

Eugenol-Enriched Alginate/Graphene Oxide Composites Crosslinked with Ba²⁺ and Fe³⁺: Synthesis and Structural Evaluation

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Eugenol-Enriched Alginate/Graphene Oxide Composites Crosslinked with Ba^{2+} and Fe^{3+} : Synthesis and Structural Evaluation

Abstract. Eugenol-enriched alginate/graphene oxide (Alg/GO) composites were successfully fabricated as Ba^{2+} -crosslinked films and Fe^{3+} -crosslinked beads using modified Hummers, chemical crosslinking, and ionic gelation methods. SEM analysis revealed that eugenol incorporation increased the surface porosity and roughness of both formulations, which may facilitate controlled release. Swelling studies demonstrated distinct pH-responsive behavior, with Ba-Alg/GO films gradually swelling and degrading in phosphate-buffered saline (pH 6.8), whereas Fe-Alg/GO beads maintained excellent structural stability under acidic conditions (pH 1.2). Although eugenol slightly reduced the tensile strength of Ba-Alg/GO films, the composites retained sufficient mechanical integrity for practical applications. These findings highlight the synergistic effect of graphene oxide and multivalent ion crosslinking in producing mechanically stable, pH-responsive biomaterials with considerable potential as carriers for the encapsulation and controlled delivery of hydrophobic bioactive compounds.

Keywords: Eugenol, Alginate, Graphene Oxide, Composites, Crosslinked

1. Introduction

The synthesis of alginate/graphene oxide (Alg/GO) composites in bead form has attracted significant interest due to their versatility in various applications. For example Alg/GO beads with Ca^{2+} ions as crosslinkers to adsorb uranium [1], Alg/GO beads with Ca^{2+} ions as crosslinkers to adsorb lysozyme [2], Alg/GO beads to determine the catalytic reaction of covalently immobilized pectinase [3], chitosan/GO/Alg-derived beads with Ca^{2+} ions as crosslinkers [4] and other studies that have produced morphology is much different than using crosslinking ions such as Ca^{2+} , Ba^{2+} , and Fe^{3+} which results in a denser and denser morphology of Alg/GO beads. The morphological differences formed indicate that the incorporation of GO into the alginate matrix and multivalent cations affects the morphology of the composite, thereby increasing the mechanical properties and stability of the composite.

Alg/GO film composite with CaCl_2 crosslinker as antibacterial [5]. Zheng et al. (2016) successfully prepared sodium alginate/graphene oxide (Na-Alg/GO) nanocomposite films crosslinked with multivalent cations (Ca^{2+} , Ba^{2+} , and Fe^{3+}). The incorporation of graphene oxide and ionic crosslinking significantly enhanced the mechanical performance and thermal stability of the composite films. However, the swelling behavior of these multivalent cation-crosslinked Alg/GO composites was not investigated, leaving their water absorption characteristics and dimensional stability under different environmental conditions to be further explored [6]. Research on M-Alg/GO composites that has been carried out in the past where the swelling power (Swelling Test) of the M-Alg/GO composite showed that the physical properties were not too high, in fact there was no physical change at all. This is evidenced by the swelling test where the low swelling ability is caused in an acidic environment. The M-Alg/GO composite is very stable so it is difficult to experience swelling. In an acidic environment, H^+ ions are not sufficiently capable to break the cross-links formed in the composite. In increasing the mechanical resistance and physical properties of Alg/GO composites, it is also necessary to enrichment of compounds that can form composite morphology with good properties such as eugenol [7]. Eugenol was chosen because it has excellent biological activity with anti-inflammatory properties as a drug delivery system and its presence in nature is quite large [8]. Shinde and Nagarsenker (2011) developed gelatin–sodium alginate microparticles containing eugenol to improve encapsulation efficiency and controlled release, taking advantage of the well-established antiseptic and analgesic properties of eugenol [9]. Likewise, Talón et al. (2019) demonstrated that the addition of encapsulated eugenol into starch-based films enhanced antioxidant activity while modifying the film microstructure and mechanical properties [10]. These studies indicate that eugenol is a promising bioactive additive capable of improving the

functional performance of biopolymer composites, although its incorporation may also alter their physicochemical characteristics. Furthermore, polymer composite beads prepared by ionotropic gelation have attracted considerable interest because they can be fabricated under mild aqueous conditions and possess tunable particle size, interconnected porous structures, high encapsulation efficiency, and adjustable physicochemical properties, making them suitable carriers for the encapsulation and controlled delivery of bioactive compounds [11].

