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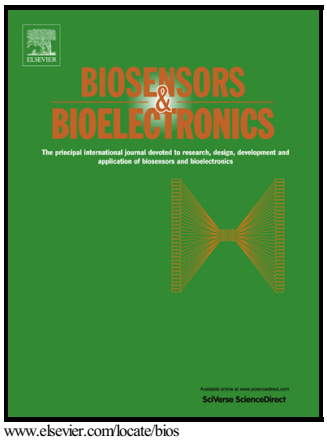

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Electrochemical biosensing platform based on amino acid ionic liquid functionalized graphene for ultrasensitive biosensing applications

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ABSTRACT

In this study, a facile non-covalent method was developed for preparing water-soluble graphene with excellent electronic conductivity. Room temperature ionic liquids (ILs) with high ionic conductivity were used for the non-covalent surface functionalization of graphene through π– π stacking interactions. Compared to other ILs used, amino acid ionic liquids (AAILs) were found to be the most effective for improving the dispersion of graphene in water phase. Electrochemical and spectroscopic results confirmed that the obtained AAIL functionalized GR can retain the excellent electronic conductivity of pristine graphene without damaging the graphene lattice. The obtained water-soluble graphene (GR-AAIL) was exemplified to fabricate an electrochemical biosensor using tyrosinase as a model enzyme, and the sensitivity (12600 mA cm^{-2} M^{-1}) of GR-AAIL based biosensor was about 17 times higher than that of graphene oxide and other nanomaterial based biosensor, displaying its unprecedented high sensitivity for

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biosensing. The detection limit for catechol (one important environmental pollutants) reached as low as 8 nM with a response time of 3 s and a linear range from 25 nM to 11100 nM. The AAIL-GR based biosensor also demonstrated good reproducibility, repeatability, selectivity, long-term stability and high recovery for catechol detection. Amino acid ionic liquid functionalized graphene proves to be a robust and versatile electrochemical biosensing platform for fabricating biosensors with excellent performance.

KEYWORDS: Biosensor, Graphene, Amino Acid Ionic liquids, Electronic conductivity, Tyrosinase

1. Introduction

Graphene (GR), the archetypal 2D nanomaterial, is a single atomic layer of carbon with extraordinary properties including exceptionally high electronic carrier mobilities, thermal conductivity, and mechanical strength. GR is expected to find potential applications in many fields such as electronics, sensors, biosensors, optoelectronics, batteries, supercapacitors, catalysis, chemical and biological sensing (Ponomarenko et al., 2008; Wu et al., 2012a; Liu et al., 2013a; Liu et al., 2013b; Gholivandn and Khodadadian, 2014). As shown with carbon nanotubes, the dispersion of nanomaterials in solution is crucial to advancing many applications (Baughman et al., 2002; Sun et al., 2002). Owing to their hydrophobic nature, the direct dispersion of GR sheets in water has been generally considered unattainable. GR sheets, which have a high specific surface area, tend to form agglomerates or even restack to form graphite through van der Waals interactions, making further processing difficult. The functionalization of GR has been

considered to be important for improving their solubility, self-assembly properties, and applications in devices. The commonly used method for dispersing GR sheets is to utilize the hydrophilic groups on GR surface. Usually water-soluble graphene oxide (GO) or functionalized graphene (e.g. carboxylic or hydroxy graphene) is used (Park and Ruoff, 2009; Zhou et al., 2013), however, it is electrically insulating or has poor electronic conductivity. To restore the electronic conductivity, GO requires complicated chemical reduction or annihilation under high temperature (Malig et al., 2012) to afford reduced graphene oxide (r-GO). Covalent functionalization of graphene (Si and Samulski, 2008) suffers from the same problem as GO (i.e. the decreased electronic contuctivity) as all these techniques use the hydroxyl or carboxylic groups on graphene surface. And these structural defects on GR surface inevitably alter the electronic conductivity of graphene. A viable alternative to form GR dispersion is the non-covalent functionalization of GR (Xu et al., 2008; Malig et al., 2012). GR might be stabilized by some amphiphilic polymers or surfactants (Lotya et al., 2009) to form a stable dispersion without destroying the intrinsic structure of GR. However, one potential risk of the above method is that the electronic conductivity of GR may be affected by the poor conductivity of remained polymers or surfactants. Using amphiphilic room temperature ionic liquids (RTILs) may be preferred for the dispersion and functionalization of GR as ionic liquids possess excellent ionic conductivity.

