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pH-Switchable Electrochemical Sensing Platform based on Chitosan-Reduced Graphene Oxide/Concanavalin A Layer for Assay of Glucose and Urea

Yonghai Song,[†] Hongyu Liu,[†] Hongliang Tan,[†] Fugang Xu,[†] Jianbo Jia,[‡] Lixue Zhang,[§] Zhuang Li,[‡] and Li Wang*[,†]

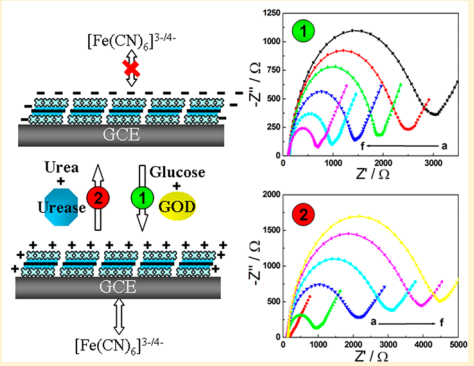
[†]College of Chemistry and Chemical Engineering, Jiangxi Normal University, 99 Ziyang Road, Nanchang 330022, China

[‡]State Key Lab of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun 130022, China

[§]Qingdao Institute of Bioenergy and Bioprocess Technology, Chinese Academy of Sciences, Qingdao 266101, China

Supporting Information

ABSTRACT: A facile and effective electrochemical sensing platform for the detection of glucose and urea in one sample without separation was developed using chitosan-reduced graphene oxide (CS-rGO)/concanavalin A (Con A) as a sensing layer. The CS-rGO/Con A with pH-dependent surface net charges exhibited pH-switchable response to negatively charged $Fe(CN)_6^{3-}$. The principle for glucose and urea detection was essentially based on in situ pH-switchable enzyme-catalyzed reaction in which the oxidation of glucose catalyzed by glucose oxidase or the hydrolyzation of urea catalyzed by urease resulted in a pH change of electrolyte solution to give different electrochemical responses toward $Fe(CN)_6^{3-}$. It was verified by cyclic voltammograms, differential pulse voltammograms, and electrochemical impedance spectroscopy. The resistance to charge transfer or amperometric current changed proportionally toward glucose concentration from 1.0 to 10.0 mM and urea concentration from 1.0 to 7.0 mM. On the basis of human serum experiments, the sensing platform was proved to be suitable for simultaneous assay of glucose and urea in a practical biosystem. This work not only gives a way to detect glucose and urea in one sample without separation but also provides a potential strategy for the detection of nonelectroactive species based on the enzyme-catalyzed reaction and pH-switchable biosensor.



As an important research area of analytical chemistry, electroanalytical chemistry has attracted more and more attention due to its low-cost instrumentation, high sensitivity, simplicity, rapid response, etc.[1−10] A long-term challenge of electroanalytical chemistry is the analysis of nonelectroactive substrates.[11] Over the past few decades, important advancements in nonelectroactive species assay have been achieved as a result of the introduction of the competitive reaction[11] and supramolecular principles,[12] which opens new opportunities for the assay of nonelectroactive species. However, a fatal weakness in those strategies is the nonrepeatability of those electrodes.

Recently, reversibly switchable biocatalysis has been introduced into electroanalytical chemistry to develop controllable biosensors and to amplify and transform signal.[13−18] The reversible switching of biocatalysis can be achieved by controlling pH,[15−18] ion,[13] temperature,[15] etc. Among various external stimuli, pH is frequently used, owing to the simplicity and accuracy.[19−22] Concanavalin A (Con A) protein has been extensively used for fabrication of the pH-switchable sensors because Con A may carry different net surface charges at different pH.[13] Most of the pH-switchable sensors were developed by layer-by-layer (LbL), assembling Con A and polyelectrolyte for immobilizing a large number of Con A to enhance the pH response.[17−19] Although it exhibits wonderful pH-switchable behavior, the high cost and complicated procedure limit the development in the practical application. Thus, some materials with large specific surface area still need be introduced to immobilize a large amount of Con A, such as graphene, which is a single layer of carbon nanosheets with an ultrahigh specific surface area of 2630 m^2 g^{-1}.[23−27]

As electrochemically inactive molecules, glucose and urea are widely distributed in the human body, whose levels in blood are usually used to obtain information on diabetes and kidney disease, respectively, which indicates that simple quantitative monitoring of glucose and urea is of crucial significance.[28−41] Although some electrochemical independent assay of glucose and urea has been developed based on indirect methods, simultaneous analysis of glucose and urea is still needed in one sample without separation since glucose and urea coexist in blood and urine and the separation from these samples is

