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Research Article

Comparison of DPPH and FRAP Assays for Determination of Antioxidant Activity of synthesized-electro Polypyrrole /graphene nanocomposite

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ABSTRACT

This study is in investigated the antioxidant effects of polypyrrole and grapheme nanocomposite on 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical and Ferric reduction activity potential (FRAP). The electrosynthesis of this nanocomposite on the tip of a graphite pencil by cyclic voltammetry (CV) in the potential range -0.4 to 1.2V at the 5 mL of phosphoric acid (H_3PO_4), in the presence of potassium chloride (KCl) electrolyte supplements as an inorganic salt and p-Toluene sulfonic acid (pTSA) as an organic salt was performed. Antioxidant capacity was determined using Uv-vis spectroscopy. Also, FT-IR Spectroscopy and scanning electron microscopy (FESEM) were used to study the surface structure and topography of nanocomposite. Polypyrrole-graphene nanocomposite has antioxidant properties and increases the absorption rate at FRAP = λmax596 nm by reducing iron ions and reducing the absorption rate in DPPH = λmax516 nm by the transition of electrons and destroying DPPH free radicals. Also, this nanocomposite in the presence of p-Toluene sulfonic acid has a higher antioxidant capacity than the presence of potassium chloride.

Keywords: Electrosynthesis; Nanocomposite; Antioxidant activity; DPPH; FRAP; CyclicVoltammetry.

1. Introduction

Conducting polymers are now widely used not only in the fabrication of electronic nanometers, sensors, catalysts, and electrolytes but also in biomedicine such as drug delivery, protein purification, tissue engineering, neural interactions, and activators. Different types with different properties, such as polypyrrole, the most common polycarbonate, with environmental stability, biocompatibility, electrochemical properties, water solubility, high electrical conductivity, and ease of synthesis of conductive polymers can be synthesized [1, 2]. Graphene, a monolayer of carbon atoms usually made the by reduction of oxides, has attracted much attention due to its high electronic and mechanical properties [3]. The results showed that the polymerization of pyrrole in the presence of small amounts of graphene nanoplates dramatically improved the electrical properties of PPy [4, 5]. Polypyrrole and graphene are nanocomposites that possess antioxidant properties and prevent free radical oxidation. Antioxidants are abundant in foods and vegetables and are divided into natural and synthetic classes in plant form, along with nutrients, mineral salts, enzymes, and polyphenols that protect against the development of heart disease and cancer [6, 7]. Synthetic antioxidants such as polyaniline and polypyrrole graphene are used in the industry to prevent side reactions and their adverse effects [8, 9]. The purpose of this study is to evaluate the antioxidant activity of electrosynthesis polypyrrole-graphene nanocomposites on 1,1-diphenyl-2-picrylhydrazyl (DPPH) and ferric reduction potential (FRAP). DPPH is inhibited by the H-atom transfer method in which antioxidants and substrates react with hydroxyl radical groups [10, 11], and FRAP is generated by an electron transfer technique associated with a color change [12, 13].

2. Experimental

Materials, Equipment, and Method

Graphene nanoplatelets (GR) (99.5%, thickness 2-18 nm, 32 layers, USA); potassium chloride (KCl); phosphoric acid (H3PO4) and p-Toluene sulfonic acid (pTSA) (Fluka Chemicals, Switzerland); Methanol (MeOH) (BDH Chemicals, UK); 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Sigma-Aldrich Chemicals, USA). Iron (III) chloride hexahydrate (Sigma-Aldrich Chemicals, USA); hydrogen chloride (HCl) and sodium acetate (C2H3NaO2) (PubChem Chemicals, USA); tripyridyl triazine (TpTz) (Sigma Chemicals, Germany). All aqueous solutions were freshly prepared using ultra-pure water from Milli-Q Plus (Millipore Corp., USA).

The electrochemical synthesis and characterization of polypyrrole-graphene nanocomposite (PPy/Gr) were carried out with compact Stat potentiostat (Ivium Technologies, Netherlands) by a computer equipped with the Ivium software package. FTIR spectrophotometer system 2000 (Bruker Tensor27, Germany); Uv-visible spectrophotometer V-500(carry, Germany), and Scanning electron microscope (SEM) (KYKY-EM3200, China) were used for structural analysis and characterization measurements. The three-electrode cell consists of pencil graphite (1.8 mm diameter) (Staedtler Lumograph, Germany) as the working electrode and a Platinum (Pt) electrode was used as the counter electrode against a pseudo-Ag/AgCl reference electrode.

