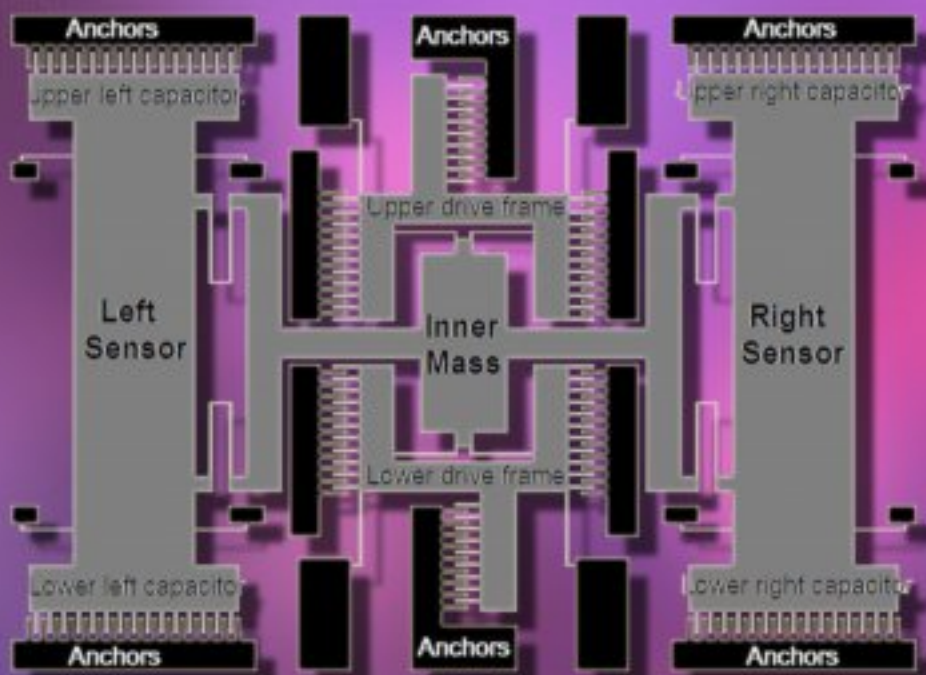


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Biomedical Applications of Modified Carbon Glassy Electrode Sensor with Nanoparticles and Dendrimers

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Abstract: We previously reported the development of a biosensor platform that is capable of measuring biometabolites and environmental sensitive species, such as peroxide and nitrate/nitrite, to concentrations in the order of ppb (parts per billion) or lower. In this investigation, we modified our platform with dendrimers to enhance its performance. Zero and second generation of dendrimers were coated on the surface of a carbon glassy electrode which was then modified with l-glutamate dehydrogenase (GDH) and α -keto glutarate. The resulting electrode was tested with ammonium solutions, concentrations ranged from 2 to 300 nM at pH 7.4; the results were satisfactory. Measurements at lower concentrations had better resolution than at higher concentrations and it is believed that the measurement limit can be lower than 2 nM. This biosensor platform was proven to be versatile and can be employed as a platform for ultrasensitive detecting devices in many biomedical and environmental applications. *Copyright* © 2011 IFSA.

Keywords: Biosensor, GDH, Ammonium, Glassy carbon electrode, PAMAM.

1. Introduction

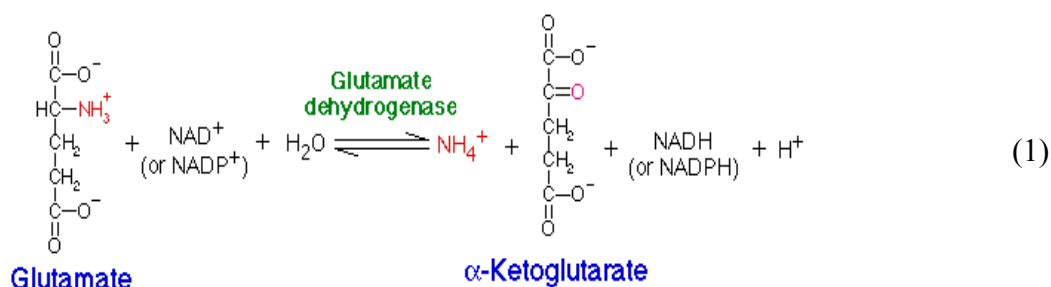
In the past several years, our research group has been developing a platform for biosensor construction that is capable of detecting target species that are in nano and subnanomolar levels [1, 2]. This sensor

platform is based on an Au electrode modified with sol-gel Au colloid and biocatalyst that is specific to the chemical species to be detected, the Au colloid comprises nano-size Au particles and cysteamine which binds strongly on the surface of the metal electrode and provide stable linkage with the nano gold particles and biocatalysts. Thus far, biometabolites and environmental sensitive chemicals such as nitrite/nitrate and peroxide were successfully detected at level of nM and below with this sensor platform.