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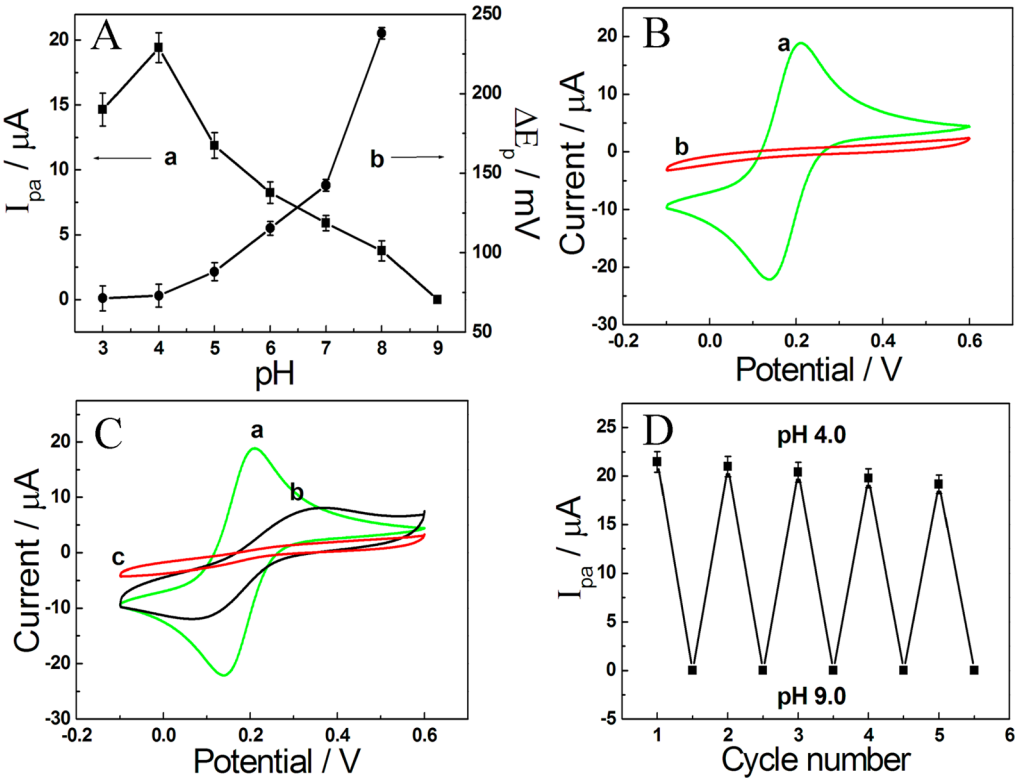

Figure 1. (A) Effect of solution pH on (a) I_{pa} and (b) ΔE_p of 1.0 mM $Fe(CN)_6^{3-}$ at a scan rate of 0.1 V s^{-1} on the Con A/CS-rGO/GCE. (B) CVs response of the Con A/CS-rGO/GCE in 0.1 M NaCl solution containing 1.0 mM $Fe(CN)_6^{3-}$ at (a) pH 4.0 and (b) pH 9.0. (C) CVs response of the (a) GCE, (b) Con A/GCE, and (c) Con A/CS-rGO/GCE in 0.1 M NaCl solution containing 1.0 mM $Fe(CN)_6^{3-}$ at pH 9.0. (D) Dependence of I_{pa} of 1.0 mM $Fe(CN)_6^{3-}$ at a scan rate of 0.1 V s^{-1} on the solution pH switched between pH 4.0 and 9.0.

always difficult and complicated. As the best-known enzyme-catalyzed reactions, glucose oxidase (GOD) can catalyze the oxidation of glucose into gluconic acid to decrease the pH,[42−47] and urea can be hydrolyzed into NH_3 catalyzed by urease to increase the solution pH.[48] This prompted us to propose a simple electrochemical strategy for estimating the concentration of glucose and urea in a sample without separation based on the reversibly pH-switchable biocatalysis, using GOD and urease.

Herein, chitosan-reduced graphene oxide (CS-rGO)/Con A protein was used as a sensing layer, where the CS-rGO with a large specific surface area was introduced to immobilize a large amount of Con A, exhibiting nice pH-switchable behaviors to $Fe(CN)_6^{3-}$. A simple and portable electrochemical biosensor for glucose and urea detection in a solution without separation was developed based on the pH-switchable behaviors of CS-rGO/Con A sensing layer toward $Fe(CN)_6^{3-}$. The change of resistance to charge transfer or amperometric current in the presence of GOD or urease resulted from the change of glucose or urea concentration. As far as we know, this is the first simultaneous detection of glucose and urea based on in situ pH-switchable enzyme-catalyzed reactions. The introduction of reversibly pH-switchable biocatalysis can overcome the fatal weakness of the nonrepeatability in previous strategies for the detection of nonelectroactive species. This work may also provide a potential reference for the design of more nonelectroactive species detection.

EXPERIMENTAL SECTION

Chemicals and Solutions. Graphite powder (99.95%, 325 mesh) was obtained from Aladdin. Concanavalin A (Con A) from Jack bean, chitosan (CS, deacetylation 75%), glucose oxidase (GOD, 140 units mg^{-1}), urease from *Canavalia ensiformis* (31.660 units mg^{-1}), and human serum were purchased from Sigma-Aldrich. Other chemicals were obtained from Beijing Chemical Reagent Factory (Beijing, China). The CS powder was ultrasonically dissolved in 1% acetic acid to obtain 0.5 wt % CS solution, and the pH was adjusted to about 6.0 using 1.0 M NaOH. 0.2 M phosphate buffer solution (PBS, pH 6.0) was used to prepare various enzyme solutions (80 μM Con A, 62.5 units mL^{-1} GOD, and 50 units mL^{-1} urease). 0.1 M NaCl solution with 1.0 mM $Fe(CN)_6^{3-}$ was used as an electrolyte and HCl or NaOH was used to adjust the pH of the electrolyte. Ultrapure water was purified by a Millipore-Q System ($\rho \geq 18.2$ MΩ cm) and used in the whole experiments.