2. Materials and Methods

2.1 Preparation of Graphene Oxide (GO)

Graphene oxide (GO) was synthesized using a Hummers oxidation method modification based on the procedures reported by Marcano et al. (2010) and Chen et al. (2022) [12, 13]. Briefly, 1 g of graphite powder and 0.5 g of sodium nitrate (NaNO_3) were added to a 250 mL Erlenmeyer flask and placed in an ice bath. While maintaining the temperature below 5 °C, 25 mL of concentrated sulfuric acid (97% H_2SO_4) was slowly added under continuous stirring. After 2 h, 3.65 g of potassium permanganate (KMnO_4) was gradually introduced, and the reaction temperature was increased to 35 °C for 2 h to promote graphite oxidation. Subsequently, 45 mL of distilled water followed by 3.5 mL of 30% hydrogen peroxide (H_2O_2) was added to terminate the oxidation reaction, producing a yellowish suspension. The synthesized GO was filtered and thoroughly washed with preheated 3% hydrochloric acid and distilled water until neutral pH was achieved, and dried in a vacuum oven at 50 °C for 24 h. Finally, 0.1 g of dried GO was dispersed in 100 mL of distilled water and ultrasonicated for 1 h to obtain a homogeneous GO suspension.

2.2 Synthesis of Fe-Alg/GO Beads Enriched with Eugenol

Fe-Alg/GO composite beads were prepared using a modified ionotropic gelation method based on Zheng et al. (2016) and Dai et al. (2018) [3, 6]. Sodium alginate (3 g) dissolved in 100 mL of water distilled and sonicated for 30 min. A 4 mL aliquot of GO suspension (0.15% w/v) was added to 20 mL of the alginate solution, followed by the addition of eugenol at varying volumes (0.1 mL, 0.2 mL, and 0.3 mL). The mixture was further sonicated for 30 min to ensure homogeneity. The GO dispersion was subsequently added dropwise to a 0.15 M FeCl_3 solution under sonication for 30 minutes to induce cross-linking. The resulting beads were filtered, rinsed, and dried at room temperature.

2.3 Synthesis of Ba-Alg/GO Film Composites Enriched with Eugenol

The Ba-Alg/GO film composite was synthesized using a modified ionic crosslinking method based on the procedures reported by Ionita et al. (2013), Zheng et al. (2016)[[6, 14]. A 3% (w/v) sodium alginate solution and a 0.15% (w/v) graphene oxide (GO) suspension were prepared and equilibrated overnight. Subsequently, 15 mL of 0.95% BaCl_2 solution was added to 100 mL of the alginate solution and sonicated for 15 min to promote homogeneous ionic interaction. Eugenol (0.6 mL) was then added dropwise under continuous stirring at 25 °C for 30 min, followed by the incorporation of 15 mL GO suspension. The mixture was further sonicated for 30 min to obtain a homogeneous colloidal dispersion. The suspension was centrifuged at 14,000 rpm for 30 min, and the resulting precipitate was cast into molds and dried in a hot-air oven at 60 °C for 12 h to obtain Ba-Alg/GO-eugenol composite films.

2.4 Characterization Methods

a. Swelling Test

Swelling behavior was assessed by immersing Ba-Alg/GO film samples (initial weight W_1) in phosphate-buffered saline (PBS, pH 6.8) for time intervals of 15, 30, and 60 minutes. The same procedure was repeated using 0.1 M hydrochloric acid (HCl, pH 1.2). After immersion, the samples were weighed (W_2), and the swelling ratio (%) was calculated using Equation (1) [15]:

$$\text{Swelling (\%)} = \frac{W_2 - W_1}{W_1} \times 100$$

Where W_1 is the initial dry weight of the sample and W_2 is the weight after swelling.

b. Stability Test

To determine chemical stability in different pH environments, Ba-Alg/GO films were immersed in solutions of HCl (pH 1.2) and NaCl (pH 6.8 and 7.3). Physical changes and disintegration were observed at 30-minute intervals until complete degradation occurred [16].

c. Tensile Strength Test

The mechanical properties of the Ba-Alg/GO composite films were evaluated using a Universal Testing Machine (UTM) in accordance with ASTM D882-18 for thin plastic films. Rectangular film specimens were tested at a crosshead speed of 2.5 mm min^{-1} , and the maximum tensile strength was recorded in megapascals (MPa). The testing procedure was adapted from previously reported methods for alginate-based composite films Benavides et al., (2012) and Wang et al., (2017) [17-18].