The electrosynthesis of PPy/Gr nanocomposite on the surface of composite 2B pencil graphite was performed in a 10 ml solution mixture of 100 µl pyrrole, 0.1 mg Gr, H3PO4 (1M), KCl (1M), and pTSA (1M) (supporting electrolyte) by cyclic voltammetry method, with the potential range -0.4 to 1.2V. Ag/AgCl, scan rate 100 mVs-1 and at 25 ×±2$_{o}$c. Antioxidants capacities of PPy/Gr nanocomposite in the scavenging of DPPH free radical and FRAP were investigated. 5 ml of freshly prepared DPPH in MeOH was reacted with 0.5 gr of the nanocomposite. The solutions were stirred and their maximum absorbance was obtained at 516 nm after 20 min. The content of antioxidants in the sample is determined as a percentage decrease in color intensity related to the negative control sample.

The FRAP reagent was prepared according to the method outlined by Benzie and Strain [14]. Acetic acid buffer (300 mM, pH=3.6) was prepared by dissolving 40.8 gr of sodium acetate in 500 ml of Milli-Q water with the aid of ultrasonic agitation. The pH of the solution was then adjusted to 3.6(±0.1) with glacial acetic acid and diluted to 1 liter with Milli-Q water, HCl (40 mM) was prepared by diluting 3.3 ml of concentrated hydrochloric acid to 1 liter with Milli-Q water. Tripyridyltriazine (TpTz) (10mM) was prepared by dispersing 62.5 mg TpTz in 20 ml of 40 mM HCl with the aid of ultrasonic agitation. Ferric chloride (20 mM) was prepared by dissolving 108.1 mg of Ferric chloride hexahydrate in 20 ml of Milli-Q water with the aid of ultrasonic agitation. The final FRAP reagent was prepared by combining 500 ml of 300 mM acetic acid buffer (pH=3.6), 20 ml of 10 mM TpTz and 20 ml of 20 mM Ferric chloride. 5 ml of freshly prepared FRAP was reacted with 0.5 gr of the PPy/Gr nanocomposite. The solutions were stirred and their maximum absorbance was obtained at 596 nm after 20 min.

3. Results and discussion

3.1. Cyclic voltammetry (CV)

Cyclic voltammetry for PPy/Gr nanocomposites fabricated in a three-electrode system which includes pencil graphite as working electrode; Ag/AgCl electrode as reference electrode and platinum electrode act as counter electrode. This CV procedure was constructed at the scan rate of 100 mVs^{-1}in the potential range from -0.4 to 1.2 V. PPy/Gr nanocomposite can be prepared in the presence of different electrolyte supplements and every time precipitated in the form of a dark layer on the working electrode. Fig.1 shows preparing PPy/Gr nanocomposite in 1 M H_3PO_4 containing 1 M KCl (inorganic electrolyte support). In Fig.2

this nanocomposite shows preparing PPy/Gr nanocomposites electro-synthesized in 1 M H_3PO_4 containing 1 M KCl and 1 M pTSA (organic electrolyte support), also Fig.3 shows preparing Py/Gr nanocomposites in 1 M H_3PO_4 containing 1 M pTSA.

Potassium chloride and p-toluenesulfonic acid are suitable for this synthesis because mono pyrrole and graphene can be polymerized in both aqueous and organic environments. The peak shifts to more negative potentials. The lower conductivity of the modified electrode compared to graphite results in a decrease in the peak synthetic flux on subsequent scales. The presence of graphene particles in the solution due to the increased contact area between the solution and the electrode leads to increased electron mobility and ultimately conductivity, thereby improving electrode properties to some extent. In the first cycle, the conjugate peak disappears in the next cycle. This peak is reported to be related to impurities in the composite bed.