Room temperature ionic liquids are compounds consisting entirely of ions that exist in the liquid state around room temperature (Lu et al., 2006; Moulthrop et al., 2005). As novel attractive green solvents, they possess unique properties such as negligible vapor pressure, wide potential windows, high thermal and chemical stability, and excellent ionic conductivity. The physical properties of RTILs, such as the oil/water partition coefficient, can be adjusted by incorporating

different organic cations and inorganic or organic anions. Some biocompatible ionic liquids, such as 1-butyl-3-methyl-imidazolium tetrafluoroborate, have been used for biosensors (Lu et al., 2006) or biocatalysis (Lou et al., 2004) with increased enzymatic activity and stability.

In this communication, we prepared stable aqueous dispersions of GR sheets without sacrificing its electronic conductivity and structure integrity by using amphiphilic anino acid ionic liquids (AAILs) as a stabilizer, since the imidazole moiety has been reported to have strong affinity with the basal plane of graphite via π-stacking. Up to date, noncovalent functionalization of GR sheets using AAILs through π-π interactions has not been addressed in the literature. Of significant is that, unlike conventional polymers or surfactants, these amphiphilic ionic liquids possess high ionic conductivity. Once these ionic liquids are purposely retained, they are expected to provide better conductivity compared to conventional polymer or surfactants stabilizer. On the other hand, these ionic liquids can be easily gotten rid of by filtration and washing if they are not needed anymore. Based on the obtained AAIL-functionalized graphene (AAIL-GR), an electrochemical tyrosinase biosensor was fabricated (tyrosinase as a model enzyme), and the unprecedented high sensitivity for biosensing was displayed. The AAIL-GR based biosensor also demonstrated good reproducibility, selectivity, long-term stability and low detection limit for catechol detection. The fabricated biosensor for real water sample detection was also investigated with high recovery. Amino acid ionic liquid functionalized graphene proves to be a robust electrochemical biosensing platform for fabricating biosensors with excellent performance.

2. Materials and Methods

2.1. Materials

GR (purity~99%) and GO (purity~99%) were from Nanjing XFNANO Materials Tech Co., Ltd (China). Chitosan (Chi, minimum 85% deacelytated) and tyrosinase (from mushroom, >1000 units mg^{-1}) were purchased from Sigma (USA). Phenol and other chemicals (analytical grade) were purchased from Tianjin Kermel Chemical Regent Company (China). Tetrabutyl phosphonium methanesulfonate, 1-Ethyl-3-methylimidazolium tetrafluoroborate, 1-Butyl-3-methylimidazolium methanesulfonate, Trihexyltetradecylphosphonium bis(trifluoromethylsulfonyl)amide (purity>98%) were purchased from Sigma-Aldrich. Other IL (purity>98%) were obtained from Dalian Institute of Chemical Physics, Chinese Academy of Sciences (China). Table 1 lists these twelve different kinds of ILs selected for investigation.

2.2. Preparation of the GR-IL nanocomposite

Twelve kinds of ILs in table 1 were tesed respectively to improve the dispersion of GR in water phase. The GR-IL nanocomposite was prepared as follows: An aliquot of GR (usually 0.4 mg) was mixed with an aqueous solution of ILs (1ml, the concentration of ILs may be adjusted from 0.25 mg ml^{-1} to 5.0 mg mL^{-1} for optimization according to the property of ILs and the dispersion results of GR) with the aid of ultrasonication and vibration for 15 min. The solution containing 0.4 mg ml^{-1} GR and 3.0 mg ml^{-1} AAILs was used for the demonstration of images in Figure 1. The solution containing 0.2 mg ml^{-1} GR and 0.25 mg ml^{-1} AAIL was used for FTIR and electrochemical impedance spectroscopy (EIS) characterization of AAIL-GR.

