The Microstructure Investigation of Thionine-Graphene Nanocomposite Using SEM

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ABSTRACT

Materials that can enhance the sensitivity and selectivity of a biosensor are greatly in demand. The nanocomposition of thionine (Th) and graphene can increase the electroconductivity of the working electrode used. Graphene is a very good electrical conductor but is also hydrophobic in nature. Composition with thionine gives it the capability to disperse well in water. Plus, thionine provides the opportunity for DNA probes to be immobilized due to the presence of the amino group in its structure. In this research, the thionine-graphene (Th-G) nanocomposite was synthesized through filtration and characterised using scanning electron microscopy (SEM) to distinguish different elements coexist in the nanocomposite and to investigate the microstructure changes of the nanocomposite to confirm the composition. Different elements were analyzed to test the presence of both thionine and graphene in the composition. Physical characterisation through SEM proved the nanocomposition was a success.

Keywords: Biosensor; Thionine; Graphene; Nanocomposite.

Introduction

The study of electrochemical biosensors emerges because of its sensitivity in detecting biological binding activities [1], [2]. The application of nanotechnology in biosensors can increases the detection sensitivity and enables rapid chemical and biological analysis because of its useful attributes such as micron size, sensitive nanosensors, nanoprobes and easy to engage in nanosystems [3].

Nanomaterials like graphene has stimulated intense research interest among researchers. This is because the two-dimensional sheet of sp^2 conjugated atomic carbon graphene is planar aromatic molecule. It has large specific surface area allowing more hybridisation of probes that can give higher sensitivity to the device. Other than that, it can also withstand high temperature, a very good electrical conductor [4] [5], great mechanical strength [2], and can be manufactured at a low cost [6]. Graphene is a very good material to be used for the preparation of electrochemical related biosensors due to its excellent conductivity and electrocatalytic activity [7] [8] [9].

Thionine, a tricyclic heteroaromatic molecule has been studied and found to have a good interaction in intercalating with DNA. It is easy to immobilise DNA onto the thionine as it has a large number of hydrophilic amino groups. Thus, the composition of graphene with thionine makes DNA-based biosensing possible. Thionine has been proven to work well with graphene as these two planar molecules produces π-π stacking force making them more stable [10]. Mixing the thionine with graphene makes it easier for graphene to disperse in water. Thionine has hydrophilic components that will cater for the

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hydrophobicity of graphene through π-π force stacking. After application, graphene does not aggregate but disperse well in water makes it easier for the fabrication of bioelectrode.

The nanocomposition method for Th-G was based on a literature by Zhu et al., (2012) [11]. According to the literature, the Th-G nanocomposite will appear as dark dispersion after reflux heating for one hour. In order to separate the Th-G from the bulk solution which contains excess thionine, centrifugation method was used. However, this method consumes time as to allow the machine to spin down between three wash intervals. Plus during the decanting of the supernatant which contains the unbound thionine, some of the Th-G pallet have the tendency to also be washed out, hence will lower the amount of Th-G obtained. In this research, the dark dispersion of Th-G was filtered using a vacuum filter (PVDF membrane, 0.22 µm pore size) instead, which requires much less washing time. The Th-G produced was characterised using SEM confirming the successfulness of the composition of graphene and thionine through physical changes shown and detection of elements nitrogen, sulfur and carbon that ideally contributed by both Th and graphene.

Nanocomposition of Thionine-Graphene

The procedure of preparing Th-G suspension was adapted from Zhu et al., (2012) [11] with a slight modification. The mixture of 5 mg of graphite oxide and 50 mL of distilled water was ultrasonicated for 40 minutes in room temperature to obtain graphene oxide (GO). After that, the mixture was centrifuged at 3000 rpm for 5 minutes to separate the undissolved graphite oxide chips or any other contaminating residues. The GO supernatant was kept and added with 10 mL of 2 mM thionine (5.75 mg thionine powder dissolved in 10 mL distilled water) and was stirred vigorously for 12 hours. 600 µL of 26% hydrazine hydrate was then dropped into the mixture. The resulting mixture was further stirred for 10 minutes to allow the mixing of the hydrazine hydrate. Here, the addition of hydrazine hydrate was to reduce the GO into graphene under the hydrothermal influence [12]. The mixture was heated under reflux in 95˚C for one hour. After heating, the stable dark dispersion obtained in the heated mixture was let to cool to room temperature.