3. Results and Discussion

3.1 Synthesis of GO, Ba-Alg/GO Film, and Fe-Alg/GO Beads Enriched with Eugenol

Graphene oxide (GO) was synthesized using a modified Hummers method, involving the oxidation of graphite powder with NaNO_3 and KMnO_4 in concentrated H_2SO_4 under ice bath conditions. This low-temperature environment is essential for controlled oxidation, preserving the aromatic structure of graphite. The intercalation of H_2SO_4 promotes the formation of reactive intermediates without disrupting the integrity of the graphene framework. A distinct color transition to dark brown during the reaction signifies successful oxidation due to the interaction of KMnO_4 and H_2SO_4 with graphite. The oxidation process introduces functional groups—hydroxyl, epoxy, carboxyl, and carbonyl—which enhance GO's hydrophilicity and facilitate its exfoliation into single-layer nanosheets. NaNO_3 aids in increasing the interlayer spacing, further improving exfoliation efficiency. After oxidation, the reaction is quenched using distilled water and H_2O_2 , which decomposes residual KMnO_4 and MnO_2 , resulting in a green-brown solution. The final product is filtered, washed with 3% HCl to remove metal impurities, dried, and sonicated to obtain a uniform GO suspension

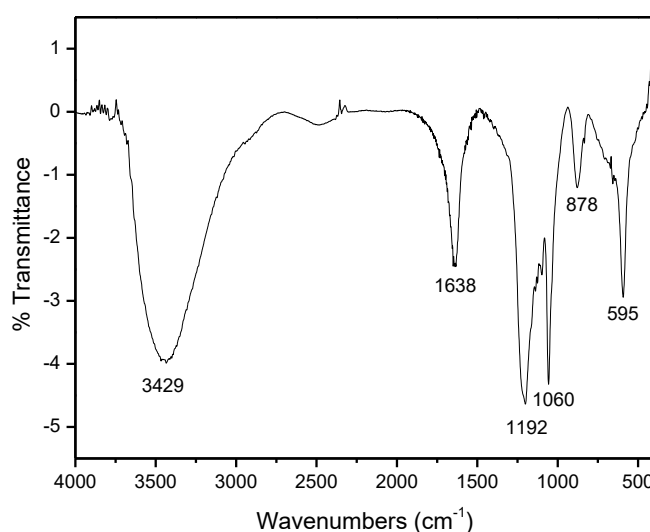


Figure 1. FTIR spectrum of graphene oxide (GO).

Based on the Fourier Transform Infrared (FTIR) spectrum of synthesized graphene oxide (GO) displays Fig.1 several characteristic absorption bands that confirm the presence of oxygen-containing functional groups, indicating successful oxidation of graphite. The broad peak observed around 3429 cm^{-1} corresponds to the O–H stretching vibration, suggesting the presence of hydroxyl groups. The band at 1638 cm^{-1} is attributed to C=O stretching from carboxylic acid or

carbonyl groups, while the peak at 1060 cm^{-1} is assigned to C–O stretching vibrations of epoxy or alkoxy groups. These findings are in good agreement with previous studies on GO [14] supporting the successful incorporation of oxygen functionalities onto the graphene sheets during the oxidation process.

The oxygen-containing functional groups of graphene oxide (GO), including hydroxyl, epoxy, and carboxyl groups, form hydrogen bonds with sodium alginate, improving GO dispersion and interfacial adhesion within the polymer matrix [14]. Upon ionic gelation, Ba^{2+} ions replace Na^+ ions in the alginate chains through the egg-box cross-linking mechanism, producing a denser and mechanically stronger network than Ca^{2+} due to their higher affinity for alginate carboxylate groups [16]. In this study, 0.15 M BaCl_2 was employed as the ionic crosslinking agent to establish a stable alginate network. Eugenol was likely retained within the Ba-Alg/GO composite through hydrogen bonding between its hydroxyl group and the oxygen-containing functional groups of alginate and graphene oxide, together with hydrophobic interactions involving its aromatic moiety [19]. Fe-Alg/GO beads enriched with eugenol were synthesized via ionic gelation using Fe^{3+} ions as the cross-linking agent. Owing to their trivalent nature, Fe^{3+} ions form a denser ionic network than Ca^{2+} -crosslinked alginate through a distinct coordination mechanism, resulting in enhanced mechanical stability and unique physicochemical properties of the hydrogel [20].

