TITLE: PLA film incorporated with amino functionalized graphene and their characterization

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ABSTRACT

Polylactic acid (PLA) films incorporating amine-functionalized graphene (AFG) were fabricated using the solution casting method to enhance their structural, thermal, and antimicrobial properties. FTIR analysis confirmed the successful integration of AFG into the PLA matrix, while XRD patterns indicated a semicrystalline nature with characteristic peaks for both PLA and AFG. TGA and DSC analyses demonstrated improved thermal stability, with AFG-PLA films exhibiting a higher glass transition temperature (62°C) and enhanced degradation resistance up to 325°C. DMA analysis revealed increased damping properties, indicating better thermo-mechanical performance. Antimicrobial evaluation showed significant activity against various bacterial strains, with MIC values as low as <0.01 µg/mL for Pseudomonas syringae. These findings suggest that AFG incorporation enhances PLA films' structural integrity, thermal stability, and antimicrobial efficacy, making them suitable for advanced biomedical and packaging applications.

Keyword *Polylactic acid (PLA) films, Antimicrobial, Antioxidant, Biodegradable films, Polymeric films*

1. INTRODUCTION

PLA is a non-toxic, fully biodegradable polymer that has been approved by the food and drug administration for use in biomedical applications. PLA doesn't have antimicrobial properties and is prone to infection during surgical implantation. Thus, PLA should be modified to improve its biocompatibility and to meet the demands of the tissue engineering materials. Xue zhao et al has surface functionalized the poly lactic film surface with zwitter poly[2-(methacryloyloxy)ethyl choline phosphate to increase its biocompatibility. Also, the antifouling property of the film was significantly increased as the modified surface could resist protein adsorption and inhibit bacterial adhesion due to zwitterionic property of PMCP and the antibacterial effect was more pronounced as the amount of PMCP grafting increased [1]. PLA is well known for its good processability, biocompatibility and biodegradability. However, some disadvantages render it inadequate for food packaging applications such as weak thermal stability, low toughness, poor gas barrier properties and low deformation at break.

Addition of natural extracts and herbal essential oils has been investigated to improve the functional properties of the PLA film in order to develop active packaging material that can inhibit microbial growth on foods [2]. Antibacterial materials based on PLA need to add antibacterial agents to the polymer matrix [3]. The cytotoxicity of the amphiphilic hybrid block copolymers was reduced by incorporating saccharide structures and also this incorporation of saccharides exhibited increased hydrophilicity, enhanced bioactivity. While the saccharides have been decorated on surfaces, the use of glycopolymers as the hydrophilic block of the amphiphilic copolymer has been less explored and the structure property relationships of these systems are not well understood. A library of HBC's composed of polyacrylamide-based glycopolymers with β -D-glucose pendant groups (hydrophilic blocks) were synthesized at varying HHB to determine the effect of the hydrophilic ratio on self-assembled morphologies and dye uptake [4].

Rachib ohib reported the synthesis of biodegradable amylose-g-PLA copolymers, which could be used as ex-situ produced compatibilizer agent in starch/PLA blends. The main objective of their work was to attain a structural control of these copolymers [5]. The drawbacks of PLA include high brittleness, narrow temperature-viscosity processing range and limited biodegradability, relative hydrophobicity, difficulty to modify. Three main approaches are known for PLA modification a). Incorporation of dispersed particles or low molecular weight and oligomeric substances. b). Copolymerization with other polymers c). Blending with other polymers. Studying the relationship between the properties of PLA and different polymers allows us to develop polymeric materials with improved properties compared to individual polymers and achieve synergy in the interaction of components that expand the scope of material application. Many researchers have tried to improve PLA properties by mixing with other polymers like Poly (ε-caprolactone), poly (glycolic acid), Poly(3-hydroxybutyrate); Poly(ethylene-co-vinylacetate). As a result of this mixing, a synergistic effect is obtained [6].

Unfortunately, most of the polymers are practically immiscible and the presence of interfacial interactions between polymer components play a important role in the structure and properties of the mixture [7]. PLA has been modified for use in biomedical, cosmetic and sanitary sectors by inserting antiseptic and anti-inflammatory nano-structured systems based on chitin-nano-fibrils, nano-lignin complexes [8]

Amine-functionalized graphene is reported to be used as a NO-generating coating on polylactic acid (PLA)-based bioresorbable stent materials [9]. Different chain length amino-functionalized graphene oxide in the PLA matrix were investigated to obtain biodegradable nanocomposite materials. The results show that the addition of modified GO to PLA improves its barrier properties and can be used in food packaging applications [10].

