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Research Article

A reflectance-based sensor for rapid and sensitive detection of carrageenan in processed food samples

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Abstract
Carrageenan's, widely utilized as food thickeners, can pose health risks at high concentrations, making precise detection crucial for quality control. This study introduces a novel reflectance spectrophotometer sensor for carrageenan analysis using a methylene blue (MB)-immobilized methylcellulose/poly n-butyl acrylate (Mc/PnBA) film. By combining the hydrophilic properties of Mc with the hydrophobicity of PnBA, we created a stable membrane for MB immobilization at room temperature. Upon interacting with carrageenan, the sensor changes color from blue to purple, indicating their presence and concentration. The reflectance intensity of MB shows a linear relationship with kappa, iota, and lambda carrageenan concentrations in the 100-1000 mg L^{-1} range, with detection limits of 80, 67, and 60 mg L^{-1}, respectively, and correlation coefficients (R^2) of 0.992, 0.972, and 0.955. Recovery experiments with spiked apple juice showed 96% and 106% results, underscoring the sensor's practical applicability. Starch, alginate, and Arabic gum were used to test the reflectance sensor, and no interference was observed from these hydrocolloids. It provides a fast, uncomplicated, and efficient alternative to intricate analytical procedures, effectively addressing significant problems regarding food safety and quality control.

Keywords: food control, reflectance spectrophotometry, polymer composite, red seaweed, carrageenan

Introduction

Carrageenan's constitute a diverse group of anionic polysaccharides sourced from red algae, renowned for their structural diversity and functional properties. These polysaccharides are commercially categorized into three primary types: kappa (κ), iota (ι), and lambda (λ). The differences between all three types of carrageenan's are their gelling ability and the properties of the gel produced. The unique gelling properties of the various carrageenan's are integral to their functionality, which determines their suitability across various applications. Kappa-carrageenan creates robust, inflexible gels with potassium ions owing to its singular sulfate group per disaccharide unit, rendering it suitable for dairy products such as cheese [1]. Iota-carrageenan, possessing two sulfate groups, creates soft, elastic gels with calcium ions and is utilized in puddings and toothpaste [2]. Lambda-carrageenan, characterized by three sulfate groups, does not gel but exhibits good solubility in water, rendering it an effective thickening agent for items such as salad dressings and sauces [3, 4]. The unique gelling properties of the various types of carrageenan's are integral to their functionality, determining their suitability across various applications. In the food industry, carrageenan's are extensively utilized for their ability to gel, thicken, and stabilize food products. Beyond food, carrageenan's have demonstrated significant value in non-food sectors, particularly in pharmaceuticals, where they serve as effective tableting excipients. Their excellent compressibility, high mechanical

strength, and persistent viscoelasticity during tablet formation make them ideal for this purpose. These properties also suggest that carrageenan's are well-suited for sustained-release formulations, enhancing their application in controlled drug delivery systems [1].

While carrageenan's are classified as "Generally Recognized as Safe (GRAS) " by regulatory authorities such as the U.S. Food and Drug Administration (FDA), concerns persist regarding potential health risks, particularly when consumed in large quantities or specific forms. Of particular concern is poligeenan, a degraded form of carrageenan, which has been associated with gastrointestinal inflammation, ulceration, and tumor promotion in animal studies [5, 6]. The lower molecular weight of poligeenan enhances the likelihood of gastrointestinal absorption and interaction with cellular pathways. In contrast, the higher molecular weight of native carrageenan limits its absorption and restricts interaction with intestinal cells, underscoring the need to evaluate poligeenan's distinct effects carefully. The reduced protein-binding capacity of poligeenan raises concerns about its potential for systemic circulation and adverse biological activities. Some studies suggest poligeenan may alter cellular communication through interactions with cell membranes, potentially triggering inflammation or modulating immune responses [7, 8].

Poligeenan is produced through the acid hydrolysis of carrageenan under highly acidic conditions (pH 0.9–1.3) and elevated temperatures exceeding 80°C over several hours. Following neutralization to a pH of approximately 7.5, the product undergoes extraction using roll-drying or spray-drying techniques. This hydrolytic process significantly reduces the molecular weight of carrageenan from its initial range of 200,000 to 800,000 Da to the much lower molecular weight of poligeenan, which falls between 10,000 and 20,000 Da [7]. As a result, poligeenan exhibits distinct biological and toxicological properties compared to its precursor.

Watson's research indicates that current risk assessments for carrageenan may only partially encompass all necessary safety evaluations, leading to potential risk misconceptions [7]. It is crucial to grasp these subtle issues to protect public health effectively. Mckim highlights the need for clarity surrounding the terminology, chemistry, and biological effects across different species related to carrageenan, contributing to misunderstandings about its safety [9, 10]. Complementary findings from a pharmacokinetic and toxicity study by Wang et al. further elucidate the effects of carrageenan and κ-carrageenan oligosaccharides (KOs). After 14 days of oral administration in mice, both compounds induced observable changes in liver and kidney biomarkers. Histological analysis revealed hepatocyte necrosis in the liver, tubular vacuolization in the kidneys, and disrupted epithelial cell structures in the colon. Notably, KOs caused more pronounced inflammatory cell infiltration compared to carrageenan. These results provide critical insights into carrageenan's pharmacokinetics, tissue distribution, and potential toxicity, contributing to the ongoing assessment of its safety and application within food and pharmaceutical industries [11].