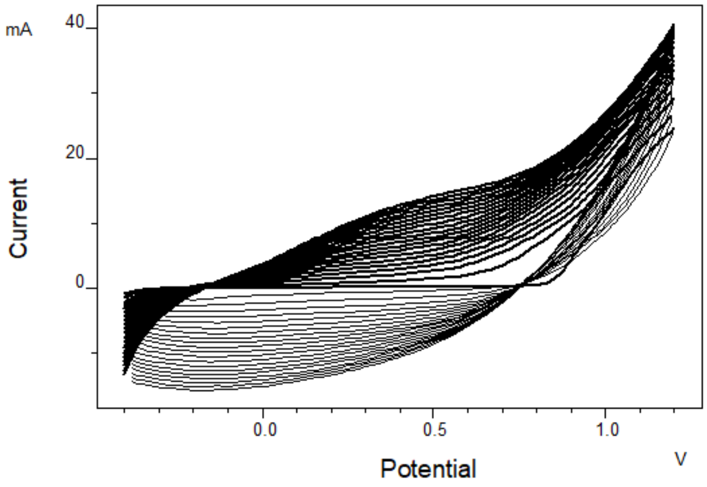

Fig1. Cyclic voltammetry curves of PPy/Gr nanocomposites electro-synthesized in 1 M H_3PO_4 containing 1 MKCl

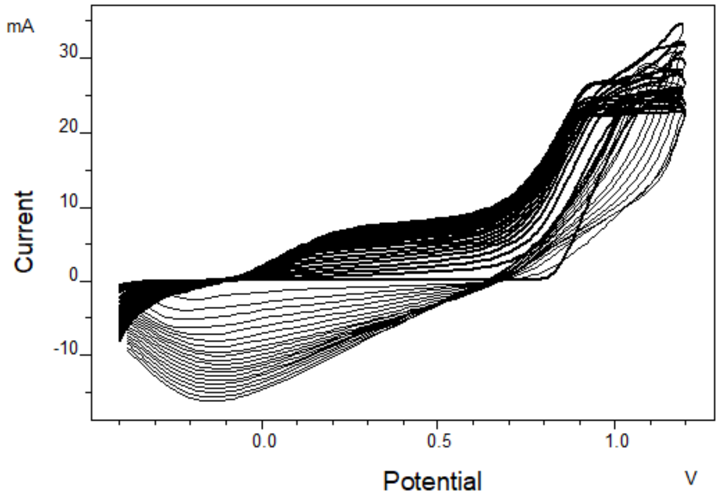

Fig2. Cyclic voltammetry curves of PPy/Gr nanocomposites electro-synthesized in 1 M H_3PO_4 containing 1 M KCl and 1 M pTSA

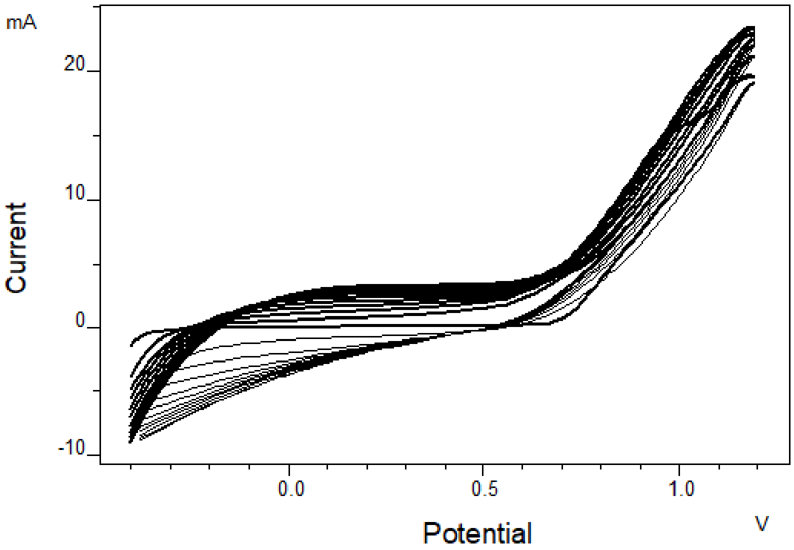

Fig3. Cyclic voltammetry curves of PPy/Gr nanocomposites electro-synthesized in 1 M H_3PO_4 containing 1 M pTSA

