

Research Article

Cellulose Acetate Antimicrobial Membranes Enabled By Thiazolodines For Potential Applications In Packaging.

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Abstract: Fabrication of new antimicrobial cellulose triacetate packaging membranes is the main focus of the present study. The integration of 2-aryl substituted benzothiazoles in a polymeric matrix of cellulose triacetate is used to fabricate these membranes. FT-IR and SEM are used to characterize these membranes. When assessed against *Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa*, the modified packaging membranes demonstrated positive antimicrobial activities.

Keywords: Thiazolodines; Antimicrobial membranes; CTA; Antibacterial Packaging.

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1. Introduction

Antimicrobial membranes are membranes that have the ability to remove bacteria and other organisms. Any microbe which is larger than the membrane's pore size will remain on the membrane's surface. The dimensions and size of particles and microorganisms removed by the membrane, rather than the form or size of the membrane pores, are associated with the elimination of microbes offered by a certain kind of membrane filter[1]. Variety of traditional microbial treatment procedures are being replaced by the Membranes. In the early 1990s several important parasites, like as Giardia and Cryptosporidia, were removed from the feed by the membranes. Coliforms, *Pseudomonas diminuta*, *E. coli*, and chlorophyll-a were among the pathogens removed by membranes as more advanced membrane techniques were developed. With the passage of time, research was conducted not only on the pore size of membranes, but also on the proper selection of membrane material or polymer [2] such as quaternary nitrogen atom polymers[3], methacrylic and acrylic polymers [4] etc. Due to biocidal surfaces [5] or having antimicrobial chemicals [6] embedded in them some membranes have ability to kill microbes on their surface, Aside from retaining microbes on the surface of the membrane. Antimicrobial membranes are created primarily for two purposes: biofouling mitigation [7] and the development of antimicrobial packaging materials to avoid fouling of the contents packed inside, such as food biofouling is prevented by food packaging[8]. Polymeric materials often are employed in the production. Polymeric packaging serves a variety of purposes, including product protection, food preservation, contamination prevention, product shelf-life extension, and product storage safety. Antimicrobial packaging involves packaging films, antimicrobial films, and antimicrobial substance coatings[8]. Antimicrobial polymeric packaging materials have been used for a variety of reasons, including food

and medical packaging. The focus of this research is on different antimicrobial polymeric membranes developed for packaging purposes.

The microbes contaminated on the polymeric materials, which then serve as a major source of cross-infections, resulting in harmful consequences. The bacteria can grow and proliferate by bio-assimilating the pollutants on the polymeric material's surface. Antimicrobial activity must be present in polymeric materials in order to prevent microbial contamination[9]. Antimicrobial packaging can reduce microbe growth rates by increasing the lag phase, which leads to the inactivation of microorganisms in the substance packed or on the package surface [10].

Antimicrobial packaging can be made by incorporating antimicrobial agents into the membranes being used for packaging, by coating antimicrobial agents on these films, or using antimicrobial polymers to make packaging materials. Antibacterial polymeric packaging is often made by mixing antimicrobial chemicals with ordinary synthetic polymers[11]. Polyethylene, poly(ethylene-co-vinyl acetate) and polypropylene, polyvinyl, polystyrene, polyamides and polyesters are among the materials used to make packaging films[12].

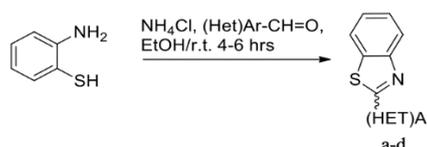
Several companies are developing antimicrobial films for surfaces that come into contact with people frequently. When bacteria come into contact with these antimicrobial films, the antimicrobial films function as a barrier, preventing the bacteria from multiplying or growing further. These antimicrobial films are applied to areas where cleanliness is critical. These films stop bacteria from spreading between the users and handlers. These can also be utilized for any type of surface that receives a lot of human contact, such as hospital interfaces. These films are effective against *MRSA*, *Aspergillus Niger*, *Streptococcus faecalis*, *Phoma Violacea Pseudomonas aeruginosa*, *E.coli* 0157Sacch, *Klebsiellaspp*, *Penicillium purpurogenum*, *Bacillus cereus* and *Cerevisiae Listeria*, according to the company that manufactures them [13].