Furthermore, sub chronic and chronic rodent studies employing doses up to 5% have not demonstrated toxicological effects or intestinal damage [8]. A recent panel review confirmed that carrageenan is not absorbed intact and found no carcinogenic, genotoxic, or prenatal toxicity effects. Despite sufficient exposure data across food categories, uncertainties in chemistry, exposure, and biological effects persist, prompting the panel to maintain a temporary acceptable daily intake (ADI) of 75 mg/kg of body weight per day for carrageenan and processed Eucheuma seaweed, with a call for further research within five years to address these gaps [8, 12, 13].

Studies with human participants have shown that adding carrageenan to food as an extra dietary fiber can help restore normal lipid metabolism in healthy individuals and those with ischemic heart disease [14]. Several suggestions have been proposed regarding the processes by which this thick polysaccharide functions. Gunness and Gidley proposed a noteworthy concept, suggesting that carrageenan hinders the process of bile salt reabsorption into the enterohepatic circulation, resulting in their expulsion through feces instead [14, 15]. This process may elucidate the advantageous benefits of carrageenan on lipid metabolism. Comprehensive evaluations of carrageenan as a food additive have been presented by McKim and Weiner. McKim critically reviewed in vitro studies, identifying potential methodological limitations and their relevance to human health and safety, while Weiner concentrated on in vivo safety assessments [8, 9].

Consequently, there is an increasing need for effective methods to monitor and regulate carrageenan levels in food products. Watson (2008) underscores the ongoing safety concerns, highlighting the pressing need for regulatory intervention [7]. By examining four public controversies, Watson reinforces the importance of implementing comprehensive regulatory frameworks to address and manage these challenges effectively. Traditional analytical methods for carrageenan, such as liquid and gas chromatography, involve complex sample preparation steps like hydrolysis, which limit their suitability for

quick and routine assessments. While spectrophotometric techniques are fast and easy, they have a limited linear range and low selectivity for high-molecular-weight carrageenan. Quantifying individual carrageenan types adds complexity to the analysis [3,4]. These challenges underline the urgent need for new, more accessible analytical techniques.

Developing sensors for rapidly and sensitively detecting carrageenan in food products marks a significant advancement in food safety and quality assurance [16]. While traditional analytical methods, such as chromatography and spectrophotometry, are known for their accuracy, they often involve time-consuming procedures, require labor-intensive preparation, and rely on sophisticated instrumentation. These limitations reduce their practicality for routine food analysis, highlighting the need for more efficient alternatives. An innovative solution involves the creation of functional films with superior mechanical stability, heightened sensitivity, and strong binding affinity for carrageenan, which enhances the performance and reliability of sensor technologies. Reflectance spectrophotometry further strengthens this approach by offering precise, non-invasive, and real-time measurements. This technique identifies subtle variations in the optical characteristics of the film, providing high sensitivity and selectivity for carrageenan detection. The novelty of this research lies in integrating advanced films with reflectance spectrophotometry, resulting in a sensor platform capable of rapid, accurate detection. This combination not only provides a practical and accessible alternative to conventional methods but also holds the potential to revolutionize food safety monitoring by enabling prompt decision-making in quality control processes.

This study focuses on developing a novel optical sensor that rapidly and sensitively detects carrageenan across various food products. The research encompasses the sensor's design, fabrication, and testing, emphasizing optimizing performance parameters to meet stringent food safety standards. The sensors were fabricated using a methylcellulose and poly(n-butyl acrylate) composite membrane, with methylene blue as the indicator. The immobilization of methylene blue via hydrogen bonding to the cellulose chain of methylcellulose is suitable for sensor applications, although methylcellulose dissolves in water, posing a challenge. Poly(n-butyl acrylate) is water-insoluble and has a low glass transition temperature, facilitating adhesion with other polymers and forming a stable membrane. This combination results in a compatible and smooth surface for methylene blue immobilization. The successful implementation of these sensors could revolutionize carrageenan detection, ensuring food products are safe for consumption and compliant with regulatory standards.

Materials and Methods

Chemicals and apparatus

This study employed the following chemicals: Tris(hydroxymethyl) aminomethane (Tris-HCl), which was acquired from Acros Organics and Duchefa Biochemie. Hydrochloric acid (37%) was supplied from Riedel-de Haen, and sodium chloride (NaCl) was bought from Sigma. The origin of methylene blue (MB) was R and M Chemicals. Sigma provided analytical-quality iota (ι) and lambda (λ) carrageenan, as well as 2-hexanediol diacrylate (HDDA). Fluka provided kappa (κ) carrageenan, 2,2-Dimethoxy-2-phenylacetophenone (DMPP), and sodium tetrakis [3,5-bis(trifluoromethyl) phenyl] borate (NaTFPB). The chemicals used in the experiment, sodium dihydrogen phosphate (NaH_2PO_4) and HEPES buffer, were obtained from Fluka and Systerm, respectively. The compound N-butyl acrylate (n-BA) was acquired from Merck. All compounds were of analytical quality and were utilized without any additional purification.

The reflectance measurements were performed using a Mikropack DH-2000-BAI UV-VIS-NIR spectrophotometer. This instrument is equipped with UV-Vis light sources, NIR, and tungsten halogen lamps that cover a spectral range from 200 to 1100 nm. A probe optical fiber with a diameter of 0.6 cm and a core diameter of 0.15 cm was utilized to direct light onto the sensor's surface. Data collection and control of instrument parameters were conducted using a personal computer and Ocean's Optic software, which facilitated the capture and processing of data.