Fabrication of CS-rGO. The CS-rGO was prepared by using CS as a reduction and functionalized agent to reduce and functionalize graphene oxide (GO), which was synthesized in accordance with Hummers' method.[49,50] Briefly, 5.0 mg GO was dispersed in 5.0 mL of ultrapure water by sonicating for 30 min. Then, 5.0 mL of 0.5 wt % CS (pH 6.0) was mixed with the above dispersion and stirred for 10 min. After that, the yellow-brown mixture was heated up to 90 °C and kept for 5 h till the color was changed to black. The resulting mixture was centrifuged and rinsed by water 3 times. Finally, 5.0 mg CS-rGO was dispersed in 10 mL of water and was sonicated for 30 min to obtain 0.5 mg mL^{-1} CS-rGO dispersion. The detailed characteristics and discussion of the CS-rGO have been provided in Figure S1 of the Supporting Information.

Preparation of Con A/CS-rGO/Glassy Carbon Electrode (GCE). First 3.0 μL of 0.5 mg mL^{-1} CS-rGO solution and 4.0 μL of 80 μM Con A solution were mixed. Then the

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mixtures were cast on the GCE surface to obtain the Con A/CS-rGO/GCE. Scanning electron microscopy was used to investigate the topography of the modified electrode, and the result was shown in Figure S2 of the Supporting Information.

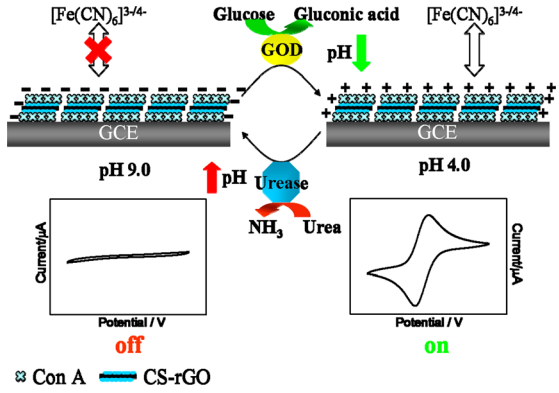
Instruments. Cyclic voltammograms (CVs), differential pulse voltammograms (DPV), and electrochemical impedance spectroscopy (EIS) measurements were carried out on an electrochemical workstation (CHI 750D) with three-electrode system. A platinum foil, the modified GCE, and a saturated calomel electrode (SCE) were used as the counter electrode, the work electrode, and the reference electrode, respectively.

RESULTS AND DISCUSSION

pH-Switchable Behaviors of Con A/CS-rGO/GCE Toward $Fe(CN)_6^{3-}$. Figure 1A shows the effect of pH on anodic peak current (I_{pa}) (curve a) and potential separation between anodic peak and cathodic peak (ΔE_p) (curve b) of 1.0 mM $Fe(CN)_6^{3-}$ on the Con A/CS-rGO/GCE at a scan rate of 0.1 V s^{-1}. The I_{pa} decreased, and the ΔE_p increased gradually as the pH of the electrolyte solution increased from 4 to 9. When pH ≥ 9.0, the I_{pa} almost decreased to 0. As shown in Figure 1B, the CVs of Con A/CS-rGO/GCE in 0.1 M NaCl solution containing 1.0 mM $Fe(CN)_6^{3-}$ at pH 4.0 exhibits a couple of typical redox peaks of $Fe(CN)_6^{3-/4-}$ (curve a),[19−21] while there is no redox peak at pH 9.0 (curve b). Therefore, the behaviors at pH 9.0 and 4.0 can be defined as the "off" and "on" states, respectively. The pH-switchable behavior should not only be attributed to the $Fe(CN)_6^{3-}$ itself because it was pH-independent of bare GCE (Figure 1C, curve a). Meanwhile, the redox current of 1.0 mM $Fe(CN)_6^{3-}$ at pH 9.0 on the Con A/GCE (Figure 1C, curve b) was lower than that on bare GCE (Figure 1C, curve a), but it was higher than that on Con A/CS-rGO/GCE (Figure 1C, curve c). It suggested that a large number of Con A was immobilized on the surface of CS-rGO, and accordingly, the Con A/CS-rGO/GCE could show a completed "off" state to the $Fe(CN)_6^{3-}$. Thus, the pH-switchable behaviors of Con A/CS-rGO/GCE might also result from the Con A molecules immobilized on the surface of CS-rGO. The Con A is an amphiprotic biomacromolecule with an isoelectric point of 5.0, and accordingly, the surface of Con A/CS-rGO/GCE should exhibit positive charges at pH < 5.0 and negative charges at pH > 5.0.[51−53] As a consequence, the Con A/CS-rGO film for $Fe(CN)_6^{3-}$ should be open at low pH and closed at high pH, owing to the electrostatic interaction between Con A and $Fe(CN)_6^{3-}$ (Scheme 1). The effects of the amount of Con A and CS-rGO on the Con A/CS-rGO/GCE have also been investigated (Figure S3 of the Supporting Information). The optimal amount of Con A and CS-rGO was 0.32 nmol and 1.5 μg, respectively. Interestingly, the "on−off" behavior was quite reversible (Figure 1D). After 10 cycles were repeated, the I_{pa} at pH 4.0 was almost maintained.