2.3. TEM and FT-IR characterization

TEM images of GR and GO were obtained with a transmission electron microscope JEM-2000EX (JEOL, Japan) with an accelerating voltage of 120 kV. FT-IR spectra of GR, GO,

EMIMAla, and GR-EMIMAla were recorded by using a Spectrum GX apparatus (Perkin--Elmer Company, USA).

2.4. Electrochemical impedance spectroscopy measurements

EIS measurements were performed by using a PGSTAT 302N (Autolab, Switzerland) workstation in a 1.0 mmol L^{-1} $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1) solution containing 0.5 mol L^{-1} KNO_3 supporting electrolyte, and the results were plotted in the form of complex plane diagrams (Nyquist plots) with a frequency range from 0.1 Hz to 10 kHz. The electrodes for EIS measurements were prepared by a similar procedure for the preparation of enzyme electrodes. The solution compositions for the preparation of different electrodes were as follows: GR-Chi/GC electrode (0.2 mg ml^{-1} GR and 0.3 mg ml^{-1} Chitosan), GO-Chi/GC (0.2 mg ml^{-1} GO and 0.3 mg ml^{-1} Chitosan), GR-EMIMAla-Chi/GC (0.2 mg ml^{-1} GR, 0.25 mg ml^{-1} EmimAla and 0.3 mg ml^{-1} Chitosan), and Chi/GC (0.3 mg ml^{-1} Chitosan).

2.5. Amperometric measurements of biosensors

Amperometric (I-t) measurements were carried out based on three electrode systems by using a CHI 440B electrochemical workstation (CHI Instruments Inc., USA). The enzyme electrodes for I-t measurements were prepared as follows. After optimization of the experimental conditions, the final solution (pH 7.0) containing tyrosinase (2.5 mg mL^{-1}), GR-EMIMAla (0.2 mg mL^{-1} GR and 0.25 mg mL^{-1}), and chitosan (1.5 mg mL^{-1}) was used for the preparation of Tyr-GR-EMIMAla-Chi/GC enzyme electrode. 5.0 μl of the above mixture was cast onto the surface of freshly polished glassy carbon electrode (GC, 3 mm diameter) to obtain the Tyr-GR-EMIMAla-Chi/GC electrode. The dried enzyme electrode was stored at 4 °C in a refrigerator when not in use. Other electrodes were prepared by using similar procedures as described above. A three-

125 electrode system consisted of the tyrosinase-based GC electrode (3 mm diameter) as the working
126 electrode, an Ag/AgCl as the reference electrode (KCl concentration: 3 mol L^{-1}), and a platinum
127 wire as the auxiliary electrode. 50 mmol L^{-1} phosphate buffer saline (PBS, pH 7.0) was used as
128 the electrolyte in the electrochemical experiments. Amperometric measurements were carried out
129 on successive additions of an aliquot volume of catechol solution (1 mmol L^{-1}) into 8 mL stirring
130 PBS at room temperature (25 °C). The potential applied to the Tyr-GR-EMIMAla-Chi/GC and
131 Tyr-GO-EMIMAla-Chi/GC working electrode was -0.1 V.

132 **3. Results and Discussion**

133 **3.1. Preparation of water-soluble graphene**

134 In our study, a commercially available Graphene (purity~99 %) from Nanjing XFNANO
135 Materials Tech Co., Ltd (Nanjing, China) was studied. Figure 1(a) shows the TEM image of GR.
136 Because of the physical preparation method, the obtained GR shows high BET surface area
137 of >900 m^2 g^{-1} and good electrical conductivity (>1000 s m^{-1}) with a single layer ratio of >80%.
138 Due to the hydrophobic nature, graphene sheets can't be dispersed in water and are inclined to
139 deposit in the bottom, as shown in Figure 1b. The dispersion of graphene in water phase is one
140 paramount challenge for chemists, which limits its wide application in many fields. Ionic liquids
141 are compounds consisting entirely of organic cations and inorganic (or organic) anions that exist
142 in the liquid state around room temperature. As novel attractive solvents, they possess unique
143 properties such as negligible vapor pressure, wide potential windows, high thermal stability, and
144 good ionic conductivity and solubility (Buzzeo et al., 2004; Lu et al., 2006). As shown in Table 1,
145 twelve different kinds of ILs were selected as stabilizer for noncovalent functionalization of GR