The acrylic polymer was synthesized via photopolymerization utilizing a UV exposure machine (RS Ltd., Cambridge, UK). This machine is fitted with four 15 W UV light tubes that emit UV radiation with a wavelength of 350 nm. The process is carried out while continuously purging with nitrogen gas. The Mc/PnBA polymer mix was chemically characterized using a Perkin Elmer Spectrum GX FTIR microscope and the KBr disc method. The composite membrane was morphologically characterized using a scanning electron microscope (SEM, LEO 1450VP). The successful attachment of MB onto the Mc/PnBA composite membrane was verified by SEM-EDS (scanning electron microscopy with energy dispersive X-ray spectroscopy), which detected the presence of MB dye in the film sample.

Preparation of reflectance membrane sensor

Poly(n-butyl acrylate) (PnBA) was synthesized with modifications to the methods described by Heng and Hall [17] and Alva et al [18]. Initially, 16.0 mg of 2,2-

Dimethoxy-2-phenylacetophenone (DMPP) was added to 1.0 ml of n-butyl acrylate. The process began with vortex mixing the precursor materials for 2 min until a milky white emulsion formed. This emulsion was then sonicated for 20 min to enhance dispersion. Subsequently, the mixture was transferred to a glass Petri dish and exposed to UV light. The photopolymerization setup included four 15-watt lamps, each emitting UV radiation at a wavelength of 350 nm. The exposure lasted for approximately 10 min under a stream of nitrogen gas, facilitating the formation of PnBA, as outlined by Ruedas-Rama and Hall [19].

The photopolymerized product was then processed further by filtration through Whatman filter paper. The retained residue was thoroughly rinsed with tetrahydrofuran (THF) to remove unreacted materials and isolate the desired polymer particles. This method ensures the production of PnBA with specific micro- and nano-scale dimensions suitable for subsequent applications.

Polymer blends utilized were methylcellulose (MC) and poly(n-butyl acrylate) (PnBA). Solutions of 2% (w/v) poly(n-BA) and methylcellulose in THF were prepared separately and contained in stoppered conical flasks. Additionally, various PnBA and MC blend compositions were prepared by mixing predetermined quantities of stock solutions to achieve ratios of 100/0, 90/10, 70/30, 50/50, 30/70, 10/90, and 0/100 % (v/v). These mixtures were stirred vigorously for one day at various temperatures (40 ºC, 60 ºC,70 ºC, and 80 ºC) to ensure thorough integration and stability.

The immobilization process involved the addition of 100 µL of methylene blue (MB) dissolved in dimethyl formamide (DMF) to 2 mL of the polymer blend solution, followed by overnight stirring. Subsequently, 20 µL of the MB-polymer blend was deposited onto a flat glass surface and allowed to dry at room temperature overnight. Post-drying, the composite membrane of the sensor was thoroughly washed with Tris-HCl buffer (pH 7) to remove residual reactants or byproducts. The cleaned composite membrane of the sensor was stored at 25 ºC in a dry, dark environment to maintain its integrity and functionality. This meticulous process ensures the preparation of effective sensors for subsequent analytical applications [20].

Results and Discussion

Morphology of Mc/PnBA composite membrane

Microscopic analysis, as depicted in **Figure 1**, was performed to evaluate the morphology and component distribution within the polymer blend. This characterization revealed that the poly(n-butyl acrylate) (PnBA) spheres ranged in size from 1 to 5 micrometers (**Figure 1a**). The hydrophilic characteristics of methylcellulose (MC) play a pivotal role in shaping the properties of composite membranes when blended with poly(n-butyl acrylate) (PnBA), a hydrophobic polymer. With its high affinity for water, MC introduces unique features into the membrane structure that can enhance or limit its functionality, depending on the intended application. A higher MC concentration can improve membrane permeability and facilitate selective filtration, making it advantageous for efficient fluid transport applications. However, excess MC may reduce the membrane's mechanical integrity and stability. A high MC content can diminish the membrane's resistance to mechanical stress, environmental degradation, and chemical exposure in applications demanding robust barrier properties or structural resilience. Thus, precise optimization of MC concentration is critical to achieving a suitable balance between durability and functionality, allowing for tailored performance characteristics in specific applications [20].

In contrast, a sample with a higher PnBA concentration, specifically at a 20/80% (v/v) MC/PnBA ratio, displayed notable agglomeration. The hydrophilic nature of Mc, which tends to dissolve in water, would create surface pores if a significant amount of Mc is present in the polymer mix. In contrast, the blended sample with a greater PnBA concentration, specifically 20/80% (v/v) Mc/PnBA, exhibited the presence of massive agglomerates. This indicates an incompatibility between Mc and PnBA, as seen in **Figure 1b, c,** and **d**. In a more 80/20% MC/PnBA ratio, the increased MC content enhances the hydrophilic nature of the composite, likely resulting in greater porosity and potentially lower water resistance compared to blends with higher PnBA proportions. While the 70/30% blend may demonstrate improved permeability and adhesion due to the MC content, it could exhibit reduced water resistance and mechanical durability relative to lower MC ratios, such as 40/60% or 60/40%, where the PnBA content enhances hydrophobicity and structural integrity.