Antimicrobial membranes are made up of several types of polymers. Cellulose triacetate (CTA) takes the top rank among them, due to properties such as high tensile strength and resistance to biodegradation and hydrolysis. [14]. CTA can be used in packaging material as it has shown antimicrobial effect in many cases. When CTA is immobilized with lysozyme it can be used in beverages packing as it can act as bacterial inhibitor especially against *M. lysodeikticus* bacteria [15-17].

To generate an antimicrobial membrane, numerous different chemicals are integrated as antimicrobial agents in the membranes. In terms of antibacterial activity, thiazolidines are a particularly important class of chemicals. Thiazolidines have been found to exhibit antibacterial properties in several investigations. Many natural items and medications contain thiazolidine and its composites, which are also found in many manufactured compounds and have the best antimicrobial activity[18]. Benzothiazoles are one of the most versatile classes of antimicrobial compounds, because of its diverse biological activity as antitubercular, anti-inflammatory, anthelmintic, antimicrobial, and antiviral agents, benzothiazole derivatives have sparked interest. [19]. Thiazolidine-2,4-dione (TZDs) molecules, which are thiazolidine derivatives, are also known to have antibacterial and antifungal properties [20]. Thiazolidines and some thiazolidine derivatives substituted at position 5 in the thiazolidine nucleus which include 5-(3-Chloro-arylidene)-thiazolidine-2,4-dione, 2-imino-4-thiazolidinone, 5-(3-Methyl-arylidene)-thiazolidine-2,4-dione, 4-thioxo-thiazolidine-2,4-dione, and 2-thioxo-1,3-thiazolidine-4-one (rhodamine are those with the most potent pharmacological properties, including antibacterial properties[21]. These compounds have been shown to have activity against Gram-positive bacteria (*Staphylococcus aureus*, *Kocuria rhizophila*, *Staphylococcus epidermis*, *Bacillus subtilis* and *Bacillus cereus*), Gram-negative bacteria (*Klebsiella pneumonia*, *E. coli* and *Pseudomonas aeruginosa*), and fungi (*Candida maltosa*, *A. fumigatus*, *A. niger*, *Cryptococcus neoformans*, and *A. flavus*) [22]. In this study substituted thiazolidines will be used as the antimicrobial agents in CTA matrix for the fabrication of antimicrobial membranes.

2. Materials and Methods

CTA (CA-436-80S, acetyl content 43.6%) from Merck was used. Graphite flakes with purity, >99.7% of Qingdao Chemical Reagent Co. Ltd., China was used. Sodium nitrate CAS 7631-99-4 | 106535 - Merck Millipore was used, sulphuric acid (98 wt. %) Acetone ($\geq 99.5\%$), NMP, $\geq 99.5\%$, 1, 4-dioxane (dioxane, $\geq 99.5\%$), acetic acid (100%), 2-NPOE, DCM from Merck were used. All solvents were of analytical reagents grade and were employed without any further purification. 2-aryl substituted benzothiazoles were prepared according to procedure reported in [23].



a: 2-(3-Nitrophenyl) benzothiazole, b: 2-(2-Nitrophenyl) benzothiazole, c: 2-(4-Bromophenyl) benzothiazole, d: 2-(4-methoxyphenyl) benzothiazole