The dark dispersion was poured into a filter bottle connected to the vacuum pump. The filtration starts right after the vacuum has been turned on. The blue supernatant was decanted. 60 mL of distilled water was poured onto the filtered substance and was stirred to wash. The vacuum pump was then turned on again to filter. The washing step was repeated three times. After that, the filtered substance was scrapped off of the filter and dissolved in 2 mL of distilled water and was stored in glass bottle for further use.

Physical Characterisation Using Scanning Electron Microscopy

SEM characterisation was done through backscattered and secondary electron scanning with accelerating voltage of 15 kV. Both characterisations were done using Hitachi TM3030 PLUS model. A dried 10 µL of Th-G sample was prepared to be observed with SEM. To compare, 10 µL of 4 mg/mL graphene dispersion was also prepared by dispersing in ethanol.

In the process of preparing Th-G nanocomposite, vacuum filter was used instead of centrifugation. Centrifugation normally was set to spin at 3000 rpm for 5 minutes. It requires few minutes to reach 3000 rpm and spins at that rate for 5 minutes and another few minutes to spin down before coming to a full stop. This step was repeated 3 times as to comply with the total washing rounds needed. Roughly, a total of 30 minutes required to finish the washing step. In this research, vacuum filtration method was used for the washing. The total time required to filter 60 mL of the Th-G dark dispersion mixture took only about 5 seconds per wash. Which requires roughly less than a minute total washing

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time through filtration. This method reduced a lot of time consumed to obtain the Th-G. After the reflux heating step, the dark dispersion was found to be suspended in a dark blue colored solution which is the unbound thionine. After filtration, the solution appears clear which indicates free from unbound thionine.

Error! Reference source not found. shows the SEM images of graphene and Th-G nanocomposite. Clear observation of the monolayer atomic carbon can be seen using transfer electron microscopy (TEM; not reported) but the bulk structure using SEM. The graphene observed under 1000 magnification appears flakey in **Error! Reference source not found.** (a). Its hydrophobicity influenced the graphene to form large aggregates as can be seen in the reported image. The Th-G nanocomposite can be observed in **Error! Reference source not found.** (b) under 1200 magnification showing one particle of the dark dispersion obtained. From the image, we can no longer see the graphene flakes as it has successfully bound with the thionine molecules. The graphene no longer appears flakey and became well-exfoliated and dissolves well in water after modified with thionine. A close up image of the nanocomposite is reported in **Error! Reference source not found.** (c) with a magnification of 6000 where we can see a bunch of soft-tissue-like substance of the Th-G. When the graphene sheets form bonds with thionine molecules, it became more hydrophilic hence more dispersive.