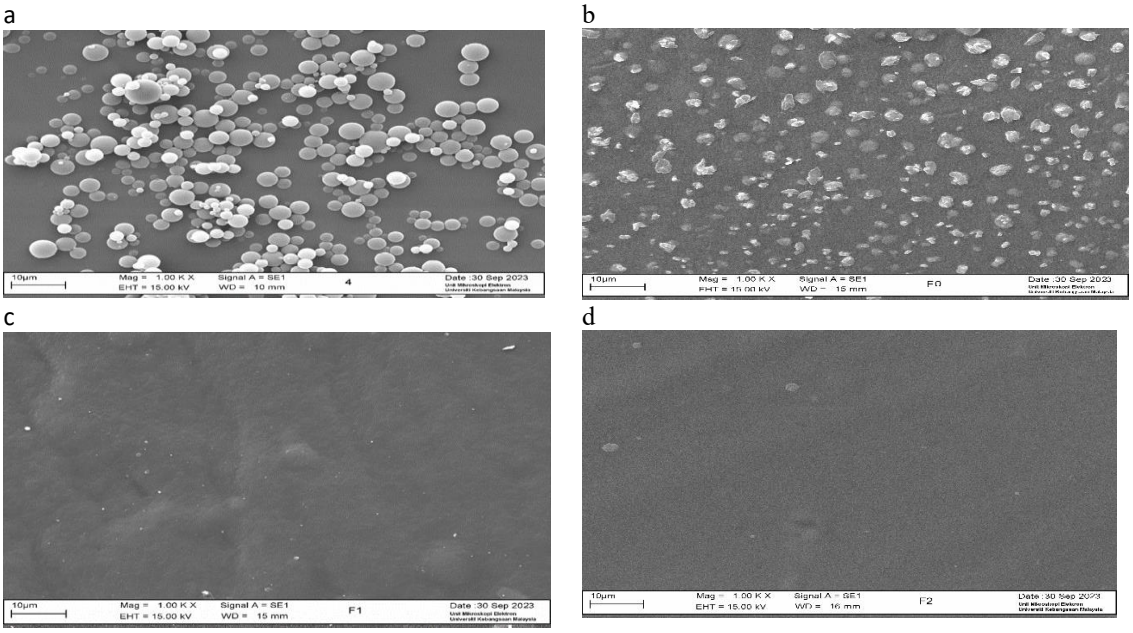


Figure 1. SEM micrographs of four different samples: (a) pure poly(n-butyl acrylate) (PnBA), (b) a polymer composite membrane containing methylcellulose (MC) and PnBA in a 40/60% (v/v) ratio, (c) a composite membrane with an MC/PnBA ratio of 60/40% (v/v), and (d) a composite membrane with an MC/PnBA ratio of 70/30% (v/v).

Incorporating methylene blue (MB) into a polymer composite with a 70% MC and 30% PnBA composition strategically utilizes MC's hydroxyl (OH) groups to foster strong physicochemical interactions. These hydroxyl groups facilitate ion exchange and complex formation mechanisms critical to MB retention. Through ion exchange, the positively charged amine groups on MB engage with the negatively charged oxygen atoms in MC's hydroxyl groups, resulting in a stable anchoring effect of MB within the polymer matrix. This electrostatic attraction is further enhanced by the high density of -OH groups present in MC, creating multiple stable interaction sites. Complementarily, complex formation mechanisms strengthen MB retention, with non-electrostatic interactions like coordinate bonding and van der Waals forces allowing MB to establish stable associations with MC's hydroxyl groups. Additionally, hydrogen bonding plays a dual role in enhancing the structural stability of the composite; intermolecular hydrogen bonds are formed between separate MC molecules or between MC and MB, which improves structural integrity and slows MB release. In contrast, intramolecular hydrogen bonds within individual MC chains contribute to the overall rigidity of the polymer matrix.

The chosen 70/30 MC to PnBA ratio optimizes the composite's mechanical properties and binding capacity for MB, where MC's hydroxyl-rich structure provides abundant binding sites. **Figure 1(d)** corresponds to the surface of the membrane, clearly showing that the surface is homogeneous and smooth. Meanwhile, PnBA introduces flexibility and hydrophobic characteristics, which counterbalance MC's rigidity and hydrophilicity. This hydrophobic component reduces water absorption, stabilizing MB retention by minimizing potential water-mediated disruptions. **Figure 2** illustrates these interactions, including ion exchange, complex formation, and hydrogen bonding sites. Altogether, the MC and PnBA combination results in a composite matrix that effectively stabilizes MB through MC's binding interactions while maintaining a flexible yet durable structure due to PnBA, making this composite suitable for applications requiring strong binding and structural adaptability.

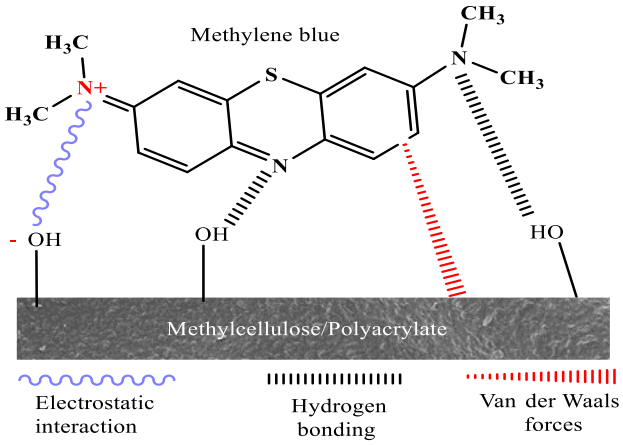


Figure 2. Schematic representation of the interaction between MB molecules and the hydroxyl groups of MC involves ion exchange and complex formation. These functional groups promote the formation of hydrogen bonds both between and within molecules.