sheets through π-π interactions and hydrophobic interaction in water phase. These ILs can be attributed to imidazolium category or phosphonium category according to their cations, and they contain amino acid, tetrafluoroborate, methanesulfonate, hexafluorophosphate, bis(trifluoromethylsulfonyl)amide, or trifluoromethanesulfonate anions. As expected, all the hydrophobic ILs proved to be not effective on improving the dispersibility of GR in water phase. On the other hand, the effect of hydrophilic ILs on the dispersibility of GR in water phase is quite different. For example, 1-ethyl-3-methylimidazolium tetrafluoroborate, which possesses good hydrophilicity (miscible with water), could improve the dispersion of GR in water phase to some extent. However, it could not make GR become completely water-soluble. Of the twelve different ILs, it is interesting to find that AAILs are the most effective ILs that can improve the dispersion of GR in water. Using amphiphilic 1-ethyl-3-methylimidazolium Alanine (EMIMAla) or 1-Ethyl-3-methylimidazolium L-Proline (EMIMPro) as stabilizer, stable aqueous dispersions of GR sheets were obtained since the negatively charged imidazole moiety has strong affinity with the basal plane of GR viaπ-stacking. Unlike GR (Figure 1b), the AAIL-functionalized GR became water-soluble and could retain stable in water phase, as shown in Figure 1 (c and d). After surface functionalization of GR by hydrophilic AAILs, the hydrophobic surface of GR became hydrophilic. Furthermore, the absorbed imidazolium cations on GR surface result in strong electrostatic repulsion interaction between GR nanosheets. Previous study revealed that electrostatic repulsion mechanism played an important role in forming stable GR or GO dispersions (Li et al., 2008). The formation of stable GR dispersions should be attributed not only to surface hydrophilicity but also electrostatic repulsion of GR after modification by charged AAILs. Typically, the dispersion of 0.1 mg ml^{-1} GR in water will need as much as 10 mg ml^{-1} sodium dodecylbenzenesulfonate (SDBS) as stabilizer (0.1 mg ml^{-1} GR vs. 10 mg ml^{-1}

SDBS). In our study, 0.4 mg ml^{-1} GR can be well dispersed in water phase with the aid of only 3.0 mg ml^{-1} AAILs (0.1 mg ml^{-1} GR vs. 0.75 mg ml^{-1} AAIL). This work, for the first time, suggests that ordinary graphene, when using AAILs as stabilizer, can be readily dispersed into water phase to form stable GR dispersion.

3.2. FT-IR characterization of GR, GO and GR-AAIL.

Conventional methods usually improve the dispersion of GR in water by introducing oxygen-functional group on the surface of GR, which sacrifices the good electronic conductivity of GR. The FT-IR spectra of GR, GR-AAIL and GO clearly displays the structural difference between GR and GO, as shown in Figure 2A. For GO, the characteristic peaks appear at 1055 cm^{-1} (C-O stretching peak), 1732 cm^{-1} (C=O), 1620 cm^{-1} (the skeletal vibrations of unoxidized graphitic domains), and 1221 cm^{-1} (C-OH stretching peak) (Xu et al., 2008; Geng et al., 2010). The FTIR of GR is quite different from that of GO. As for GR, most of the characteristic peak of oxygen functional group observed on GO disappear, and a new characteristic peak appears at 1556 cm^{-1}, corresponding to the C=C bond of the sp2 carbon networks in GR sheet. Figure 2A also shows the FTIR of GR after modification by AAIL. Almost all the characteristic peaks of EMIMAla and GR can be found in the GR-EMIMAla nanocomposite, indicating the nanocomposite is formed by physical interaction instead of chemical covalent function. The band at 1576 cm^{-1} is assigned to the characteristic absorption peaks of EMIM[Ala] (i.e., acylamide II). The C-H vibration for cyclic $EMIM^{+}$ appears at near 1167 and 623 cm^{-1}. Meanwhile, the relatively intensive characteristic peaks of EMIMAla at 1405, 843, 3080 cm^{-1} can also be found in the GR-EMIMAla nanocomposite, which confirms the presence of EMIMAla in the nanocomposite. The slight shift of the characteristic peaks of EMIMAla on the nanocomposite indicates the strong interaction between GR and EMIMAla.

