Green synthesis of graphene based manocomposite for sensing of heavy metals

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Abstract

A simple and green ecofriendly approach was revealed for the synthesis of silver-reduced graphene oxide nanocomposites (Ag@rGONCs) using jambul seed extract. The synthesized composites were characterized by UV–visible spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR), Particle size and zeta potential analysis. The surface morphology and elemental analysis of synthesized Ag@rGONCs was done using scanning electron microscopy (SEM/EDX). The modified graphene can efficiently adsorb heavy metal ions and selectivity was precise due to presence of embedded silver ions on surface. The *in situ* decoration of silver ions on graphene oxide (GO) was accomplished in single step via one pot methodology using green precursor. The catalytic action of jamun seeds helps to reduce the GO to rGO and simultaneous decoration of silver nanoparticles. It also provided capping ability to avoid aggregation. The resultant nanocomposites (NCs) shown good selectivity towards the determination of Pb^{2+}, Hg^{2+} And Cr (VI) ions with the recognition limits of 50 nM, 15 nM and 500 nM, respectively

Keywords: Graphene oxide, Green catalysis, Hevay metal sensing, Jambhul seeds.

Introduction

In the recent years the industrial pollution owing to heavy metals and dyes is a serious threat to us and is increasing day by day. This increase in environmental hazards is crucial for human, animals, fish and invertebrates.[1] The effluents from industries such as industries such as paper, pesticides, battery built-up manufacturing, fertilizer, mining, metallurgical, fossil fuel, tannery etc. were wastewater contains potential heavy metals.[2] Plastic manufacturing industries was another big hurdle in increasing the pollution day by day, which is non-biodegradable in nature. Industrial effluents come in contact with soil or water can possibly being contaminated with heavy metals, which further come in contact with animals or plants, it gets accumulated into tissue or organs.[3] A specific catalyst is required for catalyzing the free heavy metal in wastewater. The heavy metal pollutants majorly contain Pb^{2+}, Ni, Hg^{2+}, Cr and Cd. These types of heavy metals accumulate in the human body and cause serious health effects. The accumulation of heavy metals found in kidney, liver, lungs, etc. might be responsible for initiation of cancer.[4] Acute and chronic arsenic toxicity involves adverse impacts on the nervous, cardiovascular, respiratory, gastrointestinal, hepatic, renal, hematopoietic, immunological, and dermatologic systems.[5] Cadmium, lead and manganese have found a variety of uses in industry and farming owing to their physical and chemical properties. Poisoning induced by high concentration of these metals adversely influence on kidney, hematopoietic cells, nervous system, and bones.[6]

A green chemistry or synthesis methodology was emerging in 21 century due to vast demand and lesser hazardous nature of reactions. Biosynthesis of many metal nanoparticles was accomplished using many natural precursors. The green synthesis approaches can effectively substitute physical or chemical methods of synthesis.[7] Green methods were easy to enhance and can have a extensive variety of applications along with the colloidal stability of nanomaterials. Green agents from natural base comprise different kinds of

phytoconstituents, having several activity such as antioxidants and reducing, capping agents.[8]

The Jambhul or Jamun is a perennial tropical tree with scientific name *Syzygium cumini* native in Indian subcontinent and other parts of Asia. The whole plant was used for treating diabetic patients as an hyperglycemia agent.[9] The catalytic performance of seed of *Syzygium cumini* was use to biosynthetically prepare silver nanoparticles with prominent antioxidant activity.[10]

The *Syzygium cumini* is similarly known as Jambul or Jamun is family "*Myrtaceae*" It is intuitive to India. The parts of Jamun plants such as entire seeds, seed coat, and kernel are used in the traditional remedies. The phytoconstituents of jambul seed contain glycoside, jambosine, gallic acid, ellagic acid, corilagin, quercetin, and β-sitosterol.[11]

The use of plant extracts for production metallic nanoparticles is cheap, easily scaled up and environmentally benign with excellent use of green resources for production of nanoparticulate system. The use of green precursor could possibly avoid chemical catalyst or reactant and avoid production of toxic gases. The present study highlights, a simple, green and eco-friendly technique is used to synthesize Ag@rGONCs. The one-pot synthesis technique, accumulated to form graphene based silver nanocomposite in presence of direct sunlight and jambul seed. The green extract performed its role as reducing as well as capping agent to stabilize the nanocomposite. The conversion or reduction process ceases in presence of high phenolic content within aqueous extract. The phenol can efficiently reduce the metal precursor to its ion and form stable composite.