Carrageenan's negatively charged sulfate was attracted to the surface of MB-PnBA-MC, forming a complex with the MB through the electrostatic interaction. Integrating the PnBA-MC composite matrix significantly influences the interaction dynamics between carrageenan and MB within the sensor framework. Including PnBA introduces hydrophobic segments and imparts flexibility into the otherwise hydrophilic, rigid MC matrix. This composite structure creates a more adaptable interaction environment, promoting structural stability and responsiveness in the sensor's carrageenan detection capabilities. With its high hydroxyl (OH) group density, methylcellulose facilitates extensive hydrogen bonding and electrostatic interactions with MB. The flexibility introduced by PnBA enhances the mobility of carrageenan molecules toward the active MB sites, while its hydrophobic character mitigates interference from water. This feature promotes a more stable and focused interaction between carrageenan and MB. By balancing hydrophilic binding capabilities with hydrophobic structural support, the PnBA-MC matrix effectively improves the sensor's selectivity and sensitivity, optimizing the carrageenan-MB detection mechanism for heightened analytical performance.

The observed color change in the sensor from blue to purple arises from the interaction between MB and carrageenan within the PnBA-MC composite matrix. When carrageenan binds to MB, it modifies the electronic environment around the MB molecules, leading to a visible color change. As a result, the sensor provides a distinct colorimetric response that reflects carrageenan presence and concentration, making it a practical tool for qualitative and quantitative detection. The sensor measured intensity changes before and after the exposure of the sample solution. The maximum changes in the peak wavelength of the reflectance spectrophotometer sensor with carrageenan interaction with its maximum at 680 nm because the maximum change in the reflection intensity (Δ intensity) was observed at 680 nm.

Evaluation of sensor

This study developed a sensor by optimizing various factors, including response time, pH, and MB concentrations. These factors were applied to the sensor, with reflectance intensity measured at a wavelength of 680 nm, where the maximum reflectance intensity varied in response to changes in MB concentration. The relationship between the sensor and different MB concentrations is depicted in **Figure 3a**, showing that the reflectance intensities increased with rising MB concentrations. A concentration of 0.1 mM MB was selected for its optimal reflectance response. At higher concentrations of MB, the membrane matrix reaches a saturation point, where available binding sites for MB are fully occupied. This saturation reduces the effective interaction surface area, hindering the mobility of MB cations within the matrix [21,22]. The limited movement of MB within the source phase of the membrane leads to an accumulation of MB molecules near the surface, creating osmotic pressure that further drives water uptake and initiates matrix swelling. This swelling weakens the structural integrity of the matrix, making it easier for MB molecules to leach out. As a result, MB retention and overall stability within the composite are compromised, diminishing the effectiveness and durability of the membrane in maintaining MB concentration.

The pH is a critical variable that must be optimized to achieve the best response from carrageenan

[23,24,25]. The metachromatic effect depends on the pH of the medium, the presence of ionized acidic groups, and the medium's ionic strength [26]. The pH has a moderate impact on metachromaticity and is influenced by the presence of ionized acidic groups. Essentially, pH and ionic strength variations affect electrostatic interactions by altering the ionic state of the polyanion group residuals, as illustrated in **Figure 3b**. The pH level significantly modulates the interaction between MB and carrageenan due to the pH-dependent charge variations on carrageenan's acidic groups. Carrageenan molecules gain a negative charge in alkaline conditions, enhancing electrostatic attraction with MB and promoting a stronger binding interaction. Conversely, in acidic environments, the abundance of H^+ ions competes with carrageenan for bonding with MB, reducing the overall interaction. At extreme pH levels, however, the structural integrity of the poly(n-BA)-MC-MB membrane may be compromised, and the poly(n-BA)-MC may impact its capacity to bind with MB stably.

Additionally, ionic strength is a critical factor that modulates the electrostatic forces between carrageenan and MB. In low-ionic strength solutions, limited ion shielding allows for direct and strong electrostatic interactions, facilitating MB aggregation. With increasing ionic strength, additional ions shield the sulfate groups on carrageenan and MB molecules, progressively weakening their attraction. This shielding effect significantly reduces MB-carrageenan interactions in very high ionic-strength conditions.

The successful immobilization leakage of MB on poly(n-BA)/MC was verified using SEM-EDX analysis to detect the film surface bound with MB. SEM-EDX mapping was utilized to scan the film to determine the distribution of elements on the sensor's surface. The scan revealed the presence of sulfur atoms from MB, which appeared more pronounced after adding the carrageenan sample. Both MB and carrageenan, containing sulfur elements, were mapped at the microstructural level using SEM-EDS spectrum analysis for polymer composite-MB at different MB concentrations in the sensor-film designated as a sensor (**Figure 4**). The MB concentrations were optimized for the reflectance technique to ensure the best intensity for the carrageenan sensor. The SEM-EDS spectrum showed the sulfur element in the film after adding 300 mg L^{-1} ʎ-carrageenan to the sensor. The SEM-EDS spectrum indicated a stable surface of the film after adding the ʎ-carrageenan sample.

The focus on sulfur is pertinent since both MB and ʎ-carrageenan contain this element, as illustrated in **Figure 4-a**. Upon introducing the ʎ-carrageenan (MB-ʎ-carrageenan) complex, represented in **Figure 4-b**, there is a noticeable increase in the sulfur content. The interaction between MB and carrageenan results in a color change in the sensor membrane (poly(n-BA)-MC-MB), which alters the intensity measured by the reflectance technique. The immobilization leakage of MB from a membrane was assessed using UV-Vis spectrophotometry. The membrane was immersed in a 0.02 M Tris-HCl buffer for 20 min, after which the UV-Vis absorption spectrum of the buffer solution was analyzed to assess any possible release of MB. The analysis indicated no detectable MB leakage from the membrane.

