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CLAY AND MODIFIED CLAY AGAROSE-AMINOGLYCOSIDE COMPOSITE FILMS WITH ANTIBACTERIAL MINERAL RESISTANCE

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Abstract
The biopolymer films have a low thermal stability, poor gas barrier properties, low tensile strength, and low elasticity despite being environmentally friendly and biodegradable. A composite film made of clay and agarose biopolymer was developed to boost its heat stability. The film was then given glycerol to improve its flexibility. Solvent casting, clay with a weight percentage of 3 or 5, and clay that had been treated with agarose and amino glycoside medications were all used to create the films. The clay and modified clay agarose films were also characterised using thermal analysis, PXRD, and AFM methods. Two gram-positive (Staphylococcus aurous, Bacillus subtilis) and two gram-negative (Escherichia coli, Pseudomonas aeruginosa) bacteria were used to test the composite films' efficacy in treating wounds.

Key words: Bentonite clay minerals, Agarose, Aminoglycoside, Composite film, Antimicrobial resistance

1. Introduction

Over the last 10 years an accumulation of polymer based plastic products, adversely affects not only the environment but also the ecosystem, as they are slow to degrade in land, pollutes water and marine organisms etc, [1]. Recent research on biopolymer-based nanocomposite materials has been increased drastically owing to their biocompatibility, biodegradability and environment friendly [2]. Agarose is one of the bio-polymer polysaccharide materials which generally extracted from red seaweed such as *Gracilaria lemaneiformmis.* [3]. It is a linear chain polymer with a molecular weight of about ~120 kDa, and is composed of a repeating unit of agarobiose which is actually a 1,3-linked-D-galactose and 1,4-linked 3,6-anhydro-L-galactose. Agarose have a good gelling property, good stability at low pH and high temperature. However pure agarose polymer films have low tensile, thermal strength and elasticity [4]. Recently clay minerals are used as filler for polymeric material, improvement of tensile strength and thermal property [5]. Agarose with clay composite materials for food packaging film was reported by Xiaodong Li et al., [6]. In this study, we have prepared bentonite clay as filler with agarose polymer using the aminoglycoside (Streptomycin, Neomycin, and Kanamycin) drugs for antimicrobial resistance.

Materials and experimental:

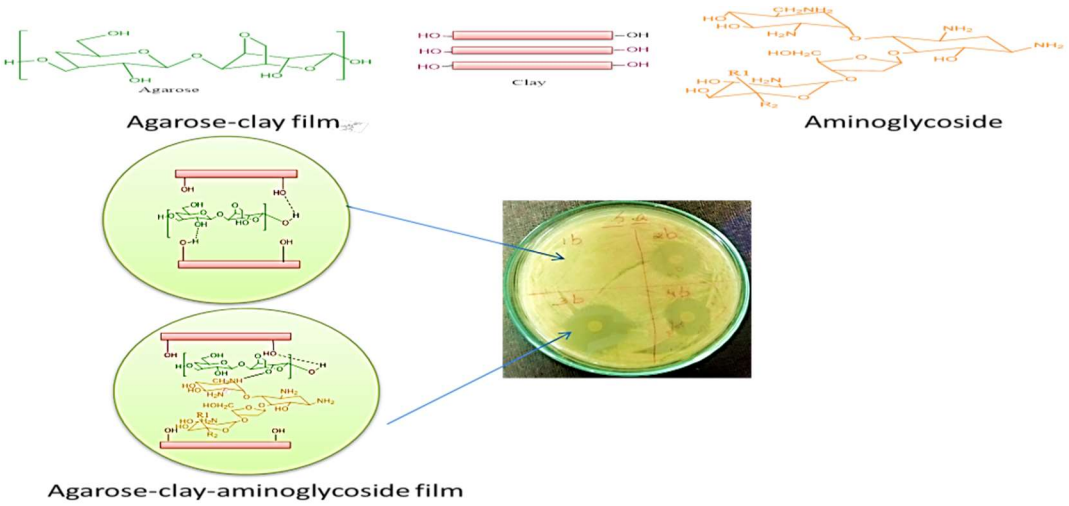

Fig.1. Graphical illustration of the experimental design used in this study

Natural Bentonite (BMT) samples were collected from Kutch, Gujarat, India. Streptomycin sulfate (Sms), Neomycin trisulfate (Nms), Kanamycin mono sulfate (Kms) and glycerol were purchased from sigma Aldrich. Germany. Agarose (A) was procured from Qualikems Laboratory, Vadodara, India.

