS, Figure S5 and associated text of discussion of results, Supporting Information).

The presented FDH bioelectrode design offers sensitivity, ultra-low clinically useful LOD, and simplicity, but it is also essential to meet selectivity criteria for clinical diagnosis of small-molecule disease markers. Figure 4 depicts the current signals for HCHO with two immediate aldehyde homologues and with acetone and two types of alcohol. The current responses confirm the high selectivity for HCHO, with ~ 25% cross-reactivity for acetaldehyde (CH_3CHO) and negligible cross-reactivity for propanaldehyde, acetone, methanol, and ethanol. The moderate cross-reactivity for acetaldehyde suggests that the

substrate binding pocket of the FDH enzyme has slight affinity to this immediate homologous member.

3.3 Stability and selectivity of the electrode

The fabricated FDH bioelectrodes were tested for film stability in PBS (pH 7.4 at 23°C) for 40 h by non-faradaic impedance spectroscopy at an applied constant frequency of 5 Hz (Figure S6, Supporting Information).³⁸ A stable impedance magnitude of 232 Ω was noted for more than 30 h. It is worth mentioning that AuSPEs are intended to be for disposable use and hence the observed stability upon continuous soaking of the modified, FDH attached SPE surface in a buffer solution is reasonably good.

Excluding the MWNT/PBA surface modification of AuSPEs, the bioelectrode design only required one step of FDH immobilization and subsequent detection and quantitation of HCHO in 10-times diluted urine. The various sample matrices used in the literature for HCHO and the reported linear dynamic range, detection limit, sensitivity, and bioelectrode stability are presented in Table 1. This comparison shows better performance of the pyrenyl-carbon nanostructure bioelectrode than simple buffer solution-based HCHO quantitation methods.

4. Conclusions

Results presented confirm the analytical detection features of pyrenyl carbon nanostructure-modified FDH bioelectrode for sensitive and selective quantitation of urine HCHO to relate to abnormal conditions that are known to result in elevated urine HCHO. The presented approach provides a viable nano-bioelectrode design for non-invasive detection of small-molecule markers of cancer and other diseases at clinically relevant ultra-low levels in complex matrices. Combining with measurements of other biomarkers and assays of significance is expected to allow successful diagnostic outcome of an abnormal condition. By appropriate immobilization of marker specific receptor molecules or enzymes coupled with a detection probe or mechanism, the proposed methodology is expected to allow broader applicability for quantitative measurement of any other small-molecule markers with selectivity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

- First pyrenyl carbon nanostructure based bioelectrode for urine formaldehyde quantitation
- Sensitivity and selectivity with a wide dynamic range and a few parts-per-billion detection limit
- Comparative analytical features of stirred-solution and flow-injection amperometric analysis
- Surface design for ultra-low measurements of small molecules present in complex sample matrices

Figure 1. (A) Schematic of the microfluidics system used in this study. (B) Fabrication steps of the AuSPEs with FDH and the reaction sequence for catalyzing HCHO and detection by flow injection or stirred solution amperometry.

Figure 2.

FTIR spectra of AuSPE coated with (a) carboxylated MWNTs, (b) after PBA stacking, and (c) after covalent immobilization of FDH.

Figure 3.

(A) Stirred solution method: (a) Amperometric responses of the AuSPE/MWNT/PBA-FDH bioelectrode for various concentrations of HCHO in 10-times diluted urine in PBS, pH 7.4, containing 5 mM NAD^+ and 1 mM Q at an applied potential of +0.35 V at 23°C and a constant stirring of solution using a magnetic stirrer at 150 rpm. Inset shows the enlarged view for lower HCHO concentrations. (b) Michaelis-Menten fit of the designed bioelectrode in oxidizing HCHO. **(B) Flow injection analysis:** (a) Amperometric responses for the AuSPE/MWNT/PBA enzyme bioelectrode upon injection of various concentrations of HCHO at a flow rate of $100 \mu\text{L min}^{-1}$. Inset shows the enlarged view for lower HCHO concentrations. (b) The corresponding Michaelis-Menten fit of the experimental data.

Figure 4. Selectivity of the designed AuSPE/MWNT/PBA-FDH bioelectrode for HCHO over other similar analytes in stirred solutions. Current signals for 5 ppm of analytes (x-axis) in 10-times diluted urine solutions in PBS are shown.

Table 1

Comparison of the present MWNT/PBA-FDH nano-bioelectrode with relevant reported studies.

Sensor type	Construction material	Sensing method	Sensing element	Matrix	Linear dynamic range/ Sensitivity/Detection limit	Ref.
Planar electrochemical transducers	Potentiometric sensor – Si ₃ N ₄ -ISFET Conductometric and amperometric sensor – gold interdigitated electrodes vacuum deposited with sintered aluminum oxide	Potentiometry, conductometry, and amperometry	Alcohol oxidase from <i>Hansenula polymorpha</i>	10 mM Phosphate buffer (pH 7.7)	150 – 9000 ppm	39
Enzyme biosensor	Si/SiO ₂ /Si ₃ N ₄ structure as physical transducers	Capacitance	NAD ⁺ and glutathione-dependent recombinant formaldehyde dehydrogenase	2.5 mM Borate buffer (pH 8.40)	0.3 – 600 ppm Sensitivity 31 mV/decade Detection limit 0.3 ppm	11
Amperometric enzyme sensor	Disks of woven graphite gauze	Amperometry	NAD ⁺ and glutathione-independent formaldehyde dehydrogenase from <i>Hyphomicrobium zavarzinii</i> strain ZV 58	0.1 M KCl, 80mM KH ₂ PO ₄ (pH 8.0)	0.5 – 15 ppm Sensitivity 0.39 µA/ppm	19
Amperometric biosensor	Platinized graphite electrode modified with low-molecular free-diffusing redox mediators or positively charged cathodic electrodeposition paints modified with Osbis-N,N'-(2,2' - bipyridyl)-chloride ([Os(bpy) ₂ Cl])	Amperometry	NAD ⁺ and glutathione-dependent formaldehyde dehydrogenase isolated from a geneengineered strain of the methylotrophic yeast <i>Hansenula polymorpha</i>	20 mM Phosphate buffer (pH 8.2)	0.3 – 3 ppm Sensitivity 358 Am ⁻² M ⁻¹ Detection limit 90 ppb	20
Electrochemical biosensor	Glassy carbon electrode containing a membrane constructed with mesoporous silica materials	Amperometry	Formaldehyde dehydrogenase from <i>Pseudomonas</i> sp.	Phosphate buffer (pH 7.4)	30 ppb – 30 ppm Detection limit 36 ppb	21
Electrochemical biosensor	Screen-printed carbon electrode modified with MWCNT	Amperometry	Formaldehyde dehydrogenase from <i>Pseudomonas putida</i>	PBS (based on <i>in situ</i> released HCHO from a prodrug treated cancer cells in PBS)	3 ppb – 3 ppm Detection limit 300 ppb	22
Electrochemical biosensor	Screen printed gold electrodes modified with MWNT/PBA-FDH via the –COOH groups of both MWNT and PBA	Amperometry	Formaldehyde dehydrogenase from <i>Pseudomonas</i> sp.	10-times diluted urine (in PBS, pH 7.4)	Stirred solution analysis: 100 ppb – 16 ppm Sensitivity (based on the initial linear range, Fig. 3) 174 nA/ppm Detection limit 73 ppb Flow injection analysis: 10 ppb – 10 ppm Sensitivity (based on the initial linear range, Fig. 3) 342 nA/ppm Detection limit 6 ppb	This work