Interaction of the Con A/CS-rGO with $Fe(CN)_6^{3-}$. UV−Vis spectroscopy has been introduced to investigate the interaction of Con A/CS-rGO with $Fe(CN)_6^{3-}$, as shown in Figure S4 of the Supporting Information. As could be observed from curve a (pH 4.0) and curve b (pH 9.0), the pure $Fe(CN)_6^{3-}$ solution exhibit the same absorption peaks which were located at 260, 302, and 422 nm, respectively. However, the adsorption peaks of the mixture of $K_3Fe(CN)_6$ and Con A/CS-rGO at pH 4.0 was shifted to 263, 305, and 424 nm, respectively (curve c). The results indicate that there is a strong affinity between Con A/CS-rGO and $Fe(CN)_6^{3-}$ at pH 4.0.[12] After NaOH solution was added into the mixture of Con A/CS-rGO and $Fe(CN)_6^{3-}$ to adjust the pH to 9.0 (curve d), the absorption peaks were moved to the original position of pure $Fe(CN)_6^{3-}$. The results further prove that the strong affinity is derived from the electrostatic attraction of positively charged Con A/CS-rGO and negatively charged $Fe(CN)_6^{3-}$. The strong electrostatic attraction between Con A/CS-rGO and $Fe(CN)_6^{3-}$ was also confirmed by the color change of the solution, as shown in the inset of Figure S4 of the Supporting Information. The color of the Con A/CS-rGO-$Fe(CN)_6^{3-}$ solution at 4.0 was brighter than that at 9.0 after it was centrifuged, demonstrating the strong affinity between $Fe(CN)_6^{3-}$ and Con A/CS-rGO that occurred at pH 4.0.

Scheme 1. Schematic Illustration of pH-Switchable Behavior of $Fe(CN)_6^{3-}$ on the Con A/CS-rGO/GCE Triggered by the Enzyme-Catalyzed Reactions



In Situ pH-Switching by Enzyme-Catalyzed Reaction. Since the Con A/CS-rGO/GCE shows nice "on−off" behavior toward $Fe(CN)_6^{3-}$ at different pH, the enzyme-catalyzed reactions have been introduced to control the solution pH so as to achieve pH-controllable biosensors for the assay of glucose and urea, as shown in Scheme 1. Scheme 1 shows two biochemical systems in which GOD−glucose and urease−urea are used to control the solution pH. After 5.0 units mL^{-1} GOD and 30.0 mM glucose were added into 0.1 M NaCl solution containing 1.0 mM $Fe(CN)_6^{3-}$ at pH 9.0, the GOD-catalyzed reaction resulted in the transformation from glucose to gluconic acid, and accordingly, the pH decreased from 9.0 to 4.0. Figure S5A of the Supporting Information shows the time-dependent pH changes triggered by GOD-catalyzed reactions in N_2- (curve a), air- (curve b) and O_2-saturated (curve c) 0.1 M NaCl + 5.0 units mL^{-1} GOD + 30.0 mM glucose + 1.0 mM $Fe(CN)_6^{3-}$ solution, respectively. The glucose was oxidized by the oxidation state of GOD (GOD_{oxd}) into gluconic acid and simultaneously GOD_{oxd} was reduced into the reduction state of GOD (GOD_{red}) (eq 1). Then GOD_{red} was oxidized back to GOD_{oxd} by O_2, accompanied by the reduction of O_2 into H_2O_2 (eq 2). Among the products, gluconic acid could give H^+ to decrease the pH of the electrolyte solution. As could be seen from Figure S5A of the Supporting Information), the pH of O_2-saturated solution decreased quickly to 4.0 in about 20 min after 30.0 mM glucose was added (curve c). The changes of pH became slow in the air-saturated solution (for curve b owing to a small quantity of O_2) and almost stopped in the N_2-saturated solution (for curve a owing to a lack of O_2). As a consequence, the pH reached to 4.0 in 50 min for the air-saturated (curve b) solution and did not reach to 4.0 for the N_2-saturated (curve a) solution. Therefore, the O_2-saturated solution will be used in the subsequent experiment. Figure S5B of the Supporting

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Information shows the pH changes with the urease-catalyzed reaction time in 0.1 M NaCl + 10.0 mM urea + 1.0 units mL^{-1} urease + 1.0 mM $Fe(CN)_6^{3-}$. The solution pH was converted from 4.0 to 9.0 in about 10 min due to the generated NH_4^+ and OH^- by the urease-catalyzed reaction (eq 3).

$$GOD_{oxd} + glucose \rightarrow GOD_{red} + gluconic\ acid \quad (1)$$

$$GOD_{red} + O_2 \rightarrow GOD_{oxd} + H_2O_2 \quad (2)$$

$$NH_2CONH_2 + 3H_2O \xrightarrow{urease} 2NH_4^+ + HCO_3^- + OH^- \quad (3)$$

Figure 2 shows the plot of pH changes with enzyme-catalyzed reaction time. The pH of 0.1 M NaCl + 5.0 units