In this study, we were developing another biosensor platform that can be used to measure biometabolites and environmental sensitive chemicals based on a non-metal glassy carbon electrode modified with sol-gel Au colloid that is similar to the sensor platform mentioned previously, except the binding polymer would be a dendrimer (polyamidoamine, PAMAM) instead of cysteamine. A dendrimer was chosen to be the linker between the electrode and Au particles/catalysts because of its low toxicity and highly branched structure that presumably can provide more anchoring sites for the Au particles and catalysts. Thus, the dendrimer can enhance the performance of the sensor platform. However, PAMAM does not have an S atom in the molecular structure and may not bind as well on the electrode's wall thus would be less durable. Performance of using cysteamine and dendrimer as a linker for the same sensor platform was compared in this study and the stability and durability of the sensor would be investigated. Ammonia, which is a major biometabolite and environmental sensitive chemical, was used as the surrogate chemical to be tested by this sensor platform [3].

1.1. Theory

As shown in the following Equation (1), glutamate and nicotinamide adenine dinucleotide (NAD^+) can be hydrolyzed to form α -keto glutarate, β -nicotinamide adenine dinucleotide reduced (NADH), and ammonium ion with the enzyme, glutamate dehydrogenase (GDH). The equilibrium constant is in favor of the formation of glutamate and thus the reverse reaction is faster kinetically [4].



For many biological reactions, the end product or metabolite is ammonium; accumulation of a high concentration of ammonium is toxic to the body. Environmentally, ammonium is a byproduct of many industrial processes, uncontrolled discharges of ammonium will lead to harmful consequence to the environment. Our goal here was to develop a sensor device that is nontoxic and can measure the ammonium ion at the lowest concentration possible; its utilities can be found in many biomedical or environmental applications. Hence, it is the reverse reaction as shown above (glutamate formation) that we utilized in this study; however, the same sensor can be used to detect α -keto glutarate or glutamate with a slight alternation of the measuring approach. Measurements of these chemical species mentioned above that were reported in literature were mostly in the range of mM [5-7]. It should be noted that the motivation of this study was to develop a sensor that can measure low concentration of the target species, more specifically, it is to relate the current measurements exerted by the reacting species in a solution (with characteristics peaks of oxidation and/or reduction) by cyclic voltammetry

to concentrations of the species, rather than to determine the reaction kinetics of the measuring species of which in many instances are already known.

2. Materials and Methods

2.1. Materials

L-glutamic dehydrogenase (GDH, from bovine liver, solution in 50 % glycerol) was purchased from Sigma-Aldrich (St. Louis, MO, USA), the concentration of GDH was 28 mg protein/mL, and 46 units/mg. Cysteamine, polyamidoamine dendrimer generation 0 (PAMAM_0), polyamidoamine dendrimer generation 2nd (PAMAM_2), α -keto glutarate, β -nicotinamide adenine dinucleotide reduced disodium salt hydrate (NADH), AuCl₃HCl·4H₂O (Au %> 48 %) and Na₃citrate were purchased from Sigma. All the other chemicals were of analytical grade or highest grade available.

2.2. Electrode Preparation

The cleaned glassy carbon electrode (GCE) was first immersed in 0.1 M cysteamine solution in darkness. The resulting monolayer-modified electrode was rinsed thoroughly with twice-distilled water and soaked in distilled water. Then, it was dipped into the colloidal gold. The gold colloid–cysteamine-modified electrode was dipped into the l-glutamate dehydrogenase (GDH) solution (pH 7.4) (or GDH solution containing NADH). In such a way, a GDH (or GDH/NADH) gold colloid–cysteamine-modified glassy carbon electrode was obtained (GCE cysteamine-Au-GDH or cysteamine-Au-GDH/NADH).

For electrode that was modified by PAMAM only, either PAMAM_0 or PAMAM_2 was used in place of cysteamine, these resulting electrodes were termed GCE PAMAM_0 or PAMAM_2-Au-GDH or GDH/NADH. In cases that both cysteamine and PAMAM were coated onto electrodes, the cleaned glassy carbon electrode was first immersed in cysteamine solution, the resulting electrode was dipped into the PAMAM solution (PAMAM_0 or PAMAM_2). The gold colloid–cysteamine/PAMAM-modified electrode was dipped into the GDH solution (pH 7.4) (or GDH solution containing NADH). In such a way, a GDH (or GDH/NADH) gold colloid–cysteamine/PAMAM-modified glassy carbon electrode was obtained (GCE cysteamine/PAMAM_0 or PAMAM_2-Au-GDH or GDH/NADH).