Experimental Methods
Materials
Jambhul seed was collected from a local market (Shirpur, Maharashtra, India). Graphite flakes was received as gift sample from Asbury Carbons, NJ, USA. Sulfuric acid, hydrochloric acid, silver nitrate and ammonia solution were purchased from Merck Specialities, Mumbai, India. Potassium permanganate and hydrogen peroxide were purchased from Loba chemie Chemicals, Mumbai, India and RFCL, Mumbai, India respectively. All other chemicals and reagents used for characterization were of analytical grade. Jambhul Seed is a natural reducing agent were be used for synthesis of Ag@rGONCs

Preparation of jambul seed extract
Preparation of Jambhul seed shells extract involved cutting the seed shells into very fine slices and accurately weighing 50 g of small pieces which were washed thoroughly with distilled water, dried, blended and boiled in distilled water (100 ml) at 80^0C for 1.5 h, followed by filtration using muslin cloth to remove insoluble material. The prepared extract was stored in a refrigerator at 4^0C for further studies. The prepared extract was tested for the presence of potential phytochemical constituents according to a reported test protocol.[12]

Synthesis of Ag@rGONCs
As in the process described elsewhere (Patil PO et al., 2017), the reaction proceed by mixing 0.1 M solution of silver ammonia solution in 1:2 (v/v) ratio of 0.1 M silver nitrate with ammonia. Start addition of 0.1 mg/mL aqueous dispersion of GO and ultrasonicate for 10 min.

The reduction process was started after addition of jambhul seed shells extract (2mL) in above solution containing predispersed GO, $AgNO_3$ and ammonia. The reaction was started once the solution allows to expose in bright sunlight. As soon as the reduction process starts, the color of the solution changes from dark brown to yellow. The slow color transformation indicates the formation of AgNPs. To accelerate the nanocomposite formation, reaction mixture was covered with aluminum foil and store in a dark area to protect it from sunlight for a few hours which helps to deposit ionized silver ions on the GO sheets. Finally, the yellowish brown colored solution was centrifuged at 4000 rpm for 10 min and the precipitate was washed three- four times with distilled water to remove any unreacted precursor. The final product was dispersed in 50 mL of deionized water and used for further studies.

Characterizations
The synthesized GO and Ag@rGONCs nanocomposite was preliminary characterized using a

UV–visible spectrophotometer (Shimadzu 1800; Japan), which recorded between 800 and 200 nm. The synthesized GO and Ag@rGONCs nanocomposite was primarily examined using a DRS 8000 IR spectrophotometer (Shimadzu 8400 s; Japan) and scanned over a wave range of 4000–400 cm^{-1}. The surface morphology and elemental analysis of the GO and Ag@rGONCs nanocomposite was examined under a scanning electron microscope (SEM) with 15 kv acceleration voltage, and

Observations were carried out using energy-dispersive X-ray spectroscopy (EDX) on Bruker 1530-2 FESEM/EDS, Germany. The particle size and zeta potential of synthesized GO and Ag@rGONCs nanocomposite were measured using the Nanoplus 3 Particulate System (Micromeritics, USA). The dynamic light scattering characterized the size and surface charge from synthesized composite.

Heavy metal sensing procedure
Spectroscopic sensing of heavy metals (Pb^{2+}, Hg^{2+}, Cr (VI)
Sensing of Pb^{2+}by Ag@rGONCs
For the detection of Pb^{2+} by UV visible spectroscopic method, typically, firstly stock solution of 1 mM PbCl$_2$in water was prepared in distilled water and also prepared a Ag@rGONCs solution (1mg/ml) in distilled water. 5, 10, 50, 100, 500, 1000, 1500 and 2000 µL ofPb^{2+} of different concentrations25 nM, 50 nM, 250 nM, 500 nM, 2500 nM, 5×10^3 nM, 75×10^2 nM and 5×10^4 nM were added in separate test tubes. Then, 2ml of Ag@rGONCs were added above different test tubes. After through shaking, the color change in the solution were observed by UV-vis absorption spectra from 800-200 nm were recorded.