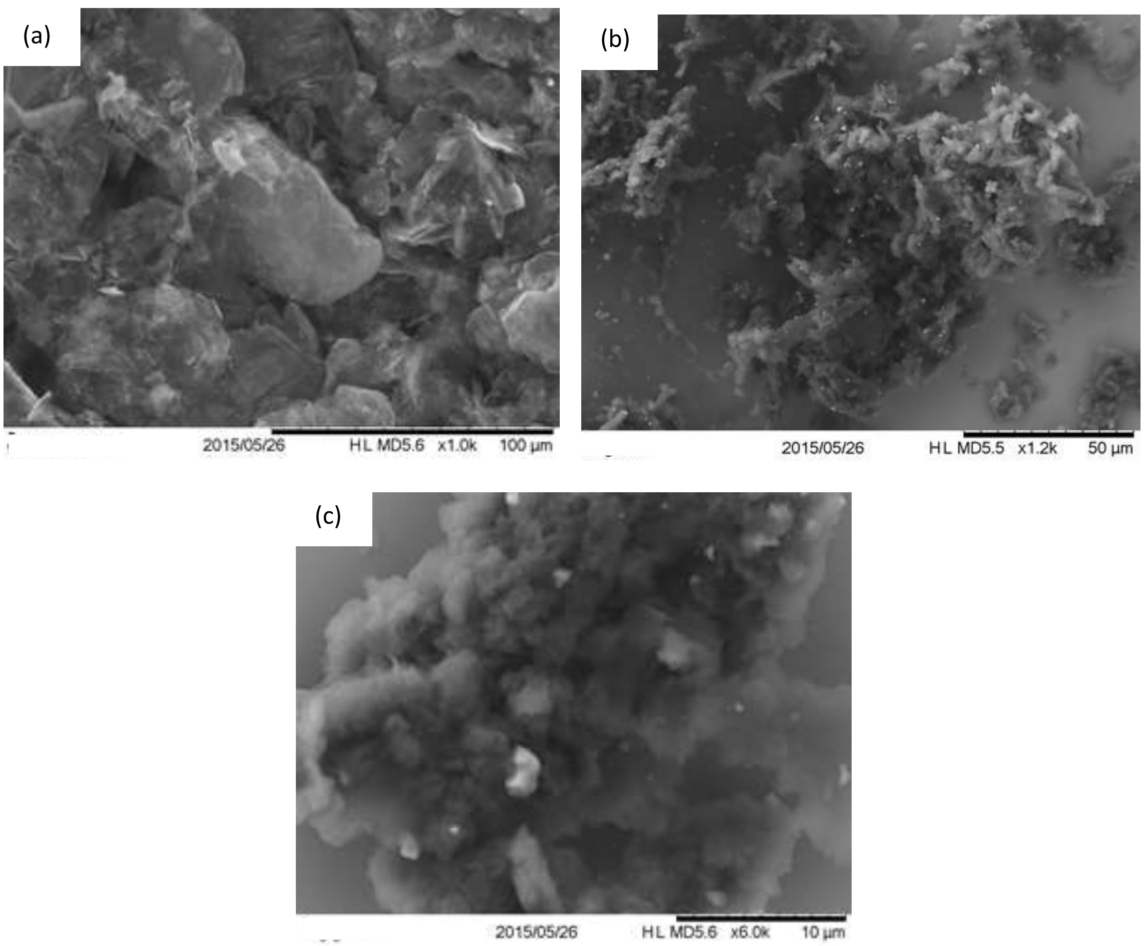

Figure 1 Images (SEM) of (a) graphene suspension, (b) Th-G nanocomposite and a (c) close up with 6000 magnification.

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The analysis of different elements present in the Th-G is shown in figure 2. Carbon trace in figure 2 (a) mostly belongs to the graphene as can be seen in its natural morphology. The presence of sulfur and nitrogen in the sample denotes for the thionine because graphene does not have any of those elements. Morphologically, graphene contains only carbon and oxygen elements which is from the carboxyl groups formed at the edge of graphene. Thionine contains sulfur and nitrogen that can be seen in the figure proves the coexistence of both graphene and thionine in the sample. Sulfur was distinguished using green label while nitrogen in blue as shown in figure 2 (b) and (c) respectively. Also in figure 2 (d) and (e) are the mixed labels as to give more visualization of the elements found in the Th-G nanocomposite. In these pictures, the elements mostly found to appear in the localized area of the Th-G particle indicated by the white arrows (inset) further proving the coexistence and the successfulness of the nanocomposition process.