2.3. Nanoparticles Solution Preparations

Nanoparticles Au was prepared by reacting HAuCl₄ with citric acid [2].

2.4. Detections

UV-VIS spectrophotometry was carried out by an Agilent diode-array spectrophotometer (Agilent Model 8453); cyclic voltammetry was conducted by using a Gamry 600 Potentiostat. Voltammetric potential was measured against a saturated chloride electrode (SCE). The experimental setup of the cyclic voltammetry is shown in Fig. 1.

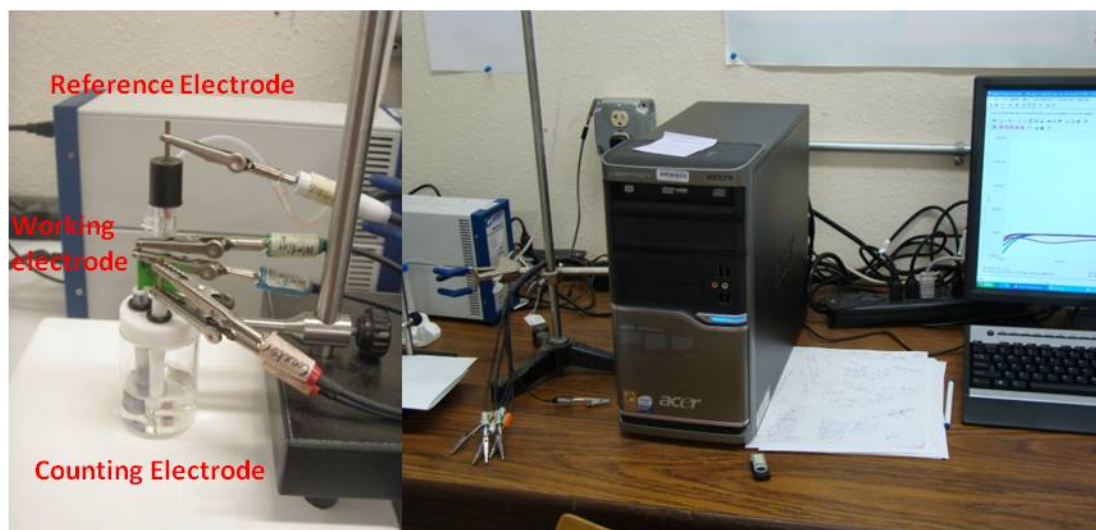


Fig. 1. Cyclic voltammetry and the sensor measurement cell.

2.5. Experimental Procedures

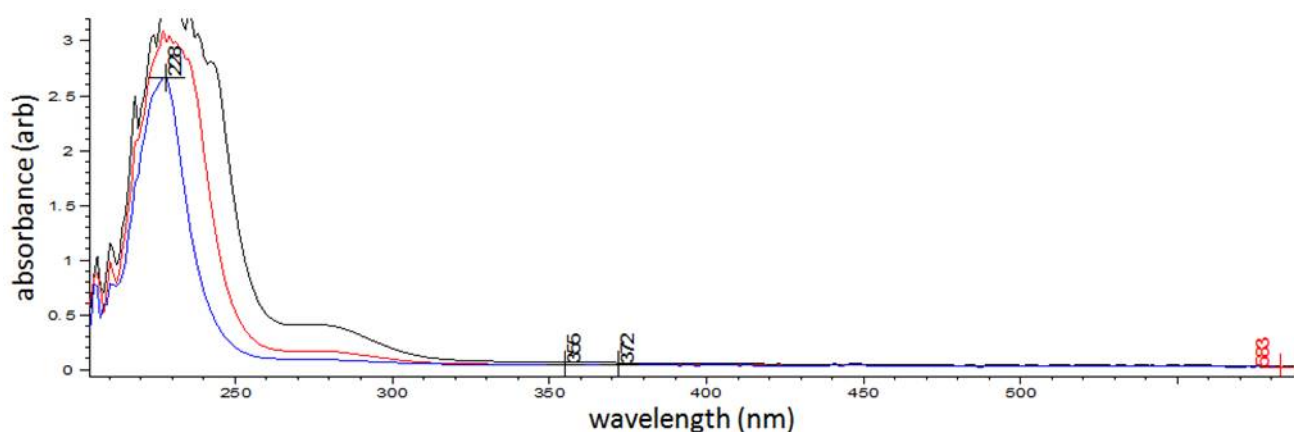
For all experiments, the measurement cell contained 5 mL of 2.5 mM of NADH, then a combined solution of α -keto glutarate and NH_4^+ was injected into the reaction cell for measurement periodically, at 60 second intervals; injection volume varied from 10 to 1500 μL depending on the concentration required. The concentration of α -keto glutarate solution was 1×10^{-6} mol/L, NH_4^+ was 1×10^{-6} mol/L. All experiments were conducted at pH 7.4 in a 0.1 M phosphate buffer solution under deoxygenated condition and all solutions were prepared with double deionized distilled water.