Sensing of Hg^{2+} by Ag@rGONCs
The sensing of heavy metals with the Ag@rGONCs was performed using UV-vis spectrophotometer. The absorption spectra of the Ag@rGONCs were recorded upon the addition of various concentrations of metal ions. For optical detection, 1ml of HgCl$_2$ (5µm)concentration solution was added into 2 mL of Ag@rGONCs solution, shaken well and subjected to 2min resting time. The changes of Ag@rGONCs absorbance band with the addition of different concentration of Hg^{2+} were monitored by recording absorption spectra from 800-200 nm. For colorimetric detection, an optimum level of higher concentration of different metals ion were added separately into the Ag@rGONCs solution, shaken well and allowed to rest and color changes were observed with bare eye.

Sensing of Cr (VI) by Ag@rGONCs
For the detection of Cr(VI) by UV visible spectroscopic method was used. Firstly, the stock solution of 1 mM K$_2$Cr$_2$O$_7$in water was prepared in distilled water and also prepared a Ag@rGONCs solution (1mg/ml) in distilled water. 5, 10, 50, 100, 500, 1000, 1500 and 2000 µL ofPb^{2+} of different concentrations25 nM, 50 nM, 250 nM, 500 nM, 2500 nM, 5×10^3 nM, 75×10^2 nM and 5×10^4 nM were added in separate test tubes. Then, 2ml of Ag@rGONCs were added above different test tubes. After through shaking, the color change in the solution were observed by UV-vis absorption spectra from 800-200 nm were recorded.

Sensing of Cr(VI) by Ag@rGONCs
The spectroscopic detection of Cr (VI) by Ag@rGONCs was carried out under direct sunlight irradiation. This detection experiments were performed on days of bright sunlight in between 10am to 2 pm. Typically, for Cr(VI) detection, Firstly prepared stock solution of K$_2$Cr$_2$O$_7$ (1mM) in distilled water as well as separately prepared a 0.5mg/ml of Ag@rGONCs in distilled water. The 25 ml of Ag@rGONCs was dispersed in 50ml of stock solution of K$_2$Cr$_2$O$_7$ and stirred for 30 min before sunlight treatment to ensure that it reaches the adsorption/desorption equilibrium. After that this reaction mixture treated with sunlight. At a given time interval of 5 min, 2.5 mL of the reaction mixture was collected and the Ag@rGONCs photocatalyst was separated from the suspension using a bar magnet. The equilibrium concentration of the supernatant of each sample was monitored in a UV-visible spectrophotometer by measuring the intensity of absorbance of Cr(VI). The residual concentration of Cr(VI) in the supernatant was evaluated using a UV/Visible spectrophotometer at a fixed wave length of 370 nm.

Ag@rGONCs solution (1 mg/ml)

2 mg of synthesized nanocomposite dissolved in 2 ml of distilled water.

Results and Discussion

Phytochemical test results: Jambhul Seed shells extracts were tested for their phytochemical contents. The phytoconstituents flavonoids, carbohydrates, alkaloids, phenols, tannins, steroids and terpenoids were present in Jambhul Seed shells extract.