Due to its hydrophobic nature, eugenol exhibits limited compatibility with aqueous alginate, making its homogeneous dispersion within the polymer matrix challenging. In the present study, the incorporation of 0.1 mL eugenol produced the most homogeneous bead morphology, whereas higher eugenol contents resulted in phase separation and structural heterogeneity, likely due to the aggregation of hydrophobic oil droplets in the hydrophilic alginate matrix. Moreover, eugenol increased the hydrophobicity of the composite and reduced water vapor permeability by limiting water diffusion through the polymer network, consistent with previous reports on essential oil-incorporated alginate and other biopolymer films [21-22].

Upon the addition of FeCl_3 , Fe^{3+} ions ionically cross-linked the carboxylate groups of alginate by replacing Na^+ ions, resulting in a denser and more compact polymer network. Owing to its high charge density and trivalent nature, Fe^{3+} forms stronger ionic interactions than divalent cations, thereby increasing the rigidity and reducing the porosity of the hydrogel [20, 23]. In addition, Fe^{3+} coordinates with oxygen-containing functional groups on GO, particularly edge carboxyl groups as well as hydroxyl, epoxy, and carbonyl groups, further reinforcing the composite network. These coordination interactions generate a highly cross-linked network that enhances the structural integrity and mechanical stability of the resulting Alg/GO biocomposite [20].