Measurement of current (i) with time was usually at about 700 mV (vs. SCE) for the reduction reaction and at near 2.0 mV for the oxidation reaction. The resulting concentrations of α -keto glutarate and NH_4^+ in the reaction cell ranged from 2 to 300 nM after each injection unless otherwise stated.

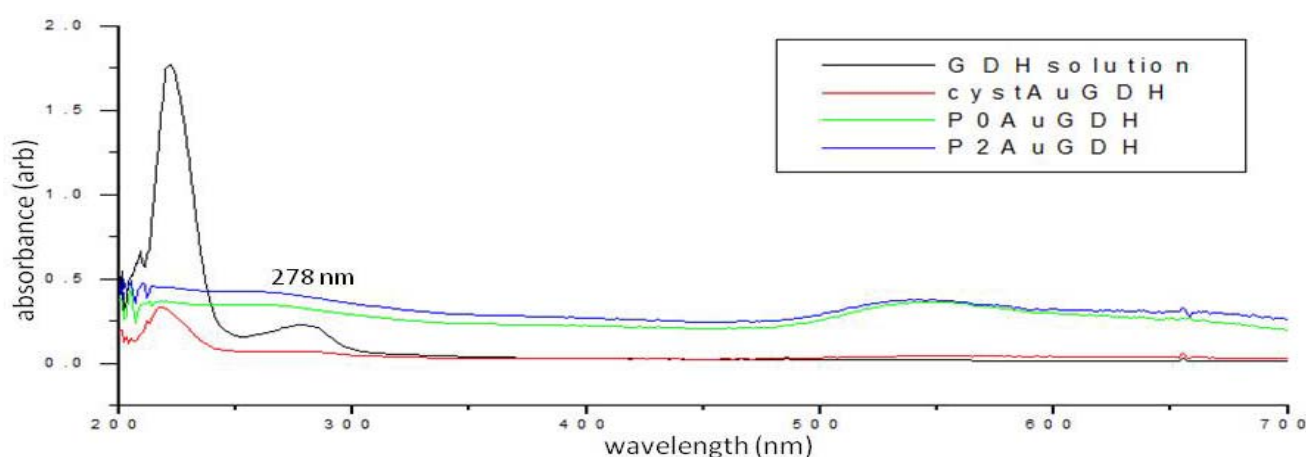
3. Results and Discussion

3.1. Identification of PAMAM Binding with GDH in Sol-Gel Au Colloid

Fig. 2(a) shows the UV-VIS spectrum of PAMAM_2 solution at various concentrations. PAMAM's in general have major absorption peak at 227 nm, but a minor peak also appears at 278 nm. The peak at 278 disappears at dilute concentration as shown in blue in Fig. 2(a). GDH solution has similar spectrum as PAMAM_2 except that its absorptivity is less intense at 278, as shown in black in Fig. 2(b). Fig. 2(b) also shows the spectra of a plastic cuvette (made for UV-VIS range) coated with cysteamine-Au-GDH, PAMAM_0-Au-GDH, and PAMAM_2-Au-GDH in red, green, and blue, respectively. The peak at near 548 nm is associated with Au particles. The peak at 227 nm would nearly disappear for the coating (sol-gel colloid) containing cysteamine (red line) after the cuvette was rinsed with deionized water numerous times, but a small peak at 278 nm would remain, that was believed to be a combined cluster layer of cysteamine and GDH situated in the Au colloid. The same can be observed if rinsing was done with the PAMAM's coating, however, the remaining cluster peak at 278 nm was much larger and the Au peak at 548 nm was accordingly higher. Hence, it can be reasoned from the magnitude of the absorbance at 278 nm that PAMAM's can bind better with the Au nanoparticles and GDH to form a colloid attached onto the electrode surface than cysteamine with Au nanoparticles and GDH in this self-assembly process.