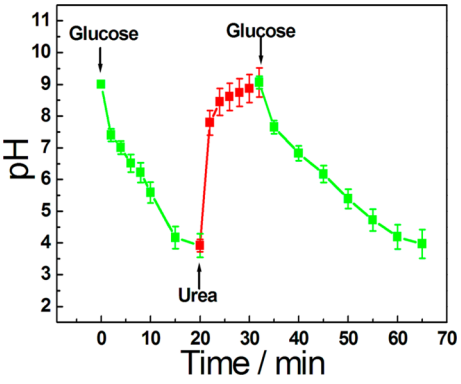

Figure 2. Time-dependent pH changes triggered by in situ enzyme-catalyzed reactions upon addition of glucose and urea. The concentration of glucose and urea are 30 and 10 mM, respectively. The pH of 0.1 M NaCl solution containing 1.0 mM $Fe(CN)_6^{3-}$, 5.0 units mL^{-1} GOD, and 1.0 units mL^{-1} urease was adjusted to 9.0 with the NaOH solution before the experiments.

mL^{-1} GOD + 1.0 units mL^{-1} urease +1.0 mM $Fe(CN)_6^{3-}$ was adjusted to 9.0 with NaOH solution. The CVs of Con A/CS-rGO/GCE did not show any redox peak of $Fe(CN)_6^{3-}$ (Figure S6, curve a, of the Supporting Information), representing a complete "off" state. After 30.0 mM glucose was added into the solution, the generated gluconic acid (eqs 1 and 2) decreased the pH of solution to 4.0 in about 20 min. And the CVs of Con A/CS-rGO/GCE show distinct electrochemical response with typical redox couples of $Fe(CN)_6^{3-/4-}$ (Figure S6, curve b, of the Supporting Information), representing a wonderful "on" state. Then, if 10.0 mM urea was added into the solution, the produced NH_4^+ and OH^- (eq 3) increased the solution pH to 9.0 in about 12 min. Meanwhile, the CVs recovered to the "off" state (Figure S6, curve c, of the Supporting Information). As shown by the curve d in Figure S6 of the Supporting Information), after another 30.0 mM glucose was added, the CVs were changed to the "on" state in about 30 min again. The pH-switching triggered by enzyme-catalyzed reaction was repeatable and could be cycled for at least two times by alternative glucose and urea, which is enough to use in the practical determination of glucose and urea in one sample. The longer time of the latter pH change may be ascribed to the buffer function of gluconic acid−NH_4^+. The switching time also depended on the enzyme concentration, and the effect of enzyme concentration on switching time was investigated and the result was listed in Table S1 of the Supporting Information.

Detection of Glucose and Urea in a Standard Solution. After different concentration glucose was added in the 0.1 M NaCl + 5.0 units mL^{-1} GOD + 1.0 mM $Fe(CN)_6^{3-}$ solution (pH 9.0) for 20 min, the CV responses of Con A/CS-rGO/GCE at 0.1 V s^{-1} were displayed in Figure 3A. The redox peaks gradually increased with the increase of glucose concentration from 1.0 to 12.0 (curves b−f). The inset is the corresponding calibration curve. The Con A/CS-rGO/GCE showed a wide linear range to the glucose concentration of 1.0−10.0 mM (R = 0.988). Meanwhile, DPV and EIS have been popularly utilized for the quantitative determination.[54−57] Figure 3C shows the DPV of Con A/CS-rGO/GCE (a) before and (b−f) after glucose (1.0−12.0 mM) was added in 0.1 M NaCl + 5.0 units mL^{-1} GOD + 1.0 mM $Fe(CN)_6^{3-}$ solution (pH 9.0) for 20 min at 0.05 V s^{-1}. The peak currents increased linearly as the glucose concentration increased in the range of 1.0−10.0 mM, as shown by the inset in Figure 3C. Figure 3E displays the EIS obtained on the Con A/CS-rGO/GCE (a) before and (b−f) after glucose (1.0−12.0 mM) was added in 0.1 M NaCl + 5.0 units mL^{-1} GOD + 5.0 mM $Fe(CN)_6^{3-/4-}$ solution (pH 9.0) for 20 min. The impedance date was fitted by the Randles circuit, in which the interfacial capacity (C_{dl}) is supposed to be parallel to the diffusion impedance (W) and the resistance to charge transfer (R_{ct}). The proposal results in a semicircle in the intermediate frequency of the plot of Z'' against Z' (left inset in Figure 3E). It clearly showed that the R_{ct} decreased from 3360 to 660 Ω as the glucose concentration increased. The right inset in Figure 3E shows the linear relationship between the ΔR ($\Delta R = R_0 - R$, where R_0 and R are the electron transfer resistances before and after glucose was added, respectively) and the glucose concentration from 2.0 to 10.0 mM. Similarly, the pH change triggered by the urease-catalyzed reaction was chosen to detect the urea concentration. As shown in Figure 3 (panels B, D, and F), the currents linearly decreased as the concentration of urea increased from 1.0 to 8.0 mM, while the ΔR of EIS proportionately increased with the increasing concentration of the urea. The detailed analytical performance of three methods and the comparison to some previous works have been listed in Table S2 of the Supporting Information. The blood glucose level of a healthy human is maintained between about 4.0 and 6.0 mM.[28] The glucose level of diabetics would be over 7.0 mM, and the concentration of urea in blood is about 2.5−7.5 mM. Thus, the linear range of 1.0−10.0 mM for glucose and 1.0−7.0 mM for urea are enough for the practical determination. Compared with previous sensors, the advantage of the sensor is the simple pretreatment of samples and the simultaneous detection of glucose and urea in one sample without separation.