PLA, and PLA incorporating cardanol and amino functionalized graphene fabricated using the solution casting method. This composite film shows multifunctional properties and also enhances the shelf life of mangoes for 15 days [11] In this work, PLA based blends were made using amino functionalized graphene. Using solvent casting method, the prepared blends were then cast into films. Chemical structure, thermo and mechanical characterization of the films was carried out and later, their biological activity was evaluated.

2. Materials and methods

PLA Ingeo 2003D was obtained from Nature Tech Private LTD, Chennai, India. The Amine functionalized graphene (AFG) (purity 99% NH_2 ratio: ~2-5, width 5-10 µm and thickness 5-10 nm) was obtained from Ad-Nano Technologies, Shimoga, Karnataka, India. Chloroform (AR grade), sodium hydroxide, and phenolphthalein (indicator) from Sisco Research Laboratories Pvt. Ltd, Mumbai, India.

2.1. Preparation of PLA and AFG composite films

PLA modified with AFG films were prepared using the solution casting method. This involved adding 3g of PLA to 100mL of chloroform solution and stirring for 3 hours. After three hours, AFG (1g) was added and stirred for 30 minutes. The solution was subjected to ultrasonication using a bath sonicator to disperse AFG uniformly in the PLA solution. The resulting mixture was cast into a glass mold (20X15cm) coated with Teflon sheet and allowed to dry at room temperature. A neat PLA film was also prepared using same method. Hereafter, the films shall be denoted as, PLA 100% (TSK-1) , PLA 99% ; AFG 1% (TSK-2), films (Table 1). The films were conditioned at room temperature for 48 hours as per ASTMD 618-00 prior to analysis. Films were analyzed for their, thermo-mechanical, thermal, characteristics. Thickness was measured by a thickness gauge meter (Mitutoyo Model: S1012X) average of 10 readings at different place are taken and reported.

2.2. ATR–Fourier transform infrared spectroscopy of PLA-based films

The ATR-FTIR analysis of film samples was performed using the transmittance mode (Perkin Elmer Spectrum 100) in the 4000cm^{-1} to 500cm^{-1} spectral region. The wavelength of the light absorbed, seen in the annotated spectrum, is a property of chemical bonding.

2.3. XRD analysis for PLA-based films

The influence of AFG on the crystalline properties of PLA films was measured with an XRD diffractometer (PANalytical Xpert Pro XRD diffractometer, Amsterdam, Netherlands). The XRD was operated at 40 kV and 30 mA.

2.4. Differential scanning calorimeter for PLA-based films

DSC analysis of PLA, PLA-AFG, composite films was performed using a TA Instrument, DSC Q100, in a heat/cool/heat mode. At a heating rate of 5ºC/min, the sample was heated from 30ºC to 250ºC, and then cooled to -20ºC at a heating rate of 5ºC/min. Glass transition (Tg), melt transition (Tm) and crystallization (Tc), temperatures were evaluated with the TA universal analysis software.

2.5 Thermalgravimetric analysis for PLA-based films

A thermogravimetric analyser (TAQ500 model) was used to determine the thermal stability of PLA and PLA - AFG inclusion films. Under nitrogen ambient conditions, the films were heated at a rate of 5°C/min from 30°C to 750°C. The sample weight for analysis was approximately 10mg.

2.6 Dynamic mechanical thermal analysis for PLA-based films

Thermo-mechanical properties of films were measured by a TA Instrument, Q800, dynamic mechanical analyzer (DMA) in dual cantilever mode. The film samples were tested at a temperature range of 30 to 120°C with a frequency of 1 Hz and at a heating rate of 5°C/min.