(a)



(b)

Fig. 2. UV-VIS spectra of PAMAM_2 and other sensor materials coated on an UV-VIS cuvette: (a) Spectra of PAMAM_2 solutions at various concentrations, characteristic peaks of PAMAM's are at 227 and 278 nm. At lower concentration, as shown in blue, PAMAM_2 only has absorption peak at 227 nm; (b) Spectrum of GDH solution and spectra of an UV-VIS plastic cuvette coated with cysteamine-Au-GDH, PAMAM_0-Au-GDH, and PAMAM_2-Au-GDH in black, red, green, and blue, respectively.

3.2. Voltammetric Responses of the Modified GCE's for NH_4^+ Detection

We have explored several combinations of sol-gel Au colloids for the sensor development to detect ammonia, these combinations were: Cysteamine/PAMAM_0-Au-GDH, cysteamine/PAMAM_2-Au-GDH, cysteamine/PAMAM_0-Au-GDH/NADH, and cysteamine/PAMAM_2-Au-GDH/NADH. We have carried out a wide-range of ammonia concentrations from 2 nM to 300 nM with all the GCE's modified with different sol-gel Au colloid combinations in order to determine the champion performer. Trial measurements of the electrodes indicated that two distinguish characteristic peaks could be used to relate proportionally the concentrations of ammonia with heights of current peaks: a reductive peak at near 700 mV and an oxidative peak at 2 mV. Fig. 3 in the following is the voltammograms for the ammonia measurements generated by using a GCE modified with PAMAM_2-Au-GDH. The blue voltammogram is measurement of the blank in phosphate buffer solution. It should be noted that the reductive peaks at 700 mV shifted to higher positive voltage with increase of ammonia concentrations. The oxidative peaks at about 2 mV were also detected but were not obviously observed from the figure.

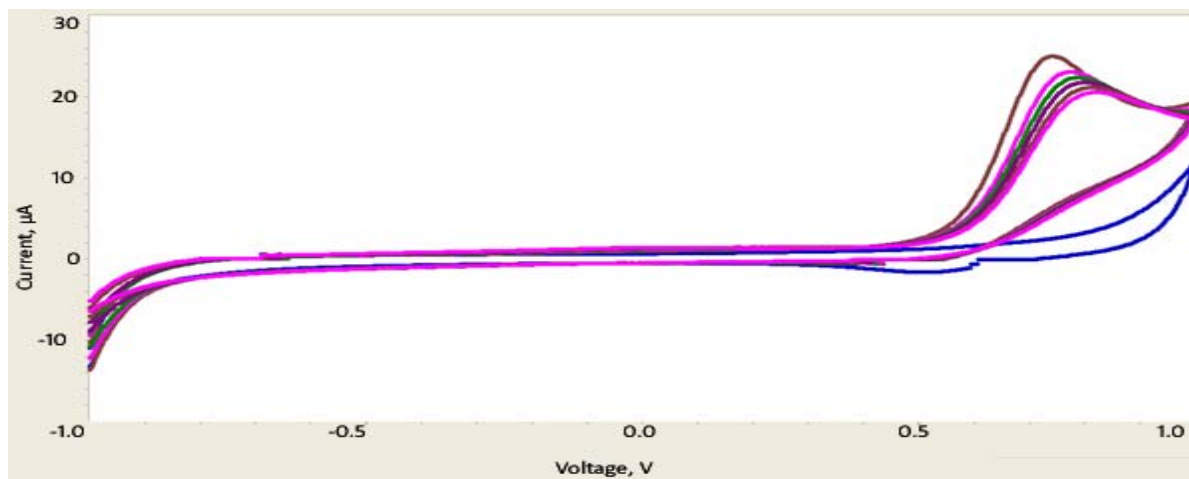
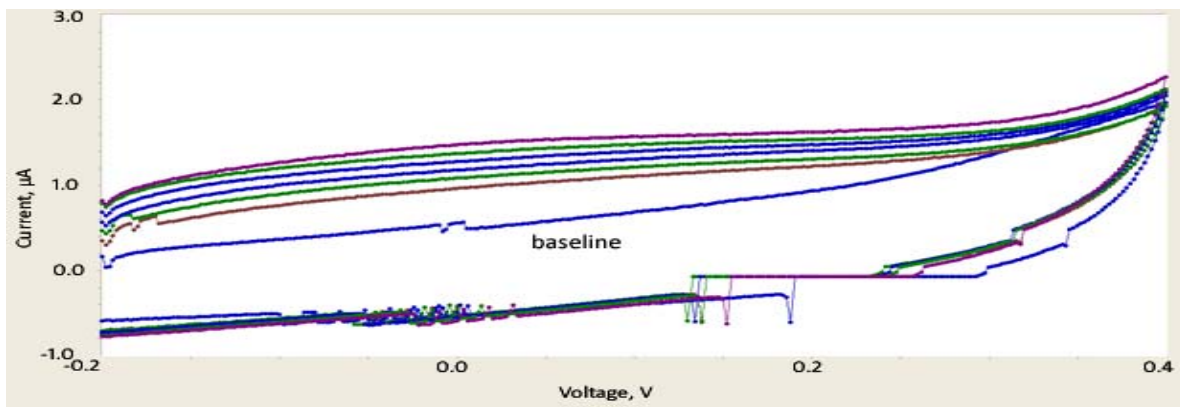