2.1 Fabrication of Antimicrobial Membrane

The plasticized CTA membranes with benzothiazole integration are made. These membranes have been proposed as possible packaging materials. The CTA membranes were made using the method described by Senhadji et al [24]. The casting solution was made by dissolving 1 gm of CTA in 100 mL of dichloromethane (DCM) at room temperature. This solution also contained 1.5 mL of 2-NPOE. After that, in DCM, solutions of various 2-Aryl Substituted Benzothiazoles were produced. To establish homogeneity, the CTA solution and the 2-Aryl Substituted Benzothiazoles (2.5ml each) were mixed together for 1 hour. For each compound, 3 separate composition solutions were prepared: 0.2 percent, 0.4 percent, and 0.6 percent. This mixture was then placed into a 2.5 cm petri dish individually and left overnight to allow the dichloromethane to evaporate. After that, the membranes were removed from the petri dish by submerging it in cold water. After that, the membranes were immersed in distilled water for a period of time. With 12 different types of benzothiazoles, 12 different membranes (numbered 1,2, 3,12) were created in this investigation. Each membrane's benzothiazole derivative is listed in the table below

Membrane	Membrane Modifier	Membrane	Membrane Modifier
1.	0.2 % 2-(3-Nitrophenyl) benzothiazole	7.	0.2 % 2-(4-Bromophenyl) benzothiazole
2.	0.4 % 2-(3-Nitrophenyl) benzothiazole	8.	0.4 % 2-(4-Bromophenyl) benzothiazole
3.	0.6 % 2-(3-Nitrophenyl) benzothiazole	9.	0.6 % 2-(4-Bromophenyl) benzothiazole
4.	0.2 % 2-(2-Nitrophenyl) benzothiazole	10	0.2 % 2-(4-methoxyphenyl) benzothiazole
5.	0.4 % 2-(2-Nitrophenyl) benzothiazole	11.	0.4 % 2-(4-methoxyphenyl) benzothiazole
6.	0.6 % 2-(2-Nitrophenyl) benzothiazole	12.	0.6 % 2-(4-methoxyphenyl) benzothiazole

2.2 Characterization of Antimicrobial membranes

FT-IR analysis of membranes

For the chemical investigation of membrane surface before and after chemical alteration, an FTIR spectrophotometer (BRUKER ALPHA-E FTIR-1005151) spectrophotometer in the attenuated total reflectance (ATR) mode in the range of 700–4000 cm^{-1} was used. The samples were vacuum dried for 24 hours at 75°C before to analysis.

2.3 Bacterial strains preparation & Assay for antibacterial activity

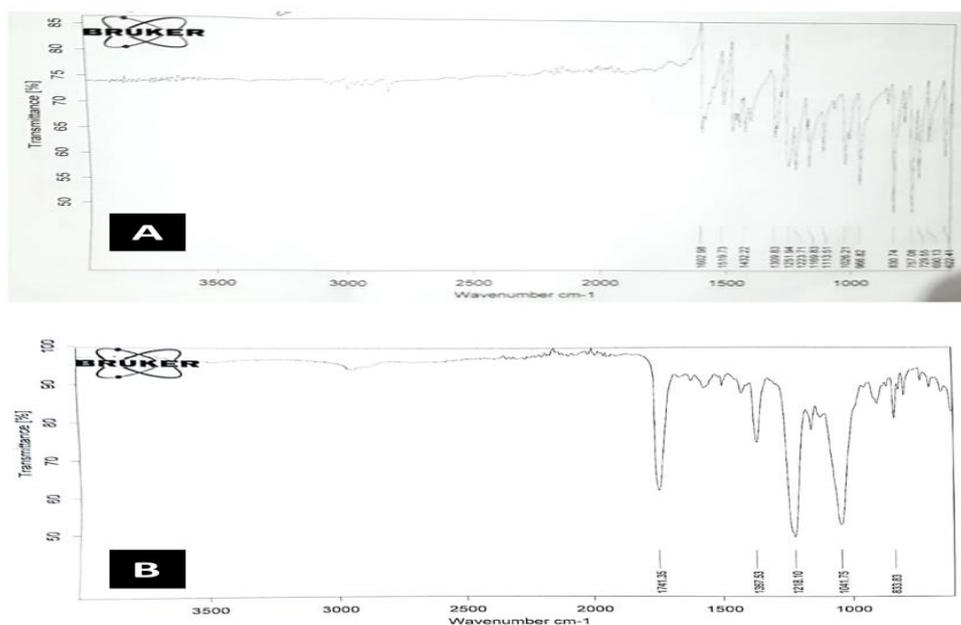
Pseudomonas aeruginosa, *Bacillus cereus*, *Escherichia coli*, and *Bacillus subtilis* were the microbiological strains used in this test. Nutrient agar slants were employed to keep these cultures at 4°C. For the introduction of loop-full of each microbe, 25ml of purified nutrient bisque in a 50ml conical flask was utilized. To activate the strains, they were placed on a rotary shaker for a 24-hour period. For the culture of these bacterial strains, Muller Hinton agar medium was utilized. The petri dishes used in the antibacterial activity test were autoclaved. Each plate was filled with 10-15ml Muller Hinton agar media and allowed to cool to room temperature. The cellular suspensions were inoculated over the surface of the agar medium using sterile cotton pads. A piece of membrane was inserted in each plate, and the plates were incubated for 24 hours at 37 degrees Celsius.