2.7 Antimicrobial activity of the PLA and PLA- AFG incorporated films

The antimicrobial activity of films was evaluated against various bacterial strains using the broth microdilution method, following standard protocols recommended by the Clinical and Laboratory Standards Institute. The tested bacterial strains included *Salmonella enterica* MTCC 1164, *Salmonella enterica* MTCC 9844, *Bacillus cereus* MTCC 1272, *Listeria monocytogenes* MTCC 657, *Staphylococcus aureus* MTCC 3103, *Clostridium perfringens* MTCC 450, and *Pseudomonas syringae* MTCC 1604, all obtained from the Microbial Type Culture Collection and Gene Bank (MTCC), CSIR-Institute of Microbial Technology, Chandigarh, India. The bacterial cultures were stored at -20°C and revived by overnight incubation before testing. Suspensions were standardized to the McFarland 0.5 scale (~1×10^8 CFU/mL) and diluted to 3×10^5 CFU/mL using sterile NaCl solution. The films were dissolved in solvent, and serial two-fold dilutions ranging from 128 µg/mL to 2 µg/mL were prepared in cation-adjusted Mueller-Hinton (CAMH) broth. Microdilution assays were performed in 96-well microplates, with each well containing 100 µL of the bacterial suspension and 100 µL of the film solution. Plates were incubated at 37°C for 24 hours. The Minimum Inhibitory Concentration (MIC) was determined by measuring the optical density at 450 nm using a microplate spectrophotometer. MIC values were defined as the lowest concentration of the film solution that inhibited bacterial growth by 50% compared to the untreated control.

2.8 Antioxidant activity of the PLA and PLA- AFG incorporated films

The antioxidant activity of the samples was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay, a standard method for evaluating the hydrogen or electron-donating potential of compounds. The samples (*TSK-1* and TSK-2) and ascorbic acid (used as a control) were dissolved in methanol to prepare appropriate stock solutions. A 0.1 mM DPPH solution was freshly prepared in methanol and protected from light. In a 96-well microplate, 100 µL of each sample at varying concentrations was mixed with an equal volume of DPPH solution. The reaction mixtures were incubated in the dark at room temperature for 30 minutes, and the absorbance was measured at 517 nm using a spectrophotometer. Methanol served as a blank, and a control without samples (containing only DPPH and methanol) was used to determine maximum absorbance. The percentage scavenging activity was calculated, and the EC_{50} values (the concentration of the sample required to scavenge 50% of the DPPH radicals) were determined from the scavenging activity versus concentration graph.

The percentage of DPPH free radical scavenging activity was calculated using the formula:

Scavenging Activity (%) = (Absorbance of Control−Absorbance of Sample) / Absorbance of Control) ×100

3. Results and discussion

Polymer along with AFG fillers into PLA to create films, which were fabricated using the solution casting method. The effects of AFG fillers on the structural, mechanical, thermal, and thermomechanical properties of PLA films were analyzed using various techniques.

Table -1: Preparation of PLA and PLA - AFG films

S.NO	PLA Wt%(g)	AFG	Code	Solvent
1	100 (3)	0	TSK-1	100ml
2	99(2.97)	1(0.03)	TSK-2	100ml

3.1 FTIR analysis of the PLA and PLA- AFG incorporated films

The FTIR analysis of the film samples was performed in the spectral region of 4000 cm^{-1} to 500 cm^{-1} to confirm the functional groups in the films. Figure 1 presents the FTIR spectra of PLA (TSK-1), *PLA- AFG*(TSK-2). The PLA film exhibits hydroxyl group absorption properties between 3500 and 3200 cm^{-1}. The peak observed between 2995 and 2945 cm^{-1} corresponds to C-H stretching vibrations. A strong peak at 1746 cm^{-1} is attributed to the ester group. The -CH_3 deformation vibrations were recorded at 1454 cm^{-1} and 1360 cm^{-1}, along with C-O stretching vibrations at 1130 cm^{-1} and 1088 cm^{-1}. Additionally, a peak at 872 cm^{-1} was corresponding to the amorphous phase of -C-C- stretching [12]. For AFG, two prominent bands appeared at 1265 and 1545 cm^{-1}, corresponding to $C_{sp}{}^{2}$–N and N–H bending vibrations. Madh [13] polymer loaded and successfully blended all composites in films.