Fig. 3. Cyclic voltammograms of reaction of NH_4^+ with α -keto glutarate at pH 7.4 for concentrations from 2 to 300 nM.

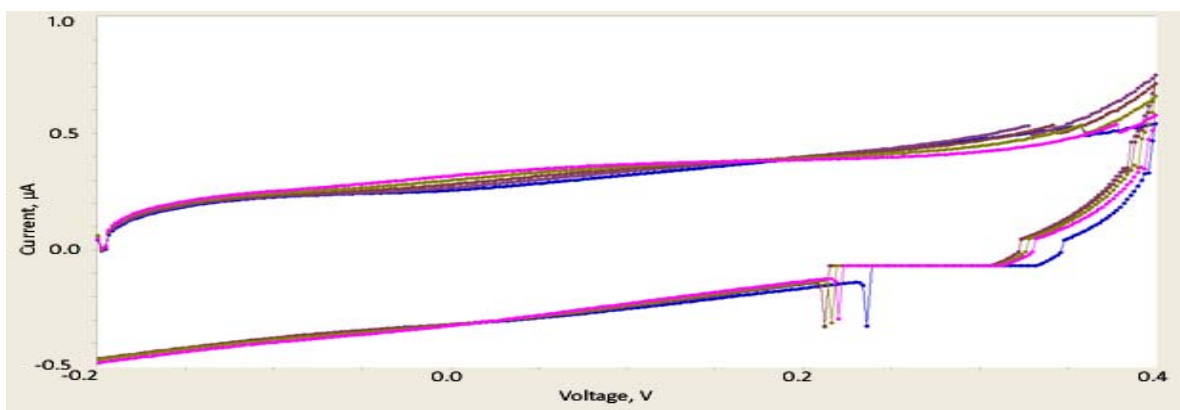
Overall, GCE modified with either PAMAM_0 or PAMAM_2-Au-GDH/NADH worked best for the detection of ammonia with α -keto glutarate, although there was no significant detection advantage by using the more branched PAMAM_2 in the sensor preparation. Detection of NH_4^+ concentrations can be better demonstrated by using the oxidative peaks at 2 mV. Voltammograms of the oxidative peaks at 2 mV of the ammonia reaction with α -keto glutarate using GCE modified by PAMAM_2-Au-GDH/NADH and cysteamine-Au-GDH are shown in Fig. 4 (a) and Fig. 4 (b) respectively.

As shown in Fig. 4(a), there was about 0.45 μA difference between the blank buffer solution (in blue) and the measurement of 2 nM NH_4^+ solution (in brown), which indicated that the sensor platform is capable of measuring NH_4^+ concentration to subnanomolar level. Fig. 4(b) is the voltammograms of the same solutions, but current increments of the oxidative peaks were relatively small, which indicates that the PAMAM_2-Au-GDH/NADH modification makes a more superior sensor for low ammonia concentration detection. The more efficient of NADH oxidation in the system could also be attributed, at least partially, by the direct binding of NADH with GDH in the Au colloid.

Fig. 5 shows the amperometric responses measured by 5 sensors using the same platform with different modifications for the reaction of NH_4^+ with α -keto glutarate at 2 mV for concentrations ranged from 2 to 300 nM. It can be observed that there were two near linear regions that can be explored to be used for sensor design: a sensitive region at concentrations less than 20 nM, and a relatively less sensitive region at concentrations larger than 40 nM. One may conclude that this non-metal biosensor platform is ideal for making sensor for ammonia detection at extremely low concentrations (>20 nM). It is unclear at this time about the mechanisms why the modified GCE's become less sensitive at the higher concentration region, it is conceivable that there is a limited quantity of Au colloid attached to the GCE surface and its ability to transfer electrons is saturated and hence turns inefficient at higher concentration.