UV spectroscopy analysis

The UV spectrum of synthesized GO showed maximum peak absorption at 229 nm (Figure 1A) which is attributed to $\pi-\pi*$ transition of the atomic C–C bonds and a shoulder peak at ~300 nm is due to n–$\pi*$ transitions of C–O group. The interaction of AgNPs on the surface of GO by in situ reduction of GO and AgNO$_3$ using JSE extracts, new band emerged at 259nm (Fig.1B).The band at 259 nm corresponding to the characteristic surface plasmon absorption band of AgNPs. The disappearance of characteristic peaks of GO and the emerging of new band at 259 nm corresponding to the reduction of GO by electrostatic conjugation that is $\pi-\pi*$ transition of aromatic C-C bond between GO and reduced GO. The process concealed of simultaneous reduction of both GO and AgNO$_3$ and the formation of Ag@rGONCs. The absorption peak at 259 nm indicating that GO was reduced successfully to reduced graphene oxide.
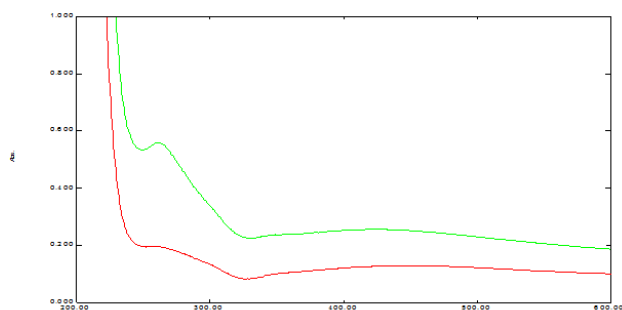

Fig. 1B: UV spectra of Ag@rGONCs using JSE

FTIR spectroscopy analysis

From the FTIR spectrum of GO (Fig. 2)shows the formation of oxygen containing groups after harsh oxidation process, such as hydroxyl(OH) stretching at

3128.54 cm^{-1}, carbonyl(C=O) at 1761.01 cm^{-1}, unstable double bond (C=C) at 1585.49 cm^{-1} and peak at 1058.92 and 1226.73 cm^{-1} due to epoxy or alkoxy group(C-O).The introduction of these oxygen-containing groups resulted in the increase of the interlayer spacing between graphite sheets but did not exfoliate them totally. The FTIR spectrum of Ag@rGONCs (Fig. 2) showed a band at 1627 cm^{-1} corresponding to stretching of C-O present in carbonyl and carboxylic group of peptide linkages. The bands observed at 2746 and 3140 cm^{-1} can be assigned to the asymmetric stretching of C-H bonds and the O-H stretching of hydroxyl group, respectively. Interestingly, the interactions between the AgNPs and GO were strong enough to ensure the nanoparticles remain attached even after the sunlight and JSE treatment. From FTIR spectra, the intensity of the oxygen containing peak of GO decreased which shows the successful formation of nanocomposite.
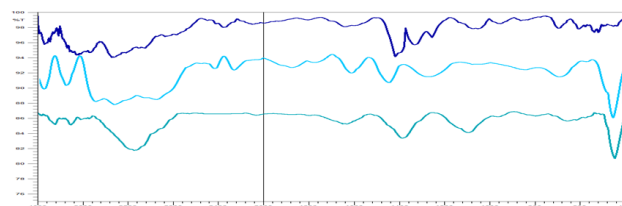

Fig. 2: FTIR spectrum of GO, Jambhul seed extract and Ag@rGONCs

Scanning electron microscopy

Fig. 3B shows surface morphology of the as-prepared Ag@rGONCs hybrids. Wherein, it illustrates that well dispersed Ag@rGONCs were deposited on to the graphene that were fabricated using JSE with the exfoliation of graphene oxide. Fig. 3B elucidates the deposition of AgNPs on to the well exfoliated graphene sheet. Interestingly, during the simultaneous reduction process, reduced graphene oxide was doped with Ag particles. The deposited Ag crystallites on rGO act as spacers between the neighboring GO sheets. Due to presence of such intermittent spacing Ag particles were randomly distributed on the graphene sheets. The reduced GO shown to be characteristic separated and exfoliated configuration. The surface morphology confirms the exfoliation of GO after oxidation process and decoration of Ag after

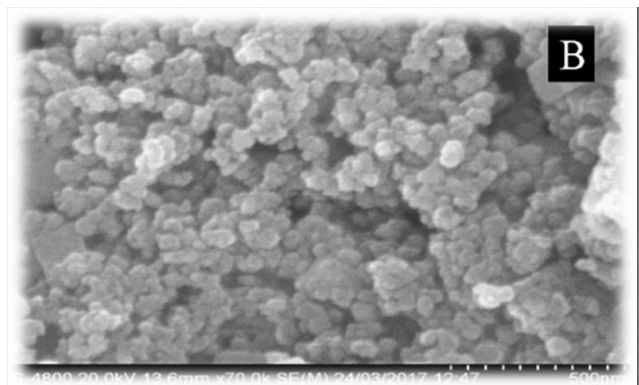green reduction strategy reveals formation of Ag@rGONCs successfully.