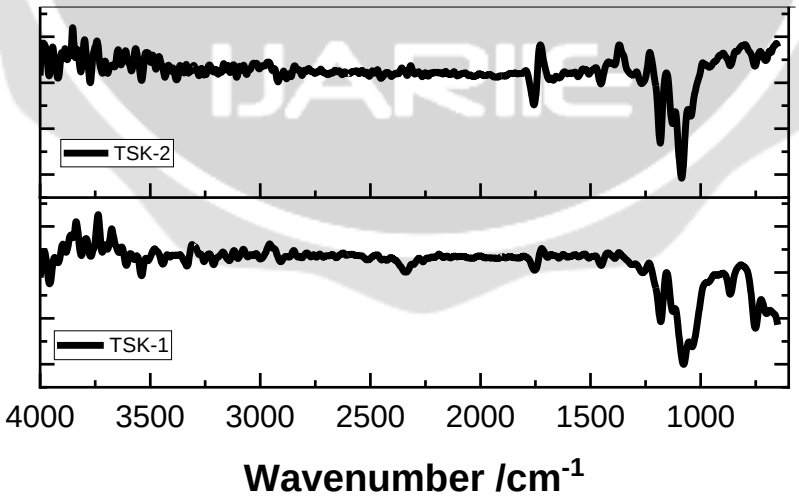

Fig-1: FTIR spectra of AFG and PLA films.

3.2 XRD analysis of the PLA and PLA- AFG incorporated films

The diffraction pattern and crystallinity of the AFG and PLA films were examined using X-ray diffractograms (XRD). As shown in Fig. 2, the XRD results indicating that PLA, as well as AFG, are semicrystalline in nature. It shows both broad and crystalline diffraction peaks. The XRD pattern of PLA exhibits its strongest diffraction peak at 2θ = 16.9°, corresponding to the (200) reflection of the α-form crystals, along with another diffraction peak at 2θ = 19.0°, which is attributed to the crystal structure of PLA [14]. Furthermore, the AFG -modified PLA films exhibit a distinct peak at 26°, corresponding to the graphene (AFG) crystalline structure, along with the PLA peak at 16.8° [15]. The XRD results, with varying diffraction peak intensities, confirm the presence of AFG, PLA, in the cast films.

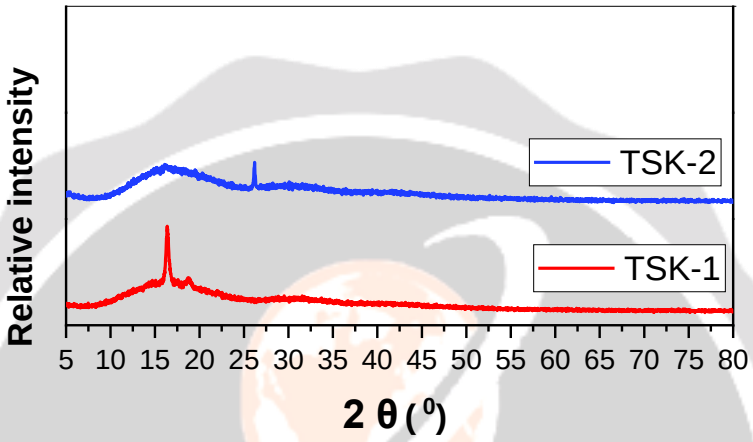

Fig-2. XRD patterns of AFG and PLA films.

3.3 TGA analysis of the PLA and PLA- AFG incorporated films

The thermal properties of pristine AFG and AFG-PLA films were analyzed using TGA, and their thermograms are shown in Fig. 3. The thermograms shown an approximate 5% weight loss around 100 °C, indicating small amount of water absorption in the films. The thermal degradation of AFG and AFG-PLA films occurs in a single step weight-loss, as evident from the DTG curve. Incorporating into the PLA matrix significantly improves the thermal stability of the blends. The thermograms of neat PLA demonstrate good thermal stability up to 250 °C. However, incorporating AFG into the PLA matrix significantly enhances thermal stability, with the modified films exhibiting excellent stability in the range of 300–325°C [16]. These results indicate that the addition of AFG effectively improves the stability of the polymer films.