3.3. Electrochemical impedance spectroscopy characterization of electronic property

The most significant advantage of the dispersion method in this study is that it does not destroy the structure integrity of GR, and meanwhile retains the excellent elctronic conductivity of GR. We carried out EIS measurements to investigate the resistance of the materials towards heterogeneous charge transfer (Rct). EIS is a very sensitive technique to investigate the ability of the material to transfer and exchange charges with surrounding molecules (Feng, et al., 2005). Such electron exchange ability is strongly influenced by the electronic conductivity of the material. If the electronic conductivity of GR reduced after modification by AAIL, it would be clearly displayed on the EIS measurement results. Because GR, GO (water-soluble) and GR-AAIL could not be steadily immobilized on the surface of GC electrode once the modified electrode was immersed in $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ electrolyte, Chitosan (Chi) was used as a film-forming polymer to immobilize the above material on the surface of the GC electrode steadily. Figure 2B shows the Nyquist diagrams for EIS measurements of GR-Chi, GO-Chi, GR-EMIMAla-Chi, and Chi modified electrodes in 1 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ containing 0.5 M KNO_3 electrolyte. The charge-transfer resistance (Rct) corresponds to the diameter of the semicircle in the Nyquist plots obtained from the measurements. As can be seen in Figure 2B, Chi shows the largest Rct. Compared to Chi, and the Rct of GO-Chi decreases only a little, indicating the poor electronic conductivity of GO. Because GO contains large amount of oxygen-containing functional group on the surface, these functional groups destroys its structure integrity and electronic conductivity. Whereas, the GR-EMIMAla-Chi shows almost a straight line and the Rct value of GR-EMIMAla-Chi is close to zero, indicating a very small interface electron resistance. This phenomenon proves that the excellent electronic conductivity of GR-EMIMAla is preserved. The Nyquist diagram of GR-EMIMAla-Chi is almost the same as that of GR-Chi,

suggesting that the presence of EMIMAla does not make the electronic conductivity of GR decrease. ILs possess excellent ionic conductivity, which contributes to the good charge transfer ability of the GR-EMIMAla nanocomposite. GR can interact with EMIMAla via the π-π, hydrophobic and electrostatic interaction, which results in a stable water-soluble GR with excellent conductivity.

3.4. Demonstration of Biosensor based on water-soluble GR with high sensitivity

Of great significance is that the successful formation of water-soluble GR enables the use of conventional solution-phase processing techniques (especially for those biomoleculars participated process) to create new GR-based materials and devices. These ionic liquids can be purposely retained or easily removed, depending on different applications. As shown in Figure 3A, the AAIL-functionalized GR was subsequently assembled with tyrosinase biomolecules (tyrosinase was chosen a model enzyme) for fabricating an electrochemical biosensor. The stable aqueous dispersion of GR is not only crucial for the assembly process, but also significant for improving the electrode to electrode reproducibility of different batches. In previous study, GO was usually used instead of GR for its good dispersion in water phase. In the present study, the tyrosinase biosensor based on EMIMAla funcctionalized GR (AAIL@GR) is compared with GO-based tyrosinase biosensor for catechol detection. As shown in Figure 3B and 3C, the detection limit of AAIL@GR based biosensor reaches as low as 8 nM with a response time of 3 s. The detection limit of the developed biosensor is significantly better than that of reported biosensor based on graphene (750 nM, Yin et al., 2011), graphene-Ag nanoparticle (100 nM, Huang et al., 2013), CNTs (7.6 μM, Perez-Lopez and Merkoçi, 2011) and Au nanoparticles (300 nM, Singh et al., 2013). The linear range reaches 3 orders of magnitude from 25 nM to 11100 nM. It should be noted that the sensitivity of AAIL@GR based biosensor (12600 mA cm^{-2} M^{-1})