(a)



(b)

Fig. 4. (a) Cyclic voltammograms of PAMAM_2-Au-GDH/NADH modified GCE for detection of NH_4^+ at pH 7.4., concentrations of NH_4^+ varied from 2 to 300 nM. The bottom reduction line in blue was measurement for a blank. (b) Same measurements with a GCE modified with cysteamine-Au-GDH.

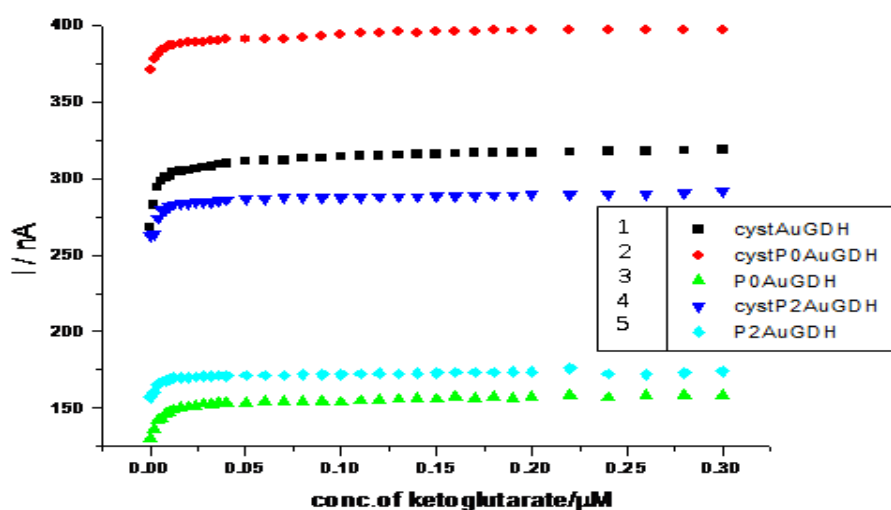
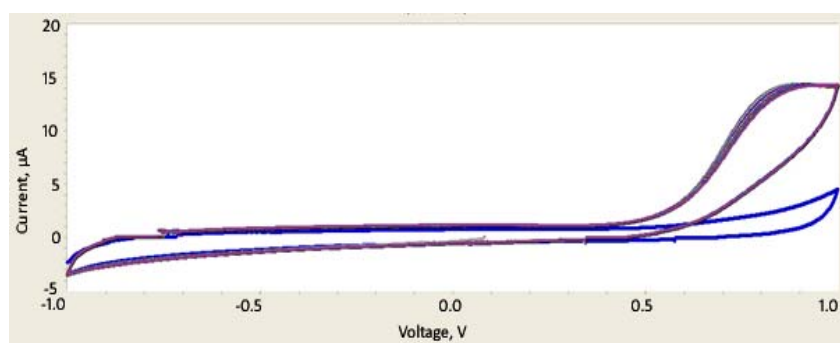


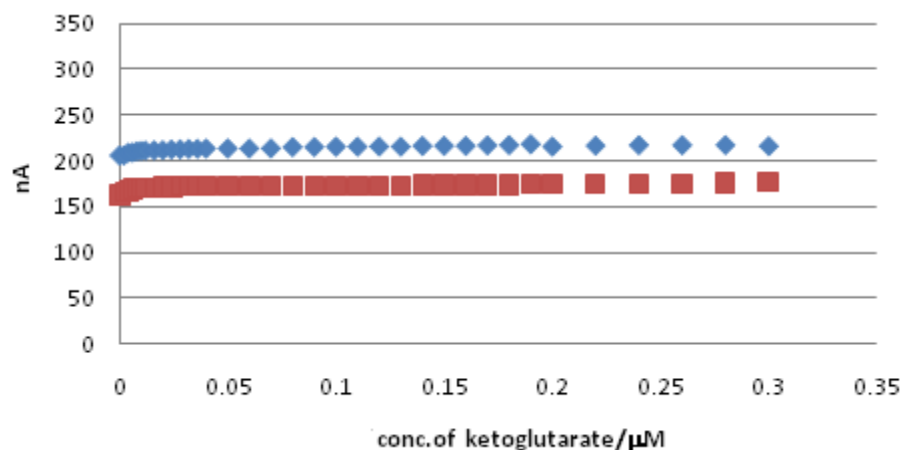
Fig. 5. Amperometric responses of ammonia reaction with α -keto glutarate from 5 different GCE sensors modified with similar platform materials: black is cysteamine-Au-GDH, red is cysteamine/PAMAM_0-Au-GDH, green is PAMAM_0-Au-GDH, blue is cysteamine/PAMAM_2-Au-GDH, light blue is PAMAM_2-Au-GDH.