BMT was purified by Stokes law of sedimentation technique as reported earlier (Patel et al, 2007) ref. number. 2% w/v raw clay slurry was made in deionized water in 2 L beaker and supernatant of less than 2 µM fractions was collected after pre-calculated time and height at room temperature. The supernatant was centrifuged on 10,000 rpm at 10 min. The slurry was dried at 70˚C for 24 h and ground to pass through 200 mesh sieve (ASTM).

BMT clay slurry was prepared by dispersion of BMT clay (3 and 5 wt%) in 20 ml water at RT for overnight. 20 ml of agarose solution (1 wt% agarose in water), which was pre-heated at 95°C for 30 min, 300 mg of glycerol (plasticizing agent) were added simultaneously to 3 and 5 wt% clay slurry. The mixture was stirred for 1 h at 90°C. In order to prepare the composite solution, 20 mg aminoglycoside drug in 5 ml water was added into the above mixture with continuous stirring at RT for 15 min. The composite solution was poured into 80*17 mm petri-dish and dried at 50°C for overnight. The film name was denoted as xBMT-A-y, where x is wt% of BMT clay, A is the Agarose and y is the drug name.

2. Experimental

2.1. Materials Required

Indian raw bentonite procured from Kutch, Gujarat, India. The aminoglycosides streptomycin (SMS), Neomycin (NMS), Kannamycin (KMS) was purchased from Sigma Aldrich, USA. Double distilled water procured from Milli Q -0225 instruments. Agarose biopolymer purchased from Sigma Aldrich, USA.

2.2. Synthesis of clay-agarose-aminoglycoside composite film

The Indian Bentonite upgrade from raw Indian bentonite from Kutch, India, by sedimentation process on previously done publication. [11]. The bentonite –agarose-aminoglycoside, composite

biofilm synthesis by solvent casting method. The 3 and 5 wt% of bentonite was swelling with 20 ml of distilled water with stirring condition at 1 h. The 1 g of agarose biopolymer stirred at 80 ºC. The aminoglycosides (Streptomycin, Neomycin, and Kannamycin) 25 mg dissolved with bentonite solution with stirring condition at more 30 min. The clay-drug solutions are added with biopolymer solutions with same stirring condition. The glycerol plastizer 2 ml was added to the biopolymer – clay-drug solutions with 30 min more stirring. The obtained polymer solutions are casting with Petridis for making biofilm.

3. Characterization

Powder XRD patterns of xBMT-A and xBMT-A-y with aminoglycoside composite films were analyzed by Miniflex II desktop X-ray diffract meter (Rigaku, Japan) curved Ni-filtered Cu-Kα radiation with a scan speed of °0.3/s in 2θ range of °2-70 at RT. Thermo gravimetric analysis (TGA**)** was carried out in the temperature range of 50-600°C at the heating rate of 10°C/min under the nitrogen flow using Mettler-Toledo (TGA/SDTA 851e). Thin films surface morphology and roughness was characterized by atomic force microscopy (AFM) (NT-MDT-N-Tegra-Aura, Russia). All polymer composite films were investigated for the antibacterial activity by disc diffusion method.

2. Results and discussion:

Powder XRD pattern of xBMT-A and xBMT-A-y are shown in Fig. 1. The intercalation of agarose biopolymer clay platelets usually increases the interlayer basal spacing as compared to the nascent BMT clay. The BMT clay depicts a diffraction peak 2θ = 6.05 and basal spacing of the interlayer 001 plane d_{001} = 1.47 nm. After the modification with biopolymer and aminoglycoside drug diffraction peak is shifted to lower 2θ value and the corresponding basal spacing (d spacing) was given in Table 1. All the composite films showed change in d_{003} plane reflection due to the interaction of agarose polymer with aminoglycoside drugs. [7-8].

Table 1. XRD patterns results

Clay composite film	2θ	Basal spacing (d_{001}) value
3BMT-A	5.04	1.86
3BMT-A-Sms	4.61	2.01
3BMT-A-Nms	2.88	3.4
3BMT-A-Kms	2.7	3.6
5BMT-A	4.76	1.87
5BMT-A-Sms	4.4	2.0
5BMT-A-Nms	4.3	2.0
5BMT-A-Kms	4.3	2.0

The thermal stability of the different percentage of clay blended with agarose film and clay agarose modified amino glycoside drug composite film was measured using TGA, and the resulting TGA and DTGA curves were shown in Fig.(2). The initial weight loss at 50-100°C in all samples was attributed to the removal of water on the surface of film. Then major weight loss was observed at 100-250°C, attributed to thermal decomposition of agarose biopolymer [9]. Further the major weight loss at 250-400°C account for elimination of aminoglycoside organic moieties [10]. The clay

mineral was decomposed above 450°C due to loss of structural hydroxyl group, reported by (Hasmukh A Patel et al) [11].