3.2. Structure analysis

Each of the absorption bands represents the presence of an element and a functional group as follows. Fig.4 shows the FTIR spectra of the polypyrrole-graphene nanocomposite. Characteristic absorption peaks were observed at 3435, 2923, 1543, and 1459 cm^{-1}, they are assigned to the N-H, C-H, C-C, C-N, and stretching vibration, respectively in the pyrrole ring. The peaks at 1291 and 1162 cm^{-1} correspond to the C-H and C-N in plan ring deformation and bending modes, respectively. The observed peak at 1110 cm^{-1}was related to the bending vibrations hydrogens in The PPy/Gr nanocomposites also show a peak at 1582 to 1588 cm^{-1} that is corresponding to the C=C stretching of the pyrrole ring, and peaks at 1189 to 1201 cm^{-1} represent C-C stretching, which were consistent with PPy characteristics, indicating the successful functionalization of Gr by PPy[15, 16].

The broad bands at 850 and 554 cm^{-1} correspond to phosphate ions. PPy/Gr nanocomposite prepared in the presence of KCl and pTSA resulted in three absorption peaks which are 340, 450, and 800 cm^{-1}. In general, the FTIR spectra of PPy/Gr nanocomposite are more similar to that of PPy. These results indicate interaction between PPy and Gr in the PPy/Gr nanocomposite.

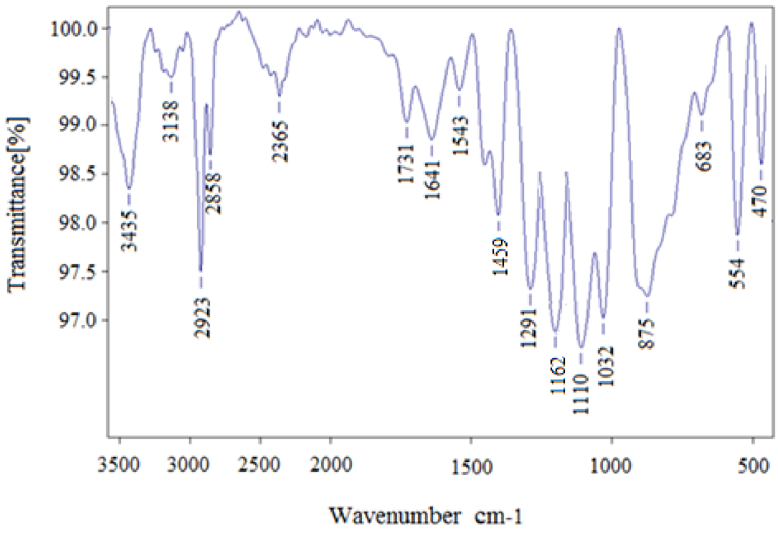

Fig4. FTIR spectra of PPy/Gr nanocomposite in 1 M H_3PO_4 containing 1 M KCl and 1 M pTSA

3.3. The antioxidant activity of PPy/Gr nanocomposite

PPy/Gr nanocomposites are synthesized separately in the presence of KCl, next time at the pTSA, and finally in the presence of both electrolytes support. After drying the nanocomposites, 0.5gr of each of them was placed in 5ml of DPPH for 30 minutes and scanned by Uv–vis spectra to investigate the antioxidant capacity of these composites on the DPPH free radical. Fig. 5 shows that the maximum absorption of DPPH is 516 nm. This nanocomposite is capable of destroying more free radicals by donating hydrogen and radical cationic inhibition. The antioxidant properties of the nanocomposite increased when synthesized in the presence of pTSA. It is evident that the existence of pTSA in electrosynthesis of PPy/Gr nanocomposite is capable of eliminating the DPPH radicals and increases the antioxidant activity of PPy/Gr nanocomposite as compared to the higher molar fraction of KCl in the electrolyte solution. This spectrum appears in the area of 200 to 800 nm.