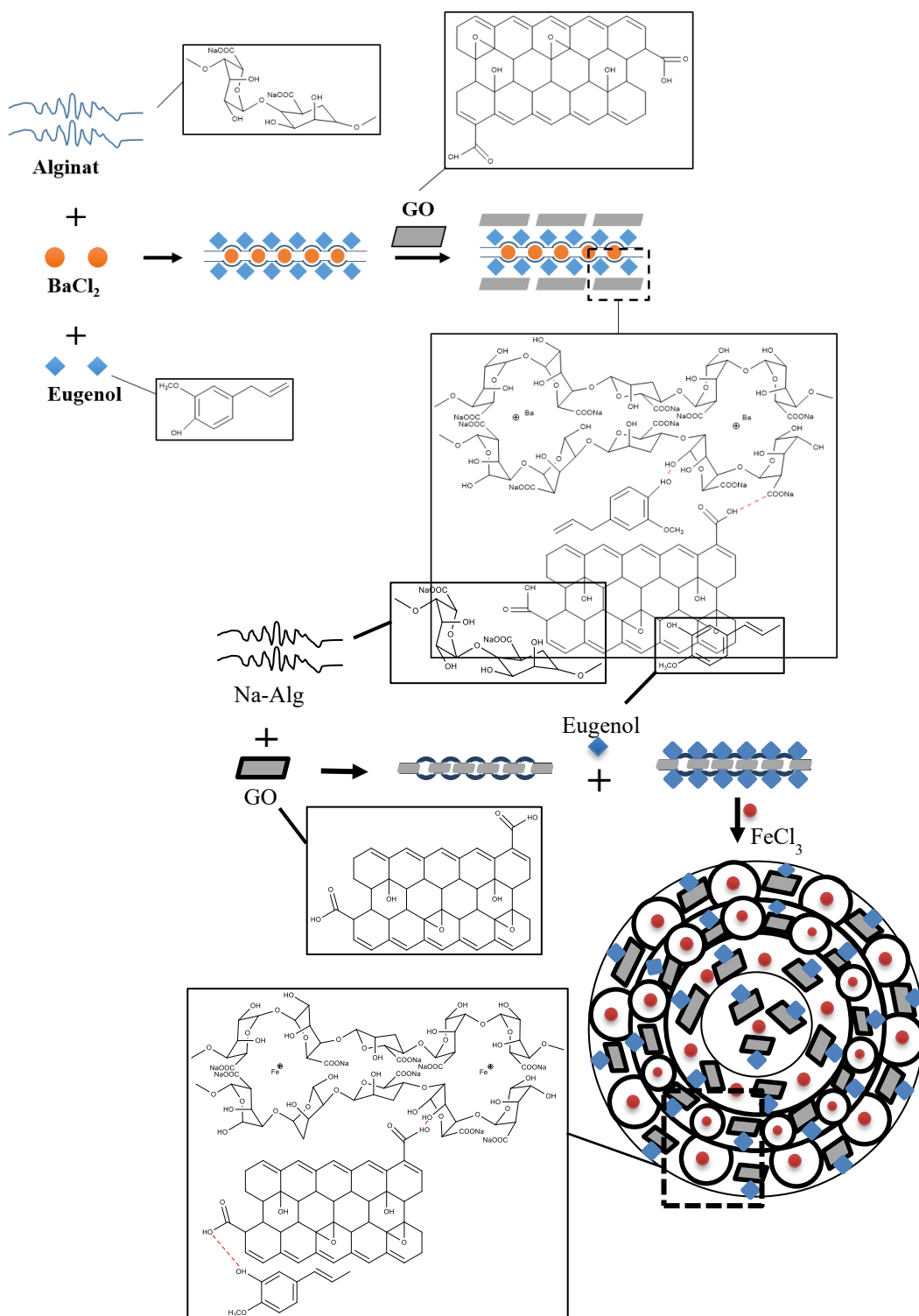


Figure 2. Proposed structural model of Fe-Alg/GO-eugenol beads and Ba-Alg/GO-eugenol films.

This schematic illustrates the proposed structural arrangement of the Fe-Alg/GO and Ba-Alg/GO composites enriched with eugenol. In the Fe-Alg/GO beads, the Fe³⁺ ions induce strong ionic crosslinking with the alginate chains, forming a stable, spherical network. The presence of eugenol, a hydrophobic molecule, introduces porosity and structural irregularities, enhancing the surface area and potentially improving loading and release characteristics. Conversely, in the Ba-Alg/GO-eugenol films, Ba²⁺ ions form a looser, more flexible cross-linked network. The film matrix appears more porous and less homogeneous due to partial dispersion of eugenol, which aligns with SEM observations. These structural models provide insight into how divalent and

trivalent cations influence the morphology and functional properties of alginate-based composite material

Characterization of GO, Composite Films, and Beads

Scanning Electron Microscopy (SEM)

Fig. 3 presents the surface morphology of the Ba-Alg/GO films with and without eugenol. The Ba-Alg/GO-eugenol films (Figures 3a–c) exhibit a less homogeneous surface characterized by the presence of visible pores, suggesting incomplete dispersion of the hydrophobic eugenol within the hydrophilic alginate matrix. Similar morphological changes have been reported following the incorporation of essential oils into biopolymer films, where limited compatibility between the hydrophobic oil phase and the hydrophilic polymer matrix leads to increased surface roughness and porosity [24]. In contrast, the Ba-Alg/GO films without eugenol (Figures 3d–f) display a smoother, denser, and more uniform surface, indicating improved structural integrity and a more compact polymer network.

Likewise, the SEM micrographs of the Fe-Alg/GO and Fe-Alg/GO-eugenol beads (not shown) revealed distinct morphological differences. The Fe-Alg/GO beads exhibited a compact and relatively non-porous surface, reflecting the formation of a dense polymer network through ionic cross-linking between Fe^{3+} ions and alginate [20, 23]. In contrast, the incorporation of eugenol resulted in a more irregular and porous surface morphology, likely due to the limited compatibility between the hydrophobic eugenol and the hydrophilic polymer matrix. Such porous structures may facilitate mass transport and promote controlled release behavior, making them attractive for drug delivery applications [21, 25].