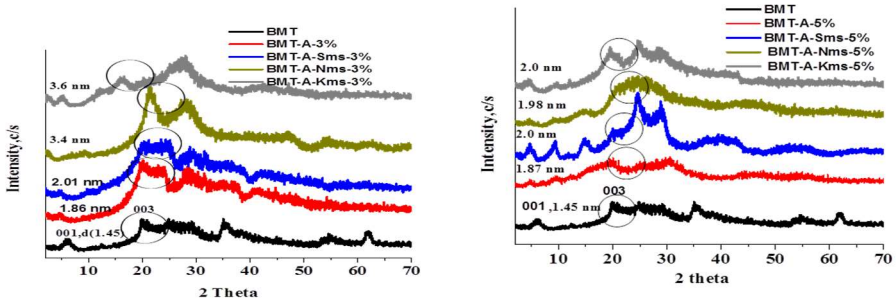

Fig.2. XRD patterns of 3 and 5 wt% polymer clay composite film and with amino glycoside drug loaded film

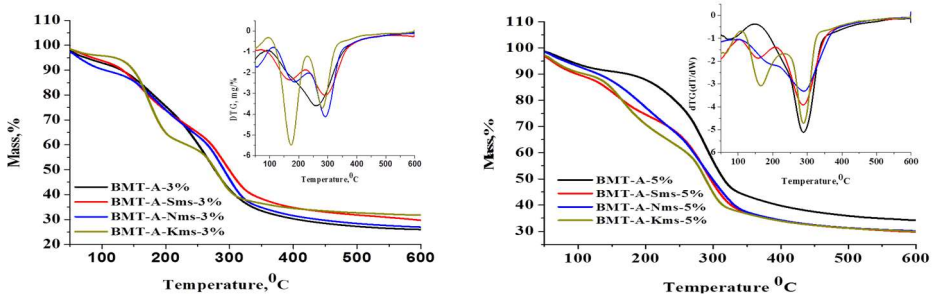

Fig.3. TGA and DTG curves 3 and 5 wt% polymer clay composite film and with amino glycoside drug loaded film

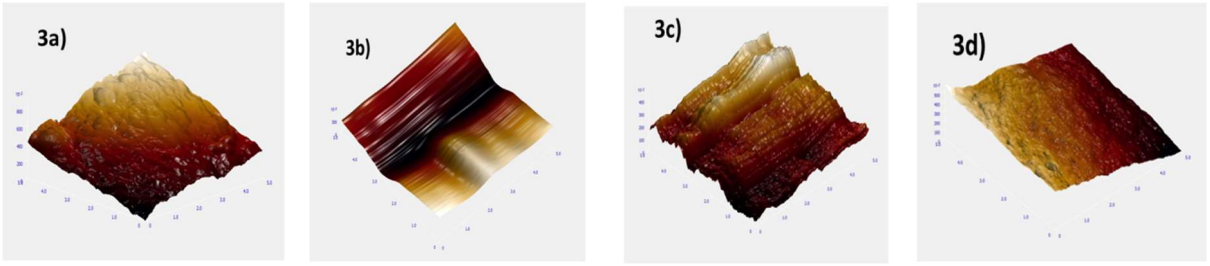

Fig.4. AFM images of 3a) 3% clay modified agarose film 3b) 3BMT-A-Sms-, 3c) 3BMT-A-Nms, 3d) 3BMT-A-Kms.

The XRD patterns clearly confirm that 3 wt% of the clay minerals platelets intercalate with drug moiety as well as with agarose polymer during composite film formation. 5wt%.. So we have choosen the 3 wt% composite films for further morphology and roughness analysis via AFM which showed the neat agar film roughness value is lesser than that of clay modified agarose film. Further the interaction of aminoglycoside organic moiety with clay modified agarose film, the roughness was decreased [12]. The 3D image of the morphology and roughness were shown in Fig. 3 and Table 2, respectively.