238 is about 17 times higher than that of GO-based biosensor (736 mA cm^{-2} M^{-1}). Unlike electrically
239 insulating GO, AAIL-functionalized GR nanosheets retain its intrinsic excellent electronic
240 conductivity of GR, which can act as electrical nanowires of enzyme electrode to capture the
241 bioelectrocatalytic signal. The sensitivity of the AAIL@GR based biosensor is about 10 times
242 higher than that of graphene-Ag nanoparticle based nanocomposite (1230 mA cm^{-2} M^{-1}, Huang
243 et al., 2013), and 2600 times higher than that of CNTs based biosensor (4.8 mA cm^{-2} M^{-1}, Perez-
244 Lopez and Merkoçi, 2011). The sensitivity of the developed biosensor is also much higher than
245 that of biosensor based on graphene (7634 mA cm^{-2} M^{-1}, Qu et al., 2013), mesocarbon (1385 mA
246 cm^{-2} M^{-1}, Wu et al., 2012) and mesocarbon-Co_3O_4 nanocomposite (6400 mA cm^{-2} M^{-1}, Wang et
247 al., 2014). Compared to conventional GR, GO, CNTs and other nanomaterials, the advantages of
248 AAIL-functionalized GR are significant. Amino acid ionic liquid functionalized graphene proves
249 to be a robust electrochemical biosensing platform for ultrasensitive biosensing applications.

250 **3.5. Reproducibility, Selectivity, Stability and real sample application of the biosensor**

251 The repeatability of the biosensor was investigated by amperometry. The relative standard
252 deviation (R.S.D.) of the biosensor response to 300 nM catechol for 7 successive measurements
253 was 2.6%, indicating good repeatbility. To evaluate the electrode-to-electrode reproducibility,
254 three biosensors were prepared under the same conditions independently. The R.S.D. of the
255 prepared biosensors was 3.5%, indicating good electrode-to-electrode reproducibility. The good
256 water phase dispersion of AAIL-functionalized graphene contributes to the good electrode-to-
257 electrode reproducibility. The influence of matrix on the biosensor response was also evaluated
258 in the PBS (pH 7.0) containing 0.3 μM catechol in the presence of 3 μM interferents. It was
259 found that ethanol, glucose, sucrose, ascorbic acid, uric acid and inorganic ions (K^+, Na^+, Ca^{2+},

NO^{3-}, PO^{4-}, Cl—, Ac^{-}, etc.) did not cause any interference. The good selectivity should be ascribed to the intrinsic selectivity of tyrosinase for catechol substrate and the applied low potential (-0.1V). The long-term stability of the biosensor was investigated over a 35-day period. When the biosensor was stored dry at 4 ^{o}C and measured intermittently (twice every week), the current response to 300 nM catechol decreased about 5% over a 35-day period. AAILs are biocompatible ionic liquids (Wu et al., 2012). The good long-term stability can be attributed to the excellent biocompatibility of the nanocomposite, which can provide a favorable microenvironment for tyrosinase to retain its bioactivity. Both biocompatibility and inherent conductivity of the nanocomposite enable it to become an excellent biosensing platform for enzymes along with good stability.

The biosensor was further used to detect real environmental water samples. The recovery test was studied by adding spiked catechol into tap water and river water. River water samples were collected from Daliao River (Liaoning). Water samples were transported to the laboratory and were stored at 4 ^{o}C in glass containers before analysis. Tap water samples were taken from our laboratory (Dalian). All samples were filtered through 0.45 μm filter membrane before use in order to remove suspended solids. When the concentrations of catechol were from 300 nM to 10000 nM, the recovery was from 90.5% to 108.6% and the RSD was from 2.2% to 6.0 % for tap water and river water. The results confirm that the fabricated biosensor is applicable for real sample detection.

4. Conclusions

In summary, a simple method was developed for preparing water-soluble graphene by using amphiphilic ILs for the non-covalent surface modification of graphene through π-π interactions.