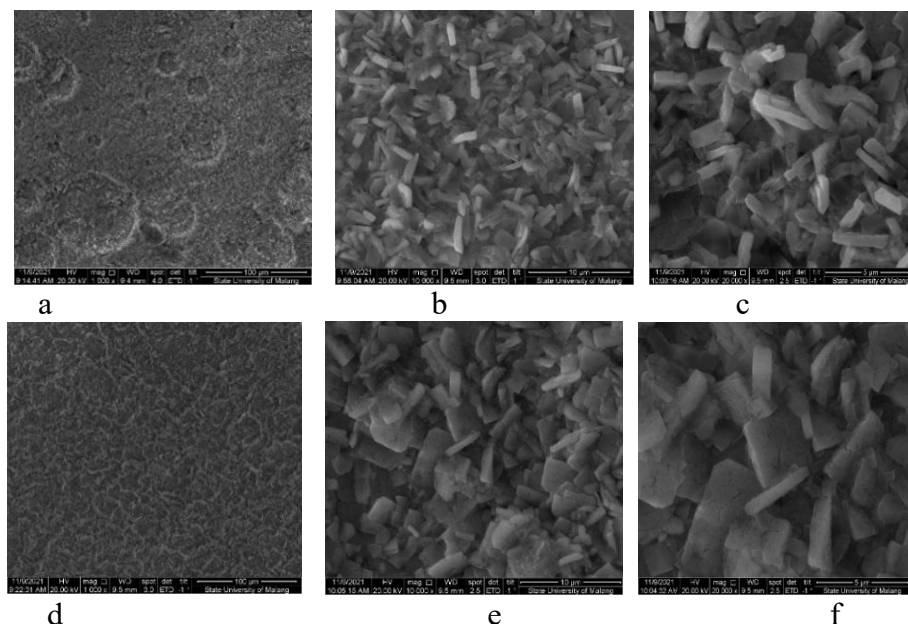


Figure 3. SEM micrographs of Ba-Alg/GO composite films. Images (a–c) show Ba-Alg/GO films enriched with eugenol, while (d–f) show Ba-Alg/GO films without eugenol, captured at magnifications of 400× and 10,000×.

The SEM images reveal distinct morphological differences between the Ba-Alg/GO films with and without eugenol. The Ba-Alg/GO-eugenol films (a–c) exhibited rougher and more porous surfaces, which are likely associated with the limited compatibility between the hydrophobic eugenol phase and the hydrophilic alginate matrix, leading to partial phase separation during film

formation. Such morphological heterogeneity may influence water permeability and diffusion-related properties, including controlled release behavior. Similar effects have been widely reported in essential oil-incorporated biopolymer films, where the dispersed hydrophobic phase disrupts matrix continuity and increases surface roughness and porosity [17, 26]. In contrast, Ba-Alg/GO films without eugenol (d–f) displayed smoother, denser, and more homogeneous surfaces, indicating improved structural integrity and better interaction between alginate and graphene oxide. The presence of graphene oxide contributes to network stabilization through hydrogen bonding and interfacial interactions, resulting in a more compact microstructure [23–24].

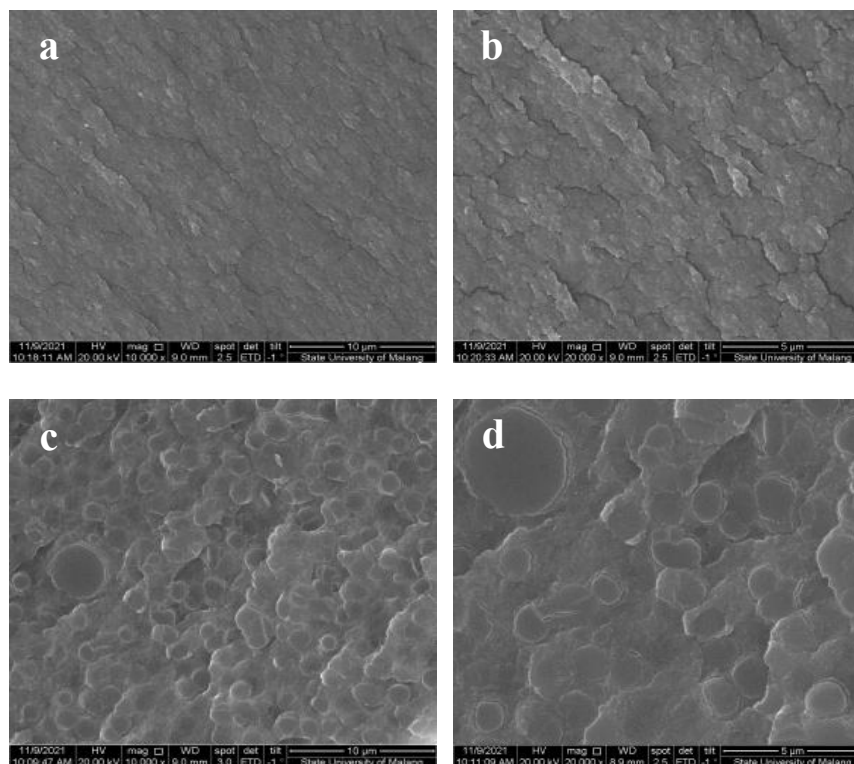


Figure 4. SEM images of (a) Fe-Alg/GO (10,000×), (b) Fe-Alg/GO (20,000×), (c) Fe-Alg/GO enriched with eugenol (10,000×), and (d) Fe-Alg/GO enriched with eugenol (20,000×).