Fig. 3B: SEM image of Ag@rGONCs

Elemental analysis by using EDX

The EDX spectra of Ag@rGONCS (Fig. 4B) shows the presence of C (11.49%), O (30.04%) and Ag (58.47%) respectively. The spectrum indicates the reduction of oxygen functionalities from GO sheets with adsorption of Ag ions. Existence of these all elements (Ag, C, O) in the nanocomposites as well as decreasing quantity oxygen concentration in nanocomposites as compare to GO demonstrates successful formation of the nanocomposites.
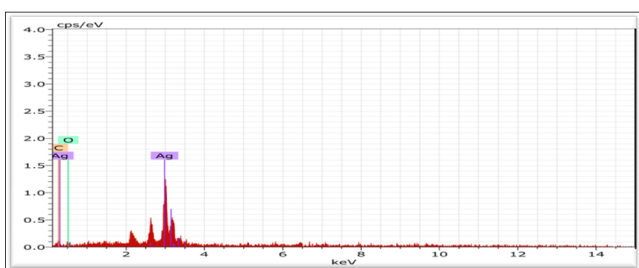
Fig. 4: EDX spectra of B) Ag@rGONCS

Particle size and zeta potential analysis

The green synthesized nanocomposites Ag@rGONCS shows particle size of 237.4 nm with respective zeta potential found to be 25.94 mV. The Fig. 5 B shows positive surface charge with 25.94 mV indicates the synthesized nanocomposite was highly stable for longer period. Due to effect of capping of JSE potentially reduces the size of composite as well as stability in aqueous environment.
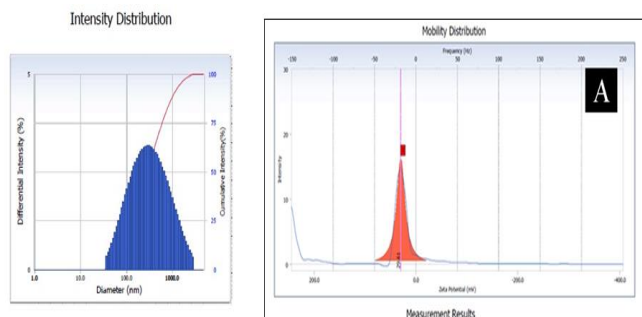

Fig. 5B: Particle size and Zeta potential of Ag@rGONCs

Spectroscopic sensing of Pb^{2+}, Hg^{2+} and Cr(VI) ions.

Sensing of Pb^{2+} ions by Ag@rGONCs

The synthesized Ag@rGONCs was tested for the spectral and optical detection of Pb^{2+} ions. In the detection of Pb^{2+}ions by Ag@rGONCs, the surface plasmon absorption peaks in UV-visible spectra was quantified on concentration basis.

As the concentration of Ag@rGONCs increases with absorption maximaPb^{2+}ions decreases was shown in Fig. 6A. The optical characteristics with deceasing peak intensity correlates with the quantitative reduction process. The point at which the absorption maxima of Pb^{2+} disappeared correlates the complete reduction of heavy metal. It is also clearly shown that the peak width went on increasing with the increased addition of Pb^{2+} ions in the Ag@rGONCs solution.
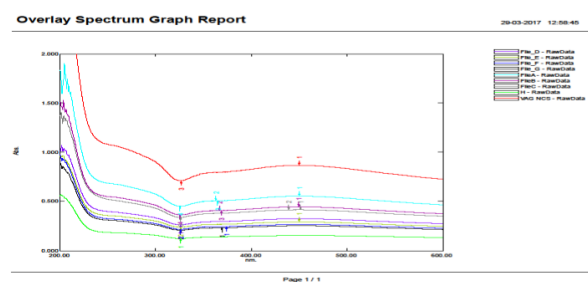

Fig. 6: A): UV-visible spectrum Ag@rGONCs synthesized using green extract B)visual detection of Pb^{2+} by Ag@rGONCs A) 25 nM, B) 50 nM, C) 250 nM, D) 500 nM, E) 2500 nM, F) 5×10^3nM, G) 75×10^2nM and H) 5×10^4nM of $PbCl_2$(1mM) solution with Ag@rGONCs.