The sensor's response time was tested in 20 min using 400 mg L^{-1} λ-carrageenan at 680 nm, in 20 mM Tris-HCl buffer at pH 7, and the response of the sensors remained constant after about 2 min. Significant changes in the sensor responses were observed in 1 min. The response changes became less after 1 min of reaction time and remained relatively constant. Thus, the response time of the λ-carrageenan sensor was approximately 2 min (**Figure 5**).

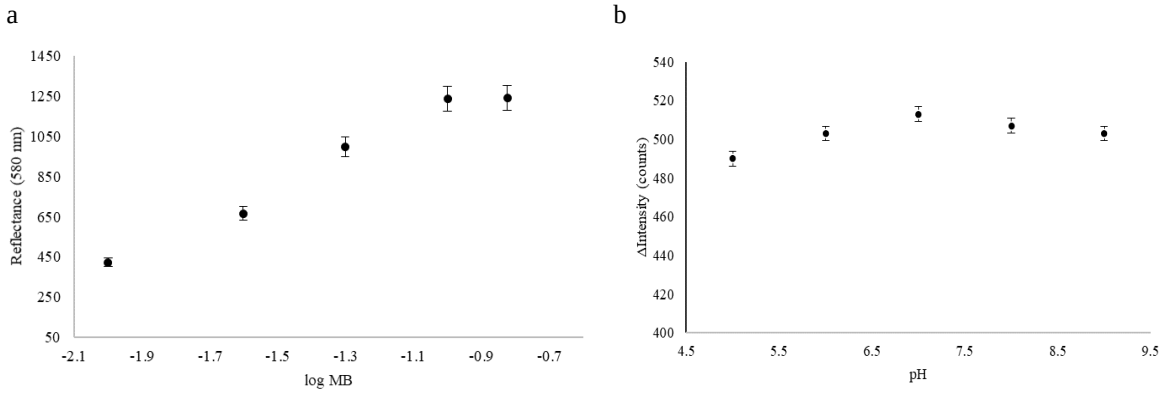
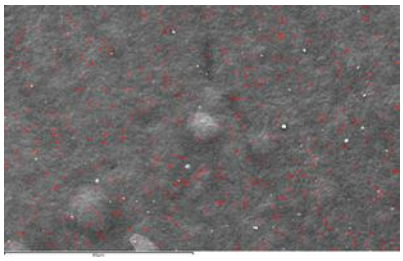
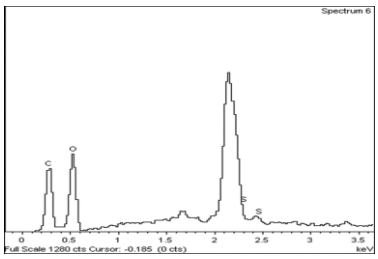


Figure 3. a) Influence of varying concentrations of MB immobilization on poly(n-BA)/MC. b) Impact of pH on sensor response to 400 mg L^{-1} λ-carrageenan in the 0.02 M Tris-HCl buffer at 680 nm

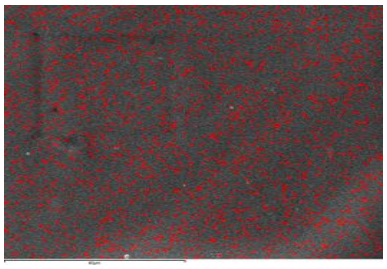
a

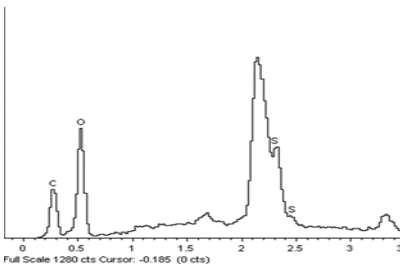




Element	Weight%	Atomic%
C K	45.04	52.30
O K	54.48	47.49
S K	0.48	0.21
Totals	100.00	

b





Element	Weight%	Atomic%
C K	39.69	48.44
O K	52.25	47.87
S K	8.06	3.68
Totals	100.00	

Figure 4. SEM-EDS spectrum examination of the poly(n-BA)–MC-MB membrane revealed the presence of C, S, and O elements after the addition of 300 mg L^{-1} λ -carrageenan, resulting in the presence of S element from the λ -carrageenan a) and b) before and after addition of 300 mg L^{-1} of ʎ-carrageenan respectively

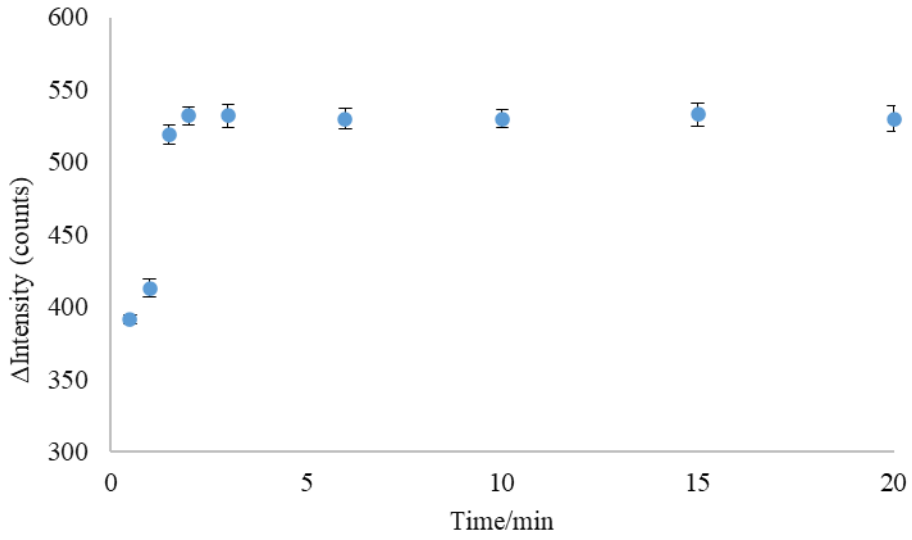


Figure 5. The sensor's response time, evaluated over 20 min using a 400 mg L^{-1} concentration of λ-carrageenan at a wavelength of 680 nm. The experiment was conducted in a 20 mM Tris-HCl buffer solution at pH 7