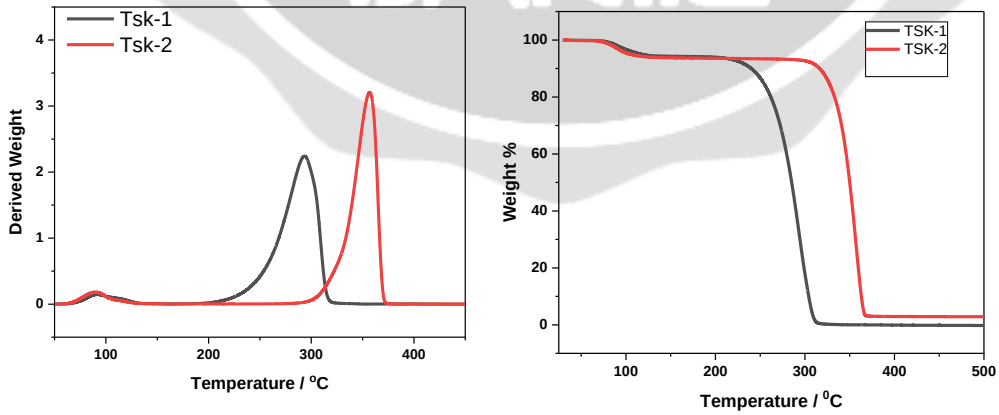

Fig-3: TGA thermograms of AFG and PLA films.

3.4 DSC analysis of the PLA and PLA- AFG incorporated films

The impacts of AFG on the thermal characteristics of PLA films were studied using DSC analysis, and the thermographs are shown in Fig. 4. The glass transition temperature (T_g) of the pure PLA film is 59 °C, while that of

the PLA-AFG film is 62 °C. The addition of AFG to the PLA film causes a slight shift in T_g to a higher temperature. This shift can be attributed to the amino groups of AFG forming strong interactions, such as hydrogen bonding or covalent linkages, with the PLA matrix. These interactions restrict the mobility of PLA polymer chains, increasing the energy required for the glass transition. Both films exhibited a crystallization temperature (T_c) in the range of 100–120 °C. The PLA exhibited two distinct melting transition temperatures (T_m) at approximately 140 °C and 150 °C due to the presence of polymorphic crystals and recrystallization during heating [17].

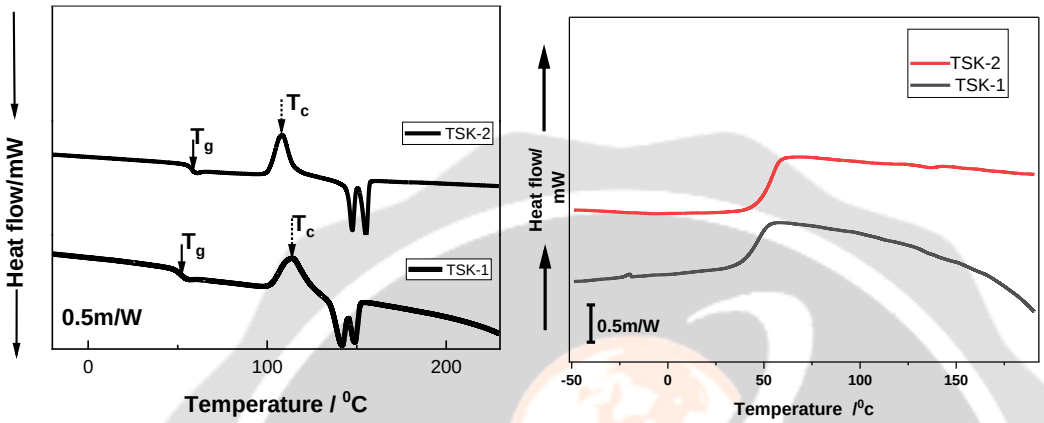

Fig-4: DSC thermograms of AFG and PLA films.