It was observed that changes in the UV spectra of Ag@rGONCs occur at 25 nM concentration of Pb^{2+}

ions. The pronounced peak at 418 nm was observed and its intensity dramatically decreased with the addition of increasing amount of Pb^{2+} ions. But rapid change was detected at 25 nM concentration. Thus 25 nM was taken as limit of detection for Pb^{2+} using Ag@rGONCs Once the addition of 10×10^4nM concentration of Pb^{2+}ions into the Ag@rGONCs solution the specific peak completely disappeared, this might be due to loss of characteristic property of nano-regiem after strong aggregation of Ag@rGONCs at this high concentration of Pb^{2+}. Upon addition of Pb^{2+} ions in the solution of Ag@rGONCs turned colorless, induced by aggregation of Ag@rGONCs, which was detected by visually and also established by UV spectroscopic as shown in Figure 6A

Sensing of Hg^{+2} ions by Ag@rGONCs
The Ag@rGONCs was tested for the spectroscopic determination of Hg^{2+}. The Ag@rGONCs shows strong absorption maxima around 418 nm in Uv-visible spectrum. The change in absorption intensity could easily monitor the variation of analyte concentration. In the determination Hg^{2+} the variable concentration of Ag@rGONCs resulted into the shifting of absorption maxima towards the red shift. Recognizing the optimal concentration, Fig. 7 demonstrates the Uv-Vis absorption intensities for correctively sensing $Hg^{2+.}$ At the optimal concentration 5 μM Hg^{2+}ions, a significant reduction in the absorption intensity was observed. The red shift on the UV band of the Ag@rGONCs indicates the limit of detection of Hg^{2+} ions from solution. The visible observations could superficially detected by change in color of the solution from faint brown to colorless. At 5μM Hg^{2+} concentration taken as limit of finding for Ag@rGONCs. The red shift and significant reduction in the absorption peak intensity was caused due to the redox interaction between the Hg^{2+} ions and Ag@rGONCs The red shift in the UV absorption band can be attributed to the adsorption of Hg^{2+} on to Ag@rGONCs Inter surface spacing between GO and Ag can efficiently detect the lowest concentration of Hg^{2+}visually, detected by change in color of the solution to colorless. The Ag@rGONCs absorption index does not affected by addition of other metal concentrates in presence or absence of Hg^{2+}.
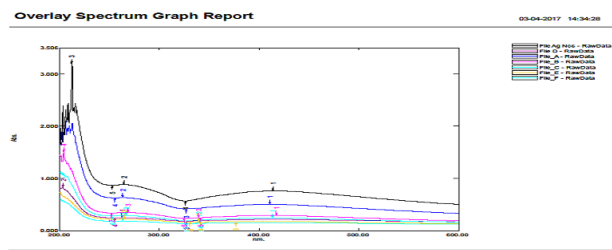

Fig. 7: UV-visible spectrum Ag@ rGONCs synthesized using JSE upon each addition of 5μM Hg^{2+} ions and Visual detection of Hg^{2+}ions