3. Results and Discussion

3.1 FT-IR characterization of the membranes

The different membranes were characterized using FTIR. The spectra of the various membranes are shown in the figures below. From the spectra, it can be deduced that all membrane constituents remained as pure constituents inside the membrane matrix, with only minor changes indicating the presence of integrated compounds. This ensures that the membrane's original character is preserved, and that the antimicrobial property is produced in the membrane as a result of the antimicrobial agents' integration. This section may be divided by subheadings. It should provide a concise and precise description of the experimental results, their interpretation, as well as the experimental conclusions that can be drawn.

The FT-IR spectra of a blank FO-CTA membrane, as well as two modified membranes impregnated with *Cis/Trans 2-(4-nitrophenyl)-1, 3-thiazolidine-4-carboxylic acid* and *Cis/Trans 2-(3-nitrophenyl)-1, 3-thiazolidine-4-carboxylic acid*, are presented in the figure below. The spectra shown that almost all of the peaks are comparable, with the exception of the modified membranes, which contain a second sharp peak at 1571-1580 cm^{-1} , indicating the existence of N-H. In *2-aryl substituted thiazolidine-4-carboxylic acids*, the N-H peak is present.



FT-IR spectra antimicrobial CTA membranes, (A) plasticized antimicrobial CTA-membrane, (B) porous antimicrobial CTA-membrane

3.2 Antimicrobial activity of membranes

The antimicrobial potential of highly reduced graphene oxide, 2-aryl substituted thiazolidine-4-carboxylic acids, and 2-Aryl Substituted Benzothiazoles impregnated membranes against *Pseudomonas aeruginosa*, *Bacillus cereus*, *Escherichia coli* and *Bacillus subtilis* have been determined. The capabilities of many membranes have been demonstrated differently. Antimicrobial plasticized membranes made for packaging applications have demonstrated to be effective against microbes. The membranes were made with four distinct 2-Aryl Substituted Benzothiazoles at three different concentrations, 0.2, 0.4, and 0.6 percent. The packaging membrane impregnated with 0.6 percent 2-(2-Nitrophenyl) benzothiazole had the best antibacterial performance. The results reveal that Nitro substituted compounds are the most efficient against all of the strains tested. Based on the results, it can be concluded that increasing the concentration of antimicrobial agents considerably improved antimicrobial activity.

Despite the fact that the antimicrobial agents in the produced membranes are in very low concentrations, they have demonstrated antibacterial action. As a result, higher antimicrobial agent concentrations in these membranes could improve their performance in the future.