Response sensor toward anionic polysaccharide

A calibration curve for the sensor was established under optimal response conditions. The sensor's response to various concentrations of anionic polysaccharide at a wavelength of 680 nm is depicted in **Figure 6**. **Table 1** lists the correlation coefficients (R^2) and the sensitivity values of the carrageenan curves. The reproducibility of the sensor, measured by relative standard deviation (RSD) values, ranged from 3.3% to 6% (n = 6). This reproducibility indicates the degree of variability in the sensor's response across multiple tests. **Figure 6** presents the calibration curve for the sensor at 680 nm. A 20 mM Tris-HCl buffer solution at pH 7 was used, with various concentrations of anionic polysaccharide added separately to evaluate the sensors' functionality. No responses were observed for starch alginate and Arabic gum, indicating no interaction with MB, likely due to similar peak overlaps.

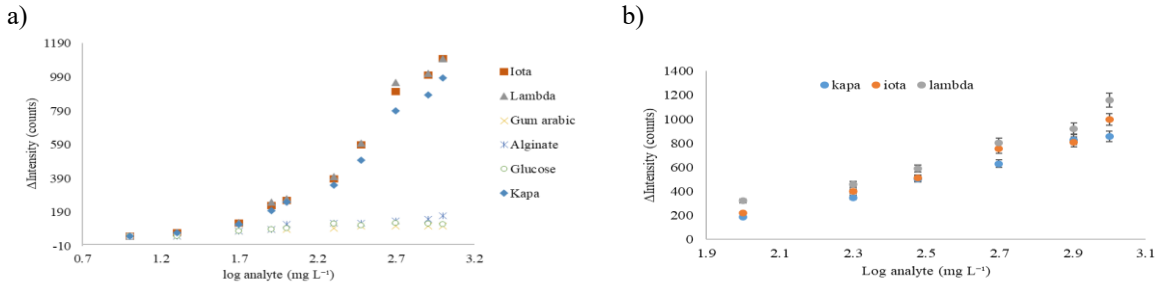


Figure 6. a) The interference study of the sensor toward some anionic polysaccharide in the range of 10-1000 mg L^{-1}, b) calibration curve of the three types of kappa, iota, and lambda carrageenan in the range of 80-1000 mg L^{-1}

Figure 6 illustrates the calibration curve for the sensor at a wavelength of 680 nm. A buffer solution of 20 mM Tris-HCl at pH 7 was selected for the experiment, where various concentrations of anionic polysaccharide were separately added to assess the sensor's functionality. The results indicated no responses for starch alginate and Arabic gum, suggesting no interactions between these substances and MB, as evidenced by the similarity in their spectral peaks. Moreover, the reflectance sensor showed slightly different response factors for κ-, ι-, and λ-carrageenan, reflecting the variations in their molar sulfate content under conditions of pH 7 with 20 mM Tris-HCl buffer. Notably, there was no change in the sensor's response to other anionic polysaccharides, such as starch, calcium alginate, and gum Arabic, at different concentrations. This indicates minimal interaction between the immobilized MB and these polysaccharides. Thus, the reflectance sensor is specific to carrageenan detection (**Figure 6b**). This selectivity is consistent with previous studies that analyzed carrageenan and other anionic hydrocolloids using the UV-Vis spectrophotometric method with MB [20, 27-30].

Table 1 presents carrageenan sensor sensitivity measurements that utilize poly(n-BA)–MC-MB for three distinct carrageenan types. The sensors demonstrated varying sensitivities, with iota- and lambda-carrageenan displaying 802.8 and 753.2 intensity/decade, respectively. In contrast, kappa-carrageenan exhibited a lower sensitivity of 698.9 intensity/decade. Furthermore, the limit of detection (LOD) in **Table 1** for lambda, followed by iota-carrageenan, was also lower than kappa-carrageenan. Lambda-carrageenan contains a sulfate group attached to the two positions of its 3-linked galactopyranosyl units, which consists of one and two extra sulfate groups than iota and kappa-carrageenan, respectively. This sulfate group improves its solubility and ability to interact with ions. This structural characteristic enhances its hydrophilic nature, facilitating improved solubility in water and more robust interactions with ionic substances. The presence of sulfate groups enhances the ability to form ionic solid connections with two-carbon alkyl chains. Lambda-carrageenan exhibit enhanced affinity towards cationic dyes such as methylene blue, potentially strengthening the complexation.

Based on previous results, the observations made with the sensor in this study are consistent with the findings of Soedjak's research [27], which utilized the UV-Vis test method. Both studies demonstrated that the sensors specifically bind to carrageenan without interference from other polyanions [16]. Furthermore, the carrageenan sensors developed in this work exhibited excellent responsiveness to carrageenan. As anticipated, the dye responded linearly to the molar sulfate content of the carrageenan, leading to distinct response factors for kappa-, iota-, and lambda-carrageenan. MB binding occurs immediately, requiring no incubation period, simplifying the method [16]. The reproducibility of the sensor was assessed by employing various carrageenan sensors and conducting a series of measurements of λ-carrageenan concentration.