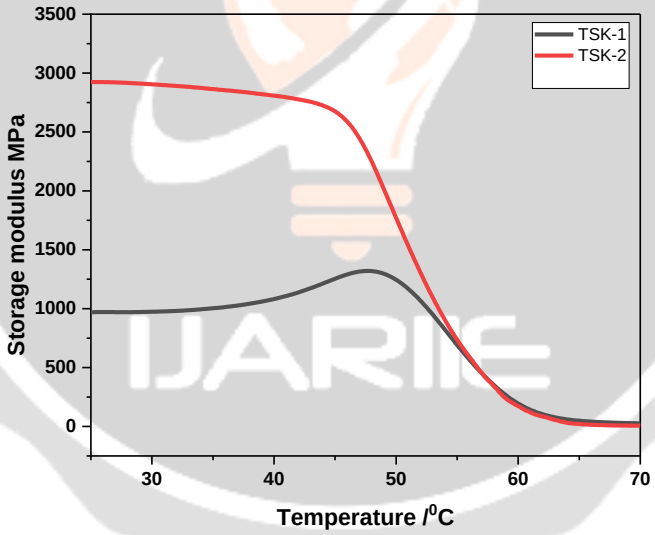

Fig-5: DMA analysis of the films TSK-1 and TSK-2

3.5 DMA analysis of the PLA and PLA- AFG incorporated films

The thermo-mechanical behavior of PLA and AFG-incorporated PLA films was determined using a DMA analyzer. Fig. 5 represents the fabricated films' temperature versus storage modulus (E'). Neat PLA film shows the glass transition temperature region at 58°C, and these results agree with the DSC thermographs (Fig 5). AFG-incorporated PLA films showed increased damping properties compared to PLA.

3.6 Antimicrobial activity of the PLA and PLA- AFG incorporated films

The antimicrobial activity of films was evaluated against various bacterial strains using the broth microdilution method, and MIC values for each bacterial strain were recorded. Results were compared to those of ciprofloxacin as

a reference standard drug. The results demonstrated that the films exhibited significant antimicrobial activity, with MIC values ranging from <0.01 µg/mL to 4 µg/mL, depending on the bacterial strain. The films showed strong activity against *Pseudomonas syringae*, with MIC values as low as <0.01 µg/mL, while relatively higher MIC values were observed for *Clostridium perfringens* and *Listeria monocytogenes* (>10 µg/mL in some cases). When compared to ciprofloxacin, the films displayed comparable or superior activity against certain strains, such as *Salmonella enterica* and *Staphylococcus aureus*. The films dissolved in methanol showed significant antimicrobial activity against a range of bacterial strains, indicating their potential as effective antimicrobial agents.

Table 2: Antimicrobial Activity of films

Pathogen	**TSK-1**	**TSK-2**	**Ciprofloxacin**
Salmonella enterica MTCC 1164	0.06	2	<0.01
Salmonella enterica MTCC 9844	<0.01	0.06	0.06
Bacillus cereus MTCC 1272	0.01	0.25	0.52
Listeria monocytogenes MTCC 657	0.25	>10	0.25
Staphylococcus aureus MTCC 3103	0.06	<0.01	0.25
Clostridium perfringens MTCC 450	0.68	>10	4
Pseudomonas syringae MTCC1604	<0.01	0.25	<0.01

3.7 Antioxidant activity of the PLA and PLA- AFG incorporated films

The antioxidant activity of the samples was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay and the corresponding results are shown in Table 3. The results revealed significant antioxidant activity among the tested samples, with EC_{50} values ranging from 25.8 µg/mL to 85.7 µg/mL. *TSK-1* (25.8 µg/mL) demonstrated the stronge antioxidant activity, comparable to ascorbic acid (30.98 µg/mL). The findings indicate that the tested samples possess varying degrees of free radical scavenging potential, with *TSK-1* showing good efficiency. These results highlight the potential application of these samples as antioxidant agents in various packaging applications.

Table 3: DPPH Radical Scavenging Activity

Sample (µg/mL)	DPPH Free radical scavenging activity EC50 Values
TSK-1	25.8
TSK-2	45.2
Ascorbic acid (Control)	30.98

4. CONCLUSIONS

PLA and AFG incorporated films were successfully prepared by solution casting method. The chemical and thermal characterization of the films was done using FT-IR, XRD, DSC, TGA, DMA. The FT-IR analysis reveals successful blending of the AFG with PLA. The XRD results confirm the presence of AFG incorporation with PLA in the cast film. The TGA curves imply that incorporation of AFG has increased the thermal stability of PLA films. The antibacterial activity and DPPH Free radical scavenging activity of the films was performed. The films dissolved in methanol showed significant antimicrobial activity against a range of bacterial strains, indicating their potential as effective antimicrobial agents. The findings indicate that the film samples possess good free radical scavenging potential. These results highlight the potential application of these samples as antioxidant agents in various packaging applications.

5. REFERENCES

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