In the figure 1 the antibacterial activity of the packaging membranes is shown

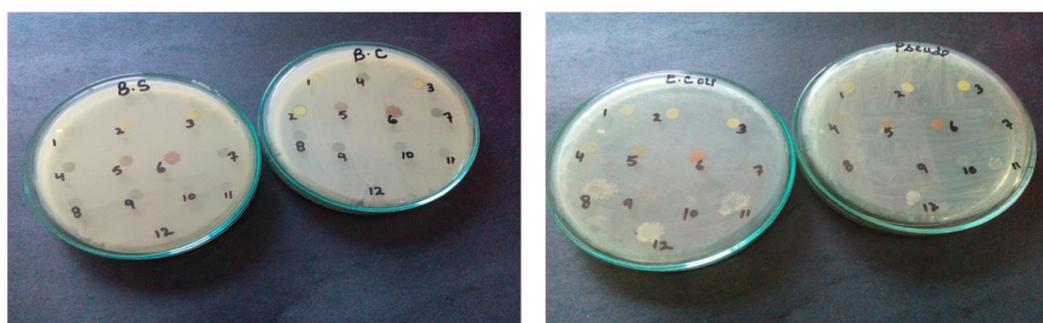


Figure 1. B.S = *Bacillus subtilis*, B.C = *Bacillus cereus*, E. Coli = *Escherichia coli*, Pseudo = *Pseudomonas aeruginosa*

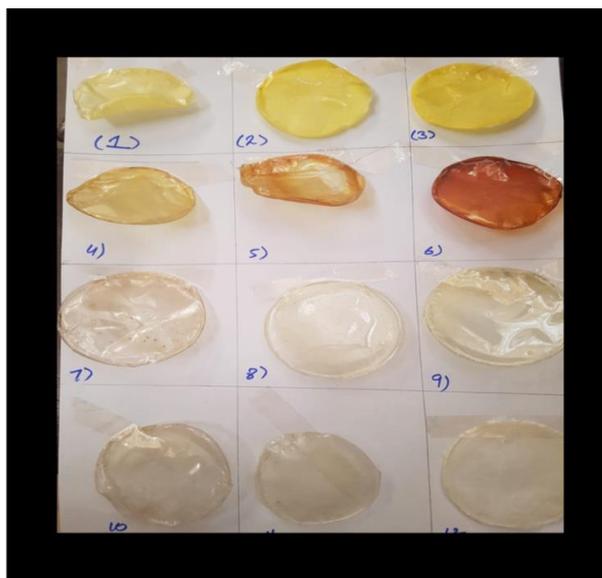


Figure 2. Fabricated antimicrobial packaging membranes

Comparative study

In the following study a comparative analysis has been made on varying types antimicrobial membranes and their activities

Antimicrobial Agents	Polymeric Matrix	Targeted Specie	Results	Ref:
Grapefruit seed extract	Agar films	Different gram-positive and gram-negative	Positive antimicrobial activity	[25]
Lysozyme	Biodegradable protein films	<i>Lactobacillus plantarum</i>	Growth inhibited	[26]
Zinc oxide nanoparticles along with niasin	Polylactic acid [27] polymer	<i>Salmonella enteric ssp.</i>	Growth inhibited	[28]
Niasin	Biodegradable protein films	<i>Lactobacillus plantarum</i>	Growth inhibited	[26]
Pediocin	Cellulose acetate	<i>Salmonella spp.</i> <i>Listeria innocua</i>	Growth retardation & inhibition	[29]
Lysozyme	Nylon 6,6 pellets	Dried <i>Micrococcus lysodeikticus</i>	Low inhibition activity	[30]
Quaternization based copolymer additive	Polyether sulfone (PES)	Anaerobic bacteria	<i>E. coli</i> killing	[31]
Bezothiazoles	CTA	<i>Bacillus subtilis</i> , <i>Bacillus cereus</i> , <i>Escherichia coli</i> , <i>Pseudomonas aeruginosa</i>	Positive antimicrobial activity	Current study

4. Conclusions

The fabrication of antimicrobial CTA packing membranes was the focus of this research. The membranes were made by incorporating 2-aryl substituted benzothiazoles into the CTA polymeric matrix. FT-IR was used to characterise these membranes afterwards. When tested against *Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa*, these packaging membranes, modified membranes demonstrated positive antibacterial action.

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