Table.2. Roughness of the 3% clay agarose film

Films	Roughness average (nm)	Roughness mean square (nm)
Agarose		
3BMT-A	0.157	0.189
3BMT-A-Sms	0.119	0.141
3BMT-A-Nms	0.071	0.087
3BMT-A-Kms	0.08	0.105

Antibacterial activity:

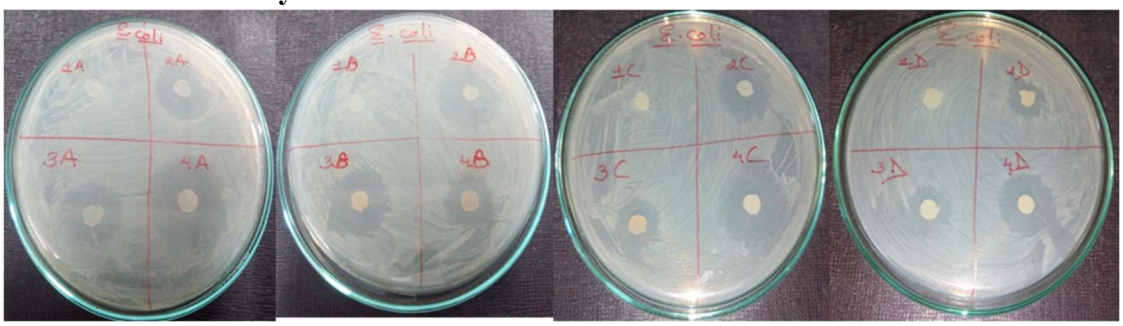

Fig.5. Anti-microbial activity zone against the E-coli bacteria

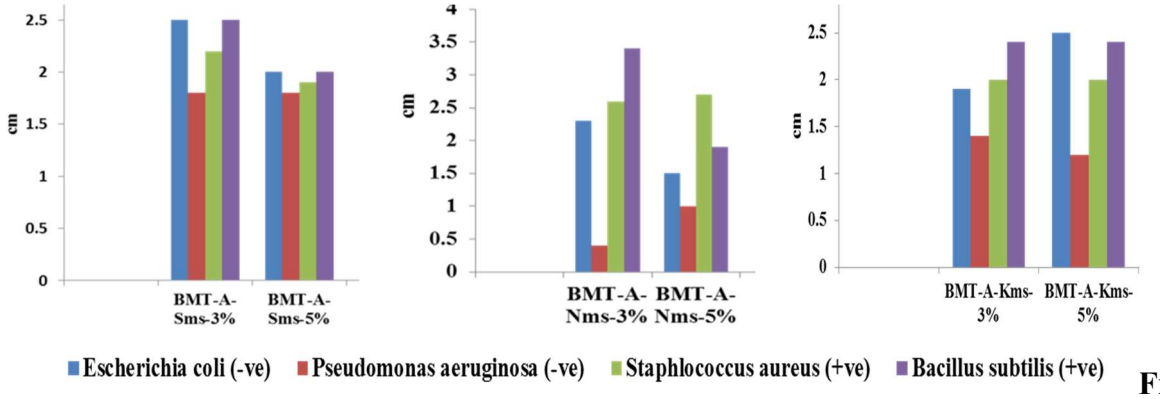

Fig.6. Antibacterial activity inhibition zone chart diagram of agarsoe clay with aminoglycoside film

0.5 mm of composite film was used to analyze against two gram negative bacteria (*Escherichia coli, Pseudomonas aeruginosa)* and two gram positive bacteria (*Staphylococcus aureus* and *Bacillus subtitles*) zone of inhibition for 24 h. Clay agarose film, before addition of drug, does not show any zone of inhibition against both type bacteria. However, when clay agarose film modified with drug showed the zone of inhibition. Among the drug modified clay agarose film composite studied, 5BMT-A-Kms accounted maximum zone of inhibition (2.5 cm) against the gram negative *Escherichia coli (-ve*) bacteria, 3 and 5% BMT-A-Sms, 3 and 5% accounted better zone of inhibition (1.8 cm) against the *Pseudomonas aeruginosa* (-ve) bacteria, 3 and 5 % BMT-A-Nms accounted better zone of inhibition against gram positive bacteria *Staphylococcus aureus (+ve)* (2.6 and 2.7

cm), and 3BMT-A-Nms film accounted against better zone of inhibition (3.4 cm) against Bacillus subtitles gram positive bacteria [10].

Conclusions

In conclusion, we prepared the bio film using naturally available Indian Bentonite clay as filler with agarose bio-polymer which intercalated with aminoglycoside antibiotic drugs. The 3BMT-A-Nms film proved the better result in combat against Bacillus subtitles gram positive bacteria.

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