After physical modification by AAILs, the hydrophobic GR became water-soluble and displayed long-term stability in water-phase without deposition. Unlike conventional method, the greatest advantage of the present method is that it can retain the excellent electronic conductivity and structure integrity of GR. Our findings make it possible to process graphene materials using low-cost solution processing techniques, opening up enormous opportunities to use this unique carbon nanostructure for many technological applications. The obtained water-soluble GR was exemplified to fabricate an electrochemical tyrosinase biosensor, and displayed its superior advantage to GR, GO and CNTs based biosensor with higher sensitivity and better detection limit. The AAIL-GR based biosensor also demonstrated good reproducibility, selectivity and long-term stability for catechol detection. The recovery for real water samples was from 90.5% to 108.6%, confirmed that the biosensor was applicable for real samples detection. The GR-AAIL nanocomposite provides a versatile and robust platform for fabricating ultrasensitive biosensors with excellent performance. The discovery in this study might inspire wide application of graphene in many fields, such as nanoelectronics, sensors, biosensors, supercapacitors, and catalysis.

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338

339 **Figure and Table Captions.**
340

341 **Table 1.** Twelve different kinds of ILs selected for investigation.

342 **Figure 1**. TEM image of graphene (a) and images of water dispersions (0.4 mg mL^{-1}) of
343 graphene (b), EMIMPro functionalized graphene (c), and EMIMAla functionalized graphene (d).
344 IL concentraton: 3.0 mg/ml.

345 **Figure 2**. (A) FT-IR spectra of pristine GR, GO, EMIMAla, and EMIMAla functionalized GR
346 (GR-EMIMAla); (B) Nyquist diagrams for EIS measurements of GR-Chi, GO-Chi, GR-
347 EMIMAla-Chi, and Chi modified electrodes. Conditions: 1 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$
348 containing 0.5 M KNO_3 supporting electrolyte. Inset: Randles equivalent circuit used for data
349 fitting.

350 **Figure 3.** (A) Schematic diagram for the preparation of AAIL-functionalized graphene and
351 subcequent assembly with tyrosinase biomolecules; (B) The typical amperometric response and
352 (C) the calibration curve of the tyrosinase biosensors based on EMIMAla-functionzalized
353 graphene (AAIL@GR) and GO for catechol detection.

354

355

356 **Table 1. Twelve different kinds of ILs selected for investigation**

ILs	ILs
1-Ethyl-3-methylimidazolium Alanine	Trihexyl(tetradecyl) phosphonium tetrafluoroborate
1-Ethyl-3-methylimidazolium L-Proline	Trihexyltetradecylphosphonium bis(trifluoromethylsulfonyl)amide
1-Ethyl-3-methylimidazolium Tetrafluoroborate	1-Benzyl-3-methylimidazolium hexafluorophosphate
1-Butyl-3-methylimidazolium methanesulfonate	Tetrabutylphosphonium methanesulfonate
1-Butyl-3-methylimidazolium trifluoromethanesulfonate	1-Benzyl-3-methylimidazolium trifluoromethanesulfonate
1-Benzyl-3-methylimidazolium tetrafluoroborate	Trihexyl(tetradecyl)phosphonium methanesulfonate

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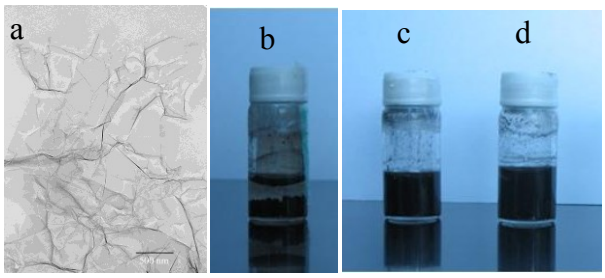

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362 **Figure 1**.

