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One-Step Enzymatic Surface Modification of Graphene Oxide

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Abstract

Graphene oxide (GO) is a material that possesses extremely particular chemical and physical properties. Graphene-based nanomaterials have spurred the advancement of flexible nanocomposites for innovative applications that demand exceptional mechanical, thermal, electrical, optical and chemical properties. These structures have the potential to be applied in various domains due to their multifunctionality. Nevertheless, GO employed have a tendency to create robust aggregate when mixed with organic components. Hence, it is necessary to alter the surfaces of polymer matrices and GO to enhance dispersion stability and compatibility. Chemical functionalization of GO allows for extensive structural change, offering a wide range of alternatives. However, chemical modifcation can lead to the utilization of ecologically harmful chemicals and substantial expenditures of energy, time and costs. Biocompatible, non-cytotoxic, target-selective biotechnological methods are being investigated for surface modification of nanoparticles to address these concerns. This work explored a new approach to modify the GO surface utilizing natural biocatalysts, specifically enzymes. The method used a one-step process where the lipase enzyme was used to modify the GO surface with the methacrylic acid. This method is conducive to mild reaction conditions, free from the generation of chemical waste, and devoid of solvent utilization, addressing the concerns associated with chemical modification methods.

Keywords: Graphene oxide, lipase, surface modification

1. Introduction

Graphene is a two-dimensional material made up of carbon atoms organized in a hexagonal pattern [1]. Because of its distinctive thermal, chemical, mechanical, optical, and electrical characteristics, it is the thinnest material currently known [2]. Because of its exceptional qualities, it has been a critical material for the twenty-first century [3]. Graphene oxide is a form of graphene that experienced chemical modification through the introduction of functional groups containing oxygen; including hydroxyl, epoxide, and carboxyl groups. [4, 5]. While GO is recognized as an oxidized layer of graphite, there is yet no precise model for its chemical structure [6, 7]. Various structural models have been suggested, such as the Lerf-Klinowski, Scholz-Boehm, Dekany, Nakajima-Matsuo, Hofmann and Ruess models. Among these models, the Lerf-Klinowski model is widely accepted configuration, with a regular lattice model containing discrete repeated units. Lerf's proposed structural model of GO consisted of sections that had unoxidized aromatic and six-membered aliphatic ring types, which contained epoxide and hydroxyl groups, as well as isolated C=C bonds. The edges of the model were terminated by COOH and OH chemical groups [6, 7, 8].

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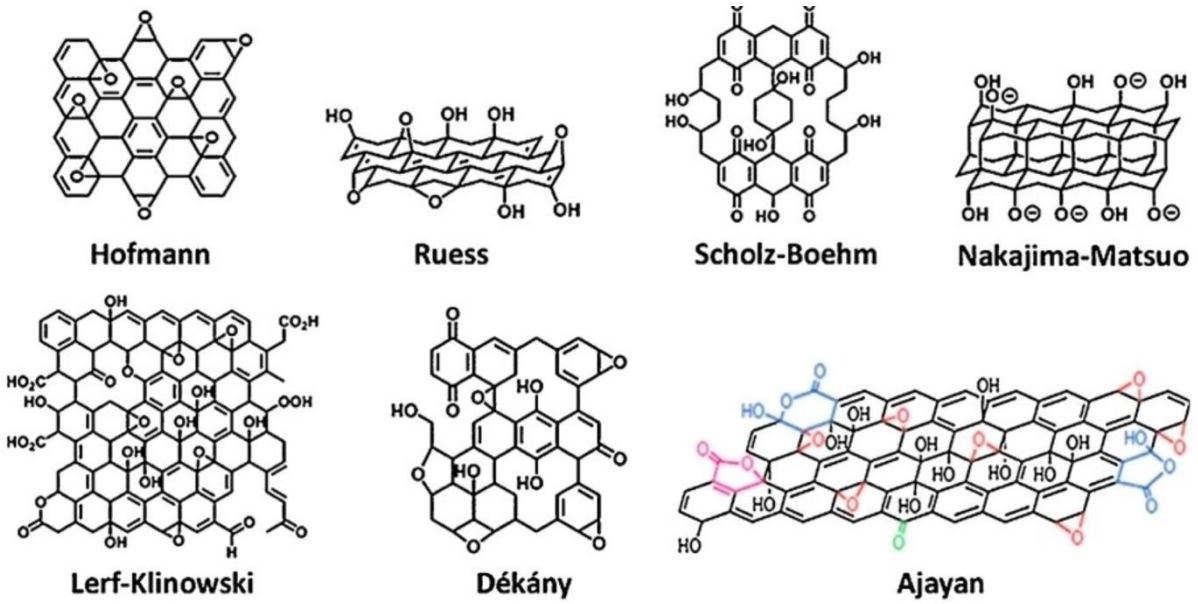