Detection of Glucose and Urea in a Practical Sample. The detection of glucose and urea in human serum samples were performed on the Con A/CS-rGO/GCE with a standard addition method, and the results were shown in Figure 4. To verify the accuracy of our methods, the original concentration of glucose and urea in human serum samples was first determined by colorimetric enzymatic method and standard rapid urease test to be 5.38 mM and 2.94 mM, respectively. Then human serum samples were diluted with 0.2 M NaCl + 2.0 mM $Fe(CN)_6^{3-}$ + 10.0 units mL^{-1} GOD or 2.0 units mL^{-1} urease (pH 9.0 or 4.0), and the concentration of glucose and urea were calculated to be 2.69 mM and 1.47 mM, respectively. Finally, the standard glucose or urea solution with different concentration was injected into the above diluted solution, and the measurements were carried out by our method. As shown in Figure 4, the detection result of practical sample was similar to the detection of standard solution and no additional signal

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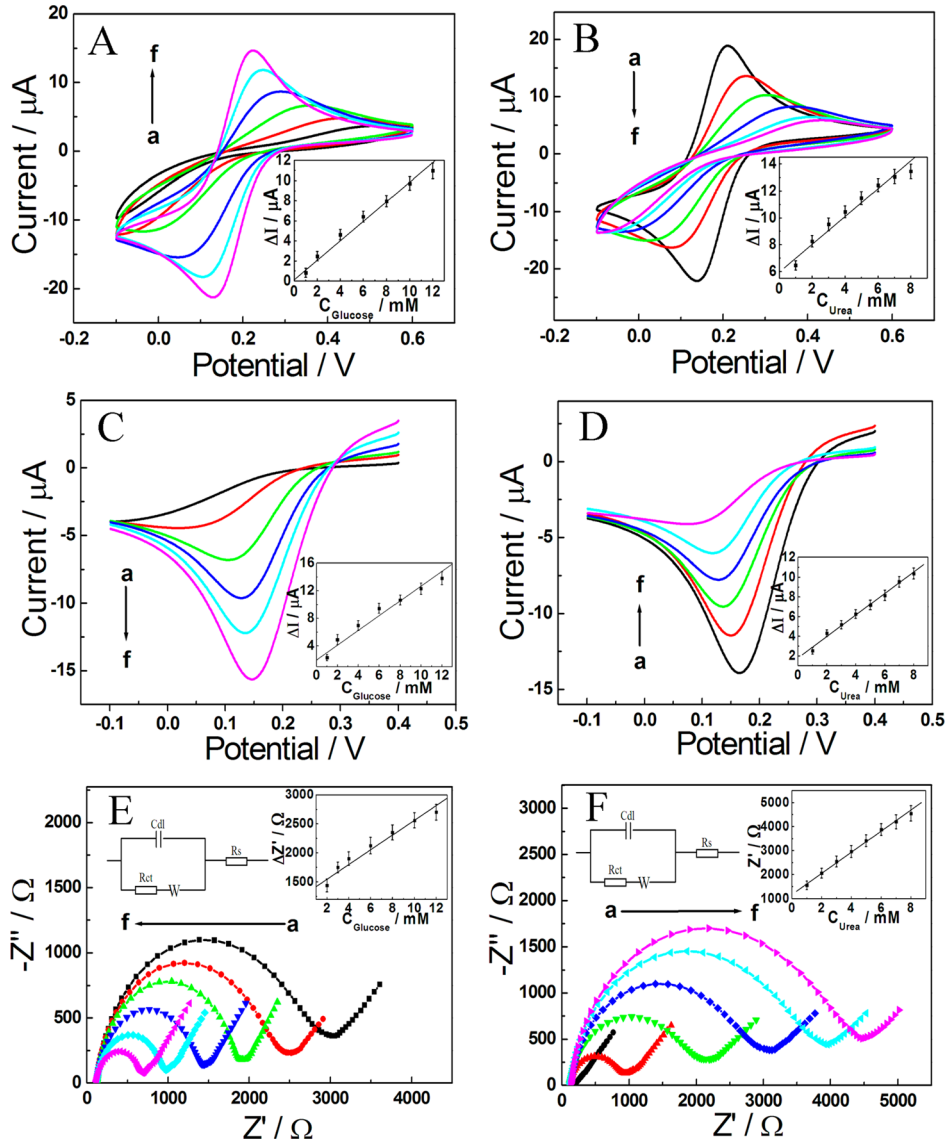