The SEM micrographs show clear morphological differences between Fe-Alg/GO beads with and without eugenol. The Fe-Alg/GO beads (a–b) exhibit a compact and smooth surface, indicating the formation of a dense polymer network due to strong Fe^{3+} –alginate coordination and high crosslinking density [6, 23]. In contrast, Fe-Alg/GO-eugenol beads (c–d) present a rougher and more porous morphology with spherical voids, likely resulting from microstructural heterogeneity induced by the hydrophobic nature of eugenol within the hydrophilic matrix. Such structural changes increase surface area and are favorable for mass transfer and controlled release applications. Similar behavior has been reported in GO–alginate hydrogel systems, where network porosity significantly influences release performance [17, 27].

Swelling Test

The swelling behavior of Ba-Alg/GO and Fe-Alg/GO composites enriched with eugenol was evaluated in PBS (pH 6.8) and HCl (pH 1.2) to simulate physiological and gastric conditions. The results are presented in Table 1

Table 1. Swelling behavior of Ba-Alg/GO and Fe-Alg/GO composites in PBS (pH 6.8) and HCl (pH 1.2)

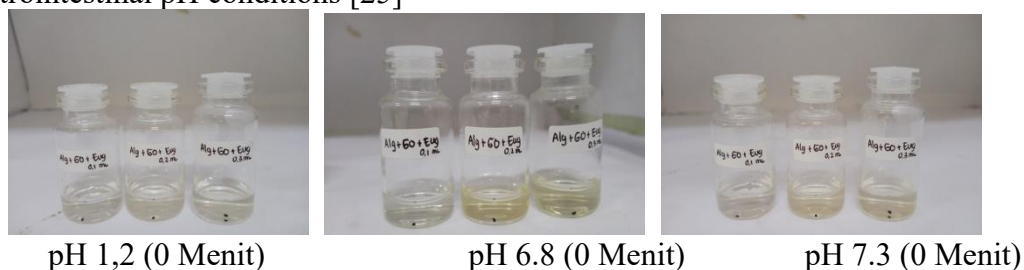
Sample	Medium	pH	Maximum Swelling (%)	Time to Max (min)
Ba-Alg/GO + eugenol	PBS	6.8	16.9	30
Ba-Alg/GO + eugenol	HCl	1.2	11.5	90
Fe-Alg/GO + 0.1 mL eugenol	PBS	6.8	8.2	330
Fe-Alg/GO (no eugenol)	PBS	6.8	4.0	330
Fe-Alg/GO + eugenol	HCl	1.2	~1.0	420

Table 1 summarizes the swelling behavior of the Ba-Alg/GO and Fe-Alg/GO composites in phosphate-buffered saline (PBS, pH 6.8) and hydrochloric acid (HCl, pH 1.2). Among all formulations, the Ba-Alg/GO composite containing eugenol exhibited the highest swelling capacity, reaching **16.9%** in PBS within **30 min**, followed by a slight stabilization at **15.9%**. The higher swelling in PBS can be attributed to the hydration of hydrophilic functional groups and partial relaxation of the Ba²⁺-cross-linked polymer network under near-neutral conditions, which facilitates water uptake. In contrast, swelling was reduced to **11.5%** in the acidic medium (pH 1.2), where protonation of alginate carboxylate groups and the stability of the ionic cross-links restrict polymer chain mobility and limit water absorption [23, 28].

The Fe-Alg/GO composites exhibited substantially lower swelling than the Ba-Alg/GO counterparts, reflecting the formation of a denser polymer network through Fe³⁺-mediated ionic cross-linking. Incorporation of eugenol increased the maximum swelling from **4.0%** to **8.2%** in PBS, suggesting that the hydrophobic oil phase generated a less compact microstructure that facilitated water penetration, consistent with the SEM observations. Under acidic conditions, the Fe-Alg/GO-eugenol composite exhibited minimal swelling (**~1.0%**) throughout the test period, indicating excellent structural stability in simulated gastric conditions. Such limited swelling in acidic media is advantageous for gastro-resistant drug delivery systems, as it minimizes premature swelling and supports the controlled release of encapsulated bioactive compounds [20, 23]