Figure 1. Structural models of GO [7]

Functionalization GO with different chemical moieties (organic, inorganic, and nanocomposites) allows for the exploration of unique and undiscovered nanoarchitectures due to its variable surface chemistry [9]. Many benefits come with surface-modified graphene oxide (GO), including exceptional optical, physico-chemical, electrical, and biocompatibility qualities [10]. Surface modification of GO is crucial for its wide applications [11, 12]. Modification of GO allows it to be processed using solvent-assisted processes, including layer-by-layer bonding and filtering methods. Additionally, it inhibits the agglomeration of graphene and preserves its intrinsic characteristics [13, 14]. Chemical functionalization of GO allows for extensive structural change, offering a wide range of alternatives for adjusting its composition. Various chemical and physical methods have been investigated to improve the stability and modification of graphene [11, 15, 16].

GO has been increasingly utilized to enhance the strength, heat resistance, and functionality of many polymers, including polymethyl methacrylate, polyvinyl alcohol, polystyrene, epoxy, and rubber. Graphene has a tendency to irreversibly accumulate together in the polymer matrix due to its extensive surface area and the strength of the van der Waals bonds between its particles [11, 17, 18]. In this instance, the process of chemically modifying graphene, along with other methods, becomes a crucial step in addressing the challenge and achieving graphene nanocomposites that possess a robust interface and a consistent dispersion of graphene sheets throughout the polymer matrix [13, 17].

Chemical procedures frequently necessitate the use of strong chemicals and rigorous reaction conditions. The use of these techniques leads to the utilization of ecologically hazardous chemicals and substantial expenditures of energy, time, and work. Also of concern is the impact of chemical wastes generated after the reaction and for their disposal [19]. Specifically, the materials used in the surface modification of biomaterials should not contain physiological toxicity and should be biocompatible [20]. Because the most important feature of biomaterials is biocompatibility and biomaterials should be materials that do not interfere with the normal changes of the surrounding tissues and do not cause unwanted reactions (inflammation, clots, etc.) in the tissue [18]. In the development of particles to be used in these areas, non-toxicity, biocompatibility, no accumulation in the human body, and no chemical pollution are the most important factors. For these reasons, biocompatible, non-cytotoxic, target-selective biotechnological methods are being investigated for the surface modification of nanoparticles [21].

Regarding the problems mentioned above, it is necessary to implement more robust procedures that apply covalent bonding, similar to chemical ways. However, it is also crucial to adopt a clean approach that avoids chemical pollution, such as physical methods. At this point, it was anticipated that these issues could be mostly prevented by utilizing enzymes, which are biocatalysts capable of facilitating chemical reactions.

This study developed an improved surface modification method that is suitable for mild reaction conditions, avoids chemical waste formation, and does not require solvents. The method leveraged natural biocatalysts, specifically enzymes, to modify the surface of GO with the methacrylic acid (MAA) through the use of a lipase enzyme.

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2. Materials and Methods

2.1. Materials

The graphene oxide supplied from Nanography Nanotechnology Inc. was utilized. The biocatalyst used in this study was lipase enzyme (Amano Lipase PS) sourced from Burkholderiacepacia. The specific activity of the enzyme, as indicated by the supplier, was 30,000 unit.g^{-1}. The lipase enzyme was purchased from Sigma-Aldrich. Methacrylic acid obtained from Sigma-Aldrich was used. The solvents, such as ethanol, acetone, hexane, and toluene were purchased from Merck and used for washing and purification of the synthesized products. Deionized water was used in all processes.

2.2. Method

As an experimental method, the method previously used by our team in hydroxyapatite surface modification was taken as a reference [22]. Figure 2 displays the experimental reaction. Since the lipase enzyme is stable and active in anhydrous medium, firstly, 0.1 g GO was pre-dried in an inert environment by vacuuming at regular intervals, and the moisture was removed. Then, the temperature was brought to 50 ˚C and 0.1 mg of lipase enzyme was added to the reaction medium and finally 0.1 mL of MAA was added to bind to the surface and the reaction was performed at 50 ˚C for 24 hours. The products obtained at the end of the reaction were washed and purified with appropriate solvents.