Figure 3. (A) CVs and (C) DPV response of the Con A/CS-rGO/GCE in 0.1 M NaCl solution (pH 9.0) + 5.0 units mL^{-1} GOD + 1.0 mM $Fe(CN)_6^{3-}$ solution (a) before and after injection of (b) 1.0, (c) 2.0, (d) 4.0, (e) 8.0, and (f) 12.0 mM glucose for 20 min. Insets are the calibration curves. Scan rates are (A) 0.1 V s^{-1} and (B) 0.05 V s^{-1}, respectively. (A) CVs and (D) DPV response of the Con A/CS-rGO/GCE in 0.1 M NaCl + 1.0 units mL^{-1} urease + 1.0 mM $Fe(CN)_6^{3-}$ solution (pH 4.0) (a) before and after injection of (b) 1.0, (c) 2.0, (d) 4.0, (e) 6.0, and (f) 8.0 mM urea for 10 min. Insets are the calibration curves. Scan rates are (C) 0.1 V s^{-1} and (D) 0.05 V s^{-1}, respectively. (E) EIS of the Con A/CS-rGO/GCE in 0.1 M NaCl + 5.0 units mL^{-1} GOD + 5.0 mM $Fe(CN)_6^{3-/4-}$ solution (pH 9.0) (a) before and after injection of (b) 1.0, (c) 2.0, (d) 4.0, (e) 8.0, and (f) 12.0 mM glucose for 20 min. Insets are the Randles circuit (left) and the calibration curve (right). (F) EIS of the Con A/CS-rGO/GCE in 0.1 M NaCl + 5.0 units mL^{-1} urease + 5.0 mM $Fe(CN)_6^{3-/4-}$ solution (pH 4.0) (a) before and after injection of (b) 1.0, (c) 2.0, (d) 4.0, (e) 6.0, and (f) 8.0 mM urea for 10 min. Insets are the Randles circuit (left) and the calibration curve (right).

appeared (Figure 4, panels A–F), which indicated the coexisted materials would not exhibit electrochemical activity in the narrow potential window. It was noticeable that the response signal in the practical sample was smaller than that in the standard solution because the human serum sample contains many complex and thick interstitial fluids, cells, and multiple kinds of protein, which would hinder the electron transfer between electrode and electrolyte solution. Therefore, the standard addition method has been used to eliminate matrix effect in the detection of glucose and urea in human serum samples. The results obtained from our method agree well with the standard method (Table S3 of the Supporting Information), suggesting that the pH-switchable biosensor is possible to apply in the human serum sample. The urine sample from a person with diabetes was also tested by our method (Figure S7 of the Supporting Information). The Con A/CS-rGO/GCE exhibited similar behavior in the urine sample with that in the standard solution. That might be attributed to the urine sample, which has no large number of complex thick materials. In view of the high concentration of urea in the urine sample,[58] the urine sample was diluted by 40 times in the detection of urea. The results obtained from our method also agree well with the standard method (Table S4 of the Supporting Information), suggesting that the pH-switchable biosensor is possible to apply in the human urine sample.

Simultaneous Detection of Glucose and Urea in One Practical Sample. As shown in Figure S8 of the Supporting Information, the simultaneous detection of glucose and urea in

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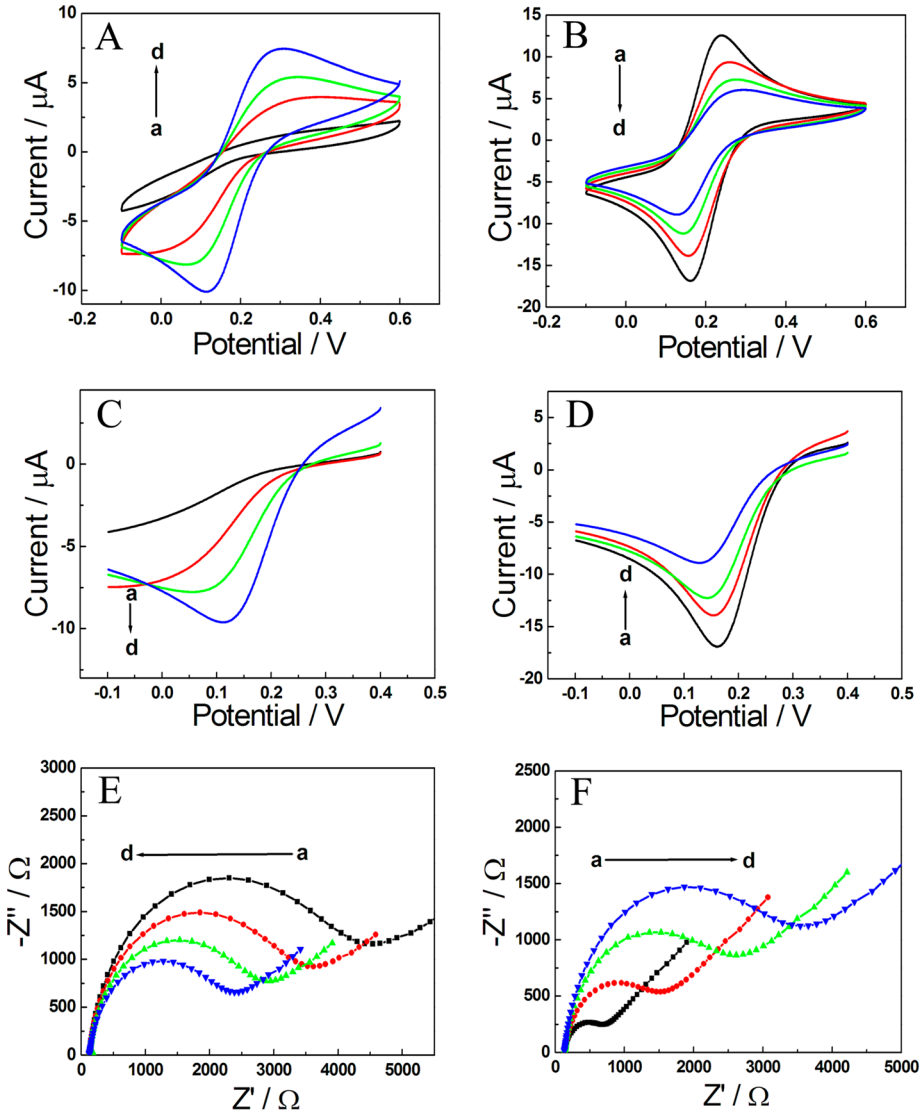