$$DPPH(purple\ ) + H - A \rightarrow DPPH - H(yellow) + A \qquad (1)$$

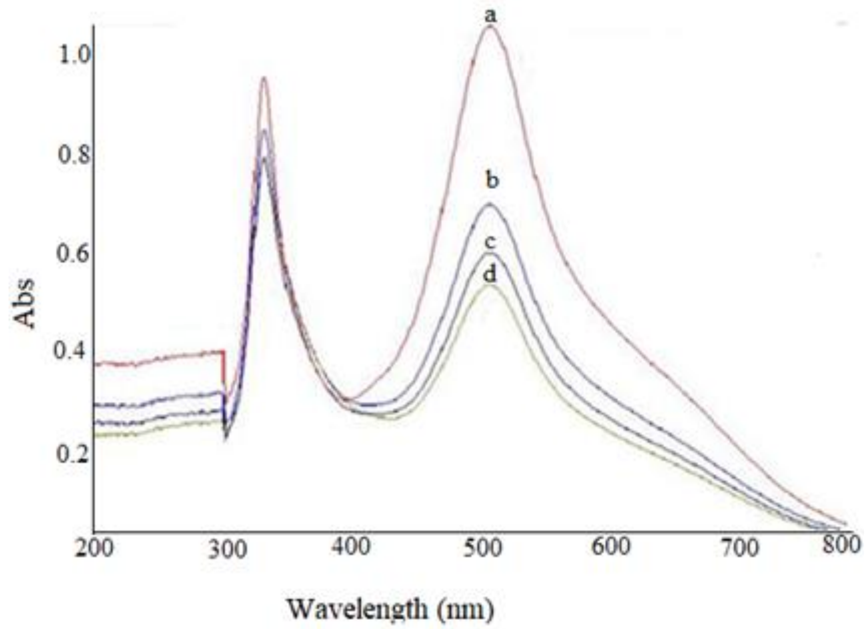

Fig5. (a) UV–visible spectra of DPPH. (b) The effect of PPy/Gr nanocomposite synthesized in 1 M H_3PO_4 containing 1 M KCl on the DPPH. (c) The effect of PPy/Gr nanocomposite synthesized in 1 M H_3PO_4 containing 1 M pTSA and 1 M KCl on the DPPH. (d) The effect of PPy/Gr nanocomposite synthesized in 1 M H_3PO_4 containing 1 M pTSA on the DPPH.

This time, 0.5 g of PPy/Gr nanocomposites prepared in the presence of different electrolytes were placed near FRAP for 30 min, after which the antioxidant capacity was examined. Figure 6 shows that FRAP has an absorption maximum of 596 nm. This nanocomposite can reduce FRAP and increase its absorption through electron transfer and conversion of Fe^{3+} to Fe^{2+} ions. This property is enhanced when nanocomposites are synthesized in the presence of pTSA.

$$Fe^{+3} - complex\ TpTz\ (colorless\ yellow) \xrightarrow{Antioxidant} Fe^{+2} - complex\ with\ TpTz\ (\ blue\ ) \qquad (2)$$

This means that the intensity of the FRAP absorption band increases by increasing the molar fraction of pTSA which supports electrolyte in the electrosynthesis of PPy/Gr nanocomposite.