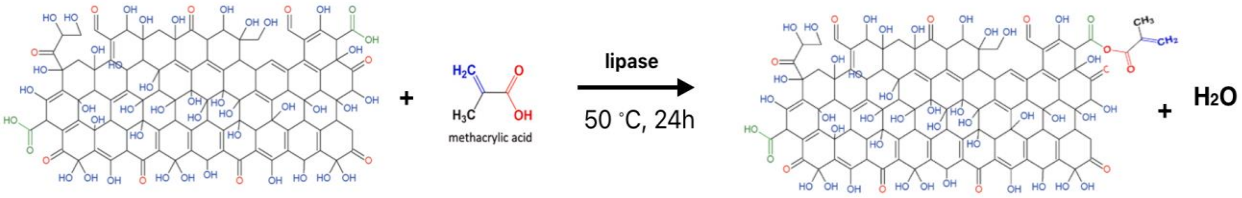

Figure 2. Schematic representation of the reaction of GO surface with MAA

2.3. Analysis

After the purified GO was dried in a vacuum oven for 24 hours, FT-IR (PerkinElmer) measurements were performed. The spectra were measured over a wavenumber range from 400 to 4000 cm^{-1}. From the spectra obtained, peaks belonging to the functional groups of the synthesized products were determined, and structural characterization was carried out in comparison with the starting materials. TG-dTG measurements of the materials were carried out in dry air between 30 °C and 700°C, with an increase of 10 ˚C per minute, and mass losses and thermal properties were determined from the thermograms obtained. SEM images (Carl Zeiss 300 VP) of the obtained particles were taken and their surface morphologies were analyzed.

GO dispersion stability in water is a critical aspect for its various applications, ranging from electronics to biomedical engineering [23]. GO exhibits hydrophilic properties because of the presence of oxygen-containing functional groups on its surface, including carboxyl (-COOH), hydroxyl (-OH) and epoxide (-O-) groups. The dispersibility of GO is influenced by the presence and density of functional groups on its surface [24]. For this reason, the agglomeration/dispersion behavior of GO in water was followed. For this, aqueous solutions of unmodified and modified GO at a concentration of 0.5 mg.mL^{-1} were prepared and dispersed in water for 1 min. in an ultrasonic bath. Then it was allowed to hang on for 30 minutes and the stability of agglomeration or dispersion in water was observed at regular intervals.

3. Results and Discussion

3.1. FT-IR Analysis

The FT-IR spectra of pure GO, surface modified GO (mGO) and MAA obtained after the reaction are given in Figure 3. The FT-IR spectra of the GO sample reveal the presence of hydroxyl bond (-OH) at 3080 cm^{-1}, carbonyl bond (C=O) at 1710 cm^{-1}, aromatic bond (C=C) at 1648 cm^{-1}, C-OH bond at 1440 cm^{-1}, and C-O bonds at approximately 1200 cm^{-1} and 1040 cm^{-1} in accordance with the literature [25, 26]. The MAA spectrum has peaks belonging to C=O and C=C bonds in the structure of MAA at 1562.8 cm-^{1} and 1601.7 cm^{-1} bands and peaks belonging to aliphatic -CHs at 2900 cm^{-1} [27]. The existence of carbonyl (C=O) and double bond (C=C) groups after the modification process does not help to clearly determine if major alterations have happened as they are already present in the GO structure. However, the increase in the intensity of the C=O band and the presence of aliphatic -CH bands at 2800 and 2950 cm^{-1} in the spectra of mGO samples are important indicators that modification has occurred [25].

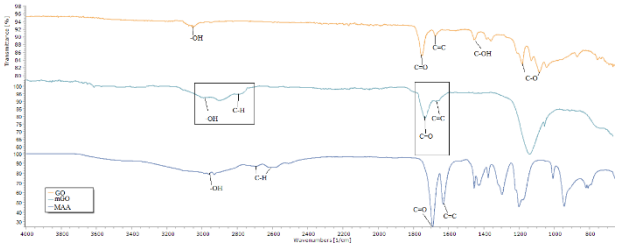

Figure 3. FT-IR spectra of GO, mGO and MAA

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3.2. TGA Analysis

TG-dTG spectra of pure GO and mGO particles are given in Figure 4. According to the TG-dTG thermograms, a mass loss of 0.946% occurred in pure GO around 42˚C. This loss is thought to belong to the water adsorbed on GO. In the thermograms of mGO obtained at the end of the reaction, the peak seen approximately at 100 ˚C is indicative of the presence of adsorbed water and the mass loss seen around 306 ˚C is thought to belong to the degradation of MAA bound to the GO surface. The total mass loss is 8.6%.