Stability Test

Figures 5 demonstrate the pH-dependent morphological stability of Ba-Alg/GO and Fe-Alg/GO composites after immersion in acidic (pH 1.2) and near-physiological (pH 6.8 and 7.3) media for up to 6 h. Overall, Ba-Alg/GO films containing eugenol retained their structural integrity with minimal visible deformation, whereas eugenol-free films showed more pronounced surface changes, indicating lower aqueous stability. This improved stability is attributed to the hydrophobic nature of eugenol, which reduces water penetration and enhances matrix compactness, thereby limiting swelling and structural disruption [17, 26]. Similarly, Fe-Alg/GO composites exhibited excellent morphological stability across all pH conditions without noticeable disintegration. This behavior is mainly governed by strong Fe³⁺-mediated ionic crosslinking, which forms a dense “egg-box” network that restricts polymer chain mobility and enhances resistance to aqueous environments [20, 23]. In addition, eugenol incorporation further improved stability by increasing hydrophobicity of the matrix, thereby reducing water uptake and swelling-related deformation. These results suggest that both Ba- and Fe-crosslinked Alg/GO systems enriched with eugenol are promising candidates for controlled drug delivery applications requiring stability under gastrointestinal pH conditions [25]



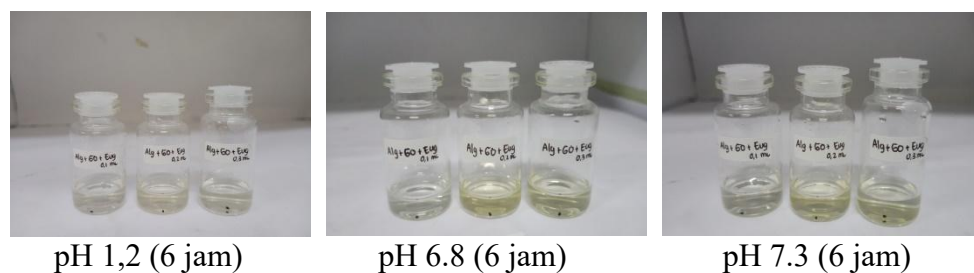


Figure 5 presents the morphological stability of the Fe-Alg/GO beads, with and without eugenol (0.1–0.3 mL), following immersion in media with pH values of 1.2, 6.8, and 7.3 for 7 h. All formulations retained their overall morphology without visible disintegration or deformation throughout the incubation period, indicating high structural stability under both acidic and near-physiological conditions.

Tensile Strength Test

The tensile strength of Ba-Alg/GO films with and without eugenol incorporation was evaluated to assess the influence of eugenol on the mechanical performance of the composite system. The results are summarized in Table 2.

Table 2. Tensile strength of Ba-Alg/GO films

Sample	Max Tensile Strength (MPa)
Ba-Alg/GO (without eugenol)	29.6
Ba-Alg/GO + eugenol	2.67

The incorporation of eugenol resulted in a pronounced decrease in the tensile strength of Ba-Alg/GO films, from 29.6 MPa (without eugenol) to 2.67 MPa (with eugenol), indicating a significant reduction in mechanical integrity. This behavior suggests that eugenol disrupts the intermolecular interactions within the alginate–graphene oxide network, leading to reduced cohesion and weaker stress-transfer efficiency. The observed reduction can be attributed to the hydrophobic nature of eugenol, which induces phase separation within the hydrophilic polymer matrix and generates structural discontinuities that act as stress concentration points during tensile loading. Similar mechanical weakening has been widely reported in alginate-based films containing essential oils, where the dispersed oil phase decreases polymer continuity and mechanical strength [17,29]. These findings confirm that although eugenol enhances functional properties such as antimicrobial activity, its incorporation significantly compromises mechanical performance, emphasizing the need for concentration optimization in biopolymer composite design.

Conclusion

Graphene oxide (GO) was successfully synthesized using the modified Hummers method and incorporated into alginate-based composites cross-linked with Ba^{2+} and Fe^{3+} ions and enriched with eugenol to form films and beads with tunable properties. SEM analysis revealed that Ba-Alg/GO-eugenol films exhibited a rough and porous morphology due to the partial hydrophobicity of eugenol, while Fe-Alg/GO-eugenol beads presented a porous structure advantageous for drug delivery. Swelling behavior was pH-dependent, with greater swelling in PBS (pH 6.8) and minimal swelling in acidic media (pH 1.2), suggesting suitability for gastro-resistant drug delivery systems. Structural stability was enhanced by Fe^{3+} cross-linking and hydrogen bonding, ensuring integrity under varying pH conditions. However, tensile strength decreased with eugenol incorporation,

likely due to disruption of hydrogen bonding and reduced matrix compatibility. Overall, these findings demonstrate that multivalent ion cross-linking combined with eugenol enrichment yields alginate-GO composites with promising potential for biomedical applications, particularly in controlled and pH-responsive drug release systems.

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