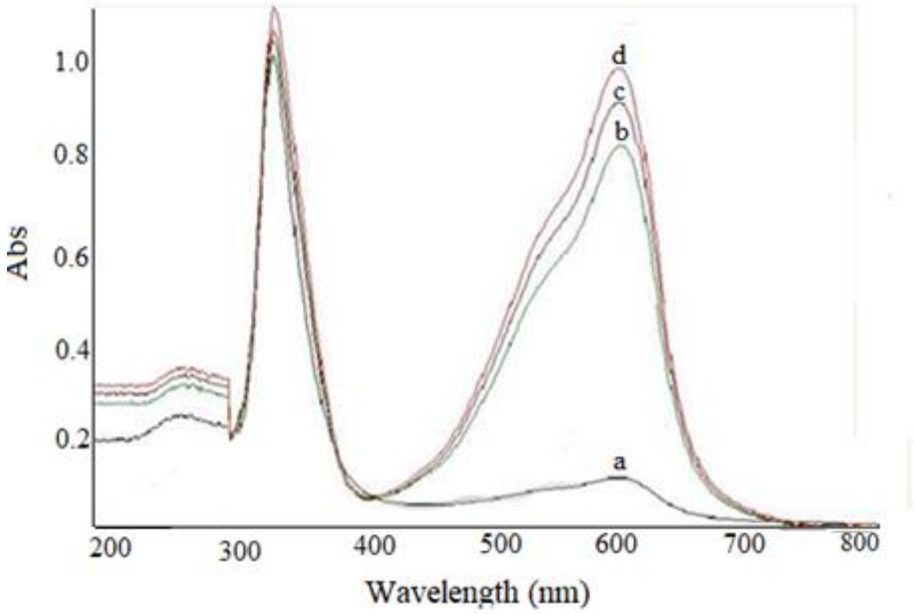

Fig6. (a) UV–visible spectra of FRAP. (b) The effect of PPy/Gr nanocomposite synthesized in 1 M H_3PO_4 containing 1 M KCl on the FRAP. (c) The effect of PPy/Gr nanocomposite synthesized in 1 M H_3PO_4 containing 1 M pTSA and 1 M KCl on the FRAP. (d) The effect of PPy/Gr nanocomposite synthesized 1 M H_3PO_4 containing 1 M pTSA on the FRAP.

3.4. Scanning Electron Microscopy (SEM)

The morphology of the PPy/Gr nanocomposites was observed by SEM. The synthesized PPy shows a large protruding sharp rocky shape and a typical rough surface of PPy as depicted. PPy/Gr nanocomposite shows in Fig.7. It was revealed that the PPy grew on the surface of the Gr and a little agglomeration of PPy is found which usually occurred during the synthesis process. This agglomeration indicates that the polymerization of pyrrole occurred on the surface of graphene owing to the π- π interaction and hydrogen bond between pyrrole and graphene. It has a large π-electron system and forms the π- π noncovalent interactions as the predominant force between the adsorbent and the adsorbate [17]. This nanocomposite could achieve high absorption. The graphene is completely covered by polypyrrole thus it forms a well-designed PPy/Gr structure with a great network. Polypyrrole nanoparticles are cumulatively composed of spherical grains on graphene nanoparticles that are plate-shaped. The presence of graphene nanoparticles in polypyrrole and graphene nanocomposites reduces the size of polypyrrole nanoparticles.

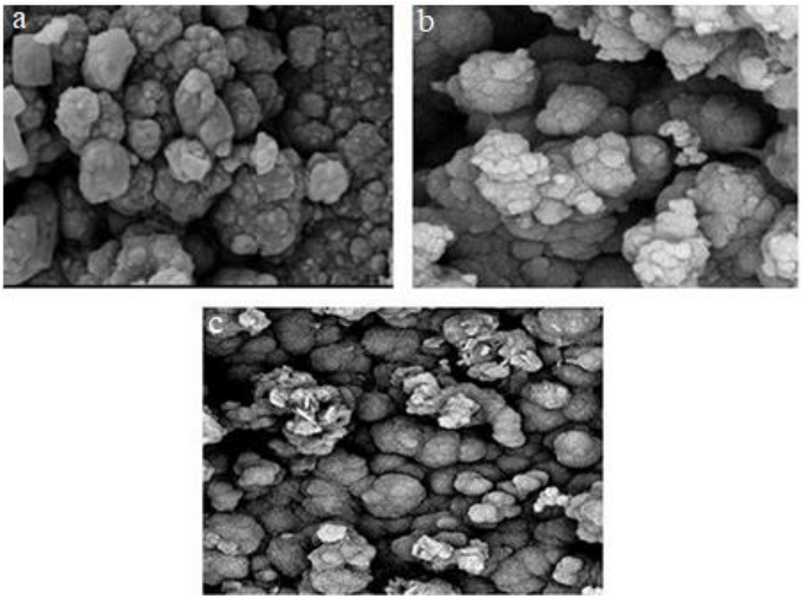

Fig7. (a) SEM analysis of electro synthesized PPy/Gr nanocomposites in 1 M H_3PO_4 containing 1 M KCl. (b) SEM analysis of electro synthesized the PPy/Gr nanocomposites in 1 M H_3PO_4 containing 1 M KCl and 1 M pTSA. (c) SEM analysis of electro synthesized the PPy/Grnanocomposites in 1 M H_3PO_4 containing 1 M pTSA

4. Conclusions

In conclusion, it has been demonstrated that PPy/Gr nanocomposite electrosynthesized at H3PO4 (1M), in the presence of pTSA (1 M) or KCl (1M) have considerable antioxidant activity on DPPH and FRAP and this property increases when the nanocomposite is synthesized in the presence of pTSA. As a result, it can be argued that paratholene particles of sulfonic acid have more antioxidant effects than those of potassium chloride while the presence of potassium chloride can also increase the conductivity and stability of the PPy/Gr nanocomposite.

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