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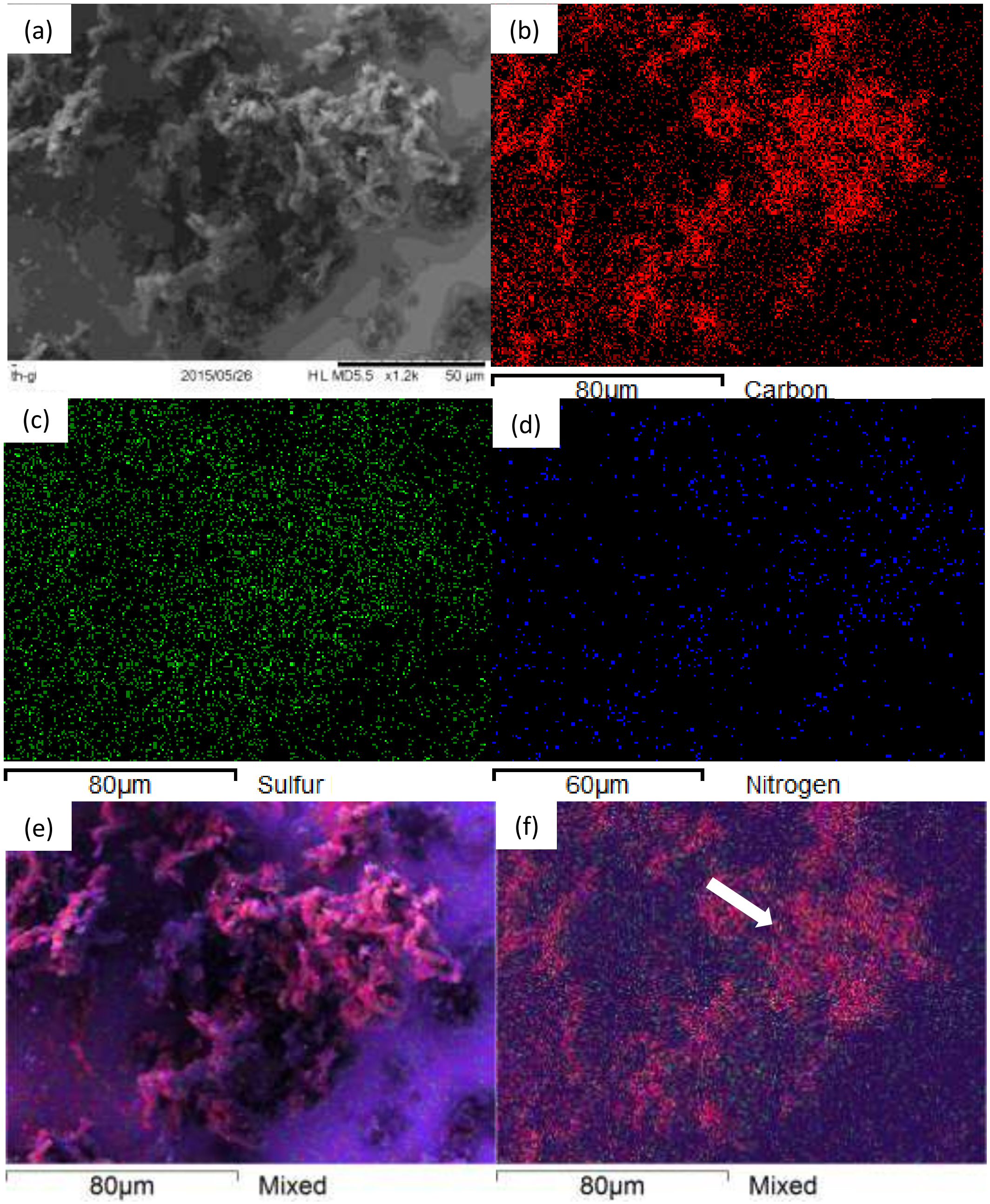

Figure 2 Different elements distinguished using SEM. (a) The original SEM image of Th-G, (b) carbon, (c) sulfur, (d) nitrogen and (e, f) mixed elements of Th-G nanocomposite.

Conclusion

Th-G synthesis was done through filtration with less production time needed. Physical characterisation of the Th-G nanocomposite has been investigated using SEM. Physical

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comparison using SEM of the graphene and modified graphene with thionine has been observed and the difference has been discussed. Graphene appears flakey but when composited with thionine, it appeared “softer” as the graphene has become more hydrophilic. The elements composition of graphene, thionine and Th-G were investigated. By observing the different elements detected, graphene and thionine were observed to coexist with the presence of nitrogen, sulfur and carbon. Hence, the nanocomposition of the Th-G is a success.

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