Sensing of Cr (VI) ions by Ag@rGONCs
The finding of Cr (VI) ion by the Ag@rGONCs using UV spectroscopic study was shown in figure 8. The UV absorption spectra of Ag@rGONCs with corresponding increasing concentration of Cr (VI) ions can be detected by change in intensity. The absorption intensity of Ag@rGONCs decreases with increasing concentration of Cr (VI), while it get disappeared at highest concentration. From figure 8, the peak projecting around 428 nm was start decreasing after addition of 25 nM concentration of Cr (VI), the significant decrease in absorption maxim correlates the limit of detection. The limit of quantification was at concentration of 10×10^4nMspecifically signifies the highest concentration at which the peak was completely disappeared. The visual observation can also determines the catalytic performance of Ag@rGONCs for detection Cr(VI), by observing color change as shown in Fig. 8B.
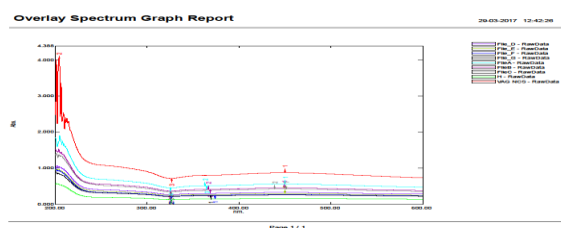

Fig. 8: Spectroscopic sensing and visual detection of Cr(VI) by Ag@rGONCs NCs, A) 25 nM, B) 50 nM, C) 250 nM, D) 500 nM, E) 2500 nM, F) 5×10^3 nM, G) 75×10^2nM and H) 5×104 nM of $K_2Cr_2O_7$ (1mM) solution with Ag@rGONCs NCs

Conclusion

The results obtained from presence hypothesis on catalytical performance encouraged us to receive the following conclusions. The fabrication methodology for Ag@rGONCs was accomplished in a single step, facile and green synthesis method, which is more economical and less time consuming. The performance of Jambhul seed extract that are usually discarded have been effectively used for the synthesis of Ag@rGONCs The heavy metals detection ability of synthesized Ag@rGONCs by spectroscopic method provides an alternative tool to conventional and expensive methods for heavy metal detection in water. Synthesized Ag@rGONCs were effectively utilized for sensing of Pb^{2+}(25 nM), Hg^{2+}(5 μM) and Cr(VI)(25nM).

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Conflict of Interest

None.

References

1. Uluturhan, E. and F. Kucuksezgin, Heavy metal contaminants in Red Pandora (Pagellus erythrinus) tissues from the eastern Aegean Sea, Turkey. *Water Res* 2007;41(6):1185-92.
2. Saeedi M, L.Y. Li, and M. Salmanzadeh, Heavy metals and polycyclic aromatic hydrocarbons: pollution and ecological risk assessment in street dust of Tehran. *J Hazardous Mater* 2012;227:9-17.
3. Merian E. and T.W Clarkson, Metals and their compounds in the environment. 1991: VCH.
4. Jiries A, Vehicular contamination of dust in Amman, Jordan. Environmentalist, 2003;23(3):205-10.
5. Okuda J. and K. Iida, Preparation of a" chronological table of main diseases in Japanese history" for pharmacy students of the 6-year program. *Yakushigaku Zasshi* 2005;40(2):137-46.
6. Inoue K, Heavy metal toxicity. *J Clinic Toxicol S* 2013;3:2161-0495.
7. Iravani S, Green synthesis of metal nanoparticles using plants. *Green Chemist* 2011;13(10):2638-50.
8. Patil P.O, Green fabrication of graphene-based silver nanocomposites using agro-waste for sensing of heavy metals. Research on Chemical Intermediates, 2017;43(7):3757-73.
9. Majtan J, Methylglyoxal—a potential risk factor of manuka honey in healing of diabetic ulcers. *Evidence-Based Complement Altern Med* 2011;2011.
10. Banerjee, J. and R. Narendhirakannan, Biosynthesis of silver nanoparticles from Syzygium cumini (L.) seed extract and evaluation of their in vitro antioxidant activities. *Dig J Nanomater Biostruct* 2011;6(3):961-8.
11. Singh K.P, Study on efficacy of herbal and copper nanoparticle ointment against wound pathogen isolated from diabetic foot ulcer. *J Pharm Res* 2018;12(4):516.
12. Kumar V, Yadav S.C, and Yadav S.K, Syzygium cumini leaf and seed extract mediated biosynthesis of silver nanoparticles and their characterization. *J Chemical Technol Biotechnol* 2010;85(10):1301-9.