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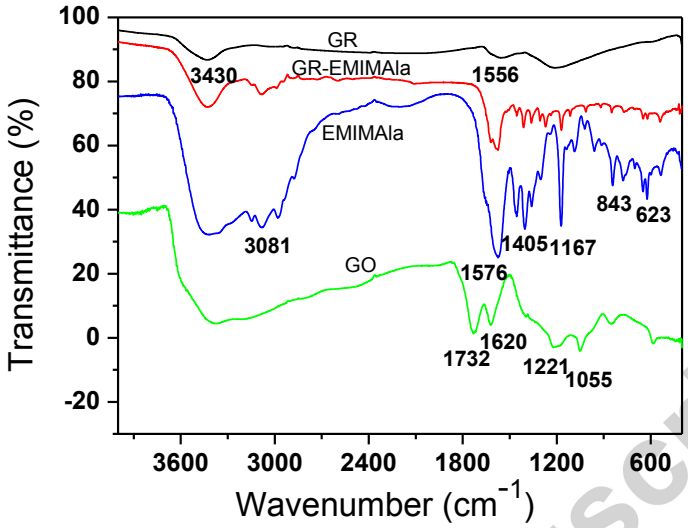

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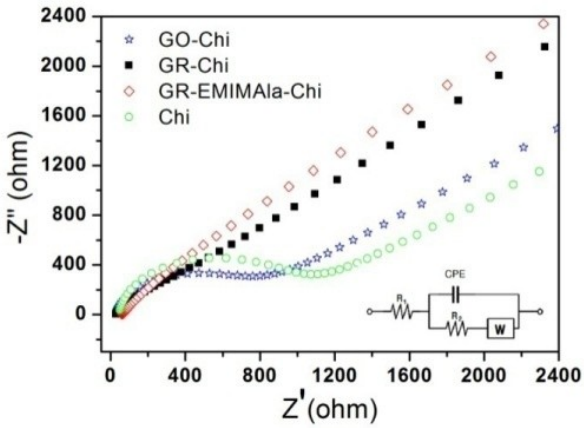
367 (A)



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369 **(B)**

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371 **Figure 2**.

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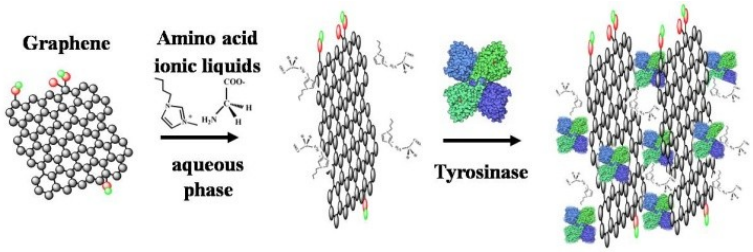
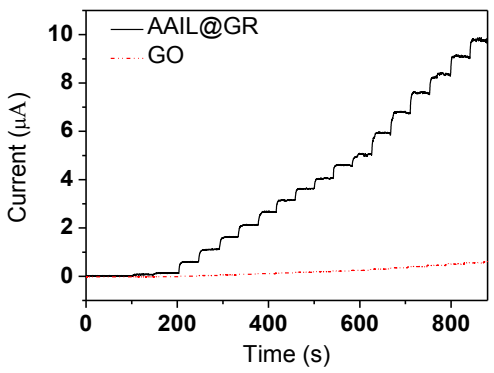
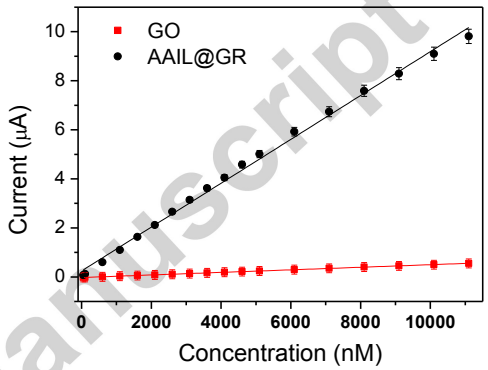


(A)





(B) **(C)**

Figure. 3.

Highlights

- A non-covalent method was developed for preparing water-soluble graphene.
- Results confirmed the water-soluble graphene retained excellent electronic conductivity.
- The graphene dispersions enable the use of conventional solution-phase processing techniques.
- A robust biosensing platform was developed based on amino acid ionic liquid functionalized graphene.
- The biosensing platform displayed excellent performance for biosensor application.