3.2. Reproducibility and Stability of the Modified GCE's

An ideal sensor platform should be able to generate measurements that are reproducible and the system is stable with time, thus the measurements are consistent and reliable. Fig. 6 (a) shows the 50 cyclic responses of a typical modified GCE in the same ammonia solution. It can be seen that some deterioration occurred (as shown by the characteristic peaks at near 700 mV) after the 50 cycles but the results are relatively consistent. Fig. 6 (b) shows the amperometric responses of a GCE modified by PAMAM_0-Au-GDH/NADH at 2.0 mV after 2 weeks stored inside a refrigerator at 4 °C, the responses are nearly identified with the same trend, the measurements indicated that the sol-gel Au colloid stabilized the GDH/NADH within the cluster and prevented the bioenzyme/biochemical from deteriorating. It is well known that these bioenzyme/biochemical are very unstable, especially under dilute concentrations as they were in this study. These results demonstrated that the biosensor platform has reasonable reproducibility and stability.



(a)



(b)

Fig. 6. (a) Voltammograms of a modified GCE with 50 measurement cycles of the same solution; (b) Amperometric responses of a GCE modified by PAMAM_0-Au-GDH/NADH at 1.8 mV, before (blue) and after (red) 2 weeks stored inside a refrigerator at 4 °C.

4. Conclusions

We successfully modified our glassy carbon electrode biosensor platform [2] for ammonium detection; we further modified the sensor platform with PAMAMs and the detection lower limit was enhanced. This highly modified electrode can detect ammonium concentrations down to 2 nM or lower. The low detection limit of this biosensor is far more superior than most available detection methods in the

public domain. This biosensor platform can be modified to be used for many measurement applications in biomedical and environmental field that require high sensitivity at low concentrations. Currently, the challenge of biosensor development is not in finding the right enzymes or conjugate reactions (Equation (1)) that govern the particular species to be detected, it is the portability, stability, and noise reduction that demand further research and development.

Acknowledgement

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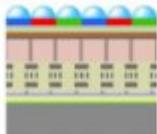
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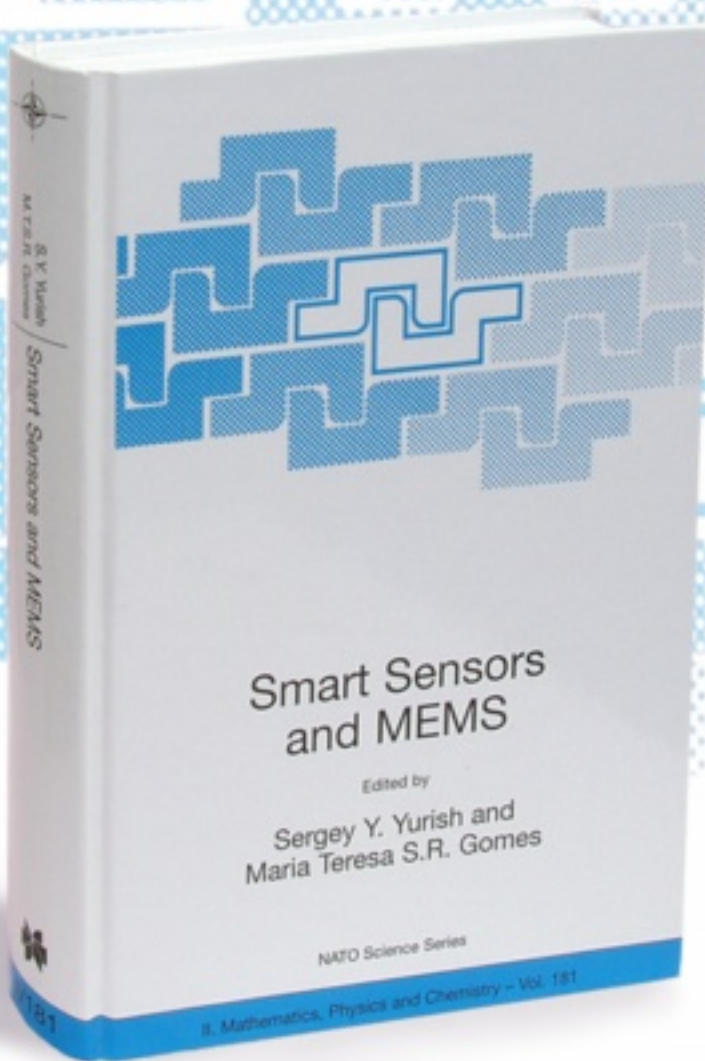
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