Figure 4. (A) CV and (C) DPV response of the Con A/CS-rGO/GCE in 5.0 mL human serum samples containing 0.1 M NaCl and 1.0 mM $Fe(CN)_6^{3-}$ (pH 9.0) (a) before and (b) after injection of 5.0 units mL^{-1} GOD as well as (c) 2.0 and (d) 4.0 mM glucose for 20 min. Scan rates are 0.1 V s^{-1}. (B) CV and (D) DPV response of the Con A/CS-rGO/GCE in 5.0 mL human serum samples containg 0.1 M NaCl and 1.0 mM $Fe(CN)_6^{3-}$ (pH 4.0) (a) before and (b) after injection of 1.0 units mL^{-1} urease as well as (c) 2.0 and (d) 4.0 mM urea for 10 min. Scan rates are 0.1 V s^{-1}. (E) EIS of the Con A/CS-rGO/GCE in 5.0 mL human serum samples containing 0.1 M NaCl and 5.0 mM $Fe(CN)_6^{3-/4-}$ (pH 9.0) (a) before and (b) after injection of 5.0 units mL^{-1} GOD as well as (c) 2.0 and (d) 4.0 mM glucose for 20 min. (F) EIS of the Con A/CS-rGO/GCE in 5.0 mL human serum samples containing 0.1 M NaCl and 5.0 mM $Fe(CN)_6^{3-/4-}$ (pH 4.0) (a) before and (b) after injection of 1.0 units mL^{-1} urease as well as (c) 2.0 and (d) 4.0 mM urea for 10 min.

human serum sample was studied by adding the GOD and urease solution successively into the human serum samples. The current increased with the addition of 5.0 units mL^{-1} GOD, indicating the existence of glucose in the human serum sample. The glucose concentration was calculated to be 5.06 mM. Then, after the addition of 1.0 units mL^{-1} urease, the current decreased. The urea concentration of original human serum sample was calculated to be 2.74 mM.

Selectivity and Stability of the pH-switchable Biosensor. Some possibly coexisted materials such as some carbohydrates, ascorbic acid (AA), and uric acid (UA) have been used to test the selectivity of the pH-switchable biosensor. The CV of Con A/CS-rGO/GCE was carried out in 0.1 M NaCl + 4.0 mM glucose + 4.0 mM urea + 1.0 mM $Fe(CN)_6^{3-}$ solution, containing those coexisted materials such as 4.0 mM UA, 4.0 mM AA, and some carbohydrates. The change of CV responses in the absence and presence of GOD or urease were compared, and the results indicated that the coexisted materials have no obvious interference for the detection of glucose and urea (Figure S9 of the Supporting Information). The high selectivity of our method might be attributed to the detection mechanism. The principle for glucose and urea detection was essentially based on the in situ pH-switchable enzyme-catalyzed reaction in which the oxidation of glucose catalyzed by glucose oxidase or hydrolyzation of urea catalyzed by urease resulted in a pH change of electrolyte solution to give different electrochemical responses toward $Fe(CN)_6^{3-}$. (The detailed discussion can be found in Supporting Information.) When the Con A/CS-rGO/GCE was stored in the refrigerator at 4 °C over 10 days, 95.70% of the initial response remained,

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indicating a quite satisfactory stability (Figure S10 of the Supporting Information). The selectivity and stability of the biosensor is obviously superior to many enzymatic and nonenzymatic sensors. The nonenzymatic sensors, using transition metal or oxide and the noble metal or alloy as the electro-catalyst, were usually used in a strong alkaline solution, which is not suitable for the direct detection of glucose and urea in blood.

CONCLUSIONS

By taking advantage of the pH-switchable Con A/CS-rGO layer, an effective strategy for the simultaneous electrochemical assay of glucose and urea in a sample based on in situ pH-switchable enzyme-catalyzed reactions was developed. The Con A/CS-rGO layer with pH-dependent surface net charges exhibits nice reversibly pH-switchable behaviors to $Fe(CN)_6^{3-}$. In the presence of GOD or urease, different concentrations of glucose or urea results in different pH changes; in other words, it gives a different electrochemical response. With human serum and urine experiments, the sensing platform was proven to be valid for the simultaneous assay of glucose and urea in a biological system. The introduction of reversibly pH-switchable biocatalysis will overcome the fatal weakness of the previous strategies for the assay of electrochemically inactive species. Thus, this work not only develops a way to detect glucose and urea in a sample without separation but also opens a new strategy for the development of electrochemically inactive species based on the pH-switchable enzyme-catalyzed reaction.

ASSOCIATED CONTENT

Supporting Information

Additional information as noted in the text. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*E-mail: lwangsy2003@hotmail.com. Tel/Fax: +86 791 88120861.

Notes

The authors declare no competing financial interest.

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