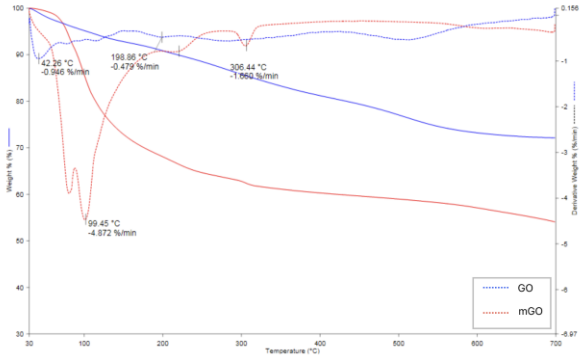

Figure 4. TG-dTG thermograms of GO and mGO

3.3. SEM Analysis

As shown in Figure 5, GO particles showed a flake-like structure in accordance with the literature [34]. When the SEM images of pure GO and mGO particles are compared, it is clearly seen that some bright regions are added between the GO layers and on the surface in the mGO images. It can be said that these regions indicate the binding of MAA to the surface as a result of surface modification. In addition, the fact that the roughness seen on the pure GO surface is reduced after modification with MAA supports that the surface is modified with MAA [28, 29, 30].

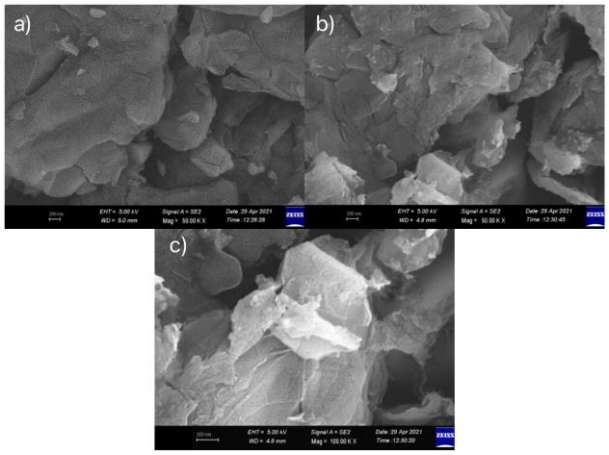

Figure 5. SEM images of (a)pure GO at mag: 50.00 kx, (b) mGO at mag: 50.00 kx , (c) mGO at mag: 100.00 **kx**

3.4. Dispersion Stability in Water

Images of the GO and mGO aqueous solutions at 1, 5, 15, 30 minutes are given in Figure 6. In the photographs, it is clearly seen that unmodified GO particles reach the bottom in a short time, whereas GO whose surface has been modified with MAA maintains its dispersion stability in water for a long time. This confirms the modification of GO and also shows that the dispersion stability of GO in water is significantly improved.

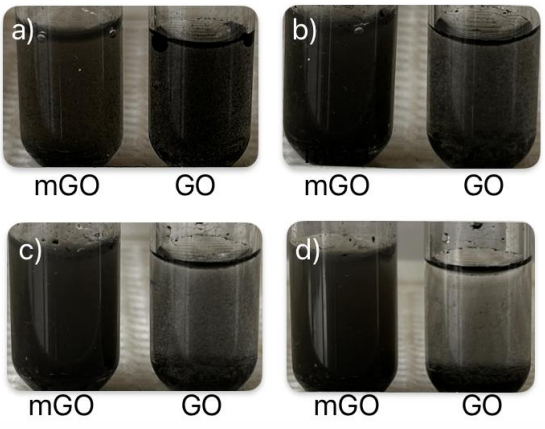

Figure 6. Images of the GO and mGO aqueous solutions at 1min. (a), 5 min. (b), 15 min. (c) and 30 min. (d)

4. Conclusion

The results obtained in this study demonstrate the feasibility of using enzymatic techniques to modify the surface of GO. In this way, by developing the enzymatic surface modification method, a reliable and ecological alternative process for surface modification has been proposed. Therefore, it is possible to readily create alternative materials that possess excellent biocompatibility and do not produce hazardous consequences; the properties of these materials can be improved and their current usage areas, especially in the field of biotechnology, can be increased. Surface modification of graphene oxide is an important area in nanomaterial research and could open the door to many more innovative applications in the future.

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Author's Contributions

Merve Danışman: Drafted and wrote the manuscript, performed the experiment and result analysis.

Ayhan Oral: Supervised the experiment's progress, result interpretation and helped in manuscript preparation.

Ethics

There are no ethical issues after the publication of this manuscript.

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