Table 1. Evaluation of the analytical performance of the carrageenan sensor 100-1000 Linear range (mg L^{-1})

Types of Carrageenan	R^2	Sensitivity*	LOD* (mg L^{-1})
kappa	0.992	698.9	80.0
iota	0.972	753.2	67.0
lambda	0.955	802.8	60.0

Note: Sensitivity= intensity/decade, LOD: limit of detection

Additionally, the stability of the carrageenan sensor was assessed by measuring the intensity differential values over 90 days using 400 mg L^{-1} λ-carrageenan at 680 nm in 20 mM Tris-HCl buffer at pH 7. The results indicated that the sensors maintained consistent performance throughout the testing period, with a relative standard deviation (RSD) of less than 5% (n = 3), demonstrating high reproducibility. These findings suggest that carrageenan-based sensors have a reliable operational lifetime under the specified conditions, making them suitable for long-term analytical applications.

Recovery study

The recovery experiment utilized three freshly prepared homemade fruit juices like pineapple, apple, and orange. Each juice was prepared by cutting the respective fruit into pieces and blending it with 400 mL of deionized water, using 200 grams of fruit per preparation. The resulting blend was filtered to remove pulp, yielding a clear juice. This clear juice was diluted with a 20 mM Tris-HCl buffer (pH 7.0) to reach a final volume of 500 mL.

A stock solution of carrageenan was prepared by dissolving 50 mg of carrageenan in 50 mL of Milli-Q water (resistance of 18 MΩ) in a water bath maintained at 50°C. After dissolving, the solution was allowed to cool to room temperature. During the experimental phase, varying concentrations of λ-carrageenan were incorporated into 100 mL portions of each fruit juice and mixed thoroughly to ensure homogeneity. The prepared samples were then stored at 4°C in a refrigerator for further analysis. The juices were then analyzed using carrageenan sensors. The results, detailed in **Table 2**, demonstrated percentage recoveries of carrageenan between 90% and 102%. These findings indicate that common additives in commercial juice products exert minimal to no interference with the carrageenan fluorescence sensor's analytical performance. In addition, sensor testing in the presence of free sulfate ions and a blank sample revealed no significant variations in intensity readings when measured via reflectance. This result underscores the sensor's high specificity, confirming its exclusive sensitivity to polysaccharides sulfated compounds while disregarding hydrolyzed sulfate groups or unbound sulfate ions.

According to **Table 2**, the results indicate that determining carrageenan in juice as a real sample is feasible for food application analysis. However, to the best of our knowledge, only one optical sensor for the determination of carrageenan was used in two different membranes, Poly(nBA) and Poly(nBA-NAS), via the reflectance method [31]. The Poly(nBA)-MC sensor comparison with the previous reflectance method of carrageenan determination is shown in **Table 3**.

Table 2. The sensor measured carrageenan-known concentrations in homemade fruit Juice prepared (n=3)

Carrageenan Added (mg L^{-1})	Found Pineapple Juice (mg L^{-1})	*R (%)	Found Apple Juice (mg L^{-1})	*R (%)	Found Orange Juice (mg L^{-1})	*R (%)
200	181	91	193	97	187	94
400	375	94	392	98	407	102
600	610	102	634	106	622	104

*R= Recovery

Table 3. Comparison of the analytical performance of λ-carrageenan sensors from this study with previously reported sensors

Parameters	Poly(nBA-NAS) [31]	Poly(nBA) [31]	This Work
Sensitivity	377.5	279.9	802.8
R^2	0.980	0.983	0.955
Dynamic linear range (mg L^{-1})	80–5000	100–5000	80–1000
Detection Limit (mg L^{-1})	80	100	60

The chemical sensor developed in this study demonstrated a lower limit of detection (LOD) than previously reported sensors, attributed to the robust polymer blend of MC and PnBA, which ensured strong bonding for MB immobilization without leakage. The hydroxyl group in methylcellulose played a key role in enhancing the surface properties of the carrageenan-based sensor. Since the sensor is designed for single use and cannot be reused or retested after its initial application, there is no fouling or regeneration to consider. Nevertheless, its performance in one-time applications shows promise for sensitive and reliable detection of carrageenan. However, future work could explore strategies for developing reusable sensors or regeneration methods to extend the life cycle of similar detection systems.

Conclusion

This study developed a reflectance sensor to detect carrageenan, utilizing MB immobilized onto a blended modified polymer (MC-PnBA). The MB was physically adsorbed within the matrix, enabling the sensor to detect carrageenan concentrations through color changes resulting from the interaction between MB and carrageenan. The sensor demonstrated strong responsiveness to carrageenan, with minimal interference from other polysaccharides, and showed notable sensitivity to the reflectance method. Under optimal conditions, the fabricated sensors exhibited a wide linear response range for carrageenan, with commendable detection limits and response times.

The sensor's selective detection of lambda (λ), iota (ι), and kappa (κ) carrageenan primarily stems from the sulfate groups in these polysaccharides. A clear linear relationship was identified between the sulfate content and the sensor response, resulting in distinct responses for each carrageenan type. Including MC-PnBA was contributory, allowing stable MB immobilization without leaching and enhancing sensor stability and reliability. Utilizing MC-PnBA microspheres provided notable repeatability and reproducibility due to their strong chemical affinity and potential for surface functionalization. This enhanced binding efficiency and contributed to consistently stable and reliable sensor performance.

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